Plankton Monitoring and Zooplankton Grazing Assessment in Vancouver Lake, WA

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Year Two Annual Report (March 2008 – February 2009)

Introduction

Since March 2007 the Vancouver Lake Watershed Partnership has provided support for the Aquatic Ecology Laboratory at Washington State University Vancouver (WSUV) to assess the plankton and water quality in Vancouver Lake, in order to better understand and quantify the factors that influence seasonal cyanobacteria blooms. Vancouver Lake has experienced numerous nuisance blooms of cyanobacteria in the past decade (e.g. Wierenga 2005, 2006) – indeed, intense cyanobacteria blooms have forced the Clark County Health Department to close the Lake to all recreational uses for several days every summer since 2006. Excessive cyanobacteria abundance may have many detrimental effects, including development of surface scums, depleted oxygen levels, and (in some cases) production of toxins that can negatively affect aquatic life and humans (Carmichael 1992, Codd 1995, Sellner et al. 2003).

During Year One of the monitoring project (2007-2008), the primary goal was to quantitatively assess the abundance, composition and lake-wide distribution of plankton over a full year in Vancouver Lake. This report summarizes the results from Year Two of the assessment (March 2008 to February 2009), which was focused on two main objectives: 1) to measure the abundance and taxonomic composition of cyanobacteria, algae and zooplankton in Vancouver Lake over a second annual cycle, and 2) to conduct experiments to quantify the growth rates of cyanobacteria and algae, as well as the grazing rates of both protozoan and metazoan zooplankton on these populations, over the course of a bloom cycle.

Background

Growth in abundance or biomass of any population over time is a function of the net population growth rate and the initial population size:

$$dN/dt = r * N$$

where N = initial population density, t = time, and r = net growth rate. The net growth rate of algal or cyanobacterial populations may be positive or negative, depending on the balance of factors promoting algal growth and factors leading to algal mortality or loss, predominantly herbivorous grazing. Thus, net algal growth "r" can be estimated using the expression:

$$r = \mu - g$$

where μ = the "intrinsic" growth rate of algae/cyanobacteria (i.e. how the population would grow without grazing pressure) and g = the grazing rate of herbivores (protozoan and metazoan zooplankton). Under typical, steady-state lake conditions, net growth rate (r) will often hover somewhat above or below zero, depending on the availability of resources that promote growth, such as nutrients and light, and the intensity of grazing pressure. In temperate, eutrophic lakes during winter, light is often limiting to algal growth such that $\mu \leq g$, and algal densities remain

low. In spring, increasing light availability allows algae and cyanobacteria to utilize the high levels of nutrients available and intrinsic growth rates increase rapidly, typically outpacing the ability of grazers to compensate, such that $\mu > g$ and a large increase in algal abundance or "bloom" occurs.

Intrinsic growth rates of cyanobacteria and algae may often be predicted based on nutrient levels, light availability, etc. However, cyanobacterial/algal losses due to grazing are highly variable and dependent upon the composition of the consumer community. Understanding how the balance of these growth and grazing rates varies over the course of a bloom cycle is important for lake management, as the relative magnitudes of μ and g will help to determine which strategies for controlling the formation and/or duration of cyanobacteria blooms may be most effective.

For instance, if μ is consistently high under a range of grazing pressures, suggesting cyanobacterial growth is not limited by grazing, then it may be most appropriate to consider management actions that limit nutrient inputs or other physical factors that promote algal growth (e.g., through land use practices). Alternatively, if g is found to be consistently low or variable, this suggests that biomanipulation strategies to promote increased grazer population size and composition may prove effective, as has been shown in a range of lake systems (e.g. Elser et al. 2000, Paterson et al. 2002, Pechar 2005, Reissig et al. 2006). Indeed, the dominant cyanobacteria taxa (*Aphanizomenon flos-aquae, Anabaena flos-aquae*) present in the Lake have been shown to be affected by zooplankton grazing (Epp 1996, Chan et al. 2004). Moreover, knowing the differential grazing impact of small (protozoan) grazers relative to larger (zooplankton) grazers, provides important information for deciding what sort of biomanipulation is most appropriate and at which trophic level to target those efforts (e.g., piscivorous fish promoting zooplankton, or planktivorous fish promoting protozoan grazers).

Methods

Plankton abundance and composition:

Results of quarterly plankton sampling at 8 stations throughout Vancouver Lake during Year One of this project (March 2007 – February 2008) demonstrated no significant geographic variability in plankton abundance or composition. Thus, in Year Two we focused our field effort to weekly (June-September), bi-weekly (April-May and October-November) and monthly (December-March) sampling from a single station (Vancouver Lake Sailing Club dock).

At each sampling time, temperature and dissolved oxygen profiles from the surface to the bottom were obtained using a YSI 85 probe. In addition, relative subsurface light penetration was estimated by measuring the depth below the surface at which a Secchi disk was no longer visible.

Triplicate water samples were collected from the surface using a clean bucket, and subsamples taken for later laboratory analyses to measure nutrient concentration and chlorophyll *a* concentration. For nutrient analysis, 50-ml aliquots were obtained using a syringe equipped with a 0.45 μm filter, stored in a plastic vial and kept chilled until analysis. For chlorophyll *a* analysis, 20-100 ml aliquots were filtered over GFF filters, and the filters wrapped in foil and immediately frozen. Additional triplicate water samples were collected from the surface and subsamples preserved in 5% acid Lugol's solution, for enumeration and identification of cyanobacteria, algae and protozoan plankton. Triplicate vertical zooplankton net tows were

conducted to capture plankton >73µm in size, with the net contents concentrated and preserved in 5-10% formalin.

Upon return to the laboratory, the water and plankton samples were processed and analyzed according to the following protocols:

Subsamples of water for nutrient analysis were sent to the Marine Chemistry Lab at the University of Washington's School of Oceanography for determination of dissolved nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), phosphate (PO_4), and silicate (SiO_4) concentrations.

In order to measure chlorophyll concentrations, thawed GFF filters were placed in vials containing 20 ml of 90% acetone for 24 hours. The concentration of chlorophyll *a* suspended in the acetone was measured on a Turner Model 10 AU fluorometer, using the acidification method (Strickland & Parsons 1972).

To determine the abundance of planktonic protists and cyanobacteria, 1-10 ml aliquots of the Lugol's preserved water samples were settled overnight in Utermohl chambers, and the chambers examined using an Olympus CK-40 inverted microscope at 200-400x to enumerate and identify unicellular plankton ~5-150 µm in size. To determine the abundance and composition of the metazoan zooplankton, 5-25 ml aliquots of the formalin-preserved zooplankton net samples were examined using a Leica MZ-6 stereo microscope to enumerate and identify the metazoan taxa (e.g. cladocerans, copepods, rotifers). Aliquot size was varied to ensure a minimum of 300 individuals were enumerated per sample. Individuals were identified to the lowest possible taxon.

Growth and grazing experiments:

Dilution experiments: Dilution experiments (Landry & Hassett 1982, Landry et al. 1995) allow for the calculation of algal and cyanobacterial intrinsic growth rates simultaneously with the grazing rates of protozoan zooplankton. Lakewater containing the planktonic assemblage was collected from the surface at the Sailing Club dock station using a clean, acid-washed bucket. Lakewater for particle-free dilutions was collected similarly, and then filtered through glass fiber filters into clean carboys. Each dilution experiment was set up with five replicated dilution levels (10, 25, 50, 75 and 100%) of natural lakewater with filtered lakewater, including bottles for initial controls. Incubation bottles were amended with added nutrients (NO_3 and PO_4) to ensure replete conditions, along with non-nutrient control bottles. Bottles were incubated for 24 hours under ambient light conditions (light:dark) on a rotating plankton wheel inside a temperature-controlled chamber. Dilution bottles were sampled for chlorophyll biomass, and algal and cyanobacterial abundance at the beginning and end of each incubation. Subsamples from each bottle for enumeration of algae and cyanobacteria, and their potential protozoan grazers, were preserved in 5% Lugol's solution and analyzed using the approach described above.

Zooplankton incubation experiments: Large-volume incubation experiments (Rollwagen-Bollens & Penry 2003, Gifford et al. 2007) using selected metazoan zooplankton taxa allow for the calculation of diet selectivity and feeding rates of zooplankton on the natural assemblage of algae and other planktonic prey present at the time of sampling. Concurrent with each dilution experiment, separate incubation experiments with adults of representative metazoan zooplankton taxa (i.e. cladocerans, copepods, rotifers) feeding upon the natural assemblage of planktonic prey were conducted. Zooplankton were collected via vertical hauls of a 73-µm plankton net, returned to the laboratory and adults of target species sorted under dim

light into holding beakers. 500-ml incubation bottles were carefully filled with lakewater containing the natural assemblage of planktonic prey obtained from the surface using a clean, acid-washed bucket. Quadriplicate bottles containing only the natural assemblage were established as initial controls. Final controls (natural assemblage only) and final treatments (assemblage plus zooplankton predators) were prepared in quadriplicate and incubated in a temperature-controlled chamber for 12 hours overnight on a slowly rotating (0.5-1 rpm) plankton wheel. All bottles were subsampled and analyzed to enumerate and identify the algae, cyanobacteria and protozoans as described above. Zooplankton ingestion rates for each category of prey were estimated according to Marin et al. (1986).

Results

During Year Two of this project, Vancouver Lake was sampled from the Sailing Club dock 35 times between March 2008 and February 2009 (Table 1).

I. 2008 Trends in Water Quality and Plankton Abundance/Composition

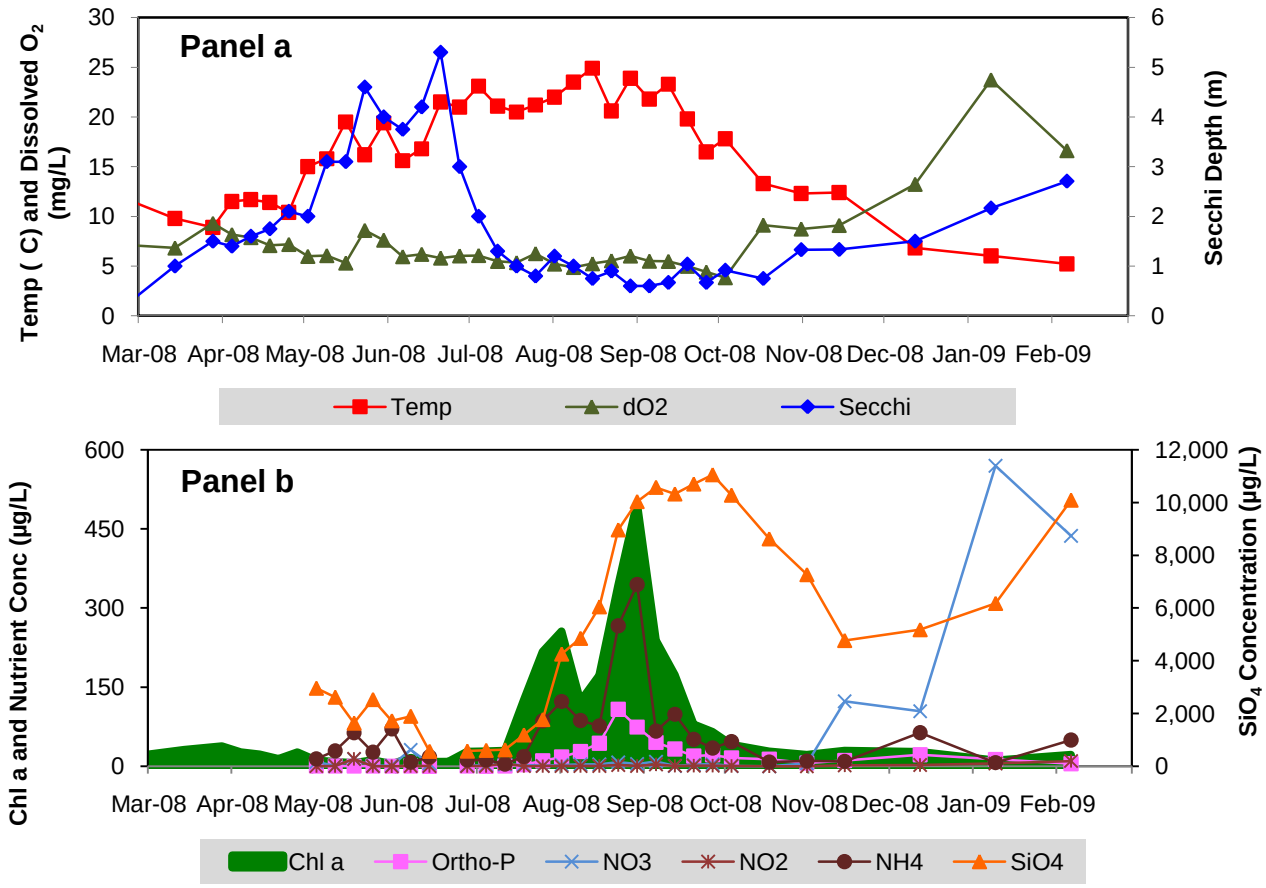

Figure 1. Panel a: Surface temperature, dissolved oxygen concentration, and Secchi depth measured from the Vancouver Lake Sailing Club dock between March 2008 and February 2009. Panel b: Mean surface chlorophyll *a* concentration and mean surface concentrations of dissolved inorganic phosphate (PO_4), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), and silicate (SiO_4) collected from the Vancouver Lake Sailing Club dock between March 2008 and February 2009.

Water quality. Surface temperatures in Vancouver Lake ranged from 5-25°C between March 2008 and February 2009, with highest values from July to September, following the typical pattern for a temperate aquatic system. Dissolved oxygen concentrations were low throughout the spring, summer and early autumn, but increased markedly from November through January 2009. Secchi depth, an indirect measure of overall water clarity, was shallow (i.e. low water clarity) in March 2008 but steadily deepened through the spring, reaching over 5 meters depth in early July. Secchi depths shallowed rapidly through July and remained <1.5 m throughout the summer and early autumn (Figure 1a, Table 1).

A seasonal signal was also evident in the pattern of chlorophyll and inorganic nutrient concentrations over the year. Chlorophyll *a* concentrations, a proxy for algal biomass, showed two summer peaks or "blooms", reaching ~280 µg/L in August and ~475 µg/L in September. Ammonium (NH_4) levels also peaked during the chlorophyll maxima, but remained low throughout the rest of the year. Silicate (SiO_4) concentrations were nearly undetectable in late June and July, but increased nearly 10-fold over the course of the chlorophyll bloom, likely due to reductions in diatom abundance and thus reduced uptake of silicate by these cells. Inorganic dissolved phosphate (PO_4) also peaked during the chlorophyll bloom in September, with nearly undetectable concentrations for the remainder of the year. By contrast, concentrations of nitrate (NO_3) were highest during the late fall and winter, and virtually undetectable during the late spring and summer (Figure 1b, Table 1).

Cyanobacteria and protist plankton abundance and composition. Two distinct peaks in the abundance of cyanobacteria were evident during 2008. Cyanobacteria abundance reached nearly $9 * 10^5$ cells/mL in late July, coincident with the first chlorophyll bloom. Cyanobacteria reached a second, higher maximum in September/October, with abundances > $1.2 * 10^6$ cells/mL. This larger peak occurred just after the second chlorophyll bloom, and cyanobacteria abundance remained elevated until November (Figure 2, Table 2).

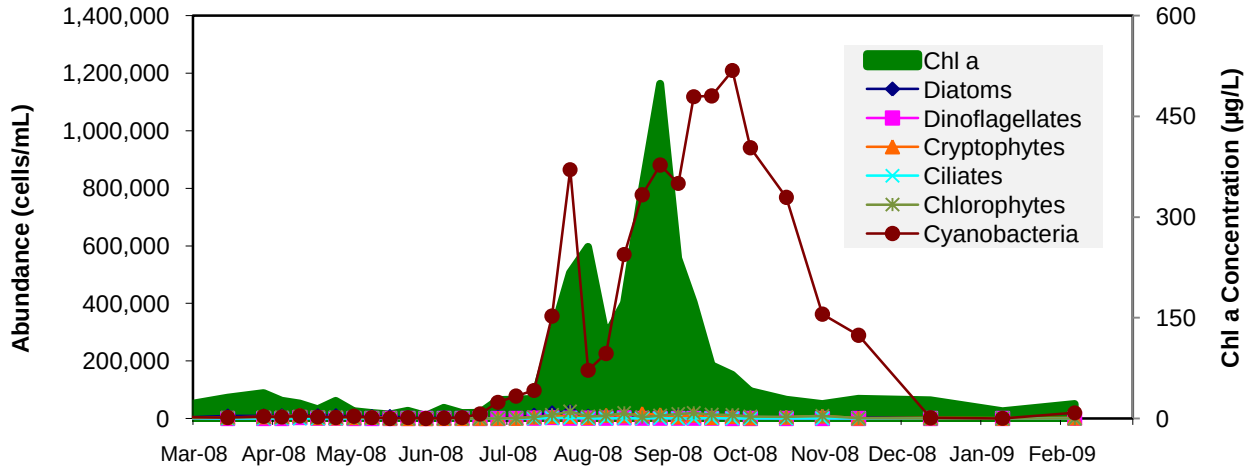

Figure 2. Mean abundance of major taxonomic groups of protist plankton and cyanobacteria collected from the Vancouver Lake Sailing Club dock between March 2008 and February 2009. Green shaded area represents mean surface chlorophyll *a* concentration measured over the same period.

Other important autotrophic protists (i.e. "algae") also showed seasonal peaks in abundance. Diatoms and flagellated algae (green algae or "chlorophytes" and cryptophytes) were abundant during spring 2008, but decreased during June and early July, a period of very low nutrient

concentrations. However, diatoms (especially *Aulacosira*, *Cyclotella* and *Nitzschia*) and chlorophytes (particularly *Scenedesmus*) both showed a very large peak (>2.5 * 10^4 cells/mL) coincident with the first chlorophyll bloom in late July. Cryptophytes, primarily *Cryptomonas*, were most abundant during the late summer chlorophyll bloom (Figure 3, Table 2).

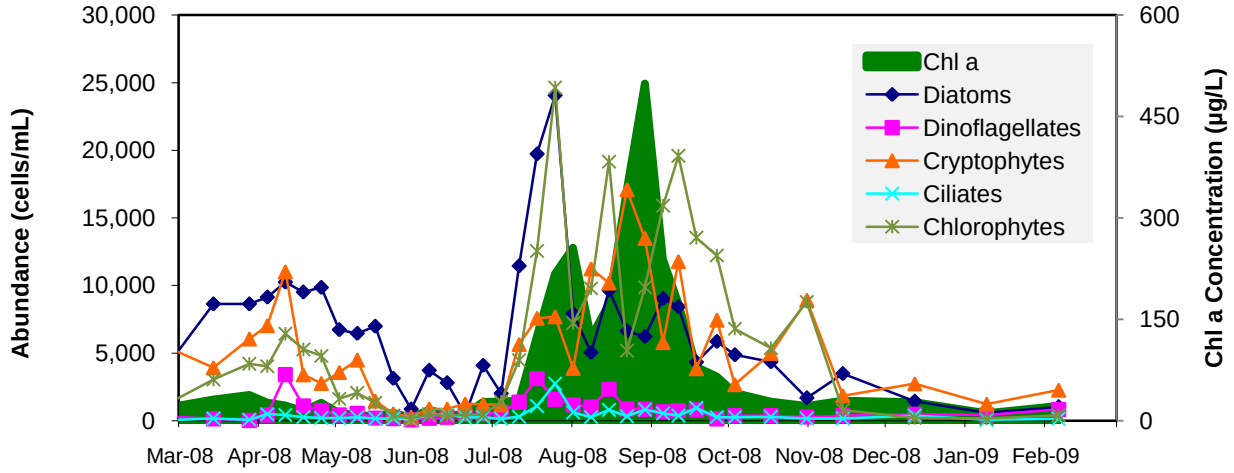

Figure 3. Mean abundance of major taxonomic groups of protist plankton (without cyanobacteria) collected from Vancouver Lake Sailing Club dock between March 2008 and February 2009.

With respect to the total carbon biomass of each group of unicellular plankton, cyanobacteria dominated the assemblage during the two chlorophyll blooms, but cyanobacteria biomass was highest in September and October, just following the second chlorophyll peak, and did not return to low levels until December (Figure 4, Table 2).

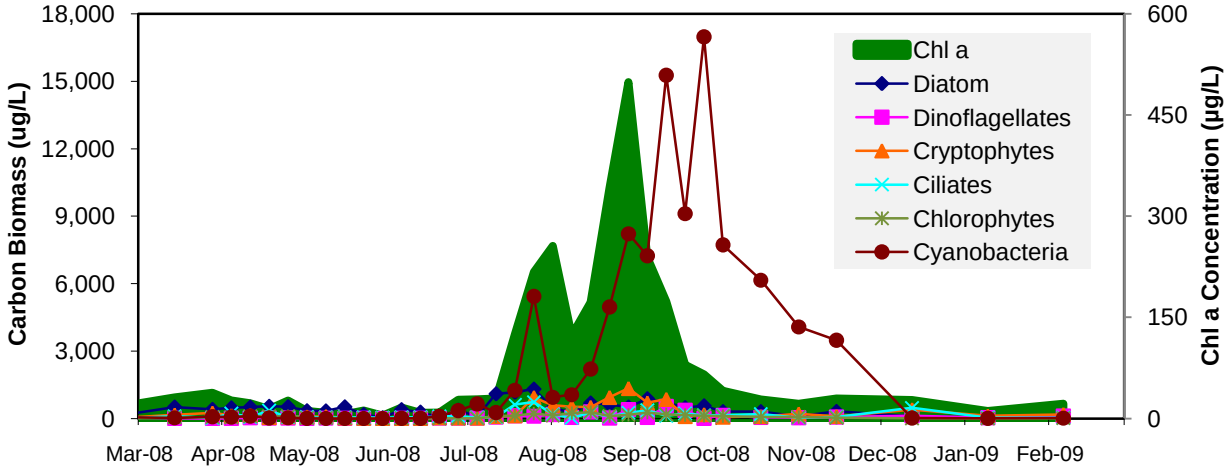

Figure 4. Mean carbon biomass of major taxonomic groups of protist plankton and cyanobacteria collected from the Vancouver Lake Sailing Club dock between March 2008 and February 2009.

The two major peaks in cyanobacterial abundance and biomass were distinctly different in composition. The July 2008 peak consisted of a mixed assemblage dominated by *Aphanizomenon flos-aquae* and a small number of less common taxa, particularly *Merismopedia*. In contrast, the second peak in late August/early September included a significant population of *Anabaena flos-aquae*. The extended period of high cyanobacteria abundance from September

through November, while initially including *Anabaena*, was strongly dominated by *Aphanizomenon* (Figures 5 and 6, Table 2). It should be noted, however, that the samples analyzed from the late fall *Aphanizomenon* bloom were extremely difficult to enumerate accurately. *Aphanizomenon flos-aquae* forms densely aggregated, filamentous colonies that make enumerating individual cells problematic. Thus, the abundance (and corresponding biomass) estimates may be overestimated in October and November, as suggested by the low chlorophyll levels during that period. In addition, the cyanobacteria cells may also have been low in chlorophyll content, indicative of the waning bloom.

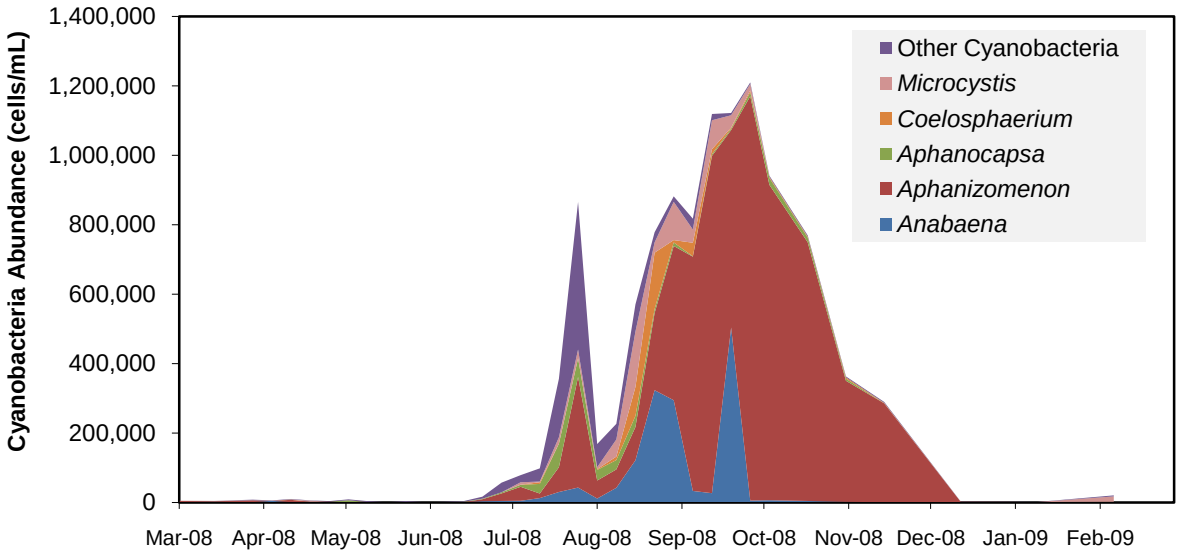

Figure 5. Mean abundance of the major cyanobacteria taxa collected from Vancouver Lake Sailing Club dock between March 2008 and February 2009.

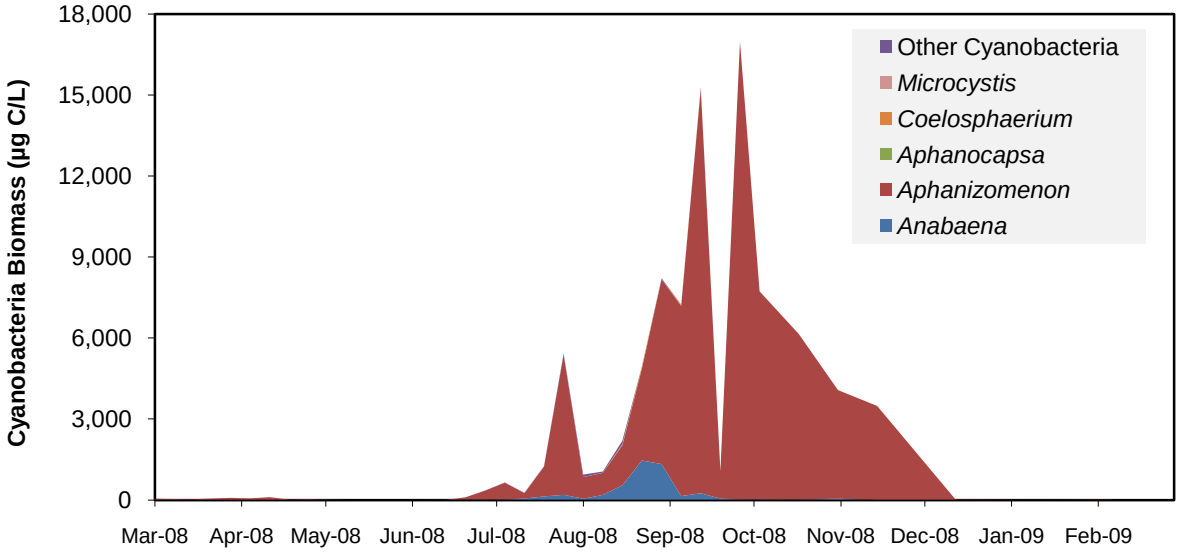

Figure 6. Mean biomass of the major cyanobacteria taxa collected from Vancouver Lake Sailing Club dock between March 2008 and February 2009.

There is clearly a compositional shift in the unicellular plankton community between winter, spring and summer in Vancouver Lake. A diverse assemblage of diatoms and flagellates in spring shifted abruptly in mid-summer to domination by cyanobacteria throughout the late summer and early autumn. Diversity increased again in winter, but to a community more dominated by cryptophytes and dinoflagellates (Figure 7a,b).

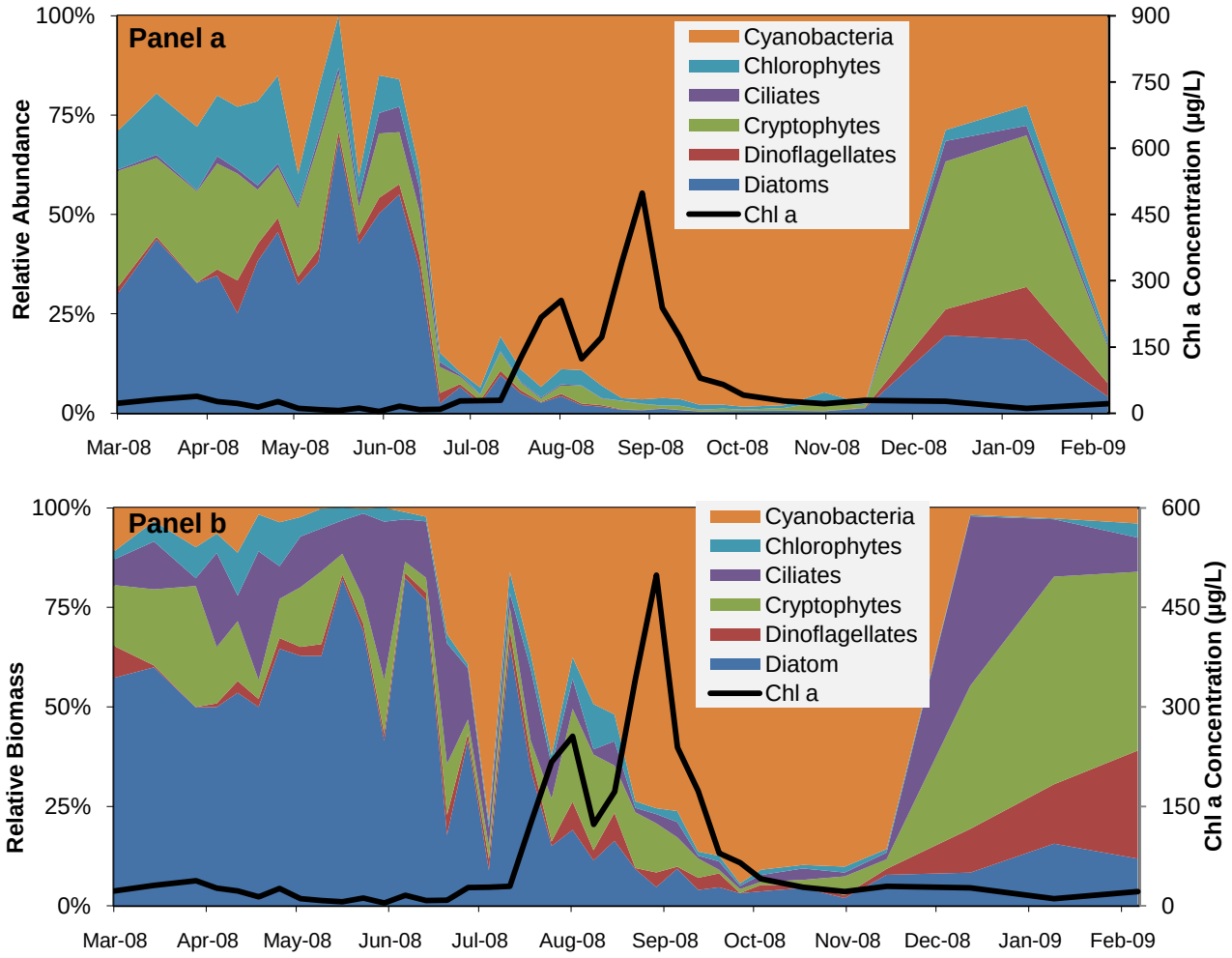

Figure 7. Panel a: Relative abundance of major taxonomic groups of protist plankton and cyanobacteria collected from Vancouver Lake Sailing Club dock between March 2008 and February 2009. Black line indicates mean chlorophyll a concentration measured over the same period. Panel b: Relative biomass of major taxonomic groups of protist plankton and cyanobacteria collected from the Vancouver Lake Sailing Club dock between March 2008 and February 2009.

Metazoan zooplankton abundance and composition. Zooplankton abundance was highly variable throughout the year, dominated numerically by small rotifers (mainly *Polyarthra*, *Asplanchna*, *Brachionus* and *Keratella*), which exhibited a "boom-bust" pattern of high vs. low abundance. The major life stages of the copepod *Diacyclops thomasi* (naupliar larvae, juvenile copepodids and adults) also showed varying peaks, often with bursts of nauplii followed by peaks in juveniles and then adults. This cohort progression was particularly evident following

the first chlorophyll bloom in late July. Notably, nearly all the metazoan zooplankton taxa (except the cladoceran *Daphnia retrocurva*) were quite low in abundance during June and early July – the period when nutrient concentrations as well as cyanobacteria and protist plankton abundances were also very low. Rotifer abundances were also notably high during the extended *Aphanizomenon flos-aquae* bloom in October and November (Figures 8 and 9, Table 3).

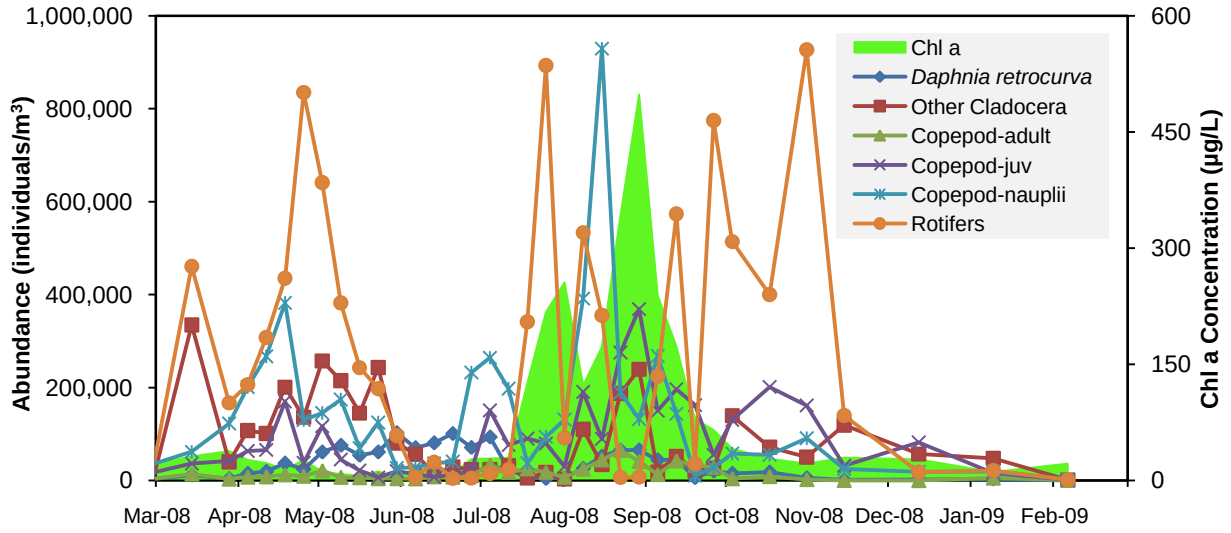

Figure 8. Mean abundance of major taxonomic groups of metazoan zooplankton collected from Vancouver Lake Sailing Club dock between March 2008 and February 2009.

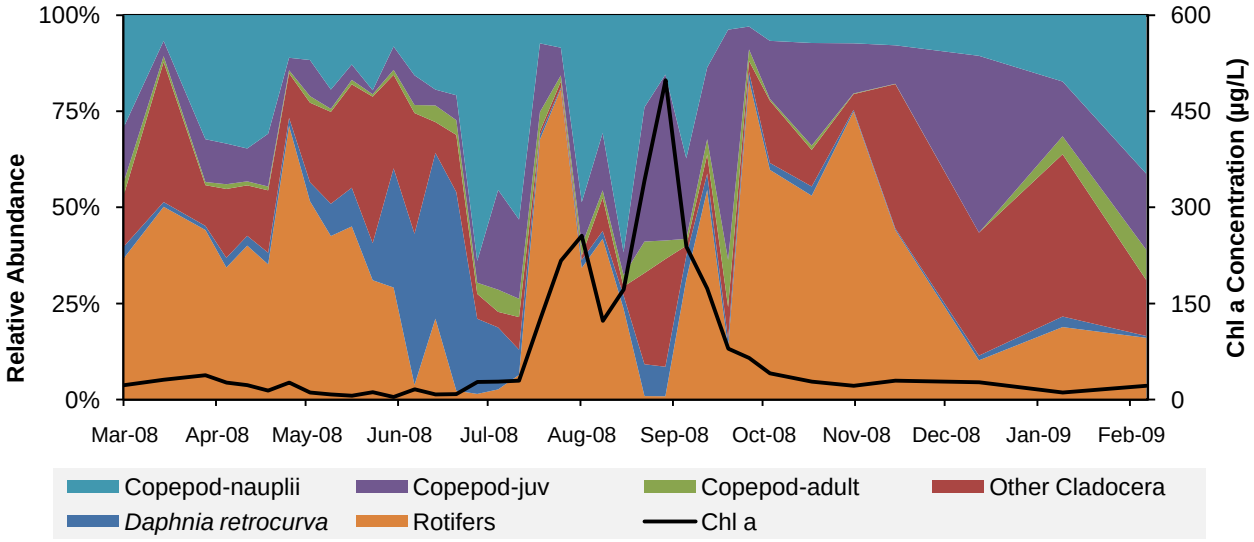

Figure 9. Relative abundance of major taxonomic groups of metazoan zooplankton collected from Vancouver Lake Sailing Club dock between March 2008 and February 2009.

II. Inter-annual Patterns (2007 – 2009)

Water quality. The most striking difference between year one (March 2007 – February 2008) and year two (March 2008 – February 2009) was the substantial deepening of the Secchi

depth during May-June 2008 compared to the spring of 2007. Vancouver Lake exhibited exceptional water clarity during this period, with Secchi depths > 2 meters for nearly three months, deepening to > 5 meters in early June (Figure 10a).

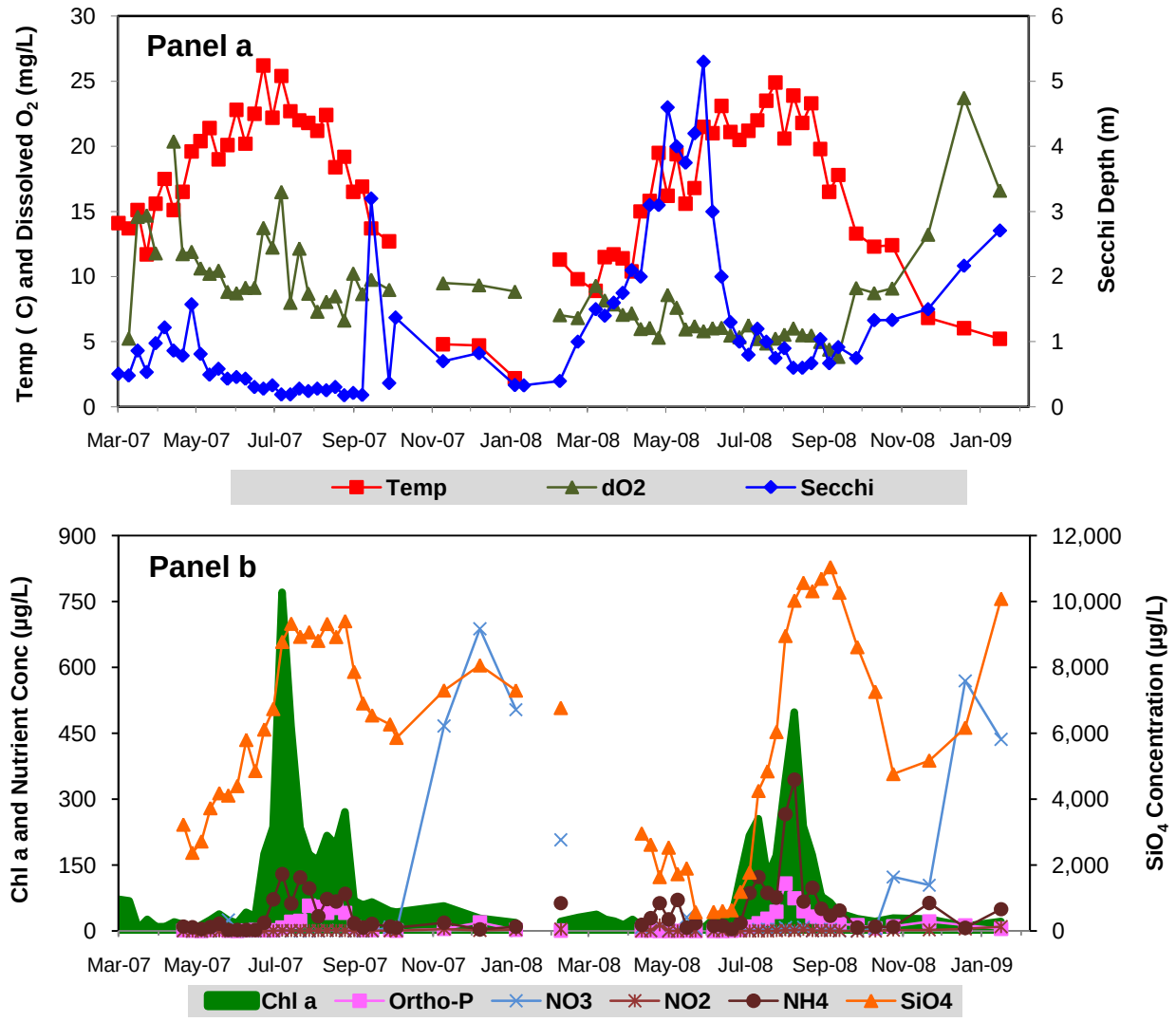

Figure 10. Panel a: Surface temperature, dissolved oxygen concentration, and Secchi depth measured from the Vancouver Lake Sailing Club dock between March 2007 and February 2009. Panel b: Mean surface chlorophyll *a* concentration and mean surface concentrations of dissolved inorganic phosphate (PO_4), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), and silicate (SiO_4) collected from the Vancouver Lake Sailing Club dock between March 2007 and February 2009.

This period of low turbidity and increased water depths coincided with very high outflows in the Columbia River, which likely increased flows into the Lake. This may also have had a diluting effect on nutrient concentrations during June 2008, as PO_4, NH_4 and especially SiO_4 concentrations were all nearly undetectable at that time (Figure 10b). By contrast, nutrient concentrations, particularly PO_4 and NH_4, were generally higher during the 2008 bloom period and peaked concurrently with the maximum chlorophyll levels in late August, compared to 2007.

Cyanobacteria and protist plankton abundance and composition. In both 2007 and 2008 cyanobacteria accounted for the two summer chlorophyll blooms that occurred in Vancouver Lake. However, in 2008 cyanobacteria abundance remained quite elevated following the chlorophyll bloom, suggesting the cyanobacteria cells were degrading and contained very low pigment concentrations (Figure 11).

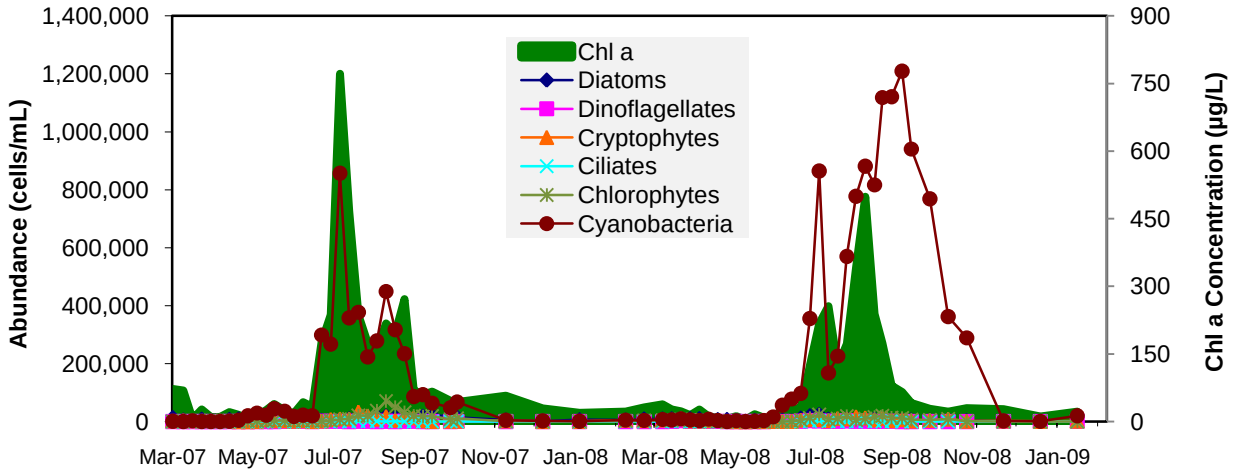

Figure 11. Mean abundance of major taxonomic groups of protist plankton and cyanobacteria collected from the Vancouver Lake Sailing Club dock between March 2007 and February 2009. Green shaded area represents mean surface chlorophyll *a* concentration measured over the same period.

Among the protist community (unicellular algae and protozoans) abundances were roughly comparable between 2007 and 2008, except for a very large pulse of chlorophytes in August 2007 that was not observed in summer 2008. However, while overall abundances did not vary substantially between years, the timing of maximal abundance of different algal groups relative to the cyanobacteria blooms differed. In 2007 the highest abundance of diatoms, chlorophytes and cryptophytes occurred after the major summer cyanobacteria peak, while in 2008 diatoms and chlorophytes were most abundant coincident with the cyanobacteria blooms (Figure 12).

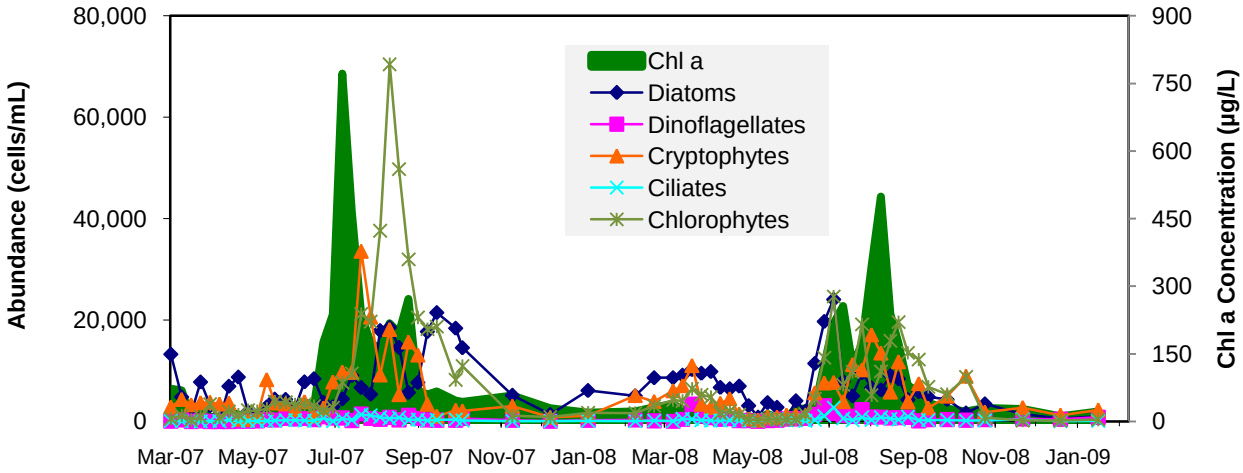

Figure 12. Mean abundance of major taxonomic groups of protist plankton (without cyanobacteria) collected from Vancouver Lake Sailing Club dock between March 2007 and February 2009.

Notably, the cyanobacteria community composition differed between the blooms of both years, with *Anabaena flos-aquae* dominant in 2007 and *Aphanizomenon flos-aquae* the primary species present in the first summer bloom of 2008. The second, larger bloom of 2008 began with a mixed assemblage of both *Anabaena* and *Aphanizomenon* in August, but became completely dominated by *Aphanizomenon* throughout the late summer and fall (Figures 13 and 14).

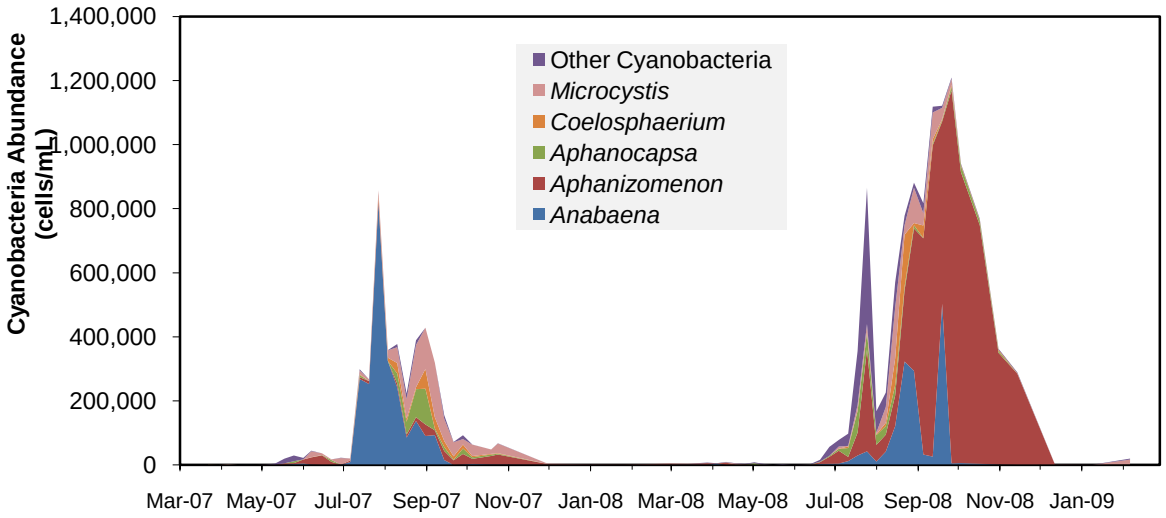

Figure 13. Mean abundance of the major cyanobacteria taxa collected from Vancouver Lake Sailing Club dock between March 2007 and February 2009.

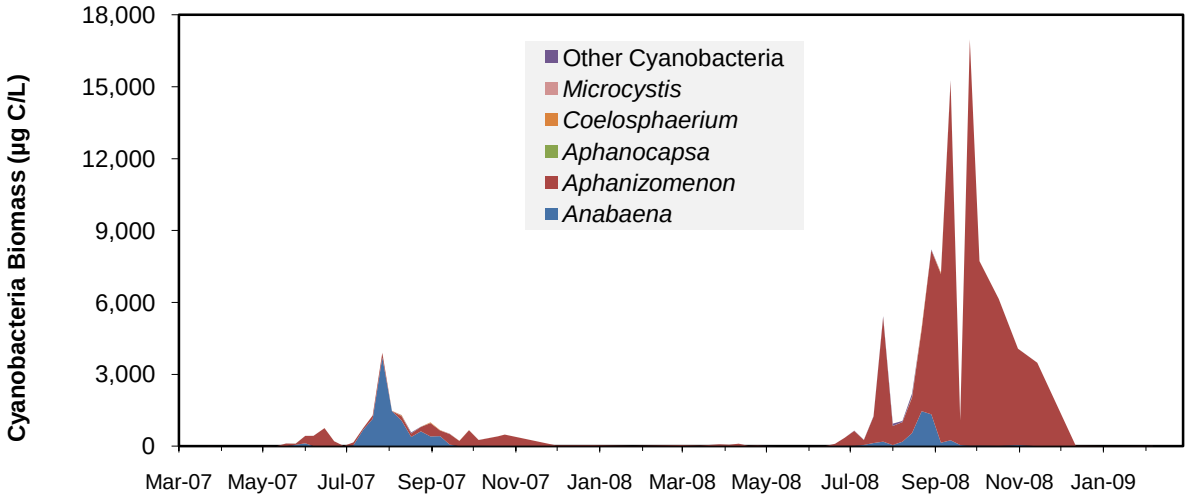

Figure 14. Mean biomass of the major cyanobacteria taxa collected from Vancouver Lake Sailing Club dock between March 2007 and February 2009.

Metazoan zooplankton abundance and composition. In summer 2007 there was a clear successional pattern among the copepod population (chiefly *Diacyclops thomasi*) from naupliar larve to juveniles to adult life stages. However, in 2008 the timing and magnitude of copepod life stage abundances were more variable. In addition, the abundance of cladoceran taxa other than *Daphnia retrocurva* (mainly *Daphnia laevis* and *Eubosmina* sp.) were much more prevalent in 2008 than in 2007, particularly in late spring and during the late summer cyanobacteria bloom.

Also, while the composition of rotifers (mainly *Polyarthra*, *Asplanchna*, *Brachionus* and *Keratella*) remained comparable between 2007 and 2008, abundances of these taxa were generally higher in 2008 and also more variable. The largest and longest period of high rotifer abundance coincided with the extended period of *Aphanizomenon* cyanobacteria abundance in late autumn (Figure 15).

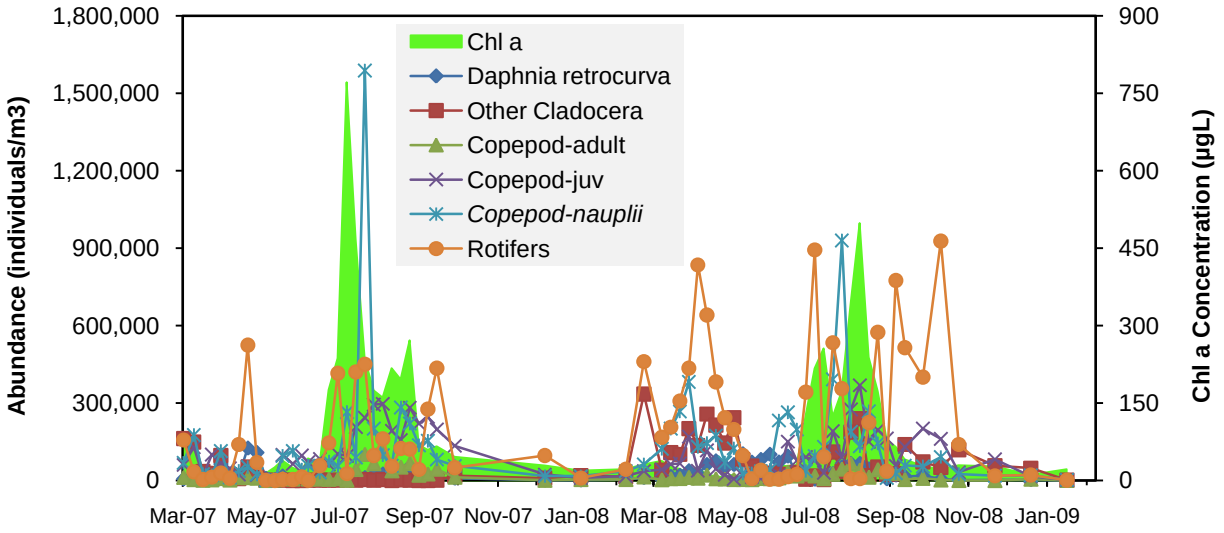

Figure 15. Mean abundance of major taxonomic groups of metazoan zooplankton collected from Vancouver Lake Sailing Club dock between March 2007 and February 2009.

Overall plankton composition patterns. The composition of the plankton in Vancouver Lake was comparable between 2007 and 2008 among major taxonomic categories, however the timing of when particular taxa were most abundant did vary between years. In summer 2007 the major bloom was dominated by *Anabaena flos-aquae* cyanobacteria, followed by relatively high numbers of various copepod life stages (nauplii, juveniles and adults). In 2008 the major blooms consisted primarily of *Aphanizomenon flos-aquae* cyanobacteria, with a higher relative abundance of cladocerans (e.g. *Daphnia* spp.) during the peak of the bloom, followed by large numbers of rotifers as well as larval and juvenile copepods (Figure 16a,b).

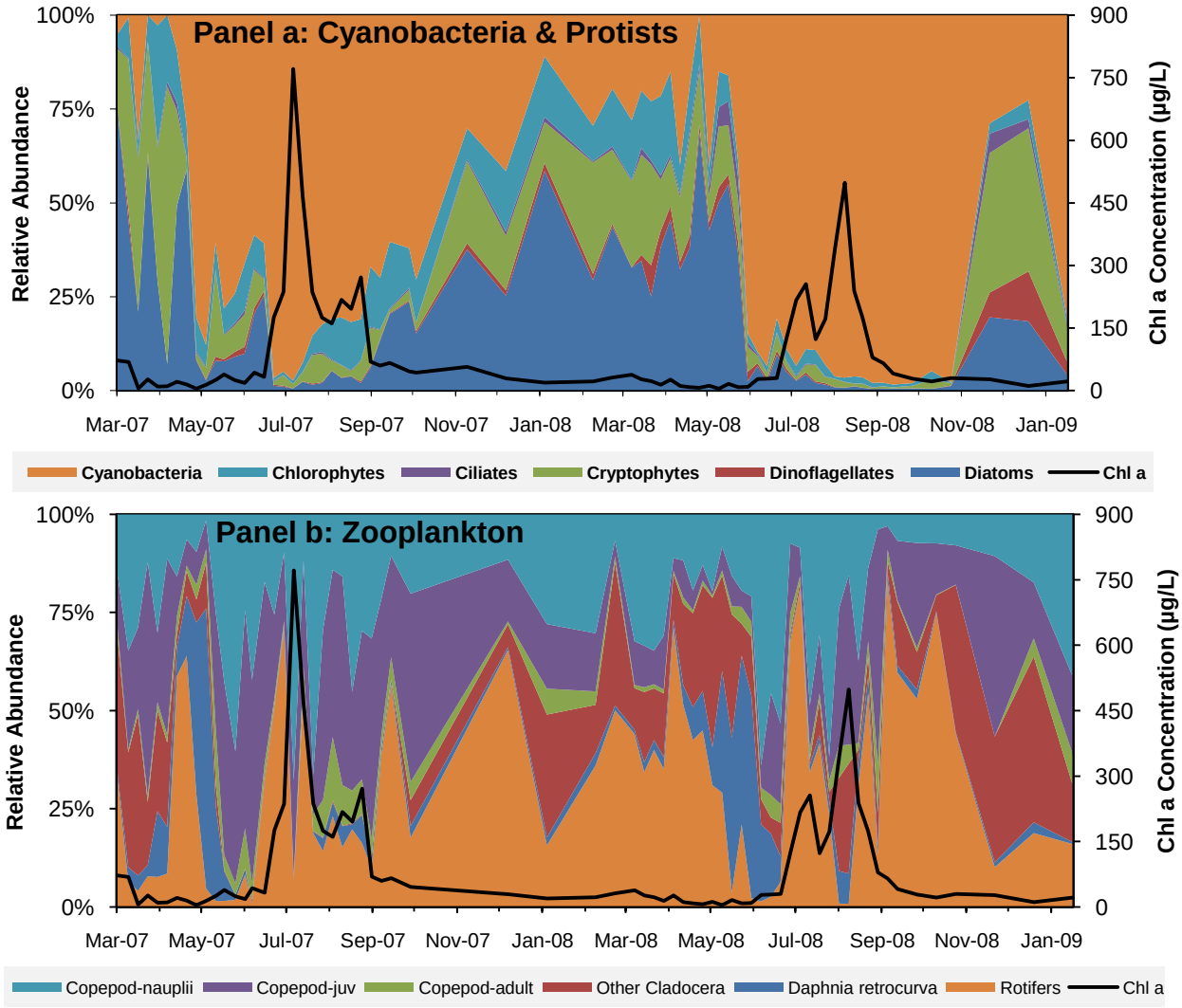

Figure 16. Relative abundance of cyanobacteria, protist and metazoan zooplankton taxa collected from the Sailing Club dock between March 2007 and February 2009. Black lines indicate chlorophyll concentration over the same period. Panel a: relative abundance of cyanobacteria and protists. Panel b: relative abundance of metazoan zooplankton.

III. Algal Growth and Zooplankton Grazing Rates

Cyanobacterial/algal growth rates and protist grazing rates. Between January 2008 and January 2009, 18 dilution experiments to determine algal community growth rates and protist community grazing rates were conducted (Table 4). Each individual graph in Figure 17 shows how net growth of photosynthetic organisms (both cyanobacteria and eukaryotic algae), as measured by increases in chlorophyll concentration over each 24-hr experimental incubation, varied over different proportions of unfiltered lakewater diluted with lakewater that had been filtered to remove all particles >1 µm in size. The lines on each graph represent the linear regression between observed net growth rates of algae vs. the fraction of unfiltered lakewater in each incubation treatment. The y-intercept (as calculated in the regression model) is the estimate of "intrinsic" algal population growth rate (i.e. what algal growth would be without the presence

of grazers). The slope of the regression line represents the community grazing rate of protist grazers. In general, the regression relationships were well-supported (mean r^2 values for all experiments > 0.5, Table 4).

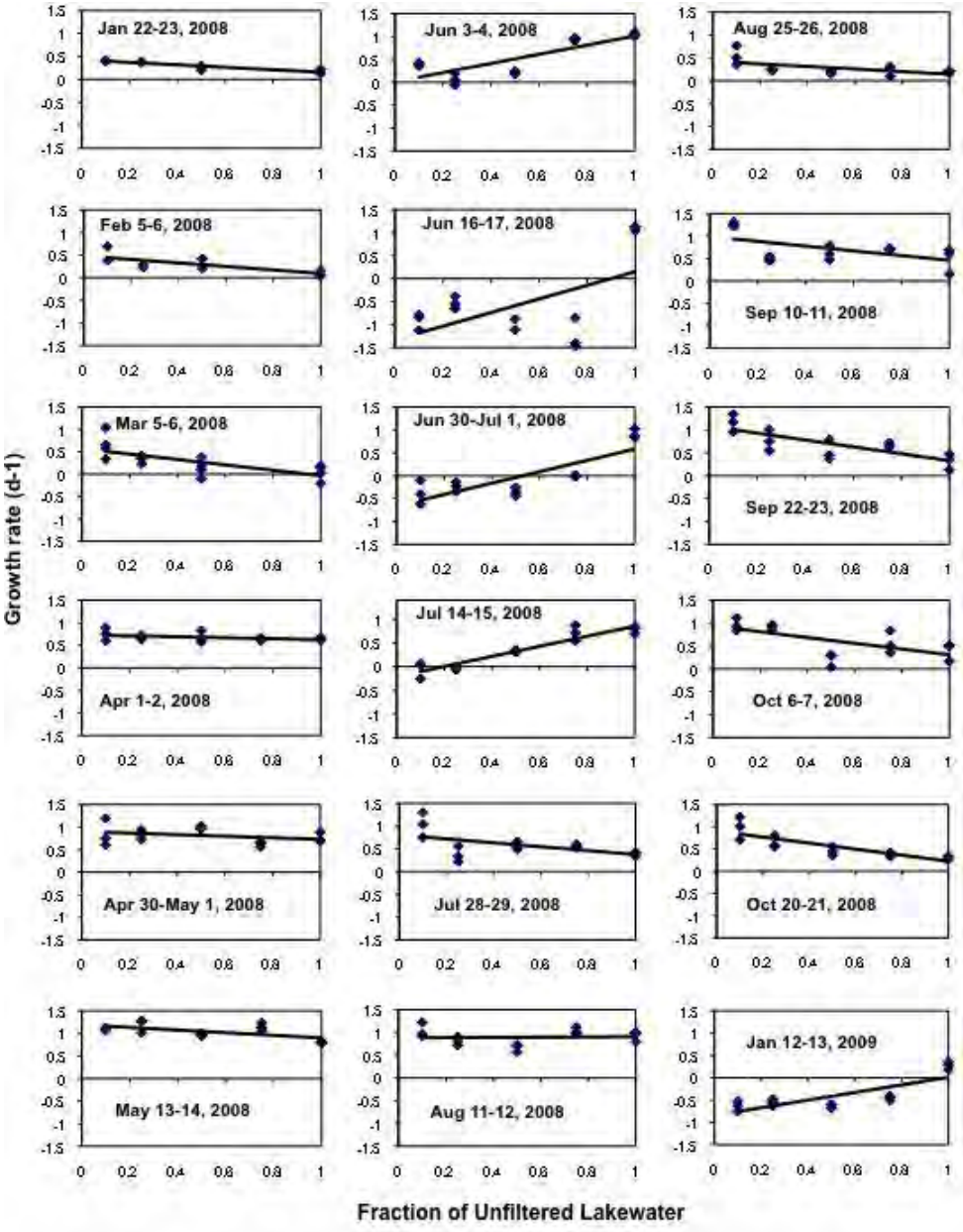

Figure 17. Plots of regression relationships between net growth rates observed in treatment bottles and degree of dilution among treatments for 18 dilution experiments conducted using water and organisms collected from the Vancouver Lake Sailing Club dock between January 2008 and January 2009.

The relationship of algal community intrinsic growth rates (units: d^{-1}) to protist community grazing rates (units: d^{-1}) from each experiment over the course of spring, summer and autumn 2008 are shown in Figure 18. From January to March 2008 algal growth and protist

grazing rates were variable, but nearly equal, suggesting the rate of protist grazing was high enough to keep pace with the rate of algal growth. However, from April to early June the relationship between algal growth rates and protist grazing rates diverged sharply, with algal growth increasing from ~0.6 d^{-1} to a maximum measured community growth rate of 1.2 d^{-1}. [For perspective, a community growth rate of 0.7 d^{-1} corresponds to a doubling of the population per day and a rate of 1.1 d^{-1} corresponds to a tripling of the population per day, assuming exponential growth.] In May 2008 the algal community (including cyanobacteria and eukaryotic algae) was therefore growing somewhat faster than a 3-fold increase per day, which is extremely quickly. On the other hand, protist grazing rates over this same period were generally decreasing, suggesting that these grazers were no longer able to consume algae as quickly as the algae were reproducing (Figure 18, Table 4). Notably chlorophyll levels in the Lake were low during spring. Thus even though the algal community was doubling or tripling in size on a daily basis, the initial population sizes were small enough that overall algal biomass (as measured by chlorophyll concentration) did not evidence a "bloom." Another consideration is the effect of larger (mesozooplankton) grazers on algal biomass, as discussed below.

Algal growth rates during the cyanobacteria bloom period from late July through October were also high (>0.7 d^{-1}), except during the maximum chlorophyll peak in early September when growth rates decreased to below 0.5 d^{-1}. However, in contrast to the late spring, protist grazing rates generally increased over the bloom period in step with the increasing algal growth rates, suggesting the protist grazer community maintained a substantial influence on algal mortality (Figure 18, Table 4).

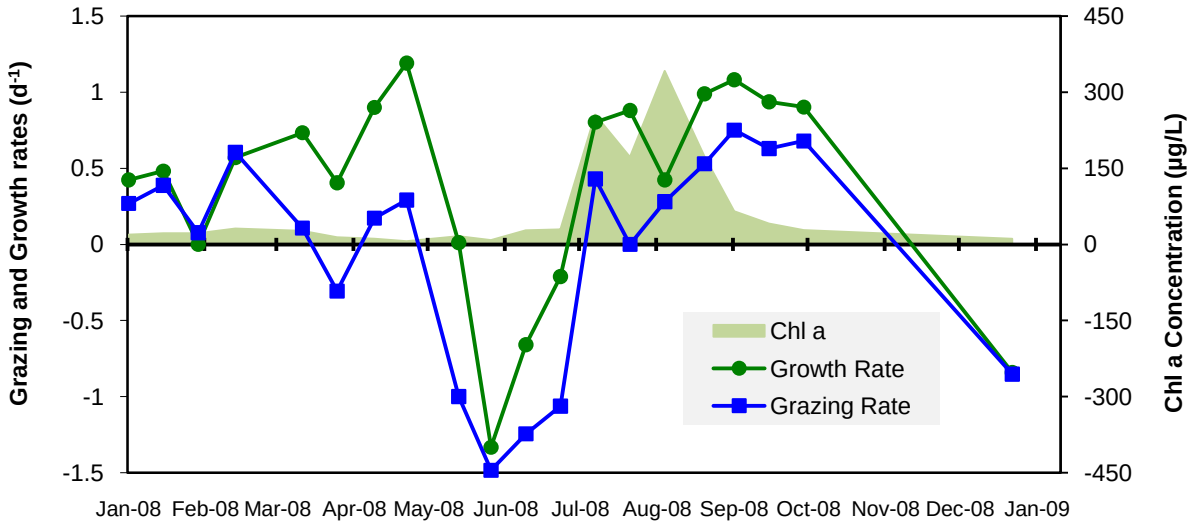

Figure 18. Algal community intrinsic growth rates (day^{-1}) and protozoan community grazing rates (day^{-1}) estimated from 18 dilution experiments conducted in Vancouver Lake between January 2008 and January 2009. Shaded area represents chlorophyll concentrations measured at each experimental sampling date.

The most notable pattern in growth and grazing rates occurred during the 5-week period from mid-June to late July preceding the explosive increase in cyanobacteria abundance and chlorophyll levels. Algal community intrinsic growth rates were negative during this period, with rates near -1.2 d^{-1} at the end of June (Figure 18, Table 4). Negative algal growth rates determined in bottle incubation experiments simply indicate that over the course of the

incubation algal abundance decreased relative to initial levels, i.e. cell losses exceeded cell gains, even when grazers were nearly absent. Such periods of negative population growth (i.e., numerical decline) are common in nature. It is less typical, however, to observe negative grazing rates. Negative protist community grazing rates calculated from dilution experiments are the result of algal cells actually growing more rapidly in the presence of grazers.

The negative grazing rates observed in Vancouver Lake during June suggest there could have been a "trophic cascading effect" occurring during the incubations, in which large protist grazers (e.g. large ciliates) could have been consuming smaller protist grazers (e.g. small dinoflagellates) who then were prevented from grazing small algae. As a result, small algae could increase in abundance over the incubation, and would grow even more rapidly in treatments with higher ratios of grazers to prey. We are pursuing this possibility by doing additional, more detailed, taxon-specific analyses of these samples. In addition to these potential trophic interactions within the protist community, the environmental conditions were also very unusual at this time. Lake levels and Secchi depths were exceptionally deep, likely due to very high Columbia River outflows, and nutrient levels (especially SiO_4, which is required by diatoms) were nearly undetectable. These physical factors could also have influenced the trophodynamics by limiting algal growth and diluting the concentrations of particles and organisms such that encounter rates between grazers and prey, and thus grazing rates, were reduced.

Zooplankton feeding incubation experiments. Feeding incubation experiments with metazoan zooplankton predators collected from Vancouver Lake were conducted in conjunction with protist community dilution experiments from July through October 2008. The zooplankton taxa selected for use as predators in each experiment were determined by observing the zooplankton community present at the time of sampling and choosing the one, and sometimes two, species that were both highly abundant and had enough individuals at or near the adult stage to ensure sufficient densities for all treatment bottles. The predator in each of the incubation experiments reported here was the copepod *Diacyclops thomasi*. In each experiment, 40 adult female copepods were incubated for 12 hours in a medium of unfiltered lakewater, and the change in prey abundances over the incubation used to calculate copepod ingestion rates and prey selectivity.

Figure 19 shows the prey preferences of *Diacyclops thomasi*, determined by comparing the relative abundance of prey taxa available at the beginning of the experiment to the relative abundance of prey taxa consumed during the incubation. In mid-July, just prior to the first summer bloom, >75% of the total prey field available to *D. thomasi* consisted of cyanobacteria cells (a mixed assemblage dominated mostly by *Aphanizomenon flos-aquae*) with diatoms and chlorophytes making up most of the remainder. However, cyanobacteria represented >95% of the total cells consumed by *D. thomasi*, suggesting copepods were preferentially targeting these prey (Figure 19a). In September, at the height of the chlorophyll bloom, cyanobacteria (mostly *Aphanizomenon*, but also *Anabaena*) accounted for nearly 99% of the total cells available to *D. thomasi*; however at this time the copepods consumed small (<15 μm in size) diatoms out of proportion to their availability. Thus, even though the majority of *D. thomasi* diet consisted of cyanobacteria, during the period of highest chlorophyll the copepods were preferentially targeting small diatoms (Figure 19b). Finally, in October when *Aphanizomenon* cyanobacteria were near the height of their extended bloom, but chlorophyll levels had decreased, *D. thomasi* were apparently avoiding the consumption of cyanobacteria altogether, and consuming

dinoflagellates and other flagellated cells (chlorophytes and cryptophytes) well out of proportion to their availability in the field (Figure 19c).

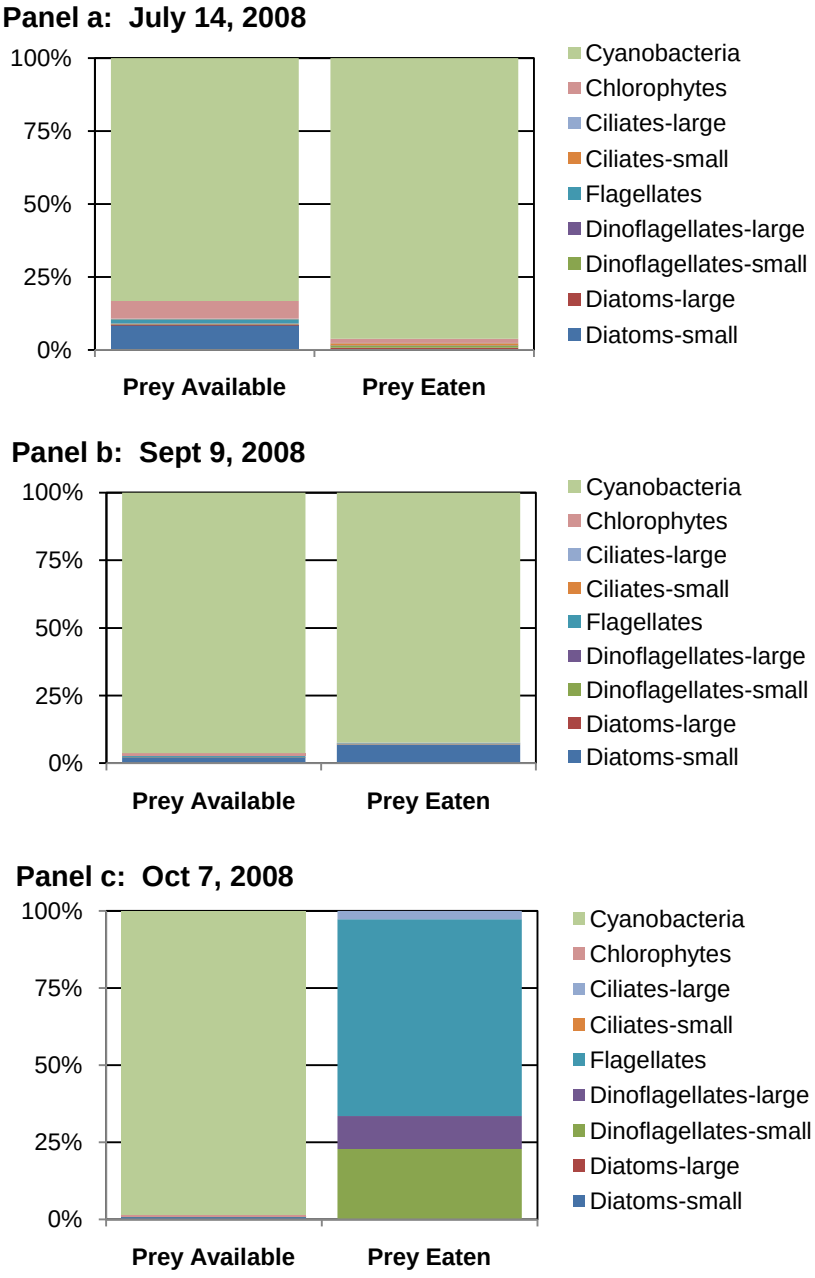

Figure 19. Proportions of major prey taxa groups available to metazoan zooplankton (*Diacyclops* sp. copepods) compared to the proportions of major prey taxa groups consumed by the grazers during three 12-hour feeding incubation experiments conducted using organisms collected in Vancouver Lake over the summer bloom period of 2008.

The measured ingestion rates of *Diacyclops thomasi* adults reflect both the apparent prey preferences of the copepod as well as the magnitude of the abundances of different prey types. In both July and September the rates of ingestion of prey carbon biomass (µg carbon consumed per predator per hour) were relatively high, with an exceptionally high ingestion rate on

cyanobacteria in September, a time when these cells were very abundant and of potentially higher nutritional quality, based on high chlorophyll concentrations, as compared to October. In July, prior to the first summer chlorophyll/cyanobacteria bloom, *D. thomasi* had highest ingestion rates on non-algal prey, namely large (>15 µm) dinoflagellates and ciliates. During the bloom in September, the copepods maintained an exceptionally high ingestion rate on cyanobacteria, but also ingested diatoms at relatively high rates. However in October, while cyanobacteria abundance was still high but chlorophyll levels were lower, *D. thomasi* ingestion rates were quite low and were exclusively targeted on dinoflagellates and small diatoms (Figure 20).

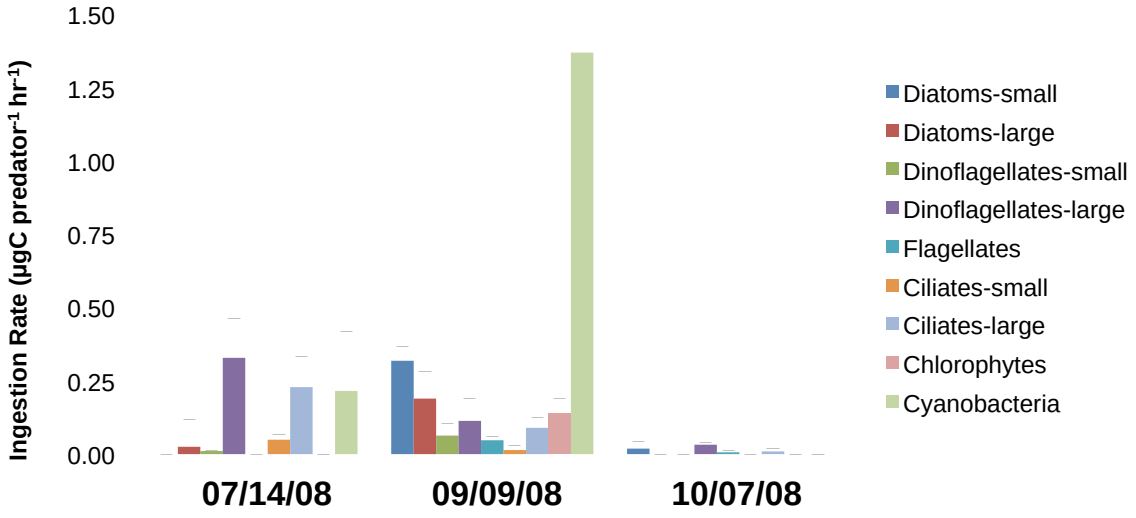

Figure 20. Mean ingestion rates of *Diacyclops thomasi* adult female copepods feeding on natural assemblages of planktonic prey in Vancouver Lake during three incubation experiments conducted using organisms collected from the Sailing Club dock. Error bars represent standard error.

IV. Grazing Impact

Protist grazing impact. The dilution experiment results produced quantitative estimates of algal community growth rates and protist community grazing rates, on a per day basis. These rates are particularly useful for assessing the pathways and fluxes of materials (i.e. carbon) through the planktonic food web. However, it is also useful to estimate the potential grazing impact of protist grazers on algal communities. The protist community grazing rates obtained in these experiments are a measurement of the proportion of the algal/cyanobacterial community removed due to grazing per day, incorporating the exponential growth on the part of the algae. By converting the calculated community grazing rates into linear units, these rates become a direct measure of the percent of algal/cyanobacterial standing stock removed due to grazing per day, referred to as grazing impact. These grazing impact results are presented in Figure 21.

Protist grazing impact was relatively high during early spring 2008, ranging from 30-75% of standing stock consumed per day. This likely held algal standing stocks at relatively low and stable levels. During late spring, protist grazing impacts were lower, consuming <30 % of algal biomass per day, likely allowing algal abundances to increase – or at least not limiting algal population growth. Interestingly, protist grazing impacts were negative during June and July, during the period of extremely low nutrient concentrations and negative measured algal growth

and protist grazing rates. As discussed above, this suggests potential trophic interactions among different types of protist grazers and algal populations, which may have resulted in the shift in algal community composition from dominance by diatoms to dominance by cyanobacteria. During the chlorophyll *a* peak from August through September, however, protist grazing impacts were again high, ranging from 50-70% of algal standing stock, and then increased further to around 100% of algal standing stock during October and November, when chlorophyll concentrations were much reduced, but cyanobacteria abundance was still very high (Figure 21).

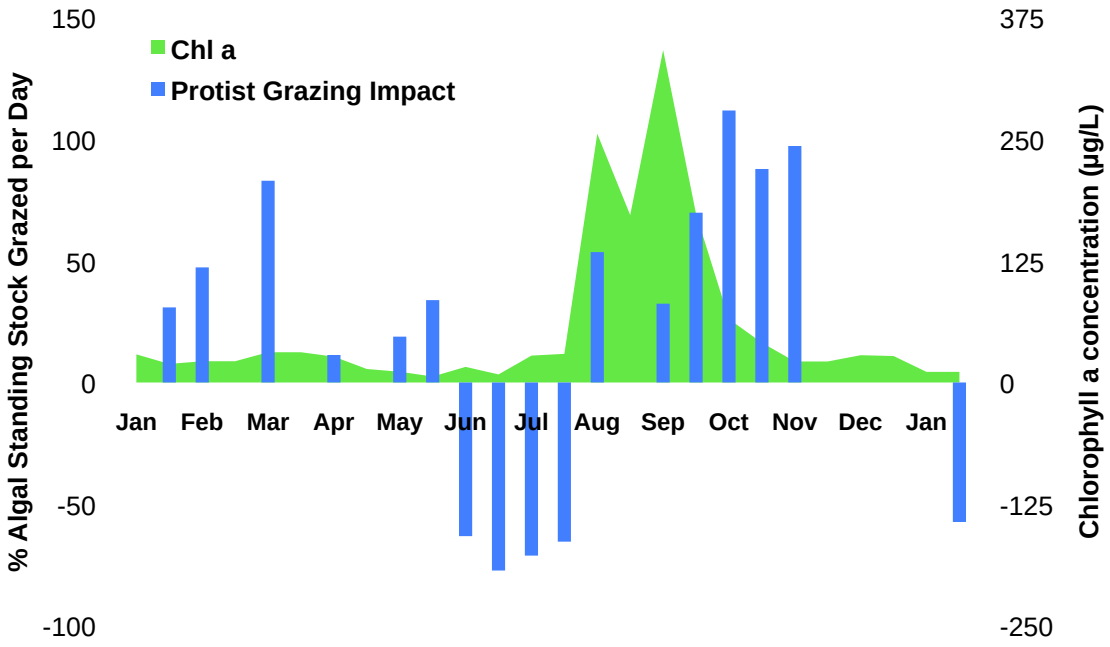

Figure 21. Protist grazing impact, measured as the % of algal/cyanobacterial standing stock consumed per day, determined from dilution experiments conducted in Vancouver Lake between January 2008 and January 2009. Green area represents chlorophyll *a* concentration over the same period.

Metazoan zooplankton grazing impact. As with the protist grazers, it is useful to estimate the potential grazing impact of the metazoan zooplankton on algae/cyanobacteria in Vancouver Lake. For these grazers, their impact on the algal community was determined by applying the empirically derived individual predator grazing rates to the densities of these predators measured in the Lake, and further comparing these density-specific ingestion rates to the abundance of algae present. Thus, grazing impact is here measured as the % of the algal community that was consumed by metazoan zooplankton per day. Note that our experiments determined the ingestion rates of only a subset of the metazoan zooplankton predators present at any one time, namely the copepod *Diacyclops thomasi*. Thus these estimates of metazoan grazing impact are likely underestimates of the total grazing impact by all zooplankton predators, but should represent the majority of the metazoan community impact.

In July, prior to the bloom, copepod grazing impact on different categories of prey taxa ranged from 10-25% of the standing stock of these groups per day, although the grazing impact on cyanobacteria was substantially higher at >60% per day. In September, at the height of the bloom, copepod grazing impacts were higher on average, particularly on diatoms, dinoflagellates

and ciliates (60-90% of standing stocks per day). Thus copepods were disproportionately impacting these taxa, while having much lower grazing impact on cyanobacteria, and possibly contributing to higher cyanobacteria abundance. In October, copepods were exhibiting virtually no grazing impact on cyanobacteria or most algae (diatoms, chlorophytes, etc.), but instead had low to moderate grazing impact focused on dinoflagellates and non-chlorophyte flagellates (Figure 22). As in September, the lack of grazing on cyanobacteria likely reduced the overall grazing pressure on these cells and contributed to the extended period of high cyanobacteria abundance.

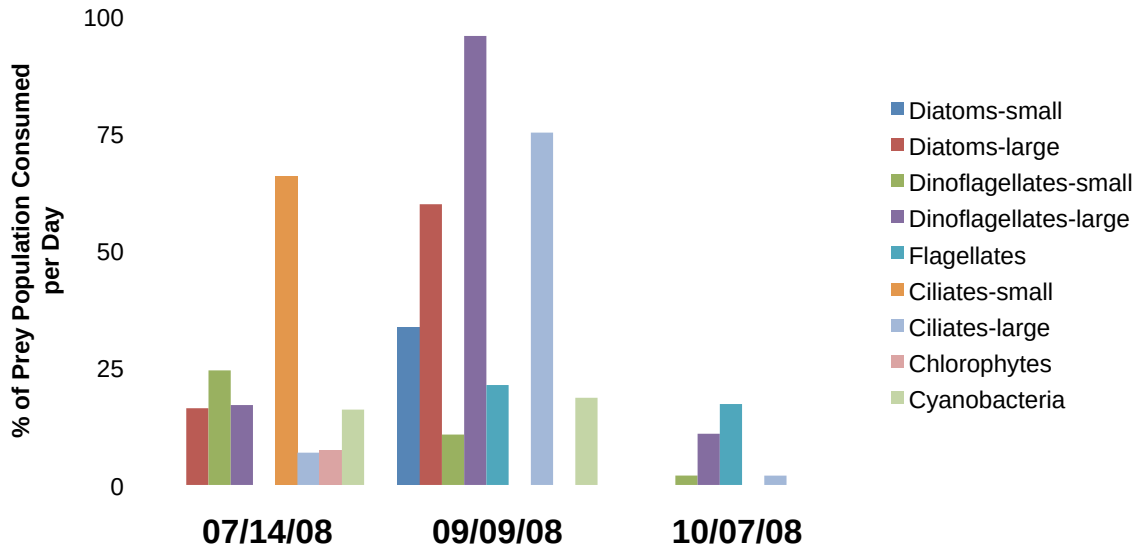

Figure 22. Copepod grazing impact, measured as % of prey population consumed per day, of *Diacyclops thomasi* adults on planktonic prey from Vancouver Lake on three sampling dates over the 2008 bloom period.

Summary and Significance

Two years of intensive sampling of the plankton in Vancouver Lake have allowed us to document several important trends in the variability of their abundance, composition and distribution over both seasonal and inter-annual time scales. On a seasonal basis in Vancouver Lake, like other temperate lakes in North America, the plankton taxonomic composition shifts rather consistently from diverse wintertime assemblages of eukaryotic algae (diatoms, chlorophytes, cryptophytes), protozoans (large dinoflagellates and ciliates), cladocerans (daphnids) and rotifers, to springtime, pre-bloom communities dominated by a smaller number of taxa including diatoms, cryptophytes, larval and juvenile stages of copepods and an increasing abundance of cyanobacteria. A cyanobacteria bloom has occurred each year beginning in late July and extended at least into September, dominated by one or the other species *Aphanizomenon flos-aquae* or *Anabaena flos-aquae*, as well as smaller peaks of diatoms and cryptophytes. Potential grazers such as ciliates, copepods and rotifers also reach maximal abundances in association with the cyanobacteria bloom. By November the plankton community has typically returned to the diverse assemblage characteristic of winter.

In Year Two we additionally focused on quantifying the algal intrinsic growth rates and planktonic consumer grazing rates, to begin to provide more specific information that may assist in decision-making for managing Vancouver Lake to reduce or avoid excessive cyanobacteria blooms. Dilution experiments to determine algal growth rates and protist grazing rates over the full course of the 2008 cyanobacteria bloom demonstrated the dynamic nature of the balance between these rate processes. In spring algal growth rates were maximal, but were largely balanced by high protist grazing rates. In mid-summer, just prior to the 2008 bloom period, unusual environmental conditions (high water levels, exceptional water clarity, very low nutrient concentrations) and a complicated set of grazer interactions may have contributed to the observation of negative algal growth and protist grazing rates – all of which likely influenced conditions that allowed for a dramatic increase in cyanobacteria abundance that occurred in late July 2008. Notably, while algal growth rates were high during the bloom, grazing rates of both protists and metazoan zooplankton (copepods) were also elevated at that time, such that overall grazing impact (% of the algal population consumed by predators per day) was consistently large, particularly on non-cyanobacteria taxa, and likely substantially influenced both the magnitude and composition of the bloom.

This first year of experimental work has allowed us to begin to quantify some of the trophic interactions among the plankton in Vancouver Lake that the on-going assessment of population abundance and composition has suggested may be important. Based on one bloom cycle during 2008, it is clear that both protozoan and zooplankton grazers have the capacity to significantly consume cyanobacteria and algae, and at various stages of the bloom may have a strong enough grazing impact on particular groups to limit their standing stocks. With a second year of experimental work currently on-going (supported by the WA Department of Ecology, Clark County, the Vancouver Lake Watershed Partnership and the WA Water Research Center) that will span another summer bloom period as well as the winter, we look forward to assessing the relative balance of algal growth and planktonic grazing over a wider range of environmental conditions. These results will need to be combined with other food web studies (especially those targeted on higher trophic levels) to provide a more explicit set of predictions about when grazing impacts may be strongest, and will help to inform the discussion of whether managing the Lake to maximize these impacts (e.g., via biomanipulation) as a means to control cyanobacteria blooms is a viable option.

References

Carmichael WW (1992) Cyanobacteria secondary metabolites – the cyanotoxins. J Appl Microbiol 72: 445-459

Chan F, Pace ML, Howarth RW, Marine RM (2004) Bloom formation in heterocystic nitrogen-fixing cyanobacteria: the dependence on colony size and zooplankton grazing. Limnol Oceanogr 49: 2171-2178

Codd GA (1995) Cyanobacterial toxins: occurrence, properties and biological significance. Wat Sci Tech 32: 149-156

Elser JJ, Sterner RW, Galford AE (and 6 others) (2000) Pelagic C:N:P stoichiometry in a eutrophied lake: responses to a whole-lake food-web manipulation. Ecosystems 3: 293-307

Epp GT (1996) Grazing on filamentous cyanobacteria by *Daphnia pulicaria*. Limnol Oceanogr 41: 560-567

Gifford SM, Rollwagen-Bollens G, Bollens SM (2007) Mesozooplankton omnivory in the upper San Francisco Estuary. Mar Ecol Progr Ser 348: 33-46

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine microzooplankton. Mar Biol 67: 283-288

Landry MR, Constantinou J, Kirshtein J (1995) Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. Deep-Sea Res 42: 657-671

Marin V, Huntley ME, Frost B (1986) Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. Mar Biol 93: 49-58

Paterson MJ, Findlay DL, Salki AG, Hendzel LL, Hesslein RH (2002) The effects of Daphnia on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophic lake. Freshw Biol 47: 1217-1233

Pechar L (1995) Long-term changes in fish pond management as 'an unplanned ecosystem experiment': importance of zooplankton structure, nutrients and light for species composition of cyanobacterial blooms. Wat Sci Tech 32: 187-196

Reissig M, Trochine C, Queimalinos C, Balseiro E, Modenutti B (2006) Impact of fish introduction on planktonic food webs in lakes of the Patagonian Plateau. Biol Conserv 132: 437-447

Rollwagen-Bollens GC, Penry DL (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). Mar Ecol Prog Ser 257: 139-158

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

Strickland JDH, Parsons TR (1972) A practical manual for seawater analysis. Fish Res Bd Can Bull 167

Wierenga RE (2005) Volunteer Monitoring Report – Vancouver Lake annual Data Summary for 2005. Clark County, WA, Department of Water Resources

Wierenga RE (2006) Volunteer Monitoring Report – Vancouver Lake annual Data Summary for 2005. Clark County, WA, Department of Water Resources