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FACTORS CONTROLLING THE ABUNDANCE AND COMPOSITION OF BLUE-GREEN ALGAE IN LAKE GRIFFIN

Final Report
to the
St. Johns River Water Management District

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FACTORS CONTROLLING THE ABUNDANCE AND COMPOSITION OF BLUE-GREEN ALGAE IN LAKE GRIFFIN

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EXECUTIVE SUMMARY

Recent research of Lake Griffin has shown that the phytoplankton community is characterized by blooms of blue-green algae (aka, cyanobacteria). It is clear from chlorophyll records for the past three decades that Lake Griffin is a nutrient-rich environment that falls into the eutrophic/hypereutrophic range under many established trophic state guidelines, including total phosphorus, total nitrogen and chlorophyll a concentration). It is therefore not surprising that the lake is subject to algal blooms. Historic data for Lake Griffin shows that chlorophyll a concentrations over 100 µg/l have been common since at least 1977. Until 1996 peak chlorophyll a concentrations were generally below 200 µg/l. In 1996, this threshold was transcended, with numerous observations of chlorophyll a concentrations higher than 250 µg/l. While the reasons for the apparent step increase in phytoplankton abundance in 1996 remain uncertain, it has become an issue of widespread public concern. The concerns just described focus attention on two important aspects of the ecology of Lake Griffin: (1) The factors that control phytoplankton standing crops in the lake and (2) The factors that contribute to domination of the phytoplankton community by blue-green algae, like *Cylindrospermopsis*. These two questions are central to the management-related question of whether the character and intensity of algal blooms in Lake Griffin can be controlled.

The goal of the research effort described in this report was to provide answers to outstanding questions about the character and causes of algal blooms in Lake Griffin that have a direct bearing on the management of the ecosystem. The research focused on three central objectives:

(1) To determine whether changes in chlorophyll concentration reflect changes in phytoplankton biomass.
(2) To determine the sensitivity of algal growth to changes in nitrogen and phosphorus concentrations.
(3) To examine key factors contributing to the ability of *Cylindrospermopsis* to dominate the phytoplankton community of Lake Griffin.

The results of the current, and a previous study of the lake, demonstrate that the abundance and species composition within the lake is relatively uniform spatially, from both a geographical and vertical perspective. The question of whether chlorophyll a concentrations are strictly proportional to phytoplankton biomass is a more complicated issue. The results of experiments dealing with the affects of light flux and nutrient regime on the growth and physiology of both natural populations and uni-algal cultures of algae reveal the potential for over two-fold differences in chlorophyll a content of cells. A direct comparison of the chlorophyll a content of three common bloom-forming species of blue-green algae, *Anabaena*, *Microcystis* and *Cylindrospermopsis*, revealed significant differences between species grown under identical light and nutrient regimes. *Cylindrospermopsis* had over two-fold higher concentrations than either *Anabaena* or *Microcystis*. The results of these experiments suggest that some portion of the temporal variability in chlorophyll a concentrations observed over the past 22 years may not fully reflect changes in phytoplankton biomass. Variations in nutrient status, light availability,

species composition and the age of bloom populations may all contribute to the changes in the concentration of chlorophyll observed in the lake.

The putative dominance of the nitrogen-fixing species of blue-green algae from the mid-1990s through mid-2002 indicates the predominance of nitrogen-limiting conditions during this time period. The importance of nitrogen limitation in Lake Griffin is corroborated by the results of nutrient limitation bioassays performed in a study of the lake from 2000 through 2002. TN/TP ratios have increased from 2001 through the current study period, ending in 2004. In addition, nutrient limitation bioassays conducted as part of this study in 2003 and 2004 showed a higher frequency of phosphorus and nitrogen/phosphorus co-limitation than in the previous study from 2000-2002. Several other observations support the hypothesis that Lake Griffin may be trending toward a greater degree of phosphorus limitation since 2001. From a biological perspective, one of the most dramatic shifts has been the rise and fall in the importance of the potentially toxic blue-green alga *Cylindrospermopsis*.

The most obvious feature of *Cylindrospermopsis* that helps to explain its domination is the ability to fix nitrogen. The results of this study clearly demonstrate that *Cylindrospermopsis* is capable of strong growth in the absence of any outside inputs of reduced nitrogen, but not more so than another common nitrogen-fixer *Anabaena*. One experiment provides preliminary evidence that *Anabaena* is more sensitive to high light and UV inhibition of growth than *Cylindrospermopsis*. The importance of this factor is accentuated in Lake Griffin because of its extremely shallow depth. The results of another experiment provide preliminary evidence that *Cylindrospermopsis* may produce and excrete biologically active substances that inhibit the growth of certain species of algae, like the blue-green alga *Microcystis incerta* and the green alga *Scenedesmus bijuga*. The results of a third experiment involving *Cylindrospermopsis* indicate that its phosphorus uptake rates are considerably higher than those of *Anabaena.*

It is clear from the results of the aforementioned experiments that *Cylindrospermopsis* has many characteristics that make it very competitive in ecosystems like Lake Griffin. This is not surprising considering the prominence of this group of algae in eutrophic lakes throughout the world. It is noteworthy that the relative decline of *Cylindrospermopsis* in the phytoplankton community in Lake Griffin after 2002 was accompanied by rise in importance of several other small-celled blue-green algae, like 1 to 2 µm diameter filaments of *Oscillatoria*. The recent major shifts in phytoplankton abundance and composition indicate that Lake Griffin is dynamic and possibly receptive to changes in nutrient load.

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I. INTRODUCTION

Recent research of Lake Griffin has shown that the phytoplankton community is characterized by blooms of blue-green algae (aka, cyanobacteria). Unfortunately, the general paucity of rigorous historical information on the lake's plankton community makes it difficult to determine how long these blooms have been a prominent feature of the system. Paleolimnological studies indicate that the rate of total phosphorus (TP) sedimentation experienced a 'sharp increase beginning about 1950' and the planktonic/benthic diatom microfossil ratio reached its highest level in the 1980's and early 1990's (Schelske 1998). Both of these paleolimnological indicators suggest an increasing frequency of algal blooms over the past 50 years, peaking over the past two decades. It is clear from chlorophyll a records for the past three decades (Fulton, personal communications) that Lake Griffin is a nutrient-rich environment that falls into the eutrophic/hypereutrophic range under many established trophic state guidelines, including total phosphorus, total nitrogen and chlorophyll a concentration (Carlson 1977). It is therefore not surprising that the lake is subject to algal blooms. Historic data for Lake Griffin shows that chlorophyll a concentrations over 100 µg/l have been common since at least 1977 (Fulton, personal communications). Until 1996 peak chlorophyll a concentrations were generally below 200 µg/l. In 1996, this threshold was transcended, with numerous observations of chlorophyll a concentrations higher than 250 µg/l. While the reasons for the apparent step increase in phytoplankton abundance in 1996 remain uncertain, it has become an issue of widespread public concern.

Another issue of concern in Lake Griffin is the prominence of potentially harmful blue-green algae species in the phytoplankton community. Among the blue-green algae that have been dominant in the lake over the past few years *Cylindrospermopsis* appears to have been the most prolific and persistent. The fact that certain species of *Cylindrospermopsis* have caused human and animal health problems in other parts of the world has precipitated considerable local concern. How far back *Cylindrospermopsis* has been a major player in Lake Griffin's phytoplankton community is uncertain. Despite the fact that some researchers have hypothesized that it is a recent addition to the

phytoplankton assemblage, there is currently insufficient data to adequately test this hypothesis. Similarly, the specific level of risk to animal health posed by the strains of *Cylindrospermopsis* found in Lake Griffin remains largely unresolved.

The current study was aimed at providing answers to outstanding questions about the structure and function of the phytoplankton community in Lake Griffin, including the basis for the extraordinary success of *Cylindrospermopsis*. Interpretation of recent historical information on the lake's phytoplankton are strongly influenced by several facts, including: (1) the past decade has been an extended period of drought for the State of Florida, interrupted by El Nino periods in 1997/98 and 2001/02, and (2) the mid-1990s was marked by significant efforts to reduce the trophic status of the Apopka chain of lakes through the employment of marsh raceways, which may have altered the nature of nutrient loading to the lakes in the Apopka chain.

II. OBJECTIVES

The concerns just described focus attention on two important aspects of the ecology of Lake Griffin: (1) The factors that control phytoplankton standing crops in the lake and (2) The factors that contribute to domination of the phytoplankton community by blue-green algae, like *Cylindrospermopsis*. These two questions are central to the management-related question of whether the character and intensity of algal blooms in Lake Griffin can be controlled.

While the current state of knowledge on phytoplankton dynamics in Lake Griffin provides a base for addressing the aforementioned questions, key voids in our understanding of driving factors remain. These voids may preclude water managers from proceeding with a sufficient level of confidence in the outcome of their actions. The goal of the research effort described in this report was to provide answers to outstanding questions about the character and causes of algal blooms in Lake Griffin that have a direct bearing on the management of the ecosystem. The research focused on three central objectives:

(1) To determine whether changes in chlorophyll a concentration reflect changes in phytoplankton biomass.
(2) To determine the sensitivity of algal growth to changes in nitrogen and phosphorus concentrations.
(3) To examine key factors contributing to the ability of *Cylindrospermopsis* to dominate the phytoplankton community of Lake Griffin.

III. RESEARCH PLAN

The objectives of this study were pursued within the context of several hypotheses associated with each objective and research tasks aimed at addressing these hypotheses:

Objective 1 – To determine whether changes in chlorophyll a concentrations at selected sampling sites accurately reflect lake wide changes in phytoplankton biomass.

Hypothesis 1a: Changes in chlorophyll a accurately reflect changes in phytoplankton biomass.

Task 1a: To test this hypothesis we examined how nutrient and light availability affect the chlorophyll a/TP, biomass/TP and chlorophyll a/biomass values for *Cylindrospermopsis* and examine whether these ratios are substantially different from two other cyanobacteria common to eutrophic lakes in Florida, i.e. *Anabaena flos-aquae* and *Microcystis incerta.* The basis for this test is the fact that nutrient and light status are the two primary environmental factors that impact the chlorophyll a concentrations in algal cells. The experiments were done with uni-algal cultures of all three species. Complementary tests of these ratios were done using whole water samples collected from Lake Griffin. Uni-algal cultures and whole water samples from Lake Griffin were subjected to light (3 light levels) and nutrient gradients (P-limited, N-limited and nutrient-rich cultures). The ratios were determined from analyses of chlorophyll a, TP and cellular biovolume content.

Hypothesis 1b: Phytoplankton in Lake Griffin is uniformly distributed around the lake and through the water column.

Task 1b: The results of recent studies of Lake Griffin (Phlips and Schelske 2004) revealed spatial and vertical stratification of phytoplankton on numerous sampling dates. In this study, a rigorous analysis was conducted of spatial heterogeneity and vertical stratification, including the influence of wind events on the distribution of phytoplankton.

Objective 2 – To determine the sensitivity of algal growth to changes in phosphorus and nitrogen concentrations.

Hypothesis 2: Phytoplankton growth in Lake Griffin is insensitive to external inputs of phosphorus and nitrogen.

Task 2: The apparent variability of limiting-nutrient status in Lake Griffin observed in recent studies in 2001 and 2002 (Phlips and Schelske 2004) raised several important issues about the sensitivity of the lakes phytoplankton to changes in phosphorus and nitrogen concentrations. Indications of phosphorus limitation of phytoplankton production suggested that the control of phosphorus inputs may be a means of reducing the frequency, intensity and even character of algal blooms in the future. However, it might be argued that 2001 was an anomalous year caused by the extreme drought conditions and exceptionally low water levels experienced during the study period. There are a number of reasons why such conditions may have led to phosphorus limitation. The most obvious potential factor is reductions in external loading of phosphorus-rich water. In this study, research on nutrient-limitation status was extended for an additional year and a half.

Objective 3 – To examine key factors contributing to the ability of *Cylindrospermopsis* to dominate the phytoplankton community of Lake Griffin.

Hypothesis 3a: *Cylindrospermopsis* is a superior competitor under conditions of low light availability and/or high light flux.

Task 3a: In freshwater ecosystems subject to hypereutrophic conditions mean light availability in the water column can be restricted due to self-shading. Phytoplankton species can adapt to this condition by regulating buoyancy or increasing the efficiency of light utilization. If the species takes the former strategy, by increasing buoyancy, it must be capable of adapting to high light flux and ultraviolet light exposure. To test the potential importance of light adaptation to the success of *Cylindrospermopsis* in Lake Griffin, the growth response of *Cylindrospermopsis, Microcystis incerta* and *Anabaena flos-aquae* to a range of light intensities was compared in indoor and outdoor experiments.

Hypothesis 3b: *Cylindrospermopsis* produces biologically active substances that inhibit the growth of other species of algae.

Task 3b: To test this hypothesis uni-algal cultures of *Cylindrospermopsis* from Lake Griffin were used to test the sensitivity *Microcystis, Anabaena*, and *Scenedesmus* using standard microbiological sensidisk procedures.

Hypothesis 3c: *Cylindrospermopsis* is a superior competitor for phosphorus.

Task 3c: The basis for this hypothesis is the suggestion that *Cylindrospermopsis* is a superior competitor for bioavailable phosphorus in Lake Griffin. As a first test of this hypothesis the rate of phosphorus

uptake and short-term growth rate was compared for *Cylindrospermopsis* and two likely competitors in the phytoplankton community of Lake Griffin, i.e. *Microcystis incerta* and *Anabaena flos-aquae*. The rates were determined for a range of phosphorus concentrations under nitrogen sufficient conditions. Time series analysis of phosphorus removal (change in ortho-phosphorus concentration) was used to compare ortho-phosphorus uptake rates for the three species.

Hypothesis 3d: *Cylindrospermopsis* is a superior competitor under nitrogen-limited conditions.

Task 3d: In any freshwater ecosystem subject to nitrogen limitation and high phosphorus loading nitrogen-fixing blue-green algae can play an important role in the phytoplankton community. In Lake Griffin the central question is why *Cylindrospermopsis* dominates rather than other nitrogen-fixers common to Florida, like *Anabaena.* To examine this question the rates of growth were compared for uni-algal cultures of *Cylindrospermopsis* and *Anabaena flos-aquae* under nitrogen-limiting conditions. Time-series analysis of algal abundance (chlorophyll a and/or cell count) was used to compare maximum growth rates.

IV. METHODS

Site description **–** Two primary sampling sites were established in Lake Griffin, one in the northern basin ('north' site) and one in the southern basin ('south' site) (Figure 1). In addition to the two primary sites, supplemental sites were collected on separate dates to evaluate spatial variability in phytoplankton standing crop (i.e. in terms of chlorophyll a) (Figure 1).

Field Measurements - A number of basic water column characteristics were measured on site. Temperature and oxygen concentration were measured at regular depth intervals using Hydrolab Surveyor units and YSI instruments. This information was used to evaluate a number of key issues related to spatial and temporal variability of water masses, including locations of vertical discontinuity layers and presence of low oxygen zones.

Quantum light flux was measured at depth intervals with Li-Cor PAR probes; 2π surface and 2π underwater downwelling. Light extinction coefficients were determined using the Beers Law equation (Wetzel 1983).

Water Sample Analyses – Water was collected at the sampling sites with a water column integrating tube that samples water evenly from the surface to 0.2 meters from the bottom. At least five replicate tube samples were combined in a mixing vessel before sub-samples were withdrawn for the various analyses

Water samples were subdivided on site into aliquots for chlorophyll a, phytoplankton composition, color, turbidity, water chemistry analysis and experimental work. Chlorophyll was used as the primary estimator of phytoplankton abundance. Chlorophyll a samples were filtered and stored frozen for subsequent analysis using standard spectrophotometric methods. Color was analyzed spectrophotometrically using a platinum cobalt standard. Turbidity was determined using a Nephelometer. Total nitrogen, nitrate (+ nitrite), ammonium, total phosphorus, soluble reactive phosphorus, silica and particulate organic carbon were analyzed using standard methods for water analysis. All methods are part of our Florida Department of Health/NELAP certified QA/QC Plan #E72883 and Project QA Plan (QAPP #200064). Analytical methods and limits of detection are provided in Appendix 5.

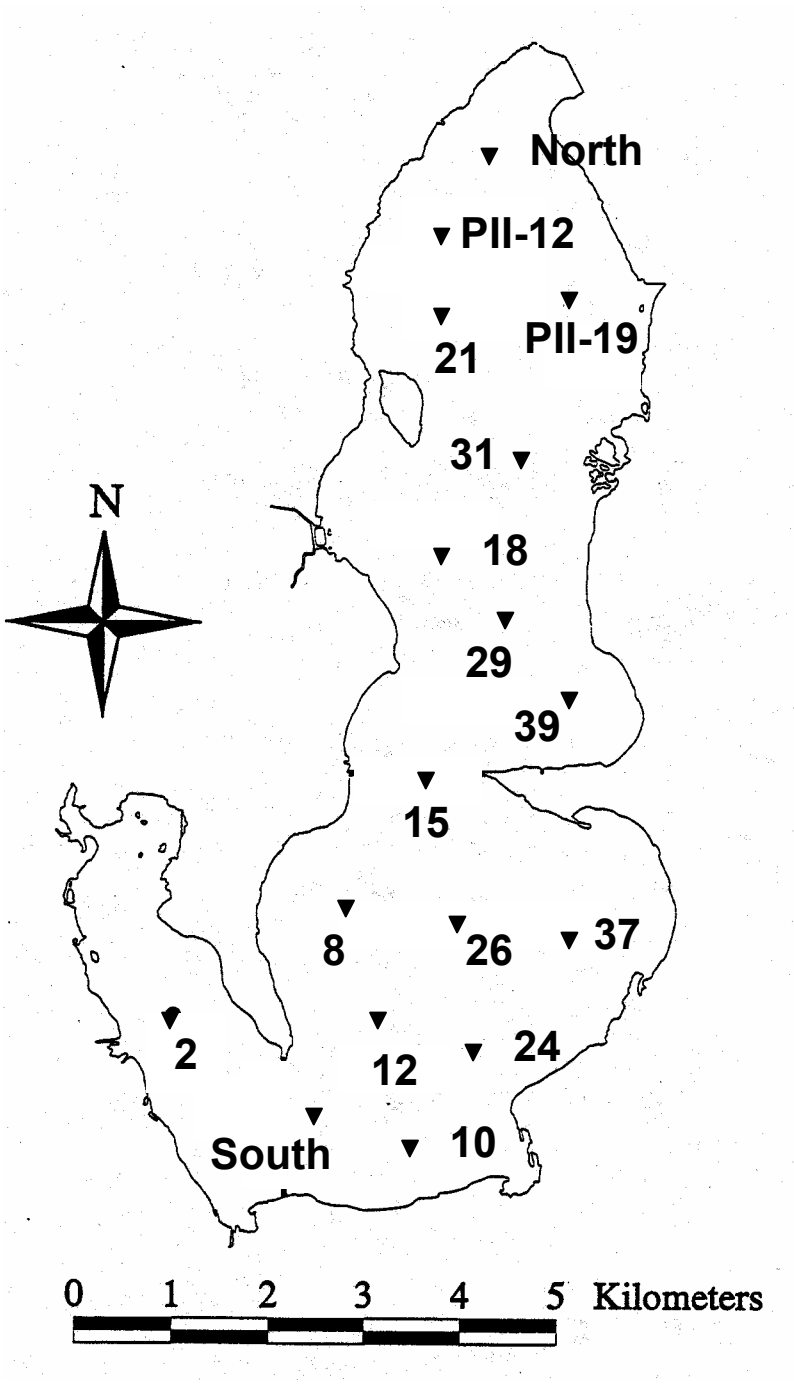

Figure 1. Locations of sampling sites in Lake Griffin.

Phytoplankton composition was analyzed microscopically using the Utermohl settling method (Utermohl 1958). Lugols solution preserved samples were settled in 19mm inner diameter cylindrical chambers. Phytoplankton cells were identified and counted at 400X and 100X with a Nikon phase contrast inverted microscope. At 400X, a minimum of 100 cells of a single taxa and 30 grids were counted. If 100 cells were not counted by 30 grids, up to a maximum of 100 grids were counted until one hundred cells of a single taxa was reached. At 100X a total bottom count was completed for taxa greater than 30 microns. Counts for individual taxa were converted to biovolume using the closest geometric shape method. Biovolume is the most direct measure of phytoplankton standing crops, and provides a physiologically meaningful way of describing the relative importance of different phytoplankton species to community structure and function. The key cyanobacterium *Cylindrospermopsis* was not assigned a species designation. Although the some of the physical characteristics of the *Cylindrospermopsis*, including the shape of heterocystes and the diameter of the vegetative cells most closely resembled the species *C. cuspis*, considerable disagreement exists about the speciation of this genus in Florida.

Zooplankton samples were acquired from the same water samples taken for phytoplankton. Zooplankton sample water was preserved with Lugols solution in the field at the time of collection. In the lab zooplankton sample water was passed through a 41 μm mesh nylon filter, the volume filtered recorded, and the filtrate rinsed into a glass amber bottle for settling and concentration to 20ml final volume. The final concentrated sample was placed in archive boxes until analysis. For analysis, sub-samples, the volume of which was determined by the zooplankton density and/or the sediment load, were withdrawn and placed in final glass bottomed settling chambers. Zooplankton were enumerated using an inverted microscope at X100 magnification. Counting was continued until 100 individuals of one taxa were enumerated and a minimum of 3ml of sample was examined. A list of taxonomic guides for phytoplankton and zooplankton is provided as part of the reference section.

Bacterioplankton were enumerated by filtering formalin preserved samples onto 0.2 μm membrane filters, staining with acridine orange, and counting at 1000X oil immersion using fluorescence microcopy.

Bioassay Experiments - Nutrient limitation/growth bioassay experiments were performed on eleven dates at the north site (Figure 1). The experimental design was based on methods described by Aldridge et al. (1995). Assays were done under laboratory conditions in 500-mL Erlenmeyer flasks. Three different test combinations were included in the bioassays: (1) Whole lake water collected on site, (2) Lake water diluted 1:10 with filtered lake water, and (3) Nutrient-depleted unialgal cultures of the green alga *Selenastrum capricornutum*. Treatments (in triplicate) included control (no additions), nitrogen (in the form of potassium nitrate) addition (final flask concentration of 400 µg N l^{-1}), P (in the form of K_2HPO_4) addition (final flask concentration of 40 µg P l^{-1}), N + P addition (final flask concentrations of 400 µg N l^{-1}+ 40 µg P l^{-1}), and N+P+Si (final flask concentrations of 400 µg N l^{-1}+ 40 µg P l^{-1}+ 400 µg Si l^{-1}).

Incubations were done in laboratory chambers containing temperature-controlled water baths with bottom illumination. Incubation temperatures were held at ambient temperatures recorded on each sampling date. Light intensity was fixed at 120 µmole photons/m^2/s. Photoperiod was 12/12 dark/light hours, respectively, from October through March and 10/14 dark/light from April through September. Algal biomass was estimated by net *in vivo* fluorescence of chlorophyll a (*IVF*) using a Turner Designs Model 10 fluorometer with a 1-cm path length at time 0, 2, 4, 6 and 8 days, or until fluorescence values peaked. Ethanol extracted chlorophyll a concentrations were determined at time 0, and at the end point of the incubation period.

A nutrient was considered limiting when the standing crop of phytoplankton in the control was lower than that observed in a nutrient addition treatment group. When algal growth occurred in the control, it was concluded that no nutrient was limiting and that some component of the phytoplankton community was growing at the time of sampling or some controlled variable, such as light, was more optimal under the assay conditions than in the natural environment. Duncan's Multiple Range Test was used to evaluate the significance of differences in response to nutrient additions using chlorophyll a values at the point of greatest resolution

Sensi-discs Growth Inhibition Experiments - *Anabaena flos-aque, Microcystis incerta, Scenedesmus bijuga* and *Cylindrospermopsis* liquid cultures were grown in

nutrient sufficient freshwater medium. Cells from the exponential phase of growth were inoculated and uniformly distributed onto agar plates made with the same nutrient sufficient freshwater medium. After two days of stabilization, sensi-disc papers were allowed to absorb different treatment solutions derived from early stationary phase *Cylindrospermopsis.* The three different *Cylindrospermopsis* extract solutions were prepared as follows:

1) Concentrated extract: (12ml+3ml) 12ml culture was centrifuged, washed with DI twice and centrifuged. 3ml of distilled water was added to the pellet and sonicated. After sonication, the suspension was centrifuged at 13,000 rpm for 5 minutes. The supernatant was used for the experiment.

2) Un-concentrated extract: (12ml+12ml) 12 ml culture was centrifuged, washed with DI twice, centrifuged, 12ml DI was added and sonicated. The suspension was centrifuged at 13,000 rpm for 5 minutes. The supernatant was used for the experiment.

3) Stationary phase medium: The suspension was centrifuged at 2,000 rpm for 5 minutes and the supernatant drawn off from the top.

Phosphorus Uptake Experiments - From batch cultures of *Cylindrospermopsis*, and *Anabaena flos-aque* appropriate amounts were taken and centrifuged. The pellets were washed with FWH-N-P (Fresh Water Hoaglands medium without nitrogen, -N, or phosphorus, -P) twice and centrifuged again. In one set of experiments these species were inoculated into FWH or FWH-N media, P concentration being 200 µg P/L (13 µM P). Since K_2HPO_4 concentration was lowered, equimolar concentration of KCl was added to the medium (For the experiments on 04.28. 2004 and 05.02.2004)(P-limited growth). In the other set of experiments, they were inoculated in FWH-P or FWH-N-P. In both cases *Anabaena* and *Cylindrospermopsis* were grown for about 7 days before the short term P uptake experiment.

At the end of the one week growth period, appropriate amounts were taken from each culture (*Anabaena* FWH, *Anabaena* FWH-N; *Cylindrospermopsis* FWH, *Cylindrospermopsis* FWH-N), centrifuged, washed with FWH-N-P twice and 10 ml of either FWH-P or FWH-N-P were added. After the filament numbers were determined from these concentrated cultures, appropriate amounts were taken and diluted to 500 ml so that there were 10^4 filaments per ml.

From each 500 ml culture, 400 ml was divided into 4 beakers. Each beaker was stirred with a magnetic bar during the uptake experiment. Before the addition of P, initial SRP (soluble reactive phosphorus) samples were taken from the cultures. This value was added to the amount of P that we added to the culture. This gave us the P concentration at time 0. There were a total of 4 different P additions: 5 µg P/L, 10 µg P/L, 15 µg P/L, and 20 µg P/L. Subsamples were taken on the 1^{st}, 5^{th}, 10^{th}, 15^{th} and 20^{th} minutes. These subsamples were immediately filtered and P-uptake was determined by measuring SRP of the subsamples.

Wind resuspension experiments – The impact of wind resuspension on phosphorus, nitrogen and phytoplankton composition in the water column was examined by collecting water samples at five sampling sites across Lake Griffin and at three depths in the water column: surface (0.3m), mid-water column and at the sediment/water interface. Initial water samples were collected during a prolonged calm period, based on an anticipated upcoming windy period. A second collection was made 12-15 hours after the initiation of a windy period. Samples were processed according to the methods outlined above and transported to the laboratory at the University of Florida for analysis.

Phytoplankton growth experiments – A number a different experiments were carried out to test the response of phytoplankton growth and physiology to differences in irradiance and nutrient regime. Indoor growth experiments used 2l fernbach flasks set up in temperature controlled incubation rooms using fluorescent lighting. Outdoor growth experiments were set up in temperature controlled water baths and natural sunlight illumination. Culture media for all experiments were based on a modified Hoaglands formula for freshwater algal culture. Nutrient concentrations were modified by the addition or omission of nitrogen, in the form of nitrate, and phosphorus, in the form of orthophosphate.

V. RESULTS AND DISCUSSION

A. TASK 1a. Chlorophyll a, Phosphorus and Cell Biovolume Relationships.

The effects of growth light flux and nutrient regime on chlorophyll a, cell biovolume and particulate phosphorus concentrations were examined for natural water samples and unialgal cultures of three common bloom-forming species of cyanobacteria: *Anabaena flos-aquae*, *Cylindrospermopsis* and *Microcystis incerta*. The central goals of these experiments were two-fold: (1) to test the null hypothesis that the relationships between the latter three parameters were unaffected by light flux or nutrient regime, and (2) to test the null hypothesis that all three species showed similar relationships between chlorophyll a, cell biovolume and particulate phosphorus concentration.

i. Light affects on natural populations

The results of experiments with natural populations of phytoplankton from Lake Griffin demonstrate that the relationships between chlorophyll a, cell biovolume and particulate phosphorus concentration are impacted by growth light flux and nutrient regime. The compensation light flux for growth of the phytoplankton community collected in March of 2003 was approximately 10 µmole photons $m^{-2}s^{-1}$ (Figure 2). Growth, in terms of chlorophyll a concentration and particulate phosphorus accumulation peaked at a light flux of 30 µmole photons $m^{-2}s^{-1}$ (Figures 2 and 3). At 140 µmole photons $m^{-2}s^{-1}$ the chlorophyll yield declined to approximately 60% of the value observed at 30 µmole photons $m^{-2}s^{-1}$. In terms of cell biovolume, concentrations continued to increase modestly from growth light fluxes of 30 to 140 µmole photons $m^{-2}s^{-1}$. These observations show that lower light fluxes can enhance chlorophyll a concentrations within natural phytoplankton communities in Lake Griffin. From a management perspective, these results also demonstrate the importance of interpreting changes in chlorophyll a concentrations within the context of major changes in light regime, which may result from prolonged periods of cloudy weather or increases in non-algal turbidity.

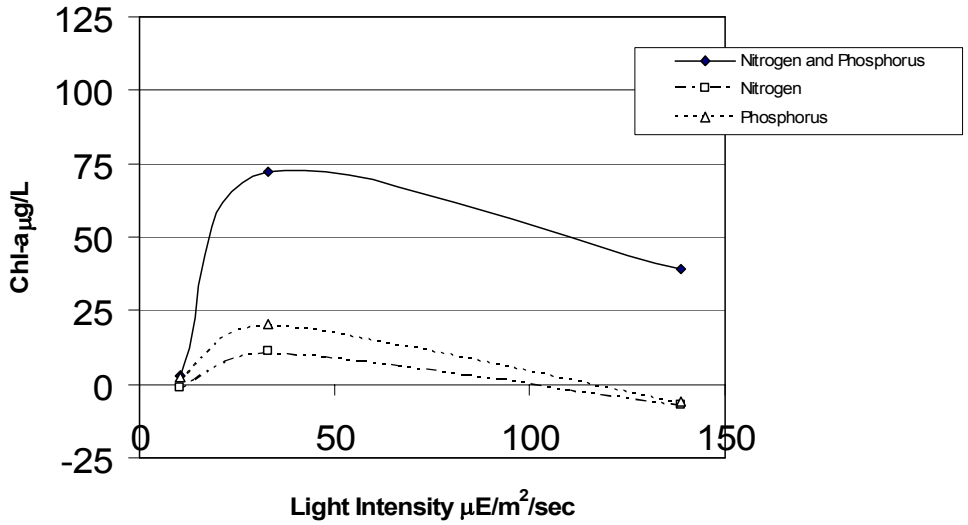

Figure 2. Chlorophyll a yield (net chlorophyll a increase) of natural phytoplankton populations from Lake Griffin grown at different irradiance levels and nutrient additions.

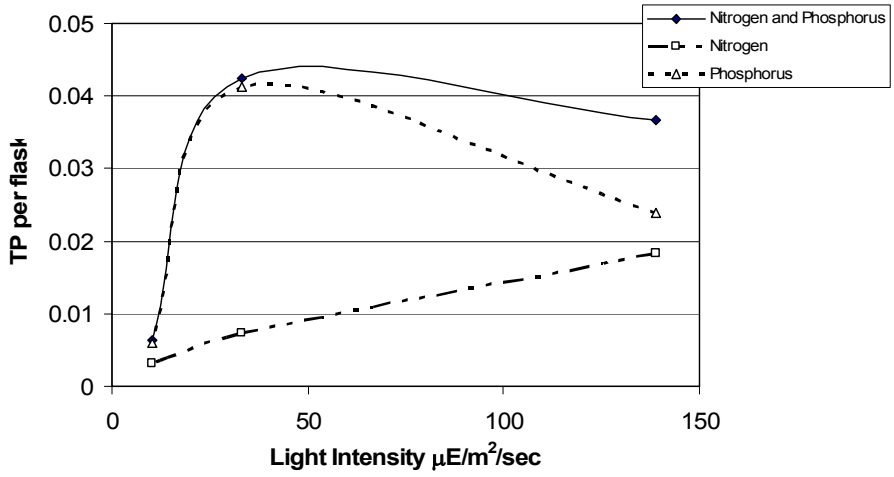

Figure 3. Particulate phosphorus yield (mg/L)(net particulate phosphorus increase) in cultures of natural phytoplankton populations from Lake Griffin grown at different irradiance levels and nutrient additions.

ii. Nutrient effects on natural populations

A comparison of chlorophyll a and particulate phosphorus yields for natural phytoplankton populations enriched with different nutrient amendments showed distinct differences in response (Figures 2 and 3). Chlorophyll a yields were dramatically increased by the addition of both nitrogen and phosphorus to the growth medium, by comparison to singular additions of nitrogen or phosphorus. In contrast, particulate phosphorus concentrations and cell biovolumes were enhanced by either nitrogen + phosphorus addition or phosphorus additions alone. These results suggest that phosphorus uptake and to a somewhat lesser extent phytoplankton growth can be sustained at lower nitrogen nutrient concentrations than chlorophyll a synthesis. From a management perspective, it appears that nutrient limitation affects different measures of phytoplankton standing crop (i.e. chlorophyll a concentration and phytoplankton biovolume) in somewhat different ways.

iii. Light effects on unialgal cultures

The impacts of light flux on chlorophyll a concentrations in unialgal cultures of bloom-forming algae were even more pronounced than for natural populations. Unlike natural populations from Lake Griffin, unialgal cultures of all three species of cyanobacteria tested (*Anabaena, Cylindrospermopsis* and *Microcystis*) grew significantly at all light fluxes included in the experiments, from 10 to 130 μmole photons $m^{-2}s^{-1}$. The ability of cultured algae to grow at low light fluxes is not uncommon and related to adaptation to the low fluxes experienced in indoor culture facilities. The adaptive response is apparent in the near two-fold higher chlorophyll a concentration in cultures grown at 10 μmole photons $m^{-2}s^{-1}$ versus 130 μmole photons $m^{-2}s^{-1}$ (Figure 4). The difference is even more pronounced in terms of the chlorophyll a content on a per unit particulate phosphorus. All three species of cyanobacteria showed a strong inverse relationship between light flux and chlorophyll a/particulate phosphorus ratio (Figure 5). The differences in the relationship were predominantly related to changes in chlorophyll concentration since particulate phosphorus concentrations remained relatively constant

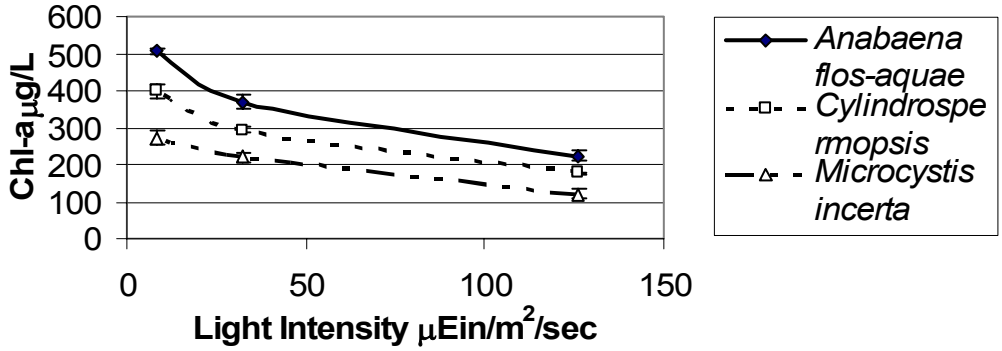

Figure 4. Chlorophyll a yields for *Anabaena, Cylindrospermopsis* and *Microcystis* cultures grown under different light regimes: 10, 36, 150 μmole photons $m^{-2}s^{-1}$. Error bars represent standard deviations.

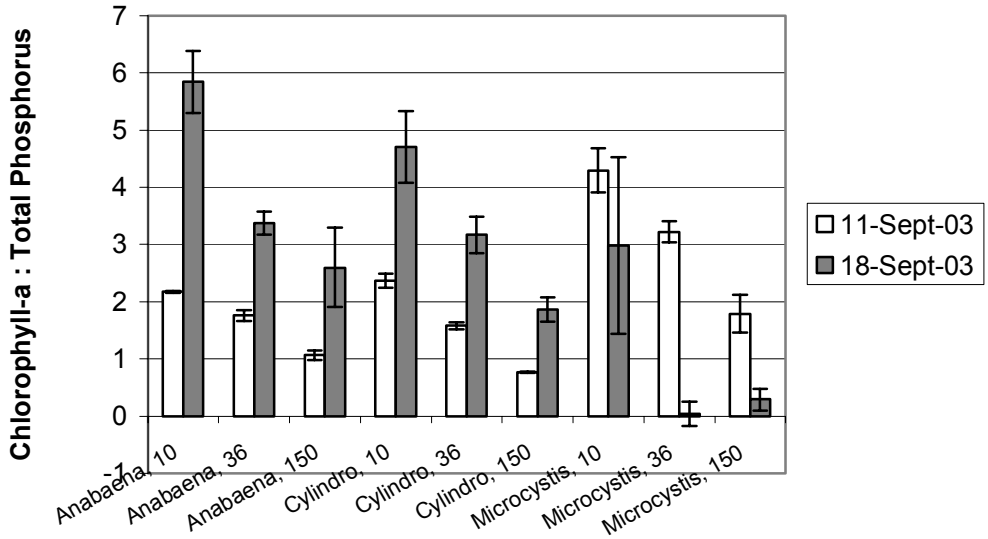

Figure 5. Chlorophyll a/total phosphorus ratios for *Anabaena, Cylindrospermopsis* and *Microcystis* cultures grown under different light regimes: 10, 36, 150 μmole photons $m^{-2}s^{-1}$. Error bars represent standard deviations.

over the entire range of light fluxes. The peak chlorophyll a/particulate phosphorus ratios were similar for all three species, although the highest ratios were observed for *Anabaena*. The ratios also varied with culture age. For both *Anabaena* and *Cylindrospermopsis* cultures the ratios after the first week of culture were less than half of the ratio after the second week of culture. This temporal pattern appears to reflect the fact that even after phosphorus uptake slowed, cell growth and chlorophyll a synthesis continued. In the case of *Microcystis*, cell growth leveled off by the end of the first week of culture, hence the peak chlorophyll a/particulate phosphorus ratios were observed before the other two species.

The results of the unialgal experiments on the affects of light flux re-enforce the observations made with natural populations of phytoplankton. The ratios of chlorophyll a/phosphorus and chlorophyll a/biovolume varied significantly with growth light flux and the age of algal cells. The variation observed in unialgal cultures was more dramatic than for natural populations of phytoplankton. The differences in the response of unialgal and natural populations are in part because the cells in unialgal cultures are more homogenous and at the same stage of development, whereas cells in natural assemblages are of mixed species composition and at widely differing stages of development. These observations illustrate the importance of keeping in mind the possibility of variations in the chlorophyll a/phosphorus and chlorophyll a/biovolume ratios when using chlorophyll a concentrations as a surrogate for algal biomass. The results of these experiments also show that the chlorophyll a/biovolume ratios for the three species are different, even at the same light and nutrient conditions, with the highest values for *Cylindrospermopsis* and the lowest for *Microcystis* (Table 1A).

iv. Nutrient effects on unialgal cultures

The effects of different nutrient regimes on cell growth, chlorophyll a/particulate phosphorus and chlorophyll a/biovolume relationships were determined for unialgal cultures of *Anabaena, Cylindrospermopsis* and *Microcystis*. Phosphorus concentrations were kept constant and different nitrogen levels were added to the cultures to induce

different forms of nutrient limitation. For the two nitrogen-fixing species, *Anabaena* and *Cylindrospermopsis*, the four nitrogen addition treatment groups all showed strong growth rates in terms of chlorophyll a increase (Figures 6 and 7). Even the nitrogen-free treatment group had growth rates as high as, or higher than, the three nitrogen addition groups, reflecting the strong nitrogen fixation capacity of *Anabaena* and *Cylindrospermopsis*. By contrast, *Microcystis* growth appeared to be related to the level of nitrogen addition (Figure 8). In terms of particulate phosphorus accumulation rates per liter, *Anabaena* and *Cylindrospermopsis* were similar and both were higher than *Microcystis*. However, converting the phosphorus accumulation to a per cell biovolume basis revealed higher mean values for *Cylindrospermopsis* and *Microcystis* than *Anabaena* (Table 1B). The latter observation provides preliminary evidence that *Cylindrospermopsis* and *Microcystis* may have superior phosphorus uptake capabilities than *Anabaena*. This observation may provide some insight into the ability of *Cylindrospermopsis* to play such a dominant role in the ecology of Lake Griffin during periods of time when bioavailable phosphorus concentrations in the water column (i.e. orthophosphate or soluble reactive phosphorus) were frequently below 10 $\mu g\ l^{-1}$ (Phlips and Schelske 2004).

A comparison of chlorophyll a/particulate phosphorus ratios for the three test species at the different nutrient addition levels revealed several trends. For *Anabaena* and *Cylindrospermopsis*, the ratios showed strong variation with cell age, increasing by over two-fold from the first to the second week of culture (Figure 9). In addition, the chlorophyll a/particulate phosphorus ratios were somewhat higher for *Anabaena* than *Cylindrospermopsis*. *Microcystis* exhibited ratios similar to *Anabaena* and *Cylindrospermopsis*, but only for the high nitrogen treatment group. The results of these experiments demonstrate the considerable range of variability exhibited by chlorophyll/particulate phosphorus ratios for all three species of cyanobacteria. Recent suggestions that high chlorophyll a/phosphorus ratios in Lake Griffin are reflective of the dominance of *Cylindrospermopsis* should be viewed in the context of the latter variability. The results of this experiment do not provide support for the hypothesis that the chlorophyll a/phosphorus ratio of *Cylindrospermopsis* is inherently higher than other cyanobacteria, like *Anabaena* and *Microcystis*.

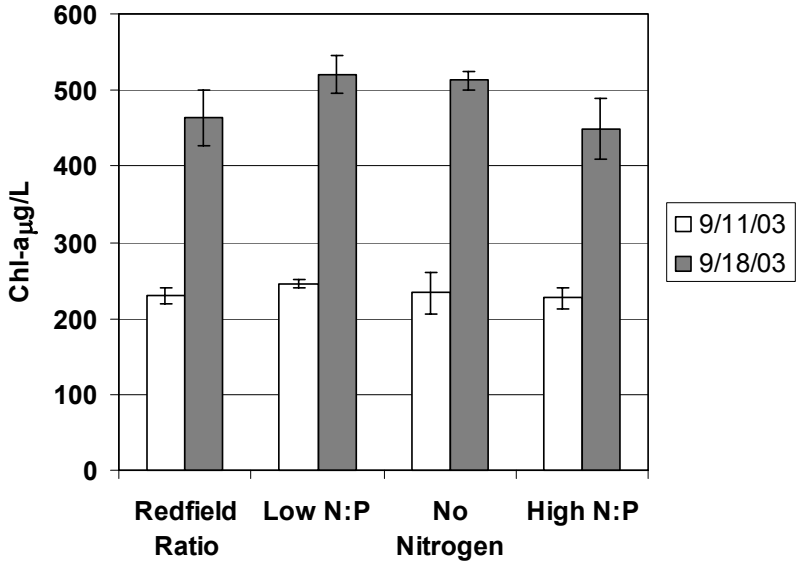

Figure 6. Chlorophyll a yield for *Anabaena* cultures grown under different nutrient regimes. Error bars represent standard deviations.

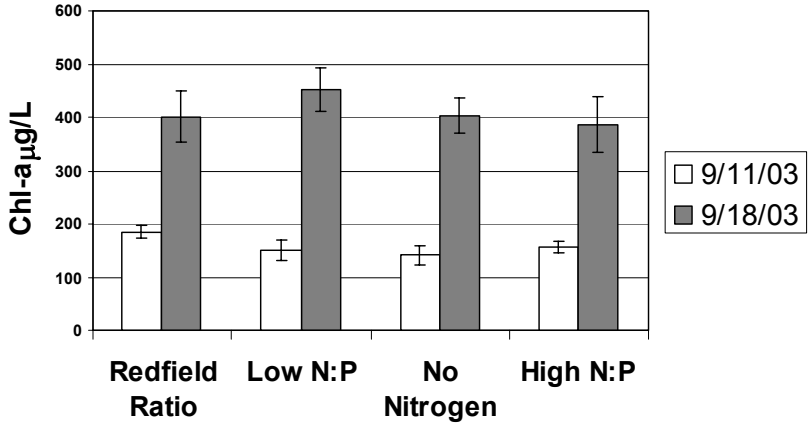

Figure 7. Chlorophyll a yield for *Cylindrospermopsis* cultures grown under different nutrient regimes. Error bars represent standard deviations.

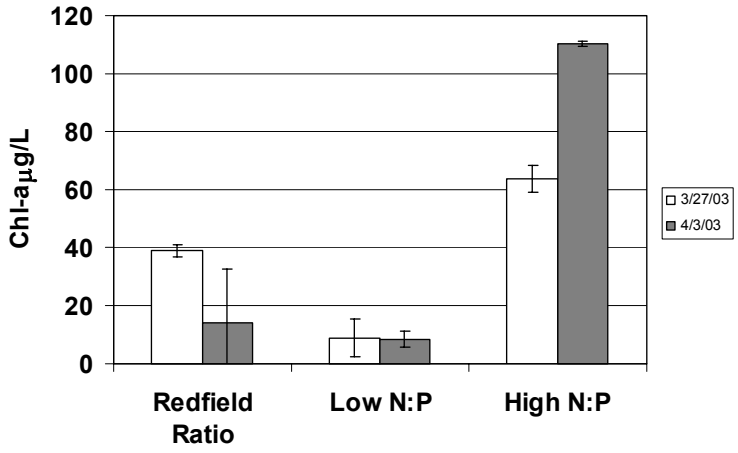

Figure 8. Chlorophyll a yield for *Microcystis* cultures grown under different nutrient regimes. Error bars represent standard deviations.

Table 1. A) Mean chlorophyll a concentration on a per unit cell biovolume basis for *Anabaena, Cylindrospermopsis* and *Microcystis* cultures grown under different growth irradiances. B) Mean particulate P concentration on a per unit cell biovolume basis for *Anabaena, Cylindrospermopsis* and *Microcystis* cultures grown at 130 μm photons m^{-2} s^{-1}. Mean values for the three species were compared at each light level using Duncans Multiple Range Test using an alpha level of 0.05 (n=3). Means with the same letter designation are not significantly different.

A)	Chlorophyll a/10^9 μm^3 Cell Biovolume	
SPECIES	10 μm photons m^{-2} s^{-1}	130 μm photons m^{-2} s^{-1}
Anabaena	3.59 (A)	0.91 (B)
Cylindrospermopsis	3.75 (A)	2.12 (A)
Microcystis	1.19 (B)	0.44 (C)

B)	Particulate P/10^9 μm^3 Cell Biovolume
Anabaena	0.85 (B)
Cylindrospermopsis	2.62 (A)
Microcystis	2.36 (A)

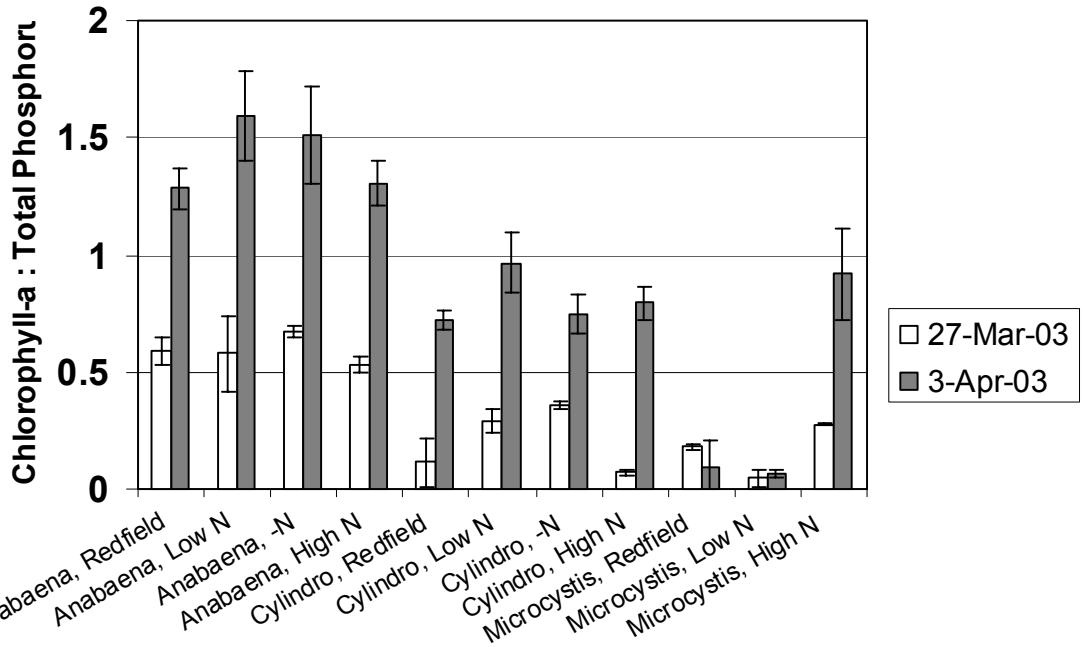

Figure 9. Chlorophyll a/total phosphorus concentrations for *Anabaena, Cylindrospermopsis* and *Microcystis* cultures grown under different nutrient addition regimes: Redfield ratio of N and P (7.2:1), low nitrogen addition (Low N), no nitrogen (-N) addition, and high nitrogen addition (High N). Error bars represent standard deviations.

B. TASK 1b. Spatial and Vertical Variability of Phytoplankton Distribution.

i. Spatial variation of phytoplankton

In a previous study of Lake Griffin, it was noted that there appeared to be some differences in mean phytoplankton standing crop between the northern and southern basins of the lake (Phlips and Schelske 2004). In the current study, these differences were statistically explored and a follow-up comparison of the basins was carried out for the 2002-2004 study period. A comparison of the chlorophyll a record for the north and south sampling sites in Lake Griffin from 2000 thru 2004 show that the northern basin frequently has lower chlorophyll a concentrations than the southern basin, particularly during periods of peak biomass in the summer months (Figure 10). The latter pattern is evident from a comparison of the mean chlorophyll a values at 17 sites throughout Lake Griffin during the summer bloom period of 2001, which extended from August through November (Figure 11). The spatial patterns of mean chlorophyll a appear to reveal a north to south gradient of increasing chlorophyll a, roughly divisible into three broad regions: north, central and south. Segregating the chlorophyll a data into the 4 northern sites, 4 central sites and 9 southern sites demonstrated significant differences between the three regions (Table 2).

The existence of a north to south gradient in phytoplankton abundance was further reinforced in the current study period when both summer mean and annual mean chlorophyll a concentrations for the southern sampling sites were significantly higher than the northern sampling site (Table 3). Despite these regional differences in chlorophyll a, the results of previous of phytoplankton community structure in 2000-2002 showed no major regional differences in the overall species composition (Phlips and Schelske 2004).

Another aspect of spatial variability examined in this study was vertical stratification. Over the past decade, the cyanobacterium *Cylindrospermopsis* has been a dominant feature of the phytoplankton community. *Cylindrospermopsis* is characterized by relatively neutral buoyancy and known for being uniformly distributed in the water

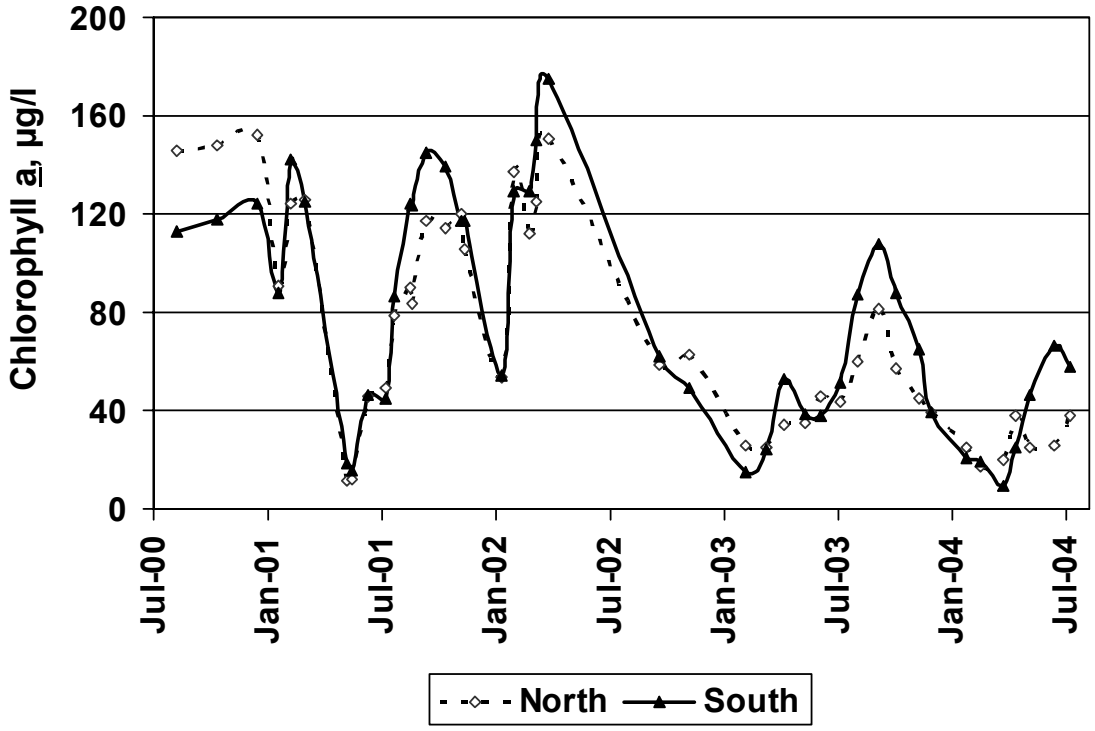

Figure 10. Chlorophyll a concentration at the north and south sampling sites from 2000 to 2004. Values for 2000 thru March of 2002 are from a previous report (Phlips and Schelske 2004).

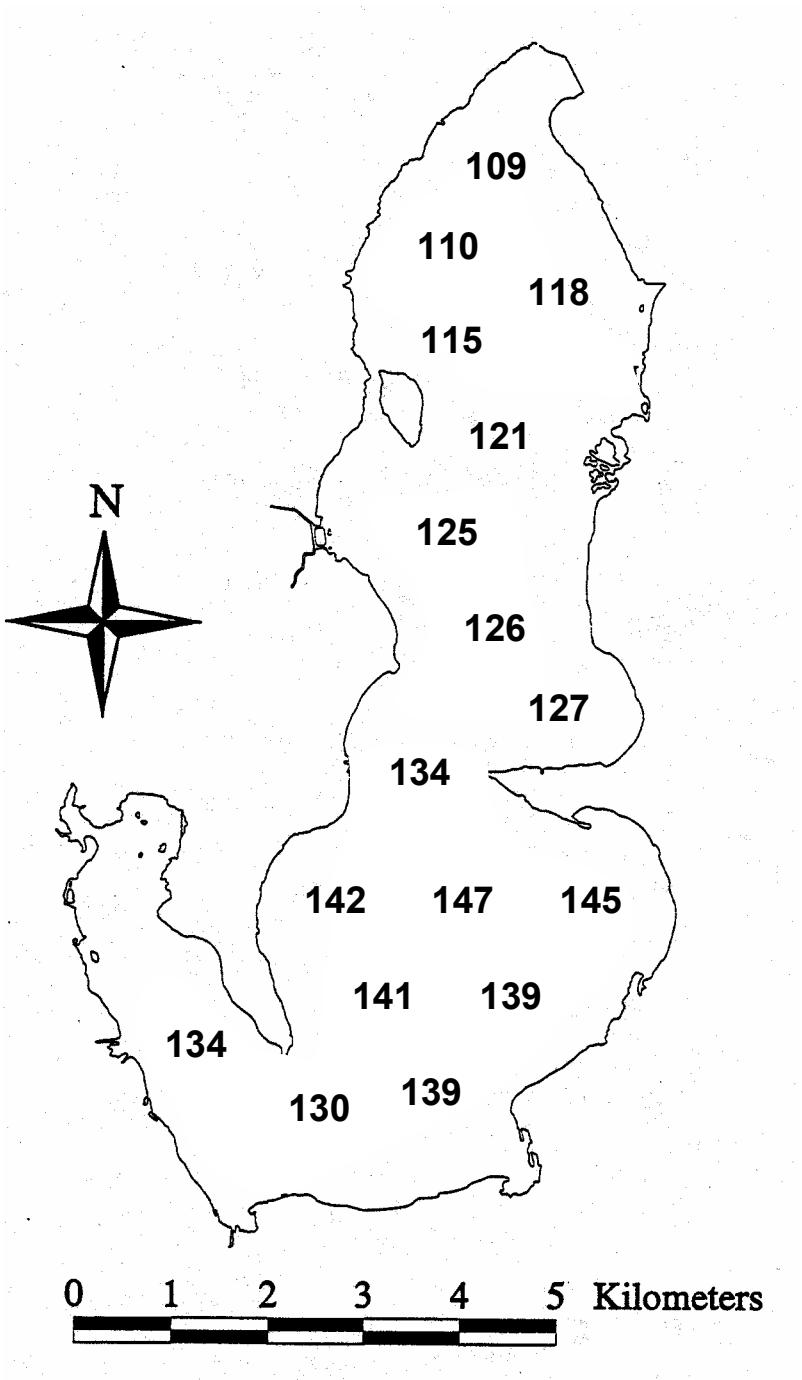

Figure 11. Mean chlorophyll a concentrations at 17 sampling sites for the bloom period of August 2001 thru November 2001.

Table 2. Regional differences in mean chlorophyll a concentration during the bloom period of August, 2001 thru November, 2001. Mean chlorophyll a values ($\mu g\ l^{-1}$) for each region are shown along with the results of Duncan's Multiple Range tests. Means with the same letter designation are not significantly different at the alpha level of 0.05.

Region	Mean	Duncan
North	111.3	C
Central	125.0	B
South	138.4	A

Table 3. Regional differences in mean chlorophyll a concentration during the study period of February, 2003 thru July, 2004. Mean chlorophyll a values ($\mu g\ l^{-1}$) for each region are shown along with the results of Duncan's Multiple Range tests. Means with the same letter designation are not significantly different at the alpha level of 0.05.

Region	Mean	Duncan
North	40.1	B
South	48.1	A

column. It is therefore not surprising that concentrations of chlorophyll a in surface and bottom samples from Lake Griffin were relatively similar (Figure 12). The greatest difference between surface and bottom concentrations occurred during a period of reduced chlorophyll a concentration (Figure 13), when the contribution of filamentous cyanobacteria to total phytoplankton standing crop, like *Cylindrospermopsis*, was diminished (Phlips and Schelske 2004). During the latter period, surface chlorophyll a was 35% lower than the bottom concentration. These vertical disparities may be in part due to preferential contributions of meroplanktonic diatoms to algal populations in the lower levels of the water column.

ii. Wind mixing and the vertical distribution of phytoplankton

An *in situ* experiment was performed to determine the impact of wind-induced vertical mixing of the water column on the distribution of nutrients, total suspended solids and phytoplankton. Initial sampling of the water column was carried out during a 24 hour period of calm wind conditions. A subsequent sampling was carried out four hours after the initiation of a continuous 8 hour period of winds in excess of 15 km/hr. Total nitrogen (TN) concentrations determined at 30 cm depth, mid-water column and bottom (i.e. approximately 0.2 m above the sediment surface) showed little vertical stratification and only minor differences between calm and wind periods (Figure 14). The latter pattern for TN held true for all five sites along the sampling transect oriented in the direction of the prevailing wind during the experiment (north to south). Total phosphorus concentrations along the sampling transect generally increased from north to south under both calm and wind conditions, and irrespective of the depth of sampling (Figure 15). The two exceptions to this pattern were spikes in TP concentration in the bottom samples from the northern-most and southern-most sites during the wind event. Total suspended solids concentrations followed a similar pattern, with elevated levels at the northern-most and southern-most sites during the wind event. These results suggest that the wind event included in this study provided sufficient energy to resuspend sediment into the bottom layers of the water column at the two shallowest sites along the transect, but was not of sufficient magnitude to result in complete mixing of bottom sediments to the surface layer.

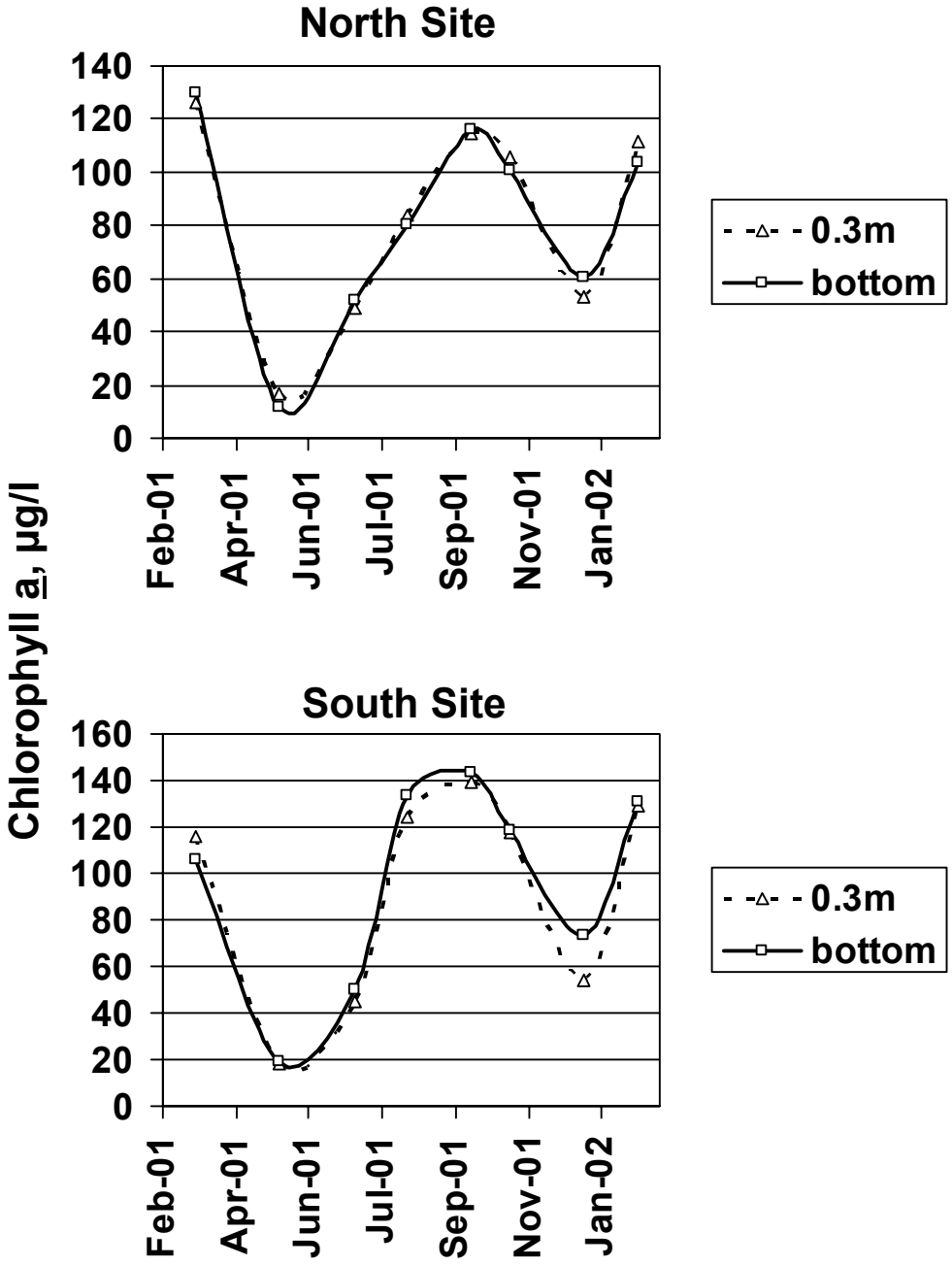

Figure 12. Chlorophyll a concentrations in surface and bottom water samples from the north (top) and south (bottom) sites.

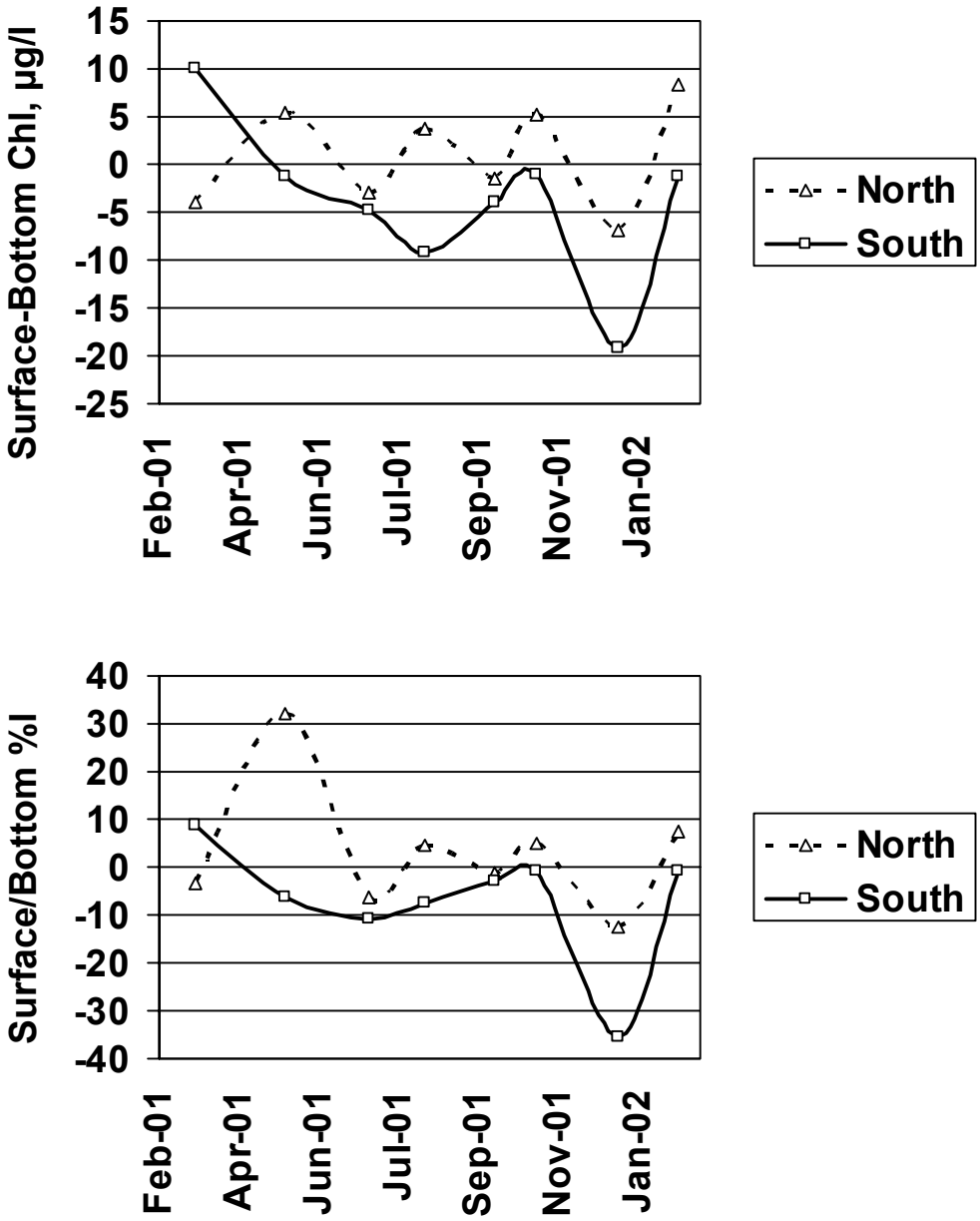

Figure 13. The net difference in the chlorophyll a concentrations between surface and bottom water samples, represented as absolute (top) and percentage values (bottom).

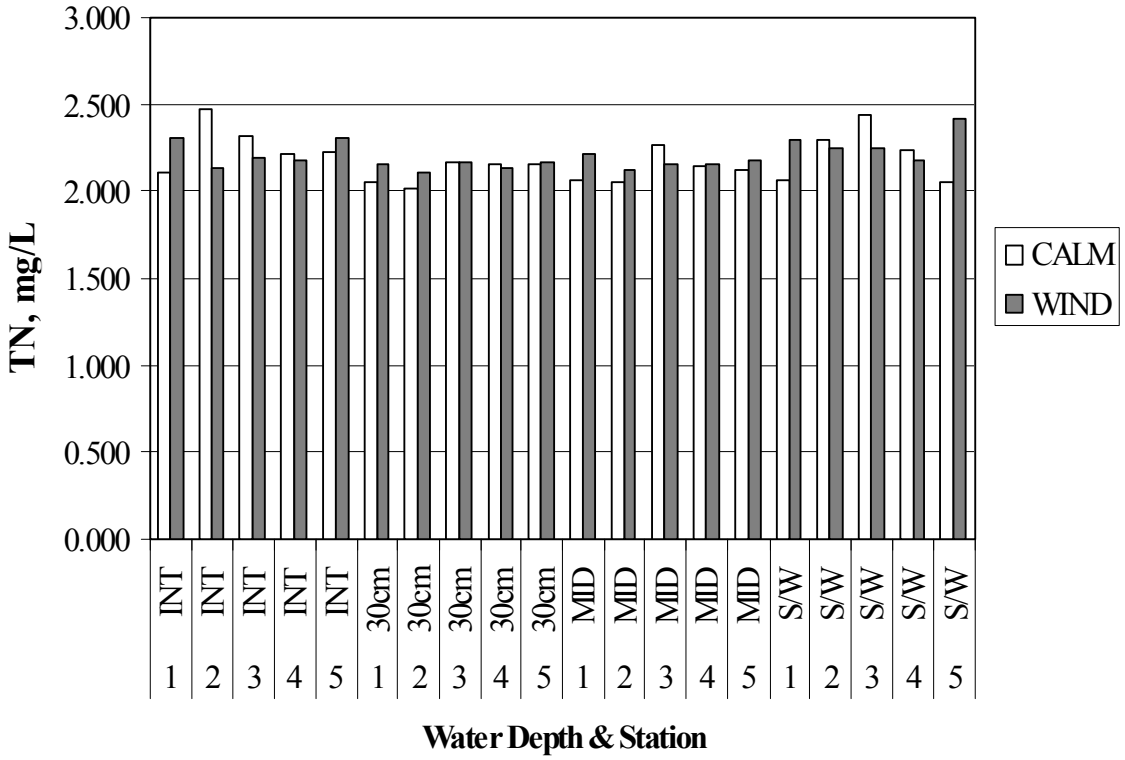

Figure 14. Surface water total nitrogen concentrations at the five transect sites of the wind experiment under calm and subsequent windy conditions. Concentrations are shown for four water column sample types: integrated, 30 cm depth, mid water column and the sediment/water interface.

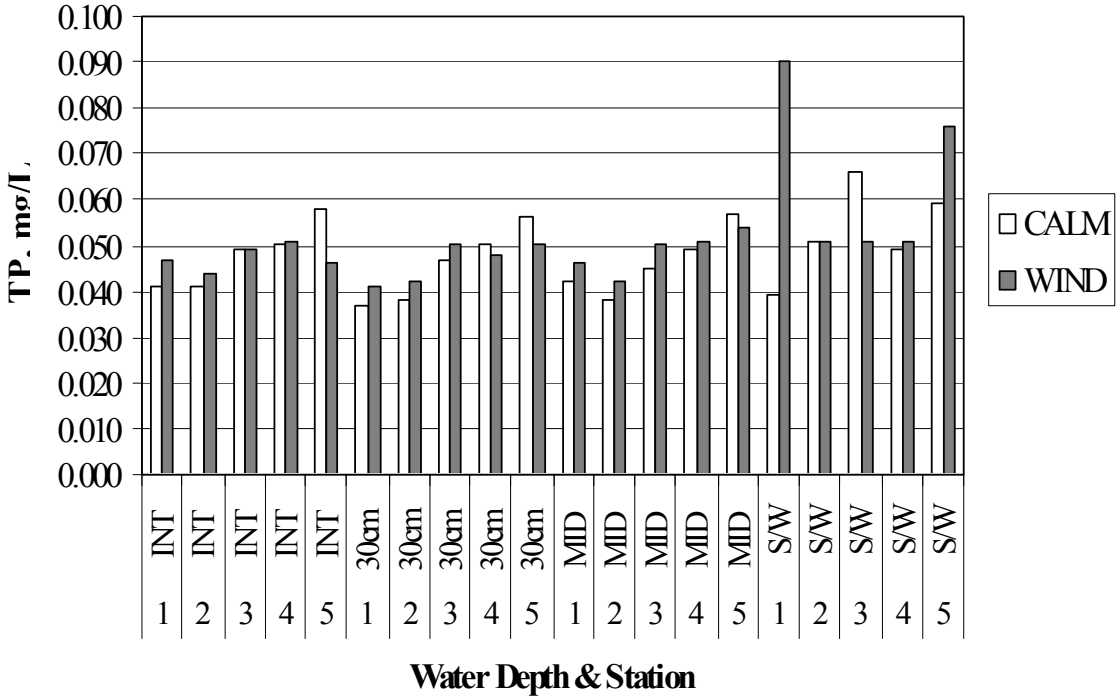

Figure 15. Surface water total phosphorus concentrations at the five transect sites of the wind experiment under calm and subsequent windy conditions. Concentrations are shown for four water column sample types: integrated, 30 cm depth, mid water column and the sediment/water interface.

A comparison of the dominant phytoplankton species in surface and bottom water during calm and subsequent windy conditions revealed fundamental differences between blue-green algal and diatom species. The dominant blue-green algae, *Oscillatoria* and *Cylindrospermopsis*, were evenly distributed through the water column under both calm and wind conditions (Table 4). The relatively neutral to slightly positive buoyancy often associated with certain strains of these blue-green algal genera enhances the relative evenness of their vertical distribution. This contrasts with certain species and strains of *Microcystis* and *Anabaena* that are well-known to form surface scums in many eutrophic lakes around the world. The relative dominance of *Oscillatoria* and *Cylindrospermopsis* in Lake Griffin (see Section C below; Phlips and Schelske 2004) helps to explain the relatively small vertical stratification of chlorophyll a.

By contrast, the dominant diatom species, *Aulacoseira*, showed a major increase of cell numbers in both the bottom and surface (30 cm) water samples (Table 5). *Aulacoseira* is a meroplanktonic species of diatom that is known to reside in the sediment/water interface until it is resuspended into the water column by vertical mixing energy. The wind-induced resuspension of *Aulacoseira* helps to explain the periodic blooms of this species observed over the current sampling period (see Section C below) and in previous research studies (Phlips and Schelske 2004).

C. TASK 2. Temporal Variability of Field Parameters, Nutrients, Phytoplankton, and Nutrient Limitation.

i. Field Parameters

Details of the values obtained for all field parameters are provided in Appendix 1. Water temperatures in Lake Griffin ranged from a winter low of 14^{o}C to a summer high of 32 ^{o}C over the sampling period (Figure 16). The north and south sampling sites exhibited similar temperature values and patterns. Vertical stratification of temperature was consistently less than 1 ^{o}C. There was a notable difference in the timing of the spring increase in water temperatures in 2003 and 2004. In 2003, water temperatures exceeded 24 ^{o}C as early as March,

Table 4. Distribution of major blue-green algal species during calm and subsequent wind period.

	CALM		WINDY	
SPECIES	SURFACE (cells/ml)	BOTTOM (cells/ml)	SURFACE (cells/ml)	BOTTOM (cells/ml)
Oscillatoria sp. A	199,949	155,337	183,174	185,441
Oscillatoria sp. B	146,491	135,964	142,100	121,265
Oscillatoria sp. C	2,021,207	1,532,113	2,105,182	1,996,215
Cylindrospermopsis	26,838	14,288	23,587	21,319

Table 5. Distribution of the meroplanktonic diatom *Aulacoseira* along the wind experiment transect during calm and subsequent wind period.

	CALM		WINDY	
SITE	SURFACE (cells/ml)	BOTTOM (cells/ml)	SURFACE (cells/ml)	BOTTOM (cells/ml)
1	0	0	756	2,570
2	0	529	454	2,419
3	0	1,134	0	1,663
4	0	151	755	1,436
5	0	0	454	2,041

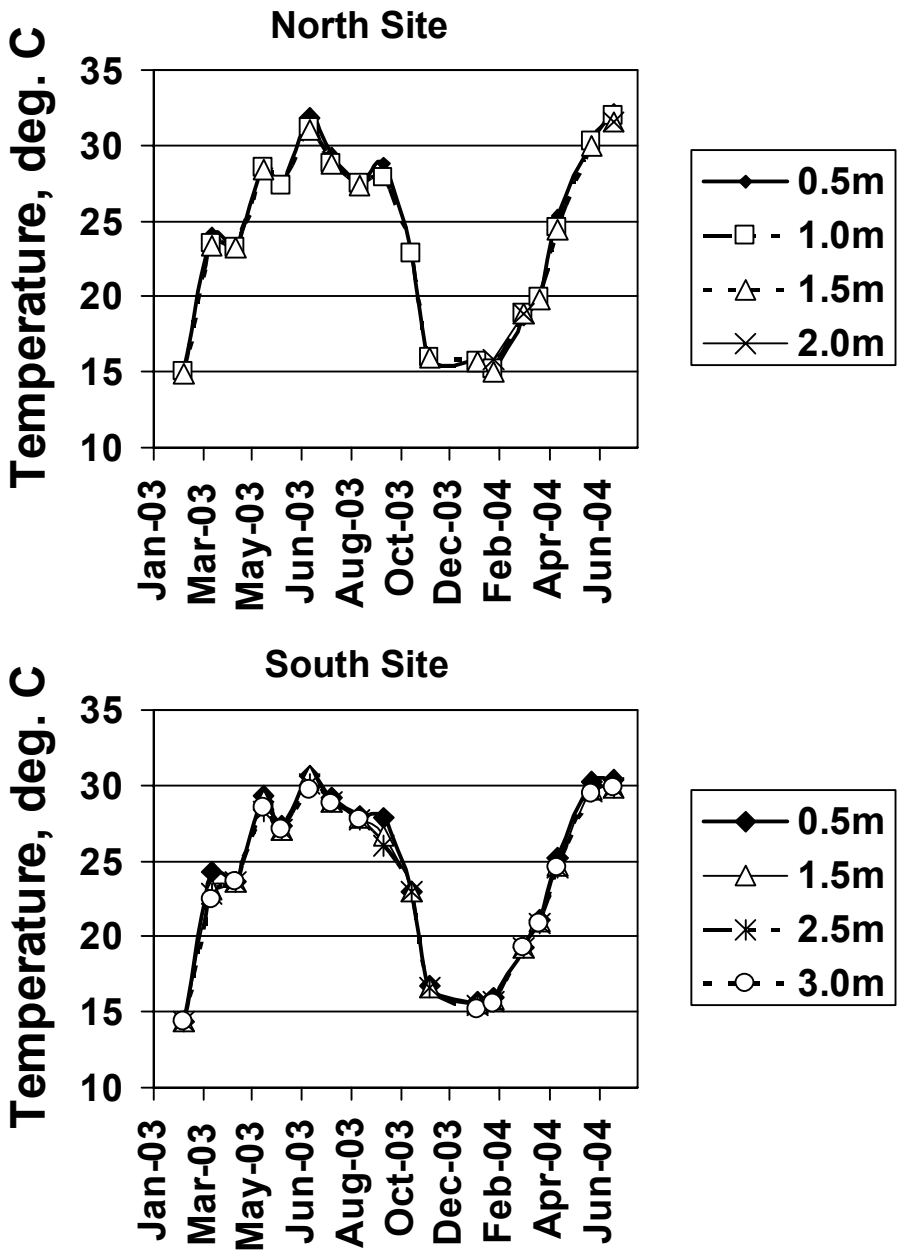

Figure 16. Temperature at different depths in the water column at the north (top) and south (bottom) sites.

while in 2004 water temperatures were not observed to exceed 24 ^{o}C until the May sampling date.

Values for pH ranged from 7.3 to 9.2 over the study period, although a majority of values were 8.0 or higher (Figure 17). The north and south sampling sites exhibited similar pH values and patterns. Vertical stratification of pH was generally less than 0.2 units, although up surface to bottom differences of up to 0.8 units were observed. In general pH values were higher in the spring-fall warm temperature period. As in the case of temperature the spring increase in pH occurred earlier in 2003 than 2004. The correlation in the timing of the spring temperature and pH increases suggest that the spring increases in temperature may stimulate primary productivity.

Surface water oxygen concentrations ranged from 5 to 12 mg l^{-1} over the sampling period (Figure 18). The majority of values fell between 7 and 9 mg l^{-1}. Vertical stratification of oxygen concentration was relatively small, less than 1 mg l^{-1}, at the northern site. However, at the southern site oxygen concentrations at the bottom of the water column were as much as 6 mg l^{-1} less than at the surface, and bottom concentrations were as low as 1.5 mg l^{-1}. The difference between the north and south site may in part be attributable to the difference in depth, the latter site ranging from 1.5-2 m and the southern site from 2.5 to 3 m.

Two measures of vertical light extinction in the water column were included in this study, Secchi disk depth and PAR (photosynthetically active radiation) light extinction coefficient. The two parameters are inversely related. Secchi depths ranged from 0.2 to 2.1 m over the study period (Figure 19), while K_e values ranges from 0.8 to 6 m^{-1}. The highest K_e and lowest Secchi depths were observed in the summer of 2003. The summer of 2003 was also characterized by the highest turbidity (Figure 20) and chlorophyll (see Section C.iii below) values of the study period, both major contributors to light attenuation.

Turbidity values ranged from 2 to 14 NTU over the study period (Figure 21). Turbidity values were similar for both the north and south sites. The highest turbidity values were observed in the summer seasons, particularly in 2003.

Color values ranged from 12 to 45 PCU over the study period (Figure 22). There were some differences in color between the south and north site, but no pattern of one site

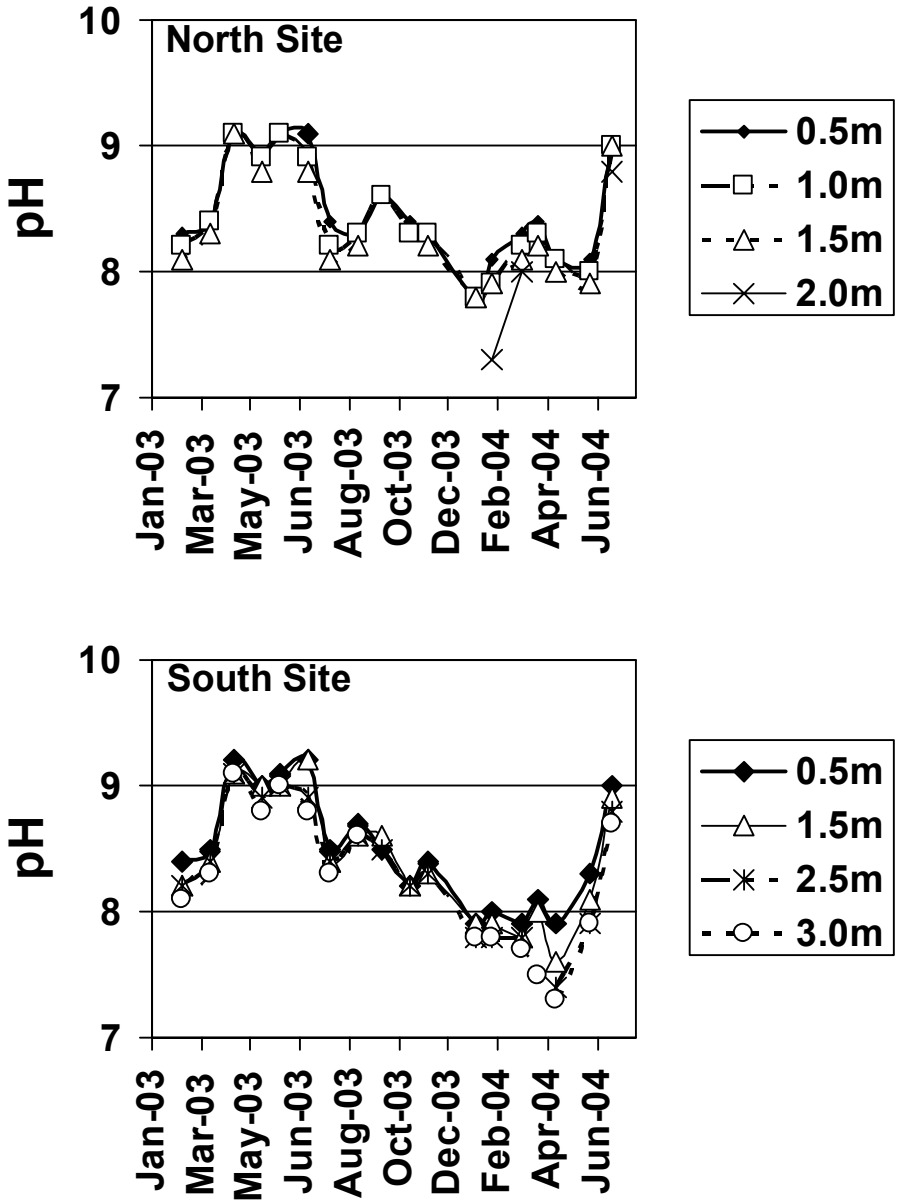

Figure 17. pH values at different depths in the water column at the north (top) and south (bottom) sites.

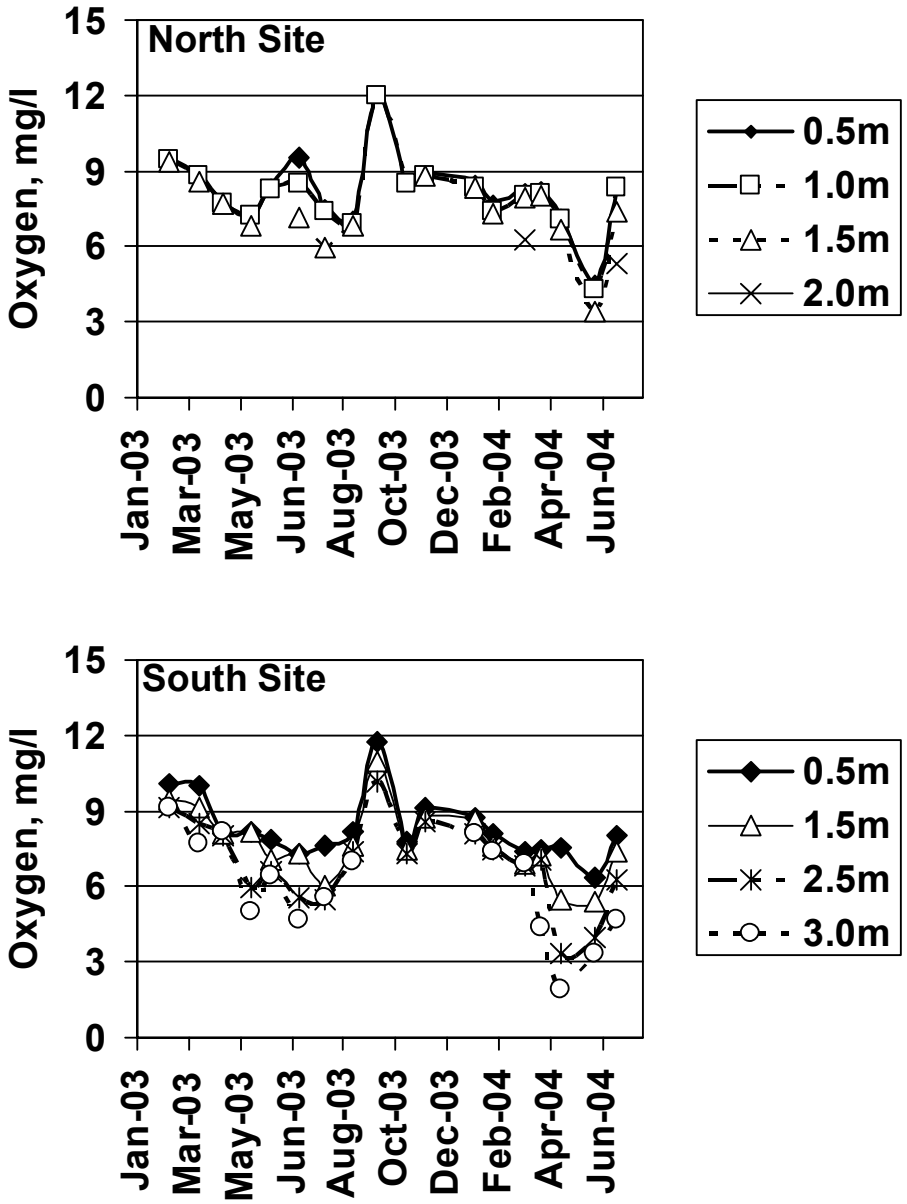

Figure 18. Dissolved oxygen concentrations at different depths in the water column at the north (top) and south (bottom) sites.

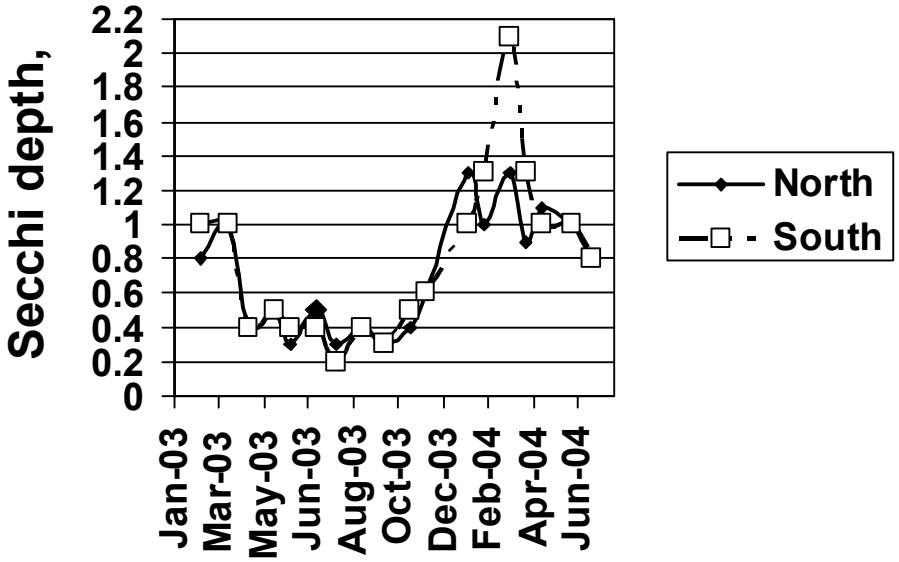

Figure 19. Secchi disk depth at the north and south sites.

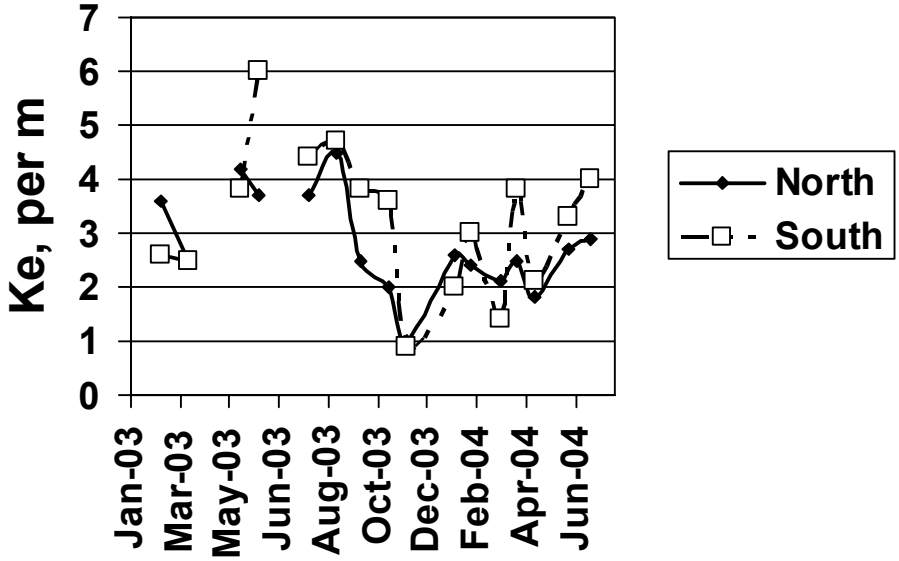

Figure 20. Vertical light extinction coefficients, K_e at the north and south sites.

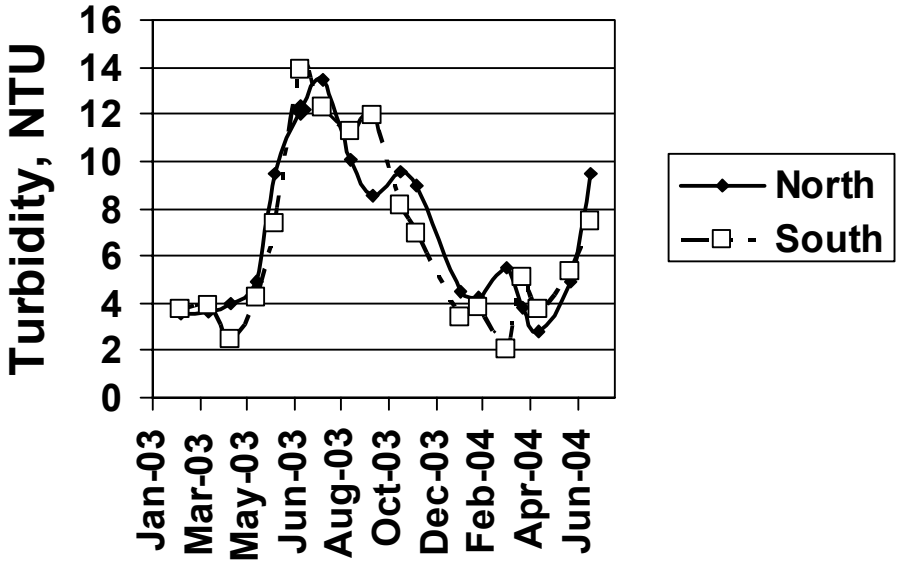

Figure 21. Turbidity at the north and south sites.

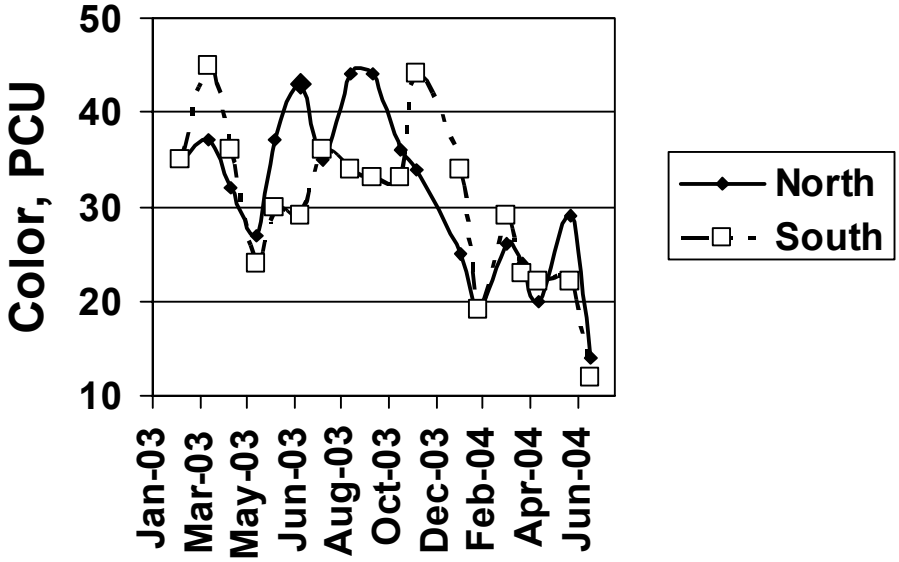

Figure 22. Color at the north and south sites.

being consistently higher than the other. Color values were generally higher in 2003 than 2004. The differences between years in color may reflect the inter-annual differences in rainfall, which was higher in 2003 than 2004.

ii. Nutrients

Total nitrogen levels in Lake Griffin were relatively stable over the study period, with the exception of a peak in July of 2003, possibly associated with a storm event prior to the sampling date (Figure 23). Values at the north site primarily ranged between 2,000 to 2,500 $\mu g\ l^{-1}$. Values at the south site were somewhat higher, with a mean for the study period of 2691 $\mu g\ l^{-1}$, compared to 2391 $\mu g\ l^{-1}$ for the north site.

Nitrate and nitrite concentrations were highly variable over the study period (Figure 24). Nitrate concentrations were typically higher than nitrite. Much of 2003 after February had nitrate and nitrite concentrations below 10 $\mu g\ l^{-1}$ at both the north and south site. Concentrations of nitrate and nitrite increased during 2004, reaching values as high as 225 $\mu g\ l^{-1}$ in May, at the south site. A similar temporal pattern was observed for ammonia, which increased to concentrations of up to 2,100 $\mu g\ l^{-1}$ in 2004 (Figure 25).

Total phosphorus concentrations ranged from 30 to 100 $\mu g\ l^{-1}$ over the study period (Figure 26). The first half of 2003 had the highest TP concentrations, particularly at the north site. Mean TP concentrations over the study period were 53.2 $\mu g\ l^{-1}$ at the north site and 54.9 $\mu g\ l^{-1}$ at the south site. Both of the latter values fall on the low side for those reported for Lake Griffin over the past 22 years, during which concentrations often exceeded 100 $\mu g\ l^{-1}$ (Personal communication SJRWMD).

Soluble reactive phosphorus concentrations ranged from 0 to 18 $\mu g\ l^{-1}$, but were generally below 5 $\mu g\ l^{-1}$, except during four months in 2003; May, September, October and December (Figure 27).

Total silica concentrations at the north and south sites were similar and ranged from 2 to 8 $mg\ l^{-1}$ (Figure 28). There was a distinct seasonal pattern in silica, with the highest concentrations in the late fall through late winter period.

Individual values for all water chemistry parameters are provided in Appendix 2.

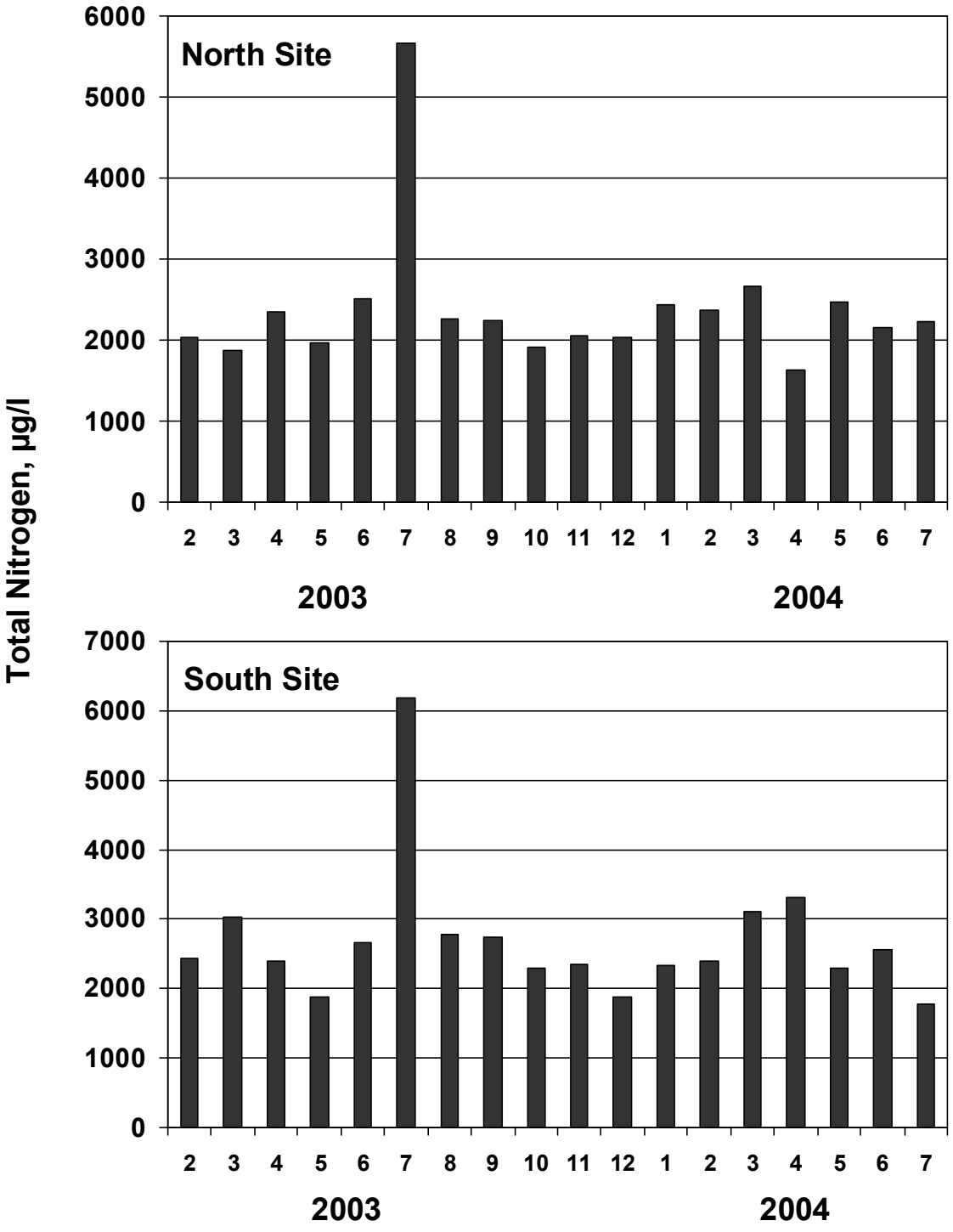

Figure 23. Total nitrogen concentrations at the north (top) and south (bottom) sites.

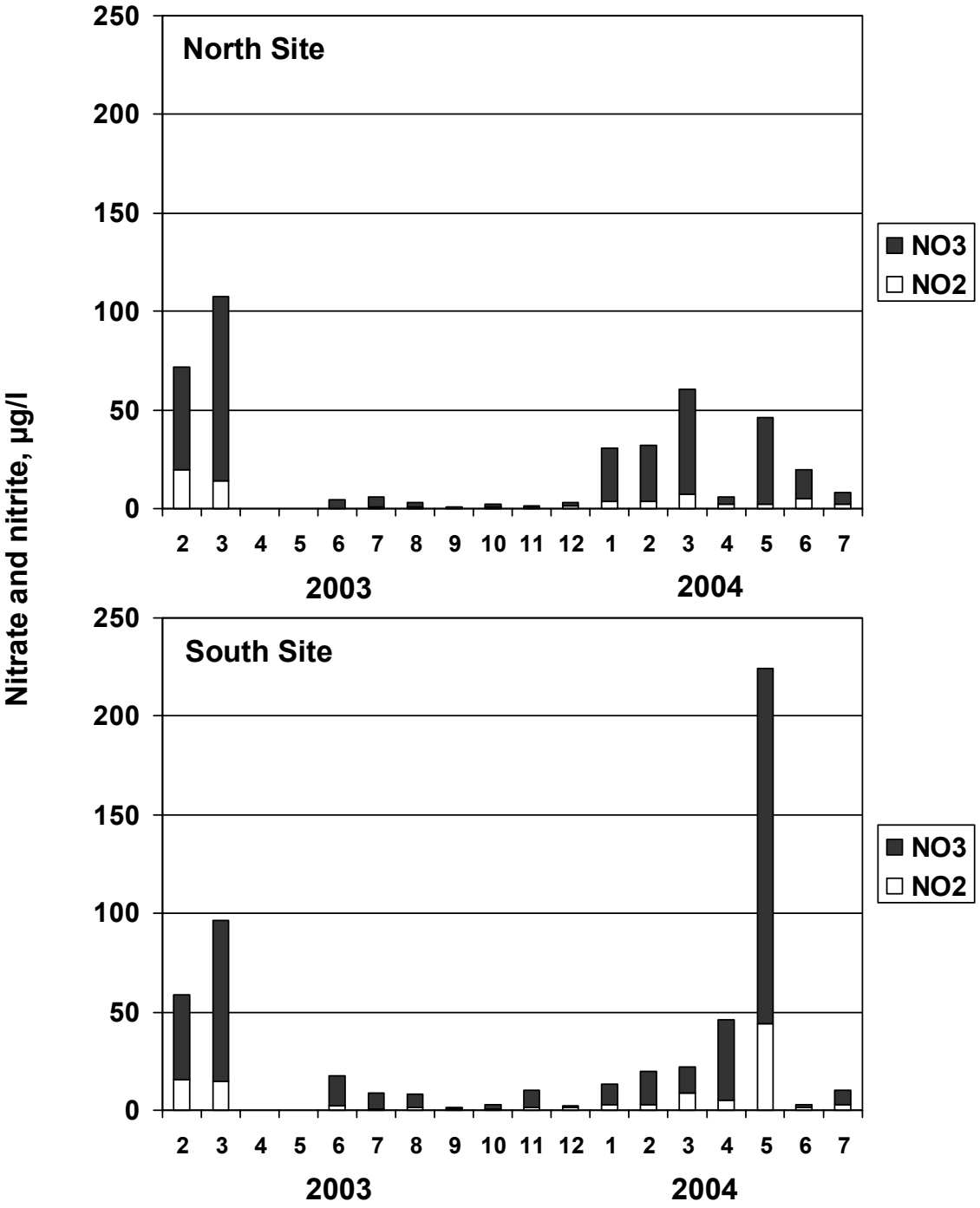

Figure 24. Nitrate and nitrite concentrations at the north (top) and south (bottom) sites.

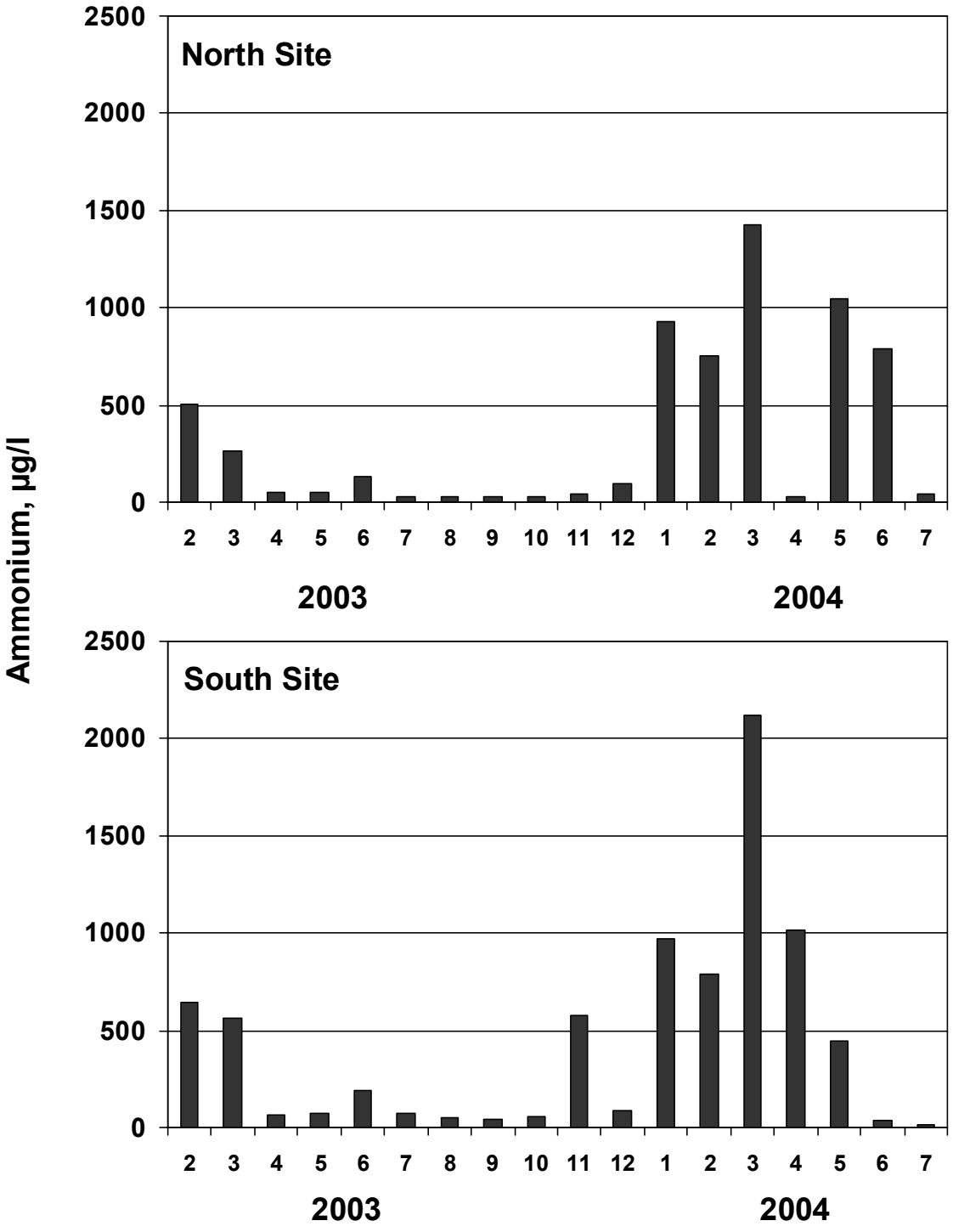

Figure 25. Ammonia concentrations at the north (top) and south (bottom) sites.

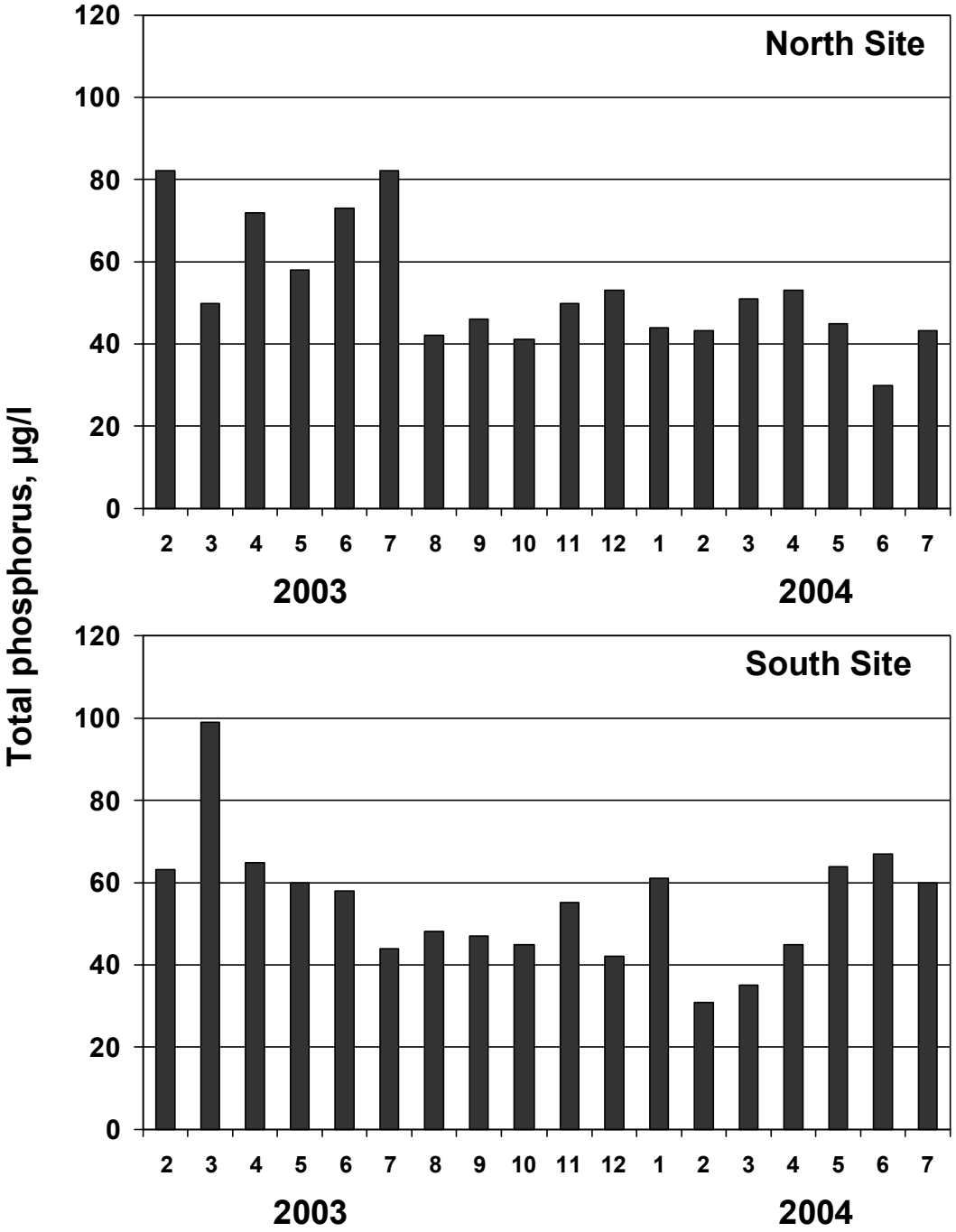

Figure 26. Total phosphorus concentrations at the north (top) and south (bottom) sites.

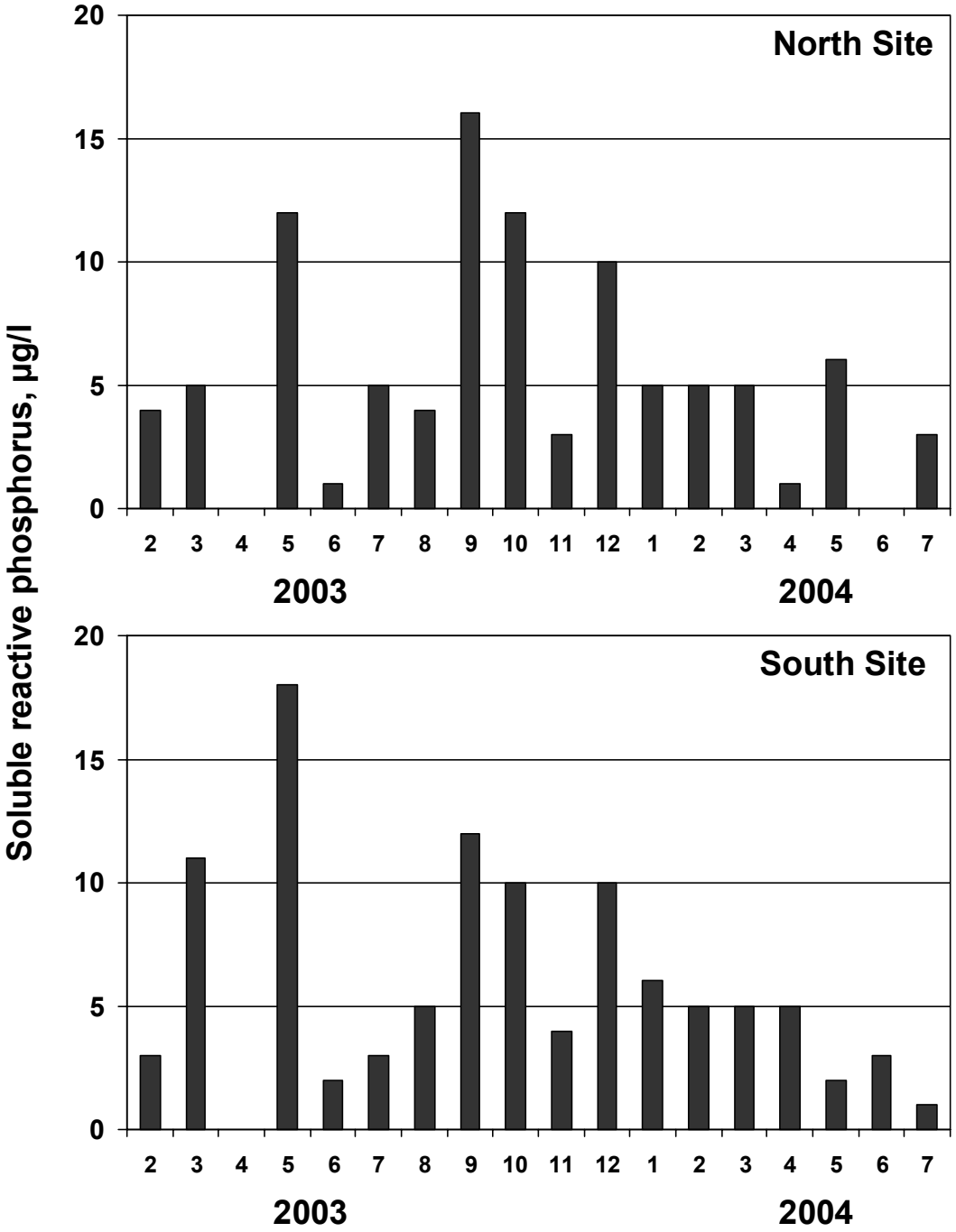

Figure 27. Soluble reactive phosphorus concentrations at the north (top) and south (bottom) sites.

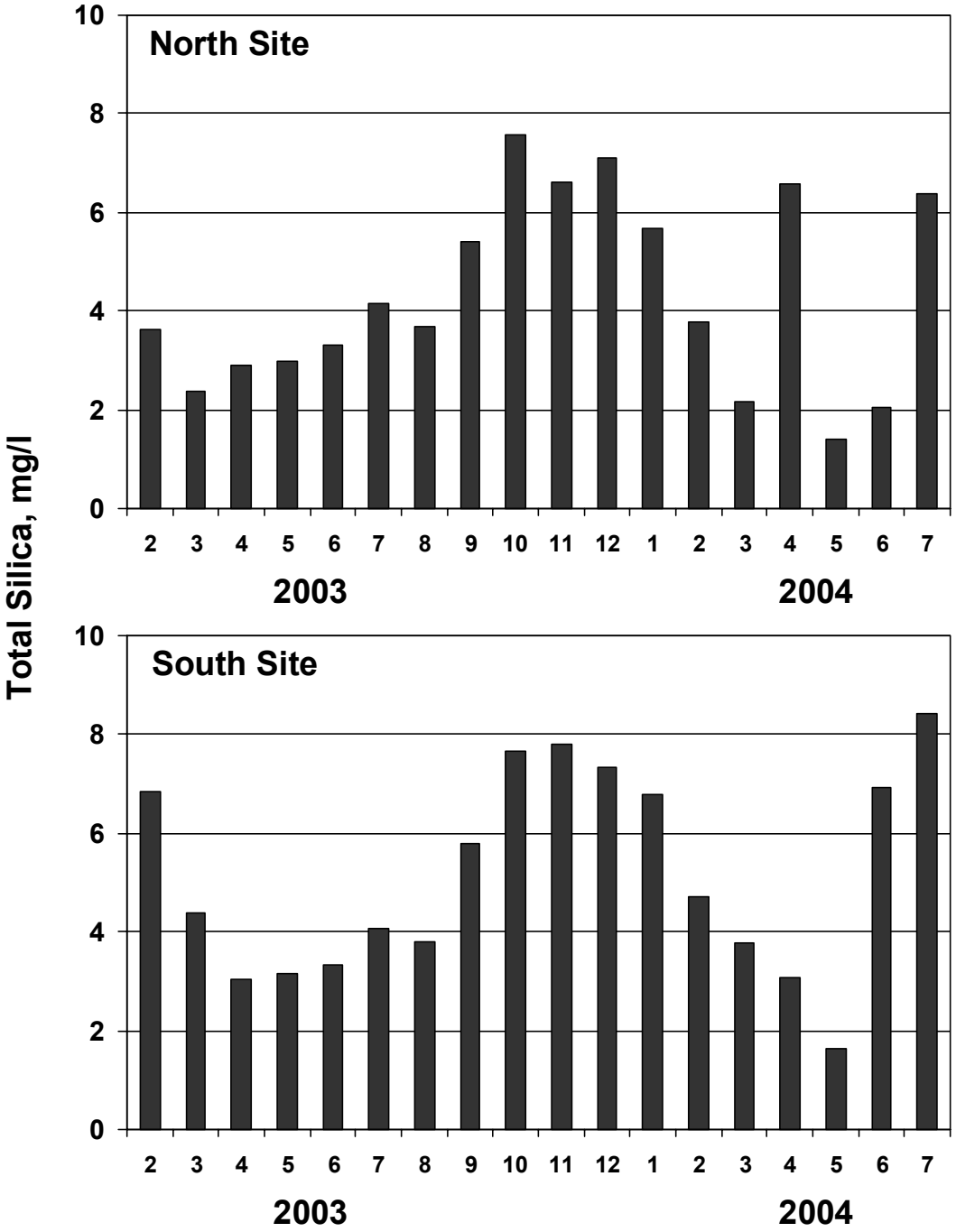

Figure 28. Total silica concentrations at the north (top) and south (bottom) sites.

iii. Chlorophyll a and pheophytin

Chlorophyll a concentrations over the study period ranged from 10 to 120 $\mu g\ l^{-1}$ (Figure 29). Pheophytin concentrations were approximately 10% of total chlorophyll a for most samples. As demonstrated in Section B.i chlorophyll a concentrations in the southern basin of the lake were generally higher than in the northern basin. There were also distinct temporal differences in chlorophyll a concentration. Concentrations were highest in the late spring through early fall and higher in 2003 than 2004. Overall, chlorophyll a concentrations were generally lower during this study period than observed in a previous study from July, 2000 to March, 2002 (Figure 10). Peak chlorophyll a concentrations during the study period were also less than half those observed during the severe bloom period from the mid- to late 1990's and more closely resembled values observed in the 1980's (Figure 30).

iv. Plankton composition

The phytoplankton community of Lake Griffin during the study period was dominated by blue-green algae on all but one sampling date (Figure 31). The one exception was the January sample in 2004, in which the meroplanktonic diatom *Aulacoseira* dominated the integrated water column sample on a biovolume basis (Figure 31). The dominant blue-green alga in a majority of samples was the non-heterocystous filamentous genus *Oscillatoria* (Figure 31). The domination of *Oscillatoria* is a deviation from the results of a previous study from July, 2000 to March, 2002, when the heterocystous blue-green alga *Cylindrospermopsis* dominated the blue-green algal component of the phytoplankton community in a majority of months (Figure 32). A species designation was not assigned to *Cylindrospermopsis* due to current uncertainties about the taxonomy of local populations of this species (See methods for further discussion. A complete list of phytoplankton taxa observed during the study period is provided in Appendix 3.

Several other blue-green algal taxa were prominent members of the phytoplankton community. Two planktonic *Lyngbya* species, *L. limnetica* and *L. contorta*, occasionally reached combined biovolumes near, or greater than $10^6\ \mu m^3 ml^{-1}$ (Figure 33). The

potentially toxic unicellular blue-green alga *Microcystis aeruginosa* was generally absent from the phytoplankton community, but did appear at levels in excess of 10^6 $\mu m^3 ml^{-1}$, in May and June of 2004. Another unicellular picoplanktonic form of cyanobacteria, *Chroococcus*, was a common feature of the phytoplankton community (Figure 34).

The phytoplankton biovolumes observed over the study period showed a distinct seasonality, with peak values in the spring through fall and minima in the winter. This pattern deviates somewhat from the results of a previous study from July, 2000 to March 2002 (Phlips and Schelske 2004), when phytoplankton biovolumes remained relatively high throughout the winter of 2000/2001 (Figure 32). Another noteworthy observation is the relative similarity in the peak biovolume levels observed during the previous study (Phlips and Schelske 2004) and the current study (Figures 31 and 32). This similarity is in contrast with the substantially higher peak chlorophyll a concentrations observed in the previous study than in the current study (Figure 10). It is apparent that the chlorophyll a concentrations per cell biovolume were higher in the former study period.

Zooplankton populations were dominated by rotifers throughout the sampling period (Figure 35). A species list of zooplankton species observed during the study period is provided in Appendix 4. Peak zooplankton densities exceeded 3000 individuals per liter in the spring seasons of 2003 and 2004, and again in July of 2004. The lowest zooplankton densities were observed in the fall through winter of 2003, often dropping below 1000 individuals per liter. Copepods were the next most abundant taxonomic group, reaching up to 40% of total zooplankton numbers in the winter of 2003. Cladocerans were generally found in low numbers, but did reach significant densities in the early spring of 2004. Based on the seasonal patterns observed in this study it did not appear that zooplankton population responded to summer peaks in phytoplankton abundance in 2003. There are some indications that spring increases in zooplankton may be a response to spring increases in phytoplankton. Overall, it does not appear that zooplankton played a dominant role in the control of phytoplankton abundance during the study period.

Bacterial densities were higher in 2003 than 2004. Spring/summer values in 2004 often exceeded 1,000,000 cells ml^{-1}, with a maximum of 2,280,000 cells ml^{-1}. In the first half of 2004 values were generally below 500,000 cells ml^{-1}.

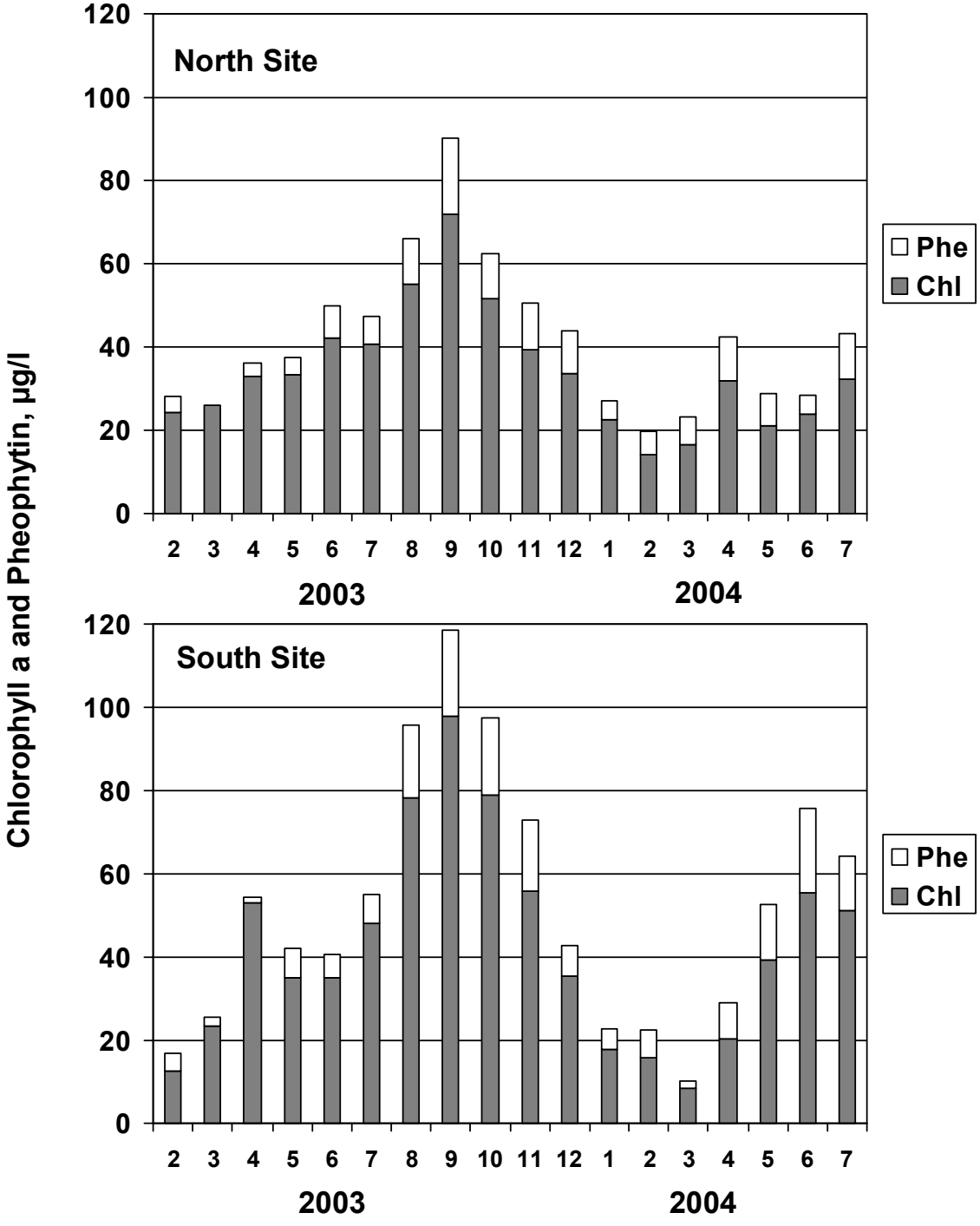

Figure 29. Chlorophyll a and pheophytin concentrations at the north (top) and south (bottom) sites.

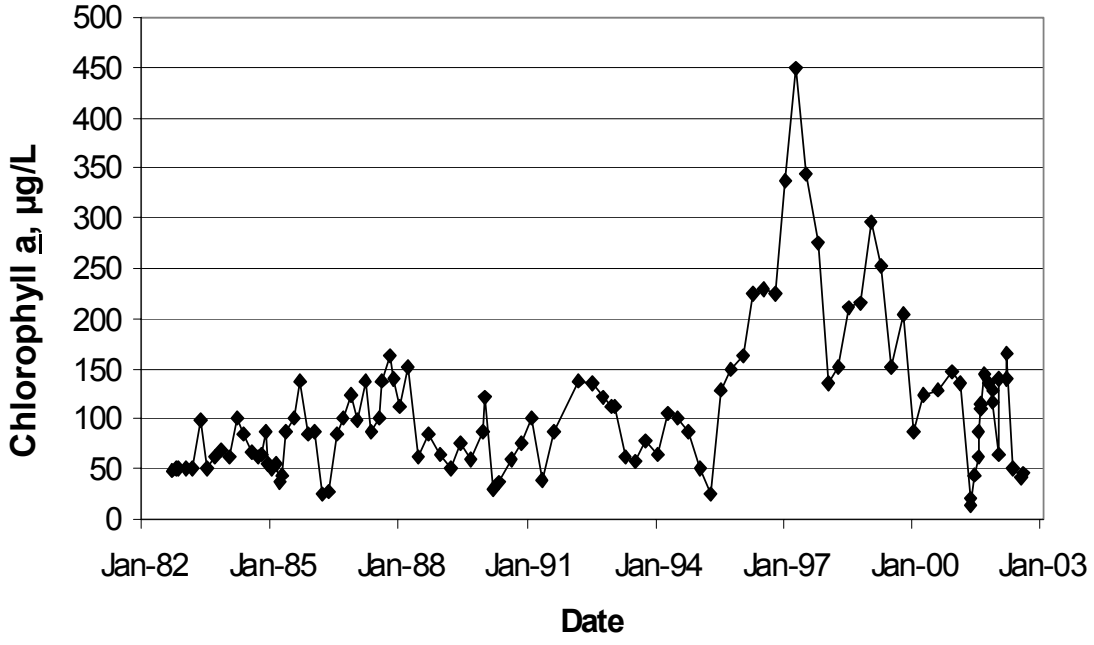

Figure 30. Chlorophyll a concentrations in Lake Griffin from1982 thru 2002 (after Phlips and Schelske 2004 and SJRWMD personal communications).

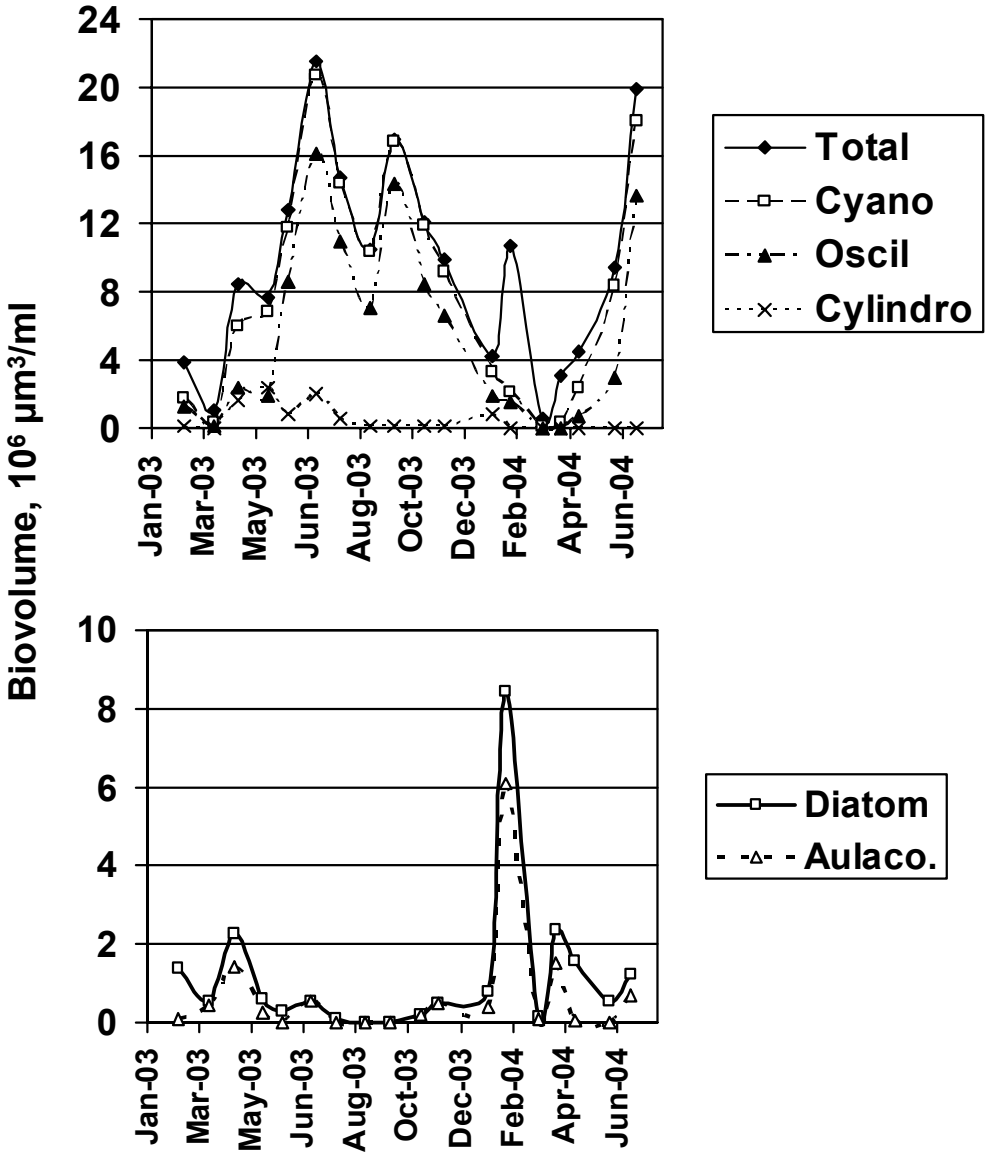

Figure 31. Total phytoplankton biovolumes (top) , biovolumes for major cyanobacteria (blue-green algal) taxa (top), and biovolumes of diatoms and the meroplanktonic diatom species Aulacosira (bottom) at the north site.

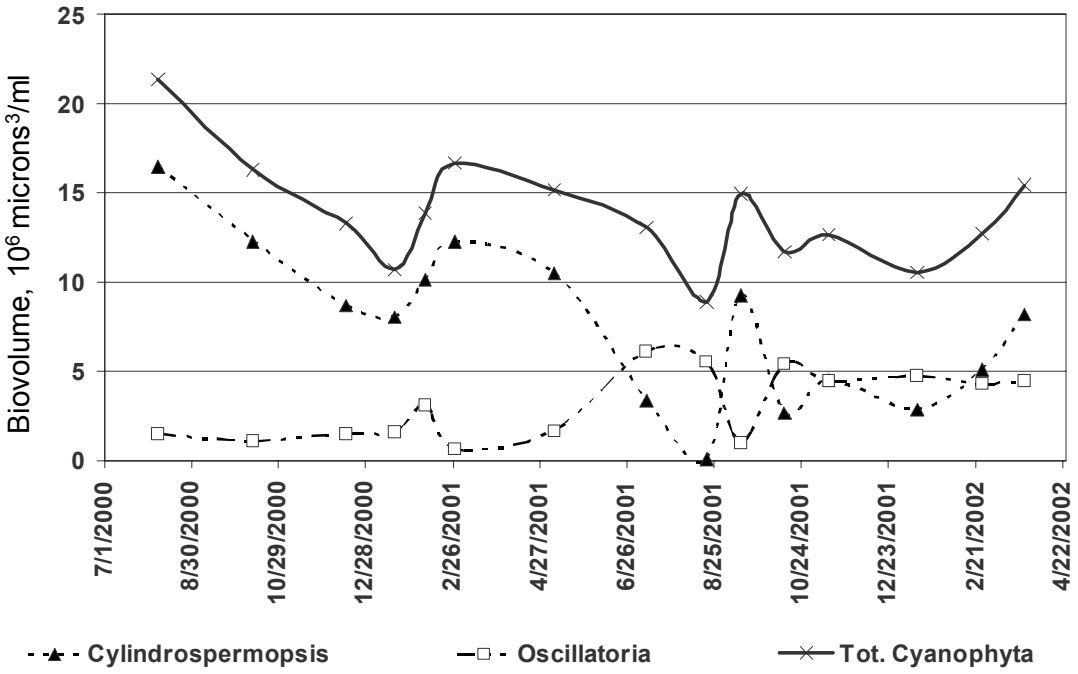

Figure 32. The relative contribution of Cylindrospermopsis to total blue-green algal biovolume in Lake Griffin July, 2000 thru March, 2002 (After Phlips and Schelske 2004).

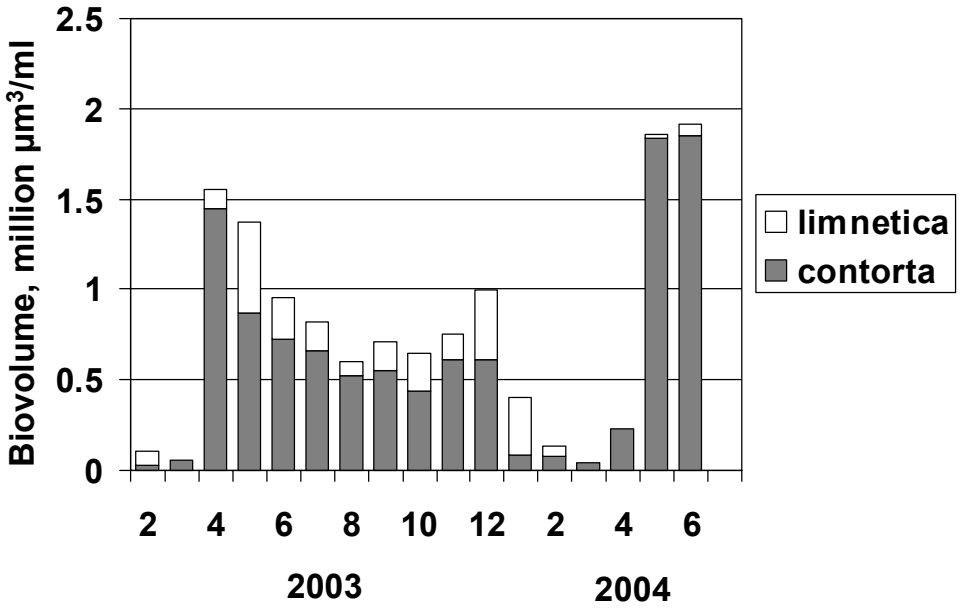

Figure 33. Biovolumes for *Lyngbya limnetica* and *Lyngbya contorta* at the north site.

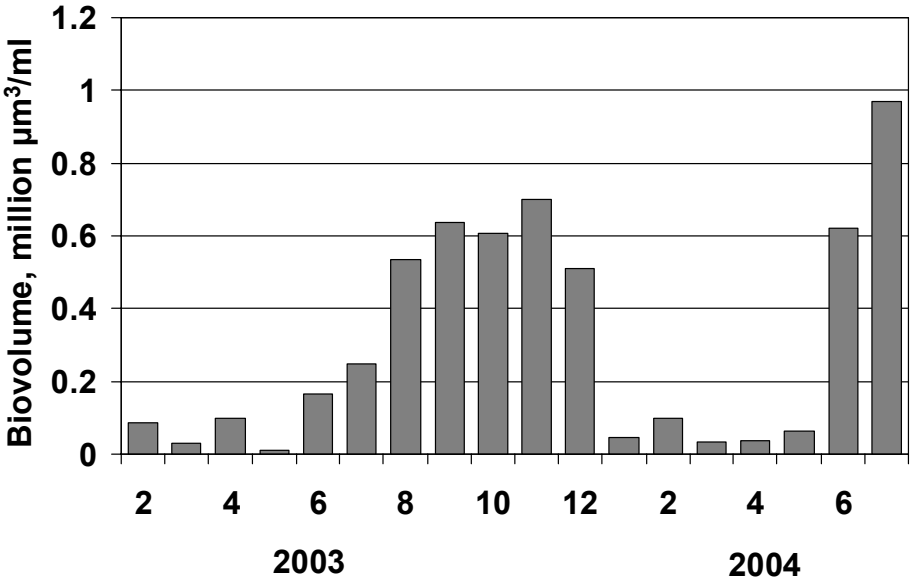

Figure 34. Biovolumes for the blue-green alga *Chroococcus* at the north site.

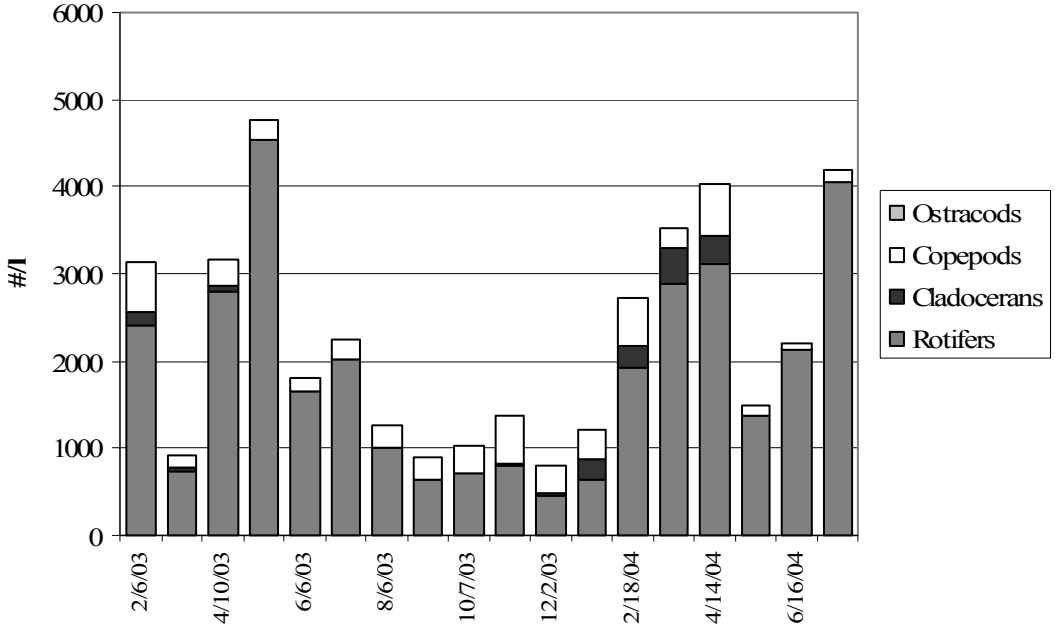

Figure 35. Density of major zooplankton groups in samples from Lake Griffin.

v. Nutrient limitation

The nutrient limitation status of phytoplankton growth in Lake Griffin was determined for eleven dates from November 2002 to July 2004. The chlorophyll yield was determined for five nutrient addition treatments (+N, +P, +N&P, +N&P&SI and +N&P&Si&trace elements) and a control, which was not given any nutrient supplements. The response to the nutrient additions was determined for three different phytoplankton groups: (1) Whole water from Lake Griffin, (2) Water from Lake Griffin diluted 10-fold with filtered lake water, and (3) Unialgal cultures of nutrient-depleted green algae (i.e. *Selenastrum capricornutum*). All three phytoplankton groups exhibited qualitatively similar patterns of response to the different nutrient addition treatments. A comparison of whole lake water and diluted lake water groups provided the greatest insight to the nutrient limitation status of Lake Griffin over the study period. In the whole-water group, all except one of the sampling dates showed significant stimulation of phytoplankton growth relative to the control group for either the +N or +P treatment (Table 6). In the case of the diluted lake water group, significant growth stimulation required the addition of both nitrogen and phosphorus for several dates (Table 7). Due to the smaller starting concentration of phytoplankton in the diluted lake water group the stimulation affects of nutrient addition were considerably larger and more distinct than in the whole water combination.

The responses of phytoplankton to nutrient additions were used to define the nutrient limitation status in Lake Griffin and therefore the potential sensitivity of primary production to nutrient load. In the whole water group, nitrogen was the primary limiting nutrient on seven of eleven bioassay dates, mostly during the summer (Table 8). Among these seven dates four showed secondary phosphorus limitation within 48 hours of the beginning of the incubation. The rapid onset of co-limitation indicates that the amount of bioavailable phosphorus present was small. Four dates showed phosphorus limitation or phosphorus/nitrogen co-limitation. The latter dates were all in the early spring or winter.

Table 6. Results of nutrient limitation bioassay experiments using whole lake water. Mean final chlorophyll a values ($\mu g\ l^{-1}$) for each treatment group are shown along with the results of Duncan's Multiple Range tests. Means with the same letter designation are not significantly different at the alpha level of 0.05.

	Treatment Group			
Date	Control	+N	+P	+N&P
Nov 2002	58.5 B	68.1 AB	69.0 AB	84.2 A
Feb 2003	98.7 B	132.9 B	186.9 A	198.3 A
Apr 2003	37.3 C	80.4 B	45.7 C	162.0 A
Jun 2003	56.3 C	105.5 B	86.2 B	153.9 A
Aug 2003	70.1 B	96.9 B	84.2 B	186.9 A
Oct 2003	57.0 C	94.1 B	61.8 C	159.7 A
Dec 2003	42.4 C	73.3 B	47.9 C	101.6 A
Feb 2004	68.8 B	66.2 B	170.9 A	203.7 A
Apr 2004	59.0 B	56.3 B	177.2 A	188.9 A
Jun 2004	50.0 C	85.8 B	47.2 C	140.9 A
Jul 2004	40.1 B	98.7 A	30.3 B	85.8 A

Table 7. Results of nutrient limitation bioassay experiments using diluted lake water. Mean final chlorophyll a ($\mu g\ l^{-1}$) values for each treatment group are shown along with the results of Duncan's Multiple Range tests. Means with the same letter designation are not significantly different at the alpha level of 0.05.

	Treatment Group			
Date	Control	+N	+P	+N&P
Nov 2002	6.0 B	8.7 B	12.3 AB	18.5 A
Feb 2003	16.2 B	23.5 B	143.1 A	179.9 A
Apr 2003	16.4 B	10.5 B	8.6 B	175.4 A
Jun 2003	11.0 B	13.0 B	9.9 B	68.9 A
Aug 2003	8.0 B	9.4 B	7.9 B	60.3 A
Oct 2003	8.3 C	15.0 B	7.1 C	34.7 A
Dec 2003	5.6 B	6.4 B	5.1 B	50.1 A
Feb 2004	16.2 B	15.2 B	156.2 A	153.9 A
Apr 2004	19.3 B	11.9 B	110.6 A	125.6 A
Jun 2004	9.8 B	9.4 B	7.5 B	114.6 A
Jul 2004	8.0 B	12.3 B	8.0 B	136.9 A

In the diluted water group only four of eleven bioassay dates showed primary nitrogen limitation (Table 9). The other seven dates exhibited phosphorus or phosphorus/nitrogen co-limitation. The results for the diluted water group support the conclusion from the whole water group that the surplus bioavailable phosphorus levels in Lake Griffin were small during most of the sampling period.

D. TASK 3a. Light Flux Tolerance of *Cylindrospermopsis, Microcystis incerta* and *Anabaena flos-aquae.*

As demonstrated in Section A.iii, *Cylindrospermopsis, Microcystis incerta* and *Anabaena flos-aquae* in laboratory cultures all demonstrated the ability to grow rapidly at the lowest light intensities included in the experiment 10 μmole photons $m^{-2}s^{-1}$, or 0.5% of midday incident irradiance in the summer in Florida. The mechanism for adapting to such low growth irradiances appears, at least in part, to be related to the synthesis of extra light absorbing chlorophyll. Among the three species, the highest chlorophyll concentrations were generated by *Anabaena* (Figure 4).

At the other end of the light flux gradient, the ability of algae to withstand high levels of PAR irradiance and UV exposure may be important in competition between species, particularly in shallow lakes, like Lake Griffin. The tolerance of *Cylindrospermopsis, Microcystis incerta* and *Anabaena flos-aquae* for high light fluxes

Table 8. Limiting nutrient status for the whole lake water samples, based on the results of bioassay experiments. The primary nutrient limitation corresponds to the nutrient addition treatment group that stimulates algal increases beyond the control group. The 'NP' designation indicates the treatment group in which both nitrogen and phosphorus were added. 'Time to secondary' limitation is the sampling interval when the secondary limiting status was first clearly visible.

	Limiting Status			
Date	Primary Limitation	Secondary Limitation	Time To Secondary	Additional Sec. Limitation
Nov 2002	N	NP	96 hours	---
Feb 2003	P	P	---	Si
Apr 2003	N	NP	120 hours	Trace
Jun 2003	N	NP	48 hours	---
Aug 2003	N	NP	48 hours	---
Oct 2003	N	NP	48 hours	---
Dec 2003	NP	NP	---	---
Feb 2004	P	NP	240 hours	Si
Apr 2004	P	P	---	Si
Jun 2004	N	NP	48 hours	---
Jul 2004	N	N	---	---

Table 9. Limiting nutrient status for the diluted lake water samples, based on the results of bioassay experiments. The primary nutrient limitation corresponds to the nutrient addition treatment group that stimulates algal increases beyond the control group. The 'NP' designation indicates the treatment group in which both nitrogen and phosphorus were added. 'Time to secondary' limitation is the sampling interval when the secondary limiting status was first clearly visible.

	Limiting Status			
Date	Primary Limitation	Secondary Limitation	Time To Separation	Additional Limitation
Nov 2002	N	NP	200 hours	---
Feb 2003	P	P	---	Si
Apr 2003	NP	NP	---	Trace
Jun 2003	NP	NP	---	---
Aug 2003	N	NP	50 hours	---
Oct 2003	NP	NP	---	Si
Dec 2003	NP	NP	---	Si
Feb 2004	P	P	---	Si
Apr 2004	P	NP	250 hours	---
Jun 2004	N	NP	50 hours	---
Jul 2004	N	N	---	---

was compared in outdoor experiments conducted during the summer, and included tests for UV exposure affects. Test mesocosms were 0.3 m deep to ensure exposure to UV and near surface light fluxes.

All three species showed initial growth at the three light treatments (full sunlight, 60% of full sunlight and 36% of full sunlight) tested in the absence of UV protection (Figure 36). The highest growth rates were observed at the lowest light flux treatment (36% of incident) for all three species. The species with the greatest high light tolerance was *Microcystis*, followed by *Cylindrospermopsis*. The maximum growth rate of *Microcystis* under full sunlight was only 20% less than at 36% of full sunlight. By contrast, the maximum growth rate of *Cylindrospermopsis* was 55% lower at full sunlight than 36% of full sunlight. *Anabaena* exhibited the least tolerance to high light flux, showing a sharp decline in standing crop after four days of exposure. The latter decline was eliminated when a UV blocking filter was added to the treatment (Figure 36). The addition of a UV block also stimulated the growth rate of *Cylindrospermopsis*. *Microcystis* was the only one of the three species which showed no difference between the growth rates under UV blocked and unblocked treatment groups.

It is clear from the results of these experiments that *Microcystis* is well-adapted for forming surface blooms at the high summer irradiances experienced in Florida. Comparing the two nitrogen-fixing species *Cylindrospermopsis* and *Anabaena*, the former appears to be more tolerant to high light fluxes, both in terms of PAR irradiance and UV exposure. Since many eutrophic lakes in Florida experience periods of nitrogen limitation due to high phosphorus loads, *Cylindrospermopsis* may be at a selective advantage under conditions of high light flux that are a common feature of very shallow lakes, like Lake Griffin.

E. TASK 3b. Inhibition of Algal Growth by *Cylindrospermopsis*.

The ability of many strains of *Cylindrospermopsis* to produce toxic and other metabolically active substances raises the question of the potential negative affect of this species on its algal competitors. This potential was examined using standard microbiological sensidisk methodologies involving three target species common to

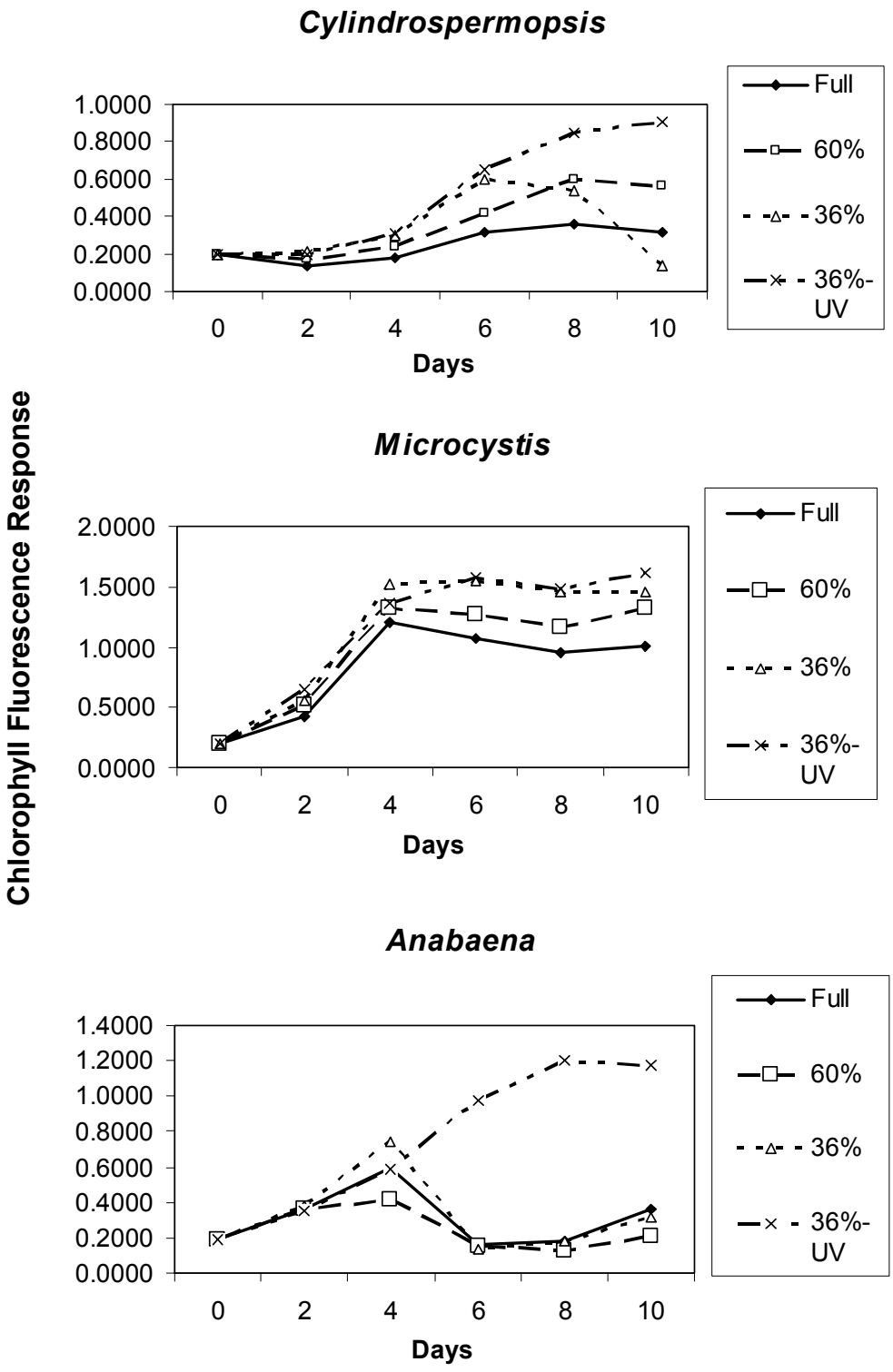

Figure 36. The growth response for the growth of three blue-green algal species under different outdoor light regimes: full sunlight, 60% full sunlight, 36% full sunlight and 36% full sunlight plus UV screen.

eutrophic Florida lakes: the green alga *Scenedesmus* and the blue-green algae *Microcystis incerta* and *Anabaena flos-aquae*. Three different treatment groups were compared for inhibitory active: (1) Concentrated extracts from lysed *Cylindrospermopsis* cells, (2) Extracts from lysed *Cylindrospermopsis* cells, and (3) Water from cultures of *Cylindrospermopsis* with cells removed. The zone of inhibition of cell growth for each species was measured for each of the three treatment groups (Table 10). A control group was included which consisted of testing the effect of *Cylindrospermopsis* extracts on the growth of *Cylindrospermopsis*, which uniformly showed no inhibitory effect.

The growth of *Anabaena* was not affected by any of the treatment types. The growth of *Scenedesmus* and *Microcystis incerta* were affected by all three treatment groups, as indicated by the presence of distinct zones of inhibition around the sensi-discs. The greatest average zone of inhibition for both species was greatest for the medium derived from the removal of cells. This observation agrees with the observations of other researchers that cells in early stationary phase of the growth cycle tend to release significant percentage of the toxin produced intra-cellularly into the environment.

F. TASK 3c. Comparative Phosphorus Uptake Rates of *Cylindrospermopsis* and *Anabaena flos-aquae.*

The phosphorus uptake rates for *Cylindrospermopsis* and *Anabaena flos-aquae* were compared in a series of three short-term dosing experiments. A series of sampling time intervals were included in the study from 1 to 20 minutes. After 5 minutes the uptake rates showed significant reduction. Therefore the 0-5 minute intervals were choosen as the most representative of the maximum uptake capacities. Within the 0-5 minute interval there was considerable variability between experiments in the observed rates of uptake. For the purpose of comparison the maximum rates observed for all experiments are shown in Figure 37. The maximum uptake rate (V_{max}) on a per liter basis were similar for both species, 4 µg P/l/min for *Anabaena* and 3 µg P/l/min for C*ylindrospermopsis*. When these values were converted to a per unit cell biovolume basis, the V_{max} for C*ylindrospermopsis* was considerably higher than for *Anabaena*, i.e.

Table 10. The magnitude of cell growth inhibition of blue-green algae in sensi-disc experiments. Values in the table represent the measured extent of the zone of inhibition from the sensi-disc in millimeters.

Microcystis incerta	**09.01.2004 Zone of inhibition (mm)**				**09.08.2004 Zone of inhibition (mm)**			
	Disc1	Disc2	Disc3	Average	Disc1	Disc2	Disc3	Average
C.cuspis concentrated extract (12ml+3ml)	0.20	0.10	0.10	0.13	0.20	0.10	0.15	0.15
C. cuspis extract (12ml+12ml)	0.10	0.10	0.20	0.13	0.10	0.10	0.20	0.13
C. cuspis extracellular medium	0.25	0.20	0.15	0.20	0.25	0.20	0.15	0.20

Secendesmus bijuga	**09.01.2004 Zone of inhibition (mm)**				**09.08.2004 Zone of inhibition (mm)**			
	Disc1	Disc2	Disc3	Average	Disc1	Disc2	Disc3	Average
C.cuspis concentrated extract (12ml+3ml)	0.10	0.25	0.10	0.15	0.10	0.30	0.10	0.16
C. cuspis extract (12ml+12ml)	<0.10	0.50	0.10		< 0.10	0.50	0.10	
C. cuspis extracellular medium	0.10	0.30	0.20	0.20	0.10	0.30	0.20	0.2

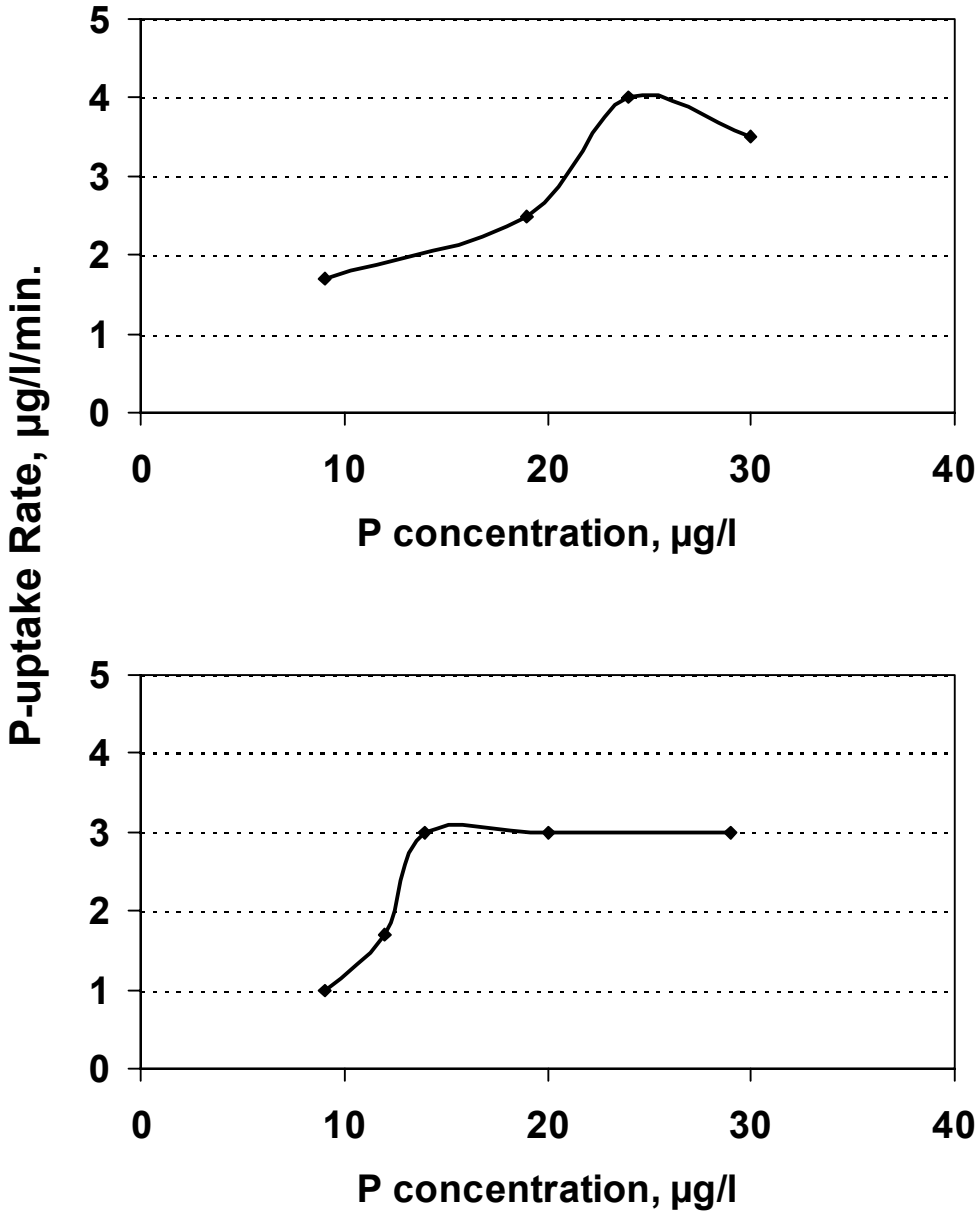

Figure 37. Comparison of maximum observed phosphorus uptake rates by *Anabaena* (top) and *Cylindrospermopsis* (bottom) at different phosphorus concentrations.

2.53 µg P/10^9 µm^3/min compared to 0.29 µg P/10^9 µm^3/min. Estimates of K_m values were 10 µg P/l C*ylindrospermopsis* and 14 µg P/l for *Anabaena*. Based on these preliminary numbers, it appears that C*ylindrospermopsis* is an excellent competitor for phosphorus, at least relative to *Anabaena***.** This conclusion supports earlier reports of high rates of phosphorus uptake in previous studies of *Cylindrospermopsis raciborski* from Lake Balaton in Hungary (Isvanovics et al. 2000). However, some caution must be taken in interpreting these results, considering the considerable variability in the rates observed in different experiments.

G. TASK 3d. Comparative Analysis of Growth Rates of *Cylindrospermopsis* and *Anabaena flos-aquae* Under Nitrogen-rich and Nitrogen Deficient Conditions

Nitrogen-limited conditions are a common feature in Lake Griffin and other eutrophic lakes in Florida, like Lake Okeechobee (Phlips and Ihnat 1995). Such conditions favor the proliferation of nitrogen-fixing species of blue-green algae. In Lake Griffin, the filamentous heterocystous blue-green alga *Cylindrospermopsis* appears to have been particularly successful at taking advantage of these nitrogen-limiting conditions. The latter observation raises the question of whether *Cylindrospermopsis* grows faster under nitrogen limited conditions than another prominent nitrogen-fixer found in Florida lakes, like *Anabaena flos-aquae*. In a series of three experiments, the growth rates of *Cylindrospermopsis* and *Anabaena flos-aquae* were compared in both nitrogen sufficient (i.e. nitrate added to the growth medium) and nitrogen-free medium (Figure 38). Both species grew rapidly in either medium, demonstrating their high nitrogen fixation capacities. The absolute growth rates (doubling times) for *Anabaena flos-aquae* were somewhat higher than for *Cylindrospermopsis*. However, it does not appear that the ability to grow under nitrogen-fixing conditions provides a significant advantage to one species over the other.

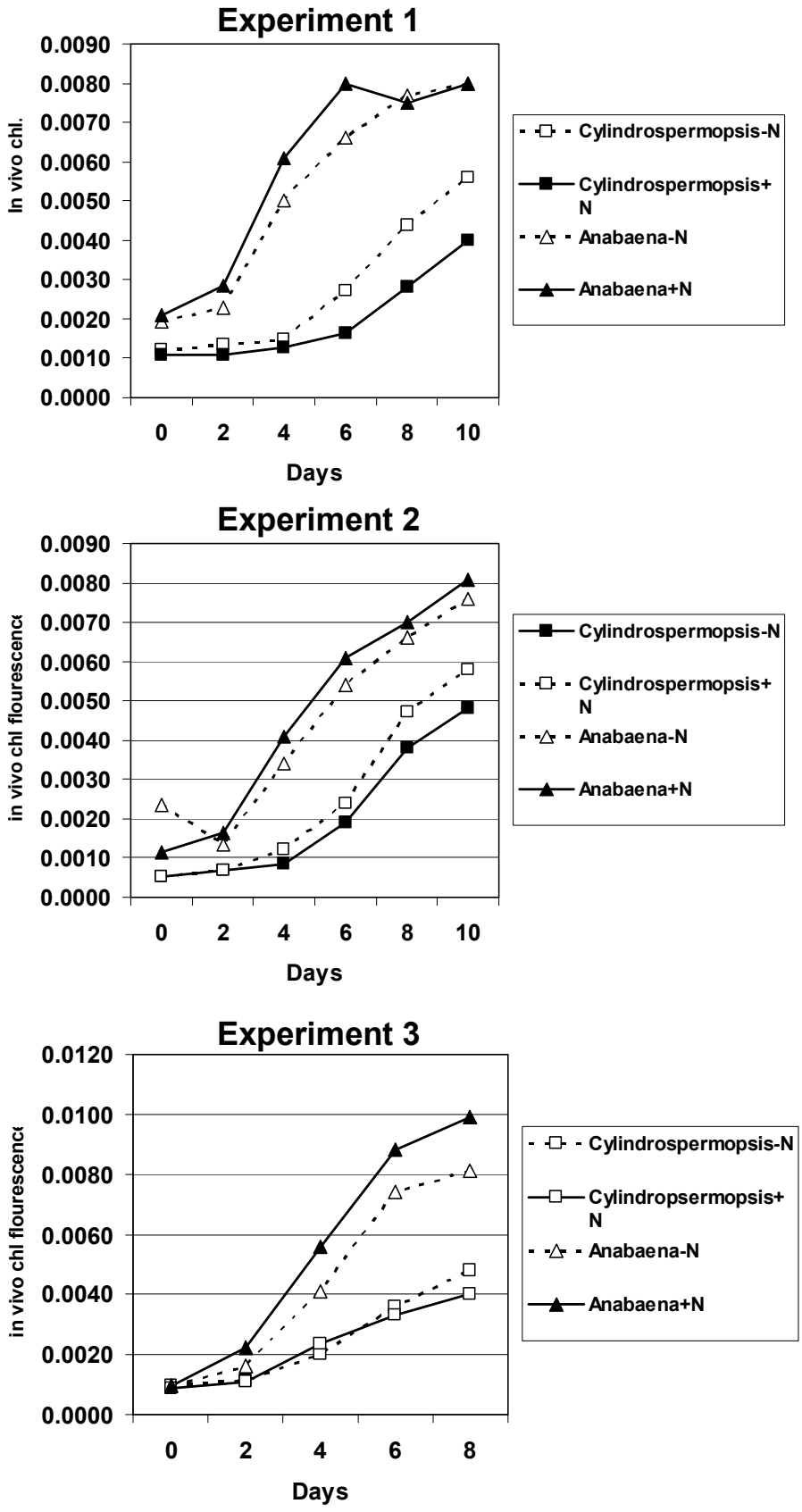

Figure 38. Growth curves for *Anabaena* and *Cylindrospermopsis* cultures grown on nitrogen sufficient (+N) and nitrogen-free (-N) media.

VI. SUMMARY AND CONCLUSIONS

In the last twenty years of the 20th century average chlorophyll a concentrations in Lake Griffin gradually increased, culminating in a dramatic 2-3 fold jump in concentration in 1996. The latter increases in chlorophyll a levels were putatively linked to a dramatic explosion of blue-green algal biomass, primarily the potentially toxic species *Cylindrospermopsis*. The potentially harmful nature of *Cylindrospermopsis* highlighted the need for a better understanding of the driving forces behind these blue-green algal blooms and more specifically the factors that encouraged the success of *Cylindrospermopsis*. The central goals of the current study were to better define the meaning of the large recent shifts in chlorophyll a concentrations in Lake Griffin and explore the reasons for the apparent success of *Cylindrospermopsis*. The fact that the observations made over this study period included a dramatic decline in both chlorophyll a and the importance of *Cylindrospermopsis* provide new insights into the phytoplankton ecology of Lake Griffin.

The central goals of this project were pursued within the context of three research objectives and seven working hypotheses, and the summary and conclusions will be presented in a complimentary manner:

Objective 1 – To determine whether changes in chlorophyll concentrations at selected sampling sites accurately reflect lake wide changes in phytoplankton biomass.

Hypothesis 1a: Changes in chlorophyll accurately reflect changes in phytoplankton biomass.

Hypothesis 1b: Phytoplankton is uniformly distributed around the lake and through the water column.

The first two hypotheses of this project focus on the question of representativeness. In other words, do the large shifts in chlorophyll a concentrations observed over the past 25 years in Lake Griffin accurately represent changes in lake wide phytoplankton biomass? The results of the current, and a previous study of the lake

(Phlips and Schelske 2004), demonstrate that the abundance and species composition within the lake is relatively uniform spatially, from both a geographical and vertical perspective. Despite the fact that there are small, but significant, differences in the average chlorophyll a concentrations between the southern and northern basins, it appears that water samples collected from most regions of the lake are representative of the overall abundance and composition of phytoplankton. In contrast, some lakes in Florida, like Lake Okeechobee, exhibit large and consistent regional disparities in phytoplankton abundance and composition (Cichra et al. 1995; Phlips et al. 1993).

The question of whether chlorophyll a concentrations are strictly proportional to phytoplankton biomass is a more complicated issue. The results of experiments dealing with the affects of light flux and nutrient regime on the growth and physiology of both natural populations and unialgal cultures of algae reveal the potential for over two-fold differences in chlorophyll a content of cells. A direct comparison of the chlorophyll a content of three common bloom-forming species of blue-green algae, *Anabaena*, *Microcystis* and *Cylindrospermopsis*, revealed significant differences between species grown under identical light and nutrient regimes. *Cylindrospermopsis* had over two-fold higher concentrations than either *Anabaena* or *Microcystis* when grown under moderate irradiance levels. By contrast, the difference in chlorophyll a concentration were less dramatic when cells were grown under low irradiance levels. The results of these experiments suggest that some portion of the temporal variability in chlorophyll a concentrations observed over the past 22 years may not fully reflect changes in phytoplankton biomass. Variations in nutrient status, light availability, species composition and the age of bloom populations may all contribute to the changes in the concentration of chlorophyll a observed in the lake. The potential for such variability is illustrated in a comparison of peak summer chlorophyll a and phytoplankton biovolume levels in 2001 and 2003. Mean summer chlorophyll a concentrations dropped significantly from 2001 to 2003, but mean biovolume values were relatively similar. The most obvious difference between the two summers was the domination of *Cylindrospermopsis* in 2001 and the shift in dominance to *Oscillatoria* in 2003. However, these differences in species composition may not be the only potential cause of the shifts in chlorophyll a/biovolume ratios. The results of nutrient limitation bioassays

conducted in both 2001 and 2003 indicate an elevated potential for nutrient deficiency in 2003 compared to 2001. It is possible that the latter differences in nutrient availability may have contributed to reduced capacity for chlorophyll a synthesis or increased rates of phytoplankton senescence in 2003. It is not possible to define the extent to which such considerations may have influenced chlorophyll a concentrations prior to 2000, because of the scarcity of phytoplankton biovolume information. It is unlikely that the very high chlorophyll a concentrations of the mid-late 1990s can be entirely attributed to such factors.

Objective 2 – To determine the sensitivity of algal growth to changes in nutrient load.

Hypothesis 2: Phytoplankton growth in Lake Griffin is sensitive to changes in nutrient load.

The observations of changes in TN/TP (Figure 38) and chlorophyll a/phosphorus (Figure 39) since 1982 manifest the possibility that both the character and degree of nutrient limitation of phytoplankton production has been subject to changes over time. The putative dominance of the nitrogen-fixing species of blue-green algae from the mid-1990s through mid-2002 indicates the predominance of nitrogen-limiting conditions during this time period, despite the fact that TN/TP ratios in Lake Griffin have been significantly higher than Redfield ratios throughout the historical record. Another indication of the increase in nitrogen limitation during the late 1990's is the drop in the TN/TP ratio that coincided with an increase in chlorophyll a/TP ratio. The driving force behind this decrease in the TN/TP ratio appears to have been an increase in phosphorus concentration rather than a change in nitrogen levels, which have remained relatively constant over the historical time period. The importance of nitrogen limitation in Lake Griffin is corroborated by the results of nutrient limitation bioassays performed

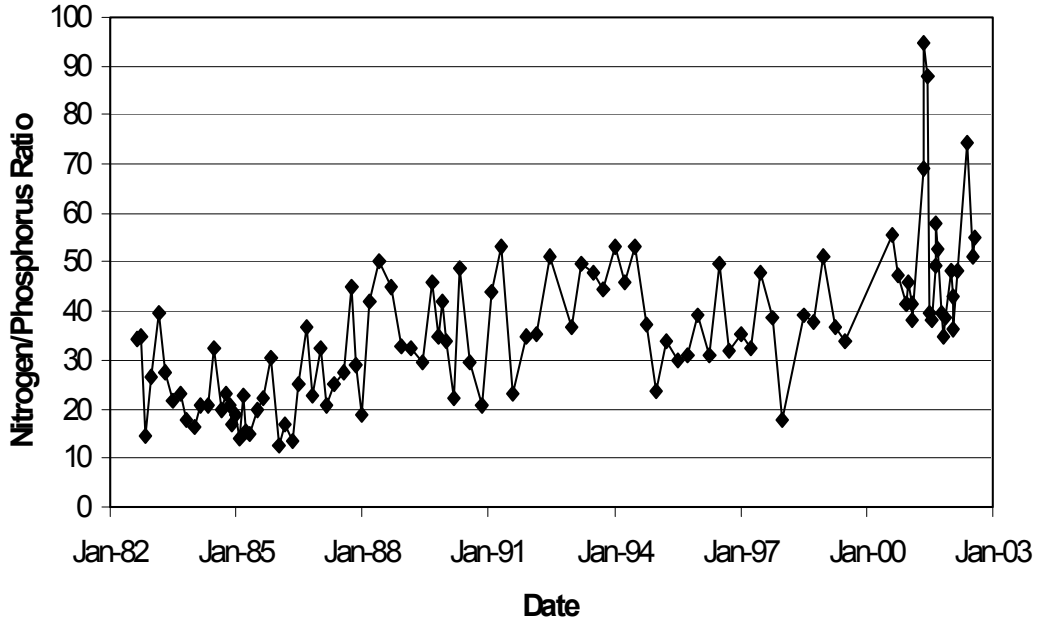

Figure 39. Record of nitrogen/phosphorus ratios for Lake Griffin from1982 thru 2002 (after Phlips and Schelske 2004 and SJRWMD personal communications).

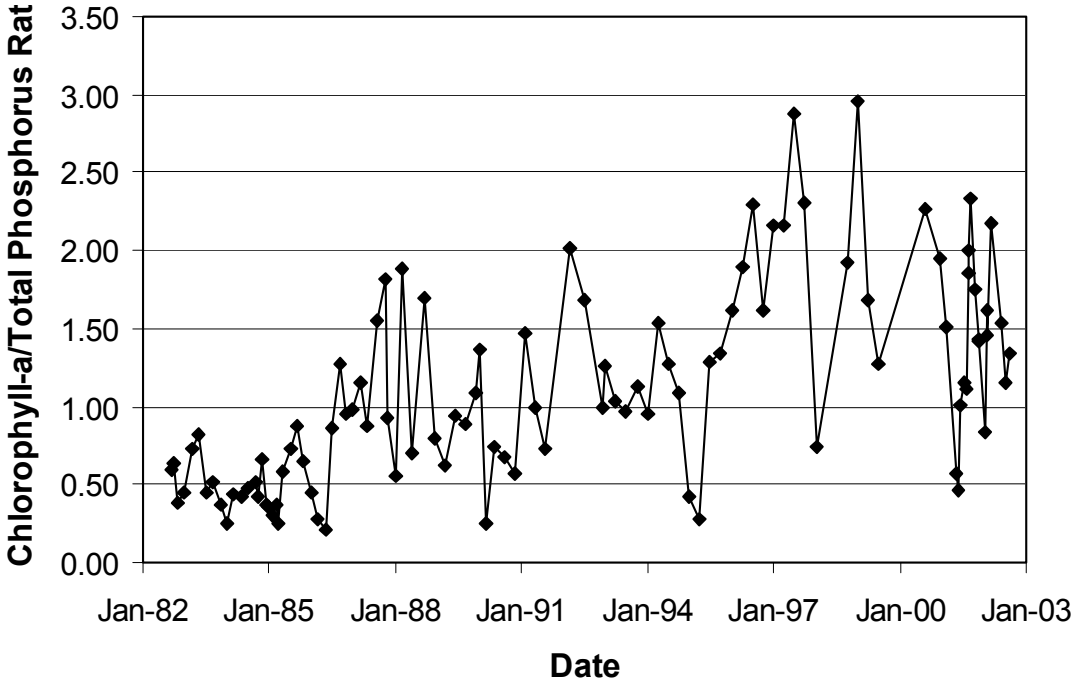

Figure 40. Record of chlorophyll a/total phosphorus ratios for Lake Griffin from1982 thru 2002 (after Phlips and Schelske 2004 and SJRWMD personal communications).

in a study of the lake from 2000 through 2002 (Phlips and Schelske 2004). However, there are also indications that the 2000-2002 time period may be the beginning of a transition toward a greater degree of phosphorus limitation. TN/TP ratios have increased from 2001 through the current study period, ending in 2004. In addition, nutrient limitation bioassays conducted as part of this study in 2003 and 2004 showed a higher frequency of phosphorus and nitrogen/phosphorus co-limitation than in the previous study from 2000-2002 (Phlips and Schelske 2004).

Several other observations support the hypothesis that Lake Griffin may be trending toward a greater degree of phosphorus limitation since 2001. During the peak chlorophyll a period of the late 1990s, highly elevated levels of chlorophyll (i.e. greater than 100 $\mu g\ l^{-1}$) were often maintained throughout the year, suggesting a relatively constant internal and/or external source of bioavailable phosphorus. After 2001, and particularly during the current study period (late 2002 thru mid 2004), chlorophyll a levels have been on the decline and much more seasonal, with distinct winter/early spring minima. The latter pattern suggests a greater dependence of phytoplankton production on external inputs of phosphorus during the summer. Perhaps an even more compelling argument for greater phosphorus limitation over the past few years is the shift in dominance of the phytoplankton population from nitrogen-fixing species, like *Cylindrospermopsis*, to non-nitrogen-fixing blue-green algae, like *Oscillatoria*, *Chroococcus* and *Lyngbya*.

Objective 3 – To examine key factors contributing to the ability of *Cylindrospermopsis* to dominate the phytoplankton community of Lake Griffin.

Hypothesis 3a: *Cylindrospermopsis* is a superior competitor under conditions of low light availability and/or high light flux.

Hypothesis 3b: *Cylindrospermopsis* produces biologically active substances that inhibit the growth of other species of algae.

Hypothesis 3c: *Cylindrospermopsis* is a superior competitor for phosphorus.

Hypothesis 3d: *Cylindrospermopsis* is a superior competitor under nitrogen-limited conditions.

The aforementioned observations associated with Objectives 1 and 2 highlight the fact that Lake Griffin has undergone shifts in both chemical and biological aspects of its ecology over the past 25 years and even over the past four years. From a biological perspective, one of the most dramatic shifts has been the rise and fall in the importance of the potentially toxic blue-green alga *Cylindrospermopsis*. Preliminary evidence indicates that *Cylindrospermopsis* was not a prominent element of the phytoplankton community of Lake Griffin in the 1960s (Chapman and Schelske 1997), although the amount of data available for this period is very limited. There is considerable evidence from a variety of sources (Bill Johnson, FWCC, personal communications), albeit only limited peer reviewed literature (Chapman and Schelske 1997), that *Cylindrospermopsis* dominated the phytoplankton community through the high chlorophyll a period of 1996 through the 2001 and to a lesser extent in 2002 (Phlips and Schelske 2004). The basis for the success of *Cylindrospermopsis* was an important component of the current research effort.

In relation to Hypothesis 3a, it is well known that the tolerances of different phytoplankton species to low and high irradiance environments vary widely (Phlips and Mitsui 1982). The comparison of the responses of *Microcystis, Anabaena* and *Cylindrospermopsis* demonstrated differences at both ends of the irradiance spectrum. At low growth irradiances, both *Anabaena* and *Cylindrospermopsis* showed the ability to generate higher chlorophyll a concentrations on a per unit cell biovolume basis than *Microcystis*. Conversely, *Microcystis* demonstrated superior tolerance to high irradiance, including UV radiation. The latter observation helps to explain the propensity of *Microcystis* to form surface scums in eutrophic lakes. In comparing *Anabaena* and *Cylindrospermopsis*, *Anabaena* showed the greatest sensitivity to high irradiance, which appears to be in part due to sensitivity to UV radiation. Hence, in a competition between *Anabaena* and *Cylindrospermopsis* in high irradiance situations, *Cylindrospermopsis* appears to have an advantage. The importance of this factor is accentuated in Lake Griffin because of its extremely shallow depth and is further exacerbated by the fact that

Florida has been in a relatively low rainfall period through the 1990s, resulting in low lake water levels throughout the state.

In relation to Hypothesis 3b, the results of another series of experiments provide preliminary evidence that *Cylindrospermopsis* may produce and excrete biologically active substances that inhibit the growth of certain species of algae, like the blue-green alga *Microcystis incerta* and the green alga *Scenedesmus bijuga*. The role of such substances in directing the outcome of competition between algal species has been reported in several other studies. The fact that *Cylindrospermopsis* from Lake Griffin has been associated with the production of toxins (Williams et al. 2001), also brings to the fore a number of issues related top-down control of phytoplankton community structure and abundance. A considerable body of evidence exists for the inhibitory role of toxin production in grazing activity (Chorus and Bartram 1999). Therefore, part of the success of *Cylindrospermopsis* may be attributable to its resistance to grazing loss.

In relation to Hypothesis 3c, the results of a third set of experiments involving indicate that its maximum phosphorus uptake rates of *Cylindrospermopsis* are considerably higher than those of *Anabaena.* This observation corroborates the exceptional phosphorus uptake rates reported for *Cylindrospermopsis raciborski* from Lake Balaton in Hungary (Isvanovics et al. 2000). From a physiological standpoint, it is possible to hypothesize that the small size (i.e. 2µm diameter) and large surface area/volume ratio characteristic of *Cylindrospermopsis* provides it with an inherent advantage in terms of rates of nutrient uptake.

In relation to the final hypothesis, '3d', the results of this study demonstrate that *Cylindrospermopsis* is capable of strong growth in the absence of any outside inputs of reduced nitrogen. However, another prominent blue-green alga in lakes throughout Florida, *Anabaena*, is equally capable of high growth rates under nitrogen deficient conditions, but is a minor component of the phytoplankton population of Lake Griffin. Therefore, the nitrogen-fixing capability of *Cylindrospermopsis* may only in part explain its ability to dominate the phytoplankton community during certain periods of the lakes recent history. The results of three other experiments included in the current study provide some additional clues about the ability of *Cylindrospermopsis* to out compete *Anabaena,* and other phytoplankton species, in Lake Griffin.

Overall, it is clear from the results of the aforementioned experiments that *Cylindrospermopsis* has many characteristics that make it very competitive in ecosystems like Lake Griffin, including: the ability to fix nitrogen, strong phosphorus uptake capacity, adaptability to low light conditions and the production of compounds inhibitory to the growth of other species. From a broader geographical perspective, the competitive ability of *Cylindrospermopsis* is demonstrated by its prominence in eutrophic lakes throughout the world (Padisak 1997). It is noteworthy that the relative disappearance of *Cylindrospermopsis* from the phytoplankton community in Lake Griffin after 2002 was accompanied by the increase of several other small-celled blue-green algae, like 1-2µm diameter filaments of *Oscillatoria*. Recent major shifts in phytoplankton abundance and composition, as well as changes in nutrient ratios, indicate that Lake Griffin is dynamic and possibly receptive to changes in nutrient load.

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Appendix 1: Site measurements										
Date	Station	Time	Depth	Secchi	D.O. -0.5	D.O. -1.0	D.O. -1.5	D.O. -2.0	D.O. -2.5	D.O.-3.0
YYMMDD			m	Disk	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
04/10/03	Mid-lake	11:15	2.8	0.4	8.09	8.06	8.14	8.14		
05/14/03	Mid-lake	11:14	2.6	0.4	8.01	7.96	7.11	6.50		
06/06/03	Mid-lake	10:03	2.3	0.5	7.97	7.72	7.28	6.95		
07/09/03	Mid-lake	11:14	2.5	0.5	9.16	8.46	7.60	7.19		
08/06/03	Mid-lake	11:02	2.4	0.3	7.30	7.05	6.71	5.99		
09/09/03	Mid-lake	10:58	2.6	0.4	7.84	7.61	7.15	6.80		
10/07/03	Mid-lake	11:16	2.4	0.3	12.64	12.30	10.91	8.28		
11/11/03	Mid-lake	11:20	2.3	0.4	8.48	8.16	8.03	7.54		
12/02/03	Mid-lake	10:50	2.6	0.6	9.01	8.81	8.71	8.63		
01/28/04	Mid-lake	12:00	2.5	1.0	8.73	8.65	8.66	8.59		
02/18/04	Mid-lake	11:40	2.5	1.3	8.06	7.69	7.61	7.61		
03/25/04	Mid-lake	11:10	2.6	1.3	7.61	7.65	7.65	7.65	6.01	
04/14/04	Mid-lake	9:35	2.6	1.2	7.56	7.52	7.56	7.53		
05/06/04	Mid-lake	11:35	2.6	1.1	6.01	6.02	5.68	3.84		
06/16/04	Mid-lake	11:36	2.5	1.0	5.75	5.33	5.01	3.37		
07/11/04	Mid-lake	10:45	2.5	0.8	8.33	7.80	6.70	6.90		
02/06/03	North	10:20	2.0	0.8	9.55	9.42	9.34			
03/13/03	North	11:32	2.0	1.0	8.82	8.82	8.57			
04/10/03	North	10:40	2.0	0.4	7.77	7.72	7.68			
05/14/03	North	10:40	1.8	0.5	7.26	7.21	6.82			
06/06/03	North	10:31	1.4	0.3	8.43	8.25				
07/09/03	North	11:50	2.0	0.5	9.51	8.53	7.15			
08/06/03	North	11:28	1.7	0.3	7.64	7.41	5.93			
09/09/03	North	11:28	1.9	0.4	7.12	6.94	6.84			
10/07/03	North	12:17	1.5	0.3	12.01	11.97				
11/11/03	North	11:45	1.5	0.4	8.48	8.47				
12/02/03	North	11:30	2.0	0.6	8.92	8.79	8.77			
01/28/04	North	11:10	2.0	1.3	8.60	8.36	8.35			
02/18/04	North	10:57	2.0	1.0	7.76	7.36	7.32	1.20		
03/25/04	North	10:40	2.0	1.3	8.29	8.05	7.92	6.29		
04/14/04	North	10:00	2.0	0.9	8.34	8.07	8.00			
05/06/04	North	12:00	2.1	1.1	7.23	7.09	6.67			
06/16/04	North	10:47	1.9	1.0	4.62	4.31	3.42			
07/11/04	North	11:16	2.0	0.8	8.26	8.35	7.35	5.31		
02/06/03	South	9:17	3.0	1.0	10.04	9.50	9.43	9.27	9.10	9.09
03/13/03	South	10:50	3.0	1.0	9.98	9.36	9.11	8.69	8.50	7.70
04/10/03	South	11:32	3.0	0.4	8.09	8.08	8.11	7.98	7.98	8.14
05/14/03	South	11:44	3.0	0.5	8.19	8.18	8.21	6.87	5.94	5.02
06/06/03	South	9:32	3.0	0.4	7.82	7.21	7.10	6.60	6.62	6.41
07/09/03	South	10:22	3.0	0.4	7.34	7.47	7.34	6.24	5.55	4.72
08/06/03	South	10:26	3.0	0.2	7.62	6.48	6.06	5.86	5.48	5.52
09/09/03	South	10:25	3.0	0.4	8.20	8.00	7.65	7.50	7.40	7.00
10/07/03	South	10:40	3.0	0.3	11.73	10.95	10.92	10.95	10.16	
11/11/03	South	10:55	3.0	0.5	7.74	7.49	7.49	7.37	7.31	
12/02/03	South	10:27	3.0	0.6	9.11	8.87	8.70	8.66	8.55	
01/28/04	South	12:25	3.0	1.0	8.70	8.70	8.62	8.43	8.06	8.06
02/18/04	South	12:05	3.0	1.3	8.11	7.75	7.61	7.52	7.48	7.40
03/25/04	South	11:30	3.6	2.1	7.39	6.97	6.91	6.89	6.84	6.87
04/14/04	South	9:05	3.0	1.3	7.44	7.36	7.25	7.26	7.06	4.39
05/06/04	South	11:10	3.6	1.0	7.51	6.05	5.46	4.15	3.36	1.87
06/16/04	South	12:02	3.0	1.0	6.38	5.84	5.36	4.38	4.00	3.37
07/11/04	South	10:13	3.0	0.8	8.03	8.02	7.40	6.55	6.27	4.70

Date	Station	Time	Temp	Temp	Temp	Temp	Temp	Temp
YYMMDD			0.5 m	1 m	1.5 m	2 m	2.5 m	3 m
04/10/03	Mid-lake	11:15	22.5	22.5	22.5	22.4		
05/14/03	Mid-lake	11:14	29.5	29.4	28.9	28.8		
06/06/03	Mid-lake	10:03	27.3	27.1	26.9	26.9		
07/09/03	Mid-lake	11:14	31.1	30.6	30.5	30.4		
08/06/03	Mid-lake	11:02	29.3	28.9	28.7	28.7		
09/09/03	Mid-lake	10:58	27.8	27.7	27.6	27.6		
10/07/03	Mid-lake	11:16	26.8	25.9	25.8	25.7		
11/11/03	Mid-lake	11:20	22.8	22.7	22.7	22.7		
12/02/03	Mid-lake	10:50	16.5	16.6	16.7	16.8		
01/28/04	Mid-lake	12:00	15.0	15.0	15.0	14.9		
02/18/04	Mid-lake	11:40	15.0	15.9	15.9	15.8		
03/25/04	Mid-lake	11:10	19.3	19.3	19.2	19.2	19.5	
04/14/04	Mid-lake	9:35	20.1	20.1	20.1	20.1		
05/06/04	Mid-lake	11:35	25.0	24.8	24.6	24.5		
06/16/04	Mid-lake	11:36	30.1	30.0	29.8	29.6		
07/11/04	Mid-lake	10:45	30.9	30.8	30.7	30.4		
02/06/03	North	10:20	15.1	15.0	14.9			
03/13/03	North	11:32	24.1	23.5	23.4			
04/10/03	North	10:40	23.2	23.2	23.2			
05/14/03	North	10:40	28.6	28.5	28.4			
06/06/03	North	10:31	27.4	27.3				
07/09/03	North	11:50	31.8	31.1	31.0			
08/06/03	North	11:28	29.5	28.8	28.8			
09/09/03	North	11:28	27.4	27.4	27.3			
10/07/03	North	12:17	28.8	27.8				
11/11/03	North	11:45	22.8	22.8				
12/02/03	North	11:30	15.9	15.9	15.9			
01/28/04	North	11:10	15.7	15.7	15.7			
02/18/04	North	10:57	15.0	15.1	15.0	15.8		
03/25/04	North	10:40	18.4	18.9	18.9	18.9		
04/14/04	North	10:00	19.9	19.9	19.8			
05/06/04	North	12:00	25.4	24.6	24.4			
06/16/04	North	10:47	30.3	30.2	30.0			
07/11/04	North	11:16	32.3	31.9	31.6	31.6		
02/06/03	South	9:17	14.3	14.3	14.3	14.3	14.3	14.3
03/13/03	South	10:50	24.3	23.4	23.1	23.0	22.8	22.5
04/10/03	South	11:32	23.6	23.6	23.6	23.6	23.6	23.6
05/14/03	South	11:44	29.3	29.2	29.1	28.7	28.3	28.5
06/06/03	South	9:32	27.3	27.1	27.1	27.1	27.1	27.1
07/09/03	South	10:22	30.7	30.6	30.6	30.5	30.1	29.7
08/06/03	South	10:26	29.2	28.4	28.9	28.8	28.9	28.8
09/09/03	South	10:25	28.0	27.8	27.8	27.8	27.7	27.7
10/07/03	South	10:40	27.8	27.0	26.7	26.5	26.0	
11/11/03	South	10:55	23.0	23.0	23.0	22.9	22.9	
12/02/03	South	10:27	16.7	16.7	16.6	16.6	16.6	
01/28/04	South	12:25	15.7	15.7	15.6	15.6	15.4	15.2
02/18/04	South	12:05	15.9	15.9	15.8	15.8	15.7	15.6
03/25/04	South	11:30	19.3	19.3	19.3	19.3	19.3	19.3
04/14/04	South	9:05	21.1	21.0	21.0	20.9	20.9	20.9
05/06/04	South	11:10	25.2	24.8	24.7	24.6	24.6	24.5
06/16/04	South	12:02	30.3	29.8	29.7	29.6	29.6	29.5
07/11/04	South	10:13	30.4	30.0	29.9	29.9	29.9	29.8

Date	Station	Time	pH	pH	pH	pH	pH	pH
YYMMDD			0.5 m	1 m	1.5 m	2 m	2.5 m	3 m
04/10/03	Mid-lake	11:15	9.2	9.2	9.2	9.2		
05/14/03	Mid-lake	11:14	9.0	9.0	9.0	8.9		
06/06/03	Mid-lake	10:03	9.1	9.1	9.0	9.0		
07/09/03	Mid-lake	11:14	9.2	9.1	9.1	9.1		
08/06/03	Mid-lake	11:02	8.7	8.6	8.5	8.3		
09/09/03	Mid-lake	10:58	8.7	8.6	8.6	8.5		
10/07/03	Mid-lake	11:16	8.6	8.6	8.5	8.4		
11/11/03	Mid-lake	11:20	8.3	8.3	8.3	8.3		
12/02/03	Mid-lake	10:50	8.4	8.3	8.3	8.3		
01/28/04	Mid-lake	12:00	7.9	7.9	7.9	7.9		
02/18/04	Mid-lake	11:40	8.2	8.1	8.0	8.0		
03/25/04	Mid-lake	11:10	8.0	8.0	8.0	8.0	7.8	
04/14/04	Mid-lake	9:35	8.2	8.1	8.1	8.0		
05/06/04	Mid-lake	11:35	7.8	7.8	7.8	7.5		
06/16/04	Mid-lake	11:36	8.2	8.2	8.1	8.0		
07/11/04	Mid-lake	10:45	9.0	9.0	8.9	8.9		
02/06/03	North	10:20	8.3	8.2	8.1			
03/13/03	North	11:32	8.4	8.4	8.3			
04/10/03	North	10:40	9.1	9.1	9.1			
05/14/03	North	10:40	8.9	8.9	8.8			
06/06/03	North	10:31	9.1	9.1				
07/09/03	North	11:50	9.1	8.9	8.8			
08/06/03	North	11:28	8.4	8.2	8.1			
09/09/03	North	11:28	8.3	8.3	8.2			
10/07/03	North	12:17	8.6	8.6				
11/11/03	North	11:45	8.4	8.3				
12/02/03	North	11:30	8.3	8.3	8.2			
01/28/04	North	11:10	7.8	7.8	7.8			
02/18/04	North	10:57	8.1	7.9	7.9	7.3		
03/25/04	North	10:40	8.3	8.2	8.1	8.0		
04/14/04	North	10:00	8.4	8.3	8.2			
05/06/04	North	12:00	8.1	8.1	8.0			
06/16/04	North	10:47	8.1	8.0	7.9			
07/11/04	North	11:16	9.0	9.0	9.0	8.8		
02/06/03	South	9:17	8.4	8.3	8.2	8.2	8.2	8.1
03/13/03	South	10:50	8.5	8.5	8.4	8.4	8.4	8.3
04/10/03	South	11:32	9.2	9.1	9.1	9.1	9.1	9.1
05/14/03	South	11:44	9.0	9.0	9.0	8.9	8.9	8.8
06/06/03	South	9:32	9.1	9.0	9.0	9.0	9.0	9.0
07/09/03	South	10:22	9.2	9.2	9.2	9.1	8.9	8.8
08/06/03	South	10:26	8.5	8.5	8.4	8.4	8.4	8.3
09/09/03	South	10:25	8.7	8.6	8.6	8.6	8.6	8.6
10/07/03	South	10:40	8.5	8.5	8.6	8.6	8.5	
11/11/03	South	10:55	8.2	8.2	8.2	8.2	8.2	
12/02/03	South	10:27	8.4	8.3	8.3	8.3	8.3	
01/28/04	South	12:25	7.9	7.9	7.9	7.9	7.8	7.8
02/18/04	South	12:05	8.0	7.9	7.9	7.9	7.8	7.8
03/25/04	South	11:30	7.9	7.8	7.8	7.8	7.8	7.7
04/14/04	South	9:05	8.1	8.2	8.0	8.0	9.0	7.5
05/06/04	South	11:10	7.9	7.7	7.6	7.5	7.4	7.3
06/16/04	South	12:02	8.3	8.2	8.1	8.0	7.9	7.9
07/11/04	South	10:13	9.0	9.0	8.9	8.9	8.8	8.7

Date	Station	Time	Light Pen	Light Pen	Light Pen	Light Pen	Light Pen	Light Pen	Light Pen	Light Pen
YYMMDD			Surf-0.5	DW-0.5	Surf-1.0	DW-1.0	Surf-1.5	DW-1.5	Surf-2.0	DW-2.0
04/10/03	Mid-lake	11:15	.	.	.	.	.	.		
05/14/03	Mid-lake	11:14	1537.0	158.6	1548.0	78.8	1564.0	18.3	1560.0	5.9
06/06/03	Mid-lake	10:03	1424.0	57.8	1415.0	34.4	1415.0	0.4	14.2	.
07/09/03	Mid-lake	11:14								
08/06/03	Mid-lake	11:02	410.5	30.0	404.5	18.5	407.0	4.1	409.8	0.8
09/09/03	Mid-lake	10:58	944.5	256.5	1205.0	79.3	1148.0	9.1	1374.0	1.1
10/07/03	Mid-lake	11:16	1238.0	88.5	1232.0	61.7	1230.0	18.0	1215.0	3.5
11/11/03	Mid-lake	11:20	751.5	446.6	752.6	84.1	751.6	24.3	751.6	5.6
12/02/03	Mid-lake	10:50	1081.0	288.6	1102.0	352.9	1108.0	.	1105.0	.
01/28/04	Mid-lake	12:00	917.0	532.2	934.7	280.5	915.9	89.2	928.6	5.1
02/18/04	Mid-lake	11:40	1543.0	100.0	1545.0	117.9	1537.0	53.6	1556.0	58.1
03/25/04	Mid-lake	11:10	2110.0	746.5	2134.0	675.3	2109.0	381.4		
04/14/04	Mid-lake	9:35	1081.0	230.4	1054.0	165.3	1037.0	79.9	1028.0	31.3
05/06/04	Mid-lake	11:35	1808.0	772.6	1818.0	482.3	1816.0	284.9	1810.0	194.7
06/16/04	Mid-lake	11:36	588.5	146.2	552.2	57.0	543.7	29.9	533.4	0.3
07/11/04	Mid-lake	10:45	921.3	280.6	924.2	139.2	884.3	27.9	862.8	5.4
02/06/03	North	10:20	588.6	196.1	620.2	90.3	614.4	17.1		
03/13/03	North	11:32	1481.0	709.9	1482.0	302.3	1488.0	124.1		
04/10/03	North	10:40	.	.	.	.	.	.		
05/14/03	North	10:40	1555.0	66.1	1500.0	76.9	1508.0	23.7		
06/06/03	North	10:31	1457.0	.	1489.0	37.3				
07/09/03	North	11:50								
08/06/03	North	11:28	1729.0	478.7	1749.0	252.8	1759.0	42.9		
09/09/03	North	11:28	1933.0	279.1	1611.0	122.4	1610.0	18.2		
10/07/03	North	12:17	1290.0	163.3	1301.0	106.1				
11/11/03	North	11:45	1476.0	279.4	1501.0	198.4				
12/02/03	North	11:30	1114.0	687.2	1134.0	528.3	1155.0	438.2		
01/28/04	North	11:10	1430.0	474.8	1364.0	254.6	1215.0	91.4		
02/18/04	North	10:57	1426.0	343.0	1376.0	212.2	1365.0	127.5	1401.0	0.0
03/25/04	North	10:40	704.6	220.5	694.7	147.4	668.1	78.2		
04/14/04	North	10:00	1171.0	141.7	1148.0	100.5	1143.0	91.0		
05/06/04	North	12:00	1871.0	712.5	1855.0	624.5	1833.0	310.2	1830.0	171.5
06/16/04	North	10:47	1597.0	639.4	1581.0	233.4	1562.0	101.9		
07/11/04	North	11:16	1544.0	382.4	1551.0	299.6	1447.0	80.8		
02/06/03	South	9:17	925.1	350.3	925.4	164.0	881.2	66.1	769.3	28.7
03/13/03	South	10:50	1345.0	522.2	1312.0	260.7	1360.0	109.2	1375.0	48.5
04/10/03	South	11:32	.	.	.	.	.	.		
05/14/03	South	11:44	1718.0	504.8	1723.0	352.7	1724.0	39.8	1711.0	22.9
06/06/03	South	9:32	1300.0	43.8	1291.0	15.5	1294.0	3.3	1272.0	.
07/09/03	South	10:22								
08/06/03	South	10:26	1329.0	165.1	1331.0	28.2	1339.0	16.7	1373.0	1.6
09/09/03	South	10:25	1183.0	146.8	1214.0	108.0	1223.0	10.8	1224.0	7.9
10/07/03	South	10:40	1313.0	131.6	1326.0	204.5	1323.0	30.8	1326.0	7.0
11/11/03	South	10:55	815.3	159.7	791.9	79.9	780.5	21.1	776.7	4.8
12/02/03	South	10:27	1063.0	321.4	1055.0	349.0	1055.0	421.2	1059.0	285.3
01/28/04	South	12:25	1359.0	826.8	1392.0	335.3	1373.0	179.5	1444.0	83.7
02/18/04	South	12:05	1613.0	112.8	1605.0	322.3	1607.0	82.2	1595.0	38.4
03/25/04	South	11:30	489.2	195.3	467.2	134.0	442.4	105.5		
04/14/04	South	9:05	809.6	74.4	839.7	30.4	831.2	19.2	793.5	12.9
05/06/04	South	11:10	1727.0		1695.0	438.4	1701.0	203.5	1681.0	99.3
06/16/04	South	12:02	1951.0	472.8	1949.0	375.4	1951.0	74.5	1947.0	18.3
07/11/04	South	10:13	1819.0	417.0	1840.0	209.4	1848.0	32.3	1865.0	13.0

Appendix 2: Analytical parameters										
Date	Station	Time	COLOR	Turbidity	TSS	POC	NO2	NO3	NH4	TN
YYMMDD			PCU	NTU	mg/L		mg/L	mg/L	mgN/L	mgN/L
04/10/03	Mid-lake	11:15	32	2.66	35.0	13.06	0.0000	0.0000	0.048	2.661
05/14/03	Mid-lake	11:14	25	2.35	20.0	9.83	0.0000	0.0000	0.142	1.941
06/06/03	Mid-lake	10:03	36	7.07	8.0	9.37	0.0001	0.0036	0.113	2.406
07/09/03	Mid-lake	11:14	44	12.80	22.0	9.82	0.0008	0.0089	0.055	5.707
08/06/03	Mid-lake	11:02	38	15.30	23.0	12.49	0.0019	0.0074	0.048	2.687
09/09/03	Mid-lake	10:58	37	12.20	21.0	13.65	0.0010	0.0005	0.050	2.797
10/07/03	Mid-lake	11:16	47	10.90	18.0	10.94	0.0003	0.0002	0.062	2.271
11/11/03	Mid-lake	11:20	32	8.06	22.0	6.41	0.0011	0.0068	0.071	2.167
12/02/03	Mid-lake	10:50	34	7.72	18.0	7.82	0.0011	0.0069	0.037	2.001
01/28/04	Mid-lake	12:00	31	3.65	8.0	4.78	0.0019	0.0095	0.966	2.379
02/18/04	Mid-lake	11:40	18	5.60	11.0	3.74	0.0031	0.0190	0.825	2.300
03/25/04	Mid-lake	11:10	26	3.23	2.0	5.60	0.0101	0.0235	1.485	2.819
04/14/04	Mid-lake	9:35	22	5.27	6.0		0.0076	0.0636	0.939	3.123
05/06/04	Mid-lake	11:35	24	2.77	6.8		0.0237	0.0882	0.843	2.241
06/16/04	Mid-lake	11:36	23	5.67	11.0		0.0018	0.0002	0.017	2.678
07/11/04	Mid-lake	10:45	14	7.92	15.2		0.0026	0.0036	0.036	1.665
02/06/03	North	10:20		3.56	14.0	3.90	0.0199	0.0515	0.501	2.044
03/13/03	North	11:32	37	3.63	3.0	4.77	0.0137	0.0941	0.266	1.883
04/10/03	North	10:40	32	3.99	35.0	10.42	0.0000	0.0000	0.052	2.355
05/14/03	North	10:40	27	4.91	14.0	8.34	0.0000	0.0000	0.049	1.959
06/06/03	North	10:31	37	9.46	17.0	8.71	0.0000	0.0041	0.129	2.514
07/09/03	North	11:50	43	12.20	21.0	10.52	0.0004	0.0058	0.030	5.673
08/06/03	North	11:28	35	13.50	18.0	10.43	0.0010	0.0022	0.031	2.256
09/09/03	North	11:28	44	10.06	24.0	11.96	0.0005	0.0000	0.026	2.238
10/07/03	North	12:17	44	8.55	19.0	9.80	0.0005	0.0016	0.027	1.909
11/11/03	North	11:45	36	9.59	19.0	9.12	0.0007	0.0007	0.046	2.048
12/02/03	North	11:30	34	9.01	24.0	10.54	0.0012	0.0018	0.095	2.038
01/28/04	North	11:10	25	4.52	.	4.43	0.0040	0.0269	0.929	2.441
02/18/04	North	10:57	19	4.27	12.0	3.99	0.0037	0.0281	0.750	2.362
03/25/04	North	10:40	26	5.51	14.0	7.45	0.0073	0.0534	1.427	2.673
04/14/04	North	10:00	24	3.79	4.0		0.0019	0.0042	0.026	1.624
05/06/04	North	12:00	20	2.82	7.4		0.0025	0.0436	1.045	2.468
06/16/04	North	10:47	29	4.87	14.0		0.0049	0.0149	0.787	2.160
07/11/04	North	11:16	14	9.45	20.4		0.0022	0.0059	0.044	2.222
02/06/03	South	9:17	35	3.69	9.0	3.31	0.0152	0.0432	0.643	2.441
03/13/03	South	10:50	45	3.88	13.0	4.80	0.0145	0.0822	0.565	3.039
04/10/03	South	11:32	36	2.45	28.0	9.86	0.0000	0.0000	0.064	2.394
05/14/03	South	11:44	24	4.24	14.0	7.85	0.0000	0.0000	0.072	1.892
06/06/03	South	9:32	30	7.36	28.0	7.93	0.0020	0.0153	0.192	2.669
07/09/03	South	10:22	29	13.90	21.0		0.0006	0.0082	0.075	6.180
08/06/03	South	10:26	36	12.30	19.0	11.73	0.0011	0.0072	0.054	2.783
09/09/03	South	10:25	34	11.30	19.0	13.19	0.0010	0.0006	0.045	2.750
10/07/03	South	10:40	33	11.90	23.0	10.31	0.0007	0.0022	0.058	2.301
11/11/03	South	10:55	33	8.13	23.0	8.52	0.0012	0.0088	0.581	2.353
12/02/03	South	10:27	44	6.94	19.0	8.07	0.0018	0.0005	0.088	1.886
01/28/04	South	12:25	34	3.36	30.0	4.41	0.0031	0.0098	0.974	2.335
02/18/04	South	12:05	19	3.83	0.0	3.48	0.0032	0.0166	0.787	2.387
03/25/04	South	11:30	29	2.04	7.0	4.90	0.0090	0.0131	2.119	3.113
04/14/04	South	9:05	23	5.05	1.0		0.0054	0.0407	1.019	3.310
05/06/04	South	11:10	22	3.70	7.6		0.0439	0.1806	0.449	2.285
06/16/04	South	12:02	22	5.35	19.0		0.0017	0.0009	0.038	2.550
07/11/04	South	10:13	12	7.49	15.6		0.0026	0.0073	0.018	1.774

Date	Station	Time	SRP	TP	Si	Chl	Phe	Total Chl	Est Chl
YYMMDD			mgP/L	mgP/L	mgSi/L	µg/l	µg/l	µg/l	µg/l
04/10/03	Mid-lake	11:15	0.000	0.076	2.891	51.1	5.0	56.1	53.1
05/14/03	Mid-lake	11:14	0.013	0.055	2.927	33.8	7.2	41.0	37.4
06/06/03	Mid-lake	10:03	0.003	0.055	3.532	40.5	5.4	45.9	42.9
07/09/03	Mid-lake	11:14	0.022	0.050	3.354	52.2	6.5	58.7	55.1
08/06/03	Mid-lake	11:02	0.005	0.047	3.158	73.7	16.8	90.5	82.1
09/09/03	Mid-lake	10:58	0.014	0.048	5.740	92.7	22.9	115.6	104.3
10/07/03	Mid-lake	11:16	0.014	0.052	7.293	80.7	19.2	99.9	90.3
11/11/03	Mid-lake	11:20	0.002	0.047	7.618	56.7	18.9	75.6	66.6
12/02/03	Mid-lake	10:50	0.007	0.040	7.713	37.4	5.6	43.0	40.0
01/28/04	Mid-lake	12:00	0.005	0.041	5.708	22.1	6.1	28.2	25.2
02/18/04	Mid-lake	11:40	0.005	0.046	4.549	17.6	6.1	23.7	20.8
03/25/04	Mid-lake	11:10	0.004	0.045	3.503	8.9	4.7	13.7	11.5
04/14/04	Mid-lake	9:35	0.005	0.040	2.516	24.6	11.2	35.8	30.6
05/06/04	Mid-lake	11:35	0.001	0.040	1.721	27.7	7.5	35.2	31.5
06/16/04	Mid-lake	11:36	0.002	0.063	6.966	47.5	15.7	63.1	55.6
07/11/04	Mid-lake	10:45	0.002	0.054	6.970	39.1	8.6	47.7	43.3
02/06/03	North	10:20	0.004	0.082	3.623	24.3	3.7	28.0	26.0
03/13/03	North	11:32	0.005	0.050	2.356	25.8	0.0	25.8	25.1
04/10/03	North	10:40	0.000	0.072	2.900	33.0	3.0	36.0	34.1
05/14/03	North	10:40	0.012	0.058	2.985	33.2	4.2	37.4	35.1
06/06/03	North	10:31	0.001	0.073	3.303	42.2	7.7	49.9	45.9
07/09/03	North	11:50	0.005	0.082	4.144	40.8	6.5	47.3	43.8
08/06/03	North	11:28	0.004	0.042	3.686	55.0	10.9	66.0	60.3
09/09/03	North	11:28	0.016	0.046	5.420	72.1	18.0	90.1	81.1
10/07/03	North	12:17	0.012	0.041	7.574	51.7	10.9	62.5	57.0
11/11/03	North	11:45	0.003	0.050	6.597	39.4	11.1	50.5	45.1
12/02/03	North	11:30	0.010	0.053	7.111	33.8	10.0	43.8	39.0
01/28/04	North	11:10	0.005	0.044	5.660	22.6	4.5	27.1	24.8
02/18/04	North	10:57	0.005	0.043	3.777	14.0	5.7	19.7	17.0
03/25/04	North	10:40	0.005	0.051	2.151	16.5	6.8	23.3	20.1
04/14/04	North	10:00	0.001	0.053	6.576	32.1	10.5	42.6	37.6
05/06/04	North	12:00	0.006	0.045	1.410	20.9	7.8	28.8	25.1
06/16/04	North	10:47	0.000	0.030	2.043	23.7	4.6	28.4	26.0
07/11/04	North	11:16	0.003	0.043	6.371	32.4	10.8	43.2	38.0
02/06/03	South	9:17	0.003	0.063	6.830	12.8	4.0	16.9	14.9
03/13/03	South	10:50	0.011	0.099	4.396	23.6	1.9	25.5	24.3
04/10/03	South	11:32	0.000	0.065	3.047	53.1	1.2	54.3	52.9
05/14/03	South	11:44	0.018	0.060	3.146	35.2	7.0	42.2	38.6
06/06/03	South	9:32	0.002	0.058	3.332	35.2	5.6	40.8	37.8
07/09/03	South	10:22	0.003	0.044	4.067	48.0	7.1	55.1	51.3
08/06/03	South	10:26	0.005	0.048	3.809	78.2	17.7	95.9	87.0
09/09/03	South	10:25	0.012	0.047	5.797	97.8	20.7	118.4	107.9
10/07/03	South	10:40	0.010	0.045	7.653	78.8	18.6	97.3	88.0
11/11/03	South	10:55	0.004	0.055	7.794	55.9	17.1	73.0	64.7
12/02/03	South	10:27	0.010	0.042	7.338	35.5	7.4	42.8	39.1
01/28/04	South	12:25	0.006	0.061	6.794	17.9	5.0	22.9	20.5
02/18/04	South	12:05	0.005	0.031	4.693	15.9	6.6	22.5	19.4
03/25/04	South	11:30	0.005	0.035	3.771	8.4	1.7	10.1	9.2
04/14/04	South	9:05	0.005	0.045	3.084	20.4	8.6	29.0	24.9
05/06/04	South	11:10	0.002	0.064	1.632	39.4	13.1	52.5	46.2
06/16/04	South	12:02	0.003	0.067	6.931	55.6	20.2	75.8	66.2
07/11/04	South	10:13	0.001	0.060	8.432	51.1	13.2	64.4	57.8

Appendix 3. Species list for phytoplankton identified at the North station in Lake Griffin, Florida, February 2003 through July 2004. Some taxa have more than one number because they appear in various size categories.

DIVISION CHLOROPHYTA (greens)

100.01 Actinastrum hantzschii
101.01 Ankistrodemus convolutus
101.02 " falcatus
101.03 " nannosolene
101.07 " falcatus var.acicularis
101.10 " #101.10
105.00 Closteriopsis
103.00 Chlamydomonas
106.01 Closterium #106.01
106.03 " cf parvulum
106.08 " #106.08
106.29 " #106.29
106.31 " #106.31
107.01 Coelastrum sphaericum
107.02 " reticulatum
107.03 " cambricum
108.01 Cosmarium $\leq$ 25µ
108.02 " > 25µ
109.01 Crucigenia tetrapedia
109.02 " crucifera
109.06 " quadrata
110.01 Dictyosphaerium pulchellum
110.02 " ehrenbergianum
112.00 Elakatothrix
117.02 Kirchneriella contorta
117.05 " subsolitaria
117.07 " obesa
119.01 Mougeotia (5µ diameter)
120.01 Nephrocytium limneticum
122.00 Oocystis
123.00 Pandorina
124.01 Pediastrum duplex
124.02 " duplex var. gracilimum
124.04 " simplex var. duodenarium
124.05 " tetras
124.07 " duplex var. reticulatum
124.10 " boryanum
124.13 " boryanum var. longicorne
124.14 " #124.14
126.03 Selenastrum minutum

127.00 Scenedesmus spp.
127.03 " quadricauda (4s)
127.04 " " (2s)
127.05 " bijuga (4s)
127.06 " " (2s)
127.08 " bicaudatus (2s)
127.13 " quadricauda var. maximus
127.14 " dimorphus
127.16 " quadricauda var. westii
127.21 " perforatus
127.39 " falcatus
127.40 " longus vaar. Naegelii
127.49 " #127.49
127.53 " #127.53
128.02 Schroederia > 50μ
129.19 Staurastrum ≤ 50μ
129.20 " > 50μ ≤ 100μ
130.02 Tetraedron minimum
130.03 " muticum
130.06 " incus
130.09 " gracile
130.10 " pentaedricum
130.12 " regulare
130.14 " trigonum var. gracile
130.18 " lunula
130.34 " #130.34
131.03 Tetrastrum staurgeniaeforme
170.00 unidentified #170
172.00 Gonatozygon
213.00 unidentified flagellate
294.00 Unidentified #294
295.00 Unidentified, non-flagellated greens ≤ 5μ
297.00 Unidentified #297
299.00 Unidentified colonial greens

DIVISION EUGLENOPHYTA
300.01 Euglena acus ≤150μ
300.11 Euglena spp ≤ 30μ
300.12 Euglena spp. > 30μ
301.00 Phacus spp.
301.02 " tortus
301.03 " longicauda
301.08 " #301.08

DIVISION PYRRHOPHYTA
400.00 small, armored dinoflagellates ≤ 25μ

400.01 armored dinoflagellated >25µ
402.01 Ceratium hirundinella
402.02 " " var. brachyceros or C. brachyceros
404.00 Gymnodinium ≤ 30µ

DIVISION CRYPTOPHYTA
500.01 cryptophytes > 5µ ≤ 15µ
500.02 " > 15µ
500.03 " ≤ 5µ
500.04 " #500.04

DIVISION CHRYSOPHYTA - non-diatoms
600.00 Dinobryon spp.
600.01 " sertularia
600.02 " bavaricum
601.00 Mallomonas
609.00 Unidentified ≤ 5µ

DIVISON CHRYSOPHYTA - diatoms
Centrics
610.04 5µ
610.05 10µ
610.09 30µ
610.10 35µ
610.11 40µ
610.12 45µ
610.21 90µ
613.00 Melosira / Aulacoseira spp.
613.02 " granulata var. angustissima
616.00 Rhizosolenia

Pennates
630.03 5m diameter, length > 150µ ≤ 200µ
630.05 length > 10µ ≤ 25µ
630.07 length > 75µ ≤ 150µ
630.08 length > 25µ ≤ 50µ
630.09 length > 50µ ≤ 75µ
630.12 5µ diameter, length >200µ ≤ 300µ
630.24 20µ diameter, length > 150µ ≤ 200µ
632.36 Nitszchia #632.36
635.02 Fragilaria crotonensis
637.00 unidentified pennate chains, each cell ≤ 50µ in length
639.00 Pinnularia
646.00 Achnanthes
651.09 Navicula >50µ ≤ 100µ

651.11 " > 25μ ≤ 50μ
660.00 pennate #660
664.00 cf Nitzschia closterium
690.02 Asterionella formosa

CYANOBACTERIA
700.00 Anabaena spp.
701.02 Anabaenopsis phillipinensis
704.00 Aphanothece
706.05 Chroococcus prescotti
706.10 " 2μ
706.12 " 5μ, heavy sheath
706.13 " 3-4μ
706.14 □ 5μ
707.02 Coelosphaerium naeglianum
708.00 Lyngbya 2μ diameter
708.01 " contorta
708.02 " limnetica
710.01 Merismopedia tenuissima
710.02 " 2μ
710.06 □ 3μ
711.01 Microcystis incerta
711.03 " aeruginosa
714.00 Oscillatoria 2μ diameter, length > 25m
714.01 Oscillatoria 2μ diameter, length ≤ 25m
714.02 " #714.02
714.06 " 5μ diameter
715.00 Raphidiopsis
718.00 single-celled, 5μ
718.01 doubles, each 5μ
718.02 single-celled, 2μ
718.03 doubles, each 2μ
722.00 Cylindrospermopsis
726.02 Dactylococcopsis Smithii
738.00 cf Dactylococcopsis
756.00 single-celled and rod shaped
759.02 Oscillatoria 1μ diameter

Misc.
908.00 a budding bacteria - Planktomyces

Appendix 4. List of zooplankton taxa, along with code numbers.

ROTIFERS

Anuraeopsis (201)
Asplanchna (202)
Brachionus angularis (203.02)
" caudatus (203.03)
" havanensis (203.01)
Colletheca (232)
Conochiloides (204)
Conochilus (205)
Filinia (206)
Hexarthra (207)
Keratella cochlearis (208.01,.03,.05,.08
Lepadella (217)
Monostyla (220)
Notommata (210)
Polyarthra (211)
Pomphloyx sulcata (253.01)
Trichocerca multicrinis (213.06)
" similis (213.02)
" spp. (213.01,.03, .05)
Unclassified Rotifera (214, 230, 242, 246)

CLADOCERANS

Alona sertulosa (312.03)
Alona sp. (312.00)
Bosmina (301)
Ceriodapnis (305)
Chydorus (313)
Diaphanosoma (302)
Daphnia lumholtzii (303.01)
" spp. (303.00)
Eubosmina (314)

COPEPODS

nauplii (401)
calanoids (404)
cyclopoids (403)

OSTRACODS (501)

Appendix 5. Analytical methods and limits of detection.

i) Parameter: Ammonium

1) Method Reference: Strickland & Parsons, (1972) A Practical Handbook of Seawater Analysis: Determination of Ammonia (Oxidation Method). Fisheries Research Board of Canada.
2) Method Description: Photometric determination of ammonia in seawater based on the oxidation reaction with hypochlorite in an alkaline medium. Results are read on a Bran-Luebbe autoanalyzer without the cadmium column.
3) Preservation Method: Samples are filtered through PALL A/E glass-fiber filters (1µm pore size) the field, stored at -20^{o}C and run within 48 hours.

ii) Parameter: Nitrite and Nitrate

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM4500-NO3-F. United Book Press, Inc. Baltimore, Maryland.
2) Method Description: A water sample is passed though a cadmium column where the nitrate is reduced to nitrite, which is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine to form a colored azo dye that is measured colorometrically on a Bran-Luebbe autoanalyzer. The procedure is the same for nitrite analysis less the cadmium column.
3) Preservation Method: Samples are filtered through PALL A/E glass-fiber filters (1µm pore size) in the field, stored at -20^{o}C and run within 48 hours.

iii) Parameter: Total Nitrogen

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM4500-Norg-D and SM4500-NO3-F. United Book Press, Inc., Baltimore, Maryland.
2) Method Description: Potassium persulfate in DI H_2O is added to sample which is then autoclaved for 30 minutes at 15 psi and cooled to room temperature. The digested sample is passed though a cadmium column where the nitrate is reduced to nitrite which is then diazotized with sulfanilamide and coupled with N-(1-naphthyl)-ethylenediamine to form a colored azo dye that is measured colorometrically on a Bran-Luebbe autoanalyzer.
3) Preservation Method: Samples are stored at -20^{o}C and run within 2 weeks.

iv) Parameter: Orthophosphate

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 4500-P-E (Ascorbic acid method). United Book Press, Inc., Baltimore, Maryland.
2) Method Description: Ammonium molybdate and potassium antimony in acid medium react with orthophosphate to form an acid that is reduced to a bright blue by ascorbic acid. Concentrations are measured on a dual-beam scanning spectrophotometer at 882 nm. The curve is read within 30 minutes.
3) Preservation Method: Samples are filtered through PALL A/E glass-fiber filters (1µm pore size) in the field and stored at 20^{o}C. Samples are run in less than 48 hours.

v) Parameter: Total Phosphorus

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 4500-

P-E (Ascorbic acid method with persulfate digestion). United Book Press, Inc., Baltimore, Maryland.

2) Method Description: Potassium persulfate in DI H_2O is added to sample which is then autoclaved for 30 minutes at 15 psi and cooled to room temperature. Ammonium molybdate and potassium antimony in acid medium are added to sample which reacts with orthophosphate to form an acid that is reduced to a bright blue by ascorbic acid. Concentrations are measured on a dual-beam scanning spectrophotometer at 882 nm. The curve is read within 30 minutes.
3) Preservation Method: Samples are stored at –20°C and run within two weeks.

vi) Parameter: Silica

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 4500-SiO_2-D. United Book Press, Inc., Baltimore, Maryland.
2) Method Description: HCl followed by ammonium molybdate are added to the sample which is then shaken. To correct for turbidity and tannin, a background sample of the water is processed similarly, but in the absence of ammonium molybdate. After 10 minutes, absorbance is measured on a dual-beam scanning spectrophotometer at 820 nm. The results are then subtracted to determine the net amount of silica.
3) Preservation Method: Samples are stored at –20°C and run within two weeks.

vii) Parameter: Chlorophyll a and Pheophytin

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 10200 H.2. United Book Press, Inc., Baltimore, Maryland. Extraction method for chlorophyll from Sartory, D. P. & Grobbelaar, J. U. 1984. *Hydrobiologia* **114,** 177-187.
2) Method Description: Samples are thawed, placed in test tubes with 95% ethanol and heated in a water bath at 78°C for 5 minutes. They are subsequently placed in the dark for 24 hours followed by centrifugation to remove particulate material. Absorbances are read on a dual-beam scanning spectrophotometer according to Standard Methods. After the initial reading, 0.2N HCl is added to the sample and re-run for pheophytin a determination. Functional chlorophyll a (FCHL_N) was determined by correcting chlorophyll for pheophytin content using the method described in Standard Methods. Chlorophyll a (CHLA_N) represents the chlorophyll a concentration, without correction for pheophytin, using a simplified equation based on the extinction coefficient for chlorophyll a in ethanol solvent.
3) Preservation Method: Samples are filtered onto PALL A/E glass-fiber filters (1µm pore size) in the field, stored in plastic bags in the dark at –20°C, and run within two weeks.

viii) Parameter: Color

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 2120B H.2. United Book Press, Inc. Baltimore, Maryland.
2) Method Description: The absorbance of the filtrate is determined at 465 nm using a dual-beam scanning spectrophotometer. Color values are measured against a platinum-cobalt standard.
3) Preservation Method: Samples are filtered through PALL A/E glass-fiber filters (1µm pore size) in the field, stored at 4°C and run within 48 hours.

ix) Parameter: Turbidity

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 2130A. United Book Press, Inc., Baltimore, Maryland.
2) Method Description: NTU values of whole water samples are measured directly in a LaMotte Model 2020 Turbidimeter.
3) Preservation Method: Whole water samples are stored at 4°C and run within 48 hours.

x) Parameter: Total Suspended Solids

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 2540D. United Book Press, Inc. Baltimore, Maryland.
2) Method Description: Aliqouts of whole water are filtered onto pre-washed and pre-weighed Whatman 934-AH glass-fiber filters (1.5 µm pore size). The filters are subsequently dried at 104°C, then placed in a desiccator until cool and re-weighed.
3) Preservation Method: Filtration is performed in the field. Filters are kept at –20°C for up to two weeks before drying.

xi) Parameter: Particulate Organic Carbon

1) Method Reference: APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Method SM 5310B. United Book Press, Inc., Baltimore, Maryland.
2) Method Description: Filters are dried at 80°C. Organic carbon concentration is determined using a coulometer against a dextrose standard.
3) Preservation Method: Aliqouts of whole water are filtered onto pre-burned (to eliminate any residual organic carbon) Whatman 934-AH glass-fiber filters (1.5 µm pore size) in the field and kept at –20°C before drying. Samples are run within one month.

Limits of Detection

Analytical Parameter	Method Detection Limits*	Dates in Use
Ammonium	0.008 mg/L as N	2003
Chlorophyll	0.01 µg/L	2003
Pheophytin a	0.01 µg/L	2003
Water Color	1 PCU	2003
Nitrite + Nitrate	0.0014 mg/L as N	2003
Nitrite	0.0014 mg/L as N	2003
Total Nitrogen	0.0014 mg/L as N	2003
Orthophosphate	0.002 mg/L as P	2003
Total Phosphorus	0.002 mg/L as P	2003
Silica	0.047 mg/L as SI	2003
Total Suspended Solids	0.1 mg/L	2003
Particulate Organic Carbon	0.01 mg/L	2003

*Method Detection Limits (MDL) are derived from the replicate samples method in APHA (American Public Health Association). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. United Book Press, Inc. Baltimore, Maryland. MDL will change with the background levels of samples; therefore, there is no constant MDL.