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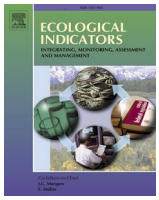

Review

State of knowledge on early warning tools for cyanobacteria detection

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ABSTRACT

The potential for cyanobacterial blooms to impact recreational and drinking water source quality is a growing concern. Numerous monitoring tools have been developed that can alert stakeholders to the onset of cyanobacterial blooms to initiate mitigation efforts for waters used for recreation or drinking water supply. Early warning monitoring systems need to consider multiple aspects of a cyanobacterial bloom: whether a bloom is occurring in the source water, whether it might be transported to drinking water intakes, whether toxin or taste and odor compound producers are present and what proportion of the cells in a bloom they comprise, and whether cells are entering a utility at concentrations above threshold levels. No single monitoring tool can provide all this information, so multi-barrier approaches are needed. Reviews of monitoring tools and their variations are available, but they are generally limited to one type of tool. Instead, a review and comparison of all the available tools is needed to inform stakeholders of them and their relative advantages and limitations. Therefore, this review covers conventional tools including microscopic enumeration, pigment extraction, qPCR, probes, and remote sensing as well as emerging techniques including next-generation sequencing, photonic systems, biosensors, drones, and applications of machine learning and discusses them primarily from a practical and operational standpoint. Moreover, a three-tier framework is proposed for designing comprehensive early warning systems that groups monitoring tools by their analytical targets: biological activity or algal biomass, cyanobacteria or cyanobacteria-related genes, and cyanobacterial metabolites. First tier tools are generally simple and inexpensive to use, including turbidity, optical density, visual inspection, drones, chlorophyll a, and adenosine triphosphate. Changes in water quality conditions detected using a first tier tool triggers the use of a second tier tools for identification and quantification of cyanobacteria by microscopy, phycocyanin, biosensors, hyperspectral remote sensing, or next-generation sequencing. If potentially harmful concentrations of cyanobacteria are confirmed, third tier tools are deployed for quantifying concentrations of cyanotoxins and taste and odor compounds or the genes that encode for them using enzyme-linked immunosorbent assays, mass spectrometry, qPCR, or other analytical methods. This framework is designed to minimize the time and cost associated with cyanobacteria monitoring without compromising the ability of stakeholders to detect the onset of a bloom.

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1. Introduction

Cyanobacteria are a group of diverse photosynthetic bacteria that can form blooms in water bodies (Bergkemper and Weisse, 2018; Seckbach, 2007). Climate change and excess nitrogen and phosphorus in water bodies promote cyanobacterial blooms, which may decrease phytoplankton diversity (Merel et al., 2013; Paerl and Paul, 2012). Moreover, toxins produced by cyanobacteria can induce serious illness or mortality in animals, including humans. Cyanobacteria can cause harmful algal blooms (HABs) and have become a serious global issue in freshwater lakes, rivers, and reservoirs (Zhou et al., 2017). Although multiple environmental factors contributing to bloom formation have been identified, the exact triggers that initiate their occurrence are still unclear (Huo et al., 2018).

Some strains of cyanobacteria can produce potent toxins that may cause acute or chronic effects. Human exposure to cyanotoxins can occur through drinking water, recreational activities, or ingestion of seafood grown in HAB-impacted waters (Merel et al., 2013; Shang et al., 2018; Zamyadi et al., 2012a). Cyanobacteria can also produce taste and odor compounds, which affect water aesthetics and consequently the perception of drinking water quality by consumers and recreational water users, although these are typically not regulated. Drinking water quality guidelines related to cyanobacteria are based on maximum acceptable concentrations of toxins in treated water supplies (Courtois et al., 2018). Among all the cyanotoxins, microcystins are the most monitored group of cyanotoxin for water quality evaluation due to their high toxicity and widespread occurrence (Banerji et al., 2019; Du et al., 2019; Lee et al., 2018). Globally, drinking water guidelines typically limit the concentration of microcystins (expressed as total microcystins, microcystin-LR, or microcystin-LR equivalents) to 1 to 2 μg/L for the general population and 0.3 to 0.4 μg/L for bottle-fed infants.

Due to the potential for HABs to cause aesthetic and regulatory issues, a wide range of techniques for monitoring cyanobacteria have been developed. However, accurately predicting cyanobacterial blooms in freshwater sources remains a challenge, so many utilities have focused on early warning systems to gain sufficient response time to react to potentially toxic HABs (Beardall and Raven, 2004; Bissinger et al., 2008; Geider and La Roche, 2002; McQuaid et al., 2011; Mohlin et al., 2012; Paerl et al., 2014). These source water monitoring programs require extensive financial resources, staff time to complete sampling and laboratory analyses, and technical expertise to interpret data. Alert levels for what constitutes a HAB are scattered throughout the literature and other sources and include different water quality parameters (Ahn et al., 2007; Chorus and Bartram, 1999; EPA Office of Water, 2015; Hazen and Sawyer, 2015; Newcombe, 2009). As a result, utilities are faced with making independent decisions on thresholds that prompt monitoring of cyanotoxins. This has resulted in an extreme diversity in the monitoring and detection tools deployed, ranging from visual identification to more sophisticated online monitoring with buoys equipped with probes. Despite these efforts, routine monitoring programs have been historically unreliable for alerting water utilities to cyanobacterial blooms (with only 53% reporting success in being notified of developing HABs) (AWWA, 2016). Furthermore, existing detection systems are often susceptible to interferences. Therefore, a synthesis of the knowledge in the literature and a summary of the advantages and limitations of current monitoring tools would benefit utilities that face HAB risks.

A variety of different water quality indicators have been proposed for early detection of cyanobacterial blooms, including cell counts (Chorus and Bartram, 1999), chlorophyll a (Izydorczyk et al., 2009), extracted phycocyanin (Ahn et al., 2007), and phycocyanin fluorescence (Almuhtaram et al., 2018). Threshold values (medium, high, very high) are often provided to prompt utilities to begin additional monitoring or treatment (Newcombe, 2009). The ability to monitor cyanobacteria promptly and accurately, as well as associated toxins and taste and odor compounds, in order to correctly identify the exceedance of an alert threshold, is thus a key factor in the implementation of a successful risk management strategy (Zamyadi et al., 2016). However, these parameters and guidelines vary with respect to sensitivity and time of analysis. Furthermore, cyanobacterial biomass and community composition are highly inconsistent in time and space; therefore, their characterization requires an analytical approach that captures this variability (Zamyadi et al., 2016).

Some parameters can be measured in real-time and in situ using water quality probes, while other parameters require laboratory space and longer turnaround times before the information can be used to make decisions. For example, many utilities continue to rely on microscopy to enumerate cyanobacteria although this may require two to five days to receive a result (Zamyadi et al., 2016), which is problematic because the doubling time of cyanobacteria populations can be as fast as 1.24 days (Moisander et al., 2009). However, relying solely on real-time monitoring tools is not a satisfactory solution because they do not identify the potential for toxin or taste and odor compound production (Zamyadi et al., 2016). Thus, early warning systems should include multiple complimentary technologies to encompass real-time cyanobacteria monitoring, cyanotoxin and taste and odor compound measurements, and forecasts for cell growth. Therefore, a review of current monitoring tools and the potential for the interaction of their measurements to aid in decision-making for drinking water and recreational water stakeholders is warranted. However, existing reviews have been limited to specific types of monitoring tools such as satellite remote sensing (Dörnhöfer and Oppelt, 2016; Yan et al., 2018), fluorescence-based probes (Bertone et al., 2018; Zamyadi et al., 2016), drones (Wu et al., 2019), biosensors (Vogiazi et al., 2019), and qPCR (Pacheco et al., 2016). Despite recent studies investigating the advantages and limitations of some of these methods, a systematic study to collect all the available techniques and information about the performance and of these methods in early warning systems is not available. He et al. (2016) reviewed several types of sensors and monitoring systems for cyanobacteria and cyanotoxins in the context of a multi-barrier management approach, including prevention, source control, monitoring, and treatment. In contrast, this review is focused on summarizing and assessing cyanobacteria monitoring technologies, especially recent advances, and identifying potential ways to combine them to create a robust early warning system.

This review proposes a three-tier cyanobacteria system comprising tools for detecting biological events in the first tier, tools for confirming cyanobacteria presence in the second tier, and tools for detecting cyanobacterial metabolites and the genes that encode for them in the third tier. In general, first-tier tools are the least expensive and may be used at high frequencies to monitor baseline biological activity. A deviation from the baseline would then trigger the use of second and third-tier tools to confirm that the increased biological activity is due to cyanobacteria, and that cyanobacteria metabolites (toxins and taste and odor compounds) are present. This is a cost-saving approach that ensures that expensive third tier monitoring tools are used strategically when the likelihood of cyanobacterial metabolites being present is highest. However, costs associated with cyanobacteria monitoring depend heavily on whether capabilities are available onsite or must be contracted to third-party labs. This distinction also significantly affects turnaround time, which, for some tests, can range from less than an hour if conducted onsite to a week if conducted by a third-party lab. Therefore, designing a cyanobacteria monitoring system is a multi-faceted problem that requires careful consideration of a utility's capabilities and, most importantly, the choice of monitoring tools.

2. First Tier: Detecting biological activity

The objective of this monitoring tier is to quantify biological activity in a water body or intake. Several tools are available for monitoring algal or biological activity in source waters and drinking water intakes including visual inspection, chlorophyll a extraction and fluorescence, adenosine triphosphate (ATP), and drones. The simplicity and low cost of these tools allows them to be used for routine monitoring at high

frequencies. By monitoring these parameters over the long term, utilities can establish alert thresholds that can be used to trigger monitoring of the second-tier tools to confirm the presence of cyanobacteria. The advantages and limitations of the first tier monitoring parameters are summarized in Table 1.

2.1. Monitoring tools for detecting biological activity

2.1.1. Visual inspection

The simplest indicator of biological activity in a water body is a change in its visual appearance. Changes to transparency and color, the presence of visible streaks, and the formation of cohesive scums of buoyant cells are indicators of blooms, surface blooms, and surface scums, respectively (Chorus and Welker, 2021). In calm, thermally stratified water, buoyant cyanobacteria such as *Dolichospermum* can float to the euphotic zone to receive light for photosynthesis while nonbuoyant phytoplankton sink, so surface streaks or scums may be due to cyanobacteria (Webster et al., 2000). However, visual inspection alone cannot be used to reliably conclude that cyanobacteria are present because some algae species also form dense algal mats similar in appearance to cyanobacterial scums (Kravtsova et al., 2014; Watson et al., 2015; Znachor and Jezberová, 2005). Therefore, visual monitoring is a first-tier tool because additional testing is needed to verify that cyanobacteria comprise an observed algal bloom.

Visual inspection suffers from other important drawbacks. First, not all cyanobacterial genera, such as *Planktothrix*, *Limnothrix*, *Raphidiopsis*, and *Pseudanabaena* form easily identifiable surface blooms or scums and instead may disperse homogeneously throughout a water body (Chorus and Welker, 2021). Other species have been reported to accumulate between thermal layers in a stratified water column and cannot be detected by visual inspection of the water surface (Konopka, 1989; Le Vu et al., 2011). This is problematic for drinking water utilities because intakes are often located deep below the water surface, and untreated cyanotoxins in water supplies can have disastrous consequences, as in the case of the Caruaru, Brazil incident in 1996 (Azevedo et al., 2002). Therefore, it is possible for a water body to appear free of algae activity at the surface but for cells to enter the plant via a deep raw water intake (Almuhtaram et al., 2018). Second, visual inspection may be less accurate depending on the time of day because surface blooms and scums formed when waters are calm, typically in the early morning, may be mixed back into the water column by wind. Third, it is not feasible for utilities to carry out visual inspection frequently if their intakes are in inaccessible locations, such as hundreds of meters offshore as is common for plants drawing water from large lakes and reservoirs. Fourth, cells may be imperceptible in open water but become visible as they are accumulated on leeward shores of water bodies by prevailing winds. Finally, visual assessments of source water quality can be subjective and limited to areas that are visually accessible. Specifically, unless a utility conducts visual inspection in benthic zones or there are floating mats in the source, visually inspecting source water surfaces may not warn a utility of benthic cyanobacteria. Nevertheless, visual inspection continues to be a useful tool for detecting biological events and is included in the guidance documents of some jurisdictions (Chorus and Welker, 2021; EPA Office of Water, 2015; Health Canada, 2016) and can be extended to screens, filters, and other parts of a drinking water treatment process in addition to source water (American Water Works Association, 2010).

2.1.2. Adenosine triphosphate (ATP)

Adenosine triphosphate (ATP) represents total viable planktonic biomass. It occurs only in living cells and although ATP cell quotas vary among species, it can be used to estimate biomass (Method 10,200 I. in Baird et al., 2017). ATP is a potential early-warning indicator of increasing planktonic biomass, but insufficient peer-reviewed research is available to support its adoption in source water monitoring. It has been used successfully for other monitoring purposes including bacterial growth in distribution systems (Delahaye et al., 2003), wastewater and surface water infiltration (Vang et al., 2014), and microbial biomass of biofilters (Singh Sidhu et al., 2018). Greenstein and Wert (2019) evaluated ATP as an early indicator of algal activity in a culture of *Microcystis aeruginosa* as well as environmental samples collected in Lake Mead, USA. ATP exhibited a strong correlation to cyanobacterial biomass ($R^2 = 0.97$) in the monoculture and a moderate correlation to extracted chlorophyll a in the environmental samples ($R^2 = 0.79$). The correlation in the environmental samples was lower than in the monoculture because of different ATP cell quotas in mixed species assemblages (Greenstein and Wert, 2019). Nevertheless, it is promising that ATP correlates to chlorophyll a, a well-established indicator of algal biomass. In another study, average cytoplasmic ATP concentrations among different species of aquatic microorganisms were reported to range from 0.62 to 1.37 mM ATP, suggesting that ATP monitoring is robust against diversity in microbial communities (Bochdansky et al., 2021). Finally, increases in the ATP content of *M. aeruginosa* and *Synechocystis* sp. have been shown to coincide with increased cell division and glycogen production, so an increase in ATP could be an early indicator for the start of bloom growth (Huang et al., 2014; Saha et al., 2016).

For ATP to be used as an early-warning indicator for algal blooms, baseline and normal fluctuations in ATP in source waters need to be understood. ATP is found in all living organisms, so a water body will always elicit an ATP response. Moreover, sudden changes in water quality can rapidly increase ATP concentrations despite the absence of a bloom. For example, a rainfall event that washes bacteria like *E. coli* from soil, animal fecal matter, and other land sources into a water body may increase ATP in the absence of algae (Bushon et al., 2009). If algae are present, their spatial distribution may be uneven, resulting in variable measurements depending on the selected sampling location in a water body. Therefore, care should be taken to select a statistically relevant and strategic locations within a drinking water supply or intake.

Commercially available ATP test kits are easy-to-use, relatively inexpensive, and have fast turnaround times (<10 min). Also, online ATP analyzers have emerged that would allow continuous measurements of source water, although they are relatively expensive. Furthermore, sampling depth can be optimized for both grab and online samples to monitor both pelagic and benthic niches. For ATP to be implemented, protocols need to be developed for determining site-specific thresholds that trigger additional monitoring or mitigative actions, but these are currently lacking due to the limited use of ATP for monitoring algal activity in drinking water sources. For example, Greenstein and Wert (2019) derived an ATP threshold of 175 pg/mL corresponding to 5 μg/L chlorophyll a in samples dominated by *Planktothrix agardhii/suspensa*, which is the standard for controlling disinfection byproduct (DBP) formation in the state of Colorado, USA. In addition, Bochdansky et al. (2021) established a correlation between molar ATP concentration and the concentration of *Thalassiosira weissflogi* in cells/mL, which suggests that a threshold ATP concentration can be derived for a cell concentration threshold for that species. Additional research is needed into using ATP to indicate the onset of blooms in source waters and drinking water intakes containing mixed assemblages of species.

2.1.3. General water quality parameters

Several general water quality parameters may be simple indicators of algal activity, including Secchi disc depth, turbidity, and optical density. Secchi disc depth is a measure of water transparency, which is impacted by phytoplankton abundance as well as turbidity and humic substances (Chorus and Welker, 2021). Waters with Secchi disc depths >2–3 m (i.e. are highly transparent) are unlikely to contain algae or cyanobacteria at concentrations that may adversely affect water quality. Secchi disc depth is the depth at which a disc is no longer visible while being submerged into a water body (Bowers et al., 2020). Thus, it simply represents water clarity, and low transparency (e.g., <2–3 m) can be used to trigger sample collection for determining whether the cause is due to

Table 1
Summary of first-tier monitoring tools for detecting biological activity.

Parameter	Analytical Method	Target	Alert Thresholds	Advantages	Disadvantages
Visual inspection	Surface water quality assessment by operators, citizen scientists, etc. Fixed cameras Drones fitted with cameras	• Visible algal or cyanobacterial scum or floating mats on the water surface; • Changes in surface water transparency and color	No established alert thresholds	• Simple to conduct; • No cost; • Rapid visual assessment of the severity and extent of a bloom • Option for continuous surface water quality surveillance and imagery data logging • Capture conditions at inaccessible locations; • Easy to deploy; • Can be equipped for sample collection; • Can be equipped with multispectral sensors to detect chlorophyll *a* or phycocyanin	• Visual inspection can be subjective; • Limited to sites that are visually accessible; • Camera installations can be challenging for some sites; • Flying drones is prohibited in some jurisdictions; • Battery capacity limits drone flight range, image quality, and payload weight; • Does not capture spatiotemporal variations in water quality; • Cyanobacteria confirmation needed • No indication of metabolite production
Water transparency or turbidity	Secchi disk	• Changes in surface water transparency and color	Secchi depth below 2 m with green discoloration indicates algal growth; (Chorus and Welker, 2021)	• Simple to conduct; • Relatively low cost	• Changes in clarity may originate from non-bloom related factors • Cyanobacteria confirmation needed • No indication of metabolite production
	Handheld Turbidimeter or online turbidity analyzers		No established alert thresholds	• Simple and rapid measurement • Relatively low cost • Online analyzers allow for high frequency real-time source water characterization	• Changes in clarity may originate from non-bloom related factors • Cyanobacteria confirmation needed • No indication of metabolite production • Susceptible to interference by turbidity;
	Optical density		No established alert thresholds	• Established surrogate for algal biomass; • Simple and rapid measurement • Relatively low cost	• Requires external calibration to estimate biomass concentration; • Cyanobacteria confirmation needed; • No indication of metabolite production
Chlorophyll *a*	Lab-based pigment extraction and analysis using spectrophotometry	• Changes in pigment concentration	**Medium**: 1 μg/L (Initiate toxin monitoring**High**: 12 μg/L (Chorus and Welker, 2021)	• Established surrogate for algal biomass; • Robust against cell morphologies and colonies; • Low detection limit	• Laborious laboratory process; • Potentially long turnaround time if outsourced • Cyanobacteria confirmation needed • No indication of metabolite production
	Fluorescence spectroscopy probes			• Online analyzers allow for high frequency real-time source water characterization • Easily deployable to assess pigments at different depths • Handheld probes are field-ready	• Susceptible to interference by turbidity; • Readings affected by cell morphology (colonial and filamentous); • Cyanobacteria confirmation needed • No indication of metabolite production
ATP	Handheld luminometers and ATP test sticks Online analyzers	• Changes in biological activity	175 pg/ml and 5 μg/L Chlorophyll *a* (Initiate additional monitoring) (Saunders et al., 2015)	• Simple and rapid assessment • Low cost; • Handheld meters and kits are field-ready • High frequency real-time source water characterization	• Non-specific measure of biological activity; • Limited adoption and little guidance available for best practices • Cyanobacteria confirmation needed • Analytical reagents can be costly for online analyzers • No indication of metabolite production

algae or cyanobacteria, or turbidity and humic substances. Turbidity measurements may be interpreted in a similar way. Drinking water utilities routinely measure turbidity in influent raw water, but elevated turbidity may be due to inorganic particulates in addition to algae (Chorus and Welker, 2021). A more specific indicator of phytoplanktonic biomass that is commonly used is optical density (absorbance) between 680 and 750 nm, although the presence of other organic compounds that absorb light at these wavelengths may interfere with this measurement (Baptista et al., 2009; Greenstein and Wert, 2019; Lv et al., 2018).

2.1.4. Chlorophyll a extraction

Chlorophyll a is a green pigment found in photosynthetic organisms that is responsible for photosynthetic activities (Weiqi Zhou et al., 2004). The concentration of chlorophyll a in a sample can be positively but not proportionally correlated with algal biovolume, so algal biomass in water bodies is typically estimated by chlorophyll a concentration (Ergun et al., 2004). Chlorophyll a extraction is widely used for this purpose because the extraction methods are well understood and relatively inexpensive and because it can be measured using spectrophotometric or fluorometric techniques (American Water Works Association, 2010). One common procedure involves measuring the absorbance of a filtered extract at 665 nm produced by dissolution in a solvent, such as methanol or acetone, or physical extraction, such as freeze-and-thaw or homogenization (Ergun et al., 2004; Iwamura et al., 1970). This process, however, can take up to 30 h to complete or longer if the measurement is made by a third-party lab (Bowling et al., 2016; Kasprzak et al., 2008). One of its main advantages is that unlike visual inspection, chlorophyll a extraction can be applied to pelagic or benthic samples to quantify algal biomass from samples collected from a raw water intake, throughout a water column, or in sediments (Smith et al., 2019).

Some jurisdictions, such as the World Health Organization (WHO), recommend alert levels for cyanobacteria based on chlorophyll a concentrations: 1 μg/L and 12 μg/L for Alert Levels 1 and 2, respectively (Chorus and Welker, 2021). It is estimated that chlorophyll a comprises 1.5% of dry algal organic matter, so algal biomass can be indirectly estimated from it (Method 10,200 I. in Baird et al. (2017). Various environmental and physiological factors affect chlorophyll a concentrations within organisms, and chlorophyll a cell quotas vary among species, making the correlation to algal or cyanobacterial biomass site-specific (Carvalho et al., 2009; Poikāne et al., 2010). A correlation may be established between chlorophyll a and cyanobacterial biovolume (Ahn et al., 2002), but the proportion of cyanobacteria in phytoplanktonic biomass can vary significantly with total biomass, so a constant conversion factor may not be reliable (Kasprzak et al., 2008). Moreover, chlorophyll a cell quotas display diurnal patterns, posing another challenge for correlating chlorophyll a to cell concentrations (Masuda et al., 2018). Consequently, a fixed concentration threshold of chlorophyll a, such as 1 μg/L, may correspond to significantly different biomasses in different water bodies. Additionally, correlations between chlorophyll a and cyanobacteria are generally weaker than correlations with more precise cyanobacteria metrics, such as phycocyanin (Ahn et al., 2007; Brient et al., 2008; Zamyadi et al., 2012b). Therefore, because chlorophyll a represents algal biomass in general it is a first-tier monitoring tool and additional testing is necessary to confirm the presence of cyanobacteria.

2.2. Remote sensing and fluorometry for detecting biological/bloom activity

2.2.1. Chlorophyll a fluorescence

Another method for quantifying chlorophyll a is using optical sensors that measure its fluorescence. chlorophyll a has excitation and emission maxima of 431 nm and 670 nm, respectively (Moberg et al., 2001). Several commercially-available products have been developed that leverage this feature to estimate chlorophyll a in situ and in real time and are reviewed in Zamyadi et al. (2016). Correlations between chlorophyll a determined by submersible fluorometers and conventional methods are generally high ($R^2 > 0.8$) but can be affected by water temperature, excitation and emission band widths, and the presence of colonial and filamentous cells (Gregor and Maršálek, 2004; Izydorczyk et al., 2009). Despite strong linearity, the slopes of such correlations are not 1, meaning that in situ fluorometry may over- or underestimate the concentration of chlorophyll a. Consequently, sensors need to be calibrated periodically using extracted chlorophyll a or otherwise be used qualitatively (Almuhtaram et al., 2021b). Regardless, chlorophyll a fluorescence, like chlorophyll a extraction, represents only total algal biomass.

2.2.2. Drones

The use of drones for source water monitoring and sample collection is an innovative approach that overcomes some of the limitations of other monitoring tools and sample collection practices. Drones can be equipped with cameras and sensors to quantify surface water conditions far from the shoreline, so while monitoring at a drinking water intake reveals the water quality entering a plant, drones can be used to assess the spatial distribution of algal blooms in a water source. Similarly, sample collection at a drinking water intake in response to elevated biological activity does not reflect the spatial and temporal variations of water quality in the source and cannot be used to forecast future conditions (Gholizadeh et al., 2016). Drones can be equipped with apparatuses for both sample collection and sensors for water quality measurement (Koparan, 2016; Koparan et al., 2018; Wu et al., 2019). For example, Kwon et al. (2020) equipped a drone with a fluorescence-based monitoring probe for in situ fluorescence measurements up to a depth of 5 m as well as sample collection. Levy et al. (2020) mapped microbial mats in the Arctic using a drone equipped with a high-resolution hyperspectral camera. For collecting surface water samples, Benson et al. (2019) 3D-printed a device to hold a conical tube and tethered it to a drone for sample collection up to 50 m away from the shore.

When employing drones for sample collection, one limitation is the thrust it can produce because it determines the payload that it can carry (Aguirre-Gómez et al., 2017; Koparan, 2016; Koparan et al., 2018). For source water monitoring, other important considerations are the compromise between battery capacity and image resolution, airspace restrictions in some jurisdictions, and undesirable weather (Wu et al., 2019). Because capturing high quality images and collecting large payloads require substantial battery power, drones may be suitable for short flights to otherwise inaccessible locations without sacrificing either aspect. A possible solution is to utilize a fleet of drones with some designed for capturing images or measuring water quality using sensors and others for sample collection, although this has yet to be reported in the literature.

Another important consideration when using drones for source water monitoring is the regulatory framework governing the use of unmanned aerial vehicles (UAVs). In some jurisdictions, it is not permitted to fly UAVs over drinking water intakes or other sensitive areas such as power stations that may be in proximity to a drinking water treatment facility. More restrictive prohibitions at the utility level might include not flying drones over open water at all to prevent the risk of losing them. In addition to local restrictions, the International Civil Aviation Organization (ICAO) is developing guidance for countries to adopt regarding UAVs that includes recommendations for registration, inspection, operator certification, and UAV operating conditions. Therefore, utilities considering using drones for source water monitor should be aware of the requirements specific to their jurisdictions.

3. Second Tier: Confirming the presence of cyanobacteria

The objective of this monitoring tier is to confirm the presence of cyanobacteria and the potential for toxin or taste and odor production to

determine whether metabolite analysis is needed. Tools for monitoring cyanobacteria include microscopic enumeration, phycocyanin extraction, real-time monitoring probes, hyperspectral remote sensing, photonic systems, biosensors, next-generation sequencing (NGS), and automated cell imaging. They may be implemented following the detection of elevated biological activity by a first-tier tool, but some can be used to take measurements at high frequencies, avoiding the need for monitoring biological activity entirely. High frequency monitoring tools, however, are associated with higher capital costs than grab sampling techniques. Cyanobacteria monitoring tools also vary significantly in the detail they provide, which ranges from basic assessments of total cyanobacterial biovolume to species identification. Monitoring probes and pigment extraction techniques measure the fluorescence or concentration of phycocyanin, which is found in all cyanobacteria, so these techniques cannot be used to determine whether taste and odor and toxin producers are present. In contrast, microscopic enumeration and automated cell imaging can identify cyanobacteria at the genus or species level and determine if the potential to produce adverse metabolites exists. NGS provides even more detail by identifying species that are otherwise difficult to differentiate visually using genetic markers.

Table 2 summarizes the advantages and disadvantages of second-tier tools. The decision to use one or more of these techniques depends on the specific monitoring needs as well as cost, onsite expertise, and usefulness of the results to other components of the monitoring system.

3.1. Analytical methods for confirming cyanobacteria presence

3.1.1. Microscopic enumeration

Cyanobacteria enumeration by light microscopy is a conventional and widespread technique employed by drinking water utilities and other stakeholders. Species are identified by their morphology and characteristics under magnification and counted following established algae counting protocols using counting chambers or hemocytometers (De Gelder and Nollet, 2013). The WHO alert level framework recommends utilities to enhance their monitoring activities for cyanotoxins if cyanobacterial biovolume exceeds 0.3 mm^3/L (Alert Level 1). Additionally, utilities should consider ways to prevent cells from entering the plant or apply measures to treat potential cyanotoxins, which may occur at the lifetime guidance value for microcystins, anatoxin-a, or saxitoxin. Alert Level 2 occurs when biovolume exceeds 4 mm^3/L and indicates there is the potential for an acute toxin risk based on the short-term guidance values for cyanotoxins in drinking water (Chorus and Welker, 2021). The alert level framework is less applicable to cylindrospermopsin as it may be actively released by cyanobacteria cells so cyanobacterial biomass is a poor indicator of this toxin. Thus, microscopic enumeration provides an unambiguous measure of microcystin, anatoxin-a, and saxitoxin risk upon which well-defined response actions are based. Consequently, this technique is the gold standard for monitoring cyanobacteria.

This method provides accurate information about the species and concentrations of cyanobacteria in a sample, but it is time consuming and requires highly qualified personnel to perform analysis, which may not be available at all utilities. Consequently, this analysis is often conducted by third-party laboratories, resulting in turnaround times of up to a week. Therefore, the results may not reflect current concentrations of cyanobacteria in a water body. Furthermore, changes in cell biovolume due to preservation by Lugol's iodine solution can cause measurement errors (Hawkins et al., 2005). Other important limitations are that light microscope methods do not provide information about cell integrity, the potential for toxin release, or the toxigenicity of cyanobacterial blooms (Fan et al., 2014; Newcombe, 2009; Zamyadi et al., 2013). Nonetheless, the results of light microscopy can be correlated with other measurements, such as pigment concentrations (Horváth et al., 2013), optical density (Greenstein and Wert, 2019), and fluorescence (Almuhtaram et al., 2018), to estimate cell counts or cyanobacterial biovolume on a more frequent basis.

3.1.2. Phycocyanin extraction

Phycocyanin is a photosynthetic pigment found in cyanobacteria species, and its concentration can be used to estimate the presence and relative abundance of cyanobacteria (Horváth et al., 2013). It also occurs, however, in rhodophytes and cryptophytes in addition to cyanobacteria, and this may result in an overestimation of cyanobacterial biomass by phycocyanin, especially when low cell concentrations of cyanobacteria are present (Zamyadi et al., 2016). Phycocyanin is extracted using techniques similar to chlorophyll a extraction, but its absorbance is measured at 615 nm and 652 nm instead of 665 nm (Hodges et al., 2018; Horváth et al., 2013; Patel et al., 2005; Soni et al., 2006). Additionally, extraction is usually achieved using a phosphate buffer (0.001 M to 0.05 M) to rupture cell walls (Furuki et al., 2003), although extraction efficiency can be significantly improved if phospholipid mixtures such as asolectin are used instead (Zimba, 2012). Like chlorophyll a, phycocyanin can be measured using spectrophotometric and fluorometric techniques, and interference caused by the presence of chlorophyll a can be corrected for (Lauceri et al., 2017). Apart from these considerations, phycocyanin extraction as a monitoring tool is characterized by the same limitations as chlorophyll a: sample analysis can take 30 h to complete or more if a third-party lab is used; it can be applied to pelagic or benthic samples; and it represents mainly total cyanobacterial biomass. Correlations between extracted phycocyanin and cyanobacterial biomass generally have higher coefficients of determination than correlations between chlorophyll a and cyanobacterial biomass over large ranges (Horváth et al., 2013). Thus, extracted phycocyanin is a better indicator of cyanobacteria than chlorophyll a.

3.1.3. Automated cell imaging

Automated cell imaging is the identification of cyanobacteria using a microscope, a camera, and image recognition software. Cyanobacteria can be discriminated from other algal species by their unique fluorescent signatures or by matching sample images to reference databases. One commercially-available system allows the pairing of different magnification levels with different flow cell depths to achieve a desired taxonomic resolution (Graham et al., 2018). For example, a high magnification (x20) paired with a shallow depth (50 μm) provides the highest taxonomic resolution, enabling the detection of picocyanobacteria, and a low magnification (x4) paired with a large depth (100 μm) provides resolution limited to the morphotype level (Graham et al., 2018). An example system might pump a sample through the flow cell at a rate of 0.03 mL/min while images are captured at 80 frames/s, by default, so thousands of images are generated for a single 0.2 mL sample, although individual system configurations may vary. A subset of the images can be selected for sorting and counting, and although identification at the species level based on the particle properties of the cells in the captured images is possible (Camoying and Yñiguez, 2016), this can be challenging in practice (Graham et al., 2018). Nonetheless, enumerating total cyanobacteria and identifying cyanobacterial genera are possible using automated cell imaging (Álvarez et al., 2012; Wang et al., 2015) and can have strong correlations to the results of traditional light microscopy (Álvarez et al., 2014; Camoying and Yñiguez, 2016; Graham et al., 2018). Although this is largely an imaging-based approach, some models come equipped with a laser for detecting chlorophyll a and phycocyanin fluorescence, providing another means to discriminate cyanobacteria from algae.

Although not commercially available, another image-based approach has been proposed that is specifically designed to measure five filamentous cyanobacterial genera: *Aphanizomenon*, *Raphidiopsis*, *Dolichospermum*, *Limnothrix*, and *Planktothrix* (Gandola et al., 2016). In this system, images of samples at x10 magnification are taken manually and pre-processed and processed using two algorithms. The resulting data are evaluated in terms of 17 numerical parameters extracted from the images using a random forest-based machine learning algorithm trained on samples with known genera (Gandola et al., 2016). Compared

Table 2
Summary of second-tier monitoring tools for detecting cyanobacteria.

Parameter	Analytical Method	Target	Alert Thresholds	Advantages	Disadvantages
Microscopic enumeration	Cell counting and biovolume methods	• Cell density and species identification	**Medium**: 0.3 mm^3/L cyanobacteria biovolume(initiate toxin monitoring) **High**: 4 mm^3/L cyanobacteria biovolume (Chorus and Welker, 2021)	• Identifies species and quantifies cell concentrations or biovolumes;	• Time-consuming; • Requires expertise in phycology; • Susceptible to errors from counting and preservation • No indication of metabolite generation
	Automated cell imaging			• Automatic identification and enumeration of cyanobacteria; • Reduces need for onsite expertise;	• Expensive; • Accuracy of identification depends on quality of calibration
Phycocyanin	Lab-based pigment extraction and analysis using spectrophotometry	• Changes in pigment concentration	**Medium**: 0.1–5 μg/L**High**: 5–40 μg/L (Izydorczyk et al., 2005; Srivastava et al., 2013; Zamyadi et al., 2016)Other site-specific thresholds based on correlations to cell counts or biovolume have been reported (Macário et al., 2015; McQuaid et al., 2011; Thomson-Laing et al., 2020)	• Established surrogate for cyanobacterial biovolume; • Specific to cyanobacteria	• Laborious multi-step procedure; • Pigment concentrations per cell are variable; • Correlations to cell concentrations are site specific • No indication of metabolite generation
	Fluorescence spectroscopy probes			• Surrogate measurement for cyanobacterial biovolume; • Real-time monitoring; • Easily deployable in the field	• Correlations to cell concentrations are site specific; • Susceptible to interference by chlorophyll *a* and turbidity • No indication of metabolite generation
	Photonic systems	• Changes in pigment concentration	• No established alert thresholds	• Simple, rapid, and near real-time analysis; • High sensitivity even with low cell concentrations;	• Not commercially available • Cannot differentiate toxic vs nontoxic species; • Cannot quantify cyanobacteria without external calibration
Remote sensing	Sentinel 3 Ocean Land Color Imager (OLCI) and other satellites	• Changes in pigment concentration	No established alert thresholds	• Satellite data is freely available; • Identifies bloom extent and movement; • Band algorithms available to determine phycocyanin and chlorophyll a concentrations	• High detection limit (e.g., ≥20,000 cells/mL); • Detects surface conditions only; • Revisit time of 1–2 days; • Pre-processed data not available for all locations • Limited to sources with large surface areas • Impacted by environmental conditions • Infrequent source characterization
Biosensors	Fluorescence plate reader	• Gene counts representing cyanobacteria or specific cyanobacteria species	No established alert thresholds	• Species-level identification of cyanobacteria; • Detection limits ranging from 10^2-10^4 cells/mL; • Can target genes that encode for cyanotoxins	• Detects species based on primer selection; • Cannot quantify cyanobacteria without external calibration
NGS	Gene sequencing platform	• Identification of all gene sequences in a sample	No established alert thresholds	• Detects all species in a sample; • Differentiates species more accurately than cell counting	• Long turnaround times; • Provides only relative abundance of cells;
Automated cell imaging	Image recognition software to process images of cells under magnification	• Identification of cell genus or species and cell counts	**Medium**: 0.3 mm^3/L cyanobacteria biovolume(initiate toxin monitoring) **High**: 4 mm^3/L cyanobacteria biovolume (Chorus and Welker, 2021)	• Automatic identification and enumeration of cyanobacteria; • Reduces need for onsite expertise;	• Expensive; • Accuracy of identification depends on quality of calibration

to conventional microscopy, the samples are accurately characterized in terms of filament lengths and widths, cell lengths, and cell abundance over the range of 5000 to 50,000 cells/mL (Gandola et al., 2016). Another system that utilizes a camera-equipped microscope uses sample fluorescence to identify and enumerate cyanobacteria cells (Jin et al., 2018). *Microcystis* and *Dolichospermum* cells could be differentiated in the presence of a non-cyanobacterial species and enumerated with a high degree of accuracy. The proposed process is as follows: a sample is excited at 365 nm, a binary classifier separates foreground and background objects in the image field of view, and seven morphological parameters are evaluated and used to classify cells into one of the three genera (Jin et al., 2018). Although promising, this approach needs to be expanded to include other important cyanobacterial genera before it can be adopted in practice.

3.2. Remote sensing and fluorometry for confirming cyanobacteria

3.2.1. Phycocyanin fluorescence

Real-time monitoring probes are available that measure the fluorescence of pigments like chlorophyll a, phycocyanin, and phycoerythrin to detect algae and cyanobacteria cells. Optical sensors make use of each pigment's unique excitation/emission spectra to accurately detect it by measuring near its peak emission wavelength. Chlorophyll a is excited using 410–430 nm wavelengths and has a peak emission at 685 nm; phycocyanin is excited by 590–630 nm light and has a maximum emission at 650 nm; and phycoerythrin is excited by 550–570 nm light and emits at 578 nm (Asai et al., 2001; Beutler et al., 2003). Despite these unique signatures, measurement errors can occur for sensors with wide measurement band widths that measure emission wavelengths from multiple pigments (Bertone et al., 2018). Light scattering by turbidity above 50 NTU has also been shown to cause errors (Bowling et al., 2013). Zamyadi et al. (2016) reviewed and quantified the impacts of sources of interference on phycocyanin fluorescence including chlorophyll a, turbidity, cell morphology, and sensor calibration. The authors show that the overlapping chlorophyll a emission spectrum is the most significant source of interference, affecting phycocyanin fluorescence by up to 600%. However, correction factors have been derived for specific probe models that limit the impact of chlorophyll a interference (Choo et al., 2019). Thus, monitoring phycocyanin fluorescence has the potential to be an especially accurate approach.

Commercially available real-time phycocyanin monitoring probes have limited sensitivity below 10^3 cyanobacteria cells/mL (Ezenarro et al., 2021). Furthermore, these systems can be costly to utilities with limited resources, creating the need for low-cost, near real-time, in situ fluorescence analysis systems. One novel system concentrates cells from field samples onto 0.2 μm nitrocellulose filters and measures phycocyanin fluorescence of retained cells via a low-cost portable apparatus comprising a filtration unit, a peristaltic pump, a control board, and a computer (Ezenarro et al., 2021). This system was reported to achieve a low detection limit of <435 cells/mL in aquaponic samples containing mixtures of cyanobacteria and other eukaryotic algae. Thus, if made commercially available, it may be a useful tool in future early warning systems as an initial rapid, low-cost cyanobacteria screening tool with high sensitivity. Such a low detection limit, however, might be susceptible to interference by other phycocyanin-producing species such as cryptophytes (Zamyadi et al., 2016).

A lab-based photonic system that overcomes this limitation employs light microscopy coupled with a hyperspectral camera to determine the reflectance of a sample in 4.69 nm band widths in the range 400 to 1000 nm (Paine et al., 2018). It was found that *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* have unique reflectance derivatives at three wavelengths (468, 509, and 628 nm) that can be used to determine whether either species is present in a sample (Paine et al., 2018). These findings are promising, but further research is needed to characterize the reflectance derivatives of other toxic and nontoxic cyanobacteria species.

Phycocyanin fluorescence is typically correlated to microscopically-enumerated cell counts or biovolume (Bertone et al., 2018; Macário et al., 2015). This correlation can be used to derive site-specific threshold values that correspond to alert levels for implementing HAB response strategies (Chorus and Welker, 2021). Attention must be given to the species present in samples used to establish such correlations because pigment concentrations vary among cyanobacteria species, so a correlation established for one cyanobacterial community may not apply to another (Chang et al., 2012; Loisa et al., 2015; Thomson-Laing et al., 2020). Consequently, periodic sample analysis is needed to ensure the correlation is valid as species dynamics change (Symes and van Ogtrop, 2016). The detection of the presence of cyanobacteria through phycocyanin analysis needs to be followed up with other monitoring tools because it does not differentiate between potential toxin-producing species and non-toxic species.

3.2.2. Applying anomaly detection algorithms to phycocyanin data

Utilities that monitor phycocyanin fluorescence but do not collect cell count data are unable to adopt the conventional threshold approach because no phycocyanin-cell count correlation can be made. A novel way to interpret phycocyanin fluorescence without the need for cell count data is to use machine learning for anomaly detection (Almuhtaram et al., 2021b). This approach involves training unsupervised machine learning algorithms on historical fluorescence data to identify anomalies in current monitoring data. Anomalies identified this way may be due to interference (i.e., false positives) or elevated cyanobacteria activity (i.e., true positives). Upon detection of an anomaly, sample collection and analysis is necessary to confirm the presence of harmful cyanobacteria and to initiate mitigative measures such as modifications to the treatment process to target cyanobacteria cell and metabolite removal. Almuhtaram et al. (2021b) demonstrated that three algorithms, One-Class Support Vector Machine, elliptic envelope, and Isolation Forest, are able to accurately identify cyanobacterial blooms in four datasets when trained on standardized historical phycocyanin data and tested on more recent data. Similarly, Cao et al. (2016) applied a multi-objective hybrid evolutionary algorithm to successfully identify the onset of cyanobacterial blooms using water quality parameters, and Chen et al. (2015) developed an autoregressive integrated moving average model to predict chlorophyll a concentrations and provide early warning of algal blooms. Thus, these and other machine learning algorithms can potentially be implemented as part of a utility's harmful algal bloom monitoring strategy. Additionally, the potential exists for such algorithms to be adopted by monitoring probe manufacturers and included directly in monitoring software.

3.2.3. Satellite remote sensing

Satellites capture images of the Earth's surface in daily, 2–3 day, or weekly intervals that can be used the same day they are captured for near real-time monitoring (Chorus and Welker, 2021). Many satellites equipped for remote sensing are operated by governments, so the data they collect is generally available to the public for free. In contrast to fluorescence-based monitoring probes, satellite sensors quantify the reflectance of a pixel (i.e., the smallest unit of surface area measured by the sensor). Satellites are equipped with various sensors that measure different spectral bands, each representing a parameter (Schlundt et al., 2010). Choosing which sensor to use requires consideration for their spatial resolutions, bands, correlated spectral resolution, turnaround time, available indices, historical data, and academic research conducted using them. Each sensor consists of different numbers of bands or different numbers of spectral ranges, which each have various functions including measuring absorbance and reflectance. The band functions of the commonly used Sentinel-3 satellite Ocean and Land Color Instrument (OLCI) sensor are summarized in Donlon et al. (2012). In addition to the bands available, another important consideration is the spectral width of the band. For example, phycocyanin has an absorption peak at 620 nm, but chlorophyll a and other accessory pigments also absorb

light near that wavelength and can potentially obscure the phycocyanin signal (Hunter et al., 2010; Mishra et al., 2009). Thus, the ability of a satellite to detect algal blooms in general or cyanobacteria specifically depend on its spectral bands and their spectral widths.

Yan et al. (2018) show that only a few satellite sensors have spectral bands centered on 620 nm with widths narrow enough (e.g., 5–10 nm) to measure phycocyanin including Sentinel-3A OLCI and the non-operational Envisat satellite with the Medium Resolution Imaging Spectrometer (MERIS) sensor. OLCI is an improvement to MERIS, with a new channel at 673 nm for better chlorophyll fluorescence measurement, improved signal to noise ratio, improved atmospheric corrections, faster revisit time, and reduced sun glint (Donlon et al., 2012; Lunetta et al., 2015). Heritage MERIS data, however, can be included in time-series analyses of OLCI data. For comparison, OLCI has a spatial resolution of 300 m and its satellite has a revisit time of less than two days while Landsat series satellites have spatial resolutions of 15 m and revisit times of 16 days. Thus, there is a tradeoff between data resolution and data frequency, although the Landsat spectral band widths are wider than those of OLCI.

Another important decision following the choice of sensor is the method for processing its spectral data (Beck et al., 2017). For cyanobacteria monitoring, the simplest approach would be to use data from a single spectral band at 620 nm, but this wavelength is affected by suspended sediment in addition to phycocyanin, so single band approaches are inadequate. Therefore, algorithms that process multiple bands have been proposed. Early algorithms used spectral band ratios but were susceptible to interference from chlorophyll a, turbidity, water absorption, and did not take into account variable chlorophyll a:phycocyanin ratios (Schalles and Yacobi, 2000; Vincent et al., 2004). Mishra et al. (2009) developed a single band ratio algorithm using 600 nm and 700 nm wavelengths to avoid the interference of chlorophyll a at 620 nm, although 600 nm wavelengths are still susceptible to interference by turbidity. This limitation was overcome by decomposing remote sensing reflectance to determine phytoplankton absorption coefficients from the 620 nm:665 nm band ratio, but this process requires adjustment and calibration (Mishra et al., 2013; Yacobi et al., 2015). Nonetheless, several promising models are capable of accurately estimating cyanobacterial biovolume or pigment concentrations (Duan et al., 2010; Hunter et al., 2010; Tebbs et al., 2013; Wolny et al., 2020; Zhang et al., 2017).

Band ratio algorithms are particularly suited for sensors with fewer bands, such as MERIS and Landsat (Sun et al., 2015), and can be used to establish strong correlations to chlorophyll a (Binding et al., 2011). Two- and three-band algorithms for MERIS data have been used to estimate chlorophyll a concentrations accurately ($R^2 > 0.94$) by minimizing the effect of scattering by inorganic particles (Gurlin et al., 2011; Yacobi et al., 2011). A hybrid model was developed that assesses each pixel in remote sensing data using three models then selects the model that results in the best chlorophyll a estimation (Matsushita et al., 2015). Other algorithms that employ atmospheric correction factors, such as the Rayleigh-corrected reflectance, can be effective (Tao et al., 2017) but are often site-specific because the corrections need to be readjusted for other datasets (Shi et al., 2019).

Beck et al. (2017) evaluated the ability of 27 algorithms to determine cyanobacterial biovolume using data from multiple sensors and showed that high accuracy can be achieved by both chlorophyll a- and phycocyanin-based algorithms, although the performance of the phycocyanin algorithms is slightly better. Yan et al. (2018) conclude that data from only the OLCI sensor, due to its narrow band width, are suitable for measuring phycocyanin in large-scale applications and stress the need for research on and validation of algorithms using OLCI data. Recently, Mishra et al. (2021) showed that the existing Cyanobacteria Index algorithm, which uses OLCI and MERIS data, is up to 84% accurate for determining cyanotoxin-producing cyanobacteria in data collected across the United States.

In general, compared with other monitoring techniques, the advantages of satellite remote sensing are that vast areas of water bodies can be covered at once; low or no costs are associated with data acquisition; no maintenance is required; reliable and long-term monitoring is possible; bloom extent and movement can be determined; and it requires comparatively less personnel training than manual sampling techniques. However, an important limitation is that it captures only surface water conditions whereas drinking water utilities typically have intakes deep below the water surface. Thus, the detection of cyanobacterial activity in a water body may not indicate that a utility drawing from that water body is at risk of experiencing cyanobacteria or cyanotoxins. Instead, sample collection and analysis by one or more of the methods described in Section 2 is necessary. In this way, the function of remote sensing data is similar to fluorescence-based monitoring probes, although there is a compromise between detecting local conditions (e.g., at a drinking water intake) and broader cyanobacteria activity (e.g., movement of a bloom through a lake or reservoir).

3.3. Molecular methods for monitoring cyanobacteria

3.3.1. Next-Generation sequencing (NGS)

The most widespread technique for identifying what species are present in a sample is to sequence 16S ribosomal RNA (rRNA) amplified using polymerase chain reaction (PCR) from genomic DNA (Deng et al., 2017). Sequencing is traditionally accomplished by measuring the fluorescence of DNA fragments size-separated by capillary gel electrophoresis, a labor-intensive process. Instead, (next-generation sequencing) NGS significantly simplifies this process while increasing the throughput by parallelizing the sequencing reactions (Deng et al., 2017). In NGS, 16S rRNA amplicons of multiple samples are analyzed at once, and oligonucleotide barcodes are added to the primers of the amplicons to identify which samples they belong to. Then, bioinformatics is used to extract detailed genetic information about the species present in a sample. Consequently, NGS is the standard technique for in-depth analyses of community compositions in microbial surveys.

A typical NGS workflow can take 2–3 days or more to complete and involves generating 16S rRNA amplicons by qPCR amplification of genomic DNA using target-specific primers (e.g., for cyanobacteria) (Kurmayer et al., 2017). Then, target regions of the 16S rRNA are amplified and purified to remove excess nucleotides. Next, a sequencing platform is used to provide reads spanning a pre-determined number of 16S rRNA base pairs. The data generated is processed using software packages to remove problematic sequences, and the remaining sequences are compared against reference databases (Balvočiūtė and Huson, 2017; Huo et al., 2018; Zamyadi et al., 2019). Thus, NGS is a full scan of all the gene sequences in a sample that identifies a wide range of species, including those that may be missed by light microscopy, such as melainabacteria and other indicators of fecal contamination (Gaget et al., 2020; E. Lee et al., 2017b; Vadde et al., 2019; Zamyadi et al., 2019).

Monitoring cyanobacteria and other bacteria to assess species diversity is based primarily on operational taxonomic units (OTUs) derived from 16 s rRNA amplicons (Casero et al., 2019). For example, Berry et al. (2017) showed that cyanobacterial blooms disturb bacterial community compositions by monitoring cyanobacterial and non-cyanobacterial OTUs over the course of a bloom. Similarly, Woodhouse et al. (2016) used OTUs to demonstrate that changes in the abundance and diversity of cyanobacteria species are linked to bacteria species, establishing that biotic factors in addition to abiotic factors affect cyanobacteria growth. NGS has also been used to identify cyanotoxin biosynthesis genes allowing researchers to determine whether the potential for toxin production exists in a sample (Kim et al., 2018; Lezcano et al., 2017; Liao et al., 2016; Scherer et al., 2017). Therefore, NGS is both a second and third-tier monitoring tool.

3.3.2. Biosensors

Using immunological techniques, a microarray-based biosensor,

CYANOCHIP, was developed for identifying 17 cyanobacteria species that occur in European waters in situ using polyclonal antibodies (Blanco et al., 2015). In this assay, environmental samples are concentrated on 3 μm polycarbonate filters and reconstituted into 1 mL of modified Tris-buffered saline Tween 20-reinforced buffer (TBSTRR). Then, 50 μL of the 1 mL solutions are added to microarray wells, which contain antibodies and air-dried bovine serum albumin, and incubated for 1 h. Next, wells are washed, and an antibody solution is added and incubated for another hour. Finally, wells are washed, dried, and scanned for fluorescence at 635 nm using a portable plate reader (Blanco et al., 2015). Important advantages of this biosensor are that it is a low-cost way to identify specific cyanobacteria species in situ with detection limits as low as 10^2 to 10^4 cells/mL, although it cannot measure cyanobacteria concentrations without a correlation to cell counts. Thus, like the photonic systems described above, cyanobacteria detected with this method may need to be validated by other means.

Detection limits as low as 50 cells/mL for *M. aeruginosa* have also been achieved by a cantilever-based 16S RNA biosensor. Johnson and Mutharasan (2013) immobilized a 27-base DNA strand complimentary to a target region of *M. aeruginosa* 16S RNA in a flow-based cantilever sensor. As a sample flows through the sensor, 16S RNA binds to DNA, increasing its effective mass. This mass change is detected by the sensor and is proportionate to an increase in *M. aeruginosa* concentrations (Johnson and Mutharasan, 2013). This procedure has a total run time of about 2–3 h.

Another biosensor for *M. aeruginosa* that has been reported utilizes the existing NanoGene assay (E.-H. Lee et al., 2017a). The authors targeted the *M. aeruginosa mcyD* gene, which encodes for microcystin production. This approach resulted in a very low detection limit of 9 cells/mL and was not impaired by the presence of up to 2×10^7 algal cells/mL (E.-H. Lee et al., 2017a). The workflow duration is likely 2–3 h long. In contrast, a flow-through biosensor for another common cyanobacteria species *Planktothrix agardhii* has been reported with a 25 min assay time after DNA extraction (Ölcer et al., 2015). The assay involves injection of DNA extracted from sample into a microfluidics integrated electrode array that is connected to a potentiostat that relays data to a computer for processing. Samples containing the target sequence of *P. aragdhii* are injected to the electrode array and immobilized by a capture probe sequence. Then, biotinylated detection probes join the immobilized target sequence to modified Au nanoparticles, emitting electrochemical signals. The signals are measured using a Real-time Electrochemical Profiling™ assay and reflect the concentration of the *P. agardhii* DNA in the sample (Ölcer et al., 2015).

4. Third Tier: Monitoring cyanobacteria metabolites

Following the detection of biological/bloom events in the first tier and the confirmation of cyanobacteria presence in the second tier, the goal of the third tier monitoring is to confirm weather the cyanobacteria species detected produce toxic or odorous metabolites or have the genes necessary to do so. Factors triggering the production of metabolites are unclear, however variability in nutrients, temperature, and light intensity can influence the production and subsequent external release of cyanotoxins and taste and odor compounds to the surrounding water (Clercin and Druschel, 2019; Oh et al., 2017; Zhang et al., 2009). Toxin production can be expressed as a function of the rate of cell division, which varies depending on the stage of bacterial growth, which is in turn impacted by environmental conditions (Orr et al., 2018). Extracellular metabolites are often released toward the later bloom growth stages i.e., stationary phase or during cell death. Therefore, it is arguable that monitoring for extracellular taste and odor compounds and cyanotoxins are not ideal parameters to be implemented as first indicators of a cyanobacterial bloom as they may be produced once the bloom is established. Monitoring for total metabolites (which includes intracellular metabolites) may provide improved early warning compared to measuring extracellular concentrations.

Various qualitative and quantitative analytical tools are available for the metabolite detection. The semi-quantitative enzyme-linked immunosorbent assay (ELISA) screening tools are often used to establish the presence or absence of cyanotoxins. Cyanotoxin and taste and odor producing genes in source water samples can also be quantified using quantitative polymerase chain reaction (qPCR) methods. Quantitative results for cyanotoxins and taste and compounds can also be obtained through liquid chromatography-mass spectrometry (LCMS) analysis.

4.1. Enzyme-Linked immunosorbent assays (ELISAs)

For semi-quantitative determination of cyanotoxin concentrations, enzyme-linked immunosorbent assays (ELISAs) are routinely used in cyanobacteria monitoring programs. Commercially available ELISAs have been developed for microcystins and other cyanotoxins (Weller, 2013). Microcystin ELISAs can be specific to the congeners that the antibodies were raised against (Zeck et al., 2001a) or broadly react to the Adda side chain, which is the type of ELISA typically used in routine monitoring (Fischer et al., 2001; Khreich et al., 2009; Zeck et al., 2001b). The Adda side chain is common to all microcystins and nodularins, so Adda ELISAs measure all congeners of both toxins, which is important in jurisdictions where health advisories are in place for total microcystins as opposed to microcystin-LR. However, the Adda ELISA has variable cross-reactivity among microcystin congeners and may be susceptible to interference due to Adda-containing chlorination byproducts (He et al., 2017; Rapala et al., 2002). Limited comparisons have been made between anatoxin-a ELISAs and high-pressure liquid chromatography (HPLC) methods, although one study reports that anatoxin-a was detected in microbial mats by ELISA but not HPLC, suggesting that the ELISA may be susceptible to matrix interference (Khomutovska et al., 2020). Similarly, significant differences have been reported between a commercially-available saxitoxin ELISA and HPLC, possibly due to poor cross-reactivity with co-occurring ciguatoxins (Gaget et al., 2017). The same study reports comparable measurements of cylindrospermopsin using ELISA and HPLC (Gaget et al., 2017), although variable cross reactivity among congeners has been reported (Loftin et al., 2016). Nevertheless, the cost and speed of the ELISA analysis make it a valuable tool for detecting the presence or absence of cyanotoxins to trigger quantification using an appropriate analytical method.

Subsequent quantification is necessary to determine molecular toxicity associated with the cyanotoxins present, which varies significantly among congeners of each class of cyanotoxins. Reported LD_{50} values (the dose that kills 50% of exposed animals) in mice subjected to intraperitoneal injection are 50 μg/kg for microcystins -LR and -LA. For [(6Z)-Adda]microcystins -LR and -RR, which result from photo-isomerization of MC-LR and MC-RR, respectively (Almuhtaram et al., 2021a), LD_{50} values are >1200 μg/kg (Chernoff et al., 2020; Jang et al., 2003). LD_{50} values in mice subjected to intraperitoneal injection for saxitoxin congeners similarly vary up to 17-fold, ranging from 0.028 to 0.480 μmol/kg (Selwood et al., 2017). As a result, some jurisdictions consider both concentration and toxicity and regulate microcystins and saxitoxins in terms of microcystin-LR and saxitoxin equivalents (Chorus and Welker, 2021).

4.2. qPCR

A well-established technique for cyanobacteria monitoring is qPCR, which has been reviewed in detail by Pacheco et al. (2016). The concept behind qPCR is that there are certain gene clusters found in cyanobacteria species that contribute to the biosynthesis of cyanotoxins including *mcy*, *cyr*, *sxt*, and *ana*. Each cluster contains several operons that can be targeted by qPCR or NGS. For example, operons *mcyA-E* and *mcyG* are known to encode enzymes that are involved in microcystins synthesis (Pacheco et al., 2016). Moreover, the number of gene copies may correlate to the concentration of toxins, although this is not always the case because the presence of a gene does not imply that the toxin is being

produced. Secondly, this may be affected by the choice of gene because primer inefficiencies may result in one gene being amplified more than another. For example, in 80% of cases where *mcyE* was reported, there was a positive correlation to total microcystins concentration, but this occurred in only 60% of studies where other *mcy* genes were amplified (Pacheco et al., 2016). These and other genes involved in cyanotoxin biosynthesis are obtained from sequenced strains that produce the corresponding toxins and are present in one copy per cell. Therefore, the detection of a gene may additionally indicate the presence of *Microcystis*, *Dolichospermum*, *Raphidiopsis*, *Lyngbya*, or *Aphanizomenon* cells (Lou et al., 2017; Orr et al., 2010; Pacheco et al., 2016). Commercially available testing kits may also quantify 16S rRNA genes, which are multiple copy genes common to cyanobacteria. Thus, the ratio of a toxin gene to 16S rRNA may indicate the proportion of cells that are toxic, although this is prone to error due to the single copy vs multiple copy natures of the toxin and 16S rRNA genes, respectively (Pacheco et al., 2016).

Primers have been developed for geosmin and 2-methylisoborneol (MIB) to quantify genes responsible for the biosynthesis of taste and odor compounds in addition to cyanotoxins. Gaget et al. (2020) developed a qPCR primer for MIB using previously reported MIB synthase gene sequences and used it to identify a potential source of MIB in an urban reservoir. Similarly, Chiu et al. (2016) developed primers for 13 sequences of MIB synthase genes and coupled them to a real-time qPCR system for rapid on-site monitoring of MIB. Other researchers have developed primers for taste and odor compound synthesis genes specific to certain producers (Devi et al., 2021). For example, Su et al. (2013) developed primers for *Dolichospermum* sp. and the geosmin synthase gene in *Dolichospermum* sp. to demonstrate the geosmin-producing potential of samples containing that species. Other primers have been developed that target geosmin in other cyanobacterial genera including *Nostoc*, *Geitlerinema*, *Aphanizomenon*, *Lyngbya*, *Phormidium*, and *Oscillatoria* (Devi et al., 2021). Species-specific approaches can be applied in water bodies that are predictably dominated by one species, but they may underestimate the taste and odor production potential in more diverse communities. Consequently, universal primers that detect geosmin and MIB in all cyanobacteria are more desirable, such as those developed by John et al. (2018) for geosmin and Gaget et al. (2020) for MIB. In summary, although the availability of qPCR primers limits their application to specific genes, it may serve as a potential early warning indicator for the onset of a toxigenic bloom as results can be obtained within several hours (Gaget et al., 2017).

4.3. Chromatography and separation methods

Several analytical techniques exist for the determination of trace concentrations of toxins and taste and odor compounds including gas and liquid chromatography, capillary electrophoresis, and immunological and antibody assays. These are described in detail in Meriluoto et al. (2016) and Kaushik and Balasubramanian (2013). In general, these techniques require the most sensitive and expensive analytical instruments to be used and consequently are not suitable for routine monitoring at high frequencies (e.g., daily). Moreover, sample preparation and concentration, calibration standards, and high quality reagents are often required. Due to practical requirements for sample collection, transportation (if contract laboratories are used), laboratory preparation and analysis, interpretation and reporting, these analytical methods may have long turnaround times. Given that cyanobacteria and the toxic and odorous metabolites present can change in abundance quite rapidly within a short period, delays in the procurement of results may subsequently delay necessary response actions to protect source and treated water quality as well as consumer health.

In many jurisdictions, drinking water utilities are required to submit samples to accredited laboratories for metabolite quantification on a weekly or biweekly basis. Sample collection at similar frequencies may also be required for recreational and agricultural waters. This frequency, however, is not high enough to capture short-lived blooms or sudden changes in the risk of exposure to cyanobacterial metabolites, such as bloom migration to an intake zone due to wind. Therefore, analytical methods need to be used strategically and in response to one or more indicators of the potential for adverse metabolite presence identified through multi-barrier early warning systems. Automated high-frequency characterization of metabolites in source and treated water are not established. Research and development are needed to establish sensitive and accurate real-time analytical tools for cyanotoxins and taste and odor compounds.

5. Need for Multi-Barrier early warning systems

Table 3 summarizes the capabilities of the cyanobacteria monitoring tools covered in this review in terms of cost, turnaround time, ability to quantify benthic or pelagic cyanobacteria, measurement accuracy, and susceptibility to interference. The cost column represents relative costs per sample, where low cost refers to a cost less than $10 USD, moderate refers to between $10 and $100, and high refers to greater than $100, although costs may vary depending on whether analyses are conducted onsite or by third party labs. Turnaround times represent the time to complete an analysis and are separated into <10 min, between 10 min and a day, and more than a day. Turnaround times can vary significantly if samples are shipped to and analyzed by third party labs due to shipping time and position in analysis queues. The pelagic or benthic column describes to which form of algae or cyanobacteria each tool can be applied. The accuracy column describes the ability of a tool to be correlated to or indicate cyanobacterial biomass. Finally, the interferences column describes any important drawbacks or sources of interference for each tool.

A comprehensive early warning system needs to include a combination of monitoring tools as no single tool can provide information about all the important aspects of a cyanobacterial bloom (Gaget et al., 2017). Spatial and temporal assessments of cyanobacteria in source waters using satellite- or drone-based remote sensing help forecast the movement of a bloom and allow drinking water utilities and other stakeholders to anticipate when they might experience it. Sample analysis by qPCR or NGS can be used to determine whether an advancing bloom is potentially toxic. Rapid raw water testing using automated imaging, probes, or photonic systems can detect the moment cyanobacteria begin entering a drinking water treatment plant or recreational area of a water body. Samples that indicate an increase compared to baseline levels may be subjected to further analysis by microscopy or pigment extraction to quantify the concentration of cells to assess whether any thresholds are exceeded. Based on this information, source water control, drinking water treatment, or agricultural and recreational water use strategies can then be implemented.

To develop a source water monitoring system, users should consider which of the parameters described in Table 3 are the most pertinent to their system, in addition to the capability of personnel to conduct the analyses (Kibuye et al., 2021). For a small utility with limited funds available to purchase specialized equipment or automated monitoring tools, a low cost, rapid tier one tool such as ATP test sticks may be suitable. Then, for confirming the presence and relative abundance of cyanobacteria, microscopic imaging may be used as this involves minimal cost and operators can be trained to identify common cyanobacteria species using reference images, which are widely available (AWWA, 2010). Approximate cell counts determined by microscopy this way can be compared to local or international thresholds like those described in the WHO alert level framework, and, if they exceed those thresholds, samples can be collected for cyanotoxin measurement. For a utility with more funds available, opting for a real-time tier two monitoring tool for phycocyanin fluorescence, such as a probe, may be suitable. This removes the need for a tier one monitoring tool and eliminates the potential error associated with multiple operators performing microscopy because significant differences in microscopy results can occur

Table 3
Summary of cyanobacteria monitoring tool capabilities.

Technology		Cost per sample	Turnaround time	Pelagic or Benthic	Accuracy	Interferences
Tier 1	Visual inspection	No cost	Immediate	Pelagic	Cannot estimate cell concentrations	Cannot distinguish species
	chlorophyll a extraction	Moderate	<1 day	Both	Moderately correlates to cyanobacteria	Low
	chlorophyll a fluorescence	Moderate	Real-time	Pelagic	Moderately correlates to cyanobacteria	Turbidity and other fluorescent compounds
	ATP	Low	<10 min orreal-time	Pelagic	Measure of biological activity	Presence of bacteria
	Drones	Low	<1 day	Pelagic	Captures surface conditions only	Poor weather, flight restrictions
	Microscopy	Low	<1 day	Both	High	Low
	Phycocyanin extraction	Moderate	<1 day	Both	Strong correlation to cyanobacteria	Cell quota varies among species; turbidity and other fluorescent compounds
	Phycocyanin fluorescence	Moderate	Real-time	Pelagic	Strong correlation to cyanobacteria	Cell quota varies among species; turbidity and other fluorescent compounds
Tier 2	Satellite remote sensing	Low	>1 day	Pelagic	Captures surface conditions only	Cloud cover, orbital period
	Photonic systems	Low	<1 day	Pelagic	Measure of phycocyanin	Turbidity and other fluorescent compounds
	Automated cell imaging	High	<10 min	Pelagic	High	Low
	Biosensors	Moderate	<1 day	Pelagic	Measures specific cyanobacteria strains or species	Low
	NGS	High	<1 day	Both	High	Low
	qPCR	Moderate	<1 day	Both	Presence of genes does not indicate metabolite production	Low
Tier 3	ELISA	Low	<1 day	Both	Variable cross-reactivity among cyanotoxin variants	Presence of partially degraded cyanotoxins; false positives may occur
	Gas/liquid chromatography-mass spectrometry	High	<1 day	Both	High	Low

if multiple microscopists are used (Vuorio et al., 2007). Phycocyanin fluorescence may be used to trigger alerts by way of correlations to independent cell counts or anomaly detection algorithms, prompting sample analysis for cyanotoxins. Finally, a utility desiring a more comprehensive cyanobacteria monitoring system may benefit from implementing multiple tools that provide complimentary information. A second-tier automated cell imaging technology may be used at the intake to monitor cell concentrations entering a plant along with qPCR to determine the potential for metabolite production. In addition, source monitoring activities may be carried out using drones equipped with multispectral cameras to anticipate algal activity before it occurs. Thus, the three-tier framework may assist stakeholders in developing early warning systems for cyanobacteria based on resource availability and monitoring requirements.

6. Future directions and research needs

This review evaluates the existing tools for monitoring cyanobacteria and organizes them into three tiers based on their measurement targets. Further research is needed on evaluating early warning systems comprising different combinations of monitoring tools as well as to advance the state of individual tools. Specifically, ATP is a promising low cost, rapid indicator of biological activity but needs to be assessed in terms of its ability to identify the onset of an algal bloom. Cell quotas for ATP vary among species and throughout growth stages, so its use as a monitoring tool must be demonstrably robust against natural fluctuations in concentration. The use of drones has emerged in recent years as an inexpensive way to rapidly assess water quality spatially, but drone designs need to be developed that balance battery life, payload size, and image quality before they can be easily adopted by drinking water utilities. Additionally, purpose-built sensors (e.g., for phycocyanin fluorescence or hyperspectral imaging) to be mounted on drones are needed. Further research is needed in applying data analytics to sensor data, such as phycocyanin fluorescence, to extract and utilize inherent patterns that occur in historical data, especially now that an increasing number of utilities have at least several years of monitoring data to analyze. Moreover, interferences caused by other parameters need to be considered to minimize false positives. Several photonic systems and biosensors have been developed and reported in the literature, but research is needed in the optimization and quality control of these systems before commercialization. Finally, existing qPCR workflows need to be standardized for different matrix types, and universal primers for biosynthesis genes for taste and odor compounds need to be evaluated in multiple water bodies to confirm their universality.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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