Development of Nutrient Enrichment Criteria for Iowa Streams

DRAFT

Iowa Department of Natural Resources

August 23, 2013

Abstract

The March 2013 Iowa Nutrient Reduction Strategy emphasizes implementation of technology-based nutrient controls and practices in the near-term, while retaining as a long-term goal the development of appropriate nutrient criteria. In 2010 the Iowa Department of Natural Resources (IDNR), with the assistance of a technical advisory committee, initiated a new project to build a technical foundation for stream nutrient criteria. This report presents results from an analysis of Iowa-specific nutrient data and a review of scientific literature and other relevant technical information. As an outgrowth of this work, nutrient enrichment criteria recommendations have been prepared for B(WW-1) and B(WW-2) wadeable, warmwater streams and B(CW1) coldwater (trout) streams. Current data are not adequate to formulate criteria recommendations for B(CW2) coldwater spring runs, B(WW-2) and B(WW-3) marginally perennial, headwater creeks of watershed area less than ten square miles, and large wadeable/nonwadeable rivers of watershed area greater than 700 square miles. IDNR continues to gather and analyze nutrient and biological-response monitoring data needed to develop nutrient criteria recommendations for all stream classifications. Going forward, a strong commitment to nutrient-related monitoring, data analysis, and reporting will be necessary to support development and implementation of nutrient criteria and the objectives of the state nutrient reduction strategy.

Executive Summary

Nutrient enrichment of rivers and streams can lead to degraded water quality conditions which threaten human health and ecosystem integrity. Nitrogen and phosphorus are the nutrients most often linked to water quality impairments. Nutrient criteria define acceptable levels of nutrients and nutrient response parameters that support beneficial water uses such as fishing, swimming, and drinking water supply. Water quality standards criteria provide an objective, scientific basis supporting water quality assessments and management decisions.

This report summarizes work completed to-date seeking to determine levels of nutrients and nutrient response parameters that are protective of Iowa's stream biological assemblages and designated aquatic life uses. Through a review of technical and scientific literature and the analysis of monitoring data from Iowa streams, this project attempted to identify benchmark values that can serve as a foundation for establishment of nutrient enrichment criteria.

Data analysis approach

Scientific and technical literature were gathered and reviewed to become familiar with findings from relevant studies in the Midwest. Readily available data from stream monitoring programs in Iowa were identified and reviewed for quality and suitability. Data collected in four monitoring projects administered by the IDNR were utilized in the analysis: 1) probabilistic (random) survey; 2) biological reference condition; 3) ambient water quality; 4) impaired streams.

Statistical analysis techniques recommended in U.S. EPA guidance were utilized to examine nutrient stressor – aquatic biological response relationships and to identify meaningful changepoints or thresholds. Data obtained in the 2002-2006 Regional Monitoring and Assessment Program (REMAP) probabilistic survey of Iowa's perennial streams were used extensively for these purposes. The project incorporated sampling for water quality parameters with sampling of biological assemblages (fish and macroinvertebrates) and stream habitat characteristics.

Conceptual diagrams were used to illustrate potential stream nutrient response pathways and organize data analysis. The conceptual models illustrate processes and steps linking nitrogen and phosphorus with biological responses that negatively impact water quality and stream ecosystem integrity. Data analysis focused on evaluating the strength of evidence connecting nutrient stressors with adverse changes in stream biological communities.

Stream classification

The examination of nutrient and nutrient-response indicator data along with previously documented patterns in biological assemblage characteristics demonstrated the importance of partitioning the data before performing changepoint/threshold analysis. Stream size, ecological region, and thermal regime were important factors distinguishing stream nutrient conditions across Iowa. Small and medium-size (wadeable) warmwater streams were separated from large (nonwadeable) rivers and streams for analysis of relationship changepoints. Likewise, streams designated as coldwater ecosystems in Iowa's water quality standards were separated from warmwater streams for data analysis.

Nutrient stressor- response relationships

Conventional statistical approaches such as correlation analysis and linear regression analysis found generally weak relationships between nutrient parameters and nutrient response parameters. For example, Total Kjeldahl Nitrogen, the nutrient parameter most strongly correlated with seston Chlorophyll A, an indicator of water column-based algal biomass, only explained roughly 30% of the variation in Chlorophyll A levels in wadeable, warmwater streams across the state. For certain nutrient parameters the directionality of correlation with Chlorophyll A was opposite of the expected direction. For example increased levels of nitrate-nitrogen, the dominant form of dissolved inorganic nitrogen in Iowa streams, were associated with decreased chlorophyll A levels. In contrast, total phosphorus and dissolved orthophosphate were correlated in a positive direction with seston chlorophyll A.

Overall, the findings from this project suggest that nitrogen and phosphorus monitoring parameters, at best, are weak predictors of nutrient response indicators such as chlorophyll A and dissolved oxygen. A stoichiometric analysis of nutrient data that examined relative levels (i.e., ratios) of carbon, nitrogen, phosphorus, and chlorophyll A suggested that neither nitrogen nor phosphorus was limiting to sestonic algal production during a large majority of sampling occasions (79%) spanning a five-year period. Sampling visits were conducted predominantly during summer and fall base flow conditions favoring algal production.

While the specific factors responsible for weak and/or counter-intuitive relationships between nutrient and nutrient-response variables were not determined in this project, other studies have observed similar weak or inconsistent relationships. Stream environmental variables such as flow, substrate, and light availability are often attributed as being among the important physical factors limiting algal production in nutrient-rich agricultural streams of the Midwest.

Nutrient stressor – biological assemblage relationships

Benthic macroinvertebrate assemblage metrics were more strongly and consistently related with nutrient response parameters than were fish assemblage metrics. This finding cannot be easily explained by any single factor. The co-variation of nutrient response parameters and stream habitat characteristics is one confounding factor. Previous work in Iowa has demonstrated that stream fish assemblages are shaped to a greater extent by macro-habitat characteristics than are benthic macroinvertebrates. The IDNR method of sampling benthic macroinvertebrates targets the collection of organisms from the most optimal micro-habitat available within a stream reach (i.e., flowing water over stable rock or wood substrates). Consequently, the effects of macro-scale site-to-site habitat differences on benthic macroinvertebrate sampling results are thought to be smaller compared with habitat influences on fish sampling results. Also, because benthic macroinvertebrates occupy a lower level in the food web, it is likely that changes in food resources and micro-habitat caused by increased algal production would more directly impact them rather than fish.

The nutrient stressor-response analysis uncovered evidence of adverse changes in stream benthic macroinvertebrate assemblages related with elevated levels of sestonic algal biomass. The main effects appeared to be a reduction in certain types of organisms (e.g.. scrapers) that obtain food resources through a specialized feeding mode. There also appeared to be an overall reduction in taxonomic diversity that is consistent with impacts often reported in scientific/technical literature. The magnitude of changes in benthic macroinvertebrate assemblage condition that appear to be directly attributable to elevated levels of seston chlorophyll A might be characterized as moderate.

The most severe nutrient-related impacts to benthic macroinvertebrate and fish assemblages were associated with the occurrence of large diel fluctuations in dissolved oxygen, particularly those resulting in the occurrence of substandard minimum levels. The causal pathway linking elevated sestonic algal biomass with the occurrence of dissolved oxygen levels that are considered stressful or lethal to aquatic organisms was the strongest pathway observed in the data analysis project. Increased growth of benthic algae on hard substrates (periphyton) or soft sediments (epiphyton) was not associated with occurrences of low dissolved oxygen in Iowa streams, but rather was positively associated with increased diel maxima in DO and increased rates of algal primary production.

Benthic chlorophyll A (attached periphyton and fine sediment-associated) relationships with benthic macroinvertebrate and fish data metrics did not show consistent linear response patterns. Optimal levels of biological assemblage metrics tended to occur in a broad middle range of benthic algal chlorophyll A levels. Levels at the low and high ends of the sampled range were not consistent with the occurrence of the highest diversity and structural/functional balance in the aquatic community. Periphyton chlorophyll A was correlated positively with habitat conditions and dissolved oxygen, both of which are beneficial to fish assemblage condition.

Criteria recommendations

Nutrient criteria recommendations are available for some, but not all stream types. Listed below is the status of nutrient criteria development for stream aquatic life uses designated in Iowa Water Quality Standards (IAC 567:61). Watershed area subdivisions are proposed for the refinement of nutrient enrichment criteria based on data analysis results described in this report.

<u>Status of stream nutrient criteria recommendations for stream designated aquatic life uses (IAC 567:61) and proposed watershed area subdivisions</u>.

Stream designation (applicable watershed size)	Status of criteria recommendations
B(CW1) - Coldwater streams supporting trout and associated aquatic community	Preliminary criteria recommendations available
B(CW2) - Coldwater, spring runs not capable of supporting trout	Criteria recommendations currently unavailable (insufficient data); Designated use currently not populated with waterbodies
B(WW1) - Warmwater, large wadeable streams and nonwadeable rivers (watershed area >700 mi^2)	Criteria recommendations currently unavailable (insufficient data, biological reference condition unavailable)
B(WW1) - Warmwater, medium-to-large wadeable streams (WA $\leq$700 mi^2)	Criteria recommendations available
B(WW2) - Warmwater, small perennial streams (WA $\geq$10 mi^2)	Criteria recommendations available
B(WW2) - Warmwater, small marginally-perennial streams (WA <10 mi^2)	Criteria recommendations currently unavailable (insufficient data, biological reference condition unavailable)
B(WW3) - Warmwater, intermittent flowing streams with perennial pools	Criteria recommendations currently unavailable (insufficient data, biological reference condition unavailable)

The nutrient stressor-biological response data analysis project revealed several benchmarks that seem promising for establishment of stream nutrient enrichment criteria. A summary of recommendations is provided at the end of this section. Data analysis findings indicated the need for establishment of a criteria framework that, at a minimum, accounts for differences in stream size and thermal regime.

Benchmark values for total Kjeldahl nitrogen and total phosphorus are best thought of as indicators of nutrient enrichment status and potential nutrient availability because they include the organically-bound fraction of nutrients contained in living and dead organic matter including algal cells. As such, these parameters are not reliable predictors of readily-available nutrients, but more as longer-term indicators of nutrient status and ecosystem productivity. Most comprehensive nutrient monitoring projects will also include sampling and analysis for dissolved inorganic nutrient forms such as nitrate and orthophosphate.

The amount of data and evidence supporting the wadeable, warmwater stream nutrient benchmarks is fairly strong and should make them eligible for immediate use for water quality assessments and reporting purposes. Based on the results of a contingency table analysis, with the exception of diel dissolved oxygen minima, the nutrient and nutrient-response benchmarks do not individually appear well-suited for use as determinants of aquatic life uses support status. Among a randomly selected group of Iowa streams, total nitrogen and total phosphorus were inaccurate predictors of nutrient responses and water quality conditions that adversely impact stream aquatic life. Predictive capability, however, was greatly increased when the nutrient benchmarks were applied in conjunction with nutrient-response indicator benchmarks.

<u>Nutrient enrichment criteria recommendations for wadeable warmwater streams.</u>

Stream Designation	Parameter	Acceptable Level	Season
B(WW1), B(WW2), (Watershed Area 10-700 mi^2)	Total Kjeldahl Nitrogen (TKN)	Median sample value $\leq$ 0.80 mg/L	June 15 – Oct. 15
	Total Phosphorus (TP)	Median sample value $\leq$ 0.10 mg/L	June 15 – Oct. 15
	Dissolved Oxygen Diel Range	Median daily range (maxima-minima) $\leq$ 5 mg/L	July 1 – Sept. 15
	Filamentous Algae Coverage Rating	Median rating $\leq$ 3 (50-75%)	June 15 – Oct. 15
	Seston Algal Chlorophyll A	Median sample value: $\leq$ 5.0 µg/L (Watershed Area $\geq$10-25 mi^2) $\leq$ 10.0 µg/L (WA >25-100 mi^2) $\leq$ 15.0 µg/L (WA >100-300 mi^2) $\leq$ 20.0 µg/L (WA >300-700 mi^2)	June 15 – Oct. 15

The stressor-response analysis found relationships linking the nutrient response variables seston chlorophyll A and diel dissolved oxygen range with diel dissolved oxygen (DO) minima. When the diel average DO minima was less than 5 mg/L, there was an increased frequency of benthic macroinvertebrate index (BMIBI) scores not attaining reference expectations.

The diel DO minima benchmark of 5 mg/L is not included as a criteria recommendation because it is equivalent to the 16-hour criterion for warmwater B(WW1) and B(WW2) streams; therefore, it is unnecessary to establish a new criterion.

Nutrient and nutrient response benchmarks from the analysis of coldwater stream data should be considered more as preliminary recommendations. Additional monitoring and data analysis work is being conducted to provide strengthen the foundation for criteria recommendations.

<u>Nutrient enrichment criteria recommendations for coldwater streams</u>

Stream Designation	Parameter	Acceptable Range	Season
B(CW1)	Total Kjeldahl Nitrogen	Median value $\leq$ 0.16 mg/L	June 15 – Oct. 15
	Total Phosphorus	Median value $\leq$ 0.08 mg/L	June 15 – Oct. 15
	Filamentous Algae Coverage Rating	Median rating $\leq$ 2 (25-50%)	June 15 – Oct. 15
	Periphyton Algal Chlorophyll A	Median value $\leq$ 15.0 µg/cm^2	June 15 – Oct. 15
	Sediment Algal Chlorophyll A	Median value $\leq$ 7.5 µg/cm^2	June 15 – Oct. 15
	Seston Algal Chlorophyll A	Median value $\leq$ 3.0 µg/L	June 15 – Oct. 15

Monitoring and assessment recommendations

Establishing sufficient capacity for monitoring and assessing nutrient conditions is essential to successful nutrient criteria implementation. The data analysis work completed thus far has called attention to ways that monitoring efforts can be improved. The 2013 Iowa Nutrient Reduction Strategy identifies local and basin-scale monitoring needs for nutrient status/trends determinations and tracking progress toward achieving nutrient reduction goals. The development and implementation of a monitoring design template that mutually supports nutrient criteria development and the information needs of the strategy is strongly recommended.

In designing nutrient monitoring systems it is important to recognize that monitoring to assess local nutrient impacts to stream aquatic communities requires a different approach than monitoring to track nutrient loads at any given point in the stream network. The basic difference is that sampling to assess local nutrient impacts is most appropriately conducted under base flow conditions during the summer and early fall when adverse responses to nutrients are typically manifested. In contrast, monitoring for nutrient load estimation needs to be conducted throughout the year and should specifically include sampling during storm events and high flow conditions when the majority of annual nutrient loads are transported.

Monitoring projects typically must strike a balance between the optimal sampling design and an acceptable design that fits within budget constraints. Designs that incorporate varying levels of sampling intensity can provide a flexible, economical way to implement nutrient monitoring and assessment. This type of approach relies on informed decision making to determine monitoring needs based on available evidence from previous sampling events and/or other sources.

Stream biological assessment sampling for benthic macroinvertebrates and fish are conducted in Iowa for many purposes and programs. At a basic level, it is recommended that each time biological assessment sampling is conducted, the list of analytes should include a standard suite of nutrient and chlorophyll A parameters. The results from this one-time sampling event can be compared to applicable nutrient benchmarks to yield a snapshot picture of nutrient status. The results also provide valuable context for the interpretation of benthic macroinvertebrate and fish assemblage sampling results.

Many stream monitoring projects focus exclusively on water quality and do not include biological assessment sampling. Sampling for these projects often is conducted on a biweekly or monthly basis. Including the standard suite of nutrient and chlorophyll A parameters would be a cost-effective way to screen for potential nutrient impacts. It would not require additional site visits. The only added costs are those of collecting and analyzing additional parameters not included in the original list of analytes. At a minimum, the list of analytes should include TKN, TP, and seston Chlorophyll A. Benthic chlorophyll A and continuous dissolved oxygen monitoring require additional equipment and labor and therefore can be considered optional at the screening monitoring level.

More intensive sampling is recommended where screening monitoring or other evidence points to the potential impairment of aquatic life uses due to nutrient over-enrichment. Most likely, this level would incorporate biweekly or more frequent monitoring for nutrient status indicators during the biological index period along with continuous monitoring of dissolved oxygen/temperature, and biological assessment sampling.

Summary of recommendations:

1. Incorporate the nutrient enrichment criteria and monitoring recommendations in the water quality assessment protocol for the biennial Section 305(b)/303(d) Integrated Report. Apply the proposed nutrient criteria and assessment guidelines to support determinations of nutrient-related stream aquatic life impairments.

2. Complete nutrient and biological monitoring and data analysis projects in-progress for small, headwater streams and large wadeable/nonwadeable rivers. The focus should continue to be documenting linkages between nutrient stressors and biological response indicators and identification of appropriate nutrient and nutrient-response benchmarks for all stream aquatic life use designations.

3. Develop and conduct nutrient monitoring projects in targeted watersheds that support the objectives of the Iowa Nutrient Reduction Strategy. With respect to development and implementation of nutrient enrichment criteria, a primary objective should be to assess the degree to which nutrient over-enrichment is contributing to stream aquatic life use impairments. Nutrient criteria-related monitoring should complement and not duplicate other monitoring efforts including stream nutrient load monitoring.

4. Continue utilizing the expertise of the stream nutrient criteria technical advisory committee (TAC). Convene the TAC annually or more frequently as needed to complete the development of criteria recommendations for all stream aquatic life use designated uses.

5. Periodically update the technical report of the stream nutrient criteria development as new data are gathered and analyzed. Address technical input from the TAC.

Abbreviations

ACPUE – fish adjusted catch per unit effort
AOV – analysis of variance
AVG – average value
AVGMAXDO – average diel maximum dissolved oxygen
AVGMINDO – average diel minimum dissolved oxygen
AVGRNGDO – average diel range of dissolved oxygen
BINVSP – number of benthic invertivore fish species
BMIBI – benthic macroinvertebrate index of biotic integrity
BTI – benthic (macroinvertebrate) tolerance index
CBI – coldwater benthic (macroinvertebrate) index
CHLR – chlorophyta (green) algae
CHLA - chlorophyll A
CP – conditional probability
CR – community respiration
CHRY – chrysophyta algae
CW – coldwater
CWA – Federal Clean Water Act
CYN – cyanobacteria (blue-green) algae
DIN – dissolved inorganic nitrogen
DINO - dinoflagellate
DO – dissolved oxygen
DOP – dissolved orthophosphate
DTM – diatom algae
EMAP – Environmental Monitoring and Assessment Program
EPA – Environmental Protection Agency
EPT – Ephemeroptera (E), Plecoptera (P), Trichoptera (T) aquatic insect orders
EUGL – euglenophyta
FA – filamentous algae
FIBI – fish index of biotic integrity
GPP – gross primary production
HABSCR – rapid habitat assessment score
IBI – index of biotic integrity
IDNR – Iowa Department of Natural Resources
L10 – logarithm base 10
MH – multi-habitat benthic macroinvertebrate sample
N - nitrogen
NHX – total ammonia nitrogen
NOX – nitrate + nitrate nitrogen
MHEPTX – multi-habitat number of EPT taxa
MHSNSTV - multi-habitat number of sensitive taxa
MHTTX – multi-habitat number of total taxa
NPP – net primary production
NTVSP – number of native fish species
ORGN – organic nitrogen
P - phosphorus

PCT – percentage value
P3ABUND – percent abundance top three dominant fish species
PBINV – percent abundance benthic invertivore fish species
PCHLA – periphyton chlorophyll A
PDELT – percent abundance fish with deformities (D), eroded fins (E), Lesions (L), Tumors (T)
PH – logarithm of the reciprocal of hydrogen ion concentration
POMNV – percent abundance omnivore fish species
PR – ratio of gross primary production to respiration
PROT - protozoa
PRTCP – particulate-bound phosphorus
PSLITH – percent abundance simple lithophil fish species
PTOPC – percent abundance top carnivore fish species
QR – quantile regression
REMAP – Regional Environmental Monitoring and Assessment Program
RT – regression tree
SCR – metric score
SCHLA – sediment chlorophyll A
SH – standard habitat benthic macroinvertebrate sample
SHCHIR – standard habitat percent abundance Chironomidae taxa
SHDFFG – standard habitat percent abundance dominant functional feeding group
SHEPHM – standard habitat percent abundance Ephemeroptera taxa
SHEPTX – standard habitat number of EPT taxa
SHL – State Hygienic Laboratory of Iowa
SHMHBI – standard habitat modified Hilsenhoff biotic index
SHP3DOM – standard habitat percent abundance top three dominant taxa
SHPEPT – standard habitat number of EPT taxa
SHSCRPR – standard habitat percent abundance scraper organisms
SHTTX – standard habitat number of total taxa
SI – stressor identification
SNSTVSP – number of sensitive fish species
SCKRSP – number of sucker fish species
TKN – total Kjeldahl nitrogen
TN – total nitrogen
TOLINDX – fish species tolerance index
TP – total phosphorus
TSS – total suspended solids
TVSS – total volatile suspended solids
UTM – universal transverse mercator geographic coordinate system
WCHLA – water column (seston) chlorophyll A (ug/L)
WM – algal wet biomass
WW - warmwater

Table of Contents

List of Tables

List of Figures

1. Introduction

Nutrients are chemical elements and compounds that allow organisms to grow and carry out essential life functions. Nutrients are often categorized as macronutrients or micronutrients. Nitrogen (N) and Phosphorus (P) are considered macronutrients because they are required in relatively large quantities compared with micronutrients such as the trace metal manganese. Nitrogen is contained in proteins such as enzymes and nucleic acids and also is a component of chlorophyll molecules. Phosphorus is essential to cell membrane stability, energy transfer, and genetic systems. In aquatic ecosystems N and/or P availability is often lower than the demand; therefore, these nutrients are considered among the ones most likely to limit productivity.

The trophic state of ecological systems refers to energy availability in the food web, which originates from autotrophic and heterotrophic processes (Dodds 2007). Trophic is derived from the Greek word 'trophikos,' which means 'to nourish.' Autotrophic energy production is obtained from the sun through photosynthesis, while heterotrophic energy is derived from the decomposition of organic matter. The relative trophic status of aquatic ecosystems is often categorized as Oligotrophic (low productivity), Mesotrophic (moderate productivity) or Eutrophic (high productivity). The trophic state of pristine aquatic ecosystems in the absence of significant human alteration ranges from Oligotrophic to Eutrophic depending upon natural factors such as precipitation, geology, and soils.

The process whereby aquatic ecosystems become more nutrient- enriched and productive over time is called eutrophication. Cultural eutrophication is the acceleration of the eutrophication process caused by increased nutrient loading from human activities including agriculture and urban development. Cultural eutrophication often leads to undesirable changes in water quality and ecosystem health. Nutrient controls and reduction efforts are designed to prevent or reverse the negative consequences of nitrogen and/or phosphorus enrichment. Iowa and other states are considering new water quality standards to protect surface waters from the harmful effects of excess nutrients. In order to establish appropriate nutrient criteria for streams, the linkages connecting nutrient stressors and biological responses must be examined and quantified.

Sources and forms of nutrients

Nutrient sources are often categorized as either point source or nonpoint source. Point sources originate from a known area or facility and are discharged to a waterbody from a discrete location, such as a pipe discharging treated wastewater into a stream. Nutrients coming from nonpoint sources are leached or carried in surface runoff from diffuse areas, such as agricultural fields and undeveloped land. Nonpoint source runoff is sometimes conveyed through subsurface tiles and constructed drainage ditches that enter streams at discrete locations; however, these discharges are not usually considered as point sources.

Point and nonpoint sources of nutrients vary significantly in their composition. Nutrient forms can be broadly categorized as dissolved or particulate-bound. Most of the nitrogen from point and nonpoint sources is delivered in dissolved forms. It is generally held that most of the phosphorus coming from point sources is dissolved, while most of the load from nonpoint sources is particulate-bound. However, nonpoint sources may also contain significant amounts of dissolved phosphorus, particularly in regions drained by subsurface drainage.

Dissolved nutrients are usually assumed to be more biologically available than particulate-bound forms; however, this is a generalization. Some particulate-bound nutrients are easily disassociated and can be readily available for uptake, while certain dissolved forms are unavailable because they are tightly held in complex dissolved organic compounds. For nitrogen, the primary bio-available forms are ammonium ($NH4^{+}$) and nitrate ($NO3^{-}$). Ammonium is preferentially taken up because it can be assimilated using less energy; however, nitrate is the most abundant form of dissolved nitrogen in oxygenated waters. Orthophosphate (PO_4^{3-}) and its conjugate ions (HPO_4^{2-}, $H_2PO_4^{-}$, H_3PO_4) are the main forms of phosphorus available for uptake in aquatic environments, although some organisms are able to utilize phosphorus contained in certain types of dissolved organic compounds.

Instream processes and nutrient cycling

A multitude of instream processes transform and cycle nutrients from one form to another, either removing or adding to the pool of biologically-available nutrients. Dissolved inorganic nutrients are assimilated into the cells of photosynthesizing plants and algae. Primary and secondary consumers utilize nutrients stored in living or dead organic matter in order to grow and reproduce. Nutrients are released to the stream once again through organism excretion and microbial decomposition. Denitrification is an important biochemical process whereby nitrate is transformed into nitrogen gas, which can then escape to the atmosphere. Certain types of Cyanobacteria (blue-green algae) are able to directly uptake or fix atmospheric nitrogen, thus eventually adding to the pool of available nutrients as these cells expire and decompose. Some of the phosphorus in a stream can be stored indefinitely in stream sediments as organic and inorganic particles settle out of the water column and are buried by other particles. Conversely, chemical oxygen and reduction processes that take place at the sediment-water interface can result in the release of biologically-available phosphorus to the overlying stream water.

Biological responses

When nutrient demand exceeds supply, the addition of nutrients will increase ecosystem productivity. Nutrients allow organisms to grow and reproduce, thus leading to an accrual of organic matter or biomass in the ecological system. Increased production can have cascading effects that alter ecosystem integrity and negatively impact beneficial water uses, such as fishing, recreation, and potable water supply. For example, nutrient enrichment of lakes, reservoirs and large rivers often results in blooms of Cyanobacteria or blue-green algae. These blooms are often associated with negative changes in biological integrity and water quality. When phytoplankton assemblages are dominated by Cyanobacteria, the aquatic food web is altered, often resulting in dominance by less desirable species that are more tolerant of poor water quality. For example, rough fish such as bullheads (*Ameiurus spp.*) and common carp (*Cyprinus* carpio) are able to thrive in turbid, nutrient-rich lakes and reservoir where they replace more desirable game fish species, such as bluegill (*Lepomis macrochirus*) or largemouth bass (*Micropturus salmoides*). Certain types of Cyanobacteria release toxins into the water column that can be hazardous to humans and other animals that come into contact with the water.

Effects on stream aquatic communities

Stream biological responses to nutrient enrichment are complex and dynamic. There is no single response or cause-effect pathway that encompasses the multitude of ways that nutrient enrichment can alter stream aquatic communities. A shift in species composition is perhaps the most commonly observed effect. The addition of nutrients in a nutrient-limited system will not result in all organisms growing and reproducing at equal rates; therefore, the composition of organisms will shift toward species that are best able to compete for and assimilate the extra nutrients. Changes in the food resource composition and abundance at lower trophic levels such as algae and microorganisms growing on substrates or suspended in the water are transferred throughout the food web to higher trophic levels of consumers such as macroinvertebrates and fish. Certain species are able to exploit the newly abundant food resources, and as they increase in abundance other organisms trying to occupy the same habitat or physical space are crowded-out. Consequently, the complexity and diversity of organisms making up the food web often decreases as the habitat becomes increasingly dominated by opportunistic species.

One of the most common changes in water quality linked to nutrient enrichment is increased dissolved oxygen fluctuation. Excessive fluctuations can be stressful or even lethal to aquatic organisms. A certain amount of dissolved oxygen fluctuation is natural because the solubility of oxygen is temperature dependent. Primary producers (autotrophs) add oxygen to the water during the day via photosynthesis, but oxygen levels fall during the night as autotrophs and heterotrophs consume oxygen through respiration. In nutrient over-enriched ecosystems, dissolved oxygen fluctuations can become greatly magnified with higher peaks during the day and lower sags at night. Increased daily fluctuation of dissolved oxygen by itself can be stressful to some aquatic organisms; however, when oxygen sags below critical levels, it can result in massive kills of fish and other aquatic organisms.

Nutrient enrichment can degrade water quality and physical habitat in several other ways. For example, water clarity may decrease from a proliferation of algal cells and other suspended organic matter. Reduced water clarity can negatively impact sight-feeding species. Levels of pH and ammonia may become stressful to aquatic organisms as a byproduct of rapid photosynthesis and organic matter decomposition. Suitable habitat for certain benthic (bottom-dwelling) species may become limiting when a stream becomes choked by dense mats of algae or plants.

Nutrient stressor–response mechanisms and pathways

A conceptual model is a useful tool for illustrating ways that a biological, chemical, or physical stressor can produce an adverse response within an aquatic ecosystem (U.S. EPA 2010a). Figure 1 shows a conceptural diagram of potential nutrient stressor – biological response mechanisms and pathways. This diagram and more detailed individual diagrams for nitrogen and phosphorus (Appendix 1(a-b)) are available from the U.S EPA's online application, Causal Analysis Diagnosis Decision Information System – CADDIS (U.S. EPA 2010b).

Conceptual diagrams are also helpful for organizing data analysis of evidence in support of a given nutrient stressor-response pathway. The data analysis approach described in this report examined stressor-response relationships and attempted to "connect-the-dots" linking nutrient variables with adverse changes in aquatic community indicators. Data analysis was conducted from the top-down, by examining relationships between nutrient parameters (e.g., TN & TP) and nutrient response indicators (e.g. chlorophyll A), and from the bottom-up, by examining relationships between biological assemblage attributes and nutrient response indicators. Although data collected from field studies are seldom able to isolate cause and effect mechanisms, the bi-directional data analysis approach used here was meant to provide more objectivity in evaluating the plausibility of hypothetical nutrient stressor-biological response relationships.

Four primary modes or pathways in which nutrient enrichment may lead to adverse impacts to stream biological communities were examined.

1. <u>Altered food web</u>

Changes in the type and amounts of algal production can cause an imbalance in the stream food web. Algae growth is stimulated under favorable growing conditions, which includes sufficient light and nutrient supplies. Nutrient enrichment may lead to increases in algal biomass in the water column (sestonic) or covering the stream bottom (benthic). Benthic macroinvertebrate and fish species have varying feeding modes and food item preferences. For example, collector-filterer macroinvertebrates feed by filtering organic particles from the water column. These species are able to capitalize on increases in sestonic algae as a food source and will increase in abundance in relation to other taxa. An increase in suspended organic matter can also cause a reduction in water clarity and decreased light penetration to the stream bottom. Benthic algae production is hindered under these conditions, and consequently macroinvertebrates and fish that feed on algae or other invertebrates that colonize the stream bottom can also be negatively impacted.

2. <u>Altered dissolved oxygen regime</u>

Under favorable growing conditions, excess nutrients may stimulate high rates of instream algae and plant growth, causing dissolved oxygen (DO) to fluctuate beyond the tolerance range of aquatic organisms. During the daytime, DO levels can become super-saturated as oxygen production during photosynthesis outpaces oxygen consumption and atmospheric loss. Conversely, during night-time oxygen levels can become depleted as the respiratory demand by primary producers and heterotrophic consumers in nutrient enriched stream ecosystems can outpace atmospheric inputs. Benthic macroinvertebrate and fish species vary in their tolerance of extreme dissolved oxygen fluctuations due to varying metabolic requirements and morphological or behavioral adaptations. Stream aquatic life may experience short-term acutely lethal DO levels or long-term exposure to stressful, sub-lethal effects that can affect growth and reproduction. Long-term chronic exposures to stressfully low DO often results in reduced species diversity, loss of sensitive species, and dominance by tolerant organisms. Short, episodic events of lethal DO concentrations may result in low overall organism abundance and/or dominance by species that are pioneering species or have short life cycles. If these episodes are rare and the stream is hydrologically connected to a colonization source area or refugia, a complete recovery can take place.

3. Water quality alterations

Besides amplifying fluctuations in dissolved oxygen, excessive levels of primary production fueled by abundant nutrient supplies may cause pH levels to elevate above thresholds that are stressful or lethal to aquatic organisms. A rapid collapse and die-off of algae or plants may cause a sudden release of stressful or lethal concentrations of un-ionized ammonia. These water quality conditions have been observed in the epilimnion (surface layer) of hyper-eutrophic Iowa lakes during periods of calm, sunny weather when algal production is near peak levels. Ammonia and pH violations caused by excessive primary production are probably less likely to occur in a mixed stream environment, but may still be possible during warm, sunny weather and low stream flow.

4. Altered benthic habitat

Excessive growth of vascular macrophytes or benthic macro-algae can negatively impact benthic (bottom-dwelling) aquatic life. Dense growth of macrophytes or filamentous algae can act as a physical barrier to limit the amount of suitable habitat for certain types of benthic macroinvertebrates and fish. In particular, organisms that live on or among rock substrates are susceptible because rock surfaces and interstitial spaces can become covered by vegetation. Dense vegetation also alters the flow of water in a stream, causing sediment and organic particles to be trapped by the vegetation. As a result of changes in the quantity and quality of benthic habitat and available food resources, benthic macroinvertebrate and fish species occupying streams having excessive growth of plants and macro-algae will become dominated by species that thrive or can tolerate these conditions. Organisms that have specialized feeding or reproduction needs that require access to unvegetated benthic habitat will decrease in abundance.

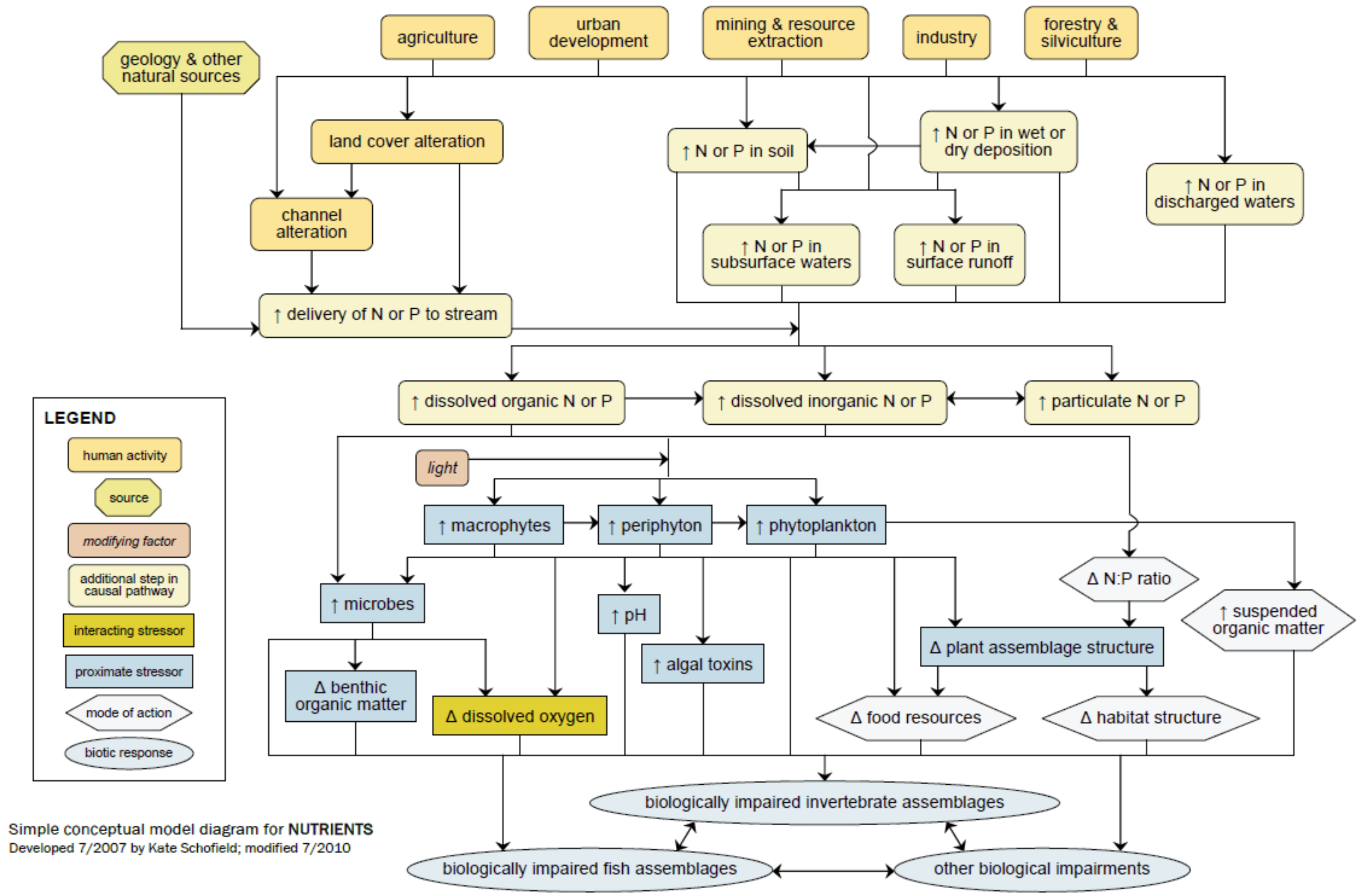


Figure 1. Conceptual model diagram of nutrient stressor pathways leading to impairment of aquatic biological communities (U.S. EPA, CADDIS 2010).

2. Stream Data

2.1. Projects and sampling parameters

A review of ongoing and completed sampling projects was completed to identify readily-available sources of data that could provide useful data for analysis of nutrient stressor-biological response relationships. Four data sets from sampling projects administered by the IDNR were identified. All of the sampling data from these projects were collected and analyzed under established standard operating procedures and protocols. Laboratory analysis of nutrient and other water quality parameters were done following U.S. EPA approved methods by the State Hygienic Laboratory (SHL) of Iowa under contractual agreement with the IDNR. Sampling data from Iowa streams collected by other agencies and institutions might prove useful for the purpose of developing stream nutrient criteria; however, for reasons of data consistency and immediate availability it was decided to begin the stressor-response analysis using data from the projects described below.

Wadeable reference stream site network

Stream reference site sampling data have been collected since 1994 to serve as a foundation for conducting stream biological assessments. Figure 2 shows the geographic distribution of 96 wadeable reference stream sites through 2010. Recent additions and deletions in the network of cold water reference sites are not reflected in the map. Surface watershed areas of reference streams range from 3 – 903 square miles. Reference sites are chosen to represent stream conditions that are least disturbed by anthropogenic disturbances. They are also selected for representativeness of the ecological region (ecoregion) in which the stream watershed is located. Iowa's land surface is covered by portions of ten Level IV ecoregions (Chapman et al. 2002).

The reference site sampling protocol includes collection of benthic macroinvertebrates and fish, as well as evaluation of stream habitat and sampling for water quality analytes. Reference sites are sampled approximately every five years in a rotational cycle. The sites are typically sampled once during the biological index period lasting from July 15 through October 15. A description of the reference stream site selection process and summarization of sampling data can be found in Wilton (2004). The reference site data set is particularly useful because they have been used to establish stream biological expectations in the form of benthic macroinvertebrate and fish metric and index scores. These quantitative measures of stream biological health provide helpful context for interpreting results from the nutrient stressor- biological response analysis.

Regional Environmental Monitoring and Assessment Program (REMAP)

The REMAP probabilistic stream survey was conducted in 2002-2006 to provide an unbiased assessment of biological and water quality conditions in perennial streams throughout Iowa. A total of 228 randomly selected stream sites were sampled for this project (Figure 3). The stream network from which the random sites were drawn encompassed perennial rivers and streams excluding the border rivers and impounded segments of large rivers. The sample sites encompassed second through seventh (Strahler) order streams having watershed areas ranging from 1.4 - 14,443 square miles.

Despite limitations in sampling frequency and duration, the REMAP project data were fairly well-suited for exploration of nutrient stressor-response relationships. Besides the advantages of providing an unbiased, random sample design, the project also provided integrated sampling of nutrient, nutrient response, and aquatic life variables within the established biological index period. Sampling details are summarized in Table 1. The large majority of water quality samples were collected during the months of June through October during baseflow, non-runoff stream flow conditions. The number of nutrient samples collected and analyzed ranged from 1-15, with a median of three samples per site. Dissolved oxygen and temperature data loggers were deployed at most sites and measurements were recorded at ten-minute intervals around-the-clock. Deployment length ranged from three days to more than two weeks; the median deployment lasted six days. Algal chlorophyll A measurements were obtained from samples of the water column (sestonic), rock or wood substrates (periphytic), and fine sediments (epipelic / epipsammic algae). Nutrient and chlorophyll A samples were collected concurrently with data logger deployment, which often overlapped sampling for benthic macroinvertebrates and fish. Biological assemblage sampling was conducted using the same procedures as reference sites (IDNR 2001). The data were entered in BioNet, IDNR's biological assessment data base, and used to calculate the Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) and Fish Index of Biotic Integrity (FIBI) (Wilton 2004).

Table 1. Sampling data utilized in the analysis of nutrient stressor-biological response relationships.

Project	Sites / spatial coverage	Sampling frequency / date range	Data Variables
Wadeable Reference	96 / statewide	Single grab during bio-sampling; July – Oct.	<u>Nutrients</u>: NHx, NOx, TKN, TN (calculated), DOP, TP <u>Nutrient Response</u>: CHLA (p, s, w) [limited coverage], TSS, TVSS, Turbidity <u>Aquatic Life:</u> Benthic macroinvertebrate, fish
REMAP / Random Survey	228 / statewide	Occasional grab during bio-sampling & data logger deployment; June – Oct.	<u>Nutrients</u>: NHx, NOx, TKN, TN (calculated), DOP, TP <u>Nutrient Response</u>: CHLA (p, s, w); CR, GPP, NPP, PR, diel DO (min, max, range); pH, TSS, TVSS, Turbidity <u>Aquatic Life</u>: Benthic macroinvertebrate, fish
Ambient Monitoring	85 / statewide	Monthly, year-round	<u>Nutrients</u>: NHx, NOX, TKN, TN (calculated), DOP, TP <u>Nutrient Response</u>: CHLA (w), pH, TSS, TVSS, Turbidity <u>Aquatic Life</u>: Benthic macroinvertebrate, fish
Impaired Waters (Stressor Identification)	26 sites, 14 streams / targeted watersheds	Biweekly or monthly; various date range, mostly ice-free months	<u>Nutrients</u>: NHx, NOx, TKN, TN (calculated), DOP, TP <u>Nutrient Response</u>: CHLA (p, s, w); CR, GPP, NPP, PR, diel DO (min, max, range); pH, TSS, TVSS, Turbidity <u>Aquatic Life</u>: Benthic macroinvertebrate, fish

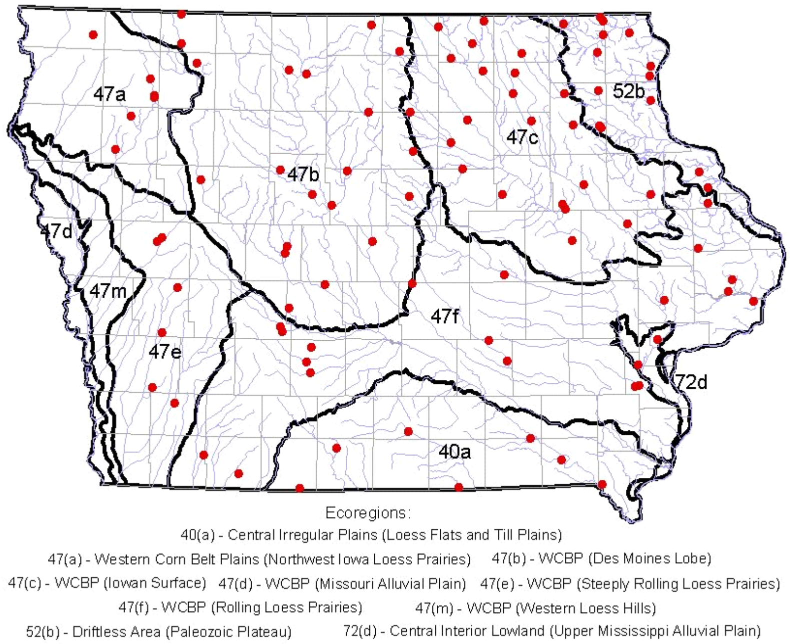


Figure 2. Wadeable stream reference sites (Wilton 2004) and ecological regions of Iowa (Chapman et al. 2004).

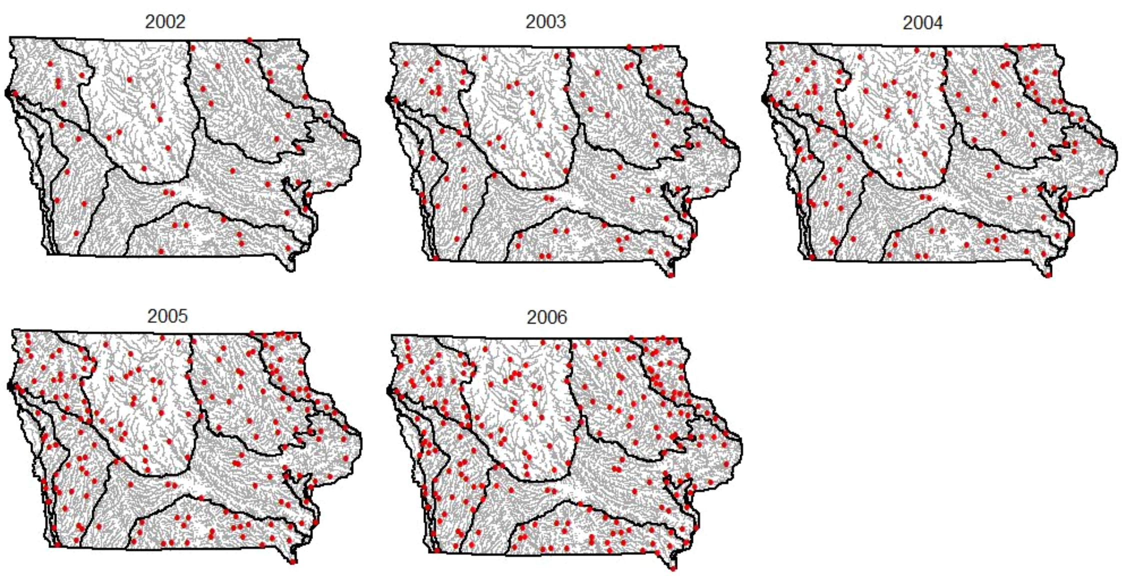


Figure 3. Annual cumulative distribution of REMAP sampling sites in relation to Level IV Ecoregion boundaries. The statewide sample size goal of 228 sites was reached in 2006. The targeted resource was perennial rivers and streams excluding large river impoundments and border rivers. Sample sites were drawn from a DNR-developed stream network derived from the statewide 1:100,000 U.S. Geological Survey map.

Ambient and upstream/downstream city water quality monitoring

The ambient stream monitoring project has a long history of providing data to evaluate water quality status and trends. The project dates back to the late 1970's and early 1980's when monthly monitoring was conducted at sixteen targeted locations. The initial locations were often strategically selected to monitor water quality downstream from several of Iowa's largest municipalities and industries. The monitoring network was redesigned in 1986 to provide broader geographic representation in medium sized and large streams throughout the state. The current enhanced project design was initiated in the fall of 1999. The enhancements included increasing sampling frequency from quarterly to monthly at 75-85 locations (Figure 4), increasing the number and types of sampling parameters, and initiating a upstream/downstream monitoring project for ten of Iowa largest municipalities.

The main usefulness of the ambient stream and city monitoring projects comes from the long-term, monthly monitoring of nutrient and other water quality parameters at fixed stream monitoring locations throughout the state.

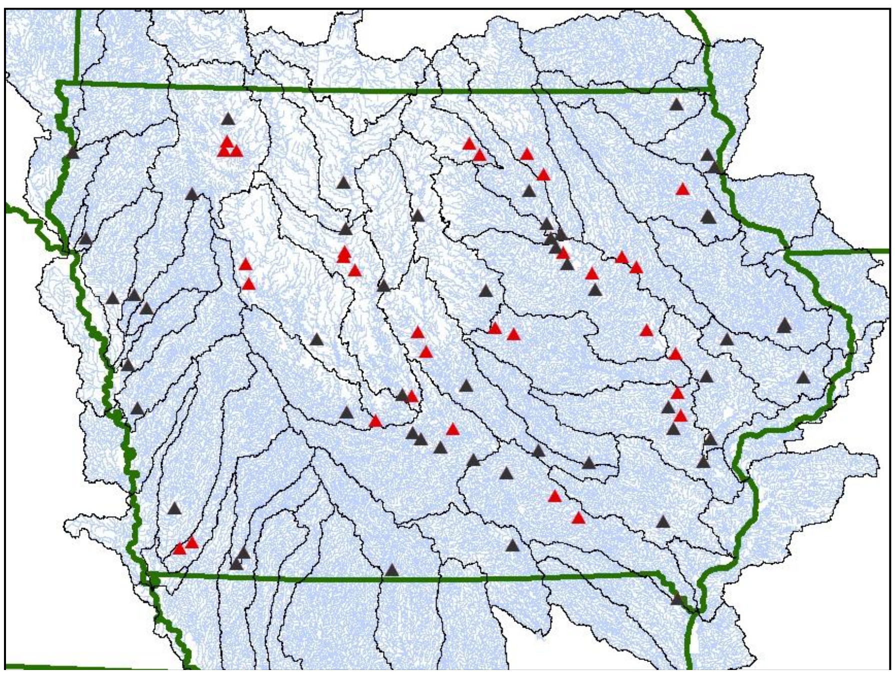


Figure 4. Locations of 85 ambient (black) and upstream/downstream city (red) stream monitoring sites, HUC8 basins and stream (NHD) network.

Unfortunately, the amount of biological sampling data from fixed monitoring sites is fairly limited. Sixteen of the sites were sampled for benthic macroinvertebrates in conjunction with early fall monthly water quality sampling visits in 2000-2002. The sites are located on medium-to-large streams and mostly exceed the size range covered by the wadeable reference site network. These biological data are useful because they have long-term water quality associated with them, and they supplement the limited sampling data from streams of similar size collected for the REMAP project.

Additional biological sampling was conducted in 2011 to provide more data to examine nutrient and biological response relationships in Iowa's large wadeable streams and nonwadeable rivers. Thirty sites representing a reasonably broad size and geographic distribution were selected for phytoplankton assemblage composition sampling and benthic macroinvertebrate assemblage sampling. The watershed areas of selected sites ranged from 408 mi^2 (Soldier River near Pisgah) to 13,412 mi^2 (Des Moines River near Ottumwa). Biological sampling was done on the same day or close to the same day as the usual monthly water quality sampling visits. All thirty sites were sampled for phytoplankton and benthic macroinvertebrates during September, and fifteen of the sites were also sampled in July for phytoplankton composition for intra-seasonal comparison purposes. Phytoplankton samples were processed and analyzed following the established SHL protocols developed for analysis of phytoplankton samples collected in the Iowa ambient lake monitoring project. Benthic macroinvertebrate samples were collected and analyzed using the established DNR/SHL protocols followed in the REMAP and wadeable reference site sampling projects.

Impaired streams monitoring

A subset of the monitoring projects conducted in impaired streams was selected for inclusion in the nutrient analysis project. Nutrient and biological response data from these streams provide a useful comparison with data collected from least disturbed reference sites and random sampling sites. The Iowa DNR initiated monitoring of impaired streams in 2001 to provide data for TMDL (Total Maximum Daily Load) development. Under Section 303(d) of the Federal Clean Water Act (CWA), the Iowa DNR must periodically identify and list impaired waterbodies that are not fully supporting the applicable designated uses (e.g., fishing/aquatic life, raw drinking water supply, recreation). In cases where a pollutant has been identified as contributing to the impairment, the CWA also requires that a TMDL be established to define the maximum acceptable pollutant load and level of reduction needed to achieve water quality standards when implementation of best available technology will not achieve compliance.

The focus and design of impaired stream monitoring projects varies depending on the type and cause of impairment. For example, monitoring to support development of a TMDL to achieve recreational uses impaired by bacteria will take a different approach than monitoring to support a TMDL for an ammonia impairment of aquatic life uses. Sampling of biological assemblages (benthic macroinvertebrates, fish, mussels) often results in the listing of streams as impaired for aquatic life uses due to unknown causes. Experience has shown that the causes of biological impairment are often complex and involve the degradation of both stream physical habitat and water quality. IDNR has employed the Stressor identification (SI) process (U.S. EPA 2000) in such cases to identify the specific causes of impairment, including any pollutants contributing to the impairment for which TMDL development will be required.

Stream monitoring projects designed to support the SI process have been included in the nutrient data analysis project. Monitoring for SI purposes has been conducted exclusively in small, wadeable stream watersheds that fall within the size range encompassed by the wadeable stream network. Data from 26 sampling sites located on 14 impaired stream segments were summarized for the nutrient analysis project. Stream nutrient and water quality parameters were sampled with frequencies ranging from monthly to weekly. Many of the streams received continuous monitoring of dissolved oxygen and temperature using the same methods employed in the REMAP project. Benthic macroinvertebrate and fish sampling as well as stream physical habitat evaluation were conducted following IDNR bioassessment standard operating procedures.

2.2. Sample collection and analysis procedures

Sampling procedures have changed relatively little since the IDNR stream bioassessment project was initiated in 1994. This consistency makes it possible to compare sampling data across projects, sites, and years. Sampling procedures are based on established protocols developed by the IDNR and the State Hygienic Laboratory of Iowa (SHL). Analysis of biological samples (benthic macroinvertebrates, fish, phytoplankton) were performed by SHL following established standard operating procedures. Water sample analyses were also performed by SHL following EPA-approved analytical methods. Analytical procedures, maximum holding times, sample preservation methods, and method detection limits for water analytes are specified in SHL laboratory standard operating procedures and IDNR monitoring project quality assurance plans.

Biological assemblages

Standardized sampling procedures are used to sample benthic macroinvertebrate and fish assemblages, thereby ensuring the data can be compared across sites and years. Sampling provides species presence/absence and proportional abundance data that are used to calculate the Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) and the Fish Index of Biotic Integrity (FIBI) (Wilton 2004). A coldwater benthic index (CBI) is also calculated to assess the condition of benthic macroinvertebrate assemblages in designated coldwater streams (SHL 2012).

Sampling area. Sampling of benthic macroinvertebrates and fish occurs within a designated length or reach of stream. The appropriate reach length is determined from criteria based on stream width and the occurrence frequency of channel features, such as riffles and channel bends. For wadeable streams, the sampling reach length should not be less than 150 meters nor greater than 350 meters.

Collection Period. Biological sampling is conducted during an index period generally lasting from July 15 through October 15. Sampling of small headwater streams is permitted in June during base stream flow conditions. Biological sampling is permitted when stream flow levels are close to base flow and the water is sufficiently clear to allow effective sampling of aquatic organisms.

To ensure sampling representativeness, the following restrictions apply:

- Sampling is prohibited during elevated stream flow or turbidity levels that reduce effectiveness;
- Sampling is prohibited during periods of extremely low flow which are stressful to stream biota;
- Sampling is prohibited within one week of a storm runoff event that may result in minor, short-term disruption of the aquatic community;

- Sampling is prohibited for one year following a major flood event that results in substantial disruption of the aquatic community;
- Sampling is prohibited for one year following a major drought that significantly depletes the aquatic community.

Sampling crew. Sampling activities are supervised by a professional aquatic biologist possessing extensive knowledge of stream ecosystems and experience applying a range of field sampling techniques. Crew members (one or more in addition to the crew leader) are trained by a professional aquatic biologist, and have at a minimum general knowledge of streams and aquatic biology, and have demonstrated competency in stream benthic macroinvertebrate and fish sampling techniques.

Benthic macroinvertebrate assemblage. Depending on habitat characteristics, either a Hess sampling net, Surber net, or Hester-Dendy artificial substrate sampling device is used to collect triplicate semi-quantitative benthic macroinvertebrate samples. Semi-quantitative samples are used to calculate data metrics that are based on the proportional abundances of different types of benthic macroinvertebrates. A modified Hess net is used in streams where there is stable riffle habitat or shallow runs containing abundant rock substrates. The Surber sampling net is used in small streams having shallow riffles and smaller amounts of streamflow. In streams lacking stable riffle habitat, a Hester-Dendy artificial substrate device is used to collect semi-quantitative samples. The device consists of a series of eight 4” X 4” wood plates mounted on a metal rod that is inserted in the stream bottom. In large, nonwadeable rivers Hester-Dendy artificial substrates are suspended underneath a float and anchored to the stream bottom. Artificial substrates are deployed for a colonization period of 4-6 weeks prior to retrieval.

A qualitative multi-habitat sample is also collected at each site to provide a more complete representation of the diversity of benthic macroinvertebrates inhabiting the sampling reach. To collect a multi-habitat sample, the designated reach is subdivided into three areas: upper, middle, and lower. A crew member is assigned to each area and macroinvertebrates are collected by handpicking or sieving organisms from all accessible types of substrates in flowing and slack water environments. The most common types of benthic substrates found include: silt, sand, muck, rock, detritus, wood, root wad, and vegetation. Sampling effort is fixed at 90 minutes combined among crew members. The samples from each area are composited into a single sample representing the entire sampling reach.

Benthic macroinvertebrate samples are preserved in 10% formalin and transported to SHL, where they are rinsed and transferred into 85% ethanol solution for storage. A random subsample of 100 organisms is obtained from each of the triplicate semi-quantitative samples for identification. All organisms in the multi-habitat sample are retained for identification.

Macroinvertebrates are identified to the "lowest practical taxonomic level." The lowest practical level varies depending on several factors. In most cases, the analysis endpoint is genus or species. Certain organisms that are difficult or time-consuming to identify (e.g., Chironomidae) are identified to family level. Factors that determine the achievable taxonomic endpoint include: 1) organism life stage and morphological integrity; 2) current status of systematics and availability of taxonomic keys; 3) time/cost recommended to make an accurate determination. Organism identification and verification is conducted by trained biologists following established SHL standard operating procedures and laboratory quality assurance/control procedures. Experts from outside scientific institutions are routinely utilized to confirm or assist with taxonomic determinations.

Fish assemblage. Fish are sampled with direct current (DC) electrofishing gear. A single battery-powered, backpack shocker is used in small streams of average width less than 15 feet. In wide and shallow streams, two or three backpack shockers are operated side-by-side to gain adequate coverage. A tow-barge electrofishing unit is used to sample deeper wadeable streams that require more power output for effective sampling. The unit consists of a six-foot fiberglass tow-boat equipped with live well, generator, electrical control box, and a maximum of three retractable, reel-mounted electrodes. Block nets are set across the stream at the downstream and upstream sampling boundaries as needed to prevent large mobile fish, such as suckers (Catostomidae), from escaping the sampling area. Block nets are not required in streams with shallow riffles that serve as barriers to fish movement.

Fish sampling is accomplished in a single pass proceeding from downstream to upstream through the designated sampling reach. All accessible types of fish habitat such as pools, riffles, woody debris snags, and undercut banks are sampled methodically in an effort to obtain a representative sample. An effort is made to collect all stunned fish in 3/16" mesh-diameter landing nets. Netted fish are transferred to plastic buckets or a live well for examination. Fish are identified to species (if practical), counted, and examined for external physical abnormalities before being released to the stream. Juvenile fish less than 25 mm in total length are excluded from the sample. Fish that cannot be identified to species in the field are preserved in 10% formalin solution and brought to the laboratory for identification. Fish voucher specimens from each site are collected and catalogued. Experts in fish identification are periodically consulted for identification assistance and verification. A reference collection of Iowa stream fishes is maintained by SHL as a resource for the IDNR stream biological assessment project.

Non-wadeable sampling adaptations. Initially, the IDNR stream bioassessment project concentrated on developing sampling protocols for wadeable streams. In order to proceed with biological sampling at ambient fixed monitoring locations and REMAP survey sites located on medium and large rivers and streams, it was necessary to adapt the wadeable sampling procedures to nonwadeable sampling environments.

With respect to semi-quantitative benthic macroinvertebrate sampling, the Hess sampling net is used at sites where riffle habitat is accessible. The riffle must be comprised of stable rock substrates and not exceed the depth or current velocity limitations for effective use of the sampling device. In streams lacking riffle habitat, the same design of Hester-Dendy multi-plate artificial substrates used in wadeable streams is used in nonwadeable streams; however, instead of deploying the substrates mounted on a steel rod that is pushed into the stream bottom, the artificial substrates are mounted on a steel eye-bolt that is suspended underneath a float and anchored to the stream bottom using a concrete block.

Stream biological assessment sampling of fish assemblages in nonwadeable rivers is done using a boat mounted DC-electrofishing unit. The boat is equipped with two circular (hoop) style electrodes in the front, a large live well and a jet propelled outboard motor that improves access to shallow areas in the river. Two sampling runs of 500 meters each are conducted along the left and right sides of the channel. The boat proceeds from upstream to downstream maneuvering along the banks and out toward the center of the river. Sampling effort is also focused around important habitat features such as woody debris snags where fish tend to congregate. Where significant areas of shallow water that cannot be accessed from the boat occur, supplemental shocking using a backpack shocker is conducted up to a maximum of 20 minutes. Fish processing is carried out following the same procedures as wadeable streams.

Continuous dissolved oxygen and temperature

Continuous monitoring of dissolved oxygen (DO) and temperature was first implemented in 2002 with the onset of the REMAP probabilistic stream survey. The sampling procedures were adjusted after the first season and applied consistently for the remainder of the REMAP project ending in 2006. Application of the procedures has since been expanded to monitoring projects supporting stressor identification and nutrient criteria development.

Continuous monitoring of DO and temperature was accomplished by deploying a battery-powered, submersible sonde/data logger in the stream for a period of two weeks or longer. The instrument was attached by cable to a steel post that was driven into the stream bottom. The instrument was situated in an area where the flow was mixed and in a manner that reduced the risk of debris or sediment accumulation. The data logger was programmed to record DO and temperature readings at 10-minute or 15-minute intervals around-the-clock. The sonde was calibrated according to manufacturer specifications prior to installation. For validation purposes, independent readings of DO and temperature were taken using a calibrated hand-held meter at the time of deployment, retrieval, and during any routine maintenance visits.

The majority of REMAP site deployments lasted two weeks during the months of June through October. Monitoring plans in recent years have narrowed the deployment season to July through mid-September in order to capture the warmest conditions when stressful DO and temperature levels are most likely to occur. For greater resolution of DO and temperature variation within the biological index period, in several cases deployments have been lengthened to one month or ten weeks.

The REMAP project deployed instruments equipped with Clark-type membrane DO probes. Experience using these units in Iowa streams found they were prone to measurement accuracy problems often due to a build-up of sediment or organic matter on the membrane. Weekly maintenance visits were conducted to check the accuracy of DO and temperature readings, and if necessary, replace the instrument with a clean, calibrated unit. The Clark-type sondes have been replaced in recent years with optical (luminescent) DO sensors. Optical sensors are less prone to fouling and perform better than membrane-type DO sensors under low water current velocity conditions. Maintenance visits are scheduled at two week intervals between deployment and retrieval in order to check battery status and measurement accuracy.

The quality and completeness of continuous DO and temperature data were reviewed prior to analysis. The data were downloaded from the sonde/data logger into an Excel spreadsheet and graphed for visual examination. The data quality evaluation must be performed by a water quality professional who is familiar with stream dynamics. The presence of erratic, abrupt changes in DO or temperature between readings are usually, but not always an indicator that a sensor is not performing correctly. The data were reviewed for suspicious patterns and compared with the independent validation readings and comments recorded by field personnel during deployment visits. DO validation readings made with the hand-held meter during the deployment, maintenance, and retrieval visits were matched as closely as possible in time with sonde/logger readings. The difference in hand-held meter and sonde/logger readings should not exceed ± 0.6 mg/L. When sonde/readings did not meet this criterion, the data set was edited to remove the measurements recorded during the period in which suspect readings were recorded. Sometimes the entire data set was discarded, but typicallly a significant portion of the data set could be salvaged after careful examination.

Physical habitat

Measurements and observations of stream physical habitat characteristics are important sampling components of stream ecological assessments. Stream habitat data provide context for the interpretation of biological sampling data and evaluation of stressors that limit attainment of aquatic life use goals. In bioassessment work, stream habitat is usually evaluated at a local, stream reach scale; however, it is important to consider that habitat characteristics are also shaped by factors at the watershed and ecoregional scales.

IDNR bioassessment sampling projects typically incorporate quantitative or qualitative habitat evaluation procedures, depending on sampling objectives. Quantitative procedures are used when sampling objectives call for a complete biological assessments that will be used for a determination of stream aquatic life use support status. Qualitative habitat assessment is used in less rigorous bioassessments that are usually conducted for initial screening purposes or when biological index sampling is restricted to just the benthic macroinvertebrate assemblage.

The IDNR quantitative protocol (IDNR 2001b) was developed for wadeable streams. It consists of taking habitat measurements and observations from ten channel cross-section transects evenly spaced in the designated sampling reach. Habitat data parameters general types of parameters include: stream dimensions, bottom substrate composition, instream cover, channel bedform features, bank condition, and riparian land use and vegetation. For bioassessment purposes, the individual habitat measurements and observations are typically used to calculate various summary statistics that represent the entire sampling reach instead of using data collected at any particular point or area within the reach.

A similar but more intensive quantitative protocol (Kaufmann et al. 1998) developed for U.S. EPA's Environmental Assessment and Monitoring Program (EMAP) was also used at 135 random stream sites as part of EPA's Wadeable Streams Assessment (WSA) and IDNR's REMAP project from 2004-2006. A plethora of habitat summary statistics were calculated from this project. The habitat metrics were used extensively in research examining relationships between habitat characteristics and Iowa stream fish assemblages (Rowe et al. 1999a), and inter-relationships between fish, stream habitat, and landscape characteristics (Rowe et al. 1999b).

For qualitative habitat assessments, the IDNR stream bioassessment program uses the guidelines and form developed for the U.S. EPA's rapid biological assessment protocol (Barbour et al. 1999). The RBP habitat assessment can be completed in less than ten minutes. It involves filling out a habitat rating form after the completion of biological sampling. The form and RBP manual provide guidelines for assigning a score to each of ten individual assessment metrics that characterize instream habitat, channel morphological conditions, and stream bank and riparian conditions. The individual scores are summed to obtain an overall habitat condition score ranging from 0 (poor) – 185 (optimal). Because the protocol can be completed quickly, the form is also filled out at sites that receive quantitative habitat sampling.

Water quality

A grab water sample is normally collected in conjunction with biological sampling to provide a snapshot picture of water quality conditions that can be useful in the interpretation of biological analysis results. The data obtained from a single sample is certainly not sufficient to fully characterize water quality at a given site; however, the results are informative when viewed in the context of how the levels compare with reference sites or a random population of sites. Occasionally, levels of water quality parameters such as dissolved oxygen or ammonia exceed water quality standards criteria, which is an indicator of a potential underlying water quality problem.

Water samples for wadeable bioassessment sites are collected by SHL personnel in an area of well-mixed flow and adequate depth for submersing the sample containers. Samples are collected prior to any other activities that might disturb the stream. Water sampling at fixed ambient and city monitoring locations is also conducted by SHL personnel in accordance with standard operating procedures and the project quality assurance project plan. The procedures for sampling wadeable fixed monitoring sites are the same as the procedures for wadeable bioassessment sites. Large nonwadeable fixed monitoring sites are sampled by lowering a sample bucket from a bridge access point into a well-mixed portion of the river. Sample containers are then individually filled from the bucket.

The specific list of water quality analytes varies depending on project objectives. Dissolved oxygen, pH, and temperature are measured in-situ using calibrated meters. Stream flow is measured at a representative cross-section using a wading rod and current velocity meter according to SHL standard operating procedures. The analytical suite of parameters typically includes several nutrient and conventional water quality parameters, including: total ammonia, nitrate+nitrite-nitrogen, total Kjeldahl nitrogen, dissolved orthophosphate, total phosphorus, specific conductance, total dissolved solids, total suspended solids, and turbidity. Other types of parameters, such as trace metals and pesticides, may be included to address site-specific water quality concerns.

3. Data Analysis Methods

The data analysis approach used in this project generally followed the process and steps described in U.S. EPA guidance on using stressor-response relationships to derive numeric nutrient criteria (U.S. EPA 2010a).

3.1. Data preparation and summarization

Biological and water quality sampling data were carefully reviewed prior to data analysis. Biological results that had been flagged as being collected under poor sampling conditions (e.g., elevated flow and turbidity) were excluded for being potentially unrepresentative. Extreme outlier values in water quality parameters were investigated. A few data values were excluded because they lacked corroboration and plausibility. Analytical results reported as being less than the minimum quantification limit (i.e., non-detects) were assigned a value equal to 99.9% of the limit. Spreadsheet tables containing reviewed data were imported into Microsoft Access™ and linked using a common identifier so that data queries could be executed efficiently. Queried data were imported into Microsoft Excel™ and various statistical analysis software programs for summarization and analysis.

Graphs and statistical summaries were prepared in order to become more familiar with the underlying characteristics of nutrient and nutrient-response variables. This is an important step because it can reveal nutrient-related patterns and also inform decisions about how to partition the data for examination of stressor-response relationships. Data summarization and graphing were done using Microsoft Excel® and the Minitab 16® statistical software program (Minitab, Inc.). Because of its unbiased sample design, the REMAP data set was best suited for this purpose.

Statistical summaries were prepared using the average sample value from each site. Box plots and scatter plots of the data were prepared for visual examination. Tabular summaries of common distributional statistics were also prepared. The data were first summarized without categorization and then grouped and summarized by categories of stream size, ecoregion, and thermal regime. Patterns in nutrient variables and biological response variables that were associated with these categories were important to consider for nutrient criteria development.

Stream size categories are defined by Strahler stream order, a commonly used indicator of stream size and network development. A small stream with no tributaries flowing into it is considered a first order stream. When two first order streams flow together, they form a second order stream. Two second order streams flowing together form a third order stream, and so on. The REMAP sampling project included perennial streams with Strahler order ranging from 2-7. The REMAP site selection design incorporated three stream order groups: small (2), medium (3-4), and large (5-7).

Iowa's land surface is covered by portions of ten Level IV ecoregions (Figure 2) (Chapman et al. 2002). Prior to analysis of ecoregion effects, the proportion of each sample site's watershed that was located within the same ecoregion as the site location was determined. Sites in which more than 50% of the watershed fell within a different ecoregion were omitted from the analysis. This filtering step was believed necessary for a true representation of the ecoregion effect on nutrient conditions. Five sites located in three small ecoregions (47d, 47m, and 72d) were also omitted from the analysis because of inadequate sample size.

Of the 227 REMAP sites with nutrient data, 179 (79%) were retained for the ecoregion analysis. A disproportionately large proportion (41%) of the omitted sites were located on large order streams. This seems logical because these sites have larger watershed areas that are more likely to overlap ecoregions. Despite the filtering bias, the blend of sites included in the ecoregion effects analysis was reasonably diverse (i.e., 16% large, 53% medium, 31% small).

Iowa water quality standards (Iowa Administrative Code Ch. 567.61) contain aquatic life use classifications for warmwater and coldwater streams. Thermal characteristics of warmwater streams are not defined in Iowa's water quality standards except in terms of limits on temperature alterations from wastewater discharges. According to the IDNR's cold water stream use assessment protocol, the ambient water temperature of coldwater streams is not to exceed 75F anytime during three consecutive years.

The REMAP project obtained nutrient data from 212 designated warmwater stream sites and 15 designated coldwater stream sites. The data were assigned to three thermal categories for data analysis: cold (ecoregion 52b); warm (52b); warm (all ecoregions except 52b). Warmwater sites in the 52b (Paleozoic Plateau) ecoregion were assigned their own category separate from warmwater sites in other ecoregions because these streams typically receive significant groundwater inputs and many times their watersheds contain stream segments that are designated as coldwater. Also, three warmwater sites and one coldwater site located in ecoregion 52b were omitted from the analysis because more than 50% of their respective watersheds fell within another ecoregion. It was thought that ecoregion effects in these specific cases might potentially obscure patterns caused by thermal effects.

Boxplots showing nutrient data distributions by stream category were prepared for visual examination. The Kruskal-Wallis, nonparametric analysis of variance (AOV) test was used to evaluate for treatment effects (i.e., stream size, ecoregion, thermal regime) on nutrient parameters. Non parametric two sample t-tests and multiple mean testing were done to identify significantly different treatment groups (e.g. small streams vs. large streams). Primarily, nonparametric tests were used because numbers of samples were often small and uneven among treatment groups. Also, many of the variables displayed skewed (non-normal) data distributions.

3.2. Biological condition indicators and benchmarks

Quantification of biological condition expectations or goals is an important part of nutrient stressor-biological response analysis. Without this step, it can be difficult to determine how much of a stressor can be tolerated while still achieving biological goals. The IDNR has established a quantitative assessment framework that utilizes the Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI), Fish Index of Biotic Integrity (FIBI) and the Coldwater Benthic Index (CBI) as indicators of stream biological condition. Given their established use, these indexes also seemed appropriate for stressor-response analysis.

The BMIBI and FIBI were developed specifically for biological assessment of Iowa's wadeable, warmwater streams. The CBI is a specifically tailored version of the BMIBI for application in designated coldwater streams of Northeast Iowa. Each of the indexes is a composite of several individual metrics (Tables 2 and 3) that convey ecologically relevant information about the biological assemblage. The metrics are individually scored and the scores are aggregated to obtain a composite score ranging from 0-100. Qualitative rating categories (i.e., excellent, good, fair, poor) were developed to assist with interpretation of BMIBI and FIBI sampling results (Tables 4 and 5). Qualitative ranges for the CBI have yet to be developed.

Qualitative ranges are useful tools for communicating relative condition; however, quantitative benchmarks are preferred when conducting biological assessments. Since 2000, the IDNR has used the 25^{th} percentiles of reference site BMIBI and FIBI scores as thresholds for biological assessments and reporting on the support status of stream aquatic life uses under Section 305(b) and 303(d) of the Federal Clean Water Act. The biological assessment criteria (BIC) vary by ecoregion and within some ecoregions by habitat classification (IDNR 2013).

Reference 25^{th} and 75^{th} percentile scores of the BMIBI and FIBI and their component metrics were selected as benchmarks for stressor-response analysis. The percentile values were calculated from 1994-2008 reference site sampling data. Statewide benchmarks were chosen instead of ecoregion-based benchmarks because a statewide analysis was thought to be more practical. There were two main reasons behind this decision. Firstly, the amount of coupled nutrient and biological response data available for stressor-response analysis was not considered adequate to support an ecoregion-specific statistical analysis. Stressor-response analysis is best performed on large data sets. There is not a defined dataset size requirement; however, experience has shown that one-hundred or more stressor-response samples are better than something like a few dozen. Secondly, it was believed that a statewide data set would provide larger gradients in nutrient-biological response relationships from which it might be easier to discern patterns and thresholds.

As described later in this section, either the 25^{th} or the 75^{th} percentile value was used depending on the statistical approach. While the 25^{th} percentile has a history of being used to define the lowest acceptable biocondition level that is still meeting aquatic life use goals, the 75^{th} percentile is more representative of optimal conditions in which effects of stressors like nutrients are minimal.

Table 2. Data metrics (and abbreviations) of the Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) and the Fish Index of Biotic Integrity (FIBI).

BMIBI Metrics	**FIBI Metrics**
MH*-taxa richness (MHTTX)	# native fish species (NTVSP)
SH*-taxa richness (SHTTX)	# sucker species (SCKRSP)
MH-EPT richness (MHEPT)	# sensitive species (SNSTVSP)
SH-EPT richness (SHEPTX)	# benthic invertivore species (BINVSP)
MH-sensitive taxa (MHSNSTV)	% 3-dominant fish species (P3ABUND)
SH-% 3-dominant taxa (SH3DOM)	% benthic invertivores (PBINV)
SH-Mod. Hilsenhoff Biotic index (SHMHBI)	% omnivores (POMNV)
SH-% EPT (SHPEPT)	% top carnivores (PTOPC)
SH-% Chironomidae (SHCHR)	% simple lithophil spawners (PSLTH)
SH-% Ephemeroptera (SHEPHM)	fish assmblg. tolerance index (TOLINDX)
SH-% Scrapers (SHSCRPR)	adjusted catch per unit effort (ACPUE)
SH-% Dom. functional feeding grp, (SHDFFG)	% fish with DELTs (PDELT)

MH, Multi-habitat sample; SH, Standard-habitat sample.

Table 3. Data metrics (and abbreviations) of the Coldwater Benthic Macroinvertebrate Index (CBI) (SHL 2012)

Biological Metric/Index	Abbrv.
MH sensitive taxa richness	MHSNSTXS
MH CW taxa richness	MHCWTXS
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	MHTNOHS
MH % tolerant taxa	MHPTOLS
SH % Dominant Functional Feeding Group (FFG)	SHPDFFGS
SH % CW individuals	SHPCWS
SH % Hydropsyche individuals	SHPHYDS
SH % Hydropsychinae individuals	SHPHYNS
SH Benthic Tolerance Index (BTI)	BTIS

Table 4. Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) qualitative scoring ranges.

Biological Condition Rating	Characteristics of Benthic Macroinvertebrate Assemblage
76-100 (Excellent)	High numbers of taxa are present, including many sensitive species. EPT taxa are very diverse and dominate the benthic macroinvertebrate assemblage in terms of abundance. Habitat and trophic specialists, such as scraper organisms, are present in good numbers. All major functional feeding groups (ffg) are represented, and no particular ffg is excessively dominant. The assemblage is diverse and reasonably balanced with respect to the abundance of each taxon.
56-75 (Good)	Taxa richness is slightly reduced from optimum levels; however, good numbers of taxa are present, including several sensitive species. EPT taxa are fairly diverse and numerically dominate the assemblage. The most-sensitive taxa and some habitat specialists may be reduced in abundance or absent. The assemblage is reasonably balanced, with no taxon excessively dominant. One ffg, often collector-filterers or collector-gatherers, may be somewhat dominant over other ffgs.
31-55 (Fair)	Levels of total taxa richness and EPT taxa richness are noticeably reduced from optimum levels; sensitive species and habitat specialists are rare; EPT taxa still may be dominant in abundance; however, the most-sensitive EPT taxa have been replaced by more-tolerant EPT taxa. The assemblage is not balanced; just a few taxa contribute to the majority of organisms. Collector-filterers or collector-gatherers often comprise more than 50% of the assemblage; representation among other ffgs is low or absent.
0-30 (Poor)	Total taxa richness and EPT taxa richness are low. Sensitive species and habitat specialists are rare or absent. EPT taxa are no longer numerically dominant. A few tolerant organisms typically dominate the assemblage. Trophic structure is unbalanced; collector-filterers or collector-gatherers are often excessively dominant; usually some ffgs are not represented. Abundance of organisms is often low.

Table 5. Fish Index of Biotic Integrity (FIBI) qualitative scoring guidelines.

71-100 (Excellent)	Fish (excluding tolerant species) are fairly abundant or abundant. A high number of native species are present, including many long-lived, habitat specialist, and sensitive species. Sensitive fish species and species of intermediate pollution tolerance are numerically-dominant. The three most abundant fish species typically comprise 50% or less of the total number of fish. Top carnivores are usually present in appropriate numbers and multiple life stages. Habitat specialists, such as benthic invertivore and simple lithophilous spawning fish are present at near optimal levels. Fish condition is good; typically less than 1% of the total number of fish exhibit external anomalies associated with disease or stress.
51-70 (Good)	Fish (excluding tolerant species) are fairly abundant to very abundant. If high numbers are present, intermediately tolerant species or tolerant species are usually dominant. A moderately high number of fish species belonging to several families are present. The three most abundant fish species typically comprise two-thirds or less of the total number of fish. Several long-lived species and benthic invertivore species are present. One to several sensitive species are usually present. Top carnivore species are usually present in low numbers and often one or more life stages is missing. Species that require silt-free, rock substrate for spawning or feeding are present in low proportion to the total number of fish. Fish condition is good; typically less than 1% of the total number of fish exhibit external anomalies associated with disease or stress.
26-50 (Fair)	Fish abundance ranges from lower than average to very abundant. If fish are abundant, tolerant species are usually dominant. Native fish species usually equal ten or more species. The three most abundant species typically comprise two-thirds or more of the total number of fish. One or more sensitive species, long-lived fish species or benthic habitat specialists such as Catostomids (suckers) are present. Top carnivore species are often, but not always present in low abundance. Species that are able to utilize a wide range of food items including plant, animal and detrital matter are usually more common than specialized feeders, such as benthic invertivore fish. Species that require silt-free, rock substrate for spawning or feeding are typically rare or absent. Fish condition is usually good; however, elevated levels of fish exhibiting external anomalies associated with disease or stress are not unusual.
0-25 (Poor)	Fish abundance is usually lower than normal or, if fish are abundant, the assemblage is dominated by a few or less tolerant species. The number of native fish species present is low. Sensitive species and habitat specialists are absent or extremely rare. The fish assemblage is dominated by just a few ubiquitous species that are tolerant of wide-ranging water quality and habitat conditions. Pioneering species, introduced species, and short-lived fish species are typically the most abundant types of fish. Elevated levels of fish with external physical anomalies are more likely to occur.

3.3. Stressor-response analysis

Graphical and quantitative techniques were used to explore nutrient stressor-biological response relationships. Stressor-response scatter plots were prepared in MINITAB™ and examined as a means to become familiar with data relationship patterns. A quantitative analysis was subsequently performed using methods available from the CADStat analysis package developed for use in the R statistical software program (Version 2.70, © R Development Core Team 2008). The CADStat package was downloaded from the U.S. EPA's Causal Analysis/Diagnosis Decision Information System (CADDIS) web site: http://www.epa.gov/caddis/.

Three methods, conditional probability (CP), quantile regression (QR), and regression tree (RT), were used to evaluate the strength of stressor-response relationships and identify relationship changepoints or thresholds. These methods provided a statistically diverse and robust analysis of stressor-response relationships. Because stressor-response relationships do not always exhibit the same pattern, any one method is not likely to perform optimally in all situations (Brenden et al. 2008). For example, some relationships can be modeled as smooth linear functions while others display a non-linear, stair-step response pattern.

Conditional probability

Conditional probability (CP) is simply the probability of observing an event when another event has occurred. CP is a useful technique for identifying non-linear relationships between two variables because it requires no assumptions about data distribution (Paul and McDonald, 2005). With respect to stressor-response analysis, for example, CP could be used to calculate the proportion of samples achieving a biological condition goal when a stressor such as total ammonia exceeds a specified level.

CP was used in this analysis both as an exploratory tool to examine the strength of nutrient stressor-biological response relationships, and also as a means to identify stressor threshold levels at which meaningful changes in biological condition occurred.

CP analysis was performed in CADStat and a follow-up contingency analysis was performed in Excel and Minitab. CP results were graphed showing the stressor variable on the horizontal axis and the response variable on the vertical axis (Figure 5a). Results are shown as a continuous function that can be viewed in "forward" or "reverse" directions through the stressor-response plot. The forward view displays the proportion of samples in which the biological response level equals or exceeds the specified goal (P_r) when stressor levels are greater than or equal to a given level along the stressor gradient axis. Conversely, the reverse view displays P_r when stressor levels are less than or equal to a given level along the axis.

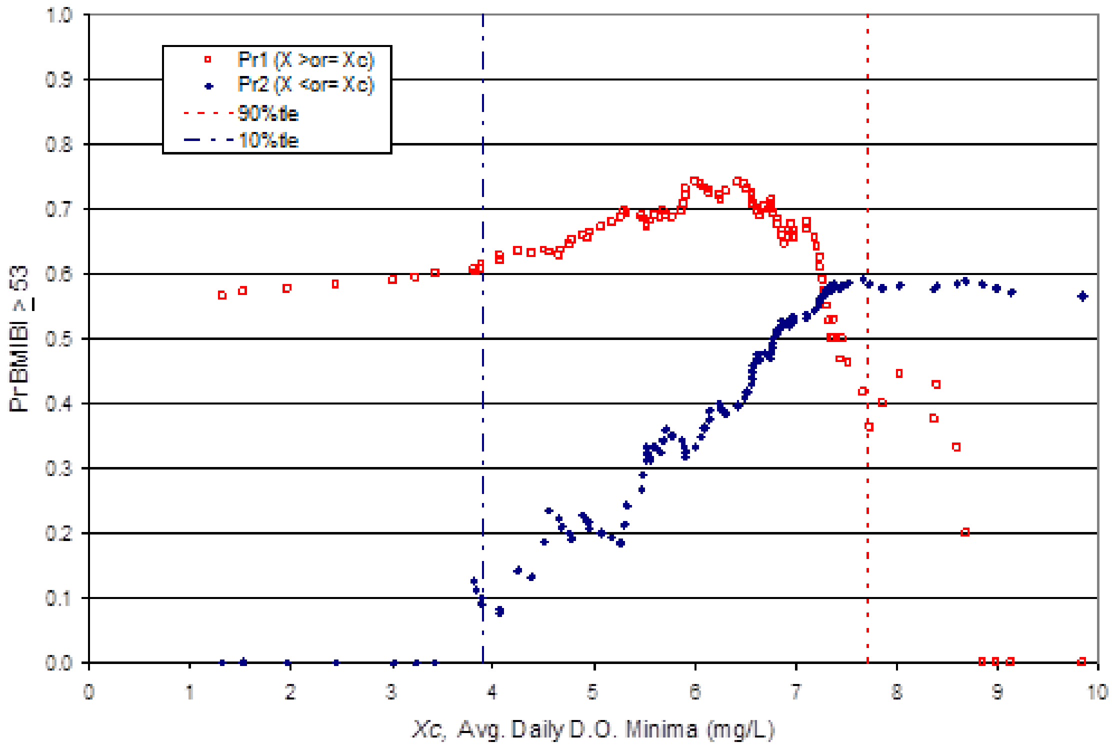


Figure 5(a) Conditional probability plot showing the proportion (*Pr*) of Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) samples equaling or exceeding the wadeable reference 25th percentile BMIBI score (53) at varying levels (*Xc*) of the stressor, Avg. Diel DO Minima. Red, open-square symbols represent the proportion of samples (Pr_1) equaling or exceeding the reference benchmark (BMIBI=53) when stressor X $\geq$ Xc (forward view); blue, filled diamond symbols represent the proportion of samples (Pr_2) equaling or exceeding the reference benchmark (BMIBI=53) when stressor X $\leq$ Xc (reverse view). Estimates of *Pr* based on relatively few samples (<10%tle reverse or >90%tle, forward) are considered unreliable.

The rate of change in P_r (i.e., proportion of samples achieving the biological response goal) can be large and behave erratically near the lower and upper ends of the stressor axis. A cautious approach is needed when interpreting the tail ends of the CP plots because the results are greatly influenced by a small number of samples. In this project, values of P_r corresponding with stressor levels below the 10th percentile or above the 90th percentile were disregarded.

Stressor-response CP analysis plots were examined in forward and reverse directions. The pattern of change and relative levels of P_r were noted as the approximate location of visually evident changepoints or thresholds. In general, a decrease of more than 15% in the proportion of samples achieving the biological response goal was considered potentially meaningful.

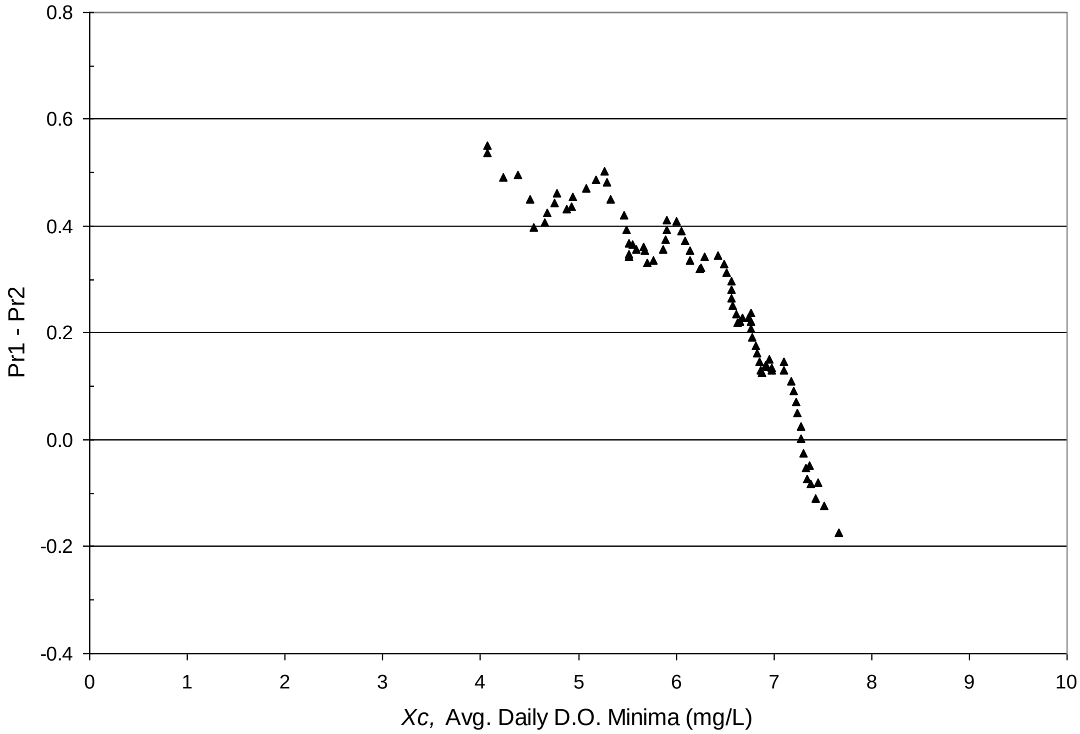


Figure 5(b). Plot showing difference in proportion of samples ($Pr_1 – Pr_2$) equaling or exceeding the reference benchmark (BMIBI=53). The plot shows the maximum difference in ($Pr_1 – Pr_2$) occurs at approximately 4 mg/L, Avg. Diel DO Minima. The percentage of BMIBI samples achieving the reference BMIBI benchmark is approximately 55% greater among stream sites having Avg. Diel DO Minima $\geq$ 4 mg/L compared with sites where Avg. Diel DO Minima < 4 mg/L. Difference estimates of *Pr* calculated for data percentiles <10% or >90% were excluded due to low numbers of samples.

After examining the CP plots, a contingency analysis was conducted for the purpose of more precisely identifying the point along the stressor axis where the rate of change in P_r was maximum. This point was considered to be a potentially meaningful changepoint or threshold in the stressor-response relationship. First, the stressor data were sorted from lowest to highest level. Then running totals of P_r were calculated in the forward and reverse directions described above: 1) stressor level greater than or equal to a given level (forward); 2) stressor level less than or equal to a given level (reverse). Next, the difference in the proportions of cases attaining the biological response goal P_r l was obtained by simple subtraction at each level of the stressor. The stressor level at which the difference was the largest (either positively or negatively) was interpreted as the most prominent changepoint. Figure 5b shows the contingency analysis output using the data displayed in Figure 5a.

The last step in the CP analysis was to conduct a Chi-square distribution test to determine whether the stressor changepoint is associated with a statistically significant change in the proportion of samples achieving the biological response goal (P_r) stressor threshold. The test considers the probability that (P_r) is the same for the forward and reverse groups.

A test result p-value $\leq$ 0.05 was interpreted as sufficient to reject the null hypothesis that the two groups had equal proportions of cases attaining the biological response goal, and therefore, the stressor changepoint/threshold was considered significant.

Quantile regression

Quantile regression (QR) allows quantiles (e.g., 95%, 75%, 50%) of a response variable to be estimated in a linear model (Cade and Noon 2003). Variance is often unevenly distributed in ecological relationships involving a predictor and a response variable. Instead of data being evenly distributed around a mean response linear regression fitted line (Figure 6a), wedge-shaped relationship patterns (Figure 6b) are often observed. In ecological studies, this pattern is often interpreted as representing the combined effects of more than one environmental factor on a response variable (Cade and Noon 2003; Brenden et al. 2008).

The upper, outer edge of the data can be interpreted as approximating the limiting effect of a stressor variable over the response variable. Data points that fall below the upper edge of the data distribution are presumed to be affected to a greater or lesser degree by interactions with one or more additional stressors. These additional stressors cannot be accounted for in a simple bivariate stressor-response model. Potentially important relationships between environmental variables and stream biological response variables can be obscured when the mean response is modeled using a conventional least square regression approach. By providing the ability to model the outside edge of the bivariate data grouping, QR offers a more powerful tool for performing limiting factor analysis, for which the objective is to determine whether or not a stressor is likely to exert a limiting influence over the biological response variable.

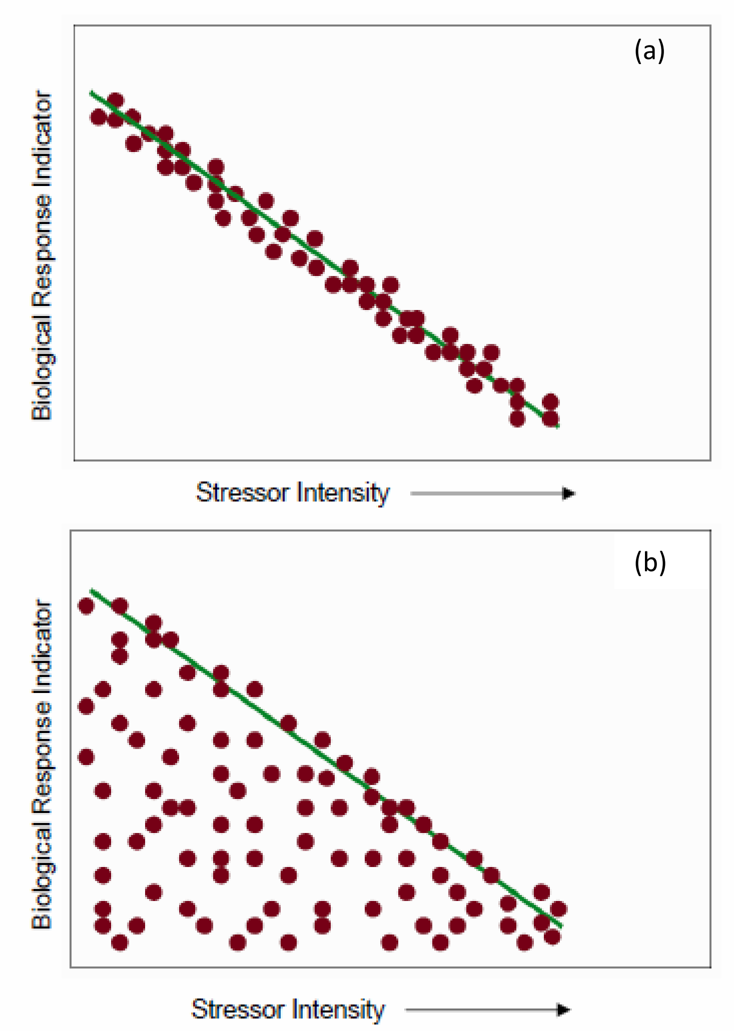


Figure 6(a-b). Two conceptual relationships between a stressor variable and a biological response variable (after Cade and Noon, 2003); (a) As the stressor variable increases in intensity, the biological response indicator decreases. Variation around the least square mean regression line is relatively minor and remains constant from low to high stressor levels. The stressor variable appears to explain most of the variation in the biological response indicator. Other stressors not included in the regression model would seem to exert very little influence over the biological response indicator. (b) A wedge-shaped pattern in the relationship between the stressor and biological response variables is observed. The hypothesized limiting influence of the stressor variable is most clearly seen along the upper edge of the plot as approximated by the 90% quantile regression line. Variation in the relationship is not constant from low to high stressor levels, thus suggesting that one or more additional stressors not included in the regression model often substantially impact the biological response variable.

QR computes a linear relationship between a predictor variable and response variable at specified percentiles of the response variable. For the nutrient response analysis, QR was primarily used as a tool to examine the likelihood that a nutrient stressor variable exerts a limiting effect over a biological response variable. In the example shown in Figure 7, the stressor variable is average seston chlorophyll A and the biological response variable is the standard habitat sample percent abundance of top three dominant benthic macroinvertebrate taxa. The analysis is focused on the upper quartile of the response variable data because this area is most likely to reflect the limiting influence of the stressor variable. A series of regressions was initially done at 5% increments (i.e., 95%, 90%, 85%, 80%, 75%) in order to evaluate the general behavior of the regression models and the amount of variability associated with predicted values for slope coefficient and y-intercept (e.g., Figure 7).

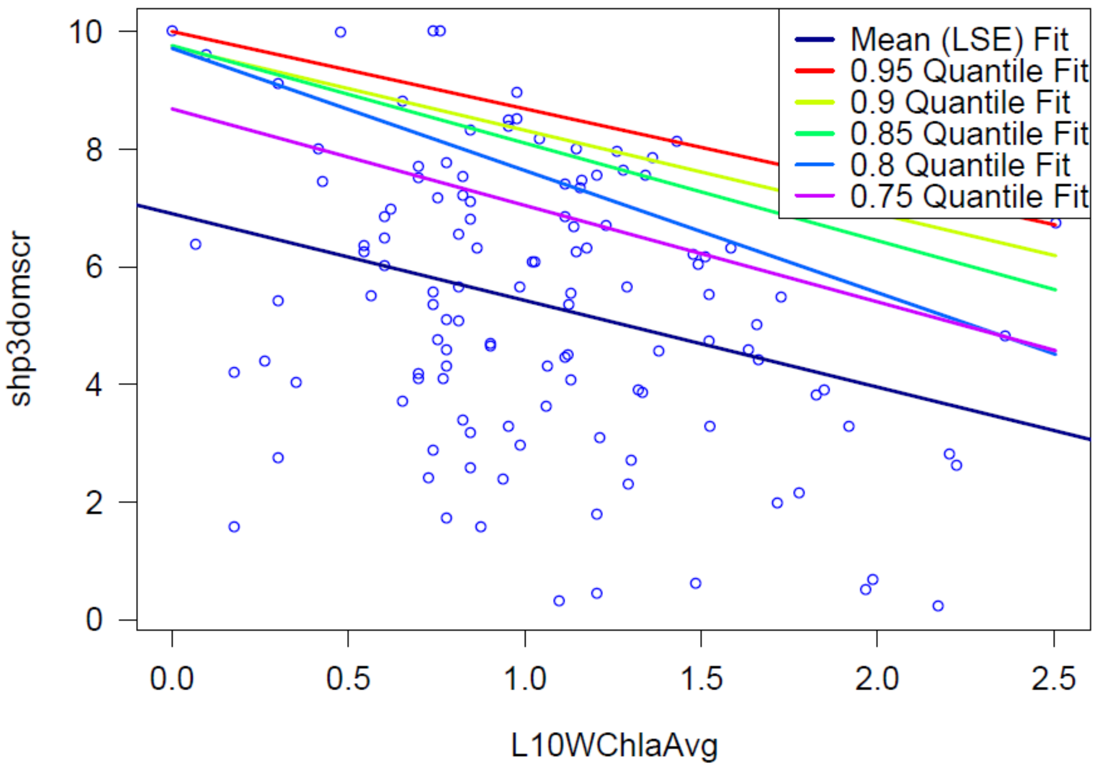


Figure 7. P95, P90, P85, P80, P75 Quantile regression fits of Log10 site-average seston chlorophyll A (L10WchlaAvg) versus standard-habitat metric score for percent abundance of top-3 dominant benthic macroinvertebrate taxa.

Based on this evaluation, the 90th percentile was chosen for limiting factor analysis. This level seemed to represent a good balance between modeling the outer edge of the data (i.e., stressor-limited) while still incorporating enough data so that the regression was not unduly influenced by a small number of outlier values.

The 95% confidence interval (CI) bracketing the QR slope coefficient were reported by the CADStat program. A regression model was considered significant when the 95% CI did not encompass zero, which would indicate a lack of linear relationship between stressor and response variables. Significant regression models were then used to calculate stressor threshold for achieving a specified biological response goal. The biological response goal was set equal to the reference 75th percentile score for the biological index or data metric of interest. QR is well suited for modeling the maximum potential of a biological response at a given stressor level. The reference 75th percentile was therefore chosen because it represents a high performing level of biological condition and a relatively small departure from optimum levels in reference condition.

Regression tree

Classification and regression tree analysis is used to obtain predictions or classifications of data cases by applying a series of if-then logical conditions to (independent) predictor variables in relationship with a (dependent) response variable (De'ath and Fabricius, 2000). The analysis can be performed with categorical (classification tree) or continuous (regression tree) types of predictor variables. Regression tree (RT) is referred to as a nonparametric deviance reduction technique that is used in disturbance threshold analysis. The threshold or changepoint is identified computationally as the stressor level where the largest variance reduction in the response variable occurs (Brenden et al. 2008).

Output from RT can be displayed in the form of a tree diagram (e.g., Figure 8) comprised of branches (splits) and leaves (resulting groupings of data cases) produced from a series of if-then logical conditions. The RT algorithm creates successive splits in the data set based upon decision rules. These are applied to each predictor variable in the model to maximize the reduction in variance or homogeneity of the dependent response variable within the resulting leaves or classes.

In this project, RT analysis was performed on each individual combination of nutrient stressor and biological response variable. The sensitivity of RT analysis can be adjusted to produce fewer or more splits. After performing some trial runs, the settings were adjusted to produce fewer splits. This was thought to be the best approach since the analysis was conducted using only one predictor variable at a time. The first (primary) split was noted as the relationship changepoint since it always represented the largest reduction in variance of the biological response variable.

A statistical test was then performed to evaluate the statistical significance of each changepoint. A two-sample t-test was performed to determine whether the mean values of the biological response variable representing each of the two data groupings (leaves) formed by the split were significantly different from each other. The specified significance level was $\alpha = 0.05$. A nonparametric t-test method (rank sum) was used in cases where the assumption of equal data variance among the two groups was not valid.

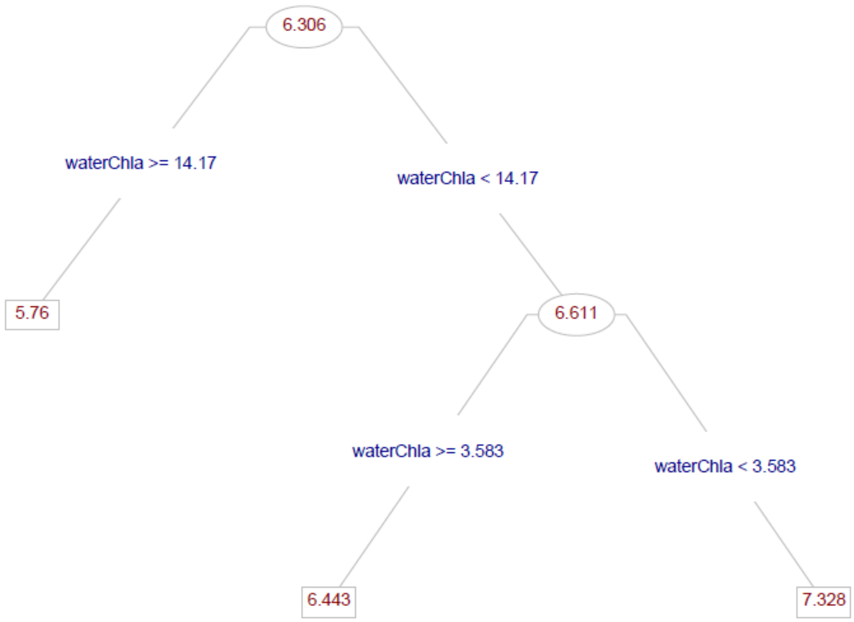


Figure 8. Regression Tree (RT) analysis of average seston chlorophyll A (waterChla) versus standard-habitat total taxa richness metric score (shttxscr). Minimum data required to split = 25% of samples; minimum bucket size = 10%; complexity parameter (cp) = 0.02. The first or primary split resulted in one data group with mean shttxscr = 5.76 and a second group with shttxscr mean score = 6.61 for sites having wtrChla $\geq$ 14.2 or < 14.2 ug/L, respectively. A secondary split was found within the group consisting of sites having wtrChla < 14.2, resulting in group means of 6.4 and 7.3 shttxscr for sites having waterChla $\geq$ 3.6 or < 3.6 ug/L, respectively.

4. Nutrients and Nutrient-Response Analysis

4.1. Nutrient analytes and calculated parameters

Nutrient sampling analytes and calculated parameters sampled in the 2002-2006 REMAP stream survey project are listed in Table 6. The sampling results are summarized in Tables 7 and 8, and shown in boxplot form in Figures 9(a-m).

Nutrient analytical results were highly variable among this statewide group of random sites. The minimum and maximum values of the five nutrient analytes varied by more than two orders of magnitude. Statistical distributions of these variables were positively skewed (i.e., mean > median) by large outlier values. Positive data skew was strong in total ammonia (NHx), dissolved orthophosphate (DOP), and total phosphorus (TP), and was less pronounced in nitrate+nitrite nitrogen (NOx) and total Kjeldahl nitrogen (TKN). The analytes each have some truncation in the lower range of their distribution caused by sample values reported as below the detection limit.

Table 6. REMAP probabilistic stream survey (2002-2006) nutrient analytes and calculated nutrient parameters.

ABBRV.	*Parameter*	*Analyte / Calculated*	*Description*
NHx	*Total Ammonia Nitrogen*	*Analyte*	*Ammonium + unionized ammonia (mg/L as N)*
NOx	*Nitrate+Nitrite Nitrogen*	*Analyte*	*Nitrate + Nitrite (mg/L as N)*
TKN	*Total Kjeldahl Nitrogen*	*Analyte*	*Ammonia + organic nitrogen (mg/L as N)*
DOP	*Dissolved Ortho- phosphate*	*Analyte*	*Dissolved Orthophosphate (mg/L as P)*
TP	*Total Phosphorus*	*Analyte*	*Total Phosphate (mg/L as P)*
DIN	*Dissolved Inorganic Nitrogen*	*Calculated*	*AMMN + NITR (mg/L as N)*
DIN:TN	*Dissolved Inorganic N:Total N ratio*	*Calculated*	*DIN / TN (unitless)*
ORGN	*Organic N*	*Calculated*	*TN – DIN (mg/L as N)*
TN	*Total Nitrogen*	*Calculated*	*NITR + TKN (mg/L as N)*
DOP:TP	*Dissolved Ortho P:Total P ratio*	*Calculated*	*DOP / TP (unitless)*
PRTCP	*Particulate P*	*Calculated*	*TP – DOP (mg/L as P)*
TN:TP	*Total N:Total P ratio*	*Calculated*	*TN / TP (unitless)*

Table 7. Summary statistics for REMAP (2002-2006) nutrient analytes.

Parameter	Total Ammonia as N	Nitrate + Nitrite as N	Total Kjeldahl N	Dissolved Ortho-phosphate as P	Total Phosphate as P
ABBRV.	NHx	NOx	TKN	DOP	TP
Units	mg/L	mg/L	mg/L	mg/L	mg/L
N	646	647	647	647	647
Min	<0.05	<0.05	<0.05	<0.02	<0.02
Q25	<0.05	1.50	0.45	0.04	0.09
Median	<0.05	4.90	0.70	0.07	0.16
Q75	<0.05	8.20	1.20	0.12	0.26
Max	12.0	26.00	20.00	20.00	30.00
Mean	0.15	5.78	1.05	0.172	0.313
Std.Dev.	0.70	5.07	1.44	1.116	1.554

Table 8. Summary statistics for REMAP (2002-2006) calculated nutrient parameters.

Parameter	Dissolved Inorganic Nitrogen	Dissolved Inorganic N:Total N ratio	Organic N	Total Nitrogen	Dissolved Ortho P:Total P ratio	Particulate P	Total N:Total P ratio
ABBRV.	DIN	DIN:TN	ORGN	TN	DOP:TP	PRTCP	TN:TP
Units	mg/L	-	mg/L	mg/L	-	mg/L	-
N	646	646	646	647	647	647	647
Min	<0.1	0.03	0.01	<0.25	0.02	0.01	0.40
Q25	1.68	0.63	0.40	2.90	0.34	0.03	12.16
Median	5.05	0.89	0.63	5.90	0.51	0.07	33.87
Q75	8.45	0.95	1.05	9.40	0.73	0.15	73.00
Max	26.05	1.00	10.79	26.53	1.00	10.00	773.33
Mean	5.94	0.76	0.89	6.82	0.54	0.14	58.14
Std.Dev.	5.05	0.27	0.96	5.00	0.25	0.49	76.35

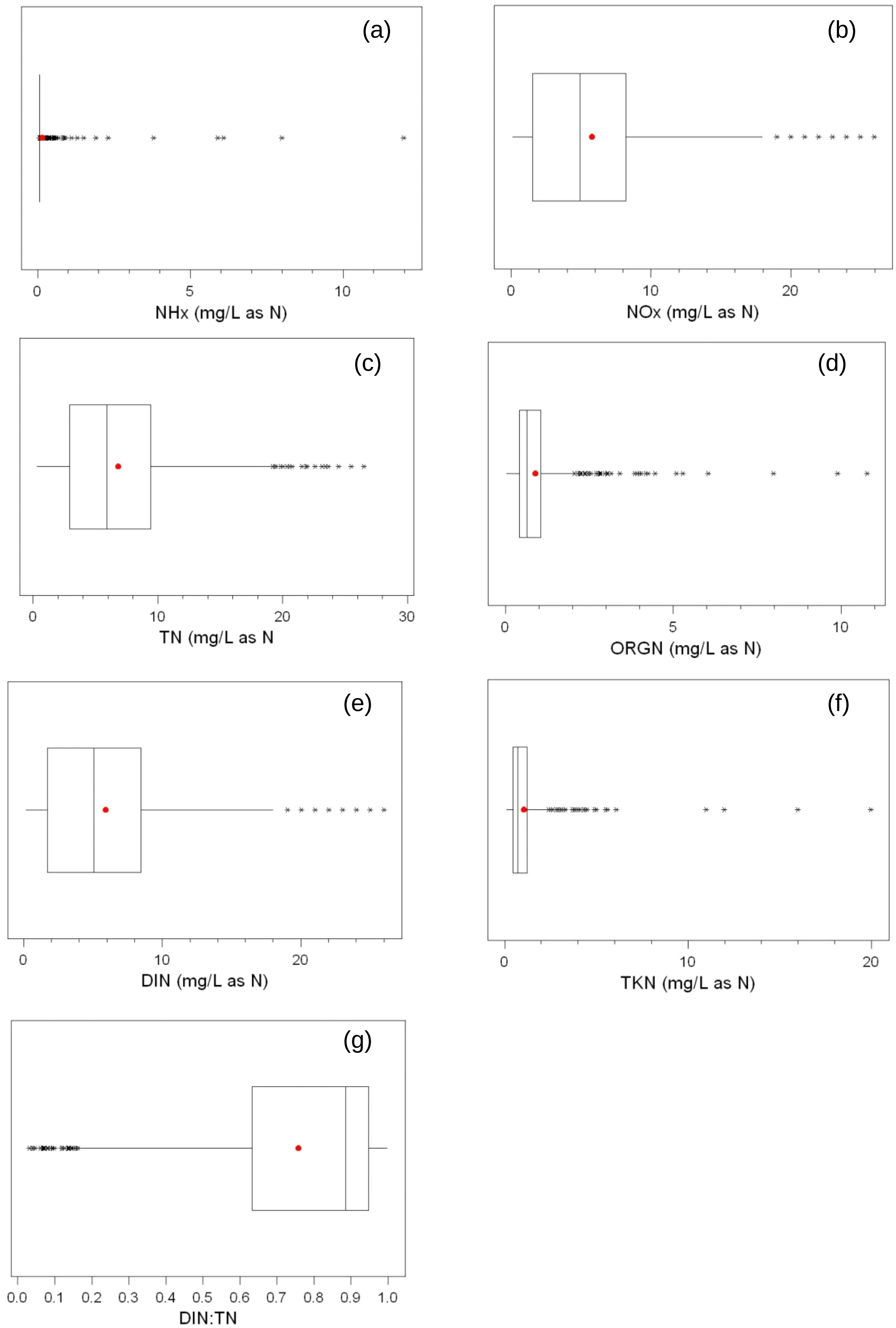


Figure 9(a-g). Boxplots showing the statistical characteristics of REMAP (2002-2006) nutrient analytes and calculated parameters.

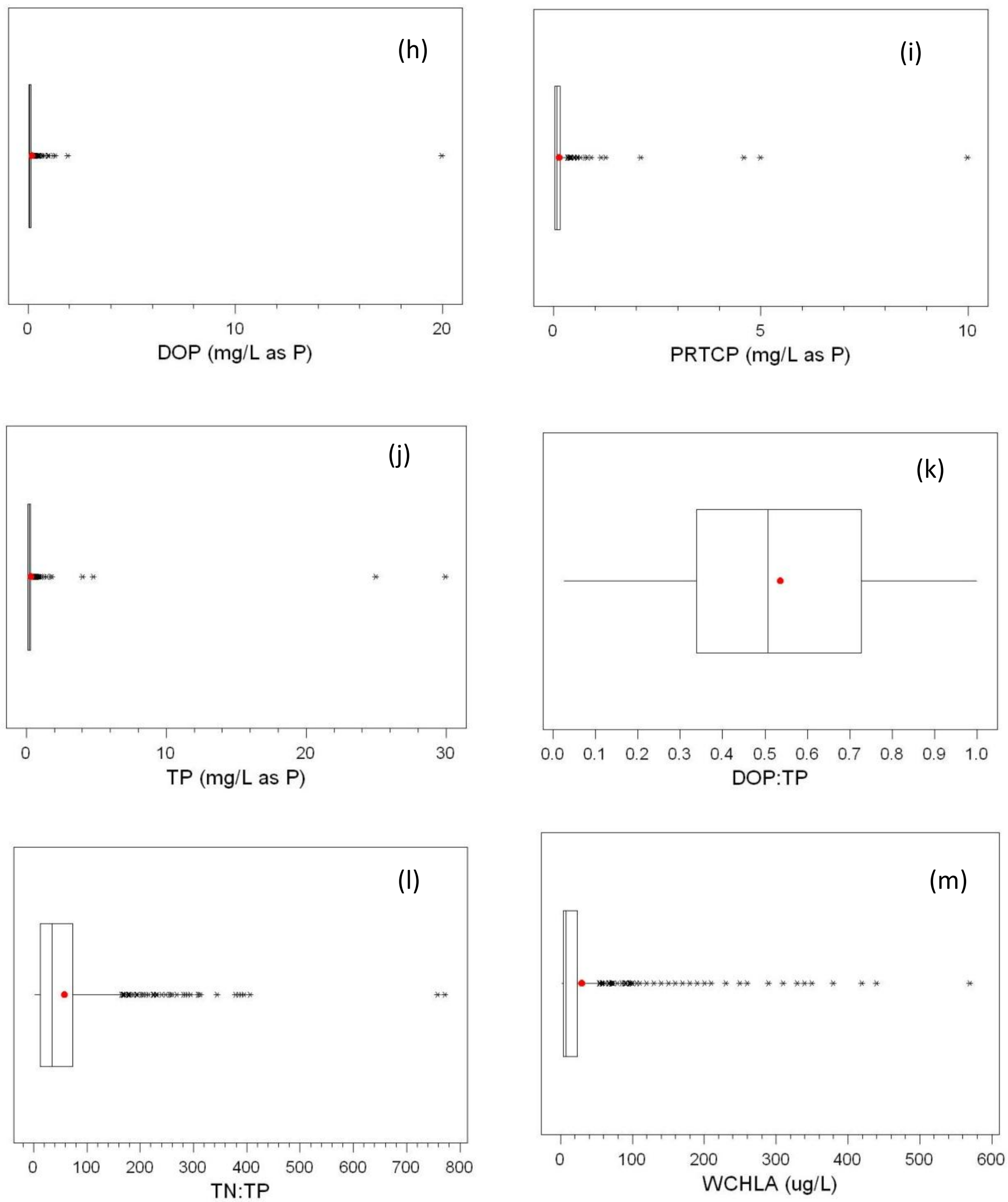


Figure 9(h-m). Boxplots showing the statistical characteristics of REMAP (2002-2006) nutrient analytes and calculated parameters.

Relationships between nutrient variables and categorical variables of stream size, ecological region (ecoregion), and thermal regime were examined to help determine the types of stream classifications that might be important for nutrient criteria development purposes. Boxplots showing nutrient data distributions by stream category were prepared for visual examination. The Krusgal-Wallis, nonparametric analysis of variance (AOV) test was used to evaluate treatment effects (e.g., ecoregion) on nutrient parameters. Two sample t-test and multiple mean testing were done to identify significantly different treatments (stream categories). Statistical tests were performed using the average sample value from each site.

Stream size

Relationships between nutrient variables and two indicators of stream size, watershed area and Strahler stream order, were evaluated. Pearson and Spearman rank (nonparametric) correlation tests were performed to examine the strength of linear relationships between nutrient variables and watershed area (Table 9). The Pearson correlation was also performed using Log10 transformed data to compensate for the non-normal data distributions observed in most sampling parameters. Linear relationships between several nutrient variables and watershed size determined by Pearson correlation were statistically significant, but relatively weak. Correlation coefficients for the Log10 transformed data, which were generally larger than coefficients from untransformed data, ranged from -0.363 (DOP:TP) to 0.263 (ORGN).

Table 9. Pearson and Spearman rank correlation coefficients for (Log10) stream watershed area versus nutrient variables from REMAP (2002-2006) sampling.

Stream Watershed Drainage Area

Pearson		Pearson (Log10)		Spearman Rank	
Parameter	r	Parameter (Log10)	r	Parameter	rho
DOPTP	-0.259**	DOPTP	-0.363**	DIN:TN	-0.593
DINTN	-0.251**	DINTN	-0.243**	TN:TP	-0.468
DIN	-0.193*	TNTP	-0.243**	NOx	-0.450
NOX	-0.187*	DIN	-0.233**	DIN	-0.431
TN	-0.165*	NHX	-0.205*	DOP:TP	-0.406
TNTP	-0.157*	TN	-0.200*	TN	-0.345
NHX	-0.033	NOX	-0.153*	DOP	-0.040
DOP	-0.025	DOP	-0.146*	NHx	0.157
TP	-0.012	TP	0.082	TP	0.341
PRTCP	0.022	TKN	0.205*	PRTCP	0.465
TKN	0.089	PRTCP	0.222**	TKN	0.557
ORGN	0.186*	ORGN	0.263**	ORGN	0.564

*p<0.05; **p<0.001

For nitrogen, the general response to increasing watershed area was decreasing dissolved inorganic nitrogen and increasing organic nitrogen (Appendix 2). Total nitrogen (TN) was inversely correlated with watershed area. The majority of TN in Iowa streams is comprised of dissolved inorganic nitrogen, particularly nitrate-nitrogen. Nitrate uptake and biological assimilation as well as nitrogen denitrification are two instream processes that probably contribute significantly to the observed pattern.

A similar pattern in phosphorus was observed. Levels of dissolved orthophosphate (DOP) decreased and particulate-based phosphorus (PRTCP) increased with increasing watershed area (Appendix 3). Overall, levels of total phosphorus (TP) did not change significantly in response to increasing watershed area; however, the ratio of dissolved orthophosphate to TP (DOP:TP) decreased. This pattern generally matches the DIN:TN pattern and probably reflects a downstream pattern of increasing nutrient uptake and biological assimilation. One possible reason the relationship of TP with watershed area did not match TN is that phosphorus lacks a process like denitrification whereby nitrate-nitrogen is converted to nitrogen gas and can be released into the atmosphere.

Boxplot summaries of REMAP nutrient data by stream order group are shown in Appendix 4. The Krusgal-Wallis AOV test did identify several nutrient variables for which the stream order group effect was significant. Significant AOV test results were followed by a nonparametric multiple mean test to identify differences among treatment groups. Mean testing results are reported in Table 10.

As would be expected, patterns in nitrogen variables across stream order groups were similar as patterns observed in nitrogen correlations with watershed area. For example, Large order streams had significantly lower mean rank of nitrate+nitrite nitrogen, dissolved inorganic nitrogen, total nitrogen, and ratio of dissolved inorganic nitrogen to total nitrogen (TN) compared with mean rank of small and medium order streams. Large order streams also had a higher mean rank of organic nitrogen than small and medium order streams.

Differences in phosphorus variables across stream order groups were also generally similar as correlations with watershed area. Mean rank of dissolved orthophosphate (DOP) and total phosphorus (TP) did not differ significantly by stream order group; however, mean rank of particulate phosphorus (PRTCP) was significantly higher in large order streams compared with small and medium order streams. Mean ranks of DOP:TP ratio and TN:TP were significantly lower than mean ranks for small and medium order streams. These results match correlation results which show DOP:TP and TN:TP decreasing with increasing watershed area.

The stream order treatment effect analysis uncovered many differences between large order streams and medium or small order streams. T-test results showed differences for eight of the twelve nutrient variables. Although medium order streams tended to have intermediate median values ranking between small order and large order streams for most of the nutrient variables, no statistically significant differences between small order and medium order streams were detected. These results indicate that medium order streams in Iowa are more similar to small order streams than large order streams, which are markedly different in many nutrient characteristics.

Table 10. Median values of nutrient variables by Strahler Order group: Small (2nd order); Medium (3rd–4th); Large (5th-7th). All nutrient parameters except ratio parameters are in mg/L. Stream order groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

Parameter	Stream Order Group Small	Medium	Large
NHx	0.05 [a]	0.05 [a]	0.05 [a]
NOx	6.05 [a]	5.48 [a]	4.02 [b]
DIN	6.65 [a]	5.55 [a]	4.21 [b]
ORGN	0.66 [b]	0.73 [b]	1.03 [a]
TKN	0.71 [a]	0.80 [a]	1.14 [a]
TN	7.57 [a]	6.67 [a]	5.15 [b]
DIN:TN	0.90 [a]	0.87 [a]	0.70 [b]
DOP	0.075 [a]	0.072 [a]	0.060 [a]
PRTCP	0.073 [b]	0.095 [b]	0.150 [a]
TP	0.191 [a]	0.166 [a]	0.220 [a]
DOP:TP	0.63 [a]	0.52 [a]	0.39 [b]
TN:TP	71.20 [a]	49.22 [a]	28.43 [b]

Ecoregion

Boxplots displaying the nutrient data grouped by ecoregion are shown in Appendix 6. As the boxplots illustrate, there is substantial amount of overlap in nutrient levels across ecoregions. Krusgal-Wallis, nonparametric analysis of variance (AOV) test results, however, did find the ecoregion treatment effect was significant on all nutrient variables. Generally, more variation in nitrogen than phosphorus variables was attributable to ecoregion. For example, ecoregion explained 32% of order-ranked total nitrogen (TN) values compared with 21% for total phosphorus (TP).

Ecoregion mean rank testing results are reported in Table 11. The Loess Flats and Till Plains ecoregion (40a) stands out for having low median levels of nitrate+nitrite nitrogen (NOx) and ratio of dissolved inorganic nitrogen to total nitrogen (DIN:TN). It also had among the highest ranking median levels of organic nitrogen (ORGN). The Paleozoic Plateau (Driftless Area) ecoregion (52b) was another fairly distinct ecoregion. It had among the lowest median levels in organic nitrogen (ORGN), particulate phosphorus (PRTCP), and it was among the highest ranking in DIN:TN ratio and dissolved orthophosphate to total phosphorus ratio (DOP:TP).

Table 11. Median values for nutrient variables by ecoregion. All nutrient variables are mg/L except unitless ratio variables. Ecoregions sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Ecoregion						
Parameter	40a	47a	47b	47c	47e	47f	52b
NHx	0.07 [a]	0.05 [ab]	0.05 [b]	0.05 [b]	0.05 [ab]	0.05 [ab]	0.05 [b]
NOx	0.23 [c]	6.82 [a]	7.67 [a]	6.78 [a]	7.65 [a]	3.30 [bc]	4.49 [ab]
DIN	0.45 [c]	6.87 [a]	7.73 [a]	6.93 [a]	7.70 [a]	3.41 [bc]	4.54 [ab]
ORGN	1.12 [a]	0.92 [a]	0.80 [ab]	0.57 [bc]	0.67 [abc]	0.75 [abc]	0.34 [c]
TKN	1.35 [a]	0.98 [ab]	0.89 [ab]	0.63 [bc]	0.72 [abc]	0.92 [abc]	0.40 [c]
TN	1.58 [c]	7.72 [a]	8.41 [ab]	7.45 [ab]	8.17 [ab]	4.27 [c]	5.09 [bc]
DIN:TN	0.32 [c]	0.86 [ab]	0.86 [ab]	0.91 [a]	0.92 [ab]	0.74 [bc]	0.92 [a]
DOP	0.06 [bc]	0.11 [a]	0.06 [bc]	0.05 [c]	0.09 [a]	0.10 [ab]	0.07 [abc]
PRTCP	0.15 [a]	0.11 [ab]	0.07 [abc]	0.05 [bc]	0.13 [a]	0.10 [ab]	0.04 [c]
TP	0.21 [ab]	0.27 [a]	0.14 [abc]	0.12 [c]	0.22 [a]	0.21 [ab]	0.11 [bc]
DOP:TP	0.44 [b]	0.53 [ab]	0.56 [ab]	0.52 [ab]	0.48 [b]	0.56 [ab]	0.71 [a]
TN:TP	9.8 [c]	49.0 [a]	56.1 [a]	81.0 [a]	36.6 [ab]	19.7 [bc]	48.1 [a]

Thermal regime

Boxplot summaries again showed substantial overlap in nutrient data among treatment groups (Appendix 7). Results from the Kruskal-Wallis AOV indicated the effect of thermal/ecoregion classification was significant for 6 of 12 nutrient variables (ORGN, TKN, DIN:TN, PRTCP, TP, DOP:TP). Median values for warmwater sites in the 52b ecoregion (Table 12) mostly fell intermediately between coldwater sites and warmwater sites of all other ecoregions.

Relative to warmwater stream sites, coldwater sites were characterized as having relatively high ratios of dissolved inorganic nutrients to total nutrients (Appendix 7(g),(k)), as well as low levels of organic N and particulate P (Appendix 7(e),(i)). The data range of nutrient variables for coldwater sites was relatively narrow for several variables (see Appendix 7(d),(f),(h-k)), From a statistical significance standpoint, however, the mean ranks for warmwater 52b sites were not distinguishable from coldwater sites for any of the six variables. The results of this limited analysis, therefore, suggest the main driver with respect to the observed differences in nutrient data appears to be more related to ecoregion characteristics than thermal characteristics. A larger sample size of coldwater and warmwater streams in ecoregion 52b might help reveal whether more subtle differences in nutrient conditions exist.

Table 12. Median values for nutrient variables by thermal/ecoregion group. All nutrient variables are mg/L except unitless ratio variables. Thermal groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Thermal/Ecoregion Group		
Parameter	Warm (not 52b)	Warm (52b)	Cold (52b
NHx	0.05 [a]	0.05 [a]	0.05 [a]
NOx	5.15 [a]	4.60 [a]	4.37 [a]
DIN	5.32 [a]	4.65 [a]	4.42 [a]
ORGN	0.80 [a]	0.43 [b]	0.22 [b]
TKN	0.90 [a]	0.48 [b]	0.27 [b]
TN	6.60 [a]	5.20 [a]	4.64 [a]
DIN:TN	0.83 [b]	0.91 [a]	0.94 [a]
DOP	0.07 [a]	0.09 [a]	0.06 [a]
PRTCP	0.10 [a]	0.06 [b]	0.03 [b]
TP	0.20 [a]	0.13 [ab]	0.11 [b]
DOP:TP	0.50 [b]	0.67 [a]	0.71 [a]
TN:TP	30.3 [a]	53.7 [a]	47.1 [a]

4.2. Nutrient response indicators

Seston chlorophyll A

Seston chlorophyll A (WCHLA) is an indicator of the amount or biomass of algal cells that are suspended in the water column. REMAP sampling data for seston chlorophyll A are summarized in Table 13.

Table 13. Summary statistics for REMAP (2002-2006) chlorophyll A sampling data.

Parameter	Periphyton Chlorophyll A	Sediment Chlorophyll A	Seston Chlorophyll A
ABBRV.	PCHLA	SCHLA	WCHLA
Units	ug/cm2	ug/cm2	ug/L
N	535	535	646
Min	<0.1	<0.1	<1
Q25	1.9	1.0	3.0
Median	4.7	2.7	7.0
Q75	10.0	6.3	23.0
Max	290.0	97.0	570.0
Mean	8.8	5.5	29.6
Std.Dev.	19.4	8.7	62.1

Average values and maximum values of seston chlorophyll A were both initially considered for use in the stressor-response data analysis. REMAP sampling data of these variables was highly correlated ($r^2 = 0.89$; Figure 10). Variation in the linear relationship became larger with increasing levels of WCHLA. Ultimately, the decision was made to use average values because they were more likely to represent long-term effects of stream algal productivity on the aquatic biological community than would a maximum value. Unless otherwise noted, site average values are reported henceforth.

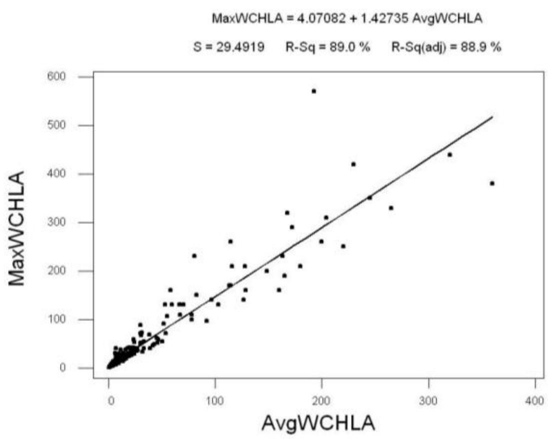


Figure 10. Linear regression of REMAP site average seston chlorophyll A concentration vs. site maximum concentration (2002-2006; most sites sampled 2-3 times in same sample year).

Like several of the nutrient variables, the statistical distribution of WCHLA was truncated at the lower end and positively skewed (Figure 11a). Log10 transformation of the data resulted in a data distribution that was closer to a normal distribution (Figure 11b). For data analysis purposes either the log-transformed or untransformed WCHLA data were used depending on the assumptions and requirements of the statistical test that was applied.

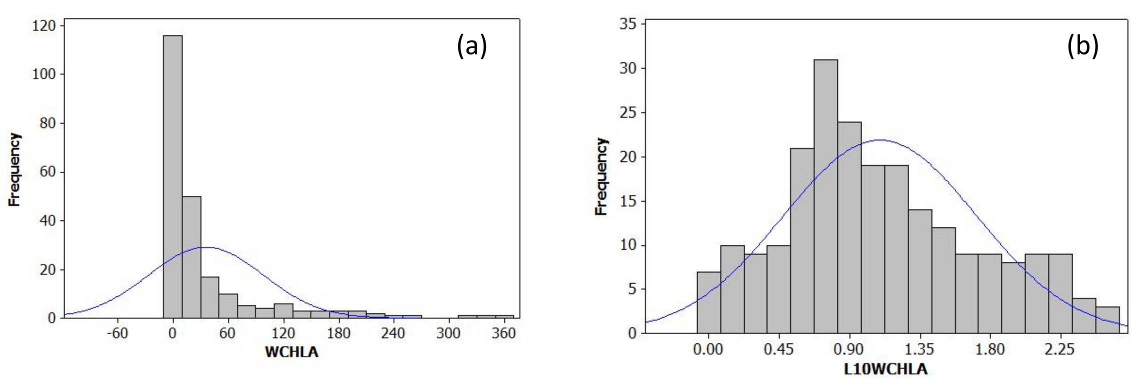


Figure 11. Histogram and approximated normal distribution curve for (a) untransformed seston chlorophyll A (WCHLA) and (b) Log10 transformed WCHLA. REMAP project data: 2002-2006..

A pattern of increasing seston chlorophyll A with increasing stream watershed area was visually evident in the Iowa REMAP data set (Figure 12). Van Nieuwenhuyse and Jones (1996) observed a positive relationship between stream watershed area and seston chlorophyll A in a synthesis analysis of global temperate stream data. This relationship had previously been observed in Iowa streams where it was attributed to physical mechanisms such as scouring and flow transport of benthic algae from upstream areas leading to an increase in planktonic algal abundance in downstream reaches (Swanson and Bachmann 1975). A similar relationship was observed in Minnesota rivers where water retention time was reported as being an important determinant of seston algal biomass (Heiskary and Markus 2003).

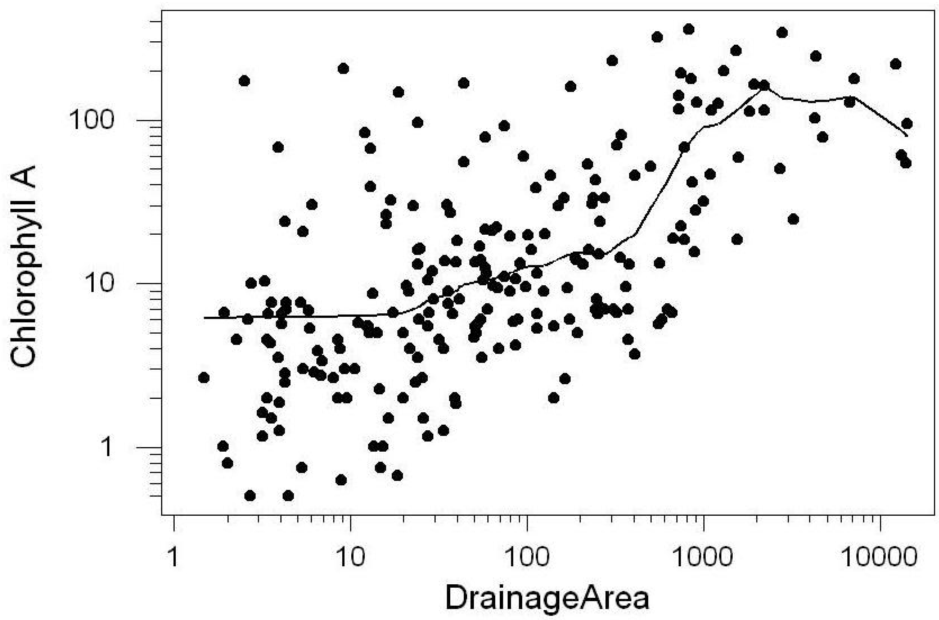


Figure 12. Scatter plot and lowess curve (locally weighted scatter plot smoothing) showing the relationship between stream site watershed drainage area (square miles) and average seston chlorophyll A (ug/L). REMAP (2002-2006) sampling data.

Regression tree (RT) analysis was performed to examine for significant splits or changepoints in the relationship between stream watershed area and seston chlorophyll A (WCHLA). RT analysis found a primary split at 700 mi^2 watershed area. This changepoint resulted in a 33.4% reduction in the total variance in the relationship. The mean WCHLA concentration was 20.8 ug/L for streams having watershed area less than 700 mi^2 compared with 119.7 ug/L for streams of larger watershed area ranging from 700-14,443 mi^2. Secondary splits were found at 291 mi^2 and 1164 mi^2. The additional reduction in variance attributed to these secondary changepoints combined was much smaller (2.7%).

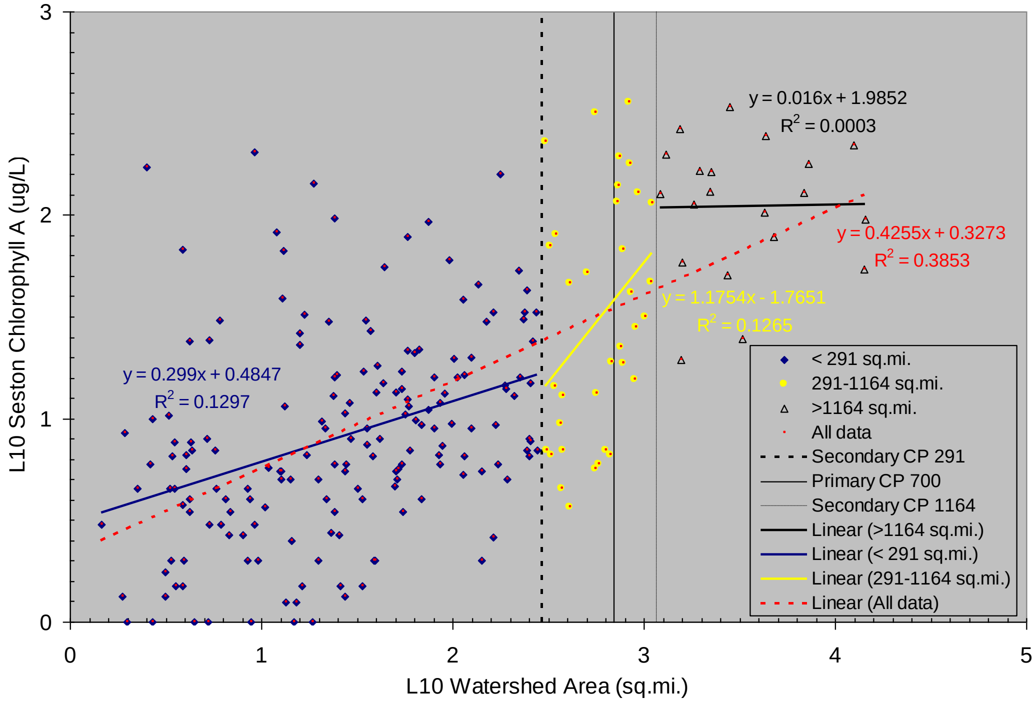


Figure 13. Piecewise linear regression of Log10 watershed drainage area versus Log10 seston chlorophyll A (WCHLA). Nodes of regression pieces were defined by Regression Tree analysis secondary splits at 291 mi^2 and 1164 mi^2 watershed area. REMAP 2002-2006 data.

A Log$_{10}$-linear regression model explained 38.5% of the variation in the stream watershed area - WCHLA relationship (Figure 13) compared with 14.4% for untransformed data. Raw data and residual values were approximately normally distributed for log-transformed data and not normally distributed for untransformed data. A piecewise regression of the log-transformed data was also conducted using the secondary splits from RT analysis to define regression nodes at 291 mi^2 and 1164 mi^2 (Figure 13). WCHLA displayed the greatest rate of increase between 291-1164 mi^2 watershed area. The lowess curve in Figure 12 shows a very similar pattern. Beyond the RT secondary changepoint at 1164 mi^2 watershed area, WCHLA levels did not show a significant increasing or decreasing trend.

Levels of seston chlorophyll A increased progressively from small order to large order streams (Table 14; Appendix 8a). The relationship between WCHLA and stream order and also watershed area fits the previously noted nutrient pattern in which levels of dissolved inorganic nutrients decreased in relation to total nutrient levels as watershed area increased. At the same vein, the increased levels of organic N and particulate-based P seen in large order streams are consistent with markedly increased WCHLA levels. These patterns strongly suggest that assimilation of nutrients by sestonic algal assemblages is an important process influencing stream nutrient characteristics, particularly during the summer and fall months.

Table 14. Median values of periphyton (PCHLA), sediment (SCHLA), and seston (WCHLA) chlorophyll A by Strahler Order group: small (2nd); medium (3rd–4th); large (5th-7th). REMAP (2002-2006) sampling data. Stream order groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Stream Order Group		
Parameter	Small	Medium	Large
PCHLA	5.0 [a]	4.9 [a]	5.9 [a]
SCHLA	2.6 [b]	3.0 [b]	6.0 [a]
WCHLA	4.5 [c]	9.4 [b]	52.0 [a]

Although the effect of ecoregion on seston chlorophyll A was statistically significant, no individual ecoregion was distinctly difference from all other ecoregions (Table 15; Appendix 8b). Some of the highest ranking levels occurred in ecoregions 40a, 47b, and 47f, while lower levels tended to occur in 47e and 52b.

Table 15. Median values of periphyton (PCHLA), sediment (SCHLA), and seston (WCHLA) chlorophyll A by ecoregion (Figure 2). REMAP (2002-2006) sampling data. Ecoregions sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Ecoregion						
Parameter	40a	47a	47b	47c	47e	47f	52b
PCHLA	1.7 [d]	6.8 [abc]	5.9 [bc]	8.3 [ab]	2.7 [cd]	4.3 [bcd]	14.6 [a]
SCHLA	2.0 [b]	3.0 [ab]	4.6 [ab]	3.0 [ab]	1.6 [ab]	3.2 [ab]	5.3 [a]
WCHLA	16.0 [a]	13.5 [ab]	14.7 [a]	9.0 [ab]	6.0 [ab]	14.7 [a]	4.5 [b]

Among thermal/ecoregion groups, coldwater stream sites had distinctly lower levels of WCHLA than warmwater sites located in ecoregions other than 52b (Table 16; Appendix 8c). Levels of WCHLA in 52b warmwater sites were intermediate and not significantly different than coldwater sites or warmwater sites in other ecoregions.

Table 16. Median values of periphyton (PCHLA), sediment (SCHLA), and seston (WCHLA) chlorophyll A by thermal/ecoregion group. REMAP (2002-2006) sampling data. Thermal/ecoregion groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Thermal/Ecoregion Group		
Parameter	Warm (not 52b)	Warm (52b)	Cold (52b)
PCHLA	4.53 [b]	14.10 [a]	15.00 [a]
SCHLA	2.93 [a]	5.15 [a]	6.30 [a]
WCHLA	11.5 [a]	6.0 [ab]	3.5 [b]

Benthic chlorophyll A

Benthic chlorophyll A is an indicator of the biomass of algae growing on the bottom of the stream or attached to hard substrates immersed in the water column. Chlorophyll A samples for the REMAP project were collected from two distinct types of benthic algae: a) periphyton (PCHLA) algae was sampled from rock or wood substrates; b) sediment (SCHLA) algae samples were collected from surface accumulations of fine sediment particles (i.e. fine sand, silt) found in depositional zones along the stream margin.

PCHLA and SCHLA sampling data from the REMAP project are summarized in Table 13. Similar to seston chlorophyll A, both benthic chlorophyll A variables displayed non-normal, positively skewed data distributions (Figures 14 and 15).

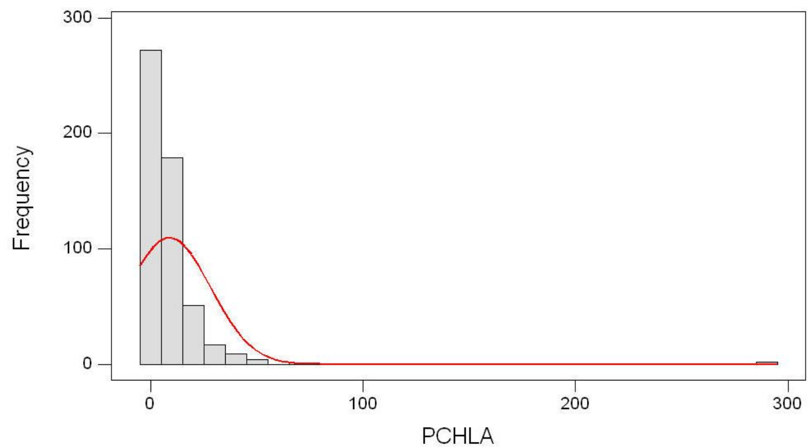


Figure 14. Frequency distribution of REMAP (2002-2006) periphyton chlorophyll A (PCHLA) sampling data. The red line shows the approximated normal distribution curve.

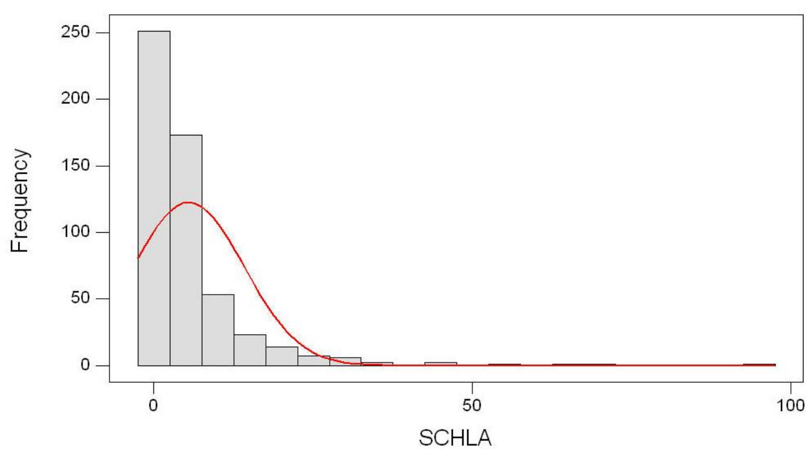


Figure 15. Frequency distribution of REMAP (2002-2006) sediment chlorophyll A (SCHLA) sampling data. The red line shows the approximated normal distribution curve.

PCHLA sample levels were not linearly related with watershed area (Figure 16). SCHLA levels did show a slight linear relationship (Figure 17) with watershed area; however, the amount of variation attributable to the relationship was small (4.4%).

Variation in benthic chlorophyll A levels by stream order group showed similar patterns as the relationships with watershed area. PCHLA levels did not vary significantly among stream order groups (Table 14; Appendix 9a). SCHLA levels representing large order (5-7) stream sites were significantly greater than SCHLA levels for medium order (3-4) or small order stream groups. Similar to the relationship with watershed area, the proportional amount of SCHLA variation explained by stream order group was relatively small (6.2%).

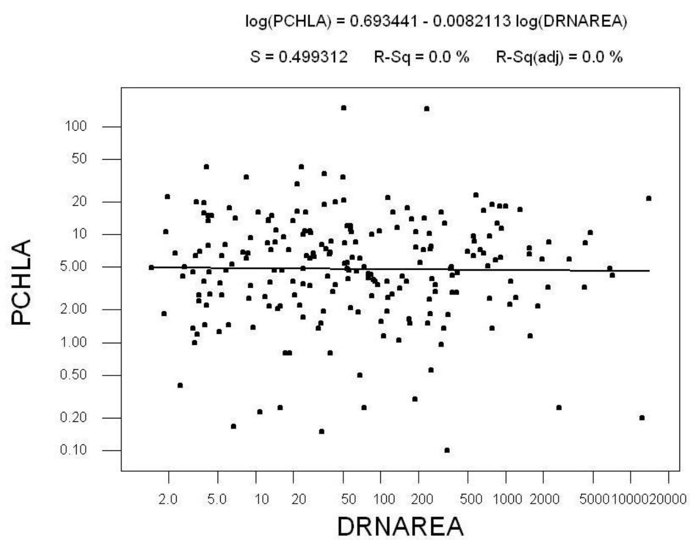


Figure 16. Simple linear regression of stream watershed drainage area (DNRAREA) (square miles) versus periphyton chlorophyll A (PCHLA) (ug/cm^2).

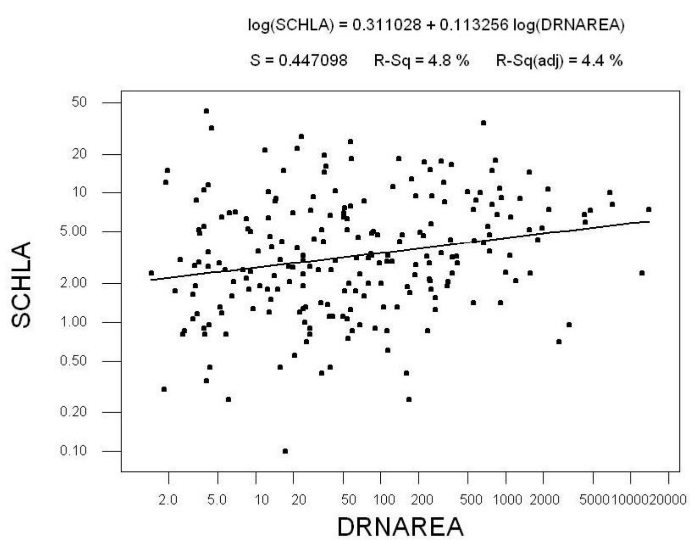


Figure 17. Simple linear regression of stream watershed drainage area (DNRAREA) (square miles) versus sediment chlorophyll A (SCHLA) (ug/cm^2).

The ecoregion treatment effect was significant on both periphyton and sediment chlorophyll A (Table 15; Appendix 9c-d). Ecoregion explained a greater amount of the variation in levels of PCHLA (34.1%) than SCHLA (8.8%). The Paleozoic Plateau ecoregion (52b) and the Loess Flats and Till Plains (40a) ecoregions again are statistically distinguishable from other ecoregions and represent opposite ends of the range in PCHLA median values.

Inherent differences in hydrology and stream habitat characteristics are probably partly responsible for the observed differences in stream periphyton and seston chlorophyll A levels between the 52b and 40a ecoregions. For example, stream reference sites located in 52b had higher mean levels of coarse substrate, proportional area of riffles, and stream gradient (i.e., channel slope) relative to mean levels of reference sites located in other ecoregions (Wilton 2004). These habitat characteristics are more likely to favor production of benthic algae than sestonic algae, which is more able to flourish in slow moving, low gradient streams like those found in the 40a ecoregion. Streams in ecoregion 52b also receive significant groundwater inputs, whereas streams in ecoregion 40a have relatively little groundwater input and are dominated by surface runoff. The more stable stream flow conditions of ecoregion 52b are more conducive to sustained growth of periphyton. Extreme flow fluctuations are known to be a limiting factor to the development of periphyton biomass.

The effect of thermal/ecoregion groups was significant for PCHLA, but not SCHLA (Table 16; Appendix 9e-f). However, the effect appears to be caused by ecoregion differences, not thermal classification. Levels of PCHLA and SCHLA did not vary significantly according to thermal classification (i.e., cold vs. warm) within the 52b ecoregion. Periphyton levels in 52b warmwater and coldwater stream sites both were significantly higher than levels in warmwater sites in other ecoregions, while levels in sediment chlorophyll A levels did not vary significantly.

Dissolved oxygen and stream metabolic rates

Instream fluctuations in dissolved oxygen are directly linked to stream metabolic processes, in particular photosynthesis and respiration. In the daytime, when the rate of photosynthetic DO production exceeds the rate of biological respiration and atmospheric exchange, DO levels will exceed the saturation point and there is a surplus. Conversely, when the rate of DO consumption by biological respiration exceeds the rate of production and atmospheric re-aeration, DO levels are under-saturated and there is a deficit. Nutrient availability can play a role in regulating the rate of oxygen production and consumption by supporting the growth of autotrophs (algae/plants) and heterotrophs (bacteria, fungi, invertebrates, fish). Generally, higher rates of oxygen production and consumption are more likely to occur under nutrient-enriched conditions than nutrient-limited conditions.

Continuous dissolved oxygen monitoring data from 189 REMAP sample sites is summarized in Table 17. The data were obtained by deploying a continuous monitoring data logger for several days at each site. A typical deployment interval lasted six days. Average diel (i.e., daily, also referred to as diurnal) values for mean, maximum, minimum, and range of DO were obtained, as well as the absolute minimum DO level recorded during the deployment. The summarized results (Table 17) show that Iowa's streams experience highly variable DO conditions from substantially under-saturated to substantially over-saturated and all levels in between.

The REMAP probabilistic survey estimated that 31% (±6%) of Iowa's perennial stream miles experienced DO levels that are below the 5 mg/L water quality standards criterion for warmwater aquatic life uses.

Table 17. REMAP diel dissolved oxygen monitoring statistical summary.

Parameter	Average Diurnal D.O.	Average Diurnal Maximum D.O.	Average Diurnal Minimum D.O.	Instantaneous Diurnal Minimum D.O.	Average Diurnal Range D.O.
ABBRV.	AVG2DO	AVGMAXDO	AVGMINDO	MIN2DO	AVGRNGDO
Units	mg/L	mg/L	mg/L	mg/L	mg/L
No. Sites	189	189	189	189	189
Min	1.35	2.99	0.36	0.01	0.44
Q25	7.30	8.83	5.52	4.74	2.07
Median	8.20	10.19	6.69	6.05	3.43
Q75	9.05	12.48	7.44	6.91	5.80
Max	13.98	22.57	11.62	10.89	18.69
Mean	8.24	10.72	6.43	5.68	4.29
Std.Dev.	1.80	2.85	1.87	2.09	3.00

The continuous dissolved oxygen and temperature monitoring data were used to calculate average diel values of several stream metabolic variables summarized in Table 18. Calculations were done using an Excel spreadsheet program (Anderson and Huggins 2003) that is based upon methods developed by Odum (1956) and also described in Hauer and Lamberti (1996).

Diel stream metabolic rates are calculated from the rate of change in dissolved oxygen after correcting for water temperature and re-aeration. A simple equation is used to describe the net primary production (NPP) rate of oxygen.

NPP (net primary production) = GPP (gross primary production) – CR (community respiration)

Although highly variable, most of the REMAP sites had negative rates of net primary production (AVGNPP). That is, the daily amount of oxygen consumption (community respiration) exceeded the amount of oxygen production (gross primary production) resulting in a negative balance or deficit in DO. The mean of AVGNPP was -2.27 g/O_2/day (Table 18). The Production to Respiration ratio (P:R) is another metabolic variable that serves as an indicator of stream energetic or trophic function. When P:R ratio > 1, a stream is mostly functioning autotrophically. In this trophic state there is a net accrual of organic matter from algae and plant growth. When P:R <1, a stream is mostly functioning heterotrophically and in order to maintain a given level of biological production, the stream must rely on external (allochthonous) organic matter inputs (e.g., leaf fall) to make up for deficiencies in instream (autochthonous) production.

Table 18. REMAP stream metabolism metric summary derived from diel dissolved oxygen monitoring data.

Parameter	Average Daily Gross Primary Production	Average Daily Community Respiration	Average Daily Net Primary Production	Average Production : Respiration Ratio
ABBRV.	AVGGPP	AVGRESP	AVGNPP	AVGPR
Units	$gO^2/m^2/d$	$gO^2/m^2/d$	$gO^2/m^2/d$	-
No. Sites	186	186	186	186
Min	-1.62	0.93	-32.89	-0.96
Q25	1.76	4.91	-4.45	0.33
Median	3.99	6.50	-2.37	0.61
Q75	7.55	9.54	0.01	1.02
Max	19.83	37.31	13.86	26.91
Mean	5.20	7.46	-2.27	0.92
Std.Dev.	4.42	4.30	4.83	2.09

Dissolved oxygen levels and stream metabolic rates among stream order groups differed significantly in eight of nine variables (Table 19). AVGRNGDO was the only variable for which the stream order effect was not significant. Generally, DO levels ranked highest in large order streams compared with medium and small order streams as displayed by box plot distributions (Appendix 10a-c). Net primary production rate (AVGNPP) and production: respiration ratio (AVGPR) were highest in large order streams and lowest in small order streams. The median level of AVGPR was 1.0, indicating that 50% of large order streams functioned autotrophically compared with median levels < 1.0 for medium and small order streams, indicating heterotrophic stream function was more typical.

Table 19. Median values of diel dissolved oxygen and stream metabolism variables by Strahler Order group: small (2nd); medium (3rd–4th); large (5th-7th). REMAP (2002-2006) sampling data. Dissolved oxygen values are in mg/L. Stream metabolism values are in $gO_2/m^2/day$ except unitless AVGPR. Stream order groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Stream Order Group		
Parameter	Small	Medium	Large
AVGDO	7.81 ^{b}	8.12 ^{b}	9.0 ^{a}
AVGMAXDO	9.55 ^{b}	10.38 ^{ab}	11.4 ^{a}
AVGMINDO	6.71 ^{ab}	6.34 ^{b}	7.2 ^{a}
INSTMINDO	6.33 ^{ab}	5.71 ^{b}	6.5 ^{a}
AVGRNGDO	2.84 ^{a}	3.94 ^{a}	3.6 ^{a}
AVGGPP	3.04 ^{b}	4.01 ^{ab}	5.5 ^{a}
AVGRESP	7.22 ^{a}	6.20 ^{ab}	5.3 ^{b}
AVGNPP	-4.25 ^{c}	-2.21 ^{b}	0.1 ^{a}
AVGPR	0.45 ^{c}	0.62 ^{b}	1.0 ^{a}

Similar to stream order, the ecoregion treatment effect was significant for all DO and stream metabolic variables except AVGRNGDO (Table 20). Similar to ecoregion differences among nutrient and chlorophyll A variables, ecoregions 40a and 52b tended to have the most distinct differences in dissolved oxygen and stream metabolism characteristics. Streams in ecoregion 40a can generally be characterized as having low DO levels (e.g., AVGDO), low net primary production rate (AVGNPP), and high community respiration rate (AVGRESP), while 52b levels in these characteristics were generally opposite ranking (Table 20; Appendix 11).

Table 20. Median values of diel dissolved oxygen and stream metabolism variables by ecoregion (Figure 2). REMAP (2002-2006) sampling data. Dissolved oxygen values are in mg/L. Stream metabolism values are in $gO_2/m^2/day$ except unitless AVGPR. Stream order groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Ecoregion						
Parameter	40a	47a	47b	47c	47e	47f	52b
AVGDO	6.67 b	8.11 ab	8.19 a	8.54 a	8.29 a	8.34 a	8.91 a
AVGMAXDO	8.11 b	11.39 a	10.61 a	10.54 a	9.31 ab	10.68 ab	10.54 a
AVGMINDO	4.77 c	5.69 bc	6.62 ab	6.86 ab	7.09 ab	6.77 ab	7.84 a
INSTMINDO	3.36 c	5.04 bc	6.04 ab	6.41 ab	6.63 ab	6.16 ab	7.23 a
AVGRNGDO	3.00 a	4.80 a	3.02 a	4.00 a	1.76 a	3.51 a	3.75 a
AVGGPP	2.92 ab	8.04 a	4.07 ab	4.48 ab	1.42 b	3.39 ab	3.50 ab
AVGRESP	8.77 a	8.34 ab	7.22 ab	6.32 ab	5.08 b	5.66 ab	5.36 b
AVGNPP	-5.05 b	-1.47 a	-2.45 a	-1.76 a	-2.93 ab	-2.33 ab	-1.14 a
AVGPR	0.29 b	0.85 a	0.53 ab	0.70 a	0.42 ab	0.46 ab	0.77 a

The effect of thermal groupings was significant for five of nine DO and stream metabolic variables (Table 21). For variables in which the treatment effect was significant, coldwater sites had significantly higher DO levels than levels in combined warmwater sites from ecoregions other than 52b. Median DO levels in warmwater streams of 52b ranked between the other two groups; however, the differences were not statistically significant. Group differences in AVGRNGDO, AVGGPP, AVGNPP, and AVGRESP were not significant. Levels of AVGPR were significantly higher in coldwater streams than median level in warmwater streams located in other ecoregions excluding 52b.

Table 21. Median values of diel dissolved oxygen and stream metabolism variables by thermal/ecoregion group. REMAP (2002-2006) sampling data. Dissolved oxygen values are in mg/L. Stream metabolism values are in $gO_2/m^2/day$ except unitless AVGPR. Stream order groups sharing the same alpha designator are not significantly different based on a nonparametric two-sample test of mean value order rank (p>0.05).

	Thermal/Ecoregion Group		
Parameter	Warm (not 52b)	Warm (52b)	Cold (52b)
AVGDO	8.07 [b]	8.15 [ab]	9.55 [a]
AVGMAXDO	10.03 [b]	9.95 [ab]	12.38 [a]
AVGMINDO	6.57 [b]	7.11 [ab]	7.99 [a]
INSTMINDO	5.86 [b]	6.49 [ab]	7.65 [a]
AVGRNGDO	3.40 [a]	2.84 [a]	4.06 [a]
AVGGPP	4.23 [a]	3.46 [a]	3.75 [a]
AVGRESP	6.98 [a]	5.86 [a]	4.82 [a]
AVGNPP	-2.56 [a]	-1.90 [a]	-0.54 [a]
AVGPR	0.58 [b]	0.57 [ab]	0.83 [a]

4.3. Biological assemblage indicators

The Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) and the Fish Index of Biotic Integrity (FIBI) are the main stream biological condition indicators used in the nutrient stressor-biological response analysis. These indexes were developed with sampling data from least disturbed reference sites and test (impacted) sites. Stream biological assessment criteria were derived based on the statistical distributions of reference site BMIBI and FIBI scores. Because the REMAP project sampling data was heavily utilized to examine nutrient stressor–biological response relationships, a comparison of REMAP and reference site BMIBI and FIBI levels was done to evaluate their similarity.

Statistical characteristics of REMAP (random) and reference data sets are summarized in Appendix 13(a-b). Results from analysis of variance (AOV) testing determined that significant amounts of the variability in BMIBI and FIBI scores were explained by site type (8.2% and 20.5%, respectively). Two-sample t-test results confirmed that the mean reference site BMIBI score (61.2, “good”) was significantly higher than the mean random site score (52.6, “fair”), and similarly, mean reference FIBI score (51.4, “good”) was higher than mean random score (34.1, “fair”).

Ecoregion explained a comparatively larger proportion of variability in IBI scores than did site type. Ecoregion accounted for 22.6% of the variability in random site BMIBI scores and 26.6% of reference score variability. For the FIBI, ecoregion accounted for 47.1% of variability in random scores and 36.2% of reference score variability. Examination of box plots (Figures 18 and 19) and statistical summary tables (Appendix 13a-b) revealed a pattern of heavier representation of random site scores in the lower areas of the BMIBI and FIBI scoring ranges, while the upper areas are generally less represented in comparison with reference scores.

Results from multiple-mean comparison testing (Tukey, family error rate = 5%) of ecoregion mean BMIBI and FIBI scores are listed in Table 22. The BMIBI test results for random sites and reference sites were identical. Ecoregions covering northeastern and northcentral Iowa (52b, 47c, 47b; see Figure 2) ranked highest in mean BMIBI score and all were significantly greater than the mean BMIBI score for ecoregion 40a in Southcentral Iowa.

A greater number of significant ecoregion differences in mean FIBI score (11, 50%) were found using random site data, compared with the number of significant differences found using reference site data (6/22, 27%). Mean random site FIBI scores from ecoregions 52b, 47c, and 47b again ranked at the top. Levels in these ecoregions each differed significantly from ecoregions 47a, 40a, and 47e, which cover northwestern, southcentral, and southwestern Iowa, respectively. Mean reference site FIBI scores for northeastern Iowa ecoregions 52b and 47c were significantly higher than mean reference scores from the southern Iowa ecoregions, 47f, 40a, and 47e.

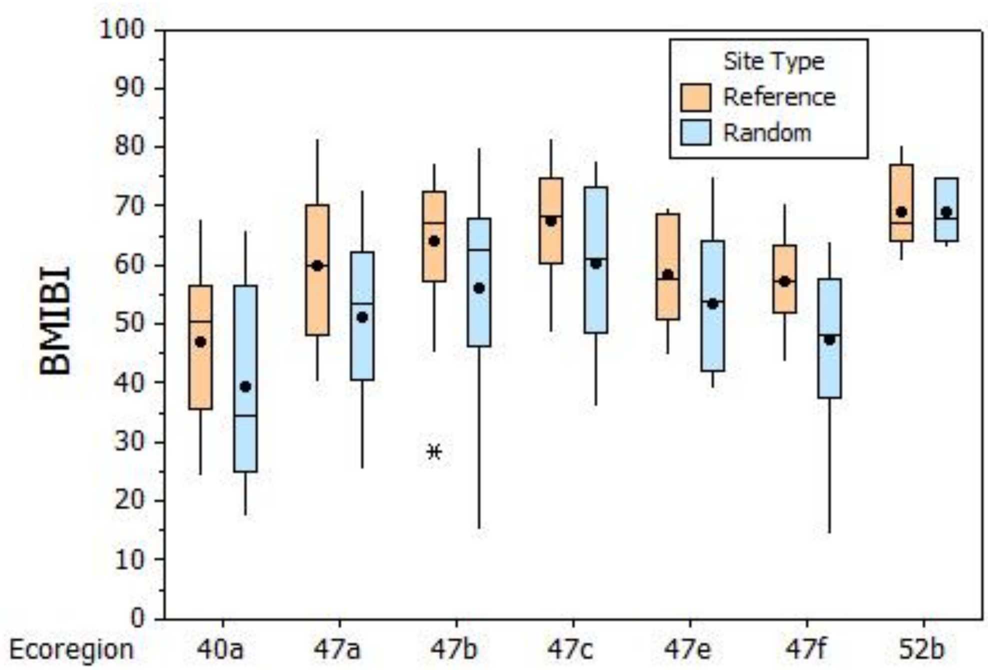


Figure 18. Box plot comparison of Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) scores sampled from wadeable reference and random (REMAP) sample sites. Only ecoregions (Figure 2) represented by more than five sample sites are included.

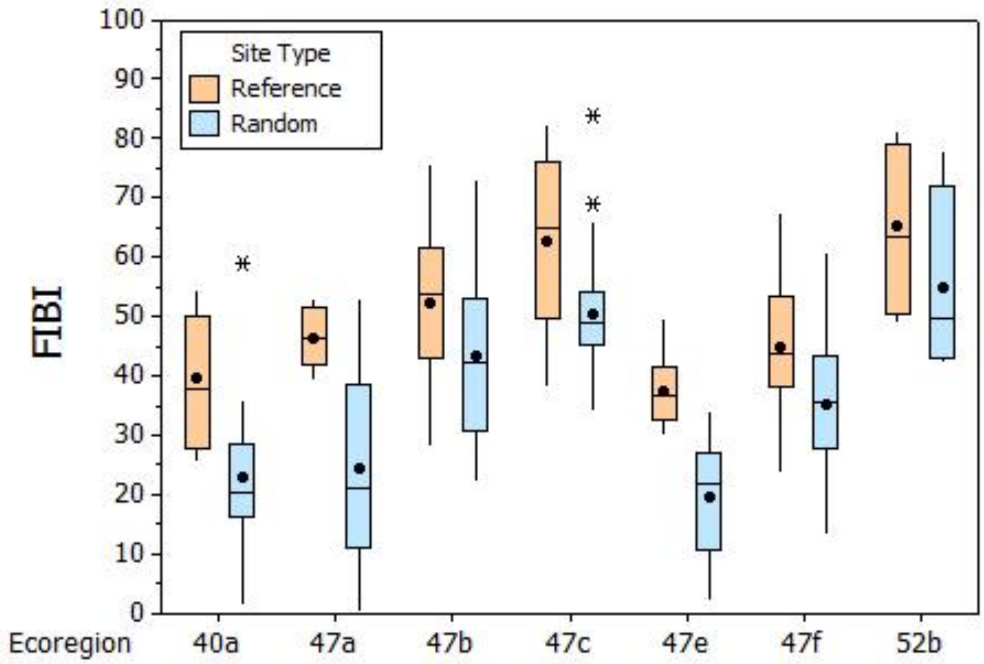


Figure 19. Box plot comparison of Fish Index of Biotic Integrity (FIBI) scores sampled from wadeable reference and random (REMAP) sample sites. Only ecoregions (Figure 2) represented by more than five sample sites are included.

Table 22. Tukey multiple two-sample t-test results from comparison of ecoregion mean scores of BMIBI and FIBI representing (REMAP) random stream sites and reference stream sites. Group means not sharing a letter in common are significantly different from each other (P<0.05).

BMIBI							
Random				Reference			
Ecoregion	N	Mean	Grouping	Ecoregion	N	Mean	Grouping
52b	5	69.3	A	52b	7	69.1	A
47c	18	60.2	A	47c	20	67.4	A
47b	18	56.2	A	47b	21	64.1	A
47e	11	53.6	AB	47a	6	59.8	AB
47a	18	51.0	AB	47e	6	58.5	AB
47f	13	47.2	AB	47f	19	57.4	AB
40a	14	39.3	B	40a	7	46.9	B

FIBI							
Random				Reference			
Ecoregion	N	Mean	Grouping	Ecoregion	N	Mean	Grouping
52b	4	54.9	A B	52b	7	65.3	A
47c	19	50.4	A	47c	20	62.6	A
47b	21	43.1	A B	47b	21	52.4	A B
47f	14	35.2	B C	47a	6	46.3	A B
47a	18	24.3	C D	47f	19	44.8	B
40a	18	22.6	C D	40a	7	39.5	B
47e	13	19.3	D	47e	6	37.4	B

4.4. Nutrient – algal response relationships

Seston algae chlorophyll A

It is often presumed that increased nutrient availability will produce a proportional increase in algal biomass. However, algal biomass may be governed by other environmental factors such as herbivore grazing, flow instability, or light limitation. In such cases, nutrient supplies can exceed demand and additional nutrient inputs will not stimulate more algal growth.

Pearson and Spearman rank (nonparametric) correlation tests were performed on REMAP sampling data to examine the linear relatedness of seston algal biomass and nutrient parameters in Iowa streams. Site average values were used in the analysis. Pearson correlation was also performed on Log_{10} transformed data to compensate for a lack of normal data distribution in most parameters. Pearson coefficients for Log_{10} transformed nutrient parameters and seston chlorophyll A (WCHLA) ranged from -0.497 (DOP:TP) to 0.555 (TKN) (Table 23).

Table 23. Correlation analysis results for sestonic chlorophyll A versus nutrient parameters. REMAP sampling data (2002-2006).

Seston Chlorophyll A

Pearson		Pearson (Log10)		Spearman Rank	
Parameter	r	Parameter (Log10)	r	Parameter	rho
DIN:TN	-0.458**	DOP:TP	-0.497**	DIN:TN	-0.593
DOP:TP	-0.406**	DIN:TN	-0.480**	TN:TP	-0.468
NOx	-0.280**	TN:TP	-0.471**	NOx	-0.450
DIN	-0.252**	NOx	-0.420**	DIN	-0.431
TN:TP	-0.217**	DIN	-0.400**	DOP:TP	-0.406
TN	-0.162**	TN	-0.282**	TN	-0.345
DOP	0.154**	DOP	-0.015	DOP	-0.040
TP	0.176**	NHx	0.163**	NHx	0.157
PRTCP	0.200**	TP	0.353**	TP	0.341
NHx	0.212**	PRTCP	0.471**	PRTCP	0.465
TKN	0.420**	ORGN	0.521**	TKN	0.557
ORGN	0.480**	TKN	0.555**	ORGN	0.564

*p<0.05; **p<0.001

Ordinary least-square linear regression was performed using REMAP individually matched sample values from the same site and day. Nutrient parameters were found to be statistically significant, yet generally weak predictors of seston chlorophyll A levels. Among nitrogen parameters, Total Kjeldahl nitrogen (TKN) had the strongest linear relationship, explaining 29.8% of the variation in WCHLA (Figure 20a). Total nitrogen (TN) and dissolved inorganic nitrogen (DIN) were inversely related with WCHLA, explaining 7.5% and 15.5% of WCHLA variability, respectively (Figure 20b-c). Organic nitrogen (ORGN) was positively related and explained 26.1% of WCHLA variability (Figure 20d).

Among phosphorus variables, particulate phosphorus (PRTCP) was more strongly related with WCHLA (21.6%) than was total phosphorus (11.9%). Dissolved orthophosphate phosphorus (DOP) was not significantly related with WCHLA (Figures 21a-c).

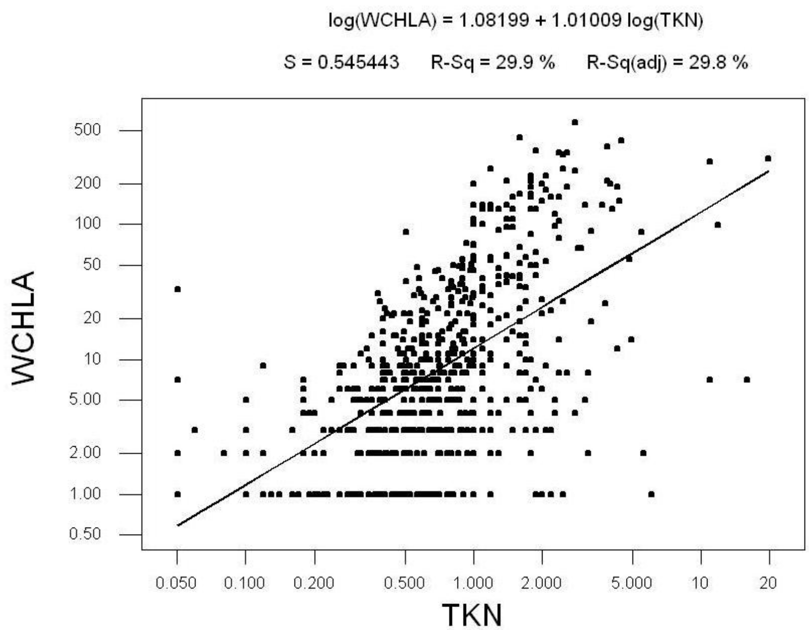


Figure 20(a). Ordinary least squares linear regression of seston chlorophyll A (WCHLA) versus (a) total Kjeldahl nitrogen (TKN). Remap sampling data (2002-2006).

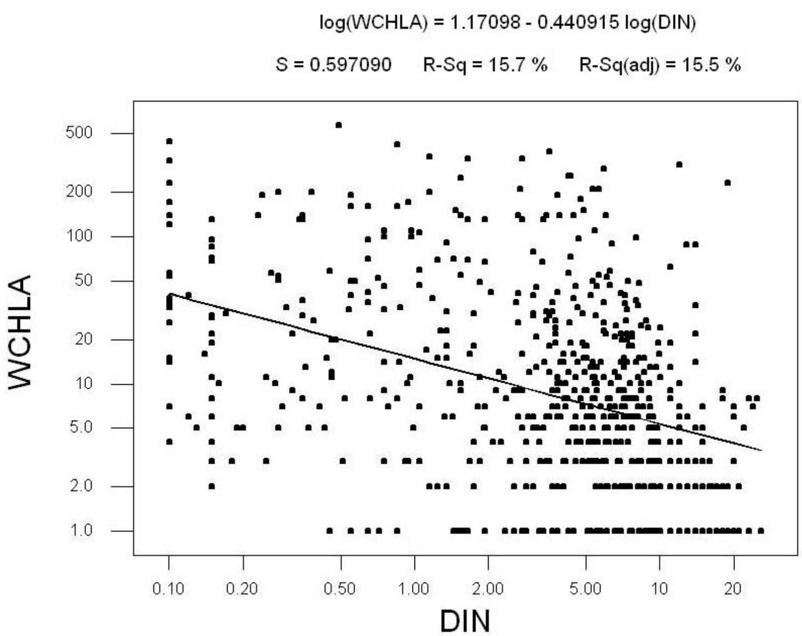


Figure 20(b). Ordinary least squares linear regression of seston chlorophyll A (WCHLA) versus dissolved inorganic nitrogen (DIN). Remap sampling data (2002-2006).

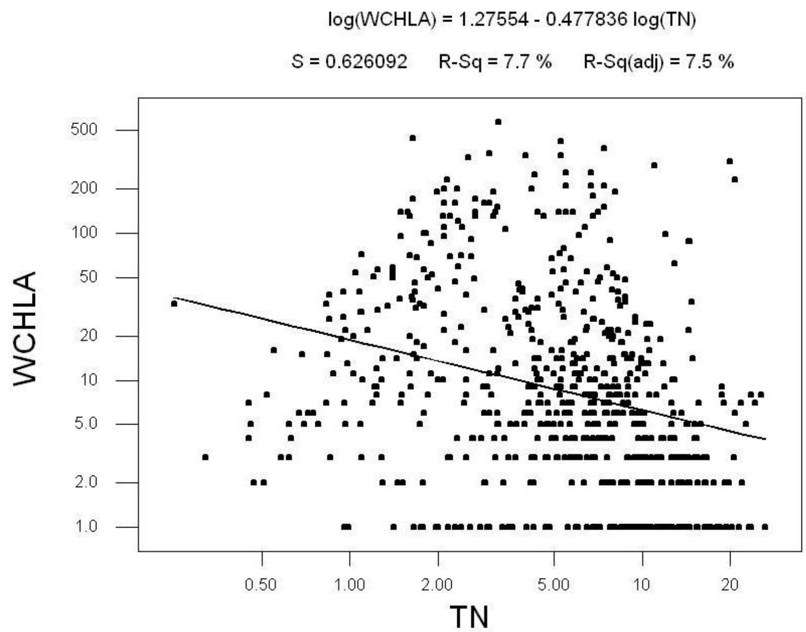


Figure 20(c). Ordinary least squares linear regression of seston chlorophyll A (WCHLA) versus total nitrogen (TN). Remap sampling data (2002-2006).

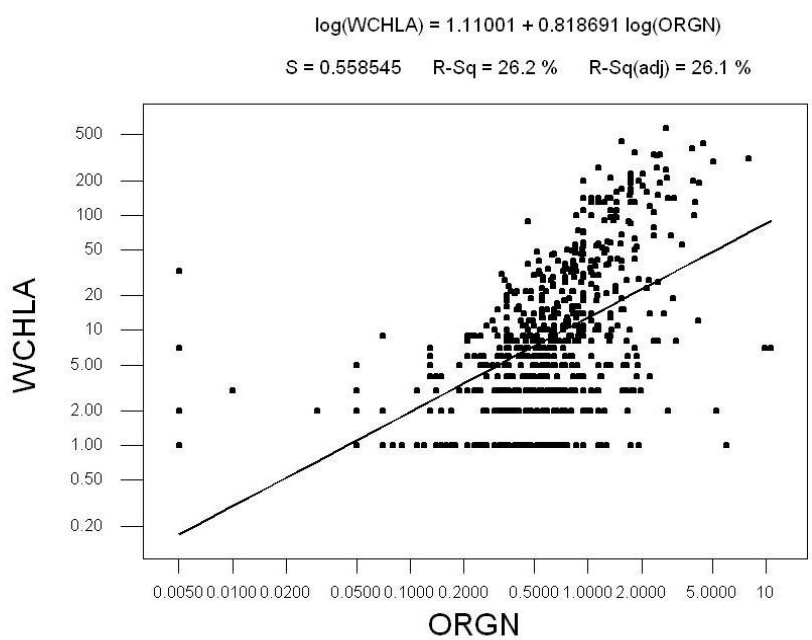


Figure 20(d). Ordinary least squares linear regression of seston chlorophyll A (WCHLA) versus organic nitrogen (ORGN). REMAP sampling data (2002-2006).

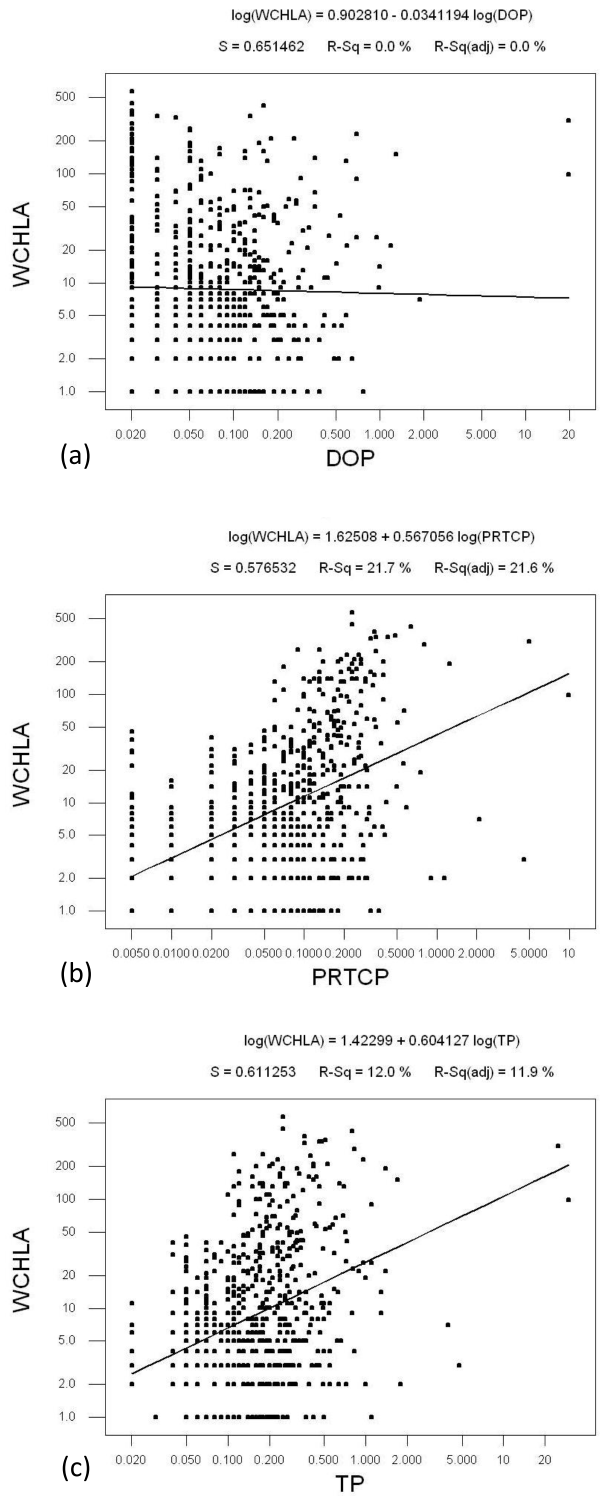


Figure 21(a-c). Ordinary least squares regression of seston chlorophyll A (WCHLA) versus (a) dissolved orthophosphate (DOP); (b) particulate phosphorus (PRTCP); (c) total phosphorus (TP). REMAP sampling data (2002-2006).

Evaluation of nutrient limitation

REMAP project (2002-2006) sampling data for nutrient parameters and seston chlorophyll A were analyzed to evaluate the potential occurrence of nutrient limitation in Iowa streams. Individually matched sample results from the same site and day were used in the analysis. Typically, two or three samples were collected at each site during the growing season The analysis approach generally involved interpreting nutrient concentrations and stoichiometric ratios in relation to levels of seston chlorophyll A following the guidelines described below.

The 'Redfield ratio' is an expression of the cellular stoichiometric ratio of carbon, nitrogen, and phosphorus in phytoplankton growing under conditions in which nutrients are not limiting (Redfield 1958; Hauer and Lamberti 1996). The Redfield ratio is 106:16:1 (C:N:P) on a molar basis and 40.1:7.2:1 on an atomic weight basis. The ratio of phytoplankton chlorophyll A to cellular carbon has been reported as ranging from 10-50 ug/L chlorophyll A to 1 mg/L C (1%-5%) depending on phytoplankton species and availability of light and nutrients (Chapra 1997). Reported levels measured in controlled experiments have ranged from near zero to 10% (Cloern et al. 1995).

After standardizing the analytical reporting units, several ratios were calculated for each REMAP water sample:

- TN:TP (total nitrogen [calculated as Kjeldahl N + nitrite+nitrate N]: total phosphorus)
- TN:WCHLA (total N: seston chlorophyll A)
- TP:WCHLA (total P: seston chlorophyll A)
- DIN:WCHLA (dissolved inorganic nitrogen [calculated as total ammonia N + nitrite+nitrate N: seston chlorophyll A)
- DOP:WCHLA (dissolved orthophosphate phosphorus: seston chlorophyll A)

Non-detect sample analysis results were initially assigned a value equal to 99.9% of the detection limit (i.e., face value). In the course of the analysis, many of these non-detects were considered on a case-by-case basis to evaluate the impact the assumed value might have with respect to the interpretation of stoichiometric ratios. Values equal to one-half and one-quarter of the detect limit were often substituted to see how different (lower) assumed levels might affect the interpretation of results.

Each sample was thought to represent a snapshot of both algal biomass (as estimated from chlorophyll A concentration) and nutrient status. As such, each individual sample was compared to a set of stoichiometric guidelines and assigned one of the following suggested nutrient status designations: a) N-limited; b) P-limited; c) N & P limited; d) not (N or P) limited.

Nutrient limitation was inferred by comparing the calculated stoichiometric ratios with Redfield ratio guidelines and literature-based phytoplankton chlorophyll A:carbon ratios. Data interpretation was done according to the following steps and rationale:

1. <u>Compare TN:WCHLA and TP:WCHLA levels to Redfield ratio guidelines.</u>
   Potential nutrient limitation was indicated when levels of TN and/or TP could almost completely be attributed to amounts contained in algal cells. These occurrences suggest that amounts of readily-available levels of N and/or P were scarce relative to the standing

crop of algae. Without additional inputs of biologically available nutrients, algal biomass was not likely to increase and might actually decrease as algal populations senesce. With subsequent cellular decomposition, nutrient availability may increase again thereby stimulating new algal growth.

2. <u>Compare DIN:WCHLA and DOP:WCHLA levels to Redfield guidelines.</u>
   This comparison followed in the same vein as the previous one by taking a closer look at levels of dissolved inorganic nutrients in relation to algal biomass. When dissolved nutrient levels were small relative to the standing crop of sestonic algae, it was assumed that additional growth and accrual of biomass was unlikely to occur.

3. <u>Examine non-detect nutrient values.</u>
   Calculating a stoichiometric ratio with a non-detect sample result adds uncertainty to its interpretation. Each ratio that included a non-detect value was examined individually. Alternative calculations were done setting the non-detect value at one-half and one-quarter of the detect limit. The alternative ratio levels were compared to the guidelines to evaluate how the interpretation of nutrient limitation status might change depending on which assumed value was assigned to a non-detect sample result.

4. <u>Assign probable nutrient limitation status designation.</u>
   The guidelines listed below were used to assign one of the following nutrient limitation designations to each matched N and P sample set: a) N-limited; b) P-limited; c) N & P limited; d) not (N or P) limited.

5. <u>Sort by nutrient limitation status and calculate summary statistics.</u>
   The samples were sorted by assigned nutrient status and summary statistics for calculated N:P ratios were obtained in Excel. Using a conventional interpretation of the Redfield ratio, N:P ratios below 7.2 would be considered indicative of potential N-limitation, N:P substantially above 7.2 indicate potential P-limitation, and N:P levels approximately equal to 7.2 are potentially N and P –limited or neither nutrient is limiting.

<u>Data interpretation guidelines:</u>

<u>N-limited:</u>

Any of the following:

- TN:WCHLA <17.5;
- TN:WCHLA 17.5 - 40.0 and DIN:WCHLA < 7;
- Total Ammonia N & Nitrate+Nitrite N analysis results are both non-detects ($\leq$ 0.15 mg/L).

<u>P-limited:</u>

Any of the following:

- TP:WCHLA < 2.4;
- DOP < 0.02 mg/L (non-detect);
- DOP & TP both are non-detects (<=0.05 mg/L);
- TP:WCHLA 2.4 - 4.0 and DOP <0.05 mg/L..

The guidelines were meant to identify sample cases in which the majority of total nitrogen and/or total phosphorus could be accounted for as contained within algal cells, and levels of dissolved inorganic nutrients were scarce relative to algal biomass. Under these circumstances, it was presumed that additional algal growth and biomass accrual would be constrained due to insufficient levels of bio-available N and/or P.

Approximately 21% of the 646 REMAP samples exhibited nutrient and sestonic chlorophyll A levels that were suggestive of nitrogen and/or phosphorus limitation. Phosphorus limitation was suggested in 12.2% of samples and was roughly four times more likely to occur than nitrogen limitation (2.9%) and twice as likely to occur as N & P co-limitation (6.2%). (Figures 21a-b; Table 24).

Interesting contrasts in statistical characteristics of chlorophyll A, nitrogen, phosphorus and TN:TP levels can be seen among the data grouped by nutrient limitation status. For example:

- Median chlorophyll A for samples identified as not N or P limited was 6 ug/L, compared with 28 ug/L (N-limited), 29 ug/L, (P-limited), and 140 ug/L (N&P-limited);

- Median TN for samples identified as N-limited was 0.97 mg/L, compared with 5.2 mg/L (P-limited), 2.1 (N&P-limited), and 6.8 mg/L (not N or P limited);

- Median TP for samples identified as P-limited was 0.07 mg/L, compared with 0.19 mg/L (N-limited), 0.24 (N&P-limited), and 0.16 mg/L (not N or P limited);

- Median N:P ratio of 5.5, determined *a posteriori* for samples categorized as N-limited, was consistent with the Redfield mass-based ratio of 7.1 representing a level below which N-limitation is suggested. Median N:P ratios of 50 for P-limited and 8.9 for N & P-limited samples generally seemed consistent with Redfield ratio representing a balanced nutrient status.

Table 24. Statistical characteristics of sample values for sestonic chlorophyll A, total nitrogen, total phosphorus, and total nitrogen: total phosphorus ratio grouped by nutrient limitation status estimated by stoichiometric analysis. REMAP stream survey 2002-2006.

		Chlorophyll A (ug/L)				
	samples	min	P25	median	P75	max
N-Limited	19 (2.9%)	2	7.5	28	34	57
P-Limited	79 (12.2%)	<1	7	29	98.5	380
N & P Limited	40 (6.2%)	7	81.8	140	200	570
Not N or P Limited	508 (78.6%)	<1	3	6	13	310
All	646 (100%)	<1	3	7	22.75	570
		Nitrogen (mg/L)				
	samples	min	P25	median	P75	max
N-Limited	19 (2.9%)	<0.25	0.57	0.97	1.32	2.10
P-Limited	79 (12.2%)	0.32	2.55	5.20	6.98	17.20
N & P Limited	40 (6.2%)	0.83	1.56	2.10	2.78	5.30
Not N or P Limited	508 (78.6%)	0.46	3.98	6.83	9.88	26.53
All	646 (100%)	<0.25	2.90	5.90	9.40	26.53
		Phosphorus (mg/L)				
	samples	min	P25	median	P75	max
N-Limited	19 (2.9%)	0.05	0.09	0.19	0.26	0.49
P-Limited	79 (12.2%)	<0.02	<0.05	0.07	0.17	0.83
N & P Limited	40 (6.2%)	<0.02	0.18	0.24	0.32	0.80
Not N or P Limited	508 (78.6%)	<0.02	0.10	0.16	0.27	30.00
All	646 (100%)	<0.02	0.09	0.16	0.26	30
		N:P ratio (mass)				
	samples	min	P25	median	P75	max
N-Limited	19 (2.9%)	1.6	4.0	5.5	8.5	12.1
P-Limited	79 (12.2%)	6.4	18.4	50.0	104.1	406.5
N & P Limited	40 (6.2%)	4.8	7.0	8.9	11.0	44.0
Not N or P Limited	508 (78.6%)	0.4	16.0	39.7	74.1	773.3
All	646 (100%)	0.4	12.2	34.0	73.0	773.3

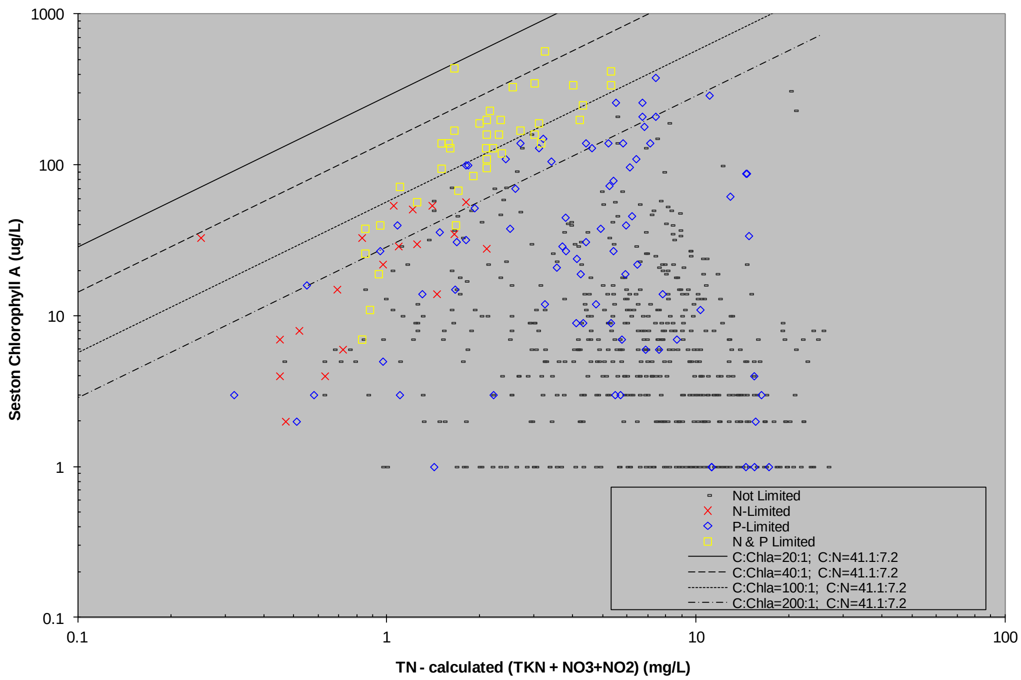


Figure 22(a).Total nitrogen versus seston chlorophyll A. Symbols denote nutrient limitation status inferred by stoichiometric analysis. Lines display varying stoichiometric ratio of carbon to chlorophyll a ranging from 20:1 (5% chlorophyll a) to 200:1 (0.5%). REMAP (2002-2006) sample data.

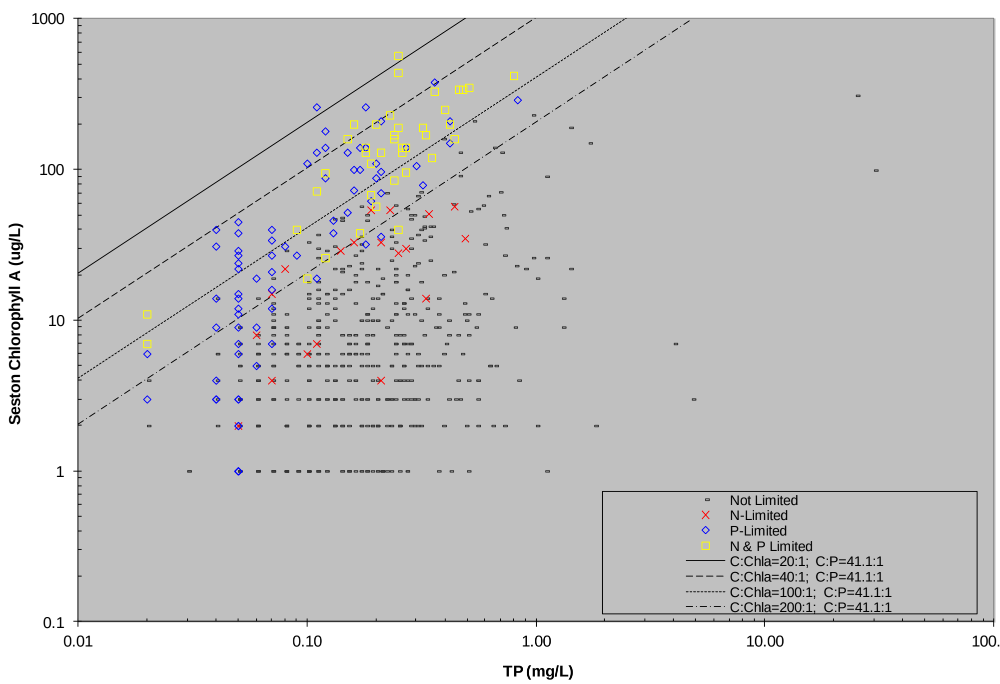


Figure 22(b).Total phosphorus versus seston chlorophyll A. Symbols denote nutrient limitation status inferred by stoichiometric analysis. Lines display varying stoichiometric ratio of carbon to chlorophyll a ranging from 20:1 (5% chlorophyll a) to 200:1 (0.5%). REMAP (2002-2006) sample data.

Nutrients and benthic algae chlorophyll A

Correlative relationships of benthic algal chlorophyll A and nutrient parameters were examined using REMAP sample data. Log-transformed periphyton chlorophyll A (PCHLA) was significantly related with all of the nutrient parameters, while log-transformed sediment chlorophyll A (SCHLA) was related with none of the parameters (Tables 25 and 26). Compared with seston chlorophyll A (WCHLA), correlations between PCHLA and nutrient parameters were weaker (e.g., mean Pearson correlation coefficient (r) absolute value WCHLA = 0.39; PCHLA = 0.19), and correlation directionality (+/-) was almost completely opposite. For example, total phosphorus was positively related with WCHLA (r = 0.353) and inversely related with PCHLA (r = -0.248). Perhaps not surprisingly, WCHLA was not significantly related with PCHLA (r = -0.031; p=0.49); however, it was weakly correlated with SCHLA (0.244; p<0.001).

Table 25. Correlation analysis results for periphyton chlorophyll A versus nutrient parameters. REMAP (2002-2006) sample data.

Periphyton Chlorophyll A

Pearson		Pearson (Log10)		Spearman Rank	
Parameter	r	Parameter (Log10)	r	Parameter	rho
ORGN	-0.052	PRTCP	-0.277**	PRTCP	-0.310
TKN	-0.044	TP	-0.248**	TP	-0.286
PRTCP	-0.036	TKN	-0.244**	TKN	-0.255
NHx	-0.024	ORGN	-0.228**	ORGN	-0.245
TP	-0.023	NHx	-0.123*	NHx	-0.230
DOP	-0.016	DOP	-0.1141*	DOP	-0.145
TN	0.032	TN	0.0932*	TN	0.059
DIN	0.040	DIN	0.162**	DIN	0.101
NOx	0.044	DOP:TP	0.177**	NOx	0.120
TN:TP	0.089*	NOx	0.192**	DOP:TP	0.192
DIN:TN	0.104*	DIN:TN	0.218**	DIN:TN	0.241
DOP:TP	0.131*	TN:TP	0.251**	TN:TP	0.270

*p<0.05; **p<0.001

Table 26. Correlation analysis results for sediment chlorophyll A versus nutrient parameters. REMAP (2002-2006) sample data.

Sediment Chlorophyll A

Pearson		Pearson (Log10)		Spearman Rank	
Parameter	r	Parameter (Log10)	r	Parameter	rho
TN	-0.030	DOP	-0.074	NOx	-0.150
DIN	-0.026	TN	-0.058	DIN	-0.145
NOx	-0.026	DOP:TP	-0.057	TN	-0.136
ORGN	-0.018	DIN	-0.044	DOP	-0.128
TKN	-0.013	TP	-0.037	DIN:TN	-0.082
PRTCP	-0.006	NOx	-0.036	NHx	-0.071
NHx	-0.004	PRTCP	-0.024	TP	-0.066
TP	0.000	TN:TP	-0.015	DOP:TP	-0.034
DOP	0.002	DIN:TN	-0.014	TN:TP	-0.034
DOP:TP	0.038	NHx	0.010	PRTCP	-0.029
DIN:TN	0.046	TKN	0.032	TKN	0.000
TN:TP	0.081	ORGN	0.034	ORGN	0.006

*p<0.05; **p<0.001

Diel dissolved oxygen and stream metabolic relationships with nutrients and algal chlorophyll A

Correlation analysis was performed to evaluate relationships among nutrient variables and chlorophyll A, dissolved oxygen, and stream metabolism nutrient response variables. Many of the variables were not normally distributed; therefore, results from the Spearman (nonparametric) correlation analysis are reported in Table 27. Pearson correlation coefficients ranked similarly in directionality and magnitude as the Spearman rank coefficients.

Table 27. Spearman rank correlation coefficients (rho) among dissolved oxygen and stream metabolism response variables and with nutrient and chlorophyll A parameters. REMAP sampling data (2002-2006).

	AVGDO	AVGMAXDO	AVGMINDO	AVGDORNG	AVGGPP	AVGCR	AVGNPP	AVGPR
AVGMAXDO	0.748							
AVGMINDO	0.688	0.158						
AVGDORNG	0.224	0.757	-0.448					
AVGGPP	0.325	0.752	-0.248	0.847				
AVGCR	-0.453	-0.029	-0.661	0.404	0.458			
AVGNPP	0.773	0.808	0.306	0.515	0.589	-0.353		
AVGPR	0.631	0.846	0.100	0.708	0.818	-0.054	0.886	
NHX	-0.360	-0.136	-0.408	0.137	-0.010	0.307	-0.283	-0.165
NOX	0.134	-0.062	0.367	-0.322	-0.199	-0.259	-0.021	-0.102
DIN	0.081	-0.086	0.313	-0.308	-0.200	-0.217	-0.068	-0.134
TKN	-0.295	-0.042	-0.494	0.239	0.193	0.375	-0.097	-0.036
ORGN	-0.278	-0.027	-0.488	0.245	0.204	0.370	-0.080	-0.024
TN	0.051	-0.069	0.241	-0.258	-0.153	-0.156	-0.067	-0.124
DINTN	0.265	0.001	0.522	-0.322	-0.226	-0.369	0.056	-0.027
DOP	-0.244	-0.196	-0.123	-0.119	-0.090	0.178	-0.264	-0.228
PRTCP	-0.236	-0.079	-0.349	0.113	0.080	0.200	-0.046	-0.061
TP	-0.283	-0.100	-0.344	0.081	0.073	0.272	-0.148	-0.115
DOPTP	0.051	-0.034	0.219	-0.130	-0.089	0.033	-0.185	-0.112
TNTP	0.298	0.093	0.442	-0.204	-0.116	-0.302	0.102	0.049
PCHLA	0.404	0.423	0.203	0.273	0.341	0.020	0.314	0.399
SCHLA	0.383	0.425	0.072	0.333	0.321	-0.023	0.355	0.353
WCHLA	0.016	0.294	-0.349	0.454	0.443	0.188	0.322	0.361

Examination of the directionality and magnitude of correlation coefficients provided useful insight into stream nutrient response relationships. Correlations among nutrient variables and average diel minimum DO (AVGMINDO) tended to be stronger than correlations between nutrient variables and other DO response variables. Average diel maximum DO (AVGMAXDO) tended to have weaker relationships with nutrient variables and stronger relationships with algal chlorophyll A variables, particularly periphyton (PCHLA) and sediment (SCHLA) chlorophyll A. Notably, seston chlorophyll A (WCHLA) was inversely correlated with AVGMINDO, which happened to be positively correlated with PCHLA.

AVGMAXDO tended to be correlated more strongly with gross primary production (AVGGPP), while AVGMINDO correlated more strongly with community respiration (AVGCR). Average diel DO range (AVGDORNG), which encompasses both diel maxima and diel minima, was relatively strongly correlated with both gross primary production rate (AVGGPP) and community respiration rate (AVGRESP), instream metabolic processes that produce and consume oxygen.

Among the strongest correlations with nitrogen parameters, AVGMINDO was inversely related with ammonia (NHx) and organic nitrogen (ORGN), and was positively related with nitrate-nitrogen (NOx) and ratio of dissolved inorganic nitrogen to total nitrogen (DIN:TN). Conversely, AVGCR was positively related with ammonia (NHx) and organic nitrogen (ORGN), and it was inversely related with NOx and ratio of dissolved inorganic nitrogen to total nitrogen (DIN:TN).

DO and stream metabolism correlations with phosphorus parameters were slightly weaker than with nitrogen parameters. AVGMINDO and AVGDO were inversely correlated with dissolved orthophosphate (DOP) and total phosphorus (TP), while these variables were positively related with AVGCR. Particulate-bound phosphorus (PRTCP) appeared to be slightly more strongly related than DOP with AVGMINDO and AVGCR. Phosphorus variables were not significantly related with either AVGDORNG or AVGGPP.

Simple linear regressions using individual nutrient and chlorophyll A variables were able to explain at best a minority ($\leq$30%) of the variability in DO and stream metabolism parameters. Examples of the best linear models include: Log10WCHLA v. AVGGPP (r^2 = 0.30); Log10WCHLA v. AVGDORNG (r^2 = 0.29) (Figures 23a-b); L10TKN v. AVGMINDO (r^2 = 0.25); L10TKN v. AVGCR (r^2 = 0.13) (Figures 24a-b).

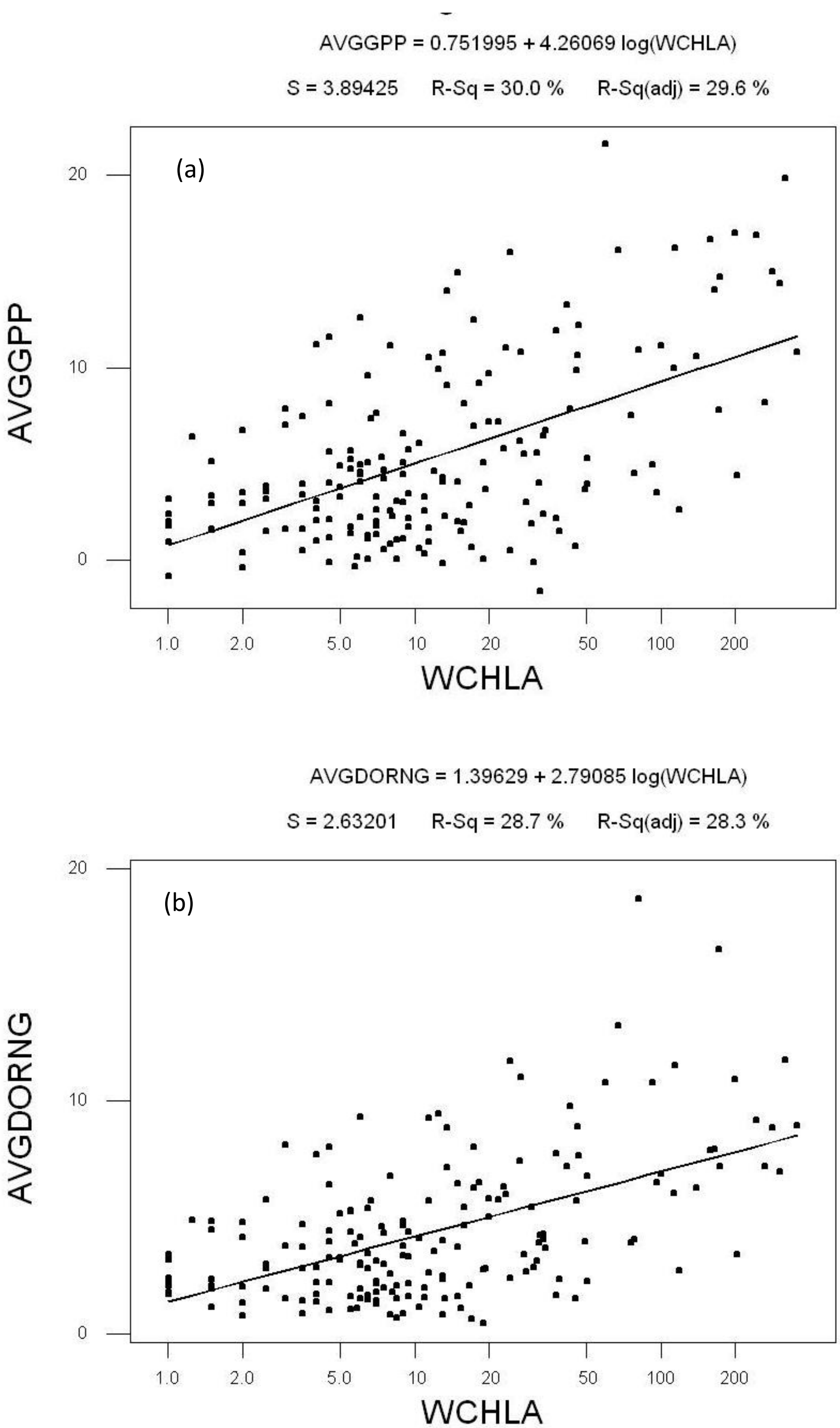
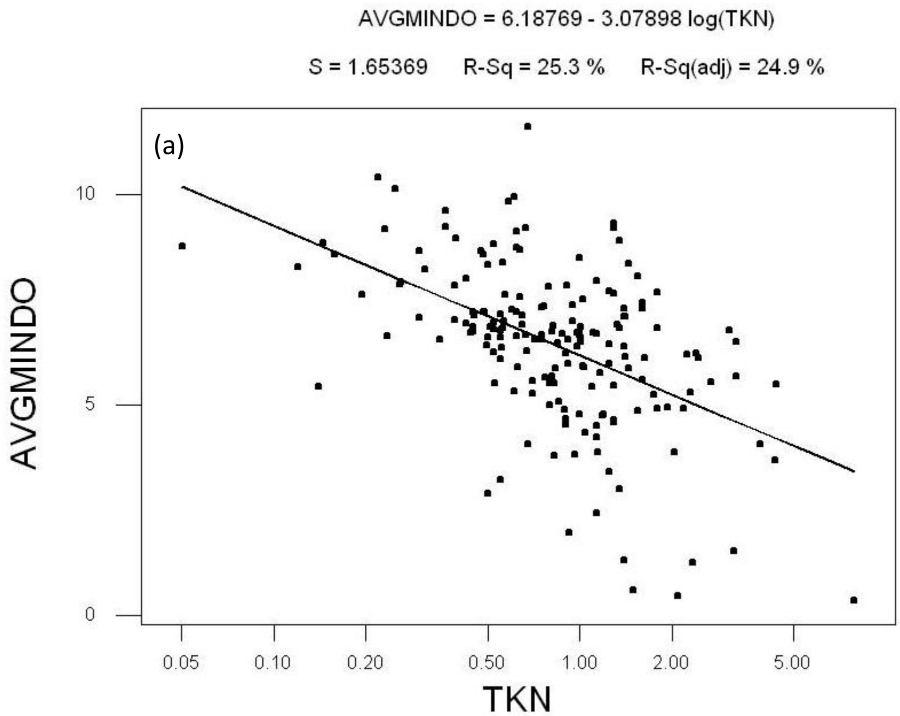


Figure 23(a-b). Ordinary least squared regression of seston chlorophyll A (WCHLA) versus (a) average diel gross primary production (AVGGPP); and (b) dissolved oxygen range (AVGDORNG). REMAP (2002-2006) sampling data.



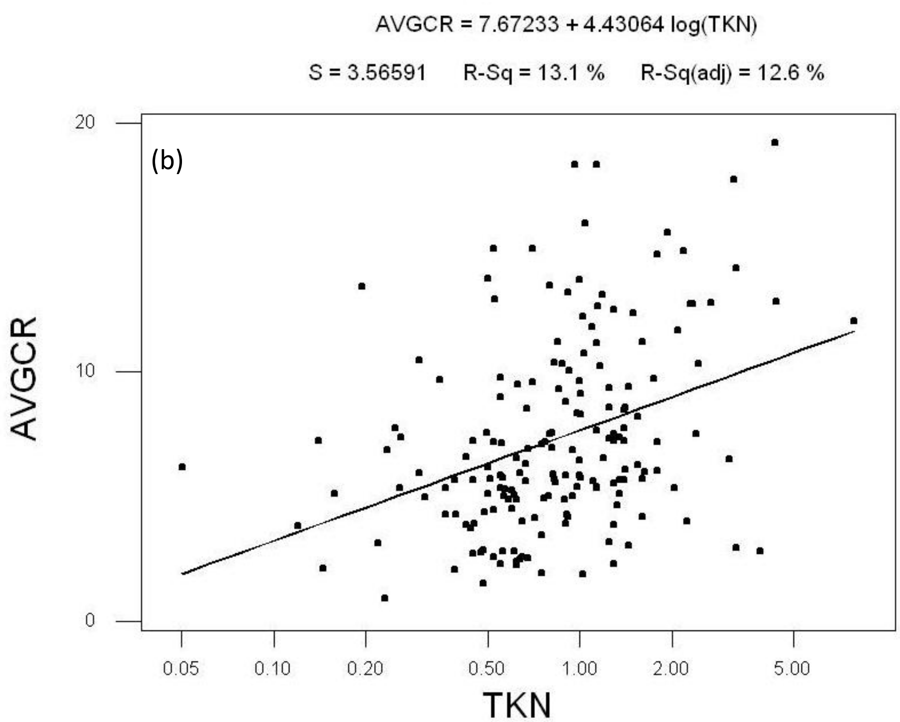


Figure 24(a-b). Ordinary least squared regression of total Kjeldahl nitrogen (TKN) versus (a) average diel minimum dissolved oxygen (AVGMINDO); and (b) average community respiration (AVGCR). REMAP (2002-2006) sampling data.

4.5. Nutrients and phytoplankton composition

To support nutrient criteria development, phytoplankton composition samples were collected at 30 stream locations during Summer 2011. The locations were chosen from a statewide network of 75 fixed stations from IDNR's ambient stream monitoring and upstream/downstream city monitoring projects. Phytoplankton sampling sites were located on medium-to-large interior rivers and streams. Surface watershed areas draining to these sites range from 408 – 13,412 mi^2 with a median area of 1,709 mi^2. The sampling locations provide broad geographic coverage within each of Iowa's five major drainage divisions. An effort was made to choose sites that would represent a broad range of environmental conditions encompassing varying influence from dams, urban development, and major wastewater treatment facilities.

Water quality monitoring is conducted monthly at IDNR fixed monitoring stations by State Hygienic Laboratory personnel. A phytoplankton composition sample was collected at each of the 30 chosen sites during the September monthly visit. To evaluate temporal changes, an additional phytoplankton sample was collected in July at 15 of the 30 sites. Phytoplankton samples were collected by filling a 1-liter sample container below the water surface in a well-mixed area of the stream. Phytoplankton sample preparation and analysis was done following established standard operating procedures.

Algal biomass was higher in September than July at almost every site sampled in both months. September levels ranked higher than July levels at 87.5% (13/15) of the sites for chlorophyll A and 100% for wet biomass. Wilcoxon signed rank test (nonparametric alternative to paired T-test) results confirmed that September levels of phytoplankton chlorophyll A and total wet mass were both significantly higher than July levels ($p=0.0026$, $p=0.0007$).

September chlorophyll A levels generally were three to four times higher than July levels. The median and range of chlorophyll A concentration in July samples were 50 and 7-150, respectively, compared with 160 and 21-480 for September samples. The subset of sites selected for July and September sampling purposely included several sites on the Cedar River where chlorophyll A levels have ranked among the highest in the ambient stream and city monitoring projects. Levels were generally lower among the other fifteen sites sampled in September that were located on streams other than the Cedar River; the chlorophyll A median and range for this group were 66 and 2-180, respectively.

Phytoplankton taxa biomass and composition (% total biomass) in July and September samples were dominated by genera from three major taxonomic divisions: Bacillariophyta (diatoms), Chlorophyta (green algae), and Cyanophyta (blue-green algae / Cyanobacteria) (Table 28; Figures 25 and 26). Other taxa were dominant at only one location (Des Moines River upstream of Ottumwa) in a July sample. Protozoa (41%) and Cryptophyta (29%) dominated this phytoplankton assemblage sample, which had a relatively low biomass level (i.e., , chlr. A = 8 ug/L wet mass = 1.1 mg/L) that was not indicative of algal "bloom" conditions. Wilcoxon signed rank testing did not reveal any major shifts in taxa composition between July and September samples. September biomass levels of taxa belonging to the Bacillariophyta, Chlorophyta, and Cyanophyta divisions all increased significantly from July levels.

Table 28. Summary statistics for nutrient and phytoplankton compositional metrics sampled at 30 large wadeable and nonwadeable streams in July and October 2011.

Variable	N	Mean	Std.Dev.		Minimum	25th %	Median	75th %	Maximum
NHX	45	0.05	0.00		0.05	0.05	0.05	0.05	0.05
NOX	45	3.75	3.15		0.10	1.10	2.50	6.55	9.70
TKN	45	1.16	0.57		0.30	0.70	1.10	1.50	2.50
TNcalc	45	4.92	2.93		0.90	2.72	4.10	7.25	10.50
DINTN	45	0.65	0.29		0.06	0.54	0.75	0.89	0.97
DOP	45	0.07	0.06		0.02	0.02	0.07	0.11	0.23
TP	45	0.21	0.07		0.06	0.15	0.20	0.26	0.39
DOPTP	45	0.35	0.24		0.07	0.14	0.30	0.50	0.88
TNTP	45	26.14	17.77		4.74	11.84	21.20	40.45	74.29
WCHLA	45	110.70	115.20		2.00	24.50	66.00	150.00	480.00
Chlr Pct.	45	18.76	13.57		0.19	10.19	17.35	23.54	80.25
Chry Pct.	45	0.36	0.75		0.00	0.00	0.00	0.32	3.05
Cryp Pct.	45	5.15	7.05		0.21	1.16	2.54	4.12	29.35
Cyn Pct.	45	27.72	22.50		0.05	11.96	23.02	34.18	95.26
Dino Pct.	45	0.07	0.21		0.00	0.00	0.00	0.02	1.38
Dtm Pct.	45	37.45	19.42		3.06	21.01	39.88	48.26	91.60
Eugl Pct.	45	1.31	3.18		0.00	0.00	0.00	0.77	16.60
Prot Pct.	45	9.18	7.92		0.00	3.17	7.60	13.07	40.73
Chlr Wtm.	45	4.64	5.03		0.00	0.83	2.41	6.20	21.02
Chry Wtm.	45	0.04	0.09		0.00	0.00	0.00	0.02	0.42
Cryp Wtm.	45	0.54	0.36		0.02	0.30	0.44	0.79	1.50
Cyn Wtm.	45	9.98	18.30		0.01	0.99	2.40	12.77	94.77
Dino Wtm.	45	0.01	0.02		0.00	0.00	0.00	0.00	0.09
Dtm Wtm.	45	8.00	7.62		0.13	1.64	5.46	11.21	26.56
Eugl Wtm.	45	0.19	0.48		0.00	0.00	0.00	0.11	2.26
Prot Wtm.	45	1.32	1.24		0.00	0.48	0.87	1.88	6.23
Total Wtm.	45	24.71	25.15		0.59	6.01	19.38	34.40	136.78

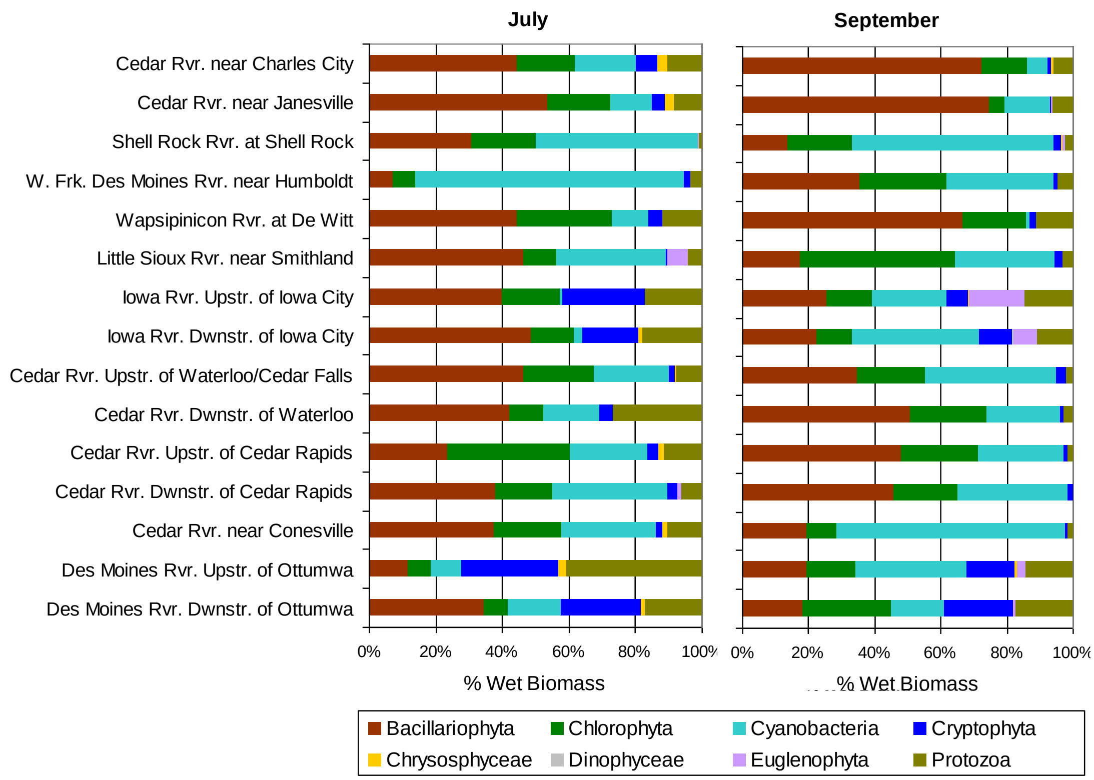


Figure 25. Phytoplankton composition (% total wet biomass) by major taxonomic division. Ambient stream and city monitoring locations sampled in July and September 2011.

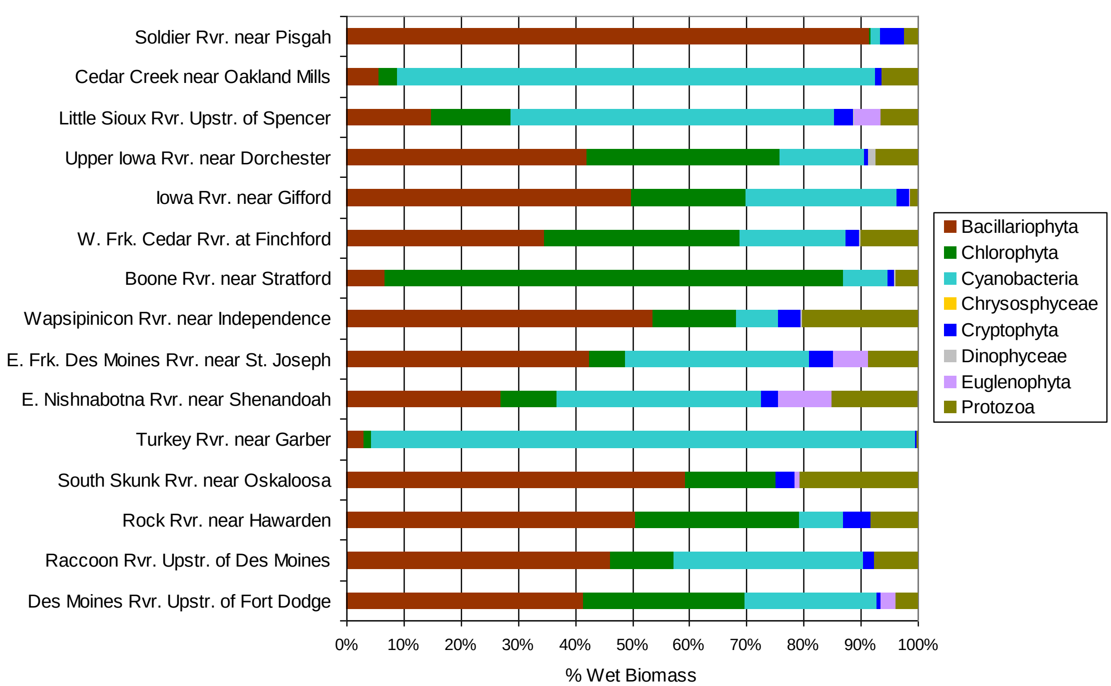


Figure 26. Phytoplankton composition (% total wet biomass) by major taxonomic division. Ambient stream and city monitoring locations sampled in September 2011.

Chlorophyll A is commonly used as a surrogate indicator of algal biomass; however, as mentioned earlier, cellular concentrations of this pigment can vary depending on algal species and environmental conditions. Chlorophyll A was significantly related with total algal wet biomass in samples collected in July and September 2011. A power regression model provided a slightly better fit than a linear model of the relationship (Figure 27). The variability in this predictive relationship increased substantially when chlorophyll A levels reached approximately 25 ug/L.

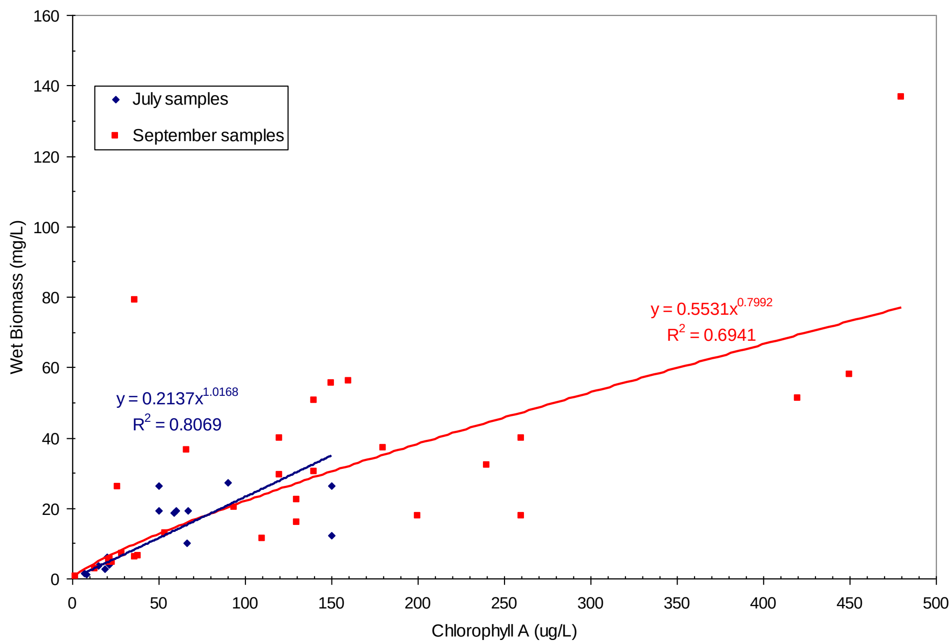


Figure 27. Power regression of algal chlorophyll A and wet biomass among stream phytoplankton samples collected in July and September, 2011.

Chlorophyll A levels in the 20-30 ug/L range have been characterized as indicative of nuisance algal bloom conditions in Minnesota lakes, (MPCA 2005). A Lowess trend analysis of data from worldwide north temperate lakes (Downing et al. 2003) shows that Cyanobacteria are likely to dominate lake phytoplankton assemblages (>50% biomass) when chlorophyll A exceeds approximately 25-30 ug/L and total algal biomass exceeds approximately 7-8 mg/L.

Similar to the lakes relationship illustrated in Downing et al. (2003), the proportional and total biomass of Cyanobacteria did increase linearly with increasing phytoplankton biomass in 45 samples collected from 30 large wadeable and nonwadeable Iowa streams (Figure 28 and 29). The bloom guidelines described above are generally consistent with an apparent threshold in the 2011 sampling data at which Cyanobacteria may become dominant in Iowa streams. For example, Cyanobacteria did not exceed 50% of total phytoplankton biomass on any of the eleven sample occasions in which chlorophyll A was less than 25 ug/L and total biomass was less than 7 mg/L (% Cyanobacteria: median, 16.1%; range, 0.8%-35.6%). In contrast, Cyanobacteria biomass was more than 50% in 6 of 34 samples (18%) that exceeded the bloom guidelines (median, 26.1%; range, 0.05%-95.3%).

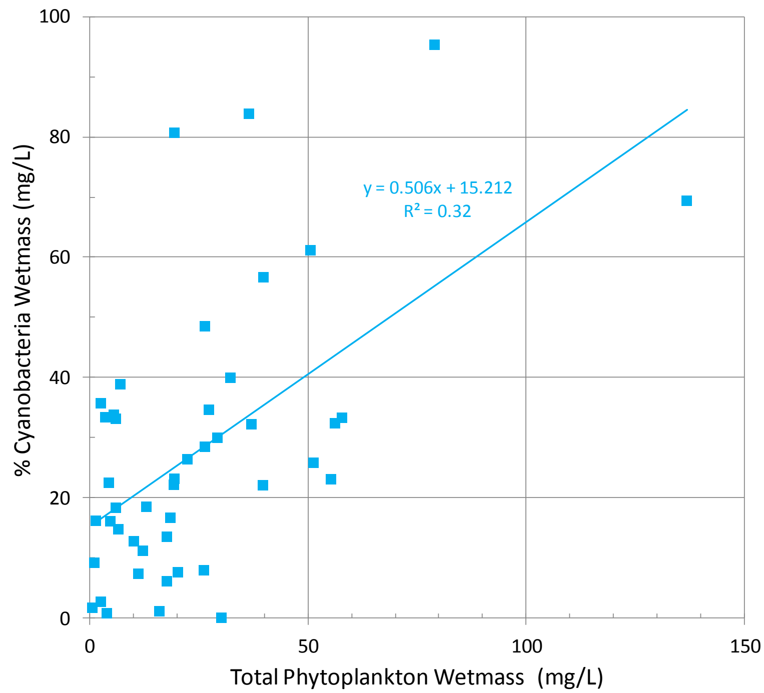


Figure 28. Total phytoplankton wetmass versus percentage of wet mass comprised of Cyanobacteria in stream phytoplankton samples collected from ambient and city monitoring locations: July and September, 2011.

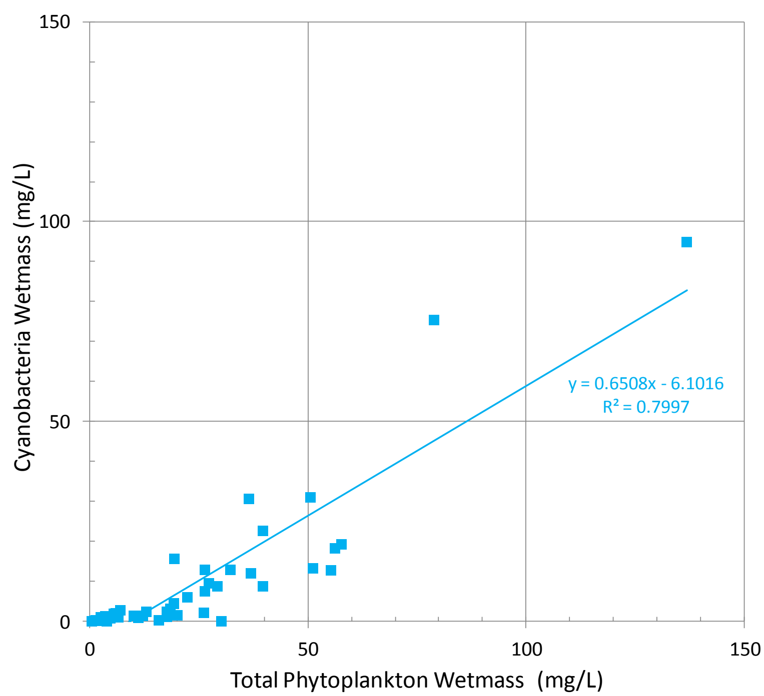


Figure 29. Total phytoplankton wetmass versus Cyanobacteria wet mass in stream phytoplankton samples collected from ambient and city monitoring locations: July and September, 2011.

Correlation analysis was performed to examine relationships among the 2011 nutrient and phytoplankton sampling variables. Results of Pearson and Spearman (nonparametric) rank correlation analysis were generally similar (Table 29 and 30). Total Kjeldahl nitrogen (TKN) and the ratio of dissolved orthophosphate phosphorus to total phosphorus (DOP:TP) were generally the nutrient variables most strongly correlated with phytoplankton composition and wetmass metrics. TKN was correlated positively with total phytoplankton wetmass, and also positively correlated with wetmass of Bacillariophyta, Chlorophyta, and Cyanophyta (Figure 30), the dominant phytoplankton divisions in 2011 sampling. TKN was inversely correlated with % wetmass of Chrysophytes, Cryptophytes, and Protozoa, which comprise a larger fraction of total wetmass at lower levels. Phytoplankton metric correlations with DOP:TP were exactly opposite in direction from those with TKN (Figure 31). DOP:TP is an indicator of the proportion of total phosphorus that is readily available for biological uptake. Similar patterns were observed in correlations with the ratio of dissolved inorganic nitrogen and total nitrogen (DIN:TN). These results, like previous correlations with seston chlorophyll A, reflect the assimilation of biologically available N and P with accrual of algal biomass. Total nitrogen (TNCALC) was inversely correlated with total phytoplankton wetmass. High levels of TN are comprised mostly of nitrate-nitrogen, which itself was inversely correlated with total wetmass. These relationships could indicate that nitrogen is usually not limiting, and that other environmental factors that occur in association with high nitrate levels are responsible for inhibiting algal growth.

Of the phytoplankton metrics, Bacillariophyta wetmass appeared to be the more strongly correlated with various nutrient variables than was Chlorophyta wetmass or Cyanobacteria wetmass. TKN was the most strongly correlated nutrient variable overall, and the correlation with Bacillariophyta wetmass was the highest ranking correlation. TN was weakly correlated with Chlorophyta wetmass. An interesting pattern was observed when TN was plotted against the phytoplankton division comprising the largest percentage of total wetmass. The pattern seems to change at levels of TN above 5 mg/L, where samples having high TN levels tend to be less dominated by a single taxonomic group (i.e., < 50%) compared with samples having lower levels of TN (Figure 32). Samples associated with high TN levels tended to have Bacillariophyta as the most abundant group compared with a greater mixture of dominant taxonomic groups associated with samples having lower TN levels. Of particular note, Cyanobacteria % wetmass only exceeded 50% in samples associated with TN less than 5 mg/L.

TP was not significantly correlated with total phytoplankton wetmass. There was no discernible pattern between total phosphorus (TP) and the relative dominance of any particular type of phytoplankton (Figure 33). DOP was inversely correlated with total phytoplankton wetmass. At the elevated levels observed in this sampling project, TP appeared to be less relevant as a nutrient predictor variable than DOP. As Figure 34 shows, the highest levels of phytoplankton wetmass tended to occur when the ratio of dissolved orthophosphate to total phosphorus was relatively low (< 0.40). More data of this kind are needed to gain a better understanding of nutrient-phytoplankton relationships.

Table 29. Pearson correlation coefficients (r) for nutrient variables versus phytoplankton percent composition (PCT) and wetmass (WM) variables sampled in July and September, 2011 at ambient monitoring locations on large wadeable and nonwadeable streams (n=45). (Bolded values are statistically significant at p<0.05.)

	NHX	NOX	TKN	TNCALC	DINTN	DOP	TP	DOPTP	TNTP
CHLRPCT	*	-0.14	-0.02	-0.15	-0.18	-0.19	-0.24	-0.14	-0.03
CHLRWM	*	**-0.43**	**0.53**	**-0.36**	**-0.61**	**-0.47**	-0.03	**-0.51**	**-0.33**
CHRYPCT	*	**0.58**	**-0.33**	**0.55**	**0.41**	0.25	-0.19	**0.46**	**0.59**
CHRYWM	*	**0.47**	-0.09	**0.49**	0.29	0.06	-0.07	0.12	**0.43**
CRYPPCT	*	**0.34**	**-0.43**	0.28	**0.37**	**0.58**	-0.08	**0.74**	0.27
CRYPWM	*	-0.20	**0.45**	-0.13	**-0.34**	-0.07	0.05	-0.14	-0.18
CYNPCT	*	-0.27	0.23	-0.25	-0.23	-0.10	0.20	-0.20	-0.29
CYNWM	*	-0.29	**0.37**	-0.23	**-0.34**	-0.29	0.17	**-0.32**	-0.23
DINOPCT	*	-0.03	-0.21	-0.07	0.12	-0.13	**-0.34**	0.00	**0.32**
DINOWM	*	-0.12	-0.12	-0.16	0.02	-0.22	**-0.31**	-0.11	0.14
DTMPCT	*	0.17	0.11	0.21	0.09	-0.22	-0.06	-0.23	0.20
DTMWM	*	**-0.42**	**0.81**	-0.29	**-0.62**	**-0.56**	0.08	**-0.64**	-0.29
EUGLPCT	*	-0.19	-0.04	-0.22	-0.04	**0.40**	0.29	0.26	**-0.32**
EUGLWM	*	-0.29	**0.38**	-0.24	**-0.37**	-0.07	0.09	-0.14	-0.27
PROTPCT	*	**0.31**	**-0.48**	0.24	**0.37**	**0.46**	-0.03	**0.57**	0.22
PROTWM	*	-0.14	0.27	-0.10	**-0.30**	-0.19	0.12	-0.26	-0.18
WCHLA	*	**-0.42**	**0.81**	-0.29	**-0.55**	**-0.55**	0.13	**-0.65**	**-0.30**
WETMASS	*	**-0.44**	**0.65**	**-0.34**	**-0.58**	**-0.48**	0.15	**-0.54**	**-0.33**

* Correlation result not available; NHX values all below detection limit

Table 30. Spearman rank correlation coefficients (rho) for nutrient variables versus phytoplankton percent composition (PCT) and wetmass (WM) variables sampled in July and September, 2011 at ambient monitoring locations on large wadeable and nonwadeable streams (n=45).

	NHX	NOX	TKN	TNCALC	DINTN	DOP	TP	DOPTP	TNTP
CHLRPCT	*	-0.06	0.09	-0.07	-0.13	-0.37	-0.27	-0.23	0.07
CHLRWM	*	-0.40	0.57	-0.29	-0.54	-0.66	-0.12	-0.64	-0.22
CHRYPCT	*	0.46	-0.34	0.42	0.50	0.40	-0.19	0.53	0.44
CHRYWM	*	0.45	-0.27	0.40	0.45	0.33	-0.18	0.45	0.42
CRYPPCT	*	0.32	-0.39	0.25	0.40	0.53	0.00	0.56	0.23
CRYPWM	*	-0.19	0.40	-0.12	-0.31	-0.16	0.10	-0.22	-0.15
CYNPCT	*	-0.27	0.28	-0.20	-0.28	-0.07	0.25	-0.19	-0.32
CYNWM	*	-0.39	0.52	-0.26	-0.47	-0.50	0.02	-0.56	-0.27
DINOPCT	*	-0.07	-0.18	-0.12	-0.01	-0.09	-0.18	0.00	0.00
DINOWM	*	-0.10	-0.16	-0.14	-0.04	-0.12	-0.19	-0.03	-0.03
DTMPCT	*	0.16	0.20	0.19	0.04	-0.24	-0.07	-0.23	0.22
DTMWM	*	-0.38	0.71	-0.24	-0.56	-0.73	-0.06	-0.76	-0.18
EUGLPCT	*	-0.26	0.11	-0.26	-0.25	0.25	0.25	0.17	-0.36
EUGLWM	*	-0.29	0.18	-0.28	-0.30	0.15	0.18	0.09	-0.34
PROTPCT	*	0.33	-0.45	0.21	0.40	0.55	0.08	0.56	0.17
PROTWM	*	-0.21	0.38	-0.18	-0.35	-0.30	0.09	-0.36	-0.20
WCHLA	*	-0.44	0.80	-0.30	-0.65	-0.78	-0.04	-0.83	-0.24
WETMASS	*	-0.51	0.65	-0.37	-0.63	-0.67	-0.03	-0.71	-0.34

* Correlation result not available; NHX values all below detection limit

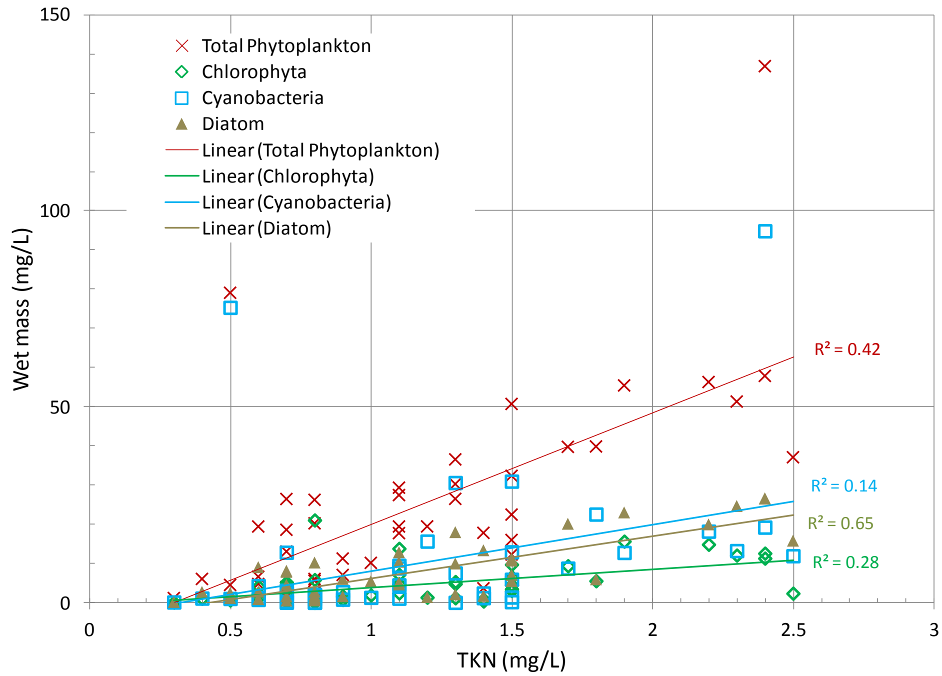


Figure 30. Linear regression of Total Kjeldahl nitrogen versus total wetmass and wetmass of three dominant phytoplankton groups (Bacillariophyta, Chlorophyta, and Cyanobacteria) sampled from large wadeable and nonwadeable stream monitoring locations in July and September 2011..

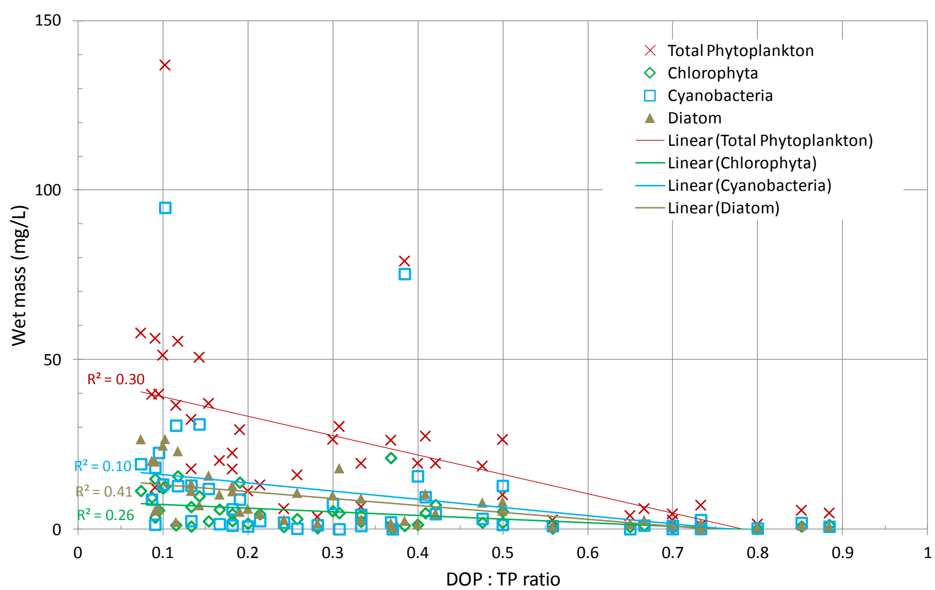


Figure 31. Linear regression of dissolved orthophosphate : total phosphorus ratio (DOP:TP) versus total wetmass and wetmass of three dominant phytoplankton groups (Bacillariophyta, Chlorophyta, and Cyanobacteria) sampled from large wadeable and nonwadeable stream monitoring locations in July and September 2011.

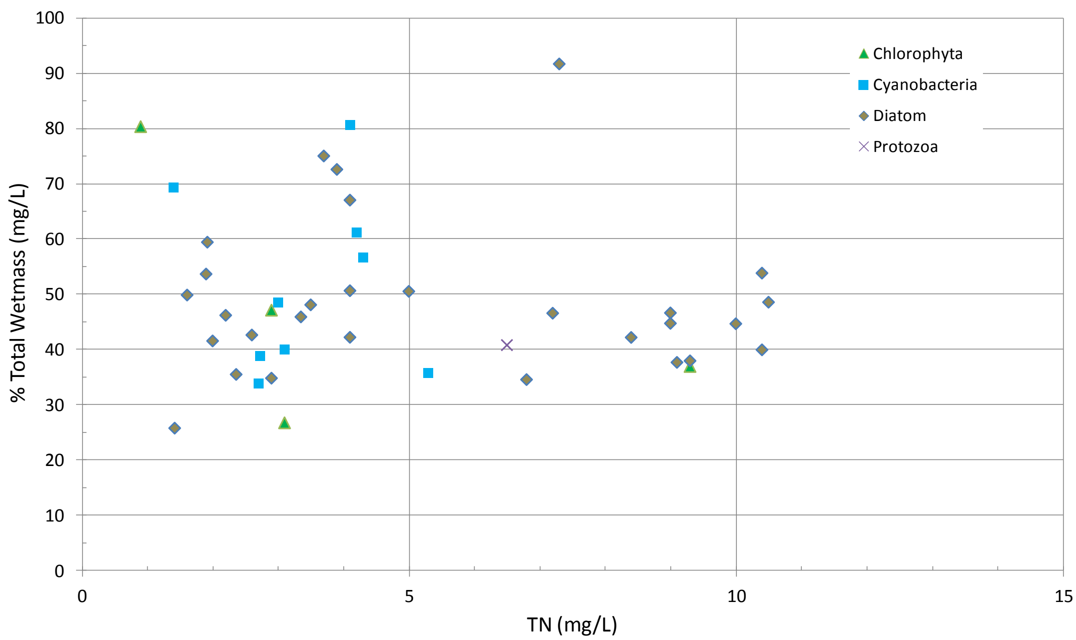


Figure 32. Total nitrogen (calculated) versus % total wetmass comprised of the most abundant phytoplankton group (Chlorophyta, Cyanobacteria, Bacillariophyta, Protozoa) in 45 samples collected from large wadeable and nonwadeable stream monitoring locations in July-September 2011.

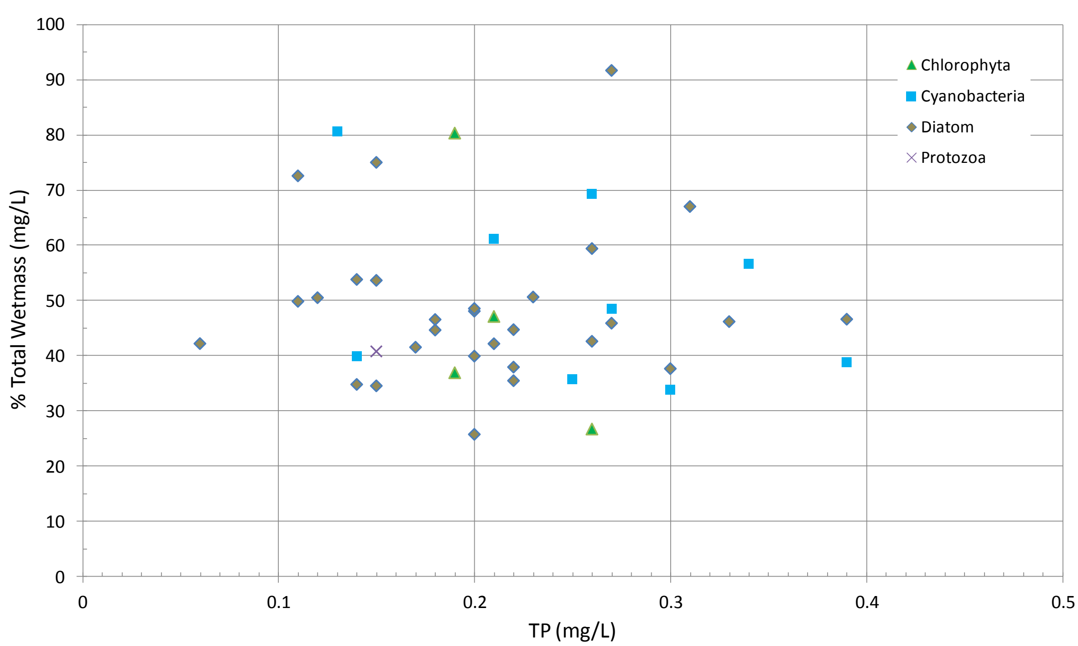


Figure 33. Total phosphorus versus % total wetmass comprised of the most abundant phytoplankton group (Chlorophyta, Cyanobacteria, Bacillariophyta, Protozoa) in 45 samples collected from large wadeable and nonwadeable stream monitoring locations in July-September 2011.

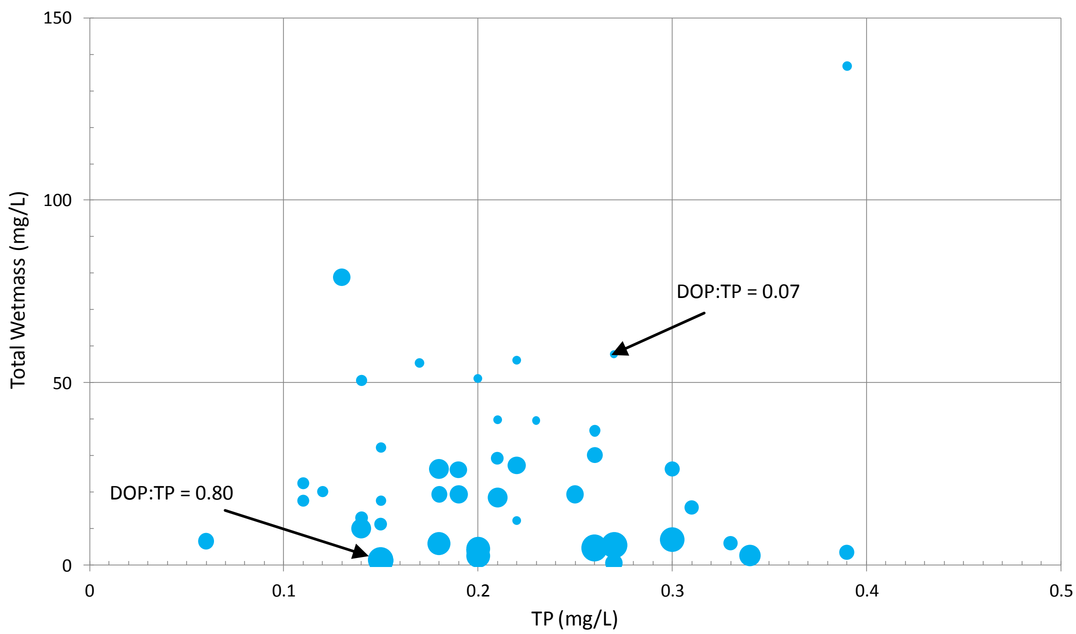


Figure 34. Total phosphorus versus total phytoplankton wetmass. Bubble size is proportional to the ratio of dissolved orthophosphate to total phosphorus (DOP : TP). 45 samples collected from large wadeable and nonwadeable stream monitoring locations in July-September 2011.

<u>Comparison of lake and stream phytoplankton assemblages</u>

The 2011 stream phytoplankton sampling data were compared with data from Iowa lakes to evaluate differences in phytoplankton assemblages representing lotic and lentic waterbodies. Phytoplankton composition sampling has been a component of the IDNR ambient lake monitoring program for several years; however, this type of data have rarely been collected from Iowa's rivers and streams. In 2011, a total of 392 phytoplankton samples were collected from 133 lakes (artificial and natural) and analyzed for taxonomic composition by the Iowa State University Limnology Laboratory. Typically, three samples were collected from the deepest area of each lake approximately one month apart between June and September.

Data analysis procedures for lake and stream samples were similar. Phytoplankton taxa were typically identified to the genus taxonomic level. A total of 83 taxonomic classifications were recorded (Appendix 14). Stream samples contained 69 taxa compared with 61 taxa from lakes. 47 taxa were found in both lakes and streams, 14 taxa were found in lakes only, and 22 taxa were found only in streams. Stream samples tended to contain more taxa than lake samples. The median number of taxa found in stream samples was 25 and the range was 16-35, compared with a median of 9 and range of 2-24 taxa in lake samples.

In stream samples, genera of Chlorophyta (green algae) were the most numerous (30), followed by Bacillariophyceae (diatoms) (25) and Cyanobacteria (blue-green algae) (16). The most frequently observed types of green algae were *Selenastrum* (98% of samples), *Ankistrodesmus* (98%), *Scenedesmus* (96%) and *Actinastrum* (91%). The most common diatoms were *Cyclotella*

(100%), *Navicula* (98%), and Melosira (93%). *Merismopedia* (88%), *Aphanocapsa* (88%), *Limnothrix* (69%),and *Microcyctis* (69%) were the most common blue-green taxa.

In lake samples, *Schroederia* (43%) and *Scenedesmus* (41%) were the most common types of green algae. *Cyclotella* (46%), *Aulacoseira* (40%) and *Synedra* (34%) were the most common diatoms, and *Microcyctis* (100%), *Aphanizomenon* (61%) and *Anabaena* (54%) were the most common types of blue-green algae.

In terms of algal biomass, levels of lake and stream samples were generally similar in the middle (interquartile) range of data; however, maximum biomass levels among lake samples greatly exceeded stream maximum levels (Figure 35). The median biomass (18.2 mg/L) of lake samples was not significantly different than the stream sample median (19.4 mg/L). The occurrence of a phytoplankton bloom (i.e., algal biomass exceeding 6 mg/L) were indicated in 89% of lake samples and 75% of stream samples. Nine phytoplankton taxa exceeded the bloom threshold in stream samples, and 20 taxa exceeded the bloom threshold in lake samples (Tables 31 and 32).

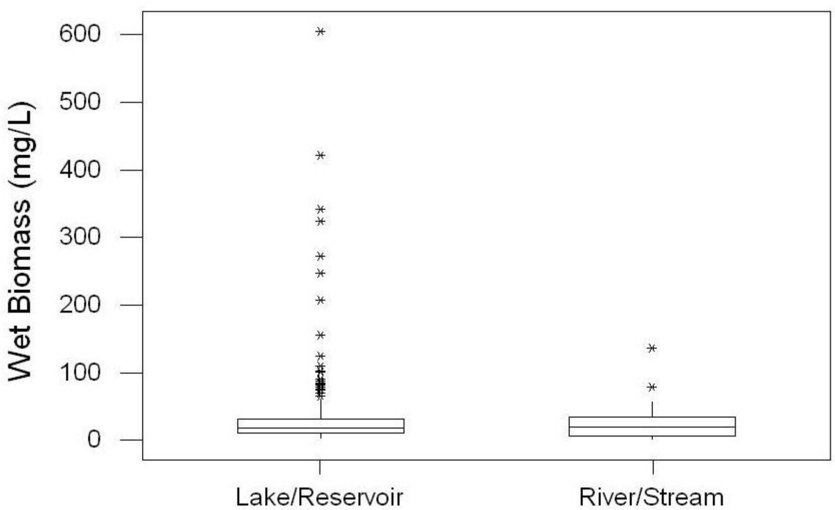


Figure 35. Algal phytoplankton biomass in stream samples (n=45) and lake samples (n=392) collected in Summer 2011.

Microcystis occurred at bloom levels in 64.8% of lake samples and 11.1% of stream samples. Other algal taxa that individually occurred at bloom levels with some frequency in streams include the diatom genera, *Cyclotella* (20.0%) and *Melosira* (11.1%), and the Cyanobacteria genus *Aphanocapsa* (15.6%). In lake samples, besides *Microcystis*, another HAB producing algal taxon, *Aphanizomenon*, occurred at bloom levels on a relatively frequent basis (20.2%).

Table 31. Phytoplankton taxa having wet biomass exceeding "nuisance bloom" levels (≥ 6 mg/L) in 45 stream phytoplankton samples collected in Summer 2011.

Stream Phytoplankton Samples (n=45)			
Taxa Division	Genus/Taxa	Maximum Wet Biomass (mg/L)	% Samples Wetmass ≥ 6mg/L
CYANOBACTERIA	Microcyctis	75.0	11.1%
CYANOBACTERIA	Aphanocapsa	50.1	15.6%
BACILLARIOPHYCEAE	Cyclotella	17.6	20.0%
CHLOROPHYTA	Dictyosphaerium	16.5	2.2%
CYANOBACTERIA	Merismopedia	15.4	4.4%
BACILLARIOPHYCEAE	Melosira	14.0	11.1%
CYANOBACTERIA	Planktothrix	13.8	4.4%
CHLOROPHYTA	Pandorina	6.3	2.2%
PROTOZOA	Protozoa	6.2	2.2%

Table 32. Phytoplankton taxa having wet biomass exceeding "nuisance bloom" levels (≥ 6 mg/L) in 392 lake phytoplankton samples collected in Summer 2011.

Lake Phytoplankton Samples (n=392)			
Taxa Division	Genus/Taxa	Maximum Wet Biomass (mg/L)	% Samples Wetmass ≥ 6mg/L
CHLOROPHYTA	Volvox	586.0	0.3%
CYANOBACTERIA	Microcystis	417.7	64.8%
CYANOBACTERIA	Planktolyngbya	168.4	7.1%
BACILLARIOPHYCEAE	Aulacoseira	134.8	1.5%
CYANOBACTERIA	Aphanizomenon	85.6	20.2%
CYANOBACTERIA	Oscillatoria	50.0	3.3%
CYANOBACTERIA	Coelosphaerium	38.5	2.6%
BACILLARIOPHYCEAE	Synedra	25.6	2.0%
BACILLARIOPHYCEAE	Stephanodiscus	20.8	0.3%
BACILLARIOPHYCEAE	Asterionella	20.1	1.5%
CRYPTOPHYTA	Cryptomonas	18.1	1.0%
CYANOBACTERIA	Cylindrospermopsis	13.8	0.3%
BACILLARIOPHYCEAE	Cyclotella	12.9	0.3%
DINOPHYCEAE	Ceratium	12.8	0.8%
BACILLARIOPHYCEAE	Fragilaria	12.1	1.3%
CYANOBACTERIA	Anabaena	12.1	1.5%
CHRYSOSPHAERELLA	Dinobryon	11.6	0.8%
BACILLARIOPHYCEAE	Cymbella	8.8	0.3%
CHLOROPHYTA	Crucigenia	7.9	0.3%
CYANOBACTERIA	Chroococcus	7.9	0.3%

A national database consisting of 5,939 records of algal taxa sampled for the USGS/NAWQA sampling program (Porter 2007) served as a resource for evaluating some of the characteristics of taxa observed in 2011 stream phytoplankton samples. According to habitat classifications assigned to alga taxa in the database, sestonic taxa (i.e., living primarily in the water column) outnumbered benthic taxa (i.e., living on or attached to the stream bottom) by 46 to 14 taxa found in 2011 stream phytoplankton samples. The other nine algal genera are of indeterminate habitat orientation because they include both benthic and sestonic species. In reference to sampling results from the NAWQA nationwide stream sampling project, Porter et al (2008) noted that algal samples from small streams tended to be dominated by benthic taxa, while larger streams were more likely to be dominated by sestonic taxa. The latter appears to be the case among the nutrient-rich, medium and large streams sampled in 2011. All of the eight taxa that occurred at nuisance bloom levels in the 2011 stream phytoplankton samples are classified as sestonic algae.

Among the 2011 lake phytoplankton samples, 38 taxa are classified as sestonic, 17 are benthic, and 6 taxa are indeterminate. Sestonic taxa were by far the most dominant among taxa occurring at nuisance bloom levels. The single exception was a ‘bloom’ in the benthic diatom genus, *Cymbella*, which was a component of a large bloom in a shallow natural lake that was dominated by *Microcystis*.

Certain types of Cyanobacteria are able to capture or ‘fix’ nitrogen from the atmosphere, thereby providing these taxa with a competitive growth advantage when dissolved inorganic forms of nitrogen are scarce. Of the 69 stream phytoplankton taxa sampled, three Cyanobacteria genera (*Anabaena, Aphanizomenon, Cylindrospermopsis*) have the ability to fix atmospheric nitrogen. None of these taxa occurred at “bloom” levels in 2011 stream samples; however, these taxa (particularly *Aphanizomenon*) were present at nuisance bloom levels in many 2011 lake phytoplankton samples (Table 32).

5. Nutrient Stressor - Biological Assemblage Response Analysis

The following sections present the results from analysis of relationships between nutrient response variables and biological condition indicators. Unless otherwise noted, the analysis was conducted using data from the 2002-2006 REMAP probabilistic stream survey. Before the analysis was conducted, the data were subdivided by thermal classification and stream size to reduce the potentially confounding influences of these environmental variables and for consistency in the application of biological condition indicators.

Coldwater streams were analyzed separately from warmwater streams because of inherent differences in stream characteristics. Coldwater streams are more influenced by groundwater, which besides the obvious influence on water temperature, also increases flow constancy and tends to reduce levels of suspended solids during base flow. As was shown earlier, patterns in nutrient and nutrient response variables also differed between coldwater and warmwater streams. Differences in hydrology, thermal regime, and water quality are reflected in strong differences in the species composition of biological assemblages (e.g., fish, macroinvertebrates). These differences are widely recognized in the Midwest as a rationale for developing separate biological indicators for the purpose of assessing the biological health of these systems (Lyons et al. 1996; Mundahl 1998; SHL 2012; Wilton 2004).

Warmwater streams were further subdivided by stream size to account for previously demonstrated patterns and relationships in nutrient variables and for consistency with the use of biological assemblage indexes in Iowa streams. As previously noted, the Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) and Fish Index of Biotic Integrity (FIBI) were calibrated using data from warm-water, wadeable reference stream sites. Watershed area, as a surrogate indicator of stream size, was used in the calibration of biological data metrics in each index. The watersheds of warmwater reference sites range in size from 10-900 mi^2. Wadeable and nonwadeable are somewhat subjective terms that are used mostly for operational purposes to distinguish streams that can be sampled effectively using wading techniques versus those that must be sampled from a boat. Years of biological sampling in Iowa streams of all sizes has shown that the transition from wadeable to nonwadeable generally occurs in stream segments of 4^{th}–6^{th} Strahler order with watershed area ranging from 500–1500 mi^2. Where the transition occurs in a given river basin depends on hydrologic and geomorphologic characteristics.

Taking into consideration the warmwater reference calibration range of the BMIBI and FIBI and the stream size-related nutrient response patterns described earlier, a decision was made to separate the analysis of nutrient stressor-biological response relationships in small and medium-size wadeable streams from large, predominantly nonwadeable streams. The size range for wadeable streams included in the analysis was 10-700 mi^2, and large nonwadeable streams were defined as those exceeding 700 mi^2 of watershed area. The data analysis results from these distinct groups are presented separately below. Unless otherwise noted, the stressor-response analysis of BMIBI and FIBI metrics was performed using the normalized metric scores (possible range 0-10) instead of the raw data metric value.

5.1. Warmwater wadeable streams

Seston chlorophyll A and benthic macroinvertebrates

In response to increasing levels of seston chlorophyll A (WCHLA), downward trending patterns were observed in several benthic macroinvertebrate (BMIBI) data metrics (e.g., MHTTXSCR, MHSNTVSCR, SHTTXSCR, SHSCRPRSCR, SHEPHSCR, SHP3DOMSCR, SHDFFGSCR) (Appendix 15a-b). The wedge-shaped response pattern discussed earlier was probably the most noticeable with the % scraper abundance metric (SHSCRPRSCR). Two data outliers representing sites having WCHLA exceeding 200 ug/L do not fit the general pattern displayed by several metrics. Also, for several metrics (e.g. SHEPTX) the bottom-edge of the data was sloped noticeably downward, thus suggesting that additional stressors by themselves or in conjunction with seston algal biomass might adversely influence benthic macroinvertebrate assemblage condition.

Correlation analysis of BMIBI metrics versus maximum seston chlorophyll A produced similar results as correlations with average levels (Table 33). Relatively weak, yet statistically significant linear relationships were found between (log10)WCHLA versus the BMIBI and six component metrics: SHTTXSCR, MHEPTXSCR, SHEPTXSCR, SHSCRPRSCR, SHP3DOMSCR, SHDFFGSCR. The amount of variation in BMIBI metric scores explained by (log10)WCHLA ranged from approximately 3%-10%. Percent abundance of top-three dominant taxa (SHP3DOMSCR) was the most strongly correlated metric (Figure 36). The relatively minor amount of variation in BMIBI metrics that can be attributed to WCHLA suggests that other stressors or environmental factors and sampling error account for most of the variation in benthic macroinvertebrate assemblage condition. For example, a previous analysis found that 23% of variation in BMIBI scores was explained by stream bottom sediment composition (Wilton 2004).

Changepoint analysis results of WCHLA and BMIBI metrics are summarized in Table 34. Conditional probability (CP) analysis found significant changepoints in relationships between WCHLA and the BMIBI, as well as four component metrics (MHSNSTVSCR, SHP3DOMSCR, SHDFFGSCR, SHEPTXSCR). WCHLA changepoints ranged from 5-46 ug/L; the average and median values were 21.9 and 19.9, respectively. In comparison to reference conditions, the percentage of sites equal to or greater than the applicable reference 25th percentile score averaged 70.4% for sites having WCHLA below the applicable changepoint compared to 43.5% for sites with WCHLA above the changepoint. Generally, a difference of at least 20% was needed in order to detect a significant difference based on a chi-squared two-sample test.

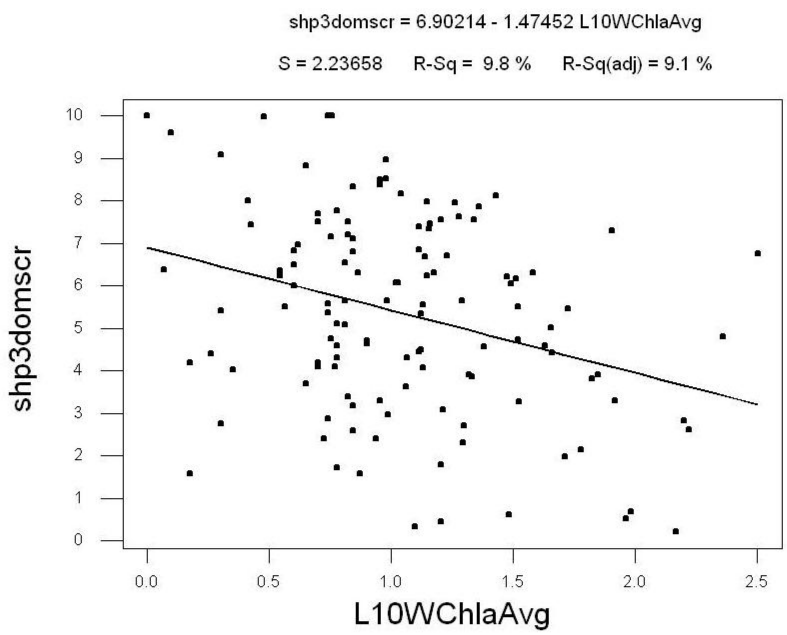


Figure 36. Least-square linear regression of log10 average seston chlorophyll A (L10WchlaAvg) and percent abundance of top-three dominant benthic macroinvertebrate taxa metric score (shp3domscr) in wadeable warmwater streams. REMAP (2002-2006) project data.

Table 33. Comparison of Pearson and Spearman correlation results for maximum and average chlorophyll A versus BMIBI metrics in wadeable warmwater streams. REMAP (2002-2006) project data.

	Pearson (r)			Spearman Rank (rho)	
BMIBI Metric	Max.	Avg.		Max.	Avg. WCHLA
SHP3DOMSC	-0.31*	-0.31*		-0.28	-0.25
SHEPTXSCR	-0.29*	-0.28*		-0.26	-0.23
SHTTXSCR	-0.26*	-0.27*		-0.23	-0.21
SHSCRPRSC	-0.24*	-0.25*		-0.21	-0.21
BMIBI	-0.25*	-0.24*		-0.21	-0.17
MHEPTXSCR	-0.18*	-0.18*		-0.17	-0.17
SHDFFGSCR	-0.15	-0.18 *		-0.07	-0.09
SHCHIRSCR	-0.16	-0.15		-0.15	-0.11
SHMBISCR	-0.16	-0.14		-0.17	-0.15
SHEPHSCR	-0.13	-0.10		-0.13	-0.10
SHPEPTSCR	-0.12	-0.09		-0.14	-0.10
MHSNSTVSC	-0.07	-0.07		-0.14	-0.13
MHTTXSCR	-0.01	0.02		0.01	0.05
* r^2 significance test, $p \leq 0.05$					

The WCHLA level above which optimal metric scores (defined as the 95% of reference site scores) were no longer observed was noted for each metric. The WCHLA level above which optimal metric levels in BMIBI metrics were no longer observed ranged from 5.8 - 320 ug/L and the median was 16.8. BMIBI scores that are considered excellent ($\geq$ 76) were not observed when average WCHLA exceeded 19 ug/L.

Quantile regression (QR) results showed significant relationships between WCHLA and three BMIBI metrics (SHP3DOMSCR, SHSCRPRSCR, AND SHTTXSCR) (Table 34). Slope coefficients and intercepts from the 90th percentile (P90) regression models were used to estimate the threshold WCHLA level above which the reference 75th percentile value of each metric was predicted to occur in fewer than 10% of samples. As discussed earlier, the 75% percentile was chosen to represent a relatively high level of biological performance that is consistent with full attainment of aquatic life use goals and a reasonably small departure from optimum levels in Iowa streams. The range of QR threshold values for WCHLA ranged from 19.7 - 27.8 ug/L; the mean and median values were 24.8 and 26.9 ug/L, respectively. The regression model for the composite BMIBI index did not result in a P90 slope coefficient that was significantly different from zero. This lack of relationship suggests that elevated levels of WCHLA, while adversely impacting a few BMIBI metrics, by itself it does not exert a strong limiting influence over the overall condition of benthic macroinvertebrate assemblages in wadeable, warmwater streams.

Table 34. Seston Chlorophyll A - Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) relationship changepoints (streams of watershed area 10-700 square miles).

	Seston Chlorophyll A (ug/L)		
Biological Metric / Index	CP*	QR	RT
MH**-taxa richness (MHTTX)	-	-	(25.5)***
SH**-taxa richness (SHTTX)	-	26.9	3.6
MH-EPT richness (MHEPTX)	-	-	10.6
SH-EPT richness (SHEPTX)	5.0	-	10.6
MH-sensitive taxa (MHSNSTV)	19.9	-	-
SH-% 3-dominant taxa (SHP3DOM)	46.0	19.7	45.8
SH-Mod. Hilsenhoff Biotic index (SHMHBI)	-	-	5.2
SH-% EPT (SHPEPT)	-	-	-
SH-% Chironomidae (SHCHR)	-	-	-
SH-% Ephemeroptera (SHEPHM)	-	-	-
SH-% Scrapers (SHSCRPR)	-	27.8	5.2
SH-% Dom. functional feeding grp, (SHDFFG)	33.5	-	49
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	5.0	-	5.2
****Range	5.0-46.0	19.7-27.8	3.6-49
Mean	21.9	24.8	18.5
Median	19.9	26.9	10.6

*Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree;
** MH, Multi-habitat sample; SH, Standard-Habitat sample.
*** ( ) indicates direction of biological response opposite of expected.
**** Range, mean, and median excluding metrics having response opposite of expected direction.

Regression Tree (RT) analysis found significant WCHLA changepoint splits in the BMIBI and seven component metrics (Table 34). The WCHLA changepoints ranged from 3.6 - 49 ug/L; the average and median values were 18.5 and 10.6 ug/L, respectively. These statistics do not include the changepoint value for MHTTXSCR, which had a changepoint relationship that was opposite of the expected response (i.e., MHTTXSCR increased with increased WCHLA). The reductions in regression model variance that were attributable to the WCHLA – BMIBI metric relationship changepoints were relatively small, ranging from 2.1% - 11.5% (Table 35).

Table 35. Regression Tree analysis of stressor (WCHLA) – response (BMIBI metric) changepoints (streams of watershed area 10-700 square miles). Changepoint results are reported only for metrics having a statistically significant mean score difference.

BMIBI metric	WCHLA Change point (ug/L)	Mean Score < Change point	Mean Score ≥ Change point	<> Differ-ence	BionetR25 metric score	Variance Reduction (%)
mheptx	10.6	5.665	4.574	1.09*	4.93	5.1
mhttxscr	25.5	6.123	6.946	-0.8*	5.61	3.5
shchirscr	19.6	7.432	6.64	0.79	7.44	3.8
shdffgscr	33.4	6.337	4.762	1.58*	5.36	6.9
shephmscr	5.2	4.326	3.374	0.95	1.64	2.1
sheptxscr	10.6	6.24	5.02	1.22*	5.57	7.4
shmhbiscr	5.2	6.672	5.419	1.25*	5.59	4.9
shpeptscr	15.5	6.088	5.165	0.92	5.28	2.6
shscrprscr	5.2	4.081	2.158	1.92**	1.15	11.5
shttxscr	3.6	7.429	6.121	1.31*	5.58	6.0
BMIBI	5.2	60.01	50.85	9.16*	53	5.7

For additional perspective, the metric mean scores representing data subgroups formed by a primary split (i.e., WCHLA < or ≥ changepoint) were compared to wadeable reference 25th and 50th percentile metric values. For MHEPTXSCR, SHP3DOMSCR, SHDFFGSCR, SHEPTXSCR, the data subgroups of sites having WCHLA below the changepoint had mean BMIBI metric scores equal to or greater than the applicable reference 25th percentile, while subgroups of sites having WCHLA above the changepoint had mean scores below applicable 25th percentiles (Table 35). The average WCHLA changepoint for the these four metrics was 29 ug/L. For three additional metrics (SHMHBISCR, SHSCRPRSCR, SHTTXSCR), the data subgroups representing sites having WCHLA below the changepoint had mean scores equal to or greater than applicable reference 50th percentiles, while mean scores of subgroups representing sites having WCHLA above the changepoint were all below the 50th percentiles. The average WCHLA changepoint was 4.6 ug/L for these three metrics. The relative difference in average changepoint levels for the groups attaining the 25th%, but not the 50th% (29 ug/L) versus the group attaining the 50th% (4.6 ug/L) hints at varying sensitivity ranges among BMIBI metrics in response to increasing levels of WCHLA.

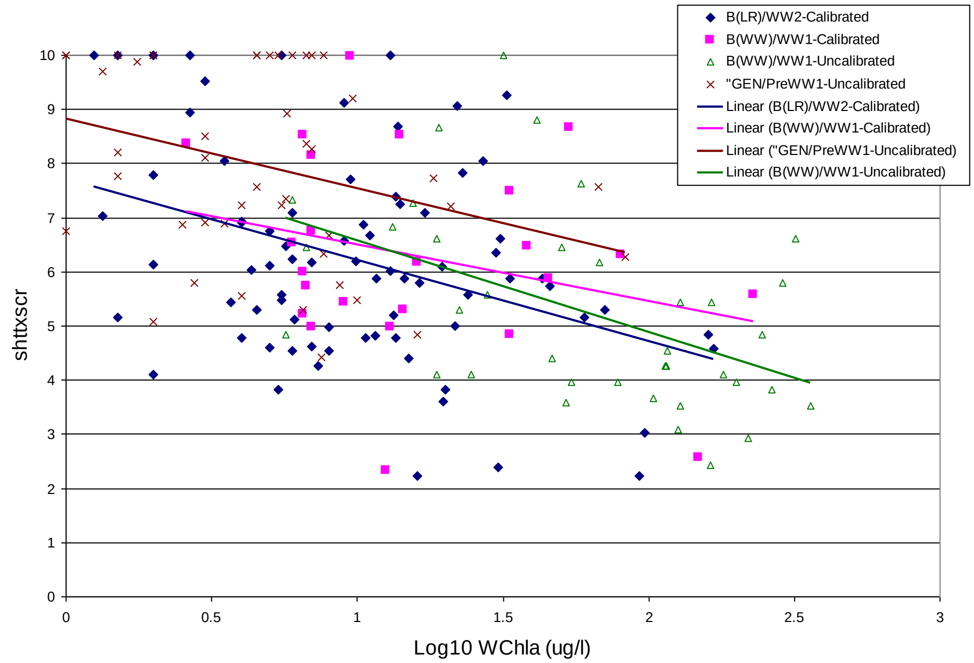
Calibrated wadeable streams

A subsequent analysis was conducted using a subset of the REMAP wadeable stream data set. The analysis was intended to provide insight to how relationship patterns and changepoints might differ among categories of streams that are defined by pre-2006 aquatic life use designations and reference calibration criteria. These designations and criteria have been used in the assessment of biological assemblage sampling data for purposes of the Clean Water Act (CWA) Sections 303(d) and 303(b) Integrated Report.

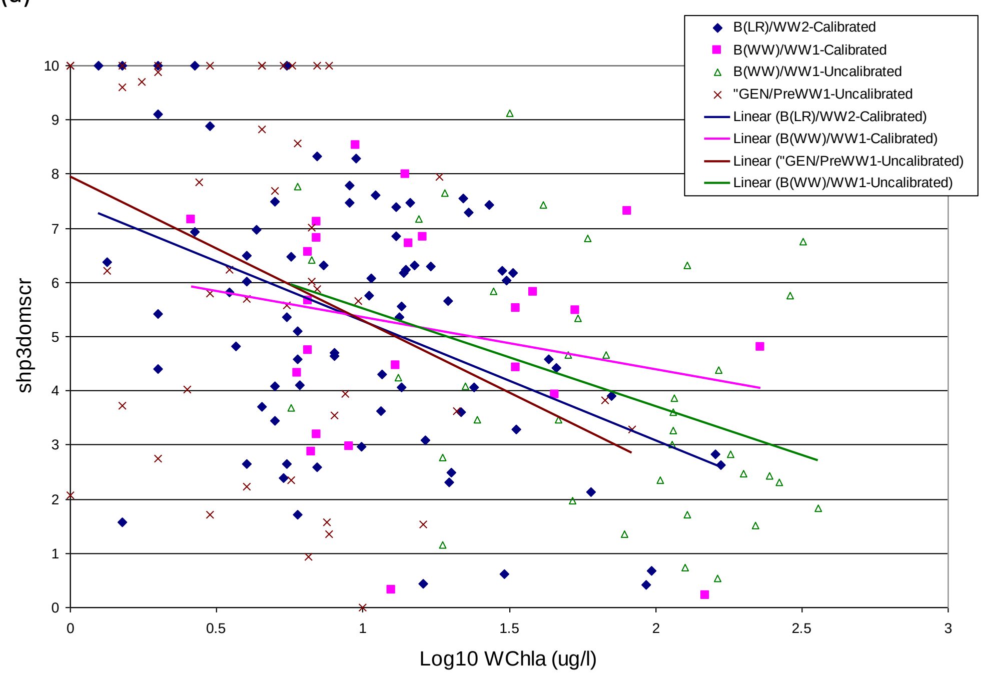
Calibrated streams are segments of streams designated prior to 2006 as B(LR)-Limited Resource or B(WW)-Significant Resource for aquatic life uses, and which have a watershed drainage area less than 500 square miles. Stream segments meeting these criteria are considered to be within the fully calibrated range of the wadeable warmwater reference network allowing the highest level of confidence in biological assessments (IDNR 2013). Uncalibrated streams consist of two groups: small headwater streams that were designated for general uses prior to 2006, and large wadeable/nonwadeable streams designated as B(WW1) and having watershed area exceeding 500 mi^2.

Since Iowa's stream aquatic use designations largely reflect a gradient from small to large perennial streams, it is important to know if nutrient response patterns behave consistently across designated use classifications. Scatter plots of the relationship between WCHLA and BMIBI metrics were prepared and examined. The relationship patterns for calibrated and uncalibrated stream categories were generally similar as examples show in Figures 37(a-b). The trajectories of the regression fitted prediction lines are directionally consistent and the mean slope coefficients were not statistically different ($p>0.05$). In addition, the upper margin of the data, which theoretically defines the limiting influence of seston chlorophyll a over the benthic macroinvertebrate metric response, is represented by several use designations, not just one. Although fewer cases of elevated WCHLA are observed in small and medium size wadeable streams, a similar pattern of response in the benthic macroinvertebrate assemblage seems to occur.

The changepoint analysis techniques reported on earlier in this section were applied again using sampling data from calibrated wadeable streams only. Changepoints obtained from the calibrated data set (Table 36) tended to be lower than changepoints obtained using the less restricted wadeable stream data set. Median changepoint values for the CP, QR, and RT calibrated analysis were 5.0, 11.2, and 5.2 ug/L, respectively compared with 19.9, 26.9, 10.6 ug/L from the initial analysis of wadeable streams. The first analysis included data from small headwater streams and from large wadeable streams that fall outside of the calibrated watershed area range. One possible reason for a shift toward lower changepoints for the calibrated data set could be the exclusion of large wadeable streams, which tended to have high WCHLA levels. Excluding these data might have caused the shift by narrowing the range of stressor levels, particularly by excluding some high values.



(a)



(b)

Figure 37(a-b). Least square linear regression of seston chlorophyll a (Log10WChla) versus (a) standard habitat total taxa richness score (shttxscr) and (b) standard habitat total taxa richness score (shp3domscr). REMAP (2002-2006) project data. Colored lines and symbols correspond with Iowa's former/current aquatic life use designations: Limited resource –B(LR)/ WW2; Significant resource-B(WW)/BWW1); General use/presumed WW1. *Calibrated* means stream segments that are within the wadeable reference stream calibration range (i.e., drainage area ≤ 500 sq.mi. and not formerly designated for general uses).

Table 36. Seston Chlorophyll A - Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) relationship changepoints. (Analysis conducted using data only from streams defined as calibrated, warmwater stream sites for purposes of Clean Water Act Sections 303(d)/305(b) reporting.)

	Seston Chlorophyll A (ug/L)		
Biological Metric / Index	CP*	QR	RT
MH**-taxa richness (MHTTX)			
SH**-taxa richness (SHTTX)		24.6	3.6
MH-EPT richness (MHEPTX)			10.6
SH-EPT richness (SHEPTX)	5.0		14.8
MH-sensitive taxa (MHSNSTV)			
SH-% 3-dominant taxa (SHP3DOM)	43.0	13.5	3.2
SH-Mod. Hilsenhoff Biotic index (SHMHBI)			
SH-% EPT (SHPEPT)			
SH-% Chironomidae (SHCHR)	5.0		5.2
SH-% Ephemeroptera (SHEPHM)			
SH-% Scrapers (SHSCRPR)		63.7	5.2
SH-% Dom. functional feeding grp, (SHDFFG)			3.2
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	3.5	10.0	
Range	3.5-43.0	10.0-63.7	3.2-14.8
Mean	14.1	28.0	7.1
Median	5.0	19.1	5.2

Suspended solids and turbidity as covariables of seston chlorophyll A

Several environmental covariables have the potential to confuse or mask the true relationship between nutrient stressors and biological response variables. Since levels of suspended solids and seston algal biomass are typically positively correlated, it is possible that biological responses could be erroneously attributed to elevated levels of sestonic algae when, in fact, they are caused by water quality conditions that are not linked to nutrient eutrophication. Suspended solids and/or turbidity can negatively impact stream aquatic communities in several ways, including: reducing visibility needed by sight-feeding species, clogging or irritating gill structures, and smothering substrates needed for feeding and reproduction.

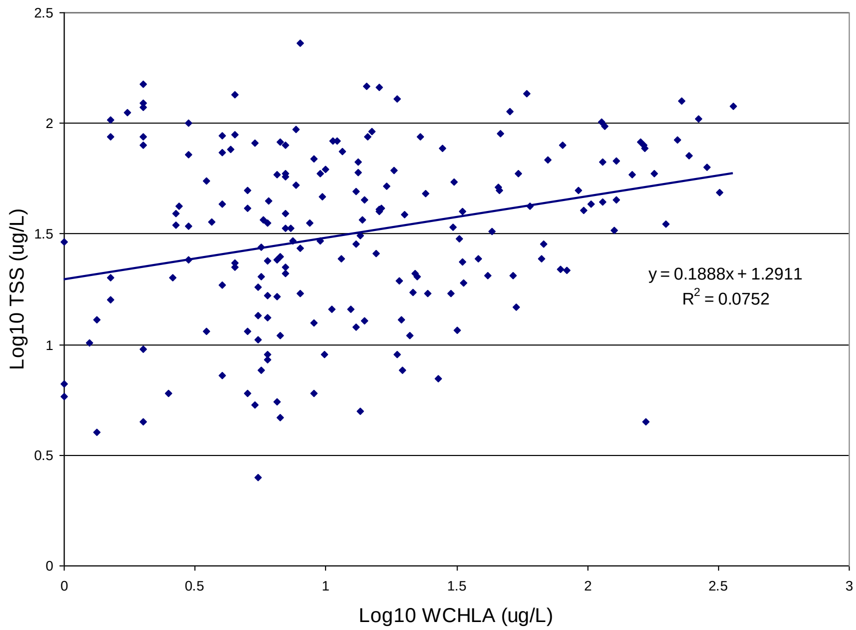
Linear relationships between WCHLA and TSS and between WCHLA and turbidity were directionally correct and statistically significant, but relatively weak (Figures 38a-b). WCHLA as a predictor variable only explained 7.5% and 4.5% of variance in TSS and turbidity levels, respectively.

Relationships between BMIBI metrics and TSS or turbidity levels were also weak or non-existent (e.g., Figures 39a-b). High levels of standard-habitat total taxa richness metric score (SHTTXSCR) were observed when TSS levels were low or high as long as WCHLA levels were low or moderately low (as indicated by smaller bubble sizes in Figure 39(a). Conversely, low levels of SHTTXSCR levels tended to occur when TSS levels were high and WCHLA levels were also high. A similar pattern was observed between standard-habitat percent abundance top-three dominant benthic macroinvertebrate taxa metric score (SHP3DOMSCR) and TSS (Figure 39(b)).

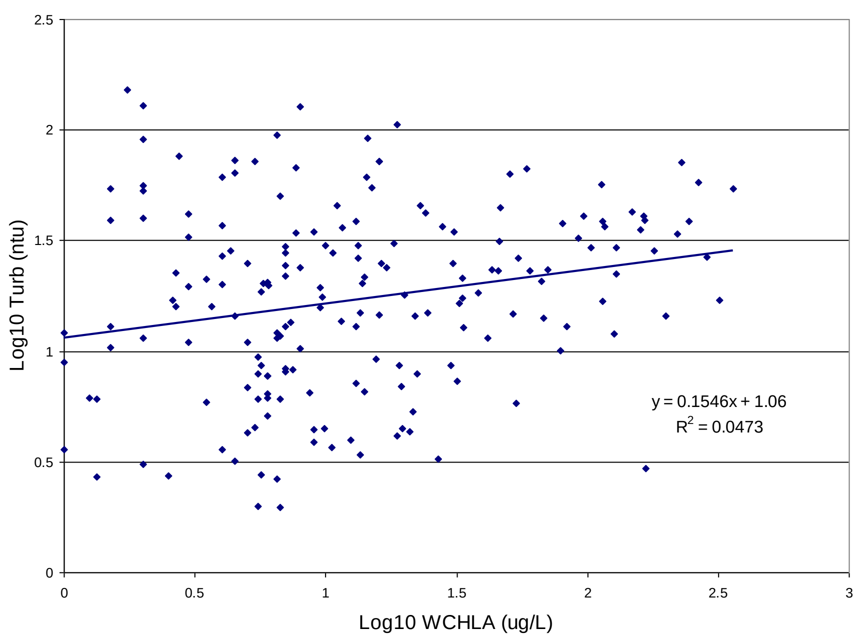
The relationship between SHTTXSCR and total volatile (organic) suspended solids (TVSS) was relatively stronger (r^2 = 0.16) than between SHTTXSCR and TSS (Figure 40). The pattern of response and bubble size distribution seems to suggest that sestonic algal biomass is the driving variable in the relationship rather than non-algal organic solids. SHTTXSCR levels fall consistently below optimal levels when (log10)TVSS levels exceed approximately 1.2 (15.8 mg/L); however, as the lower right portion of the figure shows, the lowest levels occur when WCHLA levels are highest (as indicated by large bubbles).

Turbidity, which is a measure of the degree to which light is scattered in water, is not only a function of the mass and type of suspended solids (e.g., algal cells, colloidal clays), but also the occurrence of light-attenuating dissolved constituents (e.g., tannic acid). Therefore, a somewhat difference relationship between turbidity and the response variables might be expected. As seen in Figure 41, however, as with TSS, a similar weak relationship between turbidity and SHTTXSCR was observed (r^2=0.06). Once again, the lowest levels of SHTTXSCR appear to be disproportionately associated with elevated levels of WCHLA, while optimal levels of SHTTXSCR occur along a gradient from low to high turbidity. There appears to be a potential threshold near turbidity of 50 ntu (log10 = 1.7) above which maximum levels of SHTTXSCR levels fall significantly from a maximum possible score of ten.

This preliminary examination of potential interactions of TSS and turbidity with seston chlorophyll A seems to indicate that response patterns in benthic macroinvertebrate metrics are more strongly linked to varying levels of algal biomass than other aspects of suspended solids or turbidity. A more in-depth statistical analysis should be considered, however, as it might lead to a deeper understanding of multi-variate stressor-biological response relationships in Iowa's streams.

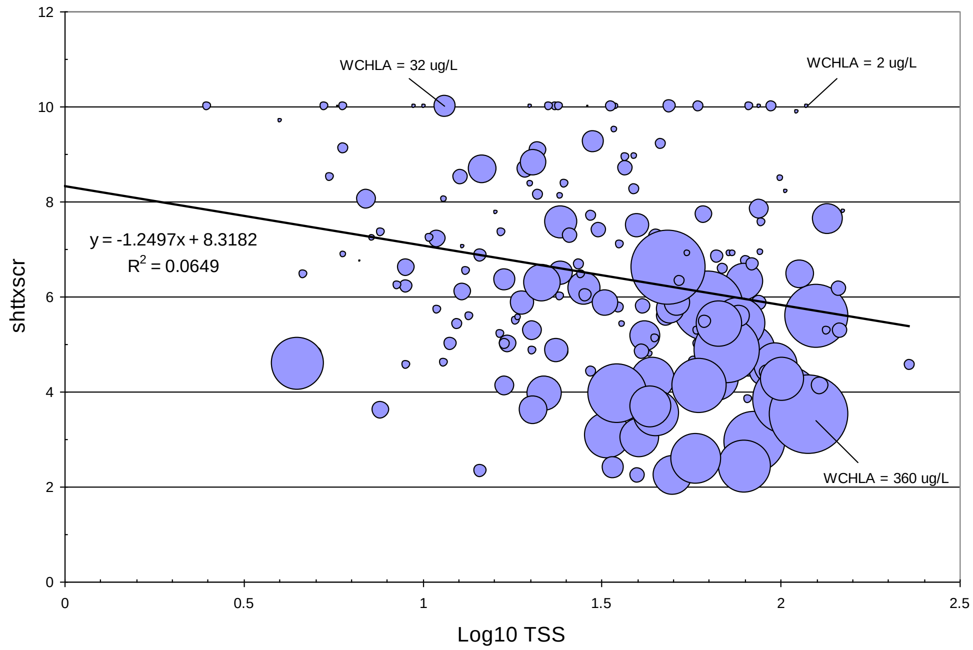


(a)

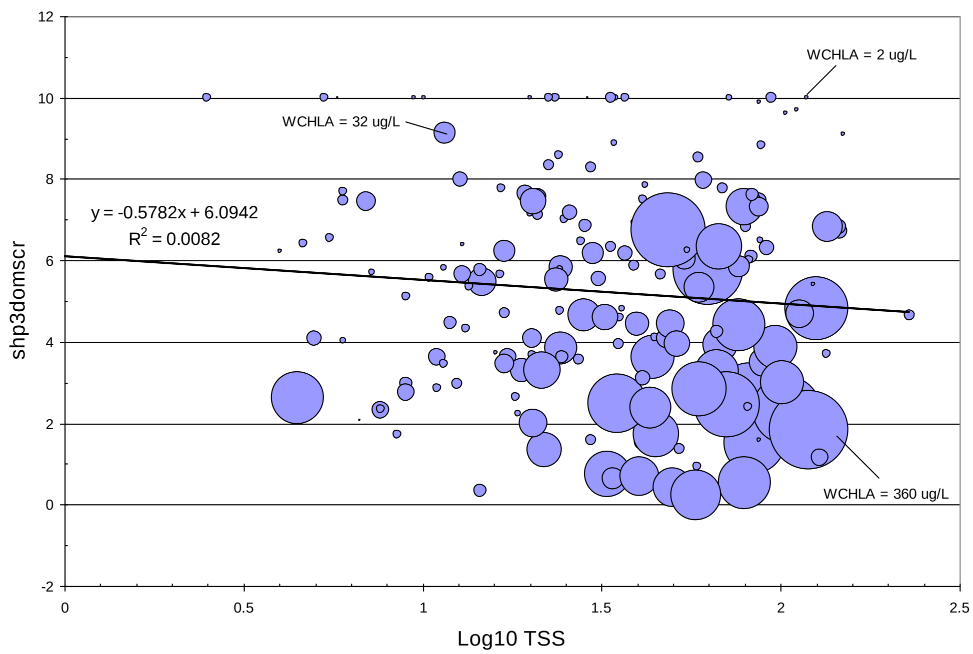


(b)

Figure 38(a-b). Least square linear regression relationship of log10 seston chlorophyll a (WCHLA) vs. (a) log10 Total Suspended Solids (TSS) and (b) log10 turbidity (ntu). REMAP (2002-2006) wadeable warmwater stream data.



(a)



(b)

Figure 39(a-b). Least square linear regression relationship of log10 total suspended solids (TSS) vs. (a) standard habitat total taxa richness metric score (SHTTXSCR) and (b) standard habitat total taxa richness score (SHP3DOMSCR). Bubble size is proportional to the concentration of seston chlorophyll A (WCHLA). REMAP (2002-2006) wadeable warmwater stream data.

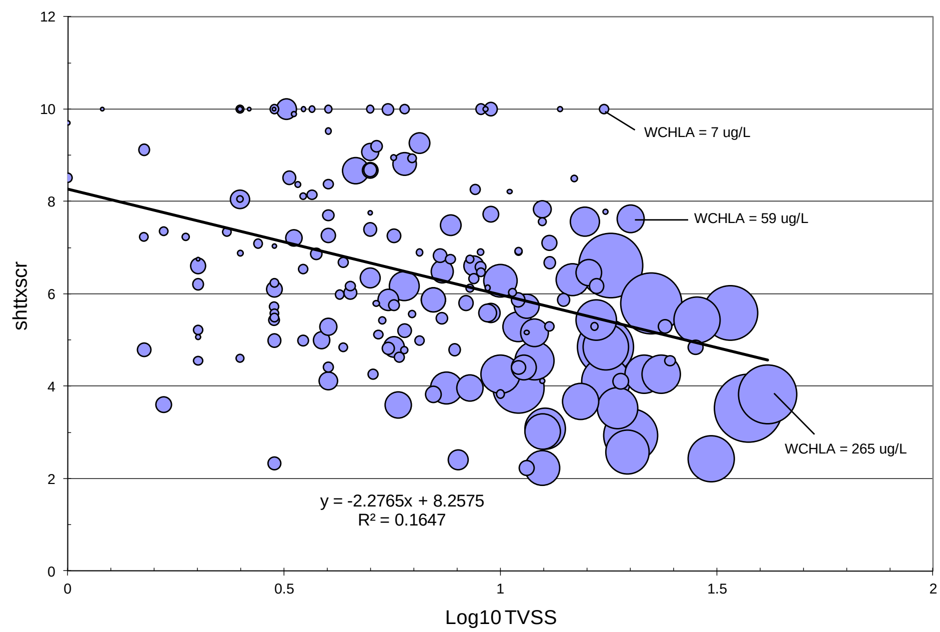


Figure 40. Least square linear regression relationship of log10 total volatile suspended solids (TVSS) vs. standard habitat total taxa richness metric score (SHTTXSCR). Bubble size is proportional to the concentration of seston chlorophyll A (WCHLA) – see labeled examples. REMAP (2002-2006) wadeable warmwater stream data.

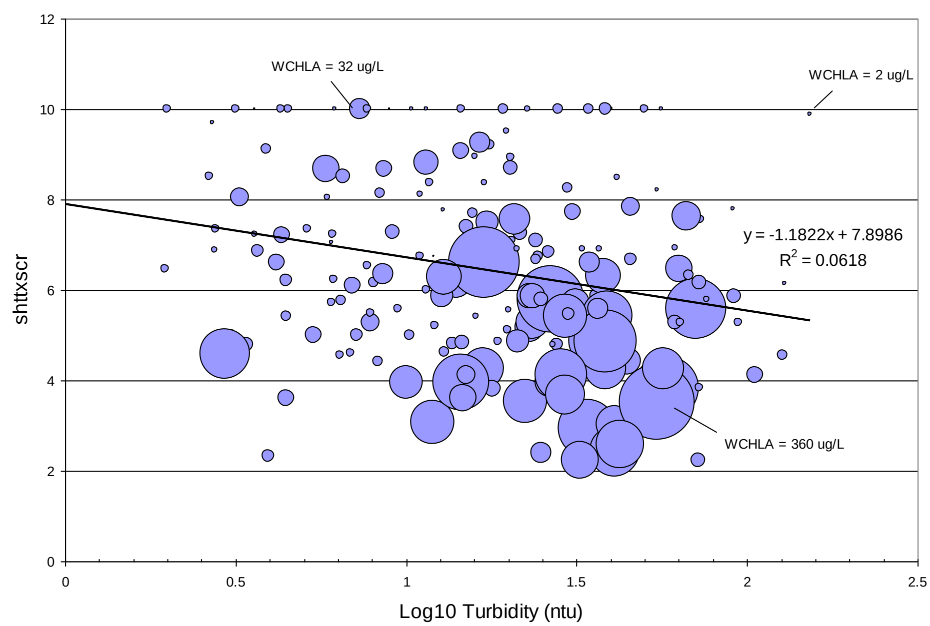


Figure 41. Least square linear regression relationship of log10 turbidity vs. standard habitat total taxa richness metric score (SHTTXSCR). Bubble size is proportional to the concentration of seston chlorophyll A (WCHLA) – see labeled examples. REMAP (2002-2006) wadeable warmwater stream data.

Seston chlorophyll A and fish

Bivariate plots showed inconsistent relationship patterns between WCHLA and Fish Index of Biotic Integrity (FIBI) metrics. (Appendix 15f). When first plotted against untransformed WCHLA data, several FIBI metrics appeared to display a wedge-shaped response pattern. Levels of several metrics (e.g., SNSTVSPSCR, PBINVSCR, PTCVSCR, PSLITHOSCR) appeared to decline slightly and suggest potential thresholds at the highest levels of WCHLA. However, when the metrics were plotted against log-transformed WCHLA, sub-optimal levels were also observed in the lower region of the WCHLA gradient. The unrecognizable or somewhat pyramid-shaped patterns displayed in most of the response plots were consistent with results from correlation analysis, in which no significant linear relationships between (log10)WCHLA and FIBI metrics were revealed.

Conditional probability (CP) analysis found significant changepoints associated with three metrics (POMNVSCR, PSLITHSCR, PTOPCSCR) (Table 37). The changepoints all occurred at the lower end of the WCHLA range (3.5 - 5.5 ug/L). The responses of PSLTHSCR and PTOPCSCR to the stressor variable were opposite of the expected direction. That is, the proportion of sites attaining the reference 25th percentile metric score was actually higher for sites where WCHLA levels were above the changepoint than for sites with WCHLA below the changepoint. POMNVSCR was the only metric that responded in the expected direction (i.e., metric score decreasing with increasing WCHLA). The changepoint found in the analysis of the FIBI composite score was not statistically significant, and therefore it is not reported in Table 37. Levels of the FIBI considered representative of excellent fish assemblage condition were observed at sites where WCHLA levels varied substantially from 1.3 - 53.3 ug/L (Appendix 15f).

Table 37. Seston Chlorophyll A – Fish Index of Biotic Integrity (FIBI) relationship changepoints (streams of watershed area 10-700 square miles).

	Seston Chlorophyll A (ug/L)		
Biological Metric / Index	CP*	QR	RT
# native fish species (NTVSP)	-	-	-
# sucker species (SCKRSP)	-	-	(35.9)**
# sensitive species (SNSTVSP)	-	-	-
# benthic invertivore species (BINVSP)	-	-	-
% 3-dominant fish species (P3ABUND)	-	-	(30.3)
% benthic invertivores (PBiNV)	-	-	(30.3)
% omnivores (POMNV)	3.5	33.2	3.6
% top carnivores (PTOPC)	(5.5)	-	(5.6)
% simple lithophil spawners (PSLTH)	(3.7)	_	(35.9)
fish assemblage tolerance index (TOLINDX)	-	-	-
adjusted catch per unit effort (ACPUE)	-	-	-
Fish Index of Biotic Integrity (FIBI)	-	-	-

*Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree
** ( ) indicates direction of biological response opposite of expected

Regression tree analysis revealed significant changepoints in relationships between WCHLA and six FIBI metrics (Table 37). Only POMNVSCR had a significant WCHLA changepoint (3.6 ug/L) response in the expected direction. The other metrics (P3ABUNDSCR, PBINVSCR, PSLITHOSCR, SCKRSCR, TOPCVSCR) had statistically significant changepoints ranging from 5.6 – 35.9 ug/L, all opposite of the expected response direction. Mean scores of these metrics were significantly higher among sites where WCHLA levels exceeded the changepoint compared to sites with WCHLA below the changepoint.

.

Quantile regression (QR) found a significant relationship between WCHLA and the percent abundance of omnivore fish (POMNVSCR). The 90th percentile regression slope coefficient and intercept were used to calculate a WCHLA threshold level of 33.2 ug/L above which levels of pomnvscr equal to or greater than the reference 75th percentile value were predicted to occur in less than 10% of samples.

Dissolved oxygen and benthic macroinvertebrates

Four dissolved oxygen (DO) monitoring variables were evaluated for inclusion in the stressor-response analysis: average diel DO maxima (AVGMAXDO), average diel DO minima (AVGMINDO), average diel DO average (AVG2DO), and average diel DO range (AVGRNGDO). Based on the examination of stressor-response plots and consideration of correlation analysis results, AVGMINDO and AVGRNGDO were selected for the nutrient stressor-biological changepoint/threshold analysis.

Scatter plots of dissolved oxygen variables and benthic macroinvertebrate (BMIBI) metrics showed mixed response patterns (Appendix 15i-j). Some plots showed a wedge-shaped pattern (e.g., AVGRNGDO v. SCRPRSCR), while others displayed a more consistent linear pattern (e.g., AVGMINDO v. MHBISCR) or no recognizable pattern (e.g., AVGMINDO v SHTTXSCR).

Correlation results generally indicated that BMIBI metrics were more strongly correlated with AVGMINDO than the other DO variables (Table 38 and 39). AVGMAXDO lacked significant relationships with all but one BMIBI metric. AVGMINDO and AVG2DO were very similarly correlated with BMIBI metrics in terms of directionality; however, correlation coefficients for AVG2DO were consistently smaller than AVGMINDO coefficients. AVGRNGDO was significantly correlated with three BMIBI metrics that AVGMINDO and AVG2DO were not. AVGMINDO and AVGRNGDO were correlated with stream metabolic rates of gross primary production and community respiration, which are influenced by environmental factors such as temperature and nutrient availability. Ultimately, AVGMINDO and AVGRNGDO were chosen for the stressor-response changepoint analysis because of their dual role as stressor variables (i.e., extreme fluctuations of DO and hypoxic conditions are stressful to aquatic organisms) and as indicators of nutrient-influenced stream ecological functions.

Table 38. Pearson correlation coefficients (r) representing diel dissolved oxygen (DO) variables and benthic macroinvertebrate metrics sampled from 106 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Bolded values are significant at p<0.05.

	AVGMAXDO	AVGMINDO	AVG2DO	AVGRNGDO
MHBISCR	0.01	**0.45**	**0.34**	**-0.24**
MHEPTXSCR	0.04	**0.35**	**0.29**	-0.16
MHSNSTVSCR	0.10	**0.31**	**0.31**	-0.10
MHTTXSCR	0.10	-0.02	0.08	0.09
SHCHIRSCR	-0.07	0.12	0.05	-0.13
SHDFFGSCR	-0.02	**0.26**	**0.19**	-0.16
SHEPHSCR	-0.17	0.13	-0.01	**-0.21**
SHEPTXSCR	-0.09	**0.39**	**0.23**	**-0.29**
SHP3DOMSCR	-0.16	0.16	0.03	**-0.23**
SHPEPTSCR	-0.05	**0.31**	**0.20**	**-0.22**
SHSCRPRSCR	**-0.19**	0.16	-0.03	**-0.25**
SHTTXSCR	-0.06	0.03	0.00	-0.07
BMIBI	-0.07	**0.35**	**0.22**	**-0.26**

Table 39. Spearman correlation coefficients (rho) representing diel dissolved oxygen (DO) variables and benthic macroinvertebrate metrics sampled from 106 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles.

	AVGMAXDO	AVGMINDO	AVG2DO	AVGRNGDO
MHBISCR	-0.06	0.38	0.23	-0.27
MHEPTXSCR	-0.01	0.34	0.21	-0.18
MHSNSTVSCR	0.04	0.34	0.24	-0.14
MHTTXSCR	0.07	0.04	0.05	0.09
SHCHIRSCR	-0.11	0.19	0.04	-0.21
SHDFFGSCR	-0.04	0.23	0.12	-0.15
SHEPHSCR	-0.20	0.18	-0.01	-0.28
SHEPTXSCR	-0.13	0.31	0.10	-0.26
SHP3DOMSCR	-0.20	0.19	-0.02	-0.22
SHPEPTSCR	-0.11	0.33	0.17	-0.30
SHSCRPRSCR	-0.18	0.30	0.06	-0.27
SHTTXSCR	-0.05	0.03	-0.07	-0.01
BMIBI	-0.10	0.33	0.15	-0.25

Results of the AVGMINDO changepoint/threshold analysis are shown in Table 40. CP and RT analysis produced similar ranges of AVGMINDO changepoints in relationships with BMIBI metrics. The mean and median values of CP changepoints were lower than the mean and median values of RT changepoints.

QR analysis did not find any significant linear relationships between AVGMINDO and 90th percentile metric scores. This finding is congruent with the lack of consistent linear patterns seen in scatter plots (Appendix 15i). Levels of several BMIBI metrics generally increased with increasing AVGMINDO and then tended to decline from optimal levels after AVGMINDO levels reached approximately 7 mg/L (e.g., see Appendix 15i; AVGMINDO v. SHEPHMSCR). This bi-directional pattern might tend to interfere with finding statistically significant QR models.

Table 40. Changepoint/threshold analysis results for diel dissolved oxygen minima and BMIBI metrics. 2002-2006 REMAP wadeable, warm-water streams.

	Diurnal D.O. Minima (mg/L)		
Metric/Index	CP*	QR	RT
MH**-taxa richness (MHTTX)	-	-	-
SH**-taxa richness (SHTTX)	-	-	-
MH-EPT richness (MHEPT)	4.1	-	5.2
SH-EPT richness (SHEPTX)	4.1	-	4.2
MH-sensitive taxa (MHSEN)	4.1	-	5.2
SH-% 3-dominant taxa (SH3DOM)	4.2	-	4.8
SH-Mod. Hilsenhoff Biotic index (SHMHBI)	4.1	-	5.3
SH-% EPT (SHEPT)	4.1	-	6.0
SH-% Chironomidae (SHCHR)	5.3	-	6.0
SH-% Ephemeroptera (SHEPH)	5.9	-	6.1
SH-% Scrapers (SHSCR)	4.1	-	6.0
SH-% Dom. functional feeding grp, (SHDFFG)	5.9	-	6.0
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	4.1	-	6.0
Range	4.1 - 5.9	-	4.2 - 6.1
Mean	4.5	-	5.5
Median	4.1	-	6.0
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** MH, Multi-habitat sample; SH, Standard-Habitat sample			

Changepoint/threshold results for relationships between AVGRNGDO and BMIBI metrics are summarized in Table 41. The ranges of changepoints from CP and RT analysis were again similar (1.5 – 5.9 mg/L), with CP analysis producing slightly lower mean and median changepoint values. QR analysis found significant linear relationships between AVGRNGDO and two BMIBI metrics: SHEPTSCR and SHSCPRSCR. The calculated AVGRNGDO thresholds from the 90th percentile regression models for these biological response metrics were 7.4 mg/L and 5.3 mg/L, respectively.

Table 41. Changepoint/threshold analysis results for diel dissolved oxygen range and BMIBI metrics. 2002-2006 REMAP wadeable, warm-water streams.

	Diurnal D.O. Range (mg/L)		
Metric/Index	CP*	QR	RT
MH**-taxa richness (MHTTX)	(1.5)***	-	(1.5)
SH**-taxa richness (SHTTX)	(1.6)	-	-
MH-EPT richness (MHEPT)	5.8	-	5.8
SH-EPT richness (SHEPTX)	2.3	-	5.8
MH-sensitive taxa (MHSEN)	5.8	-	2.8
SH-% 3-dominant taxa (SH3DOM)	2.1	-	2.2
SH-Mod. Hilsenhoff Biotic index (SHMHBI)	1.6	-	2.5
SH-% EPT (SHEPT)	2.6	7.4	2.6
SH-% Chironomidae (SHCHR)	1.6	-	2.8
SH-% Ephemeroptera (SHEPH)	1.5	-	1.6
SH-% Scrapers (SHSCR)	1.6	5.3	5.9
SH-% Dom. functional feeding grp, (SHDFFG)	2.1	-	2.1
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	2.6	-	2.6
Range	1.5 - 5.8	5.3 - 7.4	1.6 - 5.9
Mean	2.5	6.4	3.3
Median	2.1	6.4	2.6

* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree

** MH, Multi-habitat sample; SH, Standard-Habitat sample

*** ( ) indicates direction of biological response was opposite of expected

The individual effects of AVGRNGDO or AVGMINDO on the BMIBI might be confounded by co-linearity. As shown in Figure 42, AVGMINDO levels tend to decrease in response to increasing levels of AVGRNGDO. A CP test was conducted using AVGRNGDO as the “stressor” or predictor variable and AVGMINDO as the response variable. The test found a significant changepoint at 5 mg/L AVGRNGDO that corresponded with a significant increase in the proportion of deployment samples in which AVGMINDO was below the water quality standards criterion of 5 mg/L. The criterion is accepted as a threshold below which adverse changes may occur in warmwater stream aquatic communities. When AVGRNGDO was less than or equal to 5 mg/L , the proportion of samples achieving the DO criterion was 89.0% compared with 51.5% when AVGRNGDO exceeded 5 mg/L.

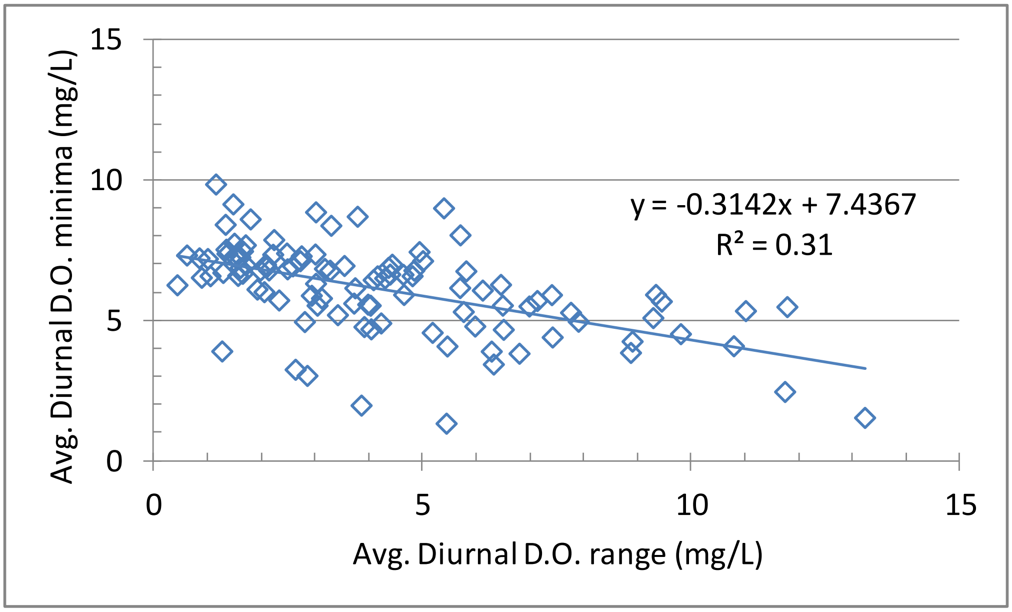


Figure 42. Simple linear regression of average diel dissolved oxygen (DO) range versus average diel dissolved oxygen (DO) minima. 2002-2006 REMAP wadeable, warm-water streams.

From this analysis, it is not clear whether or not AVGRNGDO by itself represents a stressor that is capable of impairing the benthic macroinvertebrate assemblage. As seen in Figure 43(a), increasing levels of AVGRNGDO do appear to correlate with a declining trend in the maximum response in the scraper metric as depicted by the 90th percentile regression line. However, it is also appears that substandard levels of AVGMINDO generally correspond with many of the lowest metric scores independent of AVGRNGDO level.

AVGRNGDO was not significantly related in the 90th percentile regression of BMIBI scores (Figure 43b), thus suggesting that AVGRNGDO by itself was not imparting a limiting influence over BMIBI levels. Substantially stressful levels of AVGMINDO below the instantaneous DO criterion of 4 mg/L were, however, more likely to occur at elevated levels of AVGRNGDO ($\geq$ 5 mg/L), and these levels were more likely to be associated with BMIBI levels that failed to achieve the reference benchmark score (53). When AVGMINDO was less than 4 mg/L, the proportion of BMIBI samples attaining the reference benchmark score was 7.7% compared with 63.4% when AVGMINDO was greater than 4 mg/L.

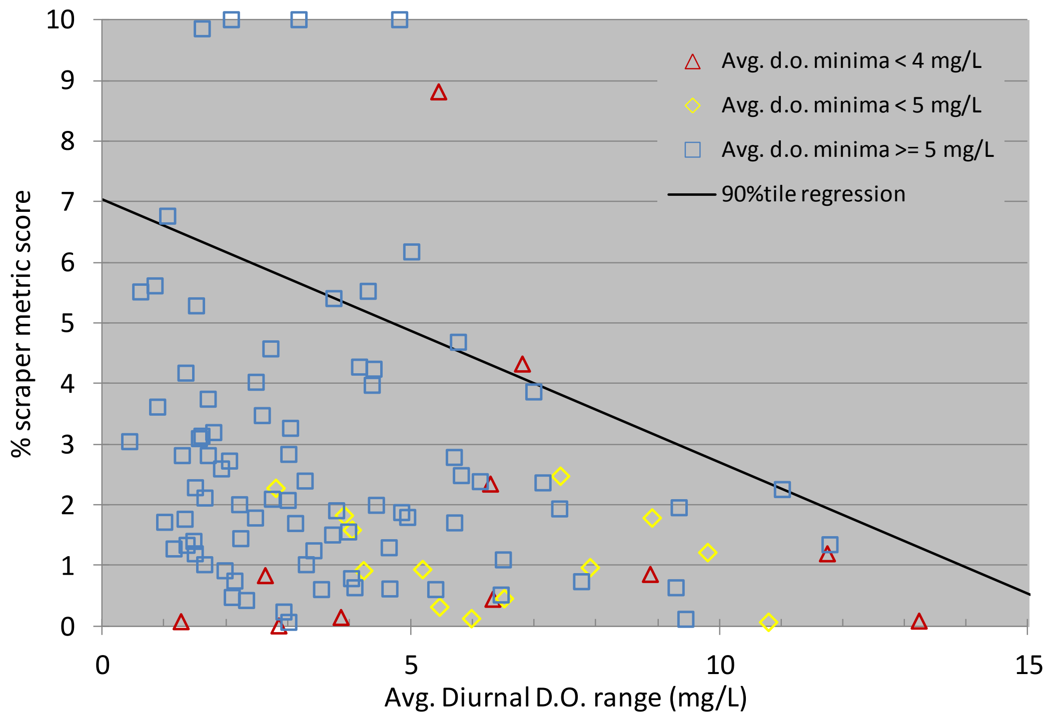


Figure 43(a). Quantile (90%) regression model of average diel dissolved oxygen (DO) range versus benthic macroinvertebrate % scraper organisms metric score. 2002-2006 REMAP wadeable warmwater streams .

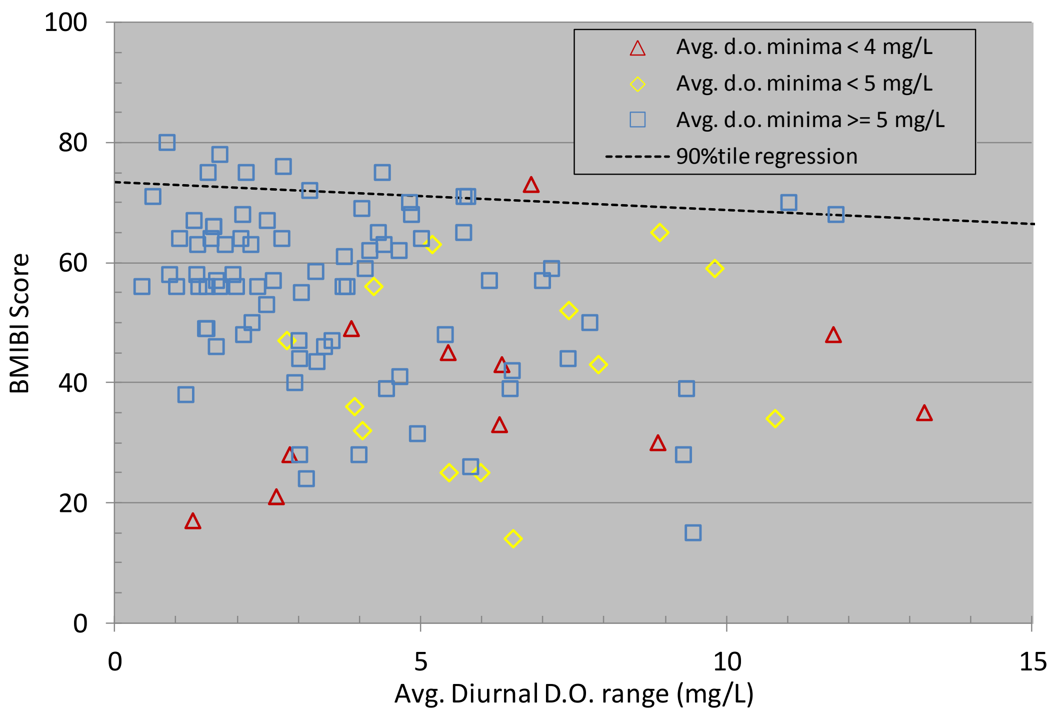


Figure 43(b). Quantile (90%) regression (not significant; p>0.05) model of average diel dissolved oxygen (DO) range versus benthic macroinvertebrate index of biotic integrity (BMIBI). 2002-2006 REMAP wadeable warmwater streams.

Dissolved oxygen and fish

Diel dissolved oxygen variables were virtually uncorrelated with Fish IBI metrics. Correlation analysis revealed two weak relationships among the 52 combinations of DO variables and FIBI metrics (Tables 42 and 43). Adjusted fish catch per unit effort (ACPUE) (i.e., total fish catch excluding tolerant species per 100 ft. stream length) increased with increasing average diel maximum DO (AVGMAXDO) and the average DO range (AVGRNGDO). ADJCPUE also increased with increasing levels of gross primary production (AVGGPP); all of these correlations signaled that high levels of fish abundance were linked to increased stream primary production.

Table 42. Pearson correlation coefficients (r) representing relationships of diel dissolved oxygen (DO) variables and fish index of biotic integrity (FIBI) metrics sampled from 112 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Bolded values indicate a significant linear relationships ($p<0.05$).

	AVGMAXDO	AVGMINDO	AVG2DO	AVGRNGDO
ACPUESCR	**0.19**	-0.10	0.06	**0.21**
BINVSPSCR	0.04	0.10	0.11	-0.03
NTVSPSCR	0.02	0.02	0.03	0.01
P3ABUNDSC	0.05	-0.02	0.03	0.05
PBINVSCR	0.02	-0.05	0.00	0.05
PDELT	-0.06	-0.06	-0.09	-0.02
POMNVSCR	-0.05	-0.01	-0.03	-0.04
PSLITHOSC	0.01	0.07	0.08	-0.03
PTCVSCR	-0.07	0.18	0.08	-0.16
SCKRSPSCR	0.10	0.03	0.11	0.07
SNSTVSPSC	0.11	-0.02	0.09	0.10
TOLINDXSC	0.12	0.10	0.17	0.05
FIBI	0.08	0.03	0.10	0.05

Table 43. Spearman correlation coefficients (rho) for relationships of diel dissolved oxygen (DO) variables and fish index of biotic integrity (FIBI) metrics sampled from 112 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles.

	AVGMAXDO	AVGMINDO	AVG2DO	AVGRNGDO
ACPUESCR	0.22	-0.04	0.12	0.22
BINVSPSCR	0.10	0.10	0.14	0.02
NTVSPSCR	0.05	-0.02	0.03	0.04
P3ABUNDSC	0.06	-0.09	-0.02	0.10
PBINVSCR	0.02	-0.12	-0.04	0.09
PDELT	0.05	-0.09	-0.06	0.08
POMNVSCR	-0.06	-0.02	-0.02	-0.01
PSLITHOSC	0.04	0.09	0.07	-0.01
PTCVSCR	-0.07	0.17	0.05	-0.16
SCKRSPSCR	0.10	0.00	0.08	0.08
SNSTVSPSC	0.17	-0.04	0.13	0.13
TOLINDXSC	0.12	0.08	0.14	0.06
FIBI	0.10	-0.01	0.08	0.09

CP and RT changepoint relationship analysis of diel DO minima (AVGMINDO) and FIBI metrics were inconsistent. One-half of the responses were opposite of the expected direction (Table 44; Appendix K). AVGMINDO changepoints identified for five metrics (SCKRSP, BINVSP, PTOPC, PSLTH, TOLINDX) ranged from 5.1-6.1 mg/L. These results were fairly consistent with changepoint results identified in the BMIBI response analysis. The other changepoints, ranging from 7.2 - 7.5 mg/L, were opposite of the expected direction and indicated less chance of attaining applicable reference benchmarks when AVGMINDO levels were highest.

Table 44. Changepoint/threshold analysis results for diel dissolved oxygen minima (AVGMINDO) and fish index of biotic integrity (FIBI) metrics sampled. REMAP (2002-2006) wadeable, warmwater streams.

	Diurnal D.O. Minima (mg/L)		
Metric/Index	CP*	QR	CART
# native fish species (NTVSP)	-	-	-
# sucker species (SCKRSP)	5.2	-	-
# sensitive species (SNSTVSP)	-	-	-
# benthic invertivore species (BINVSP)	6.3	-	-
% 3-dominant fish species (PTOP3)	(7.4)**	8.3	(8.4)
% benthic invertivores (PBINV)	(7.3)	-	(7.2)
% omnivores (POMNV)	-	-	-
% top carnivores (PTOPC)	5.2	-	5.8
% simple lithophil spawners (PSLTH)	6.1	-	-
fish assemblage tolerance index (TOLINDX)	(7.4)	-	5.1
adjusted catch per unit effort (ACPUE)		(6.5)	(7.5)
Fish Index of Biotic Integrity (FIBI)	-	-	-
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** ( ) indicates direction of biological response opposite of expected			

QR analysis found two conflicting thresholds. AVGMINDO levels below 8.3 mg/L were associated with less than 10% frequency of attaining the reference 25th percentile score for PTOP3 metric, whereas, levels above 6.5 mg/L were associated with a low chance of attaining the ACPUE reference benchmark.

CP and RT analysis results for average diel DO range (AVGRNGDO) were also inconsistent. Changepoint responses of seven FIBI metrics were opposite of the expected direction (Table 45). Changepoints were found at low and high levels of AVGRNGDO as illustrated in the relationship between AVGRNGDO and number of sensitive fish species SNTVSPSCR (Figure 44a). Optimum levels of SNTVSPSCR were observed in the middle range of AVGRNGDO between the CP and RT changepoints. CP, RT, and QR analysis all found responses in ADJCPUESCR that were opposite of the expected direction (Figure 44b), again confirming that increased fish abundance (excluding tolerant species) was associated with moderate to high levels of stream productivity.

Table 45. Changepoint/threshold analysis results for average diel dissolved oxygen range (AVGRNGDO) and fish index of biotic integrity (FIBI) metrics. REMAP (2002-2006) wadeable warmwater streams.

	Diurnal D.O. Range (mg/L)		
Metric/Index	CP*	QR	CART
# native fish species (NTVSP)	(1.4)**	-	-
# sucker species (SCKRSP)	(1.4)	-	(1.3)
# sensitive species (SNSTVSP)	(7.9)	-	(1.5)
# benthic invertivore species (BINVSP)	-	-	-
% 3-dominant fish species (PTOP3)	(1.4)	-	-
% benthic invertivores (PBINV)	(7.9)	-	(1.5)
% omnivores (POMNV)	6.3	-	-
% top carnivores (PTOPC)	2.2	-	5.3
% simple lithophil spawners (PSLTH)	-	-	-
fish assemblage tolerance index (TOLINDX)	(2.6)	-	-
adjusted catch per unit effort (ACPUE)	(2.4)	(3.6)	(2.5)
Fish Index of Biotic Integrity (FIBI)	-	-	-
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** ( ) indicates direction of biological response opposite of expected			

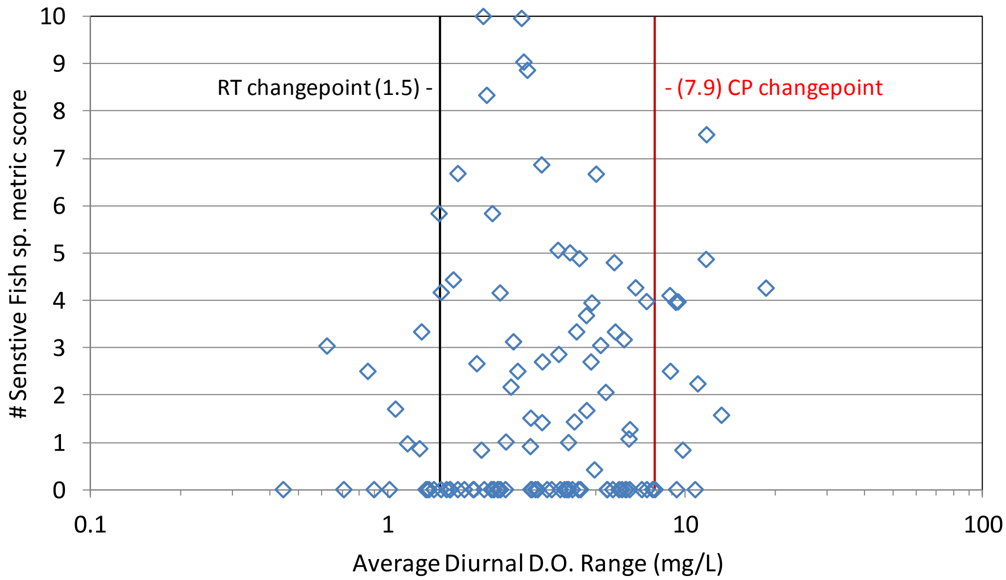


Figure 44a. Stressor-response plot of average diel dissolved oxygen (DO) range (AVGRNGDO) and number of sensitive fish species metric score (SNSTVSPSCR) including regression tree (RT) and conditional probability (CP) analysis changepoints. REMAP (2002-2006) wadeable warmwater streams.

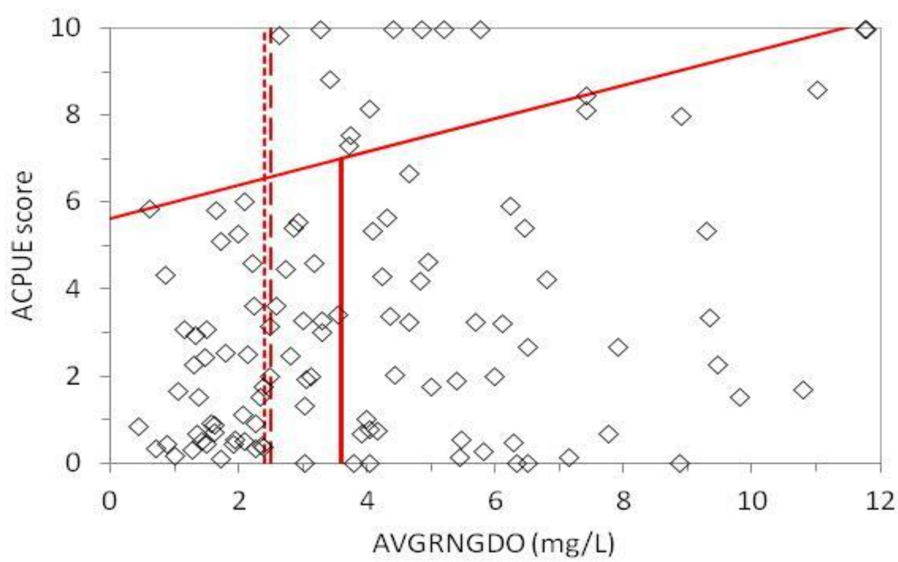


Figure 44b. Fish adjusted catch per unit effort (ACPUE) plotted against average diel dissolved oxygen range (AVGRNGDO). REMAP (2002-2006) wadeable warmwater streams. Stressor-response breakpoint/threshold analysis results are displayed as vertical lines: Conditional Probability breakpoint (vertical, short dashed); Quantile Regression threshold (vertical, thick solid); Quantile Regression 90th percentile regression (sloping, thin solid) Regression Tree (RT) breakpoint (vertical, long dashed). A red line indicates the metric response was opposite of predicted (i.e. overall metric scores improved with decreasing AVGRNGO levels).

Benthic chlorophyll A and benthic macroinvertebrates

Periphyton chlorophyll A (PCHLA) and sediment chlorophyll A (SCHLA) relationships with benthic macroinvertebrate (BMIBI) metrics were examined by correlation analysis. Results of seston chlorophyll A (WCHLA) correlations are included for comparison purposes. Chlorophyll A variables were log-transformed for Pearson correlation to improve the normality of data distributions. Pearson correlation results and nonparametric Spearman rank correlation results were generally similar with respect to ranking the magnitudes of relationships between chlorophyll A variables and BMIBI metrics (Table 46). Seston chlorophyll A (WCHLA) had a greater number of significantly correlated ($p<0.05$) BMIBI metrics (5) compared with PCHLA (3) and SCHLA (1).

Table 46. Correlation results of periphyton (PCHLA), sediment (SCHLA), and seston, water-column (WCHLA) chlorophyll A versus BMIBI metrics sampled from 106 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Chlorophyll A values were Log10 transformed for Pearson linear correlation. Bolded values are significant at $p<0.05$.

	Pearson (r)			Spearman Rank (rho)		
	L10 PCHLA	L10 SCHLA	L10 WCHLA	PCHLA	SCHLA	WCHLA
MHTTXSCR	**0.21**	0.13	0.05	0.18	0.13	0.05
SHTTXSCR	0.11	-0.08	**-0.27**	0.13	-0.07	-0.22
MHEPTXSCR	0.12	-0.01	-0.16	0.11	0.02	-0.16
SHEPTXSCR	-0.01	-0.07	**-0.27**	0.05	-0.03	-0.23
MHSNSTVSCR	**0.20**	0.13	-0.04	0.17	0.12	-0.12
SHEPHSCR	-0.17	-0.09	-0.08	-0.19	-0.08	-0.10
SHPEPTSCR	**-0.20**	-0.04	-0.09	-0.17	-0.02	-0.10
SHCHIRSCR	-0.06	-0.06	-0.14	-0.08	-0.07	-0.11
SHSCRPRSCR	-0.06	**-0.22**	**-0.24**	-0.02	-0.18	-0.21
SHP3DOMSCR	-0.10	-0.12	**-0.29**	-0.02	-0.11	-0.25
SHDFFGSCR	0.03	-0.11	**-0.20**	0.10	-0.09	-0.10
MHBISCR	-0.07	-0.09	-0.14	-0.04	-0.06	-0.15
BMIBI	-0.01	-0.07	-0.23	0.02	-0.06	-0.18

PCHLA was positively correlated with MHTTXSCR and MHSNTVSCR and inversely correlated with SHEPTSCR. SCHLA was inversely correlated with SHSCRPRSCR. Linear relationships of PCHLA and SCHLA with BMIBI metrics were weak. None of the relationships could account for more than 5% of the variation in any given BMIBI metric.

CP analysis results for PCHLA were inconsistent. Changepoint levels ranged from 1.2 – 16.0 ug/cm^2, and one-half of the changepoint responses were opposite of the expected direction (Table 47). CP results for SCHLA were slightly more consistent (Table 48). SCHLA changepoints ranged from 7.4 – 12.8 ug/cm^2 in relationships with the BMIBI and five component metrics. Changepoints at low levels of SCHLA (1.3 – 2.0 ug/cm^2) were found in relationships with all three multi-habitat (MH) BMIBI metrics.

Table 47. Changepoint/threshold analysis results for periphyton chlorophyll A (PCHLA) and benthic macroinvertebrate index of biotic integrity (BMIBI) metrics. 2002-2006 REMAP wadeable, warm-water streams.

	Periphyton Chlorophyll A (ug/L)		
Biological Metric / Index	CP*	QR	RT
MH**-taxa richness (MHTTX)	(1.3)***	(2.9)	(2.0)
SH**-taxa richness (SHTTX)	-	-	-
MH-EPT richness (MHEPT)	(2.2)		(2.3)
SH-EPT richness (SHEPTX)	-	-	-
MH-sensitive taxa (MHSEN)	(1.1)	(3.7)	(2.3)
SH-% 3-dominant taxa (SH3DOM)	-	-	-
SH-Mod. Hilsenhoff Biotic index (SHMHBI)	-	-	-
SH-% EPT (SHEPT)	1.4	-	7.9
SH-% Chironomidae (SHCHR)	1.4	-	
SH-% Ephemeroptera (SHEPH)	14.1	-	15
SH-% Scrapers (SHSCR)	-	-	-
SH-% Dom. functional feeding grp, (SHDFFG)	(16.0)	-	(16.0)
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	10.7	-	-
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** MH, Multi-habitat sample; SH, Standard-Habitat sample			
*** ( ) indicates direction of biological response opposite of expected			

Table 48. Changepoint/threshold analysis results for sediment chlorophyll A (SCHLA) and benthic macroinvertebrate index of biotic integrity (BMIBI) metrics. 2002-2006 REMAP wadeable, warm-water streams.

	Sediment Chlorophyll A (ug/L)		
Biological Metric / Index	CP*	QR	RT
MH**-taxa richness (MHTTX)	(1.3)***	(1.4)	(2.0)
SH**-taxa richness (SHTTX)	10.7		7.6
MH-EPT richness (MHEPT)	(1.5)		
SH-EPT richness (SHEPTX)			6.0
MH-sensitive taxa (MHSEN)	(2)	(2.4)	(2.4)
SH-% 3-dominant taxa (SH3DOM)	7.4		6.0
SH-Mod. Hilsenhoff Biotic index (SHMHBI)			
SH-% EPT (SHEPT)			
SH-% Chironomidae (SHCHR)			
SH-% Ephemeroptera (SHEPH)	12.8		
SH-% Scrapers (SHSCR)	12	4.3	1.2
SH-% Dom. functional feeding grp, (SHDFFG)	10.7		
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	7.6		9.8
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** MH, Multi-habitat sample; SH, Standard-Habitat sample			
*** ( ) indicates direction of biological response opposite of expected			

RT changepoint analysis results were also inconsistent. PCHLA changepoints ranged from 2.0 – 16.0 ug/cm^2, and four of six changepoint responses were opposite of the expected direction (Table 47). SCHLA changepoints ranged from 1.2 – 9.8 ug/cm^2 with one-half representing opposite responses (Table 48). Although statistically significant, the amount of metric variation in these stressor-response relationships that was attributable to any given PCHLA or SCHLA changepoint was relatively small, ranging from 4.1 – 8.1%.

QR analysis of PCHLA and SCHLA found opposite of expected response thresholds for two BMIBI metrics (MHTTXSCR, MHSNSTVSCR). Threshold levels were similar to the corresponding CP and RT changepoint values for these metrics (Tables 47 and 48). The relationship of SCHLA and percent abundance of scraper organisms (SCRPRSCR) was the only one for which a threshold (9.8 ug/cm^2) was found in the expected direction.

The variable results from changepoint/threshold analysis of PCHLA and SCHLA suggested that influences from other environmental factors such as habitat and water quality might obscure relationships involving benthic chlorophyll A and BMIBI metrics. To explore this possibility, benthic chlorophyll - BMIBI response patterns were examined for covariation or interaction with dissolved oxygen and physical habitat conditions. Data from 79 wadeable warmwater REMAP sites that included all sampling components were used for this analysis.

The question of BMIBI metrics responding oppositely of the expected direction in relation to varying levels of benthic chlorophyll A was specifically examined. The multi-habitat (MH) data metrics (MHTTXSCR, MHEPTXSCR, MHSNSTVSCR) consistently showed a pattern in which metric scores tended to be lower at sites where PCHLA and SCHLA levels were also low. It was suspected that other environmental factors such as low dissolved oxygen or poor habitat conditions could be correlated with the occurrence of low benthic algal biomass, and that these conditions might be partly responsible for the observed patterns.

Correlation analysis found the BMIBI and eight component metrics were significantly related with a qualitative habitat index score (HABSCR). The habitat index was generally more strongly correlated with MH metrics of the BMIBI compared with standard habitat (SH) metrics (Table 49a-b). Epifaunal substrate/cover (EPISUB) was the habitat metric that was correlated the most strongly with BMIBI metrics. EPISUB rates the overall abundance and diversity of benthic substrates (e.g., detritus, rock, wood, vegetation) that is available for colonization. A high score in this metric suggests that habitat conditions are favorable for supporting good abundances and diversity of benthic macroinvertebrates. Accordingly, EPISUB correlations with MH metrics ranked higher than correlations with SH metrics, which are less indicative of stream habitat complexity. PCHLA was positively correlated with the composite habitat index (HABSCR) and five component metrics, while SCHLA was not significantly related to HABSCR or any component metrics.

The potential confounding influences of DO minima and habitat quality were further explored graphically. PCHLA changepoint/threshold values (Figure 45) from CP, RT, and QR analysis of MHSNSTVSCR were clustered near the low end of the PCHLA data range (i.e., opposite of expected). The frequency of habitat quality scores interpreted as poor or marginal (78.1%) among sites where PCHLA levels were below the average changepoint/threshold value (2.4 ug/L) was significantly higher than the frequency of occurrence at sites having PCHLA greater than the changepoint (35.6%). Similarly, the proportion of sites having diel DO minima less than 5 mg/L ranked higher at sites below the average PCHLA changepoint than above the changepoint (37.5% vs. 19.0%); however this difference was not statistically significant (chi-square test; p-value = 0.12). While it appears that DO minima was a contributing factor to low MHSNSTVSCR levels at some sites, the colinearity of periphyton and habitat quality seems more likely to be a driving factor explaining the unexpected response of MHSNTVSCR to PCHLA.

Table 49. Pearson (a) and Spearman (b) correlation analysis results for relationships of a composite habitat index (HABSCR) and seven individual habitat metrics (BNKSTBL, CHNLALT, CHNLFLW, EPISUB, RPRWDTH, SEDIDEPO, BNKVEG) versus BMIBI metrics sampled from 92 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Chlorophyll A values were Log10 transformed for Pearson linear correlation; bolded, italicized values are significant at $p<0.05$.

(a) Pearson (r)

	BNKSTBL	CHNLALT	CHNLFLW	EPISUB	RPRWDTH	SEDIDEPO	BNKVEG	HABSCR
L10PCHLA	***0.24***	0.14	***0.36***	***0.40***	-0.02	***0.32***	0.06	***0.35***
L10SCHLA	0.12	-0.02	0.18	0.05	0.03	-0.05	0.05	0.04
L10WCHLA	0.06	0.13	-0.10	0.04	***0.28***	-0.01	0.14	0.13
MHTTXSCR	***0.36***	***0.25***	0.09	***0.36***	0.19	***0.27***	***0.33***	***0.42***
SHTTXSCR	***0.37***	0.05	***0.25***	***0.28***	-0.07	***0.27***	***0.23***	***0.29***
MHEPTXSCR	0.14	0.28	0.20	***0.36***	0.11	***0.35***	0.08	***0.39***
SHEPTXSCR	0.07	0.16	0.20	***0.24***	0.04	0.18	-0.01	***0.24***
MHSNSTVSCR	0.21	***0.25***	***0.21***	***0.39***	0.11	***0.31***	0.13	***0.38***
SHEPHSCR	-0.14	0.06	-0.03	0.07	-0.10	0.01	-0.19	-0.02
SHPEPTSCR	-0.17	***0.22***	0.02	0.04	0.03	-0.03	-0.18	0.06
SHCHIRSCR	0.02	***0.28***	0.11	0.15	0.09	0.05	0.03	***0.23***
SHSCRPRSCR	-0.01	0.08	0.01	***0.23***	-0.13	0.16	-0.06	0.09
SHP3DOMSCR	0.20	0.09	0.16	0.14	0.04	0.12	0.19	***0.23***
SHDFFGSCR	0.05	0.19	0.11	0.21	0.08	0.20	0.12	***0.27***
MHBISCR	-0.06	0.20	0.13	***0.24***	-0.01	0.15	-0.12	0.17
BMIBI	0.11	***0.27***	0.18	***0.34***	0.04	***0.25***	0.05	***0.34***

(b) Spearman (rho)

	BNKSTBL	CHNLALT	CHNLFLW	EPISUB	RPRWDTH	SEDIDEPO	BNKVEG	HABSCR
PCHLA	0.25	0.02	0.33	0.35	-0.10	0.22	0.05	0.27
SCHLA	0.10	0.01	0.10	0.10	-0.02	-0.06	-0.03	0.01
WCHLA	-0.02	0.15	-0.04	0.05	0.26	0.01	0.12	0.12
MHTTXSCR	0.32	0.23	0.07	0.32	0.22	0.26	0.32	0.38
SHTTXSCR	0.35	0.02	0.17	0.27	-0.07	0.29	0.25	0.28
MHEPTXSCR	0.14	0.31	0.13	0.36	0.13	0.34	0.06	0.37
SHEPTXSCR	0.05	0.18	0.14	0.22	0.06	0.17	0.01	0.25
MHSNSTVSCR	0.17	0.24	0.16	0.32	0.09	0.26	0.05	0.27
SHEPHSCR	-0.16	0.10	-0.09	0.05	-0.09	-0.01	-0.21	-0.02
SHPEPTSCR	-0.17	0.17	-0.04	0.03	0.01	-0.05	-0.16	0.02
SHCHIRSCR	0.02	0.15	0.05	0.14	0.05	0.01	0.02	0.15
SHSCRPRSCR	-0.06	0.14	0.06	0.24	-0.11	0.21	-0.10	0.10
SHP3DOMSCR	0.17	0.08	0.11	0.14	0.05	0.13	0.19	0.25
SHDFFGSCR	0.06	0.18	0.10	0.21	0.09	0.19	0.18	0.26
MHBISCR	-0.04	0.23	0.07	0.25	0.04	0.14	-0.07	0.18
BMIBI	0.12	0.30	0.16	0.37	0.06	0.28	0.06	0.35

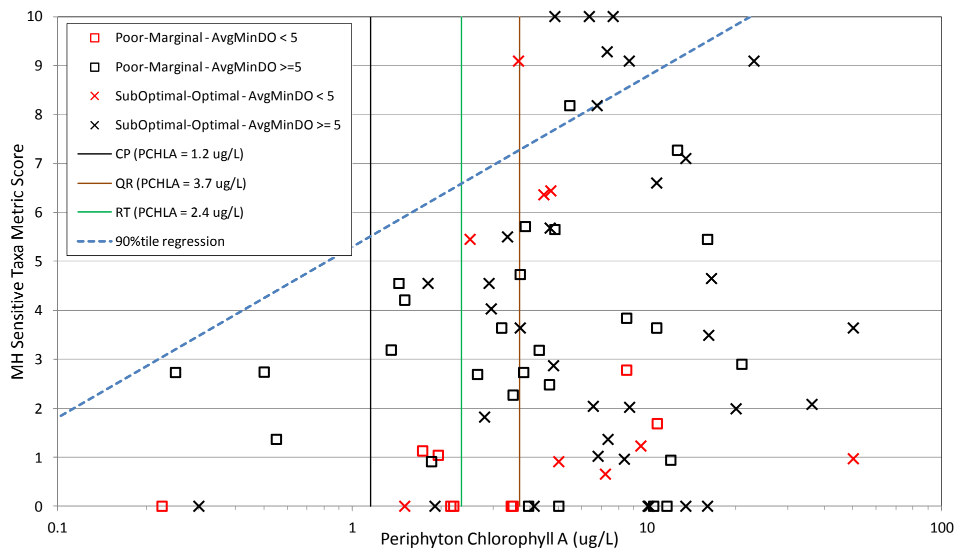


Figure 45. Response plot of multi-habitat number of sensitive benthic macroinvertebrate taxa metric score (MHSNSTVSCR) versus Log10 periphyton chlorophyll A (PCHLA). Rectangle symbols indicate sites rated as having poor-marginal (fair) habitat quality; "X" symbols indicate sites rated as having suboptimal (good) or optimal (excellent) habitat quality. Red outlined symbols denote sites where the average diel DO minima was less than the water quality standard criterion of 5 mg/L. Solid vertical lines correspond with PCHLA changepoint values from CP (conditional probability), QR (quantile regression), and RT (regression tree) analysis. Dashed line represents the 90th percentile fitted regression line from quantile regression analysis

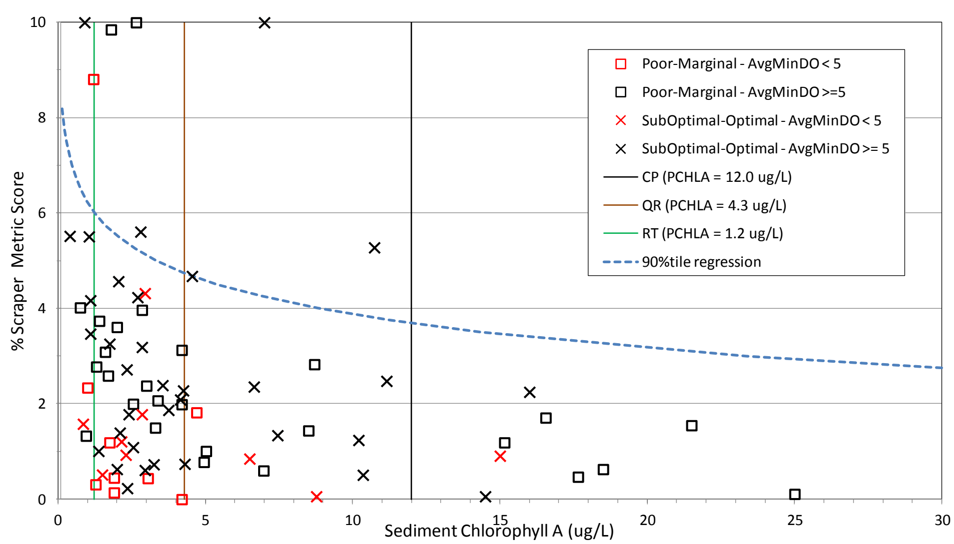


Figure 46. Response plot of standard-habitat percent abundance scraper organisms metric score (SCRPRSCR) versus Log10 sediment chlorophyll A (SCHLA). Rectangle symbols indicate sites rated as having poor-marginal (fair) habitat quality; "X" symbols indicate sites rated as having suboptimal (good) or optimal (excellent) habitat quality. Red outlined symbols denote sites where the average diel DO minima was less than the water quality standard criterion of 5 mg/L. Solid vertical lines correspond with PCHLA changepoint values from CP (conditional probability), QR (quantile regression), and RT (regression tree) analysis. Dashed line represents the 90th percentile fitted regression line from quantile regression analysis.

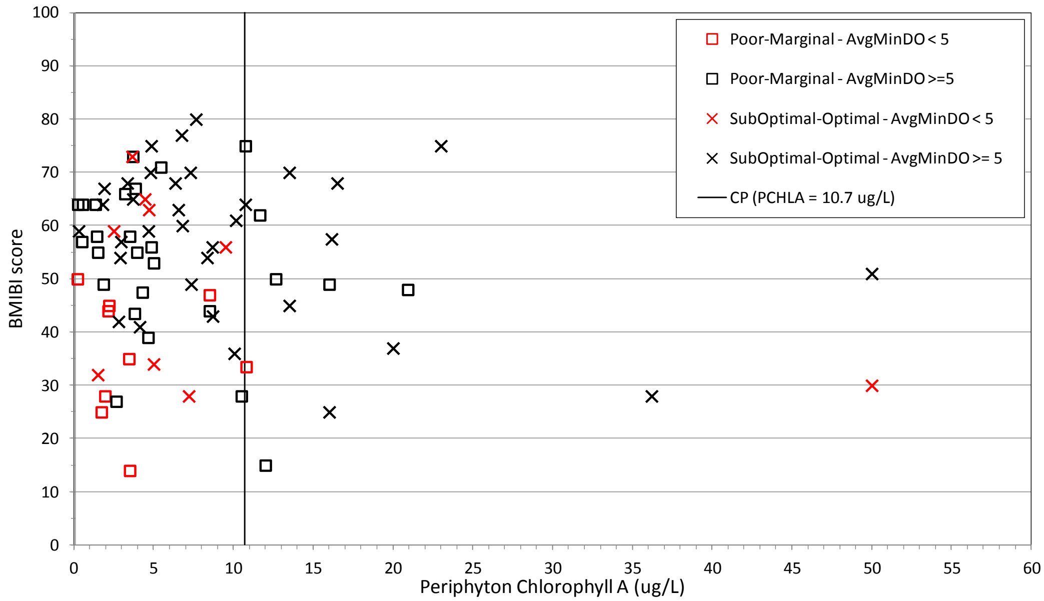


Figure 47. Response plot of BMIBI versus periphyton chlorophyll A (PCHLA). Rectangle symbols indicate sites rated as having poor-marginal (fair) habitat quality; "X" symbols indicate sites rated as having suboptimal (good) or optimal (excellent) habitat quality. Red outlined symbols denote sites where the average diel DO minima was less than the water quality standard criterion of 5 mg/L. Solid vertical lines correspond with PCHLA changepoint value from CP (conditional probability) analysis.

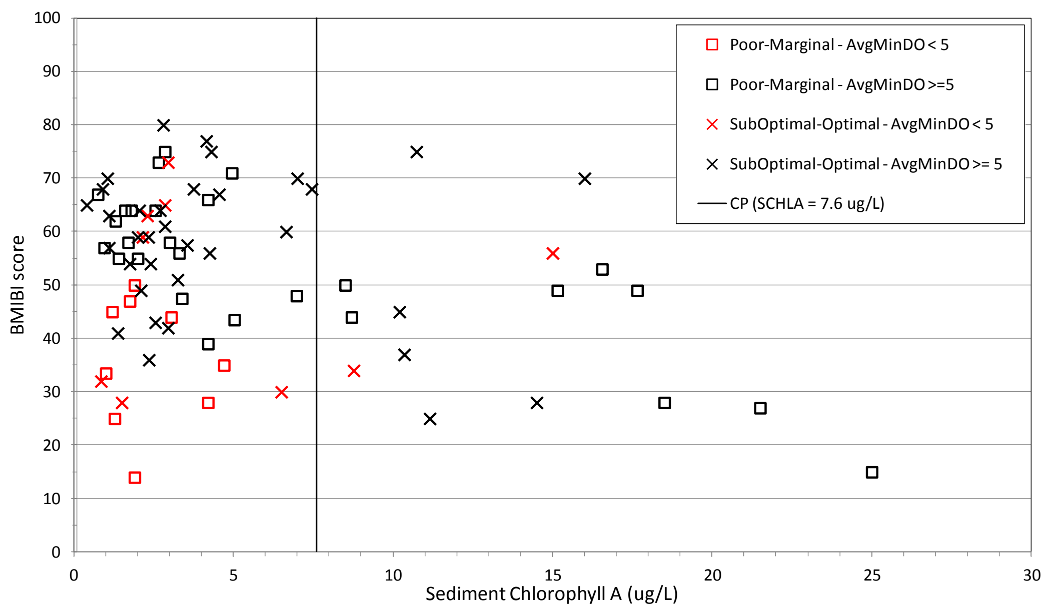


Figure 48. Response plot of BMIBI versus sediment chlorophyll A (SCHLA). Rectangle symbols indicate sites rated as having poor-marginal (fair) habitat quality; "X" symbols indicate sites rated as having suboptimal (good) or optimal (excellent) habitat quality. Red outlined symbols denote sites where the average diel DO minima was less than the water quality standard criterion of 5 mg/L. Solid vertical lines correspond with SCHLA changepoint value from CP (conditional probability) analysis.

Aside from two MH metrics, the percent abundance scraper organisms (SCRPRSCR) metric was the only SH metric of which a significant quantile regression (QR) relationship was found. Figure 46 shows the SCRPRSCR - SCHLA response pattern and the CP, QR, and RT changepoints. The 90th percentile log-linear QR fitted line shows a decline from optimal levels that occurs approximately between 1-7 ug/L of SCHLA; the QR threshold was 4.3 ug/L. Above this threshold, the reference 75th percentile SCRPRSCR metric score is not expected to be attained in more than 10% of samples.

Scraper organisms possess specialized feeding structures that allow them to harvest the thin biofilm of algae and microbes that colonize coarse substrates such as rocks and woody debris. With sedimentation and increased production of sediment-associated algal biomass, it is plausible that scraper abundance will decline and other benthic macroinvertebrates that are better equipped to exploit this habitat niche will become more prevalent. Unlike the MH metrics, the SCRPRSCR metric was not correlated with HABSCR; therefore, it is unlikely that habitat quality is the major driver in the response relationship. Sites having DO minima levels below the 5 mg/L criterion tended to cluster in the lower left region of the plot, indicating that low DO was not linearly related with SCHLA and probably not masking the QR-modeled limiting relationship between SCHLA and SCRPRSCR.

CP analysis found significant changepoints for PCHLA (10.7 ug/L) and SCHLA (7.6 ug/L) above which the frequencies of sites attaining the reference site 25th percentile BMIBI score were decreased. Visual examination of response plots (Figures 47 and 48) seemed to indicate that BMIBI levels reach maximum levels near these changepoints and then decline at elevated levels of PCHLA and SCHLA. Similar declining patterns appear to occur in several of the BMIBI component metrics, including: PCHLA vs. MHTTXSCR, SHTTXSCR, MHSNSTVSCR, SHEPHMSCR, SHSCRPRSCR, and SHMHBISCR; SCHLA vs. MHSNSTVSCR, SHEPHMSCR, SHSCRPRSCR, AND SHMHBISCR (Appendix 15c-d). Acquisition of more data particularly from sites having elevated benthic chlorophyll A levels are needed for a more robust statistical analysis of relationships with benthic macroinvertebrate assemblage metrics.

In summary, this exploratory analysis found that improved benthic macroinvertebrate assemblage condition was correlated with higher quality habitat, which was correlated with increased periphyton algal biomass (PCHLA). Low levels of PCHLA tended to be associated with poor or marginal habitat. In contrast, sediment chlorophyll A (SCHLA) levels were not significantly related to habitat characteristics. Medium-to-high levels of PCHLA and SCHLA tended to be associated with less frequent occurrences of low dissolved oxygen. The BMIBI and certain component metrics display a declining response pattern at the highest observed levels of PCHLA and SCHLA. The types of metrics involved suggest that the responses are likely to be related to changes in micro-scale substrate and food resource characteristics, rather than reach-scale habitat or dissolved oxygen limitations.

Benthic chlorophyll A and fish

Periphyton chlorophyll A (PCHLA) and sediment chlorophyll A (SCHLA) sample data were examined for relationships with fish index of biotic integrity (FIBI) metrics. Chlorophyll A variables were log-transformed to improve the normality of data distributions for Pearson linear correlation.

Pearson correlation results and nonparametric Spearman rank correlation results were generally similar with respect to ranking the magnitudes of relationships between chlorophyll A variables and FIBI metrics (Table 50). A greater number of FIBI metrics were correlated with PCHLA (4) than with SCHLA (1). All significant relationships with FIBI metrics were opposite of the expected direction (i.e., metric levels increased with increasing chlorophyll A levels). PCHLA was correlated with NTVSPSCR, SCKRSPSCR, SNSTVSPSCR, and ACPUESCR. SCHLA was positively correlated with PBINVSCR. All linear relationships were relatively weak, with none explaining more than 7.1% of the variation in any given FIBI metric.

Table 50. Correlation results of periphyton (PCHLA) and sediment (SCHLA) chlorophyll A versus FIBI metrics sampled from 131 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Chlorophyll A values were Log10 transformed for Pearson linear correlation. Bolded values are significant at $p<0.05$.

	Pearson (r)		Spearman Rank (rho)	
	L10PCHLA	L10SCHLA	PCHLA	SCHLA
NTVSPSCR	***0.19***	0.04	0.25	0.07
SCKRSPSCR	***0.19***	0.07	0.21	0.08
SNSTVSPSCR	***0.27***	0.07	0.28	0.09
BINVSPSCR	0.16	0.09	0.21	0.11
P3ABUNDSCR	0.08	0.05	0.11	0.07
PBINVSCR	0.14	***0.18***	0.16	0.15
POMNVSCR	0.04	-0.06	0.14	-0.01
PTCVSCR	-0.06	0.04	-0.05	0.04
PSLITHOSCR	-0.06	0.02	-0.03	0.03
TOLINDXSCR	0.16	0.06	0.21	0.08
ACPUESCR	***0.23***	0.08	0.25	0.08
FIBI	0.17	0.07	0.22	0.10

Conditional probability analysis results for FIBI metrics (Tables 51 and 52; Appendix 15g-h) were similar to CP results for BMIBI metrics. Changepoints in PCHLA and SCHLA relationships with FIBI metrics were clustered in the lower ends of the stressor data ranges and response directions were mostly opposite of the expected direction. A changepoint of 12.6 ug/L PCHLA for the metric, PTCVSCR, and a changepoint of 10.2 ug/L SCHLA for POMNVSCR were the only ones that were consistent with the expected response direction. Regression tree (RT) analysis results were almost identical to CP results in terms of metrics, changepoint levels, and response directions. Quantile regression (QR) thresholds were identified at PCHLA levels ranging from 1.5-15.4 ug/L (Table 51) for four of the FIBI metrics (SNSTVSPSCR, POMNVSCR, TOLINDXSCR, ACPUESCR).

Table 51. Changepoint/threshold analysis results for periphyton chlorophyll A (PCHLA) and fish index of biotic integrity (FIBI) metrics. 2002-2006 REMAP wadeable, warm-water streams.

	Periphyton Chlorophyll A (ug/L)		
Metric/Index	CP*	QR	CART
# native fish species (NTVSP)	(1.7)**		(1.7)
# sucker species (SCKRSP)	(1.7)		(1.8)
# sensitive species (SNSTVSP)	(1.7)	(15.4)	(1.9)
# benthic invertivore species (BINVSP)	(1.7)		(1.7)
% 3-dominant fish species (PTOP3)	(1.6)		(1.7)
% benthic invertivores (PBNV)	(1.7)		(1.7)
% omnivores (POMV)		(1.5)	
% top carnivores (PTOPC)	12.6		10.3
% simple lithophil spawners (PLTH)			
fish assemblage tolerance index (TOLINDX)	(1.7)	(8.8)	(2.6)
adjusted catch per unit effort (ACPUE)	(1.8)	(3.4)	(1.8)
Fish Index of Biotic Integrity (FIBI)	(1.7)		(1.8)
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** ( ) indicates direction of biological response opposite of expected			

Table 52. Changepoint/threshold analysis results for sediment chlorophyll A (SCHLA) and fish index of biotic integrity (FIBI) metrics. 2002-2006 REMAP wadeable, warm-water streams.

	Sediment Chlorophyll A (ug/L)		
Metric/Index	CP*	QR	CART
# native fish species (NTVSP)	(1.2)**		
# sucker species (SCKRSP)	(1.0)		
# sensitive species (SNSTVSP)	(1.0)		
# benthic invertivore species (BINVSP)			(0.9)
% 3-dominant fish species (PTOP3)	(1.0)	1.9	
% benthic invertivores (PBNV)		(2.9)	
% omnivores (POMV)	10.2		9.2
% top carnivores (PTOPC)	(1.2)		
% simple lithophil spawners (PLTH)			
fish assemblage tolerance index (TOLINDX)	(0.8)		(1.6)
adjusted catch per unit effort (ACPUE)	(0.8)		(1.4)
Fish Index of Biotic Integrity (FIBI)			
* Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree			
** ( ) indicates direction of biological response opposite of expected			

In the same manner as the analysis of BMIBI metric responses, the issue of FIBI metrics responding oppositely of expected in relationships with benthic chlorophyll A levels was examined in greater detail. Overall, FIBI metrics were more strongly correlated with habitat variables (Tables 53 and 54) than were BMIBI metrics (Table 49a-b). This finding is consistent with results from a previous study (Wilton 2004). Weigel and Dimick (2011) also noted that the benthic macroinvertebrate IBI they developed for use in nonwadeable Wisconsin rivers using sampling data from Hester-Dendy artificial (wood-plate) substrates is more likely to reflect water quality differences than differences in physical habitat.

That fish assemblage metrics would be more strongly correlated with habitat measures than benthic macroinvertebrate metrics makes sense because habitat evaluation protocols tend to emphasize macro-habitat (i.e., reach-scale) characteristics that are important determinants of fish assemblage structure. In contrast, many benthic macroinvertebrate sampling protocols, including IDNR's, target sampling in micro-habitats (e.g., coarse rock or wood substrates in flowing water) where the abundance and diversity of organisms is optimized. Benthic macroinvertebrate metrics, therefore, might not be correlated with habitat metrics summarized at the stream reach scale.

Table 53. Pearson correlation coefficients (r) for relationships of a composite habitat index (HABSCR) and seven individual habitat metrics (BNKSTBL, CHNLALT, CHNLFLW, EPISUB, RPRWDTH, SEDIDEPO, BNKVEG) versus FIBI metrics sampled from 100 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Chlorophyll A values were Log10 transformed for Pearson linear correlation. Bolded, italicized values are significant at $p<0.05$.

	BNKSTBL	CHNLALT	CHNLFLW	EPISUB	RPRWDTH	SEDIDEPO	BNKVEG	HABSCR
NTVSPSCR	0.13	***0.37***	0.14	***0.37***	-0.03	***0.28***	0.00	***0.36***
SCKRSPSCR	0.13	***0.36***	0.15	***0.37***	-0.02	***0.38***	-0.04	***0.39***
SNSTVSPSC	***0.27***	***0.34***	0.19	***0.54***	***0.25***	***0.48***	***0.24***	***0.58***
BINVSPSCR	0.08	***0.34***	0.10	***0.48***	0.07	***0.36***	0.05	***0.42***
P3ABUNDSC	0.13	***0.33***	-0.01	***0.35***	0.14	***0.28***	0.10	***0.37***
PBINVSCR	***0.25***	0.14	0.11	***0.39***	0.02	***0.30***	0.19	***0.32***
POMNVSCR	-0.04	***0.20***	-0.10	0.16	0.01	0.15	-0.03	0.14
PTCVSCR	0.05	***0.30***	0.08	0.12	***0.31***	0.06	0.13	***0.27***
PSLITHOSC	0.09	***0.29***	0.16	***0.34***	0.12	***0.30***	0.07	***0.37***
TOLINDXSC	0.17	***0.33***	***0.22***	***0.42***	0.13	***0.36***	0.05	***0.44***
ACPUESCR	***0.25***	***0.29***	***0.22***	***0.47***	0.10	***0.40***	0.19	***0.49***
FIBI	0.18	***0.40***	0.15	***0.49***	0.14	***0.42***	0.12	***0.51***

Table 54. Spearman rank correlation coefficients (rho) for relationships of a composite habitat index (HABSCR) and seven individual habitat metrics (BNKSTBL, CHNLALT, CHNLFLW, EPISUB, RPRWDTH, SEDIDEPO, BNKVEG) versus FIBI metrics sampled from 100 REMAP wadeable stream sites with drainage area ranging from 10-700 square miles. Chlorophyll A values were Log10 transformed for Pearson linear correlation.

	BNKSTBL	CHNLALT	CHNLFLW	EPISUB	RPRWDTH	SEDIDEPO	BNKVEG	HABSCR
NTVSPSCR	0.16	0.37	0.11	0.39	-0.03	0.31	0.02	0.39
SCKRSPSCR	0.16	0.37	0.12	0.39	-0.01	0.38	-0.01	0.39
SNSTVSPSC	0.23	0.37	0.22	0.53	0.20	0.46	0.16	0.56
BINVSPSCR	0.06	0.32	0.05	0.49	0.07	0.34	0.01	0.39
P3ABUNDSC	0.13	0.33	-0.06	0.38	0.14	0.30	0.11	0.40
PBINVSCR	0.19	0.21	0.03	0.43	0.04	0.27	0.09	0.31
POMNVSCR	0.00	0.17	-0.13	0.19	0.04	0.17	0.02	0.17
PTCVSCR	0.05	0.27	0.01	0.07	0.32	0.02	0.10	0.22
PSLITHOSC	-0.06	0.22	0.08	0.27	0.09	0.22	-0.06	0.25
TOLINDXSC	0.20	0.33	0.13	0.41	0.13	0.37	0.06	0.43
ACPUESCR	0.16	0.32	0.14	0.41	0.07	0.37	0.11	0.43
FIBI	0.19	0.40	0.09	0.49	0.12	0.41	0.10	0.49

The interrelationships between the FIBI, PCHLA, HABSCR and DO minima can be seen in Figure 49. For sites having PCHLA below the CP changepoint of 1.7 ug/L, the frequency of FIBI scores attaining the reference 25th percentile score was 0% compared with 43.8% for sites above the changepoint. For sites below the PCHLA changepoint, the frequency of sites where habitat was rated as either poor or marginal (71.4%) was significantly higher than the frequency (41.9%) for sites above the changepoint (Chi-square; p=0.04). The mean habitat score was 81.8 (marginal) for sites below PCHLA changepoint and 106.4 (suboptimal) for sites above the changepoint. Among all sites that failed to attain the FIBI reference benchmark, whether above or below the changepoint, the proportion of sites where habitat was rated as poor or marginal (61.5%) was significantly higher than the proportion (22.5%) for sites attaining the FIBI reference benchmark (Chi-square; p<0.001). The mean habitat score was 93.1(marginal) for sites below the FIBI benchmark value and 117.8 (suboptimal) for sites equal or greater than the benchmark.

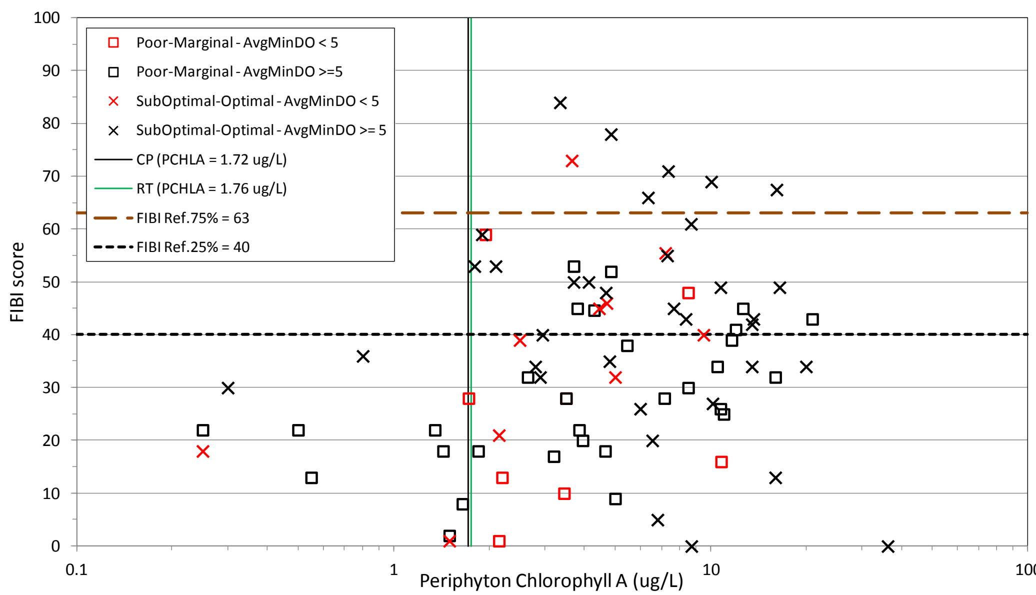


Figure 49. Response plot of Fish Index of Biotic Integrity (FIBI) versus periphyton chlorophyll A (PCHLA). Rectangle symbols indicate sites rated as having poor-marginal (fair) habitat quality; "X" symbols indicate sites rated as having suboptimal (good) or optimal (excellent) habitat quality. Red outlined symbols denote sites where the average diel DO minima was less than the water quality standard criterion of 5 mg/L. Solid vertical lines correspond with PCHLA changepoint value from CP (conditional probability) and regression tree (RT) analysis. Dashed horizontal lines show the FIBI 25th and 75th percentile scores from wadeable stream reference sites.

These analysis results further demonstrate that PCHLA and habitat condition in Iowa streams are correlated, and that this relationship confounds the interpretation of PCHLA relationships with fish and macroinvertebrate assemblages. There is a relatively low threshold in PCHLA (1.7 ug/L) below which there is a higher occurrence of scores not attaining the FIBI reference benchmark and also a higher occurrence of sites having poor or marginal habitat ratings. Among sites rated as having poor-marginal habitat the proportion of sites having AVGMINDO below the 5 mg/L criterion (21%) was similar to the proportion among sites rated as having suboptimal-optimal habitat (23%). Therefore it is unlikely that a disproportionate occurrence of low DO levels was a factor affecting the relative location of the PCHLA changepoint and unexpected (opposite) directionality in the PCHLA-FIBI relationship.

Optimal levels of the FIBI equal to or greater than the reference 75th percentile were observed when PCHLA levels were roughly between 3 – 16 ug/L. Similar to the BMIBI findings, there is a suggestion that FIBI levels decline at PCHLA levels above this range. Additional sampling data from streams having high levels of PCHLA would be helpful for a closer examination of this apparent pattern.

Periphyton qualitative ratings and benthic macroinvertebrates

An exploratory data analysis was conducted to examine relationships between qualitative periphyton observations and benthic macroinvertebrate (BMIBI) metrics. As part of the benthic macroinvertebrate sampling protocol, field personnel record visual observations about the characteristics of rock or wood substrates that are sampled for benthic macroinvertebrates. The observations include: (a) amount of periphyton coverage; (b) type of periphyton growth; (c) amount of embeddedness/sedimentation; (d) amount of benthic macroinvertebrate colonization.

Substrate type was recorded as rock or wood (i.e., Hester-Dendy style artificial substrates). Periphyton growth type was recorded as filamentous or non-filamentous. Amount ratings were recorded using the following areal coverage categories: light (0-25%); moderate (25-50%); moderately heavy (50-75%); heavy (75-100%). Appendix 16 contains a few example photographs of substrates displaying varying amounts and forms of periphyton growth.

Data from 446 sets of benthic macroinvertebrate samples collected from warmwater wadeable streams between 1994 and 2009 were included in the analysis. The dataset includes a mixture of samples including 132 sample sets from REMAP probabilistic survey sites, 139 sets from wadeable reference sites, and 198 sets collected for other purposes such as problem investigation and TMDL development.

After matching the correct substrate observations with corresponding BMIBI results, the data were visually examined for patterns and statistically tested for differences among treatment groups. An iterative approach was taken in which first the entire dataset was examined, and later the data were partitioned by substrate and periphyton growth types.

Analysis of Variance (AOV) was used to test for differences in BMIBI metric levels (dependent variables) by periphyton amount and type categories (treatment variables). The nonparametric (Kruskal-Wallis) AOV test was performed when it was determined that variance among treatment groups was unequal. Multiple mean testing of treatment group means was performed using the Fisher Least Significant Difference (LSD) and Tukey Honestly Significant Difference (HSD) multiple mean comparison tests.

AOV testing of the entire warmwater wadeable stream dataset found the periphyton amount treatment effect was significant on the BMIBI and nine component metrics ($p<0.05$). The amount of variation in BMIBI scores attributable to the treatment effect was relatively small (5.3%). Subsequent AOV testing of data subdivided by substrate type found a significant periphyton effect on rock, but not wood substrates. Generally, BMIBI scores from rock substrates evaluated as having heavy periphyton growth tended to have lower BMIBI scores than substrates evaluated as having lower periphyton amount ratings (Figure 50). The mean BMIBI score (54.6) for rock substrate samples rated as having heavy growth was significantly lower (Tukey HSD; $p<0.05$) than the mean scores for substrates rated as having moderate or moderately heavy periphyton growth (61.7 and 63.2, respectively). The difference in mean BMIBI scores between heavy (54.6) and light periphyton groups was only marginally significant (Fisher LSD; $p<0.10$).

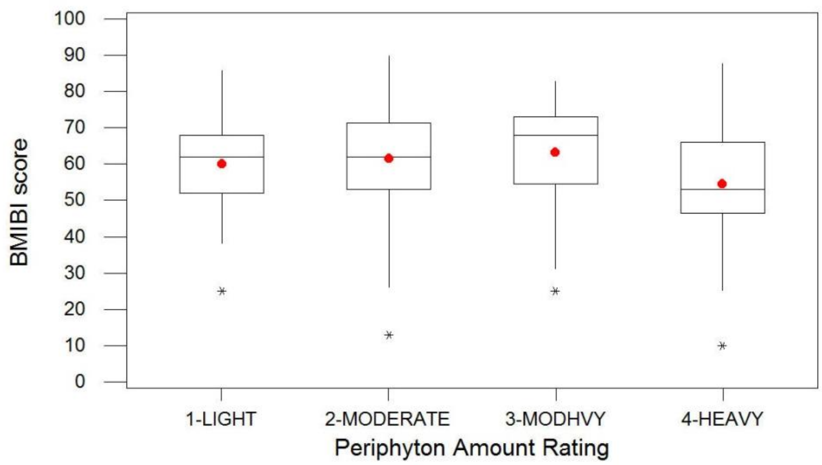


Figure 50. Benthic macroinvertebrate index of biotic integrity (BMIBI) score by rock substrate periphyton amount rating: light (0-25%); moderate (25-50%); moderately heavy (50-75%); heavy (75-100%). IDNR 1994-2009 wadeable stream bioassessment data.

Subsequent AOV testing revealed overall differences in mean rankings in the amounts of periphyton coverage, sedimentation , and macroinvertebrate colonization by substrate type. Rock substrates had significantly higher mean rankings in each of these observation categories than did wood substrates. Rock substrates were also significantly more likely to be evaluated as being dominated by filamentous algae growth (67.1%) than were wood substrates (38.8%) (Chi-square, $p<0.001$).

Substrates (either rock or wood) evaluated as being dominated by filamentous algae had higher ratings for periphyton coverage and sedimentation than those evaluated as having non-filamentous algae growth. The mean ranking of benthic macroinvertebrate colonization amount for substrates dominated by filamentous algae, however, was not significantly different than the mean ranking for substrates dominated by non-filamentous growth.

Among rock substrate samples evaluated as dominated by filamentous algae, the AOV test found a significant periphyton coverage effect ($p=0.04$) on the BMIBI score. Treatment (filamentous algae coverage rating) mean comparisons indicated that samples rated as having heavy filamentous algae growth had a lower mean BMIBI score (53.7) compared with rock substrates evaluated as having moderate or moderately heavy filamentous algae growth (BMIBI = 61.8 and 61.5, respectively). Samples evaluated as having light filamentous growth had a mean value (57.4) that was intermediate and did not differ significantly from the other treatment groups differed significantly from samples having heavy growth. AOV p-values for several of the BMIBI metrics were just slightly above the specified (α=0.05) significance level. When mean comparisons were repeated after combining samples evaluated as having moderate or moderately heavy filamentous growth, mean levels of the BMIBI and four standard-habitat metrics (sheptxscr, shephmscr, shscrprscr, shp3domscr) were significantly lower for samples evaluated as having heavy filamentous growth in comparison to mean BMIBI levels representing samples evaluated as having moderate or moderately heavy filamentous algae (Figure 51a-e).

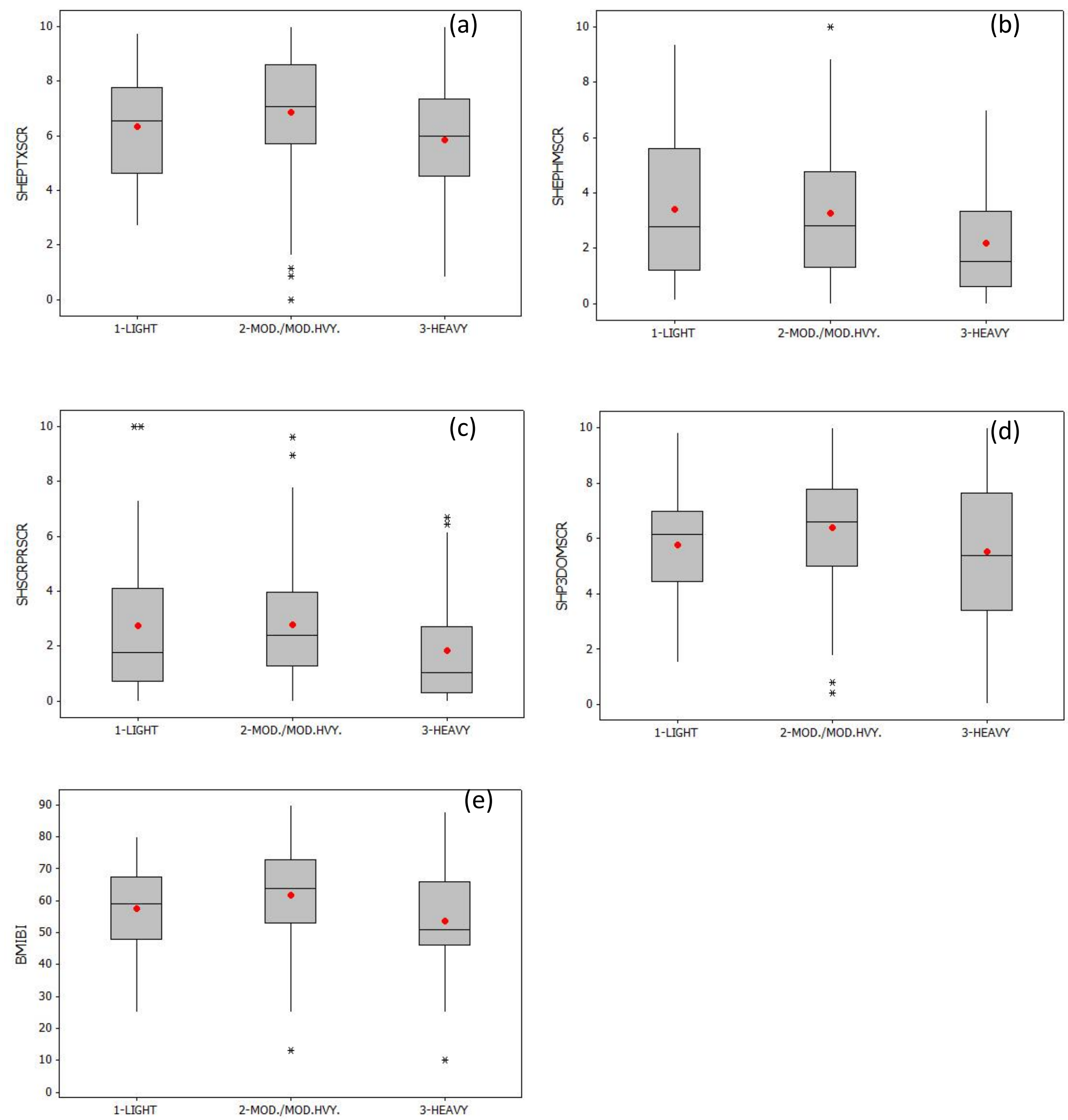


Figure 51(a-e). Box and whisker plots showing categorical rating of amount of rock substrate covered by filamentous algae: 1-Light (<25%); 2-Moderate/Moderately Heavy (25-75%); 3-Heavy (>75%) versus benthic macroinvertebrate metrics and composite index: (a) SHEPTXSCR; (b) SHEPHMSCR; (c) SHSCRPRSCR; (d) SHP3DOMSCR; (e) BMIBI.). IDNR 1994-2009 wadeable stream bioassessment data.

The metrics, SHEPHMSCR and SHSCRPRSCR, illustrate a reduction in benthic macroinvertebrate condition that occurs in the presence of heavy filamentous algae growth. As illustrated in Figures 52(a-b), maximum levels in metric scores are reduced from optimal levels in the presence of heavy filamentous growth. The reduction appears to happen independently of the occurrence of moderately heavy or heavy sedimentation observations, which have the potential to mask the benthic macroinvertebrate response to excessive algal growth.

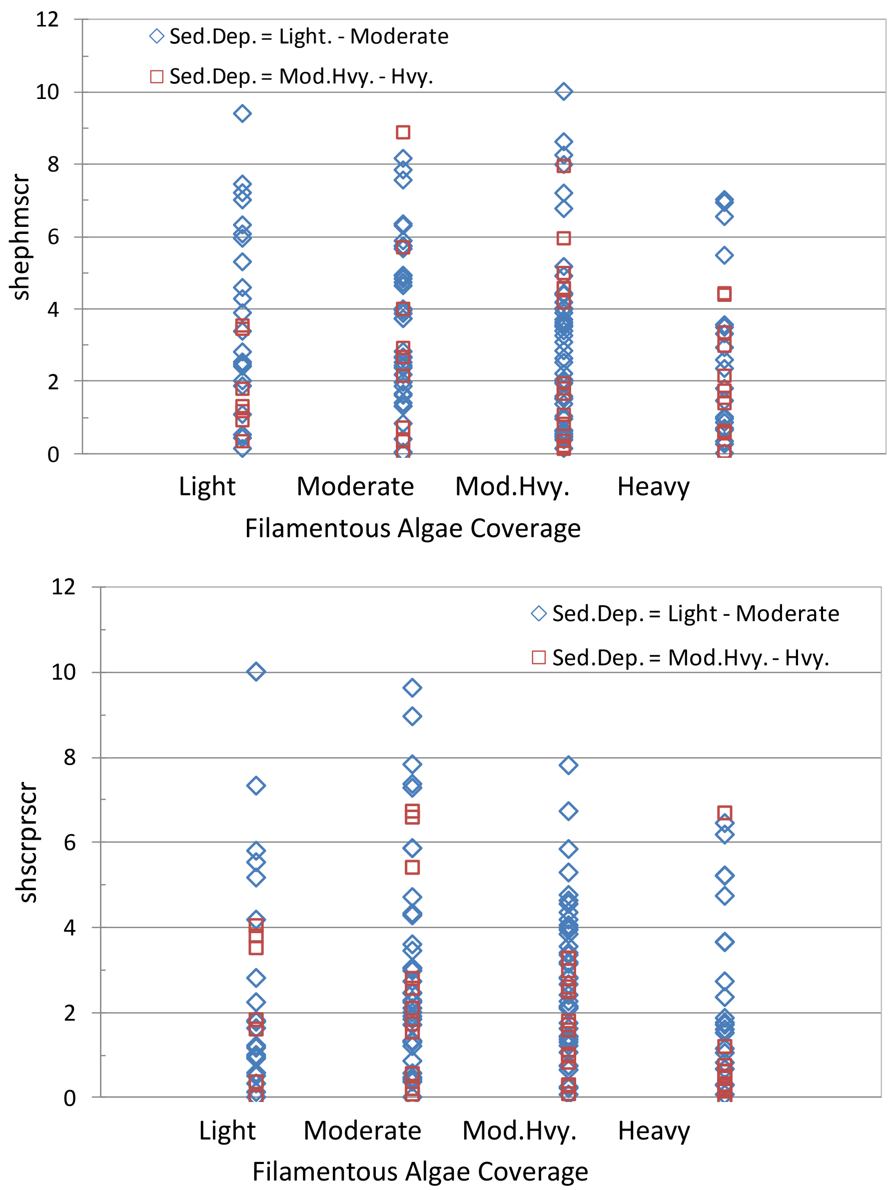


Figure 52(a-b). Categorical rating of amount of rock substrate covered by filamentous algae: 1-Light (<25%); 2-Moderate/Moderately Heavy (25-75%); 3-Heavy (>75%) versus benthic macroinvertebrate metrics (a) percent abundance Ephemeroptera (shephmscr); (b) percent abundance scraper benthic macroinvertebrates (shscrprscr). IDNR 1994-2009 wadeable stream bioassessment data.

5.2. Large wadeable streams and nonwadeable rivers

The number of REMAP sites located on large wadeable streams or nonwadeable rivers (31) was insufficient to conduct a stressor-response analysis. Therefore, the REMAP dataset was supplemented with comparable sampling data from ambient stream monitoring stations. Data were included from two intervals in which benthic macroinvertebrate assemblage samples were collected: 2000-2002 and 2011. A total of 66 BMIBI samples from 50 sites were included in the analysis. Average values of monthly nutrient and seston chlorophyll A sampling data from the July – October biological index period were also used in the analysis.

Seston chlorophyll A and benthic macroinvertebrates

Changepoint/threshold analysis results are summarized in Table 55. Scatter plots showing relationship patterns of WCHLA and benthic macroinvertebrate metrics are shown in Appendix 15(e). Conditional probability (CP) found WCHLA changepoints in relationships with the BMIBI and six component metrics. Changepoint values ranged from 32.8 – 69.8 ug/L and the median value was 67.7 ug/L. Quantile regression (QR) analysis found thresholds in relationships of (Log10)WCHLA and the BMIBI and three component metrics. QR thresholds ranged from 2.9 – 86.9 ug/L and the median was 37.3 ug/L. Regression tree (RT) analysis found WCHLA changepoints in relationships with the BMIBI and eight individual metrics. Changepoint values ranged from 44.7 – 150.4 ug/L and the median was 70.6 ug/L.

Table 55. Seston Chlorophyll A - Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) relationship changepoints (streams of watershed area ≥700 square miles).

	Seston Chlorophyll A (ug/L)		
Biological Metric / Index	CP*	QR	RT
MH**-taxa richness (MHTTX)	-	-	-
SH-taxa richness (SHTTX)	67.7	2.9	68.7
MH-EPT richness (MHEPT)	-	-	98.2
SH-EPT richness (SHEPTX)	69.8	86.9	70.6
MH-sensitive taxa (MHSEN)	-	-	98.2
SH-% 3-dominant taxa (SH3DOM)	69.8	10.7	70.6
SH-Mod. Hilsenhoff Biotic index (SHMHBI)	(163.3)***	-	(164.2)
SH-% EPT (SHEPT)	-	-	-
SH-% Chironomidae (SHCHR)	-	-	-
SH-% Ephemeroptera (SHEPH)	69.8	-	70.6
SH-% Scrapers (SHSCR)	62.8	-	150.4
SH-% Dom. functional feeding grp, (SHDFFG)	32.8	-	44.7
Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	62.8	48.7	70.6
****Range	32.8-69.8	2.9-86.9	44.7-150.4
Mean	62.2	37.3	82.5
Median	67.7	29.7	70.6

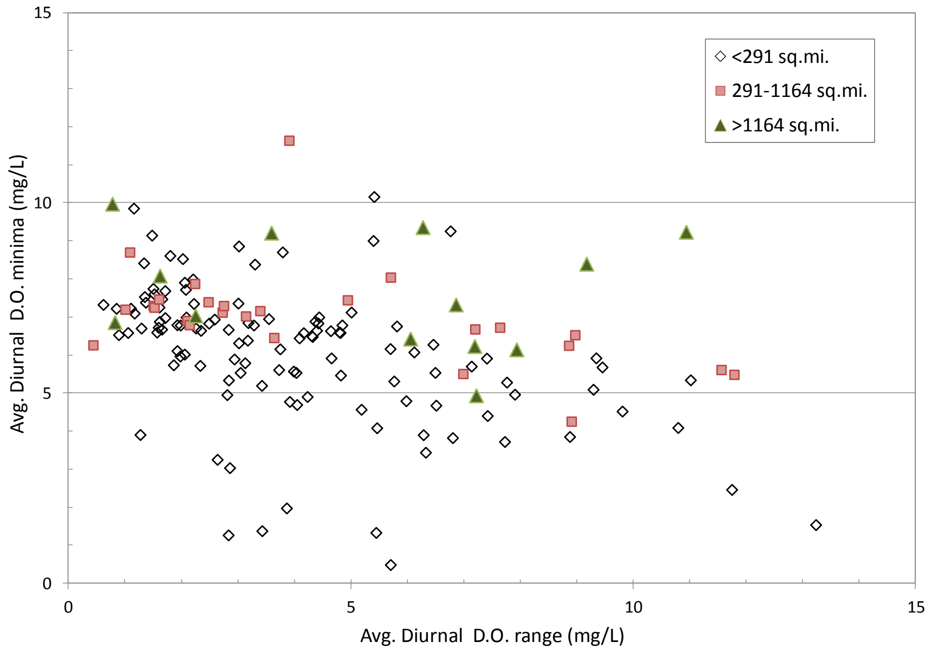
*Analysis Method: CP, Conditional Probability; QR, Quantile Regression; RT, Regression Tree;
** MH, Multi-habitat sample; SH, Standard-Habitat sample.
*** ( ) indicates direction of biological response opposite of expected direction
**** Range, mean, and median excluding metrics having response opposite of expected direction.

Dissolved oxygen and benthic macroinvertebrates

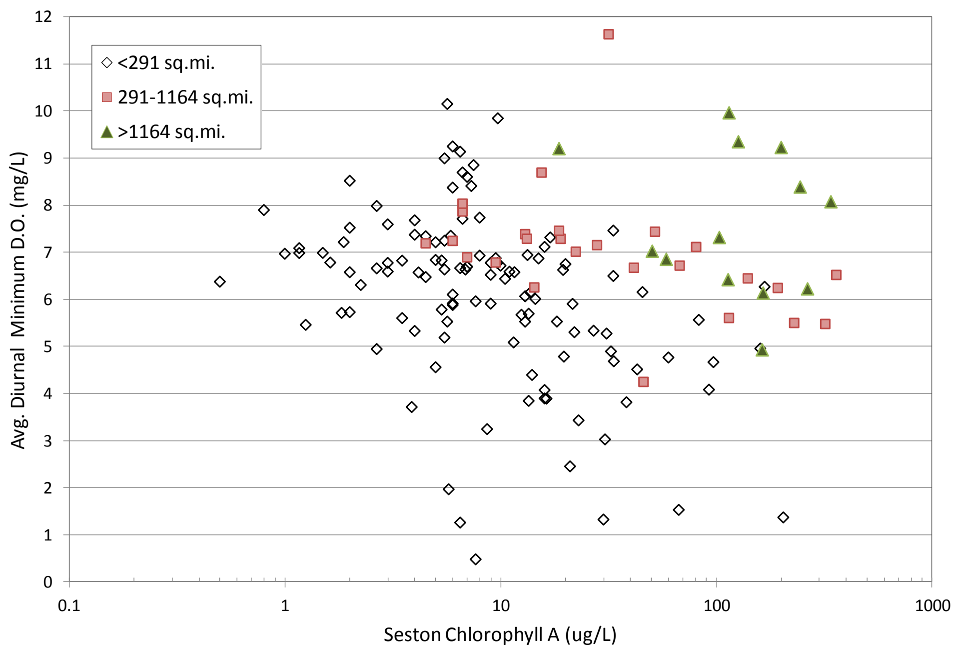
The number of large, wadeable or nonwadeable streams having diel DO monitoring data was insufficient to conduct a DO stressor – biological response changepoint analysis. The REMAP dataset, did however, provide a limited amount of diel DO monitoring data from large streams that were plotted for visual examination along with data from small and medium-size streams (Figures 53a-b).

Levels of average diel DO minima (AVGMINDO) and average diel DO range (AVGRNGDO) in wadeable streams were fairly strongly related with benthic macroinvertebrate and fish IBI metrics. Chlorophyll A levels and stream metabolic rates also correlated with these DO variables, thus demonstrating a causal pathway linking increased primary production, with low DO and/or highly variable diel fluctuations, and adverse changes in benthic macroinvertebrate assemblages.

CP changepoints of 15 ug/L WCHLA and 5 mg/L AVGRNGDO were found in the stressor-response analysis of wadeable stream data. At levels above these changepoints the occurrence frequency of low (substandard) DO (<5 mg/L) was significantly greater. While substandard DO levels were occasionally observed in medium-to-large streams, as Figures 53(a-b) show, the risk of such an occurrence seems to be lessened. One could speculate that dampening of extreme fluctuations in certain environmental factors such as flow and water temperature might contribute to reducing the risk of low DO levels; however, the exact causes of the apparent pattern are not understood. Additional monitoring of diel DO and temperature levels in large streams is being conducted to supplement the data available for analysis.



(a)



(b)

Figure 53(a-b). Comparison of REMAP (2002-2006) continuous diel dissolved oxygen monitoring results from small-medium wadeable streams (<291 mi^2), medium-large transitional streams (291-1164 mi^2), and large nonwadeable streams (>1164 mi^2). Graphs show the relationship of (a) average diel DO range vs. average diel DO minima; and (b) seston chlorophyll A vs. average diel DO minima. Typical data logger deployments were about one week. 5 mg/L is generally considered a threshold below which warmwater aquatic species experience stress.

Seston algal composition – benthic macroinvertebrates

Benthic macroinvertebrate assemblage samples were collected at fifteen of the thirty ambient stream monitoring locations sampled for phytoplankton composition in 2011. Results from the taxa composition analysis were described in Section 4.5. Although, these limited data were insufficient for a rigorous statistical analysis, they did allow for a preliminary examination of potential relationships. Sestonic algae provide a food source for many benthic macroinvertebrates and differences in the composition of algae could have cascading effects that influence the structure and condition of the benthic macroinvertebrate assemblage. Such differences might be observable as response patterns in the BMIBI and component metrics.

As described earlier, 2011 phytoplankton samples were dominated by genera from three major taxonomic divisions: Bacillariophyta (diatoms), Chlorophyta (green algae), and Cyanophyta (blue-green algae or Cyanobacteria). Among the fifteen sites that were also sampled for benthic macroinvertebrates, these algal divisions made up an average of 92.8% of the phytoplankton biomass (range: 81.0 - 99.6%). %Cyanobacteria and %Diatoms were the most strongly related of the phytoplankton composition metrics (Figure 54). %Chlorophyta did not exceed 50% in any sample and was not significantly related with either %Cyanobacteria or %Diatoms.

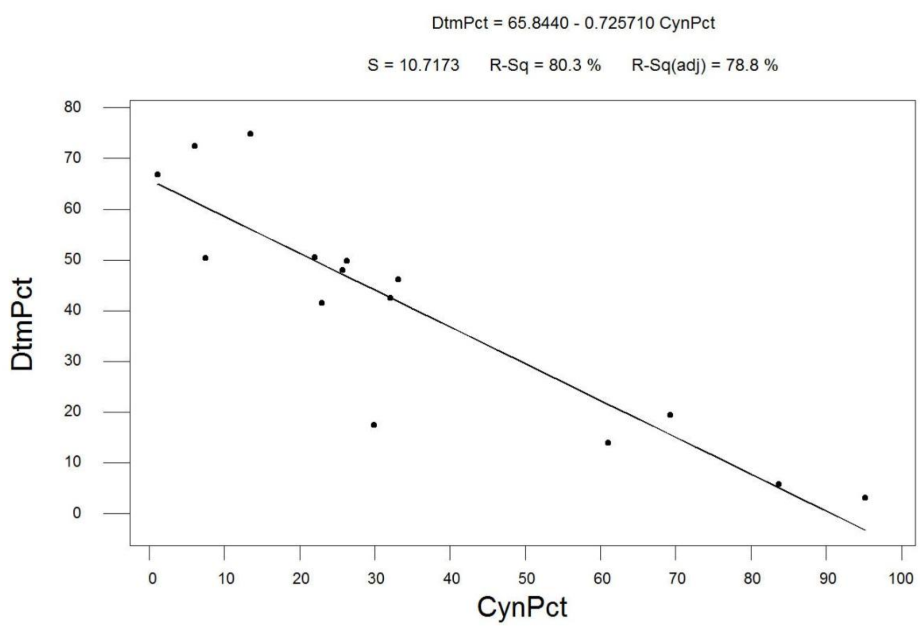


Figure 54. Least square regression of %Cyanobacteria biomass versus %Diatom biomass. Samples were collected in September 2011 at 15 ambient stream monitoring stations located on large wadeable or nonwadeable streams.

As a starting point for exploring potential relationships, various scatter plots were prepared and examined. A nonparametric Spearman rank correlation analysis (Table 56) was also conducted to evaluate the relative strength of association between algal and benthic macroinvertebrate assemblage metrics.

Table 56. Spearman correlation coefficient (rho) values representing phytoplankton metrics and benthic macroinvertebrate metrics sampled at 15 ambient monitoring stations sampled in September 2011.

	% Chlr.	% Cyn.	% Dtm.	Chlr. Wtms.	Cyn. Wtms.	Dtm. Wtms.	Totl. Wtms.
% Cyn.	-0.47						
% Dtm.	0.19	-0.89					
Chlr. Wtms.	0.75	-0.10	-0.18				
Cyn. Wtms.	-0.26	0.82	-0.82	0.30			
Dtm. Wtms.	0.24	-0.35	0.40	0.49	0.08		
Totl. Wtms.	-0.03	0.54	-0.62	0.52	0.90	0.36	
BMIBI	-0.09	-0.07	0.31	-0.33	-0.16	0.03	-0.17
HBISCR	-0.22	-0.15	0.45	-0.35	-0.19	0.30	-0.14
MHEPTXSCR	-0.25	0.43	-0.34	-0.25	0.28	-0.37	0.12
MHSNSTVSC	-0.25	0.24	-0.15	-0.29	0.16	-0.24	0.07
MHTTXSCR	-0.37	0.50	-0.36	-0.35	0.31	-0.49	0.08
SHCHIRSCR	0.11	-0.25	0.39	-0.20	-0.25	0.24	-0.19
SHDFFGSCR	0.21	0.26	-0.16	0.03	0.16	-0.01	0.13
SHEPHSCR	0.13	-0.34	0.44	-0.28	-0.43	-0.07	-0.28
SHEPTXSCR	-0.23	-0.05	0.28	-0.43	-0.13	-0.05	-0.17
SHP3DOMSC	0.17	-0.37	0.53	-0.21	-0.39	0.23	-0.30
SHPEPTSCR	-0.02	-0.13	0.34	-0.30	-0.23	0.15	-0.25
SHSCRPRSC	0.03	-0.27	0.46	-0.30	-0.37	0.15	-0.26
SHTTXSCR	-0.25	-0.16	0.34	-0.43	-0.15	0.03	-0.13
AVGSHSCR	-0.01	-0.21	0.44	-0.30	-0.29	0.18	-0.23

Despite data limitations, scatter plot evaluations and correlation analysis results suggested the existence of significant relationships between benthic macroinvertebrate assemblage and phytoplankton assemblage metrics. Among the percent composition metrics, the strongest associations tended to occur between %Diatoms and standard-habitat BMIBI metrics (Table 56). Phytoplankton assemblages in which diatom taxa were dominant or co-dominant with other phytoplankton taxa tended to occur at lower overall levels of phytoplankton biomass that tended to correspond with higher taxonomic balance (SHP3DOMSCR) and proportional abundances of scraper feeders (SHSCRPRSCR) and sensitive taxa, such as mayflies (SHEPHMSCR). By comparison, phytoplankton assemblages that were dominated by Cyanobacteria tended to be associated with high overall levels of phytoplankton biomass and tended to have opposite patterns in the above-mentioned benthic macroinvertebrate metrics (Figure 55).

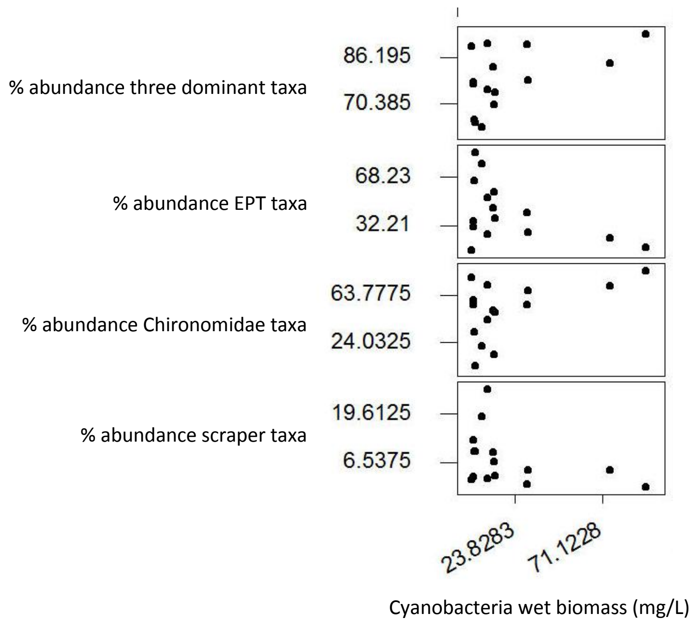


Figure 55. Scatter plots of Cyanobacteria wet biomass versus selected standard-habitat metrics of the BMIBI. Samples were collected in September 2011 at 15 ambient stream monitoring stations located on large wadeable or nonwadeable streams.

The BMIBI is a composite index of nine standard-habitat (SH) metrics and three multi-habitat (MH) metrics. Because SH metric data are collected from micro-habitats that are targeted for maximum taxa diversity, these metrics are more likely to show responses to water quality conditions, including nutrient enrichment status, than are MH metrics. Graphs of average SH metric score plotted against Cyanobacteria biomass and total biomass were examined for the occurrence of potential threshold levels in these response relationships.

A potential threshold was observed around 10 mg/L Cyanobacteria biomass (Figure 56). Above this level the average SH metric score did not exceed 5.0. An average metric score of 5.6 is consistent with attaining an overall BMIBI index score of 56, which is the lower boundary for scores qualitatively rated as “good” condition. As Figure 57 shows, average SH metric scores exceeding 5.6 were observed only in cases where phytoplankton composition was Diatom or mixed- dominant, and total phytoplankton biomass did not exceed roughly 20-40 mg/L. In none of the cases in which Cyanobacteria was dominant (i.e., >50% of total biomass), did the average standard-habitat metric score exceed 4.0, which is consistent with attaining an overall BMIBI rating of “fair” condition.

Based on this preliminary analysis, additional sampling was conducted in 2012 and more sampling is planned for 2013 to provide data for exploration of relationships between phytoplankton and benthic macroinvertebrate assemblages of large wadeable and nonwadeable streams.

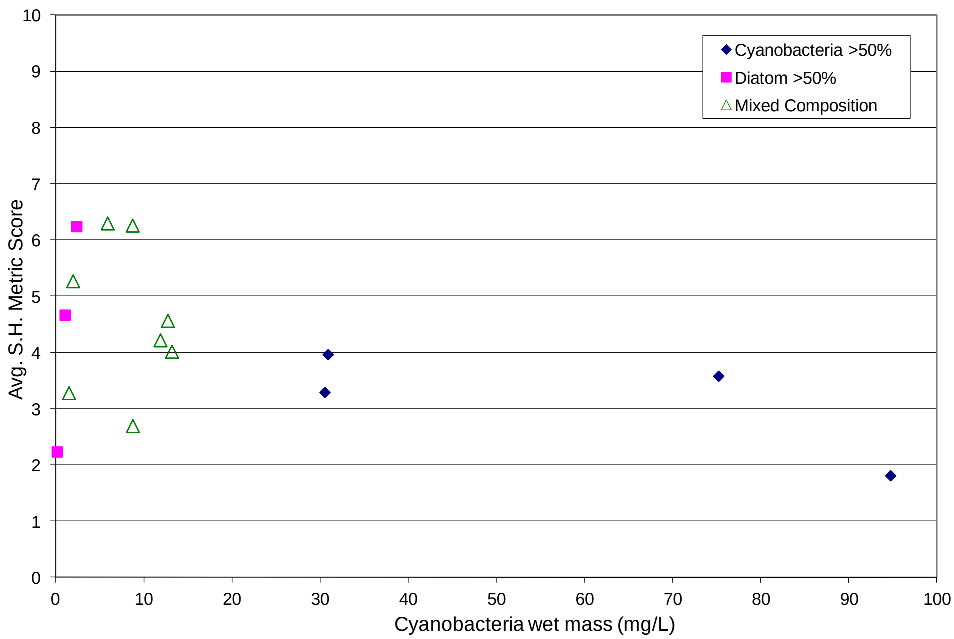


Figure 56. Relationship of Cyanobacteria wet biomass and standard-habitat metric score in 15 ambient monitoring sites on large wadeable or nonwadeable streams sampled in September 2011..

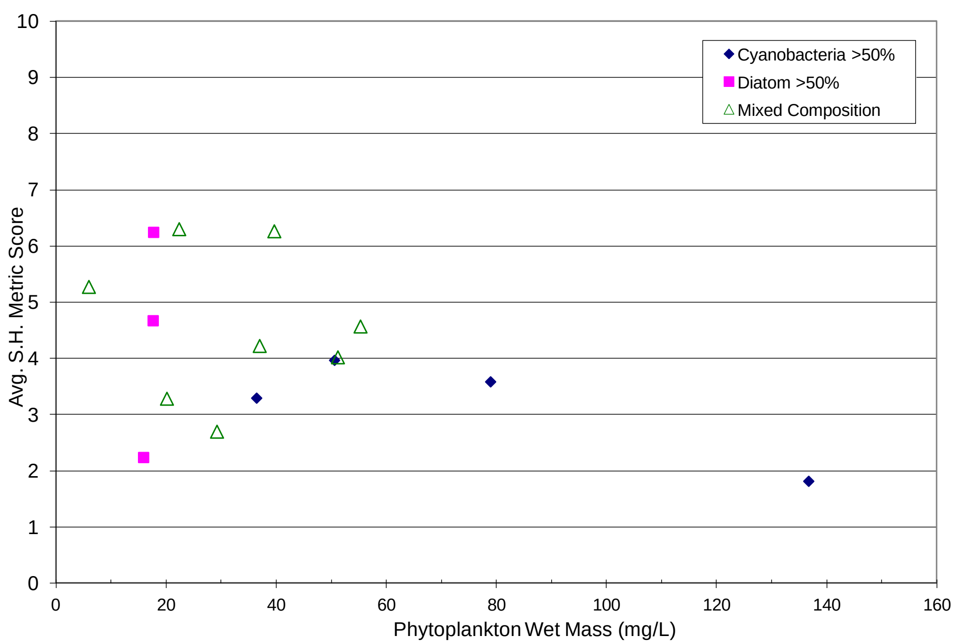


Figure 57. Relationship of total phytoplankton wet biomass and standard-habitat metric score in 15 ambient monitoring sites on large wadeable or nonwadeable streams sampled in September 2011.

5.3. Coldwater streams

Iowa's coldwater streams occur primarily in the Paleozoic Plateau (52b) ecoregion and to a smaller degree in the Iowan Surface (47c) ecoregion of Northeast Iowa (Figure 2). Groundwater inputs have a strong influence on the hydrologic and water quality characteristics of coldwater streams, more so than in warmwater streams which are influenced more by surface runoff.

Analysis results summarized earlier in this report showed how coldwater streams differed from warmwater streams in several nutrient characteristics. As was also noted earlier, there are inherent differences in aquatic species composition of coldwater and warmwater streams because of differences in thermal tolerances. For all of these distinctions, it makes sense to examine nutrient stressor–biological response patterns in coldwater streams separately from warmwater streams.

Unfortunately, the availability of matched nutrient and biological response sampling data is more limited for coldwater streams than warmwater streams. Monitoring conducted in recent years for the IDNR stream bioassessment program has helped to address this gap; however, the current data are still insufficient for a robust statistical analysis. Therefore, nutrient-biological response monitoring of coldwater streams should remain a high priority.

Coldwater stream data from random and reference sites sampled between 2002 and 2011 were used to examine nutrient stressor-biological response relationships. Random sampling data from two groups of sites were included: a) 14 sites sampled for the 2002-2006 REMAP probabilistic survey; b) 10 sites sampled in 2011 for validation of the Coldwater Benthic Index (CBI). Reference site data included in the analysis consisted of data from eight of sixteen reference sites used in the development of the CBI. Four of the reference sites are classified as Outstanding Iowa Waters (OIW). These and other coldwater OIW stream segments are afforded special (anti-degradation) protection status in Iowa's water quality standards (IAC 567:61) because of their high recreational value and ability to support natural reproduction of trout.

Random and reference site sampling results for chlorophyll A, nutrients, and other stream variables are summarized in Table 57. The summarized data represent either single grab samples or averages of samples from individual sites collected during the biological index period. The Kruskal-Wallis nonparametric AOV test was used to statistically examine differences in the mean ranks of random and reference site data values. The ability to detect significant differences was limited because of small numbers of samples.

,

Mean ranked values of WCHLA, TKN, TP, and PRTCP for random sites were significantly higher ($p<0.05$) than reference site mean ranks. Reference site mean ranks of dissolved to total nutrient ratios (DIN:TN, DOP:TP) were significantly higher than random site mean ranks. Among the other stream variables tested, random sites had significantly higher mean ranks of temperature and turbidity and lower mean ranks of flow and CBI score.

Table 57. Statistical summary of coldwater stream sampling data collected from random (RD) and reference (RF) sites (2002-2011). Summary results were derived from season averages for each site. Four of eight reference sites were sampled in more than one season. Reported P-values are from Kruskal-Wallis two-sample comparison tests of mean order-ranked values. Bolded and italicized results (p-value ≤0.05) represent statistically significant differences.

	N		Minimum		Q25		Median		Q75		Maximum		Mean		Std.Dev.		KW
Variable	RD	RF	RD	RF	RD	RF	RD	RF	RD	RF	RD	RF	RD	RF	RD	RF	p-value
PCHLA	24	9	2.5	2.2	7.0	4.6	15.0	9.9	26.5	14.7	42.0	23.0	17.4	10.3	11.2	6.7	0.075
SCHLA	24	9	0.9	0.5	3.0	2.6	6.6	4.5	11.4	7.5	27.2	11.0	8.0	5.0	6.4	3.2	0.258
WCHLA	24	12	1.0	1.0	3.0	1.2	3.9	2.0	5.8	2.6	24.2	3.4	5.5	2.0	5.3	0.7	***<0.001***
NHX	24	12	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.12	0.08	0.06	0.05	0.02	0.01	0.264
NOX	24	12	0.90	1.84	3.52	3.65	5.43	4.35	7.29	6.44	9.45	9.70	5.27	5.02	2.40	2.19	0.627
TKN	24	12	0.10	0.10	0.16	0.10	0.25	0.11	0.38	0.16	0.82	0.20	0.31	0.13	0.22	0.04	***0.001***
DIN:TN	24	12	0.74	0.95	0.92	0.98	0.96	0.99	0.98	0.99	0.99	0.99	0.94	0.98	0.06	0.01	***0.005***
TN	24	12	1.28	1.94	3.69	3.83	5.65	4.47	7.71	6.59	9.61	9.80	5.58	5.15	2.40	2.17	0.524
DOP	24	12	0.02	0.03	0.05	0.04	0.07	0.05	0.09	0.07	0.36	0.11	0.08	0.06	0.07	0.02	0.185
TP	24	12	0.04	0.04	0.07	0.05	0.11	0.06	0.14	0.08	0.42	0.13	0.12	0.07	0.08	0.03	***0.016***
DOP:TP	24	12	0.35	0.67	0.56	0.71	0.69	0.85	0.78	0.92	0.90	1.00	0.67	0.83	0.14	0.12	***0.003***
PRTCP	24	12	0.01	0.00	0.02	0.00	0.03	0.01	0.06	0.02	0.14	0.03	0.04	0.01	0.03	0.01	***<0.001***
TN:TP	24	12	8.56	38.70	30.81	55.40	43.06	67.20	92.09	105.50	161.43	147.00	61.33	80.80	43.69	36.60	0.07
DO	24	11	7.5	8.5	9.9	10.4	10.6	11.0	11.8	11.6	14.1	12.5	10.7	10.9	1.6	1.1	0.582
PH	24	11	7.8	7.4	8.0	7.6	8.3	8.2	8.4	8.4	8.7	8.4	8.2	8.0	0.2	0.4	0.188
TEMP	24	11	12.3	11.8	14.6	12.8	16.4	13.4	19.2	14.2	23.8	16.1	16.7	13.7	2.8	1.2	***0.002***
FLOW	24	11	0.4	1.9	2.1	3.5	3.2	8.3	5.5	13.6	15.7	39.3	4.9	11.6	4.4	12.3	***0.047***
TSS	24	12	1.3	1.0	3.7	3.0	7.4	4.0	14.2	6.1	33.0	7.8	10.1	4.3	8.4	1.9	0.054
TURB	24	12	1.0	1.0	2.1	1.3	3.7	1.7	7.0	3.0	21.5	3.8	5.3	2.1	5.0	1.0	***0.025***
CBI	24	12	20	48	28	55	48	69	60	81	79	91	46	69	16	14	***0.001***

Chlorophyll A and benthic macroinvertebrates

Scatter plots and correlation results were reviewed as part of the preliminary examination of nutrient stressor – biological response relationships. Similar to results for wadeable warmwater stream sites, correlations of nutrient variables and chlorophyll A variables sampled from coldwater streams were mostly weak or nonexistent (Table 58). Among nutrient correlations with benthic chlorophyll A, only DIN:TN ratio, was significantly related with periphyton chlorophyll A (PCHLA) (r= 0.46). Seston chlorophyll A (WCHLA) was somewhat more strongly correlated with TKN (r=0.57) and TP (r=0.58).

Seston chlorophyll a (WCHLA) was inversely correlated with the CBI as well as several component metrics (Table 59). Benthic chlorophyll A levels (PCHLA, SCHLA) were not significantly related with the Coldwater Benthic Index (CBI). The CBI metric percent abundance dominant functional feeding group (SHDFFGS) was correlated with PCHLA and SCHLA, which seems to indicate that increased benthic algae production was compatible with increased benthic macroinvertebrate trophic guild diversity.

Data limitations prevented use of the previously described changepoint/threshold analysis methods for analysis of nutrient stressor–biological response relationships. In lieu of these methods, a reference condition comparison approach was used in which random site data were visually compared with reference data percentile levels.

Table 58. Pearson and Spearman (nonparametric) rank correlation results of chlorophyll A parameters in relationship with nutrient parameters sampled from coldwater random and reference sampling sites (2002-2011). Parameter abbreviations are listed in Table 6. Pearson correlation analysis was performed on Log 10 transformed data. N = 33 (PCHLA, SCHLA); 41 (WCHLA). Bolded and italicized Pearson results are considered statistically significant relationships (p-value $\leq$0.05).

	Pearson (r)				Spearman (rho)		
Nutrient	PCHLA	SCHLA	WCHLA		PCHLA	SCHLA	WCHLA
NHX	-0.288	-0.137	***0.367***		-0.215	0.001	0.238
NOX	0.320	0.301	0.083		0.266	0.311	-0.045
TKN	-0.196	0.026	***0.574***		-0.129	0.057	0.573
TN	0.287	0.285	0.100		0.247	0.307	-0.011
DINTN	***0.465***	0.303	-0.119		0.256	0.111	-0.487
DOP	-0.061	-0.078	***0.497***		-0.045	-0.002	0.447
PRTCP	0.207	0.139	***0.340***		0.102	0.125	0.560
TP	-0.025	-0.019	***0.585***		-0.053	0.016	0.501
DOPTP	-0.091	-0.146	-0.211		-0.030	-0.124	-0.299
TNTP	0.226	0.220	***-0.374***		0.248	0.254	-0.368

Table 59. Pearson and Spearman (nonparametric) rank correlation results of chlorophyll A parameters in relationship with the Coldwater Benthic Index (CBI) and component metrics sampled from coldwater random and reference sampling sites (2002-2011). Pearson correlation analysis was performed on Log 10 transformed data. N = 33 (PCHLA, SCHLA); 41 (WCHLA). Bolded and italicized Pearson results are considered statistically significant relationships (p-value $\leq$0.05).

		Pearson (r)				Spearman (rho)		
Biological Metric/Index	Abbrv.	PCHLA	SCHLA	WCHLA		PCHLA	SCHLA	WCHLA
MH sensitive taxa richness	MHSNSTXS	0.201	0.131	-0.237		0.132	0.085	-0.118
MH CW taxa richness	MHCWTXS	-0.182	-0.062	***-0.345***		-0.066	0.058	-0.376
MH Trichoptera taxa richness (no Hydropsychinae & Hydroptilidae)	MHTNOHS	-0.009	0.167	***-0.338***		-0.022	0.214	-0.343
MH % tolerant taxa	MHPTOLS	0.313	0.145	-0.210		0.267	0.116	-0.208
SH % Dominant Functional Feeding Group (FFG)	SHPDFFGS	***0.379***	***0.502***	0.054		0.339	0.466	0.058
SH % CW individuals	SHPCWS	-0.114	0.134	***-0.341***		-0.133	0.051	-0.451
SH % Hydropsyche individuals	SHPHYDS	0.077	0.070	***-0.554***		-0.043	0.005	-0.364
SH % Hydropsychinae individuals	SHPHYNS	-0.120	-0.194	***-0.458***		-0.183	-0.231	-0.384
SH Benthic Tolerance Index (BTI)	BTIS	0.058	0.220	***-0.411***		0.035	0.239	-0.524
Coldwater Benthic Index (CBI)	CBI	0.092	0.187	***-0.483***		0.034	0.174	-0.478
Benthic sample type: MH, Multi-habitat; SH Standard-habitat								

Figures 58(a-c) show CBI scores for random sites and reference sites plotted against each of the chlorophyll A nutrient response variables (PCHLA, SCHLA,WCHLA). The reference site 25th percentile CBI score and the applicable reference 75th percentile chlorophyll A level are shown for comparison purposes. The majority of the reference data can be seen in the upper left regions of the graphs bounded by the reference 25th CBI score and the 75th percentile chlorophyll A value. This result is expected since these same data were used in determining the reference percentile levels. In comparison, the CBI scores from random sites mostly fell below the reference CBI 25th percentile score (60) regardless of whether chlorophyll A was above or below reference 75th percentile levels.

An outlier can be seen in the upper right areas of Figures 58a and 58b. This outlier represents a random site located on Waterloo Creek, which happens to be designated a reference stream and Outstanding Iowa Water (OIW). The occurrence of a high CBI score (78) at this site in the presence of elevated benthic chlorophyll A levels (PCHLA = 42.0 ug/cm^2, SCHLA = 27.2 ug/cm^2) suggests that a high level of benthic algal biomass by itself does not necessarily cause a reduction in the condition of the benthic macroinvertebrate assemblage. In this case, the impact might have been mitigated by more optimal, reference-level conditions in other environmental variables such as flow, substrate, and temperature.

The relationship between CBI score and seston chlorophyll A (WCHLA) appeared to be more consistent (Figure 58c). CBI scores generally show a declining trend in response to increasing levels of WCHLA. The majority of sites exceeding the reference site 75th percentile for WCHLA are clustered near or well below the reference 25th percentile CBI score.

A similar set of graphs was prepared with the Benthic Tolerance Index (BTI) plotted against chlorophyll A variables (Figures 58d-f). The BTI is a component metric of the CBI and also the warmwater BMIBI. The BTI is sensitive to the effects of organic enrichment and responds independently of stream thermal classification. The BTI score is derived from a weighted average of tolerance values assigned to the various benthic macroinvertebrate taxa contained in a standard-habitat sample. BTI response patterns to chlorophyll A variables were generally similar to CBI patterns. The most discernible pattern again was a general declining trend in BTI levels in response to increasing levels of WCHLA.

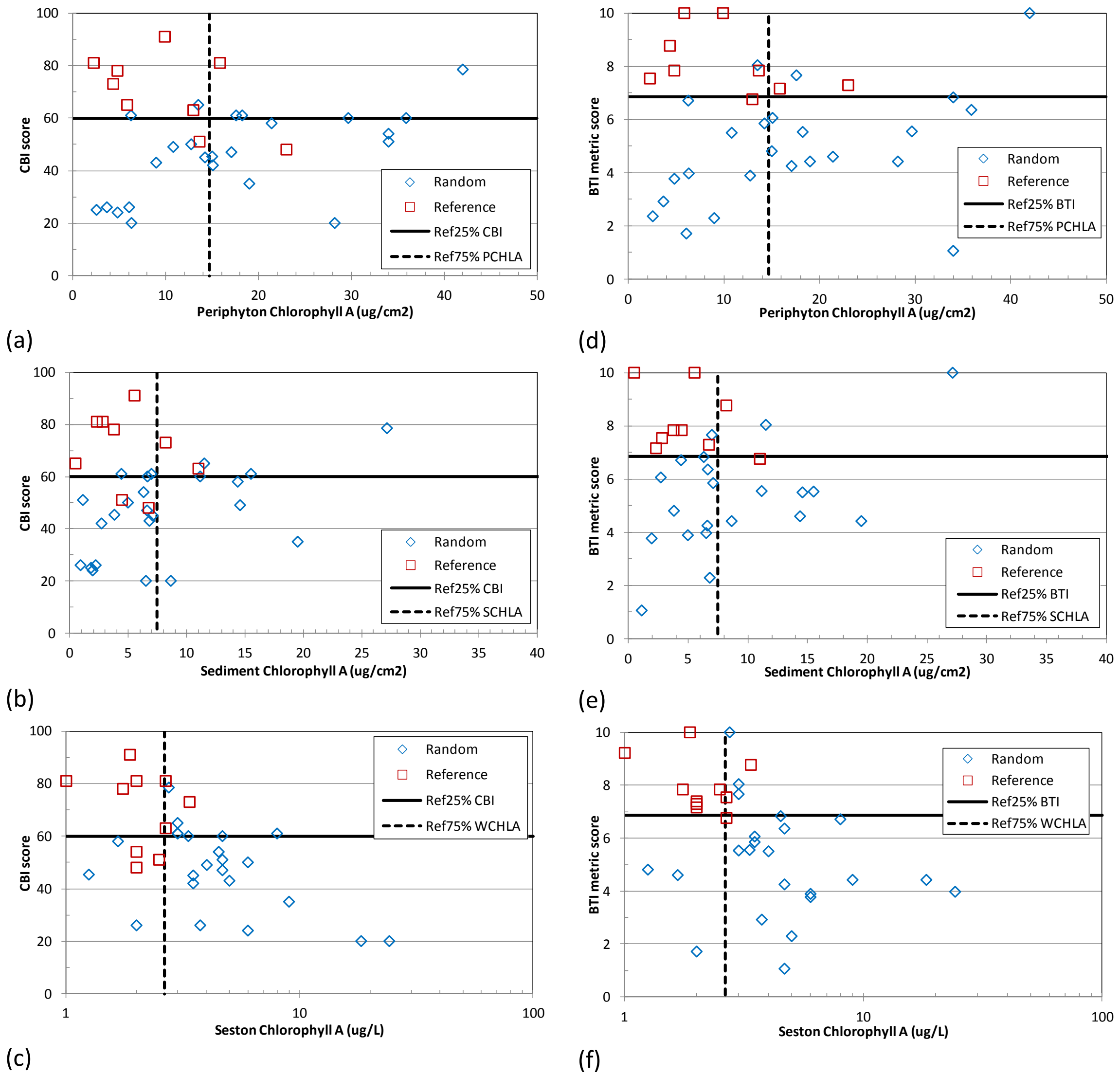


Figure 58(a-e). Periphyton, sediment, and seston chlorophyll A versus (a-c) Coldwater Benthic Index (CBI) and (d-f) Benthic Tolerance Index (BTI) metric score. Sampling data from random sites (open blue diamond) and reference sites (open red square). Reference site 25th percentile CBI/BTI value shown as dashed line; Reference 75th percentile chlorophyll A values shown as solid line.

The observance of inverse correlations between seston chlorophyll A and CBI levels lead to an exploratory analysis of benthic macroinvertebrate assemblage condition responses to relative rankings of benthic algal chlorophyll A versus sestonic algal chlorophyll A among random sampling sites. Scatter plots were made contrasting the relative site rankings in benthic algae chlorophyll A and sestonic chlorophyll A versus the CBI score (Figure 59a-b) and the BTI metric score (Figure 59c-d). The patterns seen in the graphs visually suggest that CBI and BTI levels are more affected by whether a stream site tends to rank higher in benthic algae or sestonic algae than whether the actual values of each fall above or below the applicable chlorophyll A reference site 75th percentile value.

Simple linear regression of relative rank of WCHLA–PCHLA versus CBI explained a statistically significant amount of variability in the relationship (Figure 59a) (r^2=0.394; p=0.001), and likewise between WCHLA–PCHLA versus BTI (Figure 59b) (r^2=0.192; p=0.032). Both the CBI and BTI declined as the relative rank of PCHLA within the group of random sites decreased in relation to WCHLA rank. Sites that had negative relative ranks (i.e., periphyton rank higher than seston rank) had a significantly higher CBI mean (57.8) than sites having positive relative ranks (37.5). The same was true for BTI mean scores.

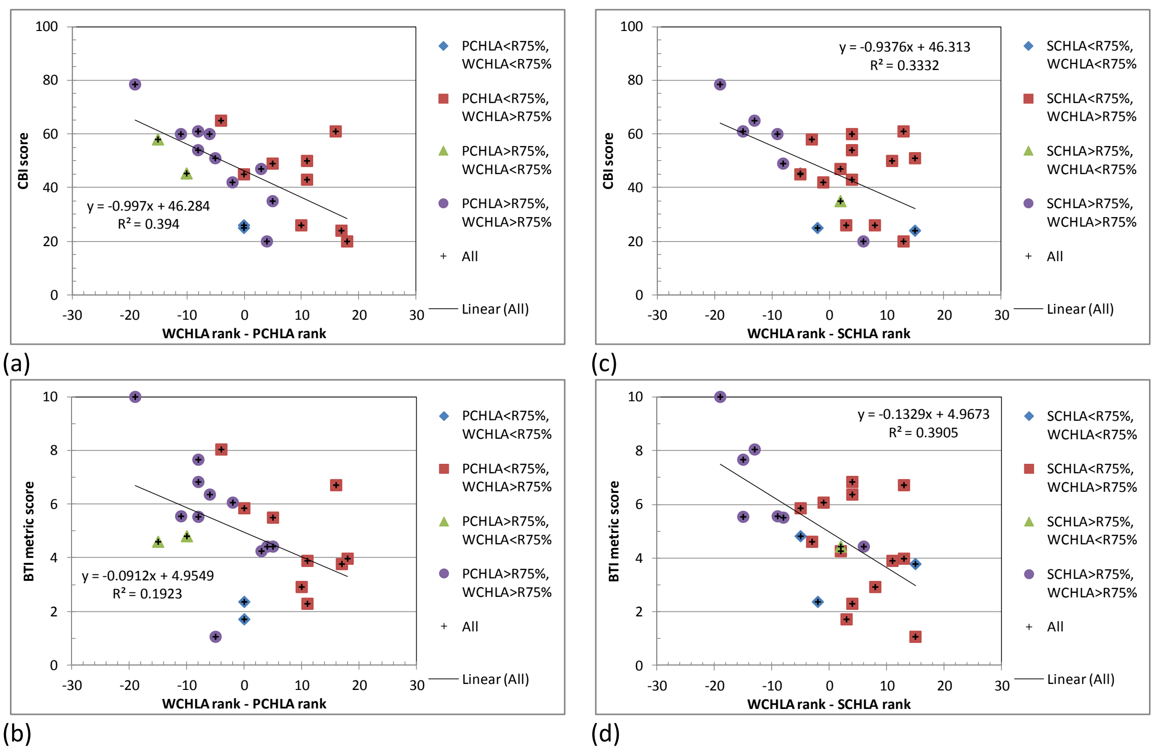


Figure 59(a-d). Difference of site ranks for seston chlorophyll A (WCHLA) and periphyton chlorophyll A (PCHLA) plotted against (a) Coldwater Benthic Index (CBI) and (b) Benthic Tolerance Index (BTI) metric score. Difference of site ranks for seston chlorophyll A (WCHLA) and sediment chlorophyll A (SCHLA) plotted against (c) Coldwater Benthic Index (CBI) and (d) Benthic Tolerance Index (BTI) metric score. Sampling data are from 24 random coldwater stream sites (2002-2011). A high positive value for WCHLA-PCHLA or WCHLA-SCHLA indicates WCHLA rank is high in relation to PCHLA or SCHLA. Colored symbols indicate site status for PCHLA, SCHLA, WCHLA in comparison to reference site 75th percentile values.

The same statistical tests were applied to relative rank of WCHLA–SCHLA. Simple linear regression of WCHLA-SCHLA relative rank versus CBI explained a statistically significant amount of variability in the relationship (Figure 59c) (r^2=0.332; p=0.003), and likewise between WCHLA–SCHLA versus BTI (Figure 59d) (r^2=0.391; p=0.001). Both the CBI and BTI declined as the relative rank of SCHLA within the group of random sites decreased in relation to WCHLA rank. Sites that had negative relative ranks (i.e., higher sediment chlorophyll A rank than seston chlorophyll A rank) had a significantly higher CBI mean (53.6) than sites having positive relative ranks (39.8). The same was true for BTI mean scores.

Diel dissolved oxygen and benthic macroinvertebrates

Continuous monitoring of dissolved oxygen and temperature was conducted at thirteen coldwater stream sites during the 2002-2006 REMAP random site survey. The median number of days with continuous monitoring was five and the range was 3-12 days. This dataset, limited as it is, can provide useful insight to the role that dissolved oxygen variables might serve as nutrient response variables in coldwater streams.

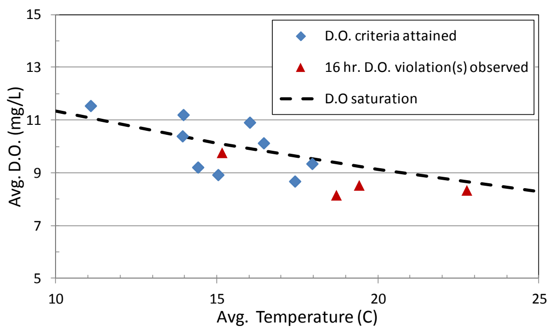
Summary statistics for dissolved oxygen and temperature variables are listed in Table 60. The instantaneous minimum DO criterion of 5 mg/L was not violated at any of the sites. The 16-hour diel criterion of 7 mg/L was violated at four sites (31%). Of these sites, the proportion of monitored days in which the 16-hour criterion was not met ranged from 8.3% - 37.5%. The Iowa DNR's coldwater stream assessment protocol states that continuous monitoring data may be used as evidence to classify a coldwater stream segment when the maximum temperature does not exceed 75 F (23.9 C) between mid-May and mid-September in three separate years. One of the thirteen monitored REMAP sites failed to meet the temperature criterion during the brief deployments.

Table 60. Summary statistics of daily (diel) dissolved oxygen (DO) and stream metabolism variables obtained from continuous monitoring at thirteen coldwater REMAP (2002-2006) random survey sites.

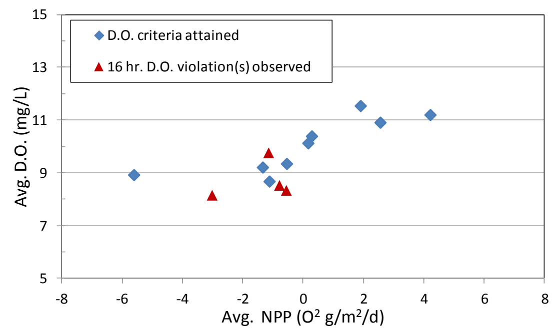
Variable	Abbrv.	Min.	Q25	Median	Q75	Max.	Mean	Std.Dev.
Deployment days	ddy	3	3	5	7.5	12	5.85	3.18
Avg. Daily Maximum DO (mg/L)	avgmaxdo	10.25	10.53	12.25	13.33	15.97	12.22	1.89
Avg. Daily Minimum DO (mg/L)	avgmindo	6.74	7.18	7.93	8.59	9.18	7.91	0.78
Deployment Minimum DO (mg/L)	min2do	6.37	6.45	7.64	8.18	9.07	7.46	0.94
Avg. Daily DO (mg/L)	avg2do	8.15	8.60	9.34	10.64	11.53	9.62	1.13
Avg. Daily Range DO (mg/L)	avgrngdo	1.44	3.34	3.96	5.35	8.05	4.31	1.71
Avg. Daily Gross Primary Production (gO2/m2/d)	avggpp	1.58	2.98	3.50	6.78	11.61	4.66	2.91
Avg. Daily Community Respiration (gO2/m2/d)	avgresp	0.94	3.30	4.32	6.09	13.43	5.04	3.08
Avg. Daily Net Primary Production (gO2/m2/d)	avgnpp	-5.60	-1.24	-0.55	1.11	4.22	-0.38	2.45
Avg. Daily Production: Respiration Ratio	avgpr	0.50	0.65	0.80	1.23	3.78	1.12	0.86

Temperature is a major determinant of dissolved oxygen concentrations in aquatic ecosystems. As water temperature increases, the DO concentration at equilibrium with atmospheric oxygen decreases. Three of the REMAP sites that experienced violations of the 16-hour DO criterion ranked at the top in diel average temperature (Figure 60a). Elevated stream temperature, therefore, may have contributed to the occurrence of DO violations. Maximum temperature levels, however, were not sufficiently high to cause dissolved oxygen violations simply by reducing DO saturation levels.

Stream metabolic processes are another important determinant of DO levels. Eight of thirteen monitored coldwater REMAP sites had average diel DO levels that were below saturation levels in relation to average diel temperature, including all four sites where the 16-hour criterion was violated (Figure 60a). DO undersaturation occurs when the rate of DO consumption exceeds the rate of DO inputs and production. The eight sites that were undersaturated with diel oxygen all had negative levels of net primary production.



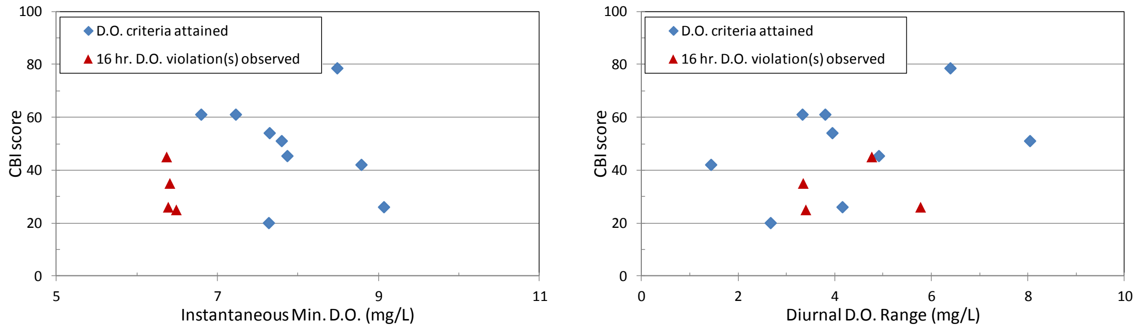
(a)



(b)

Figure 60(a-b). Average diel dissolved oxygen versus (a) diel average temperature and (b) average diel net primary production (NPP). Continuous monitoring data were obtained at 13 coldwater REMAP (2002-2006) random survey sites. Data loggers were deployed 3-12 days during summer months. Iowa water quality standards specify that DO concentrations $\geq$ 5 mg/L must be achieved always and DO concentration $\geq$7 mg/L must be achieved at least 16 hours in each day.

This initial analysis of limited data suggests that observed violations in the 16-hr DO standard were associated with increased biological respiration relative to primary production, the effect of which was exacerbated in some cases by comparatively warm stream temperatures. The four sites where the 16-hour DO criterion was violated also had relatively low ranking scores for the coldwater benthic index (CBI) (Figures 61a). Unlike warmwater streams, however, high ranking levels of diel DO flux (AVGRNGDO) in coldwater streams did not seem to increase the risk of violating the DO criteria or producing lower ranking CBI scores (Figure 61b). Additional diel DO monitoring of coldwater streams is needed for a more thorough analysis of relationships between nutrient variables, diel dissolved oxygen variables, and biological assemblage indicators. These limited data, however, suggest that the existing 16-hour criterion of 7 mg/L might represent a useful nutrient response benchmark.



(a) (b)

Figure 61(a-b). Coldwater benthic macroinvertebrate index (CBI) score plotted against (a) instantaneous dissolved oxygen minima from continuous diel monitoring and (b) average diel dissolved oxygen range. Continuous monitoring data were obtained from 13 coldwater REMAP (2002-2006) random survey sites. Data loggers were deployed 3-12 days during summer months. Iowa water quality standards specify that DO concentrations ≥ 5 mg/L must be achieved always and DO concentration ≥7 mg/L must be achieved at least 16 hours in each day.

Periphyton coverage and benthic macroinvertebrate

Relationships between periphyton coverage and coldwater benthic index (CBI) metrics were examined. As in warmwater streams, field personnel record visual observations about substrate conditions in conjunction with sampling benthic macroinvertebrates in coldwater streams. Benthic macroinvertebrates are usually sampled from rock substrates in coldwater streams because they are normally abundant. The observations made are the same as for warmwater streams: (a) amount of periphyton coverage; (b) type of periphyton growth (filamentous or non-filamentous); (c); amount of embeddedness/sedimentation; (d) amount of benthic macroinvertebrate colonization. Amount observations are made using the following categories: Light (0-25%); Moderate (25-50%), Moderately Heavy (50-75%); Heavy (75-100%).

112 sets of benthic macroinvertebrate samples and substrate observations obtained in bioassessment sampling from 1994- 2011 were available for analysis. The dataset includes a mixture of sites including 28 sample sets from random survey sites, 37 sets from reference sites, and 47 sets from test sites sampled for various purposes such as watershed assessments and TMDL development.

After matching the sampling observations with the corresponding CBI results, the data were visually and quantitatively examined. The analysis focused on the type and amount of periphyton growing on rock substrates since only 6% of all coldwater stream benthic macroinvertebrate samples were collected from artificial substrates.

Analysis of Variance (AOV) tests were done to examine for CBI differences among periphyton type and amount categories. The nonparametric (Kruskal-Wallis) AOV test was performed when it was determined that variance among treatment groups was unequal. Multiple treatment group mean testing was performed using the Tukey Honestly Significant Difference (HSD) and Fisher Least Significant Difference (LSD) methods.

AOV testing was done using data combined from random, reference and test sites. However, it is worth noting that reference sites as a group were overall of better quality than random or test sites. The reference site group had a higher mean CBI score, the frequency of rock substrates dominated by filamentous algae was lower, and the mean ranks of periphyton and substrate embeddedness ratings were lower than either random sites or test sites.

Similar to results from warmwater streams, the mean CBI score (63) from rock substrates that were dominated by nonfilamentous algae growth was significantly higher than the mean CBI sample score (48) from substrates dominated by filamentous algae growth (Figure 62).

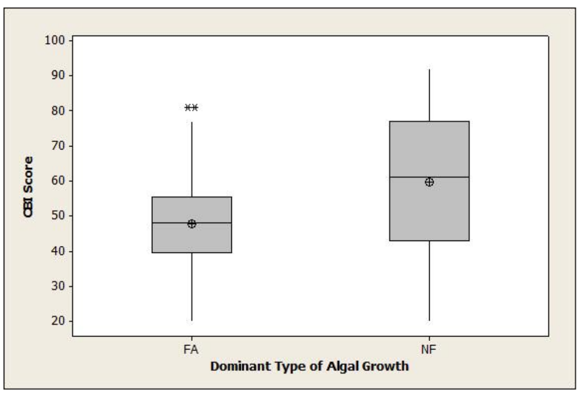


Figure 62. Boxplot of Coldwater Benthic Index (CBI) scores grouped by dominant form of algal growth (FA, Filamentous; NF, Nonfilamentous) observed on rock substrates sampled for benthic macroinvertebrates in coldwater streams 1994-2011 (n=105).

Results from AOV and group mean comparisons found significant differences in mean CBI levels among the combined categories of algal growth type and coverage. Mean CBI levels representing samples obtained from rock substrates evaluated as having moderately heavy or heavy filamentous algae growth ranked lowest among all categories and were significantly lower than mean CBI levels from samples having moderate or moderately heavy nonfilamentous algae growth (Figure 63).

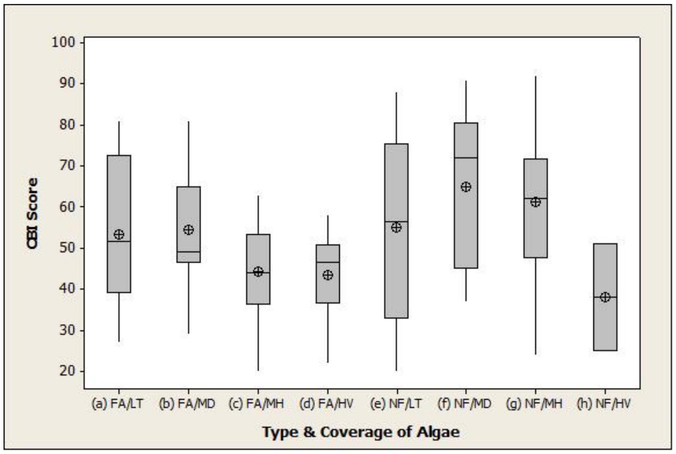


Figure 63. Boxplot of Coldwater Benthic Index (CBI) scores grouped by dominant type (FA, Filamentous; NF, Nonfilamentous) and coverage rating (LT, Light <25%; MD, Moderate-25-50%; MH, Moderately Heavy-51-75%; HV, Heavy->75%) observed on rock substrates sampled for benthic macroinvertebrates in coldwater streams 1994-2011 (n=105).

The total number of samples representing rock substrates rated as having filamentous periphyton growth. To examine the relative impact of varying levels of periphyton coverage it was useful to group samples rated as having light or moderate coverage into one category (LT-MD, $\leq$50%) and samples rated as moderately heavy and heavy coverage as another category (MH-HV >50%). After combining the data, a two sample t-test of CBI scores representing each category was performed. The mean CBI score for samples in the LT-MD filamentous algae category (55.3) was significantly higher ($p<0.05$) that the mean score for samples in the MH-HV filamentous algae category (44.0) (Figure 64). No such difference in mean CBI scores of similarly combined periphyton coverage categories was found among samples from substrates evaluated as having nonfilamentous periphyton growth.

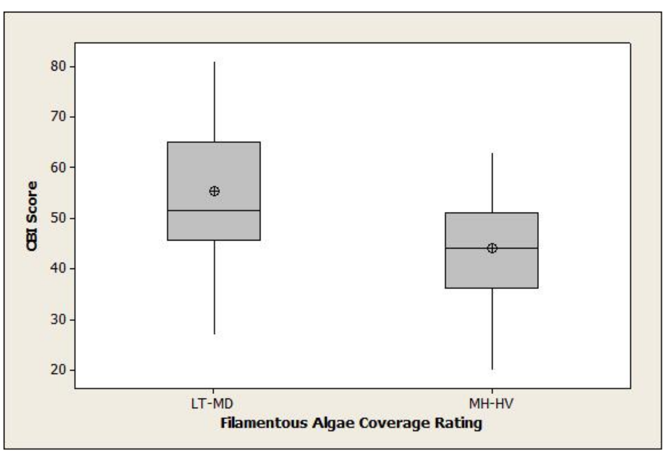


Figure 64. Boxplot of Coldwater Benthic Index (CBI) scores grouped by filamentous algae coverage rating (LT-MD, Light to Moderate <50%; MH-HV, Moderately Heavy to Heavy->50%) observed growing on rock substrates sampled for benthic macroinvertebrates in coldwater streams 1994-2011 (n=56).

Similar to observations from warmwater streams, levels of substrate embeddedness evaluated as moderately heavy or heavy (>50%) tended to cluster in samples also having moderately heavy or heavy filamentous algae growth (Figure 65). Although relatively high levels of embeddedness do appear to dampen CBI scores across a gradient from light to heavy filamentous algae coverage, embeddedness does not appear to be the only factor limiting the maximum CBI scores that occur at moderately heavy or heavy levels of filamentous coverage.

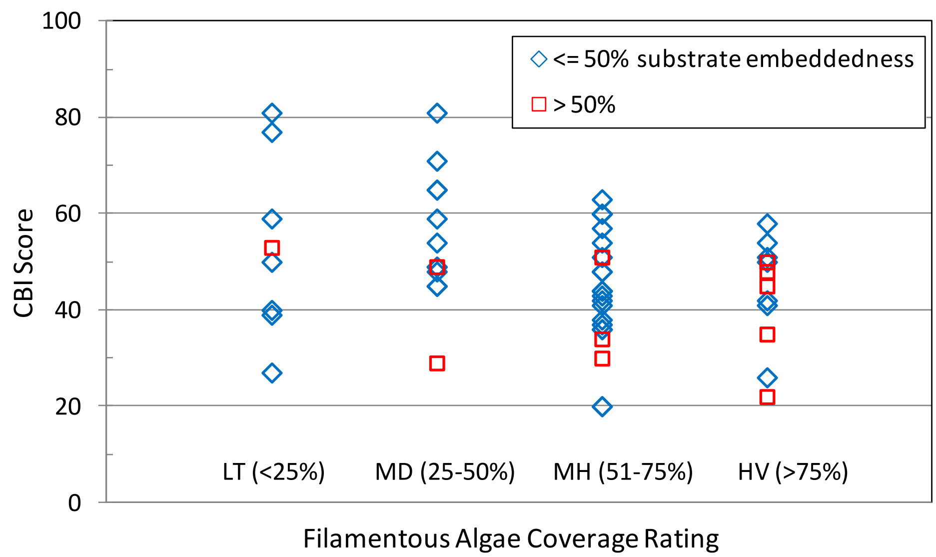


Figure 65. Coldwater Benthic Index (CBI) grouped by filamentous algae coverage rating (LT, Light <25%; MD, Moderate-25-50%; MH, Moderately Heavy-51-75%; HV, Heavy->75%) and substrate embeddedness rating (blue diamond $\leq$ 50%; red square >50%) observations taken from rock substrates sampled for benthic macroinvertebrates in coldwater streams 1994-2011 (n=56).

Dense growths of filamentous algae are easily recognized by trained sampling personnel. In a recent photograph-based exercise, field personnel were able to identify the presence or absence of heavy filamentous algae growth with a very high level of agreement (102/105 (97%) observations). Appendix 16 shows a few examples of periphyton conditions that were evaluated in the exercise. The correspondence of reduced benthic macroinvertebrate index scores and sampling substrates evaluated as having heavy filamentous algal growth in both coldwater and warmwater streams points to the potential utility of an observation-based nutrient response benchmark based on periphyton coverage amount and type.

6. Comparison of Random and Targeted Monitoring Data

To provide additional perspective on nutrient conditions in Iowa's streams, sampling data from the REMAP random stream survey were compared with monitoring data from impaired streams and least disturbed reference streams. Sampling frequency and duration was variable across these projects. For consistency, the comparison included only data collected from wadeable warmwater streams during the months of the biological sampling index period (July-October).

The impaired stream dataset included data from 26 sites located on 14 biologically-impaired stream segments. The data were specifically collected for use in the Stressor Identification (SI) process. Monitoring frequency ranged from weekly to monthly for one year or more between 2001 and 2009. The random dataset included sampling data from 161 random (REMAP) sites typically consisting of two or three samples per site during one biological index period between the years of 2002 and 2006. The reference stream dataset represents 65 least disturbed ecoregion reference sites sampled once or more between 2002-2008. The data for a typical site consists of one or two grab water quality samples collected at the time of biological sampling.

Table 61. Summary statistics for nutrient parameters grouped by type of sample site: Impaired (2001-2009); (REMAP) Random (2002-2006); Reference (2002-2008). All data represent site averages from grab samples collected in wadeable, warmwater streams during July-October. N = number of stream sites. * Kruskal-Wallis Analysis of Variance test was significant ($p<0.05$) for treatment (site type) effect. ** Results of nonparametric treatment mean (ranked values) comparisons; treatment mean ranks are statistically different ($p<0.05$) when they do not share the same letter.

	Site Type	N	Minimum	Q25	Median	Q75	Maximum	Mean Test **
NHx*	Impaired	26	<0.05	<0.05	0.06	0.14	0.65	a
	Random	161	<0.05	<0.05	<0.05	0.08	10.00	a
	Reference	65	<0.05	<0.05	<0.05	<0.05	0.48	b
NOx*	Impaired	26	0.42	3.42	5.91	8.08	11.83	a
	Random	161	<0.1	2.28	5.27	8.27	22.00	a
	Reference	65	<0.1	1.25	3.70	6.75	14.00	a
TKN*	Impaired	26	0.25	0.36	0.78	1.06	4.53	ab
	Random	161	0.14	0.55	0.77	1.07	16.00	a
	Reference	65	<0.1	0.41	0.58	0.92	2.60	b
TN*	Impaired	26	1.23	5.22	6.89	9.00	13.58	a
	Random	161	0.62	3.11	6.27	9.04	22.73	a
	Reference	65	0.56	2.00	4.60	7.43	14.50	b
TP*	Impaired	26	0.07	0.09	0.15	0.27	2.30	a
	Random	161	0.04	0.10	0.17	0.27	27.50	a
	Reference	65	0.03	0.06	0.11	0.16	0.42	b
TN:TP	Impaired	26	4.89	23.53	63.61	92.23	119.84	a
	Random	161	0.60	14.32	37.10	69.33	398.58	a
	Reference	65	3.33	12.58	50.00	78.83	303.33	a

Nutrient data from impaired, random, and reference stream sites are summarized in Table 61 and Figures 66(a-f). The nutrient parameter data ranges represented by random sites for the most part capture the ranges of data represented by impaired sites and reference sites.

Perhaps this is not too surprising since nutrient conditions found in impaired streams and reference streams would presumably be encompassed within the entire population of streams represented by the random site group.

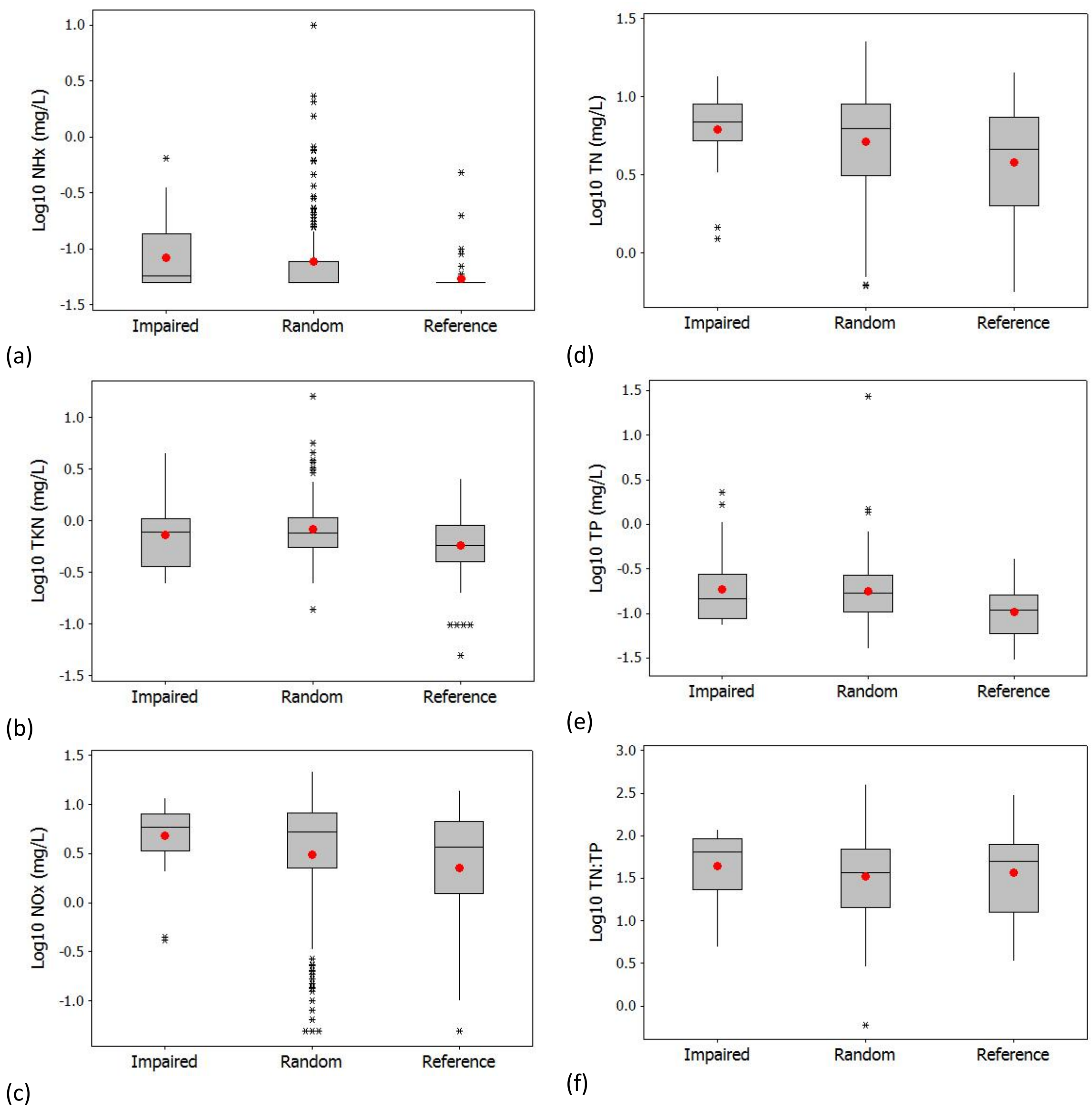


Figure 66(a-f). Comparison of nutrient sampling data grouped by type of sampling site. Box plots represent statistical distribution of log10 of sample site averages from wadeable streams sampled July through October: (a) total ammonia (NHx); (b) total Kjeldahl nitrogen (TKN); (c) nitrite+nitrate-nitrogen (NOx); (d) total nitrogen (calculated); (e) total phosphorus; (f) total nitrogen: total phosphorus ratio (TN:TP).

The summary also shows a tendency for the highest levels of nutrient parameter data (e.g., $\geq$75%tile) for impaired and random sites to extend above the highest levels in the reference data set.

The Kruskal-Wallis AOV test found a significant treatment (i.e., site type) effect for NHx, NOx, TKN, TN, and TP, but not TN:TP ratio (Table 61). The nonparametric two-sample test of order-ranked sample values found reference site mean rank was significantly lower ($p<0.05$) than impaired stream mean rank for NHx, TN, and TP. Reference site mean rank was also significantly lower than random site mean rank for NHx, TKN, TN, and TP. Impaired site mean rank did not differ significantly from random site mean rank for any of the six nutrient parameters.

6.1. Stressor identification

In 2001, IDNR began conducting Stressor Identification (SI) studies to determine the causes of impairment in streams listed as biologically impaired for aquatic life uses. U.S. EPA guidance (2000) describes SI as a process that involves gathering relevant data and information about a stream and its watershed, objectively analyzing plausible stressor-response scenarios, and reaching conclusions about which stressor(s) are the main contributor(s) to biological impairment.

SI studies have been completed for 14 impaired streams through 2012 (Table 62). Nutrient monitoring data from 26 sites located in 14 impaired SI study streams are summarized in Appendix 17. Summary statistics are shown for the entire data set, and the subset of data collected during the biological sampling index months from July–October. The data summary illustrates substantial variability in the amount of nutrient monitoring data collected for SI studies.

SI monitoring approaches have evolved and become more sophisticated since 2001. The basic approach now includes biweekly grab water sampling, storm runoff event sampling, instantaneous flow and continuous flow stage monitoring, continuous dissolved oxygen and temperature monitoring, stream habitat evaluation and biological assemblage (fish and benthic macroinvertebrates) sampling. A basic suite of conventional water quality parameters is supplemented with parameters of special concern as needed (e.g., pesticides, toxic metals). GIS-based tools are used to characterize and quantify stream and watershed characteristics such as stream sinuosity and gradient, riparian and watershed land cover, soils, and subsurface drainage.

Table 62. Streams impaired for aquatic life uses for which a Stressor Identification study has been completed (2004-2012) in order to identify the primary stressor(s) contributing to biological impairment.

Stream Name	County	Year SI Completed	Primary Causes of Impairment	CWA Sec.305(b)/303(d) Report Codes	TMDLs Prepared
Buttermilk Creek	Wright	2009	Ammonia; COD/BOD	Organic enrichment/Low DO (1200)	
Camp Creek	Polk	2004	Habitat alteration (excessive fines; poor habitat diversity); Nutrient enrichment; Suspended solids/turbidity	Siltation (1100); Nutrients (900)	Sediment
Dick Creek	Wayne	2012	Low D.O.; Low (seasonal) flow & habitat limitation	Organic enrichment/Low DO (1200)	
Dry Run Creek	Black Hawk	2008	Elevated levels of fines; Reduced macro- and micro-habitat; Excessive storm water/hydroalteration	Siltation (1100); Flow alteration (1500); Other habitat alterations (1600)	Connected impervious surfaces
Hecker Creek	Allamakee	2009	Elevated chloride/TDS; Habitat alteration; Decreased habitat complexity	Salinity/TDS/Chlorides (1300); Other habitat alterations (1600)	
Little Floyd River	Sioux, O'Brien	2004	Ammonia; Dissolved oxygen; Siltation and sediment; Channelization; Temperature	Siltation (1100); Organic enrichment/Low DO (1200)	Oxygen demand; Sediment
Long Dick Creek	Story, Hamilton	2012	Increased suspended and deposited fine sediments; Embedded rock substrates; Decreased in-stream cover and epifaunal micro-habitat	Siltation (1100); Suspended Solids (2100); Other Habitat Alterations (1600)	
Lyons Creek	Hamilton	2010	Low dissolved oxygen; Excessive suspended and bedded sediments	Siltation (1100); Organic enrichment/Low DO (1200)	
Marrowbone Creek	Carroll, Calhoun	2010	Low dissolved oxygen caused by high primary productivity and flow restrictions due to beaver dams	Organic enrichment/Low DO (1200); Algal Growth/Chlorophyll A (2210); Nutrients (900) and Flow alteration (1500)	
Mddl. Frk. So. Beaver Creek	Hardin, Grundy	2005	Low dissolved oxygen; Excess nutrients; Siltation	Siltation (1100); Organic enrichment/Low DO (1200)	Phosphorus; Sediment
Milford Creek	Dickinson	2005	Nutrient enrichment allowing excessive growth of plants and algae which are depleting dissolved oxygen supplies at night; Flow alteration; Silt/sediment deposition	Organic enrichment/Low DO (1200); Phosphorus (910); Algal Growth/Chlorophyll A (2210); Other habitat alterations (1600)	Phosphorus
North Fork Maquoketa River	Delaware, Dubuque	2006	Lethal level of unionized-ammonia; Elevated TSS/turbidity; Silt accumulation and rock substrate embeddedness; Low D.O. and extreme D.O. fluctuations; Excessive algal growth.	Unionized Ammonia (600); Phosphorus (910); Siltation (1100); Organic enrichment / Low DO (1200); Suspended Solids (2100) / Turbidity (2500); Algal Growth/Chlorophyll a (2210)	Ammonia; Phosphorus; Sediment
Silver Creek	Clayton	2007	Elevated and potentially lethal concentrations of un-ionized ammonia; Elevated levels of silt accumulation and sedimentation of rock substrates; low / potentially lethal levels of dissolved oxygen; Dewatering due to in-stream sinkholes.	Unionized Ammonia (600); Siltation (1100); Organic enrichment / Low DO (1200); Flow alteration (1500)	Ammonia; Sediment
Walnut Creek	Poweshiek	2012	Increased peak flow frequency and magnitude; Decreased macro-habitat complexity; Decreased in-stream cover and epifaunal micro-habitat; Increased suspended and deposited fine sediments	Siltation (1100); Suspended Solids (2100); Turbidity (2500); Flow Alteration (1500); Other Habitat Alterations (1600)	

6.2. Nutrient-related biological impairments

The SI process has identified a variety of physical habitat and water quality stressors contributing to aquatic life use impairment (Table 62). Nutrient enrichment was identified as a primary stressor in five streams. Nutrient parameter data and nutrient response indicator data from these streams are summarized in Tables 63 and 64.

One objective of the SI process is to determine any pollutants that contribute to biological impairment, and require establishment of a Total Maximum Daily Load (TMDL) specifying the level of pollutant reduction needed to achieve water quality standards and full attainment of applicable designated uses (i.e., aquatic life use support). The SI studies led to twelve separate TMDLs including three that establish phosphorus load reduction targets for impaired streams (Table 62). SI analysis also led to a TMDL for unionized ammonia in two impaired streams; however, the TMDL addresses ammonia from the standpoint of aquatic life toxicity rather than nutrient enrichment.

Table 63. Nutrient parameter sampling results from wadeable warmwater streams for which nutrient enrichment was identified among the primary stressors contributing to biological impairment of aquatic life uses. Summarized data represent average values from 1-3 sites per stream sampled during the July–October biological index period.

Stream	NHx mg/L	NOx mg/L	TKN mg/L	TN mg/L	DIN:TN ratio	DOP mg/L	TP mg/L	DOP:TP ratio	TN:TP ratio
Camp	0.06	5.92	0.94	6.86	0.68	0.08	0.19	0.50	41.0
Marrowbone	0.05	7.72	0.52	8.22	0.87	0.06	0.11	0.67	103.3
Mdl.Frk.So.Bvr.	0.07	7.79	1.14	8.93	0.81	0.06	0.15	0.51	76.9
Milford	0.20	3.43	1.60	5.03	0.66	1.65	1.66	1.05	4.9
No.Frk.Maq.	0.28	3.67	1.11	4.79	0.82	0.19	0.25	0.68	26.8
Median	0.07	5.92	1.11	6.86	0.81	0.08	0.19	0.67	40.96
Mean	0.13	5.71	1.06	6.76	0.77	0.41	0.47	0.68	50.59
Std.Dev.	0.10	2.11	0.39	1.85	0.09	0.70	0.67	0.22	39.43

Table 64. Nutrient response indicator sampling results from wadeable warmwater streams for which nutrient enrichment was identified among the primary stressors contributing to biological impairment of aquatic life uses. Summarized data represent average values from 1-3 sites per stream sampled during the July–October biological index period.

Stream	WCHLA ug/L	PCHLA ug/L	SCHLA ug/L	INST.MIN.DO mg/L	AVG.MIN.DO mg/L	RNG.DO mg/L
Camp	116.3	3.3	8.2	4.6	5.7	10.7
Marrowbone	11.0	13.8	5.5	0.0	3.6	3.3
Mdl.Frk.So.Bvr.	225.5	7.7	10.2	3.1	4.0	16.8
Milford	41.5	23.7	8.2	0.2	1.1	10.7
No.Frk.Maq.	26.7	8.8	7.9	5.0	6.0	8.8
Median	41.5	8.8	8.2	3.1	4.0	10.7
Mean	84.2	11.4	8.0	2.6	4.1	10.1
Std.Dev.	88.7	7.8	1.7	2.4	2.0	4.8

In the absence of nutrient criteria for wadeable streams, the evaluation of nutrient enrichment as a primary stressor was accomplished by reviewing the data from the SI stream against data collected from reference site and random site data collected from the same ecoregion. The SI review team would take into consideration the results comparison and other information in order to rank the strength of evidence supporting or not supporting the occurrence of a complete causal pathway linking elevated nutrient levels with elevated nutrient response levels and decreased levels in the biological assemblage indicators. The SI reports detailing the process by which nutrients and other stressors were identified as contributors to stream aquatic life use impairment are accessible from the IDNR Watershed Improvement Section document library at http://www.iowadnr.gov/Environment/WaterQuality/WatershedImprovement/WatershedResearchData/WaterImprovementPlans/PublicMeetingsPlans.aspx.

7. Technical Literature Review

A review of technical literature was conducted before preparing nutrient criteria recommendations. Information from several sources is summarized below. Findings and conclusions from projects and research studies conducted in the Midwest and which examined nutrient-biological response relationships were considered the most relevant to Iowa's stream nutrient criteria development project.

7.1. Nutrient – biological response relationships

Several recently published studies provide valuable perspective into nutrient relationships with biological assemblages in Midwestern streams. Using data collected for the U.S. Geological Survey's National Water-Quality Assessment Program (NAWQA), Frey et al. (2011) examined responses of biological assemblage metrics (algae, fish, invertebrates) to varying nutrient levels in wadeable streams. The study encompassed 64 streams primarily located in agricultural watersheds within three U.S. EPA (2000) Nutrient Regions: Cornbelt and Northern Great Plains (VI), Mostly Glaciated Dairy Region (VII); and Glaciated Upper Midwest and Northeast (VIII). Stream nutrient levels in Nutrient Region VI were generally three to five times higher than levels in streams of Nutrient Region VII or VIII. The nutrient-biological response analysis led to subsequent grouping of sites into a Glacial North (diatom) Ecoregion (GNE) comprised of sites located in Nutrient Regions VII and VIII, and the Central and Western Plains (diatom) Ecoregion (CWPE) comprised of sites in Nutrient Region VI.

Multivariate analysis of the biological assemblage data found significant differences between streams in the GNE from the CWPE, which indicated that a separate analysis of nutrient response relationships was needed. The nutrient data were used to statistically partition sites into low, medium, and high nutrient categories within each diatom region. In the GNE, invertebrate compositional differences across nutrient categories were greater than algae and fish differences. Algal composition differences were greater across nutrient categories of the CPWE compared with benthic macroinvertebrate or fish differences. Breakpoints in relationships between biological assemblage metrics and nutrient (N & P) concentrations were found in both diatom ecoregions. Generally, breakpoints were lower and more numerous in the (north) GNE in comparison to the CWPE (south). Breakpoints in the GNE (approximately 0.60 mg/L, TN and 0.02-0.03 mg/L, TP) were characterized as defining the division between oligotrophic and mesotrophic stream nutrient status. Breakpoints found in the CWPE were approximately three to five times higher than those of the GNE. The authors concluded that mostly nutrient-saturated conditions existing in CWPE streams prevented the detection of lower nutrient breakpoints similar to those observed in the GNE.

Black et al. (2011) examined benthic algal community relationships with nutrients and a suite of stream habitat and watershed environmental variables across a broad gradient in stream nutrient levels. The study included sampling sites located in agricultural watersheds of central and eastern Nebraska. Nutrient variables (TN and TP) were predictably related with several diatom assemblage metrics and explained the greatest amount of variation in metric levels, although several other stream and watershed variables were also important predictors. Although, nutrient levels represented in the study were often substantially higher than previously reported TN and TP thresholds in relation to limitation of algal biomass (Dodds 2002; Stevenson 2006), the authors found evidence of ecologically relevant thresholds at relatively high levels of TN (median, 1.25 mg/L; range 0.59-1.79 mg/L) and TP (median, 0.075 mg/L; range 0.03-0.28 mg/L) in relation to diatom assemblage metric responses.

The reported values represent combined threshold analysis results from fine sediment and coarse substrate algal community sampling. The authors note that TP explained a greater proportion of variability in diatom metrics than was explained by TN. Significant overlap in taxa representing high TP and TN levels might have made it more difficult to discern responses to individual nutrients. Generally poor correlations between TN and similar algal metrics were also reported by Porter et al. (2008). Benthic macroinvertebrate assemblage trophic structure metrics explained a minor amount of variation in algal composition metrics compared with nutrient variables or stream reach and basin characteristics. The authors also noted that additional work is needed to better understand relationships between the diatom assemblage metrics and environmental conditions under which nuisance growths of filamentous green algae occur.

In Wisconsin, Robertson et al. (2006) and Wang et al. (2007) examined relationships between nutrient parameters and wadeable stream biological assemblages (diatoms, benthic macroinvertebrates, fish). Nutrient threshold levels for responses of diatom indicators and benthic chlorophyll A were similar to thresholds in benthic macroinvertebrate responses, but lower than fish response thresholds. TN and TP ranged from 0.54-1.83 mg/L and 0.04-0.09 mg/L, respectively. Among other findings, the authors report that regression modeled 25th percentile reference nutrient concentrations were approximately 30-40% lower than biological response thresholds. Nutrient parameters explained a significant, but minor amount of variation in benthic macroinvertebrate metrics (22%) and fish metrics (15%). Nutrients in combination with other stream environmental variables explained approx. 50% of variation in benthic macroinvertebrate and fish metrics. The authors concluded that nutrients are “among the key factors” in determining biological health.

Weigel and Robertson (2007) examined nutrient-biological relationships in nonwadeable rivers of Wisconsin. Sampling data on fish, macroinvertebrates, and nutrients plus watershed characteristics from 41 sites on 34 nonwadeable rivers were analyzed. Regression tree analysis was used to identify breakpoints between biotic indicators and nutrient parameters. Redundancy analysis was used to identify the most important nutrient, watershed, and stressor variables in relation to biotic response variables (fish and macroinvertebrate IBIs and component metrics).

Breakpoints in TP (~0.06 mg/l) and TN (~0.64 mg/l) were somewhat higher (2-6X) than reference nutrient levels determined by other statistical analysis approaches. Environmental variables explained about 61% and 44% of variation in macroinvertebrate and fish assemblage variation, respectively. Most of the variation was explained by combinations of several environmental covariates. Nutrient parameters by themselves only explained 2% of macroinvertebrate variation and 25% of fish variation determined by partial redundancy analysis. Based on their multi-variate analysis results, the authors recommend simultaneous application of a suite of biotic and nutrient variables to determine if waters are not meeting designated uses. The study demonstrates one approach to identifying and quantifying the environmental variables, including nutrients, which are important determinants of biotic condition and assemblage structure. The observed relationships between nutrients and biological assemblage metrics are correlative, however, and by themselves do not establish the nutrient stressor-response mechanisms and pathways impacting stream biological assemblages.

The Minnesota Pollution Control Agency (MPCA) conducted a stressor-response analysis that examined relationships between nutrient variables, nutrient response variables (i.e., BOD, seston chlorophyll A, and d.o. flux), and biological assemblage metrics (benthic macroinvertebrates, fish) sampled in Minnesota rivers (Heiskary et al. 2013). The analysis sought to identify relationship breakpoints or thresholds in three River Nutrient Regions (RNR): North, Central, and South. The South region includes the portion of Minnesota located in the Western Corn Belt Plains (WCBP), a U.S. EPA Level III ecoregion covering the majority of Iowa. MPCA used additive quantile regression and regression tree statistical analysis techniques to identify threshold concentrations (TCs) in TP, CHLA, D.O. flux, and BOD at which there was a significant change in benthic macroinvertebrate and/or fish data metrics. MPCA utilized the 25th percentile of TCs as one line of evidence to consider in the development of nutrient eutrophication criteria development. The 25th percentile values from the South RNR adjoining Iowa were: TP, 145 ug/L; CHLA, 21 ug/L; D.O. flux, 3.1 mg/L; BOD, 3.1 mg/L.

7.2. Nutrient criteria derivation

Described below is a summary of nutrient criteria work relevant to Iowa stemming from work at national, regional, and state levels. A tabular summary of data analysis benchmarks and nutrient enrichment criteria recommendations is provided in Table 65.

U.S. Environmental Protection Agency

In accordance with the 1998 national strategy, the U.S. EPA issued stream nutrient criteria guidance (U.S. EPA 2000a) and numeric criteria recommendations for aggregate nutrient regions of the conterminous United States (U.S. EPA 2001b; 2001c; 2001d). Iowa is covered by parts of three aggregate regions. The guidance indicates, at a minimum, EPA expects states to adopt criteria for nitrogen and phosphorus, and at least one nutrient response variable (e.g., seston chlorophyll A). EPA used a statistical data distribution technique to estimate reference nutrient levels, and these were presented as initial nutrient criteria benchmarks for consideration by state and tribal governments. In lieu of using data from minimally disturbed reference streams, which were deemed unavailable in many regions of the U.S., the EPA chose the 25th percentile nutrient levels from all available monitoring data in the most recent ten-year period to represent reference nutrient conditions.

Table 65 lists the EPA criteria benchmark values by aggregate nutrient region and individual Level III ecoregions that apply to Iowa. Aggregate nutrient region benchmarks are typically lower than Level III ecoregion benchmarks, which are more likely to be representative of Iowa streams assuming that adequate data were available. For example, the reported total nitrogen (TN) benchmark values range from 0.54–2.18 mg/L for aggregate regions taking in Iowa and from 0.712-3.26 mg/L for Level III ecoregions. The pattern is the same for total phosphorus, which ranges from 33-76.25 ug/L among aggregate regions compared with a range from 70-118.13 ug/L for just those Level III ecoregions covering Iowa.

According to the national strategy, once the EPA issued the criteria methodology and recommendations, states could take one of three paths to establishing nutrient standards: 1) adopt the EPA nutrient criteria benchmarks; 2) supplement the EPA data and benchmarks with other data from which new criteria are derived or used to support existing state nutrient criteria; 3) use EPA's methodology or their own approach to develop criteria independently of EPA's benchmarks, so long as the approach is scientifically defensible.

Regional Technical Assistance Group (RTAG)

As part of the national strategy, EPA established a nutrient team to coordinate the development and adoption of nutrient standards. The national strategy also called for establishment of a nutrient coordinator and technical assistance group (RTAG) within each EPA region. The nutrient RTAG for EPA Region VII is comprised of the EPA's regional nutrient coordinator, technical representatives from each state (Iowa, Kansas, Missouri, Nebraska), and academic experts. The RTAG was convened in 2000 and initially focused on refining region-wide nutrient criteria benchmarks for lakes and reservoirs. The RTAG completed this work and issued a report, which included nutrient criteria recommendations for all states within Region VII. The RTAG next began work on region-wide criteria for streams and rivers. The RTAG met several times before completing a draft report and stream nutrient criteria recommendations. The report has not been finalized or released publically in draft form (personal communication, Gary Welker, U.S. EPA Region VII).

Under leadership of the Central Plains Center for Bioassessment (CPCB), and with the assistance of academic, EPA and state members, the RTAG compiled and analyzed an extensive amount of nutrient, nutrient response, and biological assemblage data. Results from a variety of data analysis techniques were combined using a weight-of-evidence approach to identify nutrient benchmarks that would be sufficiently protective of stream aquatic communities. A key difference between the RTAG approach and the EPA national approach is the RTAG approach incorporated benchmarks from the analysis of nutrient–biological assemblage relationships by the RTAG.

Although not considered final benchmarks, the RTAG nutrient benchmarks are identified strictly for context to evaluate a full range of analytical results and criteria benchmarks relevant to Iowa's streams. The draft RTAG criteria parameters and benchmark values are: TN, 0.9 mg/L; TP, 75 ug/L; Seston chlorophyll A, 8 ug/L; Benthic chlorophyll A, 40 mg/m^2.

State nutrient criteria development

States adjoining Iowa are in various stages of stream nutrient criteria development and adoption as water quality standards. Below is a state-by-state synopsis.

Illinois

The Natural Resources Working Group of the Illinois Council on Food and Agricultural Research (C-FAR) recommended a strategic research initiative (SRI) to establish a scientific foundation for the development of nutrient standards for Illinois waters and assist with the development and implementation of TMDLs. An advisory team identified research needs around a central theme which sought to examine the strength of cause and effect relationships between stream nutrient levels and biotic impairment. Four collaborative teams comprised of scientists from academic institutions and natural resource organizations were formed to investigate different research questions pertaining to the overarching research theme.

The SRI was an extensive project that resulted in publication of several peer-reviewed research papers. A final report summarizing the research was issued in 2007 (Czapar 2007). Many of the research findings relate to stream nutrient issues facing Iowa. The research showed that complex relationships involving environmental factors such as physical habitat, light availability, and hydrology were more likely to influence stream biological responses to nutrients in Illinois streams than nutrient concentrations alone. The research also concluded that uniformly high nutrient levels in Illinois streams make it difficult to observe cause-effect relationships between nutrients and algal production, because nutrient availability is rarely a limitation. While providing valuable scientific insight, the research raised additional questions and did not appear to provide a clear direction for the development of stream nutrient standards.

Minnesota

The Minnesota Pollution Control Agency (MPCA) has proposed river eutrophication standards that are based on findings from prior studies that were specifically designed to examine regional differences in nutrient conditions and relationships between nutrients and biological response variables (Heiskary 2008; Heiskary and Parson 2010; Heiskary et al. 2013). MPCA evaluated multiple lines of evidence in formulating criteria recommendations, including: a) stressor-response threshold concentrations; b) statistical data distributions of minimally impacted and reference MN streams; c) U.S. EPA ecoregional statistical data distributions; d) predicted values from regression modeling of nutrient and nutrient-response variables.

MPCA has proposed stream nutrient criteria for three River Nutrient Regions (RNR): North, Central, and South. Criteria parameters include total phosphorus (TP) and three nutrient response parameters: seston chlorophyll A, diel dissolved oxygen flux (DO flux) and 5-day biochemical oxygen demand (BOD_5). The recommendations for the agriculturally-dominated South River Nutrient Region are the most relevant to stream nutrient criteria development in Iowa. The criteria are: TP, 150 ug/L; ChlA, 35 ug/L; DO flux, 5.0 mg/L; BOD_5, 3.0 mg/L. Minnesota's proposed criteria are currently in draft water quality standards rule review.

In response to a state legislative directive, the MPCA has also developed draft criteria for nitrate-nitrogen. The criteria development work was done as part of the state's recent triennial water quality review/update of water quality standards. A technical support document (Monson 2010) details the analysis in which MPCA reviewed dose-response toxicological studies on the lethality of nitrate-nitrogen to aquatic species. They also arranged additional toxicity studies for two test organisms that were not in the national database, but are common inhabitants of Minnesota waters. Incorporating these new data, MPCA followed EPA's 1985 protocol for deriving acute and chronic water quality standards criteria.

The draft criteria specify acute and chronic nitrate exposure concentrations that are protective of aquatic communities in Minnesota. The acute (1-day exposure) criterion is 41 mg/L nitrate-N. The chronic (4-day exposure) criterion is 3.1 mg/L for designated cold waters (Class 2A) and 4.9 mg/L for all other designated (Class 2B) cool/warm waters.

Missouri

Missouri has been working toward establishment of nutrient standards for streams. A draft stream nutrient standards rule was completed in 2010. Information from meeting notes (November 16, 2010) at the MDNR nutrient standards website (http://www.dnr.mo.gov/env/wpp/wqstandards/wq_nutrient-criteria.htm) indicates the stream nutrient rule was not likely to be carried forward during the most recent triennial review period ending 2012. Recent nutrient work has focused on refining proposed lake nutrient standards.

Missouri took a regionalized approach to development of stream nutrient criteria recommendations. Recommendations were made for five nutrient criteria zones, which are combinations of ecoregions and drainage basins. The proposed criteria for Nutrient Region I covering northern Missouri adjoining Iowa and sharing river drainages are the same as U.S. EPA Region VII Nutrient RTAG benchmark recommendations (TN, 900 ug/L, TP, 75 ug/L). According to information available on the website, criteria recommendations were based on consideration of several lines of evidence including published benchmarks, RTAG nutrient-biological analysis, and reference and non-reference statistical analysis benchmarks. Scientific collaborators reviewed Missouri-specific data from the Ozark region and the Plains region using statistical techniques to examine relationships between nutrients and biological responses such as algal composition (Ozarks) and benthic macroinvertebrate tolerance index (Plains). The data summaries provided on the web (November 16, 2010) show a range of statistical analysis benchmarks. From the information provided, the analytical method or other decision process used to derive the final recommendations cannot be discerned.

Nebraska

Nebraska's 2008 nutrient criteria development plan submitted to the U.S. EPA (NDEQ 2008) indicates that nutrient stressor-response data are being obtained for streams and that stream nutrient criteria development will proceed after lake and reservoir nutrient criteria are completed. The initially proposed lake and reservoir criteria were not approved by EPA. NDEQ has indicated (Bender 2010) there is now a revised agreement with U.S. EPA to address lake/reservoir work. NDEQ apparently is working with the University of Nebraska – Lincoln to address stream data gaps involving relationships between nutrients and biological data. EPA approved revised nutrient criteria for Nebraska lakes and reservoirs in 2012. There does not appear to be a schedule for development stream criteria at this time.

South Dakota

No schedule for stream nutrient criteria is mentioned in either the EPA progress tracking site http://www.epa.gov/nandppolicy/progress.html#tabs-1 or the South Dakota WQ standards website http://denr.sd.gov/des/sw/swqstandards.aspx

Wisconsin

Wisconsin recently adopted phosphorus criteria for 46 named river segments (100 ug/L) and all other streams (75 ug/L). The U.S. EPA approved Wisconsin's phosphorus standards in 2012. An extensive amount of research and data analysis was conducted in Wisconsin prior to developing the proposed criteria. Two studies (Robertson et al. 2006; Robertson et al. 2008) that identified a suite of breakpoints or thresholds in relationships between phosphorus and biological assemblages (algae, aquatic insects, fish) were the primary basis for establishing phosphorus criteria. The rule proposal package indicates an averaging method was used to derive the final criteria values from the various data analysis benchmarks (WDNR 2010).

Other criteria derivation approaches

Herlihy and Sifneous (2008) examined three alternative methods of identifying nutrient criteria benchmarks across ecoregions of the U.S. and compared the results to the respective EPA aggregate ecoregion values. The study utilized data from 1392 random sites sampled for U.S. EPA's Wadeable Streams Assessment (WSA) project. The three methods are: a) 25th percentile of random population; b) 75th percentile of least disturbed reference sites; c) disturbance modeling. Nutrient benchmarks for aggregate ecoregions covering Iowa are summarized in Table 65. The authors found that method results were generally correlated with each other across aggregate ecoregions (i.e., aggregate regions having low nutrient benchmarks ranked consistently lower for all methods compared with aggregate ecoregions having higher nutrient benchmarks). However, they also found substantial variation in nutrient benchmarks among Level III ecoregions within any given aggregate ecoregion. For the majority of aggregate regions, WSA 25th percentile estimates for TN and TP ranked lower than corresponding EPA nationally-derived 25th percentiles. Another key finding was that population 25th percentile approaches (EPA-national and WSA) both resulted in consistently and substantially lower benchmark values than the reference 75th percentile approach. The authors concluded the 25th percentile cannot be used as a surrogate approximate for reference nutrient levels. The authors also concluded the scale of aggregate regions was too coarse upon which to derive appropriate criteria because it fails to account for significant within-region differences in stream type.

One additional factor the authors did not address was seasonality. The EPA national benchmarks were derived using all available data from all seasons. The WSA data upon which Herlihy and Sifneous derived nutrient benchmarks was collected during the summer season. In Iowa, as was most likely also the case in other states, WSA samples were typically collected during base flows that were suitable for biological sampling. The WSA season-specific and flow-specific sampling constraints could have had a significant influence over the nutrient levels that were found. Ambient stream monitoring in Iowa has documented seasonal differences in nutrient parameters. Nitrate-nitrogen levels, for example, are often highest during the spring – early summer period following fertilizer application on annual row crop fields and because subsurface drainage is substantial during the typically wet months of May and June followed by a declining pattern throughout the summer into early fall.

Herlihy and Sifneous (2008) point to fundamental differences in stream types and human disturbance displayed between Level III ecoregions of the same aggregate nutrient region as a reason for the observed variation in stream nutrient. Reference stream site evaluations and sampling data from Iowa (Wilton 2004) have also shown significant variation in human disturbance and stream characteristics, including nutrient levels, at the Level IV sub-ecoregion scale.

Other studies have employed modeling techniques to derive regional nutrient benchmarks. Dodds and Oakes (2004) used multiple regression modeling to estimate nutrient levels with land use/human disturbance indicators set equal to zero in the regression model. Smith et al. (2003) applied the SPARROW empirical model to calculate background stream loads for nitrogen and phosphorus, from which they were able to estimate the 75th percentile background nutrient concentrations for TN and TP. Generally, both approaches resulted in comparatively low nutrient benchmarks that were closer to EPA national 25th percentile benchmarks for TN and TP than to empirically-derived Herlihy and Sifneous (2008) reference 75th percentile benchmarks (Table 65).

Table 65. Selected stream nutrient data analysis benchmarks from literature review.

Source	Geographic Applicability	Derivation Method / Ecological or WQ relevance	TN (mg/L calculated)	TN (mg/L reported)	NO3+NO2 (mg/L)	TKN (mg/L)	NH4 (mg/L)	TP (ug/L)	DP (ug/L)	Seston ChlA (ug/L fluorometric)	Seston ChlA (ug/L spectrophotometric)	Seston ChlA (ug/L trichromatic)	Benthic ChlA (mg/m2)	Turbidity (NTU)	Turbidity (FTU)	Turbidity (JCU)	DO Flux (mg/L)	BOD5 (mg/L)
Camargo & Alonso 2006	Global	Review of ecological & toxicology studies; threshold to prevent toxicity & eutrophication in sensitive waters	-		0.5-1													
Camargo et al. 2005	Global	Review of toxicology studies; limit for sensitive freshwater species	-		2													
Carleton et al. 2009	Minnesota River Basin - Blue Earth River	AQUATOX modeling result; threshold to prevent nuissance seston chlorophyll & Bluegreen dominance	-	2.7				100										
Dodds and Oakes, 2004	Aggregate Nutrient Region VI (including Iowa's WCBP #47)	Regression model intercept; nutrient reference condition estimator	-	0.215				23										
Dodds and Oakes, 2004	Aggregate Nutrient Region VII (including Iowa's Driftless region #52)	Regression model intercept; nutrient reference condition estimator	-	0.565				23										
Dodds and Oakes, 2004	Aggregate Nutrient Region IX (including Iowa's CIP#40 & MRL#72)	Regression model intercept; nutrient reference condition estimator	-	0.37				31										
Dodds et al. 1998	General applicability in streams	Consensus of cited studies; nuissance level of periphyton growth	-										150					
Dodds et al. 1998	Temperate Streams Mesotrophic-Eutrophic boundary	67% of data distribution; trophic state estimator	-	1.5				75			30*		70					
Dodds et al. 1998	Temperate Streams Oligotrophic-Mesotrophic boundary	33% of data distribution; trophic state estimator	-	0.7				25			10*		20					
EPA R7 RTAG	EPA Region 7: IA, KS, MO, NE	Draft benchmarks from consideration of multiple evidence lines including: literature values, EPA nutrient region benchmarks, reference stream median, benthic macroinvertebrate data tri-section.		0.9				75		8			40					
Evans-Smith et al. 2009	KS, MO, NE	Spearman correlation & nonparametric changepoint; primary consumers	-	1				60										
Evans-Smith et al. 2009	KS, MO, NE	Spearman correlation & nonparametric changepoint; secondary consumers	-					90										
Heiskary et al. 2013	MN - Central nutrient region	Changepoint analysis nutrients v various biotic response variables						100				18					3.5	2.0
Heiskary et al. 2013	MN - North nutrient region	Changepoint analysis nutrients v various biotic response variables						50				7					3.0	1.5
Heiskary et al. 2013	MN - South nutrient region	Changepoint analysis nutrients v various biotic response variables						150				35					4.5	3.0
Heiskary et al. 2013	MN - statewide	Adapted MT aesthetic/recreation criterion											150					
Herlihy & Sifneous, 2008	Aggregate Nutrient Region IX (including Iowa's CIP#40 & MRL#72)	Wadeable Stream Assessment (WSA) 25th percentile of random site data	-	0.331				20.4										
Herlihy & Sifneous, 2008	Aggregate Nutrient Region IX (including Iowa's CIP#40 & MRL#72)	WSA 75th percentile	-	0.681				60.2										
Herlihy & Sifneous, 2008	Aggregate Nutrient Region VI (including Iowa's WCBP #47)	WSA 25th percentile	-	1.86				65.8										
Herlihy & Sifneous, 2008	Aggregate Nutrient Region VI (including Iowa's WCBP #47)	WSA 75th percentile	-	2.5				181										
Herlihy & Sifneous, 2008	Aggregate Nutrient Region VII (including Iowa's Driftless region #52)	WSA 25th percentile	-	0.581				17										
Herlihy & Sifneous, 2008	Aggregate Nutrient Region VII (including Iowa's Driftless region #52)	WSA 75th percentile	-	-				-										
Miltner & Rankin 1998	Ohio	Multiple linear regression; benthic macroinvertebrate & fish IBI	-	610				60										
Missouri DNR 2010	Stream Nutrient Criteria Zone I (Central Plains EDU's 11-17,22)	Nutrient statistical summaries & biological response (benthic macroinvertebrates)		900				75										
Missouri DNR 2010	Stream Nutrient Criteria Zone II (Ozark EDUs 26,27,29)	Nutrient statistical summaries & biological response (algae)		700				35										
Missouri DNR 2010	Stream Nutrient Criteria Zone III (Ozark EDUs 21a,23,24,25,28)	Nutrient statistical summaries & biological response (algae)		500				31										
Missouri DNR 2010	Stream Nutrient Criteria Zone IV (Ozark EDU 21b)	Nutrient statistical summaries & biological response (algae)		430				10										
Missouri DNR 2010	Stream Nutrient Criteria Zone V (Mississippi Alluvial Basin EDU's 31,32,33)	Nutrient statistical summaries		500				75										
Monson 2010	MN - cold waters	Chronic toxicity value calculated from acute value			3.1													
Monson 2010	MN - cool/warm waters	Chronic toxicity value calculated from acute value			4.9													
Porter et al. 2008	Continental U.S.	Upper quartile algal biovolume estimated-biomass distribution representing eutrophic-mesotrophic boundary											55					
Porter et al. 2008	Continental U.S.	Lower quartile algal biovolume estimated-biomass distribution representing eutrophic-mesotrophic boundary	-										21					
Robertson et al. 2006	Wisconsin (statewide - wadeable)	Regression tree analysis avg. of Benthic Macroinvert metric breakpoints	-	0.895	2.732	0.985	0.03	89	72									
Robertson et al. 2006	Wisconsin (statewide - wadeable)	Regression tree analysis average of fish indicator breakpoints	-	0.539	2.747	0.411	0.026	59	49									
Robertson et al. 2006	Wisconsin (statewide - wadeable)	Regression tree analysis of benthic chlorophyll response	-	0.918	0.187	0.31	0.04	39	20									
Robertson et al. 2006	Wisconsin (statewide - wadeable)	Regression tree analysis of Diatom Nutrient Index	-	1.216	0.381	0.745	0.21	57	26									
Robertson et al. 2006	Wisconsin (statewide - wadeable)	Regression tree analysis of seston chlorophyll	-	1.679				70										
Smith et al. 2003	U.S.	Regression model w/ instream loss rates, background nutrient yield & concentrations	-	0.02-0.5				6-80										
Smith et al. 2007	New York	Bray-curtiss, weighted average benthic macroinvertebrate tolerance value	-	0.98				65										
Stevenson et al. 2009	Mid-Atlantic Highlands	75% reference; lowess regression; regression tree	-					10-20										
Suplee et al. 2009	Montana	Public survey; benthic algae chlorophyll	-										150					
U.S. EPA Ambient WQ Criteria Recommendations	Aggregate Nutrient Region IX (including Iowa's CIP#40 & MRL#72)	25th percentile (P25) for all seasons, all data for decade	0.425	0.69	0.125	0.3		36.56		2.25	0.93	0.53	20.35	7.02	5.7	3.53		
U.S. EPA Ambient WQ Criteria Recommendations	Aggregate Nutrient Region VI (including Iowa's WCBP #47)	25th percentile (P25) for all seasons, all data for decade	1.22	2.18	0.633	0.591		76.25		2.7	7.33	6.83		9.89	6.36	10.4		
U.S. EPA Ambient WQ Criteria Recommendations	Aggregate Nutrient Region VII (including Iowa's Driftless region #52)	25th percentile (P25) for all seasons, all data for decade	0.54	0.54				33		1.54	3.5			1.7	2.32			
U.S. EPA Ambient WQ Criteria Recommendations	Level III ecoregion # 40 - CIP)	25th percentile (P25) for all seasons, all data for decade	0.855	0.712	0.23	0.625		92.5		2.75	5.488							
U.S. EPA Ambient WQ Criteria Recommendations	Level III ecoregion # 47 - WCBP)	25th percentile (P25) for all seasons, all data for decade	2.615	3.26	1.965	0.65		118.13		4.4	7.85	9.38		15	7.69	10.15		
U.S. EPA Ambient WQ Criteria Recommendations	Level III ecoregion # 52 - Driftless Region)	25th percentile (P25) for all seasons, all data for decade	1.88	1.51	1.73	0.15		70		1	2.32			3.38	2.4	4.25		
U.S. EPA Ambient WQ Criteria Recommendations	Level III ecoregion # 72 - MRL)	25th percentile (P25) for all seasons, all data for decade	0.754	1.669	0.215	0.539		83.125		1.5	5.74			15	6.263	29.75		
Wang et al. 2007	Driftless Area of Wisconsin	Avg. of regression & 25th percentile	-	1.065		0.23	0.025	45	25									
Wang et al. 2007	Wisconsin, statewide wadeable	Avg. of regression tree & Kolmogorov-Smirnoff thresholds/breakpoints based on benthic macroinvertebreate & fish metrics	-	0.988		0.644	0.03	73	55									
WDNR 2010	Wisconsin, 46 named rivers	Averaging of nutrient-biological assemblage relationship analysis benchmarks						100										
WDNR 2010	Wisconsin, all streams except 46 named rivers	Averaging of nutrient-biological assemblage relationship analysis benchmarks						75										
Weigel & Robertson 2007	Wisconsin, statewide nonwadeable	Regression tree breakpoints indicating benthic macroinvertebrate or fish impairment	-	0.64				60										

* analytical method not specified; spectrophotometric is default assumption

8. Summary and Recommendations

8.1. Review of data analysis methods

Statistical analysis methods used in the nutrient stressor-biological response data analysis were described in Section 3.3. The three primary methods, Conditional Probability (CP), Quantile Regression (QR), and Regression Tree (RT), each take a distinct approach to identifying a changepoint or threshold in the stressor-response relationship.

CP analysis finds the stressor variable level at which there is maximum rate of change in the attainment of a critical level in the response variable (e.g., reference 25th percentile). The selection of the critical level can affect where the changepoint is found along the stressor gradient and the strength of the stressor-response signal. Perhaps the greatest advantage of CP analysis, as it has been applied in this analysis, is its relevance and interpretability with respect to aquatic life use goal attainment. A potential weakness of CP and the other bivariate stressor-response models is its inability to isolate the effects of the nutrient stressor from other co-varying stressors.

QR analysis requires choosing a regression percentile (e.g., 90th percentile) that is used to model the limiting effect of the stressor variable over the response variable. It was also necessary to choose a critical response level (e.g. reference 75th percentile) in order to identify a threshold along the stressor variable gradient above or below which (depending on the direction of response to the stressor) the critical level of the response variable is rarely, if ever, attained. The biggest advantage of QR analysis was that it presumably comes closer to eliminating the effects of other stressor-covariables in modeling the limiting relationship between the stressor and response variables of interest. A disadvantage is that it models the response as a simple linear relationship, which is probably not a good assumption when changes in the response variable are abrupt (e.g., stair-step) rather than constant.

In comparison, RT analysis does not require specification of a critical response level. As such, the method is unbiased in its approach to finding a changepoint. The analysis algorithm finds a primarily split or natural changepoint in the data set at which the variation in the response variable is reduced the most. Successive splits in the data set may be identified depending on the statistical characteristics of the data set and the model specifications; however, the first (primary) split results in the largest variance reduction in the stressor-response relationship, and is usually considered the most meaningful changepoint. RT analysis has the advantage of being objective and free of any assumptions about data structure. However, because it works by analyzing the data variation surrounding a central (mean) tendency, like CP, the effects of the stressor of interest could not be isolated from other stressor co-variables in the simple bivariate model.

Nutrient data from biological reference sites and impaired sites were also summarized and compared with random stream data to provide additional insight into nutrient conditions in targeted streams displaying varying levels of biological assemblage condition ranging from excellent to poor.

8.2. Synthesis of data analysis results

Numerous breakpoints and thresholds in nutrient stressor-biological response relationships have been revealed so far. A consolidation and synthesis of these findings is necessary in order to move forward with nutrient criteria recommendations. U.S. EPA (2000) guidance recommends taking a weight-of-evidence approach to nutrient criteria derivation. Essentially this approach proposes that the foundation supporting nutrient criteria is strongest when the criteria are supported by multiple, convergent lines of evidence. Evidence lines should be science-based and represent expressions of nutrient status that are relevant and compatible with achieving a desired ecological condition and/or designated water uses.

With this guiding philosophy, a consolidation of nutrient data analysis results and synthesis of criteria recommendations was attempted. The process involved considering the relevance and strength of evidence supporting each result, and the degree to which results converged around a common level in a nutrient or biological response variable. Benchmark values from other studies provided additional perspective for issuing recommendations.

Wadeable warmwater streams

Seston chlorophyll A

Seston algal chlorophyll A (WCHLA) is a key nutrient response indicator because it directly and indirectly impacts the condition of Iowa's stream benthic macroinvertebrate assemblages. Direct impacts included reduction in benthic macroinvertebrate diversity and alteration of trophic composition. The most prominent indirect impact of WCHLA was through alteration of dissolved oxygen levels.

WCHLA results from the analysis of benthic macroinvertebrate metric responses ranged from 5.2 – 26.9 ug/L (Table 34). The median value was 15 ug/L, which also happens to be the CP analysis level above which a significant increase in the occurrence of stressfully low dissolved oxygen was observed.

Two dissolved oxygen parameters, average diel DO minima (AVGMINDO) and average diel DO range (AVGRNGDO), showed fairly strong relationships with levels of benthic macroinvertebrate and fish assemblage metrics. WCHLA was correlated with both DO variables, thus demonstrating a pathway linking increased primary production with DO stress, leading to reductions in aquatic biological condition.

As Figure 67 indicates, low DO levels were observed far less frequently in medium-to-large wadeable streams (i.e., watershed area $\geq$ 291mi^2) than in small-to-medium streams.

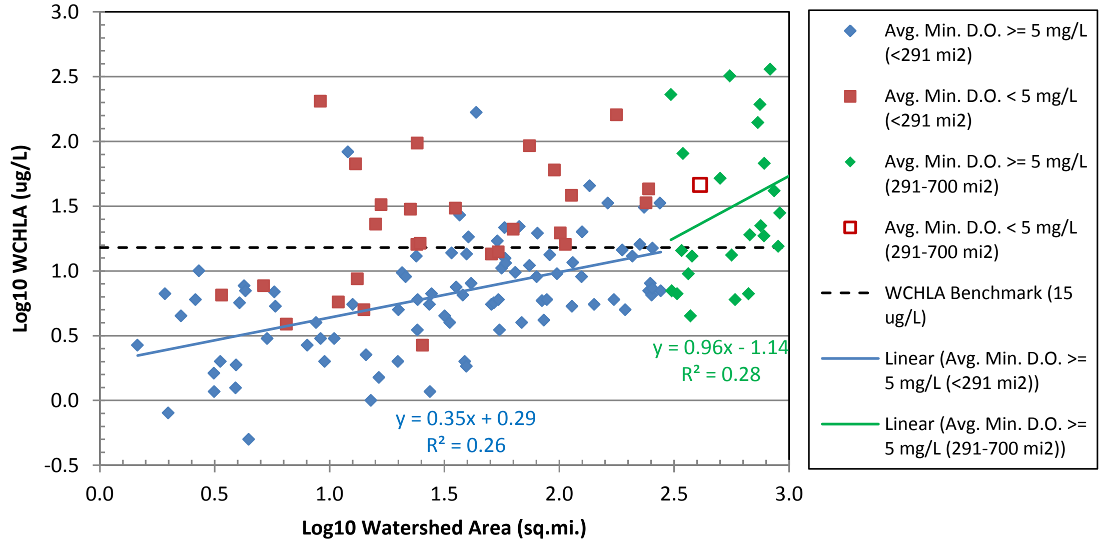


Figure 67. Linear regression of Log10 stream watershed area and Log10 seston chlorophyll A (WCHLA) for wadeable streams with average diel minimum dissolved oxygen (AVGMINDO) greater than or equal to the 5 mg/L benchmark. Blue diamond symbols represent sites having watershed area <291 mi^2; green diamonds represent sites having watershed area 291-700 mi^2). Also shown as maroon filled or open squares are sites that failed to meet the AVGMINDO benchmark.

This pattern was examined more closely to evaluate whether WCHLA recommendations might need to be adjusted by stream watershed size. The wadeable stream data were first subdivided using the watershed area - WCHLA regression tree analysis split of 291 mi^2 watershed area previously noted in Section 4.2. A least square linear regression of the partitioned WCHLA and watershed area data resulted in moderately strong relationships among sites meeting or exceeding the 5 mg/L AVGMINDO benchmark for wadeable streams (Figure 67; r^2 = 0.26, 0.28). As the graph shows, there were several instances of sites failing to meet the 5 mg/L AVGMINDO benchmark when WCHLA was near or below the 15 ug/L benchmark. These cases primarily occurred at sites having watershed area of approximately 100 mi^2 or less.

From the graph, it appeared the regression line for sites achieving the AVGMINDO benchmark and having watershed area < 291 mi^2 could serve as a means to adjust the WCHLA benchmark downward in order to minimize the risk of low DO. The occurrence of substandard DO levels was limited to one site when WCHLA levels were below the regression line. Therefore, it might be possible to use the regression equation to obtain a graduated scale in WCHLA levels. For example WCHLA levels of 4.4, 9.8, and 14.2 ug/L were calculated for watershed areas of 10, 100, and 291 mi^2, respectively.

It appears a graduated scale for WCHLA might be appropriate for small-medium streams to protect aquatic communities from low DO. Likewise, an argument can be made for adjusting the WCHLA benchmark upward for medium-large wadeable streams since low DO is a smaller risk in steams of this size. By applying the applicable regression equation, for example, WCHLA levels of 17.3, 28.2, and 39.0 ug/L were calculated for stream watershed areas of 300, 500, and 700 mi^2, respectively.

In light of other analysis findings, however, an upward adjustment of the WCHLA benchmark might not be justifiable. For example, optimal BMIBI scores were not observed in wadeable streams when WCHLA levels exceeded approximately 20 ug/L. Also, as previously noted blooms of phytoplankton are noticeable according to some guidelines at approximately 20 ug/L and can be considered a nuisance at 30 ug/L. Furthermore, preliminary data from Iowa streams suggest that phytoplankton blooms in the range of 20-30 ug/L or higher are more likely to be dominated by Cyanobacteria. In addition to other water quality concerns, the initial analysis here within suggested adverse changes in stream benthic macroinvertebrate assemblages can occur at elevated levels in Cyanobacteria biomass.

Relationships between WCHLA and fish IBI metrics were inconsistent (Table 37) and do not help to bring criteria recommendations into focus. As discussed earlier, for unknown reasons WCHLA breakpoint analysis results were often opposite of expected in relationships with FIBI metrics. There was evidence to suggest a correlation between WCHLA and the stream habitat quality index could be masking the true relationship between WCHLA and FIBI metrics. FIBI levels in random sites overall were lower than reference site FIBI levels. Data bias toward lower scores also might have hindered the ability to discern consistent relationships between WCHLA and FIBI metrics.

After taking into consideration the previously discussed findings and literature review, the following seston chlorophyll A criteria are recommended. The criteria will protect stream aquatic communities from stressful dissolved oxygen levels and prevent trophic (food web) alterations that negatively impact benthic macroinvertebrate taxa richness and balance. The criteria recommendations are also compatible with the goal of avoiding nuisance algal blooms that are dominated by Cyanobacteria.

The recommended levels for Iowa wadeable streams are lower than the proposed criterion (35 ug/L) for Minnesota rivers in the South Nutrient Region (SNR) (Table 65). However, the proposed SNR criterion reportedly was developed mostly using data from medium-to-large wadeable and nonwadeable streams of 4th Strahler order and higher (Heiskary et al. 2013). The proposed criteria for wadeable streams with watershed area < 100 mi^2 are similar to the EPA Region VII RTAG benchmark (8 ug/L), which was derived largely from wadeable stream data.

Seston algal chlorophyll A recommended criteria for wadeable, warmwater streams.

Watershed Area (mi^2)	WCHLA (ug/L)
<25	5
25-100	10
>100-300	15
>300-700	20

Dissolved oxygen

Daily fluctuations in dissolved oxygen were linked to stream metabolic processes, which, in turn, were related to algal biomass and nutrient parameters. The inter-relationships of these parameters provide evidence of a complete nutrient response pathway connecting nutrient enrichment with impairment of Iowa's stream aquatic communities.

Average diel dissolved oxygen minima (AVGMINDO) CP and RT breakpoint levels ranged from 4.1-6.1 mg/L in relationships with the benthic macroinvertebrate assemblage metrics and the composite (BMIBI) index score. AVGMINDO was inconsistently related with fish assemblage metrics (i.e., responses in some metrics were opposite of the expected direction), and the composite index (FIBI) score was not significantly related.

The mean AVGMINDO benchmark value (5 mg/L) from the benthic macroinvertebrate response analysis is equivalent to the chronic DO criterion for significant resource warmwater streams. This correspondence suggests the existing DO criterion can also function as a nutrient enrichment criterion. The most appropriate application of the criterion would be for comparison against the average diel DO minimum concentration obtained from continuous monitoring during the summer biological sampling months.

Average diel dissolved oxygen range (AVGRNGDO) was also found to be linked with nutrient-dependent stream processes and negative impacts to the aquatic community, particularly benthic macroinvertebrates. Elevated levels of AVGRNGDO provide indirect evidence of increased of stream productivity, which can stress stream aquatic communities through changes in the food web, benthic habitat, and water quality.

However, the analysis results from this study did not conclusively determine that negative impacts on Iowa stream biota could be attributed to AVGRNGDO alone because large fluctuations were also linked with increased occurrences of substandard minimum DO. As such, the AVGRNGDO benchmark is probably best suited for use in conjunction with other nutrient and nutrient response benchmarks, and not as a stand-alone indicator of nutrient impairment. The protocol used to monitor and assess AVGMINDO should also be suitable for AVGRNGDO.

The 5 mg/L AVGRNGDO benchmark is similar to Minnesota's proposed DO flux criterion (4.5 mg/L) for the South Nutrient Region. The Minnesota criterion reportedly was developed on the basis of correlation analysis and threshold response analysis of benthic macroinvertebrate and fish assemblage metrics. The Minnesota study also reports a high level of correlation (r^2 =0.94) between DO flux and DO minima (Heiskary et al. 2013; Table 16). In their extensive literature review, the authors note that while there is a general belief that large DO fluctuations are harmful to stream aquatic communities, field research studies have generally not been able to show the specific causal mechanism or isolate DO effects from other stressors.

<u>Benthic chlorophyll A</u>

The interpretation of relationships between benthic algal chlorophyll A and biological assemblage indicators was not straightforward. Further monitoring and analysis work is needed before definitive recommendations for benthic chlorophyll A can be made. The preliminary data analysis results suggested that biological assemblage responses to other environmental variables (e.g., dissolved oxygen, physical habitat) might be obscuring aquatic community relationships with benthic chlorophyll A.

Despite these inconsistencies, the analysis results did suggest that elevated levels of benthic chlorophyll A, roughly near 110 mg/m^2 for periphytic algae (PCHLA) and 80 mg/m^2 for fine sediment-associated algae (SCHLA), might negatively impact certain aspects of stream benthic macroinvertebrate assemblage condition. Also, optimal scores in the FIBI ($\geq$ 71) were not observed when PCHLA exceeded 160 mg/m^2. Additional data are being gathered for a more rigorous examination for biological assemblage responses to benthic chlorophyll A.

For comparison, the proposed statewide criterion for stream benthic chlorophyll A is 150 mg/m^2 (Heiskary et al. 2013) designed to prevent formation of nuisance blooms of green alga (*Cladophera* sp.) that interfere with stream recreational uses. The Region VII RTAG draft benchmark was 40 mg/m^2 reportedly based on a consideration of multiple evidence lines including thresholds for benthic macroinvertebrate diversity. The U.S. EPA 2000 recommended benchmark for Aggregate Nutrient Region IX encompassing South-central and Southeast Iowa was 20.35 mg/m^2 derived from the 25th percentile of available data.

Periphyton areal coverage of rock substrates

Periphyton is algae that grow attached to submersed coarse substrates such as rocks or wood. The species composition and biomass of periphyton is influential in determining the composition of benthic macroinvertebrates living on coarse substrates in streams. Alternatively, benthic macroinvertebrate grazers can also play an important role in regulating periphyton growth. Dense growth of filamentous benthic algae is considered aesthetically undesirable and a nuisance from the standpoint of interfering with stream recreation (Dodds 1998; Heiskary et al. 2013; Suplee et al. 2009).

Visual observations of periphyton coverage are recorded during the collection of benthic macroinvertebrate samples. Areal coverage of sampling substrates (rock or wood) is rated using percentile ranges (<25%, light; 25-50%, moderate; >50-75%, moderately heavy; >75%, heavy). Data analysis results indicated that levels of the BMIBI and several component metrics were reduced in samples from rock substrates evaluated as heavily covered by filamentous algae. Trained sampling personnel have demonstrated consistency in recognizing this specific condition.

In order to fully protect benthic macroinvertebrate assemblages from the negative impacts of dense periphytic algal growth, it is recommended that filamentous algae coverage on rock substrates should not exceed a level rated as moderately heavy (i.e., 50-75%). Given the observational assessment basis, the most appropriate application of the periphyton coverage benchmark is as a diagnostic indicator for evaluation of nutrient enrichment impacts on stream benthic macroinvertebrate assemblages.

Nitrogen

Total Kjeldahl Nitrogen (TKN), the recommended nitrogen nutrient criterion parameter, is the parameter that correlated directionally consistent and relatively strongly with seston chlorophyll A and dissolved oxygen response variables. TKN is dominated by organic nitrogen since total ammonia nitrogen levels usually occur at levels that are below the detection limit of 0.05 mg/L. In Iowa streams, Total Nitrogen (TN) is dominated by Nitrate-nitrogen (NOx), which typically comprises two-thirds or more of TN. Chlorophyll A and dissolved oxygen nutrient response variables were inversely correlated with TN and NOx. The stoichiometric analysis of nutrient data suggested that total nitrogen is rarely limiting and less likely than phosphorus to limit stream productivity. Environmental factors such as light availability, hydrology and sediment composition are believed more likely to limit primary production in agriculturally dominated watersheds of the Midwest.

TN and NOx sampling data will continue to be needed as part of a comprehensive stream nutrient evaluation and they are essential for nutrient load estimations and tracking progress toward nitrogen reduction goals. However, these parameters are not as useful as TKN as indicators of stream trophic status or nutrient enrichment impacts to stream aquatic communities. As indicated earlier, the Minnesota Pollution Control Agency (MPCA) recently developed draft acute and chronic nitrate criteria for protection of aquatic life (Monson 2010); however, the agency has yet to initiate water quality standards rule-making. Moving forward, the Iowa DNR will need to monitor this initiative and other research that might provide a basis and demonstrate the need for establishment of nitrate criteria for the protection of aquatic life.

The recommended TKN criterion is 0.80 mg/L, which represents the average of four analysis results ranging from 0.53 – 1.11 mg/L. CP changepoints of 0.86 and 1.11 correspond with increased frequencies of occurrence of substandard DO concentrations (< 5 mg/L) and seston chlorophyll concentration exceeding 15 ug/L, respectively. The 90% quantile regression (QR) threshold of 0.53 mg/L corresponds with an estimated 10% or less frequency of exceeding the 15 mg/L WCHLA benchmark. The fourth TKN benchmark value is 0.69 mg/L, which is the statewide median concentration among warmwater wadeable stream reference sites sampled from 2002-2006.

The proposed criterion will protect against the common occurrence of nuisance algal blooms ranging from 20-30 ug/L or higher. In REMAP random site sampling, 92% of sites having average TKN less than or equal to the criterion had average WCHLA levels below the nuisance algal bloom threshold compared with only 45% below the WCHLA nuisance threshold for sites that exceeded the recommended TKN criterion.

The statewide median TKN concentration among warmwater wadeable stream reference sites sampled from 2002-2006 was 0.69 mg/L. The reference TKN 50th percentile value of 0.69 mg/L was also equivalent to the median concentration of REMAP randomly selected sites having an average seston chlorophyll concentration less than or equal to the 15 ug/L threshold. The median value for REMAP sites exceeding the 15 mg/L threshold is 1.14 mg/L. Similarly, the median TKN concentration among random sites in which the 5 mg/L DO criterion was achieved was 0.70 mg/L compared with 1.20 mg/L TKN among sites having an average diel DO minima not achieving the criterion.

TKN has not been proposed as a nutrient criterion parameter by states surrounding Iowa. However, nutrient-biological assemblage analysis of data from Wisconsin wadeable streams can provide additional perspective. TKN breakpoints reported by Robertson et al. (2006) from Regression Tree analysis of biological indicator responses include: benthic chlorophyll A (0.31 mg/L), benthic macroinvertebrate metrics (0.985 mg/L), diatom nutrient index (0.745 mg/L), and fish (0.539 mg/L). In a subsequent related study, Wang et al. 2007 report multiple Regression Tree and Kolmogorov-Smirnoff breakpoints that average 0.644 mg/L (TKN) in relationships with benthic macroinvertebrate and fish assemblage metrics (Table 65).

<u>Phosphorus</u>

Total Phosphorus (TP) is the phosphorus criterion parameter recommended for Iowa's warmwater wadeable streams. TP provided slightly better correlation coefficients in relationships with the nutrient response variables, WCHLA and average diel minimum dissolved oxygen (AVGMINDO) in comparison to dissolved orthophosphate phosphorus (DOP). DOP levels are thought to be more temporally dynamic than TP, which is believed to be a more representative indicator of stream productivity in the long-term. Used in conjunction with TP, however, DOP is a useful diagnostic indicator that can help in the evaluation of bio-available phosphorus and the degree to which phosphorus availability may be limiting stream productivity.

The recommended TP criterion is 0.10 mg/L, which represents the average of four analysis results ranging from 0.06-0.12 mg/L. CP changepoints of 0.12 and 0.10 mg/L correspond with increased frequencies of occurrence of substandard DO concentrations (< 5 mg/L) and seston chlorophyll concentration exceeding 15 ug/L, respectively. The 90% quantile regression (QR) threshold of 0.06 mg/L corresponds with an estimated 10% or less frequency of occurrence in exceeding the 15 mg/L WCHLA benchmark. The fourth benchmark value is 0.13 mg/L, which is the statewide median TP concentration among warmwater wadeable stream reference sites sampled from 2002-2006.

The proposed criterion will protect against the common occurrence of nuisance algal blooms ranging from 20-30 ug/L or higher. In REMAP random site sampling, 87% of sites having average TP less than or equal to the criterion had average WCHLA levels below the nuisance algal bloom threshold with a maximum of 53 ug/L compared with 62% below the WCHLA nuisance threshold and a maximum of 360 ug/L for sites that exceeded the recommended TP criterion. The recommended TP criterion is also consistent with modeling results from Minnesota Rivers reported by Carleton et al. (2009). This study found a breakpoint of 0.10 mg/L TP in the multivariate regression relationship between Cyanobacteria abundance and TN, TP, and TSS. Above this breakpoint, the model indicated %Cyanobacteria rapidly increases.

The statewide median TP concentration among warmwater wadeable stream reference sites sampled from 2002-2006 was 0.13 mg/L. For additional perspective, reference site values were compared with random site values representing sites with nutrient response variables achieving or not achieving benchmark values. The reference site 50th percentile value of 0.13 mg/L happens to be similar to the median concentration (0.135 mg/L) for REMAP randomly selected sites having an average seston chlorophyll concentration less than or equal to the 15 ug/L WCHLA threshold. In comparison, TP was 0.22 mg/L for random sites exceeding the WCHLA threshold. The median TP concentration among random sites in which the 5 mg/L DO criterion was achieved was 0.16 mg/L compared with 0.23 mg/L for sites having an average diel DO minima not achieving the criterion.

Several regional and state TP benchmark/criteria values are available for comparison with the TP benchmark recommended for Iowa wadeable, warmwater streams (Table 65). While informative, it is hard to directly compare any of the values against the Iowa-specific benchmark because of differences in derivation approaches and regional variation in stream characteristics.

The approach taken by Heiskary et al. 2013 in deriving criteria recommendations for Minnesota rivers is perhaps the closest match because it involved examining multiple lines of evidence including results from breakpoint/threshold analysis of benthic macroinvertebrates and fish metrics and regression modeling of nutrient-nutrient response relationships. The proposed TP criterion for rivers in the Southern Nutrient Region (SNR) is 150 ug/L is higher than the proposed criterion for Iowa wadeable, warmwater streams; however, the criterion was derived using mostly data from medium and large streams of 4th Strahler stream order or higher. A higher seston chlorophyll A target (35 ug/L) was used in deriving the SNR criterion for TP compared to the proposed seston chlorophyll A criteria for Iowa wadeable, warmwater streams, so it is understandable that the Minnesota TP criterion would also be higher. The proposed criterion for the Central Nutrient Region (CNR) (100 ug/L) is the same as the proposed Iowa criterion and the proposed CNR seston chlorophyll A criterion (18 ug/L) is within the range (i.e., 5-20 ug/L) of the proposed criteria for Iowa's wadeable, warmwater streams.

The proposed TP criterion for Iowa wadeable, warmwater streams is equivalent to Wisconsin's TP criterion for 46 named river segments (100 ug/) and higher than the criterion for all other designated streams (75 ug/L) (Table 65). The draft TP benchmark from the Region VII RTAG data analysis and Missouri's draft criterion for Stream Nutrient Criteria Zone I that shares common watersheds with Iowa also happen to be 75 ug/L. Should Missouri proceed to adopt the 75 ug/L TP criterion as a water quality standard and Iowa adopts the proposed 0.10 mg/L criterion, there are likely to be cases in which streams meet Iowa's TP standard, but violate Missouri's standard simply by flowing across the state line. Of course, such circumstances are to be avoided by taking downstream effects into consideration in setting appropriate water quality standards criteria.

<u>Summary of data analysis benchmarks for nutrient and nutrient-response parameters</u>

Recommended nutrient and nutrient response parameter benchmarks obtained from the consolidation and synthesis process are listed in Table 66.

Table 66. Data analysis benchmarks for wadeable, warmwater stream nutrient and nutrient-response parameters.

Parameter	Benchmark
Total Kjeldahl Nitrogen (TKN)	$\leq$ 0.80 mg/L
Total Phosphorus (TP)	$\leq$ 0.10 mg/L
Dissolved Oxygen Diel Minima (AVGMINDO)	$\geq$ 5 mg/L
Dissolved Oxygen Diel Range (AVGRNGDO)	$\leq$ 5 mg/L
Seston Algal Chlorophyll A (WCHLA)	$\leq$ 5.0 (Watershed Area $\geq$10-25 mi^2) $\leq$ 10.0 (WA >25-100 mi^2) $\leq$ 15.0 (WA >100-300 mi^2) $\leq$ 20.0 (WA >300-700 mi^2)
Filamentous Algae Coverage (FAC)	$\leq$ 50-75% Rock Substrate Surface Area

Next, a contingency analysis was conducted to determine the frequencies of different outcomes or nutrient assessment scenarios resulting from a comparison of REMAP sampling data against the recommended benchmark values. The analysis looked at the frequency of occurrence of 32 unique combinations of pass/fail comparisons involving five benchmarks (TKN, TP, AVGDORNG, AVGMINDO, and WCHLA). The rock substrate filamentous algae coverage (FAC) benchmark was not used in the analysis because it was not evaluated at many of the REMAP sites.

The results of the contingency analysis are shown in Table 67. The number and percentage of sample site occurrences in each combination of nutrient benchmark pass/fail comparisons is reported along with the percent occurrence of cases in which the BMIBI and FIBI scores fail to attain the statewide 25th percentile score for wadeable reference sites. As indicated earlier, the 25th percentile values historically have been used to represent thresholds at which aquatic life condition might be considered impaired.

A total of 100 sites from wadeable, warmwater streams had complete data for the suite of nutrient and nutrient-response indicators (excluding rock substrate filamentous algae coverage rating). Twenty of the thirty-two possible scenarios contained at least one case (i.e., sample data representing a site). Seven scenarios accounted for 82% of the cases, four of which (#'s 1, 8, 16, 24) encompassed two-thirds of the total. The most common nutrient scenarios were given a closer examination.

12% of the sample sites failed to meet any of the five nutrient and nutrient-response benchmarks (#1), while at the opposite end of the spectrum, 16% of sample sites passed all five benchmarks (#32). For sites failing to meet all five nutrient enrichment benchmarks, the percentages of sites that also failed to attain the BMIBI and FIBI benchmarks was 75% and 75%, respectively, compared with 31.3% and 25%, respectively, for sites that attained all nutrient benchmarks. The differences in BMIBI and FIBI attainment percentages between the groups were statistically significant [p-value: 0.0042 (BMIBI); 0.0035 (FIBI)]. Among all sites, the failure percentages for the BMIBI and FIBI were 45% and 59%, respectively.

There was a high level of agreement in BMIBI and FIBI outcomes (83.3%) for sites failing to meet all five nutrient enrichment benchmarks. The level of agreement in attainment status of the BMIBI and FIBI thresholds for this group was also fairly strong (68.8%). The overall level of agreement among all sites was 60%.

A sizeable percentage of sites (39%) failed to meet either or both of the TP and TKN benchmarks, and yet passed all three nutrient response thresholds (#'s 8, 16, 24). Most commonly, only the TP benchmark failed (# 8, 24%), or both the TP and TKN benchmarks failed (#16, 14%). These would seem to be cases where nutrient levels are elevated yet the nutrient response pathways are decoupled or not evident.

The BMIBI failure percentage among combined sites from these two groups was 26.3%, which was significantly different than the 71% failure rate for the FIBI (Chi-square p-value <0.001). These results support the findings from the nutrient stressor-response analysis, which indicated a stronger and more consistent correspondence between nutrient response variables and BMIBI metrics compared with nutrient stressor - FIBI relationships. Judging by the elevated level of FIBI benchmark failure in cases where only TP and/or TKN levels fail to attain the benchmarks, it would appear that one or more other stressors that are correlated with TP an TKN could be impacting the fish assemblage to a greater extent than the benthic macroinvertebrate assemblage. As previous analyses have shown, TP and TKN are correlated with total suspended solids (TSS) levels and stream habitat evaluation scores. It has also been demonstrated that the FIBI is more strongly correlated with these variables than is the BMIBI.

Going forward, it is likely that stream monitoring projects will include TKN, TP, and WCHLA, but they may not include continuous diel DO monitoring since this requires special equipment and extra costs associated with equipment deployment and maintenance. Therefore, it is useful to consider the frequency of BMIBI failure in cases when just TKN, TP, WCHLA data are available to compare against applicable benchmarks.

In cases in which only TP and TKN monitoring data are available, the REMAP contingency table indicates that both benchmarks would fail to be attained at 49% sites drawn from a random sample of wadeable, warmwater streams. The BMIBI failure rate among this group (53.1%) ranked higher, but was not significantly different than the failure rate (37.2%) among the group of sites at which one or both benchmarks were attained (Chi-square p-value 0.112).

Next, assuming the available monitoring data includes TP, TKN, and WCHLA, but not diel DO monitoring data, the percentage of sites in Table 67 that fail to attain each of these benchmarks is 28% of the total. The BMIBI failure rate among sites in this group (64.3%) was significantly greater than the failure rate (37.5%) among sites at which one or more of the benchmarks was attained (Chi-square p-value 0.016). The results of this analysis suggest the prediction accuracy for nutrient-related aquatic life impairments can be improved significantly by always including seston chlorophyll A among the suite of water quality variables monitored in warmwater streams.

Table 67. Nutrient contingency table showing all possible combinations of outcomes obtained from pass (P) / fail (F) comparisons of REMAP (2002-2006) wadeable, warmwater stream site average values against applicable nutrient / nutrient-response parameter benchmarks in Table 66.

		Nutrient / Nutrient Response					Biological Assemblage Index		
Nutrient Scenario	No. Cases	TP	TKN	WCHLA	D.O. RNG	D.O. MIN	BMIBI (% fail)	FIBI (% fail)	BMIBI / FIBI (% agree)
1	12	F	F	F	F	F	75.0%	75.0%	83.3%
2	6	F	F	F	F	P	66.7%	66.7%	66.7%
3	4	F	F	F	P	F	75.0%	50.0%	75.0%
4	6	F	F	F	P	P	33.3%	50.0%	83.3%
5	3	F	F	P	F	F	66.7%	66.7%	100.0%
6	3	F	F	P	F	P	33.3%	100.0%	33.3%
7	1	F	F	P	P	F	100.0%	0.0%	0.0%
8	14	F	F	P	P	P	28.6%	85.7%	42.9%
9		F	P	F	F	F			
10	1	F	P	F	F	P	0.0%	0.0%	100.0%
11		F	P	F	P	F			
12	1	F	P	F	P	P	0.0%	100.0%	0.0%
13		F	P	P	F	F			
14	2	F	P	P	F	P	100.0%	50.0%	50.0%
15		F	P	P	P	F			
16	24	F	P	P	P	P	25.0%	62.5%	45.8%
17		P	F	F	F	F			
18		P	F	F	F	P			
19	1	P	F	F	P	F	100.0%	100.0%	100.0%
20		P	F	F	P	P			
21		P	F	P	F	F			
22	1	P	F	P	F	P	100.0%	100.0%	100.0%
23		P	F	P	P	F			
24	1	P	F	P	P	P	100.0%	0.0%	0.0%
25	1	P	P	F	F	F	100.0%	100.0%	100.0%
26	1	P	P	F	F	P	0.0%	0.0%	100.0%
27		P	P	F	P	F			
28		P	P	F	P	P			
29		P	P	P	F	F			
30	1	P	P	P	F	P	100.0%	0.0%	0.0%
31	1	P	P	P	P	F	100.0%	0.0%	0.0%
32	16	P	P	P	P	P	31.3%	25.0%	68.8%
Totals	100						45.0%	59.0%	60.0%

Large wadeable streams and nonwadeable rivers

Nutrient criteria recommendations for large wadeable / nonwadeable streams (i.e., watershed area >700mi^2) should be postponed until additional monitoring and data analysis plans have been completed. Two main obstacles make it impractical to formulate criteria recommendations at this time. The first is insufficient data. New datasets consisting of integrated sampling data for nutrients, nutrient response variables, and biological assemblages are needed. The second obstacle is a lack of quantitatively defined biological reference conditions representing least disturbed conditions for large streams. Additional monitoring to address these deficiencies is being conducted as part of the Iowa DNR's ambient monitoring program. The new data, combined with existing data, will provide a stronger foundation for nutrient stressor-biological response data analysis.

Despite current data deficiencies, the preliminary statistical analysis did reveal some noteworthy patterns. For example, seston chlorophyll A (WCHLA) breakpoint analysis results for large wadeable-nonwadeable streams tended to be higher than WCHLA breakpoint results from small-medium wadeable streams. Possible reasons include the more compressed data ranges for nutrient response and biological index variables, and the apparent reduced risk of substandard dissolved oxygen levels in large streams. Correlation analysis of phytoplankton composition variables (e.g., Cyanobacteria % abundance and biomass) and benthic macroinvertebrate IBI metrics suggested potentially important linkages that will be pursued more completely using new monitoring data from the 2012 and 2013 field seasons.

Coldwater streams

The analysis of REMAP sampling data found significant differences in nutrient levels among ecoregion and thermal classes. As a group, coldwater stream sites within the Paleozoic Plateau ecoregion (52b) showed some of the most distinct nutrient characteristics. Sampling data collected from coldwater reference stream sites provided additional perspective on nutrient conditions in least-disturbed stream habitats that support relatively healthy biological assemblages.

Data limitations prevented the use of stressor-response analysis methods like those used to identify benchmark criteria values for wadeable warmwater streams. In lieu of these methods, a reference condition comparison approach was used to identify preliminary benchmark values for nutrient and nutrient-response variables (Table 68).

With the exception of filamentous algae (rock substrate) coverage (FAC), benchmark values were derived from the 75th percentile values from coldwater reference stream site monitoring. The FAC benchmark was derived from an analysis of targeted and random coldwater stream sampling data collected for a variety of purposes under the Iowa DNR stream bioassessment program.

Table 68. Coldwater stream data analysis benchmarks.

Parameter	Benchmark
Total Kjeldahl Nitrogen (TKN)	$\leq$ 0.16
Total Phosphorus (TP)	$\leq$ 0.08
Filamentous Algae Coverage	$\leq$ 50%
Periphyton Algal Chlorophyll A	$\leq$ 15.0
Sediment Algal Chlorophyll A	$\leq$ 7.5
Seston Algal Chlorophyll A	$\leq$ 3.0

The limited amount of continuous dissolved oxygen monitoring data obtained from thirteen random sites did suggest that DO minima could be an important nutrient-related stressor in coldwater streams. Violations of the 7 mg/L, 16-hour dissolved oxygen criterion were observed at several sites. Coldwater benthic index (CBI) scores at these sites were among the lowest of random stream sites, and below the reference 25th percentile score used as a threshold to identify aquatic life use impairment. Correlation analysis and examination of stressor-response bivariate plots did not provide a clear picture of relationships between nutrient variables, dissolved oxygen variables and benthic macroinvertebrate assemblage metrics. Additional nutrient-related monitoring of coldwater streams including continuous DO monitoring should continue as a priority to provide a larger dataset for stressor-response statistical analysis.

8.3. Recommendations

Status of recommendations

The implementation of nutrient criteria will require appropriately matching the criteria with stream aquatic life use designations in Iowa's water quality standards (Iowa Administrative Code Ch. 567.61). Table 69 lists the current stream aquatic life use designations and provides a brief status description of criteria recommendations. Recommendations are available for coldwater, B(CW1) streams and warmwater, wadeable B(WW1) and B(WW2) streams. For nutrient criteria purposes, a subdivision of the WW1 warmwater stream aquatic life use based on watershed size is recommended. This subdivision is necessary to distinguish small to medium size wadeable streams from large wadeable streams and nonwadeable rivers, for which separate criteria recommendations have been issued.

Table 69. Status of nutrient criteria recommendations for stream aquatic life use designations in the Iowa Water Quality Standards (IAC 2012).

Stream designation (and applicable watershed size)	Status of criteria recommendations
B(CW1) - Coldwater streams supporting trout and associated aquatic community	Preliminary criteria recommendations available
B(CW2) - Coldwater, spring runs not capable of supporting trout	Criteria recommendations currently unavailable (insufficient data); Designated use currently not populated with waterbodies
B(WW1) - Warmwater, large wadeable streams and nonwadeable rivers (watershed area >700 mi^2)	Criteria recommendations currently unavailable (insufficient data, biological reference condition unavailable)
B(WW1) - Warmwater, medium-large wadeable streams (WA $\leq$700 mi^2)	Criteria recommendations available
B(WW2) - Warmwater, small perennial streams (WA $\geq$10 mi^2)	Criteria recommendations available
B(WW2) - Warmwater, small perennial streams (WA <10 mi^2)	Criteria recommendations currently unavailable (insufficient data, biological reference condition unavailable)
B(WW3) - Warmwater, intermittent flowing streams with perennial pools	Criteria recommendations currently unavailable (insufficient data, biological reference condition unavailable)

Sufficient nutrient data and biological reference conditions are not available to formulate draft recommendations for B(WW-1) large wadeable or nonwadeable streams of watershed area greater than 700 mi^2, small perennial streams of watershed area less than 10 mi^2, and intermittent, perennial pooled B(WW3) streams. Currently, there are no classified stream segments or nutrient sampling data representing the coldwater B(CW2) stream designation.

Criteria recommendations

Stream nutrient enrichment criteria recommendations for B(CW1) coldwater streams and wadeable B(WW-1) and B(WW-2) warmwater streams of watershed area 10-700 mi^2 are listed in Table 70. The recommendations identify a suite of nutrient and nutrient response parameters, the acceptable concentrations or ratings, and the date interval in which the benchmarks are applicable.

Table 70. Stream nutrient enrichment criteria recommendations for B(CW1) coldwater streams and wadeable B(WW-1) and B(WW-2) streams.

Stream Designation	Parameter	Acceptable Range	Interval
B(CW1)	Total Kjeldahl Nitrogen	Median sample value ≤ 0.16 mg/L	June 15 – Oct. 15
	Total Phosphorus	Median sample value ≤ 0.08 mg/L	June 15 – Oct. 15
	Filamentous Algae Coverage Rating	Median rating ≤ 2 (25-50%)	June 15 – Oct. 15
	Periphyton Algal Chlorophyll A	Median sample value ≤ 15.0 µg/cm^2	June 15 – Oct. 15
	Sediment Algal Chlorophyll A	Median sample value ≤ 7.5 µg/cm^2	June 15 – Oct. 15
	Seston Algal Chlorophyll A	Median sample value ≤ 3.0	June 15 – Oct. 15
Wadeable, B(WW1), B(WW2), (Watershed Area 10- 700 mi^2)	Total Kjeldahl Nitrogen (TKN)	Median sample value ≤ 0.80 mg/L	June 15 – Oct. 15
	Total Phosphorus (TP)	Median sample value ≤ 0.10 mg/L	June 15 – Oct. 15
	Dissolved Oxygen Diel Range	Median daily range (maxima-minima) ≤ 5 mg/L	July 1 – Sept. 15
	Filamentous Algae Coverage Rating	Median rating ≤ 3 (50-75%)	June 15 – Oct. 15
	Seston Algal Chlorophyll A	Median sample value: ≤ 5.0 µg/L (Watershed Area ≥10-25 mi^2) ≤ 10.0 µg/L (WA >25-100 mi^2) ≤ 15.0 µg/L (WA >100-300 mi^2) ≤ 20.0 µg/L (WA >300-700 mi^2)	June 15 – Oct. 15

The data analysis benchmark for average diel DO minima (5 mg/L) is not listed in Table 70. This benchmark happens to be equivalent to the 16-hour DO criterion for warmwater B(WW1) and B(WW2) streams (Table 71). Monitoring and assessment of diel DO minima and flux needs to continue as an integral part of stream nutrient status assessments; however, there is no need to establish a new diel DO minima benchmark as a nutrient enrichment criterion since it would duplicate the existing DO criterion which serves the same purpose.

The median (50^{th} percentile) sample value is recommended for comparison to the applicable nutrient enrichment criterion. Given the relatively small number of samples and non-normal distribution of data that typify previously obtained datasets, the sample median is a more appropriate indicator of central tendency than the sample mean. Sampling data collected in previous Stressor Identification (SI) projects illustrate the highly variable and non-normal data distributions characterizing nutrient and chlorophyll A parameters (see Appendix 17a-k). The parameter data are usually positively skewed by high outlier values which inflate the sample mean relative to the median. Non-normal data distribution is also often a consequence of small sample size. The number of samples collected at SI stream sites has been relatively small (i.e., $\leq$ 25 during the summer-early fall bioindex period). Using the sample median instead of the sample mean will help to avoid any statistical analysis issues associated with asymmetric data (Helsel and Hirsch 2002).

July 1 – October 15 is the recommended time interval to evaluate attainment of the proposed nutrient enrichment criteria. This interval closely coincides with the wadeable stream biological assessment index period and also the period in which the majority of the REMAP sampling project data were collected. REMAP sampling data were the primary data from which nutrient data analysis benchmarks were derived. Iowa stream flow conditions tend to stabilize and warm temperatures during summer and early fall are conducive to expression of nutrient impacts.

Table 71. Dissolved oxygen criteria for protecting designated aquatic life uses as specified in the Iowa Water Quality Standards (IAC 2012).

	B(CW1)	**B(CW2)**	**B(WW-1)**	**B(WW-2)**	**B(WW-3)**	**B(LW)**
	Coldwater streams		*Warmwater streams/rivers*			*Lake/wetland*
Minimum for 16 hours of a 24-hour period	7.0	7.0	5.0	5.0	5.0	5.0*
Minimum during a 24-hour period	5.0	5.0	5.0	4.0	4.0	5.0*

* applies only to the upper layer of stratification in lakes

Monitoring and assessment recommendations

Outlined below are general guidelines for nutrient criteria-related monitoring and assessment. Specific monitoring details will need to be tailored to site-specific circumstances and objectives. Monitoring guidelines are offered for two basic monitoring approaches: nutrient status monitoring and nutrient impairment confirmation.

Nutrient status monitoring can be thought of as entry-level monitoring designed to characterize nutrient conditions and identify streams at risk of aquatic life impairment due to nutrient over-enrichment. Nutrient impairment confirmation monitoring incorporates the basic elements of status monitoring, but also includes additional components such as continuous diel dissolved oxygen monitoring, periphyton sampling, and biological assemblage sampling. Nutrient impairment confirmation monitoring may naturally follow nutrient status monitoring, but does not have to be preceded by status monitoring if other available information suggests the presence of a nutrient impairment.

Nutrient status monitoring

- Monitoring conducted at least one year in the most recent five-year period
- Samples collected monthly or biweekly June 15 – October 15 (monitoring outside of nutrient index period is optional)
- Minimum of 10 samples collected during base flow conditions (samples from storm runoff events or extreme low flow periods are excluded)
- Nutrient and water quality parameters:
  - Temperature, pH, DO, Flow, Specific Conductance, Turbidity, TSS
  - NHx, NOx, TKN, DOP, TP
  - WCHLA, PCHLA (coldwater streams), SCHLA (coldwater streams)
  - Additional nutrient & water quality parameters (optional)

Nutrient impairment confirmation

- Monitoring conducted at least two years of most recent five-year period
- Nutrient and water quality samples collected biweekly or more frequently between June 15 and October 15 (monitoring outside of nutrient index period is optional)
- Minimum of 8 nutrient and water quality samples collected during base flow conditions (samples from storm runoff events or extreme low flow periods are excluded)
- Nutrient and water quality parameters:
  - Temperature, pH, DO, Flow, Specific Conductance, Turbidity, TSS
  - NHx, NOx, TKN, DOP, TP
  - WCHLA, PCHLA, SCHLA, Periphyton coverage and amount ratings
  - Additional nutrient & water quality parameters (optional)
- Diel dissolved oxygen and water temperature
  - July 1 – September 15 deployment interval
  - Minimum total of four weeks diel monitoring; minimum two-week consecutive deployment interval
  - Reading taken ≤15 minutes apart
- Biological assemblage (Index of Biotic Integrity - IBI)
  - Sampling conducted within applicable biological index (generally July–October)
  - Benthic macroinvertebrate IBI
  - Fish IBI (typically included for complete bioassessment; optional for nutrient assessment)

Assessment guidelines

Pursuant to Section 305(b) and Section 303(d) of the Clean Water Act, the IDNR is required to assess and report on the condition of water quality in Iowa and the degree to which designated beneficial water uses are supported. The following guidelines are offered as a framework for utilizing nutrient and nutrient response parameter sampling data in the 305(b)/303(d) Integrated Report.

As demonstrated in this project and elsewhere in the Midwest Corn Belt, the occurrence of excess levels of total nitrogen and total phosphorus are often inaccurate predictors of ecosystem responses that adversely impact stream biological assemblages. Reliance on numeric nutrient criteria for TN and TP as the sole basis for identifying nutrient-related impairment of stream aquatic life uses would likely result in numerous false positive determinations leading to water program inefficiencies and wasted financial resources. N and P criteria, therefore, are better suited for use in conjunction with nutrient response criteria for indicators such as chlorophyll A and dissolved oxygen.

As with other water quality assessments, the level of confidence in assessment of stream nutrient enrichment status and/or nutrient impairment of aquatic life uses depends on monitoring data quality and completeness. Two basic assessment confidence levels can be distinguished. The guidelines below for "evaluated" and "monitored" nutrient assessments are meant to complement existing guidelines described in the protocol for water quality assessment and reporting for the CWA Sections 305(b) and 303(d) Integrated Report (IDNR 2013).

1. Evaluated assessment – potential nutrient-related stream aquatic life use impairment:
    - Data quality and completeness requirements commensurate with "evaluated" assessments are met;
    - Failure to attain the applicable TKN criterion and/or TP criterion and at least one of the applicable nutrient response parameter criteria (e.g., seston chlorophyll A) are required to identify a potential designated stream aquatic life use impairment caused by nutrient enrichment.

2. Monitored assessment – nutrient-related stream aquatic life use impairment
    - Data quality and completeness requirements commensurate with "monitored assessments" are met;
    - Evidence of a complete nutrient stressor-response pathway is required to identify nutrient enrichment as a cause of stream aquatic life use impairment. A complete nutrient stressor-response pathway includes one or both of the following stressors:
        - Low dissolved oxygen:
            - TKN and/or TP criteria exceeded;
            - At least one nutrient response parameter criterion not attained (e.g., seston chlorophyll A, diel DO range);
            - Diel DO minima not attaining applicable 16 hour and/or 24 hour DO criteria.
        - Excessive sestonic or benthic algae growth:
            - TKN and/or TP criteria exceeded;
            - At least one chlorophyll A criterion not attained and/or filamentous algae coverage criterion exceeded;

- Benthic macroinvertebrate index (BMIBI) assessment criterion not attained.

Failure to attain the TKN, TP, and at least one nutrient response criterion indicates a potential nutrient-related impairment of stream aquatic life uses. In the absence of diel DO monitoring data showing water quality standards violations, biological confirmation of aquatic life use impairment is required to identify a "monitored" nutrient-related aquatic life use impairment. When sufficient DO monitoring data are available, the assessment category is automatically considered as "monitored" instead of "evaluated," even in the absence of biological assemblage monitoring results. Determination of the presence of a complete nutrient stressor-response pathway should be done by a professional aquatic biologist, limnologist, or water resource specialist familiar with Iowa water quality standards and water quality assessments.

Adoption of the recommended monitoring and assessment guidelines will provide valuable experience for the development and validation of nutrient criteria recommendations. New monitoring projects should be implemented in such a way that contributes to meeting the objectives of the state nutrient reduction strategy. For example, nutrient status and impairment confirmation monitoring could be done in concert with monitoring of nutrient loads to provide a comprehensive assessment of stream nutrient status in priority watersheds.

9. References

Anderson, J. and D. Huggins. 2003. Production calculator, version 1.5 operations manual: Draft June 4, 2003. Developed by Central Plains Center for Bioassessment, Kansas Biological Survey, University of Kansas for U.S. Environmental Pollution Control Agency.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Bender, J. 2010. Nutrient water quality criteria: Current status and future direction. Nebraska DEQ. PowerPoint Slides from Nutrient Management for Water Protection in Highly Productive Systems of the Heartland: Setting Regulatory Controls for Nutrients. June 10, 2010. http://www.ksre.ksu.edu/waterquality/2010/Presentations/Workshop/Bender.pdf

Black, R.W., Moran, P.W., and Frankforter, J.D., 2011, Response of algal metrics to nutrients and physical factors and identification of nutrient thresholds in agricultural streams: Environmental Monitoring and Assessment. 175:397- 417.

Brenden, T.O., L. Wang, and Z. Su, 2008. Quantitative identification of disturbance thresholds in support of aquatic resource management. Environ. Manage.42:821-832.

Cade, B.S. and B.R. Noon, 2003. A gentle introduction to quantile regression for ecologists. Front. Ecol. Environ. 1(8):412-420.

Carleton, J.N., R.A. Park, and J.S. Clough. 2009. Ecosystem modeling applied to nutrient criteria development in rivers. Environmental Management 44:485-492.

Chapman, S.S., Omernik, J.M., Griffith, G.E., Schroeder, W.A., Nigh, T.A., and Wilton, T.F., 2002. Ecoregions of Iowa and Missouri (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia, U.S. Geological Survey (map scale 1:1,8000,000).

Chapra, S.C. 1997. Lecture 28: The eutrophication problem and nutrients. 521-533 p. *In*: Surface Water Quality Modeling. McGraw Hill Companies, Inc.

Cloern, J.E., C. Grenz, and L. Vidergar-Lucas. 1995. An empirical model of the phytoplankton chlorophyll:carbon ratio – the conversion factor between productivity and growth rate. Limnol. Oceanogr. 40(7):1313-1321.

Czapar G.F. 2007. Illinois Council on Food and Agricultural Research final report of the Strategic Research Initiative (SRI) in water quality. University of Illinois Extension. November 16, 2007. 23p.

De'ath, G; and K.E. Fabricius. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. Ecology 81(11):3178-3192.

Dodds, W. K., V.H. Smith, and K. Lohman. 2002. Nitrogen and phosphorous relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Science, 59, 865–874.

Dodds, W.K.. and R.M. Oakes. 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnol. Oceanogr. Methods 2:333-341.

Dodds, W.K. 2007. Trophic state, eutrophication and nutrient criteria in streams. Trends in Ecology and Evolution 22(12):669-676.

Frey, J.W., A.H. Bell, J.A. Hambrook Berkman and D.L. Lorenz. 2011, Assessment of nutrient enrichment by use of algal-, invertebrate-, and fish-community attributes in wadeable streams in ecoregions surrounding the Great Lakes: U.S. Geological Survey Scientific Investigations Report 2011–5009, 49 p.

Greenberg, A.E., L.S. Clesceri, A.D. Eaton, eds. 1992. Standard Methods for Examination of Water and Wastewater. 18th ed. American Public Health Association. 2(1-10), 10(17-19).

Hauer, F.R. and G.A. Lamberti. 1996. Methods in stream ecology. Academic Press, New York, NY. 674 pp.

Heiskary, S. and H. Markus. 2003. Establishing relationships among in-stream nutrient concentrations, phytoplankton and periphyton abundance and composition, fish and macroinvertebrate indices, and biochemical oxygen demand in Minnesota USA rivers. Minnesota Pollution Control Agency. 100 p.

Heiskary, S. 2008. Relation of nutrient concentrations and biological responses in Minnesota streams: Applications for river nutrient criteria development. Minnesota Pollution Control Agency (MPCA), St. Paul, MN. 60p.

Heiskary, S. and K. Monson. 2010. Regionalization of Minnesota's rivers for application of river nutrient criteria. Minnesota Pollution Control Agency, Environmental Analysis and Outcomes Division. July 2010. 27p.

Heiskary, S., R.W. Bouchard, Jr., and H. Markus. 2013. Minnesota nutrient criteria development for rivers. Minnesota Pollution Control Agency, Environmental Analysis and Outcomes Division. July 2010. 176p.

Helsel, D.R. and R. M. Hirsch, 2002. Chapter 3 Describing Uncertainty. *In* Statistical Methods in Water Resources Techniques of Water Resources Investigations, Book 4, chapter A3. U.S. Geological Survey. 522 pages.

Herlihy, A.T. and J.T. Sifneos. 2008. Developing nutrient criteria and classification schemes for wadeable stream in the conterminous U.S. J. N. Am. Benthol. Soc., 2008, 27(4):932–948

IAC. 2012. Chapter 567-61: water quality standards. Iowa Administrative Code [effective date 05/16/2012].

IDNR 2013. Methodology for Iowa's 2012 water quality assessment, listing, and reporting pursuant to Sections 305(b) and 303(d) of the federal Clean Water Act. Attachment 2: Guidelines for determining Section 305(b) aquatic life use support (ALUS) using stream biocriteria sampling data. Iowa Department of Natural Resources, Environmental Protection Division, Geological Survey and Land Quality Bureau, Watershed Monitoring and Assessment Section.

Kaufmann, P.R. and E.G. Robison. 1998. Physical Habitat Characterization. pp 77-118 In: J.M. Lazorchak, D.J. Klemm and D.V. Peck (eds.). Environmental Monitoring and Assessment Program -- Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Office of Research and Development, Washington D.C.

Lyons, J., L. Wang, and T.D. Simonson. 1996. Development and validation of an index of an index of biotic integrity for coldwater streams in Wisconsin. North American Journal of Fisheries Management. 16. 241-256.

Monson, P. 2010. Aquatic life water quality standards technical support document for nitrate. Triennial water quality standards amendments to Minnesota R. Chs. 7050 and 7052. Draft for external review, November 12, 2010. Minnesota Pollution Control Agency, Saint Paul, MN. 20p.

Morgan, A.M., T.V. Royer, M.B. David, and L.E. Gentry. 2006. Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. J. Environ. Qual. 35:1110– 1117.

Mundahl, N.D. and T.P. Simon. 1998. Development and application of an index of biotic integrity for coldwater streams of the upper Midwestern United States, p. 383-415. In T.P. Simon (editor). Assessing the sustainability and biological integrity of water resource quality using fish assemblages. Lewis Publishers. Boca Raton, Florida.

NDEQ 2008. Nebraska Nutrient Criteria Development Plan. Nebraska Department of Environmental Quality. February 2008. http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/upload/NEBRASKA-NUTRIENT-CRITERIA-DEVELOPMENT-PLAN-February-2008.pdf

Odum, H.T. 1956. Primary production in flowing waters. Limnology and Oceanography 1(2): 102-117.

Paul and McDonald, 2005. Development of empirical geographically specific water quality criteria: A conditional probability analysis approach. Journal of American Water Resources Association 1211-1233.

Porter, S. D., Mueller, D. K., Spahr, N. E., Munn, M. D., & Dubrovsky, N. M. (2008). Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. Freshwater Biology, 53, 1036–1054.

Potapova, M. G., & Charles, D. F. (2007). Diatom metrics for monitoring eutrophication in rivers of the United States. Ecological Indicators, 7, 48–70.

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. American Scientist 46:205-221.

Robertson, D.M., D.J. Graczyk, P.J. Garrison, L. Wang, G. LaLiberte, and R. Bannerman. 2006. Nutrient Concentrations and Their Relations to the Biotic Integrity of Wadeable Streams in Wisconsin: U.S. Geological Survey Professional Paper 1722, 156 p.

Robertson, D.M., Weigel, B.M., and Graczyk, D.J. 2008. Nutrient Concentrations and their relations to the biotic integrity of nonwadeable rivers in Wisconsin: U.S. Geological Survey Professional Paper 1754, 81 p.

Rowe, D.C., C.L. Pierce, T.F. Wilton. 2009a. Fish assemblage relationships with physical habitat in wadeable Iowa streams. North American Journal of Fisheries Management 29:1314-1332.

Rowe, D.C., C.L. Pierce, T.F. Wilton. 2009b. Physical habitat and fish assemblage relationships with landscape variables at multiple spatial scales in wadeable Iowa streams. North American Journal of Fisheries Management 29:1333-1351.

Royer, T.V., M.B. David, L.E. Gentry, C.A. Mitchell, K.M. Starks, T. Heatherly II, M.R. Whiles. 2008. Assessment of Chlorophyll-a as a criterion for establishing nutrient standards in the streams and rivers of Illinois. J. Environ. Qual. 37:437-447.

Scheffer, M. 1998. Ecology of Shallow Lakes. Klewer Academic Publishers, Dordrecht, The Netherlands.

SHL 2012. Development of a coldwater benthic index in Iowa. State Hygienic Laboratory at the University of Iowa Limnology Section in cooperation with the Iowa Department of Resources. May 2012. Coralville, IA.

Smith, R.A., R.B. Alexander, G.B. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environmental Science & Technology 37(14):3039-3047.

Suplee, M.W., V. Watson, M. Teply, H. McKee. 2009. How green is too green? Public opinion of what constitutes undesirable algae levels in streams. Journal American Water Resources Association, 45(1):123-140

Stevenson, R. J., Rier, S. T., Riseng, C. M., Schultz, R. E.,& Wiley, M. J. (2006). Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia, 56, 149–165.

Steinman, A.D. and P.J. Mulholland. Chapter 9: Phosphorus limitation, uptake, and turnover in stream algae. 161-189 p. *In*: Methods in Stream Ecology. Edited by F.R. Hauer and G.A. Lamberti. Academic Press.

Swanson, C.D. and R.W. Bachmann, 1976. A model of algal exports in some Iowa streams. Ecology 57:1076-1080.

U.S. Environmental Protection Agency, 2000a, Nutrient criteria technical guidance manual—Rivers and streams: Washington, D.C., EPA–822–B00–002, 152 p. + appendices.

U.S. Environmental Protection Agency, 2000b, Ambient water quality criteria recommendations—Information supporting the development of state and tribal nutrient criteria-rivers and streams in Nutrient Ecoregion VI: Washington, D.C., Office of Water, EPA 822–B–00–01; 28 p. + appendices.

U.S. Environmental Protection Agency, 2000c, Ambient water quality criteria recommendations—Information supporting the development of state and tribal nutrient criteria-rivers and streams in Nutrient Ecoregion VII: Washington, D.C., Office of Water, EPA 822–B–00–018; 28 p. + appendices.

U.S. Environmental Protection Agency, 2000d, Ambient water quality criteria recommendations—Information supporting the development of state and tribal nutrient criteria-rivers and streams in Nutrient Ecoregion IX: Washington, D.C., Office of Water, EPA 822–B–00–019; 33 p. + appendices.

U.S. EPA. 2010a. Using stressor-response relationships to derive numeric nutrient criteria. EPA 820-S10-001. U.S. Environmental Protection Agency, Office of Science and Technology, Office of Water, Washington, D.C. 80 p.

U.S. EPA (Environmental Protection Agency). 2010b. Causal Analysis/Diagnosis Decision Information System (CADDIS). Office of Research and Development, Washington, DC. Available online at http://www.epa.gov/caddis. Last updated September 23, 2010.

Van Nieuwenhuyse, E.E. and J.R. Jones, 1996. Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area. Can. J. Fish Aquat. Sci. 53:99-105.

Wang, L., D.M., Robertson, P.J. Garrison. 2007. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implications to nutrient criteria development. Environmental Management 39:194-212.

WDNR 2010. Notice of public hearings. WT-25-08. Proposed rule revisions for phosphorus water quality standards criteria and WPDES effluent standards and limitations.

Weigel, B.M. and D.M. Robertson. 2007. Identifying biotic integrity and water chemistry relations in nonwadeable rivers of Wisconsin: toward the development of nutrient criteria. Environmental Management 40:691-708

Weigel, B.M. and J.J. Dimick, 2011. Development, validation, and application of a macroinvertebrate-based Index of Biotic Integrity for nonwadeable rivers of Wisconsin. Journal of the North American Benthological Society 30:665-679.

Wilton T.F. 2004. Biological assessment of Iowa's wadeable streams. Project Report. Iowa Department of Natural Resources, Environmental Protection Division, TMDL and Water Quality Assessment Section. Des Moines, Iowa. Available at: http://wqm.igsb.uiowa.edu/wqa/streambio/index.html

10. Appendices