

TMDL LAKES – BIOASSAY PROJECT



VARIABILITY OF NUTRIENT LIMITATION ON PHYTOPLANKTON GROWTH IN SMALL AND MEDIUM KANSAS LAKES

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I. INTRODUCTION

I.1. Background

Eutrophication is one of the nation's leading causes of water pollution (USEPA, 2002). It is described as the nutrient enrichment (typically nitrogen and phosphorus) of a body of water (*e.g.*, streams, ponds, and lakes) by human activities. Eutrophication is of particular concern in lakes with large watersheds that are heavily devoted to agricultural production. For example, the Kansas Department of Health and Environment (KDHE) has indicated that agricultural activities accounted for nearly 50% of the impairment in lakes within the state. As a result, many lakes require Total Maximum Daily Load (TMDL) development to achieve target water-quality standards (KDHE, 2002).

Common water quality problems associated with excess nutrients include increases in plant biomass that alter aquatic habitats and reduce light penetration, reduction in lake aesthetics and lake depth/volume from siltation (or sedimentation), and the subsequent occurrence of objectionable taste and odor conditions (Smith, 1998; deNoyelles *et al.*, 1999; KDHE, 1999; Wang *et al.*, 1999; Mankin *et al.*, 2003). For example, the major source water reservoirs for the cities of Wichita and Lawrence (Cheney Reservoir and Clinton Lake, respectively) frequently show symptoms of eutrophication such as cyanobacterial (blue-green algae) blooms, reduced water transparency, depleted levels of hypolimnetic dissolved oxygen (DO), and reoccurring taste and odor problems (Wang *et al.*, 2000; Smith *et al.*, 2001; KMU Dispatch, 2003; Wang *et al.*, 2003). The conceptual relationship between eutrophication and nutrient loads, DO, algal biomass, light penetration, and taste and odor conditions is illustrated in Figure 1.



Figure 1. Conceptualized model of lake eutrophication.

Trophic state (oligotrophic, mesotrophic, and eutrophic) is a widely used classification system that describes the degree of lake eutrophication using several key parameters [total nitrogen (TN), total phosphorus (TP), and chlorophyll *a* as well as Secchi depth (SD)] to determine phytoplankton autotrophy. Of them, chlorophyll *a* is

considered the most valuable biological criterion for trophic assessment because it provides not only an estimate of overall lake productivity, but also information regarding recreational desirability and an index of lakes and their associated watershed management. The simplest response model of chlorophyll *a* is the Trophic State Index (TSI) of Carlson (1977), which has been adopted in many states (USEPA, 1988).

From a lake management perspective, there is considerable scientific evidence suggesting that the resource supply ratio of TN and TP is a fundamental mechanism regulating phytoplankton growth and community composition in aquatic ecosystems (Elser *et al.*, 1990; Downing and McCauley, 1992; Smith and Bennett, 1999; Smith *et al.*, 1999). TN:TP ratios have been used to infer nutrient limitation in terms of which of these nutrients most likely limits phytoplankton growth in a system. This is based on the relative requirement for each nutrient by different types of plants, which for algae tends to be 10N:1P by mass. Higher ratios, particularly above 17, infer P limitation for algae, and lower ratios, particularly below 5, infer N limitation and favor N₂ fixing cyanobacteria. Nitrogen and P are considered to co-limit algal growth in waterbodies where TN:TP ratios occur between 10 and 17 (Smith, 1998).

However, numerous researchers have found that there is substantial variability in the use of TN:TP ratios to predict nutrient limitation (CEEP, 1999). Data from the Kansas Biological Survey's TMDL supplemental studies also shows that there is a high level of uncertainty associated with the determination of nutrient limitation derived from the simple calculation of TN and TP concentration ratios (KBS unpublished data). In order to provide information on the factors (biotic and abiotic) affecting phytoplankton growth in eutrophically impaired lakes and to properly guide management practices, we conducted a series of laboratory bioassay experiments. In this study, small and medium sized lakes were selected because they are numerous throughout Kansas and important to people's daily life (*i.e.*, drinking water, fisheries, and recreation), yet ecologically they are highly vulnerable to environmental impacts.

I.2. Study Methods and Approach

Nineteen lakes were sampled during the spring (April-June) and late summer/fall (August-October) periods of 2002 and 2003 (Figure 2). At each lake, a 1-L water quality sample was collected at a main basin site. Attempts were made to collect water samples from riverine sections if the lake was distinctly elongate and/or the main basin area was relatively limited in size. In addition to the collection of water samples, phytoplankton samples were collected and preserved in the field with Lugol's solution for late microscopic enumeration of blue-green algae. The water samples were analyzed for total and dissolved forms of N and P, and chlorophyll *a* according to appropriate analytical procedures. *In situ* measurement of DO, turbidity, specific conductance, pH, and air/water temperature were taken concurrently with the water sample collection using a Horiba[®] field water quality device. Water transparency was measured using a 20-cm Secchi disk with alternating black and white quadrants. Non-algal turbidity was calculated using the method developed by Walker (1986). The study parameters, analytical methods, and detection limits are outlined in the Table 1.



Figure 2. Ecoregions of Kansas and locations of 19 TMDL-listed lakes sampled during 2002 and 2003 by the Central Plains Center for BioAssessment.

Parameter	Instrument/Method	Method Detection Limit		
рН	Horiba U-10 Water Quality Checker (measured <i>in situ</i>)	Horiba, 1991 APHA, 1995; 4500-H A	0.1	
Conductivity	Horiba U-10 Water Quality Checker (measured <i>in situ</i>)	Horiba, 1991 APHA, 1995; 2510 A-B	1 μS cm ⁻¹	
Dissolved Oxygen (DO)	Horiba U-10 Water Quality Checker (measured <i>in situ</i>)	Horiba, 1991 APHA, 1995; 4500-O G	0.1 mg L ⁻¹	
Turbidity	Horiba U-10 Water Quality Checker (measured <i>in situ</i>)	Horiba, 1991 APHA, 1995; 2130 B	1.0 NTU	
Air and Water Temperature	Horiba U-10 Water Quality Checker (measured <i>in situ</i>)	Horiba, 1991 APHA, 1995; 2550 B	0.1 °C	
Total Phosphorus (TP)	Pressurized sterilizer, Lachat QuikChem 4200 Flow Injection Analyzer	Ebina <i>et al.</i> , 1983	5 μg L ⁻¹	
Total Nitrogen (TN)	Pressurized sterilizer, Lachat QuikChem 4200 Flow Injection Analyzer	Ebina <i>et al.</i> , 1983	0.01 mg L ⁻¹	
Ammonium-N (NH ₄ -N)	Lachat QuikChem 4200 Flow Injection Analyzer	APHA, 1995 4500-NH3 G	1 μg L ⁻¹	
Dissolved Reactive Phosphorus (PO ₄ -P)	Lachat QuikChem 4200 Flow Injection Analyzer	APHA, 1995 4500 P	1 μg L ⁻¹	
Nitrate-N (NO ₃ -N)	Lachat QuikChem 4200 Flow Injection Analyzer	APHA, 1995 4500-NO3 G	0.01 mg L ⁻¹	
Transparency	Secchi Disk (measured in situ)	Wetzel and Likens, 1979	-	
Chlorophyll <i>a</i>	Optical Tech. Devices, Ratio-2 System Filter Fluorometer	APHA, 1995 10200 H	1.0 μg L ⁻¹	

 Table 1.
 Water quality and biological parameters assessed in the study.

Approximately 20-liter of surface water was collected from each lake and used to test algal responses in relation to nutrient-enriched conditions. To test for algal responses to N and P enrichment, potassium nitrate (KNO₃) and potassium phosphate (KH₂PO₄) were added to the bottles at 800 μ g N L⁻¹ and 200 μ g P L⁻¹, respectively. Because responses of phytoplankton production and biomass to nutrients are closely dependent on light conditions when light levels are low (Heyman and Lundgren, 1988), two light treatments were established to examine the potential effect of turbidity on phytoplankton growth.

The nutrient and light responses were examined using a 6 X 3 experimental design (Table 2). Each of the five nutrient and light additions and controls (no nutrient additions) were run in triplicate 1-L incubation bottles. The test bottles were randomly placed on shake-like shelves that provided a 12- hr gentle stirring condition. Bottles were incubated in a growth chamber for seven to nine days at 20°C on a twelve-hour light/dark cycle using a bank of fluorescent lights. A Turner Model 10 Fluorometer was used to measure fluorescence at the initiation of the experiments and every day during the incubation period.

Treatment	Description
Control	No nutrient additions with light level at approximately 230 microeinsteins
+N (800 μ g N L ⁻¹)	N added as KNO_3 to increase the concentrations by 800 $\mu g \ N \ L^{\text{-1}}$
+P (200 μg P L ⁻¹)	P added as KH_2PO_4 to increase the concentrations by 200 $\mu g \ P \ L^{\text{-1}}$
+N, +P	N and P added as above
Low light intensity	Light level maintain at approximately 400 microeinsteins, no nutrient additions
High light intensity	Light level maintain at approximately 620 microeinsteins, no nutrient additions

Table 2. Design of nutrient bioassay experiments to be used to examine TMDL lake responses to nutrient and light alterations.

II. RESULTS AND DISCUSSION

The 19 lakes selected for this study were representative of small and medium sized lakes that required the development of TMDLs for eutrophication, pH, DO, and nuisance aquatic plant growth. Laboratory and *in situ* analyses were used to characterize the physical, chemical and biological nature of each lake's water quality. Water quality in individual lakes was in part determined by a combination of physical and hydrologic factors, daily and seasonal weather effects, and internal lake processes. Complete results of these assessments of water quality for each of the nineteen sampled lakes are presented in Appendix A.

II.1.1. Nutrients

Total nitrogen concentrations in surface water ranged from 0.52 to 5.34 mg/L. Twenty-nine of the 38 samples had TN levels below 2.0 mg/L, and the overall mean and median values were 1.53 and 1.27 mg/L, respectively (Figure 3). Organic nitrogen ranged from 0.50 to 2.32 mg/L, accounting for more than 70% of the total nitrogen in 29 of 38 total samples and 90% of the total nitrogen levels in 20 of the 38 samples. Mean nitrate levels were under 0.50 mg/L for 15 of the 19 lakes. Seven of the nine lakes studied in 2002 had nitrate and organic nitrogen levels that vary less than 0.50 mg/L between sampling dates. Mission, Hiawatha and Sunflower Lake had the highest NO₃-N and NH₄-N levels and the greatest differences in NO₃-N and organic N concentrations between sampling dates in 2002. Spring to fall fluctuations in NO₃-N and organic N were greatest in the lakes studies in 2003. Four of the ten lakes monitored in 2003 had ranges in NO₃-N concentration greater than 0.50 mg/L, and three lakes had ranges in organic N greater than 0.50 mg/L.

Lakes with a large percentage of the watershed devoted to agriculture (*i.e.*, Hiawatha, Mission) and highly artificial, urban lakes (*i.e.*, Gage Park, Newton City Park, Mingenback) had the higher levels of TP (Figure 3). Total P concentrations in surface water ranged from 25.2 to 640.7 μ g/L. Mean and median values were 146.1 and 86.2 μ g/L respectively. All lakes samples showed TP levels indicative of eutrophic status (Carlson, 1977). Seventeen of 38 lake samples had TP concentrations greater than 96 μ g/L, which Carlson suggests is an indicator of a hypereutrophic state. Organic P constituted more than 70% of the TP value in 31 of 38 lake samples and more than 90% of the TP value in 10 of 38 samples. In both years, the lakes with higher organic P levels generally had higher PO₄-P levels as well.



Figure 3. TN and TP concentrations in 19 TMDL lakes. The TN and TP concentrations of 0.70 mg/L and 35 μ g/L, identified by the blue lines, indicate the regional benchmark values set by EPA Regional Technical Assistance Group (Region VII), respectively. The TP concentration of 96 μ g/L, identified by the red line, indicates hypereutrophic conditions as per Carlson, 1977.

II.1.2. Physical Characteristics

Water temperatures within and among the lakes were observed to vary greatly due to seasonal sampling and highly differing lake depths, which ranged from 1 to 10m thus contributing to a range of surface to volume ratios. Surface water (0.25m) temperatures for all lakes ranged from 17.3 to 31.7 °C, depending on the time of year sampling was conducted. The lowest surface water temperature (9.8°C) was recorded in Pony Creek during the late fall of 2002. Most of the small and medium-sized lakes sampled in this study were well mixed and homeothermic. Shallow depths, runoff events and prevailing winds provided some of the mechanisms for this mixing process. Temperatures in the lower portion of the water columns of the deeper study lakes decreased slightly at the deeper sampling depths, but no distinct thermoclines were observed in any of the lakes. The highest temperature difference between surface water and maximum depth water was approximately 8°C, observed at the 7m-deep Bronson City Lake.

Turbidity and non-algal turbidity measurements varied widely among the study lakes, with higher turbidity and lower Secchi depth readings occurring in more shallow lakes. The highest turbidity values were recorded in Sunflower and Mingenback lakes (133 and 416 NTUs, respectively). These lakes also had the highest recorded concentrations of non-algal turbidity (7.86 and 16.49 mg/L, respectively). Deeper lakes (*i.e.*, Gardner City, Afton, Pony Creek) were relatively less turbid at the surface than the more shallow lakes. Higher turbidity values were observed on sampling dates occurring shortly after rain events and on windy days. In lakes with noticeable differences in turbidity in the water column, turbidity values were generally higher in surface water than in the water column. This is likely due to the presence of phytoplankton at or near the surface. Seasonal and weather-related influences appeared to be related to the variation in turbidity values among lakes. Samples collected in the late spring and summer were typically less turbid than those collected in the fall. Increased wind speeds, proximity of precipitation events to sampling, and natural cycles (*i.e.*, fall turnover) or a combination of these and other factors are likely the contributors to the observed differences.

Six of the studied lakes are on the KDHE 303(d) list and are recognized as high or medium priority lakes due to DO issues. Dissolved oxygen concentrations in surface water (0.25m) ranged from 3.3 to 18.4 mg/L. Anoxic conditions (0 mg/L) were observed in lower depths of Gardner City, Pony Creek and Hiawatha Lakes during the summer of 2002, and Bronson, Mingenback and Pratt County Lakes in summer 2003. Lower DO concentrations were observed in the surface waters of the shallower lakes on dates with higher turbidity and non-algal turbidity values. Increased turbidity can impact algal populations by causing light limitations in the photic zone, decreasing photosynthetic activity. Higher DO concentrations generally appeared from late spring to early fall, when warmer water temperatures and nutrient concentrations created conditions suitable for algal blooms. Additionally, the daily timing of sampling efforts could possibly impact observed DO levels. Lakes were typically sampled in the early afternoon, when DO concentrations were generally nearing their diel maximum values.

The pH and DO TMDLs are frequently listed together with eutrophication TMDLs as anthropogenic changes in both of these variables is most often associated with

elevated nutrient concentrations (Figure 4). Six of the study lakes were noted to be highly to moderately impaired by pH levels. The state of Kansas considers impaired pH levels to be either less than 6.5 or greater than 8.5. The pH values for those TMDLlisted lakes included in this study were above the 8.5 upper limit. Both the flux and concentration of pH are, in part, related to plant productivity, which in turn is related to lake eutrophication. During photosynthesis, algae use carbon dioxide from the water, which can reduce the amount of carbon dioxide that can disassociate into carbonic acid thus allowing pH values to increase. Alternately, the decomposition of organic material can produce primarily organic acids as byproducts that then contribute to a lowering of the pH. In our study lakes, values for pH ranged from 7.30 to 9.45 and exhibited similar temporal and spatial patterns as DO. Typically pH decreased with depth especially in the deeper lakes while remained fairly constant in the shallower lakes. The highest observed pH values occurred in conditions with supersaturated DO levels. It is likely that the daily sampling time impacted pH because sampling efforts were usually conducting in mid-afternoon, a time period that both pH and DO would be high and perhaps not representative of average water quality conditions.

Figure 4. pH and DO values in 19 TMDL lakes during 2002 and 2003. Lines at 5.0 mg/L of DO and 8.5 of pH represent KDHE-defined values important to the development of TMDLs.

Chlorophyll *a* is the primary photosynthetic pigment present in all algae (Wetzel, 2001). It has been widely used as an indicator of algal biomass in lacustrine water quality studies. Chlorophyll *a* levels can be impacted by nutrients, particularly nitrogen and phosphorus, non-algal turbidity, which can limit the depth of the photic zone, and water temperature. Chlorophyll *a* concentrations in the study lakes ranged from 1.6 to 110.6 μ g/L. Three categories of lakes were identified according to the trophic classification developed by Carlson (1977). The two mesotrophic lakes with average chlorophyll *a* levels between 2.3 and 7.6 μ g/L were Mission and Bronson City Lakes. Fourteen lakes were classified as eutrophic, having chlorophyll *a* levels between 7.6 and 56 μ g/L (Figure 5). The remaining three lakes (Edgerton, Gage Park and Hiawatha Lake) appeared to exhibit hypereutrophic conditions with chlorophyll *a* levels were not necessarily those with the highest total phosphorus levels. Typically algal blooms were more frequent in the summer but both fall and spring blooms were recorded.

Figure 5. Chlorophyll *a* concentrations in 19 TMDL lakes during 2002 and 2003. The lines at 7.6 and 56 μ g/L refer to the lower and upper values identified by Carlson as indicative of eutrophic status. Values below 7.6 μ g/L indicate mesotrophic status; values above 56 μ g/L indicate hypereutrophic status. The regional benchmark value, set by EPA Regional Technical Assistance Group (Region VII). is 8 μ g/L.

II.2. Bioassay – Nutrient Limitation

Several classification values have been put forward to predict nutrient limitation in lakes and reservoirs based on the calculated value of the observed TN:TP ratio (Figure 1). For example, Smith (1998) suggested that N-limitation occurs in reservoirs with TN:TP ratios below 10; N+P limitation occurs in reservoirs with TN:TP ratios between 10-17; and P-limitation occurs in reservoirs with TN:TP ratios above 17. Using bioassay experiments, we tested the ability of TN:TP ratios to accurately predict which nutrient was limiting primary productivity in a series of Kansas TMDL lakes. Using Discriminant Function Analysis (DFA) we were able to correctly separated the lakes into three groups (N-limited; N+P-limited; and P-limited) based on bioassay nutrient responses (Figure 6). Table 3 lists correlation of selected variables and canonical Variates 1 and 2 determined using stepwise approach.

Figure 6. Discriminant analysis showing the separation of N-, N+P-, and P- limited lakes using canonical Variates 1 and 2.

Variable	Canonical Variate						
	Variate 1	Variate 2					
PO ₄ -P	-0.396	0.755					
Chlorophyll a	-0.092	0.216					
TN:TP ratio	0.884	0.408					

Table 3. Correlation of selected independent variables and canonical Variates 1 and 2.

As indicated in Figure 6, canonical Variate 1 was found to be most contributable to the separation of nutrient limitation in the study lakes. The N-limited lakes were typically characterized by high PO₄-P and chlorophyll a and had low TN:TP ratios. Conversely, the P-limited lakes displayed the opposite values of these features. The overall linear discriminant model correctly predicted the nutrient limitation conditions, with an accuracy of 76%. However, value ranges alone put forth by Smith (1998) and others did not correctly predict which nutrient is limiting in Kansas reservoirs. Using the

alternative range presented below, TN:TP ratios accurately predicted nutrient limitation in 89.5% of the lakes. Based on these data, we suggest that the TN:TP ratios can be an accurate predictor of nutrient limitation in Kansas lakes and reservoirs.

There were also signs of light limitation in several Kansas lakes. For example, Mingenback Lake exhibited strong light limitation, and several other lakes including Mission, Sunflower, and Newton City Park Lakes showed signs of secondary light limitation (Table 4). These results suggest that turbidity can limit phytoplankton growth in Kansas lakes and reservoirs. Furthermore, in these lakes that exhibited at least signs of secondary light limitation, TN:TP ratios tended to not be good predictors of nutrient limitation. When we did not include lakes that showed signs of light limitation in our analysis, TN:TP ratios correctly predicted nutrient limitation in 97% of the Kansas lakes using the classification values below.

Laka	Sampling season							
Lake	Spring	Late Summer/Fall						
Afton	N+P (13)	N (5)						
Bronson	P (30)	N+P (20)						
Central Park	N+P (17)	N+P (14)						
Centralia	N+P (17)	N+P (15)						
Crystal	N+P (11)	N+P (12)						
Dillon Park	N+P (20)	P (33)						
Edgerton	N+P (10)	N+P (8)						
Gage Park	N+P (12)	N+P (10)						
Gardner City	N+P (19)	N+P (14)						
Hiawatha	P (90)	N (12)						
Kingman	N+P (19)	N+P (19)						
Mary's	N+P (12)	N+P (14)						
Mingenback	Light (7)	Light, N (4)						
Mission	N, light (13)	N, light (8)						
Mound City	N+P (15)	N+P (21)						
Newton City Park	N (4)	N, light (5)						
Pony Creek	N+P (9)	N+P (12)						
Sunflower	N+P, light (8)	N+P, light (5)						
Pratt County	N+P (11)	N+P (9)						

Table 4. Results of bioassay experiments. The nutrient that was limiting is listed for each season. The actual TN:TP ratio is presented in parenthesis. If light was limiting in a particular lake, it is also listed.

Historically, P has been considered to be the primary nutrient limiting phytoplankton growth in aquatic ecosystems. As a result, management efforts have focused mainly on controlling P inputs into lakes and reservoirs (Smith *et al.*, 2002; Havens and Walker, 2002). It is becoming increasingly clear however, that limitation by N and co-limitation by N+P are also common (Elser *et al.*, 1990; Maberly *et al.*, 2002). For example, in a bioassay study of 30 European lakes, Maberly *et al.* (2002) found that roughly 60% of the lakes were co-limited by N+P. Similarly, the majority of Kansas TMDL lakes in this study were co-limited by N+P (Table 4). For example, 76% percent of the lakes were limited by N+P, compared to only 16% limited by N, and surprisingly only 8% by P. Furthermore, most lakes were limited by the same nutrient(s) during both seasons (Table 4). These results suggest that management efforts must focus not only on controlling phosphorus, but also nitrogen inputs into lakes and reservoirs in order to control excess phytoplankton growth.

Within our study group, the majority of N-limited lakes had TN:TP ratios between 4-8; all N+P limited lakes had TN:TP ratios between 9-21; and the three Plimited lakes had TN:TP ratios between 30-90 (Figure 7). These results suggest that the criteria (TN:TP<10) from Smith (1998) correctly predicted N-limitation in Kansas lakes most of the time, although there was some overlap between lakes limited by N and those limited by N+P at TN:TP ratios between 8-10. The range of ratios where co-limitation by N+P occurred in Kansas lakes, does not fit the range suggested by Smith (1998) of 10-17. Our data suggests that co-limitation occurs in lakes with TN:TP ratios from 9-20. Furthermore, the range of TN:TP ratios where P limitation occurred was also higher in Kansas lakes than suggested by Smith (1998). For example, P limitation only occurred in lakes with TN:TP ratios above 30.

Figure 7. Relationships between TN and TP concentrations and nutrient limitation in 19 Kansas TMDL lakes. Lines represent values representing TN:TP ratios of 10:1, 20:1, and 30:1.

The classification values (Are these values or ranges really criteria) presented by Smith (1998) do not accurately predict nutrient limitation in Kansas lakes. Based on these results of our bioassay studies, we suggest that the following criteria be used for predicting nutrient limitation in Kansas lakes: 1) N-limited lakes exhibit TN:TP ratios equal to or less than 8; 2) N+P limited lakes exhibit TN:TP ratios between 9-20; and 3) P-limited lakes exhibit TN:TP ratios greater than 30. Using these criteria we were able to correctly predict nutrient limitation in 89.5% of the lakes, and 97.5% of the lakes when we did not include lakes that were light limited.

II.3. Cyanobacterial Production

On of the most detrimental byproducts of eutrophication is the development of cyanobacterial, or blue-green algae, blooms (Downing *et al.*, 1999; Smith, 2003). Nuisance cyanobacterial blooms are common in drinking water lakes and reservoirs, and have been associated with the occurrence of objectionable taste and odor events (Saadoun *et al.*, 1999; Smith *et al.*, 2002; Wang *et al.*, *in review*). Elucidating which mechanisms, or combination of mechanisms, regulate cyanobacterial production is an important first step towards developing effective lake and watershed management strategies (Smith *et al.*, 2002).

In controlling cyanobacterial production in lakes and reservoir, a majority of studies and management efforts have focused on the relative concentrations of N and P (Smith, 1983; Walker and Havens, 2003; Havens and Walker, 2002). Nutrient enrichment often leads to increases in algal biomass (Jones and Knowlton, 1983), and cyanobacterial production and dominance (Downing *et al.*, 1999). In particular, Smith (1983) suggested that low TN:TP ratios favored dominance by N-fixing cyanobacteria because they have a competitive advantage over other taxa when nitrogen is low relative to phosphorus. Additional factors including light intensity, increased water column stability, and increased temperature have also been associated with cyanobacterial blooms (reviewed by Hyenstrand *et al.*, 1998).

Quantifiable densities of cyanobacteria were present in most of the study lakes at least during one season. However, there were four lakes in which cyanobacterial populations appeared to be absent during both sampling events (Table 5). The occurrence of these two lake groups allowed us to examine the limnological characteristics of lakes with (n = 4) and without (n = 15) cyanobacteria. We conducted a multivariate analysis of the limnological data collected at each reservoir using Principal Components Analysis (PCA) to identify differences between lakes with and without cyanobacteria. The first principal component (PC) was characterized by several measures of water clarity (turbidity and Secchi depth) and quality (DO and pH), as well as nutrients (TN, TP, PO4, and TN:TP ratio). The second PC was characterized by chlorophyll *a*, pH, conductivity, and DO. Together, these two PCA components explained approximately 50% of the total variance, with 33.3% of the total variance being explained by PC 1. The four lakes without cyanobacteria tended to separate along the first PC suggesting that they have higher TP, TN, and turbidity concentrations, as

well as lower DO and pH levels than lakes with cyanobacteria during at least one sampling event (Figure 8).

Lake	Sampling season							
Lake	Spring	Late Summer/Fall						
Afton	+	+						
Bronson	-	+						
Central Park	-	+						
Centralia	+	+						
Crystal	+	+						
Dillon Park	-	+						
Edgerton	-	-						
Gage Park	+	+						
Gardner City	-	+						
Hiawatha	+	+						
Kingman	+	-						
Mary's	+	+						
Mingenback	-	-						
Mission	-	+						
Mound City	-	+						
Newton City Park	-	-						
Pony Creek	+	+						
Sunflower	-	-						
Pratt County	+	+						

Table 5. Presence and absence data for cyanobacteria in the 19 Kansas study lakes: (+) indicates that cyanobacteria were present; (-) indicates that cyanobacteria were absent during a specific sampling event.

Figure 8. Principal Components Analysis (PCA) based on the limnological characteristics of the 19 study lakes. The black dots represent the four study lakes (each sampled twice) that did not support cyanobacteria during either sampling event; the white dots represent lakes that supported cyanobacteria during at least one sampling event.

High nutrient concentrations (Downing *et al.*, 1999) and turbid conditions (see below) can facilitate cyanobacterial production; however, the four lakes without cyanobacteria had unusually high turbidity levels. For example, the average non-algal turbidity concentration in the four lakes without cyanobacteria was (6.62 ± 5.37) compared to (1.36 ± 1.27) in the lakes with cyanobacteria. As a result of this increased turbidity, most of these lakes exhibited signs of light limitation in the bioassay study (Appendix B) suggesting that unusually high levels of turbidity negatively affected cyanobacterial growth.

Using data collected from the lakes that did support populations of cyanobacteria, we were able to examine relationships between cyanobacterial biovolume and the limnological characteristics of the lakes. The water column ratio of TN:TP has often been used to predict cyanobacterial presence/absence and abundance in lakes and reservoirs (Smith, 1983; Smith, 1990). For example Smith (1990) reported that lakes with TN:TP ratios less than 22:1 tended to be dominated by cyanobacteria. Most lakes

sampled in this study had TN:TP ratios that were within this range of ratios (less than 22:1) that predicts dominance by cyanobacteria (Figure 9). However, cyanobacteria were not always present during each sampling event even though they had low TN:TP ratios (Table 5). These observations suggest that the TN:TP ratio alone may not be a good predictor of cyanobacterial presence/absence in the study lakes. Similarly, Wang *et al.* (*In review*) found that TN:TP was a poor predictor of cyanobacterial biovolume in a seasonal analysis of cyanobacterial production in Clinton Lake. Therefore, we suggest that the TN:TP ratio alone is not a useful predictor of cyanobacteria presence or abundance in Kansas lakes.

Figure 9. Relationships between cyanobacterial biovolume and TN:TP ratio in the 19 study lakes. The vertical lines represent the theoretical cutoffs for cyanobacterial dominance based on TN:TP ratios (Smith, 1983 - 29:1; Smith, 1990 - 22:1; and Smith, 1998 - 17:1).

Several limnological variables were marginally significant predictors of cyanobacterial biovolume in the study lakes. For example, the light extinction coefficient was positively correlated (R = 0.555; P = 0.005) with log cyanobacterial biovolume. These results suggest that cyanobacterial biovolume increased with decreased light intensity. Low light conditions can be more favorable for cyanobacterial growth since

some taxa have a greater tolerance for turbid conditions (Scheffer, 1998; Hyenstrand *et al.*, 1998). In addition, cyanobacteria can create a positive feedback where cyanobacterial blooms lead to lower light condition that, in turn, enhanced cyanobacterial growth (Hyenstrand *et al.*, 1998). For example, Presing (1996) found that low light conditions created by cyanobacteria blooms facilitated their persistence. Furthermore, there was also a marginally strong relationship between log cyanobacterial biovolume and log chlorophyll *a* concentration (R = 0.544; P = 0.004). As chlorophyll *a* concentrations increased so did cyanobacterial biovolume. These results likely reflect the existence of a non-causal relationship. Part of the relationship may be due to the fact that cyanobacteria tend to be larger than other algal taxa; therefore, as cyanobacterial biovolume increases there is a corresponding increase in chlorophyll *a* concentration.

Recent research suggests that several additional limnological variables, not measured in this study, may help promote cyanobacterial growth in Kansas lakes. Wang *et al. (In review)* suggested that internal nutrient recycling from the sediments during periods of anoxia helped to further facilitate cyanobacterial production in Clinton Lake (Hyenstrand *et al.*, 1998; Johnston and Jacoby, 2003). In addition, a recent study of internal nutrient regeneration conducted by the Kansas Biological Survey revealed that high concentrations of P are released from the sediments during anoxic conditions (48 mg m⁻² day⁻¹) (unpublished data, 2003). Similarly, Johnston and Jacoby (2003) hypothesized that internal nutrient release was an important factor fueling cyanobacteria blooms in a large lake in Seattle, Washington.

Furthermore, Wang *et al.* (*In review*) also found a strong negative relationship between cyanobacterial biovolume and alkalinity in Clinton Lake. Bicarbonate (and/or carbonate), measured by alkalinity, is a crucial carbon (C) source of photosynthesis in aquatic ecosystems and can affect competitive interactions between algal taxa (Caraco and Miller, 1998). Therefore, competition for C may lead to shifts in phytoplankton community composition. Cyanobacteria tend to exhibit higher growth rates under low C conditions and may be able to displace other taxa such as green algae and diatoms (Talling, 1976; Reynolds, 1984, Scheffer, 1998).

Based on the results of Wang *et al.* (*In review*) and the lack of a relationship between TN:TP ratios and cyanobacterial production observed in this study, we suggest that more research is needed in order to 1) determine the relative impact of internal nutrient recycling on the overall nutrient budget of the study lakes, and on its contribution to cyanobacterial production, and 2) determine how alkalinity affects competitive interactions between cyanobacteria and other algal taxa using a similar bioassay approach.

III. CONCLUSION

The 19 lakes selected for this study were representative of small and medium sized lakes that required the development of TMDLs to address impacts due to eutrophication, pH, DO, and nuisance aquatic plant growth. Laboratory and *in situ* analyses were used to characterize the physical, chemical and biological nature of each lake's water quality. Water quality in individual lakes was in part determined by a

combination of physical factors, seasonal weather effects, the ratio of the size of the watershed to the surface area of the lake, land use/land cover in the watershed, and internal lake processes.

Observed differences in nutrient concentrations and physical characteristics allowed the classification of the 19 study lakes into two groups independent of sampling year. One group of seven lakes had elevated nutrient and chlorophyll *a* concentrations, high turbidity and/or non-algal turbidity, and noticeably high or low pH and dissolved oxygen levels. The other twelve lakes typically had moderate and consistent nutrient concentrations and physical characteristics. Further research efforts should focus on the relationships among watershed land use, the ratio of watershed areas to lake surface areas, the relative "naturalness" or "artificialness" of the lake and water quality in small and medium sized TMDL-listed lakes.

Using bioassay experiments, we were able to determine if the surface water TN:TP ratio could be used to accurately predict nutrient limitation in the study lakes. Our data suggests that the TN:TP ratios is a good predictor of nutrient limitation when using the classification scheme developed in this study, but not when using previously reported values (*e.g.*, Smith, 1998). For example, the vast majority of study lakes were co-limited by N+P and exhibited a ranged of TN:TP ratios from 9-21. Several lakes were N-limited and had TN:TP ratios generally less than 9, and three lakes were P-limited and had TN:TP ratios greater than 30. Therefore, management efforts should focus on controlling both N and P inputs into lakes.

We were also able to look for relationships between cyanobacterial production and the water quality characteristics of the lakes. Cyanobacteria were absent from lakes that tended to have unusually high non-algal turbidity levels. Several variables were marginally significant predictors of cyanobacterial biovolume in the lakes that did supported cyanobacteria. While TN:TP ratios could accurately predict nutrient limitation, there was not a significant relationship between TN:TP ratio and cyanobacterial biovolume in the study lakes. Future research should focus on determining how alkalinity and internal nutrient recycling influence cyanobacterial production in Kansas lakes.

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APPENDIX A

Water Quality Data of 19 TMDL Lakes

Dante Lai	Lahe	Conductivity	-11	DO	Turb	Secchi	TN	TP	Chl a	тылто	тет	KDHE a	verages ¹	VS. CPCB	averages
		µ%/cm	pri	mg/L	NTU	cm	mg-N/L	μg-P/L	µg∕L	- IN/IP ISI	1 51	TP	Chla	TNTP	TSI
9/26/01	Sunflower	234	80	33	128	14	2.15	286.4	29.0	8	69	370.0	10.7		48.0
4/23/02	Sunflower	171	28	6.4	133	12	1.65	322.3	189	5	65	<u>304.3</u>	<u>24.0</u>		<u>669</u>
9/26/01	Mary's	235	8.1	4.4	26	50	0.85	738	21.6	12	66	60.4	14.8	16.8	S3 S
4/23/02	Mary's	484	7.4	6.0	20	53	1.17	80.7	2.4	14	45				
8/6/02	Mary's	360	9.1	11.6	36	47	0 <i>9</i> 6	61.6	273	16	68	<u>72.0</u>	<u>17.1</u>		<u>59.7</u>
5/7/02	Gardner City	434	86	10.6	6	131	0.77	413	198	19	రు	47.4	20.2	14.б	60.1
9/17/02	Gardner City	327	8.7	28	8	118	0 <i>.5</i> 5	38.0	192	14	65	<u>39.6</u>	<u>19.5</u>		<u>65.0</u>
6/4/02	Edgerton	264	90	76	33	30	1.56	162.7	84.6	10	80		90.4		74.8
10/2/02	Edgerton	294	79	62	77	23	1.41	177.6	742	8	78	<u>170.2</u>	<u>79.4</u>		<u>79.0</u>
6/4/02	Crystal	326	8.6	9.4	25	ర	1.16	109.9	618	11	76	65.4	49.8	10.4	689
10/2/02	Crystal	285	8.1	6.4	16	68	0.98	85.0	41.6	12	73	<u>97.5</u>	<u>51.7</u>		<u>74 5</u>
6/19/02	Pony Creek	422	8.4	75	22	62	0.66	77.0	24.1	9	67	142.1	35.1	11.8	65.5
9/5/02	Pony Creek	366	8.8	9.1	11	88	0.72	593	25.1	12	68				
10/29/02	Pony Creek	417	89	9.4	11	98	0.69	402	168	17	64	<u>58.8</u>	<u>22.0</u>		<u>662</u>
6/19/02	Centralia	291	8.6	83	41	50	1.44	83.1	30.6	17	70	157.1	48.3	19.8	63.D
9/5/02	Centralia	237	9.1	93	44	38	136	93.0	625	15	77	<u>88.0</u>	<u>46.5</u>		<u>735</u>
5/21/02	Mission	287	79	59	118	15	3.42	262.2	16	13	41	140.0	21.1		නෙය
8/21/02	Mission	267	83	63	59	34	0.99	121.7	7.7	8	56	<u>192.0</u>	<u>4.7</u>		<u>48.4</u>
6/25/02	Hiawatha	304	9.0	13.5	20	80	534	59.1	742	90	78	163.3	36.8	13.2	659
8/21/02	Hiawatha	255	83	65	76	23	2.30	188.4	889	12	80	<u>123.7</u>	<u>81.6</u>		<u>79.1</u>
5/7/03	Central Park	550	82	9.6	22	66	1.75	102.7	319	17	70	120.0	58.5		705
8/26/03	Central Park	515	8.7	8.4	67	- 38	1.48	104.2	319	14	70	<u>103.4</u>	<u>31.9</u>		<u>70.0</u>
5/7/03	Gage Park	.392	8.1	92	30	- 50	1.09	87.4	26.0	12	68	115.0	76.8		70+
8/26/03	Gage Park	860	89	13.9	74	42	236	233.9	110.6	10	82	<u>160.6</u>	<u>68.3</u>		<u>75.1</u>
5/20/03	Bronson	279	88	18.7	10	194	0.75	252	6.1	- 30	54	70.0	22.6	20	61.2
9/8/03	Bronson	239	83	8.1	12	160	0.52	26.4	63	20	54	<u>25.8</u>	<u>62</u>		<u>539</u>
5/20/03	Mound City	291	8.8	18.4	- 36	33	0.62	41.4	118	15	60	59.0	27.9	13.7	632
9/8/03	Mound City	243	78	10.3	23	-53	0.78	379	159	21	ങ	<u>39.6</u>	<u>13.9</u>		<u>63.1</u>
6/3/03	Afton	384	9.1	7.4	14	83	0.81	62.7	4.0	13	-50	114.0	25.5	45	623
9/23/03	Afton	392	8.4	6.4	52	47	0.73	143.3	119	5	60	<u>103.0</u>	<u>80</u>		<u>54 9</u>
6/3/03	Newton City Park	623	95	13.2	112	18	1.60	361.5	87.8	4	80	195.0	64.4		71.4
9/23/03	Newton City Park	538	83	7.0	96	- 30	1.70	326.6	198	5	65	<u>344.1</u>	<u>53.8</u>		<u>72.6</u>
6/16/03	Dillon Park	495	86	13.6	36	42	1.61	79,4	24.1	20	67	70.0	51.0		69.1
10/7/03	Dillon Park	432	90	28	52	42	233	71.0	143	33	62	<u>75.2</u>	<u>19.2</u>		<u>64.7</u>
6/16/03	Mingerback	186	73	4.1	380	6	4.65	636.4	69	7	55	290.0	12.8		55.6
10/7/03	Mingerback	173	7.7	4.6	416	8	2.67	640.7	8.7	4	57	<u>638.5</u>	<u>78</u>		<u>56.1</u>
7/1/03	Kingman	271	8.8	10.4	35	35	1.09	583	27.0	19	-68				
10/21/03	Kingman	359	89	78	60	45	1.54	81.8	28.0	19	69	<u>70.0</u>	<u>27.5</u>		<u>68 5</u>
7/1/03	Pratt County (South)	442	89	12.0	31	- 30	0.78	90.1	159	9	ങ	109.0	122.0	4.17	77.7
10/21/03	Pratt County (Main)	504	9.1	12.4	43	48	2.34	211.8	84.6	11	80	<u>150.9</u>	<u>50.2</u>		<u>71.3</u>

APPENDIX B

Bioassay Experiments – Nutrient Limitation

APPENDIX C

Water Chemistry Profiles of selected TMDL Lakes

