



# Management Strategy for Overwintering Cyanobacteria in Sediments Contributing to Harmful Algal Blooms (HABs)

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**PURPOSE:** Cyanobacteria that cause harmful algal blooms (HABs) can overwinter in sediments as resting cells (akinetes or vegetative colonies) and contribute to seasonal bloom resurgences. However, to date there has been limited focus on management tactics specifically targeting the control of cyanobacterial sources from sediments. Targeting resting cells in sediments for preventative management may provide a viable approach to delay onset and mitigate blooms (Calomeni et al. 2022). However, there are limited resources for this novel strategy. Given the growing global impact of HABs, there is a need to develop management strategies focused on sediments as a potential source and contributor to HABs. Therefore, the objective of this report is to provide a management strategy in terms of approaches, information, and case study examples for managing overwintering cyanobacteria in sediments with the goal of mitigating seasonal HAB occurrences.

**BACKGROUND:** The US Army Corps of Engineers (USACE) manages approximately 600 impoundments where HABs can interfere with multiple authorized purposes (e.g., potable water supply, fish and wildlife propagation, recreation, and water quality) (Linkov et al. 2009; Brooks et al. 2016). Cyanobacteria cells that settle to sediments at the end of the growing season may contribute to rapid bloom formation when environmental conditions become suitable for growth, and thus function as drivers of annual HAB resurgence in some water bodies (Kim et al. 2005; Kaplan-Levy et al. 2010; Cirés et al. 2013; Kitchens et al. 2018). However, to move toward successful proactive mitigation of resting cells that lessen HAB impacts, clearly defining the problem and identifying causal variables are needed. Additionally, given these current uncertainties of this proactive approach, multiple lines of evidence supporting overwintering cell viability may improve the likelihood of success by management actions (Calomeni et al., “Identification and Prioritization,” 2023). Therefore, given these uncertainties and the ecological complexity of blooms, an adaptive management strategy that uses iterative steps to provide a framework to plan management actions is appropriate (NRC 2004; Williams et al. 2009; CMP 2020).

**RESULTS: Adaptive Management Approach:** The proposed management strategy for targeting overwintering cyanobacteria follows an adaptive management plan to (1) clearly define the issue in a problem formulation step, (2) identify and prioritize management goals, (3) develop an action plan, (4) conduct management action, (5) measure outcomes, and (6) adapt and refine the plan based on outcomes (Figure 1). This process includes intentional outreach and communication, and collaboration with stakeholders to improve project success.

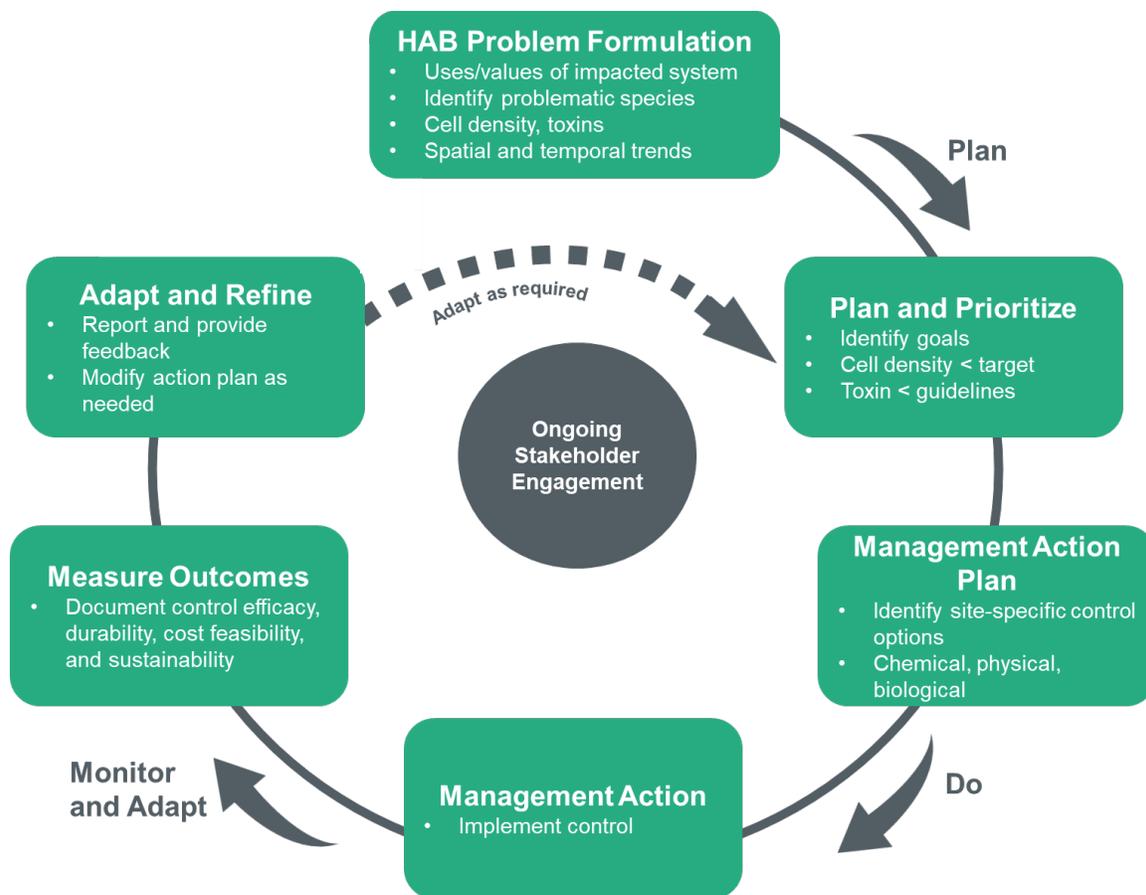


Figure 1. Harmful Algal Bloom (HAB) Adaptive Management Plan (adapted from NRC 2004; Williams et al. 2009; and CMP 2020).

**HAB Problem Formulation:** The first step in the management process is clearly defining the extent and scale of the problem in terms of the dominant HAB species; the timing, duration, intensity, and frequency of the bloom; and its negative impacts (e.g., on human health and on economic, recreational, and environmental factors). At this stage, resource managers work with stakeholders to consolidate historical data and better understand temporal and spatial trends of HABs at the location. Certain factors (e.g., cyanobacteria presence, density, and environmental conditions) may suggest that resting cells in sediments are contributing to HAB formation at a location. For example, some common HAB-producing genera such as *Aphanizomenon* (and others in the order Nostocales) have the potential to produce specialized resting cells, called akinetes, that may survive well in sediments. Others, such as *Microcystis* and *Planktothrix*, are known to overwinter in sediments as cells or colonies. Additional evidence would be a rapid and repeated resurgence of HABs annually or every few years. In the context of using a preventative strategy like managing resting cells, multiple lines of evidence are needed to predict if sediments are a source and risk driver for the impacted water resource. The lines of evidence (see Table 1) to support this step include answering three fundamental questions: (1) are overwintering cyanobacteria present in sediments at the site, (2) are the overwintering cells viable, and (3) are they present in a location that has conditions to trigger the growth and transfer of cells to the planktonic phase?

The first step is confirming whether HAB genera capable of producing akinetes or overwintering vegetative cells are present in sediments at the site (see Calomeni et al. 2022 for a full list of common genera associated with overwintering cyanobacteria). This can be done using traditional microscopic identification and enumeration methods (Figure 2). Multiple laboratories specialize in the identification of cyanobacteria and algae in water. Laboratories used for overwintering cell identification should have experience identifying and quantifying these specific cells in sediment.

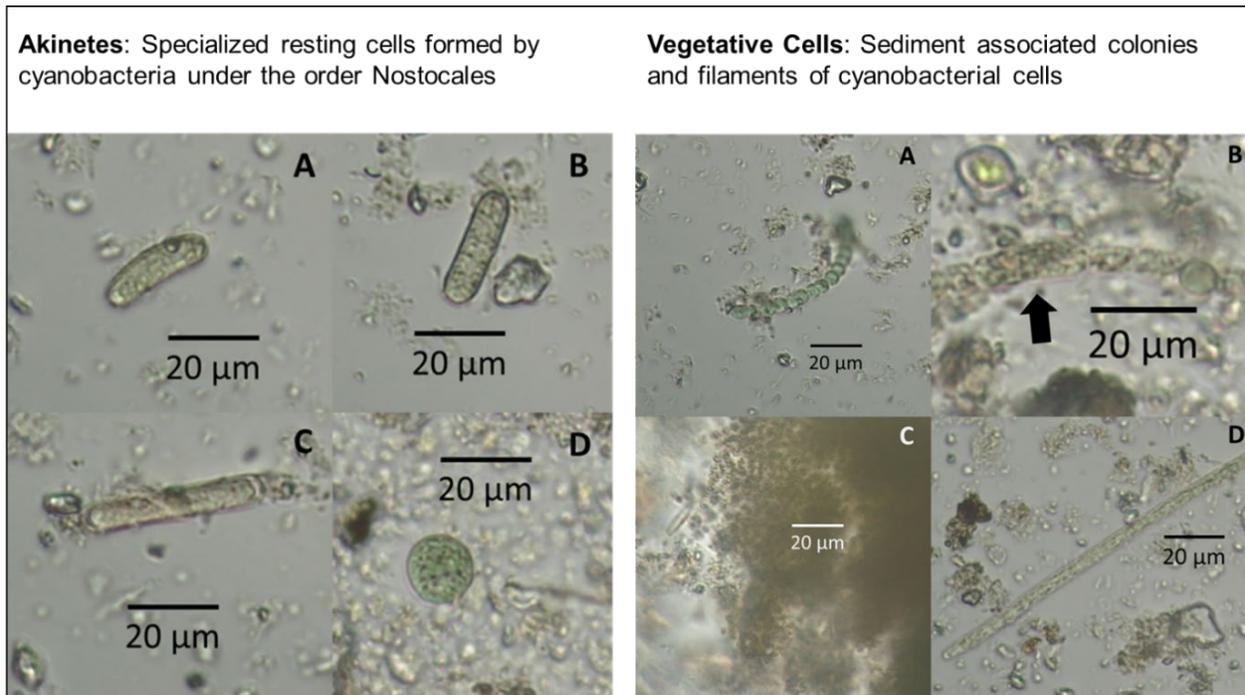


Figure 2. *Left:* examples of akinetes observed in sediment samples from three HAB-impacted water bodies: (A) arcuate akinete, possibly *Dolichospermum* (formerly *Anabaena*); (B) cylindrical akinete, possibly *Dolichospermum* (formerly *Anabaena*); (C) long cylindrical akinete, possibly *Aphanizomenon* or *Dolichospermum* (formerly *Anabaena*); and (D) spherical akinete, possibly *Dolichospermum* (formerly *Anabaena*) or *Trichormus*. *Right:* examples of overwintering vegetative cells: (A and B) *Anabaena*; (C) a *Microcystis* colony; and (D) *Planktothrix*. Arrow points to an akinete. (Images by Alyssa Calomeni, ERDC Environmental Laboratory.)

To determine whether resting cells are viable, a laboratory approach has been developed in which site-collected samples are exposed to favorable growth conditions and observed for relative growth potential (Figure 3). Briefly, sediments and filtered water collected from the site are placed in an incubator with ideal temperature and light conditions for 14 days, after which, the overlying water is observed for the presence of cyanobacteria. Site specific factors likely play a key role in the rate and extent of planktonic recruitment, including the species' environmental tolerances, light, temperature, nutrient, and mixing conditions of the sediments (Calomeni et al. 2022); management locations should be prioritized based on multiple lines of evidence (see examples in Calomeni et al., "Identification and Prioritization," 2023).

Table 1. Approaches to predicting overwintering cyanobacterial risks at impacted sites (modified from Calomeni et al. 2022).

Question	Approach
Are overwintering cells present at the site?	Dilution and separation of overwintering cells from sediment samples followed by <b>identification and enumeration</b> with microscopy
Are overwintering cells viable? At what growth potential?	<b>Laboratory incubation study</b> with site-collected sediment and water
Are cells viable when additional nutrients are added?	<b>Laboratory incubation study</b> with addition of nutrients
Which site locations have the greatest potential of reaching environmental conditions to trigger growth?	<b>Field site data</b> collection and/or review (e.g., bathymetric data, secchi disk/light attenuation, sediment and water nutrient data, etc.)

If algal toxins are an important risk driver, evidence collected in the problem formulation step can include identifying and enumerating densities of species that are known toxin producers, reviewing historical toxin data (if available), collecting toxin data at the location, and monitoring toxins in laboratory incubation studies. From a risk perspective, it is important to consider potential toxin production associated with the benthic environment as well as the water column.

Water bodies that would be strong candidates for preventative management of benthic overwintering cells would contain viable cells but have limited allochthonous sources of cyanobacteria (i.e., sediments are the primary source in the system). In cases where allochthonous sources are unknown, monitoring riverine or other inflowing cyanobacteria sources to the waterbody may be helpful. If a waterbody is identified as a candidate site for preventative management of overwintering cells, monitoring of environmental conditions including stratification and turnover, hydraulic residence time, depth and light penetration, nutrients, and temperature can be used to refine management areas/treatment zones (Calomeni et al. 2022).

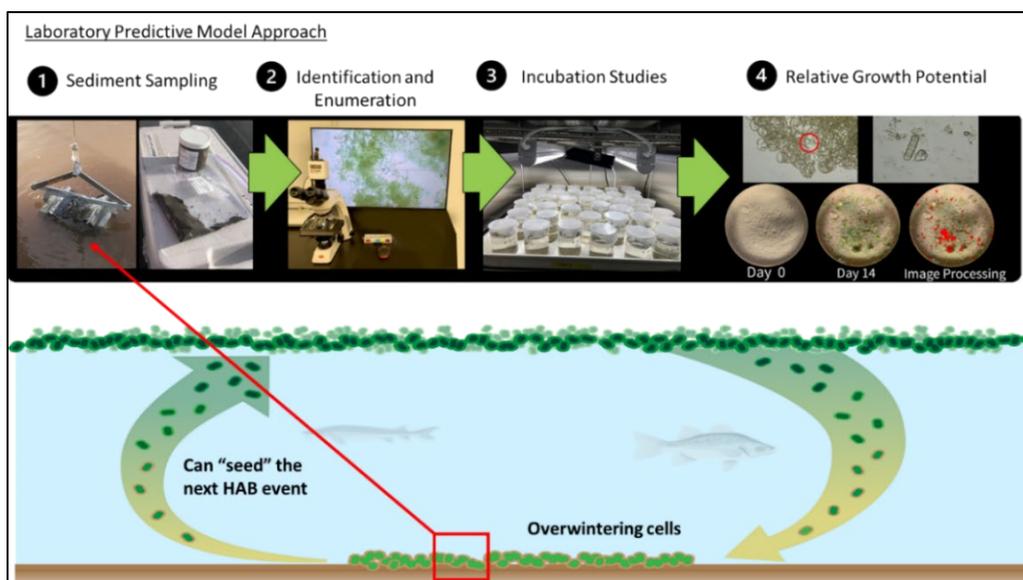


Figure 3. Approach to inform presence, viability, and relative growth potential of overwintering cyanobacteria using site-collected samples (see Calomeni et al. 2022; Calomeni et al., "Efficacy of Algaecides," 2023).

**Plan and Prioritize:** The next step is to identify site-specific control options focused on the benthic phase to target overwintering cyanobacteria. These scenarios could include chemical, physical, or biological management tactics over a range of time spans. Because this is a relatively novel strategy, limited efficacy data is available that is specially focused on the proactive treatment of overwintering cells. However, there is an extensive database demonstrating the efficacy of algaecides for the treatment of benthic cyanobacteria (Duke 2007; Bishop and Rodgers 2011; Calomeni et al. 2015; Geer et al. 2017; Anderson et al. 2019; ITRC 2021b). Many fundamental concepts that apply to algaecide treatments of benthic cyanobacteria will also apply to treatments of overwintering cells and will inform future management strategies. For example, laboratory methods that provide predictions of algaecide efficacy for the field have been well developed for benthic and planktonic algae (e.g., Bishop and Rodgers, 2011; Geer et al., 2017; Calomeni et al. 2018; Kinley-Baird et al. 2021). Laboratory studies can be used to develop site-specific predictions about algal responses to differential treatments and are expressed as exposure-response models (Calomeni et al. 2018). Appropriately designed and implemented laboratory experiments (e.g., using site water and representative samples) can result in site-specific predictions. These concepts are also readily transferable for chemical, physical, or biological treatment mechanisms. Several factors that may impact the efficacy of treatments for overwintering cells are (1) treatment efficacy in proximity to sediment (e.g., competing ligands for algaecides or other chemical treatments); (2) overwintering cells relative sensitivities to treatments; (3) timing and periodicity (e.g., repeat exposures) of preventative treatments; and (4) treatment mechanisms, concentration, and exposure times required to achieve efficacy as well as treatment application methodologies (Calomeni et al. 2022). For overwintering cyanobacteria in sediments, methods have been developed to predict treatment efficacy using incubation studies. These studies assess planktonic growth potential or the cell density in the water column after incubation of sediments containing resting cells are treated with candidate products. Efficacy data can then be used to inform selection of the appropriate active ingredient, formulation, and application rate of US Environmental Protection Agency (USEPA) registered algaecides (Calomeni et al., “Efficacy of Algaecides,” 2023).

Consideration of factors that affect efficacy during the planning stages of preventative treatments can inform treatment decisions and increase the likelihood of success. As is the case with planktonic algae, laboratory experiments can be used to discern relative sensitivities of overwintering cells to different treatments, durations of exposure, and concentrations necessary to achieve control (e.g., Figure 4). These experiments will be critical to refine candidate mitigation actions for the preventative treatment of sediment-associated overwintering cells. Published efficacy data (sourced from literature), subject matter expert recommendations, and/or laboratory-based efficacy trials are all potential sources of data to inform the treatment plan. At this stage, all the gathered information from previous stages of the adaptive management process helps inform the selection of product, application rate, scale, cost, permitting, and treatment timing. In some cases, it can be helpful to organize these (sometimes disparate) data into lines of evidence to help interpret data. For example, columns representing lines of evidence, conclusions, actions, and decisions can be arranged into a tabular format (i.e., logic table) (Chapman 1990; Suter and Cormier 2011; Calomeni et al., “Identification and Prioritization,” 2023) that can help managers prioritize treatment locations.

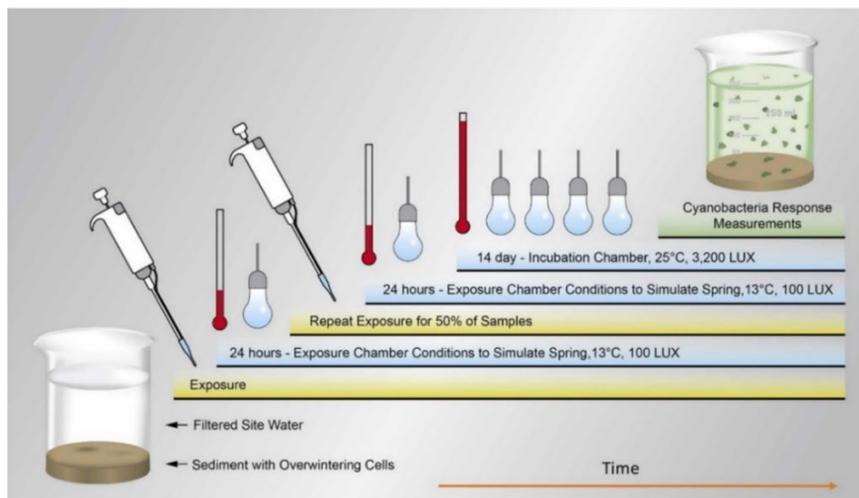


Figure 4. Example of a laboratory method designed to predict algaecide efficacy for overwintering cyanobacteria (reproduced from Calomeni et al., “Efficacy of Algaecides,” 2023).

**Management Action:** At this stage, state-licensed and certified applicators apply the treatment to the target location. The timing of preventative treatments will likely be critical for successful implementation. The current data suggest that early spring (or perhaps late winter) prior to growth are ideal times to preventatively treat the sediments. If treatments are delayed until cell densities increase at the sediment water interface, efficacy could diminish. Additionally, there are limited studies in current peer-reviewed literature to provide data on the impact of sediment exposure modifying factors on preventative treatment efficacy, relative sensitivities of overwintering cells, timing of treatments, and water temperature influences on algaecide efficacy (e.g., how colder temperatures impact efficacy). To fill these data gaps, preliminary laboratory-scale experiments will be critical to mitigate uncertainties of success (as described in the section “Plan and Prioritize”). To date, there are several technical approaches to apply algaecides to bottom sediments, including drop hoses for algaecides in solution, venturi eductors for bulk solids (e.g., powders, pellets, or flakes), or mechanical broadcast spreaders for granular products (Figure 5).

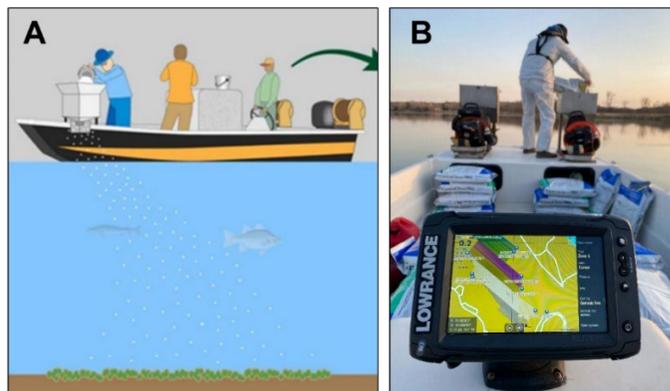


Figure 5. Examples of granular-based algaecide application methods to target sediments using a spreader applicator: (A) graphical depiction and (B) application in practice. (Photograph by Ciera Kinley-Baird, Aquatic Control, Inc.)

**Measure Outcomes:** There are numerous strategies to measure outcomes from a preventative approach. Initially, aspects of the treatment should be monitored in terms of whether the application achieved the targeted exposure parameters (e.g., concentration and duration) that were outlined in the management plan. It is also beneficial to measure immediate pretreatment conditions (if feasible) to clearly define posttreatment differences. Field monitoring considerations are outlined in Table 2.

Table 2. Considerations relevant to field monitoring of overwintering cyanobacteria.

Target Sample Location	Parameter	Approach	Reference
<b>Overwintering Algal Cells in Sediments</b>			
Sediment (0–2 cm at water–sediment interface) <sup>a</sup>	Presence of overwintering cells in sediment	Microscopic identification and enumeration of overwintering cells. Approaches: sediment dilution, particle size separation, and density separation.	Calomeni et al. 2022
Sediment (0–2 cm at water–sediment interface) <sup>a</sup> and representative water samples	Viability and planktonic growth potential of overwintering cells in sediment	14-day laboratory incubation studies followed by microscopic identification and enumeration	Calomeni et al. 2022; Calomeni et al., “Identification and Prioritization,” 2023
<b>Monitoring Environmental Conditions</b>			
Water–sediment interface and/or representative water column	Planktonic cyanobacteria and algae cell densities	Identification and enumeration of planktonic algae via microscopy, flow cytometry, genetic tools, etc. (see ITRC 2021a for the benefits and limitations of different methods)	e.g., State laboratories, universities and contract laboratories
	Concentration of algal toxins or secondary metabolites	e.g., microcystin-LR, anatoxin-a, saxitoxin, taste and odor compounds	e.g., USEPA Methods 544 (Shoemaker et al. 2015), 545, 546 (USEPA 2015, 2016) for toxins and APHA and AWWA 2022 (see 6040 A for taste and odor compounds)
	Algal pigments (chlorophyll a, phycocyanin)	Grab and/or composite samples, in situ probes	APHA and AWWA 2022
	Temperature	Grab and/or composite samples, in situ probes	APHA and AWWA 2022
	Light intensity, attenuation	In situ probes, secchi disk	e.g., Davies-Colley et al. 1993
	Turbidity	Grab and/or composite samples, in situ probes	APHA and AWWA 2022
	Nitrogen as nitrate-nitrite and ammonia	Grab and/or composite samples, in situ probes	APHA and AWWA 2022
	Phosphorus as soluble reactive phosphorus	Grab and/or composite samples	APHA and AWWA 2022

Table 2 (cont.). Considerations relevant to field monitoring of overwintering cyanobacteria.

Target Sample Location	Parameter	Approach	Reference
Water–sediment interface and/or representative water column	General water chemistry (e.g., pH, dissolved oxygen, alkalinity, hardness, conductivity; metals)	Grab and/or composite samples, in situ probes	APHA and AWWA 2022
	Parameters that represent treatment exposure (e.g., [peroxide], [copper])	Grab and/or composite samples	—
Site nontarget species	Monitor nontarget species (e.g., fish, benthic invertebrates, mussels)	—	—

<sup>a</sup>For energetic systems, akinetes from deeper sediments may be suspended; deeper sediments should be sampled.

**Adapt and Refine:** Improving overall mitigation success requires that outcomes be monitored, that managers learn from the system’s reponse to management actions, and that future actions be revised based on that learning (NRC 2004). Therefore, to ensure success, it is critically important to document outcomes, communicate transparently with stakeholders, and adapt future efforts based on the available information. As compared to a haphazard “trial and error” approach, the structured feedback inherent to the adaptive management model is more likely to produce a range of management options with clearly defined decision points that are based on scientifically defensible information (NRC 2004). This adaptive management approach is particularly useful for developing novel solutions to complex problems—in this case, to optimize strategies for treating overwintering cyanobacteria. Currently, the conceptual model of this preventative strategy targeting the source of the HAB in a system is aimed to disrupt the seasonal growth cycle of the cyanobacteria, and therefore alter the system’s impairment (Figure 6).

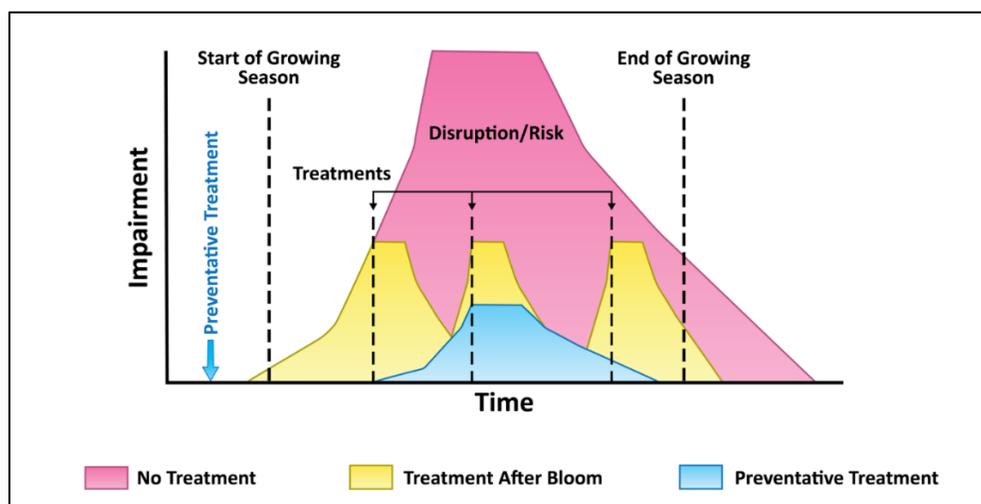


Figure 6. Conceptual model of water resource impairment (area under the curve) over a growing season.

Figure 6 is a conceptual model of water resource impairment over a growing season consisting of primary bloom formation, bloom maintenance, and collapse for multiple management scenarios: (1) *no treatment*, where a HAB peaks in intensity and persists throughout growing season; (2) *treatment after bloom*, which may require multiple concentrated treatments because of high cell densities; and (3) *preventative treatment*, in which source cells are removed, decreasing primary bloom formation and minimizing the timing and intensity. *Preventative treatments* may also require additional treatments throughout the growing season to meet performance goals, depending on the severity of impairment.

**CASE STUDY: Milford Gathering Pond, Kansas US:** A field demonstration of a preventative treatment approach targeting overwintering cyanobacteria in sediments was used at a historically HAB impacted waterbody, Milford Gathering Pond in Junction City, Kansas, US. The case study is structured to demonstrate a pertinent example of the adaptive management process in practice. Kansas Department of Health and Environment (KDHE), which operates the Kansas HAB Response Program, conducted preventive peroxide treatments as part of a large multiyear HAB mitigation effort supported by state funds. The Milford Gathering Pond is a USACE managed property, which offered a unique opportunity for collaboration among state and Federal organizations with the shared goal of conducting preventative treatment field demonstrations.

**Problem Statement:** The lake is managed by the USACE Kansas City District and is used for recreation (swimming beach) and fishing. The lake is also leased by the Kansas Department of Wildlife and Parks (KDWP) for source-water used to operate a nearby fish hatchery. The lake has a history of impairments from HABs during warmer months, with a notable event occurring in 2019 that resulted in microcystin concentrations measuring up to 400 µg/L\* based on monitoring by KDHE (2023). The total microcystin concentrations consistently surpass the recommended recreational ambient water quality criterion of 8 µg/L set by the USEPA (USEPA 2019; KDHE 2023). The presence of HABs has led to frequent closures of the swimming beach, notably in 2019 when the beach remained closed for several consecutive months throughout the summer. Due to the lake's annual HAB occurrences, there is a high likelihood of resting cells in the sediments at the site. Historical data spanning 2019–2022 indicate that prevalent HAB genera in the lake include *Aphanizomenon*, *Dolichospermum*, *Microcystis*, *Planktothrix*, and *Raphidiopsis*. These HABs are typically observed starting in June and can persist until late October, as reported by KDHE (2023). Therefore, there was a question of whether source control of overwintering cells in the sediments could alter the timing or intensity of these blooms.

**Plan and Prioritize:** Treatments were informed using historic bloom history data (sourced from KDHE and USACE), laboratory incubation studies (Calomeni et al., “Efficacy of Algaecides,” 2023), and a laboratory algaecide efficacy trial using site-collected samples (Kinley-Baird et al., 2023). Based on these results, the candidate algaecide that was selected was a granular peroxide-based algaecide (GreenClean® Pro) that showed efficacy of decreasing both density and planktonic bloom potential of overwintering cells in sediments. These data were used to scale the treatments for application to a 31.5 ha treatment zone in Milford Gathering Pond.

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\* For a full list of the spelled-out forms of the units of measure used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52, <https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf>.

**Management Action:** During 19–21 April 2022, two consecutive applications of a granular peroxide-based algaecide (GreenClean Pro) were applied with a 48 h interval between applications. The treatments were targeted at a 31.5 ha “treatment zone,” strategically separated from a 12.9 ha control zone by means of a silt curtain. The formulation of the treatment plan was guided by algaecide efficacy experiments conducted in the laboratory, utilizing samples collected from the site (Calomeni et al. 2022; Kinley-Baird et al. 2023).

**Measure Outcomes:** In this case study, performance was compared against a control zone designated in the lake using a silt curtain. The evaluation of in-lake treatment effectiveness centered on the examination of planktonic cyanobacterial densities in both the treatment and control zones. These measurements were conducted every 2 weeks over a span of 4 months, followed by monthly assessments for an additional 2 months. Ancillary evidence was gathered by analyzing resting cell densities and recruitment viability in sediments 3 days after the treatments were executed. Around 1-month posttreatment (25 May 2022), the average planktonic cell densities were 109,800 cells/mL in the treatment zone and 302,100 cells/mL in the control zone, as illustrated in Figure 7. Notably, *Microcystis* emerged as the dominant genus during the initial month of observation. Therefore, this management strategy showed measurable differences in seasonal planktonic cyanobacteria densities during the first emergence of the seasonal bloom in this system. The subsequent monitoring event in June was confounded by the fish hatchery discharging water from various rearing ponds into the treatment zone. Even with the introduction of cyanobacteria and nutrients from the hatchery discharge, where the algal assemblage was mainly comprised of *Dolichospermum*, *Microcystis*, and *Raphidiopsis*, the average cell densities in the treatment zone consistently exhibited a downward trend compared to the control zone throughout the remaining sampling events. In 9 out of 11 instances (82%) spanning from May to October, the average planktonic cyanobacterial densities demonstrated a lower trend in the treatment zone than in the control zone, ranging from 6% to 97% lower (refer to Figure 7). Therefore, the lines of evidence from this case study suggest that targeting sediment overwintering cells can have a measurable difference in seasonal planktonic blooms.

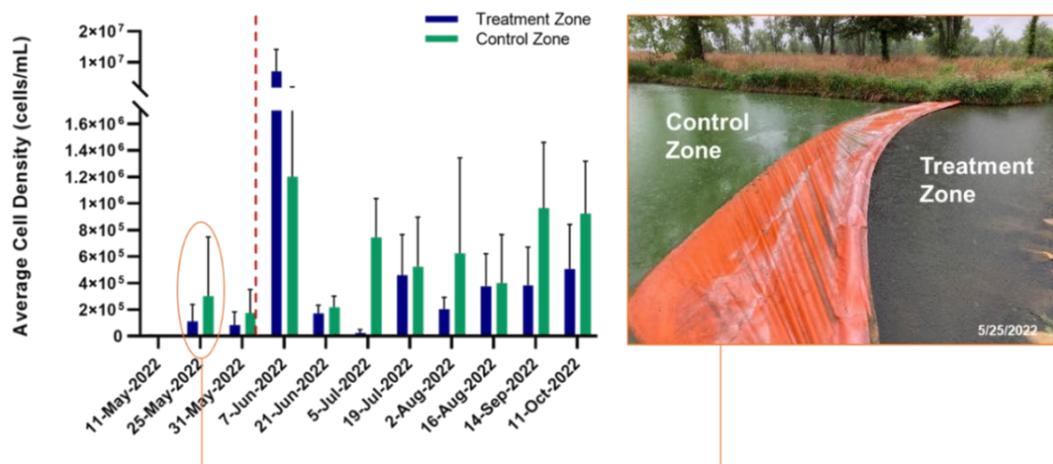


Figure 7. Average cell densities of planktonic cyanobacteria ( $n = 4$ ) in treatment and control zones in the months following treatment at Milford Gathering Pond. Red dashed line indicates date when fish hatchery released water into the treatment zone. Error bars indicate  $\pm 1$  standard deviation. (Modified with permission from Kinley-Baird et al. 2023; Photograph insert by KDHE staff.)

**Adapt and Refine:** The recruitment data from laboratory experiments comparing treatment and control zones played a crucial role in interpreting performance outcomes. Despite the treatment zone having a higher potential for recruitment and receiving hatchery discharges with cyanobacteria in late May 2022, subsequent monitoring revealed consistently lower average cell densities of cyanobacteria in the surface water of the treatment zone compared to the control zone. A significant challenge in interpreting performance data was the considerable variability within and among sample sites. Previous studies have also documented spatial heterogeneity of resting cells, such as differences between shallow and deep areas (Cirés et al. 2013, Legrand et al. 2017). Therefore, future sampling efforts should account for these variations. Additionally, future demonstrations should strive for comparable sediment characteristics and resting cell viability in both treatment and control zones to minimize confounding factors.

In 2022, Milford Gathering Pond exclusively employed preventative algaecide treatments for managing HABs. To enhance the likelihood of achieving management goals, proactive algaecide treatments could be incorporated into management plans. This entails developing a strategic monitoring plan with a defined action threshold, such as cell density, to prompt algaecide treatments early in a growth cycle (Kinley-Baird et al. 2020).

**SUMMARY AND PATH FORWARD:** Effective preventative management strategies are needed to alleviate the growing impacts of HABs to freshwater resources. There are numerous anticipated benefits if preventative management strategies are effective. Early year preventative treatments using algaecides (or other existing or emerging products or technologies) could be strategically designed to mitigate viable overwintering cells prior to germination and growth, potentially minimizing the biomass and severity of HABs that form later in the year. In turn, this should also decrease the frequency of treatments and/or total magnitude (volume and scale) necessary for treatments later in the peak growing season. This tactic could be coupled with other ongoing near-term (proactive algaecide treatments) or long-term prevention and mitigation strategies, including watershed nutrient reduction initiatives (e.g., nutrient source controls and wetland/riparian restoration). Additionally, ongoing research is examining the quantitative connections between overwintering cell abundance and growth potential with omics and quantitative molecular techniques (such as quantitative polymerase chain reaction [qPCR]) to enhance the effectiveness of predictive tools used to guide proactive sediment treatments in the future. If early detection and preventive treatment of resting cells are effective, substantial value is added by decreasing severity of bloom events, human health risks, costs associated with HAB mitigation efforts, and treatment costs.

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