MICROZOOPLANKTON GRAZING ON CYANOBACTERIA

IN VANCOUVER LAKE, WASHINGTON, USA

BY

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To the Faculty of Washington State University:

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MICROZOOPLANKTON GRAZING ON CYANOBACTERIA

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Abstract

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Many studies have examined microzooplankton (20-200 µm) grazing impacts on phytoplankton using dilution experiments in marine systems, but few have been conducted in shallow temperate lakes. I conducted 16 dilution experiments (April 2008 - January 2009) to estimate microzooplankton grazing and intrinsic phytoplankton growth rates before, during and after a cyanobacteria bloom in Vancouver Lake, Washington, USA. Intrinsic phytoplankton growth rates were low in early spring (0.41 d^{-1}), then increased over the spring to a maximum (1.19 d^{-1}) in May 2008, before declining to zero and becoming negative in June and July 2008, just prior to the bloom. Phytoplankton growth rates rose as the bloom progressed through August and September 2008, reaching rates >1.0 d^{-1}, then declined through the fall and winter. Microzooplankton grazing rates were low (0.29 d^{-1}) in the spring, then became substantially negative (–1.0 to –1.50 d^{-1}) in the month preceding the initial chlorophyll a bloom. During the bloom in late summer, grazing rates quickly increased to a maximum of 0.75 d^{-1} and remained high as the bloom declined. Microzooplankton grazing specifically on cyanobacteria was high in the spring (0.97 d^{-1}), negative just prior to the bloom (-0.66 d^{-1} to -0.97 d^{-1}) in summer, and positive but low in the fall (0.31-0.68 d^{-1}). Microzooplankton grazing appeared to influence the

formation and decline of the cyanobacteria bloom. The negative grazing on cyanobacteria in the

summer may have been due to preferential grazing on other co-occurring prey, thus enabling the

bloom to form, while higher grazing rates on cyanobacteria in the fall likely contributed to the

decline of the bloom. These findings show that microzooplankton can potentially control

cyanobacteria blooms through grazing and may help us to understand food web dynamics of

plankton assemblages in large, shallow lakes.

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CHAPTER 1

INTRODUCTION

Over the past several decades, aquatic systems have been increasingly impacted by excessive nutrient inputs from agriculture, livestock production, urbanization and industry, leading to dramatic increases in phytoplankton blooms globally, including changes in the structure and function of surface water systems (Allan, 1995; Carpenter et al., 2000; Havens, 2008; Heisler, 2008). Moreover, there have been more harmful algal blooms (HABs) of greater geographic extent, longer duration, and involving more toxic species in the past decade than in preceding decades (Smayda, 1990; Hallegraeff, 1993; Anderson et al., 2002; Glibert et al., 2005). In particular, eutrophication of shallow surface waters often leads to the formation of concentrated cyanobacteria blooms, the most pervasive and problematic noxious freshwater algal taxa worldwide (Carmichael and Falconer, 1993; Carmichael, 1997; Codd, 1998; Chorus and Bartram, 1999; Chorus and Falconer, 2000; Chorus, 2001; Heisler, 2008).

Blooms of cyanobacteria lead to increased turbidity, decreased biodiversity, oxygen depletion, and in many cases toxin production, resulting in significant degradation of the quality of water for drinking, agriculture, and wildlife habitat (Carpenter et al., 2000; Codd, 2000; Celik and Ongun, 2006; de Figueiredo et al., 2006). Cyanobacteria blooms can sometimes be fatal to wild and domestic animals (Beasley et al., 1989) and pose serious health risks to humans, including increased incidences of liver cancer (Wu et al., 2006) and a two-fold increase in respiratory problems after exposure to high levels of cyanobacteria toxins in recreational waters (Stewart et al., 2006).

Cyanobacteria blooms can occur in any nutrient-rich surface water, including lakes, ponds, roadside ditches, or areas of river overflow. Blooms are typically associated with

eutrophic, poorly flushed waters (Havens, 2008; Codd, 2000; Oliver and Ganf, 2000; Anderson et al., 2002; Heisler, 2008). The precise chemical and physical factors required for cyanobacteria blooms are uncertain and vary from habitat to habitat, but it is known that excessive nitrogen and phosphorous, low nitrogen to phosphorus ratios (cyanobacteria are scarce when nitrogen-to-phosphorus ratios exceed 29:1; Smith, 1983), a pH of 6-9, and temperatures which can support the growth of cyanobacteria (20-25 °C; de Figueiredo et al, 2006; Celik and Ongun, 2006) are typically necessary (Crayton, 1993; Elser, 1999; Oliver and Ganf, 2000; Yamamoto, 2009).

In addition to physical and chemical environmental parameters, "top-down" factors such as zooplankton grazing on cyanobacteria can also influence bloom formation. The majority of scientific literature has focused on the role of mesozooplankton (typically crustaceans >200 µm, e.g. copepods, cladocerans) grazing in controlling harmful algal blooms (Elser, 1999; Lurling, 2003; Mitra and Flynn, 2006; Wilson et al., 2006). Previous studies have found that mesozooplankton grazer survival is negatively affected in the presence of toxic cyanobacteria (namely *Microcystis*; Lurling, 2003; Wilson et al., 2006) and that filamentous cyanobacteria with low mucilage are grazed more than single-celled cyanobacteria with high mucilage (Wilson et al., 2006). Despite findings that mesozooplankton such as *Daphnia* can successfully graze on cyanobacteria and reduce cyanobacteria abundance (Epp, 1996; Elser, 1999; Paterson et al., 2002), cyanobacteria are generally considered a poor food source for mesozooplankton because cyanobacteria inhibit grazing and may produce cyanotoxins that have negative physiological effects on mesozooplankton (Hambright et al., 2001; Lurling, 2003; Wilson et al., 2006).

In addition to crustacean grazers, microzooplankton (heterotrophic protists 20-200 µm in size, e.g. dinoflagellates and ciliates) may also be influential on cyanobacteria bloom dynamics. In marine and estuarine systems, numerous studies have demonstrated the substantial grazing

impact of microzooplankton on primary producers generally (Calbet and Landry, 2004; Leising et al., 2005; Calbet, 2008), as well as on the timing, composition and potential magnitude of algal blooms, including blooms of harmful taxa (Henjes et al., 2007; Demir et al., 2008).

However, the role of microzooplankton grazing on algal bloom dynamics has rarely been considered in freshwater systems. In one study of a riverine system, Leonard and Paerl (2005) found microzooplankton grazing rates were higher on phytoplankton standing stock when cyanobacteria were abundant, demonstrating a greater microzooplankton grazing impact on phytoplankton when cyanobacteria were abundant. In addition, using laboratory-based methods, several studies have documented the role of freshwater microzooplankton in modulating the timing and rate of cyanobacteria colony formation, and observed micrograzers to consume small, solitary cyanobacteria cells which stimulated colony development, possibly as a refuge from grazing (Fialkowska and Pajdak-Stos, 2002; Jakobsen and Tang, 2002; Yang et al., 2006).

While laboratory-based studies have provided evidence that micrograzers can substantially reduce the abundance of cyanobacteria, it may not be appropriate to expect the same results in natural settings. Microzooplankton may select alternative prey based on size, abundance, or nutritional value (Dryden and Wright, 1987). Additionally, many laboratory studies are limited by the species of microzooplankton and cyanobacteria that can be successfully cultured (Dryden and Wright, 1987). Studies of microzooplankton grazing on cyanobacteria based on observations of naturally occuring scenarios can provide crucial information that may be more representative of trophic interactions occuring within lake systems compared to studies using laboratory-based methods with cultured organisms.

Very few studies have measured microzooplankton grazing on the algal community in lake systems, and of those an even smaller number have estimated microzooplankton grazing

during cyanobacteria blooms and/or specifically on cyanobacteria. Gobler et al. (2007) found that microzooplankton grazing on phytoplankton in a eutrophic temperate lake was consistent throughout a summer cyanobacteria bloom, and that microzooplankton did not appear to be affected by toxin levels. In another eutrophic lake, Tijdens et al. (2008) estimated microzooplankton grazing to have removed up to 90% of unicellular cyanobacteria production, however this was based on only four experiments conducted over a four-month period. Given the potential for micrograzers to both directly and indirectly influence cyanobacteria colony formation and bloom development based on results from the laboratory, the need for quantifying microzooplankton grazing impacts on cyanobacteria bloom formation in the field is evident.

Vancouver Lake, a large, shallow lake located in southwest Washington state, has experienced seasonal blooms of cyanobacteria for over twenty years (Wierenga, 2005). When these blooms occur, county regulations require closing the lake to the public. Private citizens along with public agencies are concerned about the state of Vancouver Lake, and have been working together with the goal of managing the lake in such a way as to prevent cyanobacteria blooms from occurring.

As part of a larger investigation into the biological factors that may influence cyanobacteria blooms in Vancouver Lake, in this study my objective was to quantify microzooplankton grazing rates on algae and cyanobacteria over the course of a summer cyanobacteria bloom cycle in order to assess the influence of microzooplankton grazing on bloom formation and decline.

CHAPTER 2

MATERIALS AND METHODS

Study area

Vancouver Lake is located in the lower Columbia River floodplain in southwest Washington state (45.68°N, 122.72°W; Fig. 1). The lake is approximately 5,681 hectares and has a mean depth of 0.6 meters (Caromile et al., 2000). Vancouver Lake is connected hydrologically to the Columbia River by Lake River to the north and receives additional input from Burnt Bridge Creek to the east. Vancouver Lake also has a flushing channel on the southwest shoreline to allow flow from the Columbia River. I collected all samples from the end of a dock at a private sailing club located on the eastern shore of the lake (Fig. 1). Previous sampling conducted at 8 different locations throughout the Lake in the year prior to my study found the abundance and taxonomic composition of the plankton at the dock station to be representative of the lake as a whole (data not shown).
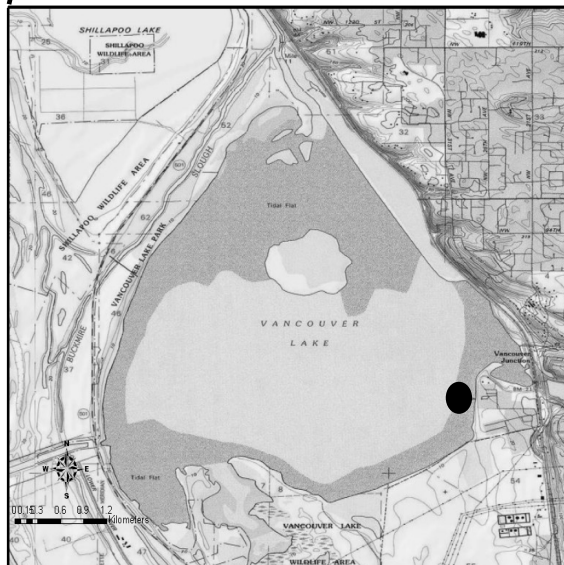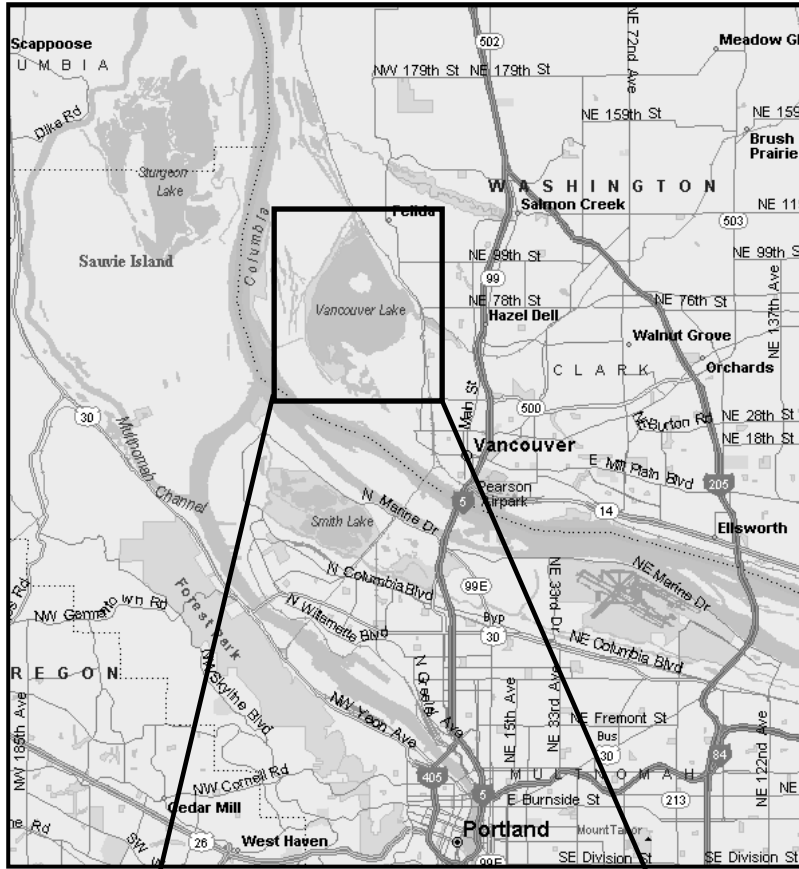
Figure 1. Map of Vancouver Lake, Vancouver, Washington, U.S., indicating sampling location (filled circle).

Field sampling

Lake sampling was conducted from April 2008 to January 2009, occurring weekly except during November through January, when it was conducted monthly. I used a YSI 95 probe to measure dissolved oxygen and temperature, and a Secchi disk to estimate water clarity. I collected samples in triplicate of surface lakewater using an acid-washed bucket. From each bucket, 3-20 mL of lakewater, depending on the period of the bloom cycle, was filtered over glass fiber filters (GFF), frozen, then extracted in 90% acetone and chlorophyll a concentration measured using a Turner Model 10 fluorometer (Strickland and Parsons, 1972). An additional 50 mL of lakewater from each bucket sample was filtered through a 0.45 µm filter, frozen and then shipped to the University of Washington, School of Oceanography for analysis of nutrient (NO_3, NO_2, NH_4, PO_4, SiO_2) concentrations using a Technicon AAII system according to the WOCE Hydrographic Program protocols. Finally, 200 mL of lakewater from each bucket sample was preserved in 5% acid Lugol's solution for microscopic analyses to identify and enumerate the plankton <200 µm in size (Gifford, 1993).

Analysis of Growth and Grazing Rates

       I conducted bi-weekly dilution experiments (Landry and Hassett 1982; Landry et al., 1995; Gobler et al., 2007) from April to October 2008 to estimate grazing rates of the microzooplankton community and growth rates of the phytoplankton community over the course of an entire cyanobacteria bloom cycle. A winter experiment was conducted in January 2009 in order to observe differences in grazing and growth rates due to the seasonal shift in plankton community composition (Table 1).

Table 1. Experiment dates with growth rates, grazing rates and r^2 values found from linear regression. Asterisks denote significant slopes (*=p<0.05, **=p<0.01, ***=p<0.001).

Experiment date	Growth rate (d^{-1})	Grazing rate (d^{-1})	r^2	F statistic	p-value
4/1/08	0.73	0.11	0.66	2.76	0.121
4/15/08	0.41	-0.31	0.37	7.53	0.017*
4/30/08	0.90	0.17	0.11	1.56	0.234
5/13/08	1.19	0.29	0.34	8.17	0.013*
6/3/08	0.01	-0.10	0.68	26.95	0.000***
6/16/08	-1.33	-1.48	0.30	5.52	0.035*
6/30/08	-0.66	-1.25	0.68	27.81	0.000***
7/14/08	-0.21	-1.06	0.89	104.8	0.000***
7/28/08	0.80	0.43	0.27	4.84	0.047*
8/11/08	0.88	0.00	0.00	0.03	0.859
8/25/08	0.42	0.29	0.33	6.52	0.024*
9/10/08	0.99	0.53	0.31	5.73	0.032*
9/22/08	1.08	0.75	0.59	18.87	0.001**
10/6/08	0.94	0.63	0.40	8.82	0.011*
10/20/08	0.90	0.68	0.69	28.89	0.000***
1/12/09	-0.84	-0.85	0.64	23.13	0.000***

For each dilution experiment, I collected surface lakewater using a clean, acid-washed carboy, then pre-filtered the lakewater through 73 µm and 30 µm mesh filters before using gravity filtration and a peristaltic pump to filter the water through 0.22 µm cellulosic filters. I then collected additional unfiltered water from the Lake in a separate clean, acid-washed carboy. The filtered and unfiltered lakewater samples were kept at lake temperatures throughout the experimental set-up.

I prepared triplicate dilutions in one-liter bottles of ambient lakewater to filtered lakewater in the following ratios: 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75, and 0.1:0.9. I then added 227 µl of 75 g/L $NaNO_3$ and 420 µl of 5.0 g/L $NaH_2PO_4 \cdot H_2O$ to ensure nutrient-replete conditions (final concentrations of 1.7 g/L $NaNO_3$ and 0.2 g/L $NaH_2PO_4 \cdot H_2O$). These concentrations were higher than maximum levels observed in a similar lake located in New York (C.J. Gobler, personal communication, data not shown). I included triplicate dilutions of the endpoints (1.0:0.0 and 0.1:0.9 ambient lakewater to filtered lakewater) without any added nutrients to serve as controls. The bottles were incubated for 24 hours under ambient light and temperature conditions on a plankton wheel revolving at 0.5 rpm. Each treatment was sampled for chlorophyll *a* concentration at the beginning and end of the 24 hour incubation.

In every other experiment (approximately once per month), I also preserved 200 mL subsamples from every incubation bottle in Lugol's iodine solution for enumeration and identification of protists <200 µm. Aliquots of 2-25 mL from the Lugol's preserved samples were settled overnight into counting chambers, then examined at 400x magnification using an Olympus CK40 inverted microscope to enumerate, size and identify all protists to the genus level, and to species where possible. For each sample, I counted at least 100 individual cells from randomly selected fields (>10 fields) along transects according to the methods described in

Kirchman (1993). Cell biovolume was calculated according to Hillebrand (1999). Carbon biomass was estimated from biovolume using formulas described in Menden-Deuer and Lessard (2000). Taxa were identified using Wehr and Sheath (2002) and Patterson (1992).

For each dilution experiment, I estimated net phytoplankton growth rates in each dilution treatment based on the change in chlorophyll *a* concentration over the course of the 24-hour incubation, assuming exponential growth. Linear regression analyses of the relationship between net growth rate of phytoplankton and the fraction of unfiltered lakewater were then conducted; the intrinsic phytoplankton growth rate was estimated as the y-intercept of the regression, and the microzooplankton grazing rate was estimated as the slope of the regression line (Landry and Hassett 1982; Landry et al., 1995; Gobler et al., 2007). I tested whether the regression slopes were significantly different from zero ($p < 0.05$) in each experiment using an F-test (Zar, 1996). For those experiments in which protists were enumerated and identified, I also estimated taxon-specific growth rates and microzooplankton grazing rates on specific prey categories and phytoplankton size classes.

CHAPTER 3

RESULTS

Water Quality and Plankton Composition

From April to May 2008, surface lake temperatures ranged from 12 to 20°C and dissolved oxygen concentrations ranged between 5.3 and 8.6 mg/L (Fig. 2A). Surface temperature peaked at 25°C in August and dissolved oxygen concentrations averaged 5.0 mg/L (Fig. 2A). Dissolved oxygen fell to a minimum of 3.9 mg/L in early October before peaking at 23.7 mg/L in January 2009. Surface temperature was also lowest (6°C) in January (Fig. 2A).

Phytoplankton biomass, as measured by chlorophyll *a* concentration, was low in the spring, ranging between 4 and 27 µg Chl *a*/L then rapidly increased to an initial peak of 256 µg Chl *a*/L in late July, followed by a much larger peak (499 µg Chl *a*/L) at the end of August. Chl a concentrations decreased rapidly in October back to low (20-30 µg Chl *a*/L) levels throughout the late fall and early winter of 2008 (Fig. 2B).

Surface concentrations of dissolved inorganic phosphate (PO_4) peaked just prior to maximum chlorophyll *a* levels, reaching 0.1 mg/L, while ammonium (NH_4) concentrations reached a maximum (0.3 mg/L) coinciding with the algal biomass peak (Fig. 2B). Nitrate (NO_3) and nitrite (NO_2) concentrations remained relatively low during this period (0.7-4.5 µg/L; Fig. 2B). N:P also remained relatively low (0.6:1 – 19:1) prior to, during, and just after the peak chlorophyll *a* then became relatively high (45:1) in the winter (data not shown). Fall and winter (November through January) saw the highest nitrate and silicate (SiO_4) concentrations, with nitrate increasing over 100-fold from low summer levels to 569 µg/L in January 2009, and silicate reaching 6.2×10^3 µg/L (Fig. 2B).
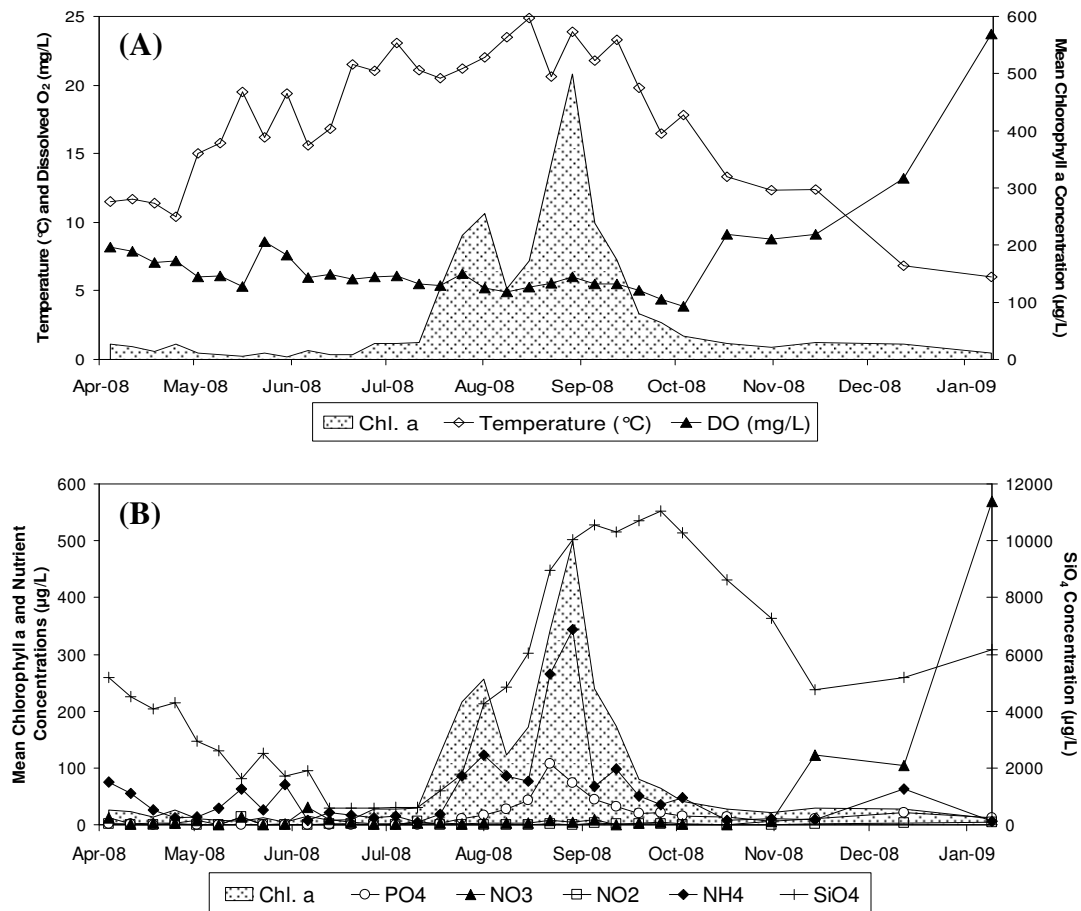
Figure 2. Seasonal trends of (A) mean surface chlorophyll *a* concentration, surface temperature and dissolved oxygen concentration, and (B) mean surface chlorophyll *a* concentration and mean surface dissolved inorganic phosphate (PO_4), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) and silicate (SiO_4) collected from Vancouver Lake April 2008 through January 2009.

The overall abundance of microplankton (plankton 20-200 µm) in the Lake was low in May but increased from July through August (Fig. 3). Diatoms and chlorophytes peaked in late July and cryptophytes peaked in mid-August. Dinoflagellates and ciliates remained relatively rare throughout the study period (Fig. 3A). Cyanobacteria reached an initial peak in abundance in late July (8.6×10^5 cells/mL), concurrent with the initial chlorophyll *a* peak, reached their highest abundance in late September (1.2×10^6 cells/mL) after the chlorophyll *a* bloom, and remained in high abundance through October (Fig. 3B). In terms of biomass, the microplankton community shifted from dominance by diatoms in the spring to cyanobacteria in the summer and fall (Fig. 4). Ciliate and cryptophyte biomass was relatively high in the winter (December and January) at nearly 40% and 50% of the total biomass, respectively, but lower during the remainder of the study period (Fig. 4).
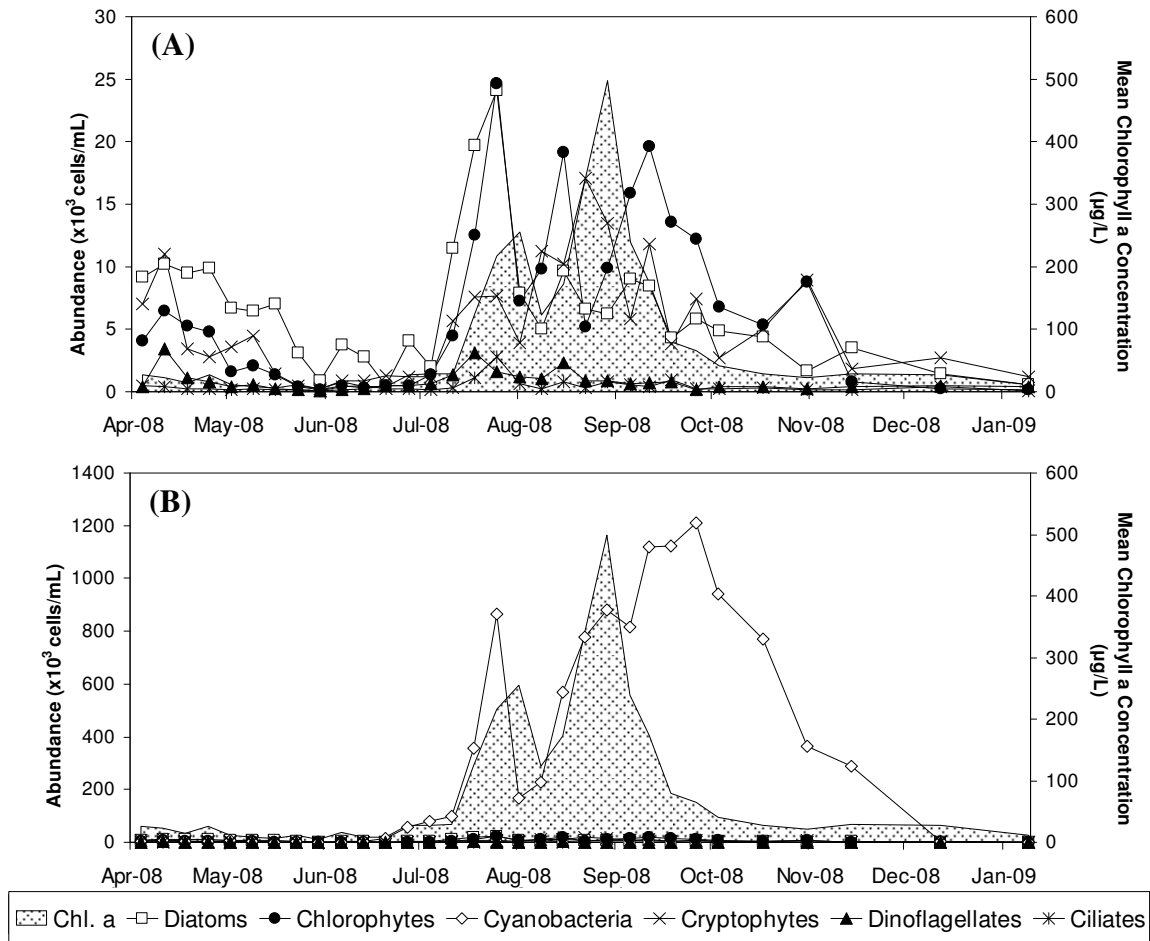
Figure 3. Mean abundance of major taxonomic groups of phytoplankton and mean surface chlorophyll *a* concentration (A) without cyanobacteria and (B) including cyanobacteria collected from Vancouver Lake from April 2008 through January 2009.
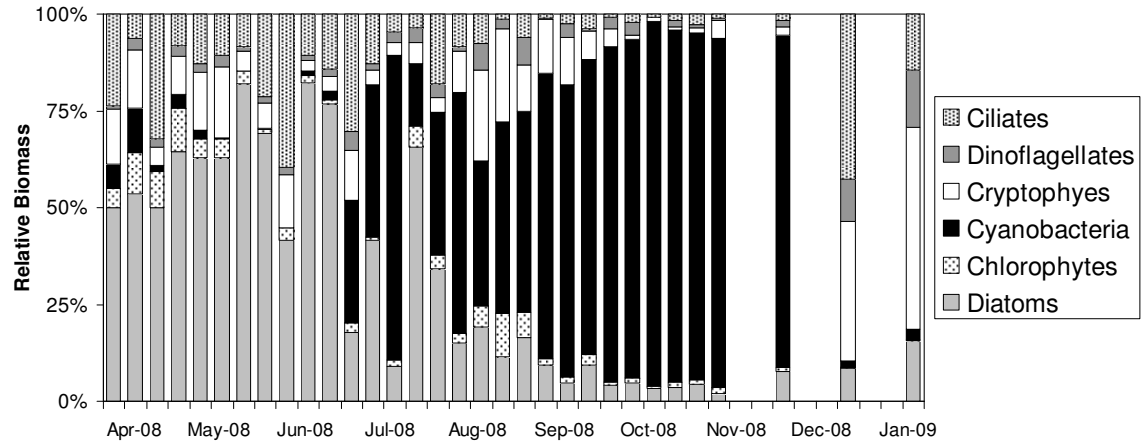
Figure 4. Relative biomass of major taxonomic groups of microplankton collected in Vancouver Lake from April 2008 through January 2009.

Taxonomic diversity of cyanobacteria was relatively high in the Lake from April through September, although *Aphanizomenon flos-aquae* was the most abundant species over nearly half of the study period (Fig. 5A). *Anabaena flos-aquae* was present in substantial amounts from April through October, and comprised over 25% of cyanobacteria biomass and over 50% of abundance in mid-August, just prior to the chlorophyll *a* bloom (Figs. 3 and 5). The dominant cyanobacteria taxa according to biomass was principally *Aphanizomenon flos-aquae,* except during May when *Synechococcus* spp., *Aphanothece* spp. and *Merismopedia* spp. were abundant (Fig. 5B).
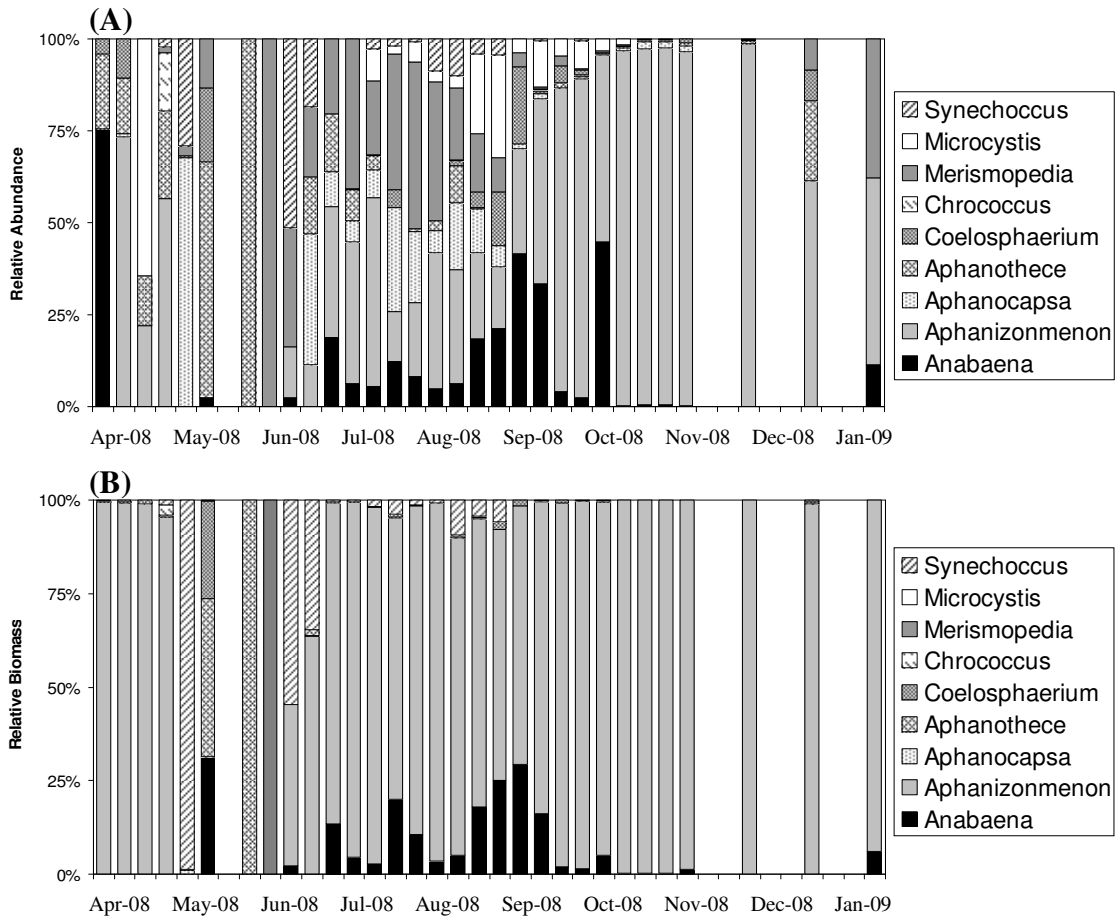
Figure 5. Relative abundance (A) and relative biomass (B) of cyanobacteria collected from Vancouver Lake from April 2008 through January 2009.

Growth and Grazing Rates

I confirmed whether I achieved the intended level of dilution of the total planktonic community (phytoplankton and microzooplankton) in the treatment bottles by microscopically determining the percent reduction in the phytoplankton community within the most dilute (10% unfiltered lakewater) treatments compared to ambient lakewater in five out of the 16 total experiments. I achieved a reduction to $10 \pm 0.5\%$ of the ambient plankton community in each case, with the exception of two experiments in which the most dilute treatments were 14.2 and 5.9% of the unfiltered community (Table 2).

Table 2. Actual dilution level of 0.1:0.9 ratio of ambient lakewater to filtered lakewater treatments based on cell enumeration.

Experiment date	Plankton population remaining (%)
5/13/08	14.2
6/16/08	9.9
7/14/08	10.7
9/10/08	5.9
10/6/08	10.2

Fig. 6 shows the relationship between net phytoplankton growth rate (d^{-1}) and dilution level for the 16 dilution experiments conducted between April 2008 and January 2009. Of the 16 experiments conducted, 13 resulted in regression slopes that were significantly different from zero (Table 1). Most of the regressions with a slope significantly different from zero showed a linear correlation between the apparent growth rate and dilution level (Table 1). Intrinsic phytoplankton growth rates estimated from the y-intercept of the significant regressions between net growth rate and dilution level were initially low but increased over the spring, from 0.41 d^{-1} in April to a maximum of 1.19 d^{-1} in May (Fig. 7). Negative intrinsic algal growth rates were observed in June through mid-July, indicating that phytoplankton were actually decreasing in biomass over the course of the 24-hour incubations. Intrinsic phytoplankton growth rates rose in September, just after the peak in chlorophyll *a* concentration, to a maximum of 1.08 d^{-1}, then declined through the fall and winter as the bloom degraded. Negative intrinsic algal growth rates occurred again in January 2009 (Fig. 7).
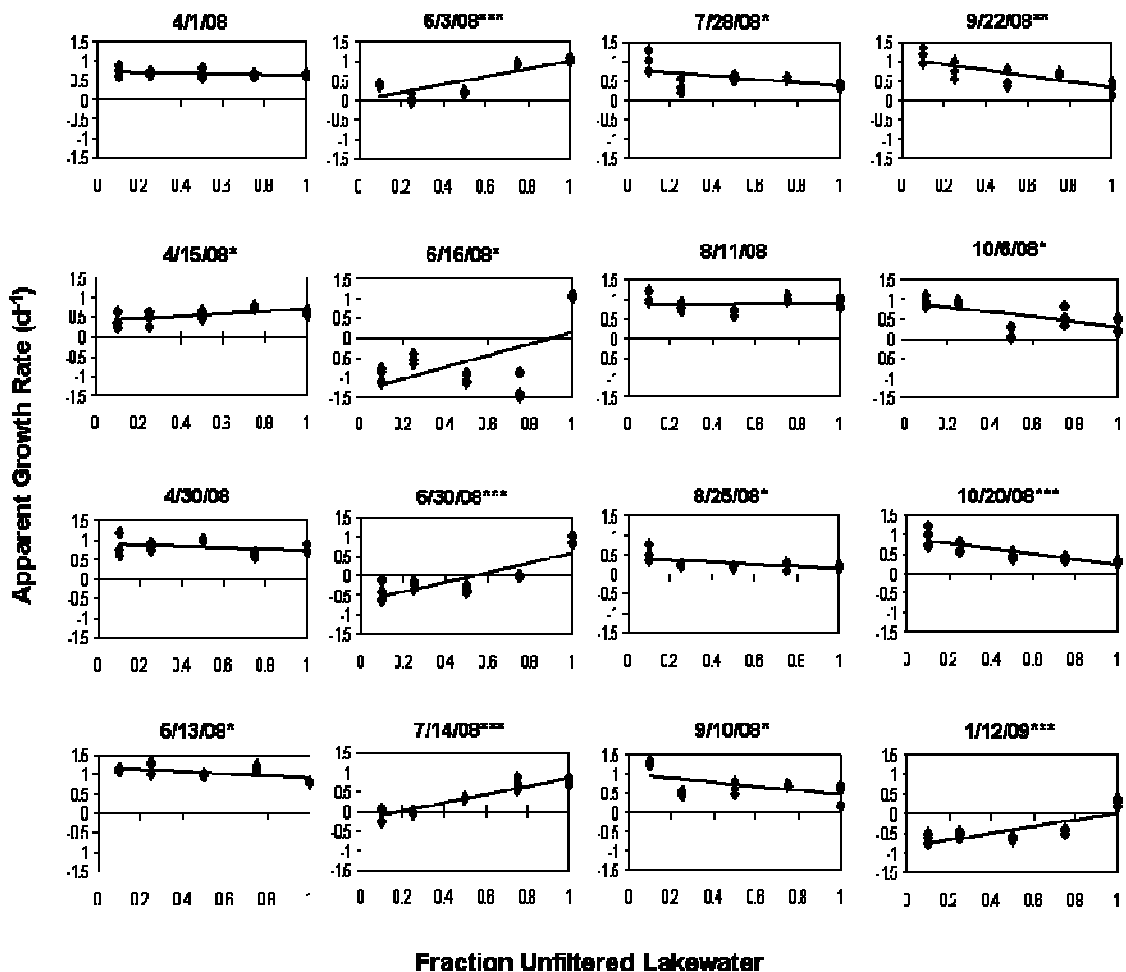
Figure 6. Dilution plots showing grazing on phytoplankton in Vancouver Lake as measured by chlorophyll *a* concentrations. Asterisks denote significant slopes (*=p<0.05, **=p<0.01, ***=p<0.001).
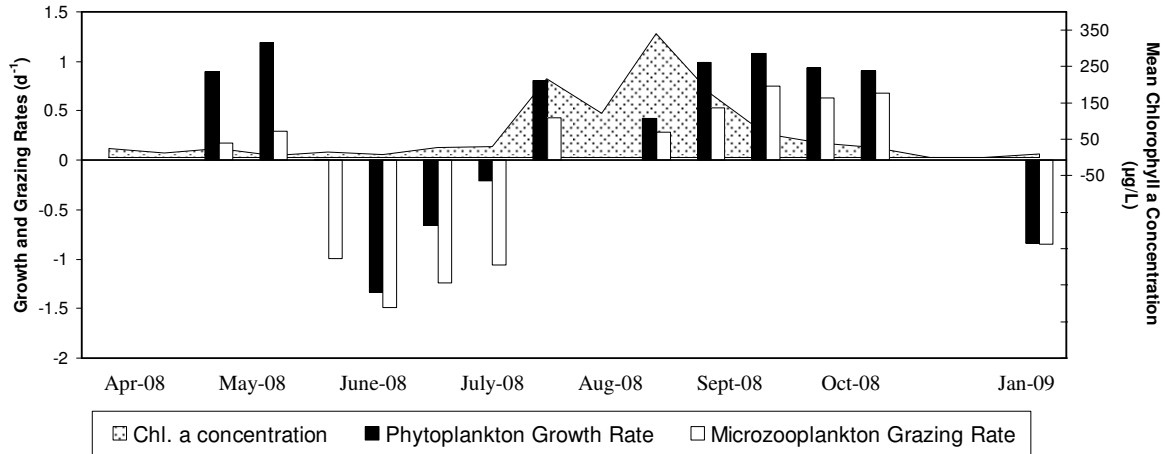
Figure 7. Intrinsic phytoplankton growth rates (d^{-1}) and microzooplankton grazing rates (d^{-1}) determined from dilution experiments conducted in Vancouver Lake from April 2008 to January 2009. Shaded area represents mean chlorophyll *a* concentrations measured on each experiment date.

Significant microzooplankton grazing rates estimated from the dilution experiments were low (0.29 d^{-1}) in the spring then became substantially negative (-1.0 to -1.50 d^{-1}) in the six weeks preceding the initial chlorophyll *a* bloom in the Lake (Fig. 7). Negative microzooplankton grazing rates indicate that net phytoplankton growth rates in each dilution treatment increased across the dilution spectrum from highest dilution (lowest relative abundance of grazers) to lowest dilution (highest abundance of grazers). In the fall experiments, microzooplankton grazing rates quickly increased, more than doubling from late August to mid-September, reaching a maximum of 0.75 d^{-1} just after the maximum peak of chlorophyll *a* and remained comparatively high (from 0.68 to 0.75 d^{-1}) through the decline of the chlorophyll *a* bloom in early October. In January 2009, microzooplankton grazing rates were again negative (Fig. 7).

Taxon-Specific Grazing Mortality Rates

In a subset of experiments, I also calculated intrinsic phytoplankton growth rates and microzooplankton grazing rates for particular prey groups based on changes in their abundance over the incubation for each dilution treatment. Algal and cyanobacteria growth rates and microzooplankton grazing rates obtained via cell enumeration of selected experiments in May, June, July, September, and October mirrored rates calculated based on chlorophyll *a* concentrations. The only exception was in July, when intrinsic phytoplankton growth rate calculated from changes in cell number was twice the magnitude of the chlorophyll *a*-based rate and the microzooplankton grazing rate was half the magnitude of the chlorophyll *a*-based rate (data not shown).

Microzooplankton grazing rates on major algal taxonomic groups based on cell enumeration showed that cyanobacteria were the taxa most heavily grazed upon in May (0.97 d^{-1}), shifting to diatoms in early summer (0.4 d^{-1}), then chlorophytes mid-summer into fall (0.7-0.9 d^{-1}; Fig. 8). Cyanobacteria were again the prey group most heavily grazed on in October (0.31 d^{-1}; Fig. 8). Conversely, cryptophytes experienced negative microzooplankton grazing mortality rates in the spring and summer experiments before the chlorophyll *a* bloom in the Lake, meaning these organisms increased in abundance over the incubation period even though grazers were present (Fig. 8). It should be noted that while the magnitude of negative grazing on cryptophytes in June was substantially higher than the other taxa, the relative abundance of cryptophytes was extremely low at that time. Cyanobacteria also experienced negative microzooplankton grazing mortality rates in the June and July experiments, with the lowest grazing rate on cyanobacteria in July (-0.97 d^{-1}) occurring directly before the chlorophyll *a* bloom. Only diatoms experienced positive microzooplankton grazing mortality in every experiment (Fig. 8).
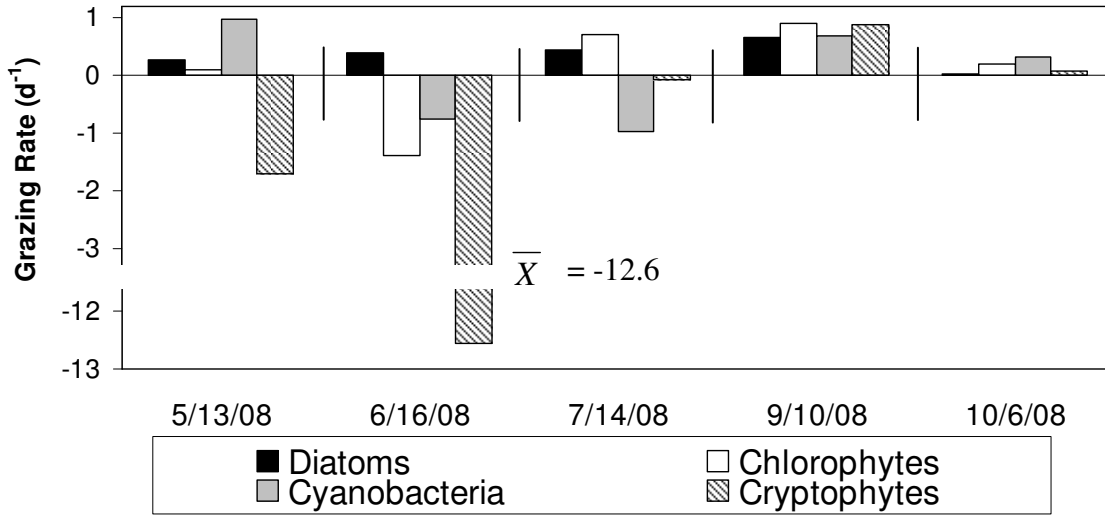
Figure 8. Microzooplankton grazing rates (d[-1]) on major prey taxonomic groups, based on cell enumeration of dilution experiments conducted in Vancouver Lake.

Microzooplankton grazing rates on different size classes of prey within each major taxonomic group, compared with their relative abundance, are shown in Fig. 9. Small-sized (<10 µm) diatoms were the most abundant prey but experienced negative grazing mortality (-3.02 d^{-1}) in the spring experiment (Fig. 9). Cyanobacteria (<10 µm) were grazed upon the most (0.96 d^{-1}) and comprised < 25% of the prey community in the spring experiment (Fig. 9). The microzooplankton grazing rate for small-sized cryptophytes in June was substantially negative (-12.61 d^{-1}) but small cryptophytes comprised a very small proportion of the total prey community (Fig. 9). Cyanobacteria (<10 µm) were the most abundant prey and experienced negative grazing mortality in the June and July experiments (-0.66 d^{-1} and -0.97 d^{-1}) just prior to the chlorophyll *a* bloom in the Lake; grazing on cyanobacteria (<10 µm) was positive but low (0.68 d^{-1} and 0.31 d^{-1}) in the fall experiments (Fig. 9).

(A)

(B)

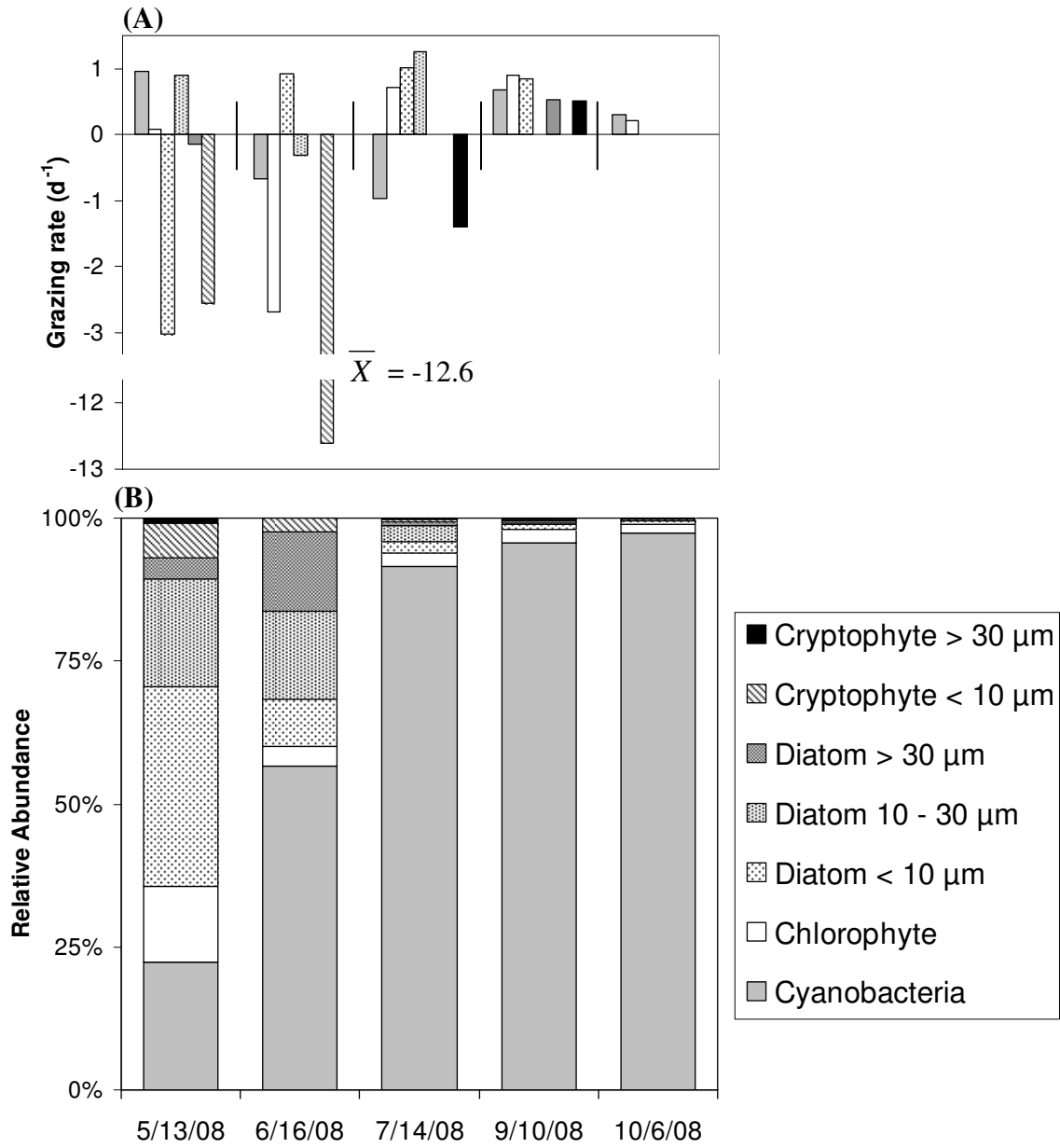Figure 9. Ranges in (A) microzooplankton grazing rates (d^{-1}) according to size classes within major prey taxonomic groups, combined across all taxa, and relative abundance (B) of major prey taxonomic groups according to size class based on cell enumeration collected from dilution experiments conducted in Vancouver Lake.

CHAPTER 4

DISCUSSION

Seasonal Pattern of Growth and Grazing Rates

The chlorophyll *a* bloom in Vancouver Lake in 2008 was intense and appeared rapidly, as demonstrated by a rise in chlorophyll *a* from very low springtime levels to extremely high levels in late summer. The chlorophyll *a* bloom was strongly dominated by cyanobacteria, and cyanobacteria abundances were so high that Vancouver Lake was closed to public use during most of August 2008. Chlorophyll *a* quickly diminished in the fall and returned to low levels in winter, but cyanobacteria abundance remained high into the fall. This may have been due to inadvertently enumerating post-bloom senescent cyanobacteria cells in which chlorophyll *a* had been degraded, in addition to actively growing cells. I saw a 3-5% relative increase in pheophytin just after the bloom which may have translated to an inflated value of cyanobacteria cells determined from cell counts compared to chlorophyll *a* analysis (data not shown).

Vancouver Lake phytoplankton growth rates were lowest during the weeks preceding the bloom and were high after the peak of the chlorophyll *a* bloom. Microzooplankton grazing rates followed a similar pattern. Microzooplankton grazing rates were lowest, becoming negative, just prior to the bloom and reached maximal rates during the latter part of the chlorophyll *a* bloom in the fall. The seasonal pattern of phytoplankton growth and microzooplankton grazing rates I observed is somewhat different from that observed in other studies. In the few studies examining phytoplankton growth and microzooplankton grazing rates in freshwater lakes, both rates were observed to increase steadily in the period leading up to an algal bloom. For instance, in a shallow, eutrophic, temperate lake in New York pre-bloom phytoplankton growth rates climbed from 1.03 to 1.22 d^{-1} and microzooplankton grazing rates rose from 0.35 to 1.04 d^{-1} (Gobler et

al., 2007). In general, summer phytoplankton growth rates and microzooplankton grazing rates in a range of temperate freshwater and estuarine systems have been observed to be positive, with growth rates ranging from 0.03-0.43 d^{-1} and grazing rates ranging from 0.24-1.14 d^{-1} (Murrell and Hollibaugh, 1998; Lignell et al., 2003).

The unusual negative phytoplankton growth rates and microzooplankton grazing rates observed in the June and July experiments were quite unexpected, and could have been the result of several factors. For instance, if microzooplankton were selectively grazing on a particular phytoplankton group which comprised only a small portion of the total abundance, this may have allowed other phytoplankton to proliferate faster than they might otherwise have done without grazers present. The resulting high growth rates of the non-grazed taxa could have exceeded the loss rate of the grazed taxa, leading to apparently higher phytoplankton growth in the undiluted treatments with more grazers (Gallegos, 1989; Landry et al., 1995; Lessard and Murrell, 1998; Murrell and Hollibaugh, 1998; Nejstgaard et al., 2001; Redden et al., 2002).

The negative grazing rates could also have been the result of a "trophic cascade" effect within the treatment bottles. The ambient assemblage of protist grazers in planktonic systems is often highly diverse, with respect to taxonomy, size and relative abundance. If during this period the protist community in the dilution treatments consisted of a three-trophic-level system, with larger, less abundant micrograzers feeding on smaller, more abundant micrograzers feeding on even higher abundance phytoplankton, then as dilution increased the larger micrograzers could have been selectively removed allowing the smaller grazers to exert higher grazing pressure on phytoplankton compared to less dilute treatments. Such "top down" trophic interactions within the protist community have been observed in a range of aquatic environments, as well as in a set of dilution experiments conducted in the Arabian Sea (reviewed in Verity and Smetacek, 1996;

Reckermann and Veldhuis, 1997). This mechanism could translate into a negative grazing rate, where net phytoplankton growth was higher in the undiluted treatments.

As the chlorophyll *a* bloom in Vancouver Lake progressed through August and September 2008, phytoplankton growth rates increased rapidly in the treatment bottles from 0.41 to 1.08 d^{-1} (Fig. 7). Microzooplankton grazing followed a similar trend, reaching the highest rate (0.75 d^{-1}) just after the peak of the chlorophyll *a* bloom (Fig. 7). The intrinsic phytoplankton growth and microzooplankton grazing rates during the bloom period were within the range found in saltwater systems and a subtropical freshwater lake, but not a similar temperate lake. In an extensive synthesis of 66 studies that used the dilution method, Calbet and Landry (2004) found average phytoplankton growth rates of 0.59 d^{-1} for marine systems, 0.67 d^{-1} for coastal systems, 0.97 d^{-1} for estuarine systems, and 0.69 d^{-1} for temperate saltwater systems overall. Microzooplankton grazing rates averaged 0.39 d^{-1} in marine systems, 0.40 d^{-1} in coastal systems, 0.53 d^{-1} in estuarine systems, and 0.41 d^{-1} in temperate saltwater systems overall (Calbet and Landry, 2004).

In other studies conducted in coastal and estuarine systems, growth rates ranged from 0.1-1.23 d^{-1} and grazing rates ranged from 0.05-1.22 d^{-1} (Murrell and Hollibaugh, 1998; Lignell et al. 2003; Loebl and Van Beusekom, 2008). Similarly, Leising et al. (2005) found phytoplankton growth rates >0.5 d^{-1} in Puget Sound, Washington during a spring phytoplankton bloom in April 2002 and Demir et al. (2008) observed phytoplankton growth rates from 0.21-1.54 d^{-1} and microzooplankton grazing rates from 0.11-0.82 d^{-1} during phytoplankton blooms in Delaware inland bays. The rates seen for the Vancouver Lake plankton were also in line with those observed in a subtropical lake. Leonard and Paerl (2005) observed a phytoplankton growth rate of 0.58 d^{-1} and microzooplankton grazing rate of 0.5 d^{-1} during a cyanobacteria bloom in

Lake George, part of the St. Johns River system in Florida. However, phytoplankton growth

rates and microzooplankton grazing rates measured in Vancouver Lake were substantially less

than those observed in a similar temperate lake in New York, where the phytoplankton growth

rate reached 1.81 d^{-1} and the microzooplankton grazing rate peaked at 1.62 d^{-1} during a

cyanobacteria bloom (Gobler et al., 2007). During the peak of the bloom in Vancouver Lake

phytoplankton biomass was extremely high, which may have led to increased competition for

light in the undiluted treatment bottles, in turn leading to lower growth rates than the rates

observed from other similar systems during the summer.

As the chlorophyll a bloom in Vancouver Lake declined in the fall, phytoplankton growth

rates in the incubation bottles remained high (0.90-0.94 d^{-1}), as did the microzooplankton grazing

rates (0.63-0.68 d^{-1}). These high growth and grazing rates contrast somewhat with those found in

other studies of temperate systems, which showed a general reduction in phytoplankton growth

and microzooplankton grazing rates as the bloom dissipated. For instance, Gobler et al. (2007)

found that phytoplankton growth rates in a New York lake fell from 1.81 d^{-1} to 0.71 d^{-1} and

microzooplankton grazing rates decreased from 1.16 d^{-1} to 0.36 d^{-1} after the peak of a

phytoplankton bloom from September to October. Loebl and Van Beusekom (2008) observed a

growth rate of 0.1 d^{-1} after a diatom bloom in a temperate shallow coastal system and Lignell et

al. (2003) saw an average post-bloom phytoplankton growth rate of 0.29 d^{-1} in a nearshore

marine environment.

However, in a subtropical freshwater system Leonard and Paerl (2005) saw relatively low

growth rates, ranging from 0.28-0.30 d^{-1}, and the highest grazing rates, ranging from 0.2-1.04 d^{-1},

in the fall after a phytoplankton bloom. The high growth rates seen in my post-bloom

experiments may be a result of persistent warm temperatures and an overall decreased abundance

of phytoplankton, which would have reduced competition for resources (light, nutrients) for remaining phytoplankton and allowed higher growth rates. Higher grazing rates may be the result of a change in the phytoplankton community as the composition began to shift away from dominance by cyanobacteria, possibly providing microzooplankton more relative availability of prey preferable to cyanobacteria.

The non-significant grazing mortality rates seen for three of the dilution experiment plots may be a result of a feeding saturation effect where a high intrinsic phytoplankton growth rate essentially masks the level of microzooplankton grazing, resulting in an underestimated grazing rate (Gallegos, 1989; Landry et al., 1995; Lessard and Murrell, 1998; Nejstgaard et al., 2001; Redden et al., 2002). The intrinsic phytoplankton growth rates were high in these experiments $(0.73\text{-}0.90 \text{ d}^{-1})$ suggesting that the grazing rates were underestimated possibly as a result of saturated feeding.

Taxon-specific Grazing Mortality Rates

The microscopical analysis of a subset of the dilution experiments yielded interesting information on potential predator-prey interactions and microzooplankton grazing impacts on particular prey species. The microzooplankton appear to have selectively grazed on the fast-growing phytoplankton and not the most abundant prey. In a similar study, Gaul and Antia (2001) found microzooplankton in the temperate northeast Atlantic selectively grazed on fast growing phytoplankton and not necessarily the most abundant taxa.

Only diatoms were grazed on consistently throughout the study period, and only small-sized diatoms showed a significant correlation between grazing mortality rate and relative abundance (p=0.01). Small-sized diatoms experienced substantially negative grazing when they were most abundant and increasingly higher grazing as they were becoming less and less abundant (when cyanobacteria began to dominate the phytoplankton community). Diatoms can be high in nutritional value (Volkman et al., 1989) and strong interactions have been seen between mesozooplankton grazers and diatoms (Hampton et al., 2006). Cyanobacteria, by contrast, have been recognized as a poor food source because their cell walls are resistant to digestion, they may contain toxins, and their filamentous colonies are difficult to graze upon (Allan, 1995). Diatoms may have been preferable even when in very low abundance because of their higher nutritional value and ease of being ingested over cyanobacteria.

Chlorophytes were grazed on just before the bloom and chlorophytes and cryptophytes were grazed on after the bloom though they were not abundant. Cyanobacteria were the dominant taxa just before and after the bloom, suggesting the microzooplankton may again have been selecting prey preferable to cyanobacteria. Hampton et al. (2006) determined that *Daphnia* feeding on cryptophytes allowed mesozooplankton to increase in abundance in a temperate lake

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and cryptophytes and chlorophytes are high in proteins, carbohydrates, and lipids (Volkman et al., 1989). Microzooplankton in Vancouver Lake may have benefited by feeding on cryptophytes and chlorophytes when cyanobacteria were abundant.

Microzooplankton Grazing on Cyanobacteria

The compositional data obtained from microscopical analysis of the dilution experiments revealed how microzooplankton grazing may have directly or indirectly affected the cyanobacteria community and ultimately the cyanobacteria bloom dynamics in the Lake.

Microzooplankton grazing on cyanobacteria was observed in the spring before the bloom and during the fall as the bloom was declining. In May cyanobacteria were the second most abundant prey taxa and experienced the highest microzooplankton grazing rate (0.97 d^{-1}). These rates are in line with Murrell and Hollibaugh's (1998) springtime measurement of microzooplankton grazing on cyanobacteria in Tomales Bay, California, which ranged between zero and 3.11 d^{-1}, and Tijdens et al.'s (2008) findings of 0.45-1.25 d^{-1} in a shallow lake in The Netherlands.

The relatively high grazing rate on cyanobacteria in spring may have been due to the availability of *Anabaena flos-aquae* during this time. *Aphanizomenon flos-aquae* forms colonies composed of long filaments, while *Anabaena flos-aquae* forms strands of spherical cells. The long, blade-like filaments of *Aphanizomenon flos-aquae* may be more difficult to graze than the differentiated "beads-on-a-string" morphology of *Anabaena flos-aquae*. Protist grazing on *Aphanizomenon* has not been observed often, but grazing on *Anabaena flos-aquae* has been documented. The ciliate *Nassula aurea* was observed grazing on small-celled filaments of *Anabaena flos-aquae* in temperate lakes in Cumbria, England and increased numbers of *Nassula aurea* were coincident with declines in *Anabaena* complexes (Canter et al., 1990). Additionally, *Nassula aurea* only grazed on bundles of *Aphanizomenon flos-aquae*, as opposed to individual filaments, such as were observed in my samples. Moreover, Tijdens et al. (2008) saw no grazing on filamentous cyanobacteria in a shallow lake in The Netherlands, but microzooplankton did

graze on unicellular cyanobacteria (0.45-1.25 d^{-1}), suggesting that microzooplankton may select for different species of cyanobacteria based on morphology.

In the weeks leading up to the dramatic increase in cyanobacteria abundance in Vancouver Lake, microzooplankton grazing rates on cyanobacteria in my experiments were negative. Hambright et al. (2001) found that mesozooplankton grazing rates in lakewater dominated by *Aphanizomenon* were 10-fold lower than grazing rates in water with no *Aphanizomenon*. Indeed, several studies have found that filamentous cyanobacteria, such as *Aphanizomenon*, interfere with zooplankton (i.e. *Daphnia*) grazing, ultimately leading to cyanobacteria dominance (Haney, 1987; Sterner, 1989; Gliwicz and Lampert, 1990; Vanni and Temte, 1990; Elser and Goldman, 1991; Kerfoot et al., 1988; Havens, 2008). It is possible that cyanobacteria morphology could similarly affect microzooplankton grazers.

The negative microzooplankton grazing on cyanobacteria during my summer experiments could also have been the result of preferential microzooplankton grazing on other co-occurring prey, namely diatoms, which could have released the cyanobacteria from grazing pressure. Modeling studies have shown microzooplankton to graze on phytoplankton other than harmful algal species even when these harmful species are the dominant species (Mitra and Flynn, 2006). In the field, Leonard and Paerl (2005) determined that microzooplankton exhibited preferential grazing, placing greater grazing pressure on co-occurring phytoplankton (diatoms and green algae) rather than toxic cyanobacteria. Preferential grazing by microzooplankton on prey other than cyanobacteria during my experiments conducted in June and July may have allowed *Aphanizomenon* to persist by releasing the cyanobacteria from grazing pressure, resulting in the formation of the bloom in Vancouver Lake.

Nutrients likely also influenced the formation of the cyanobacteria bloom. The available NO_3 was lowest during the peak of chlorophyll *a* and may have been depleted from the previous growth activity of the phytoplankton community but the limitation of NO_3 does not appear to hamper cyanobacteria growth as cyanobacteria can assimilate NH_4 instead of other available N sources (Blomqvist et al., 1994; Oliver and Ganff, 2000). In addition, low N:P values over the course of the bloom may have contributed to dominance by cyanobacteria (Smith, 1983; Crayton, 1993; Elser, 1999; Oliver and Ganf, 2000; Yamamoto, 2009).

Summary and Significance

Microzooplankton grazing appeared to play an important role in the formation and decline of the cyanobacteria bloom in Vancouver Lake. Negative microzooplankton grazing rates on phytoplankton, in particular cyanobacteria, immediately prior to the bloom suggest that the presence and/or selective feeding behavior of microzooplankton grazers could have allowed cyanobacteria to proliferate rapidly. Microzooplankton appeared to preferentially graze on the fastest growing phytoplankton taxa and to select more nutritious phytoplankton than cyanobacteria, even when cyanobacteria were the dominant taxa. Thus, microzooplankton may have a strong influence on the dynamics and timing of cyanobacteria blooms through grazing behavior.

These results provide quantitative measurements of microzooplankton grazing and phytoplankton growth rates that may be useful for comparing with those of other temperate, shallow lakes, particularly those freshwater systems plagued by frequent cyanobacteria blooms. Incorporating the biological component of microzooplankton grazing, in concert with chemical and physical factors contributing to harmful algal blooms, will assist resource managers in developing effective management strategies designed to prevent harmful algal blooms.

My findings may also be useful in understanding the overall role of microzooplankton in temperate aquatic food webs. Microzooplankton grazing on phytoplankton can lead to shifts in plankton assemblages and changes in food web dynamics, ultimately affecting fish and other organisms at higher trophic levels.

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