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Seasonal Nutrient Limitations of Cyanobacteria, Phytoplankton, and Cyanotoxins in Utah Lake

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ABSTRACT

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Excess nutrients from human activity trigger toxic cyanobacterial and algal blooms, creating expansive hypoxic dead zones in lakes, damaging ecosystems, hurting local economies, undermining food and water security, and directly harming human health. To identify when and where nutrients limit phytoplankton and cyanobacterial growth, and cyanotoxin concentrations across Utah Lake, USA we conducted four *in-situ* bioassay studies (563 cubitainers or experimental units) that experimentally added N, P or N+P over the spring, early summer, summer, late summer, and fall in lake water from the top 20 cm of the water column. For our purpose, we defined total phytoplankton as all prokaryotic or eukaryotic organisms containing chlorophyll-a. We evaluated changes in chlorophyll-a and phycocyanin concentrations; the abundance of cyanobacterial species and total phytoplankton species or divisions; cyanotoxin concentrations of the microcystin, anatoxin-a, and cylindrospermopsin; DIN, SRP, TP, and TN concentrations; and other water chemistry parameters. We found that the nutrient limitation of cyanobacteria, and to a lesser extent phytoplankton, was influenced by season and space. Cyanobacteria were often co-limited in the spring or early summer, limited by a single nutrient in the summer, and not limited by N or P in the late summer and fall. Alternatively, phytoplankton were co-limited from the summer into the fall in the main body of the lake and either N limited or co-limited continually in Provo Bay. *Microcystis, Aphanocapsa*, *Dolichospermum, Merismopedia,* and *Aphanizomenon spp.*, and *Aulacoseira* and *Desmodesmus spp.* and two taxonomical categories of algae (i.e., unicellular and colonial green algae) were primarily associated with cyanobacteria and phytoplankton nutrient limitations. Concentrations of the three cyanotoxins demonstrated a seasonal signal and loosely followed the growth of specific cyanobacteria but was not dependent on total cyanobacterial cell density. The DIN and SRP were biologically available in all water and nutrient treatments with nutrient concentrations declining over the incubation period, suggesting that nutrient levels were not oversaturated. Our results offer insights into specific nutrient targets, species, and, and cyanotoxins to consider in the future to manage Utah Lake.

Keywords: nutrient limitation, harmful algal bloom, cyanobacteria, cyanotoxins

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INTRODUCTION

Human activity has resulted in dramatic changes to local and global biogeochemical cycles, affecting nutrients, sources, removal pathways and availability (Frei et al. 2020). Nitrogen (N) and phosphorous (P) are commonly co-limiting to growth in freshwater ecosystems, and – when added in conjunction with one another – cause algae and cyanobacteria to spike in a phenomenon referred to as an algal bloom (Elser et al. 2007; Aanderud et al. 2016). Cyanobacteria and algae become dominant under specific physiochemical water conditions, generally connected to excessive P and N loading (Lewis et al. 2011; Paerl et al. 2011; Davis et al. 2015; Paerl et al. 2016; Descy et al. 2016; Song et al. 2017; Jankowiak et al. 2019;). Specific P and N pools are more bioavailable than others and nutrient chemical forms also influence HABs (Paerl et al. 2008).

Additionally, the relative abundance of cyanobacterial and phytoplankton species is governed by more than excessive N and P (Wood et al. 2017; Randall et al. 2019). Weather fluctuations (e.g., temperature, wind speed, and solar irradiance) may favor different species and influence bloom intensity and composition (Wu et al. 2016). The composition of species in a bloom is important because green algal species such as *Aulacoseira*, *Pediastrum*, and *Desmodesmus spp.* may contribute to the overall growth, however, only cyanobacteria produce cyanotoxins. Cyanotoxins are created by specific cyanobacteria species with different cyanotoxins requiring various levels of energy and N investment. The production of toxins is likely linked to intracellular C and N regulation and to a lesser extent P (Davis et al. 2009). For example, production of the neurotoxin, anatoxin-a , is inhibited by internal high C:N ratios, and mildly stimulated by low C:N ratios (Tao et al. 2020). Alternatively, microcystin synthesis tends to be upregulated following intracellular high C:N ratios, especially when extracellular NH_4^+

concentrations are low (Downing et al. 2005; Beversdorf et al. 2013). Further cyanobacterial biomass production as well as hepatotoxic microcystin and neurotoxic anatoxin production were N and P co-limited with microcystin production (Barnard et al. 2021). Still, predictors of algal blooms relating to specific species and cyanotoxin production remain highly uncertain, especially in the context of generating cyanotoxins.

Knowing which nutrient to control/regulate is key in the remediation of HABs, as the absolute and relative abundance of N and P may determine phytoplankton and cyanobacterial growth rates and abundances (Bergstrom 2010). Climate change has brought increased winter rainfall and more short, intense storms that lead to erosion and an influx of nutrient runoff into freshwater bodies (Jeppsen et al. 2009). Paired with the growing human populations and resulting increases in effluent from WWTPs, more freshwater bodies are excessively loaded with nutrients, specifically N and P (Galloway et al. 2004; Haygarth et al. 2005; Foley et al. 2011).

When N and P are available, seasonal temperatures may structure HAB responses. Primary production in nutrient-rich and warmer waters may lead to cyanobacterial dominance due to their preference for slightly warmer temperatures (Paerl et al. 2009). A multi-lake analysis revealed that nutrients rather than temperature predominantly control cyanobacterial biovolume, with certain taxa more sensitive to nutrients, and others more responsive to temperature (Rigosi et al. 2014). However, it is unclear whether cyanobacterial growth rates increase enough with higher temperatures to give these species the competitive edge over other phytoplankton, specifically green algae. Optimum growth temperatures vary between organisms; cyanobacterial growth peaks at temperatures higher than 25°C, while the temperature range for green algae is between 27–32°C, and dinoflagellates and diatoms prefer even cooler temperatures at 17–27°C (Paerl et al., 2014). When waters are cooler in the spring and fall, cyanobacterial growth rates are lower

than those of green algae potentially signaling algal dominance early in the season (Lurling et al. 2013). Further, optimum growth temperatures (30–35°C) for cyanobacteria may differ from the optimal temperatures for cyanotoxin production (≈25°C) decoupling growth from toxicity (van der Westhuizen et al. 1986; Gorham et al. 1964). Nutrient enrichment may have a more dramatic effect on cyanobacterial and algal biomass than increasing temperature (Lurling et al. 2018).

HAB biology is innately complex. They are often dominated by multiple different phytoplankton and cyanobacteria species responding to a host of environmental factors while acting as the primary producers of lake food webs (Randall et al. 2019; Wood et al., 2017). Many eukaryotic grazers prey on phytoplankton (Work 2003), but other ecological phenomena exist in lake food webs that affect cyanobacteria populations. For example, zooplankton grazing reduced N_2-fixation of filamentous cyanobacteria by 40% as filamentous length decreased and reduced the growth of cyanobacteria (Chan et al. 2004). In general, cyanobacteria are a poor nutrient source for zooplankton and may either produce toxins or contain intracellular toxins causing zooplankton to selectively graze on algae, but selective grazing may facilitate the bloom of marginalized cyanobacterial species (Work 2003). This phenomenon is known as the 'predation release' or 'ecological release' hypothesis: when a given species is freed from specific limiting factors, such as competition or grazing pressure, the species population may dramatically increase. Additionally, cyanobacterial growth form may also influence grazing potential. For example, colonial or filamentous growth of certain cyanobacterial species may render the species inedible by eukaryotic grazers because they become too large to ingest and may even disrupt feeding behavior (Gilbert & Durand 1990).

HABs are especially problematic in shallow lakes because of the close proximity of interactions among the water, land, atmosphere, and sediment (Gulati et al. 2007; Qin et al.

2007). Often, shallow lake systems transition from P limitation early in the growing season to N limitation later in the season (Xu et al. 2010; Paerl 2011; Paerl et al. 2019) providing opportunities for algal-dominated waters to transition to late-season cyanobacterial dominance due to their N-fixing capabilities. In other lakes, non-N fixing cyanobacteria may dominate throughout the bloom season, or N-fixing species may increase but may not be actively fixing N. Many HAB dynamics remain elusive, such as the exact form and amount of P or N necessary to initiate or sustain blooms in nutrient-rich waters or the extent that dissolution of nutrients from sediments alter HABs (Ogdahl et al. 2014). Certain forms of P and N may elicit, but more likely intensify specific cyanobacteria and algal species. Shallow lakes are especially prone to P release given high surface area to volume ratio, making sediment-water interactions a particularly key role in dissolved P exchange (Søndergaard et al. 2013; Xu et al. 2021). Internal P fluxes from sediments to the water column often results in time lags for shallow lake restoration after reduction in external nutrient loads (Jeppesen et al. 2005; Scheffer et al. 1993; Sharpley et al. 2013; Søndergaard et al. 2013). Increased eutrophication in shallow systems may become the norm further pressing the need to understand the ecology and nutrient relations surrounding even more intense HABs.

Utah Lake, one of the largest natural freshwater lake in the western U.S., is experiencing frequent and extensive HABs leading to lake impairment due to nutrient overloading, altered hydrology, and climate (PSOMAS 2007; Randall et al. 2019). Utah Lake is a shallow lake housing the remnant of Pleistocene Lake Bonneville with an average area of 375 km^2 and average depth of 3 m (maximum depth of 6 m) under average lake levels. The lake is located in rapidly urbanizing Utah Valley, with a population >500,000 on the east side of the lake, which is expected to double by 2050. The temporal and spatial nutrient limitation dynamics of HABs in

Utah Lake are poorly understood, but their effects are often felt in the form of recreational advisories and a few localized beach closures (https://deq.utah.gov/Divisions/dwq/health-advisory/harmful-algal-blooms/). As a basin bottom lake in a rapidly urbanizing area, Utah lake receives nutrients from agricultural runoff, wastewater effluent, natural P in the local geology, and atmospheric deposition (PSOMAS 2007). From the east, Utah Lake is bordered by seven wastewater treatment plants, three of which discharge into Provo Bay. The western portion of Utah Lake experiences much less urban influence, but continued population growth may increase the nutrient loading in the near future.

MATERIALS AND METHODS

Seasonal and Spatial Bioassay Study Design and Lake Locations

We conducted the bioassay studies with water across the three locations capturing the differences in nutrient inputs to Utah Lake (Collins 2019). The specific locations for each of the locations was as follows: main body East (40°14’16”N, 111°45’56”W), main body West (40°15’33”N, 111°50’22”W), and Provo Bay (40°10’42”N, 111°42’41”W). Nearly all urban development borders the east side of Utah Lake, providing an opportunity to evaluate HABs in relation to a gradient of N and P concentrations in the water column and legacy sediments between the east and west sides of the lake (Randall et al. 2019). Provo Bay is a unique area of the lake (Collins 2019). Provo Bay waters are poorly mixed (i.e., sheltered from the wind), highly impacted by urbanization, extremely biologically productive often leading to anaerobic conditions and potential alterations in N and P availability. Bioassay experimental unit consisted of 3L of lake water added to a 3.8 L cubitainer. For each location, the water in the cubitainers was from 180 L of lake water collected from the top 20 cm of the water column pooled into one

200 L plastic drum. The lake water used in all cubitainers was passed through a Wisconsin net (153 μm mesh size) at the time of collection to remove zooplankton potentially influencing phytoplankton and cyanobacteria. For the seasonal bioassay study each treatment (control, N, P, N+P) had three replicates for a beginning (time zero) and end (time one) timepoint. The N, P, and N+P amendments were performed by directly adding 1 mL of a specific stock solution to respective treatment cubitainers: the P amendment equaled an increase in 0.10 mg-P/L above background concentrations added as K_2HPO_4, the N amendment equaled an increase in 0.72 mg-N/L added as NH_4NO_3 to achieve a 16:1 molar ratio of DIN:SRP, and the N+P treatment was the combination of the N and P amendments. All three nutrient treatments and control received C amendments in the form of 1 ml of 221.8 mg $NaHCO_3$ to alleviate CO_2 limitation to photosynthesis, at a rate to support production of 100 ug/L chlorophyll, based on preliminary inorganic C levels in the lake. For the seasonal bioassay study there was a total of 360 replicates or cubitainers=three locations × five seasons × four treatments (control, N, P, N+P) × two time points × three replicates.

Seasonal Sampling Times, Bioassay, and HAB designation

We conducted bioassay manipulations during five time points to capture the seasonal component of HAB-nutrient interactions. The times included: spring (4-8 May 2020), early summer (15-19 June 2020), summer (22-26 July 2019), late summer (26-30 August 2019), and fall (7-11 October 2019). Cubitainers were incubated in a common water garden at the Utah Lake State Park to allow for accessibility and to maintain similar light and temperature conditions. We placed the cubitainers in the floating corrals (diameter 1.5 m) and covered the corrals with shade cloth of reduce incoming solar radiation by ≈30% to reduce light inhibition of

photosynthesis. The plastic cubitainer kept water at a common depth, exposed organisms to similar light and temperature conditions, and filtered an additional 15% of PAR (Paerl et al. 2014).

We sampled the cubitainers at an initial time zero and either 48 (bloom) or 72 (non-bloom) hours (time one) to allow adequate time for the phytoplankton and cyanobacteria to respond based on the initial bloom conditions. For Utah Lake, we defined an active bloom as the initial water conditions possessing a chlorophyll-a concentration equal or above 10 μg/L or a phycocyanin concentration equal or above 1 μg/L measured with a YSI EXO2 multi-parameter sonde (Yellow Springs Instrumentation, Yellow Springs, Ohio). The HAB status is an unofficial designation generated by the researchers over the seasons and was not determined by the UT-DWQ or the ULWQS. We selected 48 and 72 hours as appropriate response times based on results of a time series approach with the first sampling, summer, where assays were sampled at incubations times of one, two, or five days. Most cubitainers were incubated for 48 hours, while cubitainers in the spring and fall experiments in East and West were incubated for 72 hours. If there was already a bloom present when we ran the trial, we identified if N and/or P limited the responses of an active HAB.

Lake Chemistry and Nutrient Analyses

In-situ physicochemical analyses were conducted with a YSI EXO2 sonde (Yellow Springs Instrumentation, Yellow Springs, OH) immediately after opening the cubitainers to estimate of phytoplankton pigments (chlorophyll-a and phycocyanin) temperature, pH, electrical conductivity, and dissolved oxygen (see Jones et al. 2017). TP in the cubitainers was measured using a nitric acid assisted microwave digestion and determination on Thermo Scientific ICP-

OES (Thermo Electron, Madison, WI) and TN was determined using a potassium persulfate digestion followed by flow injection analysis on a rapid flow analyzer (Lachat Instruments, Loveland, CO). We calculated inorganic N as combined values for $N\text{-}NH_4^+$ (N from ammonium) and $N\text{-}NO_3^-$ (N from nitrate) again using a flow injection analysis on a rapid flow analyzer and SRP or orthophosphate using the ascorbic acid method (4500-P E, SM).

Chlorophyll-a and Phycocyanin Concentrations

We evaluated phytoplankton and cyanobacteria, a fraction of phytoplankton, as shifts in chlorophyll-a and phycocyanin and concentrations, respectively. Chlorophyll-a was analyzed via ethanol extraction and evaluation on a microplate spectrophotometer (Spectramax Plus, Molecular Devices, LLC, San Jose, CA) at a wavelength of 665 and 750 nm. Again, shifts in chlorophyll-a represented general trends in all phytoplankton taxa. Phycocyanin, a major phycobiliprotein pigment produced by cyanobacteria, was measured via a phosphate buffer extraction and spectrophotometry (Kasinak et al. 2014).

Cyanobacteria and Total Phytoplankton Determinations

We analyzed species composition (cell counts or biovolume) by direct microscopy for specific cyanobacteria species, but only a general quantitative evaluation of algae to the division level or lower. We focused on five cyanobacterial species and one general category (i.e., *Aphanizomenon, Aphanocapsa, Dolichospermum*, filamentous cyanobacteria, *Merismopedia,* and *Microcystis spp.*) that were often found in the lake. The filamentous category includes *Phormidium*, *Planktothrix*, *Leptolyngbya*, and *Psuedanabeaena spp*. For total phytoplankton, we focused on three dominant eukaryotic species (i.e., *Aulacoseira*, *Pediastrum*, and *Desmodemus*

spp.) and five categories (i.e., pennate diatoms, centric diatoms, dinoflagellates, unicellular green algae, and colonial green algae) of phytoplankton to capture total phytoplankton response to nutrient additions and dilutions. Again, total phytoplankton counts did not include cyanobacterial species, which were evaluated separately.

We completed the cell counts (cells/mL) on a Zeiss Axioplan2 upright fluorescent microscope (Ziess, New York, NY) with a PhotoFluor LM-75 light source. Water for microscopic identification/quantification was collect from the cubitainer with a sterile specimen cups, treated with a Lugol's iodine solution, and stored at room temperature until counting. Counts were performed on 20 mL of sample that was filtered onto 0.2 μm cellulose acetate membrane filter (Advantec Toyo Roshi Kaisha, Ltd., Japan). The cyanobacteria and phytoplankton on the filters were removed/washed from the filters with 2 mL of ultrapure water (milli-q). We performed counts on 100 μL of the 2 mL solution in a Palmer counting cell (volume 0.1 mL, 17.9 mm diameter) at 40x magnification. We performed counts on 20% of the slide or until 600 individual cells were counted. To convert cyanobacterial cell counts (cells/mL) to biovolume (μm^3/mL), we used an average biovolume quantified by Rushforth Phycology LLC (http://www.rushforthphycology.com) for individual species in Utah Lake (Table 1). Direct microscopy was measured in only two of the three replicates for each location, season, and treatments.

Cyanotoxin Quantification

We measured three cyanotoxins–microcystin, cylindrospermopsin, and anatoxin-a using ADDA, anatoxin-a, and cylindrospermopsin enzyme-linked immunosorbent assays or ELISA. Specific toxins were chosen based on the dominant cyanobacteria found in Utah Lake (i.e.,

Aphanizomenon, Microcystis, and Dolichospermum spp.) (Collins 2019). Water for the cyanotoxin analyses was collected from the cubitainer in ashed amber glass vials with a PTFE-lined lids. Anatoxin-a samples received a preservative immediately upon collection to prevent sample degradation. Toxins were then analyzed using the appropriate enzyme-linked immunosorbent assay kit (Eurofins Abraxis, Warminster, PA). Detection limits were as follows: 0.10 ppb microcystin, 0.10 ppb anatoxin-a, 0.04 ppb cylindrospermopsin. Just as with direct microscopy, we measured the three cyanotoxins in only two of the three replicates for each location, season, and treatment combination.

To identify potential links between cyanotoxin concentrations (μg/L) and the cell density (cells/mL) of the cyanobacteria potentially responsible for the producing the toxin, we created a series of linear regression models. cyanobacterial cell density. Specifically, we created models relating each of the three toxins to the cell density of groups of cyanobacterial taxa potentially responsible for the generation of a given toxin (i.e., anatoxin-a = *Aphanizomenon* and *Dolichospermum spp.*; cylindrospermopsin = *Aphanizomenon* and *Dolichospermum spp.,* and filamentous cyanobacteria; and microcystin = *Microcystis*, *Dolichospermum spp.,* and filamentous cyanobacteria) for each season.

Response Ratios and Statistical Analyses

We quantified responses of Cyanobacteria and phytoplankton to potential nutrient limitations as the growth response (ΔR) during the 48-hour or 72-hour incubations. An example of the calculation is as follows:

$$\Delta R = \text{mean chlorophyll-}a\text{ treatment/mean chlorophyll-a control)} \quad (1)$$

The mean chlorophyll-a and phycocyanin was calculate from all possible ratios between the three control and the three treatment replicates for a given nutrient treatment (n = 9). ΔR values above one indicates a positive response to the nutrient additions relative to the control. To identify differences among the ΔR for the nutrient treatments, we performed one-way ANOVAs in R. If a co-limitation was apparent but not significantly higher than N or P, the limitation was designated as a single nutrient limitation. We created jitterplots to demonstrate the overall variability in chlorophyll-a, phycocyanin, and cyanotoxins measurements with the 'ggplot2' package in R, all other figures were generated in SigmaPlot version 14.5.

Time Series Bioassay and Growth Rate Study Design

We evaluated the growth rates of phytoplankton and cyanobacteria to establish the most appropriate time to sample the cubitainers in the seasonal study. Growth rates were evaluated in cubitainers following the same procedure as outlined in seasonal study. The only difference was that cubitainers were evaluated across a time series from 0–96 hours. In addition to our initial measurements (T_0), we included three other time points: T_1–24, T_2–48, and T_3–96 hours. The 96-hour time allowed the potentially slower cyanobacterial species to respond and for cyanobacterial growth to catch up to chlorophyte growth. All analyses that were performed in the seasonal study were also performed on these time-series replicates. For the time series bioassay study there was a total of 144 replicates or cubitainers=three locations × four treatments (control, N, P, N+P) × four incubation time points (0, 24, 48, 96 hours) × three replicates.

Phytoplankton and cyanobacterial growth rates and statistics

We calculated phytoplankton and cyanobacterial growth as the specific growth rate (μ) based on the first-order rate law using the equation:

$$\mu = ln(N_1/N_2)/t_1 - t_0 \quad (2)$$

where N_0 was the pigment concentration per mL at time t_0 and N_1 was the pigment concentration at time t_1. As with the first study we created jittered boxplots for chlorophyll-a, phycocyanin, and cyanotoxins with the 'ggplot2' package in R, other figures were generated in SigmaPlot version 14.5.

RESULTS

HAB status Prior to Bioassay

The HAB status of water prior to incubations varied by location and season. For example, in the main body East and West location, HABs were present in the early summer and late summer based on both chlorophyll-a (> 10 μg/L) and phycocyanin (>1 μg/L; Figure 1). Conversely, Provo Bay waters were always in a bloom state, except in the spring, again based on chlorophyll-a and phycocyanin.

Initial Nutrient Concentration in Bioassay

Biologically available DIN ($N\text{-}NH_4^+ + N\text{-}NO_3^- + N\text{-}NO_2^-$), and to a lesser extent SRP, varied across seasons with loads being similar in the main lake body compared to Provo Bay. The DIN load in the lake was higher in the spring across all locations (Figure 2). DIN was also relatively

high in the fall in the main body of the lake reaching a high of 0.44 mg/L ±0.08 (mean ± standard error) in the East and 0.38 mg/L ±0.09 in the West. The concentration of SRP was relatively low across all seasons in the main body of the lake but was dramatically higher during the summer in Provo Bay (0.26 mg/L ±0.06). DIN:SRP in the main body of the lake never exceeded 0.96, while the ratio reached 10.9 in the summer and averaged 4.3 (±1.7) across the seasons in Provo Bay.

DIN and SRP After Incubation

DIN and SRP amendments initially elevated the nutrient concentrations by 0.72 mg/L and 0.10 mg/L, respectively. At the conclusion of the incubation period, the final DIN and SRP concentrations were almost always lower than the corresponding amount of DIN and/or SRP added with the treatment (Table 2). However, in several treatments (i.e., DIN addition in the N treatment in East and West during summer, and West during late summer; and SRP addition in the N+P treatment in Provo Bay during summer, and P treatment in Provo Bay during late summer) in the summer, late summer, and fall, the DIN and SRP concentrations at the conclusion of the incubation period were similar to the nutrient addition levels. The nutrient treatments followed a DIN:SRP of 16:1. Even after DIN and SRP were used by the phytoplankton and cyanobacteria, the DIN:SRP for the N+P treatment was strikingly close to 16:1. The only deviation from a final ratio of 16:1 in the N+P treatment occurred in both main body locations in the spring and early summer, in East in late summer, and in Provo Bay during the late summer and fall. After the incubation, the DIN:SRP in the N addition treatment was generally higher than 16:1, while in the P addition treatment DIN:SRP was lower than 16:1 except in the East and Provo Bay in the spring, and the West in late summer.

Water Chemistry

Lake temperature and chemistry followed consistent seasonal patterns but Provo Bay HABs actively altered dissolved oxygen levels and pH (Figure 3). Across all locations, lake temperatures in the summer and late summer were consistently higher than 28.2°C and the lowest temperatures occurred in fall with waters never reaching above 11.2°C. HAB activity in Provo Bay elevated dissolved oxygen levels by at least 31% and pH by 1.3 in summer and late summer relative to the other two locations.

Cyanobacteria Nutrient Colimitation

Nutrient colimitations of cyanobacteria occurred in spring in the main body West and in the early summer in Provo Bay. In the spring, Δ*R* for phycocyanin demonstrated that N and P co-limited cyanobacteria in the West (Figure 4). Colimitation occurred due to all of the three nutrient treatments inducing at least a 2-fold increase in phycocyanin relative to the control measured as Δ*R* (Table 2). For Provo Bay waters, cyanobacterial responses were limited by P in the spring (one-way ANOVA by treatment: F value = 4.97, $P = 0.02$, $df = 2$) but the P limitation continued into the early summer when cyanobacteria was also co-limited. The variation of phycocyanin concentrations is provided in Figure 5.

Cyanobacteria Single Nutrient Limitation in Summer

Cyanobacterial nutrient limitation was present in all three locations during the summer. In the East location, Δ*R* for phycocyanin was 50 (±15.3) demonstrating a 50-fold increase in the pigment with the P addition (one-way ANOVA by treatment: F value=5.40, $P=0.01$, $df=2$, Figure 4) resulting in the highest phycocyanin concentration measured in these waters (16.2 ±7.57,

Table 3). In Provo Bay, P also limited cyanobacteria in the summer (one-way ANOVA by treatment: F value=4.82, P=0.21, df=2) as phycocyanin concentrations increased in all treatments from the spring and early summer. Alternatively, in the West location, ΔR of phycocyanin was 3.1 (±0.44) following the N addition (ANOVA by treatment: F value=5.84, P=0.009, df=2).

No Nutrient Limitation in Late Summer and Summer

In the late summer and fall, the ΔR for phycocyanin was not above 1 or the error bars of a treatment overlapped 1, indicating that cyanobacterial responses in the nutrient treatments were not different from the control (Figure 4). During these later seasons, phycocyanin concentrations in all nutrient amendments and the control remained relatively high (Table 3).

Total Phytoplankton Nutrient Limitation

Nutrient colimitation of phytoplankton occurred in the summer, late summer, and fall in the main body of the lake, and in the late summer in Provo Bay. In the East location, the addition of N+P more than the single additions of N or P led to a higher ΔR value for chlorophyll-a ranging from 3.7 (±0.38) in the summer to 6.4 (±0.35) in the early summer (Figure 6). In the other main body location, West, ΔR for chlorophyll-a was 10 (±2.4) for the N+P treatment (one-way ANOVA by treatment: F value = 246, $P < 0.0001$, $df = 2$) resulting in the highest chlorophyll-a concentration measured in these waters (136 ±51.7, Table 2). The colimitation during the fall in the East and West locations was due to all three nutrient treatments inducing a ΔR higher than 1 but none of the treatments were significantly different from each other. In Provo Bay, chlorophyll-a concentrations were limited during every season with N limiting phytoplankton responses through the summer and into the fall. One exception to this N-limitation occurred

during the late summer as the addition of N, P, and N+P led to a ΔR of at least 2.5 when chlorophyll-a concentrations were at a maximum for the Provo Bay (Figure 7).

Cyanobacteria Cell Counts in Bioassay

The cyanobacteria associated with the nutrient limitations varied between the main body of the lake and Provo Bay, and among seasons. During the summer, *Microcystis sp.* was associated with cyanobacterial P limitation in the East (46 ±26 cell/mL) and N limitation in the West location (46 ±26 cell/mL, Figure 8). *Merismopedia sp.* (62 ±8.8 cells/mL) also contributed to the cyanobacterial response to P in East waters. The N+P colimitation in the West location was associated with predominantly *Aphanocapsa sp.* (659 ±482 cells/mL) and to a lesser extent *Microcystis* and *Merismopedia spp* in the spring. In the Provo Bay, *Aphanocapsa*, *Dolichospermum, Merismopedia,* and *Aphanizomenon spp.* were associated with the P limitation in summer and with N+P in the early summer. *Aphanocapsa* and *Dolichospermum spp.* were the most abundant taxa responding to nutrient limitations. For example, in summer under P limitation, the cell count (cell/mL) of *Aphanocapsa* was 3.18E+4 (±2.01E+4) *Dolichospermum* was 1.66E+4 (±4.18E+3), while *Merismopedia* was 7.74E+3 (±5.68E+3), and *Aphanizomenon* was 7.86E+3 (±2.05E+3).

When cyanobacterial cell counts were converted to biovolume, the cyanobacteria responding to the nutrient limitation demonstrated a similar pattern within the main body of the lake and Provo Bay, and among seasons with two distinct changes. First, *Dolichospermum sp.* became the most abundant species on Provo Bay waters regardless of treatment, in spring, early summer, summer, and late summer (Figure 9). Second, *Aphanizomenon sp.* dominated East water in late summer.

Phytoplankton Cell Counts in Bioassay

Aulacoseira and *Desmodesmus spp.* and two taxonomical categories of algae (i.e., unicellular and colonial green algae) were primarily associated with the phytoplankton nutrient limitations across Utah Lake among the seasons. Unicellular and colonial green algae were the primary phytoplankton associated with the N+P limitations in the in East and West consistently demonstrating the highest cell counts among phytoplankton. *Aulacoseira*, in the late summer, and *Desmodesmus*, across all seasons, contributed to the phytoplankton responses but to a lesser extent based on cell counts (Figure 10). In the bay Provo Bay, a similar pattern appeared with unicellular and colonial green algae, but *Desmodesmus spp.* played a more dominant role with the cell counts of this species ranging from 1.35E+4 (±1.84E+3) in the summer N treatment to 2.56E+3 (±1.06E+3) in the late summer N+P treatment. The overall concentration of phytoplankton (cells/L) following all nutrient treatment and controls was highest in Provo Bay (3.39E+5 ±2.35E+3), moderate in East (9.49E+4 ±1.62E+3), and lowest in West (8.22E+4 ±1.40E+3).

Cyanotoxin Concentrations in Bioassay

The three cyanotoxins demonstrated a seasonal signal that was not dependent on the cell density of cyanobacteria know to generate the cyanotoxin. Based on the linear regression models, which included all data from the three lake locations for each season, there was no apparent relationship between the concentrations of the three toxins and counts of cyanobacteria known to produce a given toxin (results from the fifteen linear regression models: df = 21-28, adjusted P values consistently above > 0.05, and adjusted R^2-values ranging from -0.01945 to

0.22). The three cyanotoxins demonstrated a seasonal signal that was not related to cyanobacterial cell density (Figure 9). For example, cylindrospermopsin was highest in the spring (concentration, μg/L, East = 0.082 ±0.012, West = 0.075 ±0.012, 0.08 ±0.01, Provo Bay = 0.032 ±0.014) when cyanobacteria potentially generating this cyanotoxin (i.e., *Aphanizomenon* and *Dolichospermum spp.,* and filamentous cyanobacteria) were low or non-detectable (Figure 9). Anatoxin-a concentrations were generally higher in the spring, late summer, and fall, while microcystin was more prevalent in the early summer and summer, regardless of nutrient treatment or a specific nutrient limitation to phytoplankton (Figure 9, 11).

Growth Response to Nutrient Limitation

At time zero, Provo Bay waters, relative to the other two locations, supported 312-times the phycocyanin (μg/L Provo Bay=3.1 ±0.25, East=0.01 ±0, West=0.01 ±0) and 18-times the chlorophyll-a concentrations (μg/L Provo Bay=53 ±15, East=2.9 ±0.77, West=2.9 ±0.76) and was in an active bloom, based on the researchers' designation (Table 3). The activity of phytoplankton and cyanobacteria most likely increased pH almost an order of magnitude and elevated dissolved oxygen by 52% in Provo Bay compared to the main body of the lake (Figure 13). The water temperatures decreased by more than 2°C during the incubation with the drop occurring between 24-48 hours. Temperature varied from 28.5°C ±0.18 (East T_3) to 32.5°C ±0.37 (Provo Bay T_1).

Phytoplankton and Cyanobacteria Growth Rate

The 48-hour or 72-hour incubations in the time series captured the majority of phytoplankton and cyanobacterial responses (i.e., changes in chlorophyll-a, phycocyanin, and cyanotoxin

concentrations) to DIN and/or SRP additions but the changes were most likely associated with faster- rather than slower-growing phytoplankton and cyanobacteria.

In the summer, phytoplankton growth was generally higher in the first 24 hours of the 96-hour time series and stimulated by P and N+P in the main body of the lake (Figure 14a). In the East and West water, phytoplankton growth rates were consistently stimulated by N+P even after 48 hours, but rapidly declined after 96 hours. In general, chlorophyll-a concentrations continued to climb in the N+P treatment during the 96-hour incubation. In Provo Bay any nutrient addition treatment slightly elevated phytoplankton growth rates.

The relative growth rates of cyanobacteria responded to specific nutrient additions that differed depending on lake location. For cyanobacteria, in East water, P and N+P additions enhanced growth rates in the first 24 hours and growth slowed as P was potentially consumed (Figure 14b). Alternatively, the growth rate under N addition was consistent through the 96 hours (ranging from $\mu\ T_1=0.05 \pm 0.025$ - $\mu\ T_2=0.02 \pm 0.026$, Figure 14b). In the West location, the addition of any nutrient resulted in higher cyanobacterial growth rate in the N, P, and N+P treatment than the control, but only for the first 24 hours. After the first 24 hours, the growth rates in all nutrient treatments were slightly negative in the West. In Provo Bay waters, cyanobacterial growth was stimulated by P ($\mu=0.08 \pm 0.003$) in the first 24 hours and by N ($\mu=0.09 \pm 0.005$) in the last 48 hours of the incubation; however, these values were only slightly above the control values.

The variation in the chlorophyll-a and phycocyanin concentrations during the time series is provided in Figure 15.

Phytoplankton and Cyanobacteria Cell Counts During Growth

In the main body of the lake, faster relative growth rates of phytoplankton following N+P additions were associated with different species through time. In the first 24 hours, unicellular and colonial green algae accounted for much of the phytoplankton biomass. But by 48 hours *Desmodesmus* increased in cell density, and by 96 hours pennate and centric diatoms contributed changes in phytoplankton growth (Figure 14). The effect of the nutrient treatments on phytoplankton species/categories was less apparent in Provo Bay where phytoplankton abundance (cell/mL) was orders of magnitude higher and included multiple green algae categories like *Desmodesmus* and *Aulacoseira spp*. across the entire time series.

In the main body of the lake, the cyanobacterial species that responded in the first 24 hours and accounted for the relatively high growth rates was *Microcystis sp*. (Figure 14). Further, *Microcystis* cell density increased with the addition of N or N+P in the West even after 96 hours of incubations. In Provo Bay water, three species dominated the responses to any nutrient addition: *Aphanocapsa, Dolichospermum, and Aphanocapsa spp*. *Microcystis* was almost absent in this water that supported orders of magnitude more cyanobacteria.

Cyanotoxins During Cyanobacterial Growth

Cyanotoxins loosely followed the growth of cyanobacteria, but not cyanobacterial cell density. In West waters, the enhanced cyanobacterial growth rates under P additions (P and N+P) led to higher concentrations of cyanotoxins, especially cylindrospermopsin (Figure 16). Further, of the species that potentially produce microcystin (*Aphanocapsa, Microcystis*, *Dolichospermum spp.,* and filamentous cyanobacteria)*, Microcystis sp.* contributed to the growth rates in the East and West location where microcystin was often the dominant cyanotoxin captured in the time

series. In the bay compared to other waters, the relatively higher phycocyanin concentrations (Table 3) and cell density of cyanobacteria (Figure 16) did not equate to higher concentrations of cyanotoxins (Figure 17).

DISCUSSION

Our hypothesis regarding a seasonal shift in nutrient limitation commonly found in shallow lakes was partially true for total phytoplankton in the western location of the lake. In the West location, which has few anthropogenic nutrient inputs, phytoplankton responses were limited by P in the spring and N+P in the summer, late summer, and fall. Total phytoplankton responses in East water were consistently co-limited by N as the lake warmed into the summer months. Provo Bay water, which is highly impacted by urbanization and anthropogenic nutrient inputs, was predominantly N limited, except in the spring and late summer when the phytoplankton was co-limited by N and P. *Aulacoseira* and *Desmodesmus spp.*and two taxonomical categories of algae (i.e., unicellular and colonial green algae) were primarily associated with the phytoplankton nutrient limitation across Utah Lake regardless of season. *Aulacoseira*, most likely *Aulacoseira granulate,* is a filamentous diatom that forms abundant gelatinous masses, structured communities in all seasons except spring and fall. *Aulacoseira granulata* occurs frequently across Utah Lake but the cell densities of this diatom are low. Unicellar and colonial green algae that were grouped within a general category included species such as *Crucigeniella sp.* and *Kirchneriella contorta* that commonly occur in Utah Lake with relatively high cell densities. Last, *Desmodesmus spp*., such *Desmodesmus communis*, *Desmodesmus opoliensis*, *Desmodesmus bicellularis,* and *Desodesmus bicellularis*, are common in HAB blooms across Utah Lake, especially in the bay.

Seasonal nutrient limitations for phytoplankton in the main body of Utah Lake followed similar patterns to other shallow lake systems. For example, our seasonal shift from P-limitation to co-limitation or N-limitation is consistent with phytoplankton responses documented in other shallow lake systems (Fang et al. 1993; Kolzau et al. 2014; Andersen et al. 2019). Provo Bay was mostly N-limited, transitioning to co-limitation in the summer, similar to other shallow waterbodies with P-rich sediments and high anthropogenic P-inputs where persistent N-limitation is observed (Filbrun et al. 2013; Xu et al. 2021).

Co-limitation from Biochemical and Community Structure Perspectives

Both N and P are essential elemental nutrients for total phytoplankton and cyanobacterial growth at the biochemical level. P availability is linked to microbial metabolism, cell division, and protein syntheses, and N availability is essential to synthesize proteins, DNA, and bacterial cell walls. These elements interact on a cellular level and may, thus, be biochemically co-limited (Braken et al 2015). Another type of co-limitation may exist at the community level. Communities of primary producers may be stimulated by different nutrients (Arrigo 2005) If the growth of N-fixing species is enhanced by P addition (Karl et al. 1997, Wu et al. 2000), whereas the growth of non-N-fixing species is enhanced by N addition (Suzumura and Ingall 2004) an overall co-limitation will be measured. We believe that our measured colimitation for phytoplankton was predominately biochemical. During the summer seasons across all locations, the ratio of DIN to SRP in the N+P addition treatment remained close to 16:1 in the incubation. Therefore, DIN:SRP was relatively close to 16:1 at the beginning and end of the incubation. The Redfield ratio is 16:1 and represents the consistent atomic ratio of N and P in phytoplankton

biomass. Since the ratio stays the same over the incubation, primary producers potentially utilized N and P in equal proportions to generate biomass and were biochemically co-limited.

Seasonal and Spatial Nutrient Limitation of Cyanobacteria

Our hypothesis for cyanobacteria was also partially correct. Cyanobacterial responses were controlled by P availability in the summer in East and Provo Bay water, but by N availability in the summer in the West. Further, neither P or N limited cyanobacterial response in the late summer or fall. The difference in the summer limitation was potentially linked to the cyanobacteria species residing in the different locations. During the summer, non-fixing *Microcystis sp.,* most likely *Microcystis aeruginosa*, was associated with cyanobacterial nutrient limitation in the East and West and was potentially responded to the addition of N. Alternatively, in the bay, N-fixing *Dolichospermum,* most likely *Dolichospermum circinalis*, and *Aphanizomenon spp.*, most likely *Aphanizomenon flos-aquae*, were associated with nutrient limitation in the early summer and summer and potentially responded to the addition of P. Cyanobacteria generally fare better than total phytoplankton in N-limiting conditions (Tillman et al. 1982; Heil et al. 2007) leading to a seasonal succession where cyanobacteria increased in abundance in the summer months. We found this to be true in our data. In the late summer and fall compared to spring and early summer, cyanobacterial biomass was high and non-responsive to nutrient additions, suggesting that the nutrient requirements of these bacteria were being met. Cyanobacteria may exploit nutrients that are regenerated and tightly cycle within a bloom and fix atmospheric N_2 to satisfy metabolic requirements. The N-fixing and cyanotoxin production capability are summarized in Table 5.

Generally, across the main body of the lake, *Microcystis* and *Aphanocapsa spp.* dominated the cyanobacterial community in waters from the spring to summer, while *Aphanizomenon sp.* dominated in late summer. Similarly, in Provo Bay, *Microcystis aeruginosa* was abundant in water in the spring, early summer, and fall, and *Aphanizomenon Aphanocapsa*, and *Dolichospermum sp.* dominating in the early summer, summer, and late summer. The seasonal patterns that we found for N-fixing *Aphanizomenon flos-aquae* in our treatments followed its early through late summer dominance that is common in Utah Lake (Table 6). However, the additions of P and/or N and in Cubitainers caused *Microcystis aeruginosa* to become dominant earlier in the season than usually measured across the lake. These inferences are based on direct microscopic counts performed by the UT-DWQ.

Seasonal and Spatial Cyanotoxin Levels

We hypothesized that cyanotoxin concentrations will be enhanced as cyanobacterial nutrient limitation was alleviated. We found some evidence of this. In a couple of instances, the alleviation of P or N+P limitation induced the production of cylindrospermopsin. This relationship was also visible in the nutrient dilution bioassay study. Generally, the three cyanotoxins measured demonstrated a seasonal signal that was not dependent on the cell density of cyanobacteria known to generate the cyanotoxin. Based on our linear regression models, we found no direct relationship between specific cyanotoxin concentrations and the cell counts of the cyanobacteria that may produce the cyanotoxin. Also, we found that overall cyanobacterial cell density did not equate to higher concentrations of cyanotoxins. For example, the location with the highest levels of cyanobacteria, Provo Bay, produced similar or lower levels of cyanotoxins as the main body water. If we evaluated cyanotoxin concentrations in relation to

single species, instead of groups of species, we may find connections between these two parameters.

We identified a seasonal signal associated with cyanotoxin levels where higher concentrations of specific cyanotoxins were associated spring, summer, or fall. For example, the concentration of cylindrospermopsin was highest in the spring; anatoxin-a concentration was generally higher in the spring, late summer, and fall; and microcystin was more prevalent in the early summer and summer.

Biologically Available DIN and SRP

The DIN and SRP were biologically available to the cyanobacteria and total phytoplankton with the concentrations of DIN and SRP consistently declining in treatments—the addition of N resulting in lower P concentrations and the addition of P leading to lower N concentrations. Further, during periods of high cyanobacteria and total phytoplankton activity (i.e., the summer and late summer), measured as phycocyanin and chlorophyll-a respectively, added SRP was almost completely removed, indicating that this form of P was biologically available. Based on our findings, we predict that when SRP is measured in the water column of Utah Lake that the P is available to primary producers to exploit. The SRP is not just bound in a mineral complex.

Growth Rate Differences

The 48-hour or 72-hour incubation time in the time series captured the majority of phytoplankton and cyanobacterial responses (i.e., changes in chlorophyll-a, phycocyanin, and cyanotoxin concentrations), especially for faster- rather than slower-growing species. Contrary to our hypothesis, we found cyanobacterial growth was generally higher than phytoplankton growth

in the first 48 hours and peak growth for both organisms occurred in the first 24 hours. In general, cyanobacteria often grow more slowly than green algae when waters are cooler in the spring and fall (Lurling et al 2013), but growth rates of cyanobacteria may increase in nutrient-rich and warmer waters. Our growth trials occurred in the summer under lake temperatures above 30°C and the warmer temperatures potentially stimulated cyanobacterial growth. Additionally, the low initial abundance of cyanobacteria at the beginning of the incubation potentially induced high relatively growth rates. The initial concentrations of phycocyanin were almost non-detectable. Thus, even slight increases in biomass resulted in high relative growth rates.

Incubation time is critical in lake bioassay studies. Generally, the more primary production in a lake system, the shorter the incubation period. If the incubation is too short the impact of slower-growing species may go undetected. We are aware that we missed some of the potential response of phytoplankton and cyanobacteria. For example, we observed a sequential addition of species during our 96-hour incubation. In the open lake, the faster relative growth rate of phytoplankton, following the addition of N+P, was associated with unicellular and colonial green algae in the first 24 hours; unicellular, colonial green algae, and *Desmodesmus spp.* after 48 hours; and colonial green algae, unicellular, colonial green algae, *Desmodesmus spp.*, *Aulacoseira spp.*, and pennate and centric diatoms after 96 hours. Further, *Microcystis spp*. was consistently present in the main body water in the first 24 hours and accounted for the relatively high growth rate of cyanobacteria.

Cyanotoxins and Growth Rate

As hypothesized, cyanotoxin concentrations were relatively low during the summer. For example, in the seasonal bioassay study, the concentrations of all three cyanotoxins was generally lower in the summer than during any other time. Even in waters above 25°C, cyanotoxins loosely followed cyanobacterial growth but not necessarily cyanobacterial cell density. The most striking example of this was in bay water where the orders of magnitude higher phycocyanin concentrations failed to generate orders of magnitude more cyanotoxins

CONCLUSION

The nutrient limitation of cyanobacteria and to a lesser extent total phytoplankton (e.g., chlorophytes, diatoms, and cyanobacteria) was influenced by season (i.e., spring, early summer, summer, late summer, and fall) and space (i.e., main body of the lake, East; and main body of the lake, West; and Provo Bay).DIN and SRP limited cyanobacteria in the summer across all three locations. SRP limited cyanobacterial responses (i.e., phycocyanin concentrations) in East and Provo Bay water, while DIN limited cyanobacterial responses in West water. Nutrient colimitation of cyanobacteria occurred in the early summer in Provo Bay and spring in West water. In the late summer and fall, cyanobacteria were not limited by either DIN or SRP.During the summer, *Microcystis sp.* was associated with nutrient limitation in the East and West. In the bay, *Aphanocapsa*, *Dolichospermum, Merismopedia,* and *Aphanizomenon spp.* were associated with nutrient limitation in the early summer and summer. The three cyanotoxins measured demonstrated a seasonal signal that was not dependent on the cell density of cyanobacteria know to generate the cyanotoxin. Cylindrospermopsin concentration was highest in the spring. Anatoxin-a concentration was generally higher in the spring, late summer, and fall. Microcystin

was most prevalent in the early summer and summer, regardless of nutrient treatment or a specific nutrient limitation to phytoplankton.

Nutrient colimitation (DIN and SRP) of total phytoplankton (i.e., chlorophyll-a concentrations from all prokaryotic and eukaryotic organisms, which also includes cyanobacteria) occurred in the summer, late summer, and fall in the main body of the lake, and in the late summer and spring in Provo Bay. In the relatively nutrient rich Provo Bay that supported orders of magnitude more total phytoplankton biomass than the main body East and West, phytoplankton was limited during every season with DIN generally limiting phytoplankton responses. *Aulacoseira* and *Desmodesmus spp.* and two taxonomical categories of algae (i.e., unicellular and colonial green algae) were primarily associated with phytoplankton nutrient limitation across Utah Lake regardless of season. In the summer, phytoplankton growth was generally higher in the first 24 hours of the 96-hour time series in the main body of the lake. Increases in cyanobacterial growth were dependent on the nutrient addition and lake location. In the main body, cyanobacterial growth was stimulated by nutrient addition (i.e., P and N+P addition in the East, and any treatment in the West) in the first 24 hours. There was no clear and consistent growth pattern in the bay during the incubation. In the main body of the lake, the faster relative growth rate of phytoplankton following the addition of N+P was associated with unicellular and colonial green algae in the first 24 hours; unicellular, colonial green algae, and *Desmodesmus sp.* after 48 hours; and colonial green algae, unicellular, colonial green algae, *Desmodesmus sp.*, *Aulacoseira sp.*, and pennate and centric diatoms after 96 hours. The effect of the nutrient treatments on phytoplankton was less apparent in Provo Bay where phytoplankton abundance (cell/mL) was orders of magnitude higher than the main body. In the main body of the lake, *Microcystis sp*. responded in the first 24 hours and accounted for the relatively high

growth rate of cyanobacteria. In Provo Bay waters, three species dominated the responses to the addition of N, P, and N+P: *Aphanocapsa, Dolichospermum, and Microcystis spp*. Despite supporting orders of magnitude more cyanobacteria, *Microcystis* was nearly absent in Provo Bay. Cyanotoxins only loosely followed cyanobacterial growth and toxin levels did not necessarily increase with higher cyanobacteria cell density. In West water, the higher cyanobacterial growth rate under P addition (P and N+P) led to higher concentrations of cyanotoxins, especially cylindrospermopsin; however, in the bay the relatively higher phycocyanin concentration and cell density of cyanobacteria did not equate to higher concentrations of cyanotoxins.

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FIGURES

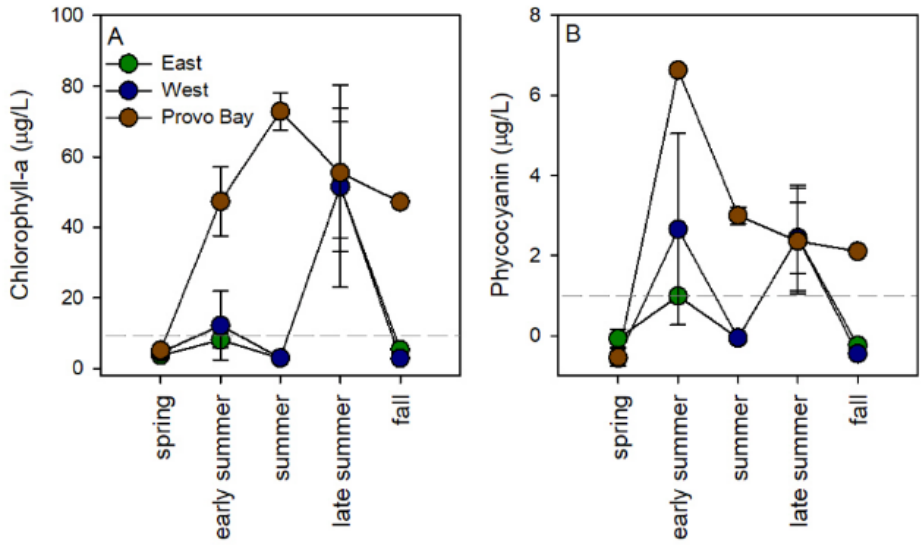

Figure 1. Baseline Chlorophyll-a and Phycocyanin Concentrations. Chlorophyll-a (A) and phycocyanin (B) concentrations in the upper 20 cm of lake water at the three locations immediately prior to the nutrient additions. Values are from YSI EXO2 sonde measurements (*n*=3) in the field during water collection. The dashed line in figures represent the threshold for waters to be designated as a HAB for Utah Lake as designated by the researchers.

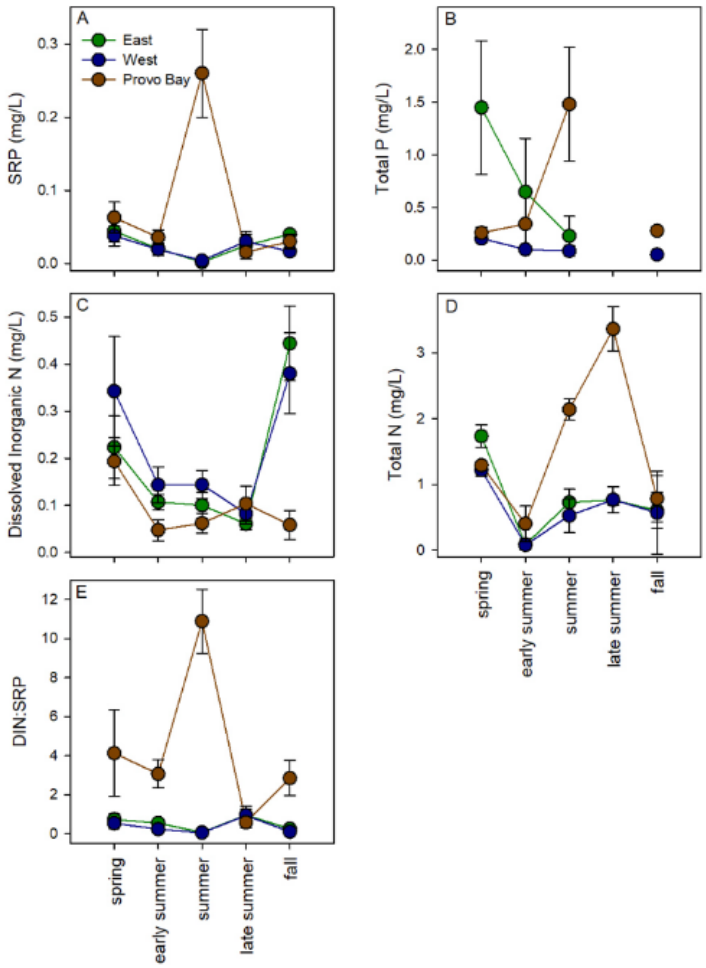

Figure 2. Total N and P, SRP, DIN, and DIN:SRP, expressed as a molar ratio, for the three lake locations in the control treatment during T_0 and T_1 of the incubation (n=6).

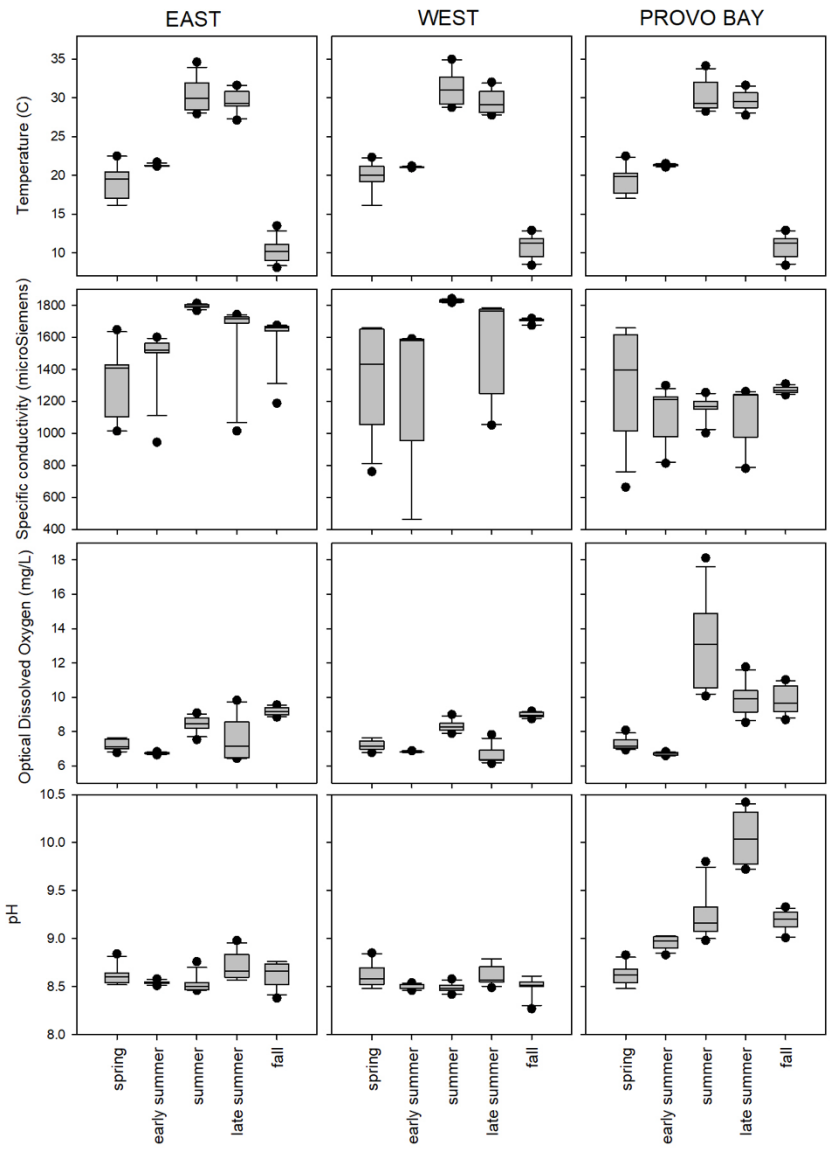

Figure 3. Boxplot of water physicochemical characteristics across the seasons in three locations. Values are from all three nutrient treatments and the control replicates following the incubation by location (*n*=12).

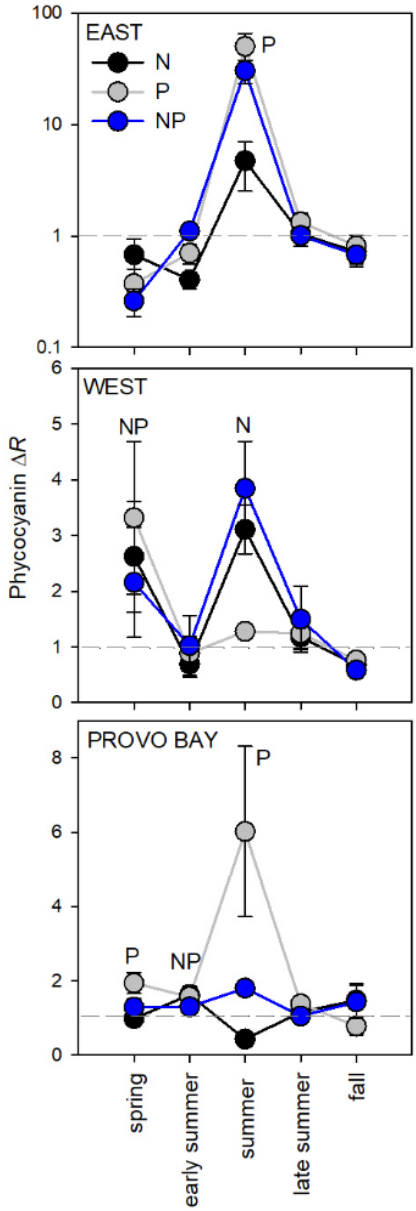

Figure 4. N, P, and NP limitation for cyanobacteria based on phycocyanin for the three locations. Limitation is expressed as response ratios or Δ*R*s following the bioassay incubation (*n*=9). Values above one (gray dashed line) indicates a positive response to the nutrient additions. Letters indicate potential nutrient limitation for each time point based on one-way ANOVA $P<0.05$. If a co-limitation was apparent but not significantly higher than N or P, the limitation was designated as a single nutrient limitation.

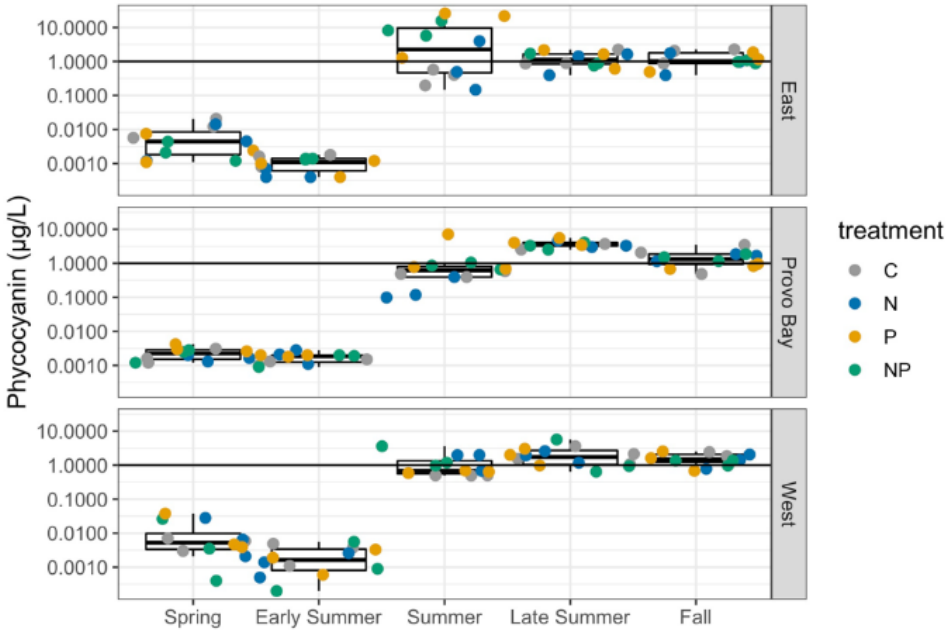

Figure 5. Seasonal variation in phycocyanin concentrations in all bioassays from the three nutrient treatments and the control replicates following the incubation by location. The values are presented as a boxplot overlaid with individual bioassay values (*n*=3).

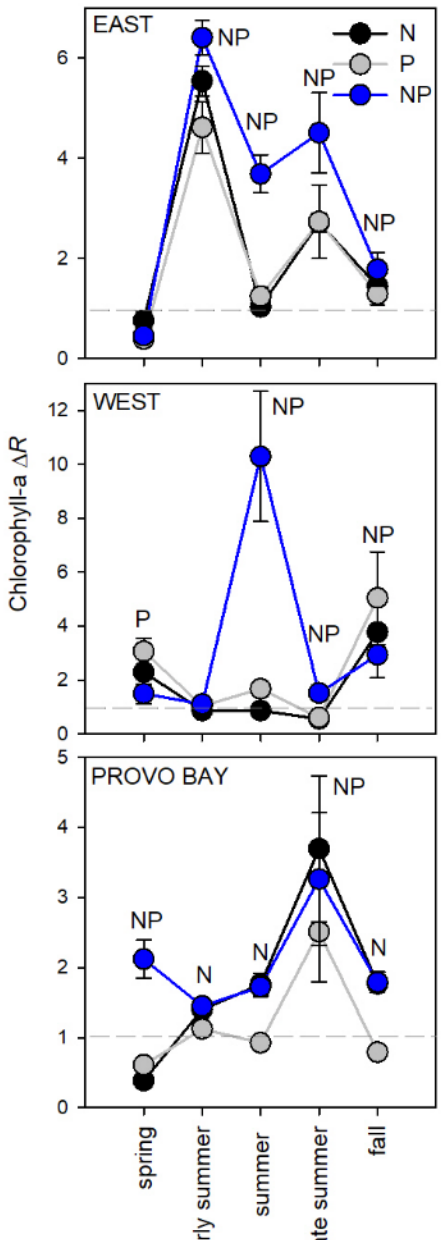

Figure 6. N, P, and N+P limitation for phytoplankton based on chlorophyll-a for the three locations. Limitation is expressed as response ratios or Δ*R*s following the bioassay incubation (*n*=9). Values above one (gray dashed line) indicates a positive response and letters indicate the limitation based on ANOVA $P<0.05$.

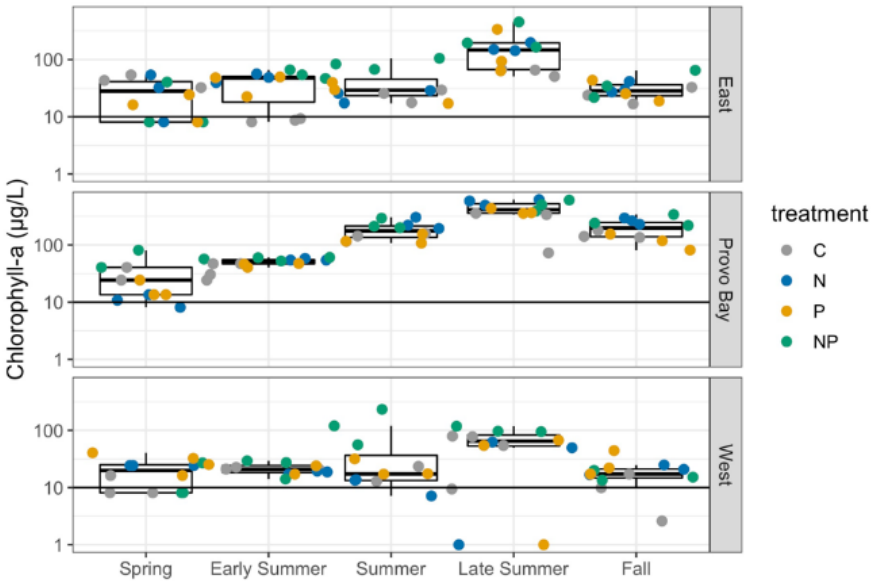

Figure 7. Seasonal variation in chlorophyll-a concentrations in all bioassays from the three nutrient treatments and the control replicates following the incubation by location. The values are presented as a boxplots containing jittered points with individual bioassay values (*n*=3).

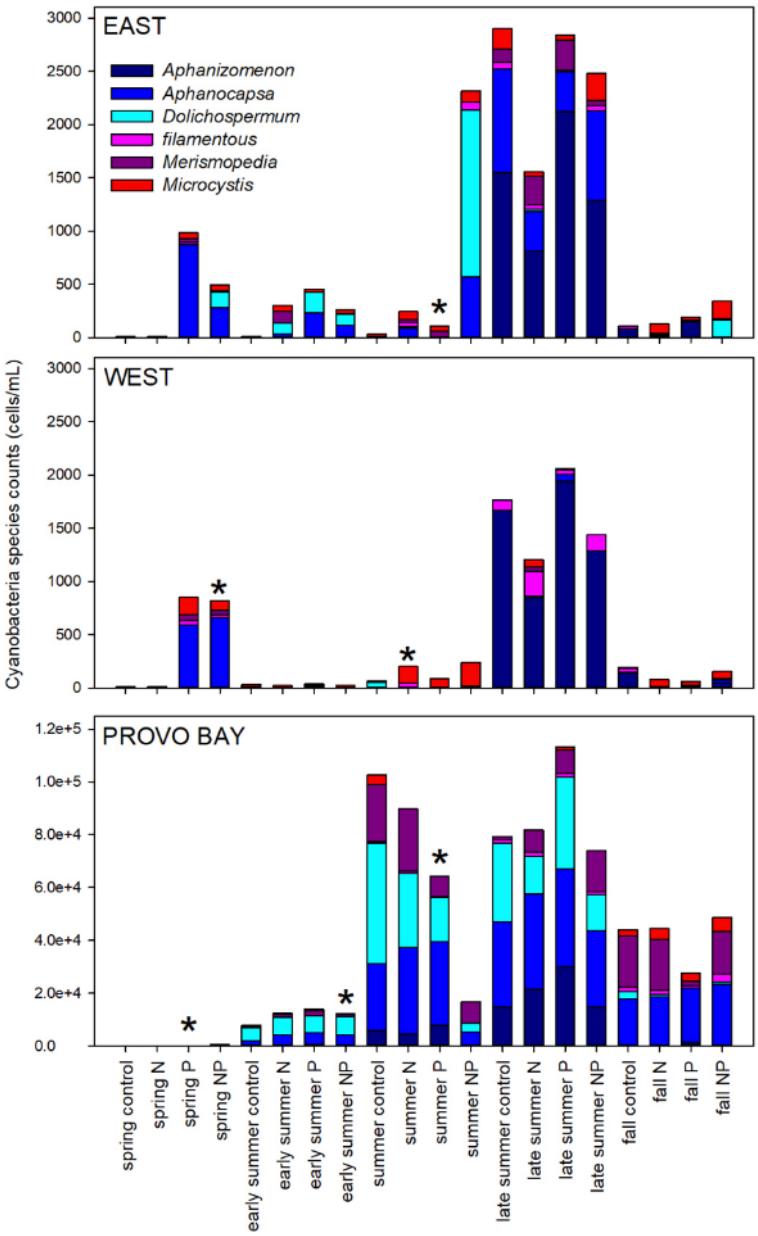

Figure 8. Cyanobacterial species abundance by season and treatment (cell/ml). The abundance (cells/mL) of cyanobacterial species in the nutrient treatments in the three locations. Values are means presented as stacked bars from direct microscopy counts (n=3). Asterisks indicate a nutrient limitation based on ΔR *of phycocyanin concentrations*.

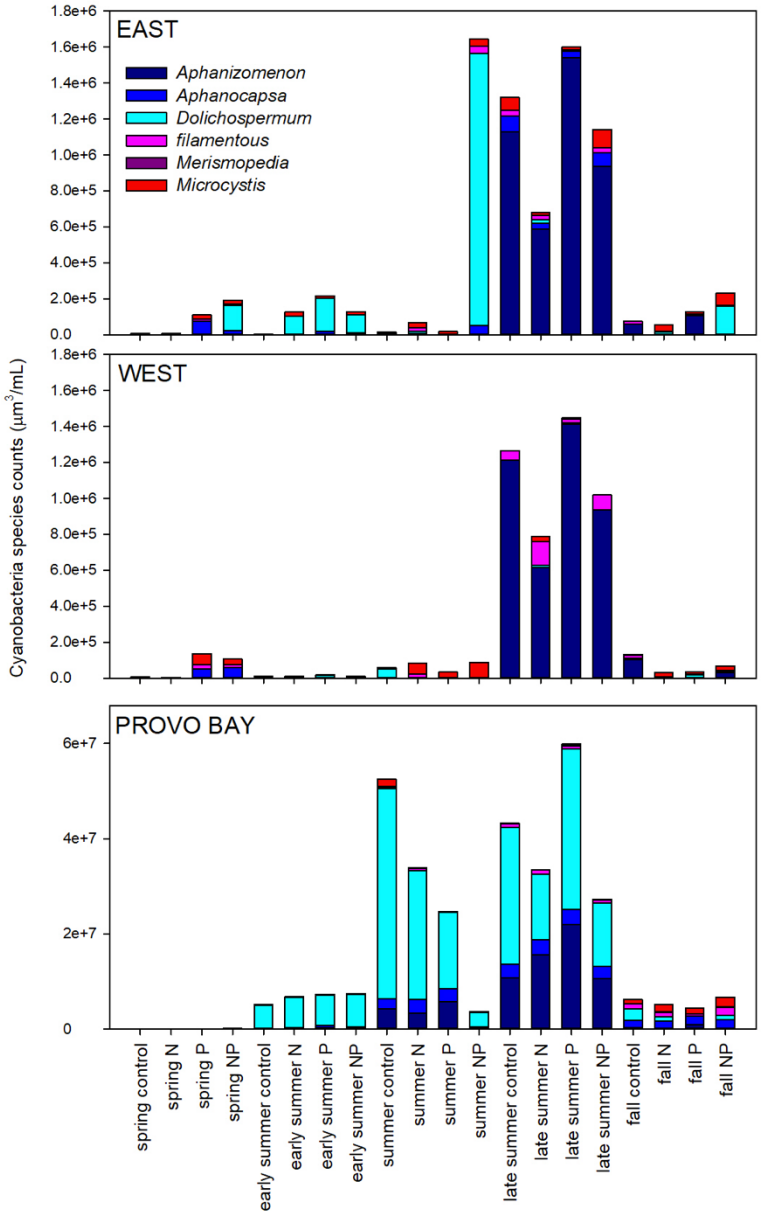

Figure 9. Cyanobacterial species abundance by season and treatment (µm^3/mL). Values are means presented as stacked bars from direct microscopy counts (*n*=3).

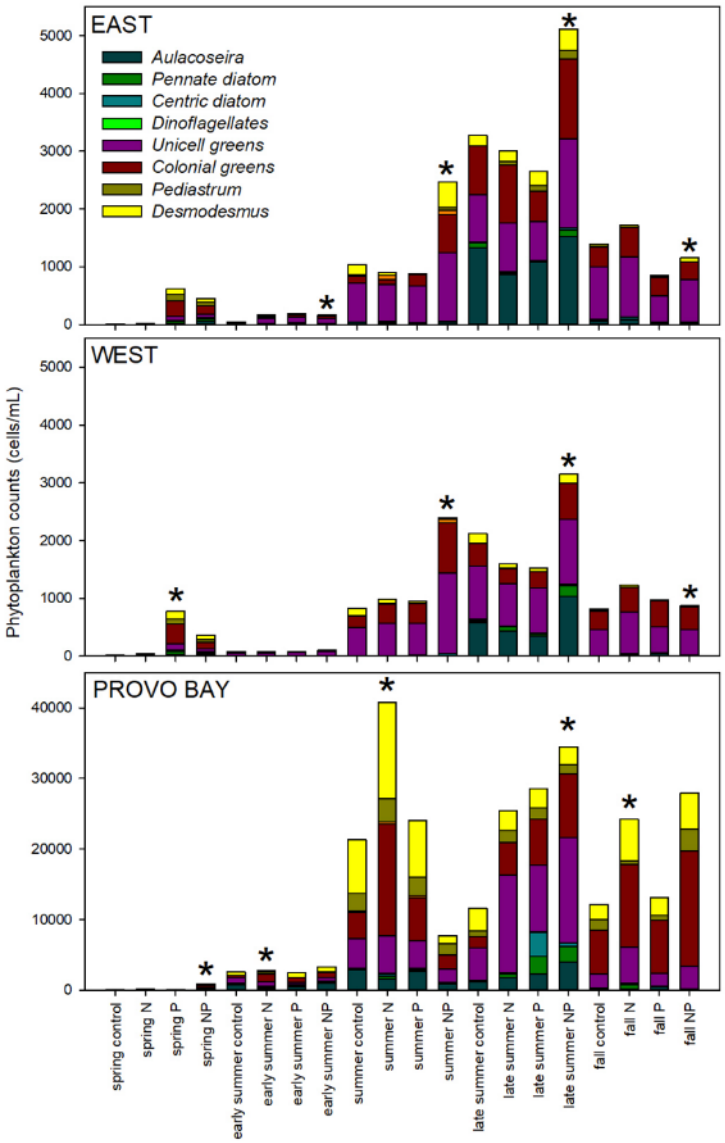

Figure 10. Total phytoplankton species abundance by season and treatment. The abundance (cells/mL) of three species and five categories of phytoplankton in the nutrient treatments in the three locations. Values are means presented as stacked bars from direct microscopy counts (n=3). Asterisks indicate a nutrient limitation based on ΔR of chlorophyll-a concentrations.

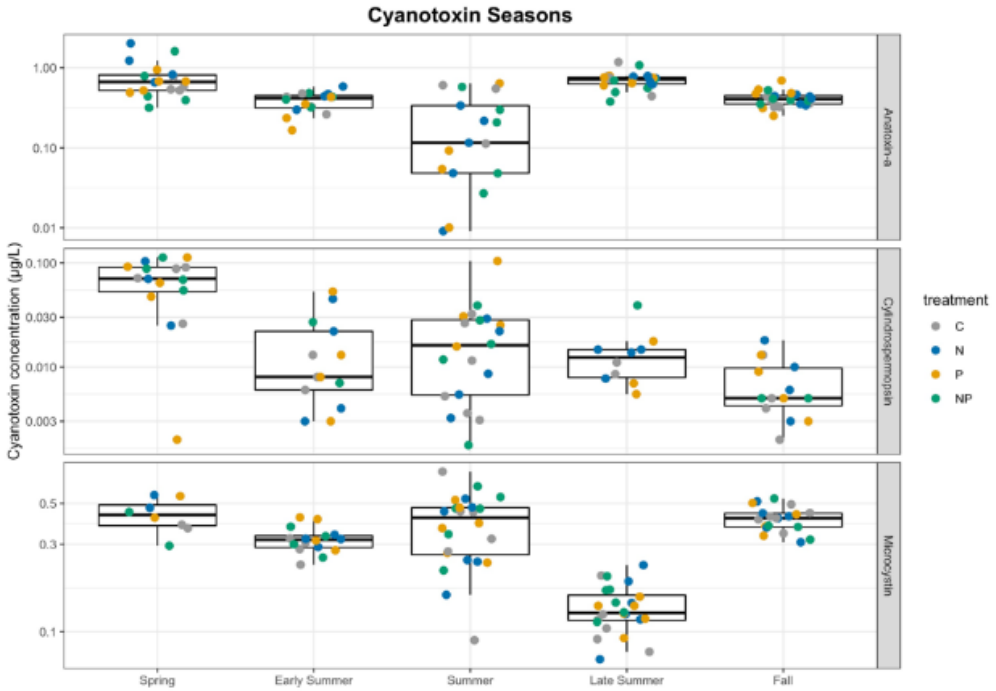

Figure 11. Cyanotoxin concentrations by season and treatment. The concentrations of anatoxin-a, cylindrospermopsin, and microcystin over the five seasons season. The values are from ELISA analyses presented as boxplots containing jittered points with individual values (*n*=24).

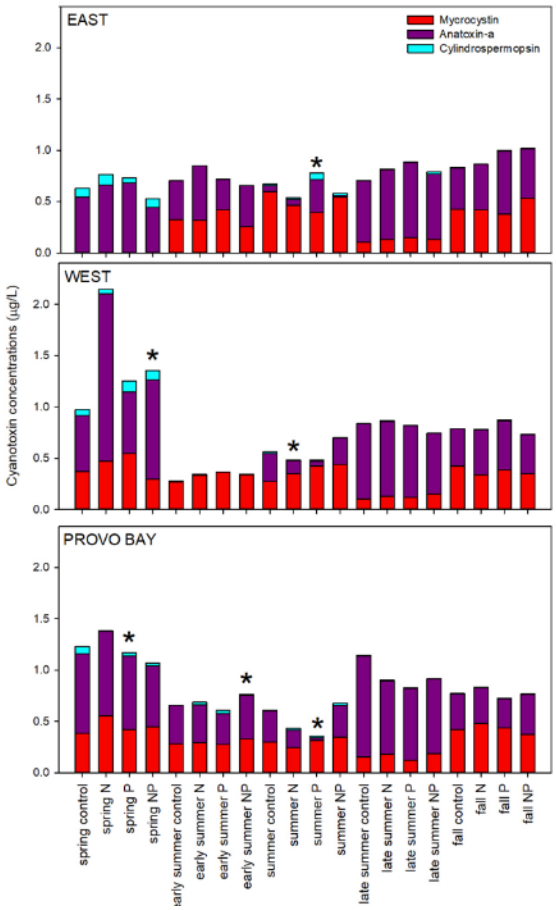

Figure 12. Anatoxin-a, cylindrospermopsin, and microcystin concentrations (µg/L) in the nutrient amendments across seasons. Values are means presented as stacked bars from direct microscopy counts (n=3). Asterisks indicate a nutrient limitation based on ΔR of phycocyanin concentrations.

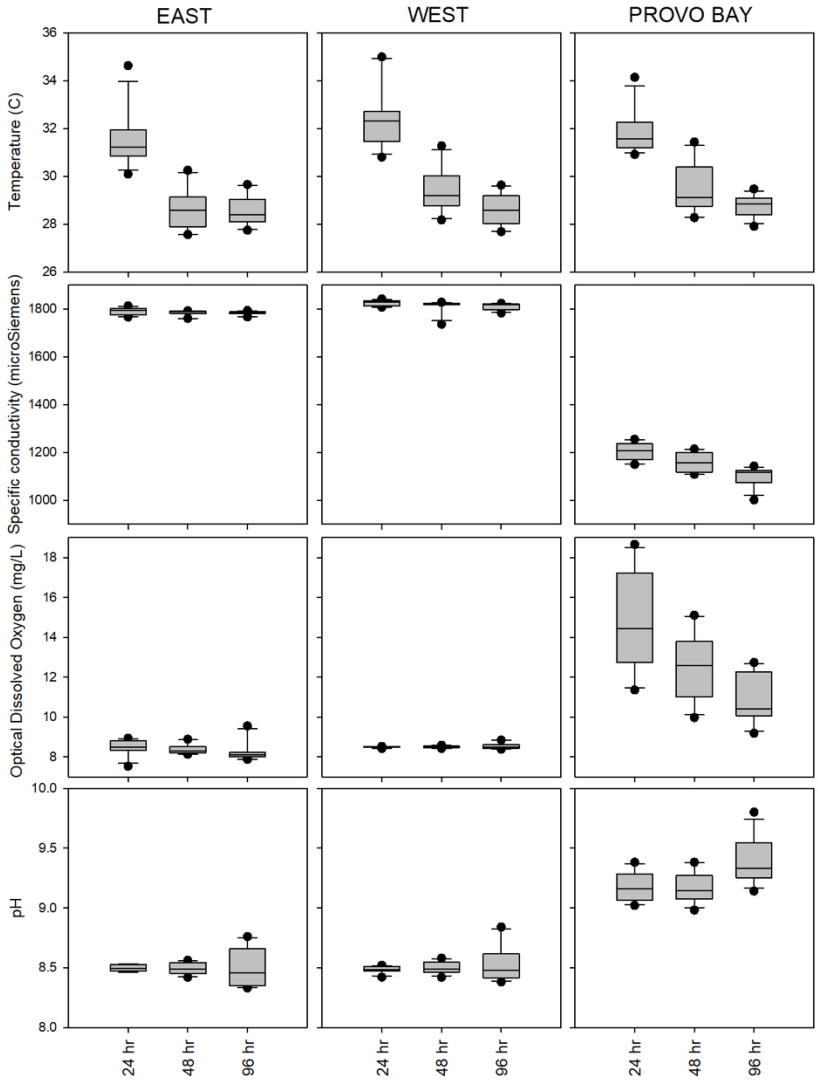

Figure 13. Boxplot of water physiochemical characteristics during the time series in three locations. Values are from all three nutrient treatments and the control replicates following the incubation location (n=12).

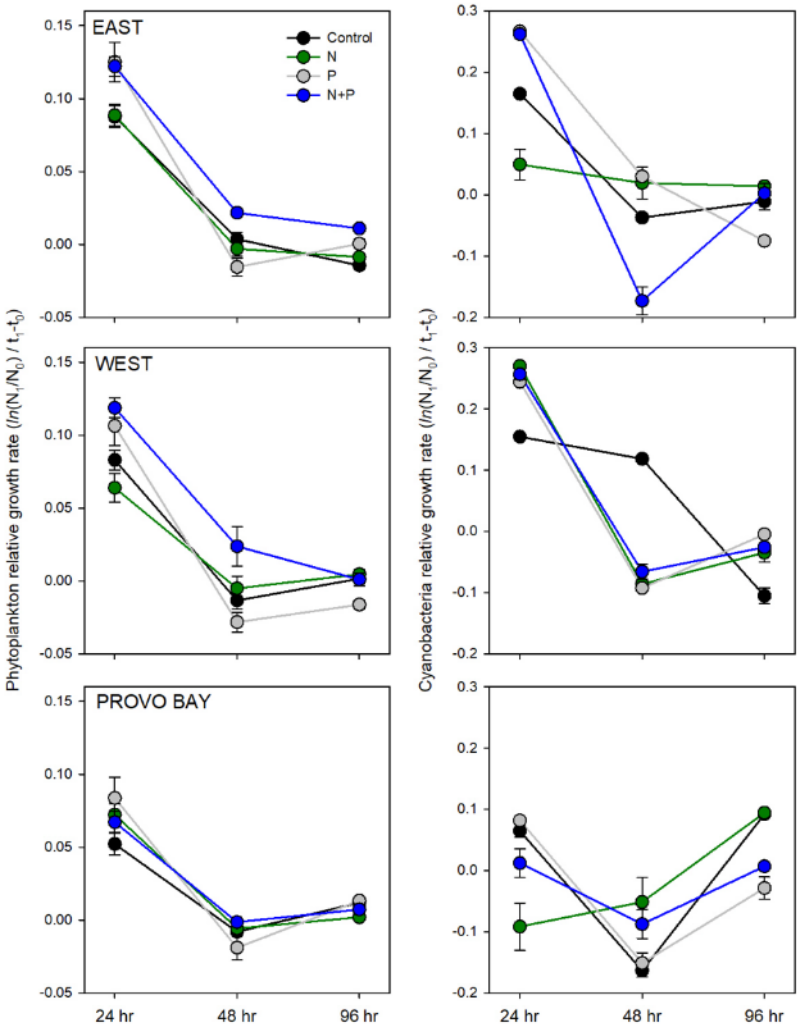

Figure 14. Relative growth rates of total phytoplankton and cyanobacteria in the different nutrient and control treatments over the 96-hour incubation. Values are means with ± standard error based on the pigments chlorophyll-a and phycocyanin from all possible replicate combinations between two time points (*n*=9).

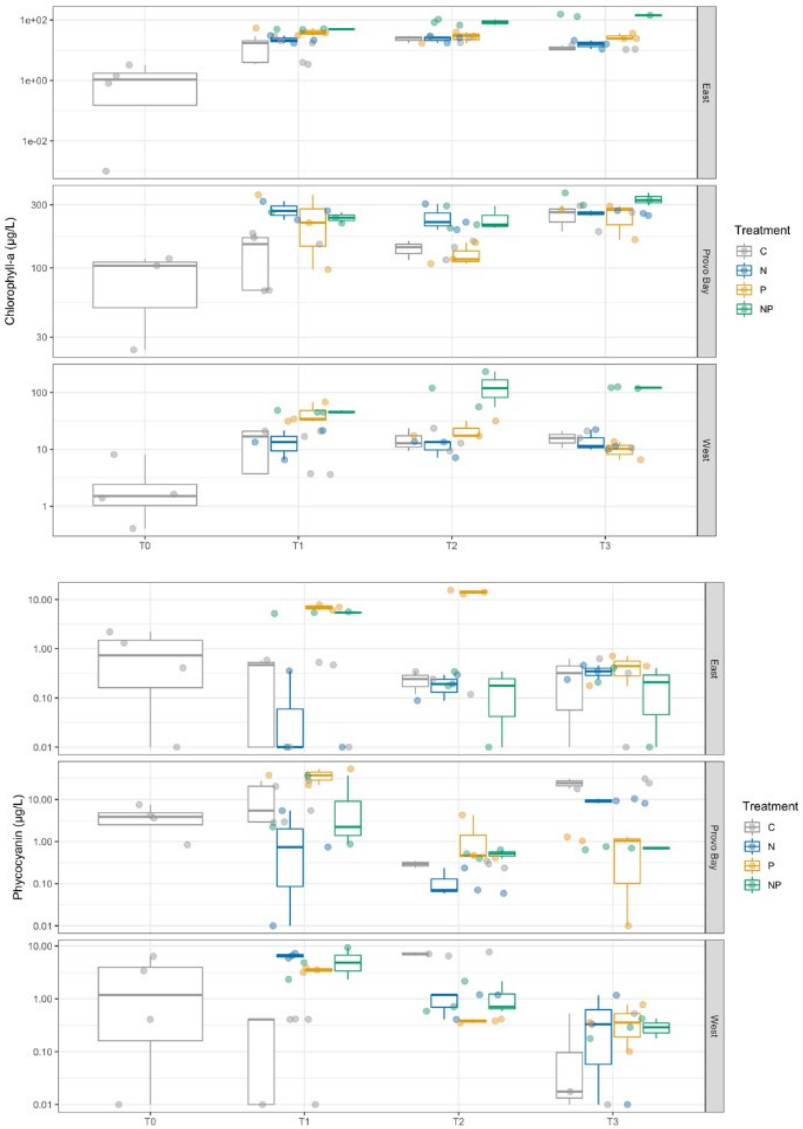

Figure 15. Chlorophyll-a and phycocyanin concentrations in the nutrient addition and the control treatments incubated over four days across three location in early summer. Concentrations were evaluated at T_0, T_1–24, T_2–48, and T_3–96 hour. Values are presented as boxplots containing jittered points with individual bioassay values (n=3).

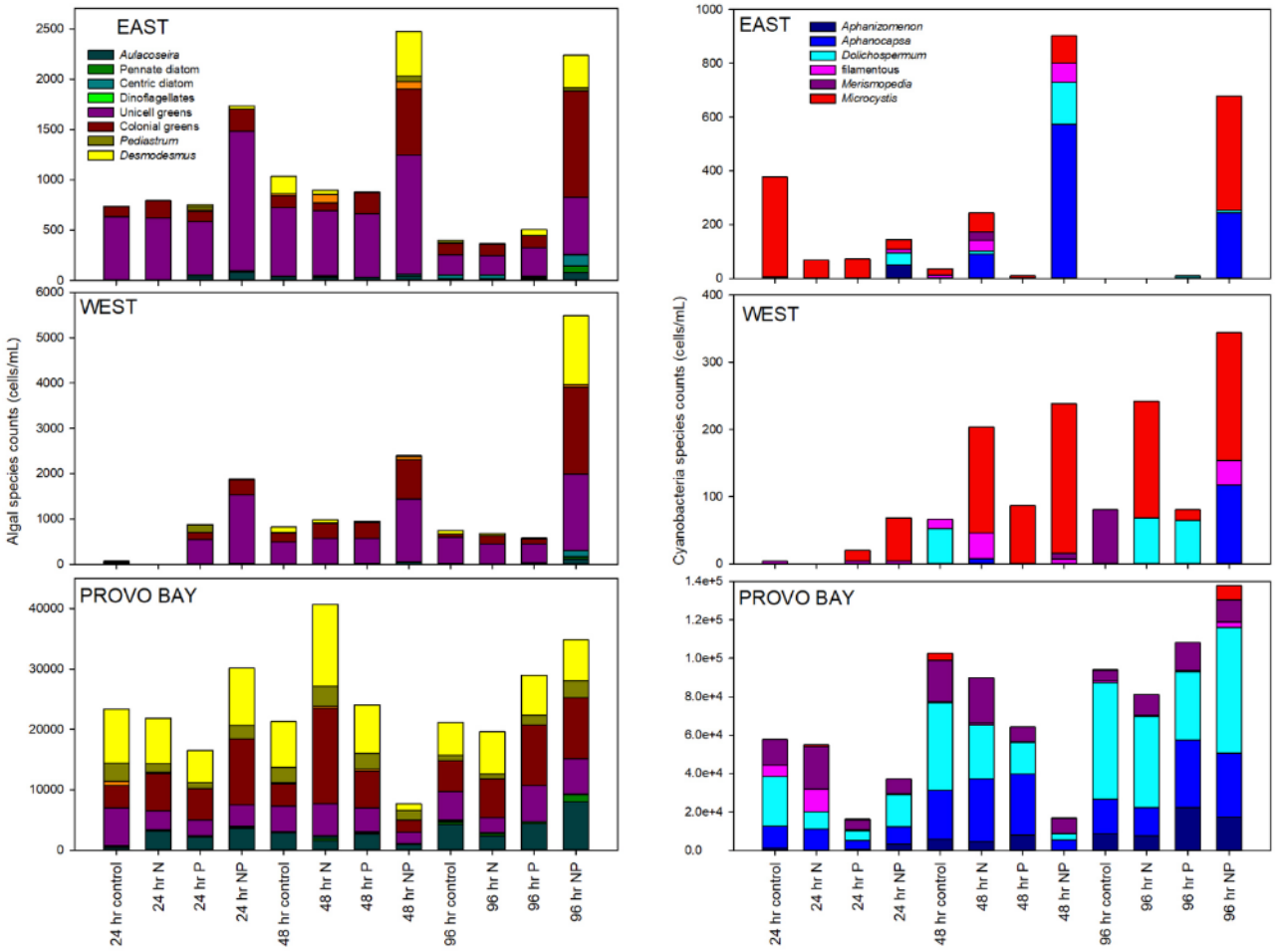

Figure 16. The abundance (cells/mL) of phytoplankton and cyanobacteria species and categories in the nutrient additions through the 96-hour time series. Values are presented as stacked bars from direct microscopy counts (n=2).

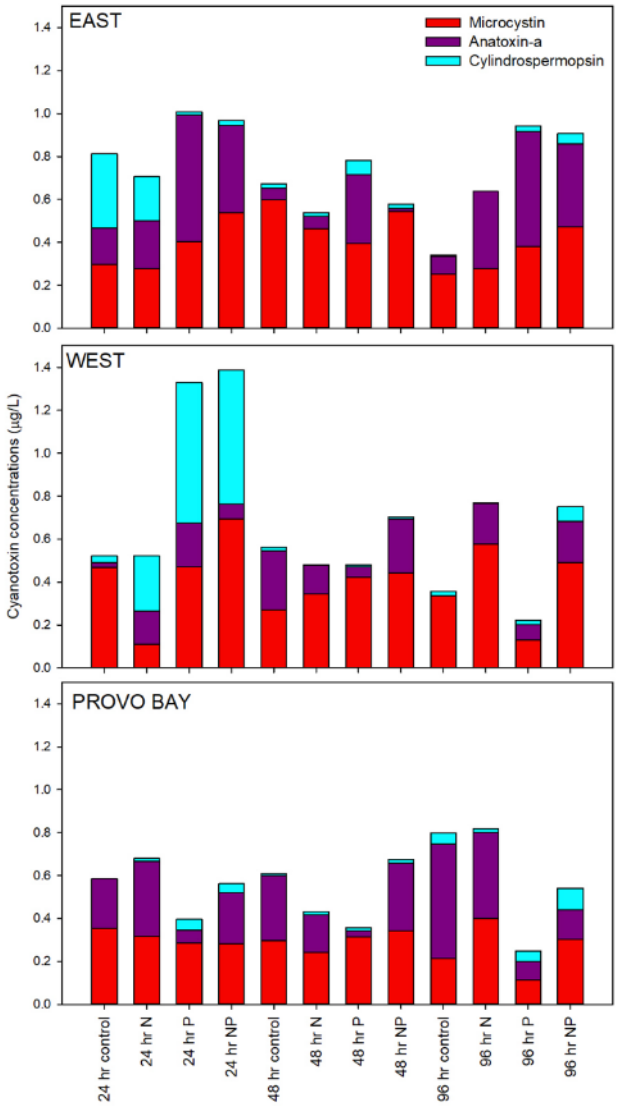

Figure 17. Microcystin, anatoxin-a, and cylindrospermopsin concentrations in the nutrient bioassay by location. Values are presented as stacked bars (*n*=2).

TABLES

Table 1. Biovolume conversions for the five cyanobacterial species and one general category. Conversion factors are based mean biovolume conversion factor from Utah Lake microscopy data collected by the UT-DWQ between 2018-2019 ($n < 20$).

Cyanobacteria	Biovolume conversion factor (cells/mL % to μm^3/mL
Aphanizomenon	727
Aphanocapsa	88.0
Dolichospermum	967
filamentous species	554
Merismopedia	6
Microcystis	382

Table 2. Final concentrations of SRP and DIN, and DIN:SRP following the incubation with N, P, and N+P additions in the three locations. Values are measured as SRP and DIN (*n*=3). The DIN:SRP is expressed as a molar ratio.

Location	Treatment	Treatment	SRP (mg/L)	DIN (mg/L)	DIN:SRP (mole:mole)
EAST	spring	N	0.013 ±0.002	0.05 ±0.02	9.22 ±3.33
		P	0.029 ±0.015	0.26 ± 0.01	32.6 ±12.8
		N+P	0.016 ±0.004	0.49 ± 0.33	55.5 ±25.5
	early summer	N	0.005 ±0.001	0.19 ±0.01	117 ±4.88
		P	0.008 ±0.003	0.07 ±0.06	16.2 ±8.66
		N+P	0.007 ±0.001	0.02 ± 0.001	5.30 ±1.25
	summer	N	0.004 ±0.002	0.86 ±0.08	800 ± 405
		P	0.100 ±0.001	0.06	1.33
		N+P	0.096 ±0.20	0.70 ±0.15	16.2 ±0.614
	late summer	N	0.031 ±0.012	0.39 ±0.06	33.5 ±7.72
		P	0.067 ±0.033	0.02 ±0.01	8.49 ±7.95
		N+P	0.037 ±0.033	0.17 ±0.06	94.1 ±53.2
	fall	N	0.008 ±0.004	1.00 ±0.06	122 ±61.5
		P	0.140 ±0.020	0.29 ±0.06	4.58 ±0.365
		N+P	0.123 ±0.021	1.18 ±0.38	12.0 ±6.45
WEST	spring	N	0.022 ±0.021	0.14 ±0.07	104 ±93.8
		P	0.084 ±0.026	0.06 ± 0.04	1.36 ±0.469
		N+P	0.117 ±0.043	0.25 ± 0.23	3.17 ±2.33
	early summer	N	0.005 ±0.002	0.28 ±0.01	372 ±278
		P	0.006 ±0.001	0.03 ±0.01	11.2 ±4.12
		N+P	0.009 ±0.002	0.23± 0.001	75.0
	summer	N	0.003 ±0.002	1.0 ±0.13	2859 ±1764
		P	0.094 ±0.002	0.14	3.43
		N+P	0.068 ±0.003	0.63 ±0.04	20.3 ±0.962
	late summer	N	0.065 ±0.037	0.75 ±0.04	13.0 ±7.78
		P	0.020 ±0.014	0.08 ±0.02	49.0 ±39.2
		N+P	0.037 ±0.021	0.50 ±0.09	19.7 ±14.3
	fall	N	0.009 ±0.006	0.96 ±0.11	913 ±712
		P	0.141 ±0.009	0.34 ±0.04	5.41 ±0.263
		N+P	0.106 ±0.003	0.96 ±0.06	20.0 ±0.836
PROVO BAY	spring	N	0.024 ±0.006	0.30 ±0.16	34.5 ±24.7
		P	0.015 ±0.002	0.31 ± 0.02	45.1 ±1.55
		N+P	0.021 ±0.006	0.14 ± 0.04	18.9 ±8.72
	early summer	N	0.012 ±0.002	0.30 ±0.16	31.4 ±14.6
		P	0.010 ±0.002	0.31 ±0.02	2.42
		N+P	0.010 ±0.002	0.14 ±0.04	17.7 ±14.1
	summer	N	0.008 ±0.001	0.14 ±0.06	41.0 ±29.1
		P	0.246 ±0.020	0.37 ±0.31	3.68 ±3.13
		N+P	0.074 ±0.018	0.26 ±0.12	11.1 ±7.11
	late summer	N	0.021 ±0.005	0.09 ±0.06	16.9 ±13.9
		P	0.114 ±0.010	0.19 ±0.06	3.72 ±1.08
		N+P	0.056 ±0.032	0.19 ±0.07	3.84 ±1.66
	fall	N	0.009 ±0.001	0.09 ±0.07	26.9 ±19.8
		P	0.084 ±0.006	0.01 ±0.001	0.257 ±0.129
		N+P	0.010 ±0.001	0.11 ±0.05	29.5 ±16.4

Table 3. Final concentrations of chlorophyll-a and phycocyanin pigments following N, P, and N+P additions in the three locations after incubation. Values are means (n=3).

Location	Season	Treatment	Chlorophyll-a (μg/L)	Phycocyanin (μg/L)
EAST	spring	Control	43.2 ±6.24	0.013 ±0.004
		N	31.5 ±13.3	0.007 ±0.004
		P	16.2 ±4.68	0.004 ± 0.002
		N+P	18.9 ±10.8	0.003 ± 0.001
	early summer	Control	8.72 ±0.344	0.0014 ±0.0003
		N	48.2 ±4.81	0.0005 ±0.0001
		P	40.2 ±8.84	0.0009 ±0.0002
		N+P	55.8 ±5.64	0.0014 ± 0.001
	summer	Control	24.3 ±3.46	0.391 ±0.108
		N	24.0 ±3.41	1.53 ±1.21
		P	28.8 ±6.53	16.2 ±7.57
		N+P	85.5 ±11.0	9.85 ±3.01
	late summer	Control	61.3 ±5.30	1.33 ±0.453
		N	164 ±17.2	1.15 ±0.381
		P	165 ±86.9	1.47 ±0.457
		N+P	272 ±92.2	1.11 ±0.294
	fall	Control	24.5 ±4.63	1.73 ±0.435
		N	32.8 ±4.45	1.04 ±0.394
		P	29.2 ±7.42	1.18 ±0.395
		N+P	40.5 ±12.8	0.975 ±0.057
WEST	spring	Control	10.8 ±2.71	0.005 ±0.001
		N	22.2 ±1.21	0.012 ±0.008
		P	29.7 ±7.15	0.015 ± 0.011
		N+P	14.4 ±6.29	0.010 ± 0.008
	early summer	Control	21.5 ±0.558	0.0033 ±0.0011
		N	18.4 ±0.649	0.0015 ±0.0006
		P	22.1 ±0.2.51	0.0019 ±0.0008
		N+P	23.6 ±4.78	0.0022 ± 0.0017
	summer	Control	15.2 ±4.22	0.498 ±0.002
		N	11.4 ±2.15	1.55 ±0.434
		P	22.1 ±4.81	0.635 ± 0.031
		N+P	136 ±51.7	1.91 ±0.838
	late summer	Control	70.3 ±7.94	2.07 ±0.777
		N	37.7 ±18.7	1.89 ±0.407
		P	40.9 ±20.3	2.00 ±0.592
		N+P	103 ±7.48	2.41 ±1.62
	fall	Control	9.90 ±4.22	2.13 ±0.162
		N	20.8 ±2.31	1.41 ±0.367
		P	27.8 ±8.32	1.61 ±0.535
		N+P	16.2 ±1.97	1.23 ±0.130
PROVO BAY	spring	Control	29.7 ±5.40	0.0020 ±0.0006
		N	10.8 ±1.56	0.0017 ±0.0002
		P	17.1 ±3.60	0.0033 ± 0.0005
		N+P	59.4 ±11.8	0.0022 ± 0.0005
	early summer	Control	41.5 ±5.57	0.0013 ±0.0001
		N	55.7 ±1.27	0.0020 ±0.0005
		P	44.8 ±2.13	0.0019 ±0.0001
		N+P	57.7 ±2.61	0.0016 ± 0.0004

summer	Control	139 ±13.0	0.488 ±0.054
	N	240 ±33.2	0.203 ±0.095
	P	126 ±14.7	2.86 ±2.13
	N+P	236 ±29.5	0.857 ±0.114
late summer	Control	257 ±92.3	3.23 ±0.357
	N	568 ±37.3	3.65 ±0.516
	P	386 ±25.1	4.34 ±0.624
	N+P	502 ±63.3	3.30 ±0.455
fall	Control	151 ±13.5	2.01 ±0.865
	N	264 ±18.9	1.56 ±0.221
	P	118 ±21.2	0.823 ±0.082
	N+P	267 ±37.4	1.51 ±0.202

Table 4. Concentrations of chlorophyll-a and phycocyanin pigments following N, P, and N+P additions at T_0, T_1=24, T_2=48, and T_3=96 hours in summer following the different incubation times. Values are means (n=3).

Location	Time	Treatment	Chlorophyll-a (μg/L)	Phycocyanin (μg/L)
EAST	T_0=0 hours	Control	2.93 ±0.775	0.010±0.0001
	T_1=24 hours	Control	22.4 ±3.66	0.526 ±0.034
		N	23.0 ±3.96	0.125 ±0.115
		P	40.6 ±6.89	6.94 ±0.521
		N+P	50.1 ±0.908	5.42 ± 0.128
	T_2=48 hours	Control	24.3 ±3.46	0.234 ±0.065
		N	24.0 ±3.41	0.192 ±0.060
		P	28.8 ±6.53	14.2 ±0.728
		N+P	85.5 ±11.0	0.176 ±0.096
	T_3=96 hours	Control	12.0 ±1.29	0.318 ±0.178
		N	16.0 ±2.97	0.349 ±0.065
		P	28.4 ±4.09	0.443 ±0.154
		N+P	143 ±4.10	0.209 ±0.115
WEST	T_0=0 hours	Control	2.92 ±0.759	0.010±0.0001
	T_1=24 hours	Control	19.6 ±1.38	0.410 ±0.002
		N	13.8 ±0.4.29	6.57 ±0.389
		P	44.5 ±11.8	3.52 ±0.210
		N+P	46.1 ±1.24	5.50 ± 2.04
	T_2=48 hours	Control	15.2 ±4.22	7.07 ±0.343
		N	11.4 ±2.15	0.928 ±0.260
		P	22.1 ±4.81	0.381 ± 0.019
		N+P	136 ±51.7	1.15 ±0.503
	T_3=96 hours	Control	15.9 ±3.05	0.185 ±0.171
		N	14.6 ±3.88	0.504 ±0.346
		P	10.1 ±2.10	0.410 ±0.196
		N+P	122 ±2.22	0.296 ±0.070
PROVO BAY	T_0=0 hours	Control	53.0 ±14.5	3.12 ±0.249
	T_1=24 hours	Control	168 ±9.19	17.9 ±6.57
		N	273 ±25.4	2.06 ±1.70
		P	224 ±74.9	37.5 ±9.00
		N+P	241 ±13.2	13.2 ± 11.7
	T_2=48 hours	Control	139 ±13.0	0.293 ±0.032
		N	240 ±33.2	0.122 ±0.057
		P	126 ±14.7	1.72 ±1.28
		N+P	236 ±29.5	0.541 ±0.068
	T_3=96 hours	Control	249 ±31.6	24.5 ±3.75
		N	260 ±6.54	9.25 ±0.657
		P	244 ±40.5	0.777 ±0.390
		N+P	331 ±20.7	0.689 ±0.034

Table 5. Summary of the N_2 fixation potential and cyanotoxin production capability of dominant cyanobacteria present in the main body of the lake and Provo Bay.

Cyanobacteria	N_2 fixation potential	microcystin	anatoxin-a	cylindrospermopsin
Aphanizomenon	Yes		Yes	Yes
Aphanocapsa		Yes		
Dolichospermum	Yes	Yes	Yes	Yes
filamentous species	Yes	Yes	Yes	
Merismopedia		Yes		
Microcystis		Yes	Yes	

Table 6. Seasonal shifts in cyanobacterial species at the three locations. Seasonal abbreviations include spring = SP, early summer = ES, summer = S, late summer = LS, and fall = F. Data was collected by the UT-DWQ between 2018-201

	EAST						WEST						PROVO BAY					
	counts (#)	richness = 18					counts (#)	richness = 15					counts (#)	richness = 12				
Species		SP	ES	S	LS	F		SP	ES	S	LS	F		SP	ES	S	LS	F
Aphanizomenon flosaquae	47,463-234,076						5,466-81,833						100,476-344,058					
Aphanocapsa grevillei	728																	
Aphanocapsa holsatica	3,528																	
Aphanocapsa planctonica	1,568-10,662						314-627											
Aphanocapsa species	2,394-10,591						532-8,512						1,862-46,075					
Calothrix species	157																	
Chroococcus species							62											
Chrococcus dispersus													3,240					
Chroococcus limeticus	101												101					
Coelosphaerium species							45						1,440					
Cyanodictyon planctonicum	336-2,688						2,520						2,700-54,000					
Dolichospermum circinalis	645-74,650						946-3,830						1,125-630,157					
Dolichospermum species													6,413					
Gomphosphaeria aponina	5,018																	
Leptolyngbya species	3,928-9,565						3,007						7,515-17,763					
Merismopedia glauca	3,472-48,288						5,555						6,535-65,596					
Microcystis aeruginosa	686						392											
Microcystis species	2,688-3,584						6,272						12,600					
Phormidium species	1,456-2,058						2,464						1,456-12,555					
Phormidium species 3	168-8,623																	
Planktothrix species	826-19,936						9,390-15,680						5,376-36,000					
Pseudanabaena species	162-1,217						324-1,966						1,620-3,035					
Snowella lacustris	784						2,867											