BUILDING A COLLABORATIVE MONITORING STRATEGY FOR A CHANGING ST. LOUIS RIVER ESTUARY:

A RECOMMENDATION REPORT

BUILDING A COLLABORATIVE MONITORING STRATEGY FOR A CHANGING ST. LOUIS RIVER ESTUARY: A RECOMMENDATION REPORT

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***Photo:** Green streaks of a cyanobacteria bloom in the St. Louis River Estuary, near Barker's island Swimming Beach on October 2, 2023.*


THE NORTH FACE

EXECUTIVE SUMMARY

The research results and monitoring recommendations presented in this report are a collaborative effort among water quality monitoring experts, scientists, and estuary caretakers. We share a motivation that stems from the anticipated 2030 delisting of the St. Louis River as a Great Lakes Area of Concern, and the pressing need to chart a path forward to develop a long-term monitoring plan. We offer this report as a first step toward building a more coordinated, efficient, and future-focused water quality monitoring program—one that supports the unique St. Louis River Estuary that serves as the foundation for the health, livelihoods, economies, and sense of place of the Twin Ports community and the broader Lake Superior watershed.

This project was led by the Lake Superior National Estuarine Research Reserve (LSNERR) and University of Minnesota's Natural Resource Research Institute (NRRI). The following document presents foundational scientific information on the environmental predictors of cyanobacteria abundance and characterizes harmful algal blooms, phytoplankton dynamics, nutrient conditions, decadal trends in water quality, and hypoxia in the SLRE. From these findings, we offer monitoring recommendations on where, when, and how to focus future efforts. Some are highly specific, such as proposed locations for monitoring stations, while others are broader programmatic considerations.

The data generated through this effort supports not only the recommendations herein, but also ongoing and future research. It will contribute to a Master's thesis (for Peter Birschbach) and is expected to inform several peer-reviewed publications.

All data generated by this project are publically available:

Nicklay, H., E.D. Reavie, C. Filstrup, P. Birschbach, and A. Knoll. 2025. St. Louis River Estuary Phytoplankton and Water Quality, 2022 - 2024 ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/cf58e8c6af8a79077bf4330d60a6032c.

ACKNOWLEDGMENTS

This work was made possible through support from the National Estuarine Research Reserve System Science Collaborative (University of Michigan, NOAA's Office for Coastal Management), which funded not only the scientific components, including data collection, processing, and analysis, but also the meaningful conversation among the collaborative team throughout the project. Additional funds from NRRI's Technology Review Board (Permanent University Trust Fund, University of Minnesota) supported additional data analysis and drafting of this report.

In addition to all our collaborative team members, several individuals were instrumental in accomplishing this work. We thank members of the NRRI's Central Analytical Laboratory, Elena Ceballos, Mia Hallet, Eva Hendrickson, Jerry Henneck, Shawnee McMillian, and Becca Marston, for their efforts in processing and analyzing water quality samples associated with this project. We also thank, from the LSNERR, Noah Pinsonnault and Addison Knoll for their significant contributions collecting and processing samples and Sam Blackburn for his contribution to the long-term data analysis.

***Photo:** Peter Birschbach sampling a cyanobacteria surface scum on the St. Louis River Estuary near the mouth of the Nemadji River on October 3, 2023.*

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Cover photo

Field crewmembers, Dane Polkinghorne and Peter Birschbach in Allouez Bay collecting an intergrated water sample.

ABSTRACT

As the St. Louis River Estuary (SLRE) approaches delisting as a Great Lakes Area of Concern (AOC), emerging stressors, particularly cyanobacteria blooms, raise new questions about the estuary's long-term condition. To investigate these concerns, we conducted intensive water quality and phytoplankton monitoring across eight sites in 2023 and 2024, selected as "hotspots" based on their susceptibility to nutrient enrichment, hypoxia, and bloom formation. Our approach combined high-frequency routine sampling with targeted analyses to identify key drivers of cyanobacteria biovolume and assess spatial and temporal redundancies in monitoring design.

We observed multiple small, ephemeral cyanobacteria blooms across both years, with diverse community composition, and documented a widespread lower estuary bloom in October 2023 dominated by *Aphanizomenon flos-aquae*. Random Forest Analysis identified low total nitrogen, elevated temperatures, low dissolved organic carbon, and high pH as the strongest predictors of cyanobacteria biovolume. Bloom development was further linked to late-summer drought conditions and calm wind conditions. Nonmetric multidimensional scaling analysis revealed spatial overlap in environmental and phytoplankton patterns, supporting a streamlined routine sampling design focused on fewer locations and parameters for regular monitoring. We recommend a shift from evenly timed, year-round sampling across all sites to a more flexible strategy that intensifies sampling during late summer at high-risk locations, with a refined set of core parameters.

By providing foundational insights into phytoplankton dynamics and evaluating monitoring design, this work supports the development of a collaborative monitoring strategy for a changing SLRE.

INTRODUCTION

The SLRE at the headwaters of Lake Superior is a bustling commercial port and popular point of connection and recreation for nearby communities and visitors. It is also the ancestral homeland of the Fond du Lac Band of Lake Superior Chippewa, who retain Ceded Territory treaty rights to hunt, fish, and gather. Over 100 years of unregulated industrial and municipal activity left a legacy of contamination and habitat degradation in the SLRE that compromised ecological and human health and treaty-protected practices. In response, remediation and restoration efforts, ranging from contaminated sediment removal to wetland and aquatic habitat restoration have been implemented. These actions have helped revitalize fish and wildlife populations and manoomin (wild rice) beds, thereby supporting Indigenous cultural practices and improving overall ecosystem function. More people are using the estuary for recreation, including activities such as walking and hiking, swimming, cultural and aesthetic appreciation, picnicking and relaxation, and wildlife viewing (Wick et al. 2024).

The SLRE is a complex urban–natural interface that falls under the stewardship of multiple city, state, federal, and tribal agencies. These entities share a vested interest in understanding the impacts of land use, urbanization, and climate change on the estuary and Lake Superior, as well as addressing existing water quality concerns. However, a lack of foundational knowledge about the causes of degraded conditions, particularly those that lead to HABs, has created a barrier to informed, coordinated management. In response to this knowledge gap, a breakout session held at the 2019 St. Louis River Summit, attended by research and management professionals from twelve regional organizations, identified the need for a "comprehensive program of observations, analyses, and public reporting" that is collaborative, transparent, and adaptive to emerging threats (Hollenhorst and Axler 2019). A key recommendation from that session was the development of a scientifically vetted, repeatable sampling design for long-term monitoring in the SLRE. Many people from that original Summit Session joined this project's collaborative team and confirmed that a recommendation report (such as this one) would serve as a valuable tool to initiate a shared, flexible monitoring program that can evolve with future conditions and needs.

Guided by our collaborative team, this project sought to address two overarching questions:

1. **How do environmental conditions contribute to degraded algal communities?**
2. **What should a future monitoring program look like?**

The St. Louis River was designated in 1987 as an AOC due to significant environmental degradation from local sources before environmental regulations existed. It is one of 43 historically contaminated areas in the Great Lakes (MPCA and WDNR 2013). Major beneficial use impairments (BUIs) included restrictions on dredging due to contaminated sediment, lost fish and wildlife habitat, excess sediment and nutrient loads, fish consumption advisories and degradation of benthos, among others. Specifically, the SLRE's Beneficial Use Impairment (BUI) #6 is titled "Excessive Loading of Sediment and Nutrients." This is a site-specific modification of the "Eutrophication or Undesirable Algae" BUI identified in the Great Lakes Water Quality Agreement. Removal of this BUI requires confirmation that nutrient and sediment levels no longer impair water quality or habitat. It also requires that these levels do not restrict recreational uses such as fishing, boating, or swimming in the SLRE and western Lake Superior. Studies show that remediation and restoration efforts, including advanced wastewater management, have improved overall water quality, especially in well-mixed portions of the SLRE (Alexson et al. 2018; Bellinger et al. 2016).

In 2020, all management actions associated with this impairment were complete and BUI #6 was removed (U.S. EPA 2020).

Unfortunately, past harms are not the only threat to the ecological health of the SLRE. Despite progress improving conditions from legacy contamination and habitat destruction, recent water quality and phytoplankton research signals ongoing and emerging issues, including estuary “hotspots” experiencing higher than average nutrient levels, cyanobacteria blooms and hypoxia (Reavie 2020). Harmful algal blooms (HABs), defined as cyanoacteria blooms that produce toxins, are one concern. HABs represent one of the greatest threats to freshwater ecosystems and human health worldwide (Brooks et al. 2016). Freshwater researchers predict that HABs will increase due to more intense rainfall events (Nepal and Parajuli 2025), extended droughts (Cantwell 2021), and warming temperatures (Paul 2008), all of which are considered modern SLRE stressors. Paleolimnological records indicate that some shallow estuary locales have increasing nutrient loads (based on diatom-inferred total phosphorus) and cyanobacteria abundance (based on subfossil pigments; Alexson et al. 2018). Further, research indicates that storm events flush nutrients and algal propagules into Lake Superior from its many estuaries and tributaries, potentially fostering cyanobacteria blooms in Lake Superior (Reinl et al. 2020; Sterner et al. 2020; Hauser 2018).

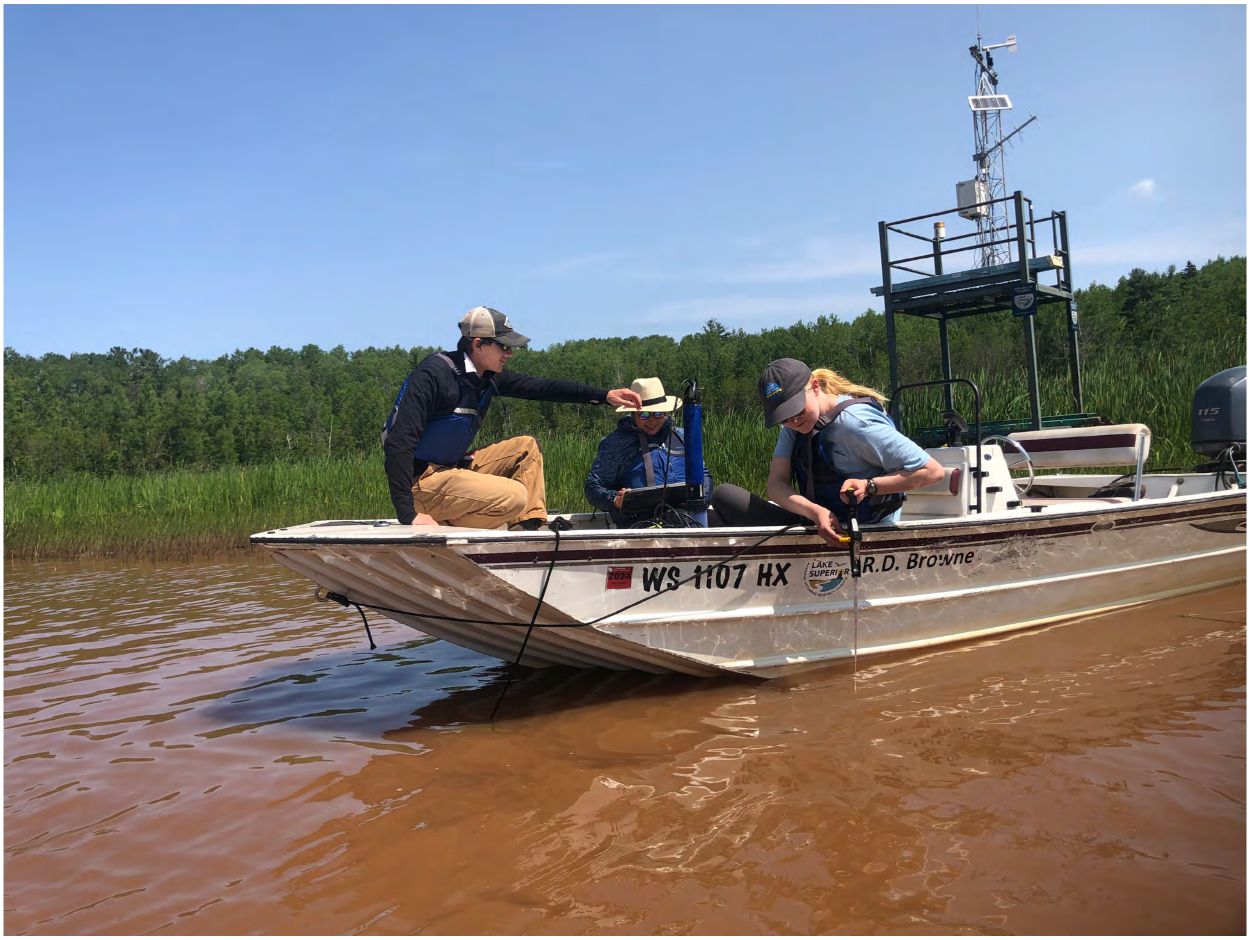
Recent observations suggest that cyanobacteria blooms in recreational areas of the SLRE are becoming more frequent (Green 2021). Because historical bloom monitoring was limited, especially for short-lived surface blooms, it is difficult to determine whether bloom frequency is truly increasing or simply being reported more often. Nonetheless, cyanobacteria blooms are clearly a current issue in the estuary and one that could worsen with climate change. Late-summer blooms have been noted in the Lower Harbor in 2018, 2021, 2023, and 2024 (Lafrancois and Reinl 2025), with the first confirmed toxic HAB detected by LSNERR staff at the Barker’s Island swimming beach in 2021. Given the SLRE’s status as a popular destination for fishing and recreation, there is substantial potential for ecological and human health impacts if HABs persist or become more frequent or more toxic. For example, cyanobacteria blooms can negatively affect fisheries by contributing to low dissolved oxygen conditions (via microbial respiration of high cyanobacteria biomass) and by lowering energy transfer to zooplankton and ultimately fish (i.e., stunted fisheries; Heathcote et al. 2016; Steinkopf et al. 2024). Despite this risk, the research community has not yet quantified or characterized the nutrient dynamics or environmental conditions that facilitate cyanobacteria blooms or HABs within the SLRE.

Climate-driven changes, such as warming temperatures and more frequent, intense rainfall events and droughts, are expected to degrade water quality and shift nutrient dynamics in ways that may favor HABs in the St. Louis River Estuary (WICCI 2021). Blooms in general are influenced by environmental factors including water temperature, thermal stratification, and light availability, and are often fueled by excess nutrients, particularly nitrogen and phosphorus. In many nutrient-rich (eutrophic) freshwater systems, nitrogen limitation during late summer can give a competitive advantage to nitrogen-fixing (diazotrophic) cyanobacteria such as *Dolichospermum* and *Aphanizomenon,* which can use atmospheric nitrogen when dissolved forms are scarce (Fogg 1971). Warm water also promotes cyanobacteria growth (Paerl and Huisman 2008), though recent studies show exceptions to this pattern (Reinl et al. 2023).

Emerging evidence also points to the potential role of seasonal hypoxia as a contributor to HAB risk in the SLRE. Low-oxygen conditions can release phosphorus and other nutrients from sediments, priming the system for bloom formation under the right conditions (Nürnberg 1984; Garono and Schooler

2015). Localized hypoxic events, especially in winter, have been observed (Garono et al. 2025), but little is known about whether these low-oxygen conditions trigger nutrient release from sediments or influence phytoplankton dynamics.

Understanding these complex physical, chemical, and biological dynamics is critical for developing a responsive, cost-effective monitoring program that can inform future management decisions for the SLRE. Other Great Lakes systems offer useful context but are not direct analogs. In western Lake Erie, substantial research has led to models that help predict HABs and mitigate risks to human health, particularly from *Microcystis*-produced microcystin (a liver toxin) in drinking water (Stumpf et al. 2016; Jetoo et al. 2015). However, the SLRE differs from Lake Erie in several important ways, including its lower overall nutrient load and different nutrient ratios. These differences highlight the need for region-specific research, monitoring strategies, and management tools.



Field crewmembers, Arianna Northbird, Noah Pinsonnault, and Addi Knoll in Pokegama Bay with the Lake Superior NERR's weather station in the background.

METHODS

This study's selected sampling locations, frequency, parameters, and analytical approaches support both foundational algal research and evaluation of monitoring efficiencies. Methods are briefly described below, but further details are located in **Appendix A**.

SITE SELECTION

Sampling locations were selected based on several "hotspot" criteria, including previously documented hypoxia (Gorano and Schooler 2015), degraded phytoplankton communities (increases in cyanobacteria and high-nutrient diatoms; Alexson et al. 2018), cyanobacteria blooms, and nutrient enrichment (Bellinger et al. 2016). Other considerations included proximity to impaired urban streams and stormwater outfalls, and recent or planned remediation and restoration projects (**Table 1**). The collaborative team listed many candidate locations based on these criteria then collectively narrowed down the selection to eight sites, ensuring they spanned anticipated gradients in SLRE water quality (**Figure 1**). Two sites, Barker's Island and Pokegama Bay are also LSNERR System-wide Monitoring Program (SWMP) sampling locations, where continuous (15-minute) water quality and monthly nutrient and chlorophyll-a samples have been collected from 2013-2024.

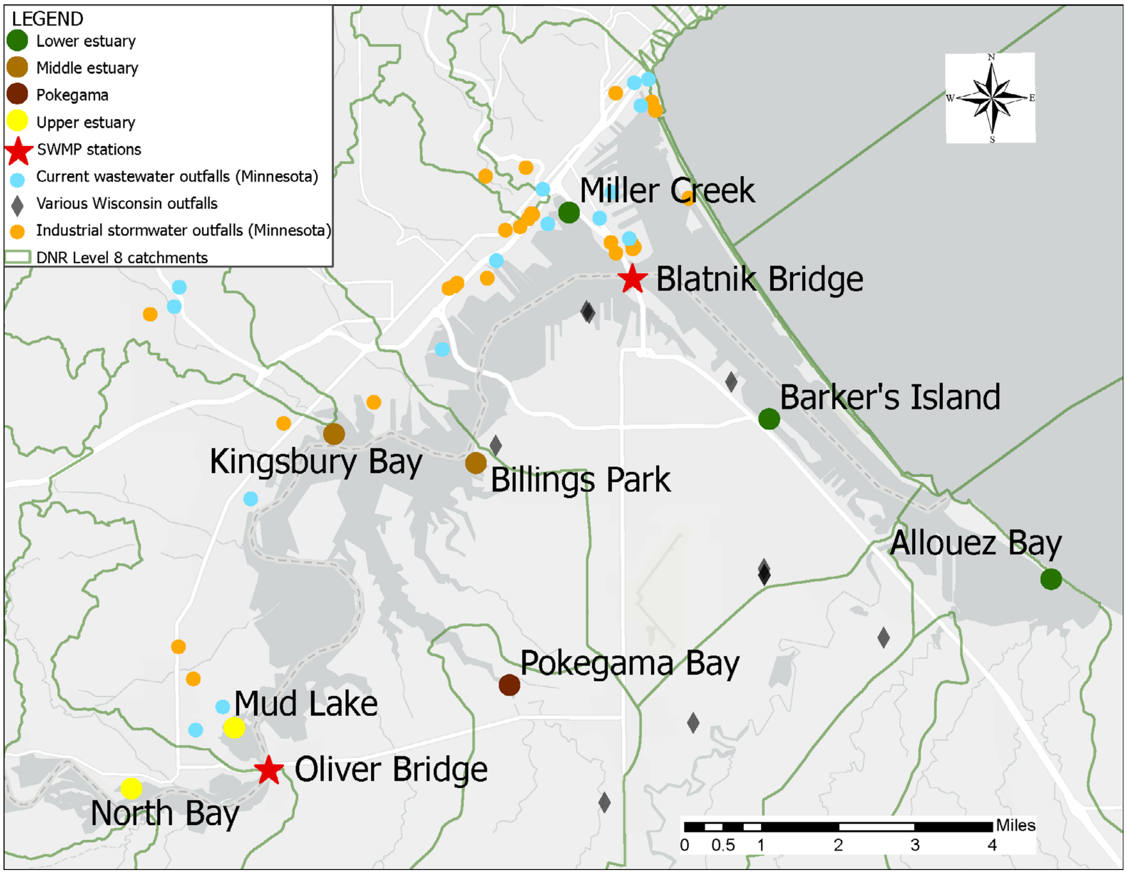


Figure 1: *St. Louis River Estuary map showing the eight project sample locations (large circles), additional long-term monitoring stations from the System-wide Monitoring Program (stars), catchment regions and documented industrial and wastewater outfalls. Outfall data were sourced from the Wisconsin DNR's Surface Water Data Viewer (***https://dnr.wisconsin.gov/topic/SurfaceWater/swdv***) and the Minnesota Natural Resource Atlas (***https://mnatlas.org***).*

Table 1: Study sites in the SLRE and their associated "hotspot" characteristics that informed site selection. Characterization is based on what was known prior to the study when site selection took place.

Sampling Location	Site Code	Hypoxia	HAB	Nutrient Enrichment	Proximal to Impaired Water	Current or Planned Remediation or Restoration
Allouez Bay	AL	Y	N	Y	N	Y
Barker's Island	BA	N	Y	Y	Y	Y
Miller Creek	MI	N	N	Y	Y	Y
Billing's Park	BI	Y	N	N	N	N
Kingsbury Bay	KI	N	N	N	Y	Y
Pokegama Bay	PO	Y	N	Y	Y	N
Mud Lake	MU	Y	N	N	Y	Y
North Bay	NO	N	N	N	Y	Y

SAMPLING DESIGN - ROUTINE AND RESPONSE

Routine sampling was conducted at all eight sites during the 2023 open water season. Sampling frequency varied with expected cyanobacteria phenology: biweekly during spring and late fall, and weekly during the bloom-prone summer months (July–early October). In 2024, open water routine sampling was reduced to biweekly through August 13. Winter sampling was planned monthly for both years and was completed in winter 2022-2023. Low ice cover in winter of 2023 to 2024 limited safe access; therefore, weekly to biweekly under ice sampling was conducted at Barker's Island and Billing's Park only. Overall, 50 routine sampling events were completed, 28 of which included all eight sites. Routine sampling events co-occurred with additional SWMP sampling at two non-hotspot locations at Blatnik Bridge and Oliver Bridge once per month during the summer (May-October). For one routine sampling event on July 11-12 2023, we sampled the eight hotspot locations and four additional thalweg sites (HWY 23, Spirit Lake, Bong Bridge, and Superior Bay) concurrent with Fond du Lac's Water Resources Program's sampling at five SLR locations within the Reservation, to provide a snapshot of larger spatial variety in the watershed. Those data will be analyzed outside of this report.

Response sampling sets were reserved for major storm events, heat waves, or visible cyanobacteria blooms. Since routine sampling often captured post-storm conditions, response sampling was used primarily for bloom events. Between 2023–2024, 19 response samples were collected across seven bloom events, triggered by visible cyanobacteria aggregations or surface scums. One additional response sample was collected due to observed high dissolved oxygen and pH levels, which turned out to be high abundances of centric diatoms and cryptophytes, not cyanobacteria.

FIELD SAMPLING

During each sampling event, we collected vertical water profiles using a calibrated sonde, measuring key water quality parameters such as temperature, dissolved oxygen, pH, conductivity, and turbidity. Light penetration depths of Photosynthetically Active Radiation (PAR) and water clarity (Secchi) were also recorded. Discrete water samples were collected at 1.5 m depth for laboratory analysis of nutrients, organic carbon, suspended solids, and chlorophyll-a. Phytoplankton were collected as integrated water column samples to ensure representativeness. Winter sampling followed the same procedures but through augered holes in the ice. To continously monitor under-ice conditions, dissolved oxygen sensors were deployed near the bottom at each site during the winter seasons. All collected parameters and their abbreviations are listed in Table 2.

Table 2: All study parameters collected or calculated and their associated abbreviation used throughout the report.

Category	Parameter	Abbreviation
Vertical Profile (YSI / EXO Sonde)	Temperature	Temp
	Dissolved Oxygen Concentration	DO
	Dissolved Oxygen Saturation	DO %
	pH	pH
	Specific Conductivity	SpCond
	Salinity	Sal
	Turbidity	Turb
Surface Sensor	Carbon Dioxide	CO2
Water Clarity & Light Attenuation	Secchi Disc Depth	Secchi
	Photosynthetically Active Radiation - Light Exinction Coefficient	PARkd
Water Chemistry (1.5m sample)	Chlorophyll-a	CHLA
	Total Suspended Solids	TSS
	Volatile Suspended Solids	VSS
	Nitrate + Nitrite	NO2+NO3
	Ammonium	NH4
	Dissolved Inorganic Nitrogen	DIN
	Total Nitrogen	TN
	Total Phosphorus	TP
	Soluble Reactive Phosphorus	SRP
	Nitrogen to Phosphorus Molar Ratio	TN:TP
	Total Organic Carbon	TOC
	Dissolved Organic Carbon	DOC
	Acid-Neutralizing Capacity / Alkalinity	ANC
Phytoplankton (depth integrated)	Phytoplankton Biovolume & Cell Density	

LABORATORY PROCEDURES

Field samples collected for dissolved nutrients and chlorophyll-a were filtered in the LSNERR laboratory within 8 hours of collection. Samples were then sent to NRRI's Central Analytical Laboratory, where they were analyzed for total nitrogen (TN), total phosphorus (TP), ammonium (NH4), nitrate plus nitrite (NO2+3), soluble reactive phosphorus (SRP), total organic carbon (TOC), dissolved organic carbon (DOC), and acid neutralizing capacity/alkalinity (ANC). Calculated parameters include dissolved inorganic nitrogen (DIN = NH4 and NO2+3) and nitrogen to phosphorus molar ratio (N:P).

Additional analyses, including measurements of total suspended solids (TSS), volatile suspended solids (VSS), and chlorophyll-a (chl-a), were completed by the LSNERR laboratory.

Phytoplankton taxonomic samples were analyzed microscopically using either the Utermöhl settling chamber method (Utermöhl 1958) or the Sedgewick-Rafter cell method depending on cell densities. Phytoplankters were identified to the highest possible taxonomic resolution, and biovolumes were calculated based on cell dimensions (e.g., length, width, diameter; Reavie et al. 2010).

DATA ANALYSIS

We applied several analytical techniques to address our first research question:

How do environmental conditions contribute to degraded algal communities?

- Summarized bloom event conditions by compiling data on weather, river discharge, water levels, and observed field conditions during bloom occurrences.
- Constructed a Kendall rank correlation matrix to assess parameter relationships and identify redundant or less relevant environmental variables for exclusion from subsequent analyses.
- Conducted Random Forest Analysis (RFA; Breiman 2001) using cyanobacteria biovolume as the response variable and a suite of physicochemical variables as predictors to rank the most important predictors of cyanobacteria biovolume. RFA was conducted with spatial and temporal subests.

To give additional context to both study questions, we characterized current and past water quality and nutrient conditions and assessed change using longer-term datasets from the SLRE.

- Performed paired t-tests to compare hotspot water quality data to the nearest thalweg SWMP sites.
- Calculated monthly anomalies for water quality parameters in 2023 and 2024 relative to long-term monthly means (2013–2024) at SWMP stations using the *SWMPr* R package.
- Applied Mann-Kendall trend analysis to detect modern (2012–2024) trends in water quality variables.
- Assessed long-term (1953–2014) trends in TP and TSS concentrations and loads using data from the Milestone Monitoring Program and SWMP, with Mann-Kendall annual trend analysis (Hamed and Rao 1998). Additional patterns in NH4, NO2+3, and DO were evaluated using non-parametric, Locally Estimated Scatterplot Smoothing (LOESS).
- Summarized hypoxia results and compared findings to previous LSNERR research.

To address our second research question:

What should a future monitoring program look like?

- Evaluated spatial redundancy using Nonmetric Multidimensional Scaling (NMDS; Shepard 1980) followed by Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson 2014). PERMANOVA, a nonparametric method selected because of non-normal distributions of sample data, used restricted permutations to test for differences among all pairwise combinations of sampling events across sites.
- We assessed temporal redundancy by comparing how much summer environmental and biological measurements (e.g., chlorophyll-a, total phytoplankton, cyanobacteria biovolume) changed between all pairs of sampling dates. We then used LOESS to find the shortest time interval needed to detect meaningful changes.
- Conducted time lagged correlation analysis using Spearman rank correlations between cyanobacteria biovolume and environmental variables lagged from 0 to 50 days to determine the time window over which predictors most strongly influence bloom occurrence.
- Prioritized parameter selection by assessing RFA predictor importance scores and correlation matrix results combined with professional judgement to identify key environmental variables for future monitoring.

FOUNDATIONAL RESEARCH RESULTS & DISCUSSION

ALGAL BLOOMS

Throughout this report, we use the term "bloom" to mean visible aggregations of cyanobacteria, either densely suspended in the water column or forming surface scums, globs, or streaks (**Figure 2**). During the study, we collected 19 bloom response samples across seven bloom events. These samples were taken either outside of our routine sampling schedule or at locations other than our designated "hotspots." We collected bloom samples at Miller Creek (n=1), Allouez Bay (n=2), Nemadji River Mouth (n=1), Superior Bay (n=1), Pokegama Bay (n=1), Lake Superior (n=1), and Barker's Island (n=12). Barker's Island likely had a bloom detection bias due to the proximity of the station to the LSNERR's dockside laboratory.

What did blooms have in common?

Calm winds

All bloom events we sampled occurred under similar wind conditions, with mild winds the day before and very calm conditions (1 to 3 mph) during the night and day of the bloom (NOAA CO-OPS n.d.). This aligns with the behavior of common bloom-forming cyanobacteria species, which can regulate their buoyancy using gas vesicles. This ability gives them a competitive advantage over other phytoplankton when vying for light and often results in visible surface blooms during calm weather (Reynolds et al.

1987; Xue et al. 2022).

Only two events, on September 20–21 and October 2–3, 2023, experienced calm winds under 7 mph for more than 24 hours, allowing for persistent and expanding surface scums. All other blooms dissipated within a day, often ending when seasonally typical afternoon winds picked up. Most blooms occurred in September, a month that occasionally brings lingering late summer calm conditions. However, such calm periods are infrequent, with wind speeds under 7 mph occurring only about 26.3 % of the time (Baker 1983). These calm periods are becoming more rare as regional wind speeds increase, especially near Lake Superior (Desai et al. 2009). This low occurrence of calm conditions may help explain why most visible surface blooms (scums) were short-lived, typically lasting only a few hours.

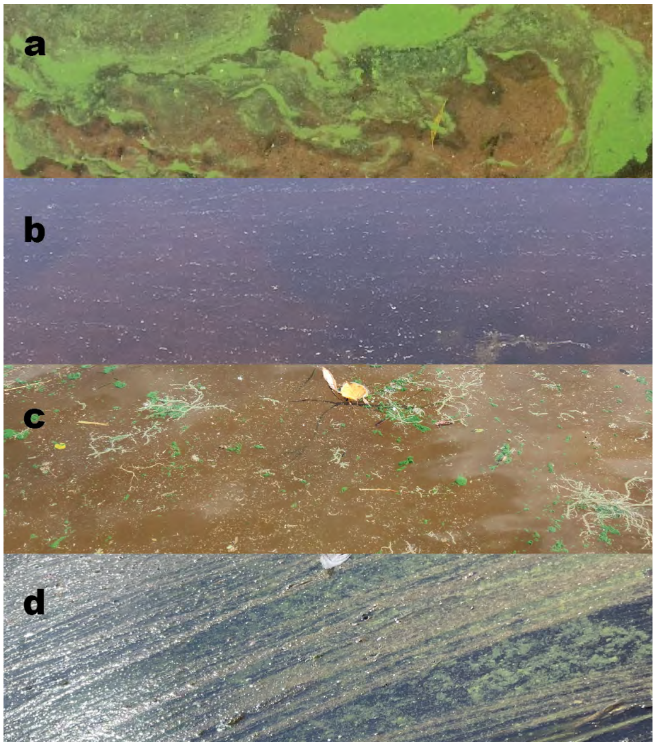


Figure 2: *Examples of cyanobacteria bloom appearance in the SLRE: a) bright streaks and swirls at Barker's Island on 9/10/2021, b) subtle streaks at Barker's Island on 8/21/2024, c) globs and spiderwebs at Nemadji River Mouth on 10/3/2023, d) thick mat with shiny sheen in Superior Bay, 8/26/24.*

Seasonal Timing

The timing of blooms was similar in 2023 (**Figure 3**) and 2024 (**Figure 4**), and aligned with previous estuary bloom timing in 2018 and 2021. In 2023, discrete, small-scale blooms were detected in sheltered areas of the lower harbor as early as August 26. This timing coincided with two other bloom events recorded on Lake Superior in 2024 (Lafrancois and Reinl 2025). In both years, by early to mid September, several small to medium-sized blooms had occurred, all short-lived (lasting just a few morning hours), similar in timing and intensity to the small but toxic bloom in the estuary that occurred September 10, 2021. However, two more extensive, multi-area blooms that persisted throughout a day or on subsequent days occurred later in the 2023 season (September 20-21 and October 2-3, 2023).

Low Flow

In 2023, St. Louis County—where most of the St. Louis River watershed is located—experienced moderate to severe drought from August 1 through September 26 (NDMC n.d.). In 2024, the county remained abnormally dry or in a moderate drought from September 10 through the end of fall (NDMC n.d.). As a result, most cyanobacterial blooms (6 out of 7) occurred during periods of low river discharge, ranging from 11.0 to 21.2 cubic meters per second (**Figure 3** & **Figure 4**). These low-flow conditions likely promoted a more stable water column, reduced flushing, and allowed nutrients to accumulate in surface waters, conditions favorable to cyanobacterial growth (Lung and Paerl 1988; Paerl 1996).

In contrast, the most spatially extensive and persistent bloom occurred on October 2–3 after two

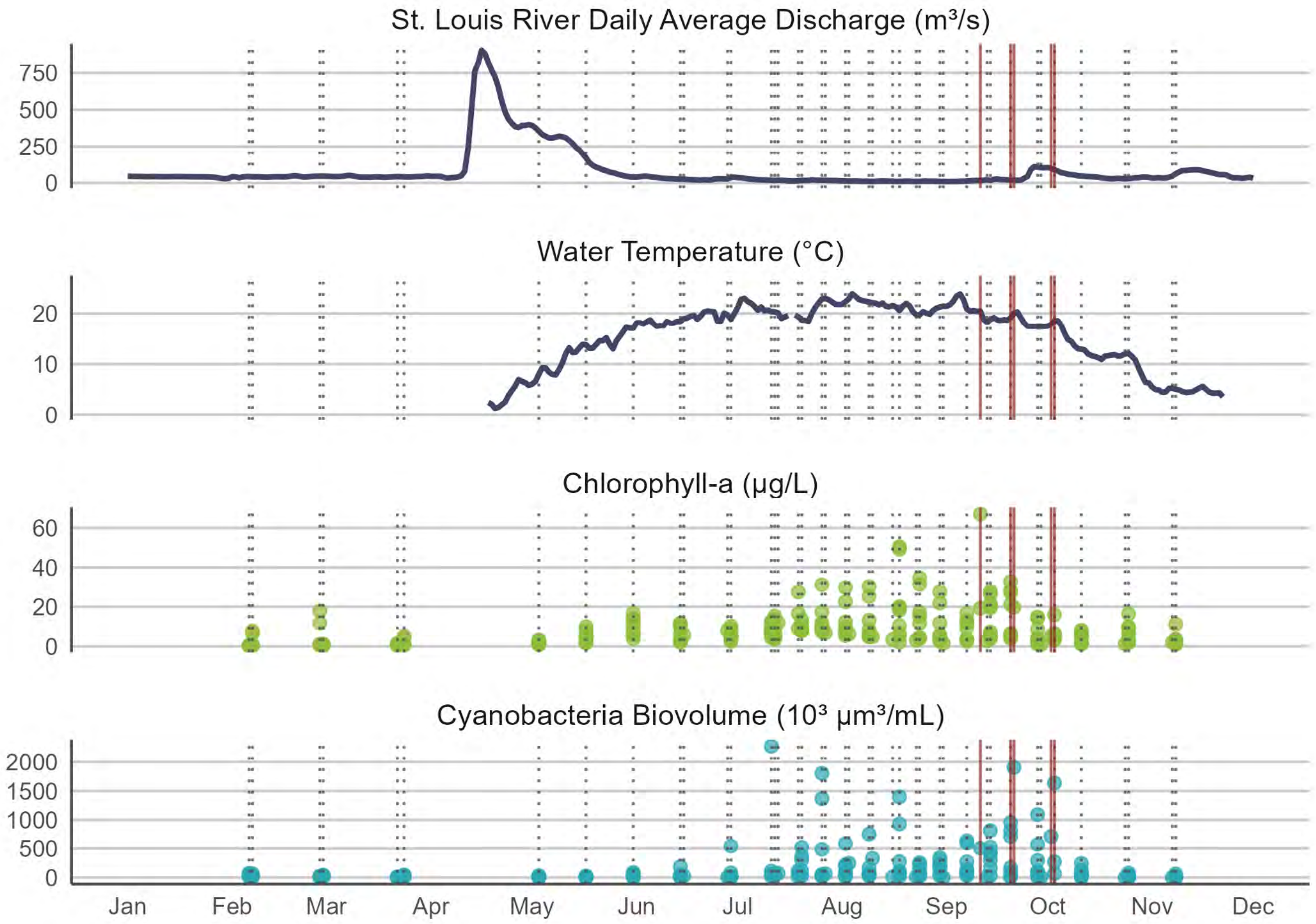


Figure 3: *2023 St. Louis River discharge at Scanlon (USGS), water temperature at Barker's Island (NOAA/NERRS 2025), chlorophyll-a ug/L and cyanobacteria biovolume across study sites. Routine sampling event timing denoted with vertical dotted lines, bloom response sampling event denoted with vertical solid red lines.*

substantial rainfall events: 1.5 inches on September 24 and 0.5 inches on September 30, 2023 (NOAA/NERRS 2025). Although September typically sees only two rainfall events over 0.5 inches (NWS 2023), the back-to-back timing of these storms produced a substantial discharge pulse (89.8 to 99.4 m³/sec) despite the preceding drought conditions. While surveying the October bloom, that extended across the lower estuary and into parts of the middle estuary, we observed turbidity plumes, floating woody debris, and decaying plant fragments intermingling with surface scums. These materials accumulated in eddies and other slow-moving areas, though rapid flushing of algal material through the Superior Canal into Lake Superior was also documented.

Although this bloom occurred during elevated river discharge, the event was preceded by an extended drought period from June through September. Similarly, the SLRE's first confirmed toxic bloom, on September 10, 2021, also followed a prolonged dry period. Two instances of bloom–drought coincidence (Figure 6) does not establish a definitive pattern, but it does suggest that hydrologic conditions play a strong role in bloom dynamics. Study results discussed later in this report also lend evidence to the importance of hydrology. Further investigation is needed to assess how extended low-flow periods may set the stage for bloom development by stabilizing water masses and concentrating nutrients,

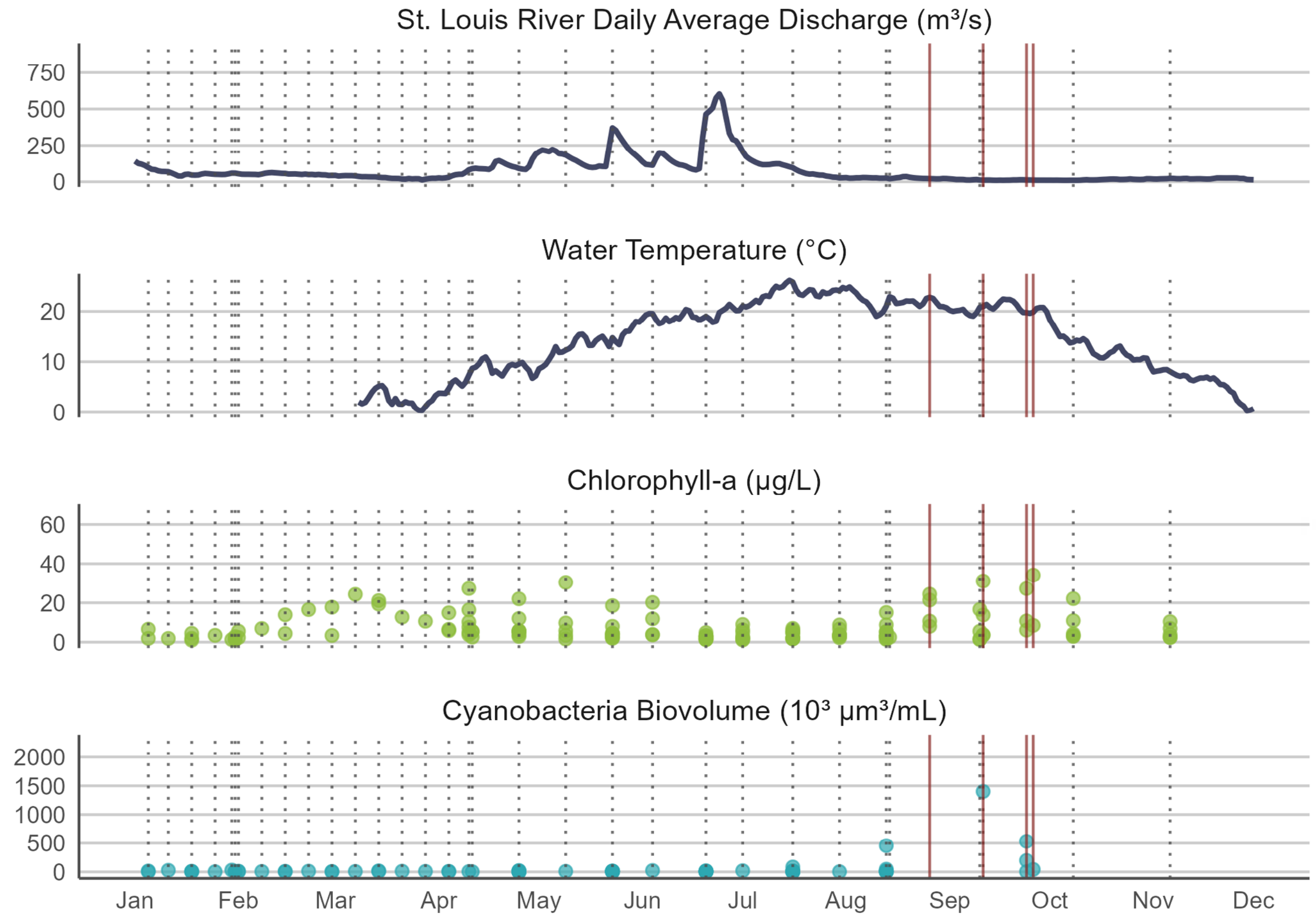


Figure 4: *2024 St. Louis River discharge at Scanlon (USGS), water temperature at Barker's Island (NOAA/NERRS 2025), chlorophyll-a ug/L and cyanobacteria biovolume across study sites. Routine sampling event timing denoted with vertical dotted lines, bloom response sampling event denoted with vertical solid red lines.*

and whether physical flushing mechanisms, such as wind, discharge, or seiche activity, can interrupt or prevent bloom formation.

We also conducted a preliminary review of whether water level fluctuations in Lake Superior, driven by seiche activity, may have acted as a flushing mechanism during the seven bloom events. Using NOAA CO-OPS data, we observed the maximum water level change that occurred during each bloom. However, seiche-driven fluctuations did not appear to exert a strong flushing effect. In several cases, blooms persisted, or even intensified, despite water level changes, including events with swings as large as 0.7 feet. These observations suggest that seiche activity alone may not be sufficient to disrupt blooms once they are established, but a more detailed hydrodynamic analysis is needed to better understand the interaction between water level variability and bloom dynamics in the estuary.

How did blooms differ?

Community composition

The composition and biovolume of bloom communities varied markedly between years (**Appendix B**). In 2023, following the aforementioned moderate to severe drought conditions, blooms were dominated by high abundances of *Aphanizomenon flos-aquae*. These blooms were preceded by dispersed, but

Bloom Highlight

October 2 - 3, 2023

Extent: Majority of Lower Estuary had persistent scums and green streaks

Type of bloom: *Aphanizomenon flos-aquae* dominant (Figure 5)

Toxins: Total microcystins = 0.21 µg/L at Barker's Island Swimming Beach; Saxitoxin not detected

Water Temperature: 17.5 - 18.3 C (63.5 - 65 F)

Weather: Extremely calm winds, < 3 mph for 48 hours, mostly sunny

Cyanobacteria bloom, *visible as bright green scum on the shoreline at Barker's Island Swimming Beach on October 3, 2023 (left) and* ***Aphanizomenon flos-aquae*** *colonies from October 3, 2023 bloom, viewed under magnification (top).*

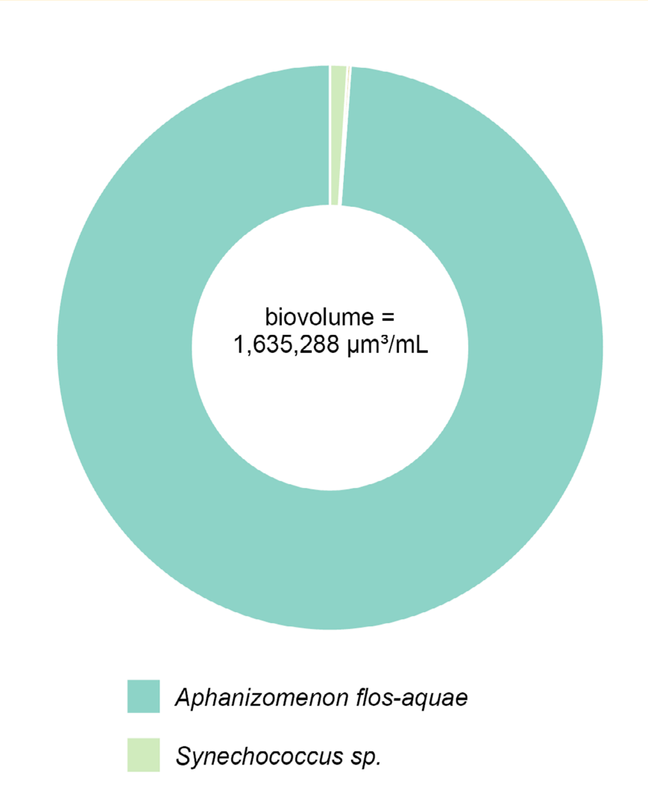


Figure 5: *Cyanobacteria species composition (proportions) of intergrated water column sample collected from Barker's Island on October 3, 2023.*

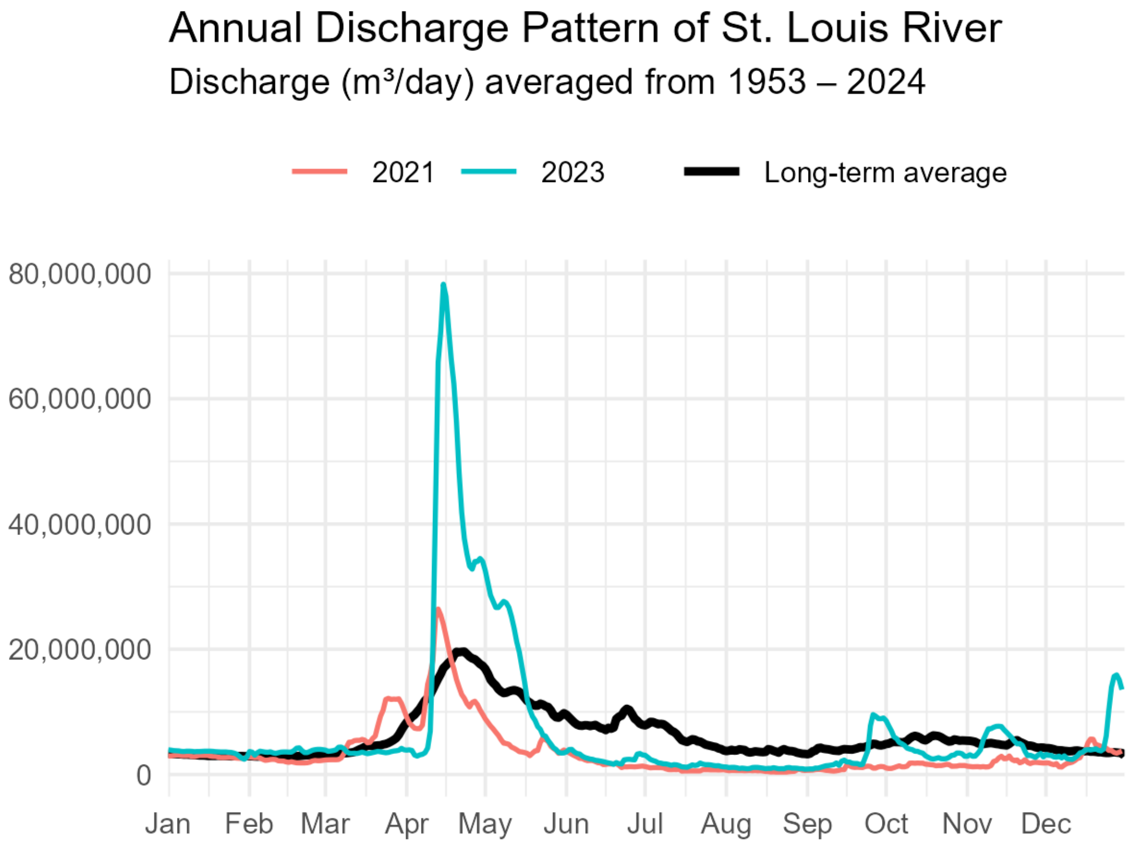


Figure 6: *St. Louis River daily discharge (m3/day) pattern calculated by averaging daily discharge from 1953 -2024 (USGS 2025), overlaid with daily discharge (m3/day) in 2021 and 2023, years with pronounced droughts and bloomy conditions in the SLRE. For an interactive version that allows you to select any year to overlay visit:* **https://lakesuperiornerr.shinyapps.io/StLouisRiverDischargeViz/**

visible planktonic *Aphanizomenon* colonies in the water column that persisted for weeks before surface scums developed. In contrast, blooms in 2024 had much lower abundances of cyanobacteria (roughly 1/3 of peak abundances in 2023) and featured a more diverse community, often including multiple *Dolichospermum* species and *Microcystis aeruginosa*. These blooms appeared more abruptly and were more localized and ephemeral compared to those in 2023.

In addition to depth-integrated water column samples for phytoplankton identification, we often collected concentrated surface scum samples and examined the live material. Surface scums often showed higher cyanobacteria diversity than the corresponding integrated samples, sometimes containing species absent from the underlying water column. Numerous factors regulate vertical distribution and scum formation by planktonic cyanobacteria in general, though prior research has focused heavily on *Microcystis aeruginosa*. These factors include hydrodynamic effects of wind and turbulence (Aparicio Medrano 2014) and complex regulation of buoyant intracellular "aerotope" structures by extracellular chemical conditions (Klemer et al. 1996; Brookes & Ganf 2001; Drugă et al. 2019) to optimize light and nutrient conditions (Paerl & Ustach 1982). These effects differ among species, contributing to distinct vertical community structures. Surface scums also exhibited striking spatial heterogeneity. For example, two surface scum samples collected ~50 m apart at the Barker's Island swimming beach in September 2024 differed in composition, with one containing exclusively *Dolichospermum* and the other containing

Bloom Highlight

September 11, 2024

Extent: Area sheltered by Barker's Island had planktonic aggregations and some shoreline scums that dissipated by the afternoon

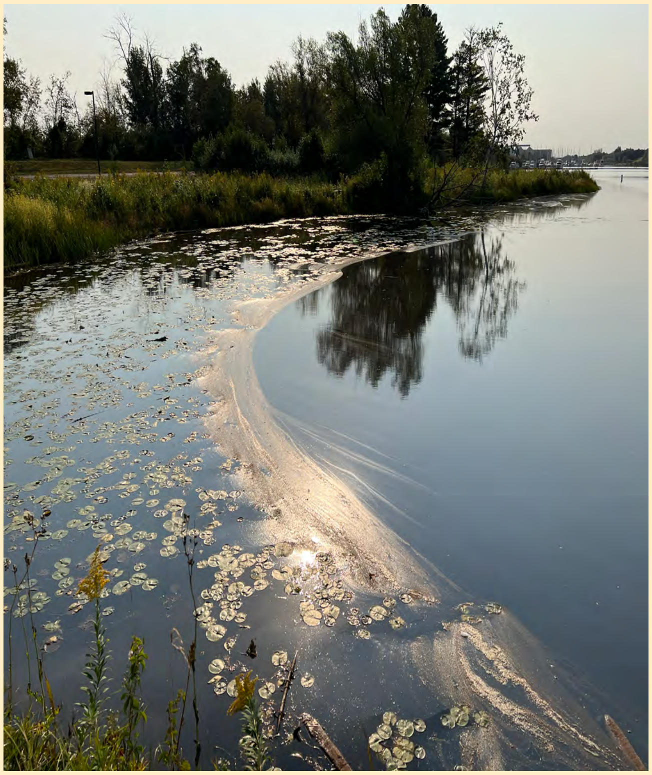
Type of bloom: *Diverse cyanobacteria* community (Figure 7)

Toxins: Not Tested

Water Temperature: 20.4 - 21 °C (68.7 – 69.8 °F)

Weather: calm morning winds <2 mph, then <7 mph in the afternoon, mostly sunny

Cyanobacteria bloom mixed with aquatic vegetation in a sheltered bay near Barker's Island Swimming Beach on September 11, 2024 (bottom) and diverse community of cyanobacteria from September 11, 2024 bloom, viewed under magnification (top).



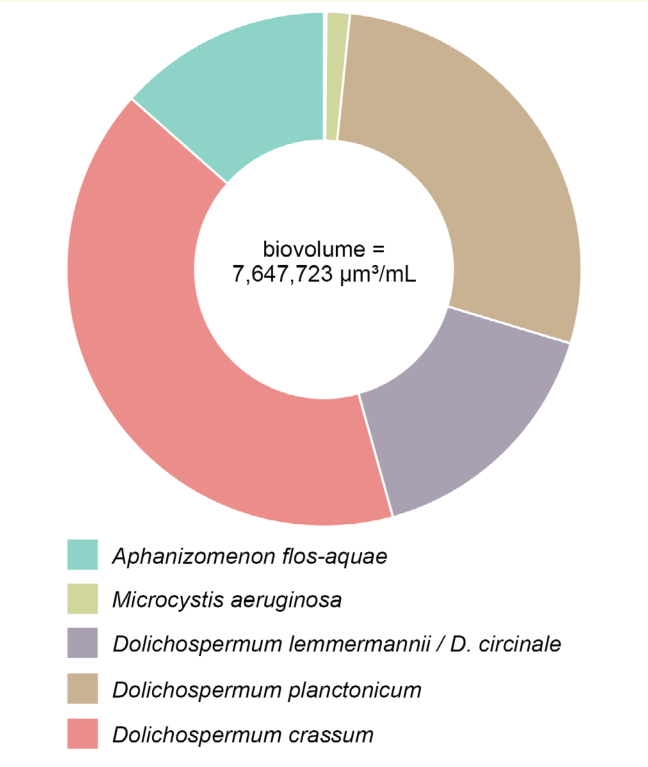


Figure 7: *Cyanobacteria community composition (species proportions) of intergrated water column sample collected from Barker's Island on September 11, 2024.*

exclusively *Microcystis*, while their depth-integrated samples had similar community compositions. Clearly, while surface scums are the most recognized cyanobacteria bloom marker, representative samples must also consider the water column to accurately characterize conditions.

In July and August of 2023, benthic filamentous cyanobacteria *Oscillatoria* sp. and *Phormidium sp.* were observed near the Barker's Island swimming beach and Miller Creek tributary mouth. In shallow waters on calm, warm, sunny afternoons, mats of filaments were densely arranged on the sediment surface. Colonies with this benthic substrate still attached were abundant as dense globs throughout the water column, likely buoyed by rapid photosynthetic oxygen production. Notably, benthic mats only occurred near sampling sites with relatively high NH4; *Oscillatoria* is known for its high NH4 affinity and has been used for nutrient removal in wastewater treatment practices (Hashimoto & Furukawa 1989). While both of these taxa can produce toxins, it is not known whether they are responsible for cyanotoxin production at these locations.

In both years, *Aulacoseira ambigua*, a known mesotrophic, filamentous diatom in the Great Lakes (Reavie et al. 2014) commonly co-occurred in cyanobacteria bloom samples. This "weedy" (Potapova et al. 2010) species had a much higher average biovolume (9,444,088 μm3/ml) in 2024 than in 2023 (5,167,714 μm3/ml). In general, heavy, silica-rich *Aulacoseira spp.* thrive in turbulent environments that can ensure suspension in the water column, suggesting they may have been favored by the increased rainfall and higher flow regimes in 2024.

Were blooms toxic?

We did not systematically assess toxin concentrations in bloom samples for this study, but four samples were tested using Wisconsin DNR-provided kits and analyzed by Wisconsin State Lab of Hygiene for total microcystins and saxitoxin. Most samples had no detectable toxins. However, low levels were detected at Barker's Island during two events (September 21 and October 3, 2023). All values were below World Health Organization recreational thresholds, but one total microcystins result exceeded U.S. EPA drinking water guidelines (**Table 3**).

Table 3: Cyanotoxin results from non-systematically collected sampling efforts in 2023 and 2024. Guidance Thresholds: Drinking Water (EPA 2018): Microcystin 0.3 μg/L (children), 1.6 μg/L (adults), no formal threshold for Saxitoxin; Recreational Water (WHO 2003): Microcystin – 8.0 μg/L; Saxitoxin 10 - 30 μg/L.

Date	Location	Total Microcystins (μg/L)	Saxitoxin (μg/L)	Drinking Water Exceedance	Recreation Exceedance
Oct 3, 2023	Barker's Island	0.21	ND	Yes – children	No
Oct 3, 2023	Lake Superior / Nemadji River	ND	ND	No	No
Sept 21, 2023	Barker's Island	1.9	0.026	Yes – adults & children	No

Were blooms similar to those on Lake Superior?

Because our study focused on the SLRE, we only sampled one Lake Superior bloom event on October 3, 2023 when algal scums were visibly discharging through the Superior Entry Canal. Algal blooms on Lake Superior have been collaboratively monitored and compiled in a public database (Lafrancois and Reinl 2025), allowing for some broad comparisons between blooms in the SLRE and those in Lake Superior.

Over the past decade, bloom activity has not consistently aligned between the SLRE and Lake Superior. Widespread, persistent blooms occurred in the lake in 2012 and 2018, while major SLRE blooms occurred in 2021 and 2023, years when Lake Superior had only small blooms in isolated locations. Bloom community composition also differs. Lake Superior blooms are dominated by *Dolichospermum spp.*, while SLRE blooms show greater taxonomic variability. Notably, no *Aphanizomenon*-dominated blooms, like those observed in the SLRE in 2023, had been recorded on Lake Superior prior to this study. However, we did observe the October 3, 2023 estuary bloom extending into Lake Superior. We sampled near the public swimming beach at the tip of Minnesota Point, and *Aphanizomenon flos-aquae* was the dominant cyanobacteria by biovolume (234,956 μm3/ml), while *Dolichospermum sp. cf circinale/lemmermannii* was present at lower levels (6,708 μm3/ml). This suggests SLRE strains may enter the lake during blooms, though Lake Superior conditions may not support their persistence or dominance, and more study is warranted.

Hannah Nicklay holds a sample bottle green with cyanobacteria from a bloom at the mouth of the Nemadji River, October 3, 2023

Overview

While targeted bloom response sampling provided snapshots of community composition during visible events, our routine sampling revealed broader patterns in phytoplankton dynamics across sites and seasons. In total, 107 unique taxa with greater than 5 observations in the dataset were retained in statistical summaries and analyses. Given that species-level identification is not always possible at 400x magnification, there are likely far more than 107 genetically distinct taxa within the SLRE. In some cases, routine samples even captured higher cyanobacteria biovolumes than bloom response events, underscoring the value of systematic sampling for understanding temporal and spatial variability; for example, a routine sample at Barker's Island

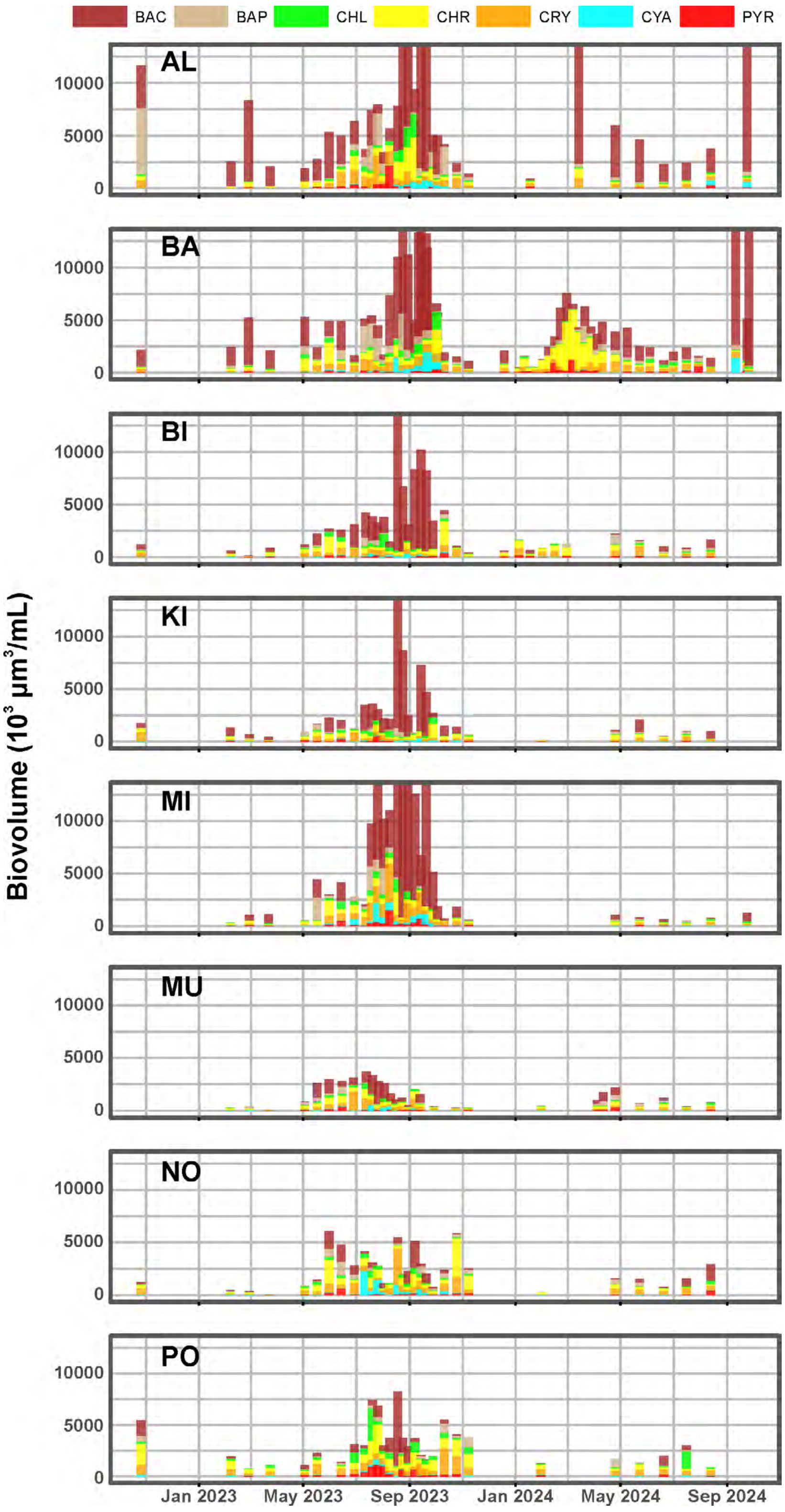


Figure 8: *Phytoplankton biovolume in the SLRE from October 2022 through October 2024. Taxon groups are color-coded and codes represent: BAC = centric diatoms, BAP = pennate diatoms, CHL = chlorophytes (green algae), CHR = chrysophytes, CRY = cryptophytes, CYA = cyanobacteria, PYR = pyrrophytes (dinoflagellates). To better enable visualization of phytoplankton types, some bars are cropped to minimize the effect of very high centric diatom abundance and retain the same scale in all the graphs. Two letter codes for the sites are in* **Table 1**. *For an interactive version visit* **https://lakesuperiornerr.shinyapps.io/SLRE_phyto_sp/**

collected on Aug 18, 2023, contained the third-highest combined cyanobacteria biovolume of all samples collected at the site, even though a bloom was not visible.

Phytoplankton data from the eight monitoring sites (**Table 1**) over the two-year study period (**Figure 8**; **https://lakesuperiornerr.shinyapps.io/SLRE_phyto_sp/**) clearly indicate that centric diatoms (especially *Aulacoseira spp.*) are the dominant phytoplankton taxonomic group in the SLRE, even during late summer periods when cyanobacteria also become abundant. Pennate diatoms including *Fragilaria spp.* and *Nitzschia spp.* also frequently dominated communities or co-dominated with the centric diatoms. Cyanobacteria are generally most prevalent in July-September, particularly in the lower SLRE. Notable summer peaks were observed at Allouez Bay and Barker's Island, while sites like North Bay and Miller Creek showed more sporadic seasonal patterns. However, even at the height of these blooms, cyanobacteria biovolume was almost always surpassed by diatoms. Because our samples represent the entire water column—not just surface waters—our data capture the full vertical structure of the phytoplankton community. As a result, even in samples collected during visible surface scums and streaks, diatoms often remained the dominant group in the water column.

Cyanobacteria biovolume was consistently low at Billing's Park, Kingsbury Bay, and Pokegama Bay. Chrysophytes ("golden algae") were prevalent and diverse across sites and seasons. They were especially abundant and dominant at Barker's Island during winter and early spring of 2024, when biovolumes of *Uroglena sp.* and the closely related synurophyte *Synura sp.* steadily rose and declined over a roughly two-month period. Though chlorophytes ("green algae"), cryptophytes ("cryptomonads"), and pyrrophytes ("dinoflagellates") rarely dominated communities, they each were often present and diverse at relatively low biovolumes in the majority of samples. Total phytoplankton biovolume was lowest at Mud Lake and North Bay, and highest in the lower SLRE where light and nutrients were generally most abundant.

Diatoms, in addition to occasionally abundant chrysophytes, chlorophytes and cryptophytes, are important food resources for aquatic invertebrates. With the exception of cyanobacteria bloom periods, the diverse, diatom-dominated communities observed throughout the SLRE reflect good water quality that supports a robust food web base. In general, the taxa observed in this study represent a temporally variable mesotrophic to eutrophic system which matches with sedimentary records describing nearshore trophic status that exceeds pre-Anthropocene conditions (Alexson et al. 2018). One of the goals of this work was, for the first time, to characterize in detail the phytoplankton communities in the SLRE so that cyanobacteria bloom periods and future monitored conditions can be placed in appropriate context. While we do not offer detailed interpretations for every taxon, below we highlight several key taxa and notable spatial and interannual patterns.

Cyanobacteria

Cyanobacteria in the SLRE were taxonomically diverse and exhibited varied spatial and seasonal distributions, including both late-summer, bloom-forming taxa and ambient taxa that occurred at low abundance during non-bloom periods.

Cyanobacteria were present in all collected samples. Small (1-2 μm) unidentified coccoid cyanophytes were common in samples, as they tend to be in Great Lakes samples (Reavie et al. 2014a), but they were removed from analyses due to the lack of knowledge around their ecological significance and the difficulty of distinguishing them from the small sediment and detritus particles that were common in SLRE samples. Other small cyanophytes such as *Synechococcus* and *Chroococcus* were also ubiquitous.

The small filamentous species *Pseudanabaena limnetica*, also common in the Great Lakes, was more abundant in cold water, and generally in middle and upper SLRE sites. Several distinct cyanobacteria species including Trichodesmium lacustre, *Cuspidothrix issatschenkoi, Snowella sp., Woronichinia sp., and Sphaerospermopsis aphanizomenoides* were observed fewer than 5 times. Even when not visibly manifested as surface scums or streaks, common members of bloom communities were present in routine samples.

In 2023, the bloom-forming *Aphanizomenon flos-aquae* was most abundant from mid to late summer, and consistently more abundant at lower SLRE sites. Within the lower SLRE, its abundance varied by year, being higher at Barker's Island and Miller Creek in 2023, and markedly higher at Allouez Bay in 2024. Although no blooms were detected at Billings Park or Kingsbury Bay, clear peaks of *Aphanizomenon flos-aquae* were observed in August 2023.

Dolichospermum crassum, a large colonial diazotroph, was notably abundant at Billings Park and Miller Creek during a single sampling event on July 26, 2023. It was also abundant in all 2024 bloom samples from Barker's Island and appeared throughout mid-summer at several upper and middle SLRE sites. *Dolichospermum sp. cf circinale/lemmermannii*, a combined taxon representing two morphologically similar species, was also most abundant in 2023 at Billings Park and Miller Creek. It made sporadic appearances in non-bloom samples across additional sites and was a frequent member of bloom communities. *Dolichospermum planctonicum* was less abundant overall but appeared in several 2024 bloom samples at Barker's Island and was the dominant taxon in a 2024 bloom at the mouth of Pokegama Bay. *Planktothrix sp. cf agardhii/rubescens*, another group of morphologically similar taxa, was detected occasionally across most sampling sites and was particularly abundant in a single sample from Barker's Island on August 18, 2024.

Centric diatoms

Centric diatoms in the SLRE comprise a taxonomically and ecologically diverse group, exhibiting broad environmental tolerances and distinct spatial-temporal patterns that reflect underlying gradients in SLRE physiochemistry and nutrient availability.

Among all taxa, the generalist *Aulacoseira ambigua* had the highest total biovolume, while its close relative *Aulacoseira granulata* ranked fifth. Both were observed across most sites, but their distributions and seasonal patterns differed. *A. ambigua* was typically more abundant in the lower SLRE and occurred year-round. It co-dominated bloom events alongside cyanobacteria, contributed substantially to spring and fall diatom pulses, and was the dominant winter species at lower estuary sites in 2023. In contrast, *A. granulata* was more common in the upper and middle SLRE and was largely restricted to the late spring through summer period. Though less persistent than *A. ambigua*, it was present fairly consistently during warmer months. A spiral morphotype of *Aulacoseira* was also documented in many samples, particularly at lower SLRE sites during the open water season, with pronounced peaks at Barker's Island and Miller Creek in summer 2023, which occasionally coincided with cyanobacteria blooms. This spiral morphotype was likely displayed by either *A. ambigua* or *A. granulata*, or both, though species could not be confidently distinguished at 400x magnification due to lack of terminal spines; however, subspecies of both species are known to rarely express this morphotype (Janse van Vuuren et al. 2018; Wang et al. 2020) as a response to "turbid, warm, and eutrophic [river] waters with medium to high mineral content."

Two filamentous centric diatoms *Melosira varians*, known for its adaptability across a broad range of environmental conditions, and *Skeletonema sp.*, a more marine-estuarine associated taxon, often co-occurred and were present primarily from spring through fall (Spaulding et al. 2021). They were found at similar levels across sites, but their presence varied over time at each site. Two eutrophication indicator taxa, *Stephanodiscus sp.* and *Cyclotella sp.*, were most common during the summer months, with notable peaks at Allouez Bay, Barker's Island, Billings Park, Miller Creek, and Mud Lake. Their large cell size contributed substantially to total biovolume during periods of high abundance. Both taxa have increased in prevalence over recent decades in some SLRE embayments (Alexson et al. 2018). In the Great Lakes, *Cyclotella spp.* have also been associated with stronger summer stratification regimes linked to atmospheric warming (Reavie et al. 2017). The centric diatoms *Rhizosolenia sp.* and *Acanthoceras sp.* also showed strong summer peaks at lower SLRE sites and at Billings Park in both years.

The centric diatoms *Rhizosolenia sp.* (synonymous here with *Urosolenia sp.*) and *Acanthoceras sp.* illustrate the dynamic and diverse nature of SLRE centric diatom communities. Both showed strong summer peaks at lower SLRE sites and at Billings Park in both years. While *Rhizosolenia sp.* is typically associated with oligotrophic, pelagic environments and is well documented in Lake Superior, *Acanthoceras sp.* is more often linked to eutrophic and high alkalinity conditions (Spaulding et al. 2021). Lastly, small unidentified centric diatoms, both unicellular and filamentous, were ubiquitous across all regions of the estuary and displayed clear peaks in spring and summer.

Pennate diatoms

Pennate diatoms were an important component of the SLRE phytoplankton community, particularly in the lower estuary, where they displayed strong seasonal patterns and species-specific variability across years.

In 2023, pennate diatom biovolume increased from spring through fall, then declined briefly before steadily rising again through winter and spring; however, 2024 abundances never reached 2023 levels. *Fragilaria crotonensis*, a large bodied, colonial pennate that is a Great Lakes generalist, had very high peaks at all lower SLRE sites throughout summer 2023, as well as notable peaks at Kingsbury Bay and North Bay, but was rarely observed in 2024. However, its close relative, *Fragilaria capucina*, had sporadic high biovolumes in both years at Barker's Island and Miller Creek especially in spring. Another colonial generalist, *Asterionella formosa*, had a spring peak followed by a stronger late summer peak in 2023, particularly at Allouez Bay and Barker's Island, and it was a common member of winter communities. Several species within the genus Diatoma, including *D. tenuis* and *D. vulgaris*, were prominent during spring diatom peaks at all sites except Pokegama Bay. They were not observed at other times of year, with the exception of a single *D. vulgaris* appearance at Miller Creek in November 2023. Numerous other pennate diatoms were documented in relatively low numbers of samples, including *Gomphonema sp., Navicula sp., Nitzschia sp., Ctenophora pulchella, Entomoneis sp., Gyrosigma sp., Surirella sp.,* and *Amphora sp.*

Chrysophytes

Chrysophytes were a seasonally dynamic component of the SLRE phytoplankton community, with both unicellular and colonial forms contributing substantially to total summer biovolume, but with certain species only abundant in winter.

Chrysophyte abundances had both summer and winter peaks. The highest chrysophyte biovolume of the entire study occurred during winter 2023 to 2024, with the extreme abundance of Synura sp. cf petersenii/uvella at Barker's Island. This taxon, representing one or both of two ecologically and morphologically similar species (Siver 1987), co-occurred with another winter-associated taxon, *Dinobryon cylindricum*. In contrast, most other chrysophyte taxa peaked during the summer months, with much higher abundances in summer 2023 than in 2024. Unidentified unicellular coccoid chrysophytes, including both flagellated and non-flagellated forms, were present in all samples and collectively accounted for the third-highest total biomass among all taxonomic groups. Some distinct unicellular chrysophytes in samples included *Chromulina sp., Ochromonas sp., Kephyrion sp., Pseudokephyrion sp., Chrysolykos sp., Pedinella sp.,* and *Chrysamoeba sp.* Several species of large colonial chrysophyte *Dinobryon spp.* are known to occur in many aquatic systems and some of them form odorous nuisance blooms. *Dinobryon spp.* were present in the SLRE; these included *D. divergens, D. bavaricum,* and *D. sertularia,* which tended to co-occur with one another and with diatoms, and *D. cylindricum*. The synurophyte *Mallomonas sp.*, which is common in the Great Lakes, showed volatile peaks at all sites throughout the summer of 2023 and to a lesser extent 2024.

Chlorophytes

Chlorophytes in the SLRE were spatially structured but temporally variable, with diversity often increasing alongside abundance, and community composition showing notable interannual differences.

Chlorophyte biovolume did not display a clear seasonal peak, but rather fluctuated sporadically at all sites in both summers. Chlorophytes were, however, spatially consistent, being more abundant at lower and middle SLRE sites than upper sites. Abundances were also nearly twice as high in 2023 than 2024. Chlorophyte diversity often tended to increase as chlorophyte abundance increased in samples. Contrary to that interannual pattern, *Ankistrodesmus spp.* was more abundant in summer 2024 than 2023. Several small chlorophyte taxa including *Monoraphidium spp., Ankistrodesmus spp.*, and *Chlamdomonas spp.* were extremely common, particularly in spring and early summer of both years. Many small but visually distinct taxa including *Scenedesmus spp., Desmodesmus spp., Cosmarium spp., Crucigenia spp., Treubaria sp., Chlorogonium elongatum, Carteria spp.*, and T*etraedron spp.*, made more sporadic appearances throughout the dataset. Large colonial chlorophytes were generally most abundant from mid through late summer, sometimes co-occurring with blooms; some of these taxa include *Pandorina sp., Eudorina sp., Coelastrum sp., Sphaerocystis sp.,* and *Pediastrum duplex.*

Cryptophytes

Cryptophytes were a consistently present and ecologically versatile component of the SLRE phytoplankton community, with biomass patterns that reflected their ability to thrive across seasons, under turbid conditions, and alongside cyanobacteria.

Cryptophyte biovolume followed a relatively consistent spatial pattern across sites and displayed similar phenology in both years, with a primary peak abundance in early July and a secondary peak abundance in fall. They were also prevalent in winter communities and under turbid conditions, including during periods of high cyanobacteria abundance. This is supported by a significant negative correlation between cryptophyte biovolume and Secchi depth (Kendall's $r = -0.16$, $p < 0.05$), and aligns with the known ecological traits of common SLRE cryptophytes, which include metabolic mixotrophy, high photosynthetic efficiency in low light, and high motility (Morgan & Kalff 1979; Grossart et al. 1997).

Rhodomonas minuta, a widespread Great Lakes species, was present in most samples and showed a steady increase from winter lows to summer peaks in both years, ultimately representing the fourth-highest total biovolume across all taxa. *Cryptomonas erosa* followed a similar seasonal trajectory in 2023 but was sparse and sporadic in 2024. *Cryptomonas reflexa* was even more sporadic, though it exhibited a comparable seasonal pattern, except at Barker's Island, where summer 2024 biovolume levels remained consistent with those in 2023.

Pyrrophytes (Dinoflagellates)

In 2023, dinoflagellate biovolume peaked during the summer at Allouez Bay, Miller Creek, and Pokegama Bay. In contrast, 2024 displayed more erratic temporal patterns, with the highest abundances occurring in winter at Barker's Island. A large *Glenodinium* species was particularly abundant during several winter sampling events at that site. Observed dinoflagellate taxa included *Gymnodinium spp., Glenodinium spp., Peridinium spp., Ceratium hirundinella*, and *Cystodinium cornifax*.

Ceratium hirundinella, notable for its large cell size that contributes substantially to total biovolume when present, exhibited a sharp and sustained peak during late July and early August 2023 at Allouez Bay, and was also detected at Miller Creek, Barker's Island, and Billings Park that year, but was entirely absent in 2024. Like many dinoflagellates and cryptophytes, *C. hirundinella* is highly motile and mixotrophic, traits that provide an advantage in low-light environments such as the turbid waters of Allouez Bay (Hansen & Calado 1999). Despite this, combined dinoflagellate biovolume did not show a significant correlation with Secchi depth (Kendall's $r = -0.04$, $p = 0.33$), and *C. hirundinella*–specific relationships were not assessed due to limited observations ($n = 10$).

Paleolimnological context

Our findings generally confirm the results from paleolimnological data on inferred nutrients at North Bay, Pokegama Bay, Billings Park, and Allouez Bay and cyanobacteria pigments at North Bay and Billings Park (Alexson et al. 2018). This suggests that recent high abundances of cyanobacteria may represent newly emerging problems since the end of the 1980s. Even though monitoring data from this study do not suggest HAB risk is high everywhere in the SLRE, they corroborate the fossil-inferred results that algal abundances, particularly cyanobacteria, are generally increasing in nearshore (non-thalweg) areas. Several of the high-nutrient diatom indicators identified as increasing at SLRE locations were also documented as abundant in the SLRE in our study. Paleolimnological observations clearly depict the SLRE's lower food web as a changing, phosphorus-driven system throughout most of the 20th century, and that current water quality and phytoplankton are the product of a nutrient-enriched and urbanized system. Our new observations indicate that the structure and dynamics of the SLRE's lower food web is now determined to some degree by nitrogen limitation (see Environmental Predictors section below). For a more direct comparison of modern diatom communities to paleolimnological data, or for specific applications involving diatom-inferred biomonitoring criteria, archived sample material from this study could be processed for greater species-level taxonomic resolution.

ENVIRONMENTAL PREDICTORS OF CYANOBACTERIA ABUNDANCE

To better understand which environmental factors most strongly predict cyanobacteria biovolume in the SLRE, we used random forest analysis (RFA). This approach is well-suited for complex ecological datasets because it can handle many variables at once, identify important patterns, and rank the most important predictors, even when relationships are not simple or linear. It then allows you to assess each parameter's individual effect on the model.

We first generated a "whole model" RFA using data from events with complete sample sets collected in 2023 and 2024 across all eight bloom hotspot sites (for analytical method details see **Appendix A**). The model had an R^2 of 0.49, with temperature, DO, TOC, and pH emerging as the top four predictors of cyanobacteria biovolume, based on their relative importance as measured by percent increase in mean squared error (%IncMSE; **Table 4**). Because the dataset spans two years, multiple seasons, and diverse sites, the ranked predictor importance reflects a blend of interannual, seasonal, and spatial gradients. These underlying gradients must be considered when interpreting the potential ecological role of each predictor as a possible driver or consequence of cyanobacteria abundance.

Table 4: Relative importance of environmental predictors in random forest models of cyanobacteria biovolume, ranked by percent increase in mean squared error (%IncMSE). Rankings are shown for the whole estuary model, combined upper estuary model, and lower estuary model. NA indicates that the predictor was removed during iterative variable reduction in the final model. Three additional models are presented using all Barker's Island (BA) data (approximately weekly sampling), and subsets of bi-weekly and monthly sampling for that site. Model parameters included are: number of sampling dates (n), mean squared error of prediction (mse), and multiple correlation coefficient (R^2).

model parameter	whole model	upper model	lower model	BA	BA bi-weekly	BA monthly
n	272	124	119	56	32	16
mse	0.29	0.27	0.29	0.25	0.29	0.39
R^2	0.49	0.46	0.58	0.66	0.4	0.22
Predictor						
temp	1	8	1	8	8	2
DO	2	6	7	10	9	1
TOC	3	5	4	2	1	7
pH	4	14	2	13	15	14
DOC	5	7	8	3	5	9
TN	6	2	3	1	2	5
NO2+NO3	7	1	6	6	4	3
spcond	8	9	9	12	11	8
ANC	9	12	5	17	10	10
TN:TP	10	NA	10	5	3	4
PAR kd	11	3	14	9	6	6
NH4	12	4	12	7	13	13

model parameter	whole model	upper model	lower model	BA	BA bi-weekly	BA monthly
DOsat	13	16	11	14	14	11
TP	14	10	18	15	NA	15
secchi	15	11	15	18	16	17
SRP	16	15	17	11	12	NA
TSS	17	NA	13	4	7	12
turbidity	18	13	16	16	NA	16

Temperature was the most important predictor (19.3 %IncMSE), with partial dependence plots (**Figure 9a**) revealing a positive relationship with cyanobacteria biovolume, with an apparent threshold near ~15°C. While temperature directly affects cyanobacteria growth rates (Richardson et al. 2018; Przytulska et al. 2017), its top rank in the model also likely reflects co-variation with other factors such as PARkd, DO, and TN—all of which have seasonal variability and show significant correlations to temperature (**Appendix E**). Thus, temperature may serve both as a direct driver and as a strong proxy for broader seasonal changes linked to cyanobacteria abundance. It is important to note that extensive blooms in the SLRE did not occur during peak summer heat but in the weeks that followed, suggesting potential lagged effects. These delayed responses, explored in the temporal redundancy section, point to temperature's role not just as an immediate driver but also as a temporal signal shaped by its potential interaction with nutrient dynamics and hydrologic conditions.

DO was the second-most important predictor (14.6 %IncMSE) and had a complex relationship with cyanobacteria biovolume, likely reflecting different underlying DO gradients over time and space. The highest predicted biovolume occurred at intermediate DO levels (7.5–11 mg/L), while very high DO (>11 mg/L) was negatively associated with cyanobacteria. This pattern likely reflects two seasonal mechanisms: cold, oxygen-rich conditions in winter and spring when cyanobacteria are scarce, and elevated DO in summer linked to photosynthetic activity of cyanobacteria and other phytoplankton. A significant, negative correlation between DO and temperature (**Appendix E**; $r=-0.49$, $p<0.05$) supports the seasonal effect interpretation. Site-specific differences to this pattern, including winter hypoxia at Allouez Bay and low summer DO at Pokegama, may also contribute to this complexity. Overall, we interpret DO as an indicator of both seasonal and biological processes, rather than a direct driver of cyanobacteria growth.

TOC was the third most important predictor (14.4 %IncMSE) and had a negative relationship with cyanobacteria biovolume, likely due to its combined strong interannual, seasonal, and spatial gradients. TOC was lower in 2023 than in 2024, declined over the 2023 season (**Appendix C**), and was lowest in the lower estuary where cyanobacteria were most abundant. These patterns help explain its model importance, with higher cyanobacteria biovolume consistently associated with lower TOC levels across years, seasons, and space. Although TOC/DOC (significantly related $r=0.93$; **Appendix E**) could have a direct impact on cyanobacteria (Klemer et al. 1996), its strong temporal gradient likely masks any discernible direct effects on SLRE abundances.

pH was the fourth most important predictor (14.1 %IncMSE) with a positive relationship with cyanobacteria biovolume. Like other important parameters in the model, pH has some seasonal and

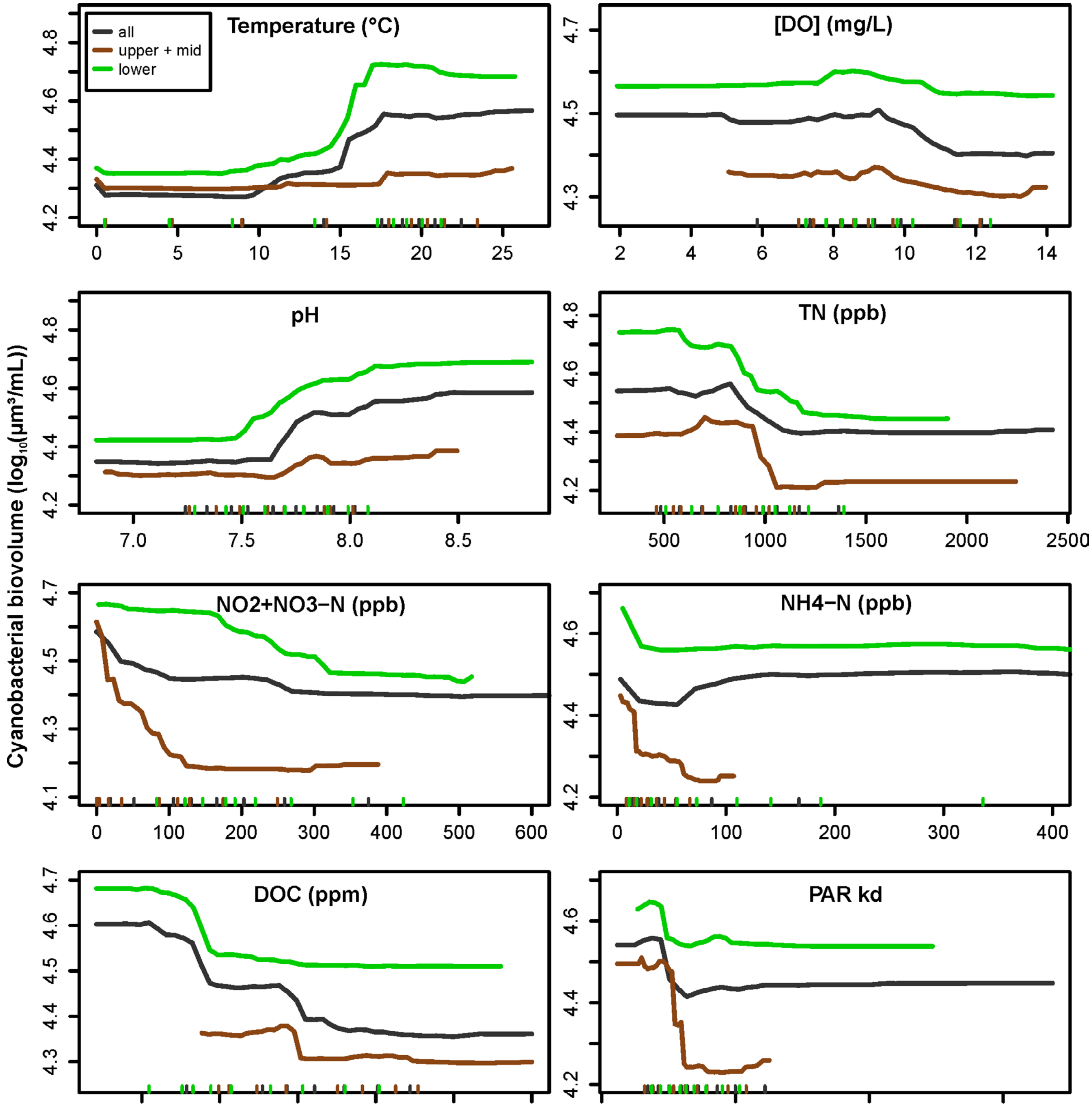


Figure 9a: *Partial dependence plots showing the marginal effects of selected environmental variables had on the predicted cyanobacteria biovolume in a random forest machine learning model. Models were separately developed for all sites and two subdivisions (upper + mid, lower SLRE;* **Figure 1***).*

spatial gradients, being higher in the lower estuary and in the fall (**Appendix C**). In general, pH has lower variability (Appendix D) when compared to the other top ranked parameters, except in the lower estuary sites where pH and cyanobacteria abundance are highest. This positive relationship between pH and cyanobacteria could be a consequence of photosynthesis-induced depletion of CO2 and subsequent increase in pH that often occurs when cyanobacteria are abundant and productivity is high (Badger and Price 2003; Paerl and Ustach 1982). At Barker's Island, late-summer pH often increases, coincident with high cyanobacteria and phytoplankton abundance (**Figure 10**). We documented high pH and CO2 concentrations below atmospheric concentrations (~430 ppm; NOAA 2025) preceding and during bloom events. The potential use of pH as a proxy for CO2 drawdown and therefore a good bloom indicator is discussed in the Monitoring Recommendations section.

We noted spatial separation in SLRE biological and water quality conditions using NMDS (see spatial redundancy section below), therefore we performed RFA on spatial subsets of data: upper model (NO, MU, KI, BI), lower model (BA, MI, AL), and Barker's Island only model (BA). These subset models allowed us

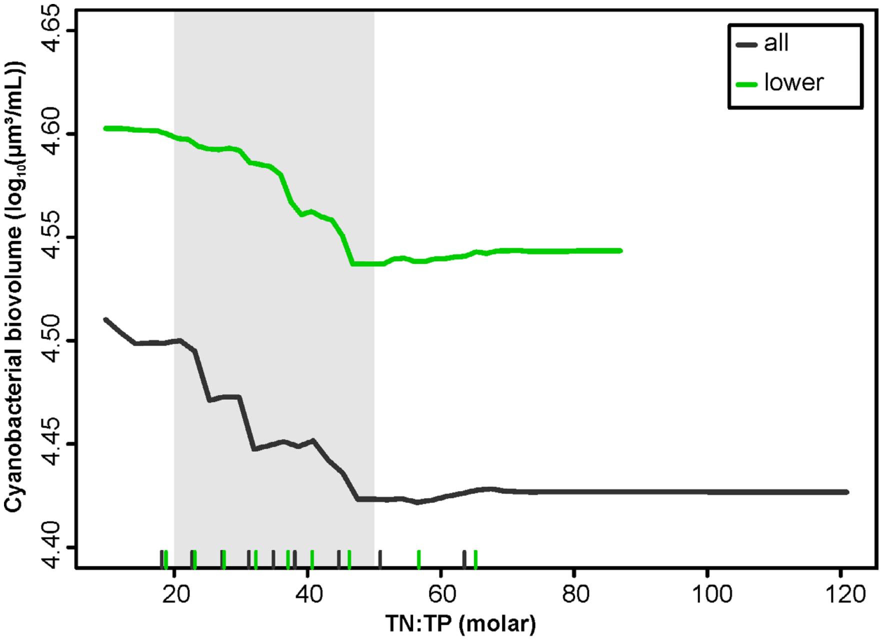


Figure 9b: *Partial dependence plot showing the marginal effects of TN:TP on the predicted cyanobacteria biovolume in a random forest machine learning model. Models were separately developed for all sites and the lower estuary. Gray shading is used to indicate the region below which nitrogen is limiting and above which phosphorus is limiting.*

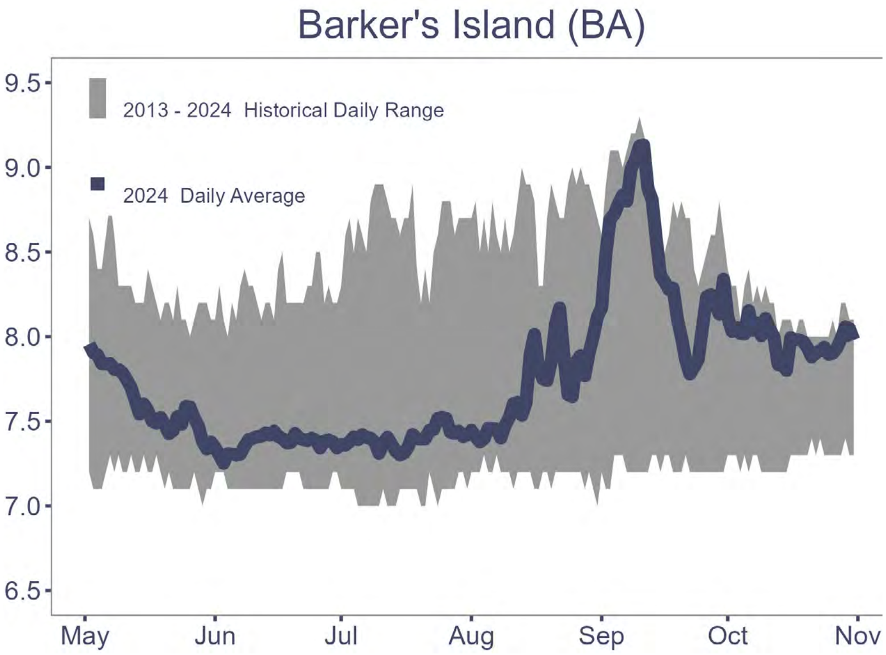


Figure 10: *Historical range (2013 - 2024) in pH overlaid with 2024 daily mean showing high pH in September. Data from SWMP Barker's Island continuous (15-minute) water quality monitoring station (NOAA 2025)*

to isolate region-specific relationships and reduce some of the spatial effects that confound identifying temporal and direct effects in the whole model. The Barker's Island-only model had the highest R^2 among all models (0.66), suggesting that performance can improve when analyses are tailored to specific locations.

Despite the reduced spatial variability, TOC remained a top predictor in all subset models. In the Barker's Island-only model, TOC and DOC ranked second and third, respectively, reinforcing their importance as excellent temporally structured predictors. DO dropped out of the top predictors in all subset models, suggesting its importance in the whole model stems from spatial variation more than temporal dynamics. pH increased in importance in the lower model, again supporting the idea that it could have a direct relationship to cyanobacteria abundance in sites that experience high productivity and therefore high photosynthesis rates that increase pH.

Temperature remained the top predictor in the lower estuary model, but dropped in importance in the upper estuary and the Barker's Island model (ranked 8th). In contrast, nitrogen forms gained importance in the subset models. TN ranked among the top three predictors in all models except the whole estuary (ranked 6th). NO2+3 and NH4 were especially important in the upper estuary model, where temperature had a lesser importance. All nitrogen forms had a consistent negative relationship with cyanobacteria biovolume (**Figure 9a**). Similar to TOC, TN showed a strong seasonal signal, declining from June through September 2023 due to low river discharge during worsening drought conditions. TN was also higher overall in 2024 (**Appendix C**), reinforcing its sensitivity to interannual hydrologic variation. The importance of nitrogen as a driver of cyanobacteria growth is described in greater detail, below.

Finally, TP and SRP consistently ranked among the least important predictors. This likely reflects their consistently high concentrations and limited variability across most sites (with the exception of Pokegama Bay and Allouez Bay), which reduced their ability to explain cyanobacteria dynamics in this dataset. This does not suggest that phosphorus is unimportant for bloom formation, in fact its foundational role in eutrophication is well established (Schindler 1974). Rather, the persistently elevated levels of phosphorus (typically >30 µg/L; **Appendix C**) indicate that it was likely non-limiting and thus exerted less influence on the models. Instead, nitrogen forms and availability were more strongly associated with cyanobacteria abundance and are therefore better predictors of blooms, an interpretation that is further supported by the consistent selection of low N:P ratios as a moderately important predictor across RFA models (**Table 4**).

Taken together, TN, TOC, and temperature were consistently important predictors of cyanobacteria biovolume across all models, and were especially important predictors in the bloom-prone lower estuary. Although they are important for prediction, we caution against interpreting them as drivers of blooms. Each reflects broader ecological conditions, particularly strong seasonal gradients. Among them, TOC appears to be a robust proxy for several bloom-favorable factors: low TOC aligns with base flow periods, high water residence times, and Lake Superior water intrusion into the lower estuary (Loken et al. 2015). These baseflow conditions, typical from late July through September, coincide with peak seasonal warming which promotes high productivity and nitrogen drawdown. This convergence of conditions helps explain TOC's predictive strength.

In 2023, these conditions were especially pronounced. A prolonged base flow period extended from June to September (Figure 6), enhancing the duration of bloom-favorable conditions. This coincided with the warmer-than-average September (NWS 2023a), which likely amplified productivity and strengthened the "denitrification pump" (Loken et al. 2016), accelerating nitrogen loss from the system. The combination of warm water, high water residence time, high productivity, and low nitrogen availability favor nitrogen-fixing cyanobacteria (Schindler 1977; Smith 1983). This likely explains the overwhelming dominance of the diazotrophic *Aphanizomenon flos aquae* during the most intense bloom events (**Appendix B)**, and aligns with ecological theory that nitrogen limitation provides a competitive advantage to diazotrophic taxa. These findings suggest that extended low-flow periods, indicated by low TOC and TN in our models, may be key precursors to bloom development in the SLRE.

While we cannot confirm a mechanistic link, additional support for nitrogen limitation comes from the relationship between cyanobacteria biovolume and N:P ratio. Although N:P was not a top predictor in the RFA models, its marginal effect showed cyanobacteria biovolume was highest under nitrogen-limited conditions and declined steadily as conditions shifted toward phosphorus limitation (Guildford and Hecky 2000;**Figure 9b**).

In contrast to the long duration of base-flow conditions in 2023 that may have led to favorable conditions for *Aphanizomenon*, 2024 had a short base flow duration (August-September) and blooms that were small and more taxonomically diverse, with greater proportions of non-diazotrophic species like *Microcystis aeruginosa, Pseudanabaena limnetica*, and *Planktothrix sp. cf agardhii/rubescens*. This shift suggests that bloom intensity and composition is highly responsive to interannual variation in hydrology and nutrient stoichiometry, further underscoring the importance of temperature, TN, and TOC as integrative indicators of bloom-favorable conditions in the SLRE.

Our results indicate that hydrologic conditions (approximated by TOC), nitrogen availability (approximated by TN), and temperature are key predictors of bloom risk, emphasizing the influence of climate-sensitive factors such as rainfall patterns and rising temperatures. These findings suggest that, if point source nutrient inputs remain unchanged, future bloom dynamics in the SLRE will likely be shaped by climate-driven shifts in temperature and hydrology.

WATER QUALITY AND NUTRIENT CHARACTERIZATION

Water quality data for the eight sampling locations over the two year period are provided in **Appendix C** and **Appendix D**. While we do not discuss each water quality parameter and trend at length in this report, specific trends associated with phytoplankton dynamics are highlighted.

Explore Further

Explore water quality site comparisons over time using our data tool:
https://lakesuperiornerr.shinyapps.io/SLRE_hot_spot/

SPATIAL TRENDS

Spatial patterns in 2023-2024 water quality generally followed expected SLRE gradients. Specific conductivity was highest in the upper estuary and decreased toward Lake Superior, with two exceptions: Pokegama Bay, which receives inputs from the Pokegama River, and Miller Creek, which receives inputs from its namesake creek and wastewater discharges. Both sites exhibited elevated conductivity relative to nearby areas. Dissolved and total carbon showed a similar pattern, with higher concentrations in the upper estuary and lower values downstream as carbon-rich waters from the SLRE were diluted by Lake Superior and urban stream inputs. In contrast, NO2+3 concentrations were higher in the lower estuary, reflecting the influence of Lake Superior's nutrient profile.

Turbidity was notably higher in Pokegama Bay and Allouez Bay, consistent with sediment transport from red clay watersheds. TN concentrations were highest at Miller Creek and Pokegama Bay, likely from point source inputs. Temperature was generally similar across sites, although in spring 2023, upper estuary locations warmed more rapidly than those closer to Lake Superior.

COMPARISONS TO LONG-TERM MONITORING DATA

System-wide Monitoring Program

The SWMP tracks long-term changes and short-term variability in estuarine water quality, weather, and biotic conditions across all thirty National Estuarine Research Reserves. In the SLRE, SWMP has operated since 2012, collecting continuous water quality measurements at 15-minute intervals and monthly samples for nutrients, chlorophyll, and suspended sediments. Four primary SWMP stations serve the SLRE: Barker's Island and Pokegama Bay, which overlap with hotspot areas identified in this project, and Oliver Bridge and Blatnik Bridge, located in deeper, well-mixed sections of the SLRE thalweg. Additional infrastructure includes a weather station at Pokegama Bay and a Lake Superior buoy. These long-term datasets provide a critical baseline for comparing hotspot sites to average estuary conditions.

Explore Further

Explore and access all SWMP data https://cdmo.baruch.sc.edu/ and https://seagull.glos.org/landing

Hotspots vs. Thalweg

To evaluate how hotspot conditions differed from the thalweg, we paired each hotspot with its nearest thalweg SWMP station (Oliver Bridge or Blatnik Bridge) and conducted paired t-tests on mean values for shared water quality parameters. Analyses were limited to the spring through fall open-water season.

In the upper and middle estuary, North Bay, Kingsbury Bay, and Billings Park had significantly ($p < 0.05$) higher NH4 concentrations than Oliver Bridge. North Bay and Billings Park also had lower dissolved oxygen. Otherwise, most shallow, sheltered sites were otherwise similar to Oliver Bridge during the open-water season. Winter data, which SWMP does not capture, revealed hypoxia at North Bay and Mud Lake. Pokegama Bay stood out from other upper and middle estuary sites, with significantly higher concentrations of TP, SRP, TN, TSS, and turbidity, and lower DO. These differences align with the expected red clay influence from its watershed.

In the lower estuary, Allouez Bay showed higher TP, TSS, and turbidity than Blatnik Bridge, again consistent with red clay inputs. Barker's Island was generally similar to Blatnik Bridge. Miller Creek differed the most from Blatnik Bridge even though geographically close, with higher TP, TN, NH4, and conductivity, likely capturing runoff from an impaired Miller Creek and wastewater discharges. The most pronounced difference was concentrations of NH4 at Miller Creek, which averaged 341 µg/L, compared to 109 µg/L at Blatnik Bridge. A full summary of mean differences is provided in **Appendix D**, with significant differences indicated by asterisks.

Anomalies and short term trends

We used continuous SWMP data to characterize 2023 and 2024 conditions relative to the entire 2013-2024 SWMP record. Anomalies were calculated by subtracting the 2013–2024 monthly mean from observed monthly means from each year. Monthly Mann-Kendall trend tests were also conducted by parameter and station. For method details see **Appendix A**.

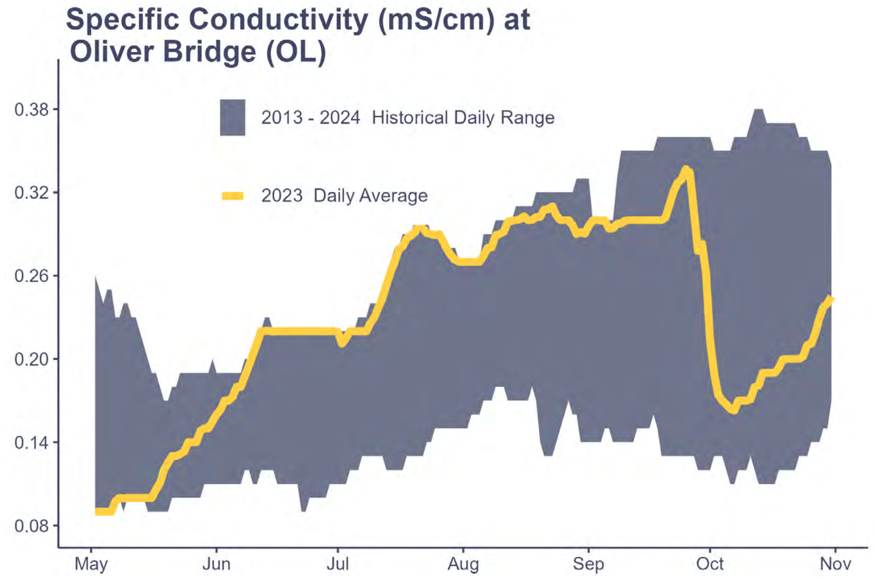


Figure 11: *Historical range (2013 - 2024) in specific conductivity (mS/cm) overlaid with 2023 daily mean, showing record breaking high values in June and July. Data from SWMP Oliver Bridge continuous (15-minute) water quality monitoring station (NOAA/NERRS 2025)*

In 2023, Duluth received approximately 8 inches of rain between May 1 and August 31, about 6 inches below average (NOAA 2023). This deficit drove severe drought conditions across the region. In the SLRE, the lack of rainfall contributed to elevated specific conductivity and unusually clear water, similar to impacts seen during the 2021 drought. Specific conductivity at Oliver Bridge exceeded 0.30 mS/cm, the Fond du Lac Reservation water quality standard threshold (Fond du Lac Band of Lake Superior Chippewa 2020), during August and September (**Figure 11**). Turbidity levels were the second-lowest on record, behind only 2021, across all SWMP stations.

In 2024, water temperatures were notably high, particularly at Barker's Island, where they exceeded the long-term average throughout the open-water season. September and October had especially above average temperatures (**Figure 12**). Elevated temperatures reflected the second warmest fall on record for air temperatures (Loverien 2024), compounded by low ice cover on Lake Superior and the estuary during the preceding winter and dry conditions in September.

Barker's Island also had higher than average pH in both 2023 and 2024, with the largest anomalies in August and September. A significant increasing trend in pH over time was observed (**Figure 13**). These high pH results may reflect broader increases in Lake Superior pH documented in western Lake Superior (Minor et al. 2019), especially during dry, low-discharge months, meaning acidic rainwater (~5.6 pH) is not added to the system. They could also reflect increased photosynthetic activity in summer months with highest overall phytoplankton biovolume.

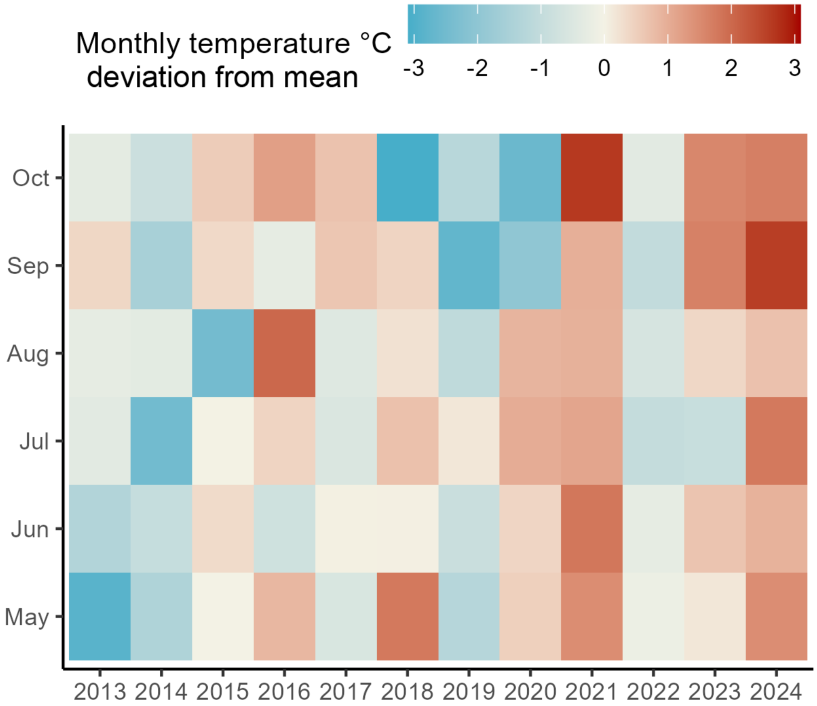


Figure 12: *Monthly water temperature (°C) anomalies - calculated as each month's deviation from historic monthly mean (2013 - 2024). Data from SWMP continuous monitoring station (NOAA/NERRS 2025)*

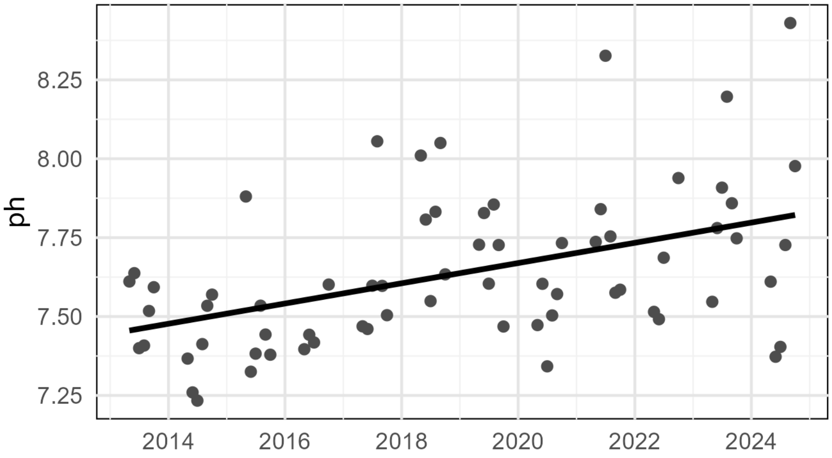


Figure 13: *Monthly mean pH values over time, with significant ($p < 0.05$) positive Mann-Kendall trend. Data from SWMP Barker's Island continuous (15-minute) water quality monitoring station (NOAA/NERRS 2025).*

Milestone Monitoring Program

The Minnesota Pollution Control Agency (MPCA) initiated the Milestone Monitoring Program (MMP) in the SLRE in the early 1970s to assess long-term trends and evaluate the effectiveness of pollution control measures. These efforts built on earlier U.S. Public Health Service monitoring, with data available as far back as 1953 (Water Quality Portal 2021). Bellinger et al. (2016) conducted a time-series analysis of nutrient and TSS data spanning 1953 to 2010, drawing on MMP and earlier datasets. Their findings showed significant decreases in TP and TSS concentrations and loads over time, contributing critical evidence toward the removal of BUI 6: Excessive Loading of Sediment and Nutrients (U.S. EPA 2020). Building on that work, we repeated the Bellinger et al. (2016) analysis, adding a decade of new data from the SWMP (2013–2024). Full methodological details are provided in **Appendix A**.

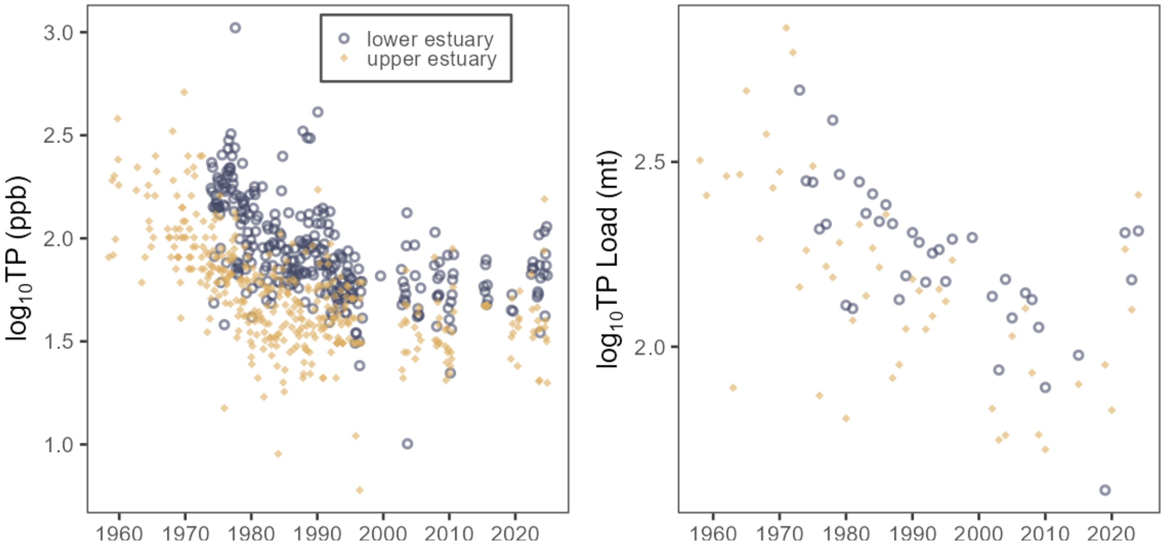


Figure 14: *Total Phosphorus (TP) monthly concentrations (left) and annual loads (right) over time. There was a significant decline in both concentrations and loads at the lower estuary station (open circle) at Blatnik Bridge and upper estuary station (diamond) at HWY23 / Oliver Bridge based on Mann-Kendall test $p<0.05$ (see* ***Appendix A*** *for details). Data from MMP accessed from the U.S. EPA Water Quality Data Portal, and the SWMP (NOAA/NERRS 2025)*

Total Phosphorus

Monthly concentrations and annual loads of TP have significantly decreased at both upper and lower estuary sites since the 1960s. This trend remains consistent after the addition of the last decade of SWMP data (**Figure 14**).

Total Suspended Solids

TSS concentrations have continued to decline over time at both sites, but the addition of data from the most recent decade has shifted the long-term trend in TSS loads at the lower estuary site (**Figure 15**). This period includes both exceptionally wet and dry years, highlighting the growing influence of hydrologic variability. These recent data suggest that TSS loads are now more strongly driven by storm

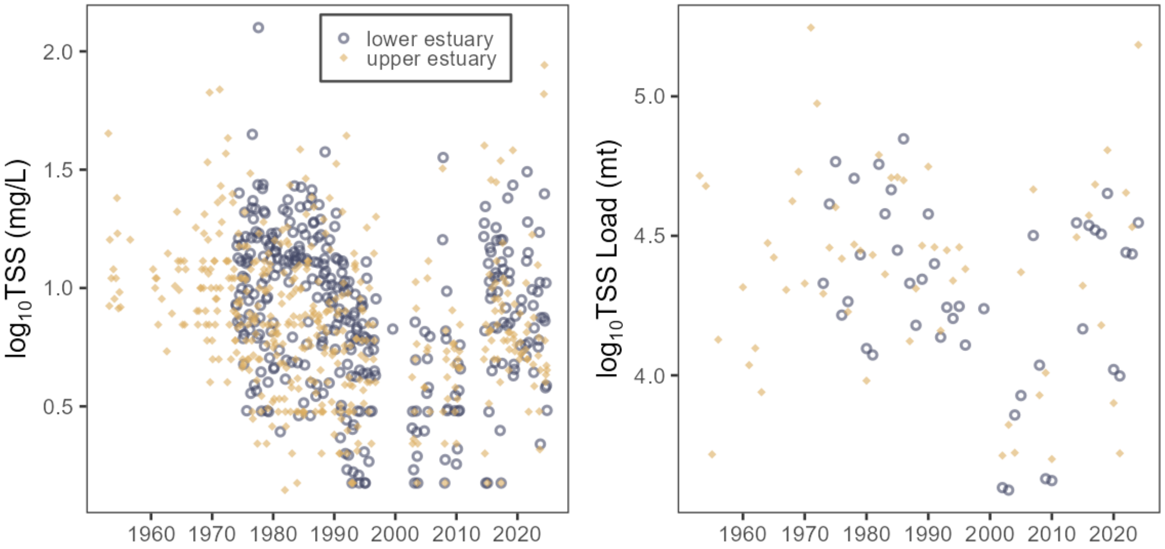


Figure 15: *Total Suspended Solids (TSS) monthly concentrations (left) and annual loads (right) over time. There was a significant decline in concentrations at both the lower estuary station (open circle) at Blatnik Bridge and upper estuary station (diamond) at HWY23 / Oliver Bridge based on Mann-Kendall test $p<0.05$ (see* **Appendix A** *for details). There was a significant decline in loads at the upper estuary station, however there was no significant trend in loads at the lower estuary station. Data from MMP accessed from the U.S. EPA Water Quality Data Portal, and the SWMP (NOAA 2025)*

events and river discharge than by historical point-source pollution from industry.

The relationship between TSS and discharge has strengthened, indicating that current sediment loads are increasingly shaped by runoff rather than legacy sources such as wood pulp or industrial waste. However, an uptick in TSS concentrations at both stations since approximately 2012 raises additional questions. Possible contributing factors include lingering mobility of sediments from the 2012 flood event (NWS 2012), changes in sediment transport dynamics, or dam operations. Because the same trend is observed in both the upper and lower estuary, the increase in TSS is unlikely to be explained by dredging activities, which do not occur upstream of the Oliver Bridge, or by wind-driven resuspension, which tends to be stronger in the open waters of the lower estuary.

Dissolved Oxygen

DO concentrations increased at both Oliver Bridge and Blatnik Bridge from 1950 to approximately 1980, reflecting water quality improvements following early pollution controls. Since then, concentrations have shown a slight decline, although dissolved oxygen percent saturation has remained stable, suggesting that temperature increases rather than declining water quality are responsible.

Nitrogen Trends

Nitrate concentrations have declined in the upper estuary but remained relatively stable in the lower SLRE over time. Our analysis confirms findings from Loken et al. (2016) and Bellinger et al. (2014) that Lake Superior is currently a substantial source of nitrate to the lower estuary, especially during late summer and fall. Although upper estuary inputs of nitrate have decreased, concentrations are likely stable in the lower estuary because some earlier point sources have been replaced by Lake Superior inputs, which have been gradually increasing due to lake-wide nitrification (Sterner et al. 2007).

Ammonia concentrations have steadily declined in the upper estuary since the 1960s, an expected trend following the reduction of direct sewage discharge; however, in the lower estuary, levels declined until the 1990s and have since increased (**Figure 16**). This reversal may be driven by changes in wastewater treatment operations or by greater accumulation of ammonia during prolonged periods of low river discharge. Supporting this, ammonia shows an inverse relationship with discharge (**Figure 17**), with concentrations typically peaking during low-flow conditions from late summer through winter. The Blatnik Bridge site, located near the Western Lake Superior Sanitary District (also known as Resource Renew) wastewater treat,emt outfall, is likely influenced by this localized point source, which may affect the interpretation of long-term trends. As such, we caution against interpreting these observations as evidence of increasing ammonia concentrations throughout the entire lower estuary. Data from this study's Miller Creek site, situated within the bay that receives treated effluent (**Figure 1**), had average NH4 concentrations of 341.4 ppb during open water sampling, substantially higher than those observed at other lower estuary sites (**Appendix D**). These elevated concentrations likely reflect limited flushing within the bay. That said, broader increases in ammonia across the lower estuary cannot be entirely ruled out. Larger-scale biogeochemical changes may also be at play, particularly processes in which nitrate, primarily supplied by Lake Superior, is increasingly converted to ammonia.

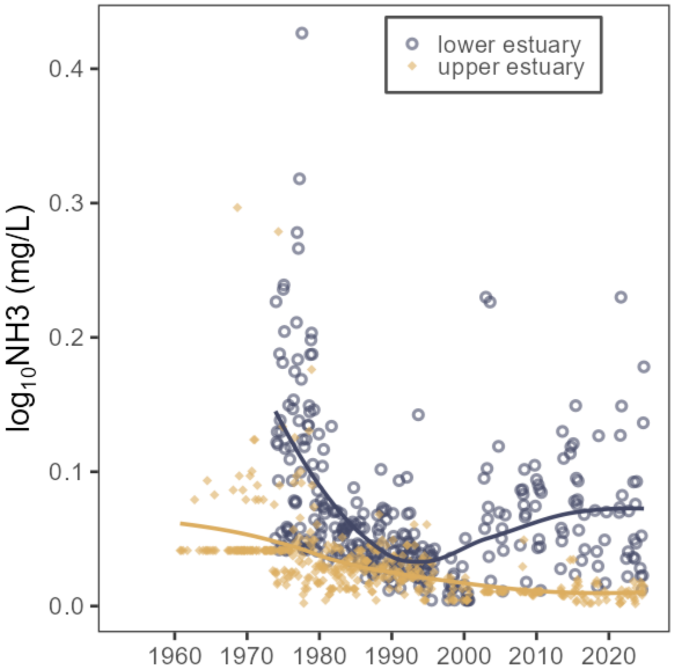


Figure 16: *Ammonia (NH3) monthly concentrations over time with LOESS fit showing declines at the upper estuary station (diamond) at HWY23 / Oliver Bridge but recent increases at the lower estuary station (open circle) at Blatnik Bridge. Data from MMP accessed from the U.S. EPA Water Quality Data Portal, and the SWMP (NOAA 2025).*

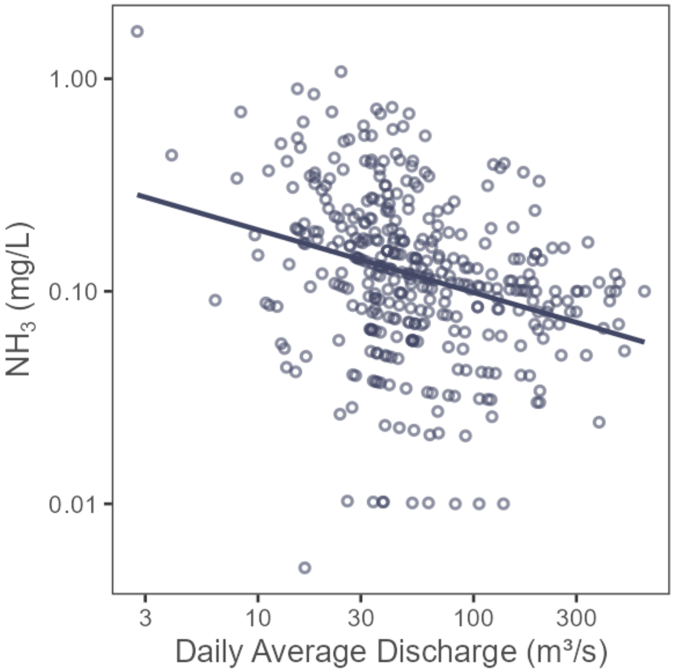


Figure 17: *Ammonia concentration related to daily river discharge at the lower estuary station (Blatnik Bridge). Data from MMP accessed from the U.S. EPA Water Quality Data Portal and St. Louis River USGS Surface Water Monitoring Site 04024000 at Scanlon (USGS 2025).*

Summary - Comparisons to Long-term Monitoring Data

By comparing our recent sampling years to long-term trends in the SLRE, we provide essential context for interpreting the RFA results. Importantly, two predictors identified by the RFA as most influential, temperature and nitrogen, are also variables undergoing potential long-term changes in the system. Temperature emerged as the most important predictor of cyanobacteria biovolume, and SWMP data confirm that both 2023 and 2024 were above-average temperature years, with 2024 being the warmest on record. Concurrently, nitrogen availability and its seasonal dynamics play a critical role in shaping bloom composition and intensity, and long-term trends suggest that hydrologic conditions likely influence the form and timing of nitrogen availability. pH also ranked among the top predictors in the RFA, and SWMP data show a significant increasing trend at Barker's Island during late summer months, likely reflecting elevated productivity.

Together, these findings indicate that warming, shifting nitrogen dynamics, and hydrology are closely intertwined in shaping bloom risk in the SLRE. As climate-driven changes continue, these interacting factors are likely to exert increasing influence over the timing, intensity, and composition of cyanobacterial blooms.

HYPOXIA RESULTS AND DISCUSSION

To better understand how low DO conditions may contribute to nutrient dynamics and bloom formation in the SLRE, we evaluated the spatial and temporal extent of hypoxia across eight sites. This section summarizes the extent and severity of hypoxia during the study period, with consideration of both winter and open-water seasons. Particular focus is given to identifying sites most vulnerable to low oxygen conditions. Additional analyses are underway and will be presented separately from this report.

Allouez Bay, Pokegama Bay, and North Bay were the most hypoxia-prone sites, with winter DO concentrations frequently falling below the 2 mg/L threshold (**Figure 18**). Although Miller Creek did not become hypoxic, it did infrequently (0.1% of the time) have DO values below 5 mg/L, a threshold commonly used for coldwater fish (Bjornn and Reiser 1991). The occurrence of hypoxia varied by year, with the three hypoxia-prone sites experiencing hypoxia more frequently in 2023 and rarely in 2024 (**Figure 19**). In 2023, Allouez Bay had the highest proportion of hypoxic observations at 12.7%, followed by Pokegama Bay and North Bay, each with 1.5%. While DO values at North Bay were more continuously distributed, Allouez Bay, and to a lesser extent Pokegama Bay, showed a bimodal pattern. Observations clustered either below 5 mg/L or above 9 mg/L, suggesting rapid shifts between oxic and hypoxic states at these locations. In 2024, Allouez Bay (19.6%) and Pokegama Bay (1.8%) still had DO concentrations below the coldwater fish threshold of 5 mg/L.

Short-term winter DO patterns also varied among sites. North Bay displayed consistent diel fluctuations, with DO levels varying by 5 mg/L or more in both years. In contrast, Allouez Bay and Pokegama Bay exhibited minimal diel variability (**Figure 20**). Preliminary analyses suggest that diel DO

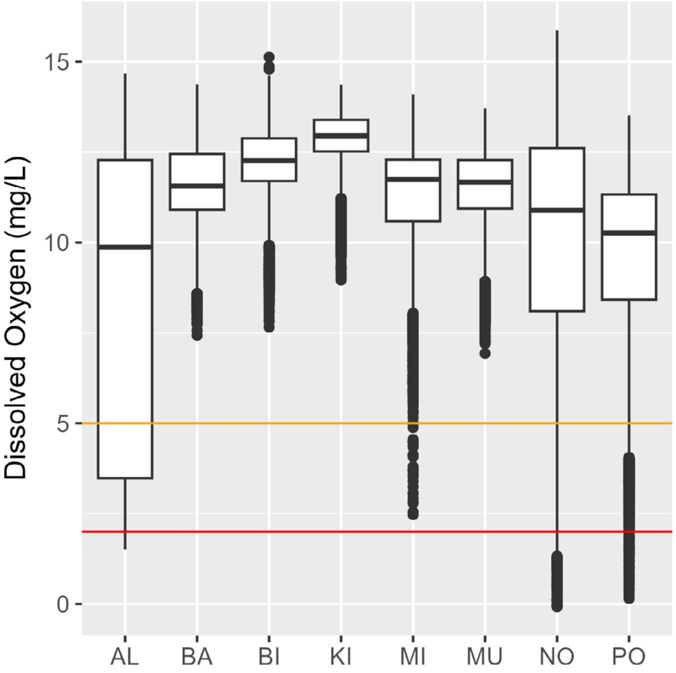


Figure 18: *Boxplots of dissolved oxygen concentrations (mg/L) by site including data from both winters of this study. Red line indicates the hypoxia threshold of 2 mg/L, the orange line indicates the stress threshold for fish at 5 mg/L.*

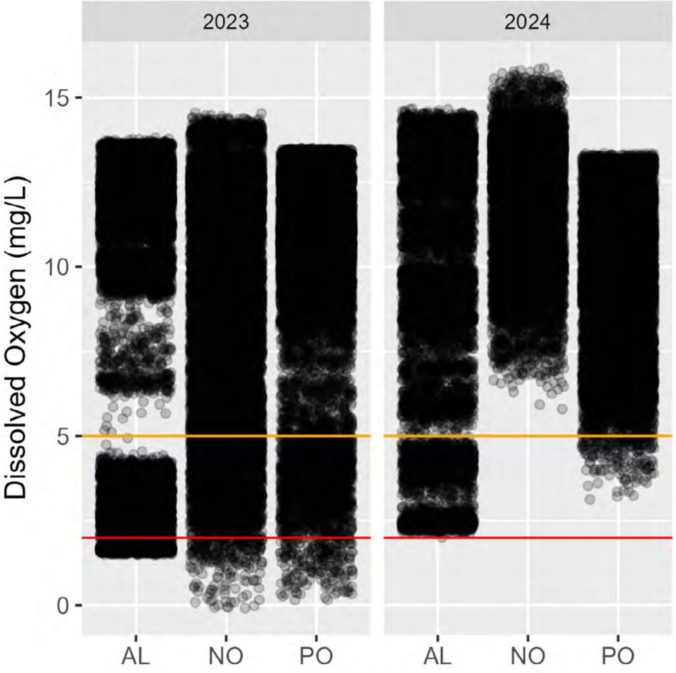


Figure 19: *Distribution of dissolved oxygen concentration (mg/L) observations by year for the three hypoxia-prone sites. Markers are slightly transparent in which dark bands and regions indicate densely distributed observations. Red line indicates the hypoxia threshold of 2 mg/L, the orange line indicates the stress threshold for fish at 5 mg/L.*

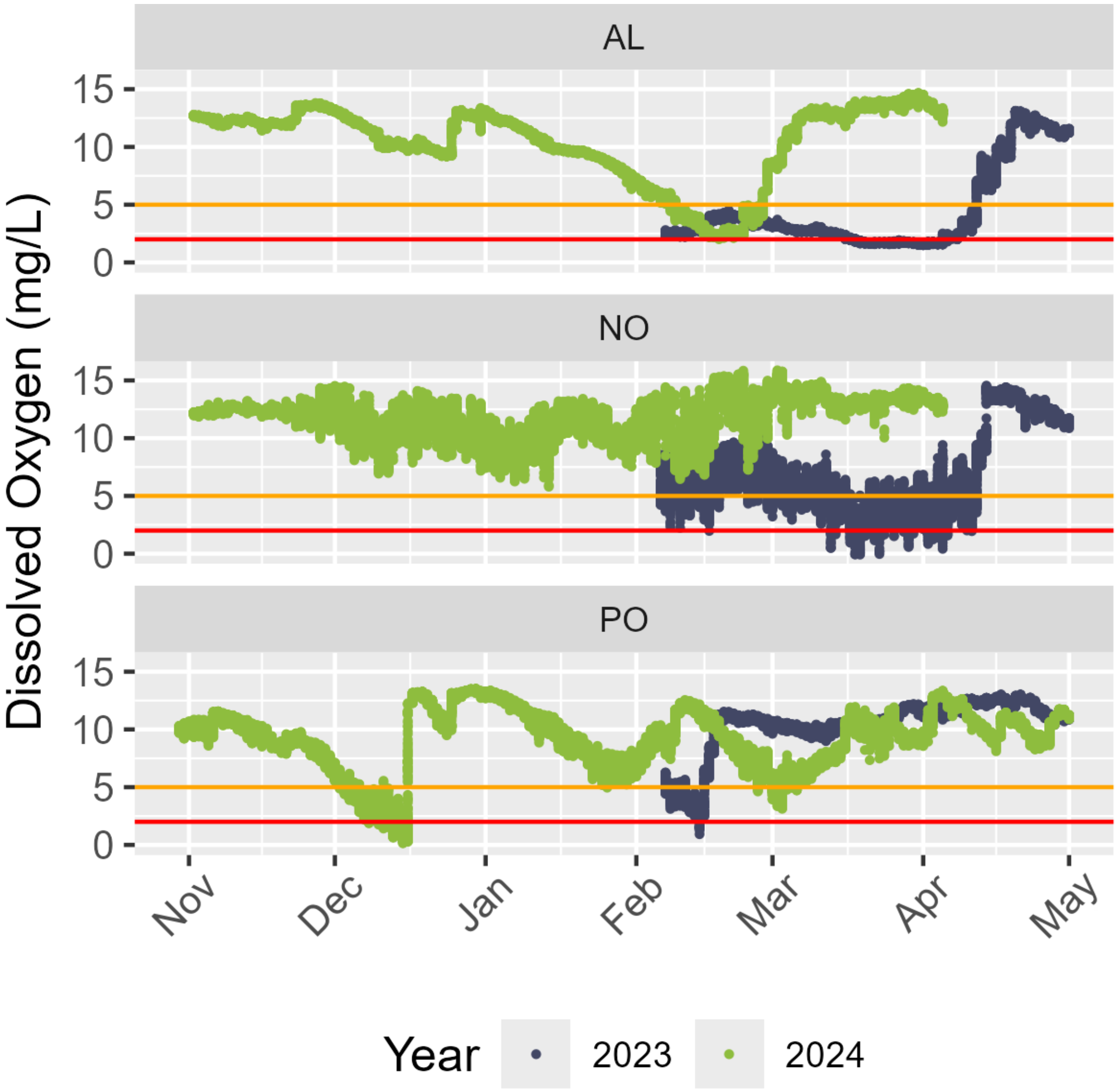


Figure 20: *Time series of continuous dissolved oxygen concentrations (mg/L) for winters of 2022 to 2023 and 2023 to 2024 for the three hypoxia-prone sites. Red line indicates the hypoxia threshold of 2 mg/L, the orange line indicates the stress threshold for fish at 5 mg/L.*

concentration and saturation signals were more pronounced across most sites in winter 2023-2024, when ice cover was thin or absent, than in 2022-2023, when ice and snow were thicker. However, this difference was least apparent at the highly turbid sites, Allouez Bay and Pokegama Bay. These short-term dynamics will be examined further in future analyses outside the scope of this report.

Our winter observations build on earlier findings by Garono and Schooler (2015), who documented recurring winter hypoxia in turbid, clay-rich bays and occasionally in sheltered, non-turbid bays of the SLRE. Their work showed that ice thickness and light transmission were associated with DO concentrations. This relationship will be revisited with the addition of data from this study in future analyses.

No hypoxic conditions were observed during routine discrete sampling in the open-water season. Continuous DO data from the SWMP station shows that Pokegama Bay consistently exhibited low DO during summer, with concentrations dropping as low as 2.7 mg/L. This is a recurring pattern. Between 2013 and 2024, Pokegama Bay recorded DO values below 2 mg/L an average of 18 days per year during the May to September period.

Winter sampling was limited due to unsafe ice conditions in 2023 to 2024. Across both winters, only two sampling events coincided with hypoxia, both in Allouez Bay. This limited overlap between hypoxic periods and nutrient or phytoplankton sampling prevents a thorough evaluation of the ecological impacts. However, it is notable that Allouez Bay had relatively high winter chlorophyll-a and centric diatom biovolume compared to other sites. This suggests that sufficient nutrients were available to support under-ice diatom abundance in February and March of 2023. Research has shown that anoxia can trigger sediment phosphorus release (Nürnberg 1984), and this remains an important and active area of study in the SLRE.

Historically, hypoxia in the SLRE was widespread due to excessive inputs of sewage, industrial waste, wood pulp, and logging debris. Reductions in industrial discharge and management actions, including the establishment of wastewater treatment facilities, led to substantial improvements in water quality. Today, hypoxia in non-turbid, sheltered bays is likely associated with the decomposition of internally produced organic matter, such as algae and aquatic plant material, combined with limited mixing due to their disconnection from main river flows. This pattern is reflected in our findings at North Bay, a sheltered bay with manoomin-supporting wetlands and otherwise good water quality that still experienced winter hypoxia. In contrast, hypoxia in turbid bays is more likely driven by the respiration of organic matter delivered from tributaries, particularly in a location like Pokegama Bay. To test these hypotheses, further research is needed to better understand the role of low DO in driving sediment nutrient release. Further field sampling, paired with controlled experiments are recommended to investigate the underlying mechanisms.

REDUNDANCY ANALYSIS

SPATIAL REDUNDANCY

To assess whether selected monitoring sites within the SLRE capture distinct environmental conditions or exhibit redundancy, we employed ordination to identify relationships among sites based on environmental dissimilarity. NMDS is well-suited for ecological datasets because it does not assume linear relationships among variables and is effective for representing complex, multivariate differences in low-dimensional space (Shepard 1980). This makes it an applicable tool for examining spatial redundancy, where the goal is to evaluate overall similarity in site characteristics based on a suite of interrelated parameters. By visualizing site separation in multivariate space and pairing it with PERMANOVA to statistically test differences, we determined which sites provide unique information and which may be redundant in the context of long-term monitoring design. We performed two types of NMDS analyses using the full dataset across all sites and years: one based on water quality data and one on phytoplankton community composition. We also conducted each NMDS using summer-only (July-October) data to explore patterns relevant to the time period that cyanobacteria are typically abundant in the SLRE.

Prior to water quality ordination, we applied correlation screening to reduce the number of environmental variables (**Appendix E**, **Appendix A)**. The final set of water quality variables included TP, TN, NO2+3, NH4, DOC, chl-a, specific conductivity, pH, temperature, secchi, and N:P. Phytoplankton NMDS was generated using total biovolume (µm3/ml) of each taxonomic division (centric diatoms, pennate diatoms, chlorophytes, chrysophytes, cryptophytes, cyanobacteria, pyrrophytes).

Results from NMDS (**Figure 21**) and PERMANOVA (**Figure 22**) using the full dataset showed that phytoplankton communities were more similar across sites than water quality conditions. Phytoplankton site-specific ellipses showed greater overlap in **Figure 21**c, and PERMANOVA confirmed that several sites were not statistically distinct (**Figure 22**a). Water quality displayed clearer separation among sites (**Figure 21**a), and PERMANOVA identified only one non-significant site pair (**Figure 22**b). This contrast likely reflects the rapid and variable nature of water quality conditions, which can fluctuate on short timescales, whereas phytoplankton communities tend to respond more slowly with some genera having broad environmental tolerances.

To explore the influence of interannual variability and examine spatial heterogeneity within the summer bloom-prone timeframe in greater detail, we repeated the NMDS using July-October 2023 data only. Summer-only NMDS (**Figure 21**b,d) revealed even greater site differentiation, particularly for water quality, illustrating the distinctly higher NH4 and other nitrogen compounds at Barker's Island and Miller Creek. In this subset, water quality redundancy increased within upper and middle estuary regions, but upper and lower estuary sites still remained statistically distinct from one another. Interestingly, phytoplankton redundancy among sites decreased in 2023, suggesting greater localized phytoplankton dynamics that may have been a result of drought conditions that minimized hydraulic connectivity among sites.

It is worth noting that in interpreting these results, some site pairs with visually overlapping ellipses in NMDS plots (axes 1 and 2; **Figure 21**a) were still statistically distinct according to PERMANOVA. This

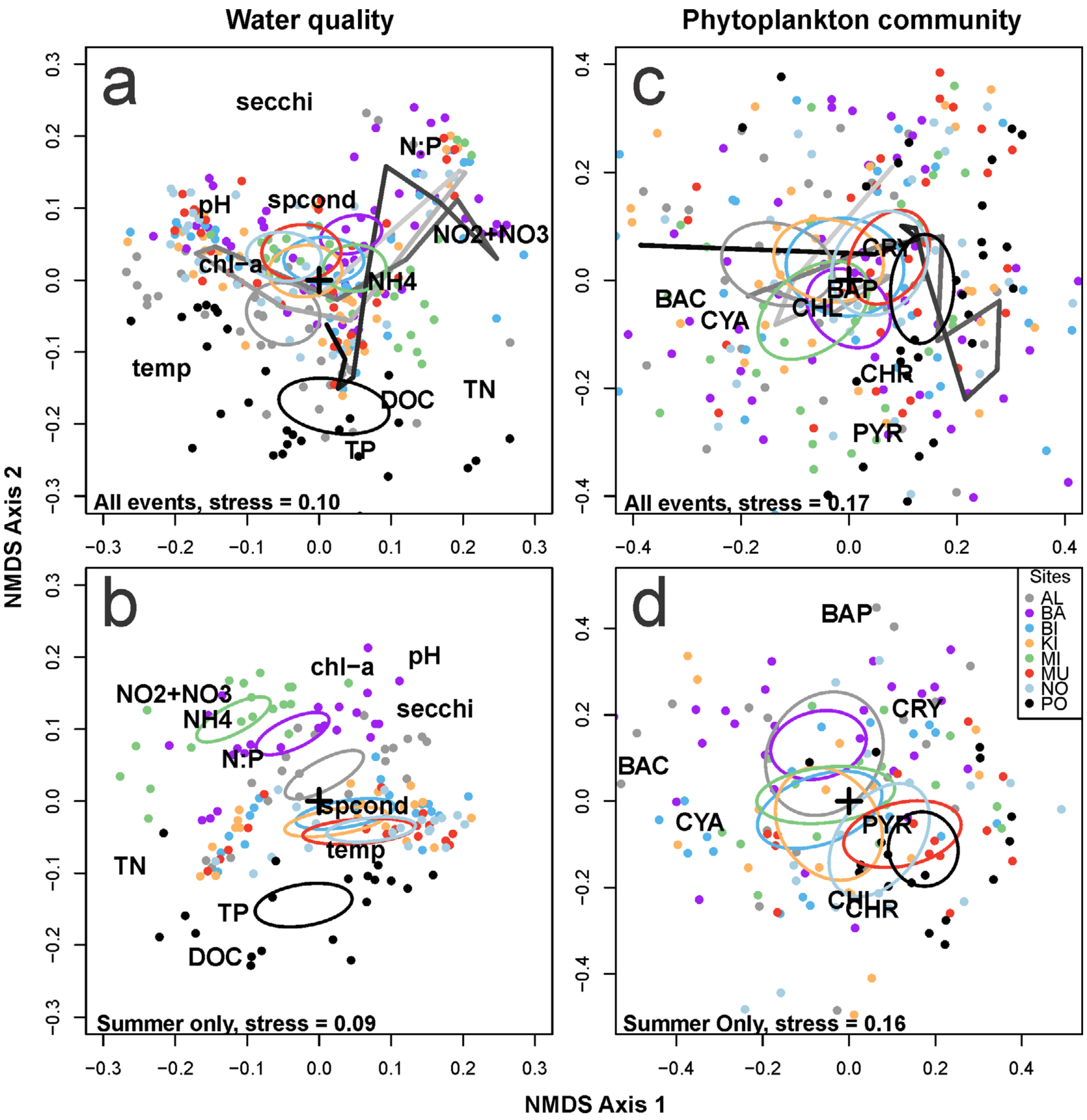


Figure 21: *Nonmetric multidimensional scaling analyses of water quality (left plots), phytoplankton (right plots), and for all data (October 2022 - October 2024; upper plots) and summer (July - October 2023; lower plots). Sample scores are shown as points while variable centroids are shown as text. Colored ellipses represent the two-dimensional, 95% confidence interval of that site's sample scores. All-event plots include monthly sample centroids connected by grayscale lines from earliest (light gray) to latest (black), to track temporal trajectory of scores. Two letter codes for the sites are in Table 1 and taxon group codes are the same as for* Figure 8.

is because PERMANOVA evaluates differences across all three NMDS axes, with some sites showing greater separation along axis 3 (**Appendix F**).

Beyond redundancy assessments, NMDS also provides insight into which variables contribute most to site uniqueness. For summer water quality (**Figure 21**b), Pokegama Bay stood out with elevated TP, TN, and DOC, while Miller Creek and Barker's Island were characterized by higher chl-a, pH, and nitrogen-related parameters (NO_2+NO_3, NH_4, N:P). For summer phytoplankton (**Figure 21**d), Allouez Bay, Barker's Island, Billings Park, and Miller Creek were more associated with cyanobacteria and centric diatoms, whereas North Bay, Mud Lake, and Pokegama Bay supported more diverse assemblages of chrysophytes, dinoflagellates, and chlorophytes. Although phytoplankton communities overall showed greater similarity among sites than water quality metrics, upper estuary sites and Pokegama Bay consistently separated from lower estuary locations.

Finally, additional ordination approaches such as Canonical Correspondence Analysis (CCA) can be used to directly link water quality gradients to phytoplankton composition and individual taxa that respond unimodally across environmental gradients. While we do not report those results here, that analysis will be explored further in Peter Birschbach's MS thesis.

TEMPORAL REDUNDANCY

To inform efficient sampling design, we conducted three related analyses: (1) temporal redundancy to evaluate how frequently sampling is needed to capture meaningful changes in water quality and biological indicators, (2) a sampling frequency reduction analysis to test how lower-frequency sampling affects model performance in predicting cyanobacteria biovolume, and (3) a lag analysis to assess whether conditions preceding bloom events can improve predictive understanding. Together, these analyses help identify where and when high-frequency sampling is most valuable.

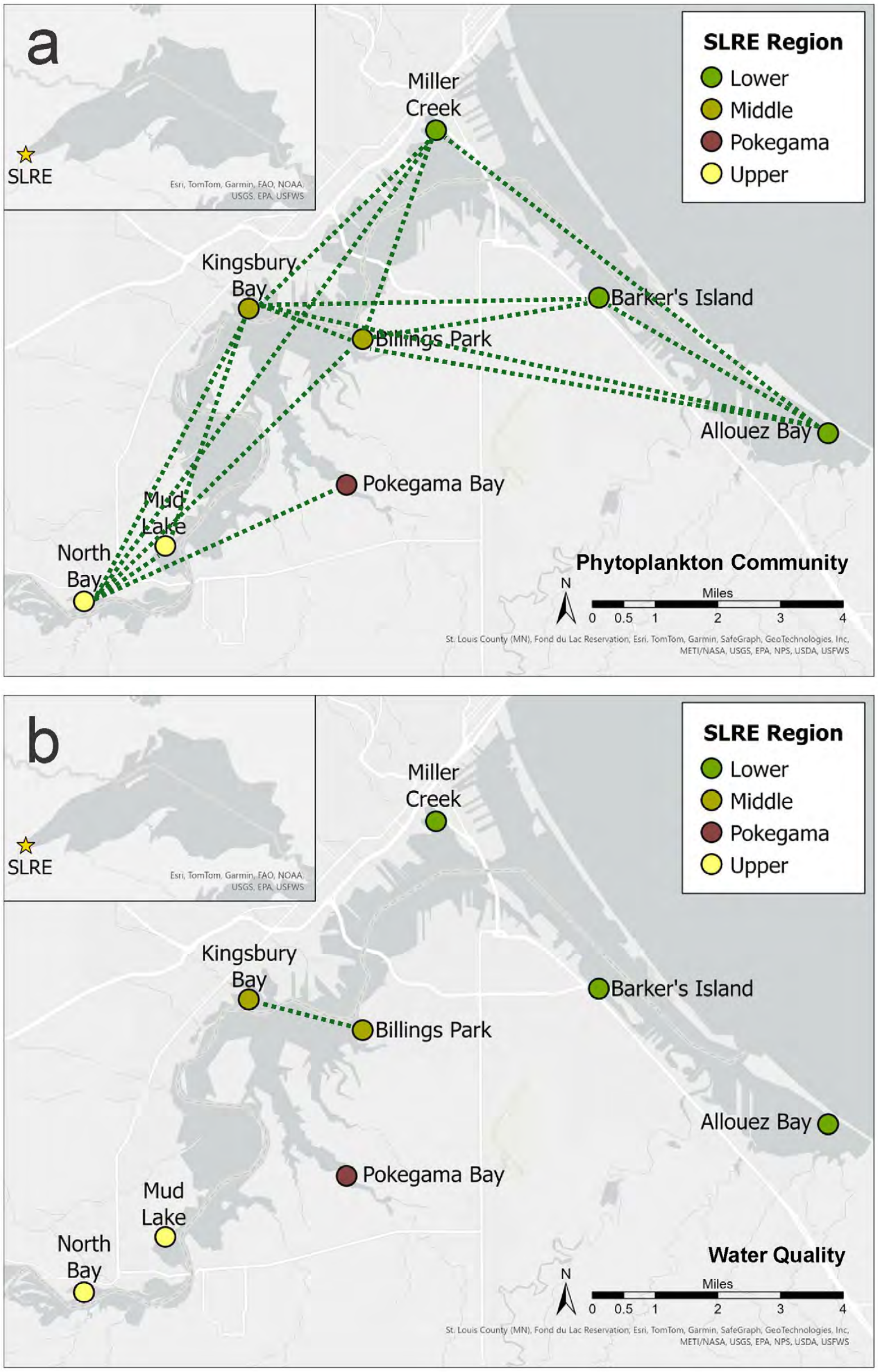


Figure 22: *Similarities among sample sites based on PERMANOVA of phytoplankton communities (a) and water quality (b). Connections by dashed line indicate no significant difference (F-test, p > 0.05) in pairwise site comparisons of NMDS sample scores.*

Sampling Interval

We hypothesized that there is a minimum time interval between sampling events needed to capture meaningful variability in water quality and biological response. Sampling more frequently than this threshold might provide little added value, while less frequent sampling risks missing important dynamics. To test this, we examined how differences in water quality and biological parameters changed as the time between sampling events increased.

Using summer (July–October) data from each site, we calculated all possible pairwise comparisons of observations for each parameter. Each point in the resulting plots represents a pairwise comparison between two sampling events, with the time between them on the x-axis and the absolute difference in observed values on the y-axis. LOESS smoothing was then applied to visualize general trends in variability as time between samples increases (**Figure 23**).

For physicochemical parameters such as temperature and nutrients, results showed no consistent minimum sampling interval. As expected, smaller differences were observed between closely spaced samples, with variability generally increasing as the time between sampling events grew. However, some physicochemical and biological parameters did not follow this pattern. In many cases, particularly for biological indicators, variability remained high regardless of the time interval, suggesting that these parameters fluctuate rapidly. This finding is consistent with coastal monitoring research from South Australia, which found that daily sampling was preferable to weekly sampling for capturing chlorophyll-a variability (Elsdon and Connell 2009).

There were also clear differences among sites. Some locations consistently showed higher variability in biological parameters across all time intervals, reflecting more dynamic conditions. For example, Allouez Bay, Barker's Island, and Miller Creek had higher overall chlorophyll-a concentrations and exhibited greater differences

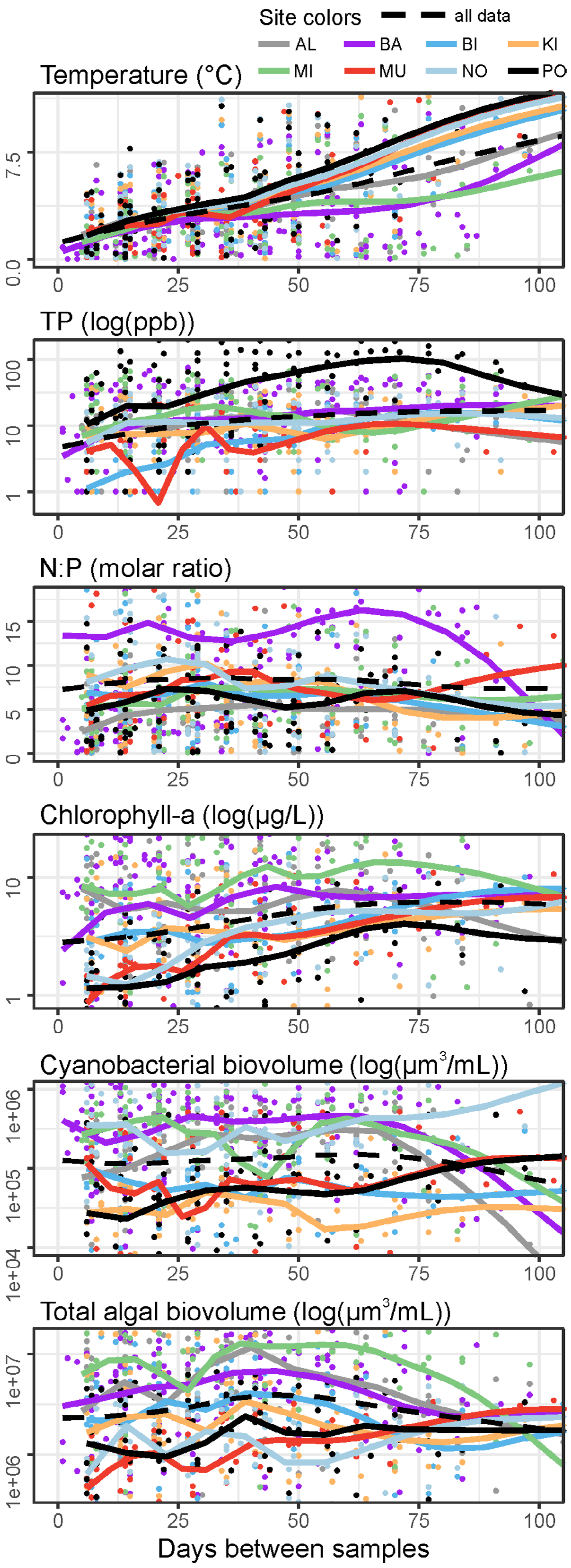


Figure 23: *(Right) Analysis of the relationship of time between samples (x-axis) versus associated differences in variable data (y-axes). Analyses include summer (July - October) data, and locally-weighted (LOESS) curves (span = 0.3) for each site and for all data combined summarize the relationships.*

between sampling events, regardless of whether those events were separated by a few days or several weeks. This suggests that sites with higher baseline concentrations also tend to have greater short-term fluctuations. These results support the idea that sampling frequency should be tailored to site-specific conditions and monitoring goals. Sites with high biological activity, such as those in the lower harbor, showed greater variability in chlorophyll-a and cyanobacteria biovolume, indicating that higher-frequency sampling is warranted to capture productivity dynamics. Conversely, if monitoring efforts are focused on regulatory nutrient parameters like TP, more frequent sampling may be more valuable at sites such as Pokegama Bay where TP variability was more pronounced. In this way, temporal redundancy plots offer a practical tool for identifying where and when high-frequency sampling is most justified, especially for tracking rapidly changing biological indicators or localized nutrient dynamics.

Effects of Sampling Frequency Reduction on Random Forest Model Performance

Because the bloom-prone Barker's Island site had the most temporally complete dataset across two years, we used it to assess how sampling frequency influences the ability of RFA to predict cyanobacteria biovolume. We evaluated three versions of the dataset: weekly (original), bi-weekly, and monthly, ensuring that each included at least one late-summer high cyanobacteria biovolume event (methodology in **Appendix A**). Unsurprisingly, RFA results (**Table 4**) show that weekly sampling yielded the highest model performance ($R^2 = 0.66$) and lowest predictive error (MSE = 0.25), indicating that higher-frequency sampling better captures variability in cyanobacterial abundance. Bi-weekly sampling showed a notable drop in R^2 (0.40), but only a slight increase in predictive error (MSE = 0.29), and shared a similar set of top predictors: total TN and TOC remained highly ranked, with temperature still relatively low in importance. This suggests that bi-weekly sampling is likely sufficient to capture major predictors of cyanobacteria biovolume, although more frequent sampling is preferred for detecting fine-scale dynamics. Monthly sampling, by contrast, resulted in temperature and DO emerging as the top predictors, reflecting a shift toward broadly more seasonal patterns rather than potential direct cyanobacteria-related nutrient conditions. Across all sampling frequencies, nitrogen-based metrics consistently ranked among the most important predictors, with low TN emerging as the strongest predictor of cyanobacterial biovolume at Barker's Island. These findings reinforce the value of higher-frequency sampling for resolving short-term dynamics and nutrient relationships that contribute to bloom formation, which may be missed by programs such as SWMP that rely on monthly sampling.

Lag Effects: Identifying Precursor Conditions for Blooms

Similar to assessing temporal redundancy, we explored whether certain conditions captured through routine monitoring were correlated with high cyanobacteria biovolume observed days or weeks later. Previous studies (e.g., Liu et al. 2022) suggest that algal blooms may respond to environmental triggers with a time delay; for example, a storm-driven nutrient pulse may lead to a bloom several days later or weeks. To investigate this, we examined whether correlations between cyanobacteria biovolume and water quality parameters improved when time lags were considered.

To explore lag effects, data from all eight stations were linearly interpolated between sampling events to generate daily values for each variable. Spearman correlations were then calculated between cyanobacteria biovolume and each water quality parameter for the day prior, then two days prior, continuing up to 50 days. These results were plotted to identify whether stronger correlations emerged

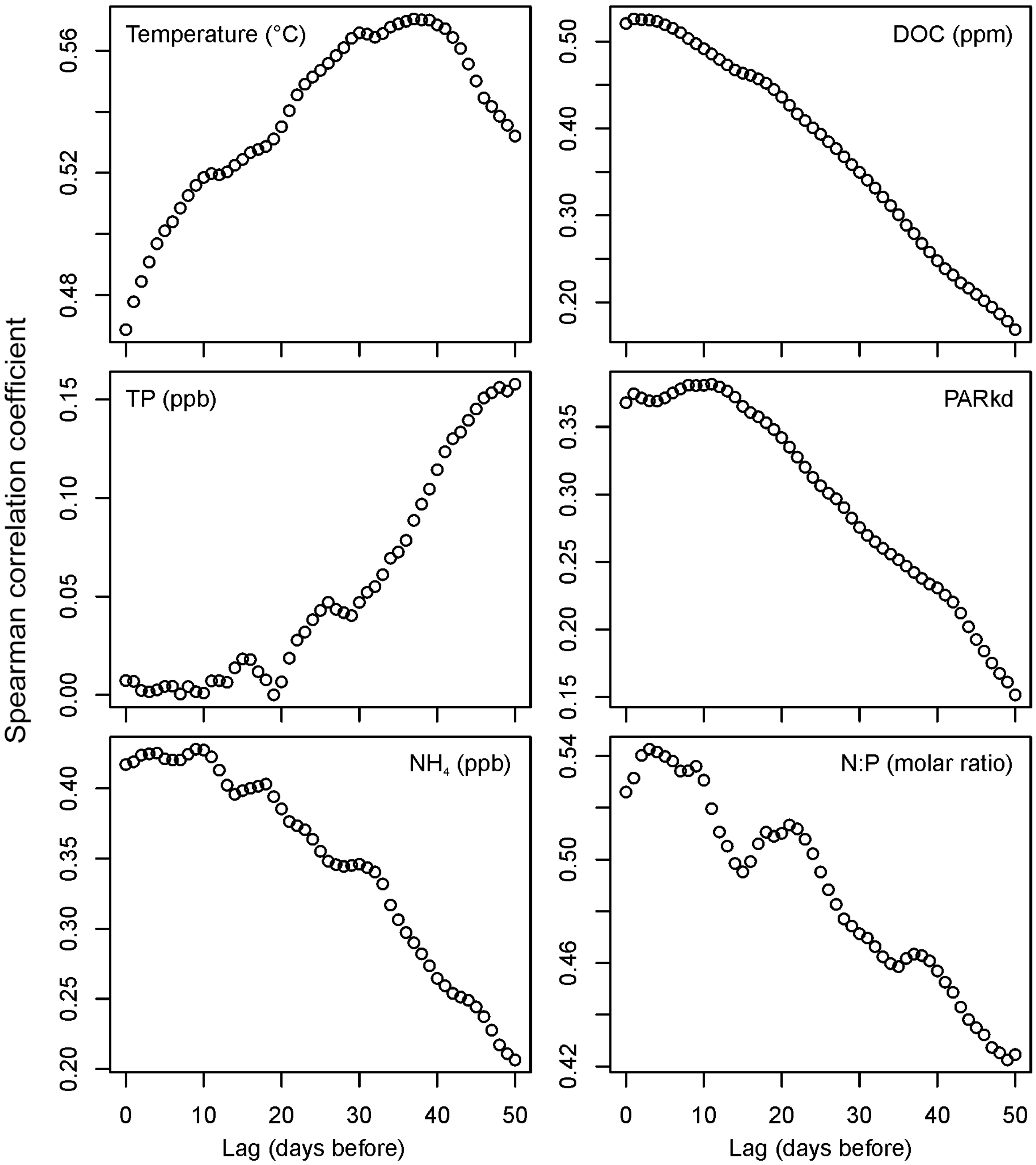


Figure 24: *Lag analysis of the relationships between selected environmental variables and cyanobacterial biovolume. Lags in environmental data measurements from zero to 50 days prior to cyanobacterial measurements were explored using the absolute value of the correlation coefficient between the variable and biovolume.*

at specific lag periods.

Because the interpolation process estimates values between discrete sampling dates based on a linear method, these findings should be interpreted with caution. For parameters that vary rapidly or nonlinearly, interpolation may not accurately reflect true conditions. For parameters that change in a predictable manner over time, such as temperature and DOC; in these cases, interpolation is more likely to reflect true values.

Most variables did not indicate a lag effect on cyanobacteria biovolume; for example, DOC correlation coefficient declined steadily (Figure 24) indicating that same-day values were most strongly related to cyanobacteria biovolume. There were notable exceptions. The optimal temperature-cyanobacteria relationship was maximized at 30–40 days prior (Figure 24), suggesting that water temperature a month ago has a stronger relationship to cyanobacteria biovolume today. Lag analyses for variables NH4, N:P, and PARkd indicate that their measurements within ~10 days prior are equally indicative of cyanobacteria abundance, with N:P possibly optimal four days prior. Lag analysis for TP showed no meaningful correlations with cyanobacteria abundance, and although correlation increased slightly from 20 to 50 days prior, the relationship remained weak.

Additional lag analyses for chlorophyll-a, total algal biovolume, and total diatom biovolume are provided in Appendix G. While our approach does not yet offer actionable early-warning thresholds, these findings highlight the potential for using early summer observations, particularly temperature and signs of nitrogen limitation, to anticipate bloom risk later in the season. Future monitoring with higher temporal resolution will help evaluate and refine the reliability of these early indicators.

MONITORING RECOMMENDATIONS

SITE SELECTION

We aimed to identify a subset of sampling locations that will provide a complete set of monitoring data that will spatially describe SLRE conditions in the future (Table 5). This selection involved eliminating redundancy among sites, considering stations with existing long-term data, and addressing spatial concerns related to cyanobacteria blooms.

Table 5: Considerations for incorporating study sites into a future monitoring strategy, including preliminary prioritization based on ecological value, bloom history, logistical feasibility, and spatial coverage.

Where	Needed? Prioritized? Why/why not?
North Bay	Yes. Low Priority. Although the upper estuary in general has low bloom-susceptibility, winter hypoxia, and climate warming trends warrant tracking a sheltered bay in the upper estuary long-term.
Mud Lake	No. Mud Lake was generally redundant with North Bay and similar to the SWMP Oliver Bridge station. It is not particularly susceptible to blooms. While winter hypoxia did occur it was more severe in North Bay.
Pokegama Bay	Yes. High Priority. As a persistent summer and winter hypoxia hotspot and current SWMP station it is a high priority to maintain monitoring at this site. Although current conditions do not foster blooms, nutrients are high and could contribute to blooms downstream.
Kingsbury Bay	Maybe. Low Priority. In general the Kingsbury Bay site was redundant with Billings Park and did not appear to be of concern for algal blooms. Based on future priorities and any expanded recreational access, Kingsbury Bay or Billings Park could be selected.

Where	Needed? Prioritized? Why/why not?
Billings Park	Maybe. Low Priority. In general the Billings Park site was redundant with Kingsbury Bay and did not appear to be of concern for algal blooms. Based on future priorities and any expanded recreational access, Kingsbury Bay or Billings Park could be selected.
Miller Creek	Yes. High Priority. This site is prone to blooms and has particularly high ammonium concentrations, giving it a unique characteristic for the SLRE. While SWMP's Blatnik Bridge station is close by, the unique water quality of the Bay where Miller Creek enters the estuary is distinct.
Barker's Island	Yes. High Priority. It is a very bloom prone site, has an existing SWMP station, and is proximal to a public swimming beach, therefore is a high priority to maintain. While SWMP collects monthly nutrients samples, we recommend high frequency routine sampling in summer and fall to act a bloom sentinel for the lower estuary.
Allouez Bay	Yes. High Priority. The bay has distinct water quality, winter hypoxia, and unique winter phytoplankton communities. While no blooms extended into Allouez Bay during the study period, the site had relatively high phytoplankton and cyanobacteria biovolume.

Based on spatial redundancy analysis of water quality data, we expect additional unique, localized coastal sites (bays, tributaries) that could be identified within the SLRE. However, we feel the occurrence of algal blooms was adequately characterized using our selected set of stations, or a subset thereof (Table 5). Algal blooms are undoubtedly occurring in other locales within the SLRE, but the consistency in timing for phytoplankton community dynamics among sites (especially in the lower estuary) suggests these sites provide good sentries for tracking and prediction of blooms.

Spirit Lake

With input from the collaborative team, we initially planned to include Spirit Lake in our sampling, near the recent US Steel Superfund remediation site, however, access was not possible due to active aquatic vegetation restoration at the site. Spirit Lake is a distinctive transitional zone within the SLRE, where the river channel broadens and flow velocities decrease. It is also an area of historic contamination and current restoration planning. While our study results do not suggest susceptibility to blooms in this region, we recommend future monitoring in Spirit Lake to improve spatial representation of the estuary and to capture the dynamics of this unique location. While Spirit Lake is a lower priority for bloom detection, including it as a routine monitoring station would complement the Fond du Lac Environmental Program's annual monitoring near Spirit Island and aid restoration and long-term management efforts, especially since extensive restoration efforts (LimnoTech 2012) have the potential to shift biogeochemistry.

Lower Estuary

In general, the lower estuary is more susceptible to blooms, especially in sheltered areas where scums can easily form and accumulate. When bloom activity was observed at Barker's Island, it was often present in other nearby sheltered areas as well. While it is not feasible to routinely monitor every bay and slip during the bloom season, we recommend a short list of locations—Loon's Foot Landing, Sand Point Yacht Club, and Park Point Boat Launch—where public exposure is more likely and where conditions can serve as sentinels of broader bloom activity in the estuary.

Clay-influenced sites

The farthest upstream bloom we detected and sampled occurred at the mouth of Pokegama Bay. This was unexpected, as cyanobacteria biovolume was low farther upstream in the Pokegama River and at other upper estuary sites. This area may be more susceptible to blooms due to the mixing of nutrient-rich Pokegama River water with St. Louis River water. We recommend including this location as a bloom response site to be checked when sentinel sites are experiencing blooms. More broadly, we suggest prioritizing monitoring at clay-influenced mixing zones, as these areas tend to have elevated nutrient concentrations, particularly TP, and often exhibit low dissolved oxygen levels.

SAMPLING EFFORT

Routine Sampling

Given the high variability and noisiness of the SLRE water quality and biological data, we were not able to successfully identify an ideal sampling frequency for a future monitoring program. Given funding limitations a recommendation to sample "as frequently as possible" is not helpful, so we recommend a tiered approach going forward using existing protocols (**Table 6**). Monthly samples to characterize SLRE water quality condition would likely suffice at well-mixed, thalweg locations to address long-term trends, with more frequent sampling (e.g., weekly) at sites with greater HABs concerns, particularly in the warm late summer months. This should continue to be supplemented with visual observations that may prompt immediate bloom sampling, including analysis for bloom toxicity. The high frequency and regularity of discrete sampling data collected at Barker's Island over all 50 project sampling events will allow us to explore temporal reduction and subsetting for analysis in further detail in future reporting, and may yield empirical insight on the frequency required to capture community variability at different times of the year at the bloom-prone site.

Table 6: A proposed, three-tiered approach to sampling at SLRE locations identified in Table 5.

Tier 1. Year-round sampling	Monthly sampling at all stations, including recommended water quality (Table 7) and preserved phytoplankton collections (can be archived or analyzed immediately if funds allow).
Tier 2. Higher resolution sampling during ice-free periods	Collect parameters as for Tier 1, but increase sampling frequency to biweekly at a subset of locations that represent SLRE conditions (Miller Creek, Barker's Island, Allouez Bay, Pokegama Bay). Continue monthly sampling at remaining locations.
Tier 3. HABs-focused and bloom-response sampling	Starting in late July every year, increase intensity of sampling to weekly at bloom-susceptible locations through October; particularly Barker's Island. If blooms are spotted, immediate response sampling should include surface scum and toxin analysis, using rapid-assessment procedures; samples with detectable toxins should be analyzed quantitatively by laboratory methods.

Because phytoplankton communities were more redundant than water quality, sampling frequency optimization was focused on capturing water quality variability; thus, by optimizing frequency to capture water quality variability, a monitoring program will inherently capture phytoplankton community variability suitably, as well as some additional implied redundancy. However, enumeration is a time-intensive process and can only be performed by trained taxonomists, so feasibility of routinely enumerating all program samples is heavily contingent on availability of personnel and funding. We recommend collecting phytoplankton samples with all sample suites, regardless of the short-term ability to perform enumeration, as phytoplankton sample collection is inexpensive and preserved samples can be archived for years prior to enumeration if necessary; this would ensure completeness, continuity, and availability of a long-term record, even if the data cannot not be generated from the samples in real time. The LSNERR has collected phytoplankton samples during monthly SWMP sampling events in recent years and outsourced enumeration to the Wisconsin State Lab of Hygiene, but as SWMP program funding streams become increasingly uncertain, non-required program parameters including phytoplankton will unfortunately be primary candidates for exclusion.

Rapid Response Sampling

Given previous indications that storm events were a likely driver of blooms in Lake Superior (Sterner et al. 2020), it was a goal of the project to ensure focused sampling around storms. We did not, however, do any targeted storm response sampling, in part because routine sampling often sufficed to capture conditions shortly after major discharge events. Targeted sampling at priority locations during peak events gives us the most useful data for tracking nutrient loads and understanding ecosystem impacts. However, as seen in the long-term loading estimates, TP and TSS have strong correlations to discharge. Thus, if we first assess a variety of discharges across many years to ensure that the relationship holds true, we can estimate concentrations and loadings of TP and TSS, which are the major water quality factors delivered by rain events, without needing to sample peak discharges. In this context, the Scanlon dam USGS-operated discharge gauge provides a highly valuable tool, and gauging more tributaries could lead to more comprehensive and accurate load estimates. Regarding discharge and sampling logistics, the peak spring discharge often occurs immediately after ice-off, a time of year with abundant floating ice chunks, woody debris, and remnant shoreline ice cover that can make boat-based or dock-based sampling challenging. To capture peak discharge loadings at primary main thalweg locations and continue the long-term record, efforts at this time of year should be focused on the SWMP's Blatnik Bridge and Oliver Bridge sites which have long-term records. As with storm events, phytoplankton sampling during peak spring melt discharge is not discouraged should the timing coincide, but it is not a distinct sampling priority in the context of our findings on what influences cyanobacteria abundance.

A rain event and associated increase in discharge occurred between the two most intense blooms of the 2023 study period (**Figure 3**), the first of which followed a prolonged drought. Both blooms were dominated by the same cyanobacteria species, *Aphanizomenon flos-aquae*, but the variable responses in water quality parameters following the rain event (**Appendix C**) make it difficult to clearly interpret the effects of discharge on bloom development. In 2024, rainfall-driven discharge events were more frequent and intense but did not correspond clearly with bloom timing (**Figure 4**), and blooms that year were generally less widespread. Although nutrient loading increases during peak discharge, phytoplankton density is often diluted. Additionally, storm-influenced samples tend to contain high TSS and organic debris, making phytoplankton enumeration more difficult and time-consuming. For these reasons, it is more valuable to monitor nutrient concentrations than to prioritize phytoplankton sampling during peak discharge events. We recommend primarily focusing monitoring efforts on visual assessments and real-time data in the days and weeks following storms. If resources allow, collecting nutrient samples can be a secondary priority, but phytoplankton sampling during the storm events themselves is not recommended.

We do recommend increasing the frequency of sampling above the aforementioned monthly baseline during the most bloom-prone timeframe extending from the third week in August to the first week of October. If sample sets are limited, this practice should be prioritized at lower SLRE sites, Barker's Island, Miller Creek, and Allouez Bay. With very limited resources, Barker's Island should be prioritized given its high public use and history of HABs. If surface scums are present at any locations, lower harbor back bays and slips should be visually assessed. This increases the likelihood of the sampling design incidentally capturing blooms should they occur, and also can provide an early warning of bloom development and what pre-bloom taxa are present at ambient levels. However, we above all else recommend that bloom response samples be prioritized above all others in the interest of public health, tracking blooms over time, and refinement of bloom prediction by modeling key environmental relationships.

Flexible bloom response sampling requires having personnel at least semi-flexible daily schedules and limited late summer travel plans, and necessitates perpetual readiness of field and lab equipment for "grab and go" situations. We suggest that real-time bloom indicators such as elevated pH, daytime DO saturation, and low wind speeds should trigger sending an individual or crew to visually survey for blooms in sheltered bays and slips with easy public access from shore (Loon's Foot Landing, Sand Point Yacht Club, and Park Point Boat Launch), and observation of macroscopic colonies or scums should trigger immediate collection of a sample suite and a rapid toxin assessment. Thinking of Barker's Island as a bloom sentinel or proxy site may be of aid. We developed an informal saying among our field crew: 'If it's blooming anywhere, it's blooming at Barker's.' This proved accurate for the vast majority of the blooms we observed. This was incredibly logistically convenient given the LSNERR's location on the island, and will facilitate quick identification and sampling of blooms going forward.

As to the daily timing of flexible blooms response sampling, our study identified early morning to be the most susceptible to scum formation coincident with calm winds that generally increase as the day progresses, so checking early can be important. When winds increase, scums tend to rapidly disperse, which is good news at surface level, particularly in light of climate data showing a local increasing wind speed trend (Austin and Coleman 2007); however, wind disturbing a scum does not indicate that cyanobacteria biovolumes or toxin concentrations have decreased, particularly in the generally shallow SLRE. This should be kept in mind when issuing beach safety warnings.

If bloom response sample sets are limited, then we recommend reducing collection of multiple samples per bloom, to one sample per bloom at one location selected to maximize both public health utility and sampling of the most visually intense bloom location. We recommend a minimum of 6-8 bloom response samples be allocated per year, but if less were available, perhaps chl-a data associated with past blooms could be used to determine a sufficient threshold that triggers sampling only during moderate to severe blooms. If the duration of the annual optimal thermal window for cyanobacteria continues to increase due to climate change, more bloom response samples may be needed in future years than in the years spanning this study.

For immediate assessments of communities during blooms in the interest of public health, we recommend that live surface scum samples from blooms in high-use areas be assessed upon collection to detect if any potentially toxic genera may be present. This would require an available microscope and an individual with at least basic cyanobacteria identification training, roughly following methods outlined herein; the LSNERR recently conducted a basic taxonomic training workshop, which equipped more local professionals with the ability to make real-time genus identifications of the most common bloom-forming cyanobacteria without professional taxonomist involvement. Citizen science tools exist for this purpose, too; portable microscopes allow anyone to magnify a drop of scum in the field, take pictomicrographs on a cell phone via Bluetooth, and upload photos to an artificial-intelligence-powered genus identification algorithm application, turning water into relevant data for public health in a matter of minutes. As a tangent to this project, we assessed the performance of one such device called the ioLight in conjunction with the software BloomOptix (bloomoptix.com), and found that it consistently identified cyanobacteria genera such as Aphanizomenon, Microcystis, and Dolichospermum moderately well in concentrated scums, but performed poorly on adjacently collected depth-integrated samples with lower densities. The up-front costs of such devices and required software subscriptions are highly variable, but at minimum typically several thousand dollars, and ongoing funding initiatives to make them more accessible to citizen scientists at low cost are in jeopardy; thus, expanding the number of local non-taxonomists who are capable of identifying potentially toxic genera by their distinct morphologies may be a more cost-effective means to this end in the current funding landscape. Additional taxonomic training workshops in the region, including workshops scaled for non-researchers would be beneficial.

Winter Sampling

Growing interest in winter limnology is being driven by critical knowledge gaps in how lakes and estuaries function under ice, particularly in light of rising temperatures and decreasing ice cover, which may significantly affect ecosystem metabolism and water quality (Ozersky et al. 2021; Cotner et al. 2022). Ice-based sampling poses greater logistical challenges than open-water sampling, including the need to keep equipment and personnel safe and warm under harsh conditions (Block et al. 2019). Most importantly, safe access depends on sufficiently thick ice cover. In the winter of 2022–2023, thick ice allowed access to all eight sites on three occasions. However, in 2023–2024, we were unable to access Miller Creek at all and reached only four other sites once each.

Given these circumstances, we decided to target accessible sites for more frequent sampling, with Barker's Island and Billings Park sampled weekly and biweekly, respectively. We recommend incorporating similar flexibility in future winter sampling designs as winter conditions are becoming increasingly unpredictable. To establish a long-term winter record, we recommend sampling under-ice from each SLRE region at least once per winter if possible, but sampling monthly would be preferred to account for interannual weather variability (for example, data collected in January in different years are not equivalent). Because dominant phytoplankton species (e.g., Aulacoseira, Synura) biovolumes were much higher in the lower SLRE in both winters and winter communities can have strong implications for subsequent open-water period conditions (Reavie et al. 2016) and communities (Hazuková et al. 2021), we also recommend that future monitoring of winter phytoplankton should prioritize additional sampling at lower SLRE locations if feasible, particularly Allouez Bay and Barker's Island.

The high-resolution DO loggers we deployed at all sites in both winters provided informative data at little cost. Even in winters in which discrete sampling is possible, high-resolution in situ logging captures more variability, which can show short-term dynamics that are not revealed by manual sampling. Because Allouez Bay was the only site that experienced extensive winter hypoxia, it should be prioritized for logger deployment; however, given the ease of logger data collection, we see no reason not to continue a long-term record at all sites, given the frequently-observed spatial heterogeneity in winter conditions and ice cover in the system.

PARAMETER CHOICE

Several factors were considered in recommending a refined set of monitoring parameters based on best professional judgment: inclusion of standard monitoring variables (especially those with regulatory significance); low-cost variables typically measured by rapid instrumentation; correlations among parameters; and RFA results identifying important predictors of cyanobacteria biovolume. Table 7 summarizes the relative importance of the environmental variables we evaluated for the SLRE. A cost- and effort-efficient set of parameters is recommended, including simple variables such as Secchi depth, which can serve as surrogates for more effort-intensive variables like PARkd. While full processing of phytoplankton community samples is expensive, such collections should continue at a minimum during blooms to identify key species of concern and be supplemented with toxicity testing when possible.

Table 7. Considerations for incorporating study parameters into a future monitoring strategy

Environmental variable	Is it needed? Why or why not?
Temperature	Yes. Important and easily/cheaply collected. High frequency continous data of high value since temperature is an important predictor of blooms
Dissolved oxygen	Yes. Important and easily/cheaply collected. High frequency continous data of high value since spikes in pH also may indicate rapid algal production during a bloom.
pH	Yes. Important and easily/cheaply collected. High frequency continous data of high values as spikes in pH also may indicate rapid algal production during a bloom.
Specific conductivity	Yes. While not a strong predictor of blooms, it is known as a tracer of pollution, is relavent to manoomin and fish health in the SLRE and is easily/cheaply collected.
Salinity	No. Not for routine sampling. It may be useful for salt pollution-related studies, but otherwise it is very low, highly correlated with specific conductivity, and is not a unique predictor of phytoplankton in the SLRE.
Secchi depth	Yes. Especially if no turbidity sensor is available. An important surrogate for turbidity and easily/cheaply collected.
Chlorophyll-a	Yes. While not directly indicative of cyanobacteria blooms, it is the most common measure of general productivity
Total phosphorus	Yes. Especially at SWMP stations to predict long term trends and loads. This is a standard nutrient variable in monitoring programs and equally if not more important as a predictor of algae abundance than orthophosphorus. While not a strong predictor of blooms in the SLRE, it is an essential nutrient that is supporting the base of the food web and is important to stoichiometric considerations.
Soluble Reactive Phosphorus	No. While this variable is considered important for phytoplankton growth generally, it was not more important than other nutrient variables as a predictor.

Environmental variable	Is it needed? Why or why not?
Total nitrogen	Yes. An important phytoplankton predictor and a standard nutrient variable in monitoring programs.
Ammonium	Yes. A known cyanobacterial resource; also a specific characteristic of certain areas of the SLRE.
Nitrates + nitrites	Yes. An important predictor of cyanobacteria especially in the middle and upper estuary
TN:TP ratio	Yes. This is a calculated ratio of two already-included variables, was strongly influenced by drought precluding 2023 blooms, and had moderate predictive importance for cyanobacteria biovolume.
Acid neutralizing capacity / Alkalinity	No. Is redundant with other parameters and has a short hold time making it laborous to analyze
Dissolved organic carbon	Yes. Is a defining spatial characteristic in the SLRE that has both high seasonal and interannual variability, and has implications on phytoplankton and physical variables such as water clarity.
Total organic carbon	No. TOC is highly correlated to DOC.
Particulate carbon	No. Although could be useful in understanding the source of TSS loads at SWMP sites
Turbidity	Maybe. But in rapid response sampling or citizen science efforts, secchi depth is an excellent surrogate.
Total suspended solids	Yes. But only needed at SWMP sites to assess long term trends in concentration and loads associated with storm events
Photosynthetically active radiation extinction	No. Related to turbidity and secchi depth which are easier and cheaper to deploy

Some particular recommendations for how and when parameters are selected are discussed below:

Secchi Disc

The Secchi disc has long been a cost-effective, easy-to-use tool for tracking long-term changes and short-term variability in water clarity (Preisendörfer 1986). Water clarity can be influenced by several factors, including fine suspended sediments, colored organic matter, and algal biomass (Bachmann et al. 2017; Brezonik et al. 2019). Because these factors are highly dynamic across time and space in the SLRE, we assessed how reliably Secchi depth reflects overall water clarity under varying conditions.

In this study, we compared Secchi disc depth measurements to other more expensive or time-intensive indicators of water clarity, including turbidity, PARkd, and TOC/DOC. To evaluate the potential of Secchi depth as a practical substitute, we calculated Spearman correlations between Secchi depth and these parameters. When combining all sites and sampling events, Secchi depth was significantly correlated with all other water clarity measures (**Appendix E**). The strongest relationship was with turbidity (Kendall's Tau, r = –0.64), followed by PARkd (r = –0.45) and TOC (r = –0.30). Correlations between Secchi depth and turbidity were consistently strong across individual sites, with coefficients ranging from –0.55 to –0.92. These findings confirm that the Secchi disc is a reliable tool for estimating water clarity in the SLRE, with the strongest and most consistent relationship to turbidity across both spatial and temporal scales.

Nutrients

To improve cost efficiency, we explored redundancy among numerous variables to identify the most informative variables. TP and SRP had low importances in RFAs; however, TP should still be monitored due to its regulatory relevance and its common role in limiting productivity in freshwater ecosystems. We do not recommend prioritizing SRP, given its weak association with cyanobacteria in the SLRE and the rapid turnover of bioavailable phosphorus. TN was important in the RFA for explaining community composition and cyanobacteria biovolume, and its relative abundance to TP (TN:TP) serves as an indicator of drought-induced changes that preceded the most intense blooms. We recommend that TN be included in future monitoring despite not having regulatory criteria for regional freshwater ecosystems. Both NO2+3 and NH4 should be retained because their year-round availability and variability, particularly during late summer and fall, has implications for cyanobacteria bloom dynamics. Unlike NO2+3, NH4 is the least energetically expensive form of dissolved nitrogen for phytoplankton to assimilate (Donald et al. 2011). The relationship between high NH4 concentrations and low river discharge (Figure 17) aligns with seasonal patterns of cyanobacteria abundance. The combination of warm temperatures, low flows, and elevated NH4 creates conditions favorable for some non-diazotroph cyanobacteria species. In the winter, NO2+3 and NH4 remained readily available in the lower SLRE; this may have contributed to the relatively high biovolumes of diatoms and chrysophytes relative to the rest of the SLRE, which will be explored more in future in-depth reporting on winter dynamics. Because of their important role in HABs, and their moderate to high importances in analyses of cyanobacteria predictors, both DIN variables should be retained.

Carbon and Carbonate System Related Variables

DOC and TOC are highly redundant in the estuary ($r = 0.93$, $p < 0.05$; **Appendix E)**, varying predictably across spatial and seasonal gradients. Given their near 1:1 relationship, we recommend prioritizing DOC, as it holds greater relevance for assessing potential direct effects on HABs. While DOC/TOC were important in the RFA, this is likely due to their alignment with broad temporal and spatial patterns. However, continued measurement of DOC may help clarify whether carbon availability influences cyanobacteria dynamics more directly, beyond its role as an environmental proxy.

CO2 was not identified as a key driver or limiting factor for SLRE HABs and is not recommended for future monitoring, particularly since pH seems to provide an effective indicator of CO2 drawdown. ANC has a short 48-hour hold time, moderate analytical cost, and weak association with HABs. Given its strong correlation with specific conductivity ($r = 0.70$, $p < 0.05$), it can be omitted from routine monitoring. If modeled or measured from conductivity, ANC could substitute for total alkalinity as an input to the CO2SYS program (Lewis & Wallace 1998), along with pH, temperature, salinity, and pressure, to estimate carbonate system variables. If this approach is pursued, the accuracy of modeled values should be validated prior to further investment.

CONTINUOUS LIVE DATA

The LSNERR currently maintains one continuous, live water quality monitoring station at Barker's Island. This station has been used to preliminarily evaluate the YSI EXO Total Algae Sensor in hopes that it could act as an early warning indicator of blooms. However, results to date suggest that this sensor is not effective at reliably tracking algal abundance or indicating bloom conditions, primarily due to interferences from fluorescent dissolved organic matter and turbidity, which affect chlorophyll and phycocyanin fluorescence readings (Dix et al. 2022). While algae sensors may still hold potential with further investment in site-specific correction factors, they remain costly, with costs exceeding $2,000 per sensor.

Given our study's findings that pH is one of the most important predictive variables for cyanobacteria biovolume, particularly at HAB-prone sites in the lower estuary, we recommend prioritizing investment in live pH data streams at locations where public exposure is most likely. The live pH data at the SWMP's Barker's Island station has already proved useful. The project team informally initiated bloom checks when pH increased above 8.5. If future recreational access points or public swimming beaches are planned in the lower estuary, these locations should be considered for real-time pH monitoring. Further modeling could help determine specific pH thresholds that could serve as an early-warning indicator and trigger to initiate bloom response sampling.

There is also growing interest in deploying low-cost sensor platforms to provide high-frequency water condition information in near real-time. Low-cost, low-power communication protocols, such as the Long Range Wide Area Network (LoRaWAN), have been used by the NRRI to share near real-time water quality conditions online to reduce drowning risks as part of the MNDNR Minnesota's Lake Superior Coastal Program-funded Drowning Hazards project. LoRaWAN can cover buoys distributed throughout the entire SLRE with the addition of a single gateway. Additionally, researchers are actively developing low-cost sensors for recording water quality observations (e.g., temperature, conductivity) that will help reduce instrumentation costs. Given the existing recreational use of Allouez Bay and Wisconsin Point—including kayak launches and swimming—this area would be a strong candidate for piloting such systems. Depending on the configuration, monitoring platforms at high-traffic areas could provide information on both dangerous physical water conditions (e.g., strong currents, cold water) and data forecasting the development of toxic cyanobacteria blooms. Integrating multiple public health and safety monitoring needs into a shared sensor platform may increase feasibility and long-term support.

CYANOTOXINS

While we collected very limited cyanotoxin samples during our study, two sampled blooms did have detectable levels of total microcystins and/or saxitoxin, highlighting the need for more regular sampling to protect human health and more research to understand environmental drivers of toxin production. While decades of research on HABs dynamics and toxin production in warm, nutrient-rich aquatic ecosystems have identified various environmental conditions favoring development of toxic HABs (Paerl & Huisman 2008; Filstrup et al. 2016; Wagner et al. 2021), the emergence of cyanobacteria blooms in colder, low-nutrient aquatic ecosystems suggests that different factors may be regulating HABs in these systems (Reinl et al. 2021; Reinl et al. 2023). Adding to this complexity, different strains of the same cyanobacteria species (i.e., morphologically identical cells) may have different capabilities to produce toxins depending on whether or not they have the full complement of toxin-producing genes (Dick et al. 2021). Further, strains containing toxin-producing genes may not be producing toxins during blooms, and the amount produced is regulated by various environmental factors (Neilan et al. 2013; Wagner et al. 2021). Because of strain-level variability in cyanobacteria's ability to produce toxins, it is challenging to assess the toxicity of blooms based on visual examination.

We recommend establishing a routine cyanotoxin monitoring program to keep people and pets safe from toxic HABs, similar to or incorporated into state (WI and MN) beach programs that monitor E. coli concentrations at popular SLRE and Lake Superior beaches. While event-driven response monitoring is better than nothing, samples typically reflect conditions after there is already a problem and people have been exposed to potentially dangerous conditions. Due to program funding limitations, many agencies only have the capacity and resources to follow this approach, so creating a regional funding model with pertinent agencies should be a priority. Additionally, microcystins are typically analyzed as the only cyanotoxin by such programs, similar to using E. coli as an indicator of fecal contamination, but may not be correlated with concentrations of other potent cyanotoxins (e.g., anatoxin-a, saxitoxin, cylindrospermopsin). Only microcystins and saxitoxin have been detected in the SLRE (Lafrancois and Reinl 2025), which make them good candidates for initial screening.

Because traditional approaches to measure cyanotoxins can be expensive and take 24-48 hours to produce results, there are several rapid test strips on the market that can provide an estimate of cyanotoxin concentrations in 30 minutes or less. Researchers at LSNERR recently evaluated the accuracy of three brands of microcystin field test strips, Gold Standard Diagnostics, Attogene, and 5Strands, under varying conditions in the SLRE. The different brands provided comparable accuracy when tested, but measured different fractions of cyanotoxins (see **Appendix H** for detailed results). Gold Standard Diagnostics measures total microcystins (intra- and extra-cellular microcystins) in samples, whereas Attogene and 5Strands measure only dissolved extra-cellular microcystins and therefore do not quantify cyanotoxins bound within cells. Additionally, it is not clear what 5Strands is actually measuring in the sample. There are currently no commercially available rapid test strips for other cyanotoxins. Agencies will need to evaluate the limitations of different rapid test strips before implementing them, but might want to consider using these as a screening tool for more quantitative laboratory analyses of toxins for samples with detectable concentrations.

REGIONAL ALIGNMENT FOR BROADER IMPACTS

This project identified bloom-prone areas within the SLRE, characterized the environmental drivers of cyanobacteria blooms, and provided nutrient trend analyses—all of which align with key priorities outlined in the Lake Superior Lakewide Action and Management Plan 2020–2024 (LAMP; ECCC and U.S. EPA 2022). Although the SLRE is a distinct system from Lake Superior, it is hydrologically and ecologically connected to the lake and influences the condition of its nearshore waters. LAMP Action #13 calls for science and monitoring to better understand both modern and historical nutrient conditions in Lake Superior and to identify the conditions and locations associated with potential algal blooms. Our findings contribute directly to this goal by improving understanding of bloom predictors and nutrient dynamics within this connected estuarine system.

To build on this alignment, we recommend that future monitoring and research efforts be intentionally coordinated across the estuary and nearshore Lake Superior. This includes developing shared bloom response protocols, harmonized nutrient monitoring strategies, and collaborative data integration to better assess how estuarine conditions may influence bloom risk in the lake. This connection is already underway through efforts like the Lake Superior Algal Bloom Subgroup and the shared bloom reporting tool (Lafrancois and Reinl 2025). Strengthening these collaborations will further support lakewide management objectives and advance protection of the broader Lake Superior ecosystem.

MONITORING LONGEVITY AND RELIABILITY

With this report, we have identified strong recommendations for the structure of a future monitoring program that optimizes the cost and effort associated with protecting environmental and human health. As a final step, the authors of this report intended to compile a resource guide outlining grant opportunities to expand monitoring efforts across the SLRE, based on spatial and temporal recommendations. However, the urgency of maintaining existing monitoring assets amid a shifting federal funding landscape has redirected our focus toward preserving our current monitoring efforts.

Currently, the most consistent and long-standing water quality monitoring effort in the SLRE is LSNERR's implementation of SWMP. In 2012, SWMP expanded upon the MPCA Milestone Monitoring Program (MMP) by extending data collection at the Oliver and Blatnik Bridges and by adding stations that represent distinct water quality conditions. Over time, SWMP has evolved to meet local management needs, including targeted sampling during algal bloom events. Programs like SWMP, which are continuous, long-term, and historically well supported, are rare and invaluable. Their consistency is essential for tracking ecological change and supporting data-driven water management decisions (Reinl et al. 2025).

LSNERR's SWMP is primarily funded through federal appropriations under the Coastal Zone Management Act and administered by NOAA. Recent and significant reductions to NOAA's discretionary budget threaten LSNERR's ability to maintain essential monitoring infrastructure and to continue its core sampling program.

In the short term, the MPCA's Environmental Analysis and Outcomes Division plans to monitor the SLRE in 2025 and 2026 at 12 thalweg locations. This effort has been coordinated with SWMP, with input from additional members of this collaborative team, to ensure compatibility between datasets. Such alignment will strengthen regional data integration and enhance cross-agency comparisons.

The longer term future is less certain. Core functions such as continuous water quality station maintenance, HAB response, and laboratory operations are increasingly vulnerable to funding instability. Other federally supported partners involved in SLRE monitoring face similar fiscal pressures. Without reliable funding, the collaborative infrastructure that supports estuary-scale monitoring could erode, leading to fragmented datasets and reduced long-term data value. Pending the outcome of SWMP funding, this collaborative team will need to consider how to creatively sustain long-term data collection efforts given the recommendations in the report.

This study's recommendations, particularly the call for intensified sampling in the lower estuary during bloom-prone periods, highlight the need for new and innovative approaches to expand current efforts. Citizen science, private foundation assistance, or low-cost sensors may help fill some of the anticipated gaps, especially in monitoring and reporting algal blooms at publicly accessible locations. Other systems have demonstrated success through similar initiatives. For example, the Courte Oreilles Lakes Association works in alignment with state protocols to help detect blooms and inform management responses (COLA n.d.), and the Cleveland Water Alliance, a public and private alliance, works together to fund real-time data platforms in Lake Erie (Cleveland Water Alliance n.d.) The SLRE already benefits from an engaged network of community organizations, including the St. Louis River Alliance, Friends of the Lake Superior Reserve, and the Lake Superior Headwaters Sustainability Partnership. These groups could play a key role in supporting or encouraging bloom monitoring when institutional capacity is limited.

Existing tools and programs offer a foundation for community-engaged science, but systematic algal bloom monitoring in the SLRE would require additional coordination and outreach to organize participants. State-supported efforts such as WDNR's Water Action Volunteer Program (WAV 2025) in partnership with UW-Madison Division of Extension as well as MPCA's volunteer water quality monitoring program (MPCA n.d.) provide useful models and points of connection. For example, integrating routine cyanobacteria toxin monitoring into Minnesota Department of Health's Beach Monitoring Program could provide routinely collected cyanobacteria toxin information to keep people safe at SLRE and Lake Superior beaches. Another promising tool is Mote Marine Laboratory's Beach Conditions Reporting System, which was recently expanded to the Great Lakes through its adoption at Old Woman Creek NERR. This digital platform allows for real-time citizen reporting of bloom conditions and could be adapted to support similar efforts in the SLRE (MOTE n.d.).

FURTHER RESEARCH

While this study has improved our understanding of bloom timing, drivers, and monitoring strategies in the SLRE, fully applying these insights requires filling several key knowledge gaps. Targeted research is needed to better predict bloom behavior, assess health risks, and guide potential mitigation. The following areas represent high-priority next steps to strengthen bloom response and long-term management:

Additional areas of future research include:

- Quantifying nutrient pools. Hypoxia and resuspension events are likely providing nutrients that contribute to blooms. Paleoecological study of previously deposited nutrients, as has been performed for Lake of the Woods (Edlund et al. 2017), would quantify the mobility of stored nutrients, enabling predictions of future internal loading.
- Pairing the monitoring dataset with more advanced hydrological modelling would better explain how hydrological phenomena (like prolonged low river discharge) affect bloom formation. Currently the monitoring data are very good predictors, but factoring in detailed hydrology would better describe the role of climate in bloom development. There is opportunity to leverage existing models (Prihoda n.d., Smith 2020) to do this work that could then incorporate this project's data.
- Genomic approaches to quantify toxic HAB risk. The genetic basis for toxin production, especially microcystins, is well-known, which provides another opportunity for assessing bloom toxicity. Genetic approaches can identify if cyanobacteria strains have the genetic machinery to produce toxins (metagenomics), as well as whether or not those genes are actively producing toxins (metatranscriptomics). New portable sequencing technologies can identify and quantify genes in the field using simple procedures. Future research is needed to determine how the number of gene copies translates to cyanotoxin concentrations.
- Quantify human health risks and feasibility of mitigation approaches. Routine monitoring of cyanotoxins at bloom-susceptible public beaches and boat landings would help quantify the frequency and severity of blooms in relation to human health guidelines. Depending on these findings, a feasibility study of common approaches used to mitigate cyanobacteria blooms tailored to characteristics of the SLRE and their associated costs is recommended.

STATE OF THE ESTUARY - FUTURE COLLABORATION

The SLRE is undergoing a pivotal transition. As collaborative remediation and restoration efforts under the AOC program begin to wind down, the focus is shifting from addressing legacy impairments to proactively managing emerging stressors. This study contributes to that transition by helping close key knowledge gaps on algal community degradation, cyanobacteria blooms, and hypoxia.

In conversations among our collaborative project team, a shared priority emerged: the need for updated, locally relevant metrics, indicators, and benchmarks that reflect current estuarine conditions plus management and regulatory goals. These indicators should encompass ecological, biological, and human-use dimensions ranging from aquatic and avian communities to water quality and public health. A long-term vision shared by many partners is the creation of a public-facing, collaborative "State of the Estuary" report, a product that brings together science, management priorities, and stakeholder perspectives to summarize estuary health.

To move toward this vision, we propose building a synthesis framework that starts with water quality, which is a rich and long-running dataset. Focusing on water quality also builds on the existing collaboration in this study. We recommend producing a comprehensive "State of the Estuary" synthesis every 5 years, with lighter "score cards" of water quality and phytoplankton health based on most recent data updated annually. This schedule aligns well with current regulatory and funding cycles and complements ongoing monitoring programs.

Below, we outline the primary programs contributing water quality data to the SLRE, highlighting their strengths, limitations, and potential for integration:

Minnesota Pollution Control Agency (MPCA)

- Cycle: 10-year Intensive Watershed Monitoring
- SLRE Coverage: Upstream St. Louis River assessed in 2019; 12 estuary monitoring sites planned for 2025–2026
- Strengths: High-frequency sampling across broad spatial coverage in thalweg sites
- Limitations: Limited to Minnesota; does not include shallow embayments or tributaries

Wisconsin Department of Natural Resources (WDNR)

- Cycle: Biennial surface water quality reporting to the EPA
- SLRE Coverage: Focus to date limited to AOC-related efforts; no systematic estuary monitoring or impairment criteria
- Strengths: Potential to align with Minnesota efforts for cross-state synthesis
- Limitations: No dedicated non-AOC SLRE monitoring effort currently planned

Lake Superior National Estuarine Research Reserve - System-Wide Monitoring Program (SWMP)

- Cycle: Year-round, monthly and continuous (15-minute intervals)
- SLRE Coverage: Two thalweg sites and two shallow hotspot locations
- Strengths: Extremely high temporal resolution; long-term continuity with historical datasets

- Limitations: Limited spatial coverage; lacks key parameters (e.g., E. coli, mercury, sulfate); unstable funding; limited winter data

Fond du Lac Environmental Program - Water Quality Monitoring

- Cycle: Annual water quality monitoring
- SLRE Coverage: Five Reservation sites providing upstream context; annual sampling in Spirit Lake
- Strengths: Long-standing and consistent program
- Limitations: Limited spatial and temporal coverage in the SLRE

U.S. EPA - National Coastal Condition Assessment (NCCA)

- Cycle: Funding available on a 5-year cycle
- SLRE Coverage: Could include the entire SLRE
- Strengths: Probabilistic, cross-jurisdictional design ideal for spatial coverage
- Limitations: Requires EPA coordination and funding

Additional Program and Research Efforts likely to have sharable water quality data

- MNDNR and WDNR restoration and remediation project site monitoring
- NCCA survey effort on Nearshore Lake Superior
- Minnesota Department of Health Beach Monitoring Program
- Wisconsin Beach Program
- Lake Superior Research Institute's Ballast Water Research Program
- State Fisheries Programs
- Great Lakes Coastal Wetland Monitoring Program
- NOAA's Sea Grant and Coastal Program funded research
- Various targeted University led research studies

Each of these programs has unique objectives, indices, and regulatory drivers. A comprehensive synthesis would weave these threads into a unified estuary-wide "State of the Estuary" report and annual score cards, communicating both detailed findings and broad patterns in an accessible, actionable format.

The first step in building this collaborative product is securing funding for facilitation, synthesis, coordinated decision making, and confirmation of roles and funding sources. The NERRS Science Collaborative offers one potential mechanism to support a transfer or collaborative grant aimed at bringing these datasets together and advancing shared indicators. Additional opportunities may exist through NOAA's Coastal Resilience and Sea Grant programs.

A key early task will be harmonizing datasets across agencies and institutions. As a starting point, this study developed a set of R scripts to streamline public data access and facilitate cross-platform integration. These tools are available in a public GitHub repository https://github.com/Lake-Superior-Reserve/SLRE-signals. Additional efforts to gather and synthesize data across Lake Superior's South Shore are currently funded and underway through a partnership with WDNR, National Park Service, UW-Madison Center for Limnology and LSNERR.

The eventual format of the "State of the Estuary" product should be driven by both audience needs. Several models provide inspiration for how to translate technical data into meaningful public communications:

- **Friends of the Mississippi River – State of the River Report** | https://fmr.org/state-river-report-report
- **Great Bay NERR Water Quality Report Card** | https://greatbay.org/science/monitoring/
- **Baltimore Water Watch – Real-Time Dashboard** | https://baltimorewaterwatch.org/current-conditions
- **Wisconsin WAV Volunteer Monitoring Dashboard** | https://connect.doit.wisc.edu/wav-dashboard/
- **Chesapeake Bay Health EcoReport Card** | https://ecoreportcard.org/report-cards/chesapeake-bay/bay-health/

These examples span from printed summaries and report cards to interactive web dashboards, providing several different models. We envision a facilitated process with end users to identify the intended audience and appropriate format.

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APPENDIX A: DETAILED METHODS

FIELD SAMPLING

At each site during each sampling event, vertical profiles of the water column were collected using a YSI/Xylem - EXO2 or EXO2s sonde, calibrated following SWMP protocols (NOAA/NERRS 2025a). Recorded parameters included temperature, dissolved oxygen concentration and saturation, pH, specific conductivity, salinity, turbidity. Parameters were recorded at 1-Hz frequency. Values reported are averages of the entire vertical profile, after verification that temperature stratification was rare (< 4%). Photosynthetically active radiation (PAR) was measured using a spherical underwater quantum sensor (Li-COR Model LI-193) slightly above the water surface, just below the water surface and at 50 cm intervals from the surface to bottom. Using the Beer-Lambert law, PAR values were used to calculate the light extinction coefficient (PARkd) to quantify how quickly light diminished as it passed through water. Secchi disc depth was measured to determine water clarity by lowering the disc into the water column until it disappeared, then raising it up until it reappeared. Values reported are an average of the disappear and reappear water depths.

Discrete water chemistry samples were collected at 1.5m depth using a horizontal style Van Dorn sampler to be consistent with sampling protocols for SWMP (NOAA/NERRS 2025b). These samples were later analyzed for chlorophyll-a, Total Suspended Solids, Volatile Suspended Solids, nitrate+nitrite, soluble reactive phosphorus, ammonium, Total Nitrogen, Total Phosphorus, Total Organic Carbon, Dissolved Organic Carbon, and Alkalinity. Water chemistry samples were stored on ice in the dark until laboratory arrival, where they were processed.

CO2 concentrations were measured using a specially modified Vaisala pCO2 sensor (Johnson et al. 2009) attached to a float for uniform depth-pressure corrections; generally, 15-30 minutes of equilibration time was required for readings to stabilize at each site prior to recording values.

Phytoplankton taxonomic samples were collected as integrated water column samples to ensure that samples were representative of phytoplankters distributed throughout the water column, including surface-scum-forming and understory low-light-adapted species. Phytoplankton samples were collected from the water's surface to the sediment or to a maximum of 2m depth. Phytoplankton samples were preserved with Lugol's solution in the field and stored at room temperature.

During winter, field observations and water samples were collected through holes in the ice following the open-water procedures described above. To monitor under-ice hypoxia at each sampling site, HOBO Dissolved Oxygen Loggers were deployed at 0.5m depth above the bottom and programmed to collect water temperature and DO concentration data at 15-minute intervals. Sensors were deployed February 2023 - May 2023 and October 2023 - April 2024, with sensors being retrieved after ice-out in both years. Barometric pressure data from SWMP weather station in Pokegama Bay (NOAA/NERRS 2025c) was used to calculate dissolved oxygen percent saturation.

LABORATORY PROCEDURES

Field samples collected for dissolved nutrients and chlorophyll-a were filtered in the LSNERR laboratory within 8 hours of collection. NRRI's Central Analytical Laboratory analyzed the following water quality variables following standard methods (Baird and Bridgewater 2017). Total nitrogen (TN) and total phosphorus (TP) were measured on unfiltered water samples following potassium persulfate digestion (SM 4500-P J) using the automated cadmium reduction (SM 4500-NO3 F) and ascorbic acid (SM 4500-P E) methods, respectively. Ammonium (4500-NH3 G) and nitrate + nitrite (4500-NO3 F) were measured on filtered water samples (0.45-μm pore size). Soluble reactive phosphorus (SRP) was measured on filtered water samples using the ascorbic acid method (SM 4500-P E). Total organic carbon (TOC) and dissolved organic carbon (DOC) were measured on unfiltered and filtered water samples (0.7-μm pore size), respectively, using a Shimadzu TOC-L analyzer (SM 5310 B). Alkalinity was measured on unfiltered water samples using the 2-endpoint method (SM 2320 B).

The following parameters were analyzed in LSNERR's laboratory. Total and volatile suspended solids (TSS/VSS) were measured as dry weight of particulates on GF/C filters (1.2-μm pore size) using the loss-on-ignition method (SM 2540 D, SM 2540 E). Chlorophyll-a was measured using the unacidified fluorometric method (SM 445.0) following a 24 hour, 90% acetone extraction.

Phytoplankton taxonomic samples were analyzed for total protists, largely phytoplankton but protozoans and zooplankton were also assessed if present. The highest possible taxonomic resolution was used for identification, which resulted in a mixture of species, genera, and lesser-defined groups, such as "ovoid cyanobacteria". Analysis followed the quantitative Utermöhl settling chamber method (Utermöhl 1958) unless protist concentrations were very high, in which a measured aliquot of sample water was placed in a Sedgewick-Rafter counting chamber, sometimes employing dilution with filtered estuary water if cell densities or debris were very high. The Utermöhl method employed settling samples for 24 hours in a chamber to concentrate protists for counting. Counting of settled samples occurred on an inverted microscope (Olympus BX51) at 400× magnification. At least 250 phytoplankton entities were counted per settled sample, and the transect area was recorded for quantitative calculations. To account for large, rare taxa (e.g., large colonies, Ceratium hirundinella), the full chamber was assessed at 100× magnification for these large entities. In calculations, the abundance of these large entities was downweighted by the actual area counted during 400× assessment. For biovolume assessments, up to the first 10 specimens of each taxon observed were measured based on visible dimensions (length, width, diameter, depth, as necessary) and biovolume calculations for each taxon were conducted based on standardized cell shape formulas for each taxon (Reavie et al. 2010).

DATA ANALYSIS

All data analysis was performed in R (R Core Team 2024, version 4.4.1 or 4.4.2)

All data is publically available:

Nicklay, H., E.D. Reavie, C. Filstrup, P. Birschbach, and A. Knoll. 2025. St. Louis River Estuary Phytoplankton and Water Quality, 2022 - 2024 ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/cf58e8c6af8a79077bf4330d60a6032c.

Correlation matrix

To evaluate relationships among water quality parameters, we generated a Kendall rank correlation matrix using observations from main sampling sites. Kendall's tau was used due to its robustness to non-normality and missing values.

The matrix was visualized using the pairs() function in R, displaying scatterplots in the lower triangle and annotated correlation coefficients with p-values in the upper triangle. Statistically significant correlations ($p < 0.05$) were color-coded: positive correlations in dark red and negative correlations in navy blue. Non-significant correlations are black.

Environmental Drivers of Cyanobacteria Blooms

We utilized random forest analysis (RFA) (Breiman 2001) to predict combined cyanobacteria biovolume from a suite of environmental variables, assess predictor importances, and evaluate marginal predictor-response relationships over predictor ranges. Random forest constructs ensembles of decision trees, with each tree built on subsets of input data and predictors, to make predictions, and then self-assesses performance through out-of-bag error estimation by making predictions for observations not used in tree construction. This approach is well-suited for complex ecological datasets because it can handle many variables at once, is fairly robust against collinearity, can identify important patterns, and rank which parameters are the most important predictors, even when relationships are not simple or linear.

RF models were fit to the whole SLRE, a combined upper and middle SLRE sites subset, and a lower SLRE sites subset using the R function randomForest in package randomForest version 4.7.1.1 (Liaw & Wiener 2002). Of 272 input samples, five samples had NA values for DOC, TOC, and ANC, and one of these five had NA secchi depth; prior to all analyses, the NA values were imputed with the randomForest function rfImpute, which first replaces NA values with variable medians, then fits an RF model to the data, and finally applies weighting to the imputed values based upon similar observations. Cyanobacteria biovolumes were log10 transformed to attain a normal distribution prior to modeling. Because random forest is fairly robust to correlated predictors, we selected a large initial suite of 18 environmental predictors. Depth was excluded because sites were specifically chosen to have fairly uniform depth. Chla was excluded as it is expected to be related to the response variable, cyanobacteria biovolume. VSS and CO_2 were excluded because they contained many zeros (VSS) and missing data (CO_2). Model arguments ntree, mtry, and nodesize were evaluated with a custom function and optimized from default when necessary. Predictor importances in each model were ranked by %incMSE, the standard metric for assessing importances (Breiman 2001), with a higher value for a predictor indicating a greater relative decrease in model performance when it is randomly permuted. The least important predictor was iteratively dropped from subsequent model versions until model performance, as assessed by OOB mean square of residuals and percent variance explained, worsened or did not improve. Partial dependence plots were then generated with the randomForest function partialPlot to assess marginal effects of environmental variables on predicted cyanobacteria biovolume, across the entire ranges of individual variables, and compare these effects among the three aforementioned models.

WATER QUALITY AND NUTRIENT CHARACTERIZATION

Hotspots vs. Thalweg

We conducted event-matched paired t-tests to compare water quality variables between thalweg and hotspot sites within two geographic groups: upper estuary (OL compared to MU, NO, KI, PO, BI) and lower estuary (BL compared to AL, BA, MI). and conducted paired t-tests on mean values for shared water quality parameters using the t.test() function from the STAT package (Bolar 2019). Analyses were limited to open-water season (May through November) when SWMP data is complete.

Anomalies and short term trends

The R package SWMPr (Beck and Cressman 2025), created specifically to ingest, quality assure and plot SWMP data was used to plot data over time, conduct annual and monthly Mann-Kendall trend analysis, and assess anomalies for all water quality parameters.

To assess temporal trends in water quality, we conducted a Mann-Kendall (MK) trend analysis using continuous monitoring data from four sites (Barker's Island, Oliver Bridge, Pokegama Bay, and Blatnik Bridge). Data were first filtered to include only months with consistent coverage (May–October), excluding year-month combinations with fewer than 2,500 records (~26 days) and omitting all data from 2012 due to incompleteness.

Each dataset was aggregated to monthly means for five parameters: temperature, dissolved oxygen concentration, pH, specific conductance, and turbidity. Annual Mann-Kendall tests were applied to the time series of monthly averages to detect monotonic trends. For additional resolution, seasonal Mann-Kendall tests were performed separately for each month across years, provided at least six years of data were available. Significant trends ($p < 0.05$) were visualized using linear smoothing over time, and key results were interpreted in the report.

To visualize parameter anomalies, we used a custom plotting function derived from SWMPr's plot_summary() function (Beck and Cressman 2025) that calculates and displays deviations from monthly long-term means. Data were restricted to the May–October seasonal window and averaged daily with no interpolation of missing points applied. Monthly means were computed across all years, forming a monthly baseline. For each year and month, anomalies were calculated by subtracting the long-term monthly mean from the observed monthly mean. These anomalies were then visualized using a diverging color scale in a heatmap format, with zero as the midpoint, enabling identification of relative increasing or decreasing patterns across years and months.

SPATIAL AND TEMPORAL REDUNDANCY

Spatial Redundancy

Spatial analysis of water quality and phytoplankton data enabled identification of redundancy among sample locations, thereby supporting site selection for future monitoring. Nonmetric multidimensional scaling (NMDS) of water quality and phytoplankton data was used to identify uniqueness among sites.

We conducted a correlation analysis (**Appendix E**) to identify and remove correlated variables prior to spatial redundancy analysis. Several variables were excluded based on either high correlation ($r>0.8$) with another single parameter or moderate ($0.3>r<0.8$), but significant ($p<0.05$) correlation with several other parameters, for example, TOC and DOC were significantly correlated ($r=0.93$) so only DOC was retained. Among water clarity metrics, TSS, turbidity, PARkd, and secchi were all moderately, yet significantly correlated, so only secchi was included. pH was moderately, but significantly, correlated to several parameters (ANC, DO %, and DO) so was retained for analysis, while the others were excluded. This filtering process resulted in a final set of variables: TP, TN, NO_2+NO_3, NH_4, DOC, chl-a, spcond, pH, temp, secchi, and N:P. Chlorophyll-a was not used in the phytoplankton NMDS.

The phytoplankton dataset, and therefore the corresponding NMDS, contained three fewer sample sets than the water quality dataset due to subsetting of open water 2024 sample enumeration. Phytoplankton biovolume (µm3/ml) by taxonomic division (BAC = centric diatoms, BAP = pennate diatoms, CHL = chlorophytes (green algae), CHR = chrysophytes, CRY = cryptophytes, CYA = cyanobacteria, PYR = pyrrophytes) was ordinated using the metaMDS function in the R package vegan version 2.6.4 (Oksanen et al. 2022). NMDS analyses were performed separately for all data from the 8 monitoring sites, and subsequently for summer data (July - October) so that spatial redundancy could be assessed during the likely period for cyanobacterial abundance. Visually, site-specific ellipses representing 95% confidence intervals of samples were used to identify uniqueness among sites. Ordinations were performed in three dimensions to minimize stress after evaluating scree plots, and Shepard stress plots were used to assess preservation of rank order and absolute distances among sample scores.

This was followed by permutational multivariate analysis of variance (PERMANOVA) on sample scores from all "complete" sampling events (at all eight sites) to statistically confirm visual differences among sites, using the vegan function adonis2. PERMANOVA is advantageous in this context because it constrains scores by a selected "blocking variable" during analysis, so that inferred differences reflect differences at given points in time, and not necessarily differences among entire site clusters of sample scores. We blocked temporally by individual sampling events, as complete events were unevenly distributed over the sampling period, and a preliminary two-way PERMANOVA demonstrated significant independent effects of sampling event on NMDS scores for water quality ($F = 33.95$, $R^2 = 0.61$, $p = 0.0001$) and for phytoplankton ($F = 2.85$, $R^2 = 0.26$, $p = 0.0001$) along with significant independent effects of site on both water quality and phytoplankton ($F = 57.44$, $R^2 = 0.27$, $p = 0.0001$; $F = 3.32$, $R^2= 0.09$, $p = 0.0001$).

Further, pairwise PERMANOVA from the R package pairwiseAdonis version 0.4 (Martinez Arbizu 2017) provides Bonferroni-corrected pairwise statistical comparisons (pseudo-F; Padj a = 0.05) among all sites, also blocked by sampling event, to aid in identifying whether certain sites are providing redundant information about water quality and/or phytoplankton. For visual depiction of pairwise PERMANOVA relationships among sites, we connected site pairs with non-redundant water quality conditions with lines on one map, and did the same for phytoplankton communities on another map.

Temporal Redundancy

To inform an efficient and cost-effective water quality monitoring strategy, we assessed temporal redundancy in physicochemical and nutrient variables, as well as indicators of algal abundance (chlorophyll-a, total algal biovolume, cyanophyte biovolume). Overly frequent sampling can yield redundant data and increase costs, while infrequent sampling may fail to capture short-term variability critical for detecting events such as algal blooms.

Sampling Interval

For each variable at each site, all possible pairwise comparisons between sampling events were used to calculate the absolute difference in time between samples and the corresponding difference in variable values. This produced a full set of combinations (e.g., 1,225 comparisons from 50 observations) across a temporal range of 0 to over 500 days. These data were then analyzed using smoothed regression (LOESS) to model the relationship between time difference and variable divergence. Resulting plots enabled visual assessment of when additional sampling yields diminishing returns, helping to guide optimized monitoring frequency for each parameter.

Effects of Sampling Frequency Reduction on Random Forest Model Performance

RFA were conducted using only Barker's Island data, following the methods described above. The full model used weekly data (n = 56), excluding winter 2022–2023 due to inconsistent sampling. Two reduced-frequency models were then developed: a biweekly model using all odd-numbered sampling events (n = 32), and a monthly model with manually selected events to ensure even seasonal coverage and inclusion of a late-summer high-cyanobacteria biovolume event (n = 16). Model performance was evaluated using R2 and mse, as well as changes in parameter importances.

Lag Effects: Identifying Precursor Conditions for Blooms

There is an interest in knowing whether algal dynamics such as blooms are subject to lag periods, a topic that has been studied using theoretical models (e.g., Liu et al. 2022). For instance, a sudden storm-induced flux of nutrients may result in an algal bloom after 10 days of chemical and biological changes in the receiving environment, meaning any attempt to understand relationships between blooms and water quality changes is hampered by a need to have water quality collections for several days leading up to a bloom. We assessed whether meaningful lags may be occurring by statistically comparing phytoplankton variables (chlorophyll-a, total algal biovolume, total cyanophyte biovolume, total diatom biovolume) with water quality variables on the day of sampling and at iterative daily intervals prior to the sampling day. For a given variable, all data from each of the eight sites were extrapolated linearly between sampling events to generate daily results. Then, Spearman correlations were calculated for data representing actual phytoplankton sample events (i.e., no lag). Phytoplankton data were then related with water quality data from the day before, then two days before, and so on until a lag period of 50 days was reached. Plotting of results allowed for the determination of any lag effect (independent) on water quality-phytoplankton correlations (dependent).

LONG-TERM WATER QUALITY TRENDS

We repeated the Bellinger et al. (2016) analysis, adding a decade of new data from the SWMP (2013–2024) to the MMP dataset. MMP data, spanning 1953-2010, were obtained from EPA Water Quality Portal (Water Quality Portal, 2021). SWMP data, spanning 2012-2024, was obtained from the Central Database Management Office (NOAA/NERRS 2025c). Streamgage data from the St. Louis River at Scanlon, MN; USGS gage #04024000, spanning 1953-2024, was also obtained (USGS 2025). The MMP and SWMP share the same sampling location at US HWY 53/Blatnik Bridge, so the data was combined into one time series, spanning 1973-2024. The SWMP's Oliver Bridge sampling location, while not in the same location as MMP's US Hwy 23 sampling location, are comparable sites, both in the upper estuary within the main river thalweg. We assessed the applicability of combining these time series using a period of MMP data where both locations were sampled. All parameters were highly and significantly correlated ($p < 0.001$, $r > 0.60$).

With the addition of 10 years of SWMP data added to the time series at both locations, we performed nearly identical analytical methodology presented in Bellinger et al. (2016). Slight deviations in the method include the use of a modified Mann-Kendall test that accounts for autocorrelation (Hamed and Rao 1998). We performed the same concentration and load adjustments for all parameters at the Blatnik Bridge location to account impacts of Lake Superior water mixing, except that we used the quadratic model presented in Sterner et al 2007 to account for increasing Lake nitrate levels over the timeseries.

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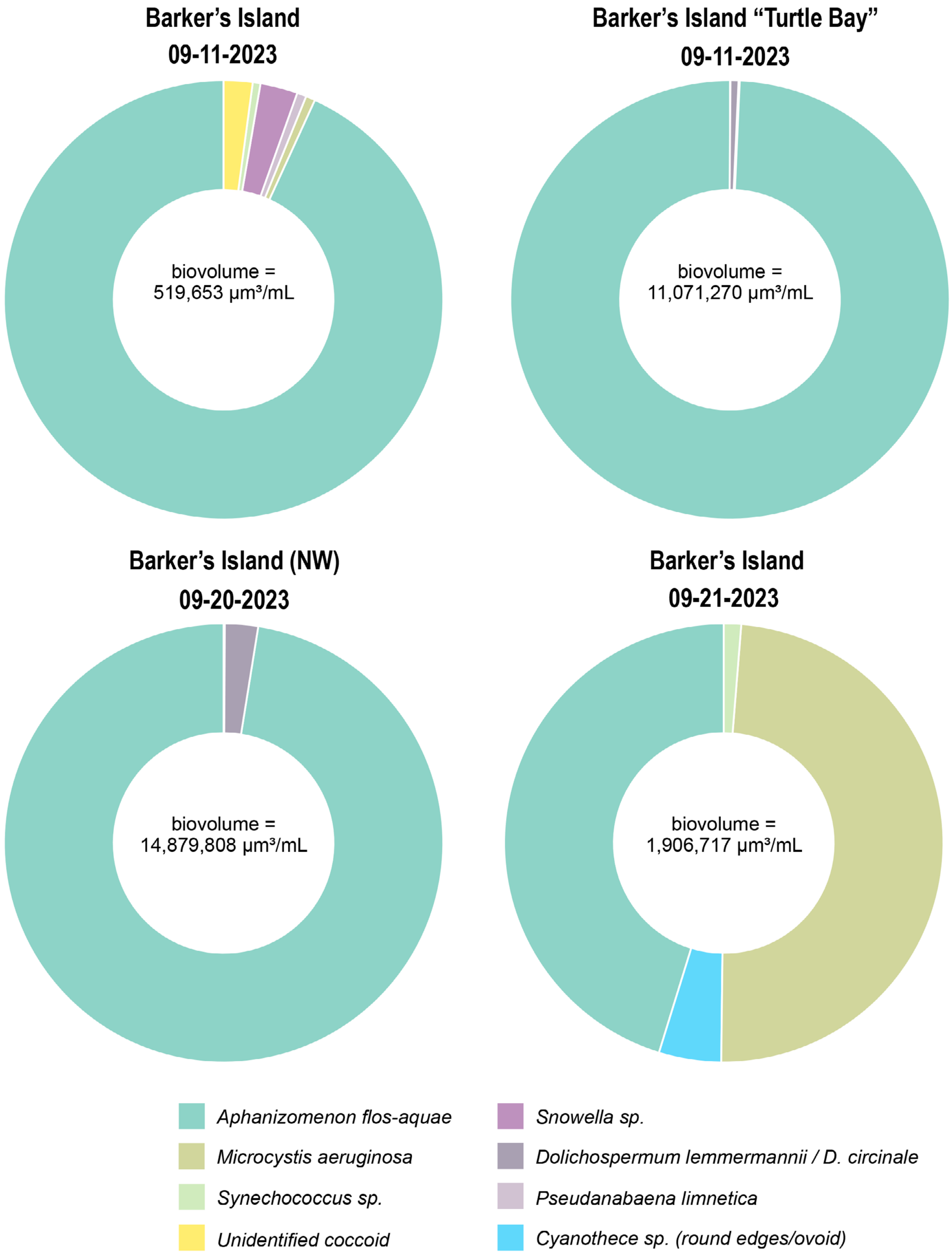
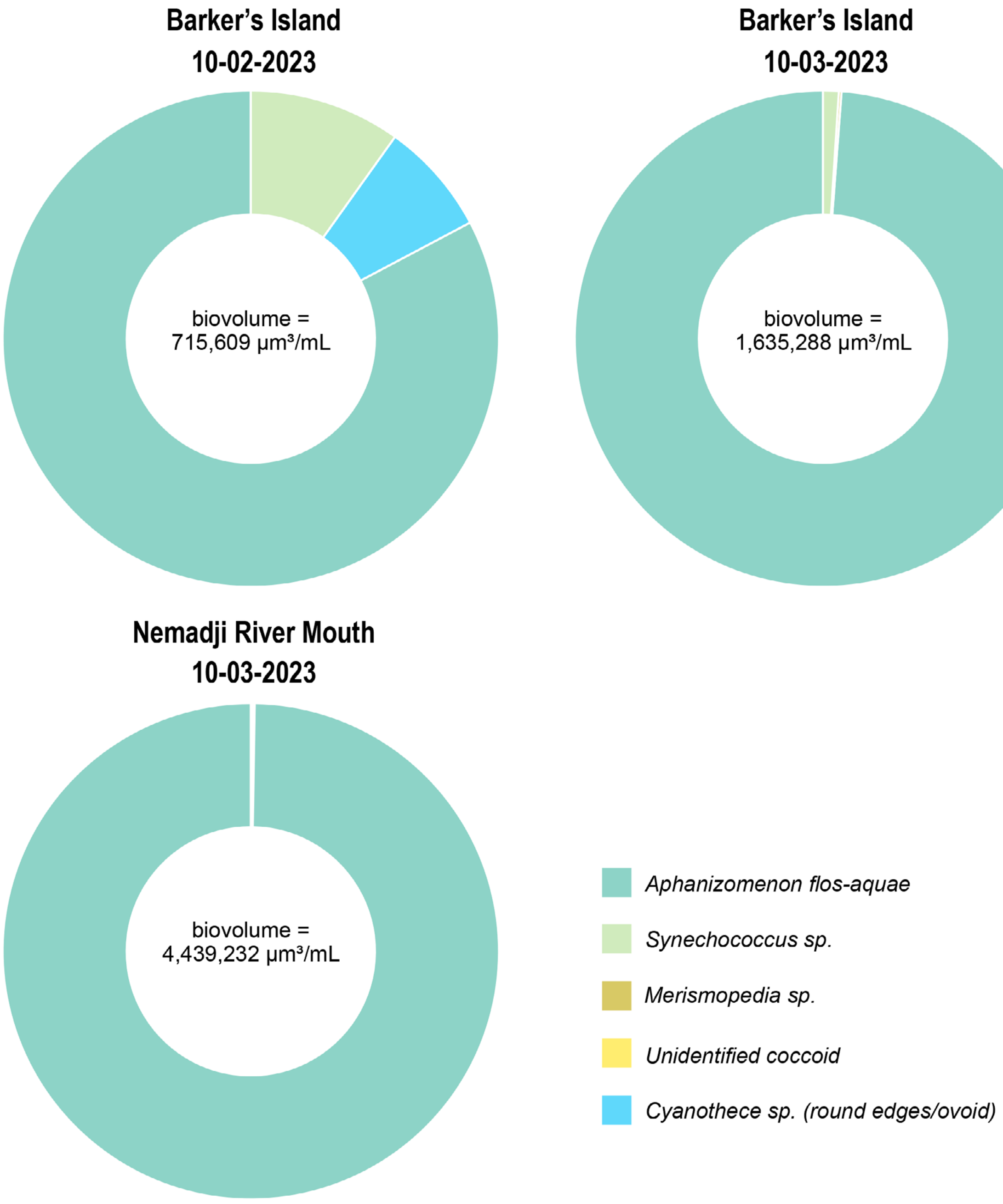
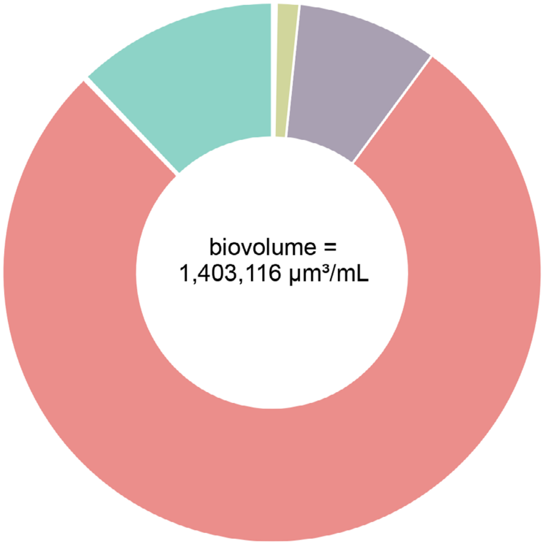
APPENDIX B: BLOOM COMPOSITION

Cyanobacteria community composition for each bloom response sample. Pie charts display each cyanobacteria species biovolume as a proportion of total cyanobacteria biovolume (µm3/ml). Note that species with <1% in any given chart are not visible but are listed in the species legend. They are organized from top to bottom by date with total cyanobacteria biovolume for each sample displayed in the center of each pie.

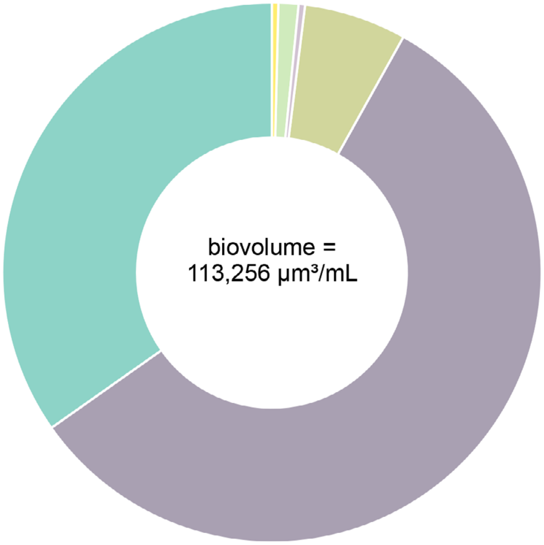



Barker's Island
10-02-2023
biovolume =
715,609 µm³/mL
Barker's Island
10-03-2023
biovolume =
1,635,288 µm³/mL
Nemadji River Mouth
10-03-2023
biovolume =
4,439,232 µm³/mL
Aphanizomenon flos-aquae
Synechococcus sp.
Merismopedia sp.
Unidentified coccoid
Cyanothece sp. (round edges/ovoid)

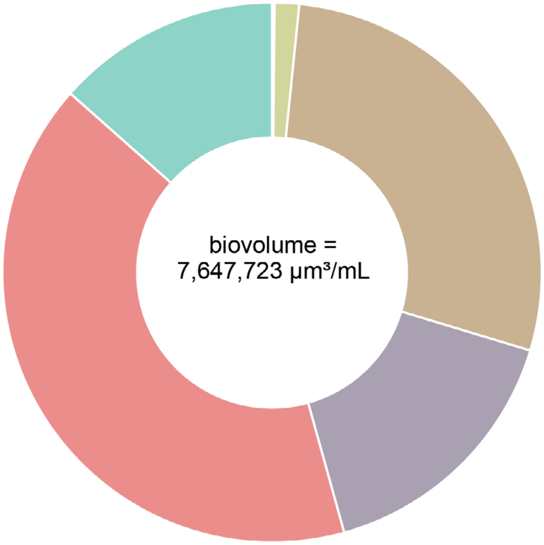
Barker's Island
09-11-2024



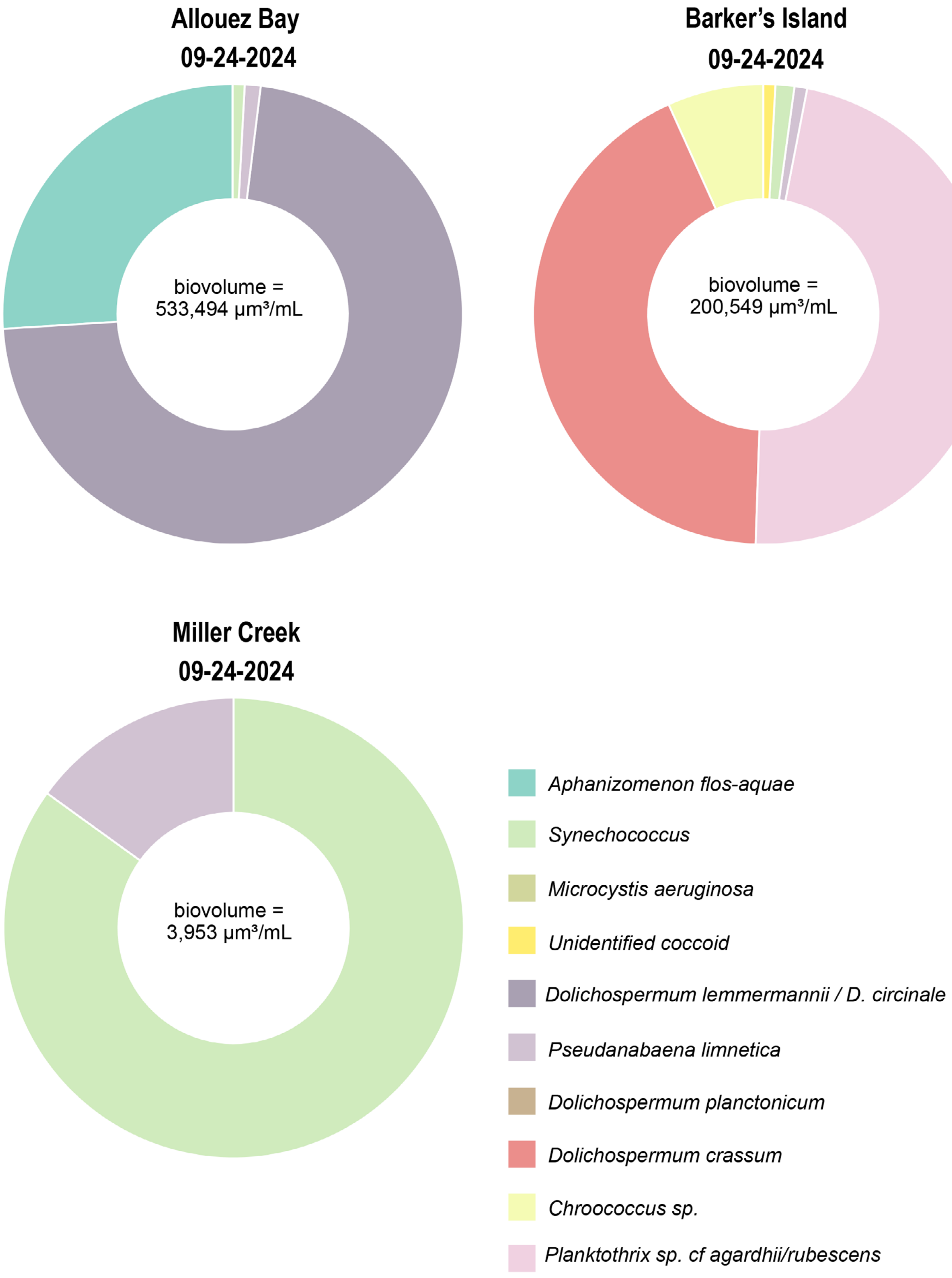
Superior Bay - near Barker's Island
09-11-2024

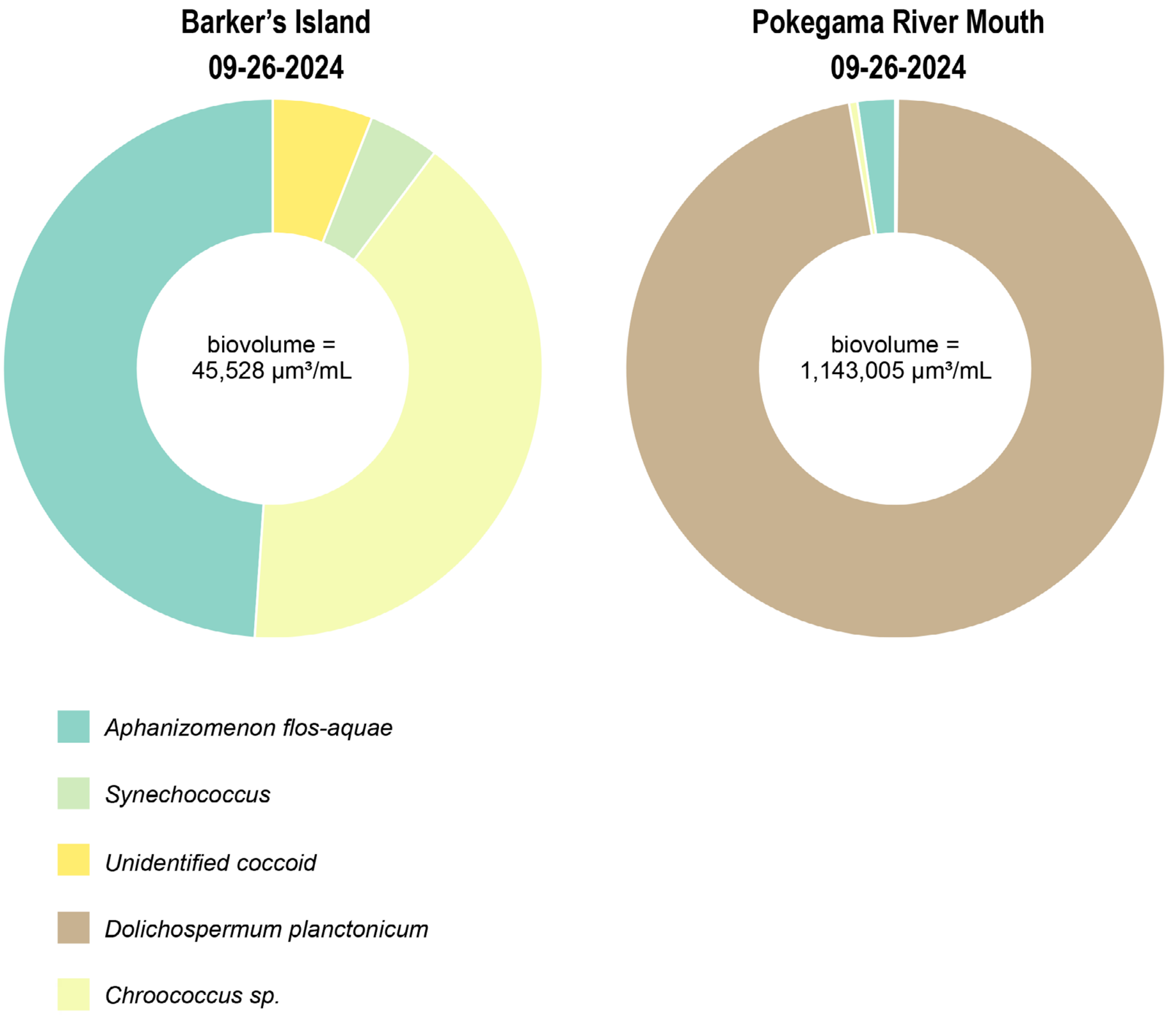


Barker's Island "Turtle Bay"
09-11-2024



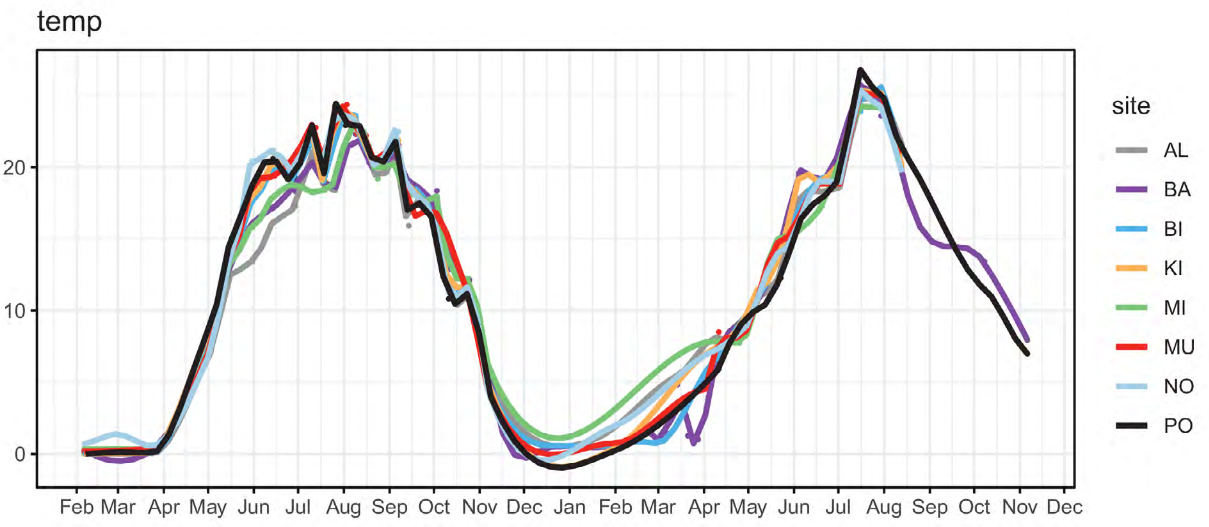
- ■ *Aphanizomenon flos-aquae*
- ■ *Synechococcus sp.*
- ■ *Microcystis aeruginosa*
- ■ *Unidentified coccoid*
- ■ *Dolichospermum lemmermannii / D. circinale*
- ■ *Pseudanabaena limnetica*
- ■ *Dolichospermum planctonicum*
- ■ *Dolichospermum crassum*
- ■ *Chroococcus sp.*

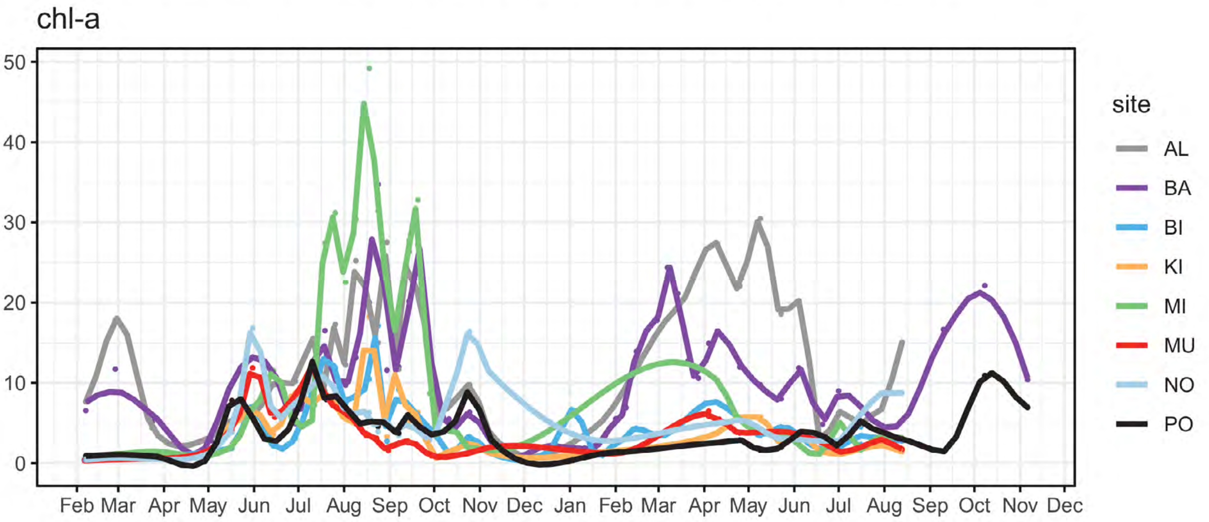

Allouez Bay
09-24-2024
biovolume =
533,494 µm³/mL
Barker's Island
09-24-2024
biovolume =
200,549 µm³/mL
Miller Creek
09-24-2024
biovolume =
3,953 µm³/mL
Aphanizomenon flos-aquae
Synechococcus
Microcystis aeruginosa
Unidentified coccoid
Dolichospermum lemmermannii / D. circinale
Pseudanabaena limnetica
Dolichospermum planctonicum
Dolichospermum crassum
Chroococcus sp.
Planktothrix sp. cf agardhii/rubescens

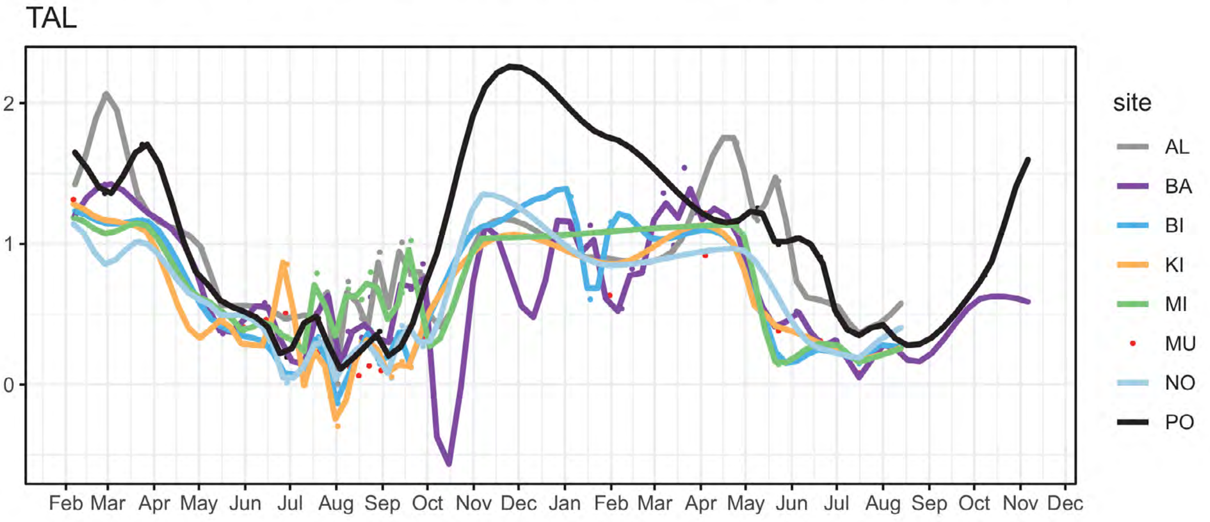

Barker's Island
09-26-2024
biovolume =
45,528 µm³/mL
Pokegama River Mouth
09-26-2024
biovolume =
1,143,005 µm³/mL
Aphanizomenon flos-aquae
Synechococcus
Unidentified coccoid
Dolichospermum planctonicum
Chroococcus sp.

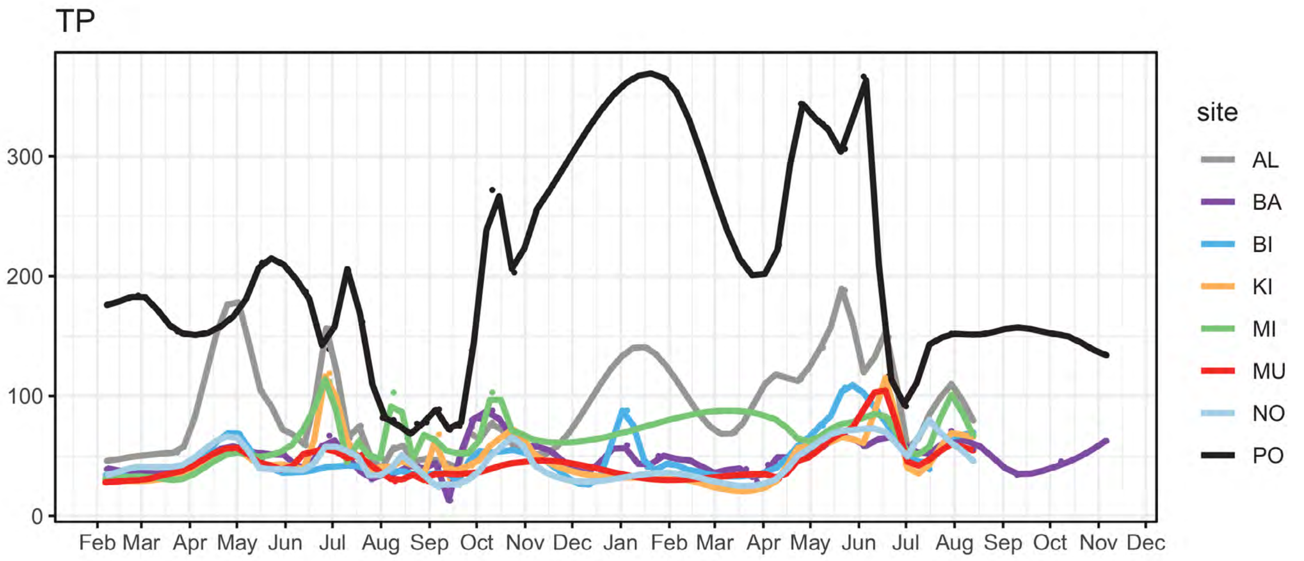
APPENDIX C: WATER QUALITY TIME SERIES

Generalized additive models of water quality variables, collected from the eight monitoring locations, 2023-2024. Variable abbreviations and units match those in **Appendix D**. For an interactive version please visit **https://lakesuperiornerr.shinyapps.io/SLRE_hot_spot/**





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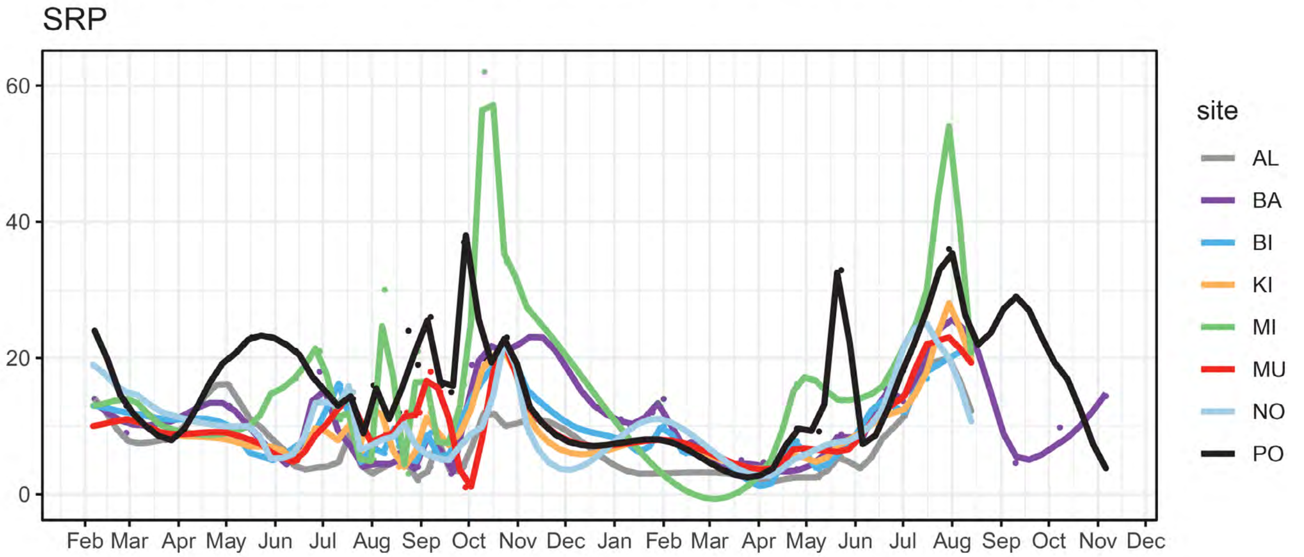
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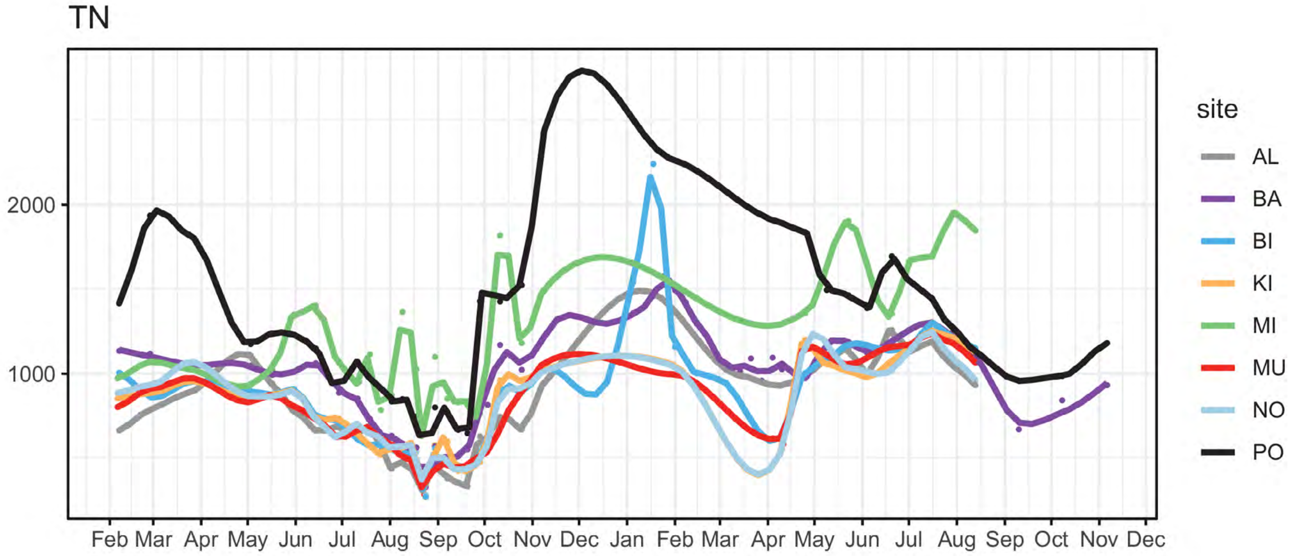
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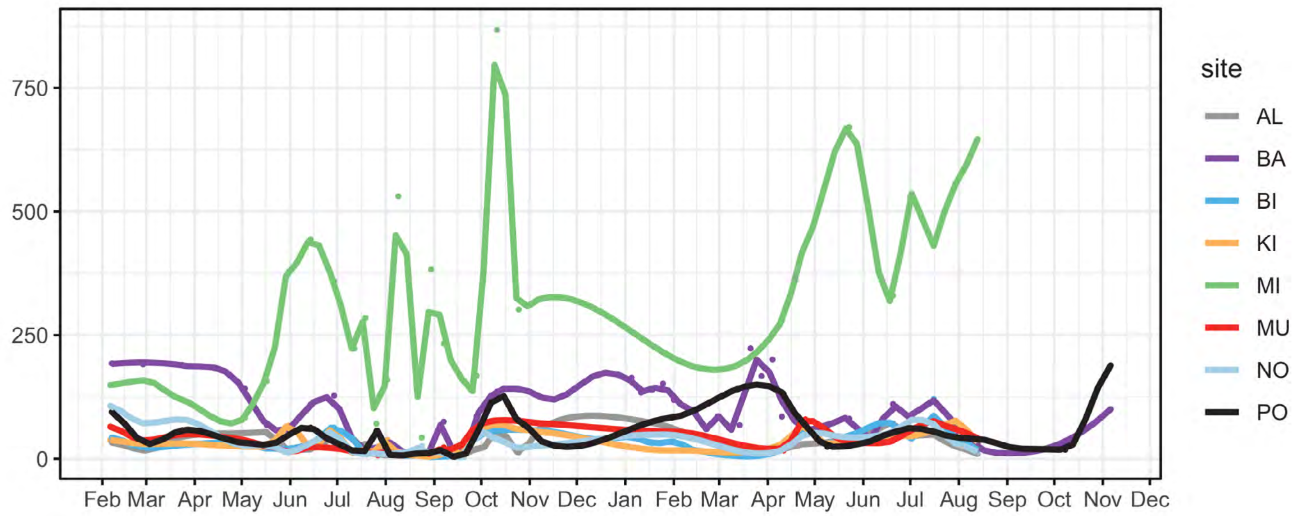

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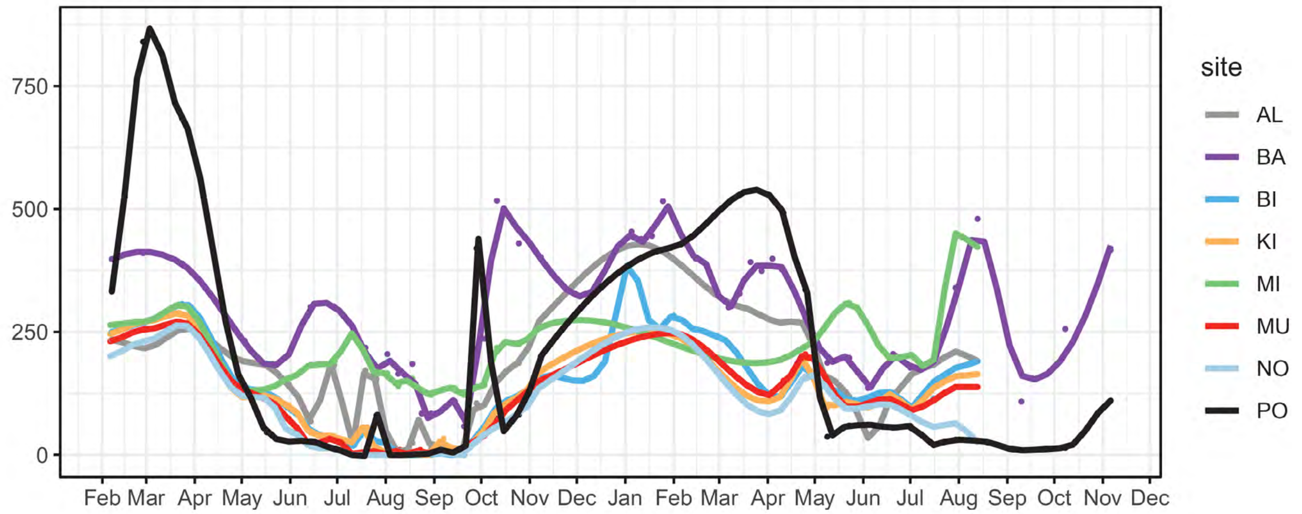

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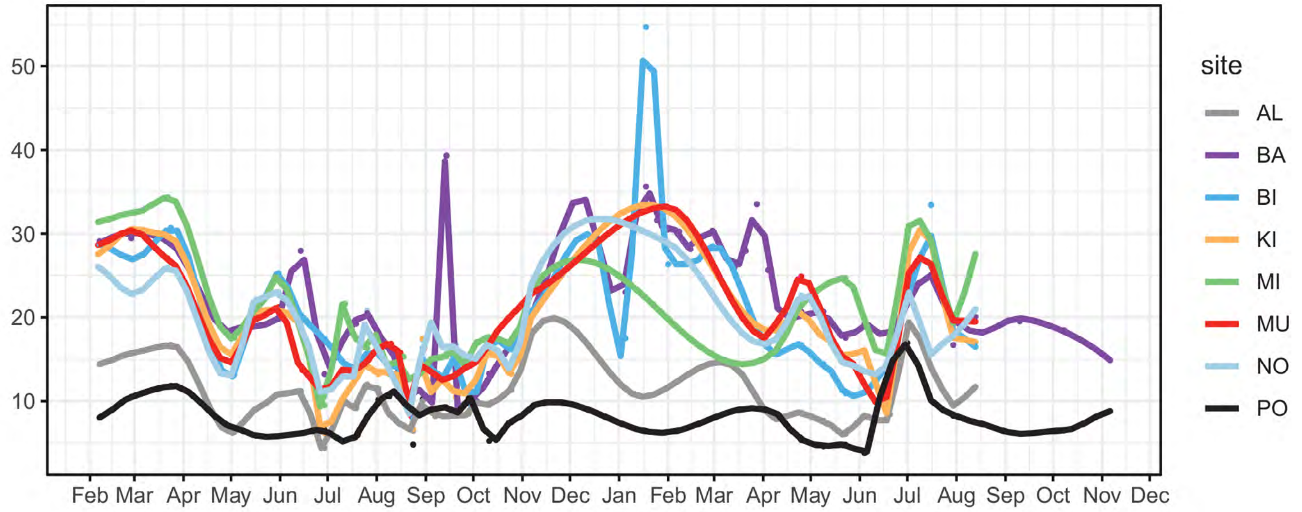

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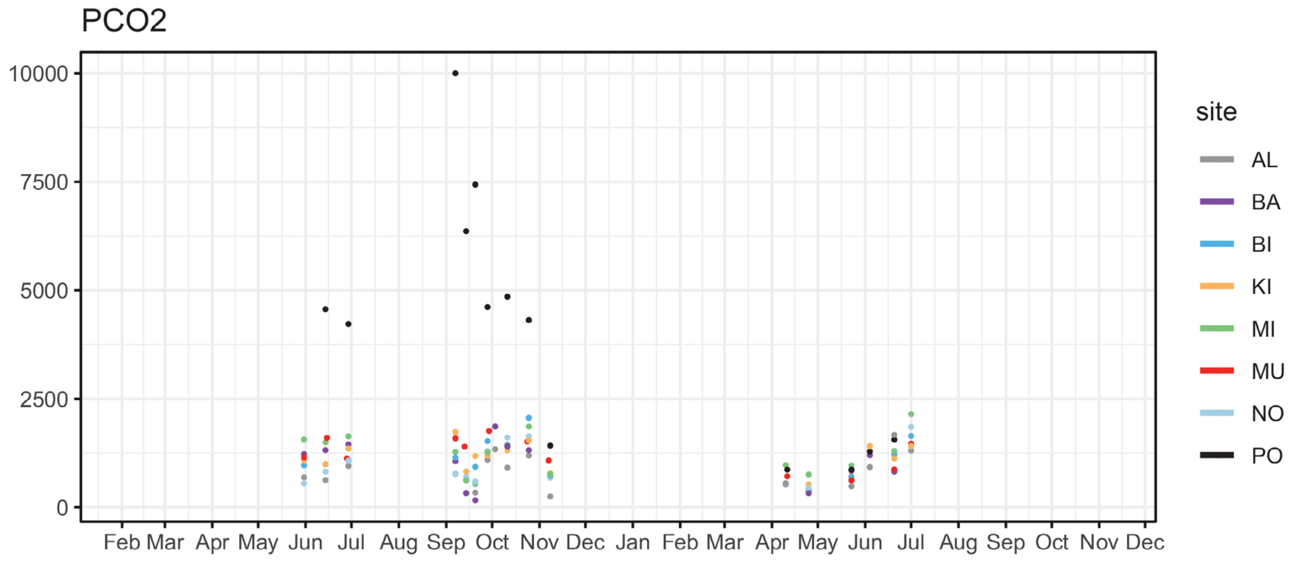
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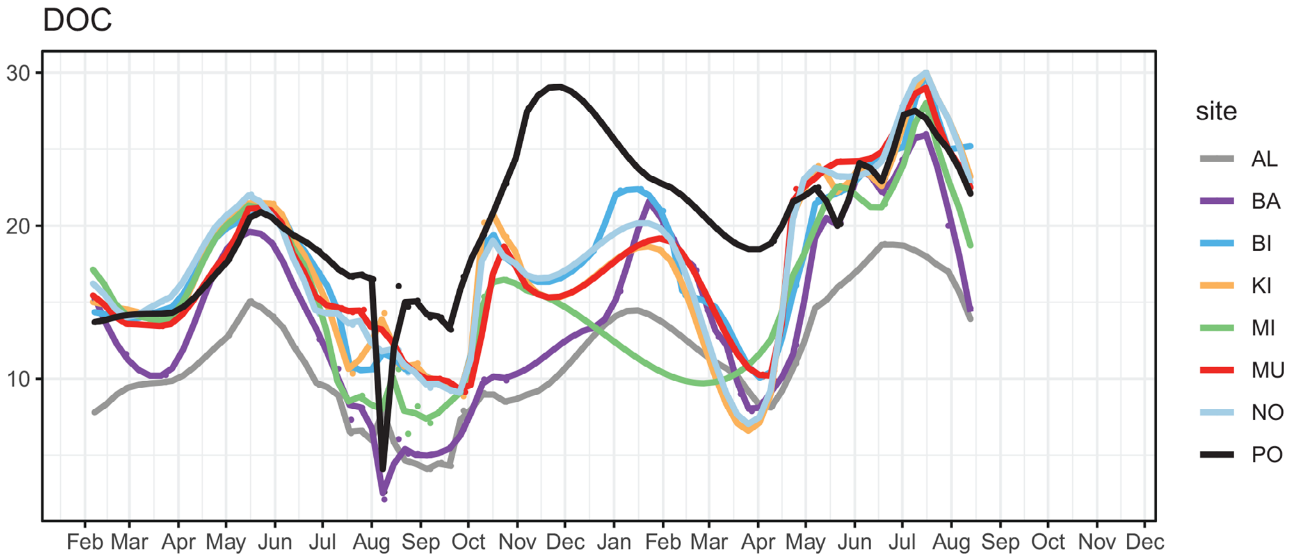
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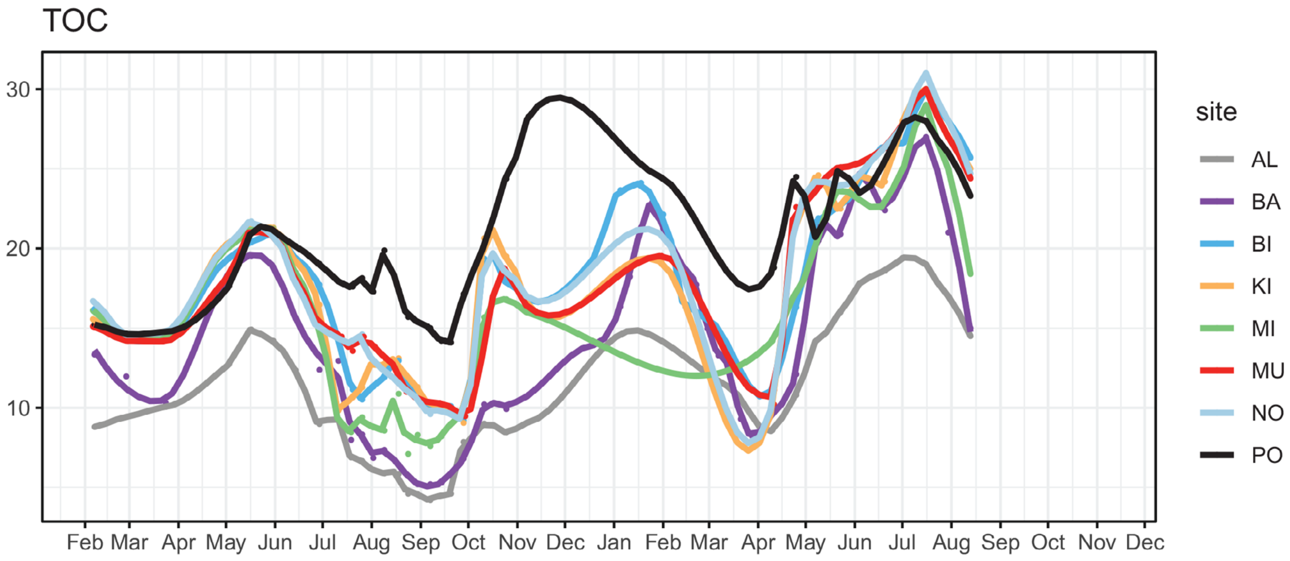
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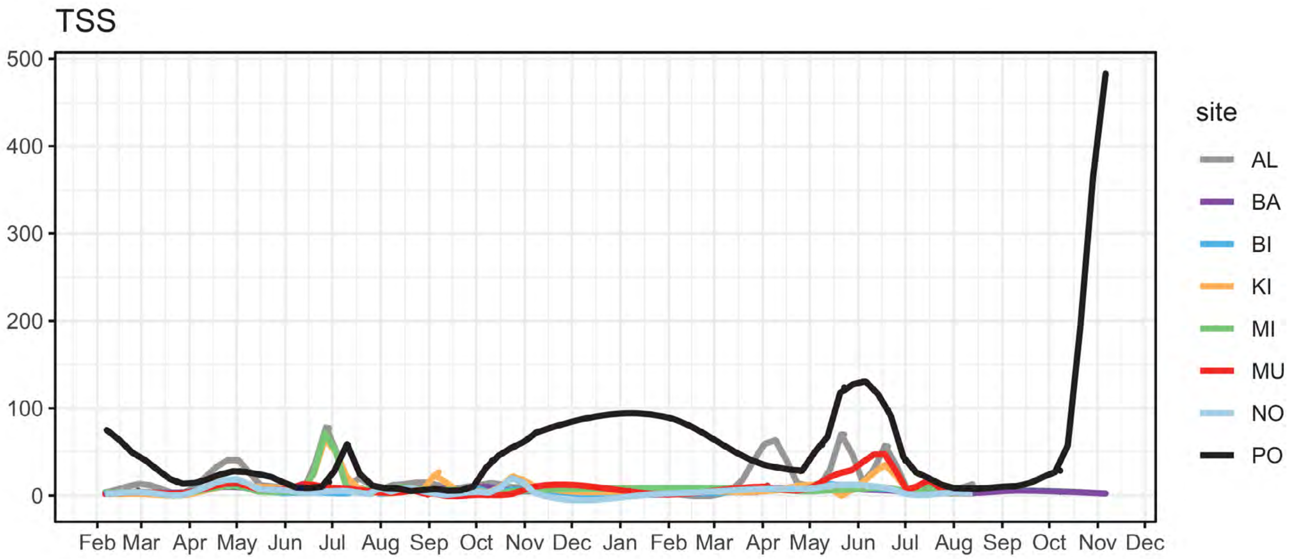
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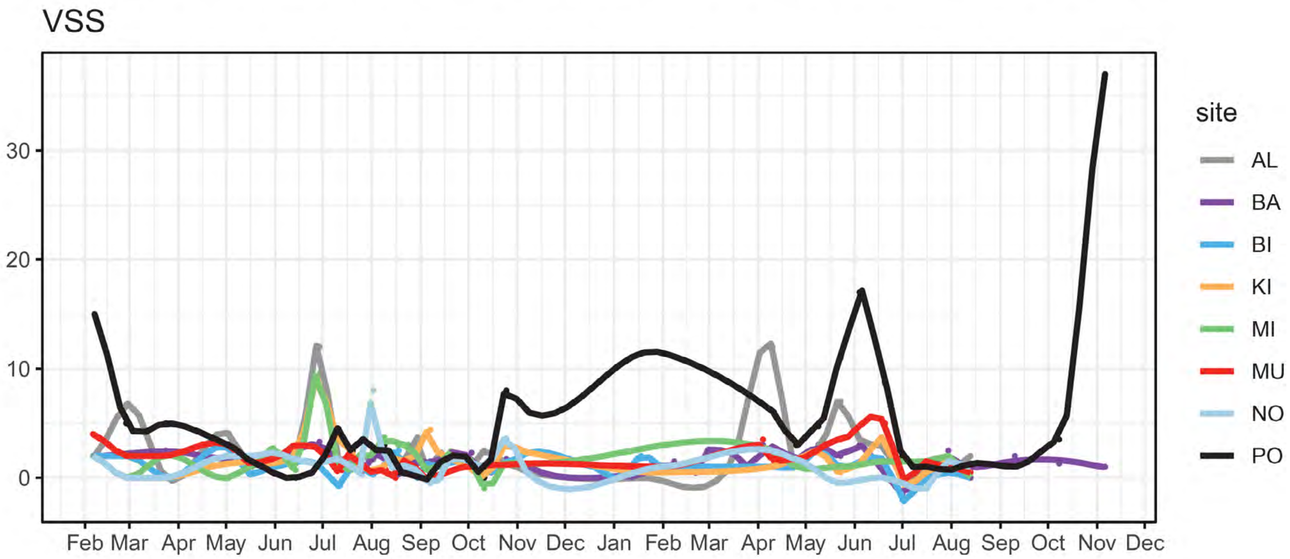
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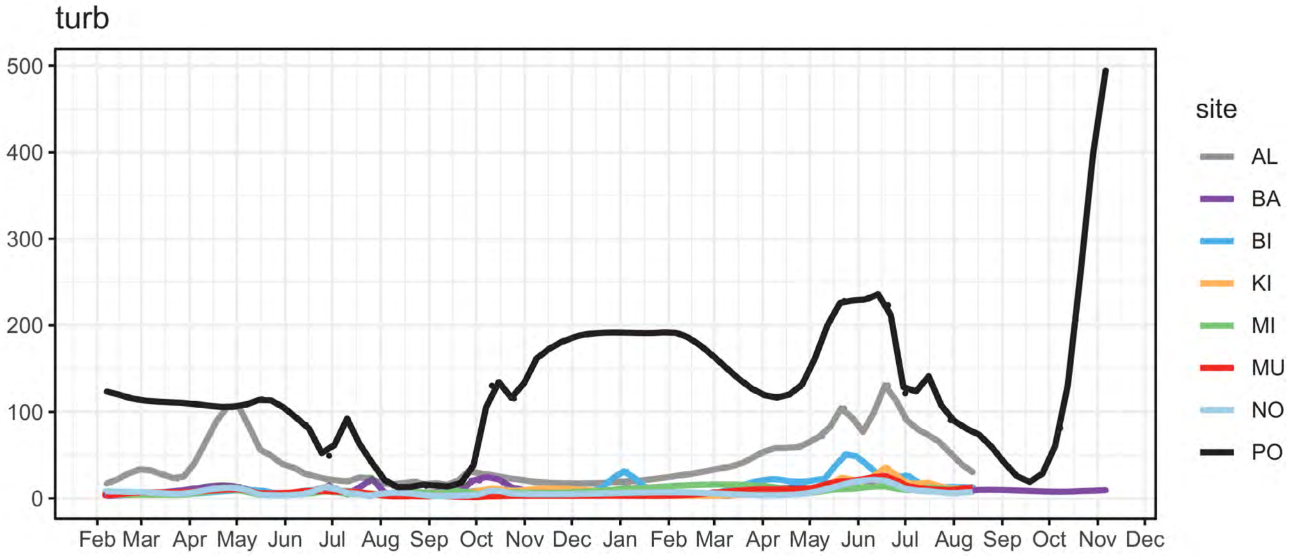
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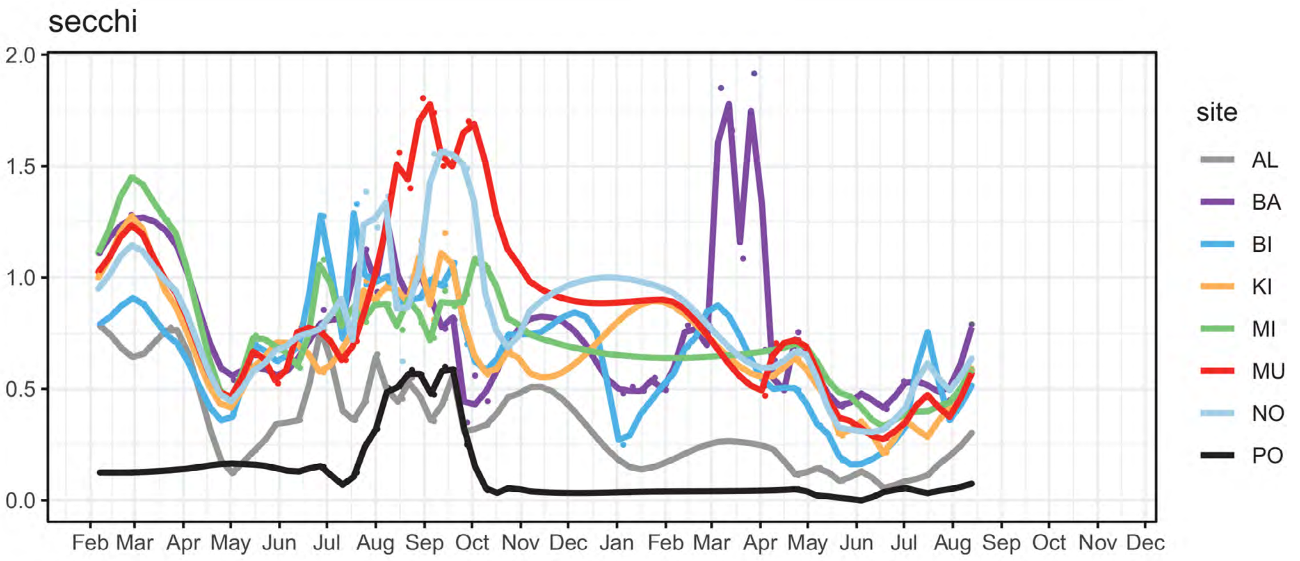
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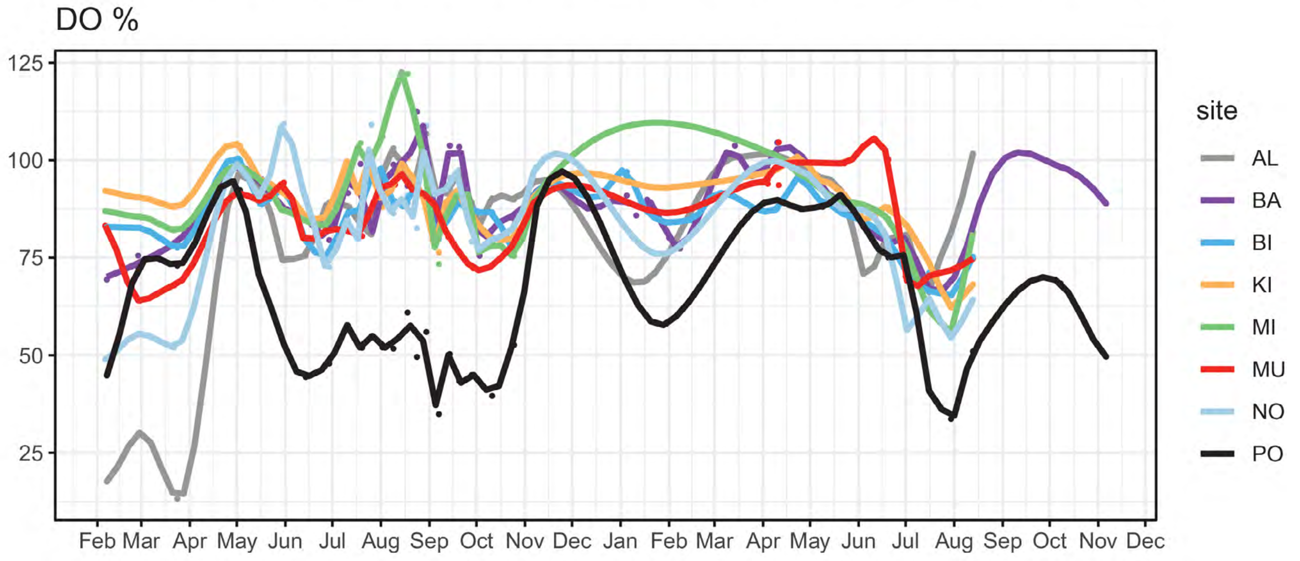
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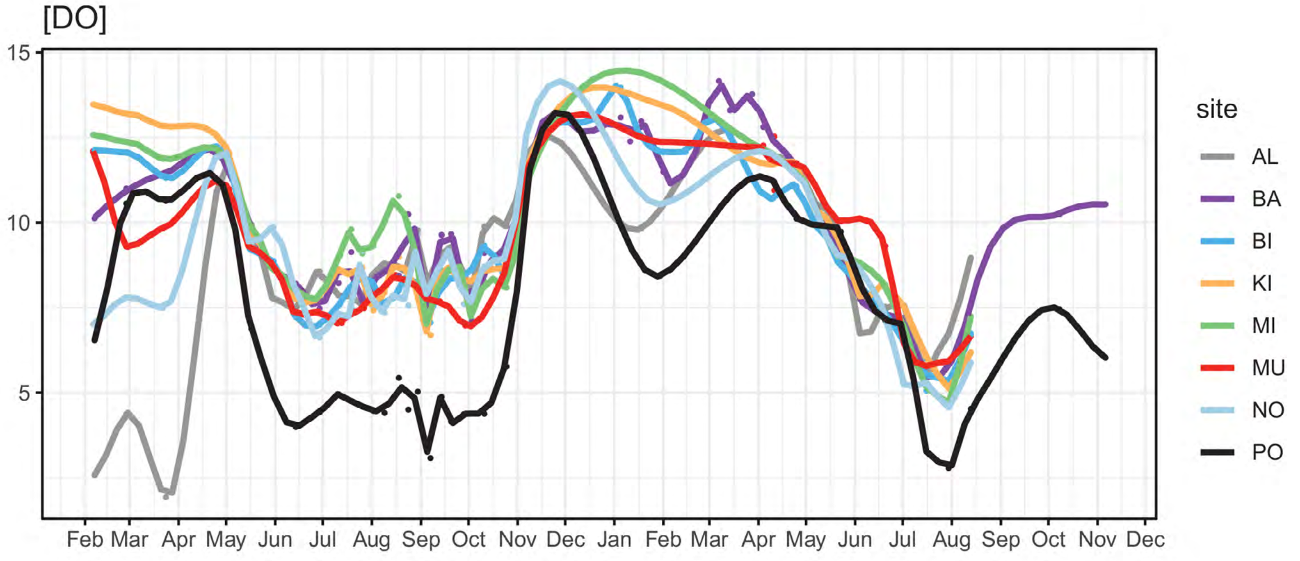
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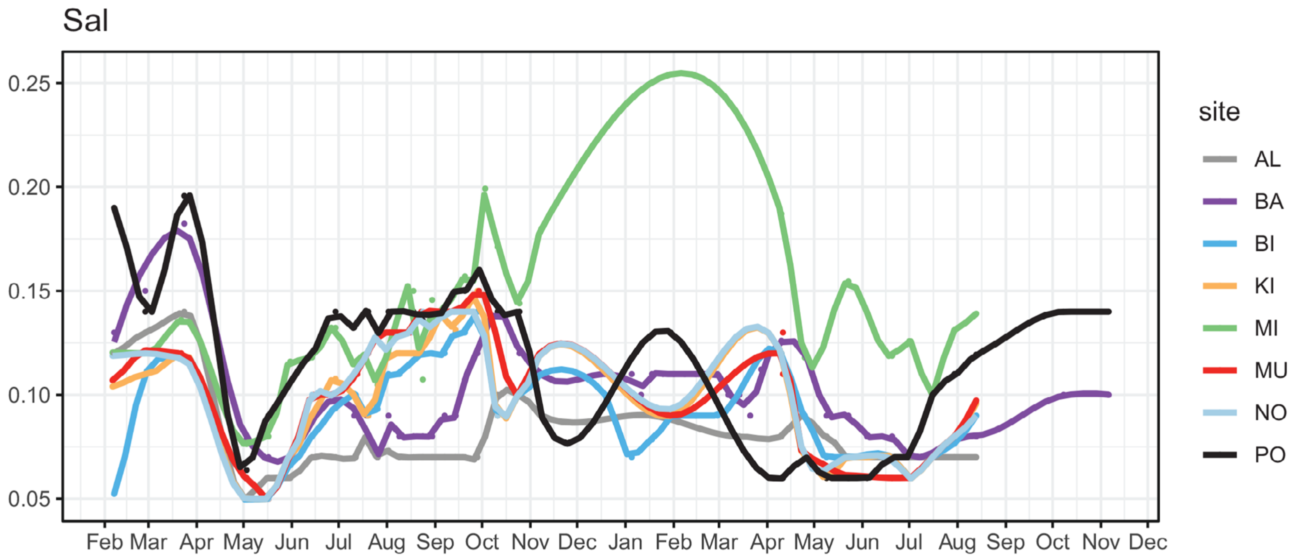
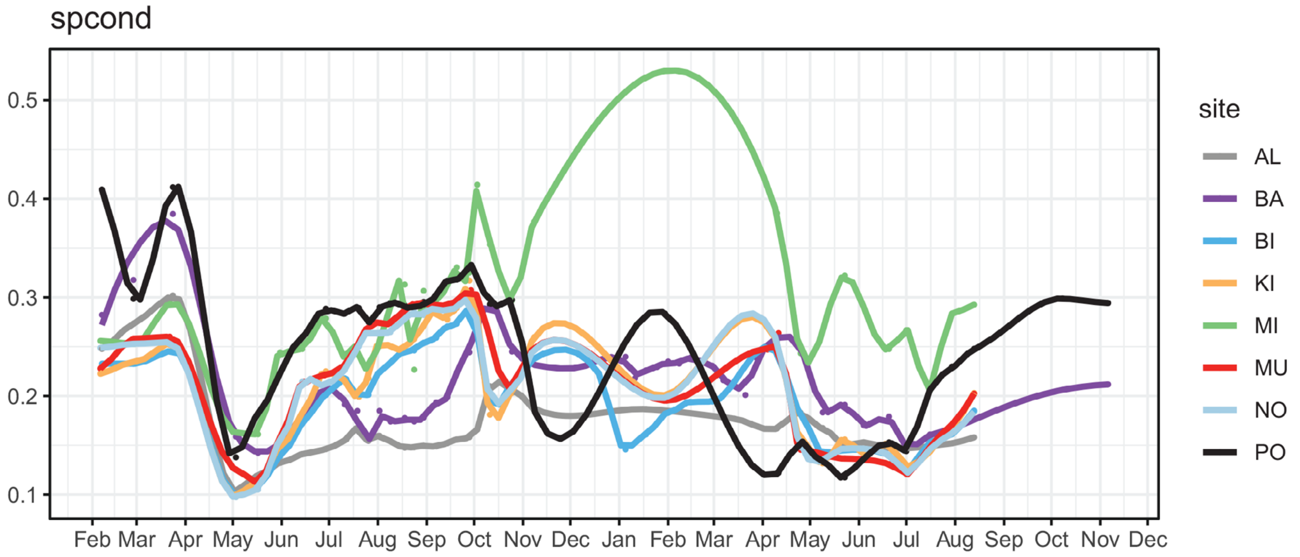
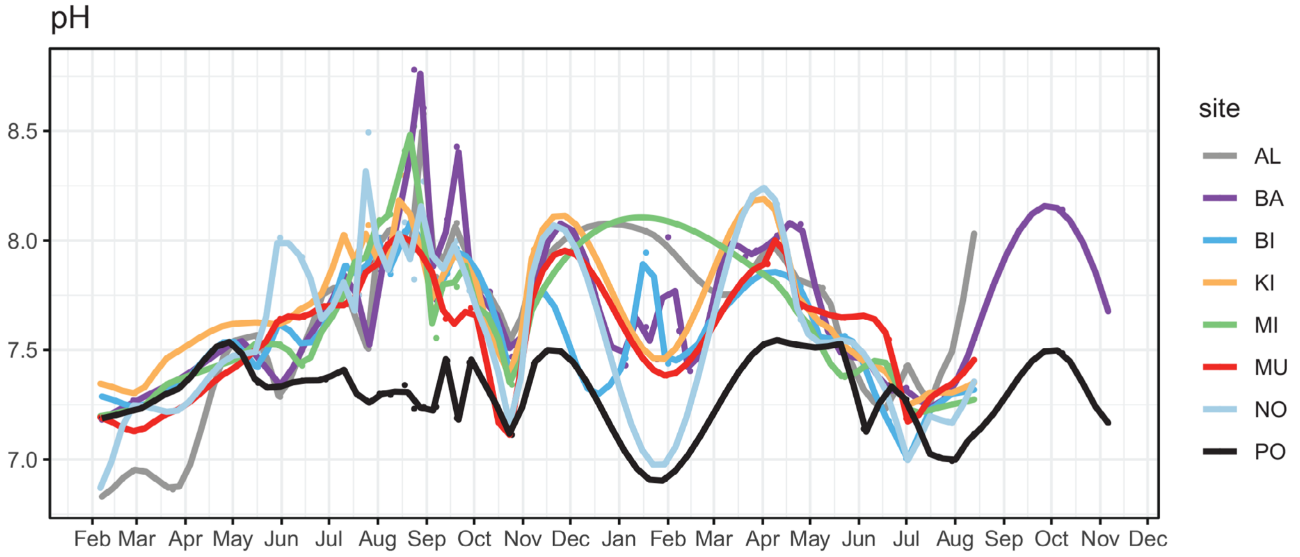
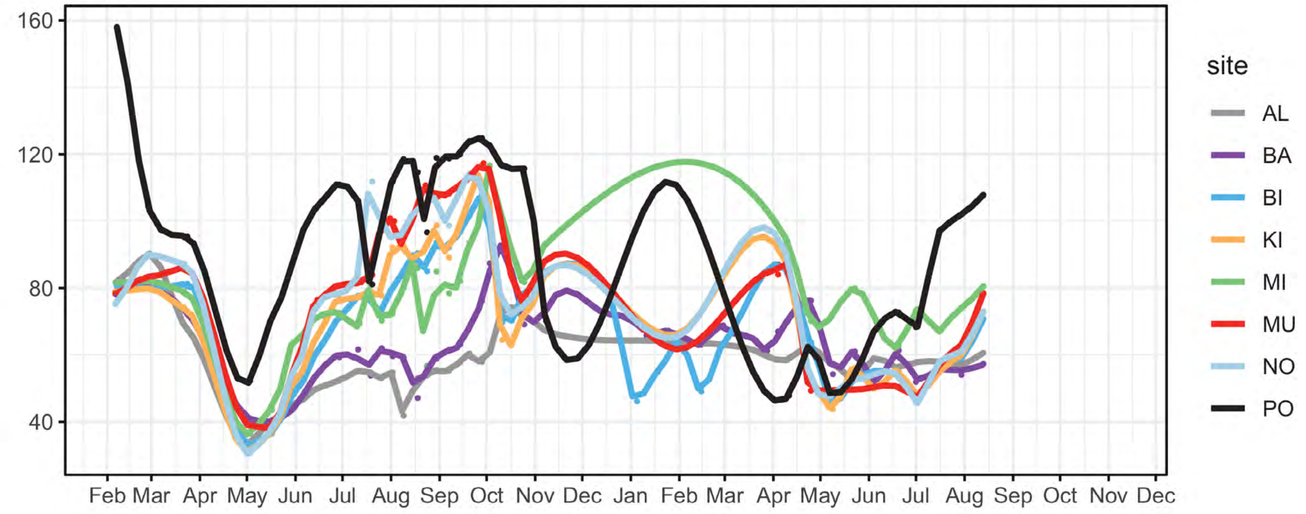

Sal

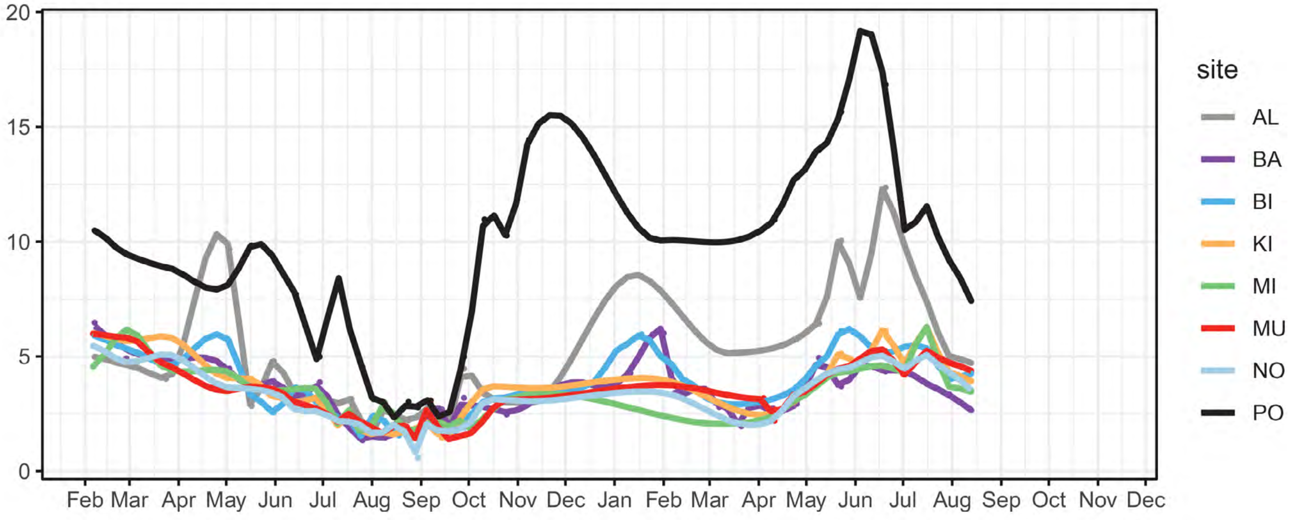

spcond


pH

ANC


160
120
80
40
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
site
AL
BA
BI
KI
MI
MU
NO
PO

PARkd


20
15
10
5
0
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
site
AL
BA
BI
KI
MI
MU
NO
PO

APPENDIX D: WATER QUALITY TABLE

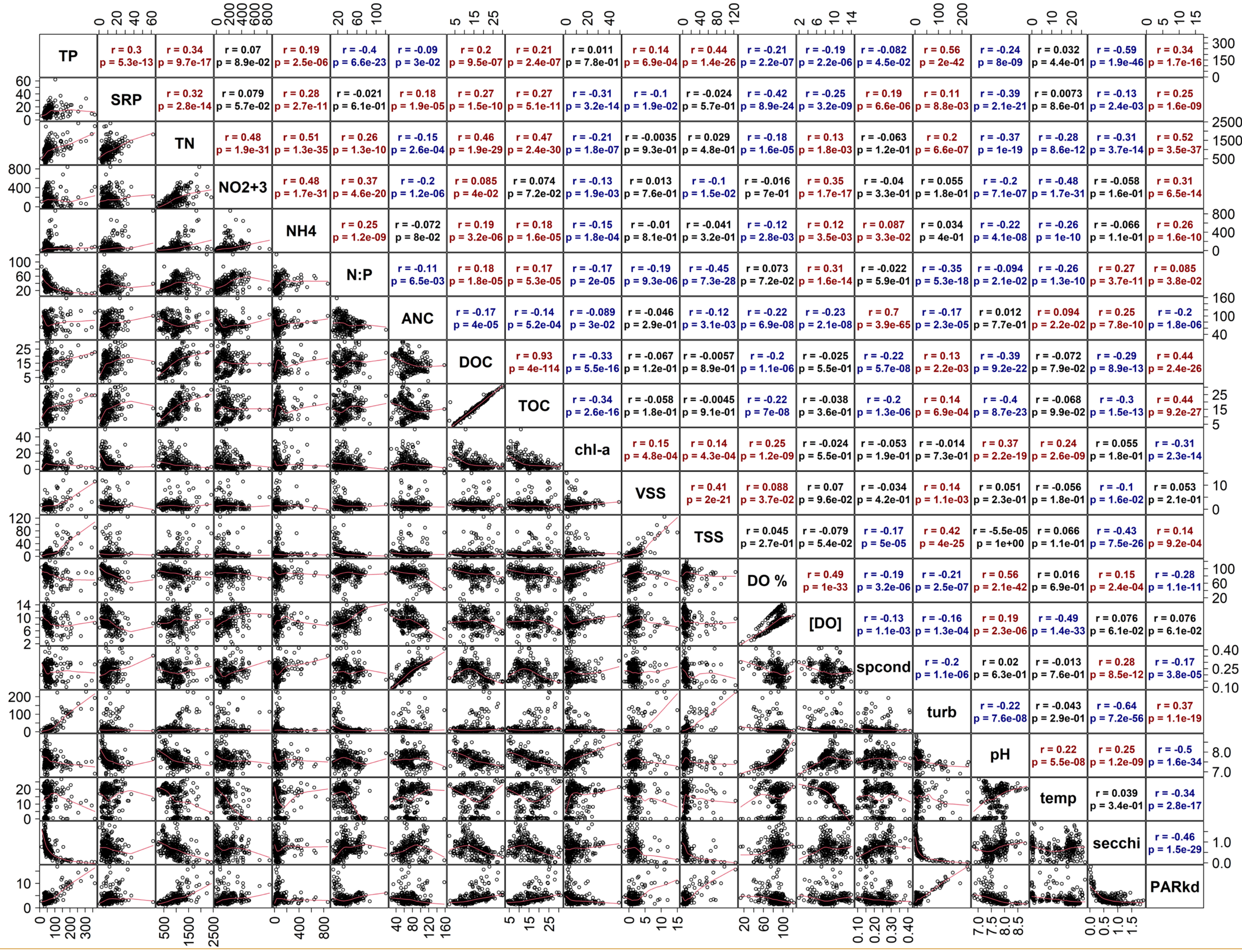
Table D. Summary water quality data for the project sample locations, for the sample period (October 2022 - November 2024), not including winter collections. Empty cells indicate no data collected for that parameter. Square parentheses indicate abbreviations used for variables throughout the report. Astrisks next to an average value indicates a pairwise t-test significance <0.05 between a site and the nearest SWMP station. Oliver Bridge paired with NO, MU, PO, KI, and BI. Blatnik Bridge paired with MI, BA, and AL.

		North Bay (NO)	Mud Lake (MU)	Pokegama Bay (PO)	Kingsbury Bay (KI)	Billings Park (BI)	Miller Creek (MI)	Barker's Island (BA)	Allouez Bay (AL)	Blatnik Bridge	Oliver Bridge
Temperature (°C) [temp]	Min	3.921	4.375	4.119	4.448	4.583	5.791	3.970	5.026	5.538	7.870
	Ave	17.451*	16.752	16.574*	17.357	17.129	17.076	16.226	16.235	15.563	17.906
	Max	25.356	25.231	26.784	25.338	25.569	24.248	25.742	24.981	23.883	24.760
Dissolved oxygen (mg/L) [DO]	Min	4.570	5.785	2.782	5.130	5.056	4.700	5.440	5.502	5.997	7.214
	Ave	8.439*	8.715	6.142*	8.650	8.407*	8.793	9.055	8.921	9.019	8.818
	Max	12.884	12.528	11.436	12.058	11.852	12.025	12.806	12.087	11.487	11.320
pH	Min	7.005	7.124	6.992	7.252	7.004	7.225	7.234	7.245	7.233	7.383
	Ave	7.715*	7.695*	7.320*	7.724	7.678*	7.693	7.779	7.736	7.643	7.724
	Max	8.494	8.084	7.545	8.297	8.101	8.522	8.780	8.579	8.076	8.099
Specific conductivity (mS/cm) [spcond]	Min	0.097	0.112	0.117	0.099	0.100	0.161	0.142	0.103	0.146	0.129
	Ave	0.216	0.221	0.241*	0.206	0.200	0.281*	0.198	0.157*	0.198	0.223
	Max	0.301	0.307	0.333	0.317	0.290	0.414	0.291	0.210	0.288	0.334
Salinity (PSU)	Min	0.050	0.050	0.060	0.050	0.049	0.076	0.070	0.050	0.070	0.060
	Ave	0.103	0.105	0.115	0.098	0.095	0.134	0.094	0.074	0.094	0.105
	Max	0.140	0.150	0.160	0.150	0.140	0.199	0.140	0.100	0.140	0.160
Secchi depth (m) [secchi]	Min	0.320	0.280	0.000	0.215	0.180	0.335	0.350	0.055		
	Ave	0.865	0.868	0.202	0.662	0.718	0.741	0.718	0.336		
	Max	1.565	1.805	0.600	1.200	1.330	1.085	1.295	0.765		
Chlorophyll-a (mg/L) [chl-a]	Min	1.3	0.9	1.1	1.1	1.2	1.0	2.8	2.5	1.1	1.3
	Ave	6.7*	4.4	5.7*	5.1	5.6	13.0	12.0	14.2	7.6	3.9
	Max	16.8	11.9	20.5	18.2	17.1	49.2	34.7	30.5	50.5	9.8
Total phosphorus (ppb) [TP]	Min	24.0	29.0	65.0	29.7	28.0	37.0	13.0	38.0	29.0	18.9
	Ave	46.3	44.9	168.1*	54.3	49.5	66.5*	50.6	83.8*	49.6	44.8
	Max	80.0	100.7	366.6	119.0	107.2	112.0	88.0	188.1	107.3	154.1
Soluble reactive phosphorus (ppb) [SRP]	Min	2.9	1.0	3.8	4.0	1.8	3.0	2.9	1.8	4.0	5.3
	Ave	10.5	10.7	18.0*	10.6	10.3	18.4	10.4	7.2	12.4	9.9
	Max	25.0	23.0	37.0	28.0	22.0	62.0	28.0	20.0	22.0	17.0
Total nitrogen (ppb) [TP]	Min	284.0	286.0	371.0	268.0	270.0	411.0	328.0	279.0	647.0	394.0
	Ave	762.1	758.1	1190.4*	797.9	793.4	1217.9*	896.3	755.6*	976.2	807.1
	Max	1246.0	1238.0	2424.0	1254.0	1302.0	1956.0	1304.0	1259.2	1304.0	1207.0

		North Bay (NO)	Mud Lake (MU)	Pokegama Bay (PO)	Kingsbury Bay (KI)	Billings Park (BI)	Miller Creek (MI)	Barker's Island (BA)	Allouez Bay (AL)	Blatnik Bridge	Oliver Bridge
Ammonium (ppb) [NH_4]	Min	3.0	7.0	4.0	4.0	3.0	43.0	9.0	5.0	22.0	4.0
	Ave	27.9*	32.6	42.5	36.1*	31.0*	341.4*	68.6	27.5*	109.4	24.5
	Max	77.9	79.7	189.0	77.0	71.0	867.0	200.9	71.6	325.0	43.0
Nitrate + nitrite (ppb) [NO2+NO3]	Min	0.0	0.0	0.0	0.0	0.0	122.0	58.0	3.0	124.1	1.0
	Ave	46.7*	73.8	71.8*	76.6	79.0	195.9*	243.7	123.8*	220.4	78.2
	Max	159.8	204.8	492.1	193.8	190.6	450.0	517.0	276.0	367.0	197.0
TN:TP ratio [N:P]	Min	7.7	8.2	3.8	6.2	7.9	9.5	8.2	4.4	10.5	7.8
	Ave	16.8*	17.1*	7.9*	15.5*	16.2*	18.7	19.0	9.7*	21.0	21.4
	Max	24.7	26.3	17.0	29.2	26.0	30.4	39.3	19.0	33.4	32.2
Acid neutralizing capacity (mg/L CaCO3) [ANC]	Min	30.6	38.1	47.0	31.1	32.8	36.5	29.7	28.1		
	Ave	80.1	79.7	91.7	71.3	70.5	77.1	60.1	54.8		
	Max	114.1	117.3	124.8	116.8	108.1	116.6	92.6	73.5		
Dissolved organic carbon (ppm) [DOC]	Min	9.1	9.1	2.6	8.9	8.9	6.4	2.1	4.1		
	Ave	16.8	16.4	19.0	17.2	16.6	14.6	12.1	10.3		
	Max	30.0	29.0	27.7	30.0	30.0	28.0	26.0	18.8		
Total organic carbon (ppm) [TOC]	Min	9.2	9.5	14.1	9.1	9.2	7.1	5.2	4.2		
	Ave	17.3	16.8	20.3	17.5	17.1	15.0	12.7	10.4		
	Max	31.0	30.0	28.3	30.0	30.0	29.0	27.0	19.4		
Particulate carbon (ppm)	Min	440.3	406.4	868.3	528.7	392.2	536.8	164.4	249.1		
	Ave	938.2	1178.2	4025.3	1110.2	1067.8	1279.2	966.9	845.3		
	Max	1851.6	1762.3	9998.5	1733.9	2061.6	2147.8	1863.6	1669.3		
Turbidity (FNU)	Min	2.6	1.4	11.5	4.0	4.1	4.1	4.1	15.2	4.2	2.2
	Ave	6.5	7.9	100.4*	10.5	12.2	7.5	10.7	43.0*	10.0	10.4
	Max	19.9	26.0	494.5	34.8	49.8	13.6	26.4	130.5	32.7	39.9
Total suspended solids [TSS]	Min	0.7	-0.7	5.0	0.5	2.0	2.0	2.5	6.0	1.5	1.0
	Ave	5.8	8.4	45.7	11.5	5.9	8.3	6.5	19.1*	6.3	14.1
	Max	20.6	44.5	483.0	66.0	16.7	72.5	11.7	77.3	24.0	86.5
Photosynthetically active radiation at water surface [PARkd]	Min	0.6	1.4	2.2	1.5	1.5	1.6	1.3	1.6		
	Ave	2.9	3.0	8.4	3.2	3.2	3.0	2.9	4.6		
	Max	5.1	5.2	19.2	6.3	6.1	6.3	4.9	12.4		

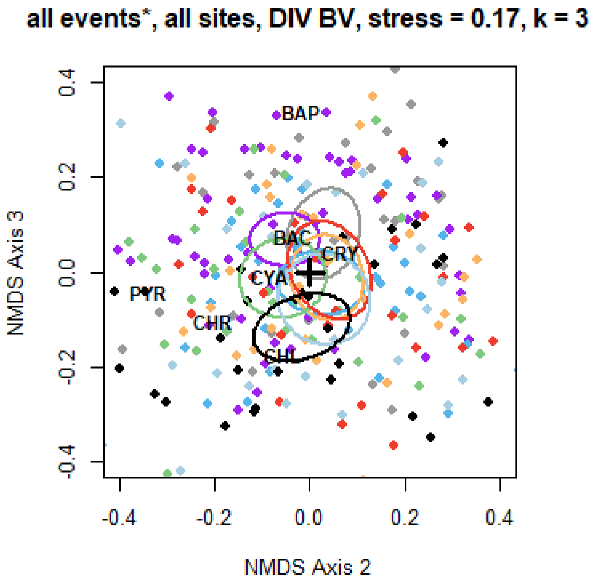
APPENDIX E: CORRELATION MATRIX

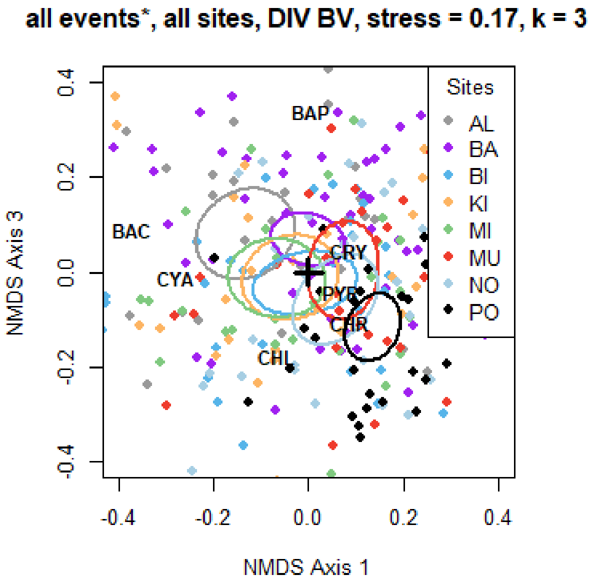
A Kendall rank correlation matrix using observations from main eight hotspot sampling sites is presented below. Scatterplots are in the lower triangle and annotated correlation coefficients with p-values are in the upper triangle. Statistically significant correlations ($p < 0.05$) were color-coded: positive correlations in dark red and negative correlations in navy blue. Non-significant correlations are black.

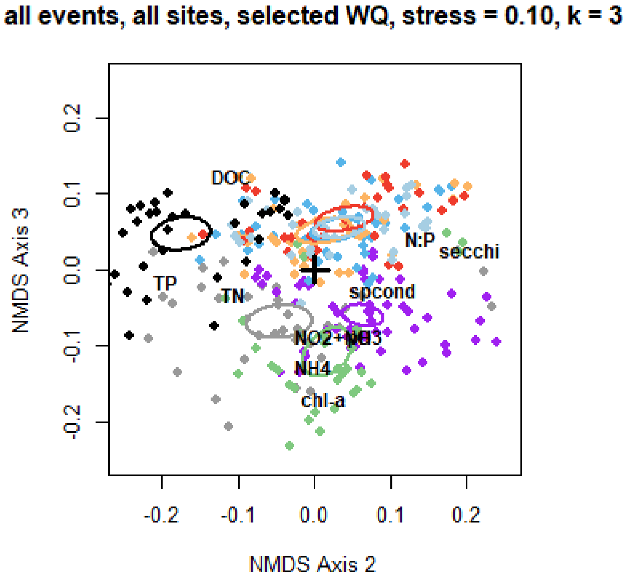


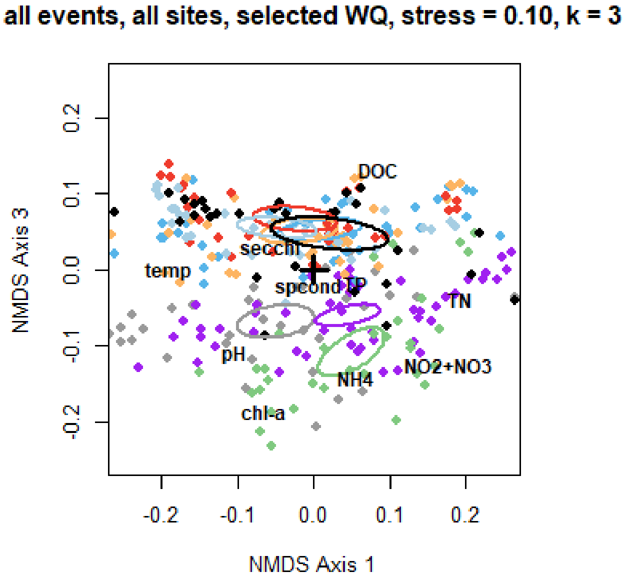
APPENDIX F: SPATIAL REDUNDANCY ANALYSIS

Visualizations of axis 3 for NMDS analysis that was used to assess spatial redundancy in water quality and phytoplankton communities (**Figure 21**).

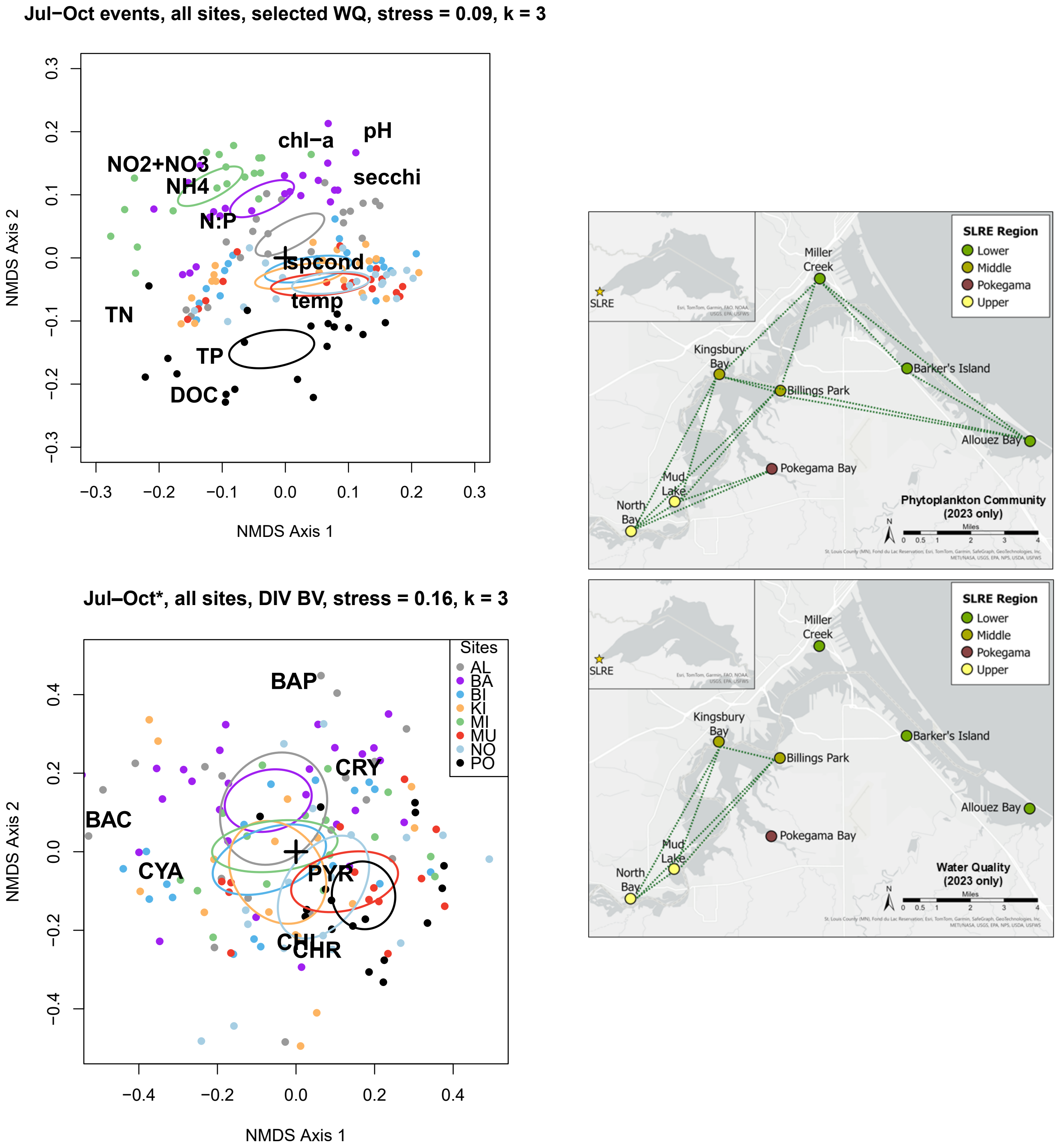

all events*, all sites, DIV BV, stress = 0.17, k = 3


all events*, all sites, DIV BV, stress = 0.17, k = 3


all events, all sites, selected WQ, stress = 0.10, k = 3

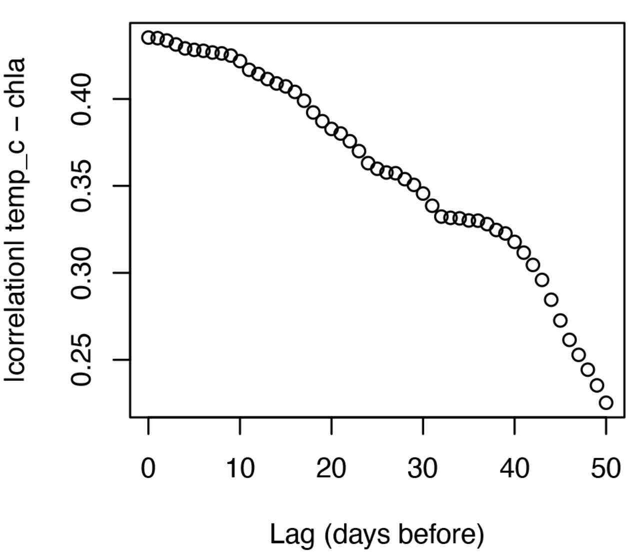

all events, all sites, selected WQ, stress = 0.10, k = 3

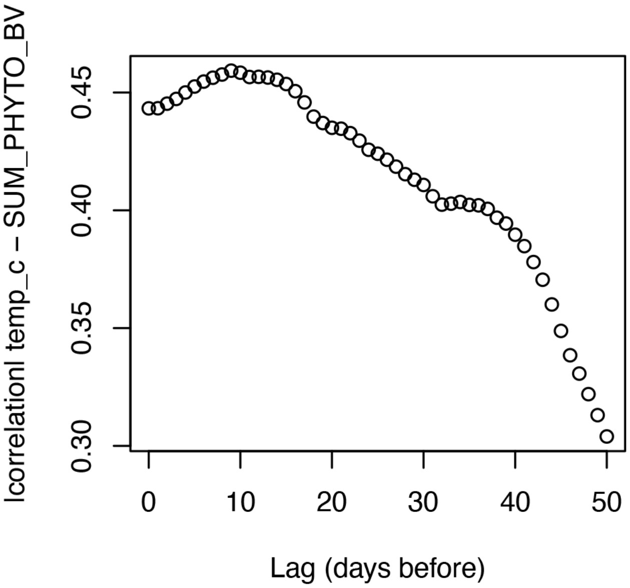
Similarities among sites based on NMDS and PERMANOVA of summer (July - October 2023) water quality (WQ) and phytoplankton biovolume (DIV BV). Connections by dashed line indicate no significant difference (F-test, p > 0.05) in pairwise site comparisons of NMDS sample scores.

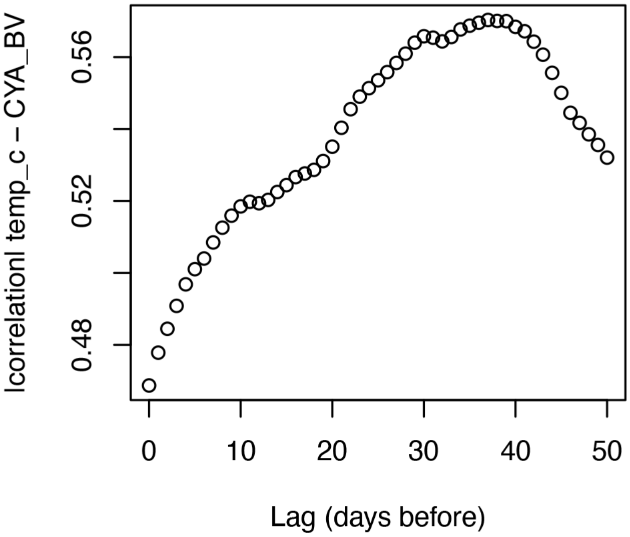


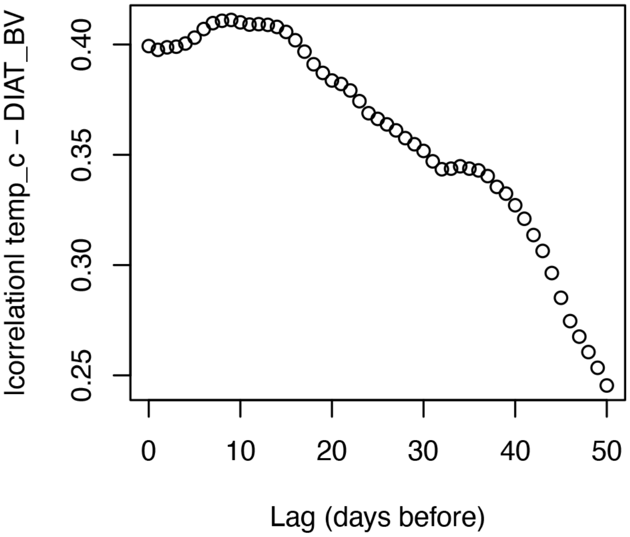
APPENDIX G: LAG EFFECTS

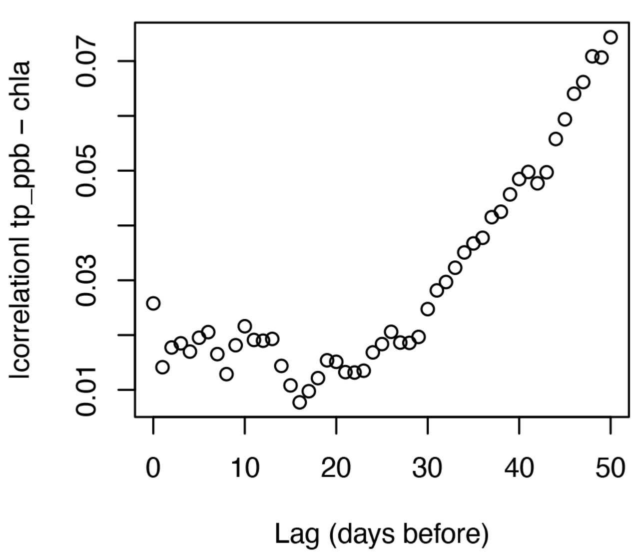
Lag analysis of the relationships between environmental variables and cyanobacterial biovolume. Lags in environmental data measurements from zero to 50 days prior to cyanobacterial measurements were explored using the absolute value of the correlation coefficient between the variable and biovolume.

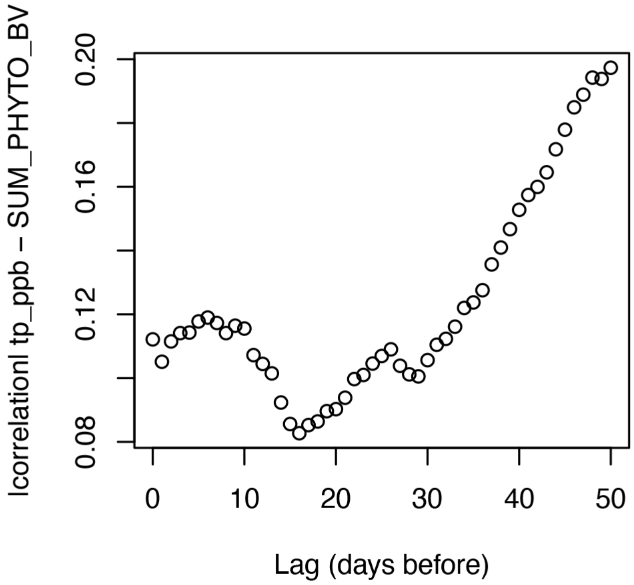


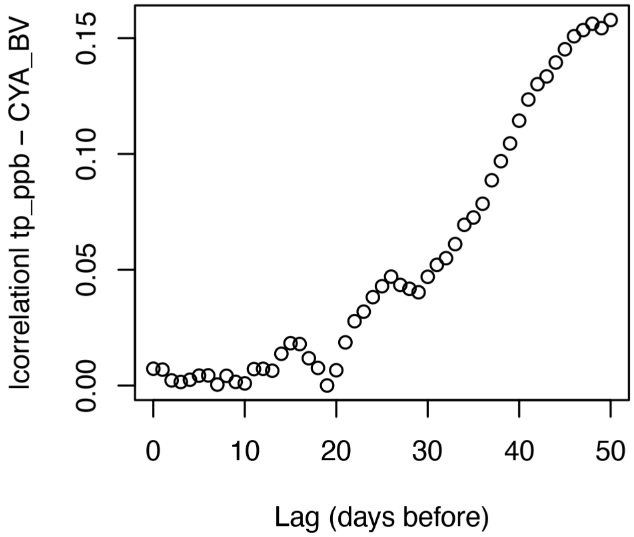


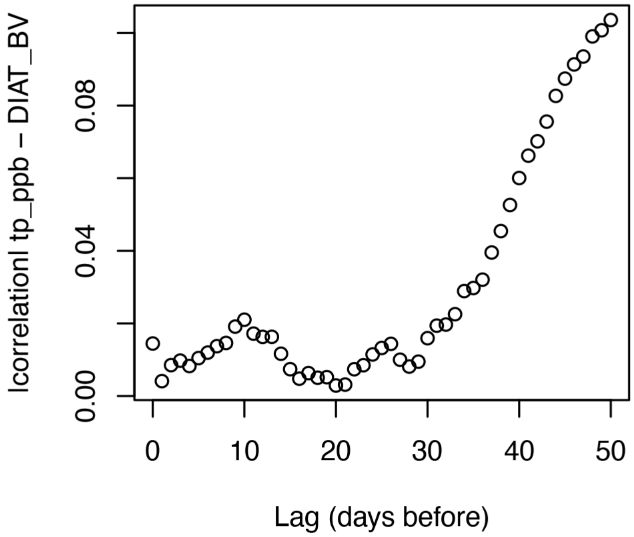


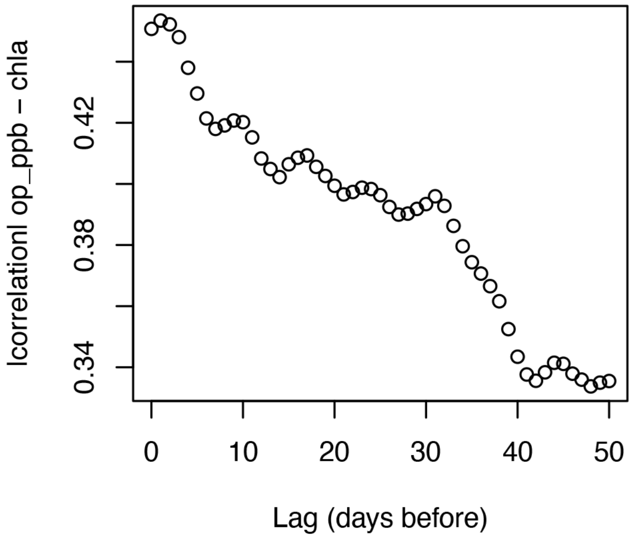



|correlation| tp_ppb – chla
0.01
0.03
0.05
0.07
0
10
20
30
40
50
Lag (days before)

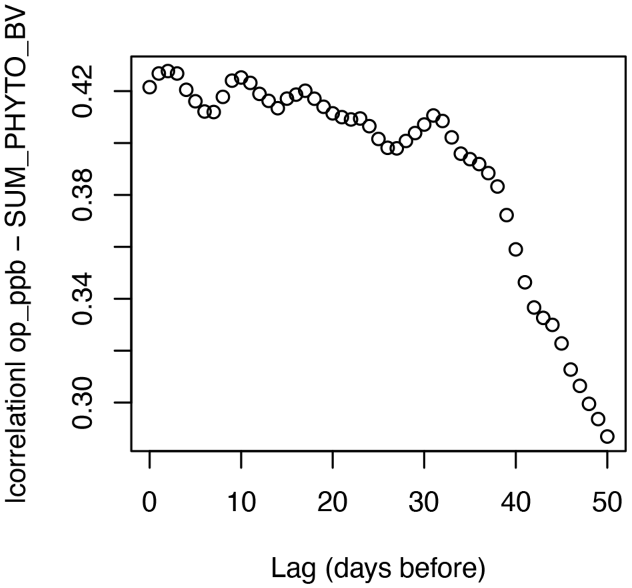

|correlation| tp_ppb – SUM_PHYTO_BV
0.08
0.12
0.16
0.20
0
10
20
30
40
50
Lag (days before)


|correlation| tp_ppb – CYA_BV
0.00
0.05
0.10
0.15
0
10
20
30
40
50
Lag (days before)

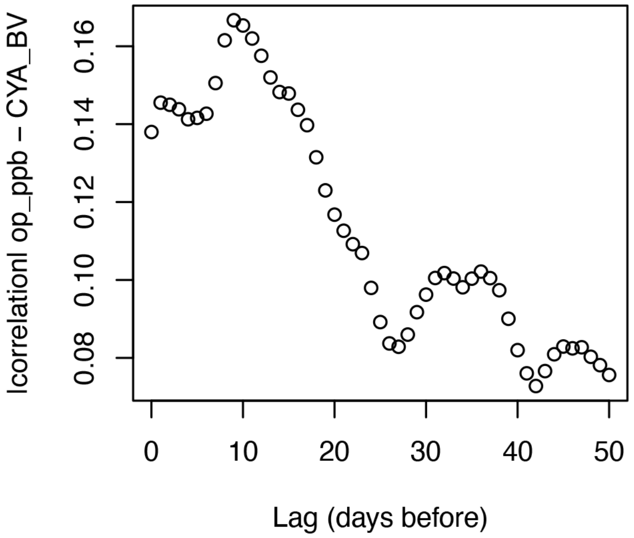

|correlation| tp_ppb – DIAT_BV
0.00
0.04
0.08
0
10
20
30
40
50
Lag (days before)



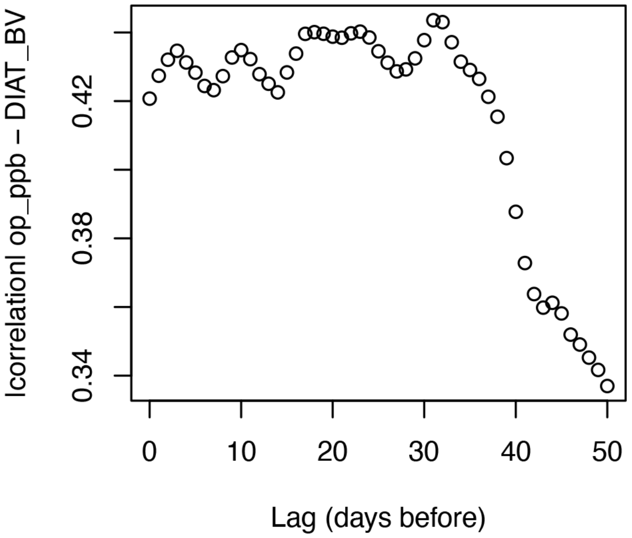
|correlation| op_ppb – chla
0.34
0.38
0.42
0
10
20
30
40
50
Lag (days before)



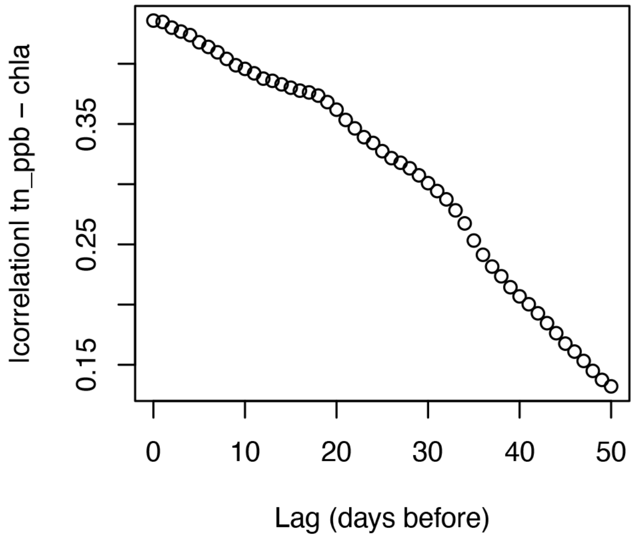
|correlation| op_ppb – SUM_PHYTO_BV
0.30
0.34
0.38
0.42
0
10
20
30
40
50
Lag (days before)

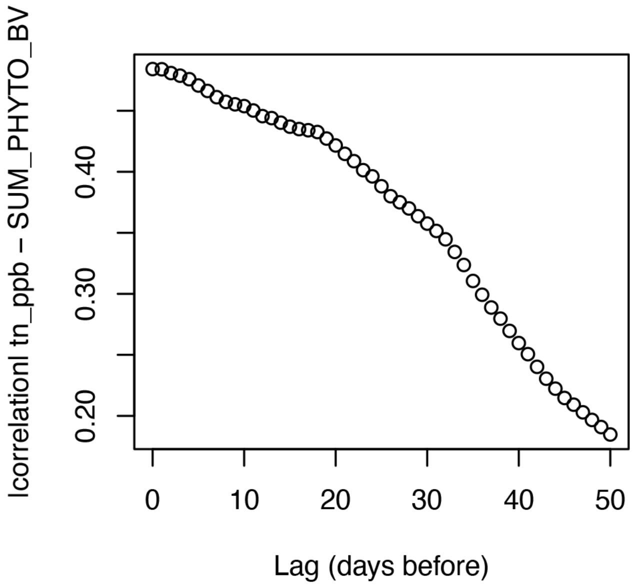


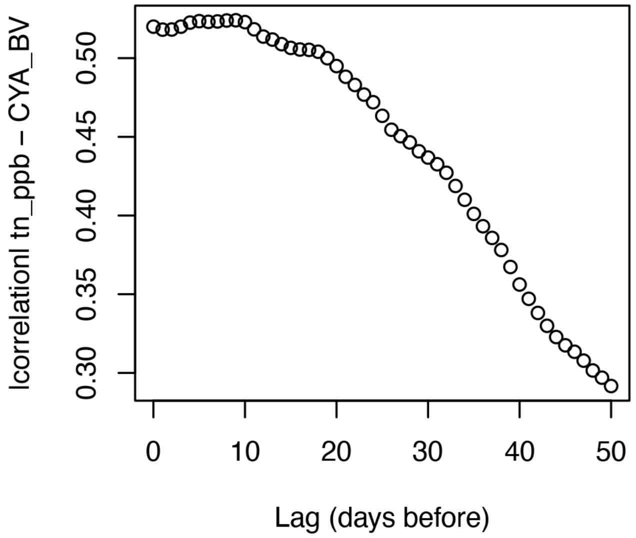
|correlation| op_ppb – CYA_BV
0.08
0.10
0.12
0.14
0.16
0
10
20
30
40
50
Lag (days before)

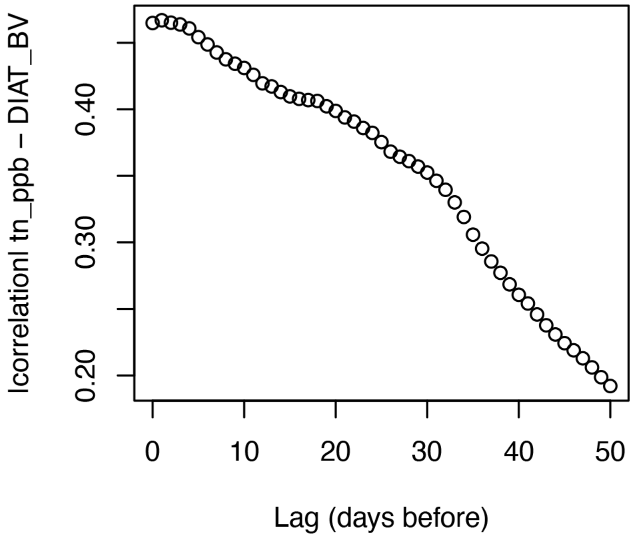


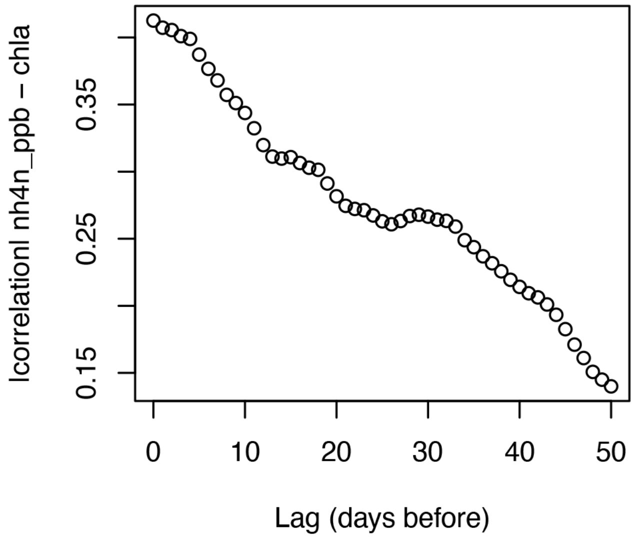
|correlation| op_ppb – DIAT_BV
0.34
0.38
0.42
0
10
20
30
40
50
Lag (days before)


|correlation| tn_ppb – chla
0.15
0.25
0.35
0
10
20
30
40
50
Lag (days before)

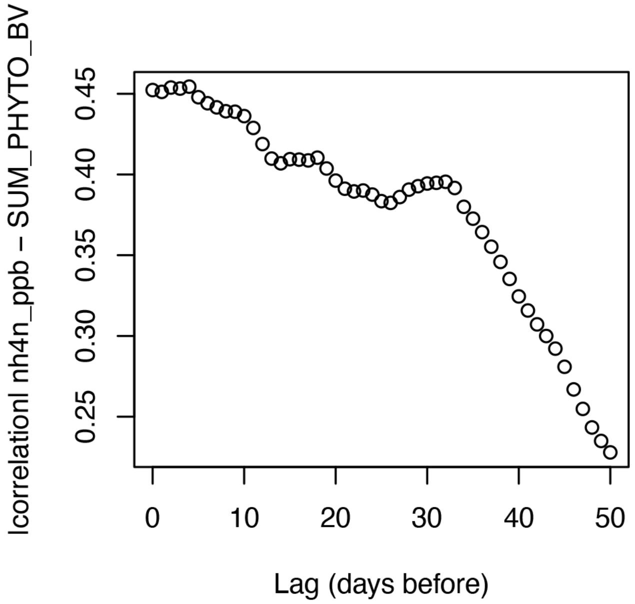

|correlation| tn_ppb – SUM_PHYTO_BV
0.20
0.30
0.40
0
10
20
30
40
50
Lag (days before)


|correlation| tn_ppb – CYA_BV
0.30
0.35
0.40
0.45
0.50
0
10
20
30
40
50
Lag (days before)

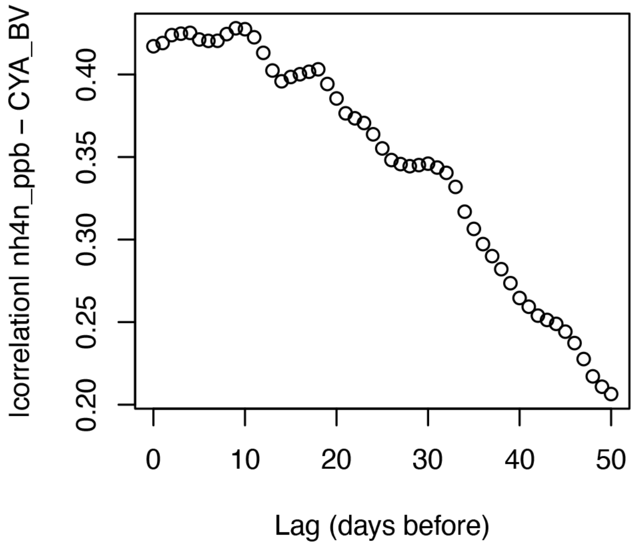

|correlation| tn_ppb – DIAT_BV
0.20
0.30
0.40
0
10
20
30
40
50
Lag (days before)



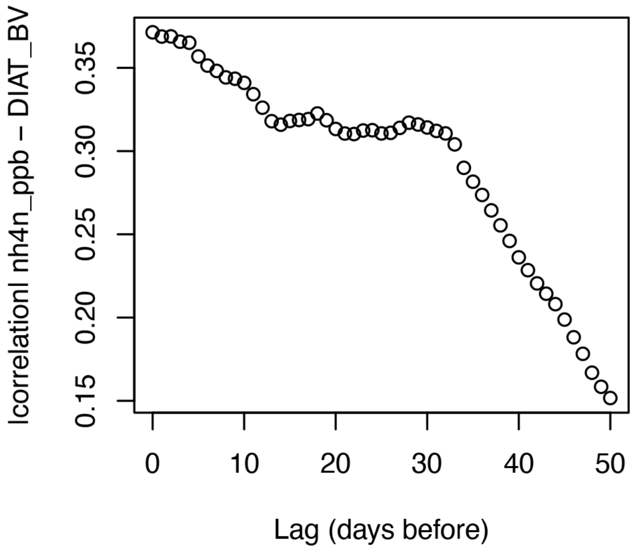
|correlation| nh4n_ppb – chla
0.15
0.25
0.35
0
10
20
30
40
50
Lag (days before)



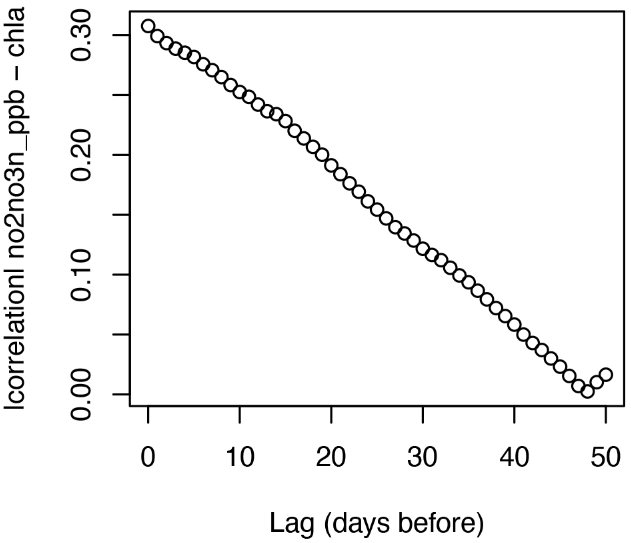
|correlation| nh4n_ppb – SUM_PHYTO_BV
0.25
0.30
0.35
0.40
0.45
0
10
20
30
40
50
Lag (days before)

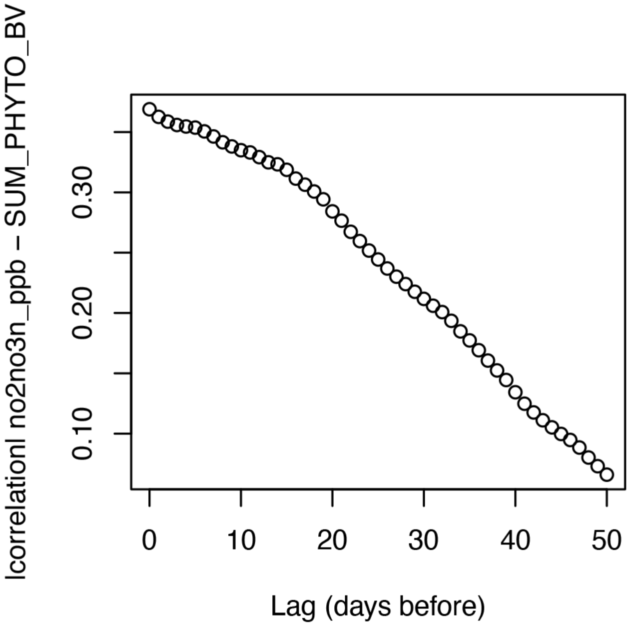


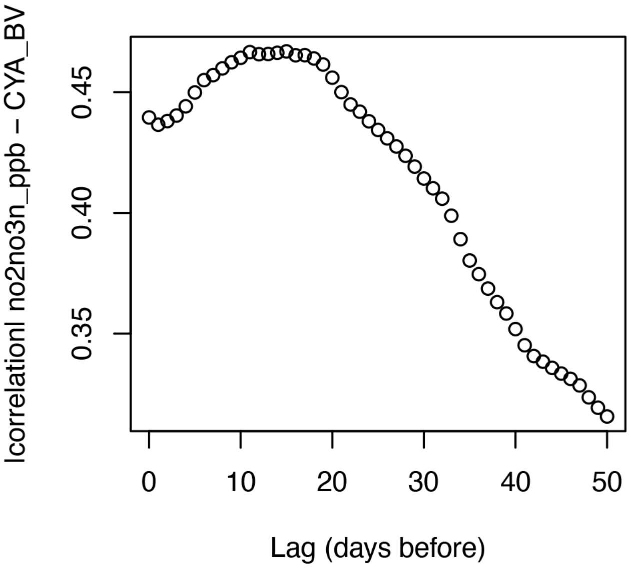
|correlation| nh4n_ppb – CYA_BV
0.20
0.25
0.30
0.35
0.40
0
10
20
30
40
50
Lag (days before)

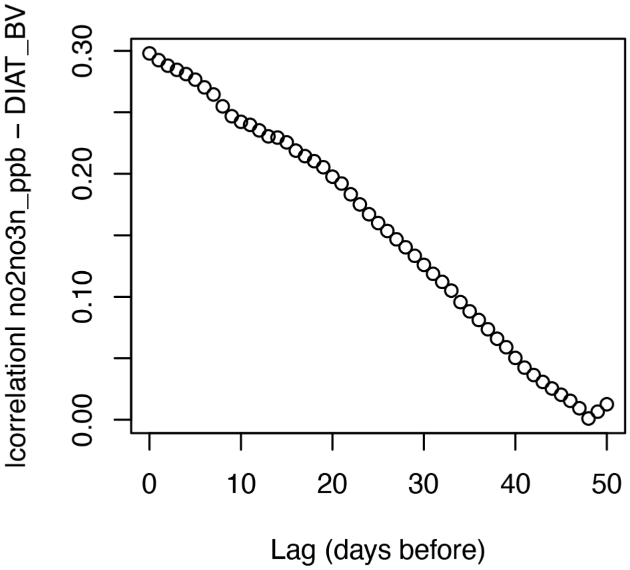


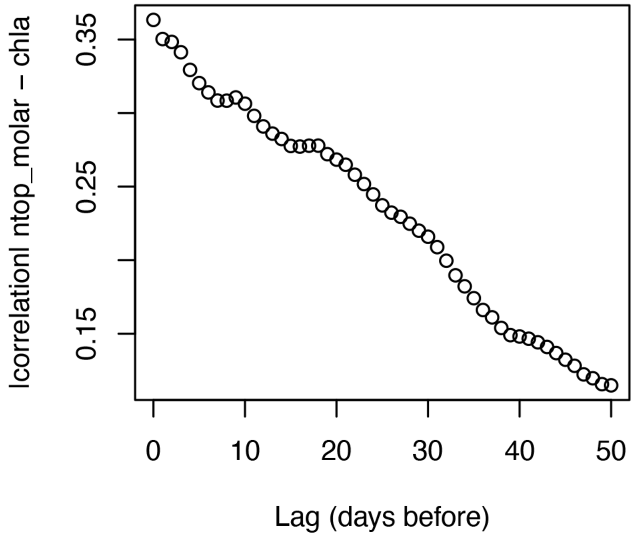
|correlation| nh4n_ppb – DIAT_BV
0.15
0.20
0.25
0.30
0.35
0
10
20
30
40
50
Lag (days before)

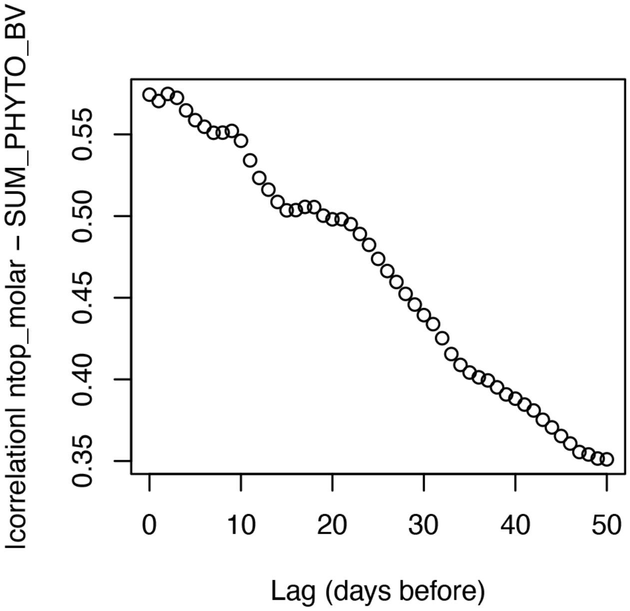

|correlation| no2no3n_ppb – chla
0.00
0.10
0.20
0.30
0
10
20
30
40
50
Lag (days before)

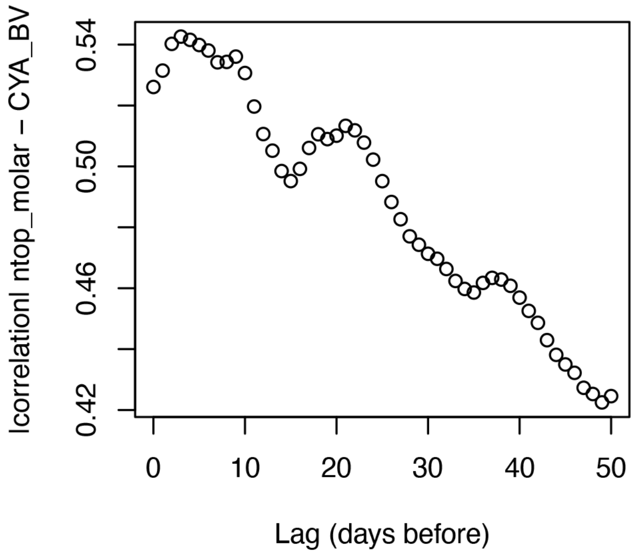

|correlation| no2no3n_ppb – SUM_PHYTO_BV
0.10
0.20
0.30
0
10
20
30
40
50
Lag (days before)

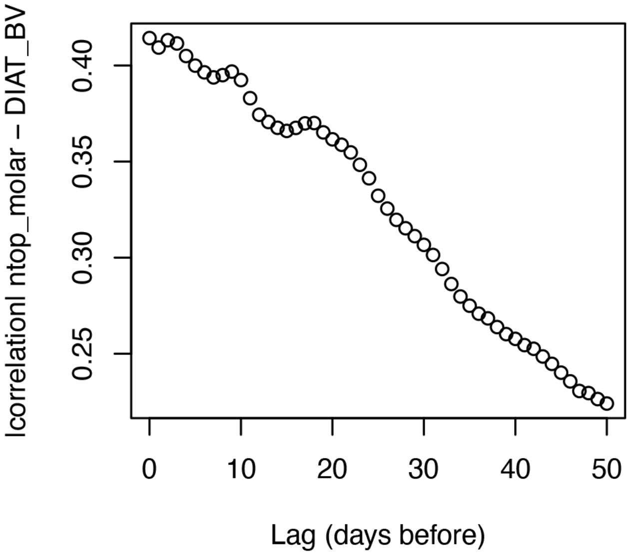

|correlation| no2no3n_ppb – CYA_BV
0.35
0.40
0.45
0
10
20
30
40
50
Lag (days before)

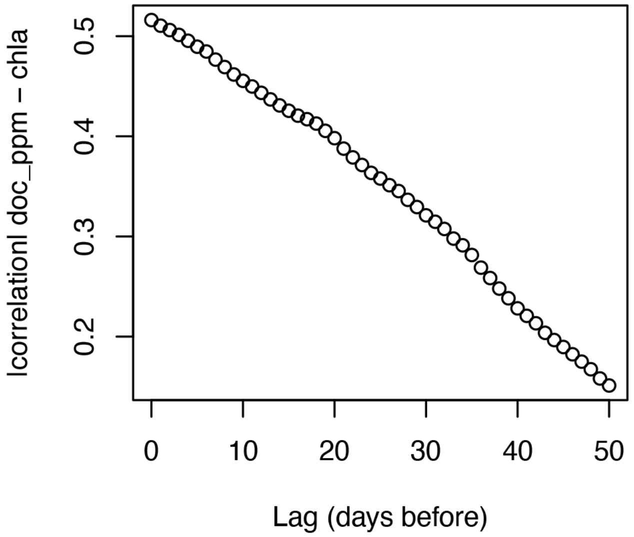

|correlation| no2no3n_ppb – DIAT_BV
0.00
0.10
0.20
0.30
0
10
20
30
40
50
Lag (days before)

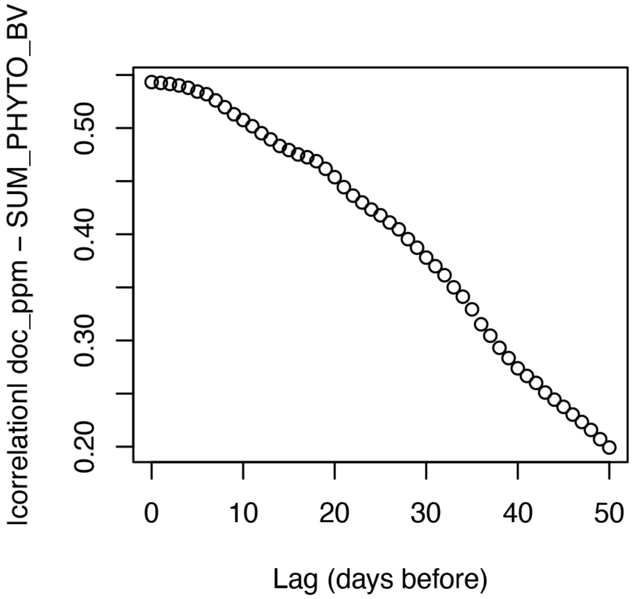

|correlation| ntop_molar – chla
0.15
0.25
0.35
0
10
20
30
40
50
Lag (days before)

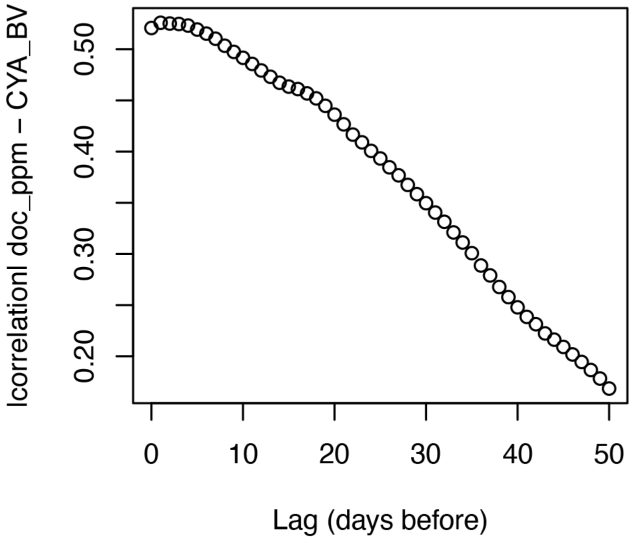

|correlation| ntop_molar – SUM_PHYTO_BV
0.35
0.40
0.45
0.50
0.55
0
10
20
30
40
50
Lag (days before)

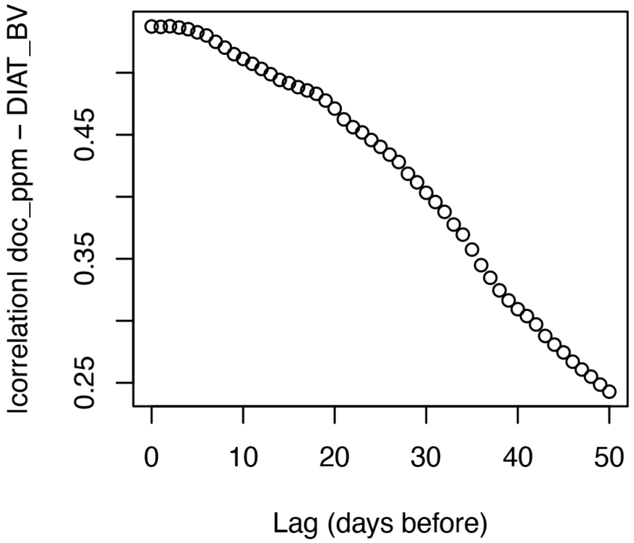

|correlation| ntop_molar – CYA_BV
0.42
0.46
0.50
0.54
0
10
20
30
40
50
Lag (days before)

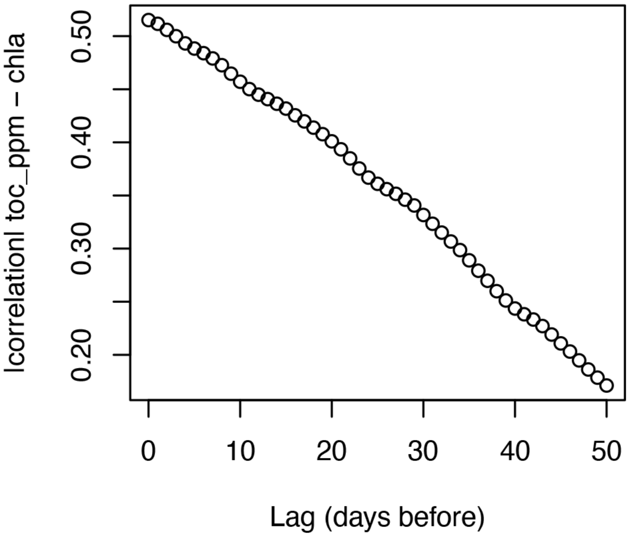

|correlation| ntop_molar – DIAT_BV
0.25
0.30
0.35
0.40
0
10
20
30
40
50
Lag (days before)

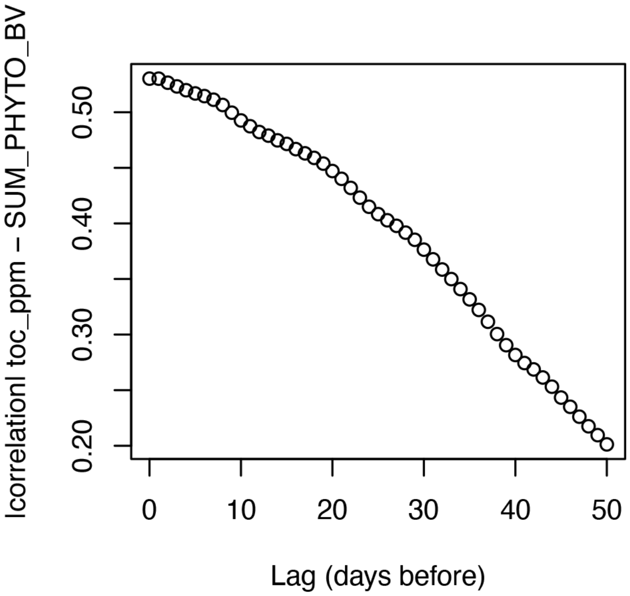

|correlation| doc_ppm – chla
0.5
0.4
0.3
0.2
0
10
20
30
40
50
Lag (days before)

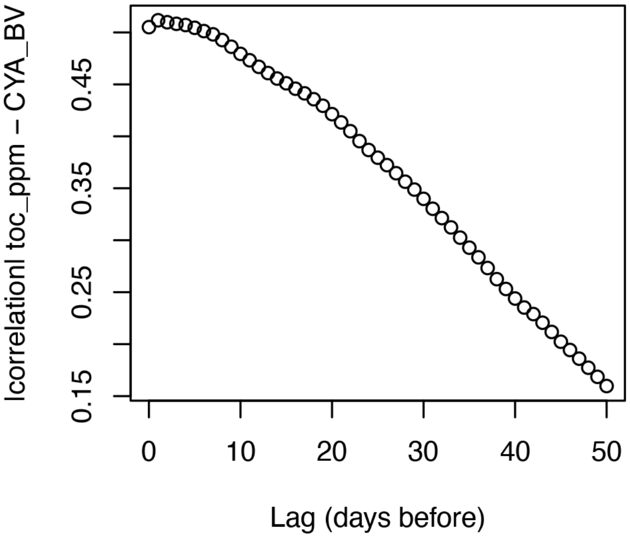

|correlation| doc_ppm – SUM_PHYTO_BV
0.50
0.40
0.30
0.20
0
10
20
30
40
50
Lag (days before)

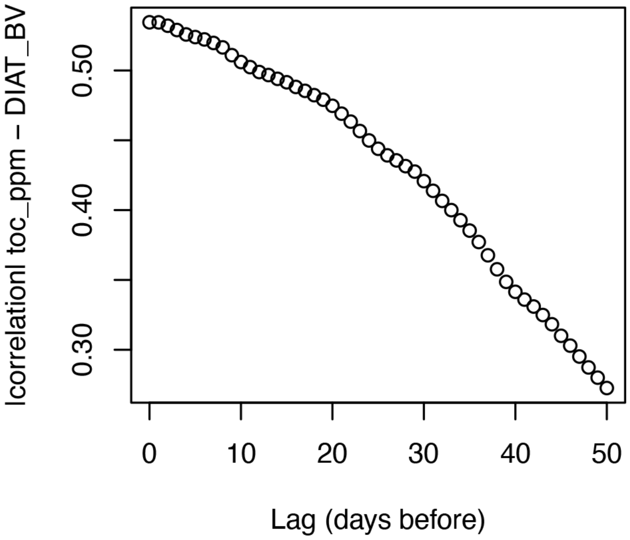

|correlation| doc_ppm – CYA_BV
0.50
0.40
0.30
0.20
0
10
20
30
40
50
Lag (days before)

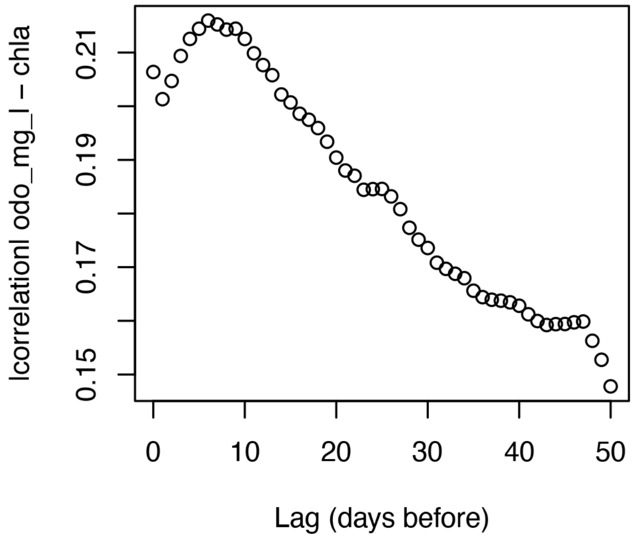

|correlation| doc_ppm – DIAT_BV
0.45
0.35
0.25
0
10
20
30
40
50
Lag (days before)

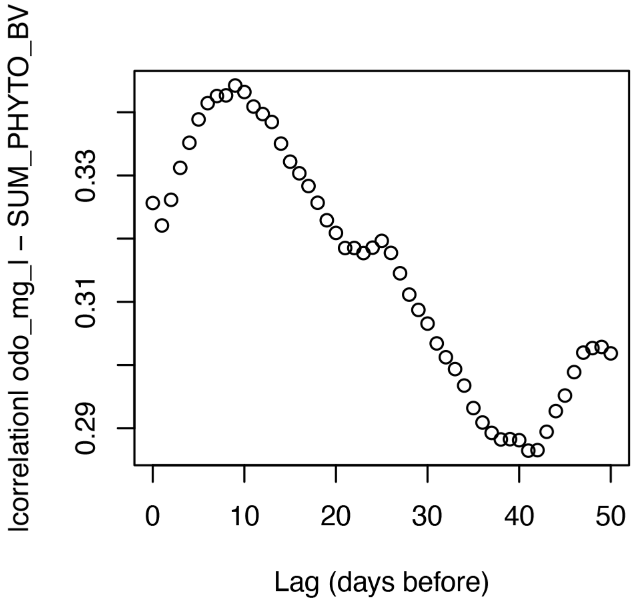

|correlation| toc_ppm – chla
0.20
0.30
0.40
0.50
0
10
20
30
40
50
Lag (days before)

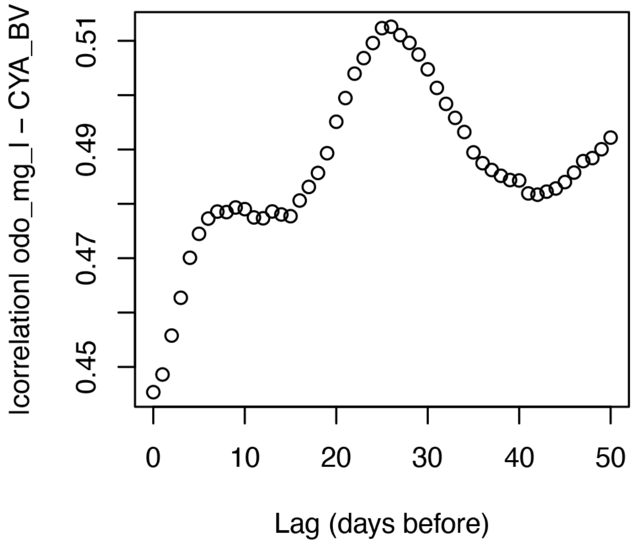

|correlation| toc_ppm – SUM_PHYTO_BV
0.20
0.30
0.40
0.50
0
10
20
30
40
50
Lag (days before)

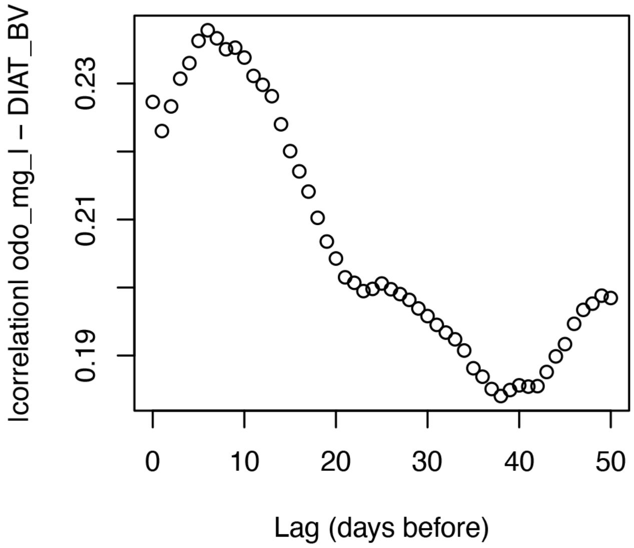

|correlation| toc_ppm – CYA_BV
0.15
0.25
0.35
0.45
0
10
20
30
40
50
Lag (days before)

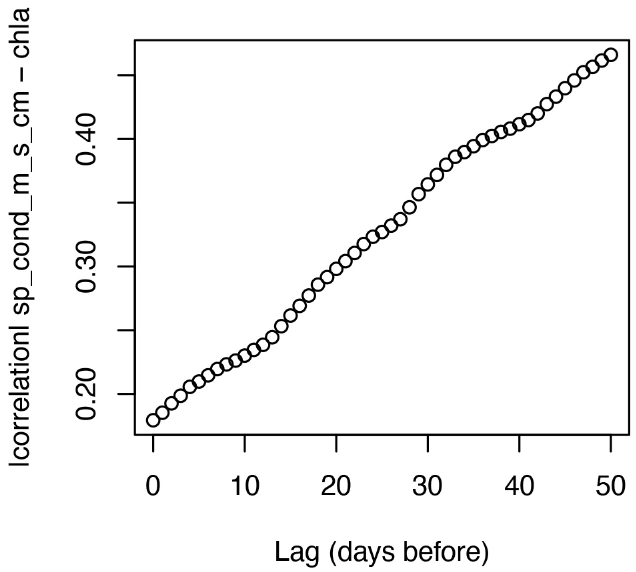

|correlation| toc_ppm – DIAT_BV
0.30
0.40
0.50
0
10
20
30
40
50
Lag (days before)

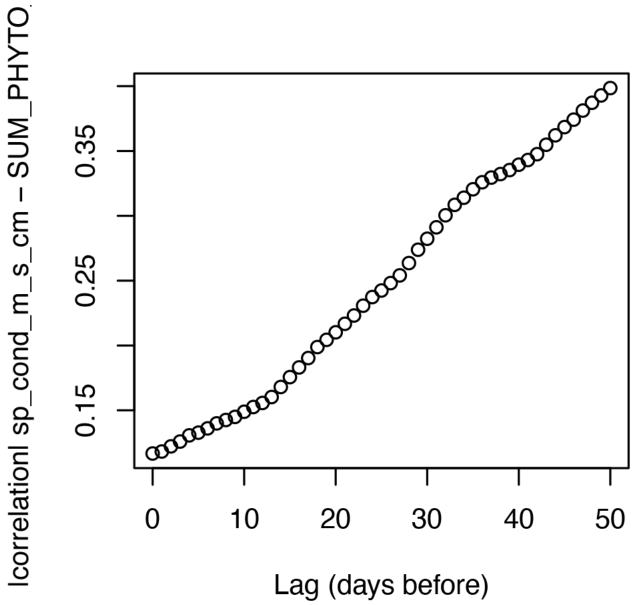

|correlation| odo_mg_l – chla
0.15
0.17
0.19
0.21
0
10
20
30
40
50
Lag (days before)

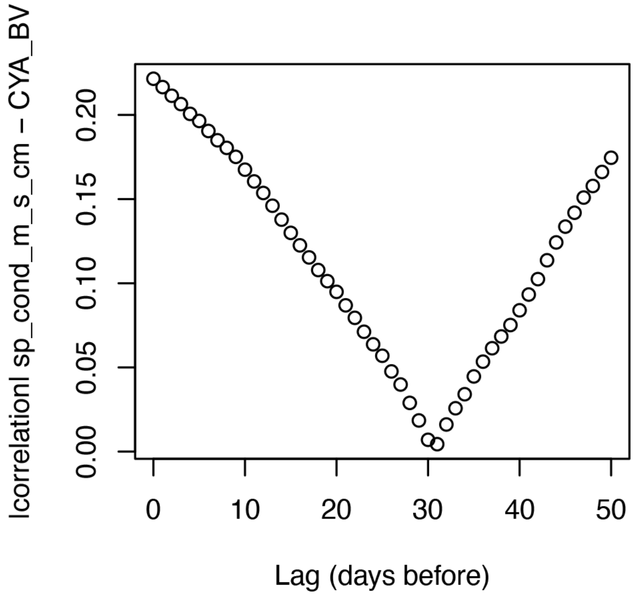

|correlation| odo_mg_l – SUM_PHYTO_BV
0.29
0.31
0.33
0
10
20
30
40
50
Lag (days before)

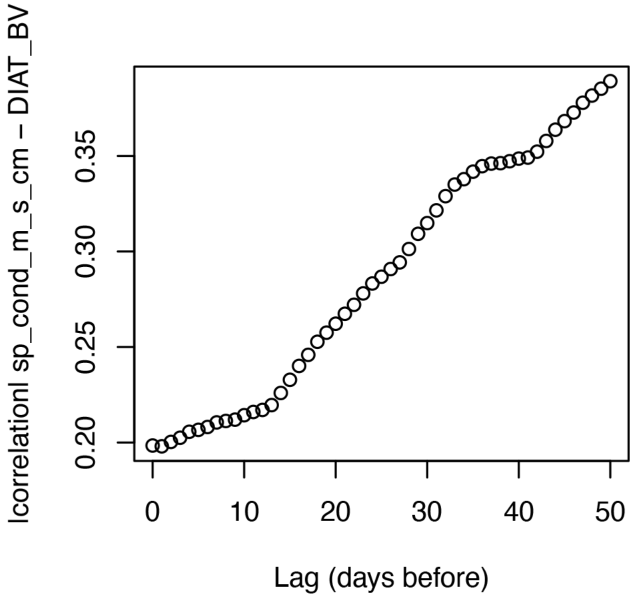

|correlation| odo_mg_l – CYA_BV
0.45
0.47
0.49
0.51
0
10
20
30
40
50
Lag (days before)

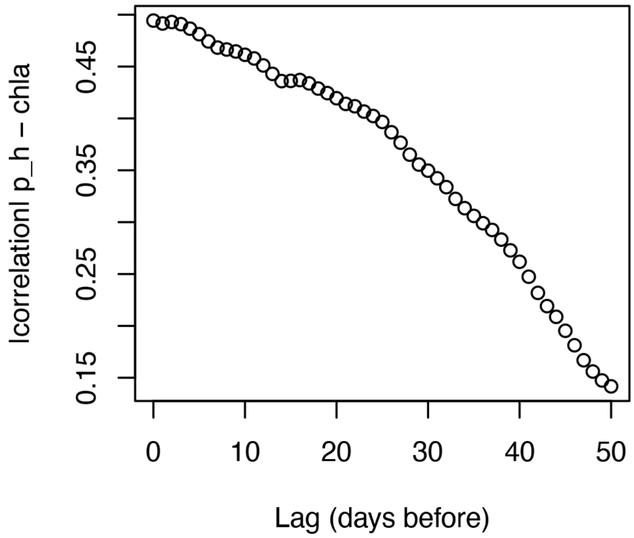

|correlation| odo_mg_l – DIAT_BV
0.19
0.21
0.23
0
10
20
30
40
50
Lag (days before)

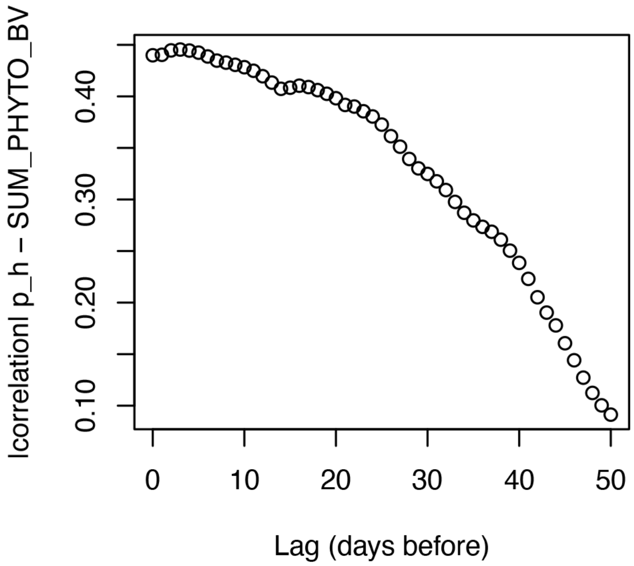

|correlation| sp_cond_m_s_cm – chla
0.20
0.30
0.40
0
10
20
30
40
50
Lag (days before)

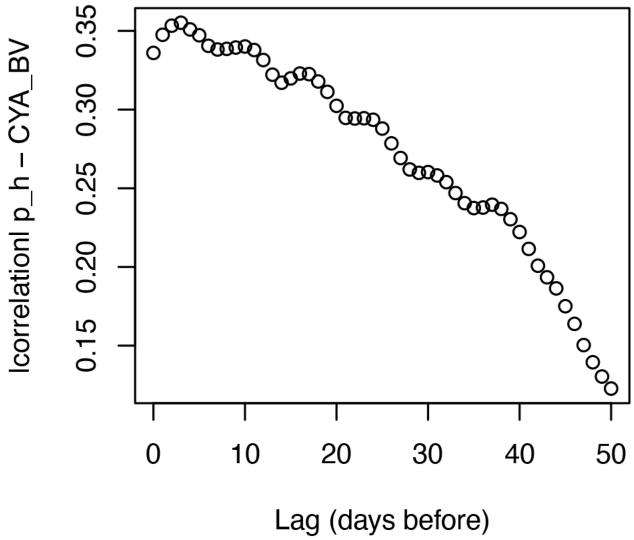

|correlation| sp_cond_m_s_cm – SUM_PHYTO
0.15
0.25
0.35
0
10
20
30
40
50
Lag (days before)

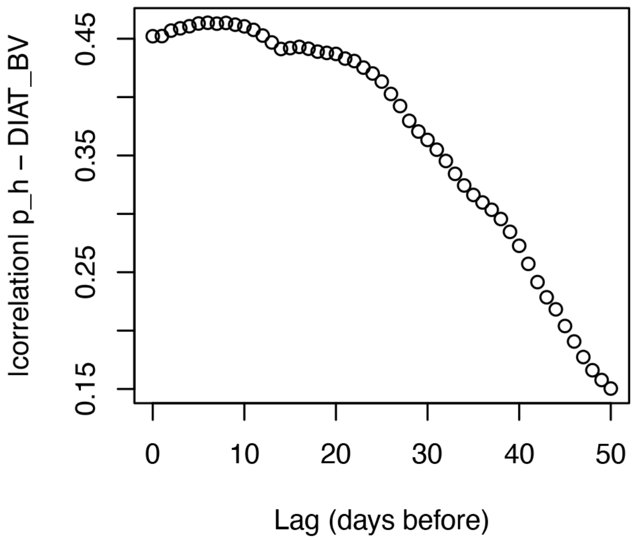

|correlation| sp_cond_m_s_cm – CYA_BV
0.00
0.05
0.10
0.15
0.20
0
10
20
30
40
50
Lag (days before)

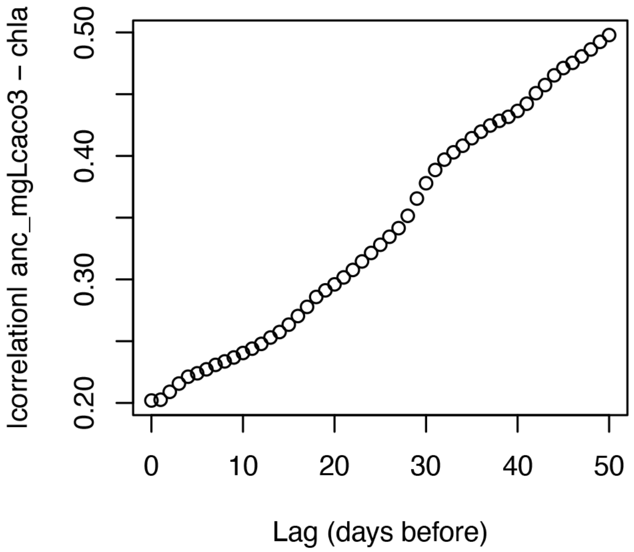

|correlation| sp_cond_m_s_cm – DIAT_BV
0.20
0.25
0.30
0.35
0
10
20
30
40
50
Lag (days before)

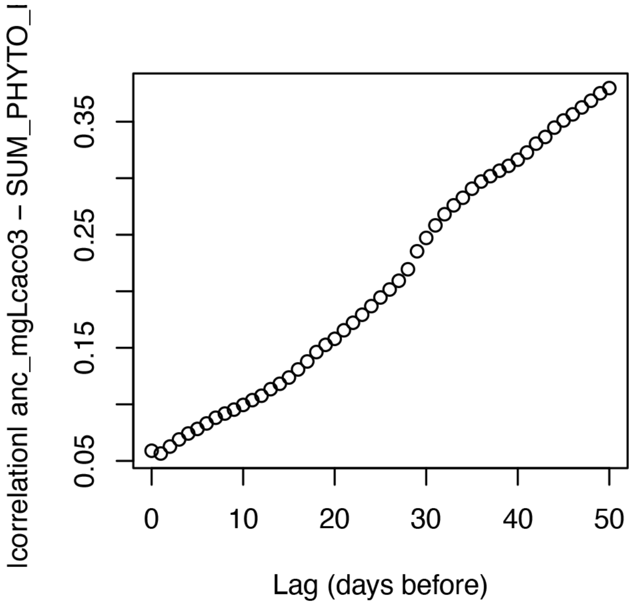

|correlation| p_h – chla
0.15
0.25
0.35
0.45
0
10
20
30
40
50
Lag (days before)

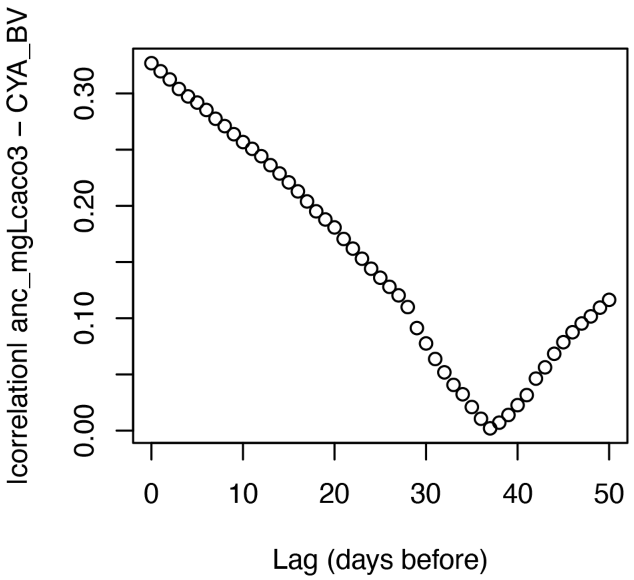

|correlation| p_h – SUM_PHYTO_BV
0.10
0.20
0.30
0.40
0
10
20
30
40
50
Lag (days before)

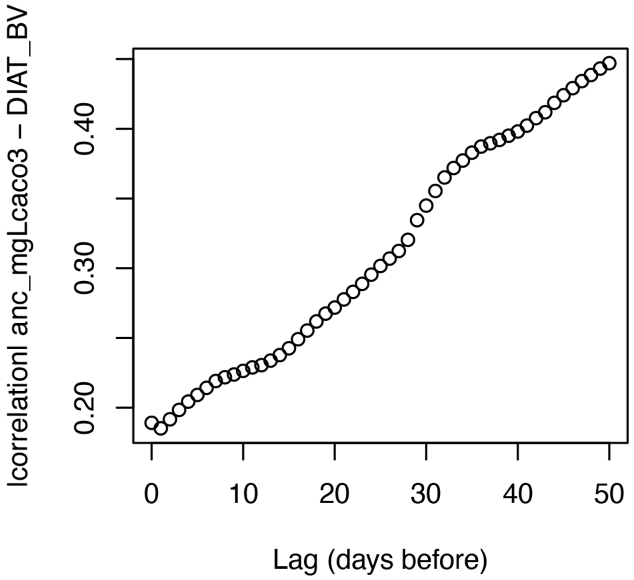

|correlation| p_h – CYA_BV
0.15
0.20
0.25
0.30
0.35
0
10
20
30
40
50
Lag (days before)

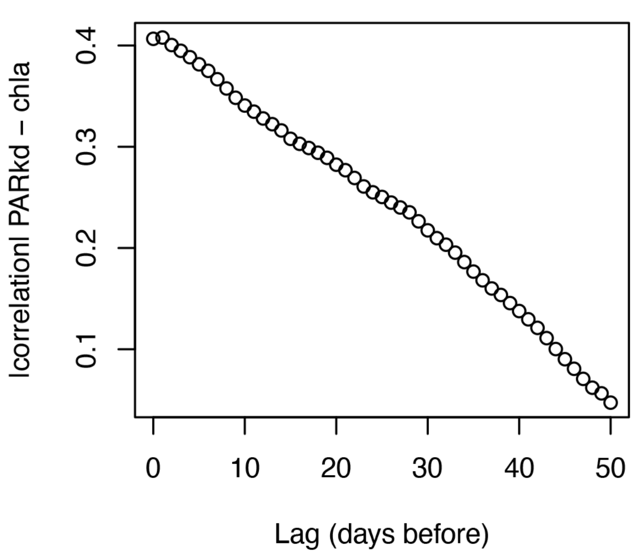

|correlation| p_h – DIAT_BV
0.15
0.25
0.35
0.45
0
10
20
30
40
50
Lag (days before)


|correlation| anc_mgLcaco3 – chla
0.20
0.30
0.40
0.50
0
10
20
30
40
50
Lag (days before)

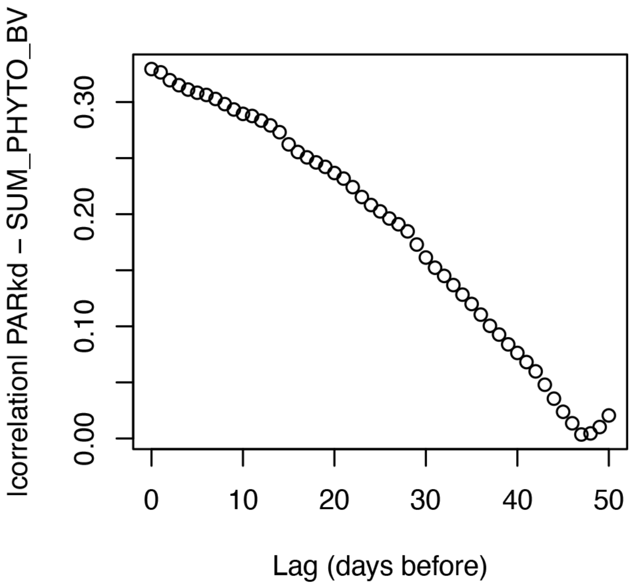

|correlation| anc_mgLcaco3 – SUM_PHYTO_I
0.05
0.15
0.25
0.35
0
10
20
30
40
50
Lag (days before)


|correlation| anc_mgLcaco3 – CYA_BV
0.00
0.10
0.20
0.30
0
10
20
30
40
50
Lag (days before)

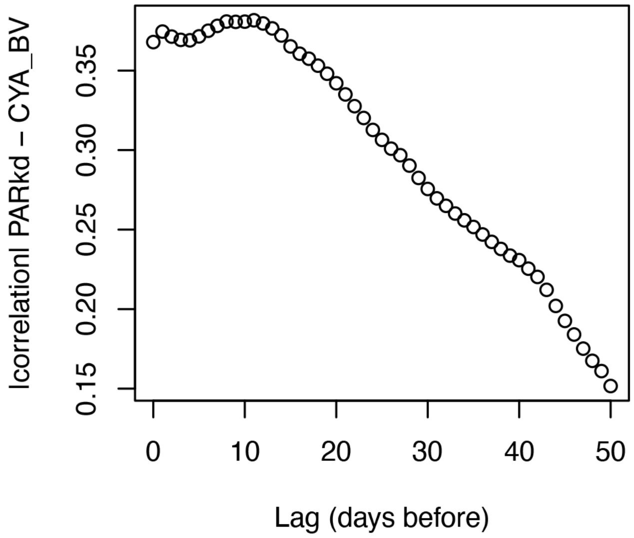

|correlation| anc_mgLcaco3 – DIAT_BV
0.20
0.30
0.40
0
10
20
30
40
50
Lag (days before)



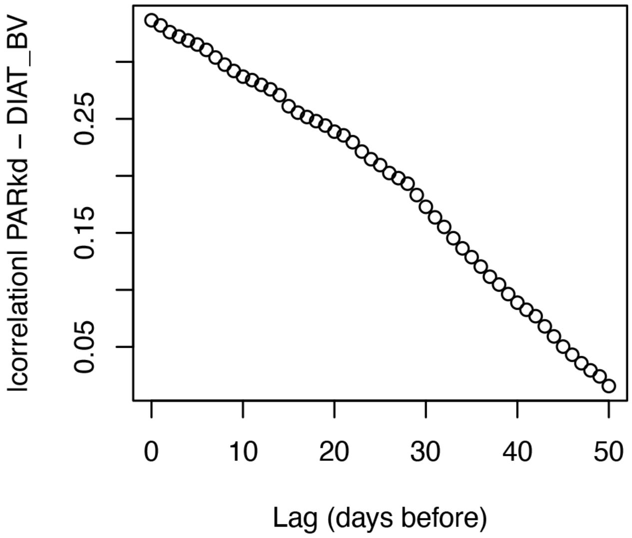
|correlation| PARkd – chla
0.1
0.2
0.3
0.4
0
10
20
30
40
50
Lag (days before)



|correlation| PARkd – SUM_PHYTO_BV
0.00
0.10
0.20
0.30
0
10
20
30
40
50
Lag (days before)



|correlation| PARkd – CYA_BV
0.15
0.20
0.25
0.30
0.35
0
10
20
30
40
50
Lag (days before)



|correlation| PARkd – DIAT_BV
0.05
0.15
0.25
0
10
20
30
40
50
Lag (days before)

APPENDIX H: RAPID CYANOTOXIN TEST STRIP EXPANDED RESULTS

Traditional methods for detecting microcystin, a common cyanotoxin, often take several days to yield results, making timely and effective management decisions, like closing public swimming beaches difficult. Although some rapid tests are available, their accuracy is not well characterized. In 2024, Janae Widiker evaluated the accuracy of three brands of microcystin field test strips—Gold Standard Diagnostics, Attogene, and 5Strands—under varying water quality conditions and microcystin concentrations, using spike solution, natural bloom samples, and live lab-grown *Microcystis aeruginosa* cultures.

Gold Standard Diagnostics strips were 86% accurate in the lab and 81% accurate in the field, with a detection limit of 2–10 µg/L. The Attogene strips were 79% accurate in the lab, with a detection limit of 4–20 µg/L; no field testing was conducted for Attogene strips, as they were incorporated into the study after the bloom season had ended. The 5Strands strips were 97% accurate in the lab and 83% accurate in the field, with a detection limit of 1–10 µg/L. All three brands produced comparable results when testing *Microcystis aeruginosa* cultures, although the exact concentration of microcystin in these samples was unknown, making direct comparisons of accuracy difficult. Furthermore, each of these brands tests for a different subset of MC congeners, some test for only extracellular toxins (Attoegene, 5Strands), and employ different analytical methods to determine toxin concentrations. Therefore, a one for one comparison is not advised for these tests. When using microcystin rapid test strips for recreational water, it is important to know whether the test detects only extracellular toxins, potentially underestimating total toxin levels if cyanobacterial cells have not lysed and continue to hold microcystins internally. Accurate risk assessment may therefore require additional methods to measure both intra- and extracellular concentrations, but rapid test strips remain valuable for preliminary screening and as a mitigation tool to support timely decisions.

All three brands effectively detected Microcystin across a range of water quality conditions and concentrations. Gold Standard Diagnostics provides specific microcystin concentrations but fails to quantify concentrations below 2 µg/L. Similarly, Attogene provides specific microcystin concentrations but cannot quantify concentrations below 4 µg/L. In contrast, 5Strands is more likely to detect low levels of microcystin but does not indicate whether concentrations exceed thresholds of concern.

These findings underscore the potential utility of rapid test strips for preliminary screening of microcystin in the field, while emphasizing the necessity of acknowledging their limitations when informing public health interventions and management strategies. It would be up to the City, County and State Health programs to decide the appropriate response to positive tests, but at least test strips could act as a tool for deciding whether to collect a sample for laboratory testing.

Sources for Purchasing

Gold Standard Diagnostics (ABRAXIS® Microcystins, 0-10(20) ppb, Recreational Water with QuikLyse® Feature)

Attogene (Microcystin Detection Kit (Rapid – Recreational Water))

5Strands (BlueGreen Algae Water Test)