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REVIEW

Identification of cyanobacteria overwintering cells and environmental conditions causing growth: Application for preventative management

Alyssa J. Calomeni-Eck[1] | Andrew D. McQueen[1] | Ciera M. Kinley-Baird[2] | Tony Clyde Jr.[3]

[1]U.S. Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, Mississippi, USA

[2]Aquatic Control, Seymour, Indiana, USA

[3]U.S. Army Corps of Engineers, Tulsa District, Programs and Project Management Division, Tulsa, Oklahoma, USA

Correspondence
Alyssa J. Calomeni-Eck
Email: alyssa.j.eck@usace.army.mil

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Abstract

1. Harmful algal blooms (HABs) formed by freshwater cyanobacteria pose risks to human and ecological health globally. Some cyanobacteria form overwintering cells that remain in the sediment during non-ideal growth conditions and provide an inoculum for HABs during the growing season, perpetuating the cyanobacterial life cycle. Preventative management targeted at decreasing the viability of overwintering cells is an attractive strategy for limiting HAB formation and decreasing risks.
2. However, this approach is novel, and information is needed for sampling, identification and enumeration of overwintering cells in sediments as well as methods for determining if overwintering cells have the potential to contribute to HAB formation. Peer reviewed literature related to these topics are available; yet these data need to be synthesized and placed into the context of management. Therefore, a strategic review was conducted to inform methods and data needs for this preventative strategy.
3. To sample overwintering cells, corers or dredges are used to collect surficial (0–2 cm) sediment layers containing overwintering cells. Identification and enumeration of overwintering cells via light microscopy are aided by dilution, sieving, or density separation of cells from sediment. Incubation studies which simulate environmental conditions triggering akinete germination and cell growth provide evidence of overwintering cell viability.
4. Critical environmental conditions are light (≥0.5 $\mu mol\, m^{-2} s^{-1}$) and temperature (20–30°C) for triggering akinete germination and growth of quiescent vegetative *Microcystis* sp. cells, respectively. Limited information was available on the roles of mixing and dissolved oxygen as environmental conditions for germination and growth.
5. Synthesized information can be used to identify potential areas of concern in which overwintering cells are contributing to HABs. Additionally, this information can be used to design incubation studies in which field collected sediments

containing overwintering cells are placed in ideal environmental conditions for germination and growth and cyanobacteria transferring to the water column are measured over time. These data inform investigations of areas that are candidates for preventative management and measurements of overwintering cell responses to management.

KEYWORDS
akinete, blue-green algae, control, harmful algal bloom, *Microcystis*, *Planktothrix*, viability

1 | INTRODUCTION

Harmful algal blooms (HABs) are visible accumulations of planktonic cyanobacteria in freshwater ecosystems, which have been recognized as a global threat (Carmichael, 2008; Chorus & Welker, 2021; Hou et al., 2022; Svirčev et al., 2019). Cyanobacteria can produce toxins that impact the liver, kidneys and nervous system. These toxins have been detected in all continents and have attributed to adverse effects and mortalities in humans, domestic animals, livestock, and ecological receptors (e.g. elephants, giraffes, deer) within all continents apart from Antarctica (Backer et al., 2013; Jungblut et al., 2006; Veerman et al., 2022; Wood, 2016). Additionally, impacts from HABs have been experienced in terms of economic losses when waterways in proximity to tourist destinations (Steffensen, 2008) and private properties (Environmental Consulting & Technology, Inc. [ECT], 2015; Paerl et al., 2018) become inundated. Significant costs have also occurred due to the need to mitigate toxin concentrations in drinking water that approach or exceed drinking water health advisories. For example, Toledo, Ohio, [United States] spent approximately $3 million to $4 million in 2013 to mitigate toxin concentrations in potable water (ECT, 2015).

To mitigate risks from HABs and their toxins, management actions are implemented at a range of spatial and temporal scales from large-scale watershed management of nutrients to small-scale management of municipal water treatment facilities where toxins are removed from potable water (Interstate Technology & Regulatory Council (ITRC), 2021; Sklenar et al., 2016; United States Environmental Protection Agency (USEPA), 2015). Part of the management solution should include prevention. A novel preventative approach is to target overwintering cells in sediments with the goal of interrupting the cyanobacterial life cycle to delay the onset and decrease the severity of HABs (Calomeni et al., 2022; Figure 1). This

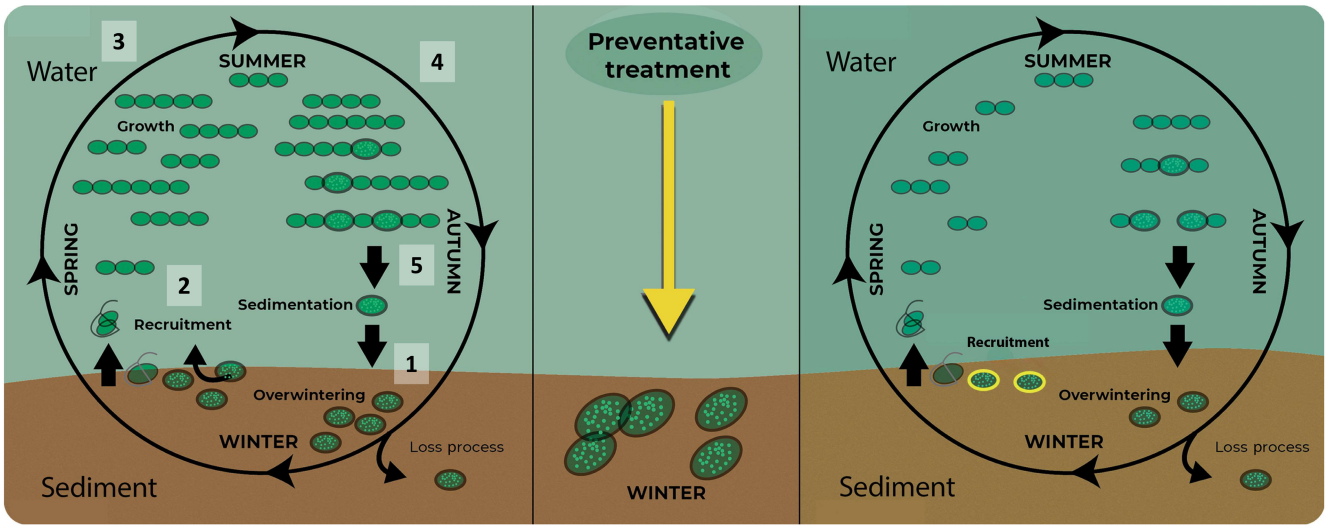

FIGURE 1 Life cycle of akinete producing cyanobacteria with preventative treatment decreasing inoculum for HABs. Briefly, the life cycle consists of a series of steps: (1) akinetes remain quiescent in sediments during non-ideal growth conditions (e.g. winter), (2) germling cells release from akinetes (i.e. germination) and suspend in the water column (i.e. recruitment) or akinetes become suspended in the water column where they germinate, (3) cyanobacteria growth produces a harmful algal bloom (HAB), (4) akinetes are produced and HAB senesces, (5) dense akinetes settle to the sediment phase (Hense & Beckmann, 2006; Kaplan-Levy et al., 2010). Preventative management may decrease the viability of akinetes in the sediment phase leading to lower germination rates. Yellow arrow indicates a preventative treatment (e.g. chemical, physical or biological) and yellow ovals surrounding akinetes in the right panel indicate cells made non-viable from preventative treatments. Cyanobacteria such as *Microcystis* sp. and *Planktothrix* sp. do not form akinetes. However, the vegetative cells remain quiescent in sediments during the winter and similarly contribute to bloom formation.

preventative approach is aimed at targeting the resting benthic stage of the life cycle, which has to date been largely ignored (Cottingham et al., 2021). Common HAB producing cyanobacteria that are known to form specialized overwintering cells or akinetes are of the order Nostocales (e.g. *Aphanizomenon* sp., *Dolichospermum* sp. [formerly *Anabaena*], *Raphidiopsis* sp. [formerly *Cylindrospermopsis*]). Other common HAB producing cyanobacteria, including *Microcystis* sp. and *Planktothrix* sp., do not produce akinetes, but overwinter as non-specialized vegetative cells that remain quiescent at depth during non-ideal growth conditions and contribute to HAB formation when conditions improve (Barbiero & Welch, 1992; Fallon & Brock, 1981; Kitchens et al., 2018; Micheletti et al., 1998; Preston et al., 1980). Akinetes and vegetative cells that remain quiescent under non-ideal growth conditions and provide a viable cyanobacterial inoculum for planktonic HABs are collectively termed overwintering cells throughout this manuscript. Therefore, overwintering cells refers to both akinetes and quiescent vegetative cells, akinetes refers to the specialized cells produced by the cyanobacteria order Nostocales, and quiescent vegetative cells refers to non-specialized resting cells in this manuscript.

Because preventative management of overwintering cells is novel, there are limited resources available to provide guidance for implementation. There are three preliminary knowledge gaps that need to be addressed, (1) how to sample, detect and identify overwintering cells, (2) what environmental conditions trigger germination and growth of overwintering cells and (3) how to determine if overwintering cells are viable and have the potential to contribute to HAB formation. Since overwintering cells are quiescent, they are challenging to detect and often evade traditional monitoring protocols that tend to focus on HABs in the planktonic phase (e.g. visible scums, blooms; Wood et al., 2020). Identification of overwintering cells provides a line of evidence for potential inocula contributing to HAB formation. However, the presence of overwintering cells does not equate to viability, germination, and growth. In fact, overwintering cells (i.e. akinetes) could remain quiescent in sediments for more than 50 years (Livingstone & Jaworski, 1980; Wood et al., 2008, 2021) and reportedly after 1800 years (Legrand et al., 2019) in sediment cores before germinating under suitable environmental conditions. Germination refers to the event where a germling (i.e. early stage of a cyanobacterial cell) emerges from the envelop that surrounds the akinete and could contribute to a HAB. Identifying environmental conditions that lead to overwintering cell germination and growth will be critical to provide additional lines of evidence for areas that have the potential to provide an inoculum for HABs. Sediment zones that contain viable overwintering cells and suitable environmental conditions for overwintering cell germination and growth are termed areas of concern and would be candidates for preventative management (Calomeni et al., 2022).

The aim of this manuscript is to conduct a strategic literature review to inform field monitoring programs and the preventative management of overwintering cells. Specifically, the objectives of this review were to (1) determine methods for sampling, detecting and identifying overwintering cells within sediments, (2) identify environmental conditions (e.g. light, temperature, nutrients, mixing [movement of cells from sediment to water column] and dissolved oxygen) necessary for the germination and growth of overwintering cells and (3) discuss how environmental conditions can be used to inform identification of areas of concern and responses of overwintering cells to management action.

2 | STRATEGIC LITERATURE REVIEWS

2.1 | Sampling and identification methods for overwintering cells

A strategic literature review was conducted of Google Scholar for articles, with the following search terms: akinetes, cyanobacteria, detect, identification, *Microcystis*, overwintering, resting, sample, sediment. Articles included those published from 1974 to 2021. Titles and abstracts were reviewed to determine if manuscripts fit within the scope of this review. Mainly, manuscripts were excluded from this review if their focus was not on overwintering cells of freshwater cyanobacteria (e.g. cyanophages, bacteria, toxins). The focus of this literature review was on overwintering cells, collectively, as this information may be used to inform the identification of areas of concern for which akinetes and quiescent vegetative cells can serve as inocula for blooms. A secondary review of methods was conducted for manuscripts retained during this initial review. Manuscripts included in this study were selected on the basis that relatively standard and common methods were used that could be implemented in laboratories that conduct enumeration of planktonic algal cells using light microscopy with few modifications for enumeration of benthic overwintering cells. Therefore, methods included in this manuscript use widely available tools and have a minimal cost barrier.

2.2 | Environmental conditions for overwintering cells

Environmental conditions for akinetes germination and quiescent vegetative cell growth were defined a priori as light, temperature, nutrients (i.e. nitrate, ammonium and phosphate), mixing and dissolved oxygen. Strategic literature reviews were performed separately for akinetes and quiescent vegetative cells as these cell types are different in terms of both structure and function and may have different environmental conditions leading to germination and growth, respectively. Akinetes differ from vegetative cells as they are specialized and are often larger and denser (Adams & Duggan, 1999; Sukenik et al., 2012). Akinetes also contain additional energy storage molecules (i.e. glycogen and cyanophycin) and are surrounded by a thickened cell envelop to remain viable during unfavourable growth conditions (Fay, 1988; Sukenik et al., 2012). Quiescent vegetative cells are morphologically like vegetative cells present in HABs with a few exceptions (Reynolds et al., 1981; Verspagen et al., 2004).

Quiescent vegetative cells of *Microcystis* sp. have been observed with aerotopes (i.e. gas vesicles), chlorophyll-*a* and glycogen granules. However, the photosynthetic efficiency and the number of energy storage molecules are decreased.

2.3 | Environmental conditions for germination of akinetes

To identify environmental conditions needed for the germination of akinetes a search of Google Scholar was conducted, using the following search terms: akinete, cyanobacteria, germination, recruitment, resting in combination with specific search terms for environmental conditions of interest. These search terms were light, temperature, nutrients, ammonia, nitrate, phosphate, mixing, recruitment, wind, wave, migration and dissolved oxygen (DO). Manuscripts included those published from 1976 to 2021. Titles and abstracts were initially reviewed to ensure that only genera that are commonly associated with HABs were included (Beaver et al., 2018; Graham et al., 2008, 2020; Rosen & St. Amand, 2015).

A secondary review of methods was conducted to determine if experimental designs were unconfounded. Laboratory and mesocosm studies were included in this review as they allow for the isolation of individual environmental conditions or independent variables while other conditions remain constant (i.e. unconfounded; Graney et al., 1995). Results from field studies were used as supporting information when unconfounded data were limited and these studies were specifically identified in subsequent sections. The response measurement for akinete germination was visual confirmation via light microscopy. Visualization of a newly emerged cyanobacteria cell from the akinete envelop by light microscopy has been reported by multiple authors (Baker & Bellifemine, 2000; Kim et al., 2005; Rolland & Vincent, 2014).

2.4 | Environmental conditions for growth of quiescent vegetative cells

A separate Google Scholar search was performed for quiescent vegetative cells and search terms were: overwintering, resting, quiescent, *Aphanocapsa*, *Microcystis*, *Planktontrix*, *Pseudanabaena* and *Woronichinia* with specific search terms for environmental conditions of interest as previously listed. Manuscripts included those published from 1975 to 2021. The methods were reviewed to (1) determine if experimental designs were unconfounded (i.e. laboratory and mesocosm studies) and (2) discern that the measurement endpoint was inclusive of growth and not identification of cells alone. Identification of cells at one time point may overestimate the number of quiescent vegetative cells capable of growth as some of these cells may later degrade. Therefore, response measurements needed to include measurements over multiple time points. Only studies that presented results of vegetative cells over time were included in this review.

3 | SAMPLING, DETECTON AND IDENTIFICATION OF OVERWINTERING CELLS

3.1 | Sampling

When planktonic HABs senesce, overwintering cells will begin to settle and become impacted by the same physical forces (e.g. resuspension, water velocity) that spatially distribute detritus and sediment. For example, *Dolichospermum* spp. akinete sizes range from 5 to 50 μm with most akinetes less than 20 μm (e.g. Komárek & Zapomělová, 2007; Figure 2). Based on Stokes' law, it is anticipated that akinetes of these size classes would be associated with silt (4 to 63 μm) size classes. Higher densities of akinetes have been measured

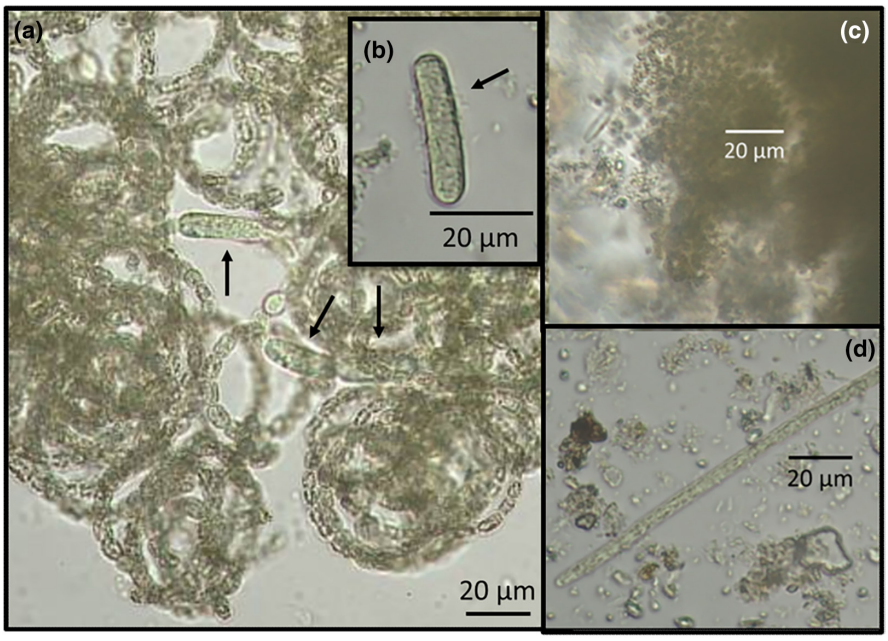

FIGURE 2 Microscopic images of overwintering cells. Akinetes in planktonic *Dolichospermum* (formerly *Anabaena*) in a water sample (a) and in a diluted sediment sample (b). Arrows point to akinetes. Quiescent vegetative cells of *Microcystis* sp. (c) and *Planktothrix* sp. (d) in diluted sediment samples.

in sediments dominated by clay (<4 μm) and silt (Huber, 1984; Kravchuk & Ivanova, 2009). Similarly, high densities of akinetes have been reported in areas with slower water velocities allowing smaller particles to settle such as in deposition zones upstream of a dam (Cirés et al., 2013) and areas with dense submerged aquatic vegetation (Kravchuk & Ivanova, 2009). Higher densities of overwintering cells have also been reported in areas with deeper water columns (Cirés et al., 2013; Legrand et al., 2017; Reynolds et al., 1981).

Within a sediment profile, higher densities of overwintering cells are at approximately 0 cm to 2 cm from the sediment–water interface (Kravchuk & Ivanova, 2009; Takamura et al., 1984; Tsujimura & Okubo, 2003). Densities of overwintering cells in sediment range by 5 orders of magnitude (Cirés et al., 2013). Units used to express overwintering cell densities range and are represented in terms of cells or akinetes per weight or volume of sediment or surface area (Brunberg & Blomqvist, 2002; Cirés et al., 2013; Kravchuk & Ivanova, 2009; Ramm et al., 2012). Sampling of sediments containing overwintering cells along the shoreline at wadable depths can be conducted with hand-held devices such as a shovel or hand core (Calomeni et al., 2022). Sampling at greater depths can be performed with a remotely activated device such as Wildco [Wildco Supply Company®, FL, USA], Eckman or Ponar grab samplers (Calomeni et al., 2018, 2022; Lind, 1974).

3.2 | Detection and identification

Common HAB genera that produce akinetes include (*Anabaenopsis*, *Aphanizomenon*, *Dolichospermum* [formerly *Anabaena*]), *Nodularia* and *Raphidiopsis*. Common HAB producing genera that can have quiescent vegetative cells include *Microcystis*, *Planktothrix* and *Woronichinia* (Table 1). To identify and enumerate overwintering cells using light microscopy, cells are separated from the sediment matrix. Effective methods for separating cells from sediment are dilution, particle size and density separation. Dilution is a relatively simple method for separating overwintering cells and researchers have utilized 1:9, 1:20, 1:50 and 1:100 g sediment to ml water (e.g. filtered site water or distilled water to remove planktonic algae; Bunting et al., 2016; Calomeni et al., 2023; Cirés et al., 2013; Eilers et al., 2004; Huber, 1984; Rücker et al., 2009; Tsujimura & Okubo, 2003). Filtration has been used to separate overwintering cells from sediments using size differences (Cirés et al., 2013; Rücker et al., 2009). However, knowledge of the approximate size of the target cell is necessary and with a mixed assemblage collected from the field, this may be challenging. Density separation with relatively inert polyvinylpyrrolidone coated silica sols have also been used (Borges et al., 2016; Cirés et al., 2013; Legrand et al., 2017; Verspagen et al., 2004). Sonication may be necessary for disaggregating overwintering cells from sediments that are tightly bound (Legrand

TABLE 1 Order, genus, potential toxin target (i.e. organ or organ system) and overwintering cells of common HAB-producing cyanobacteria (citations for common HAB-producing cyanobacteria: Beaver et al., 2018; Graham et al., 2008, 2020; Rosen & St. Amand, 2015).

Order	Genus	Potential toxin target	Overwintering cell type	Reference[c]
Chroococcales	*Microcystis*	Liver	Vegetative	Preston et al. (1980), Reynolds et al. (1981) and Kitchens et al. (2018)
Nostocales	*Anabaenopsis*	Liver	Akinete	Komárek (2010)
	Aphanizomenon	Liver, Neuro	Akinete	Komárek (2010)
	Cuspidothrix	Neuro	Akinete	Komárek (2010)
	Cylindrospermopsis	Liver	Akinete	Komárek (2010)
	Dolichospermum[a]	Liver, Neuro	Akinete	Wacklin et al. (2009)
	Gloeotrichia	Liver	Akinete	Komárek and Mareš (2012)
	Nodularia	Liver	Akinete	Komárek and Mareš (2012)
	Nostoc	Liver	Akinete	Rajaniemi et al. (2005)
	Raphidiopsis	Liver, Neuro	Akinete	Komárek (2010)
	Sphaerospermopsis[b]	Liver, Neuro	Akinete	Komárek and Mareš (2012)
Oscillatoriales	*Planktothrix*	Liver, Neuro	Vegetative	Holland and Walsby (2008)
Synechococcales	*Aphanocapsa*	Liver	Vegetative[d]	Rolland and Vincent (2014), Roos et al. (1991)
	Pseudanabaena	Liver	Vegetative[d]	Agha et al. (2016)
	Woronichinia	Liver	Vegetative	Trimbee and Harris (1984) and Head et al. (1999)

[a]Formerly planktonic species of *Anabaena*.

[b]Formerly *Anabaena* and *Aphanizomenon*.

[c]Reference associated with documented overwintering cell type.

[d]Planktonic and benthic populations have been observed. The extent that benthic populations overwinter and transfer to the planktonic phase has not been reported in the literature identified during this review.

et al., 2017; Wood et al., 2021) prior to dilution, filtration or density separation techniques. However, careful consideration of sonication duration and intensity is important to minimize or avoid damage to cells (Rajasekhar et al., 2012).

4 | ENVIRONMENTAL CONDITIONS TRIGGERING GERMINATION OF AKINETES

4.1 | Light

There is consensus among the published literature that light is an important driver for the germination of akinetes (Huber, 1985; Karlsson-Elfgren et al., 2004; Kim et al., 2005; Rai & Pandey, 1981; Sutherland et al., 1985; Yamamoto, 1976). This is evident as no germination or low (6%) germination percentages has been found to occur in darkness (Huber, 1985; Karlsson-Elfgren et al., 2004; Kim et al., 2005; Yamamoto, 1976) and relatively low illuminances (100 LUX) and photosynthetic photon flux density (PPFDs) (0.5 $\mu mol\, m^{-2}\, s^{-1}$) were necessary for germination (Table 2). For context, the illuminance measured in daylight can range from 100 LUX (approximately 2 $\mu mol\, m^{-2}\, s^{-1}$) on an overcast day to 130,000 LUX (approximately 2500 $\mu mol\, m^{-2}\, s^{-1}$) in direct sunlight (Hänel et al., 2018) and depends on latitude and season. The recommended PPFD by the USEPA for algal growth studies is 86 $\mu mol\, m^{-2}\, s^{-1}$ (illuminance of 4306 LUX; United States Environmental Protection Agency (USEPA), 2002) and is approximately 170 times greater than the lowest reported PPFD that resulted in akinete germination. Maximum germination percentages among the reviewed studies ranged between 20% and 96% corresponding with illuminances of 1200 LUX to 3000 LUX and 50% to 90% germination corresponding with photosynthetic photon flux densities (PPFDs) of 5 to 100 $\mu mol\, m^{-2}\, s^{-1}$ (Table 2).

4.2 | Temperature

Germination occurred over a wide range of temperatures from 5°C to 35°C (Table 2; Huber, 1985; Karlsson-Elfgren et al., 2004; Kim et al., 2005; Park et al., 2018; Yamamoto, 1976); however, the reviewed data indicate that maximum germination occurred at 22°C to 27°C for most cyanobacteria investigated (e.g. *Nodularia spumigena*, *Anabaena cylindrica* and *Dolichospermum circinale*). The exception to this temperature range occurred in germination of *Dolichospermum flos-aquae* akinetes from a Korean reservoir which had maximum growth at approximately 10°C in the laboratory and at comparable temperatures in situ (Kim et al., 2005). Although there may be some cyanobacteria (e.g. *Dolichospermum flos-aquae*) that are suited for lower temperatures (i.e. 10°C), these results suggest that for multiple genera, the maximum germination of akinetes would be induced when temperatures reach 22°C to 27°C for 3 to 5-day consecutively (Fay, 1988; Huber, 1985; Park et al., 2018; Yamamoto, 1976).

4.3 | Nutrients

The reviewed data suggest that nutrient concentrations (e.g. nitrate and phosphate) are unlikely to play a critical role in triggering akinete germination. To produce known nutrient concentrations for experimentation, studies used culture media with nitrate, ammonium and phosphate salts added as nominal concentrations (Huber, 1985; Myers et al., 2010; Park et al., 2018; Rai & Pandey, 1981; Sutherland et al., 1985; Yamamoto, 1976). Multiple studies reported germination with no added nutrient sources (i.e. no sediment and nutrient free culture media; Table 2; Huber, 1985; Park et al., 2018; Sutherland et al., 1985; Yamamoto, 1976). However, akinetes may germinate at higher percentages when nutrient concentrations are within specific ranges. For instance, one study reported that phosphate concentrations between 27 $\mu g\, P\, L^{-1}$ as K_2HPO_4 and 6937 $\mu g\, P\, L^{-1}$ as K_2HPO_4 as nominal concentrations had no impact on the germination percentage for *Nodularia spumigena* akinetes (Huber, 1985). Another study reports a dose–response relationship between phosphate concentrations and percent germination of *Nodularia spumigena* akinetes (Myers et al., 2010). The greatest germination percentage (40%) occurred at nominal concentrations of 1500 to 2500 $\mu g\, P\, L^{-1}$ as K_2HPO_4 (Myers et al., 2010). Similarly, high germination rates (90%–95%) occurred with no known addition of nitrate to concentrations of nitrate of 9996 $\mu g\, N\, L^{-1}$ as $NaNO_3$ (Huber, 1985.) Alternatively, a dose–response relationship was observed for nominal concentrations of nitrate and germination in Myers et al. (2010). The greatest percent germination (i.e. 17% to 25%) was observed at nitrate amendments from 1500 to 3000 $\mu g\, N\, L^{-1}$ as $NaNO_3$.

4.4 | Mixing

Mixing or the movement of cyanobacteria cells from the sediment to the water column is necessary for the formation of HABs from benthic overwintering populations. This movement can occur from passive suspension by thermocline or wind driven turnover as well as active suspension from formation of gas vacuoles (Karlsson-Elfgren et al., 2004). Karlsson-Elfgren et al. (2004), conducted laboratory experiments with test tubes containing filtered site water (25 mL) and site-collected sediment (3 mL collected with a 7 cm diameter core sampler) with *Gloeotrichia enchinulata* akinetes. When sediments were intentionally mixed with surficial water over the course of a 20-day experiment, cyanobacteria in the water column were identified earlier (2–4 days) relative to unmixed treatments. Greater cyanobacteria biovolumes (e.g. in one treatment two orders of magnitude greater) were measured in the water column in mixed treatments relative to the unmixed treatments. In theory, mixing surficial sediments may expose more viable overwintering cells to suitable environmental conditions for germination (e.g. light) that may have been sequestered by overlying sediment. Yet, there are relatively limited data investigating the

TABLE 2 Reported environmental conditions (light, temperature, nutrients, mixing, and dissolved oxygen) influencing akinete germination.

Environmental condition	Range for germination	Value for maximum germination[a]	Maximum percent germination	Units	Reference
Light[b]	100–8000	1200-3000	20%–96%	LUX	Yamamoto (1976), Rai and Pandey (1981) and Sutherland et al. (1985)
	0.5–100	5–100	50%–90%	$\mu mol\, m^{-2}\, s^{-1}$	Huber (1985), Kim et al. (2005) and Myers et al. (2010)
Temperature	5–35	10–27	20%–85%	°C	Yamamoto (1976), Huber (1985), Fay (1988), Kim et al. (2005) and Park et al. (2018)
Nutrients					
Nitrate	No nitrate source—9996	No nitrate source—9,996[c]	90%–95%	$\mu g\, N\, L^{-1}$ as $NaNO_3$	Yamamoto (1976), Rai and Pandey (1981), Huber (1985), Sutherland et al. (1985), Myers et al. (2010) and Park et al. (2018)
Ammonium	No ammonium source to 630[d]	No added to 630	60%–85%	$\mu g\, N\, L^{-1}$ as NH_4Cl	Huber (1985)
Phosphate	No phosphate source—6937	27–6,937[c]	75%–95%	$\mu g\, P\, L^{-1}$ as K_2HPO_4	Rai and Pandey (1981), Huber (1985), Sutherland et al. (1985), Myers et al. (2010) and Park et al. (2018)
Mixing	Mixing or no mixing	Mixing	Germination occurred earlier and had a larger biovolume in the aqueous phase with mixed samples	Not applicable	Karlsson-Elfgren et al. (2004)
Dissolved oxygen	Insufficient data				

[a]The value of the environmental conditions that resulted in the maximum germination response from each study (where applicable) is reported as a range.

[b]Units for illuminance and photosynthetic photon flux density (PPFD) were not converted among studies. Illuminances and PPFDs represent those that were reported in the individual study.

[c]Myers et al. (2010) observed a positive correlation between nitrate and phosphate concentrations and akinete germination. Maximum germination (10%–25% for nitrate and 20%–45% for phosphate) was observed at 1500 and 3000 $\mu g\, N\, L^{-1}$ as $NaNO_3$ and 1500 and 2500 $\mu g\, P\, L^{-1}$ as K_2HPO_4.

[d]Percent germination declined with ammonium concentrations in excess of 630 $\mu g\, N\, L^{-1}$ as NH_4Cl.

specific causal mechanisms of turbulent mixing at the sediment–water interface and relationship to akinete germination.

4.5 | Dissolved oxygen

Additionally, there are limited data from controlled laboratory experiments on the role of dissolved oxygen concentrations in akinete germination. Kim et al. (2005) measured akinete germination with different environmental conditions in controlled laboratory experiments and within an aquatic system (Seokchon Reservoir, Seoul, Korea). However, dissolved oxygen concentrations were not evaluated in the laboratory, but monitored in the field; therefore, the impact of oxygen concentrations could not be isolated. Within Seokchon Reservoir, dissolved oxygen concentrations remained aerobic (7 mg O_2/L to saturation limit) throughout the monitoring period and no correlations were observed between akinete germination and oxygen concentrations (Kim et al., 2005).

5 | ENVIRONMENTAL TRIGGERS FOR GROWTH OF QUIESCENT VEGETATIVE CELLS

The published literature related to the impacts of environmental conditions on quiescent vegetative cell growth largely pertains to the cyanobacterial genus *Microcystis* (Borges et al., 2016; Misson & Latour, 2012; Reynolds et al., 1981; Yang et al., 2020). Therefore, this summary focuses on environmental conditions impacting the growth of quiescent vegetative cells of *Microcystis* sp. associated with the sediment phase.

5.1 | Light

The impacts of light on quiescent vegetative *Microcystis* sp. growth ranged at a function of sample location within and among freshwater ecosystems (Borges et al., 2016; Misson & Latour, 2012; Reynolds et al., 1981). Light ranging from 600 to 1800 LUX positively impacted

TABLE 3 Reported environmental conditions (light, temperature, nutrients, mixing, and dissolved oxygen) influencing growth of quiescent vegetative cells of *Microcystis* sp.

Environmental condition	Range evaluated	Value for maximum response	Maximum response	Units	Reference
Light	Dark control—1800	600–1800	Colony concentration 1.4 times greater than dark control	LUX	Reynolds et al. (1981)
	Dark control—100	100	No impact on cell density to 1.4 times greater cell density[a] relative to dark control	$\mu mol\, m^{-2} s^{-1}$	Misson & Latour, 2012 and Borges et al. (2016)
Temperature	4–35	20–30	Increased growth rate observed[b]	°C	Reynolds and Walsby (1975), Borges et al. (2016), Yang et al. (2020) and Cai et al. (2021)
Nutrients					
Nitrate and ammonium[c]	40–1900	500	Order of magnitude increase in *Microcystis* number enclosure^{-1} relative to other concentrations evaluated	$\mu g\, N\, L^{-1}$ as nitrate and ammonia	Ståhl-Delbanco et al. (2003)
Total phosphorus[c]	30–230	130		$\mu g\, P\, L^{-1}$ as total phosphorus	
Ammonium	No ammonium source—5000	100–500	No impact on cell density to 2.0 times greater cell density relative to other concentrations evaluated	$\mu g\, N\, L^{-1}$ as NH_4Cl	Borges et al. (2016)
Mixing	Mixing or no mixing	Mixing	Statistically significant ($p < 0.01$) increase in the ratio of cells in the water column to cells in the sediment relative to the treatment without mixing	Not applicable	Misson and Latour (2012)
Dissolved oxygen	Insufficient data				

[a]Sediments were collected from near-shore and mid-lake. Light impacted cell densities for mid-lake samples and not near-shore samples (Borges et al., 2016).

[b]Growth rate significantly greater ($p < 0.05$) at temperatures from 15 to 35°C relative to 4–14°C (Yang et al., 2020).

[c]Amendments of N (amended as $Ca(NO_3)_2$ and measured as nitrate and ammonium) and P (amended as KH_2PO_4 and measured as total phosphorus) added to the same enclosures in situ (Ståhl-Delbanco et al., 2003). The impact of N and P as separate nutrients cannot be discerned from this study.

the growth of quiescent vegetative *Microcystis* sp. from a Lake located in England (Reynolds et al., 1981). In Reynolds et al. (1981), colony densities (colony mL^{-1}) were greater at 600 and 1800 LUX relative to dark controls from 10 to 31 days. Thirty-one days post experiment initiation, colony densities were approximately 1.4 times greater at 600 and 1800 LUX relative to dark controls. No significant differences in terms of percent recruitment (2.3%–3.3%) were reported by Misson and Latour (2012) at relatively low light intensities ($10\, \mu mol\, m^{-2} s^{-1}$) and in dark controls using sediments containing quiescent vegetative *Microcystis* sp. from a lake in France. In Borges et al. (2016), two sediment samples were collected from different locations, near-shore and mid-lake, within a lake in New Zealand. For near-shore samples, planktonic cell densities remained low ($<2000\, cells\, mL^{-1}$) and were similar in dark controls and a series of 4 increasing light intensities ranging from 1.5 to $100\, \mu mol\, m^{-2} s^{-1}$. In contrast, for sediments collected from near-shore, planktonic cell densities were greater in treatments with light ($50–100\, \mu mol\, m^{-2} s^{-1}$) relative to dark controls. At 12 days, planktonic cell densities were 1.4 times greater with a light intensity of $100\, \mu mol\, m^{-2} s^{-1}$ relative to dark controls (Table 3).

5.2 | Temperature

The growth of quiescent vegetative cells of *Microcystis* sp. was observed over a wide range of temperatures (4°C–35°C; Borges et al., 2016; Reynolds & Walsby, 1975; Yang et al., 2020; Table 3). Yang et al. (2020) reported rapid growth rates at temperatures ranging from approximately 15°C to 35°C, with the highest rate occurring at temperatures ranging from 20°C to 30°C in the laboratory. Elevated phycocyanin concentrations also occurred in the field at temperatures ranging from 24°C to 28°C (Yang et al., 2020). Borges et al. (2016) observed rapid growth rates at temperatures ranging from 16°C to 25°C in the laboratory setting. Reynolds and Walsby (1975) report initial bloom appearance in the field at temperatures ranging from 15°C to 18°C.

5.3 | Nutrients

Studies demonstrating the responses of quiescent vegetative cells of *Microcystis* sp. to nutrient concentrations are limited (Table 3). In

Ståhl-Delbanco et al. (2003), nutrient cycling between the sediment and water were evaluated using enclosures inserted into sediments maintaining contact between sediment and water (filtered site water) phases. Nutrient concentrations were measured following additions of nitrate and phosphate to the enclosures and the number of *Microcystis* sp. cells enclosure^{-1} was measured. *Microcystis* sp. in the unamended enclosures was less than 0.1×10^8 cells enclosure^{-1}. *Microcystis* sp. was an order of magnitude (1.1 to 1.6×10^8 cells enclosure^{-1}) greater in the enclosures with 500 μg N L^{-1} as nitrate and ammonium and 130 μg P L^{-1} as total phosphorus relative to all other enclosures. In a different study, lake sediments from two locations (near-shore and mid-lake) were collected with a Ponar grab sampler and placed in experimental chambers with milli-Q water prior to amendments with ammonium chloride (Borges et al., 2016). Relatively low cell densities were measured (≤2000 cells mL^{-1}) in unamended controls and in all amendments (series of 5 amendments from 100 to 5000 μg N L^{-1} as NH_4Cl) using near-shore sediment samples. For the mid-lake sediment sample, cell densities doubled at 100 to 500 μg N L^{-1} as NH_4Cl relative to the unamended control and 1000 to 5000 μg N L^{-1} as NH_4Cl. These results suggest that there is a relationship between *Microcystis* sp. cell densities and concentrations of nitrate, ammonia and phosphate.

5.4 | Mixing

Authors have discussed the role of the physical transfer of quiescent vegetative cells of *Microcystis* sp. from the sediment to the water in forming planktonic blooms (Borges et al., 2016; Misson & Latour, 2012; Verspagen et al., 2004). The transfer of quiescent vegetative cells of *Microcystis* sp. from the sediment to air-water interface may occur through passive processes (e.g. thermocline turnover), active processes (e.g. changes in density), or a combination (Verspagen et al., 2004). Active cell-mediated processes leading to the transfer of quiescent vegetative cells of *Microcystis* sp. have been explored using field collected sediments containing overwintering colonies. Verspagen et al. (2004) and Borges et al. (2016) report changes in cell buoyancy throughout the year. In terms of the impact of physical mixing of sediments and how that could relate to increased growth rates of *Microcystis* sp. cells, there are limited data. Misson and Latour (2012) discerned greater ratios of *Microcystis* sp. cells in overlying water relative to sediments in treatments where experimental chambers containing field collected sediment samples and filtered site water were inverted relative to chambers where intentional mixing did not occur. Therefore, these data suggest that mixing at the sediment–water interface may increase growth rates of quiescent vegetative cells of *Microcystis* sp.

5.5 | Dissolved oxygen

Data relevant to dissolved oxygen concentrations as drivers for growth of quiescent vegetative cells of *Microcystis* sp. are limited. Harris and Trimbee (1986) observed a correlation between a decrease in dissolved oxygen (minimum of 0–1 mg O_2/L) concentrations followed 10-day later by a shift in algal abundance to mostly *Microcystis* sp. Initial dissolved oxygen concentrations of 1 to 4 mg O_2/L resulted in the greatest rate of increase in colony densities ([colony/mL]/day) of *Microcystis* sp. observed by Reynolds et al. (1981).

6 | APPLICATION TO MANAGEMENT

6.1 | Identification of areas of concern

Areas of concern refer to sediments containing overwintering cells that have the potential to provide an inoculum for a HAB. Areas of concern are defined as locations in which viable overwintering cells are present and suitable environmental conditions exist at the sediment–water interface to trigger germination and growth (Calomeni et al., 2022; Rolland & Vincent, 2014; Figure 3).

To identify areas of concern for akinetes, a critical environmental condition to monitor would be light intensities (Huber, 1985; Karlsson-Elfgren et al., 2004; Kim et al., 2005; Rai & Pandey, 1981; Sutherland et al., 1985; Yamamoto, 1976). Ramm et al. (2017) similarly hypothesized that although akinete abundance increased with depth, there was a maximum water column depth in which sufficient light reached akinetes allowing for germination and akinetes located at greater depths were incapable of germination. Results from this literature review can be used to inform in situ monitoring by understanding ranges of light intensities that may result in germination. However, to transfer results from the laboratory to in situ monitoring, light attenuation needs to be considered. In the laboratory, the experimental chambers had small water volumes (i.e. μL to mL) and consequently light attenuation would be low. Therefore, light intensities reported in the laboratory experiments are likely directly comparable to light measured at the sediment–water interface and would not require correction for light attenuation in the water column if measured at this location. Alternatively, if light is measured at the air-water interface, correction for light attenuation through the water column is necessary.

For quiescent vegetative cells of *Microcystis* sp., temperature at the sediment–water interface could be used to inform observations of increases in cell densities in situ (Borges et al., 2016; Reynolds & Walsby, 1975; Yang et al., 2020). In situ, an increased growth rate in *Microcystis* sp. cell densities would be anticipated at temperatures ranging from 15°C to 35°C; with the greatest growth rates occurring at 20°C to 30°C (Yang et al., 2020). Measurements of the nutrients, nitrate, ammonia and phosphate and light at the sediment–water interface may provide predictive capability for growth rates of quiescent vegetative cells of *Microcystis* sp.

There were numerous studies pertaining to light and temperature as drivers for overwintering cell germination and growth. In contrast, data pertaining to nutrient concentrations were limited for both akinetes and quiescent vegetative cells. The identified ranges of environmental conditions can be used to inform in situ monitoring by identifying potential zones that may contribute to HABs. However, environmental conditions outside of these ranges may also result

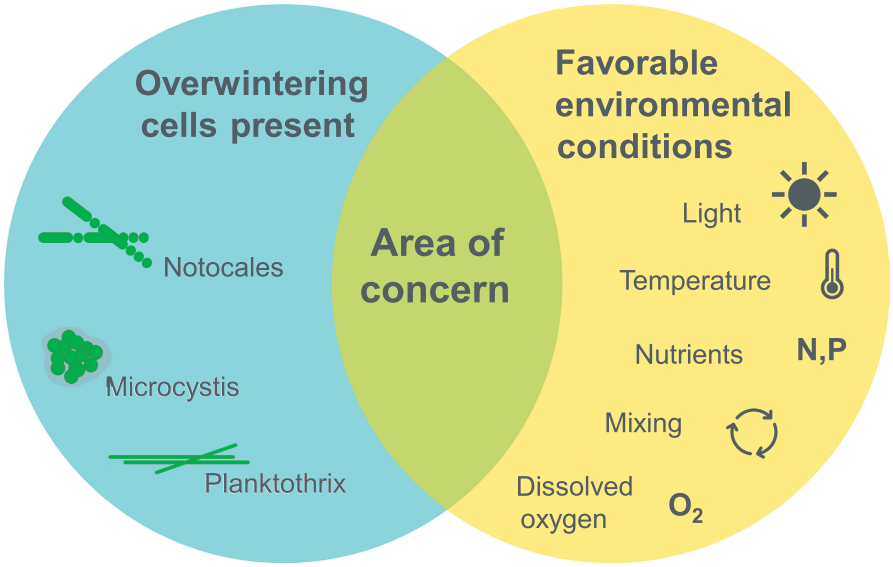

FIGURE 3 Characteristics of areas of concern or areas that may serve as an inoculum for harmful algal bloom formation. Areas of concern would contain overwintering cells (i.e. akinetes of Nostocales and quiescent vegetative cells of cyanobacteria such as *Microcystis* sp. and *Planktothix* sp.) and have favourable environmental conditions co-occurring.

in overwintering cell germination and growth. Responses of cyanobacteria to conditions in their environment may be species, site and ecotype specific. This was evident for *Dolichospermum flos-aquae* akinete germination, as one study identified maximum germination at 10°C (Kim et al., 2005) while another study identified maximum *Dolichospermum cicinale* akinete germination at 22°C (Fay, 1988). Ranges identified in the peer-reviewed literature and summarized here can be used to develop hypotheses regarding potential zones for HAB development, while further monitoring and experimentation can be used to refine ranges at specific sites.

6.2 | Measuring responses to management

For overwintering cyanobacteria, presence does not equate to the potential for germination, growth, transfer to the water column and HAB formation. Identification and enumeration of overwintering cells provides an estimate of the number of cells that are present at one point in time. A proportion of the identified overwintering cells may later degrade without transferring to the water column as vegetative cells (Ramm et al., 2017). To provide evidence of the growth potential of overwintering cells, laboratory experiments can be performed in which site collected sediments containing overwintering cells are placed in environmental chambers under conditions suitable for growth, termed incubation (Calomeni et al., 2023; Gangi et al., 2020; Rolland & Vincent, 2014; Wood et al., 2008). Following an incubation period (e.g. 10–14 days), overwintering cell densities are measured in the sediment and water phases to discern the potential for growth. These studies can be used to ask several questions. These questions include, (1) what cyanobacteria genera currently have the potential to transfer to the water column?, (2) how does planktonic growth potential at a site change throughout a year or following a growing season?, (3) how does planktonic growth potential change throughout a water body?, (4) how does the planktonic growth potential at a site change following management actions?

TABLE 4 Future research questions or data gaps related to preventative management of overwintering cyanobacteria.

Topic	Future research questions/data gaps
Problem formulation/ characterization	How prevalent are overwintering cells in HAB impacted water bodies?
	What is the relative contribution of overwintering cells from sediments versus allochthonous sources in HAB formation?
Planning and prioritization	How do you identify candidate sites for preventative management?
	How do you prioritize candidate sites for preventative management?
Management	What chemical, physical, or biological techniques are effective at managing overwintering cells?
	How can overwintering cells be practically managed in the field?
	What scale is appropriate for the management of overwintering cells?

Environmental conditions used for incubation studies should range as a function of the research question. If the goal is to understand if management action was capable of decreasing planktonic growth rates, environmental conditions resulting in maximum germination and growth that are realistic for the site should be used. As an example, targeted environmental conditions for akinetes could be 100 $\mu mol\, m^{-2} s^{-1}$ and 27°C. Since the results from this literature review suggest that nutrients are an unlikely trigger for germination, site collected sediments and water may be used without additional nutrient amendment.

7 | FUTURE RESEARCH NEEDS

To preventatively manage overwintering cyanobacteria in sediments within freshwater resources that experience seasonal HABs, additional data gaps need to be addressed (Table 4). Data are needed to improve our understanding of the extent in which overwintering cells can impact HAB formation. To plan and prioritize candidate sites for management, decision criteria are needed to inform site selection. To manage overwintering cell, experiments designed to understand the efficacy of physical, chemical and biological mitigation techniques are needed.

8 | CONCLUSIONS

Preventative management to decrease the planktonic growth of overwintering cyanobacteria in sediment prior to HAB formation is an attractive strategy to lessen human and ecological risks and economic impacts. This literature review informs methods for quantifying overwintering cells in sediment and assessing their potential to transfer to the water column where they could contribute to HAB formation. Methods for sampling overwintering cells are like those used for sediment collection and can be conducted using equipment such as an Eckman or Ponar dredge. Overwintering cells can be separated from sediments for identification and enumeration using dilution, particle and density separation.

Environmental conditions that trigger the germination of akinetes and the growth of quiescent vegetative cells of *Microcystis* sp. are critical to understand to assess the potential for cells to germinate (i.e. for akinetes) and grow. Relatively low light intensities (e.g. 0.5 $\mu mol\, m^{-2}\, s^{-}1$) at the sediment–water interface are a critical trigger for akinete germination while, temperatures between 20°C to 30°C resulted in rapid growth rates of overwintering *Microcystis* sp. These data can be used to inform incubation studies designed to discern the potential for overwintering cells to transfer to the water column as well as discern responses of overwintering cells to management.

AUTHOR CONTRIBUTIONS

Andrew D. McQueen, Ciera M. Kinley-Baird, Alyssa J. Calomeni-Eck and Tony Clyde Jr. conceived the ideas and designed methodology; Andrew D. McQueen and Alyssa J. Calomeni-Eck collected the data; Andrew D. McQueen and Alyssa J. Calomeni-Eck analysed the data; Andrew D. McQueen and Alyssa J. Calomeni-Eck led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The authors confirm that this review article does not involve the collection, generation or analysis of original data. The information presented herein is based on a literature review and DOIs are provided for manuscripts included in this review in the references section.

ORCID

Alyssa J. Calomeni-Eck https://orcid.org/0000-0002-4101-7197
Ciera M. Kinley-Baird https://orcid.org/0000-0002-6724-0442
Tony Clyde Jr. https://orcid.org/0000-0001-8863-411X

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