Utah Lake Bioassay Final Report- Nutrient Limitation of Total Phytoplankton, Cyanobacteria and Cyanotoxins

Version 6 April 2021

Prepared by the Utah Lake Bioassay Team

Contents

List of Contributors

Professors in order of overall contribution
PI: Dr. Zachary T. Aanderud, Brigham Young University
Co-PI: Dr. Ben W. Abbott, Brigham Young University
Co-PI: Dr. Michelle A. Baker, Utah State University

Graduate Students in order of overall contribution
Gabriella M. Lawson, Brigham Young University (MS candidate)
Dr. Erin F. Jones, Brigham Young University
Samuel P. Bratsman, Brigham Young University (MS candidate)
Rachel Buck, Utah State University (PhD candidate)

List of Reviewers from the Utah Lake Quality Study Science Panel

Scott Daly, Utah Department of Environmental Quality-Water Quality
Dr. Kateri Salk-Gunderen, Tetra Tech
Dr. Hans W. Paerl, University of North Carolina at Chapel Hill
Dr. Ryan King, Baylor University
Dr. Michael J. Paul Tetra Tech
Dr. Mitch Hogsett, Forsgren Associates Inc.

Revision History of the Final Report

January 16, 2021	Initial release to the Utah Department of Environmental Quality-Water Quality and the Utah Lake Quality Study Science Panel
February 16, 2021	Second version —edited, revised, and returned to the Utah Department of Environmental Quality-Water Quality
February 22, 2021	Third version—edited, revised, and returned to the Utah Department of Environmental Quality-Water Quality and the Utah Lake Quality Study Science Panel
March 11, 2021	Fourth version—edited, revised, and returned to the Utah Department of Environmental Quality-Water Quality and the Utah Lake Quality Study Science Panel
April 14, 2021	Fifth version—edited, revised, and returned to the Utah Department of Environmental Quality-Water Quality and the Utah Lake Quality Study Science Panel
April 26, 2021	Sixth version—edited, revised, and returned to the Utah Department of Environmental Quality-Water Quality and the Utah Lake Quality Study Science Panel

Acknowledgements

The work was supported by the Utah Department of Environmental Quality-Water Quality. Any recommendations expressed here are those of the author(s) and do not necessarily reflect the views of the Utah Department of Environmental Quality.

Acronyms/Abbreviations

C	carbon
DIN	Dissolved inorganic nitrogen (NH_4^+-N + NO_3^--N + NO_2^--N)
HAB	Harmful algal bloom
N	nitrogen
P	phosphorus
PAR	Photosynthetically active radiation
SRP	Soluble reactive phosphorus or orthophosphate
TN	Total nitrogen
TP	Total phosphorus
UT-DWQ	Utah Department of Environmental Quality-Water Quality
ULWQS	Utah Lake Water Quality Study Science Panel
WWTP	Wastewater Treatment Plants

1. Utah Lake Management Highlights

Study 1: Seasonal and Spatial Nutrient Limitation

Cyanobacteria Responses

- The nutrient limitation of cyanobacteria and to a lesser extent total phytoplankton (e.g., chlorophytes, diatoms, and cyanobacteria) were 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 cyanotoxin producing cyanobacteria.
- 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 total phytoplankton.

Total Phytoplankton Responses

- Nutrient colimitation (DIN and SRP) of total phytoplankton (i.e., chlorophyll-a concentrations from all prokaryotic and eukaryotic organisms) 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, total phytoplankton was limited during every season with DIN generally limiting total phytoplankton responses.
- *Aulacoseira* and *Desmodesmus spp.* and unicellular and colonial green algae were primarily associated with total phytoplankton nutrient limitation across Utah Lake regardless of season.

Summary of Nutrient Limitation

Variable and Location	spring	early summer	summer	late summer	fall
Cyanobacteria nutrient limitation					
East	No limitation	No limitation	P	No limitation	No limitation
West	N+P	No limitation	N	No limitation	No limitation
Provo Bay	P	N+P	P	No limitation	No limitation
Total phytoplankton nutrient limitation					
East	No limitation	N+P	N+P	N+P	N+P
West	P	No limitation	N+P	N+P	N+P
Provo Bay	N+P	N	N	N+P	N

DIN and SRP Concentrations

- DIN and SRP in the water were biologically available to the cyanobacteria and total phytoplankton as species sequestered the N and P to new biomass containing phycocyanin and chlorophyll-a—the addition of N resulted in lower P concentrations and the addition of P leading to lower N concentrations.
- During the summer seasons, across all locations, the ratio of DIN to SRP in the N+P addition remained close to 16:1 (Redfield ratio of the consistent atomic ratio of N and P in total phytoplankton biomass), suggesting primary producers potentially utilized N and P in equal proportions to generate biomass.

N_2 Fixation in Early Summer

- N_2 fixation increased 7.7-fold in N+P relative to the control (N+P=9.41 ng N/L/hour ±4.27, control=1.23 ng N/L/hour ±0.523) in East water. The addition of P alone did not elevate N_2 fixation.
- In Provo Bay, N_2 fixation rates were at least 4-times higher than in East but were not influenced by any nutrient addition. N_2 fixation was non-detectable in West water.

Study 2: Growth Rate Response to Nutrient Limitation

- The 48-hour or 72-hour incubation in Study 1 captured the majority of total phytoplankton and cyanobacterial responses (i.e., changes in chlorophyll-a, phycocyanin, and cyanotoxin concentrations) to DIN and/or SRP additions, but the responses were most likely associated with faster- rather than slower-growing total phytoplankton and cyanobacteria.
- In the summer, total 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 location in the lake. 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 total 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 total phytoplankton was less apparent in Provo Bay where total 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, three cyanobacterial species dominated the responses to the addition of N, P, and N+P: *Aphanocapsa, Dolichospermum, and Microcystis spp*.
- Cyanotoxins loosely followed cyanobacterial growth but 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 Provo bay the relatively higher phycocyanin

concentration and cell density of cyanobacteria did not translate into higher concentrations of cyanotoxins.

Study 3: Nutrient Dilutions to Determine Threshold Response (Spring)

- Overall, a target DIN concentration < 0.14 mg/L and a SRP concentration ≤ 0.005 mg/L may reduce HAB potential.
- In the spring, the nutrient levels needed to reduce total phytoplankton activity, measured as chlorophyll-a concentrations, were a DIN concentration < 0.14 mg/L, and a SRP concentration < 0.06 mg/L.
- The nutrient level needed to reduce cyanobacterial activity or growth, measured as phycocyanin concentrations, is a SRP concentration ≤ 0.005 mg/L.
- Microcystin was detected after 120 hours and N+P additions supported the highest concentrations. Cylindrospermopsin was most abundant in the first 48 hours of the dilution; dilutions that received relatively high nutrient inputs of N and/or P supported the highest cylindrospermopsin concentrations. Anatoxin-a was consistently high through time and was often the most abundant of the three toxins evaluated.
- We strongly suggest that managers of Utah Lake create a dual management strategy to successfully reduce eutrophication and HAB potentials.

Study 4: Grazing of Primary Producers

- In the main body of the lake, in the early summer, microzooplankton grazed total phytoplankton and cyanobacteria, but in the bay, microzooplankton grazers demonstrated a selective feeding preference for cyanobacteria.
- Microzooplankton grazing was measured in the absence of microzooplankton and macroinvertebrate predation and reflect an incomplete foodweb. The inclusion of a complete aquatic foodweb will reduce the impact of microzooplankton grazing.
- In the main body of the lake, microzooplankton grazed cyanobacteria, measured as phycocyanin concentrations, to almost non-detectable levels.
- In Provo Bay water, the inclusion of microzooplankton led to an increase in chlorophyll-a concentrations across all treatments and the control.

2. Abstract

The overarching purpose of our bioassay studies was to identify when and where nutrients limited total phytoplankton, cyanobacteria, and cyanotoxins across Utah Lake, USA, and to determine the N and P concentration thresholds needed to curb HAB formation. Here, we summarize the findings from four *in-situ* bioassay studies that experimentally added or diluted 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. Lake water was examined from three locations (i.e., main body of the lake, East; and main body of the lake, West; and Provo Bay) that vary in anthropogenic nutrient loading and physiochemistry. 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 mycrocystin, anatoxin-a, and cylindrospermopsin; DIN, SRP, TP, and TN concentrations; and other water chemistry parameters. We also evaluated N_2 fixation (acetylene reduction) rates in the spring and early summer. We found that nutrient limitation of cyanobacteria, and to a lesser extent total total phytoplankton (including all prokaryotic or eukaryotic organism containing chlorophyll-a), were influenced by season and depended on lake location. 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, total 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. Based on our spring dilution bioassay, the nutrient levels needed to limit total phytoplankton growth are a DIN concentration < 0.14 mg/L combined with a SRP concentration < 0.06 mg/L. The nutrient level needed to reduce cyanobacterial growth is a SRP concentration ≤ 0.005 mg/L. The species primarily associated with nutrient limitation were cyanobacteria *Microcystis*, *Aphanocapsa*, *Dolichospermum*, *Merismopedia*, and *Aphanizomenon spp.*, and total phytoplankton *Aulacoseira* and *Desmodesmus spp.* and two taxonomical categories of algae (i.e., unicellular and colonial green algae). 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. DIN and SRP were biologically available in the bioassay experiments with nutrient concentrations declining over the incubation period. In the main body of the lake, in the early summer, zooplankton grazed total phytoplankton and cyanobacteria, but in the bay, zooplankton demonstrated a selective feeding preference for cyanobacteria. Zooplanktivory decreased phycocyanin concentrations to almost non-detection levels, demonstrating that microzooplankton grazing has the potential to regulate cyanobacterial growth in the absence of macrozooplankton and macroinvertebrate predation. Our results offer insights into specific nutrient targets, species, and, and cyanotoxins to consider in the future to manage Utah Lake.

3. Introduction

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 (Brooks et al 2016). The inception of blooms is linked to the appropriate conditions allowing photosynthetic organisms to break dormancy and become abundant (Aanderud et al 2016). Cyanobacteria and algae become dominant under specific physiochemical water conditions, generally connected to excessive TP and TN 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 pools within TP and TN are more bioavailable than others and nutrient chemical forms also influence HABs (Paerl et al 2008). Additionally, the relative abundance of cyanobacterial and total phytoplankton species is governed by more than excessive N and P (Wood et al 2017; Randall et al 2019). For example, 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 chlorophyte 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, the neurotoxin anatoxin-a production 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 along 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 total phytoplankton and cyanobacterial growth rates and abundances (Bergstrom 2010). Climate change has brought 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 and nonpoint nutrient sources (i.e., stormwater and agricultural inputs), more freshwater bodies are excessively loaded with nutrients, specifically N and P (Galloway et al. 2004; Haygarth et al. 2005; Seitzinger et al 2010; Foley et al. 2011; Pinay et al 2015). Because water from lakes with active HABs often supply nutrients in excess of total phytoplankton and cyanobacteria demands, N and P need to be diluted to identify the loads necessary to deter bloom formation (Xu et al 2015; Paerl and Bowles 1987). Dilution bioassays are extremely helpful in examining total phytoplankton and cyanobacterial responses to lower nutrient levels and generating thresholds that may curb HABs.

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 total 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).

The biology of a HAB is inherently complicated. HABs are often dominated by multiple different total phytoplankton and cyanobacteria species responding to a cadre of environmental factors while acting as the primary producers of lake food webs (Randall et al 2019; Wood et al 2017). Multiple eukaryotic grazers prey on total phytoplankton (Work, 2003), but other ecological interactions may 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. The ecological release hypothesis states that 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 and 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 intensify specific cyanobacteria and algal species. Shallow lakes are 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.

4. Purpose Statement

The overarching purpose of our research was to identify when and where N, P, or N+P limit total phytoplankton or cyanobacteria across Utah Lake, and to determine the N and P concentration thresholds needed to control HAB formation. Here, we summarize the findings from four bioassays studies that evaluated the nutrient limitation of total phytoplankton and cyanobacteria growth, specific HAB taxa responses, and cyanotoxin production to help describe the current state of the lake with respect to nutrient levels, trophic state, and ecology. For our purposes, we define total phytoplankton as all prokaryotic or eukaryotic organism containing chlorophyll-a (e.g., chlorophytes, diatoms, and cyanobacteria). Cyanobacteria determinations are a subset of total phytoplankton but are evaluated separately due to the importance of these species in HAB processes such as N_2 fixation and cyanotoxin production. Further, special attention was given to cyanobacteria and cyanotoxins since cyanobacteria cell density is the current cause of Utah Lake being listed as impaired and toxins contribute to partial lake closures and health advisories.

5. Study Objectives, Experiments, and Hypotheses

The objective of this research was to address the following topics identified by the ULWQS as critical to understanding the current state of Utah Lake with respect to nutrient interactions and primary producers. All studies were conducted in *in-situ* bioassays with lake water from the upper 20 cm of the water column.

5.1 Study 1: Seasonal and Spatial Nutrient Limitation

- Study Objective: Determine the extent that seasonal (i.e., spring, early summer, summer, late summer, and fall) and spatial (i.e., main body of the lake, East; and main body of the lake, West; and Provo Bay) components drive nutrient limitation of total phytoplankton and cyanobacteria species. Nutrient limitation may include N or P individually or a combined co-limitation of total phytoplankton and cyanobacteria.

- Bioassay Experiments: We conducted three nutrient additions (i.e., N, P and N+P addition) and a control in a bioassay study over five seasonal time periods (i.e., spring in May, early summer in June, summer in July, late summer in August, and fall in October) across three main locations in Utah Lake. Specifically, the nutrient additions followed a DIN: SRP molar ratio equal to 16:1 and were applied to directly to surface water from main body East (East), main body West (West), and Provo Bay. Although the same nutrient additions were applied for every season, the resulting DIN:SRP in the bioassay may have fluctuated due to seasonal differences in lake water nutrient loads. The potential N, P, and/or N+P limitation of total phytoplankton and cyanobacteria (a subset of total phytoplankton) was evaluated as a response ratio of chlorophyll-a and phycocyanin pigment concentrations relative to the control. We also evaluated cyanotoxin concentrations, N_2 fixation rates, and lake chemistry to help explain our findings.

- Hypotheses: We hypothesized that: Utah Lake total phytoplankton and cyanobacteria will follow a similar nutrient limitation pattern present in other shallow lakes. Total phytoplankton will be P-limited in the spring and early summer and

switch to N-limited in the summer and fall. Cyanobacteria will also be P-limited in the spring and summer but will stay P-limited due to the ability of some of these species to fix atmospheric N_2. We also hypothesized that cyanotoxin concentrations will be enhanced as cyanobacterial nutrient limitation is removed.

5.2 Study 2: Growth Rate Response to Nutrient Limitation

- Study Objective: Determine the potential for N, P, and/or N+P limitation to influence the primary production/growth of total phytoplankton and cyanobacteria across the lake.

- Bioassay Experiment: We evaluated the growth rates of total phytoplankton and cyanobacteria to establish the most appropriate time to sample replicates across the lake locations in the summer (July). Growth rates were evaluated as the relative growth rates of total phytoplankton and cyanobacteria across time (0, 24, 48, and 96 hours) and were following the same treatments, procedures, and analyses as in Study 1.

- Hypotheses: We hypothesized that the warmer lake temperatures in summer will favor cyanobacterial growth but cyanobacteria relative to total phytoplankton will demonstrate a slightly slower growth rate and a time lag before reaching peak growth even when N, P, or N+P limitation are removed. We also hypothesized that cyanotoxins production will be minimal due to the optimal toxicity temperatures potentially being around 25°C.

5.3 Study 3: Nutrient Dilutions to Determine Threshold Response

- Study Objective: Determine the level of N and/or P needed to control total phytoplankton and cyanobacteria bloom formation.

- Bioassay Experiment: To examine the N and P thresholds that may limit HAB formation, we performed a dilution bioassay following the same design outlined for Study 1, except that we sequentially reduced nutrient levels. The dilution study was completed in spring (May) in only the main body East location. We evaluated total phytoplankton and cyanobacteria responses, species distribution, and cyanotoxin production in the same manner as Study 1.

- Hypotheses: We hypothesize that nutrient thresholds will be reached for total phytoplankton and cyanobacteria individually in early spring since primary producers are extremely sensitive to DIN and SRP levels.

5.4 Study 4: Grazing of Primary Producers

- Study Objective: Determine the potential for zooplankton to graze total phytoplankton and cyanobacteria.

- Bioassay Experiment: In the early summer (June), we evaluated the impact of zooplanktivory on total phytoplankton and cyanobacteria responding to nutrient additions. We created treatments that included and excluded zooplankton grazers across the nutrient treatments and lake locations.

- Hypotheses: We hypothesized that if zooplanktonic grazers are present in early summer waters, zooplanktivory or grazing will decrease total phytoplankton more than cyanobacteria due to the presence of cyanotoxins in cyanobacteria cells.

6. Methods

6.1 Study 1: Seasonal and Spatial Nutrient Limitation

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); Map 1). 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). The bay waters are generally 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 total 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.

Map 1. Map of the three Utah Lake locations: main body West, and main body East, and Provo Bay

Seasonal Sampling Times and Bioassay

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 maintain similar light and temperature conditions. We placed the Cubitainers in the floating corrals (diameter 1.5 m, Photo 1) 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 total phytoplankton and cyanobacteria to respond based on the initial bloom conditions. For Utah Lake, we defined an active bloom as the initial water conditions having 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.

Photo 1. Corrals and Cubitainers deployed in boat slip at Utah Lake State Park

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 total 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 NH_4^+-N (N from ammonium) and NO_3^--N (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.F.; Koenig et al 2014).

Chlorophyll-a and Phycocyanin Concentrations

We evaluated total phytoplankton and cyanobacteria, a fraction of total 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 total 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. The conversion factor we used to change the cyanobacteria cell counts (cells/mL) of each species to biovolume (μm^3/mL) is presented in Table 1.

Table 1. The 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

We conducted the cell counts (cells/mL) on a Zeiss Axioplan2 upright fluorescent microscope (Zeiss, 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 total 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. The convert cyanobacterial cell counts to biovolume, we used an average biovolume quantified by Rushforth Phycology LLC (http://www.rushforthphycology.com) for individual species in Utah Lake. 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 = *Aphanocapsa, Microcystis, Dolichospermum spp.*, and filamentous cyanobacteria) for each season.

N_2 Fixation Rates

In the spring and early summer, we performed acetylene reduction assays to quantify N_2 fixation rates (ng N/L/hour). We measured N_2 fixation rates in three locations × one time point × four treatments (control, N, P, N+P) × two seasonal times (spring and early summer) × three replicates = 72 total samples. The acetylene reduction assay was an indirect method for estimating fixation by measuring the activity of the nitrogenase enzyme, which cyanobacteria use to fix N_2 to NH_4^+. We followed the method outlined by Marcarelli & Wurtsbaugh (2009). We measured nitrogenase enzyme activity in a gas-tight 800 mL chamber filled completely with lake water and fitted with a gas tight syringe. A latex balloon containing acetylene was added to the chamber as the chamber was filled with lake water. The balloon was popped with a needle introducing 200 mL of acetylene. We measured the amount of ethylene produced over three hours. In this assay, the biota was saturated with acetylene gas, which is converted to ethylene gas at a rate related to the potential N_2 fixation rate. The amount of ethylene formed was measured with a gas chromatograph (GC; Shimadzu GC-8A) equipped with a flame ionization detector (FID), Porapak N column (80/100 mesh, 1/80OD669), and integrator (Hewlett Packard 3396) with N^2 as the carrier gas (25 mL/min low rate).

Response Ratios and Statistical Analyses

We quantified responses of Cyanobacteria and total phytoplankton to potential nutrient limitation 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 jitter plots 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.

6.2 Study 2: Growth Rate Response to Nutrient Limitation

Time Series Bioassay and Growth Rate Study Design

We evaluated the growth rates of total phytoplankton and cyanobacteria, a subset of total phytoplankton, individually to establish the most appropriate time to sample the Cubitainers in the Study 1. Growth rates were evaluated in Cubitainers following the same procedure as outlined in the Study 1. 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 Study 1 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.

Total Phytoplankton and Cyanobacterial Growth Rates and Statistics

We calculated total phytoplankton and cyanobacterial growth as specific growth rates (μ) over multiple time steps based on the first-order rate law using the equation:

$$\mu = ln\,(N_F/N_I) / T_F - T_I \qquad (2)$$

where N_I was the pigment concentration per mL at an initial time point (T_I, as either T_0, T_1, or T_2) and N_F was the pigment concentration at the next time step (T_F as either T_1, T_2, or T_3). 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.

6.3 Study 3: Nutrient Dilutions to Determine Threshold Response

Dilution Bioassay Study Design

To examine the N and P thresholds that may limit HAB formation, we performed an *in-situ* dilution bioassay experiment following the same design outlined for Study 1, except for some key changes in the nutrient addition treatments and the duration of the incubation. The week following the spring bioassay study (May 25–29), we performed the nutrient dilution using lake water from the top 20 cm of the water column from water in the main body East Location. We selected the East location due to its proximity to WWTP and a growing urban population and with the intent to capture a more general response of total phytoplankton and cyanobacteria to lower nutrient levels. The water collection, cubitainer deployment, and resulting samples were treated and analyzed in the same way as with other bioassay campaigns (Study 1). Dilution replicates consisted of 50% lake water and 50% synthetic ion solution mimicking Utah Lake major ion chemistry of the East location minus N and P (Table 2). The dilution of the lake water with the synthetic solution reduced the inherent available levels of N and P by 50%. Based on previous N and P data for the East location in spring (Study 1), the ambient water concentration of DIN=0.28 and SRP=0.01 mg/L. The nutrient dilutions were heavily influenced by the methods of Xu et al 2021, especially for guidance on the creation of the synthetic water.

Nutrient Dilution Treatments and Nutrient Concentrations

After the addition of the synthetic water, dilution Cubitainers were subjected to three different nutrient amendment treatments. First, we created a "dilution low" treatment that received nutrient additions similar to the design outlined in in Study 1 resulting in the final concentrations (lake and experimental N and P combine) of N=0.86 mg/L as NH_4NO_3 and/or P=0.105 mg/L as K_2HPO_4 depending on nutrient treatment (N, P, or N+P). For the dilution low treatment, the reduction of N and P was only due to the 50% reduction of the inherent levels of lake water and not the nutrient additions. Second, we created a "dilution medium" treatment that received 50% of the N and P amendments used in Study 1 resulting in the final concentration of N=0.50 mg/L as NH_4NO_3 and/or P=0.06 mg/L as K_2HPO_4 depending on nutrient treatment (N, P, or N+P). Third, we created a "dilution high" treatment that did not receive any nutrient amendments resulting in one set of Cubitainers with a final concentration of N=0.14 mg/L as NH_4NO_3 and/or P=0.005 mg/L as K_2HPO_4. Finally, we added a 100% lake water control to ensure that our 50% ion solution didn't influence HAB responses. To capture the potential responses of both total phytoplankton and cyanobacteria to these nutrient dilutions we incubated the Cubitainers over two time points (48 and 120 hours). For the dilution bioassay study there was a total 83 replicates=main Body East location × three incubation time points (0, 48, 120 hours) × varying levels of treatments depending on dilution [(2 dilution treatments (low and medium with N, P, N+P addition); and 2 dilution treatments (high and control with no additions)] × three replicates.

Table 2. Chemical composition and concentration of the major ions in the synthetic solution used in the dilution bioassay experiment. The recipe is based on ICP-MS data generated from water chemistry analyses in the main body East location or close to the East location in Utah Lake (n = 15).

Chemical form	Final concentration of the major ion solution used to dilute the assays (mg/L or element)
Si^{4+} as Na_2SiO_3 9H2O	0.037
Ca^{2+} as $CaCl_2$ $2H_2O$	44.0
Mg^{2+} as $MgSO_4$ $7H_2O$	77.0
Na^+ as Na_2SO_4	50.0
K^+ as K_2SO_4	10.6
SO_4^{2-} as $MgSO_4$ $7H_2O$	304
Cl^- as $CaCl_2$ $2H_2O$	165

6.4 Study 4: Grazing of Primary Producers

Grazer Exclusion Study Design

In the early summer, we attempted to evaluate the impact of zooplanktivory on total phytoplankton and cyanobacteria responses. In addition to the bioassay from Study 1, we used another full set of Cubitainers for the main body and the bay water that included zooplankton grazers (plus grazer treatment) and the nutrient additions. The lake water for this bioassay study was passed through a 153 μm Wisconsin net (WaterMark, Forestry Suppliers Inc. Jackson, MS 39201) to remove zooplankton. Typical mesh size for filtering macrozooplankton varies from 65 μm to 200 μm (Kolzau et al., 2014; Vanni et al., 2006; Xu et al., 2010). We compared these grazer inclusion replicates to the seasonal bioassays in the early summer. Cubitainers from the seasonal bioassay study represented the exclusion of grazers (minus grazer treatment). The impact of grazers was measured as changes in chlorophyll-a and phycocyanin concentrations between the minus and plus grazer Cubitainers. All other experimental parameters were the same as Study 1. The grazer inclusion bioassay study represented a total of 24 replicates=three locations × four treatments (control, N, P, N+P) × two replicates.

7. Results—Study 1: Seasonal and Spatial Nutrient Limitation

7.1 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.

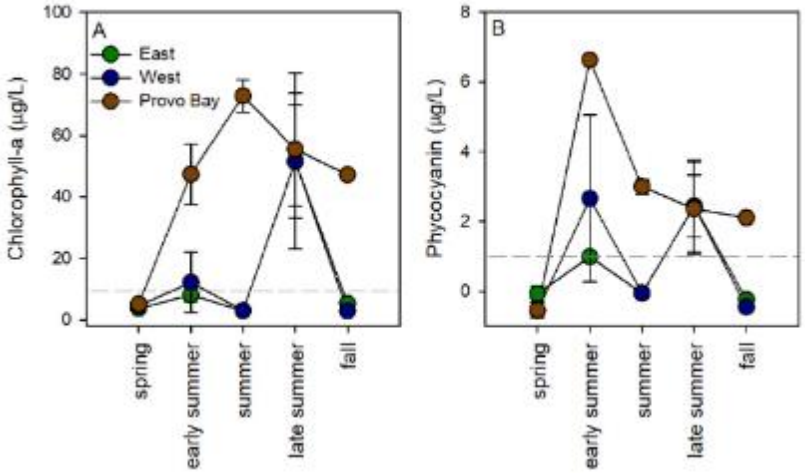
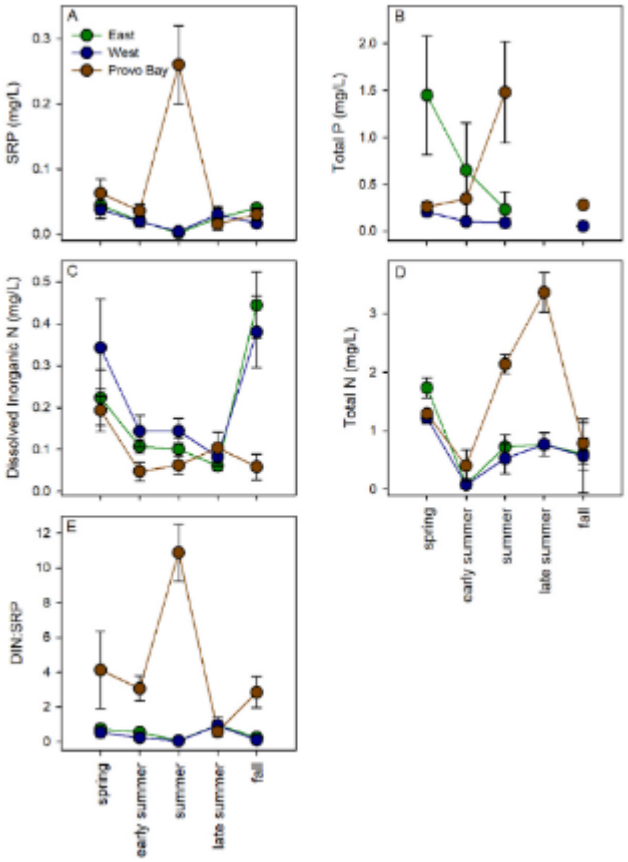


Figure 1. Chlorophyll-a (A) and phycocyanin (B) concentrations in the upper 20 cm of lake water at the three locations immediately prior to the nutrient additions. Values are from YSI EXO2 sonde measurements (*n*=3) in the field during water collection. The dashed line in figures represent the threshold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

6.2 Initial Nutrient Concentration by Season and Location

Biologically available DIN (NH_4^+-N + NO_3^--N + NO_2^--N), and to a lesser extent SRP, varied across seasons with loads being similar in the main lake body compared to Provo Bay. DIN 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.

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).



7.3 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 3). 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 total 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.

Table 3. 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

7.4 Water Chemistry

Lake water 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.

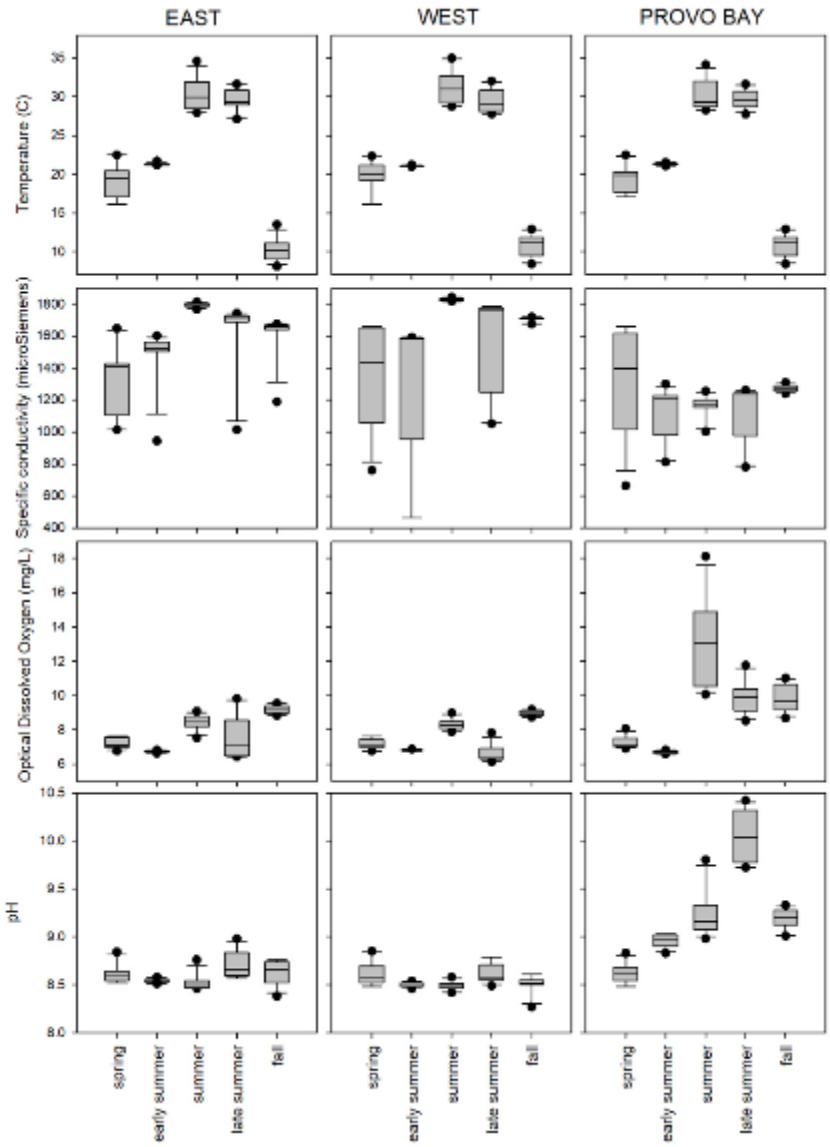


Figure 3. Boxplot of water physicochemical characteristics across the seasons in three locations. Values are from all three nutrient treatments and the control replicates following the incubation by location (*n*=12).

7.5 Cyanobacterial 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 4). 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, Δ*R* of phycocyanin was 3.1 (±0.44) following the N addition (ANOVA by treatment: F value=5.84, *P*=0.009, *df*=2).

7.6 Cyanobacterial Nutrient Co-limitation

Nutrient colimitation (i.e., the response limited by both N and P) 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.

7.7 Nutrient Limitation in Late Summer and Summer

In the late summer and fall, the Δ*R* 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 4).

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.

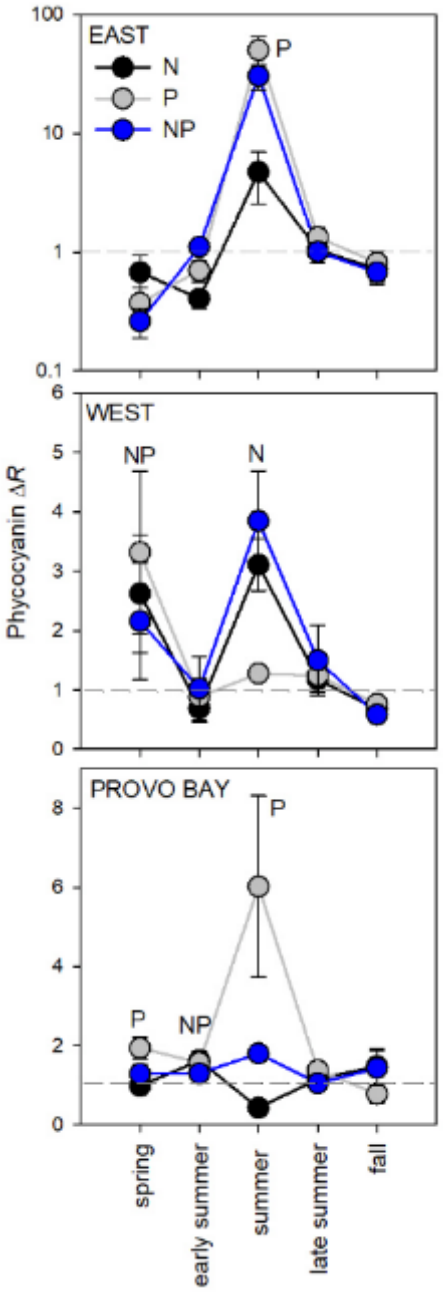


Table 4. Final concentrations of chlorophyll-a and phycocyanin piments following N, P, and N+P additions in the three locations after incubation. Values are means (*n*=3).

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

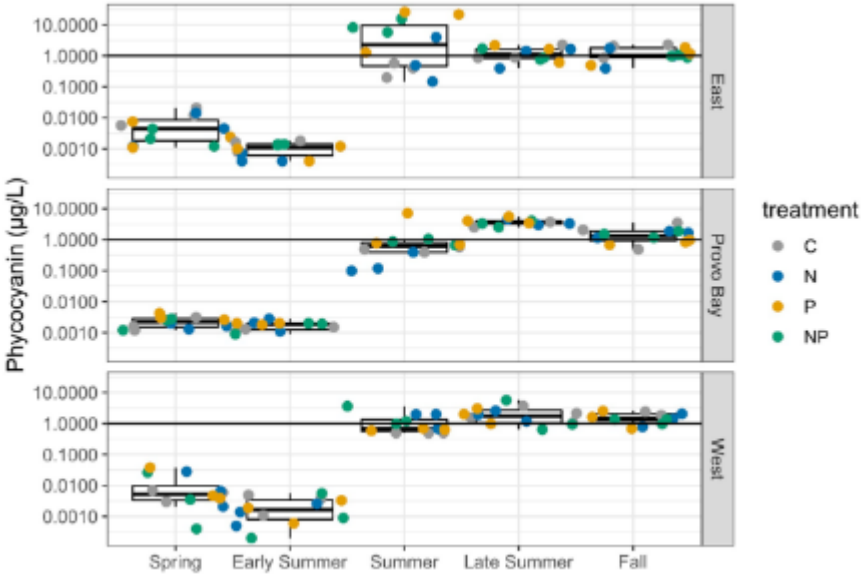


Figure 5. Variation in phycocyanin concentrations in all bioassays from the three nutrient treatments and the control replicates following the incubation by location. The values are presented as a jitter plot containing box plots overlayed with individual bioassay values (*n*=3).

7.8 Total Phytoplankton Nutrient Limitation

Nutrient colimitation of total 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 Δ*R* 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, Δ*R* 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 4). The co-limitation during the fall in the East and West locations was due to all three nutrient treatments inducing a Δ*R* 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 total 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 Δ*R* of at least 2.5 when chlorophyll-a concentrations were at a maximum for the Provo Bay. The variation in treatment phycocyanin concentrations is provided in Figure 7.

Figure 6. N, P, and N+P limitation for total 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.

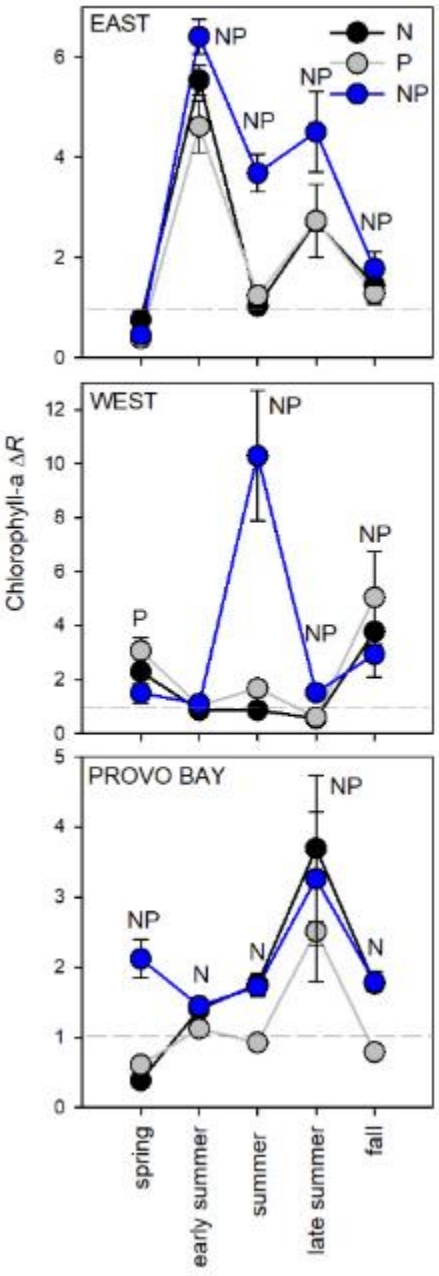
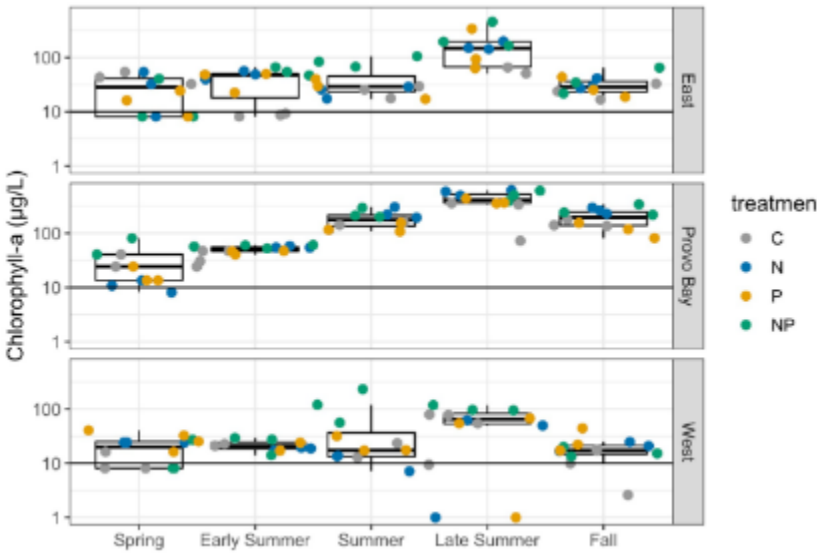


Figure 7. Variation in chlorophyll-a concentrations in all bioassays from the three nutrient treatments and the control replicates following the incubation by location. The values are presented as a jitter plot containing box plots overlayed with individual bioassay values (n=3).



7.9 Cyanobacteria Cell Counts in Bioassay

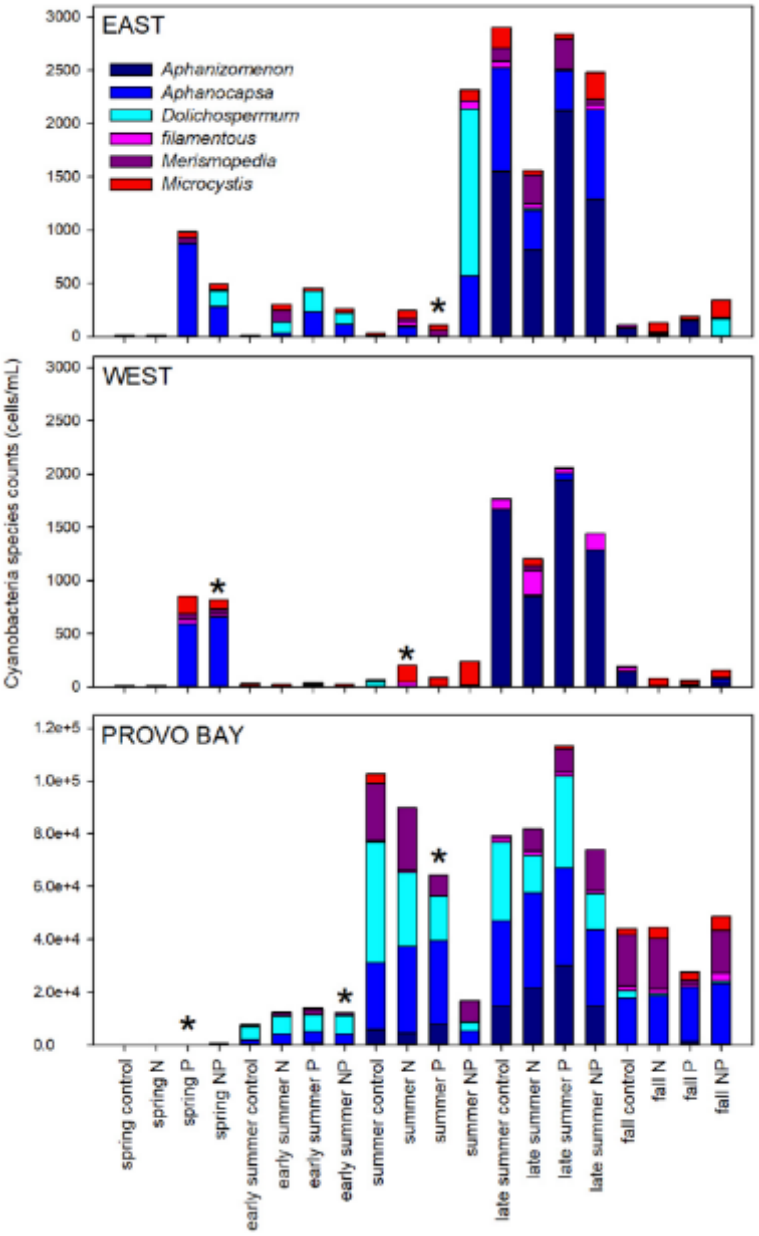


Figure 8. The abundance (cells/mL) of cyanobacterial species in the nutrient treatments in the three locations. Values are means presented as stacked bars from direct microscopy counts (*n*=3). Asterisks indicate a nutrient limitation based on Δ*R of phycocyanin concentrations*.

The cyanobacteria associated with the nutrient limitation 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 co-limitation 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 P limitation in summer and with N+P in the early summer. *Aphanocapsa* and *Dolichospermum spp.* were the most abundant taxa responding to nutrient limitation. 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).

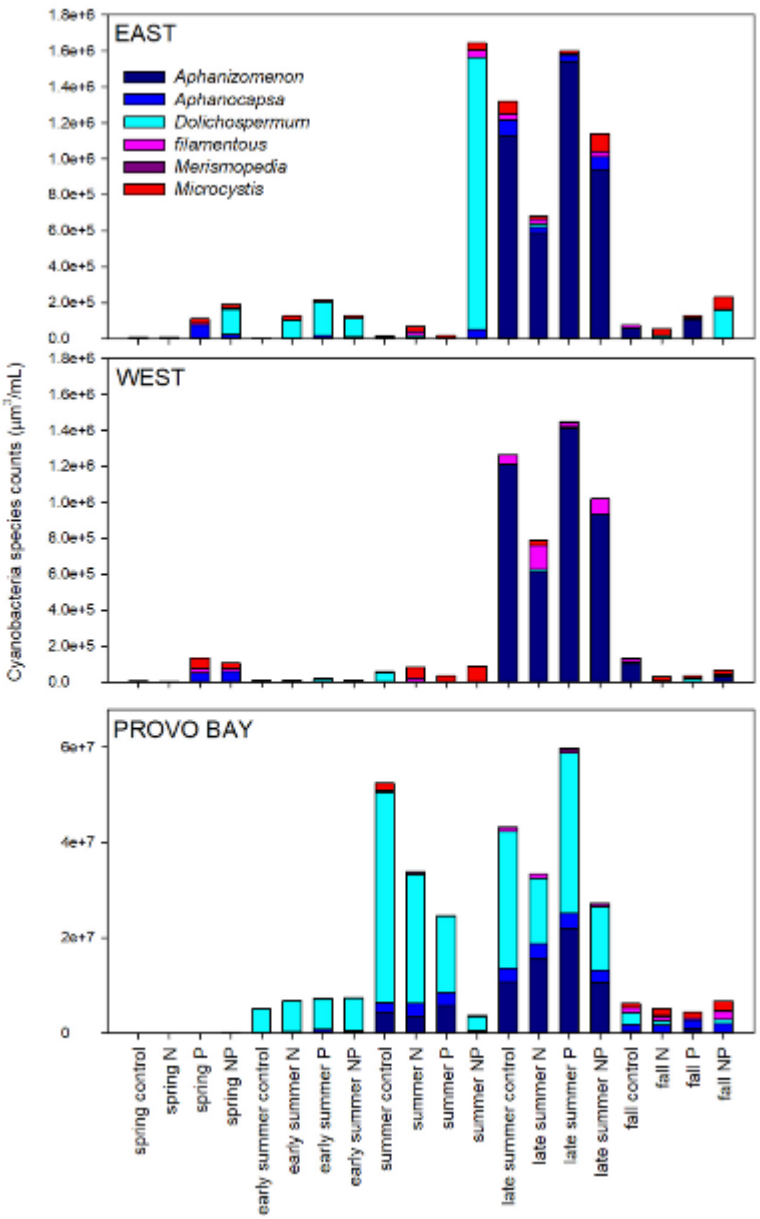


Figure 9. The abundance (µm³/mL) of cyanobacterial species in the nutrient treatments in the three locations. Values are means presented as stacked bars from direct microscopy counts (*n*=3).

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.

7.10 Total Phytoplankton Cell Counts in Bioassay

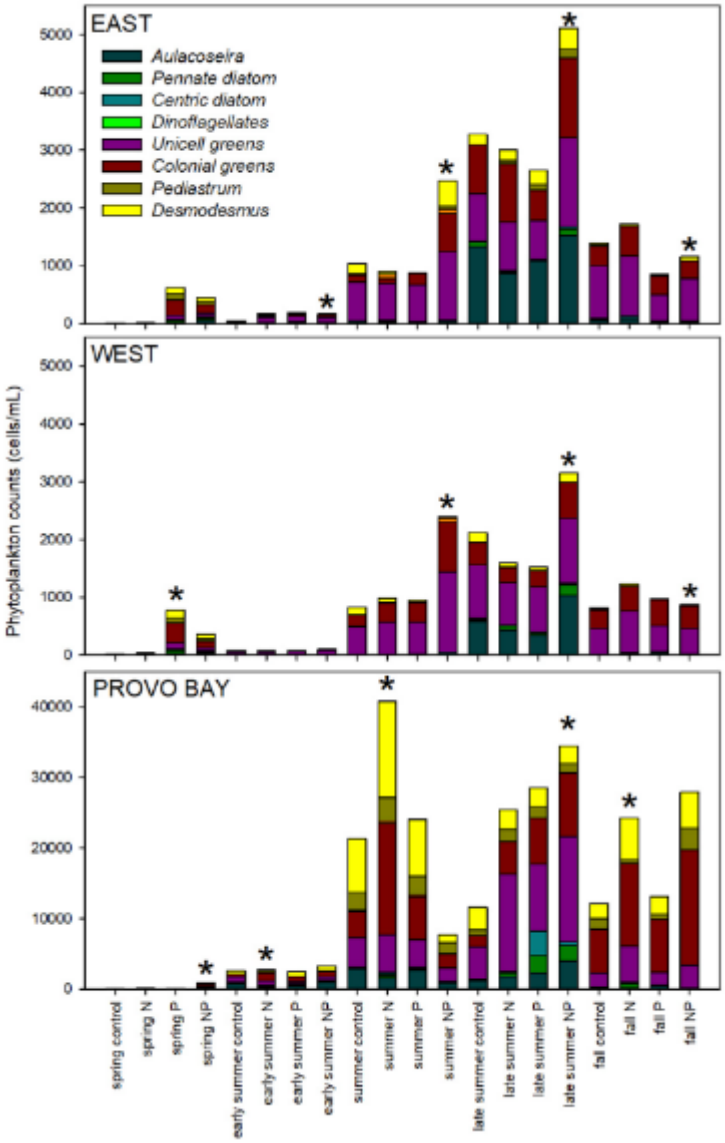


Figure 10. The abundance (cells/mL) of three species and five categories of total phytoplankton (phytoplankton) in the nutrient treatments in the three locations. Values are means presented as stacked bars from direct microscopy counts (*n*=3). Asterisks indicate a nutrient limitation based on Δ*R* of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

Aulacoseira and *Desmodesmus spp.* and two taxonomical categories of algae (i.e., unicellular and colonial green algae) were primarily associated with the total phytoplankton nutrient limitation across Utah Lake among the seasons. Unicellular and colonial green algae were the primary total phytoplankton associated with the N+P limitation in the in East and West consistently demonstrating the highest cell counts among total phytoplankton. Also, *Aulacoseira*, in the late summer, and *Desmodesmus*, across all seasons, contributed to the total phytoplankton responses but to a lesser extent based on cell counts (Figure 10). In 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 total 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).

7.11 Cyanotoxins in Bioassay by Season and Location

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 12). 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 12). 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 total phytoplankton. (Figure 11, 12)

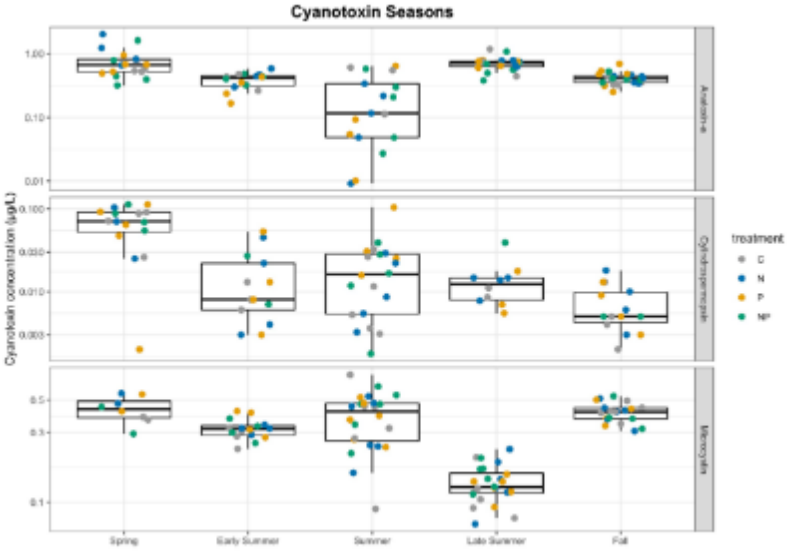


Figure 11. The concentrations of anatoxin-a, cylindrospermopsin, and microcystin over the five seasons season. The values are from ELISA analyses presented as a jitter plot containing box plots overlayed with individual values (*n*=24).

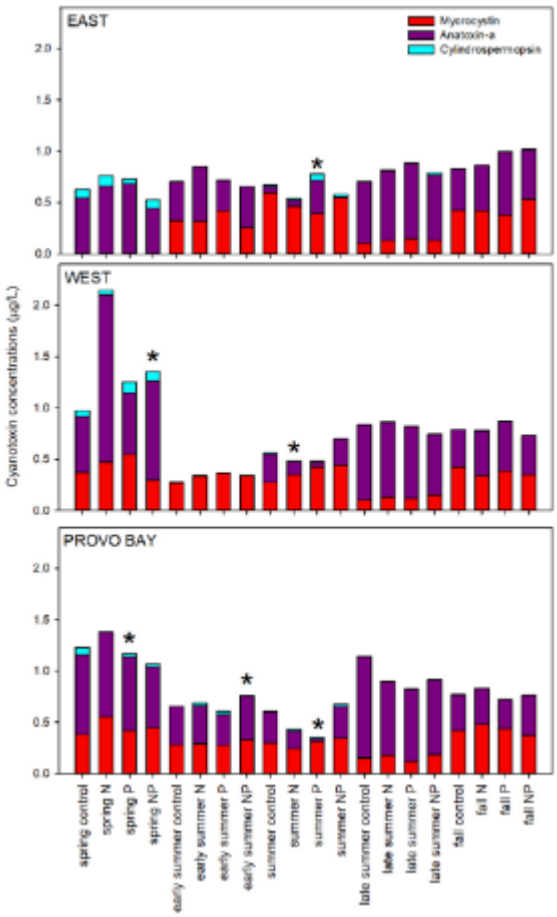


Figure 12. Anatoxin-a, cylindrospermopsin, and microcystin concentrations (µg/L) in the nutrient amendments across seasons. Values are means presented as stacked bars from direct microscopy counts (*n*=3). Asterisks indicate a nutrient limitation based on Δ*R* of phycocyanin concentrations.

7.12 N_2 Fixation in Bioassay by Season and Location

N_2 fixation rates (ng N/L/hour) were quantified in the early summer when the cyanobacteria in Provo Bay was co-limited by N and P. In bay water, the addition of N+P relative to the control increased fixation 2.1-fold, but the difference was not significant (one-way ANOVA by treatment: F value=0.81, *P*=0.53, *df*=3; Figure 13). Fixation in the West was non-detectable in the early summer. In East water, following the addition of N+P (9.41 ng N/L/hour ±4.27) compared to the control (1.23 ng N/L/hour ±0.523), fixation was 7.7-times higher and marginally significant (one-way ANOVA by treatment: F value=2.3, *P*=0.16, *df*=3). There was no measurable N_2 fixation in the West location. After standardizing N_2 fixation rates by phycocyanin concentrations, an approximation of cyanobacteria biomass, there were no significant differences among the treatments based on one-way ANOVA.

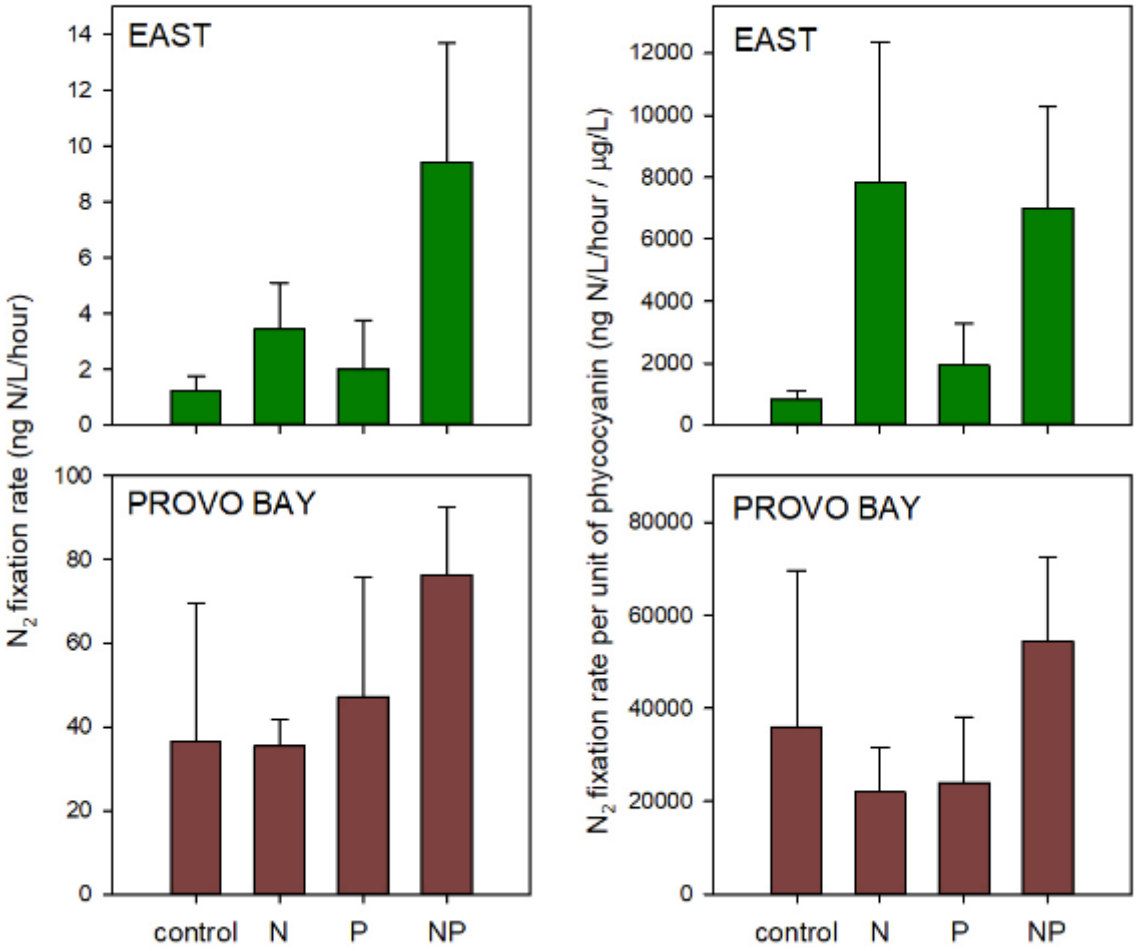


Figure 13. N_2 fixation rates in the three nutrient treatments and a control across the three lake locations in the early summer. Values are based on acetylene reduction assay (*n*=3). Values are expressed as a rate ng N/L/hour and standardized by phycocyanin concentrations.

8. Results—Study 2: Growth Rate Response to Nutrient Limitation

8.1 HAB status Prior to Bioassay and Lake Chemistry

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 (Table 5). The activity of total 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 14). 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).

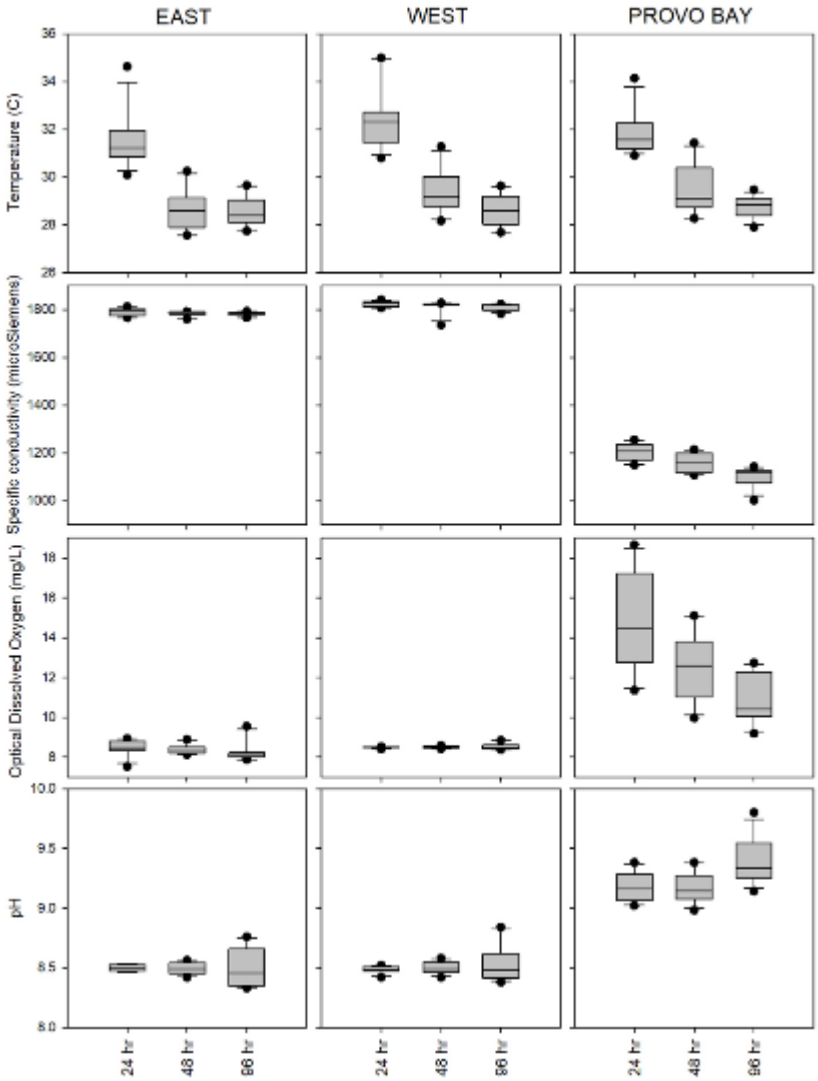


Figure 14. Boxplot of water physicochemical characteristics during the time series in three locations. Values are from all three nutrient treatments and the control replicates following the incubation by location (*n*=12).

Table 5. 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

8.2 Total Phytoplankton and Cyanobacteria Growth Rate

The 48-hour or 72-hour incubations in Study 1 captured the majority of total 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 total phytoplankton and cyanobacteria.

In the summer, total 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 13). In the East and West water, total 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 total 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 (Figure 15). Alternatively, the growth rate under N addition was consistent through the 96 hours (ranging from μ T_1=0.05 ±0.025 - μ T_2=0.02 ±0.026, Figure 4). 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 (μ=0.08 ±0.003) in the first 24 hours and by N (μ=0.09 ±0.005) in the last 48 hours of the incubation; however, these values were only slightly above the control values.

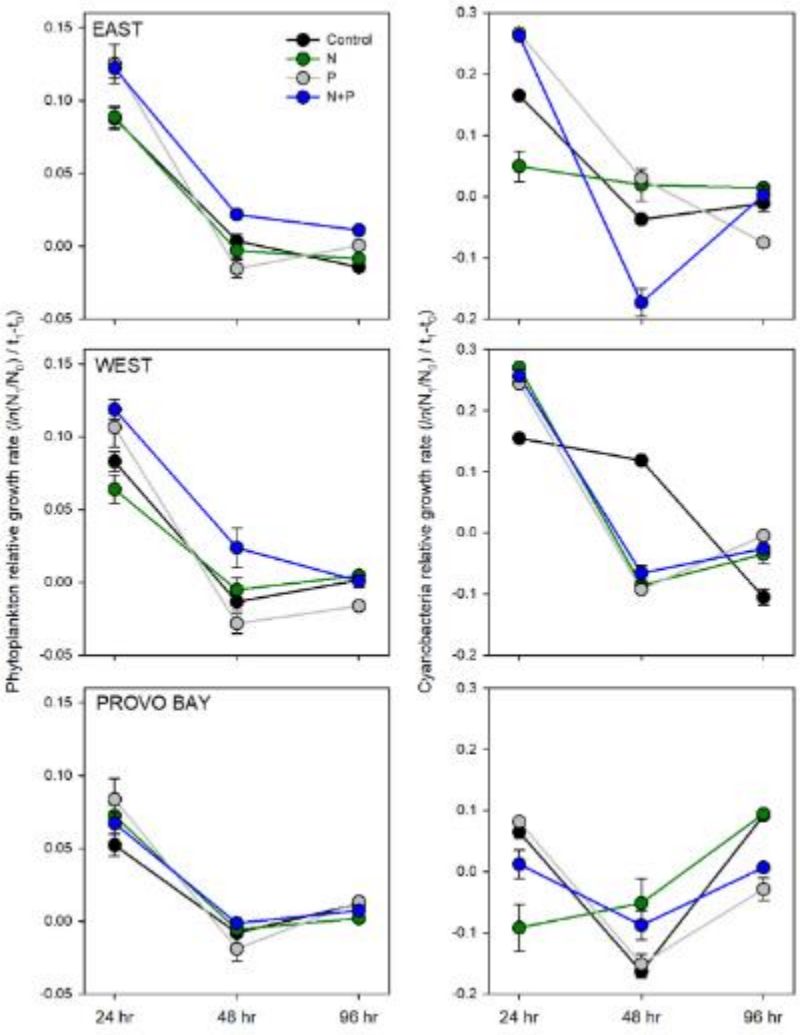
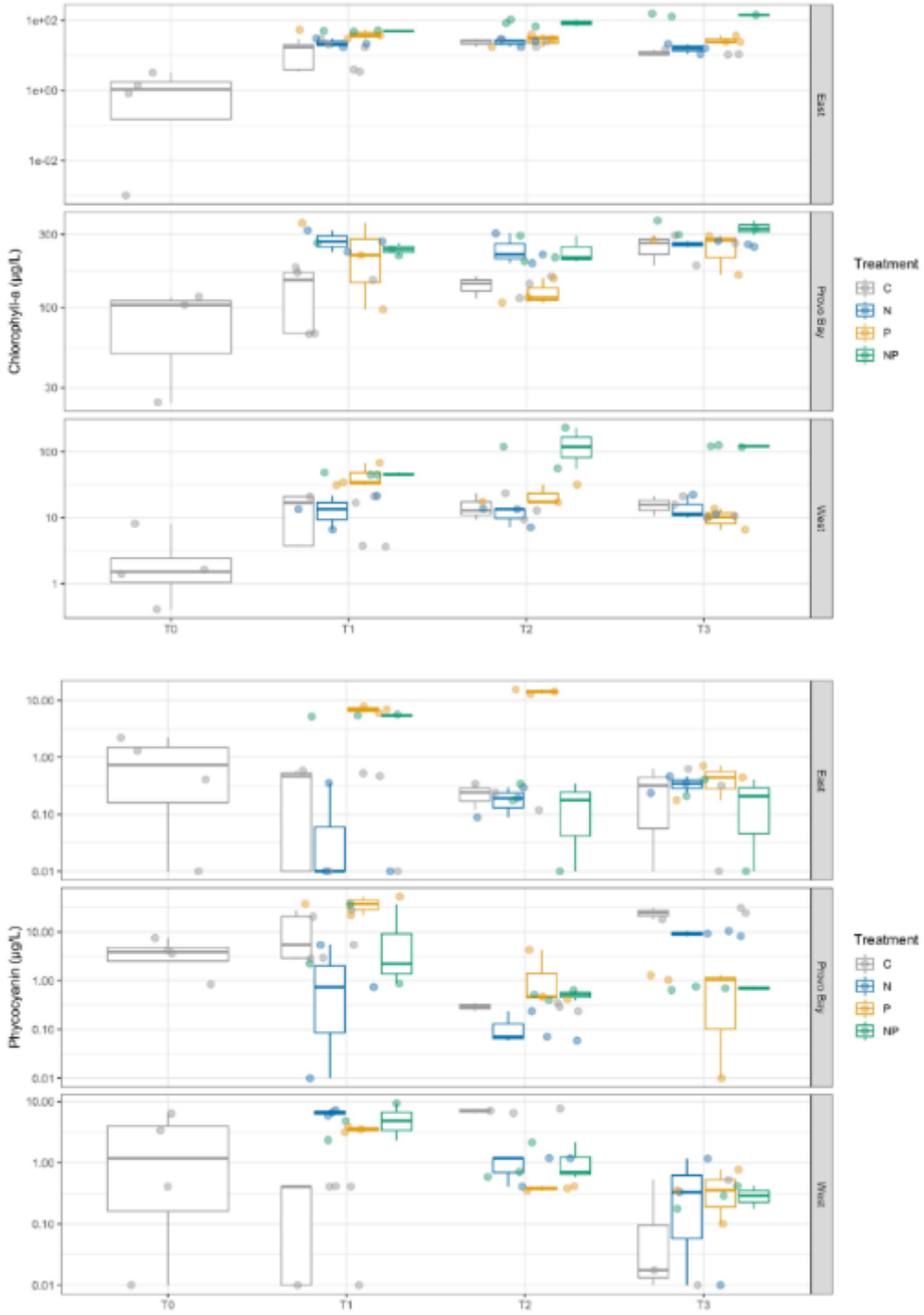


Figure 15. Relative growth rates of total phytoplankton (phytoplankton) and cyanobacteria in the different nutrient and control treatments over the 96-hour incubation. Values are means with ± standard error based on the pigments chlorophyll-a and phycocyanin from all possible replicate combinations between two time points (*n*=9).

The variation in the chlorophyll-a and phycocyanin concentrations during the time series is provided in Figure 16.

Figure 16. 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 a jitter plot containing box plots overlayed with individual bioassay values (*n*=3).



8.3 Total Phytoplankton and Cyanobacteria Cell Counts During Growth

In the main body of the lake, faster relative growth rates of total 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 total phytoplankton biomass. But by 48 hours *Desmodesmus* increased in cell density, and by 96 hours pennate and centric diatoms contributed changes in total phytoplankton growth (Figure 17). The effect of the nutrient treatments on total phytoplankton species/categories was less apparent in Provo Bay where total 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.

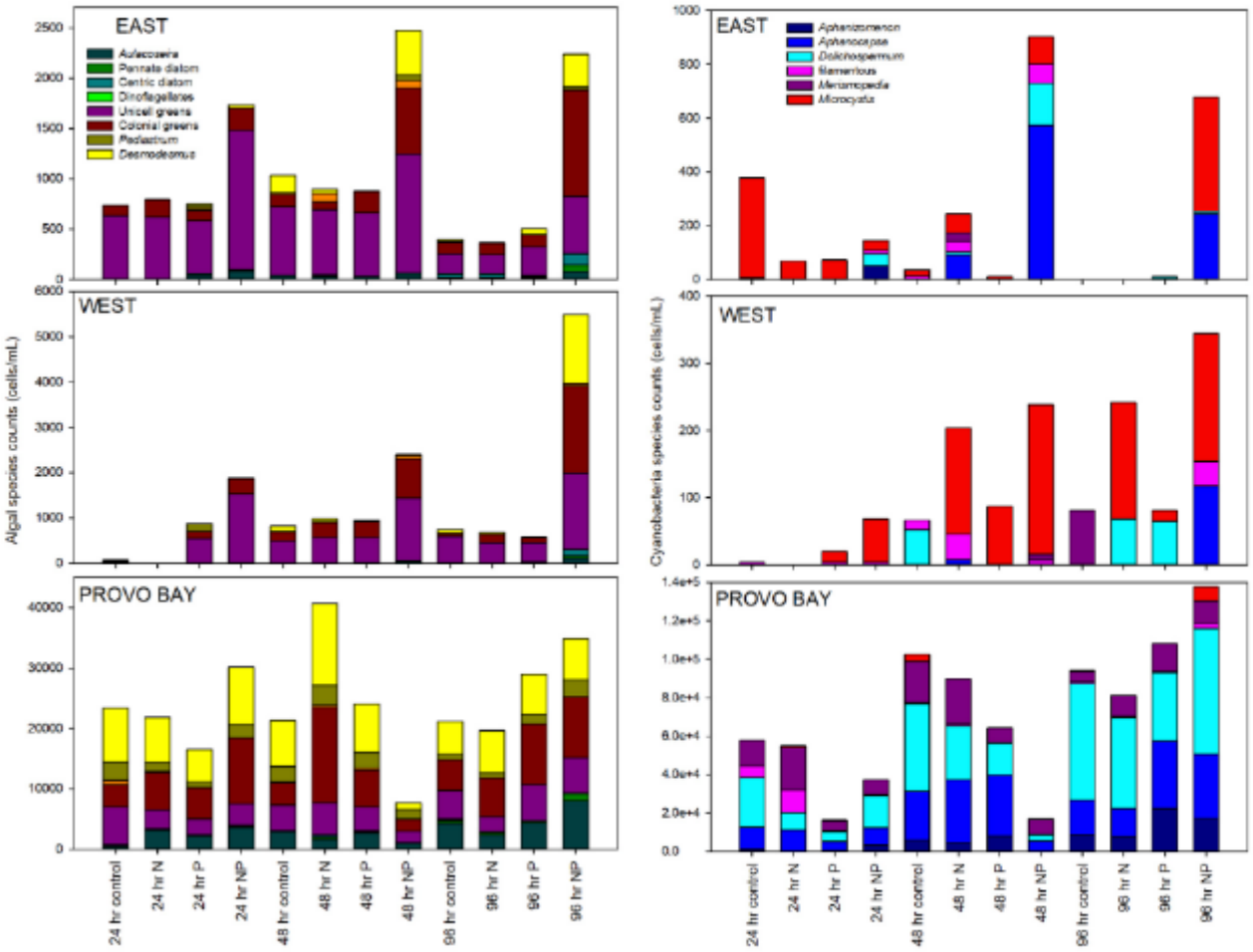


Figure 17. The abundance (cells/mL) of total phytoplankton (algal species count) and cyanobacteria species and categories in the nutrient additions through the 96-hour time series. Values are presented as stacked bars from direct microscopy counts (*n*=2).

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.

8.4 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 18). 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 5) and cell density of cyanobacteria (Figure 17) did not equate to higher concentrations of cyanotoxins.

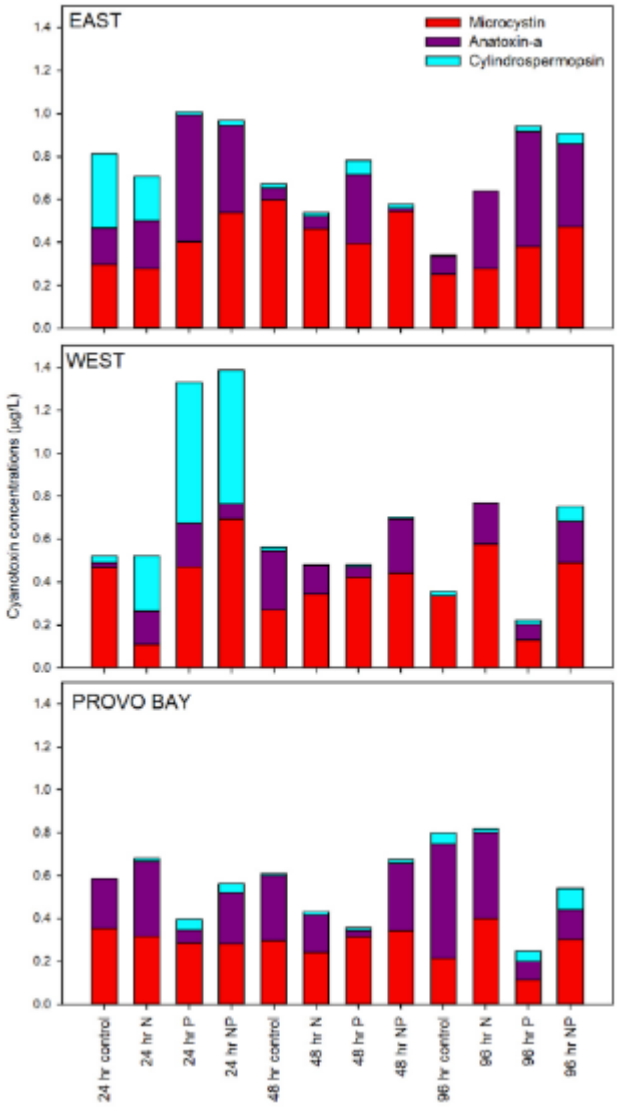


Figure 18. Microcystin, anatoxin-a, and cylindrospermopsin concentrations in the nutrient bioassay by location. Values are presented as stacked bars (*n*=2).

9. Results—Study 3: Nutrient Dilutions to Determine Threshold Response

9.1 HAB status Prior to Bioassay and Lake Chemistry after Nutrient Dilution

Prior to the nutrient dilution bioassay, the water in East during the spring contained a chlorophyll-a concentration of 3.7 µg/L (±0.51) and phycocyanin concentration was non-detectable (Table 5). The dilution of East water and the introduction of the synthetic solution resulted in a similar specific conductivity and dissolved oxygen level (Figure 19). The dilution with the synthetic solution slightly elevated the pH from 8.45 (±0.022) in the control to 8.65 (±0.034) in all of the dilution treatments.

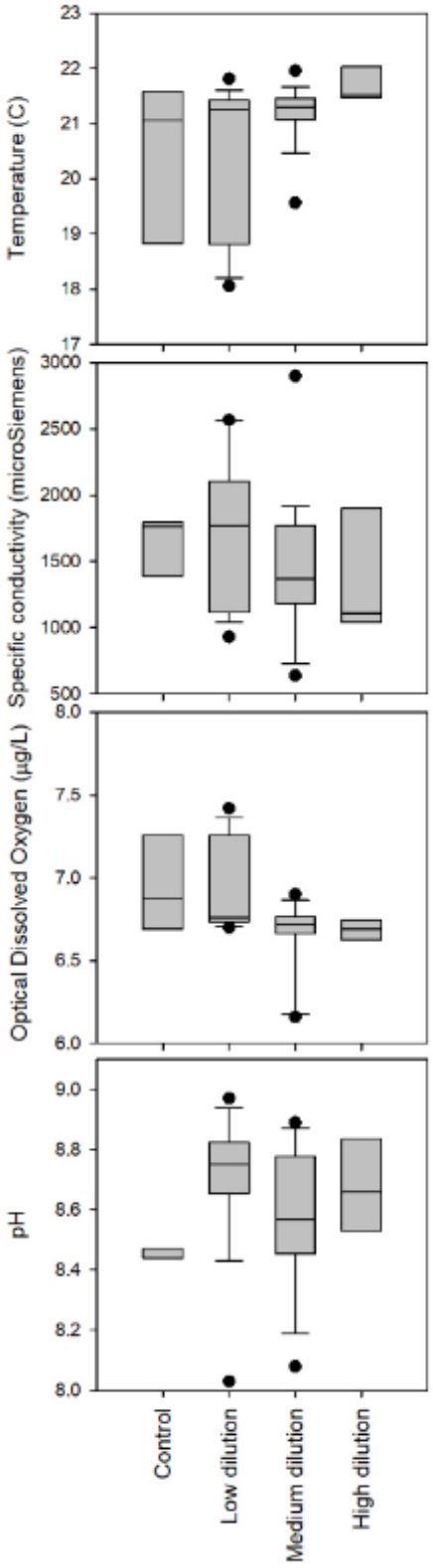


Figure 19. Boxplot of water physicochemical characteristics during the nutrient dilution experiment in the East. Values are from all three nutrient treatments and the control replicates following the incubation for both 48 and 120 hours (*n*=5-18).

9.2 Total Phytoplankton and Nutrient Thresholds

Based on the dilution bioassay, the nutrient levels needed to curb total phytoplankton growth are a DIN concentration < 0.14 mg/L combined with an SRP concentration < 0.06 mg/L. A natural break in the concentration of chlorophyll-a in the Cubitainers occurred at a chlorophyll-a concentration of 3.5 mg/L (Figure 18). This break, and the initial concentrations of DIN and SRP in the Cubitainers, guided the determination of nutrient thresholds. For example, chlorophyll-a concentrations only slightly increased (120-hour chlorophyll-a concentration < 2.27) in low dilution N, medium dilution N, and high dilution N. Therefore, DIN below 0.14 mg/L, in conjunction with SRP of 0.005 mg/L controlled chlorophyll-a concentrations. Further, any dilution receiving a P amendment above 0.06 mg/L SRP stimulated total phytoplankton (120-hour chlorophyll-a concentration > 4.63) even when DIN was relatively low (0.14 mg/L). The exception was for the medium dilution N+P with a DIN of 0.50 and SRP of 0.06 mg/L. Chlorophyll-a concentration in this treatment experienced an initial increase at 48 hours and subsequently declined after 120 hours. Only in the control water (100% East lake water) did total phytoplankton induce a bloom based on the researchers' designation of a HAB for Utah Lake (chlorophyll-a > 10 μg/L).

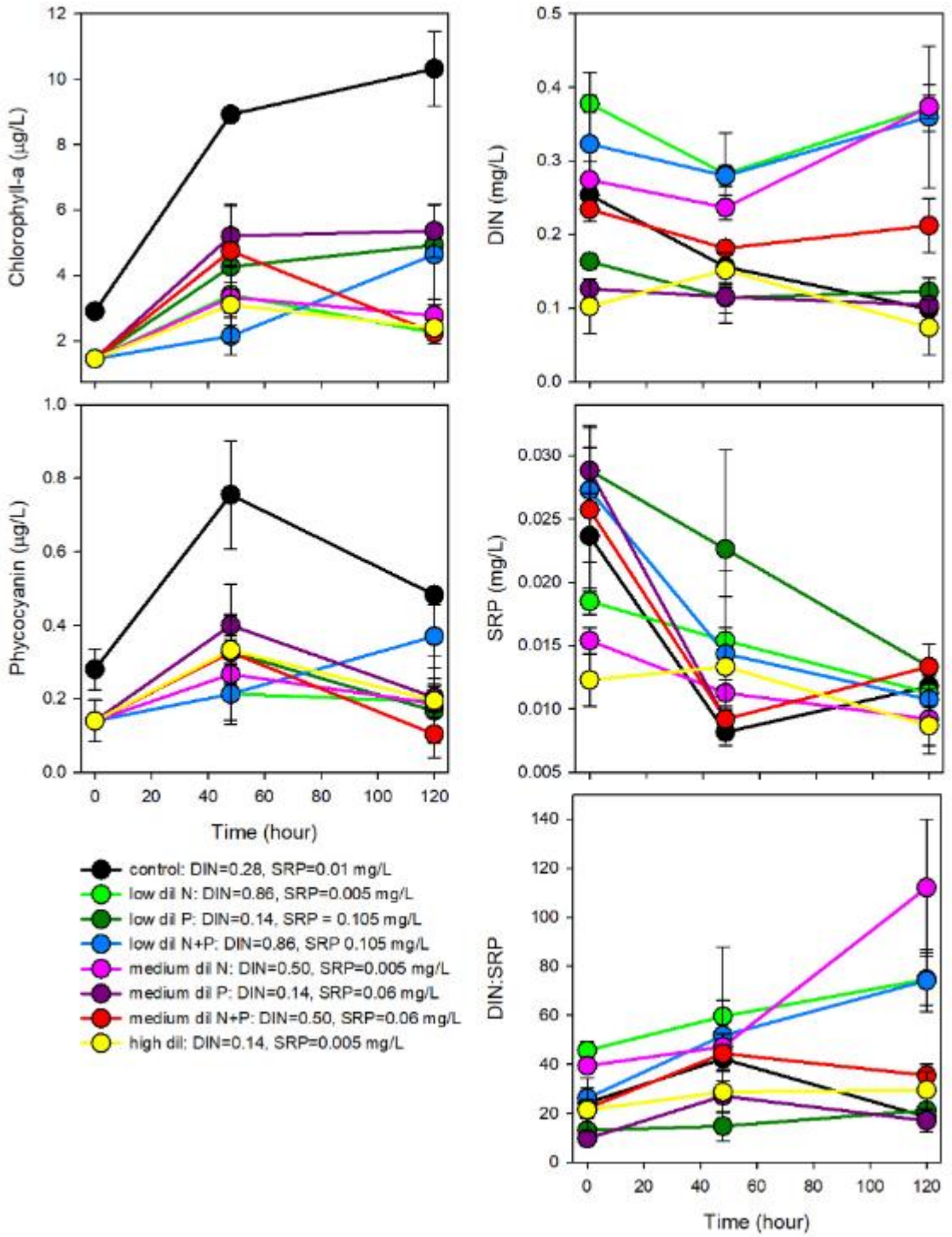
9.3 Cyanobacteria-Nutrient Thresholds

Based on the dilution bioassay, the nutrient level needed to curb cyanobacteria is an SRP concentration ≤ 0.005 mg/L. Unlike with chlorophyll-a where a natural break occurred, the phycocyanin concentration in two nutrient treatments (low dilution N and medium dilution N) helped define the threshold (Figure 20). The phycocyanin concentration in these two treatments was basically the same between the two time points. The initial concentration of N in these dilutions was the same 0.86 or 0.50 mg/L, while SRP equaled 0.005 mg/L in the low dilution N and medium dilution N treatments. Therefore, the decline in SRP, when DIN was presumably available, caused a decline in phycocyanin concentrations and potentially cyanobacteria.

9.4 DIN and SRP under Dilution Conditions

All dilution treatments and the control experienced a decline in SRP levels, while decreases in DIN were dependent on whether the treatment received an addition of N. For example, SRP concentrations declined at least 30% in all dilution treatments from 0 to 120 hours, regardless of the initial concentration (Figure 20). Conversely, DIN concentrations did not decrease in dilution treatments that received an addition of N such as the low dilution N, low dilution N+P and medium dilution N treatments. However, DIN did decrease by upwards of 17% from 0-120 hours in treatments that received no N addition (i.e., medium dilution P, low dilution P, high dilution, and the control). The changes in SRP and DIN were also reflected in the DIN:SRP. DIN:SRP increased in all treatments, ranging from 7.3 in the medium dilution P to 48 in the low dilution N+P.

Figure 20. Chlorophyll-a and phycocyanin, DIN, and SRP concentrations and DIN:SRP in the dilution bioassays through time—0, 48, and 120 hours. Values are means from all nutrient dilution and a control (*n*=3). The initial DIN and SRP concentration for all treatments is provided in the legend.



9.5 N_2 Fixation in the Dilution Bioassay

In the dilution bioassay, regardless of treatment, N_2 fixation dramatically increased at least 5.5-fold from 48 to 120 hours (Figure 21). The relatively high rate was even apparent in the low dilution N treatment that experienced very little change in phycocyanin concentrations and in replicates that received a N addition.

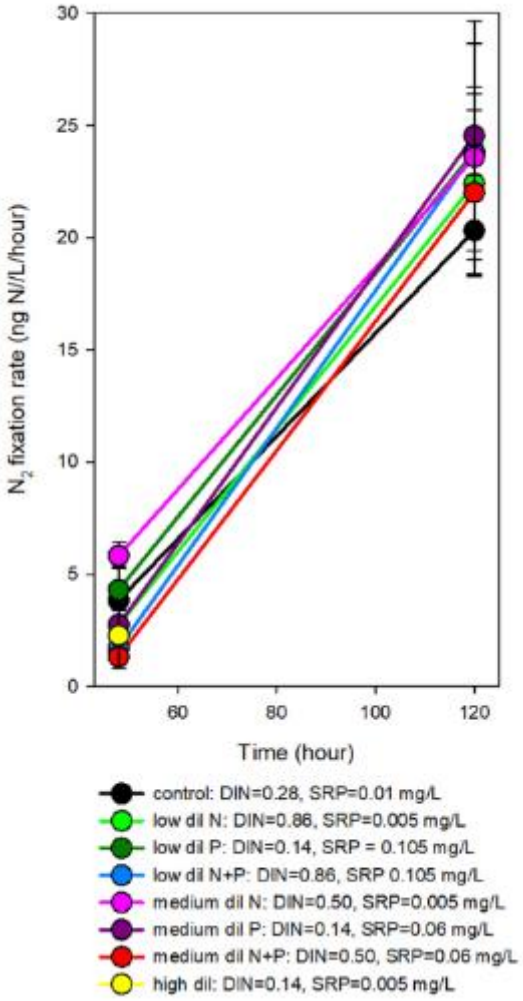


Figure 21. N_2 fixation rates in the nutrient dilution and control bioassays in the spring.

9.6 Cyanotoxins in the Dilution Bioassay

Cyanotoxins varied by dilution treatment and time. Generally, mycrocystin was undetectable after 48 hours but apparent in most treatments after 120 hours (Table 6). Dilutions amended with N+P supported the highest mycrocystin concentrations. Cylindrospermopsin was most abundant in the first 48 hours of the dilution. The highest concentrations of cylindrospermopsin occurred in the three low dilution treatments, which received relatively high nutrient inputs of N and/or P. Anatoxin-a concentrations were consistently high through time and this cyanotoxin was often the most abundant of the three toxins evaluated.

Table 6. Concentrations (µg/L) of the three cyanotoxins in the nutrient dilution assays. Values are means (*n*=3).

Treatment	Time (hours)	microcystin	anatoxin-a	cylindrospermopsin
Control	48	0	0.202 ±0.106	0
	120	0.268 ±0.044	0.299 ±0.018	0
Low dilution N	48	0	0.568 ±0.303	0.107 ±0.008
	120	0	0.342 ±0.060	0.027 ±0.023
Low dilution P	48	0	0.885 ±0.129	0.083 ± 0.001
	120	0.106 ±0.105	0.409 ±0.171	0
Low dilution N+P	48	0	0.549 ±0.107	0.070 ±0.013
	120	0.345 ±0.080	0.143 ±0.014	0.014 ±0.013
Medium dilution N	48	0	0.561 ±0.043	0
	120	0.301 ±0.077	0.386 ±0.033	0.031 ±0.030
Medium dilution P	48	0	0.151 ±0.150	0.016 ±0.015
	120	0.290 ±0.019	0.484 ±0.123	0.011 ±0.010
Medium dilution N+P	48	0	0.410 ±0.021	0.209 ±0.115
	120	0.329 ±0.022	0.639 ±0.196	0.010±0.0001
High dilution	48	0	0.443 ±0.070	0
	120	0.257 ±0.015	0.468 ±0.037	0.013 ±0.012

10. Results—Study 4: Grazing of Primary Producers

10.1 Chlorophyll-a and Phycocyanin in the Presence or Absence of Grazers

In the main body of the lake, zooplankton grazed total phytoplankton and cyanobacteria, but in Provo Bay, zooplankton demonstrated a selective feeding preference for cyanobacteria. In the main body of the lake, the inclusion (grazer plus) relative to the exclusion of zooplankton (grazer minus) caused chlorophyll-a and phycocyanin concentrations to decrease by at least 3.8 and 54-fold respectively (Table 7). In the East and West water, zooplankton grazed the cyanobacteria, measured as phycocyanin concentrations, to almost non-detection levels. Alternatively, in Provo Bay water, the inclusion of zooplankton led to an increase in chlorophyll-a concentrations across all treatments and the control. For cyanobacteria in the bay, the inclusion of zooplankton induced at least a 1.8-fold decrease phycocyanin concentrations.

Table 7. Concentrations (μg/L) of chlorophyll-a and phycocyanin with zooplanktonic grazers included and excluded in the nutrient treatments. Values are means for grazers excluded (n=3) and included (n=2) from a EXO2 multi-parameter sonde.

		Chlorophyll-a		Phycocyanin	
Location	**Treatment**	**plus grazers**	**minus grazers**	**plus grazers**	**minus grazers**
EAST	Control	2.28 ±0.870	8.72 ±0.344	0.01 ±0.005	0.540 ±0.56
	N	2.48 ±1.07	48.2 ±4.81	0	2.62 ±0.254
	P	4.84 ±3.44	40.2 ±8.84	0.01 ±0.035	2.08 ±0.344
	N+P	3.90 ±2.49	55.8 ±5.64	0.01 ±0.045	2.64 ±0.333
WEST	Control	2.56 ±1.17	21.5 ±0.558	0.01 ±0.01	0.960 ±0.051
	N	2.41 ±1.01	18.4 ±0.649	0.01 ±0.005	0.870 ±0.006
	P	4.49 ±3.09	22.1 ±2.51	0.01 ±0.055	0.953 ±0.087
	N+P	3.97 ±2.57	23.6 ±4.78	0.01 ±0.050	0.990 ±0.107
PROVO BAY	Control	78.2 ±10.4	41.5 ±5.57	3.27 ±0.340	5.21 ±2.00
	N	101 ±12.9	55.7 ±2.61	4.26 ±0.645	7.71 ±0.254
	P	76.5 ±12.1	44.8 ±2.13	3.22 ±0.390	7.26 ±0.155
	N+P	89.3 ±0.660	57.7 ±2.61	3.76 ±0.145	7.09 ±0.274

11. Discussion—Study 1: Seasonal and Spatial Nutrient Limitation

11.1 Study Objective and hypotheses

Determine the extent that seasonal (i.e., spring, early summer, summer, late summer, and fall) and spatial (i.e., main body of the lake, East; and main body of the lake, West; and Provo Bay) components drive nutrient limitation of total phytoplankton and cyanobacteria species. We hypothesized that: Utah Lake total phytoplankton and cyanobacteria will follow a similar seasonal nutrient limitation pattern present in other shallow lakes. The pattern being that total phytoplankton are P-limited in the spring and early summer and switch to N-limited in the summer and fall, and cyanobacteria will also be P-limited in the spring and summer but will remain P-limited due to the ability of some of these species to fix atmospheric N_2. We also hypothesized that cyanotoxin concentrations will be enhanced as cyanobacterial nutrient limitation is removed.

11.1 Seasonal and Spatial Nutrient Limitation of Total Phytoplankton

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, total 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 total 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 total 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 total 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 total 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).

11.2 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 total 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 total 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.

11.3 Seasonal and Spatial Nutrient Limitation of Cyanobacteria

Our hypothesis for cyanobacteria was also only 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 responses in the late summer or fall. The difference in the summer limitation was potentially linked to the cyanobacterial 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 8.

Table 8. 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	

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 9). 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.

11.4 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 did find 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.

Table 9. 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											
Chroococcus 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											

11.5 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.

11.6 N_2 Fixation and Nutrient Addition, Lake Location, and Time of Incubation

In Utah Lake, the addition of N+P, higher cyanobacterial biomass, and longer incubation times elevated N_2 fixation. In the early summer, the addition of N+P increased N_2 fixation 7.7-fold in East water. The stimulation of fixation by N+P instead of P alone was unexpected. Usually, N_2 fixation is stimulated by SRP additions. For example, meta-transcriptomic analyses reveal that expressions of genes involved in N_2 fixation (*nifDKH*) by P-scavenging cyanobacteria were significantly upregulated during HABs in an agricultural impacted lake, Harsha Lake, in southwestern Ohio (Lu et al. 2019). However, not all cyanobacteria are extremely responsive to SRP. Under N-fixing conditions, *Dolichospermum* had a higher maximal growth rate, a greater affinity for P, and higher N_2 fixation activity than *Aphanizomenon* (DeNobel 1997). *Dolichospermum sp.* was most likely predominantly responsible for N_2 fixation in East and Provo Bay waters in the early summer (Table 8 and Figure 9). N_2 fixation in terms of heterocyst density, nitrogenase activity, and *nif*H expression increased in *Dolichospermum flos-aquae*

following P enrichment, but the addition of DIN also enhanced the expression of these same parameters in *Dolichospermum flos-aquae* (Wang et al 2018). Therefore, N as well as P may stimulate fixation by this specific species.

Lastly, in all dilution treatments, N_2 fixation dramatically increased at least 5.5-fold from 48 to 120 hours in the early spring. The explanation for the elevation in rates with time is potentially due to nutrient regeneration as total phytoplankton and cyanobacteria died-off, biomass decomposed, and responding cyanobacteria utilized SRP and fixed N_2. Moisander et al. (2007) found that *Nodularia spumigena* and *Aphanizomenon sp.* elevated their N_2 fixation rates by utilizing periodic pulses of P at night from potentially dead and decomposing biomass. We may have a similar situation where nutrients were being regenerated and reused.

12. Discussion—Study 2: Growth Rate Response to Nutrient Limitation

12.1 Study Objective and Hypotheses

Determine the potential for N, P, and/or N+P limitation to influence the growth of phytoplankton and cyanobacteria across the lake. We hypothesized that the warmer lake temperatures in summer will favor cyanobacterial growth but cyanobacteria relative to phytoplankton will demonstrate a slightly slower growth rate and a time delay before reaching peak growth even when N, P, or N+P limitation are removed. We also hypothesized that cyanotoxins production will be minimal due to the optimal toxicity temperatures potentially being around 25°C.

12.2 Growth Rate Differences

The 48-hour or 72-hour incubation time in Study 1 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.

In lake bioassay studies, the incubation time is critical. 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 sp.* after 48 hours; and colonial green algae, unicellular, colonial green algae, *Desmodesmus sp.*, *Aulacoseira sp.*, and pennate and centric diatoms after 96 hours. Further, *Microcystis sp*. was consistently present in the main body water in the first 24 hours and accounted for the relatively high growth rate of cyanobacteria.

12.3Cyanotoxins and Growth Rate

As hypothesized, cyanotoxin concentration was 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.

13. Discussion—Study 3: Nutrient Dilutions to Determine Threshold Response

13.1 Study Objective and hypothesis

Determine the level of N and/or P needed to control phytoplankton and cyanobacteria bloom formation. We hypothesize that nutrient thresholds will be reached for both phytoplankton and cyanobacteria in early spring, since primary producers are extremely sensitive to DIN and SRP levels.

13.2Nutrient Thresholds for DIN and SRP

As hypothesized, we found nutrient thresholds for DIN and SRP that may control total phytoplankton and cyanobacterial growth. Overall, a target DIN concentration < 0.14 mg/L and a SRP concentration ≤ 0.005 mg/L may deter HABs from forming. Based on the dilution bioassay study, in the spring, the nutrient concentrations needed to curb total phytoplankton responses was a DIN concentration < 0.14 mg/L combined with a SRP concentrations ≤ 0.06 mg/L. The nutrient concentrations needed to curb cyanobacteria was a SRP concentration ≤ 0.005 mg/L. Therefore, thresholds for DIN and SRP are necessary to regulate phytoplankton; however, SRP may exert more control over cyanobacteria. In the bioassay studies DIN and SRP were biologically available in the water column and were not necessarily bound to mineral complexes in the column. For example, in the dilutions, SRP declined, regardless of receiving various levels of SRP. DIN also declined but only in treatments that did not receive an N addition.

Our nutrient thresholds (DIN concentration < 0.14 mg/L and a SRP concentration ≤ 0.005 mg/L) are compatible with other lake systems that are attempting to deter phytoplankton and cyanobacteria blooms. For example, Andersen et al (2019) found a significant threshold relationship at 0.56 mg/L DIN and a marginally significant relationship at 0.005 mg SRP/L for the total phytoplankton (i.e., chlorophytes, cyanobacteria, and diatoms) in a hypertrophic reservoir, Acton Lake, in southwestern Ohio, USA. Further, Xu et al (2015) employed dilution bioassays to identify thresholds for TN (0.80 mg/L) and TP (<0.05 mg/L) to limit the growth of *Microcystis* dominated blooms in a eutrophying shallow lake, Lake Taihu, in China. Using the annual average of TN and DIN and TP and SRP for Lake Taihu, we may convert these thresholds for TN and TP to approximate thresholds for DIN at 0.39 mg/L and SRP at 0.005 mg/L that may deter cyanobacterial dominated HABs. Our DIN threshold is a lower than other reported values and may need to be further investigated, but the SRP threshold seems appropriate. We strongly suggest that managers of Utah Lake create a dual management strategy to successfully reduce eutrophication in our already eutrophying lake. This dual strategy focusing on DIN and SRP is suggested and implemented elsewhere across the globe (Paerl et al 2011; Wurtsbaugh et al 2019).

13.3Cyanotoxins in Nutrient Thresholds

Only cylindrospermopsin demonstrated a response to nutrient dilution. Cylindrospermopsin was most abundant in the first 48 hours of the dilution and treatments that received relatively high nutrient inputs of N and/or P supported the highest concentrations.

14. Discussion—Study 4: Grazing of Primary Producers

14.1 Study Objective and Hypothesis

Determine the potential for zooplankton to reduce phytoplankton and cyanobacteria. We hypothesized that if eukaryotic grazers are present in early summer waters, zooplanktivory or grazing will decrease phytoplankton more than cyanobacteria due to the presence of cyanotoxins in cyanobacteria cells.

14.2 Grazing of phytoplankton and cyanobacteria

Zooplanktivory may dramatically reduce cyanobacteria and a lesser extent phytoplankton depending on lake location. Contrary to our hypothesis, the inclusion of eukaryotic grazers dramatically decreased both chlorophyll-a and phycocyanin concentrations in the main body of the lake. For example, in the East and West water, zooplankton grazed the cyanobacteria, measured as phycocyanin concentrations, to almost non-detectable levels. However, in Provo Bay, zooplankton seemed to selectively feed on cyanobacteria instead of phytoplankton. In the bay inclusion of zooplankton actually increased chlorophyll-a concentrations across all treatments and the control, while phycocyanin concentrations steadily declined. In a previous study we conducted on Utah Lake across multiple seasons and locations (Collins et al 2019), the decline of total cyanobacterial abundance was influenced by Monogononta, a rotifer species. Often successful grazing depends on prey selection and the ability to avoid ingesting toxins or toxic cells if the zooplankton does not have tolerance to the toxin (Gilbert and Durand, 1990). Rotifers are sensitive to toxins and selectively graze the non-toxic species (Kirk and Gilbert, 1992). Potentially the Monogononta either avoided toxic cyanobacterial cells but still grazed cyanobacteria indiscriminately or many of our cyanobacterial species were mostly non-toxic.

Zooplankton species are common across all waters, while other grazers are more common in specific Utah Lake locations. Based on recent phytoplankton community evaluations at the family taxonomical level in early summer (i.e., June), Cyclopidae (copepod), Diaptomidae (calanoid copepod), and Daphnidae (cladocerans) are abundant across all waters (Richards 2019). However, Provo Bay grazers also include rotifers of the family Bosminidae (0.41 mm), such as *Bosmina longirostris*. In East water, small rotifers within the *Brachionus* genus (0.18 mm) and in the West, even smaller rotifers such as grazers from the Sididae family (<0.1 mm) are prominent early summer taxa (Richards 2019). Utah Lake has unusually low zooplankton species richness, with zooplankton that are often smaller than those found in nearby waterbodies. The paucity in zooplankton richness may result from the unnatural top-down effect of introduced planktivorous fish feeding on zooplankton communities (Sondergard et al 2008). Utah Lake has numerous introduced planktivorous fish species whose annual abundances are measured in the tons. When planktivorous fish are more abundant, large zooplankton species decrease in abundance and small species become dominant (Gophen 1990). These effects from an unnaturally high abundance of planktivorous fish may cascade through the trophic web and affect phytoplankton community composition, algal bloom, and HAB prevalence.

Even with zooplankton included in the lake, zooplankton still coexist with HABs across Utah Lake. Our cubitainers only captured microzooplankton like rotifers. We did not see microzooplankton in our Cubitainers like Cladocera and Copepods and macroinvertebrates. These small crustaceans are common in freshwater lakes (Wetzel et al 1983). Also, hatches of

aquatic insects and other macroinvertebrates are present in Utah waterbodies (https://extension.usu.edu/waterquality/files-ou/whats-in-your-water/aquatic_macroinvertebrates/pond_macroinvertebrate_guide.pdf) . Our Cubitainers did not contain the entire aquatic foodweb. Thus, our measured impact of grazers on total phytoplankton and cyanobacteria is most likely inflated since we did not include species that potentially ate our microzooplankton preying on primary producers.

14.3 Unfortunately, No Grazer Species Identified or Quantified

We were not budgeted to quantify the biomass or species composition of grazers in the Cubitainers. Techniques to accurately evaluate grazer biomass involve costly and time-consuming methods. One common method for estimating grazer biomass is through microscopy and calculations based on the identity and dimensions of grazers (Dumont 1975; Yuan & Pollard 2018). Another method is to preserves DNA from aquatic systems and perform 16S/18S target metagenomics (Tuorto et al 2015). We are capable of doing both of these analyses, but we did not budget to evaluate the biomass of grazers beyond a simple filtering metric.

15. References

Aanderud, Z.T. et al. (2016). Bacterial dormancy is more prevalent in freshwater than hypersaline lakes. *Frontiers in Microbiology*. doi: 10.3389/fmicb.2016.00853.

Andersen, I.M., Williamson T.J., Gonzales M.J., Vanni M.J. (2019). Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir. *Limnology and Oceanography* 9999: 1-17.

Arrigo, K.R. (2005). Marine microorganisms and global nutrient cycles. *Nature* 437: 349-355.

Baird, R., Bridgewater, L. (2017). 4500-P B, E PHOSPHORUS. In *Standard Methods for the Examination of Water and Wastewater* (Vol. 1–0). 23rd edition. Washington D.C.: American Public Health Association. doi: 10.2105/SMWW.2882.093.

Barnard, M.A. et al. (2021). Roles of Nutrient Limitation on Western Lake Erie CyanoHAB toxin production. *Toxins* 13, 47. doi:10.3390/toxins13010047

Beversdorf, L.J., Miller, T.R., & McMahon, K.D. (2013). The role of nitrogen fixation in cyanobacterial bloom toxicity in a temperate, eutrophic lake. *PLoS ONE*, 8(2), e56103, doi: 10.137/journal.pone.0056103.

Bracken, M.E.S. et al. (2014) Signatures of nutrient limitation and co-limitation: response of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos* 124:2 113-121. doi: 10.1111/oik.01215.

Chan, F., Pace, M.L., Howarth, R.W., & Marino, R.M. (2004). Bloom formation in heterocystic nitrogen-fixing cyanobacteria: The dependence on colony size and zooplankton grazing. *Limnology and Oceanography*. 49, 2171-2178. doi: 10.4319/lo.2004.49.6.2171.

Cohen, M.J. et al. (2013). Diel phosphorus variation and the stoichiometry of ecosystem metabolism in a large spring-fed river. *Ecological Monographs* 82:155-176.

Collins, S. (2019). More biologically available phosphorus, less eukaryotic grazing, and warmer temperatures may intensify harmful algal blooms. PhD Dissertation, BYU

Davis, T.W., Berry, D.L., Boyer, G.L., & Gobler, C.J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae*, 8, 715-725. doi:10.1016/j.hal.2009.02.004.

Davis, T. W. et al. (2015). Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. *Environmental Science & Technology*, 49(12), 7197-7207. doi: /10.1021/acs.est.5b00799

DeNobel, W.T. et al. Competition for phosphorus between the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. *FEMS Microbiology Ecology* 24(3) 259-267.

Descy, J.P. et al. (2016). Identifying the factors determining blooms of cyanobacteria in a set of shallow lakes. *Ecological Informatics*, 34, 129-138.

Downing, T.G., C. Meyer, M.M. Gehringer, & M. van de Venter. (2005). Microcystin content of Microcystis aeruginosa is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. *Environmental Toxicology*. 20: 257-262

Dumont, Henri, Isabella Van de Velde, & Simonne Dumont. (1975). The Dry Weight Estimate of Biomass in a Selection of Cladocera, Copepoda and Rotifera from the Plankton, Periphyton and Benthos of Continental Waters. *Oecologia* 19: 75-97. doi: /10.1007/BF00377592.

Fang, P., Zedler, J.B. & Donohoe, R.M. (1993). Nitrogen vs. phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnology and Oceanography*, 38(5) 906-923. doi.org/10.4319/lo.1993.38.5.0906.

Filbrun, J.E., J.D., Conroy, & D.A. Culver. (2013). Understanding seasonal phosphorus dynamics to guide effective management of shallow, hypereutrophic Grand Lake St. Marys, Ohio. *Lake and Reservoir Management*, 29(3), 165-178. doi.org/10.1080/10402381.2013.823469.

Foley, J.A. et al. (2011). Solutions for a cultivated planet. *Nature* 478, 337-342.

Galloway, J.N. et al. (2004). Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry* 70: 153-226.

Giannuzzi, L. (2018). Cyanobacteria Growth Kinetics. *Algae*. doi: /10.5772/intechopen.81545

Gilbert, J. J., & Durand, M. W. (1990). Effect of *Anabaena flos-aquae* on the abilities of *Daphnia* and *Keratella* to feed and reproduce on unicellular algae. *Freshwater Biology* 24, 577-596. doi: 10.1111/j.1365-2427.1990.tb00734.x.

Gophen, M. (1990). Biomanipulation: retrospective and future development. *Hydrobiologia* 200/201: pp. 1-11

Haygarth P.M. et al. (2005). The phosphorus transfer continuum: Linking source to impact with an interdisciplinary and multi-scaled approach. *Science of The Total Environment* 344: 5-14.

Jankowiak, J., Hattenrath-Lehmann, T., Kramer, B.J., Ladds, M., & Gobler, C.J. (2019). Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie. *Limnology and Oceanography*, *64*(3), 1347–1370. doi: 10.1002/lno.11120

Karl, D. et al. (1997). The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* 388: 533-538.

Kasinak, J.E., Holt, B.M., Chislock, M.F., Wilson, A.E. (2015). Benchtop fluorometry of phycocyanin as a rapid approach for estimating cyanobacterial biovolume. *Journal of Plankton Research* 37:248-257.

Koenig, L.E. et al. (2014) Improving automated phosphorus measuremnts in freshwater: an analytical approach to eliminate silica interference. *Limnology and Oceanography: Methods* 12: 223-231.

Kolzau, S. et al. (2014). Seasonal patterns of nitrogen and phosphorus limitation in four German Lakes and the predictability of limitation status from ambient nutrient concentrations. *PLoS ONE,* 9(4), e96065.

Lürling, M., Eshetu, F., Faassen, E.J., Kosten, S., and Huszar, V.L.M. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology* 58, 552-559. doi: 10.1111/j.1365-2427.2012.02866.

Marcarelli, A.M., Wurtsbaugh, W.A. (2009). Nitrogen fixation varies spatially and seasonally in linked stream-lake ecosystems. *Biogeochemistry* 94:95-110.

Nürnberg G.K. (1996). Trophic state of clear and colored, soft- and hard-water lakes with special consideration of nutrients, anoxia, phytoplankton and fish. *Lakes and Reservoir Management* 12: 432-47.

Paerl, H.W. & Bowles, N.D. (1987). Dilution bioassays: Their application to assessments of nutrient limitation in hypereutrophic waters. *Hydrobiologia* **146,** 265-273 (1987). doi: 10.1007/BF00016348

Paerl, H.W. (2008). Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. *Advances in Experimental Medicine and Biology*, 619, 217–237. doi: 10.1007/978-0-387-75865-7_10

Paerl H.W. (2014). Mitigating harmful cyanobacterial blooms in a human-and climatically-impacted world. *Life* 4(4):988-1012. doi: 10.3390/life4040988

Paerl, H.W. et al. (2011). Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N&P) management strategy. *Water Research* 45: 1973-1983.

Paerl, H.W. et al. (2014). Nutrient limitation dynamics examined on a multi-annual scale in Lake Taihu, China: implications for controlling eutrophication and harmful algal blooms. *Journal of Freshwater Ecology* 30(1): 5-24. doi:10.1080/02705060.2014.994047.

Lewis, W.M., Wurtsbaugh, W.A., & Paerl, H.W. (2011). Rationale for control of

anthropogenic nitrogen and phosphorus in inland waters. *Environmental Science & Technology* doi: 45:10030-10035.

Lu J et al. (2019). Nitrogen-phosphorus-associated metabolic activities during the development of a cyanobacterial bloom revealed by metatranscriptomics. *Nature Scientific Reports* 9, 2480.

Moisander P.H. et al. (2007). Phosphorus limitation and diel control of nitrogen-fixing cyanobacteria in the Baltic Sea. *Marine Ecology Progress Series* 345:41-50.

Paerl, H.W. et al. (2016). It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environmental Science & Technology* 50: 10805-10813.

Paerl, H.W. et al. (2019). Mitigating eutrophication and toxic cyanobacterial blooms in large lakes: The evolution of a dual nutrient (N and P) reduction paradigm. *Hydrobiologia*. doi.org/10.1007/s10750-019-04087-y.

Pinay, G.S., Peiffer, & De Dreuzy, J.R. (2015). Upscaling nitrogen removal capacity from local hotspots to low stream orders' drainage basins. *Ecosystems*: 18, 1101-1120.

PSOMAS. (2007). Utah Lake TMDL: pollutant loading assessment and designated beneficial use impairment assessment. 88 State of Utah Division of Water Quality.

Randall, M.C. et al. (2019). Sediment potentially controls in-lake phosphorus cycling and harmful cyanobacteria in shallow, eutrophic Utah Lake. *PLoS ONE* 14(2): e0212238.

Richards, D. C. (2019). *Utah Lake Zooplankton Assemblages.* doi: 10.13140/RG.2.2.21464.96004

Seitzinger, S.P. et al. (2010). Global river nutrient export: a scenario analysis of past and future trends. Global *Biogeochemical Cycles* 24, GB0A08.

Sliwinska-Wilczewska, S., Maculewicz, J., Felpeto, A.B., & Latala., A. (2018). Allelopathic and bloom-forming picocyanobacterial in a changing world. *Toxins* 10:48

Steffen, W. et al. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science* 347, 1259855.

Seitzinger, S.P. et al. (2010). Global river nutrient export: A scenario analysis of past and future trends. *Global Biogeochemistry Cycles* 24, GB0A08.

Song, H. et al. (2017). Biological and chemical factors driving the temporal distribution of cyanobacteria and heterotrophic bacteria in a eutrophic lake (West Lake, China). *Applied Micro. and Biotech*., vol. 101, no. 4, pp. 1685-1696.

Suzumura, M. & E.D. Ingall. (2004). Distribution and dynamics of various forms of phosphorus in seawater: insights from field observations in the Pacific Ocean and a laboratory experiment. *Deep Sea Research* 51:1113-1130.

Sondergaard, M., A.R. Pedersen, L., Liboriussen, & Jeppesen, E. (2008). *Ecosystems* 11(8): 1291-1305.

Tao, S., Wang, S., Song, L., & Gan, N. (2020). Understanding the differences in the growth and toxin production of anatoxin-producing *Cuspidothrix issatschenkoi* cultured with inorganic and organic N sources from a new perspective: carbon/nitrogen metabolic balance. *Toxins*, *12*(11). doi: 10.3390/toxins12110724.

Tuorto, S.J. et al. (2015). BioDry: an inexpensive, low-power method to preserve aquatic microbial biomass at room temperature. *PLoS ONE* 10 (12): e0144686. doi 10.1371/journal.pone.0144686.

Turk, K.A. et al. (2011). Nitrogen fixation and nitrogenase (*nif*H) expression in tropical waters of the eastern North Atlantic. *ISME Journal* 5:1201-1212.

Vanni, M. J. et al (2006). Nutrient and light limitation of reservoir phytoplankton in relation to storm-mediated pulses in stream discharge. *Archiv fur Hydrobiologie* 167(1-4):421-445.

Wang S. et al. (2018) Mutual dependence of nitrogen and phosphorus as key nutrient elements: one facilitates *Dolichospermum flos-aquae* to overcome the limitation of the other. *Environmental Science Technology* 52:10 5653-5661.

Wetzel, R.G. 1983. Limnology. Second Edition. Saunders College Publishing. page 860.

Wood, A. et al. (2017). Contrasting cyanobacterial communities and microcystin concentrations in summers with extreme weather events: Insights into potential effects of climate change. *Hydrobiologia*, vol. 785, no. 1, pp. 71-89.

Work, K. A. (2003). Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake. *Journal of Planktonic Research* 25, 1301-1306. doi:10.1093/plankt/fbg092.

Wu, J. et al. (2000). Phosphate depletion in the western North Atlantic Ocean. Science 289: 759-762.

Wu, Y. et al. (2016). Patterns of succession between bloom-forming cyanobacteria *Aphanizomenon flos-aquae* and *Microcystis* and related environmental factors in large, shallow Dianchi Lake, China. *Hydrobiologia*, vol. 765, no. 1, pp. 1-13.

Wurtsbaugh, W.A., Paerl, H.W., & Dodds, W.K. (2019). Nutrients, eutrophication, and harmful algal blooms along the freshwater to marine continuum. WIREs Water 6. doi:10.1002/wat2.1373.

Xu, H., H. W. Paerl, B. Qin, G. Zhu, G. Gao. (2010). Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnology and Oceanography* 55:420-432.

Xu, H. et al. (2015). Determining Critical Nutrient Thresholds Needed to Control Harmful Cyanobacterial Blooms in Eutrophic Lake Taihu, China. *Environmental Science & Technology*, 49(2), 1051-1059.

Xu, H. et al. (2021). Contributions of external nutrient loading and internal cycling to cyanobacterial bloom dynamics in Lake Taihu, China: Implications for nutrient management. *Limnology and Oceanography*. doi: 10.1002/lno.11700.

Yuan, L.L., & Amina I.P. (2018). Changes in the Relationship between Zooplankton and Phytoplankton Biomasses across a Eutrophication Gradient. *Limnology and Oceanography* 63 (6): 2493-2507. doi: /10.1002/lno.10955.