Novel high throughput sequencing - fluorometric approach demonstrates *Microcystis* blooms across western Lake Erie are promoted by grazing resistance and nutrient enhanced growth

Megan Ladds [a], Jennifer Jankowiak [a], Christopher J. Gobler [a,*]

[a] School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, NY, USA

ARTICLE INFO

ABSTRACT

Cyanobacterial harmful algal blooms (CHABs) are a global public health threat. While CHABs are often promoted by nutrients, an important and often overlooked influence on bloom dynamics is zooplankton grazing. In the present study, zooplankton grazing and nutrient enrichment experiments were combined with next generation sequencing and fluorometric analyses to quantify differential grazing and nutrient effects on specific cyanobacterial genera across the western basin of Lake Erie. Grazing by two different sized daphnids, *Daphnia magna* and *Daphnia pulex*, was compared to protozooplankton grazing effects assessed via a dilution approach at sites within the Maumee and Sandusky Bays where *Planktothrix, Microcystis, Synechococcus*, and *Dolichospermum* were the dominant genera. Daphnid grazing significantly reduced *Synechococcus* net growth rates at most sites as well as *Planktothrix* net growth in Sandusky Bay and *Dolichospermum* in Maumee Bay. Dilution resulted in significant growth increase of *Synechococcus* at half of the sites and *Planktothrix* at most sites evidencing substantial grazing pressure by the protozooplankton community on these genera. In contrast, *Microcystis* populations were largely unaffected by daphnids and protozooplankton grazing but benefitted from nutrient enrichment more than other CHAB genera. When diatoms were present in moderate abundance, grazing rates by daphnids on diatoms were significantly greater than grazing rates on cyanobacteria. The novel approach used in this study established differences in grazing pressure and nutrient effects on differing taxa and revealed that, while many taxa were grazed by multiple classes of zooplankton (e.g. *Planktothrix, Synechococcus, Dolichospermum*, diatoms), the lack of grazing pressure on *Microcystis* coupled with nutrient-enhanced growth in western Lake Erie promotes the occurrence of CHABs of this genus.

1. Introduction

Harmful algal blooms (HABs) have been globally recognized as a significant environmental and public health threat in recent decades (Sukenik et al., 2015; Carmichael and Boyer, 2016; Huisman et al., 2018) with the primary HABs in freshwater systems being toxic cyanobacterial blooms (CHABs) (Carmichael and Boyer, 2016). Cyanobacteria can produce a variety of hepatotoxins and neurotoxins that can have deleterious effects on animals and humans (Carmichael, 1992, 2001; World Heath Organization, 1999; Kaebernick and Neilan, 2001). The distribution and intensity of CHABs have expanded in recent decades (Paerl and Paul, 2012; Harke et al., 2016) and are expected to continue to increase in the future given their ability to flourish in environments

with elevated temperatures and nutrients (Elliott et al., 2006; Paerl and Huisman, 2009; Carey et al., 2012) and their ability to deter grazing (Gobler et al., 2007; Paerl and Paul, 2012; Ger, Urrutia-Cordero, et al., 2016).

Lake Erie is the smallest of the North American Great Lakes, but is socioeconomically important as a drinking resource, for recreation, and for supporting fisheries (Fuller et al., 2002). The western basin of Lake Erie has been prone to CHABs for decades, with the 2014 bloom causing contamination of the drinking water supply in the city of Toledo (Carmichael and Boyer, 2016; Steffen et al., 2017). Blooms are generally thought to be facilitated by nutrient inputs from the surrounding river watersheds (Baker et al., 2014; Kast et al., 2021). CHABs in Lake Erie are dominated by *Microcystis* and *Planktothrix*, both of which have the

potential to produce microcystins (Sivonen and Jones, 1999) with blooms of these genera typically occurring in Maumee Bay and Sandusky Bay, respectively (Rinta-Kanto and Wilhelm, 2006; Jankowiak et al., 2019).

One factor that is often overlooked in the occurrence of CHABs is top-down control by zooplankton (Ger et al., 2016; Urrutia-Cordero et al., 2016). Lowered zooplankton grazing pressure on cyanobacteria relative to other algae has been attributed to many factors (Wilson et al., 2006; Lürling, 2020) including secondary metabolite production (Rohrlack et al., 1999, 2004; Lürling, 2003; Pawlik-Skowrońska et al., 2019), colony/filament formation (Fulton, 1988; Gliwicz and Lampert, 1990; Epp, 1996; Bednarska et al., 2014), and poor nutritional quality (Von Elert et al., 2003; Martin-Creuzburg et al., 2005, 2008; Ravet and Brett, 2006). Moreover, climate change is likely to further alter zooplankton grazing as increased temperatures shift zooplankton communities to smaller and more selective species (Strecker et al., 2004; Cremona et al., 2020; Zhou et al., 2020) potentially reducing grazer impact on CHABs. In addition, zooplankton susceptibility to cyanotoxins increases with increased temperature (Heitala et al., 1997; Claska and Gilbert, 1998) suggesting that grazing suppression during CHABs could become more common in the future.

Daphnids are non-selective grazers (DeMott and Moxter, 1991), generally consuming plankton based on size and availability (Burns, 1968; DeMott et al., 2001). CHABs have often been associated with a decrease in the abundance of larger daphnids, which are adversely impacted by cyanobacteria and are often replaced by smaller zooplankton grazers (smaller daphnids, copepods, and protozooplankton) (Ghadouani et al., 2003; Rollwagen-Bollens et al., 2013; Jiang et al., 2017). *Daphnia* spp., however can adapt to cyanobacteria and their toxins and pass those traits on to offspring, which may eventually mitigate negative effects (Hairston Jr. et al., 1999, 2001; Sarnelle and Wilson, 2005). Copepods are selective grazers that can avoid toxic prey sometimes allowing them to co-exist with CHABs (Kirk and Gilbert, 1992; Ger et al., 2010, 2016) and/or promote blooms (Wang et al., 2010).

Protozoan grazers or protozooplankton are known to consume the majority of primary production in aquatic ecosystems (Calbet and Landry, 2004) including Lake Erie (Gobler et al., 2008). Protozooplankton are generally less affected by cyanobacterial grazing deterrents than larger, metazoan zooplankton and have been shown to be in high abundance and to actively graze during CHABs (Gobler et al., 2007; Davis et al., 2012). There is, however, evidence of selection for more palatable prey by some protozooplankton, that could, in turn, prolong CHABs (Kirk and Gilbert, 1992; Boyer et al., 2011). In addition, protozooplankton can be important prey items, especially for copepods (Bec et al., 2006) and this relationship may become more important as lakes become increasingly eutrophied (Burns and Schallenberg, 2001). It has been shown that reduced or selective grazing pressure can contribute toward CHAB development especially when systems are highly eutrophied (Wang et al., 2010). Collectively, the relationship between zooplankton and toxic cyanobacteria is complex, with studies supporting the idea that grazing can depend on multiple environmental and biological factors and can indirectly cause the intensification of CHABs.

Cyanobacteria are notoriously difficult to quantify microscopically. Filamentous types are frequently misidentified or self-obscuring making accurate quantification of cells in colonies and large filaments a challenge (Wilson et al., 2000; Zhang et al., 2014). Many protozooplankton are also difficult or impossible to identify microscopically (Finlay and Esteban, 1998; Carr et al., 2017). Next generation sequencing and the use of metabarcoding targeting the ribosomal subunits 16S (for prokaryotes) and 18S (for eukaryotes) has allowed for a more detailed understanding of plankton communities (de Vargas et al., 2015; Otten and Paerl, 2015; Jankowiak et al., 2019). This technology can be combined with traditional experimental techniques to address questions related to the changes in community composition with greater accuracy

and depth compared to use of microscopy alone. Many studies have examined protozooplankton grazing or mesozooplankton grazers alone, yet few if any have performed traditional grazing experiments exploring multiple grazer size classes in tandem with next generation sequencing to assess differential grazing on plankton communities.

The purpose of this study was, therefore, to combine high throughput sequence analyses (16S, 18S) with traditional analyses (fluorometry) and experiments to determine how specific groups of cyanobacteria as well as other eukaryotic phytoplankton (such as diatoms) were affected by grazer manipulations within Lake Erie. Experiments were performed within regions of Maumee Bay and Sandusky Bay with differing cyanobacterial abundances to assess responses of differing plankton communities. The combined use of fluorometry coupled with high throughput sequencing provided a novel means for quantification of the effect of grazing by different zooplankton populations and nutrients on all genera of cyanobacteria present during blooms yielding a unique CHAB data set. We hypothesized that *Microcystis* colonies would be poorly grazed by *Daphnia* spp. compared to filamentous and unicellular cyanobacteria that are generally more palatable to many zooplankton. We further hypothesized that protozooplankton would graze all cyanobacteria equally. Finally, we hypothesized that larger cyanobacteria would be more nutrient limited than small unicellular cyanobacteria.

2. Methods

2.1. Study site and transects

In August of 2015, the western basin of Lake Erie experienced a dense cyanobacterial bloom that continued into September 2015. MODIS Satellite imagery (Stumpf et al., 2012; Wynne et al., 2013) of this CHAB from September 2015 indicated Sandusky Bay and Maumee Bay within the western basin were the epicenters of two physically distinct blooms. Transects were performed across these two bloom locations going from the densest bloom area to more dilute regions further from the river mouths on the *R/V* Erie Monitor (The Ohio State University) in order to sample a gradient in biomass and plankton community composition (Rinta-Kanto and Wilhelm, 2006; Davis et al., 2015; Jankowiak et al., 2019). A BBE Fluoroprobe was used to quantify cyanobacteria, brown algae (diatoms and dinoflagellates), as well as green algae across transects using differences in pigment abundance and ratios (Beutler et al., 2002). The Sandusky Bay transect had six sampling locations (S1-S6) and the Maumee Bay transect had five (M1-M5). Surface temperature and dissolved oxygen was measured using a YSI sonde. For each transect at each location, surface water samples were obtained for high throughput sequencing by filtering 30 ml whole bloom water onto 0.22 μm polycarbonate filters in triplicate, flash freezing in liquid nitrogen, and then storing in -80 °C until DNA extraction. Whole water samples were preserved with Lugol's iodine solution (5% v/v) for microscopic analyses. Transect data are presented in Jankowiak et al. (2019).

2.2. Grazing experiments

To assess grazing pressures on bloom communities, experiments were performed at two sample locations across both the Sandusky Bay and Maumee Bay transects. Within each bay, one sampling location within the epicenter of the bloom that had high cyanobacterial abundance (Fig. 1, S3 & M2) and one location on the periphery of the bloom that had lower cyanobacterial abundance and greater diversity of other phytoplankton (e.g. diatoms; Fig. 1, S5 & M4) were chosen.

Two types of experiments were performed at each sampling site. The first was a dilution experiment following the protocol of Landry et al. (1995) with triplicate, 250 ml polycarbonate bottles of 100% whole water with and without replete nutrient enrichment (20 μmol L^{-1} NO_3^-, 1.5 μmol L^{-1} K_2HPO_4) as well as 75%, 50%, and 25% whole water diluted with 0.2 μm filtered bloom water all with nutrient enrichment.
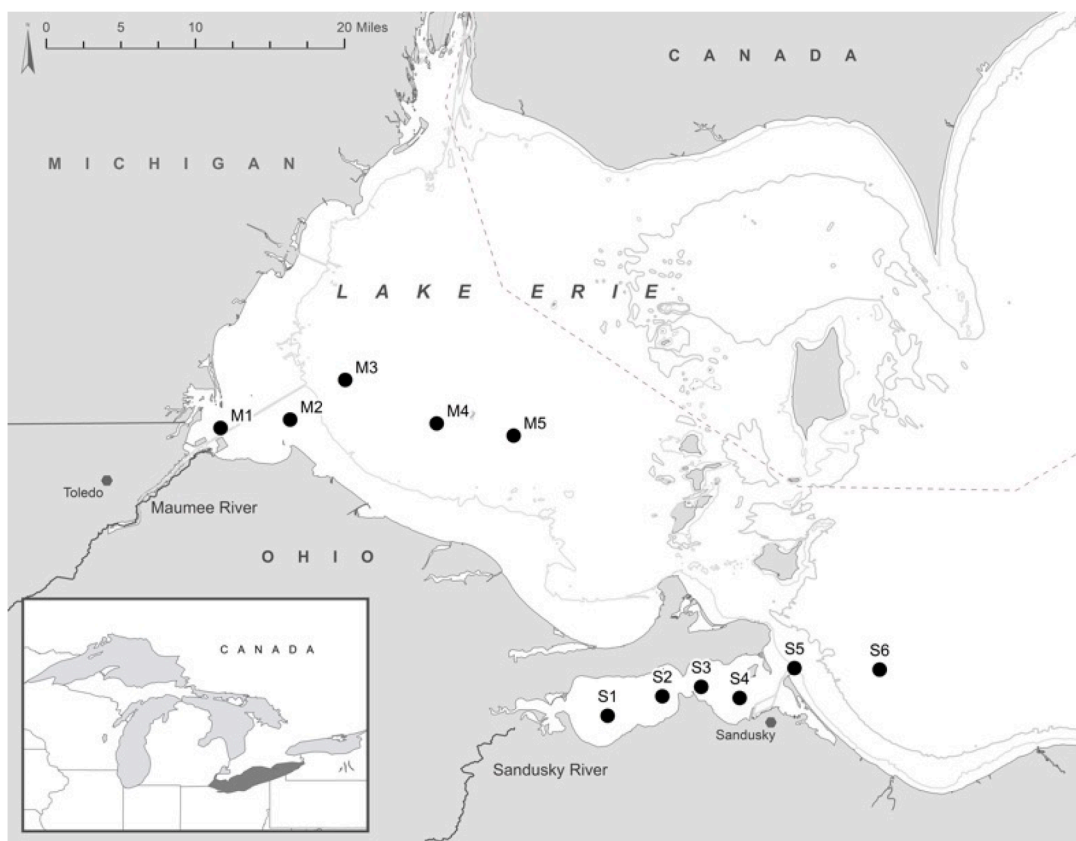
Fig. 1. Map of the western basin of Lake Erie showing transect sites at Maumee Bay (M1-M5) and Sandusky Bay (S1-S6). Experiments were performed at sites M2, M4, S2, and S5.

The theoretical basis for dilution experiments is that if nutrients are in excess, as whole water is diluted the encounter rates of plankton are reduced, decreasing grazing by abundant zooplankton (i.e. proto-zooplankton) linearly as a function of dilution and thus, growth rates of phytoplankton that are under grazing pressure increase. The second type of experiment was a daphnid addition experiment where two different sized daphnids were added to whole bloom water amended with replete nutrients (as above) to assess potential grazing impacts. *Daphnia magna* (~3 mm) was used to represent a large mesozooplankton while *Daphnia pulex* (~1 mm) was used to represent a smaller mesozooplankton grazer. *D. pulex* and *D. magna* (Aquatic Research Organisms, New Hampshire, USA) were cultured in mineral water and fed a diet of *Scenedesmus* sp. (grown on BG-11 media) *ad libitum.* Daphnids were naïve, not exposed to cyanobacteria or cyanotoxins prior to experiments. Individual *D. pulex* and *D. magna* were transferred to clean mineral water 24 h prior to the experiments to minimize contamination with *Scenedesmus* sp. Daphnids were added at levels found during previous bloom events within lakes (100 individuals L^{-1} for *D. pulex* (Threlkeld, 1979; Sellner et al., 1993; Camacho and Thacker, 2006) and 40 individuals L^{-1} for *D . magna* Lürling (2003)) to triplicate 250 ml polycarbonate bottles. Our sequencing efforts demonstrated that *D. magna* was present within the CHABs in western Lake Erie. The triplicate set of nutrient replete bottles without added grazers served as the control. All bottles were incubated for 24 h in a flow through chamber located in the top 0.5 m of Put-in-Bay, in the Western Basin, providing ambient temperature and light conditions. Initial and final samples were obtained as described for the transect for Fluoroprobe analyses, high throughput sequence ana-lyses, and preservation of plankton with Lugol's iodine. In addition, samples were filtered onto glass fiber filters in triplicate and frozen in -20 °C for fluorometric chlorophyll *a* analyses (Welschmeyer, 1994). *Daphnia* spp. were shown to have survived during the experiment. The

net growth rates were calculated using the equation: $g = [\ln(C_t/C_i)]/t$ where g is the net growth rate per day, C_t is the final cell concentration or pigment value, C_i is the initial cell concentration or pigment value, and t is the time in days Frost (1972). Pigment data (BBE fluoroprobe and chlorophyll *a*) were evaluated in regression analyses for the dilution series following Landry et al. (1995) to obtain protozooplankton grazing rates on total phytoplankton (chlorophyll *a*), classes of phytoplankton (Fluoroprobe), and identified taxa (16S, 18S), as well as phytoplankton growth of these groups in the absence of grazers. Comparisons between growth were made between the nutrient amended control to the daph-nid grazer additions, the 25% dilution, and no nutrient control.

2.3. DNA isolation, sequencing, and analyses

For the metabarcoding analyses, nucleic acids were first extracted from samples (excluding added daphnids) using the cetyltrimethyl ammonium bromide (CTAB) method (Dempster et al., 1999). Frozen samples were placed into 1 ml CTAB lysis buffer with beta-mercaptoethanol, warmed to 50 °C for 30 min then re-frozen at -80 °C. Next, a chloroform extraction and isopropanol/sodium chloride precipitation were performed to isolate the nucleic acids. Extracted DNA was quantified on a Qubit fluorometer with a dsDNA BR Assay and samples were normalized to the sample with the lowest quantity of DNA. PCR amplification and amplicon sequencing were performed at Molec-ular Research Laboratories in Shallowater, TX, USA.

To assess cyanobacterial community composition, the 16S rRNA gene was amplified using a cyanobacterial specific primer set that allows for differentiation between genera. The forward primer used was CYA106F: 5'-CGG ACG GGT GAG TAA CGC GTG A-3' and the reverse primer was 530R: 5'-CCG CNG CNG CTG GCA C-3' (Nübel et al., 1997; Usher et al., 2004; Y. Wang & Qian, 2009). To assess eukaryotic

community composition, an 18S rRNA primer set was utilized. The primers were chosen to target the V7 region of the small subunit of 18S rRNA. The forward primer used was 1183F: 5'-AAT TTG ACT CAA CAC GGG-3' and the reverse primer was 1631R: 5'- TAC AAA GGG CAG GGA CG (Starke et al., 2016). Before PCR amplification each sample was given an identifying barcode on the forward primer. Amplification was conducted using the HotStarTaq Plus Master Mix Kit with the following cycling conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. Amplification success was determined by visualization of band intensity using a 2% agarose gel. Samples were then pooled and purified using Ampure XP beads with the products used to prepare a DNA library following Illumina TruSeq DNA library preparation protocol and then sequenced on an Illumina MiSeq platform for paired end reads (2 × 300) following the manufacturers guidelines.

All sequence data were processed using the Quantitative Insights into Microbial Ecology (QIIME) 2 pipeline (Bokulich et al., 2018; Bolyen et al., 2018). The QIIME2 pipeline begins by extraction of barcodes, then demultiplexing, and denoising creating amplicon sequence variants (ASVs). Then reference reads were extracted from a reference database using the same primer sets as sequencing, which are then made into a classifier using a naïve Bayes methodology (Bokulich et al., 2018). The Greengenes 97% sequence identity database was used as the reference database for the 16S rRNA sequences and Silva database 99% sequence identity was used for the 18S rRNA sequences. The classifier was then applied to the sequences and taxonomy is assigned based on 100% similarity resulting in taxonomically identified ASVs. Using the Greengenes database 130 unique ASVs were produced and all chloroplast sequences were removed. After filtering there were 26 different cyanobacterial ASVs from three known classes of cyanobacteria as well as two ASVs maximally identified to the Cyanobacteria phylum and family *Nostocaceae* (unresolved *Nostococeae*). Each experiment was analyzed individually and any ASV that was present in less than 10% of the samples was removed. After filtering the Maumee Bay epicenter site had 17 ASVs, the Maumee Bay periphery site had 10 ASVs, the Sandusky Bay epicenter site had 14 ASVs, and Sandusky Bay periphery site had 17 ASVs. The relative abundance data found via sequencing was multiplied by Fluoroprobe pigment data for cyanobacteria (μg L^{-1}) to produce absolute concentrations of each taxa following the methods of Lusty and Gobler (2020). Net growth rates of each taxa were then determined as described above for the pigment data.

For 18S analyses, using the Silva database, 296 different ASVs were identified. All suspected contaminate ASVs, fungi, and any ASV that was present in less than 10% of samples was removed from analyses leaving 133 unique ASVs. 18S ASVs belonged to 16 different phyla; Amoebozoa, Chlorophyta, Heliozoa, Cryptista, Euglenozoa, Haptophyta, Sulcozea, Choanozoa, Arthropoda, Mollusca, Rotifera, Ciliophora, Miozoa, Cercozoa, Bigyra, and Ochrophyta. There were also three ASVs that were only classified to higher classifications; Opisthokonta, Stramenopiles, Alveolata, and one classified simply to Eukaryota (unresolved Eukaryota). For the one site that had Fluoroprobe data for the diatoms (Maumee Bay periphery site, M5), the relative abundance data for identified diatoms via sequencing was combined with Fluoroprobe data for brown algae (μg L^{-1}) to produce absolute concentrations of each taxa. Net growth rates of each taxa were then determined as described above for the pigment data. Sequence data from this study have been deposited in GenBank (accession number PRJNA753822).

2.4. Statistical analyses

Statistical analyses were performed using R software 1.3.1056 (R Core Team, 2020). Data were tested with a Shapiro-Wilk test for normality and log transformed to normal when non-normal. For the grazer addition experiments one-way analysis of variance (ANOVAs) were performed to test for significant differences between phytoplankton growth in the amended control compared to the daphnid additions. A Tukey post hoc test was used following the ANOVA to determine significant differences among groups. T-tests were performed comparing the growth rates of each taxa or pigment in the whole water control with added nutrients and the 25% dilution with nutrients to assess relative protozooplankton grazing pressure. T-tests were also used to compare the no nutrient controls in Maumee Bay to the whole water control with added nutrients to assess changes in growth rates due to nutrient additions. In addition regressions were preformed to quantify grazing rates of protozooplankton following traditional methods (Landry and Hassett, 1982; Landry et al., 2008). In 32 out of 35 cases (92%), experiments displaying significant correlations between dilution of lake water and net growth rates of a given taxa calculated using four dilution levels (25%, 50%, 75%, 100% lake water; Landry and Hassett (1982)), also showed significant correlations between dilution of lake water and net growth rates calculated using two dilution levels (25% & 100% lake water; Landry et al. (2008)), and a significant difference between the net growth rates in the 25% dilution treatment and the nutrient amended control as calculated using the growth formula stated above (Supplementary Table 1). Hence, while the results section focuses on net growth rates differences between the 25% dilution treatment and the nutrient amended control, such differences are largely (92%) consistent with differences in grazing rates as determined using Landry dilution experimental approaches (Landry and Hassett, 1982; Landry et al., 2008).

3. Results

Temperatures were between 19 °C and 21 °C across western Lake Erie during September 2015 (Table 1). Cyanobacteria were, fluorometrically, the dominant phytoplankton groups within all sampling locations, while green algae were fluorometrically below detection limits at all sites. Brown algae were fluorometrically detected at the Maumee Bay periphery site only and microscopic analyses revealed these were almost exclusively diatoms. Initial fluorometric concentrations of cyanobacteria within Maumee Bay were 58.7 μg L^{-1} at the epicenter and 10.9 μg L^{-1} at the periphery while levels in Sandusky Bay were 128 μg L^{-1} at the epicenter and 40.7 μg L^{-1} at the periphery (Table 1). Brown algae concentrations were 16.6 μg L^{-1} at the Maumee Bay periphery (Table 1). Beyond differences in the absolute levels of cyanobacteria, cyanobacterial community composition and abundances differed across sites (Fig. 2). Sandusky Bay was dominated by *Planktothrix* (periphery: 45.2%, 16.8 μg L^{-1}; epicenter: 53%, 46.1 μg L^{-1}) and *Synechococcus* (periphery: 29%, 10.4 μg L^{-1}; epicenter: 37.7%, 32.8 μg L^{-1}) with *Dolichospermum* appearing at higher concentrations at the peripheral site (8%, 3 μg L^{-1}) (Fig. 2). In Maumee Bay, *Planktothrix* dominated at the epicenter (33%, 14.7 μg L^{-1}) while *Microcystis* (43.5%, 0.5 μg L^{-1}) was the most abundant ASV at the periphery site (Figs. 2). *Synechococcus* (epicenter: 41%, 18.3 μg L^{-1}; periphery 24.6%, 0.3 μg L^{-1}) and *Dolichospermum* (epicenter 6%, 2.7 μg L^{-1}; periphery 17.8%, 0.2 μg L^{-1}) were also abundant at both Maumee Bay sites (Figs. 2). Regarding eukaryotes, at the Maumee Bay epicenter site the top eukaryotic phyla were Ochrophyta (34.6%), Arthropoda (28.5%), unresolved Eukaryota (6.9%), Rotifera (6.7%), and Cryptista (5.7%) with all other phyla comprising 18% (Fig. 3). At the Maumee Bay periphery, the top five eukaryotic phyla were Ochrophyta (46.7%), Cryptista (8.8%), Rotifera (7.9%), unresolved Eukaryota (7.1%), and Ciliophora (6.6%), with other phyla making up 23% (Fig. 3). At the Sandusky Bay epicenter, the top eukaryotic phyla were Arthropoda (33%), Cryptista (25.6%), Ciliophora (13.6%), Ochrophyta (10.3%), and Miozoa (5%), with all others comprised 12.5% (Fig. 3). At the Sandusky Bay periphery, the top eukaryotic phyla were Arthropoda (57.2%), Ochrophyta (9.6%), Cryptista (8.7%), Ciliophora (6.3%), and Mollusca (3.1%) with all others representing 15% (Fig. 3). Within the *Arthropoda* phylum copepods (cyclopoid and calanoid) were dominant, but *D. magna* were also shown to be present at all sites.

Table 1

Concentration of phytoplankton pigments and temperatures at each study site. ND is not detected. The ± are standard deviations.

Experimental Site	Cyanobacteria (μg L⁻¹)	Diatoms (μg L⁻¹)	Chlorophyll *a* (μg L⁻¹)	Latitude	Longitude	Temperature (°C)
Maumee Bay epicenter	58.7 ±0.75	ND	32.3 ±1.22	41.73	-83.34	19
Maumee Bay periphery	10.9 ±1.2	16.6 ±2.8	22.42 ±0.15	41.73	-83.15	20.9
Sandusky Bay epicenter	128 ±0.52	ND	44.32 ± 8.8	41.48	-82.8	19.1
Sandusky Bay periphery	40.7 ±0.35	ND	20.95 ±0.43	41.5	-82.68	19.3
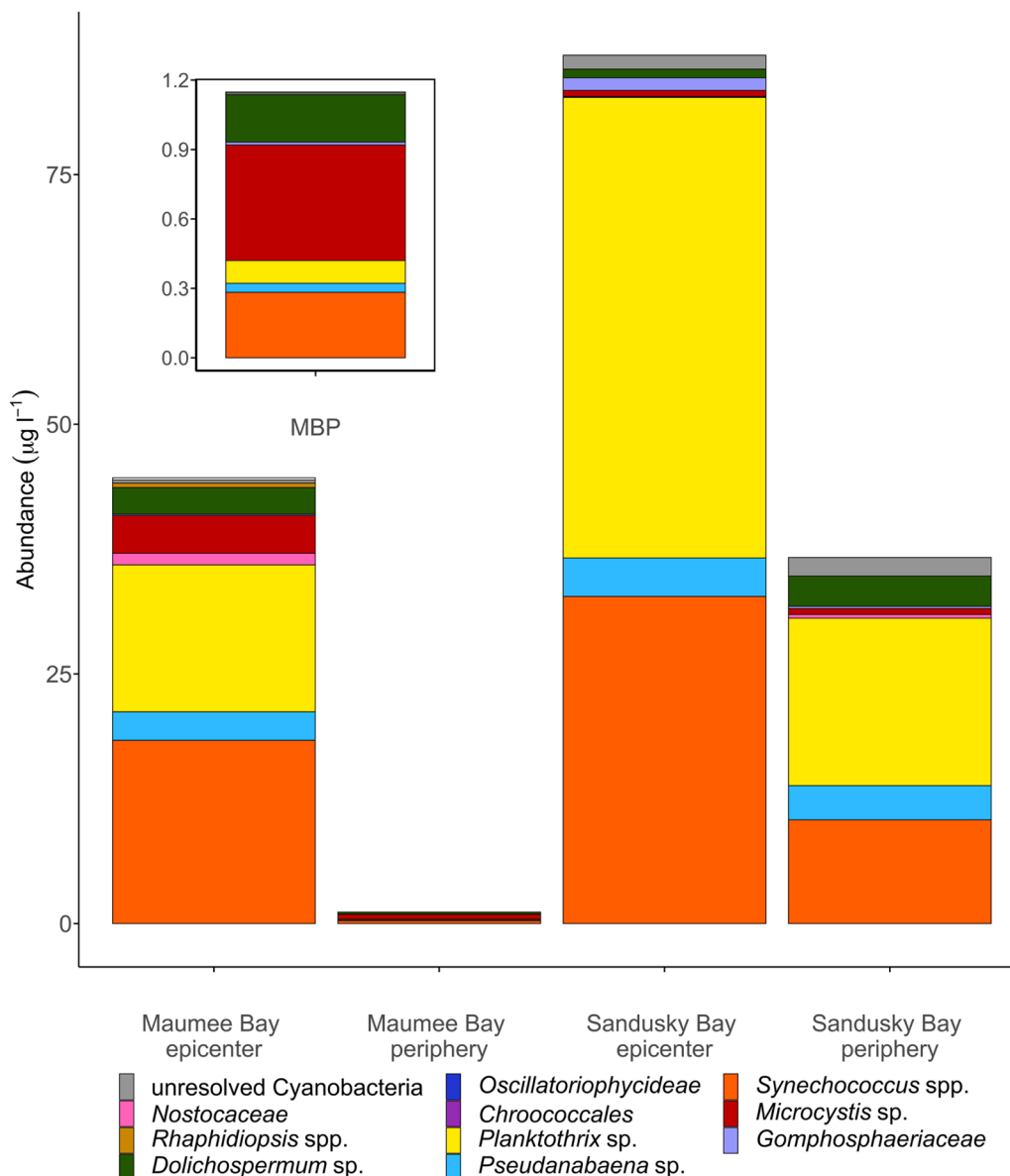
Fig. 2. Cyanobacterial density using the 16S sequence and fluoroprobe combined data for all sites; Maumee Bay epicenter (M2), Sandusky Bay epicenter (S2) and Sandusky Bay periphery (S5; Fig. 1). The inset represents an expanded view of Maumee Bay periphery (M4; Fig. 1).

3.1. Maumee Bay epicenter experiment

While net growth rates of cyanobacteria and the total phytoplankton community (chlorophyll *a*) were ~0.25 per day in the nutrient amended control during the Maumee Bay epicenter experiment, these growth rates became significantly lower and negative in the *D. magna* and *D. pulex* treatments (Fig. 4A). While dilution of protozooplankton had no effect on cyanobacterial growth rates, chlorophyll *a*-based growth rates were significantly higher than the nutrient amended control in the dilution treatment (Fig. 4A). The addition of nutrients also significantly

enhanced the growth of both cyanobacterial and chlorophyll *a* pigments relative to the unamended control (Fig. 4A). In addition, there was significant enhancement of the net growth rates of *Microcystis, Pseudanabaena, Synechococcus,* and unresolved *Nostocaceae* with the addition of nutrients compared to the unamended control treatment (*p*<0.05 for all; Fig. 4B).

The top cyanobacterial ASVs at the Maumee Bay epicenter were *Planktothrix* (32.9%), *Synechococcus* (41.1%), *Microcystis* (8.5%), *Pseudanabaena* (6.4%), and *Dolichospermum* (6%). Almost all groups, with the exception of *Dolichospermum*, displayed positive growth rates within
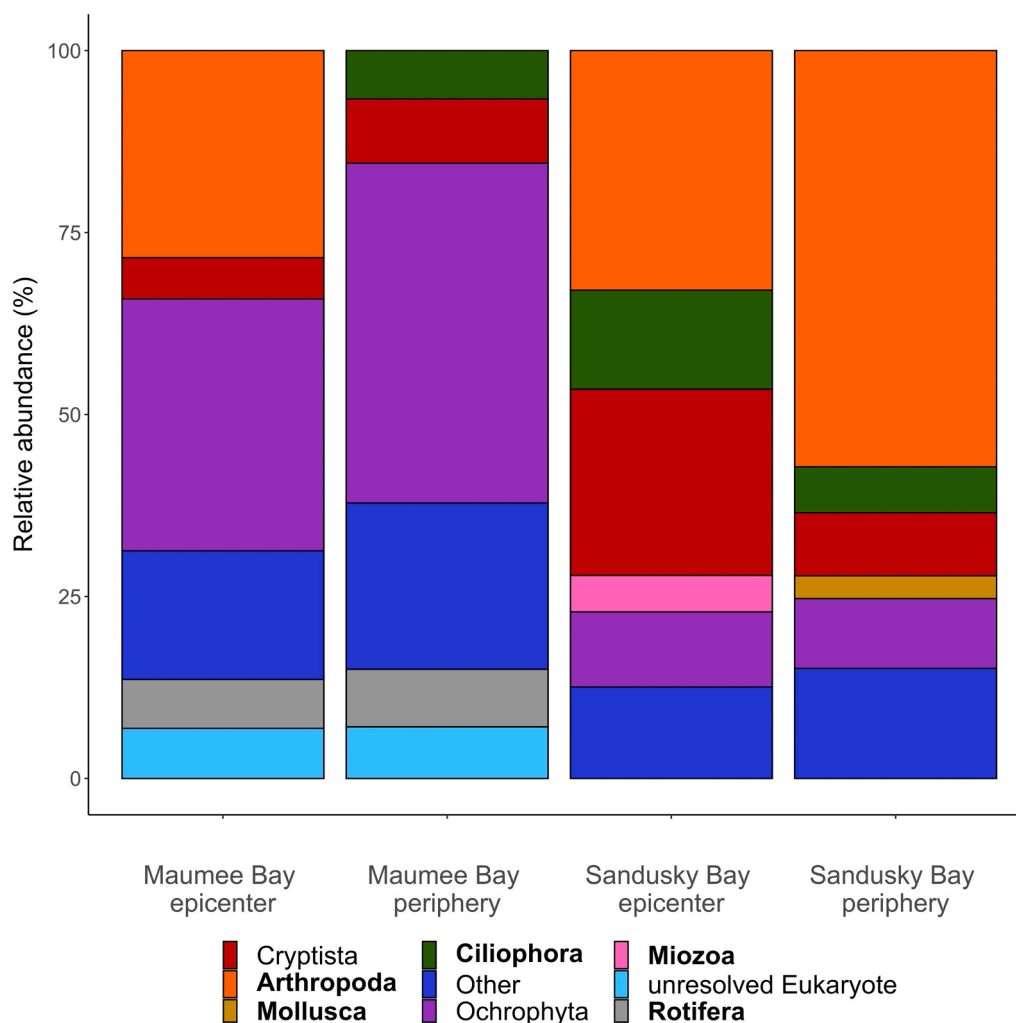
Fig. 3. 18S sequence data relative abundances for the top six phyla for all sites; Maumee Bay epicenter (M2), Sandusky Bay epicenter (S2) and Sandusky Bay periphery (S5). The inset represents an expanded view of Maumee Bay periphery (M4; Fig. 1). Bolded phyla represent predominately heterotrophic phyla.

the nutrient amended control treatment ranging from 0.02 – 0.92 per day (Fig. 4B). The addition of *D. magna* resulted in a significant decrease in, and sharply negative, growth rates for *Raphidiopsis* (=*Cylindrospermopsis*) and unresolved *Nostocacaea* (-0.34 and -0.77 per day respectively; Fig. 4B). Although *Planktothrix, Synechococcus, Psuedanabaena,* and *Dolichospermum* were abundant taxa, the addition of *D. magna* had no significant effect on growth of these taxa. While the addition of *D. pulex* resulted in a significantly lowered and strongly negative growth rate for *Synechococcus* (-0.44 per day; *p* < 0.05, Fig. 4B), it had no significant effect on growth rate of *Planktothrix, Pseudanabaena,* or *Dolichospermum* at this site (Fig. 4B). In contrast, following protozooplankton dilution, *Dolichospermum, Planktothrix,* and *Synechococcus* all experienced significantly higher growth rates compared to the nutrient amended control (*p* < 0.05 for all, Fig. 4B). Among these four genera, *Planktothrix* and *Synechococcus* experienced the largest (>1 per day) and most significant increases in growth within the 25% dilution treatment (Fig. 4B). Despite being one of the most abundant taxa at the Maumee Bay epicenter site, the addition of either daphnid or the dilution of grazers did not significantly alter the growth rates of *Microcystis*.

3.2. Maumee Bay periphery experiment

For the Maumee Bay periphery experiment, net growth rates of cyanobacteria, brown algae, and chlorophyll *a* were -0.03, 0.22, and 0.34 per day in the nutrient amended control (Fig. 5A). Within the

D. magna and *D. pulex* treatments, growth rates of all three groups became negative and were significantly lower than the nutrient amended control (*p* < 0.001 for all), with the declines in brown algal growth rates being significantly larger than those of cyanobacteria (*p* < 0.001; Fig. 5A). Net growth rates of brown algae in the *D. magna* treatment were significantly lower than those in the *D. pulex* treatment (*p* < 0.05; Fig. 5A). Within the protozooplankton dilution treatment, brown algal growth rates significantly increased (*p* < 0.001) whereas cyanobacterial growth significantly decreased (*p* < 0.05) and chlorophyll *a*-based growth did not differ from the nutrient amended control (Fig. 5A). Brown algal growth also significantly increased with nutrient addition compared to the unamended control (*p* < 0.05) while total cyanobacterial and chlorophyll *a* did not. The addition of nutrients did, however, significantly enhance the net growth rates of *Microcystis, Dolichospermum, Planktothrix,* and *Synechococcus* relative to the unamended control (*p* < 0.05 for all; Fig. 5B).

The top ASVs at the Maumee Bay periphery were *Microcystis* (43.5%), *Synechococcus* (24.6%), *Dolichospermum* (17.8%), and *Planktothrix* (8.6%). Of these, both *Dolichospermum* and *Synechococcus* displayed significantly lower growth rates relative to the nutrient amended control treatment when *D. magna* and *D. pulex* were added (*p* < 0.05 for all, Fig. 5B). Growth rates of *Dolichospermum* were ~0.5 per day in the nutrient amended control but declined to near zero in both daphnid treatments while *Synechococcus* growth rates were ~1.3 per day in the control and declined to ~0.3 in the daphnid treatments (Fig. 5B). In
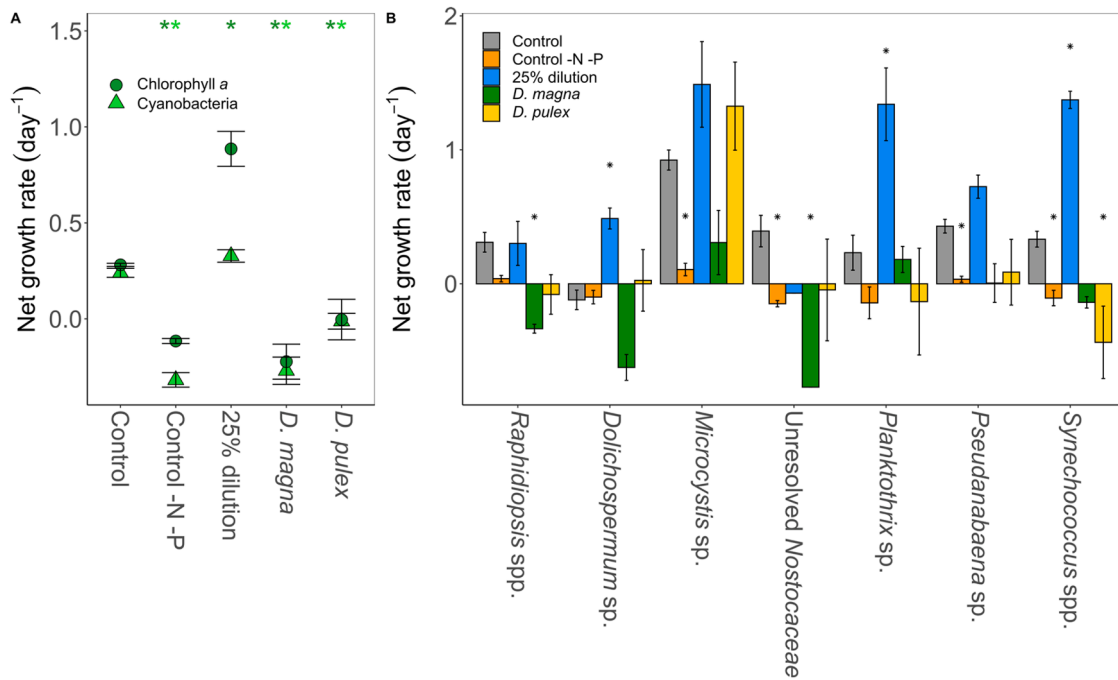
Fig. 4. A) Net growth rates of cyanobacterial pigments and chlorophyll *a* when *Daphnia* spp. were added and during the 25% dilution at the Maumee Bay epicenter (station M2, Fig. 1). Asterisks indicate rates that were significantly different from the nutrient amended control and color indicates which pigment was significant. B) Net growth rates of cyanobacterial genera that had one treatment that was significantly different from the nutrient amended control using the combined 16S sequence and fluoroprobe data for Maumee Bay epicenter. Asterisks represent treatments that were significantly different from the nutrient amended control.
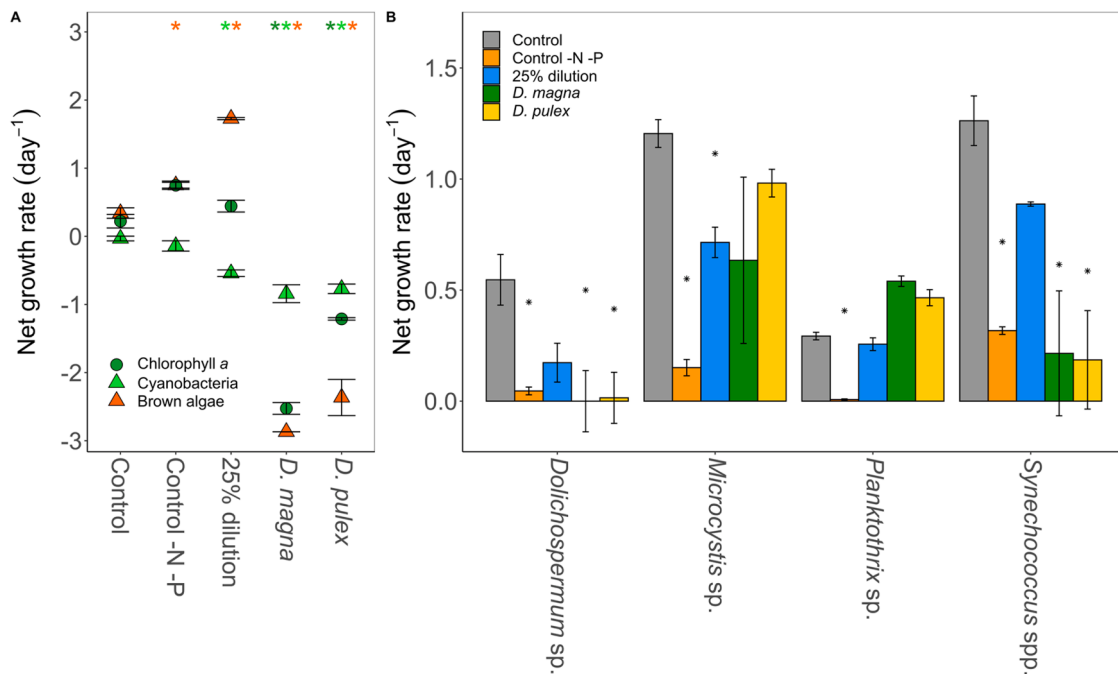

Fig. 5. A) Net growth rates of cyanobacterial, chlorophyll *a*, and brown algae pigments when *Daphnia* spp. were added as well as when water was diluted to 25% at the Maumee Bay periphery (station M4, Fig. 1). Asterisks indicate significance from the nutrient amended control and color indicated which pigment was significant. B) Net growth rates of each cyanobacterial genus that had one treatment that was significantly different from the nutrient amended control using the combined 16S sequence and fluoroprobe data for Maumee Bay periphery. Asterisks represent treatments that were significantly different from the nutrient amended control.

contrast, *Microcystis,* despite being the most abundant cyanobacteria (43.5%) was unaffected by either daphnid but displayed significantly lower growth rates compared to the nutrient amended control when the water was diluted to 25% ($p < 0.05$, Fig. 5B). *Planktothrix* was not significantly affected by any treatment (Fig. 5B).

The Maumee Bay periphery site was the only locale with fluorometrically measurable levels of brown algae, allowing the growth responses of individual diatom taxa to be assessed. Growth was positive in the nutrient amendment control for *Nitzschia, Actinocyclus,* and *Aulacoseira,* but negative for *Thalassiosira* and *Skeletonema.* When either

D. pulex and *D. magna* was added, *Nitzschia, Skeletonema, Thalassiosira, Actinocyclus,* and *Aulacoseira* all displayed negative and significantly lowered growth rates relative to the nutrient amended control ($p < 0.05$ for all, Fig. 7), with the growth rates *Actinocyclus,* and *Aulacoseira* declining to $\leq$ -1.5 per day, but those of *Nitzschia, Skeletonema*, and *Thalassiosira* declining only modestly (Fig. 7). Responses to the dilution of lake water were more modest with both *Thalassiosira* and *Skeletonema* displaying significantly increased growth rates in this treatment compared to the nutrient amended control ($p < 0.05$ for both; Fig. 6).

3.3. Sandusky Bay epicenter experiment

During the Sandusky Bay bloom epicenter experiment, the growth rates of total cyanobacteria and total phytoplankton based on pigments were ~ 0.2 per day in the nutrient amended control treatment (Fig. 7A). When *D. pulex* or *D. magna* were added, the growth rates of cyanobacteria and total phytoplankton became negative and were significantly lower than the amended control (Fig. 7A). Lake water dilution slightly increased net growth rates for both groups, but not significantly (Fig. 7A).

The growth rates of the three most abundant cyanobacterial ASVs in the nutrient amended control at the Sandusky Bay epicenter site, *Planktothrix* (53%), *Synechococcus* (37.7%), and *Pseudanabaena* (4.4%) were 0.45 d^{-1}, 0.6 d^{-1}, and 0.27 d^{-1}, respectively (Fig. 7B). The growth rate of *Planktothrix* became significantly lower than the nutrient amended control in the *D. pulex* and *D. magna* treatments ($p < 0.0001$ for both) with a large decrease of -2.3 per day when *D. magna* were added (Fig. 7B). In contrast there was no significant change in growth with the addition of either daphnid for *Pseudanabaena* or *Synechococcus* (Fig. 7B). There were significantly higher growth rates for *Planktothrix, Synechococcus*, and *Pseudanabaena* in the 25% dilution treatment relative to the nutrient amended control ($p < 0.05$, Fig. 7B) with the net growth rates of *Planktothrix* and *Synechococcus* exceeding 1.5 per day and the net growth of *Pseudanabaena* reaching ~ 1 per day (Fig. 7B). The addition of either

daphnid and the dilution of grazers did not significantly alter the growth rates of *Microcystis* or *Dolichospermum*.

3.4. Sandusky Bay periphery experiment

While cyanobacterial and chlorophyll *a*-based growth rates were 0.22 and 0.25 per day within the nutrient amended control treatment at the Sandusky Bay periphery site, the addition of either daphnid species caused these rates to become negative and significantly lower than this control treatment ($p < 0.05$ for all; Fig. 8A). Dilution of water from the Sandusky Bay periphery site resulted in a significant increase in chlorophyll *a*-based growth rates compared to the nutrient amended control ($p < 0.05$) but did not alter cyanobacterial growth rates (Fig. 8A).

Within the Sandusky Bay periphery site, altering grazing pressure caused eight different cyanobacterial taxa to experience significantly different growth rates relative to the amended control (Fig. 8B). The addition of *D. pulex* and *D. magna* caused the growth rates of *Planktothrix, Synechococcus, Pseudanabaena*, and unresolved Cyanobacteria to all become strongly negative and significantly lower than the nutrient amended control treatment ($p < 0.005$ for all; Fig. 8B). These taxa were also some of the most abundant at this site (45%, 29%, 9%, and 5% respectively). The addition of *D. pulex* and *D. magna* also reduced the growth of unresolved *Nostocaceae* to a level significantly lower than the nutrient amended control, although the resulting growth rates were slightly positive and slightly negative, respectively ($p < 0.05$ for all; Fig. 8B). *D. pulex* reduced the growth rate of *Microcystis* to levels significantly lower than the nutrient amended control ($p < 0.005$), but still higher than all other cyanobacteria within this treatment (Fig. 8B), while the addition of *D. magna* or dilution to 25% had no significant effect on this genus. *Planktothrix* and *Dolichospermum* experienced growth rates significantly greater than the nutrient amended control when the bloom was diluted to 25% ($p < 0.05$ for all), with growth rates for *Planktothrix* going from negative in the control to greater than one per day in this treatment (Fig. 8B).
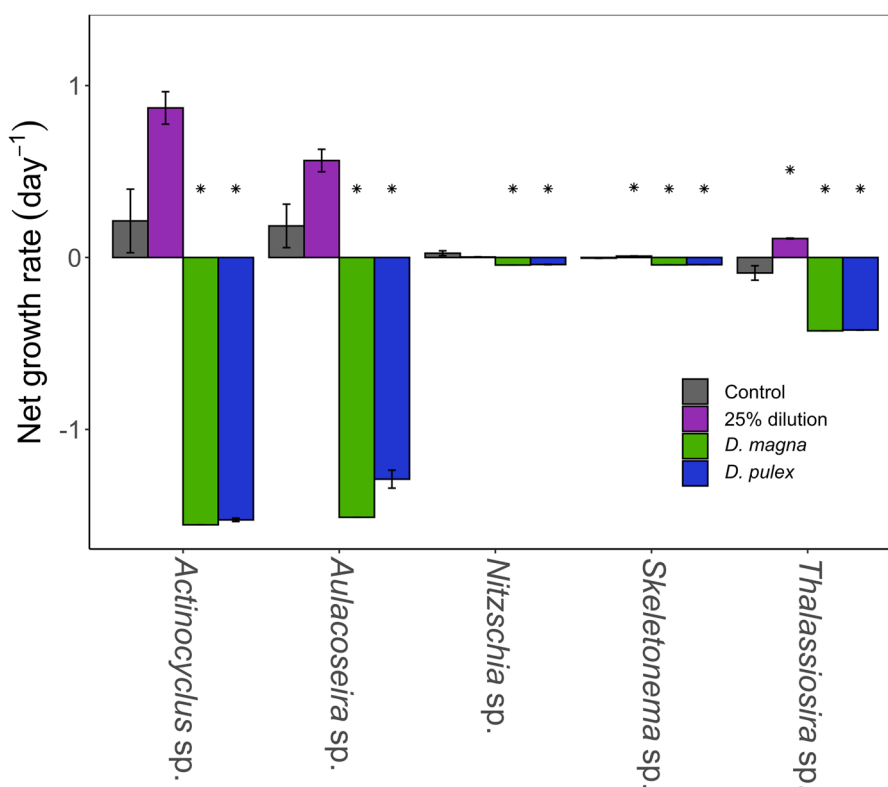

Fig. 6. Net growth rates of different diatom genera when *D. pulex* or *D.magna* were added or water was diluted to 25% whole water at the Maumee Bay periphery (station M4, Fig. 1). Asterisks represent treatments that were significantly different from the nutrient amended control.
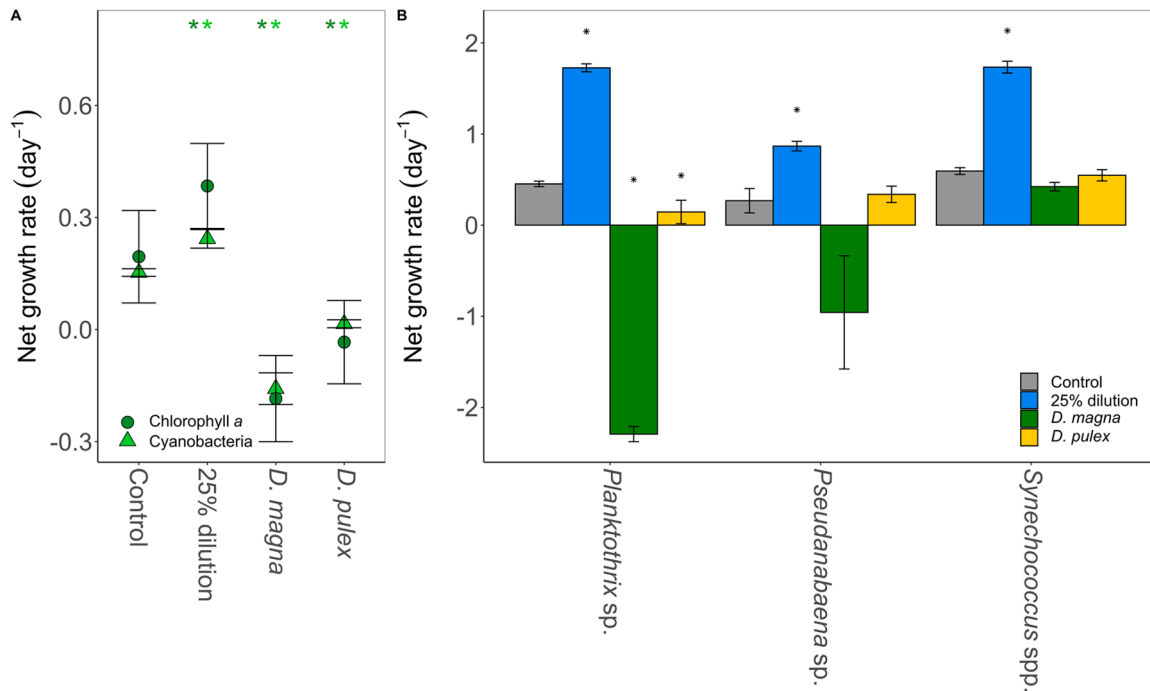
Fig. 7. A) Net growth of cyanobacterial and chlorophyll *a* pigments when *Daphnia* spp. were added as well as when water was diluted to 25% at the Sandusky Bay epicenter (station S2, Fig 1). Asterisks indicate significance from the nutrient amended control and color indicated which pigment was significant. B) Net growth of each cyanobacterial genus that had one treatment that was significantly different from the nutrient amended control using the combined 16S sequence and fluoroprobe data for Sandusky Bay epicenter. Asterisks represent treatments that were significantly different from the nutrient amended control.
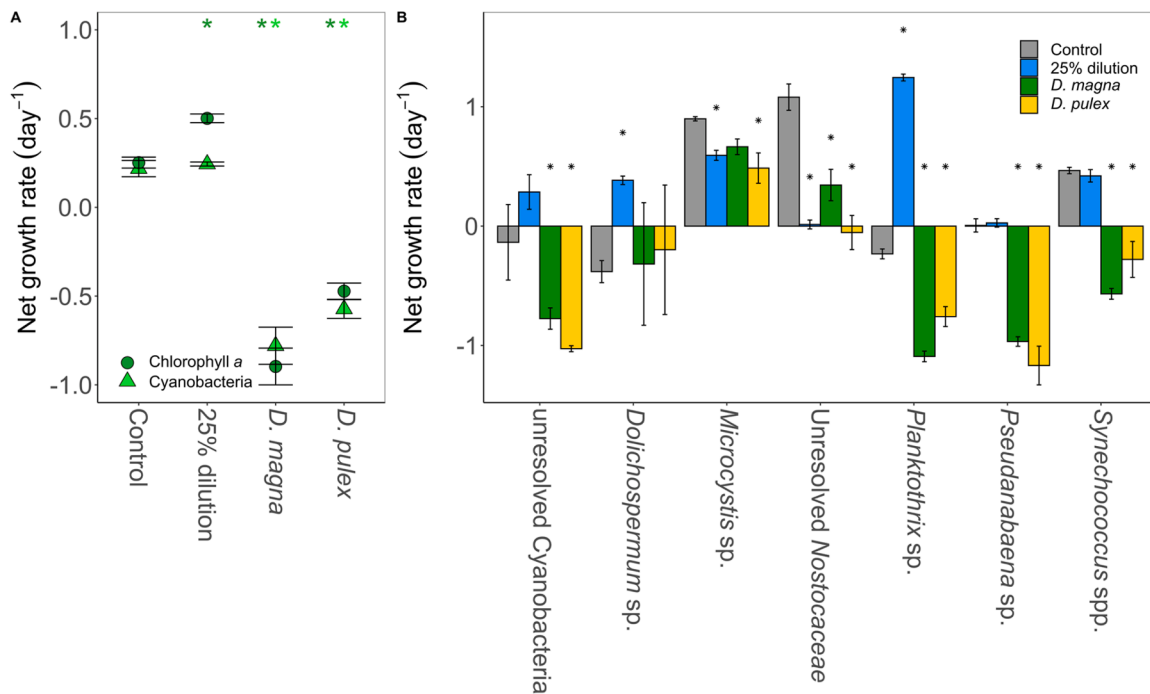

Fig. 8. A) Net growth of cyanobacterial and chlorophyll *a* pigments when daphnia were added as well as when water was diluted to 25% at the Sandusky bay periphery (station S5, Fig 1). Asterisks indicate significance from the nutrient amended control and color indicated which pigment was significant. B) Net growth of each cyanobacterial genus that had one treatment that was significantly different from the nutrient amended control using the combined 16S sequence and fluoroprobe data for Sandusky Bay periphery. Asterisks represent treatments that were significantly different from the nutrient amended control.

4. Discussion

This study used a novel approach that combined fluorometry, high throughput sequencing, and incubation experiments to assess differential effects of zooplankton grazing and nutrients on multiple cyanobacterial genera in Lake Erie. The ability to quantify net growth rates of individual genera of phytoplankton, regardless of size or abundance, under differing nutrient and grazing pressure scenarios

provided unique insight regarding factors driving the dominance of specific cyanobacterial genera during blooms. While multiple genera were commonly grazed by daphnids and protozooplankton and benefited from nutrient enrichment during this study, *Microcystis* was distinguished as the cyanobacteria genus that was the least commonly grazed by all classes of zooplankton and also most commonly benefited from nutrient enrichment, providing new perspective on the dominance of this CHAB in Lake Erie.

4.1. Nutrient effects on cyanobacteria genera in Maumee Bay

Nutrient enrichment enhanced the growth of the cyanobacteria at the Maumee Bay epicenter site, with *Microcystis* and *Synechococcus* being the genera that grew significantly faster in the presence of excess nutrients. At the Maumee periphery site, nutrient loading yielded significant increases in *Microcystis, Dolichospermum, Planktothrix,* and *Synechococcus*. These results are consistent with prior nutrient CHAB studies (Paerl et al., 2015), including those in Maumee Bay (Chaffin et al., 2013, 2014) indicating that nutrient loading can selectively promote the growth of specific genera of toxic cyanobacteria. Among the genera considered in this study, *Microcystis* was the only potentially toxic genus that benefited from nutrient loading in all experiments where effects on multiple genera were considered. While there were strong and consistent changes in phytoplankton growth rates due to altered grazing pressure across all experiments, the response of specific taxa was more nuanced.

4.2. Zooplankton grazing on phytoplankton communities

This study explored the dynamics of the lower levels of the food web, specifically zooplankton grazing, that are often oversimplified when considering CHAB occurrence (Sigee et al., 1999; Ger et al., 2014). While changes in the abundance of algal groups may have been caused directly or indirectly by the intensification (i.e. daphnid additions) or relaxation (i.e. dilution) of grazing pressure, the approach used here demonstrated the differential susceptibility of distinct algal groups and genera to zooplankton grazing in western Lake Erie.

Cyanobacterial and chlorophyll *a*-based growth rates significantly declined in all eight experimental instances when *Daphnia* spp. were added across western Lake Erie. Beyond direct grazing by these daphnids, trophic cascades, prey switching, and changes in the structure of microbial food webs could have also contributed to these trends (Carpenter et al., 1985; Polis and Strong, 1996; Becks et al., 2005). Responses of specific cyanobacterial taxa to experimental manipulation of grazing pressure, in some cases, differed across sites, an outcome that could also have been influenced by the relative importance of these other processes at each site as well as density dependent predation (Munawar et al., 1999; Lavrentyev et al., 2004; Heath et al., 2010). Trophic cascades have been well-studied in terms of CHAB dynamics, although most such studies have concentrated on the effects of fish predation on larger daphnids (Dawidowicz, 1990; Carpenter et al., 1995; Ekvall et al., 2014; Ersoy et al., 2017). Since the effects of trophic cascades are most commonly observed two trophic levels below the level of disturbance and since daphnids always caused a decrease, and not an increase, in algal abundance during this study, it would seem that the consistent decline in phytoplankton in experiments with daphnids was likely due to direct predation and not a trophic cascade. In support of this notion, larger daphnids such as the species used here are more effective at controlling plankton populations and preventing CHABs than smaller daphnids (Christoffersen et al., 1993).

Diatoms were present at all sites but were detectable via fluorescence only at the Maumee Bay periphery site where other phytoplankton fluorometrically detected as brown algae such as dinoflagellates and raphidophytes (Jankowiak et al., 2019) were absent. In this experiment, brown algae and cyanobacteria were at similar abundances (17 μg L^{-1} and 11 μg L^{-1} respectively), but daphnid grazing caused a significantly

larger decline in brown algae net growth rates compared to cyanobacteria. In addition the large diatoms (12 – 50 μm) *Actinocyclus* and *Aulacoseira,* were more intensely grazed by daphnids than other diatom genera. This indicates diatoms were likely the most palatable prey for daphnids relative to cyanobacteria at the Maumee Bay periphery site and perhaps elsewhere, a conclusion consistent with prior studies (Fulton, 1988; Epp, 1996; Work and Havens, 2003). Diatoms are also known to be a preferred prey of protozooplankton (Leonard and Paerl, 2005; Boyer et al., 2011). The net growth rates of brown algae (inclusive of diatoms) significantly increased when lake water was diluted and became significantly greater than cyanobacteria (-0.54 d^{-1} versus 1.73 d^{-1}) suggesting the protozooplankton community was exerting heavy grazing pressure on diatoms but were not consuming cyanobacteria which did not experience enhanced net growth after dilution. While larger diatoms were ingested more by daphnids, it was the smaller diatoms, *Skeletonema* and *Thalassiosira,* that experienced significantly increased net growth upon dilution and thus were seemingly preferentially consumed by protozooplankton (Suzuki et al., 2002; Boyer et al., 2011).

4.3. Grazing effects on cyanobacterial genera

Synechococcus is one of the most abundant cyanobacteria on the planet and represented a large percentage (25–41%) of the cyanobacterial sequences present in Lake Erie. This genus was significantly affected by at least one zooplankton grazing treatment during all experiments. Regarding daphnids, *Synechococcus* displayed significantly decreased growth in two of four *D. magna* treatments and in three of four *D. pulex* treatments (Table 2). *Daphnia* spp. are thought to be generalist grazers, consuming prey within their edible size range in ratios similar to their natural abundances (DeMott, 1982; Ger et al., 2018) and, as stated above, *Synechococcus* was ubiquitously abundant during this study. *Daphnia* spp. can consume and survive on *Synechococcus* (Lampert, 1981; Callieri et al., 2004; Martin-Creuzburg et al., 2008), *D. pulex* has been shown to feed on *Synechococcus* in Lake Erie (Davis et al., 2012), and *D. magna* can efficiently feed on particles as small as 0.6 μm (Geller and Müller, 1981). Given this and that facilitation of decreased growth of *Synechococcus* via a trophic cascade would require changes across at least three levels of the food chain, it would seem the daphnids were directly grazing on *Synechococcus*. There were significant increases in net growth of *Synechococcus* when water was diluted at bloom epicenter sites suggesting that *Synechococcus* was under significant grazing pressure by the protozooplankton communities in the areas with the densest cyanobacterial blooms. This finding is consistent with literature demonstrating that protozooplankton preferentially graze *Synechococcus* at rates that match their growth in the Great Lakes (Fahnenstiel et al., 1991; Gobler et al., 2008; Davis et al., 2012) but also suggests that this grazing pressure may be more pronounced during denser CHABs perhaps due to the absence of other preferred prey items (Boyer et al., 2011).

Planktothrix comprised the largest portion of the sequenced cyanobacterial community in three of the four sites studied here (all but Maumee Bay periphery) and is a public health concern due to its ability to produce microcystin (Sivonen and Jones, 1999; Davis et al., 2015; Steffen et al., 2015). While *Planktothrix* is known to be the dominant cyanobacterial taxa in Sandusky Bay (Harke et al., 2016), in this study it was found to be the dominant cyanobacteria in both bays. This deviation could be explained by *Planktothrix* dominance shown in the Maumee River (Kutovaya et al., 2012) and potential differences in river flow during this year that allowed for this population to further enter and establish within the bay. *Daphnia* spp. have been shown to be poor grazers of this filamentous algae, experiencing declining grazing with increasing filament length of *Planktothrix* (Oberhaus et al., 2007) and a heightened sensitivity to harmful secondary metabolites produced by this genus (Blom et al., 2003; Rohrlack et al., 2005; Schwarzenberger et al., 2020). *D. magna* can experience reduced survival and disrupted

Table 2
The change in net growth rate with each treatment relative to the control for each taxa and pigment at each site. Up arrows indicate growth that was higher than the control and down arrows indicate growth that was lower than the control. The arrows are significant changes, the dash indicates no significant change and ND indicates that taxa or pigment was not detected at that site.

	Maumee Bay epicenter			Maumee Bay periphery			Sandusky Bay epicenter			Sanduky Bay periphery		
	D. pulex	*D. magna*	25% dilution	*D. pulex*	*D. magna*	25% dilution	*D. pulex*	*D. magna*	25% dilution	*D. pulex*	*D. magna*	25% dilution
Pigment												
Chlorophyll *a*	↓	↓	–	↓	↓	↑	↓	↓	↑	↓	↓	↑
Cyanobacteria	↓	↓	↓	↓	↓	–	↓	↓	↑	↓	↓	–
Brown algae	↓	↓	↑	ND	ND	ND	ND	ND	ND	ND	ND	ND
Taxa												
Unresolved Cyanobacteria	–	–	–	–	–	–	–	–	–	↓	↓	–
Unresolved Nostocaceae	–	↓	–	ND	ND	ND	ND	ND	ND	↓	↓	↓
Dolichospermum sp.	–	–	↑	↓	↓	–	–	–	–	–	–	↑
Microcystis sp.	–	–	–	–	–	↓	–	–	–	↓	–	↓
Planktothrix sp.	–	–	↑	–	–	–	↓	↓	↑	↓	↓	↑
Pseudanabaena sp.	–	–	–	–	–	–	–	–	↑	↓	↓	–
Synechococcus spp.	↓	–	↑	↓	↓	–	–	–	↑	↓	↓	–
Raphidiopsis spp.	–	↓	–	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nitzschia sp.	ND	ND	ND	↓	↓	–	ND	ND	ND	ND	ND	ND
Skeletonema sp.	ND	ND	ND	↓	↓	↑	ND	ND	ND	ND	ND	ND
Thalassiosira sp.	ND	ND	ND	↓	↓	↑	ND	ND	ND	ND	ND	ND
Actinocyclus sp.	ND	ND	ND	↓	↓	–	ND	ND	ND	ND	ND	ND
Aulacoseira sp.	ND	ND	ND	↓	↓	–	ND	ND	ND	ND	ND	ND

reproduction when fed *Planktothrix* spp. (Dao et al., 2016). Still, during this study both daphnid species were capable of significantly reducing the abundances of *Planktothrix* in Sandusky Bay where this genus comprised ~50% of cyanobacterial sequences, but not in Maumee Bay where it was less abundant (8–33%). This suggests the grazing pressure of daphnids on *Planktothrix* may have been partly dependent on its relative abundance, a conclusion consistent with the concept of daphnids being generalist predators (DeMott, 1982; Ger et al., 2018). While filament size was not measured, it is also possible that the formation of larger thicker, filaments within Maumee Bay provided a grazing refuge against daphnids (Kurmayer and Jüttner, 1999; Oberhaus et al., 2007). In addition chytrids, which although not in high abundance were present within the sequences, are known to facilitate grazing on cyanobacteria, specifically filamentous forms via shortening of filaments (Agha et al., 2016; Frenken et al., 2020) and may have aided in the daphnid grazing seen in Sandusky Bay. In contrast to the daphnid treatments, *Planktothrix* net growth rates increased across all sites in the dilution treatments with the increase being statistically significant in three of four experiments, suggesting that the protozooplankton community exerted substantial grazing pressure on these cyanobacteria. Among the protozooplankton, ciliates are known to effectively graze *Planktothrix* (Combes et al., 2013; Dazley, 2018) and there was a high relative abundance of ciliates (i.e. *Ciliophora*) at most of these sites (5–14% of eukaryotic sequences). Copepods are also known to prey on ciliates, specifically in highly eutrophic conditions (Burns and Schallenberg, 2001) which may in turn account for the higher copepod abundances (*Arthropoda* 28–57% of 18S sequences) at the three sites where *Planktothrix* was in high abundance. Regardless, among the bloom-forming cyanobacteria in Lake Erie, *Planktothrix* was the most consistently grazed genera.

Dolichospermum (*Anabaena*) was present at modest abundances across most sites during this study (1–17% of cyanobacteria sequences; 0.2–3 µg L^{-1}) and is suspected of producing anatoxin-a within western Lake Erie (Steffen et al., 2015). Although anatoxin-a producing strains of *Dolichospermum* can have negative effects on *Daphnia* spp. (DeMott et al., 1991; Kirk and Gilbert, 1992; Claska and Gilbert, 1998), this chain-forming alga experienced reduced growth at three sites when *Daphnia* spp. were added, but significantly so only at the Maumee Bay periphery site. This is consistent with the idea that some *Daphnia* spp. are able to graze *Dolichospermum* effectively, although rates can be

strain-dependent (Gilbert and Durand, 1990; Epp, 1996; Urrutia-Cordero et al., 2016). In addition, like *Planktothrix*, filament length, size, or form could also account for differences in grazing pressures between sites. *Dolichospermum* growth increased following lake water dilution at three of four sites with increases being significant at two sites suggesting that the protozooplankton community was exerting significant grazing pressure on *Dolichospermum*. While some types of protozooplankton are able to graze *Dolichospermum* (*Anabaena*) species (Dryden and Wright, 1987; Gobler et al., 2007), others cannot (Boyer et al., 2011). Regardless, *Dolichospermum* was seemingly grazed by both daphnids and protozooplankton at multiple locations across western Lake Erie during this study.

Raphidiopsis was only detected at the Maumee Bay epicenter site, where *D. magna* caused a significant reduction of its net growth rates, suggesting this daphnid was able to consume this genus despite potential deterrents (toxicity, filaments; Nogueira et al., 2004; Ferrão-Filho et al., 2014). While this finding contrasts with prior studies that have highlighted the negative effects of this filamentous cyanobacteria on *Daphnia* spp. growth (Nogueira et al., 2004; Bednarska et al., 2011, 2014) and grazing (Panosso & Lürling, 2010), those studies examined grazing at high densities of *Raphidiopsis* (> 5 × 10^5 cells ml^{-1}). If the effects of *Raphidiopsis* on *D. magna* are dose-dependent, the low absolute and relative abundance (<1%) in Maumee Bay would have lessened its inhibitory effects. Again, filament length was not measured in this study but it is also plausible that *Raphidiopsis* was present as smaller, more consumable filaments (Bednarska et al., 2014).

Pseudanabaena forms thin (~1 µm), solitary filaments that are less of a morphological hinderance for zooplankton compared to cyanobacteria that form large filamentous colonies (Oberhaus et al., 2007). Like *Planktothrix* and *Synechococcus*, there was significant grazing pressure on *Pseudanabaena* by the protozooplankton community at the bloom epicenter sites but not at the periphery site in Sandusky Bay. This suggests protozooplankton grazing, or perhaps the detection of zooplankton grazing, of this genera was, in part, density dependent, and that protozooplankton tend to graze *Pseudanabaena* in plankton mixed communities (Liu et al., 2019). Conversely, *Pseudanabaena* net growth rates were significantly reduced at the Sandusky Bay periphery site when *Daphnia* spp. were added. While *D. magna* is able to consume *Pseudanabaena* (Olvera-Ramírez et al., 2010), extracts from *Pseudanabaena* can be harmful to this daphnid (Olvera-Ramírez et al., 2010; Nguyen et al.,

2020). Similar to the case of *Raphidiopsis*, since *Pseudanabaena* was in relatively low abundance (8%) at this site, it is possible densities were below the threshold of harm for this daphnid.

Patterns of zooplankton grazing on *Microcystis* differed from all other cyanobacteria genera. *Microcystis* formed a large portion of the cyanobacterial community in the Maumee Bay (8–44%, 3.8–0.5 μg L^{-1}), but was a smaller portion (~1%) in Sandusky Bay. Across all locations, however, *Microcystis* was the cyanobacterial genera most resistant to grazing during this study. Zooplankton grazing of this genus was detected in only one of 12 experiments (Sandusky Bay epicenter with *D. pulex*) and its decline in net growth rate when exposed to *D. pulex* was significantly smaller than the five other cyanobacterial genera that also experienced declines in this same experiment. The complete absence of detectable grazing effects on this genera during the 11 other experiments was consistent with our hypothesis and prior studies showing that this genus is largely grazing resistant (DeMott, 1999; Harke et al., 2016). *Microcystis* causes reductions in reproductive output, survival, and ingestion rate in many daphnids (Laurén-Määttä et al., 1997; DeMott, 1999; Rohrlack et al., 2001) including *D. pulex* (Reinikainen et al., 1994; Hietala et al., 1997) and *D. magna* (Sarnelle et al., 2010). It is generally accepted that microcystin is not the major causative agent of harm to *Daphnia* spp. (Jungmann and Benndorf, 1994; Wilson et al., 2006), as non-microcystin producing strains also reduce *Daphnia* spp. survival (Lürling, 2003; Lürling and Esther, 2003). *Microcystis* produces many harmful metabolites (Carmichael, 1992; Huang and Zimba, 2019) including a suite of protease inhibitors (Weckesser et al., 1996) that negatively impact the digestive systems of *Daphnia* spp. (Agrawal et al., 2005; Chen et al., 2005) and reduce grazing rates (DeMott, 1999). The proteases trypsins and chymotrypsins (Von Elert et al., 2004) have specifically been shown to have negative effects on *D. magna* digestive systems causing reduced growth (Agrawal et al., 2005). Harke et al (2017) demonstrated that *Microcystis* upregulates biochemical pathways associated with colony formation to deter grazing by *D. pulex* and *D. magna*. Still, *D. pulex* can be an effective grazer of *Microcystis*, especially when a bloom is in decline or when the ratio of *Daphnia* to *Microcystis* is larger (Gobler et al., 2007; Harke et al., 2017), perhaps accounting for the minor decline in *Microcystis* caused by *D. pulex* at the Sandusky Bay periphery site. Furthermore, excretion and nutrient recycling via zooplankton grazing can also enhance the growth of *Microcystis* (Lehman, 1980).

While *Microcystis* is well-known for deterring grazing by larger zooplankton, protozooplankton are thought to be less affected by cyanobacterial deterrents and are more effective grazers of cyanobacteria (Fulton and Paerl, 1987; Kim et al., 2006; Wilken et al., 2010) including *Microcystis* (Gobler et al., 2007; Davis et al., 2012). During this study, however, contrary to our hypothesis, *Microcystis* net growth rates were unaffected by dilution at the two epicenter sites and net growth rates actually significantly decreased due to dilution at both periphery sites, suggesting the protozooplankton community was not exerting significant control on this genus, a finding consistent with prior studies (Davis and Gobler, 2011). Protozooplankton have been shown to exhibit selectivity against cyanobacteria in favor of other prey (such as diatoms), which could aid in promotion of the CHAB due to reduced grazer pressure (Leonard and Paerl, 2005; Boyer et al., 2011). The ability of *Microcystis* to upregulate biochemical pathways associated with colony formation in response to zooplankton (Harke et al., 2017) could also create size mismatches between some protozooplankton and *Microcystis* (Long et al., 2007).

Differences in *Daphnia* spp. clones and genetic diversity has been shown to be important when considering resistance to cyanobacterial deterrents (Lemaire et al., 2012; Schwarzenberger et al., 2012; Kuster and Von Elert, 2013). Eutrophic water bodies are more likely to experience CHABs and daphnids from eutrophic systems that regularly experience CHABs are more resistant to cyanobacterial deterrents (Hairston Jr. et al., 1999, 2001; Gustafsson and Hansson, 2004; Sarnelle and Wilson, 2005) and are more able to readily graze CHABs

(Gustafsson and Hansson, 2004; Chislock et al., 2013). In addition, resistance can be passed down through maternal traits enabling successive generation to be better adapted to consuming CHABs (Gustafsson et al., 2005; Jiang et al., 2013; Lyu et al., 2016; Akbar et al., 2017). Since the current experiments were performed using naïve daphnia (never exposed to CHABs prior to experiments), their grazing patterns may have differed from native populations (Davis and Gobler, 2011) that might be more resistant to any deleterious effects (Lemaire et al., 2012). The results of these experiments, however, demonstrate that even naïve daphnids can consistently graze many cyanobacterial taxa, even without prior exposure.

4.4. Methodological considerations

The combined use of fluorometry, high throughput sequencing, and experiments generated a unique data set regarding cyanobacterial responses to environmental drivers in Lake Erie. One of the benefits of the approach was the quantification of changes in growth rates of low abundance taxa within larger groups. Since generalist grazers, such as daphnids, consume the most abundant prey (DeMott, 1982; Ger et al., 2018), quantifying grazing rates on low abundance taxa can be a challenge. In addition to density dependent effects on trophic interactions, analytical detection limits may prohibit detection of changes in growth rates. This was certainly the case with fluorometric methods used here, as the Fluoroprobe did not detect green algae nor brown algae at most locations, despite their presence at low levels. Given the ability of high throughput sequencing to resolve rare taxa, it would seem fluorometric detection will be the process that controls the absolute detection limit of the novel method used here. Still, rare taxa may not be equally represented in sequencing sample replicates increasing variance among samples (Elbrecht and Leese, 2015). Hence, high throughput sequencing approaches often cannot resolve the least abundant taxa (Kring et al., 2014). Nevertheless, this study demonstrated that the novel, combined method used here was able to make significant ecological conclusions regarding low abundance taxa (e.g. *Raphidiopsis, < 1%* of cyanobacteria) as well as taxa that are abundant but difficult to resolve microscopically due to their small size (e.g. *Synechococcus, Pseudanabaena*) or sometimes complex, three-dimensional morphology (e.g. *Microcystis, Dolichospermum*). In addition, significant changes in growth rates of specific taxa were resolved, even when the overall total cyanobacterial growth rates measured using fluorometry alone were unchanged.

4. 5 Conclusions

This study has shown that the use of next generation sequencing in tandem with fluorometry and experimental techniques can identify patterns of differential growth and grazing by multiple classes of zooplankton on multiple, co-occurring taxa of phytoplankton and cyanobacteria as well as the effects of nutrient enrichment. This novel methodological approach specifically identified *Microcystis* as the genus of cyanobacteria in Lake Erie that most benefited from nutrient enrichment and was least commonly grazed upon by zooplankton. Next generation sequencing can identify species that may be in low abundance, small, or otherwise misidentified. This is especially relevant for cyanobacteria as smaller genera (e.g. *Synechococcus, Pseudanabaena*) comprised 30 – 40% of the total sequenced population but are frequently overlooked in cyanobacterial studies of Lake Erie. Similarly, the protozooplankton community, which may exert significant grazing pressure on CHABs, can be difficult to identify by microscopy alone, but comprised a significant portion of ribosomal sequences in western Lake Erie. Moving forward the number of organisms in sequence databases will continue to increase, sequencing costs will continue to decline, and informatic approaches will hasten and refine sample analyses, making approaches like those presented here more usable and useful for the investigation of CHABs.

Declaration of Competing Interest

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

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2021.102126.

References

Agha, R., Saebelfeld, M., Manthey, C., Rohrlack, T., Wolinska, J., 2016. Chytrid parasitism facilitates trophic transfer between bloom-forming cyanobacteria and zooplankton (Daphnia). Sci. Rep. 6 (October), 1–9. https://doi.org/10.1038/srep35039.

Agrawal, M.K., Zitt, A., Bagchi, D., Bagchi, S.N., Elert, E.Von, 2005. Characterization of proteases in guts of daphnia magna and their inhibition by microcystis aeruginosa PCC 7806. Environ. Toxicol. 20, 314–322. https://doi.org/10.1002/tox.20123.

Akbar, S., Du, J., Lin, H., Kong, X., Sun, S., Tian, X., 2017. Understanding interactive inducible defenses of Daphnia and its phytoplankton prey. Harmful Algae 66, 47–56. https://doi.org/10.1016/j.hal.2017.05.003.

Baker, D.B., Confesor, R., Ewing, D.E., Johnson, L.T., Kramer, J.W., Merryfield, B.J., 2014. Phosphorus loading to Lake Erie from the Maumee, Sandusky and Cuyahoga rivers: the importance of bioavailability. J. Great Lakes Res. 40 (3), 502–517. https://doi.org/10.1016/j.jglr.2014.05.001.

Bec, A., Martin-Creuzburg, D., Von Elert, E, 2006. Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate Paraphysomonas sp. Limnol. Oceanogr. 51 (4), 1699–1707. https://doi.org/10.4319/lo.2006.51.4.1699.

Becks, L., Hilker, F.M., Malchow, H., Jürgens, K., Arndt, H., 2005. Experimental demonstration of chaos in a microbial food web. Nature 435 (7046), 1226–1229. https://doi.org/10.1038/nature03627.

Bednarska, A., Los, J., Dawidowicz, P., 2011. Temperature-dependent effect of filamentous cyanobacteria on daphnia magna life history traits. J. Limnol. 70 (2), 353–358. https://doi.org/10.3274/JL11-70-2-19.

Bednarska, A., Pietrzak, B., Pijanowska, J., 2014. Effect of poor manageability and low nutritional value of cyanobacteria on Daphnia magna life history performance. J. Plankton Res. 36 (3), 838–847. https://doi.org/10.1093/plankt/fbu009.

Beutler, M., Wiltshire, K.H., Meyer, B., Moldaenke, C., Lüring, C., Meyerhöfer, M., Dau, H., 2002. A fluorometric method for the differentiation of algal populations in vivo and in situ. Photosynth. Res. 72, 39–53.

Blom, J.F., Bister, B., Bischoff, D., Nicholson, G., Jung, G., Süssmuth, R.D., Jüttner, F., 2003. Oscillapeptin J, a new grazer toxin of the freshwater cyanobacterium Planktothrix rubescens. J. Nat. Prod. 66 (3), 431–434. https://doi.org/10.1021/np020397f.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., Caporaso, J.G., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2 ' s q2-feature-classifier plugin. Microbiome 6 (90), 1–17.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Caporaso1, J.G., 2018. QIIME 2: Reproducible, Interactive, Scalable, and Extensible Microbiome Data Science. Peerj Preprints.

Boyer, J., Rollwagen-bollens, G., Bollens, S.M., 2011. Microzooplankton grazing before, during and after a cyanobacterial bloom in Vancouver Lake, Washington, USA. Aquat. Microb. Ecol. 64, 163–174. https://doi.org/10.3354/ame01514.

Burns, C.W., 1968. The relationship between body size of filter-feeding cladocera and the maximum size of particle ingested. Limnol. Oceanogr. 13 (4), 675–678. https://doi.org/10.4319/lo.1968.13.4.0675.

Burns, C.W., Schallenberg, M., 2001. Calanoid copepods versus cladocerans : consumer effects on protozoa in lakes of different trophic status. Limnol. Oceanogr. 46 (6), 1558–1565.

Calbet, A., Landry, M.R., 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol. Oceanogr. 49 (1), 51–57. https://doi.org/10.4319/lo.2004.49.1.0051.

Callieri, C., Balseiro, E., Bertoni, R., Modenutti, B., 2004. Picocyanobacterial photosynthetic efficiency under Daphnia grazing pressure. J. Plankton Res. 26 (12), 1471–1477. https://doi.org/10.1093/plankt/fbh134.

Camacho, F.A., Thacker, R.W., 2006. Amphipod herbivory on the freshwater cyanobacterium Lyngbya wollei: chemical stimulants and morphological defenses. Limnol. Oceanogr. 51 (4), 1870–1875. https://doi.org/10.4319/lo.2006.51.4.1870.

Carey, C.C., Ibelings, B.W., Hoffmann, E.P., Hamilton, D.P., Brookes, J.D., 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Res. 46 (5), 1394–1407. https://doi.org/10.1016/j.watres.2011.12.016.

Carmichael, W.W., 1992. Cyanobacteria secondary metabolites—the cyanotoxins. J. Appl. Bacteriol. 72 (6), 445–459. https://doi.org/10.1111/j.1365-2672.1992.tb01858.x.

Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs. Hum. Ecol. Risk Assess. 7 (5), 1393–1407.

Carmichael, W.W., Boyer, G.L., 2016. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. Harmful Algae 54, 194–212. https://doi.org/10.1016/j.hal.2016.02.002.

Carpenter, S.R., Christensen, D.L., Cole, J.J., Cottingham, K.L., He, X., Hodgson, J.R., Kitchell, J.F., Knight, S.E., Pace, M.L., Post, D.M., Schindler, D.E., Voichick, N., 1995. Biological control of eutrophication in lakes. Environ. Sci. Technol. 29 (3), 784–786. https://doi.org/10.1021/es00003a028.

Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., 1985. Cascading trophic interactions and lake productivity. Bioscience 35 (10), 634–639.

Carr, M., Richter, D.J., Fozouni, P., Smith, T.J., Jeuck, A., Leadbeater, B.S.C., Nitsche, F., 2017. A six-gene phylogeny provides new insights into choanoflagellate evolution. Mol. Phylogenet Evol. 107, 166–178. https://doi.org/10.1016/j.ympev.2016.10.011.

Chaffin, J.D., Bridgeman, T.B., Bade, D.L., 2013. Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. Adv. Microbiol. 03 (06), 16–26. https://doi.org/10.4236/aim.2013.36a003.

Chaffin, J.D., Bridgeman, T.B., Bade, D.L., Mobilian, C.N., 2014. Summer phytoplankton nutrient limitation in Maumee Bay of Lake Erie during high-flow and low-flow years. J. Great Lakes Res. 40 (3), 524–531. https://doi.org/10.1016/j.jglr.2014.04.009.

Chen, W., Song, L., Ou, D., and Gan, N. (2005). Chronic toxicity and responses of several important enzymes in daphnia magna on exposure to sublethal microcystin-LR. 323–330. 10.1002/tox.20108.

Chislock, M.F., Sarnelle, O., Jernigan, L.M., Wilson, A.E., 2013. Do high concentrations of microcystin prevent Daphnia control of phytoplankton? Water Res. 47 (6), 1961–1970. https://doi.org/10.1016/j.watres.2012.12.038.

Christoffersen, K., Riemann, B., Klysner, A., Søndergaard, M., 1993. Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. Limnol. Oceanogr. 38 (3), 561–573. https://doi.org/10.4319/lo.1993.38.3.0561.

Claska, M.E., Gilbert, J.J., 1998. The effect of temperature on the response of Daphnia to toxic cyanobacteria. Freshwater Biol. 39 (2), 221–232. https://doi.org/10.1046/j.1365-2427.1998.00276.x.

Combes, A., Dellinger, M., CadelSix, S., Amand, S., Comte, K., 2013. Ciliate Nassula sp. grazing on a microcystin-producing cyanobacterium (Planktothrix agardhii): Impact on cell growth and in the microcystin fractions. Aquatic. Toxicol. 126, 435–441. https://doi.org/10.1016/j.aquatox.2012.08.018.

Cremona, F., Agasild, H., Haberman, J., Zingel, P., Nõges, P., Nõges, T., Laas, A., 2020. How warming and other stressors affect zooplankton abundance, biomass and community composition in shallow eutrophic lakes. Clim. Change 159 (4), 565–580. https://doi.org/10.1007/s10584-020-02698-2.

Dao, T.S., Vo, T.-M.-C., Pham, T.-L., 2016. First report on chronic effects of non-microcystin producing cyanobacteria, cylindrospermopsis curvispora and planktothrix sp., on daphnia magna. Environ. Manage. Sustain. Develop. 5 (2), 118. https://doi.org/10.5296/emsd.v5i2.9867.

Davis, T.W., Bullerjahn, G.S., Tuttle, T., Mckay, R.M., Watson, S.B., 2015. Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during Planktothrix Blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol. 49, 7197–7207. https://doi.org/10.1021/acs.est.5b00799.

Davis, T.W., Gobler, C.J., 2011. Grazing by mesozooplankton and microzooplankton on toxic and non-toxic strains of Microcystis in the Transquaking River, a tributary of Chesapeake Bay. J. Plankton Res. 33 (3), 415–430. https://doi.org/10.1093/plankt/fbq109.

Davis, T.W., Koch, F., Marcoval, M.A., Wilhelm, S.W., Gobler, C.J., 2012. Mesozooplankton and microzooplankton grazing during cyanobacterial blooms in the western basin of Lake Erie. Harmful Algae 15, 26–35. https://doi.org/10.1016/j.hal.2011.11.002.

Dazley, J. (2018). Ciliated Protozoa and Zooplankton as Potential Grazers of Freshwater Cyanobacteria. March. 10.13140/RG.2.2.33574.37446.

Dawidowicz, Piotr, 1990. Effectiveness of phytoplankton control by large-bodied and small-bodied zooplankton. Hydrobiologia 200-201, 43–47. https://doi.org/10.1007/BF02530327.

de Vargas, Colomban, Audic, Stéphane, Henry, Nicolas, Decelle, Johan, Mahé, Frédéric, Ramiro, Logares, Lara, Enrique, Berney, Cédric, Le Bescot, Noan, Probert, Ian, Carmichael, Margaux, Poulain, Julie, Romac, Sarah, Colin, Sébastien, Aury, Jean-Marc, Bittner, Lucie, Chaffron, Samuel, Dunthorn, Micah, Engelen, Stefan, Flegontova, Olga, Guidi, Lionel, Horák, Aleš, Jaillon, Olivier, Lima-Mendez, Gipsi, Lukeš, Julius, Malviya, Shruti, Morard, Raphael, Mulot, Matthieu, Scalco, Eleonora, Siano, Raffaele, Vincent, Flora, Zingone, Adriana, Dimier, Céline, Picheral, Marc, Searson, Sarah, Kandels-Lewis, Stefanie, Acinas, Silvia G., Bork, Peter, Bowler, Chris, Gorsky, Gabriel, Grimsley, Nigel, Hingamp, Pascal, Iudicone, Daniele, Not, Fabrice, Ogata, Hiroyuki, Pesant, Stephane, Raes, Jeroen, Sieracki, Michael E.,

Speich, Sabrina, Stemmann, Lars, Sunagawa, Shinichi, Weissenbach, Jean, Wincker, Patrick, Karsenti, Eric, 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348 (6237). https://doi.org/10.1126/science.1261605.

DeMott, W.R., 1982. Feeding selectivities and relative ingestion rates of Daphnia and Bosmina. Limnol. Oceanogr. 27 (3), 518–527.

DeMott, W.R., 1999. Foraging strategies and growth inhibition in five daphnids feeding on mixtures of a toxic cyanobacterium and a green alga. Freshwater Biol. 42 (2), 263–274. https://doi.org/10.1046/j.1365-2427.1999.444494.x.

DeMott, W.R., Gulati, R.D., Van Donk, E., 2001. Daphnia food limitation in three hypereutrophic Dutch lakes: evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. Limnol. Oceanogr. 46 (8), 2054–2060. https://doi.org/10.4319/lo.2001.46.8.2054.

DeMott, W.R., Moxter, F., 1991. Foraging on cyanobacteria by copepods: responses to chemical defenses and resource abundance. Ecol. Soc. America 72 (5), 1820–1834.

DeMott, W.R., Zhang, Q.-X., Carmichael, W.W., 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of Daphnia. Limnol. Oceanogr. 36 (7), 1346–1357.

Dempster, E.L., Pryor, K.V., Francis, D., Young, J.E., Rogers, H.J., 1999. Rapid DNA extraction from ferns for PCR- based analyses. BioTechniques 27 (1), 66–68.

Dryden, R.C., Wright, S.J.L., 1987. Predation of cyanobacteria by protozoa. Can. J. Microbiol. 33 (6), 471–482. https://doi.org/10.1139/m87-080.

Ekvall, M.K., Urrutia-Cordero, P., Hansson, L.A., 2014. Linking cascading effects of fish predation and zooplankton grazing to reduced cyanobacterial biomass and toxin levels following biomanipulation. PLoS One 9 (11). https://doi.org/10.1371/journal.pone.0112956.

Elbrecht, V., Leese, F., 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. PLoS One 10 (7). https://doi.org/10.1371/journal.pone.0130324.

Elliott, J.A., Jones, I.D., Thackeray, S.J., 2006. Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559, 401–411. https://doi.org/10.1007/s10750-005-1233-y.

Epp, G.T., 1996. Grazing on filamentous cyanobacteria by Daphnia pulicaria. Limnol. Oceanogr. 41 (3), 560–567. https://doi.org/10.4319/lo.1996.41.3.0560.

Ersoy, Z., Jeppesen, E., Sgarzi, S., Arranz, I., Cañedo-Argüelles, M., Quintana, X.D., Landkildehus, F., Lauridsen, T.L., Bartrons, M., Brucet, S., 2017. Size-based interactions and trophic transfer efficiency are modified by fish predation and cyanobacteria blooms in Lake Mývatn, Iceland. Freshwater Biol. 62 (11), 1942–1952. https://doi.org/10.1111/fwb.13039.

Fahnenstiel, G.L., Carrick, H.J., Iturriaga, R., 1991. Physiological characteristics and food-web dynamics of Synechococcus in Lakes Huron and Michigan. Limnol. Oceanogr. 36 (2), 219–234. https://doi.org/10.4319/lo.1991.36.2.0219.

Ferrão-Filho, A.S., Soares, M.C.S., Lima, R.S., Magalhães, V.F., 2014. Effects of Cylindrospermopsis raciborskii (cyanobacteria) on the swimming behavior of Daphnia (cladocera). Environ. Toxicol. Chem. 33 (1), 223–229. https://doi.org/10.1002/etc.2420.

Finlay, B.J., Esteban, G.F., 1998. Freshwater protozoa: biodiversity and ecological function. Biodivers. Conserv. 7 (9), 1163–1186. https://doi.org/10.1023/A:1008879616066.

Frenken, T., Wolinska, J., Tao, Y., Rohrlack, T., Agha, R., 2020. Infection of filamentous phytoplankton by fungal parasites enhances herbivory in pelagic food webs. Limnol. Oceanogr. 65 (11), 2618–2626. https://doi.org/10.1002/lno.11474.

Frost, B. W. (1972). Effect of size and concentration of food particles on the feeding behavior of the marine planktonic copepod Calanus pacificus. 17(6), 805–815.

Fuller, K., Shear, H., Wittig, J., 2002. The Great Lakes: An Environmental Atlas and Resource Book. Government of Canada United States Environmental Protection Agency (US EPA/The Government of Canada). In Cartography, Toronto, ON.

Fulton, R.S., 1988. Grazing on filamentous algae by herbivorous zooplankton. Freshwater Biol. 20 (2), 263–271. https://doi.org/10.1111/j.1365-2427.1988.tb00450.x.

Fulton, R.S., Paerl, H.W., 1987. Toxic and inhibitory effects of the blue-green alga Microcystis aeruginosa on herbivorous zooplankton. J. Plankton Res. 9 (5), 837–855.

Geller, W., Müller, H., 1981. The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. Oecologia 49 (3), 316–321. https://doi.org/10.1007/BF00347591.

Ger, K.A., Arneson, P., Goldman, C.R., Teh, S.J., 2010. Species specific differences in the ingestion of Microcystis cells by the calanoid copepods Eurytemora affinis and Pseudodiaptomus forbesi. J. Plankton Res. 32 (10), 1479–1484. https://doi.org/10.1093/plankt/fbq071.

Ger, K.A., Hansson, L.-A., Lürling, M., 2014. Understanding cyanobacteria-zooplankton interactions in a more eutrophic world. Freshwater Biol. 59, 1783–1798. https://doi.org/10.1111/fwb.12393.

Ger, K.A., Leitao, E., Panosso, R., Havens, K., 2016. Potential mechanisms for the tropical copepod Notodiaptomus to tolerate Microcystis toxicity. J. Plankton Res. 38 (4), 843–854. https://doi.org/10.1093/plankt/fbw036.

Ger, K.A., Naus-wiezer, S., Meester, L.De, Lürling, M, 2018. Zooplankton grazing selectivity regulates herbivory and dominance of toxic phytoplankton over multiple prey generations. Limnol. Oceanogr. 9999, 1–14. https://doi.org/10.1002/lno.11108.

Ger, K.A., Urrutia-Cordero, P., Frost, P.C., Hansson, L.A., Sarnelle, O., Wilson, A.E., Lürling, M., 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic world. Harmful Algae 54, 128–144. https://doi.org/10.1016/j.hal.2015.12.005.

Ghadouani, A., Pinel-Alloul, B., Prepas, E.E., 2003. Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshwater Biol. 48, 363–381.

Gilbert, John J., Durand, Mark 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. https://doi.org/10.1111/j.1365-2427.1990.tb00734.x.

Gliwicz, Z.M., Lampert, W., 1990. Food thresholds in Daphnia species in the absence and presence of blue-green filaments. Ecology 71 (2), 691–702.

Gobler, C.J., Davis, T.W., Coyne, K.J., Boyer, G.L., 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. Harmful Algae 6 (1), 119–133. https://doi.org/10.1016/j.hal.2006.08.003.

Gobler, C.J., Davis, T.W., Deonarine, S.N., Saxton, M.A., Lavrentyev, P.J., Jochem, F.J., Wilhelm, S.W., 2008. Grazing and virus-induced mortality of microbial populations before and during the onset of annual hypoxia in Lake Erie. Aquat. Microb. Ecol. 51, 117–128. https://doi.org/10.3354/ame01180.

Gustafsson, S., Hansson, L.A., 2004. Development of tolerance against toxic cyanobacteria in Daphnia. Aquat. Ecol. 38 (1), 37–44. https://doi.org/10.1023/B:AECO.0000020985.47348.5e.

Gustafsson, S., Rengefors, K., Hansson, L.A., 2005. Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. Ecology 86 (10), 2561–2567. https://doi.org/10.1890/04-1710.

Hairston Jr., N.G., Holtmeier, C.L., Lampert, W., Weider, L.J., Post, D.M., Fischer, J.M., Caceres, C.E., Fox, J.A., Gaedke, U, 2001. Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity. Evolution 55 (11), 2203–2214.

Hairston Jr., N.G., Lampert, W., Caceres, C.E., Holtmeier, C.L., Weider, L.J., Gaedke, U., Fischer, J.M., Fox, J.A., Post, D.M, 1999. Rapid evolution revealed by dormant eggs. Nature 401, 446.

Harke, M.J., Jankowiak, J.G., Morrell, B.K., Gobler, C.J., 2017. Transcriptomic responses in the bloom-forming cyanobacterium Microcytis induced during exposure to zooplankton. Appl. Environ. Microbiol. 83 (5), 1–15.

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl, H. W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, Microcystis spp. Harmful Algae 54, 4–20. https://doi.org/10.1016/j.hal.2015.12.007.

Heath, R.T., Hwang, S.-J., Munawar, M., 2010. A hypothesis for the assessment of the importance of microbial food web linkages in nearshore and offshore habitats of the Laurentian Great Lakes. Aquat. Ecosyst. Health Manage. 6 (3), 231–239.

Heitala, J., Laurén-Määttä, C., Walls, M., 1997. Sensitivity of Daphnia to toxic cyanobacteria: effects of genotype and temperature. Freshwater Biol. 37, 229–306.

Hietala, J., Laurén-Määttä, C., Walls, M., 1997. Life history responses of Daphnia clones to toxic Microcystis at different food levels. J. Plankton Res. 19 (7), 917–926. https://doi.org/10.1093/plankt/19.7.917.

Huang, I.S., Zimba, P.V., 2019. Cyanobacterial bioactive metabolites-A review of their chemistry and biology. Harmful Algae 86, 139–209. https://doi.org/10.1016/j.hal.2019.05.001.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16 (8), 471–483. https://doi.org/10.1038/s41579-018-0040-1.

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 intensi fi cation, diversity, and toxicity in western Lake Erie. Limnol. Oceanogr. 64, 1347–1370. https://doi.org/10.1002/lno.11120.

Jiang, X., Xie, J., Xu, Y., Zhong, W., Zhu, X., Zhu, C., 2017. Increasing dominance of small zooplankton with toxic cyanobacteria. Freshwater Biol. 62 (2), 429–443. https://doi.org/10.1111/fwb.12877.

Jiang, X., Yang, W., Zhao, S., Liang, H., Zhao, Y., Chen, L., Li, R., 2013. Maternal effects of inducible tolerance against the toxic cyanobacterium Microcystis aeruginosa in the grazer Daphnia carinata. Environ. Pollut. 178, 142–146. https://doi.org/10.1016/j.envpol.2013.03.017.

Jungmann, D., Benndorf, J., 1994. Toxicity to Daphnia of a compound extracted from laboratory and natural Microcystis spp. and the role of microcystins. Freshwater Biol. 32, 13–20.

Kaebernick, M., Neilan, B.A., 2001. Ecological and molecular Microbiol, investigations of cyano-toxin production. FEMS Microbiol. Ecol. 35, 1–9.

Kast, J.B., Apostel, A.M., Kalcic, M.M., Muenich, R.L., Dagnew, A., Long, C.M., Evenson, G., Martin, J.F., 2021. Source contribution to phosphorus loads from the Maumee River watershed to Lake Erie. J. Environ. Manage. 279, 111803 https://doi.org/10.1016/j.jenvman.2020.111803.

Kim, B.-R., Nakano, S., Kim, B.-H., Han, M.-S., 2006. Grazing and growth of the heterotrophic flagellate Diphylleia rotans on the cyanobacterium Microcystis aeruginosa. Aquat. Microb. Ecol. 45, 163–170.

Kirk, K.L., Gilbert, J.J., 1992. Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. Ecology 73 (6), 2208–2217.

Kring, S.A., Figary, S.E., Boyer, G.L., Watson, S.B., Twiss, M.R., 2014. Rapid in situ measures of phytoplankton communities using the bbe FluoroProbe: Evaluation of spectral calibration, instrument intercompatibility, and performance range. Can. J. Fish. Aquat.Sci. 71 (7), 1087–1095. https://doi.org/10.1139/cjfas-2013-0599.

Kurmayer, R., Jüttner, F., 1999. Strategies for the co-existence of zooplankton with the toxic cyanobacterium Planktothrix rubescens in Lake Zürich. J. Plankton Res. 21 (4), 659–683.

Kuster, C.J., Von Elert, E, 2013. Interspecific differences between D. pulex and D. magna in tolerance to Cyanobacteria with protease inhibitors. PLoS One 8 (5), 1–8. https://doi.org/10.1371/journal.pone.0062658.

Kutovaya, O.A., McKay, R.M.L., Beall, B.F.N., Wilhelm, S.W., Kane, D.D., Chaffin, J.D., Bridgeman, T.B., Bullerjahn, G.S., 2012. Evidence against fluvial seeding of recurrent toxic blooms of Microcystis spp. in Lake Erie's western basin. Harmful Algae 15, 71–77. https://doi.org/10.1016/j.hal.2011.11.007.

Lampert, W., 1981. Inhibitory and toxic effects of blue-green algae on daphnia. Int. Revue Hydrobiol. 66 (3), 285–298.

Landry, M.R., Decima, M., Simmons, M.P., Hannides, C.C.S., Daniels, E., 2008. Mesozooplankton biomass and grazing responses to Cyclone Opal, a subtropical mesoscale eddy. Deep-Sea Res. II 55, 1378–1388. https://doi.org/10.1016/j.dsr2.2008.01.005.

Landry, M.R., Hassett, R.P., 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. 67 (3), 283–288. https://doi.org/10.1007/BF00397668.

Laurén-Määttä, Camilla, Hietala, Jaana, Walls, Mari, 1997. Responses of Daphnia pulex populations to toxic cyanobacteria. Freshwater Biology 37, 635–647. https://doi.org/10.1046/j.1365-2427.1997.00189.x.

Lavrentyev, P.J., McCarthy, M.J., Klarer, D.M., Jochem, F., Gardner, W.S., 2004. Estuarine microbial food web patterns in a Lake Erie coastal wetland. Microb. Ecol. 48 (4), 567–577. https://doi.org/10.1007/s00248-004-0250-0.

Lehman, J.T., 1980. Release and cycling of nutrients between planktonic algae and herbivores. Limnol. Oceanogr. 25 (4), 620–632. https://doi.org/10.4319/lo.1980.25.4.0620.

Lemaire, V., Brusciotti, S., van Gremberghe, I., Vyverman, W., Vanoverbeke, J., De Meester, L., 2012. Genotype×genotype interactions between the toxic cyanobacterium Microcystis and its grazer, the waterflea Daphnia. Evol. Appl. 5 (2), 168–182. https://doi.org/10.1111/j.1752-4571.2011.00225.x.

Leonard, J.A., Paerl, H.W., 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns river system, Florida as influenced by the toxic cyanobacterium Cylindrospermopsis raciborskii. Hydrobiologia 537 (1–3), 89–97. https://doi.org/10.1007/s10750-004-2483-9.

Liu, P., Wang, L., Xia, X., Zeng, L., Zhou, Q., Liu, B., He, F., Wu, Z., 2019. Microzooplankton grazing and phytoplankton growth in a Chinese lake. Polish J. Environ. Studies 28 (1), 225–235. https://doi.org/10.15244/pjoes/83689.

Long, J.D., Smalley, G.W., Barsby, T., Anderson, J.T., Hay, M.E., 2007. Chemical cues induce consumer-specific defenses in a bloom-forming marine phytoplankton. PNAS 104 (25), 10512–10517. https://doi.org/10.1073/pnas.0611600104.

Lürling, M., 2003. Effects of microcystin-free and microcystin- containing strains of the cyanobacterium microcystis aeruginosa on growth of the grazer daphnia magna. Environ. Toxicol. 18, 202–210. https://doi.org/10.1002/tox.10.1002/tox.10115.

Lürling, M., 2020. Grazing resistance in phytoplankton. Hydrobiologia 3, 237–249. https://doi.org/10.1007/s10750-020-04370-3.

Lürling, M., Esther, van der G, 2003. Life-history characteristics of Daphnia exposed to dissolved microcystin-LR and to the cyanobacterium Microcystis aeruginosa with and without microcystins. Environ. Toxicol. Chem. 22 (6), 1281–1287.

Lusty, M.W., Gobler, C.J., 2020. The efficacy of hydrogen peroxide in mitigating cyanobacterial blooms and altering microbial communities across Four Lakes in NY, USA. Toxins 12 (7). https://doi.org/10.3390/toxins12070428.

Lyu, K., Guan, H., Wu, C., Wang, X., Wilson, A.E., Yang, Z., 2016. Maternal consumption of non-toxic Microcystis by Daphnia magna induces tolerance to toxic Microcystis in offspring. Freshwater Biol. 61, 219–228. https://doi.org/10.1111/fwb.12695.

Martin-Creuzburg, D., Von Elert, E., Hoffmann, K.H., 2008. Nutritional constraints at the cyanobacteria – Daphnia magna interface : The role of sterols. Limnol. Oceanogr. 53 (2), 456–468.

Martin-Creuzburg, D., Wacker, A., Von Elert, E, 2005. Life history consequences of sterol availability in the aquatic keystone species Daphnia. Oecologia 144 (3), 362–372. https://doi.org/10.1007/s00442-005-0090-8.

Munawar, M., Munawar, I. F., Weisse, T., vanStam, H., Fitzpatrick, M., Lorimer, J., Wenghofer, C., and Carou, S. (1999). Probing microbial food-web structure in Lake Erie. State of Lake Erie: Past, Present and Future, January, 417–439.

Nguyen, T.D., Ngo, X.Q., Pham, T.L., Dao, T.S., 2020. Ecotoxicological investigation of cyanobacterial crude extracts to daphnia magna under subchronic test conditions. Turkish J. Zool. 44 (6), 498–507. https://doi.org/10.3906/zoo-2005-35.

Nogueira, I.C.G., Saker, M.L., Pflugmacher, S., Wiegand, C., Vasconcelos, V.M., 2004. Toxicity of the cyanobacterium Cylindrospermopsis raciborskii to Daphnia magna. Environ. Toxicol. 19 (5), 453–459. https://doi.org/10.1002/tox.20050.

Nübel, U., Garcia-Pichel, F., Muyzer, G., 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microbiol. 63 (8), 3327–3332.

Oberhaus, L., Gélinas, M., Pinel-Alloul, B., Ghadouani, A., Humbert, J.F., 2007. Grazing of two toxic Planktothrix species by Daphnia pulicaria: potential for bloom control and transfer of microcystins. J. Plankton Res. 29 (10), 827–838. https://doi.org/10.1093/plankt/fbm062.

Olvera-Ramírez, R., Centeno-Ramos, C., Martínez-Jerónimo, F., 2010. Toxic effects of Pseudanabaena tenuis (cyanobacteria) on the cladocerans Daphnia magna and Ceriodaphnia dubia. Hidrobiologica 20 (3), 203–212.

Otten, T.G., Paerl, H.W., 2015. Health effects of toxic cyanobacteria in US. Drinking and recreational waters: our current understanding and proposed direction. Curr. Environ. Health Rep. 2 (1), 75–84. https://doi.org/10.1007/s40572-014-0041-9.

Paerl, H.W., Huisman, J., 2009. Climate change : a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Reports 1 (1), 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x.

Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46 (5), 1349–1363. https://doi.org/10.1016/j.watres.2011.08.002.

Paerl, H.W., Xu, H., Hall, N.S., Rossignol, K.L., Joyner, A.R., Zhu, G., Qin, B., 2015. Nutrient limitation dynamics examined on a multi-annual scale in Lake Taihu, China: implications for controlling eutrophication and harmful algal blooms. J. Freshwater Ecol. 30 (1), 5–24. https://doi.org/10.1080/02705060.2014.994047.

Panosso, R., Lürling, M., 2010. Daphnia magna feeding on Cylindrospermopsis raciborskii: The role of food composition, filament length and body size. J. Plankton Res. 32 (10), 1393–1404. https://doi.org/10.1093/plankt/fbq057.

Pawlik-Skowrońska, B., Toporowska, M., Mazur-Marzec, H., 2019. Effects of secondary metabolites produced by different cyanobacterial populations on the freshwater zooplankters Brachionus calyciflorus and Daphnia pulex. Environ. Sci. Pollut. Res. 26 (12), 11793–11804. https://doi.org/10.1007/s11356-019-04543-1.

Polis, G.A., Strong, D.R., 1996. Food web complexity and community dynamics. Am. Nat. 147 (5), 813–846. https://doi.org/10.1086/285880.

R Core Team. (2020). R: A Language and Environment for Statistical Computing.

Ravet, J.L., Brett, M.T., 2006. Phytoplankton essential fatty acid and phosphorus content constraints on Daphnia somatic growth and reproduction. Limnol. Oceanogr. 51 (5), 2438–2452. https://doi.org/10.4319/lo.2006.51.5.2438.

Reinikainen, M., Ketola, M., Walls, M., 1994. Effects of the concentrations of toxic Microcystis aeruginosa and an alternative food on the survival of Daphnia pulex. Limnol. Oceanogr. 39 (2), 424–432. https://doi.org/10.4319/lo.1994.39.2.0424.

Rinta-Kanto, Johanna M., Wilhelm, Steven W., 2006. Diversity of Microcystin-Producing Cyanobacteriain Spatially Isolated Regions of Lake Erie. Applied and Environmental Microbiology 72 (7), 5083–5085. https://doi.org/10.1128/AEM.00312-06.

Rohrlack, T., Christoffersen, K., Friberg-Jensen, U., 2005. Frequency of inhibitors of daphnid trypsin in the widely distributed cyanobacterial genus Planktothrix. Environ. Microbiol. 7 (10), 1667–1669. https://doi.org/10.1111/j.1462-2920.2005.00877.x.

Rohrlack, T., Christoffersen, K., Kaebernick, M., Neilan, B.A., 2004. Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in Daphnia pulicaria. Appl. Environ. Microbiol. 70 (8), 5047–5050. https://doi.org/10.1128/AEM.70.8.5047-5050.2004.

Rohrlack, T., Dittmann, E., Börner, T., Christoffersen, K., 2001. Effects of cell-bound microcystins on survival and feeding of daphnia spp. Appl. Environ. Microbiol. 67 (8), 3523–3529. https://doi.org/10.1128/AEM.67.8.3523-3529.2001.

Rohrlack, T., Dittmann, E., Henning, M., Börner, T., Kohl, J.G., 1999. Role of microcystins in poisoning and food ingestion inhibition of Daphnia galeata caused by the cyanobacterium Microcystis aeruginosa. Appl. Environ. Microbiol. 65 (2), 737–739.

Rollwagen-Bollens, G., Bollens, S.M., Gonzalez, A., Zimmerman, J., Lee, T., Emerson, J., 2013. Feeding dynamics of the copepod Diacyclops thomasi before, during and following filamentous cyanobacteria blooms in a large, shallow temperate lake. Hydrobiologia 705 (1), 101–118. https://doi.org/10.1007/s10750-012-1385-5.

Sarnelle, O., Gustafsson, S., Hansson, L.A., 2010. Effects of cyanobacteria on fitness components of the herbivore Daphnia. J. Plankton Res. 32 (4), 471–477. https://doi.org/10.1093/plankt/fbp151.

Sarnelle, O., Wilson, A.E., 2005. Local adaptation of Daphnia pulicaria to toxic cyanobacteria. Limnol. Oceanogr. 50 (5), 1565–1570. https://doi.org/10.4319/lo.2005.50.5.1565.

Schwarzenberger, A., Kurmayer, R., Martin-Creuzburg, D., 2020. Toward disentangling the multiple nutritional constraints imposed by planktothrix: the significance of harmful secondary metabolites and sterol limitation. Front. Microbiol. 11 (October), 1–14. https://doi.org/10.3389/fmicb.2020.586120.

Schwarzenberger, A., Kuster, C.J., Von Elert, E, 2012. Molecular mechanisms of tolerance to cyanobacterial protease inhibitors revealed by clonal differences in Daphnia magna. Mol. Ecol. 21 (19), 4898–4911. https://doi.org/10.1111/j.1365-294X.2012.05753.x.

Sellner, K.G., Brownlee, D.C., Bundy, M.H., Brownlee, S.G., Braun, K.R., 1993. Zooplankton grazing in a Potomac River cyanobacteria bloom. Estuaries 16 (4), 859–872. https://doi.org/10.2307/1352445.

Sigee, D.C., Glenn, R., Andrews, M.J., Bellinger, E.G., Butler, R.D., Epton, H.A.S., D, H.R, 1999. Biological control of Cyanobacteria: principles and possabilities. Hydrobiologia 395, 161–172.

Sivonen, K., Jones, G., 1999. Cyanobacterial toxins. Toxic Cyanobact. Water 1, 43–112.

Starke, R., Kermer, R., Ullmann-Zeunert, L., Baldwin, I.T., Seifert, J., Bastida, F., von Bergen, M., Jehmlich, N., 2016. Bacteria dominate the short-term assimilation of plant-derived N in soil. Soil Biol. Biochem. 96 (March), 30–38. https://doi.org/10.1016/j.soilbio.2016.01.009.

Steffen, M.M., Belisle, B.S., Watson, S.B., Boyer, G.L., Bourbonniere, R.A., Wilhelm, S.W., 2015. Metatranscriptomic evidence for co-occurring top-down and bottom-up controls on toxic cyanobacterial communities. Appl. Environ. Microbiol. 81 (9), 3268–3276. https://doi.org/10.1128/AEM.04101-14.

Steffen, M.M., Davis, T.W., McKay, R.M.L., Bullerjahn, G.S., Krausfeldt, L.E., Stough, J.M. A., Neitzey, M.L., Gilbert, N.E., Boyer, G.L., Johengen, T.H., Gossiaux, D.C., Burtner, A.M., Palladino, D., Rowe, M.D., Dick, G.J., Meyer, K.A., Levy, S., Boone, B. E., Stumpf, R.P., Wilhelm, S.W., 2017. Ecophysiological examination of the lake erie microcystis bloom in 2014: linkages between biology and the water supply shutdown of Toledo, OH. Environ. Sci. Technol. 51 (12), 6745–6755. https://doi.org/10.1021/acs.est.7b00856.

Strecker, A.L., Cobb, T.P., Vinebrooke, R.D., 2004. Effects of experimental greenhouse warming on phytoplankton and zooplankton communities in fishless alpine ponds. Limnol. Oceanogr. 49 (4 I), 1182–1190. https://doi.org/10.4319/lo.2004.49.4.1182.

Stumpf, R.P., Wynne, T.T., Baker, D.B., Fahnenstiel, G.L., 2012. Interannual variability of cyanobacterial blooms in Lake Erie. PLoS One 7 (8). https://doi.org/10.1371/journal.pone.0042444.

Sukenik, A., Quesada, A., Salmaso, N., 2015. Global expansion of toxic and non-toxic cyanobacteria: effect on ecosystem functioning. Biodivers. Conserv. 24 (4), 889–908. https://doi.org/10.1007/s10531-015-0905-9.

Suzuki, K., Tsuda, A., Kiyosawa, H., Takeda, S., Nishioka, J., Saino, T., Takahashi, M., Wong, C.S., 2002. Grazing impact of microzooplankton on a diatom bloom in a mesocosm as estimated by pigment-specific dilution technique. J. Exp. Mar. Biol. Ecol. 271 (1), 99–120. https://doi.org/10.1016/S0022-0981(02)00038-2.

Threlkeld, S.T., 1979. The midsummer dynamics of two Daphnia species in Wintergreen Lake, Michigan. Ecology 60 (1), 165–179.

Urrutia-Cordero, P., Ekvall, M.K., Hansson, L.A., 2016. Controlling harmful cyanobacteria: taxa-specific responses of cyanobacteria to grazing by large-bodied Daphnia in a biomanipulation scenario. PLoS One 11 (4), 1–14. https://doi.org/10.1371/journal.pone.0153032.

Usher, K.M., Fromont, J., Sutton, D.C., Toze, S., 2004. The biogeography and phylogeny of unicellular cyanobacterial symbionts in sponges from Australis and the Mediterranean. Microb. Ecol. 48 (2), 167–177. https://doi.org/10.1007/s00248-003-1062-3.

Von Elert, E., Agrawal, M.K., Gebauer, C., Jaensch, H., Bauer, U., Zitt, A., 2004. Protease activity in gut of Daphnia magna: Evidence for trypsin and chymotrypsin enzymes. Compar. Biochem. Physiol. B Biochem. Mole. Biol. 137 (3), 287–296. https://doi.org/10.1016/j.cbpc.2003.11.008.

Von Elert, E., Martin-Creuzburg, D., Le Coz, J.R., 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (Daphnia galeata). Proc. Royal Soc. B 270 (1520), 1209–1214. https://doi.org/10.1098/rspb.2003.2357.

Wang, X., Qin, B., Gao, G., Paerl, H.W., 2010. Nutrient enrichment and selective predation by zooplankton promote Microcystis (Cyanobacteria) bloom formation. J. Plankton Res. 32 (4), 457–470. https://doi.org/10.1093/plankt/fbp143.

Wang, Y., Qian, P., 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4 (10). https://doi.org/10.1371/journal.pone.0007401.

Weckesser, J., Martin, C., Jakobi, C., 1996. Cyanopeptolins, depsipeptides from cyanobacteria. Syst. Appl. Microbiol. 19 (2), 133–138. https://doi.org/10.1016/S0723-2020(96)80038-5.

Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol. Oceanogr. 39 (8), 1985–1992. https://doi.org/10.4319/lo.1994.39.8.1985.

Wilken, S., Wiezer, S., Huisman, J., Van Donk, E., 2010. Microcystins do not provide anti-herbivore defence against mixotrophic flagellates. Aquat. Microb. Ecol. 59 (3), 207–216. https://doi.org/10.3354/ame01395.

Wilson, A.E., Sarnelle, O., Tillmanns, A.R., 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: meta-analyses of laboratory experiments. Limnol. Oceanogr. 51 (4), 1915–1924. https://doi.org/10.4319/lo.2006.51.4.1915.

Wilson, K.M., Schembri, M.A., Baker, P.D., Saint, C.P., 2000. Molecular characterization of the toxic cyanobacterium Cylindrospermopsis raciborskii and design of a species-specific PCR. Appl. Environ. Microbiol. 66 (1), 332–338. https://doi.org/10.1128/AEM.66.1.332-338.2000.

Work, K.A., Havens, K.E., 2003. Zooplankton grazing on bacteria and cyanobacteria in a eutrophic lake. J. Plankton Res. 25 (10), 1301–1306. https://doi.org/10.1093/plankt/fbg092.

World Heath Organization. (1999). Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. 10.1046/j.1365-2427.2003.01107.x.

Wynne, T. T., Stumpf, R. P., Tomlinson, M. C., Fahnenstiel, G. L., Dyble, J., Schwab, D. J., and Joseph, S. (2013). Evolution of a cyanobacterial bloom forecast system in western Lake Erie : Development and initial evaluation. J. Great Lakes Res.. 10.1016/j.jglr.2012.10.003.

Zhang, W., Lou, I., Ung, W.K., Kong, Y., Mok, K.M., 2014. Application of PCR and real-time PCR for monitoring cyanobacteria, Microcystis spp. and Cylindrospermopsis raciborskii in Macau freshwater reservoir. Front. Earth Sci. 8 (2), 291–301. https://doi.org/10.1007/s11707-013-0409-4.

Zhou, J., Qin, B., Zhu, G., Zhang, Y., Gao, G., 2020. Long-term variation of zooplankton communities in a large, heterogenous lake: Implications for future environmental change scenarios. Environ. Res. 187, 109704 https://doi.org/10.1016/j.envres.2020.109704.