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Effect of Nutrient and Temperature Conditions on the Production of Microcystins From Cyanobacteria in Pineview Reservoir

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EFFECT OF NUTRIENT AND TEMPERATURE CONDITIONS ON THE PRODUCTION OF MICROCYSTINS FROM CYANOBACTERIA IN PINEVIEW RESERVOIR

by

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ABSTRACT

Effect of Nutrient and Temperature Conditions on the Production of Microcystins from Cyanobacteria in Pineview Reservoir

by

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Utah State University, 2024

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The objective of this project was to determine the effects of three environmental factors: added phosphorus concentrations, added molar nitrogen to phosphorus ratios, and changing water temperature on the production of microcystins during harmful algal blooms using water and cyanobacteria from Pineview Reservoir, Utah.

Surface water was taken from Pineview Reservoir and used to grow toxin-producing cyanobacteria, along with other aquatic organisms, at 25ºC in a medium selected for cyanobacteria. DNA analysis of the cyanobacteria culture determined what cyanobacteria and cyanotoxin producing genes were present. Cultured organisms were centrifuged and inoculated into filtered Pineview Reservoir water in an experimental program to assess changing environmental growth conditions on the production of the cyanotoxin microcystin. The environmental variables were 1) phosphorus added (low levels at 0.015 mg/L and high levels at 0.085 mg/L) 2) nitrogen:phosphorus ratio (dissolved nitrogen was added to achieve ratios of 4:1 and

25:1), and 3) rapid temperature change (either leave temperature at 25°C or reduce the temperature from 25°C to 16°C). Different combinations of nutrients were replicated so that four cultures were grown at 25ºC and at 16ºC in triplicate. This experimental design was used in four different blocks and analyzed first by lumping all of the blocks together, assuming each block had the same environment, testing if there was significant increase in microcystin production. Blocks were then analyzed separately, under the assumption each block had a different environment, determining if the levels of environmental factors caused a significant increase in microcystin production.

Blocks were statistically different from one another varying in microcystin quota, microcystin production, total phosphorus, total nitrogen, and pH. Decreasing water temperature from 25ºC to 16ºC, low added dissolved phosphorus concentrations (0.015 mg/L), and a low dissolved N:P ratio (4:1) did not significantly increase or decrease concentration of toxins. Even though no change significant increase in toxin production was observed a positive correlation between total phosphorus concentrations and toxin concentration was seen. Also, as the total molar N:P ratio decreased an increase in toxin production was seen. Key words in this document include cyanobacteria, cyanotoxin, microcystin, phosphorus, nitrogen, temperature, and factorial experiment.

(177 pages)

PUBLIC ABSTRACT

Effect of Nutrient and Temperature Conditions on the Production of Microcystins from Cyanobacteria in Pineview Reservoir

Brent Jacobson

Cyanobacteria, sometimes known as harmful algae, are an aquatic bacteria capable of producing toxic compounds. Cyanobacteria are found worldwide in both saltwater and freshwater environments. Depending on the environment, toxic cyanobacteria species can outcompete other aquatic species, grow in large numbers, and produce these toxic compounds. Further understanding of what environmental conditions promote the production of these harmful bacteria and toxins is needed to protect and inform the public.

In order to understand why cyanobacteria produce toxins in certain environments, samples containing cyanobacteria were taken from a Pineview Reservoir, Utah, and cultured at the Utah State Water Research Laboratory. Cyanobacteria from these cultures were subjected to different nutrient concentrations (phosphorus and nitrogen) and water temperature (25ºC and 16ºC) conditions to test whether these conditions resulted in an increase in toxin production.

Results from the experiments show that lowering water temperature, a low dissolved phosphorus concentration, and the dissolved N:P ratio did not increase the production of toxins during the experiments. Even though no increase in toxin production was seen in the environmental factors tested, it was seen that as total

phosphorus concentrations increased so did microcystin concentration. It was also seen that as the total N:P ratio decreased an increase in toxin production was seen.

ACKNOWLEDGMENTS

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Introduction

Cyanobacteria, otherwise known as blue-green algae or harmful algal blooms (HABs), are abundant in water systems throughout Utah (Table 1). People use waterbodies across Utah for irrigation, recreation, and drinking water purposes causing possible exposure to toxins produced from toxigenic cyanobacteria. Several waterbodies were considered as a focus in this study but one (Pineview Reservoir) was chosen because it provides drinking water and is a popular recreation area close to major cities where potential for exposure to toxic cyanobacteria is high.

Differing exposure routes to different cyanotoxins have varying health effects. Swimming in a toxic cyanobacteria bloom can lead to dermal exposure (causing itching) as well as incidental ingestion of cyanobacteria toxins (Carmichael & Boyer, 2016). Exposure can also come from consuming toxin infected fish. The toxins can be taken up by the fish in muscle tissue then, if consumed, transferred to the consumer (Cazenave et al., 2006). Exposure to cyanobacteria and their toxins can even occur away from a cyanobacterial bloom by inhaling aerosolized cyanobacteria (Facciponte et al., 2018). Facciponte et al. (2018) study showed that there was no correlation between the number of aerosolized cyanobacteria found and the time of year or even the subject's proximity to a waterbody.

Table 1

Primary Cyanotoxins in Utah Waterbodies and Their Health Effects (UDWQ, 2022a)

Cyanotoxin	Acute Health Effects in Humans	Most Common Cyanobacteria Producing These Toxins in Utah
Microcystin-LR	Abdominal pain, headache, sore throat, vomiting and nausea, dry cough, diarrhea, blistering around the mouth, and pneumonia	*Microcystis, Anabaena, Nodularia, Planktothrix, Fischerella, Nostoc, Oscillatoria, Gloeotrichia, and Dolichospermum*
Cylindrospermopsin	Fever, headache, vomiting, bloody diarrhea, liver inflammation, and kidney damage	*Cylindrospermopsis raciborskii, Aphanizomenon flos-aquae, Aphanizomenon gracile, Aphanizomenon ovalisporum, Umezakia natans, Anabaena bergii, Anabaena lapponica, Anabaena planctonica, Lyngbya wollei, Rhaphidiopsis curvata, and Rhaphidiopsis mediterranea*
Anatoxin-a group	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death	*Chrysosporum (Aphanizomenon) ovalisporum, Cuspidothrix, Cylindrospermopsis, Cylindrospermum, Dolichospermum, Microcystis, Oscillatoria, Planktothrix, Phormidium, Anabaena flos-aquae, A. lemmermannii Raphidiopsis mediterranea (strain of Cylindrospermospsis raciborskii), Tychonema and Woronichinia*

Exposure to cyanotoxins over time have been shown to cause acute health effects (Table 1) along with chronic effects such as gastroenteritis (Drobac et al., 2013). Health advisories, provided by the Environmental Protection Agency (EPA), are provided for two toxins produced by cyanobacteria over a 10-day exposure period (Table 2) (Environmental Protection Agency [EPA], 2021a). Incidentally ingesting

water above the health advisory over the designated exposure period can have negative consequences on humans as well as wildlife.

Table 2

Cyanotoxin Drinking Water Advisories for Cyanobacteria Species (EPA, 2021a)

Cyanotoxin	Drinking Water Health Advisory (10-day)	
	Bottle-fed Infants and pre-school children	School-age Children and Adults
Cylindrospermopsin	0.7 µg/L	3 µg/L
Microcystin	0.3 µg/L	1.6 µg/L

In July of 2020; a pet dog died from drinking water from the North Fork of the Virgin River in Zion National Park, Utah (Wink, 2020). The dog reportedly started having convulsions an hour after ingestion consistent with cyanotoxin (anatoxin-a) exposure (Mean & Maffly, 2020). To reduce the risks of cyanotoxin exposure to humans and animals alike, predicting when cyanobacteria blooms produce toxins is important to protecting the public from exposure.

Being able to anticipate cyanobacteria toxin production is important for water resource managers to protect the public and animals from the toxins. Utah has focused on the reduction of nutrients by implementing site specific measures, discussed in the Pineview Reservoir section of the literature review. Large growths of algae and cyanobacteria occur when a waterbody is overloaded with nutrients causing the depletion of dissolved oxygen and fish kills (UDWQ, 2021). The experiments conducted in this work aim to measure the impacts of different phosphorus and nitrogen concentrations along with decreasing water temperatures on the production of cyanotoxins.

Problem Statement

Toxin-producing cyanobacteria are problematic in waterbodies across Utah. Predicting when cyanobacteria produce toxins will aid in preventing cyanobacteria and cyanotoxins from entering water treatment facilities and preventing public exposure in waterbodies. Cyanobacteria entering a water treatment facility have the potential to infect drinking water with cyanotoxins in addition to creating clean-up issues in the plants themselves (EPA, 2022a).

Literature Review

Cyanobacteria differ depending on the environmental conditions in the water body and the contributing watershed. According to the Utah Division of Water Quality (UDWQ), the most common cyanobacteria genera in Utah are *Aphanizomenon, Dolichospermum, Anabaena, Cylindrospermopsis,* and *Microcystis* (UDWQ, 2019).

This section provides background information on cyanobacteria blooms involving these genera of cyanobacteria.

Cyanobacteria

Aphanizomenon.

Aphanizomenon is a freshwater and saltwater genus of cyanobacteria commonly found in Utah waterbodies. *Aphanizomenon* is a filamentous shaped cyanobacteria (Figure 1) with varying individual cell lengths depending on the species. Single *Aphanizomenon flos–aqua* cells can range anywhere from 4 to 12.1 µm in length while colonies can reach up to 2 cm long. Individual *Aphanizomenon flos–aqua* are 3.6 to 5.6 µm wide making them visible without a microscope (Ryu et al., 2016). Many colonized *Aphanizomenon* species appear like grass clippings floating in the water column. The formation of colonies is not *Aphanizomenon* specific, but the grass-like appearance is (Figure 2).

Figure 1

Aphanizomenon sp. Under a Microscope (magnification unknown) (Baker, 2012)

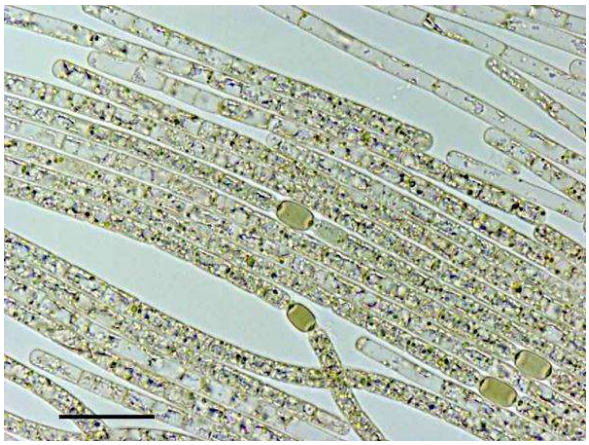


Figure 2

Utah Aphanizomenon Bloom (UDWQ, 2022b)

Aphanizomenon has the ability to fix nitrogen from the environment using heterocysts (Garcia-Pichel, 2009), which appear as an oblong shape in the middle of individual cells (Figure 1). Heterocysts vary in length and width according to the species of *Aphanizomenon*. *Aphanizomenon flos-aquae* heterocysts range from 6.6 to 8.5 μm long and 3.3 to 3.9 μm wide (Ryu et al., 2016). The narrow nature of *Aphanizomenon* makes it more susceptible to shear than algae under turbulent conditions (Wang & Lan, 2018).

Aphanizomenon flos-aquae is the most common *Aphanizomenon* species associated with HABs (Matthews, 2014). Environmental conditions that suppress the growth of *Aphanizomenon flos-aquae* are pH values less than 7.1, water temperature under 11 ºC, and a 10-to-14-hour light to dark period (Yamamoto & Nakahara, 2005). The optimal temperature for *Aphanizomenon flos-aqua* growth is between 23 and 29°C, however growth can occur at temperatures as low as 8ºC (Tsujimura et al., 2001).

Cyanotoxin release from *Aphanizomenon flos-aqua* has been linked to water temperature and light intensity. Preußel et al. (2009) subjected two different sub-species of *Aphanizomenon flos-aqua* to different combinations of light intensities (10 to 60 $\mu E/m^2/sec$) and temperatures (16, 20, 25°C). Results showed extracellular cylindrospermopsin concentrations highest at 16ºC along with the lowest growth rates, but total toxin production (cylindrospermopsin) was highest at 20°C.

Nitrogen is an important nutrient in the synthesis of cyanotoxins which many contain amino acids in their chemical makeup. Environments where nitrogen is

abundant showed similar toxin concentration patterns as a phosphorus deficient environment (Preußel et al., 2014). Nitrogen-deficient cultures tended to produce intracellular toxins, while phosphorus concentrations have been found to be an important factor in the production of total cyanotoxins. When *Aphanizomenon ovalisporum* were subjected to deprived phosphorus concentrations in the growth medium used, cyanotoxin production increased (Bar-Yosef et al., 2010).

Dolichospermum* and *Anabaena.

Dolichospermum and *Anabaena* are two similar cyanobacteria genera capable of producing cyanotoxins depending on environmental conditions (Table 1). *Anabaena* and *Dolichospermum* look similar under a microscope (Figure 3) and are difficult to distinguish. Figure 3 compares a straight specie of *Anabaena* to a straight specie of *Dolichospermum*. Both genera have large akinetes which are resistant to the cold and used as a growth base for when optimal conditions are once again present and both have heterocysts capable of nitrogen fixation.

Figure 3

Dolichospermum and Anabaena Under a Microscope (Magnification unknown) (Matthews, 2022a, 2022b)

Early studies refer to *Anabaena* and *Dolichospermum* as the same cyanobacteria genus until later distinguishing them as two separate cyanobacteria genera (Wacklin et al., 2009). The main difference between *Anabaena* and *Dolichospermum* is that *Anabaena* do not have gas vesicles used to regulate buoyancy while *Dolichospermum* do. Because of the lack of gas vesicles, *Anabaena* prefer a benthic environment where light is limited. With the inability to move in the water column, *Anabaena* prefer a shallow eutrophic lake in order to take in light.

Both of these cyanobacteria can form dense colonies. Individual cyanobacteria lengths vary from species to species. The width of cyanobacteria cells in *Anabaena flos-aqua* range from 4 to 7 µm (Komárek & Zapomělová, 2007). *Dolichospermum* and

Anabaena have similar widths to that of *Aphanizomenon* but do not have an enveloping sheath to make them filamentous (Wu, 2023).

Total phosphorus levels and water temperatures play a factor whether *Dolichospermum* dominates blooms or other cyanobacteria such as *Microcystis*. One study observed under conditions of low phosphorus and temperatures below 17ºC, *Dolichospermum* outcompeted *Microcystis* (Zhang et al., 2020). Zhang's et al. (2020) study also found when temperatures exceeded 17 ºC, *Microcystis* dominated. This study did not distinguish *Dolichospermum* from *Anabaena* instead referred to *Dolichospermum* at the start of the study as *Dolichospermum* (*Anabaena*).

Optimal temperature conditions for *Anabaena sp.* growth are between 28 and 32ºC with a sharp decrease in growth rates at 35ºC (Nalewajko & Murphy, 2001), although a different species of *Anabaena* showed optimal growth rates around 20ºC (Rapala & Sivonen, 1998). Growth rates in Rapala and Sivonen's 1998 study showed a general trend of increasing growth rates with an increase in light intensity from 7 to 42 µmol m^{-2} sec^{-1}. pH values between 5 and 8 did not affect the growth rate of *Anabaena*, but growth rates decreased when the pH increased above 9 (Peters et al., 1980).

Field data show the concentrations of dissolved PO_4^{-} and NO_3^{-} are the main difference between differing cyanobacteria in HABs (Rapala & Sivonen 1998). Hepatotoxic *Anabaena* blooms were associated with low dissolved concentrations of phosphorus (1-12 µg/L), while the non-toxic species favored higher dissolved phosphorus concentrations (2-40 µg/L). Along with dissolved phosphorus and nitrogen

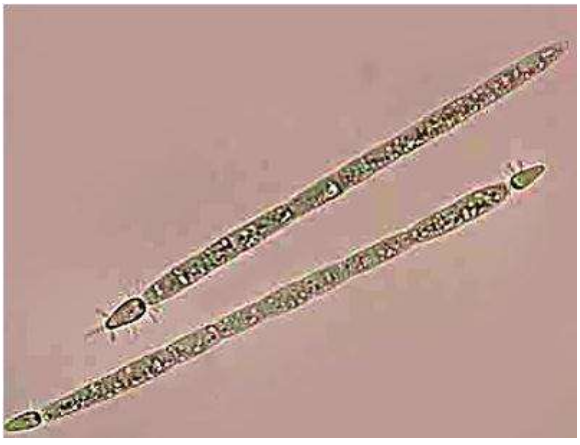
concentrations, the production of anatoxin-a (cyanotoxin) from neurotoxic species of *Microcystis* and *Anabaena* was linked with suboptimal temperature conditions (13-22ºC).

Cylindrospermopsis.

Cylindrospermopsis is a freshwater toxin-producing cyanobacteria known for producing cyanotoxins (anatoxin-a and cylindrospermopsin) in Utah waterbodies (Table 1). The genus, *Cylindrospermopsis*, shares physical similarities to *Cylindrospermum* and *Aphanizomenon*. Figure 4 shows two *Cylindrospermopsis* side by side. *Aphanizomenon* differs physically from *Cylindrospermopsis* because *Cylindrospermopsis* has the heterocysts (Figure 4) , capable of nitrogen fixation, at the end of the vegetative cell (Garcia-Pichel, 2009).

Figure 4

Two Cylindrospermopsis Cells Side by Side (Baker, 2012)



Both *Cylindrospermopsis* and *Cylindrospermum* have terminal heterocysts but *Cylindrospermopsis* has heterocysts teardrop shaped and are not near the akinetes. Individual *Cylindrospermopsis* cells are cylindrical with a diameter less than 4 μm (Raju, 2018) and can be curved or straight depending on the specie. Under a microscope individual cells may appear yellowish, brown, or pale blue green (Matthews, 2022c).

Cylindrospermopsis raciborskii has an optimal temperature for growth higher than other cyanobacteria discussed in the literature review ranging from 25.5 to 32.7ºC in monomictic and mesotrophic lakes (Recknagel, Orr, and Cao 2014). *Cylindrospermopsis raciborskii* can also uptake and convert phosphorus more quickly than other common toxic cyanobacteria such as *Aphanizomenon flos-aqua* and *Microcystis aeruginosa* (Wu et al., 2009). *Cylindrospermopsis raciborskii* can also use differing forms of dissolved organic phosphorus (DOP) to facilitate growth other cyanobacteria cannot use (Bai et al., 2014) giving it a competitive advantage when phosphorus resources are low.

The ratio of nitrogen to phosphorus (N:P) is not an important factor when considering the growth of *Cylindrospermopsis*. *Cylindrospermopsis* dominates under both low and high N:P ratios even though there was no effect on growth. Even though the N:P ratio does not play a significant factor in the growth of *Cylindrospermopsis*, toxin (saxitoxin) concentrations were higher under higher N:P ratios for this genus (Chislock et al., 2014)

Microcystis.

The genus of *Microcystis* has both toxin and non-toxin producing species all of which are spherical in appearance (Figure 5) and range in sizes from 2.5 to 5 µm in diameter (Matthews, 2022d). *Microcystis* has a higher tolerance to shear than other cyanobacteria focused on in the thesis. *Microcystis aeruginosa* has the greatest growth rate at a flow velocity of 0.5 m/s with a static-equivalent flow velocity of 0.47 m/s (Song et al., 2018). *Microcystis* colonies clump together to form larger clumps which can be seen in Figures 5 and 6 under low shear environments.

Figure 5

Microcystis Bloom from Matt Warner Reservoir at 100x Magnification

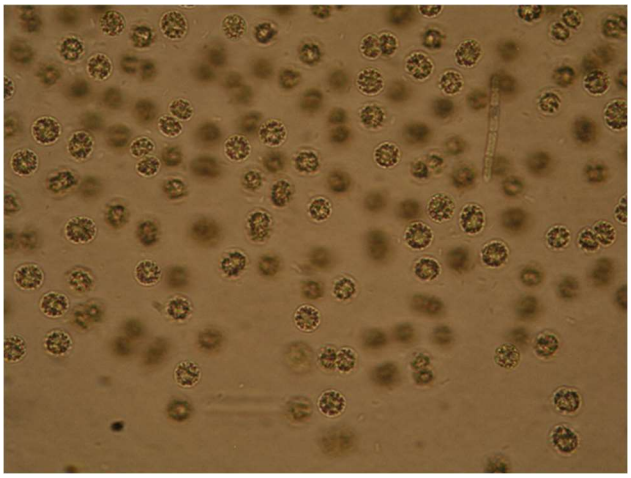


Figure 6

Microcystis Bloom from Matt Warner Reservoir

Environmental data found a correlation between the number of toxic *Microcystis* cells and the amount of microcystins (Davis et al., 2009). Davis et al. (2009) study suggest that as temperature and phosphorus concentrations increase, the number of toxic *Microcystis* cells also increase to produce more toxins. Even with an increase in *Microcystis* concentration, this does not always equate to larger amounts of toxin being produced. Another study found that the production of microcystins was not related to the growth rate (Wilson et al., 2006).

Growth for *Microcystis aeruginosa* at 25 ºC showed after a lag phase of roughly 4 days rapid growth occurred from days 4 to 10. After this rapid growth little growth was seen from day 10 to day 20 (Giannuzzi, 2019). Measuring the growth of toxigenic

Microcystis can be done by measuring the number of toxigenic genes. *Microcystis* cells, depending on the specie, on average range from 0.858 to 1.338 microcystin producing gene per cell of toxigenic *Microcystis* (K. H. Oh et al., 2013).

Light intensity and temperature have been shown to impact the production of microcystins from *Microcystis* species. Song et al. (1998) found *Microcystis viridis* produced the most microcystins at 25ºC and lower light intensities (40 – 50 µmol m^{-2} sec^{-1}). At even lower temperatures (15ºC), *Microcystis aeruginosa* growth rate and cell viability decreases but growth is still viable (Yi et al., 2017). As water temperatures decreased, the growth rate of *Microcystis* species decreases but the production of microcystins was found to increase (Martin et al., 2020). Martin et al. (2020) found that by decreasing temperature from 26ºC to 19ºC, in a pure culture of *Microcystis aeruginosa*, intracellular production of microcystins nearly doubled.

The Redfield ratio gives the ratio of carbon to nitrogen to phosphorus (C:N:P) in order for typical biomass accumulation in phytoplankton. The Redfield ratio is 106 moles of carbon to 16 moles of nitrogen to 1 mole of phosphorus (Tyrrell, 2001). Carbon is assumed to be in sufficient supply because cyanobacteria are photoautotrophs capable of gaining carbon through photosynthesis. Since carbon is assumed to be in sufficient supply, the ratio of nitrogen to phosphorus is only considered in the experiments. The Redfield ratio is often used to show which nutrient is limiting. In environments where the N:P ratio is less than 15:1, *Microcystis* blooms are likely to occur along with the production of toxins (Paerl & Fulton, 2006).

Unlike the other cyanobacteria genre discussed, *Microcystis* does not have heterocysts capable of fixing nitrogen from the atmosphere (Figure 5). Even so, *Microcystis* does have the ability to uptake nitrogen more efficiently from urea and ammonium than other species of algae (Paerl & Fulton, 2006). *Microcystis* also shows the ability to gain nitrogen from other cyanobacteria that have the ability to fix nitrogen. Often, *Aphanizomenon* and *Microcystis* are codominant in cyanobacteria blooms. *Microcystis* blooms often follow *Aphanizomenon* blooms using nitrogen *Aphanizomenon* have fixed from the atmosphere (Bartram et al., 1999).

The growth of cyanobacteria and other organisms is often limited by the amount of bioavailable phosphorus of which the most common form is phosphate (PO_4^{3-}) (EPA, 2021b). Reducing bioavailable phosphorus sources could drive the dominance of toxigenic *Microcystis* strains over the non-toxic strains (Hellweger et al., 2022). Models used in Hellweger et al. (2022) study predicted a lower biomass with a reduction of phosphorus, but also make nitrogen and light more available for uptake by nitrogen fixing toxigenic cyanobacteria.

Comparison of Cyanobacteria.

The most common toxigenic cyanobacteria genera in Utah are compared in Table 3 according to their capabilities to produce specific toxins, fix atmospheric nitrogen, and control buoyancy (Echard, 2021). Each of these capabilities give a specific advantage over other competing aquatic species depending on the environment. Further discussion on the toxins found in this table are found in the cyanotoxin section of the thesis.

Table 3

Cyanobacterial Qualities and Toxin Production (Carmichael, 2001; Echard, 2021; EPA, 2020a; Paerl et al., 2001; UDWQ, 2020)

Cyanobacteria	N_2 Fixation?	Buoyancy Control?	Neurotoxins		Hepatotoxins	
			Ana.	Sax.	Cyl.	Mc
Aphanizomenon	Yes	Yes	✓	✓	✓	
Cylindrospermopsis	Yes	No	✓	✓	✓	
Dolichospermum	Yes	Yes	✓	✓	✓	✓
Anabaena	Yes	No	✓	✓	✓	✓
Microcystis	No	Yes	✓			✓

Note. Anatoxin-a is represented as Ana., saxitoxin is represented as Sax., cylindrospermopsin is represented as Cyl., microcystins is represented as Mc, and blank spaces mean the cyanobacteria does not produce the specific toxin.

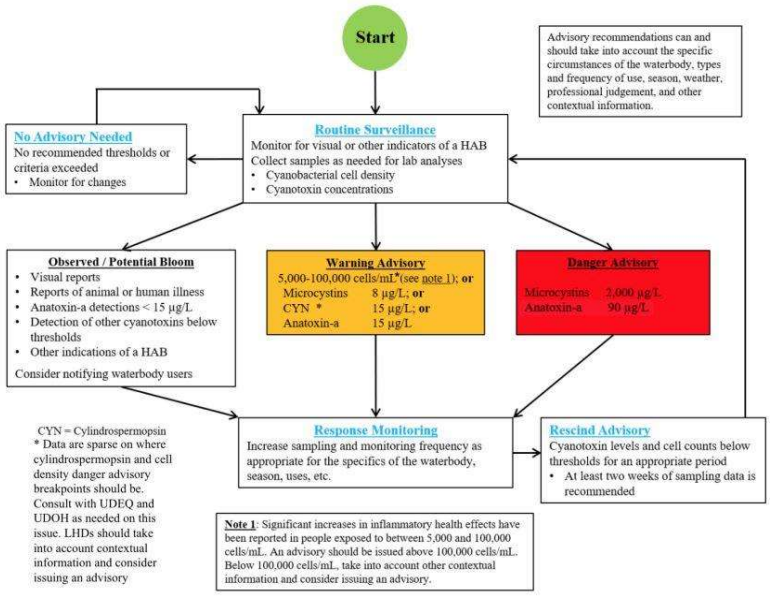
Cyanotoxins

The cyanotoxins found in Utah, and most commonly in the United States, are microcystins, anatoxins, cylindrospermopsin, and saxitoxin (EPA, 2021c). The toxicity, health advisory limits, and the occurrence of cyanotoxins across Utah waterbodies according to monitoring data provided by UDWQ are discussed in this

section. The UDWQ monitors 62 lakes and reservoirs across Utah for cyanotoxins with a system to inform the public when there is significant danger of cyanotoxins in a lake or reservoir (Figure 7).

Figure 7

Warning Advisory System from UDWQ (UDWQ, 2022c)



Warning and danger advisories include cell counts along with the concentrations of microcystins, anatoxin-a, and total cyanotoxin concentrations.

There is no way of telling if a cyanobacteria bloom is toxic by visual cues, for this reason UDWQ conducts cyanotoxin measurements across the state to warn the public when toxins are found.

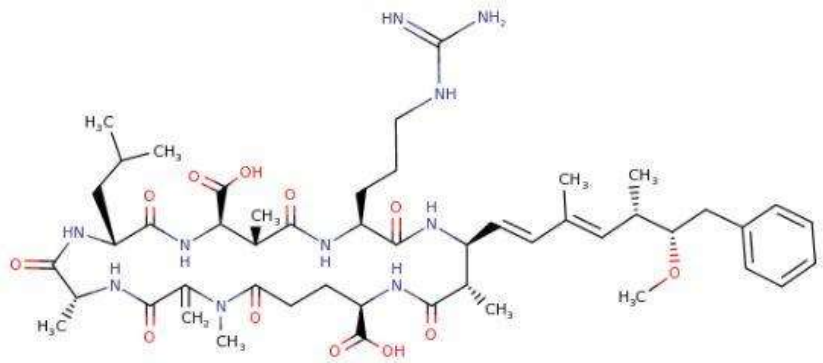
Microcystins.

Microcystins represent a class of more than 75 hepatotoxic compounds (Svrcek & Smith, 2004). Hepatotoxins, in high enough concentrations, cause harm to the liver. When referring to the toxicity, the mean lethal dose (LD_{50}) is used for reference. The LD_{50} value refers to the mean amount of the toxin required to kill 50% of the test subjects (generally mice). The reported LD_{50} for *microcystis* species range from 0.05 mg/kg (mg toxin/kg subject body weight) to 1.2 mg/kg depending on the microcystin produced (Bartram et al., 1999).

The most common seven main congeners of microcystins: microcystin LR, LA, YR, LW, LY, LF, and RR. The letters represent the type of amino acid side chain in the compound. The most common and most studied microcystin across Utah is microcystin LR. The chemical formula for microcystin LR is $C_{49}H_{74}N_{10}O_{12}$ (Figure 8) (EPA, 2017). Measurements for microcystins in this study measured all of the possible microcystin congeners.

Figure 8

Chemical Structure of Microcystin LR (EPA, 2017)



Note. Blue shows the nitrogen and the red shows the oxygen.

Drinking water health advisories for total microcystins are 0.3 µg/L for bottle-fed infants and preschool children and 1.6 µg/L for school-age children and adults (Table 2). The warning advisory concentration is 8 µg/L and the danger advisory is 2,000 µg/L (Figure 7). Incidental ingestion of microcystin concentration at the warning and danger advisory level could lead to serious health effects (Table 1). Microcystins are the most common cyanotoxin associated with HABs found across Utah waterbodies according to monitoring data provided by UDWQ.

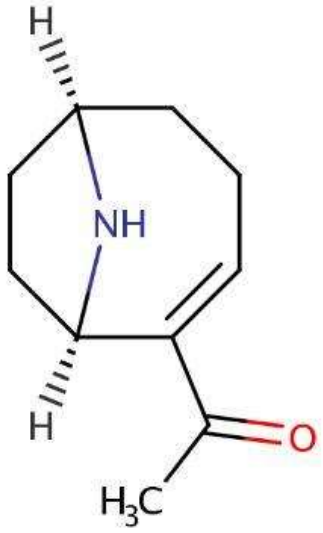
Anatoxin-a.

Anatoxin-a is a neurotoxin produced by several different species of cyanobacteria (Table 1). Anatoxin-a has three different homologs: Homoanatoxin-a, Dihydroanatoxin-a, and Dihydrohomoanatoxin-a. Anatoxin-a and its homologs have

differing structures making each unique. The molecular formula for anatoxin-a is $C_{10}H_{15}NO$ (Figure 9) (EPA, 2020b). Nitrogen is a necessary nutrient in the formation of anatoxin-a and its homologs.

Figure 9

Chemical Structure of Anatoxin-a (EPA, 2020b)



Note. Blue shows the nitrogen and the red shows the oxygen.

Both anatoxin-a and its homologs have a LD_{50} ranging from 200 to 250 µg/kg (Farrer et al., 2015). Anatoxin-a has the ability to be agonists to muscular neuronal

nicotinic acetylcholine receptors (Aráoz et al., 2010). This causes muscle spasms and death to the victim quickly. Mice used to conduct toxicity studies died within 2 to 5 minutes after being injected with anatoxin-a resulting in anatoxin-a being known as having a very fast death (Pike, 1977).

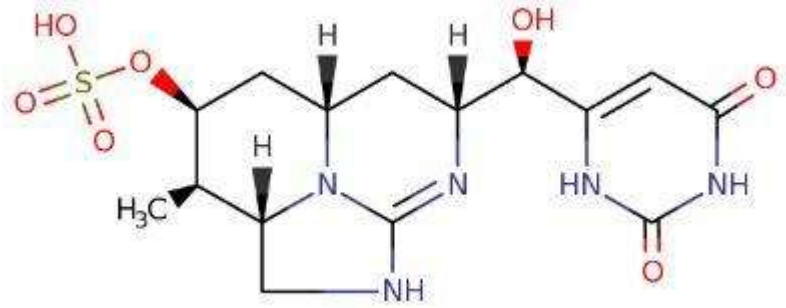
Despite possible health risks, the EPA does not have drinking water advisories for anatoxin-a (EPA, 2021a). Anatoxin-a degrades rapidly in sunlight and at pH values above 7. The half-life of anatoxin-a in water is 1 to 2 hours when pH values are between 8 and 9, but slows in dark conditions (EPA, 2015). UDWQ monitors anatoxin-a with warning and danger advisories given to the public when concentrations are above 15 µg/L and 90 µg/L (Figure 7). Even with Utah waterbodies having higher pH values (~8), anatoxin-a is commonly found in high concentrations.

Cylindrospermopsin.

Cylindrospermopsin is a hepatotoxin produced by cyanobacteria in the genera of *Anabaena*, *Cylindrospermopsis,* and *Aphanizomenon* (Table 1). The mean lethal dose of cylindrospermopsin is 2.1 mg/kg which is higher than the other cyanotoxins discussed in this report making it the least toxic. The molecular formula for cylindrospermopsin is $C_{15}H_{21}N_5O_7S$ (Figure 10).

Figure 10

Chemical Structure of Cylindrospermopsin (EPA, 2022b)



Note. Blue shows the nitrogen and the red shows the oxygen

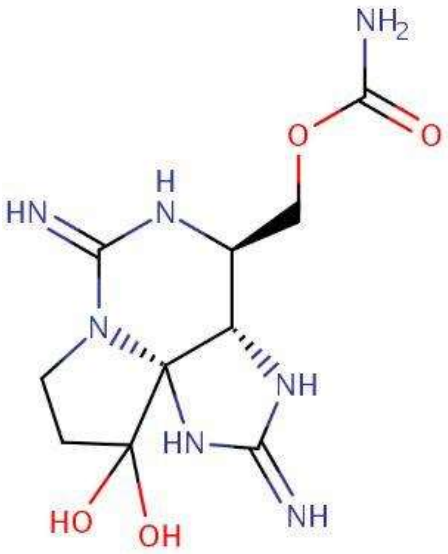
Along with microcystins, there are drinking water health advisories for cylindrospermopsin. The health advisory for bottle-fed infants and pre-school children is 0.7 µg/L and for school-age children and adults is 3 µg/L (Table 2). Utah issues a warning if cylindrospermopsin is found in concentrations greater than 15 µg/L. Cylindrospermopsin is monitored by the state but was rarely recorded in the dataset provided by UDWQ. There is no concentration of cylindrospermopsin to warrant a danger advisory in Utah (Figure 7).

Saxitoxin.

Saxitoxin consists of over 25 naturally occurring homologs (Robillot & Llewellyn, 2005). The molecular formula for saxitoxin is $C_{10}H_{17}N_7O_4$ (Figure 11).

Figure 11

Chemical Structure of Saxitoxin (EPA, 2022c)



Note. Blue shows the nitrogen and the red shows the oxygen.

The toxicity of saxitoxin and its homologs vary depending on the variant. The LD_{50} for saxitoxin and it's homologs range from 0.005 mg/kg to 0.01 mg/kg (Ostlund & Ballenger, 1974). Saxitoxin has the lowest LD_{50} of all the toxins discussed in this thesis but is also the rarest. Saxitoxins block sodium channels leading to neuromuscular paralysis and respiratory failure. The EPA does not have a health advisory or a warning advisory if saxitoxin is detected.

Saxitoxin and its homologs are known for being primarily in marine environments, although some freshwater cyanobacteria such as *Aphanizomenon*,

Anabaena, and *Cylindrospermospis* have been known to produce this toxin (Aráoz et al., 2010; Farrer et al., 2015). Cyanobacteria blooms with saxitoxins were reported in the North Fork of the Virgin River in Zion National Park, which is the only location in Utah where saxitoxin has been detected.

Comparison of Toxins.

Toxins discussed in this thesis each cause health problems to humans and animals alike. The LD_{50} compares how much of the toxin, if injected, causes death to the subject. Saxitoxins have the lowest LD_{50} (Table 4), but also are the rarest occurring cyanotoxin in Utah. Among microcystins, anatoxin-a, and cylindrospermopsin; microcystins have potentially the lowest LD_{50} among the three and, according to data provided by UDWQ, are the most abundant and found in highest concentration in Utah waterbodies. For this reason, microcystins are the measured toxins in the experiments discussed in this thesis. Table 4 compares symptoms and the LD_{50} for microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin.

Table 4

Comparison of Toxins Produced from Cyanobacteria (Center for Disease Control [CDC], 2018; EPA, 2021d)

Toxin	LD_{50} (mg/kg)	Type	Symptoms
Microcystin	0.05 – 1.2	Hepatotoxin	Abdominal pain, headache, sore throat, vomiting and nausea, dry cough, diarrhea, blistering around the mouth, and pneumonia
Anatoxin-a	0.25	Neurotoxin	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death
Cylindrospermopsin	2.1	Hepatotoxin	Fever, headache, vomiting, bloody diarrhea
Saxitoxin	0.005 – 0.01	Neurotoxin	Nausea, vomiting, cranial nerve dysfunction, floating sensation, headache, muscle weakness, paresthesia and vertigo

Pineview Reservoir

Pineview Reservoir is located near Huntsville, Utah in Weber County, east of the City of Ogden. Pineview Reservoir is officially designated as a cold-water fishery but is managed as a warm water fishery with recreation opportunities, and is classified as a lower elevation reservoir at 4,900 ft above sea level (Table 5) (Whitehead & Judd, 2002). The maximum lake physical characteristics are recorded in Table 5 but do not represent the current conditions at Pineview Reservoir due to recent drought. As of May 4, 2022 the storage in the lake according to the Bureau of Reclamation (2015) was 59,791 acre-ft, 54% of full capacity.

Table 5

Pineview Reservoir Lake Characteristics (Whitehead & Judd, 2002; EPA, 1977)

Characteristics	Value
Elevation	1,493 m (4,900 ft)
Dam Height	41.76 m (137 ft)
Maximum Surface Area	1,163 ha (2,874 ac)
Maximum Volume	135,868,000 m^3 (110,150 ac-ft)
Maximum Depth	24.7 m (81.04 ft)
Mean Annual Drawdown	32,330,085 m^3 (26,210 ac-ft)
Average Retention Time	248 days

Reservoir levels are dependent on the inflows (precipitation, runoff, groundwater, etc.) and the outflows (dam, evaporation, etc.). During irrigation months (April 15 to October 15), Pineview water is used for drinking water and irrigation. During this time, toxic cyanobacteria often occur with the potential for toxins and biomass to enter the drinking water treatment facility.

Environmental conditions impairing Pineview Reservoir include water temperature, phosphorus and nitrogen concentrations, and dissolved oxygen (DO) levels. The target phosphorus concentration in Utah lakes is under 0.025 mg/L (UDWQ, 2014). Target phosphorus concentrations exceeded 0.025 mg/L in Pineview Reservoir close to the dam in 1996, 1998, and 2000. The molar N:P ratio in Pineview Reservoir is approximately 20:1 mole. Phosphorus and nitrogen loading is consistent throughout the year at this ratio (Whitehead & Judd, 2002).

The impact of nutrient loading into waterbodies is an issue focused on by UDWQ with an emphasis on phosphorus reductions. The UDWQ has introduced and implemented plans to reduce the phosphorus levels for waterbodies across Utah. Phosphorus concentrations across Utah vary, with 43% of the lakes in Utah being nutrient impaired (UDWQ, 2014). High phosphorus concentrations are defined, for management purposes, as those greater than 0.075 mg/L, medium phosphorus concentrations are between 0.025 mg/L and 0.075 mg/L, and low concentrations are 0.025 mg/L or less.

Pineview Reservoir stratifies in the summer months resulting in warmer surface water and a colder lower level (Whitehead & Judd, 2002) especially near the dam where the water is deeper. Water temperature in Pineview Reservoir varies with depth and time of year. The temperature in the euphotic zone can be as high as 26.7°C in summer months (Pineview Reservoir, 2022). Water temperatures start to decline in August and September when, coincidentally, cyanotoxin levels increase. During a bloom in 2019, once temperature dipped beneath 15°C, cyanotoxin production decreased as cyanobacteria concentrations continued to increase (Table 6). Even though cyanobacteria concentrations were increasing, the total toxin quota (femtogram of cyanotoxin per cyanobacteria cell) decreased along with temperature under 15ºC. This could be an indication that temperature has an effect on the production of cyanotoxins (Pineview Reservoir, 2022) (Table 6).

Table 6

Pineview Reservoir Cyanobacteria Bloom in 2019 Located at 41.2687 and -111.8186 (Pineview Reservoir, 2022)

Sample Date	Genera	(Cells/mL)	Microcystins	Anatoxin	Temp. (ºC)	Toxin Quota (fg/cell)
10/4/19	*Aphanizomenon*	8,602				
10/4/19	*Dolichospermum*	13,956				
10/4/19	Sum (cells/mL)	22,558	49	0.16	15	2,179.4
10/11/19	*Aphanizomenon*	77,476				
10/11/19	*Dolichospermum*	2,082,257				
10/11/19	*Microcystis*	222,959				
10/11/19	Sum (cells/mL)	2,382,692	19	0.12	14	8.0
10/25/19	*Aphanizomenon*	1,931,598				
10/25/19	*Dolichospermum*	921,896				
10/25/19	*Microcystis*	47,142				
10/25/19	Sum (cells/mL)	2,900,636	4	0	8	1.3

Past toxic cyanobacteria blooms in Pineview Reservoir from 2018 and 2019 show presence of three of the five genera of cyanobacteria focused on in this thesis. Cyanobacteria found in Pineview Reservoir include several species of *Aphanizomenon*, *Microcystis*, and *Dolichospermum* (Tables 6 and 7). The UDWQ sent the collected samples to the Utah Public Health Laboratories (UPHL) for toxin analysis. UPHL used the ELISA procedure (EPA Method 546) to estimate total cyanotoxin production. The cyanobacteria blooms during August and October produced both microcystins and anatoxins but never cylindrospermopsin (Table 7). The highest level of microcystins in Pineview Reservoir in 2018 and 2019 was 49 µg/L causing a warning advisory by UDWQ. The drinking water health advisory for

microcystins (Table 2) was exceeded eight times during 2019 and two times during 2018 in September and October. Because of the high concentrations of microcystin found in Pineview Reservoir and across the state according to data provided by UDWQ, microcystin is the measured cyanotoxin for the experiments described in this thesis.

Cyanobacteria blooms in Pineview Reservoir occur at different times of the year and under differing environmental conditions. Several cyanobacteria species occur in blooms in differing populations. The bloom in 2018 (Table 7) occurred one month earlier than the bloom in 2019 (Table 6) and in a different part of the lake. Unlike the bloom in 2019, cyanotoxin production decreased with decreasing cyanobacteria cell concentration, but showed an increase in the total toxin quota as the water temperature cooled to 18ºC.

Table 7

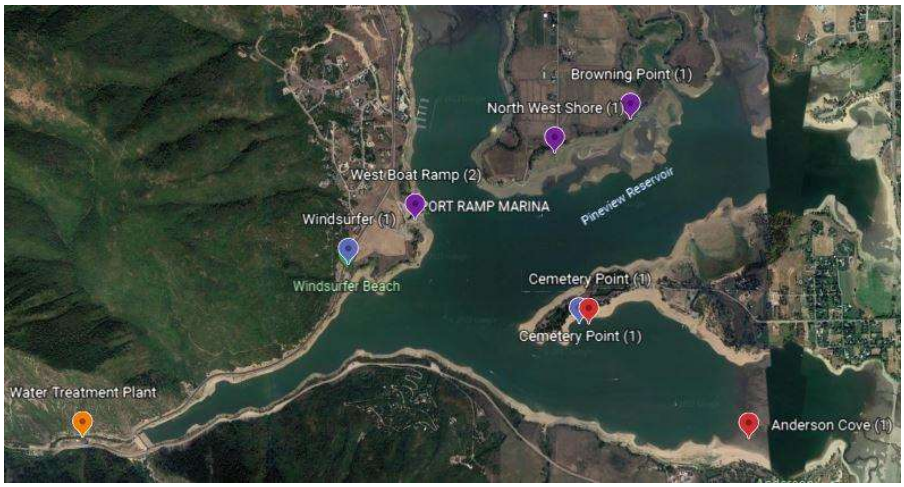
Pineview Reservoir Cyanobacteria Bloom in 2018 Located at 41.27226 and -111.80637 (Pineview Reservoir UT, 2022)

Sample Date	Genera	(Cells/mL)	Microcystins	Anatoxin	Temp. (ºC)	Toxin Quota (fg/cell)
9/4/18	Aphanizomenon	5,699,146				
9/4/18	Dolichospermum	819,310				
9/4/18	Microcystis	18,572				
9/4/18	Sum (cells/mL)	6,537,027	11	0.12	21	1.7
9/10/18	Aphanizomenon	4,905				
9/10/18	Dolichospermum	1,283				
9/10/18	Microcystis	794				
9/10/18	Sum (cells/mL)	6,982	0.20	0	20	28.6
9/17/18	Aphanizomenon	9				
9/17/18	Dolichospermum	373				
9/17/18	Microcystis	32				
9/17/18	Sum (cells/mL)	414	0.17	0	18	410.5

Anatoxin-a concentrations found in Pineview Reservoir from 2018 and 2019 never exceeded 0.25 µg/L. Anatoxin-a concentrations were found in conjunction with microcystins on four different occasions. Three of the four occasions were in 2019 at the end of August and the start of October (data not included). Figure 12 shows the locations of where cyanotoxins occurred in Pineview Reservoir. The purple marker represents locations where both anatoxin-a and microcystins exceeded 1.6 ug/L. Blue markers and red markers represent locations where only microcystins and anatoxin-a occurred above 1.6 µg/L according to data provided by UDWQ. It is notable that the locations where toxin levels were higher coincided with heavily used marinas, beaches, and other human access points.

Figure 12

Cyanotoxin Locations in Pineview Reservoir (2018-2019)



Note. Purple markers represent bloom of both microcystins (>1.6 µg/L) and anatoxin-a bloom. Red markers represent only anatoxin-a bloom and blue represent only microcystin blooms (>1.6 µg/L).

Objectives

Environmental conditions influencing the production and release of toxins include water temperature, differing nutrient levels, pH, light intensity, predation, presence of other aquatic organisms including other toxic cyanobacteria and their toxins, and the nature of the water (i.e., waterbody). The toxin of concern in this thesis are microcystins because of the high concentrations found in Pineview Reservoir and across Utah waterbodies. The objective of the thesis is to determine the effects of decreasing water temperature, added dissolved phosphorus concentrations, added dissolved molar N:P

ratios on the production of microcystins during cyanobacterial blooms using Pineview Reservoir water. Specifically, the study will assess the following:

1. The effect of sudden decrease in temperature on the production of microcystins in non-axenic culture conditions.
2. The effect of low and high added phosphorus concentration on the production of microcystins in non-axenic culture conditions.
3. The effect of low and high molar N:P ratios on the production of microcystins from non-axenic culture conditions.
4. The effect of how factors 1-3 interact with one another.

These objectives were chosen in order to further the understanding of water temperature, low added phosphorus concentrations, low added molar N:P ratio, and their interactions on the production of microcystins in waterbodies. Formally, if

$$CT = CT_0 + \alpha_i + \beta_j + \gamma_k + \alpha_i\beta_j + \alpha_i\gamma_k + \beta_j\gamma_k + \alpha_i\beta_j\gamma_k + \varepsilon_{ijk}$$

where CT is the cyanotoxin production, CT_0 is the mean cyanotoxin production, α_i is the effect of temperature change, β_j is the effect of added phosphorus concentrations, γ_k is the effect of added molar N:P ratios, and ε_{ijk} is experimental error. The combined terms represent the interaction effects.

Hypothesis 1

Decreasing water temperature from 25°C to 16°C will increase microcystin production. The null hypothesis is that there will be no difference in microcystin

production between leaving the cultures at 25ºC and transferring them to 16ºC, or $\alpha = 0$.

Hypothesis 2

Low added phosphorus levels (0.015 mg/L) will decrease biovolume but increase the production of microcystins compared to high added phosphorus levels (0.085 mg/L). The null hypothesis is that the addition of 0.015 mg/L phosphorus will show no statistical difference in microcystin production from those at 0.085 mg/L phosphorus added, or $\beta = 0$.

Hypothesis 3

Microcystin production under low added dissolved N:P (4:1) conditions will increase microcystin production compared to that of adding a molar ratio of (25:1). The null hypothesis is there will be no statistical difference in microcystin production between the two molar N:P ratios, or $\gamma = 0$.

Methods and Materials

Experimental Design

A three-factor factorial statistical design at two levels (2^3) is used to determine significance of decreasing water temperatures, dissolved phosphorus additions, and the dissolved molar N:P ratios in producing microcystins. The two temperatures chosen for the experiments were 25°C and 16°C according to surface water and thermocline temperatures found in Pineview Reservoir in the summer (July - August). The two dissolved phosphorus concentrations chosen for the experiments are set 0.01 mg/L

below the lower indicator level of 0.025 mg/L (UDWQ, 2014), and 0.01 mg/L above the upper indicator level of 0.075 mg/L, or 0.015 and 0.085 mg/L. Because of the variability in total phosphorus content of each of the cyanobacterial cultures, the factor is defined as the dissolved phosphorus concentration added.

The experimental factor of N:P ratio is independent of the factor of P addition. This means that the added dissolved nitrogen concentrations in the study are based on the added dissolved phosphorus concentration in solution. Low N:P molar ratios in the environment are 4:1 while high ratios are 25:1 with Pineview Reservoir having a total molar N:P ratio of 20:1 (Whitehead & Judd, 2002). Low molar N:P ratios tend to have cyanobacteria, such as *Microcystis sp.,* dominate and when N:P ratios increase to 25:1 an increase in green algae often occurs (Patel, 2019). For this reason, 4:1 and 25:1 N:P molar ratios were selected.

The factorial experimental design is shown in Table 8 with the main response variable of microcystin concentrations represented as $CT_{(A-H)}$. Other environmental variables were measured or analyzed (pH, water temperature, and PAR (photosynthetic active radiation)), but the focus is on microcystin production. There will be eight experimental conditions assigned letters A through H representing the eight combinations of factors.

Table 8

Experimental Setup with Objectives (Temperature, Added Phosphorus Levels, and Molar N:P Ratio) and Response Variables (Toxin Production)

Test Cultures	Temperature	P level (mg/L)	N:P ratio (molar)	Toxin Production	N level (mg/L)
A	25	0.015	4:1	CT_A	0.060
B	25	0.015	25:1	CT_B	0.375
C	25	0.085	4:1	CT_C	0.340
D	25	0.085	25:1	CT_D	2.125
E	25 to 16	0.015	4:1	CT_E	0.060
F	25 to 16	0.015	25:1	CT_F	0.375
G	25 to 16	0.085	4:1	CT_G	0.340
H	25 to 16	0.085	25:1	CT_H	2.125

Note. The nitrogen level is the calculated amount of nitrogen needed to achieve the corresponding N:P ratio.

The term test culture refers to cultures with the assigned environmental factors in Table 8. Each of the test cultures was given a label such as, A11, and run in triplicate per block in four blocks for a total of twelve runs for each test culture (Table 9). Experiments were run in blocks because there were not enough cyanobacteria to run all test cultures at once. The letter represents the assigned experimental condition

(Table 8) according to water temperature, added dissolved phosphorus concentrations, and the added dissolved molar N:P ratio. The first number after the letter represents the test block for that test culture. The second number represents which of the 3 triplicate test cultures are of the associated block. The triplicate number has no meaning besides keeping track of which samples are which.

Table 9

Experimental Setup for Each Block for the Experimental Conditions Shown in Table 8

Test Cultures	Block 1	Block 2	Block 3	Block 4
A	A11	A21	A31	A41
	A12	A22	A32	A42
	A13	A23	A33	A43
B	B11	B21	B31	B41
	B12	B22	B32	B42
	B13	B23	B33	B43
C	C11	C21	C31	C41
	C12	C22	C32	C42
	C13	C23	C33	C43
D	D11	D21	D31	D41
	D12	D22	D32	D42
	D13	D23	D33	D43
E	E11	E21	E31	E41
	E12	E22	E32	E42
	E13	E23	E33	E43
F	F11	F21	F31	F41
	F12	F22	F32	F42
	F13	F23	F33	F43
G	G11	G21	G31	G41
	G12	G22	G32	G42
	G13	G23	G33	G43
H	H11	H21	H31	H41
	H12	H22	H32	H42
	H13	H23	H33	H43

Note. Each letter and number combination (i.e. A11) represents a test culture in a given block.

Materials

Laboratory equipment necessary for one block of experiments included 30 Pyrex Erlenmeyer flasks capable of holding a volume of 125 mL. These are used as to hold the Pineview water and concentrated aquatic organisms (cyanobacteria, algae, etc.) from a stock culture of *Microcystis*. Concentrating the organisms from the stock culture required 56 plastic centrifuge tubes capable of holding 50 mL volume. Two different types of plastic centrifuge tubes, Teflon and polypropylene, were used in Block 1 inadvertently. The mistake was noted and the polypropylene plastic was used for the remainder of the blocks. In the first block both the centrifuge tubes and Erlenmeyer flasks were soaked in 50% HCl and rinsed with distilled/deionized water to eliminate any accompanying nutrients. In addition to this, the Erlenmeyer flasks were autoclaved at 121ºC for 20 minutes to deactivate any possible accompanying organisms. To sterilize the centrifuge tubes in-between use, 10% sulfuric acid is used to eliminate any accompanying organisms.

For total phosphorus and nitrogen analysis, 50 mL clear glass sampling tubes were acid rinsed in 10% HCl overnight to eliminate any nutrient contamination from the glassware. For one block of experiments, 48 sampling tubes were required for taking samples and preparing quality control parameters for the analysis. Sampling for DNA required 50 mL glass sampling tubes soaked in 10% sulfuric acid to eliminate contaminating DNA from the containers. Pre-sterilized nylon filters with a 0.2 µm pore size was used to filter Pineview Reservoir water to rid the water of accompanying organisms capable of skewing results. Other laboratory equipment required is outlined in the standard operating procedures (SOPs) for the analyses. A total of 108 microcystin

samples (used also for pH and temperature measurements), 108 samples for total phosphorus and total nitrogen, and 2 samples from the stock cultures for DNA analysis were obtained.

Experimental Methods

Reservoir Selection

Several waterbodies across Utah were considered as a focus in the study based on frequent HAB incidence. The waterbodies considered for selection were Scofield Reservoir, Pineview Reservoir, and Matt Warner Reservoir. Pineview Reservoir was selected due to the proximity of the drinking water facility, abundance of past HABs, and the proximity to the UWRL for sampling.

Stock Culture Conditions

Cyanobacteria samples were collected according to the recommended standard procedures outlined by UDWQ (UDWQ, 2016) in October 2022 from Pineview Reservoir. The cyanobacteria samples collected were grown in cultures using Zarrouk medium (Z8). Z8 medium was used to maintain and cultivate cyanobacteria strains along with BG-11 and 2 other media by Blue Biotechnology and Ecotoxicology Culture Collection (Ramos et al., 2018). BG-11 medium was initially tried as a growth medium for samples provided by UDWQ from different waterbodies across Utah, but the cyanobacteria did not grow well in the BG-11 medium. The Z8 medium was tried and found to be more effective in growing the cyanobacteria and was therefore chosen as an alternative due to the high growth of algae and cyanobacteria alike in the cultures. The recipe for Z8 medium is found in Appendix A (Cyanosite, 2022).

To cultivate cyanobacteria, ten mL of the cyanobacteria sample from Pineview Reservoir was added to 40 mL of Z8 medium and placed on a shaker table (100 rpm) at 25ºC with a light intensity of ~50 µmol $m^{-2}sec^{-1}$ (Figures 13 and 14). White LED lights (Ultra-thin LED Grow Light, White) were used as a light source with 50 µmol $m^{-2}sec^{-1}$ PAR achieved by adjusting the distance the light source is from the culture. Cyanobacteria counts using the Sedgewick Rafter counting chamber (Hausser Scientific, Horsham, PA) was used to ensure cyanobacteria numbers were increasing. The volume in the cultures was increased incrementally from 50 mL (Figure 13) to 2 liters (Figure 14) according to increasing cell counts.

Figure 13

Starting Cultures (50 mL) on a Shaker Table (100 rpm)

Figure 14

Two-Liter Cultures on a Shaker Table (100 rpm) in 25ºC Room

Due to limitations in the size and weight, culture sizes greater than two liters could not be placed on a shaker table. Growing cyanobacteria stock cultures larger than 2 liters required larger space and volume so a clear, plastic, 42-liter sterilite bin without constant mixing was used (Figure 15). The 2-liter volume was then added to ~10 liters of distilled water with the addition of 1 liter of Z8 medium. Then, the addition of ~500 mL of the Z8 medium was added to the clear plastic bins every week to ensure fresh cyanobacteria continued growing. Dead cyanobacteria and other organisms sink to the

bottom of the clear plastic bins and the healthy cyanobacteria remained elevated in the water column. Dead organisms at the bottom were removed with a hydraulic pump.

Figure 15

Stock Culture Growing in a Large Plastic Bin in the 25ºC Room

To ensure growing cyanobacteria, subcultures were taken from stock cultures and the put back into a smaller volume of medium every two to three weeks. This procedure was repeated until the start of the experiment so that active cyanobacteria

are used in the experiment. There was no visual evidence that sub-culturing selects for specific organisms. Upon visual examination, the genus of *Microcystis* was most abundant in the cultures.

Experimental Procedure

Preparation.

This section covers the steps taken to prepare the environmental conditions in the test cultures (Table 8). Pineview water for the test cultures was prepared by first filtering the water through a sterilized 0.2 µm filter to eliminate any accompanying organisms taken during sampling. A minimum of three liters was filtered in order to have sufficient volume for the test cultures. Containers for Pineview water were rinsed with 50% HCL to rid the container of possible nutrient contamination.

Organisms from the stock culture were prepared by first conducting cell counts from the stock culture using a Sedgewick Rafter counting chamber. This is done to ensure a minimum number of approximately 250,000 cells were inoculated from the stock culture into the test cultures. Once the stock culture samples were taken, the samples were centrifuged at 8,500 rpm for 10 minutes. Residual stock water was removed and the centrifuged biomass was then reconstituted with Pineview Reservoir water.

To prepare the nutrient conditions for the test cultures found in Table 8, nutrient concentrations were prepared by making a 10 mg P/L solution of KH_2PO4 and a 100 mg N/L solution of $NaNO_3$. From the 10 mg/L KH_2PO_4 solution, 150 and 850 µl were added to achieve 0.015 and 0.085 mg phosphorus per liter in the test cultures eventually

filled to 100 mL with Pineview Reservoir water. To achieve a concentration of 60, 375, 340, and 2,125 μg nitrogen per liter from the 100 mg N/L $NaNO_3$, volumes of 60, 375, 340, and 2,125 μl were added to the corresponding test cultures.

Preparation of the test cultures included taking acid rinsed and autoclaved 125 mL Erlenmeyer flasks and filling them with 90 mL filtered Pineview water, the corresponding phosphorus and nitrogen concentrations, and the centrifuged stock culture organisms. This procedure was done in the 25ºC room for test cultures A through D and then the needed materials were transferred to the 16ºC room where the procedure was repeated for test cultures E through H. Additional Pineview water was added, if necessary, to equal 100 mL in the test cultures. The flasks were then plugged with sterile cotton to reduce contamination during the experiment.

Once the experiment started, samples were mixed by hand by swirling the test cultures for five seconds once every 24 hours. The cyanobacteria were expected to take up the dissolved nutrient within the first two days (based on preliminary experiments, Appendix B) and then go into a nutrient deficient environment where toxin production could increase.

Controls were used in each block consisting of 100 mL of Pineview water. Controls consisted of filtered Pineview Lake water (0.2-micron membrane filter, ThermoFisher/Waltham, MA) without any nutrient addition or cyanobacteria from the stock cultures. The filtered control ensured that no toxin producing organisms were able to get through the filters and grow during the allotted time period of the

experiment. This ensured that all toxin producing organisms came from the stock culture.

Sampling Schedule.

Total nitrogen and phosphorus samples were taken on day 0 after cyanobacteria were inoculated into the test cultures along with 10 mL samples for DNA analysis. The 10 mL samples for DNA were not analyzed. These samples took 20 mL from each test culture leaving 80 mL in each of the test cultures during the 4-day experimental period. Sampling on day 4 included 80 mL to be tested for microcystins, pH, and water temperature. Measuring the pH and water temperature was done before centrifuging the 80 mL sample down to 5 mL for microcystin analysis. The sampling occurred one to two hours before the end of the light period and start of the dark period with sterilized 10 mL pipettes. The data and observations were recorded in a lab notebook and then transferred into a database for analysis. A schedule for all samples taken during the experiment is given in Table 10.

Table 10

Schedule and Amount of Sample Taken

Samples	Day 0	Day 1-3	Day 4
Total Phosphorus and Total Nitrogen	10 mL	0	0
Microcystins	0	0	80 mL
pH/Water Temp.	0	0	80 mL
DNA	10 mL	0	0
Light intensity	yes	No	yes

Note. The same 80 mL sample is used for microcystins, pH, and water temperature.

Nutrient Analysis

Ten mL samples were required for the analysis of both total phosphorus and total nitrogen. In addition to the 10 mL sample, 2 mL of the prepared digestion reagent which included a mixture of was added and then autoclaved at 100ºC for 90 minutes to digest the sample for both total nitrogen and total phosphorus (Valderrama, 1980). After digestion, total phosphorus was then analyzed using the ascorbic acid method, standard method 4500-P (O'Dell, 1993a), with a Genesys 10 VIS spectrophotometer set at a wavelength of 880 nm. Total nitrogen was measured with an AQ2 autoanalyzer (Seal Analytical, Mequon, WI), using a cadmium reduction method with analysis of nitrite by the azo dye (standard methods 4500-NO_2 E.) (O'Dell, 1993b). Procedures are outlined for setting up the AQ2 instrument for analysis of total nitrogen or nitrate by the manufacturer (AQ2 - USEPA Approved Methods, 2022).

Toxin and Cell Quantification

A preliminary toxin analysis of the stock culture using toxin test strips (Golden Standard Diagnostics, 2023a) determined the presence of microcystins. Based on preliminary analysis of the stock culture, the minimum cyanobacteria required to reach 0.3 µg/L microcystins is 250,000 cells. To ensure this number of cells was put into the test cultures, a Sedgewick Rafter counting chamber (Hausser Scientific, Horsham, PA) was used to find the concentration of *Microcystis* cells in the stock culture (Catherine et al., 2017). Once the concentration of *Microcystis* cells was found in the stock culture, an amount of stock culture was then sampled, centrifuged, and the biomass was inoculated into the test cultures to provide the target 250,000 cells. Cell concentrations in the test cultures on day 0 were calculated from concentration of *Microcystis* cells in the stock culture determined one day prior to starting the experiment and the volume taken from the stock culture.

Identification of microcystin producing genes from cyanobacteria genera was done using polymerase chain reaction assays (PCR) (T100 Thermo Cycler, Cat #1861096 Bio-Rad) on samples taken from the stock culture. DNA samples taken during the experiment were not analyzed due to lack of volume needed for the analysis. DNA extraction kits include detailed instructions on the purification and extraction of DNA (DNeasy PowerBiofilm Kit, 2023). Sequences and probes in Table 11 were used to identify genes capable of producing microcystins from *Microcystis* (mcyE MC), *Anabaena* (mcyE AB), and *Oscillatoria* (mcyE OS). Sequences and probes for genes capable of producing anatoxin-a, cylindrospermospin, and saxitoxin are provided to see if these were present as well.

Table 11

Primers and Probes Provided by UDWQ Used for PCR

Genes	F sequence	R Sequence	Probe
mcyE (*Microcystis*)	CGGAATGCCCAGTGCTTATC	ATTTGATTATGGACAACTTGACGGG	[6FAM]TGAAAATGCCTTTCAACAGTTAATTCAACGCCATGAAA[BHQ1]
mcyE (*Anabaena*)	ACAAATGCAACACGGAATTGGT	AGCGACTCGTTCTACACCTG	[Cyanine5]GGAATGCAGTCTAATATTGCAGCAGAAACAGCT [BHQ2]
mcyE (*Oscillatoria*)	CGGACATTCTCTGATGCTTTCG	AAACGGCTAATCCGGCAATG	[HEX]TAACCCACGTTCATAAAGAATTAAATGTATCGGTAAAATTGGC[BHQ1]
Anatoxin-a	ATCTGGTATTCAGTCCCCTCTATTC	GGGAATATGCACCATCAACTGA	[6FAM]AGAACCATTTTGTTTGCGGGTGAAGTTTT [BHQ1]
Saxitoxin	TGGCGTGTATTCCATGTCGG	CCGTAAGGCATATCGCTGCT	[HEX]CAGCTTACGTGCGTCTGGCAAAAGAG[BHQ1]
Cylindrospermopsin	CAGATCGCCCCATCAAAGAGG	GGCAGAACATAGGCATCTCATCG	[Cyanine5]CTCTTCATGGATAACGGTTGGCAATTCATCG [BHQ2]

For total microcystins, 80 mL samples were taken and centrifuged down to 5 ml with the excess 75 mL, without biomass, discarded. The remaining 5 mL is then tested using the microcystin/nodularins 96-test kit from Golden Standard Diagnostics (Lot P23F1409). Since the sample size was downsized from 80 to 5 mL through centrifugation prior to the instrument analysis, microcystin values from the instrument were adjusted to represent the microcystin concentrations in 80 mL.

Sample preservation was followed according to the procedures outlined by Golden Standard Diagnostics (Budapest) (Eurofins, 2022) (Golden Standard Diagnostics, 2023b). Kits do not measure the specific microcystins (LR, LA, YR, etc.), rather, the concentration of all microcystins. Cell lysis was induced by using the freeze/thaw method to release cyanotoxins held within the cyanobacteria cells so that a measure of total microcystins was conducted. Since 75 mL was discarded without biomass, it is noted that some external microcystins could have been discarded with the 75 mL.

PAR, Water Temperature, and pH

PAR was measured using a light meter (Apogee, model MQ-500, Logan, UT). White LED lights (Ultra-thin LED Grow Light, White) were used as a light source for growth. Adjusting the distance from the light source to the test flasks was done to obtain the desired light reading (50 µmol m^{-2} sec^{-1}). Light and dark periods were each 12 hours with PAR and pH readings along with microcystin samples taken two hours before the dark period began, and manually recorded in lab notebooks. PAR decreases ~10 µmol m^{-2} sec^{-1} as it passes through glass so organisms were exposed to ~40 µmol m^{-2} sec^{-1}. Cyanobacteria species like *Microcystis* and *Anabaena* grow best under low light conditions (25 µmol m^{-2} sec^{-1}) (Muhetaer et al., 2020), for this reason a lower light intensity was chosen.

The pH was expected to remain constant because of the high alkalinity of Pineview Reservoir water, but cyanobacteria can cause an increase pH (Zepernick et al., 2021). Readings for pH (Fisher Scientific XL25) (American Public Health

Association [APHA], n.d.) and water temperature were taken with microcystin samples on day 4. After each set of pH readings, the probe was rinsed with distilled water to ensure no contamination between readings. Water temperature readings were taken with an analog thermometer on one random sample in the 25ºC room and on one random sample in the 16ºC room to reduce possible contamination between test flasks. It was assumed water temperatures across the test cultures in the constant temperature rooms were the same.

Summary of the Analyses

Table 12 summarizes sample volume, instruments, and detection ranges for the associated analysis. A total of 10 mL is required for the analysis of both total phosphorus and total nitrogen. The same sample used for toxin analysis (microcystins) will be used for water temperature and pH measurements. After measuring pH and water temperature, sample volume for microcystins was reduced from 80 ml to 5 ml after centrifugation with the excess 75 mL discarded.

Table 12

Summary of Experimental Analyses (APHA, n.d.; Catherine, 2017; O'Dell, 1993a; O'Dell, 1993b)

Analysis	Detection range	Instrument	Sample Amount (mL)	Method Name
Total Phosphorus	0.005 - 0.5 mg/L	Spectrophotometer	10	Ascorbic acid method after digestion, 4500-P
Total Nitrogen	0.015 - 15 mg/L	AQ2	10	Cadmium reduction method, 4500 -NO_3 F
Microcystin	0.15 – 5 ppb	ELISA	80	Microcystin/Nodularin kits
pH		Fisher Scientific XL25	80	Method 4500-H^+
Water Temperature		Analog Thermometer	80	
Light Intensity		Apogee, model MQ-500		
DNA		PCR	10	DNeasy PowerBiofilm Kit & QIAquick PCR Purification Kit
Cell count		Sedgewick Rafter Counting Chamber	1	Abundance Estimation Using Counting Chambers

Data management, QA/QC, and statistical analysis

Samples from the experiment were tracked by assigning a tracking identifier on the sample storage container, with the sample source (which experimental run from Table 8), sampling date, intended analyses, and expiration date. These samples were entered into lab notebooks at the time of sampling and recorded in spreadsheets. Analysis results were either entered manually (pH, temperature, total phosphorus) or by transcribing the results provided by the AQ2 and ELISA in a pdf form into a

spreadsheet. This transcription was checked by a third party before use in analysis. Each set of results were then added to a relational database for safe archiving.

Quality control included spiking distilled water at both phosphorus concentrations (0.015 and 0.085 mg/L), all nitrogen concentrations (0.06, 0.340, 0.375, and 2.125 mg/L), and a spike of 0.5 μg/L of microcystins. Concurrent Calibration Verifications (CCVs) were prepared to ensure an instrument is measuring the correct concentration from a known standard. Spikes were analyzed according to percent recovery along with CCVs at defined concentrations. The data quality objectives in this study were to be within 30% of the actual value. To account for human error an additional 5% was granted from the standard deviation of 25% to account for human error.

Pineview water was also analyzed for total nitrogen and phosphorus to determine how much nitrogen and phosphorus was added with the Pineview water. Blanks were run to ensure no contamination occurred. These quality control measures were used to track data quality during the experiments so that any analytical problems could be addressed.

The data from the factorial experimental design was transferred to statistical software R (R foundation for Statistical Computing, 2022) for analysis using the analysis of variance (aov(…)) and linear regression (lm(…)) to determine the statistical significance and magnitudes of the effect of each of the three factors and their interactions on the production of microcystins. A nominal α value of 0.05 was used to help determine significance. If residuals were not normally distributed with constant

variance (NIID(0,σ^2)), suitable transformations were sought to ensure statistical results are valid. Even after transformation, differences were seen among the blocks, so analysis of the blocks was also done individually. All R results are included in Appendix D of the thesis.

Results and Discussion

Data Validation and Quality Control

Microcystins/Cell Counts/Microcystins Quota.

As described above, the cyanotoxin, microcystin, was measured on all study samples using ELISA. The data includes blanks, control spikes (microcystins spiked into Pineview water), and blank spikes to determine the quality of the data taken from each analysis. Table 13 shows the percent recoveries for each of the spikes from each block. One spike into distilled water and Pineview water was done in every block. The instrument takes two measurements on the one sample and the average of those two measurements is used in calculating the percent recovery.

Table 13

Percent Recoveries for Microcystin Spiked into Distilled Water (DISpk 0.5) and Pineview Water Control recovery (PineSpk 0.5)

Spike	Block 1 (%Recovery)	Block 2 (%Recovery)	Block 3 (%Recovery)	Block 4 (%Recovery)
PineSpk0.5	67.6	73.6	80.6	98.2
DISpk 0.5	67.4	76.4	66.4	90.4

There were three spikes that were not within the designated 30% percent of the actual value two of which were in Block 1. Block 1 was still analyzed even though the spikes did not pass the data quality objective. Block 4 had the best percent recovery for the blank and the Pineview water control spike at 90.4 and 98.2%. One absorbance reading was discarded in Block 3 because the ELISA instrument stopped and had to be restarted due to difficulties maintaining pressure. The sample read above 5 µg/L which was much higher than other measurements in that block. The duplicate measurement for this test culture read a microcystin concentration of 1.78 µg/L which was more typical of other observations so the concentration was kept for analysis.

Absorbance values for microcystins from the ELISA were taken and compiled into a dataset according to the treatments (A-H). Microcystin concentrations were then regenerated in R using the standard curve from the experiments in every block. Microcystin concentrations provided by the ELISA were confirmed using this process.

Microcystin concentrations for the blanks and controls (Pineview water (Con.)) were below the detection limit and less than the microcystin concentrations reported for the treatments except for one control sample. The control sample (Con 12) resulted in a microcystin concentration greater than 5 μg/L. None of the test cultures (A-H) tested as high as this control so it is assumed no contamination from the Pineview water occurred in the test cultures, instead it is assumed an instrument malfunction occurred.

Total Phosphorus.

Quality control measures for total phosphorus in each block consisted of spikes into distilled water of 0.015 and 0.085 mg P/L, Pineview water control spikes (0.2 mg P/L), CCVs (independently pre prepared standards measured to check for instrument drift) (0.5 mg P/L), and blanks to ensure no phosphorus contamination occurred during the procedure. Percent recoveries on blank spikes, Pineview water control spikes (Con Spike), and CCVs are found in Table 14. Block 4 had a CCV of 0.2 mg P/L instead of 0.5 mg P/L and Block 1 did not have a Pineview water control spike.

Table 14

Percent Recovery for Spikes and CCVs for Total Phosphorus Analysis

Sample ID	Block 1 (%Recovery)	Block 2 (%Recovery)	Block 3 (%Recovery)	Block 4 (%Recovery)
15P60N	235.9	72		85.3
15P340N	223.3	125.3		72
85P375N	119.7	90.4	111.6	78.6
85P2125N	170.9	66.8		85.6
blkspk (200)	92.7	75.5	110.7	105.5
CCV (500)	92	79	123.3	90.7

Note. Sample IDs with 15P and 85P represent the spikes of 0.015 and 0.085 mg P/L.

Calibration curves were measured using with a spectrophotometer for all blocks, except Block 2, which resulted in R^2 values of at least 0.999. Block 2 did not have a calibration curve generated along with the samples because reagents were incorrectly added to the standards resulting in an unusable calibration curve. In an effort to estimate total phosphorus for Block 2, Block 2 absorbance values were converted to concentrations using the calibration curve from Block 4. Percent recoveries for blank spikes in Block 2 varied but only one spike did was not within 30% of the intended concentration at 66.8%. CCVs and Pineview water spikes showed passing percent recoveries for all blocks being within 30% of the estimated values (Table 14).

Zeroing the spectrophotometer during Block 3, using the blank, was not performed. As a result, blank measurements had absorbance readings above the lower detection limit (0.005 mg P/L). To account for not zeroing the spectrophotometer, absorbance readings for each measurement were reduced by the absorbance value of the blank. A calibration curve with an R^2 value of 1 was generated using this procedure. Due to glassware breaking, only one spike was measured along with the CCV and the Pineview water control spike passing the quality control objective being within 30% of the actual concentration.

High levels of phosphorus were found in the blank in Block 1, but the quantity was not measured. A calibration curve was instead made using distilled water as a blank measurement. Both of the 0.015 mg P/L spikes and one 0.085 mg P/L spike did not pass quality control parameters being within 30% of the calculated value (Table 14). These high spiking concentrations were high possibly due to phosphorus contamination, but with other measurements passing quality control parameters (CCV and Pineview water spike) it is unclear why there were inconsistent spike values. It is possible that Block 1 did not have consistent phosphorus spiking into the test cultures.

Total Nitrogen.

Total nitrogen quality control consisted of spikes into distilled water at nitrogen concentrations of 0.06, 0.34, 0.375, and 2.125 mg N/L along with CCVs, spiked Pineview water control concentrations, and a higher concentration blank spike. Some blocks had different nitrogen concentrations for CCVs, spiked Pineview water control concentrations, and the other blank spikes. This was not done on purpose; spikes should

have been the same across all blocks. Block 1 did not have any CCVs or Pineview water control spike due to glassware breakage. The higher concentration blank spike, even after dilution by the instrument in Block 1, kept increasing in total nitrogen concentration indicating possible nitrogen contamination. This higher concentration blank spike was assumed to be contaminated and was not recorded in Table 15. Percent recoveries for Pineview water control spikes, higher concentration blank spikes, and CCVs across the different blocks are recorded in Table 15 with the calibration curves for each block found in Appendix C. Percent recoveries for nitrogen should be within 30% of the target concentration to pass quality control.

Table 15

Percent Recoveries for Spikes, the Average CCV, and Pineview Water Control Spike for Total Nitrogen

Sample ID	Block 1 (%Recovery)	Block 2 (%Recovery)	Block 3 (%Recovery)	Block 4 (%Recovery)
15P60N	203.3	141.6		538.3
15P340N	123.8	30.9		129.1
85P375N	127.2	150.1		124
85P2.125N	111.5	97.2	91.9	57.4
blkspk		94.3	109.3	56.6
Average CCV		96.4	91.1	55.1
Con Spk		134.2	75.3	52.6

Note. Spikes with 60N, 340N, 375N, and 2.125N represent nitrogen spikes of 0.06, 0.34, 0.375, and 2.125 mg N/L. Blank spaces represent no recorded percent recovery for the corresponding Sample ID.

Block 4 showed the lowest percent recoveries of nitrogen, even with an acceptable standard curve, out of all of the blocks for the average CCV, blank spike, and the Pineview water control spike not passing quality control checks. Since values for this block had poor recoveries for the CCV, the higher concentration blank spike, and the Pineview water spike total nitrogen values in this block are not used for analysis. It is unclear why recoveries for these were low but two out of the four nitrogen spikes were within the 30% of the expected spiked value passing the quality control. Block 2 and 3 showed passing CCVs, higher concentration blank spike, and Pineview water control spikes being within 30% of the expected value.

Spike values of 0.06, 0.34, 0.375, and 2.125 mg/L varied between blocks with none of the 0.06 mg/L spikes being within 30% of the expected value. The spikes of 0.375 mg N/L were consistently higher than the expected value across all of the blocks. Each block had at least one spike value of 0.34, 0.375, and 2.125 mg N/L be within 30% of the expected value. Since some spikes passed and others didn't there seem to be inconsistent spiking of nitrogen done across all of the blocks.

A mistake was made on Block 3 when the wrong top nitrogen standard was used for making spikes, the CCV, and the top standard resulting in a calibration curve with lower absorption values than were common for the remainder of the analyses. This resulted in values from the AQ2 being higher than expected, so the calibration curve from Block 4 was used to estimate the Block 3 nitrogen concentrations. Taking the absorption values from Block 3 and applying them to the Block 4 calibration curve, total nitrogen values were more consistent with those seen in other blocks. Block 3 only had one nitrogen spike of 2.125 mg N/L due to glassware breaking which ended up being within 30% of the expected value passing quality control.

Water Temperature/PAR/pH.

Water temperatures were measured using an analog thermometer after the temperature stabilized in only one of the test cultures in every block. It is assumed that the water temperature in all the test cultures were not statistically different from one another. PAR readings did not change throughout the experiment indicating that constant light intensity was provided during the duration of the blocks. Light intensities did not exceed 55 µmol m^{-2} sec^{-1} or drop below 45 µmol m^{-2} sec^{-1}. While measuring

pH, the probe was rinsed with organic-free distilled/deionized water after every sample. The instrument was calibrated every ten samples to ensure instrument response remained constant throughout the experiment. There was no drift seen in the pH probe.

DNA.

Quality control for PCR included a blank and a spike from one of the primers. There was no response to the blank indicating no DNA contamination through the procedure (Figure 20). The spikes from primers showed light (DNA) appearing in the gel indicating a successful spike (Figure 20).

Blocking Effects

Cell Concentrations/Microcystins/Microcystins Quota.

Seventy mL of stock culture was taken, washed, and reinoculated from the stock culture for Blocks 1,2, and 3 and sixty-five mL for Block 4 to account for the higher concentration of cells in the stock culture at the time. Calculated cell concentrations at the start of the experiment differed between the blocks with Block 1 having the lowest calculated concentration of 5,289,867 cells/L followed by Block 2, Block 3, and then Block 4 (Table 16). The procedure for obtaining the cell culture to add to the flasks was replicated throughout, however it is recognized that the number of cells added to each flask varied randomly. It is assumed the same concentration of cells, in each of the blocks, are in each of the test cultures. Microcystin concentrations from each block can be found in Table and Figure 16.

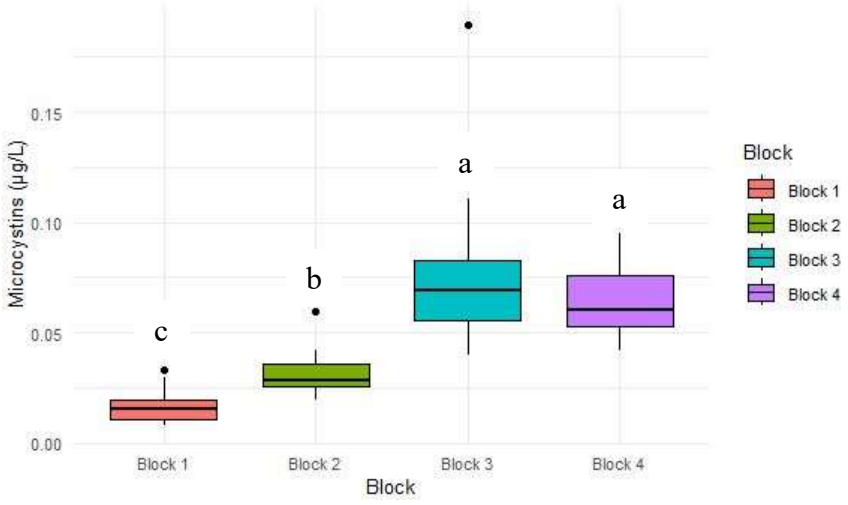
Table 16

Microcystin Concentrations with 95% Confidence Intervals and Results from Post Hoc Comparison Test

Measurements	Block 1	Block 2	Block 3	Block 4
Microcystins (µg/L)	0.017 ± 0.0035c	0.031 ± 0.0037b	0.073 ± 0.013a	0.063 ± 0.0070a

Figure 16

Microcystin Concentrations vs Block with Results from Post Hoc Comparison Test

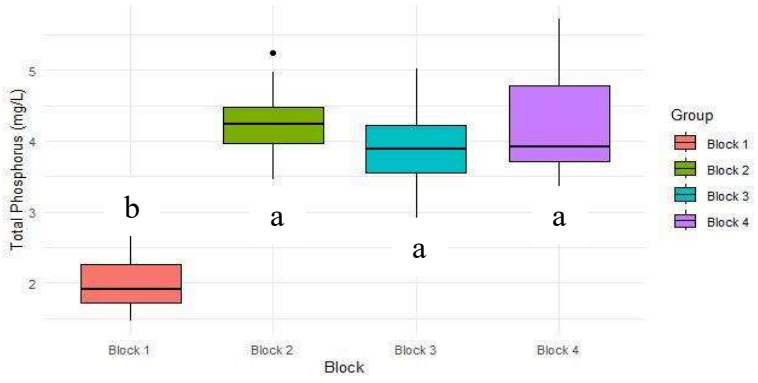


Total Phosphorus.

Total phosphorus concentrations varied in the test cultures depending upon the block (Figure 17). Block 1 had the lowest concentration of total phosphorus averaging 2.01 ± 0.17 mg P/L. Blocks 2, 3, and 4 averaged more than double that of Block 1 measuring 4.24 ± 0.19, 3.93 ± 0.22 and, 4.21 ± 0.30 mg/L. Block 1 was statistically different than all the other blocks while Blocks 2,3, and 4 were not significantly different from one another (Figure D.12). Phosphorus was dosed into the microcosms at low level (0.015 mg/L) and high level (0.085 mg/L) as bioavailable phosphate. The background concentration of total P was due to the addition of the cyanobacteria inoculum where the cyanobacteria's capability of luxury phosphorus uptake in the stock culture.

Figure 17

Box and Whisker Plot of Total Phosphorus Results for Every Block with Results from Post Hoc Comparison Test



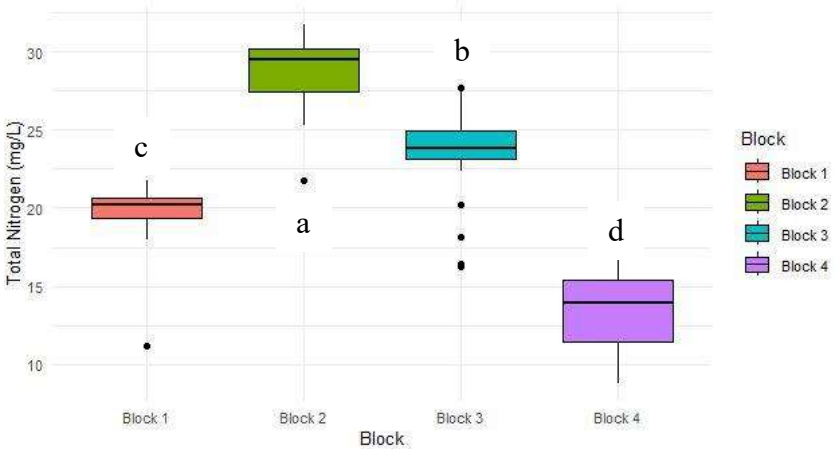
A similar trend occurred with total phosphorus in the Pineview water. In Block 1, Pineview water averaged a total phosphorus measurement of 0.0076 µg/L. Blocks 2, 3, and 4 averaged 0.024, 0.020, and 0.023 mg/L. It is unclear why total phosphorus values were lower in Block 1 since the same Pineview water sample was drawn from in each of the blocks.

Total Nitrogen.

Figure 18 shows the differences among each block according to total nitrogen measurements. Blocks 1, 2, and 3 consisted of total nitrogen values ranging from 20 to 32 mg/L as N with some individual total nitrogen concentrations measurements dropping into the teens. Block 4 had the lowest total nitrogen values ranging from 8 to 16 mg/L. Block 2 averaged the highest total nitrogen out of all the blocks at 28.8 mg/L. All blocks were statistically different from one another (Figure D.13).

Figure 18

Box and Whisker Plot for Total Nitrogen Results in Every Block with Results from Post Hoc Comparison Test



Total nitrogen values in Pineview water were consistent throughout the blocks averaging 0.56 mg/L but ranged anywhere from 0.29 to 0.97 mg/L with no trend over time.

Water Temperature/PAR/pH.

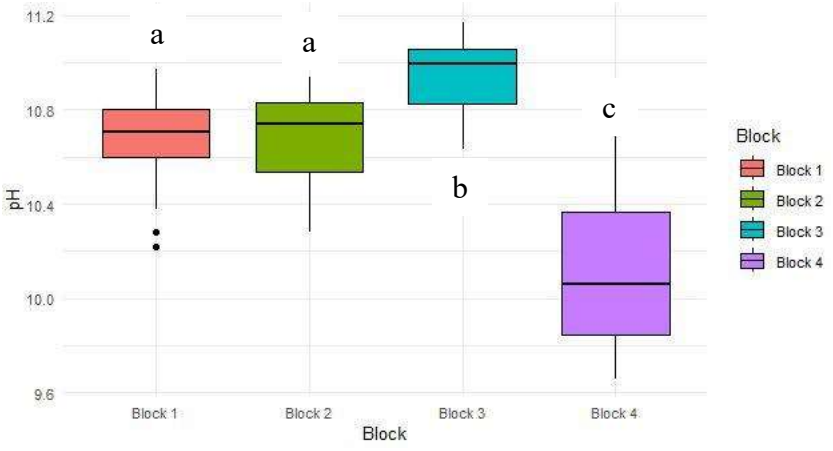
Water temperatures on day 4 were higher than the nominal ambient temperature of 25ºC and 16ºC likely due to the heat coming from the lamps or from biological activity. Water temperature ranged from 25.8 to 28ºC in the 25ºC room and 17.8 to

18ºC in the 16ºC room across all four blocks. PAR readings remained unchanged during the duration of the experiment for all 4 blocks at 50 $\pm$5 µmol m^{-2} sec^{-1}.

Values of pH for the test cultures on day 4 ranged from 9.66 to 11.17 depending on the block (Figure 19). Block 3 had the highest pH values, some of which ranged above 11, and the lowest pH values occurred in Block 4 with some of the readings under 10. Blocks 1 and 2 were not statistically different one from another while all of the other blocks were statistically different one from another (Figure D.14).

Figure 19

Box and Whisker Plot of pH Results for Every Block with Results from Post Hoc Comparison Test

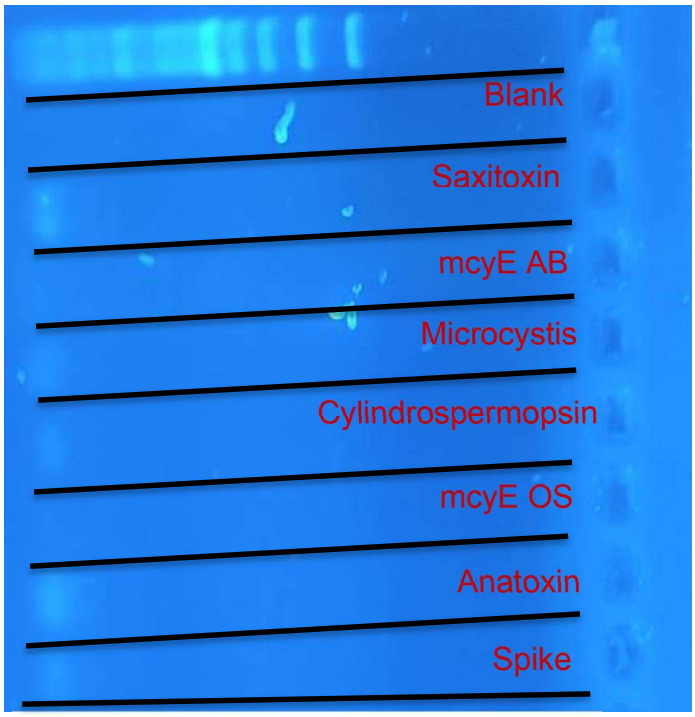


DNA.

DNA samples were taken from the stock culture and then analyzed using PCR to confirm the presence of three cyanotoxin producing genes (cylindrospermopsin, anatoxin-a, saxitoxin) identifying microcystin genes specific to *Microcystis* (mcyE MC*)*, *Anabaena* (mcyE AB*)*, *Oscillatoria* (mcyE OS*)* (Table 11). Figure 20 is a picture of the gel where a square light appears on the left, this confirms the presence the DNA for what was tested. Analysis showed the presence of (mcyE AB), although very dim in the picture was visible, and (mcyE MC) genes, but not the presence of (mcyE OS*)* (Figure 20). PCR also confirmed the presence of genes capable of producing microcystins, anatoxin-a, and saxitoxin in the stock culture.

Figure 20

DNA Results from PCR Procedure



Response Variable Effect on Microcystin Production

Microcystin concentrations varied from block to block (Table 16). The analysis of variance residuals, using microcystin concentrations in µg/L (without transformation), were analyzed using a normal Q-Q plot and a residuals vs fit plot (Figure D.1). There was clear evidence of non-normality and non-constant variance in the residuals, key assumptions for ANOVA tests, in these plots so microcystin

concentrations across all blocks were transformed using the Box-Cox (Box & Cox, 1964) transformation

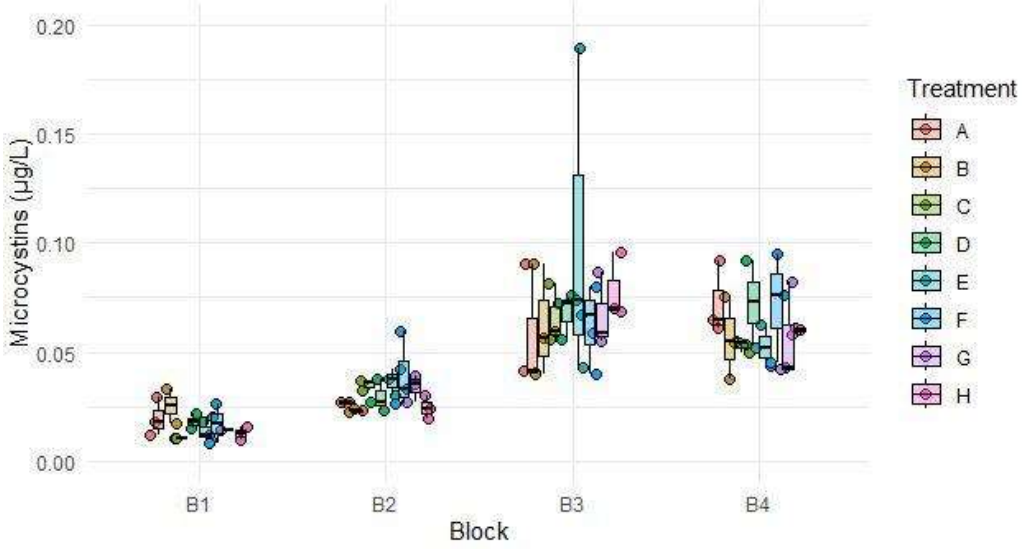
$$y_{i,t} = \frac{y_i^{\lambda} - 1}{\lambda}$$

where y_i is the measured microcystin concentration in µg/L, y_{it} is the transformed value, and λ is the transformation parameter that normalizes the residuals and stabilizes the residual variance. The transformation was applied to each of the four blocks using the same λ value of 0.228. The lambda value was determined by combining untransformed microcystin concentrations into one dataset and then transforming the data using the boxcox() procedure in R.

The purpose of this transformation is to ensure the ANOVA test requirements for the residuals are met, mainly they are normally distributed with constant variance. Figure 21 shows a boxplot of microcystin data (without transformation) where the variability of the observed microcystin concentrations is seen to increase with the median for each block. Figure 22 displays the transformed microcystin data in a boxplot showing variability is more consistent across all blocks.

Figure 21

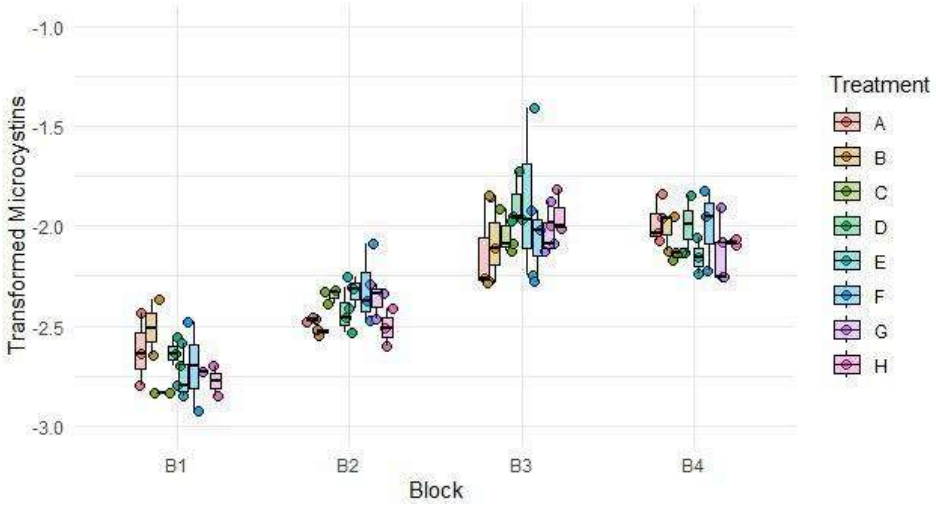
Microcystin Concentrations According to Block (No Transformation)



Note. Individual observations are plotted by jittering (shifting slightly) in the *x* direction so that all can be seen. Blocks 1 through 4 are represented on the x-axis as B1, B2, B3 and B4. Treatments A, B, E, F received low added phosphorus (0.015 mg/L) and treatments C, D, G, H received high added phosphorus (0.085 mg/L). Treatments A, C, E, and G received nitrogen addition resulting in a molar N:P of 4:1 while treatments B, D, F, and H received nitrogen addition to achieve a molar N:P ratio of 25:1. Treatments A, B, C, D were in the 25ºC room during the experiment while treatments E, F, G, and H were in the 16ºC room.

Figure 22

Microcystins According to Block (Box-Cox Transformation with $\lambda = 0.228$*)*



Note. Individual observations are plotted by jittering (shifting slightly) in the *x* direction so that all can be seen. Blocks 1 through 4 are represented on the x-axis as B1, B2, B3 and B4. . Treatments A, B, E, F received low added phosphorus (0.015 mg/L) and treatments C, D, G, H received high added phosphorus (0.085 mg/L). Treatments A, C, E, and G received nitrogen addition resulting in a molar N:P of 4:1 while treatments B, D, F, and H received nitrogen addition to achieve a molar N:P ratio of 25:1. Treatments A, B, C, D were in the 25ºC room during the experiment while treatments E, F, G, and H were in the 16ºC room.

The transformed data resulted in an improvement in normality of the residuals (Figure D.2) and a stabilized variance so the transformed microcystin concentrations were used in the final ANOVA analysis. An ANOVA table was generated to determine whether the mean microcystin concentration of each block results differed, after accounting for the treatment effects. With all transformed microcystin

concentrations evaluated together, there were no differences seen among treatments but all blocks (Figure D.3 ANOVA Table) (Figure 22). Blocks 3 and 4 were the only similar blocks (Figure D.4 Tukey table)

Other parameters are significantly different from one another such as microcystin cell quotas (Figure D.11 Tukey table) indicating a different environment was present in each of the different blocks. More evidence of a different environment in each of the different blocks include all blocks significantly different for total nitrogen (Figure D.13 Tukey table), Block 1 was statistically different than all the other blocks for total phosphorus (Figure D.12 Tukey table), and Blocks 3 and 4 were statistically different than Blocks 1 and 2 in regards to pH (Figure D.14 Tukey table). Since each block had a statistically different environment, blocks were also analyzed individually and transformed using the same λ value of 0.228 as above, to determine the significance of the response variables. A linear regression model

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_3 x_{i3} + \beta_{12} x_{i1} x_{i2} + \beta_{13} x_{i1} x_{i3} + \beta_{23} x_{i2} x_{i3} + \beta_{123} x_{i1} x_{i2} x_{i3} + \varepsilon_i$$

where y_i is the transformed response for experiment, x_{ij}, j=1,2,3 are the coded levels of the j experimental factors, and ε_i is the residual for experimental i and the β values measured the change in y_i when x_{ij} is varied from 0 to 1, was fitted to the results using R for the different blocks and each block showed different results. Response variables with p-values less than 0.05 were considered statistically significant.

Results from the ANOVA analyses (using the same combined lambda (λ) value of 0.228) are summarized in Table 17 from statistical tables in the appendix

(Figures D.3, D.5, D.6, D.7, and D.8 in Appendix D). By using the same λ value for the transformation, blocks can be compared one to another.

Table 17

Significance of Response Variables Analyzed According to Blocks

Effect Type	Response Variable	Block 1	Block 2	Block 3	Block 4	All Blocks Together
Main Effects	Phosphorus	x	x	x	x	x
	N:P ratio	x	x	x	x	x
	Water Temperature	x	x	x	x	x
2 Factor Interactions	Phosphorus: N:P ratio	x	x	x	x	x
	Phosphorus: Water Temperature	x	✓	x	x	x
	N:P ratio: Water Temperature	x	x	x	x	x
3 Factor Interaction	Phosphorus: N:P ratio: Water Temperature	x	x	x	x	x

✓ $p < 0.05$. x $p > 0.1$. *$0.05 < p < 0.1$.

Analyzing all the blocks together showed no significant results, and blocks analyzed individually resulted in no significant increase or decrease in microcystin

production. By assuming the block effects are independent of the test variables significant test variables can be hidden when this is not the case. In this case no difference between the analysis of the blocks together or individual analysis was seen.

In Block 2, one interaction term was significant and that being between water temperature and phosphorus showed a significant *p*-value but is not discussed in the discussion section of the thesis. This interaction was not found to be significant in the literature and was only found to be significant in one of the blocks in this study.

Phosphorus.

The experiments in this study do not support the hypothesis that changing added dissolved phosphorus concentration from 0.015 to 0.085 mg/L would increase the production of microcystins in non-axenic culture conditions. No blocks, analyzed individually and together, showed significance for the increase or decrease in microcystin concentration under this added phosphorus concentration.

One paper studied this effect of phosphorus deprivation on *Microcystis aeruginosa* using differing levels of phosphorus. Wei et al. (2021) found higher phosphorus levels (5.4 mg/L) resulted in higher microcystin production while lower phosphorus concentrations (0.054 to 0 mg/L) inhibited the growth of *Microcystis aeruginosa* so the expression of microcystin producing genes was reduced. In contrast, Pimental and Giani (2014) saw an increase in microcystin production and microcystin producing genes as dissolved phosphorus levels decreased 10- and 100-fold (0.475 and 0.0475 mg/L). The increase in toxin production in lower nutrient concentrations was said to be from oxidative stress from nutrient limited conditions.

Differences in the way the cyanobacteria were cultured prior to the start of the experiments could explain why some papers saw an increase in toxin production while another saw a decrease in toxin production. Cyanobacteria have the capability to uptake phosphorus in excess and store it to account for conditions where phosphorus concentrations fluctuate (Solovchenko et al., 2020). Wei et al. (2021) starved the cyanobacteria one week before starting experiments to deplete the phosphorus reserves found in cyanobacteria cells. Pimentel and Giani (2014) did not indicate this starvation occurred prior to their experiments. This extra phosphorus in the cells' reserves could be used towards toxin production or cell maintenance when dissolved phosphorus concentrations are low in the surrounding environment.

Prior to the start of the experiments in Blocks 1, 3, and 4, the stock culture was given medium for growth two days before. Block 2, unlike Blocks 1, 3, and 4, was fed 4 days prior to the start of the experiment. This did not seem to affect the production of microcystins in the experiments done in this study. It is suspected that the high levels of total phosphorus, both dissolved and inside the cells, ranging from 2 to 5 mg/L in the test cultures, was high enough to not cause the cyanobacteria cells to enter into a state of nutrient deprivation causing the increase or decrease in toxin production. This amount of total phosphorus in the test cultures could have come from not washing the cells or the uptake of excess phosphorus by cyanobacteria in the stock culture.

Phosphorus inputs to surface waters can come from a variety of external sources, such as for Pineview Reservoir, including groundwater, runoff from tributaries, animal waste, and onsite wastewater treatment systems (Whitehead & Judd, 2002). Every lake has a different mix of nutrient sources and all of these need to be

monitored to reduce phosphorus buildup in a reservoir. Over time, both internal and external phosphorus can settle and accumulate in the sediment. This stored phosphorus in the sediments can release back into the environment for aquatic organisms to uptake again, especially in stratified waterbodies; this is known as internal loading (DataStream Initiative, 2021). Reducing phosphorus inputs will not have an immediate effect on reducing HABs because of internal loading, but over time, internal sources will be depleted if external inputs are reduced. A method for reducing phosphorus inputs include relocating grazing, by cattle and other animals, away from the sides of lakes and tributaries entering the waterbody.

N:P ratio.

The dissolved N:P ratio was not a significant factor in any of the blocks. A dissolved N:P ratio beneath the Redfield ratio is an indicator of a nitrogen deficient environment (Reynolds, 2006). During a bloom event, the reduction of nitrogen can induce the production of microcystins. One paper saw that as *Microcystis* cells experienced nitrogen starvation, with phosphorus present, microcystins were produced (Zhou & Wang, 2022). In another observational study, microcystin production was highest when both nitrogen and phosphorus were depleted (Barnard et al., 2021). Not seeing an increase in toxin production during this study from a low dissolved molar N:P ratio (4:1) could be because there was already a sufficient amount of nitrogen in the test cultures causing the test cultures to not be nitrogen deficient in the present conditions. Future experiments should take into consideration the levels of total nitrogen present and decrease the total concentration to determine if there are any effects of a lower dissolved N:P ratio on the production of microcystins.

Higher nitrogen concentrations could select for non-nitrogen fixing cyanobacteria in a natural environment such as *Microcystis* and *Planktothrix* (Gobler et al., 2016). Even though lowering nitrogen inputs could select for nitrogen fixing cyanobacteria, lowering total nutrient concentrations should lower biomass reducing overall toxin production potential. One study found a 40% reduction in nutrient concentrations resulted in a decrease in biomass and toxin production (Barnard et al., 2021). To reduce both nitrogen and phosphorus loading, water utility managers need to understand where nutrient loading can be reduced and work with the corresponding organizations to implement plans to reduce the entry of the pollutants. Pineview Reservoir, as previously mentioned, has nutrient inputs from wastewater treatment systems (septic tanks) so updating these systems could reduce the input of nutrients into Pineview Reservoir. The reduction of nutrient inputs will not have an immediate effect on the reducing HABs due to the nutrient cycling of nitrogen (Hoffman et al., 2022) causing non-nitrogen fixing cyanobacteria, such as *microcystis*, to continue dominating.

Water temperature.

Results from this experiment do not support temperature reduction being a significant factor in the production of microcystins. Water temperature is a key environmental element determining the growth rate of cyanobacteria. Cyanobacteria grow in the springtime even when temperature conditions are not optimal (11ºC to 14ºC) and increase growth rates when temperatures are higher (20ºC to 30ºC). An increase in water temperature above 30ºC showed an increase in microcystin concentrations with an increased expression of microcystin producing genes (Yang et

al., 2020), while Martin et al. (2020) found as temperature decreased from 26ºC to 19ºC cyanotoxin production starting increasing after 2 days.

The water temperature values in this experiment decreased on average across all of the blocks from 27.2ºC to 17.9ºC. Unlike the literature, microcystin concentrations did not double in concentration when the temperature decreased like that found in the study done by Martin et al. (2020) even with similar temperatures.

Water utility managers should monitor water temperatures close to the intake, especially if HABs can potentially enter a drinking water facility. Even though this experiment did not confirm that decreasing water temperatures from optimal to suboptimal can induce a temperature stress increasing microcystin production, decreasing water temperature has been shown to increase external toxin concentration (Preußel et al., 2009). Protecting the plant from cyanobacteria biomass will not necessarily rid the water of the toxins external to cyanobacteria cells. Further research on the decrease in temperature increasing external toxin concentrations is needed. It is advised additional treatment is done to ensure toxins do not enter the water supply or contaminate plant surfaces.

Non-Response Variable effect on Microcystin Production

Cell concentrations/ Microcystin Quota.

The stock culture is a non-axenic culture where multiple organisms can grow including algae and other types of photosynthetic organisms. The stock culture contains microcystin-producing cyanobacteria (*Microcystis* and *Anabaena)* but may also contain other *Microcystis* species not capable of producing toxins which are

indistinguishable under a microscope from the *Microcystis* cells that can produce toxins. *Microcystis* cells were the most abundant cyanobacteria in the stock culture and was the only cyanobacteria counted in the cell counts.

Using both the average microcystin concentrations and the cell counts from the stock culture, toxicity-per-cell measurements for the test cultures was determined by dividing the average microcystin concentration per block by the calculated concentration of cells in the test cultures at the start of the experiment. This measurement is referred to as microcystin quota and has units of femtogram (10^{-15} grams) of microcystin per cell. This measurement assumes cell concentration did not change significantly over the four-day test period or by treatment. Blocks differed greatly in regards to the toxicity of individual cells with each block being statistically different one from another (Figure D.11 Tukey Table). According to the microcystin quota, the most toxic experimental block was Block 3, followed by Block 4, Block 2, and then Block 1 was the least toxic experimental blocks (Table 18).

Table 18.

Cell Concentrations and Microcystin Quotas with 95% Confidence Intervals and Results from Post Hoc Comparison Test

Measurements	Block 1	Block 2	Block 3	Block 4
Cell Concentration (cell/L)	5,289,867	6,435,217	7,070,343	8,965,122
Microcystin/cell (fg/cell)	3.16 ± 0.67d	4.82 ± 0.58c	10.26 ± 1.88a	7.02 ± 0.78b

As the calculated starting cell concentration increased from Block 1 to 4, an increase in the average microcystin concentration was seen (Table 18) except for in Blocks 3 and 4. Blocks 4 averaged lower microcystin concentrations than Block 3 but had a higher calculated cell concentration.

In previous studies, microcystin concentrations have been positively correlated with more biomass (Dolman et al., 2012). This conclusion supports the actions taken by UDEQ to decrease nutrient inputs to reduce biomass, but more biomass does not always equate to more toxins (Table 16). A study in New Zealand (Wood et al., 2021) did an analysis of two eutrophic lakes that experience HABs yearly. A strong relationship between cell and microcystin concentration occurred in all parts of both lakes except for one bay that had high cyanobacteria biomass but no toxin production.

Microcystin quotas are measurements aimed at determining the toxicity of each cell in a bloom. Some key assumptions made in order to calculate the Microcystin quota

are that all the test cultures in each block contained the same concentration of cyanobacteria and did not significantly change from the start of the experiment until the end of the experiment. The assumptions do not account for how the test cultures could have changed or how the cultures could have grown according to the different nutrient and temperature conditions from the start of the experiment until the end. These assumptions are made to calculate an estimated microcystin quota to aid in understanding what could be occurring in the test cultures in every block.

Microcystin quotas could vary depending on the number of toxin producing cells, specie of microcystin producing cyanobacteria, stage of growth (Orr & Jones, 1998), and the conditions in the stock culture prior to the start of each experimental block. There were significant differences in microcystin quotas between every block (Figure D.11) indicating a different toxicity level environment in each of the blocks. Microcystin quota data were transformed using a Box-Cox transformation with a λ of 0.065 because there was evidence of non-normality and non-constant variance in the residuals (Figure D.9), and after transformation residual plots improved (Figure D.10).

Orr and Jones (1998) found microcystin quotas sixteen times higher than those found in this thesis. Orr and Jones (1998) noted that during different stages of growth, in non-axenic cultures, the microcystin quota was different. During the late and early growth phase, in the Orr and Jones (1998) study, microcystin quotas ranged from 145 to 165 fg cell^{-1}). Orr and Jones (1998) also recorded lower microcystin quotas (56 ±10 fg cell^{-1}) in the late stationary phase (late maintenance phase) of growth. Microcystin quotas calculated in this study, using the microcystin concentrations, saw lower microcystin quotas than those found in the Orr and Jones (1998) with Block 3 having

the highest microcystin quota averaging 10.26 fg cell^{-1} and Block 1 had the lowest microcystin quota averaging 3.16 fg cell^{-1} (Table 16). With each block having a statistically different microcystin quota, it is possible that the test cultures could have been in different growth phases.

For each waterbody, monitoring where HABs are likely to occur and drift (wind direction) is important to ensure no cyanobacteria biomass, regardless of the microcystin quota, can enter a drinking water facility. Since there is a strong linkage of biomass and microcystins, water managers need to be aware of when cyanobacteria blooms are likely to occur (April through October for Utah) and what cyanobacteria biomass looks like (UDWQ, 2022b) in order to assess the risk of a bloom entering a treatment facility. Conventional surface water treatment is not sufficient to rid the water of accompanying toxins, especially if toxins have been transported from the cell interior into the water column. Additional treatment steps, such as activated carbon adsorption or membrane treatment, would likely be needed to rid the water of the toxins.

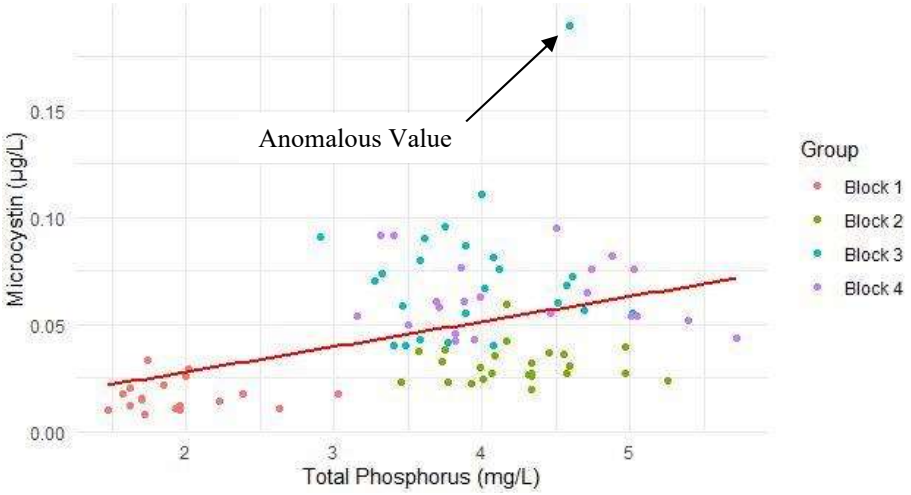
Total Phosphorus.

Phosphorus is a nutrient used for growth, photosynthesis, and is a key ingredient in ATP which is for transferring and storing energy in cells. Studies have shown correlation between total phosphorus and the production of microcystins (H.M. Oh et al., 2000; Rinta-Kanto et al., 2009). This correlation is applicable to the total phosphorus data obtained in this study. Block 1 averaged the lowest total phosphorus levels (2.01 ± 0.17 mg/L) and averaged the lowest microcystin concentration (0.017 ± 0.0035 µg/L). Microcystin concentrations were plotted against their corresponding

total phosphorus concentrations from the different test cultures to see if this held true across all the blocks (Figure 23). There was a positive correlation between total phosphorus concentrations and microcystin concentrations across all blocks, with a positive Pearson's correlation coefficient of 0.42. The *r* increases slightly to 0.43 when the anomalous microcystin value of 0.1894 µg/L was not included.

Figure 23

*Total Phosphorus vs Microcystin Concentration*s



Total phosphorus levels were higher than expected in the test cultures. These higher levels of total phosphorus concentrations in the test cultures could be due to cyanobacteria cells not being washed with distilled water after centrifugation. It is possible some dissolved nutrient carryover occurred from the stock culture into the test cultures. Another explanation could be cyanobacteria's capability to uptake phosphorus in excess to account for the variability of available phosphorus (Solovchenko et al., 2020). The medium used to cultivate the cyanobacteria contains phosphorus; the cyanobacteria could have taken up all the available phosphorus from the medium and stored it within the cells. These are two possible explanations for why total phosphorus was higher than what is seen Utah waterbodies.

Total Molar N:P Ratio and Total Nitrogen.

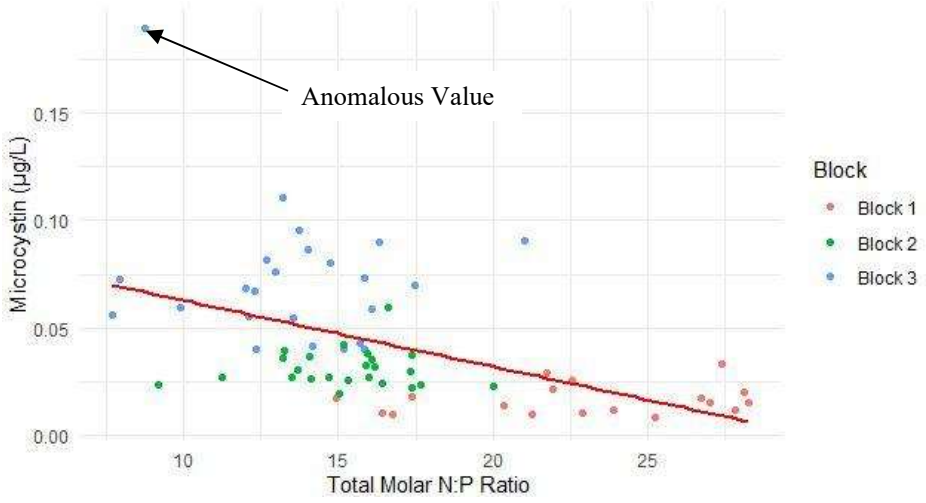
Total molar N:P ratios have also been correlated with microcystin production. An observational study compiled data across 10 years of cyanobacteria blooms in Canadian lakes reporting maximum concentrations of microcystins when the total molar N:P ratio was less than 23:1 (Orihel et al., 2012). This is supported by Paerl and Fulton (2006) from the literature review who found that *Microcystis* was more likely to dominate when the molar N:P ratio is less than 15:1.

Even though the total molar N:P ratio was not a response variable in this project, total nitrogen and phosphorus were measured at time 0 as an estimate of the amount of total nutrients in the test cultures. It was assumed that there was no significant increase or decrease in phosphorus and nitrogen concentrations in the test cultures from day 0 till day 4. The total molar N:P ratio in the test cultures decreased from 22.38 ± 1.8 to

13.56 ± 1.3 from Blocks 1 to 3 coinciding with the increase in microcystin concentration and microcystin quotas supporting previous studies (Figure 24). Total nitrogen values from Block 4 were excluded from this analysis because they did not pass quality control parameters. The calculated *r* in Figure 24 is -0.50 and without including the anomalous microcystin value, the *r* is -0.47.

Figure 24

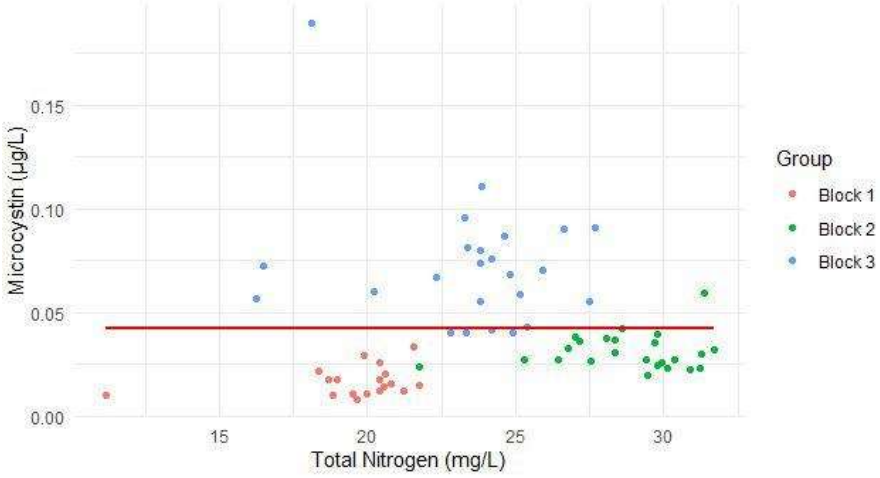
The Total Molar N:P ratio vs Microcystin Concentration (µg/L)



High total nitrogen levels have been positively correlated with microcystin concentrations at a national scale (Yuan & Pollard, 2017), but this is not supported in the results from this experiment. Total nitrogen and microcystin concentration showed no correlation with a *r* value of -0.003 (Figure 25).

Figure 25

Total Nitrogen vs. Microcystin Concentration

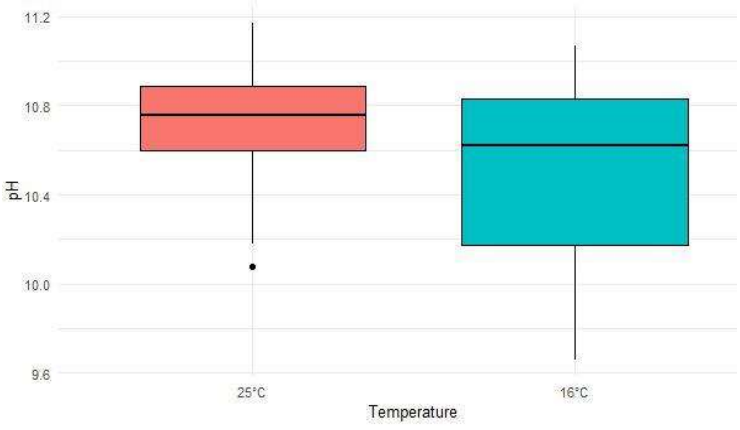


Water Temperature and pH.

The pH in the test cultures on Day 4 averaged 10.73 and 10.5 in the 25ºC and 16ºC rooms (Figure 26) with variance seen between blocks (Figure 18). Assuming a starting pH of 8.79 across the test cultures, the pH of the test cultures increased by approximately 2 standard units. Measurements for pH were taken near the end of the light period during which the uptake of CO_2 is occurring causing the higher pH (Figure 26). Measurements for pH during the dark period were not taken so it is unclear how much, or if, the pH dropped during respiration. The pH of the test cultures was statistically different one from another (Figure D.15) with the 25ºC room cultures averaging higher than the test cultures in the 16ºC room on day 4 (Figure 26).

Figure 26

Box and Whisker Plot Comparing pH and Water Temperature



Differences in pH values between 25ºC and 16ºC could be because the solubility of CO_2 is greater at lower temperatures and the slowed microbial activity. Growth rates and photosynthetic activity of cyanobacteria are higher at 25ºC than 16ºC. Decreasing the water temperature from ~25ºC to ~16ºC could have slowed growth but did not have an impact of the production of microcystins in the test cultures.

The added dissolved phosphorus concentrations of 0.015 mg P/L and 0.085 mg P/L seemed to have a significant impact on pH levels as well. The test cultures with the addition of 0.015 mg P/L were statistically higher on average (10.73) than the test cultures with the addition of 0.085 mg P/L (10.57) (Figure D.16). Unlike the addition of different dissolved phosphorus concentrations, the added dissolved molar N:P ratios of 4:1 and 25:1 did not see a significant difference in pH (Figure D.17).

Summary and Conclusions

This research was undertaken to better understand how nutrients and water temperature effect the production of toxins in HABs. Water taken from Pineview, a northern Utah reservoir, was inoculated with an active cyanobacteria culture, predominantly *Microcystis*, and exposed to varying environmental conditions in a 2^3 factorial experiment in four blocks, each replicated three times. After incubation for four days, microcystin concentrations were determined along with measurements of pH and water temperature. Total nitrogen and phosphorus samples were taken at the start of the experiment and measured. Data was compiled and analyzed in R using analysis

of variance on the transformed microcystin concentrations to determine significance of the experimental factors and their interactions.

Low added dissolved phosphorus did not play a significant factor in the production of microcystins but increasing total phosphorus concentrations did positively correlate with an increase in microcystin concentrations (Figure 23). The dissolved N:P ratio did not show any significant change in microcystin production although the total N:P ratio negatively correlated with microcystin concentrations. UDWQ has focused on reducing nutrient loading into Utah waterbodies to prevent eutrophication. This strategy of reducing total nutrients concentrations should decrease potential biomass in waterbodies overtime decreasing the potential for HABs.

Decreasing water temperatures in the test cultures found that a cold stress response did not increase toxin production as was expected. Even though there was no significant increase in the production of microcystins, the literature supports that decreasing temperature could cause cells to release toxins within the cells into the water. Further research on this is needed to understand why this occurs.

Cyanobacteria biomass has been positively correlated with microcystin production but it is not the case for all HABs. Microcystin quotas are a way of measuring the toxicity of a bloom and vary depending on the environmental conditions. Each HAB is unique and should be treated as if it was toxic until further analysis can be done.

Recommendations

UDWQ has focused on reducing phosphorus loading into Utah reservoirs to prevent eutrophication. This reduction in total phosphorus will decrease potential biomass in waterbodies possibly decreasing the potential for HABs from occurring. This study saw a positive correlation between total phosphorus and microcystin concentration, so reducing phosphorus inputs could aid in reducing the production of toxins. Further monitoring of total phosphorus could help predict when toxin production increases or decreases.

Decreasing nitrogen inputs is important to decrease the growth of cyanobacteria and not select for a non-nitrogen fixing species of cyanobacteria. Each waterbody has its own environment which could promote various aquatic organism. More environmental factors should be considered, other than the ones set forth in the thesis, to determine an acceptable amount of nutrient loading to minimize cyanobacteria growth and situations where toxin production can increase in Pineview Reservoir.

Even though a decrease in water temperature from 25ºC to 16ºC saw no increase microcystin production there is evidence in the literature that external toxin levels increase at lower temperature. More research into this is needed, but it is recommended that water temperatures are monitored.

For future research, it is important to test stock culture growth for what phase of growth the cyanobacteria could possibly be in. Testing for microcystin quotas and doing regular cell counting can aid in understanding where the stock culture is in regards to its growth curve. Future studies studying the impact of lower nutrient levels

on the production of toxins should consider the levels of total nutrients present because, if in high enough concentrations, test cultures could not be under nutrient stress conditions. To reduce the total nutrient concentration carried over from the stock culture, washing the cells in distilled water would reduce the total nutrient inputs.

Engineering Significance

As populations increase, the need for clean water and agriculture production will also increase. The increase in agriculture production can lead to increased nutrient loading into waterbodies causing the eutrophication of current and future drinking water and recreational resources. Understanding the role nutrients, such as phosphorus and nitrogen, and water temperature have on cyanotoxin production is important to predict and prevent HABs from occurring in the future. Understanding which environmental factors cause the production of toxins aids in predicting and preventing exposure.

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APPENDICIES

Appendix A. Composition of Z8 Medium for Cyanobacteria Culturing

$MgSO_4 \cdot 7H_2O$	0.25 g
$NaNO_3$	0.467 g
$Ca(NO_3)_2 \cdot 4H_2O$	59 mg
NH_4Cl	31 mg
Na_2CO_3	0.02 g
FeEDTA solution	10 mL
Gaffron micronutrients	1.0 mL
Deionized water to	1.0 L

FeEDTA solution:
Made in two solutions:
Solution A - 2.8 g $FeCl_3$ in 100 mL 0.1 N HCl
Solution B - 3.9 g $EDTANa_2$ in 100 mL 0.1 N NaOH
Add 10 mL solution A and 9.5 mL solution B plus water to 1 L.

Gaffron micronutrients:

H_3BO_3	3.1 g
$MnSO_4 \cdot 4H_2O$	2.23 g
$ZnSO_4 \cdot 7H_2O$	0.22 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.088 g
$Co(NO_3)_2 \cdot 6H_2O$	0.146 g
$VOSO_4 \cdot 6H_2O$	0.054 g
$Al_2(SO_4)_3K_2SO_4 \cdot 2H_2O$	0.474 g
$NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$	0.198 g
$Cd(NO_3)_2 \cdot 4H_2O$	0.154 g
$Cr(NO_3)_3 \cdot 7H_2O$	0.037 g
$Na_2WO_4 \cdot 2H_2O$	0.033 g
KBr	0.119 g
KI	0.083 g
Deionized water to	1 L

Appendix B. Growth Kinetics

The growth kinetics of *Microcystis* are given here and used to estimate substrate uptake, growth rates, and possible cyanotoxin production from the culture. Growth kinetics are dependent on temperature, light intensities, and nutrient concentrations, and cyanobacteria species. Growth rate across 32 different *Microcystis* species varied from 0.13 to 0.46 day^{-1} with an average of 0.27 day^{-1} (Wilson et al., 2006). Another paper found a similar growth rate as the first paper for *Microcystis* species at 0.27 day^{-1} with a doubling time of 2.8 days at a light intensity of 30 to 60 μmole/m^2sec. The same paper estimated half saturation constant (K_s) is 10.7 μg/L. (Ghaffar et al., 2017).

The substrate to biomass conversion in *Microcystis aeruginosa* is needed to calculate the uptake of substrate converting into biomass. The maximum biomass yield is the conversion of biomass to substrate. One paper found the substrate to biomass conversion in *Microcystis aeruginosa* to be between 0.017 and 0.042 gram PO_4 per gram of biomass with an average of 0.0295 (Palabhanvi et al., 2014). Taking the reciprocal of the average value gives the biomass yield ($Y_{x/s}$) for *Microcystis aeruginosa* giving a value of 33.90-gram biomass per gram of PO_4. The production of microcystins per biomass ($Y_{p/x}$) is 0.24 ng microcystin per μg biomass. This value can be used to calculate the total amount of microcystins produced per liter (Wilson et al., 2006).

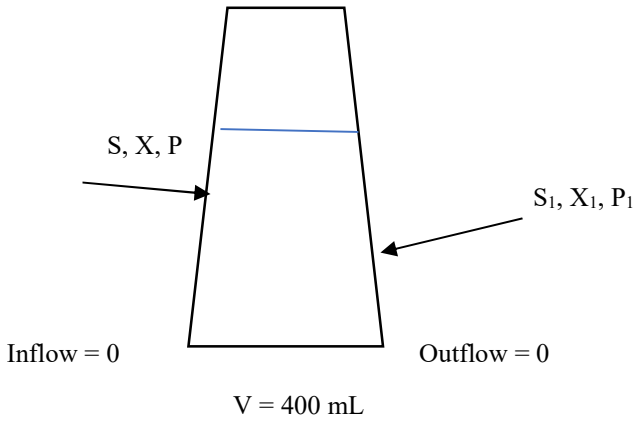
To help predict the levels of microcystins likely to be observed in the experiments, growth kinetics for microcystis are used from the literature review

section to analyze for the total production of microcystins. An initial biomass of 100,000 cells per mL in 400 mL.

Microcystis aeruginosa has a dry weight of $2.24*10^{-11}$ g/cell (Hu, 2014). The temperature of the water in the reactors are 25ºC with a light intensity of 50 μmole/m^2sec. Figure B.1 is a process flow diagram of the batch reactor in the experiment with the flows and inputs into the reactor. The blue line across the reactor represents the water line in the reactor. Initial substrate, phosphorus concentration, is represented as a S. Initial Biomass, cell concentration, is represented with an X. Initial product, microcystin concentration, is represented by a P. The S_1, X_1, and P_1 represent the substrate, biomass and product concentration in the batch reactor at a given time interval.

Figure B.1

Process flow diagram for the batch reactor in experiment



The first equation represents a mass balance on the batch reactor system in Figure B.1 written out. The following equation represents the mass balance in terms of V, X, the accumulation term, $\frac{dx}{dt}$, and the reaction term represented by the rate constant (k).

$$Accumulation = In - out \pm reaction$$

$$\frac{dx}{dt}V = kX^1V$$

Further simplifying the previous equation yields the equation below.

$$\frac{dx}{dt} = kX$$

The previous equation can then be further simplified by plugging the first equation below into the equation on the previous line which in turn yields the equation below equaling μ_g.

$$k = \mu_g = \frac{\mu_m * S}{K_s + S}$$

$$\frac{dx}{dt} = \mu_g = \frac{\mu_m * S}{K_s + S}$$

Values for μ_m and K_s are previously given as 0.27 day^{-1} and 10.7 µg/L. Using the literature values and the equation equaling μ_g, biomass on day 1 and day 2 can be calculated according to the amount of substrate left. The specific growth rate decreases as substrate decreases in the reactor. The number of cells per mL is converted to microgram of cells using the dry weight of a single microcystis cells ($2.24*10^{-11}$ g/cell). Calculating substrate consumption is done by using another equation written below

where initial substrate and biomass are represented by S_o and X_o. The biomass yield ($Y_{x/s}$) used is 33.90-gram biomass per gram PO_4.

$$S = S_o - \frac{X - X_o}{Y_{\frac{x}{s}}}$$

Table B.1

Substrate uptake and biomass accumulation along with the specific growth rate.

Time (days)	Substrate (µg/L)	Cells/mL	µg cells/L (X)	Specific Growth Rate
0	15	100000	2240	0.16
1	3.7	117068	2622	0.07
2	0.0	125515	2812	0.00

The rate of product formation and can be calculated using the $Y_{p/x}$ value of 0.24 ng microcystins per µg cells found in literature review. The cyanobacteria according to Table B.1 will have a biomass concentration of 2,812 µg cells per liter after 2 days with no substrate available for uptake and growth. Multiplying $Y_{p/x}$ by the biomass concentration will give a microcystin concentration on day 2. The amount of microcystins in the cells on day 2 is estimated to be 675 ng microcystins per liter.

Appendix C. Data

Block 1.

Microcystins.

Figure C.1

Microcystin results (Block 1) from ELISA instrument with blank spike (BLKSPK0.5) and Pineview water control spike (Con13SPK0.5) of 0.5 µg/L included

Assay Information

Assay Name: MICROCYSTINS ADDA
Version: 2
Temperature: Room Temperature
Last Modified By: Security disabled
Units: µg/L
Assay Description: PN 520011
Assay Substances: Controls:
MCT LRB (0.000-0.300)
MCT QCS (0.5625-0.9375)
Standards:
MCT Std 0, Concentration = 0.000, Minimum number to use: 2
MCT Std 1, Concentration = 0.150, Minimum number to use: 2
MCT Std 2, Concentration = 0.400, Minimum number to use: 2
MCT Std 3, Concentration = 1.000, Minimum number to use: 2
MCT Std 4, Concentration = 2.000, Minimum number to use: 2
MCT Std 5, Concentration = 5.000, Minimum number to use: 2
Curve valid interval: 1 days 0 hours
Axis Mode: Y = Abs, X = Log(Conc)

Assay Mode: 4-Parameter Logistic Weight by:None
Well Type: Flat bottom
Last Modified On: 7/25/2019 1:53:38 PM
Normal: 0.300 - 5.000
# of decimals: 3
Kit Lot Number: P23C0589

Assay Calibration Current Calibration Status: " "

Name	Absorbance	Concentration	Interpretation	Position
7/3/2023 6:36:25 PM				
MCT Std 0	1.048 Abs		R^2=0.99418, 102.444 %Abs	RK1:23->A01@2
MCT Std 0	0.997 Abs [1.0225] {3.5 CV}		R^2=0.99418, 97.458 %Abs	RK1:23->B01@2
MCT Std 1	0.785 Abs		R^2=0.99418, 76.735 %Abs	RK1:24->C01@2
MCT Std 1	0.802 Abs [0.7935] {1.5 CV}		R^2=0.99418, 78.397 %Abs	RK1:24->D01@2
MCT Std 2	0.613 Abs		R^2=0.99418, 59.922 %Abs	RK1:25->E01@2
MCT Std 2	0.560 Abs [0.5865] {6.4 CV}		R^2=0.99418, 54.741 %Abs	RK1:25->F01@3
MCT Std 3	0.401 Abs		R^2=0.99418, 39.198 %Abs	RK1:26->G01@3
MCT Std 3	0.348 Abs [0.3745] {10.0 CV		R^2=0.99418, 34.018 %Abs	RK1:26->H01@3
MCT Std 4	0.353 Abs		R^2=0.99418, 34.506 %Abs	RK1:27->A02@2
MCT Std 4	0.318 Abs [0.3355] {7.4 CV}		R^2=0.99418, 31.085 %Abs	RK1:27->B02@2
MCT Std 5	0.194 Abs		18.964 %Abs	RK1:28->C02@2
MCT Std 5	0.190 Abs [0.1920] {1.5 CV}		18.573 %Abs	RK1:28->D02@2
++++++++++++++++++++++++++++++++	++++++++++++++++++++		++++++++++++++++++++	++++++++++++++++
7/3/2023 6:36:25 PM				
MCT LRB (0.000-0.300)	0.938 Abs		91.691 %Abs	RK1:10->E02@2
MCT LRB (0.000-0.300)	0.939 Abs [0.9385] {0.1 CV}		91.789 %Abs [91.740 %Abs]	RK1:10->F02@3
MCT QCS (0.5625-0.9375)	0.523 Abs		51.124 %Abs	RK1:29->G02@3
MCT QCS (0.5625-0.9375)	0.491 Abs [0.5070] {4.5 CV}		47.996 %Abs [49.560 %Abs]	RK1:29->H02@3
++++++++++++++++++++++++++++++++	++++++++++++++++++++	++++++++++++++++++++	++++++++++++++++++++	++++++++++++++++

Statistic				
MCT Std 0 [MEAN]	1.0225			
MCT Std 0 [SD]	0.0361			
MCT Std 0 [%CV]	3.5269			
MCT Std 1 [MEAN]	0.7935			
MCT Std 1 [SD]	0.0120			
MCT Std 1 [%CV]	1.5149			
MCT Std 1 [%DIFF]				
MCT Std 2 [MEAN]	0.5865			
MCT Std 2 [SD]	0.0375			
MCT Std 2 [%CV]	6.3899			
MCT Std 2 [%DIFF]				
MCT Std 3 [MEAN]	0.3745			
MCT Std 3 [SD]	0.0375			
MCT Std 3 [%CV]	10.0071			
MCT Std 3 [%DIFF]				
MCT Std 4 [MEAN]	0.3355			
MCT Std 4 [SD]	0.0247			
MCT Std 4 [%CV]	7.3767			
MCT Std 4 [%DIFF]				

*Generated by software version (6.4.1.1065/1019/1.00/0.95) 7/3/2023 6:48:18 PM

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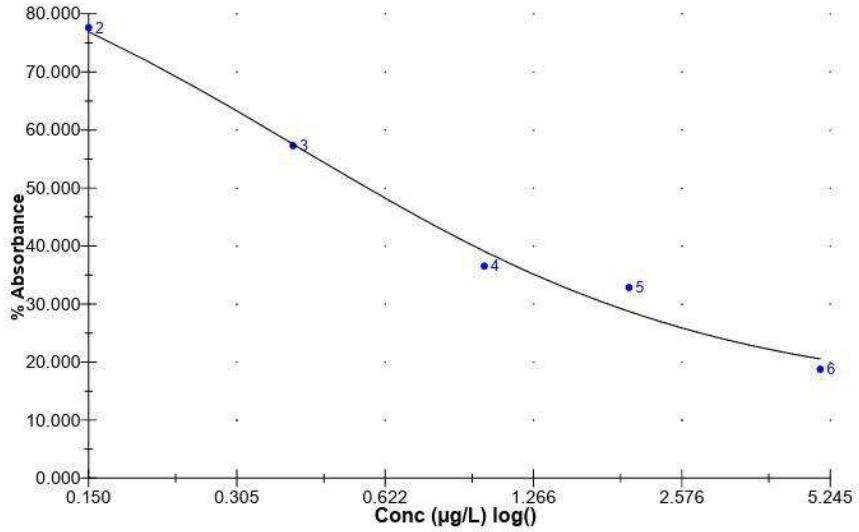
MICROCYSTINS ADDA - Assay Calibration Report

Name	Absorbance	Concentration	Interpretation	Position
MCT Std 5 [MEAN]	0.1920			
MCT Std 5 [SD]	0.0028			
MCT Std 5 [%CV]	1.4731			
MCT LRB (0.000-0.300) [MEAN]	0.9385			
MCT LRB (0.000-0.300) [SD]	0.0007			
MCT LRB (0.000-0.300) [%CV]	0.0753			
MCT QCS (0.5625-0.9375) [MEAN]	0.5070			
MCT QCS (0.5625-0.9375) [SD]	0.0226			
MCT QCS (0.5625-0.9375) [%CV]	4.4630			

Assay Curve

y = (A-D)/(1+(x/C)^B) + D
Weight: NONE
A = 1.0250
B = 0.99093
C = 0.40975
D = 0.14200
R2 coef = 0.99418
50% = 0.571



Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
A11	MICROCYSTINS ADDA	0.766 Abs	0.169 µg/L	LOW, 74.878 %ABS	0.300 - 5.000	P23C0589
A11	MICROCYSTINS ADDA	0.727 Abs [0.7465] (3.7 CV)	0.207 µg/L [0.188] [1	LOW, 71.065 %ABS	0.300 - 5.000	P23C0589
D11	MICROCYSTINS ADDA	0.644 Abs	0.310 µg/L	52.952 %Abs	0.300 - 5.000	P23C0589
D11	MICROCYSTINS ADDA	0.600 Abs [0.6220] (5.0 CV)	0.380 µg/L [0.345] [1	58.651 %Abs [60.802	0.300 - 5.000	P23C0589
E11	MICROCYSTINS ADDA	0.788 Abs	0.149 µg/L	LOW, 77.028 %ABS	0.300 - 5.000	P23C0589
E11	MICROCYSTINS ADDA	0.775 Abs [0.7815] (1.2 CV)	0.160 µg/L [0.155] [5	LOW, 75.758 %ABS	0.300 - 5.000	P23C0589
F11	MICROCYSTINS ADDA	0.612 Abs	0.380 µg/L	59.824 %Abs	0.300 - 5.000	P23C0589
F11	MICROCYSTINS ADDA	0.553 Abs [0.5825] (7.2 CV)	0.471 µg/L [0.415] [1	54.057 %Abs [56.940	0.300 - 5.000	P23C0589
G11	MICROCYSTINS ADDA	0.706 Abs	0.232 µg/L	LOW, 68.915 %ABS	0.300 - 5.000	P23C0589
G11	MICROCYSTINS ADDA	0.718 Abs [0.7115] (1.3 CV)	0.217 µg/L [0.225] [4	LOW, 70.186 %ABS	0.300 - 5.000	P23C0589
H11	MICROCYSTINS ADDA	0.783 Abs	0.153 µg/L	LOW, 76.540 %ABS	0.300 - 5.000	P23C0589
H11	MICROCYSTINS ADDA	0.768 Abs [0.7755] (1.4 CV)	0.167 µg/L [0.160] [5	LOW, 75.073 %ABS	0.300 - 5.000	P23C0589
A12	MICROCYSTINS ADDA	0.625 Abs	0.339 µg/L	51.095 %Abs	0.300 - 5.000	P23C0589
A12	MICROCYSTINS ADDA	0.501 Abs [0.5630] (15.6 CV)	0.600 µg/L [0.470] [3	48.974 %Abs [55.034	0.300 - 5.000	P23C0589
B12	MICROCYSTINS ADDA	0.507 Abs	0.583 µg/L	49.560 %Abs	0.300 - 5.000	P23C0589
B12	MICROCYSTINS ADDA	0.550 Abs [0.5285] (5.8 CV)	0.478 µg/L [0.530] [1	53.763 %Abs [51.662	0.300 - 5.000	P23C0589
CON13SPK0.5	MICROCYSTINS ADDA	0.655 Abs	0.295 µg/L	LOW, 64.027 %ABS	0.300 - 5.000	P23C0589
CON13SPK0.5	MICROCYSTINS ADDA	0.599 Abs [0.6270] (6.3 CV)	0.382 µg/L [0.338] [1	58.553 %Abs [61.200	0.300 - 5.000	P23C0589
BLK2	MICROCYSTINS ADDA	0.999 Abs	0.012 µg/L	LOW, 97.654 %ABS	0.300 - 5.000	P23C0589
BLK2	MICROCYSTINS ADDA	1.009 Abs [1.0040] (0.7 CV)	0.007 µg/L [0.010] [5	LOW, 98.631 %ABS	0.300 - 5.000	P23C0589
C12	MICROCYSTINS ADDA	0.758 Abs	0.176 µg/L	LOW, 74.096 %ABS	0.300 - 5.000	P23C0589
C12	MICROCYSTINS ADDA	0.779 Abs [0.7685] (1.9 CV)	0.157 µg/L [0.167] [9	LOW, 76.149 %ABS	0.300 - 5.000	P23C0589
B13	MICROCYSTINS ADDA	0.689 Abs	0.251 µg/L	LOW, 67.351 %ABS	0.300 - 5.000	P23C0589
B13	MICROCYSTINS ADDA	0.646 Abs [0.6875] (4.8 CV)	0.307 µg/L [0.279] [1	63.148 %ABS [LOW	0.300 - 5.000	P23C0589
CON13	MICROCYSTINS ADDA	0.967 Abs	0.028 µg/L	LOW, 94.528 %ABS	0.300 - 5.000	P23C0589
CON13	MICROCYSTINS ADDA	1.007 Abs [0.9870] (2.9 CV)	0.008 µg/L [0.018] [7	LOW, 98.436 %ABS	0.300 - 5.000	P23C0589
CON12	MICROCYSTINS ADDA	0.187 Abs	> 5.000 µg/L	18.280 %Abs, Out(LR	0.300 - 5.000	P23C0589
CON12	MICROCYSTINS ADDA	0.124 Abs [0.1555] (28.6 CV)	> 5.000 µg/L	12.121 %Abs, Out(LR	0.300 - 5.000	P23C0589
A13	MICROCYSTINS ADDA	0.676 Abs	0.267 µg/L	LOW, 66.080 %ABS	0.300 - 5.000	P23C0589
A13	MICROCYSTINS ADDA	0.649 Abs [0.6625] (2.9 CV)	0.303 µg/L [0.285] [5	63.441 %ABS [LOW	0.300 - 5.000	P23C0589
C13	MICROCYSTINS ADDA	0.775 Abs	0.160 µg/L	LOW, 75.758 %ABS	0.300 - 5.000	P23C0589
C13	MICROCYSTINS ADDA	0.763 Abs [0.7690] (1.1 CV)	0.172 µg/L [0.166] [5	LOW, 74.585 %ABS	0.300 - 5.000	P23C0589
D13	MICROCYSTINS ADDA	0.683 Abs	0.264 µg/L	LOW, 64.809 %ABS	0.300 - 5.000	P23C0589
D13	MICROCYSTINS ADDA	0.685 Abs [0.6840] (0.2 CV)	0.261 µg/L [0.262] [5	LOW, 65.005 %ABS	0.300 - 5.000	P23C0589
D12	MICROCYSTINS ADDA	0.696 Abs	0.243 µg/L	LOW, 67.937 %ABS	0.300 - 5.000	P23C0589
D12	MICROCYSTINS ADDA	0.697 Abs [0.6960] (0.2 CV)	0.241 µg/L [0.242] [5	LOW, 68.133 %ABS	0.300 - 5.000	P23C0589
G13 Deleted	MICROCYSTINS ADDA	0.909 Abs	0.081 µg/L	LOW, 88.856 %ABS	0.300 - 5.000	P23C0589
G13 Deleted	MICROCYSTINS ADDA	0.866 Abs [0.8875] (3.4 CV)	0.089 µg/L [0.075] [2	LOW, 84.653 %ABS	0.300 - 5.000	P23C0589
BLKSPK0.5	MICROCYSTINS ADDA	0.678 Abs	0.267 µg/L	LOW, 66.080 %ABS	0.300 - 5.000	P23C0589
BLKSPK0.5	MICROCYSTINS ADDA	0.585 Abs [0.6305] (10.2 CV)	0.407 µg/L [0.337] [2	57.185 %Abs [61.632	0.300 - 5.000	P23C0589
H13 Deleted	MICROCYSTINS ADDA	0.857 Abs	0.095 µg/L	LOW, 83.773 %ABS	0.300 - 5.000	P23C0589
H13 Deleted	MICROCYSTINS ADDA	0.838 Abs [0.8475] (1.6 CV)	0.109 µg/L [0.102] [5	LOW, 81.916 %ABS	0.300 - 5.000	P23C0589
E12	MICROCYSTINS ADDA	0.643 Abs	0.312 µg/L	62.854 %Abs	0.300 - 5.000	P23C0589
E12	MICROCYSTINS ADDA	0.627 Abs [0.6350] (1.8 CV)	0.336 µg/L [0.324] [5	61.290 %Abs [62.072	0.300 - 5.000	P23C0589
E13	MICROCYSTINS ADDA	0.724 Abs	0.211 µg/L	LOW, 70.772 %ABS	0.300 - 5.000	P23C0589
E13	MICROCYSTINS ADDA	0.772 Abs [0.7480] (4.5 CV)	0.163 µg/L [0.187] [1	LOW, 75.464 %ABS	0.300 - 5.000	P23C0589
F12	MICROCYSTINS ADDA	0.831 Abs	0.114 µg/L	LOW, 81.232 %ABS	0.300 - 5.000	P23C0589
F12	MICROCYSTINS ADDA	0.791 Abs [0.8110] (3.5 CV)	0.146 µg/L [0.130] [1	LOW, 77.322 %ABS	0.300 - 5.000	P23C0589
CON11	MICROCYSTINS ADDA	1.162 Abs	0.000 µg/L	LOW, 113.587 %ABS	0.300 - 5.000	P23C0589
CON11	MICROCYSTINS ADDA	1.026 Abs [1.0940] (9.8 CV)	0.000 µg/L [0.000]	LOW, 100.293 %ABS	0.300 - 5.000	P23C0589
H12	MICROCYSTINS ADDA	0.730 Abs	0.204 µg/L	LOW, 71.359 %ABS	0.300 - 5.000	P23C0589
H12	MICROCYSTINS ADDA	0.659 Abs [0.6945] (7.2 CV)	0.289 µg/L [0.247] [2	LOW, 64.418 %ABS	0.300 - 5.000	P23C0589

*A - Abs > 3 , IA - Initial Abs; DA - Delta Abs; SD - SD of Abs; LR - Linear Range; [ ] - Mean result of duplicate tests
*Generated by software version (5.6.1.1065*101SP1.00G.90) 7/3/2023 6:47:38 PM

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Test Report (by Request)

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
BL3	MICROCYSTINS ADDA	1.009 Abs	0.007 µg/L	LOW, 98.631 %ABS	0.300 - 5.000	P23C0589
BL3	MICROCYSTINS ADDA	0.994 Abs [1.0015] (1.1 CV)	0.014 µg/L [0.011] [4	LOW, 97.165 %ABS	0.300 - 5.000	P23C0589

Table C.1

Microcystin results accounting for concentrating the sample from 80 mL to 5 mL.

Sample ID	Microcystin
A11	0.0118
A12	0.0294
A13	0.0178
B11	NA
B12	0.0331
B13	0.0174
C11	NA
C12	0.0104
C13	0.0104
D11	0.0216
D12	0.0151
D13	0.0176
E11	0.0097
E12	0.0203
E13	0.0117
F11	0.0259
F12	0.0081
F13	NA
G11	0.0141
G12	NA
G13	NA
H11	0.0100
H12	0.0154
H13	NA

Total Phosphorus.

Figure C.2

Standard curve for total phosphorus analysis (Block 1)

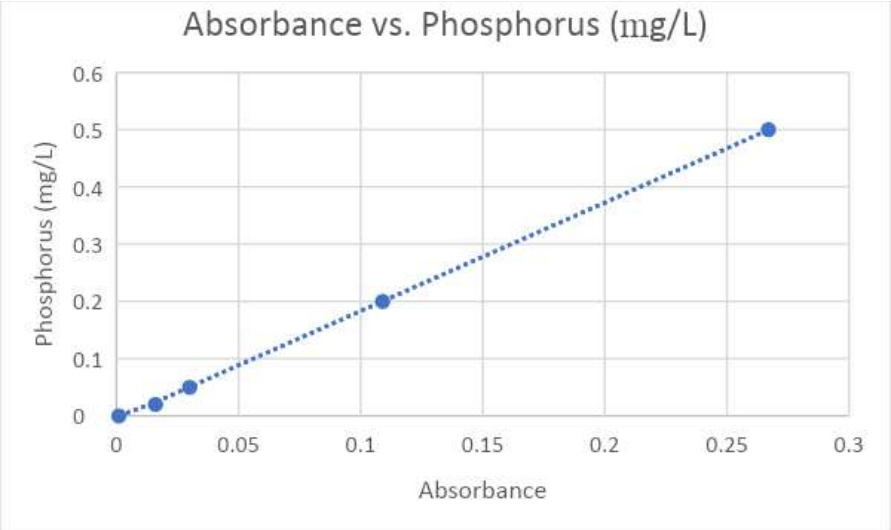


Table C.2

Calculated total phosphorus concentration for each test culture (Block 1). Samples were diluted 1:10 before analysis.

Sample ID	Absorbance	Calculated value (mg/L)
A11	0.107	1.96
A12	0.101	1.85
A13	0.081	1.47
B11	0.109	2.00
B12	0.121	2.23
B13	0.107	1.96
C11	0.11	2.02
C12	0.095	1.74
C13	0.105	1.93
D11	0.163	3.03
D12	0.129	2.38
D13	0.142	2.63
E11	0.086	1.57
E12	0.093	1.70
E13	0.089	1.62
F11	0.089	1.62
F12	0.094	1.72
F13	0.093	1.70
G11	0.103	1.89
G12	0.094	1.72
G13	0.101	1.85
H11	0.136	2.51
H12	0.134	2.48
H13	0.139	2.57

Table C3

Total phosphorus quality control parameters and Pineview water (Con 11, Con12, Con13) (Block 1)

Sample ID	Absorbance	Calculated value (µg/L)	% Recovery
15P60N	0.022	35.4	235.9
15P340N	0.021	33.5	223.3
85P375N	0.057	101.7	119.7
85P2N	0.08	145.3	170.9
CON11	0.004	1.3	/
CON12	0.008	8.9	/
CON13	0.01	12.6	/
blkspk(500)	0.248	463.6	92.7
CCV (500)	0.246	459.8	92.0
Blank			

Total Nitrogen.

Figure C.3

Total nitrogen results (Block 1) from AQ2

AQ2 Tray Report

SEAL Analytical

Software Version:	2.1.4
Report Requested By:	uwrllab
Date & Time:	2023-06-30 17:57:52
Tray Number:	71
Tray Name:	firstA11Brent

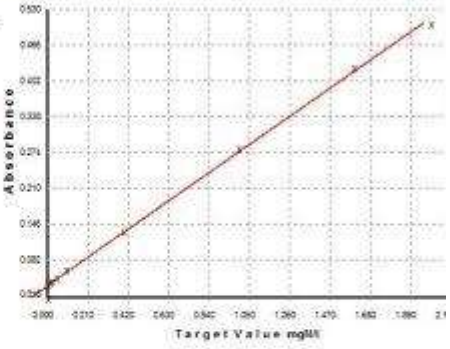
NO3+NO2

Calibration Chart

Type	Absorbance	Calc mgN/l	Target mgN/l	% Error
S1	0.0183	-0.089	0.000	
S90	0.0415	0.008	0.000	
S91	0.0452	0.024	0.012	98.98
S92	0.0474	0.033	0.020	65.43
S93	0.0546	0.063	0.052	21.75
S94	0.0679	0.119	0.100	19.26
S95	0.1388	0.409	0.400	2.35
S96	0.2635	1.027	1.000	2.71
S97	0.4268	1.630	1.800	1.90
S98	0.5048	1.959	2.000	-2.06
S0	0.0434	0.016	0.000	

Polynomial Order:	1
Correlation Coefficient:	0.9988
Carryover(%):	5.1
Calibration equation:	y = bx + a
y =:	Concentration mgN/l
x =:	Measured absorbance
a =:	-1.665426E-001
b =:	4.210314E+000
Date & Time:	2023-06-30 15:31:44

Calibration Graph



Reagents

Name	Batch	Prepared By	Expiry Date
NO3 working buf		uwrllab	
NO3 SULF-NED		uwrllab	
Digested Blanks (Blank)		uwrllab	

blank spike should be 1 mg/L

Test Results

Cup	Type	ID	Result	Units	QC Pro	Raw Data	Auto Dil.	Man Dil.	User	Time/Date
	S1	Standard 1	0.018			0.018338			uw	2023-06-30 15:14:06
	S90	Standard 0	0.041			0.041478			uw	2023-06-30 15:15:52
	S91	Standard 0.01	0.045			0.045227			uw	2023-06-30 15:17:37
	S92	Standard 0.02	0.047			0.047414			uw	2023-06-30 15:19:23
	S93	Standard 0.05	0.055			0.054593			uw	2023-06-30 15:21:09
	S94	Standard 0.1	0.068			0.067881			uw	2023-06-30 15:22:54
	S95	Standard 0.4	0.137			0.136795			uw	2023-06-30 15:24:40
	S96	Standard 1.0	0.284			0.283508			uw	2023-06-30 15:26:26
	S97	Standard 1.8	0.427			0.426801			uw	2023-06-30 15:28:11
	S98	Standard 2.0	0.505			0.504787			uw	2023-06-30 15:29:57
	S0	Standard 0	0.043			0.043378			uw	2023-06-30 15:31:43
4	U1	NO2	1.331	mgN/l		0.355647			uw	2023-06-30 15:33:30
5	U2	NO3	1.032	mgN/l		0.284624			uw	2023-06-30 15:35:16
6	U3	A11	21.205ELH	mgN/l		0.543202	x10.0000		uw	2023-06-30 16:55:08
6	U3	A11	10.951	mgN/l		2.840629			uw	2023-06-30 15:37:02
7	U4	C13	19.993ELH	mgN/l		0.514422	x10.0000		uw	2023-06-30 16:56:57

7	U4	C13	10.932	mgN\l	2.836034		uw	2023-06-30 15:38:48
8	U5	F12	19.648ELH	mgN\l	0.506208	x10.0000	uw	2023-06-30 15:58:48
8	U5	F12	10.932	mgN\l	2.836034		uw	2023-06-30 15:40:35
9	U6	CON11	0.472	mgN\l	0.151689		uw	2023-06-30 15:42:21
10	U7	A12	18.368	mgN\l	0.475771	x10.0000	uw	2023-06-30 17:00:35
10	U7	A12	10.875	mgN\l	2.822531		uw	2023-06-30 15:44:08
11	U8	D11	20.428ELH	mgN\l	0.524736	x10.0000	uw	2023-06-30 17:02:24
11	U8	D11	10.913	mgN\l	2.831486		uw	2023-06-30 15:45:55
12	U9	F13	20.780ELH	mgN\l	0.533095	x10.0000	uw	2023-06-30 17:04:14
12	U9	F13	10.894	mgN\l	2.828986		uw	2023-06-30 15:47:42
13	U10	CON12	0.555	mgN\l	0.171325		uw	2023-06-30 15:49:28
14	U11	A13	11.150	mgN\l	0.304383	x10.0000	uw	2023-06-30 17:06:03
14	U11	A13	10.894	mgN\l	2.828986		uw	2023-06-30 15:51:15
15	U12	D12	18.708	mgN\l	0.483842	x10.0000	uw	2023-06-30 17:07:53
15	U12	D12	10.913	mgN\l	2.831486		uw	2023-06-30 15:53:02
16	CCV	CCV	1.076	mgN\l	0.295110		uw	2023-06-30 15:54:48
17	U13	Blank	0.000	mgN\l	0.039509		uw	2023-06-30 15:56:34
18	U14	CON13	0.520	mgN\l	0.163108		uw	2023-06-30 15:58:20
19	U15	B11	20.411ELH	mgN\l	0.524347	x10.0000	uw	2023-06-30 17:09:42
19	U15	B11	10.051	mgN\l	2.840629		uw	2023-06-30 16:00:06
20	U16	D13	19.521	mgN\l	0.503201	x10.0000	uw	2023-06-30 17:11:32
20	U16	D13	10.894	mgN\l	2.828986		uw	2023-06-30 16:01:52
21	U17	15P80N	0.122	mgN\l	0.068574		uw	2023-06-30 16:03:38
22	U18	G12	20.630ELH	mgN\l	0.529539	x10.0000	uw	2023-06-30 17:13:21
22	U18	G12	10.913	mgN\l	2.831486		uw	2023-06-30 16:05:24
23	U19	G11	19.778ELH	mgN\l	0.509304	x10.0000	uw	2023-06-30 17:15:11
23	U19	G11	10.894	mgN\l	2.828986		uw	2023-06-30 16:07:10
24	U20	15P340N	0.421	mgN\l	0.139585		uw	2023-06-30 16:08:56
25	U21	G13	19.824ELH	mgN\l	0.510398	x10.0000	uw	2023-06-30 17:17:00
25	U21	G13	10.894	mgN\l	2.828986		uw	2023-06-30 16:10:41
26	U22	B12	20.532ELH	mgN\l	0.527219	x10.0000	uw	2023-06-30 17:18:50
26	U22	B12	10.913	mgN\l	2.831486		uw	2023-06-30 16:12:27
27	U23	E11	18.968	mgN\l	0.490069	x10.0000	uw	2023-06-30 17:20:39
27	U23	E11	10.894	mgN\l	2.828986		uw	2023-06-30 16:14:12
28	U24	Blank	0.028	mgN\l	0.046237		uw	2023-06-30 16:15:58
29	U25	Blank Spike	77.614ELH	mgN\l	1.862975	x10.0000	uw	2023-06-30 17:22:29
29	U25	Blank Spike	10.971	mgN\l	2.845274		uw	2023-06-30 16:17:44
30	U26	B13	18.841	mgN\l	0.487042	x10.0000	uw	2023-06-30 17:24:18
30	U26	B13	10.894	mgN\l	2.828986		uw	2023-06-30 16:19:29
31	U27	85P375N	0.477	mgN\l	0.152889		uw	2023-06-30 16:21:15
32	U28	H11	17.978	mgN\l	0.466545	x10.0000	uw	2023-06-30 17:25:15
32	U28	H11	10.857	mgN\l	2.818122		uw	2023-06-30 16:23:00
33	U29	E12	21.738ELH	mgN\l	0.555863	x10.0000	uw	2023-06-30 17:26:08
33	U29	E12	10.932	mgN\l	2.836034		uw	2023-06-30 16:24:45
34	U30	85P2.125N	2.428	mgN\l	0.097235	x10.0000	uw	2023-06-30 17:27:01
34	U30	85P2.125N	2.370	mgN\l	0.802483		uw	2023-06-30 16:25:42
35	U31	C11	19.859ELH	mgN\l	0.511221	x10.0000	uw	2023-06-30 17:27:54
35	U31	C11	10.894	mgN\l	2.828986		uw	2023-06-30 16:26:35
36	U32	E13	20.601ELH	mgN\l	0.528859	x10.0000	uw	2023-06-30 17:28:47
36	U32	E13	10.932	mgN\l	2.836034		uw	2023-06-30 16:27:29
37	U33	C12	21.553ELH	mgN\l	0.551478	x10.0000	uw	2023-06-30 17:29:41
37	U33	C12	10.894	mgN\l	2.828986		uw	2023-06-30 16:28:22
38	U34	H12	21.143ELH	mgN\l	0.541728	x10.0000	uw	2023-06-30 17:30:34
38	U34	H12	10.913	mgN\l	2.831486		uw	2023-06-30 16:29:15
39	CCV	CCV	1.071	mgN\l	0.293902		uw	2023-06-30 16:30:08
40	U35	Blank Spike	96.550ELH	mgN\l	2.332724	x10.0000	uw	2023-06-30 17:31:27
40	U35	Blank Spike	10.051	mgN\l	2.840629		uw	2023-06-30 16:31:02
41	U36	F11	20.402ELH	mgN\l	0.524135	x10.0000	uw	2023-06-30 17:32:20
41	U36	F11	10.894	mgN\l	2.828986		uw	2023-06-30 16:31:55
42	U37	H13	21.104ELH	mgN\l	0.540809	x10.0000	uw	2023-06-30 17:33:13
42	U37	H13	10.913	mgN\l	2.831486		uw	2023-06-30 16:32:48
43	U38	Blank	0.047	mgN\l	0.050609		uw	2023-06-30 16:33:41

PAR, Water Temperature, and pH.

Table C.4

Results for PAR, pH, and water temperature from Block 1 including Pineview water (Con11, Con12, Con13)

Sample ID	PAR (µmol*m^2*sec^{-1}) (Day 0 and Day 4)	pH	Water Temperature (°C)
A11	51	10.63	25.8
A12	47	10.76	25.8
A13	50	10.93	25.8
B11	50	10.79	25.8
B12	47	10.59	25.8
B13	49	10.6	25.8
C11	50	10.68	25.8
C12	47	10.75	25.8
C13	49	10.8	25.8
D11	51	10.38	25.8
D12	50	10.55	25.8
D13	48	10.63	25.8
E11	50	10.67	18
E12	47	10.97	18
E13	49	10.91	18
F11	48	10.76	18
F12	47	10.73	18
F13	51	10.91	18
G11	47	10.82	18
G12	47	10.65	18
G13	51	10.86	18
H11	49	10.22	18
H12	47	10.28	18
H13	48	10.44	18
CON11	50	8.76	25.8
CON12	49	8.74	25.8
CON13	49	8.74	25.8

Block 2.

Microcystins.

Figure C.4

Microcystin results (Block 2) from ELISA with blank spike (BLKSPK0.5) and Pineview water control spike (Con11SPK0.5) of 0.5 µg/L included

Assay Information

Assay Name: MICROCYSTINS ADDA
Version: 2
Temperature: Room Temperature
Last Modified By: Security disabled
Units: µg/L
Assay Description: PN 520011
Assay Substances: Controls:
MCT LRB (0.000-0.300)
MCT QCS (0.5625-0.9375)
Standards:
MCT Std 0, Concentration = 0.000, Minimum number to use: 2
MCT Std 1, Concentration = 0.150, Minimum number to use: 2
MCT Std 2, Concentration = 0.400, Minimum number to use: 2
MCT Std 3, Concentration = 1.000, Minimum number to use: 2
MCT Std 4, Concentration = 2.000, Minimum number to use: 2
MCT Std 5, Concentration = 5.000, Minimum number to use: 2
Curve valid interval: 1 days 0 hours
Axis Mode: Y = Abs, X = Log(Conc)

Assay Mode: 4-Parameter Logistic Weight by:None
Well Type: Flat bottom
Last Modified On: 7/25/2019 1:53:38 PM
Normal: 0.300 - 5.000
# of decimals: 3
Kit Lot Number: P23F1409

Assay Calibration Current Calibration Status: " "

Name	Absorbance	Concentration	Interpretation	Position
7/15/2023 4:37:09 PM				
MCT Std 0	1.381 Abs		R^2=0.99782, 100.803 %Abs	RK1:23->A01@2
MCT Std 0	1.359 Abs [1.3700] {1.1 CV}		R^2=0.99782, 99.197 %Abs	RK1:23->B01@2
MCT Std 1	1.166 Abs		R^2=0.99782, 85.109 %Abs	RK1:24->C01@2
MCT Std 1	1.178 Abs [1.1720] {0.7 CV}		R^2=0.99782, 85.985 %Abs	RK1:24->D01@2
MCT Std 2	0.890 Abs		R^2=0.99782, 64.964 %Abs	RK1:25->E01@2
MCT Std 2	0.831 Abs [0.8605] {4.8 CV}		R^2=0.99782, 60.657 %Abs	RK1:25->F01@3
MCT Std 3	0.568 Abs		R^2=0.99782, 41.460 %Abs	RK1:26->G01@3
MCT Std 3	0.553 Abs [0.5605] {1.9 CV}		R^2=0.99782, 40.365 %Abs	RK1:26->H01@3
MCT Std 4	0.450 Abs		R^2=0.99782, 32.847 %Abs	RK1:27->A02@2
MCT Std 4	0.454 Abs [0.4520] {0.6 CV}		R^2=0.99782, 33.139 %Abs	RK1:27->B02@2
MCT Std 5	0.296 Abs		21.606 %Abs	RK1:28->C02@2
MCT Std 5	0.304 Abs [0.3000] {1.9 CV}		22.190 %Abs	RK1:28->D02@2
7/15/2023 4:37:09 PM				
MCT LRB (0.000-0.300)	1.308 Abs		95.474 %Abs	RK1:10->E02@2
MCT LRB (0.000-0.300)	1.314 Abs [1.3110] {0.3 CV}		95.912 %Abs [95.693 %Abs]	RK1:10->F02@3
MCT QCS (0.5625-0.9375)	0.702 Abs		51.241 %Abs	RK1:29->G02@3
MCT QCS (0.5625-0.9375)	0.633 Abs [0.6675] {7.3 CV}		46.204 %Abs [48.723 %Abs]	RK1:29->H02@3

Statistic				
MCT Std 0 [MEAN]	1.3700			
MCT Std 0 [SD]	0.0156			
MCT Std 0 [%CV]	1.1355			
MCT Std 1 [MEAN]	1.1720			
MCT Std 1 [SD]	0.0085			
MCT Std 1 [%CV]	0.7240			
MCT Std 1 [%DIFF]				
MCT Std 2 [MEAN]	0.8605			
MCT Std 2 [SD]	0.0417			
MCT Std 2 [%CV]	4.8483			
MCT Std 2 [%DIFF]				
MCT Std 3 [MEAN]	0.5605			
MCT Std 3 [SD]	0.0106			
MCT Std 3 [%CV]	1.8924			
MCT Std 3 [%DIFF]				
MCT Std 4 [MEAN]	0.4520			
MCT Std 4 [SD]	0.0028			
MCT Std 4 [%CV]	0.6258			
MCT Std 4 [%DIFF]				

*Generated by software version (6.4.1.1065/1019/1.00/0.95) 7/15/2023 4:43:19 PM

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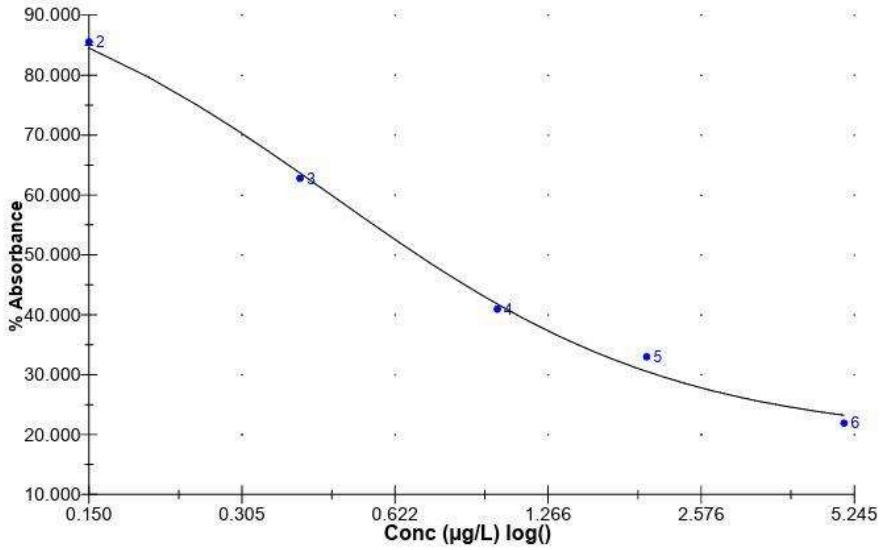
MICROCYSTINS ADDA - Assay Calibration Report

Name	Absorbance	Concentration	Interpretation	Position
MCT Std 5 [MEAN]	0.3000			
MCT Std 5 [SD]	0.0057			
MCT Std 5 [%CV]	1.8856			
MCT LRB (0.000-0.300) [MEAN]	1.3110			
MCT LRB (0.000-0.300) [SD]	0.0042			
MCT LRB (0.000-0.300) [%CV]	0.3236			
MCT QCS (0.5625-0.9375) [MEAN]	0.6675			
MCT QCS (0.5625-0.9375) [SD]	0.0488			
MCT QCS (0.5625-0.9375) [%CV]	7.3094			

Assay Curve

y = (A-D)/(1+(x/C)^B) + D
Weight: NONE
A = 1.3751
B = 1.2509
C = 0.46532
D = 0.26433
R2 coef = 0.99782
50% = 0.691



Test Information

Request: 7/15/2023 4:37:09 PM
Date: 7/15/2023

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
MCT Std 0	MICROCYSTINS ADDA	1.381 Abs	0.000 µg/L	R^2=0.99782, 100.80		P23F1409
MCT Std 0	MICROCYSTINS ADDA	1.359 Abs [1.3700] {1.1 CV}	0.016 µg/L [0.008] {1	R^2=0.99782, 99.197		P23F1409
MCT Std 1	MICROCYSTINS ADDA	1.166 Abs	0.145 µg/L	R^2=0.99782, 85.109		P23F1409
MCT Std 1	MICROCYSTINS ADDA	1.178 Abs [1.1720] {0.7 CV}	0.137 µg/L [0.141] {4	R^2=0.99782, 85.985		P23F1409
MCT Std 2	MICROCYSTINS ADDA	0.890 Abs	0.380 µg/L	R^2=0.99782, 64.964		P23F1409
MCT Std 2	MICROCYSTINS ADDA	0.831 Abs [0.8605] {4.8 CV}	0.450 µg/L [0.415] {1	R^2=0.99782, 60.657		P23F1409
MCT Std 3	MICROCYSTINS ADDA	0.568 Abs	1.016 µg/L	R^2=0.99782, 41.460		P23F1409
MCT Std 3	MICROCYSTINS ADDA	0.553 Abs [0.5605] {1.9 CV}	1.074 µg/L [1.045] {3	R^2=0.99782, 40.365		P23F1409
MCT Std 4	MICROCYSTINS ADDA	0.450 Abs	1.680 µg/L	R^2=0.99782, 32.847		P23F1409
MCT Std 4	MICROCYSTINS ADDA	0.454 Abs [0.4520] {0.6 CV}	1.646 µg/L [1.663] {1	R^2=0.99782, 33.139		P23F1409
MCT Std 5	MICROCYSTINS ADDA	0.296 Abs	> 5.000 µg/L	21.606 %Abs		P23F1409
MCT Std 5	MICROCYSTINS ADDA	0.304 Abs [0.3000] {1.9 CV}	> 5.000 µg/L	22.190 %Abs		P23F1409
MCT LRB (0.000-0.300)	MICROCYSTINS ADDA	1.308 Abs	0.052 µg/L	95.474 %Abs		P23F1409
MCT LRB (0.000-0.300)	MICROCYSTINS ADDA	1.314 Abs [1.3110] {0.3 CV}	0.048 µg/L [0.050] {9	95.912 %Abs [95.693		P23F1409
MCT QCS (0.5625-0.9375)	MICROCYSTINS ADDA	0.702 Abs	0.656 µg/L	51.241 %Abs		P23F1409
MCT QCS (0.5625-0.9375)	MICROCYSTINS ADDA	0.633 Abs [0.6675] {7.3 CV}	0.814 µg/L [0.735] {1	46.204 %Abs [48.723		P23F1409

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
blnk	MICROCYSTINS ADDA	1.471 Abs	0.000 µg/L	**LOW, 107.372 %ABS**	0.300 - 5.000	P23F1409
blnk	MICROCYSTINS ADDA	1.426 Abs [1.4485] {2.2 CV}	0.000 µg/L [0.000]	**LOW, 104.088 %ABS**	0.300 - 5.000	P23F1409
A21	MICROCYSTINS ADDA	0.860 Abs	0.414 µg/L	62.774 %Abs	0.300 - 5.000	P23F1409
A21	MICROCYSTINS ADDA	0.838 Abs [0.8490] {1.8 CV}	0.441 µg/L [0.428] {4	61.168 %Abs [61.971	0.300 - 5.000	P23F1409
B21	MICROCYSTINS ADDA	0.903 Abs	0.365 µg/L	65.912 %Abs	0.300 - 5.000	P23F1409
B21	MICROCYSTINS ADDA	0.890 Abs [0.8965] {1.0 CV}	0.380 µg/L [0.373] {2	64.964 %Abs [65.438	0.300 - 5.000	P23F1409
C21	MICROCYSTINS ADDA	0.749 Abs	0.571 µg/L	54.672 %Abs	0.300 - 5.000	P23F1409
C21	MICROCYSTINS ADDA	0.747 Abs [0.7480] {0.2 CV}	0.574 µg/L [0.572] {0	54.526 %Abs [54.599	0.300 - 5.000	P23F1409
D21	MICROCYSTINS ADDA	0.751 Abs	0.568 µg/L	54.818 %Abs	0.300 - 5.000	P23F1409
D21	MICROCYSTINS ADDA	0.708 Abs [0.7295] {4.2 CV}	0.645 µg/L [0.607] {9	51.679 %Abs [53.248	0.300 - 5.000	P23F1409
E21	MICROCYSTINS ADDA	0.777 Abs	0.526 µg/L	56.715 %Abs	0.300 - 5.000	P23F1409
E21	MICROCYSTINS ADDA	0.697 Abs [0.7370] {7.7 CV}	0.666 µg/L [0.596] {1	50.876 %Abs [53.796	0.300 - 5.000	P23F1409
F21	MICROCYSTINS ADDA	0.771 Abs	0.536 µg/L	56.277 %Abs	0.300 - 5.000	P23F1409
F21	MICROCYSTINS ADDA	0.785 Abs [0.7780] {1.3 CV}	0.514 µg/L [0.525] {3	57.299 %Abs [56.788	0.300 - 5.000	P23F1409
G21	MICROCYSTINS ADDA	0.729 Abs	0.606 µg/L	53.212 %Abs	0.300 - 5.000	P23F1409
G21	MICROCYSTINS ADDA	0.773 Abs [0.7510] {4.1 CV}	0.532 µg/L [0.569] {9	56.423 %Abs [54.818	0.300 - 5.000	P23F1409
H21	MICROCYSTINS ADDA	0.811 Abs	0.477 µg/L	59.197 %Abs	0.300 - 5.000	P23F1409
H21	MICROCYSTINS ADDA	0.800 Abs [0.8055] {1.0 CV}	0.492 µg/L [0.484] {2	58.394 %Abs [58.796	0.300 - 5.000	P23F1409
A22	MICROCYSTINS ADDA	0.908 Abs	0.360 µg/L	66.277 %Abs	0.300 - 5.000	P23F1409
A22	MICROCYSTINS ADDA	0.821 Abs [0.8645] {7.1 CV}	0.464 µg/L [0.412] {1	59.927 %Abs [63.102	0.300 - 5.000	P23F1409
B22	MICROCYSTINS ADDA	0.938 Abs	0.329 µg/L	68.467 %Abs	0.300 - 5.000	P23F1409
B22	MICROCYSTINS ADDA	0.889 Abs [0.9135] {3.8 CV}	0.381 µg/L [0.355] {1	64.891 %Abs [66.679	0.300 - 5.000	P23F1409
BLKSPK0.5	MICROCYSTINS ADDA	0.932 Abs	0.335 µg/L	68.029 %Abs	0.300 - 5.000	P23F1409
BLKSPK0.5	MICROCYSTINS ADDA	0.849 Abs [0.8905] {6.6 CV}	0.428 µg/L [0.382] {1	61.971 %Abs [65.000	0.300 - 5.000	P23F1409
C22	MICROCYSTINS ADDA	0.837 Abs	0.443 µg/L	61.095 %Abs	0.300 - 5.000	P23F1409
C22	MICROCYSTINS ADDA	0.743 Abs [0.7900] {8.4 CV}	0.581 µg/L [0.512] {1	54.234 %Abs [57.664	0.300 - 5.000	P23F1409
D22	MICROCYSTINS ADDA	0.929 Abs	0.338 µg/L	67.810 %Abs	0.300 - 5.000	P23F1409
D22	MICROCYSTINS ADDA	0.875 Abs [0.9020] {4.2 CV}	0.397 µg/L [0.368] {1	63.869 %Abs [65.839	0.300 - 5.000	P23F1409
E22	MICROCYSTINS ADDA	0.702 Abs	0.656 µg/L	51.241 %Abs	0.300 - 5.000	P23F1409
E22	MICROCYSTINS ADDA	0.685 Abs [0.6935] {1.7 CV}	0.691 µg/L [0.674] {3	50.000 %Abs [50.620	0.300 - 5.000	P23F1409
F22	MICROCYSTINS ADDA	0.870 Abs	0.402 µg/L	63.504 %Abs	0.300 - 5.000	P23F1409
F22	MICROCYSTINS ADDA	0.846 Abs [0.8580] {2.0 CV}	0.431 µg/L [0.417] {4	61.752 %Abs [62.628	0.300 - 5.000	P23F1409
G22	MICROCYSTINS ADDA	0.895 Abs	0.374 µg/L	65.328 %Abs	0.300 - 5.000	P23F1409
G22	MICROCYSTINS ADDA	0.807 Abs [0.8510] {7.3 CV}	0.483 µg/L [0.428] {1	58.905 %Abs [62.117	0.300 - 5.000	P23F1409
H22	MICROCYSTINS ADDA	0.956 Abs	0.312 µg/L	69.781 %Abs	0.300 - 5.000	P23F1409
H22	MICROCYSTINS ADDA	0.965 Abs [0.9605] {0.7 CV}	0.303 µg/L [0.308] {2	70.438 %Abs [70.109	0.300 - 5.000	P23F1409
A23	MICROCYSTINS ADDA	0.834 Abs	0.447 µg/L	60.876 %Abs	0.300 - 5.000	P23F1409
A23	MICROCYSTINS ADDA	0.858 Abs [0.8460] {2.0 CV}	0.417 µg/L [0.432] {4	62.628 %Abs [61.752	0.300 - 5.000	P23F1409

B23	MICROCYSTINS ADDA	0.883 Abs	0.387 µg/L	64.453 %Abs	0.300 - 5.000	P23F1409
B23	MICROCYSTINS ADDA	0.897 Abs [0.8900] {1.1 CV}	0.372 µg/L [0.380] {2	65.474 %Abs [64.964	0.300 - 5.000	P23F1409
C23	MICROCYSTINS ADDA	0.794 Abs	0.501 µg/L	57.956 %Abs	0.300 - 5.000	P23F1409
C23	MICROCYSTINS ADDA	0.694 Abs [0.7440] {9.5 CV}	0.672 µg/L [0.586] {2	50.657 %Abs [54.307	0.300 - 5.000	P23F1409
D23	MICROCYSTINS ADDA	0.867 Abs	0.406 µg/L	63.285 %Abs	0.300 - 5.000	P23F1409
D23	MICROCYSTINS ADDA	0.818 Abs [0.8425] {4.1 CV}	0.468 µg/L [0.437] {1	59.708 %Abs [61.496	0.300 - 5.000	P23F1409
CON11SPK0.5	MICROCYSTINS ADDA	0.922 Abs	0.345 µg/L	67.299 %Abs	0.300 - 5.000	P23F1409
CON11SPK0.5	MICROCYSTINS ADDA	0.879 Abs [0.9005] {3.4 CV}	0.392 µg/L [0.368] {9	64.161 %Abs [65.730	0.300 - 5.000	P23F1409
E23	MICROCYSTINS ADDA	0.828 Abs	0.454 µg/L	60.438 %Abs	0.300 - 5.000	P23F1409
E23	MICROCYSTINS ADDA	0.793 Abs [0.8105] {3.1 CV}	0.503 µg/L [0.479] {7	57.883 %Abs [59.161	0.300 - 5.000	P23F1409
F23	MICROCYSTINS ADDA	0.609 Abs	0.881 µg/L	44.453 %Abs	0.300 - 5.000	P23F1409
F23	MICROCYSTINS ADDA	0.568 Abs [0.5885] {4.9 CV}	1.016 µg/L [0.949] {1	41.460 %Abs [42.956	0.300 - 5.000	P23F1409
G23	MICROCYSTINS ADDA	0.768 Abs	0.540 µg/L	56.058 %Abs	0.300 - 5.000	P23F1409
G23	MICROCYSTINS ADDA	0.673 Abs [0.7205] {9.3 CV}	0.717 µg/L [0.628] {1	49.124 %Abs [52.597	0.300 - 5.000	P23F1409

* A - Abs > 3; IA - Initial Abs; DA - Delta Abs; SD - SD of Abs; LR - Linear Range; [...] - Mean result of duplicate tests
* Generated by software version (6.4.1.1065/1019/1.00/0.95) 7/15/2023 4:47:08 PM

eurofins | Abraxis

Test Report (by Request)

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
H23	MICROCYSTINS ADDA	0.910 Abs	0.358 µg/L	66.423 %Abs	0.300 - 5.000	P23F1409
H23	MICROCYSTINS ADDA	0.862 Abs [0.8860] {3.8 CV}	0.412 µg/L [0.385] {9	62.920 %Abs [64.672	0.300 - 5.000	P23F1409
CON23	MICROCYSTINS ADDA	1.429 Abs	0.000 µg/L	**LOW, 104.307 %ABS**	0.300 - 5.000	P23F1409
CON23	MICROCYSTINS ADDA	1.400 Abs [1.4145] {1.4 CV}	0.000 µg/L [0.000]	**LOW, 102.190 %ABS**	0.300 - 5.000	P23F1409
CON22	MICROCYSTINS ADDA	1.412 Abs	0.000 µg/L	**LOW, 103.066 %ABS**	0.300 - 5.000	P23F1409
CON22	MICROCYSTINS ADDA	1.328 Abs [1.3700] {4.3 CV}	0.038 µg/L [0.019] {1	**LOW, 96.934 %ABS**	0.300 - 5.000	P23F1409
CON11	MICROCYSTINS ADDA	1.508 Abs	0.000 µg/L	**LOW, 110.073 %ABS**	0.300 - 5.000	P23F1409
CON11	MICROCYSTINS ADDA	1.426 Abs [1.4670] {4.0 CV}	0.000 µg/L [0.000]	**LOW, 104.088 %ABS**	0.300 - 5.000	P23F1409
BLK	MICROCYSTINS ADDA	1.460 Abs	0.000 µg/L	**LOW, 106.569 %ABS**	0.300 - 5.000	P23F1409
BLK	MICROCYSTINS ADDA	1.459 Abs [1.4595] {0.0 CV}	0.000 µg/L [0.000]	**LOW, 106.496 %ABS**	0.300 - 5.000	P23F1409

Table C.5

Microcystin results accounting for concentrating the sample from 80 mL to 5 mL.

Sample ID	Microcystin
A21	0.0268
A22	0.0258
A23	0.0270
B21	0.0233
B22	0.0222
B23	0.0238
C21	0.0358
C22	0.0320
C23	0.0366
D21	0.0379
D22	0.0230
D23	0.0273
E21	0.0373
E22	0.0421
E23	0.0299
F21	0.0328
F22	0.0261
F23	0.0593
G21	0.0356
G22	0.0268
G23	0.0393
H21	0.0303
H22	0.0193
H23	0.0241

Total Phosphorus.

Figure C.5

Standard curve for total phosphorus analysis from Block 4 used to calculate the total phosphorus concentrations in Block 2

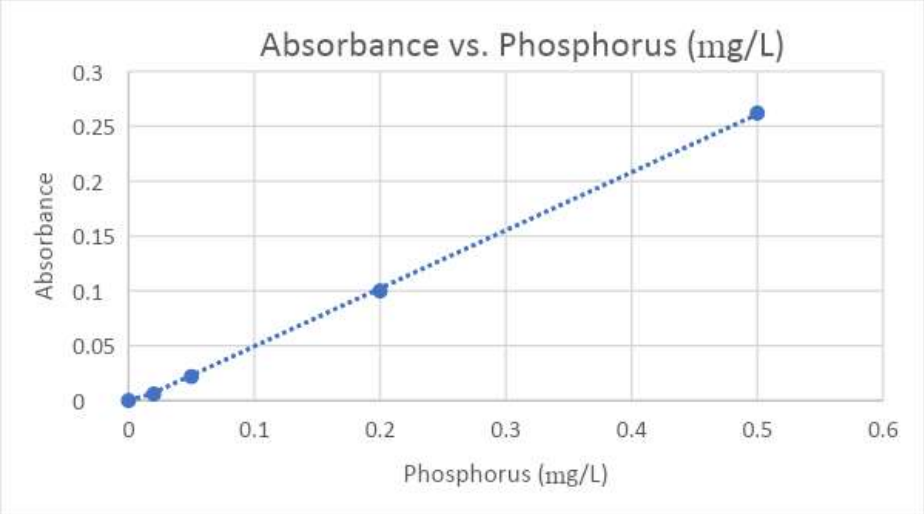


Table C.6

Calculated total phosphorus concentration for each test culture (Block 2)

Sample ID	Absorbance	Calculated value (mg/L)
A21	0.245	4.97
A22	0.185	3.77
A23	0.224	4.55
B21	0.184	3.75
B22	0.175	3.57
B23	0.183	3.73
C21	0.201	4.09
C22	0.226	4.59
C23	0.213	4.33
D21	0.193	3.93
D22	0.213	4.33
D23	0.169	3.45
E21	0.205	4.17
E22	0.212	4.31
E23	0.213	4.33
F21	0.213	4.33
F22	0.225	4.57
F23	0.259	5.25
G21	0.219	4.45
G22	0.2	4.07
G23	0.196	3.99
H21	0.205	4.17
H22	0.245	4.97
H23	0.197	4.01

Note. Samples were diluted 1:10 before analysis.

Table C.7

Total phosphorus quality control parameters and Pineview water (Con21, Con22, Con23) (Block 2)

Sample ID	Absorbance	Concentration (ug/L)	% Recovery
15P60N	0.002	10.8	72.0
15P340N	0.006	18.8	125.3
85P375N	0.035	76.8	90.4
85P2.125N	0.025	56.8	66.8
CON21	0.012	30.8	/
CON22	0.007	20.8	/
CON23	0.007	20.8	/
Con22Spk(200)	0.08	166.8	75.5
CCV (500)	0.194	394.80	79.0
Blank	0.000	0	

Total Nitrogen.

Figure C.6

Total nitrogen results (Block 2) from AQ2. Note the ID for the samples should have a 2 instead of a 1 on the first number after the letter

AQ2 Tray Report

SEAL Analytical

Software Version:	2.1.4
Report Requested By:	uwrllab
Date & Time:	2023-07-12 17:30:06
Tray Number:	79
Tray Name:	block2TN15Brent

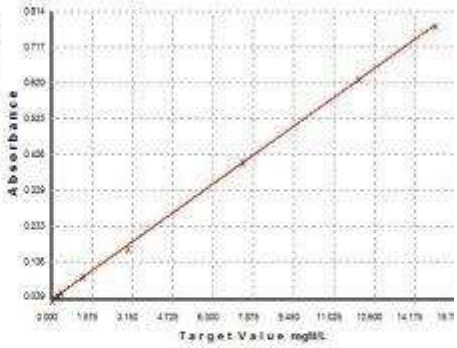
NO3_NO2 15

Calibration Chart

Type	Absorbance	Calc mgN/L	Target mgN/L	% Error
S1	0.0388	-0.017	0.000	
S90	0.0518	0.248	0.250	-0.69
S91	0.0597	0.409	0.375	9.09
S92	0.1051	1.331	1.250	6.45
S93	0.1798	2.849	3.000	-5.05
S94	0.4104	7.538	7.500	0.50
S95	0.6333	12.068	12.000	0.57
S96	0.7751	14.949	15.000	-0.34
S0	0.0381	-0.030	0.000	

Polynomial Order:	1
Correlation Coefficient:	0.9999
Carryover(%):	-0.1
Calibration equation:	y = bx + a
y =:	Concentration mgN/L
x =:	Measured absorbance
a =:	-8.052723E-001
b =:	2.032675E+001
Date & Time:	2023-07-12 15:25:40

Calibration Graph



Reagents

Name	Batch	Prepared By	Expiry Date
NO3 working buf		uwrllab	
NO3 SULF-NED		uwrllab	
Digested Blanks (Blank)		uwrllab	

CCV is supposed to be 5 mg/L and blkspk is supposed to equal 6 mg/L
Conspk was spiked with 1 mg/L

Test Results

Cup	Type	ID	Result	Units	QC Pro	Raw Data	Auto Dil.	Man Dil.	User	Time/Date
	S1	Standard 1	0.039			0.038799			uw	2023-07-12 15:11:36
	S90	Standard 90	0.052			0.051830			uw	2023-07-12 15:13:21
	S91	Standard 91	0.060			0.059742			uw	2023-07-12 15:15:06
	S92	Standard 92	0.105			0.105075			uw	2023-07-12 15:16:52
	S93	Standard 93	0.180			0.179753			uw	2023-07-12 15:18:37
	S94	Standard 94	0.410			0.410435			uw	2023-07-12 15:20:23
	S95	Standard 95	0.633			0.633328			uw	2023-07-12 15:22:08
	S96	Standard 96	0.775			0.775071			uw	2023-07-12 15:23:54
	S0	Standard 0	0.038			0.038117			uw	2023-07-12 15:25:40
4	U1	no2	-0.089ELL	mgN/L		0.035245			uw	2023-07-12 15:27:26
5	U2	no3	1.562	mgN/L		0.116473			uw	2023-07-12 15:29:12
6	U3	blank	-0.040ELL	mgN/L		0.037658			uw	2023-07-12 15:30:58
7	CCV	CCV	4.731	mgN/L		0.272386			uw	2023-07-12 15:32:44
8	U4	a11	25.267	mgN/L		0.288220	x5.0000		uw	2023-07-12 16:51:38
8	U4	a11	26.099	mgN/L		1.320850			uw	2023-07-12 15:34:30
9	U5	b11	27.001	mgN/L		0.305284	x5.0000		uw	2023-07-12 16:53:28
9	U5	b11	27.876	mgN/L		1.411017			uw	2023-07-12 15:36:17
10	U6	c11	29.699	mgN/L		0.331838	x5.0000		uw	2023-07-12 16:55:17

10	U6	c11	29.678	mg/kL	1.499681		uw	2023-07-12 15:38:03
11	U7	d11	30.893	mg/kL	0.343579	±5.0000	uw	2023-07-12 16:57:06
11	U7	d11	30.048	mg/kL	1.517750		uw	2023-07-12 15:39:49
12	U8	e11	28.585	mg/kL	0.320875	±5.0000	uw	2023-07-12 16:58:55
12	U8	e11	29.019	mg/kL	1.467229		uw	2023-07-12 15:41:38
13	U9	f11	29.470	mg/kL	0.329675	±5.0000	uw	2023-07-12 17:00:44
13	U9	f11	29.981	mg/kL	1.514568		uw	2023-07-12 15:43:22
14	U10	g11	28.350	mg/kL	0.318559	±5.0000	uw	2023-07-12 17:02:34
14	U10	g11	29.541	mg/kL	1.492926		uw	2023-07-12 15:45:09
15	U11	h11	31.356	mg/kL	0.348141	±5.0000	uw	2023-07-12 17:04:23
15	U11	h11	32.433	mg/kL	1.635184		uw	2023-07-12 15:46:55
16	U12	a12	30.098	mg/kL	0.335738	±5.0000	uw	2023-07-12 17:06:12
16	U12	a12	30.845	mg/kL	1.557089		uw	2023-07-12 15:48:41
17	U13	b12	28.089	mg/kL	0.315987	±5.0000	uw	2023-07-12 17:08:02
17	U13	b12	27.511	mg/kL	1.393077		uw	2023-07-12 15:50:27
18	U14	blank	-0.007	mg/kL	0.036282		uw	2023-07-12 15:52:13
19	CCV	CCV	4.963	mg/kL	0.283788		uw	2023-07-12 15:53:59
20	U15	blkspk	5.565	mg/kL	0.313453		uw	2023-07-12 15:55:45
21	U16	c12	28.355	mg/kL	0.318804	±5.0000	uw	2023-07-12 17:09:51
21	U16	c12	29.103	mg/kL	1.471354		uw	2023-07-12 15:57:31
22	U17	d12	31.709	mg/kL	0.351612	±5.0000	uw	2023-07-12 17:11:41
22	U17	d12	31.856	mg/kL	1.606836		uw	2023-07-12 15:59:16
23	U18	e12	27.537	mg/kL	0.310558	±5.0000	uw	2023-07-12 17:13:31
23	U18	e12	27.916	mg/kL	1.412984		uw	2023-07-12 16:01:02
24	U19	f12	30.377	mg/kL	0.338500	±5.0000	uw	2023-07-12 17:15:21
24	U19	f12	31.110	mg/kL	1.570101		uw	2023-07-12 16:02:47
25	U20	g12	29.425	mg/kL	0.329138	±5.0000	uw	2023-07-12 17:17:11
25	U20	g12	30.075	mg/kL	1.519171		uw	2023-07-12 16:04:33
26	U21	h12	29.791	mg/kL	0.332739	±5.0000	uw	2023-07-12 17:18:08
26	U21	h12	30.429	mg/kL	1.536804		uw	2023-07-12 16:06:18
27	U22	a13	27.140	mg/kL	0.306856	±5.0000	uw	2023-07-12 17:19:01
27	U22	a13	27.017	mg/kL	1.368742		uw	2023-07-12 16:08:03
28	U23	b13	26.785	mg/kL	0.303157	±5.0000	uw	2023-07-12 17:19:54
28	U23	b13	27.012	mg/kL	1.368490		uw	2023-07-12 16:09:48
29	U24	c13	29.952	mg/kL	0.334317	±5.0000	uw	2023-07-12 17:20:47
29	U24	c13	31.005	mg/kL	1.564929		uw	2023-07-12 16:11:34
30	U25	d13	31.210	mg/kL	0.346703	±5.0000	uw	2023-07-12 17:21:40
30	U25	d13	30.735	mg/kL	1.551685		uw	2023-07-12 16:13:19
31	U26	blnksppk.6mgL	5.753	mg/kL	0.322637		uw	2023-07-12 16:15:04
32	CCV	CCV	4.767	mg/kL	0.274124		uw	2023-07-12 16:16:49
33	U27	e13	26.423	mg/kL	0.299600	±5.0000	uw	2023-07-12 17:22:33
33	U27	e13	29.924	mg/kL	1.511760		uw	2023-07-12 16:18:35
34	U28	f13	21.768	mg/kL	0.253793	±5.0000	uw	2023-07-12 17:23:26
34	U28	f13	22.152	mg/kL	1.130878		uw	2023-07-12 16:20:20
35	U29	g13	31.257	mg/kL	0.347157	±5.0000	uw	2023-07-12 17:24:19
35	U29	g13	30.581	mg/kL	1.544079		uw	2023-07-12 16:22:05
36	U30	h13	29.812	mg/kL	0.332947	±5.0000	uw	2023-07-12 17:25:12
36	U30	h13	31.151	mg/kL	1.572108		uw	2023-07-12 16:23:51
37	US1	con11	0.679	mg/kL	0.073006		uw	2023-07-12 16:24:48
38	US2	con12	0.486	mg/kL	0.063540		uw	2023-07-12 16:25:41
39	US3	con13	0.293	mg/kL	0.054008		uw	2023-07-12 16:26:34
40	US4	con13spk1mgN	1.635	mg/kL	0.120051		uw	2023-07-12 16:27:27
41	U35	blank	-0.074ELL	mg/kL	0.035958		uw	2023-07-12 16:28:20
42	U36	15p60n	-0.085ELL	mg/kL	0.035456		uw	2023-07-12 16:29:13
43	U37	15p340n	0.105	mg/kL	0.044776		uw	2023-07-12 16:30:06
44	U38	85p375n	0.553	mg/kL	0.066807		uw	2023-07-12 16:30:59
45	U39	8502.125n	2.055	mg/kL	0.140714		uw	2023-07-12 16:31:52

PAR, Water Temperature, and pH.

Table C.8

Results for PAR, pH, and water temperature from Block 2 including Pineview water (Con21, Con22, Con23)

Sample ID	PAR (µmol*m^2*sec^{-1}) (Day 0 and Day 4)	pH	Water Temperature (°C)
A21	49	10.74	27.1
A22	48	10.74	27.1
A23	51	10.83	27.1
B21	49	10.84	27.1
B22	51	10.75	27.1
B23	49	10.84	27.1
C21	49	10.81	27.1
C22	51	10.76	27.1
C23	49	10.93	27.1
D21	50	10.74	27.1
D22	48	10.87	27.1
D23	51	10.85	27.1
E21	48	10.61	17.8
E22	50	10.54	17.8
E23	52	10.52	17.8
F21	47	10.43	17.8
F22	48	10.94	17.8
F23	49	10.51	17.8
G21	49	10.76	17.8
G22	50	10.59	17.8
G23	49	10.73	17.8
H21	50	10.49	17.8
H22	51	10.44	17.8
H23	47	10.28	17.8
CON21	49	8.85	27.1
CON22	51	9.11	27.1
CON23	47	9.06	27.1

Block 3.

Microcystins.

Figure C.7

Microcystin results (Block 3) from ELISA with blank spike (BLKSPK0.5) and Pineview water control spike (Con32SPK0.5) of 0.5 µg/L included

Assay Information

Assay Name: MICROCYSTINS ADDA
Version: 2
Temperature: Room Temperature
Last Modified By: Security disabled
Units: µg/L
Assay Description: PN 520011
Assay Substances: Controls:
MCT LRB (0.000-0.300)
MCT QCS (0.5625-0.9375)
Standards:
MCT Std 0, Concentration = 0.000, Minimum number to use: 2
MCT Std 1, Concentration = 0.150, Minimum number to use: 2
MCT Std 2, Concentration = 0.400, Minimum number to use: 2
MCT Std 3, Concentration = 1.000, Minimum number to use: 2
MCT Std 4, Concentration = 2.000, Minimum number to use: 2
MCT Std 5, Concentration = 5.000, Minimum number to use: 2
Curve valid interval: 1 days 0 hours
Axis Mode: Y = Abs, X = Log(Conc)

Assay Mode: 4-Parameter Logistic Weight by:None
Well Type: Flat bottom
Last Modified On: 7/25/2019 1:53:38 PM
Normal: 0.300 - 5.000
# of decimals: 3
Kit Lot Number: P23F1409

Assay Calibration Current Calibration Status: " "

Name	Absorbance	Concentration	Interpretation	Position
8/1/2023 8:12:15 PM				
MCT Std 0	1.228 Abs		R^2=0.99646, 103.454 %Abs	RK1:23->A01@2
MCT Std 0	1.146 Abs [1.1870] {4.9 CV}		R^2=0.99646, 96.546 %Abs	RK1:23->B01@2
MCT Std 1	1.019 Abs		R^2=0.99646, 85.847 %Abs	RK1:24->C01@2
MCT Std 1	0.986 Abs [1.0025] {2.3 CV}		R^2=0.99646, 83.067 %Abs	RK1:24->D01@2
MCT Std 2	0.862 Abs		R^2=0.99646, 72.620 %Abs	RK1:25->E01@2
MCT Std 2	0.821 Abs [0.8415] {3.4 CV}		R^2=0.99646, 69.166 %Abs	RK1:25->F01@3
MCT Std 3	0.570 Abs		R^2=0.99646, 48.020 %Abs	RK1:26->G01@3
MCT Std 3	0.526 Abs [0.5480] {5.7 CV}		R^2=0.99646, 44.313 %Abs	RK1:26->H01@3
MCT Std 4	0.491 Abs		R^2=0.99646, 41.365 %Abs	RK1:27->A02@2
MCT Std 4	0.385 Abs [0.4380] {17.1 CV		R^2=0.99646, 32.435 %Abs	RK1:27->B02@2
MCT Std 5	0.256 Abs		21.567 %Abs	RK1:28->C02@2
MCT Std 5	0.250 Abs [0.2530] {1.7 CV}		21.061 %Abs	RK1:28->D02@2
8/1/2023 8:12:15 PM				
MCT LRB (0.000-0.300)	1.119 Abs		94.271 %Abs	RK1:10->E02@2
MCT LRB (0.000-0.300)	1.187 Abs [1.1530] {4.2 CV}		100.000 %Abs [97.136 %Abs	RK1:10->F02@3
MCT QCS (0.5625-0.9375)	0.615 Abs		51.811 %Abs	RK1:29->G02@3
MCT QCS (0.5625-0.9375)	0.632 Abs [0.6235] {1.9 CV}		53.243 %Abs [52.527 %Abs]	RK1:29->H02@3

Statistic				
MCT Std 0 [MEAN]	1.1870			
MCT Std 0 [SD]	0.0580			
MCT Std 0 [%CV]	4.8848			
MCT Std 1 [MEAN]	1.0025			
MCT Std 1 [SD]	0.0233			
MCT Std 1 [%CV]	2.3276			
MCT Std 1 [%DIFF]				
MCT Std 2 [MEAN]	0.8415			
MCT Std 2 [SD]	0.0290			
MCT Std 2 [%CV]	3.4452			
MCT Std 2 [%DIFF]				
MCT Std 3 [MEAN]	0.5480			
MCT Std 3 [SD]	0.0311			
MCT Std 3 [%CV]	5.6775			
MCT Std 3 [%DIFF]				
MCT Std 4 [MEAN]	0.4380			
MCT Std 4 [SD]	0.0750			
MCT Std 4 [%CV]	17.1126			
MCT Std 4 [%DIFF]				

*Generated by software version (6.4.1.1065/1019/1.00/0.95) 8/1/2023 8:21:32 PM

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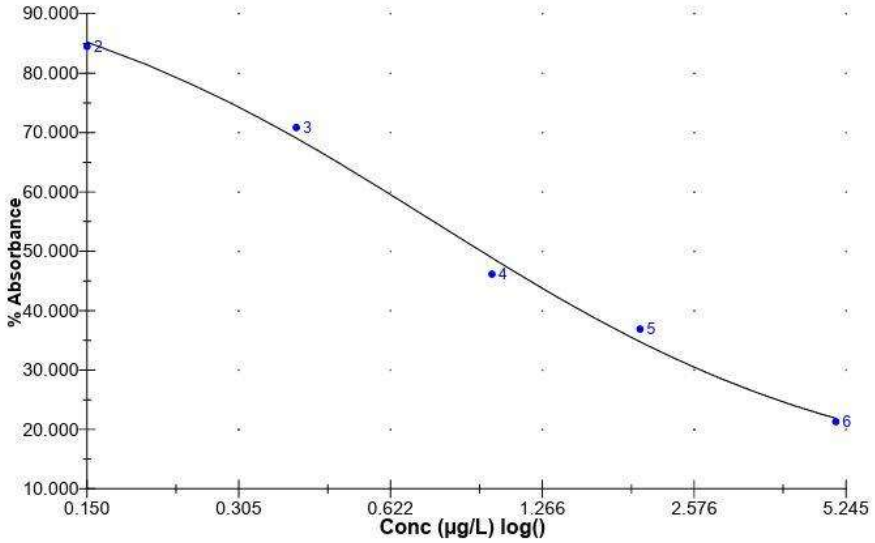
MICROCYSTINS ADDA - Assay Calibration Report

Name	Absorbance	Concentration	Interpretation	Position
MCT Std 5 [MEAN]	0.2530			
MCT Std 5 [SD]	0.0042			
MCT Std 5 [%CV]	1.6769			
MCT LRB (0.000-0.300) [MEAN]	1.1530			
MCT LRB (0.000-0.300) [SD]	0.0481			
MCT LRB (0.000-0.300) [%CV]	4.1703			
MCT QCS (0.5625-0.9375) [MEAN]	0.6235			
MCT QCS (0.5625-0.9375) [SD]	0.0120			
MCT QCS (0.5625-0.9375) [%CV]	1.9280			

Assay Curve

y = (A-D)/(1+(x/C)^B) + D
Weight: NONE
A = 1.1860
B = 1.0046
C = 0.76085
D = 0.12003
R2 coef = 0.99646
50% = 0.951



Test Information

Request: 8/1/2023 8:12:15 PM
Date: 8/1/2023

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
MCT Std 0	MICROCYSTINS ADDA	1.228 Abs	0.000 µg/L	R^2=0.99646, 103.45		P23F1409
MCT Std 0	MICROCYSTINS ADDA	1.146 Abs [1.1870] {4.9 CV}	0.030 µg/L [0.015] {1	R^2=0.99646, 96.546		P23F1409
MCT Std 1	MICROCYSTINS ADDA	1.019 Abs	0.142 µg/L	R^2=0.99646, 85.847		P23F1409
MCT Std 1	MICROCYSTINS ADDA	0.986 Abs [1.0025] {2.3 CV}	0.177 µg/L [0.160] {1	R^2=0.99646, 83.067		P23F1409
MCT Std 2	MICROCYSTINS ADDA	0.862 Abs	0.334 µg/L	R^2=0.99646, 72.620		P23F1409
MCT Std 2	MICROCYSTINS ADDA	0.821 Abs [0.8415] {3.4 CV}	0.397 µg/L [0.366] {1	R^2=0.99646, 69.166		P23F1409
MCT Std 3	MICROCYSTINS ADDA	0.570 Abs	1.040 µg/L	R^2=0.99646, 48.020		P23F1409
MCT Std 3	MICROCYSTINS ADDA	0.526 Abs [0.5480] {5.7 CV}	1.234 µg/L [1.137] {1	R^2=0.99646, 44.313		P23F1409
MCT Std 4	MICROCYSTINS ADDA	0.491 Abs	1.421 µg/L	R^2=0.99646, 41.365		P23F1409
MCT Std 4	MICROCYSTINS ADDA	0.385 Abs [0.4380] {17.1 C\	2.289 µg/L [1.855] {3	R^2=0.99646, 32.435		P23F1409
MCT Std 5	MICROCYSTINS ADDA	0.256 Abs	> 5.000 µg/L	21.567 %Abs		P23F1409
MCT Std 5	MICROCYSTINS ADDA	0.250 Abs [0.2530] {1.7 CV}	> 5.000 µg/L	21.061 %Abs		P23F1409
MCT LRB (0.000-0.300)	MICROCYSTINS ADDA	1.119 Abs	0.052 µg/L	94.271 %Abs		P23F1409
MCT LRB (0.000-0.300)	MICROCYSTINS ADDA	1.187 Abs [1.1530] {4.2 CV}	0.000 µg/L [0.026] {1	100.000 %Abs [97.13		P23F1409
MCT QCS (0.5625-0.9375)	MICROCYSTINS ADDA	0.615 Abs	0.877 µg/L	51.811 %Abs		P23F1409
MCT QCS (0.5625-0.9375)	MICROCYSTINS ADDA	0.632 Abs [0.6235] {1.9 CV}	0.823 µg/L [0.850] {4	53.243 %Abs [52.527		P23F1409

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
blk	MICROCYSTINS ADDA	1.168 Abs	0.013 µg/L	**LOW, 98.399 %ABS**	0.300 - 5.000	P23F1409
blk	MICROCYSTINS ADDA	1.138 Abs [1.1530] {1.8 CV}	0.036 µg/L [0.024] {6	**LOW, 95.872 %ABS**	0.300 - 5.000	P23F1409
A31	MICROCYSTINS ADDA	0.501 Abs	1.364 µg/L	42.207 %Abs	0.300 - 5.000	P23F1409
A31	MICROCYSTINS ADDA	0.475 Abs [0.4880] {3.8 CV}	1.519 µg/L [1.441] {7	40.017 %Abs [41.112	0.300 - 5.000	P23F1409
B31	MICROCYSTINS ADDA	0.473 Abs	1.532 µg/L	39.848 %Abs	0.300 - 5.000	P23F1409
B31	MICROCYSTINS ADDA	0.500 Abs [0.4865] {3.9 CV}	1.370 µg/L [1.451] {7	42.123 %Abs [40.986	0.300 - 5.000	P23F1409
C31	MICROCYSTINS ADDA	0.505 Abs	1.342 µg/L	42.544 %Abs	0.300 - 5.000	P23F1409
C31	MICROCYSTINS ADDA	0.521 Abs [0.5130] {2.2 CV}	1.259 µg/L [1.300] {4	43.892 %Abs [43.218	0.300 - 5.000	P23F1409
D31	MICROCYSTINS ADDA	0.439 Abs	1.775 µg/L	36.984 %Abs	0.300 - 5.000	P23F1409
D31	MICROCYSTINS ADDA	0.259 Abs [0.3490] {36.5 C\	> 5.000 µg/L [1.775]	21.820 %Abs, Out(LR	0.300 - 5.000	P23F1409
E31	MICROCYSTINS ADDA	0.291 Abs	3.953 µg/L	24.516 %Abs	0.300 - 5.000	P23F1409
E31	MICROCYSTINS ADDA	0.402 Abs [0.3465] {22.7 C\	2.106 µg/L [3.030] {4	33.867 %Abs [29.197	0.300 - 5.000	P23F1409
F31	MICROCYSTINS ADDA	0.519 Abs	1.269 µg/L	43.724 %Abs	0.300 - 5.000	P23F1409
F31	MICROCYSTINS ADDA	0.515 Abs [0.5170] {0.5 CV}	1.290 µg/L [1.280] {1	43.387 %Abs [43.555	0.300 - 5.000	P23F1409
G31	MICROCYSTINS ADDA	0.500 Abs	1.370 µg/L	42.123 %Abs	0.300 - 5.000	P23F1409
G31	MICROCYSTINS ADDA	0.496 Abs [0.4980] {0.6 CV}	1.393 µg/L [1.382] {1	41.786 %Abs [41.955	0.300 - 5.000	P23F1409
H31	MICROCYSTINS ADDA	0.491 Abs	1.421 µg/L	41.365 %Abs	0.300 - 5.000	P23F1409
H31	MICROCYSTINS ADDA	0.457 Abs [0.4740] {5.1 CV}	1.640 µg/L [1.530] {1	38.500 %Abs [39.933	0.300 - 5.000	P23F1409
A32	MICROCYSTINS ADDA	0.702 Abs	0.633 µg/L	59.141 %Abs	0.300 - 5.000	P23F1409
A32	MICROCYSTINS ADDA	0.678 Abs [0.6900] {2.5 CV}	0.693 µg/L [0.663] {6	57.119 %Abs [58.130	0.300 - 5.000	P23F1409
B32	MICROCYSTINS ADDA	0.618 Abs	0.867 µg/L	52.064 %Abs	0.300 - 5.000	P23F1409
B32	MICROCYSTINS ADDA	0.598 Abs [0.6080] {2.3 CV}	0.935 µg/L [0.901] {5	50.379 %Abs [51.222	0.300 - 5.000	P23F1409
BLKSPK0.5	MICROCYSTINS ADDA	0.891 Abs	0.292 µg/L	**LOW, 75.063 %ABS**	0.300 - 5.000	P23F1409
BLKSPK0.5	MICROCYSTINS ADDA	0.836 Abs [0.8635] {4.5 CV}	0.373 µg/L [0.332] {1	70.430 %Abs [72.746	0.300 - 5.000	P23F1409
C32	MICROCYSTINS ADDA	0.672 Abs	0.709 µg/L	56.613 %Abs	0.300 - 5.000	P23F1409
C32	MICROCYSTINS ADDA	0.563 Abs [0.6175] {12.5 C\	1.068 µg/L [0.888] {2	47.430 %Abs [52.022	0.300 - 5.000	P23F1409
D32	MICROCYSTINS ADDA	0.544 Abs	1.150 µg/L	45.830 %Abs	0.300 - 5.000	P23F1409
D32	MICROCYSTINS ADDA	0.517 Abs [0.5305] {3.6 CV}	1.279 µg/L [1.214] {7	43.555 %Abs [44.693	0.300 - 5.000	P23F1409
E32	MICROCYSTINS ADDA	0.672 Abs	0.709 µg/L	56.613 %Abs	0.300 - 5.000	P23F1409
E32	MICROCYSTINS ADDA	0.689 Abs [0.6805] {1.8 CV}	0.665 µg/L [0.687] {4	58.045 %Abs [57.329	0.300 - 5.000	P23F1409
F32	MICROCYSTINS ADDA	0.682 Abs	0.683 µg/L	57.456 %Abs	0.300 - 5.000	P23F1409
F32	MICROCYSTINS ADDA	0.715 Abs [0.6985] {3.3 CV}	0.603 µg/L [0.643] {8	60.236 %Abs [58.846	0.300 - 5.000	P23F1409
G32	MICROCYSTINS ADDA	0.619 Abs	0.864 µg/L	52.148 %Abs	0.300 - 5.000	P23F1409
G32	MICROCYSTINS ADDA	0.576 Abs [0.5975] {5.1 CV}	1.017 µg/L [0.941] {1	48.526 %Abs [50.337	0.300 - 5.000	P23F1409
H32	MICROCYSTINS ADDA	0.593 Abs	0.953 µg/L	49.958 %Abs	0.300 - 5.000	P23F1409
H32	MICROCYSTINS ADDA	0.525 Abs [0.5590] {8.6 CV}	1.239 µg/L [1.096] {1	44.229 %Abs [47.094	0.300 - 5.000	P23F1409
A33	MICROCYSTINS ADDA	0.682 Abs	0.683 µg/L	57.456 %Abs	0.300 - 5.000	P23F1409
A33	MICROCYSTINS ADDA	0.713 Abs [0.6975] {3.1 CV}	0.608 µg/L [0.646] {8	60.067 %Abs [58.762	0.300 - 5.000	P23F1409

B33	MICROCYSTINS ADDA	0.697 Abs	0.645 µg/L	58.719 %Abs	0.300 - 5.000	P23F1409
B33	MICROCYSTINS ADDA	0.702 Abs [0.6995] {0.5 CV}	0.633 µg/L [0.639] {1	59.141 %Abs [58.93(	0.300 - 5.000	P23F1409
C33	MICROCYSTINS ADDA	0.636 Abs	0.811 µg/L	53.580 %Abs	0.300 - 5.000	P23F1409
C33	MICROCYSTINS ADDA	0.556 Abs [0.5960] {9.5 CV}	1.098 µg/L [0.955] {2	46.841 %Abs [50.21(	0.300 - 5.000	P23F1409
D33	MICROCYSTINS ADDA	0.548 Abs	1.132 µg/L	46.167 %Abs	0.300 - 5.000	P23F1409
D33	MICROCYSTINS ADDA	0.537 Abs [0.5425] {1.4 CV}	1.182 µg/L [1.157] {3	45.240 %Abs [45.70(	0.300 - 5.000	P23F1409
CON32SPK0.5	MICROCYSTINS ADDA	0.834 Abs	0.376 µg/L	70.261 %Abs	0.300 - 5.000	P23F1409
CON32SPK0.5	MICROCYSTINS ADDA	0.801 Abs [0.8175] {2.9 CV}	0.431 µg/L [0.403] {9	67.481 %Abs [68.87(	0.300 - 5.000	P23F1409
CON32	MICROCYSTINS ADDA	1.294 Abs	0.000 µg/L	**LOW, 109.014 %ABS**	0.300 - 5.000	P23F1409
CON32	MICROCYSTINS ADDA	1.305 Abs [1.2995] {0.6 CV}	0.000 µg/L [0.000]	**LOW, 109.941 %ABS**	0.300 - 5.000	P23F1409
E33	MICROCYSTINS ADDA	0.575 Abs	1.020 µg/L	48.441 %Abs	0.300 - 5.000	P23F1409
E33	MICROCYSTINS ADDA	0.507 Abs [0.5410] {8.9 CV}	1.332 µg/L [1.176] {1	42.713 %Abs [45.57(	0.300 - 5.000	P23F1409
CON31	MICROCYSTINS ADDA	1.327 Abs	0.000 µg/L	**LOW, 111.794 %ABS**	0.300 - 5.000	P23F1409
CON31	MICROCYSTINS ADDA	1.367 Abs [1.3470] {2.1 CV}	0.000 µg/L [0.000]	**LOW, 115.164 %ABS**	0.300 - 5.000	P23F1409

* A - Abs > 3; IA - Initial Abs; DA - Delta Abs; SD - SD of Abs; LR - Linear Range; [...] - Mean result of duplicate tests
* Generated by software version (6.4.1.1065/1019/1.00/0.95) 8/1/2023 8:24:36 PM

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Test Report (by Request)

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
F33	MICROCYSTINS ADDA	0.568 Abs	1.048 µg/L	47.852 %Abs	0.300 - 5.000	P23F1409
F33	MICROCYSTINS ADDA	0.557 Abs [0.5625] {1.4 CV}	1.093 µg/L [1.071] {3	46.925 %Abs [47.38(	0.300 - 5.000	P23F1409
CON33	MICROCYSTINS ADDA	1.414 Abs	0.000 µg/L	**LOW, 119.124 %ABS**	0.300 - 5.000	P23F1409
CON33	MICROCYSTINS ADDA	1.441 Abs [1.4275] {1.3 CV}	0.000 µg/L [0.000]	**LOW, 121.398 %ABS**	0.300 - 5.000	P23F1409
G33	MICROCYSTINS ADDA	0.661 Abs	0.739 µg/L	55.687 %Abs	0.300 - 5.000	P23F1409
G33	MICROCYSTINS ADDA	0.576 Abs [0.6185] {9.7 CV}	1.017 µg/L [0.878] {2	48.526 %Abs [52.10(	0.300 - 5.000	P23F1409
H33	MICROCYSTINS ADDA	0.569 Abs	1.044 µg/L	47.936 %Abs	0.300 - 5.000	P23F1409
H33	MICROCYSTINS ADDA	0.534 Abs [0.5515] {4.5 CV}	1.196 µg/L [1.120] {9	44.987 %Abs [46.46(	0.300 - 5.000	P23F1409
BLK	MICROCYSTINS ADDA	1.532 Abs	0.000 µg/L	**LOW, 129.065 %ABS**	0.300 - 5.000	P23F1409
BLK	MICROCYSTINS ADDA	1.458 Abs [1.4950] {3.5 CV}	0.000 µg/L [0.000]	**LOW, 122.831 %ABS**	0.300 - 5.000	P23F1409

Table C.9.

Microcystin results accounting for concentrating the sample from 80 mL to 5 mL.

Sample ID	Microcystin
A31	0.0901
A32	0.0414
A33	0.0404
B31	0.0907
B32	0.0563
B33	0.0399
C31	0.0813
C32	0.0555
C33	0.0597
D31	0.1109
D32	0.0759
D33	0.0723
E31	0.1894
E32	0.0429
E33	0.0735
F31	0.0800
F32	0.0402
F33	0.0669
G31	0.0864
G32	0.0588
G33	0.0549
H31	0.0956
H32	0.0685
H33	0.0700

Total Phosphorus.

Figure C.8.

Standard curve for total phosphorus analysis in Block 3

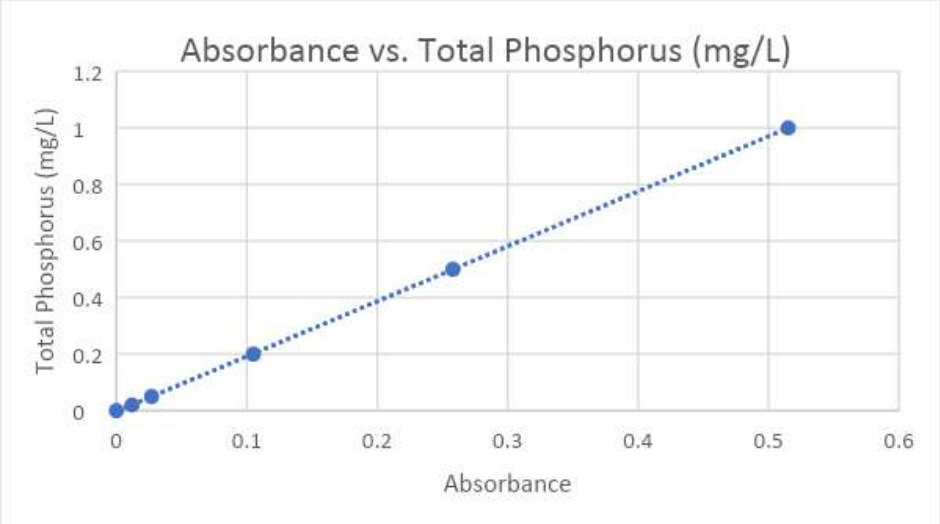


Table C.10

Calculated total phosphorus concentration and original absorbance measurements for each test culture (Block 3)

Sample ID	Absorbance		Calculated Value (mg/L)
A31	0.249	0.187	3.61
A32	0.213	0.151	2.91
A33	0.273	0.211	4.08
B31	0.269	0.207	4.00
B32	0.299	0.237	4.59
B33	0.247	0.185	3.58
C31	0.263	0.201	3.89
C32	0.256	0.194	3.75
C33	0.257	0.195	3.77
D31	0.304	0.242	4.69
D32	0.321	0.259	5.02
D33	0.275	0.213	4.12
E31	0.247	0.185	3.58
E32	0.242	0.18	3.48
E33	0.241	0.179	3.46
F31	0.298	0.236	4.57
F32	0.273	0.211	4.08
F33	0.238	0.176	3.40
G31	0.295	0.233	4.51
G32	0.3	0.238	4.61
G33	0.234	0.172	3.32
H31	0.27	0.208	4.02
H32	0.263	0.201	3.89
H33	0.232	0.17	3.28

Note. Original absorbance values were adjusted according to the absorbance found in the blank. Samples were diluted 1:10 before analysis.

Table C.11

Total phosphorus quality control parameters and Pineview water (Con31, Con32, Con33) (Block 3)

Sample ID	Absorbance		Calculated Value (ug/L)	% Recovery
85P2.125N	0.112	0.05	94.9	111.6
Con12spk200	0.115	0.115	221.4	110.7
CCV (500)	0.19	0.128	246.7	123.3
con31	0.074	0.012	21.0	
con32	0.069	0.007	11.2	
con33	0.077	0.015	26.8	
Blank	0.062	0.000	-2.40	

Total Nitrogen.

Figure C.9

Total nitrogen results (Block 3) from AQ2

AQ2 Tray Report

SEAL Analytical

Software Version:	2.1.4
Report Requested By:	uwrllab
Date & Time:	2023-07-26 16:20:20
Tray Number:	81
Tray Name:	BrentBlck3TN

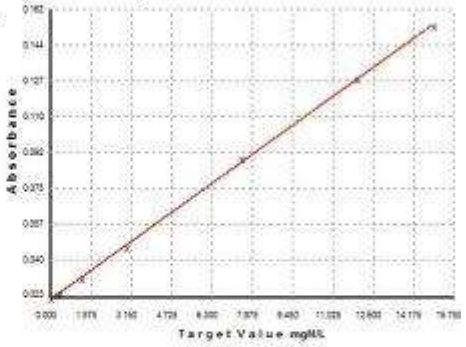
NO3_NO2 15

Calibration Chart

Type	Absorbance	Calc mgN/L	Target mgN/L	% Error
S1	0.0227	0.102	0.000	
S90	0.0243	0.289	0.250	15.69
S91	0.0247	0.335	0.375	-10.70
S92	0.0321	1.184	1.250	-6.89
S93	0.0469	2.840	3.000	-5.34
S94	0.0900	7.699	7.500	2.65
S95	0.1264	12.032	12.000	0.27
S96	0.1540	14.915	15.000	-0.57
S0	0.0216	-0.014	0.000	

Polynomial Order:	1
Correlation Coefficient:	0.9998
Carryover(%):	-0.8
Calibration equation:	y = bx + a
y = :	Concentration mgN/L
x = :	Measured absorbance
a = :	-2.454739E+000
b = :	1.127832E+002
Date & Time:	2023-07-26 14:13:05

Calibration Graph



Reagents

Name	Batch	Prepared By	Expiry Date
NO3 working buf		uwrllab	
NO3 SULF-NED		uwrllab	
Digested Blanks (Blank)		uwrllab	

CCV and blkspk are supposed to equal 6 mg/L
Con33 spk was spiked with 1 mg/L

Test Results

Cup	Type	ID	Result	Units	QC Pro	Raw Data	Auto Dil.	Man Dil.	User	Time/Date
	S1	Standard 1	0.023			0.022670			uw	2023-07-26 13:59:01
	S90	Standard 90	0.024			0.024330			uw	2023-07-26 14:00:46
	S91	Standard 91	0.025			0.024734			uw	2023-07-26 14:02:32
	S92	Standard 92	0.032			0.032084			uw	2023-07-26 14:04:17
	S93	Standard 93	0.047			0.046943			uw	2023-07-26 14:06:03
	S94	Standard 94	0.090			0.090027			uw	2023-07-26 14:07:48
	S95	Standard 95	0.126			0.126448			uw	2023-07-26 14:09:34
	S96	Standard 96	0.154			0.154008			uw	2023-07-26 14:11:19
	S0	Standard 0	0.022			0.021641			uw	2023-07-26 14:13:05
4	U1	7.5NO3	46.864	mgN/L		0.104514	x5.0000		uw	2023-07-26 15:37:18
4	U1	7.5NO3	42.765	mgN/L		0.400948			uw	2023-07-26 14:14:51
5	U2	7.5NO2	62.564	mgN/L		0.132711	x5.0000		uw	2023-07-26 15:39:07
5	U2	7.5NO2	52.112	mgN/L		0.483824			uw	2023-07-26 14:16:37
6	CCV	CCV	5.573	mgN/L		0.071175			uw	2023-07-26 14:18:23
7	U3	blkspk	6.595	mgN/L		0.079885			uw	2023-07-26 14:20:09
8	U4	blank	0.416	mgN/L		0.025455			uw	2023-07-26 14:21:55
9	U5	A31	148.465ELH	mgN/L		0.285041	x5.0000		uw	2023-07-26 15:40:56
9	U5	A31	147.751	mgN/L		1.331812			uw	2023-07-26 14:23:42

10	U6	B31	131.231ELH	mg/L	0.254479	x5.0000	uw	2023-07-26 15:42:45
10	U6	B31	138.947	mg/L	1.248431		uw	2023-07-26 14:25:28
11	U7	C31	135.937ELH	mg/L	0.262824	x5.0000	uw	2023-07-26 15:44:34
11	U7	C31	146.884	mg/L	1.324125		uw	2023-07-26 14:27:14
12	U8	D31	84.266ELH	mg/L	0.171247	x5.0000	uw	2023-07-26 15:46:23
12	U8	D31	93.607	mg/L	0.851738		uw	2023-07-26 14:29:01
13	U9	E31	140.701ELH	mg/L	0.271273	x5.0000	uw	2023-07-26 15:48:12
13	U9	E31	140.651	mg/L	1.268859		uw	2023-07-26 14:30:47
14	U10	F31	137.268ELH	mg/L	0.265185	x5.0000	uw	2023-07-26 15:50:02
14	U10	F31	151.603	mg/L	1.365966		uw	2023-07-26 14:32:34
15	U11	G31	108.831ELH	mg/L	0.214757	x5.0000	uw	2023-07-26 15:51:51
15	U11	G31	117.044	mg/L	1.059541		uw	2023-07-26 14:34:20
16	U12	H31	121.916ELH	mg/L	0.237560	x5.0000	uw	2023-07-26 15:53:40
16	U12	H31	131.688	mg/L	1.189382		uw	2023-07-26 14:36:06
17	U13	A32	154.854ELH	mg/L	0.296370	x5.0000	uw	2023-07-26 15:55:30
17	U13	A32	158.784	mg/L	1.429635		uw	2023-07-26 14:37:52
18	U14	B32	95.934ELH	mg/L	0.191887	x5.0000	uw	2023-07-26 15:57:19
18	U14	B32	95.882	mg/L	0.871907		uw	2023-07-26 14:39:38
19	U15	BLK	0.518	mg/L	0.026357		uw	2023-07-26 14:41:24
20	CCV	CCV	5.360	mg/L	0.069269		uw	2023-07-26 14:43:09
21	U16	C32	127.788ELH	mg/L	0.248337	x5.0000	uw	2023-07-26 15:59:09
21	U16	C32	121.276	mg/L	1.097084		uw	2023-07-26 14:44:56
22	U17	D32	153.719ELH	mg/L	0.294356	x5.0000	uw	2023-07-26 16:00:58
22	U17	D32	175.705	mg/L	1.579667		uw	2023-07-26 14:46:41
23	U18	E32	137.633ELH	mg/L	0.265831	x5.0000	uw	2023-07-26 16:02:48
23	U18	E32	152.191	mg/L	1.371179		uw	2023-07-26 14:48:27
24	U19	F32	124.782ELH	mg/L	0.243043	x5.0000	uw	2023-07-26 16:04:38
24	U19	F32	134.904	mg/L	1.217904		uw	2023-07-26 14:50:12
25	U20	G32	85.695ELH	mg/L	0.173728	x5.0000	uw	2023-07-26 16:06:28
25	U20	G32	92.051	mg/L	0.838205		uw	2023-07-26 14:51:58
26	U21	H32	130.908ELH	mg/L	0.253908	x5.0000	uw	2023-07-26 16:07:25
26	U21	H32	139.769	mg/L	1.261037		uw	2023-07-26 14:53:43
27	U22	A33	128.310ELH	mg/L	0.249298	x5.0000	uw	2023-07-26 16:08:18
27	U22	A33	132.022	mg/L	1.192350		uw	2023-07-26 14:55:28
28	U23	B33	131.069ELH	mg/L	0.254192	x5.0000	uw	2023-07-26 16:09:11
28	U23	B33	150.803	mg/L	1.358869		uw	2023-07-26 14:57:13
29	U24	C33	132.929ELH	mg/L	0.257313	x5.0000	uw	2023-07-26 16:10:04
29	U24	C33	139.182	mg/L	1.255836		uw	2023-07-26 14:58:59
30	U25	D33	133.187ELH	mg/L	0.257947	x5.0000	uw	2023-07-26 16:10:57
30	U25	D33	150.666	mg/L	1.357657		uw	2023-07-26 15:00:44
31	U26	CONSPK1	3.575	mg/L	0.053467		uw	2023-07-26 15:02:29
32	U27	BLANK	-0.036ELL	mg/L	0.021429		uw	2023-07-26 15:04:14
33	U28	E33	139.362ELH	mg/L	0.268898	x5.0000	uw	2023-07-26 16:11:50
33	U28	E33	151.271	mg/L	1.363015		uw	2023-07-26 15:05:59
34	U29	F33	128.108ELH	mg/L	0.248940	x5.0000	uw	2023-07-26 16:12:43
34	U29	F33	137.526	mg/L	1.241151		uw	2023-07-26 15:07:44
35	U30	G33	154.061ELH	mg/L	0.294964	x5.0000	uw	2023-07-26 16:13:37
35	U30	G33	164.921	mg/L	1.484050		uw	2023-07-26 15:09:29
36	U31	H33	144.070ELH	mg/L	0.277246	x5.0000	uw	2023-07-26 16:14:30
36	U31	H33	148.087	mg/L	1.334787		uw	2023-07-26 15:10:26
37	U32	[redacted]	2.125 2.157	mg/L	0.040889		uw	2023-07-26 15:11:19
38	U33	BSP2.125N	1.952	mg/L	0.039072		uw	2023-07-26 15:12:13
39	U34	DI	1.735	mg/L	0.037147		uw	2023-07-26 15:13:06
40	U35	CON31	0.860	mg/L	0.029393		uw	2023-07-26 15:13:59
41	U36	CON32	2.814	mg/L	0.046718		uw	2023-07-26 15:14:52
42	U37	CON33	-0.334ELL	mg/L	0.018803		uw	2023-07-26 15:15:45
43	CCV	[redacted]	6.288	mg/L	0.077516		uw	2023-07-26 15:16:38
44	U39	BLK	-0.087ELL	mg/L	0.020994		uw	2023-07-26 15:17:31

Figure C.10.

Total nitrogen standard curve from Block 4

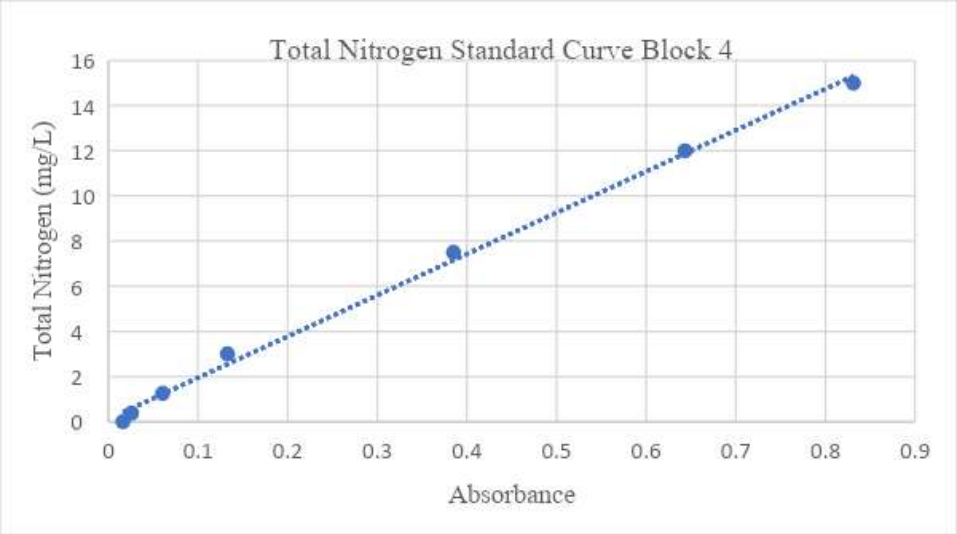


Table C.12

Total nitrogen results using the standard curve from Block 4

Sample ID	Absorbance	Total Nitrogen (mg/L)
A31	0.2850	26.64
A32	0.2964	27.68
A33	0.2493	23.38
B31	0.2545	23.85
B32	0.1919	18.13
B33	0.2542	23.83
C31	0.2628	24.62
C32	0.2483	23.29
C33	0.2579	24.17
D31	0.1712	16.25
D32	0.2944	27.50
D33	0.2579	24.17
E31	0.2713	25.39
E32	0.2658	24.89
E33	0.2689	25.17
F31	0.2652	24.83
F32	0.2430	22.81
F33	0.2489	23.35
G31	0.2148	20.22
G32	0.1737	16.47
G33	0.2539	23.80
H31	0.2380	22.34
H32	0.2539	23.80
H33	0.2772	25.93
Con31	0.0294	0.66
Con32	0.0467	0.97
Con33	0.0188	0.46

PAR, Water Temperature, and pH.

Table C.13

Results for PAR, pH, and water temperature from Block 3 including Pineview water (Con31, Con32, Con33)

Sample ID	PAR (µmol*m^2*sec^{-1}) (Day 0 and Day 4)	pH	Water Temperature (°C)
A31	*52*	*11.17*	*28*
A32	*50*	*11.15*	*28*
A33	*51*	*11.06*	*28*
B31	*49*	*11.03*	*28*
B32	*50*	*11.06*	*28*
B33	*49*	*11.06*	*28*
C31	*51*	*11.03*	*28*
C32	*50*	*11.15*	*28*
C33	*48*	*10.83*	*28*
D31	*49*	*11.02*	*28*
D32	*52*	*10.77*	*28*
D33	*49*	*11.17*	*28*
E31	*50*	*11.01*	*17.8*
E32	*49*	*10.98*	*17.8*
E33	*51*	*10.71*	*17.8*
F31	*49*	*10.86*	*17.8*
F32	*51*	*10.92*	*17.8*
F33	*51*	*11.07*	*17.8*
G31	*47*	*10.8*	*17.8*
G32	*47*	*10.76*	*17.8*
G33	*52*	*10.95*	*17.8*
H31	*48*	*10.8*	*17.8*
H32	*50*	*10.88*	*17.8*
H33	*49*	*10.63*	*17.8*
CON31	*51*	*8.78*	*28*
CON32	*50*	*8.79*	*28*
CON33	*51*	*8.64*	*28*

Block 4.

Microcystins.

Figure C.11.

Microcystin results (Block 4) from ELISA with blank spike and Pineview water control spike (Con42SPK0.5) of 0.5 µg/L included

Assay Information

Assay Name: MICROCYSTINS ADDA
Version: 2
Temperature: Room Temperature
Last Modified By: Security disabled
Units: µg/L
Assay Description: PN 520011
Assay Substances: Controls:
MCT LRB (0.000-0.300)
MCT QCS (0.5625-0.9375)
Standards:
MCT Std 0, Concentration = 0.000, Minimum number to use: 2
MCT Std 1, Concentration = 0.150, Minimum number to use: 2
MCT Std 2, Concentration = 0.400, Minimum number to use: 2
MCT Std 3, Concentration = 1.000, Minimum number to use: 2
MCT Std 4, Concentration = 2.000, Minimum number to use: 2
MCT Std 5, Concentration = 5.000, Minimum number to use: 2
Curve valid interval: 1 days 0 hours
Axis Mode: Y = Abs, X = Log(Conc)

Assay Mode: 4-Parameter Logistic Weight by:None
Well Type: Flat bottom
Last Modified On: 7/25/2019 1:53:38 PM
Normal: 0.300 - 5.000
# of decimals: 3
Kit Lot Number: P23F1409

Assay Calibration **Current Calibration Status: "** "

Name	Absorbance	Concentration	Interpretation	Position
8/15/2023 4:11:26 PM				
MCT Std 0	1.216 Abs		R^2=0.99724, 96.738 %Abs	RK1:23->A01@2
MCT Std 0	1.298 Abs [1.2570] {4.6 CV}		R^2=0.99724, 103.262 %Abs	RK1:23->B01@2
MCT Std 1	1.073 Abs		R^2=0.99724, 85.362 %Abs	RK1:24->C01@2
MCT Std 1	1.075 Abs [1.0740] {0.1 CV}		R^2=0.99724, 85.521 %Abs	RK1:24->D01@2
MCT Std 2	0.799 Abs		R^2=0.99724, 63.564 %Abs	RK1:25->E01@2
MCT Std 2	0.755 Abs [0.7770] {4.0 CV}		R^2=0.99724, 60.064 %Abs	RK1:25->F01@3
MCT Std 3	0.513 Abs		R^2=0.99724, 40.811 %Abs	RK1:26->G01@3
MCT Std 3	0.520 Abs [0.5165] {1.0 CV}		R^2=0.99724, 41.368 %Abs	RK1:26->H01@3
MCT Std 4	0.434 Abs		R^2=0.99724, 34.527 %Abs	RK1:27->A02@2
MCT Std 4	0.390 Abs [0.4120] {7.6 CV}		R^2=0.99724, 31.026 %Abs	RK1:27->B02@2
MCT Std 5	0.264 Abs		21.002 %Abs	RK1:28->C02@2
MCT Std 5	0.269 Abs [0.2665] {1.3 CV}		21.400 %Abs	RK1:28->D02@2
8/15/2023 4:11:26 PM				
MCT LRB (0.000-0.300)	1.190 Abs		94.670 %Abs	RK1:10->E02@2
MCT LRB (0.000-0.300)	1.181 Abs [1.1855] {0.5 CV}		93.954 %Abs [94.312 %Abs]	RK1:10->F02@3
MCT QCS (0.5625-0.9375)	0.657 Abs		52.267 %Abs	RK1:29->G02@3
MCT QCS (0.5625-0.9375)	0.610 Abs [0.6335] {5.2 CV}		48.528 %Abs [50.398 %Abs]	RK1:29->H02@3

Statistic				
MCT Std 0 [MEAN]	1.2570			
MCT Std 0 [SD]	0.0580			
MCT Std 0 [%CV]	4.6128			
MCT Std 1 [MEAN]	1.0740			
MCT Std 1 [SD]	0.0014			
MCT Std 1 [%CV]	0.1317			
MCT Std 1 [%DIFF]				
MCT Std 2 [MEAN]	0.7770			
MCT Std 2 [SD]	0.0311			
MCT Std 2 [%CV]	4.0042			
MCT Std 2 [%DIFF]				
MCT Std 3 [MEAN]	0.5165			
MCT Std 3 [SD]	0.0049			
MCT Std 3 [%CV]	0.9583			
MCT Std 3 [%DIFF]				
MCT Std 4 [MEAN]	0.4120			
MCT Std 4 [SD]	0.0311			
MCT Std 4 [%CV]	7.5516			
MCT Std 4 [%DIFF]				

*Generated by software version (6.4.1.1065/1019/1.00/0.95) 8/15/2023 4:19:12 PM

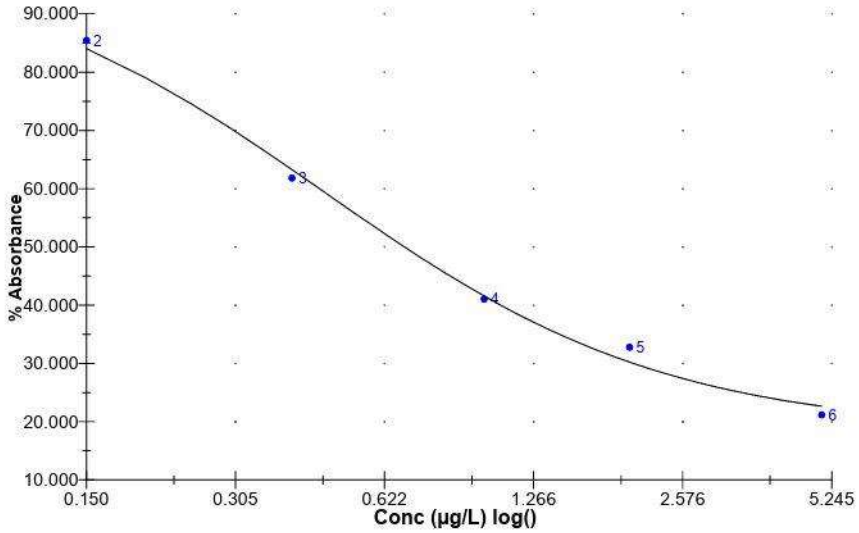
eurofins | Abraxis

MICROCYSTINS ADDA - Assay Calibration Report

Name	Absorbance	Concentration	Interpretation	Position
MCT Std 5 [MEAN]	0.2665			
MCT Std 5 [SD]	0.0035			
MCT Std 5 [%CV]	1.3267			
MCT LRB (0.000-0.300) [MEAN]	1.1855			
MCT LRB (0.000-0.300) [SD]	0.0064			
MCT LRB (0.000-0.300) [%CV]	0.5368			
MCT QCS (0.5625-0.9375) [MEAN]	0.6335			
MCT QCS (0.5625-0.9375) [SD]	0.0332			
MCT QCS (0.5625-0.9375) [%CV]	5.2461			

Assay Curve

y = (A-D)/(1+(x/C)^B) + D
Weight: NONE
A = 1.2629
B = 1.2212
C = 0.46639
D = 0.23089
R2 coef = 0.99724
50% = 0.684



Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
B1	MICROCYSTINS ADDA	1.265 Abs	0.000 µg/L	**LOW, 100.636 %ABS**	0.300 - 5.000	P23F1409
B1	MICROCYSTINS ADDA	1.289 Abs [1.2770] {1.3 CV}	0.000 µg/L [0.000]	**LOW, 102.546 %ABS**	0.300 - 5.000	P23F1409
A41	MICROCYSTINS ADDA	0.535 Abs	0.953 µg/L	42.562 %Abs	0.300 - 5.000	P23F1409
A41	MICROCYSTINS ADDA	0.495 Abs [0.5150] {5.5 CV}	1.118 µg/L [1.036] {1	39.379 %Abs [40.97	0.300 - 5.000	P23F1409
B41	MICROCYSTINS ADDA	0.476 Abs	1.212 µg/L	37.868 %Abs	0.300 - 5.000	P23F1409
B41	MICROCYSTINS ADDA			Fluid not detected (00		P23F1409
C41	MICROCYSTINS ADDA	0.549 Abs	0.904 µg/L	43.675 %Abs	0.300 - 5.000	P23F1409
C41	MICROCYSTINS ADDA	0.573 Abs [0.5610] {3.0 CV}	0.828 µg/L [0.866] {6	45.585 %Abs [44.63	0.300 - 5.000	P23F1409
D41	MICROCYSTINS ADDA	0.585 Abs	0.794 µg/L	46.539 %Abs	0.300 - 5.000	P23F1409
D41	MICROCYSTINS ADDA	0.544 Abs [0.5645] {5.1 CV}	0.921 µg/L [0.858] {1	43.278 %Abs [44.90	0.300 - 5.000	P23F1409
E41	MICROCYSTINS ADDA	0.608 Abs	0.733 µg/L	48.369 %Abs	0.300 - 5.000	P23F1409
E41	MICROCYSTINS ADDA	0.545 Abs [0.5765] {7.7 CV}	0.918 µg/L [0.826] {1	43.357 %Abs [45.86	0.300 - 5.000	P23F1409
F41	MICROCYSTINS ADDA	0.435 Abs	1.468 µg/L	34.606 %Abs	0.300 - 5.000	P23F1409
F41	MICROCYSTINS ADDA	0.422 Abs [0.4285] {2.1 CV}	1.569 µg/L [1.519] {4	33.572 %Abs [34.08	0.300 - 5.000	P23F1409
G41	MICROCYSTINS ADDA	0.638 Abs	0.662 µg/L	50.756 %Abs	0.300 - 5.000	P23F1409
G41	MICROCYSTINS ADDA	0.622 Abs [0.6300] {1.8 CV}	0.699 µg/L [0.681] {3	49.483 %Abs [50.11	0.300 - 5.000	P23F1409
H41	MICROCYSTINS ADDA	0.558 Abs	0.875 µg/L	44.391 %Abs	0.300 - 5.000	P23F1409
H41	MICROCYSTINS ADDA	0.530 Abs [0.5440] {3.6 CV}	0.972 µg/L [0.924] {7	42.164 %Abs [43.27	0.300 - 5.000	P23F1409
CON41	MICROCYSTINS ADDA	1.129 Abs	0.098 µg/L	**LOW, 89.817 %ABS**	0.300 - 5.000	P23F1409
CON41	MICROCYSTINS ADDA	1.135 Abs [1.1320] {0.4 CV}	0.094 µg/L [0.096] {2	**LOW, 90.294 %ABS**	0.300 - 5.000	P23F1409
A42	MICROCYSTINS ADDA	0.431 Abs	1.498 µg/L	34.288 %Abs	0.300 - 5.000	P23F1409
A42	MICROCYSTINS ADDA	0.439 Abs [0.4350] {1.3 CV}	1.439 µg/L [1.469] {2	34.924 %Abs [34.60	0.300 - 5.000	P23F1409
B2	MICROCYSTINS ADDA	1.134 Abs	0.095 µg/L	**LOW, 90.215 %ABS**	0.300 - 5.000	P23F1409
B2	MICROCYSTINS ADDA	1.202 Abs [1.1680] {4.1 CV}	0.048 µg/L [0.072] {4	**LOW, 95.625 %ABS**	0.300 - 5.000	P23F1409
B42	MICROCYSTINS ADDA	0.602 Abs	0.748 µg/L	47.892 %Abs	0.300 - 5.000	P23F1409
B42	MICROCYSTINS ADDA	0.519 Abs [0.5605] {10.5 CV}	1.014 µg/L [0.881] {2	41.289 %Abs [44.59	0.300 - 5.000	P23F1409
C42	MICROCYSTINS ADDA	0.574 Abs	0.825 µg/L	45.664 %Abs	0.300 - 5.000	P23F1409
C42	MICROCYSTINS ADDA	0.551 Abs [0.5625] {2.9 CV}	0.897 µg/L [0.861] {5	43.835 %Abs [44.74	0.300 - 5.000	P23F1409
D42	MICROCYSTINS ADDA	0.783 Abs	0.416 µg/L	62.291 %Abs	0.300 - 5.000	P23F1409
D42 Blank spike	MICROCYSTINS ADDA	0.732 Abs [0.7575] {4.8 CV}	0.489 µg/L [0.452] {1	58.234 %Abs [60.26	0.300 - 5.000	P23F1409
E42	MICROCYSTINS ADDA	0.636 Abs	0.667 µg/L	50.597 %Abs	0.300 - 5.000	P23F1409
E42	MICROCYSTINS ADDA	0.614 Abs [0.6250] {2.5 CV}	0.718 µg/L [0.692] {5	48.846 %Abs [49.72	0.300 - 5.000	P23F1409
F42	MICROCYSTINS ADDA	0.514 Abs	1.034 µg/L	40.891 %Abs	0.300 - 5.000	P23F1409
F42	MICROCYSTINS ADDA	0.444 Abs [0.4790] {10.3 CV}	1.404 µg/L [1.219] {2	35.322 %Abs [38.10	0.300 - 5.000	P23F1409
G42	MICROCYSTINS ADDA	0.462 Abs	1.290 µg/L	36.754 %Abs	0.300 - 5.000	P23F1409
G42	MICROCYSTINS ADDA	0.454 Abs [0.4580] {1.2 CV}	1.339 µg/L [1.314] {2	36.118 %Abs [36.43	0.300 - 5.000	P23F1409
H42	MICROCYSTINS ADDA	0.543 Abs	0.925 µg/L	43.198 %Abs	0.300 - 5.000	P23F1409
H42	MICROCYSTINS ADDA	0.516 Abs [0.5295] {3.6 CV}	1.026 µg/L [0.975] {7	41.050 %Abs [42.12	0.300 - 5.000	P23F1409
A43	MICROCYSTINS ADDA	0.534 Abs	0.957 µg/L	42.482 %Abs	0.300 - 5.000	P23F1409
A43	MICROCYSTINS ADDA	0.526 Abs [0.5300] {1.1 CV}	0.987 µg/L [0.972] {2	41.846 %Abs [42.16	0.300 - 5.000	P23F1409
B43	MICROCYSTINS ADDA	0.529 Abs	0.975 µg/L	42.084 %Abs	0.300 - 5.000	P23F1409
B43	MICROCYSTINS ADDA	0.439 Abs [0.4840] {13.1 CV}	1.439 µg/L [1.207] {2	34.924 %Abs [38.50	0.300 - 5.000	P23F1409
CON43	MICROCYSTINS ADDA	1.220 Abs	0.036 µg/L	**LOW, 97.056 %ABS**	0.300 - 5.000	P23F1409
CON43	MICROCYSTINS ADDA	1.228 Abs [1.2240] {0.5 CV}	0.030 µg/L [0.033] {1	**LOW, 97.693 %ABS**	0.300 - 5.000	P23F1409
CON41SPK0.5	MICROCYSTINS ADDA	0.754 Abs	0.456 µg/L	59.984 %Abs	0.300 - 5.000	P23F1409
CON41SPK0.5	MICROCYSTINS ADDA	0.709 Abs [0.7315] {4.3 CV}	0.526 µg/L [0.491] {1	56.404 %Abs [58.19	0.300 - 5.000	P23F1409
C43	MICROCYSTINS ADDA	0.585 Abs	0.794 µg/L	46.539 %Abs	0.300 - 5.000	P23F1409
C43	MICROCYSTINS ADDA	0.583 Abs [0.5840] {0.2 CV}	0.799 µg/L [0.797] {0	46.380 %Abs [46.46	0.300 - 5.000	P23F1409
D43	MICROCYSTINS ADDA	0.470 Abs	1.245 µg/L	37.391 %Abs	0.300 - 5.000	P23F1409
D43	MICROCYSTINS ADDA	0.408 Abs [0.4390] {10.0 CV}	1.693 µg/L [1.469] {2	32.458 %Abs [34.92	0.300 - 5.000	P23F1409
E43	MICROCYSTINS ADDA	0.549 Abs	0.904 µg/L	43.675 %Abs	0.300 - 5.000	P23F1409
E43	MICROCYSTINS ADDA	0.500 Abs [0.5245] {6.6 CV}	1.095 µg/L [1.000] {1	39.777 %Abs [41.72	0.300 - 5.000	P23F1409

* A - Abs × 3; IA - Initial Abs; DA - Delta Abs; SD - SD of Abs; LR - Linear Range; [...] - Mean result of duplicate tests
* Generated by software version [6.4.1.1085/1019/1.00/0.95] 8/15/2023 4:23:49 PM

eurofins | Abraxis

Test Report (by Request)

Name/ID	Assay	Absorbance	Concentration	Interpretation	Reference	Lot #
F43	MICROCYSTINS ADDA	0.641 Abs	0.656 µg/L	50.994 %Abs	0.300 - 5.000	P23F1409
F43	MICROCYSTINS ADDA	0.586 Abs [0.6135] {6.3 CV}	0.791 µg/L [0.724] {1	46.619 %Abs [48.80	0.300 - 5.000	P23F1409
G43	MICROCYSTINS ADDA	0.641 Abs	0.656 µg/L	50.994 %Abs	0.300 - 5.000	P23F1409
G43	MICROCYSTINS ADDA	0.626 Abs [0.6335] {1.7 CV}	0.690 µg/L [0.673] {3	49.801 %Abs [50.39	0.300 - 5.000	P23F1409
H43	MICROCYSTINS ADDA	0.578 Abs	0.814 µg/L	45.982 %Abs	0.300 - 5.000	P23F1409
H43	MICROCYSTINS ADDA	0.494 Abs [0.5360] {11.1 CV}	1.122 µg/L [0.968] {2	39.300 %Abs [42.64	0.300 - 5.000	P23F1409
CON42	MICROCYSTINS ADDA	1.260 Abs	0.004 µg/L	**LOW, 100.239 %ABS**	0.300 - 5.000	P23F1409
CON42	MICROCYSTINS ADDA	1.248 Abs [1.2540] {0.7 CV}	0.015 µg/L [0.009] {8	**LOW, 99.284 %ABS**	0.300 - 5.000	P23F1409
B3	MICROCYSTINS ADDA	1.306 Abs	0.000 µg/L	**LOW, 103.898 %ABS**	0.300 - 5.000	P23F1409
B3	MICROCYSTINS ADDA	1.301 Abs [1.3035] {0.3 CV}	0.000 µg/L [0.000]	**LOW, 103.500 %ABS**	0.300 - 5.000	P23F1409

Table C.14

Microcystin results accounting for concentrating the sample from 80 mL to 5 mL

Sample ID	Microcystin
A41	0.0649
A42	0.0918
A43	0.0608
B41	0.0758
B42	0.0551
B43	0.0754
C41	0.0541
C42	0.0538
C43	0.0498
D41	0.0536
D42	
D43	0.0918
E41	0.0516
E42	0.0433
E43	0.0625
F41	0.0949
F42	0.0762
F43	0.0453
G41	0.0426
G42	0.0821
G43	0.0421
H41	0.0578
H42	0.0609
H43	0.0605

Total Phosphorus.

Figure C.12

Standard curve for total phosphorus analysis (Block 4)

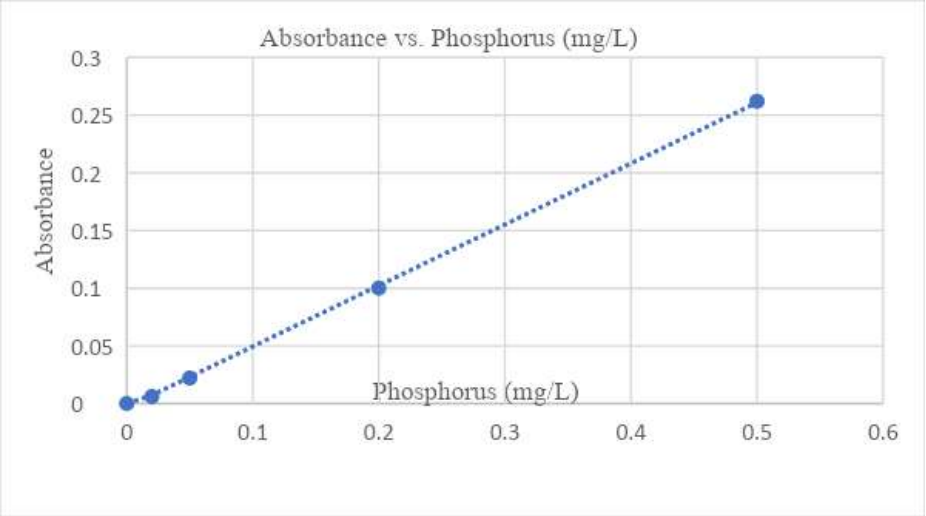


Table C.15

Calculated total phosphorus concentration for each test culture (Block 4)

Sample ID	Absorbance	Calculated Value (mg/L)
A41	0.246	4.71
A42	0.248	4.74
A43	0.164	3.16
B41	0.262	5.01
B42	0.282	5.39
B43	0.235	4.50
C41	0.206	3.95
C42	0.193	3.71
C43	0.172	3.31
D41	0.233	4.46
D42	0.264	5.05
D43	0.3	5.72
E41	0.201	3.86
E42	0.255	4.88
E43	0.202	3.88
F41	0.202	3.88
F42	0.263	5.03
F43	0.182	3.50
G41	0.177	3.40
G42	0.208	3.99
G43	0.199	3.82
H41	0.199	3.82
H42	0.192	3.69
H43	0.188	3.61

Note. Samples were diluted 1:10 before analysis.

Table C.16

Total phosphorus quality control parameters and Pineview water (Con41, Con42, Con43) (Block 4)

Sample ID	Absorbance	Calculated Value (ug/L)	% Recovery
85P375N	0.03	66.80	78.6
85P2.125N	0.033	72.80	85.6
15P60	0.003	12.80	85.3
15P340	0.002	10.80	72
con41	0.008	21.5	
con42	0.009	23.4	
con43	0.009	23.4	
CCV (200ug)	0.102	210.80	105.4
Con41spk200	0.097	200.80	90.7
Blank	0.000	0	

Total Nitrogen.

Figure C.13.

Total nitrogen results (Block 4) from AQ2

AQ2 Tray Report

SEAL Analytical

Software Version:	2.1.4
Report Requested By:	uwrllab
Date & Time:	2023-08-16 14:46:32
Tray Number:	85
Tray Name:	bjbLOCK4

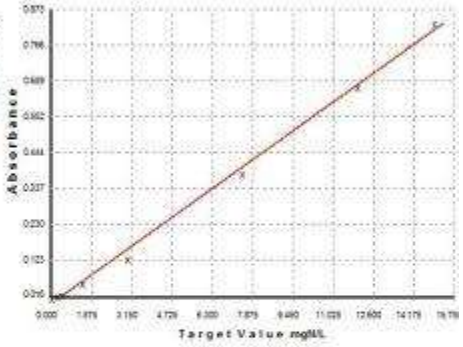
NO3_NO2 15

Calibration Chart

Type	Absorbance	Calc mgN/L	Target mgN/L	% Error
S1	0.0182	0.340	0.000	
S90	0.0233	0.471	0.250	88.37
S91	0.0257	0.516	0.375	37.58
S92	0.0605	1.157	1.250	-7.43
S93	0.1328	2.489	3.000	-17.05
S94	0.3851	7.140	7.500	-4.80
S95	0.6433	11.900	12.000	-0.84
S96	0.8312	15.363	15.000	2.42
S0	0.0184	0.380	0.000	

Polynomial Order:	1
Correlation Coefficient:	0.9985
Carryover(%):	0.3
Calibration equation:	y = bx + a
y =:	Concentration mgN/L
x =:	Measured absorbance
a =:	4.145734E-002
b =:	1.843218E+001
Date & Time:	2023-08-16 13:17:11

Calibration Graph



Reagents

Name	Batch	Prepared By	Expiry Date
NO3 working buf		uwrllab	
NO3 SULF-NED		uwrllab	
Digested Blanks (Blank)		uwrllab	

CCV and Blank spikes are supposed to have values of 5mg/L
Con42spk should have a concentration of 6 mg/L

Test Results

Cup	Type	ID	Result	Units	QC Pro	Raw Data	Auto Dil.	Man Dil.	User	Time/Date
	S1	Standard 1	0.016			0.018184			uw	2023-08-16 13:03:06
	S90	Standard 90	0.023			0.023299			uw	2023-08-16 13:04:52
	S91	Standard 91	0.026			0.025742			uw	2023-08-16 13:06:37
	S92	Standard 92	0.061			0.060531			uw	2023-08-16 13:08:23
	S93	Standard 93	0.133			0.132765			uw	2023-08-16 13:10:08
	S94	Standard 94	0.385			0.385100			uw	2023-08-16 13:11:53
	S95	Standard 95	0.643			0.643349			uw	2023-08-16 13:13:39
	S96	Standard 96	0.831			0.831248			uw	2023-08-16 13:15:25
	S0	Standard 0	0.018			0.018355			uw	2023-08-16 13:17:11
4	U1	NITRITE	7.871	mgN/L		0.424774			uw	2023-08-16 13:18:56
5	U2	NITRATE	1.885	mgN/L		0.100040			uw	2023-08-16 13:20:42
6	U3	B	0.382	mgN/L		0.018454			uw	2023-08-16 13:22:28
7	U4	CCV	2.808	mgN/L		0.150011			uw	2023-08-16 13:24:15
8	U5	A41	9.296	mgN/L		0.502100			uw	2023-08-16 13:26:01
9	U6	B41	11.649	mgN/L		0.629728			uw	2023-08-16 13:27:47
10	U7	C41	13.965	mgN/L		0.755405			uw	2023-08-16 13:29:33
11	U8	D41	13.285	mgN/L		0.718487			uw	2023-08-16 13:31:20
12	U9	E41	13.252	mgN/L		0.716718			uw	2023-08-16 13:33:06

13	U10	F41	13.928	mg/L	0.753359		uw	2023-08-16 13:34:53
14	U11	G41	10.802	mg/L	0.114961	x5.0000	uw	2023-08-16 14:38:41
14	U11	G41	15.524	mg/L	0.839950		uw	2023-08-16 13:38:39
15	U12	H41	11.787	mg/L	0.125849	x5.0000	uw	2023-08-16 14:39:34
15	U12	H41	16.551	mg/L	0.895687		uw	2023-08-16 13:38:25
16	U13	CON41	0.644	mg/L	0.032663		uw	2023-08-16 13:40:12
17	U14	A42	8.898	mg/L	0.480502		uw	2023-08-16 13:41:57
18	U15	B	0.277ELL	mg/L	0.012779		uw	2023-08-16 13:43:43
19	U16	BSPKS	2.830	mg/L	0.151269		uw	2023-08-16 13:45:29
20	U17	B42	9.998	mg/L	0.540057		uw	2023-08-16 13:47:15
21	U18	C42	10.925	mg/L	0.116288	x5.0000	uw	2023-08-16 14:40:27
21	U18	C42	15.467	mg/L	0.837978		uw	2023-08-16 13:49:00
22	U19	D42	11.610	mg/L	0.827611		uw	2023-08-16 13:50:46
23	U20	E42	9.385	mg/L	0.506939		uw	2023-08-16 13:52:31
24	U21	F42	10.988	mg/L	0.592663		uw	2023-08-16 13:54:17
25	U22	G42	10.342	mg/L	0.109967	x5.0000	uw	2023-08-16 14:41:21
25	U22	G42	15.408	mg/L	0.833695		uw	2023-08-16 13:56:02
26	U23	H42	11.218	mg/L	0.119458	x5.0000	uw	2023-08-16 14:42:14
26	U23	H42	15.897	mg/L	0.860195		uw	2023-08-16 13:57:48
27	U24	CON42	0.514	mg/L	0.025564		uw	2023-08-16 13:59:33
28	U25	A43	14.913	mg/L	0.806814		uw	2023-08-16 14:01:18
29	U26	B43	13.868	mg/L	0.739305		uw	2023-08-16 14:03:04
30	U27	B	0.316ELL	mg/L	0.014922		uw	2023-08-16 14:04:49
31	CCV	CCV	2.704	mg/L	0.144437		uw	2023-08-16 14:06:34
32	U28	C43	10.990	mg/L	0.116995	x5.0000	uw	2023-08-16 14:43:07
32	U28	C43	15.869	mg/L	0.847860		uw	2023-08-16 14:08:20
33	U29	D43	8.815	mg/L	0.476011		uw	2023-08-16 14:10:05
34	U30	E43	13.875	mg/L	0.750488		uw	2023-08-16 14:11:50
35	U31	F43	14.875	mg/L	0.804782		uw	2023-08-16 14:13:35
36	U32	G43	14.348	mg/L	0.776154		uw	2023-08-16 14:14:32
37	U33	H43	10.883	mg/L	0.115624	x5.0000	uw	2023-08-16 14:44:00
37	U33	H43	16.885	mg/L	0.913801		uw	2023-08-16 14:15:25
38	U34	BGN	0.323ELL	mg/L	0.015251		uw	2023-08-16 14:16:18
39	U35	375	0.465	mg/L	0.022987		uw	2023-08-16 14:17:11
40	U36	340	0.439	mg/L	0.021571		uw	2023-08-16 14:18:04
41	U37	2.125N	1.219	mg/L	0.063879		uw	2023-08-16 14:18:58
42	U38	CON42SPKS	3.667	mg/L	0.196717		uw	2023-08-16 14:19:51
43	U39	CON43	0.447	mg/L	0.021994		uw	2023-08-16 14:20:44
44	U40	B	0.300ELL	mg/L	0.014013		uw	2023-08-16 14:21:37

PAR, Water Temperature, and pH.

Table C.17

Results for PAR, pH, and water temperature from Block 4 including Pineview water (Con41, Con42, Con43)

Sample ID	PAR (µmol*m^2*sec^{-1}) (Day 0 and Day 4)	pH	Water Temperature (°C)
A41	46	10.21	27.8
A42	48	10.55	27.8
A43	48	10.7	27.8
B41	47	10.33	27.8
B42	47	10.31	27.8
B43	47	10.65	27.8
C41	47	10.08	27.8
C42	47	10.48	27.8
C43	48	10.48	27.8
D41	47	10.6	27.8
D42	47	10.18	27.8
D43	47	10.33	27.8
E41	46	9.81	17.9
E42	48	9.78	17.9
E43	48	10.04	17.9
F41	46	9.85	17.9
F42	50	9.66	17.9
F43	52	10	17.9
G41	51	9.8	17.9
G42	48	9.84	17.9
G43	48	9.92	17.9
H41	47	9.83	17.9
H42	47	10	17.9
H43	51	9.92	17.9
CON41	46	8.68	27.8
CON42	48	8.66	27.8
CON43	47	8.64	27.8

Appendix D. Statistical Analysis

Figure D.1

Residuals plot for untransformed microcystin concentrations

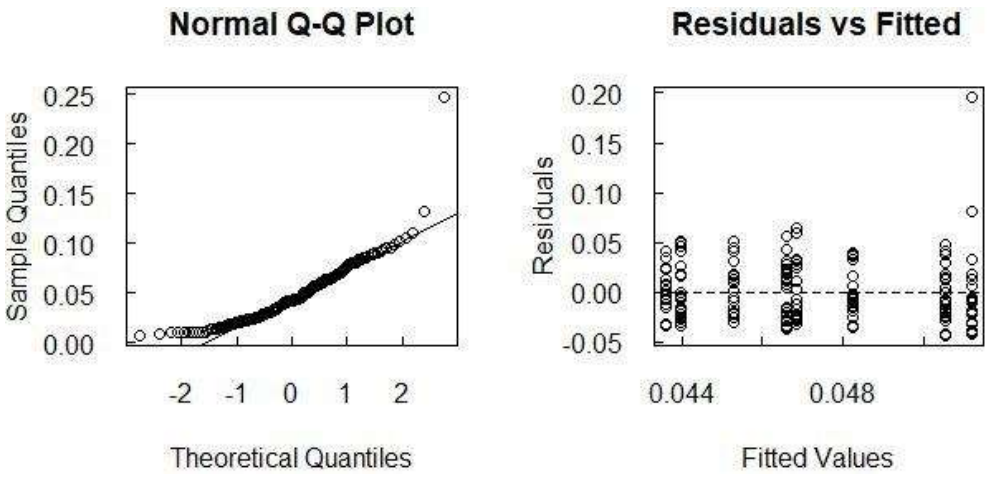


Figure D.2

Residuals plot of transformed microcystin concentrations with λ value of 0.228

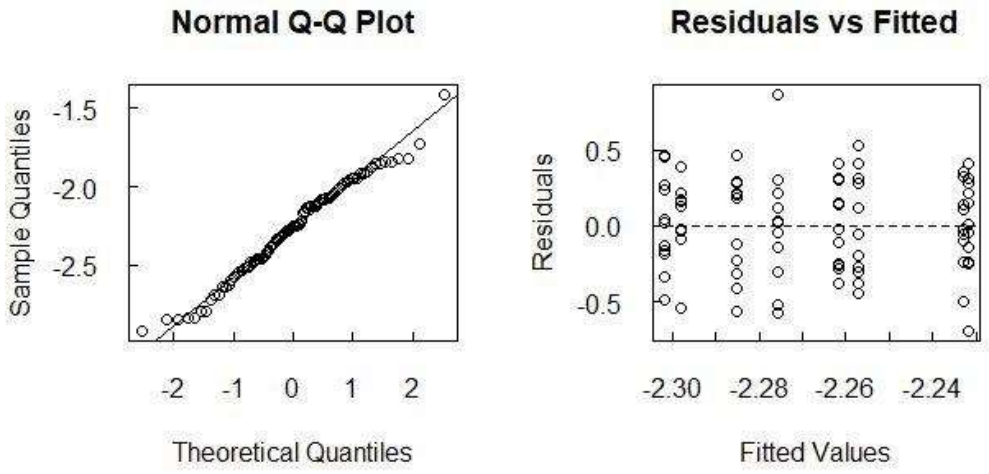


Figure D.3

ANOVA table for transformed microcystin concentrations from all 4 four blocks combined including blocks being a variable

```
Call:
lm(formula = TransAdjustMC ~ (x1 + x2 + x3)^3 + Block, data = output_average)

Residuals:
     Min       1Q   Median       3Q      Max
-0.29604 -0.09806 -0.01061  0.10568  0.58016

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.688297   0.038243 -70.295  < 2e-16 ***
x1          -0.007167   0.017140  -0.418    0.677
x2           0.013628   0.017159   0.794    0.429
x3          -0.002204   0.017152  -0.128    0.898
BlockB2      0.276325   0.050389   5.484 4.97e-07 ***
BlockB3      0.680557   0.050334  13.521  < 2e-16 ***
BlockB4      0.624990   0.050869  12.286  < 2e-16 ***
x1:x2        0.010648   0.017162   0.620    0.537
x1:x3       -0.016131   0.017152  -0.940    0.350
x2:x3       -0.006485   0.017141  -0.378    0.706
x1:x2:x3    -0.007357   0.017141  -0.429    0.669
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1607 on 78 degrees of freedom
  (7 observations deleted due to missingness)
Multiple R-squared:  0.7577,    Adjusted R-squared:  0.7266
F-statistic: 24.39 on 10 and 78 DF,  p-value: < 2.2e-16
```

Note. Response variables are represented as x1 (phosphorus), x2 (N:P ratio), and x3 (water temperature) with a lambda value of 0.228 used.

Figure D.4

Comparison of transformed microcystin concentration using a Tukey test to see differences between blocks

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = TransMC ~ Block, data = all_blocks_dataAMC)

$Block
                       diff        lwr        upr     p adj
Block 2-Block 1  0.27536081  0.1477239 0.40299770 0.0000012
Block 3-Block 1  0.68046203  0.5528251 0.80809893 0.0000000
Block 4-Block 1  0.62188306  0.4930625 0.75070363 0.0000000
Block 3-Block 2  0.40510123  0.2869324 0.52327004 0.0000000
Block 4-Block 2  0.34652225  0.2270759 0.46596860 0.0000000
Block 4-Block 3 -0.05857898 -0.1780253 0.06086737 0.5748297
```

Figure D.5

ANOVA table for transformed microcystin values from Block 1

```
Call:
lm(formula = TransAdjustMC ~ (x1 + x2 + x3)^3, data = output_average[output_average
$Block ==
    "B1", ])

Residuals:
     Min       1Q   Median       3Q      Max
-0.22180 -0.09352 -0.02156  0.12271  0.22180

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.68671    0.04383 -61.297 3.25e-14 ***
x1          -0.04227    0.04383  -0.964    0.358
x2           0.02357    0.04383   0.538    0.603
x3          -0.05072    0.04383  -1.157    0.274
x1:x2       -0.01594    0.04383  -0.364    0.724
x1:x3        0.02862    0.04383   0.653    0.529
x2:x3       -0.02432    0.04383  -0.555    0.591
x1:x2:x3    -0.00615    0.04383  -0.140    0.891
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1753 on 10 degrees of freedom
  (6 observations deleted due to missingness)
Multiple R-squared:  0.2723,    Adjusted R-squared:  -0.237
F-statistic: 0.5346 on 7 and 10 DF,  p-value: 0.7907
```

Note. Response variables are represented as x1 (phosphorus), x2 (N:P ratio), and x3 (water temperature) with a lambda value of 0.228 used.

Figure D.6

ANOVA table for transformed microcystin values from Block 2

```
Call:
lm(formula = TransAdjustMC ~ (x1 + x2 + x3)^3, data = output_average[output_average
$Block ==
    "B2", ])

Residuals:
      Min        1Q    Median        3Q       Max
-0.165197 -0.044931  0.004722  0.024689  0.227385

Coefficients:
            Estimate Std. Error  t value Pr(>|t|)
(Intercept) -2.411972   0.020448 -117.959   <2e-16 ***
x1          -0.001840   0.020448   -0.090   0.9294
x2          -0.033292   0.020448   -1.628   0.1230
x3           0.033156   0.020448    1.622   0.1244
x1:x2       -0.023137   0.020448   -1.132   0.2745
x1:x3       -0.056478   0.020448   -2.762   0.0139 *
x2:x3        0.002101   0.020448    0.103   0.9194
x1:x2:x3    -0.017114   0.020448   -0.837   0.4149
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1002 on 16 degrees of freedom
Multiple R-squared:  0.4824,    Adjusted R-squared:  0.2559
F-statistic:  2.13 on 7 and 16 DF,  p-value: 0.09975
```

Note. Response variables are represented as x1 (phosphorus), x2 (N:P ratio), and x3 (water temperature) with a lambda value of 0.228 used.

Figure D.7

ANOVA table for transformed microcystin values from Block

```
Call:
lm(formula = TransAdjustMC ~ (x1 + x2 + x3)^3, data = output_average[output_average
$Block ==
    "B3", ])

Residuals:
     Min       1Q   Median       3Q      Max
-0.37050 -0.09206 -0.03697  0.11209  0.46537

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.01453    0.04433 -45.441   <2e-16 ***
x1           0.02515    0.04433   0.567    0.578
x2           0.02420    0.04433   0.546    0.593
x3           0.01932    0.04433   0.436    0.669
x1:x2        0.06053    0.04433   1.365    0.191
x1:x3       -0.04635    0.04433  -1.046    0.311
x2:x3       -0.02741    0.04433  -0.618    0.545
x1:x2:x3     0.03456    0.04433   0.779    0.447
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2139 on 16 degrees of freedom
Multiple R-squared:  0.233,     Adjusted R-squared:  -0.1026
F-statistic: 0.6943 on 7 and 16 DF,  p-value: 0.6764
```

Note. Response variables are represented as x1 (phosphorus), x2 (N:P ratio), and x3 (water temperature) with a lambda value of 0.228 used.

Figure D.8

ANOVA table for transformed microcystin values from Block 4

```
Call:
lm(formula = TransAdjustMC ~ (x1 + x2 + x3)^3, data = output_average[output_average
$Block ==
    "B4", ])

Residuals:
      Min        1Q    Median        3Q       Max
-0.223817 -0.088741  0.003863  0.056823  0.232754

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.062346   0.028535 -72.275   <2e-16 ***
x1          -0.026149   0.028535  -0.916    0.374
x2           0.041848   0.028535   1.467    0.163
x3          -0.029716   0.028535  -1.041    0.314
x1:x2        0.010748   0.028535   0.377    0.712
x1:x3        0.009267   0.028535   0.325    0.750
x2:x3        0.010602   0.028535   0.372    0.715
x1:x2:x3    -0.035091   0.028535  -1.230    0.238
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1356 on 15 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared:  0.2876,    Adjusted R-squared:  -0.04479
F-statistic: 0.8653 on 7 and 15 DF,  p-value: 0.5544
```

Note. Response variables are represented as x1 (phosphorus), x2 (N:P ratio), and x3 (water temperature) with a lambda value of 0.228 used.

Figure D.9

Residual plots for microcystin quotas before transformation

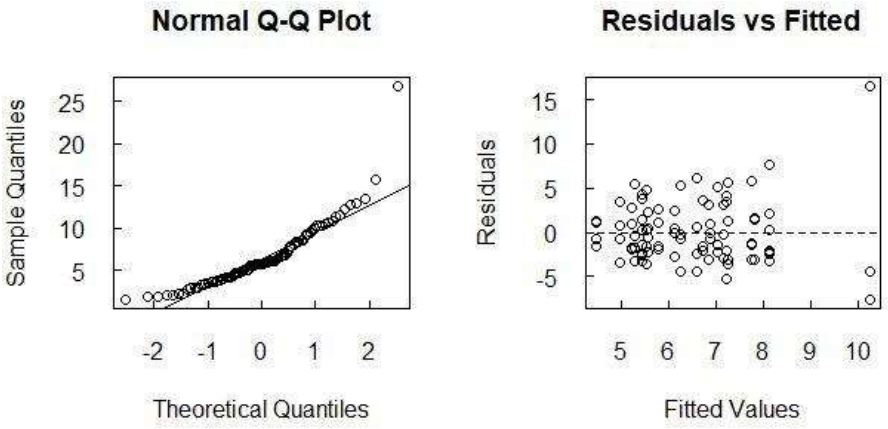


Figure D.10

Residuals plot of transformed microcystin quotas with ⌊ value of 0.065

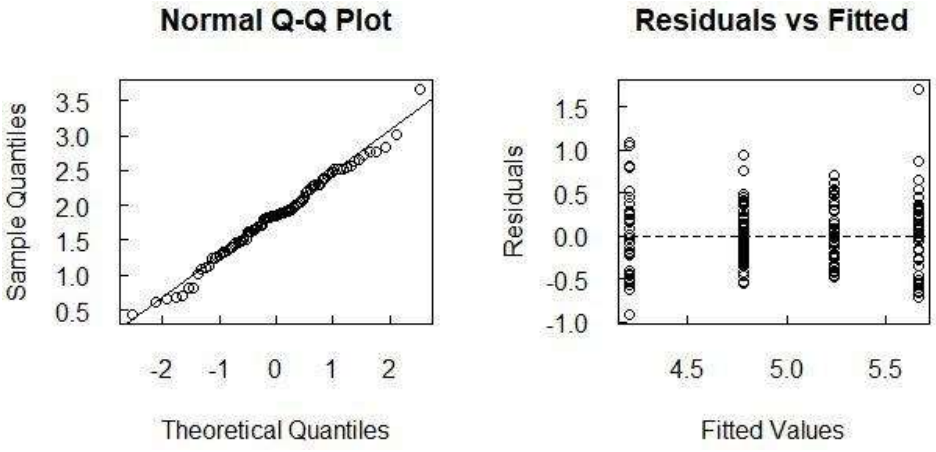


Figure D.11

Comparison of microcystin quotas (fg/cell) using a Tukey test to see differences between blocks

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = TransformedData ~ Group, data = data_df)

$Group
            diff        lwr        upr     p adj
B2-B1  0.5108823  0.2154067  0.8063579 0.0001106
B3-B1  1.3254322  1.0299566  1.6209078 0.0000000
B4-B1  0.9336184  0.6354026  1.2318342 0.0000000
B3-B2  0.8145500  0.5409927  1.0881072 0.0000000
B4-B2  0.4227361  0.1462214  0.6992508 0.0007482
B4-B3 -0.3918139 -0.6683286 -0.1152991 0.0020336
```

Figure D.12

Comparison of total phosphorus concentrations using a Tukey test to see differences between blocks

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = ValuesTP ~ Block, data = combined_TP_data)

$Block
                       diff        lwr        upr     p adj
Block 2-Block 1  2.23041667  1.8270373 2.63379602 0.0000000
Block 3-Block 1  1.91916667  1.5157873 2.32254602 0.0000000
Block 4-Block 1  2.20500000  1.8016207 2.60837935 0.0000000
Block 3-Block 2 -0.31125000 -0.7146293 0.09212935 0.1885341
Block 4-Block 2 -0.02541667 -0.4287960 0.37796268 0.9983942
Block 4-Block 3  0.28583333 -0.1175460 0.68921268 0.2551251
```

Figure D.13

Comparison of total nitrogen concentrations using a Tukey test to see differences between blocks.

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = ValuesTN ~ Block, data = combined_TN_data)

$Block
                       diff        lwr        upr   p adj
Block 2-Block 1   9.050875   7.162200  10.939550 0.0e+00
Block 3-Block 1   3.722958   1.834284   5.611633 8.4e-06
Block 4-Block 1  -6.458917  -8.347591  -4.570242 0.0e+00
Block 3-Block 2  -5.327917  -7.216591  -3.439242 0.0e+00
Block 4-Block 2 -15.509792 -17.398466 -13.621117 0.0e+00
Block 4-Block 3 -10.181875 -12.070550  -8.293200 0.0e+00
```

Figure D.14

Comparison of pH values using a Tukey test to see differences between blocks

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = ValuespH ~ Block, data = combined_pH_data)

$Block
                        diff         lwr        upr     p adj
Block 2-Block 1  0.009583333 -0.15697931  0.1761460 0.9987752
Block 3-Block 1  0.273333333  0.10677069  0.4398960 0.0002507
Block 4-Block 1 -0.540000000 -0.70656264 -0.3734374 0.0000000
Block 3-Block 2  0.263750000  0.09718736  0.4303126 0.0004355
Block 4-Block 2 -0.549583333 -0.71614598 -0.3830207 0.0000000
Block 4-Block 3 -0.813333333 -0.97989598 -0.6467707 0.0000000
```

Figure D.15

Comparison of pH measurements at 25ºC and 16ºC using a t-test

```
        Welch Two Sample t-test

data:  group1 and group2
t = 3.2948, df = 80.389, p-value = 0.001467
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.09331585 0.37793415
sample estimates:
mean of x mean of y
 10.73312  10.49750
```

Figure D.16

Comparison of pH measurements at added dissolved phosphorus concentrations of 0.015 mg P/L and 0.085 mg P/L

```
        Welch Two Sample t-test

data:  group1 and group2
t = 2.5024, df = 83.914, p-value = 0.01428
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.03233632 0.28266368
sample estimates:
mean of x mean of y
 10.73167  10.57417
```

Figure D.17

Comparison of pH measurements at added dissolved N:P ratios of 4:1 and 25:1

```
        Welch Two Sample t-test

data:  group1 and group2
t = 0.9674, df = 93.998, p-value = 0.3358
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.0765208  0.2219375
sample estimates:
mean of x mean of y
 10.65167  10.57896
```