FINAL REPORT:

Interactive effects of nutrients and grazing on the control of cyanobacteria blooms: a comparison across a eutrophication gradient in freshwater systems in Washington state

2014WA381B

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Problem and Research Objectives

Seasonal blooms of cyanobacteria and other algae are natural occurrences in lakes of varying morphology and location, and may naturally increase in frequency as lakes evolve from clean and unproductive states to more shallow and eutrophic conditions (Hutchinson 1973). However, increasing evidence demonstrates that the eutrophication process in lakes is being accelerated by human activity, through sewage and fertilizer inputs, deforestation, road construction, real estate development and other disturbances in lake watersheds, and is contributing to an increase in frequency and intensity of cyanobacteria blooms (e.g. Sellner et al. 2003, Paerl 2008). Moreover, several recent studies suggest that increased eutrophication under conditions of a warming climate may result in increased dominance of cyanobacteria in aquatic systems (Kosten et al. 2012, O'Neill et al. 2012) and may actually favor harmful cyanobacterial taxa over non-toxic forms (Paerl & Huisman 2009).

Excessive abundance of cyanobacteria may have detrimental effects on lake ecosystems and water quality, including development of surface scums and depleted oxygen levels (Sellner et al. 2003). In addition, many cyanobacteria species can produce potent hepatotoxins that can negatively affect aquatic life and in particular cause harm or even death to humans and other mammals (Chorus et al. 2000, Codd et al. 2005). This phenomenon is of great concern to water resource managers in Washington State, since cyanobacteria blooms have become an increasing problem in the Pacific Northwest (Jacoby & Kann 2007), and pose particular challenges to human health. For example, the Washington Department of Ecology Freshwater Algae Bloom Monitoring Program Harmful reported that, between 2007 and 2013, 81 Washington lakes had at least one instance in which cyanotoxin levels exceeded maximum thresholds considered safe for human contact (www.nwtoxicalgae.org). Thus cyanobacteria blooms are of concern to the public, as well, since their use and enjoyment of valued aquatic environments may be prohibited as a result.

Since 2007 we have been monitoring water quality and plankton abundance and diversity in Vancouver Lake, located in Clark County, WA, in the floodplain of the lower Columbia River. Vancouver Lake has experienced numerous summertime blooms of *Anabaena* and *Aphanizomenon* cyanobacteria over the past 20 years, however the blooms have been variable in intensity from year to year (Lee et al. in press). In addition to monitoring the plankton in Vancouver Lake, from 2007-2010 we also investigated the biotic and abiotic factors that influence the late summer cyanobacteria blooms. Our research has revealed that both small (<20 µm), single-celled planktonic grazers ("microzooplankton") and large (1-5 mm), crustacean grazers ("mesozooplankton" such as copepods and cladocerans) have measurable impact on the timing of bloom onset and decline in Vancouver Lake, but do not prevent the blooms from occurring. Specifically, mesozooplankton feeding on microzooplankton in the weeks just prior to a cyanobacteria bloom event sets up a "trophic cascade" in which those microzooplankton are prevented from grazing cyanobacteria, allowing cyanobacteria to increase rapidly in abundance (Rollwagen-Bollens et al. 2013). After the cyanobacteria bloom reaches its peak, mesozooplankton grazers appear to be inhibited, and grazing by microzooplankton increases to maximal levels, contributing to a rapid decline in cyanobacteria abundance (Boyer et al. 2011).

Also, in a multivariate statistical analysis conducted on weekly measurements of water quality (e.g. temperature, turbidity, pH, dissolved oxygen), inorganic nutrient concentration (e.g. nitrate, nitrite, ammonia, orthophosphate), and the abundance and taxonomic composition of algae and cyanobacteria from 2007-2010 in Vancouver Lake, we found that the environmental factors most strongly associated with cyanobacteria blooms were orthophosphate and ammonium concentrations. However, nutrient availability could only explain ~35-50% of the variance in algal abundance (Lee et al. in press).

Our results in Vancouver Lake, coupled with observations in other temperate lakes, strongly suggest that both nutrient availability and zooplankton grazing influence cyanobacteria bloom dynamics, in a combination of “bottom up” and “top down” forces; but the interactive effects of these forcings have not been examined in Pacific Northwest lakes or reservoirs. Moreover, it is completely unknown how the relative impacts of enhanced nutrient concentrations and zooplankton grazing may differ in lakes and reservoirs of variable trophic status (eutrophic vs. oligotrophic).

In this project our overall goals were to better understand the interactive effects of nutrient availability and zooplankton grazing on cyanobacteria bloom dynamics in Washington freshwater lakes and reservoirs across a gradient of eutrophication, and to share our results and interpretations with resource managers to better inform decision-making on steps to prevent and/or mitigate freshwater cyanobacteria blooms in the state.

We focused our research on four lakes/reservoirs in Washington state that represent a range of trophic state, from highly to moderately eutrophic (Vancouver Lake and Lacamas Lake) to more oligotrophic (Lake Merwin and Cle Elum Lake) (Figure 1).

Figure 1. Locations of four lakes/reservoirs in Washington state that were sampled three times over the course of the summer algal bloom period in 2014.

Our objectives for achieving the project goals were as follows:

Objective 1: Conduct cyanobacterial/algal growth experiments three times over the bloom cycle in each of four lakes/reservoirs along a gradient of eutrophication, using natural unfiltered water collected from each lake in a 2x2 factorial design with a control and three treatments (Fig. 2): 1) lakewater containing the natural assemblage of algae/cyanobacteria (control), 2) lakewater with added nutrients (orthophosphate), 3) lakewater with added zooplankton grazers (copepods), 4) lakewater with added nutrients and zooplankton grazers.

Objective 2: Measure a suite of water quality variables (e.g., nutrients, temperature, dissolved oxygen, etc.) in each lake/reservoir.

Objective 3: Conduct multivariate statistical analyses of field data to assess the relative importance of bottom-up (nutrient availability) and top-down (grazing) factors in influencing cyanobacteria abundance and composition.

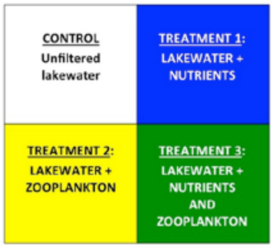
Objective 4: Combine the experimental results (objective 1) with the field results (objectives 2 and 3) to develop predictions of how increased eutrophication will likely impact lakes that currently experience cyanobacteria blooms and those that as yet do not suffer from these blooms.

Methodology

Field and experimental program. Field sampling and experiments were conducted in each of four lakes/reservoirs from May to October 2014. Each lake/reservoir was sampled for water quality variables and plankton once per month and algal/cyanobacterial growth rate experiments were conducted three times over the sampling period: in June, prior to any summer bloom, in August during the bloom peak, and in October following any bloom's decline. All analyses were conducted at WSU Vancouver.

Approach 1: Field sampling for water quality and plankton. At each sampling time, temperature and dissolved oxygen profiles was obtained using a YSI 85 probe, and relative water clarity estimated by measuring the Secchi depth. In addition, water samples were collected from the surface using a clean, acid-washed bucket, and subsamples taken for later laboratory analyses of chlorophyll *a* concentration (Strickland & Parsons, 1972). In addition, bucket samples were collected from the surface, and subsamples preserved in 5% acid Lugol's solution, for enumeration and identification of cyanobacteria and all protist (i.e. unicellular eukaryotic) plankton. Finally, triplicate vertical tows were conducted with a 0.5-m diameter, 73-µm mesh zooplankton net, and the contents concentrated and preserved in 5-10% buffered formalin. Abundance and composition of plankton were assessed via light microscopy following the approach outlined in Rollwagen-Bollens et al. (2013).

Approach 2: Lakewater incubations to measure cyanobacterial/algal growth rates under variable conditions of nutrient availability and zooplankton grazing pressure. Growth experiments were conducted following a 2 x 2 factorial design with a control and three treatments: lakewater plus added nutrients, lakewater plus added zooplankton grazers, and lakewater plus nutrients and grazers (Figure 2).



CONTROL Unfiltered lakewater	TREATMENT 1: LAKEWATER + NUTRIENTS
TREATMENT 2: LAKEWATER + ZOOPLANKTON	TREATMENT 3: LAKEWATER + NUTRIENTS AND ZOOPLANKTON

Figure 2. Diagram illustrating the 2 x 2 factorial design for the cyanobacteria/algal growth experiments.

Experimental protocols were slightly modified from the methods described in Rollwagen-Bollens et al. (2013), to include multiple treatments. Each treatment consisted of four replicate 500-ml polycarbonate incubation containers filled with unfiltered lakewater containing the natural assemblage of plankton obtained from the surface using a clean, acid-washed bucket. Four bottles containing only the natural assemblage were established as Initial Controls, and immediately preserved and subsampled. Sixteen additional bottles were filled with unfiltered lakewater and then supplemented as follows: four bottles were sealed to serve as Final Controls, four bottles received amendments of inorganic nutrients (orthophosphate and nitrate) as

Treatment 1, four bottles received additions of adult zooplankton representing five times the ambient abundance of the dominant taxon present as Treatment 2, and the remaining four bottles received both added nutrients and zooplankton as Treatment 3. Zooplankton were collected via vertical hauls of a 73-µm plankton net and adults of target species sorted under dim light into holding beakers before being added to incubation bottles. All final control and treatment bottles were incubated in a temperature-controlled chamber for 24 hours on a slowly rotating (0.5-1 rpm) plankton wheel under natural light-dark conditions. All bottles were subsampled and analyzed to enumerate and identify the algae and cyanobacteria as described above (Approach 1). Cyanobacteria and algal growth rates were estimated according to Frost (1972).

Approach 3. Statistical analyses to assess interactive effects of nutrients and zooplankton on cyanobacteria abundance and blooms. We are employing an ordination technique called non-metric multidimensional scaling (NMDS) to identify the relationships between nutrient concentrations, zooplankton abundance, and other environmental variables (e.g. temperature, turbidity) and cyanobacteria community composition and abundance. Ordination techniques are very useful in community ecology because they can detect numerous relationships between species, or assemblages of species, and environmental data. NMS is an effective ordination technique for these data since it can be used with non-normal and discontinuous distributions and does not assume linear or modal relationships (McCune & Grace 2002).

Principal Findings

Each lake (Vancouver, Lacamas, Merwin, Cle Elum) was sampled for water quality variables once per month from May to October, 2014. Experiments were conducted in each lake during June (pre-bloom), August (bloom), and October (post-bloom).

The lakes varied in a range of physical and chemical factors over the 2014 sampling period (Fig. 3). Vancouver Lake, located within the city limits of Vancouver, WA, was the shallowest

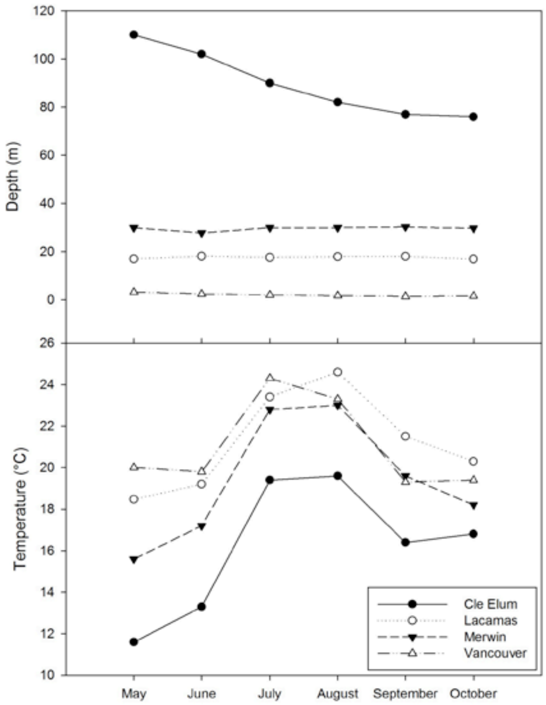


Figure 3. Variations in lake depth (upper panel) and surface temperature (lower panel) from May to October 2014, in four Washington lakes.

lake sampled, averaging ~2 m over the summer, with variations associated with changes in the tidal phase and water levels in the Columbia River. Lacamas Lake, located ~10 miles east of Vancouver Lake, is a reservoir of Lacamas Creek, where lake levels were maintained at ~17 m depth throughout the summer. Merwin and Cle Elum lakes are also reservoirs, along the East Fork Lewis River and Yakima River, respectively. Water depths in Lake Merwin averaged ~29 m and remained steady over the sampling period, however water depth in Cle Elum Lake decreased consistently over the summer from a high of 110 m in May to a low of 76 m in October, 2014 (Fig. 3 upper).

Surface temperatures varied with change in season in all four lakes in a similar pattern: the lowest temperatures were observed during May and June, ranging from 12-15 °C in the deeper lakes (Merwin and Cle Elum) and 18-20 °C in the shallower lakes (Vancouver and Lacamas); the highest temperatures were observed in July and August, where three lakes (Vancouver, Lacamas and Merwin) recorded temperatures between 23-25 °C and Cle Elum reached 19.6 °C; and temperatures in all four lakes fell to moderate levels in October, between 16-20 °C (Fig 3 lower).

Algal/cyanobacterial biomass, as measured by chlorophyll (chl) *a* concentration, was lowest in the two large river reservoirs, Lake Merwin and Cle Elum Lake, from May to October 2014 (Fig 4 upper). Chl *a* concentration did not vary measurably in these two reservoirs over the sampling period (0.1-3 µg/L). Whereas chl a concentrations varied significantly in Vancouver Lake, from lows of 20-25 µg/L in May and October, to peak levels of 150 µg/L observed in August 2014. Chl *a* concentrations were moderately high and variable (range 15-41 µg/L) in Lacamas Lake from May to October (Fig 4 upper).

Changes in water clarity (as measured by Secchi depth) were substantial in Lake Merwin and Cle Elum Lake over the 2014 sampling period, ranging from a low of 4-6 m in May to a maximum of 11 m in August in Lake Merwin. Both lakes showed similar water clarity of ~8 m in October. In contrast, the shallow lakes (Vancouver and Lacamas) had lower water clarity that did not vary much from May to October (Fig 4 lower).

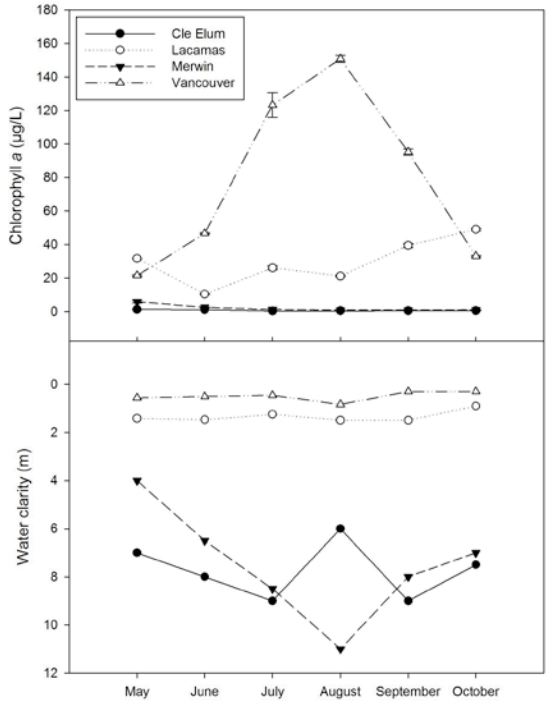


Figure 4. Variations in surface chlorophyll *a* concentration (upper panel) and water clarity (lower panel) from May to October 2014, in four Washington lakes.

A total of 12 growth rate experiments were conducted over the 2014 sampling period, 3 experiments per lake. Preliminary analyses of changes in chl *a* concentration in the experimental bottles indicate that the effects of nutrients and zooplankton grazers on algal/cyanobacterial growth rates differed between the four lakes and over the summer growth season.

In June 2014, preliminary results from the growth experiments suggested that nutrients (phosphate) were limiting to algae/cyanobacteria in Lacamas Lake and Lake Merwin, since addition of phosphate resulted in significantly higher growth rates relative to controls. And while the addition of 5x the ambient abundance of the dominant zooplankton species did not result in reduced algal growth when added to lakewater, this zooplankton addition did prevent algal growth from exceeding the controls when nutrients were also amended ("Both" treatment). A similar pattern, although quite muted, was observed in Vancouver Lake, while in Cle Elum the addition of both phosphate and zooplankton resulted in substantially lower growth rates, suggesting strong grazer influence (Fig. 5).

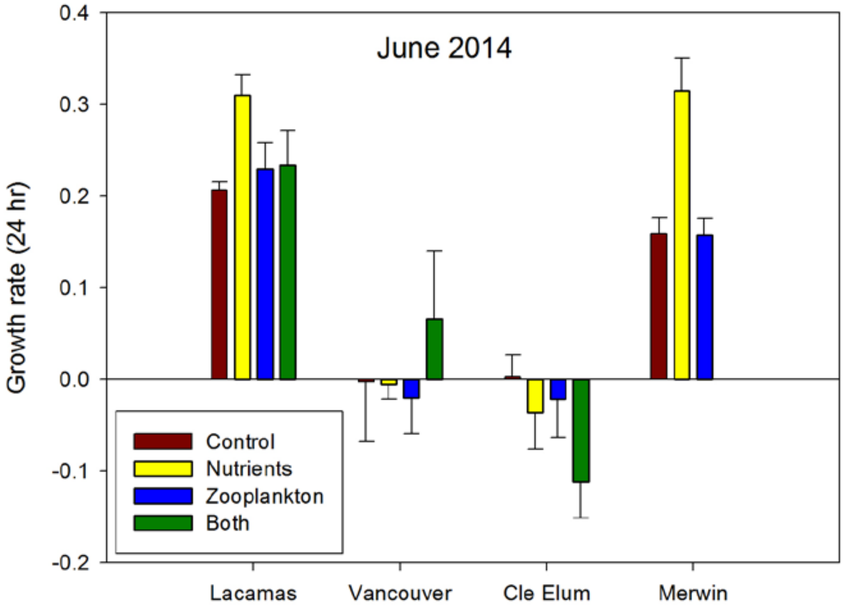


Figure 5. Growth rates (day^{-1}) of algae/ cyanobacteria in experiments conducted during June 2014 in four Washington lakes. Control = lakewater alone; Nutrients = amended with phosphate; Zooplankton = amended with 5x ambient levels of dominant grazer; Both = amended with phosphate and 5x ambient zooplankton.

During August, the addition of zooplankton grazers resulted in highly significant reductions in algal/cyanobacterial growth rates in the two deep, oligotrohic lakes (Merwin and Cle Elum), while the addition of phosphate did not result in significant changes in growth rate. However, in the two eutrophic lakes (Vancouver and Lacamas) the addition of phosphate appeared to enhance algal growth (Fig. 6). This was particularly true in Vancouver Lake, which was experiencing a substantial phytoplankton bloom at the time, and suggests that phosphate had become limiting. Moreover, algal/cyanobacterial growth rates were quite low in Vancouver Lake in August, lending further weight to the conclusion that the bloom was waning.

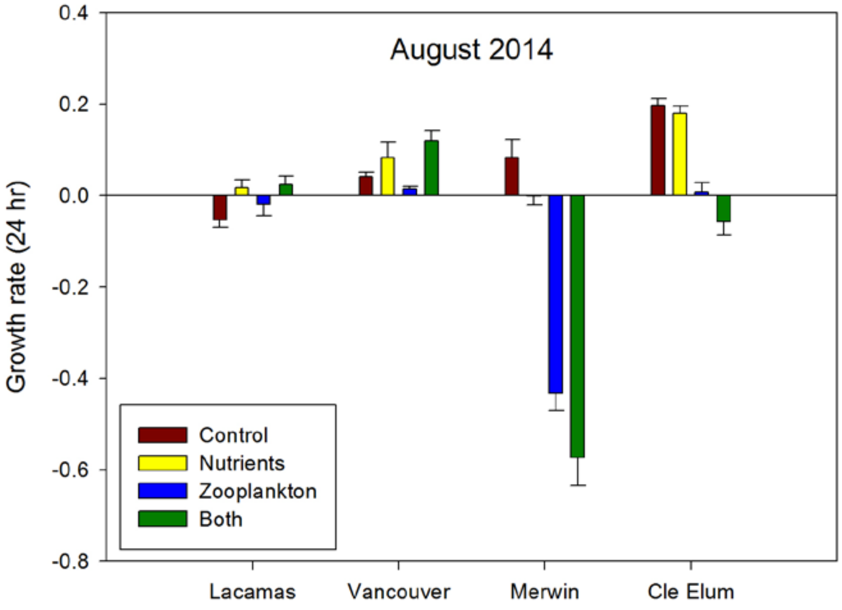


Figure 6. Growth rates (day^{-1}) of algae/ cyanobacteria in experiments conducted during August 2014 in four Washington lakes. Control = lakewater alone; Nutrients = amended with phosphate; Zooplankton = amended with 5x ambient levels of dominant grazer; Both = amended with phosphate and 5x ambient zooplankton.

Finally, during October the individual and interactive effects of added nutrients and zooplankton grazers did not appear to influence the growth rates of algae/cyanobacteria in Lacamas Lake nor Cle Elum Lake, as there were no significant differences in growth rates among the four treatments in the experiments conducted in these lakes. However, the addition of nutrients resulted in a significant increase in growth rate in Vancouver Lake, while the addition of zooplankton grazers resulted in a significant reduction in growth rate in Lake Merwin (Fig. 7).

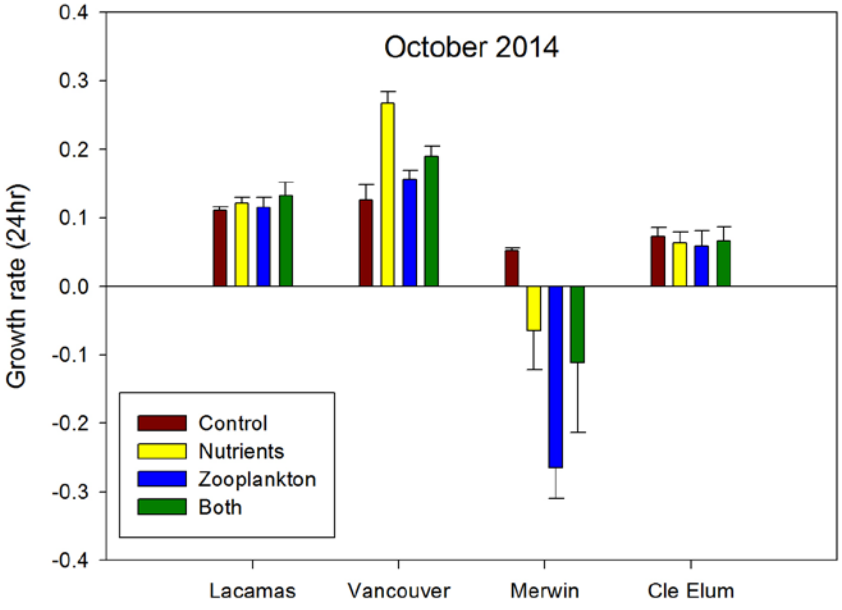


Figure 7. Growth rates (day^{-1}) of algae/ cyanobacteria in experiments conducted during October 2014 in four Washington lakes. Control = lakewater alone; Nutrients = amended with phosphate; Zooplankton = amended with 5x ambient levels of dominant grazer; Both = amended with phosphate and 5x ambient zooplankton.

Analyses to be completed

We have estimated algal/cyanobacterial growth rates based on changes in chl a concentration in the experimental treatments of each experiment in each of the four lakes/reservoirs. This provides us with a good estimate of bulk community dynamics, however the next step is to microscopically examine subsamples from each experimental treatment replicate to identify and enumerate the particular algal and cyanobacteria taxa present in each experiment. This will allow us to calculate taxon-specific growth rates to determine which components of the phytoplankton community were responding to the effects of nutrient amendment, zooplankton grazing, and their interactive effects. These analyses are on-going, but extremely time consuming, and we anticipate completing the microscopy work over the summer and fall of 2015.

Having a complete picture of the taxonomic composition of the phytoplankton community will also allow us to assess the potential impacts of other, indirect effects of added nutrients and grazing, such as the effect of "trophic cascades" in each experimental bottle. The added zooplankton grazers may be selectively consuming large algae or protozoans, which could provide a more favorable environment for smaller algae or cyanobacteria to thrive. This would lead to paradoxically higher algal/cyanobacteria growth rates in treatments with added grazers, as estimated based only on changes in chl a concentration. But our taxon-specific growth rates will help to remove this masking effect and potentially reveal the indirect, cascading effects of selective grazing.

Finally, the taxon-specific growth rate analyses will allow us to examine specifically the impacts of nutrients and grazers on cyanobacteria in particular. Harmful cyanobacteria blooms are already a problem in Vancouver and Lacamas lakes. Merwin and Cle Elum lakes have not experienced substantial blooms, but cyanobacteria abundance in both lakes has been variable in recent years, and the potential for nutrient increases due to land use change in their surrounding watersheds warrants concern for cyanobacteria to become a nuisance in these systems, as well.

Significance

Toxic cyanobacteria blooms in freshwater lakes are an increasing problem worldwide, that are also impacting lakes in Washington and the Pacific Northwest. Results from this research will provide novel information about the dynamics of toxic cyanobacteria blooms in lakes across a eutrophication gradient in Washington state, which will be applicable to temperate freshwater systems more generally. In particular, this research will address the interactive effects of nutrients (bottom-up) and grazing (top-down) on controlling the timing and magnitude of toxic cyanobacteria blooms in both eutrophic and oligotrophic systems.

Finally, identifying the potential biotic and/or abiotic factors associated with cyanobacteria bloom dynamics will provide critical information for natural resource managers to develop strategies for managing blooms based on empirical evidence. For example, if nutrients are found to be a primary factor associated with toxic cyanobacteria blooms in Washington state lakes, then a focus on measures to reduce nutrient loading into the lakes may be most effective for mitigating these blooms. Similarly, if a major control of cyanobacteria blooms is grazing impact from zooplankton consumers, then efforts to manipulate the system to maximize grazing pressure (e.g., via biomanipulation of fish stocks and cascading trophic effects) may be a possible approach to reduce toxic blooms. These results will therefore benefit state and county agencies as they make decisions about our four lakes/reservoirs, but will also be applicable to regional and national resource management agencies who face similar challenges with cyanobacteria blooms in other temperate aquatic systems.

References

Boyer, J, G Rollwagen Bollens, and S M Bollens. (2011) Microzooplankton grazing before, during and following a cyanobacteria bloom in Vancouver Lake, Washington, USA. Aquatic Microbial Ecology. 64: 163-174.

Chorus I, Falconer I, Salas H, Bartram J (2000) Health risks caused by freshwater cyanobacteria in recreational waters. J Tox Env Health Part B 3: 323-347

Codd G, Morrison L, Metcalf J (2005) Cyanobacterial toxins: risk management for health protection. Tox Appl Pharm 203: 264-272

Frost B (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17: 805-815.

Hutchinson GE (1973) Eutrophication. Am Sci 61: 269-279

Jacoby J, Kann J (2007) The occurrence and response to toxic cyanobacteria in the Pacific Northwest, North America. Lake Res Management 23:123-143

Kosten, S., V.L.M. Huszar, E. Becares, L.S. Costa, and 12 others, 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology 18: 118-126.

Lee T, Rollwagen-Bollens G, Bollens S (In press) The influence of water quality variables on cyanobacteria blooms and phytoplankton community composition in a shallow temperate lake. Environmental Monitoring and Assessment.

McCune B, Grace J (2002) Analysis of ecological communities. MjM Software Design.

O'Neill, J.M., T.W. Davis, M.A. Burford, & C.J. Gobler, 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14: 313-334.

Paerl HW (2008) Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. From: Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Advances in Experimental Medicine and Biology. Ed. H.K. Hudnell. Springer, Triangle Park, NC.

Paerl HW, Huisman J (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Envir Microbiol Reports 1: 27-37

Rollwagen-Bollens G, Bollens S, Gonzalez A, Zimmerman J, Lee T, Emerson J. (2013) Copepod grazing impact before, during and following filamentous cyanobacteria blooms in a large, shallow temperate lake (Vancouver Lake, Washington, USA). Hydrobiologia, 705: 101-118.

Sellner KG, Doucette GJ, Kirkpatrick GJ (2003) Harmful algal blooms: causes, impacts and detection. J Ind Microbiol Biotechnol 30: 383-406

Strickland, J.D.H. & T.R. Parsons, 1972. A practical manual for seawater analysis. Fisheries Research Board of Canada Bulletin 167.

Students supported

Ms. Vanessa Rose, MS candidate in Environmental Science (degree expected Spring 2016).

Publications resulting from this award

None as yet.

Notable awards and achievements

Lane Graduate Research Fellowship in Environmental Science and *Boeing Graduate Research Fellowship in Environmental Science*, both awarded from the School of the Environment to Vanessa Rose, Spring 2015.