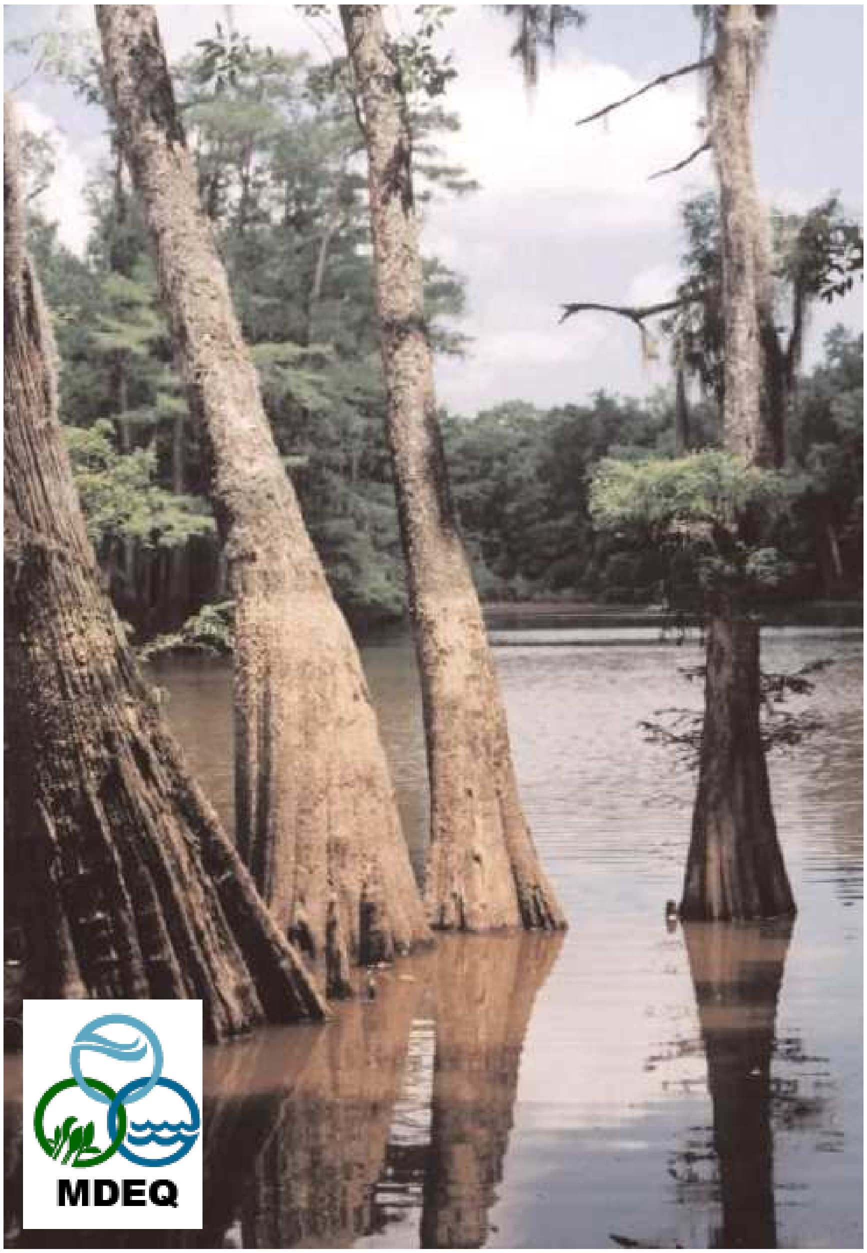
Nutrient Criteria to Protect Aquatic Life Uses in Mississippi Non-Tidal Streams and Rivers



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June 8, 2009

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Contractual Agreement Number MDEQ-01-ID-0001FTN/WLBE between Mississippi Department of Environmental Quality (MDEQ) and FTN/WLBE Joint Venture

MDEQ Work Order No. 06-02FTN/WLBE-008**, Tetra Tech Project No. 20758**

June 8, 2009

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ACKNOWLEDGEMENTS

The project was completed for the state of Mississippi Department of Environmental Quality (MDEQ) under Contractual Agreement Number MDEQ-06-ID-02FTN/WLBE between the MDEQ and FTN/WLBE Joint Venture (work order number 06-02FTN/WLBE-008). The primary authors are Lei Zheng and Michael J. Paul of Tetra Tech, Inc., Center for Ecological Sciences in Owings Mills, MD. Benjamin K. Jessup and James B. Stribling from Tetra Tech, Inc. were also involved in part of the work. Kim Caviness, Valerie Alley, and Natalie Segrest provided solid technical input and review. All field work was performed by MDEQ staff.

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EXECUTIVE SUMMARY

In response to the environmental threat posed by nutrients, EPA has requested that states develop numeric criteria to protect designated uses from impairment due to excessive nutrients. The State of Mississippi implemented this project to aid in the development of numeric nutrient criteria for non-tidal wadeable and non-wadeable streams within the State. EPA recommended three methods to establish nutrient criteria (USEPA 2000): a frequency distribution reference-based approach, a stressor-response approach, and literature-derived values. In this report, we used a weight of evidence approach, combining these three methods to derive candidate nutrient criteria from we selected recommended criteria based on the currently available data.

First, we collected and compiled data for streams in Mississippi available from seven different sources. These datasets included nutrients and other related water quality parameters, as well as biological assemblage information, i.e., algal, benthic macroinvertebrate, and fish biomass and composition. Appropriate QA/QC was further performed to assess the quality of the data and condense the data into three separate datasets for wadeable streams [the Mississippi Benthic Index of Stream Quality (M-BISQ) project dataset, Mississippi Department of Environmental Quality (MDEQ) WADES database dataset, and a combined M-BISQ and WADES dataset] and one dataset for non-wadeable streams. Due to a limited number of algal data, macroinvertebrate data for M-BISQ development was used as the primary biological response data for stressor response analyses. Other datasets were used to derive benchmarks using frequency distribution reference approaches.

We classified streams in the State based on bioregional classification to reduce variability. Preliminary analysis indicated that bioregional classification provided better resolution than level III ecoregions. Also, bioregional classification provided more reference sites for ecoregion 75 and thus strengthened the criteria development for this region. The most important advantage was that biological criteria (i.e., M-BISQ scores) have been determined for these bioregions, so stressor criteria could be linked to designated uses for each bioregion.

Three reference condition groups were defined according to Stoddard et al. (2006). The minimally disturbed condition (MDC) was extrapolated using regression equations of nutrient concentrations and human land uses in a watershed (Dodds and Oaks 2004). The least disturbed condition (LDC) represents a baseline that should protect assigned designated uses. For the purpose of nutrient criteria development, we used three different approaches to define the LDC. First, LDC was defined using the same criteria used to develop the M-BISQ, which was based on regional land use, stream physical habitat, and chemical characteristics. However, we excluded nutrient parameters. The second LDC set was selected based on land use in the surrounding watershed, stream buffer, and local habitat alone. These selection criteria eliminated anthropogenic nutrient loadings from land use/land cover changes, but treated other environmental stressors as natural. When information about the LDC was not available for a dataset, we used a third LDC method: the 25th percentile of a distribution of samples from the entire population of waterbodies within a given physical classification, which served as a surrogate for the 75th percentile of a sample distribution from LDC sites. The third reference condition set, best attainable condition (BAC), was defined using the biological criteria defined by M-BISQ scores for each bioregion (lower quartile of M-BISQ07 scores). Sites attaining the biological criteria were defined as the BAC group.

Nutrient benchmarks derived from different reference approaches varied across different bioregions. Generally speaking, nutrient benchmarks of MDC derived from land use extrapolation were much lower than that of LDC and BAC conditions. Although the criteria-based reference approach is preferred to define LD reference conditions, it was restricted by the availability of LD sites in a region. Benchmarks of BAC were similar to that of LDC in most regions, and in some cases are a little higher than LDC, as we expected. As for development of recommended nutrient criteria, criteria values should likely not exceed the BAC benchmarks.

Stressor-response relationships are a critical part of criteria development as they provide direct links to use measures. Algal biomass (Chl *a* in water column) in streams did not respond to elevated nutrient concentrations. However, macroinvertebrate metrics, which provide an indirect measure of nutrient effects, did. After a strong correlation was found between macroinvertebrate metrics and nutrient parameters, we used a conditional probability approach (CPA) to idently changes in the biological community along stressor gradients (Paul and MacDonald, 2005). We also used nonparametric deviance reduction (change point analysis) to identify ecological thresholds (Qian et al. 2003). According to the change point analysis, thresholds in M-BISQ index response to nutrient concentrations were similar to thresholds derived from CPA. We used the lower 95^{th} confidence interval of the change point estimate as benchmark for nutrient criteria development since the lower confidence limits reflected a conservative estimate of the change point.

Literature derived nutrient criteria were mostly within the same range of criteria from our analyses. Relatively few studies have been conducted in the state of Mississippi. Ray Montgomery and Associates (RMA, 2005) conducted a Nutrient Data Analysis for Pascagoula TMDLs under a MDEQ contract based on the M-BISQ 2001 dataset, and recommended the use of a TP range from 0.07 – 0.11 mg/l as the preliminary target. Other states in the same ecoregion also conducted similar studies to derive nutrient endpoints for TMDLs, but only Tennessee has developed statewide nutrient criteria. Their recommended criteria for the same region were similar to TP benchmarks developed here.

The different approaches resulted in similar candidate nutrient criteria in various regions of Mississippi. In regions with relatively large sample sizes and biological responses, e.g. East Bioregion, TN and TP criteria are likely more reliable due to the high degree of agreement among different approaches. For regions with relatively small sample sizes, we recommended a range of nutrient concentrations and recommend strengthening the criteria when more data become available.

- East Bioregion - **TN: 0.65 mg/L and TP: 0.050 mg/L**
- Southeast Bioregion - **TN: 0.540 mg/L and TP: 0.035 mg/L**
- West Bioregion
    - ecogroup 1 – **TN: 0.700-0.800 mg/L and TP: 0.080-0.100 mg/L**
    - ecogroup 5 – **TN: 0.533-0.800 mg/L and TP: 0.060 mg/L**
- South Bluff Bioregion - **TN: 0.582-0.810 mg/L and TP: 0.060-0.080 mg/L**

- Non-Wadeable streams - **TN: 0.900 mg/L and TP: 0.090 mg/L**

We developed nutrient benchmarks by combining results of several approaches recommended by EPA and adopted by various States and regions to derive nutrient criteria. However, we stress that these benchmarks were derived using indirect response indicators. Due to data limitations, direct causal response variables could not be used at this time.

1.0 BACKGROUND

Nutrients are a natural component of healthy ecosystems. In natural concentrations, essential nutrients help maintain the structure and function of ecosystems. However, in excessive quantities, nutrients can destabilize natural ecosystems leading to a variety of problems including nuisance plant growth, hypoxia and anoxia, species loss, and risks to human health.

Nutrients affect aquatic systems in diverse ways. The direct effects are on the primary producers, namely, algal and macrophyte production and species composition. The effects on most non-primary producer aquatic life are indirect (Figure 1.1).

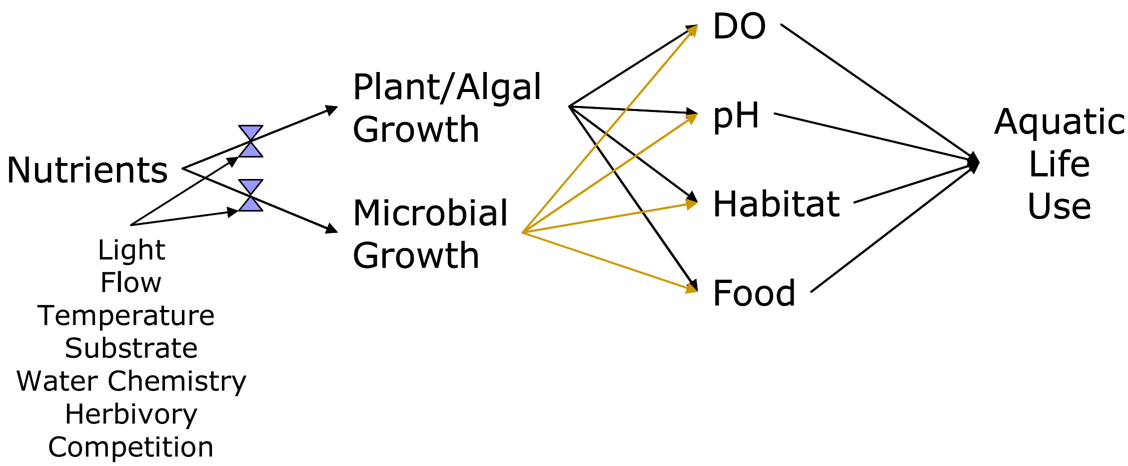

Figure 1.1 Simplified diagram illustrating the causal pathway between nutrients and aquatic life use impacts. Nutrients enrich both plant/algal as well as microbial assemblages, which lead to changes in the physical/chemical habitat and food quality o of streams. These effects directly impact the insect and fish assemblages. The effects of nutrients are influenced by a number of other factors as well, such as light, flow, and temperature.

Nutrients increase the growth of primary producers and decomposers which leads to changes in the physical and chemical stream environment (e.g., reduced oxygen, loss of reproductive habitat, alteration of the food base for aquatic animals, etc.). These effects result in changes to the biological stream community (e.g., loss disturbance to sensitive taxa), and can ultimately impair the use of a stream for aquatic life.

In response to the environmental threat of nutrient overenrichment, EPA has requested that states develop numeric nutrient criteria to protect designated uses from impairment due to excessive nutrients. Nutrient criteria are developed to protect designated uses and, as such, the applicable designated uses are integral to guiding the appropriate criteria. Nutrients principally threaten aquatic life, recreational, and drinking water uses. Aquatic life uses are threatened when nutrients actually impair plant communities and result in the proliferation of nuisance or invasive taxa or cause excessive growth of algae, which alters the habitat (physical habitat, dissolved oxygen, etc.) for other aquatic life. Recreational uses are threatened when nutrients cause growth of plant taxa that interfere with fishing, swimming, or other recreational uses of streams and rivers. Lastly, drinking water uses are impaired when nutrients cause the proliferation of taxa that generate taste and odor problems in drinking water, produce toxic compounds, or, potentially, overwhelm filtration systems.

EPA has developed recommended regional nutrient criteria, but they have encouraged states to pursue their own nutrient criteria development programs. The state of Mississippi has committed to the development of scientifically defensible nutrient criteria to protect designated uses in its waterbodies. The objective of this study is to recommend criteria that are protective of aquatic life uses for streams and rivers in Mississippi based on the data currently available.

2.0. CURRENT STATUS OF NUTRIENT CRITERIA FOR STREAMS AND RIVERS IN MISSISSIPPI

The U.S. Environmental Protection Agency (EPA), in its recommendations for nutrient criteria development, specified that "ecoregional nutrient criteria will be developed to account for the natural variation existing within various parts of the country" (USEPA, 2000). They go on to explain the importance of ecoregions:

"Ecoregions serve as a framework for evaluating and managing natural resources. The ecoregional classification system developed by Omernik (1987) is based on multiple geographic characteristics (e.g., soils, climate, vegetation, geology, land use) that are believed to cause or reflect the differences in the mosaic of ecosystems."

Ecoregions denote areas of general similarity in ecosystems and in the type, quality, and quantity of environmental resources. They are designed to serve as a spatial framework for the research, assessment, management, and monitoring of ecosystems and ecosystem components. There are 4 level III ecoregions [Southeastern Plains (65), Mississippi Alluvial Plain (73), Mississippi Valley Loess Plains (74), and Southern Coastal Plain (75)] and 21 level IV ecoregions in Mississippi (Figure 2.1). Most of these ecoregions continue into ecologically similar parts of adjacent states. Ecological and biological diversity within Mississippi is great. The state contains barrier islands and coastal lowlands, large river floodplain forests, rolling and hilly coastal plains with evergreen and deciduous forests, and a variety of aquatic habitats (ftp://ftp.epa.gov/wed/ecoregions/ms/ms_eco.html). Mississippi Alluvial Plain (73) has very special geographic and land use patterns; therefore, this ecoregion was not included within this stage of nutrient criteria development.

MDEQ conducted statewide biological monitoring using benthic macroinvertebrates as an indicator of biological integrity for wadeable streams (MDEQ, 2003a). The primary intent of this effort was the development of a credible and scientifically-defensible biological assessment tool to be used in the assessment of Mississippi's wadeable streams and rivers, the Mississippi Benthic Index of Stream Quality (M-BISQ). This index was then used in the biological assessment of the State's wadeable streams and rivers. It should also be noted that this index is not applicable to wadeable streams within Ecoregion 73.

Recently, Tetra Tech, Inc. conducted a new analysis to recalibrate the M-BISQ (MDEQ 2007a). In this round of analysis, the State was divided into 4 different bioregions according to macroinvertebrate assemblages (Figure 2.1). These bioregions encompass seven different ecogroups with different environmental characteristics. At the same time, biological indicators were also developed for non-wadeable streams (MDEQ 2007b). These efforts will be useful for linking nutrient concentrations to biological responses. To protect biological integrity within each bioregion, a nutrient criterion should be established for each bioregion so that nutrient criteria are related to aquatic life uses within each bioregion.

Currently, the state of Mississippi has no numeric or narrative criteria for total nitrogen or total phosphorus. Two nutrient compounds are regulated by MS Water Quality Standards (WQS): Ammonia and Nitrate (MDEQ 2003b). Ammonia can be potentially toxic to aquatic life under different pH and temperature levels and Mississippi uses the USEPA recommended ammonia criteria to protect aquatic life. Nitrate concentration above 10 mg/L is associated with increased risk of methemoglobinemia in human infants. As a result, the human health criterion for nitrate is 10mg/L for public water supply. In addition to these nutrient compounds, Mississippi's WQS also contain turbidity and dissolved oxygen criteria for all waterbodies. Further, MDEQ (2007e) has developed a nutrient criteria development plan for waters within the State.

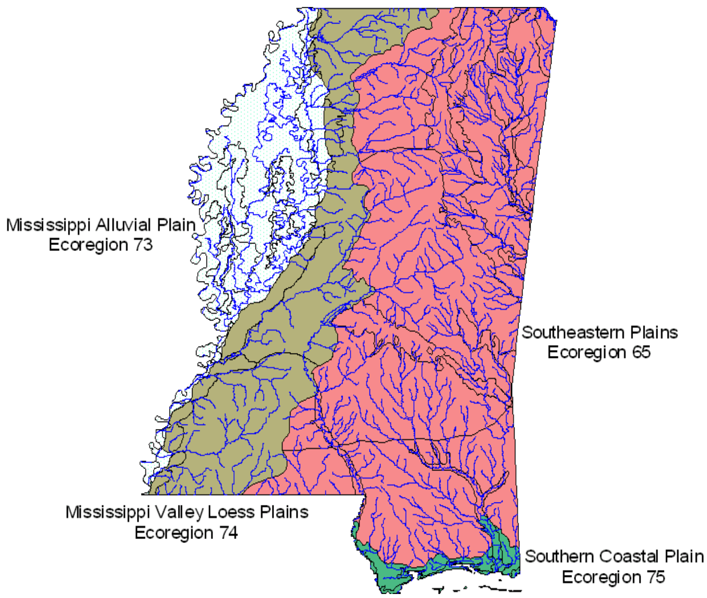

Figure 2.1 Major (Level III) ecoregions and new proposed bioregions within the State of Mississippi.

In addition to existing state water quality criteria, the USEPA have also recommended regional nutrient criteria for ecoregions in Mississippi. USEPA aggregated level III ecoregions into relatively homogeneous nutrient ecoregions according to background nutrient concentrations. The three level III ecoregions in Mississippi (excluding ecoregion 73) were aggregated into one single nutrient ecoregion: Region IX (Southeastern Temperate Forested Plains and Hills). The recommended nutrient criteria for streams in this region are: TP 0.0366 mg/L and TN 0.69 mg/L (USEPA 2000b).

In addition to the state water quality standards, nutrient TMDLs have been developed for individual basins (e.g. Pascagoula watershed). A preliminary study to develop TP targets for the Pascagoula Basin in Mississippi has been conducted and proposed (RMA 2005). Although this study primarily focused on the Pascagoula Basin and utilized only 2001 M-BISQ data, it examined the statewide nutrient classification strategies and developed a TP target using a reference-based approach. A TP concentration of 0.07 – 0.11 mg/l was proposed as the preliminary target.

3.0 APPROACHES

Traditionally, water quality criteria to protect aquatic life use were developed using toxicological approaches. Such approaches have been applied for a range of pollutants to develop water quality criteria. However, as explained above, nutrient enrichment does not have a direct toxicological effect. Instead, nutrients alter the diversity and composition of algal and plant aquatic life. For insects, fish, and other aquatic life, the mode of action of nutrients is indirect and through a causal pathway that involves alteration of physical, chemical, and biological attributes of their habitat. As a result, traditional toxicological approaches are not appropriate.

EPA has recommended three methods to establish numeric nutrient criteria (USEPA 2000): a reference-based approach, a stressor-response approach, and literature-derived values. The reference approach uses two principal methods. The first method is to derive criteria from the population of ambient nutrient concentrations observed in reference sites. This first method has been commonly used to develop biocriteria and nutrient criteria, including EPA recommended regional nutrient criteria (Dodds et al. 1998, USEPA 2000, Seip et al. 2000, Dodds and Welch 2000, Rohm et al. 2002, Ice and Binkley 2003). The second reference approach method is to estimate reference conditions by empirical modeling either through land cover – nutrient concentration models solved for the condition of zero percent human land cover (Dodds and Oaks 2004), or building reference condition regression models based on multiple natural predictors (Smith et al. 2003, Sheeder and Evans 2004). Either reference approach method requires appropriate classification in order to establish appropriate criteria for different waterbodies (Detenbeck et al. 2004, Snelder et al. 2004, Wickham et al. 2005).

The second approach establishes nutrient criteria based on stressor-response relationships. It has been argued that reference approaches using a percentile of ambient nutrient concentrations within a waterbody class alone to establish criteria can lack a direct linkage to designated use protection (Dodds and Welch 2000, McMahon et al. 2003). Aquatic life uses are one of the uses most commonly targeted for protection by numeric nutrient criteria. Stressor-response approaches derive criteria based on relationships between aquatic life measures and nutrient concentrations. Fortunately, biological assessment has been shown to be an efficient way to protect aquatic life uses (Barbour et al. 1996, 1999, 2000, King and Richardson 2003), and the indicators that are developed provide a direct measure of aquatic life use condition. As a result, correlation or regression analyses that directly relate eutrophication (stressor) to biological indicators or other valued aquatic life use attributes provide strong justification for ecologically meaningful criteria. Establishment of nutrient criteria using stressor-response approaches has relied on algal biomass and algal community indicators, among others (Welch 1988, Stevenson 1997, Biggs et al. 2000, Havens 2003). In addition, experimental approaches have been used to establish or verify the cause and effect relations between algal assemblages and potential nutrient endpoints (Havens and Aumen 2000). There is no reason that other response measures related to other uses could not also be used in such an analytical framework. For instance, indicators derived from recreational user perception surveys can also be related to nutrient concentrations and stressor response analysis used to develop criteria that protect recreational use.

The third approach is based on deriving criteria from existing literature for the same or similar regions. This approach recognizes that the limnology with regards to nutrient impacts of many systems has been well investigated in the research literature and that this literature provides another important source of guidance in developing protective nutrient criteria. This approach also includes the use of mechanistic models to develop nutrient criteria. In many regions, nutrient data are limited or not available, and interactions among multiple factors are difficult to incorporate into statistical models. In this case, mechanistic modeling approaches can be applied to establish water quality criteria for many streams and lakes (Somlyody 1997, 1998, Dodds et al. 2002, Reckhow et al. 2005). The modeling approach has been principally used for site specific criteria, since site specific predictors are generally used.

We used a weight of evidence approach that incorporates all three approaches to develop suggested nutrient criteria. The weight of evidence approach is actually the recommended strategy for states to develop scientifically defensible criteria (USEPA 2000). The endpoints derived from each method are weighed for the strength of each analysis, based on data quality and relevance, using professional judgment. A recommended candidate criterion is selected that balances these weights with the provision that the candidate criterion is assured to protect the use, and the process is documented so as to be transparent.

4.0 DATA ISSUES

4.1 Inventory of existing data for wadeable streams

To optimally utilize existing resources for nutrient criteria development for streams in Mississippi, a database with existing nutrient variables and other parameters was developed. This database (nutrient_database.mdb) includes nutrient data within the state of Mississippi from 6 different sources (Appendix A).

- EPA nutrient database: The National Nutrient Database stores and analyzes nutrient water quality data and serves as an information resource for states, tribes, and others in establishing scientifically defensible numeric nutrient criteria. It contains ambient data from the Legacy STOrage and RETrieval (STORET) data system, the US Geological Survey's National Stream Quality Accounting Network (NASQAN) data and National Water Quality Assessment (NAWQA) data, and other relevant sources such as universities and states/tribes. Data included in this table were from Jan.2, 1990 to June 29, 1997. The dataset had redundant records with the USGS Natural Water Information System (NWIS) database.

- USGS NAWQA program: The U.S. Geological Survey (USGS) began its NAWQA (National Water Quality Assessment) program in 1991, systematically collecting chemical, biological, and physical water quality data across the nation. The data warehouse contained data up through 9/30/2006. The most important data from Mississippi in this database were the biological community data for fish (29 samples from 11 sites), algae (28 samples from 3 sites) and invertebrates (19 samples from 3 sites). Concurrent water chemistry measurements were also available with biological data in the NAWQA program, but more water quality for these sites was stored in the USGS NWIS system.

- USGIS NWIS data: The United States Geological Survey (USGS) collected water-resources data at 1874 sites across the state of Mississippi and stored them in NWIS. The USGS NWIS data were collected from 1943 to 2005 by various USGS programs. Variables varied among different programs, and data quality (e.g. detection limits) differed among different programs.

- USEPA STORET (short for STOrage and RETrieval) is a repository for water quality, biological, and physical data and is used by state environmental agencies, EPA and other federal agencies, universities, private citizens, and many others. It contained water quality information from a variety of organizations across the country, from small volunteer watershed groups to State and Federal environmental agencies. The majority of MS's historical water quality data is found in the STORET Legacy Data Center, and includes data collected from 1977 to 1998. Unfortunately, although more than 2000 sites in Mississippi were found in the database, only 21 of them had nutrient data available.

- Mississippi DEQ M-BISQ database: The M-BISQ database developed for storing biological monitoring data contained statewide macroinvertebrate, habitat, chemistry, and land use data for over 600 sites stored in an Access database. Data included in this dataset were collected from 2001 to 2004. This dataset was used as the main source of stressor-response data for nutrient criteria development.

- WADES database: MDEQ's surface monitoring program includes a number of monitoring networks and special studies. This dataset contained nutrient parameters from over 2339 sites from 1978 to 2005 and included the following:

    - Ambient Fixed Station Network: In this statewide monitoring network 61 stream stations were sampled monthly. Forty-one stations in reservoirs and estuaries were sampled on a quarterly basis. Biological sampling was carried out at 25 stations.
    - Basin Wide Network: Waterbodies in each Mississippi basin were sampled on a rotating five-year cycle. One of the five basin groups was targeted annually and sampling was carried out at an average of 80 sampling stations for each basin group. In addition, a one-time biological sample was collected for each basin group.
    - Beach Monitoring Network: Twenty coastal water quality stations were sampled on a routine basis. Nutrient data were collected along with fecal coliform data.
    - Special Monitoring Studies: Special monitoring was provided by funding from the Sections 104(b), 106, 604(b), 319, and the Gulf of Mexico Program Office. In addition, a five year program was underway to monitor coastal and estuarine marine waters under the Coastal 2000 program.

As described above, the nutrient_database.mdb contains all the data used in this report on nutrients and other water quality parameters, as well as biological assemblages, i.e., algal, benthic macroinvertebrate, and fish biomass and composition from the above 6 sources. Data quality of these datasets was not consistent across the different data sources. It was also difficult to identify the appropriate protocols and detection limits for the parameters measured over different years and regions. Therefore, appropriate QA/QC was needed before applying all these datasets to nutrient criteria development.

4.2 Data Quality Control/Quality Assessment

We assessed the quality of the datasets and decided to weigh our analysis mostly on the M-BISQ dataset since it was the most complete and documented dataset and contained the best biological information for stressor response analysis. We applied the following rules to control the quality of our datasets:

1. Only the most recent 15 years of data (from 1992 to 2006) were used for nutrient criteria development;
2. Minimum detection limits (MDLs) were frequently reported and samples flagged if they were below the MDL in the original datasets. Values below MDLs were analyzed as at the MDL which is one of the most common practices for similar statistical analysis;

3. Macroinvertebrate data from the M-BISQ database were the primary biological response variable for the stressor response approach. Macroinvertebrate metrics and new M-BISQ scores (MDEQ 2007a) were imported from the M-BISQ database. Related water chemistry data were extracted from the WADES database for sites with benthic macroinvertebrate samples. Land use, physical habitat, and other characteristics were imported from the M-BISQ database. Since the M-BISQ project had the most consistent data quality, it was considered the most valuable source for nutrient criteria development (650 sites) for the stressor-response approach.
4. Stream water chemistry samples in the WADES database (885 sites after excluding M-BISQ project data) from a variety of projects were extracted for use in the reference-based approach.
5. Since the other four datasets (NWIS, STORET, NAWQA, EPA Nutrient database) had redundant records and multiple sources, they were further assessed and combined into one single dataset and were used strictly for the reference-based approach. Data redundant with the WADES database were excluded, which left only 195 unique sites in this dataset.

In summary, we based our analyses on three separated databases, MDEQ M-BISQ dataset, the WADES dataset, and a multi-agency combined dataset.

The primary variables considered for nutrient criteria development were water column concentrations of TN, TP, water column and benthic algal biomass as chl *a*, and turbidity. Due to a lack of benthic algal biomass measurements, only TN, TP and turbidity were gathered during the data collection/database building. TN and TP are the primary causal variables most closely related to response variables in streams. Nitrate and Nitrite and orthophosphate were also considered in our analysis. However, due to a lack of strong correlation between these two variables and biological responses, they were not considered for criteria development. TN and TP were log-transformed in most circumstance in order to obtain normal distributed data. Algal biomass, as represented by chlorophyll *a* and turbidity, was also log-transformed. Although turbidity is not commonly used as an index of eutrophication in either lakes or streams, it nonetheless should increase in streams with increasing algal biomass due to nutrient enrichment. It was also log-transformed.

Indices employing macroinvertebrates as indicators of nutrient pollution have great potential because they are the most reliable and frequently used organisms for water quality assessment. Individual macroinvertebrate taxa respond to enrichment, and some are particularly sensitive. Individual metrics, such as EPT taxa, were used as response variables. The richness metrics were log-transformed if necessary and percent metrics were arcsine square-root transformed. M-BISQ scores were standardized values, and were not transformed in most circumstances.

4.3 M-BISQ Data

Given the importance of M-BISQ data to this analysis, it is worth reviewing those data. Interested readers should also consult MDEQ 2003a and 2007a.

4.3.1 Land use analysis

Land cover was calculated using a GIS analysis conducted by MDEQ. Land uses were discerned in seven categories using National Land Cover Database (NLCD) 2001 data as the source. The categories included water, forest, wetland, pasture/grass, cropland, scrub/barren, and urban. Water, forest, and wetland areas were considered natural land uses.

Areas upstream of the sampling location were delineated in four spatial arrangements: the whole catchment of the sampling location, the buffer zone 100m to either side of the stream channel throughout the upstream catchment, the buffer zone 100m to either side of the stream channel for a distance of one km upstream of the sampling point, and the buffer zone 50m to either side of the stream channel for a distance of one km upstream of the sampling point. Delineation was automated and corrected as needed so that sampling location coordinates always fell in the appropriate stream channel. USGS 12-digit subwatersheds were the starting point for upstream delineations. By cutting the subwatershed containing the sampling point along ridges determined by MDEQ 10 meter digital elevation models (DEMs), the near site drainage was delineated. All subwatersheds upstream from this were then selected and merged to delineate the entire catchment.

To simplify the analysis, land use characteristics upstream of each sampling location were summarized in two ways: as percentages of land uses in the entire watershed and as a weighted average of land uses in the three buffer zones. The weighting was accomplished by averaging the land use percentages over the three buffer zones. The closest buffer zone (less than 1km from the site and less than 50 meters from the stream) is part of all three buffer zones and is thus included three times in a simple average, giving it more weight than the buffer zones of intermediate (within 100m of a stream also within 1km of point outside of 100m buffer) or longest extent (Within 100m of a stream throughout the whole upstream catchment). Likewise, the intermediate buffer zone is part of the larger buffer zone and is therefore double-counted when included in the average. Land uses in the 100 m buffer more than 1 km upstream were only included with the largest buffer zone and therefore carry less weight in the average.

4.3.2 Physicochemical measurements

Data from a total of 650 discrete river and stream sampling stations across the State of Mississippi (excluding the Mississippi Alluvial Plain or MS Delta) were used. These stations were identified to represent a range of stream reaches according to biological health status, geographic location (selected to account for ecoregion, bioregion, basin and geologic variability) and streams that potentially receive non-point source pollution from urban, agricultural and silviculture lands as well as point source pollution from NPDES permitted facilities. Data included:

- Qualitative (visual observations) habitat assessment scores. Data were collected in winter from 2001 to 2004 according to MDEQ Standard Operating Procedures (2007c) and are described in detail in the Quality Assurance Project Plan (QAPP) for 303(d) List

Assessment and Calibration of the Index of Biological Integrity for Wadeable Streams in Mississippi (MDEQ, 2001). Ten habitat parameters describing instream habitat, bank, and riparian conditions were visually assessed and rated on a scale from 0 to 20 with 0 being the poorest habitat and 20 being optimal. Habitat assessments were performed on the same 100-meter reach from which macroinvertebrate samples were collected. Duplicate and repeat habitat assessments were performed at 70 randomly chosen sites. Individual habitat parameters were also summed into three subcategories describing stream characteristics including in-stream, morphological, and riparian habitat conditions. Sediment particle size was measured using a modified 100-particle Wolman pebble count (MDEQ, 2001). Resulting data are presented as the percent of silt/clay, sand, gravel, cobble, boulder, and/or hardpan to total particle size.

- Physicochemical measurements. Field physicochemical data (dissolved oxygen, pH, temperature, specific conductance, TDS, and turbidity) were collected using a multi-probe and turbidimeter. Water chemistry grab samples were collected at the same time macroinvertebrate samples were collected. These data were collected in the winters of 2001 to 2003 (one sampling event taken at the time of biological sample collection) with additional chemical sampling in the spring and fall of 2004 (spring season = mid-March through all of April, fall season = mid-August through all of September). The 2004 data collection strategy included two sampling events in the spring season and two sampling events in the fall season. The most recent chemistry sample which has a correspondent macroinvertebrate sample from each station was selected to represent the most contemporaneous environmental condition. Sampling times varied from 7 am through 6 pm. Nutrient parameter concentrations included ammonia, TKN, Nitrate + Nitrite, Total Nitrogen (TKN + Nitrate/Nitrite), Orthophosphate, and Total Phosphorus. Data were collected according to MDEQ Standard Operating Procedures and are described in detail in the Quality Assurance Project Plan (QAPP) for 303(d) List Assessment and Calibration of the Index of Biological Integrity for Wadeable Streams in Mississippi (MDEQ, 2001). Procedures used to conduct laboratory analysis of water samples for various physical and chemical water quality parameters were also performed as noted in the QAPP according to MDEQ Analytical Chemistry Lab Methods (MDEQ, 2001). Various physical and chemical parameters were measured using EPA-approved methods.

4.3.3 Benthic macroinvertebrate metrics

Benthic macroinvertebrate community metrics, including the overall M-BISQ score, were calculated in the MDEQ EDAS database (MDEQ, 2003a). Macroinvertebrate data were collected and samples were processed in the winter of 2001-2004 according to MDEQ Standard Operating Procedures and are described in detail in the Quality Assurance Project Plan (QAPP) for 303(d) List Assessment and Calibration of the Index of Biological Integrity for Wadeable Streams in Mississippi (MDEQ, 2001). Benthic macroinvertebrate specimens were identified, tallied, and recorded. Over 60 different biological metrics that describe various characteristics of the macroinvertebrate population were derived from the resulting macroinvertebrate taxonomic data. A suite of regionally specific metrics were used to calculate an overall M-BISQ score according to methods outlined in the M-BISQ QAPP (MDEQ, 2001). Tetra Tech, Inc recently updated and recalculated the M_BISQ for the state of Mississippi (Tetra Tech, Inc. 2007a). The new M-BISQ and candidate metrics for the new M-BISQ were used for the analyses.

4.4 Data for non-wadeable streams

A database for non-wadeable streams has been developed using a Microsoft Access database with records collected in 2005 and 2006. This database allowed efficient storage and analysis of data in a format comparable to the Ecological Data Analysis System (EDAS) used by MDEQ for wadeable streams (MDEQ 2007c). Protocols for field sampling in the large rivers in Mississippi were developed in response to the recognition that protocols in use for wadeable streams and rivers were inadequate (MDEQ 2002). The data collected included habitat (rapid assessment and physical measures), water quality, and benthic macroinvertebrates. Data were collected from July to September in 2005 and 2006. In addition, information about the watersheds was collected remotely through GIS analysis.

Site locations were targeted to represent flowing water portions along the longitudinal gradients in three rivers, the Big Black, Tombigbee, and Pascagoula. At each location, the site was defined as a 500m length of river, over which six transects were evenly spaced. Habitat information was collected across the transects and within shallow-water, bank and riparian plots at each end of the transects. Benthic macroinvertebrate samples were collected from the shallow-water plots at both ends of the transects. Water quality readings were taken using a multi-probe at one point within the flowing water of the main channel. Nutrient samples were also collected simultaneously with macroinvertebrate samples.

4.4.1 Benthic macroinvertebrates

In each of the sampling plots located along the 6 transects, two 20-second jab samples were taken along the margin habitat using a modified kick net (595/600 µm mesh). In addition to the jab samples, hard substrates within the sampling plot were selected and the invertebrates picked off of them for 5 minutes and added to the jab samples. Samples were composited over the 6 transects and preserved in 80% ethanol.

4.4.2 Habitat measures

At each of 6 transects, channel dimension profiles were taken and consisted of: channel width, depths (at least 10 measurement made in the channel), bankfull width, bankfull height (estimated), height of first terrace or incision height, and the dimensions of any lateral or mid-channel bars in either direction. Bank conditions were assessed by estimating the bank angle and ranking the degree of erosion and bank exposure along a stretch of bank, 25m in either direction of the transect.

In the same 25 m stretch along each bank and extending 10m into the channel, fish habitat (cover) was assessed by visually estimating the presence and percent cover of filamentous algae, aquatic macrophytes, large woody debris, other woody debris, overhanging vegetation, undercut banks, leaf packs, exposed rootwads, and artificial substrates. Woody debris was tallied within the shallow water sample area and as it was encountered across the transect. A riparian plot extending 25m into the floodplain was evaluated using measures of the canopy and other vegetation.

General habitat condition was estimated by summing the visual ratings of 7 variables, including bank stability, bottom deposition, thalweg substrate, large woody debris, aquatic vegetation, off-channel habitat, and riparian width. Each of these variables was rated on a numeric scale associated with narrative conditions ranging from poor to excellent.

4.4.3 Water quality

Multi-probes were used to collect *in situ* water quality data for 5 variables, including dissolved oxygen, temperature, conductivity, and pH. Turbidity and total dissolved solids were analyzed from samples taken from the same locations. Nutrient samples were also collected following field protocols and analyzed in the lab following standard methods (MDEP field protocols).

Quality control (QC) checks of the field protocols, biological sample processing, and taxonomic identification were completed on 10% of the data. The QC focused on field sampling precision, sample repeatability over time, sample sorting efficiency, and taxonomic accuracy.

5.0 REFERENCE APPROACHES TO DEVELOP NUTRIENT CRITERIA (WADEABLE)

The "reference site approach" (Hughes 1995, Bailey et al. 2004) was developed originally to quantify the biological condition at a set of sites that are either minimally or least disturbed by human activity. This approach is the most common approach for estimating the various reference states and is a scientifically sound method for setting expectations, provided that the form of reference condition that the reference sites represent is clearly defined. These reference states fall within the biological condition gradient as described by Davies and Jackson (2006) (Figure 5.1).

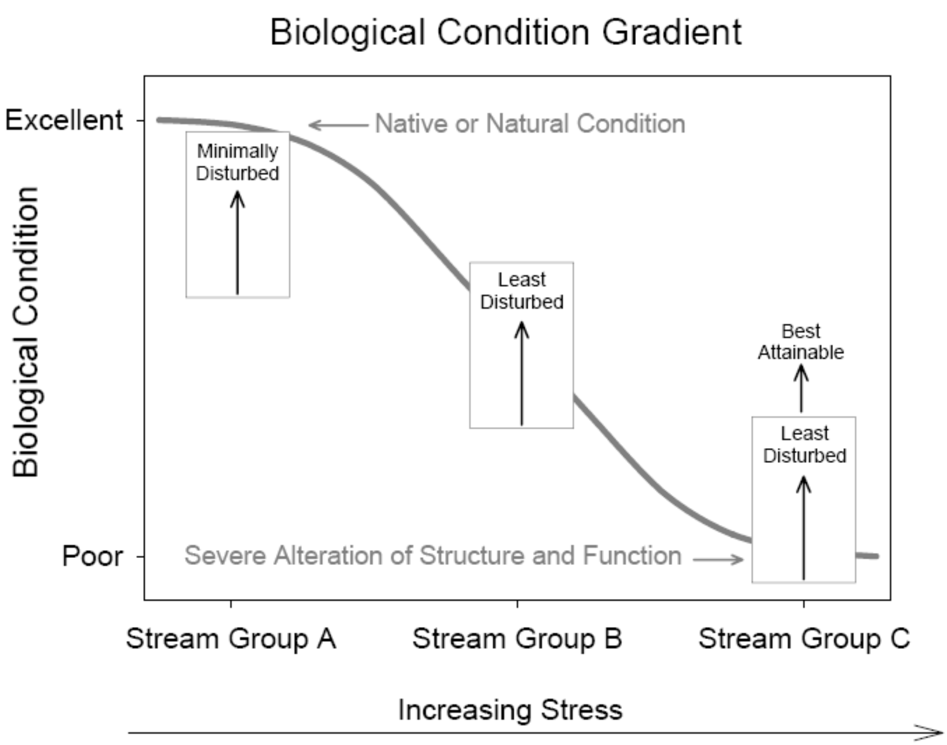

Figure 5.1 A conceptual model of how biological conditions might decline with increasing stress (Stoddard et al. 2006). Groups of streams (e.g., in different size classes, or different ecological regions) display a range of conditions that is dependent on their position on this Biological Condition Gradient. As a result, the least-disturbed streams in each group display very different states of "reference condition," varying from true minimally-disturbed condition, to a least-disturbed condition that is considerably lower than what might be attained with best management practices.

Stoddard et al. (2006) described various reference condition definitions and called for consistency in use of the term "Reference condition". In that paper, they define reference condition of biological integrity (RC-BI) as the natural biological condition that is ideal but may never be attainable. They also defined several more practical terms: Minimally Disturbed Condition (MDC), Least Disturbed Condition (LDC), and Best Attainable Condition (BAC).

- Minimally Disturbed Condition (MDC): this term describes the condition of streams in the absence of significant human disturbance, which is the best approximation or estimate of biotic integrity. For example, natural forest reserves fall into this category. In situations without minimally disturbed sites, empirical models derived from associations between biological indicators and human disturbance gradients can be extrapolated to infer conditions in the absence of human disturbance (e.g., Karr and Chu 1999).

- Least Disturbed Condition (LDC): A preferred approach is to establish a set of criteria that, in total, describe the characteristics of sites in a region that are the least exposed to stressors. This is also the most widely applied approach.

- Best Attainable Condition (BAC): When best possible management practices were in use for some period of time, many stream sites could attain the expected ecological condition of least disturbed sites.

Stoddard et al. (2006) also describe current methods by which these expectations are estimated including: the reference site approach (condition at minimally or least disturbed sites); best professional judgment; interpretation of historical condition; extrapolation of empirical models; and evaluation of ambient distributions.

In this study, we used three reference approaches. First, by applying an empirical modeling approach, we estimated nutrient concentrations under the MDC. Second, we used the population of sites defined by the M-BISQ development as reference sites as the LDC reference population. Lastly, we modified the BAC concept and defined best attained condition (BAC) as all sites that currently meet MDEQ biological criteria.

We decided to classify streams in the State based on bioregional classification to reduce natural variability (Appendix C). However, due to relatively small sample size for some regions, merging regions into fewer classes would provide sufficient data to derive criteria. For example, the LDC reference site sample size for the South Bluff bioregion was not sufficient to derive population-based benchmarks. However, by examining LDC sites from the entire ecoregion 74, an estimate for this region could be developed.

5.1 Minimally Disturbed Condition (MDC): Extrapolating Reference Condition from Empirical Models

Dodds and Oakes (2004) proposed a regression approach to estimate reference conditions by extrapolating nutrient concentrations to those existing under no human land cover disturbance. They used a multiple linear regression approach to predict TP or TN concentrations using multiple land use predictors. Statistical analyses for that study were accomplished in two steps. Analysis of covariance (ANCOVA) was first used to test for significant differences among regions (ecoregions or bioregions) while accounting for the effects of land use variables on water column nutrients. If across region effects were not significantly different ($P > 0.05$) as determined by ANCOVA, multiple linear regression was used to establish relationships between land use and nutrient concentrations. If there were significant differences among regions, then multiple linear regression was used to predict nutrient - land use relationships for each ecoregion separately.

We developed predictive models using TN and TP concentrations as response variables and percent human land uses as predictors using M-BISQ dataset. Although we expected that nutrient concentrations would most likely vary among ecoregions, we realize that macroinvertebrate assemblages were also expected to vary more strongly across bioregions. Therefore, we developed models for both bioregion and ecoregion classes.

5.1.1 Bioregion models

The first step was to determine if significant interactions existed between bioregions and land use effects. We examined the interactions among bioregions and land uses using ANCOVA. Interactions among bioregions and land uses had a significant impact on TN and TP concentrations in the State of Mississippi (Table 5.1). Both TN and TP models showed that the three way interactions among % urban land use, % pasture and grassland, and bioregions were significant. Therefore, bioregion classification was necessary to further reduce variation associated with natural geographic difference in nutrient concentrations due to geology, hydrology, and other factors.

Table 5.1. Analysis of covariance (ANCOVA) of log transformed total N and P, with four Mississippi bioregions (BIOREG) as categorical predictors and with percentages of urban (PCTURBAN), cropland (PCTCROPLAND, and pasture and grass land (PCTPASTGRAS) as the covariates.

TN ANCOVA model N: 552 R^2: 0.513 p=0.001

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
BIOREG	0.135	3	0.045	1.049	0.371
PCTCROPLAND	0.084	1	0.084	1.948	0.163
PCTURBAN	0.079	1	0.079	1.827	0.177
PCTPASTGRAS	0.065	1	0.065	1.521	0.218
BIOREG*PCTPASTGRAS	0.121	3	0.04	0.936	0.423
BIOREG*PCTURBAN	0.193	3	0.064	1.5	0.214
BIOREG*PCTCROPLAND	0.337	3	0.112	2.616	**0.05**
BIOREG*PCTCROPLAND*PCTURBAN	0.21	3	0.07	1.63	0.182
BIOREG*PCTCROPLAND*PCTPASTGRAS	0.286	3	0.095	2.216	0.085
BIOREG*PCTPASTGRAS*PCTURBAN	0.5	3	0.167	3.875	**0.009**
PCTURBAN*PCTCROPLAND*PCTPASTGRAS	0.158	1	0.158	3.679	0.056
BIOREG*PCTURBAN*PCTCROPLAND*PCTPASTGRAS	0.398	3	0.133	3.083	**0.027**
Error	22.485	523	0.043		

TP ANCOVA model N: 552 R^2: 0.409 p=0.000

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
BIOREG	0.69	3	0.23	2.519	0.057
PCTCROPLAND	0.474	1	0.474	5.194	**0.023**
PCTURBAN	0.039	1	0.039	0.422	0.516

Table 5.1. Continued.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
PCTPASTGRAS	0.163	1	0.163	1.781	0.183
BIOREG*PCTPASTGRAS	0.904	3	0.301	3.299	**0.02**
BIOREG*PCTURBAN	0.761	3	0.254	2.78	**0.041**
BIOREG*PCTCROPLAND	0.135	3	0.045	0.491	0.689
BIOREG*PCTCROPLAND*PCTURBAN	0.245	3	0.082	0.893	0.444
BIOREG*PCTCROPLAND*PCTPASTGRAS	0.607	3	0.202	2.218	0.085
BIOREG*PCTPASTGRAS*PCTURBAN	1.672	3	0.557	6.103	**0.01**
PCTURBAN*PCTCROPLAND*PCTPASTGRAS	0.517	1	0.517	5.669	**0.018**
BIOREG*PCTURBAN*PCTCROPLAND*PCTPASTGRAS	0.923	3	0.308	3.37	**0.018**
Error	47.745	523	0.091		

We further examined differences in land uses and nutrient concentrations among different bioregions (Figure 5.2). The most noticeable difference in land uses was that the West Bioregion had relatively lower pasture and grassland ($p<0.05$) than other regions. Although TN concentrations were not significantly different among bioregions, TP concentrations in the Southeast and East bioregions were significantly lower ($p<0.05$) than in the West and South Bluff bioregions (ecoregion 74) (Figure 5.2).

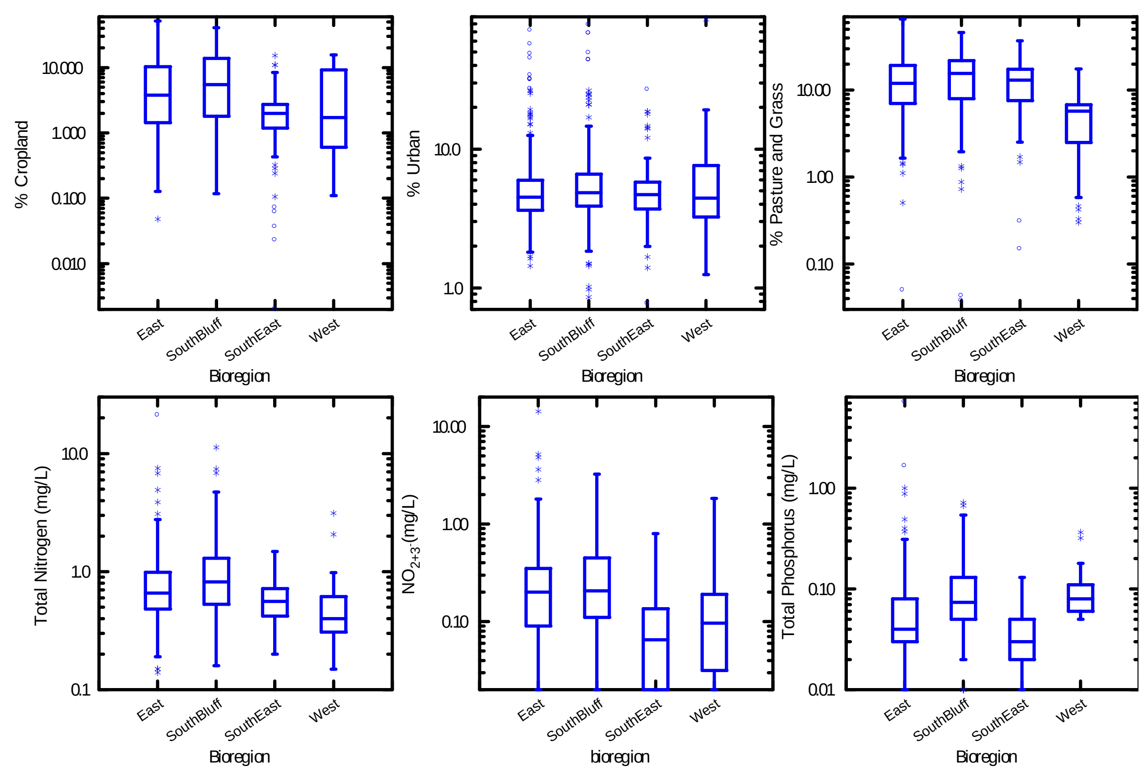

Figure 5.2. Box plots of cropland, pasture and grassland, urban land, TP, TN, and NOx for M-BISQ sites in Mississippi in different bioregions. Lines in center of boxes are the medians, tops and bottoms of boxes are 75th and 25th percentiles, respectively. Bars are 95% confidence intervals, and outliers are plotted as open points.

Since there were significant interactions between bioregions and land use classes, separate multiple regressions were performed for each individual bioregion (Table 5.2). In the East Bioregion, % pasture and grassland and % urban land contributed significantly to both TN and TP concentrations. The TN model for this region explained 37% of the total variance, better than the TP model (28.3%). The TN model in the West bioregion was stronger than in the East Bioregion, explaining more than half of the total variance. Also, all three land use categories, including % cropland, contributed to predicting nutrient concentrations in this bioregion. Both TN and TP models for the Southeast region were weak (R^2 = 0.188 and 0.070) probably due to short nutrient gradients in this region. The regression models for the South Bluff bioregion are significant but were based on relatively small sample sizes.

Table 5.2. Best model regression results for total N and total P in four bioregions in Mississippi. Both TN and TP concentrations were log transformed. A backward selection was used to choose variables (p<0.15). Abbreviations for land use as above.

Effect	Coefficient	Standard Error	Standard Coefficient	Tolerance	t	P (2 Tail)
East Bioregion Dependent Variable: TN N: 283 R^2: 0.370 p<=0.001						
CONSTANT	-0.714	0.04	0	.	-17.814	0.000
PCTURBAN	1.074	0.109	0.409	0.994	9.813	0.000
PCTPASTGRAS	0.791	0.083	0.398	0.994	9.545	0.000
Southeast Bioregion Dependent Variable: TN N: 79 R^2: 0.188						
CONSTANT	-0.549	0.076	0	.	-7.215	0
PCTURBAN	0.795	0.266	0.31	0.989	2.986	0.004
PCTCROPLAND	0.837	0.258	0.337	0.989	3.247	0.002
South Bluff Bioregion Dependent Variable: TN N: 28 R^2: 0.436						
CONSTANT	-0.713	0.101	0	.	-7.062	0
PCTURBAN	0.525	0.237	0.337	0.973	2.215	0.036
PCTCROPLAND	1.519	0.37	0.625	0.973	4.104	0
West Bioregion Dependent Variable: TN N: 162 R^2: 0.555						
CONSTANT	-0.738	0.055	0	.	-13.438	0
PCTURBAN	0.89	0.112	0.422	0.997	7.932	0
PCTPASTGRAS	0.684	0.127	0.323	0.779	5.371	0
PCTCROPLAND	0.693	0.11	0.378	0.777	6.278	0
East Bioregion Dependent Variable: TP N: 283 R^2: 0.227						
CONSTANT	-1.887	0.068	0	.	-27.726	0
PCTURBAN	0.934	0.179	0.275	0.995	5.216	0
PCTPASTGRAS	0.997	0.142	0.37	0.995	7.023	0
Southeast Bioregion Dependent Variable: TP N: 79 R^2: 0.07						
CONSTANT	-1.799	0.108	0	.	-16.716	0
PCTURBAN	1.056	0.44	0.264	1	2.399	0.019
South Bluff Bioregion Dependent Variable: TP N: 28 R^2: 0.256						
CONSTANT	-1.197	0.064	0	.	-18.799	0
PCTURBAN	0.59	0.197	0.506	1	2.991	0.006

Table 5.2. Continued.

Effect	Coefficient	Standard Error	Standard Coefficient	Tolerance	t	P (2 Tail)
West Bioregion Dependent Variable: TP N: 162 R^2: 0.296						
CONSTANT	-1.657	0.079	0	.	-20.992	0
PCTURBAN	0.778	0.161	0.323	0.997	4.828	0
PCTPASTGRAS	0.584	0.183	0.241	0.779	3.189	0.002
PCTCROPLAND	0.543	0.159	0.259	0.777	3.419	0.001

5.1.2 Ecoregion Models

Interactions among ecoregion and land uses also had a significant impact on TN and TP concentrations in the State of Mississippi (Table 5.3). The three way interactions among % urban land use, % pasture and grassland, and ecoregion were significant in both TN and TP models, indicating ecoregion effect could have contributed to differences in TN and TP concentrations among different regions. Therefore, ecoregion classification was necessary to reduce natural variability in the nutrient – land use relationships.

Table 5.3. Analysis of covariance of log transformed total N and P, with three Mississippi ecoregions as categorical predictors and with percentages of urban, cropland, and pasture and grass land as the covariates. Abbreviations for land use as above.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TN ANCOVA model N: 552 R^2: 0.482 p=0.001					
ECOREG	0.215	2	0.108	2.388	0.093
PCTCROP	0.027	1	0.027	0.6	0.439
PCTURBAN	0.279	1	0.279	6.191	**0.013**
PAST&GRAS	0.306	1	0.306	6.795	**0.009**
ECOREG* PCTPASTGRAS	0.34	2	0.17	3.773	**0.024**
ECOREG*PCTURBAN	0.139	2	0.069	1.538	0.216
ECOREG*PCTCROP	0.063	2	0.031	0.698	0.498
ECOREG*PCTCROP*PCTURBAN	0.073	2	0.036	0.805	0.448
ECOREG*PCTCROP* PCTPASTGRAS	0.135	2	0.067	1.496	0.225
ECOREG*PAST&GRAS*PCTURBAN	0.344	2	0.172	3.821	**0.023**
PCTURBAN*PCTCROP* PCTPASTGRAS	0.015	1	0.015	0.33	0.566
ECOREG*PCTURBAN*PCTCROP* PCTPASTGRAS	0.096	2	0.048	1.071	0.344
Error	23.912	531	0.045		

Table 5.3. Continued.

TP ANCOVA model N: 552 R^2: 0.351 p=0.000

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
ECOREG	0.295	2	0.148	1.495	0.225
PCTCROP	0.102	1	0.102	1.028	0.311
PCTURBAN	0.121	1	0.121	1.227	0.269
PCTPASTGRAS	0.239	1	0.239	2.423	0.12
ECOREG* PCTPASTGRAS	1.023	2	0.511	5.176	**0.006**
ECOREG*PCTURBAN	0.762	2	0.381	3.854	**0.022**
ECOREG*PCTCROP	0.083	2	0.041	0.419	0.658
ECOREG*PCTCROP*PCTURBAN	0.175	2	0.087	0.884	0.414
ECOREG*PCTCROP* PCTPASTGRAS	0.482	2	0.241	2.437	0.088
ECOREG* PCTPASTGRAS *PCTURBAN	1.684	2	0.842	8.52	**0.000**
PCTURBAN*PCTCROP* PCTPASTGRAS	0.062	1	0.062	0.626	0.429
ECOREG*PCTURBAN*PCTCROP* PCTPASTGRAS	0.521	2	0.261	2.636	0.073
Error	52.473	531	0.099		

Differences in land uses and nutrient concentrations were also examined (Figure 5.3). Although ecoregion 75 (Southern Coastal Plain) had relatively higher urban land uses than other ecoregions, agricultural land uses were not significantly different among different ecoregions (Figure 5.3). Ecoregion 74 (Mississippi Valley Loess Plains) had, on average, significantly higher TP concentrations than ecoregions 65 (Southeastern Plains) and 75 ($p<0.05$). It should be noted that ecoregion 75 had only a small number of sites (<20) in the study.

Since differences in nutrient concentrations could be provided by different land use predictors in different ecoregions, multiple regressions were performed for each individual ecoregion separately (Table 5.4). In ecoregion 65, % pasture and grassland and % urban land both contributed significantly to TN and TP concentrations. Overall, both TN and TP models were significant, but the TN model (R^2 =0.351) explained more variance than the TP model (20.2%). The TN and TP models in ecoregion 74 explained more water variance than in ecoregion 65. The TN model explained more than half of the total variance. Also, all three land use categories, including cropland, contributed to predicting nutrient concentrations in this ecoregion. No significant relation was found between TN concentrations and land use predictors in ecoregion 75, probability due to small sample size (N=11). However, the TP model for this region was significant (R^2 = 0.744), even though no one land use variable was able to significantly predict TP concentrations (Table 5.4). In addition, the sample size was very small for this region (N=11), so caution is advised in interpreting this model since the risk of over-fitting was high.

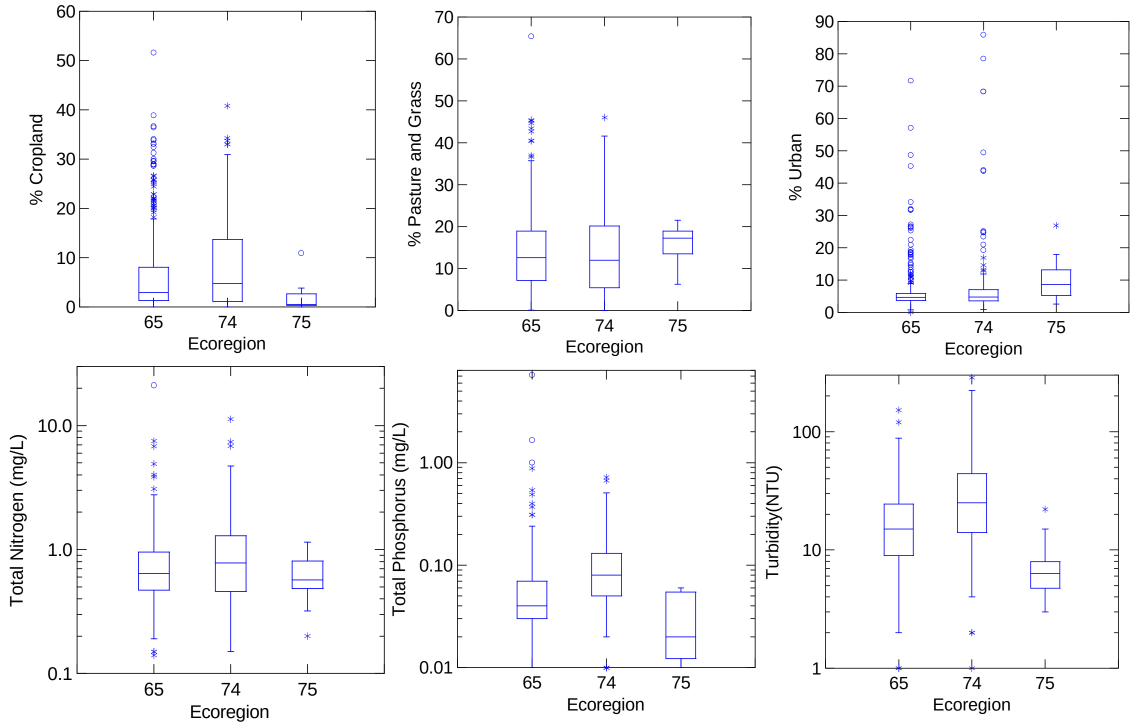

Figure 5.3. Box plots of cropland, pasture and grassland, urban land, TP, TN, and turbidity for M-BISQ sites in Mississippi by level III ecoregion. The lines in the center of boxes are the medians, and the tops and bottoms of boxes are the 75th and 25th percentiles, respectively. Whiskers are 95% confidence intervals, and outliers are plotted as open points.

Table 5.4. Best model regression results for total N and total P in ecoregion 65, 74 and 75. Both TN and TP concentrations were log transformed. A backward selection was used to choose variables (p<0.15). Abbreviations for land use as above.

Ecoregion 65 Dependent Variable: TN N: 379 R^2: 0.351 p<=0.001						
Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-0.714	0.04	0	.	-17.814	0.000
PCTURBAN	1.074	0.109	0.409	0.994	9.813	0.000
PCTPASTGRAS	0.791	0.083	0.398	0.994	9.545	0.000
Ecoregion 65 Dependent Variable: TP N: 379 R^2: 0.202 p<=0.001						
Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-1.912	0.062	0	.	-30.887	0.000
PCTURBAN	1.005	0.169	0.275	0.994	5.946	0.000
PCTPASTGRAS	0.927	0.128	0.335	0.994	7.242	0.000
Ecoregion 74 Dependent Variable: TN N: 162 R^2: 0.568 p<=0.001						
Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-0.751	0.051	0	.	-14.698	0.000
PCTURBAN	0.783	0.108	0.379	1	7.255	0.000
PCTPASTGRAS	0.772	0.138	0.361	0.655	5.594	0.000
PCTCROPLAND	0.704	0.124	0.368	0.655	5.691	0.000

Table 5.4. Continued.

Ecoregion 74 Dependent Variable: TP N: 162 R^2: 0.263 p<=0.001						
Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-1.523	0.068	0	.	-22.533	0.000
PCTURBAN	0.75	0.143	0.358	1	5.246	0.000
PCTPASTGRAS	0.481	0.183	0.222	0.655	2.631	0.009
PCTCROPLAND	0.362	0.164	0.186	0.655	2.209	0.029
Ecoregion 75 Dependent Variable: TP N: 11 R^2: 0.744 p<=0.001						
Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-1.757	0.048	0	.	-36.266	0.000
PCTURBAN	0.919	0.119	0.297	0.997	7.75	0.098
PCTPASTGRAS	0.526	0.116	0.195	0.784	4.523	0.100
PCTCROPLAND	0.367	0.1	0.159	0.786	3.681	0.084

5.1.3. Nutrient endpoints based on MDC

Using the regression models, the intercepts (constant) of the regression were used to estimate those nutrient concentrations when human land uses were all equal to zero. The confidence intervals of the intercept were also calculated from the regression models (Table 5.5). According to this approach, the natural background TN concentration was approximately 0.193 mg/L in ecoregion 65 and 0.177 mg/L in ecoregion 74. The natural TP concentration in ecoregion 65 (0.012 mg/L) was much lower than ecoregion 74 (0.030 mg/L). Due to relatively small sample size, the estimated natural TP concentration in ecoregion 75 had a larger confidence interval (ranged from 0-0.021 mg/L, which covered the range of TP concentrations in ecoregion 65).

We also ran these models by bioregion. The East and Southeast bioregions include the same regions as ecoregion 65 and 75. Both bioregion and ecoregion models had similar behavior, namely that TN and TP models for the Southeast bioregion explained less variance, and models lacked sufficient sample size. As a result, it would be more powerful to combine the entire region as one single region for the extrapolation. Moreover, the extrapolated TN and TP concentrations for the East bioregion were very similar to the Southeast bioregion, indicating that the background nutrient concentrations could be similar in these two bioregions.

Table 5.5. Results of regression extrapolation from multiple regression models.

Ecoregion	Nutrient Parameter	Mean	Lower 95th CI	Higher 95th CI
East	TN	0.194	0.157	0.238
	TP	0.013	0.010	0.018
Southeast	TN	0.282	0.200	0.398
	TP	0.016	0.010	0.026
South Bluff	TN	0.194	0.123	0.305
	TP	0.064	0.048	0.085
West	TN	0.183	0.143	0.234
	TP	0.022	0.015	0.031

Table 5.5. Continued.

Ecoregion	Nutrient Parameter	Mean	Lower 95th CI	Higher 95th CI
65	TN	0.193	0.161	0.231
	TP	0.012	0.009	0.016
74	TN	0.177	0.141	0.223
	TP	0.030	0.022	0.041
75	TN	n/a	n/a	n/a
	TP	0.002	0.0002	0.021

5.2 Least Disturbed Condition (LDC)

5.2.1 Criteria based on LDC 75th Percentiles

Conditions that represent least disturbance provide a baseline that should represent the best current estimate at chemical and biological integrity and should protect assigned designated uses. The statewide dataset for M-BISQ development provided an ideal dataset to derive nutrient criteria using the LDC approach. Least disturbed stations were identified based on regional land use, stream physical habitat, and chemical characteristics in the M-BISQ development process. The M-BISQ recalibration refined the selection criteria for LDC streams in the state. For the purpose of nutrient criteria development, we used two different selection criteria for LDC (See appendix D for detail analysis). The first LDC was developed to be consistent with the M-BISQ development process (Table 5.6). To avoid circularity, however, we removed nutrient parameters in the selection criteria, which led to only one additional site to the original M-BISQ LDC site list. The second LDC set was selected solely based on land use in the surrounding watershed, stream buffer, and local habitat. Two important factors were considered in this LDC set. First, geographic distribution of stream sites was not considered in selecting these LDC sites, therefore, regions with more natural land had more LDC sites. Second, these selection criteria eliminated anthropogenic nutrient loadings from land use/land cover changes but did not exclude potential impact from other environmental stressors that co-varied with nutrients. In other words, other environmental stressors (e.g., pH and conductivity) in this LD set were considered as natural stressors.

Table 5.6. Reference site selection criteria for LDC group 1 and LDC group 2. (Ag = agriculture, NPDES = distance to permitted discharge).

LDC1 criteria

Ecogroup	%Natural	%Natural Buffer	Habitat Score	Chloride	NPDES
1 or 2	>50	>60	>100	<10	>5km
3	>70	>80	>110	<10	>5km
4	>70	>80	>110	<10	>5km
5	>70	>80	>110	<30	>5km
6	>70	>80	>100	<30	>5km

LDC2 criteria

%Ag	%Ag Buffer	%Urban	% Urban Buffer	Habitat	NPDES
<20	15	<5	<3	>100	>5km

The two selected LDC sets were identified in the MS_nutrient.mdb database and are listed in Appendix B. The LDC1 selection criteria were more conservative than the LDC2 criteria and resulted in a smaller number of LDC sites (117 sites vs. 157 sites). The LDC1 sites were also more evenly distributed across the state than LDC2, since regional difference was used as one of the selection criteria for LDC1. The LDC2 had more sites in the Southeast bioregion and West bioregion (ecogroup 5) where streams with relatively low surrounding human land uses were dominant. However, even with these two selection criteria, there were no LDC sites within ecoregion 75 (Southern Coastal Plain).

EPA's Technical Guidance Manual for Developing Nutrient Criteria for Streams and Rivers (USEPA, 2000) advocates selecting the 75th percentile of a distribution of reference condition values as a recommended target for a sufficiently protective value that provides an appropriate margin of safety.

To estimate the 75th percentile of a distribution requires a relatively large sample size. From a biological survey standard point, a sample size of 30 is considered a minimum for estimating means and variances. Since percentile distribution is very sensitive to sample size, we required at least 20 sites with one class to estimate a percentile for a distribution.

Although we intended to develop nutrient benchmarks for each bioregion to protect aquatic life uses in these regions, the sample size of LDC sites limited our ability to identify nutrient benchmarks in some regions. For example, the sample size of LDC1 for the South Bluff bioregion was too small (only 7 sites) to derive a reasonable benchmark. Also, biological criteria for ecogroup 1 and ecogroup 5 of the West bioregion were derived separately because of differences in land use between these two ecogroups within that region. As a result, it was felt that separate nutrient benchmarks should at least be explored for these two ecogroups. Therefore, we examined percentile distributions of nutrient variables in each bioregion and ecoregion, and in addition, for ecogroups 1 and 5. The distribution of LDC1 for TN, TP, nitrite/nitrate concentrations, and turbidity were summarized for different regions (Table 5.7, Figure 5.4).

Table 5.7. LDC1 Percentile distribution and reference nutrient concentrations

	Bioregion				***Ecoregion***		***Ecogroup***		
	East	**South Bluff**	**West**	**Southeast**	**65**	**74**	**5+6**	**5**	**1**
TN (mg/L)									
Min	0.150	0.240	0.160	0.220	0.150	0.160	0.160	0.160	0.360
25th	0.363	0.360	0.405	0.363	0.365	0.360	0.313	0.293	0.650
median	0.490	0.390	0.525	0.420	0.480	0.500	0.405	0.455	0.815
mean	0.564	0.450	0.574	0.479	0.545	0.544	0.437	0.436	0.854
75th	**0.693**	**0.520**	**0.785**	**0.620**	**0.655**	**0.753**	**0.533**	**0.533**	**0.903**
max	1.59	0.76	1.03	0.85	1.59	1.03	0.780	0.78	2.07
N	68	7	20	18	87	26	18	10	12
TP (mg/L)									
Min	0.010	0.060	0.010	0.010	0.010	0.010	0.010	0.010	0.050
25th	0.024	0.075	0.050	0.010	0.020	0.060	0.050	0.042	0.073
median	0.040	0.100	0.062	0.019	0.030	0.075	0.060	0.050	0.100
mean	0.048	0.109	0.091	0.023	0.043	0.097	0.076	0.056	0.119

Table 5.7. Continued.

	Bioregion				*Ecoregion*		*Ecogroup*		
	East	**South Bluff**	**West**	**Southeast**	**65**	**74**	**5+6**	**5**	**1**
75th	**0.050**	**0.137**	**0.111**	**0.030**	**0.050**	**0.115**	**0.095**	**0.060**	**0.119**
max	0.4	0.18	0.375	0.05	0.4	0.375	0.180	0.140	0.375
N	68	7	20	18	87	26	18	10	12
Turbidity (NTU)									
Min	3.000	1.000	5.000	2.000	2.000	1.000	1.000	5.000	7.000
25th	10.750	4.000	13.800	4.000	8.500	9.390	7.000	9.000	39.625
median	15.500	9.120	25.500	5.500	13.300	21.300	16.500	18.500	45.600
mean	18.408	13.674	35.235	5.832	15.669	30.535	20.140	25.980	44.783
75th	**21.925**	**19.300**	**48.625**	**7.750**	**18.000**	**44.900**	**22.150**	**23.000**	**48.625**
max	77	39	104	12	77	104	82.80	82.80	104
N	68	7	20	18	87	26	18	10	12

Nutrient concentrations varied among different regions according to LDC1 reference site distribution (Figure 5.4, Table 5.7). TN and TP benchmarks in East and Southeast bioregions were very similar, though a small sample size for the South bioregion questions the final benchmark for this region. TN and TP benchmarks for the West and South Bluff bioregions were much higher, but the South Bluff had a small sample population. TN and TP benchmarks for ecoregion 74 may be used as a surrogate for South Bluff bioregion.

The LDC2 criteria resulted in more sites in the Southeast bioregion and ecogroup 5 of the West bioregion than any other regions (Figure 5.5, Table 5.8). Also, LDC2 criteria excluded many sites from ecogroup 1 of the West bioregion. As a result of this increased sample size, benchmarks for ecogroup 5 and the Southeast bioregion could be determined. However, ecogroup 1 was left with only 4 sites. TN benchmarks derived from LDC2 sites were marginally higher in the East but lower in the West than those derived from LDC1 sites (Tables 5.7 and 5.8). The benchmarks derived from these two LDC datasets indicated that benchmarks are dependent on how reference sites were selected.

5.2.2 Criteria Based on All Sites 25th Percentile

When information about "least disturbed sites" is not available for a state or region, EPA's technical guidance suggests using the 25th percentile of a distribution of site values from the entire population of waterbodies within a given physical classification (e.g., an ecoregion) (USEPA, 2000). According to this guidance, the 25th percentile of a sample distribution from the entire population roughly approximates the 75th percentile of a sample distribution from LDC sites.

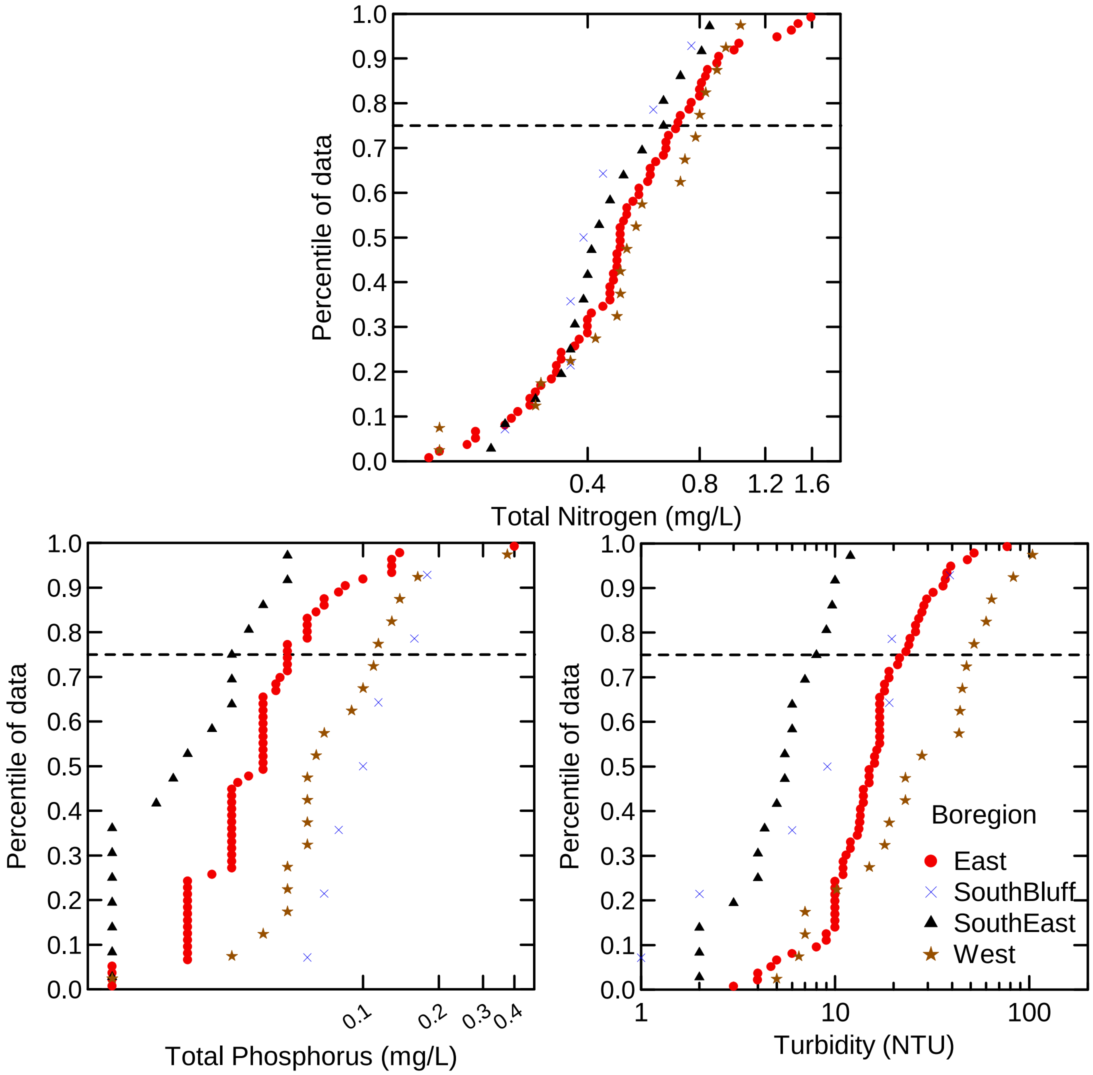

Figure 5.4. Percentile distribution and reference nutrient concentrations from LDC1 sites.

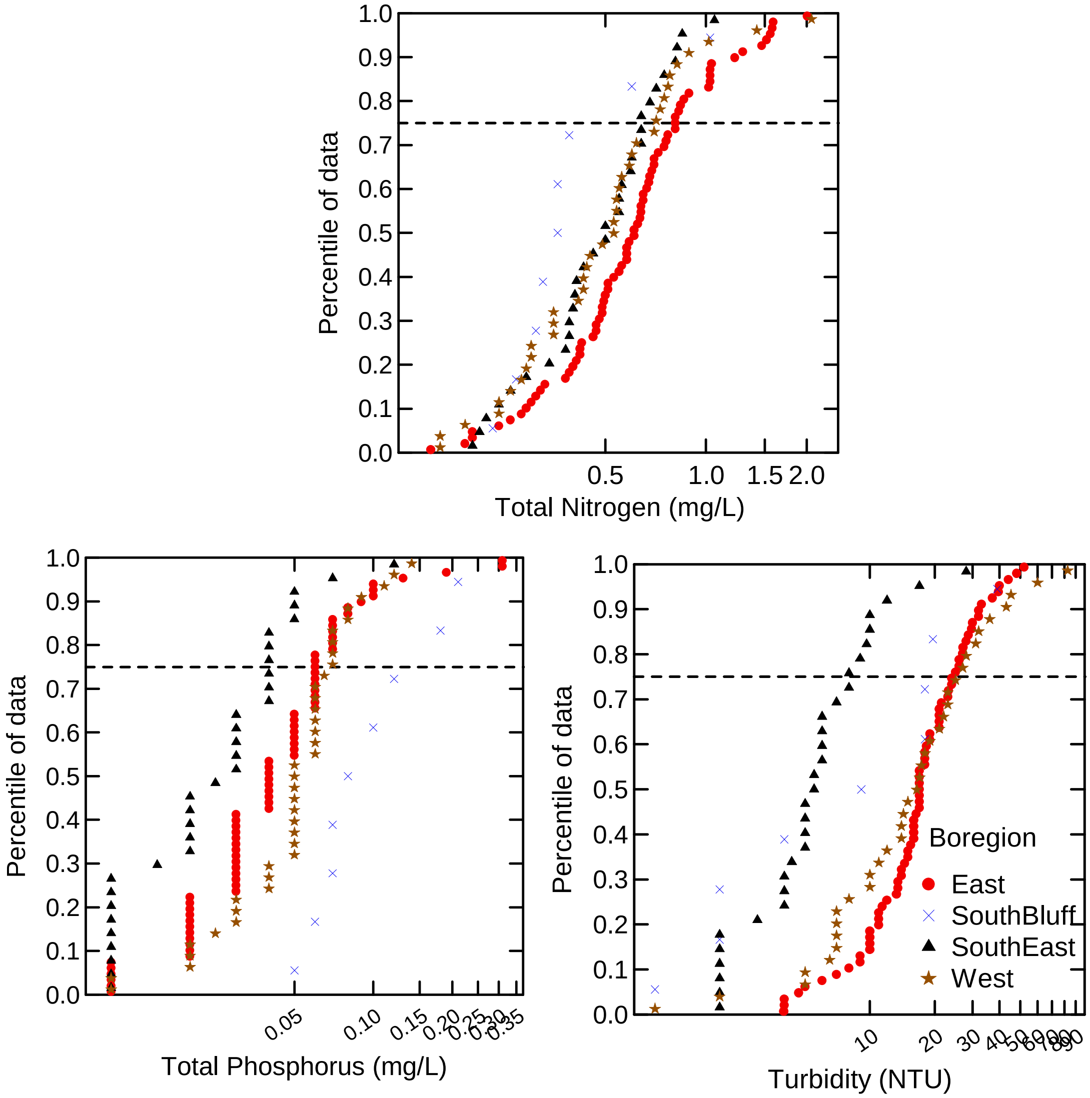

Figure 5.5. LDC2 Cumulative percent distribution of reference sites.

Table 5.8. LDC2 Percentile distribution and reference nutrient concentrations

	Bioregion				*Ecoregion*		*Ecogroups*		
	East	***South Bluff***	***West***	***Southeast***	***65***	***74***	***5+6***	***5***	***1***
				TN (mg/L)					
Min	0.150	0.230	0.160	0.200	0.150	0.160	0.160	0.160	0.560
25th	0.420	0.284	0.300	0.381	0.400	0.293	0.288	0.293	0.648
median	0.545	0.355	0.448	0.500	0.530	0.425	0.375	0.437	0.704
mean	0.626	0.358	0.523	0.511	0.591	0.484	0.433	0.458	1.009
75th	**0.768**	**0.383**	**0.658**	**0.640**	**0.710**	**0.590**	**0.540**	**0.585**	**1.065**
max	1.59	0.6	2.07	1.06	1.59	2.07	0.975	0.975	2.070
N	78	10	34	35	113	42	40	30	4

Table 5.8. Continued.

	Bioregion				Ecoregion		Ecogroups		
	East	***South Bluff***	***West***	***Southeast***	***65***	***74***	***5+6***	***5***	***1***
				TP (mg/L)					
Min	0.010	0.050	0.010	0.010	0.010	0.010	0.010	0.010	0.050
25th	0.025	0.062	0.040	0.012	0.020	0.040	0.040	0.032	0.057
median	0.040	0.075	0.050	0.025	0.035	0.050	0.050	0.050	0.060
mean	0.050	0.088	0.050	0.031	0.044	0.059	0.058	0.049	0.062
75th	**0.060**	**0.104**	**0.060**	**0.040**	**0.060**	**0.070**	**0.070**	**0.060**	**0.064**
max	0.31	0.18	0.14	0.12	0.31	0.18	0.180	0.140	0.077
N	78	10	34	35	113	42	40	30	4
				Turbidity (NTU)					
Min	1.000	1.000	2.000	2.000	1.000	1.000	1.000	2.000	7.000
25th	12.000	2.500	10.750	4.000	6.250	7.750	7.250	10.750	22.750
median	17.000	8.060	17.167	5.750	14.000	16.750	15.750	16.750	36.600
mean	19.139	11.972	22.223	6.720	15.335	19.446	18.544	20.890	31.550
75th	**24.000**	**18.000**	**27.250**	**8.000**	**21.000**	**23.500**	**22.500**	**23.500**	**45.400**
max	52	39	82.8	28	52	82.8	82.800	82.80	46
N	77	10	32	34	111	40	38	28	4

The advantage of the population distribution driven estimate was that we could fully utilize the entire dataset. Sample sizes for each region were therefore significantly improved. Three independent databases were used to derive nutrient benchmarks according to this approach. They were M-BISQ project nutrient data, WADES dataset, and combined dataset.

M-BISQ project nutrient data

At least 20 sites were found in each bioregion (Table 5.9, Figure 5.6). The 25th percentiles of nutrient distributions were mostly lower than the LDC benchmarks. TN benchmarks derived from this approach were similar among all regions except ecogroup 1, which had a higher TN benchmark. TP benchmarks were highest in the South Bluff and ecogroup 1.

Table 5.9. Percentile distribution and reference nutrient concentrations based on whole population of M-BISQ project nutrient samples.

	Bioregion				Ecoregion		Ecogroups		
	East	***South Bluff***	***West***	***Southeast***	***65***	***74***	***5+6***	***5***	***1***
				TN (mg/L)					
Min	0.14	0	0.16	0.16	0.14	0.16	0.160	0.160	0.220
25th	**0.46**	**0.312**	**0.52**	**0.41**	**0.46**	**0.45**	**0.350**	**0.350**	**0.860**
median	0.64	0.4	0.8	0.56	0.62	0.77	0.540	0.540	1.210
mean	0.919	0.483	1.198	0.608	0.86	1.15	0.772	0.772	1.613
75th	0.985	0.607	1.32	0.7525	0.92	1.31	0.775	0.775	1.855
max	21.1	0.98	11.26	1.485	21.10	11.26	6.14	6.14	11.26
N	303	26	173	80	401	170	60	60	87

Table 5.9. Continued.

	Bioregion				*Ecoregion*		*Ecogroups*		
	East	***South Bluff***	***West***	***Southeast***	***65***	***74***	***5+6***	***5***	***1***
TP (mg/L)									
Min	0.01	0	0.01	0.01	0.01	0.01	0.010	0.010	0.020
25th	**0.03**	**0.062**	**0.05**	**0.02**	**0.03**	**0.05**	**0.034**	**0.034**	**0.060**
median	0.04	0.085	0.07	0.03	0.04	0.07	0.050	0.050	0.120
mean	0.096	0.105	0.116	0.035	0.09	0.12	0.063	0.063	0.162
75th	0.07	0.1175	0.13	0.05	0.07	0.14	0.070	0.070	0.210
max	7.18	0.32	1.14	0.13	7.18	1.14	0.35	0.35	1.14
N	303	26	173	80	401	170	60	60	87
Turbidity (NTU)									
Min	3	0	1	1	1.00	2.00	2.0	2.0	6.0
25th	**12**	**6**	**11.25**	**4**	**9.00**	**14.00**	**12.5**	**12.5**	**28.0**
median	18	18	25	6	15.00	28.90	20.0	20.0	42.5
mean	23.1	18.1	34.7	7.1	19.09	36.77	24.1	24.1	51.6
75th	27.3	28.4	44	9	24.00	45.00	32.0	32.0	65.0
max	146	41.2	286	28	146.00	286.00	82.8	82.8	286
N	265	23	158	79	360	153	55	55	78

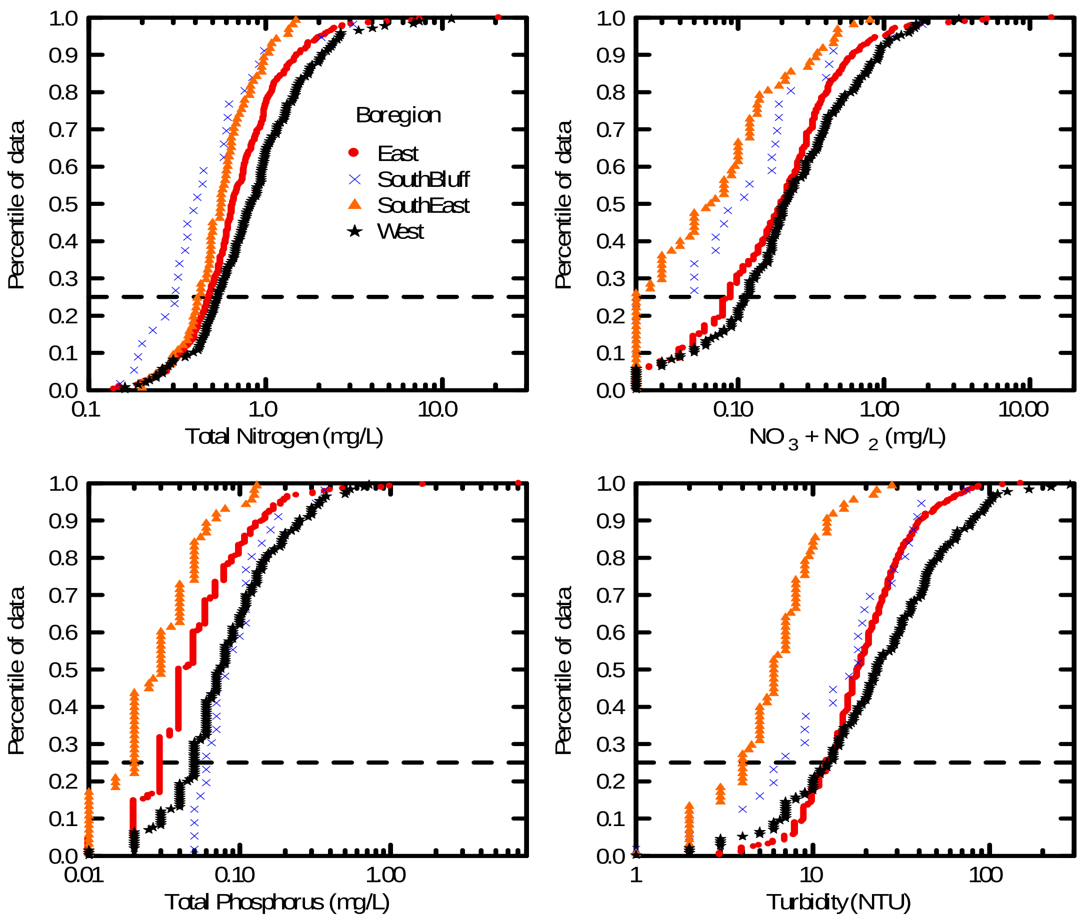

Figure 5.6. Cumulative frequency distributions of TN, TP concentrations and turbidity measured for the M-BISQ project in three ecoregions in the State of Mississippi

WADES dataset

The WADES dataset was the largest dataset (885 sites excluding the M-BISQ sites) (Figure 5.7, Table 5.10). Sample size for each bioregion was again adequate to estimate percentiles. Although the TN and TP benchmarks were slightly different from that derived from the M-BISQ dataset, the patterns were very similar. That is, TN benchmarks for East and Southeast bioregions were slightly lower than that for South Bluff and West bioregions (Figure 5.7), while TP benchmarks were much higher in the South Bluff and ecogroup 1(Table 5.10).

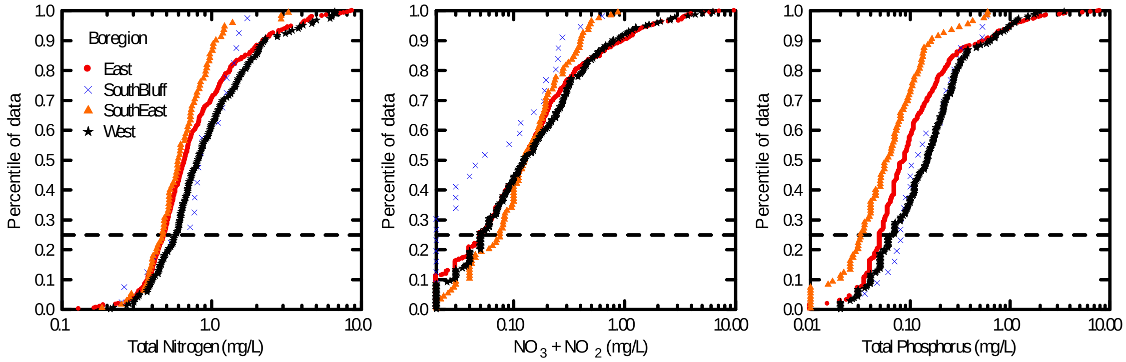

Figure 5.7. Cumulative frequency distributions of TN, TP concentrations and turbidity measured for the WADES database in four bioregions in the State of Mississippi.

Table 5.10. Percentile distribution of nutrient parameters from WADES data

	Bioregion				***Ecoregion***			***Ecogroup***		
	East	**South Bluff**	**West**	**Southeast**	**65**	**74**	**75**	**5+6**	**5**	**1**
					TN (mg/L)					
Min	0.130	0.243	0.210	0.185	0.130	0.210	0.375	0.238	0.238	0.210
25th	**0.483**	**0.582**	**0.591**	**0.480**	**0.480**	**0.589**	**0.544**	**0.440**	**0.439**	**0.694**
median	0.670	0.808	0.846	0.615	0.660	0.833	0.629	0.620	0.600	1.110
mean	1.290	0.893	1.414	0.768	1.198	1.363	0.679	1.234	0.954	1.681
75th	1.162	1.234	1.465	0.837	1.078	1.443	0.842	1.160	0.740	1.775
max	36.7025	1.73	13.68	3.31	36.7025	13.68	1.01	13.68	13.68	10.9
N	408	18	166	101	497	184	12	79	61	105
					TP (mg/L)					
Min	0.010	0.010	0.020	0.010	0.010	0.010	0.010	0.010	0.020	0.020
25th	**0.050**	**0.080**	**0.068**	**0.035**	**0.050**	**0.070**	**0.055**	**0.050**	**0.042**	**0.100**
median	0.085	0.111	0.149	0.065	0.080	0.145	0.092	0.080	0.069	0.180
mean	0.271	0.189	0.252	0.101	0.239	0.244	0.140	0.238	0.144	0.305
75th	0.176	0.260	0.260	0.118	0.162	0.260	0.139	0.190	0.143	0.310
max	7.97	0.58	3.5	0.61	7.97	3.5	0.597	1.415	1.415	3.5
N	470	26	194	117	575	220	12	90	64	130

Combined dataset from NWIS, STORET, and EPA nutrient Center

The combined dataset was weighed less for deriving nutrient benchmarks since it contained values of inconsistent data quality (Table 5.11). Consequently, there was insufficient sample size in many of the regions to derive a population based nutrient benchmark. The South Bluff and Southeast bioregions had less than 10 sites in the dataset; while the West bioregion had only 30 sites, which made it difficult to split the West into two ecogroups. The TN benchmarks from this dataset were lower than those from the other two datasets. TP benchmarks, however, were higher than those observed from the other two datasets.

In summary, nutrient benchmarks from the population distribution driven approach varied by data source and sample size. Data from a probabilistic design would be ideal for population derived benchmarks. However, in the absence of such a design, datasets representing the full spectrum of human disturbance gradients and geological distribution can be used for criteria development.

Table 5.11 Percentile distribution of a combined dataset from USGS NWIS, EPA STORET database, and EPA nutrient database. Only data after 1991 were used.

	Bioregion					Ecoregion		
	All	**East**	**South Bluff**	**West**	**Southeast**	**65**	**74**	**75**
TN (mg/L)								
Min	0.02	0.02	0.055	0.069	0.405	0.02	0.055	0.405
25th	**0.159**	**0.165**	**0.075**	**0.141**	**0.572**	**0.183**	**0.136**	**0.576**
median	0.468	0.329	0.094	0.472	0.67	0.365	0.466	0.599
mean	1.638	2.054	0.097	1.078	3.346	2.280	0.988	1.129
75th	0.689	0.700	0.117	0.673	4.5	0.82	0.650	0.677
max	31	31	0.140	13.1	12.2	31	13.1	5.9
N	80	40	3	30	7	43	33	4
TP (mg/L)								
Min	0.012	0.021	0.123	0.075	0.012	0.021	0.075	0.012
25th	**0.083**	**0.087**	**0.129**	**0.102**	**0.020**	**0.084**	**0.104**	**0.042**
median	0.110	0.107	0.135	0.137	0.032	0.103	0.136	0.061
mean	0.178	0.243	0.136	0.154	0.034	0.236	0.152	0.059
75th	0.175	0.254	0.143	0.180	0.046	0.253	0.175	0.080
max	1.79	1.79	0.151	0.32	0.061	1.79	0.32	0.086
N	68	33	3	28	4	34	31	3
Turbidity (NTU)								
Min	1.56	5.79		1.56	1.75	1.75	1.56	5.01
25th	**10.81**	**15.667**		**6**	**3.38**	**13.333**	**6**	**5.01**
median	20.983	35.333		12.433	5.01	29.667	12.433	5.01
mean	35.156	47.381		15.740	6.153	44.129	15.740	5.01
75th	55.5	62.917		20.625	8.355	61	20.625	5.01
max	164	164		45	11.7	164	45	5.01
N	36	23	0	10	3	25	10	1

5.3 Best Attainable Condition (BAC).

Using biological criteria defined by M-BISQ scores for each bioregion (the lower quartile of M-BISQ07 reference site, Table 5.12), we identified 214 sites attaining the biological criterion.

This population of sites (Appendix B) were used to define the BAC and we derived nutrient benchmarks for different bioregions using this BAC population.

Table 5.12. Selection criteria for BAC based on M-BISQ scores

Bioregion	M-BISQ score
East	>65.7
South Bluff	>55.9
South East	>66
West Bioregion - ecogroup 1	>38.5
West Bioregion - ecogroup 5	>52.3

TN benchmarks estimated using BAC sites were mostly similar to each other among different bioregions, except ecogroup 1 which was higher than the other bioregions (Figure 5.8). TP concentrations and turbidity varied more among regions. Generally, ecogroup 1 in the West bioregion and the South Bluff bioregions had higher TP concentrations than the other bioregions.

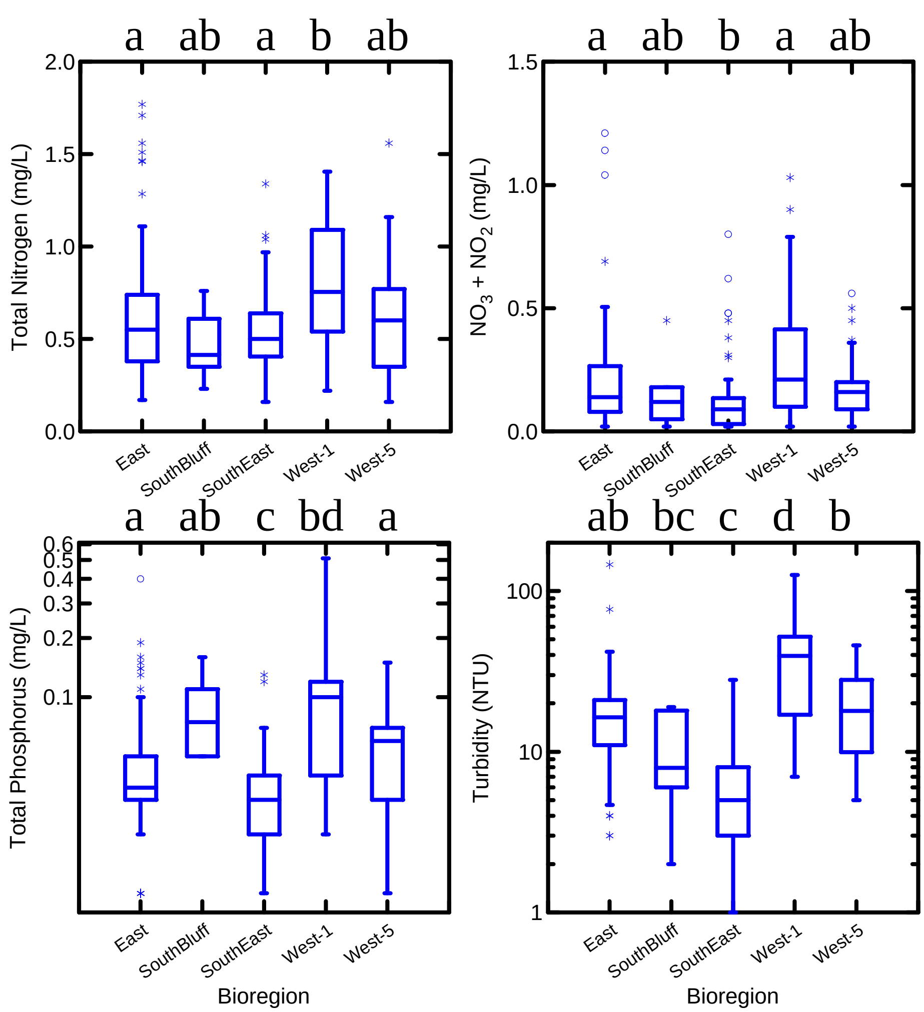

Figure 5.8. Best attainable condition (BAC) in four bioregions in the State of Mississippi. Values for regions sharing the same letter above were not significantly different (p>0.05), for example for total nitrogen, East,

SouthBluff, SouthEast, and West-5 all have the letter "a" and are therefore not significantly different, but the West-1 was significantly higher than the East and SouthEast, but similar to the other two regions and are all labeled with "b".

The percentiles estimated from BAC sites and nutrient benchmarks for each bioregion and ecoregion are listed in Table 5.13. The West bioregion was split into two ecogroups (ecogroup 1 in the North and ecogroup 5 in the South) because of different biological criteria were developed for these two ecogroups. Using the 75th percentile of BAC, TN benchmarks were highest in the West bioregion (1.12 mg/L in ecogroup 1 and 0.770 mg/L in ecogroup 5). South Bluff bioregion had only 6 BAC sites, so it would be more appropriate to either adopt benchmarks from ecogroup 5 (adjacent neighbor) or combine the data into the whole ecoregion 74 (TN=0.0.843 mg/L). Similarly, TP benchmarks were highest in ecogroup 1 (0.120 mg/L) and lowest in the Southeast bioregion (0.040 mg/L). The TP benchmark in ecogroup 5 was 0.070 mg/L. The TP benchmark for the South Bluff bioregion would be 0.087 mg/L if it was combined into the whole ecoregion 74.

Table 5.13. Percentile distribution of BAC nutrient concentrations.

	Bioregion				***Ecoregion***			***Ecogroup***		
	East	***South Bluff***	***West***	***Southeast***	***65***	***74***	***75***	***5+6***	***5***	***1***
					TN (mg/L)					
Min	0.170	0.230	0.160	0.160	0.170	0.160	0.160	0.160	0.160	0.220
25th	0.383	0.360	0.427	0.405	0.400	0.416	0.360	0.350	0.350	0.560
median	0.550	0.415	0.660	0.500	0.530	0.650	0.485	0.540	0.600	0.780
mean	0.607	0.463	0.761	0.551	0.593	0.740	0.462	0.596	0.620	0.982
75th	**0.730**	**0.567**	**0.925**	**0.640**	**0.690**	**0.843**	**0.497**	**0.755**	**0.770**	**1.120**
max	1.77	0.76	4.72	1.34	1.77	4.72	0.82	1.560	1.560	4.720
N	98	6	54	53	145	58	6	39.000	33	21
					TP (mg/L)					
Min	0.010	0.050	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020
25th	0.030	0.055	0.040	0.020	0.020	0.040	0.012	0.040	0.030	0.040
median	0.035	0.075	0.060	0.030	0.030	0.060	0.020	0.060	0.060	0.100
mean	0.049	0.087	0.073	0.033	0.045	0.075	0.023	0.058	0.053	0.104
75th	**0.050**	**0.102**	**0.080**	**0.040**	**0.050**	**0.087**	**0.020**	**0.070**	**0.070**	**0.120**
max	0.4	0.16	0.51	0.13	0.4	0.51	0.06	0.160	0.150	0.510
N	98	6	54	53	145	58	6	39	33	21
					Turbidity (NTU)					
Min	3.0	2.0	5.0	1.0	1.0	2.0	3.0	2.0	5.0	7.0
25th	11.0	6.2	13.0	3.0	7.0	10.4	3.5	8.2	10.5	17.7
median	16.4	8.0	22.0	5.0	11.0	19.0	5.0	16.0	18.0	39.5
mean	18.9	10.2	28.0	6.2	14.7	26.1	5.0	18.1	19.8	40.9
75th	**21.0**	**15.7**	**36.0**	**8.0**	**18.1**	**34.5**	**6.5**	**25.7**	**27.5**	**51.2**
max	146	19	126	28	146	126	7	46	46	126
N	93	6	46	53	139	50	7	34	28	18

5.4 Summary of nutrient benchmarks based on reference approaches

Nutrient benchmarks derived from different reference approaches varied across different bioregions (Table 5.14). Generally speaking, nutrient benchmarks derived using MDC were much lower than those using LDC and BAC conditions.

Table 5.14. Summary of nutrient benchmarks from different reference condition approaches. Sample sizes less or equal than 30 are listed in the parentheses. Benchmarks considered in the final criteria recommendations are in bold.

		Bioregions				Ecogroup	
Population	**Data**	**East**	**Southeast**	**South Bluff**	**West**	**1**	**5**
				TN (mg/L)			
MDC	M-BISQ	**0.194**	**0.183**	**0.194**	**0.282**	**0.282**	**0.282**
LDC	M-BISQ LDC1	**0.693**	**0.620**	0.520 (7)	0.785	0.903 (4)	**0.533**
	M-BISQ LDC2	0.768	0.64	0.383(10)	0.66	1.065 (4)	0.585 (30)
ENTIRE	M-BISQ	0.46	0.41	0.312 (26)	0.52	**0.860**	0.350
	WADES	0.483	0.480	**0.582**	0.591	**0.694**	0.439
	Other	0.165	0.572 (7)	0.075 (3)	0.141 (30)		
BAC	M-BISQ	**0.730**	**0.640**	0.685 (6)	0.925	**1.120 (21)**	**0.770**
				TP (mg/L)			
MDC	M-BISQ	**0.013**	**0.016**	**0.064**	**0.022**	**0.022**	**0.022**
LDC	M-BISQ LDC1	**0.050**	**0.030**	0.137 (7)	0.111	0.119(4)	**0.060**
	M-BISQ LDC2	0.060	0.030	0.104 (10)	0.060	0.064 (4)	0.060
ENTIRE	M-BISQ	0.030	0.020	0.062 (26)	0.050	0.060	0.034
	WADES	0.050	0.035	**0.080**	0.068	**0.100**	0.042
	Other	0.087	0.020 (7)	0.129 (3)	0.102 (28)		
BAC	M-BISQ	**0.050**	**0.040**	0.105 (6)	0.080	**0.120 (21)**	**0.070**

Nutrient benchmarks derived using LDC were most consistent with current state approaches. Of the six datasets used to derive nutrient benchmarks in these analyses, the population of sites in the M-BISQ LDC1 was most consistent with the population of sites used for M-BISQ biological criteria development. As a result, benchmarks derived from this dataset were heavily weighted for criteria recommendations. M-BISQ LDC2 had similar nutrient benchmarks to those derived using M-BISQ LDC1. They were used to compare and evaluate regional differences in background nutrient concentrations when land uses in different regions were similar.

Although the LDC1 approach was preferred, the small sample size restricted its utility in some regions. For example, only seven LDC1 sites for the South Bluff bioregion and four LD sites for Ecogroup 1 were available, and it would be less accurate to derive nutrient criteria based on such a small sample size. One option would be to use entire population derived estimates for these regions. The WADES dataset and M-BISQ dataset had sufficient sample sizes and wide spatial distribution in these regions. Generally, nutrient benchmarks from the 25^{th} percentile of the whole population distribution were lower than those from the 75^{th} percentile of LDC1 sites.

The BAC population generally resulted in higher TN benchmarks than MDC and LDC populations. In contrast, benchmarks for TP using BAC were similar to that of LDC in most regions; but in some cases were a little higher. Again, sample size for the South Bluff bioregion was too small to derive a population based benchmark for this region. One alternative would be to use benchmarks for the West bioregion as a surrogate before more samples were collected in this region. As for final criteria development, we would recommend that nutrient criteria not exceed benchmarks based on BAC.

6.0 STRESSOR- RESPONSE APPROACH (WADEABLE STREAMS)

Algal biomass in streams responds to elevated nutrient concentrations, therefore, they are commonly used indicators of stream eutrophication and impairment. In addition, other biological indicators, such as macroinvertebrate metrics, which may not directly respond to nutrient enrichment, indirectly respond to nutrient related impact. We analyzed both response variables, to the extent possible to develop candidate nutrient endpoints.

6.1. Correlations among chemical variables

We first examined correlations among water chemistry parameters that might potentially contribute to biological degradation (Table 6.1). We were particularly interested in nutrient related parameters, such as dissolved oxygen (DO) concentrations and turbidity since these variables are directly linked to aquatic life uses in streams. We did not find significant relations between DO and other chemical parameters but found strong correlations between turbidity and both TN and TP concentrations. Another common stressor, specific conductance, was strongly correlated with Cl concentrations, pH, alkalinity, and TP.

Table 6.1 Spearman Correlation metrics among environmental variables in M-BISQ water chemistry data. Bold font indicates significant correlations ($p<0.05$). NH4 – Ammonium, COD- chemical oxygen demand, Cl – Chloride, COND – Conductivity, ALK – Alkalinity. DO – Dissolved Oxygen, TDS- total dissolved solids, TKN- Total Kjeldahl Nitrogen, TOC-Total Organic carbon, TURB - Turbidity.

Variables	TN	NH4	COD	Cl	NO_{2+3}	pH	COND	FLOW	ALK	DO	TDS	TKN	TOC	TP
TN	1													
NH4	0.362	1												
COD	0.361	0.147	1											
Cl	0.264	0.245	0.244	1										
NO_{2+3}	**0.64**	0.328	-0.07	0.141	1									
pH	0.208	-0.004	-0.11	0.302	0.23	1								
COND	0.318	0.245	0.125	**0.735**	0.223	**0.603**	1							
FLOW	0.03	0.06	-0.01	-0.11	-0.01	-0.2	-0.24	1						
ALK	0.314	0.099	-0.01	**0.459**	0.256	**0.722**	**0.756**	-0.24	1					
DO	-0.15	-0.099	-0.23	0.01	0.063	0.283	0.155	-0.28	0.181	1				
TDS	0.318	0.245	0.117	**0.737**	0.226	**0.598**	1	-0.24	**0.754**	0.148	1			
TKN	**0.824**	0.291	**0.559**	0.252	0.193	0.127	0.278	0.063	0.238	-0.23	0.28	1		
TOC	0.388	0.306	**0.744**	0.246	-0.11	-0.16	0.153	0.088	-0.02	-0.32	0.15	**0.64**	1	
TP	**0.564**	0.229	0.276	0.394	0.353	0.407	**0.531**	-0.09	0.513	-0.01	0.53	**0.57**	0.31	1
TURB	**0.446**	0.13	0.158	0.077	0.235	0.311	0.317	-0.05	0.313	0.022	0.32	**0.47**	0.28	**0.49**

6.2 Algal-nutrient relationships

Deviations from the Redfield ratio (41:7:1 by weight or 106:16:1 molar) are frequently used to determine N and P limitation (Redfield 1958). High N:P ratios indicate P is limiting growth, and low N:P ratios suggests that N is limiting growth. As discovered in the reference approach analysis, the molar N:P ratio in the study region in the EDAS database ranged from 4 to 245 and averaged 41 in ecoregion 65 and 28 in ecoregion 74 (Table 5.9). As a result, we considered streams as potentially both/either N- and/or P-limited.

There was no measurement of benthic algal biomass available in Mississippi for effective causal response analysis between nutrient enrichment and algal growth. However, water column chlorophyll measurements were available from several different sources (EPA nutrient database, STORET, and NWIS data). The average water column algal biomass was plotted against nutrient concentrations in the water column (Figure 6.1). However, we were not able to find an algal biomass–nutrient relationship. A number of factors may have limited our ability to detect a strong relationship between water column chlorophyll and nutrient concentrations, including water velocity, light irradiance at the water surface, water clarity (turbidity), temperature, algal settling rate, and grazing.

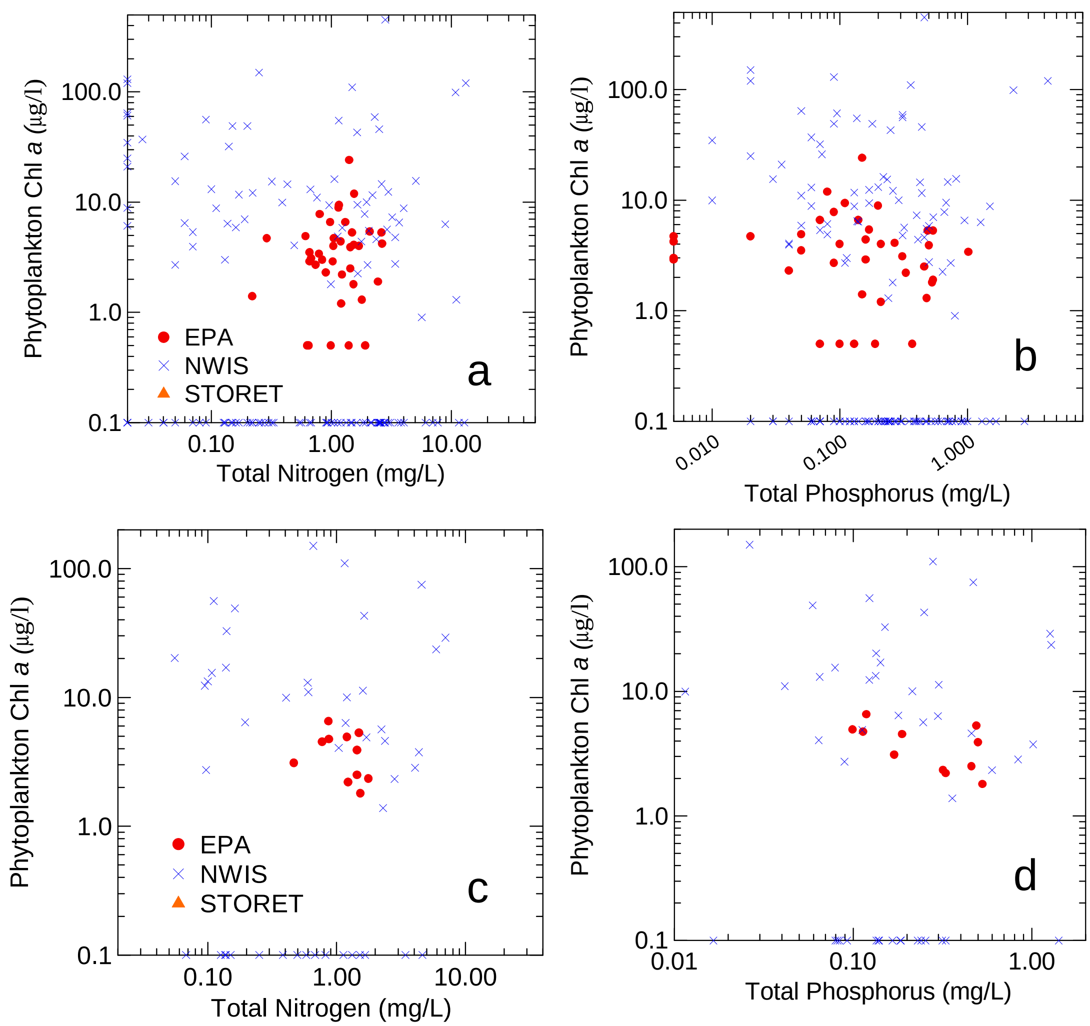

Figure 6.1. Phytoplankton biomass and total nutrient concentrations in the combined database. Figure a and b are all samples, c and d show site averages.

6.3 Macroinvertebrate Metrics and Nutrient Concentrations

In the absence of a direct linkage between nutrient concentrations and direct response variables (e.g., algal biomass and species compositions), indirect response variables, such as macroinvertebrate metrics, were used to delineate possible thresholds of responses to nutrient concentrations. After strong correlations were found between macroinvertebrate indices and metrics and nutrient parameters, we used visual plots to further explore the relationships. We then used a conditional probability approach (Paul and MacDonald, 2005) to examine changes in the biological community along stressor gradients. We also used nonparametric deviance reduction (change point analysis) to identify ecological thresholds (Qian et al. 2003). Detailed statistical methods are presented in Appendix E. The data analyses based on previous M-BISQ03 scores and selected macroinvertebrate metrics for each ecoregion are also attached in appendix E.

6.3.1 Correlations of macroinvertebrate metrics with nutrients

We used data collected from the M-BISQ program to examine relationships between macroinvertebrate indices and metrics and nutrient parameters. Correlation analysis identifies apparent linkages between biological condition and environmental variables. It may or may not indicate the real relationship between biological condition (biological indices) and environmental characteristics. A number of nutrient related environmental variables were strongly correlated with M-BISQ scores and composite metrics in each bioregion (Table 6.2). Overall, M-BISQ scores were strongly correlated with main environmental variables ($p<0.05$) in most bioregions except the South Bluff ecoregion. TN concentration was a better predictor of macroinvertebrate index than NO_{2+3} concentration most of the time. Therefore, we intend to develop TN criteria instead of NO_{2+3} criteria for the state.

We selected correlations of interest (Table 6.2) and performed visual scatter plots to further examine the relationships. We used either linear regression or a locally weighted average regression line to examine trends along environmental gradients. Due to regional differences, we examined the relationships for each bioregion.

The East bioregion had the largest sample size (280 sites) and exhibited strong biological responses to nutrient gradients (Figure 6.2). M-BISQ scores for the East Bioregion not only declined with increased TN (R2=0.157, $p<0.001$) and TP concentrations (R2=0.109, $p<0.001$), but also presented a threshold. According the LOWESS regression lines, when TN approaches 0.60 mg/L and TP approaches 0.040 mg/L, M-BISQ scores declined sharply. M-BISQ scores also declined linearly along turbidity gradients.

Macroinvertebrate responses to nutrient gradients in the West bioregion were more complicated than in other regions (Figure 6.3). The northern part of the West bioregion was dominated by high agricultural land uses while the southern part was characterized by greater natural land use. M-BISQ scores in the Southern part were generally higher than in the northern part (Figure 6.3). Therefore, the designated use protection in the north was less strict than in the southern part of the bioregion. M-BISQ criteria are 38.5 for the northern and 52.3 for the southern part of the bioregion. Similar to the East bioregion, macroinvertebrate M-BISQ scores declined with increased nutrient concentrations (linear models, $R^2=0.308$ for TN 0.284 for TP, $p<0.001$) and also exhibited thresholds as shown in Figure 6.3.

Table 6.2 Spearman Correlation matrix between macroinvertebrate metrics and selected environmental variables. Bold fonts indicate significant correlations ($p<0.05$). COC2ChiOct - % (Cricotopus + Orthocladius + Chironomus) of Chironomidae, Chemical abbreviations as above

West Bioregion

Variables	M-BISQ	Total Taxa	% Sensitive EPT	% Sensitive Coleoptera	Beck's Index	Tolerant % of Taxa
TN	**-0.570**	**-0.340**	**-0.465**	**-0.260**	**-0.566**	**0.387**
TP	**-0.492**	**-0.249**	**-0.451**	**-0.178**	**-0.493**	**0.370**
NO_{2+3}	**-0.374**	**-0.232**	**-0.182**	**-0.293**	**-0.384**	**0.319**
TKN	**-0.450**	**-0.251**	**-0.499**	-0.095	**-0.443**	**0.266**
TURB	**-0.461**	**-0.200**	**-0.413**	**-0.273**	**-0.450**	**0.437**
DO	-0.095	0.057	0.008	-0.110	-0.103	**0.372**
pH	**-0.325**	**-0.206**	-0.141	**-0.235**	**-0.364**	**0.476**
COND	**-0.481**	**-0.179**	**-0.439**	**-0.167**	**-0.541**	**0.663**
FLOW	**0.393**	0.157	**0.566**	**0.175**	**0.400**	**-0.391**

East Bioregion

Variables	M-BISQ	Total Taxa	EPT Taxa	% Sensitive EPT	COC2ChiPct	Shredder Taxa	Hilsenhoff's Index
TN	**-0.420**	**-0.235**	**-0.405**	**-0.377**	**0.198**	**-0.260**	**0.382**
TP	**-0.360**	**-0.176**	**-0.340**	**-0.331**	**0.203**	**-0.286**	**0.329**
NO_{2+3}	**-0.248**	**-0.153**	**-0.193**	**-0.167**	**0.226**	**-0.147**	**0.263**
TKN	**-0.356**	**-0.180**	**-0.388**	**-0.376**	0.091	**-0.243**	**0.292**
TURB	**-0.251**	-0.112	**-0.237**	**-0.256**	**0.199**	-0.049	**0.360**
DO	**0.141**	0.050	**0.131**	**0.149**	-0.059	0.061	-0.039
pH	**-0.365**	**-0.367**	**-0.221**	-0.097	**0.222**	**-0.296**	**0.406**
COND	**-0.635**	**-0.484**	**-0.530**	**-0.428**	**0.386**	**-0.451**	**0.666**
FLOW	**0.190**	**0.150**	**0.291**	**0.279**	0.014	**0.138**	**-0.199**

Southeast Bioregion

Variables	M-BISQ	Total Taxa	COC2ChiPct	% Non-Insect	% Filter	Sprawler Taxa	Hilsenhoff's Index
TN	**-0.409**	**-0.244**	**0.231**	**0.388**	0.042	**-0.326**	**0.307**
TP	**-0.238**	-0.224	0.069	**0.299**	0.062	**-0.301**	0.210
NO_{2+3}	0.201	0.212	-0.063	-0.188	**0.231**	-0.036	**-0.279**
TKN	**-0.530**	**-0.270**	**0.264**	**0.528**	-0.059	**-0.276**	**0.456**
TURB	**-0.542**	**-0.261**	**0.324**	**0.395**	**-0.252**	-0.196	**0.511**
DO	**0.235**	0.182	-0.094	-0.167	0.078	0.188	-0.081
pH	**-0.240**	**-0.292**	**0.368**	-0.025	0.193	**-0.280**	0.193
COND	**-0.260**	-0.108	**0.391**	0.127	-0.047	-0.149	**0.231**
FLOW	-0.013	0.081	0.225	-0.107	0.123	0.182	0.196

South Bluff Bioregion

Variables	M-BISQ	% Sensitive EPT	% Crustacea and Mollusks	Oligochaeta Taxa	% Odonata	Collector Taxa	% Swimmer
TN	0.174	0.238	0.147	-0.055	-0.219	0.039	0.133
TP	0.165	0.181	0.015	0.051	-0.196	0.034	-0.214
NO_{2+3}	0.044	-0.133	0.050	-0.101	0.325	0.179	-0.057
TKN	0.150	**0.472**	0.187	0.108	**-0.456**	0.082	0.224
TURB	-0.146	0.032	0.186	**0.449**	0.009	0.296	0.210
DO	-0.005	0.112	0.186	0.032	**-0.444**	0.014	0.105
pH	0.030	0.384	-0.267	0.071	-0.297	-0.371	0.186
COND	0.075	0.085	-0.221	-0.156	0.076	**-0.472**	-0.022
FLOW	-0.309	0.210	-0.050	0.025	0.294	0.249	**0.540**

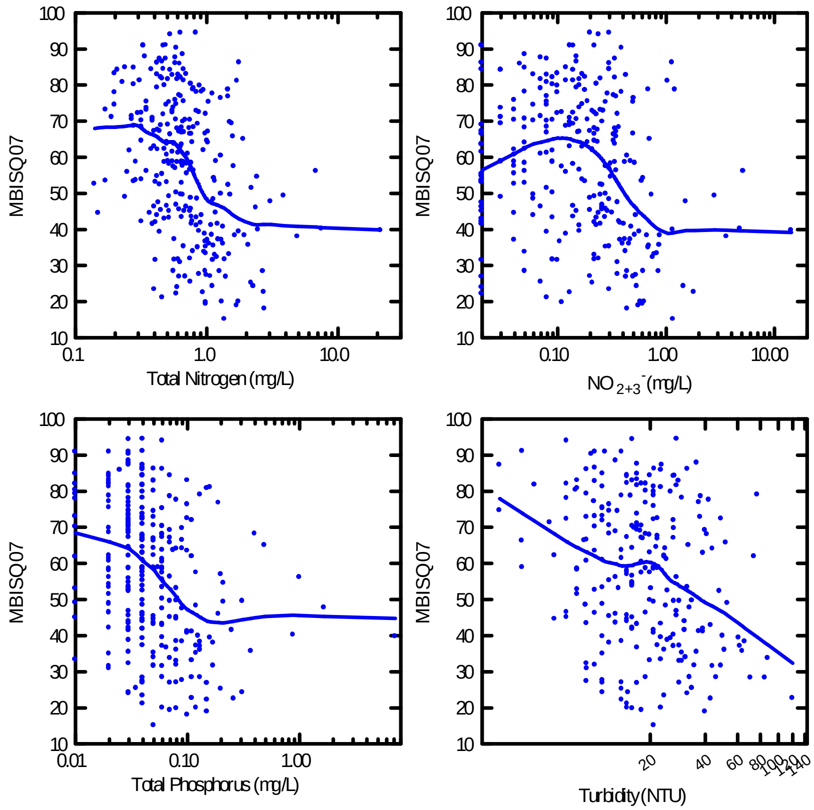

Figure 6.2 Responses of M-BISQ07 score to nutrient parameters in East Bioregion. The solid lines are LOWESS lines.

The South Bluff and West bioregion belong to the same ecoregion (ecoregion 74). However, the South Bluff bioregion had a relatively small sample size for gradient analysis. As a result, in the South Bluff region and correlation analyses (Table 6.2) exhibited no observable responses of macroinvertbrate metrics (M-BISQ scores and metrics) to either TN or TP gradients (p>0.05). Therefore, we calculated M-BISQ scores according to the West bioregion metrics for samples in the South Bluff bioregion and analyzed macroinvertebrate responses at the ecoregion level (Figure 6.3). Although combining the South Bluff and West Bioregion sites increased statistical power, it added more variation to the regression models between M-BISQ scores and log transformed nutrient concentrations (R^2 declined from 0.308 to 0.194 for TN model, from 0.284 to 0.242 for TP model). According to the LOWESS fits, when TN approached 0.7 mg/L and TP approached 0.100 mg/L, M-BISQ scores declined. M-BISQ scores exhibited more of a linear response to turbidity in this region.

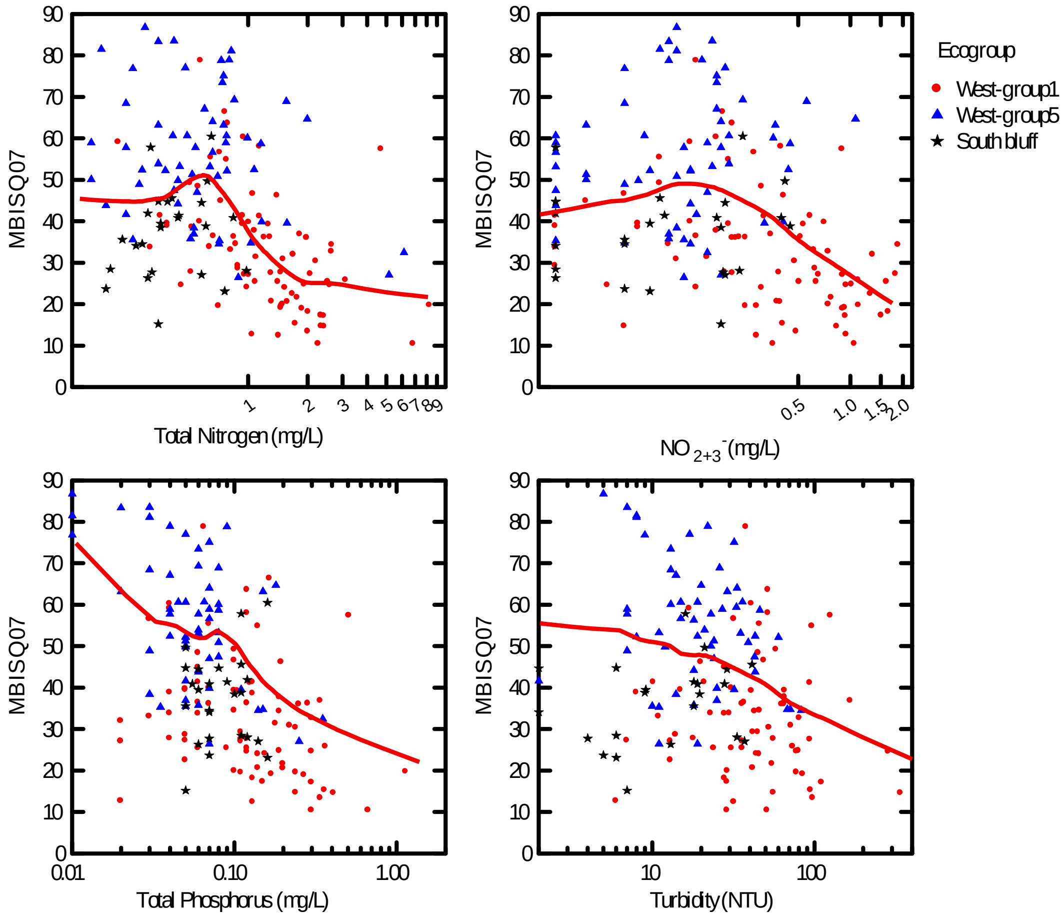

Figure 6.3 Responses of M-BISQ07 scores to nutrient parameters in West and South Bluff Bioregions. Blue triangles are sites within ecogroup 5 and red dots are sites within ecogroup 1. Black stars are sites within South Bluff bioregion and are scored according to West bioregion index.

Regression models for M-BISQ scores and TN and TP gradients in the ecogroups within the regions were much weaker compared to the whole region models. TN and TP models in ecogroup 1 (83 sites) were significant but explained little variance (TN: $R^2 = 0.242$, $p<0.001$, TP: $R^2 = 0.083$, $p=0.008$). TN models in ecogroup 5 (58 sites) were not significant (TN: $R^2 = 0.059$, $p=0.066$) and the TP model was also weak (TP: $R^2 = 0.264$, $p<0.001$).

Macroinvertebrate responses to nutrient gradients in the Southeast bioregion were weaker than in the East and West regions, perhaps due to smaller sample sizes (n=72) and reduced nutrient gradients (Figure 6.4). M-BSIQ responses to TN were significant (p=0.002), but TP models were not (p=0.132). However, the LOWESS fits to both TN and TP gradient responses still showed that macroinvertebrate M-BISQ score declined with increased TN and TP concentrations and also presented thresholds (Figure 6.4). When TN concentration was approximately 0.6-0.8 mg/L and TP concentrations 0.060 mg/L, M-BISQ scores declined sharply. M-BISQ scores also declined linearly with turbidity.

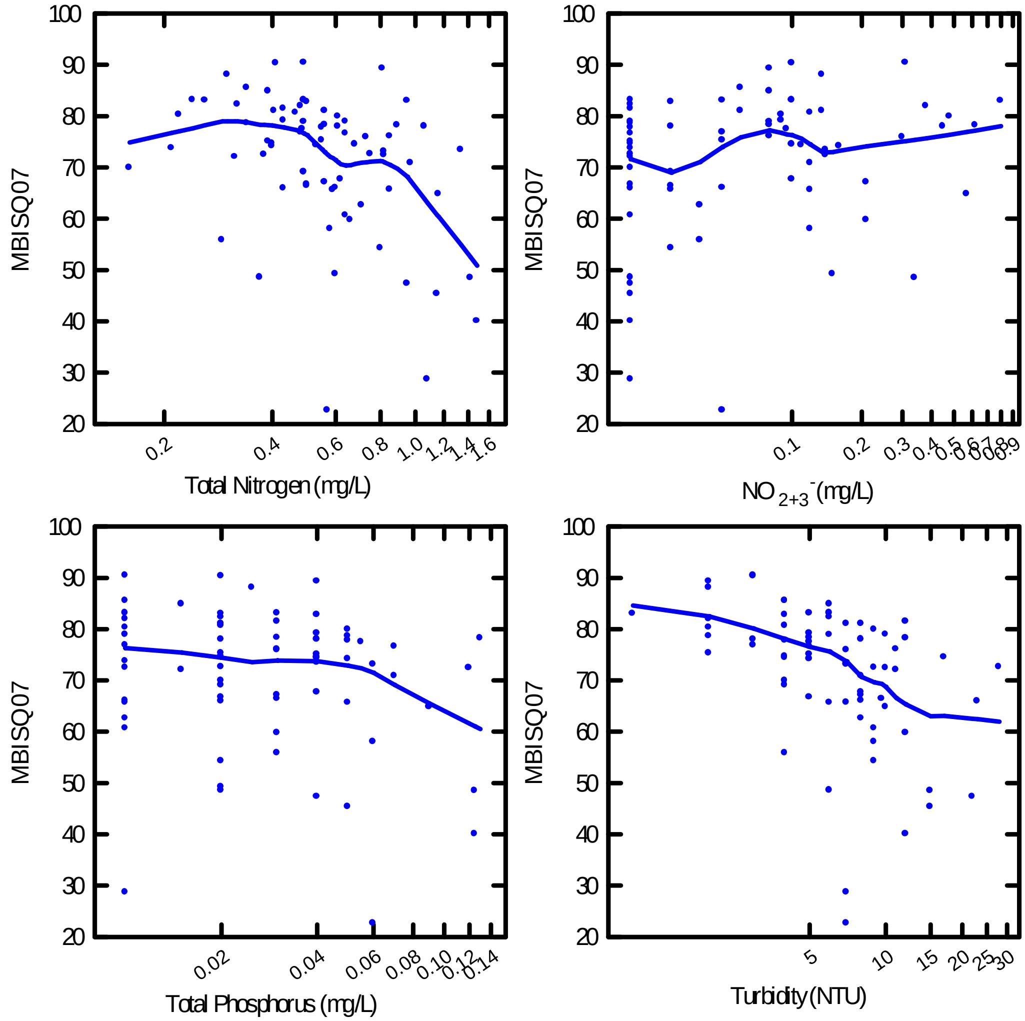

Figure 6.4 Responses of M-BISQ07 score to nutrient parameters in Southeast Bioregion. The solid lines are LOWESS lines.

6.3.2 Conditional probability analysis (CP)

A conditional probability approach (Paul and MacDonald, 2005) allows analysis of changes in the macroinvertebrate community along stressor gradients without assuming a causal-response relationship. Conditional probability is the likelihood of an event when it is known that some other event has occurred. A conditional probability statement provides the likelihood (probability) of a predefined response (e.g., M-BISQ scores < 66), if the value of a pollutant stressor (e.g. TP>0.05) is exceeded. All observed stressor values (in this example, all observed values of total phosphorous) were used to develop a curve of conditional probability (Paul and MacDonald, 2005). (See appendix D for more details and analyses at ecoregion levels).

Conditional Probability (CP) analyses for both East and Southeast bioregions revealed that probability of impairment increased with elevated nutrient concentrations (Figure 6.5). The probability of impairment (M-BISQ<65.7) in the East bioregion was relatively low when TN concentration was less than 0.5 mg/L. With increased TN concentrations, the probability of impairment sharply rose to 90% when TN was above 1 mg/L. CP also increased along TP gradients in the East bioregion and rose higher when TP increased above 0.03 mg/L. The M-BISQ score–TP concentration relationship was not significant in the Southeast bioregion due to the relatively short TP gradient, but CP indicated that the CP of macroinvertebrate impairment (M-BISQ<66) increased along the TP gradient. The CP of MBISQ impairment exhibited a stronger response to TN in the Southeast region. The CP of impairment began to increase at 0.4 mg/L TN. This visual threshold for this region was from .5 to 0.8 mg/L TN and approximately 0.04 mg/L TP.

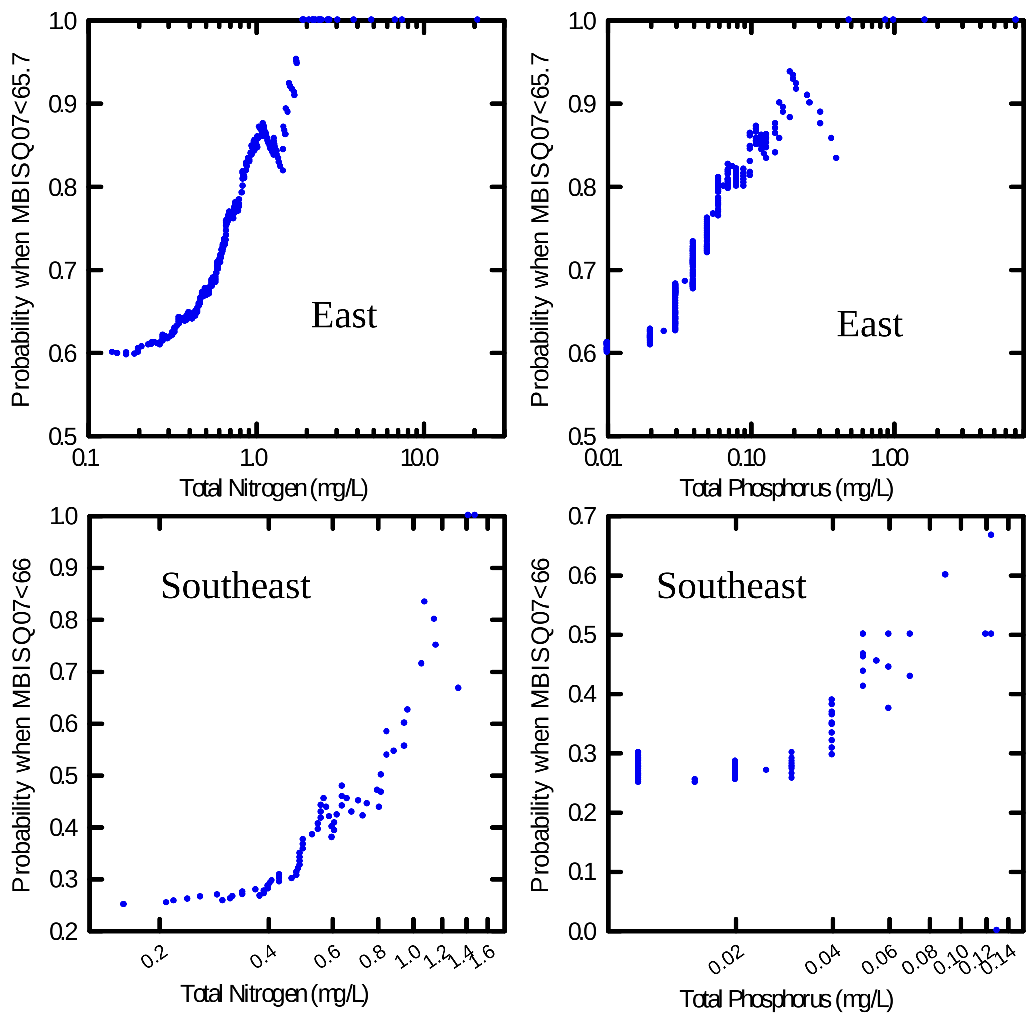

Figure 6.5. Conditional probability analysis showing the probability of impairment (biological condition less than expected values, i.e., MBISQ<65.7) increases with increased total nitrogen and total phosphorus concentrations in the East Bioregon and Southeast Bioregion.

As pointed out earlier, since different biological criteria were defined for the northern (ecogroup 1) and southern part (ecogroup 5) of the West Bioregion, we performed conditional probability analyses on the entire West bioregion (M-BISQ criterion 43) as well as the two separate ecogroups (impairment threshold: M-BISQ score <38.5 for ecogroup 1 and <52.3 for ecogroup 5) (Figure 6.6). When the West Bioregion was treated as a whole nutrient region, the thresholds were around 0.800 mg/L TN and 0.060 mg/L TP. When ecogroup 1 and ecogroup 5 were analyzed separately, these two regions had different nutrient thresholds. The nutrient thresholds were approximately 1 mg/L TN and 0.1 mg/L TP in ecogroup 1, compared to 0.8 mg/L TN and 0.07 mg/L TP in ecogroup 5.

6.3.3. Change point analyses (CPA)

Lastly, we used nonparametric deviance reduction (change point analysis) to identify specific ecological thresholds (Qian et al. 2003). This technique is based on regression tree models, which are used to predict the value of a continuous variable from one or more continuous variables. The change point in this application is the first split of a tree model when there is only a single predictor variable. When the split in the data minimizes the deviance, a threshold is identified. This approach has been used to detect ecological changes along environmental gradients (Qian *et al.*, 2003). Uncertainty in the deviance reduction changepoint (95 percent CIs) was estimated from empirical percentiles of a bootstrap distribution from resampling 1,000 times. We used both M-BISQ index values and conditional probabilities as response variables, and TN or TP as predictor to determine nutrient change points. A more detail explanation on analyses performed at ecoregion levels is described in Appendix D.

According to change point analysis, thresholds of M-BISQ responses to nutrient concentrations (Table 6.3) were similar to visual thresholds identified at the CPA. The only exceptions were the changepoints based on TN and TP concentrations for ecogroup 5 of the West bioregion. This was likely due to weak relationships of M-BISQ to TN and TP concentrations. We propose using the lower boundary of the 95^{th} confidence limit of the change point as the benchmark for nutrient criteria development since the lower confidence limits reflects a conservative estimate of the change point.

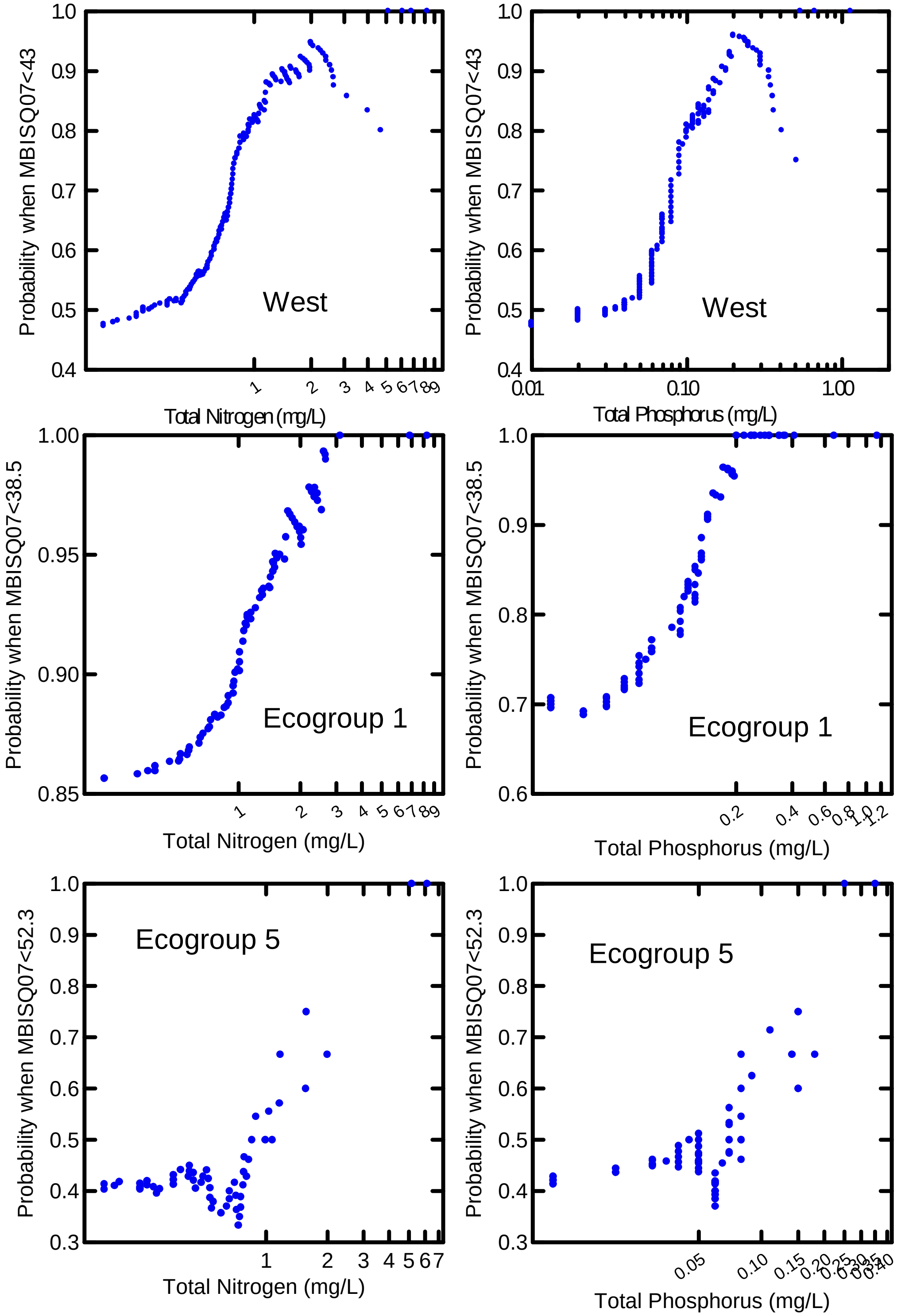

Figure 6.6. Conditional probability analysis showing the probability of impairment (biological condition less than expected values) increases with increased TN and TP concentrations in the West Bioregon and separated two ecogroups (1 and 5) of West Bioregions.

Table 6.3 Nutrient thresholds for each bioregion derived using change point analysis of raw M-BISQ scores (M-BISQ) as well as conditional probabilities (CP) of MBISQ scores being less than biological criteria using the revised MBISQ biological criteria.

		TN			**TP**		
	Response Variable	Median	Lower 95th CI	Upper 95th CI	TP	Lower 95th CI	Upper 95th CI
East	M-BISQ	0.840	**0.632**	0.980	0.060	**0.033**	0.108
	CP	0.800	**0.670**	0.820	0.053	**0.050**	0.055
Southeast	M-BISQ	0.570	**0.495**	1.070	N/A	N/A	N/A
	CP	0.835	**0.540**	1.015	0.040	**0.035**	0.045
West	M-BISQ	0.890	**0.810**	1.249	0.098	**0.095**	0.185
	CP	0.773	**0.760**	0.790	0.080	**0.080**	0.085
West_eco1	M-BISQ	1.295	**0.800**	1.438	0.135	**0.080**	0.292
	CP	0.945	**0.778**	0.975	0.115	**0.095**	0.128
West_eco5	M-BISQ	0.855	**0.265**	1.265	0.032	**0.025**	0.115
	CP	1.075	**0.811**	1.565	0.080	**0.067**	0.110
South Bluff	M-BISQ	N/A	N/A	N/A	N/A	N/A	N/A
	CP	N/A	N/A	N/A	0.070	0.065	0.115

7.0 NUTRIENT CRITERIA FOR NON-WADEABLE STREAMS

7.1 Reference approach

Due to the relatively small sample size (43 sites in three basins) and cross-regional characteristics of large rivers, we could not classify non-wadeable streams into different bioregions. MDEQ (2007b) identified 17 least disturbed (LD) sites based on five environmental variables: natural land cover in the watershed (%), conductivity, turbidity, habitat quality, and dissolved oxygen. These selection criteria did not include direct nutrient parameters and, therefore, did not lead to circularity for deriving nutrient benchmarks. The LD site distribution percentiles and all site distribution of TN and TP concentrations state-wide were used to identify nutrient benchmarks for non-wadeable streams (Table 7.1).

Table 7.1 Percentile distribution and BAC reference nutrient concentrations

	LDC Sites		Entire Site Population	
Parameters	**TN**	**TP**	**TN**	**TP**
Min	0.43	0.03	0.38	0.03
25th	0.59	0.04	**0.65**	**0.06**
median	0.712	0.07	0.891	0.115
mean	1.209	0.124	1.235	0.191
75th	**1.27**	**0.12**	1.34	0.23
max	7.36	0.48	7.36	1.21
n	17	17	42	42

The 75th percentile of LD for TN was 1.27 mg/L and for TP was 0.12 mg/L. In contrast to the 25th percentile of all sites for TN was 0.65 mg/L and for TP was 0.06 mg/L. The differences between these two sets of benchmarks were extremely large and the LD reference population may not represent the best nutrient concentrations for these sites. Due to the relatively small sample size (<20 sites), the reference approach was less reliable for nutrient criteria recommendation.

7.2 Stressor response approach

Macroinvertebrate index scores responded to nutrient parameters in different ways in non-wadeable streams (Figure 7.1). Index scores declined with both TN (R^2=0.133, p=0.018) and TP (R^2=0.227, p=0.001) concentrations, but the TP model was better than the TN model. Index scores also declined along the turbidity gradient (R^2=0.264, p=0.001) but not the NO_{2+3} gradient (p>0.05). LOWESS fits to the scatter plots revealed that index response to TN was more likely linear, while response to TP might exhibit a threshold. The metrics composing the non-wadeable stream index were plotted against TN and TP concentrations in Appendix E.

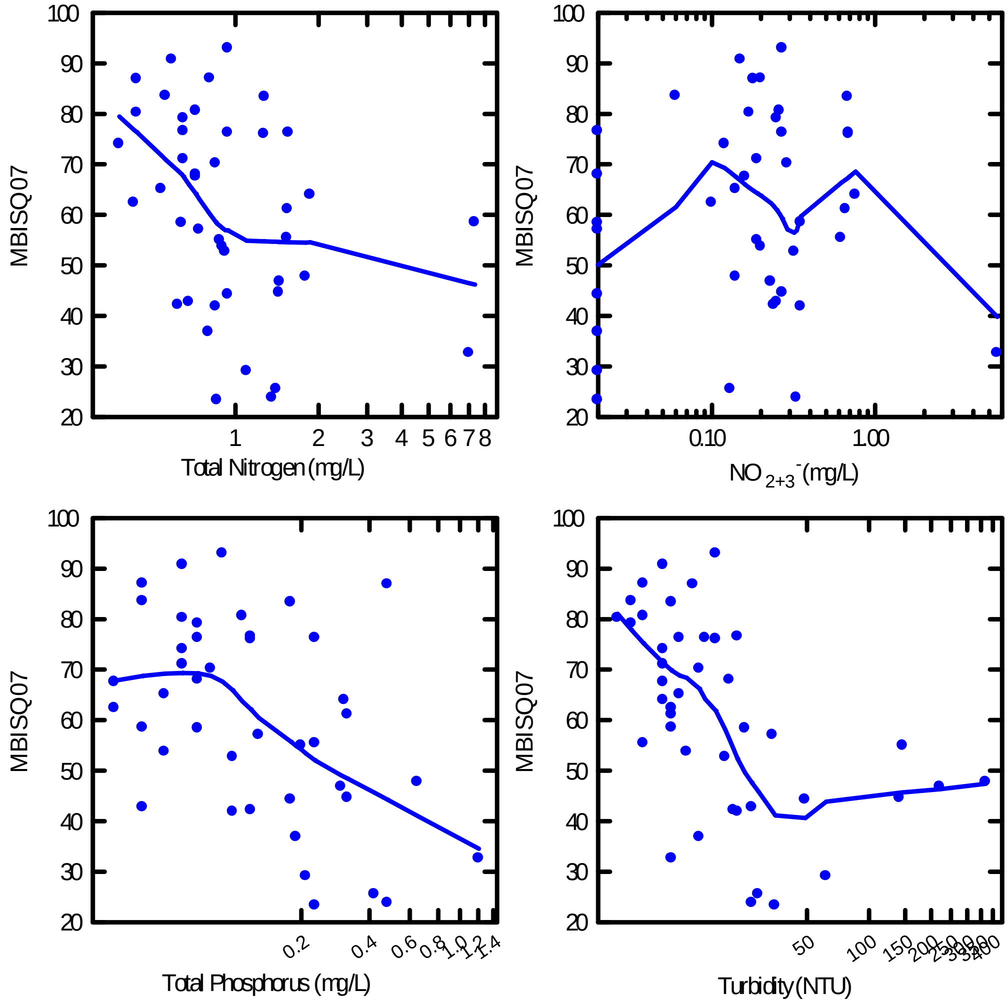

Figure 7.1. Responses of large river macroinvertebrate index to nutrient parameters.

A biological criterion was recommended for non-wadeable streams in the state (index score>67.1). We used this criterion as an impairment threshold for conditional probability analysis of non-wadeable streams (Figure 7.2). Contrary to the linear response of index score to TN (Figure 7.1), the CP of macroinvertebrate impairment increased with TN concentration and exhibited a threshold at 0.9 mg/L TN concentration (Figure 7.2). The CP of biological impairment also increased with TP concentration and exhibited a threshold at 0.10 mg/L.

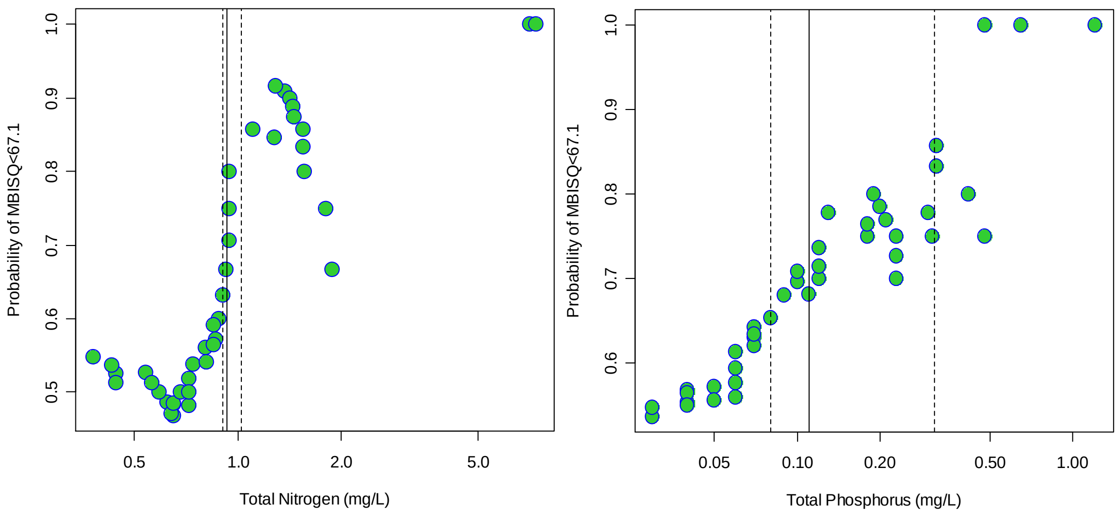

Figure 7.2. Conditional probability analysis showing the probability of impairment (biological condition less than expected values, i.e., index <67.1) increases with increased total nitrogen and total phosphorus concentrations in large rivers. Solid lines are the change points and the dashed lines are the upper and lower 95th confidence limits for the change points.

Change point analyses for the two pairs of relations resulted in slightly different thresholds than the visual CP method (Table 7.2). The change point in response of raw index scores to TN (0.760 mg/L) was lower and the lower confidence interval lower (0.605) than that identified using conditional probabilities. On the other hand, the change point in response of raw index scores to TP (0.125 mg/L) was higher (0.110 mg/L) as was the lower confidence interval (0.09 mg/L) than the conditional probability estimate.

Table 7.2. Nutrient thresholds derived from stressor-response approach and change point analysis for each non-wadeable streams. Thresholds were developed based on both raw non-wadeable MBISQ scores as well as the conditional probability of raw scores < 67.1.

	TN (mg/L)			*TP (mg/L)*		
	Threshold	**Lower CI**	**Upper CI**	**Threshold**	**Lower CI**	**Upper CI**
Raw MBISQ	0.760	0.605	1.320	0.125	0.090	0.132
CP	0.930	0.900	1.020	0.110	0.080	0.315

8.0 LITERATURE REVIEWS TO DERIVE CRITERIA

8.1 Studies and benchmarks in neighboring states and regions.

Relatively few studies have been conducted in the state of Mississippi to address nutrient related problems. Ray Montgomery and Associates (RMA, 2005) conducted a nutrient analysis for the Pascagoula under a MDEQ contract using M-BISQ 2001 data. This study considered total phosphorous (TP) as the limiting nutrient in and focused on TP targets (Thomann and Mueller, 1987). The report recommended the use of a TP range from 0.07 – 0.11 mg/l as a preliminary target. This range was based on the 75th to 90th percentiles of TP concentrations for fully attaining sites in the East Bioregion which includes the current Southeast bioregion and part of the East bioregion).

Other states in similar ecoregions have also conducted studies to derive nutrient endpoints for TMDLs, but only Tennessee has developed statewide nutrient criteria. In 2001, the Tennessee Division of Water Pollution Control, Department of Environmental Conservation (DEC) published a document entitled, *Development of Regionally-based Interpretations of Tennessee's Narrative Nutrient Criterion*. The report documented the 75$_{th}$ and 90$_{th}$ percentiles of total phosphorus and nitrate+nitrite data from each subecoregion within the state. The 75th percentiles of NO_{2+3} concentrations for ecoregion 65 was 0.24 mg/L, and for ecoregion 74 was 0.35 mg/L; TP was 0.030 mg/L for ecoregion 65, and was 0.080 mg/L for ecoregion 74. They recommended using the 90th percentiles as criteria (Table 8.1).

Table 8.1 Critical TP and NO_{2+3} benchmarks for important subecoregions within the State of Tennessee.

Region	Sample Size	75th Percentile	90th Percentile	Recommended Criterion	Revised Guidance
			TP		
74a	27	0.098	0.117	0.12	
74b	42	0.060	0.182	0.10	0.11
65a,b,i	12	0.040	0.191	0.04	
65e	55	0.030	0.040	0.04	
65j	53	0.009	0.032	0.04	
			NO_{2+3}		
74a	27	0.150	0.216	0.22	
74b	42	0.830	1.189	1.19	1.10
65a,b,i	12	0.230	0.361	0.34	
65e	55	0.278	0.340	0.34	
65j	53	0.190	0.220	0.22	

In 2002, Tennessee compared nutrient levels, periphyton densities, and dissolved oxygen concentrations in test and reference streams in 15 ecological subregions (Arnwine and Sparks 2003, Arnwine et al. 2003, Arnwine et al. 2005). Data from that study were used during the 2003 triennial review of water quality standards to refine existing dissolved oxygen and nutrient criteria. In 2004, Tennessee conducted another study to characterize nutrient, DO, habitat, and macroinvertebrates in each subecoregion. One of the goals of the 2004 study was to characterize non-wadeable streams that cross ecoregions in west Tennessee. They noticed that many of the non-wadeable rivers originate in the Southeastern Plains, crossed into the Loess Plains, and entered the Northern Mississippi Alluvial Plain on their way to the Mississippi River (TDEC 2004). They found that data for non-wadeable streams were generally not directly comparable to existing wadeable stream data and recommended developing TP and NOx criteria for non-wadeable streams in different ecoregions. The report listed several benchmarks for non-wadeable streams. For example, the 90th percentile TP concentrations of nonwadeable ecoregion 65e streams was 0.13 mg/L, for streams across several regions was 0.28 mg/L. However, these benchmarks were based on a very limited sample size (<6).

Other adjacent states had less data available to derive nutrient criteria. Alabama conducted a pilot study (ADEM 2005) to evaluate algal bioassessment techniques for nutrient enrichment in streams. They surveyed 20 impaired sites and 14 reference sites and suggested that periphyton chl *a*, total chl *a*. and percent cover of suitable substrate effectively detected nutrient enrichnment problems. The 75th percentiles of TN and TP concentrations for their reference condition streams were 0.698 mg/L and 0.043 mg/L respectively. However, these studies were conducted within ecoregions 67 and 68 which were different from MBISQ ecoregions.

The state of Florida set forth to develop nutrient criteria for each of their four bioregions (Peninsula, Panhandle, Northeast, and Everglades) (Weaver and Frydenberg 2006). Currently, they have conducted a pilot study focused on the Peninsula bioregion, which is within ecoregion 75. They adopted the reference condition approach to derive the nutrient targets by calculating the 75th percentile of data from a stringently defined reference set (Landscape disturbance index< 2.0) in the Peninsula (FDEP 2007). However, a 90th percentile was finally used due to the extensive multi-step verification of the candidate reference sites. Application of this method gave potential benchmarks of 1.7 mg/l for TN and 113 μg/L for TP.

The State of Kentucky has not developed nutrient criteria statewide yet. However, algal indicators have been developed to delineate nutrient thresholds in the state. Statewide nutrient endpoints were 1.20-1.47 mg/L TN and <20 μg/L TP based on diatom responses to nutrient concentrations (Panayotoff et al. 2006).

Lastly, Robertsen et al. (2001) used the reference approach as well as biological indicator responses to derive nutrient thresholds for different bioregions, including the Mississippi Valley-Interior River Lowland (MVIR) bioregion (including part of ecoregion 74 (Table 8.2). These benchmark values were similar to EPA recommended values.

Table 8.2. Robertson et al. 2001 An alternative regionalization scheme for defining nutrient criteria for rivers and streams. (USGS)

Sources	TN (mg/L)	TP (mg/L)
EPA guidance	0.690	0.037
USGS Environmental nutrient zones	0.510 – 0.670	0.020-0.050
reference dist 75th	0.920	0.055
all chems 25th	0.570	0.024
prob sites 25th	0.929	0.093
nCPA thresholds		0.030 - 0.040
LOWESS contours		0.020 - 0.030
Biocriteria approach	0.9 – 1.4	

8.2 Nutrient thresholds from other regions

A literature review was conducted by the Virginia Water Resources Research Center of nutrient criteria development. According to this report (VWRRC 2006), only two states, Arizona (River specific criteria) and Hawaii have developed total nitrogen criteria. Hawaii's criteria for its inland streams is 0.25 mg/L TN and 0.050 mg/L TP in the wet season (from Nov. 1 to April 30), and 0.18 mg/L TN and 0.030 mg/L TP in the dry season (from May 1 to October 30). Many more states have developed TP criteria for waterbodies. Most TP criteria were set at 0.1 mg/L TP (Nevada, New Jersey, New Mexico, North Dakota) or lower (Illinois and Utah 0.05 mg/L). Seasonal average, maximum, or monthly median values were used during different periods (low flow, summer, or growing seasons).

TMDLs have been developed for nutrient related problems all over the country. One classic example was the Clark Fork River, Montana. The Tri-State Implementation Council overseeing the Clark Fork River TMDL set mean targets at 0.300 mg/L for TN, 0.020 mg/L for TP upstream of Missoula, and 0.039 mg/L for TP below Missoula (TIC 1996). These values were based on multiple lines of evidence. In Oregon, a series of algal growth studies was performed to determine a TP target that would achieve Oregon's planktonic Chl-a criterion of 15 µg/L. According to these studies, algal growth was noticeably reduced at 0.100 mg/L of TP and was low at 0.050 mg/L of TP. Using this information and input from stakeholders, a TP target of 0.070 mg/L was set for the Tualatin River in Oregon (U.S. EPA 1999).

According to the summary of the VWRRC report, studies based on changes in the algal community generally suggested a TP threshold between 0.020 – 0.060 mg/L. Dodds and Welch (2000) conducted meta-analysis to derive empirical models between algal biomass and TP concentrations. From these studies, they concluded that water column TN concentrations should remain below 0.470 mg/L and TP concentrations below 0.060 mg/L to keep benthic mean Chl-a values around 50 mg/m^2 (thereby ensuring that Chl-*a* values stayed below 100 mg/m^2 most of the time). In later work using breakpoint regression and a two-dimensional Kolmogorov-Smirnov statistical technique, Dodds et al. (2002) suggested a much lower breakpoint for TN (0.040 mg/L) and a TP breakpoint of 0.030 mg/L to keep mean benthic Chl-*a* values low. In another study, Chételat et al. (1999) found that the filamentous green algae, *Cladophora*, dominated in streams exceeding 0.020 mg/L TP. In a study conducted in Missouri, Lohman et al. (1992) investigated

22 streams designated as "low enrichment," "moderate enrichment," and "high enrichment" based on mean "annual" (March – November) TP concentrations and land use. Their results suggested that to keep Chl *a* values below 150 mg/m^2 between 80% to 90% of the time during the summer months, the stream TN concentrations should be kept below about 0.800 mg/L. Based on observed changes in the diatom assemblages found in 37 streams in four ecoregions of Virginia during the fall of 2004, Ponader et al. (2005) propose a NO_3-N threshold of 0.5 mg/L and a TP level of 0.05 mg/L.

Studies of changes in benthic macroinvertebrate and fish communities generally suggest TP threshold levels higher than the 0.020 – 0.060 mg/L range cited above and a TN threshold somewhere between 0.35 mg/L and 0.90 mg/L. Laboratory studies by Lemly (2000) and Lemly and King (2000) demonstrated a direct linkage between bacterial growth on benthic macroinvertebrates and macroinvertebrate mortality. In the study by Lemly and King (2000), a stream classified as unenriched had mean TN concentrations between 0.715 – 1.97 mg/L and mean TP concentrations less than 0.200 mg/L (range of mean TP: 0.054 – 0.198 mg/L) and macroinvertebrates that were free of bacterial contaminations. Rankin et al. (1999) reported that macroinvertebrate ICI and fish IBI scores were typically *good* (40 – 49) in waters with TP concentrations between 0.10 and 0.20 mg/L and tended to be *exceptional* (50 – 60) when TP concentrations were below 0.10 mg/L.

Hill and Devlin (2003) found that a set of 18 reference reaches in Virginia without macroinvertebrate impairment had a mean TP concentration of 0.06 mg/L (median = 0.07 mg/L, n = 59) and a mean TN concentration of 0.33 mg/L(median= 0.34 mg/L, n = 59), whereas 19 sites with benthic impairments had a mean TN concentration of 1.82 mg/L (median = 0.90 mg/L, n = 69) and a mean TP value of 0.28 mg/L (median = 0.10 mg/L, n = 69) (Hill and Devlin 2003).

9.0 OTHER CONSIDERATIONS

9.1 Seasonality Issues

Since our analyses to derive nutrient criteria were mostly based on data collected from winter index period (M-BISQ project), it is necessary to address concerns about seasonal variation of nutrient concentrations. A separate analysis has been conducted to address the seasonality issue in the study regions (Appendix E). Three approaches were used to compare seasonal nutrient concentrations in different months/seasons. First, nutrient concentrations were compared over different months/seasons for each ecoregion and ecogroup. The analysis did not find strong seasonal patterns to nutrient concentrations in the water column for wadeable streams. Also, there was no evidence that nutrient concentrations in the winter index period were lower or higher than that in other seasons. The second analysis compared nutrient concentrations in least disturbed sites between winter and summer seasons. The results also did not reveal a significant difference between summer and winter nutrient concentrations in LD sites. The third approach selected one representative station from each of the six ecogroups. These stations have been sampled at least 40 times; therefore, multiple samples from each month were available for comparison. Although slight differences in nutrient concentrations were observed among different months, no overall pattern of nutrient fluctuation were observed along different seasons. In other words, these differences might be due to random monthly differences rather than seasonality. Also, nutrient concentrations in the winter index period were not lower than other seasons.

We expect that our approaches to derive nutrient endpoints were less vulnerable to seasonal/monthly variability since neither algal biomass nor other seasonally sensitive response variable were involved during the analysis. The main response variable we used to predict nutrient concentrations was a macroinvertebrate index, which is likely less sensitive to change in seasonal nutrient concentrations. Fore et al.(2007) compared the stream condition index (SCI, range from 0-100) in Florida between summer and winter index periods and found that on average, SCI values were 3.5 points higher in winter than in summer. Since disturbance level tended to be higher in summer than in spring, this difference might or might not have been caused by higher summer nutrient concentrations. Therefore, we expect differences in nutrient concentrations among different seasons in our study not to be a large issue.

9.2. Downstream Uses

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

Our analysis has not taken into account designated uses of downstream waters. The nutrient criteria for wadeable streams developed from our approaches would have to incorporate non-wadeable stream, lake, and estuarine nutrient criteria and designated uses in these waterbodies.

Our analyses has ensured that nutrient criteria for wadeable streams would be lower than those in non-wadeable streams throughout the state.

Nutrient criteria for streams feeding into lakes would have to satisfy nutrient criteria for lakes as well. The U.S. EPA's technical guidance manual (2000) specifically suggested that more stringent nutrient criteria might be required for streams that feed into lakes. For example, van Nieuwenhuyse and Jones (1996) suggest that the average abundance of sestonic algae per unit TP tends to be lower in streams than in lakes. Thus, nutrient concentrations that cause no problems in streams may cause nuisance levels of algae in lakes. Coordinated efforts with lake nutrient criteria development teams will help resolve the issue.

The more complicated issue is the impact of nutrients in streams and rivers draining to estuarine waters. Mississippi's streams and rivers drain into the Gulf of Mexico. While many of the stream systems are considered P limited, nitrogen is considered the major limiting factor in coastal and estuarine systems or coastal systems exhibit seasonal shifts in nutrient limitation with spring P limitation and summer N limitation (EPA 2001). Currently no estuarine nutrient criteria have been developed for waters in the State. Therefore, it is difficult to establish nutrient criteria that consider downstream estuarine waters at this time. Future refinement of stream nutrient criteria should be further evaluated based on estuarine uses designations.

10.0 SUMMARY OF RECOMMENDED NUTRIENT CRITERIA

We developed nutrient benchmarks using several approaches recommended by EPA that have been used by others to derive nutrient criteria for various states and regions. These benchmarks were based on reference approaches, stressor response approaches, and relevant literature values. The stressor response analyses were based on indirect invertebrate responses. Due to limitation of data, direct causal response variables could not be used at this time. The benchmarks derived from different approaches provided similar values of nutrient concentrations in various regions of Mississippi (Table 10.1.). In regions with relatively large sample sizes and available biological response data, e.g. East Bioregion, TN and TP criteria were stronger due to a high degree of agreement among the different approaches and tight confidence intervals from these approaches. For regions with relatively small sample size and larger confidence intervals, we recommend a range of nutrient concentrations and recommend refining criteria when more data become available.

Table 10.1. Summary of candidate criteria for each of the analytical approaches discussed. Values in bold were weighed more than others.

Approach		TN (mg/L)	TP (mg/L)
East Bioregion			
Reference	Minimally disturbed condition (MDC)	**0.194**	**0.013**
Approach	Least disturbed condition (LDC)	**0.693**	**0.050**
	Best attainable condition (BAC)	**0.730**	**0.050**
Stressor	Change point – Raw M-BISQ	**0.632**-0.840-0.980	**0.033**-0.06-0.108
Response	Change Point - CP M-BISQ	**0.670**-0.800-0.820	**0.050**-0.053-0.055
Literature	Tennessee and Mississippi		0.04-0.07
Southeast Bioregion			
Reference	Minimally disturbed condition (MDC)	**0.183**	**0.016**
Approach	Least disturbed condition (LDC)	**0.620**	**0.030**
	Best attainable condition (BAC)	**0.640**	**0.040**
Stressor	Change point – Raw M-BISQ	**0.495**-0.570-1.07	NA
Response	Change Point - CP M-BISQ	**0.540**-0.835-1.015	**0.035**-0.04-0.045
Literature	Florida Ecoregion 75	1.7	0.113
South Bluff Bioregion			
Reference	Minimally disturbed condition (MDC)	**0.194**	**0.064**
Approach	Least disturbed condition (LDC)	**0.075-0.582**	**0.062-0.137**
	Best attainable condition (BAC)	0.685 (6)	0.105 (6)
Stressor	Change point – Raw M-BISQ	NA	NA
Response	Change Point - CP M-BISQ	NA	0.065-0.07-0.115
Literature	Tennessee	NOx 0.22	0.12

Table 10.1. Continued.

	Approach	TN (mg/L)	TP (mg/L)
	West Bioregion		
Reference Approach	Minimally disturbed condition (MDC)	**0.282**	**0.022**
	Least disturbed condition (LDC)	0.785	0.111
	Best attainable condition (BAC)	0.925	0.080
Stressor Response	Change point – M-BISQ	0.81-0.89-1.249	0.095-0.098-0.185
	Conditional Probability – M-BISQ	0.76-0.773-0.79	0.08-0.08-0.085
Literature	Kentucky	0.510-1.4	0.020-0.093
	West Bioregion – Ecogroup 1		
Reference Approach	Minimally disturbed condition (MDC)	**0.282**	**0.022**
	Least disturbed condition (LDC)	**0.694-0.860**	**0.100**
	Best attainable condition (BAC)	**1.120 (21)**	**0.120 (21)**
Stressor Response	Change point – Raw M-BISQ	**0.8-**1.295-1.438	**0.08-**0.135-0.292
	Change Point - CP M-BISQ	**0.778-**0.945-0.975	**0.095-**0.115-0.128
Literature	Tennessee	1.1	0.11
	West Bioregion-Ecogroup 5		
Reference Approach	Minimally disturbed condition (MDC)	**0.282**	**0.022**
	Least disturbed condition (LDC)	**0.533**	**0.060**
	Best attainable condition (BAC)	**0.770**	**0.070**
Stressor Response	Change point – Raw M-BISQ	0.265-0.855-1.265	0.025-0.032-0.115
	Change Point - CP M-BISQ	**0.811-**1.075-1.565	**0.067-**0.08-0.11
	Non-wadeable streams		
Reference Approach	Minimally disturbed condition (MDC)		
	Least disturbed condition (LDC)	**1.27**	**0.12**
Stressor Response	Change point – Raw M-BISQ	0.605-0.760-1.320	**0.090-**0.125-0.132
	Change Point - CP M-BISQ	**0.90-**0.93-1.02	0.080-0.110-0.315
Literature	Tennessee		0.13

Our recommended nutrient criteria are as follows:

- East Bioregion - **TN: 0.65 mg/L TP: 0.050 mg/L**

The East bioregion, composed of two ecogroups, is the largest of the four bioregions. Total nitrogen and phosphorus concentrations were generally higher in the southern part of the region (Figures 10.1 and 10.2) than the Northern part (excluding the Black Belt). The highest nutrient enrichment site was Town Creek (site 200), which had extremely high TN (21 mg/L) and TP (7.2 mg/L). This stream is located in the Black Belt region (ecoregion 65a), which was dominated by flat agricultural lands, catfish ponds, and channelized highly entrenched streams.

Among all the regions, the East bioregion was the easiest for which to recommend nutrient criteria since all approaches came to similar results. TN benchmarks had a tight range from 0.632-0.693 mg/L and we weighed stressor response results the most. TP benchmarks from different approaches almost unanimously pointed to 0.050 mg/L. These benchmarks were also in agreement with literature criteria developed from the same region.

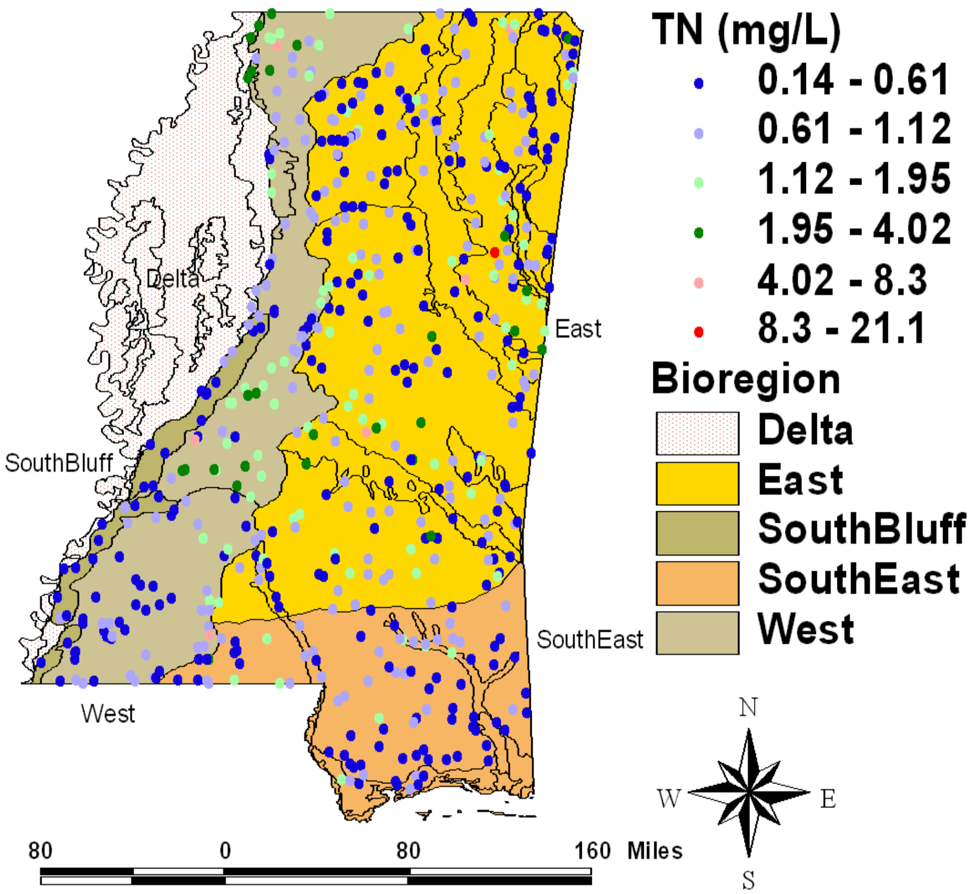

Figure 10.1. Site distribution map and total nitrogen concentrations in streams from M-BISQ project.

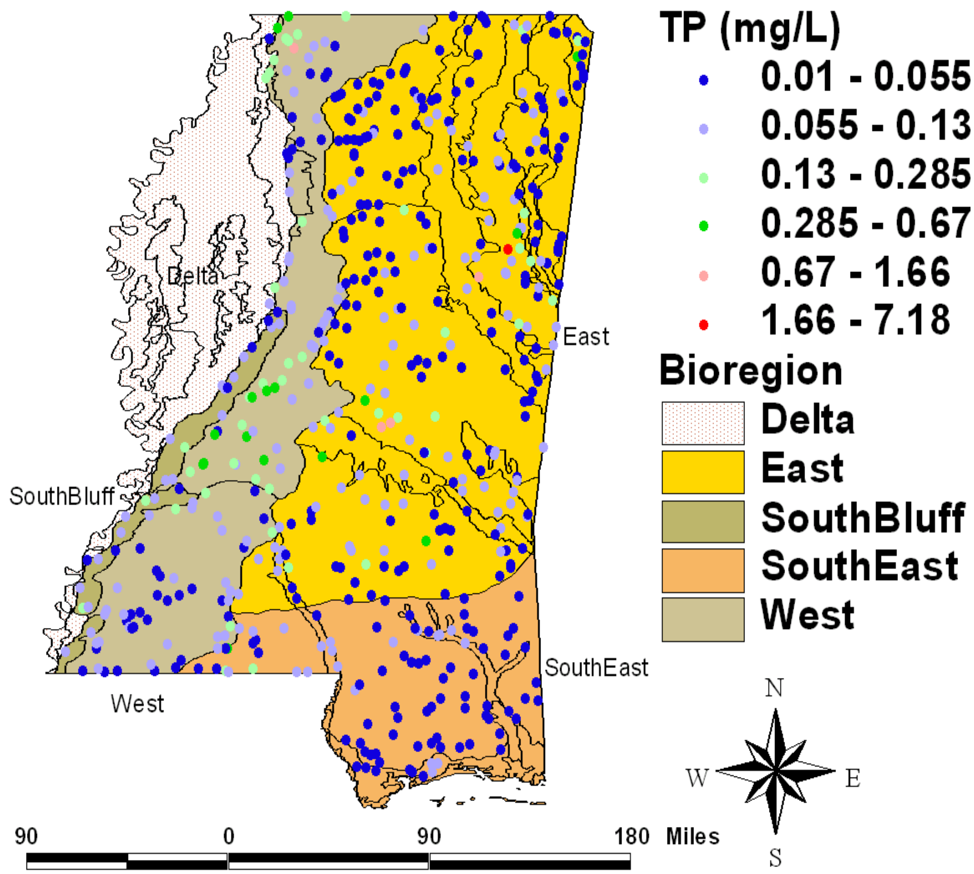

Figure 10.2. Site distribution map and total phosphorus concentrations in streams from M-BISQ project.

- Southeast Bioregion – **TN 0.540 mg/L TP: 0.035 mg/L**

  The Southeast bioregion is characterized by an abundance of low pH blackwater streams. Surrounding natural land uses were more abundant and physical habitat was of higher quality in this bioregion, therefore, low nutrient sites were more abundant than any other bioregions (Figure 10.1, 10.2). It was thus expected that nutrient criteria would be more stringent than any of the other regions.

  We again weighed most heavily those benchmarks derived from the stressor response approach. According to this approach, when TN concentration was above 0.570 mg/L, macroinvertebrate M-BISQ scores declined. The conditional probability approach identified a change point of 0.835 mg/L TN but the lower confidence limit was 0.540 mg/L TN. The TN benchmark from reference approaches in the Southeast bioregion was a little higher than these two values. After evaluating these benchmarks and examining the stressor response curves, we recommend TN 0.540 mg/L as the criterion. The TP criterion was determined based on stressor response results since the reference approaches yielded similar values.

- West Bioregion
  - ecogroup 1 - **TN 0.700-0.800 mg/L TP: 0.080-0.100 mg/L**
  - ecogroup 5 - **TN 0.533-0.800 mg/L TP: 0.060 mg/L**

The West bioregion is represented by ecoregions 74b and 74c. The northern part of this bioregion (ecogroup 1 or ecoregion 74b) was more heavily affected by human land uses, especially in the form of agricultural land use. Therefore, nutrient concentrations in the North were generally much higher than in the South (Figure 10.1 and 10.2). The sample size for LD sites in ecogroup 1 was small, therefore, the benchmarks derived from reference approaches were based on a small population distribution in this region. Depending on different sources of data, the TN benchmarks from reference approaches varied (0.694-0.860 mg/L) for ecogroup 1.

Macroinvertebrate composition also shifted along the South-North gradient in accordance with human disturbance gradient. Although biological assemblages in LDC in the north were similar to that in the south of the west bioregion, biological criteria were different for these two ecogroups (TetraTech 2007a). On average, macroinvertebrate index (M-BISQ) scores in the North were lower than that in the South. However, when the West bioregion was broken down into two ecogroups, the pattern of declines of biological integrity, along the nutrient gradients was not as strong as observed for the whole region due to abbreviated stressor gradients. It also led to larger confidence intervals around the change points. Criteria for both ecogroups can be refined and strengthened with additional data collection.

- South Bluff Bioregion - **TN 0.582-0.810 mg/L TP: 0.060-0.080 mg/L**

The South Bluff bioregion was designated as part of the West bioregion in the 2003 bioregion delineation. The new round of analysis (MDEQ 2007a) split it into a new bioregion. However, limited data for this bioregion make it difficult to define nutrient criteria. Benchmarks varied from 0.075 to 0.582 mg/L TN and from 0.062 to 0.137 mg/L TP based on reference approaches. Limited macroinvertebrate responses were observed along the short nutrient gradients, and therefore no benchmarks could be determined based on this approach. Alternative strategies were either to apply benchmarks derived from the West bioregion or from the nearest neighbor (ecogroup 5). We used ranges of these two alternatives as our recommended criteria.

- Non-Wadeable streams - **TN 0.900 mg/L TP: 0.090 mg/L**

Non-wadeable streams were sampled from statewide streams without considering ecoregional differences. Less than 20 least disturbed sites were identified from the sample selection. Therefore, we had little confidence in the nutrient benchmarks from the reference approach. Although we recommend using TN and TP benchmarks derived from stressor-response approaches for non-wadeable streams at this time, we emphasized that these criteria were based on limited data and no classification.

RECOMMENDATIONS

- We strongly recommend that Mississippi start to collect phytoplankton and periphyton biomass samples (i.e., chl *a*) to help refine the nutrient criteria. Algae are direct indicators of nutrient enrichment and excess algae is a common problem associated with nutrient enrichment. Collecting and analyzing algal biomass will require minimum field and laboratory time and will strengthen nutrient criteria.

- We also recommend that Mississippi start to collect periphyton species composition samples. These samples can be preserved for a long time and can be analyzed upon funding availability. Periphyton species composition is a sensitive nutrient indicator and has been very useful for nutrient criteria development.

- Additional least disturbed sites in several regions should be identified and nutrient and macroinvertebrate data from these new sites should be collected to refine nutrient criteria. These regions include the South Bluff bioregion and West bioregion ecogroup 5.

- Although we did not find a strong seasonal pattern of nutrient concentrations in streams, it was based on limited data for LD sites. Seasonal sampling of nutrients in LD sites would help to further explore seasonality and set criteria for nutrient criteria during different seasons.

- More sites and samples are needed to fully explore classifications and develop more defensible nutrient criteria for non-wadeable streams.

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Appendix A – Relevant Water Quality Data Available

Nutrient and biological parameters have been collected from a number of streams in the state of Mississippi by various programs. The detection limits for nutrient variables may vary according to sampling date, sampling methods, different projects and different agencies. NAWQA chemistry stations are included in the NWIS databases. Part of the EPA nutrient database is from the USGS NWIS database (4626 of a total of 7171 samples). MDEQ has more recent macroinvertebrate data (2004) than water chemistry data in the database.

	EPA Nutrient Database		Modern STORET		USGS NWIS		NAQWA		MDEQ EDAS		MDEQ WADES	
Characteristic Name	Sites	Samples	Sites	Samples	Sites	Samples	Sites	Samples	Sites	Samples	Sites	Samples
Dissolved Oxygen	146	4568	8	79					615	782	885	3093
Total Suspended Solids	7	151	20	87								
pH			21	169					614	781		
Turbidity	179	2082	21	54					614	776		
Nitrogen, ammonia as N	289	5753	17	38	514	6695		333			826	3082
Nitrogen, Kjeldahl	253	3014	6	29	579	5637		333	570	651	702	2489
Total Nitrogen	9	151	6	29	700	8618		333			701	2497
Nitrite (NO2)	81	3849	1		376	5236		333				
Nitrate (NO3)	58	509	13	29	354	2688		333				
Nitrite + Nitrate	204	5981	13	29	686	8538		333	619	747	813	3062
Phosphorus	32	6911	3	15	627	8647		333	619	747	815	3071
Orthophosphate as P	7	162	2	7	148	1888		333	48	94		
Phytoplankton, (cells/ml)					11	448						
Phytoplankton (chlorophyll a)					80	367			49	96		
Periphyton (ash-free dry mass)					18	32						
Periphyton (chlorophyll a)					20	23						
Periphyton (species)							6	29				
Macroinvertebrates	46	311	12	33			4	19	666	1042		
Fish							13	28				
Sampling Period	1/90-7/97		12/96 -11/04		10/43-9/05		2/96- 8/05		1/01-2/03		1/92-10/05	

Appendix B – Reference Sites

Reference sites developed based on different reference criteria. Least disturbed criteria sites (LDC1) were based on criteria defined by Tetra Tech, (2007a) and criteria exclude nutrient variables (added only one site, station ID 3). Least disturbed criteria II sites were based on land use and habitat, and best attainable condition sites (BAC) were based on biological criteria (M-BISQ scores) for each bioregion.

Station ID	Level III Ecoregion	Level IV Ecoregion	Ecogroup	Bioregion	Waterbody	LDC1	LDC2	BAC
112	65	65e	2	East	Yocona River			*
114	65	65e	2	East	Yocona River	*		
115	65	65e	2	East	Turkey Creek	*	*	*
120	65	65e	2	East	Cowpen Creek	*		
127	65	65e	2	East	Goodfood Creek	*		
141	65	65b	2	East	Green Creek	*		*
143	65	65i	2	East	Bull Mnt Creek	*		*
146	65	65i	2	East	Smith Creek	*	*	*
149	65	65p	2	East	Weaver Creek	*	*	*
153	65	65b	2	East	Halfway Creek	*	*	*
185	65	65b	2	East	Line Creek			*
191	65	65b	2	East	Cypress Creek	*	*	
196	65	65a	2	East	Spring Creek	*	*	
205	65	65b	2	East	Yellow Creek	*	*	
206	65	65b	2	East	Yellow Creek	*	*	
214	65	65i	2	East	Kincaid Creek	*	*	
280	65	65b	2	East	Macedonia Cree	*	*	*
287	65	65b	2	East	Wahalak Creek	*	*	
290	65	65b	2	East	Bodka Creek		*	
33	65	65e	2	East	Oak Chewalla C	*		*
34	65	65e	2	East	Little Spring	*	*	*
35	65	65e	2	East	Big Spring Cre			*
36	65	65e	2	East	Grahm Mill Cre			*
37	65	65e	2	East	Lee Creek	*		*
39	65	65e	2	East	Mill Creek			*
44	65	65e	2	East	Hurricane Cree			*
45	65	65e	2	East	Puskus Creek	*	*	*
46	65	65e	2	East	Cypress Creek	*	*	
49	65	65e	2	East	Porters Creek	*	*	*
51	65	65e	2	East	Shelby Creek	*	*	*
547	65	65e	2	East	Hatchie River			*
55	65	65e	2	East	Little Tallaha			*
555	65	65i	2	East	Bull Mnt Creek	*	*	*
556	65	65b	2	East	Sucarnoochee R	*	*	*
566	65	65b	2	East	Scooba Creek	*	*	

Station ID	*Level III Ecoregion*	*Level IV Ecoregion*	*Ecogroup*	*Bioregion*	*Waterbody*	*LDC1*	*LDC2*	*BAC*
58	65	65e	2	East	Chambers Creek			*
60	65	65j	2	East	Picken's Branc	*		*
63	65	65i	2	East	Caney Creek	*	*	*
64	65	65i	2	East	Little Yellow			*
65	65	65j	2	East	unnamed trib t	*		*
67	65	65j	2	East	Mill Creek	*		*
69	65	65j	2	East	Little Cripple	*		*
70	65	65j	2	East	Pennywinkle Cr	*		*
704	65	65e	2	East	Bearman Creek			*
708	65	65e	2	East	Upper Hatchie			*
73	65	65j	2	East	Cripple Deer C	*		
74	65	65j	2	East	Bear Creek			*
747	65	65e	2	East	Turkey Creek	*		*
75	65	65j	2	East	Bear Creek			*
76	65	65j	2	East	unnamed trib t	*	*	*
79	65	65i	2	East	Rock Creek	*		
81	65	65b	2	East	Big Brown Cree			*
816	65	65e	2	East	Hoke Creek	*	*	
819	65	65e	2	East	Courtney Creek			*
82	65	65b	2	East	Little Brown C	*		*
820	65	65e	2	East	Humphreys Cree			*
821	65	65e	2	East	Goodwin Creek			*
83	65	65b	2	East	Mackey's Creek	*	*	*
843	65	65b	2	East	Hasuqua Creek	*	*	*
86	65	65e	2	East	Clear Creek			*
875	65	65b	2	East				*
876	65	65b	2	East	Big Brown Cree			*
899	65	65b	2	East	Little Tallaha			*
921	65	65e	2	East	Courtney Creek			*
940	65	65i	2	East	Mayhew Creek			*
121	65	65d	3	East	Johnson-Coles		*	
167	65	65d	3	East	Little Topisha		*	
171	65	65d	3	East	Wolf Creek		*	
177	65	65d	3	East	Big Bywy Canal			*
178	65	65d	3	East	McCurtain Cree	*	*	*
179	65	65d	3	East	Poplar Creek			*
180	65	65d	3	East	unnamed trib t	*	*	
184	65	65d	3	East	Spring Creek		*	
240	65	65d	3	East	Senesha Creek			*
242	65	65d	3	East	Rambo Creek	*	*	*
247	65	65d	3	East	Scoobachita Cr	*	*	*

Station ID	*Level III Ecoregion*	*Level IV Ecoregion*	*Ecogroup*	*Bioregion*	*Waterbody*	*LDC1*	*LDC2*	*BAC*
248	65	65d	3	East	Zilpha Creek		*	*
249	65	65d	3	East	Yockanookany R			*
250	65	65d	3	East	Lobutcha Creek	*	*	*
252	65	65d	3	East	Tibby Creek		*	*
253	65	65d	3	East	Atwood Creek	*	*	*
254	65	65d	3	East	Lobutcha Creek	*	*	
256	65	65d	3	East	Lobutcha Creek	*	*	*
257	65	65d	3	East	Lukfapa Creek	*	*	*
262	65	65d	3	East	Standing Pine			*
263	65	65d	3	East	Noxubee River			*
272	65	65d	3	East	Pinishook Cree		*	
288	65	65d	3	East	Straight Creek	*	*	*
311	65	65r	3	East	Coffee Bogue	*	*	
319	65	65d	3	East	Strong River		*	
322	65	65d	3	East	Sipsey Creek			*
326	65	65r	3	East	Sugar Bogue	*	*	
328	65	65r	3	East	Cedar Creek	*	*	
330	65	65r	3	East	Caney Creek	*	*	
331	65	65d	3	East	Okatibbee Cree		*	*
332	65	65d	3	East	Houston Creek	*	*	
341	65	65q	3	East	Chunky River			*
344	65	65d	3	East	Big Red Creek			*
345	65	65d	3	East	Blackwater Cre	*	*	*
346	65	65d	3	East	Piwticfaw Cree	*	*	
348	65	65d	3	East	Alamuchee Cree	*	*	*
349	65	65d	3	East	Irby Mill Cree	*	*	*
350	65	65d	3	East	Long Creek	*	*	*
379	65	65d	3	East	Dabbs Creek		*	
380	65	65d	3	East	Campbell Creek	*	*	
381	65	65d	3	East	Limestone Cree	*	*	*
382	65	65d	3	East	Big Creek		*	*
383	65	65d	3	East	Riles Creek		*	*
384	65	65d	3	East	Riles Creek		*	
388	65	65p	3	East	Pegies Creek		*	
393	65	65d	3	East	Bowie Creek			*
395	65	65p	3	East	Fair River		*	
396	65	65d	3	East	Pretty Branch			*
399	65	65d	3	East	Oakahay Creek	*	*	
400	65	65d	3	East	Leaf River		*	*
401	65	65d	3	East	West Tallahala		*	
403	65	65d	3	East	Keys Mill Cree	*	*	*
404	65	65d	3	East	Okatoma Creek			*
405	65	65d	3	East	Leonards Mill			*
406	65	65d	3	East	Oakahay Creek		*	*

Station ID	*Level III Ecoregion*	*Level IV Ecoregion*	*Ecogroup*	*Bioregion*	*Waterbody*	*LDC1*	*LDC2*	*BAC*
407	65	65d	3	East	Okatoma Creek			*
408	65	65d	3	East	Oakey Woods Cr			*
410	65	65d	3	East	Souinlovey Cre		*	
412	65	65r	3	East	Castaffa Creek	*		*
413	65	65d	3	East	Tallahala Cree	*	*	*
416	65	65d	3	East	Tallahoma Cree		*	
418	65	65d	3	East	Buckatunna Cre		*	*
420	65	65d	3	East	Five Mile Cree		*	
421	65	65d	3	East	Hortons Mill C			*
422	65	65d	3	East	Coldwater Cree	*	*	*
423	65	65d	3	East	Yellow Creek			*
464	65	65d	3	East	Tilton Creek		*	
549	65	65d	3	East	Bowie Creek			*
550	65	65r	3	East	Chickasawhay R			*
700	65	65d	3	East	Kentawka Canal			*
701	65	65d	3	East	Kentawka Canal			*
710	65	65d	3	East	Big Creek			*
715	65	65d	3	East	Station Creek			*
716	65	65d	3	East	Tallahata Cree		*	
721	65	65d	3	East	Cascade Creek	*	*	
724	65	65d	3	East	Irving Creek	*	*	
808	65	65d	3	East	Big Black Rive			*
864	65	65r	3	East	Eucutta Creek		*	*
878	65	65d	3	East	Sowashee Creek			*
890	65	65d	3	East	Patton Creek t			*
221	74	74a	6	SouthBluff	Short Creek	*		*
291	74	74a	6	SouthBluff	Bliss Creek			*
295	74	74a	6	SouthBluff	Big Sand Creek		*	*
301	74	74a	6	SouthBluff	Bear Creek	*	*	
353	73	73a	6	SouthBluff	Annas Bottom	*	*	*
354	74	74a	6	SouthBluff	Fairchild's Cr		*	
356	74	74a	6	SouthBluff	Kennison Creek	*	*	
359	74	74a	6	SouthBluff	James Creek	*	*	
362	74	74a	6	SouthBluff	Dowd Creek	*	*	
431	74	74a	6	SouthBluff	Millbrook Cree		*	
560	74	74a	6	SouthBluff	Whites Creek		*	
836	74	74a	6	SouthBluff	Willis Creek	*		*
837	74	74a	6	SouthBluff	Jim's Bayou	*	*	*
469	65	65p	4	SouthEast	Lower Little C		*	
472	65	65p	4	SouthEast	Clear Creek		*	
475	65	65f	4	SouthEast	Shelton Creek			*

Station ID	Level III Ecoregion	Level IV Ecoregion	Ecogroup	Bioregion	Waterbody	LDC1	LDC2	BAC
476	65	65f	4	SouthEast	Bowie Creek			*
477	65	65f	4	SouthEast	Monroe Creek		*	*
479	65	65f	4	SouthEast	Lower Little C		*	*
480	65	65f	4	SouthEast	Black Creek			*
481	65	65f	4	SouthEast	Big Creek		*	*
482	65	65f	4	SouthEast	Beaver Dam Bra			*
483	65	65f	4	SouthEast	Little Black C			*
484	65	65f	4	SouthEast	Black Creek			*
489	65	65f	4	SouthEast	West Little Th	*	*	*
492	65	65f	4	SouthEast	Thompson Creek	*	*	
493	65	65p	4	SouthEast	Bogue Homo Cre		*	*
495	65	65f	4	SouthEast	Thompson Creek	*	*	
496	65	65f	4	SouthEast	Gaines Creek	*	*	
497	65	65f	4	SouthEast	Atkinson Creek		*	*
498	65	65f	4	SouthEast	Cypress Creek	*	*	
500	65	65f	4	SouthEast	Beaver Dam Cre	*	*	*
502	65	65f	4	SouthEast	Whisky Creek	*	*	
504	65	65f	4	SouthEast	Mason Creek	*	*	
505	65	65p	4	SouthEast	Meadow Creek	*	*	*
506	65	65f	4	SouthEast	Big Creek	*	*	*
507	65	65f	4	SouthEast	Brushy Creek			*
508	65	65f	4	SouthEast	Little Hell Cr		*	*
510	65	65f	4	SouthEast	W. Hobolochitt		*	*
511	65	65f	4	SouthEast	Murder Creek			*
514	65	65f	4	SouthEast	Moran Creek		*	*
515	75	75a	4	SouthEast	West Hobolochi			*
516	65	65f	4	SouthEast	Crane Creek			*
517	65	65f	4	SouthEast	East Hobolochi			*
520	75	75a	4	SouthEast	Catahoula Cree			*
522	65	65f	4	SouthEast	Black Creek			*
523	65	65f	4	SouthEast	Red Creek			*
524	65	65f	4	SouthEast	Flint Creek		*	*
526	65	65f	4	SouthEast	Wolf River		*	*
527	65	65f	4	SouthEast	Tenmile Creek	*	*	*
529	65	65f	4	SouthEast	Tchoutacabouff	*	*	*
530	65	65f	4	SouthEast	Biloxi River		*	*
532	65	65f	4	SouthEast	Tuxachanie Cre	*	*	*
533	65	65f	4	SouthEast	Little Biloxi		*	*
535	75	75a	4	SouthEast	Bernard Bayou			*
538	65	65f	4	SouthEast	Black Creek			*
539	65	65f	4	SouthEast	Little Cedar C			*
540	65	65f	4	SouthEast	Red Creek		*	*

Station ID	*Level III Ecoregion*	*Level IV Ecoregion*	*Ecogroup*	*Bioregion*	*Waterbody*	*LDC1*	*LDC2*	*BAC*
541	75	75a	4	SouthEast	Big Cedar Cree			*
542	65	65f	4	SouthEast	Indian Creek			*
543	65	65f	4	SouthEast	Moungers Creek	*		
544	75	75a	4	SouthEast	Bluff Creek			*
551	65	65f	4	SouthEast	Escatawpa Rive	*	*	*
565	65	65f	4	SouthEast	Terry's Creek		*	
600	65	65f	4	SouthEast	Hickory Creek			*
709	65	65f	4	SouthEast	Big Creek	*	*	*
711	65	65f	4	SouthEast	Kittrell Mill	*	*	*
719	65	65f	4	SouthEast	Big Branch		*	*
749	75	75a	4	SouthEast	Bayou Delisle			*
858	65	65p	4	SouthEast	Leaf River			*
860	65	65p	4	SouthEast	Reese Creek			*
865	65	65f	4	SouthEast	Catahoula Cree			*
867	65	65f	4	SouthEast	Big Creek			*
870	65	65p	4	SouthEast	Tallahalla Cre			*
883	65	65f	4	SouthEast	Tiger Creek	*	*	*
941	65	65f	4	SouthEast	Little Red Cre		*	*
952	65	65f	4	SouthEast	Black Creek			*
1041	74	74b	1	West	Hickahala Cree			*
1042	74	74b	1	West	Long Creek			*
1045	74	74b	1	West	Kyle Creek			*
157	74	74b	1	West	Batupan Bogue			*
158	74	74b	1	West	Cane Creek	*		
159	74	74b	1	West	Potacocowa Cre	*		
160	73	73b	1	West	Pelucia Creek	*	*	*
161	74	74b	1	West	Abiaca Creek			*
162	74	74b	1	West	Coila Creek	*		*
164	74	74b	1	West	Peachahala Cre			*
229	74	74b	1	West	Bophumpa Creek	*	*	*
230	74	74b	1	West	Fannegusha Cre	*		*
233	74	74b	1	West	Howard Creek			*
237	74	74b	1	West	Box Creek/Gree	*		*
244	74	74b	1	West	Hobuck Creek	*	*	*
28	74	74b	1	West	Grays Creek			*
293	74	74b	1	West	Hamer Bayou	*		*
3	74	74a	1	West	White's Creek	*	*	
30	74	74b	1	West	Coldwater Rive			*
300	74	74b	1	West	Porter Creek			*
318	74	74b	1	West	Steen Creek			*
702	74	74b	1	West	Bear Creek			*
703	74	74b	1	West	Doaks Creek			*
706	74	74b	1	West	Roberson			*

Station ID	*Level III Ecoregion*	*Level IV Ecoregion*	*Ecogroup*	*Bioregion*	*Waterbody*	*LDC1*	*LDC2*	*BAC*
					Creek			
763	74	74b	1	West	Unnamed Tribut	*		
811	74	74b	1	West	Mt. Tenna Cree			*
835	73	73a	1	West	Spring Branch	*		
92	74	74b	1	West	Long Creek			*
1047	74	74c	5	West	East Fork Amit		*	*
327	74	74c	5	West	Ford's Creek	*		
357	74	74c	5	West	Bayou Pierre (		*	
358	74	74c	5	West	unnamed trib t	*	*	
363	74	74c	5	West	South Fork Col		*	
364	74	74c	5	West	North Fork Col	*	*	*
365	74	74c	5	West	Middle Fork Ho		*	
367	74	74c	5	West	Fifteen Mile C	*	*	*
370	74	74c	5	West	Turkey Creek			*
371	74	74c	5	West	Brushy Creek			*
373	74	74c	5	West	Bayou Pierre (			*
375	74	74c	5	West	Bahala Creek (		*	
385	74	74c	5	West	Copiah Creek			*
427	74	74c	5	West	Sandy Creek		*	*
429	74	74c	5	West	Crooked Creek		*	
430	74	74c	5	West	Buffalo River		*	
434	74	74c	5	West	Bayou Sara		*	
438	74	74c	5	West	Mcgehee Creek		*	*
440	74	74c	5	West	Middle Fork Ho			*
441	74	74c	5	West	Dry Creek	*	*	
444	74	74c	5	West	Tar Creek	*	*	*
445	74	74c	5	West	Ziegler Creek	*	*	
446	74	74c	5	West	Brushy Creek			*
447	74	74c	5	West	Caston Creek	*	*	*
448	74	74c	5	West	West Fork Amit			*
449	74	74c	5	West	Cars Creek		*	
450	74	74c	5	West	Thompson Creek		*	
452	74	74c	5	West	Bogue Chitto			*
454	74	74c	5	West	Bogue Chitto			*
456	74	74c	5	West	Little Tangipa			*
553	74	74c	5	West	East Fork Amit		*	*
559	74	74c	5	West	Bates Creek		*	*
561	74	74c	5	West	Cypress Creek	*	*	*
729	74	74c	5	West	Foster Creek			*
730	74	74c	5	West	Little Beaver			*
731	74	74c	5	West	McCall Creek		*	
732	74	74c	5	West	Pretty Creek		*	
733	74	74c	5	West	Tangipahoa Riv			*

Station ID	Level III Ecoregion	Level IV Ecoregion	Ecogroup	Bioregion	Waterbody	LDC1	LDC2	BAC
848	74	74c	5	West	Beaver Creek		*	*
849	74	74c	5	West	Middle Fork Th			*
850	74	74c	5	West	West Fork Thom			*
851	74	74c	5	West	Dry Creek			*
852	74	74c	5	West	Brushy Creek		*	*
873	74	74c	5	West	Redding Creek		*	*
874	74	74c	5	West	Redding Creek		*	*
887	74	74c	5	West	Fords Creek	*		*
888	74	74c	5	West	Fords Creek		*	*
949	74	74c	5	West	Porter Creek		*	*
950	74	74c	5	West	Beaver Creek			*

Appendix C - Stream Classification

EPA guidance for nutrient criteria development recommends classification of waterbodies to reduce variability associated with natural geographic differences in nutrient concentrations due to geology, hydrology, and other factors. Nutrient dynamics in different regions could be distinct due to natural factors as well as other factors. Therefore, natural variability in the physical and chemical site characteristics of sites was investigated to identify potential classification schemes for the State of Mississippi, and six site classes (ecogroups) were identified (Figure C.1, Tetra Tech, Inc. 2007a). EPA level 3 and level 4 ecoregions delineated areas of similar climate, geology, soils, vegetation, topography, and hydrology and have been accepted as a geographic framework for delineating regions of relatively homogeneous natural condition (Figure C.2). Lastly, Mississippi bioregions were derived to classify streams for biocriteria development (Tetra Tech 2003). The M-BISQ indices were recalibrated and a new bioregion classification theme has been proposed (MDEQ 2007a) (Figure C.3).

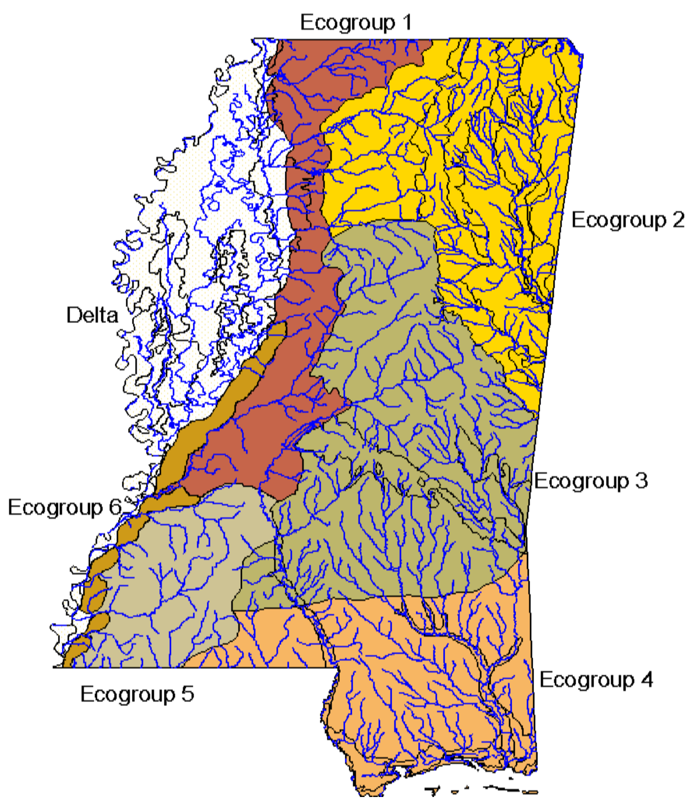


Figure C.1. Classification of site classes (ecogroups) in the State of Mississippi based on physical and chemical characteristics.

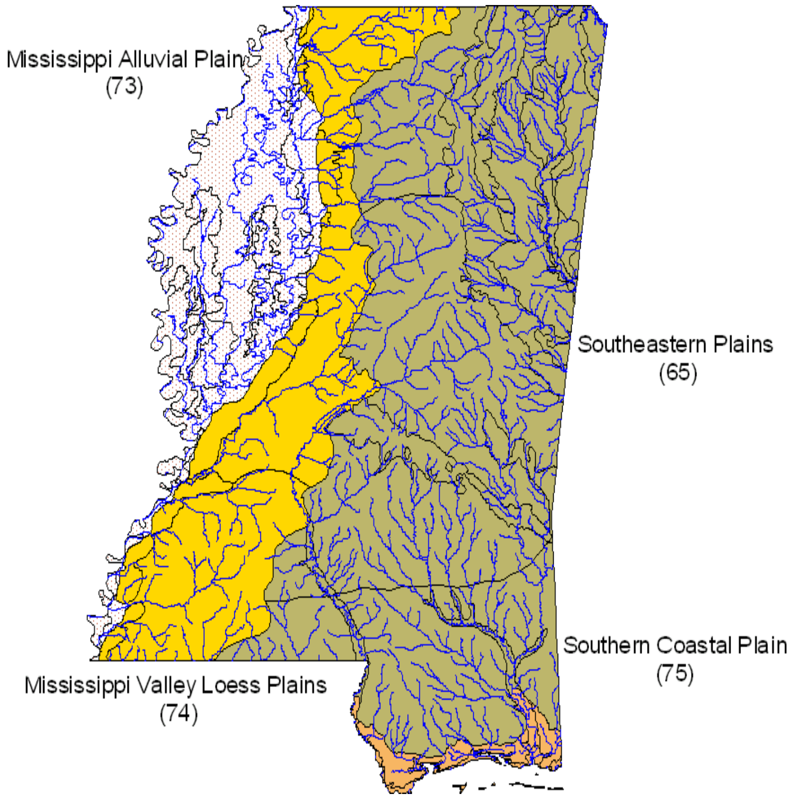


Figure C.2. Ecoregional classification within the state of Mississippi.

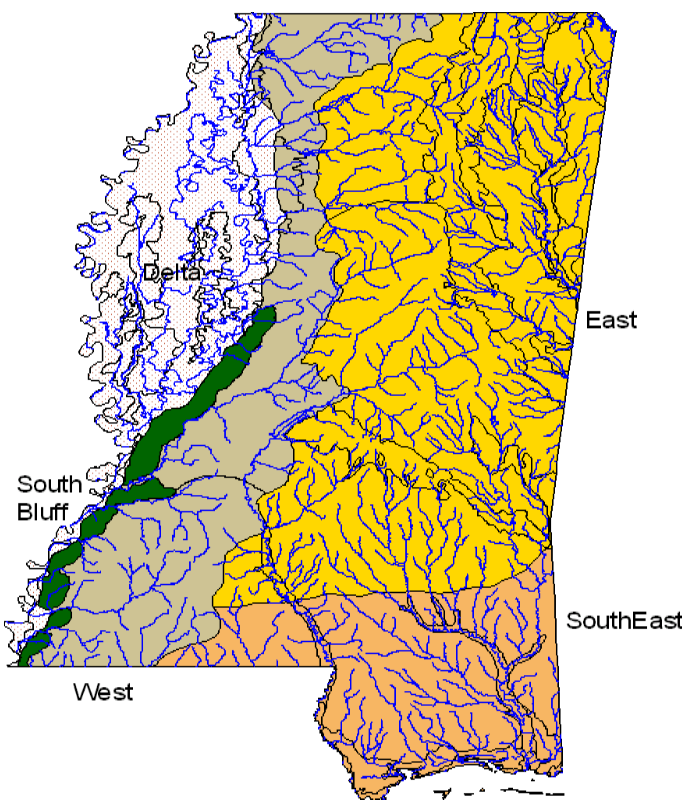


Figure C.3. Bioregional classification for the State of Mississippi.

Waterbodies in the State could be classified according to any of the above 3 schemes. Using the bioregion classification has a number of advantages:

- Biological criteria have been set to protect macroinvertebrate integrity (M-BISQ);
- Nutrient criteria are being developed to protect aquatic life, and using bioregions would be consistent with the the M-BISQ development process;
- LD Reference conditions have been determined for bioregions.

Ecoregion classification also has its advantages:

- It is nationally based;
- Factors considered include geology, physiography, vegetation, climate, soils, land use, wildlife, and hydrology;
- EPA based their nutrient regions for recommended nutrient criteria on ecoregions.

We used the bioregional classification and our effort to develop a classification scheme was consistent with the effort to develop preliminary site classes for bioregion development as used in Tetra Tech's M-BISQ (2003, 2007) studies.

Two sets of selection criteria were applied to define least disturbed condition (LDC) sites, The LDC1 selection criteria were selected to be consistent with the M-BISQ development process. According to this selection process, a certain number of least disturbed sites from each preliminary site class (ecogroup) were evaluated and selected into the LD set (Table C.1). The only difference between the LDC1 criteria for this study and those used to selected least disturbed sites for the M-BISQ development were that nutrient variables and dissolved oxygen (because it is influenced by eutrophication) were excluded to reduce circularity.

The second LDC sites (LDC2) were selected solely based on human land uses in the surrounding watershed and stream buffer and stream habitat scores (Table C.1). These selection criteria eliminated anthropogenic nutrient loadings from land use/land cover changes but did not exclude other factors that potentially contributed to impairment, such as chloride loadings. Nutrient inputs, as well as other stressors in these sites were mostly likely from natural sources in the watersheds. Biological impairment in these reference sites, if any, were less likely impacted by anthropogenic nutrient loadings. We expected that LDC2 sites from different regions would have a similar extent of nutrient loading (if any) from human land uses and would be useful for comparing background nutrient concentrations in different regions.

Table C.1 Reference site selection criteria for LDC group 1 and LDC group 2. (Ag = agriculture, NPDES = distance to permitted discharge).

LDC1 criteria

Ecogroup	%Natural	%Natural Buffer	Habitat Score	Chloride	NPDES
1 or 2	>50	>60	>100	<10	>5km
3	>70	>80	>110	<10	>5km
4	>70	>80	>110	<10	>5km
5	>70	>80	>110	<30	>5km
6	>70	>80	>100	<30	>5km

LDC2 criteria

%Ag	%Ag Buffer	%Urban	% Urban Buffer	Habitat	NPDES
<20	15	<5	<3	>100	>5km

The LDC1 selection criteria were somewhat more conservative than the LDC2 criteria and resulted in fewer reference sites (104 versus 156). The LDC1 sites were also more evenly distributed around all regions than LDC2, since selection criteria for LDC1 did not set the same standard for different ecoregions.

In order to explore the first classification scheme, nutrient concentrations from LDC sites (LDC1) were compared among different sub-ecoregions, bioregions, and ecoregions (Figure C.4). No significant differences in TN concentrations were found among different classification units ($p>0.05$). TP concentrations, however, were significantly different among sub-ecoregions (ANOVA $p<0.05$) (Figure C.4b). As illustrated in Figure C.4b, this difference was due to relatively high TP concentrations in 74a (South Bluff bioregion) and 74b (West bioregion, ecogroup 1). Sub-ecoregion 74c (ecogroup 5) also had lower nutrient concentrations than 74b ($p<0.05$), and therefore, was considered as a separated group. The southeast bioregion also had relatively lower TP concentrations than the rest of ecoregion 65, though the difference in TP concentrations was not significant ($p>0.05$). No ecoregion 75 sites were selected as LDC1 sites.

As mentioned, no significant differences in TN concentrations were found among different bioregions ($p>0.05$). However, two of the four bioregions, South Bluff and West bioregion (which compose ecoregion 74) had significantly higher TP concentrations than the East and Southeast regions (ecoregion 65 and 75). West and South Bluff bioregions had similar TP concentrations.

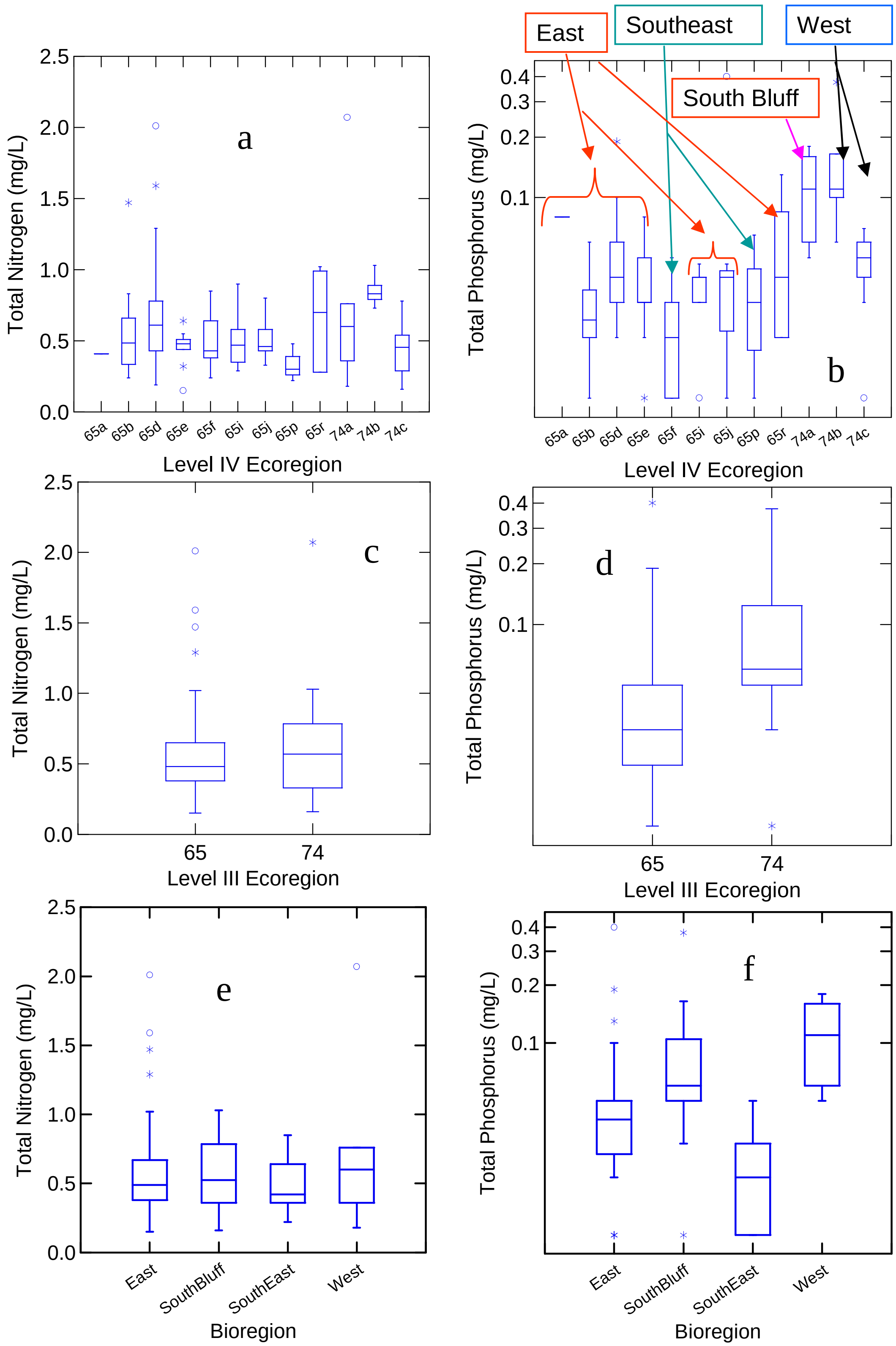


Figure C.4. TN and TP distributions in least disturbed condition sites (LDC1) from M-BISQ database in Level IV bioregions (a,b) and (c,d) and III ecoregions (e,f).

Background nutrient concentrations were also compared among different regions using the LDC2 sites (Figure C.5). The unified criteria for all regions allowed selection of the best sites across the state. As a result of using strictly land use restrictions on this LD set, only two LDC sites were found in subecoregion 74b (ecogroup 1). Background TP concentrations were still higher in ecoregion 74 than other regions ($p=0.008$), but the only significant difference was between 65f and 74 ($p=0.012$). TN concentrations were significantly higher in ecoregion 65 than in ecoregion 74 ($p=0.008$). The South Bluff bioregion had the lowest background TN concentrations and highest TP concentrations among all four bioregions ($p<0.05$). The Southeast bioregion had the lowest background TP concentrations of all regions.

In summary, LDC1 and LDC2 served different purposes but nutrient concentrations, especially TP concentrations, for both LDC1 and LDC2 were significantly different among different regions. Classification of streams would depend on the goal of protection and might not necessarily be based on background nutrient concentrations. Therefore, bioregional classification made more sense for the purpose of protecting aquatic life uses in a region. However, this effort was important in exploring potential nutrient differences among regions for classification purposes. It was also important to compare two sets of criteria to explore whether isolating land use factors alone would improve or change the classification, reducing some of the constraints imposed by using water quality criteria.

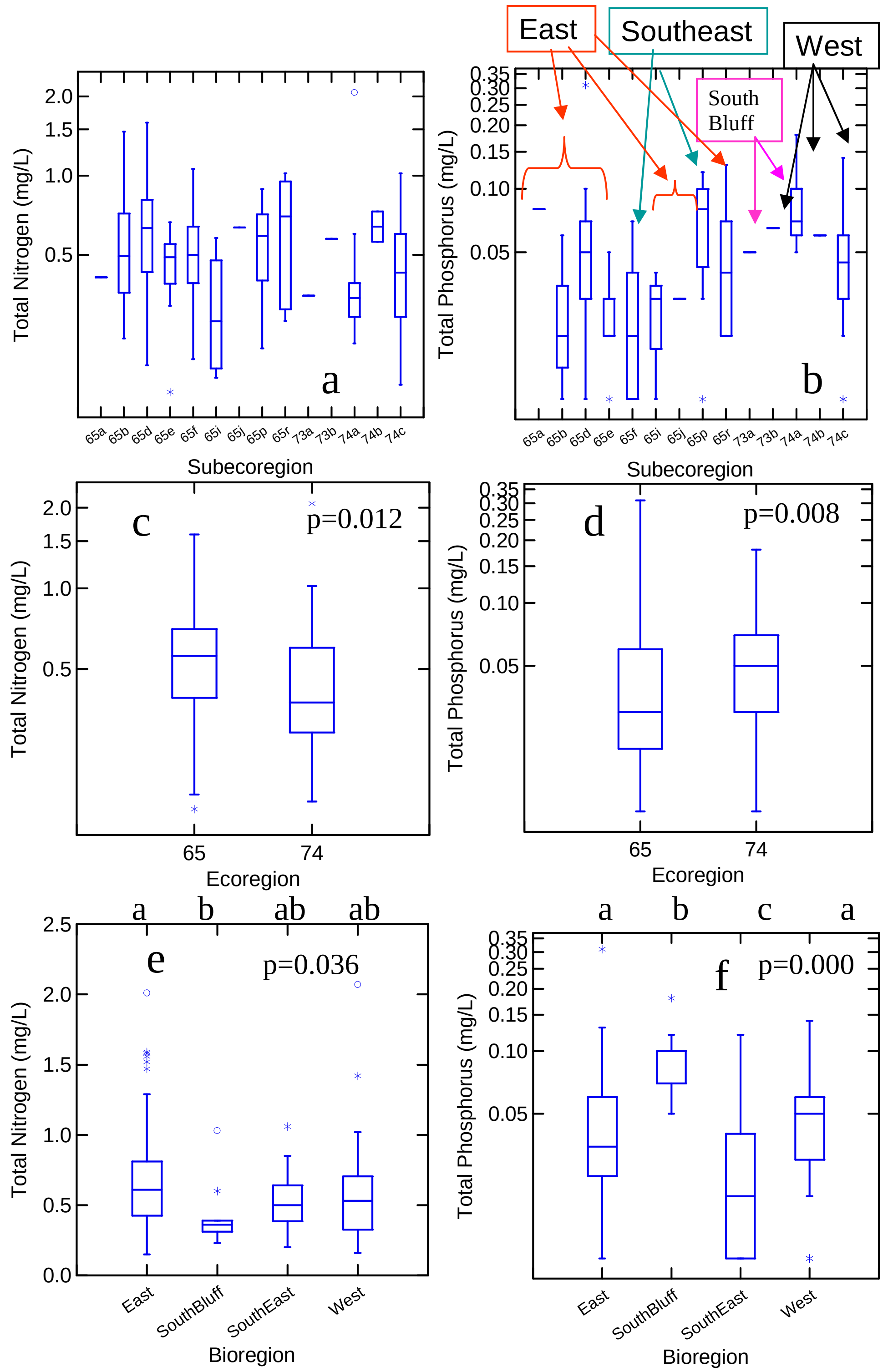


Figure C.5. TN and TP distributions in least disturbed condition (LDC2) sites among bioregions (a,b) and level III (c,d) and level 4 (e,f) ecoregions in Mississippi.

Appendix D - Seasonal Variation of Nutrient Concentrations in Mississippi

All stations

Seasonal samples of nutrient parameters have been collected from many of the wadeable stream sites in Mississippi and are included in the WADES database. However, no least disturbed sites were identified in this dataset. We compiled nutrient data from each of six site classes (ecogroups) and plotted their values to examine possible seasonality among different ecogroups (Figures D-1 and D-2).

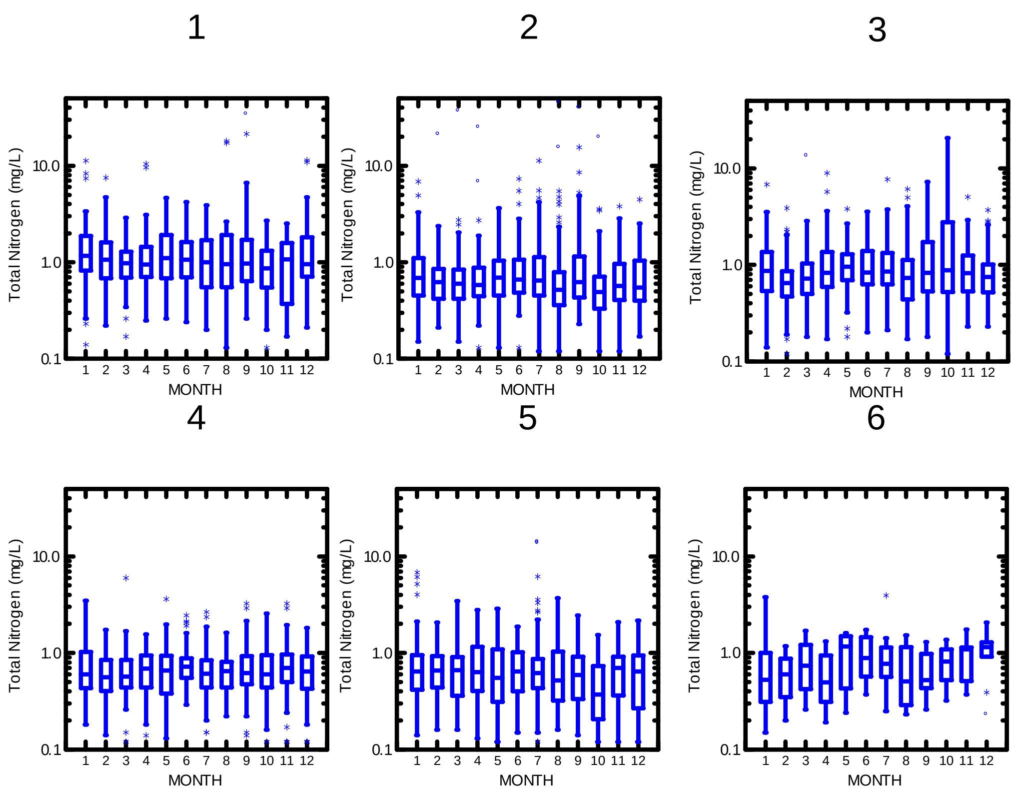


Figure D.1. Seasonal total nitrogen concentrations at six ecogroups in Mississippi. No significant difference was detected among different seasons (months)

No significant differences in TN concentrations were observed among different months in all ecogroups (ANOVA $p>0.05$). The median values of TN were stable among months for most of the ecogroups except ecogroup 6 (South Bluff) (Figure D. 1). Although one would expect TN concentrations to be highest in the spring and summer as observed elsewhere, there was no consistent pattern of change in median values among different months for all ecogroups. As a matter of fact, the highest 25th percentile and median TN values were both found in December for ecogroup 6, indicating nutrient concentrations in winter months might not be lower than in the summer months.

Similar to TN, no significant differences in TP concentrations were found among different months in any ecogroup (ANOVA $p>0.05$; Figure D.2). Median TP concentrations for ecogroups 2 and 4 were slightly higher in the spring (4-6) than in the winter (1-3), but this observation was not consistent across regions.

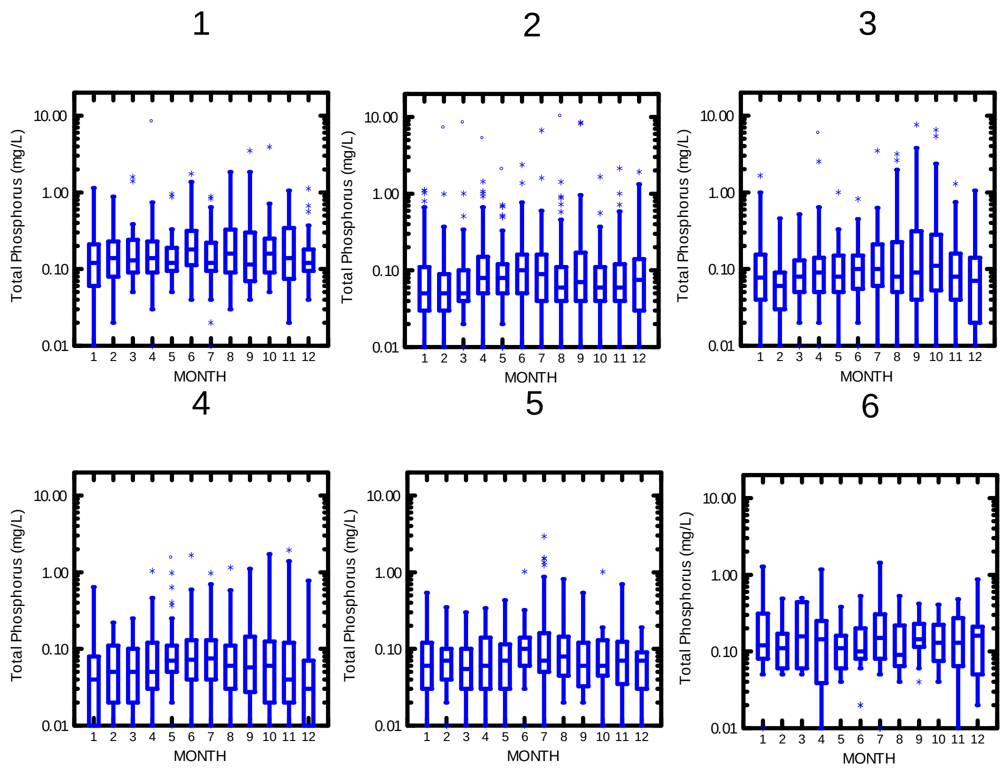


Figure D.2. Seasonal total phosphorus concentrations at six ecogroups in Mississippi. No significant difference was detected among different seasons (months).

Nutrient concentrations were also grouped according to ecoregional classification (Figure D.3). Similarly, no seasonal patterns in TN and TP concentrations were found in ecoregion 65 and 74. However, TP concentrations varied among different months in ecoregion 75 (p=0.013). However, the detected difference may be due to small sample size and an unbalanced data distribution.

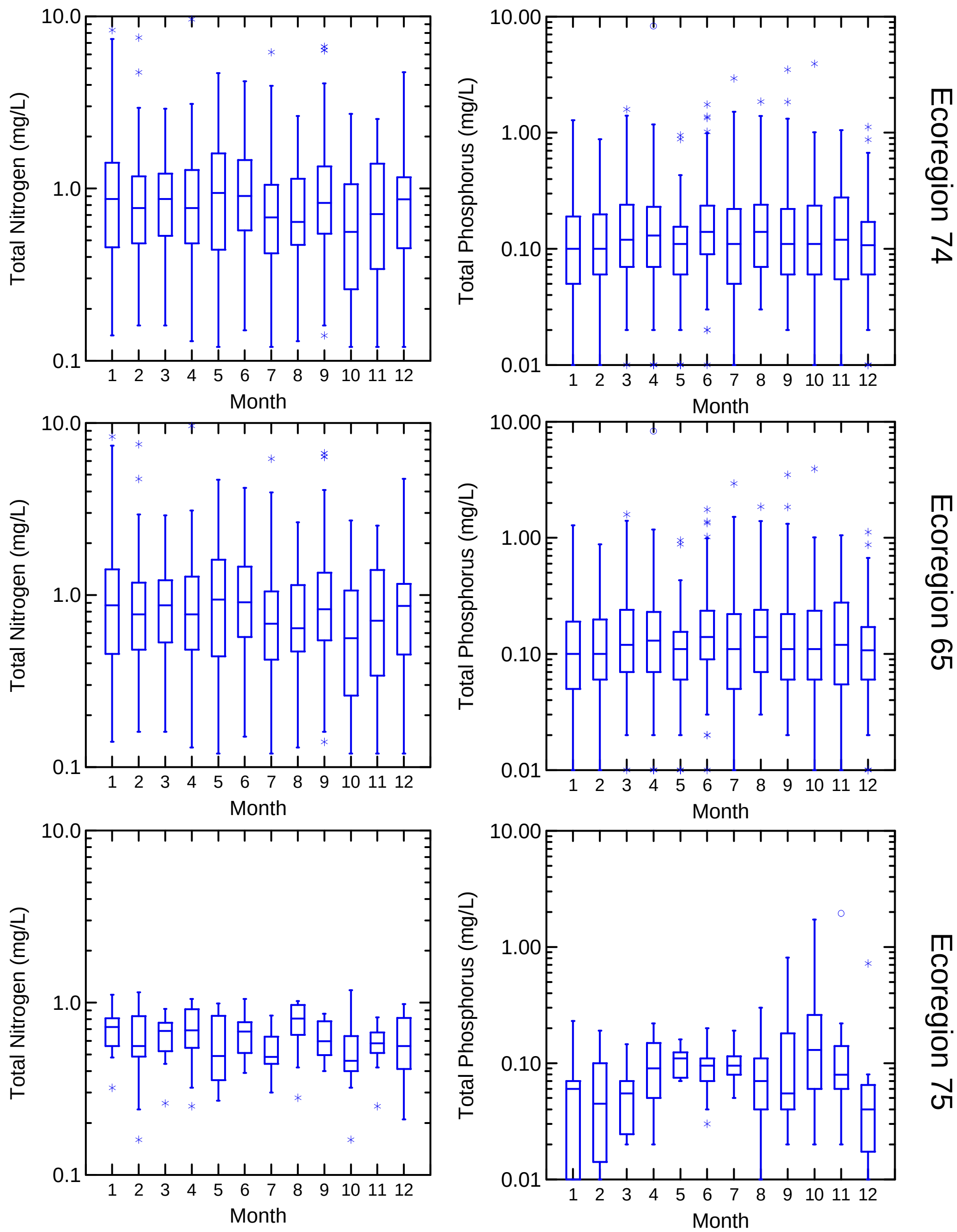


Figure D.3. Seasonal nutrient concentrations across three ecoregions in Mississippi.

Least disturbed sites

We also compared seasonal TN and TP concentrations at least disturbed sites (LDC1) from the M-BISQ database (Figure D.4). Relative fewer samples were collected outside of the winter index period. Only a limited number of samples were collected during summer (August and September) for a few stations. No seasonal data were available for least disturbed sites in ecoregion 75. Still, we did not find significant difference in TN and TP concentrations between winter and summer index periods in these LDC sites in either ecoregion 74 or 65 (Figure D.4).

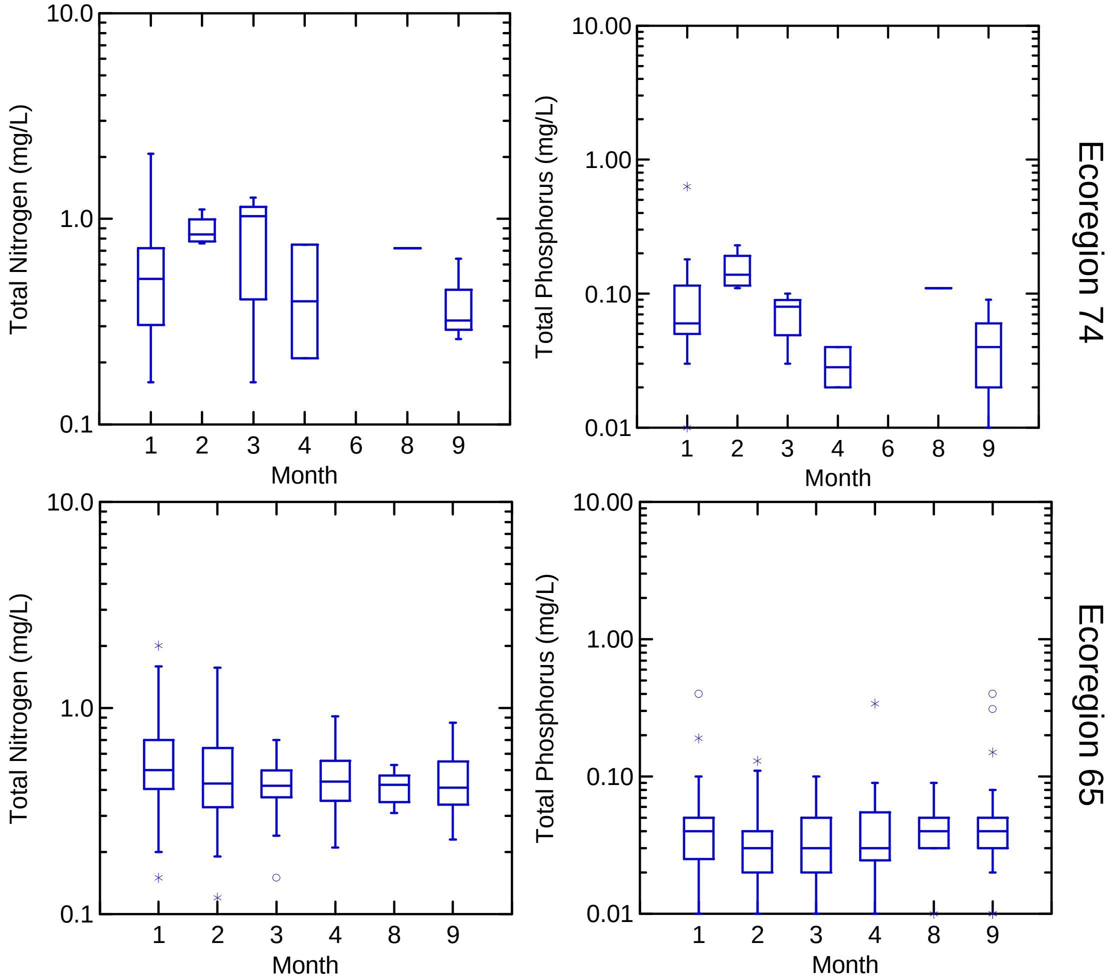


Figure D.4. Seasonality of nutrient concentration in least disturbed sites in two ecoregions of Mississippi. Data were imported from WADES dataset but LD sites were defined according to LDC1 criteria.

Site specific seasonal variation

We selected one site with multiple month samples from each site class (ecogroup) to determine seasonality of specific sites (Figures D.5 and D.6). These sites were sampled at least 31 times.

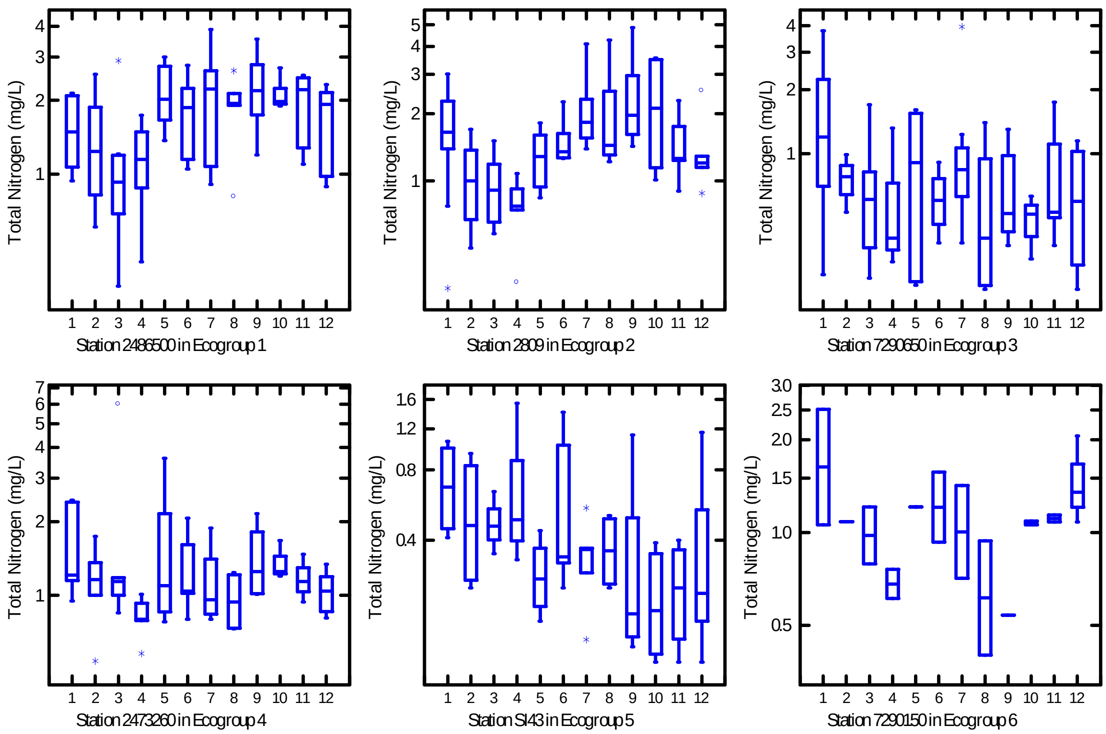


Figure D.5. Seasonal variation of TN concentration from one representative station in each ecogroup.

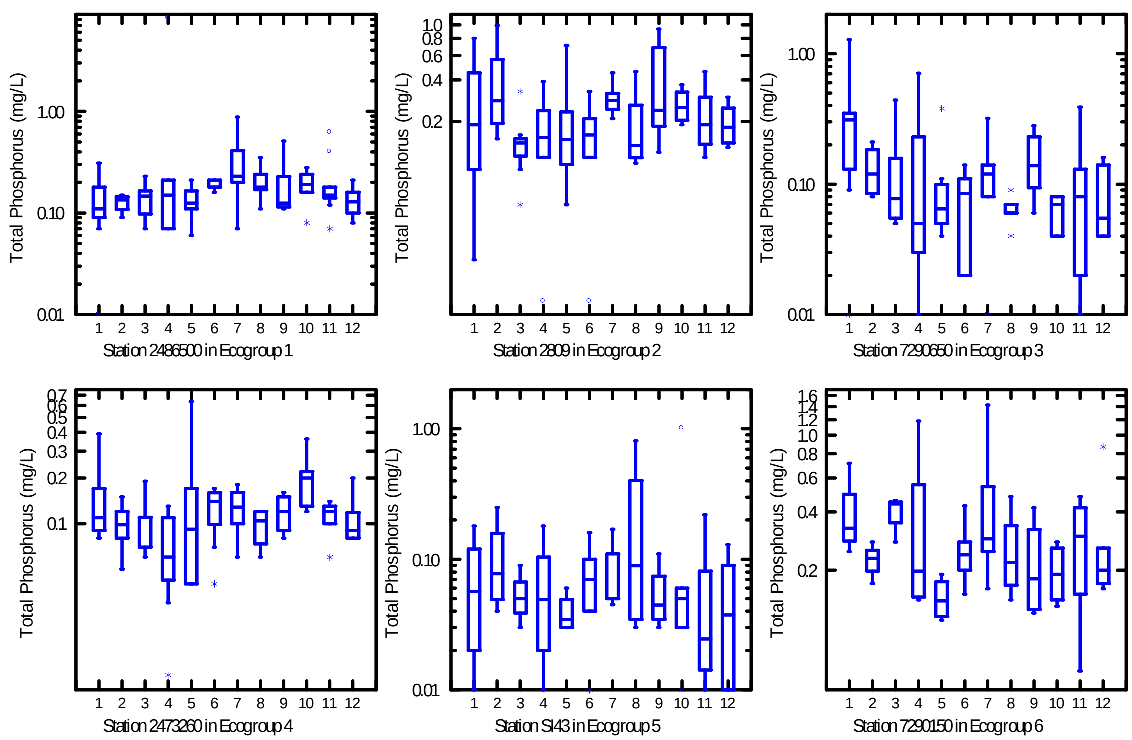


Figure D.6. Seasonal variation of TP concentration from one representative site in each ecogroup.

As shown in Figure D.5, most stations had high TN concentration in January and started to decline and reached the lowest level in the early spring. Summer TN concentrations were highest at two stations (ecogroup 1 and 2) but not significantly higher than winter sampling seasons. In several stations, median TN concentrations were highest in January when macroinvertebrates were sampled. Station SI43 in ecogroup 5 showed a pattern of decline in TN concentrations from January to December.

There was not a seasonal pattern in TP concentrations at all sites (Figure D.6). TP concentrations in winter were not significantly different from any other season in the sites examined ($p>0.05$).

Summary

TN and TP concentrations did not show consistent seasonal patterns across different regions in the State of Mississippi according to limited seasonal data sources. Nutrient concentrations in the winter index period were not different from other sampling seasons from our analysis. Therefore, although nutrient criteria were mostly developed based on data from the winter sampling period, it is possible to expand the criteria to other seasons. Further investigation of seasonality should be conducted to confirm the seasonal variation for least disturbed sites.

Appendix E - Determining Thresholds Using Change Point Analysis

1. Statistical inference

We performed a number of correlation analyses between nutrient parameters and macroinvertebrate metrics, selected correlations of interest, and examined visual scatter plots to identify relationships of interest. We used either linear regression or a locally weighted average (LOWESS) regression line to examine the trend of change along nutrient gradients. The LOWESS technique (Cleveland 1979) was designed to address nonlinear relationships where linear methods do not perform well. LOWESS combines the simplicity of linear least squares regression with the flexibility of nonlinear regression. It achieves this by fitting simple models to localized subsets of the data to construct a function that describes the deterministic part of the variation in the data, point by point. This method does not require specification of a global function to fit a model to the data; it just fits segments of the data to the model. For our LOWESS analysis, tension was set at 0.50.

We used a conditional probability approach (Paul and MacDonald, 2005) to examine changes in the biological community along stressor gradients. A conditional probability statement provides the likelihood (probability) of a predefined response, if the value of a pollutant stressor (condition) is exceeded. Conditional probability is the likelihood of an event when it is known that some other event has occurred. To estimate conditional probability of an impairment, we first define impairment as a specific value for a response variable (e.g., M-BISQ score < 66). The analysis asks the question: for a given threshold of a stressor, what is the cumulative probability of impairment? For example, if the total phosphorous concentration is greater than 0.2 mg/L, what is the probability of biological impairment for each site under consideration? All observed stressor values (in this example, all observed values of total phosphorous) are used to develop a curve of conditional probability (Paul and MacDonald, 2005).

We used nonparametric deviance reduction (change point analysis) to quantitatively identify nutrient thresholds in associated with dealing in biological condition (Qian et al. 2003). This technique is based on regression tree models, which are used to predict the value of a variable from one or more continuous predictors. The change point is the first split of a tree model when there is only a single predictor variable. Deviance is defined as

$$D = \sum_{k=1}^{n} (y_k - \mu)^2$$

where D is the deviance, N is the sample size, y_k is the response variable, and μ is the mean of n observations of y_k. When the data are divided into two groups, the sum of the deviance for the two subgroups is always less than or equal to the deviance for the entire data set. The point that results in the greatest reduction in deviance is defined as the threshold. Uncertainty in the deviance reduction changepoint (95 percent CIs) can be estimated from empirical percentiles of a bootstrap distribution based on 1,000 resampling events (Manly, 1997).

2. Data sets

Approximately 60 macroinvertebrate community metrics, including M-BISQ scores, Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, Intolerant taxa percent, tolerant taxa, Non-insect taxa, Beck's biotic Index, and Hilsenhoff's Biotic Index (HBI), were calculated using an EDAS database for various programs. Rather than examining all possible biological indicators, we selected the seven benthic macroinvertebrate indicators and used for the conditional probability and change point analysis.

To apply conditional probability analysis and change point analysis, a threshold of biological impact had to be determined. Least disturbed reference sites were identified for the M-BISQ03 study (Tetra Tech, Inc. 2003). We used the 75th percentile of reference sites as the biological impact threshold for each ecoregion (Data for ecoregion 75 is not available for this analysis).

3. Stressor-response relationships

Two of the most important metrics that responded to various human impacts in Mississippi were the overall M-BISQ score and EPT taxa richness (Tetra Tech, 2003). We therefore, selected these two metrics to examine the effect of nutrient enrichment on biological integrity and sensitive taxa (Figure E.1). M-BISQ and EPT taxa richness both declined with increasing nutrient concentrations in all three ecoregions. According to the LOWESS lines, thresholds occurred when total nitrogen concentrations exceeded 0.5-0.8 mg/L; both M-BISQ and EPT taxa richness started to decline sharply in all three ecoregions after these concentrations. M-BISQ and EPT taxa consistently declined along the TP gradient as well.

Two biotic indices, Beck's Biotic Index (BBI) and Hilsenhoff's Biotic Index (HBI), revealed the same pattern along nutrient gradients as M-BISQ scores and EPT taxa richness (Figure E.2). A higher HBI score indicates worse biological condition and a higher BBI score indicates a better biological condition. HBI scores were much lower and BBI score were much higher when TN concentrations were lower than 0.7-1 mg/L and TP concentrations were lower than 0.100 mg/L in all three regions.

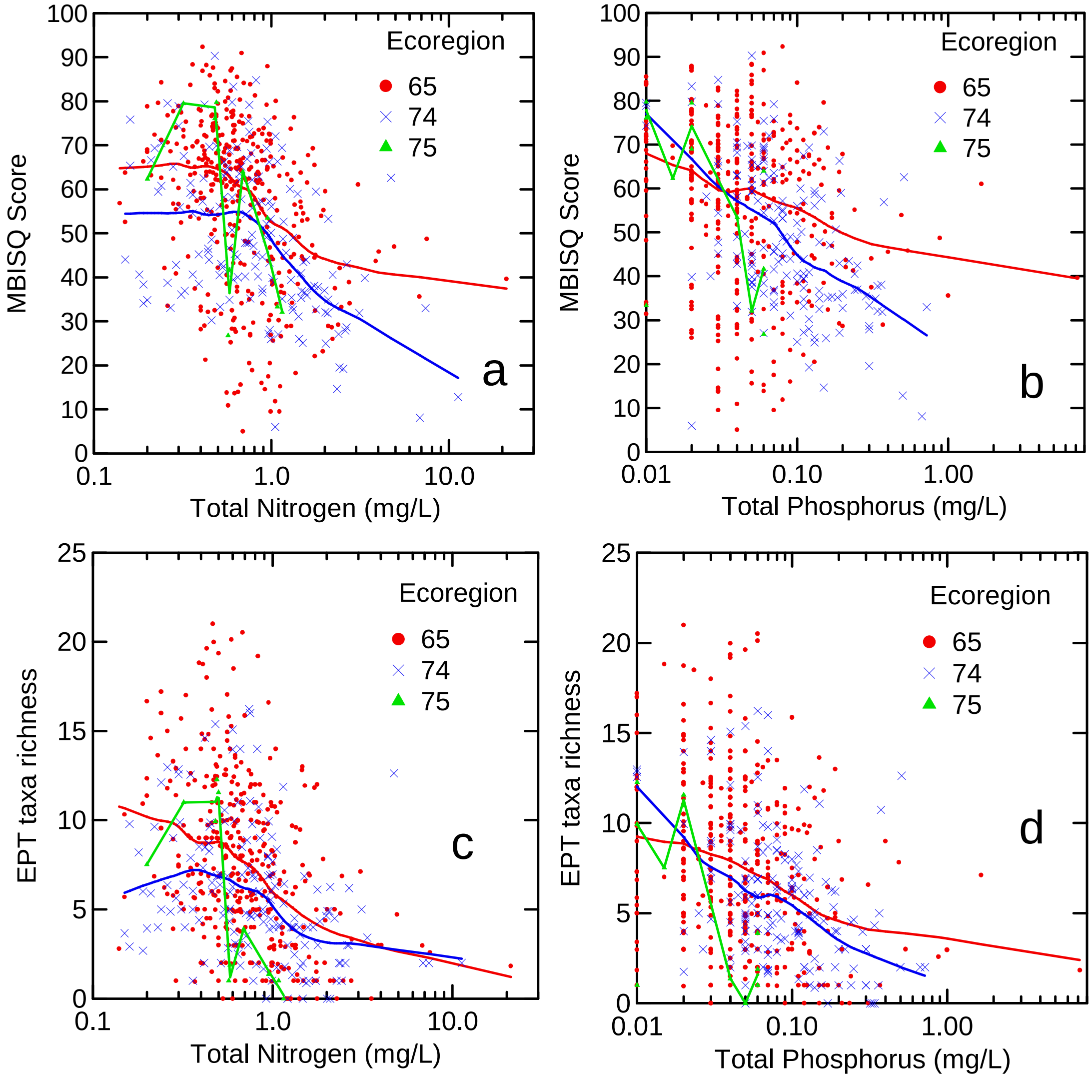


Figure E.1 Scatterplot of MBISQ03 scores, and EPT taxa vs. nutrient concentrations in three ecoregions in the State of Mississippi. Solid lines are the LOWESS lines.

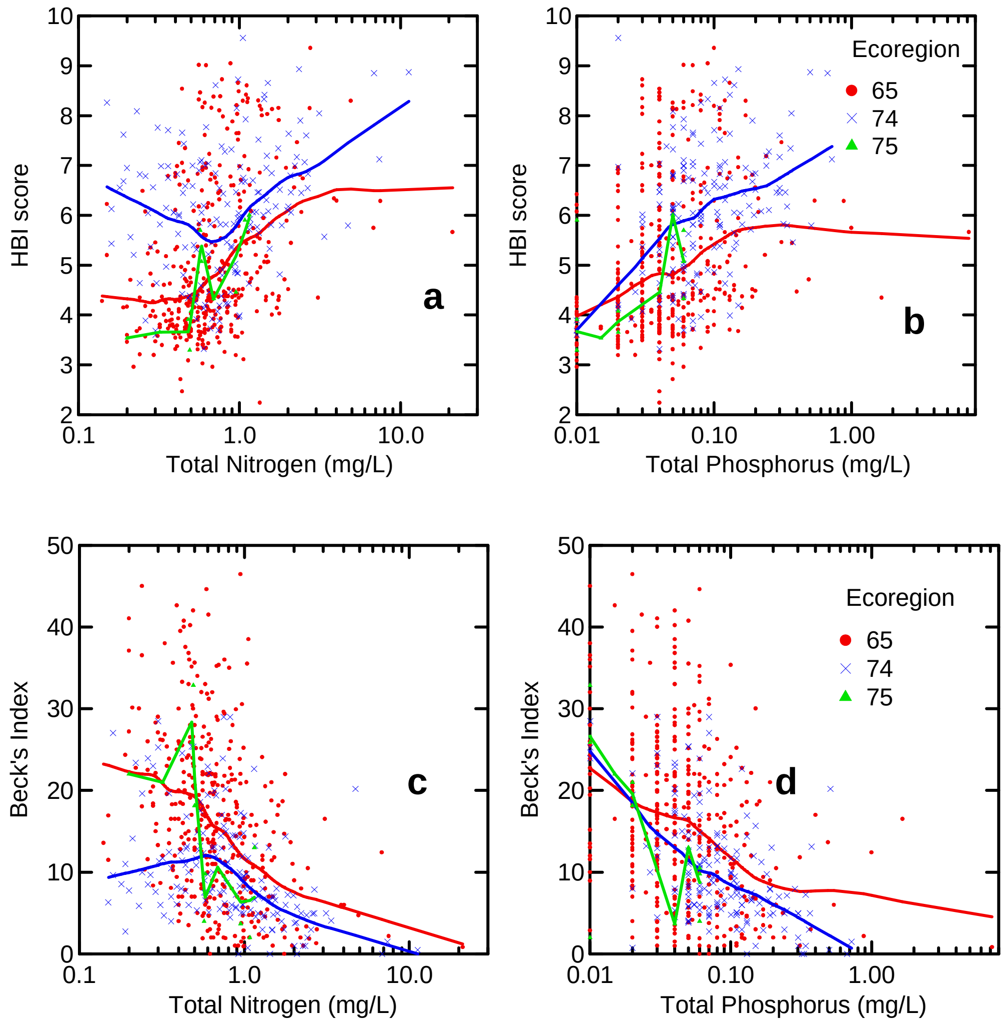


Figure E.2. Scatterplot of Hilsenhoff Biotic Index (HBI) and Beck's Biotic Index scores vs. nutrient concentrations in three ecoregions in the State of Mississippi. Solid lines are the LOWESS lines.

Other tolerant and intolerant taxa metrics revealed similar patterns in the three ecoregions (Figure E.3). With an increase in nutrient concentrations, intolerant taxa decreased in abundance, tolerant taxa became dominant, and non-insect taxa richness increased. Apparently, when nutrient enrichment increased (TN > 0.5 mg/L, TP >0.04 mg/L), native and intolerant taxa declined, and macroinvertebrate communities become dominated by tolerant, invasive taxa.

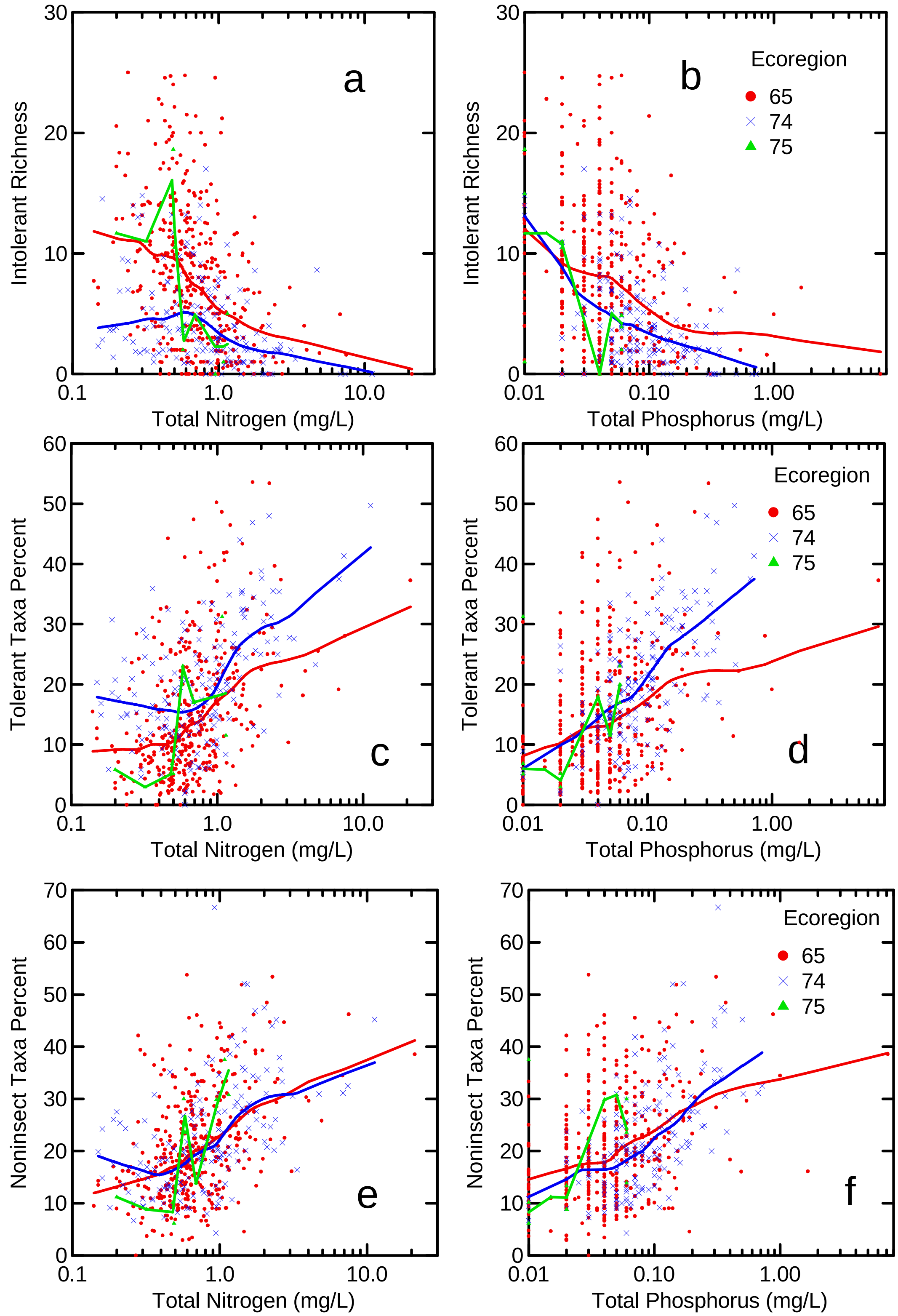


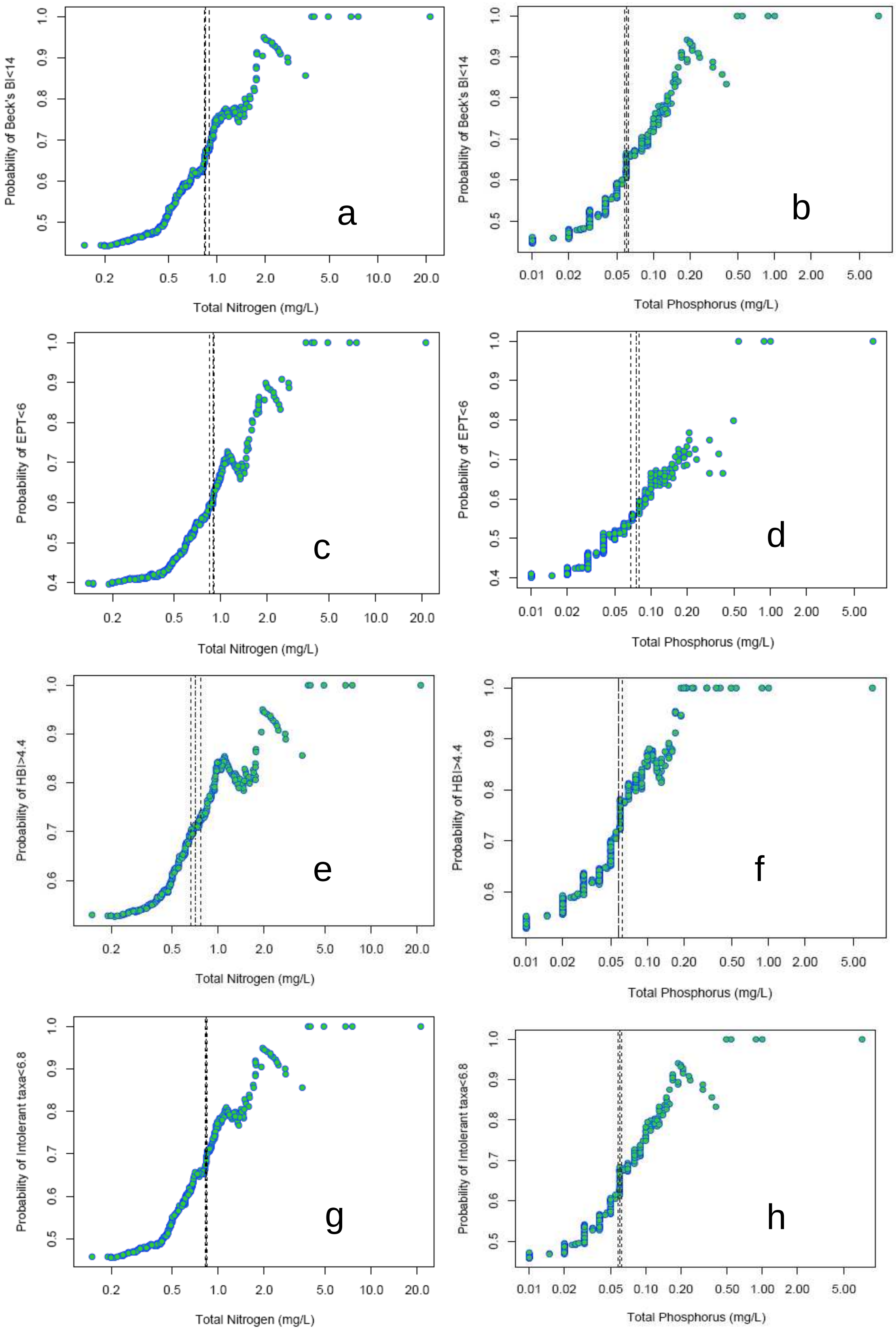
Figure E.3. Scatterplot of intolerant taxa richness, tolerant taxa percent, and non-insect taxa percent vs. nutrient concentrations in three ecoregions in the State of Mississippi. Solid lines are the LOWESS lines.

4. Conditional probability analysis and change points

Conditional probabilities for all the metrics were calculated and plotted against TN and TP concentrations for each ecoregion (Figure E.4, E.5, Table E.1). Solid lines are the change points for the conditional probabilities, and the dashed lines represented the 95 percent confidence limits of the change points.

Table E.1. Thresholds in biological response to total nitrogen and total phosphorus and their confidence intervals for different ecoregions in Mississippi using conditional probabilities. Conditional probability thresholds were based on criteria used in scoring these metrics for the M-BISQ.

	Biotic Response	Conditional Probability Threshold	TN Change Point	TN 95th Confidence Interval	TP Change Point	TP 95th Confidence Interval
Ecoregion 65	M-BISQ	<57	0.85	0.845-0.865	0.107	0.092-0.145
	EPT Taxa	<6	0.897	0.85-0.905	0.075	0.067-0.08
	Non-insect Taxa	>21	0.89	0.877-0.922	0.052	0.05-0.07
	Intolerant Taxa	<6.8	0.835	0.825-0.845	0.06	0.057-0.062
	Tolerant Taxa	>13.7	0.84	0.82-0.845	0.06	0.057-0.07
	Beck's Biotic Index	<14	0.845	0.835-0.893	0.06	0.057-0.062
	HBI	>4.4	0.71	0.66-0.768	0.057	0.057-0.062
Ecoregion 74	M-BISQ	<46	0.94	0.82-0.957	0.082	0.08-0.087
	EPT	<4.2	0.845	0.816-0.87	0.095	0.09-0.105
	Noninsect	>25	0.95	0.89-0.967	0.105	0.102-0.108
	Intolerant Taxa	<2.5	0.87	0.772-0.975	0.105	0.085-0.108
	Tolerant Taxa	>22.1	0.94	0.89-0.957	0.082	0.08-0.095
	Beck's biotic Index	<7.4	0.78	0.755-0.835	0.09	0.082-0.103
	HBI	>6.7	1.244	0.993-1.88	0.33	0.161-0.352


Probability of Beck's BI<14
Total Nitrogen (mg/L)
a
Probability of Beck's BI<14
Total Phosphorus (mg/L)
b
Probability of EPT<6
Total Nitrogen (mg/L)
c
Probability of EPT<6
Total Phosphorus (mg/L)
d
Probability of HBI>4.4
Total Nitrogen (mg/L)
e
Probability of HBI>4.4
Total Phosphorus (mg/L)
f
Probability of Intolerant taxa<6.8
Total Nitrogen (mg/L)
g
Probability of Intolerant taxa<6.8
Total Phosphorus (mg/L)
h
0.2 0.5 1.0 2.0 5.0 10.0 20.0
0.01 0.02 0.05 0.10 0.20 0.50 1.00 2.00 5.00

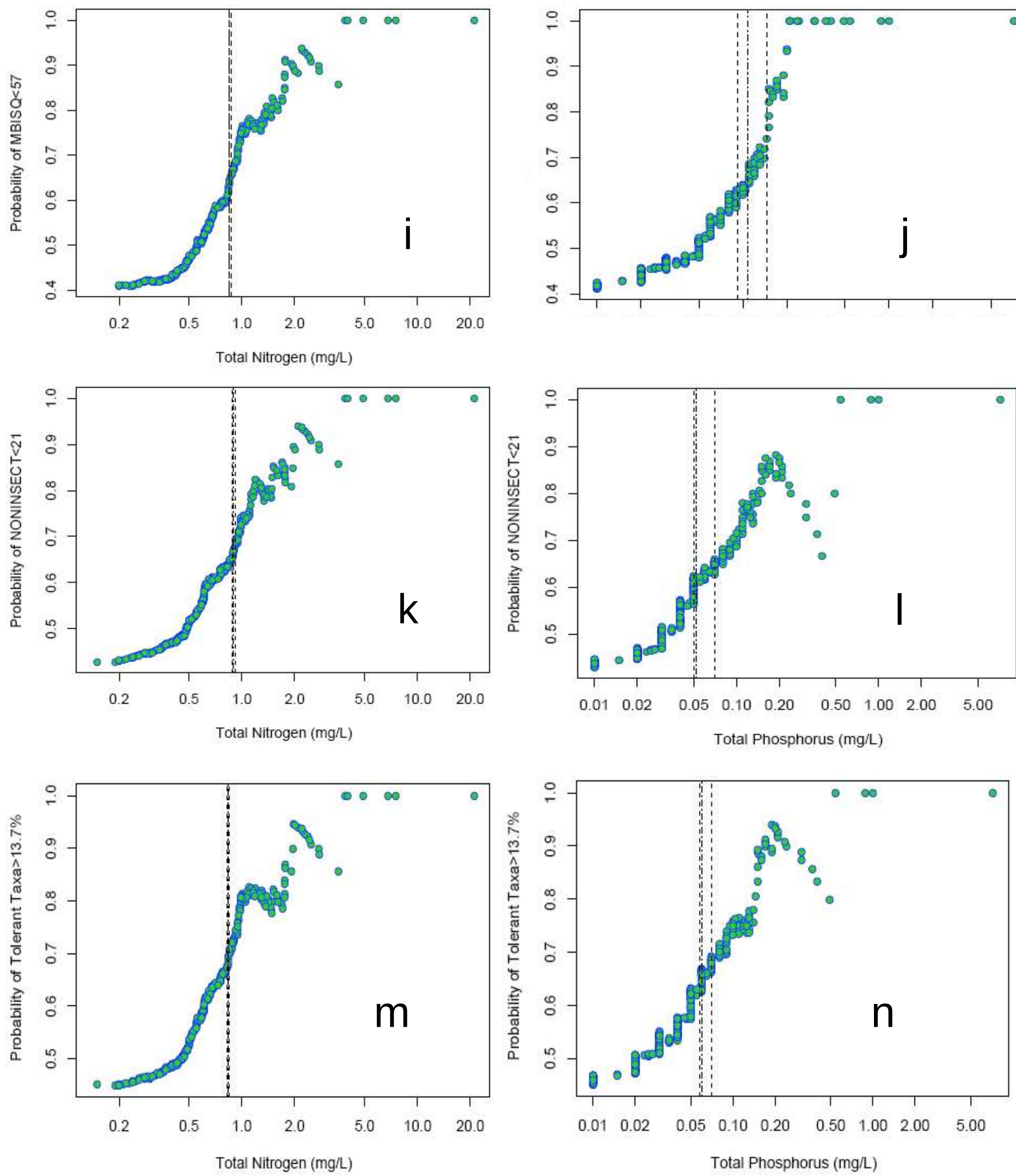
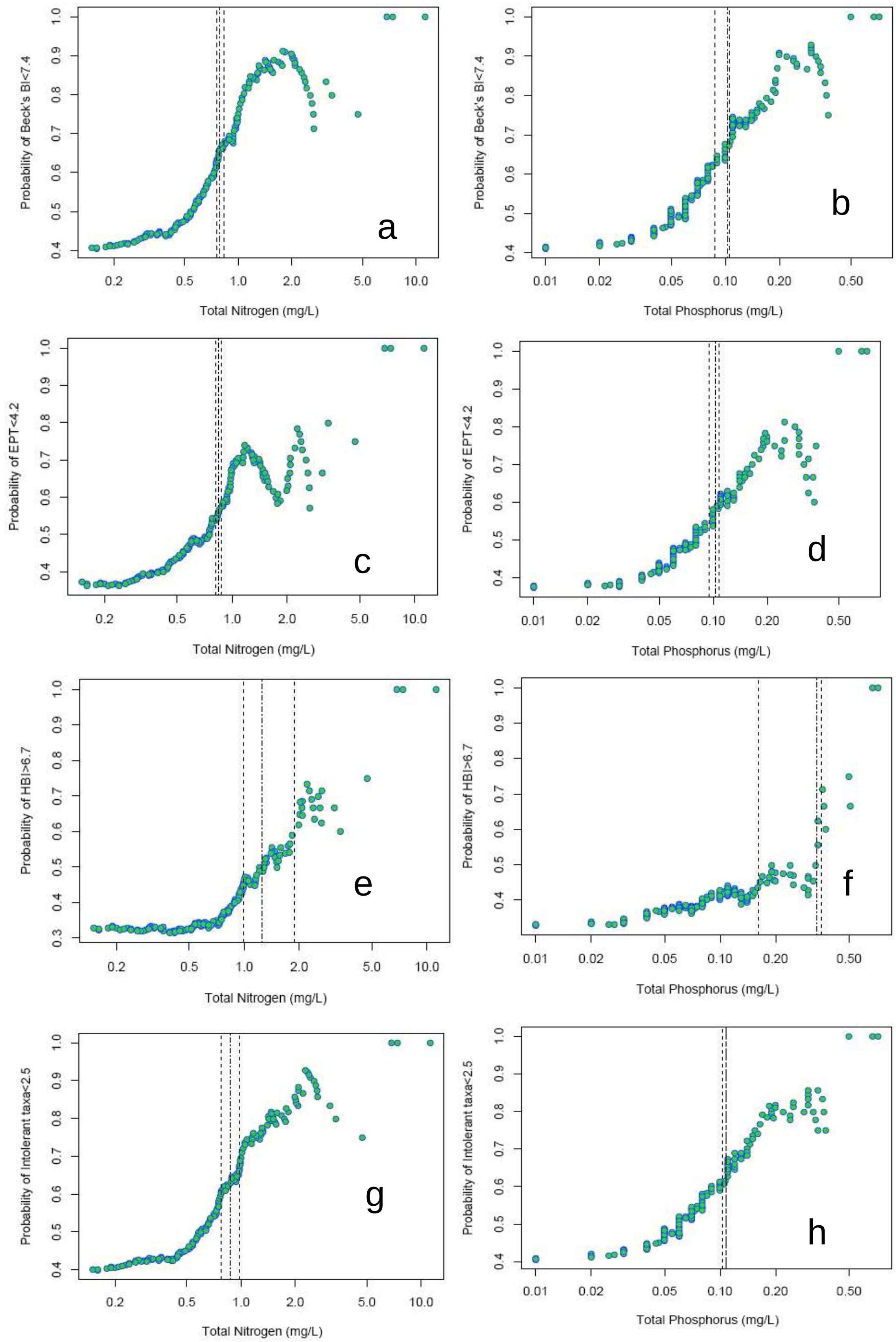


Figure E.4. Change-points in the conditional probability of biological impact (when a metric is less or greater than the conditional probability thresholds) along the total nitrogen and total phosphorus gradients. Dashed lines are 95 percent confidence intervals of the estimated change-points.



Probability of Beck's BI<7.4
Total Nitrogen (mg/L)
a
Probability of Beck's BI<7.4
Total Phosphorus (mg/L)
b
Probability of EPT<4.2
Total Nitrogen (mg/L)
c
Probability of EPT<4.2
Total Phosphorus (mg/L)
d
Probability of HBI>6.7
Total Nitrogen (mg/L)
e
Probability of HBI>6.7
Total Phosphorus (mg/L)
f
Probability of Intolerant taxa<2.5
Total Nitrogen (mg/L)
g
Probability of Intolerant taxa<2.5
Total Phosphorus (mg/L)
h

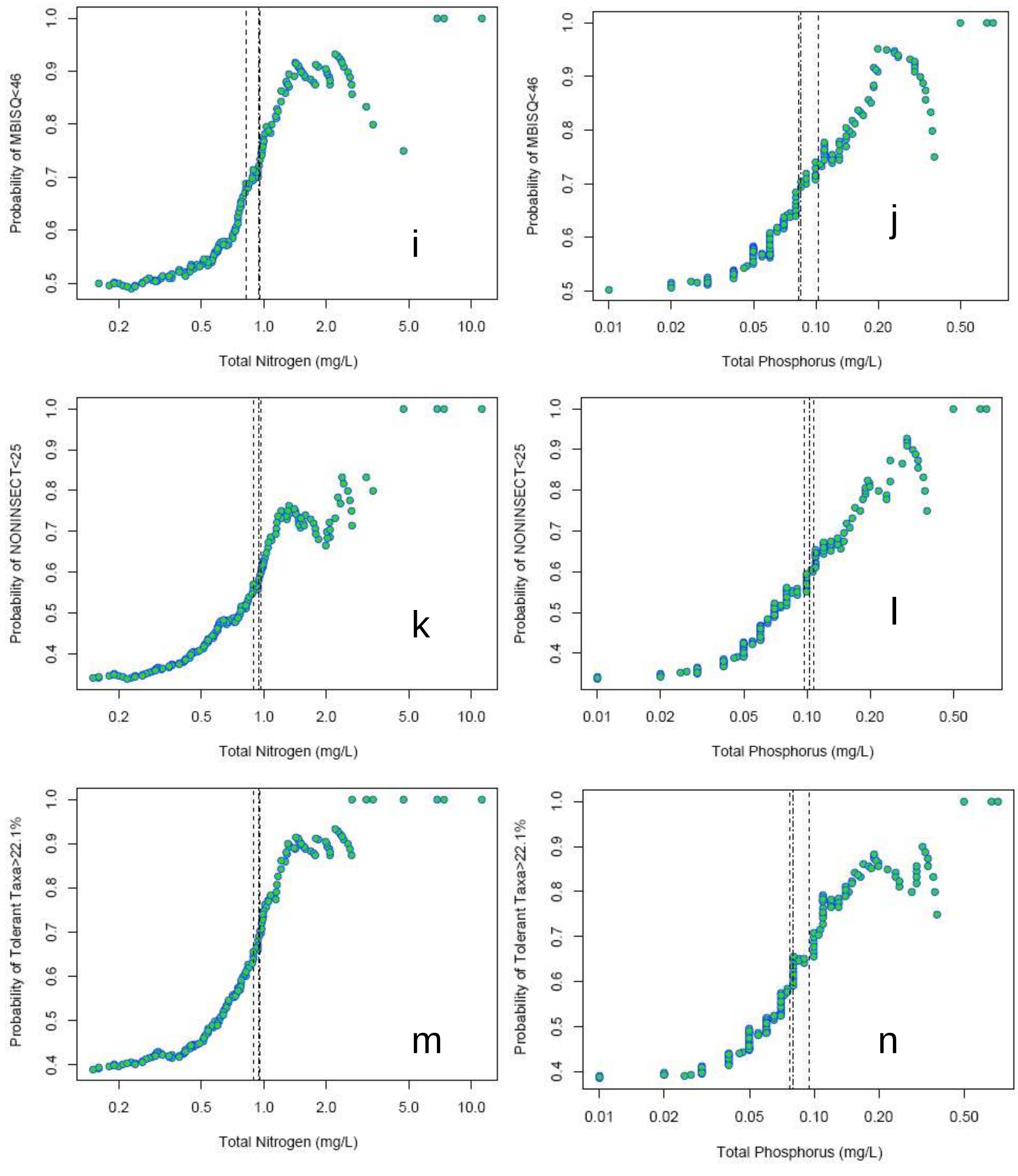


Figure E.5. Conditional probability of impairment (when a metric is less or greater than certain value) along the total nitrogen and total phosphorus gradients in Ecoregion 74. Dashed lines are the 95 percent confidence intervals of the change-point.

Appendix F - Large River Metrics and TN and TP Responses

The six metrics that compose the large river benthic index of stream quality were plotted against TN and TP concentrations for non-wadeable streams (Figure F.1 and F.2). The Spearman correlation matrix is shown in Table F.1.

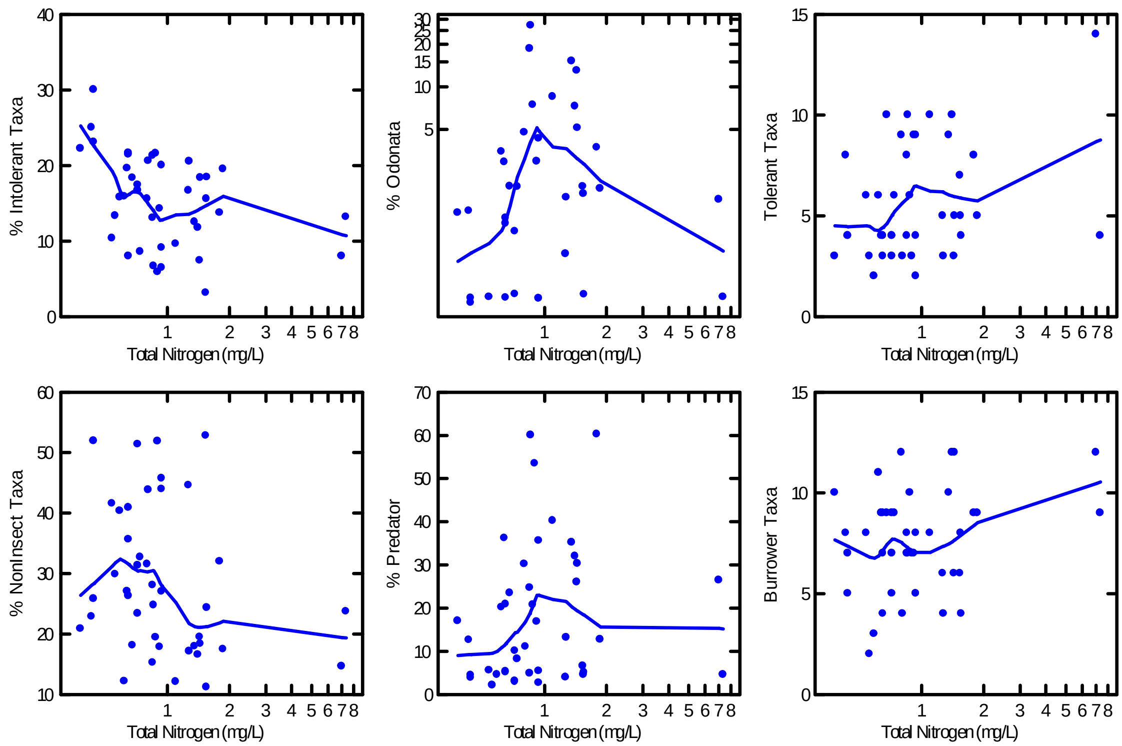


Figure F.1. The response of the six metrics to TN concentrations. LOWESS lines were plotted to chacracterize the response.

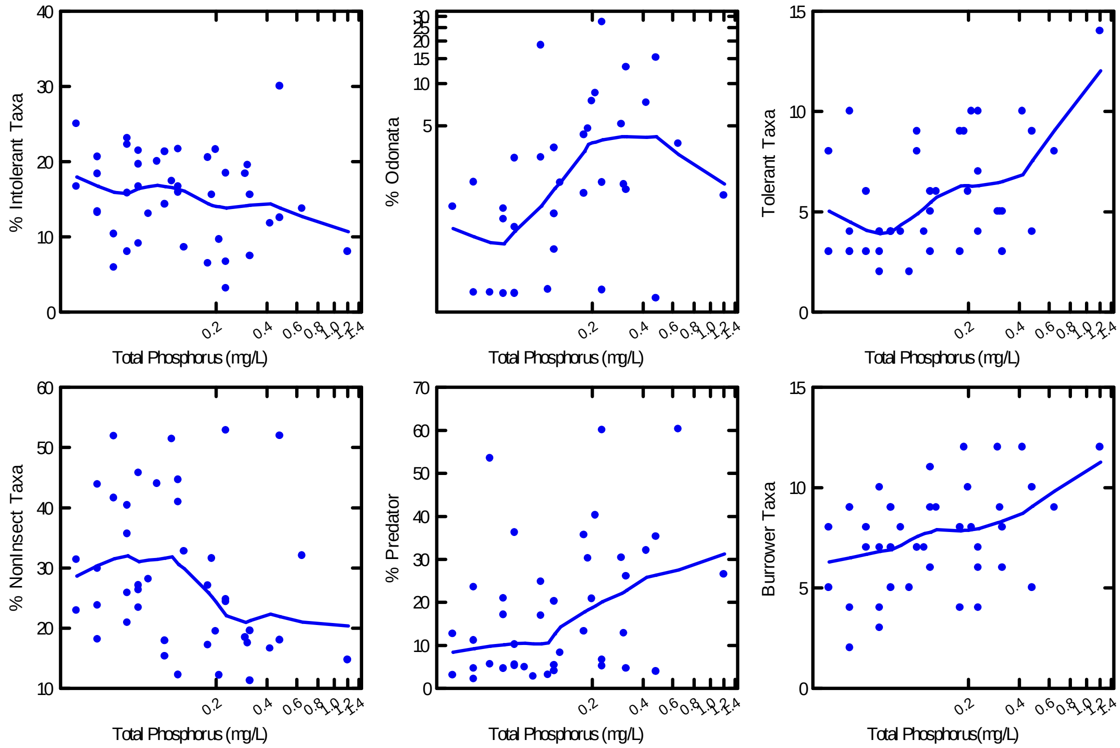


Figure F.2. The response of the six metrics to TP concentration. LOWESS lines were plotted to chacracterize the response.

Table F.1 Spearman Correlation matrix among six macroinvertebrate metrics and the overall index and TN and TP concentrations. Significant correlations are shown in bold.

	Log_{10}(TN)	Log_{10}(TP)	TURBIDITY
Tolerant Taxa	-0.274	**-0.435**	**-0.464**
% Intolerant Taxa	**-0.41**	-0.209	-0.224
% Odonata	**-0.337**	**-0.594**	**-0.688**
% Non-insect Taxa	0.006	0.023	-0.123
Burrower taxa	-0.146	**-0.325**	**-0.462**
% Predator	-0.26	**-0.363**	**-0.619**
M-BISQ Index	**-0.381**	**-0.435**	**-0.633**