Blind Spots in CyanoHAB Monitoring

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Introduction

Cyanobacterial harmful algal blooms (CyanoHABs) are made up of microscopic photosynthetic microorganisms and are most recognizable as bright green or blue-green masses on the water surface of lakes. Not only are these blooms unsightly and odorous, but they can also produce toxins that contaminate drinking water supplies, make pets and livestock sick, and in extreme cases, cause fatality. Currently, there is no universal trigger for CyanoHABs or cyanotoxin production. However, there are some known drivers of cyanobacteria accumulation, which may include excess nutrients and inorganic carbon, warm temperatures, and a stable thermal structure within the water column.

Cyanobacteria can gain competitive advantage over other phytoplankton by regulating their buoyancy, persisting in warm temperature, fixating nitrogen, and producing cyanotoxins. Studies indicate that cyanobacteria produce toxins, like microcystin, to compete with other aquatic microorganisms, e.g., phytoplankton and zooplankton, for dominance within the aquatic ecosystem.

Furthermore, microcystin may aid in regulation of intracellular inorganic carbon during periods of ambient low carbon conditions (Jahnichen et al. 2007), inhibit metabolic activities of other microorganisms, or maintain colony formation through promotion of extracellular polysaccharide production, which aids in buoyancy regulation and predation avoidance (Gan et al. 2012). Because of these competitive advantages, cyanobacteria outcompete other phytoplankton and grow to large densities; consequently, adequate methods to monitor their dynamics and impacts on water quality are of the upmost importance.

Cyanobacteria are ubiquitously found in lakes, reservoirs, rivers, and stormwater ponds. Accordingly, CyanoHABs are monitored by lake managers, water treatment plants, industrial dischargers, municipalities, lake associations, and citizen scientists. CyanoHABs can be monitored on different spatial and temporal time scales. Spatial monitoring can include whole-lake scales monitored via satellite imagery, to finer scales, such as monitoring beach sites designated for swimming. Temporally, CyanoHABs can be monitored on a response basis or near-continuous resolution. There are many types of technologies available for CyanoHAB monitoring, which can be biomass- or toxin-based. For biomass-based methods, the options include microscopy, in situ probes, and satellites. For toxin-based methods, the options range from simple test strips to complex lab analyses able to detect many different types of cyanotoxins. The wide variety of options can make CyanoHAB monitoring intimidating and overwhelming for water resource managers. This article outlines common blind spots in CyanoHAB monitoring and recommendations for overcoming CyanoHAB monitoring challenges.

Common Blind Spots

Equipment selection and procurement may be the largest challenge for many lake managers to overcome for CyanoHAB monitoring, depending on available resources. In addition to questions on the equipment selection, some of the other most common questions for CyanoHAB monitoring include:

- What parameters should be monitored?
- When and how should equipment be calibrated?
- What is the best spatial sampling strategy?
- What is the most appropriate temporal sampling frequency?

Chlorophyll vs phycocyanin

There is no known relationship between chlorophyll and phycocyanin pigment concentrations in lakes. Cyanobacteria (aka, blue-green algae) can contain several different photosynthetic compounds, including chlorophyll and phycocyanin. Chlorophyll-*a* is a common pigment found in all photosynthetic aquatic organisms which facilitates absorption of sunlight. Phycocyanin, on the other hand, is a pigment that is specific to cyanobacteria. In fact, it is the compound which gives blue green algae its name. Chlorophyll-*a* is often used as a proxy for phytoplankton biomass. It is often regulated in lakes and is typically already part of an existing monitoring plan. However, since chlorophyll-*a* is not specific to cyanobacteria, relying on chlorophyll-*a* data alone can lead to inaccurate assumptions of cyanobacteria biomass. For example, phytoplankton assemblages are usually comprised of diverse taxa of algae, all of which can produce chlorophyll-*a*. Conversely, some cyanobacteria only produce small concentrations of chlorophyll-*a* but will produce large concentrations of phycocyanin; therefore, low chlorophyll-*a* concentrations do not always indicate the absence of cyanobacteria. We have often observed cases where chlorophyll-*a* concentrations are relatively low, but concurrent taxonomic data revealed a high density of cyanobacteria. Consequently, it is important to be aware of this potential monitoring blind spot. Where resources allow, chlorophyll-*a* data should be

corroborated with taxonomic measurements or phycocyanin measurements to more accurately assess CyanoHAB conditions.

Toxins vs biomass

The true risk to human health is the cyanotoxins and not the cyanobacteria cells themselves, and there is no universal correlation between cyanobacteria biomass and cyanotoxins. The World Health Organization (WHO) has developed advisories for cyanobacteria cell concentrations as a proxy for cyanotoxin likelihood; however, these recommendations have not been adopted by the United States Environmental Protection Agency (U.S. EPA). Cyanobacteria do not consistently produce toxins, and there is currently no way to accurately predict when cyanobacteria will produce toxins. Additionally, most algal blooms are made up of several genera of cyanobacteria that produce different toxins at varying rates. For example, *Microcystis sp.* is a cyanobacteria capable of producing high concentrations of microcystin. It does not, however, produce as many different types of cyanotoxins, as does *Aphanizomenon sp.,* which can produce cylindrospermopsin, anatoxin, and saxitoxins, among others. Thus, an advisory based on cell counts does not account for the species composition of the cyanobacteria and can therefore misrepresent the cyanotoxin risk.

Cyanobacteria biomass can be measured in several different ways: phycocyanin, microscopic identification, or dry weight. Cyanotoxins can be measured using qualitative test strips, enzyme-linked immunosorbent assay (ELISA), or more precise laboratory methods like liquid chromatography combined with mass spectrometry (LC MS). Though there have been correlations between biomass and cyanotoxins (Wilkinson 2020), they are lake- and assemblage-specific.

Some major disadvantages of cyanotoxin analysis includes the costs and turnaround time for results, which can be a week or more. Biomass methods, especially those with a calibrated probe can provide some level of risk assessment more quickly and at a lower cost, but it is important to communicate the level of uncertainty with using biomass-only results. So why measure biomass at all? It depends on the goal of the monitoring plan, but understanding the accumulation of cyanobacteria biomass, and ideally the taxonomic identification of cyanobacteria, can illuminate management strategies and risk management. Also, management strategy effectiveness can vary between different cyanobacteria species. For instance, artificial mixing management would not be as effective on low-buoyant/low-light dependent cyanobacteria like *Planktothrix sp.* because they are adapted to and thrive in well-mixed conditions. Understanding the cyanobacteria composition and characteristics of dominant species can inform management and monitoring strategies. Thus, as budgets allow, monitoring plans should include both cyanotoxin and cyanobacteria biomass measurements. While there is no universal correlation between cyanotoxins and cyanobacteria, documenting local trends can help with risk management on a lake-specific basis.

Phycocyanin probe calibration

As discussed above, phycocyanin is a photosynthetic pigment specific to cyanobacteria. There are several options for phycocyanin analysis, including bench scale laboratory analysis and phycocyanin probes for collecting in situ measurements. Phycocyanin probes are a great way to get quick qualitative cyanobacteria assessment and can sometimes be added to multiparameter sondes. Phycocyanin probe data should be used as a qualitative measurement to understand relative changes in cyanobacteria biomass spatially and temporally. Phycocyanin probe data can be affected by turbidity, color, and cyanobacterial community. The default unit for the phycocyanin probes is relative florescence units (RFU), and these data can often be calibrated with rhodium, extracted phycocyanin, or cyanobacteria enumeration to establish site-specific relationships. It is difficult to compare phycocyanin probe measurements between different lakes or even different years within the same lake because of the water column conditions stated above. Thus, it is recommended that if phycocyanin probes are used as a proxy for cyanobacteria biomass, they should be calibrated using cyanobacteria biomass enumeration from the lake each year to establish site-specific relationships for trend analysis. These lake and seasonal relationships adjust the phycocyanin data to compensate for geographic and seasonal water column conditions that can affect the phycocyanin probe measurements. After the data are adjusted, phycocyanin data can be compared amongst different lakes and different years.

So, what parameters should be used to calibrate the phycocyanin probe? Calibrants that are direct measurements of cyanobacteria concentrations are the most representative for CyanoHAB conditions (e.g., cell concentration, biovolume [BV], dry weight). Since cyanobacteria have different morphologies (Figure 1) and produce phycocyanin at different rates, using BV is the best calibrant (Wilkinson 2019). BV can be analyzed by microscopy and is a normalizing parameter amongst different cyanobacteria, as it is a measure of the cellular volume and captures the variable morphologies of the cyanobacteria genera.

Spatial heterogeneity

CyanoHAB presence and density can vary vertically and horizontally within the lake. Most cyanobacteria can regulate their buoyancy which allows them to move throughout the water column seeking favorable conditions like nutrients and light. It is important to understand the spatial variability within the lake, when designing monitoring plans so that CyanoHAB presence and risk is not underestimated. Horizontal variability can be assessed through different methods including satellites, drones, citizen scientists, historic accounts, shoreline inspections, in situ measurements, and wind analysis. Vertical variability is driven by mixing conditions and density gradients within the water column (Wilkinson 2019 and 2020). Overall water column stability acts as a scaffold for cyanobacteria accumulation, allowing us to predict if cyanobacteria are mixed throughout the water column or can accumulate in the epilimnion. The driving force for vertical heterogeneity of cyanobacteria within the epilimnion is wind-mixing and surface water temperature, which determines if

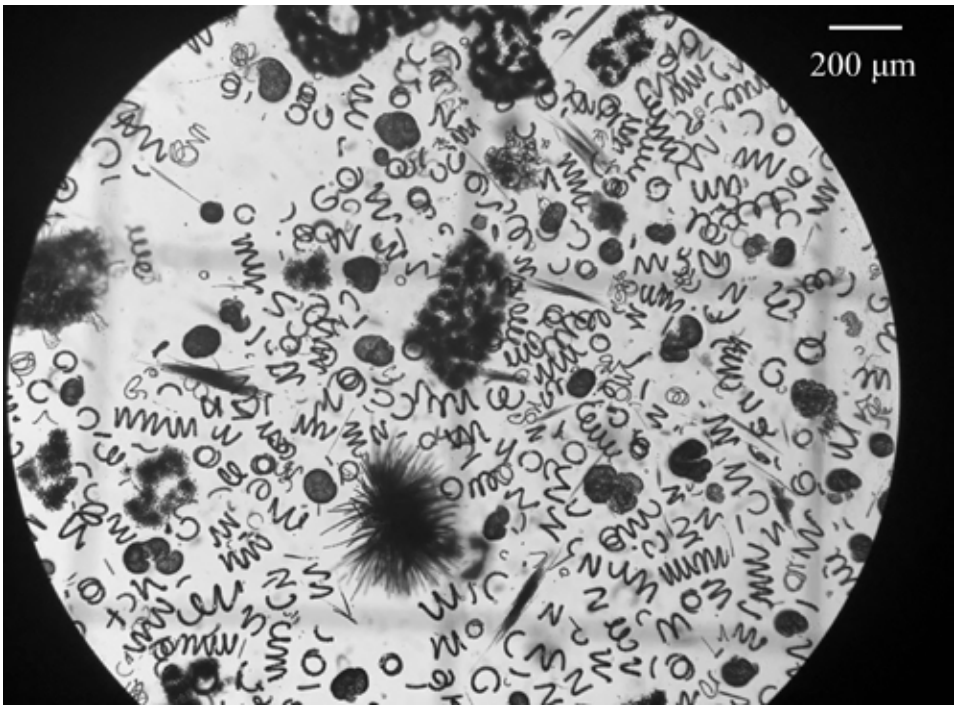

Figure 1. Micrograph of cyanobacteria assemblage.

cyanobacteria are uniformly mixed above the thermocline or whether they form distinct peaks throughout the epilimnion.

Understanding the stability of the water column and local mixing conditions dictates the monitoring depth(s) so that cyanobacteria concentration is not underrepresented. For instance, in the case of a thermally unstable water column, cyanobacteria are uniformly mixed throughout the entire water column (Figure 2a). Thus, cyanobacteria sample collected from anywhere within the water column will likely be representative. If the water column is stable but the wind is high, the cyanobacteria are expected to be well-mixed in the epilimnion (Figure 2b). Likewise, any monitoring depth within the epilimnion will be representative. However, if the water column is stable and the wind is low, cyanobacteria can form local maxima (Figure 2c). Multiple monitoring depths within the epilimnion are therefore necessary to capture the variation in community composition and density.

Depending on the goals of the monitoring plan, it is possible that only one location is appropriate for risk assessment, such as at water treatment intakes or swimming beaches. However, if predictive models based on observed data are being developed to achieve early warnings for CyanoHAB formation, it is imperative that representative samples are captured to accurately predict the risk (Figure 3).

Monitoring frequency

Cyanobacteria blooms are temporally transient because of their competitive advantages, aggressive accumulation potential, and susceptibility to mixing, as discussed above. High monitoring frequency is paramount to predictive modeling accuracy. Monitoring gaps can lead to missed or inaccurate conclusions. For example, cyanobacteria can reach exponential growth in five days, thus even weekly monitoring is potentially not frequent enough to capture bloom dynamics (Wilkinson 2016). Thus, high-frequency monitoring of CyanoHABs and toxins is really needed to accurately assess public health risk; however, lower frequency monitoring of CyanoHABs is often necessary due to resource limitations.

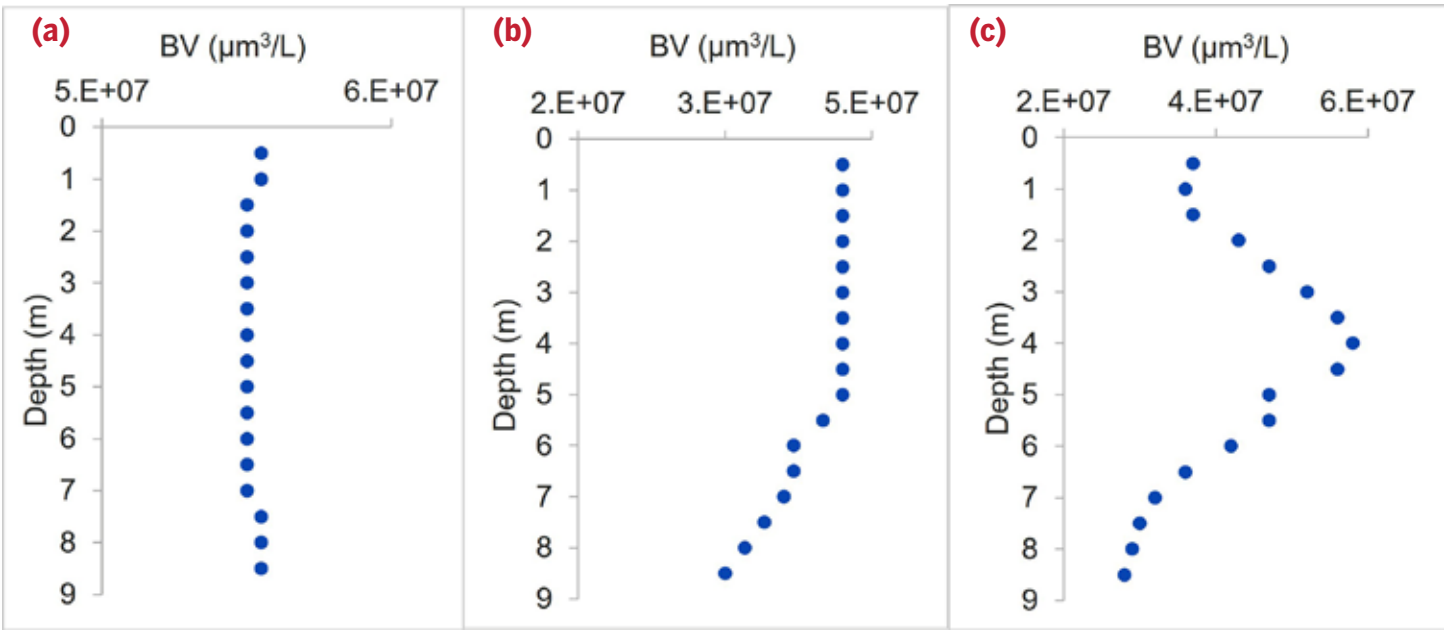

Figure 2. Examples of cyanobacteria vertical distribution under different stability and mixing conditions.

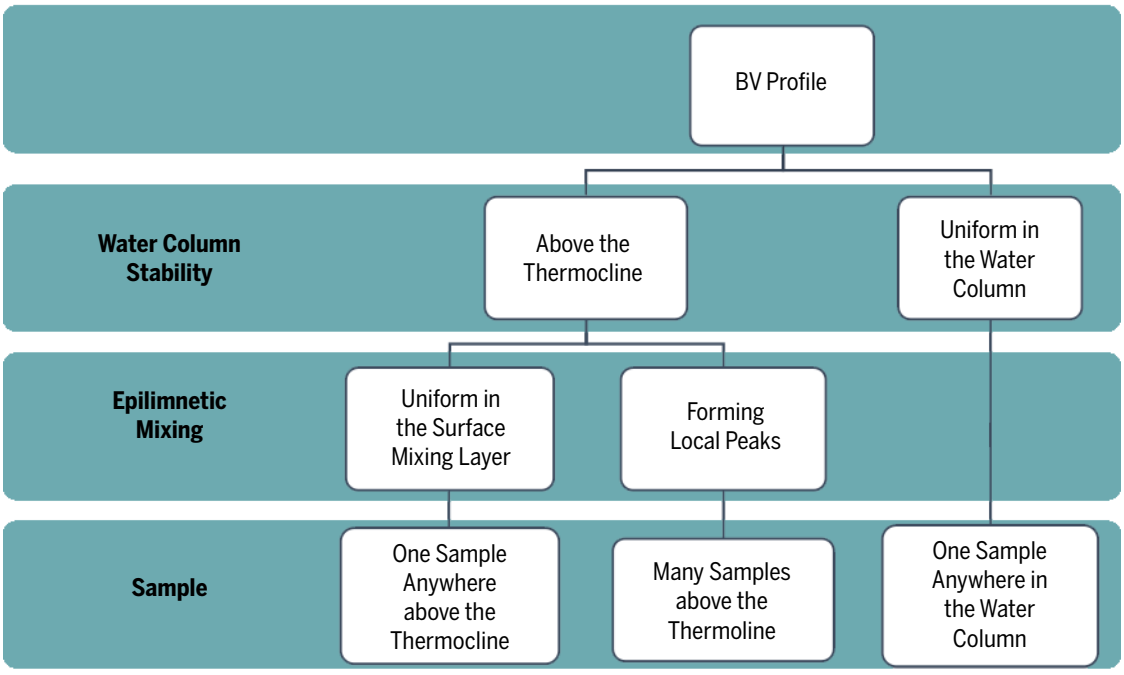

Figure 3. Monitoring depth decision tree.

Consequences of blind spots

The consequences of monitoring blind spots include over or underestimating CyanoHAB or toxin risk, errors in assessing feasibility of in-lake management actions, and errors in prediction accuracy. Underestimating risk can lead to missed opportunities for effective management, misallocation of resources, and damage to the ecosystem or public health. Though these blind spots exist, any of the methods discussed above can still be used based on the goal of the monitoring plan and if the manager is aware of the blind spots in the interpretation of the data. For example, one monitoring location at the intake of a drinking water treatment plant can alert managers of conditions at the inlet, to help prepare for possible algal blooms within the source water. In large drinking water systems, additional monitoring locations and highly resolved data could aid in development of predictive models, which can be used to predict when a bloom might reach the water intake thereby giving water treatment managers more time to respond and implement HAB protection protocols (e.g., alter depth of water intake or utilize more expensive treatment options).

Recommended monitoring plan

Not everyone has the same resources or goals for their monitoring plans. Below are three options for monitoring plans, based on cost and staff effort (Table 1). The options range from tests for presence/absence of cyanobacteria only (Tier 1) to continuous, high-frequency monitoring, with quantitative cyanotoxins (Tier 3). Each tier has a varying degree of analysis outcomes and costs for implementation. Tier 1 is a low-cost, qualitative option for determining if cyanobacteria are present. Temporal variability of CyanoHABs can be recorded if Tier 1 is performed routinely. Samples collected can be preserved indefinitely and analyzed in the future if funds become available. Tier 2 includes qualitative cyanotoxin analysis, which can be performed in the field, and includes cyanobacteria identification. The cyanobacteria identification can inform the potential for other cyanotoxins that may be present and could guide the need to measure toxins. Where resources allow, Tier 3 analysis includes algae identification, phycocyanin profiles, quantitative laboratory cyanotoxin concentration, and cyanobacteria identification. Tier 3 analyses allow for high resolution of vertical variability of cyanobacteria distribution and can determine the exact concentrations of cyanotoxins to better inform the risk to the environment and public health.

Conclusion

The complexity and uncertainty of CyanoHABs can lead to blind spots in monitoring plans including monitoring parameters, equipment calibration, sampling location, and monitoring frequency. Additionally, the many options for CyanoHAB monitoring can be overwhelming for resource managers. However, understanding the potential blind spots and how to interpret monitoring results based on of those blind spots is key to achieve successful CyanoHAB management within your waterbody.

References

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Table 1. Recommended Monitoring Plan Options.

Tier	Analysis	Equipment	Costs/Staff Commitment	Analysis Description	Data Analysis Outcomes
1	***Jar Test***	Bottles, preservative, eye dropper, test tubes	Staff time and equipment	***Jar Test:*** Presence/absence of cyanobacteria, does not inform level of toxicity/ toxins	***Answers:*** Presence of CyanoHAB and temporal variability (if test is performed routinely). Allows for preservation of sample for Tier 2 analysis in the future (if desired).
2	***Jar Test*** (Tier 1) ***Cyanobacteria identification*** ***Cyanotoxin Test Strip***	Bottles, preservative, eye dropper, test tubes, cyanotoxin test strips	Staff time and equipment $100-300/sample for Cyanobacteria Identification $10-$50/test strip	***Jar Test:*** See Tier 1 ***Cyanobacteria Identification:*** cyanobacteria concentrations and community composition ***Test Strips:*** qualitative toxin concentration	***Answers:*** See Tier 1 ***Answers:*** What is the cyanotoxin concentration range? ***Answers:*** What is the potential for other cyanotoxins? What triggers for CyanoHAB may be present in the lake which can help inform management?
3	***Cyanobacteria identification*** (Tier 2), ***Phycocyanin in-situ profiles,*** ***Laboratory Cyanotoxin test*** Note: no tier 1 necessary	Bottles, preservative, eye dropper, test tubes, Phycocyanin probe	$100-300 per sample for Cyanobacteria Identification $3,000-$15.000 for phycocyanin probe $200-600 sample for toxin analysis	***Cyanobacteria Identification:*** See Tier 2 ***In-situ profiles:*** vertical distribution of cyanobacteria ***Lab Test:*** specific concentration of the 3 most common cyanotoxins	***Answers:***: See Tier 2 ***Answers:*** Are the cyanobacteria accumulating at specific depths? ***Answers:*** What is the exact concentration of the cyanotoxins driving the HAB toxicity? This will better inform risk to animals and recreation and management efforts.

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