Development of Lake-specific Numerical Nutrient Criteria for Water Quality Standards in Fond du Lac Reservation Lakes: Analysis of the Phytoplankton Rapid Assay Results 1998-2012 compared to Southern MN lakes.

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Fond du Lac Reservation Office of Water Protection

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Executive Summary

This technical report is a continuation of a previous study establishing lake-specific nutrient criteria (Soranno 2011) for nine fisheries lakes in the Fond du Lac reservation (FDL). Both studies are part of an effort to replace narrative nutrient criteria with numeric nutrient criteria. The original report used water quality data from FDL, the Grand Portage Reservation and a 29 lake reference database from the Northern Lakes and Forests Ecoregion (same ecoregion that Fond du Lac is located in, provided originally by Steve Heiskary, MN Water Pollution Control Agency) as a comparison. Soranno (2011) described a method for determining lake-specific nutrient criteria based on the unique, colored and shallow state of the FDL lakes. In this report, Phytoplankton Rapid Assay (PRA) data dating from 1998 to 2012 was used to assess current biological state of the FDL fish lakes in an effort to substantiate the unique character and higher water quality of the FDL lakes. Two other study sites were included that contained a variety of lakes and productivities. Data were supplied by the Minneapolis Parks and Recreation Board (MRP, 2003-2012, 207.52 km SW of FDL) and the Minnehaha Creek Water District (MCWD, 2006-2012, 215.8 km SW of FDL). The larger databases allowed for analysis of a total of 47 lakes (1292 algal samples, 2202 water quality samples) to assess if the FDL lakes were stable ecologically with relatively high water quality in their algal community and how they compared to lakes of varying productivity. Both the MPR and MCWD study sites are more highly impacted by human activity, and are in a slightly different ecoregion to the southwest (North Central Hardwood Forest). The lakes clustered into six statistically significant groups which were consolidated into five Lake Groups. The FDL lakes did cluster uniquely within the larger database of 47 lakes (Multidimensional Scaling, Primer e6) along with several of the shallower, colored MPR lakes. Characteristics of the different study sites along with each cluster are discussed, with special reference to the differences in local climate, the relative percent of important algal functional groups and other water quality data. The FDL fish lakes all clustered within the highest water quality group (Group 1), consistent with results from Soranno's (2011) results. A calibration study was also conducted over three sampling dates in 2014 comparing the Phytoplankton Rapid Assay on FDL fisheries lakes from two independent taxonomists (Elaine Ruzycki, Natural Resources Research Institute, University MN-Duluth and Ann St. Amand, PhycoTech) taken from May-October, 2014. Algal counts with biovolume estimates and relative cell concentration were also compared to the PRA for the same set of samples (since this is the origin of the FDL algal database) on PhycoTech generated data and compared as well. Relative biovolume compared well with the PRA data. In addition, both counters compared exceptionally well to each other, resulting in the same data interpretation for the FDL data set among all 2014 samples.

List of Tables:

List of Appendices:

List of Figures:

Figure 42. Air Temperature (^{o}C) for FDL and MPR/MWCD study sites. FDL air temperature (Mean=20.2^{0}C, SD=5.272) is significantly lower than the southern study sites (Mean=24.8^{0}C, SD=5.441), Mann-Whitney Rank Sum Test, $p<0.001$.

Figure 43: Yearly ice out dates for lakes within each study area (lake specific data was not available for all lakes).

Figure 44. Total rainfall in FDL and MPR/MWCD geographic areas.

Figure 45. Rainfall events that exceed 1, 2, 3 and 4 inches in a) FDL and b) MPR/MWCD study sites (June through October).

Introduction

In 2011, Soranno produced a technical report that described an approach to develop numerical nutrient criteria for each of the Fond du Lac (FDL) lakes using lake nutrient monitoring data for each lake (Soranno 2011, Appendix 1). That report described the nutrient levels in the lakes, quantified lake-specific nutrient criteria, and found that the FDL lakes were minimally impacted by human activity. The purpose of this technical report is to evaluate the effectiveness of those criteria in relation to phytoplankton, a biological characteristic of the lakes. Phytoplankton have been measured in FDL lakes using the Phytoplankton Rapid Assay (PRA) method and chlorophyll concentrations to determine whether the biological data support the finding of the 2011 technical report that the lakes are minimally impacted by human activity. Algae are generally considered one of the most sensitive indicators of changes to nutrient loading and have several good indicators at either high (Chrysophytes, Diatoms, Cryptophytes) or low (toxin producing Cyanobacteria or HAB taxa) water quality (AWWA 2010). Other divisions also convey additional information about water quality including high organic matter (Euglenoids), low pH (Desmids, Cryptophytes, Dinoflagellates) or higher nitrogen (Chlorophytes in general).

The purpose of developing nutrient criteria, either as an ecoregion or on a lake specific basis, is to ensure all lakes meet their designated uses (USEPA 2000). With the exception of one lake (Third Lake, Soranno 2011), all FDL lakes are currently fully meeting their designated use which includes warm water fisheries and other various uses, despite having relatively high total phosphorus and Chlorophyll *a* on occasion.

For the 2011 technical report, Soranno compared the FDL lakes to other minimally-impacted lakes in the Northern Lakes and Forest ecoregion. In this report, I compared the FDL lakes to another group of MN lakes that are moderately to highly impacted by human activity, both recreational and agricultural to examine whether there is evidence that the FDL lakes are impacted by human activities as we know these other MN lakes are. I compared PRA data in the FDL lakes to PRA data in lakes from the Minneapolis Parks and Recreation study site and the Minnehaha Creek Water District study site The moderately-impacted lake comparative database does not cover all years of the FDL database (1998-present), but is inclusive (2003-present). 1292 algal samples were used in the final analysis.

A second goal of this technical report is to validate the phytoplankton PRA method against full algal counts with biovolume estimates and relative cell concentration, as an alternative to use when funds and time are limiting. The PRA was developed by the MN DNR to provide a rapid response to potential algal threats from toxin producing Cyanobacteria in state monitored waters (Lindon and Heiskary, 2007). This technique provides a semi-quantitative estimate of the relative biomass of the phytoplankton community focusing on the dominant algal taxa and was originally described by Dr. Ed Swain and Carolyn Dindorf, Minnesota Pollution Control Agency (6/16/1989). The advantages of the assay are that it can be done on a wet sample, and is relatively fast to complete. The disadvantages are that (a) it still requires an expert to do the species identifications who is very familiar with biovolume measurements and is an expert taxonomist; and (b) it also does not measure density per se, so there is no real measure of abundance. However, PRA results can be very useful in determining overall status or

waterbody type, and in detecting changes among sentinel algal groups. Particularly helpful in the FDL data analysis was the use of a single taxonomist during the course of the monitoring project (Elaine Ruzycki, Natural Resources Research Institute, University MN-Duluth). Both of the comparative moderately-impacted lake datasets (hereafter, the comparative lakes) used in this report also had a single taxonomist, Ann St. Amand, PhycoTech, which made harmonization between the two databases much more accurate. Both taxonomists used an expanded version of the PRA, where all taxa encountered were noted rather than just noting dominant taxa and divisions, although there were slight differences in identifications.

Background

Study Sites, Morphometric and Watershed Characteristics

The FDL lakes are located in Northeastern Minnesota near Lake Superior (Figure 1). The comparative lakes are located approximately 200 km southwest of FDL (207 km, MPR and 215 km, MCWD, Figure 1) near Minneapolis, Minnesota. FDL lakes (Figure 2) are characterized as minimally impacted and highly colored (Soranno 2011), with a high proportion of forested land and little human activity. Third lake is the most impacted system with a history of livestock (Horse Farm) activity in its watershed which has been mitigated over the last several years including developing a more effective manure management plan and an alum treatment in 2012 (K. Hedin, Fond du Lac Tribe, Personal Communication). Both groups of lakes in the comparative dataset are highly impacted by human activity. MPR (total area: 50852.8 ha) includes lakes in and near the city of Minneapolis that have a variety of settings from urban to relatively remote, and include some colored waters (Figure 3). MCWD lakes are all in the main watershed of Lake Minnetonka (total watershed 31897.3 ha), but are characterized as a series of isolated bays and lakes that tend to act independently from the main lake body (Figure 4). Table 1 identifies lakes, locations, Ecoregion, morphometry and watershed information. Several of the MCWD lakes have significant agriculture in their watersheds. Both MPR and MCWD provide a wide range of comparative lakes to the FDL lakes. Watersheds over all three projects ranged from 0.8 ha to over 46,878 ha, and lake surface areas ranged from 1.2 ha to well over 400 ha. Maximum depth ranges from approximately 0. 46 m to over 23 m, which for the most part represents shallow systems similar to the FDL lakes. Some of the lakes in Central MN in the North Central Hardwood Forest ecoregion are eutrophic/hypereutrophic which provide a valuable contrast to the FDL and higher water quality MPR lakes (Lindon and Heiskary, 2007).

Materials and Methods

Table 2 indicates data availability and data type for each of the three study sites. The most common data type is the PRA, which spans all three projects for all samples. Most of the algal samples

had synoptic water quality data to some extent which included Total Nitrogen (TN), Total Phosphorus (TP), Chlorophyll *a* (Chla), Water Temperature (WT), Dissolved Oxygen (DO), Turbidity, Temperature, DOC/Color, Secchi, and pH. All water quality data was provided by the originating organization (FDL, MPR or MCWD, respectively). TN/TP ratios, summary statistics, Kruskal–Wallis one-way analysis of variance by ranks, Tukey's HSD, and Mann Whitney U were calculated using SigmaPlot 12.0 and Systat 11 (Systat Software, Inc., San Jose, CA). Unfortunately, TSI could not be calculated because required variables were not measured in all lakes in all years. Color, DOC, Turbidity and Secchi Depth were not universally available in all three of the study sites.

Samples were pooled for a lake with multiple basins if there was strong physical connection between basins. FDL had three lakes with combined data as North and South Basins (Big Lake, Perch Lake and West Twin Lake). Lakes with multiple basins were treated as multiple samples per lake, no samples were averaged. MDS allows for uneven sample design. MCWD was dominated by multiple basins within Lake Minnetonka and some individual lakes. An initial MDS analysis was used to determine which MCWD basins should be combined and which should be treated as separate systems. Many of the basins have very little connection to the main lake or to each other (K. Dooley, MCWD, Personal Communication). Based on the MDS analysis, several basins from Lake Minnetonka were combined into two main lake basins: LM2 (Carman Bay, Smithtown Bay, West Upper Bay) and LM3: (Grays Bay, Lower Lake North, Lower Lake South, Wayzata Bay). West Arm Bay and Jennings Bay were combined into West Arm/Jennings Bay. All other MCWD bays and lakes were treated as separate systems. All MPR lakes were single basin lakes.

The PRA was developed by the MN DNR to provide a rapid response to potential algal threats from toxin producing Cyanobacteria and other indicators of poor water quality in state monitored waters (Lindon and Heiskary, 2007). This technique provides a semi-quantitative estimate of the relative biomass of the phytoplankton community focusing on the dominant algal taxa and was originally described by Dr. Ed Swain and Carolyn Dindorf, Minnesota Pollution Control Agency, 6/16/1989. PRA was reported as relative percent concentration although it is a hybrid of relative biovolume/relative concentration. Both Ruzycki and St. Amand modify the standard MN PRA by noting all taxa encountered. Ruzycki marked them as present and provided a summary category for non-tallied species. The summary category was evenly split among the rare taxa that were marked "present." St. Amand assigned a standard 1% to rare taxa which was modified to 0.1% for this analysis (MDS analysis was run for 0.1, 0.01, and 0.001% assigned to rare taxa with the same result). Ruzycki PRA analysis was completed on a wet sample (concentrated). St. Amand uses a HPMA permanent mounting technique (see below). Final PRA data was analyzed by Multidimensional scaling (MDS) and cluster analysis using Primer 6 (PRIMER-E Ltd., United Kingdom). All data used for MDS was log transformed and double standardized (C. Vanier, UNLV, Personal Communication, Yoshioka 2008).

During harmonization between the three projects and based on initial MDS results, (joint effort between E. Ruzycki and A. St. Amand), the decision was made to roll the taxonomy back to the functional group level to accommodate for slight differences in taxonomic identification both over time and between taxonomists. Table 3 lists functional group classifications (FGC), which are slightly different than used at the MN state level. The full harmonization table is located in Appendix 2. All

taxonomy was harmonized to Algaebase (Guiry, M.D. & Guiry, G.M. 2015. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 21 January 2015.) Most FGCs correspond to division, however there are several important differences. Chrysophytes and planktonic diatoms are closely related in their pigments and ecology, so the two groups were rolled together as DY. Although *Chrysochromulina parva* is a Haptophyte, its ecology is very similar to spring and fall blooming motile Chrysophytes, so that taxa is included with the DY taxa. Cryptomonads and Dinoflagellates, with the exception of *Ceratium hirundinella*, often co-occur in more highly colored systems during the summer season, irrespective of nutrient concentrations. FGC CP includes these two groups. CP1 corresponds to only *Ceratium hirundinella* as it appears to be somewhat independent in its bloom dynamics. FGC E/O includes all uncertain taxa (very few taxa were classified as uncertain) and Euglenoids. Both *Gonyostomum* species were also included in the Euglenoids due to their close co-occurrence. FGC HAB includes all heterocystic Cyanobacteria capable of producing toxins. FGC HAB1 includes non-heterocystic Cyanobacteria capable of producing toxins. Only taxa capable of or demonstrated to produce toxins were included in the HAB and HAB1 functional group classifications, not taste and odor producing taxa. In practice, HAB and HAB1 were combined in the analysis. Toxin data was not available for any of the study sites. FGC BG contains all other Cyanobacteria and G corresponds to all Chlorophytes. Although Soranno (2011) omitted outliers from the original analysis, similar PRA samples were not eliminated from this analysis. In all cases, corresponding PRA sample data either corresponded well with the previous or next sampling date or was not clearly out of range. Only PRA samples with sparse data or extremely low density that precluded determining relative percent contribution of the taxa present were omitted from the analysis (Table 4).

Growing season will most greatly influence designated use and perceived water quality, especially as it relates to increased risk of low dissolved oxygen at depth, Cyanobacterial blooms and associated toxin events. MDS was used to confirm growing season using the southern lakes comparison data set since sampling went from April through November on a monthly basis (Figure 5). April, May, and November are all statistically separate from the main summer growing season. In addition, algae were not sampled in June for the FDL lakes (which was also more distant from latter summer months), so sampling season was restricted to July through October. October was included in the data analysis because of its close clustering with the main part of the summer growing season, and the likelihood that toxin producing Cyanobacteria often bloom and produce toxins well into October.

Samples for quantitative phytoplankton analyses were analyzed from the same water used for the routine samples sent to Ruzycki (University of MN). Samples were preserved with Lugol's solution. Dr. Ann St. Amand, PhycoTech, Inc., St. Joseph, Michigan, performed identification, enumeration and biovolume, volume and area calculations for the phytoplankton. The HPMA method for producing algal sample slides provided an optically clear background while permanently infiltrating and preserving the sample for archival purposes (APHA 2012, St. Amand 1990). It offers minimal distortion and allows the use of epifluorescence on the sample while counting, which can dramatically improve the final results, especially when picoplankton or high particulates are present. St. Amand counted a minimum of 400 natural units and 15 fields at 500x. In addition, larger taxa were counted at 300x (minimum of 30 fields. Fields were counted evenly over the three slides provided for each sample (i.e. 30 total fields, 10 fields

per slide). Counting was completed when the standard error of the mean of the total number of natural units per field was less than 10%. This counting method using multiple slides, a procedure which encourages random distribution and a stratified counting strategy, combined with using a single analyst produces high quality data (Vuorio et al. 2007). Generally, 10-15 representatives of each taxon were made (up to 28 dimensions on each taxon based on the complexity of the colony or cell) unless the taxon was rare and then number encountered was measured. Additionally, cells that distort during preservation or were very consistent received a standard measurement. Biovolume, Volume and Area calculations were based on combinations of geometric figures found in Olrik et al. (1998), Hillebrand et al. (1999) or developed in-house. Biovolume (μm^3) was calculated on the living protoplasm exclusive of setae and sheath, volume (μm^3) and area (μm^2) were calculated for each natural unit including setae and sheath. All algal data was entered directly into ASA System, a Foxpro 9 driven laboratory information management system that cataloged all count data, measurements and completed all algal-related calculations. No algal taxa were excluded from the analysis.

Results

Chlorophyll *a* and PRA Results

FDL

Chla data on a yearly basis are presented in Figure 6 for the FDL lakes. Data is unavailable for 1998, which was the first year sampled. Soranno (2011) presented cumulative box and whisker plots for the Chla data by lake which indicated lakewide summary statistics; however, it is important to also look at yearly variation and how the lakes relate to one another within and among years. Third Lake has the highest Chla among the FDL lakes until 2010, with Simian being the next most productive lake. Third Lake has also had a significant amount of watershed and in-lake management activity, and has experienced large fluctuations in water depth due to drought (K. Hedin, Fond du Lac Tribe, Personal Communication). The tribe has been working with the owner of a horse farm to move manure piles away from the lake shore and there was an in-lake alum treatment to seal nutrient rich sediments in 2012 (K. Hedin, Fond du Lac Tribe, Personal Communication). The FDL lakes had the lowest Chla among all three study sites rarely reaching 20 µg/L, with the highest Chla well below 50 µg/L.

PRA results are presented in Figure 7 for each of the nine FDL lakes. Although there are some differences among the lakes, most lakes and years are dominated by non-HAB taxa. Third Lake (mentioned above) had a history of HAB blooms from 2000-2007, but conditions improved in 2008 that continue through to 2014 (data presented in the calibration section below). Pat Martin Lake has a relatively high contribution from G taxa (primarily *Quadrigula* and *Botryococcus*), especially in the late 2000s (and also relatively high transparency, Soranno 2011). Big, Lost, Simian, Sofie, Perch, Joe Martin, and West Twin Lakes are dominated by CP and DY taxa most years (often including colonial motile taxa such as *Chrysosphaeralla*, *Uroglena, Dinobryon* and *Synura* as well as the single celled *Chrysochromulina)*. BG taxa are consistent contributors but rarely dominate. CP and DY groups are also

important in Pat Martin Lake and Third Lake following drought years ending in 2007. CP taxa and large colonial Chrysophytes often dominate highly colored, kettle-hole lakes.

MPR

Chla data for MPR lakes are presented in Figure 8. MPR lakes are consistently more productive than FDL lakes, with Chla commonly between and 30-40 µg/L, reaching 180 µg/L at the highest. Data are not available for all lakes and years. Diamond, Spring and Grass Lakes often had the highest Chla. These three lakes are also shallow, with extensive emergent weed beds, and are classified as wetland systems (MPRB 2011). Additionally, Spring Lake's basin has a floating mat of cattails that has been expanding over the last several decades (R. Cross, MPRB, Personal Communication). Several of the wetland lakes receive untreated storm water (MPRB 2011). Loring and Hiawatha Lakes often also had Chla measurements above 40 µg/L, and Hiawatha has been slowly degrading over time. Wirth Lake, with intermediate Chla, has been meeting Minnesota Pollution Control Agency (MPCA) guidelines for TP, Chla and Secchi depth for most the last 15 years.

PRA results are quite interesting for the MPR lakes (Figure 9). Powderhorn Lake has had several years of Barley Straw treatments (MPRB 2011). Powderhorn shifted to a CP/DY dominated assemblage shortly after treatment started and remains consistently improved (interpreted as limited HAB and HAB1 taxa throughout the growing season). The three wetland dominated, shallow lakes (Diamond, Grass and Spring Lakes) are all dominated by CP/DY algae, with varying, sporadic contributions by G and HAB taxa. Wirth Lake, which is consistently meeting MPCA guidelines, has a recurring population of *Ceratium hirundinella*, which commonly co-occurs with HAB taxa. Loring Lake has a diverse community of algae, with strong G, CP and DY algal percentages and lessor contributions by HAB or BG taxa. Lake of the Isles and Cedar Lake are slowly improving as HAB taxa decrease and other groups increase in relative contributions. Calhoun, Harriet and Cedar are also slowly improving, as HAB taxa decrease slightly and the assemblages become marginally more diverse with increased contributions by G, CP1, CP and DY taxa.

MCWD

MCWD Chla data are presented in Figure 10. Although the MCWD lakes individually don't reach over 140 µg/L, there are several lakes that are consistently high (Jennings/West Arm, Halsted, Langdon, Parley Lakes). As in the MPR lakes, data are not available for all MCWD lakes and years. Although the spikes are not as high as the MPR lakes, overall Chla is consistently higher in the MCWD lakes. The MCWD study site is more complex and has significant agriculture in the watersheds of several of the lakes and basins (Figure 4). Lake Minnetonka is a series of strongly to mildly connected basins. Chla data is relatively complete for the years that there is also algal data (2006-2012) however, lake/basin algal data is more sporadic.

Figure 11 indicates the sparser nature of the MCWD algal data set. Some basins/lakes have 4-6 years of data (Halsted, Jennings/West Arm, St. Albans Bays and Gleason Lake). All of the other basins/lakes have 2-3 years of data each at varying points in the 2006-2012 sampling period. There are significant, ongoing watershed management activities in the MCWD study site, especially near the

western basins of Lake Minnetonka where there is agricultural activity. PRA results indicate that the lakes/basins with higher agricultural activity (Jennings/WestArm, Stubbs, Parley, Piersons, Minnewashta Lake, Schultz Lake) also have greater dominance by HAB taxa. There is a slightly different, more diverse mix of taxa in the MCWD lakes than in the HAB dominated MPR lakes, especially among *Dolichopermum* and *Aphanizomenon* species. Although there are some bays/lakes that are more balanced among the FGCs (St. Albans, Pierson, Gleason, LM2, LM3, and Christmas Lake), there are no CP/DY dominated systems in the MCWD study site as there are in the FDL and MPR sites.

MDS/Cluster Results

FDL

MDS analysis was first completed on PRA results using the only FDL lakes averaged by year (Figure 12) and lake (Figure 13). The year averaged MDS indicated that certain years were clustering (significant clusters are denoted by a solid line, $p<0.01$), but there was not a consistent year trend with increasing or decreasing water quality from 1998 to 2012. PRA MDS results, averaged by lake, indicated that there were no significant differences among the nine FDL lakes, with Pat Martin and Third Lakes diverging the most. This is consistent with Soranno's (2011) findings that the FDL lakes are related in several characteristics which influence higher water quality: low human impacts, and shallow, colored waters. All nine lakes fall within the Northern Lake Forest ecoregion and have a high percent forest cover in their watersheds as well. This is also consistent with FDL timeline graphs above (Figure 7) which indicated most of the FDL lakes were dominated by a combination of CP/DY taxa, with much lower, sporadic influence by HAB taxa, especially from 2008-2012.

Combined Study Sites

MDS results for all study sites combined (47 bays/lakes, 1292 samples total) indicate that there is more of a linear trend among years with 1998-2002 and 2003-2012 clustering significantly (Figure 14), although the individual years are not always clustering together. As in the MDS of just FDL lakes, the MDS for the combined systems averaged on lake clustered all FDL lakes together within one statistically significant cluster, with the FDL lake assemblages at least 80% similar (Figure 15, FDL lakes are denoted by black arrows). Figure 16 indicates five clusters/groups used in this analysis. Four of the clusters were statistically significant 1, 2, 3, and 5 ($p<001$). Group 4 was defined as a combination of 2 smaller statistically significant clusters with 2 lakes each that were closely related. Group 1 (significant) contained all FDL lakes as well as Loring, Grass, Powerderhorn, Spring, Webber and Hiawatha Lakes (all in the MPR study site). Group 2 (significant) contained LM2, LM3, St. Albans Bay, Christmas Lake, Calhoun Lake, Crystal Bay, Cedar Lake, Piersons Lake, Harriet Lake and Minnewashta Lake (both MPR and MCWD study sites). Group 3 (significant) includes Lake of the Isles, Nokomis Lake, Gleason Lake, Langdon Lake and Brownie Lake (both MPR and MCWD study sites). Group 4 (2 significant clusters) includes Lake Virginia, Wirth, Brownie and Langdon Lakes (both MPR and MCWD study sites). Group 5 (significant) includes Wasserman, Parley, and Dutch Lakes and Stubbs, Halsted and Jennings/West Arm Bays (all in the MCWD study site).

Water Quality Indicators by Lake Group

Chla graphed as box plots highlights Group similarities and differences (Figure 17), statistically significant differences from Group 1 are indicated by an *. Groups were uneven for sample number and variance, so Kruskal–Wallis one-way analysis of variance by ranks and Tukey's HSD was used to determine statistical difference between Group 1 and the other four groups. Group 2 has the lowest median and variance of all five groups. Group 5 has the highest median, but Group 4 has the highest variance among all five groups. Groups 2, 3, 4, and 5 are statistically different from Group 1, but not necessarily from each other. Figure 18 indicates the relative percent algal composition by FGC for the five lake groups, averaged by group. Each cluster or group has unique characteristics that are not apparent by looking at Chla results alone. Group 1, the largest group of lakes (16), is characterized by a high proportion of CP and DY taxa, moderately low G, BG and HAB taxa, and low E/O taxa. Group 2, the second largest group of lakes (10), has higher concentrations of both BG and HAB taxa. HAB taxa represent approximately 50% of the biovolume in the Group 2 lakes. There are much lower proportions of CP, DY, G and CP1 taxa cumulatively accounting for only 45-50% of the assemblage. There are almost no E/O taxa. Group 3 is characterized by 70-80% HAB taxa, with 20-25% CP, DY, G and CP1 taxa. There are very few BG taxa in Group 3. Group 4 has similar HAB composition to Group 2, but has a large population of CP1 or *Ceratium hirundinella*. There are low relative concentrations of CP, DY, BG and G taxa in Group 4 as well. Group 5 is the most productive group of lakes, with over 80-90% HAB taxa and generally less than 10% all other FGCs combined. Group 5 lakes exceed all MN nutrient criteria for the NCHF ecoregion for Shallow lakes (Heiskary and Wilson 2008). Summary statistics for each group and FGC is presented in Table 5.

Figures 19-24 are MDS plots of the 5 lake groups with bubble overlays of the different FGC s. Group 5 has the largest HAB percentage (Figure 19). CP is most prevalent in Group 1, accounting for over 20% of the group composition (Figure 20). Group 4's most unique feature is the relatively high proportion of CP1 or *Ceratium hirundinella* (Figure 21). DY is also most prevalent in Group 1, accounting for about 20% of the assemblage. Groups 2, 3, 4 have similar concentrations of DY and G taxa (Figures 22 and 23, 10-15%). Conversely, Group 5 has low concentrations of all groups other than HAB taxa. Lastly, although below 10%, E/O is only present in consistent proportions in Group 1, E/O is below 1% in all other Groups (Figure 24).

Boxplots of the TP within each Lake Group over all years is shown in Figure 25. Groups 1-4 have similar medians (just below 60 µg/L), while Group 5 is statistically higher with a median near 140 µg/L (Kruskal-Wallis One Way Analysis on Ranks, SigmaPlot 12). TN indicates similar, but more variable results as Groups 1-4 have overlapping TN levels (Figure 26, 700-1200 µg/L median values) with Group 5 being the highest with a median near 2000 µg/L (Kruskal-Wallis One Way Analysis on Ranks, SigmaPlot 12). Despite the greater variability, Groups 2, 3, 4 and 5 were all statistically different from Group 1. As would be expected by systems dominated by HAB taxa, Group 5 has the lowest median value for TN:TP ratio (Figure 27). Group 4 is also lower (below 20), while Groups 1-3 have the highest and most variable TN:TP values. Groups 1-3 also have a higher contribution by Chlorophyte algae as well. TN:TP is only moderately useful in the epiliminion during mid-summer. The ratio is often most important at depth at the sediment water interface where many HAB taxa start growth and uptake luxury phosphorus prior to

rising in the water column in early summer (Welch and Jacoby 2004). Consistent with Soranno (2011), nutrients and Chla alone do not reflect the higher, more consistent water quality of the FDL lakes. Phytoplankton data as interpreted by the PRA is the most sensitive indicator of water quality available in all three MN study sites.

Calibration Study: Phytoplankton Rapid Assay versus Counts with Biovolume Estimates and Relative Cell Concentration.

The calibration study was designed to 1) compare commonly used numeric response variables to the PRA, and 2) to compare independent analysts as a measure of QA/QC over the years. Percent biovolume, PRA, and percent cell concentration are shown in Figures 28 through 30 for each sampling date in 2014, respectively on a per lake basis. 2014 averaged over all sample dates (Figure 31) is also presented. Figures 28-31 represent data generated by St. Amand at PhycoTech for the quantitative (percent biovolume, percent cell concentration) and PRA data from the same HPMA mounting process. The PRA is intended as a surrogate for percent biovolume, requiring the analyst to assess relative contribution of each taxa not just by abundance, but by biovolume as well. Therefore, percent cell concentration is generally a poor match, especially where colonial taxa dominate or the assemblage is diverse. The best correspondence between all three response variables occurs when there is an overwhelming dominant, and taxa present are similarly sized (see Figure 28, Joe Martin Lake). For the most part, however, there is poor agreement between the PRA and percent cell concentration (See Figures 29 and 30, most lakes). Percent biovolume is a consistently closer match to, and has a good general overall agreement on a per lake basis over all dates with the PRA among all FGCs. This corroborates the PRA as described by Swain and Carolyn Dindorf as a surrogate for percent biovolume. Averaging data over multiple dates improves the relationship between percent biovolume and PRA (Figure 32). It is important to note that there is not a perfect correlation between percent biovolume and the PRA (See Figure 30, Lost, Perch and West Twin Lakes), but that ecological interpretation would not change between the percent biovolume and PRA considering how the FGCs are related to one another among different lake productivities.

Calibration Study: Percent Biovolume versus Phytoplankton Rapid Assay among independent analysts.

The second goal of the calibration study was to address QA/QC issues by comparing independent analysts (St. Amand, PhycoTech and Ruzycki, University of Minnesota). Both analysts have over 20 years of experience counting Northern Midwestern algal assemblages. Data compared by analyst (including not only PRA but Percent Biovolume as well) for each lake and date May/June (Figure 32), August (Figure 33), September/October (Figure 34) show exceptionally good agreement among the response variables and analysts. Again, higher diversity among FGCs is not as close in agreement among response variables and analysts as lower diversity lakes and dates with more similarly sized taxa, irrespective of productivity (Figure 33, Simian Lake, and Figure 34, Perch Lake). Although each analyst has personal tendencies towards certain count patterns (St. Amand tends to sometimes overestimate CP in comparison with Percent Biovolume, Ruzycki tends to overestimate BG in comparison with St. Amand and Percent Biovolume), there is excellent correspondence overall. Averaging data among sample dates (Growing season, Figure 35 and 2014, all sample dates, Figure 36) provides the best correspondence

among analysts and response variables. These comparative results indicate that there is good confidence in using the entire algal database (1998 to present) to look year trends and assessing changes in water quality with time.

In terms of differences in clustering among different analysts for the growing season samples (August-October 2014), cluster results for the FDL lakes are presented in Figure 37 (Percent Biovolume, St. Amand), Figure 38 (PRA, St. Amand), and Figure 39 (PRA, Ruzycki). None of the response variables produced significantly different clusters, consistent with the analysis of the larger database above. Relative biovolume showed a slightly different cluster pattern, however, there were strong similarities among all three of the comparisons (Percent biovolume, PRA St. Amand, PRA Ruzycki): Big Lake, Lost Lake and West Twin Lake clustered together, Perch Lake and Joe Martin Lake also clustered together in all three comparisons. Relative biovolume clustered the remaining four lakes (Sofie, Third, Pat Martin and Simian Lakes) together, while St. Amand and Ruzycki resulted in slightly separating out Sofie from the other three lakes. Again, these are all subtle differences in clustering proximity which did not result in any statistically significant clusters.

Discussion

The PRA can be a valuable tool in differentiating between lake systems with different ecologies, and in detecting changes in water quality. Because of the wide range of high water quality lakes with varying nutrient levels, researchers have recommended that more sensitive indicators of biological status be used to help determine what appropriate nutrient criteria should be (Reckhow et al 2005, Soranno et al 2008). Additional biological indicators also help to track potential changes in water quality associated with exceeding those criteria. The FDL lakes are unique in their location within the NLF ecoregion, being minimally impacted by human activity, as well being highly colored and shallow systems. Chlorophyll *a* is a commonly and easily measured water quality indicator (APHA 2012), however different algal divisions have distinct differences in pigment composition (Graham, Graham and Wilcox 2008, Paerl and Sandgren 1998). Chlorophyll *a* measurements in lakes with different relative percents of contrasting algal divisions would not be as sensitive an indicator of system change, as would the relative changes among those divisions measured directly. MDS analysis allows the inclusion of multiple taxa or taxa groups within each sample so that subtle adjustments to community composition that accompany changes in water quality can be assessed prior to detectable shifts in more cumulative indicators such as Chla. FDL lakes are already considered to be minimally impacted, thus the PRA provides a good baseline.

FDL lakes cluster with similar shallow, colored systems in the MPR site in terms of FGC composition, but differ dramatically in Chla levels (Table 6, Figures 40 and 41). Nutrients also differ among the FDL and MPR lakes in the Group 1 cluster. MPCB established TP and Chla nutrient criteria for NCHF is quite a bit higher for MPR shallow lakes (TP-60 µg/L, Chla-20 µg/L, Heiskary and Wilson 2008) than for the reference NLF lakes (TP-30 µg/L, Chla-9 µg/L, Heiskary and Wilson 2008). Soranno (2011) suggests lake-specific criteria based on the unique character of FDL lakes, which are also in the NLF ecoregion, that are higher than the more generalized MPCB criteria based on the larger ecoregion. This is due not only to the shallow nature, but also the highly colored open water conditions of the FDL lakes

which is corroborated by the PRA results. This suggests that the best way to track subtle changes in water quality involves multiple indicators, especially when we are trying to manage nutrients before water quality noticeably degrades.

There are 2 examples of how the PRA can assist in the determination of improvement or degradation of lake waters within the Group 1 cluster of the analysis (Figure 42). Third Lake, FDL study site, and Powerderhorn Lake, MPR study site, have both experienced in-lake management activities within the last several years. Powderhorn has had several years of barley straw additions to improve water quality and clarity (2007-present), and Third Lake, which is also susceptible to water level changes during drought periods, has had manure management near the lake and an alum treatment in 2012. Third Lake water quality is also highly susceptible to lower water levels and improved considerably following recovery from several drought years in 2008. Although Powerderhorn Lake water quality improved considerably following barley straw treatment (MPRB 2011), see Figure 9, Chla and TP have not dropped dramatically. Prior to treatment, Powederhorn Lake had much more substantial HAB blooms and very few CP or DY taxa. Following treatment, CP and DY taxa increased and have remained higher into 2012. Third Lake water quality improved in 2008 following release from drought, and has remained relatively high with few HAB taxa (Figure 7), despite fluctuating TP and Chla levels. An alum treatment was completed in 2012 as well, and water quality remains improved into 2014 with dominance by both CP and DY FGCs.

Yearly climate variation (temperature, rainfall, date of ice out) and associated ground water levels should be the most variable driver on the FDL lakes, with the exception of Third Lake which has had some management activity. Due to the more Northern location and proximity to Lake Superior of the FDL lakes, there are several climatic drivers which provide an additional buffer for these lakes over the more southern NCHF lakes. The air temperature (June through October) near the FDL lakes (NOAA, Duluth, Appendix 3) averaged 4.6 ^{o}C lower than the more southern systems near Minneapolis ((NOAA, Minneapolis, Appendix 3)), Figure 43). Climate information was gathered from references listed in Appendix 3. This was a statistically significant difference. Ice out also happens 5-15 days later than the southern lakes sites most years (MDNR, Ice Out, Figure 44). This lower average growing season temperature, coupled with later ice out buffers the FDL lakes with a shorter growing season, especially considering that HAB taxa do best at temperatures above 25 ^{o}C and a relatively long spring/early summer window**.** Total rainfall (Figure 45a), as well as "high volume" rainfall events (Figure 45b) vary between the FDL and southern MPR/MCWD study sites as well. Although the yearly clusters were somewhat random for just the FDL lakes, it was more a linear pattern with all lakes combined as contiguous years clustered together. Part of this may be due to the larger number of high rain events in the early and late 2000's (especially 2012) in the FDL study site and in the 2005-2007 period in the southern study sites. High rain years will often have a profound effect on the next growing season as nutrients from the surrounding watershed get washed into the lake and groundwater levels rise. Timing of high rain events (ie. early or late in the growing season) balances movement of materials into the lake versus dilution from higher lake levels.

Calibration results were extremely informative considering that no quantitative studies have been completed comparing the results of the PRA to either full numeric quantitative counts replicated

by the same analyst, or comparing the results of the PRA among independent analysts outside of the Minnesota Pollution Control Agency since its original description in 1989. Interestingly, both analysts independently expanded the PRA to include all taxa encountered (although via slightly different techniques) which dramatically improved the usefulness of the PRA. The use of FGCs helped normalize the data for professional identification differences among the analysts. Salmosa et al (2015) indicate that FGCs are most useful in interpreting ecological function and classification when taxonomy is accurate and incorporated into the classification scheme. Similar to the MFG (Morpho-Functional Group) functional classification discussed by Salmosa et al (2015) that incorporates taxonomy and function, the PRA FGCs as determined by both St. Amand and Ruzycki would be a suitable tool for assessing water quality changes over time and the importance of different environmental drivers. This includes the higher relative contribution by the CP group in highly colored waters typical of the the FDL fish lakes and some shallow colored lakes in the MPR study site, and the potential increase in HAB/HAB1 taxa associated with Cyanobacteria driven harmful algal blooms in water dominated by high nutrient agricultural runoff typical of the MCWD study site. The PRA accurately tracked increases in water quality among the different lakes given in lake and watershed mitigation when other water quality indicators did not (e.g. Powderhorn Lake, MPR, and Third Lake, FDL, Figure 41).

Summary

Soranno (2011) described a method for determining lake-specific nutrient criteria based on the unique, colored and shallow state of the FDL lakes. In this report, PRA data dating from 1998 to 2012 was used to assess past and current biological state, and substantiated the unique, higher water quality character of the FDL lakes. Two other study sites from the Northern Central Hardwood Forest ecoregion were used for comparison that included that contained a variety of lakes and productivities, bracketing the FDL lakes. Using the PRA, the FDL lakes were stable ecologically in their algal community response over the entire study period, and did not statistically significantly cluster within the FDL study site. Based on MDS analysis, the lakes from all three study sites were combined into five Lake Groups, which were a combination of six statistically significant clusters. The FDL lakes did cluster together uniquely within the the larger database of 47 lakes along with several of the shallower, more colored MPR lakes of higher water quality (Group 1). Group 1 lakes were characterized by approximately 50% CP, DY taxa and a mix of G, BG and E/O taxa, with a relative low percent of HAB/HAB1 taxa. Group 5, the most productive group of lakes, was characterized by over 80% HAB/HAB1 taxa, which were entirely within the MCWD study site (high percent agricultural runoff). Results from the PRA algal data corroborate Soranno's approach of lake specific nutrient criteria for the FDL fish lakes. As such, algal data remains the most sensitive indicator of water quality in the FDL lakes. A calibration study was conducted in 2014 comparing the quantitative counts with biovolume estimates to the PRA, since this is the origin of the entire FDL algal database (St. Amand). The calibration study also compared PRA results on FDL fisheries lakes from two independent taxonomists (Elaine Ruzycki, Natural Resources Research Institute, University MN-Duluth and Ann St. Amand, PhycoTech) taken from May-October, 2014. The results of the quantitative portion of the calibration study indicated that the percent biovolume from quantitative counts tracked well with the PRA from each FDL lake in data generated by St. Amand. There was also excellent agreement among independent analysts (St. Amand and Ruzycki) for the 2014 data as well, resulting in similar clustering for all FDL lakes. This high correspondence between analysts and among numeric response variables leads to a high level of confidence in the use of the PRA as a valid clustering technique.

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FDL Tables, Figures, and Appendices

Figure 1. Map of Minnesota with study sites marked.

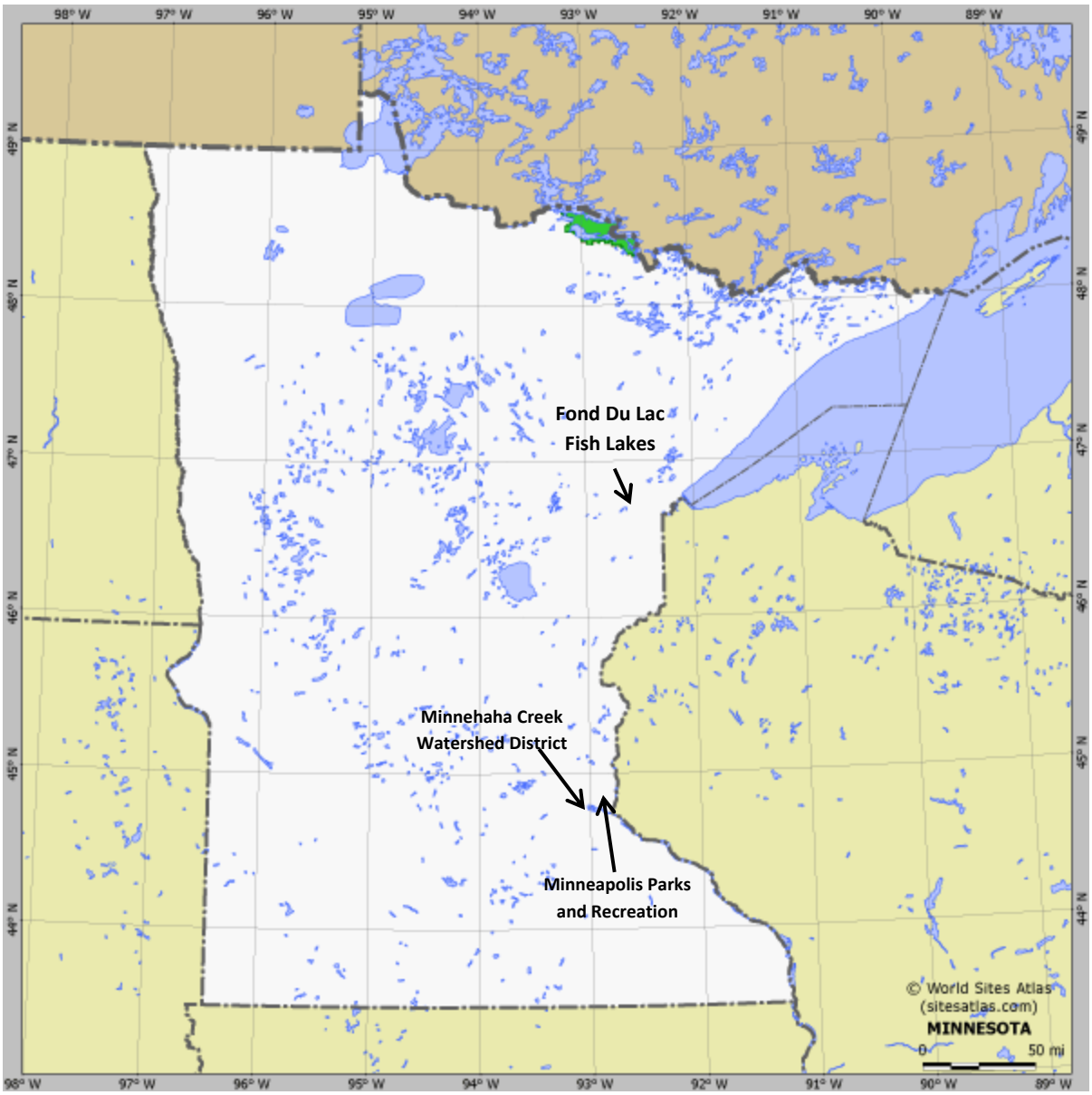


Figure 2. Map of Fond du Lac fish lakes.

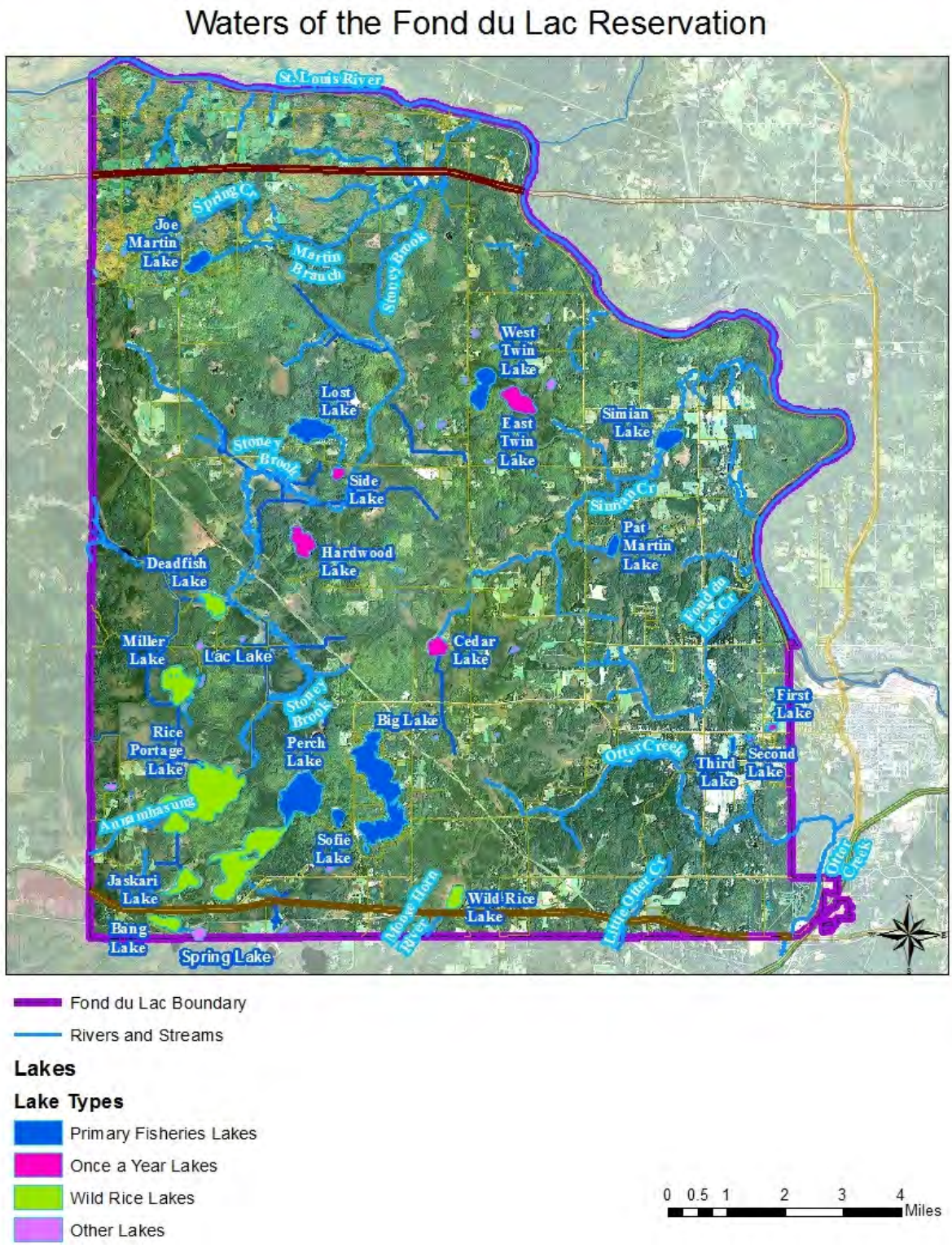


Figure 3. Map of Minneapolis Parks and Recreation lakes.

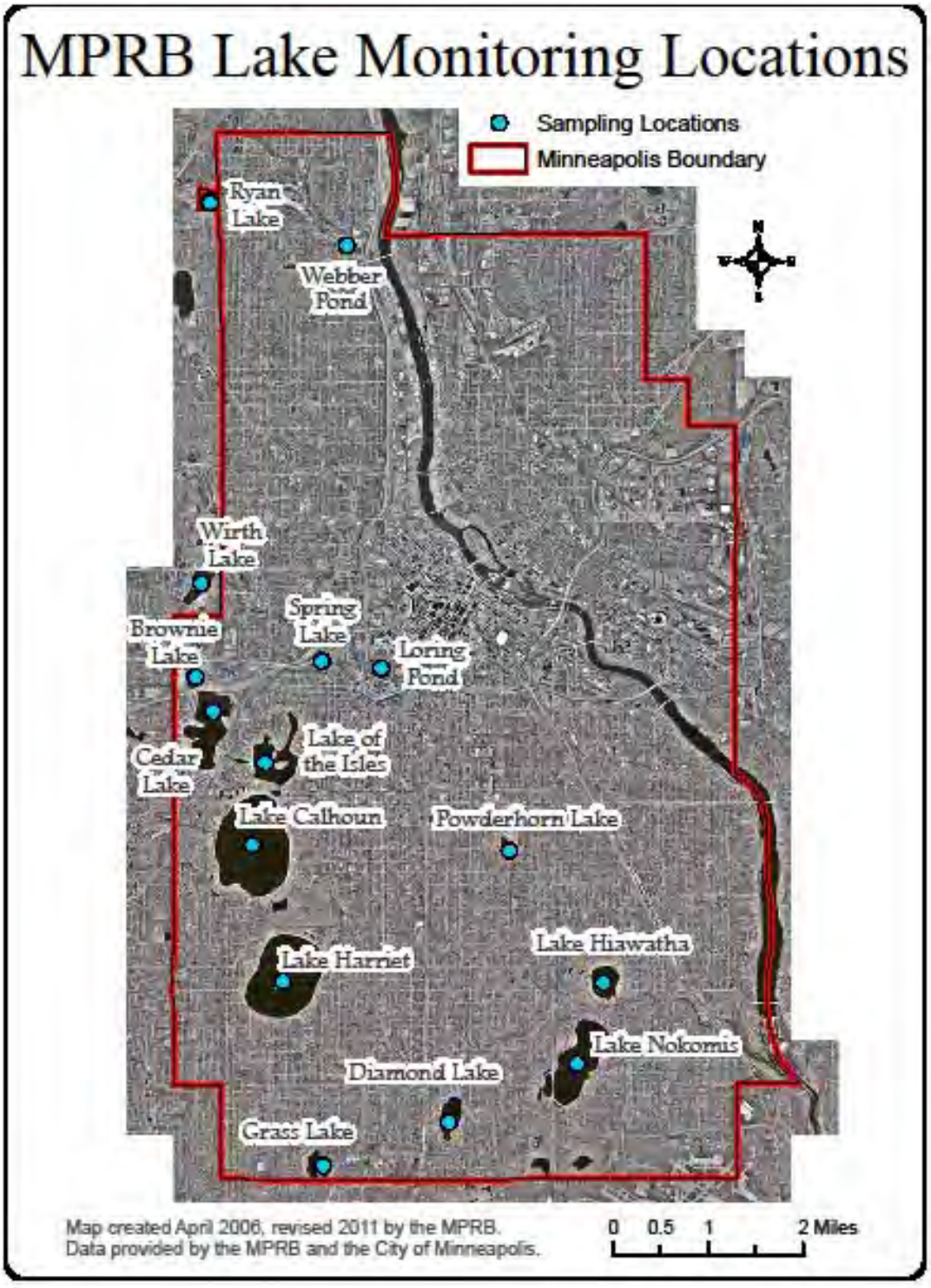


Figure 4. Map of Minnehaha Creek Watershed District Lakes.

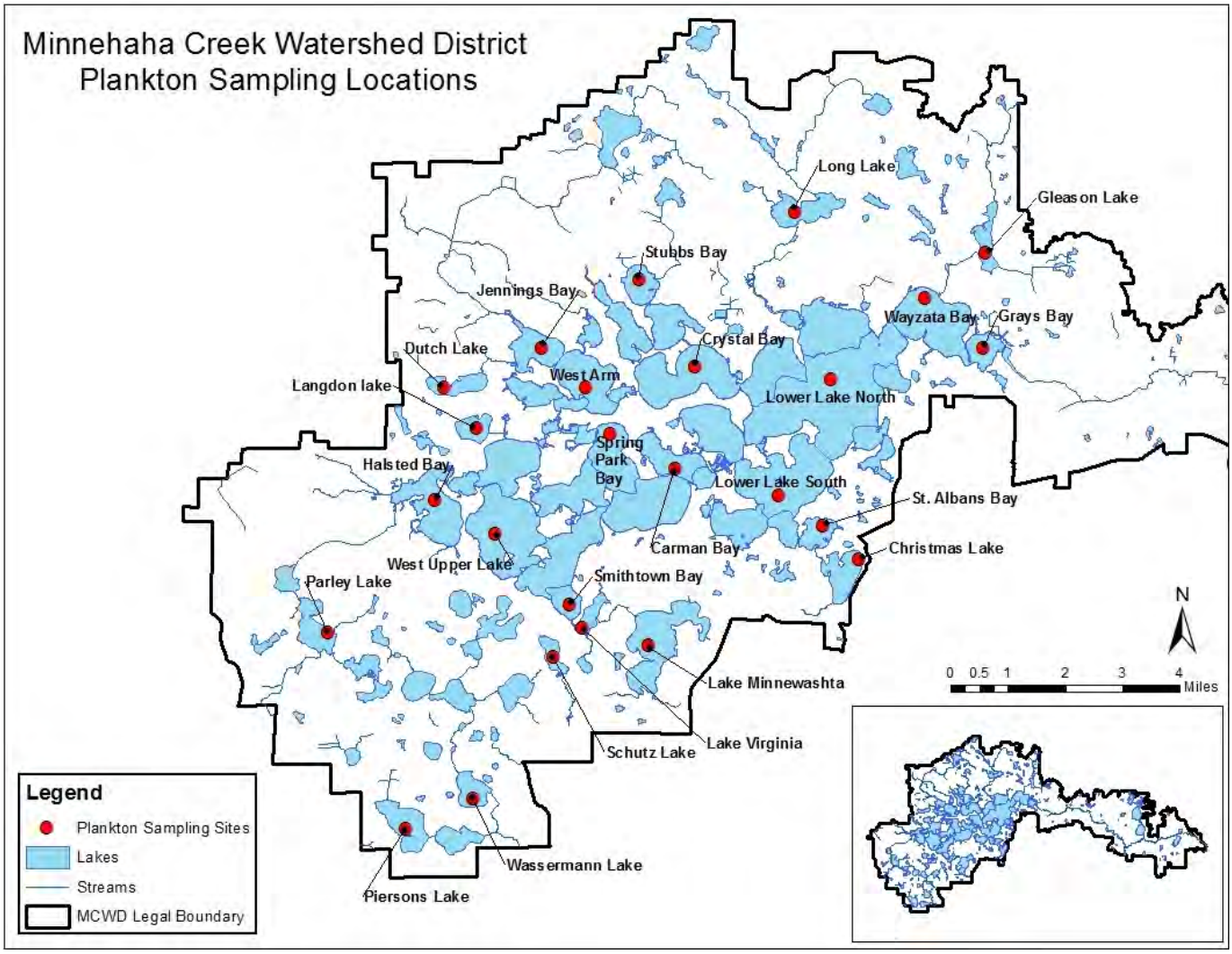


Table 1. Lake Characteristics. Appendix 2 lists all sources for watershed data. NCHF=Northern Central Hardwood Forest, NLF=Northern Lakes Forest.

Lake Name	Management District	MN Ecoregion	Max Depth, m	Surface Area, ha	Watershed Area, ha	Percent Ag
Brownie	MPR	NCHF	4.63	4.9	149.3	0
Calhoun	MPR	NCHF	8.35	165.1	1210.8	0
Cedar	MPR	NCHF	4.72	68.8	791.6	0
Diamond	MPR	NCHF	0.64	21.9	270.7	0
Grass	MPR	NCHF	0.46	10.9	156.2	0
Harriet	MPR	NCHF	7.62	142.9	460.9	0
Hiawatha	MPR	NCHF	2.13	21.9	46878.8	0
Isles	MPR	NCHF	2.87	41.7	297.4	0
Loring	MPR	NCHF	1.62	3.2	9.7	0
Nokomis	MPR	NCHF	3.08	82.6	351.7	0
Powderhorn	MPR	NCHF	1.86	4.5	115.7	0
Spring	MPR	NCHF	2.59	1.2	18.2	0
Webber	MPR	NCHF	0.61	1.2	0.8**	0
Wirth	MPR	NCHF	2.41	15.8	140.8	0
Christmas Lake	MCWD	NCHF	8.23	111.7	300.3	0
Dutch Lake	MCWD	NCHF	3.96	64.7	764.0	5
Gleason Lake	MCWD	NCHF	1.52	63.1	1524.0	0.2
Minnewashta Lake	MCWD	NCHF	6.40	265.5	688.0	50
Lake Virginia	MCWD	NCHF	3.05	40.1	140.4	5.6
Langdon Lake	MCWD	NCHF	3.66	58.3	426.9	9
Long Lake	MCWD	NCHF	3.05	58.3	3325.7	9
Parley Lake	MCWD	NCHF	1.52	100.0	333.2	50
Piersons Lake	MCWD	NCHF	3.66	112.1	485.5	50
Schultz Lake	MCWD	NCHF	4.57	42.5	392.1	22.9
Wassermann Lake	MCWD	NCHF	3.66	61.9	390.6	50
Carmen Bay	MCWD	NCHF	5.49	163.1	3276.1	0
Crystal Bay	MCWD	NCHF	7.62	325.8	2296.1	0
Gray's Bay	MCWD	NCHF	2.74	74.5	9726.6	0
Halsted Bay	MCWD	NCHF	3.35	230.7	6891.8	29.5
Jennings Bay	MCWD	NCHF	2.44	121.4	3507.4	17.4
Lower Lake North	MCWD	NCHF	7.62	407.9	8932.6	0
Lower Lake South	MCWD	NCHF	7.32	432.6	1085.6	0
Smithtown Bay	MCWD	NCHF	6.40	179*	1920.5	0
St. Albans Bay	MCWD	NCHF	3.66	64.7	153.4	0
Stubbs Bay	MCWD	NCHF	3.05	79.7	707.8	12
Wayzata Bay	MCWD	NCHF	4.88	303.9	9431.5	0
West Arm	MCWD	NCHF	3.66	234.7	1109.5	0
West Upper Lake	MCWD	NCHF	7.32	355.7	1920.5	0
Big Lake	FDL	NLF	6.1	212.1	507.0	0

Lake Name	Management District	MN Ecoregion	Max Depth, m	Surface Area, ha	Watershed Area, ha	Percent Ag
Joe Martin Lake	FDL	NLF	23.5	27.1	1808.0	0
Lost Lake	FDL	NLF	3.40	55.0	122.0	0
Pat Martin Lake	FDL	NLF	4.6	14.2	5314.0	0
Perch Lake	FDL	NLF	5.2	89.0	1832.0	0
Simian Lake	FDL	NLF	3.70	33.2	5314.0	0
Sofie Lake	FDL	NLF	4.90	14.2	85.0	0
Third Lake	FDL	NLF	6.10	6.1	50.0	0
West Twin Lake	FDL	NLF	5.50	49.0	245.0	0

Table 2. Data availability for Fond du Lac (FDL), Minneapolis Parks and Recreation (MPR), and Minnehaha Creek Watershed District (MCWD) Study Sites.

	FDL		MPR		MCWD	
Parameter	Data Range	Missing Years	Data Range	Missing Years	Data Range	Missing Years
Algae	1998-2012		2003-2012		2006-2012	
Chlorophyll-A	1999-2012		2003-2012		2006-2012	
Secchi	1999-2008	2009-2012	2003-2012		2009-2013	2006-2008
Dissolved Oxygen	1999-2012		2003-2012		2006-2012	
Water Temperature	1999-2012		2003-2012		2006-2012	
pH	1999-2012		2003-2012		2006-2012	
Turbidity	1999-2012		2007-2012	2003-2007		2006-2012
Total Phosphorus	1999-2012		2003-2012		2006-2012	
Total Nitrogen	1999-2012		2003-2012		2006-2012	
TN:TP	1999-2012		2003-2012		2006-2012	
DOC/Color	1999-2009	2010-2012		2003-2012		2006-2012

Table 3. Algal Composition of the Functional Group Classifications (FGC).

Functional Group Classification	Taxa/Division
BG	Non-toxin producing Cyanobacteria
HAB	Toxin producing heterocystic Cyanobacteria
HAB1	Toxin producing non-heterocystic Cyanobacteria
CP	Cryptophytes/Dinoflagellates
CP1	*Ceratium hirundinella*
DY	Chrysophytes/Diatoms
G	Chlorophytes
E	Euglenophytes
O	Other/Miscellaneous

Table 4. Sparse Samples Eliminated from Analysis

Study Site	Lake	PhycoTech Tracking ID	Year	Date
MPR				
	Spring	030090	2003	14-Jul
	Webber	030091	2003	9-Jul
	Wirth	030092	2003	8-Jul
	Calhoun	030103	2003	22-Jul
	Cedar	030104	2003	22-Jul
	Harriet	030105	2003	23-Jul
	Hiawatha	030106	2003	24-Jul
	Isles	030107	2003	22-Jul
	Loring	030108	2003	27-Jul
	Wirth	040061	2004	11-Jun
	Grass	100013	2010	5-May
	Calhoun	130001	2013	12-Feb
	Calhoun	130002	2013	6-May
	Webber	130049	2013	11-Jun
MCWD				
	Christmas	060001	2006	17-Apr
	Christmas	060002	2006	16-May
	Stubbs	070050	2007	2-Aug
	Grays	120046	2012	28-Jun
	Wayzata	120058	2012	28-Jun
	Smithtown	130040	2013	13-Aug
	Lower Lake South	140005	2014	5-May
	Grays	140057	2014	4-Aug
	St. Albans	140061	2014	6-Aug
FDL				
	Big Lake	NA	2001	6-Aug
	Lost Lake	NA	2001	7-Aug
	Third Lake	NA	2001	11-Oct
	West Twin Lake	NA	2001	6-Aug
	Joe Martin Lake	NA	2003	6-Aug
	Big Lake	NA	2004	9-Aug
	Perch Lake North	NA	2007	7-Aug
	Perch Lake South	NA	2012	10-Oct

Figure 5. Monthly MDS using MPR and MCWD lakes.

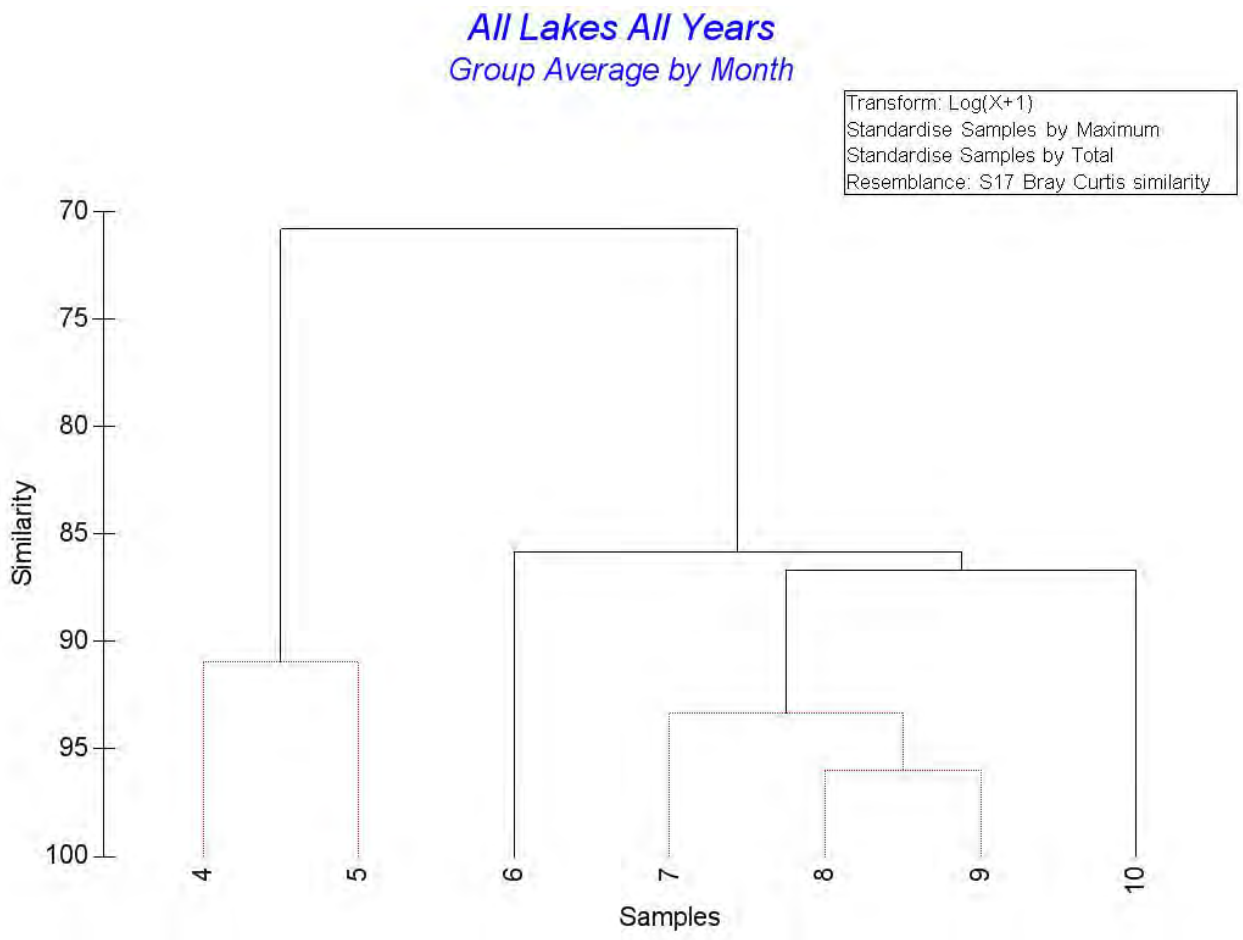


Figure 6. Chlorophyll a concentration (µg/L) of the Fond du Lac lakes, averaged by lake (July through October).

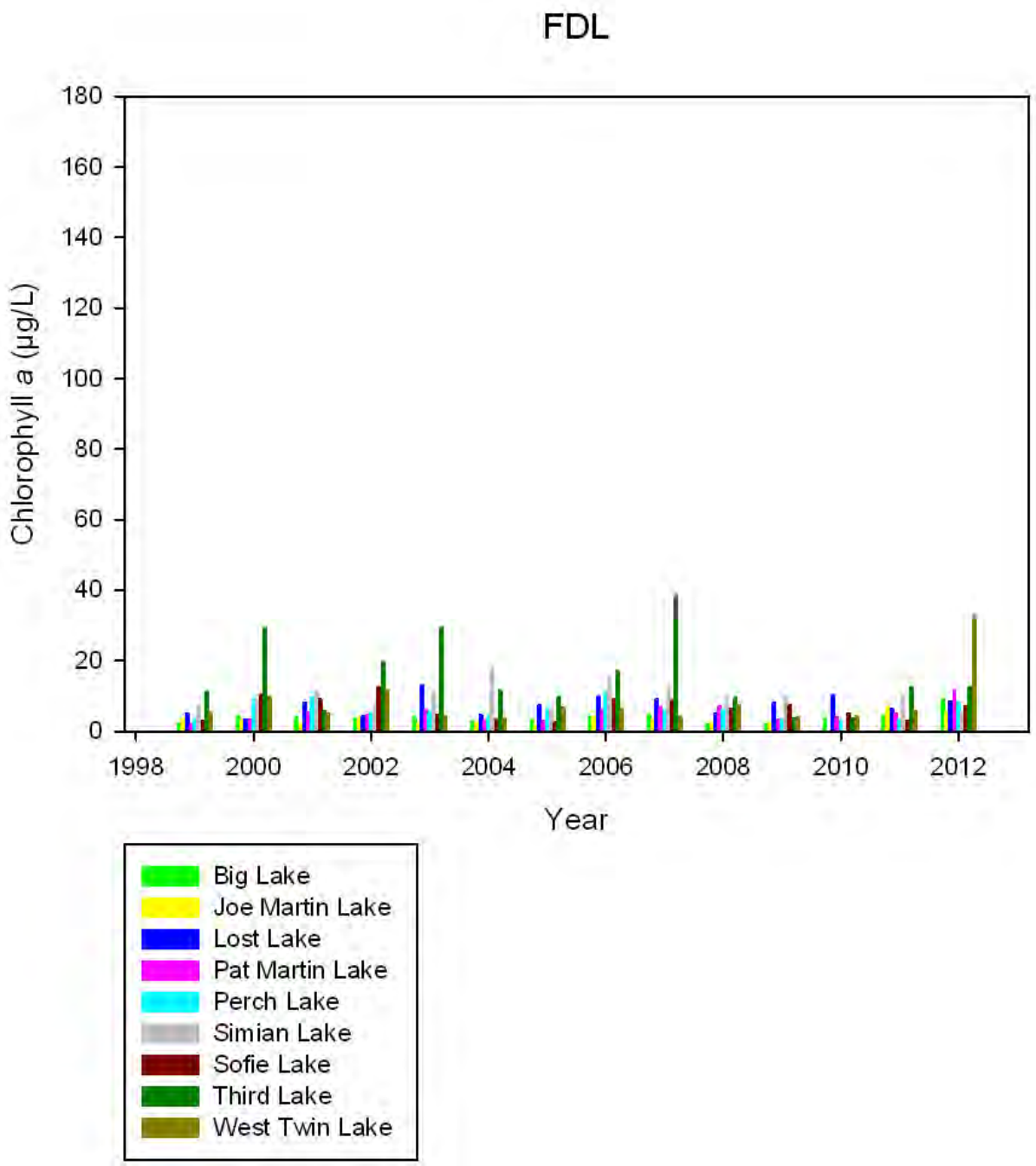


Figure 7. Relative percent algal composition by functional group of the Fond du Lac lakes, averaged by year (July through October).

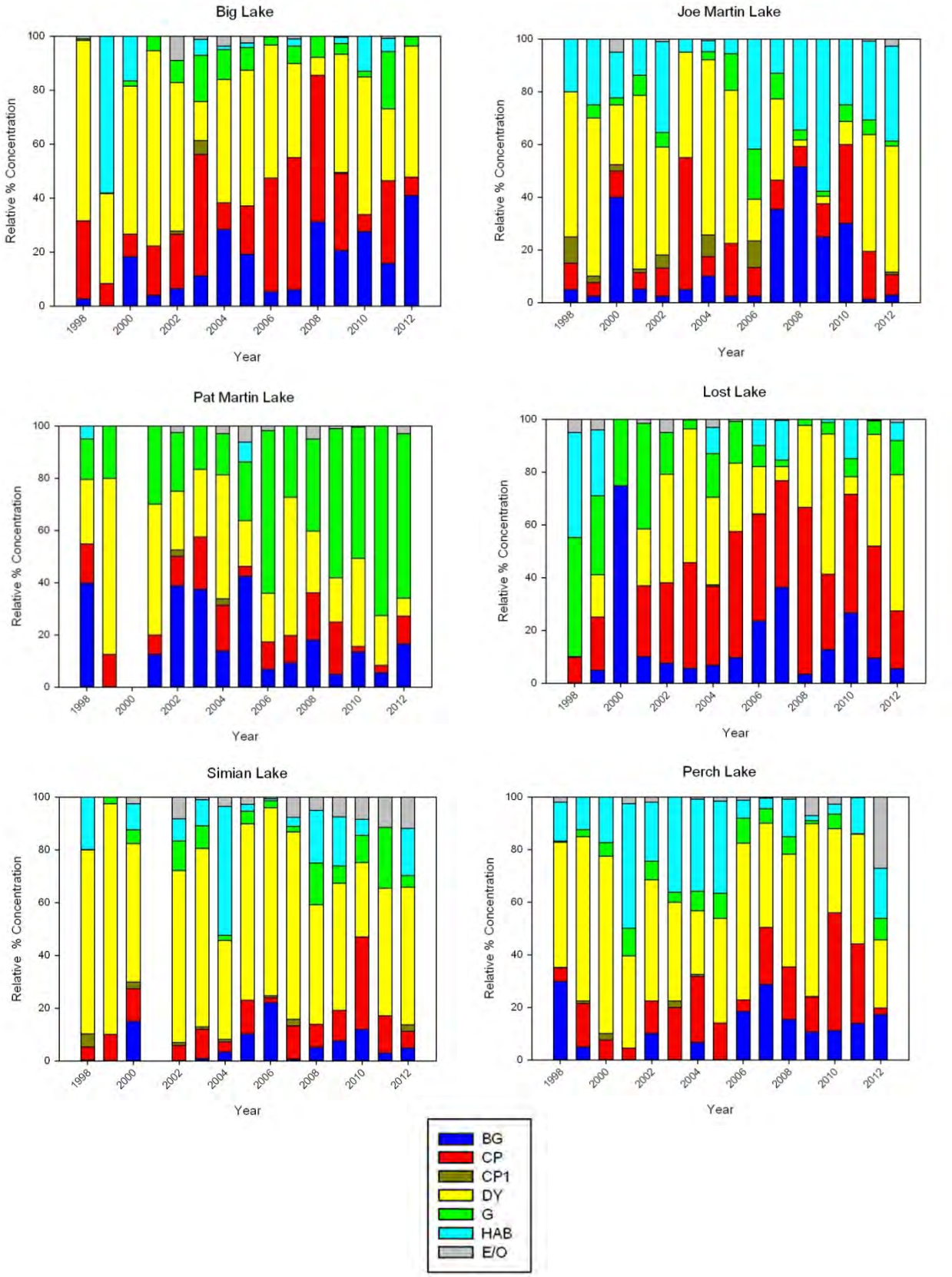


Figure 7. Relative percent algal composition by functional group of the Fond du Lac lakes, averaged by year (July through October). (continued)

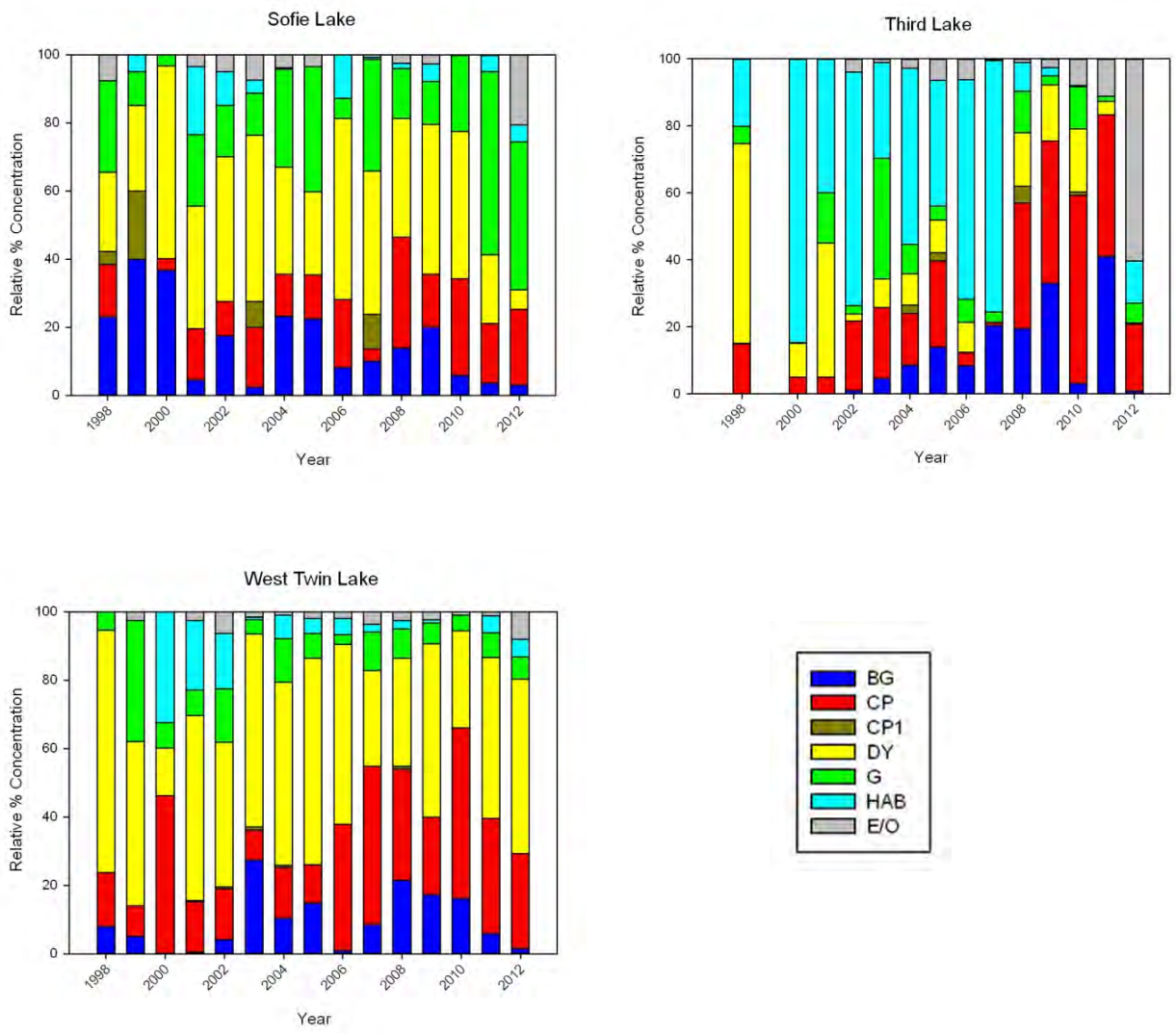


Figure 8. Chlorophyll a concentration (µg/L) of the Minneapolis Parks and Recreation lakes, averaged by lake (July through October).

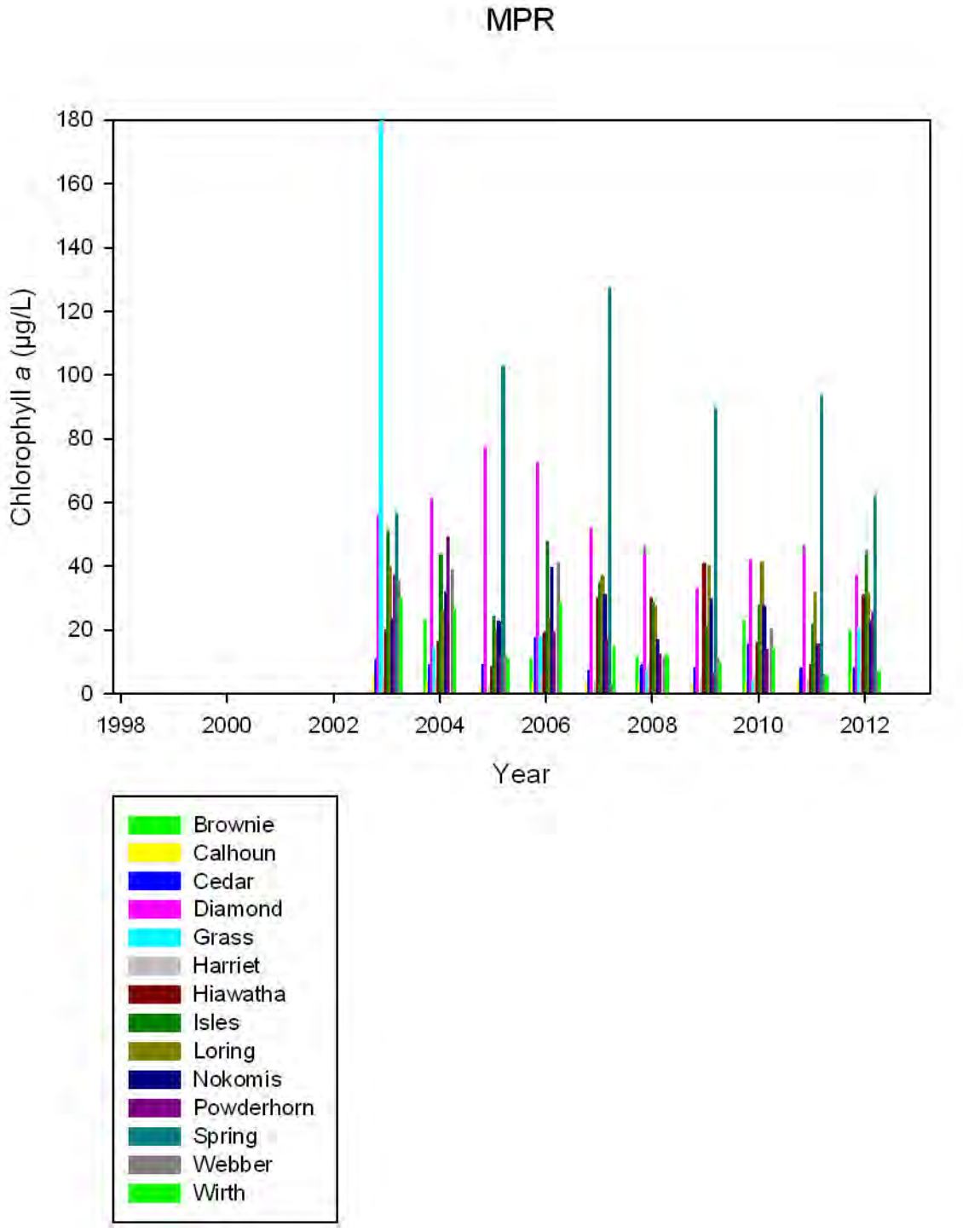


Figure 9. Relative percent algal composition by functional group of the Minneapolis Parks and Recreation Lakes, averaged by year (July through October).

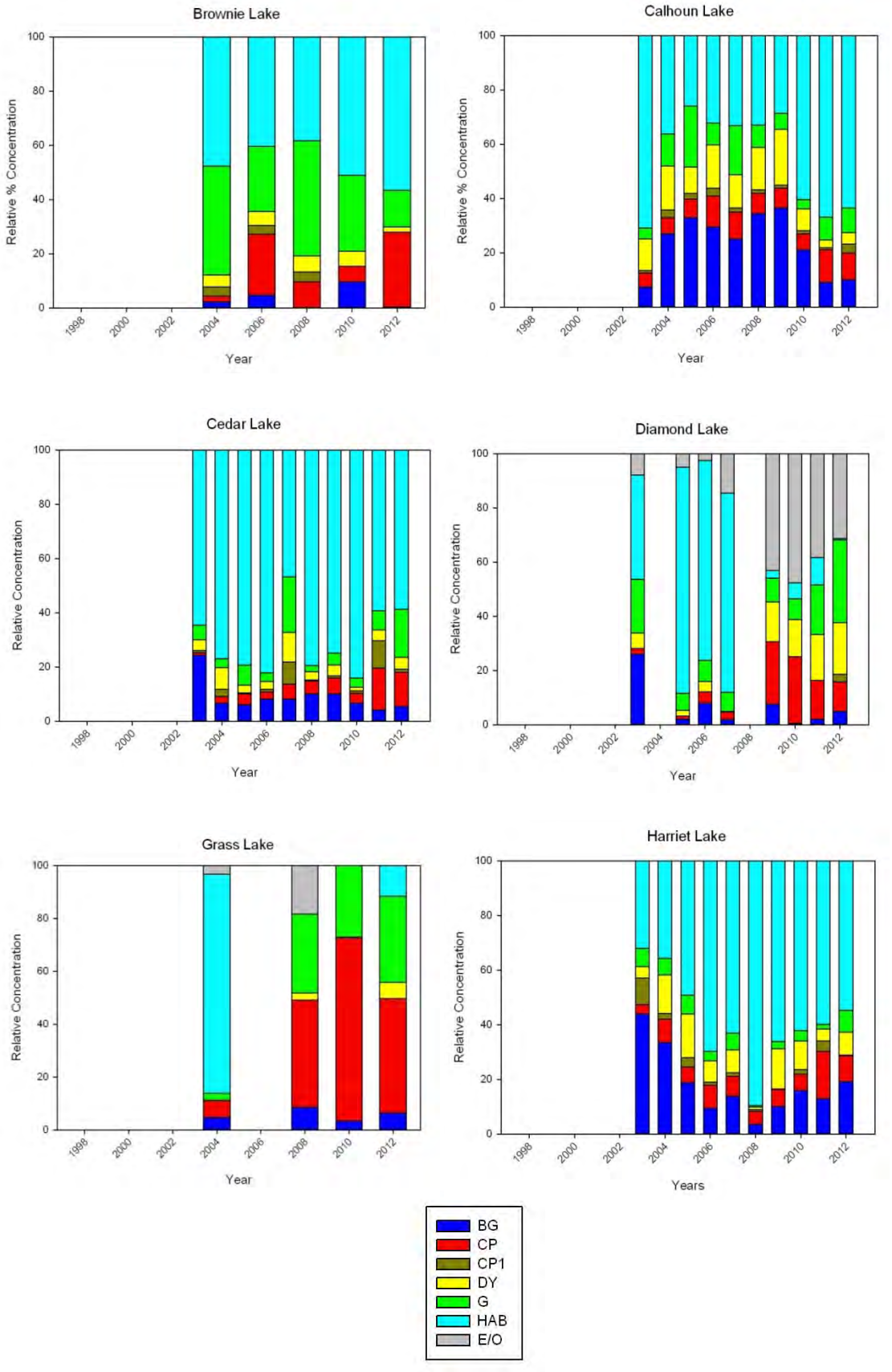


Figure 9. Relative percent algal composition by functional group of the Minneapolis Parks and Recreation Lakes, averaged by year (July through October). (continued)

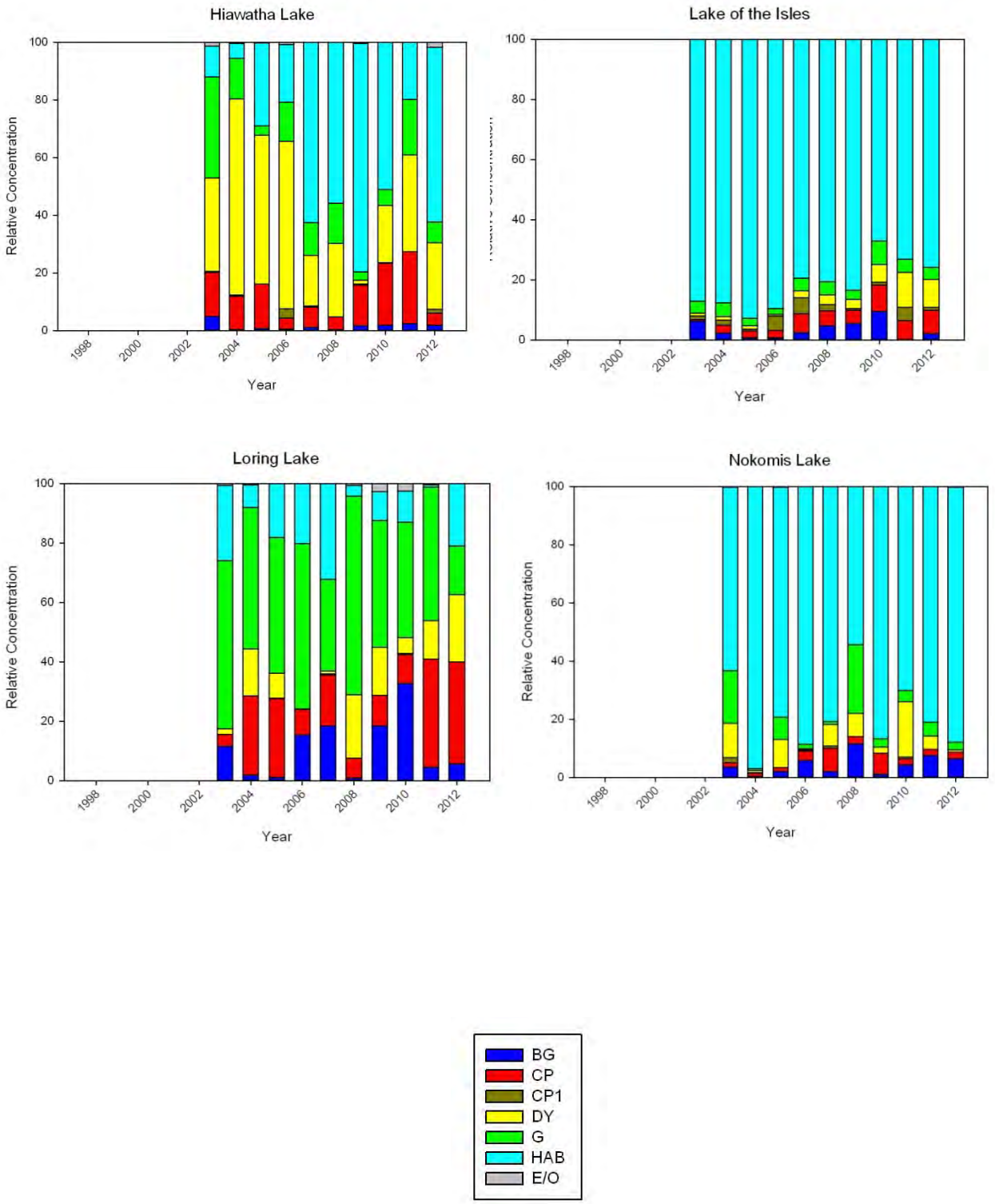


Figure 9. Relative percent algal composition by functional group of the Minneapolis Parks and Recreation Lakes, averaged by year (July through October). (continued)

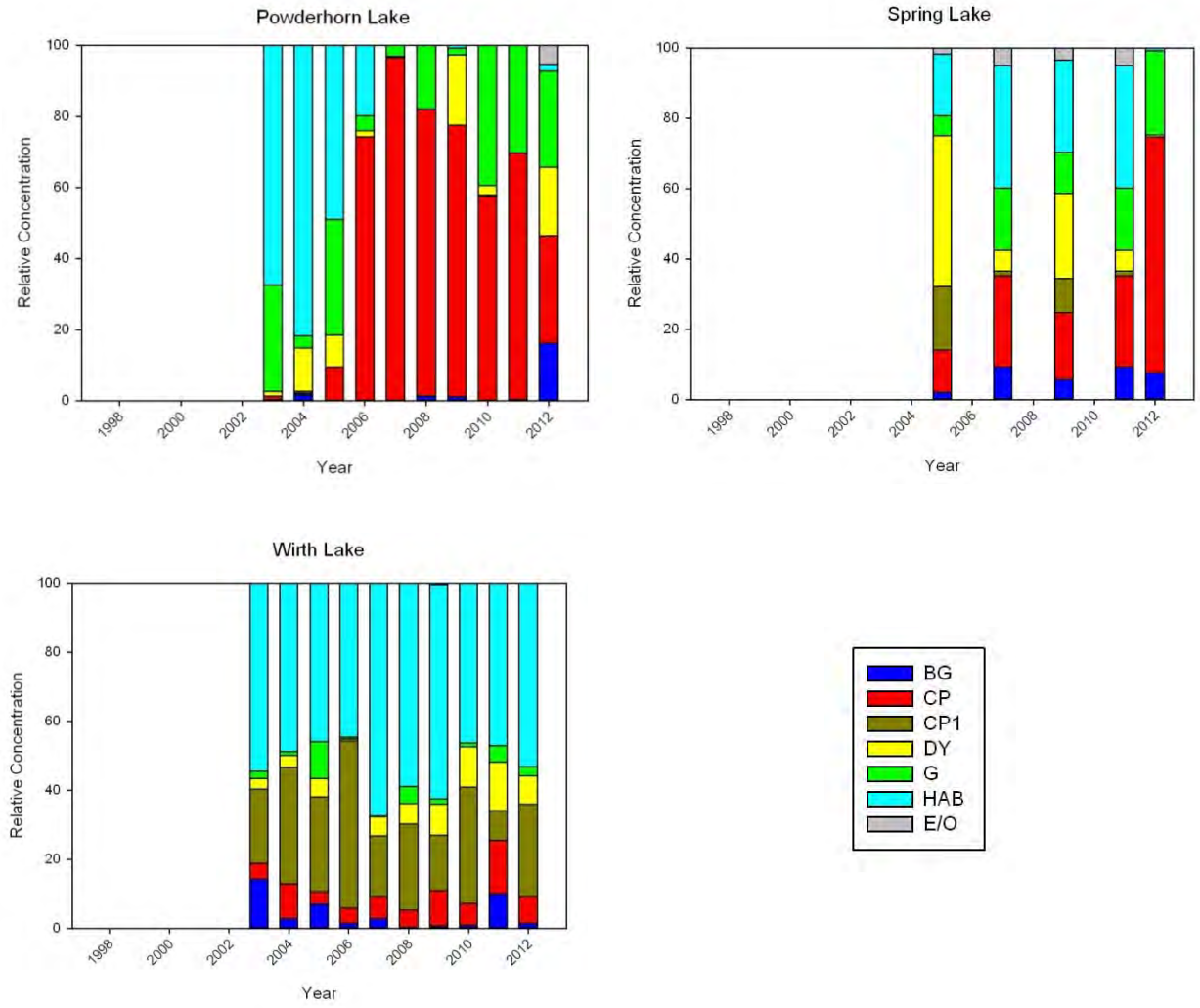


Figure 10. Chlorophyll a concentration (µg/L) of the Minnehaha Water Creek District Lake Minnetonka Bays and Lakes, averaged by lake (July through October).

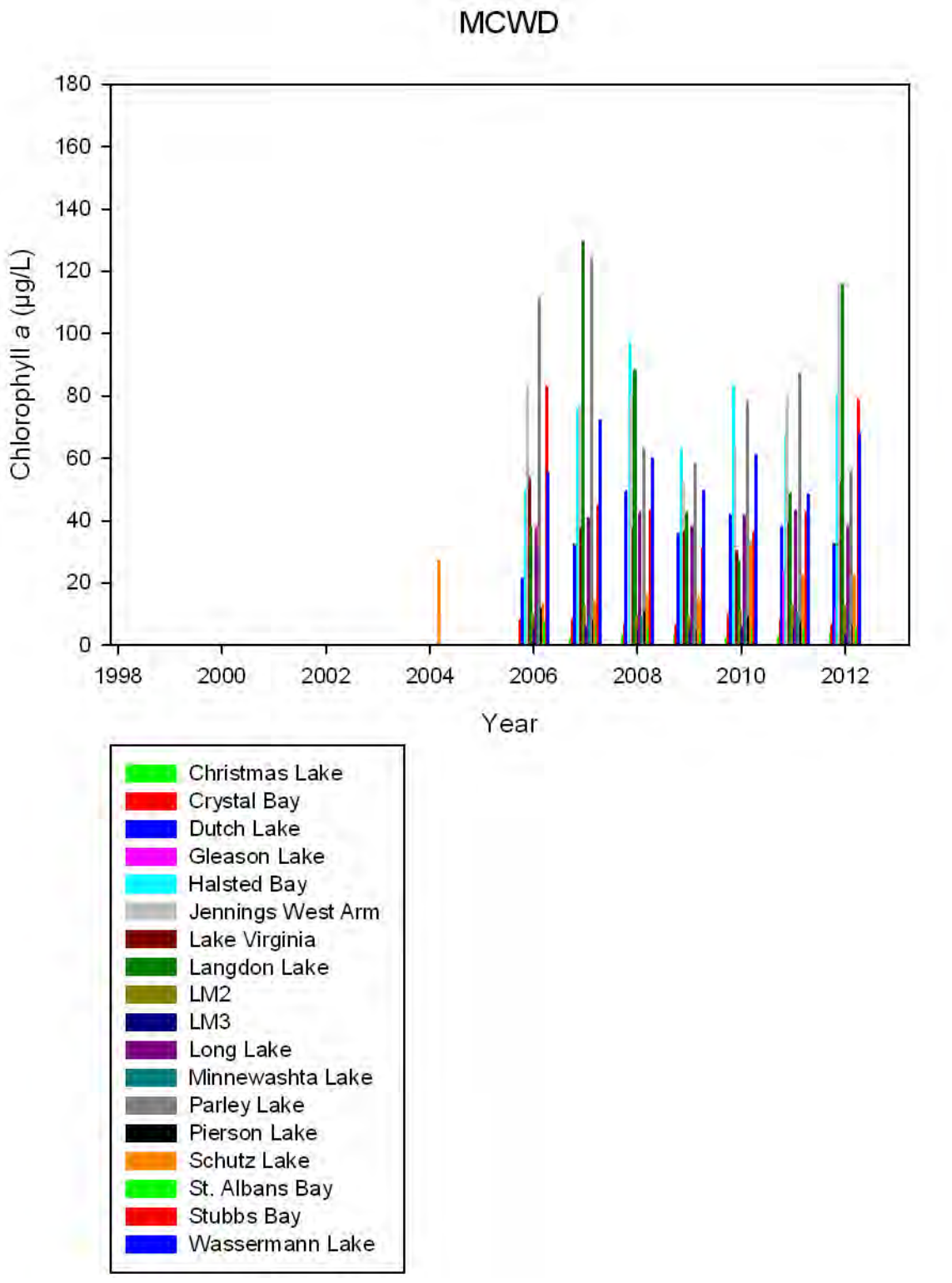


Figure 11. Relative percent algal composition by functional group of the Minnehaha Water Creek District Lakes, averaged by year (July through October).

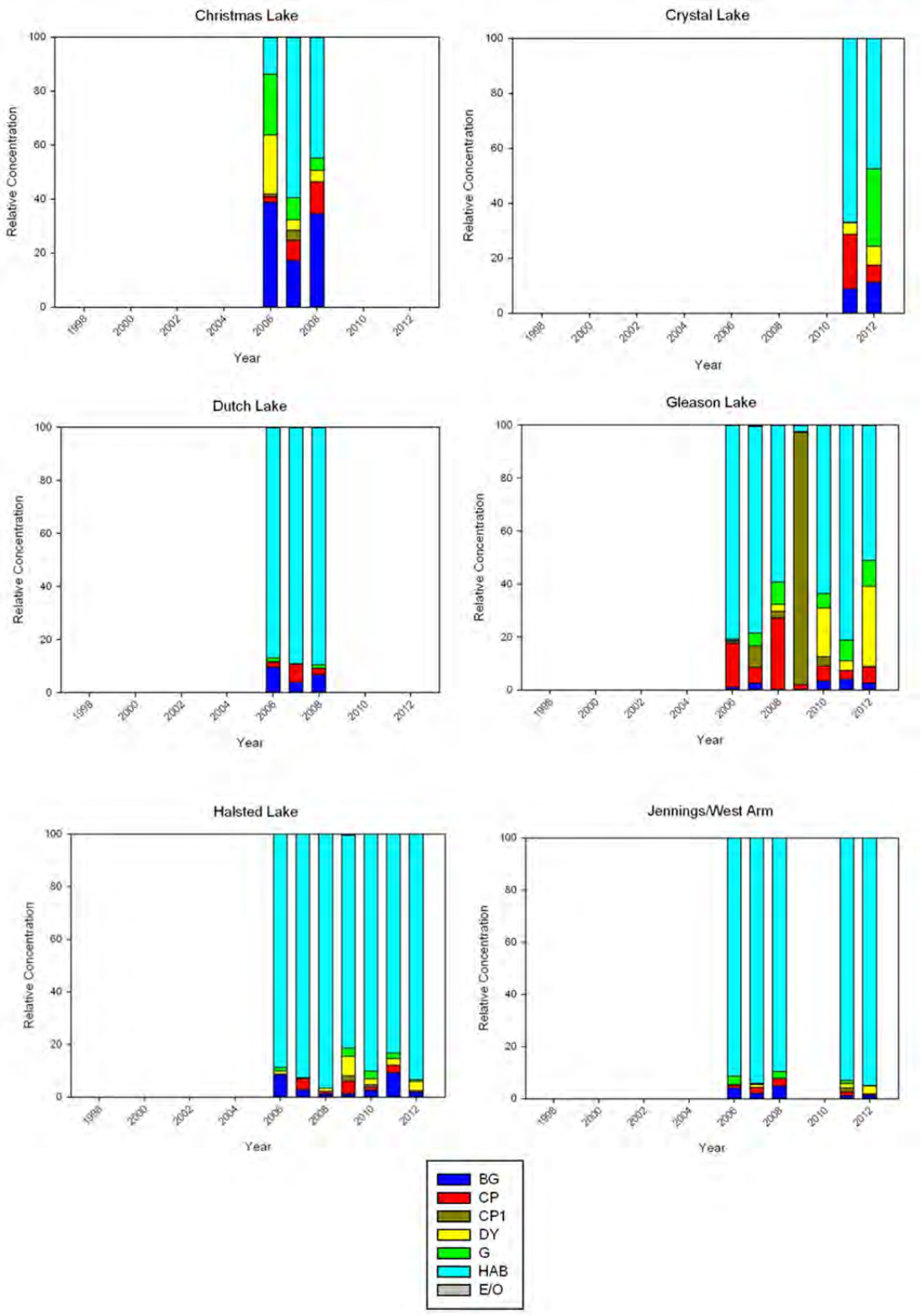


Figure 11. Relative percent algal composition by functional group of the Minnehaha Water Creek District Lakes, averaged by year (July through October). (continued)

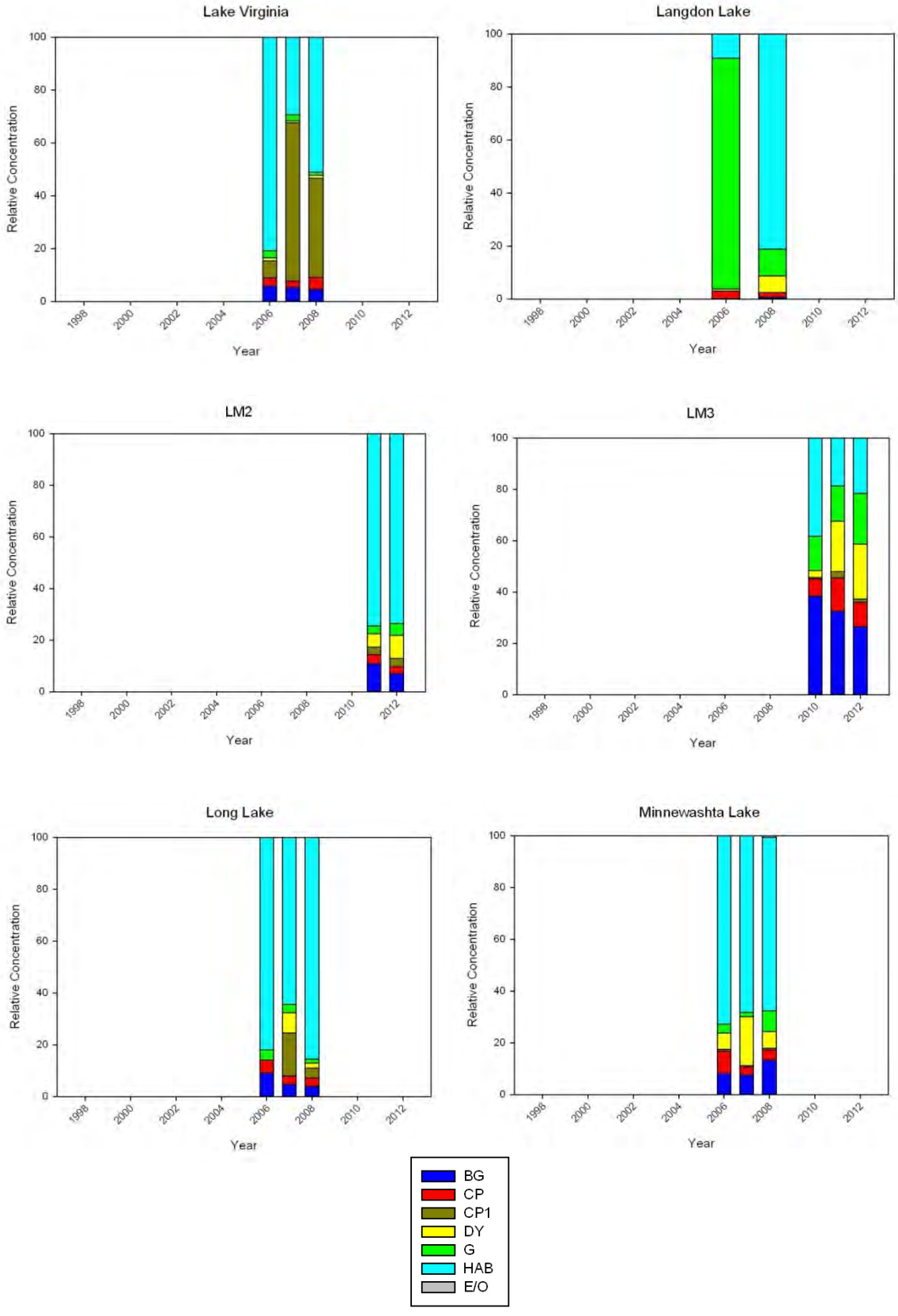


Figure 11. Relative percent algal composition by functional group of the Minnehaha Water Creek District Lakes, averaged by year (July through October). (continued)

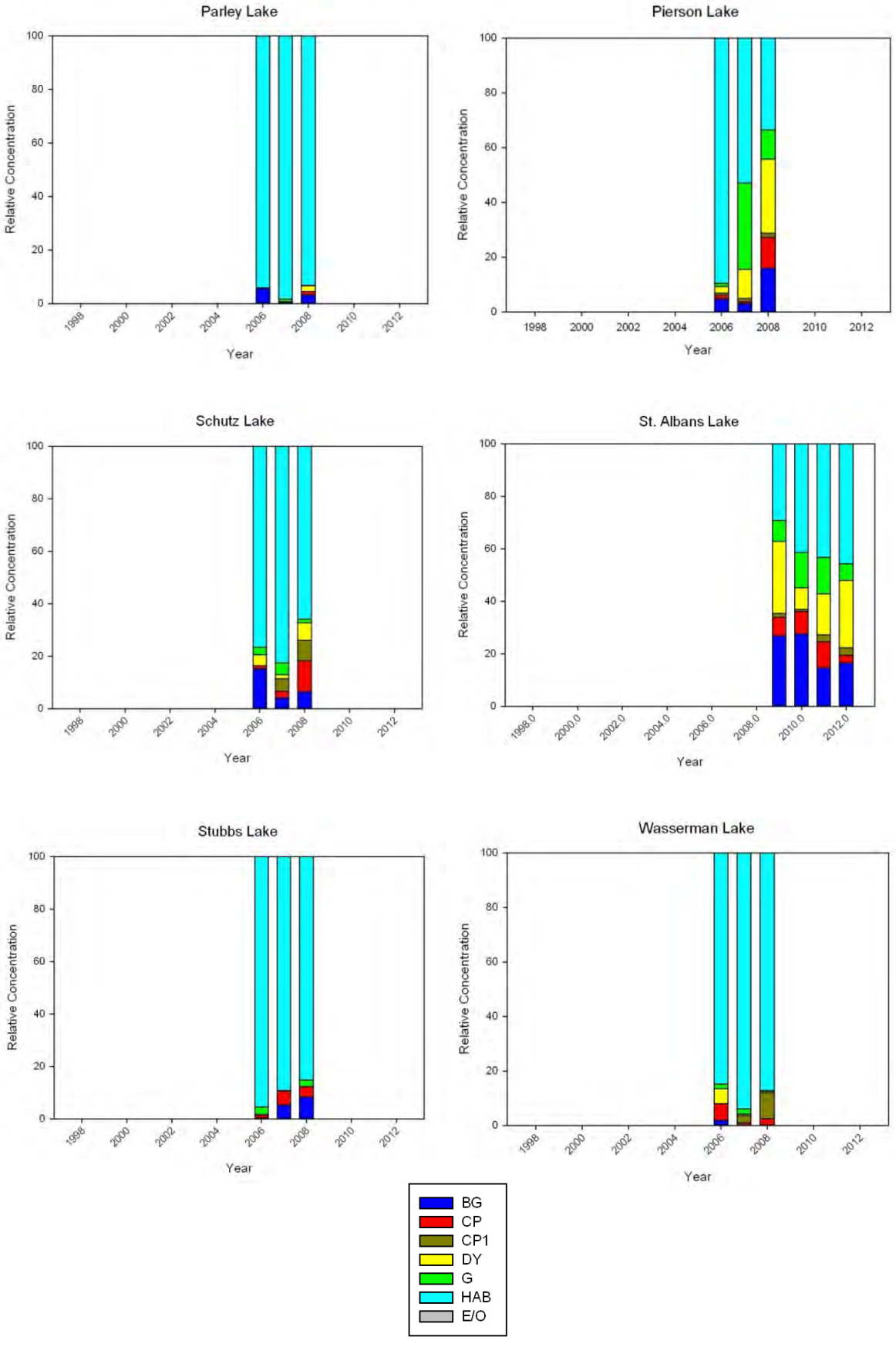


Figure 12. Cluster Diagram of all lakes from Fond du Lac, averaged by year, July through October Samples. Significance is denoted by a solid black line (p<0.01).

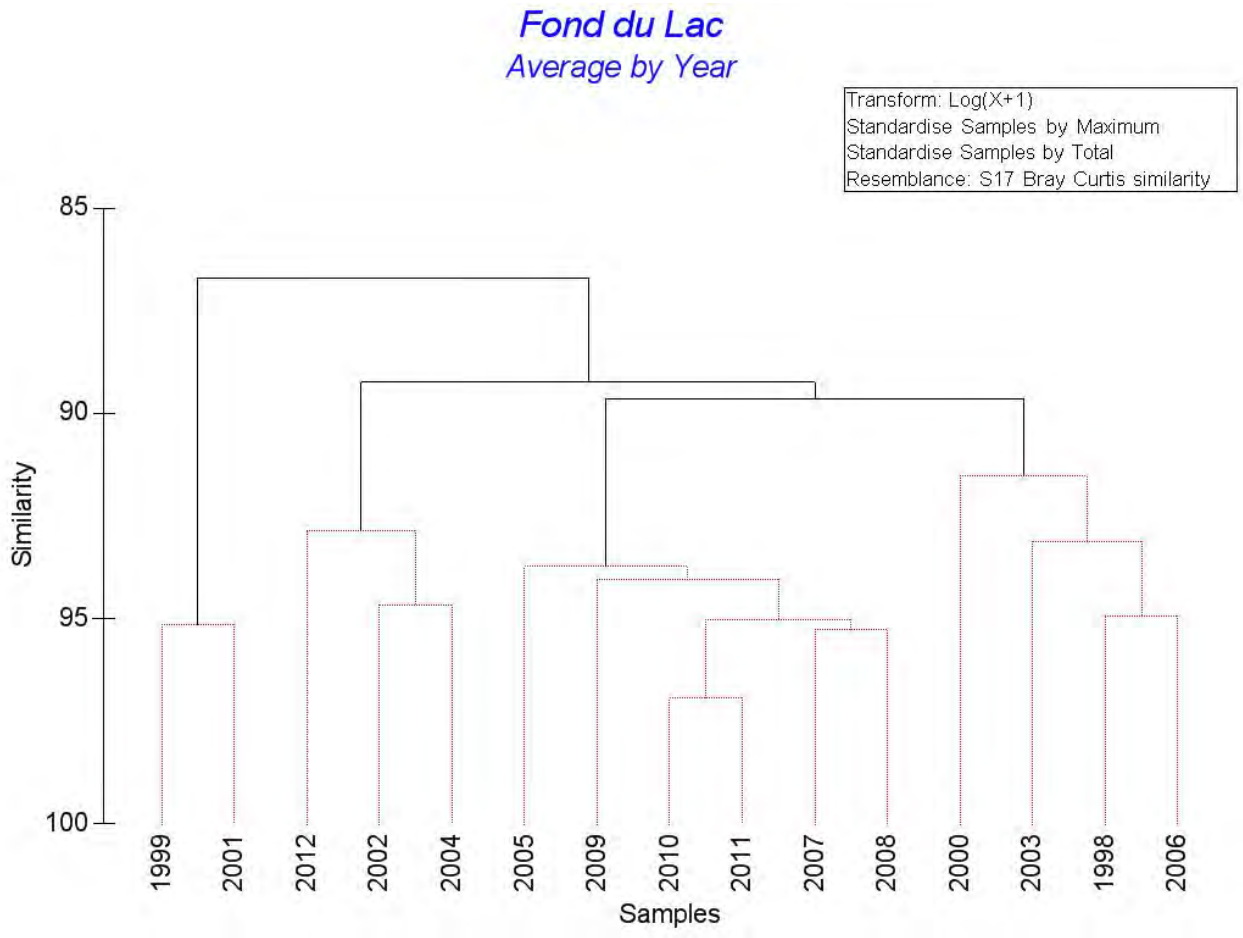


Figure 13. Cluster Diagram of all fish lakes from Fond du Lac, averaged by lake, July through October Samples. Significance is denoted by a solid black line (p<0.01).

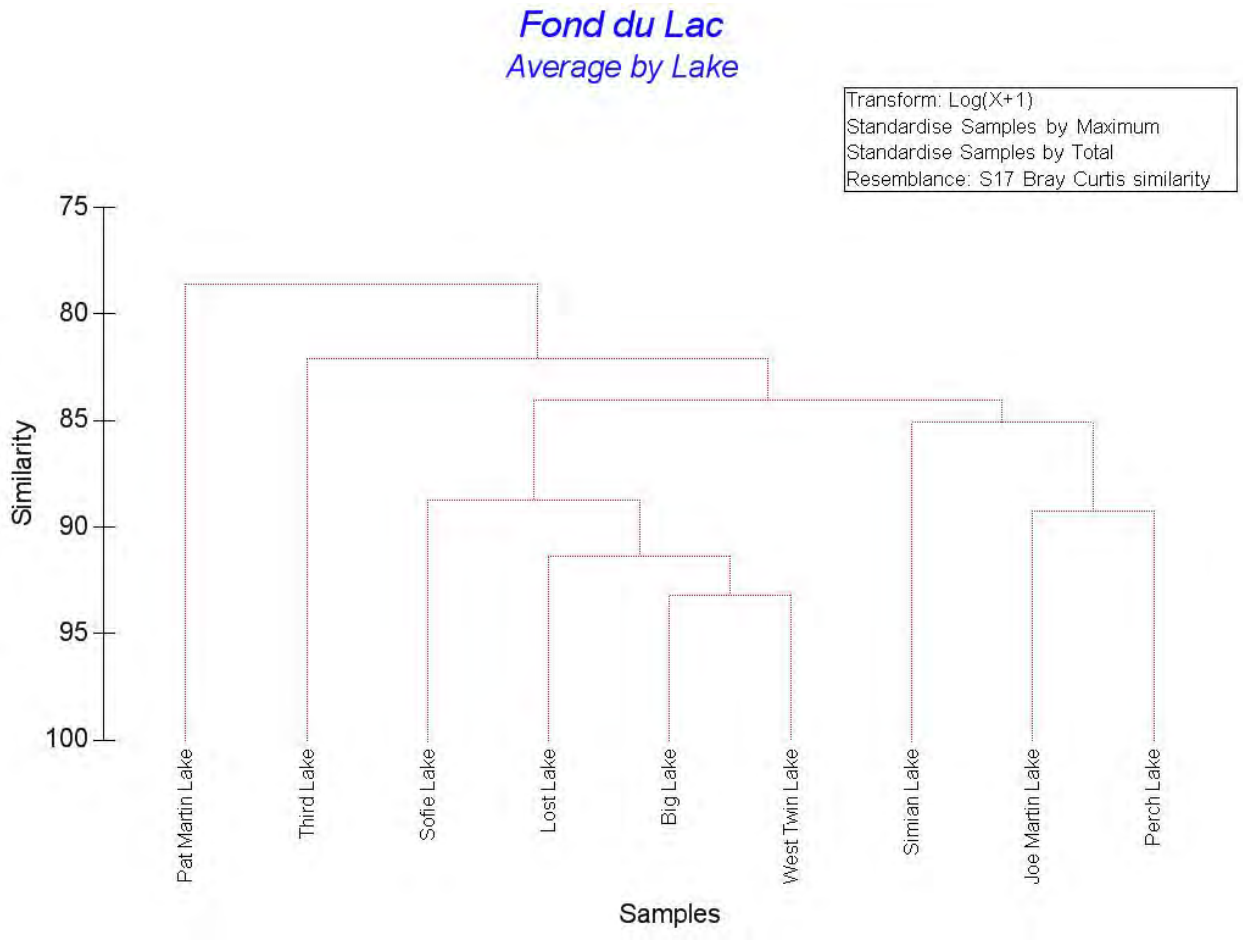


Figure 14. Cluster Diagram of all lakes from Fond du Lac, Minneapolis Parks and Recreation and Minnehaha Creek Watershed District, July through October Samples, averaged by year. Significance is denoted by a solid black line (p<0.01).

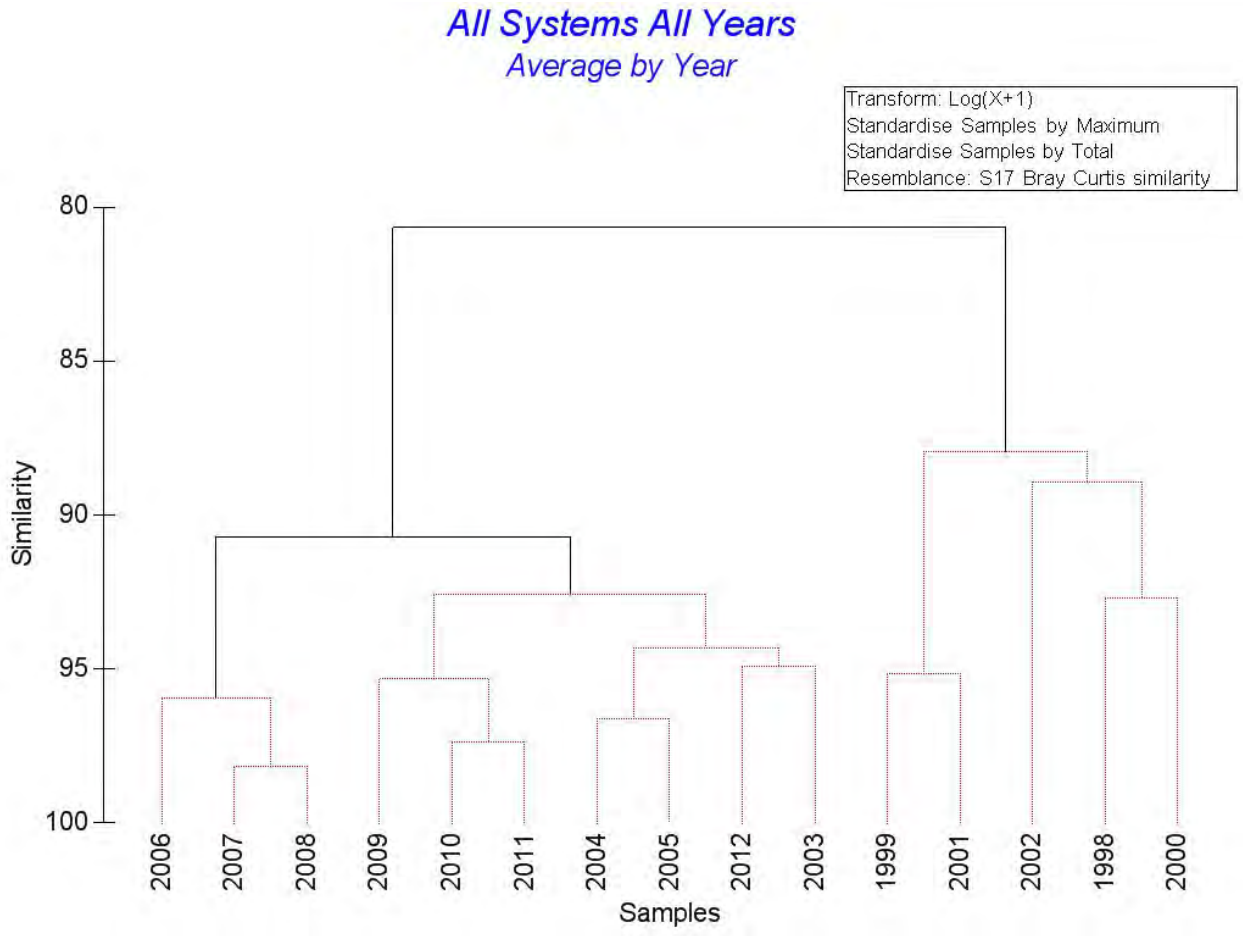


Figure 15. Cluster Diagram of all lakes from Fond du Lac, Minneapolis Parks and Recreation and Minnehaha Creek Watershed District, July through October Samples, averaged by lake. Significance is denoted by a solid black line (p<0.01). Blue box indicates cluster containing Fond du Lac lakes (Black arrows)

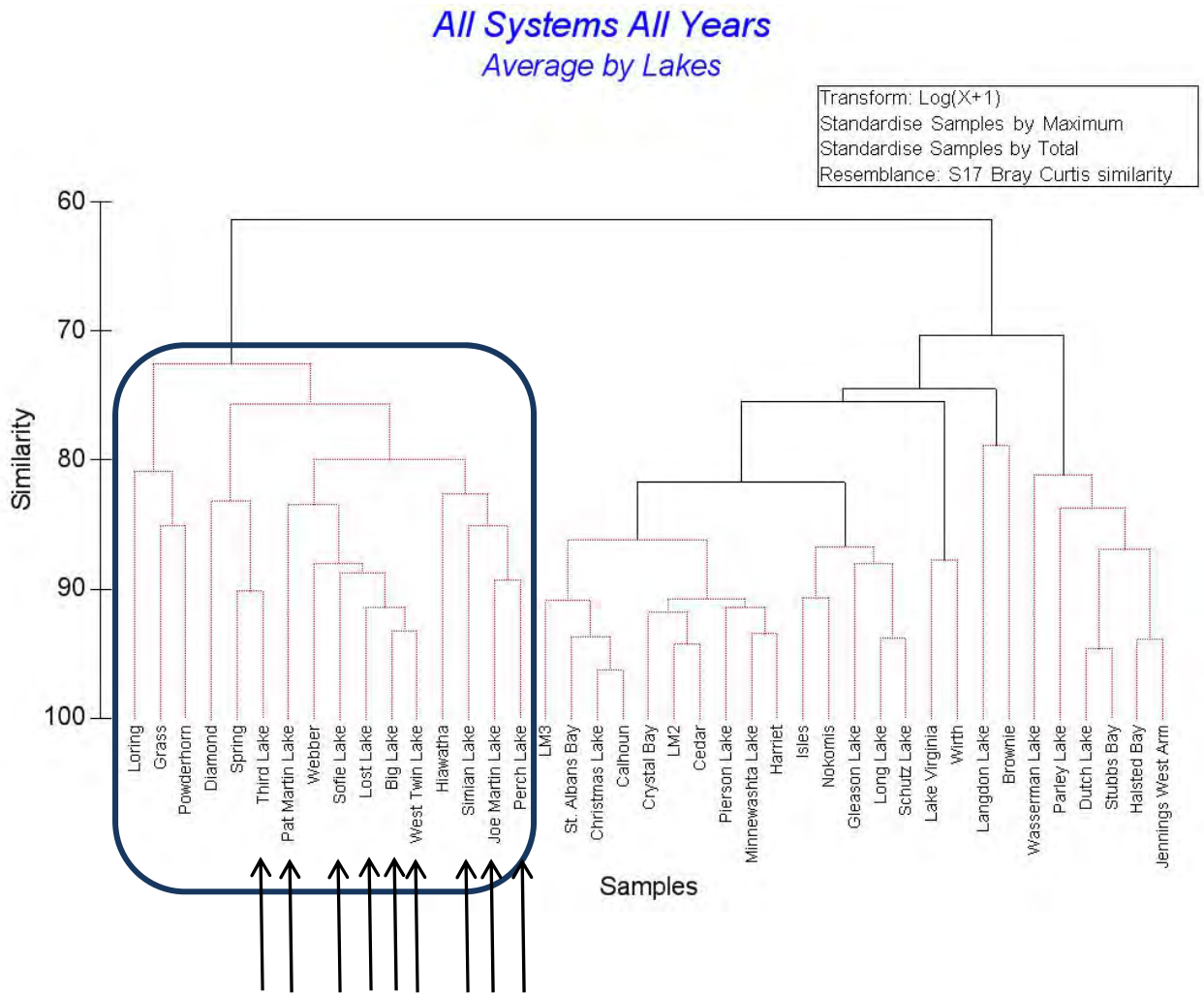


Figure 16. Cluster Diagram of all lakes from Fond du Lac, Minneapolis Parks and Recreation and Minnehaha Creek Watershed District, July through October Samples, averaged by lake. Significance is denoted by a solid black line (p<0.01). Boxes indicate 5 significant clusters/groups used in this analysis. Groups 1, 2, 3, and 5 are each significant clusters while group 4 is the combination of 2 related clusters.

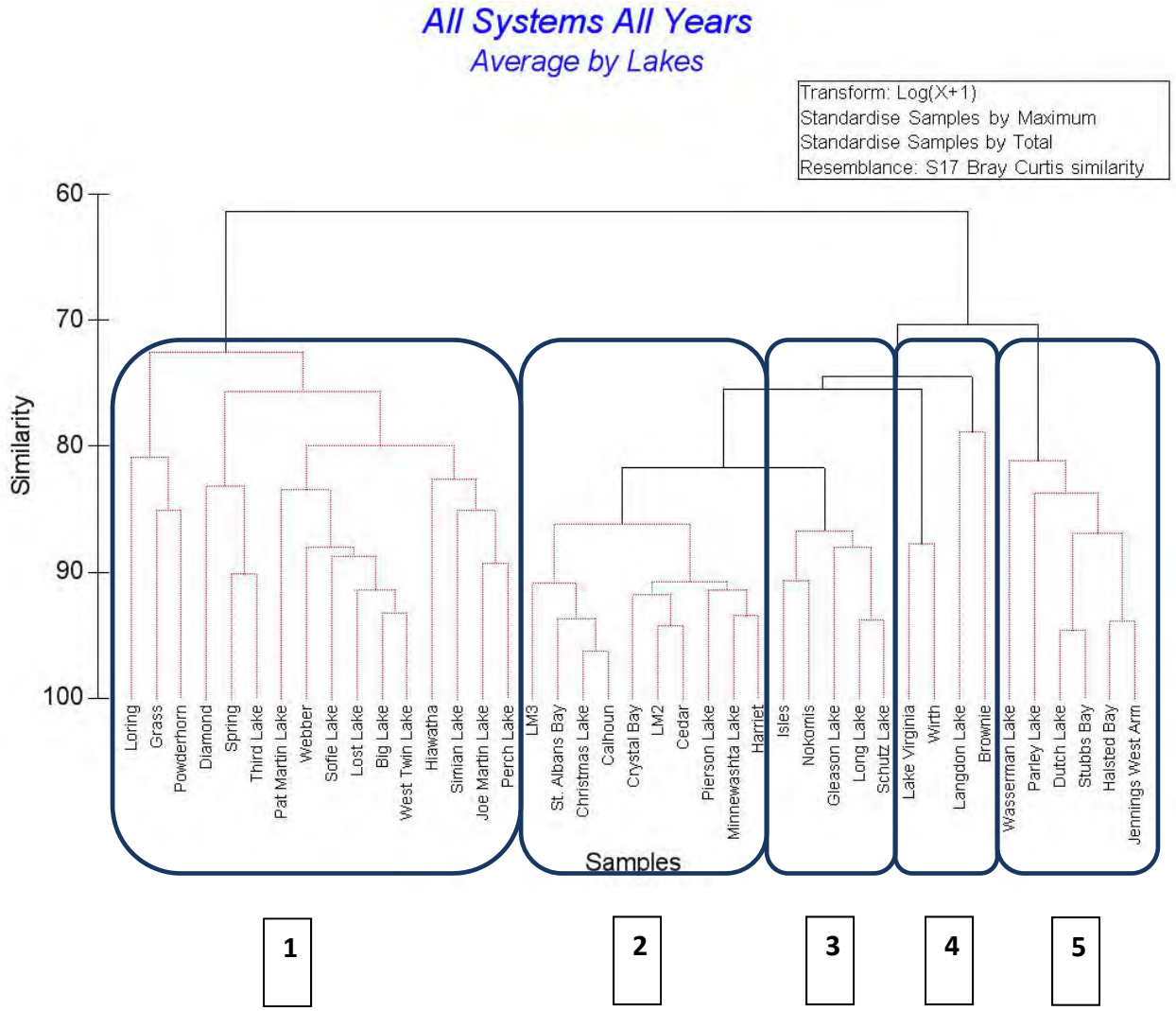


Figure 17. Box Plot of Chlorophyll *a* for the five lake groups. Median (line), 25% and 75% quartiles (upper and lower box limits) and minimum/maximum data range (exclusive of outliers, bars), and outliers as solid dots. Fond du Lac lakes all cluster in Group 1. Stars denote statically significant differences from Group 1.

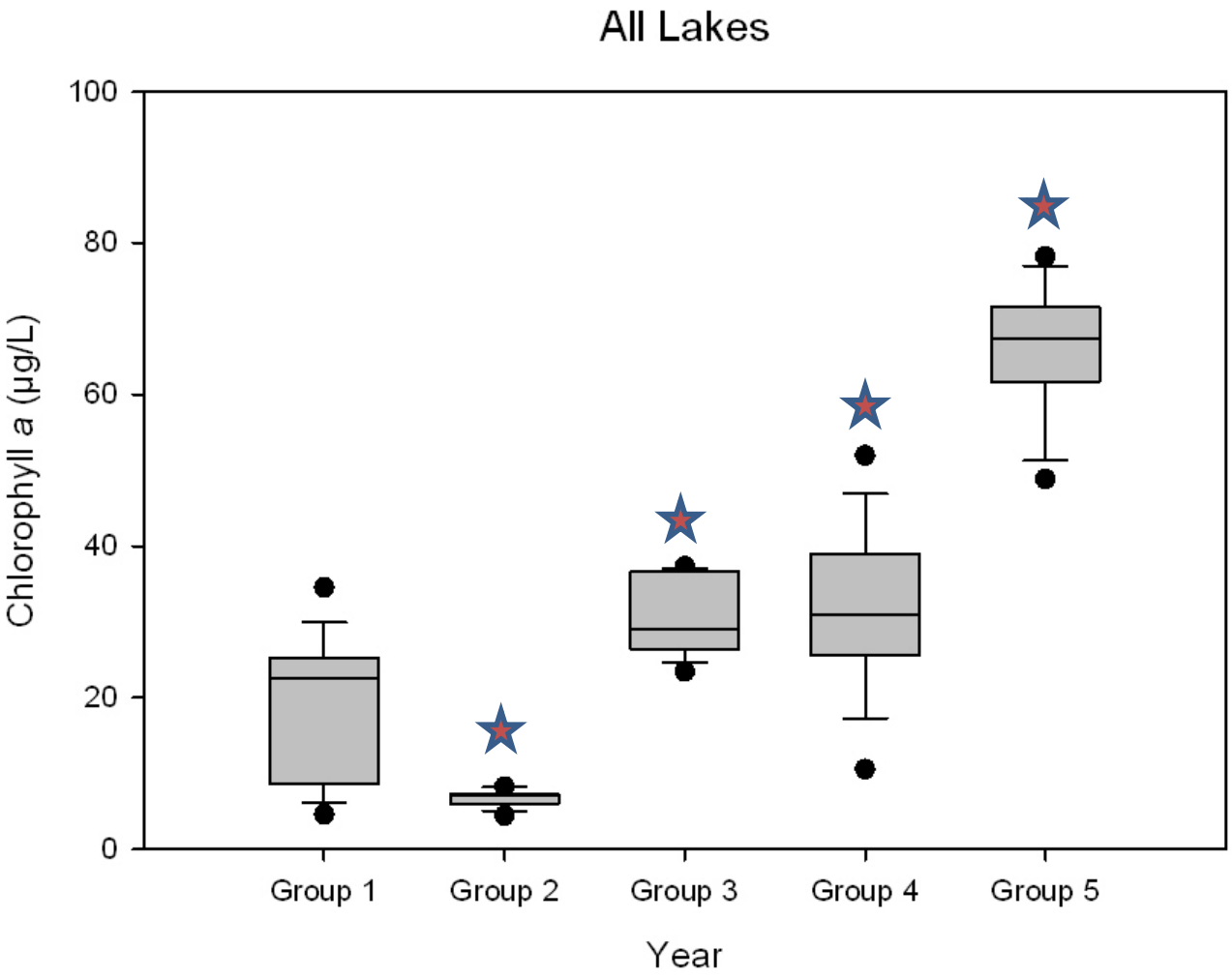


Figure 18. Relative percent algal composition by functional group classification for the five lake groups, averaged over all samples and years by Group. Group 1, n=613: Group 2, n=316, Group 3, n=182, Group 4, n=99, Group 5, n=82.

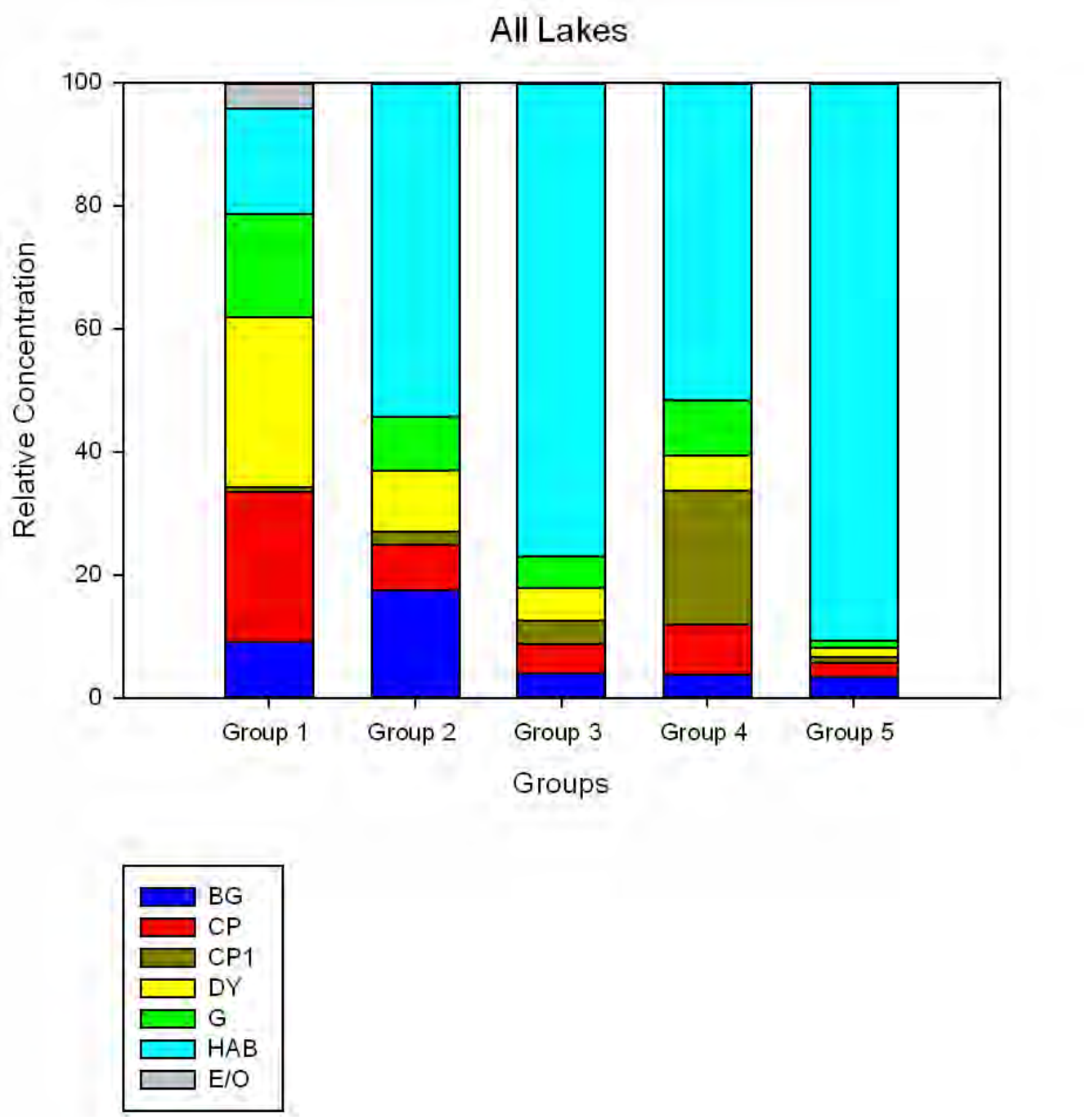


Table 5 . Relative percent algal composition by functional group classification for the five lake groups, mean, median, minimum, maximum, standard deviation. Group 1, n=613: Group 2, n=316, Group 3, n=182, Group 4, n=99, Group 5, n=82.

FGC	Cluster	Mean	Median	Minimum	Maximum	Std Deviation
BG	Group 1	8.6	2.0	0.0	90.0	14.7
	Group 2	16.3	11.3	0.0	70.1	14.9
	Group 3	3.7	1.0	0.0	30.1	5.9
	Group 4	3.5	0.2	0.0	54.0	7.7
	Group 5	3.3	1.8	0.0	29.1	5.2
CP	Group 1	23.2	11.2	0.0	100.0	26.6
	Group 2	6.8	4.1	0.0	89.0	11.5
	Group 3	4.5	2.1	0.0	74.0	7.6
	Group 4	7.8	3.6	0.0	98.5	14.4
	Group 5	2.1	2.0	0.0	18.0	3.0
CP1	Group 1	0.7	0.0	0.0	40.0	3.1
	Group 2	1.9	0.0	0.0	45.0	4.7
	Group 3	3.4	0.0	0.0	95.0	12.9
	Group 4	20.3	10.0	0.0	90.5	24.2
	Group 5	0.8	0.0	0.0	14.0	2.4
DY	Group 1	26.3	15.5	0.0	100.0	27.8
	Group 2	9.2	6.0	0.0	63.0	10.8
	Group 3	5.2	1.6	0.0	82.0	11.0
	Group 4	5.4	2.5	0.0	52.1	8.4
	Group 5	1.4	0.1	0.0	27.1	3.7
G	Group 1	15.8	7.5	0.0	97.1	19.8
	Group 2	8.3	4.5	0.0	82.4	11.4
	Group 3	4.8	2.1	0.0	76.3	9.8
	Group 4	8.5	0.6	0.0	90.8	19.4
	Group 5	1.2	0.3	0.0	9.6	2.2
HAB	Group 1	16.3	3.1	0.0	96.0	25.1
	Group 2	50.3	55.1	0.1	94.1	25.4
	Group 3	72.0	79.1	0.0	98.0	23.0
	Group 4	48.6	51.1	0.0	93.5	25.1
	Group 5	85.7	88.2	51.4	97.6	9.5
E/O	Group 1	3.9	0.1	0.0	81.6	11.2
	Group 2	0.0	0.0	0.0	2.0	0.1
	Group 3	0.1	0.0	0.0	2.1	0.3
	Group 4	0.0	0.0	0.0	2.0	0.2
	Group 5	0.0	0.0	0.0	1.5	0.2

Figure 19. MDS Bubble plot overlay of Harmful Algal Bloom (HAB) Taxa on lake groups. Fond du Lac lakes all cluster in Group 1.

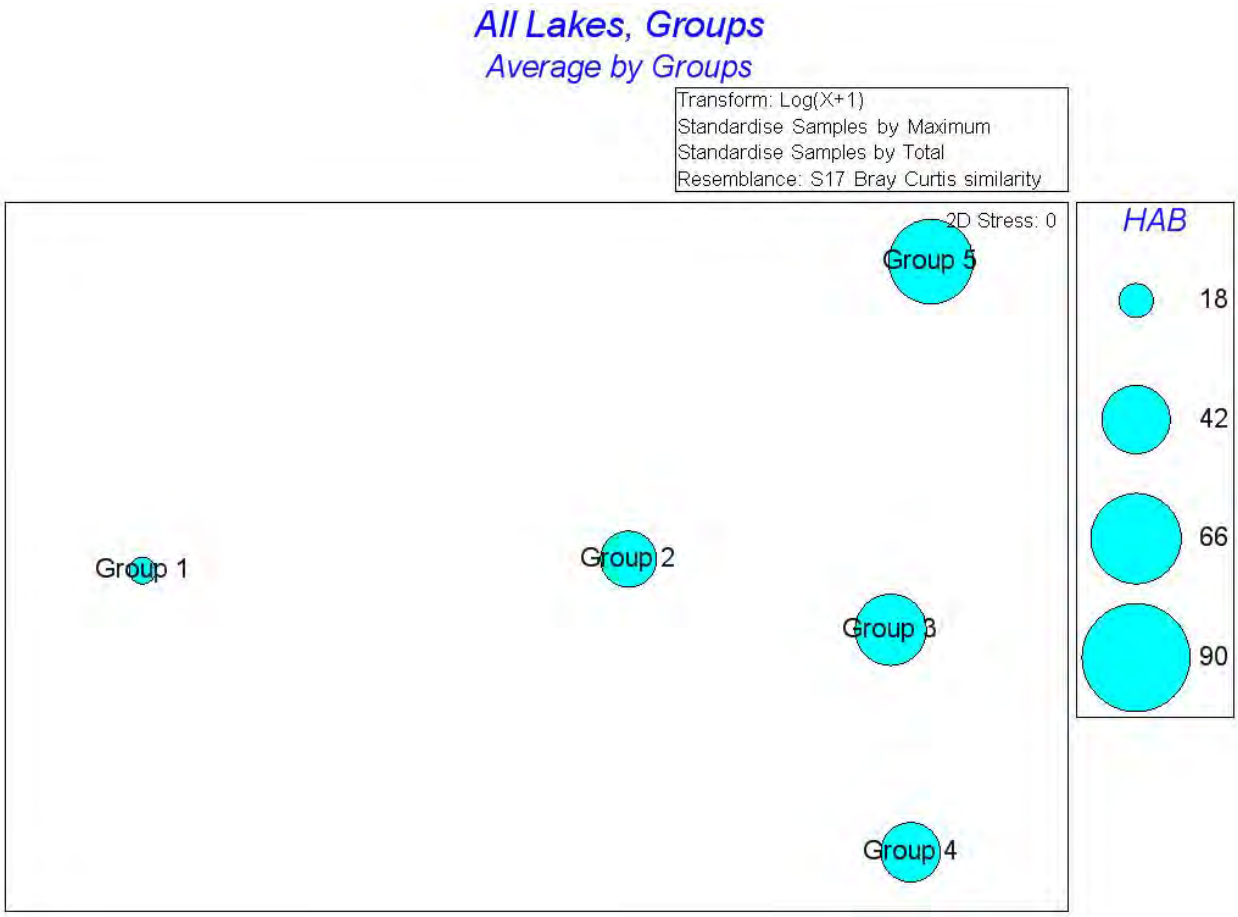


Figure 20. Bubble plot overlay of Cryptophyte/Pyrrophyta (CP) Taxa on lake groups. Fond du Lac lakes all cluster in Group 1.

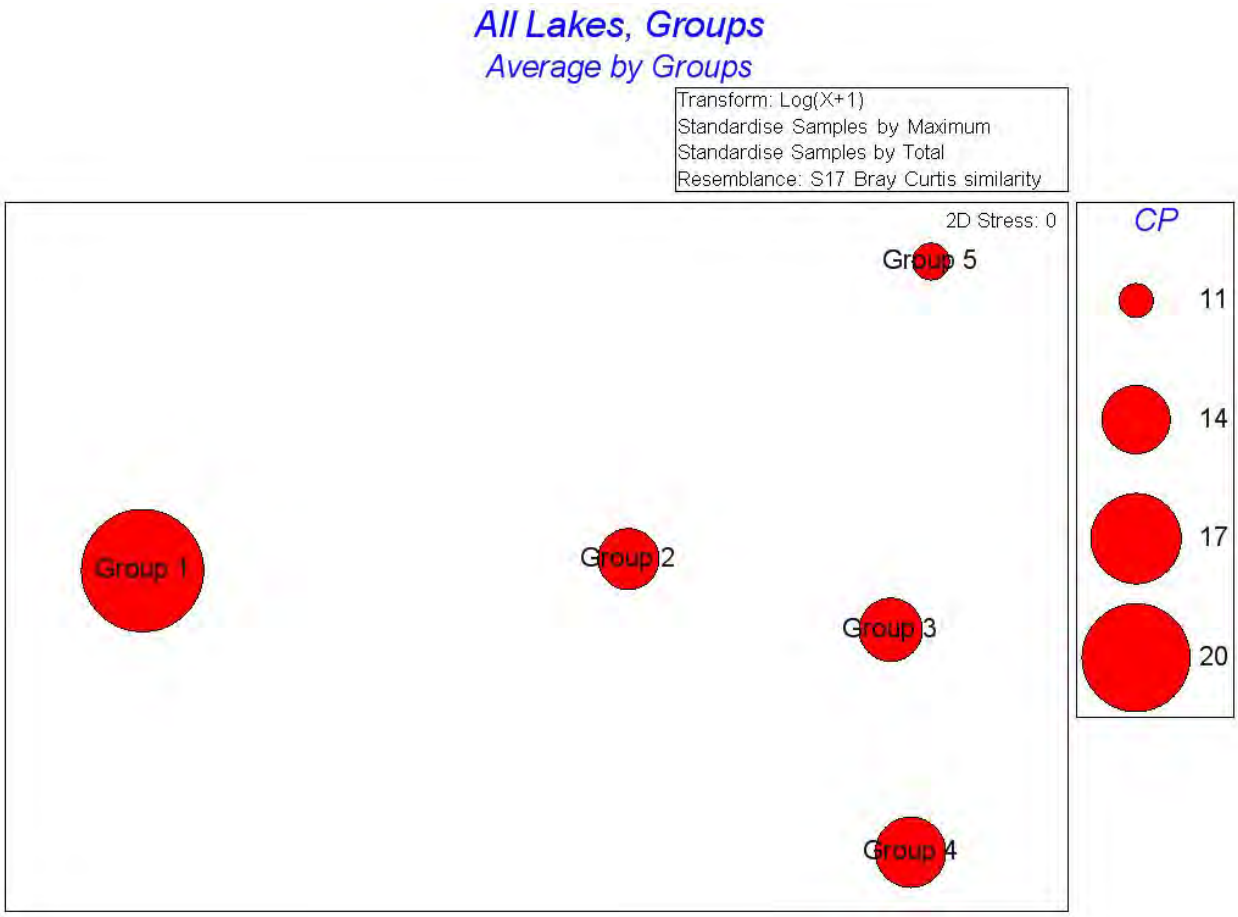


Figure 21. Bubble plot overlay of *Ceratium hirundinella* (CP1) on lake groups. Fond du Lac lakes all cluster in Group 1.

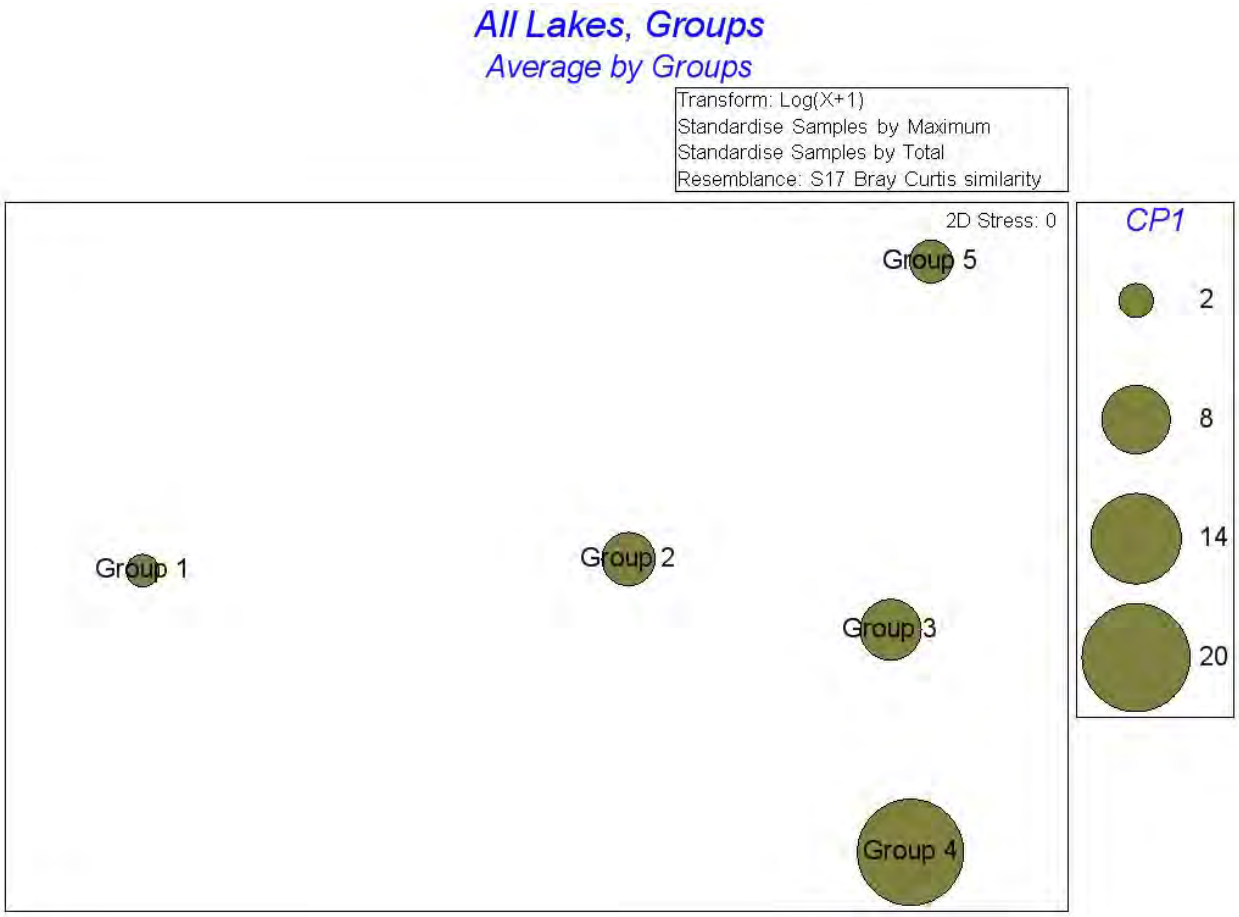


Figure 22. Bubble plot overlay of Chrysophyta/Bacillariophyta (DY) on lake groups. Fond du Lac lakes all cluster in Group 1.

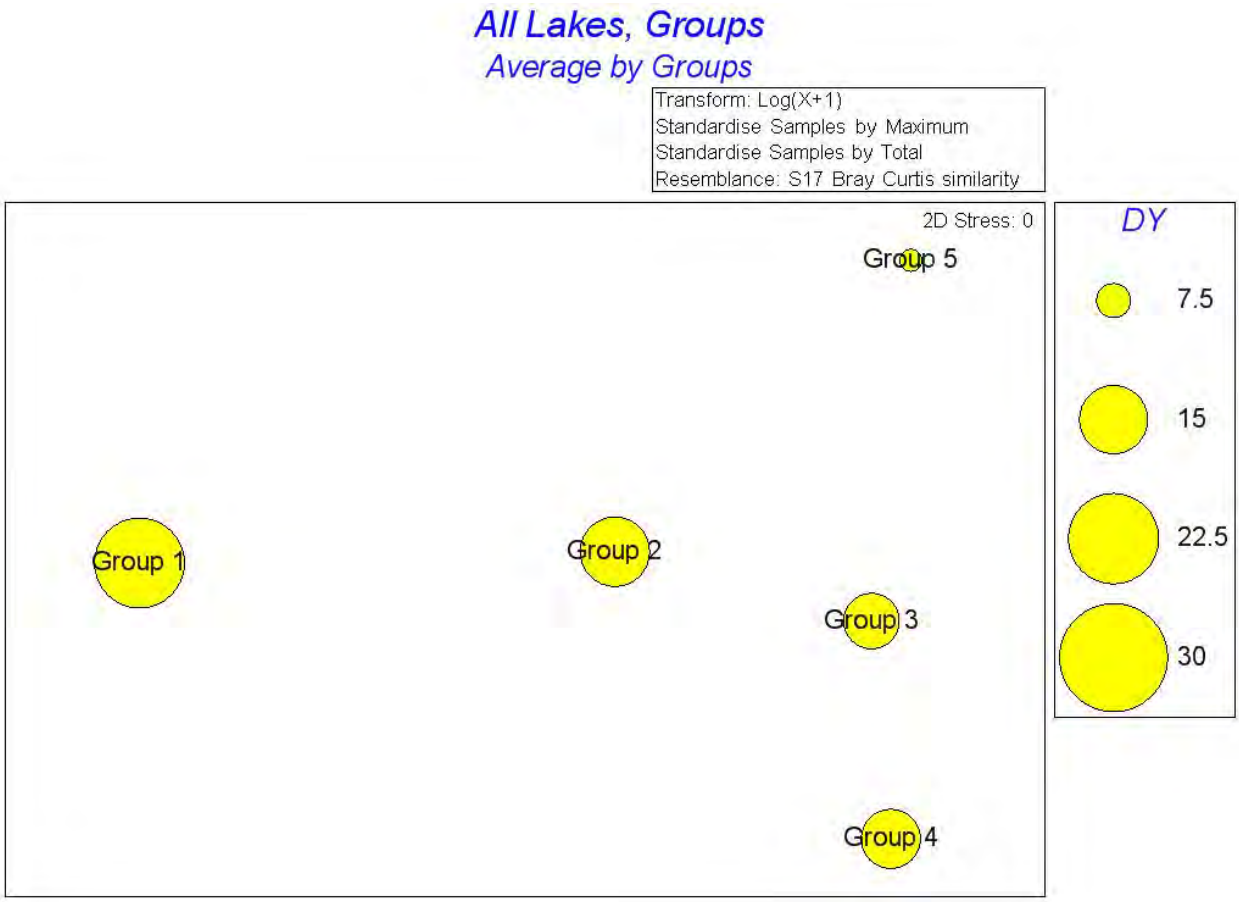


Figure 23. Bubble plot overlay of Chlorophyta (G) on lake groups. Fond du Lac lakes all cluster in Group 1.

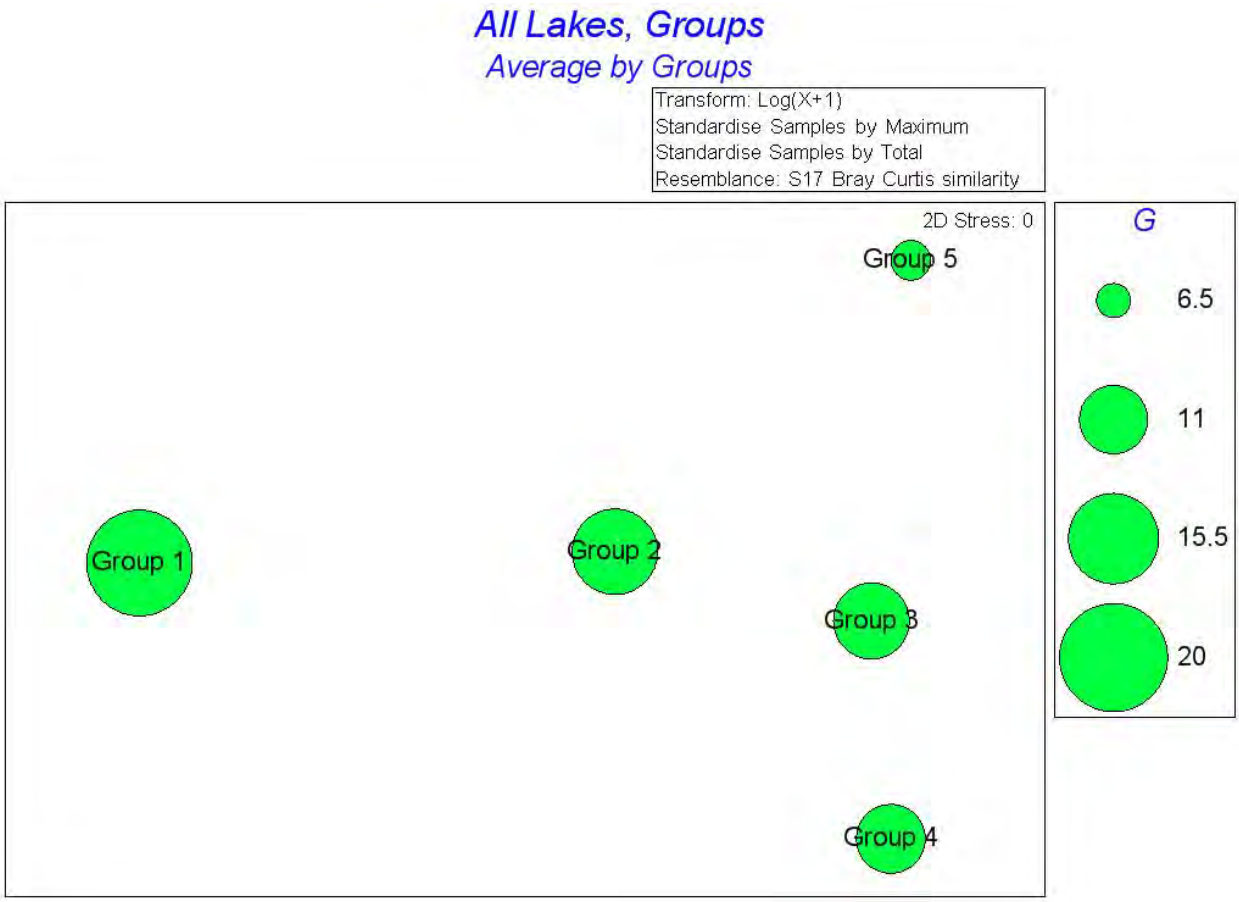


Figure 24. Bubble plot overlay of Euglenophyta/Other (E/O) on lake groups. Fond du Lac lakes all cluster in Group 1.

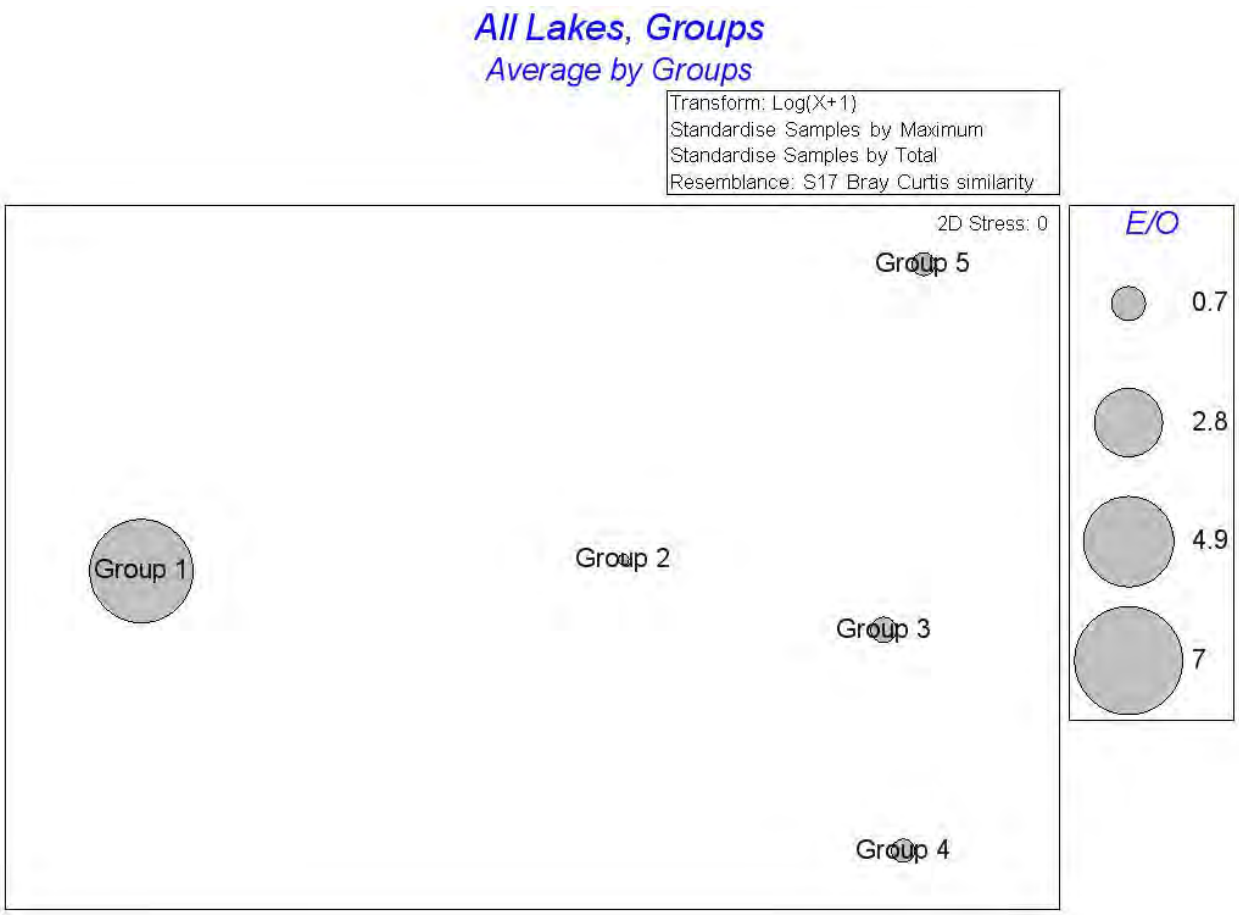


Figure 25. Box Plot of TP on lake groups. Median (line), 25% and 75% quartiles (upper and lower box limits) and minimum/maximum data range (exclusive of outliers, bars), and outliers as solid dots. Fond du Lac lakes all cluster in Group 1. Stars denote statically significant differences from Group 1.

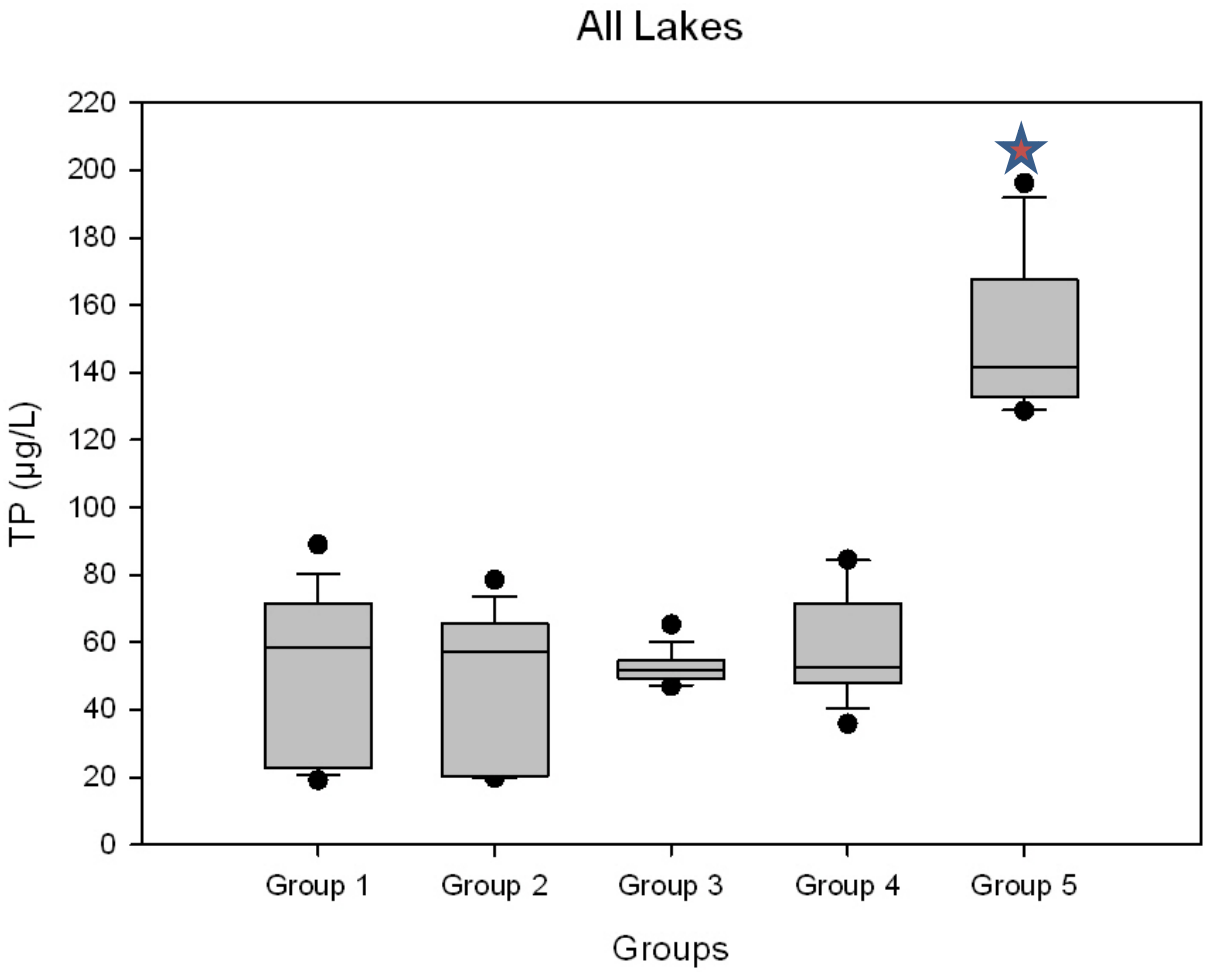


Figure 26. Box Plot of TN on lake groups. Median (line), 25% and 75% quartiles (upper and lower box limits) and minimum/maximum data range (exclusive of outliers, bars), and outliers as solid dots. Fond du Lac lakes all cluster in Group 1. Stars denote statically significant differences from Group 1.

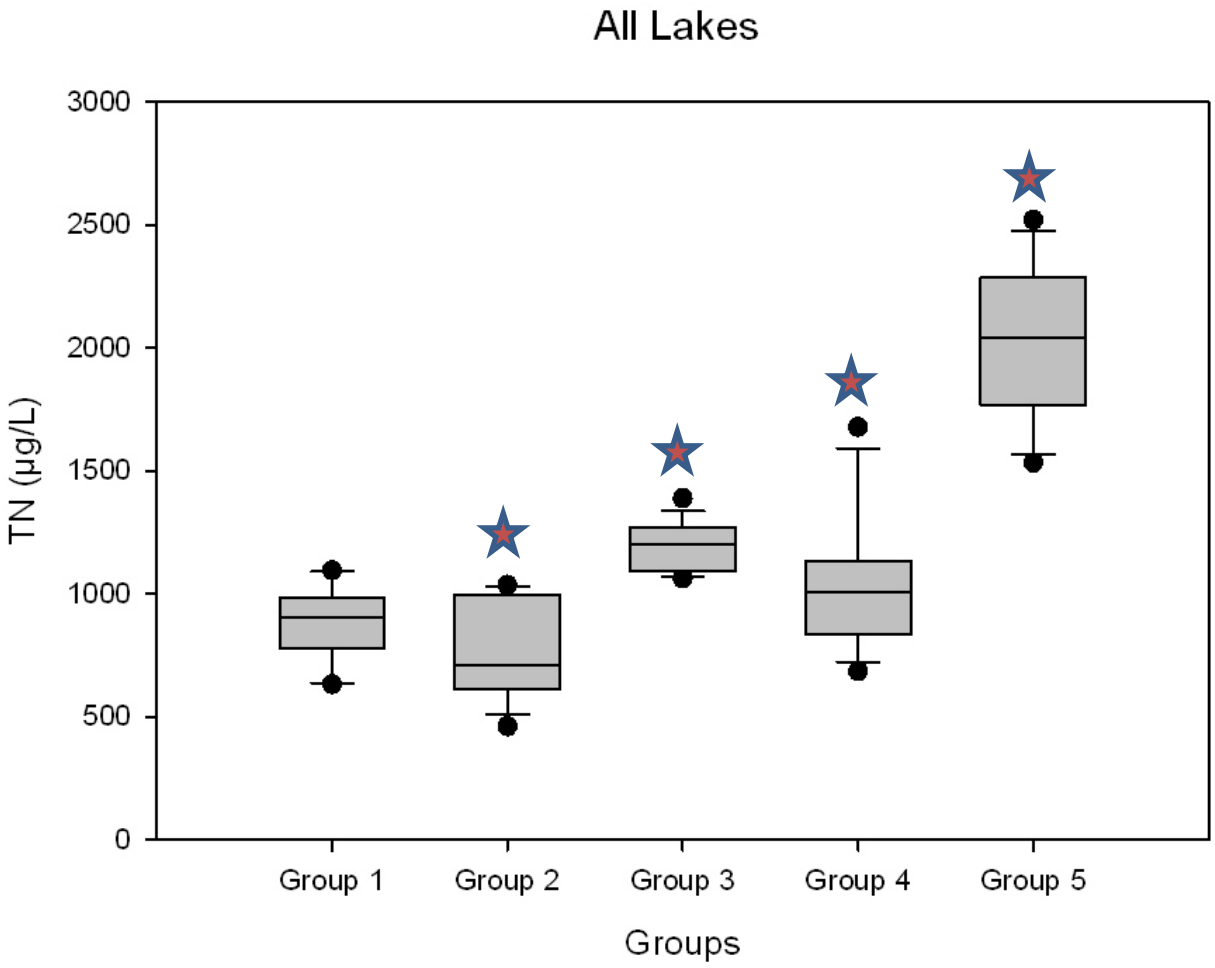


Figure 27. Box Plot of TN: TP on lake groups. Median (line), 25% and 75% quartiles (upper and lower box limits) and minimum/maximum data range (exclusive of outliers, bars), and outliers as solid dots. Fond du Lac lakes all cluster in Group 1. Stars denote statically significant differences from Group 1.

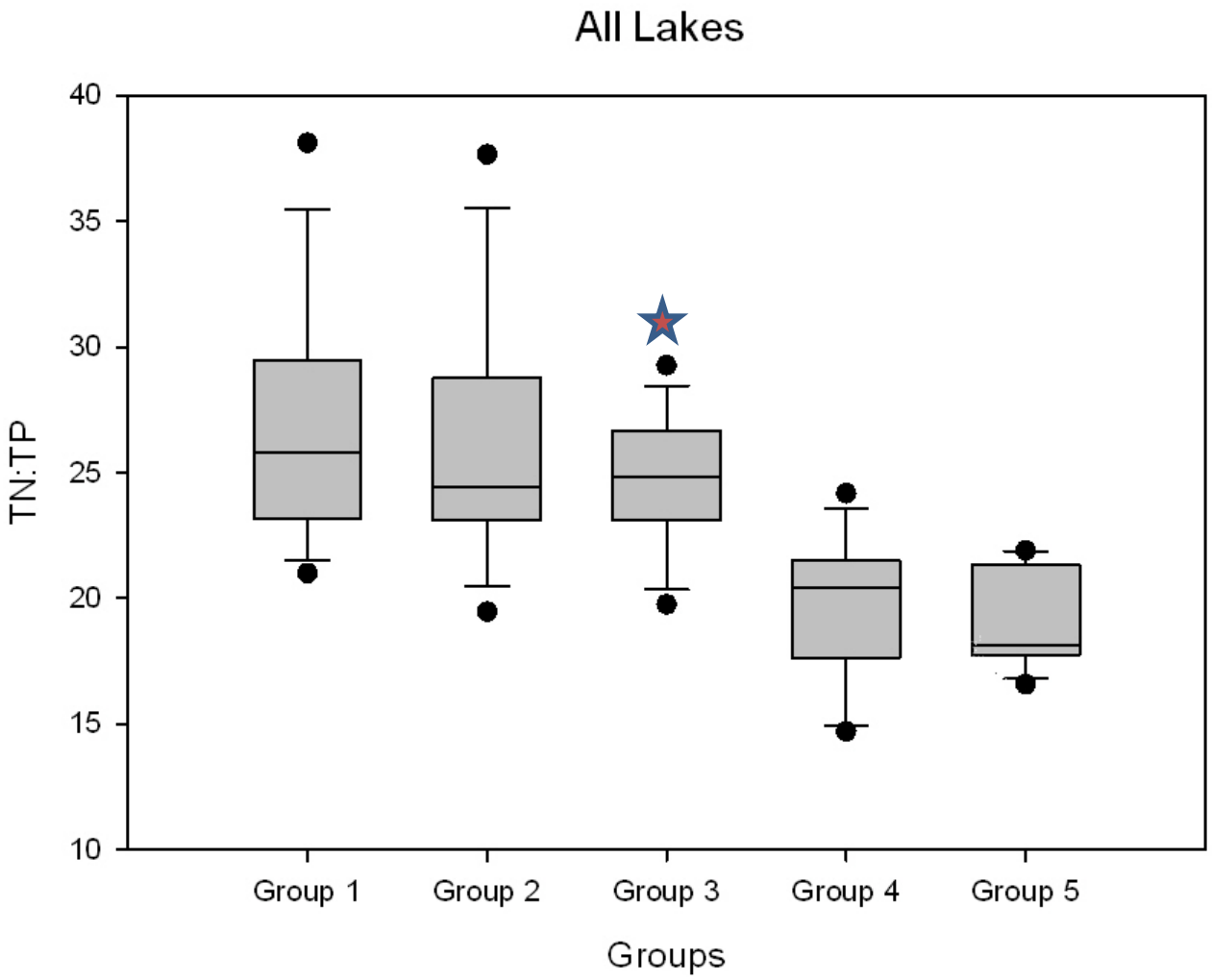


Figure 28. Relative percent algal biovolume (Rel Bio), PhycoTech PRA (PRA), and Relative percent concentration (Rel Cell Conc) by functional group classification for the nine FDL lakes for the May/June 2014 sampling date.

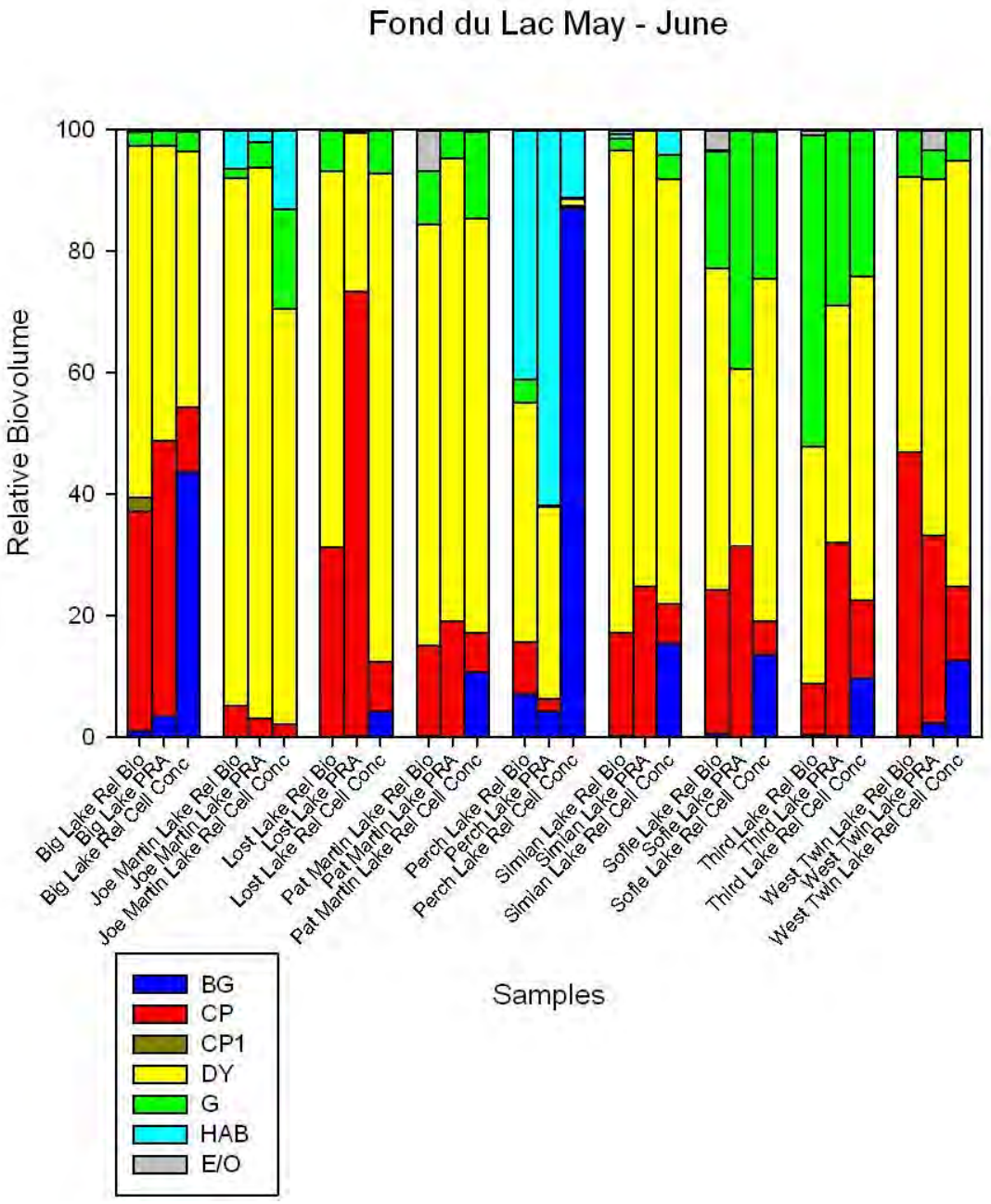


Figure 29. Relative percent algal biovolume (Rel Bio), PhycoTech PRA (PRA), and Relative percent concentration (Rel Cell Conc) by functional group classification for the nine FDL lakes for the August 2014 sampling date.

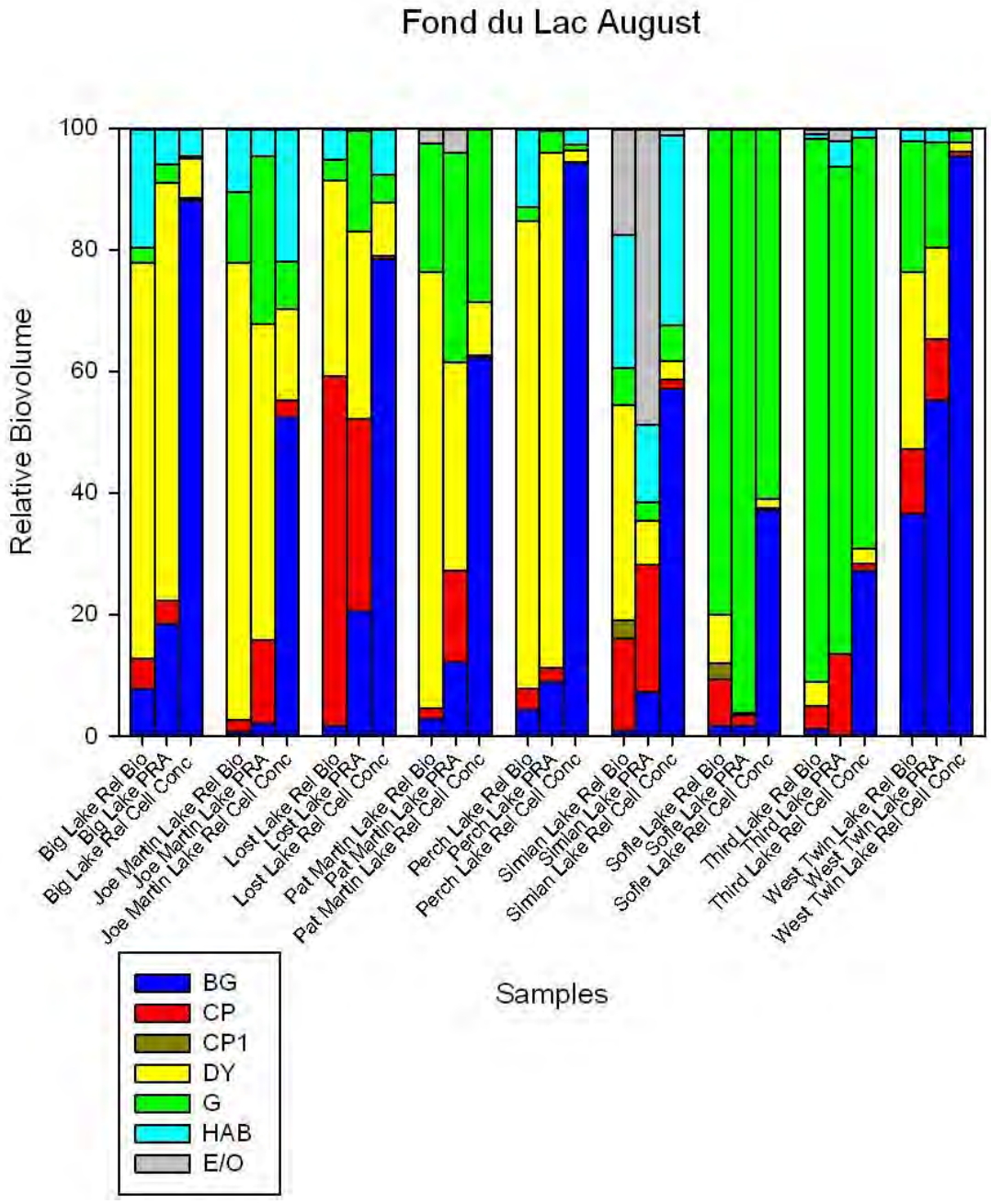


Figure 30. Relative percent algal biovolume (Rel Bio), PhycoTech PRA (PRA), and Relative percent concentration (Rel Cell Conc) by functional group classification for the nine FDL lakes for the September/October 2014 sampling date.

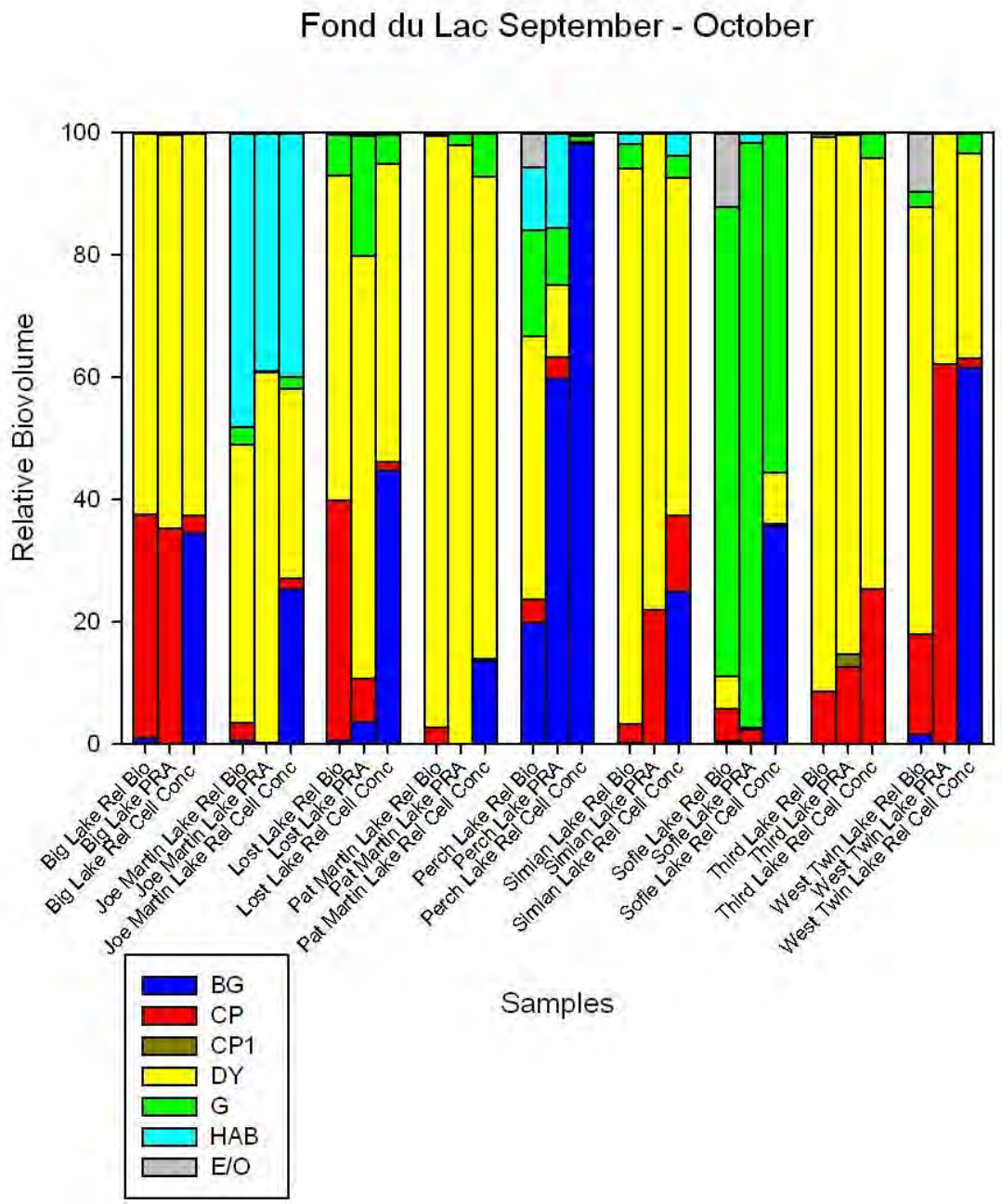


Figure 31. Relative percent algal biovolume (Rel Bio), PhycoTech PRA (PRA), and Relative percent concentration (Rel Cell Conc) by functional group classification for the nine FDL lakes for the average of all 2014 sampling dates.

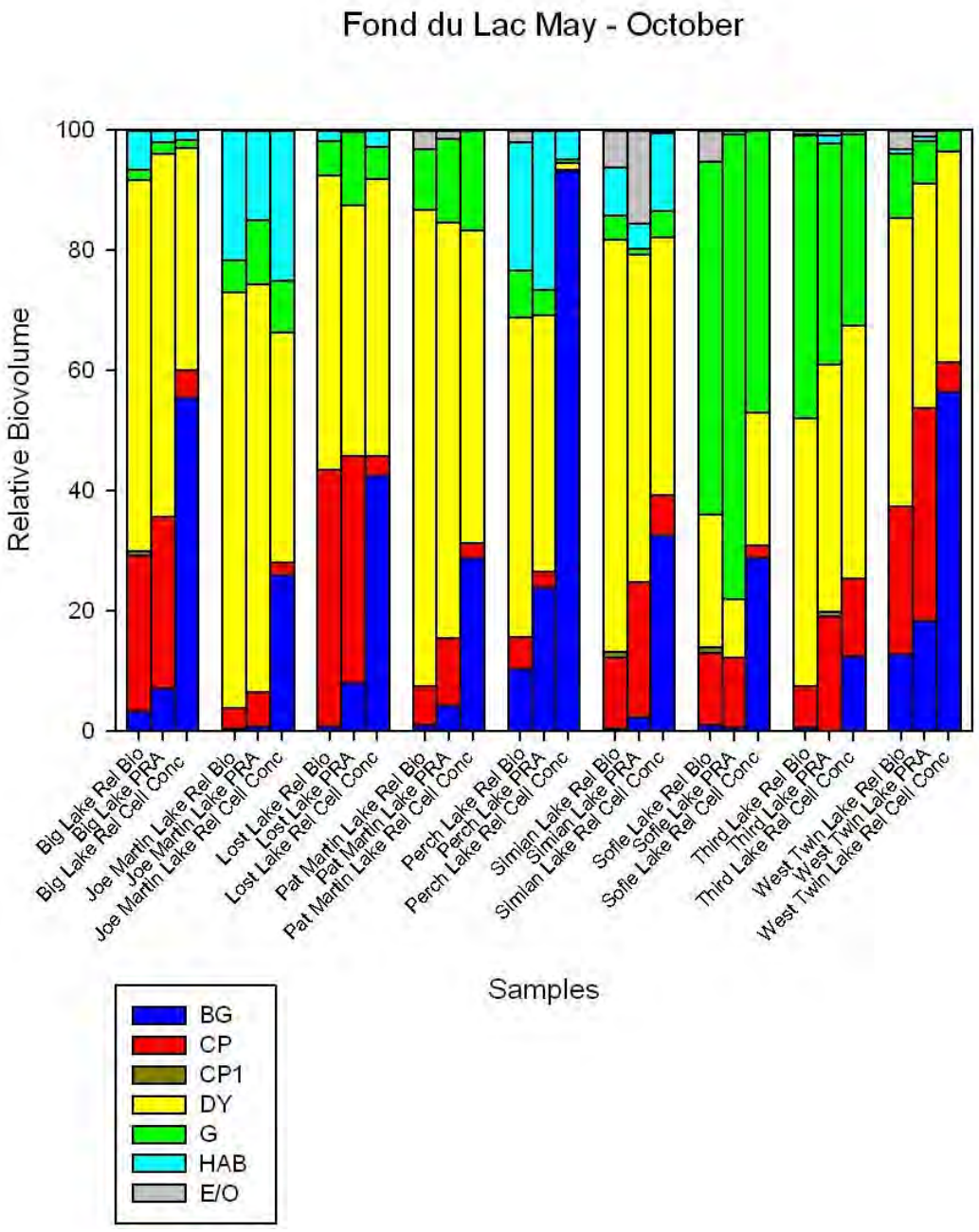


Figure 32. Relative percent algal biovolume (Quant), PhycoTech PRA (PRA), and UMN PRA (UMN) by functional group classification for the nine FDL lakes for May/June 2014 sampling date.

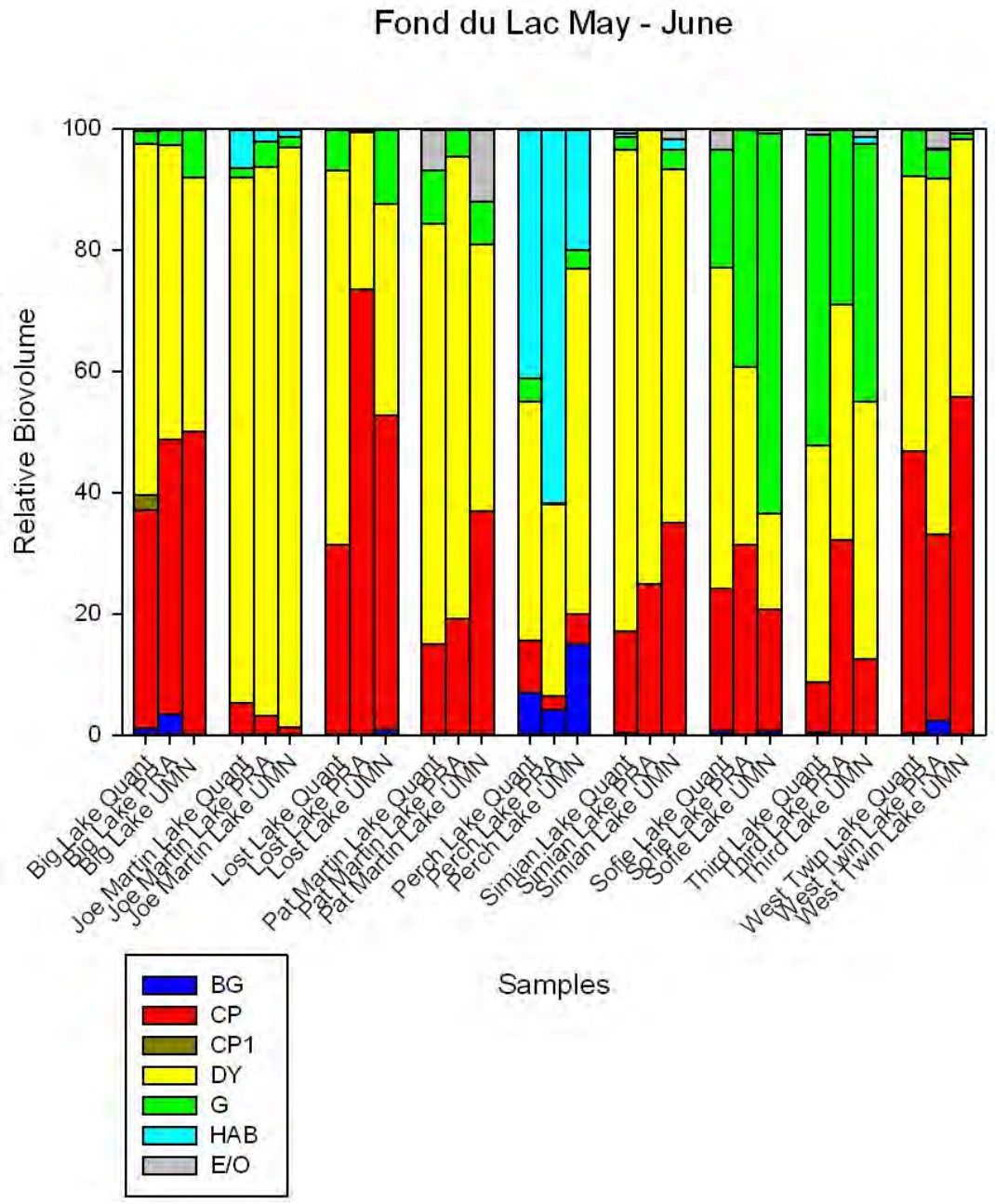


Figure 33. Relative percent algal biovolume (Quant), PhycoTech PRA (PRA), and UMN PRA (UMN) by functional group classification for the nine FDL lakes for August 2014 sampling date.

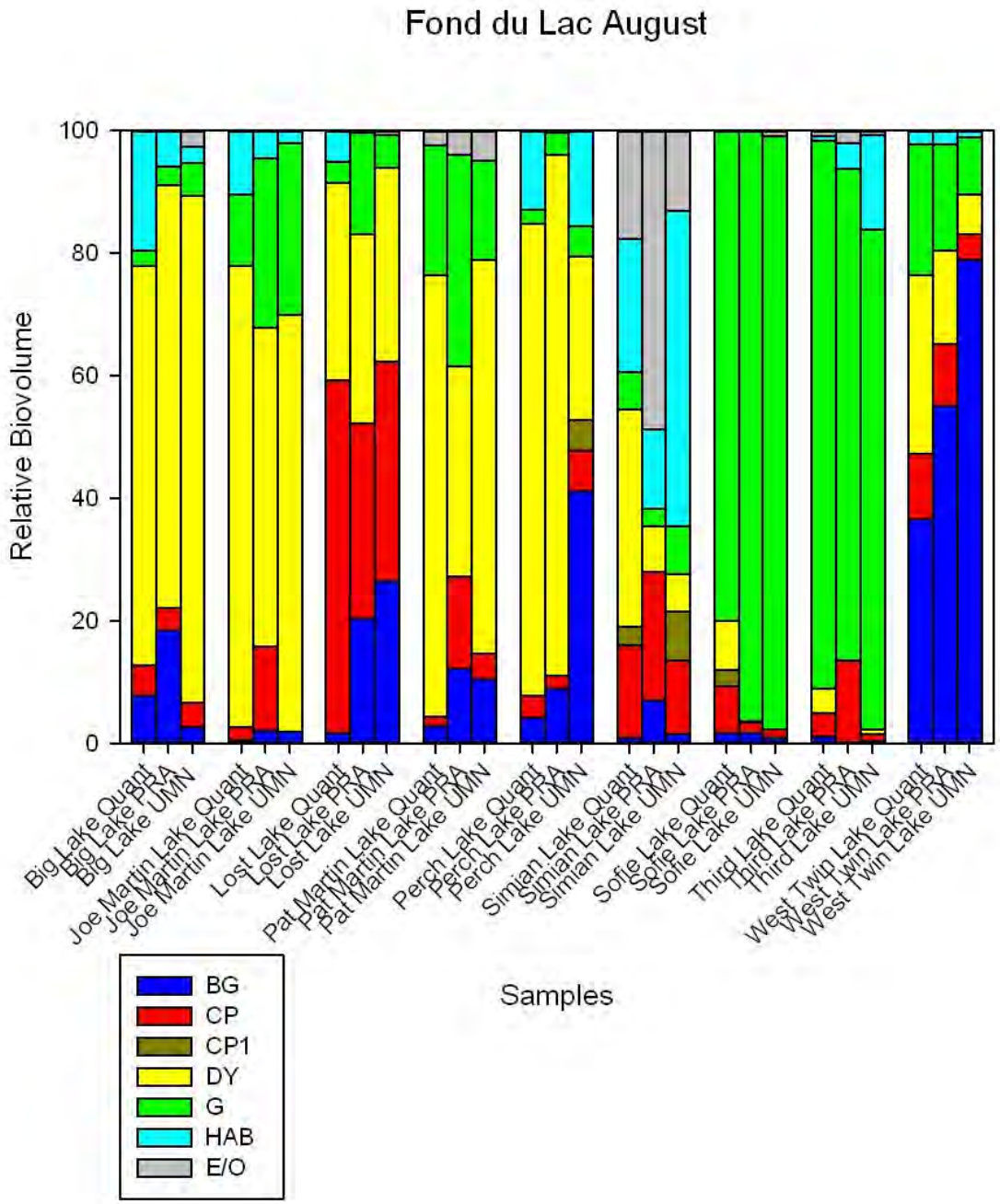


Figure 34. Relative percent algal biovolume (Quant), PhycoTech PRA (PRA), and UMN PRA (UMN) by functional group classification for the nine FDL lakes for September/October 2014 sampling date.

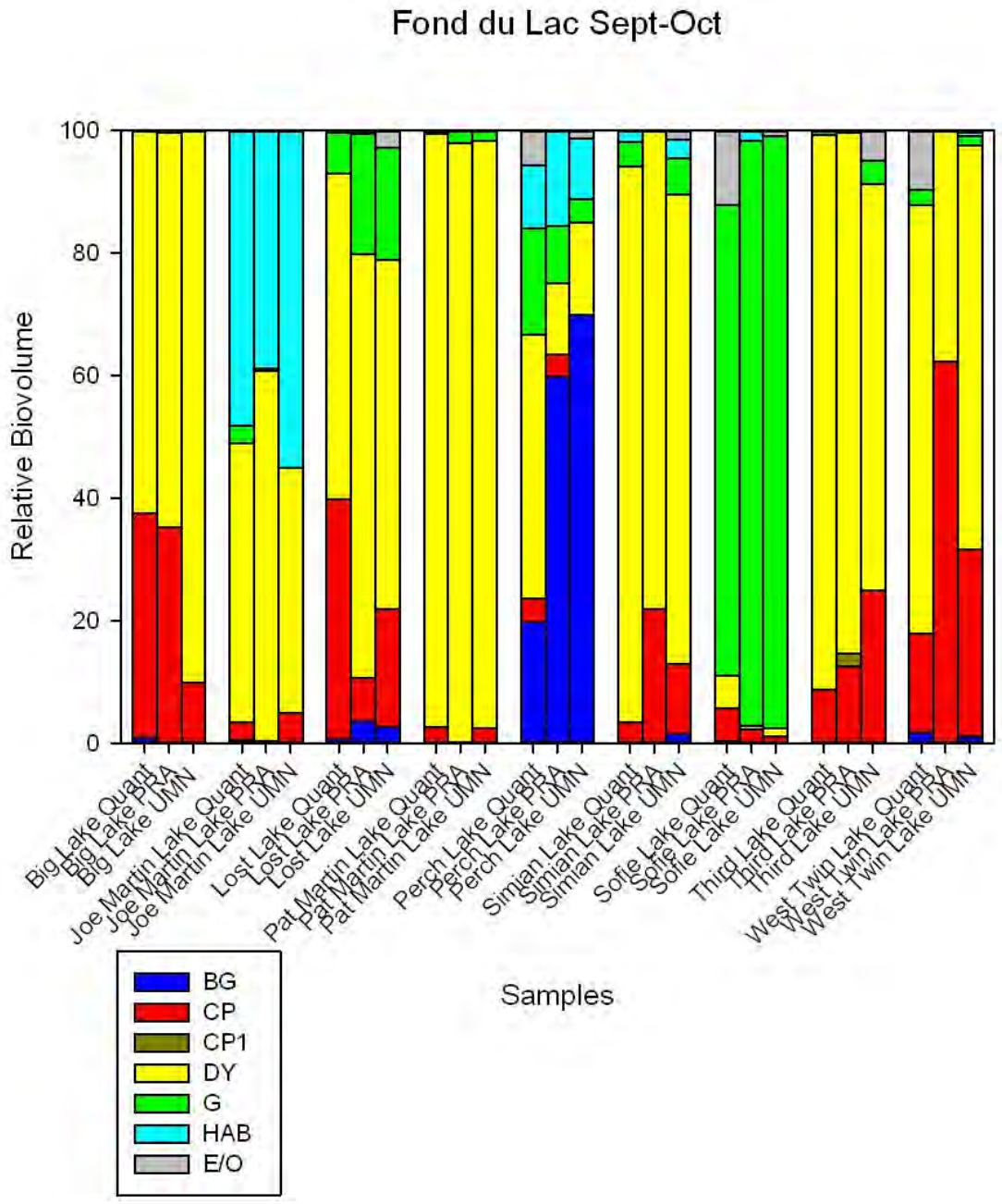


Figure 35. Relative percent algal biovolume (Quant), PhycoTech PRA (PRA), and UMN PRA (UMN) by functional group classification for the nine FDL lakes for Summer 2014 sampling dates.

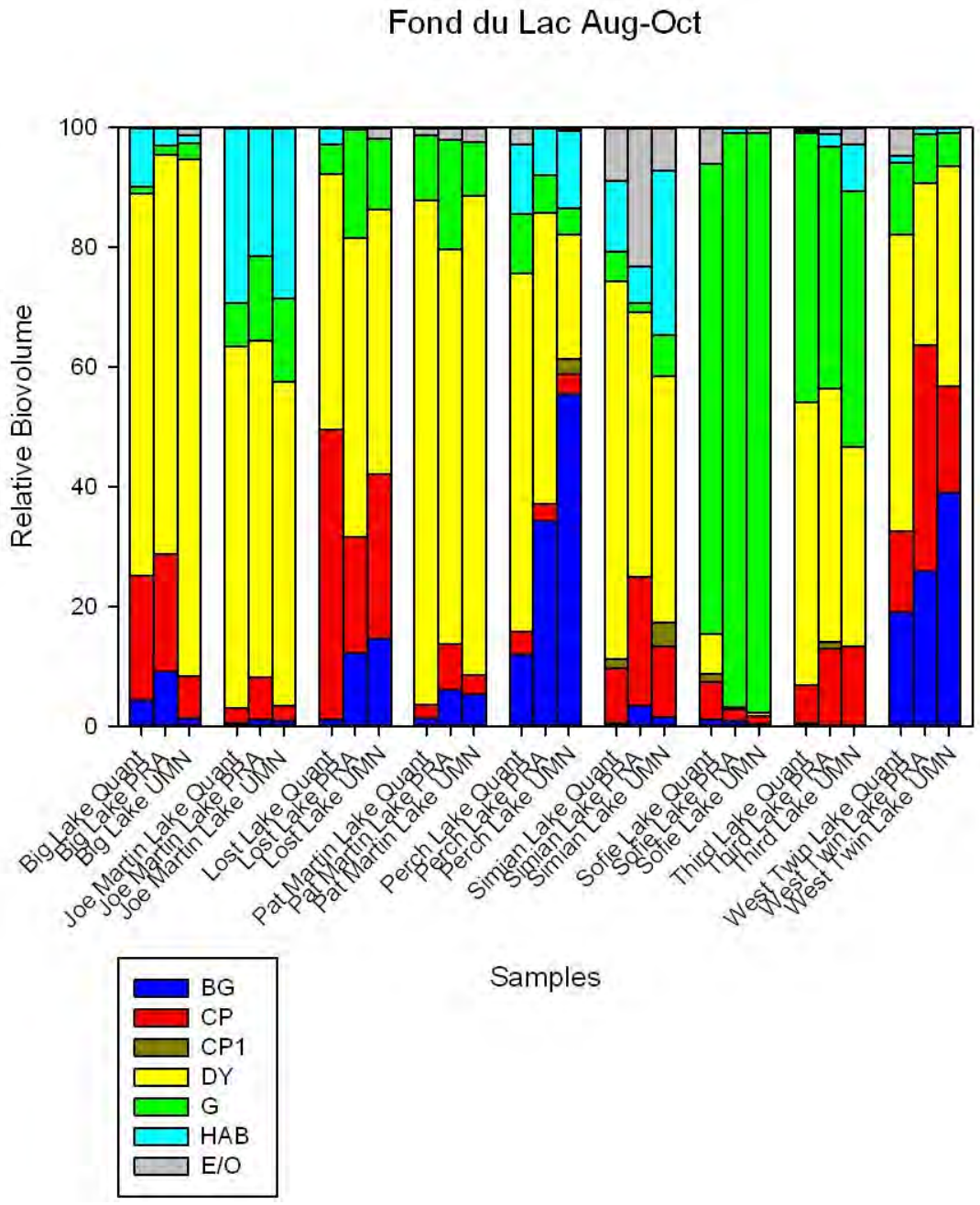


Figure 36. Relative percent algal biovolume (Quant), PhycoTech PRA (PRA), and UMN PRA (UMN) by functional group classification for the nine FDL lakes averaged for all 2014 sampling dates.

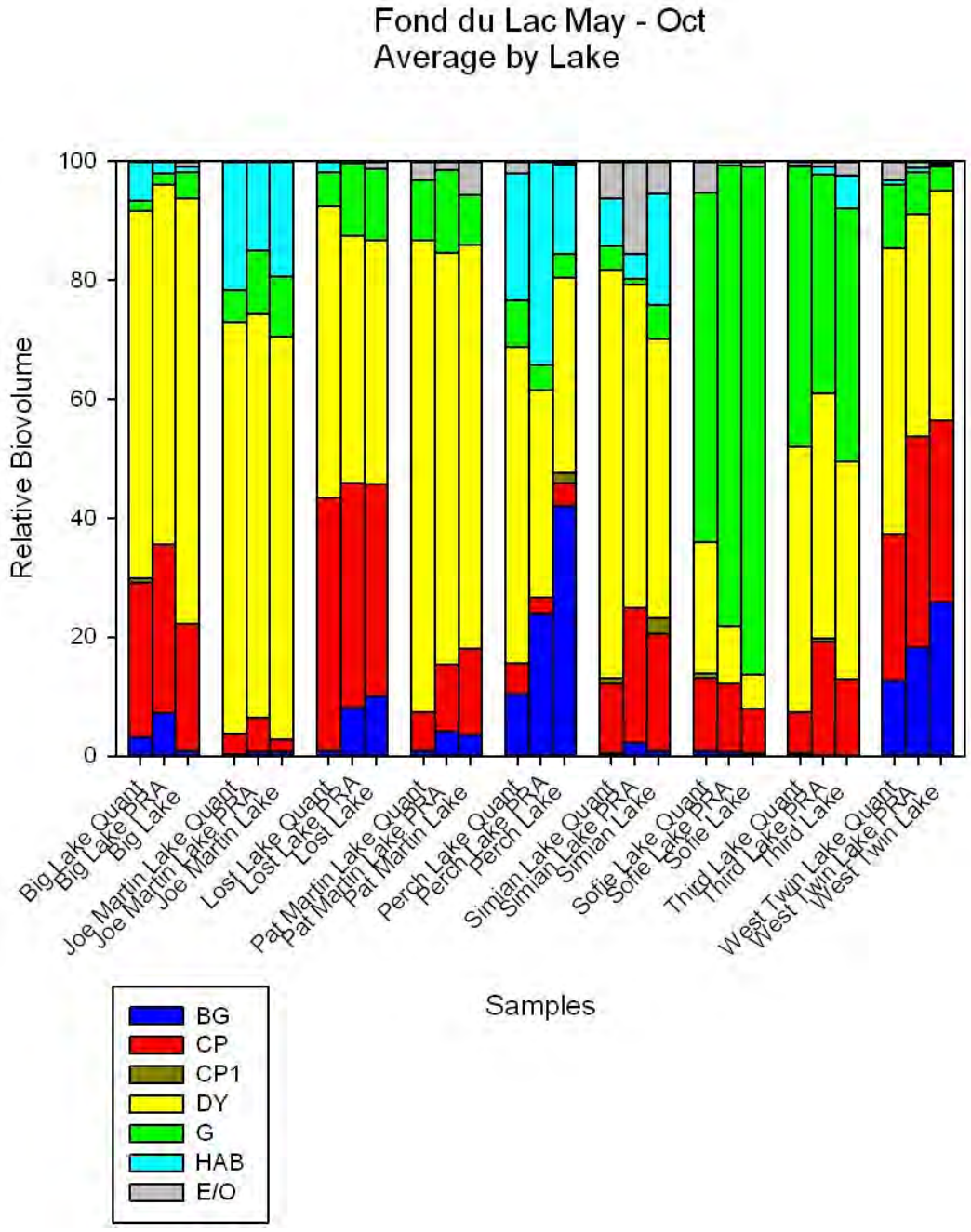


Figure 37. Cluster Diagram of all fish lakes from Fond du Lac, PhycoTech Relative Biovolume, averaged by lake, July through October Samples. Significance is denoted by a solid black line (p<0.01).

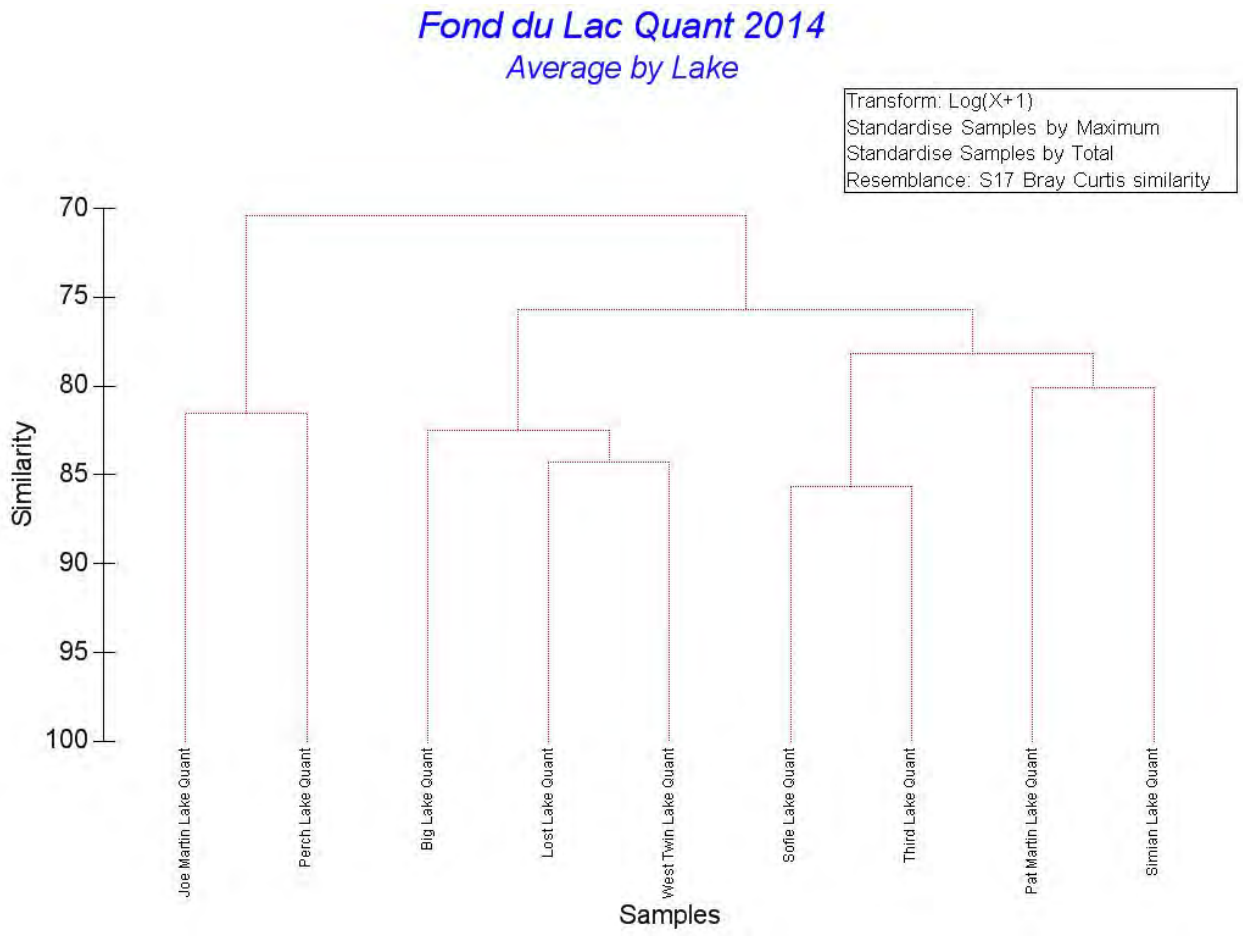


Figure 38. Cluster Diagram of all fish lakes from Fond du Lac, PhycoTech PRA, averaged by lake, July through October Samples. Significance is denoted by a solid black line (p<0.01).

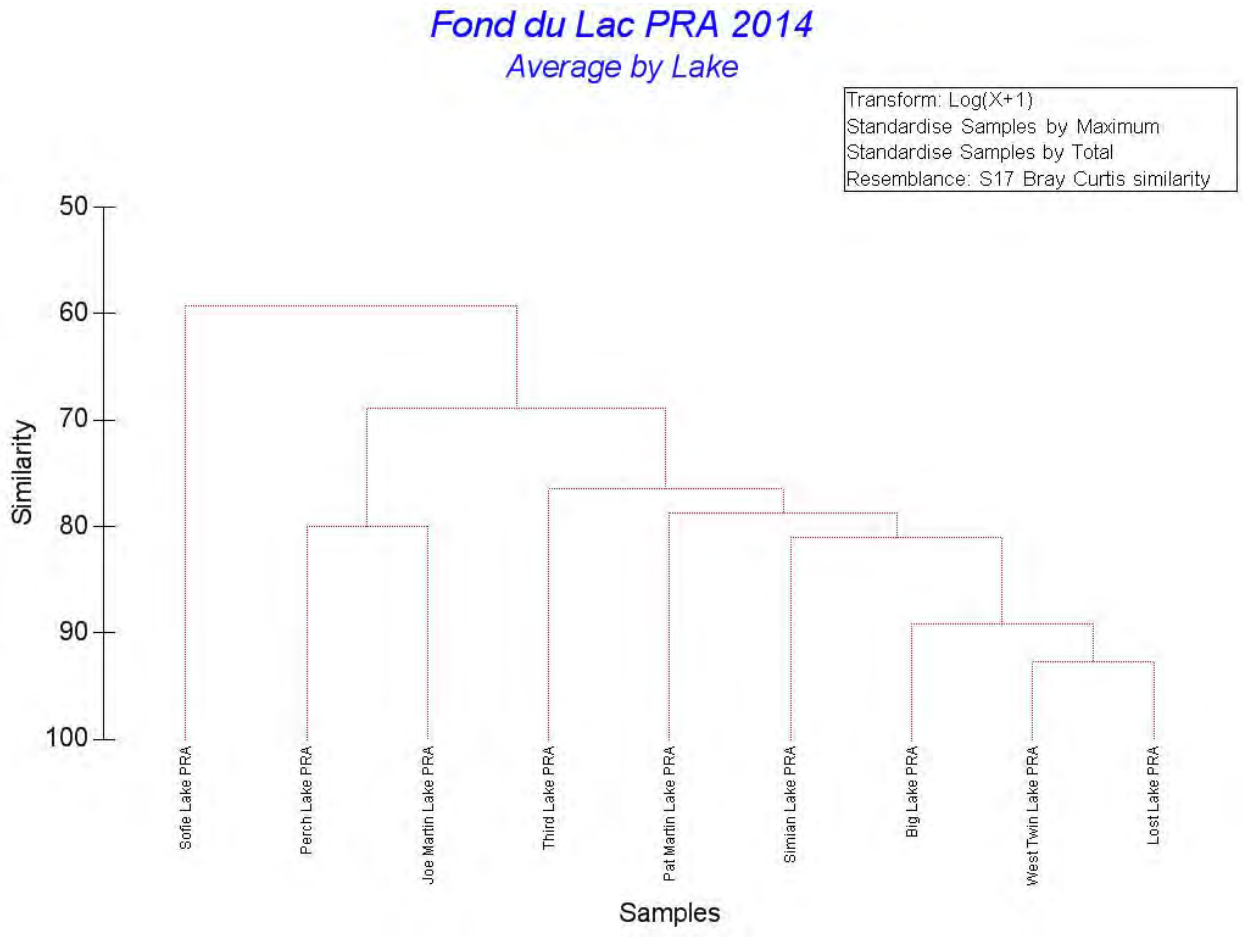


Figure 39. Cluster Diagram of all fish lakes from Fond du Lac, University of Minnesota PRA, averaged by lake, July through October Samples. Significance is denoted by a solid black line (p<0.01).

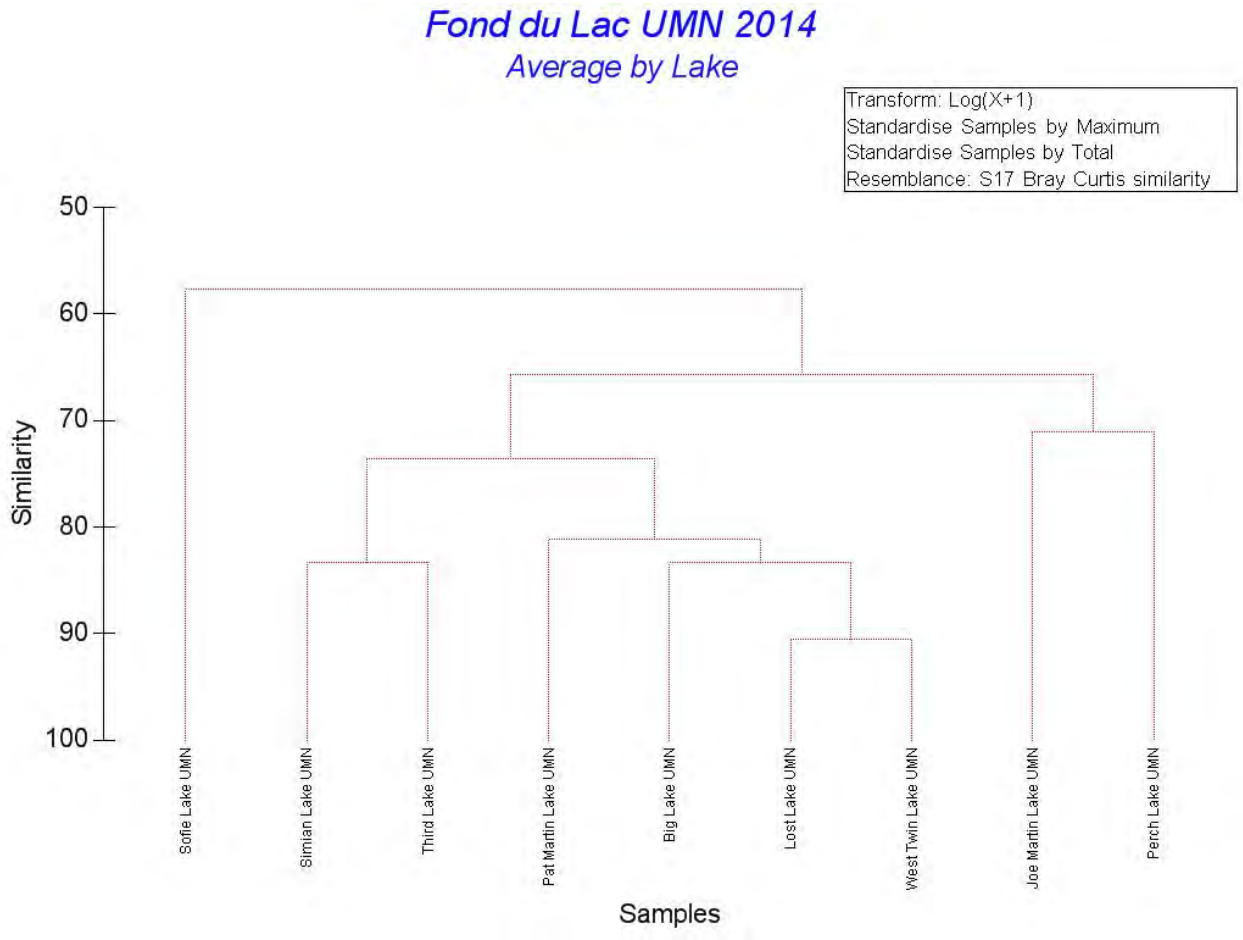


Table 6. Study Site Wide Statistics for common water quality data.

Study Site Wide Statistics			
Parameter	**Study Site**	**Mean**	**Standard Deviation**
Chlorophyll *a*	FDL	6.66	6.23
	MPR	24.59	31.41
	MCWD	29.66	35.96
Total Phosphorus	FDL	21.25	9.93
	MPR	64.5	63.82
	MCWD	91.54	92.19
Total Nitrogen	FDL	731.63	302.7
	MPR	907.44	559.99
	MCWD	1347.7	750.38
TN:TP	FDL	36.77	14.70
	MPR	18.98	16.27
	MCWD	28.29	72.89

Figure 40. Group 1 Average Chla, TN, TP and TN:TP for MPR and FDL lakes.

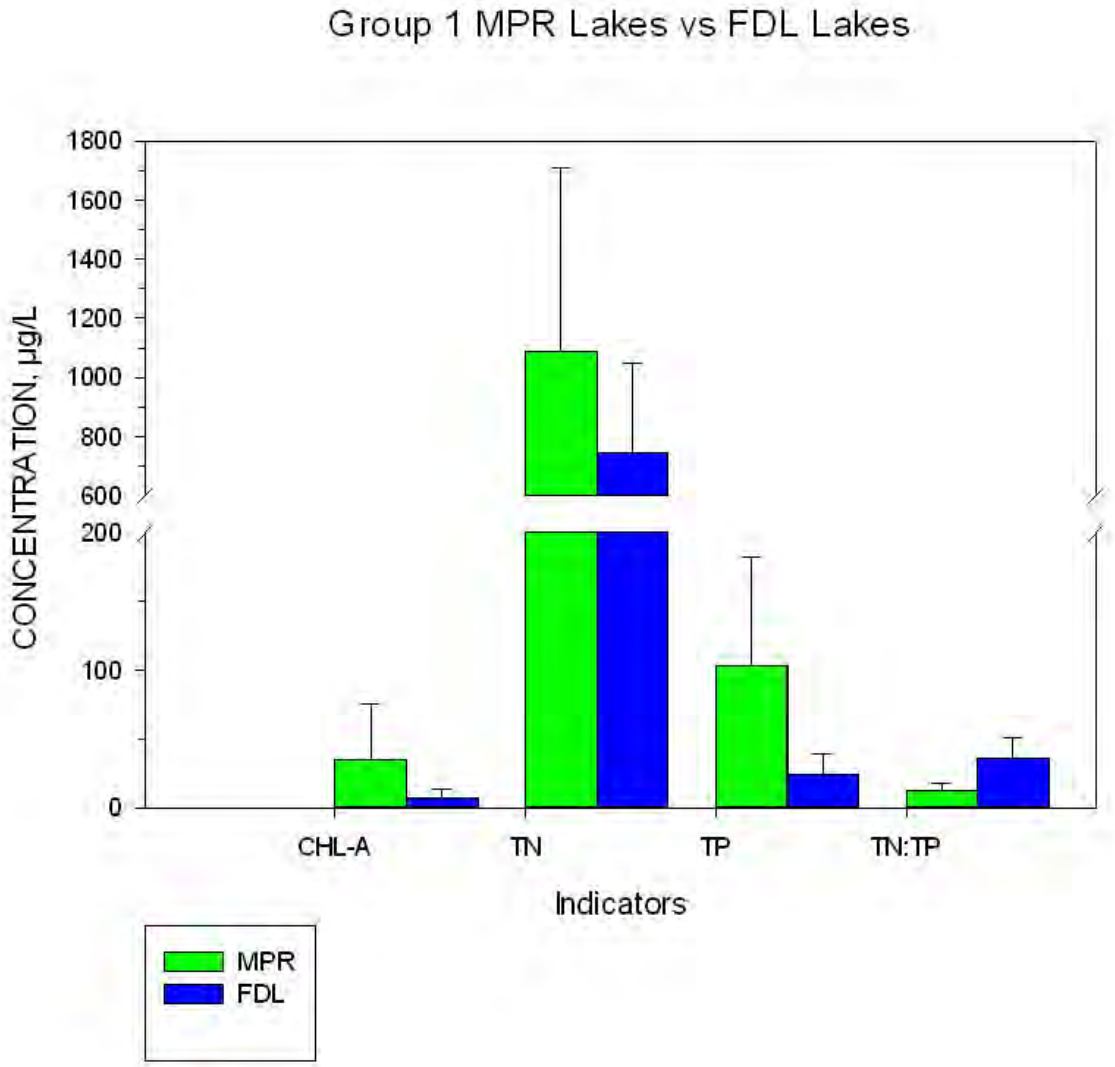
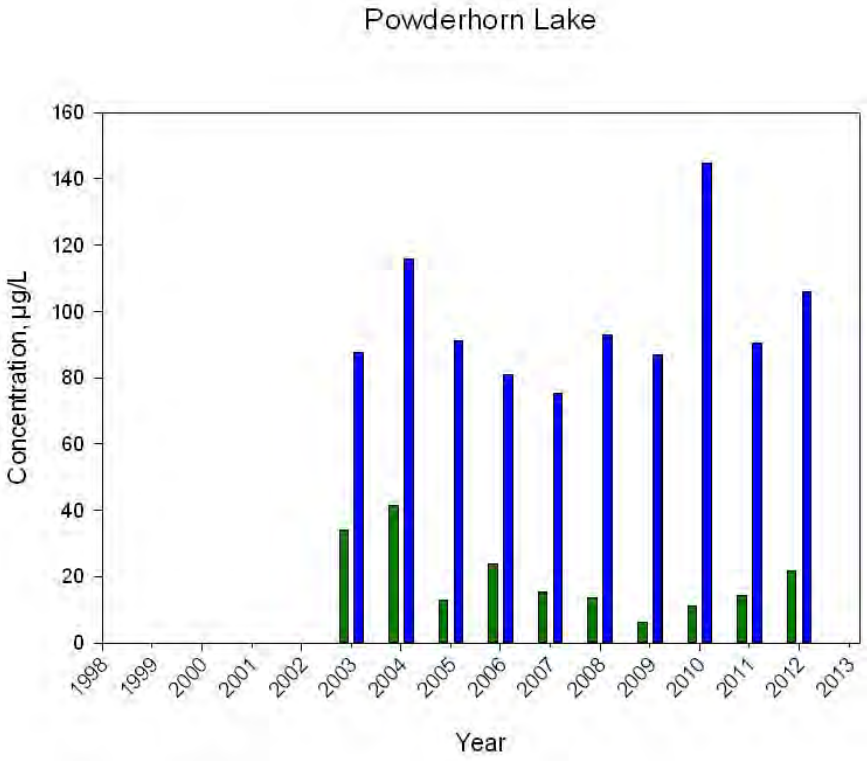


Figure 41. Chla and TP averaged by year for a) Powderhorn Lake (MPR) and b) Third Lake (FDL).

a.



b.

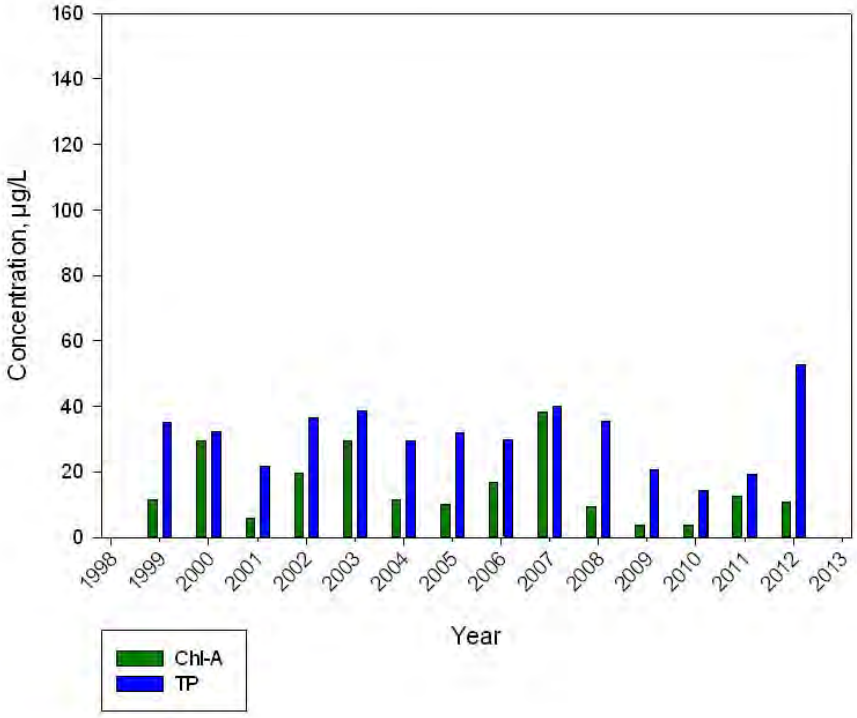


Figure 42. Air Temperature (^{o}C) for FDL and MPR/MWCD study sites. FDL air temperature (Mean=20.2^{0}C, SD=5.272) is significantly lower than the southern study sites (Mean=24.8^{0}C, SD=5.441), Mann-Whitney Rank Sum Test, p<0.001.

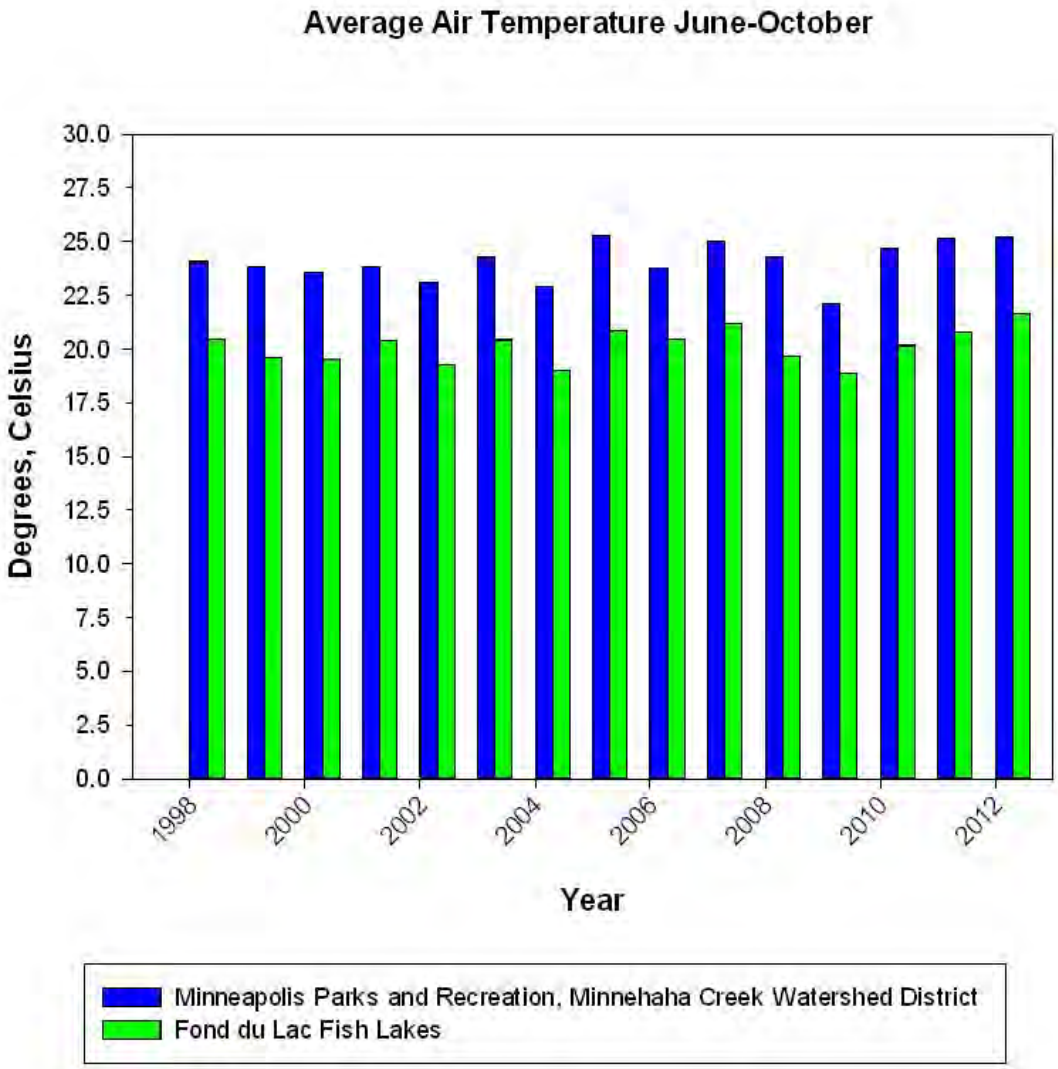


Figure 43: Yearly ice out dates for lakes within each study area (lake specific data was not available for all lakes).

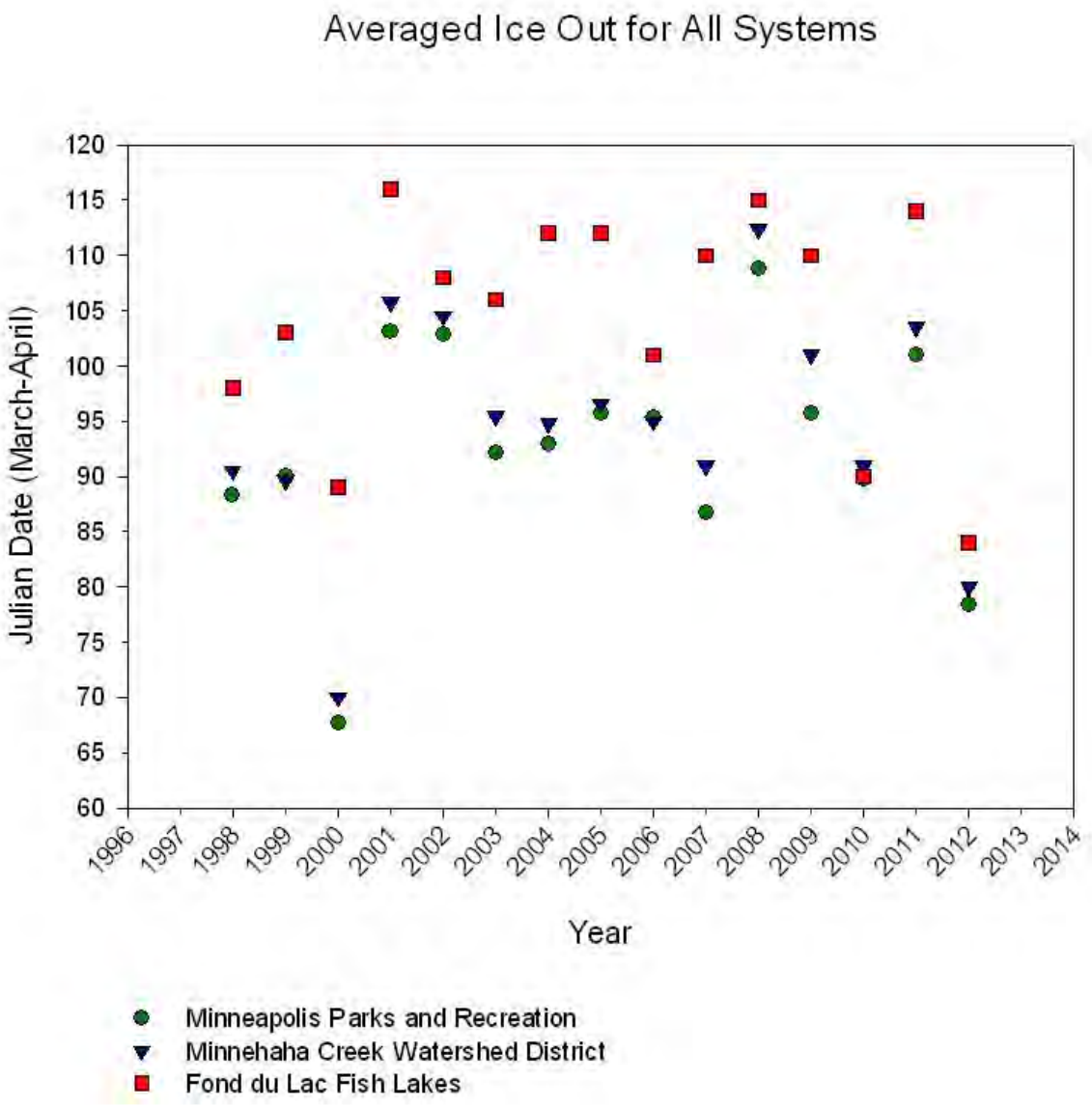


Figure 44. Total rainfall in FDL and MPR/MWCD geographic areas.

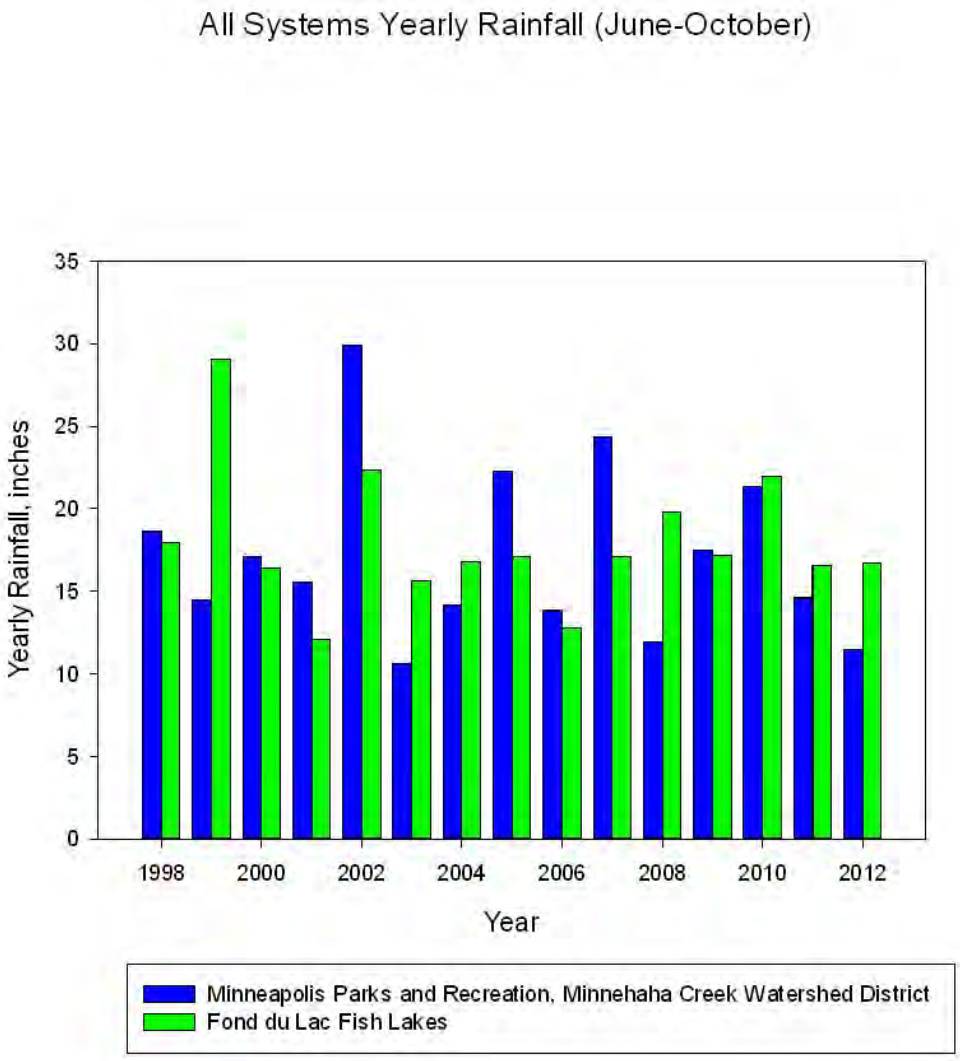
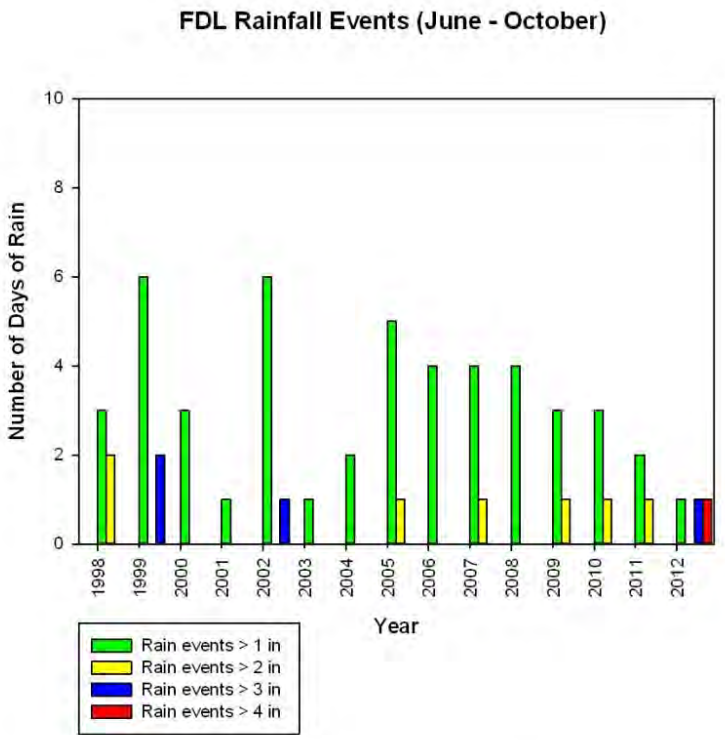


Figure 45. Rainfall events that exceed 1, 2, 3 and 4 inches in a) FDL and b) MPR/MWCD study sites (June through October).

a.



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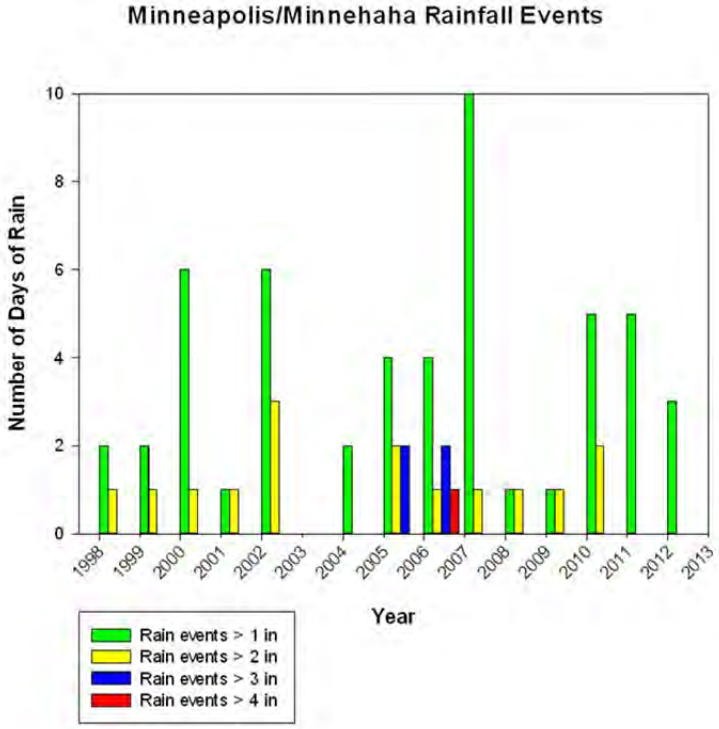

Development of Lake-specific Numerical Nutrient Criteria for Water Quality Standards in Reservation Lakes

Report submitted to:

Fond du Lac Reservation Office of Water Protection

and

Grand Portage Reservation Water Quality

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Preface

This technical report describes the approach for establishing lake-specific numeric nutrient criteria in tribal lakes in the Fond du Lac and Grand Portage reservations. Fond du Lac and Grand Portage Reservations (hereafter FDL and GP) have federally approved Water Quality Standards. Presently, both reservations are working towards USEPA's request to replace narrative nutrient criteria with numeric nutrient criteria. This report describes the approach for the 9 fisheries lakes of FDL and all 15 of the GP lakes. I also include data and analyses for comparison purposes from the 29-lake reference lake database from the Northern Lakes and Forests Ecoregion (the ecoregion for which both reservations are located within) sampled by the MN Water Pollution Control Agency and provided to me in August 2010 by Steve Heiskary.

Introduction

Background: Developing nutrients criteria in lakes

The USEPA has requested that scientifically defensible numerical nutrient criteria be developed to protect designated uses of water bodies (USEPA 2000). Because designated uses themselves are difficult to directly measure, nutrient levels of water bodies have been suggested to be important indicators of designated uses. However, nutrient concentrations alone do not directly measure designated uses. For example, for the designated use of 'supporting aquatic life' in lakes, it is not clear what concentration of phosphorus or nitrogen would indicate that this use is or is not being supported. This result occurs, in part, because 'healthy' biological communities have been found to exist in lakes with total phosphorus concentrations of 5 ug/L or 50 ug/L depending on the type of lake, the landscape setting, the lake depth, etc. To address this issue, practitioners have recommended that biological responses be used to measure the 'aquatic life' designated use such that if the biological response changes when nutrients increase, then it is an indication that the designated use is being threatened or not being supported (Stevenson et al. 2004, Reckhow et al. 2005, Heiskary and Wilson 2008, Soranno et al. 2008). However, it has also been noted that it is critical to consider the natural hydrogeomorphic setting of the lakes and use some sort of quantitative classification to ensure natural lake to lake variation is taken into account when determining whether an important biological change has occurred (Heiskary and Wilson 2008, Soranno et al. 2008, Bachmann et al. *in press*).

An important step in establishing nutrient criteria is to relate nutrient concentrations to biological responses in lakes. Much research that has been conducted in this area for purposes other than nutrient criteria development can inform any criteria development program (see citations in Soranno et al. 2008). However, the vast majority of such studies have been conducted in relatively large, deep, stratified, clear lakes. There certainly are studies conducted on shallow lakes, but such studies often contain lakes with relatively low to only moderate levels of water color, although water color is not always reported (e.g., Jeppesen et al. 2000). I would argue, that even the relationship between nutrients and algae, arguably one of the most well-studied relationships in limnology, has not been well studied in highly stained deep, or highly stained shallow lakes (but see Nurnberg and Shaw 1999 and Bachmann et al 2003). In addition, much less research related to nutrient criteria development has occurred for lakes that are not deep, stratified, and clear (but see Bachmann et al. (a,b,c) *in press*). The fundamental nature of the

hydrologic, chemical and physical characteristics of lakes that are shallow, unstratified, and colored is so different from deep, stratified lakes (Nurnberg and Shaw 1999; Webster et al. 2008). As a result, many of our commonly held assumptions regarding the basic limnological relationships need to be evaluated for such lakes.

Another critical factor to consider is the natural hydrogeomorphic setting of lakes because it sets the stage for establishing the natural, or 'expected conditions' of nutrients. 'Expected conditions' are defined as the concentrations of nutrients in a lake in its least disturbed condition given the state of today's landscape (Stoddard et al. 2006, Soranno et al. 2008). Quantifying the expected conditions in water bodies is complicated for lakes that are currently subjected to human disturbance, in which case, the expected condition cannot simply be measured by taking samples in present-day. Several possible approaches have been proposed to address this challenge (summarized in Soranno et al. 2008). However, for the situation where lakes are presently experiencing relatively low human impacts and have not changed significantly from historical levels, then measurements taken from present-day can be used as a measure of expected condition (Lafrancois et al. 2009, Bachmann et al. *in press*).

Establishing criteria in tribal waters

The fundamental scientific underpinnings, approaches, and assumptions for establishing nutrient criteria should be no different in tribal waters compared to state waters. Thus, strategies from other states or countries can be applied to establishing nutrient criteria in tribal waters. However, there are two critical differences between establishing numerical nutrient criteria in state waters compared to tribal waters is: (1) the difference in the number of water bodies for which the criteria will be applied, and (2) the type and availability of data to robustly quantify numerical criteria. In a perfect world, we would have and use the exact same data for both state and tribal waters. The reality is that because reservations typically have fewer water bodies, they can devote greater resources per water body and sample each one (or the majority of them) on a regular basis to be able to measure how they change through time. This wealth of data is rarely if ever available for state waters, although certainly some states with fewer lakes have data that are available on any given lake through time to varying degrees. However, for states that have thousands of lakes, an even greater challenge is to effectively capture the large lake-to-lake variation that exists across the state, which may in fact swamp the changes in nutrients that occur

from year to year. This fundamental difference in data availability and numbers of water bodies to be managed calls for a different approach for establishing nutrient criteria in tribal waters compared to state approaches, and consideration of how these data can be most effectively used to support the protection of these waters under the Clean Water Act.

In this report, I will describe an approach to quantify numeric nutrient criteria in tribal lands that incorporates some components of existing approaches, but that recognizes the above critical issues. I apply this strategy to lakes in two reservations: the Fond du Lac Reservation (hereafter FDL) and the Grand Portage Reservation (hereafter GP). The approach requires long-term lake data for nutrients and water color or dissolved organic carbon (to assess inter-annual variability), which is rarely available for all state lakes that must be managed and protected under the Clean Water Act.

Comparison and Analysis of the Available Approaches to Develop Numeric Nutrient Criteria

There have been several recent efforts to develop approaches for establishing numerical nutrient criteria (e.g., Dodds and Oakes 2004, Reckhow et al. 2005, Heiskary et al. 2008, Soranno et al. 2008, Bachmann et al. (c) *in press*). In Table 1, I summarize three of the approaches developed to date, as well as the approach I describe in this report. I explore the relative strengths and weaknesses of each approach to help inform the approach that I developed for these tribal waters. The three U.S. states for which criteria have been developed are large in area (incorporating 3-5 Omernik ecoregions within their boundaries), have > 6,500 lakes, and wide ranges of lake sizes (Table 1). Two of the states (MI and MN) have large numbers of different types of lakes ranging from shallow to deep, clear to colored, although the majority are clear-water; the other state (FL) has more similar lakes (shallow, colored, and seepage), but with still large variation in nutrients across the state (Table 1).

States address the challenge of managing thousands of lakes by sampling as many of their often thousands of lakes that they are responsible for at least one time (typically during the same index period). There are two main ways that such data have been then used to establish nutrient criteria: (a) by using or creating ecoregions or nutrient zones (Heiskary and Wilson 2008, Bachmann et al. (c) *in press*) that assume all lakes within a given ecoregion or zone are more similar to each other than to lakes in other zones, (b) use statistical modeling of the local or regional landscape features that are hypothesized to be most related to lake nutrients (Soranno et

al. 2008). These approaches are briefly described in Table 5, as are the pros and cons of each approach. They have been developed to try to effectively capture as much variation in nutrients across lakes within the large, heterogeneous states and to establish criteria for different lake types or regions. The advantages of these approaches are that they can be applied at large spatial scales, they can be used for states that have data from many lakes, but with limited temporal sampling, and the approaches use a variety of strategies to incorporate biological condition to inform or relate to criteria and ultimately designated uses (Table 1).

The implicit assumption in these approaches is that a single-time sample during an 'index' period captures the 'average' conditions of the lakes and that variation among individual lakes and different lake types is greater than temporal variation. In other words, temporal variation is assumed negligible. Although most practitioners recognize that this assumption is not always met, if at all, it is the best that can be done with present data. In fact, the integration of spatial and temporal variation is an important research gap that is needed to be addressed to help inform nutrient criteria development across the nation.

Although these approaches are all well-thought out and appear to work effectively for the states for which they were developed, there are weaknesses with any approach, especially when considering their use in tribes or states with few lakes and extensive long-term monitoring data. For example, for both the Michigan and Florida approaches, the models that explain TP variation across the states only account for ~40% of variation in TP among lakes. Thus, much unexplained variation in lake TP remains that likely leads to errors in applying estimated criteria to individual lakes. For the Minnesota approach, the amount of variation accounted for by their approach cannot be explicitly calculated because their criteria are not based on a single model as the other two approaches are. However, we can evaluate the variation in nutrients in lakes in the reference lake database used to develop the criteria for the Northern Lakes and Forest Ecoregion (NLF, Heiskary and Wilson 2008) and compare those values to the current nutrient concentrations in the tribal waters for the GP reservation (Lafrancios et al. 2009), there are large differences. Nutrient concentrations in GP are much higher than the reference lake database. Considering both groups of lakes are in a minimally-disturbed state, then there would be large errors in applying the NLF ecoregion criteria to GP lakes (Lafrancois et al. 2009).

For the above reasons, the approaches for establishing nutrient criteria in tribal waters must be different from the approaches that have been developed for states with large spatial

extents (e.g., 3-5 Omernik ecoregions) and large numbers of different types of lakes. In developing an approach to establish nutrient criteria in tribal waters, I have incorporated some of the ideas, concepts, and steps from past approaches, because most of the basic ideas apply. However, strategies that take into account temporal variation must be incorporated. It is hoped that the approach developed here can serve as a template for other tribal waters or states that have comparable data and small numbers of lakes to be managed.

Overview: A New Approach for Establishing Numeric Criteria for Sites with Small Numbers of Lakes and Long-term Monitoring Data

There are 8 main steps in this approach to quantify numeric nutrient criteria (Table 2). I first describe the approach in general in this section. In later sections, I describe the application of this approach to the FDL and GP lakes.

Step 1. The first step with any approach to quantify nutrient criteria is an assessment of designated uses for each water body. The most restrictive designated use is then identified and noted for each lake. This approach assumes that the lakes are currently in a minimally-impacted state. If lakes are currently experiencing significant human impacts, then an alternative approach needs to be developed. The most restrictive designated use is then used to guide the criteria development.

Step 2. All available nutrient data are compiled from as many lakes within the area to be managed. Preferred data include: lake nutrients such as total phosphorus (TP) and total nitrogen (TN), any measure of organic carbon (such as water color or dissolved organic carbon (DOC)), additional measures of water clarity such as Secchi depth, and algal measures (such as chlorophyll concentrations or algal biomass).

Step 3: The data in the database should be plotted to identify any outliers and determine if trends are present in the data. If trends are present then the likely causes should be explored. However, the remaining steps assume that there are few if any quantifiable trends in the data. Because shallow lakes mix more frequently, they may be subject to larger numbers of outlier data points in which a sample taken during a mixing event could be substantially different than a sample taken during a short-term stratification event. Outliers should be noted and monitored in the future. However, they are removed from the remaining analyses to estimate expected condition of the lakes. Because there are no universally accepted mathematical definitions of

outliers, I used a variety of approaches to identify and remove outliers. First, I plot the data through time to identify candidate outliers that are greatly different from most data points. Then, I plot well-known relationships among the variables to identify data points that fail to fit common limnological relationships. I removed data points from further analyses only after a data point appeared as an outlier from both plots. Evaluation of common limnological relationships allows an assessment of the underlying processes that control lake nutrients and algal communities. This step is important because it determines which published studies can be used to evaluate the lakes. However, often, such relationships have been primarily developed for either spring, or summer periods, and so the seasonality needs to be considered in this step as well.

Step 4. Because the lakes are currently in a minimally impacted state, then the current biological conditions can be assumed to be indicative of lakes meeting designated uses. Biological data from such lakes cannot be used to quantify thresholds in human disturbance, because there would be no lakes that are the high end of the gradient of human use; therefore if a gradient approach were to be taken, data from other sites would have to be used. However, biological data can be treated similar to the nutrient data and be used to set recommended values to support designated uses.

Step 5. Because of large seasonality for water bodies, criteria need to be determined by season (either one or more). Therefore, if samples are taken more than one time per season, or across seasons, then these data need to be accounted for. In addition, the season of most importance for establishing criteria needs to be established and decided.

Step 6. Using the nutrient database for each lake, the 'expected condition' of the lakes for nutrients, clarity and algae can be calculated as the full range of expected concentrations for each variable and each lake. At this point, another examination of outliers should be conducted on a lake-by-lake basis.

Step 7. The final step is to use the above expected conditions to calculate numeric nutrient criteria for each lake to protect designated uses. The nutrient criterion for each nutrient in each lake is calculated to be the upper 90^{th} percentile of the samples within a season across all years.

Lake Descriptions and Designated Uses

Fond du Lac and Grand Portage Lakes

FDL has nine lakes for which criteria are being established in this report. These lakes are the 'primary fisheries' lakes that range in surface area from 6 – 212 ha, and maximum depth from 3.4 – 23.5 (Table FDL1). The lakes have high amounts of natural land cover in their watersheds including forest, grassland/shrubland or wetlands (Table FDL1). The lakes have the designated uses described in Table FDL2, the most restrictive being aquatic life uses.

GP has 15 lakes for which criteria are being established in this report. These include most of the major lakes in GP and they range in surface area from 1 – 144 ha, and they range in maximum depth from .9 – 7.6. The lakes have high amounts of natural land cover in their watersheds (Lafrancois et al. 2009), and have designated uses as described in Table GP2, with aquatic life being the most restrictive.

Using several lines of evidence, I assume that the lakes in both reservations are for the most part in minimally-impacted condition. Edlund et al. (2007, 2009) show results from lake sediment cores taken in two of the GP lakes and found no difference between historic and present-day diatoms and in diatom-inferred TP. In addition, human land use/cover in both reservations is very low, with the maximum % cover of human-dominated land use/cover of 12% in the Big Lake watershed in FDL, although most lakes have human land use/cover < 5% in FDL (Table FDL1), and even lower levels in GP (Lafrancois et al. 2009). The acknowledgement of these lakes being in a minimally-impacted state is important because they presently have relatively high nutrient concentrations relative to lakes in the NLF ecoregion (Lafrancois et al. 2009). However, these high nutrient levels can be attributed to shallow depth of the water bodies, and high DOC concentrations in the lakes (Lafrancois et al. 2009). Other recent efforts to develop criteria for shallow, colored lakes have also arrived at high values for both TP and TN criteria (FL, Table 1).

Comparison of reservation lakes to reference lakes in the NLF ecoregion

There are important similarities AND differences among the three groups of lakes. In comparing the sites, I focus on differences that appear to be ecologically important rather than statistically important because the NLF dataset medians are calculated across lakes with individual data points, and the GP and FDL dataset medians are calculated across lakes and

across time. Thus, error estimates would be biased due to the different nature of the dataset structures.

First, I discuss the characteristics across the sites that are similar. Based on median values from 1998-2009 for FDL and GP (Table 3), it appears that FDL lakes are similar to the NLF lakes in the reference database for: TP, chlorophyll *a* (although not at the 90th percentile level), and chl *a*:TP ratio (although not at the 90th percentile level). For GP lakes, there are similar ranges to NLF lakes for TP at all percentiles except the 75th and 90%. In addition, chlorophyll *a* is similar for all percentiles except the 90th, with GP lakes having lower chlorophyll than NLF lakes. The chl *a*:TP ratio is also similar for the 25th and median percentiles, but the ratio is higher in GP lakes for the 75th and 90th percentiles.

Second, I discuss the characteristics across the sites that differ. Both FDL and GP lakes differ from these NLF lakes in both Secchi depth and color – both measures of water clarity such that water clarity is much lower in FDL, and even lower still in GP compared to the NLF lakes. There are differences between GP and NLF lakes for TP at the 75th and 90th percentiles (Table 3), which means the lakes that have high TP in GP have higher TP than the lakes in the NLF that have the highest values. In addition, TN is higher in both FDL and GP than NLF at all levels, with GP being higher than FDL as well.

There is an interesting pattern with chlorophyll *a*. In general, past studies have found that chlorophyll concentrations are often higher in lakes with high color (Nurnberg and Shaw 1999, Webster et al. 2008). Examining the medians across all lakes at FDL and GP, it does not appear that these more highly colored lakes have higher chlorophyll than the NLF lakes (Table 3). Perhaps the fact that lakes in both sites (especially GP) are mostly shallow, especially compared to the NLF dataset as well as the datasets cited above. In shallow lakes in Florida, for example, lakes with macrophyte cover have somewhat lower chlorophyll than laeks without macrophytes, although the relationship was noisy (Bachmann et al. 2002). Thus, the effect of higher chlorophyll in colored lakes that typically occurs could be offset by shallow depths in these lakes (and plant cover) that limits phytoplankton growth and keeps chlorophyll in some of the lakes relatively low.

Applying This Approach to Tribal Waters

Development of numeric nutrient criteria in the 9 FDL fisheries lakes

Steps 1-3: For the FDL fisheries lakes, nutrient, chlorophyll, and clarity data were collected monthly from 1999-2009 during the open-water season (typically May to October). Designated uses for each lake are described in Table FDL2 and the most restrictive use (Aquatic life) was used for criteria development for all lakes. I plotted the data through time for each lake and found no evidence for significant trends in the datasets (Appendix 1A). I plotted nutrients, clarity, and algal variations against each other to help to identify outliers in the data. A few outliers were removed based on evaluation of these common limnological relationships.

The fisheries lakes in FDL fall well within common patterns observed in other north temperate lakes, although some of the relationships are not as strong as observed in other studies. For example, TP is positively related to chlorophyll (Figure 1), however, the strength of the relationship is somewhat lower than other studies. The less strong relationship is most likely due to the fact that FDL lakes tend to be shallower and more colored relative to most lakes that are part of studies examining TP vs CHL relationships. FDL lakes appear to be more limited by phosphorus compared to nitrogen as the relationships between chlorophyll and TP is stronger than the relationship between chlorophyll and TN (Figure 1). Importantly, water color is also positively related to chlorophyll, although the slope is shallow and the amount of variation explained is low, but significant. Other studies have found that chlorophyll concentration is in fact higher in colored lakes compared to clearer lakes for the same TP levels (Webster et al. 2008). The reason for this pattern has not been conclusively identified, but some have argued that high color forces phytoplankton into a smaller volume of water near the surface. The fact that chlorophyll is not elevated compared to NLF lakes, suggests that this effect is not large (see Table 3). However, because shallow lakes can have lower chlorophyll concentrations, perhaps the water color effect is being offset by the shallow depths of these lakes. Nutrients themselves are correlated in these lakes. Plots of TP vs TN are similar to other studies. TP and water color are also positively correlated, as is TN and water color, both of which have been found elsewhere.

Step 4: COMING SOON. I will use the biological data that have been collected by the tribes to assess the current biological condition of the lakes. Similar to the nutrients, given that it is assumed the lakes are in minimally-impacted state, the biological condition should reflect that

condition as well. The biological conditions measured to date could be used as a benchmark for achieving the aquatic life designated uses given the nutrient concentrations during the same time period. Metrics to be quantified for FDL include: LIST METRICS HERE.

Step 5: Because most research and nutrient criteria development has been conducted using data from the summer index period, I selected the summer months of July, August, and September as the index period to more easily compare to other studies. In addition, this period is the time of maximum primary and secondary production in lakes. I calculated a range of percentiles for the data (including the median, the 50th percentile) to compare the two reservation sites to each other and to the 29-lake reference lake database for lakes within the Northern Lakes and Forest ecoregion (NLF) compiled by S. Heiskary for MN's nutrient criteria development (Table 3). I used this dataset for comparison because these NLF lakes were considered to be in a minimally-impacted state and because both FDL and GP are within the NLF ecoregion. FDL lakes are relatively similar to NLF lakes for TP at all percentiles. On the other hand, TN is higher in FDL lakes relative to NLF lakes for all percentiles. Chlorophyll is remarkably similar across all three sites. In addition, the Chl *a*:TP ratio is very similar between NLF and FDL lakes except for the 90th percentile lakes where FDL lakes that have the highest ratio are higher than the highest observed ratios in NLF lakes. Because for a given TP concentration, more highly stained lakes have somewhat higher chlorophyll, some of this difference may be due to the higher water color in the FDL lakes compared to the NLF lakes, especially in the upper percentiles (Table 3). This overall higher water color in FDL also leads to overall shallower Secchi depths in FDL compared to NLF lakes.

FDL and GP lakes have some similarity, but also important differences. The hydrogeomorphic settings of the two reservations differs substantially, which may be the reason for the fairly large differences in nutrient concentrations and water color. TP is higher in general in GP, primarily in the higher percentiles (Table 3). Whereas, TN is consistently higher at all percentiles, as is water color. Interestingly, despite such differences, the chlorophyll concentrations are remarkably similar across FDL and GP, as well as NLF lakes. However, given the large differences among the three groups of lakes, I would argue that chlorophyll may be limited by different factors in the different groups of lakes.

Step 6: The box plots show that both interannual variability within the lakes, and variability across the lakes is quite large in FDL and must be taken into account when setting

nutrient criteria (Figure 3a-b). A single criterion that applies to all lakes would not be recommended due to the diversity of lakes in FDL. When examining the conditions of the lakes individually, there is very large inter-lake variability in most variables except perhaps chlorophyll. The medians of TP range from between 10 and 20 ug/L to greater than 30 ug/L in a couple lakes (Figure 3a). TN has a wider range across the lakes, which chlorophyll is actually consistently below 10 ug/L except for Third Lake, which has been noted to be partially supporting its designated uses in the past due to the presence of algal blooms. Further examination of this lake is needed and is ongoing (N. Schuldt, personal comm.). Secchi depths are relatively shallow reflecting the high water color in these lakes and these two variables are inversely related as is commonly the case (Figure 3b).

To determine the expected conditions of the lakes for each nutrient, I removed any remaining outliers in the dataset (ones that were not selected using the above approaches). For this step, I defined the outliers statistically as the 'far outside values' that are beyond 3.5 times the interquartile range of the data (Systat 11.0 software). These points are shown as open circles in Figure 3a-b. I removed these outliers because they represent extremely high values that are rare across the 10 year sampling period so would be likely to bias the nutrient criteria calculations in the next step. In FDL, I deleted 2 values for TP, 3 values for TN and 3 values for chlorophyll as shown in Figure 3a.

Step 7: I then took the TP, TN and chlorophyll datasets for each lake for the samples in July, August, and September from 1999-2009 (with outliers removed) and calculated the 90th percentile value for each lake. This value is the lake-specific nutrient or chlorophyll criterion. In Table 4b, the criteria are shown for all lakes with the outliers removed as per Step 6. In brackets, I show the number of samples that were used to estimate the criterion. At the bottom of the table, I calculated the median criterion (e.g., the median of all the lake TP criteria from FDL lakes only) to compare it to the median criterion in GP lakes as well as to the criterion that has been recommended for NLF ecoregion lakes. For TP, the median FDL criterion is less than the NLF criterion, by 7 ug/L. However, an important point is that the lake to lake variation within FDL is very high such that if just the median value was used (i.e., 23) for all lakes, there are lakes with 'expected conditions' well ABOVE or BELOW that value by ecologically relevant amounts (e.g., one lake is 24 ug/L over the median, and another lake is 8 ug/L below the median). These results highlight the importance of capturing lake-to-lake variation in setting nutrient criteria.

The values for TN cannot be compared to NLF because TN criterion were not estimated. However, the chlorophyll criterion is identical to the NLF criterion. However, again, the lake-to-lake variation within FDL is large such that some lakes are 6 ug/L less than the median criterion, or 35 units above it. The very large chlorophyll criterion for Third Lake suggests that perhaps it is experiencing human impact that has not been quantified yet. Finally, to more easily compare across FDL, GP and NLF lakes, I plotted the data from Table 4b (Figure 5). The plots show that the FDL lakes fall above and below the NLF criterion, but are lower in general than the GP criteria for nutrients.

Table 4b represents the recommended nutrient criteria for the FDL lakes. However, I include Table 4a to show the effect of removal of the outlier points. In Table 4b, I highlighted in yellow, those criteria that changed once the outliers were removed. Criteria decreased after removal of outliers for no lakes for TP, for only 3 lakes for TN, and for 1 lake for chlorophyll. The differences were ecologically relevant in some cases, and not large in others. However, as the outliers that were removed were relatively rare data points, I think the dataset with outliers removed is a better reflection of expected conditions. Nevertheless, it would be worth examining the outliers in relation to other conditions (such as sampling conditions, lack of stratification, etc.) that might explain these occasional high values.

Development of numeric nutrient criteria in 15 GP lakes

Steps 1-3: For the GP lakes, nutrient, chlorophyll, and clarity data were collected monthly from 1999-2009 every other year during the open-water season (typically May to October). Designated uses for each lake are described in Table GP2 and the most restrictive use (Aquatic life) was used for criteria development for all lakes. I plotted the data through time for each lake and found no evidence for significant trends in the datasets (Appendix 1**B**). I plotted nutrients, clarity, and algal variations against each other to help to identify outliers in the data. A few outliers were removed based on evaluation of these common limnological relationships.

The GP do not seem to follow patterns observed in other north temperate lakes. For example, TP is only very weakly positively related to chlorophyll (Figure 2). The lack of a relationship is most likely due to the fact that GP lakes are even shallower and more colored than FDL lakes and much more so than most lakes that are part of studies examining TP vs CHL relationships. There is little evidence that GP lake chlorophyll is limited by either phosphorus or

nitrogen (Figure 2), as the relationships between TP or TN and chlorophyll are significant, but with extremely low R^2. And, similar to FDL, GP lake chlorophyll is only weakly, but positively related to water color (as measured by DOC). Other studies have found that chlorophyll concentration is in fact higher in colored lakes compared to clearer lakes for the same TP levels (Webster et al. 2008). The reason for this pattern has not been conclusively identified, but some have argued that high color forces phytoplankton into a smaller volume of water near the surface. The fact that chlorophyll is not elevated compared to NLF lakes, suggests that this effect is not large (see Table 3). However, because shallow lakes can have lower chlorophyll concentrations, perhaps the water color effect is being offset by the shallow depths of these lakes. Nutrients themselves are correlated in these lakes, but again, much weaker than other north temperate lakes and weaker than FDL lakes.

Step 4: COMING SOON. I will use the biological data that have been collected by the tribes to assess the current biological condition of the lakes. Similar to the nutrients, given that it is assumed the lakes are in minimally-impacted state, the biological condition should reflect that condition as well. The biological conditions measured to date could be used as a benchmark for achieving the aquatic life designated uses given the nutrient concentrations during the same time period. Metrics to be quantified for FDL include: LIST METRICS HERE.

Step 5: Because most research and nutrient criteria development has been conducted using data from the summer index period, I selected the summer months of July, August, and September as the index period to more easily compare to other studies. In addition, this period is the time of maximum primary and secondary production in lakes. I calculated a range of percentiles for the data (including the median, the 50^{th} percentile) to compare the two reservation sites to each other and to the 29-lake reference lake database for lakes within the Northern Lakes and Forest ecoregion (NLF) compiled by S. Heiskary for MN's nutrient criteria development (Table 3). I used this dataset for comparison because these NLF lakes were considered to be in a minimally-impacted state and because both FDL and GP are within the NLF ecoregion. GP lakes differ from the NLF lakes for almost all variables except chlorophyll. In fact, GP is more different to the NLF dataset than the FDL are (Table 3). TP is generally higher than NLF lakes, but only by small amounts in the higher percentile ranges. TN is much larger in GP lakes than both NLF and FDL lakes. Whereas, chlorophyll is similar across all sites. Finally, both Secchi depth and DOC are very different in GP lakes compared to NLF lakes, with FDL lakes being

intermediate between NLF and GP lakes for both. The hydrogeomorphic settings of the two reservations differs substantially, which may be the reason for the fairly large differences in nutrient concentrations and water color. Interestingly, despite such differences, the chlorophyll concentrations are remarkably similar across FDL and GP, as well as NLF lakes. However, given the large differences among the three groups of lakes, I would argue that chlorophyll may be limited by different factors in the different groups of lakes.

Step 6: The box plots show that both interannual variability within the lakes, and variability across the lakes is quite large in GP and must be taken into account when setting nutrient criteria (Figure 4a-b). GP lakes have larger ranges in TP compared to FDL, but about the same ranges for CHL and TN (although the absolute levels of TN is higher in GP). A single criterion that applies to all lakes would not be recommended due to the diversity of lakes in GP. When examining the conditions of the lakes individually, there is very large inter-lake variability in most variables except perhaps chlorophyll. The medians of TP range more across the 15 lakes than the FDL lakes (Figure 4a). Secchi depths are relatively shallow reflecting the high water color in these lakes and these two variables are inversely related as is commonly the case (Figure 4b). Although Secchi depth is relatively deep in two lakes – Trout and Taylor, and DOC concentrations are also low in these lakes (Figure 4b).

To determine the expected conditions of the lakes for each nutrient, I removed any remaining outliers in the dataset (ones that were not selected using the above approaches). For this step, I defined the outliers statistically as the 'far outside values' that are beyond 3.5 times the interquartile range of the data (Systat 11.0 software). These points are shown as open circles in Figure 4a-b. I removed these outliers because they represent extremely high values that are rare across the 10 year sampling period so would be likely to bias the nutrient criteria calculations in the next step. In GP, I deleted 14 values for TP, 4 values for TN and 5 values for chlorophyll as shown in Figure 4a. It appears that GP had more datapoints that were classified as outliers compared to FDL. One possible explanation for this result is that because GP lakes in general are more shallow than FDL lakes, they may mix more frequently leading to more events of sediment resuspension that can lead to higher pulses of nutrients and possibly algal cells that have settled to low light areas. This idea could be tested by looking at temperature profiles during these sampling events and total suspended solids to see if it is elevated on days that these outliers were present. If you remove the two deepest lakes from GP and the one deep lake in

FDL and calculate the averages of the lake depths, the average of the maximum depths for GP is 2.6 m and for FDL is 4.9 m. Nevertheless, these GP data points still met the criteria for outliers and were removed from further analyses.

Step 7: I then took the TP, TN and chlorophyll datasets for each lake for the samples in July, August, and September from 1999-2009 (with outliers removed) and calculated the 90th percentile value for each lake. This value is the lake-specific nutrient or chlorophyll criterion. In Table 4b, the criteria are shown for all lakes with the outliers removed as per Step 6. In brackets, I show the number of samples that were used to estimate the criterion. At the bottom of the table, I calculated the median criterion (e.g., the median of all the lake TP criteria from GP lakes only) to compare it to the median criterion in FDL lakes as well as to the criterion that has been recommended for NLF ecoregion lakes.

For TP, the median GP criterion is nearly identical to the NLF criterion. However, an important point is that the lake to lake variation within GP is very high such that if just the median value was used (i.e., 31) for all lakes, there are lakes with 'expected conditions' well ABOVE or BELOW that value by ecologically relevant amounts (e.g., one lake is 59 ug/L over the median amount, and another lake is 18 ug/L below the median value). These results highlight the importance of capturing lake-to-lake variation in setting nutrient criteria. The values for TN cannot be compared to NLF because TN criterion were not estimated. However, the chlorophyll criterion is identical to the NLF criterion. However, again, the lake-to-lake variation within FDL is large such that some lakes are 4 ug/L less than the median criterion, or 12 ug/L above it. Finally, to more easily compare across GP, FDL and NLF lakes, I plotted the data from Table 4b (Figure 5). The plots show that the GP lakes fall almost equally above and below the NLF median for chlorophyll, they have a much wider range in TP than in chlorophyll around the NLF median, and have higher TN 90th percentiles (and median) compared to FDL lakes.

Table 4b represents the recommended nutrient criteria for the GP lakes. However, I include Table 4a to show the effect of removal of the outlier points. In Table 4b, I highlighted in yellow, those criteria that changed once the outliers were removed. Criteria decreased after removal of outliers for 11 lakes for TP, for only 2 lakes for TN, and for 3 lakes for chlorophyll. Again, given that GP has more outliers than FDL, it is not surprising that more of the criteria changed once outliers were removed. The differences were ecologically relevant in some cases, and not in others. However, as the outliers that were removed were relatively rare data points, I

think the dataset with outliers removed is a better reflection of expected conditions. Nevertheless, it would be worth examining the outliers in relation to other conditions (such as sampling conditions, lack of stratification, etc.) that might explain these occasional high values.

Assumptions for this approach and evidence supporting these assumptions

There are several important assumptions in this approach to develop numeric nutrient criteria. First, I assume that the lakes are at minimal levels of human impact and exist in some form of 'reference' state (see above for evidence). Second, I assume that the condition of the lakes from the period of collection of the nutrient database (e.g.. for the lakes in this report, 1998-2009) is indicative of both past and future conditions of minimal human impact. Third, I assume that the nutrients in the lakes from the sampling period are at a level to support the 'Aquatic life' designated use, which is the most restrictive of the uses for the lakes. Unfortunately, the point at which an increase in nutrients will cause this use to not be supported is not known precisely or even in general because too little research has been conducted on such lakes with high color and that are very shallow. Therefore, I use the frequent sampling of nutrients from a 10 year time period for each lake to set the criterion for the nutrient level that incorporates interannual variability, as well as the biological sampling that shows communities of high biological integrity. Fourth, I assume that the climate that the lakes experienced during the time period of nutrient sampling is representative of future climate. Thus, the nutrient criteria should be valid as long as climate does not change dramatically from this period of record.

The role of projected climate change is a concern that applies to any approach for quantifying nutrient criteria. If climate does change significantly, there could be important changes in both hydrology and DOC in these lakes that likely will influence both nutrients and algal communities. For example, it is possible to develop scenarios that lead to increases OR decreases in DOC depending on changes in climate, which would likely have important effects on lake nutrients and ultimately chlorophyll. For example, it could be that with declining DOC, nutrients might also decrease, which in lakes with current minimal human disturbance is not a desired endpoint. Because DOC levels in lakes in both reservations is moderate to high, the relationship between climate, DOC, nutrients and algal response is important in these lakes.

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Edlund e tal. 2009

Bachmann et al. 2002

APPENDIX 1. I can add plots for the variables below. Takes up lots of space to do for each lake, but can easily be made if it would help.

(A) FDL Nutrient, chlorophyll, and clarity data by lake, through time.
(B) GP Nutrient, chlorophyll, and clarity data by lake, through time.

Table 1. Comparison of different approaches for estimating nutrient criteria from published studies.

Citation	Location & calculated TP criteria ranges (ug/L)	# Omernik ecoreg's	# Lakes applied to*	Max. lake area (ha)	Types of lakes	Approach	Pros	Cons
Soranno et al. 2008	Michigan **8 - 34**	5	6,595	8,000	-Most deep -Most clear -Drainage & seepage	**BTPM**: Multiple regression using landscape variables to predict expected condition; and, biological gradient analysis to determine benchmarks (ie. criteria)	-Quantifis expected condition from any lake using landscape variables -Uses biological condition to set benchmarks to inform criteria	-The model accounts for relatively low amount of variation in TP (~60% remains unexplained)
Heiskary & Wilson 2008	Minnesota **12 - 90**	4	11,842^	116,000	-Most deep -Most clear -Drainage & seepage	**Ecoregion, plus lake type & use classification:** Also factored in gradient analysis of chl, bloom frequency, and user perception.	-Predicts criterion based on ecoregion and lake type -Uses biological condition to inform criteria	-Much lake-to-lake variation within ecoregions and lake types not taken into account
Bachmann et al. *in press*	Florida **9 - 359**	3	7,700[t]	189,000	-Shallow -Colored -Seepage, 70%	**Six phosphorus zones (regions):** Clustered the lakes based on TP concentrations. Set criteria based on 90% percentile of TP within each [P] zone. -And, site-specific criteria for oligo. lakes	-Predicts criterion for any lake based on [P] zone it is in *NOTE:* Could not find obvious biological thresholds, so did not use them.	-The zones account for relatively low amount of variation in TP (~60% unexplained).
This report	Fond du Lac Res'n. **15 - 47**	<1	9	212 (median lake area = 33 ha)	-Shallow (1 deep) -Clear & colored -Drainage and some seepage	**Lake-specific temporal variation of minimally-disturbed lakes:** Use 10 yrs of monitoring data for nutrient and chlorophyll from the summer index period to calculate the criteria as the 90th percentiles for each lake for TP, TN, Chl.	-Accounts for interannual variability for individual lakes -These large datasets could be used to inform efforts at the state-scale with less data	-Need sustained long-term monitoring data for each lake
This report	G. Portage Res'n. **13 - 90**	< 1	15	144 (median lake area = 9 ha)	-Shallow -Most colored -Drainage, 100%	Same as above	Same as above	Same as above

* Lakes > 4 ha (~10 acres)

^ http://www.dnr.state.mn.us/faq/mnfacts/water.html

[t] http://www.stateofflorida.com/Portal/DesktopDefault.aspx?tabid=95

Table 2. Overview of the major steps for developing nutrient criteria for sites with interannual and seasonal water body data, and for which are presently in a minimally-impacted state. Note that this approach could be applied to lakes, wetlands or streams with minor modifications specific to each water body type. The text below is written specifically for lakes.

Step 1. Compile the designated uses for each water body: Determine the designated uses for each water body, identify the most restrictive use for relating to the criteria. Decide if the lakes are currently in the minimally-impacted state. If they are, then proceed to step 2. If not, then alternate approach required.

Step 2. Create the nutrient and algal database: Compile the nutrient criteria database that includes data on all available nutrient, clarity, and algal data for each lake through seasons and years.

Step 3. Evaluate the dataset and the controlling factors of the lakes: Make plots of all nutrient, clarity, and algal data through time to identify outliers and determine if any trends are present in the data. If trends are present, then the likely causes of the trends should be investigated. For either case, proceed to the next step. In addition, quantify common limnological relationships among water bodies (e.g., total phosphorus vs chlorophyll) using values for all lakes. At this step, data points that are clearly outliers that fall beyond common relationships or fall extremely far outside of the rest of the dataset should be removed.

Step 4. Quantify biological condition: Using available biological data, quantify the biological condition of the lakes to determine the range of acceptable condition for meeting designated uses.

Step 5. Identify the index period: Select the period within each year from which samples will be used for remaining steps that are deemed most appropriate for nutrient criteria development. Calculate the median level for the index period and compare to any other available data within the region for comparison purposes.

Step 6. Calculate lake-specific 'expected conditions' for nutrients and chlorophyll through time for the index period: Using monitoring data for the index period, determine what the 'expected condition' should be for each lake for each variable. The expected conditions includes the full range of concentrations for each variable for each lake, but with careful consideration of outliers.

Step 7. Derive lake-specific nutrient criteria: Using the expected conditions from the previous step to quantify lake-specific nutrient criteria for each nutrient, clarity, or algal variable using the 90^{th} percentile of the samples from the index period across the entire sampling period.

Table 3. Nutrient, clarity, and organic carbon characteristics of lakes in GP, the fisheries lakes in FDL, and the Northern Lakes and Forest (NLF) ecoregion reference database of 29 lakes provided by S. Heiskary (August 2010). Data from GP and FDL are calculated as medians of samples from July, August, and September from all years the lakes were sampled for each variable. Note, this table was created after removing outliers as defined by points being 'far outside values' (beyond 3 times the inter-quartile ranges (Systat, inc.) [(c)], and the Cleveland method for quantifying percentiles was used.

Variable	Location	25th	Median	75th	90th
TP (ug/L)	GP	10	20	30	52
	FDL	15	19	25	37
	NLF	14	17	26	38
TN (ug/L)	GP	700	900	1200	1600
	FDL	530	720	868	1130
	NLF	412	550	748	986
Chl *a* (ug/L)	GP	2.0	4.0	7.0	10.0
	FDL	2.9	4.9	7.8	13.0
	NLF	3.0	4.1	7.0	13.7
Chl *a*:TP	GP	0.10	0.20	0.40	0.67
	FDL	0.18	0.25	0.38	0.51
	NLF	0.19	0.25	0.34	0.37
Secchi (m) [(a)]	GP	1.3	0.8	0.7	0.5
	FDL	2.4	1.7	1.3	0.9
	NLF	4.4	3.3	2.8	1.6
Color (ptCo)	GP[(b)]	66	138	184	277
	FDL	22	41	72	129
	NLF	10	17	34	55

[(a)] The axis for Secchi depth was reversed such that the 25th percentile represents the values for which Secchi are deeper and the 90% percentile are for values in which Secchi depth is shallow to line up with the other parameters, such as nutrients.
[(b)] DOC data was converted to color using a regression equation derived from concurrent samples taken for DOC and water color in 2009 in all GP lakes (M. Watkins). The regression resulted in a R^2 of 0.925.
[(c)] The Cleveland method was used to calculate the percentiles.

Table FDL1. Lake and watershed descriptions including lake and catchment morphometry and land use/cover in lake watersheds of FDL lakes.

	Watershed and lake characteristics:				**Land use/cover:**					**Dominant human uses:**	
Lake Name	Watershed area (ha)	Lake area (ha)	WS area: LK area	Max depth (m)	All forest types	Human use	All wetlands	Grassland and shrubland	Open water	Forest cut-overs	Other rural devl.
Big Lake	507	212	2	6.1	36.0%	12.1%	6.4%	4.1%	41.4%	0.0%	11.8%
Lost Lake	122	55	2	3.4	38.6%	3.1%	6.9%	4.4%	47.1%	2.8%	0.3%
Joe Martin Lk.	1808	27	66	23.5	50.6%	0.9%	9.7%	36.5%	2.2%	0.8%	0.1%
Pat Martin Lk.	5314	14	369	4.6	35.6%	2.7%	34.3%	25.0%	2.3%	1.6%	0.2%
Perch Lake-No.	1832	89	21	5.2	46.8%	0.5%	31.5%	6.0%	15.3%	0.0%	0.3%
Simian Lake	5314	33	162	3.7	35.6%	2.7%	34.3%	25.0%	2.3%	1.6%	0.2%
Sofie Lake	85	14	6	4.9	79.0%	0.4%	0.0%	2.0%	19.0%	0.0%	0.4%
Third Lake	50	6	8	6.1	28.0%	2.0%	3.0%	58.0%	10.0%	0.0%	0.0%
West Twin Lk.	245	49	5	5.5	48.9%	4.7%	17.9%	6.1%	22.3%	3.2%	1.5%

Table FDL2. Designated uses of water bodies in FDL. All lakes are deemed to be fully supporting designated uses at this time except for Third Lake, which is classified as partially supporting due to the presence of algal blooms in the summer.

Lake Name	Aqu. Life, Cold water fisheries	Aqu. Life, Warm water fisheries	Wildlife	Recreation, primary contact	Recreation, secondary contact	Cultural, Wild rice areas	Cultural, Aesthetic waters	Agricultural	Navigation	Commercial
Big Lake		1	1	1	1	1		1	1	1
Lost Lake		1	1	1	1			1	1	1
Joe Martin Lake	1	1	1	1	1		1	1	1	1
Pat Martin Lake		1	1	1		1		1	1	1
Perch Lake-No.		1	1	1		1	1	1	1	1
Simian Lake		1	1	1		1		1	1	1
Sofie Lake		1	1	1				1	1	1
Third Lake		1	1	1	1			1	1	1
West Twin Lake		1	1	1	1	1		1	1	1

Table GP1. Lake and watershed morphometry of lakes in GP. Due to a lack of GIS coverages of lake watersheds, land use/cover percentages by lake are not available. GP is representative of the Boreal Shield landscape and is characterized by rugged topography, nutrient poor glacial soils, extensive forests, and abundant lakes and wetlands (Lafrancois et al. 2009). There is minimal human disturbance around lakes, except for forest logging that occurs to varying degrees.

Lake Name	Watershed area (ha)	Lake area (ha)	WS area: LK area	Max depth (m)
Center Lake	587	14	41	3.4
Chevans Lake	1839	4	472	1.2
Cuffs Lake	587	6	101	1.5
Dutchman Lake	335	19	18	4.3
Helmer Nelson Lake	587	9	65	2.4
Little Lake	430	1	717	0.9
Loon Lake	184	14	13	2.4
Mt. Maud Lake	550	3	162	2.4
North Lake	45	2	20	2.1
Swamp Lake	1458	144	10	5.8
Swede Lake	32	2	20	1.8
Taylor Lake	673	13	52	7.6
Teal Lake	344	29	12	2.1
Trout Lake	114	26	4	6.4
Turtle Lake	41	3	16	3.7

Table GP2. Designated uses of water bodies in GP. All lakes are deemed to be fully supporting designated uses at this time.

Lake Name	Aqu. Life, Cold water fisheries	Aqu. Life, Warm water fisheries	Aqu. Life, Wetland (e.g., wildlife, biodiv.)	Wildlife	Recreation, primary contact, moderate use	Recreation, primary contact, infrequent use	Cultural, Wild rice areas	Forestry	Navigation	Industrial
Center Lake	1		1	1		1	1	1	1	1
Chevans Lake		1	1	1		1		1	1	1
Cuffs Lake		1	1	1		1	1	1	1	1
Dutchman Lake		1	1	1		1		1	1	1
Helmer Nelson Lake		1	1	1		1	1	1	1	1
Little Lake	1		1	1		1		1	1	1
Loon Lake		1	1	1		1	1	1	1	1
Mt. Maud Lake		1	1	1		1	1	1	1	1
North Lake		1	1	1		1	1	1	1	1
Swamp Lake	1		1	1	1		1	1	1	1
Swede Lake		1	1	1		1		1	1	1
Taylor Lake	1		1	1	1			1	1	1
Teal Lake		1	1	1		1	1	1	1	1
Trout Lake	1	1	1	1	1			1	1	1
Turtle Lake		1	1	1	1			1	1	1

Table 4. Estimated lake-specific criteria for TP, TN and chlorophyll *a* (Chl) in GP and FDL, and criteria for the NLF ecoregion lakes developed by the state of Minnesota (Heiskary and Wilson 2008). The criteria are calculated as the 90th percentiles of the summer samples (July-September) any samples taken from 1999-2009 in each lake. Note, these criteria have been calculated without removal of outlier points, except for the extreme points detected from plots of the common limnological relationships. This table is provided for comparison purposes only. I recommend that **Table 4a** be used to establish nutrient criteria.

		Criteria (90th Percentile)		
Location	Lake Name	TP (ug/L)	TN (ug/L)	Chl (ug/L)
FDL	Big Lake - North	18	770	6
	Big Lake - South	21	830	7
	Lost Lake	23	1025	13
	Joe Martin Lake	15	618	3
	Pat Martin Lake	21	739	7
	Perch Lake - North	32	944	18
	Perch Lake - South	44	1686	8
	Simian Lake	47	1352	16
	Sofie Lake	36	854	33
	Third Lake	44	1548	44
	West Twin Lake - North	22	830	10
	West Twin Lake - South	24	812	11
GP	Center Lake	76	1540	67
	Chevans Lake	67	2041	9
	Cuffs Lake	70	1780	9
	Dutchman Lake	31	1820	11
	Helmer Nelson Lake	97	2180	55
	Little Lake	29	1905	8
	Loon Lake	60	1400	10
	Mt. Maud Lake	78	2070	13
	North Lake	40	1000	4
	Swamp Lake	40	1624	10
	Swede Lake	40	1440	12
	Taylor Lake	50	1220	6
	Teal Lake	40	1600	8
	Trout Lake	30	1400	7
	Turtle Lake	90	1820	9
	Median GP criteria	50	1624	9
	Median FDL criteria	23	842	10
	NLF criterion	30	--	9

Table 4b. With outliers removed (See table 3 for description of outlier removal)**.** Estimated lake-specific criteria for TP, TN and chlorophyll *a* (Chl) in GP and FDL, and criteria for the NLF ecoregion lakes developed by the state of Minnesota (Heiskary and Wilson 2008). The criteria are calculated as the 90th percentiles of the summer samples (July-September) any samples taken from 1999-2009 in each lake. The values in brackets are the number of samples for which the percentiles are calculated. The values highlighted in yellow are the values that have changed once outliers were removed (see Table 4a for what the previous values were).

		Criteria (90th Percentile)		
Location	Lake Name	TP (ug/L)	TN (ug/L)	Chl (ug/L)
FDL	Big Lake - North	18 *[17]*	770 *[17]*	6 *[9]*
	Big Lake - South	21 *[17]*	830 *[17]*	7 *[9]*
	Lost Lake	23 *[17]*	1025 *[16]*	13 *[9]*
	Joe Martin Lake	15 *[17]*	520 *[16]*	3 *[9]*
	Pat Martin Lake	21 *[17]*	739 *[16]*	7 *[9]*
	Perch Lake - North	32 *[17]*	944 *[17]*	18 *[9]*
	Perch Lake - South	44 *[17]*	1686 *[17]*	8 *[2]*
	Simian Lake	47 *[17]*	1314 *[16]*	16 *[9]*
	Sofie Lake	36 *[17]*	830 *[16]*	9 *[8]*
	Third Lake	44 *[17]*	1548 *[17]*	44 *[9]*
	West Twin Lake - North	22 *[17]*	830 *[17]*	10 *[9]*
	West Twin Lake - South	24 *[17]*	812 *[17]*	11 *[9]*
GP	Center Lake	66 *[17]*	1540 *[18]*	21 *[16]*
	Chevans Lake	56 *[17]*	2041 *[18]*	9 *[18]*
	Cuffs Lake	52 *[14]*	1348 *[14]*	9 *[15]*
	Dutchman Lake	31 *[18]*	1820 *[18]*	11 *[16]*
	Helmer Nelson Lake	89 *[17]*	2180 *[18]*	15 *[13]*
	Little Lake	20 *[15]*	1905 *[16]*	5 *[15]*
	Loon Lake	31 *[13]*	1400 *[15]*	10 *[14]*
	Mt. Maud Lake	68 *[16]*	2070 *[18]*	13 *[18]*
	North Lake	18 *[13]*	1000 *[15]*	4 *[15]*
	Swamp Lake	40 *[14]*	1624 *[13]*	10 *[15]*
	Swede Lake	28 *[14]*	1422 *[14]*	12 *[15]*
	Taylor Lake	13 *[13]*	1220 *[14]*	6 *[15]*
	Teal Lake	14 *[13]*	1600 *[15]*	8 *[15]*
	Trout Lake	30 *[14]*	1400 *[15]*	7 *[15]*
	Turtle Lake	90 *[17]*	1820 *[17]*	9 *[16]*
	Median GP criteria	31 *[15]*	1600 *[15]*	9 *[15]*
	Median FDL criteria	23 *[12]*	830 *[12]*	9 *[12]*
	NLF criterion	30	--	9

Figure 1. Relationships between nutrients, chlorophyll, and water color in FDL fisheries lakes. Data points are individual sampling events from all open-water months and all years sampled from 1999-2009.

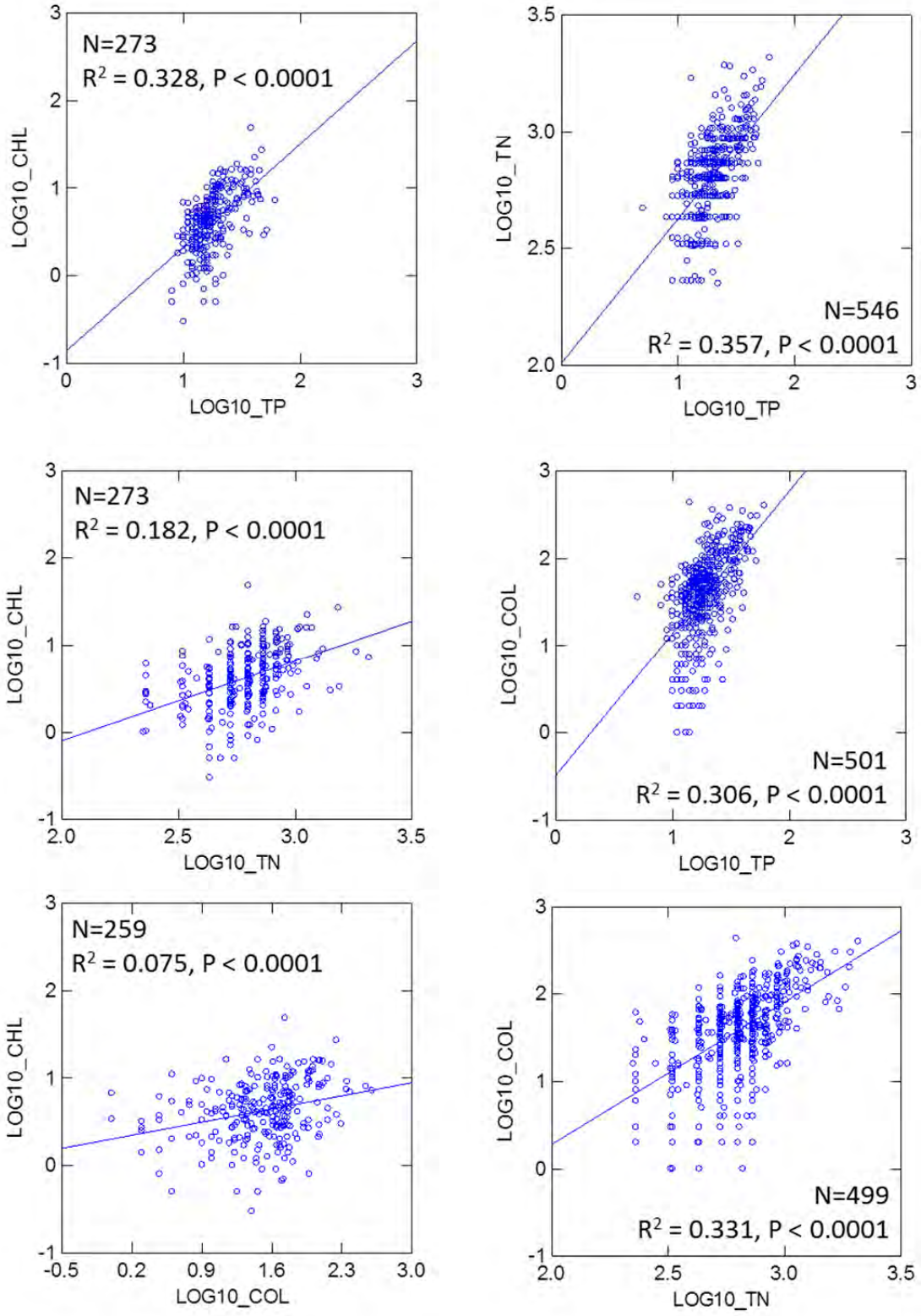


Figure 2. Relationships between nutrients, chlorophyll, and water color in GP lakes. Data points are individual sampling events from all open-water months and all years sampled from 1999-2009.

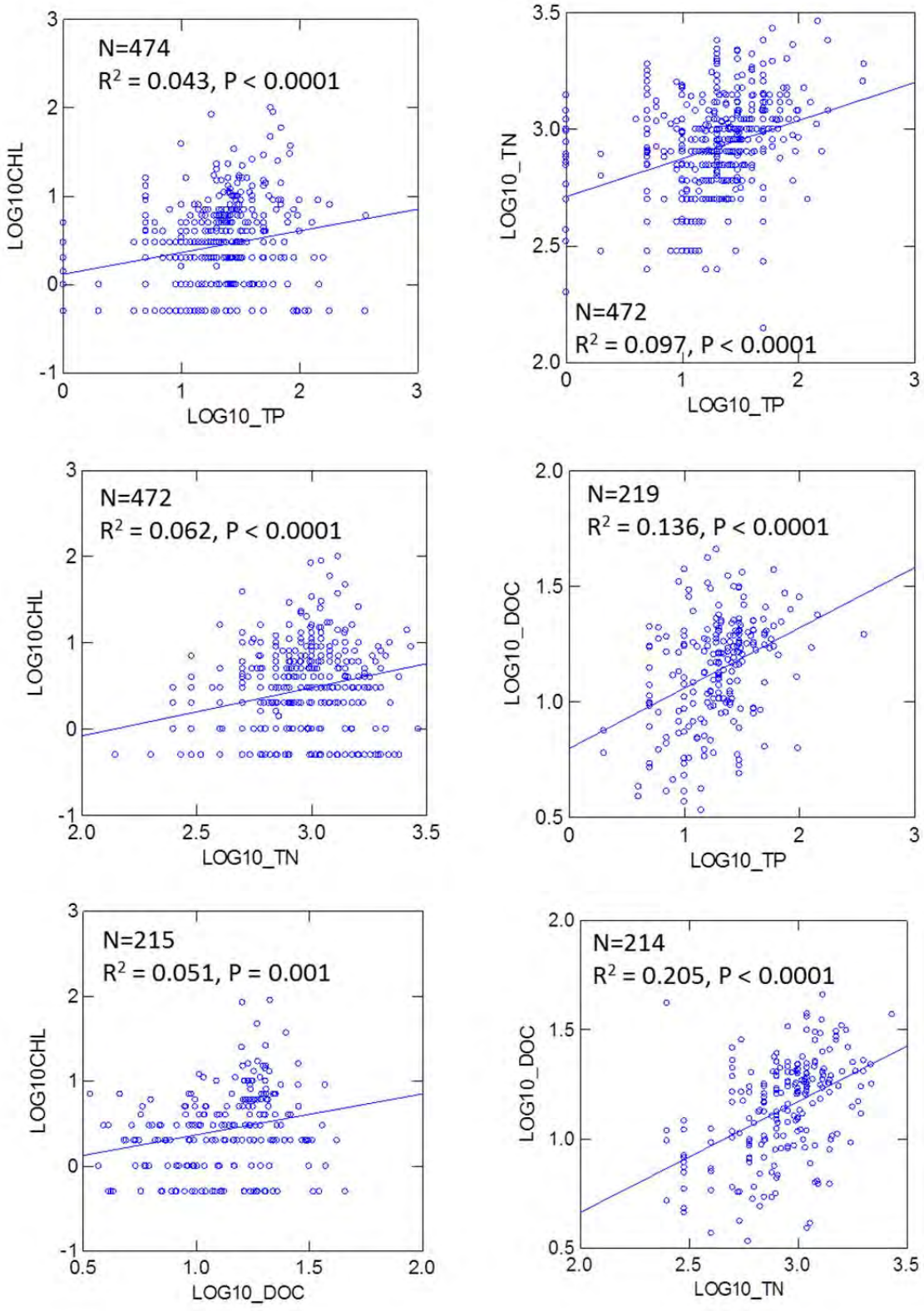


Figure 3a. Box plots of all data points for all months and all sampled years for each lake in FDL for TP, TN and chlorophyll concentrations. Note, the open circle points were defined as 'far outside values' and removed from the analysis to calculate percentiles for all tables and nutrient criteria calculation.

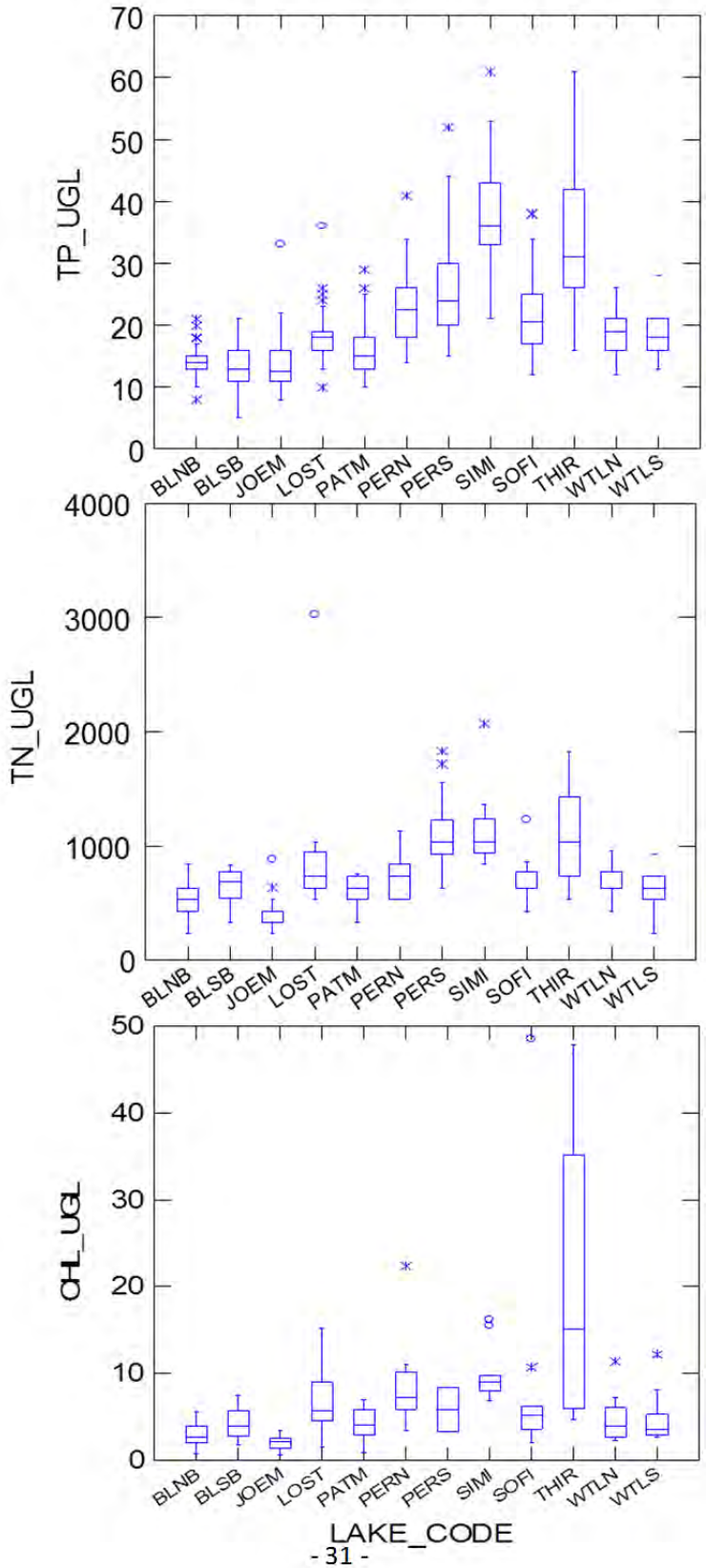


Figure 3b. Box plots of all data points for all months and all sampled years for each lake in FDL for Secchi depth and water color. Outliers were not removed for these two variables.

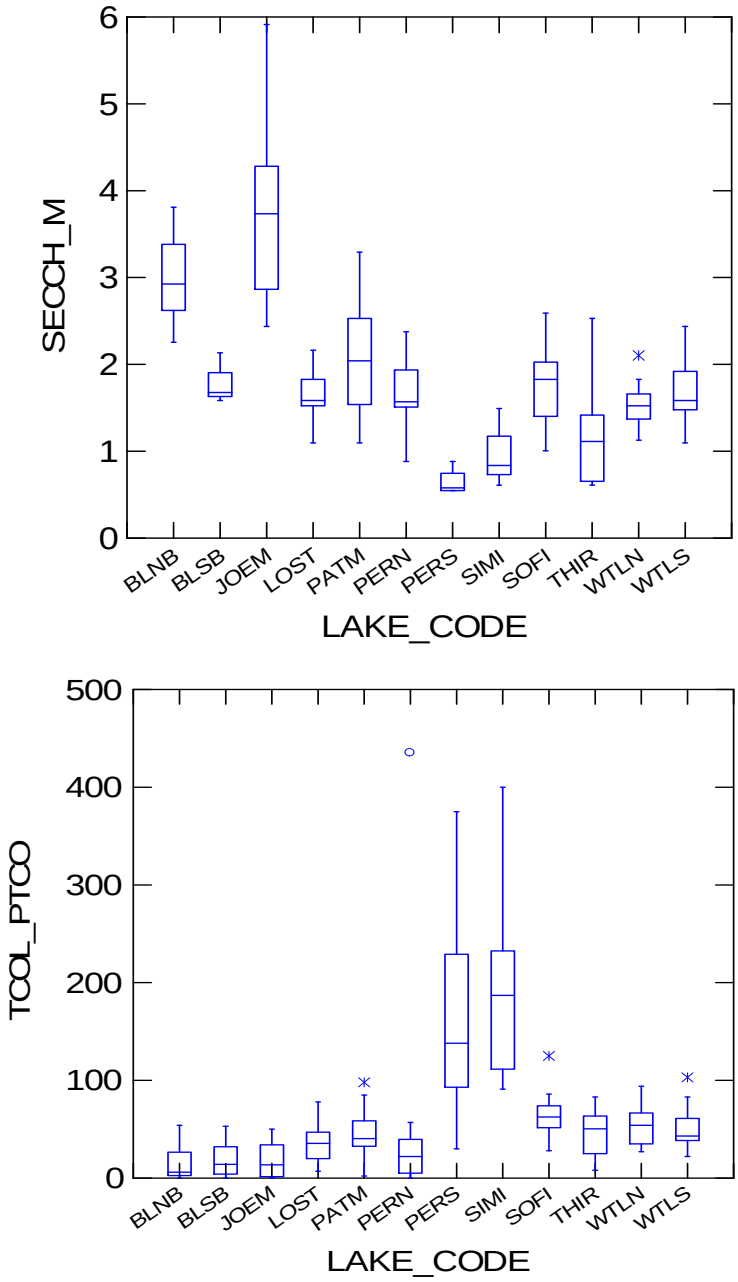


Figure 4a. Box plots of all data points for all months and all sampled years for each lake in GP for TP, TN and chlorophyll concentrations. Note, the open circle points were defined as 'far outside values' and removed from the analysis to calculate percentiles for all tables and nutrient criteria calculation.

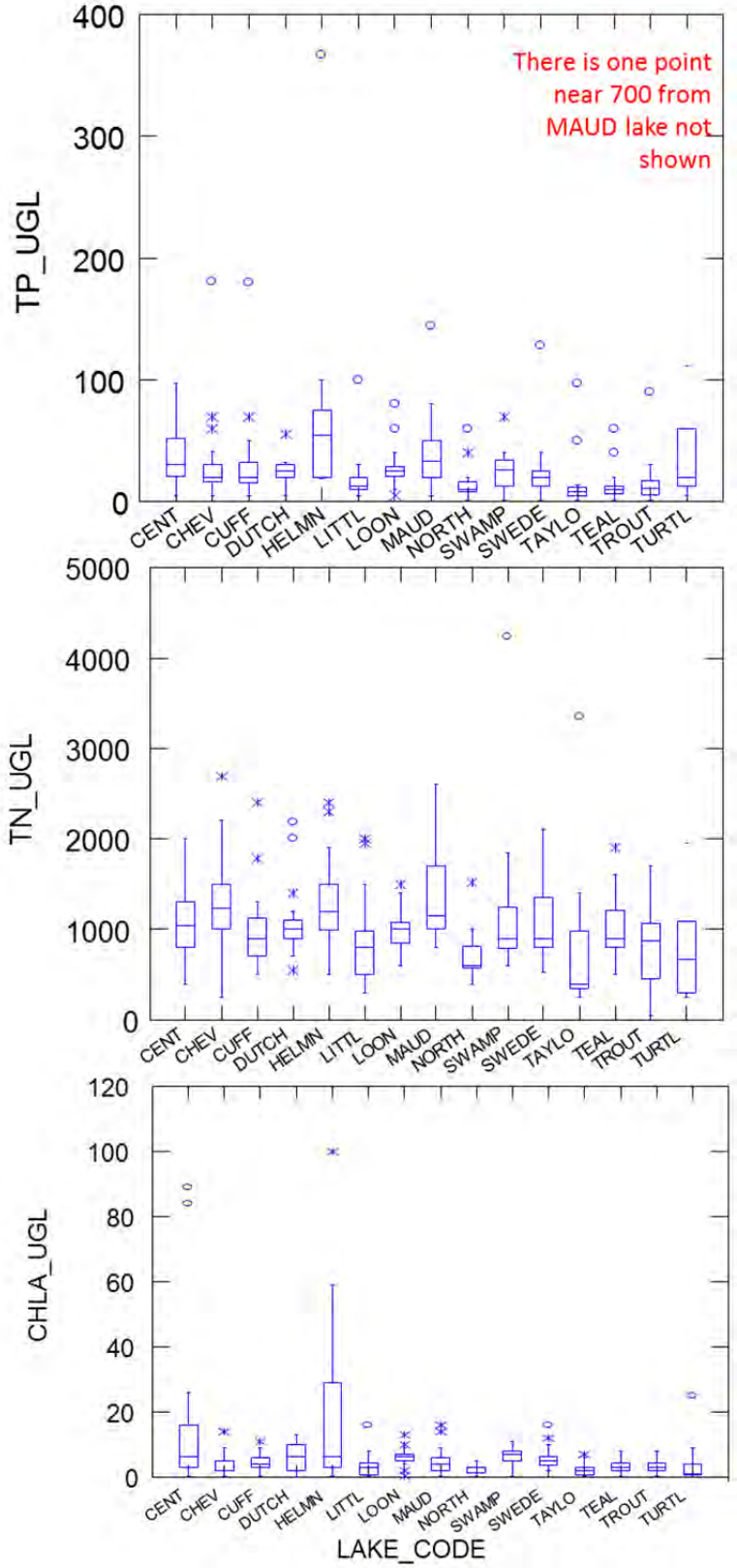


Figure 4b. Box plots of all data points for all months and all sampled years for each lake in GP for Secchi depth and DOC concentration. Outliers were not removed for these two variables.

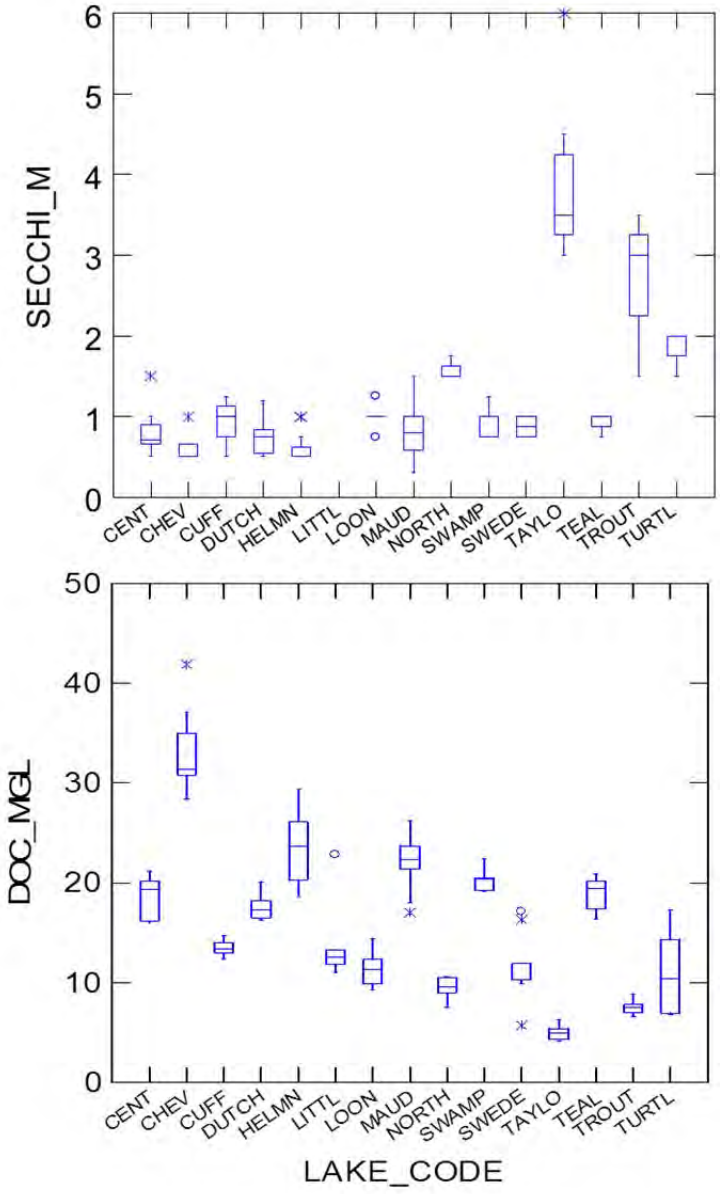
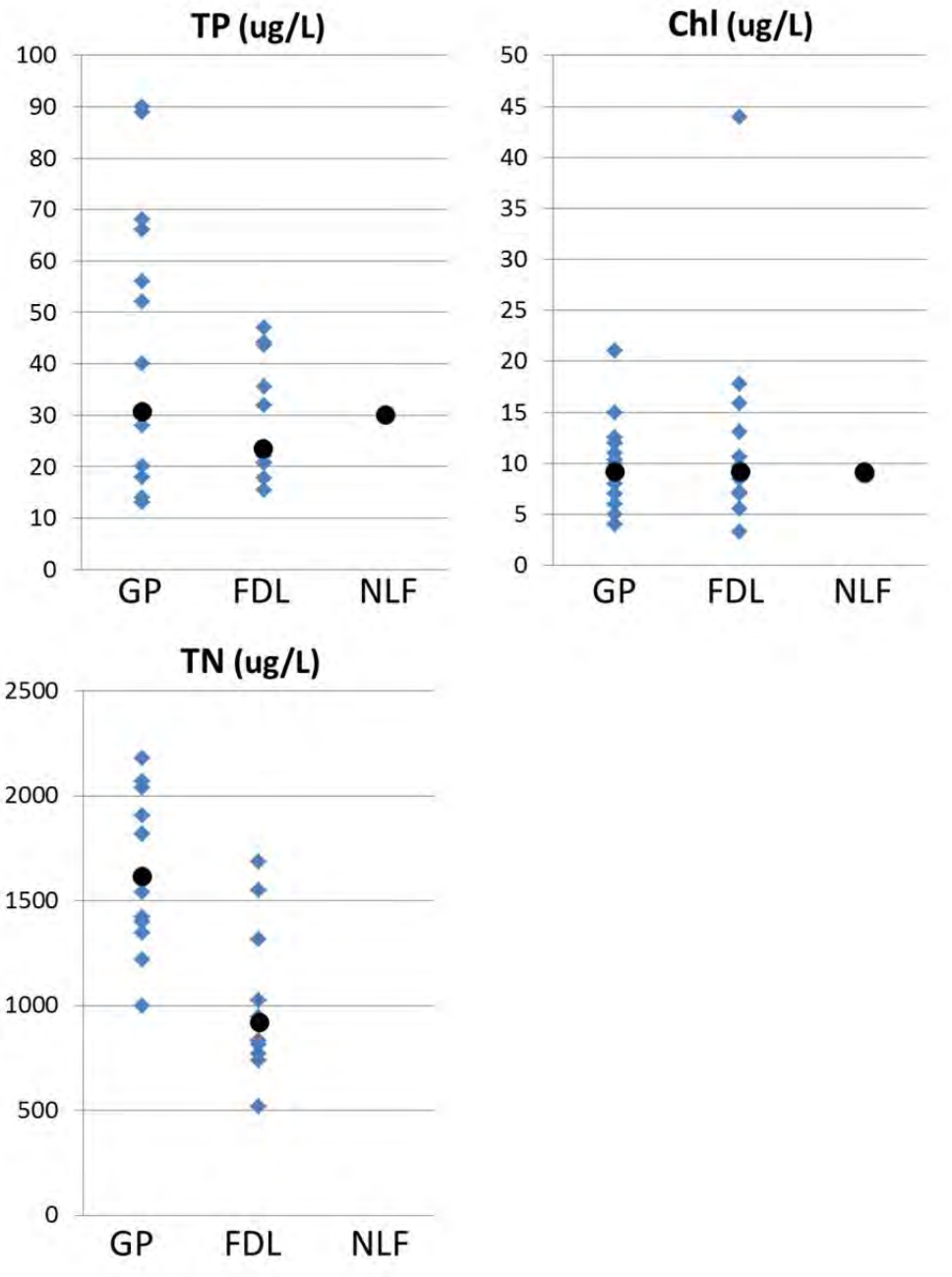


Figure 5. Plots showing the distribution of lake-specific nutrient criteria calculated for each of the lakes in each site (blue diamonds), as well as criteria estimated for the NLF ecoregion for comparison (Heiskary and Wilson 2008). These data are also provided in Table 4. The large black circles are either the median across lakes (for GP and FDL) or the value for the NLF ecoregion.



Appendix 2: . Algal Composition of all lakes combined and assigned Functional Group Classifications, Harmonization Table.

Functional Group Classification	Taxa
BG	Anabaena aequalis
BG	Anabaena inaequalis
BG	Anabaena lapponica
BG	Anabaenopsis circularis
BG	Anabaenopsis elenkinii
BG	Anabaenopsis sp.
BG	Aphanocapsa koordersi
BG	Aphanocapsa delicatissima
BG	Aphanocapsa elachista
BG	Aphanocapsa holsatica
BG	Aphanocapsa incerta
BG	Aphanothece clathrata
BG	Aphanothece nidulans
BG	Aphanothece saxicola
BG	Aphanothece stagnina
BG	Arthrospira sp.
BG	Calothrix sp.
BG	Chroococcus limneticus
BG	Chroococcus minimus
BG	Chroococcus minutus
BG	Chroococcus prescottii
BG	Chroococcus sp
BG	Chroococcus turgidus
BG	Coelosphaerium kuetzingianum
BG	Cyanocatena planctonica
BG	Cyanogranis ferruginea
BG	Cyanonephron styloides
BG	Cylindrospermum sp.
BG	Dactylococcopsis irreguaris
BG	Dactylococcopsis sp.
BG	Dolichospermum affine
BG	Geitlerinema amphibia
BG	Geitlerinema sp.
BG	Geitlerinema splendidum
BG	Heteroleibleinia sp.
BG	Jaaginema angustissimum
BG	Jaaginema sp.

Functional Group Classification	Taxa
BG	Leptolyngbya subtililissima
BG	Limnotrhix redekei
BG	Lyngbya birgei
BG	Lyngbya sp.
BG	Lyngbya sp. 15-20 µm
BG	Merismopedia cf danubiana
BG	Merismopedia punctata
BG	Merismopedia tenuissima
BG	Merismopedia trolleri
BG	Merismopedia warmingiana
BG	Myxobaktron salinum
BG	Non-motile Cyanobacteria - sph <1 µm
BG	Non-motile Cyanobacteria - sph >=2 µm
BG	Non-motile Cyanobacteria - sph >1 µm
BG	Nostoc sp
BG	Oscillatoria cf subbrevis
BG	Oscillatoria sp.
BG	Oscillatoria tenuis
BG	Planktolyngbya contorta
BG	Planktolyngbya lagerheimia
BG	Planktolyngbya limnetica
BG	Planktolyngbya sp
BG	Pseudanabaena acicularis
BG	Pseudanabaena biceps
BG	Pseudanabaena galeata
BG	Pseudanabaena limnetica
BG	Pseudanabaena limnetica f 2
BG	Pseudanabaena limnetica acicularis
BG	Pseudanabaena mucicola
BG	Pseudanabaena sp
BG	Radiocystis geminata
BG	Raphidiopsis curvata
BG	Rhabdoderma lineare
BG	Rhabdogloea scenedesmoides
BG	Romeria leopoliensis
BG	Romeria okensis (coil)
BG	Romeria sp.
BG	Snowella litoralis
BG	Spirulina sp.
BG	Spirulina subsalsa
BG	Synechococcus elongatus
BG	Synechococcus sp.
BG	Synechococcus sp. 1 <1.2 µm

Functional Group Classification	Taxa
BG	Trichodesmium lacustre
BG	Trichormus variabilis
BG	Gomphosphaeria aponina
BG	Snowella lacustris
CP	Chroomonas sp.
CP	Cryptomonas erosa
CP	Cryptomonas gracilis
CP	Cryptomonas lucens
CP	Cryptomonas ovata
CP	Cryptomonas ovata
CP	Cryptomonas rostrata
CP	Cryptomonas sp.
CP	Cyst (Dinoflagellate)
CP	Glenodinium quadridens
CP	Glenodinium sp.
CP	Gymnodinium sp. 1
CP	Gymnodinium sp. 2
CP	Gymnodinium sp. 3
CP	Peridinium bipes
CP	Peridinium cinctum
CP	Peridinium cunningtonii
CP	Peridinium inconspicuum
CP	Peridinium polonicum
CP	Peridinium sp.
CP	Peridinium umbonatum
CP	Peridinium willei
CP	Peridinium wisconsinense
CP	Rhodomonas minuta
CP	Rhodomonas minuta v. nannoplanctica
CP1	Ceratium hirundinella
DY	Acanthoceras zachariasi
DY	Achnanthidium exiguum
DY	Achnanthidium minutissimum
DY	Actinocyclus normanii
DY	Amphipleura sp.
DY	Amphora ovalis
DY	Amphora pediculus
DY	Amphora veneta
DY	Anomoeoneis vitrea
DY	Asterionella formosa
DY	Aulacoseira ambigua
DY	Aulacoseira distans
DY	Aulacoseira granulata

Functional Group Classification	Taxa
DY	Aulacoseira granulata CURL
DY	Aulacoseira granulata STRT
DY	Aulacoseira islandica
DY	Aulacoseira italica
DY	Aulacoseira muzzanensis
DY	Aulacoseira sp.
DY	Caloneis amphisbaena
DY	Caloneis permagna
DY	Caloneis sp.
DY	Centratractus belonophorus
DY	Chlorocloster pyrenigera
DY	Chlorocloster sp.
DY	Chromulina sp.
DY	Chromulina sp.
DY	Chrysochromulina parva
DY	Chrysococcus minutus
DY	Chrysolykos planctonicus
DY	Cocconeis pediculus
DY	Cocconeis placentula var. lineata
DY	Craticula halophila
DY	Ctenophora puchella
DY	Cyclostephanos damasii
DY	Cyclostephanos invisitatus
DY	Cyclostephanos tholiformis
DY	Cyclotella atomus
DY	Cyclotella bodanica
DY	Cyclotella cf ocellata
DY	Cyclotella comensis (1)
DY	Cyclotella comta bodanica
DY	Cyclotella cyclopum
DY	Cyclotella distinguenda
DY	Cyclotella meneghiniana
DY	Cyclotella ocellata
DY	Cyclotella pseudostelligera
DY	Cyclotella sp 1
DY	Cyclotella sp.
DY	Cyclotella stelligera
DY	Cymbella cf cistula
DY	Cymbella cistula
DY	Cymbella microcephala
DY	Cymbella silesiaca
DY	Cymbella sp.
DY	Cyst (Chrysophyte)

Functional Group Classification	Taxa
DY	Desmarella sp.
DY	Diatoma tenue
DY	Diatoma vulgaris
DY	Dinobryon bavaricum
DY	Dinobryon cylindricum
DY	Dinobryon cyst
DY	Dinobryon divergens
DY	Dinobryon monads
DY	Dinobryon sertularia
DY	Dinobryon sociale
DY	Dinobryon sp.
DY	Diploneis cf puella - <13 µm
DY	Ellipsoidion pachydermum
DY	Ellipsoidion sp.
DY	Entomoneis ornata
DY	Epithemia sorex
DY	Epithemia sorex sorex
DY	Epithemia sp.
DY	Epithemia turgida
DY	Epithemia turgida westermannii
DY	Eunotia sp.
DY	Fistulifera pelliculosa
DY	Fragilaria capucina
DY	Fragilaria capucina mesolypta
DY	Fragilaria capucina vaucheriae
DY	Fragilaria crotonensis
DY	Fragilariforma virescens
DY	Geissleria decussis
DY	Gomphonema olivaceum
DY	Gomphonema parvulum
DY	Gomphonema sp.
DY	Goniochloris fallax
DY	Goniochloris sp.
DY	Gyrosigma sp.
DY	Kephyrion gracile
DY	Kephyrion planctonimcum
DY	Kephyrion rubi-claustri
DY	Kephyrion sp.
DY	Mallomonas akrokomos
DY	Mallomonas caudata
DY	Mallomonas crassisquama
DY	Mallomonas sp.
DY	Melosira varians

Functional Group Classification	Taxa
DY	Monodus sp.
DY	Navicula capitata v. capitata
DY	Navicula cf. lacunolaciniata
DY	Navicula cryptocephala
DY	Navicula cryptotenella
DY	Navicula gregaria
DY	Navicula minima
DY	Navicula radiosafallax
DY	Navicula salinarum
DY	Navicula sp.
DY	Navicula tripuncta
DY	Navicula viridula var. germainii
DY	Nitzschia acicularis
DY	Nitzschia amphibia
DY	Nitzschia fonticola
DY	Nitzschia frustulum
DY	Nitzschia fruticosa
DY	Nitzschia gracilis
DY	Nitzschia incerta
DY	Nitzschia inconspicua
DY	Nitzschia intermedia
DY	Nitzschia linearis
DY	Nitzschia palea
DY	Nitzschia recta
DY	Nitzschia sigma
DY	Nitzschia sigmoidea
DY	Nitzschia sp.
DY	Nitzschia subacicularis
DY	Non motile Chrysophytes >1 µm spherical
DY	Ochromonas sp.
DY	Ophiocytium capitatum
DY	Ophiocytium sp.
DY	Pinnularia major
DY	Pinnularia sp.
DY	Polygoniochloris circularis
DY	Pseudostaurosira brevistriata
DY	Pseudostaurosira elliptica
DY	Rhoicosphenia curvata
DY	Rhopalodia gibba
DY	Sellaphora pupula
DY	Skeletonema potamos
DY	Spiniferomonas sp.

Functional Group Classification	Taxa
DY	Stauroneis phoenicenteron
DY	Stauroneis sp.
DY	Staurosira construens v. binodis
DY	Staurosira construens v. construens
DY	Staurosira construens v. venter
DY	Staurosirella pinnata
DY	Staurosirella pinnata v. pinnata
DY	Stephanodiscus alpinus
DY	Stephanodiscus hantzscia
DY	Stephanodiscus medius
DY	Stephanodiscus minutulus
DY	Stephanodiscus niagara
DY	Stephanodiscus parvus
DY	Stephanodiscus sp.
DY	Stichogloea olivacea
DY	Stichogloea sp.
DY	Surirella minuta
DY	Surirella sp.
DY	Synedra acus
DY	Synedra arcus
DY	Synedra delicatula
DY	Synedra filiformis
DY	Synedra nana
DY	Synedra radians
DY	Synedra sp.
DY	Synedra tenera
DY	Synedra ulna
DY	Synedra ulna acus
DY	Synedra ulna ulna
DY	Synura sp.
DY	Tabellaria fenestrata
DY	Tabellaria flocculosa
DY	Tribonema sp.
DY	Tryblionella kuetzingii
DY	Uroglena sp.
DY	Urosolenia
DY	Urosolenia longiseta
E	Euglena acus
E	Euglena gracilis
E	Euglena gracilis-form 1
E	Euglena sp.
E	Gonyostomum ovatum
E	Gonyostomum semen

Functional Group Classification	Taxa
E	Lepocinclis fusiformis
E	Lepocinclis glabra
E	Lepocinclis sp.
E	Phacus cf. swirenkoi
E	Phacus helikoides
E	Phacus horridus
E	Phacus sp.
E	Phacus swirenkoi
E	Strombomonas sp.
E	Trachelomonas armata
E	Trachelomonas hispida
E	Trachelomonas horrida
E	Trachelomonas sp.
E	Trachelomonas volvocina
G	Actinastrum hantzschii
G	Acutodesmus dimorphus
G	Acutodesmus obliquus
G	Acutodesmus obliquus v. alternans
G	Ankyra judayi
G	Arthrodesmus sp.
G	Asterococcus limnectus
G	Botryococcus braunii
G	Carteria globulosa
G	Carteria platyrhyncha
G	Carteria sp.
G	Characium ambiguum
G	Characium limneticum
G	Chlamydomonas globosa
G	Chlamydomonas incerta
G	Chlamydomonas platystigma
G	Chlamydomonas sp.
G	Chlorella vulgaris
G	Chlorogonium fusiforme
G	Chlorogonium sp.
G	Chlorolobion braunii
G	Chloromonas pumilio
G	Closteriopsis longissima
G	Closterium moniliferum
G	Closterium sp. - lunate
G	Closterium sp. - strt
G	Coelastrum astroideum
G	Coelastrum cambricum
G	Coelastrum microporum

Functional Group Classification	Taxa
G	Coelastrum proboscum
G	Coelastrum pseudomicroporum
G	Coelastrum pulchrum
G	Coelastrum reticulatum
G	Coelastrum sp.
G	Coleochaete sp.
G	Cosmarium formulosum
G	Cosmarium sp.
G	Cosmarium tenue
G	Crucigenia fenestrata
G	Crucigenia quadrata
G	Crucigenia tetrapedia
G	Cyst (Chlorophyte)
G	Cystomonas starrii
G	Deasonia gigantica
G	Desmodesmus abundans
G	Desmodesmus bicaudatus
G	Desmodesmus brasiliensis
G	Desmodesmus communis
G	Desmodesmus dispar
G	Desmodesmus intermedius
G	Desmodesmus opoliensis v. carinatus
G	Desmodesmus perforatus
G	Desmodesmus serratus
G	Diacanthos belanophorus
G	Dichotomococcus bacillaris
G	Dichotomococcus lunatis
G	Dictyosphaerium chlorelloides
G	Dictyosphaerium pulchellum
G	Didymogenes anomola
G	Dimorphococcus lunatus
G	Dispora crucigenia
G	Dispora sp.
G	Echinosphaerella limnetica
G	Elakatothrix gelatinosa
G	Euastrum sp.
G	Eudorina elegans
G	Franceia droescheri
G	Geminella minor
G	Geminella sp.
G	Glaucocystis sp.
G	Gloeococcus minor
G	Gloeocystis ampla

Functional Group Classification	Taxa
G	Gloeocystis sp.
G	Gloeocystis vesiculosa
G	Golenkeniopsis parvula
G	Golenkinia paucispina
G	Golenkinia radiata
G	Gonium pectorale
G	Gonium sociale
G	Gonium sp.
G	Gregiochloris lacustris
G	Helicodictyon sp.
G	Kirchneriella lunaris
G	Kirchneriella lunaris v. irregularis
G	Kirchneriella obesa
G	Kirchneriella sp.
G	Kirchneriella subsolitaria
G	Lagerheimia ciliata
G	Lagerheimia quadriseta
G	Lagerheimia subsalsa
G	Lagerheimiella longiseta
G	Lobomonas cf verrucosa
G	Lobomonas sp.
G	Micractinium pusillum
G	Microspora sp.
G	Monactinus simplex
G	Monactinus simplex var. echinulatum
G	Monomastix astigmata
G	Monomastix minuta
G	Monoraphidium arcuatum
G	Monoraphidium capricorn
G	Monoraphidium convolutum
G	Monoraphidium griffithii
G	Monoraphidium minutum
G	Nannochloris atomus
G	Nannochloris sp.
G	Nephrochlamys sp.
G	Nephrocytium agardhi
G	Nephrocytium limneticum
G	Nephrocytium sp.
G	Nephroselmis olivacea
G	Non-motile Chlorophyte - sph >10 µm
G	Non-motile Chlorophyte - sph 2-9.9 µm
G	Non-Motile Chlorophyte -ovoid
G	Oedogonium sp.

Functional Group Classification	Taxa
G	Oocystis lacustris
G	Oocystis parva
G	Oocystis pusilla
G	Pandorina morum
G	Paulschulzia tenera
G	Pediastrum boryanum
G	Pediastrum duplex
G	Pediastrum sp.
G	Phacotus lendneri
G	Phacotus sp.
G	Planctonema lauterbornii
G	Pyramichlamys cordiformis
G	Pyramichlamys dissecta
G	Pyramimonas sp.
G	Quadrigula chodatti
G	Quadrigula closterioides
G	Rhizoclonium sp.
G	Scenedesmus balatonicus
G	Scenedesmus bijuga
G	Scenedesmus bijuga v. alternans
G	Scenedesmus denticulatus
G	Scenedesmus longispina
G	Scenedesmus parisiensis
G	Scenedesmus producto-capitatus
G	Scenedesmus quadrispina
G	Scenedesmus semipulcher
G	Scenedesmus sp.
G	Schizochlamys sp.
G	Schroederia setigera
G	Selanastrum gracile
G	Selenastrum sp.
G	Spermatozopsis exsultans
G	Sphaerellopsis sp.
G	Sphaerocystis schroeteri
G	Spirogyra sp.
G	Staurastrum cingulum
G	Staurastrum hexacerum
G	Staurastrum iotanum
G	Staurastrum natator
G	Staurastrum paradoxum
G	Staurastrum sp.
G	Stauridium tetras
G	Staurodesmus dejectus

Functional Group Classification	Taxa
G	Staurodesmus sp.
G	Stichococcus pelagicus
G	Stichococcus sp.
G	Teilingia granulata
G	Tetracystis pulchra
G	Tetraedron caudatum
G	Tetraedron gracile
G	Tetraëdron incus
G	Tetraedron minimum
G	Tetraedron muticum
G	Tetraedron trigonum
G	Tetrallantos sp.
G	Tetrastrum glabrum
G	Tetrastrum heteracanthum
G	Tetrastrum staurogeniaeforme
G	Treubaria schmidlei
G	Treubaria setigera
G	Ulothrix sp.
G	Verrucodesmus verrucosus
G	Volvox sp.
G	Westella botryoides
G	Willea apiculata
G	Willea crucifera
G	Willea rectangularis
G	Willea truncata
G	Zygnema sp.
HAB	Anabaena augstumalis
HAB	Anabaena eucompacta
HAB	Anabaena oscillarioides
HAB	Aphanizomenon flos-aquae
HAB	Aphanizomenon gracile
HAB	Aphanizomenon yezeonse
HAB	Chrysosporum ovalisporum
HAB	Cuspidothrix issatschenkoi
HAB	Cylindrospermopsis raciborskii
HAB	Cylindrospermopsis raciborskii (curled)
HAB	Dolichospermum circinale
HAB	Dolichospermum compactum
HAB	Dolichospermum crassa
HAB	Dolichospermum flos-aquae
HAB	Dolichospermum lemmermannii
HAB	Dolichospermum macrosporum
HAB	Dolichospermum mendotae

Functional Group Classification	Taxa
HAB	Dolichospermum no sheath
HAB	Dolichospermum planctonicum
HAB	Dolichospermum sp.
HAB	Dolichospermum spiroides
HAB	Sphaerospermopsis aphanizomenoides
HAB1	Gloeotrichia echinulata
HAB1	Microcystis aeruginosa
HAB1	Microcystis firma
HAB1	Microcystis flosaquae
HAB1	Microcystis novacekii
HAB1	Microcystis viridis
HAB1	Microcystis wesenbergii
HAB1	Planktothrix agardhii
HAB1	Planktothrix isothrix
HAB1	Woronchinia naegeliana
O	Fungi
O	Misc. Microflagellates (St Amand)
O	Misc. Unicells (Ruzycki)

Appendix 3: Online sources for climate related data (Rainfall, Air Temperature and Ice Out dates).

"Minnesota Lake Ice Out Dates: Minnesota DNR." *Minnesota Lake Ice Out Dates: Minnesota DNR*. Web. 9 Jan. 2015. <http://www.dnr.state.mn.us/ice_out/index.html>.

The following websites were used to obtain temperature and precipitation data for FDL, MPR, and MCWD. Minneapolis was used for both MPR and MCWD.

NOAA Satellite and Information Service. National Climatic Data Center. "Local Climatological Data Publication - Duluth." *Local Climatological Data Publication.* National Climatic Data Center. Web. 26 Jan. 2015. <http://www.ncdc.noaa.gov/IPS/lcd/lcd.html;jsessionid=57D500497A6ABD80646C71B793738694?_page=1&state=MN&stationID=14913&_target2=Next>.

NOAA Satellite and Information Service. National Climatic Data Center. "Local Climatological Data Publication - Minneapolis." *Local Climatological Data Publication*. National Climatic Data Center. Web. 26 Jan. 2015. <http://www.ncdc.noaa.gov/IPS/lcd/lcd.html?_page=1&state=MN&stationID=14922&_target2=Next>.