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PRIMARY RESEARCH PAPER

Interactive effects of phosphorus and zooplankton grazing on cyanobacterial blooms in a shallow temperate lake

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Abstract Cyanobacterial blooms are becoming increasingly common worldwide. These blooms can be moderated by grazing and/or nutrient availability, but the interactive effects of these processes are not well understood. We examined the interactive effects of phosphate and copepods on growth of phytoplankton (algae and cyanobacteria) in a shallow temperate lake. Field sampling of nutrient and chlorophyll *a* concentrations was conducted weekly from May through October, 2013. Five two-factorial experiments, spanning pre-, mid-, and post-bloom periods, were conducted with unfiltered lake water incubated with amended copepods, phosphate, or both. Changes in chlorophyll *a* concentration were used to calculate net phytoplankton biomass growth rates, and cell counts were performed on selected experiments to calculate growth rates of six microplanktonic taxonomic groups. Field data revealed cyanobacterial bloom development in July and decline in September. Experimental results indicated that phytoplankton growth increased with added phosphate pre-bloom, and decreased with added copepod grazers post-bloom, but that a more complex interactive (phosphorus x copepods) effect was observed immediately prior to and during peak bloom times. More specifically, the addition of phosphate and copepods enhanced ciliate growth pre-bloom, while selective grazing by copepods reduced dinoflagellate growth mid-bloom, possibly enhancing cyanobacterial growth and bloom duration via trophic cascade effects.

Keywords Harmful algal bloom · Grazing · Trophic cascade · Phosphate · Cyanobacteria · Interactive effects

Introduction

Temperate lakes worldwide are experiencing harmful algal blooms (HABs) more frequently compared to the past, posing a serious threat to humans and/or livestock that utilize these systems for recreation or drinking water and, therefore, the use and sustainability of freshwater resources around the globe (Anderson et al., 2002; Glibert et al., 2005; Paerl et al., 2011). Cyanobacteria-dominated HABs (CyanoHABs) are particularly hazardous, as many species are capable of producing potent toxins that can affect many populations, including humans (Chorus & Bartram, 1999),

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and more broadly, ecosystem health and integrity (Carmichael, 2001; Landsberg, 2002; Paerl et al., 2011). Increased CyanoHAB occurrence is thought to be largely due to eutrophication, as runoff and wastewater effluent contain high levels of nitrogen (N) and phosphorus (P) (Conley et al., 2009; Paerl et al., 2011). However, zooplankton grazing has also been shown to influence CyanoHAB formation, duration and decline (Chan et al., 2004; Gobler et al., 2007; Davis et al., 2012; Ger et al., 2014). In addition, CyanoHAB frequency and intensity are predicted to increase with future climate change, as increasing temperatures, lower pH, altered mixing patterns, and shifts in the timing of heavy precipitation events or evaporation may support increased bloom occurrence (Moore et al., 2008).

Ecological research into planktonic community dynamics often focuses on a "bottom-up" (e.g., mixing or nutrients) or a "top-down" (i.e., grazing or predation) approach in an effort to determine which factor(s) is primarily controlling abundance at a given trophic level. Growth of algae and cyanobacteria (henceforth referred to as phytoplankton), for example, can be controlled by the concentration of bioavailable nutrients—considered a bottom-up mechanism of control on a population (Begon et al., 1996). Historically, P was considered the primary limiting nutrient for phytoplankton growth in freshwater systems (Schindler et al., 2008), leading to management efforts focused on reducing inputs of this single nutrient. However, more recent research has stressed the importance of both N and P in freshwater systems affected by eutrophication and associated cyanobacterial blooms (Conley et al., 2009). P may limit phytoplankton growth in lakes where the community is dominated by N_2-fixing cyanobacteria (Reynolds, 2006), but the role of N becomes more important where the dominant species is a non-N_2-fixer, such as *Microcystis* (Conley et al., 2009; Paerl et al., 2011). In addition, N limitation may occur where fixation alone is not enough to meet the biological demands of high phytoplankton concentrations (Scott & McCarthy, 2010) or due to other conditions, such as imbalances of nutrient inputs or unique internal storage dynamics (Scott & McCarthy, 2010; Paerl et al., 2011). While recognizing the importance of dual nutrient management of freshwater systems, the following study focuses primarily on the role of P due to previous research in Vancouver Lake that showed associations between this nutrient and summertime cyanobacterial blooms (Lee et al., 2015a).

In contrast, "top-down" control of planktonic food web dynamics has led to the idea that zooplankton grazing may be used to control phytoplankton abundance (With & Wright, 1984), and therefore, possibly, cyanobacterial blooms. In addition to consuming planktonic algae, freshwater crustacean zooplankton (e.g., cladocerans and copepods) have been known to consume colonial and filamentous cyanobacteria (Work & Havens, 2003; Oberhaus et al., 2007; Panosso & Lurling, 2010; Kâ et al., 2011). While this may enable zooplankton to limit cyanobacterial growth, feeding selectivity must also be considered. Even large cladoceran taxa (e.g., *Daphnia*), often deemed non-selective feeders that are negatively affected by consumption of toxic cyanobacteria (Hansson et al., 2007), have exhibited a species-specific preference for certain strains of filamentous cyanobacteria (Knisely & Geller, 1986; Epp, 1996).

Copepods also exhibit feeding selectivity based on prey size (Frost, 1972), quality (DeMott, 1986; Cowles et al., 1988), and toxin content (Teegarden, 1999). These preferences may cause copepods to select against some species of cyanobacteria when present in a mixed phytoplankton assemblage (Kirk & Gilbert, 1992; Burns & Hegarty, 1994). Other studies indicate that copepods can, at times, selectively consume heterotrophic and mixotrophic microplankton (cells <200 μm in size) rather than phytoplankton (Rollwagen-Bollens & Penry, 2003; Olson et al., 2006; Gifford et al., 2007), potentially causing indirect trophic cascade events by reducing effects of microzooplankton grazing on phytoplankton (Stibor et al., 2004; Olson et al., 2006; Sommer & Sommer, 2006; Boyer et al., 2011). These studies make evident the ability of zooplankton grazers to directly limit phytoplankton growth, but also to indirectly affect the phytoplankton community by altering food web structure.

Vancouver Lake, located in southwest Washington state, USA, is a highly valued recreational resource in the region. Cyanobacteria, including the genera *Aphanizomenon*, *Dolichospermum* (formerly *Anabaena*), and *Microcystis*, have been observed for many years in Vancouver Lake (Boyer et al., 2011; Lee et al., 2015a), and recent evidence demonstrated that *Microcystis* produces toxins in the lake under certain

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environmental conditions (Lee et al., 2015b). Cyano-HABs in Vancouver Lake have become more common over the past 20 years, occasionally necessitating lake closure when concentrations of cyanobacteria exceed World Health Organization safety limits (Lee et al., 2015a, b).

The metazoan zooplankton community in Vancouver Lake includes rotifers, copepods, and cladocerans (Rollwagen-Bollens et al., 2013; Lee et al., 2016); and the microplankton community consists of diatoms, ciliates, dinoflagellates, chlorophytes, cryptophytes, and cyanobacteria (Boyer et al., 2011; Lee et al., 2015a, b). A previous study revealed high levels of N and P in summer months, and a notable elevation of orthophosphate in July and August that coincided with peak cyanobacterial abundance (Lee et al., 2015a). Likewise, the effects of grazing by microzooplankton (Boyer et al., 2011) and mesozooplankton (Rollwagen-Bollens et al., 2013) in Vancouver Lake have been studied over multiple cyanobacterial bloom cycles. However, the interactive effects of phosphate and grazing pressure on phytoplankton growth during multiple stages of a bloom in Vancouver Lake have not been previously examined. Indeed, such interactive effects of nutrients and zooplankton grazing on cyanobacterial bloom dynamics have only rarely been examined experimentally in any aquatic ecosystem (Hambright et al., 2001; Gobler et al., 2007).

In the following study, we tested the overarching hypothesis that phosphate and mesozooplankton grazing exert independent and interactive effects on phytoplankton growth at different stages of a cyanobacterial bloom cycle. Specifically, we hypothesized that in Vancouver Lake (1) enhanced phosphate increases phytoplankton growth rates and extends cyanobacterial bloom duration, (2) enhanced grazing by mesozooplankton (copepods) decreases phytoplankton growth and reduces bloom duration, and (3) these two factors (added phosphate and copepod grazing) have interactive effects that serve to maintain a cyanobacterial bloom. By conducting two-factorial experiments during pre-, mid-, and post-bloom periods, we were able to examine the temporal variation in the effects of these factors on cyanobacterial blooms in Vancouver Lake. By doing so, we hoped to shed light on the temporal (seasonal) dynamics of cyanobacterial blooms in freshwater systems more generally.

Materials and methods

Study site

Vancouver Lake (45.66°N, 122.72°W) is a shallow, temperate, freshwater lake located in the lower Columbia River floodplain in southwest Washington state, USA. The lake is approximately 5,680 ha with an average depth of ~1 m and is connected to the Columbia River by Lake River to the north, Burnt Bridge Creek to the east, and a gated channel on the southwest shore which allows inflow (but not outflow) of water directly from the Columbia River at high tide (Boyer et al., 2011; Sheibley et al., 2014). All water sampling and collection of plankton was carried out from a dock located on the southeast shore of the lake (Fig. 1). In a previous study conducted in 2007, quarterly sampling of plankton composition and

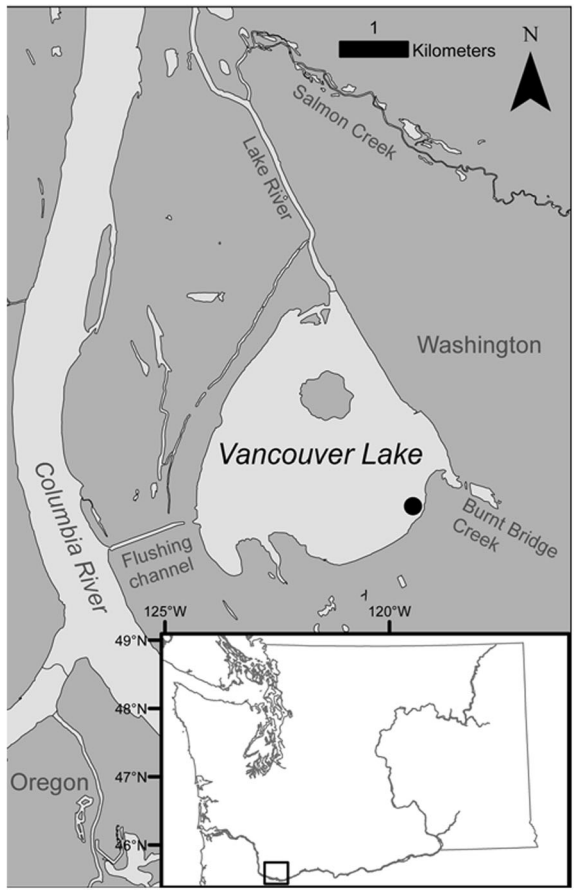

Fig. 1 Sampling location (symbolized by *filled circle*) at Vancouver Lake, Washington

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abundance at eight stations broadly distributed throughout the lake (littoral, limnetic, and dock) showed no significant spatial variability [Kendall's tau (τ_b), $n = 10$ site^{-1}, $P > 0.05$, (Bollens & Rollwagen-Bollens, 2009)]. In a separate study, water quality data taken from different in-lake locations between 2010 and 2012 were found to be similar, indicating that the lake was well mixed overall (Sheibley et al., 2014).

Field sampling

Lake sampling was conducted weekly from May 31 through October 25, 2013. Dissolved oxygen (DO) and temperature were measured just below the water surface using a YSI85 probe. Water depth and clarity (Secchi disk depth) were also measured. Surface water was collected in triplicate using a clean bucket lowered from the end of the dock. Each surface water collection was subsampled for determination of chlorophyll (chl) *a* and nutrient concentrations using procedures described below.

Surface water subsamples were filtered through a 0.45-μm filter, refrigerated, and sent to the University of Washington's Marine Chemistry Laboratory for analysis of nutrient concentrations (PO_4, NH_4, NO_3, NO_2, SiO_4) using a Technicon AAII system, according to the WOCE Hydrographic Program protocols. For measurement of chl *a*, an aliquot of 15–25 ml from each surface water sample was filtered through Whatman GF/F filters upon return to the lab. The filters were wrapped in foil and frozen for at least 24 h (but no longer than 7 days) before being extracted in 20 ml of 90% acetone for 24 h. After extraction, the concentration of chl *a* was measured on a Turner Model 10 AU fluorometer using the acidification method (Strickland & Parsons, 1972).

Experiments

Grazing and nutrient addition incubation experiments were conducted five times over the course of the 2013 bloom cycle: on July 4, July 18, August 2, August 15, and October 10. These experiments allowed for the assessment of phytoplankton growth in response to treatments during the pre-, mid-, and post-bloom periods. Phosphate was chosen for nutrient addition because of previous research showing that elevated concentrations coincided with high cyanobacterial abundance (Lee et al., 2015a). The cyclopoid copepod *Acanthocyclops robustus* (Sars, 1863) was selected as the metazoan zooplankton grazer for addition due to the known omnivory of this and related cyclopoid copepods (Adrian & Frost, 1993; Brandl, 2005; Hopp & Maier, 2005; Rollwagen-Bollens et al., 2013), and because of their high abundance in the lake during the summer of 2013. Only adult non-gravid females were selected for use in experiments as a means of avoiding variation in grazing rates between sex and life-stage. Zooplankton were collected from the lake using a 73-μm mesh, 0.5-m diameter plankton net that was towed vertically from just above the lake bottom to the surface. Upon return to the laboratory at Washington State University Vancouver (7.6 miles from the lake), copepods were sorted into holding beakers containing filtered lake water, and held from 4 to 6 h to allow for acclimation and emptying of their guts (Dam & Peterson, 1988; Irigoien et al., 1998). Enough copepods were collected to ensure an amended concentration of 80 l^{-1} in incubation bottles. This density, while high, often occurs in Vancouver Lake (Rollwagen-Bollens et al., 2013; Lee et al., 2016), and was sufficient to ensure adequate consumption and reduction of prey (20–50%) for the influence of copepods to be observable, without eliminating rare prey species during each incubation (Rollwagen-Bollens & Penry, 2003; Gifford et al., 2007).

For each experiment, twenty 500-ml bottles were filled with unfiltered lake water, obtained from the lake surface using an acid-washed carboy. Lake water was not prefiltered, as the filtering process can damage fragile microplankton, which could disrupt composition of the ambient prey community in each incubation bottle. Thus, ambient grazing conditions were present in all treatment and control bottles, and the addition of copepods and/or nutrients to treatment bottles allowed us to measure the effects of these main factors and their interaction on phytoplankton growth. Prior to the incubation, four replicate initial control bottles containing only lake water and the natural assemblage of plankton were subsampled both for chl *a* concentration (as described above for field collections) and for microplankton (200-ml subsamples preserved in 5% Lugol's acid solution). One set of quadruplicate final control bottles and three sets of quadruplicate treatment bottles were prepared as follows: "Control" bottles contained only the natural assemblage of plankton in surface lake water; "Copepod" bottles

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contained the natural assemblage of plankton plus an addition of copepod grazers; "Nutrient" bottles contained the natural assemblage of plankton with an addition of phosphate (PO_4); and "Copepod plus nutrient" bottles contained the natural assemblage plus additions of both copepod grazers and phosphate. Control and treatment bottles were topped off with unfiltered lake water and sealed with Parafilm and a lid to prevent air spaces and turbulence-causing bubbles (Rollwagen-Bollens et al., 2013).

Nutrient amendments were made with NaH_2PO_4-H_2O for an increase of 100 μg l^{-1} phosphate in incubation bottles. This value was based on the highest average values of phosphate concentration recorded in Vancouver Lake between 2008 and 2009 (Lee et al., 2015a). Bottles were incubated at ambient lake temperatures for 12 h overnight on a slowly rotating (0.5–1 rpm) plankton wheel in the laboratory. Incubation temperatures were set to 23°C (July 4), 21°C (July 18 and August 2), 24°C (August 15), and 14°C (October 10) to match ambient lake conditions at the time of sampling. At the end of each incubation period, all final bottles were subsampled for chl *a* and microplankton, as described above for the initial controls.

Cell counts were performed using three replicates for three of the five experiments (July 18, August 15, and October 10) to capture community dynamics in the pre-, mid-, and post-bloom periods. Identification and enumeration of microplankton were determined by settling aliquots of 2–4 ml from Lugol's preserved samples for 12 h prior to microscopic examination with a Leica DMI 4000B inverted microscope at 400×. For each settled sample, we counted at least 300 organisms (>5 μm) from randomly selected fields along transects, as described by Kirchman (1993). Plankton cells were enumerated, sized, and identified to genus or species, where possible, using Wehr & Sheath (2002) and Patterson (1992). In order to keep units consistent for comparison among all phytoplankton taxa (e.g., algae and cyanobacteria), our counting unit was individual cells rather than colonies (APHA et al., 2012). We included cyanobacteria in our cell counts even though individual cyanobacterial cells were <5 μm in size, since the cells were arranged in filaments and colonies larger than 5 μm, and thus were potentially consumable by copepod grazers. Organisms were categorized into the following six taxonomic groups for comparison in experiments: diatoms, dinoflagellates, flagellates, ciliates, chlorophytes, and cyanobacteria.

Analysis of net growth rates

Net phytoplankton biomass growth rates (r, day^{-1}) for all five experiments were determined based on changes in chl *a* concentration between the initial samples and the final control and treatments, assuming exponential growth, using the following equation: $r = [\ln(C_f/C_i)/t]$, where C_f is concentration at the end of the incubation, C_i is concentration at the start of the incubation, and t is incubation period (0.5 day) (Frost, 1972). In addition, the same approach was used to calculate growth rates for particular microplankton taxonomic groups based on changes in cell abundance during each of the pre-, mid- and post-bloom experiments. A two-way ANOVA was used to compare the differences in growth rates based on chl *a* concentration or cell abundance within each experiment, testing for the effect of 'copepods' and 'nutrients' on the response variable 'net growth rate,' as well as the interaction of these two factors. Normality and homoscedasticity were verified with Shapiro–Wilk and Brown–Forsythe tests, respectively. In two instances the group being measured did not meet those assumptions, and a cube root transformation was used to meet statistical assumptions. Statistics were performed using Systat software, version 10.0, and $\alpha = 0.05$.

Results

Environmental conditions

Phytoplankton biomass, as measured by chl *a*, was moderately high in Vancouver Lake from June to late July, ranging from 78 to 180 μg chl *a* l^{-1} (Fig. 2). A phytoplankton bloom formed in late July, indicated by a peak in chl *a* of 927 μg l^{-1} early in August. After a brief decline, a second peak of 1000 μg chl *a* l^{-1} occurred in late August. Levels of chl *a* decreased to around 200 μg l^{-1} in early September, indicating bloom decline, then fell further and remained below 100 μg l^{-1} through late September and October. Microscopic enumeration of microplankton revealed community dominance by the filamentous cyanobacteria *Dolichospermum flos-aquae* (Bréb. ex Bornet &

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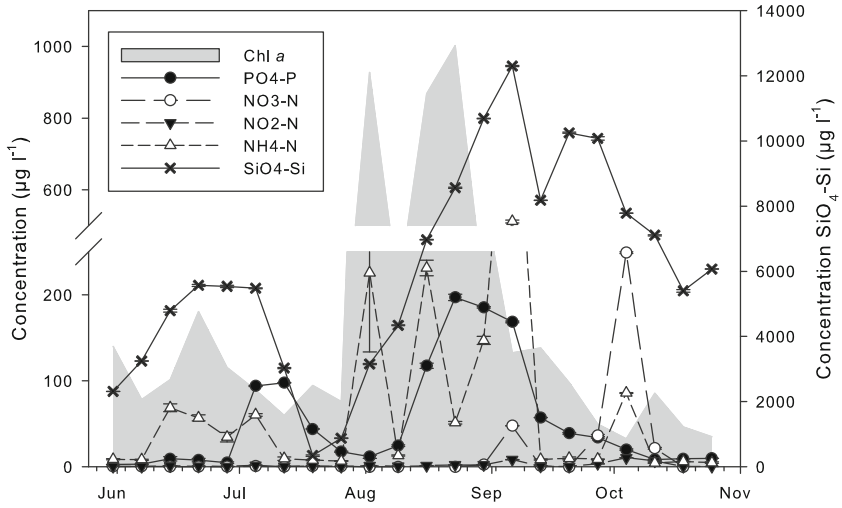

Fig. 2 Mean (±SE) concentrations of nutrients (PO_4-P, NH_4-N, NO_3-N, NO_2-N, SiO_4-Si) and chl *a* measured in Vancouver Lake during June–October, 2013

Flahault; Wacklin, L. Hoffm. & Komárek) in August, coniciding with high levels of chl *a*.

Dissolved inorganic phosphate (PO_4) reached 93.9 µg l^{-1} in early July, just prior to the first spike in chl *a,* and a high value of 197 µg l^{-1} that coincided with maximum chl *a* concentrations in late August (Fig. 2). Ammonium (NH_4) concentration increased in a pattern that lagged just behind, but closely mirrored peaks in chl *a*, reaching maxima of 225, 231, and 512 µg l^{-1} in late July, mid-August, and early September, respectively. Nitrate (NO_3) remained fairly low over the summer, but reached two peaks near the end of the bloom cycle, in early September (47.7 µg l^{-1}) and early October (249 µg l^{-1}). Silicate (SiO_4) decreased to a minimum of 345 µg l^{-1} in mid-July and then rose to a maximum of 12,300 µg l^{-1} in early September.

Surface water temperature in Vancouver Lake was high throughout the bloom cycle, ranging between 20 and 24°C (Fig. 3). DO concentrations decreased early in the summer, reaching a low value of 2.12 mg l^{-1} in mid-July before increasing, coincident with chl *a,* to a maximum of 7.95 mg l^{-1} in mid-August. Surface measures of dissolved oxygen then declined from mid-August to late September, and increased from late September through October (2.60–8.63 mg l^{-1}; Fig. 3). Water clarity, as measured using Secchi disk, declined from early June (0.66 m) to late August (0.15 m), coincident with the peak in chl *a* concentration (Fig. 3). Lake depth shoaled as the summer progressed (from 2.6 m in late May to 1.1 m in late September).

The six microplankton categories enumerated via microscopy fluctuated in abundance in experiments spanning the course of the bloom cycle (Fig. 4). On July 18 (pre-bloom), the phytoplankton community was dominated by diatoms, followed closely by cyanobacteria and chlorophytes. On August 15 (mid-bloom), cyanobacteria were dramatically more abundant and dominated the microplankton community. On October 10 (post-bloom), cyanobacteria abundance was lower compared to mid-bloom, though still predominant, with a greater proportional representation of chlorophytes, flagellates, and diatoms.

Grazing and nutrient addition experiments

Phytoplankton biomass growth rates calculated from the change in chl *a* concentration during each experiment were variable over the course of the bloom period (Fig. 5). Highest growth rates were observed in the nutrient treatment on July 4, before the bloom, and in the control and the nutrient treatment on August 2, at the height of the *Dolichospermum* bloom. Net growth rates were lowest, and in some cases negative, for all three treatments in the experiment on July 18, just prior to the bloom.

Two-way ANOVA tests performed on these growth rate data revealed that the addition of phosphate significantly increased phytoplankton growth early in the pre-bloom period on July 4 ($P = 0.001$; Table 1, Fig. 5). Later in the pre-bloom period, on July 18, the addition of copepods, and the addition of both copepods and phosphate, significantly reduced

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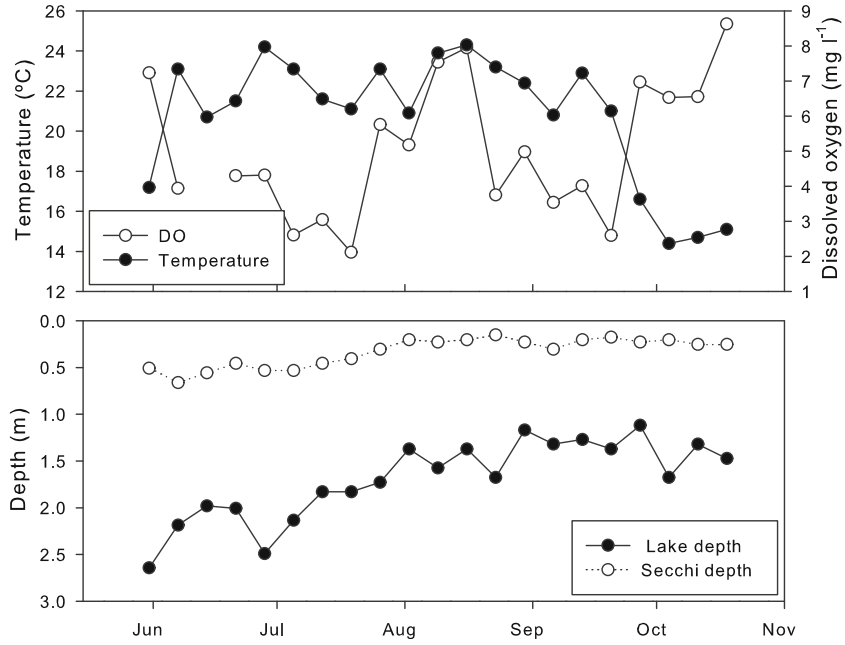

Fig. 3 Surface temperature (°C), dissolved oxygen (mg l^{-1}), lake depth (m), and Secchi disk depth (m) measured in Vancouver Lake during June–October, 2013

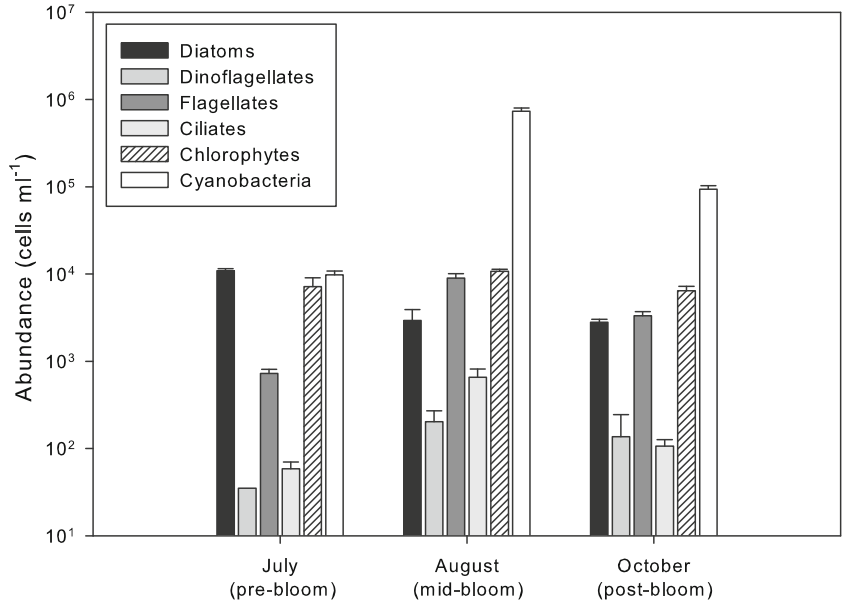

Fig. 4 Log abundance of microplankton (cells ml^{-1}) in the initial control bottles for experiments conducted on July 18, August 15, and October 10, 2013

phytoplankton growth rates ($P = 0.001$ and $P < 0.001$, respectively). Post hoc analysis (Tukey's HSD) showed that copepod grazing reduced chl *a* growth rates in both treatments; however, the reduction in growth rate was less pronounced in the treatment with added copepods and phosphate. On August 15 (mid-bloom), phosphate addition, and the combination of added copepods and phosphate, significantly increased phytoplankton growth rates ($P = 0.01$ and $P = 0.003$, respectively). This is opposite to the effects seen 4 weeks earlier, on July 18, just prior to the bloom. On August 15, added phosphate increased growth, but the effect was reduced when combined with copepod grazers. On October 10 (post-bloom), copepod grazing significantly reduced phytoplankton growth ($P < 0.001$).

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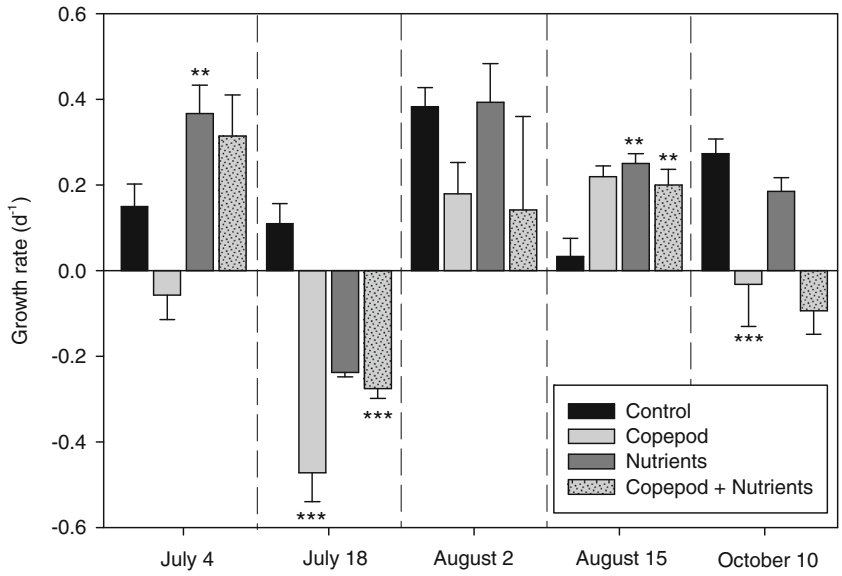

Fig. 5 Mean (±SE) net growth rate (day^{-1}) of phytoplankton biomass in the controls and all treatments from each experiment conducted over the 2013 bloom cycle (*asterisks* indicate significance of the treatments 'copepods,' 'nutrients,' and 'copepods + nutrients,' as analyzed by two-way ANOVA: $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***))

Table 1 Levels of significance (P values) from two-way ANOVAs on chl *a* data from five experiments conducted during the 2013 bloom cycle in Vancouver Lake

	July 4	July 18	Aug 2	Aug 15	Oct 10
Factor					
Copepod	0.089	**0.001**	0.143	0.058	**<0.001**
Nutrients	**0.001**	0.812	0.394	**0.010**	0.242
Copepod + nutrients	0.294	**<0.001**	0.561	**0.003**	0.834

Bold text indicates significant values ($P < 0.05$)

Net growth rates of specific microplankton groups also varied considerably over the course of our experiments (Fig. 6). Cyanobacterial growth rates were generally high in all three treatments of the July 18 (pre-bloom) experiment, reaching a maximum of 1.5 day^{-1} in the treatment with added phosphate. Ciliates also exhibited their highest growth rates in all treatments of the pre-bloom experiment on July 18, whereas ciliate growth rates were strongly negative in the October 10 (post-bloom) experiment. Growth of dinoflagellates was positive pre- and post-bloom, but strongly negative in all but the phosphate treatment of the August 15 (mid-bloom) experiment. Diatoms exhibited positive growth rates pre- and post-bloom, but negative growth mid-bloom. Growth rates of flagellates were positive pre- and post-bloom, but lowest mid-bloom. Chlorophyte growth showed a similar trend to flagellates: positive growth both pre- and post-bloom, but low or negative growth during mid-bloom.

Two-way ANOVAs of net growth rates for each microplankton group revealed that on July 18 (pre-bloom), the addition of phosphate significantly increased net growth of ciliates ($P = 0.035$); the addition of copepods also appeared to increase ciliate growth rate, but while close ($P = 0.052$) did not quite meet our criterion for statistical significance. In the treatment with both copepods and phosphate added, ciliate growth rates were significantly higher compared to controls ($P = 0.034$), indicating an interactive effect of both factors (Fig. 6; Table 2). Also, on July 18, chlorophyte net growth was significantly increased by the addition of copepods ($P = 0.032$), and the combination of added copepods and phosphate ($P = 0.008$; Fig. 6, Table 2). During the August 15 (mid-bloom) experiment, net growth of dinoflagellates

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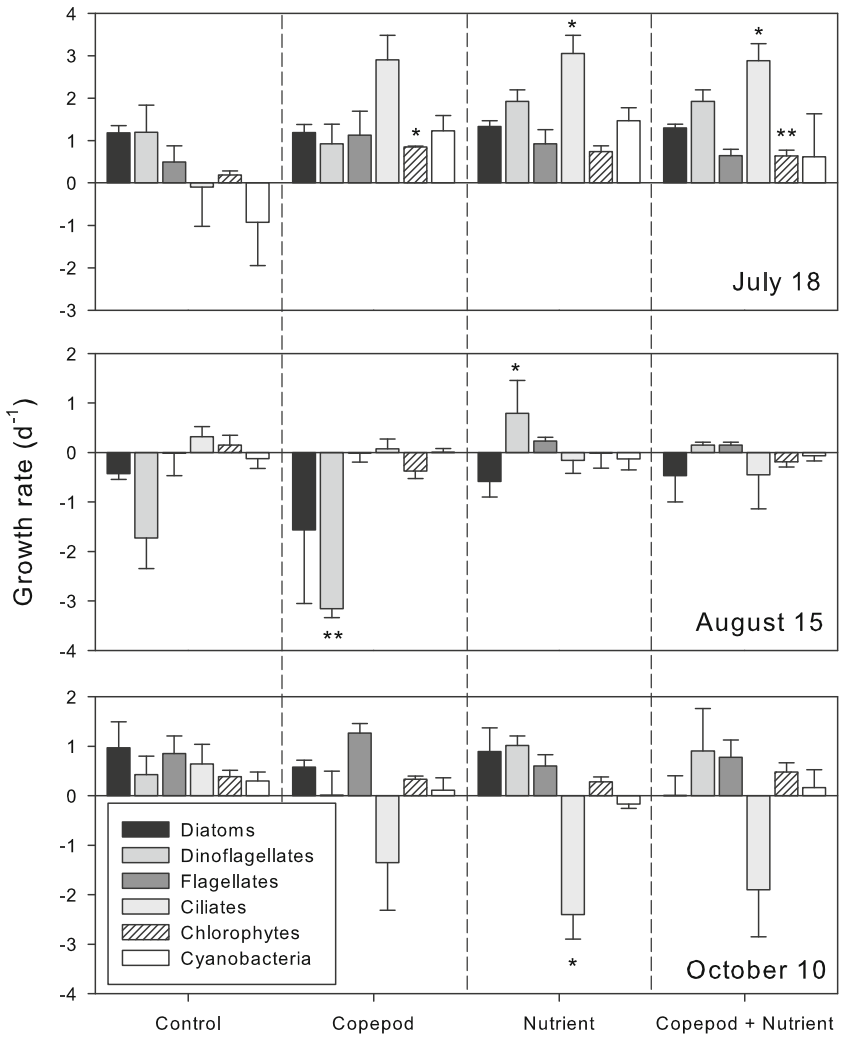

Fig. 6 Mean (±SE) net growth rate (day^{-1}) for microplankton groups enumerated in the controls and all treatments of experiments conducted on July 18, August 15, and October 10 (*asterisks* indicate significance of the treatments 'copepods,' 'nutrients,' and 'copepods + nutrients,' as analyzed by two-way ANOVA: $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***))

was significantly decreased by copepod grazing ($P = 0.003$), and increased by phosphate addition ($P = 0.010$). In the October 10 (post-bloom) experiment, net growth of ciliates was significantly decreased in the phosphate treatment ($P = 0.043$; Table 2; Fig. 6).

Discussion

Cyanobacterial bloom formation and decline in Vancouver Lake

A significant bloom of *Dolichospermum flos-aquae* cyanobacteria formed in Vancouver Lake in July and persisted into early September of 2013. In August 2013, the City of Vancouver issued an advisory cautioning the public against water contact due to high abundance of cyanobacteria in the lake. The bloom was associated with increases in phosphate, ammonium, DO, and temperature, as well as decreases in water clarity and lake depth. A similar pattern of rapid bloom initiation occurred during the summers of 2007, 2008, and 2009, associated with decreased water depth and clarity, as well as elevated water temperatures (Boyer et al., 2011; Rollwagen-Bollens et al., 2013; Lee et al., 2015a, b).

Nutrient concentrations in Vancouver Lake throughout the summer of 2013 resembled fluctuations seen previously. In 2013, a large increase in silicate was associated with a change in the phytoplankton community from dominance by diatoms in spring and

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Table 2 *P* values from two-way ANOVAs of all microplankton growth rates from pre-bloom (July 18), mid-bloom (August 15), and post-bloom (October 10) experiments conducted during the 2013 bloom cycle in Vancouver Lake

Microplankton	Copepod	Nutrients	Copepod + nutrients
July 18			
Diatoms	0.926	0.417	0.889
Dinoflagellates	0.766	0.084	0.766
Ciliates	0.052	**0.035**	**0.034**
Flagellates	0.651	0.943	0.269
Chlorophytes	**0.032**	0.158	**0.008**
Cyanobacteria	0.412	0.273	0.081
August 15			
Diatoms	0.612	0.867	0.824
Dinoflagellates	**0.003**	**0.010**	0.160
Ciliates	0.824	0.573	0.501
Flagellates	0.873	0.440	0.857
Chlorophytes	0.123	0.947	0.425
Cyanobacteria	0.574	0.815	0.849
October 10			
Diatoms	0.162	0.458	0.565
Dinoflagellates	0.639	0.205	0.788
Ciliates	0.347	**0.043**	0.134
Flagellates	0.341	0.233	0.693
Chlorophytes	0.585	0.888	0.365
Cyanobacteria	0.772	0.410	0.311

Bold text indicates significant values ($P < 0.05$)

early summer to dominance by cyanobacteria in late summer, while high levels of phosphate and ammonium were associated with the summer CyanoHAB (Lee et al., 2015a, b). This aligns with our results in which phosphate concentration peaked in early July, just prior to rapid cyanobacterial bloom formation, while ammonium increased just after the peaks of chl *a*, and nitrate remained at very low concentrations throughout the summer. This suggests that phosphate availability may have played a role in the initiation of the *Dolichospermum* sp. bloom of 2013, as it has in previous summers. However, it is also worth noting that the role of N in the cyanobacterial bloom should not be overlooked and may warrant future study, particularly as *Dolichospermum* is a N_2-fixer able to thrive during periods of N limitation and because levels of N:P, despite being frequently elevated, may at times have dropped below the Redfield ratio during the 2013 bloom cycle (Fig. 2).

The cyanobacterial bloom declined rapidly in September in a pattern that again closely mirrored that of 2007–2009 (Lee et al., 2015a). Ammonium was elevated during and immediately following sharp reductions in chl *a*, corresponding with periods of low DO concentration. Similarly, water temperatures and phosphate decreased as the bloom declined, while DO concentrations, although low early in September, steadily increased in the latter half of the month.

Experimental effects of added phosphate and copepods on growth rates

Our experiments over the course of the 2013 bloom cycle provide evidence of both bottom-up and top-down control of the phytoplankton community. Phosphate addition significantly increased phytoplankton biomass (chl *a*) growth rates early in the pre-bloom period (July 4), and copepod grazing significantly reduced phytoplankton biomass growth rate in the post-bloom period (October 10). This suggests a direct relationship between both factors (phosphate and copepods) on phytoplankton community biomass, albeit at different stages of the cyanobacterial bloom cycle. Interestingly, the interactive effects of phosphate and copepod addition were also significant late in the pre-bloom season (July 18) and at the height of the bloom (August 15), but not in the post-bloom period.

On July 18, just prior to the cyanobacterial bloom, the addition of copepods significantly reduced phytoplankton biomass growth rate, and the addition of phosphate had no significant effect. However, when copepods and phosphate were added together, phytoplankton biomass growth rates were again significantly reduced relative to the controls, but to a lesser extent compared to the addition of copepods alone, suggesting that the addition of phosphate may have stimulated growth of some phytoplankton groups and offset the grazing effect of the copepods. In contrast, on August 15, at the height of the bloom, the addition of phosphate resulted in a significant increase in phytoplankton biomass growth relative to the controls, but the addition of copepods had no significant effect. When both copepods and phosphate were added together in August, phytoplankton growth rates significantly increased, but to a slightly lower degree compared to when phosphate alone was added, suggesting a slight dampening effect of copepod grazers on growth rates in the presence of added nutrients.

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These experimental results indicate that phosphate appears to be the dominant factor increasing growth of phytoplankton biomass pre-bloom, and copepod grazing reduces growth post-bloom. However, the interactive effect of added nutrients and grazers creates conditions that reduce the growth of phytoplankton biomass prior to the bloom, but have the opposite effect at the bloom's height. Examination of the effect of copepod grazing and phosphate addition on taxon-specific growth rates provides additional insight into these patterns.

Net growth rates of particular microplankton taxonomic groups, calculated using changes in cell abundance during three experiments conducted pre-, during, and post-bloom, revealed interesting patterns of community dynamics over the cyanobacterial bloom cycle. Pre-bloom (July 18) results indicate that added phosphate and the interaction of added copepods and phosphate significantly increased ciliate net growth relative to unamended controls (and the addition of copepods alone also appeared to increase ciliate growth rate, but not quite to a significant degree [$P = 0.052$]). Somewhat paradoxically, this indicates that ciliates may have benefited from copepod and phosphate addition. This could have been the result of two (or more) possibilities: first, the addition of copepods could have increased nutrient cycling within incubation bottles which indirectly benefited ciliates by fertilizing some consumable phytoplankton taxa; and/or, second, the added copepod predators could have selectively consumed some competitor or predator of ciliates, allowing ciliates to become competitively dominant through classical "trophic cascade" effects (Carpenter et al., 1985, 1987, 1996). Indeed, during this pre-bloom experiment, chlorophyte growth was also significantly increased by the presence of copepods and the interaction of copepods and phosphate. These results suggest that higher concentrations of copepods may actually promote growth of select microplankton taxa under certain conditions.

During the cyanobacterial bloom (August 15), the addition of copepods caused substantial reduction in dinoflagellate growth rates, while the addition of phosphate led to enhanced dinoflagellate growth. Dinoflagellate growth rates were also negative in the controls, suggesting reductions at ambient grazing levels, but were markedly more negative in the copepod treatments. This clearly suggests that copepods were strongly predating dinoflagellates at the height of the bloom. This finding aligns well with the established literature, as copepods have been known to consume dinoflagellates and other microzooplankton taxa (e.g., Stibor et al., 2004; Sommer & Sommer, 2006; Rollwagen-Bollens et al., 2013). Notably, growth rates for all microplankton groups were fairly low during this mid-bloom (August) period, when cyanobacteria, primarily *D. flos-aquae*, were extremely abundant and overwhelmingly dominant.

In the experiment conducted after the bloom subsided (October 10), ciliate growth was negative and significantly lower compared to the controls when phosphate was added—an effect opposite to that seen pre-bloom. While it is reasonable to expect ciliates to decline when copepod grazers are added (as seen in our copepod and copepod plus nutrient treatments), it is less clear why they would experience such decline when only phosphate was added.

We also note that the microscopical analyses, to quantify changes in abundance of particular taxonomic groups during the incubation experiments, were limited to cells >5 μm in size, since the focus of our experiments was the grazing effect of mesozooplankton (i.e., copepods) which do not effectively consume cells smaller than this threshold (Rollwagen-Bollens & Penry, 2003 and references therein). However, many microplankton consumers (i.e., ciliates and heterotrophic dinoflagellates) are capable of capturing and ingesting phytoplankton cells as small as 2–3 μm in diameter. Thus, our measurements of taxon-specific population growth rates based on cell counts did not include this smallest size fraction (2–5 μm), and therefore did not capture the changes in small phytoplankton that were included in the total phytoplankton biomass growth rates based on changes in chl *a* concentration. This could explain why total phytoplankton biomass was reduced in every treatment on July 18, while ciliate growth concurrently increased. Similarly, grazing of copepods on heterotrophic dinoflagellates in the copepod treatment bottles may also explain some of the increase in chl *a* biomass observed mid-bloom (August 15).

These results lend support to an effect of trophic cascades in phytoplankton community dynamics in Vancouver Lake during the 2013 cyanobacterial bloom cycle. Ciliates and dinoflagellates are known to be considerable grazers of small phytoplankton (Sherr & Sherr, 1994; Tjildens et al., 2008), including cyanobacteria (Dryden & Wright, 1987; Canter et al.,

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1990; Sigee et al., 1999; Jeong et al., 2005). In fact, in Vancouver Lake, these microzooplankton have been shown to consume cyanobacteria in late summer and early autumn (Boyer et al., 2011). Therefore, by selectively consuming dinoflagellates rather than other algal species, copepods likely contributed to maintaining the cyanobacterial bloom, through cascading effects that reduced top-down control of cyanobacteria by microzooplankton during the mid-bloom (August 15) period.

Top-down trophic cascades have been documented in several other studies in which phytoplankton abundance was indirectly linked to feeding selectivity of mesozooplankton. Calbet & Landry (1999) found that grazing by mesozooplankton enhanced growth rates of phytoplankton and heterotrophic bacteria by decreasing abundances of microzooplankton and nanoheterotrophs. Schnetzer & Caron (2005) found that copepods added to incubations of natural phytoplankton assemblages preferentially consumed ciliates, and indirectly caused an increase in biomass of nanoplankton. Further examples exist from both marine and freshwater systems that support the ability of copepods to create trophic cascades by feeding on microzooplankton (Fessenden & Cowles, 1994; Nejstgaard et al., 2001; Rollwagen-Bollens & Penry, 2003; Stibor et al., 2004; Leising et al., 2005; Sommer & Sommer, 2006; Gifford et al., 2007; Rollwagen-Bollens et al., 2013).

Some of the variation in growth responses may also be explained by the ambient level of grazing by micro- and mesozooplankton in the controls and all treatments. As unfiltered lake water was used for all incubations, we anticipated that the ambient community would be similar in the controls and treatments of each experiment. However, because the zooplankton community was not assessed in each incubation bottle, it cannot be ruled out that variation in predator abundance may have contributed to some of the observed growth responses.

Bottom-up effects of nutrient enrichment, including phosphate, are widely known to promote phytoplankton growth (Chu, 1946; Wetzel, 1992; Reynolds, 2006) and cyanobacterial blooms (Anderson et al., 2002; Conley et al., 2009). Knowing this, phytoplankton response to our experimental phosphate additions was not always as clear as we had anticipated. It is possible that we would have observed higher phytoplankton growth rates with longer incubation periods, and/or with inclusion of a light period during the incubation. However, while some studies report that nutrient uptake kinetics are greatest during the daylight, in parallel with cellular growth and biomass increase (Azad & Borchardt, 1970; Rivkin & Swift, 1982), other studies reveal that uptake continues to occur in dark periods, but at lower rates compared to the light period (Perry & Eppley, 1981; Nakamura & Watanabe, 1983). In addition, rates of phosphorus uptake and subsequent biomass increase vary by species (Litchman et al., 2004; Reynolds, 2006), and may occur rapidly when nutrient concentrations are high (Grobbelaar, 2004; Reynolds, 2006). Although longer incubations that included a light period may have resulted in greater utilization of phosphate for growth and biomass increase by phytoplankton, we nevertheless saw some effect (although perhaps muted) from nighttime fertilization of phosphate on a diverse phytoplankton community. More specifically, our results imply that phosphate increased overall phytoplankton growth pre-bloom, with less of an effect mid- and post-bloom, when cyanobacterial abundance was already high.

Our results lead to several conclusions in support of our hypotheses about the influence of phosphate and copepods on cyanobacterial bloom dynamics in Vancouver Lake, and which likely apply more broadly to other shallow eutrophic lakes in temperate regions. Phosphate addition had a stronger effect on the phytoplankton community in the pre-bloom period (as seen in the results of our July 4 experiment). Copepod grazing was able to limit phytoplankton growth after the bloom (October), but likely indirectly affected cyanobacteria abundances via trophic cascade effects on mixotrophic/heterotrophic protists (e.g., dinoflagellates and ciliates) during both pre- and mid-bloom periods (late July and late August, respectively). While nutrients (phosphate) and grazing (copepods) have traditionally been thought to have opposing effects on phytoplankton bloom dynamics, it appears that as a result of indirect trophic cascade events, they may at times be acting simultaneously to maintain and even extend cyanobacterial blooms. From these results, we may also conclude that the individual and interactive effects of phosphate and copepods on phytoplankton growth vary considerably with time (season).

Understanding the roles of multiple causal factors on CyanoHAB dynamics, including the individual and

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interactive effects of nutrient availability and zooplankton grazing, will greatly assist resource managers as they seek to mitigate these increasingly common and problematic events (e.g., via reductions in nutrient input, aeration, sediment dredging, or biomanipulation). As our experimental results indicate, a combination of abiotic environmental conditions and trophic interactions promote and sustain cyanobacterial blooms in Vancouver Lake, with the effect of nutrient availability (phosphate) dominant during pre-bloom periods, interactive effects of copepods and phosphate dominant just prior to and during the bloom, and the effect of grazing (copepods) the dominant factor influencing phytoplankton growth post-bloom.

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