Senior Honors Thesis:

Cyanobacterial Dynamics of Ford Lake, MI

Chapter 1: Field Observations
Chapter 2: Laboratory Analysis and Field Experiments

Kahli E. McDonald
Department of Ecology and Evolutionary Biology
Natural Science Building
University of Michigan
Ann Arbor, MI 48109-1048 USA
kaheliza@umich.edu

Adviser: Dr. J. T. Lehman
Honors Committee Members
Dr. M. D. Hunter
Dr. Y. L. Qiu

March 25, 2009

Abstract

Eutrophic temperate lakes tend to follow typical warm weather growth patterns in which eukaryotic diatom blooms occur in the spring and are replaced with nuisance cyanobacterial blooms in the mid to late summer, due to the formation of a persisting thermocline that results in hypolimnion anoxia. Ford Lake of SE Michigan follows such a pattern, but the cyanobacterial bloom itself experiences a succession of a filamentous heterocytous species (*Aphanizomenon flos-aquae)* to a toxic colony-forming species (*Microcystis aeruginosa*) in the late summer. This thesis concerns the factors that drive this regular succession and explores a management option.

Aphanizomenon is capable of fixing N_2, and because of this it has a selective advantage over eukaryotic competitors when the lake stratifies and nutrients become trapped in the anoxic layers of the lake. However, when NO_3^- levels in the lake rise and the N:P ratio shifts to higher levels, *Microcystis* is able to out compete *Aphanizomenon*. This study found that *Microcystis* grows best with an N:P ratio of 103 (mol:mol) while *Aphanizomenon* predominates with an N:P ratio of 53. *Microcystis* cellular stoichiometry measured in this study confirms that *Microcystis* cells have N:P ratios of 46. Phycocyanin, which has traditionally served as a proxy for general cyanobacteria growth, was shown to be an adequate proxy for *Aphanizomenon* biovolume but correlates poorly with *Microcystis* biovolume. Microcystin toxin was shown to be somewhat independent of biovolume, although none of the environmental factors encompassed in this study were shown to drive its production.

Withdrawing water from the hypolimnion is proposed to destabilize the water column, thus preventing the summer thermocline. In 2006, this technique was demonstrated to successfully reduce cyanobacterial blooms and greatly impacted *Aphanizomenon* biovolume without affecting *Microcystis* biovolume or microcystin toxin levels. This experiment was repeated in 2008, with results varying from 2006. Despite the variations in results, the cyanobacterial blooms in 2008 were significantly temporally delayed; thus selective withdrawal seems to be a viable management option.

Chapter 1: Field Observations

1. Introduction

Seasonal succession from spring diatom communities to summer blooms of cyanobacteria is a pattern long recognized in temperate zone lakes worldwide (Sommer et al. 1986). This succession has generally been attributed to termination of diatom dominance owing to accelerated sinking losses and depletion of Si, P, or both. Moreover, N depletion and low N:P ratios have been linked to succession by heterocystous cyanobacteria (Smith, 1983, Hyenstrand et al., 1998). Comprehensive taxon specific analyses have demonstrated nearly opposite growth responses of diatoms and cyanobacteria to various environmental factors (Lehman et al., 2004). This paper reports results from a four-year study of summer cyanobacterial populations.

Seasonal occurrence of algal communities in Ford Lake follows a predictable pattern. Diatoms typically achieve maximum abundance in late April or early May, then subside and are replaced by cyanobacteria from July through September. The cyanobacteria develop high biomass, cover the majority of the lake's 4 km^2 surface area, release microcystin toxins (Lehman, 2007), interfere with recreation, and are regarded as a major nuisance problem. One notable feature of the cyanobacterial blooms is the reproducible pattern of species succession that occurs within them. Typically, *Aphanizomenon flos-aquae* dominates from July to early August and then is replaced by *Microcystis aeruginosa*, which dominates from the end of August through September. The two bloom species rarely coexist at mutually high biovolumes.

The purpose of this research was to explore the environmental conditions supporting optimal growth for *Aphanizomenon* versus *Microcystis*. We also examined the presence of microcystin toxin in Ford Lake, to try to determine what factors may promote its production. Finally, we also examined the relationship between cyanobacterial biovolume and pigment concentrations to determine their value as proxy measures for one another.

Accordingly, we proposed the following hypotheses:

1) *Aphanizomenon* biovolume is correlated with phycocyanin (PC) and chlorophyll *a* (Chl *a*) concentrations

2) *Microcystis* biovolume is correlated with PC and Chl *a* concentrations

3) Microcystin toxin is correlated with *Microcystis* biovolume

4) Seasonal succession from *Aphanizomenon* to *Microcystis* is associated with changes in N and/or P concentrations

5) Reduced biovolume of *Microcystis* will lead to reduced toxicity (dependent on results from hypothesis #3)

Acknowledgements– Sample collection and water chemistry analyses were performed in part by the author, in collaboration with Professor J. T. Lehman and students working in his laboratory from 2005 to 2008, including D. Bell, J. Ferris, J. Gunnell, G. Horst, E. Lehman, R. Platte, and E. Rourke. All cyanobacterial cell counts were performed by the author. The author is grateful to Dr. Y. L. Qui and Dr. M. D. Hunter for serving as readers and providing advice during the writing process. In addition, the author would like to thank Professor Lehman for serving as an advisor and providing two years of invaluable support and guidance. This work was assisted by summer support to the author from Dr. N. E. Alger through the Underwood-Alger scholarship.

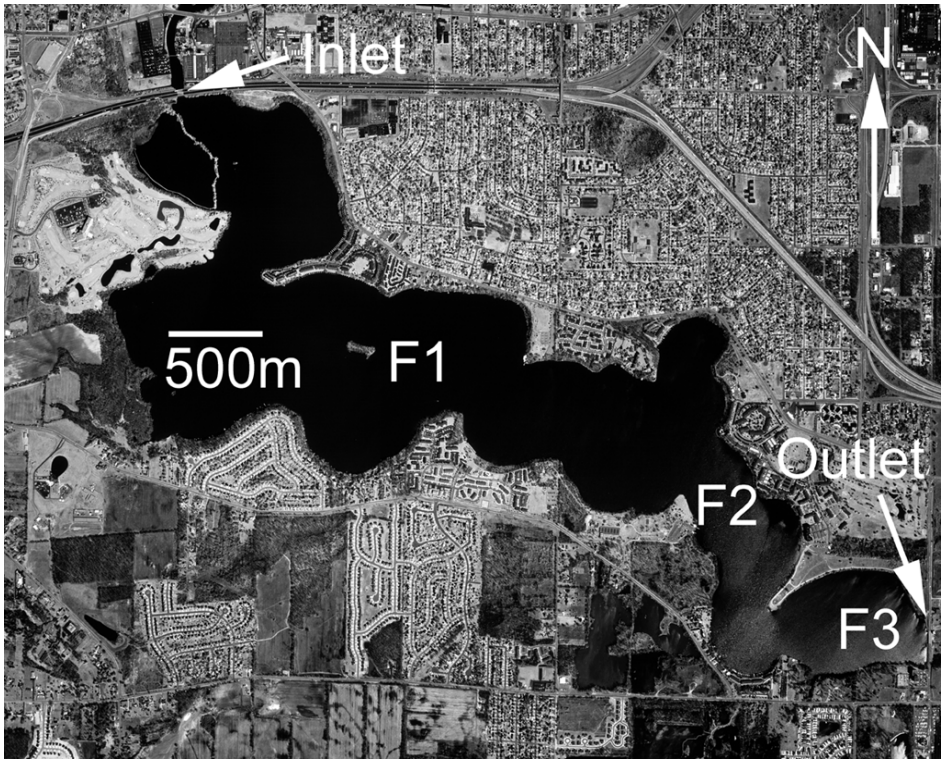
2. Materials and Methods

2.1. Study site

Ford Lake (42.221 N, 83.581W; Figure 1) lies in the middle reach of the Huron River catchment in southeastern Michigan. Ford Lake is a eutrophic man-made impoundment initially constructed by Henry Ford in 1932 to supply hydroelectric power to his Motor Company. The dam and its turbine-driven generators continue to supply power commercially. The impoundment is operated as "run of the river," meaning that stage height is regulated so that outflow matches inflow. The hydroelectric turbines draw water from the topmost 5 m, but they have limited volumetric capacity. When river discharge exceeds the

capacity of the turbines, six hydraulic gates can be opened at the base of the dam, at 11 m maximum water depth, to maintain constant lake stage height. River flow tends to slacken during the summer, and then all discharge water is directed through the turbines to maximize power generation. This practice eliminates advective renewal of hypolimnetic water, and anoxia develops along with depletion of nitrate and release of ammonia and phosphate from anaerobic lake sediments.

Figure 1. Satellite image of Ford Lake with sample sites marked.



2.2. Field sampling

From May to October during 2005 to 2008, water was collected on a weekly to biweekly basis at three stations (denoted F1, F2, and F3; Figure 1) located respectively near the western inlet (5.5 m depth), the center (7.5 m depth), and near the eastern outlet dam (10.8 m depth). Raw water was collected at each site from 0 to 5 m using an integrative tube sampler and filtered on site for nutrient analysis using Millipore™ disposable filter capsules of nominal 0.45 μm

pore size. Unfiltered samples for phytoplankton counts and pigment analyses were also collected from 0 to 5 m.

2.3. Water Chemistry

Water chemistry data are available through the Huron River Project Website (http://www.umich.edu/~hrstudy). Detailed descriptions of water chemistry methods are reported by Lehman (2007) and Ferris and Lehman (2007).

2.3.1. Nitrogen

Nitrate (NO_3^-) was measured by second derivative UV spectroscopy (Crumpton et al., 1992) by scanning filtrate from 260 to 200 nm at 0.5-nm intervals using a 1-cm quartz cuvette. Dissolved nitrogen (DN) was measured using 10 ml filtrate. For particulate nitrogen (PN), 100 ml of raw water was filtered through 25-mm Whatman^{TM} GF/C filters and placed in 10 ml deionized water. DN and PN samples were treated with alkaline persulfate oxidant, heated to 105°C for 6 h, and neutralized with HCl according to D'Elia et al. (1977). Nitrate in the resulting digests was then measured by second derivative UV spectroscopy as above after first removing the macerated glass fibers by filtration in the case of PN. Total nitrogen (TN) was calculated as the sum of DN and PN.

2.3.2. Colored Dissolved Organic Matter

Colored dissolved organic matter (CDOM) was measured as UV absorbance at 254 nm. Ferris and Lehman (2008) demonstrated that CDOM was a statistically significant predictor of both dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in the Huron River.

2.3.3 Ammonium

Ammonium (NH_4^+) was measured from raw water samples according to Holmes et al. (1999). Three-milliliter aliquots of working reagent (comprised of sodium sulfite, borate buffer, and OPA solution) were added to 20-ml samples and incubated in the dark for 2 h. Sample and reference standard fluorescence, as well as sample CDOM color blanks, were measured with a

Turner Designs Model 10 fluorometer using the long wavelength UV filter kit (P/N 10-303R) with 310 to 390 nm excitation filter and 410 to 600 nm emission filter.

2.3.4. Phosphorus

Soluble reactive P (SRP) was measured from filtrate according to Strickland and Parsons (1972). Dissolved P (DP) and total P (TP) were measured from filtrate and raw water, respectively, by treating 40-ml samples with 0.4 g potassium persulfate and heating at 105°C for 2 h. After cooling to room temperature, samples were processed as SRP. Sample absorbance was measured at 885 nm, using a 10-cm path-length cylindrical cell. PP was either calculated as the difference between TP and DP or measured directly by filtering 20 ml of raw water through 25-mm Whatman™ GF/C filters. Filters were placed in 40 ml of deionized water and treated with the same method as DP and TP; macerated glass fibers were removed by filtration prior to colorimetric analysis.

2.3.5. Soluble Reactive Silica

Soluble reactive silica (SRSi) was measured from filtrate according to Stainton et al. (1977). Silicate was reduced to silicomolybdate blue and read in a 1-cm cell at both 660 and 815 nm.

2.4. Algal Pigments

We analyzed both Chl *a* and PC. Raw water was filtered through Whatman-AH filters (100 ml for Chl *a*; 250 ml for PC). Filters were frozen over silica gel desiccant until extraction. Chlorophyll *a* was extracted by macerating filters in ice-cold 90% v/v acetone by tissue grinder, then filtering the slurry through a Whatman GF/D filter. Pigment was measured fluorometrically using a Turner Designs TD700 fluorometer with 436-nm excitation filter and 680-nm emission filter. Phycocyanin was extracted overnight in 0.05 M pH 7 phosphate buffer followed by tissue grinding. Phycocyanin was detected fluorometrically using a Turner Designs TD700 fluorometer with a 630-nm excitation filter and 660-nm emission filter.

2.5. Algal Biovolume

Water samples taken with the integrated tube sampler (0 to 5 m depth) were preserved with Lugol's iodine. Fixed samples were placed in a cylindrical settling chamber 1.6 cm high and 2.3 cm in diameter for at least 1 h prior to counting with an Olympus IMT-2 inverted light microscope. Previously performed time series tests demonstrated that longer settling times did not yield higher cell counts (Ferris and Lehman, 2007). Cyanobacteria were measured using a Whipple grid eyepiece, calibrated with a stage micrometer. Cell diameters were measured using a calibrated eyepiece micrometer. *Aphanizomenon* cells were assumed to be cylindrical, and *Microcystis* cells were assumed to be spherical.

Aphanizomenon trichomes were counted using the method of Olson (1950) whereby the number of intersections of individual trichomes with Whipple grid lines is counted, and biovolume was calculated based on the geometric expectations for a cylinder. Twenty fields per sample were measured to estimate mean population biovolume, expressed as mm^3 biovolume per liter of lake water for each sampling site and date.

Heterocysts within *Aphanizomenon* trichomes were readily identifiable (Adams and Duggan, 1999). While quantifying *Aphanizomenon* biovolume, heterocysts were also counted (Figure 2). Our limit of detection was 106 heterocysts ml^{-1}, which corresponds to one heterocysts being noted over the entire search volume of a single typical sample.

Unlike *Aphanizomenon* trichomes, which were often randomly distributed throughout the settling chamber, *Microcystis* colonies were patchier, thus, we increased the number of replicate fields searched to 30. *Microcystis* was quantified by counting the number of squares in the Whipple grid occupied by the vertical projection of each colony. A square that appeared to have greater than or equal to 50% coverage was considered filled. Assuming the colonies were roughly spherical, the area (A, μm^2) covered was calculated from the number of squares within a field filled by *Microcystis* colonies. Biovolume (BV, μm^3 per field, where a field is treated as a colony) was estimated as:

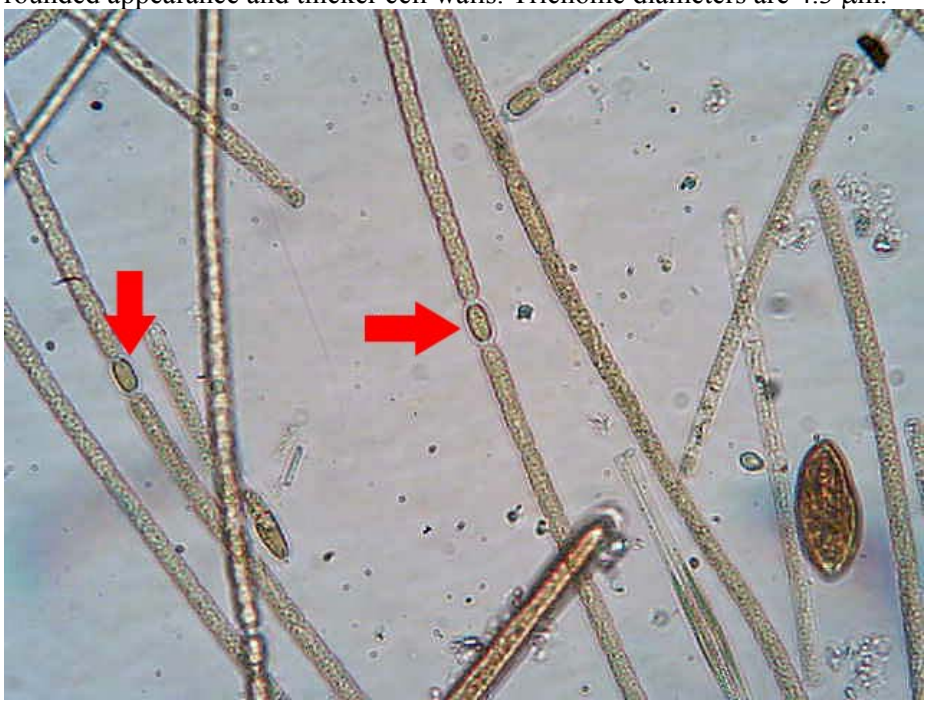
$$BV = A^{1.5} \quad (1)$$

This estimate was consistent with the visual organization of the colonies, with larger colonies tending to have larger vertical focal depths. Joung et al. (2006) determined that colony volume could be used to predict cell numbers:

$$N=1950000*BV + 1731 \quad (2)$$

where N is the estimated number of cells per colony and BV is biovolume (μm^3 per field, again where a field is treated as a colony). Thus, multiplying cell volume by N yields biovolume for *Microcystis*, excluding intercellular space within the colonial matrix.

Figure 2. Surface water sample from F2 taken October 24, 2008. Dominant species in the sample is *Aphanizomenon* and two heterocysts present on the trichomes are indicated by arrows. Note the rounded appearance and thicker cell walls. Trichome diameters are 4.3 μm.



2.6. Microcystin Toxin

Microcystin toxin was measured using competitive enzyme-linked immunosorbent assay (ELISA) by colorimetric assay (Lehman, 2007). The method is sensitive for low levels of microcystin (Pyo et al., 2005) and can be applied to aqueous samples without filtration or extraction. All assays were performed on duplicate samples that were frozen and thawed prior to analysis.

Sample concentrations were deduced by reference to standard concentrations of 0, 0.4, 0.5, 1.0, 2.5, or 3.0 nM microcystin (EnviroLogix, Inc), or serial dilutions of these, using a previously derived dose-response curve for competitive ELISA (Lehman, 2007):

$$A = 1/(\alpha * X + \beta) \quad (3)$$

where A is the difference between optical absorbance (1-cm path-length) at 450 nm and 650 nm, and α and β are statistically determined parameters. The method detection limit (MDL) for this ELISA test is 0.06 nM (Lehman, 2007). Parameter values were obtained by application of the Microsoft Excel^{TM} Solver routine and by the nonlinear regression option of SYSTAT.

2.7. Statistical Methods

All statistical analyses were performed using SYSTAT version 10.2 (SYSTAT, Inc., Evanston, IL). Prior to calculating T-tests and AOV, data were log-transformed to obtain normal distributions. P-values for statistical tests were considered significant at $\alpha = 0.05$.

2.7.1. Aphanizomenon vs. Microcystis

Differences in water chemistry were compared between periods of *Aphanizomenon* and *Microcystis* dominance by T-tests. Thresholds for elevated biovolumes were based on natural breakpoints in the frequency distributions of in situ biovolume for each species. Dates were considered elevated for *Aphanizomenon* if biovolume was greater than 2.0 $mm^3\ L^{-1}$ and *Aphanizomenon* accounted for at least 80% of combined cyanobacterial biovolume. The same

criteria were applied to *Microcystis*. Only *Aphanizomenon* and *Microcystis* were quantified because other genera of cyanobacteria were inconsequential components of total biovolume. The only other cyanobacteria occasionally encountered were *Anabaena* in the midsummer and *Planktothrix* (formerly *Oscillatoria*) late in the year, but never at levels comparable to the other two species. In addition to comparing environmental conditions on the date of each elevated abundance, we repeated the T-tests comparing conditions one week prior to each date of elevated abundance.

We also created a third category defined as dates of successful coexistence. Here, at least one genus had to satisfy the biovolume requirement stated above (2.0 mm^3 L^{-1}), but it accounted for < 80% of the joint total biovolume. One-way analysis of variance (AOV) was used to compare environmental conditions during cyanobacterial blooms (≥ 2.0 mm^3 L^{-1}) characterized by (a) *Aphanizomenon* dominance, (b) *Microcystis* dominance or (c) coexistence. Our four-year data set included a total of 266 data points. Cyanobacterial dominance and coexistence dates accounted for 28.2% of the full data set (N= 75): *Aphanizomenon* peaks accounted for 16.9% (N= 45), *Microcystis* peaks accounted for 5.6% (N= 15), and periods of coexistence accounted for 5.6% (N= 15).

2.7.2. Heterocysts

Additional T-tests were applied to differences in water chemistry during *Aphanizomenon* blooms with abundant heterocysts versus periods with an undetectable level of heterocysts. Comparisons were restricted to dates when *Aphanizomenon* biovolume was ≥ 2.0 mm^3 L^{-1}. Samples with ≥ 200 heterocysts ml^{-1} were classified as abundant, and those with 0 heterocysts ml^{-1} were classified as absent. We did not include samples containing more than 0 but fewer than 200 heterocysts ml^{-1} in this analysis, as we were attempting to classify environmental conditions associated with abundance versus absence.

2.7.3. Microcystin toxin

We examined whether microcystin toxin responded to environmental conditions independently of *Microcystis* biovolume. To do so, we conducted T-tests comparing

concentrations of NO_3^-, NH_4^+, DN, TN, SRP, DP, TP, SRSi and CDOM during periods when toxin was undetectable (defined as < 0.1 nM) to those when toxin was present (defined as ≥ 0.1 nM, a level just above our MDL for measuring microcystin by ELISA). We limited the data set to dates when *Microcystis* was detected, which yielded a sample size of 67.

2.7.4. Proxy Variables

Correlations between cyanobacterial biovolume and pigment concentrations, as well as *Microcystis* biovolume and microcystin toxin levels, were calculated as Pearson's correlation coefficients.

2.8. Hypolimnetic Withdrawal

During the sampling seasons of 2006 and 2008, hypolimnetic water was discharged from the bottom of the outlet dam in order to destabilize the water column, promote vertical mixing, and prevent the deep water from becoming anoxic. Details of the procedure as well as a detailed treatment of the results are presented in Chapter 2.

3. Results

3.1 Seasonal Succession of Cyanobacterial Populations

Epilimnetic chemistry was statistically different during periods of *Aphanizomenon* or *Microcystis* dominance as well as during periods of coexistence (Table 1). AOV identified differences ($P \leq 0.05$) for NH_4^+, NO_3^-, DN, TN:TP, and CDOM. Further post hoc analysis revealed that *Aphanizomenon* dominance was associated with NO_3^- concentrations one-third less than the levels during *Microcystis* dominance (28.4 μM vs. 41.6 μM; $P = 0.012$). This suggests that *Aphanizomenon* requires or tolerates low NO_3^- better than *Microcystis*, whereas the latter genus can out compete at higher NO_3^- levels (Figure 3). NH_4^+ concentrations were also significantly higher during *Microcystis* blooms ($P = 0.045$). In addition, TN:TP ratios (mol:mol) were significantly different during *Aphanizomenon* and *Microcystis* blooms (51:1 and 103:1 respectively; $P < 0.001$). There is a trend whereby periods of coexistence exhibit chemistry intermediate between periods of genus-specific dominance. These data suggest that

nitrogen, particularly in the form of NO_3^-, may be the most influential nutrient in Ford Lake cyanobacterial dynamics. It should be noted, however, that a complex interaction of nitrogen and phosphorus, and possibly other compounds not monitored during this study, all contribute to the success of one species or the other.

Environmental conditions one week prior to each peak date were likewise significantly different among taxa (Table 1). Analysis of variance identified significant differences for NH_4^+ and DN. Concentrations of NH_4^+ preceding *Microcystis* blooms (8.2 μM) were almost twice those preceding *Aphanizomenon* blooms (4.6 μM; P= 0.002). DN was significantly lower when *Aphanizomenon* was dominant (64.8 μM) than when *Microcystis* dominated (76.6 μM, P= 0.002). TN:TP ratios were significantly higher preceding *Microcystis* dominance than preceding *Aphanizomenon* dominance (M= 82:1, A= 60:1; P= 0.002).

We identified 11 sampling dates during July through August of 2005 to 2008 when *Aphanizomenon* abundance exceeded our threshold level of 2.0 $mm^3 L^{-1}$ and heterocyst abundance likewise exceeded a threshold of 200 ml^{-1}. We compared these with 38 sampling dates for the same period when heterocysts were not observed, but *Aphanizomenon* biovolume remained above 2.0 $mm^3 L^{-1}$ (Table 2). Periods of heterocyst abundance were characterized by significantly lower NH_4^+ (P= 0.0080), NO_3^- (P= 0.0212), DN (P= 0.0247) and significantly higher TP (P= 0.0181).

3.2 Microcystin Toxicity

We conducted T-tests comparing mean water chemistry analyte concentrations between periods of low to absent toxin (< 0.10 nM) with periods of elevated toxin (≥ 0.10 nM) for dates when *Microcystis* was detected in biovolume counts (N= 67). Concentrations of TN and PN were significantly higher during periods of elevated toxin levels (N= 61 for each analyte, P= 0.019 and P= 0.026 respectively). Not surprisingly, *Microcystis* biovolume was significantly greater (P= 0.008) at times of elevated toxin. The average *Microcystis* biovolume was 4.5 $mm^3 L^{-1}$ when toxin levels were elevated and 1.1 $mm^3 L^{-1}$ when toxin was undetectable.

3.3 Correlations

3.3.1 Correlation between Biovolume and Pigments

Correlations between biovolume and pigments were different for the two dominant cyanobacteria (Figure 4). Samples dominated by *Aphanizomenon* from July through September (N= 45) contrasted with those from August through September dominated by *Microcystis* (N= 15). *Aphanizomenon* biovolume explained approximately 67.1% of the variance observed in PC and 66.0% of the variance observed in Chl *a* ($P< 0.001$ for both pigments). *Microcystis* biovolume was not significantly correlated with either Chl *a* or PC (r^2 values were both less than 1%), revealing both pigments to be an inappropriate proxy measure for *Microcystis* biovolume in this study.

3.3.2 Microcystin Correlation

For the same dates that microcystin toxicity was examined, we also performed linear regression analysis to determine if there was correlation between toxin levels and other lake analytes. Microcystin toxin concentration had significant positive correlations with PN, TN, and PP concentrations (r^2 = 7.9%; 13.1%; 7.5% respectively). Microcystin was also correlated with *Microcystis* biovolume ($P= 0.002$), though this correlation explained only 13.2% of the observed toxin variability. Because PN makes up a portion of TN, and because PN and PP are commonly used as proxies for algal biovolume, it is likely that all of the correlations are simply indicative of a relationship between toxin and biovolume.

Table 1. Mean water chemistry differences during periods of *Aphanizomenon* dominance, *Microcystis* dominance, and coexistence. *P*-values for AOV and Tukey post-hoc analysis are presented. *P*-values greater than 0.05 are denoted as ns (not significant).

Relative Time	Analyte	Mean (A)	Mean (M)	Mean (A & M)	AOV *P*	*P* (*A* vs. A&*M*)	*P* (*M* vs. A&*M*)	*P* (*A* vs. *M*)
Week of Peak	**SRSi (N=43,8,14)**	105.9	101.6	79.0	ns	ns	ns	ns
	NH_4^+ (N=41,15,15)	3.1	7.6	4.4	**0.034**	ns	ns	**0.045**
	NO_3^- (N=45,15,15)	28.4	41.6	35.6	**0.010**	ns	ns	**0.012**
	DN (N=42,15,11)	60.2	86.0	80.5	**< 0.001**	**0.002**	ns	**< 0.001**
	TN (N=42,15,11)	94.4	108.3	108.5	**0.043**	ns	ns	ns
	SRP (N=45,15,15)	0.272	0.269	0.227	ns	ns	ns	ns
	DP (N=45,15,15)	0.734	0.622	0.612	ns	ns	ns	ns
	TP (N=45,15,15)	2.1	1.8	2.3	ns	ns	ns	ns
	TN:TP (N=42,15,11)	50.6	102.7	69.3	**< 0.001**	ns	ns	**< 0.001**
	Abs254 (N=45,15,15)	0.194	0.153	0.175	**< 0.001**	**0.047**	**0.040**	**< 0.001**
Week Before Peak	**SRSi (N= 40,12,13)**	99.1	82.7	96.7	ns	ns	ns	ns
	NH_4^+ (N= 42,14,14)	4.6	8.2	11.8	**0.001**	**0.016**	ns	**0.002**
	NO_3^- (N= 42,14,14)	26.8	27.0	34.5	ns	ns	ns	ns
	DN (N= 42,14,11)	64.8	76.6	82.2	**0.001**	ns	ns	**0.002**
	TN (N= 42,11,14)	94.4	94.9	101.4	ns	ns	ns	ns
	SRP(N= 42,14,14)	0.3	0.4	0.4	ns	ns	ns	ns
	DP (N= 42,14,14)	0.7	0.9	0.7	ns	ns	ns	ns
	TP (N= 42,14,14)	2.0	2.0	1.8	ns	ns	ns	ns
	TN:TP (N= 42,14,11)	60.5	81.8	103.1	**0.003**	ns	ns	**0.002**
	Abs254 (N= 45,15,15)	0.205	0.179	0.160	**< 0.001**	ns	ns	**< 0.001**

Table 2. Mean water chemistry differences during periods of *Aphanizomenon* dominance when heterocysts are abundant versus absent. P-values for independent samples T-tests are presented. *P*-values greater than 0.05 are denoted as ns (not significant).

Analyte	Mean (Absent*) N= 38	Mean (Abundant*) N= 12	T-test *P*
NH_4 (µM)	4.0	1.3	**0.008**
NO_3^- (µM)	33.4	18.2	**0.021**
DN (µM)	68.0	53.3	**0.025**
TN (µM)	94.1	108.2	ns
SRP (µM)	0.25	0.17	ns
DP (µM)	0.71	0.60	ns
TP (µM)	2.0	2.5	**0.018**
N:P	49.9	44.8	ns

*Abundant = 200+ heterocysts ml^{-1}, absent = below limit of detection

Figure 3. *Aphanizomenon* and *Microcystis* biovolume and NO_3^- concentrations, from all three sampling sites over all four years of study.

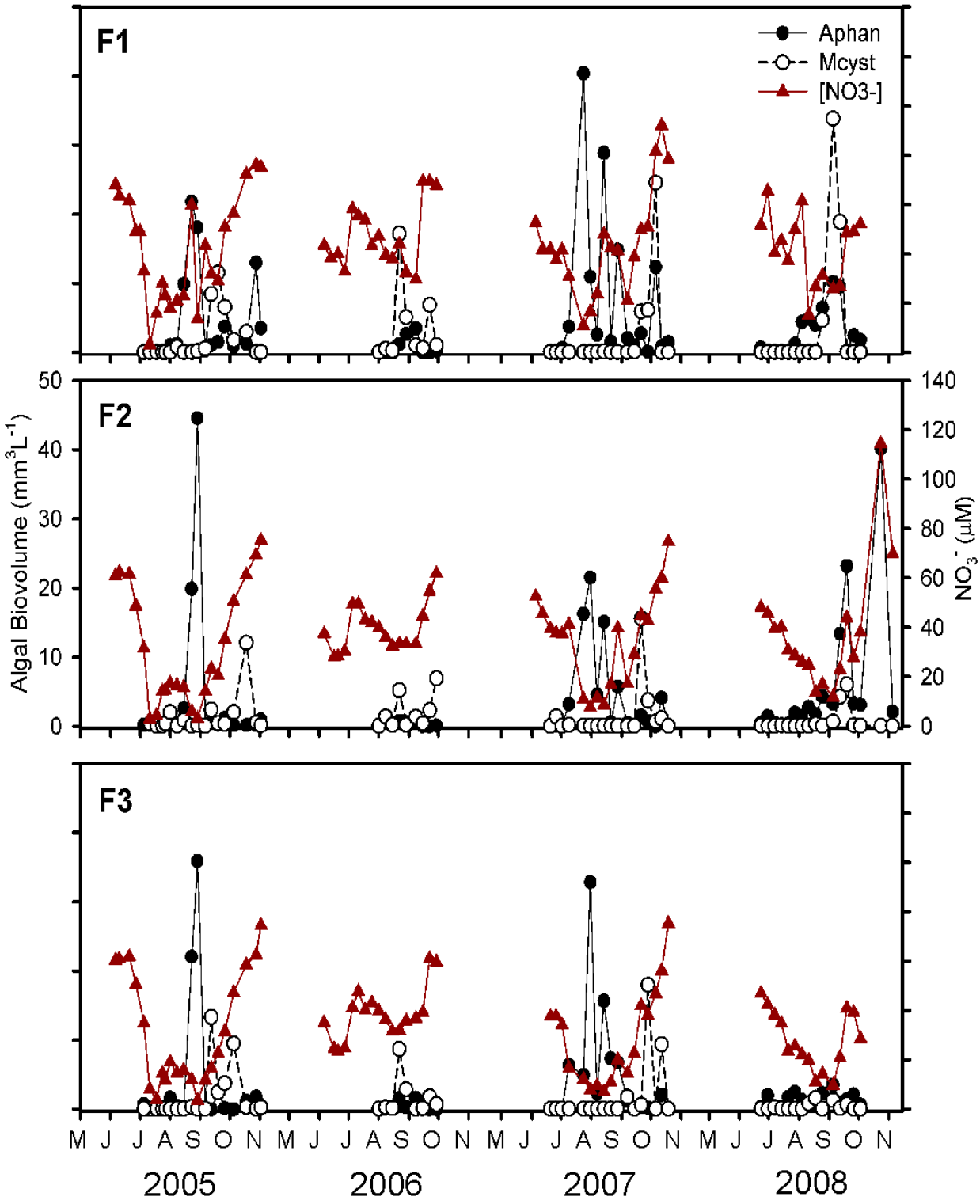
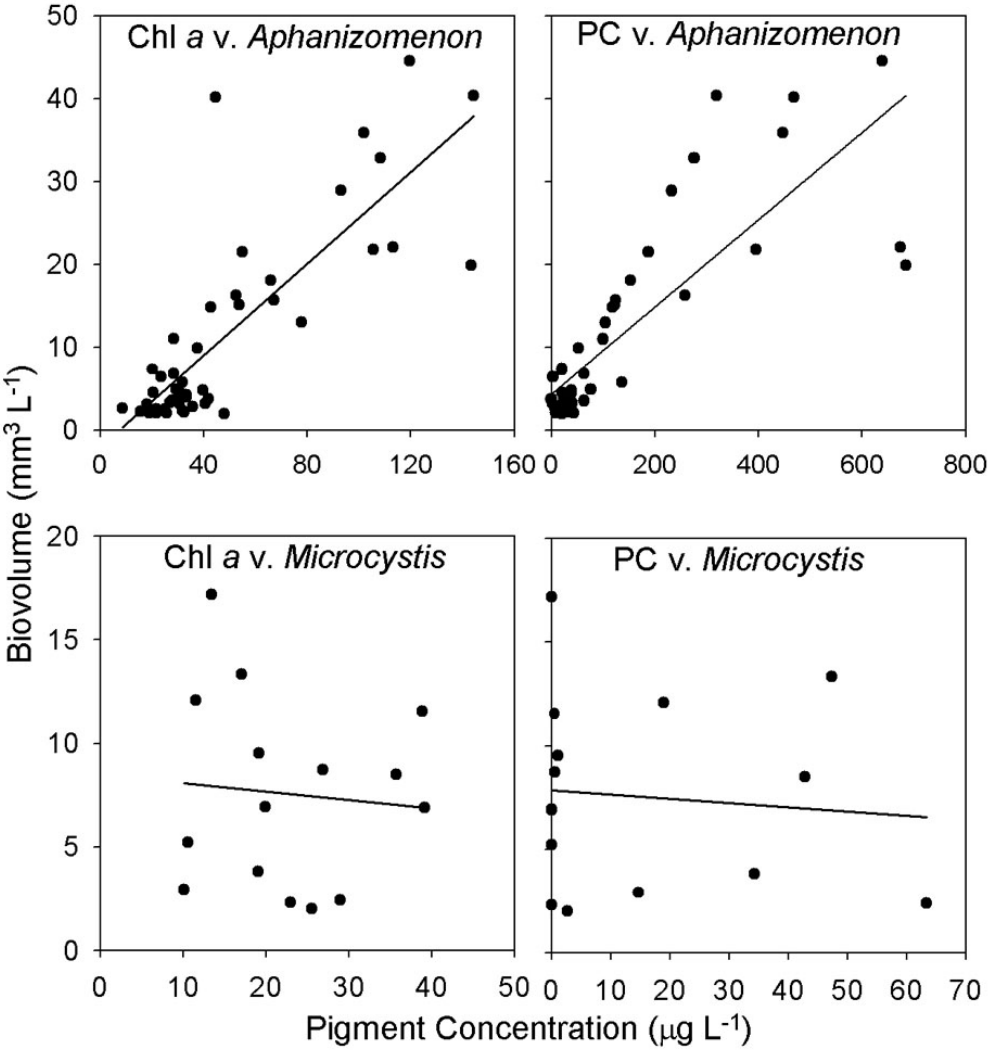


Figure 4. Biovolume of *Microcystis* and *Aphanizomenon* versus pigment concentrations and corresponding regression lines. Points include only the dates when each species is dominant ($\geq$ 2 mm^3 L^{-1}; $\geq$ 80% of total cyanobacterial population). For Chl *a* v. *Aphanizomenon*, r^2= 0.660 (P< 0.001), for PC v. *Aphanizomenon*, r^2=0.671 (P< 0.001). Neither pigment exhibited a significant linear relationship with *Microcystis* biovolume.



4. Discussion

4.1. Seasonal Succession of Cyanobacterial Populations

Phytoplankton dynamics in Ford Lake are dominated by a seasonal pattern that involves a consistent progression: a spring diatom bloom, a mid-summer *Aphanizomenon* bloom, and a

late summer/early fall *Microcystis* bloom. Previous work has examined the diatom bloom in detail (Ferris and Lehman, 2007). The purpose of this study was to examine the dynamics governing the rise of *Aphanizomenon* and the subsequent shift to *Microcystis*. The results of our research implicate N and P not only in the transition from diatoms to Cyanophyta, but within cyanobacterial communities themselves.

Periods of *Aphanizomenon* versus *Microcystis* dominance differ significantly in NH_4^+, NO_3^-, DN, and N:P ratios. *Microcystis* shows greater success during periods of higher NO_3^- and NH_4^+ concentrations. This observation is consistent with expectations based on the physiological adaptation of *Aphanizomenon* to low N concentrations, largely due to its ability to form heterocysts and fix N_2 (de Figueiredo et al, 2006; Levine and Schindler, 1999). The heterocyst concentrations we found are consistent with heterocyst levels found for *Aphanizomenon* populations in Clear Lake, California that were shown to be fixing nitrogen (Horne et. al, 1972). Our comparisons of environmental conditions when heterocysts are abundant versus when they are rare certainly show that periods of heterocyst abundance are associated with low levels of NH_4^+ and NO_3^-. Moreover, environmental conditions during periods of *Microcystis* dominance are not significantly different from those during coexistence with *Aphanizomenon*. In contrast, DN levels are significantly lower during *Aphanizomenon* dominance than levels associated with coexistence, suggesting that coexistence is typically a time of transition, and that *Aphanizomenon* requires a strict low level of DN to maintain its selective advantage over *Microcystis*.

Differences were not identified with respect to P, except in tandem with N, in that periods of dominance by each species were marked by significantly different average N:P. *Aphanizomenon* blooms were characterized by a mean ratio of 51:1, whereas *Microcystis* thrived with a mean ratio of 103:1. The comparatively higher N:P ratio during *Microcystis* blooms has been noted in other studies (Abrantes et al, 2006; Downing et al, 2005; Levine and Schindler, 1999).

Average CDOM levels were significantly higher when *Aphanizomenon* was present, versus *Microcystis*, but this study cannot determine whether this is because *Aphanizomenon* exhibits a positive response to higher levels of dissolved organic matter, or whether *Aphanizomenon* is responsible for leaking organic matter into the water. However, Haggard (2009, Masters Dissertation) demonstrated that *Aphanizomenon* from Upper Klamath Lake, Oregon exhibited growth inhibition due to humic matter, and the level of inhibition was strongly correlated with levels of CDOM. Thus it seems unlikely that CDOM is spurring *Aphanizomenon* growth in Ford Lake.

Abrantes et al. (2006) note that *Aphanizomenon* populations dominate in Vela Lake, Portugal when N concentrations are lowest. They are then succeeded by *Microcystis* when NH_4^+ levels rise and total P levels drop, resulting in an increased N:P ratio. The authors suggest that *Microcystis* is better adapted to conditions with greater N and an otherwise limiting level of P, because it has superior ability to store P and thus is not affected when P concentrations drop. Laboratory experiments done in conjunction with these field observations also support the notion that *Microcystis* may be better suited to deal with lower levels of P (see Chapter 2).

To evaluate factors that may promote biomass development, we examined conditions the week preceding each peak or coexistence date. Once again, key nutrients included NH_4^+ and DN, and statistical tests identified higher N:P ratios preceding *Microcystis* (60:1) than *Aphanizomenon* (82:1). Once again, DN significantly differs the week before periods of coexistence and the week before *Aphanizomenon* dominance, while water chemistry is not significantly different one week before coexistence versus one week before *Microcystis* dominance.

4.2. Microcystin Toxin

We observed spatial variation in toxin levels within and across years at our three sampling stations that was not completely explained by variation in biovolume. Microcystin toxin was positively correlated with *Microcystis* biovolume, but only 13.2% of the variance was explained. The phenomenon of toxin levels functioning somewhat independently from

biovolume has been observed by others (Jacoby, et al 2000; Kotak, et al 1995; Mankiewicz-Boczek, et al 2006; Watanabe, et al 1989). Of course, it is also possible that this inconsistency is driven by changing dynamics within the *Microcystis* bloom between strains that produce toxins and those that do not. Such heterogeneity within *Microcystis* blooms has been observed by others (Carmichael and Gorham, 1981; Kotak, et al 1995; Watanabe, et al 1991).

Toxin levels were not positively correlated with any environmental factors encompassed in this study, except those shown to serve as proxies for algal biovolume. However, there are many documented cases of a correlation between NO_3^- levels and toxin production. This is consistent with Inamori, et al. (2003) and Yoshida, et al. (2007) who found higher toxin levels associated with higher concentrations of NO_3^-. In Ford Lake, during the years of 2005 to 2007, T-tests found a significant association between higher levels of NO_3^- and higher concentrations of toxin (Lehman et al., 2008), but 2008 data diverged from this pattern. One reason may be that 2008 hypolimnetic withdrawal experiments were particularly successful in stimulating the growth of diatoms all summer long to the extent that nitrate levels were significantly depressed (Figure 3) by the time experiments were concluded in August and the community changed to cyanobacteria. The pattern of NO_3^- consumption was substantially different than in 2006 when the selective withdrawal experiments were more limited and episodic. Furthermore, *Microcystis* dominance in 2008 was considerably delayed compared to previous years, leading to a very different pattern of dynamics (see Chapter 2). Jacoby, et al. (2000) found SRP significantly associated with higher microcystin toxin levels, but aside from PP, we observed no relationship between toxin levels and P.

Others have suggested that biovolume and toxin levels of *Microcystis* may be dependent on cell growth phase (Inamori et al., 2003; Kotak et al., 1995). The possibility that toxin production is associated with bloom senescence has been suggested by lag times between biovolume peaks and toxin peaks (Inamori et al. 2003; Lehman, 2007). It is also possible that the conditions optimal for cell growth are not identical to those optimal for toxin production. These observations are by no means universal and certainly warrant additional study (Lee et

al., 2000; Long et al., 2001). It is also likely that microcystin toxin may be affected by other chemical factors, such as Fe, or physical factors that were not documented in this study. For example, Jiang et al, 2008, found that a complex interaction of NO_3^-, light, and Fe affected toxin production.

4.3. Correlating Biovolume, Pigment, and Toxin

Significant positive correlations were found between *Aphanizomenon* and the pigments Chl *a* and PC. *Aphanizomenon* biovolume explained approximately 67% of the variance observed in PC and 66% of the variance observed in Chl *a* while *Aphanizomenon* was the dominant species in the water column. This is similar to, but slightly lower than, the correlation found by Brient, et al (2008) who reported an r^2 of 0.73 for PC. Brient et al. examined a heterogeneous filamentous cyanobacterial community in aggregate, and one might expect variation by species. Note that *Microcystis* correlated poorly with both Chl *a* and PC in our analyses. Other studies have also found poor correlations between *Microcystis* and pigments (Bañares-España et al., 2007; Mankiewicz-Boczek et al., 2006). Bañares-España, et al. (2007) demonstrated that different genetic strains of *M. aeruginosa* grown under the same environmental conditions produced varying amounts of PC and Chl *a*. Such variability could even occur within a single colony. This might explain the poor correlation of biovolume to pigment concentration in our study, but we cannot test this hypothesis because we did not genotype our *Microcystis* samples.

Conversely, Ahn, et al. (2004) reported a much higher correlation between *Microcystis* cell counts and PC concentration (r^2= 0.632). Their estimates, however, were calculated from mixed species samples. *Microcystis* and *Anabaena* coexisted in virtually equal amounts at all times when PC levels were measured. Conclusions regarding the correlation between *Microcystis* and PC cannot be disentangled from pigment levels of *Anabaena*. It is our conclusion, therefore, that unlike with *Aphanizomenon*, PC is an inappropriate indicator for *Microcystis* biovolume even if the bloom is known to be homogeneous.

5. Conclusions

The dynamics of *Aphanizomenon* and *Microcystis* blooms in Ford Lake appear to be driven largely by NO_3^- concentrations, with higher levels shifting the selective advantage to *Microcystis*. *Aphanizomenon* was most successful with a mean N:P ratio (mol:mol) of 51, whereas *Microcystis* thrived with a mean ratio of 103. Successful elimination of both blooms would likely require a dramatic increase in N:P ratio with elevated nitrogen levels throughout the summer, so that the initial phase of cyanobacterial dominance is never attained. However, microcystin toxin may respond to factors not encompassed in this study, and understanding the key to disrupting toxin production will require further studies, both at ecosystem and cell culture scales. To fully address the problem of nuisance and toxic algal blooms in Ford Lake, an integrated approach is required that will target cyanobacterial biovolume dynamics as well as conditions optimally suited for toxin production. This study also demonstrates that, despite its association with cyanobacteria, PC may not be a good proxy for predicting *Microcystis* blooms.

The next chapter of this study explores an experimental attempt to manipulate cyanobacterial dynamics at the whole ecosystem scale and further explores some of the biological and physiological factors of the algal community of Ford Lake.

References

Adams, D.G. and P.S., Duggan. 1999. Heterocyst and akinete differentiation in cyanobacteria, *New Phytol.* 144**:** 3–33.

Ahn, C.Y., A.S. Chung, and H.M. Oh. 2004. Rainfall, phycocyanin, and N:P ratios related to cyanobacterial blooms in a Korean large reservoir. *Hydrobiologia* 474: 117–124.

Bañares-España, E., V. López-Rodas, E. Costas, C. Salgado, and A. Flores-Moya. 2007. Genetic variability associated with photosynthetic pigment concentration, and photochemical and nonphotochemical quenching, in strains of the cyanobacterium *Microcystis aeruginosa*. *FEMS Microbiol. Ecol.* 60: 449–455.

Brient, L., M. Lengronne, and E. Bertrand. 2008. A phycocyanin probe as a tool for monitoring cyanobacteria in freshwater bodies. *J. Environ. Monit.* 10 248–255.

Carmichael, W.W. and P.R. Gorham. 1981. The mosaic nature of toxic blooms of cyanobacteria. In: W.W. Carmichael, Editor, *The Water Environment: Algal Toxins and Health*, Plenum, New York. pp. 161–172.

Crumpton, W.G., T.M. Isenhart, and P.D. Mitchell. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. *Limnol. Oceanogr.* 37: 907–913.

De Figueiredo, D.R., A.S.S. Reboleira, S.C. Antunes, N. Abrantes, U. Azeiteiro, F. Gonçalves, and M. J. Pereira. 2006. The effect of environmental parameters and cyanobacterial blooms on phytoplankton dynamics of a Portuguese temperate lake. *Hydrobiologia* 568: 145–157.

D'Elia, C.F., P.A. Steudler, and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22: 760–764.

Downing, T.G., C.S. Sember, and M.M. Gehringer. 2005. Medium N:P ratios and specific growth rate comodulate microcystin and protein content in *Mycrocystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. *Microb. Ecol.* 49: 468–473.

Ferris, J.A. and J.T. Lehman. 2007. Interannual variation in diatom bloom dynamics: roles of hydrology, nutrient limitation, sinking, and whole lake manipulation. *Water Res.* 41: 2551–2562.

Ferris, J.A. and J. T. Lehman. 2008. Nutrient budgets and river impoundments: Interannual variation and implication for detecting future changes. *Lake and Reservoir Management* 24:273-281.

Haggard, K.G. 2008. Response of the Cyanobacterium *Aphanizomenon flos-aquae* to Vascular Plant Decomposition Products. Oregon State University Scholars Archive. Accessed 4 March 2009. http://hdl.handle.net/1957/9965

Holmes, R.M., A. Aminot, R. Kerouel, B.A. Hooker, and B.J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.* 56: 1801–1808.

Horne, A.J. and C.R. Goldman. 1972. Nitrogen fixation in Clear Lake, California. 1. Seasonal variation and role of heterocysts. *Limnol. Oceanogr.* 17: 678–692.

Hyenstrand, P., P. Blomquist, and A. Pettersson. 1998. Factors determining cyanobacterial success in aquatic systems: a literature review. *Ergebn. Limnol.* 51: 41–62.

Inamori, Y., K. Kameyama, T. Maekawa, and N. Sugiura. 2003. Characteristics of microcystin production in the cell cycle of *Microcystis viridis*. *Environ. Toxicol.* 19: 20–25.

Jacoby, J.M., D.C. Collier, E.B. Welch, F.J. Hardy, and M. Crayton. 2000. Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*. *Can. J. Fish. Aquat. Sci.* 57: 231–240.

Joung, S.H., C.J. Kim, C.Y. Ahn, K.Y. Jang, S.M. Boo, and H.M. Oh. 2006. Simple method for a cell count of the colonial cyanobacterium. *Microcystis sp*, *J. Microbiol.* 44: 562–565.

Kotak, B.G., A.K.Y. Lam, E.E. Prepas, L. Kenefick, and S.E. Hrudey. 1995. Variability of the hepatotoxin microcystin-LR in hypereutrophic drinking water lakes, *J. Phycol.* 31: 248–263.

Lee, S.J., M.H. Jang, and H.S. Kim. 2000. Variation of microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. *J. Appl. Microbiol.* 89: 323–329.

Lehman, E.M. 2007. Seasonal occurrence and toxicity of Microcystis in impoundments of the Huron River, Michigan, USA, *Water Res.* 41: 795–802.

Lehman, E.M., K.E. McDonald, and J.T. Lehman. 2009. Whole lake selective withdrawal experiment to control harmful cyanobacteria in an urban impoundment. *Water Research*, 43: 1187-1198.

Lehman, J.T., S.E.B. Abella, A.H. Litt, and W.T. Edmondson. 2004. Fingerprints of biocomplexity: taxon-specific growth of phytoplankton in relation to environmental factors. *Limnol. Oceanogr.* 49: 1446–1456.

Levine, S.N. and D.W. Schindler. 1999. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. *Can J. Fish. Aquat. Sci.* 56: 451–466.

Long, B.M., G.J. Jones, and P.T. Orr. 2001. Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate, *Appl. Environ. Microbiol.* 67: 278–283.

Mankiewicz-Boczek, J., K. Izydorczyk, Z. Romanowska-Duda, T. Jurczak, K. Stefaniak, and M. Kokocinski. 2006. Detection and monitoring toxigenicity of cyanobacteria by application of molecular methods. *Environ. Toxicol.* 21: 380–387.

Olson, S.C.W. 1950 Quantitative estimates of filamentous algae. *Trans. Am. Microsc. Soc.* 59: 272–279.

Pyo, D., J. Lee, and E. Choi. 2005. Trace analysis of microcystins in water using enzyme-linked immunosorbent assay. *Microchem. J.* 80:165–169.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by cyanobacteria in lake phytoplankton. *Science* 22: 669–671.

Sommer, U., Z.M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.* 106 433–471.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. The Chemical Analysis of Freshwater. Fisheries and Environment Canada, Fisheries and Marine Service Miscellaneous special publication no. 25 (second ed.). Freshwater Institute. Winnipeg, Canada.

Strickland, J.D.H., and T.R. Parsons. 1972. The practical handbook of seawater analysis, *Bull. Fish. Res. Bd. Can.* 167.

Watanabe, M.F., K.I. Harada, and K. Matsuura. 1989. Heptapeptide toxin production during the batch culture of two *Microcystis* species (Cyanobacteria), *J. Appl. Phycol.* 1: 161–165.

Watanabe, M.F., M. Watanabe, T. Kato, K.I. Harada, and M. Suzuki. 1991. Composition of cyclic peptide toxins among strains of Microcystis aeruginosa (blue–green algae, cyanobacteria). *Bot. Mag.* 104 (1991), pp. 49–57.

Yoshida, M., T. Yoshida, Y. Takashima, N. Hosoda, and S. Hiroishi. 2007. Dynamics of microcystin-producing and non-microcystin-producing Microcystis populations is correlated with nitrate concentration in a Japanese lake. *FEMS Microbiol. Lett.* 266: 49–53.

Chapter 2: Laboratory Analysis and Field Experiments

1. Introduction

In the first chapter of this study, we examined the environmental factors that influence cyanobacterial dynamics in Ford Lake, MI. The second half of this study has two goals: to test the hypotheses from the first chapter through experimental manipulations of populations of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* and to test a viable method of managing and reducing their biovolume. Ford Lake is one of the most productive warm water fisheries in Michigan and is used extensively for recreation, so there is great interest in reducing the duration of nuisance blooms.

Prior to the onset of *Aphanizomenon* blooms each summer, Ford Lake becomes thermally stratified and the bottom water becomes anoxic, resulting in plentiful inorganic nutrients that can nourish cyanobacteria if stirred up to the surface by strong winds or a summer storm. This stratification is detrimental for spring diatom blooms, which rely on mixing for access to surface light and avoidance of sinking losses (Lehman et al. 2004). Purposeful discharge of hypolimnetic water would allow the water column to destabilize and would keep the bottom oxygenated. In 2006, experimental hypolimnetic withdrawal caused a dramatic reduction in *Aphanizomenon* without significantly affecting *Microcystis* biovolume (Lehman et al., 2009). Hypolimnetic withdrawal was again performed in 2008, and this study seeks to determine if the results from 2006 are repeatable, and thus to evaluate the effectiveness of selective withdrawal as a management option.

In tandem with selective withdrawal experiments, we performed 3 bioassay experiments during late summer in which we collected water samples and manipulated them with P or N additions (N as both NO_3^- and NH_4^+). We then quantified the cyanobacterial response (including production of heterocysts and microcystin) and also used silica as a proxy for diatom response. Although diatoms are not normally present in Ford Lake during the late summer, a successful replication of the 2006 experiment would allow them to remain as a

significant presence in the lake. Given the field observations reported in the previous chapter, we made a priori predictions about how nutrient additions would affect overall algal dynamics in Ford Lake. Primarily, we predict that a lower N:P ratio would cause *Aphanizomenon* to flourish over either *Microcystis* or diatoms, and there would be a correlating increase in heterocyst formation. Although Chapter 1 did not provide any insights into toxin production, Lehman et al. (2009) did find a correlation between NO_3^- concentration and toxin levels, and several studies have found evidence of such a relationship (Kameyama et al. 2002, Yoshida et al., 2007). Thus, NO_3^- additions could potentially lead to higher microcystin concentrations in any experiments in which *Microcystis* is present.

Finally, we also analyzed the stoichiometry of a toxic strain of *Microcystis*. Redfield (1958) defines an intracellular C:N:P ratio of 106:16:1 by moles as the optimal ratio for phytoplankton. Because our field observations indicate that *Microcystis* grows best in Ford Lake at a high N:P ratio (103:1), we hypothesize that the physiology of *Microcystis* may deviate from the Redfield ratio, and specifically might have a higher N:P ratio than 16 to 1.

Thus, we proposed several hypotheses:

1) Selective withdrawal of hypolimnetic water will destabilize the water column and prevent bottom water anoxia. This will keep NO_3^- concentrations high throughout the summer, extending the diatom bloom and significantly reducing overall *Aphanizomenon* biovolume.
2) Phycocyanin concentrations will also be reduced during the period of the selective withdrawal experiment, as PC was shown in Chapter 1 to correlate strongly to *Aphanizomenon* biovolume.
3) Selective withdrawal of hypolimnetic water will not have a significant effect (either positive or negative) on either *Microcystis* biovolume or microcystin toxin.
4) During bioassay experiments, lower N:P ratios will allow *Aphanizomenon* to gain dominance.
5) Lower N:P ratios will likewise result in an increased frequency of heterocysts.

6) High N:P ratios will result in dominance of either *Microcystis* or diatoms, depending on which had a higher presence in the water prior to the onset of each experiment.
7) *Microcystis* cells will exhibit an N:P ratio exceeding that predicted by the Redfield ratio.

2. Methods and Materials

2.1 Bioassay Experiments

Experiments were carried out in August and September of 2008 and designated as B1, B2, or B3, based on the order in which they were performed. Twenty liters of water were collected from the surface of Ford Lake at station F3 for experiments B1 and B2 and at site F2 for experiment B3. The water was dispensed to 17 1-L polycarbonate bottles. Remaining water was set aside for analysis. Four of the bottles received 200 μL of 0.1 M KNO_3. Four bottles received 200 μL of 0.1 M NH_4Cl, four more received 310 μL of 1.61 mM Na_2HPO_4, and the last four bottles received no nutrient additions. These treatments raised the nominal concentrations of NO_3^-, NH_4^+ and PO_4^{2-} by 20 μM, 20 μM, and 0.5 μM, respectively. In addition, the KNO_3, NH_4Cl, and Na_2HPO_4 amendments were added to a single 1-L bottle that was immediately analyzed to ensure that the actual increase in concentration of each added nutrient was approximately equal to the expected value. Each of the nutrient amendments was chosen to approximately match the highest naturally occurring concentrations observed in the lake over the course of this study (note: occasionally values of these nutrients have exceeded the chosen concentrations, but such events are considered anomalous due to construction accidents or discharges upstream of Ford Lake, such as during the Great Blackout of August 2003).

The bottles with the added nutrients, as well as the four control bottles, were incubated under an average photon flux density of 88 μE m^{-2} s^{-1} and a 16 h L:8 h D photoperiod at 22ºC. In experiment B1, water was collected on the morning of 28 July 2008 and the experiment was broken down on the morning of 1 August 2008, for a duration of 4 days. In experiment B2, water was collected the morning of 18 August 2008 and the experiment was broken down the

morning of 22 August 2008, for a total elapsed time of 5 days. During experiment B3, water was collected on the morning of 19 September 2008 and broken down the morning of 23 September 2008, for a total elapsed time of 5 days. Bottles were arranged randomly and rearranged daily to reduce any unintentional systematic variations in light climate during the course of the experiment.

At experiment breakdown, NO_3^-, NH_4^+ CDOM, SRP, SRSi, Chl *a*, PC and microcystin toxin concentrations were measured. Small samples of unfiltered water were preserved with acid-Lugol's. *Microcystis* biovolume, *Aphanizomenon* biovolume and number of heterocysts were also measured (see section 2.5).

2.2 Microcystis Stoichiometry

Two 1-L bottles of J Media, prepared by UTEX Media on 25 August 2008, were inoculated with UTEX B 2666 *Microcystis* stock culture on 26 Aug 2008. The bottles, designated J1 and J2, were clear polycarbonate bottles, capped and grown with a 16:8 h photoperiod under an average photon flux density of 88 $\mu E\ m^{-2}\ s^{-1}$ until 10 Oct 2008. Approximately once a week the caps were loosened to allow air exchange, ensuring the algae received an adequate supply of CO_2. Bottles were also periodically vigorously shaken to prevent clumping or the formation of a surface film.

Prior to breaking down the experiment, a small sample of water was examined under an Olympus BHA compound microscope to ensure that the cultures remained unialgal. Each bottle was sampled and Chl *a*, PC, PP and PN were measured. Small samples of unfiltered water were preserved with acid-Lugol's and later used to quantify *Microcystis* biovolume (see section 2.5). An additional small sample of water was frozen, and later used to measure concentration of microcystin toxin. Calculations were used to express pmol of each analyte per cell, and to determine the cellular ratios of N:P and PC:Chl *a* (mol:mol).

2.3 Selective Withdrawal Experiment

During 2006 and 2008, we experimentally curtailed epilimnetic discharge through the turbines at Ford Lake dam so that water could be released from the hypolimnion by opening gates at the base of the dam. The objectives were (1) to replenish oxygen to the hypolimnion by forced advection, and (2) to destabilize the water column by weakening the thermal gradient and to deepen the mixed layer. In 2006, three selective withdrawal experiments were performed: June 22 to 30, July 14 to 21, and July 28 to August 4. In the first experiment, 300,000 m^3 per day was withdrawn from the hypolimnion. In the second experiment, 150,000 m^3 per day was withdrawn, and in the third experiment, 300,000 m^3 per day was again withdrawn. In 2008, just one experiment was performed from June 30 to August 13, and 300,000 m^3 per day was withdrawn until August 6, with successively smaller volumes each day for the remaining week. The duration of the 2008 experiment was limited by low levels of precipitation and runoff, which curtailed river flow below the amount sufficient for the experiment to continue past mid-August without lowering the surface level of Ford Lake.

2.4 Water Chemistry

All analytes (including algal pigments) were measured as described in Chapter 1, sections 2.3 and 2.4.

2.5 Algal Biovolume

Methods of quantifying algal biovolume reported in Chapter 1 section 2.5 were modified for the stoichiometry experiment and nutrient addition experiments.

2.5.1 Microcystis stoichiometry

Microcystis populations grown in culture did not form colonies, thus it was possible to count individual cells. These counts were performed with a hemocytometer, counting the number of algal cells per $1/16^{th}$ gridded chamber cells, which have an area of 0.0625 mm^2 and a height of 0.1 mm. Counts were performed on an Olympus BHA compound microscope at 250X magnification. Typically, between 20 and 30 replicate counts were performed. A cell in any stage of division was counted as one cell, unless it was so far progressed that a well-defined

cleavage furrow was present. A cell was counted as being in a grid square as long as at least 50% of the cell was inside of the lines defining the square. Volume of individual non-dividing cells was measured using a calibrated eyepiece micrometer. Cells were assumed to be spherical. Biovolume was then expressed as $mm^3\ L^{-1}$.

2.5.2. Nutrient Uptake Experiments

Microcystis colonies and *Aphanizomenon* heterocysts were both present in very small amounts in experiments B1 and B2. To better elucidate significant differences across experimental treatments, search area was expanded in all experiments (including the third, despite significantly higher levels of both entities). *Microcystis* was searched for in 40 replicate fields, instead of 30 as used in field observations (Chapter 1). Heterocysts were also searched for in 40 replicate fields, and heterocysts were searched for within the entire viewing field, not just the area defined by the Whipple grid. Diameter of the viewing field was measured using a calibrated eyepiece micrometer, and the viewing field was treated as perfectly circular.

2.6 Microcystin Toxin

Toxin levels were measured by ELISA, as per the technique described in Chapter 1 section 2.6.

2.7 Statistical Analysis

All statistical analyses were performed using SYSTAT version 10.2 (SYSTAT, Inc., Evanston, IL). Prior to calculating T-tests and one-way Analysis of Variance (AOV), data were log-transformed to obtain normal distributions. P-values for statistical tests were considered significant at $\alpha = 0.05$.

2.7.1 Bioassay Experiment Analyses

For each experiment, AOV was applied to each analyte to compare the differences across each treatment (N= 16). Tukey post-hoc analysis was used on any analyte that returned a significant AOV *P* value to compare each of the three nutrient treatments to the control.

2.7.2. Effect of Experimental Manipulation

Paired T-tests and repeated measures AOV were applied to differences in water chemistry and algal biovolume across years. We compared 14 weeks each year with nearly coincident sampling dates from the end of June through the end of September. The first sampling date in each year's data set coincides with the sampling date preceding the first experiment in 2006 or 2008. For microcystin toxin, however, we compared only 6 weeks because the toxin never appears before August.

3. Results

3.1 Bioassay Experiments

3.1.1 Nitrogen response

In all three experiments, average NO_3^- levels remained significantly higher than control in bottles with NO_3^- added; clearly this effect is residual and added nitrate was not entirely used up during any of the experiments (Figure 1). In B2 and B3, NO_3^- levels were also significantly higher than the control when NH_4^+ was added (P= 0.0209 for B2, P= 0.0027 for B3). During experiments B1 and B2, NO_3^- levels were low with respect to control after PO_4^{2-} additions ($P \leq 0.0001$, for both B1 and B2), but this effect was not noted in the third experiment. NH_4^+ levels likewise retained residual elevated concentrations in B1 and B2, but were not significantly higher than the control in B3. NH_4^+ concentrations were never significantly higher than control following NO_3^- additions.

3.1.2 Qualitative Algal Composition

In B1, Chl *a* levels were significantly higher than the control for each of the nutrient additions, and were much higher when PO_4^{2-} was added (P= 0.0060, P= 0.0180, $P< 0.0001$, for NO_3^-, NH_4^+, PO_4^{2-} additions respectively; Figure 2). PC was significantly higher than control when PO_4^{2-} was added, and SRSi was significantly lower (P= 0.0019, $P \leq 0.0001$). In B2, neither SRSi nor Chl *a* varied significantly from the control. PC was significantly lower than the control after NO_3^- addition (P= 0.0326) and significantly higher with respect to the PO_4^{2-} addition (P= 0.0008). In B3, SRSi was significantly higher following PO_4^{2-} additions

(*P*= 0.0054). Chl *a* was significantly higher after NH_4^+ additions (*P*= 0.0112) and PC showed no significant response.

3.1.3 Quantitative Algal Response

Average *Aphanizomenon* biovolume was significantly higher than the control in the NO_3^- addition during B1 (*P*= 0.0489, Figure 3). Heterocysts were not detectable in either of the N additions, but were present in the PO_4^{2-} addition (17,209 het L^{-1}) and the control (782 het L^{-1}). In B2, heterocysts were not present in either of the N additions or the control, but were present in the PO_4^{2-} additions (10,169 het L^{-1}). *Aphanizomenon* did not exhibit a significant biovolume response to any of the nutrient treatments. In B3, *Aphanizomenon* once again did not vary significantly among any of the nutrient additions, but it should be noted that biovolume increased approximately 6-fold from conditions at the beginning of the experiment (initial BV= 2.7 mm^3 L^{-1}). Heterocysts were present in each of the nutrient additions and the control; average concentration in NO_3^- additions was 14080 het L^{-1}, 3,129 het L^{-1} in NH_4^+ additions, 21,902 het L^{-1} in PO_4^{2-} additions and 11,733 het L^{-1} in the control.

Microcystis did not show a significant response during B1 or B2 (Figure 3). Biovolume was extremely low at the onset and end of both experiments, often below the level of detection. *Microcystis* biovolume was significantly higher during B3, and showed a significant positive response to NO_3^- (*P*= 0.0285). Again it should be noted that in all of the treatments *Microcystis* increased from its initial biovolume (4.7 mm^3 L^{-1}), but the degree to which biovolume increased was much less than that of *Aphanizomenon* biovolume. Microcystin toxin did not exhibit any significant differences across any of the treatments in any of the experiments.

Figure 1. Final concentrations of NO_3^- and NH_4^+ by treatment for each experiment. * designates a significantly different value than control; (*) designates a significant difference in concentration that is residual, from initial nutrient conditions. Black bars represent the error bars (given as the sample standard deviation over the square root of n).

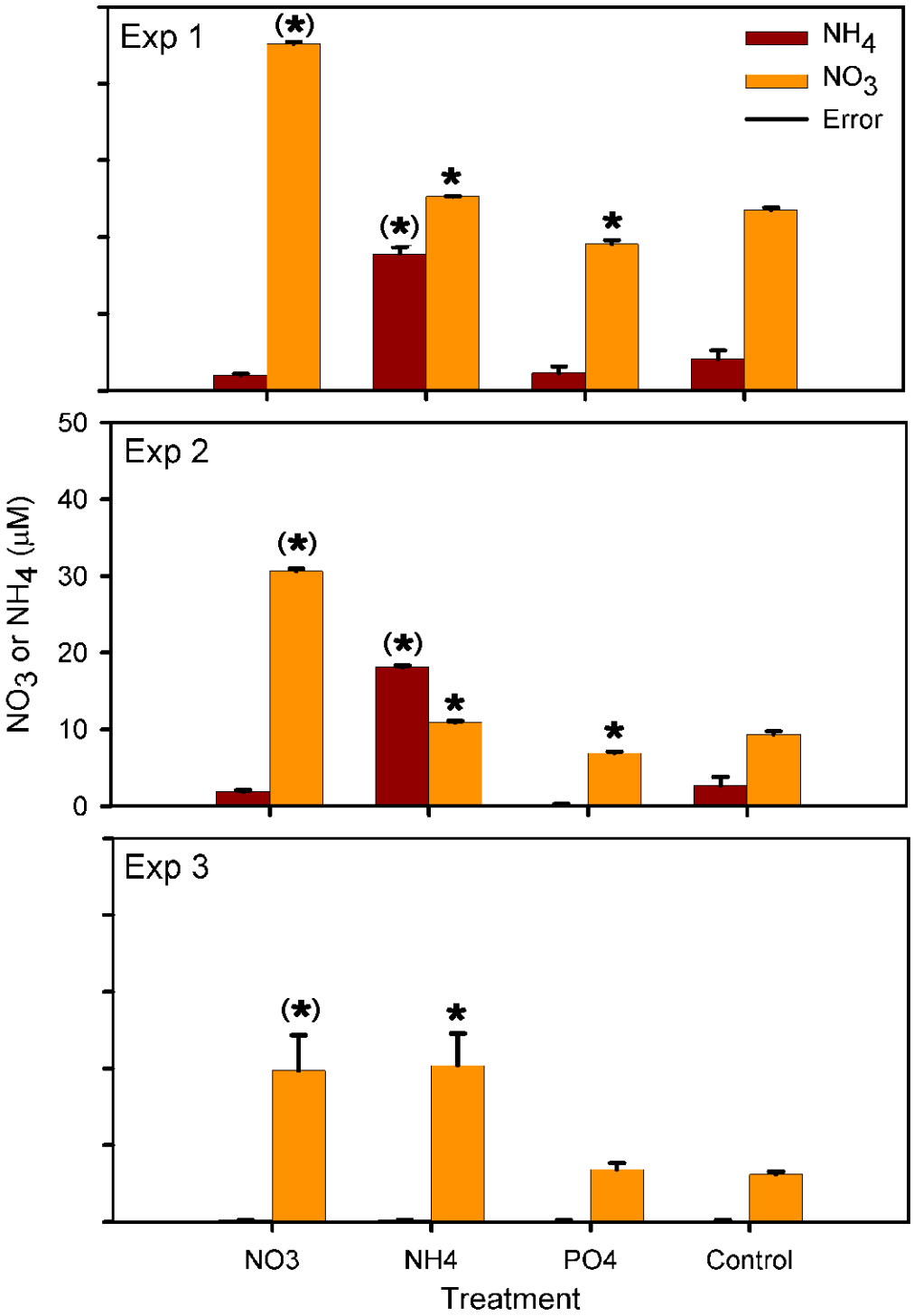


Figure 2. Final concentrations of pigments (Chl a and PC) and SRSi by treatment for each experiment. * designates a significantly different value than control. Black bars represent the error bars (given as the sample standard deviation over the square root of n).

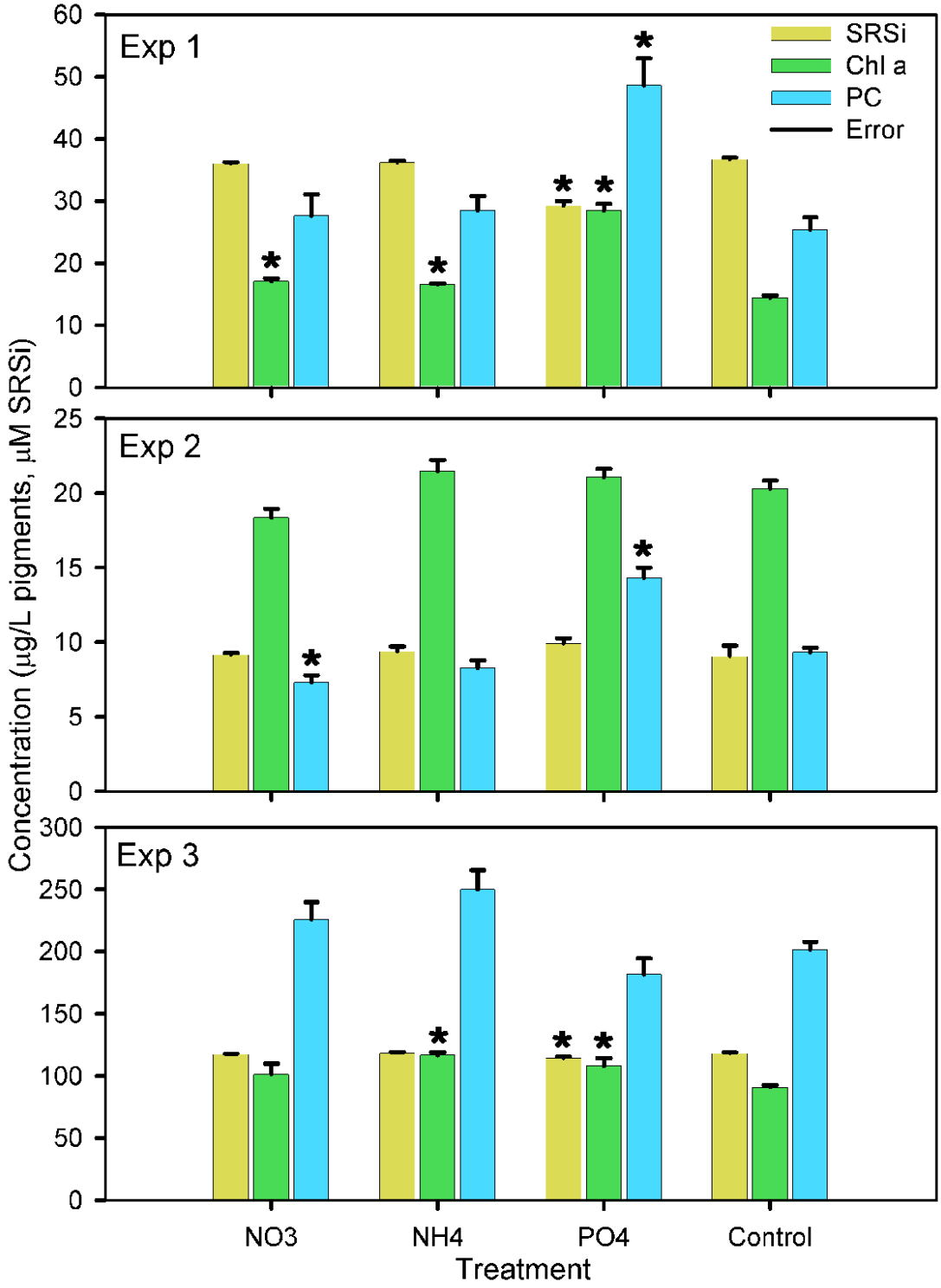
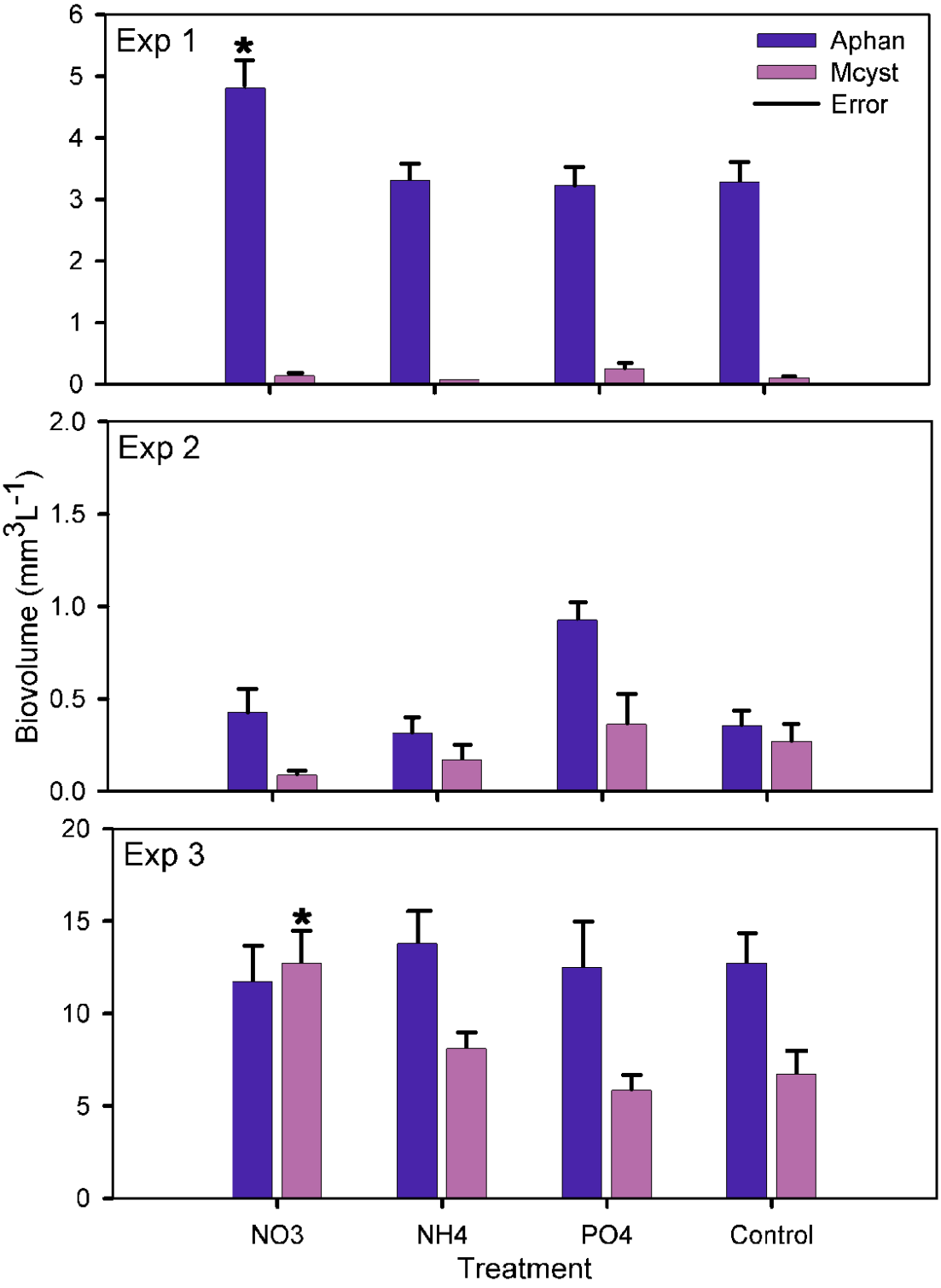


Figure 3. Final biovolume of *Microcystis* and *Aphanizomenon* by treatment for each experiment. * designates a significantly different value than control. Black bars represent the error bars (given as the sample standard deviation over the square root of n).



3.2 **Microcystis** *Stoichiometry*

Bottle J1 had a PN concentration of 50.1 μM, a PP concentration of 1.12 μM, a Chl *a* concentration of 3.66 μg L^{-1}, a PC concentration of 52.7 μg L^{-1}, and a toxin concentration of 27.19 nM. The overall biovolume was 14.4 mm^3 L^{-1}. Bottle J2 had a PN concentration of 55.7 μM, a PP concentration of 1.16 μM, a Chl *a* concentration of 4.15 μg L^{-1}, a PC concentration of 65.12 μg L^{-1}, and a toxin concentration of 26.69 nM. The overall biovolume was 22.3 mm^3 L^{-1}. Average cell volume was found to be 3.20 x 10^{-8} mm^3. Thus the average concentrations of N and P per cell are 0.0957 pmol and 0.00208 pmol respectively, with a N:P ratio of 46.0 (mol:mol). Average concentrations of Chl *a* and PC were 0.00705 pg and 0.105 pg per cell respectively, with a Chl *a*:PC ratio of 0.0669 (g:g). The molecular weight of Chl *a* is 893.5 and the molecular weight of PC is approximately 28,500 (Sigma Aldrich, Kao and Berns, 1969), so the molar ratio of Chl *a*:PC is 2.13. Average cellular amount of toxin was 4.94 x 10^{-5} pmol.

3.3 Selective Withdrawal Experiment

Our original analysis, performed at the close of the 2007 sampling season, revealed that the selective withdrawal experiment in 2006 produced statistically significant differences from the control years (2005 and 2007), while the control years exhibited very few differences from one another (Lehman et al., 2009). It was somewhat surprising to discover that the 2008 experiment year data did not duplicate 2006 in being significantly different from the control years, nor did it closely resemble the 2006 experimental year in terms of water chemistry or biovolume response.

Table 1. Mean concentrations of various analytes at each sampling site for all four years. P-values for AOV and paired t-tests are present (T-tests only performed on analytes that had a significant p-value by AOV). P-values over 0.05 are marked as ns (not significant). Note: table continues to next page. (n=14 samples each year for a total of 56 data points unless otherwise indicated; *n=12, **n=13, ♦n=9, ◊n=6)

Analyte	Station	Mean 2005	Mean 2006	Mean 2007	Mean 2008	AOV *P* (2005-2005)	Paired T-test *P* (2005-2006)	Paired T-test *P* (2005-2007)	Paired T-test *P* (2005-2008)	Paired T-test *P* (2006-2007)	Paired T-test *P* (2006-2008)	Paired T-test *P* (2007-2008)
$NH4_+$ µM	F1	3.3	4.0	4.8	4.5	ns						
	F2	6.1	4.2	5.1	3.4	ns						
	F3	7.7	4.7	5.3	3.8	ns						
NO_3^- µM	F1	29.3	48.7	33.5	41.1	0.0059	0.0084	ns	ns	0.0076	ns	ns
	F2	18.2	41.8	26.0	30.2	0.0001	0.0004	ns	0.0074	0.0014	0.0027	NS
	F3	18.7	40.7	20.9	27.9	<0.0001	0.0004	ns	0.0130	0.0001	0.0031	0.0254
DN µM	F1*	69.3	85.8	70.0	76.5	0.0088	0.0047	ns	ns	0.0003	ns	ns
	F2*	61.1	75.1	63.3	58.8	0.0034	0.0099	ns	ns	0.0036	0.0025	ns
	F3*	63.8	75.7	62.4	64.8	0.0068	0.0141	ns	ns	0.0028	0.0130	ns
PN µM	F1*	31.0	14.9	31.4	12.2	ns						
	F2*	24.5	12.8	25.6	15.2	ns						
	F3*	16.1	13.4	18.1	10.7	0.0148	ns	ns	0.0321	ns	ns	0.0015
TN µM	F1*	100.4	100.7	201.8	88.7	<0.0001	ns	>0.0001	ns	0.0000	ns	<0.0001
	F2*	85.7	87.9	90.1	74.0	0.0201	ns	ns	ns	ns	0.0209	0.0006
	F3*	79.9	89.2	80.4	75.5	0.0370	ns	ns	ns	ns	0.0400	0.0384
Abs254	F1	0.159	0.184	0.206	0.224	0.0000	0.0027	<0.0001	<0.0001	<0.0001	0.0005	ns
	F2	0.159	0.185	0.208	0.221	0.0000	0.0011	<0.0001	<0.0001	<0.0001	0.0001	ns
	F3	0.159	0.186	0.211	0.219	0.0000	0.0003	<0.0001	<0.0001	<0.0001	0.0003	ns
SRP µM	F1	0.21	0.17	0.32	0.39	0.0022	ns	ns	0.0020	ns	0.0013	ns
	F2	0.18	0.19	0.25	0.19	ns						
	F3	0.23	0.19	0.27	0.16	ns						
DP µM	F1**	0.74	0.59	0.73	0.88	ns						
	F2	0.60	0.59	0.65	0.62	ns						
	F3	0.60	0.56	0.62	0.56	ns						
TP µM	F1**	2.54	1.60	2.11	2.03	0.0342	0.0066	ns	ns	ns	0.0269	ns
	F2	2.05	1.23	1.61	1.782	0.0143	0.0096	ns	ns	0.0487	0.0133	ns
	F3	1.53	1.39	1.33	1.19	ns						

Table 1 continued.

Analyte	Station	Mean 2005	Mean 2006	Mean 2007	Mean 2008	AOV *P* (2005-2005)	Paired T-test *P* (2005-2006)	Paired T-test *P* (2005-2007)	Paired T-test *P* (2005-2008)	Paired T-test *P* (2006-2007)	Paired T-test *P* (2006-2008)	Paired T-test *P* (2007-2008)
PP mM	F1**	1.80	1.01	1.37	1.26	0.0338	0.0045	ns	ns	ns	ns	ns
	F2	1.44	0.63	0.96	1.16	0.0098	0.0087	ns	ns	0.0396	0.0115	ns
	F3	0.93	0.82	0.70	0.63	ns						
N:P mol:mol	F1*	48.4	67.0	53.6	53.3	0.0019	0.0064	ns	ns	0.0477	0.0128	ns
	F2*	50.9	74.6	59.5	49.9	0.0010	0.0047	ns	ns	0.0220	0.0025	ns
	F3*	62.1	79.5	64.1	69.7	ns						
SRSi µM	F1**	111.5	87.4	127.2	80.1	0.0003	0.0464	0.0173	0.0078	0.0143	0.86878	0.0008
	F2**	109.6	83.5	123.9	62.9	<0.0001	0.0377	0.0113	0.0012	0.0155	0.0833	0.0004
	F3**	109.7	83.1	121.8	56.7	ns						
Chl a μgL^{-1}	F1	36.9	19.3	41.6	37.3	ns						
	F2	37.4	17.5	31.3	42.1	ns						
	F3**	32.2	16.2	31.8	24.3	ns						
PC μgL^{-1}	F1	51.5	3.6	81.8	41.3	<0.0001	0.0003	ns	ns	0.0005	<0.0001	ns
	F2	105.1	2.7	64.3	70.0	<0.0001	0.0008	ns	ns	0.0002	0.0003	ns
	F3**	89.5	1.5	49.4	17.9	<0.0001	0.0012	ns	ns	0.0010	0.0002	ns
Aphan mm^3L^{-1}	F1♦	4.5	0.9	8.4	3.2	0.0001	0.0048	ns	ns	0.0120	0.0031	ns
	F2♦	5.6	0.4	5.4	4.2	0.0176	0.0021	ns	ns	0.0199	0.0051	ns
	F3◊	5.0	0.6	6.1	1.5	ns						
Mcyst mm^3L^{-1}	F1♦	2.2	3.7	1.0	4.2	ns						
	F2♦	0.6	2.0	1.7	0.9	ns						
	F3♦	1.6	1.7	1.6	0.4	ns						
MC nM	F1◊	2.9	0.4	1.1	0.6	ns						
	F2◊	0.4	0.8	1.1	1.1	ns						
	F3◊	0.3	1.2	0.3	0.3	ns						

3.3.1 Water Chemistry

N levels seemed much more sensitive to hypolimnetic withdrawal experiments than did P levels. Interannual differences emerged for NO_3^-, DN, and TN at all three sampling sites. NO_3^- was significantly higher in 2006 than in either of the control years at all three sites. (Table 1). In 2008, NO_3^- was significantly higher than in 2005 at F2 and F3. At F3, NO_3^- was significantly higher in 2008 than in 2007; however, it was significantly lower in 2008 compared to 2006 at sites F2 and F3. Thus 2008 was characterized by intermediate values of NO_3^-; typically 2008 NO_3^- concentrations were lower than in 2006 but higher than in control years. DN was significantly higher in 2006 than both of the control years across all three sites, but 2008 did not differ from control years and DN was significantly lower than in 2006 at F2 and F3. TN levels in 2008 were low compared to 2006 and 2007. TN was significantly lower in 2008 than in 2007 at all three sites, and was lower than in 2006 at F2 and F3. TN levels in 2005 and 2006 were not significantly different from each other.

No statistically significantly differences were discernible for DP. TP and PP levels were significantly higher in 2005 than in 2006 at F1 and F2. TP and PP levels were lower in 2006 than in 2008 and in 2007 at F3. TP was also significantly lower during 2006 compared to 2008 at F1. The N:P ratio was significantly higher in 2006 than in any other year at sites F1 and F2. The N:P ratio was not significantly different between 2008 and the two control years.

3.3.2 Algal Communities and Biovolume

Qualitative assessment of eukaryotic versus cyanobacterial algal communities was performed by examining SRSi, Chl *a*, and PC (Figure 4) while cyanobacterial populations were concurrently quantified. Mean Chl *a* levels were moderately lower in 2006, suggesting the experiment reduced overall algal biomass, but the differences across the years were not statistically significant ($P > 0.05$). PC, however, showed significant interannual differences at all three sampling stations (Table 1). Although PC levels were moderately lower on average in 2008 than in either of the control years, weekly variation did not produce statistically significant differences between 2008 and either of these years. However, PC levels were significantly lower during 2006 than in 2008 and both control years. This drop in PC also

corresponded with significantly less *Aphanizomenon* during 2006 at F1 compared to all three other years, with biovolumes reduced in 2006 almost to the limit of detection (average of 0.9 $mm^3 L^{-1}$ in 2006 compared to 4.5 $mm^3 L^{-1}$ in 2005, 8.4 $mm^3 L^{-1}$ in 2007 and 3.2 $mm^3 L^{-1}$ in 2008). A similar pattern was seen at F2; average concentrations were 0.4 $mm^3 L^{-1}$ in 2006 and over ten times higher in all other years. Although *Aphanizomenon* populations were extremely low during 2006 at all three sites, F3 did not return a significant AOV due to weekly variations.

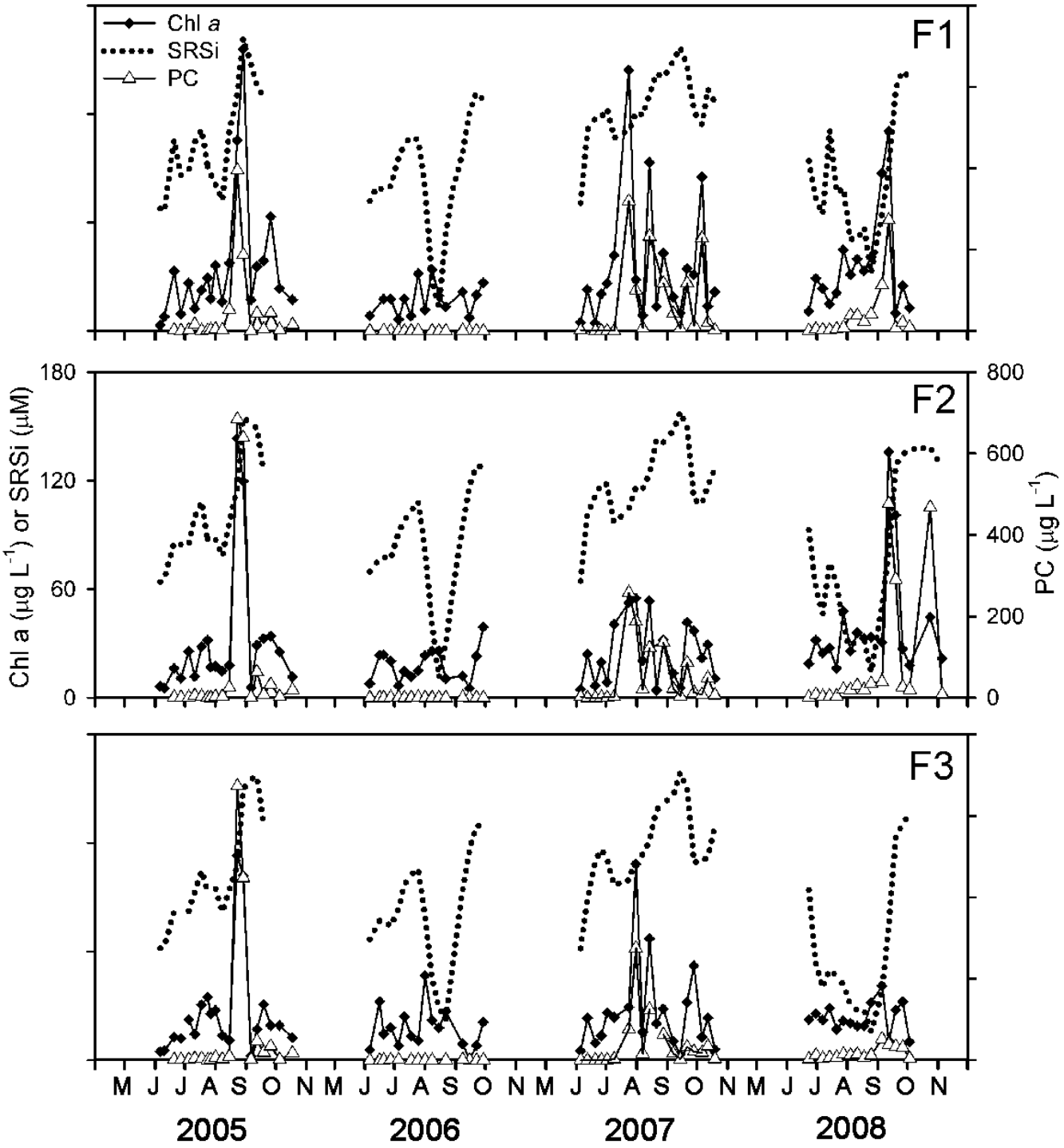
Although PC levels were not significantly lower during 2008, it is important to note that the onset of *Aphanizomenon* blooms occurred much later in 2008 compared to control years. Biovolume did not exceed 2.0 $mm^3 L^{-1}$ at any site until the very last week of July (compared to the first week of July in 2007), and did not form visible surface scum until September. Although *Microcystis* was present in the lake, the late onset of the *Aphanizomenon* bloom meant that the second species was never able to obtain dominance; in 2008 there were no dates at any of the sampling sites when *Microcystis* made up over eighty percent by biovolume of the algal community.

Microcystis did not follow the patterns exhibited by *Aphanizomenon* (Table 1). In fact, variation among years was not statistically significant for any sampling station. There seems to be no clear pattern across either the years or across the stations.

SRSi decreased notably in both 2006 and 2008 (Table 1), indicating greater uptake by diatoms during the summer months, an occurrence not seen in control years (F1: $P = 0.0003$, F2: $P < 0.0001$) [1]. Considered together, these data indicate overall algal biomass was not seriously reduced during the experimental years, but the composition was significantly different, with control years dominated by cyanobacterial communities and experimental years marked by a reduced or at least delayed cyanobacterial bloom coupled with an extended diatom bloom.

[1] See Ferris and Lehman (2007) for a detailed analysis of the extended diatom bloom attributed to the selective withdrawal experiment.

Figure 4. Chl *a*, PC, SRSi concentrations across three sampling sites over four years. Note positive relationship between SRSi and PC.



3.3.3 Microcystin Toxicity

Consistent with lack of interannual variation in *Microcystis* biovolume, microcystin toxin likewise did not exhibit low levels in the experimental years compared with 2005 and 2007 (Table 1). Statistically significant interannual differences were not identified; trends in toxin concentrations were not consistent across sampling stations. A very high level was observed at F1 in 2005 (2.5 nM), followed by lower concentrations for 2006, 2007 and 2008 (0.4 nM, 1.1 nM, 0.6 nM respectively). Station F2 exhibited a low concentration in 2005 (0.4 nM) and a moderate increase in 2006 that was repeated in 2007, but did not continue to increase in 2008 (0.8 nM, 1.1 nM, and 1.1 nM respectively). F3 also showed a low level in 2005 (0.3 nM) that was reproduced in 2007 and 2008 (0.3 nM both years), but was significantly increased in 2006 (1.1 nM).

4. Discussion

4.1 Nutrient Addition Experiments

Our experiments demonstrate that nutrient concentrations in Ford Lake change throughout the summer. These varying concentrations produce different limitations that result in unique algal composition and growth patterns. These experiments also elucidate consistent patterns in the cyanobacterial dynamics of Ford Lake.

In the experiment B1, Chl *a* indicated a positive growth response in algal biomass to each of the nutrients, but the effect was most pronounced with the addition of PO_4^{2-}, suggesting the lake was nearing P limitation at that time. The depressed NO_3^- concentration in the +P treatment also signaled an especially large overall increase in algal biomass following the PO_4^{2-} addition. Increased PC and decreased SRSi indicate the biovolume of both cyanobacteria and diatoms increased. However, *Aphanizomenon* biovolume seemed to respond to increased NO_3^- levels (4.81 $mm^3\ L^{-1}$, vs. 3.29 $mm^3\ L^{-1}$ in control). This is a somewhat surprising result for a couple of reasons. As *Microcystis* is the only other cyanobacterial species present in noteworthy amounts, and it was not present this early in the season, one would expect that elevated PC levels would correlate with an *Aphanizomenon*

growth response. Perhaps the additional PO_4^{2-} allows cyanobacteria to invest in more pigment and the increased pigment concentration does not correlate to an overall increase in biovolume. P additions have been documented to increase intercellular concentration of phycocyanin in cyanobacteria (Pandley and Pandley, 2007). A similar effect is noted in experiment B2, as PC once again exhibits a significant positive response following the +P treatment, but neither cyanobacterial species have a correlated increase in biovolume. These data also suggest that in B1, *Aphanizomenon* was not limited by PO_4^{2-} but rather by NO_3^-, although diatoms clearly showed the opposite growth response. This is unexpected given *Aphanizomenon's* capacity to fix nitrogen; however, it is important to note that decreased SRSi simply indicates diatoms responded. Diatom biovolume was not quantified in this experiment, but *Aphanizomenon* biovolume remained above levels considered significant in our observational studies of Ford Lake; diatoms in no way out competed or outgrew *Aphanizomenon*. Of course, heterocysts are energetically expensive to produce (Turpin et al., 1985, Maier, 2004). This experiment demonstrates that *Aphanizomenon* populations can grow more abundant when they are not required to produce heterocysts, so long as N_2 fixation is not necessary to provide *Aphanizomenon* with a selective advantage.

In the second experiment (B2), *Aphanizomenon* did not exhibit a significant response in any treatment. Overall biovolume levels of *Aphanizomenon* were very low compared to the first experiment, while extremely low SRSi levels suggest a high abundance of diatoms. Unpublished data collected by Goldenberg (2009 Senior Honors Thesis) confirms that Ford Lake algal biovolume was primarily dominated by the diatom species *Fragilaria* at the onset of B2.

In experiment B3, SRSi levels were extremely high. Although SRSi levels were slightly lower following the PO_4^{2-} addition, visual inspection confirms that cyanobacteria clearly dominated the community. A large increase in cyanobacterial biovolume occurred across all four treatments, suggesting that phytoplankton were not nutrient limited. This large supply of nutrients and rapid growth demonstrates how cyanobacteria are able to attain nuisance population sizes in a short period of time in Ford Lake.

Microcystis biovolume was significantly higher after the NO_3^- addition than in the control, and this was the only instance when *Microcystis* biovolume exceeded *Aphanizomenon* biovolume. These data are consistent with observational data from Ford Lake; *Microcystis* gains a selective advantage over *Aphanizomenon* when it is not N limited. However, toxin levels were not particularly high following the NO_3^- addition, meaning they did not correlate well with *Microcystis* biovolume, nor do higher levels of NO_3^- seem to lead to higher toxin production, as some studies have suggested.

In all of the bioassay experiments, NO_3^- levels remained higher than the control in NH_4^+ additions, suggesting that algae will preferentially take up NH_4^+ as a N source. This observation is not surprising, as NH_4^+ is the most reduced form of N, and thus requires the smallest amount of energy expenditure to be converted into biological products such as protein. Studies have shown that polluting water with chemically reduced forms of N, such as NH_4^+ and urea, can rapidly lead to massive cyanobacterial blooms (Glibert, 2006). The third experiment also demonstrates that NH_4^+ suppresses the formation of heterocysts more so than does NO_3^-. One would expect that both forms of DIN have a suppressive effect on heterocyst formation. Turpin et al. (1985) observed heterocysts growing in a medium with high levels of NO_3^-, although heterocyst levels were considerably lower than when *Aphanizomenon* relied solely on N_2 as an N source. They note that cells that employ a combination of NO_3^- reduction and N_2 fixation have an intermediate growth rate when compared to *Aphanizomenon* using NH_4^+ as its primary N source as well as trichomes that fix N_2 alone. It is possible that in this study, the effects of NH_4^+ suppressing heterocyst formation simply occurred in a much shorter duration than the effects of NO_3^-, and the effects of NO_3^- were not discernable after 5 days.

4.2 **Microcystis *Stoichiometry***

An N:P ratio of 46:1 as found in the cultures of *Microcystis* would seem to indicate a P deficiency when compared to the expected intracellular ratio of 16:1. However, the recipe for the UTEX prepared J Media (http://www.utex.org/) includes 0.05 mM Na_2HPO_4 and 0.22 mM K_2HPO_4, for a total of 0.27 mM SRP in solution upon inoculation. The concentration of

PP in the solution when the bottles were broken down was 1.16μM, or 0.00116 mM. Thus the majority of SRP remained in solution and the *Microcystis* cultures were not P limited by their surroundings. Downing et al. (2004) reported that, in growing *Microcystis* cultures with a cellular N:P ratio ranging from 1 to 83, the maximum specific growth rate occurred in those cells with an N:P ratio of 44. This relatively high N:P ratio may explain why naturally occurring *Microcystis* is so easily overwhelmed by *Aphanizomenon* in Ford Lake when the N:P ratio and DIN levels drop. Nalewajko and Murphy (2001) noted that *Anabaena*, a heterocyst-producing cyanobacterial species, outgrew *Microcystis* in N limited water (N:P of 10:1). They noted that *Microcystis* grew optimally at an N:P ratio of 100:1 and was able to out compete *Anabaena*, although they concluded *Microcystis* fairs poorly in extremely P-limited conditions.

Otsuki et al. (1993) demonstrated a linear relationship between Chl *a* and PC that breaks down when Chl *a* levels exceed 250 μg L^{-1}, but the g:g ratio was approximately 0.176 in a pure culture of *Microcystis*, approximately 2.6 times higher than the ratio found in this experiment. This would correspond to a Chl *a*:PC ratio of approximately 5.6 (mol:mol). Raps et al. (1983) found a Chl *a*:PC ratio ranging from 1.12-1.56 (mol:mol) as long as light intensity ranged from 20-240 μE m^{-2} s^{-1}. Higher light intensities led to a significantly lower ratio. Thus the Chl *a*:PC ratio obtained experimentally here is similar to that found in other studies, however, such a ratio was not observed during field studies when *Microcystis* was dominant in Ford Lake. This may be due to other species being present in the lake, albeit in small amounts. It has been demonstrated that pigment ratios within cyanobacteria can be highly variable due to several factors, including genetic variability within colonies and the absence or presence of gas vacuoles (Banares et Al., 2007, Jost and Porter, 2004).

Microcystin levels of 4.94 x 10^{-5} pmol $cell^{-1}$ correspond to approximately 49.1 fg $cell^{-1}$, which is within the range of other cellular quotas reported for pure toxic cultures (Lyck and Christofferson, 2003, Weidner et al., 2003). However, Weidner et al. note considerable variation caused by variations in light intensity. Many other factors also have been reported to affect the cellular quota of microcystin, such as N concentrations (Downing et al., 2005).

4.3 Selective Withdrawal Experiments

Further support for our findings regarding the dynamics of *Aphanizomenon* and *Microcystis* comes from purposeful destabilization of the lake during summer 2006 and 2008. Both experiments were able to elevate the NO_3^- levels to at least some degree (the 2006 experiment was clearly more effective), and both experiments destabilized the water column. The combined effects were profound on *Aphanizomenon* blooms; in 2006 the bloom was effectively eliminated, and in 2008 the bloom was delayed and did not reach nuisance levels until the very end of the summer. Diatoms simultaneously enjoyed an extended growing season.

Microcystis biovolume and microcystin toxin production were not quantitatively affected by the experiments. This response is consistent with the hypothesis that *Microcystis* is better adapted to a range of N:P ratios that allows it to compete with eukaryotic as well as cyanobacterial communities. Levine and Schindler (1999) suggest that decreasing P instead of increasing N may be the best approach to changing N:P ratios to favor eukaryotic communities. They predict an increase in N may drive a species shift from *Aphanizomenon* to *Microcystis*, and indeed we observed that in 2006. However, in 2008, we also observed that by delaying the *Aphanizomenon* bloom, *Microcystis* never achieved dominance in the lake.

It is important to note that these experiments are dependent on rainfall. Our intentions were to allow the hypolimnetic withdrawal to continue through the month of August, but because Ford Lake is operated as run of the river, reduced rainfall caused an early termination to the experiment in 2008, and very quickly afterward the lake assumed a late summer thermocline and the bottom layer became anoxic. The combination of stability and eutrophication then led to late summer cyanobacterial blooms.

5. Conclusions

Cyanobacterial dynamics in Ford Lake seem to be primarily driven by nitrogen, but experimental evidence shows that these relationships are complicated by a variety of other factors as well, such as the source of N, limiting effects of other nutrients, and competition from non-Cyanophyta. However, results from the third bioassay experiment emphasize the importance of N, particularly in the form of NO_3^-, in *Microcystis* and *Aphanizomenon* growth patterns. Unfortunately, the experiments were unable to provide further insight into microcystin toxin production, and the bioassay experiments particularly emphasized the lack of correlation between microcystin levels and NO_3^-.

Selective withdrawal appears to be a viable management option for improving water quality under certain circumstances. It would be best complemented by a toxin-management approach, and more work must be done to identify the environmental and molecular causes of toxin production. Environmental sustainability of the management approach will require additional experiments and testing of management theory over longer periods of time. The question of socio-political sustainability of management options exists in a different academic sphere from science and will require evaluations by the local and municipal governments charged with overseeing such operations in the context of their other commitments and priorities.

References

Banares, E. E., R.V. Lopez, E. Costas, C. Salgado, and M.A. Flores. 2007. Genetic variability associated with photosynthetic pigment concentration, and photochemical and nonphotochemical quenching, in strains of the cyanobacterium *Microcystis aeruginosa*. *Fems Microbiology Ecology*. 60: 449-445.

Downing, T.G., C. Meyer, M.M. Gehringer, and M. van de Venter. 2005. Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. *Environmental Toxicology*. 20: 257-262.

Downing, T.G., W. Sember, M.M. Gehringer, and W. Leukes. 2004. Medium N:P Ratios and Specific Growth Rate ComodulateMicrocystin and Protein Content in *Microcystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. *Microbial Ecology*. 49, 468-473.

Glibert, P. M., J. Harrison, C. Heil, and S. Seitzinger. 2006. Escalating Worldwide use of Urea – A Global Change Contributing to Coastal Eutrophication. *Biogeochemistry*. 77: 441-463.

Goldenberg, S. Z. The diatoms of Ford Lake: Bloom dynamics and whole lake manipulation in a eutrophic urban impoundment. 2009. Senior Honors Thesis, University of Michigan, Ann Arbor.

Jost, M. and J. Porter. 2004. Physiological effects of the presence and absence of gas vacuoles in the blue-green alga, *Microcystis aeruginosa* Kuetz. emend. Elenkin. *Biomedical and Life Sciences*. 110: 225-231.

Kameyama, K., N. Sugiura, H. Isoda, Y. Inamori, and T. Maekawa. 2002. Effect of nitrate and phosphate concentration on production of microcystins by Microcystis viridis NIES 102. *Aquatic Ecosystem Health and Management*. 5: 443-449.

Kao, O. and D.S. Berns. 1969. Molecular weight and dimensions of phycocyanin monomer and aggregates. *Biopolymers.* 8, 293-295.

Lehman, E.M., K.E. McDonald, and J.T. Lehman. 2009. Whole lake selective withdrawal experiment to control harmful cyanobacteria in an urban impoundment. *Water Research*, 43: 1187-1198.

Levine, S.N. and D.W. Schindler. 1999. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. *Can J. Fish. Aquat. Sci.* 56: 451–466.

Lyck, S. and K. Christoffersen. 2003. Microcystin quota, cell division and microcystin net production of preculture. *Phycologia*. 42:667-674.

Maier, R.J. 2004. Nitrogen Fixation and respiration: Two Processes linked by the energetic demands of Nitrogenase. *Respiration in Archaea and Bacteria.* Springer Netherlands. 16: 101-120.

Otsuki, A., T. Omi, S. Hashiomoto, M. Aizaki, and N., Takamura. 1993. HPLC fluorometric determination of natural phytoplankton phycocyanin and its usefulness as cyanobacterial biomass in highly eutrophic shallow lake. *Water, Air, and Soil Pollution*. 76, 383-396.

Pandley, U. and J. Pandley. 2007. Enhanced production of biomass, pigments and antioxidant capacity of a nutritionally important cyanobacterium *Nostochopsis lobatus*. *Bioresource Technology*. 99: 4520-4523.

Raps, S., K. Wyman, H.W. Siegelman, and P.G. Falkowski. 1983. Adaptation of the Cyanobacterium *Microcystis aeruginosa* to Light Intensity. *Plant Physiology* 72:829-832

Redfield, A.C. 1958. The biological control of chemical factors in the environment**.** *American Scientist*. 46:205–222

Sigma Aldrich 2008. Chlorophyll *a* from a cyanobacteria species. Jan 2008 Catalogue. http://www.sigmaaldrich.com/catalog/ProductDetail.do?N4=44014|FLUKA&N5=Product%20No.|BRAND_KEY&F=SPEC

Turpin, D. H., D.B. Layzell, and I.R. Elrifi. 1985. Modeling the C Economy of *Anabaena flos-aquae*: Estimates of Establishment, Maintenance, and Active Costs Associated with Growth on NH_3, NO_3^-, and N_2. *Plant Phyiology*. 78: 748-752.

Wiedner C., P.M. Visser, J. Fastner, J.S. Metcalf, G.A. Codd, and L.R. Mur. 2003. Effects of light on the microcystin content of Microcystis strain PCC 7806. *Applied and Environmental Microbiology*. 69: 1475-1481.

Yoshida, M., T. Yoshida, Y. Takashima, N. Hosoda, and S. Hiroishi. 2007. Dynamics of microcystin-producing and non-microcystin-producing *Microcystis* populations is correlated with nitrate concentration in a Japanese lake. *FEMS Microbiol. Lett.* 266**:**49–53