



Innovative multiplex qPCR method for rapid and reliable detection of microcystin-producing genes during harmful algal blooms: Insights from Utah Reservoirs

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ABSTRACT

Cyanobacterial harmful algal blooms (cyanoHABs) have the potential to produce cyanotoxins, which pose significant health risks to both humans and animals. The gold standard methods for monitoring cyanoHABs involve enzyme-linked immunosorbent assay (ELISA), liquid chromatography combined with triple quadrupole mass spectrometry (LC-MS/MS) and manual cell counting under microscopy. However, these techniques, while effective, are costly and time-consuming, which may not be optimal for timely decision-making to safeguard public health. Quantitative polymerase chain reaction (qPCR) offers a complementary approach that serves as an indicator of the potential for toxin production. It provides accurate results with a rapid turnaround time and high throughput capacity, and greater affordability. To assess the reliability of qPCR in predicting toxin production and determining when toxin levels exceed recreational advisory thresholds, we conducted experiments utilizing two DNA extraction methods for qPCR testing: RapidDNA and ClassicDNA. Sampling was conducted across nine water bodies in Utah throughout the recreational season from June 1 to October 31, 2023. We targeted cyanotoxin-encoding genes mainly associated with microcystins, the dominant cyanotoxin reported for these water bodies, for qPCR analysis. Toxin levels were measured using both ELISA and LC-MS/MS with cyanobacteria cell counting conducted as a reference. Out of nine water bodies studied, cyanoHABs were detected in five (i.e., Utah Lake, and Deer Creek, Echo, Schofield, and Pineview Reservoirs). Analysis of the data revealed a significant linear relationship between both the qPCR results of *mcyE* (associated with microcystin production) obtained from RapidDNA and ClassicDNA methods, and the levels of microcystins measured by ELISA and LC-MS/MS. RapidDNA qPCR methods offer a potential warning tool for indicating toxin production during blooming events, though this method is not suitable for determining risk during the pre-blooming period. Conversely, ClassicDNA methods can be utilized during the pre-blooming period to prepare for potential blooms. These results provide insight into the genetic potential of blooms around the state to produce microcystins. Findings can be implemented in both Recreational Water Quality and Drinking Water programs nationally.

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1. Introduction

Harmful algal blooms (HABs) are a global environmental phenomenon raising major concerns because of their harmful effects on aquatic ecosystems, human health, and public economy. Cyanobacterial HABs (cyanoHABs) occur in a wide range of aquatic environments due to rapid proliferation of cyanobacterial populations. CyanoHABs are commonly found in waterbodies including large freshwater lakes, smaller inland lakes, rivers, reservoirs, as well as marine coastal areas and estuaries (Brooks et al., 2016; Schmale et al. 2019). Cyanobacteria can produce high concentration of toxins such as microcystins (MCs), cylindrospermopsin (CYN), anatoxins, nodularins, and saxitoxins (Munoz et al. 2019). These toxins can induce acute and chronic health effects on both humans and animals, affecting the liver, lungs, kidneys, nervous system, gastrointestinal tract, and immune system (Funari and Testai 2008). Among these toxins, MCs are the most common cyanotoxins found both globally and in Utah. MCs have been responsible for contaminating drinking water sources, causing illness through recreational water exposure, and contributing to large-scale fish and wildlife die-offs (Landsberg et al. 2020). Conventional drinking water treatment facilities may struggle to effectively remove cyanotoxins (He et al. 2016), leaving water systems facing challenges in providing safe drinking water during severe HAB events in source waters. Effective source water management strategies for water systems rely on understanding seasonal cyanobacterial growth patterns, species dynamics, and toxin properties to select suitable treatment strategies for cyanotoxin removal (Brooke et al. 2006; Dixon et al. 2011). Additionally, water system personnel should select appropriate monitoring methods capable of rapidly and accurately assessing the efficaciousness of treatment processes so they may be both validated and optimized as necessary. Hence, there is a demand for new tools and technologies to swiftly detect, characterize, and address cyanoHABs posing threats to water security.

The U.S. EPA has set 10-day health advisory levels for MCs and CYN in drinking water. Children under 6 years old have an advisory level of 0.3 µg/L for MCs, while school-age children (6 years and older) have an advisory level of 1.6 µg/L. For CYN, the advisory level is 0.7 µg/L for children under 6 years old and 3 µg/L for older ones. The EPA recommends a threshold of 8 µg/L for MCs and 15 µg/L for CYN as the basis for issuing a recreational exposure advisory (USEPA 2019). These thresholds guide state governments in responding to cyanoHABs. Traditionally, many state governments have relied on cyanotoxin analysis via enzyme-linked immunosorbent assay (ELISA), liquid chromatography combined with triple quadrupole mass spectrometry (LC-MS/MS) and microscopic analysis of cyanobacteria cell counts to drive decisions (Luo et al. 2006; Qian et al. 2015). LC-MS/MS offers high specificity, sensitivity, and the ability to detect multiple toxins simultaneously, but it is expensive, requires skilled operators, and can be impacted by matrix effects. ELISA is faster, more accessible, and suitable for screening specific toxins, but it can suffer from cross-reactivity, lower sensitivity, and less precise quantification influenced by competitive inhibition (Liu et al. 2022; Rivas-Villar et al. 2021). Moreover, cyanobacteria microscopic analysis is susceptible to human error and bias, such as misidentification and the overlooking of cryptic species (Zhang et al. 2023).

With the increasing frequency of cyanoHABs nationwide, the need to explore fast and easy monitoring approaches becomes more urgent. Genetic monitoring tools, such as quantitative polymerase chain reaction (qPCR) (Duan et al. 2022; Lu et al. 2020; Zhang et al. 2023), may offer faster, more comprehensive, and potentially cost-effective results, thereby enhancing states' assessment of health risks associated with cyanoHABs and increasing their ability to respond rapidly. qPCR offers a promising early warning system by detecting the genetic potential for toxin production, guiding the timing of EPA-required chemical analyses like ELISA. This approach integrates molecular and chemical methods for a comprehensive cyanoHAB monitoring framework. A major

limitation is that identifying cyanobacteria species doesn't always indicate whether they produce toxins, as some members of the same species may have toxin-producing genes, while others do not. This makes it difficult to assess toxin risk based on species identification alone. Novel monitoring strategies are essential for protecting public health in recreational waters. Quantitative polymerase chain reaction (qPCR) is increasingly favored as a tool for monitoring cyanoHABs, with several states (e.g., Ohio and Florida) incorporating it into their monitoring programs (Kramer et al. 2018; Raymond 2016). MCs are the dominant freshwater cyanotoxins, other common ones include CYN, anatoxins, and saxitoxins (Heisler et al. 2008). Microcystin synthetase gene E (*mcyE*) qPCR assay covers most MC producers including cyanobacteria genera *Dolichospermum* (formerly known as *Anabaena*), *Microcystis*, and *Planktothrix* (Dreher et al. 2022; Rantala et al. 2006). For other toxins, qPCR assays have successfully targeted the *anaC* (anatoxin-a), *cyrA* (cylindrospermopsin), and *sxtA* (saxitoxins) genes (Al-Tebrineh et al. 2012; Sabart et al. 2015). Some assays target amplification of cyanobacteria but not all cyanobacteria produce toxins (Zhang et al. 2023; Zuo et al. 2018). Multiplex qPCR provides simultaneous detection and quantification of multiple target cyanotoxin-encoding genes in a single reaction, expanding the capabilities of traditional qPCR reactions (Feist and Lance 2021). However, optimizing multiplex qPCR assays to accurately identify and quantify multiple cyanotoxin-encoding genes is an ongoing challenge as designing multiplex assays can be complex due to potential cross-reactivity between primers and probes, and variations in amplification efficiencies. Moreover, the above-mentioned qPCR assays require the purity and integrity of environmental genomic DNA. The classic DNA-extracting method includes environmental sample preparation (enrichment), DNA extraction, precipitation, washing and purification (Duan et al. 2022; Lever et al. 2015). Variations in sample characteristics, differences in DNA extraction efficiency, and failure to collect extracellular DNA can all impact the accuracy and comparability of qPCR results across various laboratories and studies (Lever et al. 2015). These classic methods are often associated with high costs, lengthy and labor-intensive processing times, and a lack of certified labs to conduct the analysis. Thus, a fast and cost-effective DNA extraction method is preferred for applying in conjunction with the qPCR process.

The necessity of DNA purification in conventional DNA extraction was re-visited for PCR assays for environmental soil samples and plant stems (Bou Orm et al. 2023; Lim et al. 2017). Lim et al. (2017) found that utilizing bead beating and centrifugation alone as the baseline method for DNA extraction was effective for soil samples with low concentration of humic acids, clay, and Mg²⁺ (Lim et al. 2017). However, the necessity of DNA purification during DNA extraction for cyanoHABs qPCR assay has not been investigated previously. Bead beating followed by centrifugation (without traditional purification), may provide DNA of sufficient quality for qPCR assays. Additionally, combining bead beating with a commercially available qPCR mix that suppresses the inhibitory effects of common environmental factors leads to a successful rapid assay. Thus, in this study, we developed a multiplex qPCR assay and compared two extraction methods: one using bead beating followed by centrifugation without DNA purification (RapidDNA method), and the other employing classic DNA extraction processes (ClassicDNA method). In this way, we evaluate the effectiveness of the RapidDNA method in identifying and quantifying cyanotoxin-encoding genes and describe a fast and affordable method for quantifying cyanoHABs. By comparing the results of qPCR assays from two extraction methods, we can validate the accuracy and precision of the RapidDNA method by assessing how closely the gene expression levels match those obtained using the ClassicDNA method.

Since this is a novel approach, it is crucial to integrate reference methods, such as microscopy for cell identification and ELISA or LC-MS/MS for toxin quantification to validate the results of the qPCR assays. By comparing the cyanobacterial species identified through microscopy with the toxin genes detected by the qPCR assays, we can confirm that

the qPCR results accurately reflect the presence of toxin-producing strains. Additionally, using ELISA and LC-MS/MS for toxin quantification allows us to assess whether the qPCR assays provide results comparable to traditional methods and potentially enable earlier detection of toxin production. All samples in this study are collected from real-world environmental surface water, allowing us to demonstrate the robustness and practical applicability of the qPCR assays under natural conditions.

In summary, this study aims to: (1) compare the RapidDNA and ClassicDNA methods for detecting genes producing major classes of cyanotoxins, such as MCs, CYN, anatoxin-a, and saxitoxins, using genuine environmental surface water samples; (2) establish correlations between RapidDNA and ClassicDNA qPCR results and actual toxin levels and cyanobacteria measured by standard methods (i.e., LC-MS/MS, ELISA, and microscopy-based toxin-producing cell counts); and (3) evaluate the ability of both RapidDNA and ClassicDNA methods to indicate toxin production and predict occurrences of cyanoHABs even in

non-blooming conditions. We anticipate that these objectives will aid in establishing a rapid and dependable approach for monitoring cyanoHABs. The outcomes of this research are essential for protecting public health by safeguarding drinking water supplies and recreational areas, while also providing fast, accurate tools to help the public avoid exposure to harmful toxins.

2. Materials and methods

2.1. Field sampling and physiochemical analysis

Nine water bodies in Utah, each with a history of cyanoHAB occurrences, were selected for this study. These waterbodies include Echo Reservoir, Rockport Reservoir, Jordanelle Reservoir, Deer Creek Reservoir, Pineview Reservoir, Scofield Reservoir, Grassy Trail Reservoir, Utah Lake, and North Fork Virgin River (Fig. 1). These water bodies are utilized for both recreational purposes and drinking water sources with

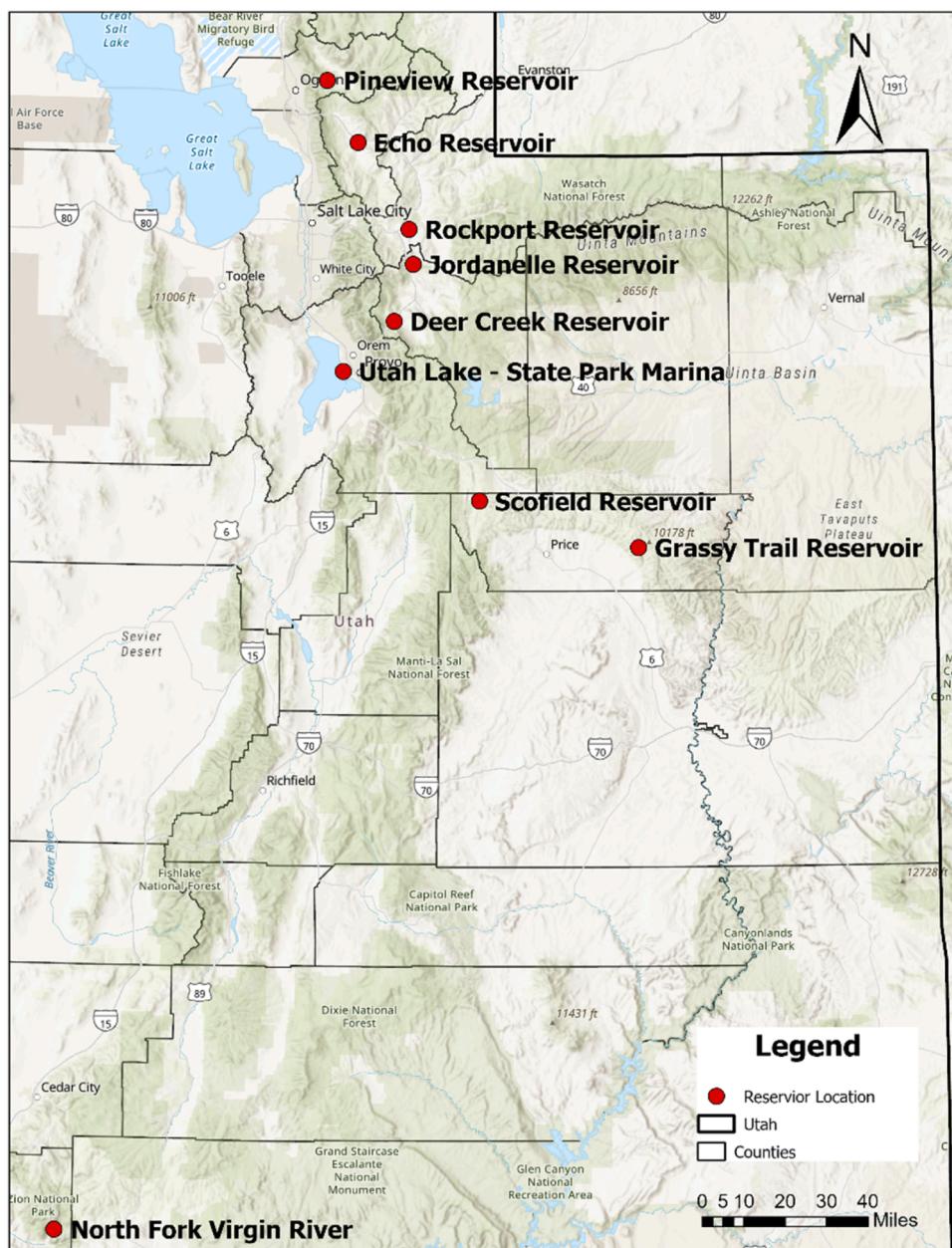


Fig. 1. A map of sampling locations.

the exception of Utah Lake (recreation only). To ensure the collection of representative water samples and to align with the routine cyanoHABs sampling conducted by the Utah Department of Environmental Quality, robust sampling protocols were developed. These protocols encompassed the collection of four types of samples from each water body: surface samples (targeted samples collected directly from the water surface when cyanoHAB surface scum is present), composite samples (integrated sample using three samples from elbow-depth to surface, collected 10 ft apart and combined in bucket), near intake samples (composite samples near the drinking water intake), and intake samples (the influent water to the drinking water treatment plant). To ensure data accuracy and reliability, field blanks and samples (for ELISA, cyanobacterial cell counts, and microbiology samples) were collected for each sampling date. Sampling locations, including latitude and longitude, are provided as Table S1. Sampling was conducted from June 1 to October 31, 2023. Monthly sampling was conducted for each sample type in the four water bodies without observed blooms (Rockport, Jordanelle, North Fork Virgin River, and Grassy Trail), while weekly sampling was carried out in water bodies with observed blooms (Utah Lake, Deer Creek, Echo, Scofield, and Pineview).

For cyanobacterial HAB cell counts, 250 mL water samples were collected in polypropylene (PP) bottles and sent to PhycoTech (MI, USA) for analysis using Imaging FlowCytobot (IFCB) to determine cyanobacteria cell counts and taxon composition. Additional 250 mL water samples were collected in amber glass bottles for ELISA (Eurofins Abraxis plate kits) testing for microcystins, anatoxin-a, cylindrospermopsin, and saxitoxin at the Utah Department of Agriculture and Food (UDAF) laboratory. The rest of the collected samples were transported from sampling sites to the Utah Water Research Laboratory for analysis. For LC-MS/MS analysis of MCs, 100 mL water samples were gathered in amber PTGE bottles pre-filled with preservation chemicals (USEPA 2017). Triplicate surface water samples (500 mL each) were collected at each site in PP bottles that had been sterilized for qPCR analysis. All water samples were kept on ice for same-day or overnight

shipping. Samples for the RapidDNA method were processed on the same day as receipt. Water samples for the ClassicDNA method can be held for extended periods post-filtration and stored at -70 °C before analysis.

2.2. Genomic DNA extraction

The RapidDNA method involved DNA extraction and centrifugation (Fig. 2). The extraction process began by adding 1.5 mL of the water sample into 2 mL microcentrifuge tubes (BioSpec Products, Inc. OK, USA) pre-filled with approximately 0.35 mL of 0.5 mm diameter Zirconia/Silica beads. These water samples were premeasured using OD600 and needed to be diluted to an OD600 of <0.5 to prevent excessively high cell concentrations. The water samples in the tubes underwent cell lysis using the FastPrep-24™ Classic instrument at 6 m/s for 40 s, with four repetitions and 15-second breaks between each repetition to prevent overheating. This process facilitated efficient cell disruption, releasing genomic DNA into the solution. The resulting lysate was then centrifuged at 13,000 × g for 5 min to separate the supernatant containing the extracted DNA. The supernatant was subsequently used for qPCR analysis.

For each site, water samples ranging from 50 to 500 mL were filtered through a 0.22 µm mixed cellulose esters filter (Millipore) to collect DNA for the ClassicDNA method (Fig. 2). The volume of water samples for filtration depended on the cell density, and these volumes were recorded for later calculations of gene copies. Genomic DNA was extracted from the filters based on our previous method adapted from the manufacturer's instructions of Fast DNA Spin Kit for soil (MP Biomedicals, Solon, Ohio) (Hou et al. 2022). Briefly, the membrane filters for each sample were cut into small pieces and transferred into a Lysing Matrix E tube, to which Sodium Phosphate Buffer and MT Buffer from the Kit were added. The FastPrep-24™ Classic instrument was utilized to homogenize the sample at a speed of 6.0 m/s for 40 s. Following homogenization, the sample underwent multiple rounds of centrifugation, with the addition

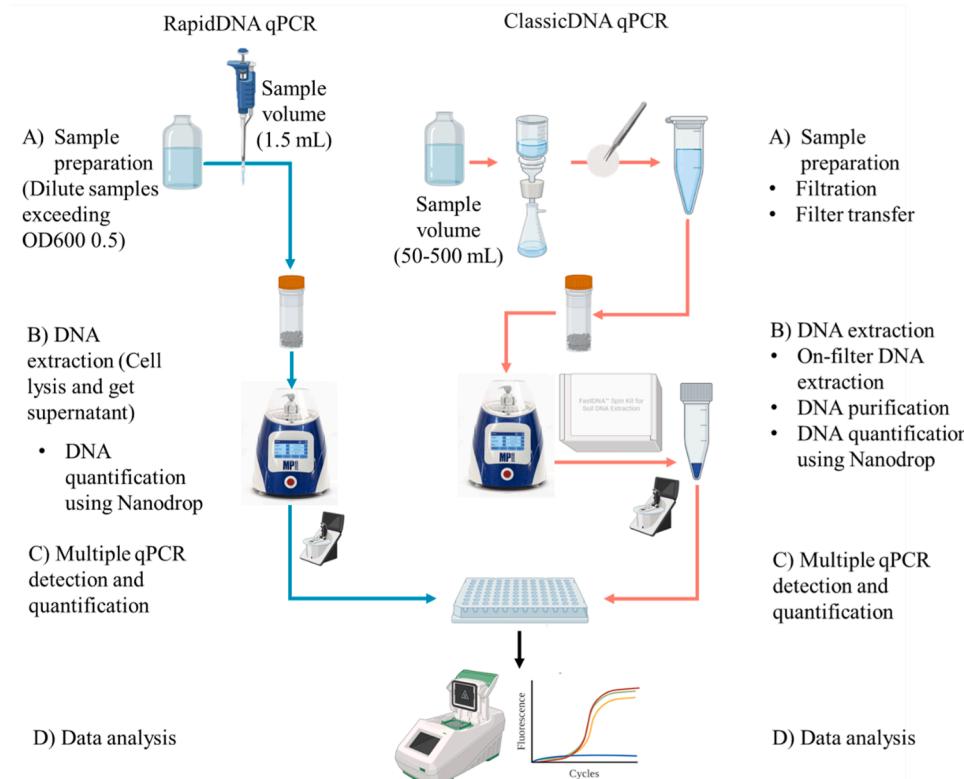


Fig. 2. A comparison between the flowcharts of RapidDNA (blue line) and ClassicDNA (red line) methods.

of various solutions provided in the kits for removal of proteins and other impurities. The purified DNA was then eluted in sterilized, nuclease-free water, yielding a DNA solution suitable for qPCR analysis. Following both extraction methods, the quality and quantity of the extracted DNA were verified using a NanoDrop ND1000 spectrophotometer. NanoDrop analysis was performed for both methods, but for RapidDNA, the results did not affect subsequent steps, as 2 µL from the 1.5 mL extraction was directly used for each qPCR assay.

2.3. Multiplex qPCR protocols for cyanotoxin-encoding genes

Following the two DNA extraction methods, qPCR analysis was conducted to quantify 16S rRNA and cyanotoxin-encoding genes, specifically targeting anatoxin-a, saxitoxin, cylindrospermopsin, and microcystin producing genes from three genera (*Microcystis* (MC), *Anabaena* (AB; currently termed *Dolichospermum*), *Oscillatoria* (OS)) (Table 1). For each sample, two multiplex reactions targeting six target genes and 16S rRNA gene were set up. Reaction 1 included 16S rRNA, *anaC*, *sxtA*, and *cyrA* gene, while reaction 2 included 16S rRNA, *mcyE* MC, *mcyE* AB, and *mcyE* OS gene. Primers and probes for *mcyE* assays (MS, OS, AB) were specifically designed using *in silico* testing (NCBI Primer-BLAST) and tested individually and in multiplex to confirm minimal cross-talk. The multiplex design and thermodynamic optimization ensured target-specific amplification and robust results. The cyanobacterium-specific internal control 16S rRNA gene was contained in both systems to check for consistency. The final qPCR reaction volume (20 µL) contained 10 µL of 2 × qPCR PerfeCta® MultiPlex qPCR ToughMix, low ROX™ (QuantaBio, MA, USA), 0.3 µM primers (Table 1), 0.125 µM probe, and 2 µL of template DNA. In the RapidDNA method, 2 µL of the supernatant was directly used for qPCR analysis. However, for the ClassicDNA method, the sample's DNA concentration was diluted to 1 ng/µL before qPCR analysis. The thermal cycling conditions included 95 °C for 3 min, 40 cycles of 95 °C for 15 s, the annealing temperature of 55 °C for 60 s. A 96-well plate format was used for all assays and run using an CFX96 Touch Real-Time PCR Detection System with CFX Maestro™ software (Bio-Rad Laboratories, CA, USA). To generate multiplex standard curves (Table S2), plasmids containing target genes were diluted from 1 to 1×10^{-6} ng/µL for each target gene and analyzed in triplicate using the multiplex assay. The standard curves were done biweekly with environmental samples. A quantification cycle (Cq) threshold was manually set for all gene targets after an automatic adjustment of the baseline. The relationship between the Log10 of the number of copies of each gene and Cq values was used to create a trendline equation for each gene. Standard curves were generated using MS Excel 2007, ensuring that the inverse slope remained between -3.10 and -3.58. Furthermore, standard curve efficiencies, calculated using the equation $E = (-1 + 10^{(-1/\text{inverse slope})}) \times 100$, were maintained within the range of 90 % to 110 % from the calculated value (Table S2). Each plate includes two positive controls for both reactions to establish the Cq threshold for consistency, along with two negative controls of sterilized, DNA-free water to determine the presence of contamination. This resulted in each DNA sample being measured for each gene in triplicates, except for 16S rRNA gene, which was replicated six times.

The calculation of gene copies for each method is outlined below:

For RapidDNA:

$$\text{Copies} / \text{mL} = \frac{\text{Numbers of copies}}{0.002 \text{ mL}}$$

For ClassicDNA:

$$\text{Copies} / \text{mL} = \frac{\text{Numbers of copies}}{\text{DNA weight in the qPCR reaction (ng)}} \times \frac{\text{Total DNA weight extracted from the sample (ng)}}{\text{Total volume of the sample used for DNA extraciton (mL)}}$$

The total gene copy concentration of *mcyE* is the sum of the gene

Table 1
Primers and probes used in qPCR Experiment.

Genes	Forward primer sequence	Reverse primer sequence	Probe with fluorophore and quencher	Reference
16S rRNA	AGCCACACTGGGACTGAGACA	TCCGCCATTTCGGGAA	[ROX]AGACTCTTACGGAGGGCACT [INHS/3' IBHQ]	(Al-Refaih et al. 2010)
<i>anaC</i>	ATCTGGATTAGTCATCCCTATTC	GGGAATATGACCATCAAATCGA	[6FAM]AGAACCATTTTGTGGGTGAAGTTT [ZEN/3' IBHQ]	This study
<i>sxtA</i>	TGGGTGTTATTCGTTATCGCTGG	CCGTAAAGGCATATGCTGTGCT	[HEX]CACTCTAGCTGGCTGCAAAGAG [ZEN/3' IBHQ]	This study
<i>cyrA</i>	CAGATCGCCCATCAAAGAGG	GGCAGAACATAGGCCATCTCATCG	[Cyanine5]CTCTTCATGGATAACCGTTAACATTGCAATTGCTAAGCT [TAO/ 3' IBHQ]	This study
<i>mcyE</i> MC*	CGGAATGCCCACTGCTTATTC	ATTGGATTATGGAGAACITGACGGG	[6FAM]TGAAAATGCCCTAACAGTTAACATTGCAAGAAACAGCT [TAO/ 3' IBHQ]	This study
<i>mcyE</i> AB*	AAGATGAAACAGGAAATGGT	AGGGACTCGGTCTACACCTG	[Cyanine5]GGAATGCCCACTGCTTATTCGAGCAACGTT [HEX]TAAACGCCAGTCTAACAAATGGC [TAO/ 3' IBHQ]	This study
<i>mcyE</i> OS*	CGGACATTCCTGATGCTTCG	AAACGGCTAAATCCGGCAATG	[HEX]TAAACGCCAGTCTAACAAATGGC [TAO/ 3' IBHQ]	This study

* *mcyE* *Microcystis* (*mcyE* MC), *mcyE* *Anabaena* (currently termed *Dolichospermum*), *mcyE* AB), *mcyE* Oscillatoria (*mcyE* OS).

copies of *mcyE* MC, *mcyE* AB, and *mcyE* OS.

2.4. Cyanobacterial cell counts and taxon analysis

We utilized a commercial laboratory, PhycoTech, Inc. for cyanoHAB identification and enumeration. PhycoTech ran samples through a tailored Imaging FlowCytobot (IFCB) which captured images and automatically identified particles as they passed through a flow-cell. The resultant data provided cell counts of toxicogenic cyanobacteria, divided to the genus or species level, in submitted samples. IFCB results were quality-checked by PhycoTech staff and manual enumeration as needed. Most samples were submitted to PhycoTech live. When samples could not be submitted within 24 h, they were preserved with glutaraldehyde.

2.5. LC-MS/MS and ELISA toxin measurement

2.5.1. LC-MS/MS

Seven microcystin congeners observed in cyanoHABs in Utah (MC-YR, MC-LR, MC-RR, MC-LA, MC-LY, MC-LW, MC-LF) plus nodularin were investigated following EPA methodology (USEPA 2017). Water samples were extracted by two methods, differentiated by sample clarity. Transparent water samples underwent filtration through a 0.8 µm Nucleopore polycarbonate filter. Prior to filtration, 20 µl of a 50 ng/µl of ethylated MC-LR, d5(C2D5-MC-LR) (Cambridge Isotope) was added to each 100 ml sample for determination of extraction efficiency. After filtration, filters were placed into a solution of 80:20 methanol: water and held at -20 °C for one hour to release intracellular MCs. This solution was combined with the filtrate. Opaque samples (10 mL) were spiked in the isotopically labeled extraction standard then combined with 10 mL methanol and held at -20 °C for two hours, then centrifuged and filtered. The filtrate was diluted with water. For both sample preparations, the aqueous solutions were passed through a Waters Oasis HLB, 150 mg, 6 cc divinylbenzene N-vinylpyrrolidone copolymer solid phase extraction cartridge. The MCs were eluted from the cartridge with 90:10 methanol:water then evaporated to dryness using a TurboVap system at 60 °C and reconstituted in 1 mL 90:10 methanol:water. An internal standard (cyclosporin-A 13C2, d4, Toronto Research Chemicals) was added to all samples, QC samples and standards before analysis to ensure accurate quantification.

An Agilent Infinity 1290 liquid chromatograph (LC) interfaced with an Agilent 6490 triple quadrupole mass spectrometer/mass spectrometer (MS/MS) was used for quantifying the selected MC congeners plus nodularin in the water samples following EPA methodology (USEPA 2017), with specified quality control including field and trip blanks, laboratory reagent method blanks, laboratory fortified blanks, continuing calibration check samples, and use of an internal standard and extraction standard to evaluate instrument and method performance. Standard curves consisting of the eight analytes purchased from Enzo Biochem (Heptatotox set 1) were generated for each sample batch. The determined reporting limit with accounting for sample concentration was 0.001 µg/L for all analytes except for MC-LR with a reporting limit of 0.006 µg/L. Analytes were separated using ThermoScientific Synchronis C8, 2.1 mm ID, 100 mm length, 1.7 µm particle size column at a flow rate of 9.4 mL/min. The mobile phase solution A was 20 % mM ammonium formate and solution B was 100 % methanol. LC conditions and MS/MS parameters are given in Table S3 and S4.

2.5.2. ELISA toxin measurement

The ELISA method, an immunoassay that quantifies congener-independent MCs and nodularin, was performed by the Utah Department of Agriculture and Food (UDAF) laboratory. ELISA analysis was also used to evaluate the presence of anatoxin-a, cylindrospermopsin, and saxitoxin in several selected samples. UDAF used Abraxis test kits specific to the toxin analysis requested and followed standard kit analysis instructions (Fischer et al. 2001). Toxin concentrations were determined using a standard color curve constructed with each run.

MCs, the primary ELISA result reported, had a detection limit of 0.3 µg/L. Samples were either analyzed within 24 h or kept frozen until analysis.

2.6. Data analysis

All plots and statistical analyses were performed using statistical software R (R Core Team, 2024). Various approaches were used to analyze the data. For data handling, the concentration of MC measured by LC-MS/MS was calculated as the sum of the concentrations of seven microcystin congeners (MC-YR, MC-LR, MC-RR, MC-LA, MC-LY, MC-LW, and MC-LF). The concentration of *mcyE* gene was determined by summing the gene copies per milliliter (copies/mL) for three targets: *mcyE* MC, *mcyE* AB, and *mcyE* OS. Values below the qPCR detection limitation ($C_q > 34$) were designated as not available (NA). When comparing different target genes using the RapidDNA and ClassicDNA qPCR methods and visually presenting the changes in gene copies/mL over time, NA values were treated as zero copies/mL. A paired *t*-test was used to compare the differences in gene concentrations between the two DNA extraction methods.

ELISA results reported as "not detected" when the MC concentration was below 0.3 µg/L. In LC-MS/MS, results below the reporting limit were recorded as $< 1 \times 10^{-3}$ µg/L. qPCR results with C_q values above 34 were recorded as NA. To address the question of how accurately we can predict the MC content of a sample based on the qPCR results of gene concentrations of target genes that produce MC, we included censored data from qPCR, ELISA, and LC-MS/MS in the regression analysis. Special approaches were needed to carry out regressions without losing substantial fractions of data and introducing bias if those censored observations were simply excluded from the analysis or set equal to a fixed value. Two approaches were used - one of them (Akritas-Theil-Sen or ATS method) is a nonparametric method that accounts for the slopes between all individual observation pairs and the slope of the ATS line is just the median of these slopes. A second method is a maximum likelihood estimation method commonly known as Tobit regression, that assumes a log-normal error distribution and forms a maximum likelihood equation as a function of the slope and intercept and variance of the regression line (Helsel 2005; Lee and Lee 2015). This method manipulates their values until the likelihood is maximized. This method allows for only the 'Y' value being censored when all values for the 'X' variable are known but this isn't required. The ATS method is used when both 'X' and 'Y' have some censored observations. R^2 is calculated based on the linear regression model (Regression line). To evaluate the fit of non-linear and censored models when both 'X' and 'Y' include censored observations, McFadden's R^2 was calculated for fitting Tobit regression. This analysis was performed exclusively on RapidDNA and ClassicDNA results in comparison with ELISA MC measurements. The McFadden's R^2 calculation were conducted using the censReg package in R.

To evaluate the accuracy of the RapidDNA and ClassicDNA qPCR methods in real-world conditions, true positive, true negative, false positive, and false negative cases were summarized and calculated by comparing the results to toxin levels measured by LC-MS/MS and ELISA. True positive cases were summarized when toxin level measured by LC-MS/MS or ELISA was above or equal to 0.3 µg/L, and the value of *mcyE* gene concentration (copies/mL) is above zero. False negative cases were calculated when toxin level measured by LC-MS/MS or ELISA was above or equal to 0.3 µg/L, and the value of *mcyE* gene concentration (copies/mL) is zero. True negative cases were identified when the value of *mcyE* gene concentration (copies/mL) were lower than a defined *mcyE* concentration, and toxin level measured by LC-MS/MS or ELISA was below 0.3 µg/L. False positive cases were summarized when the value of *mcyE* gene concentration (copies/mL) were higher than a defined *mcyE* concentration, and LC-MS/MS or ELISA was still below 0.3 µg/L. The defined *mcyE* concentration was calculated based on the linear regression relationship between *mcyE* gene concentration, measured by the two methods, and MC concentration at 0.3 µg/L, measured by LC-MS/

MS or ELISA (Table 2). For instance, the *mcyE* gene concentration for RapidDNA was $10^{7.22}$ (LC-MS/MS) or $10^{6.96}$ (ELISA) when MC was equal to 0.3 µg/L, as shown in Table 2 ($Y = -7.228 + 0.929X$; $Y = -6.948 + 0.923X$). The *mcyE* gene concentration for ClassicDNA was $10^{5.75}$ (LC-MS/MS) or $10^{5.7}$ (ELISA) when MC was equal to 0.3 µg/L (Table 2; $Y = -5.866 + 0.930X$; $Y = -6.875 + 1.115X$). The percentages of true positive, false negative, true negative, and false positive cases for the RapidDNA and ClassicDNA methods were calculated by dividing the number of each case type by the total number of cases.

3. Results

3.1. Comparison between RapidDNA and ClassicDNA qPCR methods

A total of 177 samples were collected from nine reservoirs for qPCR analysis using both extraction methods. Among the seven target genes, including 16S rRNA, *anaC*, *cyrA*, *mcyE* (*mcyE* MC, *mcyE* AB, and *mcyE* OS) and *sxtA*, all genes were detected using the ClassicDNA method but not the RapidDNA method, which did not detect *mcyE* AB, *mcyE* OS, *anaC*, and *cyrA*. For other genes that can be detected by RapidDNA methods (e.g., 16S rRNA, and *mcyE* MC), the gene concentrations measured by the RapidDNA method were significantly higher than quantified by the ClassicDNA qPCR method ($P < 0.05$, Fig. 3a). The gene concentration of 16S rRNA ranged from 4.41×10^5 to 4.28×10^{12} copies/mL for RapidDNA and from 2.95×10^3 to 6.20×10^{10} copies/mL for ClassicDNA method. The average gene concentration of *mcyE* was 7.50×10^9 copies/mL for RapidDNA and 1.39×10^7 copies/mL for ClassicDNA method. The majority of *mcyE* genes were contributed by *Microcystis* harboring the *mcyE* MC gene, as confirmed by microscopy-based taxon identification of cyanoHABs, with *Microcystis* being identified as one of the dominant genera (Fig. S1). Histograms illustrating the frequency distributions of *mcyE* gene concentration in the samples from different reservoirs over time (Fig. 3b and c) revealed that both methods exhibit a significant number of undetected cases ($C_q > 34$ for qPCR results; $\log_{10}(\text{mcyE gene copies/mL})$ recorded as 0 when *mcyE*

gene copies/mL is NA). However, the ClassicDNA method demonstrates fewer undetected cases compared to the RapidDNA method. There were more cases where *mcyE* was detectable with the ClassicDNA ($n = 87$) method but not with the RapidDNA method ($n = 37$). For the ClassicDNA method, the *mcyE* gene is most frequently detected around 10^5 copies/mL, whereas for the RapidDNA method, it is predominantly detected around 10^8 copies/mL. The average concentrations of *anaC*, *cyrA*, and *sxtA* using the ClassicDNA method were 1.65×10^4 , 1.50×10^4 , and 5.63×10^3 copies/mL, respectively.

There are linear relationships between the ClassicDNA and RapidDNA for \log_{10} -transformed *mcyE* gene concentrations and 16S rRNA gene concentrations (Figs. 3d and 3e). The plots demonstrate positive correlations between these two methods, with an R^2 value of 0.43 for *mcyE* and 0.66 for 16S rRNA concentrations. These results imply that while the RapidDNA method can be used to determine gene concentrations, caution should be exercised as it may yield different absolute values compared to ClassicDNA methods. Additionally, RapidDNA method may struggle to detect genes with low abundances in the water samples or when the cyanoHAB is in the early stages of development. Further calibration or adjustment might be necessary for specific applications to ensure accurate and reliable results.

3.2. Correlation between qPCR results and MC levels and cell densities

Although there were occasional positive results for anatoxin and its associated genes using ELISA, the analysis focuses on MC and *mcyE* due to the limited amount of data available for other toxins. To compare the toxin production-related gene concentrations measured by RapidDNA and ClassicDNA methods with other standard methods, the correlations between RapidDNA and ClassicDNA results and those obtained from LC-MS/MS, ELISA, and microscopy-based toxin-producing cell counts were investigated. Both RapidDNA and ClassicDNA methods showed correlations with MC concentrations (both LC-MS/MS and ELISA) and cyanobacterial cell density (Fig. 4). However, the RapidDNA method demonstrated a stronger correlation with LC-MS/MS MC measurements and cyanobacterial cell density compared to the ClassicDNA method, as indicated by the higher R^2 value of 0.819 and 0.572, respectively (Fig. 4a, b, e and f). The lower R^2 values indicate increased scatter in the *mcyE* gene concentration when using the ClassicDNA method. This may be tentatively attributed to the additional extraction and purification steps involved in the ClassicDNA method compared to the RapidDNA method. In addition, the correlations with ELISA, despite not having defined values, show consistent slopes, indicating that both RapidDNA and ClassicDNA methods reliably reflect MC levels (Figs. 4c and 4d). The RapidDNA method demonstrates a better fit with the ELISA MC measurements (Figs. 4c and 4d), as evidenced by a McFadden's R^2 of 0.24, compared to 0.14 for the ClassicDNA method. This indicates that the RapidDNA method explains approximately 24 % of the variation in the ELISA MC measurements, whereas the ClassicDNA method accounts for only 14 %. We observed that in four of the six regression cases (Fig. 4a, b, e, and f), the ATS and Tobin slopes matched to the same decimal places, while in the other two cases (Fig. 4c and d), the slopes were close in magnitude. This consistency lends confidence to the reliability of the slope results. LC-MS/MS and ELISA were both used to measure MC concentrations, with LC-MS/MS generally considered more precise and specific. By using ATS and Tobit regression, the censored observations can be considered with generating good linear relationships. In addition, the relationships between *mcyE* gene concentrations (both RapidDNA and ClassicDNA) and cyanobacterial cell density suggest that these genetic methods can effectively estimate cyanobacterial biomass (Fig. 4e and f). The detailed correlations among gene concentrations, cell concentrations, and MC levels were investigated using Pearson correlation coefficients (Fig. S2 and S3).

Using the linear regression equations developed from Fig. 4 (Table 2), we can estimate the MC content of a sample based on the qPCR results for the total *mcyE* gene copies responsible for producing

Table 2
Tobit regression model equations for predicting MC concentrations.

Regression equations			
	Y (\log_{10} (MC µg/L) or \log_{10} (cyanoHAB cell density (cells/mL)); X (\log_{10} (<i>mcyE</i> gene copies/mL))		
LC-MS/MS MC (µg/L)	$Y = -7.228 + 0.929X$ $R^2 = 0.819$	$Y = -5.866 + 0.930X$ $R^2 = 0.598$	
	$Y_{UCL} = -6.079 + 0.929X$	$Y_{UCL} = -3.965 + 0.930X$	
	$Y_{LCL} = -8.378 + 0.929X$	$Y_{LCL} = -7.767 + 0.930X$	
ELISA MC (µg/L)	$Y = -6.948 + 0.923X$ $R^2 = 0.24$	$Y = -6.875 + 1.115X$ $R^2 = 0.14$	McFadden's
	$Y_{UCL} = -4.889 + 0.895X$	$Y_{UCL} = -4.381 + 1.115X$	
	$Y_{LCL} = -8.380 + 0.895X$	$Y_{LCL} = -9.362 + 1.115X$	
cyanoHAB cell density (cells/ mL)	$Y = -2.856 + 0.913X$ $R^2 = 0.572$	$Y = -3.1735 + 1.154X$ $R^2 = 0.465$	
	$Y_{UCL} = -0.795 + 0.913X$	$Y_{UCL} = -0.315 + 1.154X$	
	$Y_{LCL} = -4.918 + 0.913X$	$Y_{LCL} = -6.032 + 1.154X$	

UCL: Upper Control Limit, representing the upper boundary for predicted values at the 95 % confidence level. LCL: Lower Control Limit, representing the lower boundary for predicted values at the 95 % confidence level.

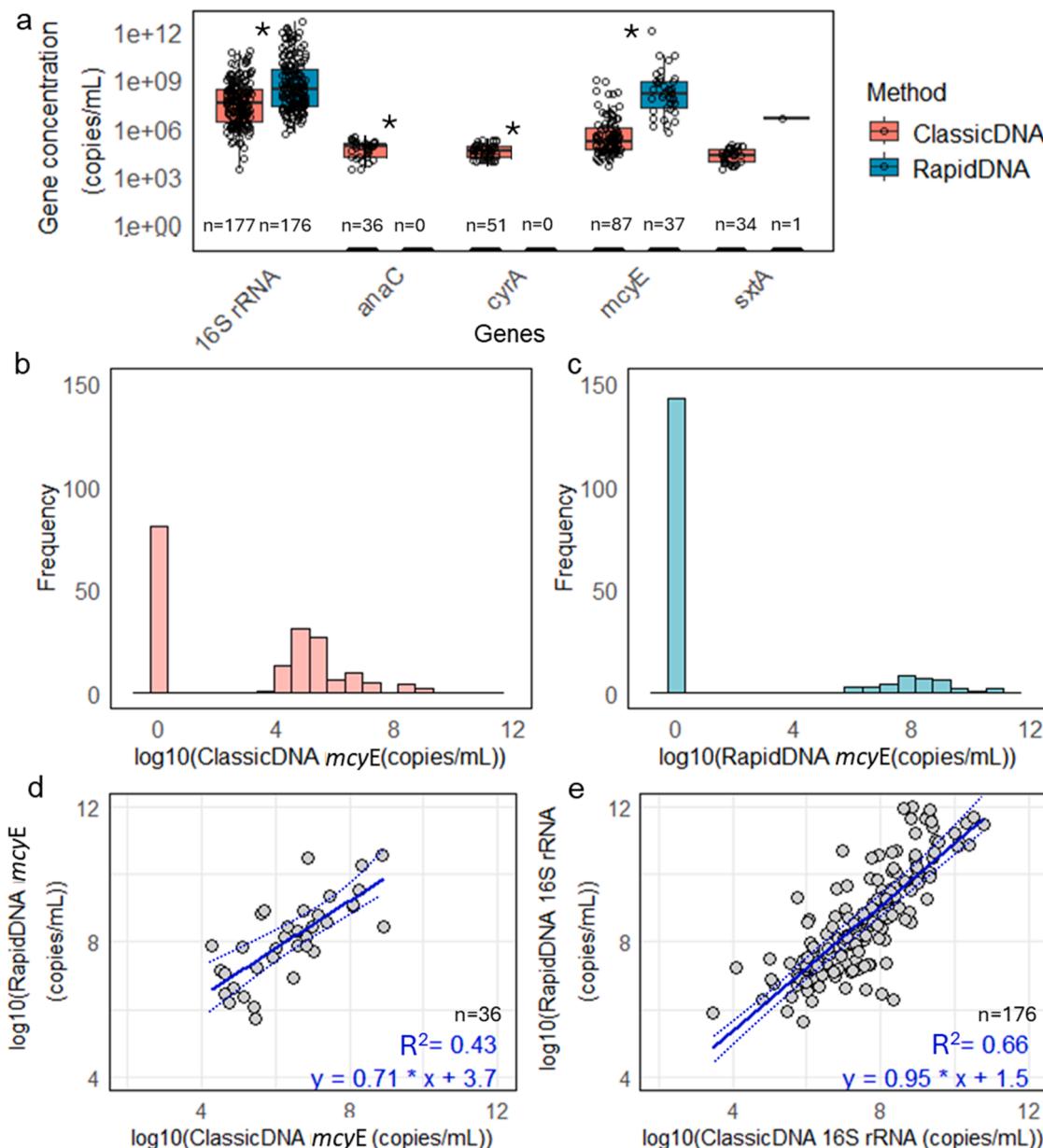


Fig. 3. (a) Box and whiskers plot comparing target gene concentration (copies/mL) using ClassicDNA (red) and RapidDNA (blue) qPCR methods; Asterisks (*) indicates significant differences at $P < 0.05$ using a paired t-test between two methods. The sample data size (n, representing the number of detected observations) for each gene and method is indicated next to the x-axis. (b-c) Histogram plots show the distribution of *mcyE* gene concentration (copies/mL) measured by ClassicDNA (red) and RapidDNA (blue) qPCR methods. Linear relationship between *mcyE* (d) and *16S rRNA* (e) measured by RapidDNA and ClassicDNA qPCR methods. The blue line represents the regression line, showing the linear relationship between log-transformed ClassicDNA and RapidDNA results. The dashed lines indicate the 95 % Confidence Interval, representing the range where the true regression line likely lies.

MC. To predict MC concentrations, an 'X' value representing *mcyE* copies/mL was integrated into the equation from Table 2, providing a 'Y' value (in the log phase) that indicated the MC concentration, depending on the selected equation for either ELISA or LC-MS/MS. The equations can also be used in reverse to estimate *mcyE* gene concentrations from known MC levels. The linear relationship indicates that when the concentrations of *mcyE* were approximately 10^9 copies/mL for the RapidDNA method and 10^7 copies/mL for the ClassicDNA method, the total MC levels exceeded 8 µg/L, reaching the no-swimming or recreational advisory thresholds (Figs. 4c and 4d, red lines). When the *mcyE* gene concentration was approximately 10^7 copies/mL for the RapidDNA method and 10^5 copies/mL for the ClassicDNA method, the MC concentration reached 0.3 µg/L, which corresponds to the Drinking Water Health Advisory for bottle-fed infants and pre-school children

(Figs. 4c and 4d, red dashed lines).

3.3. Temporal dynamics and comparative analysis of *mcyE* using two methods across different reservoirs

Among the nine reservoirs monitored regularly, cyanoHABs were reported in only five during 2023: Utah Lake, Deer Creek Reservoir, Echo Reservoir, Pineview Reservoir and Scofield Reservoir. Pineview Reservoir was excluded from the main analysis because of consistently low MC concentrations measured by LC-MS/MS (< 0.3 µg/L) and the limited number of data points available for both ELISA and qPCR, making it difficult to draw reliable conclusions for this site. Among the other four reservoirs, the *mcyE* genes, which are associated with microcystin toxin production, were the most frequently detected. Deer

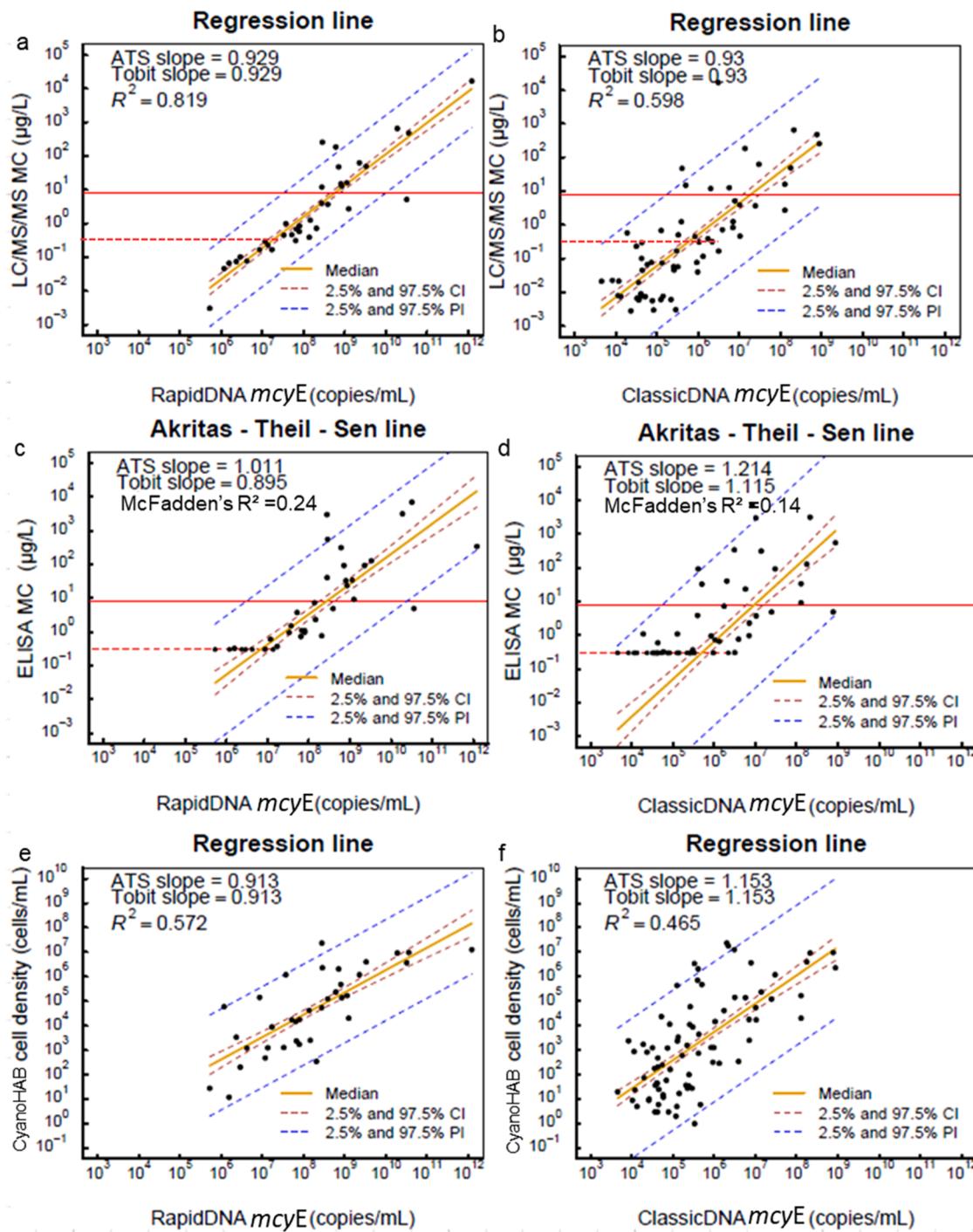


Fig. 4. Censored regression using both simple linear regression and Akritas Theil-Sen (ATS) models to elucidate the correlation between *mcyE* gene concentrations measured via RapidDNA and ClassicDNA qPCR methods, MC levels measured by LC-MS/MS or ELISA, as well as cyanobacteria cell density. Comparisons are made between RapidDNA (a) and ClassicDNA (b) and LC-MS/MS MC, RapidDNA (c) and ClassicDNA (d) and ELISA MC, RapidDNA (e) and ClassicDNA (f) and cyanobacteria cell density. The blue lines represent 97.5 % prediction intervals (PI) lines, indicating the range where 97.5 % of Y values are expected to fall for a given X value. Brown lines depict 95 % confidence intervals (CI) lines, indicating a 95 % probability that the true best-fit line lies within the confidence interval, with the median lines representing the median of CI. Red horizontal lines in panels (a-d) denote the water quality guidelines for total MC, indicating a threshold for swimming advisories at 8 $\mu\text{g/L}$. Red dashed lines (a-d) denote the Drinking Water Health Advisory for bottle-fed infants and pre-school children at 0.3 $\mu\text{g/L}$.

Creek Reservoir experienced its first bloom on July 11, 2023, followed by our specific sampling site on Utah Lake on August 15, 2023, and Scofield Reservoir (August 23, 2023). Echo Reservoir did not exhibit blooms until October 4, 2023. Initial blooms were identified by experienced sampling team members and confirmed through cyanobacteria cell counts and identification. The first date when MC concentrations measured by either ELISA or LC-MS/MS exceeded 0.3 $\mu\text{g/L}$ is indicated

by grey dashed lines in Figs. 5a-d (see also Figs. S4 and S5). The blooms in Utah Lake and Deer Creek persisted until late October. In contrast, blooms in Echo Reservoir and Scofield Reservoir were short-lived, with maximum MC concentrations remaining below 8 $\mu\text{g/L}$. Figs. 5a-d illustrates significant temporal variations in *mcyE* gene concentrations measured using both ClassicDNA and RapidDNA methods across the different reservoirs. Before the onset of blooms, *mcyE* genes were

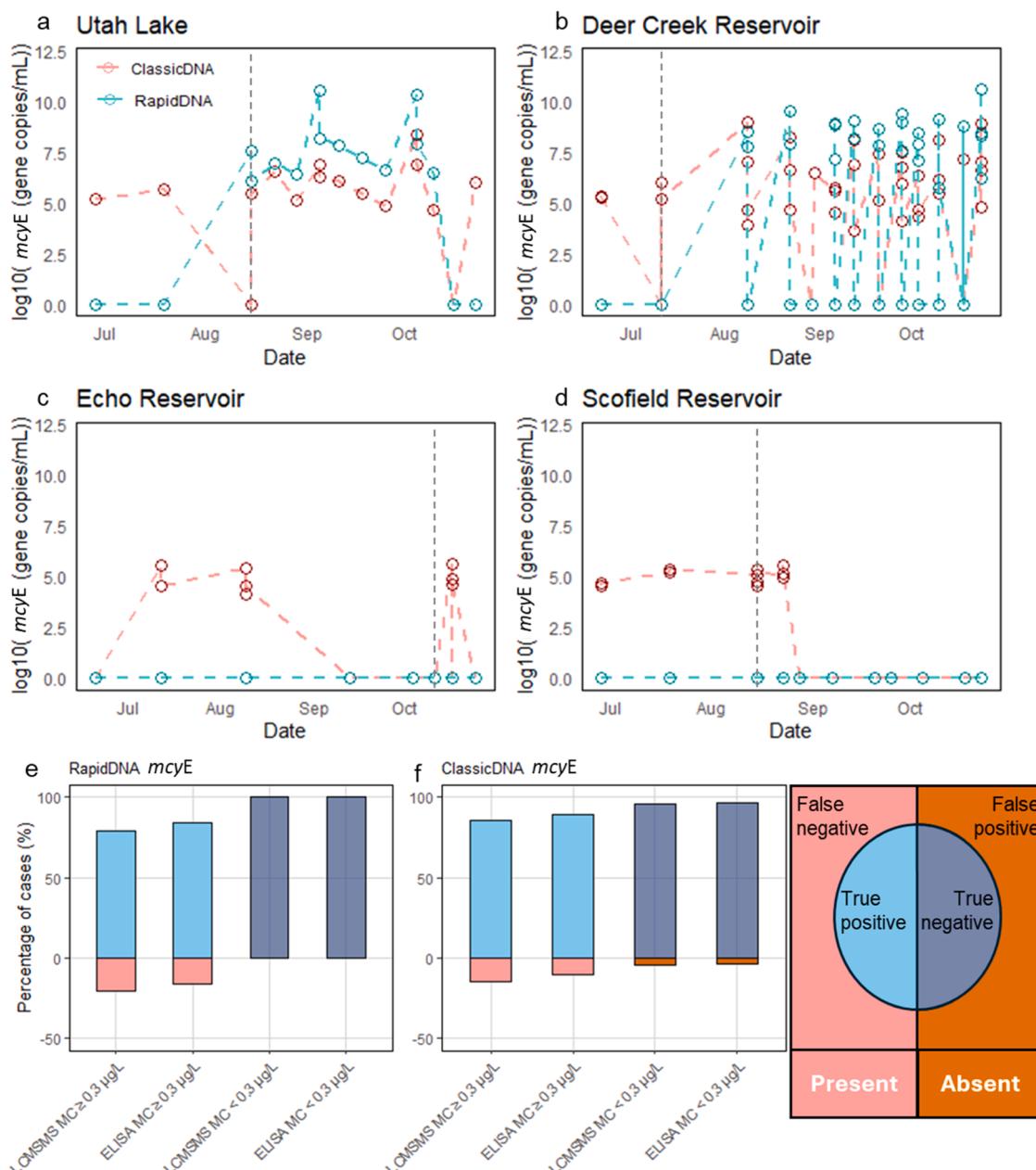


Fig. 5. Temporal dynamics of *mcyE* gene concentration in four reservoirs detected by RapidDNA (red) and ClassicDNA (blue) qPCR methods: Utah Lake (a), Deer Creek Reservoir (b), Echo Reservoir (c), and Scofield Reservoir (d). Multiple circles on the same day represent various types (surface, composite, near intake, intake samples) collected concurrently. The grey dashed lines indicate the first date when cyanobHAB was observed and MC was detected above or equal to 0.3 µg/L using LC-MS/MS. The stacked bar chart shows the percentage of true positive, false negative, true negative, and false positive cases for RapidDNA (e) and ClassicDNA (f) qPCR methods.

detected in all four reservoirs using the ClassicDNA method at around 10^5 copies/mL. Following the initiation of blooms, both methods detected *mcyE* genes in Utah Lake and Deer Creek Reservoir. However, in Echo Reservoir and Scofield Reservoir, *mcyE* genes were only detected using the ClassicDNA method after the blooms had begun. The toxin levels in both reservoirs remained low (around 0.3 µg/L; see Fig. S4) following the onset of blooms, which may explain the inability to detect them using the RapidDNA method when gene abundance was low. This suggests that both methods are effective in detecting blooms after cyanobHABs occur. However, only the ClassicDNA method is suitable for indicating or predicting cyanobHABs at an early stage (when cyanobacteria start to proliferate but have not yet reached high cell densities or produced significant levels of toxins), as the RapidDNA method may not detect the gene when its concentration is relatively low. The variability

in values (zero vs. detectable *mcyE*) on the same day using RapidDNA method for Deer Creek Reservoir was attributed to differences in water sample types, including surface, composite, near-intake, and intake samples. In many cases, intake and near-intake samples did not show detectable toxin-related genes due to their sampling positions. These observations emphasize how sampling location and water type can influence the detection of toxin-related genes, underscoring the importance of site selection in qPCR-based monitoring studies.

To understand the detection reliability of the two methods, we investigated the percentage of true positive and true negative cases in overall detection cases for MC and related genes (Fig. 5e and 5f). Although other toxins and genes were detected in some samples, the lack of a comparable dataset prevented the establishment of a linear regression curve, making it difficult to draw solid conclusions for other

toxins. When the MC concentration determined by LC-MS/MS and ELISA is higher than or equal to 0.3 µg/L, 79.4 % and 84.2 % of the detection cases showed positive detection of the *mcyE* genes using the RapidDNA method. In comparison, 85.3 % (LC-MS/MS ≥ 0.3 µg/L) and 89.4 % (ELISA ≥ 0.3 µg/L) of the detection cases showed positive detection of the *mcyE* genes using the ClassicDNA method. When the MC concentration determined by LC-MS/MS and ELISA is lower than 0.3 µg/L, 100 % of the detection cases showed *mcyE* concentrations lower than 10^{7.22} (LC-MS/MS) or 10^{6.96} (ELISA) copies/mL using the RapidDNA qPCR method. In contrast, 95.7 % and 96.7 % of the detection cases showed *mcyE* concentrations lower than 10^{5.57} (LC-MS/MS) or 10^{5.7} (ELISA) copies/mL using the ClassicDNA method. The true positive and true negative results demonstrate the reliability of both methods, indicating that both have a high accuracy in detecting cyanoHABs. In addition, the presence of false positives and negatives for RapidDNA and ClassicDNA qPCR methods suggests the potential limitations and the need for method optimization or complementary use of two methods to ensure accurate monitoring. RapidDNA qPCR method frequently detects higher toxin-related gene abundances compared to the ClassicDNA method.

4. Discussion

To evaluate the suitability of RapidDNA methods for quantifying cyanoHAB gene concentrations and potentially adopting a rapid and cost-effective DNA extraction approach, we initially compared these methods with ClassicDNA techniques. Our findings indicate that the RapidDNA method can effectively determine gene concentrations associated with cyanoHABs, albeit yielding higher values than those obtained using ClassicDNA. For instance, in a prior study on Harsha Lake utilizing traditional DNA extraction methods (Duan et al. 2022), *mcyE* gene concentrations ranged from 0 to 10^{5.5} copies/mL. Similarly, in the water-sediment interface of Rio Grande II reservoir (Antioquia, Colombia), *mcyE* concentrations ranged from 0 to 10^{7.5} copies/mL (Arismendi-González et al. 2021). Our ClassicDNA results for the *mcyE* gene ranged from 0 to 10⁷ copies/mL, consistent with these findings. However, in our study, RapidDNA results reported concentrations ranging from 0 to 10⁹ copies/mL, notably higher than those reported previously. This discrepancy may be attributed to the RapidDNA method's capability to capture free DNA released from cells in addition to DNA extracted from intact cells (Feist and Lance 2021). Additionally, in laboratory mixed cultures producing MC, Ngwa et al. (2014) reported *mcyE* gene copies ranging from 4.32 × 10⁶ to 4.75 × 10⁹ copies/mL (Ngwa et al. 2014).

It was observed in this study that when the concentrations of target genes are relatively low (around 10⁴ copies/mL using the ClassicDNA method), the RapidDNA method encountered difficulties in detecting these genes in the water samples. This may be due to the limitations of the RapidDNA method: when cyanoHAB cell concentrations are low in the sample, less DNA is released into the solution, leading to uneven distribution in the 1.5 mL cell lysis solution. Additionally, the lack of a purification process may result in the presence of inhibitors that interfere with DNA detection. Despite these challenges, the overall trends in *mcyE* and 16S rRNA concentrations detected using both ClassicDNA and RapidDNA methods for the same samples were similar, and the results from two methods showed significant linear relationships (Fig. 3). This indicates that RapidDNA qPCR method is a promising molecular tool for assessing cyanoHABs of natural surface waterbodies.

Despite this, both methods demonstrated relatively high accuracy and reliability in indicating MC production, as evidenced by the percentages of true negative and positive cases. It is crucial to understand the frequency of false positives—where *mcyE* gene concentrations indicate a problem but no or low toxins are detected (MC concentration < 0.3 µg/L)—and false negatives—where toxins are present (MC > 0.3 µg/L) but no or low *mcyE* gene concentrations were found, which is the worst scenario. In our study, there were no false positive cases for the RapidDNA method, meaning that when MC concentrations were low (<

0.3 µg/L), no *mcyE* gene concentrations were detected using this method. However, false positive cases for the ClassicDNA methods accounted for 3–4 % of overall cases. This occurred because the ClassicDNA method was able to detect *mcyE* genes even when MC concentrations were below 0.3 µg/L and the *mcyE* gene abundance was low. This likely reflects the detection of *mcyE* genes in cyanobacteria populations that were present but not actively producing toxins. Toxin production is known to occur only under certain environmental conditions, which are not yet well defined. As a result, the presence of the *mcyE* gene does not always correlate with measurable microcystin concentrations, particularly when these conditions for toxin production are absent.

The ClassicDNA method with fewer of false negative cases demonstrated better accuracy and reliability when the bloom was developing and producing low concentrations of cyanotoxins. Meanwhile, the percentage of false negatives for the RapidDNA method was relatively higher, ranging from 16 % to 21 % for ELISA and LC-MS/MS, respectively, compared to 11 % to 15 % for the ClassicDNA method. This indicates that the RapidDNA method would not detect the *mcyE* gene when toxin level measured by LC-MS/MS or ELISA was above or equal to 0.3 µg/L, which may pose the risk for incorrect response decisions. A recent study that compared bench-top and handheld qPCR devices involving DNA filtration and purification showed that the percentage of false negatives ranged from 4 % to 12.5 %, while the percentage of false positives ranged from 25 % to 40 % when the MC threshold was set at >4 µg/L (Wang et al. 2024). Additionally, as MC threshold concentrations increased, the percentage of true positives and true negatives increased (Wang et al. 2024). Our results showed increased true positives and true negatives cases as MC threshold increased to 8 µg/L. The low percentage of false positive cases indicated the accuracy and reliability of the RapidDNA method during active blooms. Both RapidDNA and ClassicDNA methods have their strengths and weaknesses in detecting cyanotoxins. The results highlight the importance of selecting appropriate detection methods based on the specific needs of water management programs. In addition to the high percentages of true positive and true negative cases for both methods in distinguishing high-toxin versus low-toxin samples, linear regression patterns showed strong correlations between gene levels and toxin levels, as well as with cyanoHAB cell density (Fig. 4).

While multiple studies have reported models for predicting MC levels using *mcy* genes (Pacheco et al. 2016), these models exhibit considerable variability due to differences in qPCR assays, environmental samples, and cyanotoxin types. Additionally, the use of multiplex qPCR assays in MC level prediction models is rare (Pacheco et al. 2016). Some of the previous models lack applicability due to the absence of detailed linear regression equations (Ha et al. 2009; Otten et al. 2012), small sample sizes in a few cases (Fortin et al. 2010), and failure to consider samples below the lower detection limits or use censored data in some studies (Pacheco et al. 2016; Wang et al. 2024). In a previous study on 29 Alpine lakes and 18 rivers, qPCR-based *mcyE* concentrations determined by traditional methods involving filtration and DNA extraction were found to be quantitatively related to MC concentrations in plankton samples ($Y = 0.01 + 0.64X$, $R^2 = 0.61$) (Jablonska et al. 2024). Unlike this study, the *mcyE* results from the current RapidDNA method with multiplex qPCR assays, conducted without filtration and DNA purification and extraction, yielded even better quantitative results with MC concentrations ($Y = -7.228 + 0.929X$; $R^2 = 0.819$; Table 2). Our results indicated that samples with *mcyE* concentration of approximately 10^{7.0} copies/mL (RapidDNA method) and 10⁶ copies/mL (ClassicDNA method) were likely to have a corresponding low toxin concentration (< 0.3 µg/L) (Table 2). Conversely, samples with *mcyE* concentrations of around 10⁹ copies/mL (RapidDNA method) and 10⁷ copies/mL (ClassicDNA method) were likely to have a corresponding high toxin concentration (~8 µg/L). In a previous study of cyanoHABs in Cayuga Lake, NY, the *mcyA* qPCR assays showed good performance for screening high toxin blooms (~8 µg/L), with a quantifiable level of the *mcyA* gene (~10³–10⁴

copies/mL) (Wang et al. 2024). Additionally, Scherer et al. (2017) reported that the samples had toxin concentrations ranging from 4 to 8 µg/L, with *mcyB* gene concentrations ranging from 3×10^5 to 7×10^5 copies/mL in two South German lakes (Scherer et al. 2017). Both studies applied classic DNA extraction, filtration, and purification steps. Their reported *mcy* gene concentrations corresponding to MC concentrations are much lower than what the current study reported. The differences may be due to variation in locations and target genes.

A previous study has demonstrated that qPCR can be a useful numerical tool to assess phytoplankton biomass, as qPCR- and microscope-based phytoplankton biomass showed a significant positive linear correlation (Zhang et al. 2023). In our study, the linear regression analysis between the log of cyanoHAB cell density (cell/mL) and the log of copy number of *mcyE* gene (copies/mL) showed a good fit for both methods. Compared to other studies that show relatively low R² values when relating the copy number of *mcy* gene to the cell density of cyanobacteria (Arismendi-González et al. 2021), our results demonstrate significantly higher R² values for environmental samples.

Understanding the temporal dynamics of cyanobacterial gene concentrations is crucial for managing water quality and public health risks associated with cyanotoxins. The choice of method can influence the detection sensitivity and the interpretation of cyanobacterial bloom dynamics, guiding timely and effective intervention measures. Both RapidDNA and ClassicDNA methods offer valuable insights, with their respective strengths making them suitable for different monitoring contexts. RapidDNA method was ineffective at tracking HAB-related genes at low concentrations, such as during the pre-bloom period, potentially missing early signs of bloom development or toxin production. It may also yield false negatives or be affected by inhibitors present in environmental samples. According to data from the Utah Department of Environmental Quality's cyanoHABs monitoring program (<https://deq.utah.gov/water-quality/recreational-health-advisory-guidance-for-harmful-algal-blooms>), Utah Lake and Deer Creek Reservoir have a long history of cyanoHABs and frequently experience blooms dominated by *Microcystis*, which produces MC at high levels (Li 2021; Penrod and 2017). Similarly, in our study, we observed that *mcyE* and MC were frequently detected as the dominant toxin-related gene and cyanotoxins. When *Microcystis* is the dominant MC producer, it might suppress other cyanobacteria species that produce anatoxin-a, cylindrospermopsin, and saxitoxin (Chia et al. 2018; Duan et al. 2022). This may explain the low abundance or infrequent detection of other genes, as well as the low levels of other toxins, in the current study. Out of the nine reservoirs studied, only five experienced blooms (Fig. 5). During the pre-bloom period, only the ClassicDNA method was able to detect the *mcyE* gene when cyanoHAB cell concentrations were relatively low. However, both methods were effective at detecting blooms once cyanoHABs had occurred. Although the ClassicDNA method detected the target genes during the pre-bloom period of Echo and Scofield Reservoir at a concentration of 10^5 copies/mL, the bloom did not reach concerning levels of MC concentrations later, with MC consistently measured below 8 µg/L (Fig. S2). In Scofield and Echo Reservoirs, microscopy-based cell taxon analysis revealed that *Aphanizomenon* was the dominant species, which is known to produce toxins such as anatoxins, CYN, and saxitoxins (Dalu and Wasserman 2018). Given that other toxins were measured in only a few samples and showed low concentrations via ELISA, it is possible that different types of toxins were present, contributing to the low abundance of *mcyE*. This suggests that the dominant species significantly influence toxin profiles, affecting the detection and interpretation of target genes and cyanotoxins. While using the *mcyE* gene as a marker was effective for predicting MC levels at certain reservoirs (e.g., Utah Lake, Deer Creek), it did not adequately capture the dynamics at Scofield or Echo, where other species and toxins may have played a more significant role. In addition, the influence of various factors on the occurrence of surface water cyanoHABs, including nutrient levels (nitrogen and phosphorus), seasonal fluctuations (light, temperature, and wind), and hydrodynamic conditions (surface flushing, pulsed inflow, artificial mixing) should be

considered. (Schindler et al. 2016; Summers and Ryder 2023; Tanvir et al. 2021). In addition, South American strains of *Cylindrospermopsis raciborskii* have demonstrated an evolutionary shift in toxin production from saxitoxins to cylindrospermopsins (Hoff-Rissetti et al. 2013), illustrating the role of geographical and evolutionary factors in regulating cyanotoxin gene expression. In our study, we examined nine Utah waterbodies, leveraging historical HAB data to design assays targeting relevant toxin-related genes. Our results revealed a linear relationship between *mcyE* abundance and microcystin concentrations in these waterbodies, demonstrating the utility of this approach for this region. This method may require adjustment for other waterbodies, where historical HAB data and environmental factors may differ. Thus, while genetic methods like qPCR offer rapid screening for potential toxin production, complementary chemical analyses (ELISA and LC-MS/MS) are indispensable for confirming toxin presence.

These findings support the integration of genetic methods (*mcy* gene detection via RapidDNA and ClassicDNA qPCR methods) with traditional toxin measurements (LC-MS/MS, ELISA and microscopy-based cyanobacteria identification) for an economic monitoring strategy. Both methods can be used to indicate toxin production during blooming events. For proactive and timely actions against cyanobacterial HABs, the extended process of DNA extraction and purification has emerged as a significant bottleneck. Perhaps it is time to re-visit the appropriateness and necessity of DNA purification in qPCR assay for surface water microbiology. RapidDNA methods offer a promising alternative, eliminating the need for additional purification steps, as a potential rapid screening tool for high-toxin blooms, and is suitable for lakes experiencing MC-producing blooms and similar issues. It requires minimal specialized training and equipment, enabling straightforward implementation with relatively lower cost. It features a fast turnaround time (3–4 h) and can target multiple microcystin-producing cyanobacterial species or toxin genes simultaneously in a single reaction, thereby enhancing throughput and efficiency. This is important for effectively managing public health risks associated with cyanoHABs. Conversely, ClassicDNA methods with its sensitivity can be utilized during the pre-blooming period to prepare for potential blooms. However, the ClassicDNA qPCR instrumentation and reagents for DNA extraction and purification can be costly. Additionally, the reliability and sensitivity of the genes targeted (other cyanotoxin genes) but not observed to bloom in this study require further evaluation. Additionally, we propose future research directions to expand this approach to diverse environmental conditions. This integrative approach will enhance the reliability and applicability of HAB monitoring across varied ecosystems.

5. Conclusions

Among the nine reservoirs, cyanoHABs were observed in five: Utah Lake, Deer Creek, Scofield, Pineview, and Echo. In these reservoirs, *mcyE* genes were the most frequently detected and had higher abundance, with *Microcystis* being the predominant species in the cyanoHABs at Utah Lake and Deer Creek Reservoir. Both ClassicDNA and RapidDNA methods effectively detected blooms, with RapidDNA yielding higher gene copy values. However, only the ClassicDNA method was capable of detecting the gene at low cyanoHAB cell concentrations during the pre-bloom period, providing an early warning for potential blooms. Both methods demonstrated high reliability, as indicated by high percentages of true positive and true negative cases. Additionally, *mcyE* gene levels showed strong linear correlations with toxin levels and cyanoHAB cell density. Using ATS and Tobit regression models, it was predicted that samples with *mcyE* concentrations around 10^7 copies/mL (RapidDNA) and 10^6 copies/mL (ClassicDNA) were likely to have low toxin levels (< 0.3 µg/L), while concentrations near 10^9 copies/mL (RapidDNA) and 10^7 copies/mL (ClassicDNA) were associated with higher toxin levels (~8 µg/L). The speed and simplicity of the RapidDNA method make it ideal for monitoring cyanoHABs during active bloom events and in resource-limited settings, such as remote field sites. Meanwhile, the

sensitivity of the ClassicDNA method allows for its use in the pre-bloom period to anticipate potential blooms. Integrating both methods can strengthen cyanoHAB monitoring programs and enhance efforts to protect public health.

CRediT authorship contribution statement

Peerzada Madany: Writing – original draft, Visualization, Formal analysis. **Donald Olsen:** Investigation. **Sarah Osama Makled:** Investigation. **Erik Cram:** Methodology, Investigation, Conceptualization. **Sarah Page:** Supervision, Resources, Methodology. **Hannah Bonner:** Supervision, Resources, Methodology. **Joan E. McLean:** Supervision, Resources, Funding acquisition. **David Stevens:** Supervision, Resources, Funding acquisition. **Mingyue Li:** Writing – review & editing. **Liyuan Hou:** Writing – review & editing, Validation, Software, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Liyuan Hou reports was provided by Utah State University. Sarah Page reports a relationship with Utah Department of Environmental Quality that includes: funding grants. If there are other authors, they 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.watres.2025.123322](https://doi.org/10.1016/j.watres.2025.123322).

Data availability

Data will be made available on request.

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