

M.L. 2016 Project Abstract

For the Period Ending June 30, 2019

PROJECT TITLE: Tracking and Preventing Harmful Algal Blooms

PROJECT MANAGER: Daniel R. Engstrom

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04a and M.L. 2015, Chp. 76, Sec. 2, Subd. 10

APPROPRIATION AMOUNT: \$593,000

AMOUNT SPENT: \$593,000

AMOUNT REMAINING: \$0

Sound bite of Project Outcomes and Results

This project provides comprehensive data on the prevalence and toxicity of Harmful Algal Blooms (HABs) in Minnesota lakes today and in the past. By combining these data with updated modeling techniques, we provide a framework for predicting the timing and composition of HABs that can be tailored to individual lakes.

Overall Project Outcome and Results

Lakes are one of Minnesota's most precious resources and harmful algal blooms (HABs) threaten them both from an ecological and economic standpoint. This provides a survey of the current prevalence and toxicity of harmful algal blooms (HABs) in a subset of Minnesota lakes, determines if these blooms are increasing in frequency, and develops and refines modeling techniques that could be used to predict HABs in lakes across Minnesota. To this end, we intensively monitored five lakes in southwest and central Minnesota over 2 years for all major water chemistry parameters, algal biomass, and four cyanotoxins. In these lakes, and five additional lakes in northern Minnesota, we collected and dated sediment cores where fossil cyanobacterial pigments could be measured to track the occurrence of Cyanobacteria over the last 150 years. Finally, we chose one of the intensively monitored lake as a pilot study where we developed a watershed model (SWAT) and an in-lake hydrodynamic model (CE-QUAL-W2) to predict annual cyanobacterial bloom patterns. As a result of this project, we determined that in lakes which are already eutrophic, internal loading dynamics will play a key role in determining the size and toxicity of the bloom. Importantly, we found that even in shallow lakes (less than 16 ft maximum depth), temperature and oxygen dynamics are critical in terms of bloom timing and toxicity. Cyanobacteria pigment data from our sediment cores showed increasing HABs in some lakes over the 20th Century, but also demonstrate that conditions may have been even worse in the early to mid- 20th Century before the passage of the Clean Water Act. Our modeling results provide a framework for resource managers to predict seasonal bloom formation and persistence in lakes across the state using publicly available and widely used modeling techniques.

Project Results Use and Dissemination

Throughout this project we have provided numerous public updates on progress via the Science Museum of Minnesota's website and the St. Croix Watershed Research Station's blog, "Field Notes" including:

- "Featured Research Project" on SCWRS website: <https://www.smm.org/scwrs/research/hab>
- "Watching When, Where and Why Harmful Algae Happen in Minnesota Lakes" describing the beginning of the project: <https://www.smm.org/scwrs/fieldnotes/watching-when-where-and-why-harmful-algae-happen-minnesota-lakes>

- A primer on the “5 super powers of Cyanobacteria”: <https://www.smm.org/scwrs/fieldnotes/five-super-powers-cyanobacteria>

We provided our expertise in major statewide news coverage of HABs over the course of this project, including:

- “Dogs as sentinels: Blue-green algae brings toxic mystery to Minn. Waters”: <https://www.mprnews.org/story/2016/05/24/water-toxic-algae-dogs-climate-change>
- Two evening news spots on FOX21 Duluth in June of 2017 where reporters accompanied us in the field
- “Researchers search for clues to toxic algae blooms”: <https://www.mprnews.org/story/2017/08/17/researchers-search-for-clues-to-toxic-algae-blooms>
- Participated in public call-in show on MPR for Minnesotans with questions about their lakes: <https://www.mprnews.org/story/2018/04/03/water-month-state-of-minnesotas-lakes>
- Participated in MPRs Climate Cast on the topic of HABs: <https://www.mprnews.org/episode/2019/07/19/conditions-ripe-for-a-record-number-of-algae-blooms?fbclid=IwAR19XRU6hUPGjlt9-9d0Pj8H5pRfPLEYWSr-SpdE-g7yTIAANwQmZU7laqQ>

We co-organized two public workshops on HABs in cooperation with the University of Minnesota St. Anthony Falls Laboratory that were held in March of 2017 and 2018 which were each attended by ~70 people, including state agency personnel, local water district managers, academic researchers, private environmental consultants, and interested members of the public.

Major research results from this project were also presented at two separate meetings of the Association for the Sciences of Limnology and Oceanography in June of 2018 and February of 2019 using in-kind funding provided by the Science Museum of Minnesota. This is the largest meeting dedicated to aquatic science in the world and is held once a year. A PDF of the scientific poster presented in 2018 and the powerpoint presented in 2019 are included as a supplemental attachment to this report.

Additional Attachments include fact sheets created by SCWRS for HABs on Pearl Lake and the Madison Lake SWAT model and a report on the CE-QUAL-W2 model produced by USGS.



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2016 Work Plan Final Report

Date of Report: August 16, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Tracking and Preventing Harmful Algal Blooms

Project Manager: Daniel R. Engstrom

Organization: St. Croix Watershed Research Station
Science Museum of Minnesota

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Location: Statewide

Total ENRTF Project Budget:	M.L. 2015, Chp. 76, Sec. 2, Subd. 10 Emerging Issues Account \$	M.L. 2016, Chp. 186, Sec. 2, Subd. 04a Work Plan \$
ENRTF Appropriation:	\$93,000	\$500,000
Amount Spent:	<u>\$93,000</u>	<u>\$500,000</u>
Balance:	\$0	\$0

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04a

M.L. 2015, Chp. 76, Sec. 2, Subd. 10

Appropriation Language :

M.L. 2016, Chp. 186, Sec. 2, Subd. 04a

\$500,000 the second year is from the trust fund to the Science Museum of Minnesota for the St. Croix Watershed Research Station to identify species composition and timing of harmful algal blooms, understand the causes of bloom development in individual lakes, and determine how nutrients and climate interact to increase harmful algae outbreaks. This work must be done in cooperation with the University of Minnesota and the Minnesota Pollution Control Agency. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

M.L. 2015, Chp. 76, Sec. 2, Subd. 10

\$1,000,000 the first year is from the trust fund to an emerging issues account authorized in Minnesota Statutes, section 116P.08, subdivision 4, paragraph (d).

I. PROJECT TITLE: Tracking and Preventing Harmful Algal Blooms

II. PROJECT STATEMENT:

Harmful algal blooms (HABs), especially those caused by toxin-producing blue-green algae (Cyanobacteria), significantly reduce the recreational and ecological value of Minnesota lakes. They negatively impact water quality, degrade fisheries, and are a health concern for humans and domesticated animals. The duration, frequency, and extent of harmful algal blooms are increasing worldwide. New evidence points to similar changes in some Minnesota lakes, yet little information is available on historical trends in blooms or the present-day composition of algae associated with bloom formation and toxin production. Harmful algal blooms occur as discrete events and are known to relate to phosphorus concentration. However, the seasonality, water-quality conditions, and sediment-water interactions that drive these events are not well understood. A better understanding of the lake characteristics and nutrient-climate interactions that stimulate harmful algal blooms would facilitate new corrective measures and better allocation of management resources.

This project will address three key questions regarding the occurrence, composition, and causes of HABs: (1) when do they occur, in which type of lakes, and which species and toxins are present; (2) are they increasing in Minnesota, and if so, in which lakes; and (3) what are the main environmental factors causing bloom formation and toxin production? To answer the first question, we will intensively monitor a set of five lakes on a bimonthly (twice per month) basis for composition and abundance of algae, their associated toxins, and key water-quality variables including nutrients (N and P), temperature, dissolved oxygen levels, chlorophyll a, and phycocyanin. We will also deploy temperature and oxygen recorders to continuously monitor key variables that affect in-lake nutrient cycling, along with sediment traps to track seasonal changes in algal composition and abundance. This sampling will be done in collaboration with the DNR and PCA as part of their long-term Sentinel Lakes monitoring program. The second question will be addressed by analysis of dated sediment cores from 10 lakes for fossil algal pigments to assess historical changes in the abundance of cyanobacteria and other algae. This will allow us to determine where and when HABs have increased and link them to possible drivers such as land-use change and temperature increases. These lakes will also be selected from among those in the Minnesota Sentinel Lakes program and will cover a range of lake types (trophic status, lake depth, and size) and ecoregions of the state. The third question will focus on a single Sentinel Lake with known bloom problems. Here we will pair results from the monitoring and sediment cores with watershed and in-lake models of phosphorus loading to determine the critical factors leading to bloom development including watershed inputs, recycling from sediments, and changing lake temperatures.

This work will be done in collaboration with the University of Minnesota in their complementary project (038-B), "Increasing Harmful Algal Blooms in Minnesota Lakes". The two research teams will coordinate monitoring effort on an overlapping set of lakes to extend the reach of this work from intensive laboratory studies to a broad range of observable field conditions in Minnesota lakes. Both research teams will regularly share data and results and coordinate the collection of samples when practical, and both groups will work jointly with the Minnesota Interagency Workgroup on Blue-Green Algae (MPCA, MDNR, MDH, MVMA) to update the agencies on our latest findings, coordinate research, response, and outreach efforts, and evaluate any emerging issues.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of July 1, 2016:

We have finalized the list of five Sentinel Lakes in Minnesota after receiving input from MPCA, MNDNR, and the St. Anthony Falls Laboratory (SAFL) Research group (who are working on a complementary LCCMR-funded HABs study). These lakes (Madison, St. James, Pearl, South Center, and Shaokatan) will undergo two years of monitoring for water quality, HABs, and cyanotoxins. We have coordinated with the MPCA to ensure all five lakes will be visited twice a month throughout the ice-free seasons of 2016 and 2017 (once each by both SCWRS and MPCA personnel).

In preparation for the first (2016) sampling season, we have acquired a YSI EXO2 multi-parameter sonde, capable of measuring vertical lake profiles for temperature, oxygen, conductivity, pH, turbidity, total algae, and Cyanobacteria. This instrument will be key for determining how HABs develop throughout the water column over the year, and what physio-chemical conditions accompany these blooms. Additionally, we have purchased and constructed five buoy arrays to be deployed in each of the study lakes. These arrays include both temperature and oxygen sensors and will record lake conditions throughout the ice-free season at 30-minute intervals. These data will help bridge the gap between bi-monthly sampling events and will record any major events that may lead to HABs fueled by internal loading of nutrients (i.e., wind-induced mixing of nutrient-rich anoxic bottom waters). Additionally, we have purchased all necessary miscellaneous supplies for the upcoming field season (i.e., bottles, chemistry reagents, calibration solutions).

Finally, thanks to the availability of the Emerging Issues funding, we were able to jump-start our sampling season shortly after ice-out in May, sampling all 5 lakes and installing the buoy arrays. We returned to sample all the lakes in June. In total, 251 water samples were collected from all 5 lakes including all major chemical, biological, and physical measures of interest (e.g., TP/TN, NO_x/NH₄, SRP, DIC, DOC, Chlorophyll *a*, phytoplankton, and four unique cyanotoxins [microcystin, cylindrospermopsin, anatoxin-a, saxitoxin]). An equivalent number of samples were also collected on alternate weeks over this time period by MPCA personnel, including phytoplankton and cyanotoxin samples to be later transferred to and analyzed by SCWRS.

Project Status as of January 1, 2017:

We have completed the first season of field sampling on the five selected sentinel lakes. Each lake was visited at least twice a month from May through October by either SCWRS or MPCA personnel. All analytes were collected for each sampling events and no events or samples were canceled or lost. In total, 524 water samples were collected across all lakes by SCWRS with a comparable number collected by MPCA in alternating weeks. We also successfully retrieved the buoy arrays from all 5 lakes in October and were able to upload all of the data that had been recorded over the previous 6 months (110,336 data points from South Center alone).

Water chemistry analysis has nearly been completed by the SCWRS Chemistry Laboratory. All analyses other than dissolved nitrogen species (NH₄ and NO_x) have been completed prior to January 1. Phytoplankton samples have all been preserved, settled, and condensed and are ready to be enumerated. We have purchased and installed an ABRAXIS Microplate reader for the purpose of analyzing our cyanotoxin samples via the ELISA plate method. This work will begin and proceed rapidly following a staff training and certification for the procedure by ABRAXIS scheduled in March 2017.

Project Status as of July 1 2017:

This project is currently right on track with the completion of one field season and the analysis of all water quality samples and the collection and processing of sediment cores from 4 of 10 lakes in this study. The remainder of the sediment cores will be collected in the Fall/Winter of 2017-2018.

We are currently in the middle of our second field season. Each of the five study lakes has been sampled bi-monthly, in cooperation with MPCA, since May of 2017. During our first sampling event, we also re-deployed temperature and oxygen sensors, as well as sediment traps in each of the 5 lakes, which will monitor the status of the lakes every 30 minutes throughout the entire open-water season.

Cyanotoxin samples analyzed by our newly purchased and installed ABRAXIS ELISA plate reader have provided the first systematic measurements of the cyanotoxins anatoxin-a, cylindrospermopsin, and saxitoxin in the state of Minnesota. All toxins were detected in at least one lake in 2016, coinciding with the peak bloom periods of July-September.

In March of 2017, we co-hosted a HABs workshop in collaborating with the St. Anthony Falls Laboratory, University of Minnesota Extension, and Minnesota Sea Grant. This day-long workshop focused on the issues of research, monitoring, and outreach as they pertain to HABs. This workshop focused on bringing together scientists, agency personnel, consulting companies and private citizens who were interested in these issues and

included administrators of state and federal agencies (MPCA, NOAA Sea Grant, MN DNR). The results of this workshop helped to foster further collaboration among HABs researchers in the State, as well as pinpoint future directions of HABs research that will be important to the citizens of Minnesota. We also invited participants to participate in coordinating a larger workshop in the future where the results from our study, and others in the State, can be presented to the general public.

Project Status as of January 1 2018:

This project continues to be on track with all activities. In November of 2017, we completed our second and final monitoring season for the five Sentinel Lakes selected for this project in Activity 2 (St. James Lake, Madison Lake, South Center Lake, Lake Shaokatan, and Pearl Lake). This included collecting water chemistry, cyanotoxin, and algae community samples twice a month from each lake in cooperation with the MPCA. All of the 2016 water quality samples have been analyzed and all water chemistry from 2017 has been analyzed in the SCWRS lab.

For Activity 3, dating has been completed on sediment cores collected from Madison Lake, Pearl Lake, Trout Lake, and Lake Shaokatan. Cores have been collected and are in the process of being analyzed for St. James Lake, Elk Lake, and Portage Lake. The remaining 3 cores (Carlos, Cedar, and South Center) will be collected before the end of the 2018 ice season (~mid-March) and are the top priority for analysis in the SCWRS laboratory. Algal pigment samples for the completed cores have also been shipped to Dr. Peter Leavitt at the University of Regina and are in the process of being analyzed.

For Activity 4, our partners at the USGS Water Science Center have completed their CE-QUAL2 models of both Madison and Pearl Lake which will be integrated with watershed models to be completed by SCWRS staff.

Project Status as of July 1 2018:

This project continues to be on track with all activities. Activity 1 has been completed and all of the sample collection and nearly all of the internal laboratory analyses have been completed for Activities 2 and 3.

For Activity 2, the final phase of analysis includes the phytoplankton samples which will be analyzed via microscopy. We have completed an initial assessment of the dominant taxa in these samples and are in the process of doing our more quantitative biovolume assessments. We have begun to synthesize the field data collected from 2016 and 2017, which includes all of the water chemistry, cyanotoxins, and the buoy data (temperature and oxygen profiles).

For Activity 3, we have completed collection and analysis of sediment cores from all the lakes in this study. This includes all geochemical measurements (TP, BSi) as well as 210-Pb dating models. We have also completed pigment analysis on three of the cores (Pearl, Madison, Shaokatan) and the remaining core samples are in the queue for analysis at the University of Regina.

For Activity 4, we continue to partner with USGS to refine their predictive model for HABs in Madison Lake and they are currently working with the data provided by us from our monitoring efforts and buoys deployed in 2016. This model will be paired with a newly produced SWAT model that will be produced by SCWRS staff in the final year of this project.

Project Status as of January 1, 2019:

This project continues to move along with final analyses and synthesis in Activities 2 through 4 (Activity 1 has been completed).

For Activity 2, we have analyzed and synthesized all water chemistry data collected in both field seasons (2016 & 2017). We have completed all cyanotoxin analyses that were budgeted for, but have stored 131 additional toxin samples collected by SCWRS and MPCA in 2016 and 2017 for later analysis pending our amendment request (below). We have developed our phytoplankton enumeration and measurement technique using the CHARM laboratory inverted microscope and imaging software, and completing these samples will be a primary focus of this activity for the remainder of the project.

For Activity 3, all algae pigment analysis at the University of Regina have either been completed or are in the final stages of analysis. Diatom samples for creating diatom inferred-total phosphorus reconstructions is ongoing. We have completed diatom counts on Madison, Trout, St. James, and Pearl. Data from Pearl were not usable due to very poor diatom preservation, likely due to silica dissolution in highly alkaline waters. Comparison of finalized diatom and pigment data with the completed geochemical profiles will be the primary focus of the final synthesis for this activity.

For Activity 4, USGS has compiled stream and lake data for Madison Lake from agency sources as well as data collected as part of Activity 2 of this project. They have begun to use these data to calibrate a preliminary in-lake nutrient/algae model with an expanded taxonomic resolution for Cyanobacterial groups. This model will be refined and incorporated with SWAT modeling of Madison lake which is to be completed in the final period of this project.

Amendment Request (January 31, 2019):

We request a change in the budget for Activity 2 to increase the supplies budget from \$5,000 to \$10,500. This will allow us to purchase additional ELISA kits for analysis of 131 additional cyanotoxin samples that were collected in 2016 and 2017, but were not analyzed due to budgetary constraints. These samples were frozen after collection and are still viable for our ELISA toxin method. To pay for these analyses, we would like to shift remaining funds originally budgeted for Travel in Activity 2 (\$4,000) and 3 (\$1,500). This would increase the Supplies budget for Activity 2 to a total of \$10,500 (up from \$5,000). We had considerable savings in travel costs by combining trips for these two activities whenever possible, and by keeping our field team on site for multiple days rather than returning to SCWRS daily.

Amendment Approved by LCCMR 2/13/2019.

Overall Project Outcomes and Results:

Lakes are one of Minnesota's most precious resources and harmful algal blooms (HABs) threaten them both from an ecological and economic standpoint. This provides a survey of the current prevalence and toxicity of harmful algal blooms (HABs) in a subset of Minnesota lakes, determines if these blooms are increasing in frequency, and develops and refines modeling techniques that could be used to predict HABs in lakes across Minnesota. To this end, we intensively monitored five lakes in southwest and central Minnesota over 2 years for all major water chemistry parameters, algal biomass, and four cyanotoxins. In these lakes, and five additional lakes in northern Minnesota, we collected and dated sediment cores where fossil cyanobacterial pigments could be measured to track the occurrence of Cyanobacteria over the last 150 years. Finally, we chose one of the intensively monitored lake as a pilot study where we developed a watershed model (SWAT) and an in-lake hydrodynamic model (CE-QUAL-W2) to predict annual cyanobacterial bloom patterns. As a result of this project, we determined that in lakes which are already eutrophic, internal loading dynamics will play a key role in determining the size and toxicity of the bloom. Importantly, we found that even in shallow lakes (less than 16 ft maximum depth), temperature and oxygen dynamics are critical in terms of bloom timing and toxicity. Cyanobacteria pigment data from our sediment cores showed increasing HABs in some lakes over the 20th Century, but also demonstrate that conditions may have been even worse in the early to mid- 20th Century before the passage of the Clean Water Act. Our modeling results provide a framework for resource managers to

predict seasonal bloom formation and persistence in lakes across the state using publicly available and widely used modeling techniques.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Jump-start lake monitoring program

Description: We will begin monitoring of algal blooms and associated limnological conditions at the onset of open water conditions in the first year of the project (April 2016). HABs typically appear in mid- to late-summer, but the conditions leading up to bloom formation cannot be understood without spring and early summer monitoring. Monitoring will be conducted at five Sentinel Lakes on a twice-monthly basis as described under Activity 2. In addition, we will instrument the five lakes with recording temperature and oxygen probes to continuously monitor chemical and physical lake conditions and sediment traps to track seasonal changes in algal composition and abundance.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 93,000
Amount Spent: \$ 93,000
Balance: \$ 0

Outcome	Completion Date
1. Accelerate the lake monitoring program by beginning in year-1 of the project (April, 2016) and extend the monitoring period to 7 months (April-October) for each of two years	December 2016
2. Instrument five lake with recording oxygen and temperature probes and sediment traps to continuously track changes in water column condition and algal composition and abundance	December 2016

Activity Status as of July 1, 2016:

Sampling sites were finalized in coordination with MPCA and the SAFL research group. We selected sites that were 1) Sentinel Lakes and 2) had a documented history of HABs according to past MPCA/MNDNR Sentinel Lakes monitoring records. We also selected lakes over a range of depths to include both polymictic (mix continuously) and dimictic (mix only in Spring and Fall) systems. The final list of lakes for monitoring is Madison Lake (SW MN; dimictic), St. James Lake (SW MN; polymictic), Lake Shaokatan (W Minnesota; polymictic), South Center Lake (Central MN; dimictic), and Pearl Lake (NC MN; polymictic). We coordinated with the MPCA Citizen Lake Monitoring Program to distribute a letter to their volunteers on each of these lakes to make them aware of the work that was ongoing and also the presence of the submerged buoys over the next 2 years. We hoped by targeting citizen stakeholders around the lake, we can both encourage them with the knowledge of work being done on “their lake” and help to dissuade any concerns if our field crew or buoys are spotted by residents or visitors to the lake.

All necessary equipment for the field season was purchased. We purchased a YSI EXO2 Sonde for taking vertical profiles of physio-chemical variables, total algae, and total cyanobacteria. We also purchased temperature, dissolved oxygen (DO), and pressure sensors to instrument the submerged buoy arrays for each of the study lakes. These were set up to measure temperature every 0.5-1 meter and DO at the bottom and surface of each lake. In extremely shallow systems (i.e., Shaokatan and St. James), only the bottom DO sensor was deployed to prevent accidental damage of vandalism to sensors placed too close to the lake surface. Sediment traps were constructed at SCWRS and deployed in lakes deep enough to contain them (South Center, Madison, and Pearl) to test their effectiveness at integrating Cyanobacteria and total algae production over the season. In addition, we purchased all other necessary supplies (sample bottles, reagents, calibration standards) to cover the first two months (May and June) of sampling prior to the initiation of Activity 2 on July 1, 2016.

Two complete sampling events were carried out in May and June of 2016 on all five study lakes. In the first event, we also deployed the submerged buoy arrays in each lake and programmed them to record temperature and oxygen concentrations every 30 minutes for the entire field season (May through October). All buoys were successfully deployed and real-time corrected GPS coordinates were taken for each of their locations. Water samples were collected for TP/TN, NO_x/NH₄, SRP, DIC, DOC, Chlorophyll *a*, phytoplankton and cyanotoxins. In total, 251 unique samples were collected and transferred back to SCWRS for later analysis in our chemistry or CHARM (Center for Harmful Algal Research in Minnesota) laboratories. Additionally, vertical profiles were collected at each visit using the YSI EXO2 sonde for water column physio-chemical and biological variables (conductivity, temperature, DO, pH, turbidity, total algae, and total Cyanobacteria).

Activity Status as of January 1, 2017:

No activity during this reporting period

Activity Status as of July 1 2017:

This portion of the project has been completed. All necessary equipment was purchased and all samples from the early portion have been collected and analyzed.

Activity Status as of January 1 2018:

No activity during this reporting period

Activity Status as of July 1 2018:

No activity during this reporting period

Activity Status as of January 1, 2019:

No activity during this reporting period

Final Report Summary:

The prevalence and severity of harmful algal blooms (HABs) in Minnesota was one of the most pressing issues identified by both State agencies and the general public in Minnesota at the onset of this project. Because of this, the LCCMR decided to use Emerging Issues funding to jump-start this project a season ahead of when it would have normally started. Thanks to this timely support, we were able to formalize our sampling design, purchase all necessary equipment, complete construction of temperature and oxygen buoys, and begin our water quality sampling in May of 2016. In effect, this gave us an additional full field season for this project, doubling the amount of data that could be collected and analyzed over the course of this project. Importantly, this additional sampling year also gave us some insight into inter-annual variability of HABs in the lakes of this study and the range of timing and severity of HABs from year to year.

ACTIVITY 2: Identify species composition and timing of harmful algal blooms

Description: We will assess the relationship between algal communities and water quality in a representative group of Minnesota lakes to determine the distribution, abundance, and seasonality of bloom-forming species. Current water quality monitoring of five Sentinel Lakes (carried out by the MN DNR and MPCA) will be amended to include a twice-monthly algae sampling during the ice-free period over two years that will be analyzed by the St. Croix Watershed Research Station's CHARM Laboratory (Center for Harmful Algal Research in Minnesota), established with prior ENRTF support. Cyanobacteria that are detected will be quantified in terms of biomass

(bloom vs. non-bloom), danger to public health (toxin producing vs. non-toxin producing), and provenance (invasive vs. historically occurring).

Sampling will be done on an alternating 2-week basis for those lakes scheduled for routine monthly monitoring by DNR/MPCA field staff. Each site visit will include the collection of algal samples from near-surface and thermocline depths along with samples (epilimnion and hypolimnion) for water chemistry – total-N, total-P, nitrate/ammonia, soluble reactive P, dissolved organic and inorganic carbon – and algal toxins. Depth profiles of temperature, conductivity, pH, dissolved oxygen, chlorophyll *a*, and phycocyanin (a photosynthetic pigment specific to cyanobacteria) will be made with a YSI water-quality sonde specifically acquired for the project.

Soft algae (including cyanobacteria) will be identified and enumerated on a specialized “inverted” microscope recently acquired through ENRTF funding of our ongoing project, “Watershed-Scale Monitoring of Long-Term Best-Management Practice Effectiveness”. Algal toxins, including microcystin, anatoxin-a, and cylindrospermopsin, will be analyzed by ELISA microplate reader.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 165,800
Amount Spent: \$ 165,800
Balance: \$ 0

Outcome	Completion Date
1. <i>A quantification of the seasonality of harmful algal blooms across a representative sampling of Minnesota lakes</i>	June 2018
2. <i>The identification of bloom-forming species, the associated risk for toxin production, and the occurrence of invasive blue-green algae</i>	June 2018

Activity Status as of January 1, 2017:

We completed the field season, begun in May, in November. All 5 study lakes were visited twice monthly between May and October of 2016 (once by SCWRS and once by MPCA). We collected all necessary water samples and YSI profiles for each visit (detailed in Activity 1). We added one additional sampling trip to South Center Lake in November of 2016, due to the lake still being stratified during our October visit. Unfortunately, the lake was still stratified at this time, though we observed a considerable amount of hypolimnetic erosion in our final sampling trip (full turnover likely occurred within days of our visit). Samples collected by MPCA that were to be analyzed by SCWRS (phytoplankton and cyanotoxins) were transferred to us after the season was completed. Water chemistry data and YSI profiles collected by MPCA will be shared with SCWRS once they have been completed.

YSI profiles from the year show clear connections between lake physics and the occurrence of Cyanobacteria blooms. Madison Lake provided one of the clearest examples of this (Fig. 1). Madison Lake was still mixed following Spring turnover when we visited in May and the entire water column was well oxygenated with low algae abundance and high clarity. On our subsequent visit in June, the lake had stratified and already driven oxygen down near 0 mg/L in the hypolimnetic bottom waters (Fig. 1). We observed a rapid accumulation of sediment P in the hypolimnion during this anoxic period, rising to 490 ug/L as compared to 84 ug/L in the surface waters. This stratification and anoxic hypolimnetic state was maintained through August. In August, stratification was rapidly broken, likely due to a series of storm events with high winds in this month, and the lake mixed once again releasing that P into the upper water column. A large Cyanobacteria bloom followed this mixing event and persisted through October (Figure 2). South Center Lake’s hypolimnion also went hypoxic during the Summer, however the lake never mixed until late November leading to less abundant Cyanobacteria and better water clarity throughout the ice-free season. St. James and Pearl both were polymictic and had Cyanobacteria blooms from mid-Summer through October. Shaokatan remained in a clear-water, macrophyte-dominated, state throughout the season and never produced a Cyanobacterial bloom.

Water chemistry samples collected by SCWRS from the previous field season are being analyzed in the chemistry laboratory and all but the dissolved nitrogen species (NO_x and NH₄) have been completed. An ABRAXIS micro-plate reader has been purchased and installed for conducting cyanotoxin enzyme assays using the ELISA method. These samples are currently preserved via freezing, and will begin to be analyzed following a scheduled staff training and certification workshop scheduled in March of 2017.

Finally, all submerged buoy arrays were recovered from the study lakes in October and the data from them were recovered successfully. These data provided us 30-minute resolution on the condition of each of the lakes over the entire field season. In our dimictic systems (i.e., South Center), these data showed the onset and duration of stratification throughout the year and the accompanying oxygen depletion in the bottom waters (Fig. 3). In others, these results were more surprising. Pearl Lake, one of our polymictic systems, actually demonstrated sustained periods of anoxia (~1 month) in the late summer (Fig. 4).

Activity Status as of July 1 2017:

We have nearly completed the analysis of toxin and water chemistry collected from the five intensively monitored lakes in 2016. These data are shown in Figure 5 over the entire sampling period. These data represent only the samples collected by SCWRS, as the MPCA samples are still being processed and formatted; those additional data will be incorporated into our analysis once they become available. These results provide the first systematic survey of all four major cyanotoxins in the state of Minnesota (Figure 5) and are plotted with a red-dashed reference line that corresponds to the minimum drinking standard as set by the state of Minnesota (microcystin, anatoxin-a), US EPA (cylindrospermopsin), or as recommended by the peer-reviewed literature (saxitoxin). We can now confirm the detectable presence of all four toxins in the study lakes as well as concentrations in excess of safe drinking standards for humans (and pets) for both microcystin and anatoxin-a. These toxins show strong seasonal trends, with the highest concentrations in late summer and fall, and also seem to persist for weeks to months within the water column. These peaks appear to be correlated to declining N:P ratios and algal biomass.

We began our second field season in May of 2017. This included the re-deployment of temperature and oxygen sensors in each of the five intensively studied Sentinel Lakes (Madison, St. James, Pearl, South Center, and Shaokatan). We also deployed sediment traps for the collection of silt and algae in the deeper lakes (Madison, Pearl, and South Center). These sensors will collect data on the temperature and oxygen stratification of the lakes every 30 minutes from May through October.

As laid out in our workplan, we have visited each of these five lakes bi-monthly, in cooperation with MPCA, in May and June, and will continue to monitor them through October of 2017. For 2017 we have added an additional hypolimnetic sample in Pearl lake (only surface water was collected in 2016) based on the observation of short periods of stratification and hypoxia in 2016. This lake was thought to remain mixed throughout the open-water season, and only through the installation of our sensors in 2016, were we able to determine it might be susceptible to anoxic nutrient release from the sediments.

Activity Status as of January 1 2018:

We have successfully completed both proposed monitoring seasons for the five lakes selected in this study. All lakes were visited twice monthly by SCWRS staff for both the 2016 and 2017 open water seasons (May – October).

We have now completed analyses on all water quality and toxin samples collected in the 2016 season and have finished analyzing all water chemistry samples from the 2017 season. Additionally, we successfully retrieved all

five oxygen and temperature buoys and sediment traps that were deployed in the lakes and have processed those data into isopleth charts. Notably, we extended the field season on South Center into November of 2017 so that we could capture the fall-mixing event that occurred too late in the season in 2016 (Figure 6).

In total, we have collected and analyzed 1,371 water quality samples in the 2016 and 2017 field seasons and recorded over 80,000 vertical profile measurements of temperature and oxygen from our five buoy arrays. These data will provide one of the most comprehensive syntheses of the environmental conditions that lead up to and follow HABs in Minnesota lakes.

Activity Status as of July 1 2018:

We have begun to synthesize the monitoring data collected in 2016 and 2017 and look at the major drivers for bloom formation and toxin production. Major findings thus far include:

- *South Center (dimictic)*: gradual increase in TP and chl-*a*, both decreasing into the fall (Fig. 7A, C).
 - Saxitoxin and anatoxin-a increased, peaked, and subsided in parallel with *Dolichospermum* and *Aphanizomenon flos-aquae* biomass (Fig. 7G,H and 8A).
 - Cylindrospermopsin peaked in fall with low abundance of *A. flos-aquae* and *Cylindrospermopsis raciborskii* (Fig. 7F).
- *Madison Lake (polymictic)*: rapid increase in TP and chl-*a* following summer mixing events (Fig. 7A,C).
 - First mixing event led to a spike in saxitoxin and anatoxin-a from *A. flos-aquae* (Fig. 7G,H).
 - Second successive mixing events produced anatoxin-a and cylindrospermopsin and more complex blooms (*A. flos-aquae*, *C. raciborskii*, *Dolichospermum* sp.; Fig. 7F,H and 8C).
- *Pearl Lake (polymictic)*: largest increase in TP and chl-*a* following late summer stratification and anoxia (Fig. 7A,C).
 - Mixing of anoxic waters in late summer led to fall blooms of *Microcystis* and *Dolichospermum* and peak concentrations of total microcystins (Fig. 7E and 8B).
- *St. James Lake (polymictic)*: never stratified and chl-*a* increased with TP, peaking in mid-to-late Summer (Fig. 7A,C).
 - Total microcystin concentrations paralleled *Microcystis* biomass and decreased into the fall (Fig. 7E).
 - Decreasing TN (Fig. 7B) and increased light limitation from DOC (Fig. 7D) may have contributed to the decline in the toxic bloom in the fall, despite elevated TP (Fig. 7A).
- *Lake Shaokatan (polymictic)*: lowest TP throughout the summer, but spiked to the highest TP concentrations in the fall. Despite this spike, chl-*a* never increased to levels seen in the other lakes and no bloom was observed (Fig. 7A, C).
 - No significant trends in cyanotoxins or bloom formation were observed throughout the year.
 - Despite a history of CyanoHABS, Shaokatan flipped to a macrophyte dominated state. The fall peak in TP corresponded to senescence of the dominant macrophytes, *Ceratophyllum demersum* and *Myriophyllum*; both are efficient users of phosphorus.

In summary, the lakes in this study which had the highest variability in stratification showed marked changes (Madison, Pearl) in both the cyanobacterial community and toxin production following mixing events. This included blooms into the late fall and temporal asynchrony of saxitoxin and anatoxin-a, likely produced by multiple different genera. Lakes that never mixed, or never stratified for long enough to produce anaerobic conditions, did produce CyanoHABS, but they followed more predictable seasonal patterns (unimodal peaks in

mid-summer). These results indicate the importance of understanding inter-annual variability in lake physical structure to predict the duration and toxicity of HABs.

Activity Status as of January 1, 2019:

All water chemistry data from 2016 and 2017 have now been compiled and we are in the process of final analysis and synthesis of these data. Trends for both water chemistry and cyanotoxins for both years are shown in Figure 10. We note that whereas certain toxins showed a consistent seasonal pattern across years, other (i.e., saxitoxin and cylindrospermopsin) were quite different. We hope to better understand this contrast through analysis of additional archived cyanotoxin samples as well as completion of the phytoplankton samples.

Final Report Summary:

This project represents the most intensive sampling of Cyanobacteria and their toxins conducted in the State of Minnesota to date. In cooperation with the MPCA, five lakes in southwest and central Minnesota were sampled every 2 weeks for water quality parameters. Additionally, each lake was instrumented with temperature and oxygen buoys which collected information on the physical stratification of the lake at 30-minute intervals from May through at least October (South Center Lake was monitored through November due to the longer stratification period and its proximity to SCWRS).

The role of temperature and oxygen on producing HABs

Although the connection between nutrients (primarily Phosphorus [P]) and HABs has long been known, there is still considerable uncertainty about how the timing of release may trigger more severe cyanobacterial blooms or the production of toxins. The use of buoys to measure thermal and oxygen structure in a lake represented a new technique for understanding what may be driving the most toxic Cyanobacteria blooms in Minnesota lakes. These buoys give us high-frequency (every 30 minutes) measurements of the lake's thermal structure and are necessary for understanding the potential role of internal loading of P from the sediments.

Internal loading of P to lakes from their sediments has been shown to increase by up to 40 times in the absence of oxygen (anoxia). Therefore, understanding if and when it occurs in lakes is critical when measuring the potential for blooms fueled by this sediment-bound P. It is often assumed that these internal loads are only important in weakly stratified lakes which are of intermediate depth. This is because shallow systems were thought to always be oxygenated due to continuous wind-mixing, and deep lakes are assumed to only mix twice a year when the water is cold and the photoperiod is still relatively short. To test these assumptions, we selected lakes along a depth gradient that ranged from 3 to 18 meters to monitor the presence of anoxia, and how it affected nutrient concentrations in the lake, as well as cyanobacterial abundance and toxicity.

The shallowest lakes in this study, St. James and Shoakatan, both behaved as expected with bottom waters super-saturated with oxygen for the majority of the year due to high rates of primary production by algae (St. James) and aquatic plants (Shaokatan) (Figure 11). The deepest lake, South Center, also behaved as assumed, with stratification beginning in the spring (May) and lasting well until the fall (November; Figure 3 and 6). In both cases, these results demonstrate that in extremely shallow systems (3m or less) and deep lakes (>15m), internal loading of nutrients is probably minimal. In these lakes fluxes of nutrients from sediments are either extremely slow due to oxygenated conditions (shallow lakes), or, timed during the part of the year where conditions are unfavorable for blooms due to cold water and short photoperiods (deep lakes). This indicates that bloom prediction and management strategies should focus on the timing and intensity of external loads from the watershed.

As expected, our lake of intermediate depth, Madison Lake (~10m), proved very susceptible to internal loading due to early mixing in August and then repeated mixing until the fall of 2016 (Figure 12). This prolonged anoxia

allowed for the accumulation of sediment P in the hypolimnion and the early mixing event in August caused that P to be mixed throughout the water column where it would be available to Cyanobacteria. This likely provided a second pulse of nutrients to the system in addition to the traditional external load associated with snow melt and elevated precipitation in the Spring (freshet). Because of this substantial potential for internal loading of P, the timing of this early mixing event (or whether it occurs at all) between years will be a key factor in determining the size of HABs and how long they persist into the fall.

Finally, perhaps the most surprising result from the buoy deployment was found in Pearl Lake. This lake is relatively shallow (5m) and was not expected to stratify due to its depth and round shape (high fetch relative to surface area). However, we found that not only did this lake stratify for a considerable amount of time in 2016 (>2 weeks), it rapidly developed anoxic conditions over this time period (Figure 4). This points to a previously unconsidered source of nutrients to lakes of similar depth (5-10m) that is important in predicting the timing and persistence of blooms between years (See “What’s causing HABs in Pearl Lake?” fact sheet attached in Supplementary Material for more information).

The results from the temperature and oxygen buoys deployed for this study have revealed additional factors that may play a role in producing HABs in lakes of intermediate depths. As expected, internal loading of P is a major player in lakes of intermediate depth (10-15m), however it must also be considered in relatively shallow lakes that were previously thought to mix continuously (5-10m).

Water Quality, Phytoplankton, and Toxin Monitoring

In order to understand the mechanisms that underlie HAB formation, intensive monitoring of a wide array of environmental parameters is necessary. In addition to characterizing the physical structure of a lake (i.e., temperature and oxygen stratification outlined above), we collected chemical, biological, and toxicity data on each of the five lakes at two-week intervals over 2 years (2016, 2017). Importantly, these data represent one of the first systematic survey of all four major cyanotoxins (microcystin, anatoxin-a, saxitoxin, cylindrospermopsin) in Minnesota. Figures 13 through 17 show a summary of all water chemistry data collected for the five lakes in this study over the 2 years.

Total phosphorus concentrations increased linearly throughout the spring and early summer in St. James which receives run-off from a largely agricultural watershed as well as the adjacent city of St. James, MN (population: ~4,600). No secondary pulses of nutrients were detected, consistent with the absence of internal loading described above. The peak cyanobacteria bloom (measured as chlorophyll-a) occurred in late-July and early August of both years and coincided with the highest TP concentrations. Both TP and algal biomass declined into the Fall. Microcystin concentrations also peaked in St. James, following the same pattern as TP and algal biomass (Figure 18). Microcystin concentrations exceeded the EPA’s safe contact standard (8 ug/L) in St. James in both 2016 and 2017, during which period the genus *Microcystis* was the dominant algae found in the lake. Detectable concentrations of both anatoxin-a (Figure 19) in the Spring and Fall, and cylindrospermopsin (Figure 20) in the mid-Summer and Fall were also found in St. James. Both anatoxin-a and cylindrospermopsin were found in samples where the genus *Dolichospermum* was the most abundant Cyanobacteria.

South Center also had linear increases in TP and total algae from spring until mid-summer which tapered off into the Fall. Unlike St. James, South Center did have a secondary pulse of internally derived nutrients in the late fall that was likely due to internal loading as the lake began to mix. However, because this event occurred late in the Fall as waters had already cooled, no secondary bloom of Cyanobacteria occurred in either year. All water chemistry data for South Center Lake are summarized in Figure 18. All four toxins were detected in South Center Lake over the course of the 2 years of sampling, however they were asynchronous and likely related to turnover in the dominant species of Cyanobacteria. Microcystin peaked in the late summer about a month after peak Cyanobacterial biomass and during a period where both TP and algal biomass was declining (Figure 18). This indicates that this toxin was likely being released from cells as they senesced during the cooler fall

temperatures. The genera *Dolichospermum* and *Aphanizomenon* were the most abundant during peak microcystin concentrations. Both genera were also likely responsible for the production of saxitoxin (Figure 21) and anatoxin-a (Figure 19) in both years, although the production of these toxins were asynchronous with saxitoxin peaking first, followed by anatoxin-a. Anatoxin-a concentrations exceeded drinking water standards in 2016 and saxitoxin concentrations exceeded standards in 2017 although both years had relatively similar bloom concentrations and timing (Figure 18). *Cylindrospermopsis* was detected in the later summer and fall (Figure 20) and was correlated to the appearance of the exotic species *Cylindrospermopsis raciborskii* in phytoplankton samples.

Shaokatan had very low concentrations of TP and total algae throughout most of the season in both years. Shaokatan was unique in that it was completely covered in aquatic plants (macrophytes) in both years. The MPCA had documented the flip of Shaokatan from a turbid, algae-dominated state to a clear-water, macrophyte dominated state prior to this study beginning. In 2016, after the macrophytes had died off we measured TP concentrations in Shaokatan ~150 µg/L, the highest measured in any lake during this study (Figure 15). The lack of an associated HAB demonstrates the effectiveness of macrophytes in locking up P in this system and reinforces the importance of protecting and reintroducing aquatic plants to shallow lakes in order to manage for HABs. As would be expected, toxin concentrations were very low in Shaokatan, although all 4 toxins were detected on at least one occasion over the 2 years (Figures 18-21). Very few Cyanobacteria were seen in any phytoplankton samples collected from Shaokatan, with only *Dolichospermum* being present in very low concentrations. Because Shaokatan is very shallow, it is possible that some of these toxins were being produced by benthic or epiphytic Cyanobacteria which would not have been collected in our water column samples.

Pearl TP and algal concentrations increased similar to St. James for the first part of 2016, but following a multi-week anoxic period (described in the previous section) TP and algae both increased at a more rapid pace (Figure 16). This secondary bloom was likely triggered by internally loaded sediment P that became unbound when the bottom waters become anoxic. This triggered both the largest biomass of Cyanobacteria and the highest concentrations of toxins, including microcystin concentrations exceeding the safe contact standard of 8 µg/L (Figure 18). This bloom was composed of primarily *Microcystis*, however, *Dolichospermum* was also abundant.

Madison TP and algal concentrations increased gradually in the first part of both years but jumped up in the late Summer due to early mixing events. Measured concentrations of TP in the hypolimnion exceeded 600 µg/l prior to the mixing event, almost 10x higher than was measured in the surface water (68 µg/l). Once this huge pool of nutrients was mixed in August of 2016 and 2017 the Cyanobacterial bloom peaked. In Madison, this bloom was primarily composed of the genus *Aphanizomenon* which produced a peak of anatoxin-a (Figure 19) coincident with the bloom and released microcystin (Figure 18) as the bloom senesced. Both toxins were observed in concentrations exceeding Minnesota Department of Health drinking water standards.

ACTIVITY 3: Reconstruct frequency of algal blooms relative to natural conditions

Description: We will determine where and when bloom-forming algae have increased in Minnesota lakes over the last century to better understand the causes and susceptibility of individual lakes to bloom development. We will use sediment paleolimnological methods to reconstruct the frequency and severity of cyanobacterial blooms in 10 lakes selected from the Sentinel Lakes monitoring program. Sediment cores will be collected from each lake and dated using radioisotopes at the St. Croix Watershed Research Station to establish a continuous history of lake condition over the last 150 years. Dated sections will be analyzed for fossil algal pigments, including those unique to blue-green algae, to determine presence, abundance, and frequency of harmful algal blooms in a historical context.

To obtain the sediment chronology, cores will be radiometrically dated by ²¹⁰Pb methods, supplemented as needed by identifying the 1963 ¹³⁷Cs peak that is remnant from the atmospheric testing of nuclear bombs. Based on typical sediment accumulation rates in Minnesota lakes, it should be possible to obtain reliable dates back to the mid to early 1800s in all lakes. Dating resolution will be roughly decadal overall, but more detailed (approximately 5-year) for the most recent 2-3 decades.

The sediment cores will be analyzed for a suite of components to assess changes in algal abundance and composition as well as nutrient levels that contribute to the development of HABs. Fossil pigments specific to cyanobacteria along with those produced by other algal groups will be the primary tool for reconstructing changes in HABs and overall lake productivity. In concert with pigment analyses, lake-water phosphorus content over time will be estimated by analysis of the remains of diatoms, a group of algae with certain species that are diagnostic of phosphorus content in the water in which they live. General algal productivity will be assessed by the accumulation of biogenic silica, which is largely composed of the glass cell walls of these diatoms. The phosphorus content (both total and extractable fractions) of the sediment will determine apparent loads of this essential nutrient.

Ultimately, core reconstructions will be compared with local land-use history, nitrogen deposition trends, and meteorological records (temperature, wind speed, precipitation) to determine whether any of these potential drivers of limnological change are correlated with shifts in lake productivity and, in particular, the abundance of HAB-forming algae.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 171,900
Amount Spent: \$ 171,900
Balance: \$ 0

Outcome	Completion Date
1. <i>A comparison of historical changes in harmful algae among a large suite of Sentinel lakes to determine the geographic extent and timing of the problem</i>	January 2018
2. <i>An assessment of the likely drivers of increasing harmful algae by comparison of trends in lake sediment cores with changes in landscape, land-use, and climate over the period of record</i>	December 2018

Activity Status as of January 1, 2017:

We have selected five additional Sentinel Lakes with more infrequent or no history of Cyanobacterial blooms (Trout, Elk, Portage, Carlos, and Cedar) to complement the study lakes described in Activity 1 and 2. These lakes were selected to fill out the range of lake-types present in Minnesota and to give a clear picture of the historical occurrence and persistence of HABs across the state. Field work for coring these lakes is scheduled to begin after January 1, 2017, once ice conditions allow us to access these lakes.

Activity Status as of July 1 2017:

In February of 2017, we collected sediment cores from St. James, Madison, and Shaokatan through the ice. We have successfully dated the cores from Shaokatan and Madison. Due to the known high sedimentation rate of Madison, based on earlier work, over 3 meters of sediment were extracted from this lake! Our St. James core was found to contain glacial clays (dating back to ~11,000 years ago) after less than 50 cm down in the core. This could possibly be due to a hiatus in sedimentation at our coring site. We will recore St. James in a different area during the Fall of 2017. Loss-on-ignition profiles for Madison, Pearl and Shaokatan sediment cores have all been completed.

Activity Status as of January 1 2018:

We have completed dating and LOI for Madison, Pearl, and Shaokatan. We have also collected and completed dating on Trout Lake. Cores from Elk and Portage lakes have been collected and are in the process of being analyzed in the SCWRS laboratory. St. James Lake was re-cored in September of 2017 and this new sediment is currently being dated. South Center Lake will be cored in February of 2018 in conjunction with a Winter Ecology

undergraduate course which is being hosted by SCWRS. Carlos and Cedar lake cores will be collected before the end of the ice season in 2018 (early March).

Pigment samples have been preserved and shipped to Dr. Peter Leavitt at the University of Regina for all cores which have completed dating models (Madison, Pearl, Shaokatan, and Trout). We expect the results from these analyses no later than March of 2018 and we will continue to select and ship samples from the remaining cores as the dating models are completed.

Activity Status as of July 1 2018:

All ten lakes in this project have been cored and those cores have been dated and analyzed for all major geochemical elements at SCWRS. We have received completed pigment analyses from University of Regina on 3 of the cores and the other 7 remaining are currently being processed. Preliminary pigment analyses from Madison, Pearl, and Shaokatan are shown in Figure 9. These pigment data show a fairly diverse history of blooms across the three lakes. Madison may have had more intense, but less toxic, blooms in the past. Blooms in Shaokatan had gotten progressively worse up until the major shift to macrophyte dominance as a result of lake restoration in the last ~5 years. The concentration of Cyano pigments in Pearl Lake has remained relatively constant through time, with a small peak in the 1950s prior to the Clean Water Act.

Activity Status as of January 1, 2019:

We have received completed pigment profiles for 6 of the 10 lakes and the final 4 lakes are nearing completion at the University of Regina.

We have completed diatom counts on the sediment cores from Madison, St. James, and Trout. Diatom counting from Pearl Lake was halted due to very poor preservation of fossil diatoms in this core, likely due to silica dissolution. Pearl is a Chara-dominated lake with high alkalinity, which probably led to these preservation issues. We have prepared diatom slides for the final six lakes and counting is underway on those cores. Once completed, these data will be used to produce the diatom-inferred phosphorus model which will serve as an indicator of past water quality conditions. Pairing these data with our nearly completed pigment profiles will provide the best historical context for the prevalence and causes of HABs in these lakes.

Final Report Summary:

One of the biggest questions surrounding HABs is if they are actually increasing in frequency or if the public and researchers are just becoming more aware of them. In the absence of good long-term monitoring records, ²¹⁰Pb-dated sediment cores provide a means of measuring changes in a lake over the last 150 years. In the case of HABs, this can be done using fossil pigments left behind by algae that are unique to Cyanobacteria. Depending on the concentration and type of pigments present, we can tell reconstruct the abundance and types of Cyanobacteria that were in the lake historically. Additionally, other algae whose remains are preserved, in this case diatoms, can tell us about the conditions of the lake over that same time period based on what we know about the water quality preferences of those species in contemporary lake samples.

Cyanobacterial pigments

The concentration of fossil pigments from Cyanobacteria were measured in 15 samples from each of the ten MN DNR Sentinel Lakes selected as part of this study. These lakes ranged from shallow hypereutrophic lakes in SW Minnesota to deep oligotrophic lakes in NE Minnesota. As was expected given the wide range in lake type and geography, pigment results showed differing patterns across the state (Figure 22). It is important to note that pigment preservation in sediment cores is lake-specific, so patterns should be contextualized based on the current condition of that specific lake. For example, if a currently eutrophic lake shows no change in pigment

concentrations throughout the core, that would indicate that it was likely historically eutrophic (no change), likewise, if an oligotrophic lake showed the same pattern it would mean that it was historically oligotrophic (also no change).

Three of the five intensively monitored lakes, all of which were known to be eutrophic to hypereutrophic, had similar patterns of increasing Cyanobacteria through time. Both Shaokatan and South Center showed increasing Echinone (a pigment present in all Cyanobacteria) through time. St. James had a similar pattern, however, due to this lake being dredged in the 1970s, we only were able to collect sediment from that time forward. This pattern reflects the gradual accumulation of nutrients from non-point source pollution over the 20th Century. Pearl shows fairly constant Echinone throughout the last 150 years, indicating that this lake was likely fairly naturally productive. In contrast, Madison actually shows a fairly steep reduction in Cyanobacterial pigments with peaks in the early to mid- 20th Century. Because this lake is adjacent to the city of Madison Lake, this is likely due to the history of this lake being used for point-source waste-water disposal prior to the passage of the Clean Water Act in 1972. Unfortunately, it appears that Cyanobacterial pigments have begun to increase again in Madison since ~1990, indicating the possibility of increasing problems in this lake.

The remaining five lakes are all meso- to oligotrophic lakes in north and northeastern MN. Trout, which acts as our control, is a nearly pristine lake on the edge of the Boundary Waters. It showed no significant change in Cyanobacterial pigments, as we would expect. Portage, which is located just north of Park Rapids, MN is a meso to eutrophic lake which shows little change in total Cyanobacterial abundance, but an increase in Canthaxanthin which is associated with the benthic Cyanobacteria *Nostoc*. Elk, which is located in Itasca State Park, shows the disappearance of Aphanizophyll, associated with N-fixing Cyanobacteria, after 1900 and very small increases in total Cyanobacteria (Echinone) as well as colonial Cyanobacteria (Myxoxanthophyll). Carlos, which is located near Alexandria, MN, shows gradual increases in Cyano pigments over the 20th Century, associated with the establishment of resorts and cottages in this popular recreational area. Interestingly, these pigments decline recently which could reflect the establishment of zebra mussels in this lake (first detected in 2009). Cedar Lake (in Morrison County, MN) shows little change in Cyanobacteria over time besides an increasing occurrence of the pigment Aphanizophyll in the late 20th Century which could indicate a switch in the lake to being N-limited.

In general, the Cyanobacterial pigments support data that HABs are becoming more frequent in our most disturbed lakes in Southern and Central Minnesota. There is evidence, however, that some lakes which may have received point-source pollution prior to the passage of the Clean Water Act have actually significantly improved (i.e., Madison) though they still remain impaired today. The less disturbed lakes in northern MN in this study show little change in the abundance of Cyanobacteria, but there is evidence of changes to the types of Cyanobacteria present in these systems.

Diatom reconstructions of past conditions

Due to the lack of long-term monitoring data available for nearly all lakes in Minnesota, we instead rely on using sediment cores to reconstruct the history of these systems. One of the most effective tools for this are diatoms, which are very sensitive to environmental conditions and have known optima and tolerance ranges for nutrients, especially phosphorus. Below are the results of this analysis from Madison, Shaokatan, and St. James.

The stratigraphic diagram for Madison showed the predominant diatoms that were driving the shifts in the community assemblages, as well as the results of the constrained cluster analysis, and the percentage of plankton throughout the core (Figure 23). The samples from the 1800s were dominated by *Aulacoseira ambigua* and *A. granulata*; these species are characteristic of nutrient-rich and turbid, wind-swept conditions. In the early 1900s, through the 1930s, the assemblage shifted to predominantly small *Stephanodiscus* species (*S. minutulus*, *S. hantzschii*, and *S. parvus*), which are also indicative of nutrient enrichment. From the 1940s to the 1960s there was another shift, characterized again by the small *Stephanodiscus* species (although in lower abundance than the early 1900s) and an increase in *Fragilaria mesolepta*, a planktonic mesotrophic indicator

which can be indicative of nitrogen enrichment. The assemblage from the 1970s to the core top was again dominated by *Aulacoseira ambigua* and *A. granulata*, with the addition of *Fragilaria crotonensis*, another eutrophic indicator. According to the constrained cluster analysis (Figure 23), the two largest breaks in the samples occurred between 1962 and 1973, and between 1883 and 1905.

Passive plotting of the Madison Lake core on the MN calibration set showed that there has been change in the diatom community assemblage in the core relative to the diatom communities in the calibration set samples (Figure 24). However, in the MN calibration set, TP is correlated with axis 1, and the major shifts in the diatom community occurred along axis 2, which is correlated with lake color. This suggests that TP is not the main driver of these shifts. The low λ_r/λ_p value of 0.12 also supports the conclusion that TP has not been the predominant driver of change in Madison Lake during the period of study.

In contrast to the more abrupt shifts observed in the diatom community in the Madison core (Figure 23), the diatom assemblage in Shaokatan showed much more subtle changes over time (Figure 25). The presence of *Aulacoseira ambigua*, *A. granulata*, and *Stephanodiscus minutulus* throughout the core indicate nutrient enrichment throughout the period of study, although all three of these species are very low in the topmost sample, which could indicate the start of a decline in nutrient levels in the lake and is consistent with the recent flip to a macrophyte-dominated clear state. The samples from the late 1970s through 2015 also showed a rise in two benthic species, *Cocconeis placentula* var. *lineata* and *Gomphonema sarcophagus*, which also suggests a rise in water clarity. The samples at the core top (2009 and 2015) showed an increase in *Fragilaria mesolepta*, a planktonic mesotrophic indicator which can be indicative of nitrogen enrichment.

As with Madison Lake, projection of the Shaokatan core on the MN calibration set showed change that is orthogonal to the TP axis; again, suggesting that TP was not the predominant driver of diatom community turnover in the lake and that the change was more aligned with lake color (Figure 26). The λ_r/λ_p value of 0.40 also suggests that although TP may have played a role in diatom community change, it was not the most important driver.

With the exception of the uppermost sample (2012), the diatom community in St. James Lake was dominated by planktonic eutrophic indicators such as *Aulacoseira ambigua*, *A. granulata*, and *Stephanodiscus minutulus* (Figure 27). The tychoplanktonic species *Staurosira construens* and *Staurosirella pinnata* were also abundant; these species are primarily benthic, but are often swept-up and suspended into the water column; many of these species are adapted to live on fine-grained sediments, such as those found in shallow lakes. The sample from 2012 showed a shift in the community, and was dominated by the eutrophic indicator *Fragilaria crotonensis* and by the planktonic diatom *Lindavia ocellata*. There is also a slight rise in *Asterionella formosa* and *Fragilaria crotonensis* in this sample, which could indicate nitrogen enrichment. However, these differences between the uppermost sample and the rest of the core could be the result of diagenetic processes. Again, none of the shifts in the diatom community were significant when compared to a broken stick model.

As with Madison and Shaokatan Lakes, projection onto the MN calibration set showed the change in St. James Lake was more closely aligned with axis 2, again suggesting drivers other than TP, such as lake color (Figure 28). However, the shift in the topmost sample (2012) shows some movement along axis 1, and this is reflected in a higher λ_r/λ_p value of 0.73.

ACTIVITY 4: Determine how nutrients and climate interact to favor harmful algae

Description: We will quantify phosphorus inputs and cycling in an intensively monitored sentinel lake to determine the critical factors leading to bloom development including watershed inputs, recycling from sediments, and changing lake temperatures. The study lake will be among those sampled in Activities 1 and 2, thus allowing us to pair monitoring of harmful algae blooms with mechanistic models that describe watershed inputs and internal recycling of nutrients. We will measure potential for in-lake recycling of legacy nutrients (phosphorus) by determining the fraction of labile phosphorus in the lake sediment. We will monitor bloom

formation by quantifying harmful algae in sediment traps and water column samples, along with potential environmental controls, including water chemistry, lake temperatures, and oxygen depletion of bottom waters.

These results will be paired with watershed and in-lake models to better understand the factors contributing to bloom formation. Specifically, we will use the Soil and Water Assessment Tool (SWAT), a watershed modeling program developed by the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA) to estimate watershed nutrient loads, present-day and in the past. Model construction requires inputs of hydrography, topography, soils, land cover, and agricultural management practices. For the study watershed, a SWAT model will be calibrated to current (2000-2010 average) land-use and climate conditions. In particular, the model will be constrained to match the sediment and phosphorus loads inferred from the sediment core data for this recent time period. Then, the model will be run to simulate sediment and phosphorus loads for selected periods in the past and tested against the sediment core data for these past periods. These model runs will build the mechanistic relationship between the erosional and fertilization history of the terrestrial watershed and how this history is recorded in the lake sediments.

To complete the analysis and infer the impact of watershed land use on lake-water quality, a coupled hydrodynamic – nutrient cycling model, CE-QUAL-W2, will be used to simulate algal and nutrient dynamics within the lake as well as the water-column physical parameters (temperature, dissolved oxygen) that govern them. A primary goal of in-lake modeling will be to partition the loading of phosphorus between external (watershed) and internal (sediment) sources. A secondary goal will be to use SWAT-model inferred nutrient loading for a selected past time period and see if a calibrated CE-QUAL-W2 model can predict the algal community as determined in the sediment core.

Summary Budget Information for Activity 4:

ENRTF Budget: \$ 162,300
Amount Spent: \$ 162,300
Balance: \$

Outcome	Completion Date
1. <i>A mechanistic understanding of drivers of harmful algal blooms based on intensive monitoring of algae phenology and in-lake processes</i>	June 2019
2. <i>A determination of the relative importance of external loading vs. the internal recycling of phosphorus in terms of driving harmful algal blooms across lakes</i>	June 2019
3. <i>A predictive framework linking internal and external nutrient loads to the occurrence of harmful algal blooms in Minnesota lakes</i>	June 2019

Activity Status as of January 1, 2017:

Based on our first year of data, and in collaboration with the USGS Water Science Center personnel, we have decided on Madison Lake as the study lake for Activity 4. We picked this lake because of its sensitivity to changes in thermal stratification as well as the noxious Cyanobacteria blooms that were observed there in 2016. Additionally, Madison Lake was the site of the SAFL HABs Research Group's buoy, which provides an additional source of high-frequency data that could be used in our modeling efforts.

Activity Status as of July 1 2017:

No activity during this reporting period

Activity Status as of January 1 2018:

Our USGS partners have completed their CE-QUAL2 model for Madison Lake which will be integrated with the watershed modeling still to be completed by SCWRS. The completed report is located online at

<https://pubs.usgs.gov/sir/2017/5056/sir20175056.pdf>. The USGS' model is to be further refined based on Cyanobacterial response experiments to be conducted by USGS in the coming year.

Activity Status as of July 1 2018:

No activity during this reporting period

Activity Status as of January 1, 2019:

USGS compiled the available data for Madison Lake from the 2016 open-water season. Sentinel Lakes program water quality data for Madison Lake were available for nine dates in 2016. Available data included nutrients, algal biomass estimates, phytoplankton group biomass distribution, and temperature and dissolved oxygen profile data. In addition to lake water quality data, inflow water quality data were available for up to five dates for the two tributaries to Madison Lake.

Data were used to develop a draft calibration of the Madison Lake CE-QUAL W2 model for the ice-free season of 2016. The new version of the model increased the number of algal groups from four to five by distributing cyanophyte biomass into two sub-groups: non-nitrogen fixing morpho-types and heterocyst-forming filamentous morpho-types. Each algal group had its own specific nutrient-dependent growth physiology incorporated into the model calibration.

The resulting draft water quality model for 2016 had water balance and heat budget calibrations within acceptable ranges as defined in a previous publication of the model (linked in Jan. 1, 2018 update). Predicted component temperature and dissolved oxygen profiles from the model compared favorably to actual field data from the nine sampled dates in 2016. Predicted total algal biomass estimates from the model, based on the cumulative output from the five modeled algal groups, were also in general agreement with the chlorophyll *a* data from the lake water quality dataset. Nutrient calibrations were acceptable for phosphorus and some species of nitrogen, but the draft model currently underestimates the observed total nitrogen in the lake. This may result from the failure of the model to account for nitrogen fixation in the water column.

Calibration efforts will continue into the next quarter with a final model result, with estimates of internal versus external loads, by the end of the project.

Final Report Summary:

This activity produced predictive models for external inputs (SWAT) which could then be fed into a mechanistic in-lake model (CE-QUAL W2) to provide a predictive framework for HABs in Minnesota lakes. Madison Lake was chosen as the pilot lake for this project due to data availability and the dynamic nature of its stratification (i.e., early mixing).

Watershed Modeling

A computer model of the Madison Lake watershed can help identify sources and transport of nonpoint-source (NP-S) pollutants (sediment, phosphorus, and nitrogen), thus informing management decisions on how to clean up these pollutants and reduce noxious algal blooms in Madison Lake. These NP-S pollutants commonly come from row-cropped fields of corn and soybeans because of fertilizer applications and tillage practices that leave fields without living cover for much of the year. The modeling program applied to the Madison Lake watershed is called the Soil and Water Assessment Tool, or SWAT for short. SWAT was developed by the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) to help understand and predict loads of NP-S pollutants from large river basins over long periods of time.

Input to the SWAT model relies on readily-available data from government-agency web sites. Topography was taken from LiDAR digital elevation models (DEMs) made available by the Minnesota Department of Natural Resources (MDNR) at a 3-m horizontal resolution. The DEM was hydro-modified to include drainage features (e.g., culverts) that correct for the false water impoundment by roads and other embankments. Soils data were taken from the SSURGO database made available by the USDA, which is the most spatially detailed soil data available. Land cover and crop types were taken from the USDA's crop data layer (CDL) datasets for 2014-18. This 5-year sequence of crops on the ground, at 30-m spatial resolution, provided an objective method for inferring typical crop areas, rotations, and locations in the watershed. Corn and soybeans accounted for nearly all the crop acreage, with minor areas of alfalfa and small grains (Figure 29). Representative amounts of inorganic fertilizer were added to all crops at the time of planting. Conservation tillage was assumed for all cropland, consisting of fall chisel plowing followed by spring disking or field cultivation. Weather data (daily precipitation and temperature) were taken from six weather stations (Amboy, Le Sueur, Mankato, St. Peter, Waseca, and Faribault) and averaged for the watershed centroid by simple inverse-distance weighting. The model was calibrated against measured daily outflow from Madison Lake for 2015-16, with Nash-Sutcliffe coefficient of efficiencies of 0.65 and 0.76 for 2015 and 2016, respectively, indicating very good model fits.

The resulting SWAT model identified which of the 197 modeled subbasins of the Madison Lake watershed were hot-spots of high sediment and nutrient yields (Figure 30). A *yield* is a mass per unit area of a selected land unit, per unit time, for example, tons per hectare per year (t/ha/yr) or kilograms per hectare per year (kg/ha/yr). In the Madison Lake watershed, high sediment, phosphorus, and nitrogen yields are consistent with each other and are driven primarily by sources, namely, the location of corn and soybean fields. Wetland, forest, and grasslands produce minimal yields of these NP-S pollutants. The cropland hot-spots of high yields are areas to target for remediation by alternative farming practices that reduce soil erosion and nutrient loss. The next steps would be to simulate possible remediation scenarios to see which ones will most efficiently reduce these pollutants while increasing landscape biodiversity and habitat, without placing undue burden on the farmers who are stewards of the land.

A fact-sheet, further detailing the SWAT portion of this Activity is included as a Supplemental File to this Report ("Construction and Calibration of a Computer Model of the Madison Lake Watershed").

In-lake modeling

A previously developed CE-QUAL-W2 model for Madison Lake, Minnesota, simulated the algal community dynamics, water quality, and fish habitat suitability of Madison Lake under recent (2014) meteorological conditions. Additionally, this earlier model simulated the complex interplay between external nutrient loading, internal nutrient loading from sediment release of phosphorus, and the organic matter decomposition of the algal biomass. However, the partitioning of cyanobacteria within the modeling framework was simplified to one group and did not account for how different cyanobacteria populations are affected by light conditions, the usage of nitrogen, temperature growth ranges, and differences in settling rates. To get a better handle on the proliferation of cyanobacteria in Madison Lake, the model required updates to at least partition the cyanobacteria into a group that fixed nitrogen and a second, more buoyant cyanobacteria group, that did not independently fix nitrogen.

To address the shortcomings of simulating cyanobacteria in the earlier model, the U.S. Geological Survey (USGS), in cooperation with the St. Croix Watershed Research Station (Science Museum of Minnesota), updated the Madison Lake CE-QUAL-W2 model to better characterize cyanobacteria into two groups. In addition to updating the cyanobacteria group differentiation, the entire portion of the model that handles the simulation of algal community dynamics was updated while preserving the model's predictive capabilities for nutrients, water temperature, and dissolved oxygen. The calibration and validation of the model was done under recent meteorological conditions with large and persistent cyanobacteria blooms (2014 and 2016). Overall, the model simulations predicted the persistently large total phosphorus concentrations in Madison Lake's hypolimnion, key

differences in nutrient concentrations between the two years, and cyanobacteria bloom persistence. As a product of this Activity, USGS produced a report which is currently being finalized. The most current draft of this report is attached as a supplemental file (Smith and Kiesling, 2019, Updates to the Madison Lake (Minnesota) CE-QUAL-W2 Water Quality Model for Assessing Algal Community Dynamics).

V. DISSEMINATION:

Description: We will collaborate with the Minnesota Interagency Workgroup on Blue-Green Algae (MPCA, MDNR, MDH, MVMA) to update the agencies on our latest findings, coordinate research, response, and outreach efforts, and evaluate any emerging issues. The Workgroup currently meets twice each year.

In addition, we will distill results from this study into compelling, accessible, and readable stories that will be widely distributed through electronic communications channels. This will include feature articles focusing on specific research results; regular short blog posts about the methods, activities, and people behind the study, and a quarterly email newsletter, "*Field Notes*", with links to the articles and blog posts.

A final project report will document all findings for reference by state personnel, presentations at regional meetings will apprise stakeholders of our methods and results, and publications in peer-reviewed journals will inform the wider academic research community.

Status as of July 1, 2016:

No activity during this reporting period

Status as of January 1, 2017:

No activity during this reporting period

Status as of July 1 2017:

We co-hosted a workshop at the St. Anthony Falls Laboratory's St. Paul campus entitled, "Freshwater Cyanobacteria - Harmful Algal Blooms (HABs) in Minnesota: Past, Present, and Future", which invited researchers, agency personnel, and private consultants working on HABs in Minnesota. This all-day workshop was held on March 28th with representation from the University of MN-Twin Cities, University of MN-Duluth, University of MN Extension, MN DNR, MPCA, MN Department of Health, Minnesota Sea Grant, Minnesota Natural Resources Research Institute, USGS Minnesota Water Science Center, the Science Museum of Minnesota, and others.

We have produced two full-length "Field Notes" articles that have been disseminated via our website and social media and have received thousands of page views since being published. The first article, "Watching When, Where and Why Harmful Algae Happen in Minnesota Lakes," introduced the public to our project and highlighted the need for this research in Minnesota. The second article, "Five super powers of Cyanobacteria", described the evolutionary backdrop that allows Cyanobacteria to produce harmful blooms. These articles continue to be available online at: <https://www.smm.org/scwrs/fieldnotes/watching-when-where-and-why-harmful-algae-happen-minnesota-lakes> and <https://www.smm.org/scwrs/fieldnotes/five-super-powers-cyanobacteria>.

Finally, we continue to use social media, traditional media, and outreach events to reach the broadest possible audiences with our message about awareness, understanding, detection, and prevention of HABs. In the last year our work has attracted the attention of both radio and television outlets. A Minnesota Public Radio story included radio play and a web video:

<http://www.mprnews.org/story/2016/05/24/water-toxic-algae-dogs-climate-change>

and a field interview with Duluth Fox 21 News resulted in two evening news special reports:

<http://www.fox21online.com/news/local-news/water-worries-investigating-toxic-water-on-minnesota-lakes/40394034>

<http://www.fox21online.com/news/local-news/Water-Worries-Researchers-Look-For-Answers/40413736>

Public outreach has included a St. Croix Watershed Research Station “Friends” event in July 2017 where project leader Dr. Adam Heathcote and graduate student David Burge teamed to present a public talk on “Harmful Algal Blooms: When Good Algae Go Bad”) and show attendees what good algae and HABs look like under the research microscope. A “Members Behind the Scenes” event held at the Science Museum of Minnesota gave over 500 museum guests and families a chance to learn about the superpowers of cyanobacteria and see them under the microscope.

Status as of January 1 2018:

We have continued to share our research highlights with the public via social media, including coverage of our coring trips to Trout Lake and Itasca State Park (Elk and Portage Lakes). Additionally, we have been featured on an MPR story regarding HABs in August of 2017 (<https://www.mprnews.org/story/2017/08/17/researchers-search-for-clues-to-toxic-algae-blooms>).

We are also co-sponsors and co-organizers of the upcoming second HABs workshop, to be held at the SAFL auditorium on March 29th. This workshop is open to the general public and hopes to attract researchers, agency personnel, and interested citizens to come learn about and share their own knowledge on HABs in Minnesota.

Status as of July 1 2018:

We continue to publicize the importance of this ENRTF-supported work through our social media channels. We co-organized and participated in the 2nd Minnesota HABs workshop on March 29th, 2018. Adam Heathcote served as the program moderator and also participated as part of an expert panel which took questions from the ~70 registrants who attended the meeting. On April 3rd, 2018, Adam Heathcote was one of two experts asked to spend an hour on MPR taking questions on Minnesota lakes, many of which revolved around the problems with HABs (<https://www.mprnews.org/story/2018/04/03/water-month-state-of-minnesotas-lakes>).

Status as of January 1, 2019:

We participated in an open forum for questions surrounding HABs in Minnesota at the 2018 Minnesota Water Resources Conference. Together with our partners at USGS (Richard Kiesling), and our collaborators at St. Anthony Falls Laboratory (Shahram Missaghi), we provided a brief background on the issues of HABs in Minnesota, highlighted the outreach efforts by the Science Museum and University of Minnesota Extension, and fielded questions from the audience. We hope to parlay this effort into a special session on HABs at the 2019 MWR Conference (application pending).

Final Report Summary:

In the process of completing this project we have created the Minnesota HABs Working Group, which is a collaboration between universities, agencies, and consulting firms working on HABs in Minnesota. With this group we hosted workshops in 2017 and 2018 that were open to the public and drew near capacity registrants in both years. We also participated in a Q&A panel on HABs at the Minnesota Water Resources Conference, which included Dr. Adam Heathcote (SCWRS), Dr. Sharahm Missaghi (University of Minnesota Extension), and Dr. Richard Kiesling (USGS). From these collaborations, we have developed an entire special session devoted to HABs which will be held at the 2019 Minnesota Water Resources Conference.

The research produced from this project has been featured prominently on traditional media including multiple interviews with MPR and local Minnesota TV affiliates. Additionally, we have produced a number of articles meant for the general public about our work on HABs which were featured on the Science Museum of Minnesota's website and have given public presentations intended for the general public. Major research results from this project were also presented at two separate meetings of the Association for the Sciences of Limnology and Oceanography in June of 2018 and February of 2019 using in-kind funding provided by the Science Museum of Minnesota. This is the largest meeting dedicated to aquatic science in the world and is held once a year.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 384,100	1 sediment geochemist at 8% FTE for 2 years (\$21,600); 1 algal and diatom analyst at 50% FTE for 2.5 years (\$122,400); 1 algal toxin specialist and data analyst at 50% FTE for 3.5 years (\$128,400); 1 hydrologist/watershed modeler at 35% FTE for 2 years (\$70,100); 1 field technician at 75% FTE for 2 years (\$41,600)
Professional/Technical/Service Contracts:	\$ 68,800	USGS CE-QUAL modeling of in-lake process and field monitoring of discharge over 3 years (\$50,000); Fossil pigment analysis by specialized external lab (\$18,800)
Equipment/Tools/Supplies:	\$ 35,500	Dissolved oxygen and temperature recording probes (\$24,000); Field supplies including sediment traps, sample bottles, vials & reagents (\$11,500)
Capital Expenditures over \$5,000:	\$ 30,000	YSI Water-quality sonde (\$20,000); ELISA microplate reader (\$10,000);
Travel Expenses in MN:	\$ 14,100	Field work: sediment core collection and twice-monthly lake sampling
Other: Analytical Services	\$ 60,500	Lab analysis of water samples (N, P, DOC, DIC) and sediment cores: radiometric dating (Lead-210, Cesium-137); biogenic silica; loss-on-ignition, sediment phosphorus and metals
TOTAL ENRTF BUDGET:	\$593,000	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: A dedicated water-quality sonde with sensors for chlorophyll a and phycocyanin is required for the intensive (twice-monthly) lake monitoring as outlined under Activity 1. An ELISA microplate reader for analysis of cyanobacterial toxins as described under Activity 2.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 5.36

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
Science Museum of Minnesota	\$ 215,000	\$ 215,000 as of Jul 1, 2019	Unrecovered support services (lab & equipment maintenance, infrastructure, project administration), 43% of direct costs
State			
DNR & MPCA (in-kind)	\$ 105,000	\$ 105,000 as of Jul 1, 2019	Support in collecting water and phytoplankton samples from Sentinel Lakes
TOTAL OTHER FUNDS:	\$ 320,000	\$ 320,000	

VII. PROJECT STRATEGY:

A. Project Partners: DNR/MPCA (Sentinel lakes monitoring)
U.S. Geological Survey (CE-QUAL-W2 modeling)

B. Project Impact and Long-term Strategy:

This project will provide a statewide assessment of whether the threat of HABs is increasing in Minnesota and, if so, it will help identify the factors most likely contributing to that change. There has been only limited, short-term monitoring of HABs in Minnesota lakes, and evidence for changes in bloom frequency and severity is largely anecdotal. The reconstruction of past algal abundance from sediment cores, as outlined in this study, will provide a solid historical context for the present-day condition of Minnesota lakes. While excess nutrients, particularly phosphorus have long been known to stimulate algal growth, there are other factors that may play an equally important role; these include changes in the thermal structure of lakes (duration and stability of stratification), surface water temperatures and length of the growing season, atmospheric deposition of reactive nitrogen, invasive species such as the common carp, and internal feedback from the growth and senescence of the algal blooms themselves.

This study will improve our ability to predict when HABs occur, when they produce toxins, and how long those toxins persist. Again, monitoring of algal blooms and their toxins has been largely discontinuous and non-systematic so that we have only limited information about the seasonality, abundance, and composition of HABs or their associated toxins in our lakes. Because algal blooms and toxin production are relatively short-term events, high-frequency, systematic monitoring and modeling of both algae and associated physico-chemical conditions is needed to understand the risk posed by HABs and the factors contributing to their development.

This project integrates an extensive package of watershed monitoring data, sediment analytical results, watershed modeling, and in-lake modeling in a way that will engender mechanistic understanding of how and why harmful algal blooms occur. A key benefit of the project is the transferability of the results. Models are inherently flexible in their application, and the lessons learned in calibrating the models to our study site can be passed along in fitting the models to other sites. Furthermore, the calibrated models can be run with possible future land use or climate data, thus giving tremendous predictive power to infer potential impacts on our lakes.

Finally, as a long-term strategy, this study will establish infrastructure and capacity to identify harmful algae and toxins within the state of Minnesota; our state agencies currently outsource much of this work. The research staff who will carry out this project already possess expertise in algal identification and ecology. The work carried out here will help hone those skills, particularly with cyanobacteria and other soft algae, which are taxonomically difficult and environmentally complex. We anticipate that this study will raise additional questions about HABs and that solutions to the problem will involve long-term research investment beyond that outlined here.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
MPCA (Lake of the Woods nutrient mass-balance study)	January 2012 -- July 2016	\$ 300,000
ENRTF (M.L. 2014, Chp. 226, Sec. 2, Subd. 03g; "Watershed-Scale Monitoring of Long-Term Best-Management Practice Effectiveness") to establish Center for Harmful Algae Research in Minnesota (CHARM lab)	July 2014 -- June 2017	\$ 900,000
ENRTF (M.L. 2009, Chap 143, Sect 2, Subd 05c "Cooperative Habitat Research in Deep Lakes") MN DNR subcontract to SMM	July 2010 -- June 2013	\$ 90,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached figures

X. RESEARCH ADDENDUM: See attached Research Addendum

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than the end of the months of July 2016, January 2017, July 2017, January 2018, July 2018, and January 2019. A final report and associated products will be submitted between June 30 and August 16, 2019.



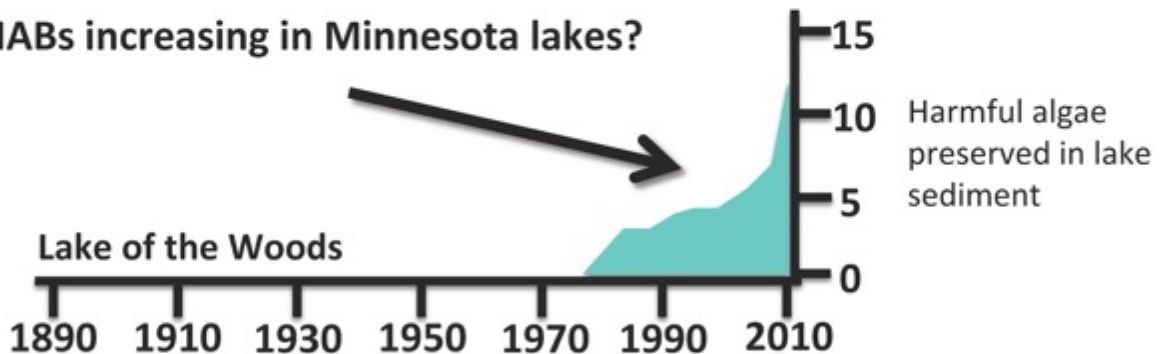
What's going on with Harmful Algal Blooms (HABs) in Minnesota lakes?

- What algae are present, when do they bloom, and are they harmful?



HABs to the public:
a soupy green mess

- HABs increasing in Minnesota lakes?



- Excess phosphorus causes HABs, but which is the bigger problem?

? Watershed inputs

? In-lake recycling

The Ghost of Phosphorus Past



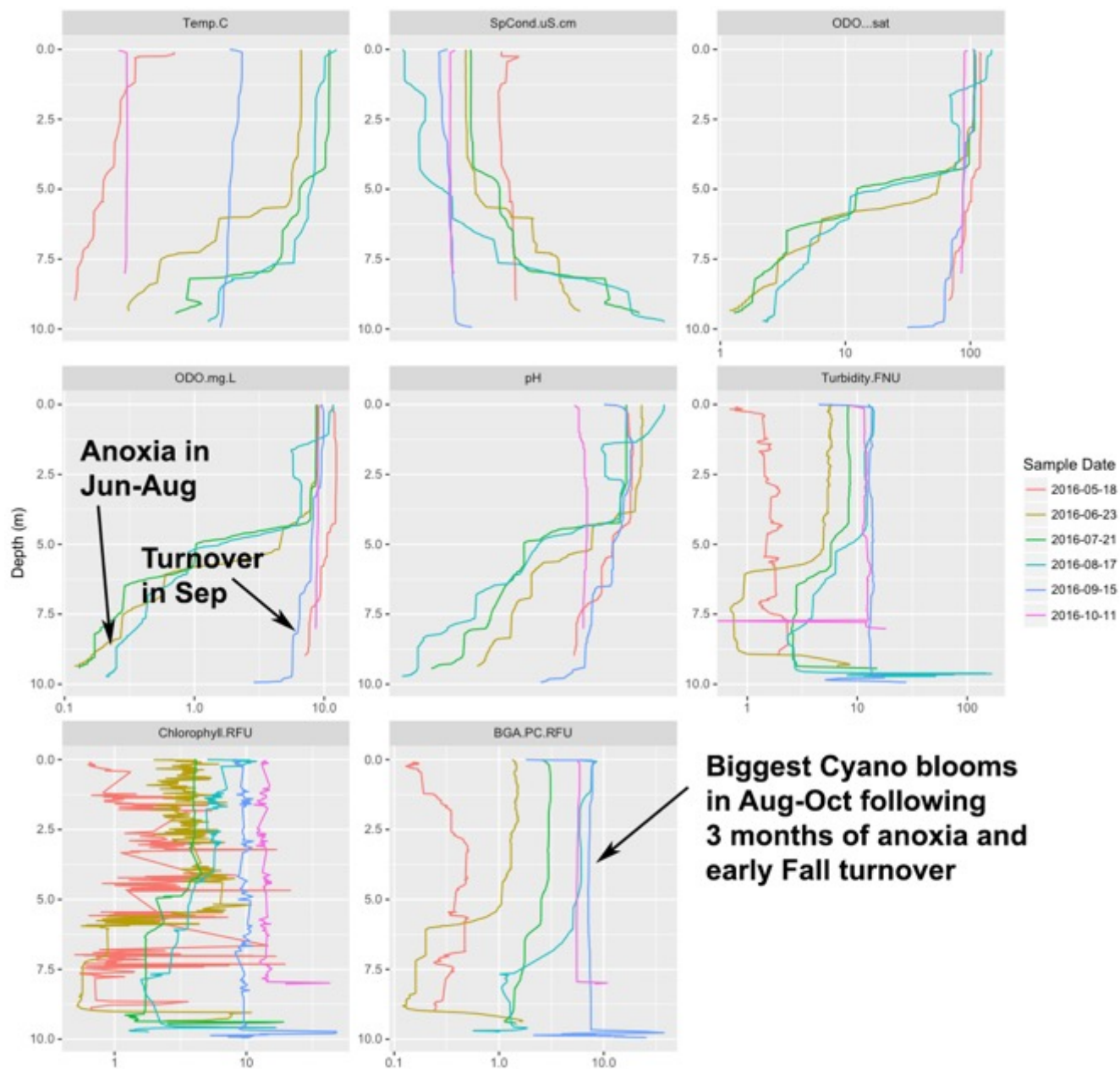


Figure 1. Example of YSI profiles collected from Madison Lake over the 2016 field season. Oxygen concentration profiles (ODO) show rapid anoxia occurring after stratification begins in June and persists until the lake turned over in September. Phycocyanin profiles (BGA.PC) show peak Cyanobacteria abundance following the turnover event, likely due to the release of sediment nutrients.

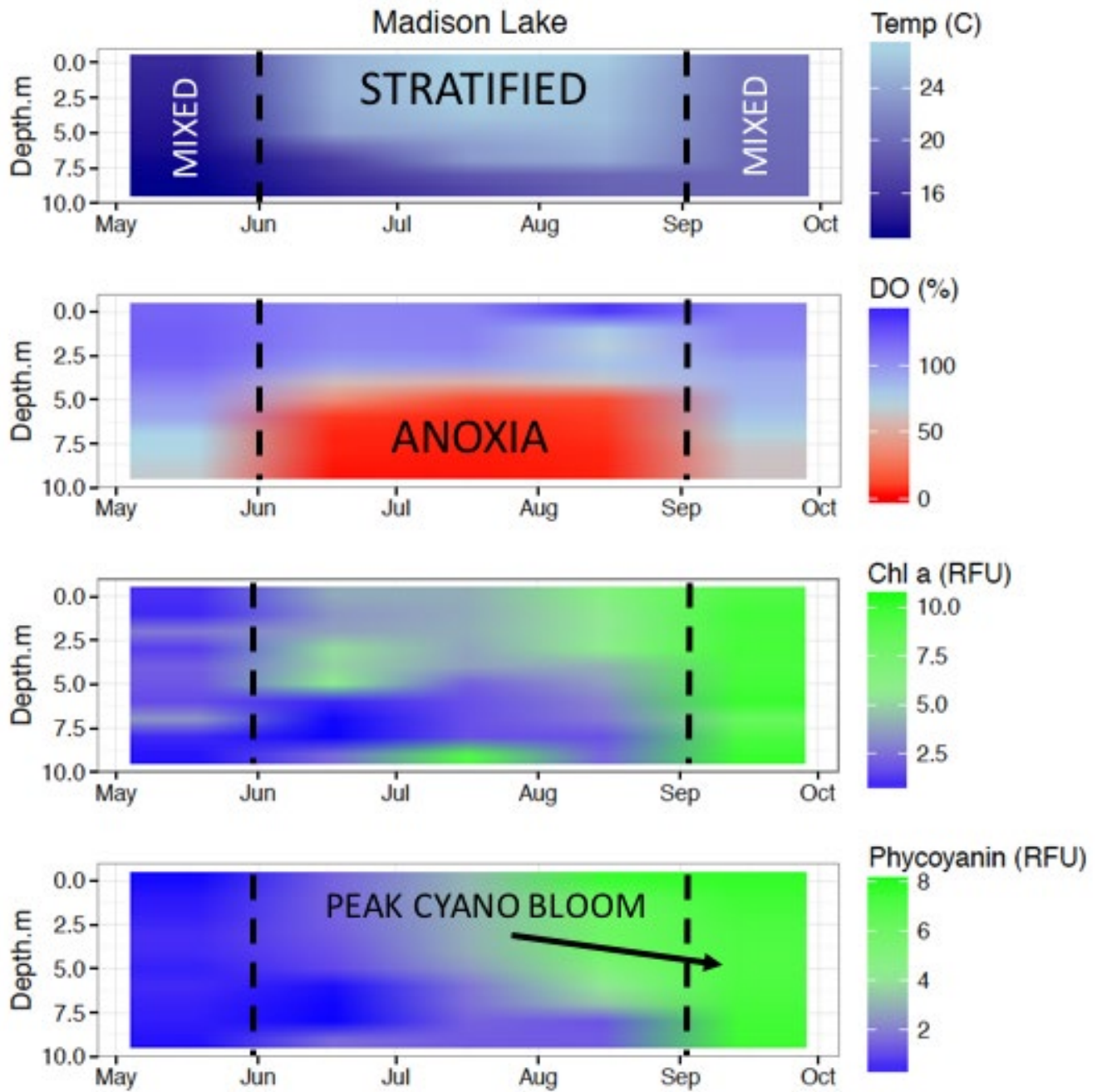


Figure 2. Interpolation of YSI profiles over the entire field season show the relationship between temperature stratification, oxygen and Cyanobacteria blooms. The largest blooms of the year in Madison Lake occurred followed a period of anoxic hypolimnetic conditions that allowed sediment P to accumulate in the bottom waters. This P was then released to the entire water column following a rapid turnover event in September.

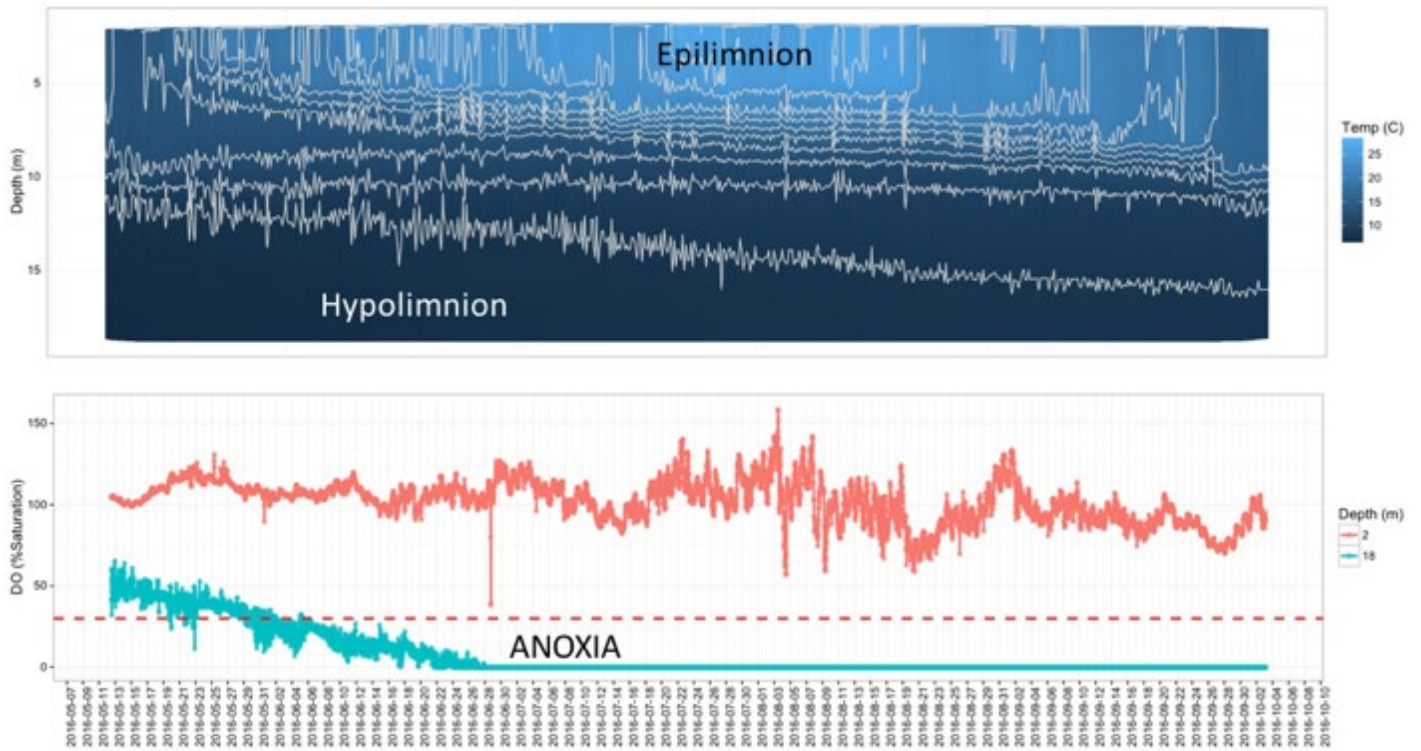


Figure 3. Temperature and dissolved oxygen data from the submerged buoy array at South Center Lake. These data show a typical dimictic lake that remained stratified throughout the entire ice-free season. Complete oxygen depletion (anoxia) occurred by the end of June and was maintained through November.

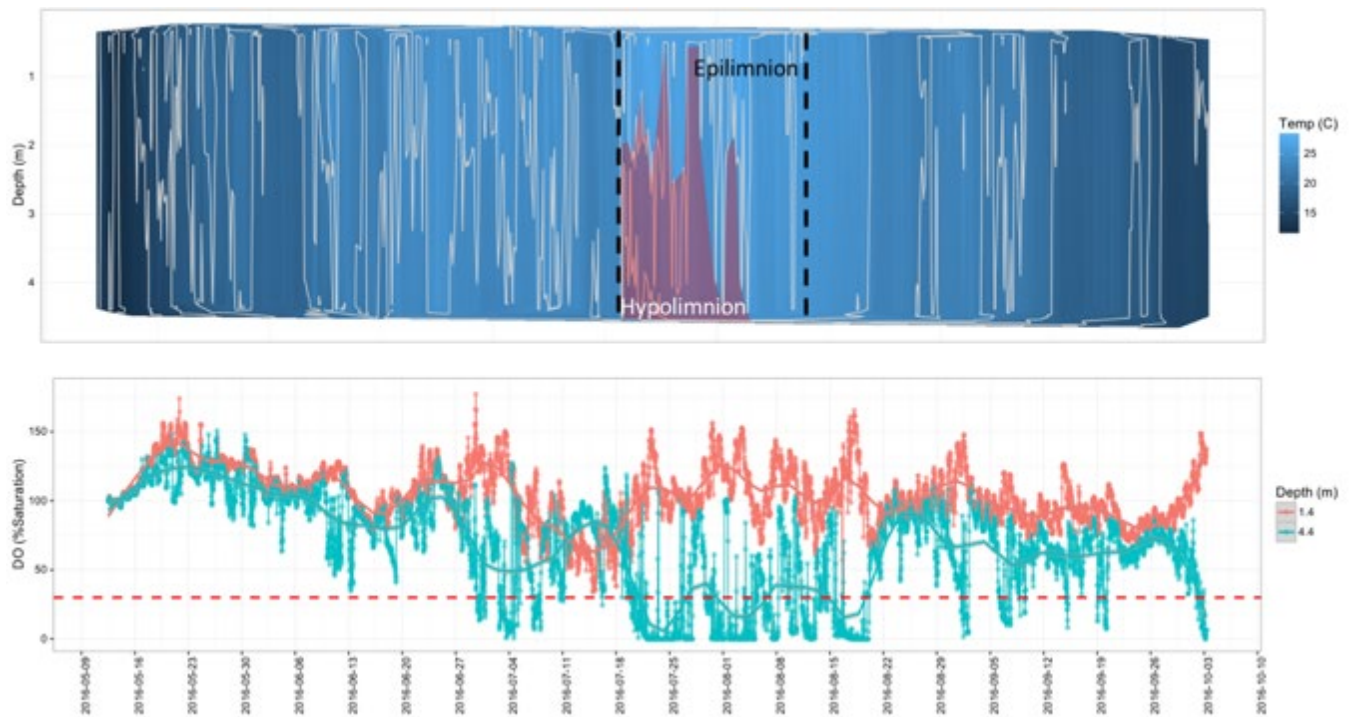


Figure 4. Temperature and dissolved oxygen data from Pearl Lake that show a surprisingly persistent stratification event occurring from mid-July through mid-August (red-shaded region in top panel). This event led to anoxic conditions and the likely release of sediment P into the water column following complete mixing at the end of August.

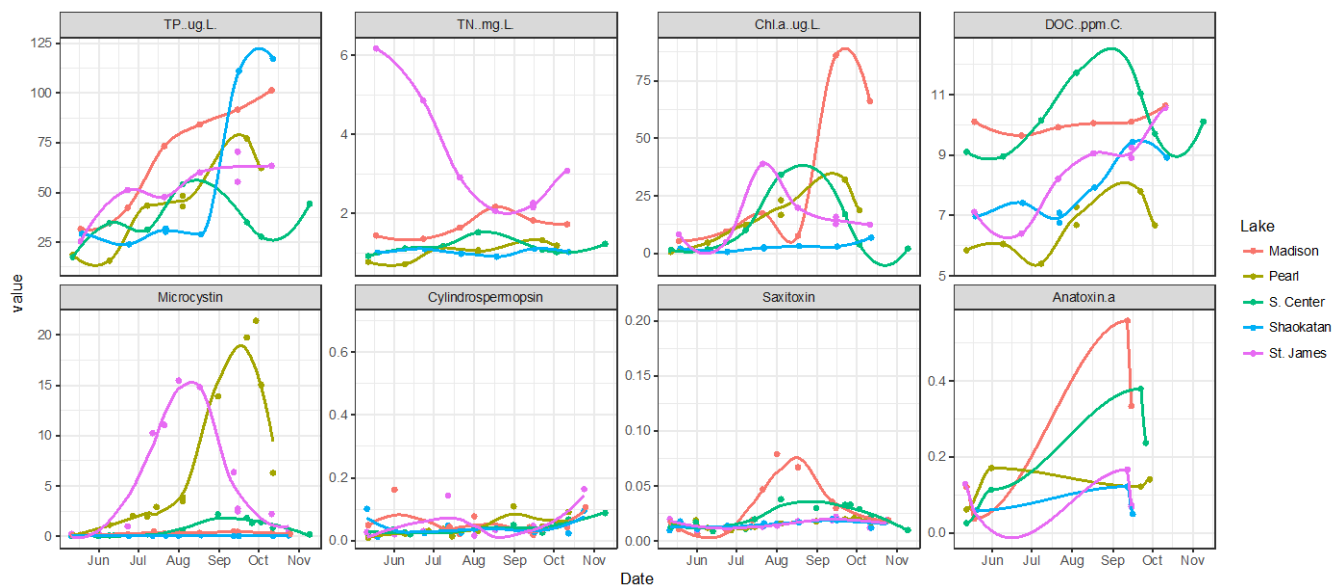


Figure 5. Water chemistry and cyanotoxin concentrations measured in the five intensively studied Minnesota lakes in 2016. Line colors correspond to each of the lakes (see legend) and dashed red line corresponds to the minimum safe drinking water concentration.

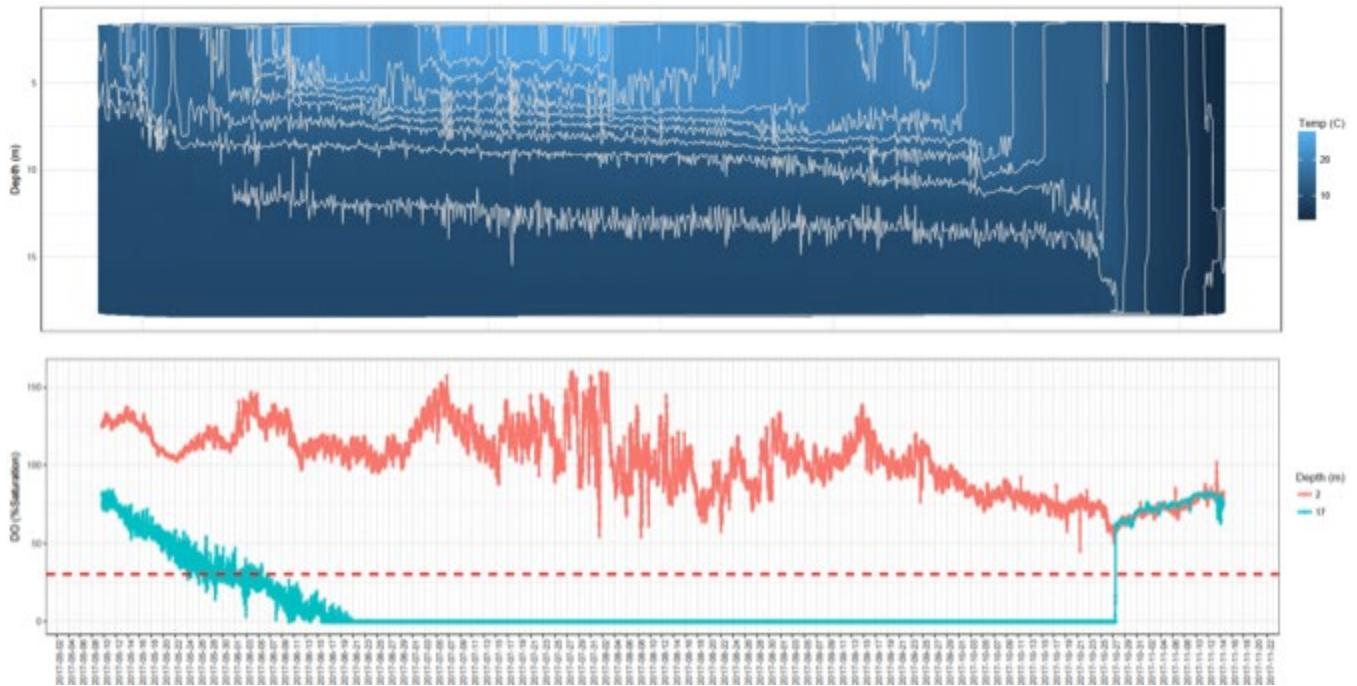


Figure 6. Isopleths of temperature over the 2017 field season in South Center Lake (top) and dissolved oxygen concentrations at the surface (orange) and bottom (teal) of the lake over the same time period. These data include measurements of temperature and oxygen collected every 30 minutes from the beginning of May until mid-November. We hope these data will illustrate the importance of stable stratification on reducing the impact of internal loading from sediment-bound nutrients. South Center Lake will serve as a strong contrast to the other polymictic lakes selected for this study which may be mixing sediment nutrients constantly, or periodically, throughout the open-water season.

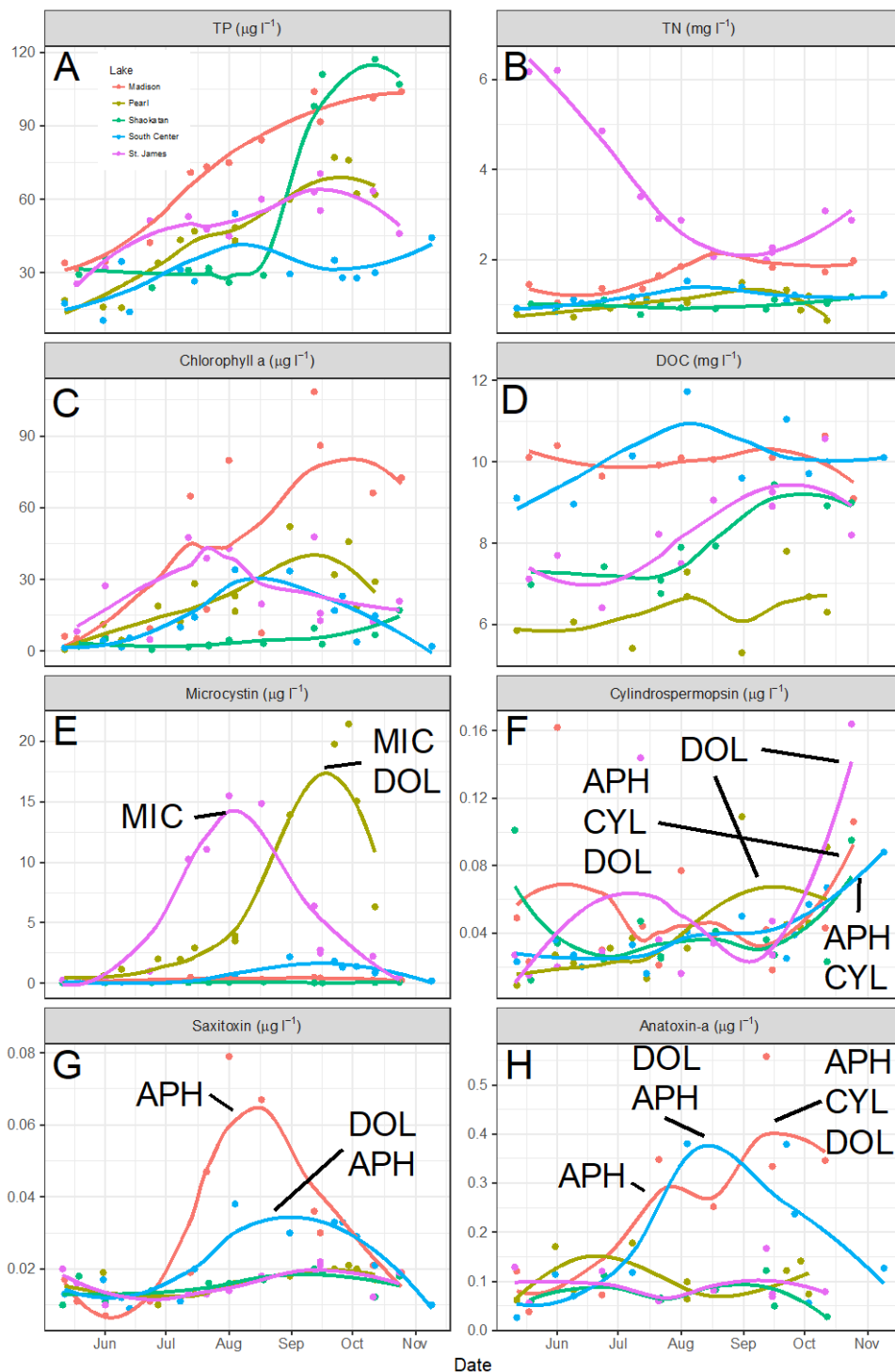


Figure 7. Water quality (A-D) and cyanotoxin (E-H) concentrations from the five lakes in this study. Colored lines correspond to different lakes (see legend). Three letter abbreviations correspond to the dominant Cyanobacteria genera in the sample (MIC: Microcystis, DOL: *Dolichospermum*, APH: *Aphanizomenon*, CYL: *Cylindrospermopsis*)

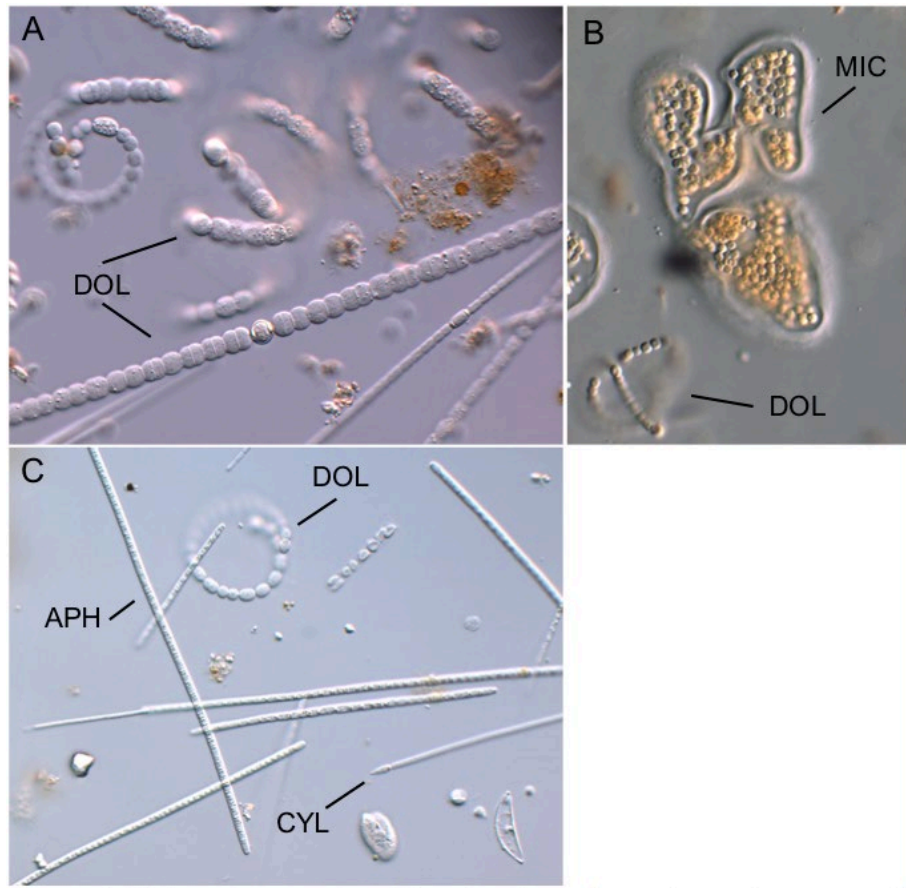


Figure 8. Light micrographs of the dominant Cyanobacteria community in selected samples. A) *Dolichospermum* and *Aphanizomenon*, South Center (8/4/2016), B) *Microcystis*, Pearl (9/29/16), C) *Aphanizomenon*, *Cylindrospermopsis*, *Dolichospermum*, Madison (9/21/16). MIC: *Microcystis*, DOL: *Dolichospermum*, APH: *Aphanizomenon*, CYL: *Cylindrospermopsis*

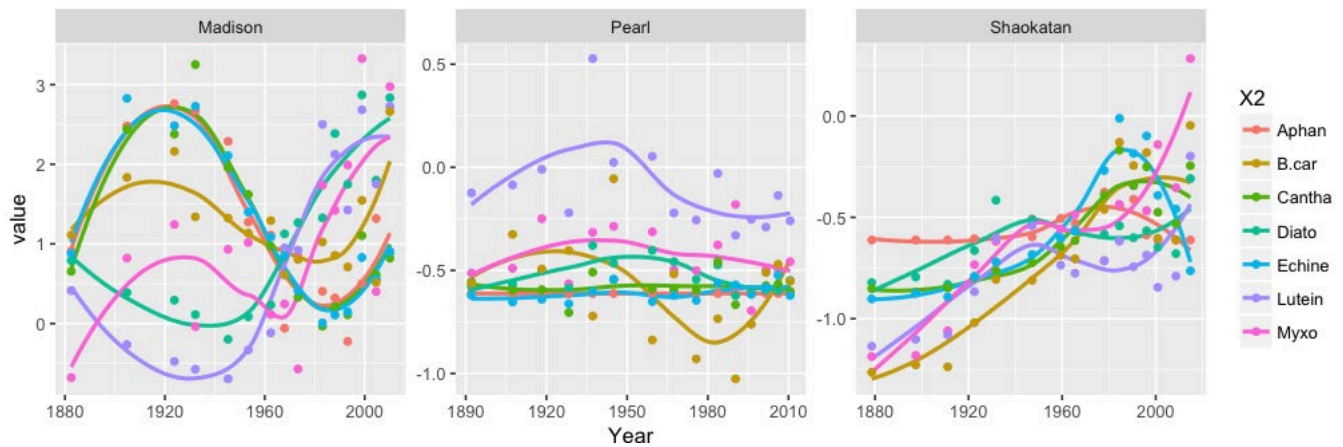


Figure 9. Algal pigment concentrations through time for three of the lakes in this study. Pigment values have been scaled to unit variance so they can be shown on the same graph. Madison and Shaokatan both show major increases in the pigment associated with colony-forming toxic Cyanobacteria (Myxo). Madison shows peak concentrations of many of the pigments associated with Cyanos (Aphan, Cantha, Echine) may have actually peaked in the 1920s and declined until recently (with the exception of Myxo). Total algae production (B.car) also follows this pattern. This may indicate that whereas algae blooms were present, and perhaps more intense, in the past; these blooms are currently made up of more toxic forms. Shaokatan shows increases in total algal production and most of the Cyanobacteria pigments that is consistent with an increasingly eutrophic system. Pearl shows less of a clear pattern in pigment concentrations, with most pigments remaining relatively flat over the entire time period or even decreasing.

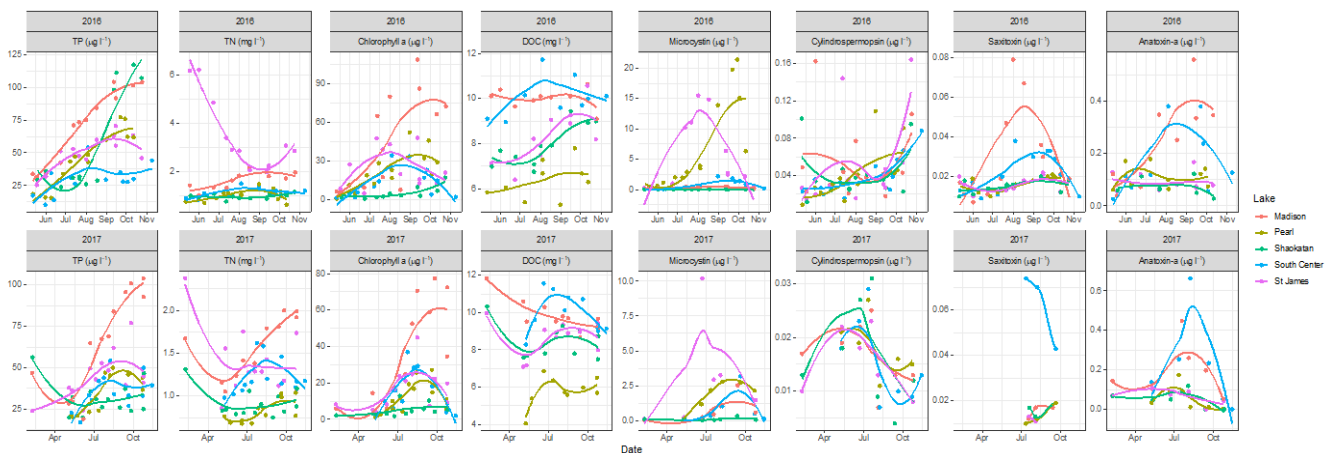


Figure 10. Major water chemistry and cyanotoxin concentrations for all five lakes in this study in 2016 and 2017. Algae density (as Chlorophyll a), microcystin, and anatoxin-a concentrations seemed to follow similar patterns across both years in the lake of this study, however, cylindrospermopsin and saxitoxin results were very different.

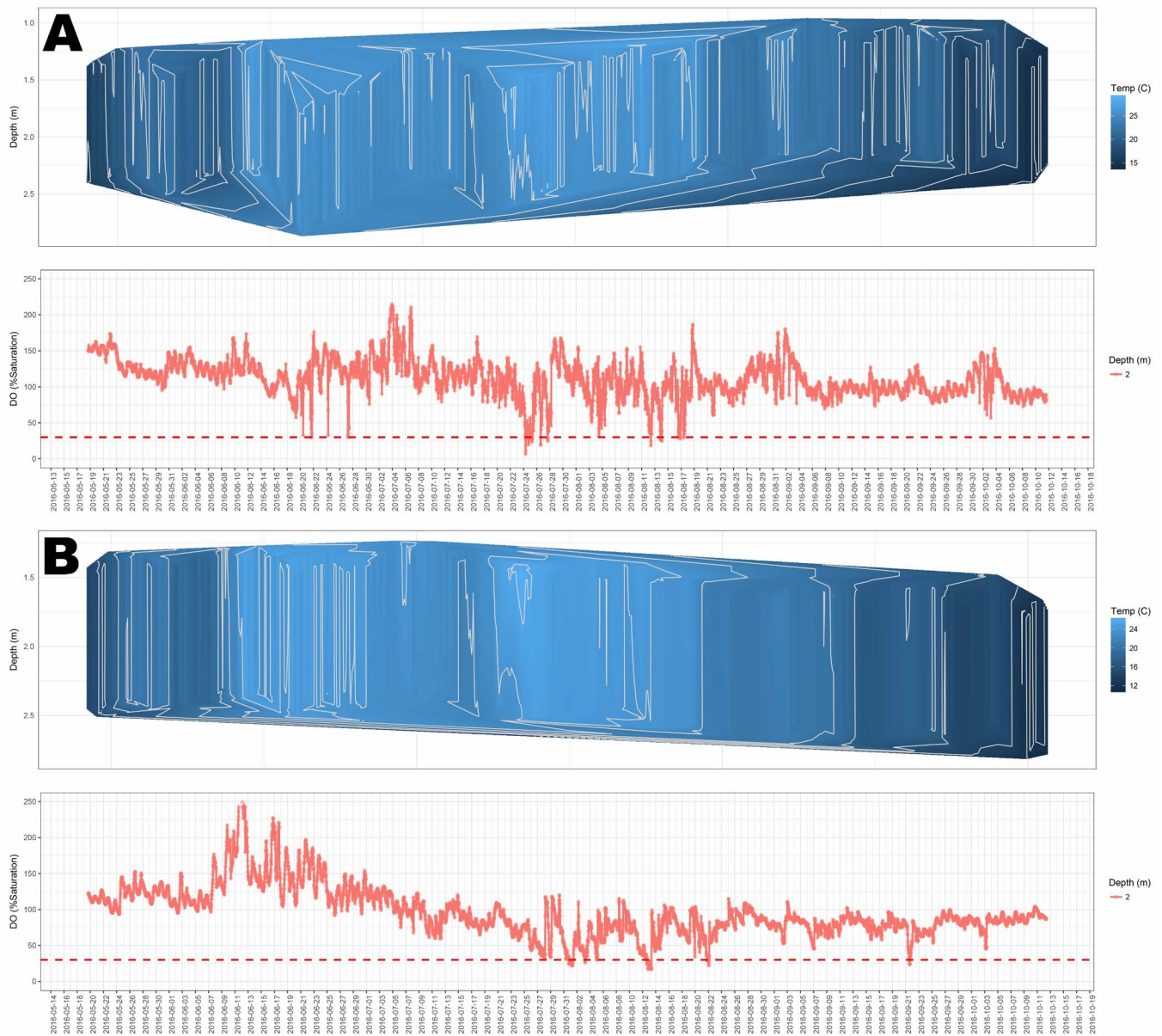


Figure 11. Temperature isopleths and bottom dissolved oxygen (DO) concentrations from buoy data collected in 2016 for Lake St. James (A) and Lake Shaokatan (B). Both lakes were mixed for almost the entire open-water season, with only very brief periods (~1-2 days) of oxygen depletion. Red dashed line represented hypoxic concentrations.

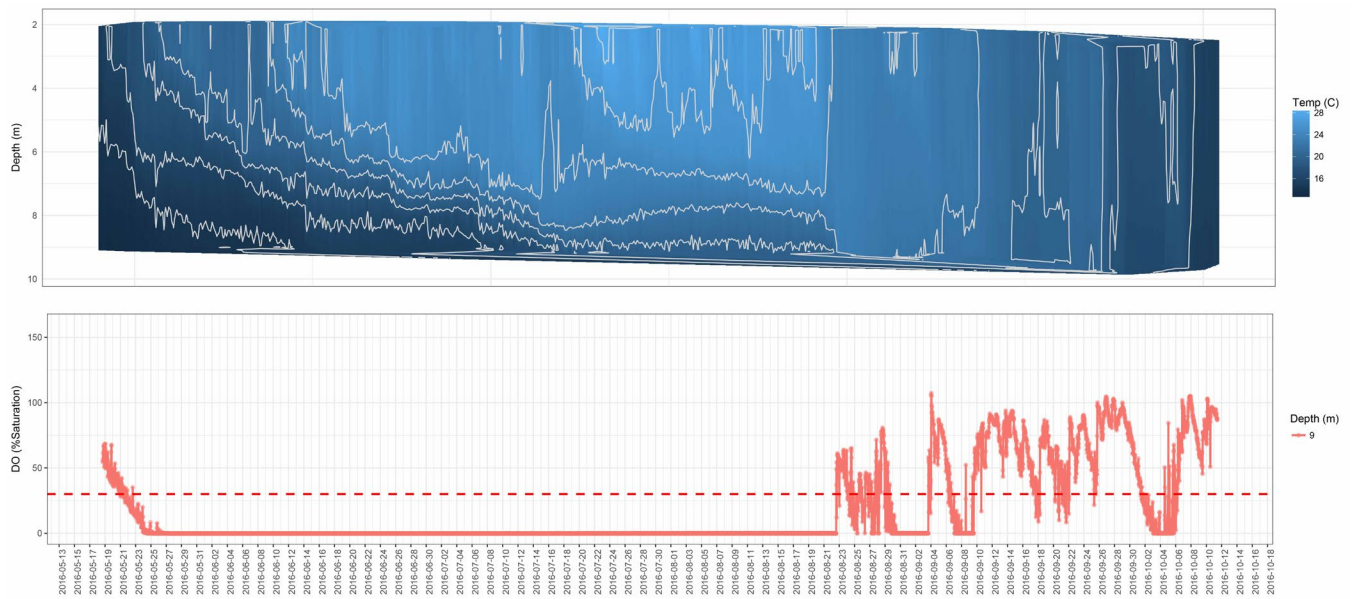


Figure 12. Temperature isopleths and bottom dissolved oxygen concentrations for Madison Lake in 2016. Data show early stratification in May followed by prolonged anoxia in the hypolimnion until a mixing event in late August. The lake then alternated between stratified and mixed for the rest of the ice-free season. Red dashed line represents the hypoxic zone.

St. James

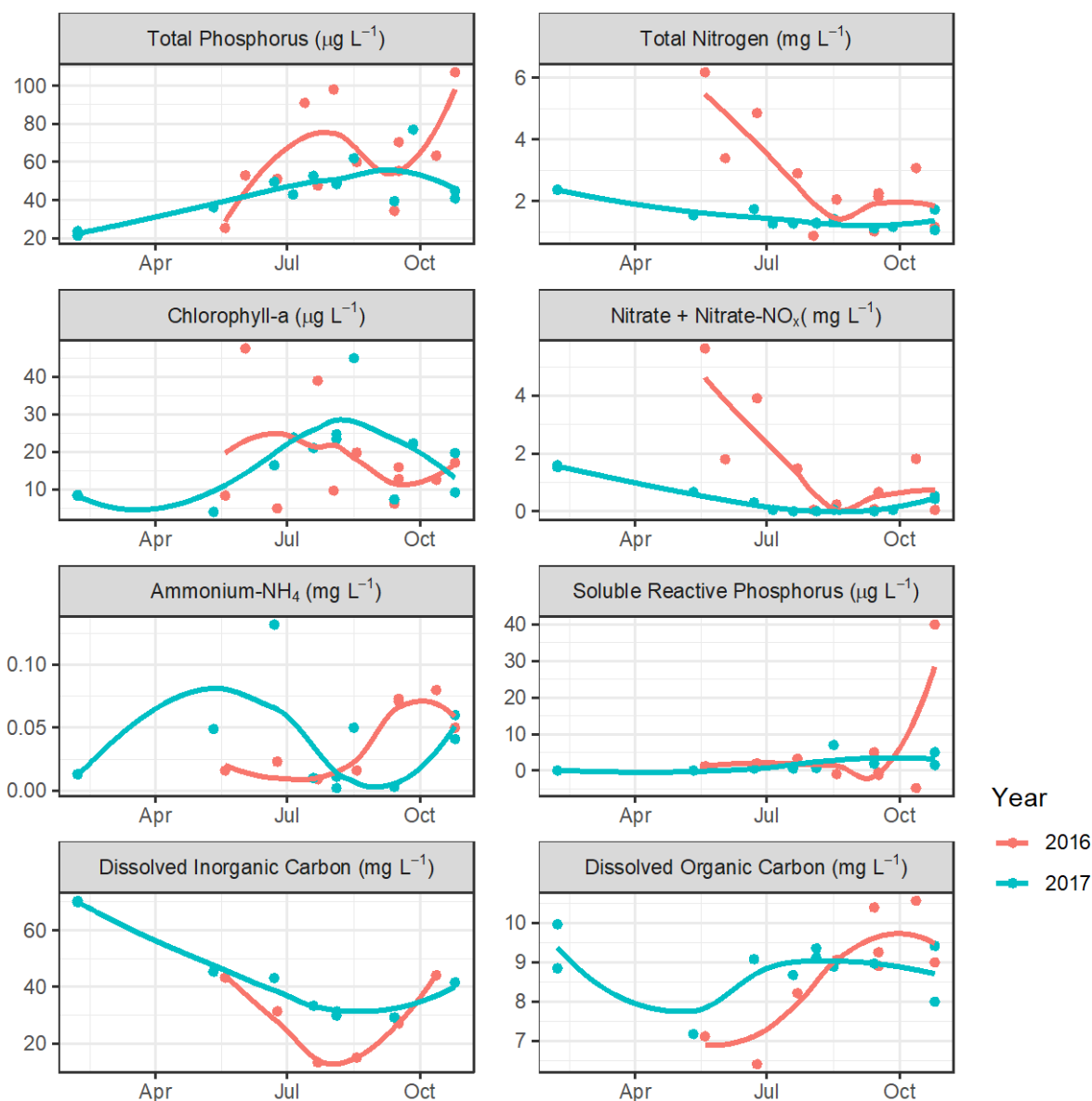


Figure 13. Water chemistry summary for all analytes collected on St. James Lake in 2016 (orange) and 2017 (turquoise). Lines represent locally weighted polynomial regression (LOWESS) smoothers of the data to show general trends.

South Center

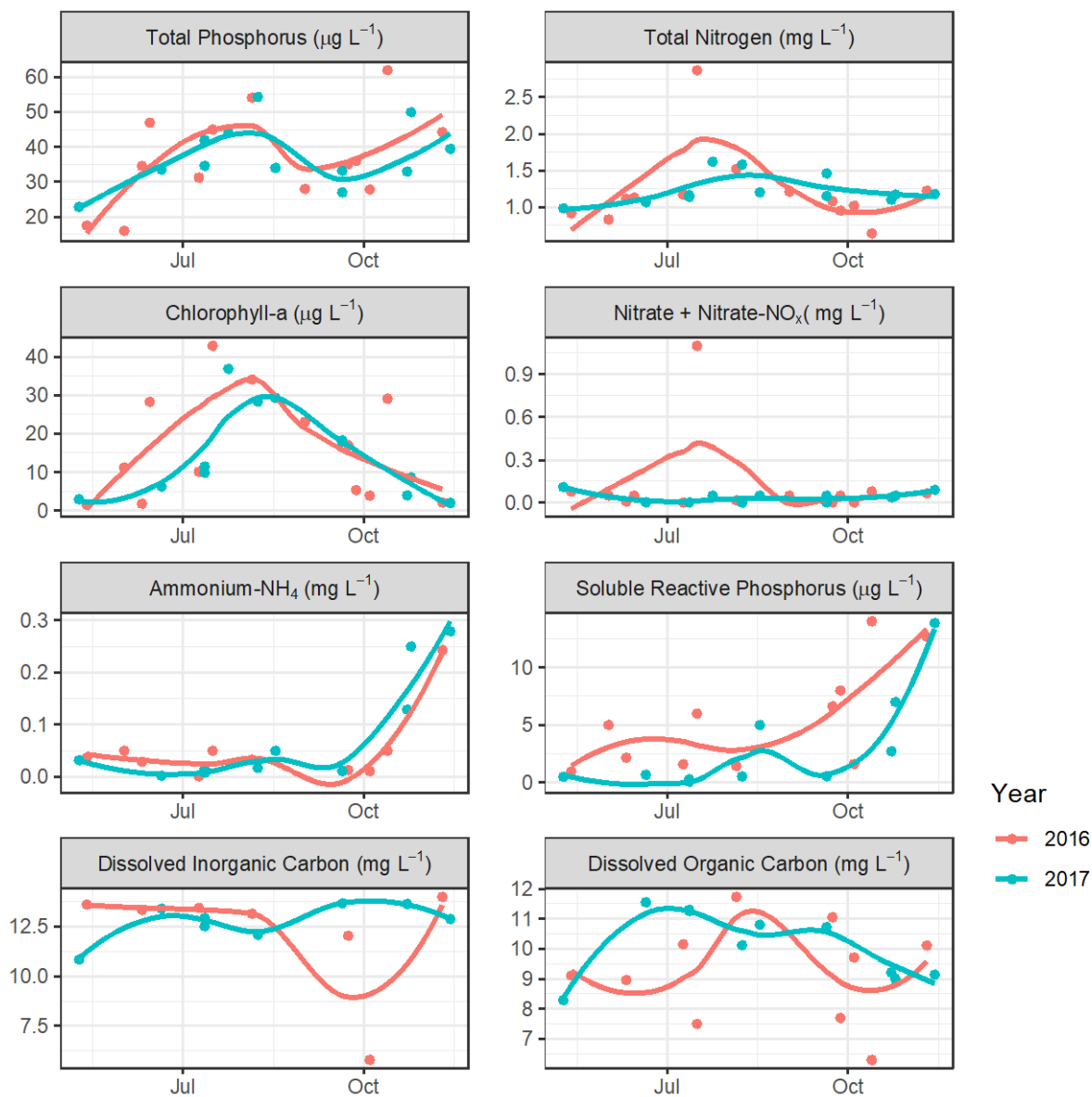


Figure 14. Water chemistry summary for all analytes collected on South Center Lake in 2016 (orange) and 2017 (turquoise). Lines represent locally weighted polynomial regression (LOWESS) smoothers of the data to show general trends.

Shaokatan

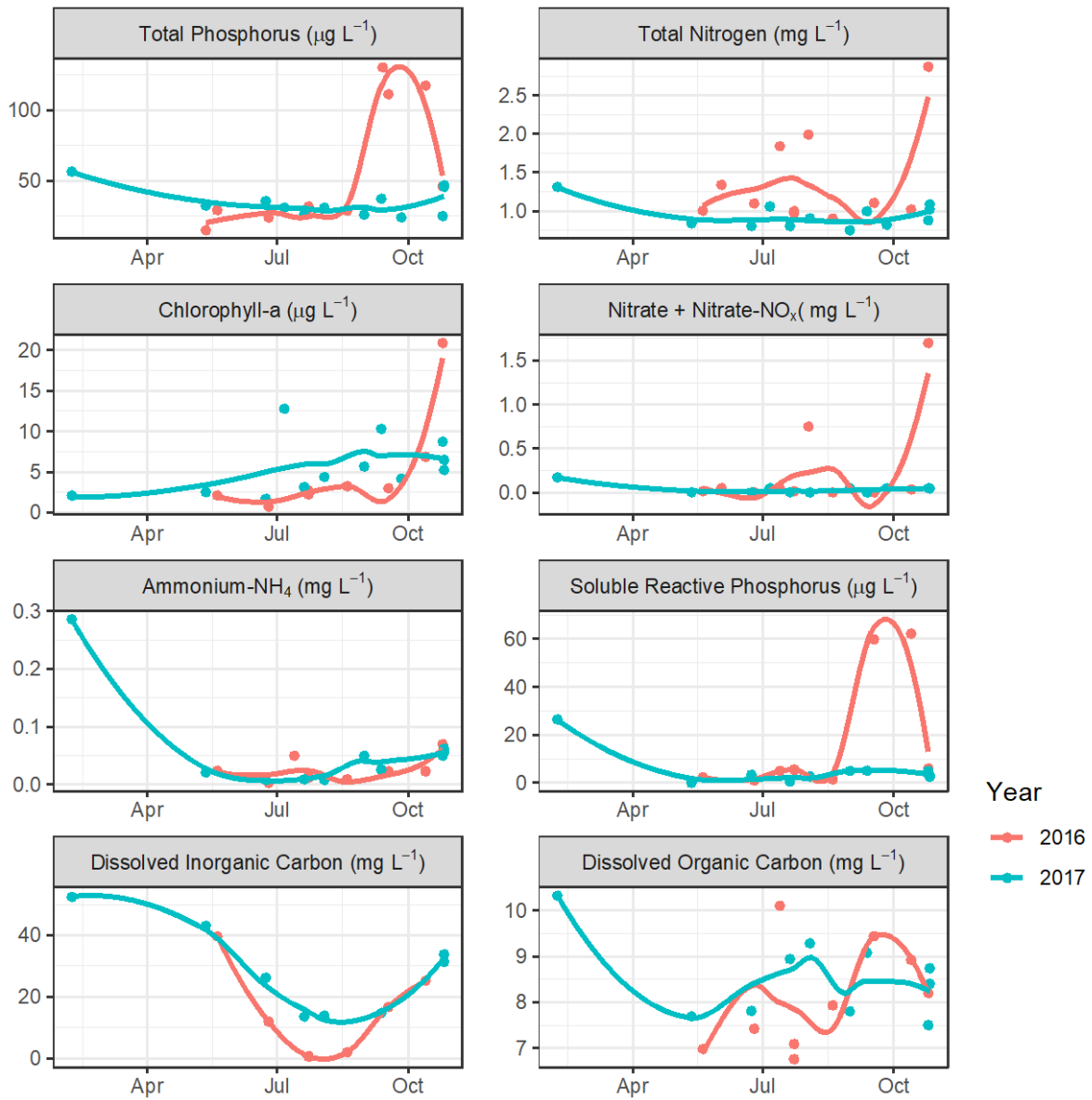


Figure 15. Water chemistry summary for all analytes collected on Lake Shaokatan in 2016 (orange) and 2017 (turquoise). Lines represent locally weighted polynomial regression (LOWESS) smoothers of the data to show general trends.

Pearl Lake

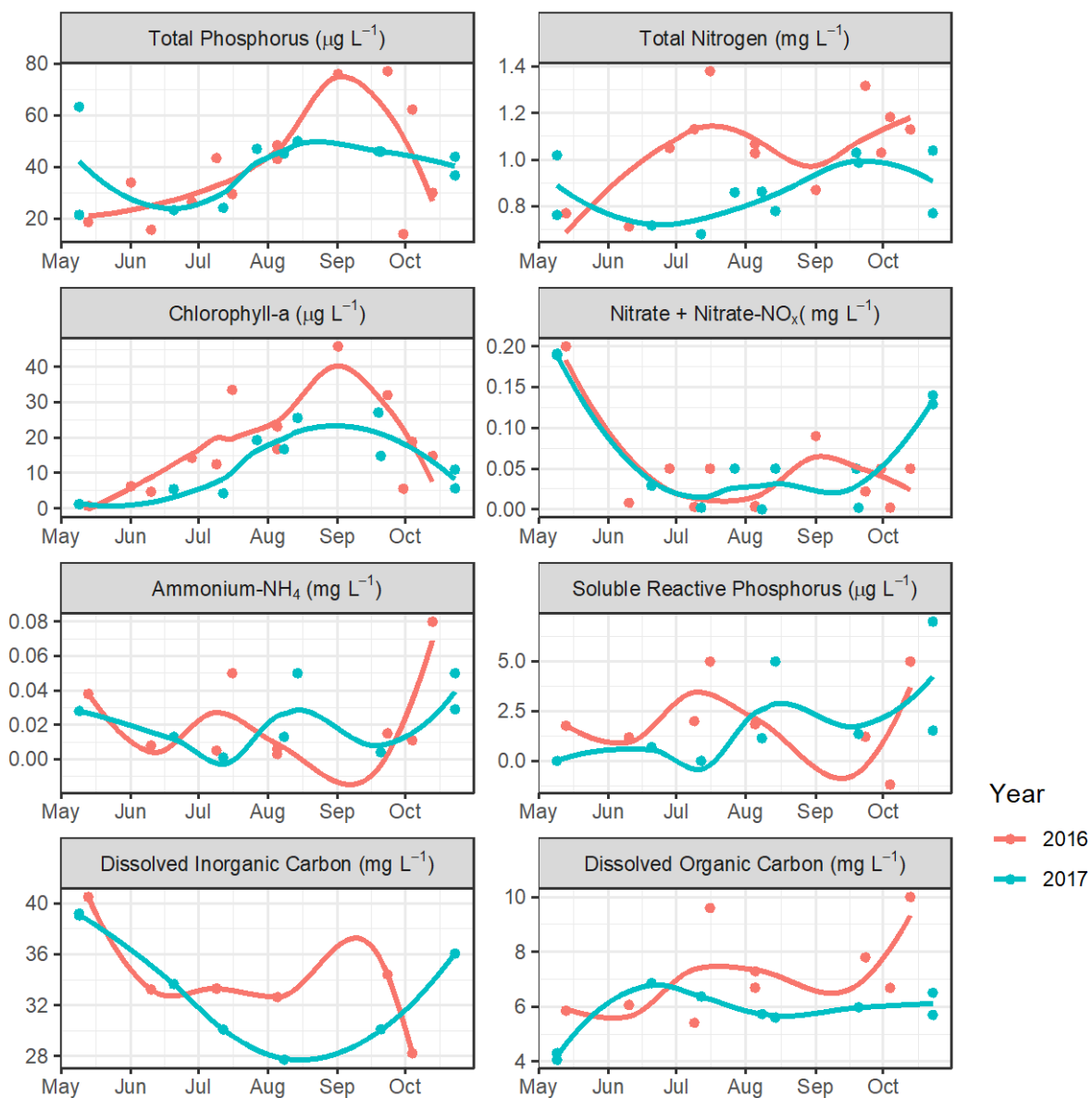


Figure 16. Water chemistry summary for all analytes collected on Pearl Lake in 2016 (orange) and 2017 (turquoise). Lines represent locally weighted polynomial regression (LOWESS) smoothers of the data to show general trends.

Madison Lake

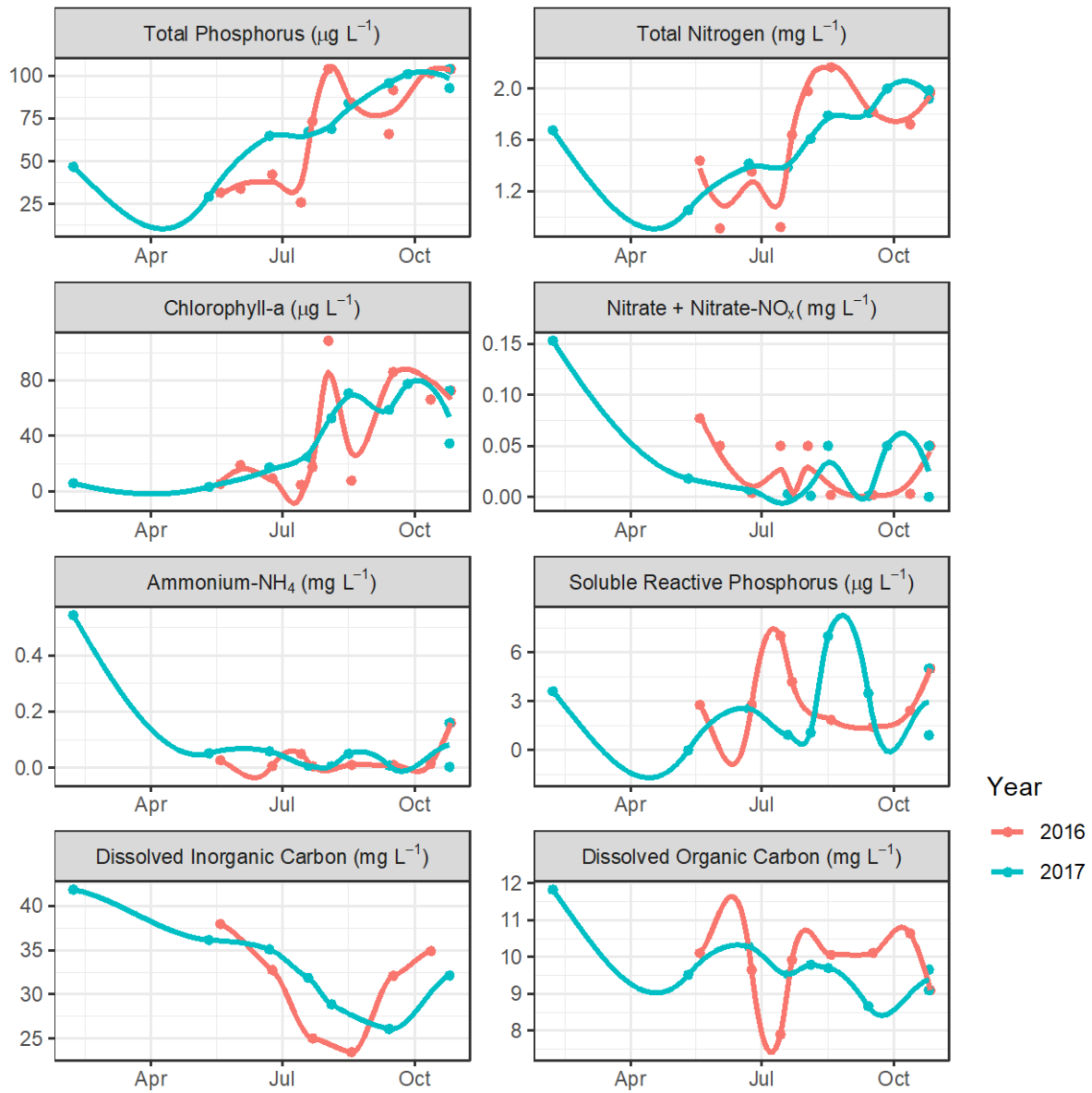


Figure 17. Water chemistry summary for all analytes collected on Madison Lake in 2016 (orange) and 2017 (turquoise). Lines represent locally weighted polynomial regression (LOWESS) smoothers of the data to show general trends.

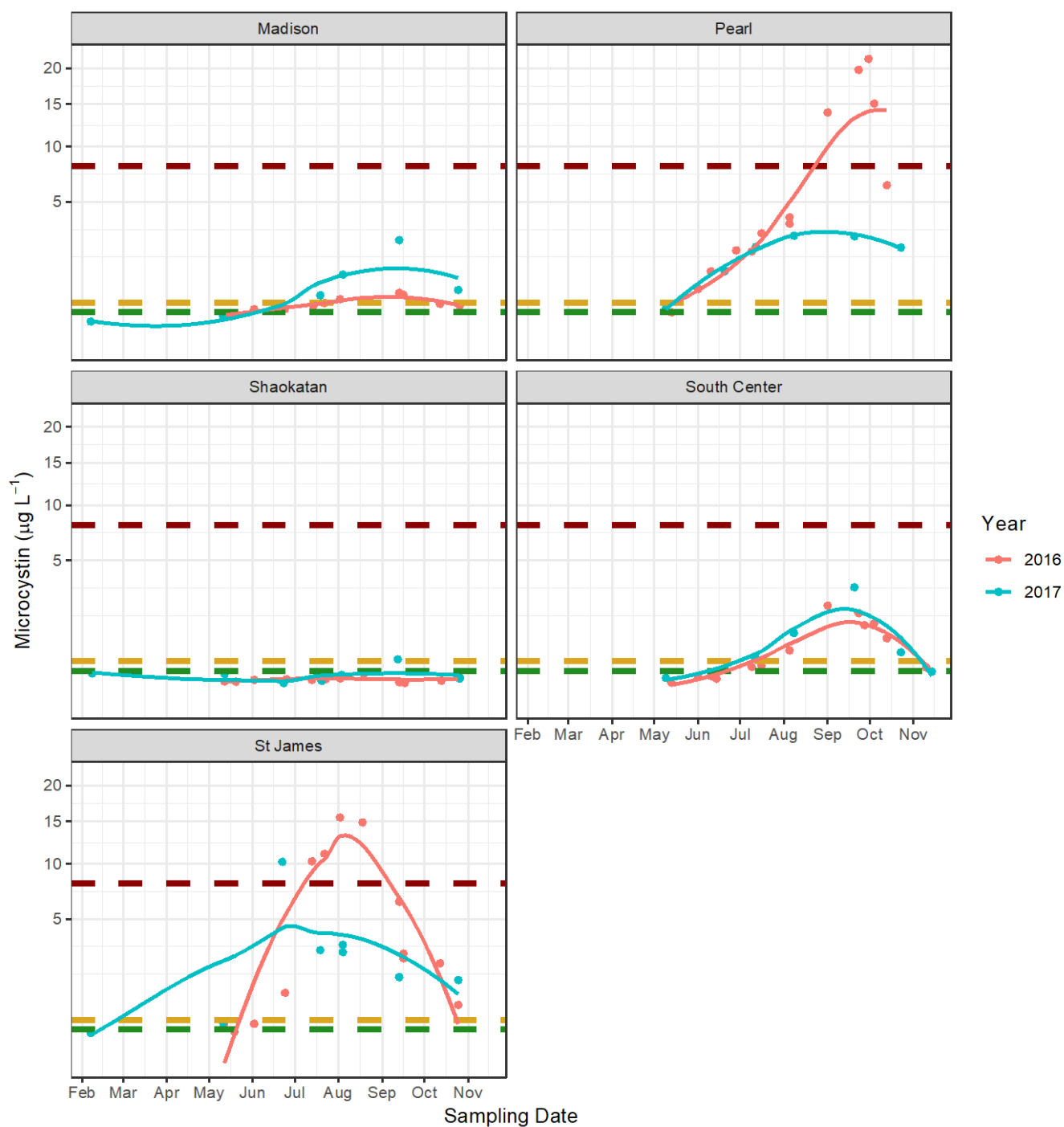


Figure 18. Microcystin concentrations in all five lakes in this study in 2016 (orange) and 2017 (turquoise). Green dashed line represents instrument detection limits (0.15 $\mu\text{g/L}$), yellow dashed line represents minimum safe drinking water standard (0.3 $\mu\text{g/L}$; EPA, MDH), and red dashed line represents recreational contact standard (8 $\mu\text{g/L}$; EPA).

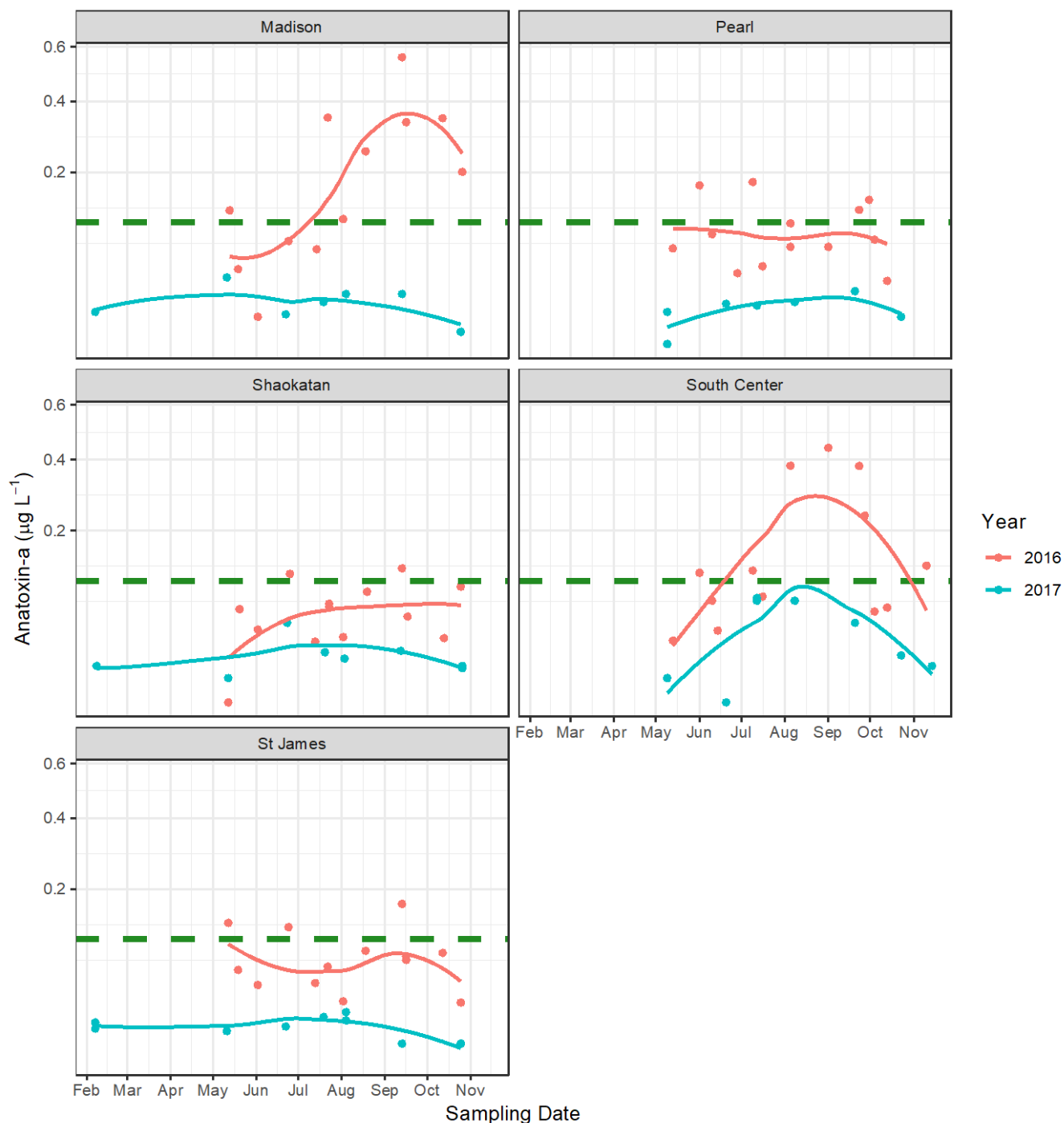


Figure 19. Anatoxin-a concentrations in all five lakes in this study in 2016 (orange) and 2017 (turquoise). Green dashed line represents instrument detection limit, which is the same as the MDH minimum drinking standard (0.1 µg/L).

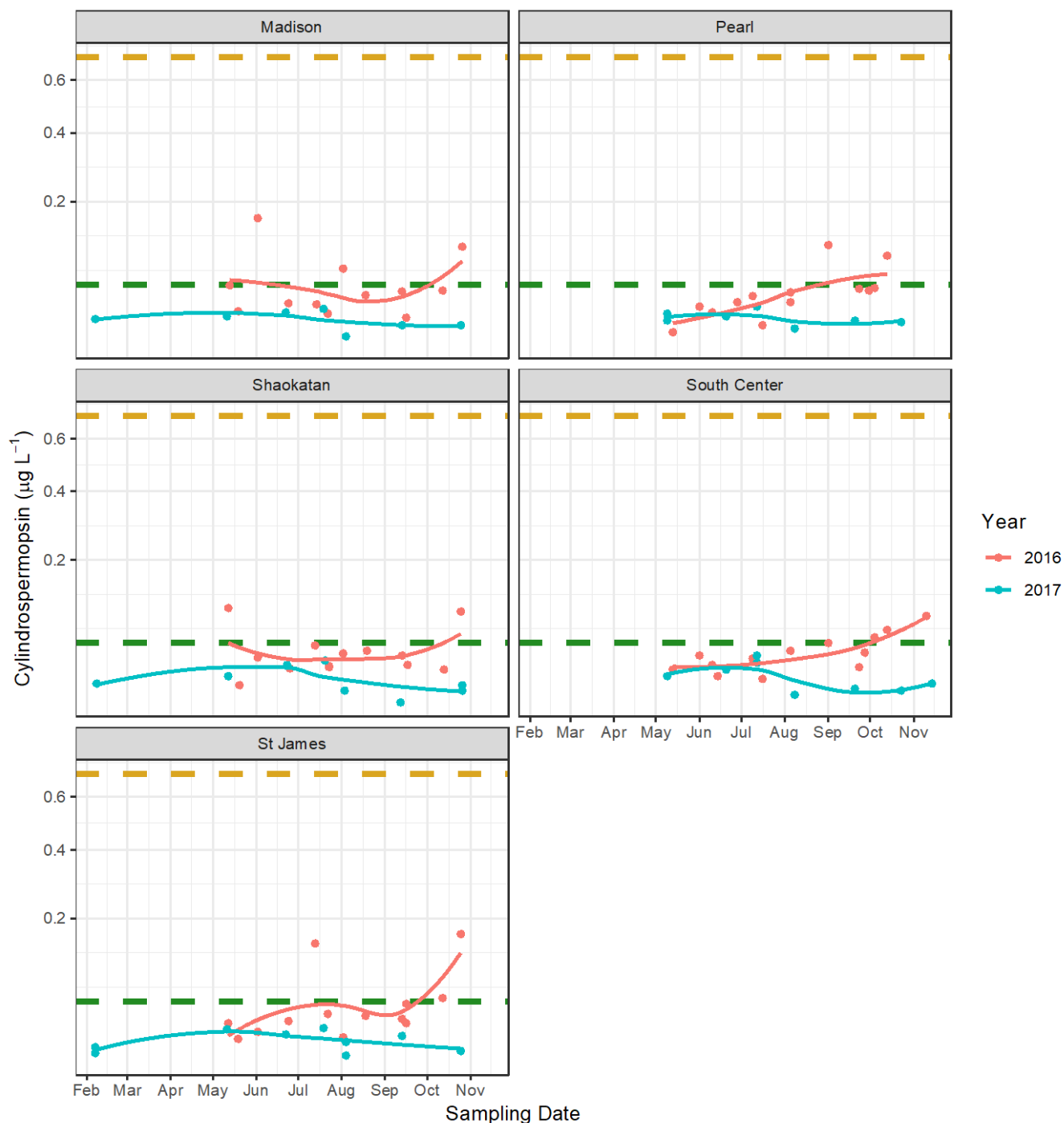


Figure 20. Cylindrospermopsin concentrations in all five lakes in this study in 2016 (orange) and 2017 (turquoise). Green dashed line represents instrument detection limits (0.05 µg/L) and yellow dashed line represents minimum safe drinking water standard (0.7 µg/L; EPA).

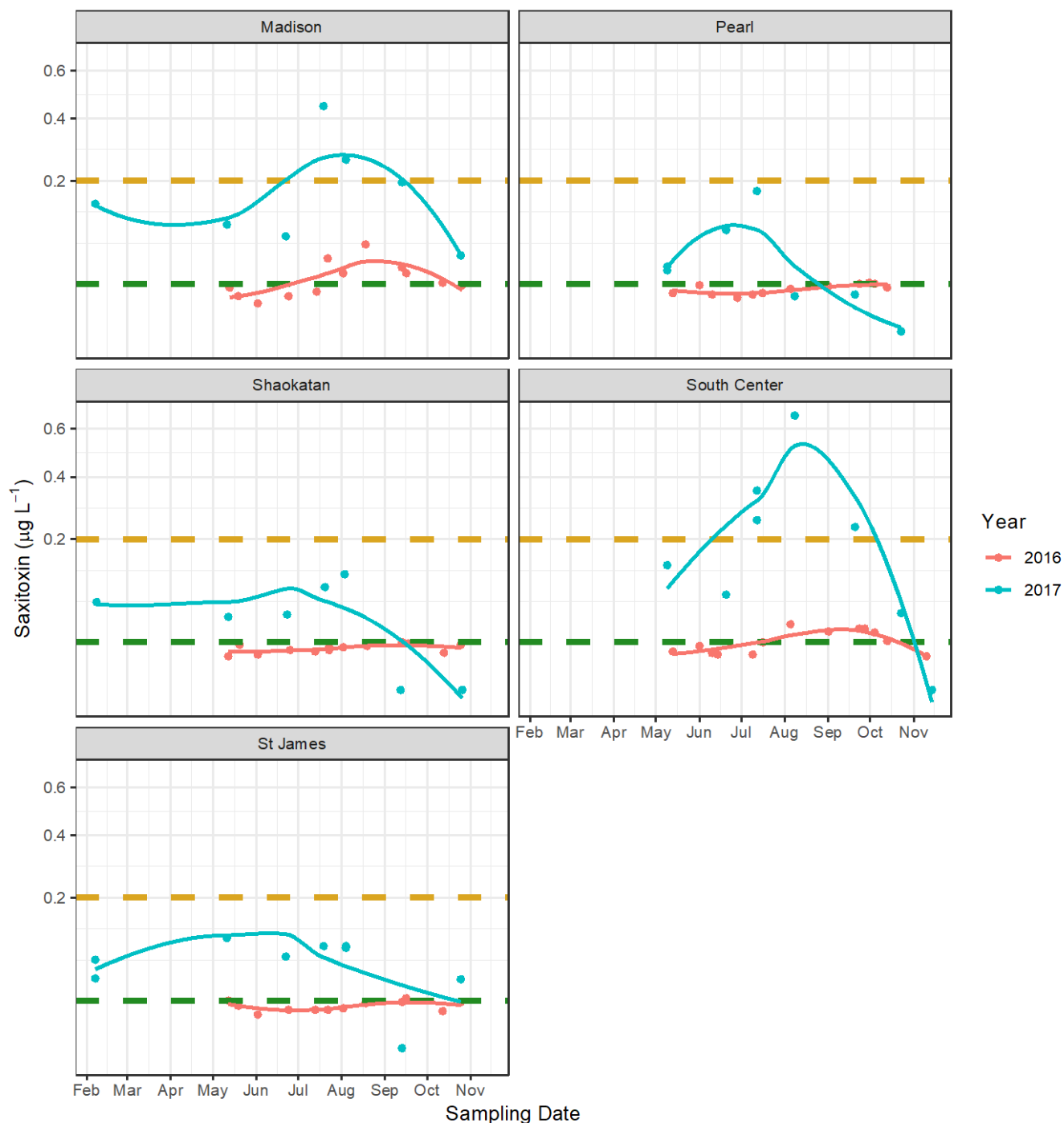


Figure 21. Saxitoxin concentrations in all five lakes in this study in 2016 (orange) and 2017 (turquoise). Green dashed line represents instrument detection limits (0.02 µg/L) and yellow dashed line represents minimum safe drinking water standard from the Ohio Department of Health (0.2 µg/l) due to a lack of any state or **federal** standards in Minnesota.

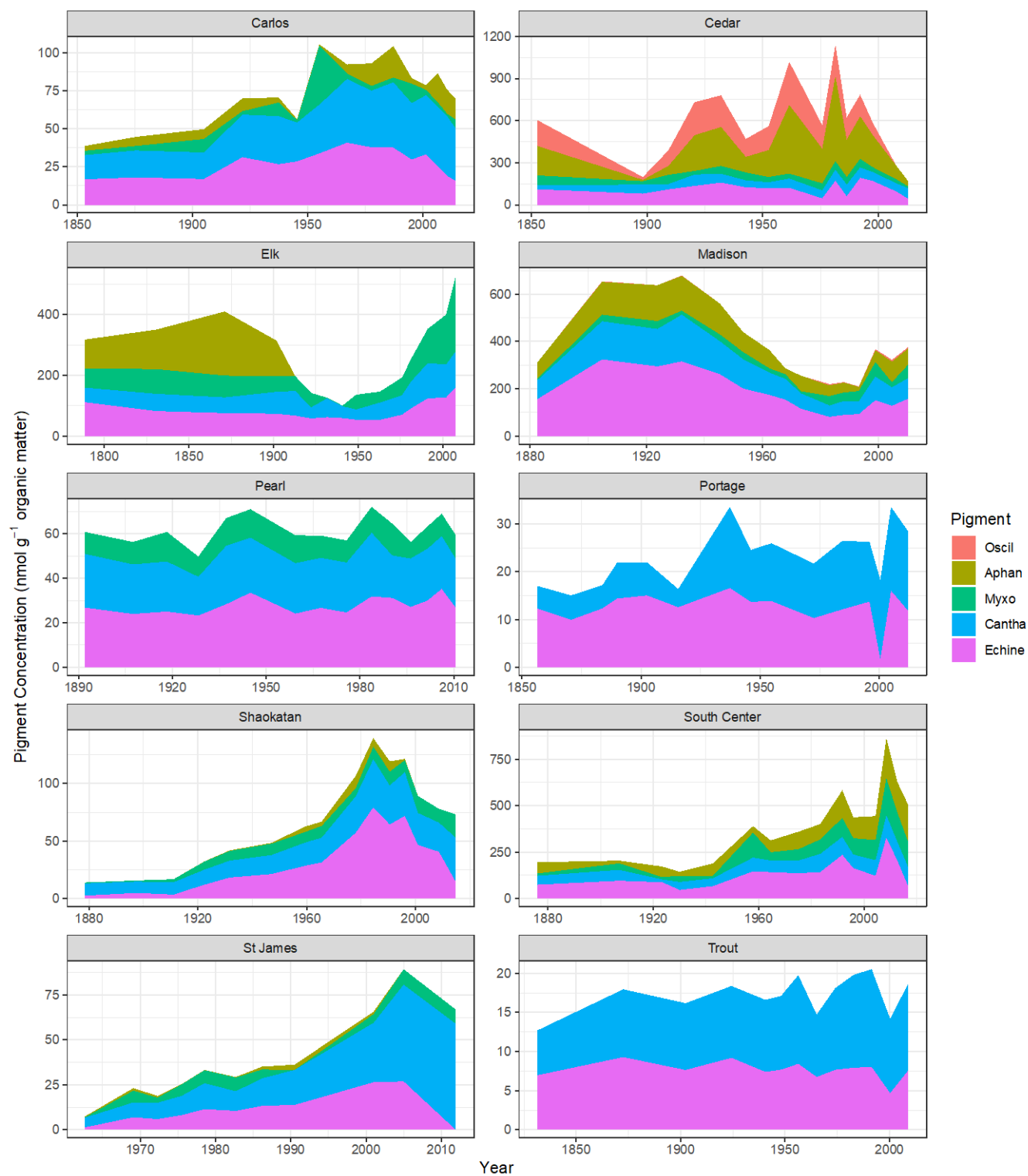


Figure 22. Fossil Cyanobacterial pigments from sediment cores in ten lakes of this study.

Madison Lake

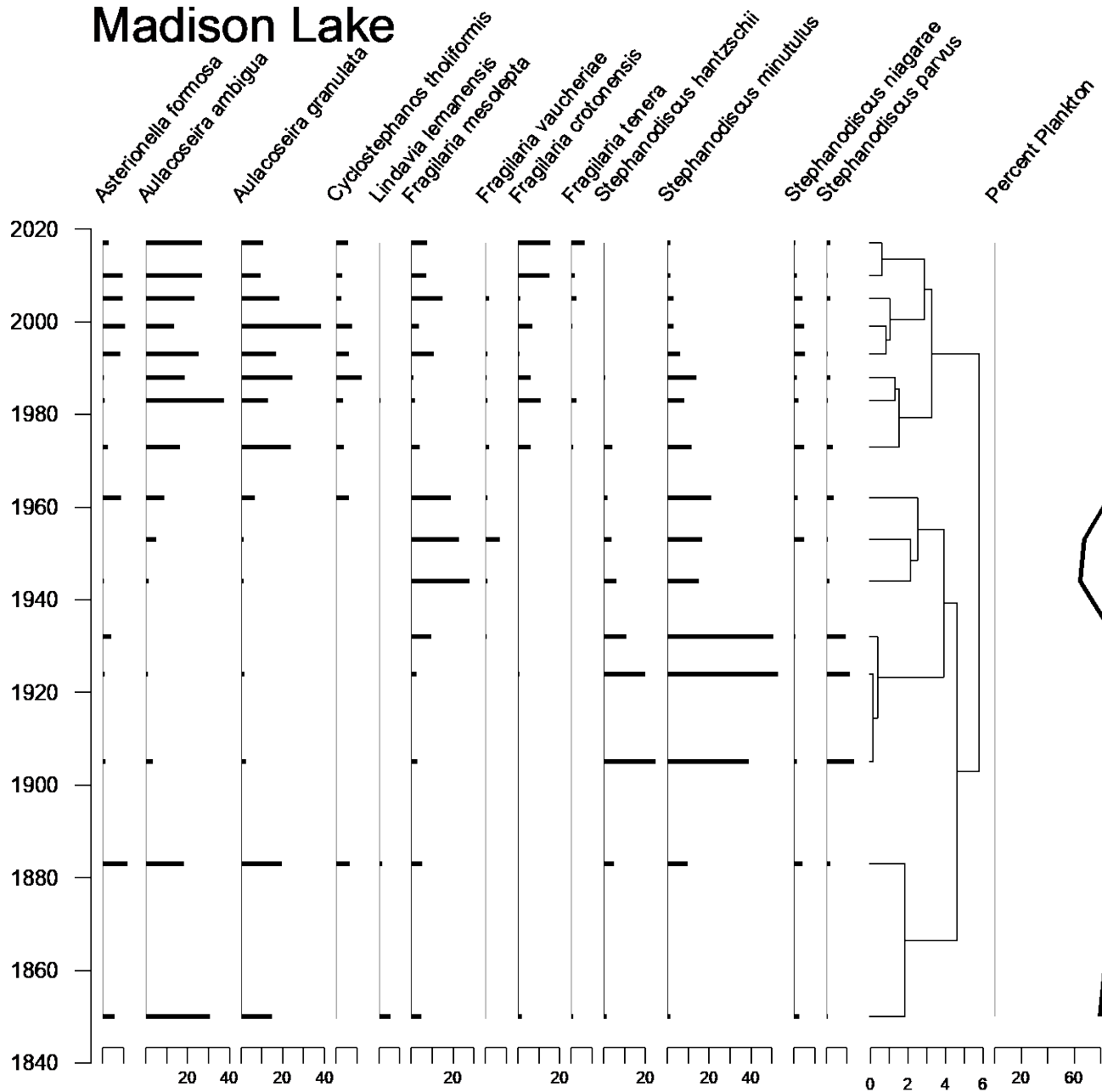


Figure 23. Stratigraphic plot of diatom abundances in Madison Lake. Dendrogram represents a constrained hierarchical clustering (CONISS) which separates distinct time periods based on the diatom flora.

CCA, 89 MN Lakes, Madison Lake fossil data

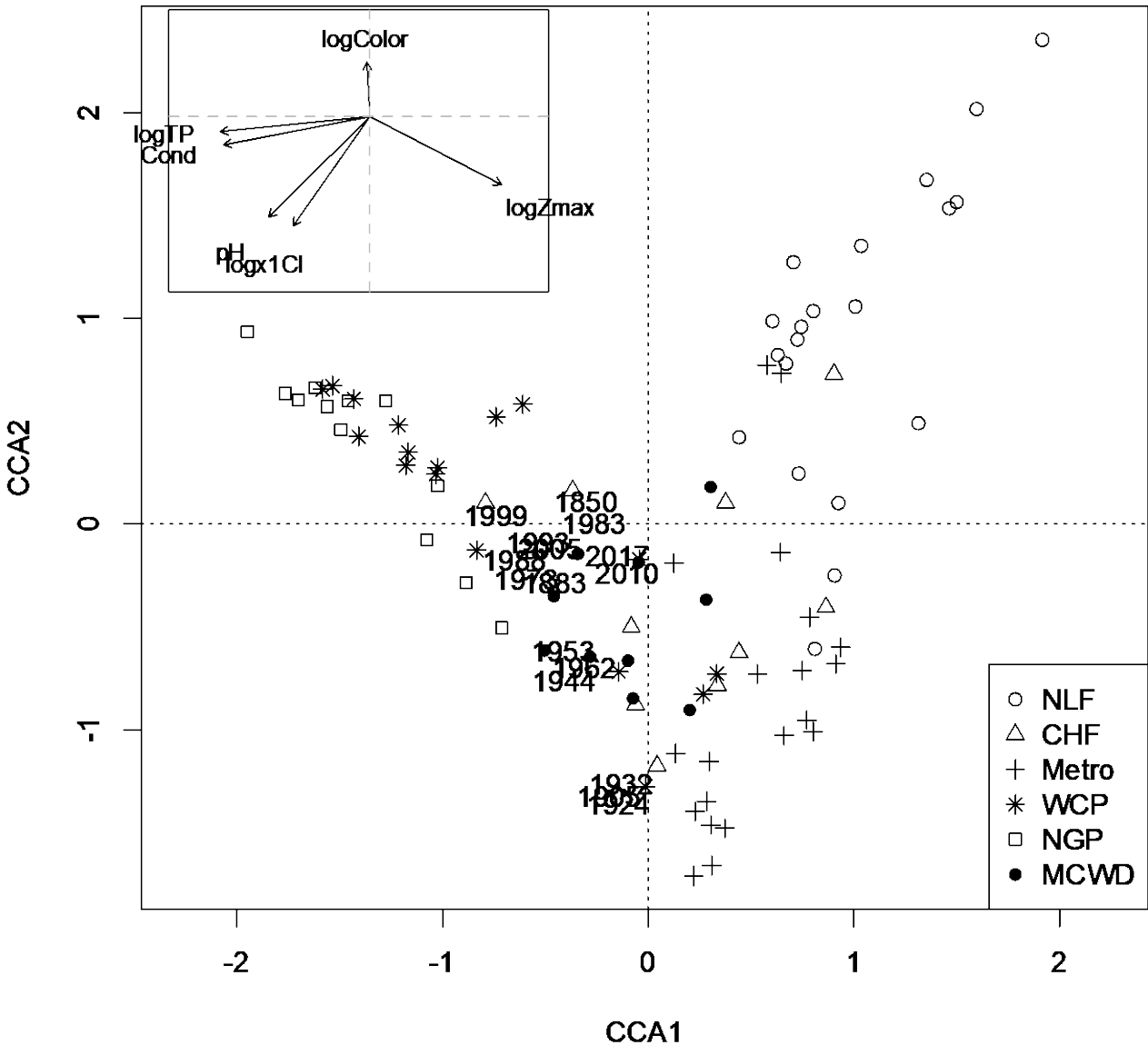


Figure 24. Constrained Coordinates Analysis (CCA) ordination of the 89-lake surface training set in Minnesota. This training set represents diatom populations in lakes all across the state and is organized along water quality axes show in the top-left inset. Diatom samples from Madison Lake are passively plotted (represented by the date of the section) onto the ordination to show how they move along these major water quality axes through time.

Shaokatan Lake

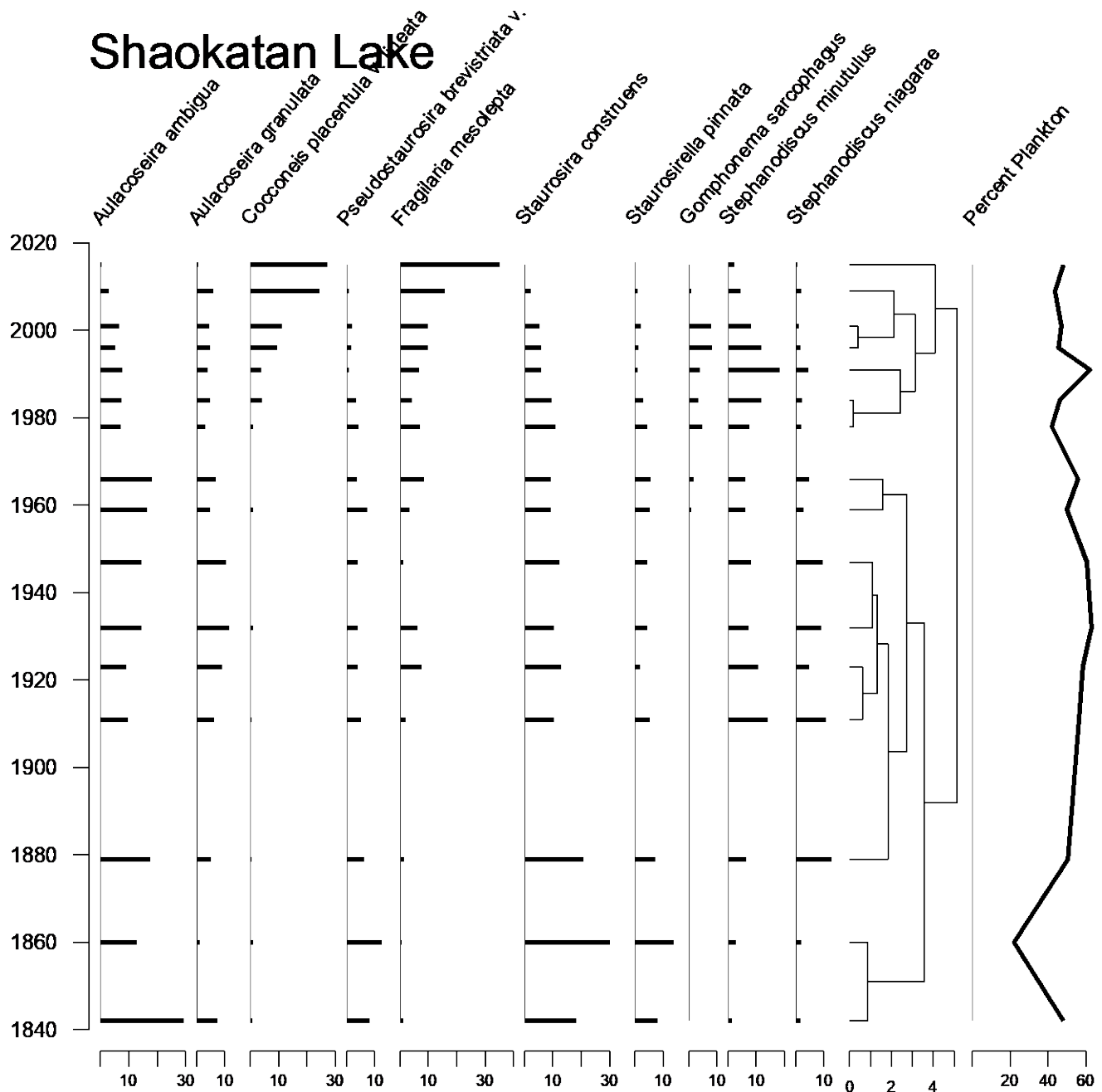


Figure 25. Stratigraphic plot of diatom **abundances** in Lake Shaokatan. Dendrogram represents a constrained hierarchical clustering (CONISS) which separates distinct time periods based on the diatom flora.

CCA, 89 MN Lakes, Shaokatan Lake fossil data

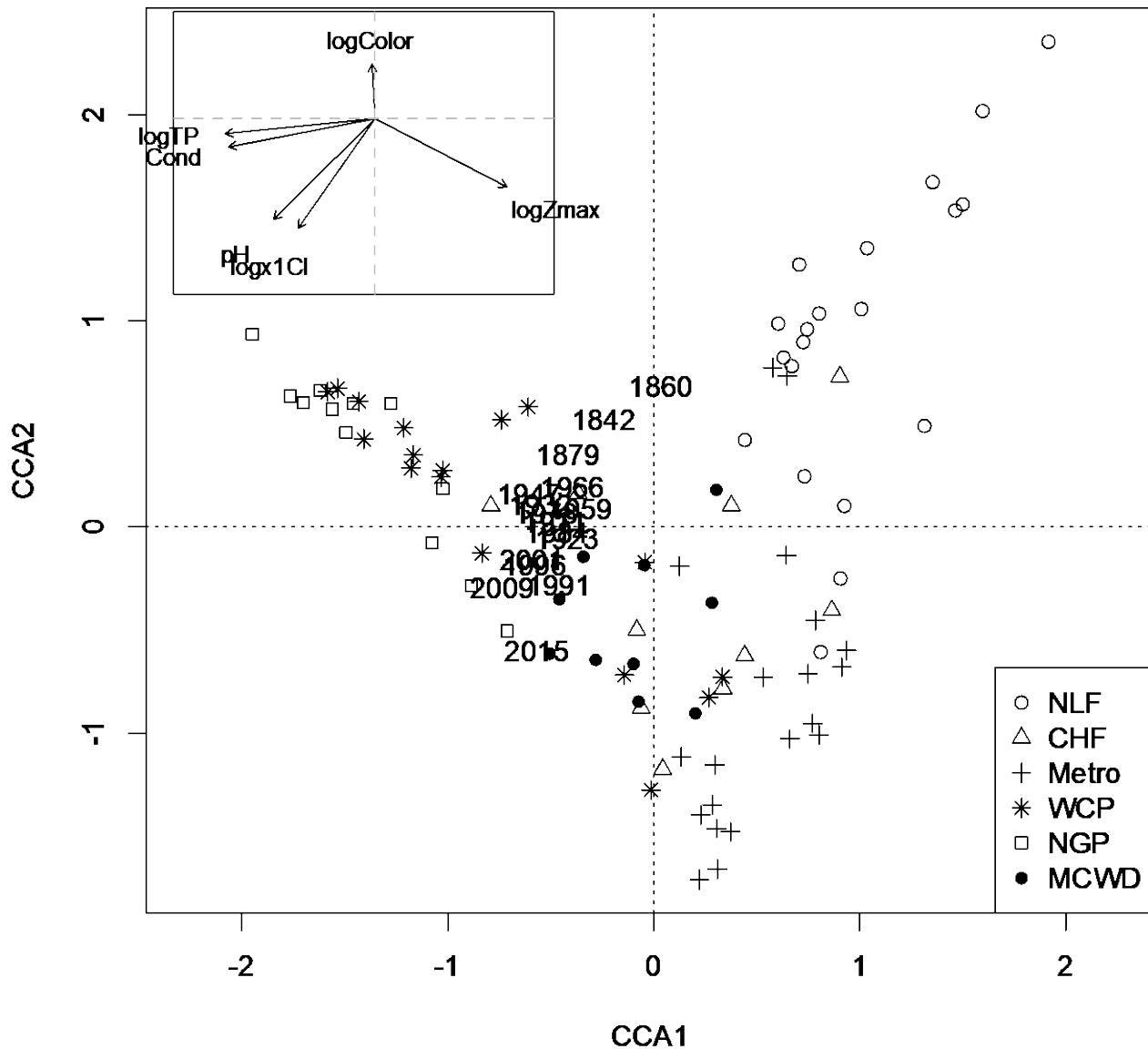


Figure 26. Constrained Coordinates Analysis (CCA) ordination of the 89-lake surface training set in Minnesota. This training set represents diatom populations in lakes all across the state and is organized **along** water quality axes show in the top-left inset. Diatom samples from Lake Shaokatan are passively plotted (represented by the date of the section) onto the ordination to show how they move along these major water quality axes through time.

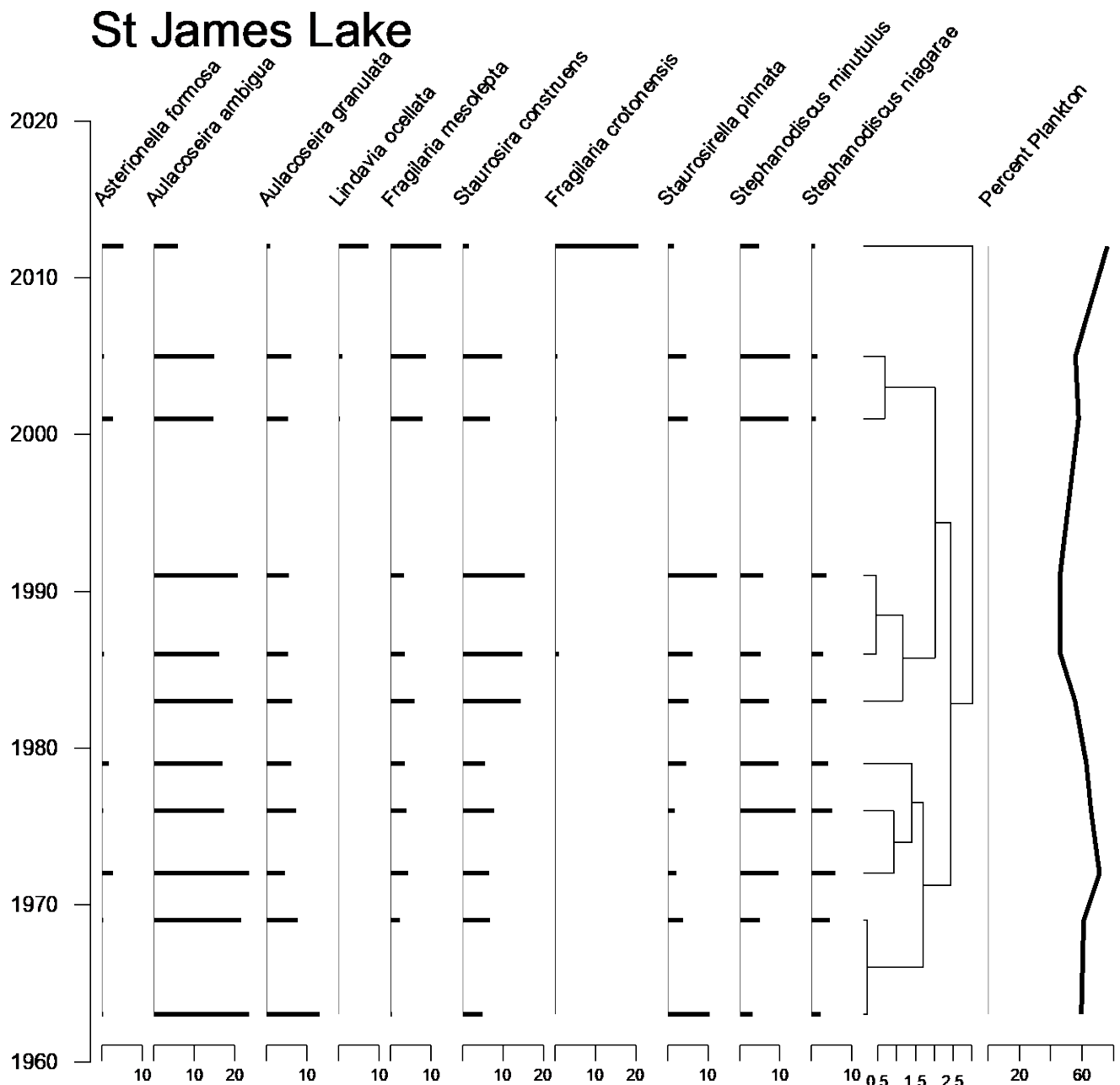


Figure 27. Stratigraphic plot of diatom abundances in Lake St. James. Dendrogram represents a constrained hierarchical clustering (CONISS) which separates distinct time periods based on the diatom flora.

CCA, 89 MN Lakes, St James Lake fossil data

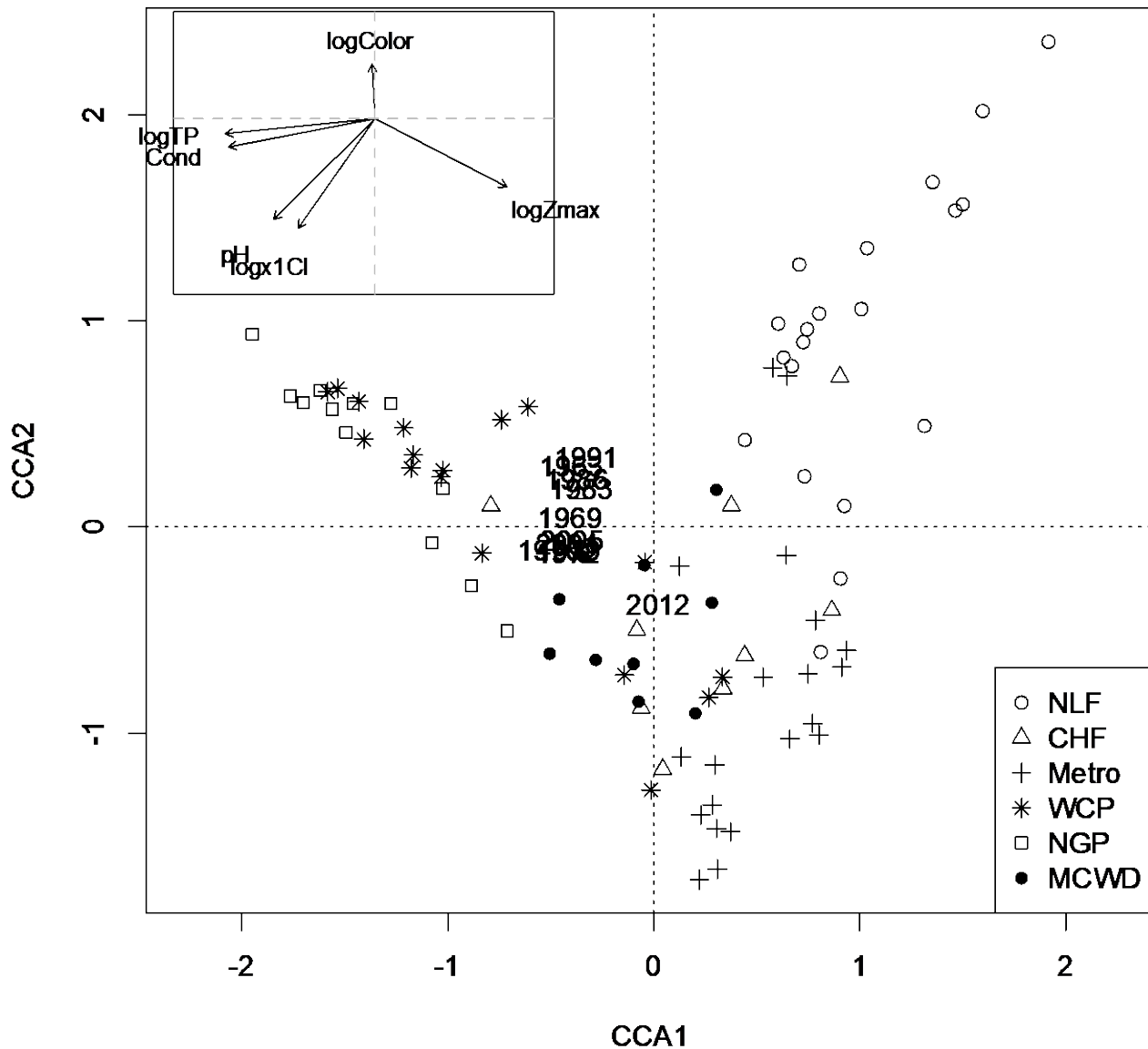


Figure 28. Constrained Coordinates Analysis (CCA) ordination of the 89-lake surface training set in Minnesota. This training set represents **diatom** populations in lakes all across the state and is organized along water quality axes show in the top-left inset. Diatom samples from Lake St. James are passively plotted (represented by the date of the section) onto the ordination to show how they move along these major water quality axes through time.

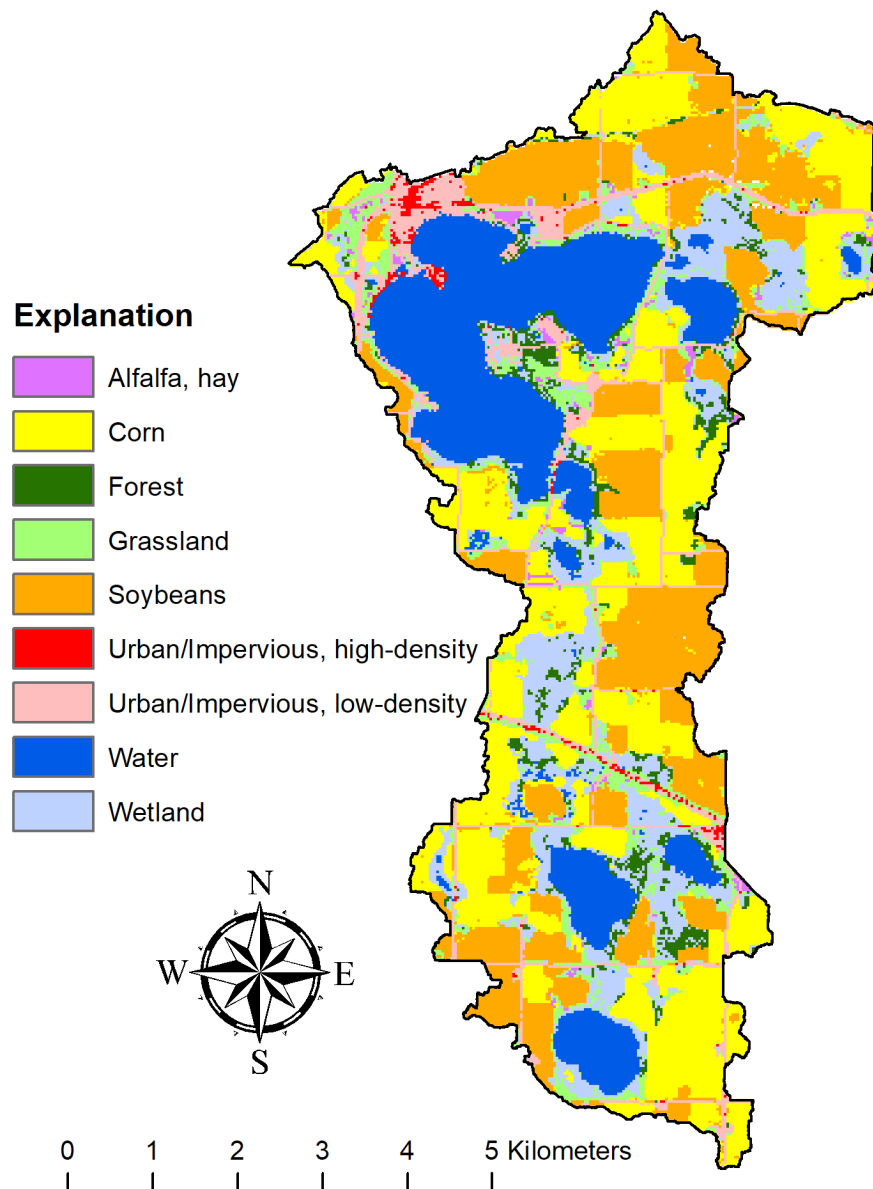


Figure 29. Land-use information for the Madison Lake watershed. Madison Lake is located in the NW corner of the map.

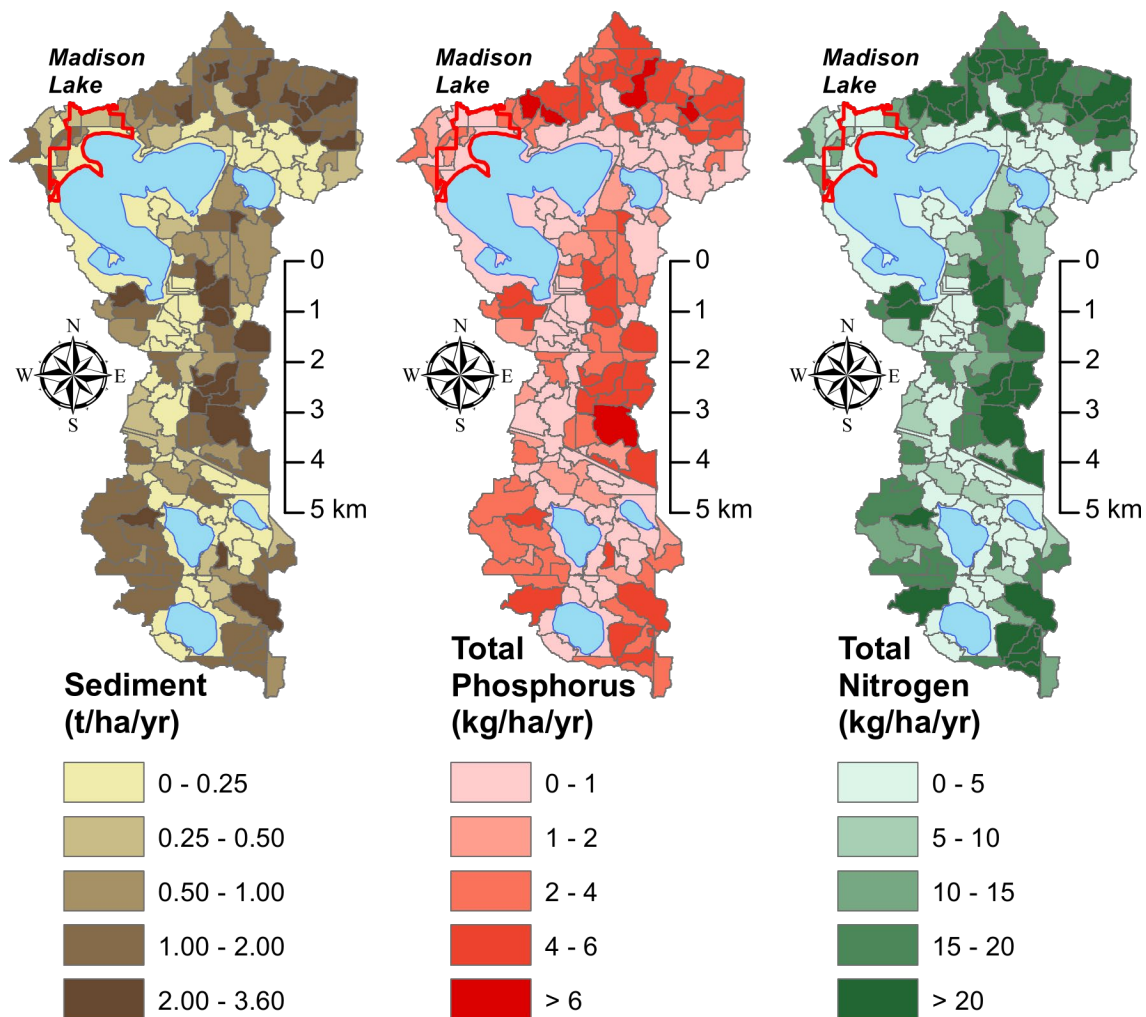


Figure 30. Soil and Water Assessment Tool (SWAT) output for annual sediment, phosphorus and nitrogen fluxes from each sub-basin within the Madison Lake watershed.

Environment and Natural Resources Trust Fund
Final Attachment A (Budget Sheet): Budget Detail for M.L. 2016 Environment and Natural Resources Trust Fund Projects



Project Title: Tracking and Preventing Harmful Algal Blooms
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04a
Project Manager: Daniel R. Engstrom
Organization: St. Croix Watershed Research Station, Science Museum of Minnesota
M.L. 2016 ENRTF Appropriation: \$ 593,000
Project Length and Completion Date: 3.5 Years, June 30, 2019
Date of Report: August 16, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 budget	Amount Spent	Activity 3 Balance	Activity 4 Budget	Amount Spent	Activity 4 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	M.L. 2015, Chp. 76, Sec. 2, Subd. 10Emerging Issues Account - Jump-start lake monitoring program			Identify species composition and timing of harmful algal blooms				Reconstruct frequency of algal blooms relative to natural conditions				Determine how nutrients and climate interact to favor harmful algae		
Personnel (Wages and Benefits)	\$42,900	\$42,900	\$0	\$127,200	\$127,200	\$0	\$107,200	\$107,200	\$0	\$106,800	\$106,800	\$0	\$384,100	\$0
Engstrom, Research Director: Sediment dating; 8% FTE for 2 yr; Salary=77%, Benefits=23% (\$21,600)														
Edlund, Senior Scientist (1 of 2); Diatom & BG algae analyses; 50% FTE for 2.5 yr; Salary=77%, Benefits=23% (\$122,400)														
Almendinger, Senior Scientist (1 of 2); SWAT modeling; 35% FTE for 2 yr; Salary=77%, Benefits=23% (\$70,100)														
Heathcote, Asst. Scientist; BG algae and toxins; data synthesis; 50% FTE for 3.5 yr; Salary=77%, Benefits=23% (\$128,400)														
Field Technician; Lakea monitoring and sampling; 75% FTE for 2 yr; Salalry=77%, Benefits=23% (\$41,600)														
Professional/Technical/Service Contracts														
U.S. Geological Survey (for CE-QUAL-W2 modeling of lake hydrodynamics and phosphorus cycling)										\$50,000	\$50,000	\$0	\$50,000	\$0
University of Regina (for analysis of fossil plant pigments in sediment cores)							\$18,800	\$18,800	\$0				\$18,800	\$0
Equipment/Tools/Supplies														
Field supplies (sediment traps, sample bottles & vials, reagents)	\$1,000	\$1,000	\$0	\$10,500	\$10,500	\$0							\$11,500	\$0
Dissolved oxygen and temperature recording probes	\$18,500	\$18,500	\$0							\$5,500	\$5,500	\$0	\$24,000	\$0
Capital Expenditures Over \$5,000														
YSI multi-parameter sonde for water-column measurements	\$20,000	\$20,000	\$0										\$20,000	\$0
ELISA micoplate reader and supplies for analysis of algal toxins				\$10,000	\$10,000	\$0							\$10,000	\$0
Travel expenses in Minnesota	\$6,500	\$6,500	\$0	\$6,500	\$6,500	\$0	\$1,100	\$1,100	\$0				\$14,100	\$0
Lake monitoring & coring (mileage and gas, ~70 trips)														
\$11,500														
Lake monitoring & coring (meals) \$6,200														
Lake monitoring & coring (lodging) \$1,900														
Other														
Lab analysis of water samples (N, P, DOC, DIC) and sediment cores: radiometric dating (Lead-210, Cesium-137); biogenic silica; loss-on-ignition, sediment phosphorus and metals	\$4,100	\$4,100	\$0	\$11,600	\$11,600	\$0	\$44,800	\$44,800	\$0				\$60,500	\$0
COLUMN TOTAL	\$93,000	\$93,000	\$0	\$165,800	\$165,800	\$0	\$171,900	\$171,900	\$0	\$162,300	\$162,300	\$0	\$593,000	\$0