M.L. 2018 Project Abstract
For the Period Ending June 30, 2022

PROJECT TITLE: Determining Risk of Toxic Alga in Minnesota Lakes
PROJECT MANAGER: Adam J. Heathcote
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

APPROPRIATION AMOUNT: $200,000
AMOUNT SPENT: $197,099
AMOUNT REMAINING: $2,901

Sound bite of Project Outcomes and Results

This project produced the first systematic survey of Minnesota's Sentinel Lakes for the toxic invasive algae *Cylindrospermopsis raciborskii* (Cylindro). Cylindro was contained to the 2 lakes where it was initially found and did not produce toxins in measurable amounts. Sediment records indicated that Cylindro has appeared in the last 10 years and has not spread statewide.

Overall Project Outcome and Results

Cylindro is a subtropical invasive species of Cyanobacteria that has been invading lakes in the Upper Midwest since the early 2000s. Cylindro is of particular concern because it is known to produce a potent liver toxin and the presence of its blooms can be difficult to identify. Cylindro was first discovered in two Minnesota lakes in 2013 and that discovery led to the design of this statewide survey of the Minnesota Sentinel Lakes to better understand the spread and invasion history of this species. Our monitoring results, based on DNA and microscopy, show that Cylindro is currently limited to the 2 lakes where it was initially found, and sediment cores indicate that it has been present in those lakes for <10 years. Additionally, even in lakes where Cylindro was present, there was no evidence of toxin production in detectable amounts, minimizing the public and wildlife health threat of this species. Thankfully, these results suggest that the threat of Cylindro invasion in Minnesota lakes is currently low, though continued monitoring for this species is important given the trend of warming lake waters across the state.

Project Results Use and Dissemination

We have shared the progress and results from this project widely over the duration of the project. This includes both articles written by our staff, shared with traditional and social media, and peer reviewed papers. These efforts are summarized below in chronological order:

- "Invisible" species of exotic algae threatens to poison Minnesota lakes, posted to SMM.org on 11/6/2018, (PDF attached in supplemental materials)
- "Conditions ripe for a record number of algae blooms", Minnesota Public Radio Climate Cast segment on 7/19/2019,

- “Why good algae go bad”, talk at the Marine Community Library by Adam Heathcote on 7/14/2019, picture of event on Twitter
- Adam Heathcote Co-chaired special session on Harmful Algal Blooms at the Minnesota Water Conference and organized a panel of experts to take questions from conference attendees in St. Paul, MN on 10/16/2019
- During the pandemic we provided information on harmful algal blooms for the public on the Science Museum website in our “Learn From Home” section, posted on 7/7/2020.
- Preliminary results from this study were presented to the Minnesota Inter-agency HABs group at their Winter Workshop on 1/25/2021. Attendees includes representatives from the Minnesota DNR, MPCA, MDH and the MVMA
- Results from this study were published in the peer-reviewed journal PLOS ONE on 3/21/2022. PDF of paper is attached in the supplemental materials.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2018 ENRTF Work Plan Final Report (Main Document)

Today's Date: August 15, 2022

Final Report

Date of Work Plan Approval: 06/05/2018

Project Completion Date: June 30, 2022

PROJECT TITLE: Determining Risk of Toxic Alga in Minnesota Lakes

Project Manager:	Adam J. Heathcote
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Location: Statewide

Total Project Budget: $200,000

Amount Spent: $197,099

Balance: $2,901

Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

Appropriation Language: $200,000 the second year is from the trust fund to the Science Museum of Minnesota for the St. Croix Watershed Research Station to determine the historical distribution, abundance, and toxicity of the invasive blue-green alga, Cylindrospermopsis raciborskii, in about 20 lakes across Minnesota and inform managers and the public about the alga's spread and health risks. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

I. PROJECT STATEMENT:

Invasive microbes are easily spread, but difficult to detect, control, or reliably assess for their environmental and public health risk. Cyanobacteria (blue-green algae) are one of the most abundant and obvious microbes in lakes, and recent work suggests a shift to more toxic forms – including the invasive species, *Cylindrospermopsis raciborskii* (*Cylindro*) – with consequences including dog deaths, human illness, and reduced natural resource value. Minnesota is outside the native range of *Cylindro*, but its arrival has recently been confirmed by ENRTF supported surveys carried out by the St. Croix Watershed Research Station (SCWRS) and Minnesota Pollution Control Agency (MPCS). It is likely that recent years with warmer summer temperatures and increased nutrient pollution have provided a new niche for this species to invade.

There is little information on *Cylindro* in Minnesota and no data on the presence of toxins it produces. This information is particularly important to the State of Minnesota, because unlike other Cyanobacteria, *Cylindro* may bloom several feet below the lake surface, making it difficult to visually assess the quality and safety of waters where it is present. This project will be the first systematic survey of Minnesota for the occurrence of *Cylindro* and its cyanotoxins in Minnesota lakes – in both the water and bottom sediments. This study will determine present-day distribution and toxicity of *Cylindro*, its historic introduction and spread across the state, and develop predictive models for bloom occurrence, seasonality, toxicity, and invasion risk. These data are the critical first step in understanding and addressing the spread of any invasive species.

This project leverages current ENRTF funding for harmful algal bloom (HABs) research on the Sentinel Lakes that were selected by the DNR as a representative sample of Minnesota's lakes. It would provide an additional year of monitoring for HABs on 20 of the Sentinel Lakes during the peak bloom season and allow us to determine the historical presence of *Cylindro* through the occurrence of its toxins in those same lakes using sediment cores.

II. OVERALL PROJECT STATUS UPDATES:

First Update January 31, 2019

We surveyed all 20 target lakes in August and September of 2018, collecting water quality and algae samples described in Activity 1. Water quality samples are currently being analyzed in the SCWRS laboratory and will be completed before the beginning of the second field season in May 2019. In addition to the survey, sediment core samples were collected on 10 of the 20 lakes for Activity 2. These sediment cores have been sectioned and are currently undergoing 210-Pb dating analyses in the SCWRS laboratory. Finally, in concert with the beginning of this study, we published a new story and infographic on the toxic algae, *Cylindrospermopsis raciborskii*, which was shared through our website and social media outlets. The story can be found here:
https://www.smm.org/scwrs/fieldnotes/invisible-species-exotic-algae-threatens-poison-minnesota-lakes

Second Update June 30, 2019

All water quality samples collected as part of Activity 1 in 2018 have been analyzed in the laboratory. We have also collected an additional 5 sediment cores for Activity 2 (5 remain to be collected). All new sediment cores have been sectioned and we continue to work on 210-Pb dating and other sediment analyses in the laboratory. We continue to share our work with the public, including mention of Cylindro on Minnesota Public Radio's Climate Cast (archived here: https://www.mprnews.org/episode/2019/07/19/conditions-ripe-for-a-record-number-of-algae-blooms).

Third Update January 31, 2020

All sediment cores have been collected and are in the process of being analyzed in the SCWRS laboratory. Currently we have dated and completed geochemistry (loss-on-ignition) on 5 of 10 cores and the remainder are being processed. We have begun perfecting our sediment toxin analysis for microcystin and cylindrospermopsin and have completed sediment toxin analyses on one of the ten cores. We presented information from this study as well as previous LCCMR-funded work to the public at the 2019 Minnesota Water Conference as part of a special session on Harmful Algal Blooms.

The final year and a half of this study will focus on completing all of the sediment toxin analyses and then synthesizing the results of both the water quality (Activity 1) and historical record (sediment cores; Activity 2) of Cylindro in Minnesota.

Fourth Update June 30, 2020

We have completed geochemistry (loss-on-ignition) and 210-Pb dating on all newly collected cores from this study and have used previous dating models to interpolate 210-Pb dates for sediment cores from lakes that were collected for previous ENRTF-funded research. This has produced dating models for sediment cores from all 20 Sentinel Lakes sampled as part of this process.

We planned to be well into our sediment toxin extractions at this point, however, due to the unforeseen COVID-19 pandemic, most of the Science Museum of Minnesota, including all scientific and technical staff at the SCWRS were temporarily laid off from the beginning of April through most of June. On July 27, the SCWRS laboratory began re-opening and is currently assessing the backlog of analytical work that accumulated over the shutdown. We are hopeful that this project can still be completed within its original timeline, as the bulk of the labwork had already been completed before the shutdown occurred.

Fifth Update January 31, 2021

We have primarily been working on data analysis of the water chemistry and phytoplankton samples which were collected as part of Activity 1 for this project, including preparing preliminary data for a presentation to the Minnesota Inter-Agency Panel on Harmful Algal Blooms on January 25th, 2021. Additionally, we have been working with our analytical chemistry laboratory to continue developing the sediment toxin extraction technique from our previously collected and dated sediment cores. We are still hoping to complete this work during the original project timeline, however, due to the setback of our laboratory being closed for most of the spring and summer of 2020 we requested a 1-year extension for this project in order to ensure we have enough time to complete and synthesize these results.

Project extended to June 30, 2022 by LCCMR 6/30/21 as a result of M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18, legislative extension criteria being met.

Sixth Update June 30, 2021:

We are grateful for the extension provided by the legislature to allow us to complete the final analyses on this project. During the last period we have worked primarily on preparing a synthesis of Cyanobacterial sediment DNA collected by the SCWRS' Environmental Research Fellow who was funded as a field and laboratory technician on this project. We have completed a peer-reviewed publication entitled "Diversity and distribution of sediment bacteria across an ecological and trophic gradient", which will be submitted to the journal PLOS ONE this month.

Seventh Update January 31, 2022:

We continue to work on final synthesis of the sediment toxin work as well as production of a peer-reviewed manuscript from the results of this study. On November 3rd we received positive reviews our manuscript from journal PLOS ONE and were asked to address the reviewer comments and resubmit. We revised the manuscript accordingly and resubmitted it on December 16th, 2021. Based on the initial positive response we anticipate this manuscript will be accepted for publication and are awaiting final decision from the editors.

Final Update June 30, 2022

Cylindro is a subtropical invasive species of Cyanobacteria that has been invading lakes in the Upper Midwest since the early 2000s. Cylindro is of particular concern because it is known to produce a potent liver toxin and the presence of its blooms can be difficult to identify. Cylindro was first discovered in two Minnesota lakes in 2013 and that discovery led to the design of this statewide survey of the Minnesota Sentinel Lakes to better understand the spread and invasion history of this species. Our monitoring results, based on DNA and microscopy, show that Cylindro is currently limited to the 2 lakes where it was initially found, and sediment cores indicate that it has been present in those lakes for <10 years. Additionally, even in lakes where Cylindro was present, there was no evidence of toxin production in detectable amounts, minimizing the public and wildlife health threat of this species. Thankfully, these results suggest that the threat of Cylindro invasion in Minnesota lakes is currently low, though continued monitoring for this species is important given the trend of warming lake waters across the state.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Survey Minnesota lakes for *Cylindro* and the conditions associated with its presence

Description: Current HABs monitoring by the St. Croix Watershed Research Station (SCWRS) will be enhanced by an additional year of monitoring on an expanded set of 20 Sentinel Lakes. SCWRS personnel will collect water quality, algae, and cyanotoxin samples during the peak bloom season (August-September) in 2018. Algae samples will be analyzed for the occurrence and abundance of *Cylindro* through standard microscopy techniques, and cyanotoxins will be measured using ELISA enzyme assays. All analyses will occur at the SCWRS CHARM Laboratory (Center for Harmful Algal Research in Minnesota). SCWRS personnel will develop a spatially explicit predictive model for the invasive spread and bloom risk of *Cylindro* for the major Minnesota lake regions using readily available geographical, weather, and water quality data.

ENRTF BUDGET: $72,705

Outcome	Completion Date
1. We will collect phytoplankton and water quality samples from the 20 Sentinel Lakes during peak bloom season to determine the presence and toxicity of *Cylindro*	October 2018
2. We will develop predictive criteria for the invasive spread of *Cylindro* that can be applied to Minnesota lakes based on its occurrence, abundance, and toxin production coincident with lake, water, and weather conditions	June 2021

First Update January 31, 2019

We visited all 20 study lakes in August and September of 2018 to conduct our statewide survey for the toxic algae Cylindro. We collected basic water-quality measurements and water chemistry samples as well as algal community and cyanotoxin samples to assess for the presence of this exotic species across the state. Water quality samples were collected for total and dissolved nitrogen and phosphorus, dissolved inorganic and organic carbon, and chlorophyll *a*. Water quality profiles were measured using our multi-parameter sonde (purchased with previous LCCMR funding) for water temperature, dissolved oxygen, pH, phycocyanin (cyanobacterial pigment), chlorophyll *a*, turbidity, and conductivity. Additionally, we collected a sample of the total algal community as well as samples for the toxin produced by Cylindro (cylindrospermopsin) and a toxin produced by other Cyanobacteria (microcystin).

All water quality samples are being processed by the SCWRS laboratory. All dissolved water chemistry (phosphorus, nitrogen, carbon) have been analyzed and total phosphorus and nitrogen will be completed in the next month. All algal toxin samples have been frozen and will be run in large batches to maximize efficient use of the ELISA kits. Phytoplankton community samples have been preserved and concentrated to be counted at a later date.

Second Update June 30, 2019

We have completed the water quality samples collected in the later summer and early fall of 2018. We have summarized some of the results in Figure 2. Which shows the detection of the cyanotoxins cylindrospermopsin (CYN) and microcystin (MC) across all the lakes in this study.

CYN, which is the toxin produced by Cylindrospermopsin was in very low concentrations or absent in all lakes in this study. This includes lakes with known populations of Cylindro (confirmed via microscope identification), Madison and South Center. These are promising results, which would support the hypothesis that the population of Cylindro in the Great Lakes states is not particularly toxic, but this will need to be confirmed via our sediment toxin analyses (Activity 2). Also, of note, the only lake where CYN concentrations were above our detection limit was Red Sand Lake. Cylindro has not been previously found in this lake, so we will need to check the phytoplankton sample to confirm if it is present. Alternatively, the detected CYN could have been produced by another species of Cyanobacteria.

To compare CYN levels to more commonly produced toxins we also measured MC, a toxin commonly produced by other Cyanobacteria, in all lakes. Unlike CYN, MC was detected in all but three lakes (Greenwood, Trout, White Iron) and always exceeded Minnesota's minimum drinking water standard

(0.1 ug/L). It should be noted that none of the lakes exceeded EPA recommended recreational concentrations (10.0 ug/L), and only St. James even approached that higher limit (8.5 ug/L). These results indicate the presence of toxin-producing Cyanobacteria in even some of the most protected and relatively clean Minnesota lakes (See Figure 2 top panels for total phosphorus and chlorophyll-a concentrations), although concentrations of MC are still not a concern for non-drinking use.

Third Update January 31, 2020
No activity during this period.

Fourth Update June 30, 2020
No activity during this period.

Fifth Update January 31, 2021
No activity during this period.

Sixth Update June 30, 2021:
No activity during this period.

Seventh Update January 31, 2022:
No activity during this period.

Final Update June 30, 2022

Cylindrospermopsis raciborskii (Cylindro) is an exotic species of Cyanobacteria that is native to subtropical lakes in the southeastern United States (USA). It has been documented in a growing number of states in the Midwest USA since the early 2000's and was first documented in two of Minnesota's Sentinel Lakes as part of a routine phytoplankton survey by the Minnesota Pollution Control Agency (MPCA) in 2013. The expansion of this species into temperate lakes has been hypothesized to be due to these systems becoming more hospitable to Cylindro as average summer water temperatures rise in this region.

The expansion of Cylindro in Minnesota is a concern primarily because of its ability to produce the potent hepatotoxin cylindrospermopsin (CYN), which is potentially deadly to both humans and wildlife. Additionally, unlike many other Cyanobacteria species, Cylindro often forms sub-surface blooms which are more difficult to detect and circumvent traditional public health messaging on the safety of lakes for recreation (i.e., "When in doubt, stay out"). An infographic for the public on Cylindro was created as part of this project (Figure 1).

This project represents the first systematic survey for Cylindro in Minnesota using the already established network of the Minnesota Sentinel Lakes which span all of Minnesota's ecoregions and major lake types.

Cylindro, water quality, and cyanotoxin survey of Minnesota

To detect the presence of Cylindro, we surveyed a subset of 20 of Minnesota's Sentinel Lakes in the late summer of 2018 when harmful algae blooms (HABs) are most prevalent. For each lake we visited, we collected a full suite of water chemistry data, including total phosphorus (TP), total nitrogen (TN), chlorophyll *a*, nitrate+nitrite (NOx), ammonia (NH4), soluble reactive phosphorus (SRP), dissolved inorganic carbon (DIC), and dissolved organic carbon (DOC). We also collected samples for the cyanotoxin produced by Cylindro, CYN, as well as a cyanotoxin that is commonly produced by other Cyanobacteria in Minnesota HABs, microcystin (MCY). All samples were collected from an integrated surface sample of the epilimnion (epi) and if the lake was stratified a second sample was collected from the hypolimnion (hypo). The full results from this survey are presented in Table 1.

Phytoplankton samples were collected from each lake to confirm the presence of Cylindro through microscopy, however, we opted to use a new DNA-based approach to collect a more comprehensive sample of the Cyanobacteria present in a lake. For this approach, we sequenced DNA in the surface sediments of each lake, which integrate 1-2 years of phytoplankton and benthic algae rather than a single snapshot in time. This method is also less skewed towards the more easily identifiable large colonial Cyanobacteria and gives a more comprehensive picture of the community. DNA samples were extracted from the surface sediments and sequenced at the University of Minnesota Genomic Center. Figure 5 shows the results from this work and the distribution of Cyanobacteria genera across the lakes in this study. Cylindro DNA was only detected in two samples, Madison Lake and South Center, which already had previously confirmed occurrences. Microscopic analyses of phytoplankton samples also confirmed the presence of Cylindro in these two lakes (Figure 6).

In addition to the presence of Cylindro DNA, our survey gave us a broader understanding of the microbial diversity of the Sentinel Lakes. This includes a significant gradient in both richness (number of species) and diversity (evenness of species) (Figure 3). Somewhat surprisingly, the highest richness and diversity in microorganisms was found in the lakes in the Cornbelt Plains and the lowest diversity in the Canadian Shield. We also characterized the diversity at the phyla level of microbial organisms in surface sediments of all 20 of the Sentinel Lakes, representing the first microbial survey of the Sentinel Lakes. For more details see Sauer *et al*. (2022) in the peer-reviewed journal PLOS ONE, included in the supplementary materials of this report.

Geographic trends in nutrient concentrations (TP), algal productivity (chl-*a*), and cyanotoxins from this survey are shown in Figure 2. The highest concentrations of TP and chl-*a* occurred in the agricultural southern portion of Minnesota (Cornbelt Plains ecoregion) and the metro area surrounding the Twin Cities. CYN, which is the toxin produced by Cylindro, was undetected in the water of all but one lake in this study. CYN was not detected in either of the lakes with known populations of Cylindro (South Center and Madison) but was detected in low concentration in Red Sand Lake. This indicates a relatively low risk of CYN toxicity in Minnesota, due to its absence or extremely low concentration even in lakes where this species is present. Unlike CYN, MCY was detected in all but three lakes (Greenwood, Trout, White Iron) and always exceeded Minnesota's minimum drinking water standard (0.1 ug/L). It should be noted that none of the lakes exceeded EPA recommended recreational concentrations (10.0 ug/L), and only St. James in southwestern Minnesota even approached that higher limit (8.5 ug/L). These results indicate the presence of toxin-producing Cyanobacteria in even some of the most protected and relatively clean Minnesota lakes in the late summer.

Predicting the spread and toxicity of Cylindro

The results of this study indicate that among the Sentinel Lakes, a representative sampling of all Minnesota Lakes, Cylindro is confined to only two lakes. This is good news in that the extent of the Cylindro invasion in Minnesota was completely unknown prior to this study. However, the isolated occurrences make it difficult to draw conclusions about what causes the spread or proliferation among Minnesota lakes in general terms. There are no obvious commonalities between the two lakes, as South Center and Madison, respectively, differ in ecoregion (Central Hardwood Forest and Cornbelt Plains), maximum depth (33 and 10 m), nutrient concentrations (21 and 89 µg/L TP), and trophic state (mesotrophic and eutrophic). Both lakes are popular for angling and recreation, but there is no obvious link between visitors of the two lakes as they are separated by nearly 200 km.

In both lakes where Cylindro was observed, no detectable concentration of the toxin CYN was found. There are two reasons that this may have occurred, 1) Cylindro was not present in high enough abundance to produce detectable toxin, and 2) the population of Cylindro in Minnesota do not possess the toxin-producing gene, as has been observed in other states in the Upper Midwest. Further genetic study of the Cylindro populations in Madison and South Center Lake could help answer this question by determining the presence of the *cyr* gene complex which is responsible for producing CYN. Although preliminary amplicon sequencing was completed as part of this study, a more detailed metagenomic approach would be required to confirm the presence of this and other genes responsible for toxin-production.

ACTIVITY 2: Using sediment cores to reconstruct the invasion history of *Cylindro* in Minnesota

Description: The exact timing and extent of the invasion of *Cylindro* in Minnesota is currently unknown. This activity would provide a statewide distribution and history of *Cylindro* invasion and spread using paleolimnological techniques. Sediment cores would be dated and analyzed for the toxin produced by *Cylindro*, cylindrospermopsin. Based on our sediment core analysis, the invasion history will be compared to known patterns of land-use, eutrophication, and climate and will be used to predict the limitations that dispersal may play in moderating the invasion front.

ENRTF BUDGET: $127,295

Outcome	Completion Date
1. We will collect and date sediment cores from the 20 Sentinel lakes and measure the *Cylindro* toxin (cylindrospermopsin) and the general Cyanobacteria toxin (microcystin) to determine when *Cylindro* arrived in Minnesota	February 2020
2. We will compare patterns of introduction and expansion of *Cylindro* to long-term weather data to assess the role of warming lake temperatures on range expansion vs. alternative invasion scenarios (i.e., eutrophication, human transport)	June 2021

First Update January 31, 2019

While conducting our statewide survey in Activity 1, we collected sediment cores from ten of the twenty study lakes (Trout, Greenwood, White Iron, Red Sand, Elk, South Twin, Artichoke, Shaokatan, Portage, Peltier). These cores have been sectioned in 1-2 cm intervals and are currently being analyzed by the SCWRS laboratory. Core samples are being processed for loss-on-ignition and then freeze-dried to begin the 210-Pb dating process. Once dating has been completed, samples can be selected for fossil toxin analyses. The remaining cores will be collected Feb-March (through the ice) or May-Oct (open water) of 2019 to leave ample time for all laboratory analyses to occur.

Second Update June 30, 2019
We collected five additional cores through the ice during Jan-March of 2019. These included Carlos, Cedar, Pearl, South Center, and Hill. We have completed geochemistry analysis on 14 of 15 and are in the process of optimizing our cyanotoxin extraction and measurement techniques for both CYN and MC. Once optimized, SCWRS will be the first laboratory in Minnesota to have demonstrated the capacity to accurately measure cyanotoxins in lake sediments. The remaining five sediment cores will be collected during the 2019 open-water season.

Third Update January 31, 2020

We have focused on completing 210-Pb dating and geochemistry on the ten new cores collected as part of this activity. We have completed this work on 5 of the lakes (Carrie, Belle, Hill, St. Olaf, Artichoke) and the remaining five lake cores are currently being worked on by our 210-Pb laboratory. We have processed the new cores collected for the ten lakes which were part of the LCCMR-funded Tracking and Preventing Harmful Algal Blooms project and we will apply previously determined 210-Pb dating models to those cores.

We have begun work on extracting and measuring toxins in the sediment of the cores from this study. This is a cutting edge method and we are still working at perfecting and enhancing the efficiency of the method. We have completed our first full core for the cylindrospermopsin toxin (CYN) on Hill lake and found no detections of the toxin within the sediments. We will next test the method on a lake known to contain Cylindrospermopsis (South Center Lake), with detectable toxin concentrations in lake water in previous years, and see if the toxin will also be detected in the sediments.

Fourth Update June 30, 2020

We have completed loss-on-ignition and 210-Pb dating on the remaining 5 new cores for this project (South Twin, White Iron, Greenwood, Peltier, Red Sand, Hill) and are in the process of producing interpolated dating models from the sediment cores that were dated in previous ENRTF-funded research (Portage, Elk, Trout, Shaokatan, South Center, Cedar, Pearl, Carlos, Madison, St. James) using the completed loss-on-ignition profiles from the 10 newly collected cores.

We have not progressed as planned on our cyanotoxin extractions due to the unforeseen shutdown of the Science Museum of Minnesota and SCWRS due to the COVID-19 pandemic as outlined in the overall status update for this period. We continue to work with the expectation that this project can be completed within its original timeline.

Fifth Update January 31, 2021

We continue to work on developing our cyanotoxin extraction method as this is the final analysis left to be completed for this project. We have successfully extracted the cylindrospermopsin toxin from the Hill Lake core and plan to apply this method to the remaining 19 cores in this data set. We continue to develop the microcystin extraction method.

Sixth Update June 30, 2021:
The SCWRS' Environmental Research Fellow, Hailey Saur (now a PhD student at the University of Minnesota) has completed the synthesis of her work on bacterial (including Cyanobacterial algae) DNA

collected in the sediment cores as a part of this project. Over the last 6 months she has worked with Adam Heathcote to complete her manuscript, entitled "Diversity and distribution of sediment bacteria across an ecological and trophic gradient" which sequenced DNA found in the top 2 sections of sediment cores from the MNDNR Sentinel Lakes collected and preserved as a part of this project. This included a survey of richness and diversity of the microbial community in lake sediments (Figure 3) as well as a phyla level survey of all microbial organisms present in the MNDNR Sentinel Lakes (Figure 4). This manuscript will be submitted for review to the peer-revied scientific journal PLOS ONE in September of 2021.

Seventh Update January 31, 2022:

We are working on finishing the final sediment core work required for this project which includes finalizing 210-Pb dating models and completing cylindrospermopsin and microcistin toxin extractions from the sediment cores collected in this study. In November of 2021, we received positive peer reviewer comments from the manuscript that was submitted in the previous update and were encouraged by the editor of the journal PLOS ONE to address these comments and resubmit our publication. After going through a thorough revision process the article was resubmitted in December of 2021. We are still awaiting the final decision of the editor.

Final Update June 30, 2022

Cylindro was first confirmed in Minnesota in 2013, however, there was considerable uncertainty around the timing of the invasion due to very limited phytoplankton surveys in the state and no concerted effort to look for it prior to the initiation of this study. Further, there was at least one earlier report of Cyilndro in Minnesota dating back to 1969, although it was collected by an amateur phycologist and not independently verified. To this end, we designed a paleolimnological survey of a representative sampling of 20 Minnesota lakes, including the only two lakes known to be infested by Cylindro. To look for the presence of Cylindro over the last century, we measured the occurrence of the CYN toxin in sediment, a technique that has been used to confirm the presence of Cylindro for thousands of years in Florida lakes where it is native. By measuring the occurrence of the CYN toxin in dated sediment cores, we aimed to better understand the timing of the invasion in any lakes where Cylindro was confirmed to occur.

Sediment core collection and dating

Sediment cores were collected from all 20 lakes in this study throughout the open-water and ice seasons of 2018 and 2019. Loss-on-ignition, a measure of the geochemical composition (percent organic, percent $CaCO_3$, and percent inorganic) of the sediment, was completed on all cores. New ^{210}Pb dating models were produced for ten of the lakes in this study and the remaining lakes were dated by correlating geochemical sediment markers to cores that were collected from previous MN ENRTF-funded work (M.L. 2016-186-2-04a: Tracking and Preventing Harmful Algal Blooms). Figure 7 shows an example of how this core correlation was completed on Cedar Lake (Morrison Co.) to leverage previously funded work. Age-depth profiles were successfully completed for all 20 lakes in this study and results are summarized in Table 2.

Sediment cyanotoxin extraction

In order to reconstruct the history of toxic HABs and Cylindro in Minnesota lakes, we measured the concentration of both MCY (a toxin produced by several common HABs producing Cyanobacteria) and CYN (a toxin produced by Cylindro) in dated sediment sections in all 20 lakes from this study. This work was done in collaboration with Dr. Matthew Waters at Auburn University, whose laboratory developed these techniques.

Both MCY and CYN were extracted from sediment sections into solution using methanol and then the concentration of each toxin was measured using standard ELISA kits developed by Eurofins Abraxis, Inc. This study represents the first time sediment toxins have been measured in dated sediment cores in Minnesota and is a demonstration of the viability of this method for future work.

MCY was found in the sediments of all but 2 of the lakes in this study (White Iron and Trout), both located in the Canadian Shield, demonstrating its suitability as a proxy for general Cyanobacteria toxicity across a wide variety of lakes (Figure 8). In many of the lakes, MCY was detected for 70-100+ years prior to core collection and patterns followed general trends of eutrophication (increased productivity) of lakes in Minnesota, with the largest increases seen after 1950, concurrent with widespread agricultural intensification and urbanization in the state. Concentrations ranged from 0 to >30,000 ng/g organic matter across all 20 lakes, with the highest concentrations found in the surface sediments where benthic or sedimented Cyanobacteria were likely still involved in active toxin production at the time of core collection.

CYN concentrations were much lower in the lake sediments of this study and were only detected in 40% of the lakes (Figure 9). In all cases, CYN was measured in much lower concentrations than MCY, ranging from 0 to 25 ng/g organic matter. CYN was detected in the surface sediments from both lakes where Cylindro is known to occur (Madison and South Center), however it was also seen in an additional 6 lakes where Cylindro was not detected in this study. Those lakes are spread across three ecoregions and include St. Olaf, Shaokatan, and Carrie from the Cornbelt Plains; Cedar, Peltier, from the Central Hardwood Forest; and Elk from the Northern Lakes and Forests. It is possible that Cylindro is or was present in these systems, however it was not detected via microscopy or from our DNA sequencing analysis. CYN is also known to be produced in low concentrations by the Cyanobacteria genera *Aphanizomenon* and *Dolichospermum*, both of which commonly occur in Minnesota lakes and provides an alternative explanation for the occurrence of these toxins in lakes where Cylindro was not identified.

Cylindro invasion history in Minnesota

The results from this study confirm that the invasion of Cylindro in Minnesota is both very recent and limited to a small number of lakes. No additional lakes were found to contain Cylindro among the 20 Sentinel Lakes selected for this study and evidence for its occurrence was only found in the surface sediments. The next most recent samples for Madison and South Center were dated to 2006 and 2011, respectively, and no toxins were detected at those times. Given this single data point, we cannot yet begin to analyze any trend in occurrence related to temperature or other environmental factors. Unless Cylindro is documented in more locations in subsequent years, it would be more informative to focus on these two confirmed populations to correlate intra- and inter-annual variability in abundance and toxcitiy in these two lakes with climatic and environmental variables.

Results from this study indicate that Cylindro was first detected in Minnesota shortly after its arrival in the Sentinel Lakes and that its movement across the state has been negligible. Currently, the importance of Cylindro in terms of ecological and public health is secondary to the more general issue of toxic HABs in Minnesota lakes. This finding could change, however, as Minnesota lakes continue to warm and phytoplankton monitoring projects which incorporate both microscopy and DNA sequencing will be an important tool to identify the potential spread of Cylindro in the future.

IV. DISSEMINATION:

Description: We will collaborate with our existing state agency partners at MPCA and MN DNR to provide these data in a form that will be publicly available. We will develop scientific reports and factsheets intended to inform managers and lay-persons on the spread of *Cylindro* in Minnesota and its environmental and public-health impacts. Research Station scientists will highlight this work at "Behind the Scenes" events, hosted three times a year by the Science Museum of Minnesota, which is open to all of the Museum's thousands of daily visitors. We will publicize the progress and results of this project via the Research Station's news releases and social media presence as well as through our ongoing collaboration with the University of Minnesota Extension HABs outreach efforts.

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.

First Update January 31, 2019

In cooperation with our communications specialist, Greg Seitz, we produced a short blog on November 6, 2018, to raise awareness of Cylindro and this newly-funded ENRTF project. This blog included a newly produced infographic on Cylindro produced by our Environmental Research Fellow Hailey Sauer (Figure 1), photographs from our Summer 2019 fieldwork, and general information on the issue. This article is available at: https://www.smm.org/scwrs/fieldnotes/invisible-species-exotic-algae-threatens-poison-minnesota-lakes

Second Update June 30, 2019

We continue to engage the public using both social media and conventional media outlets. We regularly use both Twitter and Facebook to share photos regarding the ongoing Cylindo fieldwork. Additionally, Adam Heathcote was asked to participate in a special edition of Minnesota Public Radio's Climate Cast on July 19, 2019 that focused on harmful algal blooms and climate change (https://www.mprnews.org/episode/2019/07/19/conditions-ripe-for-a-record-number-of-algae-blooms). Heathcote also participated in a public event hosted by the Marine Community Library (Marine on St. Croix, MN) where he presented on harmful algal blooms in Minnesota and acknowledged the past and current support of the ENRTF on this topic (see photo at https://twitter.com/AJ_Heathcote/status/1150751280182288389).

Third Update January 31, 2020

Results from this study and previous LCCMR-funded harmful algal blooms work were show-cased at the Minnesota Water Resources Conference held on October 15-16, 2019. PI for this project, Adam

Heathcote, co-chaired a special session on Harmful Algae Blooms at the conference, which ran for the entire afternoon of October 16th. This included Heathcote giving and introductory seminar on Cyanobacteria in Minnesota as well as participating in an expert panel answering audience questions on Cyanobacteria in lakes and streams in the State.

Fourth Update June 30, 2020

Although most of the museum was shutdown for the majority of this reporting period, we did use our online social media platforms to raise public awareness about harmful algal blooms in Minnesota using Twitter and the Museum's website: https://new.smm.org/learn/media/algal-blooms

Fifth Update January 31, 2021

We provided a presentation with preliminary results from this study to the Winter HABs Inter-agency Workshop on January 25th, 2021. This workshop included agency personnel from Minnesota Pollution Control Agency, Minnesota Department of Natural Resources, Minnesota Department of Health, and the Minnesota Veterinary Medical Association and deals specifically with environmental and health concerns about harmful algal blooms.

Sixth Update June 30, 2021:

We produced a journal article that will be submitted in September 2021 for review at PLOS ONE. If accepted, a pdf of the journal article will be included in the next update.

Seventh Update January 31, 2022:

We received comments on a journal article and submitted a revised version to the journal PLOS ONE in December of 2021.

Final Update June 30, 2022:

We have shared the progress and results from this project widely over the duration of the project. This includes both articles written by our staff, shared with traditional and social media, and peer reviewed papers. These efforts are summarized below in chronological order:

- "Invisible" species of exotic algae threatens to poison Minnesota lakes, posted to SMM.org on 11/6/2018, (PDF attached in supplemental materials)
- "Conditions ripe for a record number of algae blooms", Minnesota Public Radio Climate Cast segment on 7/19/2019,
- "Why good algae go bad", talk at the Marine Community Library by Adam Heathcote on 7/14/2019, picture of event on Twitter
- Adam Heathcote Co-chaired special session on Harmful Algal Blooms at the Minnesota Water Conference and organized a panel of experts to take questions from conference attendees in St. Paul, MN on 10/16/2019

- During the pandemic we provided information on harmful algal blooms for the public on the Science Museum website in our "Learn From Home" section, posted on 7/7/2020.
- Preliminary results from this study were presented to the Minnesota Inter-agency HABs group at their Winter Workshop on 1/25/2021. Attendees includes representatives from the Minnesota DNR, MPCA, MDH and the MVMA
- Results from this study were published in the peer-reviewed journal PLOS ONE on 3/21/2022. PDF of paper is attached in the supplemental materials.

V. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview: See attached spreadsheet

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff: N/A

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

Enter Total Estimated Personnel Hours: 3609	Divide by 2,080 = TOTAL FTE: 1.74

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

Enter Total Estimated Personnel Hours:	Divide by 2,080 = TOTAL FTE:

B. Other Funds:

SOURCE OF AND USE OF OTHER FUNDS	Amount Proposed	Amount Spent	Status and Timeframe
Other Non-State $ To Be Applied To Project During Project Period:			
Indirect costs at 40.83% waived by the Science Museum of Minnesota (in-kind)	$ 81,660	$ 80,476	Secured
Other State $ To Be Applied To Project During Project Period:			
	$	$	
Past and Current ENRTF Appropriation:			
M.L. 2016-186-2-04a: Tracking and Preventing Harmful Algal Blooms	$ 500,000	$ 500,000	Spent
M.L. 2015-76-2-10 "Tracking and Preventing Harmful Algal Blooms"	$ 93,000	$ 93,000	Spent
Other Funding History:			
M.L. 2015-76-2-10 "Tracking and Preventing Harmful Algal Blooms"	$ 93,000	$ 93,000	Spent

M.L. 2014-226-2-3g: Watershed-Scale Monitoring of Long-Term Best-Management Practice	$ 900,000	$ 900,000	Spent

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role

VII. LONG-TERM IMPLEMENTATION AND FUNDING:

This project will provide the first baseline data on the distribution of the toxin-producing aquatic invasive species *Cylindro* through space and time. The St. Croix Watershed Research Station is currently collaborating with other research groups in Minnesota on HABs, including the St. Anthony Falls Hydraulics Laboratory, University of Minnesota Extension, and the Natural Resources Research Institute. We will continue that effort so that data collected for this and other ongoing projects will be shared collectively (including standardized protocols and inter-laboratory quality control) to provide the best possible scientific product for the people of Minnesota.

VIII. REPORTING REQUIREMENTS:

- **The project is for 4 years, will begin on 07/01/2018, and end on 06/30/2022.**
- **Periodic project status update reports will be submitted 01/31 and 06/30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2022.**

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet- N/A
D. Acquisition, Easements, and Restoration Requirements- N/A
E. Research Addendum

Figures and Tables

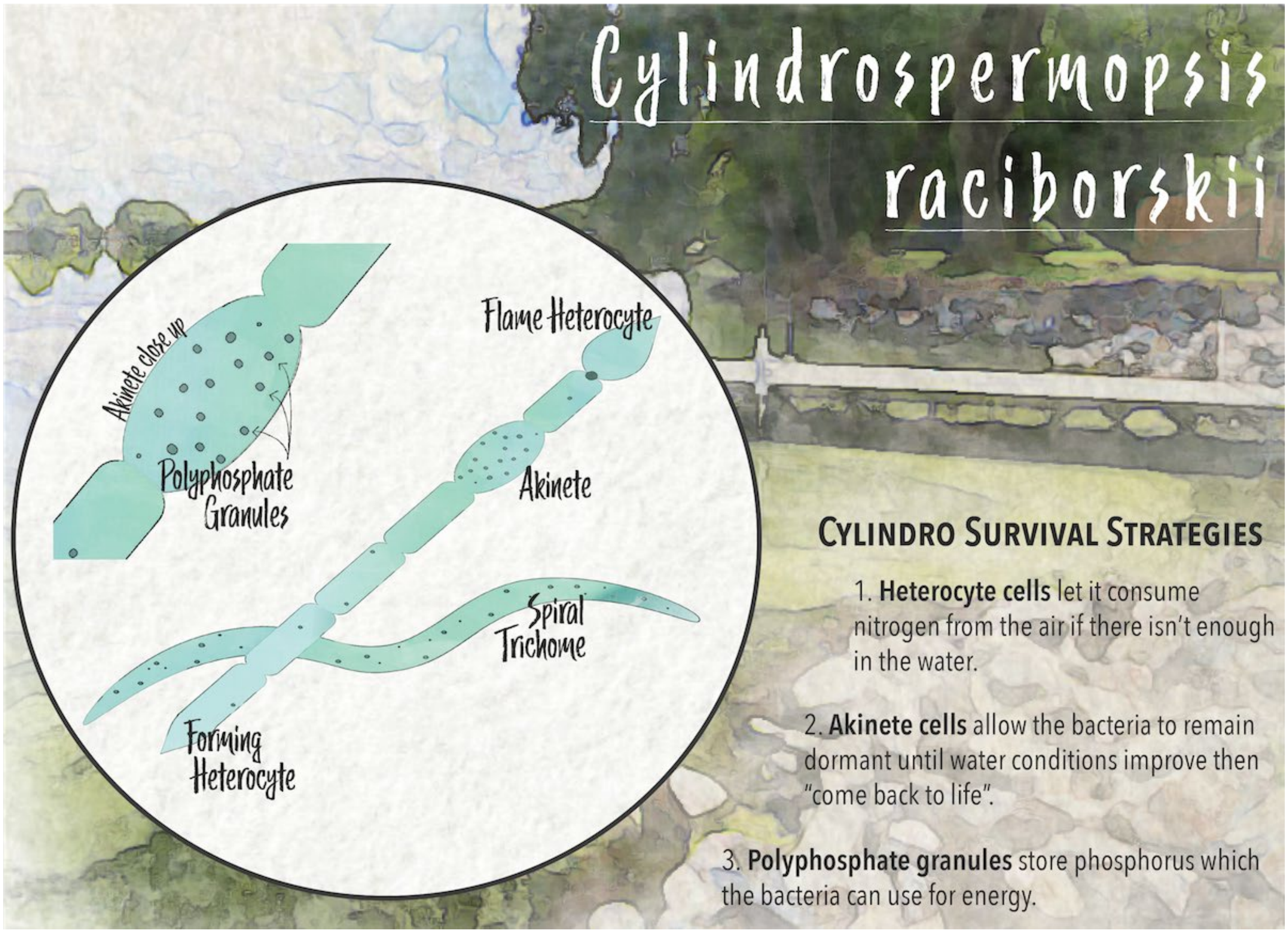


Figure 1. Infographic on the exotic algae *Cylindrospermopsis raciborskii* (Cylindro). This graphic was used in our blog that introduced the project and issue of this exotic algae in Minnesota. Original art was produced by SCWRS' Environmental Research Intern Hailey Sauer.

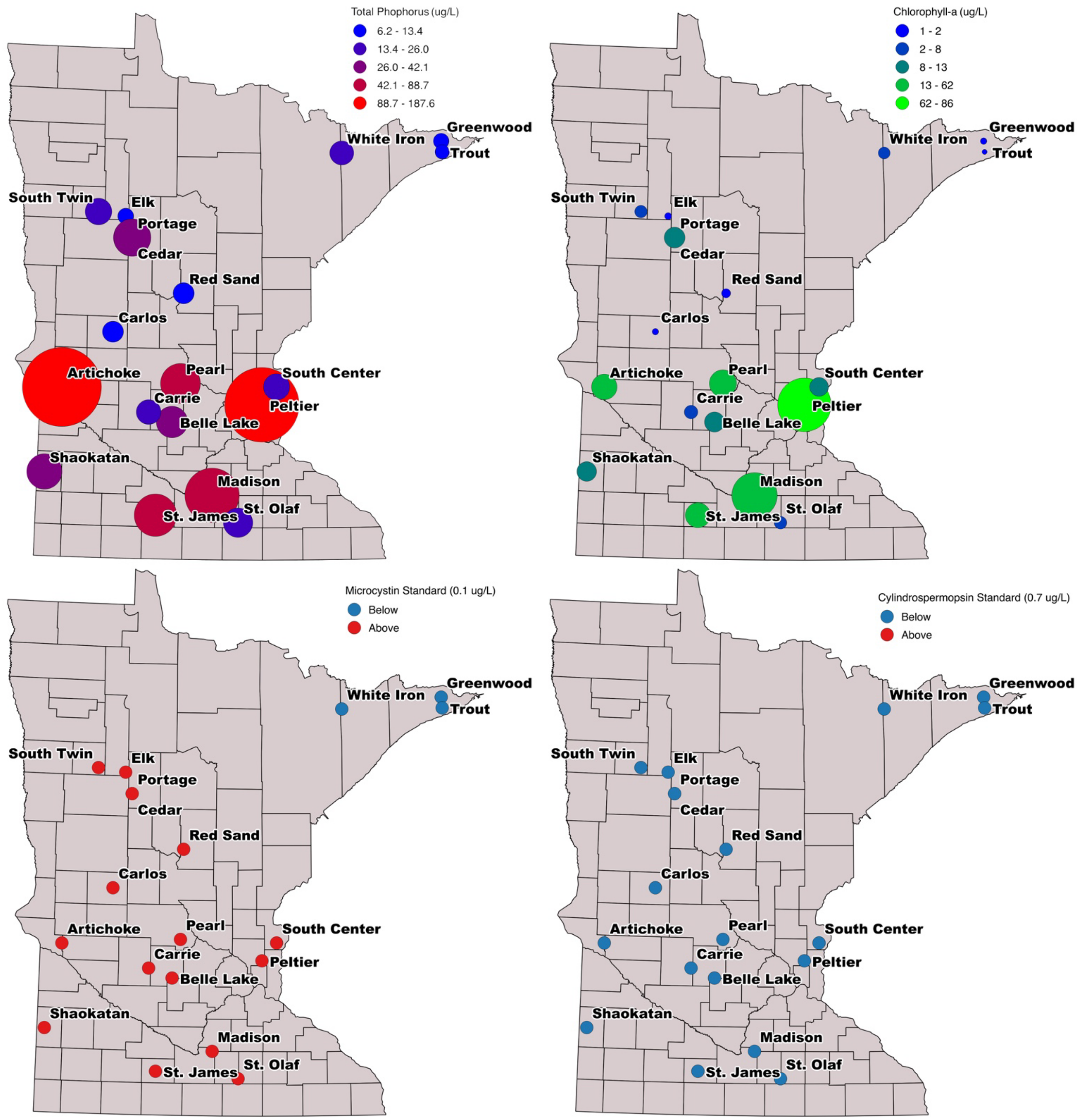


Figure 2. Map of the 20 MN DNR Sentinel Lakes included in this study and water quality parameters measured during August and September of 2018. Top left: total phosphorus concentrations which are a measure of the amount of nutrients available for algae growth; top right: chlorophyll-a concentrations which are a measure of the total biomass of algae in the lake; bottom left: detection of microcystin toxin above or below the minimum drinking standard for Minnesota (0.1 ug/L); bottom right: cylindrospermopsin toxin above or below the EPA minimum drinking standard (0.7 ug/L).

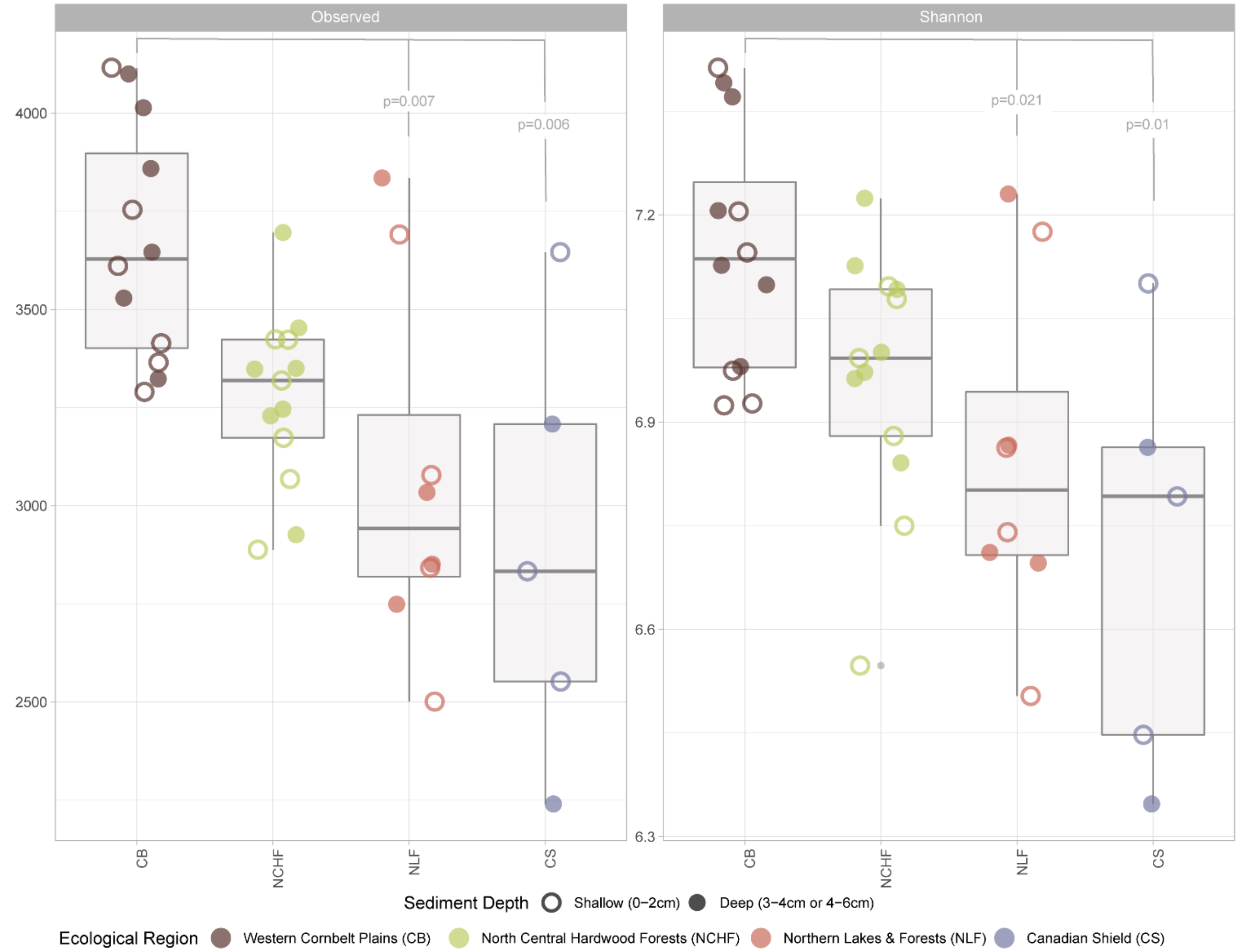


Figure 3. Microbial richness (Observed) and diversity (Shannon) for the surface (shallow, open circle) and adjacent (deep, filled circle) section of sediment cores collected from 20 MNDNR Sentinel Lakes as part of this project. Lakes are colored by their Minnesota ecoregion. Contrary to expectations, lake sediments in the traditionally more impaired Western Cornbelt Plains lakes had the highest microbial richness and diversity whereas lakes in the least impacted Canadian Shield ecoregion had the lowest.

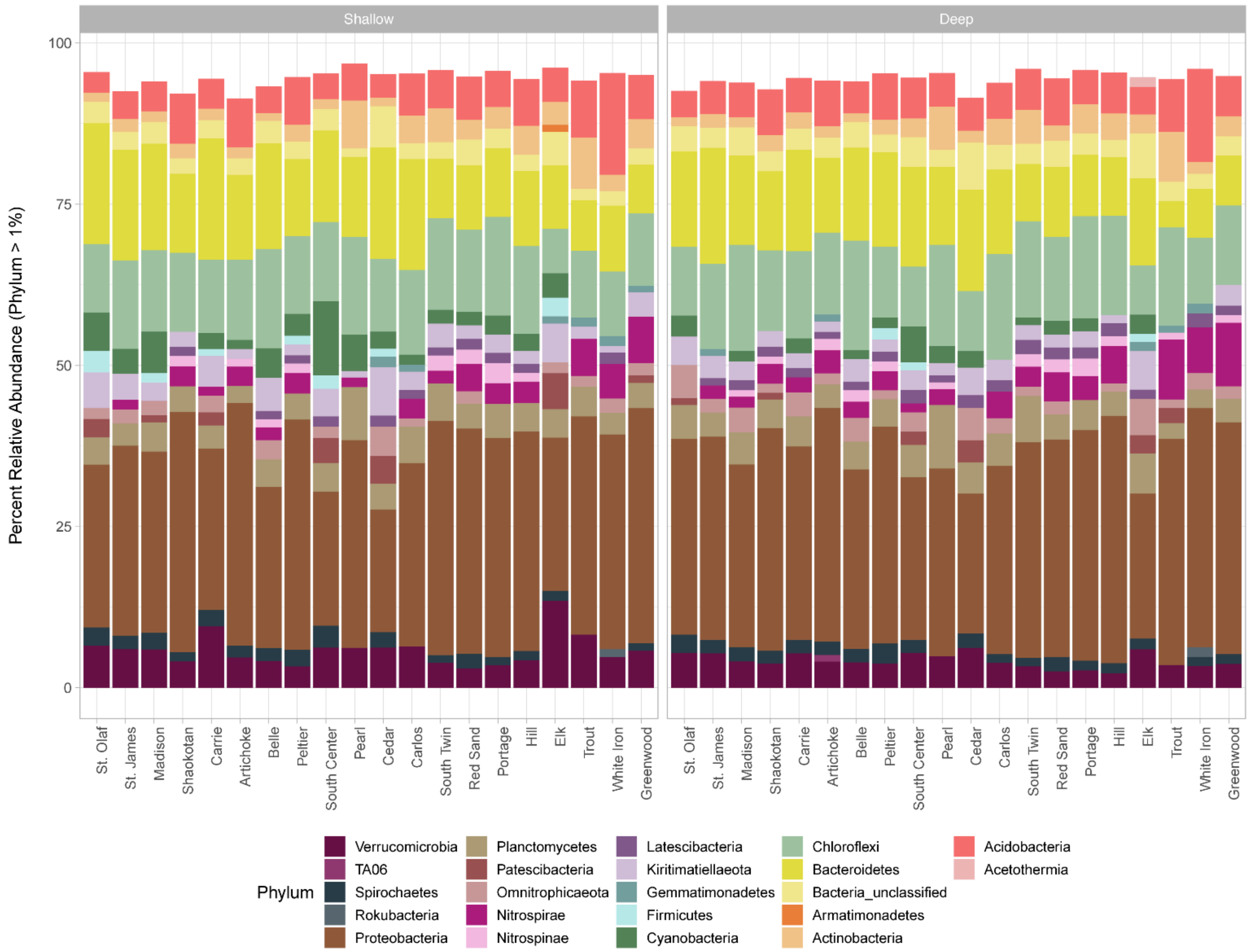


Figure 4. Relative abundance of all microbial phyla found in the surface (shallow) and deeper sediments of the 20 MN DNR Sentinel Lakes sampled as part of this project. This includes the Cyanobacteria phylum (dark green), which includes the genus *Cylindrospermopsis*. This represents the first microbial survey of lake sediments in the Sentinel Lakes.

Figure 5. 16S amplicon sequencing results for Cyanobacteria DNA in surface sediments collected from the Sentinel Lakes in this study. OTUs are assigned to genera using the SILVA freshwater algae database. Species from the genus *Cylindrospermopsis* were only present in the two lakes from this study where *C. raciborskii* had been previously seen (Madison and South Center). No data from White Iron Lake are included because Cyanobacteria DNA was not present in the sample.

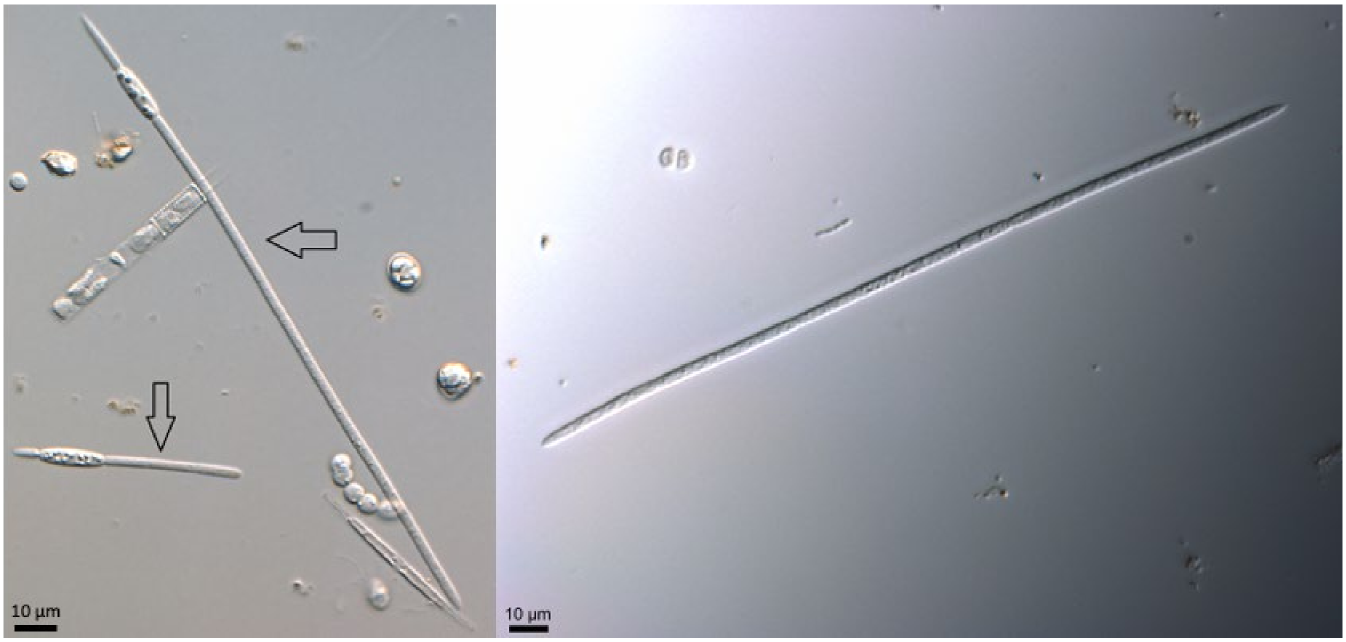


Figure 6. Light micrographs from phytoplankton samples collected from Madison Lake (left, black arrows) and South Center Lake (right) confirming the presence of *Cylindrospermopsis raciborskii* trichomes. Both samples show the typical "flame-tipped" heterocysts that are indicative of Cylindro. Akinetes are also present in the Madison lake population.

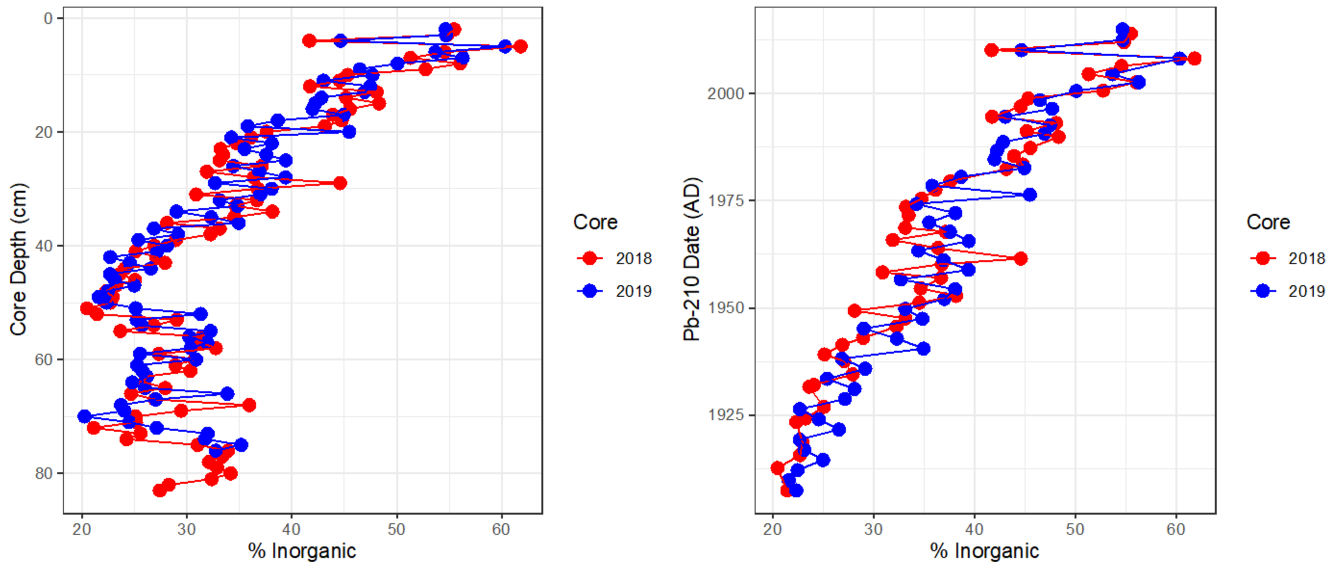


Figure 7. Example of core correlation preformed on the ten cores from this study which had been previously dated using MN ENRTF funding. The 2018 core (red) from Cedar Lake (Morrison Co.) was collected and dated using 210-Pb and the 2019 core (blue) was compared to it using common patterns across both cores in the percent inorganic matter measured via loss-on-ignition.

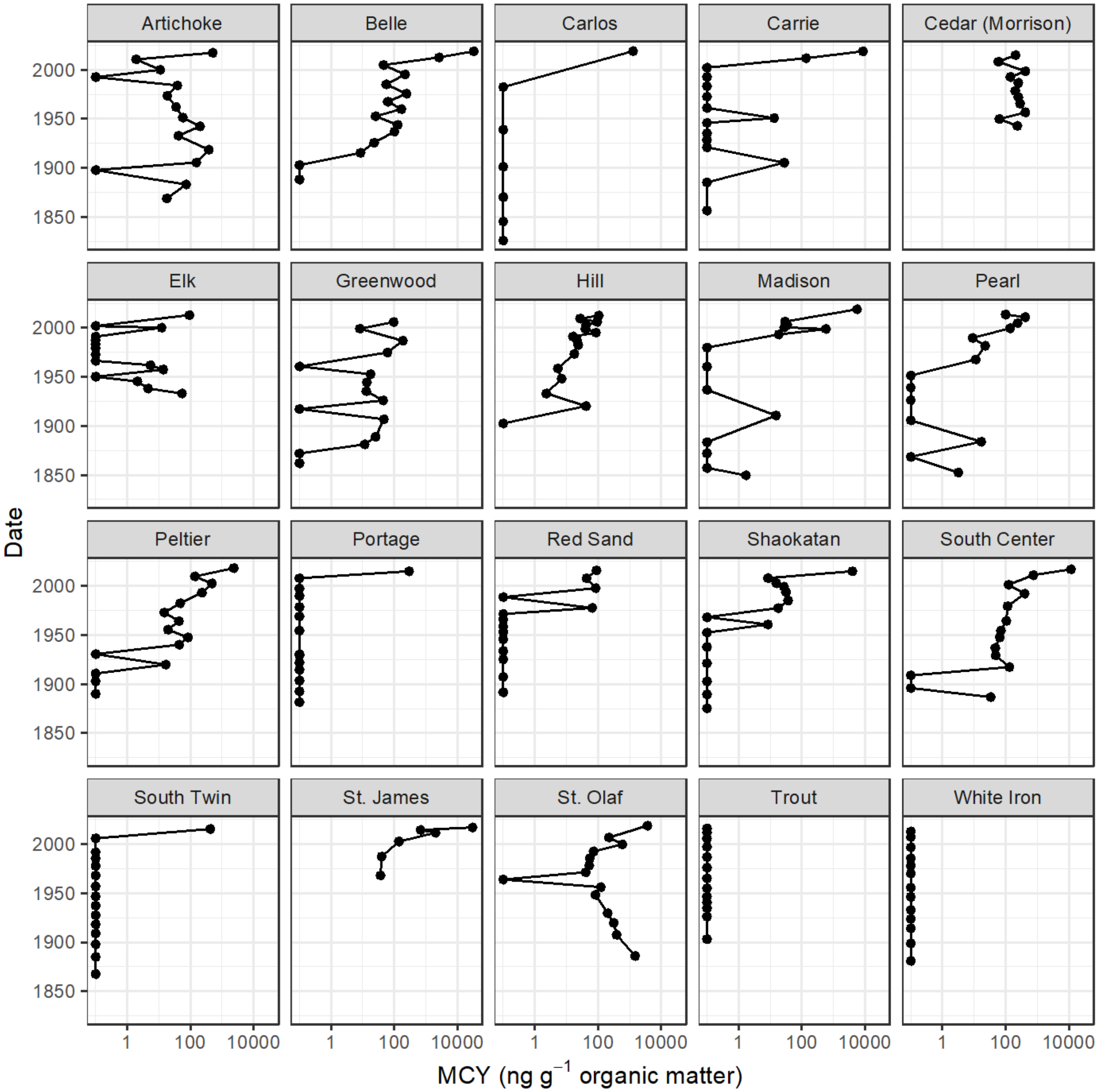


Figure 8. Concentration of the cyanotoxin Microcystin (MCY) in dated sediment cores from all lakes in this study.

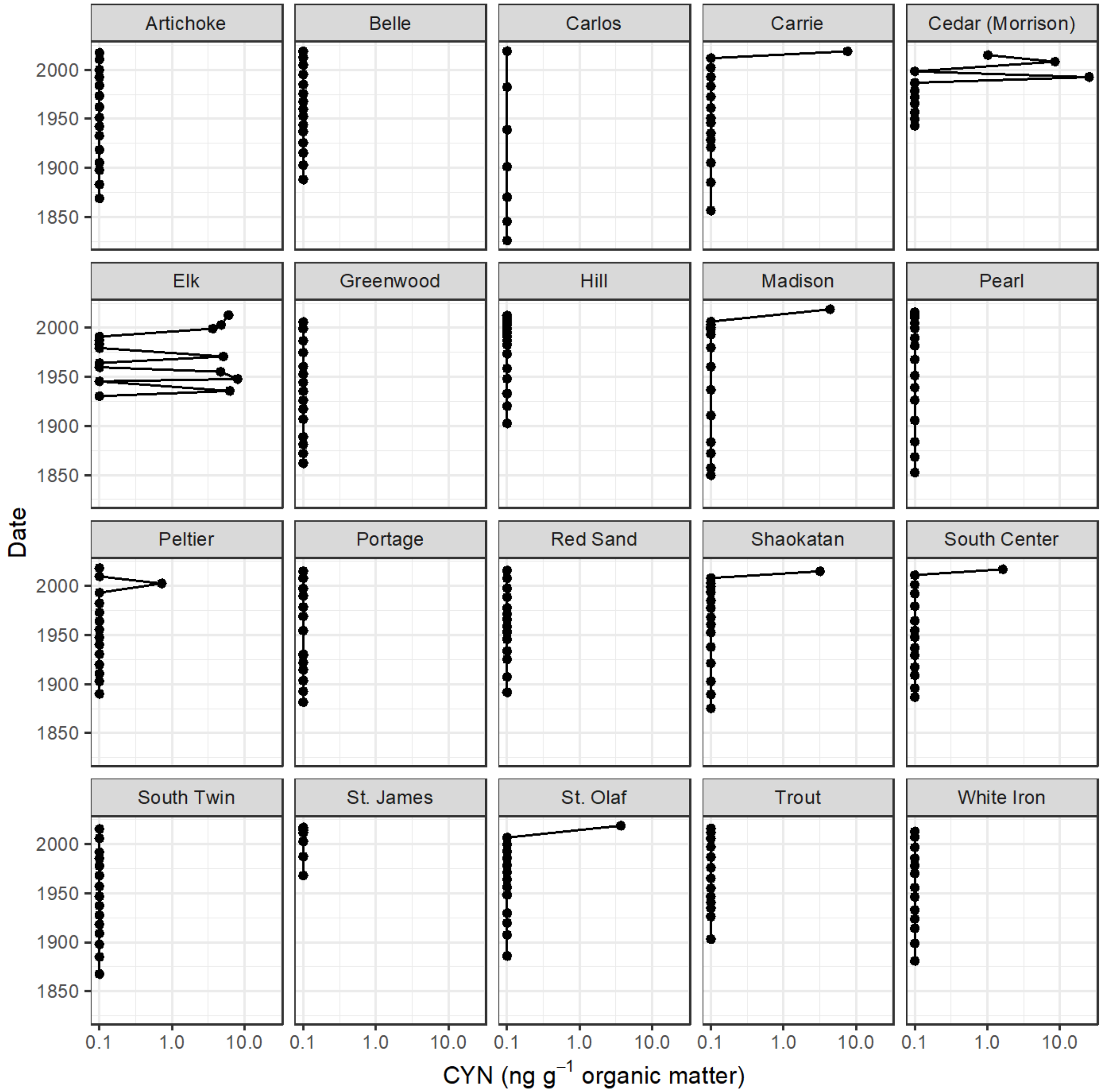


Figure 9. Concentrations of the cyanotoxin cylindrospermopsin (CYN) in dated sediment cores from all lakes in this study.

Table 1. Late summer 2018 water quality and cyanotoxin results from the 20 Sentinel Lakes included in this study. <MDL indicates the result was less than the detection limit.

Lake	Sample	TP (µg/L)	TN (mg/L)	Chl-a (µg/L)	NOx (mg/L)	NH4 (mg/L)	SRP (µg/L)	DIC (mg/L)	DOC (mg/L)	MCY (µg/L)	CYN (µg/L)
Cedar	Epi	12.9	0.6	1.5	<MDL	0.01	<MDL	32.1	6.3	0.1	<MDL
	Hypo	355.9	2.2	0.7	0.00	1.31	311.0	49.5	6.4	0.2	<MDL
Carlos	Epi	13.3	0.6	1.2	<MDL	<MDL	<MDL	34.4	7.0	0.3	<MDL
	Hypo	9.9	0.7	0.3	0.07	0.05	<MDL	38.5	6.5	0.4	<MDL
Pearl	Epi	47.8	0.9	22.1	<MDL	<MDL	<MDL	33.2	5.7	3.6	<MDL
Red Sand	Epi	13.4	0.7	2.5	<MDL	0.01	40.4	10.7	8.2	0.4	0.1
Portage	Epi	42.1	1.0	13.0	<MDL	<MDL	43.2	28.5	6.2	2.8	<MDL
South Twin	Epi	21.3	0.7	4.6	<MDL	<MDL	22.0	27.9	7.2	0.7	<MDL
Elk	Epi	7.7	0.5	1.4	<MDL	<MDL	34.9	33.1	6.7	0.2	<MDL
	Hypo	57.9	1.1	1.6	0.01	0.54	32.7	40.6	6.6	0.2	<MDL
Hill	Epi	18.0	0.7	7.6	<MDL	<MDL	8.1	29.9	9.3	0.3	<MDL
	Hypo	112.7	0.8	1.2	<MDL	0.22	69.6	37.6	8.2	0.3	<MDL
Peltier	Epi	166.7	2.3	85.9	<MDL	<MDL	5.0	26.7	14.2	0.9	<MDL
South Center	Epi	20.9	1.1	10.6	<MDL	<MDL	<MDL	11.1	9.6	0.7	<MDL
	Hypo	293.5	2.0	0.9	0.01	0.91	167.5	16.2	10.6	0.4	<MDL
White Iron	Epi	17.3	0.5	4.2	0.03	0.01	<MDL	4.9	14.3	<MDL	<MDL
	Hypo	22.3	0.7	0.7	0.13	0.02	6.0	6.2	14.7	<MDL	<MDL
Trout	Epi	6.2	0.1	0.8	<MDL	0.01	<MDL	4.3	4.0	<MDL	<MDL
	Hypo	10.2	0.3	0.9	0.04	0.01	<MDL	4.3	3.5	<MDL	<MDL
Greenwood	Epi	7.4	0.2	1.3	<MDL	0.01	<MDL	2.3	5.2	<MDL	<MDL
	Hypo	9.9	0.3	0.6	0.02	0.00	<MDL	2.5	4.6	<MDL	<MDL
Belle	Epi	29.8	1.0	12.1	<MDL	0.00	<MDL	31.5	10.0	3.9	<MDL
Carrie	Epi	18.3	2.3	5.5	1.20	0.31	<MDL	47.8	7.8	1.6	<MDL
Artichoke	Epi	187.6	1.6	19.5	0.01	0.03	114.6	46.2	15.1	0.3	<MDL
Shaokatan	Epi	37.8	1.0	11.6	<MDL	<MDL	<MDL	38.3	9.1	0.2	<MDL
St. James	Epi	54.1	1.4	18.8	0.07	0.02	<MDL	35.5	9.0	8.8	<MDL
Madison	Epi	88.7	1.6	61.9	<MDL	<MDL	3.1	31.0	10.0	0.9	<MDL
St. Olaf	Epi	26.0	0.7	4.8	<MDL	<MDL	<MDL	23.7	7.0	0.3	<MDL
	Hypo	460.4	6.5	20.3	<MDL	4.23	183.2	46.2	10.7	2.1	<MDL

Table 2. Summary of core-dating results for the 20 lakes from this study.

Lake	Dating Method	Oldest Age (AD)	Depth of Oldest Age (cm)
Artichoke	210-Pb	1845	66
Belle	210-Pb	1859	120
Carlos	Correlation	1836	36
Carrie	210-Pb	1833	150
Cedar	Correlation	1907	50
Elk	Correlation	1853	55
Greenwood	210-Pb	1852	21
Hill	210-Pb	1869	35
Madison	Correlation	1842	186
Pearl	Correlation	1836	41
Peltier	210-Pb	1860	188
Portage	Correlation	1865	49
Red Sand	210-Pb	1859	48
Shaokatan	Correlation	1860	44
South Center	Correlation	1887	92
South Twin	210-Pb	1731	46
St. James	Correlation	1972	14
St. Olaf	210-Pb	1824	168
Trout	Correlation	1831	20
White Iron	210-Pb	1804	43

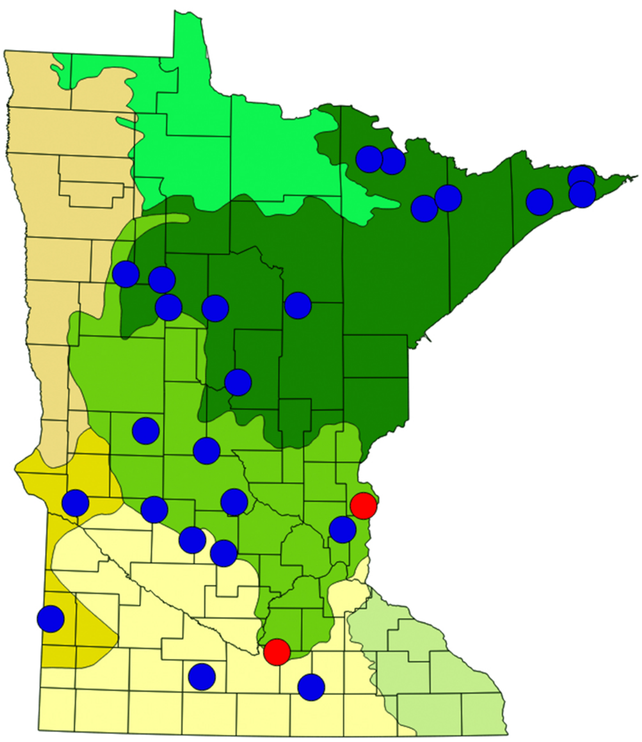
Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Budget Spreadsheet-Final

Project Title: Determining Minnesota's risk of a toxic algal invader
Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06f
Project Manager: Adam Heathcote
Organization: Science Museum of Minnesota
College/Department/Division: St. Croix Watershed Research Station
M.L. 2018 ENRTF Appropriation: $200,000
Project Length and Completion Date: 4 years, June 20, 2022
Date of Report: 8/15/2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	TOTAL BUDGET	TOTAL SPENT	TOTAL BALANCE
BUDGET ITEM			
Personnel (Wages and Benefits)	$115,800	$115,800	$0
Heathcote, Associate Scientist: Phyto & Cylindro toxins; 33% FTE for 2 yrs; Salary=71%, Benefits=29% ($56,300 over 2 years); this is a grant-funded position			
Edlund, Senior Scientist: Phyto & Cylindro toxins; 33% FTE for 1.5 yrs; Salary=71%, Benefits=29% ($48,400 over 1.5 years); this is a grant-funded position			
Field and Laboratory Technician: Field work and lab analyses; 25% FTE for 1 yr; Salary=71%, Benefits=29% ($11,100 over 1 years); this is a temporary position			
Professional/Technical/Service Contracts			
Equipment/Tools/Supplies			
Field and laboratory supplies: including bottles, reagents, calibration solutions, core tubes, sample cups	$4,002	$4,002	$0
Cyanotoxin ELISA kits: Cylindrospermopsin (22 @ $600 = $13,200) Microcystin (22 @ $600 = $13,200)	$26,400	$26,400	$0
Capital Expenditures Over $5,000			
Printing			
Travel expenses in Minnesota			
Field travel to 20 lakes for monitoring Hotel: 2 persons for 8 nights @ $76.50 = $1,224 Per diem: 2 persons for 8 days @ $36 = $576 Mileage & gas: 3,530 miles @ $0.545 = $1,924	$3,724	$3,724	$0
Field travel to 20 lakes for paleolimnology Hotel: 2 persons for 12 nights @ $76.50 = $1,836 Per diem: 2 persons for 12 days @ $36 = $864 Mileage & gas: 3,530 miles @ $0.545 = $1,924	$4,624	$1,723	$2,901
Other			
Lab analysis of water samples: TN/TP: 20 @ $36 DIN/SRP: 20 @ $36 DOC: 20 @ $20 DIC: 20 @ $15 Fe: 20 @ $36 SO4: 20 @ $36 CYN toxin: 20 @ $50 MC-LR toxin: 20 @ $44	$5,460	$5,460	$0
Lab analysis of sediment samples: 210-Pb (dating): 10 @ $2,250 ($22,500) loss-on-ignition: 20 @ $800 ($16,000)	$38,500	$38,500	$0
QA/QC of ELISA cylindrospermopsin sediment samples via HPLC (inter-lab comparison via University of Minnesota or competitive bid) ($1490)	$1,490	$1,490	$0
COLUMN TOTAL	**$200,000**	**$197,099**	**$2,901**

Cylindro: Minnesota's microscopic lake invader

What is the range, abundance, and toxicity of *Cylindro*?



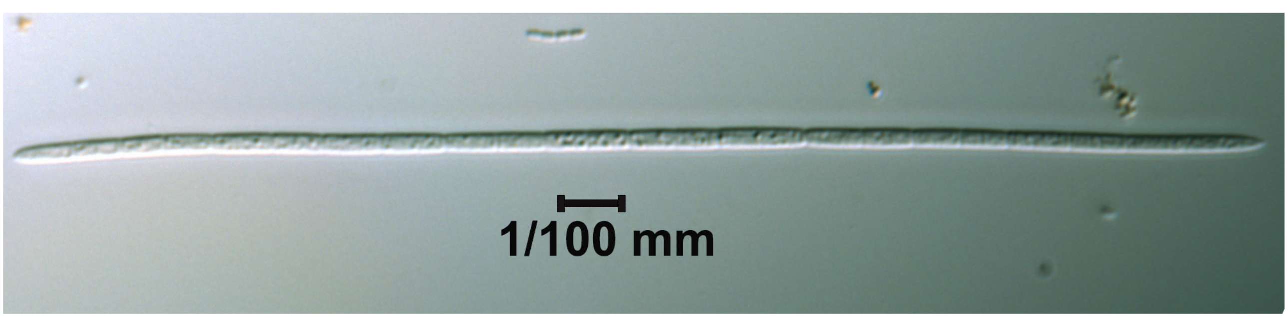
What is *Cylindro*?

a toxin-producing blue-green alga that has invaded MN lakes

Where has it been found?

Cylindro found in two lakes in 2013 and 2014 (red dots). Blue dots show other lakes to be surveyed by this project

CYN Toxin: Hepatotoxin produced by *Cylindro* is harmful to humans and pets



Cylindrospermopsis raciborskii: It may not look like much, but *Cylindro* (above) is an exotic species known to produce four different toxins. This specimen was seen by Research Station scientists in a Chisago County lake in 2015