ECOLOGICAL CONDITION OF ALGAE AND NUTRIENTS IN FLORIDA SPRINGS: THE SYNTHESIS REPORT

Submitted to the Florida Department of Environmental Protection

October 31, 2007

Authors:

R. Jan Stevenson
Agnieszka Pinowska
Andrea Albertin
James O. Sickman

DEP Grant Number S0291
Ecological Condition of Algae and Nutrients in Florida Springs

Submitted to:

Russel Frydenborg
Environmental Assessment Section
Florida Department of Environmental Protection
2600 Blair Stone Rd. MS 6511
Tallahassee, FL 32399
Phone 850-245-8063
Fax 850-245-8082

Submitted by:

R. Jan Stevenson
Department of Zoology
203 Natural Science Building
Michigan State University
East Lansing, MI 48824
Phone 517-432-8083
Fax 517-432-2789

ACKNOWLEDGEMENTS

This research was supported by the Florida Department of Environmental Protection. Russel Frydenborg oversaw the project for FDEP and provided invaluable information and logistical support for the project.

Denise Miller supervised the budget process for FDEP and facilitated completion of the project. Randy Sheets and Sharon Nichols managed the budget process at Michigan State University (MSU). Kelly Lewis and Cheryl Stephens managed the budget process at the University of Florida.

Many members of the Algal Ecology Laboratory in the Department of Zoology at MSU assisted with the project: Lei Zheng, Julianne Heinlein, Vanessa Lougheed, Scott L. Rollins, Lara Panayotoff, Kalina Manoylov, Colleen McLean, Daniel Wieserich, Elizabeth Mullen, Kevin Geyer, Amy Messick, Nicole Hedquist, Jan Seidler, and Scott Fullbright.

Many students and staff at the University of Florida also assisted with the project: Mi Youn Ahn, Delores Lucero, Francisco (Chico) Alvarado, Eric Ostmark, Martin Sandquist, Alyson Dagang, Solomon Haile, Yu Wang, Gavin Wilson, Yubao Cao and Xiaowei Gu. Marty Anderson, a co-author on other reports, provided field and GIS support.

Silver River State Park in Florida graciously provided housing during the experimental streams study. We especially want to thank Robert LaMont, Steven Logan and a crew at Silver River State Park for their help in setting up experiments and changing flat tires in the middle of Ocala State Forest. Silver River State Park, Rainbow River State Park and Wakulla Springs State Park provided boat support. We also thank Tom Frick and other staff with FDEP in Tallahassee and with the State Parks for their assistance in this study.

This report, as the other reports submitted for this project, was extensively peer-reviewed. We thank those reviewers for their valuable comments.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

1. Surveys of Florida springs indicated that almost all springs had macroscopic algae growing in them, an average of 50% of the spring bottoms were covered by macroalgae, and thickness of macroalgal mats was commonly 0.5 m and as thick as 2 m in one spring boil.
2. *Lyngbya wollei* and *Vaucheria* spp. were the two most common taxa of macroalgae that occurred in extensive growths in the studied springs, however 23 different macroalgal taxa were observed in the spring survey.
3. Abundance of *Vaucheria* spp. in surveys of springs was positively related to nitrogen and phosphorus concentrations with a clear threshold increase at 0.59 mg total nitrogen/L.
4. Abundance of *Lyngbya wollei* in surveys of springs was not related well to either nitrogen or phosphorus concentrations in spring water, however it was related to sediment phosphorus concentrations and indices of human activities within 1000 m of sampling sites.
5. Interstitial waters of macroalgal mats had higher nutrient concentrations than overlying waters, indicating entrained particulate matter, long term growth and decomposition, or groundwater may supply nutrients to supplement water column supplies.
6. Seasonal variation in macroalgae was largely related to flooding and resulting turbidity and sedimentation that reduce macroalgal biomass.
7. Macroalgal removal experiments showed that *Vaucheria* spp. recovered rapidly after removal, whereas *Lyngbya wollei* recovered slowly and incompletely after removal.
8. Laboratory experiments showed that conductivity, iron, and light as well as nutrients affected growth of both *Vaucheria* spp. and *Lyngbya wollei*.
9. Nutrient enrichment experiments showed that:
   9.1. the range in nitrate and phosphate concentrations in Florida springs could regulate growth rates of both *Vaucheria* spp. and *Lyngbya wollei*.
   9.2. algae grew in almost all nutrient treatments, even at very low nutrient concentrations, and showed a logistic response to nutrients, versus a simple asymptotic response;
   9.3. when phosphate was manipulated and nitrate was maintained in luxury supply, growth rates of *Lyngbya wollei* did not respond more than 10% below 0.011 mg PO_4-P/L, increased substantially from 0.011-0.028 mg PO_4-P/L, and did not respond more than 10% to further phosphate concentration increases above 0.028 mg PO_4-P/L (in microcentrifuge microcosms that provided the most accurate control of nutrient concentrations);
   9.4 when nitrate concentrations were manipulated and phosphate was maintained in luxury supply, growth rates of *Lyngbya wollei* did not respond more than 10% below 0.034 mg NO_3-N/L, increased substantially from 0.034-0.230 mg NO_3-N/L, and did not respond more than 10% to further nitrate concentration increases above 0.230 mg NO_3-N/L (in microcentrifuge microcosms that provided the most accurate control of nutrient concentrations);
   9.5 growth of *Lyngbya wollei* increased with nitrogen concentrations, even if phosphorus concentrations were very low;
   9.6 when nitrate concentrations were manipulated and phosphate was maintained in very low supply, growth rates of *Lyngbya wollei* did not respond more than 10% below 0.015 mg NO_3-N/L, increased substantially from 0.015-0.110 mg NO_3-N/L, and did not respond more than 10% to further nitrate concentration increases above 0.100 mg NO_3-N/L (in

microcentrifuge microcosms that provided the most accurate control of nutrient concentrations);

9.7 when phosphate was manipulated and nitrate was maintained in luxury supply, growth rates of *Vaucheria* did not respond more than 10% below 0.006 mg PO_4-P/L, increased substantially from 0.006-0.022 mg PO_4-P/L, and did not respond more than 10% to further phosphate concentration increases above 0.022 mg PO_4-P/L (in microcentrifuge microcosms that provided the most accurate control of nutrient concentrations);

9.8 when nitrate concentrations were manipulated and phosphate was maintained in luxury supply, growth rates of *Vaucheria* did not respond more than 10% below 0.069 mg NO_3-N/L, increased substantially from 0.069-0.644 mg NO_3-N/L, and did not respond more than 10% to further nitrate concentration increases above 0.644 mg NO_3-N/L, but this is probably an overestimate of regulating nitrate concentrations because it was determined in raceway microcosms in which substantial nutrient depletion occurred and could not be accounted for; and

9.9 fastest algal growth rates in high nutrient concentrations were usually 2 or more times greater than in low nutrient concentrations, which caused great differences in algal biomass accumulation of a 30 day colonization period.

10. Simple mathematical models show that reductions in algal growth rates from maximum rates to 33 or 66 % lower rates with nutrient load reductions should have great benefits for controlling macroalgal accumulation.
11. Reductions in TN and TP concentrations to less than 0.591 or 0.026 mg/L, respectively, should reduce the extent of cover of spring bottoms by *Vaucheria* spp. in Florida springs; however greater reductions in TN and TP will likely be necessary to substantially reduce *Vaucheria* cover.
12. Reductions in TN and TP concentrations to less than 0.250 or 0.033 mg/L respectively, should reduce the extent of cover of spring bottoms by *Lyngbya wollei* in Florida springs; however greater reductions in TN and TP will likely be necessary to substantially reduce *Lyngbya wollei* cover.
13. In many springs, nitrogen reductions may be the only practical restoration strategy because natural phosphorus concentrations may be higher than the concentrations that constrain algal growth.

1 - INTRODUCTION

Proliferations of nuisance and hazardous algae have become a common problem in freshwater and marine habitats throughout the world (Carpenter et al. 1998, Lembi 2003). Blooms of phytoplankton reduce water clarity and aesthetic quality of lakes, rivers, and coastal waters. Great accumulations of macroscopic algae on the bottom of these waters by both native and non-native species smother habitats once occupied by other organisms in streams, lakes, coastal embayments, and coral reefs. Biofilms of algae fowl the bottoms of ships and many underwater surfaces. Excessive accumulations of phytoplankton and benthic macroalgae can reduce oxygen concentrations and produce toxins that reduce both human and ecological health. These nutrient problems are so extensive that the USEPA has established a program with guidance manuals for nutrient regulations for all waterbodies throughout the United States (USEPA 2000).

In many cases these proliferations of phytoplankton and benthic macroalgae have been related to nutrients added to waters by human activities. Fertilizers, human and domesticated animal waste, and simply clearing trees and grasses from nearby lands can increase nutrient loading into surface waters, either directly by runoff into surface water or indirectly via groundwater (Carpenter et al. 1998). Nutrients (both N and P) flowing into the groundwater through soils can be transported to surface waters. Natural sources of nutrients, such as rain, soils, and bedrock also contribute to the nutrient supply of streams and lakes and these contributions vary among regions depending on climate and geology. However, natural sources of nutrients are usually much less than human sources, to the extent that excessive algal growths are rare when human sources of nutrients do not exist (Stevenson et al. 2006). This report describes a study of the causes of excessive accumulations of benthic macroalgae in the springs of Florida and their likely relations to human activities.

Florida springs are highly valued resources for their aesthetic and recreational value as well as their support of biodiversity (Bonn and Bell 2003). Geologists estimate as many as 600 springs occur in the state of Florida, which may be the highest density of springs in the world (Scott et al. 2004). Millions of people visit these springs each year for swimming and boating because of their clear waters. Marjory Stoneman Douglas, a revered author and environmentalist in Florida, observed that “Springs are bowls of liquid light.” Thousands use water from the springs for drinking. The network of waterways through caves and surface channels also support a unique biota, including the manatee.

Environmental problems in the Florida springs raised sufficient concern that David Struhs, then Secretary of the Florida Department of Environmental Protection, called for the organization of a multi-agency group, the Florida Springs Task Force. The Florida Springs Task Force (2000) identified known threats to the springs and a series of strategies for protecting and restoring the springs. The most common and important threats were increased nitrate concentrations, flow reductions, nuisance plant and macroalgal accumulations, and microbial contamination from human and animal wastes. Strategies for protecting and restoring Florida springs were grouped into five categories: outreach, information, management, regulation, and funding. The increased funding resulting from Florida Springs Task Force (2000) report, through the Florida Department of Environmental Protection, supported the nuisance algae-nutrient research in our report. Our report will provide information that will help resource managers develop nutrient reduction

strategies and regulations that will reduce risk of problems from excessive accumulations of benthic macroalgae in springs.

Benthic macroalgae cause many problems related to ecological and human health, as well as quality of life. *Caulerpa* in coastal embayments and on coral reefs and *Cladophora* in streams and lakes smother habitat occupied by other organisms and can reduce oxygen levels in the water (Dudley et al. 1986, Meinesz 1999). When these algae detach from substrates, they can wash ashore, foul beaches and shorelines, and thereby reduce recreational quality of these resources. In addition, recent reports suggest macroalgae provide habitat and nutrition to bacteria that may include pathogens (Ksoll et al. 2007). Either through contact while swimming or on shorelines, human health risks are an issue. Macroalgae proliferations may increase the frequency, intensity, and duration of beach closing due to microbial contamination. Macroalgae fowl boat props. The macroalga *Lyngbya*, a cyanbacterium, produces toxins that may affect biota in springs and cause skin irritations to humans (Carmichael et al. 1997, Onodera et al. 1997, Yin et al. 1997, Camacho and Thacker 2006).

Benthic macroalgae are a diverse group of algae that are large enough that individual filaments, colonies, or thalluses of algae are visible without a microscope. Problems in freshwaters are usually caused by cyanobacteria and green algae. Most nuisance macroalgae in flowing waters are filamentous, that is cells attach end to end in long strings that branch in some species and not in others. At one end of the filament they attach directly to a bottom substratum or become entangled with it. As they grow, they cover greater portions of the bottom by attachment of new cells or filaments to new substrate locations and by growth and extension of pre-existing filaments. These two processes that enable increased accumulation of algae are called colonization (immigration) and growth. Physical and biological disturbance of algae, herbivory, and death due to aging or even disease cause losses of benthic algae. The specific interactions between the algae and their environment, regulated through these accumulation and loss processes, vary greatly among species of algae.

Nitrogen (N) and phosphorus (P) are the two most common nutrients associated with pollution that regulate the growth of algae in freshwater and marine habitats. In theory, only one of these nutrients regulates growth of an alga at a time. Overall, marine habitats are most commonly limited by nitrogen and freshwater ecosystems are usually limited by phosphorus. However, reviews of nutrient-enrichment experiments in streams showed that algae in about 30-35% of streams studied were nutrient limited and of those, almost half were N-limited and about half were P limited, whereas a few were limited by both N and P (Dodds and Welch 2000, Francoeur 2001). Different supply rates of N and P by both natural and human sources were the cause of these differences among streams. A reason that growth of algae in a stream can be co-limited by N and P, and still be consistent with the single nutrient limitation hypothesis (Leibig's Law of the Minimum), is that many species of algae occur in a stream and some are limited by N and others by P.

The overarching goal of our research was to determine the relationship between macroalgal proliferations and nutrients in Florida springs in a way that managers could assess the benefits of different nutrient regulations. Historically, nitrate concentrations have increased greatly in springs at the same time that population density in sourcesheds has increased. During the same

period, macroalgae have increased in the springs (Figure 1.1). We developed a research plan that utilized both survey and experimental methods following the model of research in the Everglades (McCormick and Odell 1996, Stevenson et al. 2002) and many recommendations from USEPA nutrient criteria guidelines (USEPA 2000). The surveys of macroalgae and nutrients in springs were designed to show the threshold in nitrogen or phosphorus concentrations associated with proliferations of macroalgae. The experiments were intended to confirm the cause-effect relationships between nitrate and phosphate and macroalgal growth.

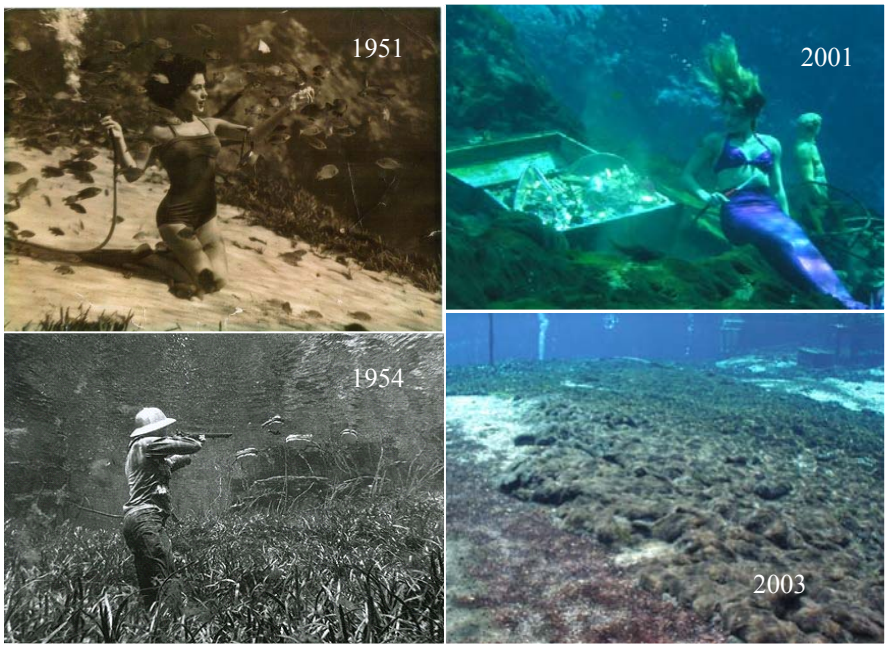


Figure 1.1. Pictures of mermaids and underwater hunters in the Weeki Wachee show in the past when no benthic macroalgae are visible (1951 & 1954) and during the last 6 years when macroalgae (*Lyngbya wollei*) are abundant (2001 & 2003). Photos from 1951, 1954, and 2001 were provided by John Athanason, Weeki Wachee. Photo from 2003 by Pinowska.

Preliminary results from the surveys of macroalgal cover and water-column nutrient concentrations did not show clear patterns between nitrogen or phosphorus concentrations and macroalgal growth, even though they were thorough and the most extensive spring assessments of their kind (Stevenson et al. 2004). Further research was planned to better understand relationships between both nitrogen and phosphorus and macroalgal growth in Florida springs. We evaluated seasonal variation, internal fluxes of nutrients in macroalgal mats, and sediment sources of nutrients to explain why macroalgal proliferations were not related well to nutrient concentrations in the overlying water of the spring runs. In addition, we conducted extensive experiments in multiple experimental settings to determine relationships between macroalgal growth and nitrogen and phosphorus concentrations. This report summarizes the findings from the many aspects of this research program that have been conducted during the last four years.

Our results show great abundances of macroalgae in some spring runs. They also show sufficient reduction of either phosphorus or nitrogen could reduce risk of nuisance levels of macroalgae in Florida springs. Natural concentrations of nitrate in springs, recorded to have been as low as 0.05 mg/L (Scott et al. 2003), were below thresholds that stimulate growth of the two most common macroalgae in Florida springs, *Vaucheria* spp. and *Lyngbya wollei*. Nitrate has now accumulated to the point that phosphorus is the most limiting nutrient for macroalgal

growth, but current levels of phosphorus in many springs where we have macroalgal proliferations are not much higher than natural concentrations. Several reasons explain how thick mats of macroalgae can accumulate in spring runs with low nutrient concentrations in the water column. These macroalgae may entrain nutrients from the water column and sediment sources that sustain slow growth. Without disturbance by physical or biological processes, including humans, macroalgae can slowly accumulate over extensive proportions of spring runs and to great thickness. We also found one macroalgae, *Lyngbya wollei*, can grow faster if given more nitrate even if phosphorus is in relatively short supply. Two of these findings contradict prevailing wisdom about relationships between algae and nutrients.

This report is organized by the different studies that we used to understand the macroalgal accumulations in Florida springs which are related to specific hypotheses tested, but also somewhat chronological in association with the successive sets of new observations and alternative hypotheses that needed to be tested. First, we describe the results of the spring surveys and then seasonal patterns in macroalgae in two springs and nutrient fluxes in macroalgal mats; finally we describe the experiments that we conducted on macroalgal recovery after disturbance and the many nutrient experiments manipulating both nitrate and phosphate concentrations. At the end, we summarize the multiple lines of evidence for specific effects of specific nutrient concentrations on macroalgae in Florida springs to provide information for the development of nutrient criteria.

Detailed descriptions of most aspects of this synthesis report can be found in one of seven reports submitted to FDEP that provide more information about our subprojects. Stevenson et al. (2004) described the preliminary results of the Florida springs survey, which has been updated and expanded in Pinowska et al. (2007a). Stevenson and Pinowska (2007) described the relationship of diatoms to nutrients in Florida springs and use of them as indicators of nutrient conditions. Albertin et al. (2007a) described the stable isotope patterns in water and algae among springs to track nutrient sources and better understand algal metabolic processes. Sickman et al. (2007) described the seasonal variation in macroalgae and nutrient conditions within macroalgal mats. Pinowska et al. (2007b) and Albertin et al. (2007b) describe the extensive experiments evaluating effects of nutrients on *Lyngbya wollei* and *Vaucheria*.

2 - SURVEYS OF MACROALGAE AND NUTRIENTS IN FLORIDA SPRINGS

2.1 – Introduction

Survey methods enable observing relationships between the nutrient concentrations in a habitat, the response of the ecosystem to observed concentrations, and linking these observations to human activities in sourcesheds. In the surveys of macroalgae and nutrients that we conducted, we hypothesized thresholds in macroalgal accumulation at specific nutrient concentrations that released macroalgal accumulation from constraints by nutrient concentrations. These responses often appear as thresholds, because once accumulation is unconstrained by resources or herbivory, macroalgae can accumulate either slowly or rapidly to a peak biomass that is constrained by other processes (Stevenson 1997).

Peak biomass, i.e. the maximum biomass that can accumulate in a habitat, can be regulated by current and nutrient availability within the mat (Stevenson 1996). Previous work indicates nutrient supply within benthic algal mats is positively related to to current velocity, because nutrient uptake rates by algae can exceed the rate at which nutrient-depleted waters within mats are replaced (Stevenson and Glover 1993). As benthic algae accumulate more thickly on a substratum, current velocity, nutrient supply, waste removal, and light for algae at the bottom of the mat decrease and cause these algae to senesce (Peterson 1996). As algae at the mat bottom senesce and decompose, the bond between algae and substratum weakens and increases the risk of current washing loosened algal mats downstream. Current positively affects nutrient supply, but it also creates drag on mats that can remove them from substrata and wash them downstream. Thus, peak biomass of algae is typically observed at high nutrient concentrations and intermediate current velocities (Stevenson 1996).

The benefit of using results of surveys for nutrient criteria development is the direct transferability to implementation of criteria based on the nutrient concentrations observed in water bodies, such as springs, that support intended uses. Whether a water body meets or does not meet nutrient criteria is evaluated, at least in part, on nutrient concentrations measured in the water body. Total phosphorus and total nitrogen are recommended by USEPA (2000) for developing and assessing compliance with nutrient criteria. The USEPA also suggests a weight of evidence approach in which response variables are also used to determine whether goals of nutrient criteria have been met. The results of experiments provide valuable information about the relationships between algal growth and other measurable processes and the nutrient concentrations that are manipulated, but experimental systems usually do not include all the complexities of ecological processes affecting nutrient concentrations in natural water bodies. These considerations have been taken into account in the conclusions where we have integrated the results of our work in experimental settings and the spring surveys.

In this section of the report we describe the results of surveys of macroalgal biomass plus several indicators of nutrient concentrations at as many as 60 spring sites in 29 first magnitude springs (i.e. springs with greater than 100 cfs, Scott et al. 2003). Surveys were conducted to identify the kinds of macroalgae in Florida springs, the percentage of the spring bottoms covered by macroalgae, the thickness of the macroalgal mats, and nutrient concentrations and sources of nutrients in the mats. Land use in sourcesheds and riparian zones was related to nutrient concentrations to distinguish anthropogenic from natural sources of nutrients in springs. A variety of statistical methods were used to relate percent cover and thickness of benthic macroalgae to nutrient concentrations in springs. Details of the methods and results can be found in reports by Pinowska et al. (2007a), Albertin et al. (2007a) and Sickman et al. (2007).

2.2 – Overview of Methods

2.2.1 Field Sampling and Laboratory Analyses

Seventy-one sites in 29 first and second magnitude springs (Appendix 1) were sampled throughout central and northern Florida during 3 seasons, spring and fall of 2003 and winter of 2006 (Figure 2.1). Only 48 sites were sampled during spring 2003 because of flooding in the Suwannee basin. During fall 2003 60 sites within 28 springs were sampled. Sixty-three sites

were sampled during winter 2006 with addition of new sites in springs for within spring run gradient studies and exclusion of eight sites that were previously sampled.

At each site, researchers designated nine transects perpendicular to flow across the spring run and spaced 10 m apart to define the sampling reach (stream section) for the site (Figure 2.2). At most sites the first transect crossed the most upstream, boil area and the following transects were located downstream from the most upstream boil. Nine observation points were spaced along each transect, resulting in a total of 81 potential observation points at each site. Channel dimensions, bank condition, canopy cover, and riparian buffer vegetation were characterized at transects. Algal attributes, current velocity, substrate type, were characterized at each of the 81 observation points.

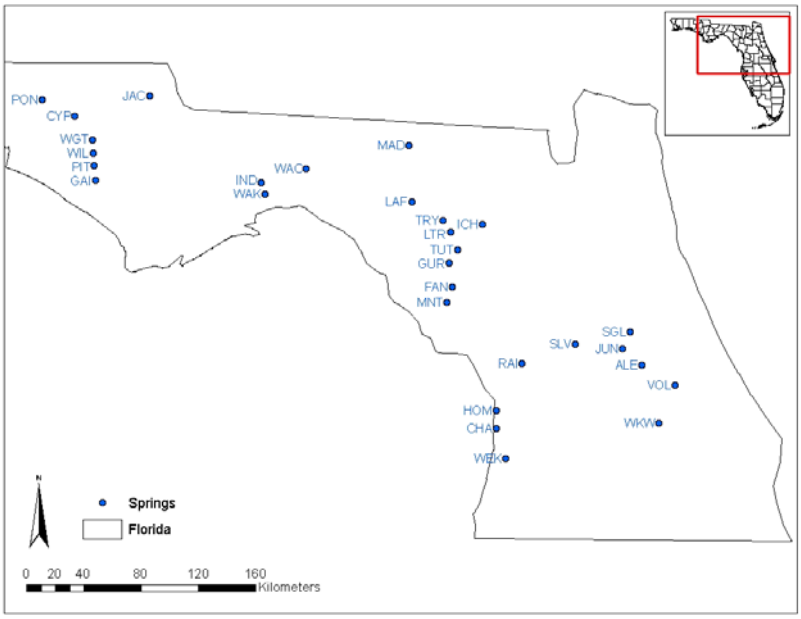


Figure 2.1. Location of 23 springs in Northern Florida that were sampled during the survey.

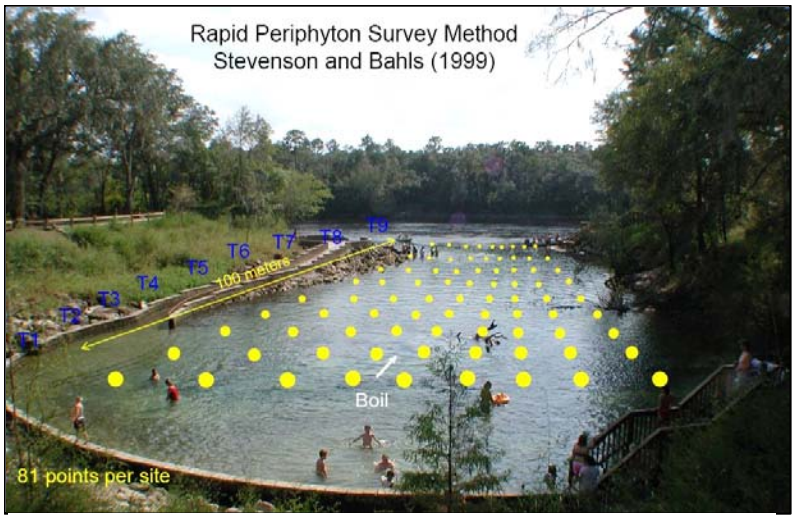


Figure 2.2. Rapid periphyton survey pattern. Macroalgae, substrate, and water conditions were characterized at nine points along each of nine transects in a section of spring run.

At each of the study sites a modified Rapid Periphyton Assessment (Stevenson and Bahls 1999) was conducted during spring and fall 2003. The presence and taxonomic identity of macrophytes and macroalgae and the thickness of the macroalgal mats were recorded at each of the 81 observation points. The percent cover of plants, macroalgae, and different substratum types was calculated as the percent of sampled points where particular plant, macroalgae, or substratum was observed. See Stevenson et al. (2004) for details of these methods. The protocol for sampling was modified during winter 2006 because of financial and resource constraints such that macroalgal assessments were only conducted at sites were

substantial differences were evident since 2003 sampling or if sites had not been assessed before (see Pinowska et al. 2007a).

Landuse was provided by FDEP for 100 m and 1000 m regions circumscribed around the sampling sites. Percent of watersheds disturbed by human activities and Landscape Development Intensity Index (LDI) scores were calculated for each site for the 100 m and 1000 m zones. Percent watershed disturbed included all urban, agricultural, and industrial land uses. Landuse Development Intensity Index (LDI) for sourcesheds of each spring was calculated using weights for different land uses reflecting the potential of each for effects (Brown and Vivas 2005). Spring discharge was based on USGS data (Scott et al. 2003). The distance from boil was measured by using the tracking tool along the river run in Microsoft Streets & Trips 2000.

A macroalgal sample was collected from each transect to confirm the accuracy of field identification (198 samples in the spring and 463 samples in the fall of 2003). At each site, a composite macroalgal sample was also collected from which a subsample was taken for algal mat total nitrogen and total phosphorus analyses (analyzed by FDEP). Subsamples of macroalgal mats were also taken to measure chlorophyll a, ash-free dry mass, fresh mass, and macroalgal volume and estimate areal algal biomass with these parameters based on measurements of % cover and thickness of macroalgae.

Microalgal samples were collected to characterize biological condition and infer nutrient conditions with diatom indicators. Microalgal samples were collected from plants (epiphytes) and sediments using quantitative methods to enable determination of algal biomass as well as species composition. Chlorophyll a was extracted with 90% ethanol and analyzed on a Turner Designs TD-700 fluorometer (APHA 1998). AFDM was determined after drying and ashing using standard methods (APHA 1998). Non-diatom species composition and diatom species composition were determined using a Palmer-Maloney counting chamber and cleaned-diatoms mounted in NAPHRAX, respectively. Subsamples of macroalgae were digested to determine N and P content of algal cells. Again, see Stevenson et al. (2004) and Pinowska et al. (2007a) for details.

Water temperature, pH, conductivity and dissolved oxygen (DO) were measured at each site at upstream and downstream ends of the reach. Water clarity was measured using a LICOR LI-250. Water samples were collected for analysis of alkalinity, ammonia nitrogen, calcium, chloride, iron, magnesium, Kjeldahl nitrogen, nitrates, soluble reactive phosphorus (indicating phosphate), total phosphorus, potassium, silica, sodium, strontium and sulfate by FDEP. When the concentration of a compound was below the detection limit of the laboratory method, the method detection limit was reported. Algal growth potential and limiting nutrient bioassays were conducted by FDEP for 15 sites in the spring and 12 sites in the fall.

Isotopic analyses of water and algal tissues from springs were conducted to (1) assess nitrate sources to spring water using the dual-isotopic analysis of nitrate (δ^{15}N-NO_3 and δ^{18}O-NO_3), (2) determine the δ^{15}N and δ^{13}C composition of macroalgae and spring sediments through one-time surveys at a regional scale and along four spring river runs, and (3) assess the relationships between the δ^{15}N and δ^{13}C of macroalgae and indicators of nutrient availability and sources at the regional scale. The dual isotope analysis of nitrate in water (δ^{15}N-NO_3 and δ^{18}O-NO_3) was

used to further differentiate sources of nitrate when $\delta^{15}N$ ranges overlap (Kendall, 1998), such as NO_3 fertilizer from soil nitrogen and NH_4 in fertilizer and rain. Details of this analysis can be found in Albertin et al. (2007a).

2.2.2 Data Transformations and Analyses

Land use and some landscape attributes were related to nutrient concentrations to determine likely contributions by natural and human sources. Latitude and longitude were used as simple variables to characterize region. Stepwise forward multiple regression analysis was used to evaluate covariation among variables and thereby determine whether relationships could be explained or masked by other factors.

Table 2.1. Dependent and independent variables in correlations relating macroalgae to environmental characteristics in Florida springs. Sediment chemistry was determined for sediments under mats and in open areas of springs.

Dependent Variables	Independent Variables		
	Water Chemistry	Sediment Chemistry	Habitat and Land Use
Avg % cover	Ammonia	%N	Depth
Avg cover thickness	Kjeldahl nitrogen	%C	Current
Avg *Lyngbya* % cover (dominant)	Nitrates	TP (mg/g)	Transect Length
Avg *Lyngbya* % cover (presence)	Phosphate	TP LOI %	Longitude
Avg *Lyngbya* mat thickness	Total Phosphorus	Available P (µg/g)	Latitude
Avg *Vaucheria* % cover (dominant)	Total Nitrogen	Available N (µg/g)	Riparian Trees
Avg *Vaucheria* % cover (presence)	Temperature	Available NH4 (µg/g)	Riparian Shrubs
Avg *Vaucheria* mat thickness	DO	Available NO3 (µg/g)	Riparian Herbs
Max % cover	Conductivity	% water	Riparian Bare
Max cover thickness	pH	% ash	Riparian Canopy
Max *Lyngbya* % cover (dominant)	Diatom Indicators of Nutrients		Riparian Buffer
Max *Lyngbya* % cover (presence)			Percent Watershed Disturbed
Max *Lyngbya* mat thickness			
Max *Vaucheria* % cover (dominant)			
Max *Vaucheria* % cover (presence)			Landscape Disturbance Index
Max *Vaucheria* mat thickness			

Algal biomass was characterized for all macroalgae and for two dominant macroalgal species independently, *Vaucheria* spp. and *Lyngbya wollei*. "Species" level analysis was important because different species could respond differently to nutrients and interact with other water chemistry parameters, such as conductivity, which varies greatly among springs and often has

significant effects on algal species composition (Table 2.1). Algal biomass was characterized by the % cover of the bottom and by thickness. Average and maximum values for both % cover and thickness were determined, where average and maximums were determined across seasons. Percent cover for *Lyngbya wollei* and *Vaucheria* was determined two ways: as percent of observation points at which either alga was dominant or just present. Therefore one set of statistics were run relating environmental parameters to macroalgal abundances where it was dominant, and the same statistics were run when abundance was based on it just being present. Both percent cover variables were evaluated to ensure thorough analysis of the data. In many cases, macroalgae were quite abundant, even if they were not the dominant alga at the site.

In addition to measured water chemistry parameters, nutrient concentrations were inferred using diatom indicators of nutrient conditions. Diatom indicators of nutrient concentrations are important, because they can more precisely and accurately measure nutrient availability than one time sampling of nutrient concentrations (Stevenson 2006). In this study, where sediments may be an important source of nutrients, epiphytic diatoms may provide an indication of nutrient availability to algae that are not reflected in measured nutrient concentrations in the daytime water column. Different species of diatom grow faster in low and high nutrients (Manoylov and Stevenson 2006). The nutrient concentrations in which diatom species have highest relative abundances can be characterized and used as traits for each species that indicate the so-called "optimum" nutrient concentration for species (Potapova et al. 2004). Nutrient concentrations in water at a site can be inferred subsequently by summing the products of relative abundances and nutrient optima of individual diatom species and correcting for lack of information about some species. Multiple indicators of nutrient conditions were calculated using diatom species nutrient optima that were derived with data from the Florida springs and from other studies. Diatom species composition responded much more to changes in total phosphorus than total nitrogen among springs. Therefore, diatom indicators of nutrients more precisely inferred nutrient concentrations for TP than TN. Details of this analysis and these results can be found in a report to FDEP by Stevenson and Pinowska (2007).

Correlations were calculated between all measures of algal biomass and all measures of nutrient concentrations resulting from laboratory analysis of water samples and diatom inference models. In addition, correlations were calculated between all measures of algal biomass and non-nutrient water parameters, stable isotopes, sediment parameters, riparian parameters, and land use parameters (Table 2.1).

All correlations that were statistically significant were graphed and analyzed with quantile regression and regression tree analysis to further explore factors that regulate macroalgal biomass in Florida springs (see Pinowska et al. 2007a for these figures and results). Lowess smoothing was used to evaluate relationships between highly correlated variables without a priori assumptions of the shape of relationships. Quantile regression enabled evaluation of the potential for algal proliferation when confounding factors constrain abundance of algae in subsets of springs with specific environmental conditions. Regression tree analysis evaluated non-linear relationships and helped identify algal response thresholds along nutrient gradients.

2.3 – Overview of Results

2.3.1 Region, land use, and nutrients

Nutrient concentrations were related to both region of the state and land use indicating both natural and anthropogenic sources of nutrients. Nitrogen concentrations (NO_x and TN) were significantly correlated to land use within 1000 m, but not 100 m of the site or directly to region (Table 2.2). After effects of land use were accounted for with multiple regression, both TN and NO_x were positively correlated to latitude ($p<0.05$). Total dissolved phosphorus (TDN) was correlated to land use within 100 m of sites, but stepwise multiple regression indicated that the correlation was confounded with regional variation. Phosphorus measures (SRP, TDN, and TP) were significantly related to both latitude and longitude. After accounting for covariation with multiple linear regression, SRP was positively correlated with latitude and longitude, TP was positively correlated with longitude, and TDN was negatively correlated with latitude. The only evidence of a P relationship with land use was after variation in SRP related to latitude and longitude was accounted for using multiple regression analysis; then SRP was positively, but poorly correlated to land use ($p=0.168$).

Table 2.2. Correlations between nutrient concentrations, land use metrics, latitude, and longitude. Nutrients include (ammonia, nitrate-nitrate, total dissolved nitrogen, total nitrogen, soluble reactive phosphorus, total dissolved phosphorus, and total phosphorus). Nutrient and landuse metrics were log transformed before analysis. Correlation coefficients in bold type have a $p<0.05$. PWD_100m and PWD_1000m are the percent watershed disturbed within 100 m and 1000 m of sampling sites. LDI_100m and LDI_1000m are the landscape disturbance intensity index within 100 m and 1000 m of sampling sites.

Nutrient	PWD_100m	PWD_1000m	LDI_100M	LDI_1000M	Latitude	Longitude
ln(NH3)	0.107	0.106	0.200	**0.291**	-0.054	-0.063
ln(NOx)	0.238	**0.454**	0.276	**0.469**	0.044	-0.148
TDN	-0.114	-0.044	-0.114	0.010	-0.173	0.266
ln(TN)	0.257	**0.434**	0.274	**0.458**	0.000	-0.058
ln(SRP)	0.019	0.038	-0.207	-0.073	-0.130	**0.416**
ln(TDP)	**0.298**	0.109	**0.284**	0.193	**-0.561**	**0.379**
ln(TP)	0.183	0.158	-0.038	0.091	***-0.414**	**0.600**

No relationship was found between stable isotope ratios and land use to help distinguish sources of nutrients (Albertin et al. 2007a). However, the consistent relationship between $\delta^{18}O\text{-}NO_3$ and $\delta^{15}N\text{-}NO_3$ indicated that fertilizer was the primarly source of nitrogen in springs and denitrification of the fertilizer explained variation among springs. Correlations between $\delta^{15}N$ or $\delta^{13}C$ and either diatom indicators of nutrients, pH, or nitrogen concentrations in springs were hypothesized to be caused by complex relationships between N and P supply rates, water hardness, and P transport via $CaPO_4$. However, no conclusions about nutrient sources and effects on algae could be drawn from these latter correlations.

2.3.2 Algae and relationships to nutrient concentrations

Macroalgae were found at 59 of the 60 sampled sites, and on average, covered almost half of the bottoms of springs at those sites (Stevenson et al. 2004). Twenty-four taxa and 23 genera of macroalgae were found from five major groups of algae: the cyanobacteria (five taxa), red algae (five taxa), diatoms (three taxa), Xanthophyceae (one taxon), and green algae (10 taxa). Thick algal mats were formed by four taxa: *Lyngbya wollei*, *Vaucheria* spp., *Compsopogon* spp., and *Dichotomosiphon* spp. Species of most genera were not identified because diagnostic features of sexual reproductive structures were not common. *Lyngbya wollei* and *Vaucheria* spp. were found in many more springs than the other mat-forming macroalgae and therefore, became the focus of most of our research.

Both nitrogen and phosphorus concentrations in spring water ranged greatly among springs, but positive relationships were only observed between *Vaucheria* and nitrogen and phosphorus concentrations. Percent cover of spring bottoms by all macroalgae ranged from close to zero to almost 100% and was not related to TN, TP, or soluble nutrient concentrations (Table 2.3). Average mat thickness on spring bottoms by all macroalgae ranged from near zero to 0.25 m and also was not related to TN, TP, or soluble nutrient concentrations. Percent cover of spring bottoms by *Lyngbya wollei* ranged from zero to almost 80% and its mat thickness ranged from zero to 0.25 m, but neither were related to TN, TP, or soluble nutrient concentrations (Table 2.3).

Table 2.3. Pearson correlation matrix for macroalgae abundance and water chemistry ($p<0.05$ in bold, Pinowska et al. 2007a).

Macroalgae abundance	Ammonia	Kjeldahl nitrogen	Nitrates	Phosphate	Total Phosphorus	Total Nitrogen
Avg % cover of all macroalgae	0.161	0.061	0.022	0.080	0.098	0.042
Avg cover thickness all macroalgae	-0.021	-0.095	-0.005	0.065	0.105	-0.023
Avg *Lyngbya* % cover (dominant)	0.186	**-0.281**	-0.073	0.116	0.089	-0.058
Avg *Lyngbya* % cover (presence)	0.204	**-0.266**	-0.052	0.138	0.119	-0.041
Avg *Lyngbya* mat thickness	0.166	**-0.337**	-0.099	0.112	0.106	-0.101
Avg *Vaucheria* % cover (dominant)	0.182	0.215	**0.288**	0.193	**0.246**	**0.310**
Avg *Vaucheria* % cover (presence)	0.194	0.198	**0.304**	0.220	**0.275**	**0.325**
Avg *Vaucheria* mat thickness	0.151	0.093	**0.257**	**0.253**	**0.302**	**0.261**
Max % cover	0.126	-0.025	-0.004	0.161	0.173	0.007
Max cover thickness	-0.120	-0.190	-0.006	0.078	0.123	-0.037
Max *Lyngbya* % cover (dominant)	0.175	**-0.338**	-0.056	0.146	0.122	-0.052
Max *Lyngbya* % cover (presence)	0.197	**-0.317**	-0.033	0.179	0.162	-0.032
Max *Lyngbya* mat thickness	0.152	**-0.371**	-0.101	0.129	0.124	-0.111
Max *Vaucheria* % cover (dominant)	0.121	0.184	**0.258**	0.203	**0.269**	**0.273**
Max *Vaucheria* % cover (presence)	0.145	0.169	**0.277**	**0.229**	**0.298**	**0.289**
Max *Vaucheria* mat thickness	0.115	0.057	**0.238**	**0.259**	**0.309**	**0.238**

Percent cover of spring bottoms by *Vaucheria* ranged from zero to 80 % and its mat thickness ranged from zero to 0.20 m and both were related positively to N and P concentrations in spring

water (Figures 2.3). Non-linear models of *Vaucheria* % cover and thickness along the TN and nitrate gradients explained substantially more variation than a linear model, with thresholds in *Vaucheria* response at 0.454 and 0.591 mg N/L as nitrate and as TN, respectively. Average *Vaucheria* cover was only 2.3 % below the 0.591 mg TN/L threshold.

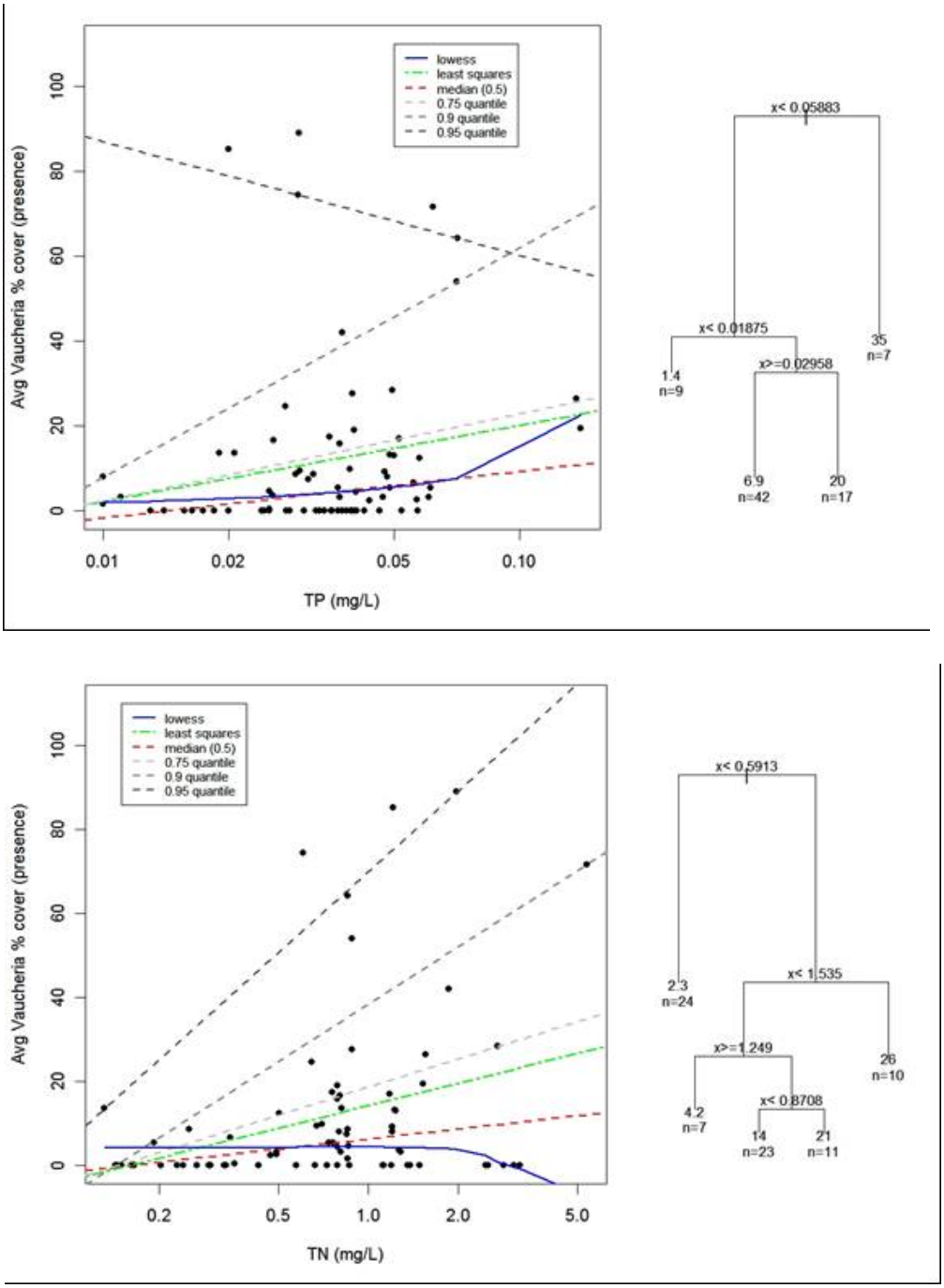


Figure 2.3. Relationship between concentrations of total nitrogen (TN) and total phosphorus (TP) and *Vaucheria* percent cover at sites in 23 springs across northern Florida. See Pinowska et al. (2007a) for details.

Despite the stronger correlations between macroalgae and N concentrations in spring water, laboratory bioassays using water from the springs indicated that algae were limited by P at 56% of spring sites, by N at 19%, and by both N and P at 22%.

The percent cover and mat thickness of *Lyngbya wollei* and *Vaucheria* were positively correlated to cellular N and P concentrations (Pinowska et al. 2007a). Lyngbya *wollei* % cover and mat thickness were positively correlated to sediment phosphorus concentrations, both when the sediment P was measured under mats and in open areas (Figure 2.4). *Lyngbya wollei* % cover was also related to nitrate concentrations in sediments below mats, but not in open areas. *Vaucheria* % cover was correlated with % carbon in sediments under mats, but not to nutrient conditions in sediments (Pinowska et al. 2007a).

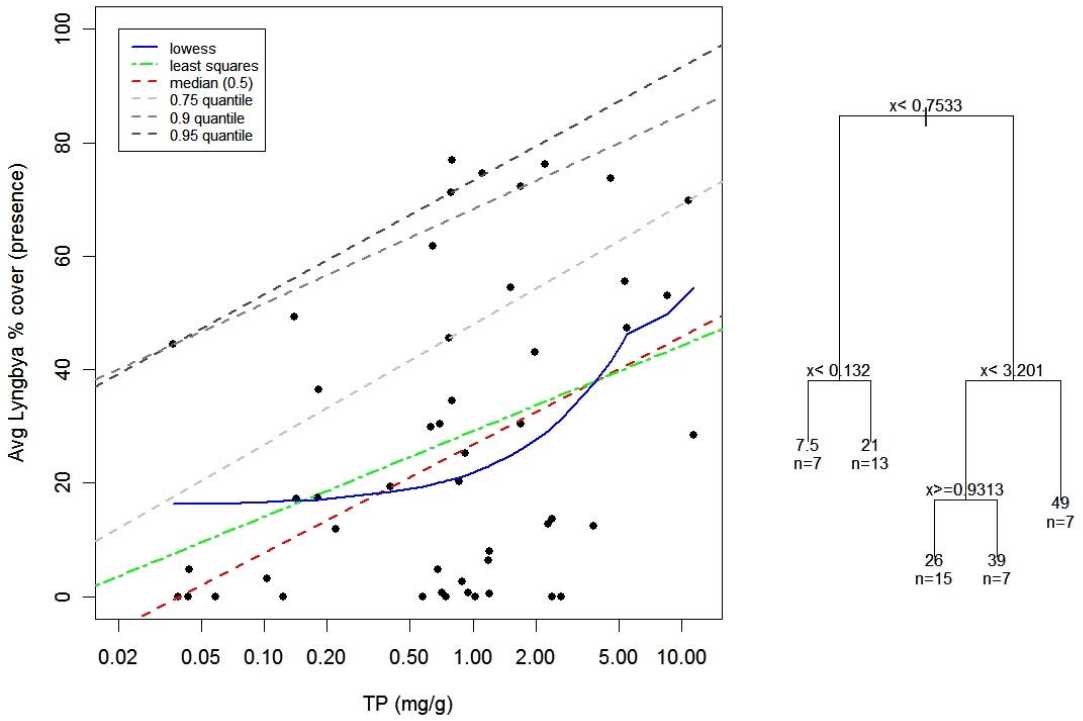


Figure 2.4. Average *Lyngbya* % cover (presence) as a function of sediment TP including lowess smooth, least square regression, quantile regression and CART (Pinowska et al. 2007a).

Diatom indicators of nutrient concentrations explain more variation in macroalgal biomass and thickness than measured nutrient concentrations in Florida springs (Table 2.4). Whereas *Lyngbya wollei* % cover was not related to nutrient concentrations, it was related to diatom indicators of nutrient concentrations and particularly to indicators of P concentrations. Percent cover of *Vaucheria* was usually correlated to N and P concentrations with correlation coefficients 0.2-0.3, but it was related to diatom indicators of nutrient concentrations with coefficients usually ranging from 0.3-0.4. This could be due to epiphytic diatoms better reflecting nutrient supply from sediments and interstitial waters than water column nutrients. Plants take up nutrients from sediments, which then leak through the epidermis of leaves (Burkholder et al. 1990) which would cause changes in diatom species composition. These

changes in species composition therefore could reflect the sediment nutrient supplies that affect *Lyngbya wollei* and *Vaucheria.*

Percent cover measures of *Lyngbya wollei* were more highly correlated to land use around sampling sites than *Vaucheria* (Table 2.5). Percent cover of *Lyngbya wollei* was correlated to LDI in the 100 m and 1000 m zone around sites. Percent cover of *Vaucheria* was not significantly correlated with percent of the watershed disturbed or with LDI in either the 100 m or the 1000 m zone around sites. These correlations indicated *Lyngbya wollei* abundances in springs were associated with human activities.

Table 2.4. Pearson correlation matrix for macroalgal abundance and diatom indicators of nutrient concentrations ($p<0.05$ in bold, Pinowska et al. 2007a).

Macroalgal Taxon	Diatom Indicator of Nutrient Conditions	Trait Dat Source	Avg % cover (present)	Avg mat thickness	Max % cover (present)	Max mat thickness
Lyngbya	*FLS* Nutrient Index	Spring Epi	0.246	**0.259**	**0.269**	**0.267**
Lyngbya	*FLS* Nutrient Index	F&S Epi	0.241	0.216	**0.268**	0.220
Lyngbya	*FLS* TP Index	F&S Epi	**0.275**	0.252	**0.285**	0.255
Lyngbya	% High P Individuals	all datasets	**0.318**	**0.264**	**0.314**	0.256
Lyngbya	*FLS* Nutrient Index	Spring Epi	0.253	**0.255**	**0.261**	**0.262**
Lyngbya	*FLS* Nutrient Index	F&S Epi	0.160	0.099	0.170	0.100
Lyngbya	*FLS* TP Index	F&S Epi	0.199	0.169	0.186	0.160
Vaucheria	*FLS* Nutrient Index	Spring Epi	**0.273**	**0.353**	**0.302**	**0.364**
Vaucheria	*FLS* Nutrient Index	F&S Epi	**0.370**	**0.386**	**0.353**	**0.359**
Vaucheria	*FLS* TP Index	F&S Epi	**0.320**	**0.398**	**0.338**	**0.380**
Vaucheria	% High P Individuals	all datasets	0.117	0.138	0.096	0.101
Vaucheria	*FLS* Nutrient Index	Spring Epi	**0.354**	**0.434**	**0.378**	**0.445**
Vaucheria	*FLS* Nutrient Index	F&S Epi	**0.357**	**0.355**	**0.340**	**0.335**
Vaucheria	*FLS* TP Index	F&S Epi	**0.360**	**0.396**	**0.358**	**0.372**

2.3.3 Algae and relationships to local and regional environmental factors

Some natural environmental factors that could affect macroalgae varied greatly among springs. Conductivity ranged from 113 to 3872 µS/cm and dissolved oxygen ranged from 0.27 to 9.98 mg/L). Water temperature and pH had relatively narrow ranges, which were assumed to be related to natural factors, from 20.06 to 23.93°C and from 7.13 to 8.33, respectively.

Lyngbya wollei % cover and mat thickness were positively correlated with water temperature, DO concentration, and pH, but were not related to conductivity. The relationship between *Lyngbya wollei* and temperature was non-linear, with a threshold observed at 21.5°C. In contrast, *Vaucheria* % cover and thickness were negatively correlated to DO, conductivity, and pH, but were not related to temperature. Note: Hand (unpublished results, FDEP) shows that temperatures of many springs have increased during the last 40-50 years. Many of these factors

may covary with other factors, but additional analysis of covariation was beyond the scope of this study.

Table 2.5. Pearson's correlation matrix for macroalgae abundance, percent disturbed land and LDI scores in 100m radius around sampling location, 1000 m radius around sampling location, and in the whole springshed for a particular spring system ($p<0.05$ in bold, Pinowska et al. 2007a).

Macroalgae abundance metric	PW_ 100 m	PW_ 1000 m	PW springshed	LDI 100 m	LDI 1000 m	LDI springshed
Avg % cover	0.155	0.106	0.177	0.170	0.174	0.132
Avg cover thickness	0.171	-0.028	0.135	0.136	0.050	0.134
Avg *Lyngbya* % cover (dominant)	**0.315**	**0.245**	0.255	**0.294**	**0.231**	0.219
Avg *Lyngbya* % cover (presence)	**0.323**	**0.279**	0.293	**0.294**	**0.268**	**0.293**
Avg *Lyngbya* mat thickness	**0.299**	0.200	0.225	**0.275**	0.195	**0.259**
Avg *Vaucheria* % cover (dominant)	-0.101	-0.074	0.106	-0.193	-0.054	**0.279**
Avg *Vaucheria* % cover (presence)	-0.098	-0.083	0.133	-0.195	-0.066	**0.316**
Avg *Vaucheria* mat thickness	-0.119	-0.016	0.115	-0.206	-0.002	**0.306**
Max % cover	0.202	0.127	0.207	0.189	0.173	0.138
Max cover thickness	0.215	-0.029	0.212	0.221	0.034	0.167
Max *Lyngbya* % cover (dominant)	**0.332**	**0.262**	0.277	**0.308**	**0.239**	0.229
Max *Lyngbya* % cover (presence)	**0.329**	**0.288**	**0.307**	**0.290**	**0.266**	**0.305**
Max *Lyngbya* mat thickness	**0.303**	0.185	0.216	**0.276**	0.178	**0.260**
Max *Vaucheria* % cover (dominant)	-0.119	-0.091	0.083	-0.203	-0.075	**0.263**
Max *Vaucheria* % cover (presence)	-0.103	-0.087	0.122	-0.194	-0.072	**0.311**
Max *Vaucheria* mat thickness	-0.124	-0.032	0.096	-0.207	-0.021	**0.282**

Percent cover and thickness of *Lyngbya wollei* and *Vaucheria* mats were not related well to local factors such as depth, canopy cover, and channel width. *Vaucheria* was positively related to higher current velocity. Few attributes measured as part of the habitat characterization for the riparian buffer were correlated to macroalgal attributes.

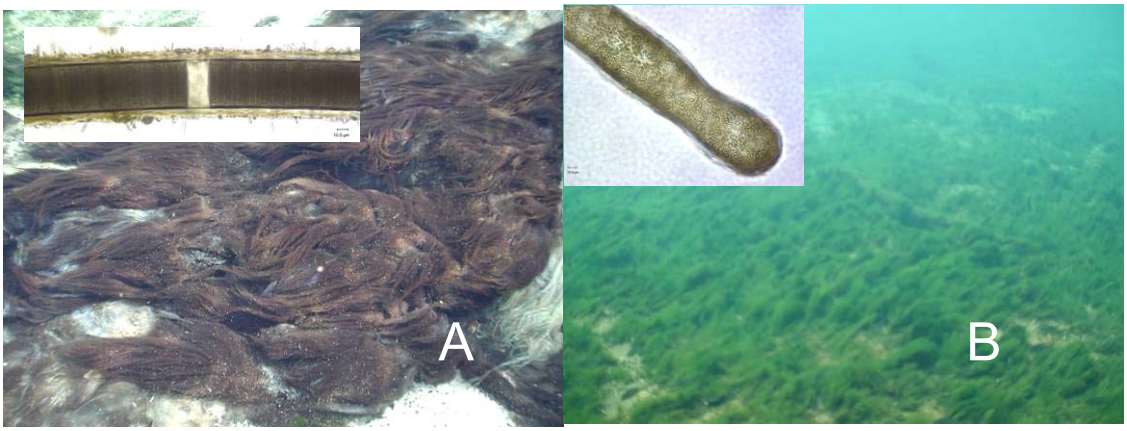
Percent cover and thickness of *Lyngbya wollei* mats were negatively correlated to both latitude and positively related to longitude. Only *Vaucheria* mat thickness was positively correlated to a regional factor, and that was to longitude. *Lyngbya wollei* % cover and thickness were non-linearly related to longitude, with a threshold around -83° W. This may be related to phosphorus-rich geology in specific regions of the state.

Details of these analyses and more thorough report of relationships between all dependent and independent variables can be found in Stevenson et al. (2004) and Pinowska et al. (2007a).

2.4 – Discussion

Lyngbya wollei and *Vaucheria*, the two most abundant macroalgae in Florida springs are very different algae. *Lyngbya wollei* is a filamentous cyanobacteria with cells approaching 0.5 mm wide, which is very wide compared to most other filamentous cyanobacteria (Fig. 2.5A). It has a thick firm mucilaginous sheet around the trichome of cells. It grows in entangled masses without a specialized method of holding onto a substratum. *Lyngbya wollei* can produce a variety of toxins (Carmichael et al. 1997, Onodera et al. 1997, Yin et al. 1997). *Vaucheria* is approximately the same diameter, but is a coenocytic filament in which the cytoplasm is not separated into small cells by cross-walls. Its multinucleate cytoplasm is arranged in a tube around a large central vacuole (Fig. 2.5B). These filaments grow in a thick turf that is connected and expanding by rhizoids. Different species of *Vaucheria* could not be identified because because sexual reproductive structures, the diacritical characteristics, were not observed in most cases.

Figure 2.5. Lyngbya wollei (A) and Vaucheria (B). Insets show photomicrographs of the algae. Larger pictures show masses growing on spring bottom.



The taxonomy of the *Lyngbya wollei* (Farlow ex Gomont) Speciale and Dyck is complicated because of its morphological and ecological variability. Historically this taxon was probably identified as *Plectonema wollei* Farlow ex Gomont in Whitford's (1957) survey of Florida springs. In Stevenson et al. (2004), we referred to it as *Lyngbya majuscula* Harvey ex Gomont. Speciale and Dyck (1992) renamed *Plectonema wollei* Farlow ex Gomont as *Lyngbya wollei* based on morphological considerations and rejected *Lyngbya majuscula* Harvey ex Gomont as a correct name, because the latter is from brackish and marine habitats. Ecology and morphology are important characteristics in modern taxonomy of the cyanobacteria. Joyner (2004) shows this alga in Florida springs is genetically different than *Lyngbya majuscula*. *Lyngbya wollei* is currently the most commonly used name for this alga in freshwater ecosystems. Therefore, we use this name for this extremely large-celled filamentous cyanobacterium.

The extent of *Lyngbya wollei* and *Vaucheria* occurrence seems to be much greater than in the past. Both taxa were observed by Whitford (1957) in his study of algae in Florida springs. He noted *Plectonema wollei* Farlow "forms abundant mats in the fresh-water springs." Pinowska found a collection of *Lyngbya* at Harvard's Farlow Herbarium, which was collected from Silver Springs in 1939. Her examination of that material showed that it looked like the alga that we now identify as *Lyngbya wollei*. However, Odum (1956a, 1956b) does not identify *Lyngbya wollei* or *Vaucheria* as important primary producers in this work in Silver Springs or eleven other Florida springs. Odum characterized most springs being dominated by aquatic vascular plants. Photographic documentation of Weeki Wachee also indicates great loses of aquatic vascular plants and increases in macroalgae from the 1950s to present (Figure 1.1). Quinlan et al. (submitted) concluded that the epiphytic and algal mat biomass in Silver River Springs is higher today than reported by Odum (1957a).

Increases in nitrogen concentrations in Florida springs were clearly related to the increases in population density over time (Scott et al. 2003) and increases in human activities among springs at present (our analyses). Total nitrogen, nitrate, and ammonia, increased with indicators of human activities, where as increases in phosphorus were less significantly related to humans. However, the high P concentrations, sometimes similar to natural background concentrations, seem sufficient to stimulate growth of macroalgae, such as *Cladophora* in streams of the Montana and the Midwest where 0.015-0.030 mg TP/L regulate growth (Dodds et al. 1997, Stevenson et al. 2006). Historic levels of nitrogen in Florida springs, which were predicted to have been around 0.050 mg/L, would have constrained growth of both *Lyngbya wollei* and *Vaucheria* (Albertin et al. 2007b).

Only % cover and thickness of *Vaucheria* mats were related to nitrogen and phosphorus concentrations in spring water. A clear threshold in *Vaucheria* response to TN at 0.591 mg/L provides a benchmark for nitrogen criteria that should prevent nuisance levels of *Vaucheria*.

The other common macroalga, *Lyngbya wollei*, was not correlated to either nitrogen or phosphorus concentration in the water column. However, percent cover and thickness of *Lyngbya wollei* mats, as well as *Vaucheria* mats, were positively related to phosphorus in spring sediments, nutrient concentrations in macroalgal cells, diatom indicators of nutrient concentrations, and land use around sampling sites. N:P ratios in the water column and algal growth assays indicated phosphorus is the most limiting nutrient in many Florida springs.

Could *Lyngbya wollei* be getting its nutrient supply from sediment sources? Would nitrogen reductions have any effect on *Lyngbya wollei* in Florida springs if phosphorus is the limiting resource? The research presented in the following sections addresses these questions. What are nutrient concentrations and fluxes in mats of *Vaucheria* and *Lyngbya wollei* compared to sediments and the water column? What nutrient concentrations affect the growth of *Vaucheria* as well as *Lyngbya wollei*? Do N:P ratios dictate responses of *Vaucheria* and *Lyngbya wollei* to nutrient reductions?

3 – NUTRIENT CONCENTRATIONS ABOVE, WITHIN, AND BELOW *LYNGBYA WOLLEI* AND *VAUCHERIA* MATS

3.1 – Introduction

The effects of nutrients on benthic algae may vary greatly with density of those algae in a habitat. The phosphorus and nitrogen concentrations required to produce maximum growth rates of diatom-dominated benthic algae increases with increasing biomass of algae on substrata (Bothwell 1989, Rier and Stevenson 2006). This density-dependent demand for nutrients has been explained by reduced mixing of nutrients from overlying surface waters into mats coupled with high cellular uptake rates as biomass of diatom-dominated mats increases. Therefore, we would expect higher nutrient concentrations to be required to generate thick than thin macroalgal mats if the same relationship between mixing and cellular uptake rates exists for macroalgae as microalgae.

However, macroalgae accumulate to produce much greater depths than the 2-3 cm of a diatom-dominated mat. Typically, cells of filamentous macroalgae are not packed as densely as microalgae. Other processes, such as entrainment of particulate nutrients and decomposition, may be more important as thickness of mats increases so greatly. Thus, entrainment and decomposition with slow interstitial flux rates may enable support of thick algal mats without high nutrient supply rates, if algae can grow slowly without great disturbance for long periods of time. As mats thicken and age, lower and interior portions of the mats die, which has been attributed to reductions in nutrient supply and light in diatom-dominated benthic algae. The same resource limitation weakens health of macroalgae in the interior and lower portions of mats and makes them more susceptible to bacterial attack and decomposition. In addition, particulate organic nitrogen and phosphorus from upstream may become entrained within mats and decompose. If mixing rates between interstitial waters of the mat and overlying waters are less than decomposition rates, decomposition could become an important source of nutrients to support deep accumulations of benthic macroalgae. Several studies show that nitrogen regeneration may help sustain benthic macroalgal productivity and some systems may function as self-regenerating through this recycling of nitrogen (McGlathery et al. 1997; Stimson and Larned 2000; Trimmer et al. 2000; Sundbäck et al. 2003).

Groundwater may be another source of nutrients that is not accounted for when only measuring surface water nutrient concentrations. Hendricks and White (2000) describe how mounds of the macroalga *Chara* in sandy streams alter surface and hyporheic flows and could increase nutrient availability. Macroalgal accumulation in Florida springs could also be supported by groundwater sources of nitrogen or phosphorus that become entrained in mats or groundwater. Such sources could provide a partial explanation for how we observe extensive growths of *Lyngbya wollei* mats in low nutrient springs. Groundwater nutrient sources could be just enough to support macroalgal growth in streams, yet not enough to increase measured nutrient concentrations in overlying spring water because of dilution.

In this study benthic macroalgal mats dominated by *Lyngbya wollei* and *Vaucheria* spp. in Florida springs were evaluated to determine whether nutrients accumulated within mats. Nutrient profiles, isotopic composition of algae, and advective water movement were measured

within, below, and above algal mats at Weeki Wachee, Manatee and Silver Glen Springs to characterize nutrient profiles of *Lyngbya wollei* and *Vaucheria* spp. mats, to determine if higher isotopic signatures indicated nutrient turnover in mats, and to estimate advective movement of dissolved nutrients out of mats. Results of this study will help us determine whether nutrient depletion within macroalgal mats could constrain growth or whether groundwater, sediments, entrainment, and decomposition may be sources of nutrients that sustain accumulation of thick mats of macroalgae. Details about the methods and results of this research can be found in Sickman et al. (2007).

3.2 – Overview of Methods

Interstitial water samplers, known as multisamplers, were used to collect water above, within, and below macroalgal mats on two dates and in three springs: in Weeki Wachee and Manatee Springs on April and August 2006 and in Silver Glen Springs (August 2006). The multisamplers were sampled at different times of the year to observe nutrient cycling during different stages of algal growth cycles and environmental conditions. Weeki Wachee had the thickest *Lyngbya wollei* mats and Manatee Springs the thickest *Vaucheria spp.* mats observed during surveys conducted in January 2006. Silver Glen was added as a comparison site to Weeki Wachee because it also has thick *Lyngbya wollei* mats, but the water chemistry varied between the two sites. Duplicate multisamplers were placed where the algal mats were thickest.

The multisamplers were 1.5 m tall PVC pipes with small holes drilled every 10 cm (modified from Martin et al. 2003)(Figure 3.1). Two layers of 500 µm nylon screen were glued to the outside of each hole to filter particulates. Tygon tubing (0.25 inch ID) was glued to the inner surface of each hole and ran the length of the water sampler. The multisamplers were inserted vertically through the thickest part of the algal mat to a depth of approximately 20-30 cm into the sediment. After a week of equilibration, water samples were drawn with a syringe from specific depths in the mats by using tubes connected to different holes in the multisampler. Both total and soluble nitrogen and phosphorus concentrations, dissolved organic carbon (DOC), and trace metals were measured in the water samples.

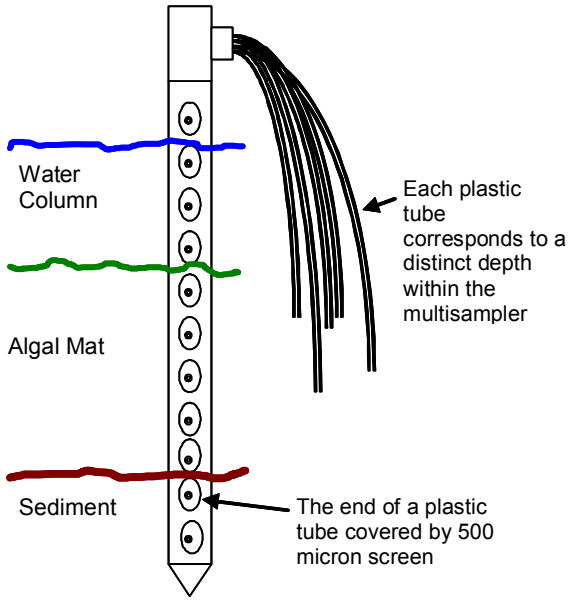


Figure 3.1. Multisampler device used to collect water column, algal mat and sediment interstitial waters.

Additionally, algal and sediment samples were taken from the algae/sediment interface, in the outer (surface) portion of the mat, and in the deeper portions. They were analyzed for % C, N and P as described in section 2.2.

Subsamples for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis were also collected and submitted for analysis by the Stable Isotope Laboratory at UC Davis.

To estimate advective movement of water and nutrients into and out of thick algal mats, a tracer study was conducted in August 2006 at Weeki Wachee, Manatee and Silver Glen springs using multisamplers. NaCl tracer solution was injected along the edge of replicate multisamplers facing the boil (i.e., what we interpreted to be the "upstream" side of the sampler). Water samples were collected 5 minutes, 15 minutes, 30 minutes and 60 minutes after injection. The samples were then analyzed using a conductivity meter to quantify NaCl tracer. The rate of decrease of NaCl tracer, expressed as the percent change, was computed for each multisampler port to indicate the rate of advective water movement through the mat.

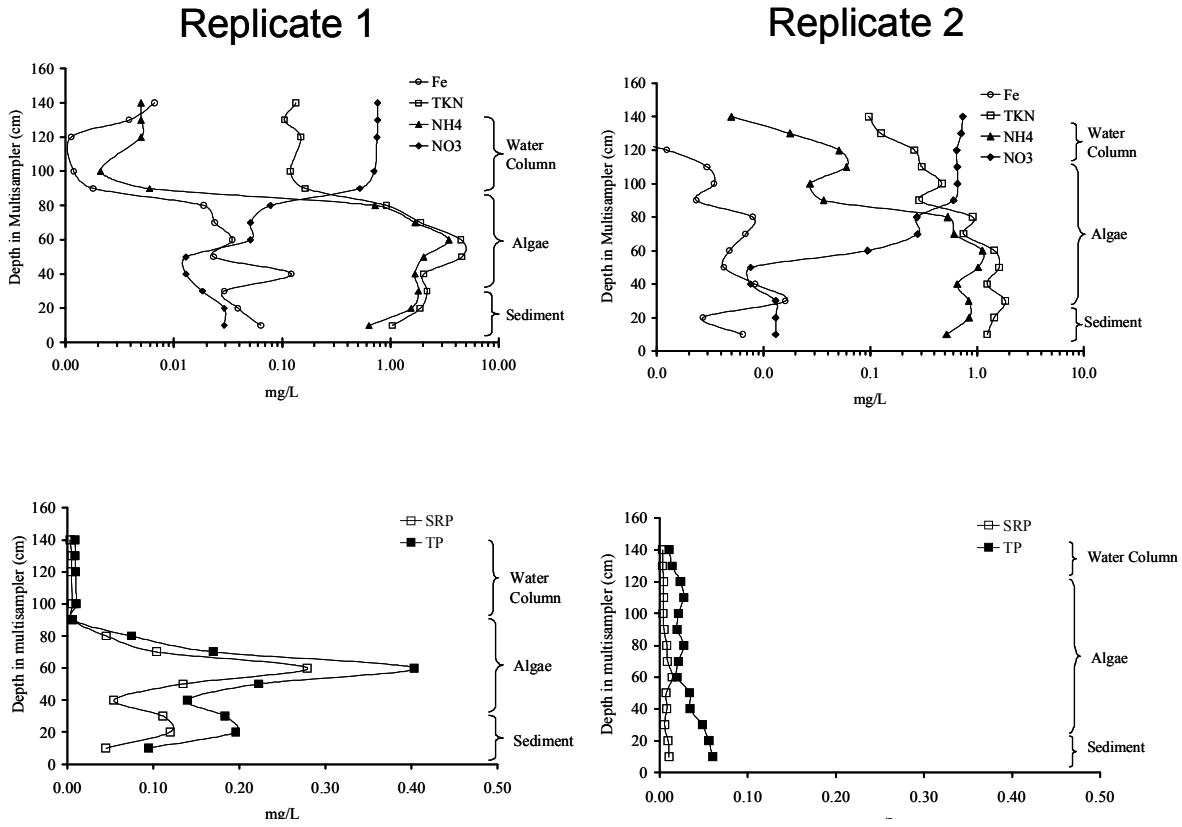


Figure 3.2. Chemical profiles measured by multisamplers through *Lyngbya wollei* on dates indicated below for two replicate samplers. Approximate depth zones of sediment, algae and water column are indicated along the right side of each figure. For panels showing N species and Fe the x-axes are logarithmic (Sickman et al. 2007).

3.3 – Overview of Results and Discussion

Nutrient concentrations varied greatly in vertical profiles through the algal mats, and patterns varied to some degree among springs and macroalgal taxon. But overall, nutrient concentrations were higher in interstitial waters within algal mats and sediments below algal mats than in the spring water above algal mats (Figure 3.2-3.3). Total phosphorus, soluble reactive phosphorus, total Kjeldahl nitrogen, and ammonia were consistently higher in water samples drawn from multisamplers at depths within the algal mats and sediments than in overlying waters. Only concentrations of nitrate decreased within algal mats, which may be attributed to the development of anoxic conditions within the mats, which were indicated by high iron concentrations.

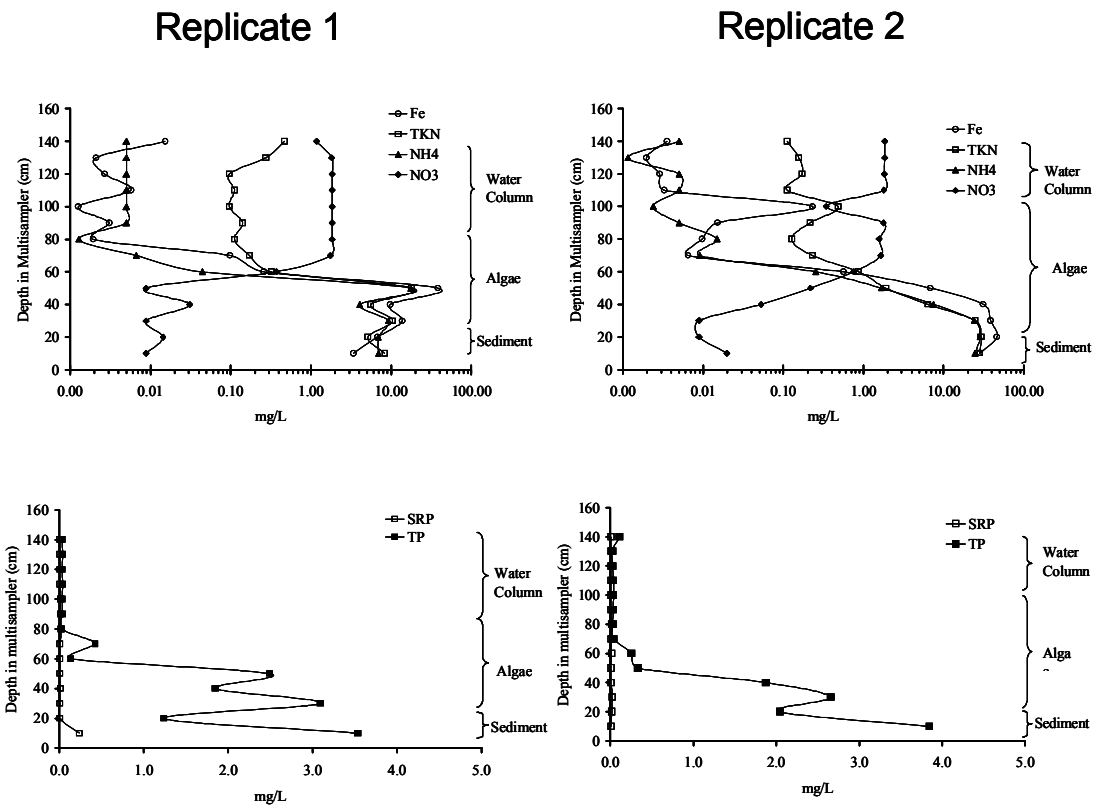


Figure 3.3. Chemical profiles measured by multisamplers through *Vaucheria* mats on dates indicated below and for two replicate samplers. Approximate depth zones of sediment, algae and water column are indicated along the right side of each figure. For panels showing N species and Fe the x-axes are logarithmic (Sickman et al. 2007).

High nutrient concentrations relative to overlying spring water occurred higher in the *Lyngbya wollei* mats of Weeki Wachee than *Lyngbya wollei* mats of Silver Glen or *Vaucheria* mats of Manatee Spring. Elevated nutrient concentrations in mats tended to be toward the bottom and

sediments in Silver Glen and Manatee Spring. Although it is tempting to relate these differences to nutrient concentrations in springs and type of macroalgae, our sampling design does not allow testing those hypotheses without confounding variables associated with other factors in the different springs.

Trends in stable isotope ratios of macroalgae with depth through mats indicated that heavier isotopes proportions increased with depth in the mat, however these patterns were not highly consistent across springs. The $\delta^{13}C$ values increased with depth in the Lyngbya mats of Weeki Wachee, but $\delta^{15}N$ values varied unpredictably with mat depth. In Silver Glen Lyngbya mats, $\delta^{13}C$ values varied little with depth, but $\delta^{15}N$ values increased with depth. Stable isotope ratios should increase with greater age and turnover of N and C in cells, but this pattern was not observed.

Mat C:N:P ratios indicated that macroalgae in Weeki Wachee and Silver Glen were highly phosphorus limited, but little consistent difference occurred with depth in mat chemistries. Element ratios for C:P and N:P ranged from 126-971:1 and 12-73:1, respectively. When compared to the Redfield benchmarks for C:P and N:P ratios of 106:1 and 16:1, phosphorus is in relatively short supply compared to both carbon and nitrogen. Although C:N, N:P, and C:P increased with depth into mats in Weeki Wachee, with the highest values occurring toward the bottoms of the mats, no pattern in elemental ratios was observed in Silver Glen. Thus we could not conclude that nutrient depletion usually increased with depth in macroalgal mats. More extensive study of more mats over longer time periods would be valuable in future investigations.

Percent change in tracer concentrations suggested that advective flow varied as a function of the distance above the spring bottom (Figure 3.4), with lowest flow at mid-depths of the mat. Higher advective flow at the bottom than the middle of the mats could indicate water movement out of sandy bottom sediments. Higher advective flow at the top than the middle of the mats indicated mixing of overlying surface waters with interstitial waters at the top of the mat.

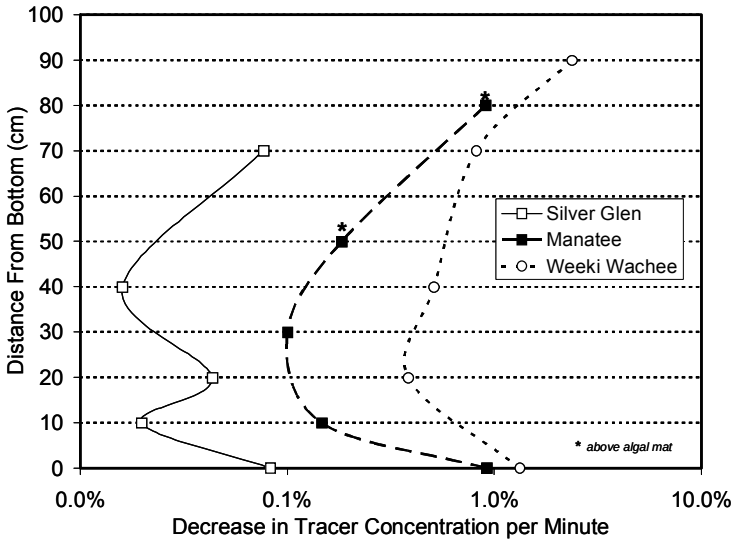


Figure 3.4. Profiles of tracer dilution at three springs. The rate of decrease of NaCl tracer (measured in units of conductivity) expressed as percent change from peak concentration are plotted on the x-axis (Sickman et al. 2007).

While it is difficult to translate the tracer data into

actual water velocities, we made a first approximation of rates of water movement by making assumptions about the distribution of tracer around the multisampler and by treating the algal mat as a porous medium like soil. Based on these assumptions, discharge through the mats ranged from 4.0×10^{-8} m s^{-1} at Silver Glen to 9.0×10^{-7} m s^{-1} at Weeki Wachee. For comparison, hydraulic conductivity through silty sand ranges from approximately 10^{-3} to 10^{-7} m s^{-1}. This compares to current velocities in overlying waters above mats commonly ranging from 0.01-0.5 m/s.

Thus, considerable evidence indicated that macroalgal mats function very differently than mats of diatom-dominated microalgae. Nutrient concentrations were not lower in interstitial mat waters than in overlying waters as predicted based on benthic microalgal ecology; they were usually higher in mats. This may be due to many factors. The macroalgae in Florida springs grow on soft sediments with varying organic matter content, which may supply nutrients to the macroalgae. Groundwater may supply nutrients to macroalgae in Florida springs through the sandy substratum. Nutrient rich silt and particulate organic matter may become entrained in mats as it is transported downstream. Low redox conditions in mats may release phosphorus bound to inorganic particles. Inorganic nutrients taken up from the water column by the mats may become entrained mats. Decomposition of accumulating organic matter may provide a source of nutrients in mats. The low advective flows in mats indicate that mineralized nitrogen and phosphorus would have long periods of time to be taken up by cells with the algal mats. Thus, thicker mats of macroalgae may not require higher nutrient concentrations than thinner mats if loss and disturbance rates are low. More information is certainly needed to better understand these processes before broad conclusions can be drawn.

4 – SEASONAL VARIABILITY IN MACROALGAE, DISCHARGE, AND NUTRIENTS IN FLORIDA SPRINGS

4.1 – Introduction and Overview of Methods

Seasonal variability in macroalgal cover has not been documented to determine the times of year in which problems may be greatest and whether seasonal factors could help us understand macroalgal ecology. Thus we monitored the monthly variability in discharge, nutrients, and macroalgal cover in Manatee and Ichetucknee Springs for a year to document seasonal variability. In addition, we compared the amount of nutrients in macroalgal mats to downstream nutrient flux to determine the potential of macroalgae for depleting nutrient supplies in overlying waters of springs. The same methods were used in this study as the large springs survey (Pinowska et al. 2007a), except that water chemistry was analyzed at the University of Florida versus the FDEP. Details of this study are described in Sickman et al. (2007).

4.2 – Overview of Results and Discussion

Vaucheria mats covered a majority of the bottom of Manatee Springs in August 2005, while *Lyngbya wollei* mats at Ichetucknee covered less than 50% of the bottom. The thickest (40-50 cm) and most extensive algal mats at Ichetucknee formed in the lee of the swim fence where water velocity was relatively low. In contrast, at Manatee Springs a semi-continuous mat

stretching along nearly the entire length of the spring run was observed with peak thickness of 50-60 cm.

The area of spring bottom covered by macroalgae and the mean thickness of algal mats in the Manatee and Ichetucknee Springs varied considerably during the study period (Figure 4.1). At Manatee spring, macroalgal area varied from near zero in the late spring and early summer of 2005 to a peak of 3500 m^2 in late winter 2006. Over the course of the study, mean thickness varied from 0.03 to 0.18 m and gross volume ranged from near zero to 518 m^3. Both algal area and thickness appeared to respond strongly to water levels in the Suwannee River. Low algal biomass in June and July of 2005 was likely a result of high water levels in the Suwannee River, which caused flooding of Manatee spring by dark tannic waters and thereby reduced sunlight available for algal growth. After the flood water receded, water clarity improved, algal growth increased rapidly and stayed relatively high until spring 2006 (Figure 4.1).

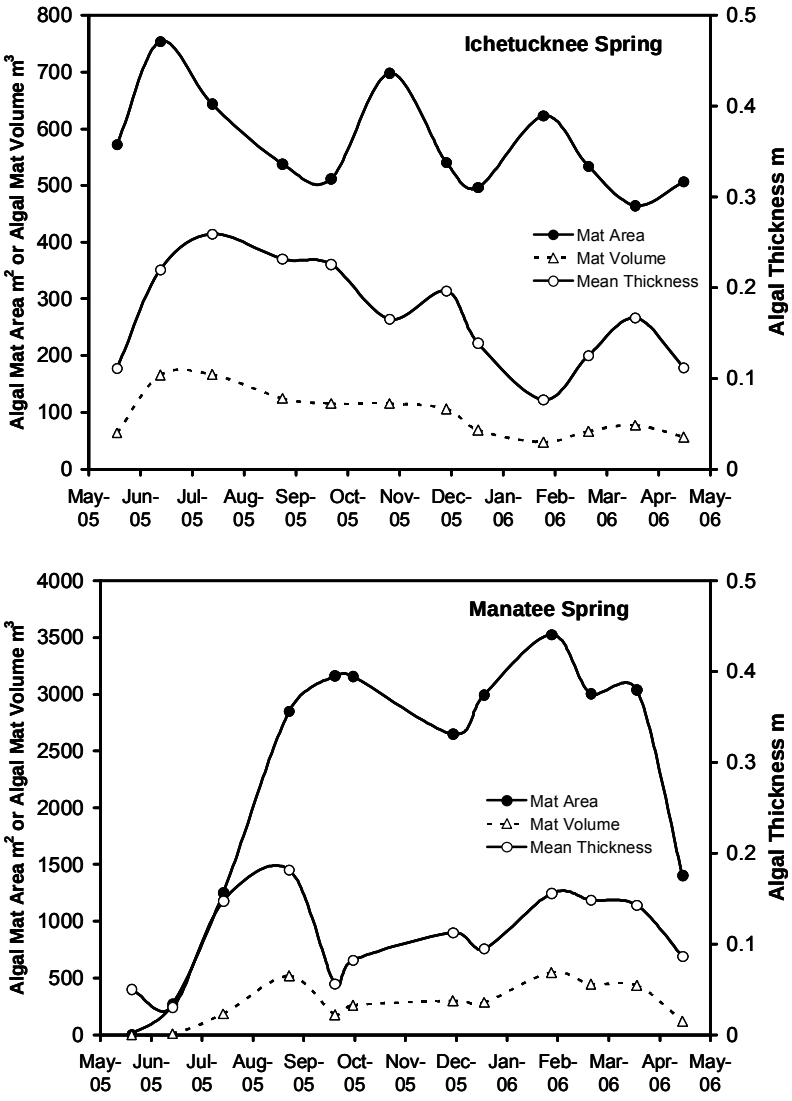


Figure 4.1. Variation in area of spring bottom covered by a *Lyngbya*-dominated mat at Ichetucknee Spring and a *Vaucheria*-dominated mat at Manatee Spring (Sickman et al. 2007).

Algal mat dynamics at Ichetucknee spring exhibited less variability than at Manatee spring (Figure 4.1). Over the year-long study there was a gradual decline in algal area, thickness and volume. The areal extent of algal cover varied between 465 to 754 m^2. Patterns of mean algal thickness often trended inversely with algal area and ranged from 0.08 to 0.26 m. Gross algal mat volume at Ichetucknee ranged from 48 to 166 m^3.

At Ichetucknee Blue Hole, discharge from the boil ranged from 6.0 – 6.9 m^3 s^{-1} between May 2005 and May 2006, and total Kjeldahl nitrogen (TKN) and total phosphorus (TP) concentrations

ranged from 0.09-0.23 mg L^{-1} and 0.03-0.05 mg L^{-1}, respectively. Discharge from the boil of Manatee Spring ranged from 5.7 – 7.8 m^3 s^{-1} between May 2005 and May 2006 and TKN and TP concentrations ranged from 0.09-0.29 mg L^{-1} and 0.02-0.04 mg L^{-1}, respectively.

For both Springs the amount of C, N, and P contained in algal biomass was typically in the range of 100-300 kg C, 10-150 kg N and <1-5 kg P. Daily C, N and P fluxes from the boils at Manatee and Ichetucknee Springs exceeded the amount contained in algal biomass on all dates. At Ichetucknee Springs mean C, N and P flux in boil water were 436 kg-C d^{-1}, 80 kg-N d^{-1}, 23 kg-P d^{-1}. At Manatee Springs, mean fluxes of C, N and P were 864 kg-C d^{-1}, 107 kg-N d^{-1}, 16 kg-P d^{-1} Comparing algal mat and boil flux, we observed that the amount of C, N and P contained in algal biomass represented less than one-day's flux from the boils. These data suggest that the entire carbon and macronutrient content of even the largest algal mats observed in Manatee and Ichetucknee contained no more than 24 hours worth of nutrient flux from boil water.

5 – EXPERIMENTAL ASSESSMENT OF *LYNGBYA WOLLEI* AND *VAUCHERIA* RECOLONIZATION AFTER PHYSICAL DISTURBANCE

5.1 – Introduction

Disturbance and recolonization is a natural cycle in benthic algal ecology. Benthic algae can be removed from substrata by floods and waves that disturb substrata, sloughing during senescence stages of community development, and bioturbation by animals. Disturbance of benthic macroalgae in Florida springs results as larger rivers backup into springs, carrying turbid water and silts that shade and bury algae (Sickman et al. 2007).

Recolonization of macroalgae in Florida springs after disturbance is poorly understood. Such events have not been studied for macroalgae as much as microalgae, and especially *Lyngbya wollei* or *Vaucheria*. Recolonization of diatom-dominated periphyton has been studied rather thoroughly in streams. In these assemblages, the roles of persistence of cells through disturbance events, immigration of new cells, reproduction, death, grazing, disease, competition, and sloughing have been characterized. Colonization by macroalgae is more complex than unicellular microalgae such as diatoms, because immigration occurs by filament fragments or by spores, which are special products of pre-existing cells. *Vaucheria* produces spores both asexually and sexually. *Lyngbya* can recolonize by fragments of filaments. Thus, their recolonization is probably regulated by the rates of settling of new spores or filament fragments into the space opened by disturbance and subsequent reproduction of these new cells and cells that persisted through the disturbance.

The objective of this study was to determine the rates of recovery of *Vaucheria* and *Lyngbya wollei* in Florida springs. In this study we removed algae from areas of spring bottoms in which these algae occurred naturally and monitored recolonization.

5.2 – Overview of Methods

The macroalgal recolonization experiments were conducted in Manatee Spring and the Blue Hole of the Ichetucknee Spring system in which, respectively, *Vaucheria* and *Lyngbya wollei* dominated. Theses sites were chosen because of differences in dominant algae and sufficiently high nutrient concentrations that regrowth of algae would not be limited. All algae were removed for 2 plots (each 50 cm x 50 cm) with a rake. The percent cover and thickness of macroalgae in removal plots and control plots were monitored every 2-4 weeks, depending on time after the disturbance and recolonization rates. The water depth, dissolved oxygen (DO), pH, conductivity and temperature were also measured. Data were square root transformed to reduce deviations from normality and homoscedasticity. Data were analyzed using repeated measures ANOVA with LS means test with SAS statistical software (SAS 2000) and R statistical software (R Development Core Team 2006).

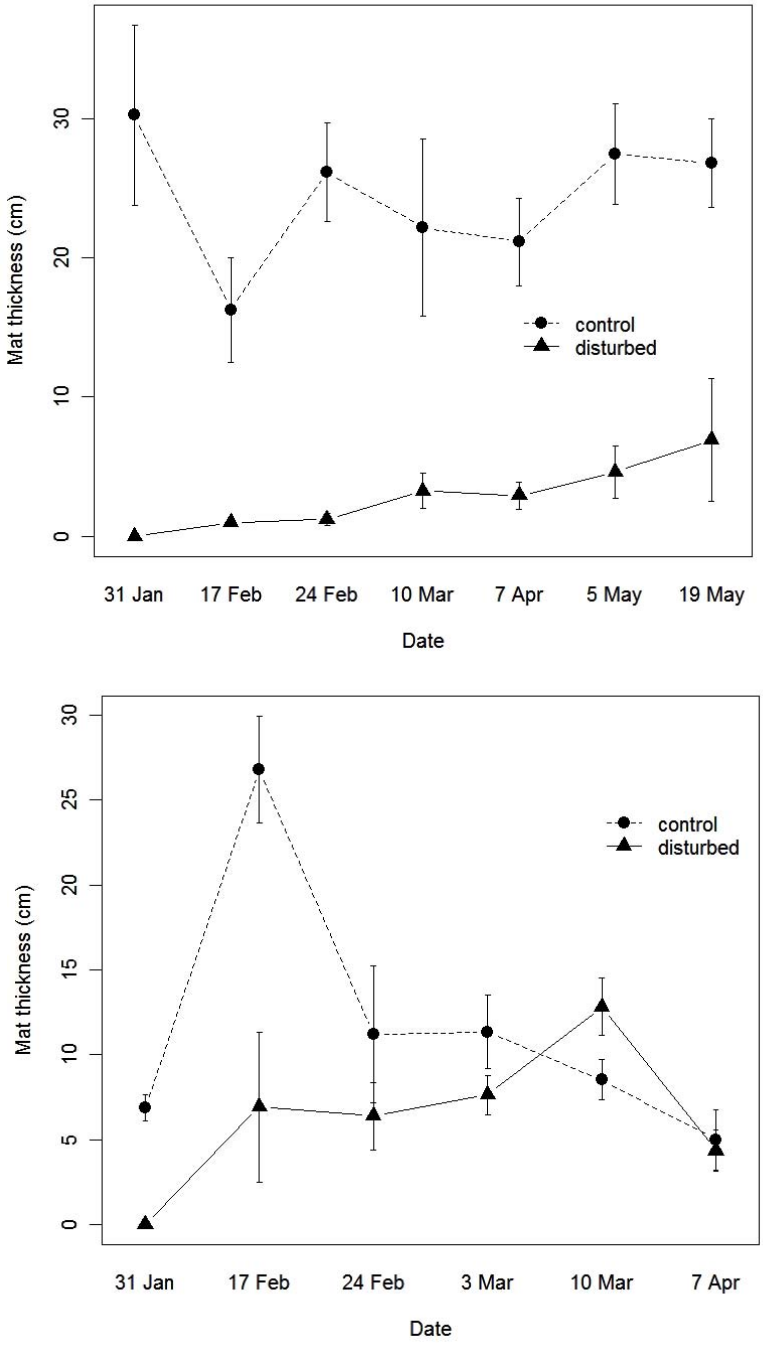


Figure 5.1. Left - Average thickness of the mats of *Lyngbya wollei* at Blue Hole in Ichetucknee springs. Disturbed sites were completely scraped on the 31 of January and control sites were undisturbed. Right - Average thickness of the mats of *Vaucheria* sp. at Manatee spring. Disturbed sites were completely scraped on January 31 and control sites were undisturbed.

5.3 – Overview of Results and Discussion

Mat thickness of *Lyngbya wollei* in control plots did not change during the course of this study (Figure 5.1). Disturbed plots of *Lyngbya*

wollei slowly grew back but did not recover from the disturbance even after 108 days (repeated measures ANOVA: time effect $p<0.0013$, disturbance effect $p< 0.0021$). Mat thickness of *Vaucheria* spp. in the control plots was greatest on February 17 but decreased in April as a result of senescing and decomposing. *Vaucheria* spp. recovered very quickly in disturbed plots; after 24 days there was no difference in the thickness of the mat at the disturbed and control sites (LS means for 24 Feb $p=0.2067$, for 3 Mar $p=0.3108$, for 10 Mar $p=0.2600$, 7 Apr $p=0.8269$).

The two sites studied had similar water quality conditions, however Manatee springs was characterized by a higher variability in conductivity and dissolved oxygen

The difference in recolonization rates of *Vaucheria* sp. and *Lyngbya wollei* in the two springs was probably related to the dispersal mechanisms of the two taxa. Both taxa had similar growth rates in experiments (Pinowska et al. 2007b). Nutrient concentrations in these two springs should have been high enough to support rapid growth. Mechanisms of dispersal vary greatly. *Vaucheria* disperses by fragmentation of mats, by asexual production of zoospores, akinetes and aplanospores, and by spores from sexual reproduction (Starmach 1972, Johanson 2005). *Lyngbya* reproduces only by fragmentation of trichomes (Komárek and Anagnostidis 2005). Thus *Vaucheria* has the capability of dispersing much more rapidly than *Lyngbya*, which could explain more rapid recolonization after disturbance. This probably explains why *Vaucheria* is the dominant macroalga in springs along the Suwannee River where disturbance is high. Manatee, Fanning and Guaranto springs are dominated by mats of *Vaucheria* sp. These springs experience frequent intrusions of turbid waters from the Suwannee River during floods.

6 – EXPERIMENTAL ASSESSMENT OF *LYNGBYA* AND *VAUCHERIA* GROWTH RATES ALONG NITRATE AND PHOSPHATE CONCENTRATION GRADIENTS

6.1 – Introduction

Slow accumulation rates with low loss rates could explain the great abundances of *Lyngbya wollei* and *Vaucheria* biomass in low nutrient as well as high nutrient springs (Pinowska et al. 2007a, Section 2 of this report). Accumulation processes are important for understanding the spatial and temporal variability in benthic algal biomass, but these processes are complexly related to environmental factors (Stevenson et al. 1996, Stevenson 1997, Biggs 2000). Immigration positively affects algal accrual. Grazing, disturbance by physical or biological processes, and death by senescence, disease, and decomposition also are regulated by a variety of factors. Reproduction, another accumulation process, is regulated by light and nutrients, the latter with is the focus of this study. Without significant loss by grazing or physical disturbance, large algal biomass could accumulate over time, even if reproduction rates are low.

Little is actually known about growth of macroalgae in different nutrient conditions and interactions with other environmental factors, such as light, micronutrient concentrations, and conductivity. Many of these factors are known to affect algal growth, but precise quantitative relationships are not well defined. To assess effects of nutrients on algal growth, we should determine those effects in conditions that are otherwise the best for algal growth. Therefore, we

conducted a set of preliminary experiments to determine effects of light, conductivity, and Fe, which was suspected to be an important micronutrient for *Lyngbya wollei* growth.

Growth rates of algae increase rapidly with increasing levels of nutrients when nutrients are in relatively low supply, but then saturate (reach an asymptotic maximum) at high nutrient concentrations. Thus, incremental effects of nutrient increases and decreases have different effects at low and high nutrient concentrations, which is an important consideration for nutrient management strategies. A Monod model is usually used to predict response of growth rates to increasing phosphate or nitrate concentrations when the other resources do not constrain growth (Figure 6.1). Saturating nutrient concentrations for many algae are less than 0.030 mg PO_4-P/L and 0.250 mg NO_3-N/L (Bothwell 1990, Rier and Stevenson 2006, Stevenson et al. 2006). As you will see in this research, the Monod model was only partially sufficient to explain response of algae to increasing nutrient concentrations.

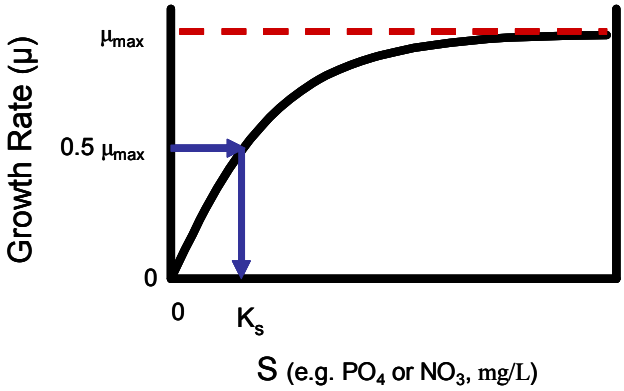


Figure 6.1 The Monod Model. Here growth rate (μ) increases asymptotically with increasing nutrient concentration (S) such that half of the maximum growth rate (μ_{max}) occurs at the half-saturation constant (K_s). Here, $\mu=\mu_{max}(S/(K_s+S))$.

Competition is one of the most important factors affecting growth of algae and affecting maximum biomass accrual. As algae accumulate in the water column or on substrata, they can consume nutrients faster than nutrients are supplied. Nutrients have been assumed to be supplied primarily by mixing from overlying surface waters with interstitial waters in benthic mats. In benthic algae, mixing of overlying surface water around cells decreases as benthic algae density on substrata increase (Carlton and Wetzel 1988). Therefore, nutrient transport into mats decreases because uptake by overlying cells exceeds the mixing rate (Stevenson and Glover 1993). Thus, we would expect that the nutrient concentrations producing maximum algal growth rate would be lower than the nutrient concentrations needed to produce peak algal biomass. This historic model of competition by algae for nutrients may be too simple for macroalgae, as we showed in Section 3 (Sickman et al. 2007). Decomposition or groundwater upwelling in and under mats may also be important sources of nutrients.

Another critical question is "Does nitrogen or phosphorus limit growth of macroalgae in Florida springs?" Historic trends in nitrogen and phosphorus concentrations show that nitrogen has increased much more than phosphorus in Florida springs. Relationships between human activities around sites and nutrient concentrations indicate much greater effect on nitrogen than phosphorus. Thus, reductions in nitrogen loading with best management practices may be much more practical than reductions in phosphorus concentration. However, N:P ratios and algal growth potential assays indicate that P alone or both N and P limit algal growth in many Florida springs.

Is it possible that changes in nitrogen alone could regulate the growth of benthic macroalgae in Florida springs, even if P supply is indicated to be relatively low supply? "Not in many springs," according to Leibig's Law of the minimum, which holds that only one resource at a time limits growth of an alga. If nutrient concentrations are low enough to limit growth of algae, then ratios of nutrients should determine whether nitrogen or phosphorus reductions will lower growth rates. Leibig's Law of the Minimum holds that only one resource at time limits growth of a species. According to current N:P ratios in Florida springs and the Redfield ratio benchmark of 16N:1P, macroalgal growth should be limited by phosphorus rather than nitrogen (Stevenson et al. 2004, Pinowska et al. 2007a). However Stevenson and Pan (1995) show that Liebig's Law of the Minimum may not hold. We observed growth of a diatom species being stimulated by both nitrate and phosphate when the other nutrient was presumed to be in limiting supply. Could the same be true for macroalgae in Florida springs?

The objectives of research presented in this section were to determine effects of nutrients on *Vaucheria* and *Lyngbya wollei* growth rates in highly controlled laboratory experiments. Our goal was to determine the nutrient concentrations, both nitrate and phosphate, that severely limited growth and that saturated growth rates (i.e. that produced no further increase in growth rates) of both macroalgal taxa. We conducted a series of experiments to determine the best conditions for assessing nitrate and phosphate regulation of growth of these macroalgae. We then conducted experiments to determine the limiting and saturating concentrations along nutrient gradients in which all other nutrients were in luxury supply. In these experiments, we varied light, micronutrient concentrations, iron, and conductivity, which have been shown to be important in experiments with other alga and other species of *Lyngbya* (e.g. Ahern et al. 2006, Watkinson et al. 2005). Finally, we conducted experiments to determine the limiting and saturating nitrate concentration for *Lyngbya wollei* and *Vaucheria* when phosphorus was in very low concentrations, as in many Florida springs. We conducted these experiments in multiple experimental systems to confirm that we would find similar relationships in all experimental systems.

6.2 – Overview of Methods

Experiments were conducted in three types of experimental systems to address slightly different hypotheses and to confirm results of the same experimental treatments in two or more settings (Figure 6.2-6.3). The small microcosms were 1.5 mL microcentrifuge tubes filled with growth media and placed on a shaker under lights in a cold room (22.5°C) at Michigan State University. The large microcosms were a set of 49 circular raceways (donut-shaped) made of clear plexiglass, filled with 100 mL of media, and placed on a shaker table under lights in a cold room (22.5°C) at Michigan State University. The mesocosms were rectangular loops of 5 cm PVC pipe, 122 cm long and 91 cm tall, oriented vertically, in which water was circulated; the top of the upper section of the rectangular loop was cut off to allow light to reach the water and algae; and 20 PVC loops were oriented vertically in water baths for cooling in a greenhouse at the University of Florida. Details about these experimental systems can be found in Pinowska et al. (2007b) and Albertin et al. (2007b).

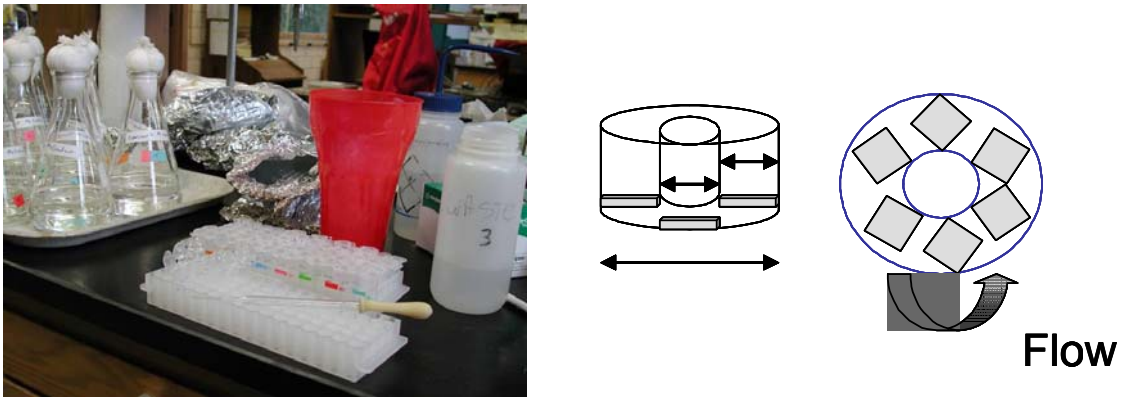


Figure 6.2. The microcentrifuge and donut mesoscosms.

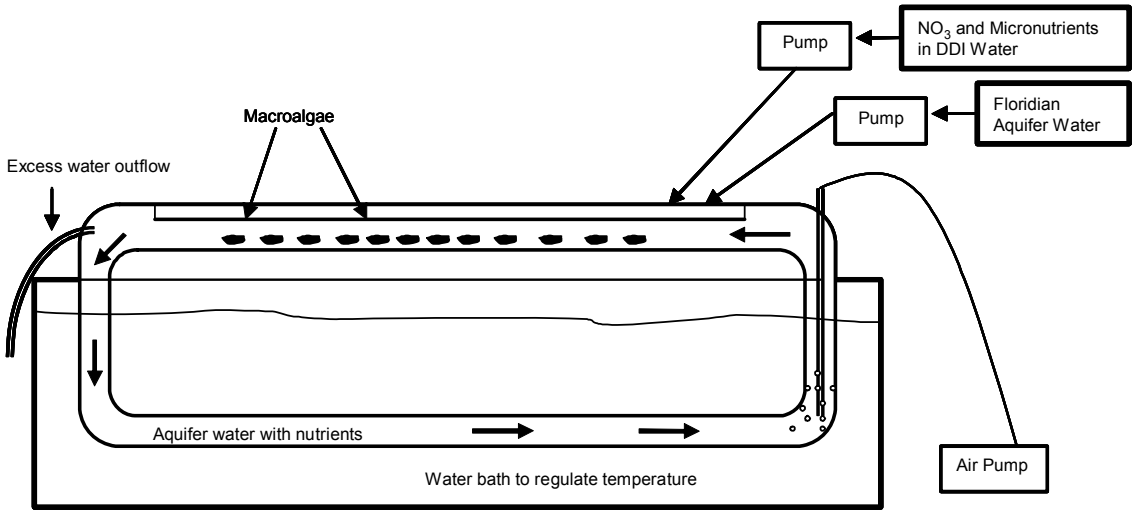


Figure 6.3. The recirculating stream mesoscosm.

Nine and three experiments were conducted in small and large microcosms, respectively, to evaluate good non-nutrient growth conditions and effects of nitrate, phosphate, and ammonia on *Lyngbya wollei* and *Vaucheria* growth. The nutrient levels used in the experiments reflected phosphate and nitrate concentrations observed in Florida springs water. Ammonia concentrations used in the experiments were higher than those observed in Florida springs water but in the range of those observed inside the *L. wollei* mats (Sickman et al. 2007). The small microcosms were valuable for high replication and use of individual filaments. The large microcosms enabled manipulation of biomass in highly controlled experimental settings. The mesocosms were used to reflect the most natural conditions with natural groundwater. To enable enrichment with nutrients to create treatment levels, we used groundwater with very low nutrient

concentrations. As a result, nutrient concentrations in the groundwater used could have been less than natural background nutrient concentrations in some regions of Florida.

6.2.1 Microcosm Experiments

Individual filaments of *Lyngbya wollei* and *Vaucheria* spp. were placed in dishes under the dissecting scope, cut with a scalpel, and then grown in microcentrifuge tubes for 11-12 days. Each filament was photographed before and after incubation in the microcentrifuge tubes to determine changes in filament lengths, which were used to calculate their growth rates. The growth medium was changed every 48 hours to replenish nutrients supplies depleted by the algae. Temperature was maintained in the incubation room at 22.5°C to simulate the average temperature of water in Florida springs. The Z8 medium (Kotai 1972) was modified to simulate the median chemistry of macronutrients in Florida spring water (referred to as medium Z8Fl). The formula for the growth medium was actually the study of preliminary experiments, which will be described subsequently. Ammonia nitrogen, Kjeldahl nitrogen, nitrite-nitrate nitrogen, soluble reactive phosphorus, and total phosphorus concentrations were analyzed routinely by DEP. Water conductivity was measured using YSI 556 MSP. Details about the experimental set-up, media, and treatments for the microcosm experiments can be found in Pinowska et al. (2007b).

6.2.1.1 Microcosm experiments to determine good non-nutrient conditions for growth. Five experiments were conducted using small algal microcosms to determine good non-nutrient conditions for growth. The first experiment compared growth of *Lyngbya wollei* and *Vaucheria* in 3 media and with 2 biomass levels: water from Alexander Spring, water from Silver Glen spring, and Z8Fl medium with either 1 or 5 filaments of macroalgae per centrifuge tube. The second experiment evaluated effects of conductivity where major cation and anion concentrations were varied in the medium to produce conductivities of 100, 300, 500, 1000 and 3000 µS/cm. Both conductivity and light were varied in experiment 3, with 3 conductivity levels (100, 300, 1000 µS/cm) and 5 light levels (1, 2.5, 5, 25, 250 µmol/m^2/s). Experiment 4 was relatively simple, with just 2 treatments: with and without micronutrients. Experiment 5 determined the effect of iron on macroalgal growth with 4 iron treatments (0, 20, 200, 600 µg Fe/L).

The results of these experiments showed that *Lyngbya wollei* and *Vaucheria* grew best in Z8Fl medium with single filaments in microcentrifuge tubes. High conductivity, about 3000 µS/cm, had a negative effect on growth of both macroalgae compared to lower conductivity treatments, which indicated that both taxa were freshwater algae, and not of marine origin. Micronutrients had a negative effect on *Lyngbya wollei* and no effect on *Vaucheria*. Iron concentration in the range of 0.020-0.200 mg/L had optimal effect on growth of both macroalgae, with negative effects at higher and lower treatment levels. Both macroalgae grew better in 25 µmol quanta m^{-2} s^{-1} than in higher and lower light intensities.

6.2.1.2 Microcosm experiments to determine nutrient effects on growth. Single filaments of *Lyngbya wollei* and *Vaucheria* from Florida springs were grown in Z8FL medium-filled microcentrifuge tubes under 25 µmol/m^2/s of light, at 22.5°C, and intermediate iron and conductivity levels. Typically each treatment was replicated 10 times, or 5 times if two

populations (from 2 different springs) were used. Each experiment was designed to evaluate effects of 1 nutrient when other nutrients (macro- and micronutrients) were in luxury supply. The experiments conducted for *Lyngbya wollei* and *Vaucheria* were very much the same, but slight differences should be noted. Experiment numbers in the following list reflect numbers associated with tasks in the project proposal. They are meant to identify the experiments, but do not reflect their order of presentation. Nutrient concentrations are stated in the following methods in µg/L to make numbers easier to read, whereas they are in mg/L in the results and discussion to match the rest of the reports.

The following experiments were conducted with *Lyngbya wollei*:

- Experiments 1, 2 and 3 had a 2 factor design with 10 PO_4 treatments (1, 5, 10, 20, 30, 40, 60, 80, 100 and 250 µg P/L) and 2 population location treatments. The NO_3-N concentrations in experiments 1, 2 and 3 were 1000 µg/L, which was assumed to be a luxury supply.
- Experiment 5 had a one factor design with 11 NO_3-N concentrations: 10, 30, 60, 125, 250, 500, 750, 1000, 1500, 2500 and 5000 µg N/L. The PO_4-P concentrations in experiment 5 was 100 µg/L, which was assumed to be a luxury supply.
- Experiments 6, 7 and 8 had a 2 factor design with 10 NO_3-N concentrations (10, 30, 60, 125, 250, 500, 750, 1000, 2000 and 5000 µg N/L) and 2 population location treatments. The PO_4-P concentrations in experiments 6, 7, and 8 were 100 µg/L, which was assumed to be a luxury supply.
- Experiment 10 had a 1 factor design with 11 NH_4 (10, 30, 60, 125, 250, 500, 750, 1000, 1500, 2500 and 5000 µg N/L). The PO_4-P concentration in experiment 10 was 100 µg/L, which was assumed to be a luxury supply.
- Experiment 12 had a 2 factor design with 16 nutrient treatments: a combination of 4 ammonia concentrations (20, 100, 500 and 1000 µg N/L) and 4 nitrate concentrations (20, 100, 500 and 1000 µg N/L).

The following experiments were conducted with *Vaucheria*:

- Experiment 1 had a two-factor design with 10 PO_4 treatments (1, 5, 10, 20, 30, 40, 60, 80, 100 and 250 µg P/L) and 2 population location treatments. The NO_3-N concentration in experiment 1 was 1000 µg/L.
- Experiment 3 had a two-factor design with 10 NO_3 treatments (10, 30, 60, 125, 250, 500, 750, 1000, 2000 and 5000 µg N/L) and 2 population location treatments. The PO_4-P concentration in experiment 3 was 100 µg/L.
- Experiment 5 had a one-factor design with 11 nutrient treatments (NH_4-N concentrations: 10, 30, 60, 125, 250, 500, 750, 1000, 1500, 2500 and 5000 µg N/L). The PO_4-P concentration in experiment 5 was 100 µg/L.
- Experiment 7 had a two-factor design with 16 nutrient treatments: a combination of 4 ammonia concentrations (20, 100, 500 and 1000 µg N/L) and 4 nitrate concentrations (20, 100, 500 and 1000 µg N/L). The PO_4-P concentration in experiment 7 was 100 µg/L.

6.2.2 Large Microcosm (Donut) Experiments

The large microcosm experiments were conducted under 150 µmol/m^2/s of light. Each microcosm was filled with 100 ml of growth medium, which was changed daily. Small

fragments of *Lyngbya wollei* mats were grown in the large microcosms. Changes in their fresh mass and dry mass were determined to calculate growth rates. All experiments had a complete factorial ANOVA design with two experimental factors: nutrient concentration and initial biomass.

- Experiment 4 had 15 treatments which were a combination of five phosphate concentrations (PO_4-P concentrations: 2, 10, 30, 60, 150 µg P/L) and three initial fresh mass levels (0.01 g, 0.05 g, 0.2 g). The NO_3-N concentration in experiment 4 was 1000 µg/L.
- Experiment 9 had a total of 15 treatments which were a combination of five nitrate concentrations (NO_3-N concentrations: 20, 125, 500, 1000, 2500 µg N/L) and three initial fresh mass levels (0.01 g, 0.05 g, 0.2 g). The PO_4-P concentration in experiment 9 was 100 µg/L.
- Experiment 11 had 15 treatments which were a combination of five ammonia concentrations (NH_4-N concentrations: 20, 125, 500, 1000, 2500 µg N/L) and three initial fresh mass levels (0.01 g, 0.05 g, 0.2 g). The PO_4-P concentration in experiment 11 was 100 µg/L.

6.2.3 Mesocosm Experiments

Two mesocosm experiments were conducted consecutively to evaluate effects of nitrate enrichment when phosphorus concentration was low. Mesocosms were located in a climate controlled greenhouse on the University of Florida, Gainesville. Groundwater from the Floridan Aquifer, nitrate, and micronutrients were added continuously to the channels with peristaltic pumps. The groundwater had low nutrient concentrations ($NO_3 <$ 0.001 mg N/L, PO_4-P = 0.009 mg P/L) and was intended to simulate spring water. Water temperature in the recirculating stream channels averaged 21°C during Experiment 1. The pH ranged from 6.27 to 8.76, the dissolved oxygen ranged 8.28 to 12.00 mg/L, and the conductivity range was 292 to 357 µS/cm. During Experiment 2, water temperature in the recirculating stream channels averaged 20.5°C. The pH ranged from 7.03-8.75 and the dissolved oxygen averaged 7.01 mg/L. Conductivity ranged from 228 to 323 µS/cm. Light averaged 650 µmol m^{-2} s^{-1} and varied with cloudiness during both experiments. Details about the experimental set-up are in Albertin et al. (2007b).

The first experiment ran for 28 days and had seven treatments: 2 treatments had no nitrate addition (Control A and Control B) and 5 treatments of nitrate additions (ranging from 0.001to 5.000 mg N/L) in the form of $NaNO_3$. All treatments except Control A received a Z8Fl micronutrient supplement. Phosphorus was not added to any treatment. Experiment 1 was designed to identify the range of nitrate concentrations in which threshold responses in macroalgal growth rates would be observed, which would be investigated more thoroughly in Experiment 2. Experiment 2 ran for 21 days and had seven treatments: one control treatment with no nitrate addition and six nitrate treatments ranging from 0.025 to 0.750 mg NO_3-N/L. All seven treatments in Experiment 2 received the Z8Fl micronutrient supplement. No phosphorus was added to any treatment. In both experiments, phosphate was depleted rapidly in the mesocosm from groundwater concentrations of 0.009 to less than 0.005 mg/L PO_4-P. Phosphate concentrations were usually 0.002-0.003 mg/L PO_4-P.

Vaucheria spp. and *Lyngbya wollei* from Florida springs were collected for mesocosm experiments. Fresh mass of small fragments of algal mats of each species were determined before and after incubation in the mesocosms. Six mat fragments each of *Vaucheria* spp. and *Lyngbya wollei* were randomly placed in each channel for Experiment 1. Nine mat fragments per genus were placed in each stream channel for Experiment 2.

During Experiments 1 and 2, temperature, conductivity, pH, and dissolved oxygen were measured every three days in every stream channel. Water samples for chemical analyses were taken five times during Experiment 1 (Days 0, 7, 14, 21 and 28) and four times during Experiment 2 (Days 0, 7, 14 and 21). The samples were analyzed for total Kjeldahl nitrogen, total phosphorus, soluble reactive phosphorus, nitrate, ammonium, dissolved organic carbon, cations, and trace metals. Nitrogen and phosphorus in algal mats was also determined.

6.2.4 Data Transformations and Analyses

Algal relative growth rate (RGR) in the small microcosm experiments was calculated using the following formula (Hunt 1990):

$$\text{RGR} = \frac{\ln(\text{Final Length}) - \ln(\text{Initial Length})}{\#\text{ of days}}$$

Data were analyzed using R statistical software (R Development Core Team 2006). If necessary, data were square root or 1/square root transformed to meet the assumptions of normality and variance homogeneity.

To determine threshold values of phosphate and nitrate concentrations causing increased macroalgal growth, the data from several experiments with the same treatments were combined. For example, data were combined for all experiments in microcosms in which PO_4 was manipulated and nitrate was in luxury concentration. A comparison of the experiments listed above and results in Table 6.1 below show how data were combined; details can be found in Pinowska et al. (2007b) and Albertin et al. (2007b). Response of both *Lyngbya wollei* and *Vaucheria* growth showed a response in which growth rates were only affected at intermediate nutrient concentrations. Neither a linear or Monod model fit this response shape, which seemed more like a logistic curve. Therefore, the data were analyzed using a four-parameter logistics model in the drc (dose response curve) package of R statistical software (Ritz and Streibig 2005).

Only statistical results that were statistically significant at the $P<0.05$ level of attained significance are described in this report, unless otherwise noted. This means that differences related to treatments could be observed by chance less than 1 in 20 times if the experiment were repeated.

6.3 – Overview of Results and Discussion

Nitrate, phosphate, and ammonia stimulated growth of *Lyngbya wollei* and *Vaucheria* spp. (Figures 6.4-6.7). In nitrate and phosphate experiments, both macroalgae were able to maintain low growth rates, around 0.10-0.15 ($\ln(\text{mm mm}^{-1})\ \text{d}^{-1}$ or $\ln(\text{g g}^{-1})\ \text{d}^{-1}$), at very low nutrient concentrations. As nitrate and phosphate increased past a threshold, growth of the macroalgae

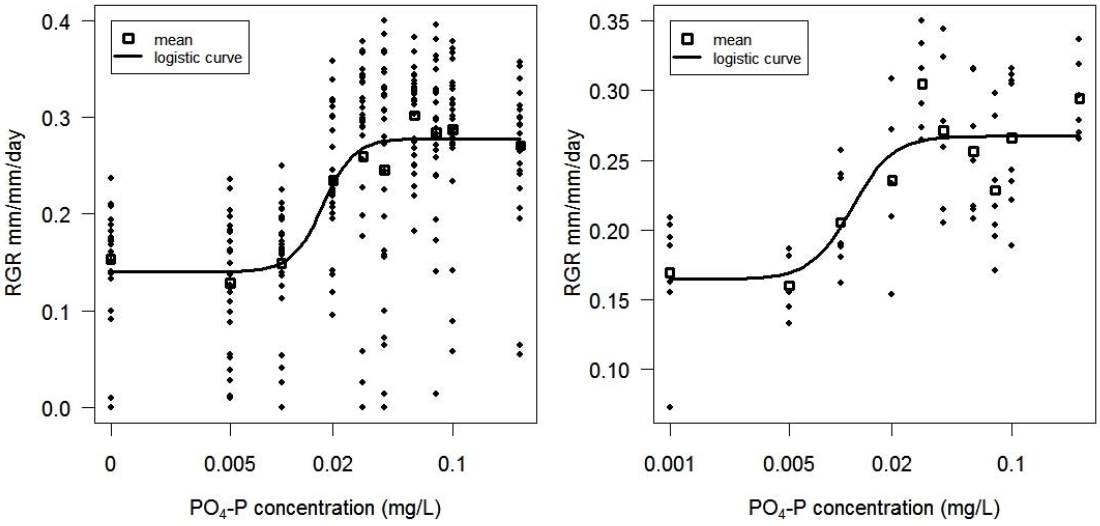


Figure 6.4. Growth rates (RGR) of *Lyngbya wollei* (left) and *Vaucheria* spp. (right) at different phosphorus concentrations in microcentrifuge tubes when nitrate concentrations were high.

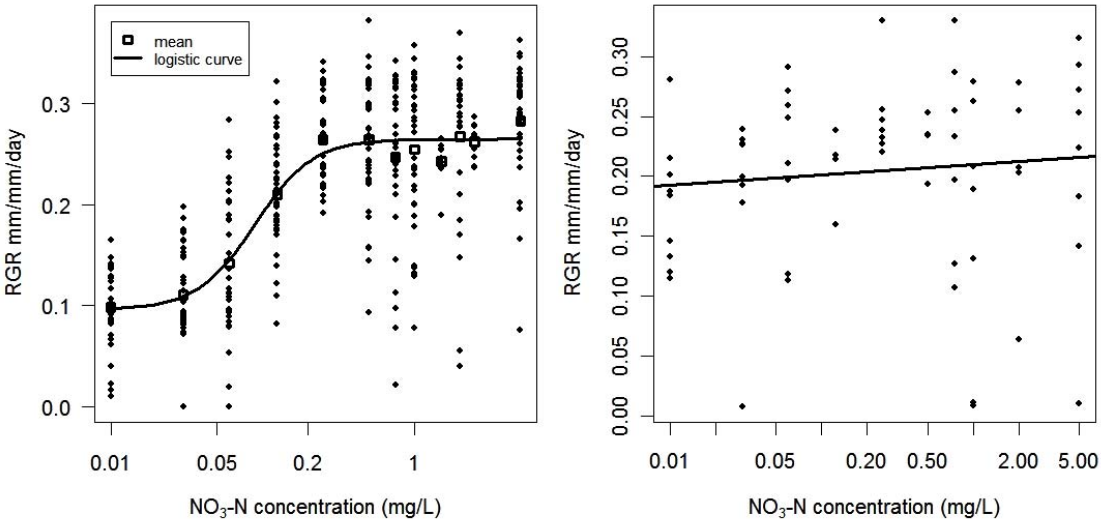


Figure 6.5. Growth rates (RGR) of *Lyngbya wollei* (left) and *Vaucheria* spp. (right) at different nitrate concentrations in microcentrifuge tubes when phosphate concentrations were high.

were stimulated until peak growth rates were reached and additional nutrient supply had no effect on growth. Ammonia, however, did have a negative effect on growth rates of both macroalgae in the high concentration treatments, which were the same as observed in some thick macroalgal mats (Sickman et al. 2007).

Response of the macroalgae to nitrate and phosphate enrichment followed a classic logistic dose-response curve observed in many toxicological studies, but instead of a negative effect of the contaminant, nutrients had a positive effect on growth rate. Using parameters from the dose response curve statistics, several parameters could be estimated that have value for macroalgal management (Table 6.1):

- minimum growth rates (r_{min});
- maximum growth rates (r_{max});
- the increase in growth rate with nutrient enrichment (r_{inc});
- constraining concentrations: the nutrient concentration at which growth was predicted to be elevated by 10%, below which growth of the macroalgae is constrained about as much as possible (ed10 – effective dose with 10 % effect); and
- saturating concentrations: the nutrient concentration at which growth was predicted to be elevated by 90%, above which no effects of nutrient reduction would be expected (ed90 – effective dose with 90 % effect).
- the nutrient concentration at which growth was predicted to be elevated by 50% of the predicted change between r_{min} and r_{max} (ed50).

In all cases reported in the text of these results, there were significant increases in algal growth with nitrate and phosphate concentrations. Ammonia concentrations also stimulated algal growth at low concentration ranges, but high ammonia concentration had a negative effect. This presentation of results will focus on nitrate and phosphate as management targets and the nutrient concentrations that delineate the range of concentrations in which effects are observed.

Before evaluating the specific nutrient concentrations affecting algal growth, it is important to examine the relative consistency in parameters differences among experiments. The nutrient concentrations at which effects were observed (ed10, ed50, and ed90) were lower for microcentrifuge tubes than donuts, which may have been due to less depletion of nutrients in microcentrifuge tubes than in donuts. Decreases in nutrient concentrations during the incubation period were greater for donuts than the microcentrifuge microcosms (Pinowska et al. 2007b). Increases in biomass in donuts increased the ed50 and ed90, consistently decreased r_{max}, and usually decreased r_{min} for *Vaucheria.* Increased biomass had a negative effect on *Lyngbya wollei* growth rates, but the response of *Lyngbya wollei* to nutrients could not be expressed with parameters of the logistic model because nutrient responses were reduced greatly in high *Lyngbya wollei* biomass treatments. This too may have been related to the greater depletion of nutrients when more algae were present to levels that were below saturation levels. No effects on growth rate were related to the spring from which the algae came, i.e. to either preconditioning or genetic differences among populations.

Lyngbya wollei in mescosms responded to NO_3 at lower concentrations, with lower growth rates, and with smaller increases in growth rates than in microcosms or donuts. This was probably related to the nature in which filaments were bundled and P concentrations were much lower in mesocosms than both microcosm types. Filaments were bundled together in relatively tight masses, which would have reduced light penetration and mixing of water and nutrients into the masses. This reduction in mixing is known to reduce nutrient concentrations in some microalgal mats, such as those dominated by diatoms (Stevenson and Glover 1993). However, our work did

show that this is not always the case when mats become thicker and other processes become important in producing nutrients in interstitial waters of mats (Sickman et al. 2007).

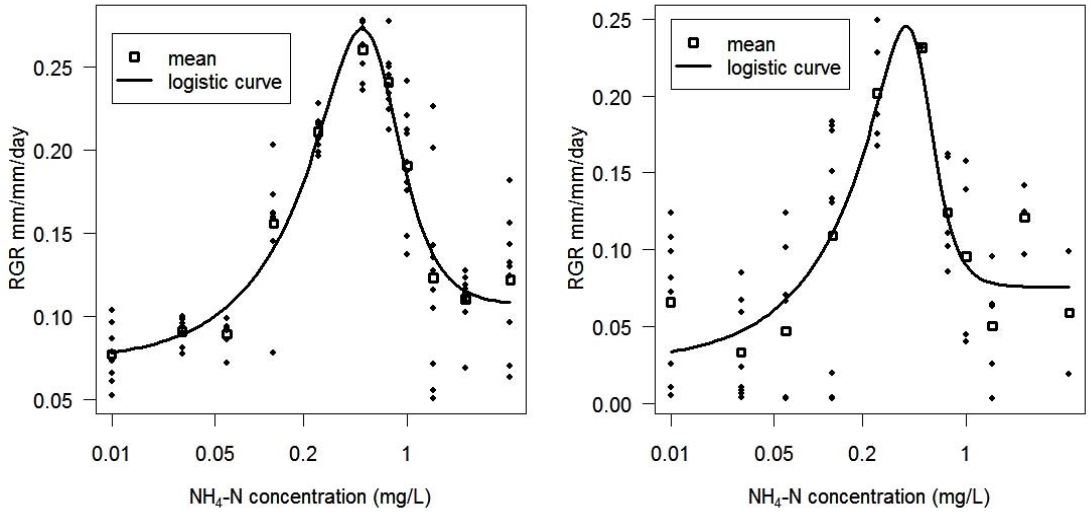


Figure 6.6. Growth rates (RGR) of *Lyngbya wollei* (left) and *Vaucheria* spp. (right) at different ammonia concentrations in microcentrifuge tubes when phosphate concentrations were high.

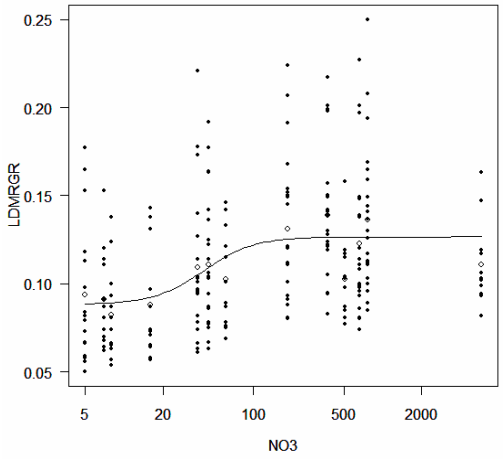


Figure 6.7. Growth rates (RGR) of *Lyngbya wollei* in mesocosms at different nitrate concentrations (µg/L) when phosphate concentrations were high.

The range of nutrient concentrations that constrained and saturated growth of *Lyngbya wollei* and *Vaucheria* spp. were very similar in experiments. R_{max} often ranged from 0.1-0.3 d^{-1}, depending largely on biomass and nutrient depletion in the experiment. Peak growth rates of many algae are 0.3 (Stevenson 1984), and higher growth rates have been observed (Manoylov and Stevenson. 2006, Rier and Stevenson 2006). Even though we optimized as many conditions as possible in experiments, experimental handling may have reduced potential growth rates.

Table 6.1. Summary of macroalgal responses to nutrient (PO_4 or NO_3) enrichment in different experiments, in different systems (small microcosms – microcosm, large microcosms – donuts, and mesocosms). Biomass indicates the amount of algae in the microcentrifuge tube, donut, and mesocosm. Model parameters for the logistic dose response curve and their column heading codes are described in the text above. Model parameters are omitted for sets of experiments in which a logistic response could not be fit. Model parameters are in bold type if they were determined to be statistically significant. ed10, ed50, and ed90 are expressed in mg/L. In the case of the ed10 and ed90, which were harder to estimate, if the standard error of estimates was less than half of the parameter, then they are in bold type.

Taxon	Exp System	Biomass	Nutrient		ed10	ed50	ed90	r(min)	r(max)	r increase
Lyngbya	Microcosm	1 filament	PO4		**0.011**	**0.017**	**0.028**	**0.14**	**0.28**	0.14
Lyngbya	Donuts	0.01g	PO4		**0.024**	**0.049**	**0.101**	**0.15**	**0.23**	0.08
Lyngbya	Donuts	0.05g	PO4	x						
Lyngbya	Donuts	0.2 g	PO4	x						
Vaucheria	Microcosm	1 filament	PO4		**0.006**	**0.012**	**0.022**	**0.16**	**0.27**	0.11
Vaucheria	Donuts	0.01g	PO4		**0.017**	**0.037**	0.078	0.02	**0.15**	0.13
Vaucheria	Donuts	0.05g	PO4		0.015		0.076	**0.06**	**0.11**	0.05
Vaucheria	Donuts	0.2 g	PO4	x						
Lyngbya	Microcosm	1 filament	NO3		**0.034**	**0.088**	**0.230**	**0.10**	**0.26**	0.16
Lyngbya	Donuts	0.01g	NO3		**0.327**	**0.519**	**0.821**	**0.15**	**0.27**	0.12
Lyngbya	Donuts	0.05g	NO3		0.087	**0.546**	3.440	**0.11**	**0.24**	0.13
Lyngbya	Donuts	0.2 g	NO3		0.058	0.808	11.283	**0.11**	**0.15**	0.04
Lyngbya	Mesocosm	masses	NO3		**0.015**	**0.042**	**0.110**	**0.09**	**0.13**	0.04
Vaucheria	Microcosm	1 filament	NO3	x						
Vaucheria	Donuts	0.01g	NO3		**0.069**	**0.210**	**0.644**	**0.10**	**0.25**	0.15
Vaucheria	Donuts	0.05g	NO3		0.036	**0.208**	1.200	**0.07**	**0.19**	0.12
Vaucheria	Donuts	0.2 g	NO3		0.293	**0.499**	0.850	**0.05**	**0.11**	0.06
Vaucheria	Mesocosm	masses	NO3	x						

However, recolonization of disturbed areas of springs was relatively slow (Pinowska et al. 2007b), even after initial colonization of filaments, which indicated that growth of these macroalgae was relatively slow in springs.

The constraining nutrient concentration (ed10) for PO_4 for both *Lyngbya wollei* and *Vaucheria* ranged from 0.006-0.015 mg PO_4-P/L and was generally lowest for the microcentrifuge treatments in which nutrient depletion was least of all experimental settings. In donuts, when nutrients were depleted, the actual nutrient concentrations were much less than the treatment level that was used to characterize exposure level of algae to the nutrients, plus the low number of treatment levels constrained precision in estimates of ed10 and ed90. Since nutrients are not depleted within the macroalgal mats (Sickman et al. 2007) and nutrient depletion in donut experiments was great, results from the microcentrifuge tube experiments should be assigned more significance when predicting the nutrient concentrations in springs that regulate macroalgal growth.

The growth-saturating nutrient concentrations (ed90) for PO_4-P were 0.028 and 0.022 mg/L in microcentrifuge tubes for *Lyngbya wollei* and *Vaucheria*, respectively. Growth-saturating nutrient concentrations were many times higher in low biomass treatments in donuts. The ed90 concentrations from microcentrifuge tube experiments were higher than observed for low-biomass diatom-dominated periphyton, which have been reported to reach peak growth rates around 0.001 mg PO_4-P/L (Bothwell 1989) and 0.016 mg PO_4-P/L by Rier and Stevenson (2006).

The NO_3-N ed10 and ed90 for *Lyngbya wollei* were 0.034 and 0.230 mg/L, respectively, in microcentrifuge tubes. Unfortunately, *Vaucheria* did not respond to nitrate manipulations in the microcentrifuge tube experiments. However, the NO_3-N ed10 and ed90 for *Vaucheria* were 0.069 and 0.644 mg/L in the low biomass treatments in donuts, which would be predicted to be much lower in microcentrifuge tubes because nutrients would not have been depleted. Few other studies have estimated limiting and saturating nutrient concentrations of nitrate for algae. Rier and Stevenson (2006) found growth of low-biomass diatom-dominated periphyton was saturated by dissolved inorganic nitrogen (mostly nitrate) at 0.086 mg/L. Cowles and Dawes (2004) found *Lyngbya wollei* growth rates were similar between 0.070 and 0.300 mg NO_3-N/L, but substantially greater and likely saturated at 0.600 mg/L. Likely reasons for higher estimates of limiting nutrient concentrations by Cowles and Dawes (2004) is that they did not have lower NO_3 treatments than 0.070 mg/L and they incubated masses of algae, rather than individual filaments, so nutrient depletion may have occurred even though they continuously supplied new medium to cultures.

Given historic records of NO_3 being around 0.050 mg/L, our experimental results indicate that nitrate loading into springs from human sources has elevated growth rates of macroalgae from a range of concentrations that severely constrained growth of *Lyngbya wollei* and *Vaucheria* spp. to concentrations that commonly saturate their nitrogen requirements.

The response of *Lyngbya wollei* to nitrate enrichment in the mesocosm experiments, in which no phosphate was added, provides insight into how nitrate additions in all springs can stimulate algal growth, whether spring phosphorus is high or low. We usually assume that either nitrate or

phosphate, or some other nutrient limits growth of a species of algae (i.e. Leibig's Law of the Minimum). But in this experiment, when all indications were that phosphorus should have been the primary limiting nutrient, nitrate enrichment stimulated growth of *Lyngbya wollei*. The following conditions indicate that phosphorus should have been the primary limiting nutrient: phosphate concentrations were low in the mesocosm water, and both N:P and C:P ratios of *Lyngbya wollei* were well above the 16:1 and 106:1 ratios that indicate relative cell requirements for these elements. Therefore, stored phosphorus in cells did not provide an unmeasured supply of phosphorus for cell growth. This appears to be a clear violation of Leibig's Law of the Minimum. Such a violation has also been observed for a diatom species, *Synedra ulna*, but tissue nutrient concentrations were not available in that study to eliminate the hypothesis that intracellular phosphorus stores supported growth (Stevenson and Pan 1995).

Table 6.2. Initial and final C:N, C:P and N:P molar ratios of *Lyngbya wollei* and *Vaucheria* spp. in Experiment 1. Target treatment concentrations (NO_3 mg/L) are shown below treatment numbers.

			Treatment						
			1	2	3	4	5	6	7
Species	Molar Ratio	Day	Cont A	Cont B	0.0005	0.005	0.05	0.5	5
Lyngbya wollei	C:N	0	7	7	7	7	7	7	7
		28	12	15	15	16	15	10	11
	C:P	0	198	198	198	198	198	198	198
		28	286	319	299	345	436	543	630
	N:P	0	29	29	29	29	29	29	29
		28	23	22	20	22	30	52	57
Vaucheria spp.	C:N	0	9	9	9	9	9	9	9
		28	17	17	17	19	20	13	14
	C:P	0	163	163	163	163	163	163	163
		28	293	341	330	351	451	606	672
	N:P	0	18	18	18	18	18	18	18
		28	17	20	19	19	23	45	51

7 - OVERALL DISCUSSION AND CONCLUSIONS

Macroalgae have increased in abundance in Florida springs during the last 50 years, which has corresponded with increases in nitrate concentrations associated with human activities. Macroalgal abundance in Florida springs is now extensive, with almost all springs having macroalgae in them and an average of 50% of spring bottoms being covered by macroalgae. Photographic documentation and historical accounts from many long-time observers record the increase in algae during the last 30-50 years, which corresponds with measured increases in nitrate in springs (Scott et al. 2003). In a review of springs nutrient data collected during the past 50-70 years, Hand (unpublished data, FDEP) observed that significant increases in nitrate

concentration were observed in 48% of Florida's springs and they correspond with increases in chloride concentration, an indicator of human sources of pollutants. Phosphate concentrations decreased in 60% of Florida springs according to Hand's review; however, they did increase in 25% of the springs.

We found that nitrogen concentrations of spring waters were related to the extent and intensity of human activities in the region around sampling sites and abundance of one common macroalga, *Vaucheria*. Abundance of *Lyngbya wollei*, the second of the two most common macroalgae in Florida springs, was not related to spring water nutrient concentrations, however it was positively related to the human activities around sampling sites and other indicators of nutrient supply.

Water column nutrient concentrations may only tell part of the story about nutrient supply to these large macroalgae. The correlation between abundance of *Lyngbya wollei* and phosphorus in sediments both under and throughout the spring reach indicate that either groundwater or accumulations of organic matter in sediments are contributing phosphorus that may also support growth of *Lyngbya wollei* (Pinowska et al. 2007a). These focused sources of nutrients may become sequestered in algae that cover sediments. Low to moderate levels of groundwater nutrient loading could be difficult to detect in simple measurements of surface water chemistry because they would be greatly diluted by the relatively large quantities of overlying spring waters. The positive relationship between diatom indicators of phosphorus concentration indicates that nutrient supply rate was related to abundance of *Lyngbya wollei*. The source of the phosphorus in these sediments should be investigated. Is it transported through the shallow groundwater pathways from watershed sources in dissolved organic or inorganic form or as calcium phosphate? Does P in sediments arise from mat accumulation and burial? Although the correlation between *Lyngbya wollei* % cover and sediment P in exposed sediments as well as under mats suggested that P was coming from the groundwater, exposed sediments may have become contaminated by previous cover by mats or shallow flows in the hyporheic zone.

High biomasses of benthic algae in low nutrient habitats could also be explained by slow accumulation of disturbance resistant algae over prolonged periods. This is the likely explanation for extensive calcareous algal mats in the oligotrophic waters of the Everglades. The algae in the calcareous mats are relatively resistant to disturbance by wind, rain, or grazers. *Vaucheria* and *Lynbgya wollei* mats accumulate during long periods, usually much longer than a month (Sickman et al. 2007). With even slow growth in low nutrient concentrations high accumulations could accumulate over time given growth rates measured in our experiments and low loss rates. This would explain how sediments could become contaminated even if nutrient supply in both ground and surface waters were low.

Another mechanism for producing large mats of macroalgae in relatively low nutrient concentrations is they could become self-sustaining as they accumulate. Nutrient concentrations were high within the macroalgal mats (Sickman et al. 2007). This indicates that sequestration and production of nutrients is greater than efflux and uptake of nutrients. In addition to uptake of dissolved inorganic nutrients by the algae, dissolved and particulate organic matter and particulate inorganic matter could get entrained in mats. After decomposition, this material could be an important nutrient source for surrounding algae. Research to further explore the

metabolic processes within these macroalgal mats will provide a more complete understanding of how such large accumulations of algae can occur even in low nutrient habitats.

Another potential source of nitrogen to *Lyngbya wollei* is nitrogen fixation (conversion of atmospheric N_2 to the bioavailable NH_3). *Lyngbya wollei* has the gene and the capability to convert the unlimited supply of N_2 into a bioavailable form (Phlips et al. 1992). But response of *Lyngbya wollei* growth to nitrate in experiments indicates that its growth is not saturated by atmospheric sources of N_2. Thus nitrate reductions in springs could be effective in reducing both *Lyngbya wollei* and *Vaucheria* growth if reductions are sufficient.

Both nitrogen and phosphorus reductions in Florida springs should reduce macroalgal accumulation because it will slow growth of macroalgae. Such reductions should be effective as long as target concentrations (e.g. nutrient criteria or pollution load reduction goals) are below saturating nutrient concentrations. What are saturating concentrations in the springs? That may depend on a lot of factors and may be difficult to pinpoint exactly. Our most accurate and conservative experimental results, those from microcentrifuge tube experiments, suggest that nutrient concentrations less than 0.028 mg PO_4-P/L and 0.230 mg NO_3-N/L are needed to slow growth (i.e. ed90) of *Lyngbya wollei,* and concentrations less than 0.022 mg PO_4-P/L are needed to slow growth of *Vaucheria* spp. The same experiments show that further reductions to as low as 0.011 mg PO_4-P/L and 0.034 mg NO_3-N/L will continue to slow growth (i.e. ed10) of *Lyngbya wollei* and concentrations to as low as 0.006 mg PO_4-P/L will continue to slow growth of *Vaucheria.* Unfortunately, *Vaucheria* spp. did not respond to nitrate enrichments in the microcentrifuge tube experiments, but it did in low biomass donut experiments. If we use other algae-nutrient combinations to develop ratios for ed90 and ed10 between microcentrifuge and low biomass donut experiments, the ratio for ed90s is about 0.25 and the ratio for ed10s is 0.35-0.46 (Table 6.1, excluding the unusually high ed10 for *Lyngbya wollei* in the low biomass donut nitrate experiments). If we use these ratios to convert responses of *Vaucheria* growth in low biomass donut experiments to comparable benchmarks for nitrate, the ed10 would be 0.017 and the ed90 would be 0.225-0.296 (mean 0.261) mg NO_3-N/L.

To convert these benchmarks from microcentrifuge tube experiments to total nitrogen and total phosphorus concentrations for nutrient criteria in Florida springs, we need to account for the differences in nutrient forms. The benchmarks from the microcentrifuge tubes were in dissolved inorganic form. Therefore, if we determine the difference between nitrate and TN concentrations or phosphate and TP concentrations in Florida springs, we can calculate factors that could convert dissolved nutrient benchmarks to benchmarks in the form of total nutrient concentrations. Average TP and TN concentrations in Florida springs were 0.043 and 0.983 mg/L, respectively, during the 2003 surveys. The average difference between phosphate concentration and TP was 0.007 mg P/L (± 0.009 standard deviation) in our 2003 survey results. The average difference between nitrate concentration and TN was 0.080 mg N/L (± 0.009 standard deviation) in our 2003 survey results. These differences represented 16 and 8 % of the total TP and TN fractions, respectively. Thus dissolved inorganic fractions of total nutrient concentrations are high in Florida springs, probably due to the relatively short distances and large discharges that limit accumulation of particulate matter. Note, dissolved inorganic fractions were variable among springs, with as little as 6 % and 64 % of phosphorus and nitrogen being particulate fractions in some streams. Since ratios of dissolved inorganic to total nutrients

should be relatively consistent within streams, such variations in fractions could be taken into account to develop spring-specific nutrient criteria. Given average TN:NO_x and TP:SRP ratios for Florida springs, the benchmarks for ed10 for *Lyngbya wollei* are 0.013 mg TP/L and 0.037 mg TN/L and for ed90 are 0.033 mg TP/L and 0.250 mg TN/L (Table 7.1). Given average TN:NO_x and TP:SRP ratios for Florida springs, the benchmarks for ed10 for *Vaucheria* are 0.007 mg TP/L and 0.018 mg TN/L and for ed90 are 0.026 mg TP/L and 0.284 mg TN/L, respectively (Table 7.1).

Table 7.1. Nutrient parameter benchmarks for phosphate (PO_4-P) and nitrate (NO_3-N) from microcentrifuge tube experiments (MTM Expt) and their conversion to total phosphorus and total nitrogen benchmarks for nutrient criteria based on average particulate phosphorus and nitrogen fractions in Florida springs.

		Benchmark			
Macroalgae	Logistic Parameter	MTM Expt PO4 (mg/L)	MTM Expt NO3 (mg/L)	TP (mg/L)	TN (mg/L)
Lyngbya	ed10	0.011	0.034	0.013	0.037
Vaucheria	ed10	0.006	0.017	0.007	0.018
Lyngbya	ed90	0.028	0.230	0.033	0.250
Vaucheria	ed90	0.022	0.261	0.026	0.284

Current, light, and mat thickness as well as whether nutrients are supplied from groundwater or surface water will affect the effectiveness of a target concentration. Water column nutrient concentrations, especially expressed as total nitrogen and total phosphorus, are only indicators of actual nutrient supplies supporting algal growth. However, the range of saturating nitrate and phosphate concentrations from experiments, especially microcentrifuge experiments, provide valuable benchmarks for consideration in nutrient load reduction goals. The certainty of macroalgal reductions should be greater if nutrient loads are reduced sufficiently that nutrient concentrations in spring waters are less than the saturating concentrations indicated in the experiments and Table 7.1. Of course the certainty of restoration will increase with greater reductions until we reach concentrations that limit algal growth as much as possible. This concentration is likely less than even the ed10, given the many sources of nutrients and factors affecting nutrient-growth relationships.

The relationship between the extensive results from experiments and limited results from field surveys also provide insight into how results from experiments can be applied in nutrient criteria development. The threshold response in *Vaucheria* biomass at 0.454 mg NO_3-N/L and 0.591 mg TN/L in the spring survey (Pinowska et al. 2007a) are greater than the benchmarks established using the microcentrifuge tube microcosm experiments (Table 7.1). Using the same rationale, the ed90s could be assumed to be reasonable benchmarks for first-stage nutrient criteria development, i.e. interim goals for long-term nutrient load reductions. However, the ed90 benchmarks for nitrate are substantially higher than the nitrogen pollution load reduction goals proposed by the St. Johns River Water Management District (SJRWMD) for the Wekiva River (Mattson et al. 2006), which was 0.156 mg NO_3-N/L; but our ed90 benchmarks for phosphate are about half of the Wekiva River phosphate goal, 0.047 mg PO4-P/L. The higher phosphate goal for the Wekiva River may be due to high natural concentrations of phosphate in the groundwater.

Our phosphorus benchmarks are in the range of the 0.030 mg TP/L benchmark for control of *Cladophora* (Dodds et al. 1997, Stevenson et al. 2006). No detailed studies of nitrogen effects on macroalgae have been done to evaluate the nitrogen benchmarks provided in this study and by SJRWMD.

Reducing nutrient loads sufficiently to stop versus slow growth of the macroalgae may be unrealistic for many reasons. First, results from our experiments show these algae seem to be able to continue to grow, even in very low nutrient concentrations. Second, natural phosphate levels in many Florida springs may be too high to stop their growth and controlling nitrate sufficiently may be impractical. Evidence indicates these macroalgae likely occurred naturally in Florida springs, but not in the excessive abundance as today. *Vaucheria* is reported in spring seeps in areas around the world with very limited human activity. Historic records of *Lyngbya wollei* exist from Silver Spring (Pinowska et al. 2007a). But pictures of these Florida springs show no nuisance algal growths prior to the last 20-30 years.

The continued growth of both *Lyngbya wollei* and *Vaucheria*, even when we added no nitrate or no phosphate to cultures, is unusual, contradicts theory, and is difficult to explain. We observed a relationship between growth and nutrient concentrations that fit in most cases, fit a logistic model rather than a Monod model. Our results were similar to the Monod model in that nutrients stimulated algal growth within a specific range of nutrient concentrations, and above that range, additional increases in nutrients had little effect on growth rates. Unlike predictions of the Monod model, both macroalgae were able to sustain low growth rates when no and low concentrations of nutrients were added to the culture media. Although it does not seem likely that intracellular nutrient stores were sufficient to sustain growth rates, that does seem to be the only reasonable explanation. Cells must have reallocated nutrients to support the most essential processes to maintain sustained growth with no new nutrient allocations. Perhaps cells allocated elemental resources to different processes at different times during the growth cycle to continue to produce new cells with lower and lower nitrogen and/or phosphorus concentrations relative to carbon. More long-term experiments are needed to explore these processes and the seemingly indisputable assumption that algae will not grow if nutrient concentrations are sufficiently low for a sufficiently long time. Regardless of these theoretical considerations, the observation that *Lyngbya wollei* and *Vaucheria* were able to continue to grow in experiments with very low nutrient supplies is also indisputable and is another reason that nutrient load reductions can not be expected to stop accumulation of these macroalgae.

Reducing macroalgal growth rates by reducing nitrate and phosphate loading in springs should have substantial effects on the frequency, intensity, and duration of nuisance macroalgal growths. Slight differences in growth rates produce great differences in how rapidly macroalgae accumulate. To illustrate this, a very simple model of algal accumulation was used simulate algal accumulation with growth rates ranging from 0.01 to 0.30 g g^{-1} d^{-1}. If we started with 1.0 gram of biomass on day 1, we would have very different biomasses after just a month of accumulation, which is a short recovery period given recovery and seasonal persistence patterns observed in our disturbance experiment and the seasonal monitoring after a flood disturbance. Assuming no losses and no other sources of macroalgae (such as algae settling on the bottom), 1, 20, and 8104 grams of biomass would be present if growth rates were 0.01, 0.10, and 0.30 g g^{-1} d^{-1}. Reducing macroalgal growth rates from the common r_{max} near 0.30 to just 0.20 would

reduce biomass accrual over 30 days from 8104 g to 403 g – almost a 20 fold reduction. The benefits of nutrient reduction on algal accrual increase with the length of time that algal mats accumulate. Thus decreases in nutrient concentrations in Florida springs could greatly reduce nuisance macroalgae problems.

8 – REFERENCES

Ahern, K.S., J.M. O'Neil, J.W. Udy, and S. Albert. 2006. Effects of iron additions on filament growth and productivity of the cyanobacterium *Lyngbya majuscula*. Marine and Freshwater Research 57:167-176.

Albertin, A., J. O. Sickman, M. Anderson, A. Pinowska, and R. J. Stevenson. 2007a. Stable isotope compositions of macroalgae, sediment and nitrate in Florida springs. Florida Department of Environmental Protection, Tallahassee, Florida.

Albertin, A., J. O. Sickman, M. Anderson, A. Pinowska, and R. J. Stevenson. 2007. Macroalgal responses to nitrate additions under conditions of low phosphorus in recirculating stream channels. Florida Department of Environmental Protection, Tallahassee, Florida.

APHA. 1998. Standard methods for the evaluation of water and wastewater, 20th edition. American Public Health Association, Washington, DC.

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient- chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19:17-31.

Bonn, M. A., and F. W. Bell. 2003. Economic impact of selected Florida springs on surrounding local areas. http://www.dep.state.fl.us/springs/reports/EconomicImpactStudy.doc, Florida Department of Environmental Protection, Tallahassee, Florida.

Bothwell, M. L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences 46:1293-1301.

Brown, M. T., and M. B. Vivas. 2005. Landscape development intensity index. Environmental Monitoring and Assessment 101:289-309.

Burkholder, J. M., R. G. Wetzel, and K. L. Klomparens. 1990. Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intact biofilm matrix. Applied and Environmental Microbiology 56:2882-2890.

Camacho, F.A. and R.W. Thacker. 2006. Amphipod herbivory on the freshwater cyanobacterium *Lyngbya wollei*: chemical stimulants and morphological defenses. Limnology and Oceanography 51: 1870–1875.

Carlton, R., and R. G. Wetzel. 1988. Phosphorus flux in lake sediments: effect of epipelic algal oxygen production. Limnology and Oceanography 33:562-570.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8:559-568.

Carmichael, W.W., W.R. Evans, Q.Q. Yin, P. Bell, and E. Moczyydlowski. 1997. Evidence for paralytic shellfish poisons in the freshwater cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb. nov. Applied Environmental Microbiology 63:3104–3110.

Dodds, W. K., V. H. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: A case study of the Clark Fork River. Water Research 31:1738-1750.

Dodds, W. K., and E. B. Welch. 2000. Establishing Nutrient Criteria in Streams. Journal North American Benthological Society 19:186-196.
Dudley, T. L., S. D. Cooper, and N. Hemphill. 1986. Effects of macroalgae on a stream invertebrate community. Journal of the North American Benthological Society 5:93-106.
Florida Springs Task Force. 2000. Florida's springs: strategies for protection & restoration. http://www.dep.state.fl.us/springs/reports/index.htm, Florida Springs Report., Florida Department of Environmental Protection, Tallahassee, Florida.
Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of North American Benthological Society 20:358-368.
Gaiser, E. E., D. L. Childers, R. D. Jones, J. H. Richards, and L. J. Scinto. 2006. Periphyton responses to eutrophication in the Florida Everglades: cross-system patterns of strucural and composition changes. Limnology & Oceanography 51:617-630.
Hunt, R. 1990. Basic growth analysis. Unvwin Hyman Ltd., London.
Johanson, L. R. 2005. Phylum Xanthophyta (yellow-green algae). Pages 245-270 *in* D. M. John, B. A. Whitton, and A. J. Brook, editors. The freshwater algal flora of the British Isles. Cambridge University Press, Cambridge.
Joyner, A. J. J. 2004. Molecular techniques applied to environmental samples of the freshwater nitrogen-fixing cyanobacterium, *Lyngbya wollei*. In: ASLO Summer Meeting Abstracts
Kendall, C. 1998. Tracing Nitrogen Sources and Cycling in Catchments. Pages 519-576 *in* C. Kendall, and J. J. McDonnell editors. Isotope Tracers in Catchment Hydrology. Elsevier, Lausanne.
Komárek, J., and K. Anagnostidis. 2005. Cyanoprokaryota. 2. Teil: Oscillatoriales. Gustav Fischer, Jena.
Kotai, J. 1972. Instructions for preparation of modified nutrient solution Z8 for algae. Norwegian Institute for Water Research, publication B-11/69., Blindern, Oslo.
Ksoll, W. B., S. Ishii, M. J. Sadowsky, and R. E. Hicks. 2007. Presence and sources of fecal coliform bacteria in epilithic periphyton communities of Lake Superior. Applied and Environmental Microbiology 73:3771-3778.
Lembi, C. A. 2003. Control of nuisance algae. Pages 805-834 *in* J. D. Wehr and R. G. Sheath, editors. Freshwater Algae of North America. Academic Press, New York, NY.
Manoylov, K. M., and R. J. Stevenson. 2006. Density-dependent algal growth along N and P nutrient gradients in artificial streams. In:. Pages 333-352 *in* N. Ognjanova-Rumenova and K. Manoylov, editors. Advances in Phycological Studies. Pensoft Publishers., Moscow, Russia.
Martin, J., K. Hartl, D. Reide Corbett, and P. Swarzenski. 2003. A multi-level pore-water sampler for permeable sediments. Journal of Sedimentary Research 73:128-132.
Mattson, R. A., E. F. Lowe, C. L. Lippincott, D. Jian, and L. Battoe. 2006. Wekiva River and Rock Springs Run Pollution Load Reduction Goals. St. Johns River Water Management District, Palatka, Florida.
McCormick, P. V. and M. B. Odell. 1996. Quantifying periphyton responses to phosphorus in the Florida Everglades: A synoptic-experimental approach. Journal of the North American Benthological Society 15:450-468.
McGlathery, K.J., D. Krause-Jensen, D. Rysgaard, P.B. Christensen. 1997. Patterns of NH+ uptake within dense mats of the filamentous macroalga *Chaetomorpha linum*. Aquatic Botany 59:99-115.
Meinesz, A. 1999. Killer Algae. The University of Chicago Press, Chicago, Illinois.

Onodera, H., M. Satake, Y. Oshima, T. Yasumoto, W.W. Carmichael. 1997. New saxitoxin analogues from the filamentous cyanobacterium Lyngbya wollei. Natural Toxins 5: 146–151.

Peterson, C. G. 1996. Response of benthic algal communities to natural physical disturbance. Pages 375-402 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, California.

Phlips, E. J., J. Ihnat, and M. Conroy. 1992. Nitrogen fixation by the benthic freshwater cyanobacterium *Lyngbya wollei*. Hydrobiologia 234:59-64.

Pinowska, A., R. J. Stevenson, J. O. Sickman, A. Albertin, and M. Anderson. 2007a. Integrated interpretation of survey for determining nutrient thresholds for macroalgae in Florida Springs: Macroalgal relationships to water, sediment and macroalgae nutrients, diatom indicators and land use. Florida Department of Environmental Protection, Tallahassee, Florida.

Pinowska, A., R. J. Stevenson, J. O. Sickman, A. Albertin, and M. Anderson. 2007b. Integrated interpretation of survey and experimental approaches for determining nutrient thresholds for macroalgae in Florida Springs: Laboratory experiments and disturbance study. Florida Department of Environmental Protection, Tallahassee, Florida.

Potapova, M. G., D. F. Charles, K. C. Ponader, and D. M. Winter. 2004. Quatifying species indicator values for trophic diatom indices: a comparison of approaches. Hydrobiologia 517:25-41.

Quinlan, E. L., E. J. Phlips, K. A. Donnelly, C. H. Jett, P. Sleszynski, and S. Keller. 2007. Estimating Primary Producer Biomass in Silver Springs, Florida, USA. Journal of Applied Ecology.

R Development Core Team. 2006. R: a language and environment for statistical computing. *in*, Vienna, Austria.

Rier, S. T., and R. J. Stevenson. 2006. Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. Hydrobiologia 561:131-147.

Ritz, C., and J.C. Streibig. 2005. Bioassay analysis using R. Journal of Statistical Software 12:1-22.

SAS. 2000. SAS/STAT. *in*. SAS Institute, Cary, N.C.

Scott, T. M., G. H. Means, R. P. Meegan, R. C. Means, S. B. Upchurch, R. E. Copeland, J. Jones, T. Roberts, and A. Willet. 2004. Springs of Florida. Florida Geological Survey, Tallahassee, Florida.

Sickman, J. O., M. Anderson, A. Albertin, A. Pinowska, and R. J. Stevenson. 2007. Estimation of Autochthonous Sources of C, N, and P in Florida Springs. Florida Department of Environmental Protection, Tallahassee, Florida.

Starmach, K. 1972. Chlorophyta III. Zielenice nitkowate: Ulotrhichales, Ulvales, Prasiolales, Shaeropleales, Cladophorales, Chaetophorales, Trentepohliales, Siphonales, Dichotomosiphonales. Panstwowe Wydawnictwo Naukowe, Kraków.

Stevenson, R. J. 1984. How currents on different sides of substrates in streams affect mechanisms of benthic algal accumulation. Internationale Revue gesamten Hydrobiologie 69:241-262.

Stevenson, R. J. 1996. The stimulation and drag of current. Pages pp. 321-340 *in* R. J. Stevenson, M. Bothwell, and R. L. Lowe, editors. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego, CA.

Stevenson, R. J. 1997. Resource thresholds and stream ecosystem sustainability. Journal of the North American Benthological Society 16:410-424.
Stevenson, R. J., and L. L. Bahls. 1999. Periphyton protocols. Pages 6-1 through 6-22 *in* M. T. Barbour, J. Gerritsen, and B. D. Snyder, editors. Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition. U. S. Environmental Protection Agency, Washington, D.C.
Stevenson, R. J., M. L. Bothwell, and R. L. Lowe, editors. 1996. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.
Stevenson, R. J., and R. Glover. 1993. Effects of algal density and current on ion transport through periphyton communities. Limnology & Oceanography 38:1276-1281.
Stevenson, R. J. 1996. The stimulation and drag of current. Pages pp. 321-340 *in* R. J. Stevenson, M. Bothwell, and R. L. Lowe, editors. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego, CA.
Stevenson, R. J., S. T. Rier, C. M. Riseng, R. E. Schultz, and M. J. Wiley. 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia 561:140-165.
Stevenson, R. J., and Y. Pan. 1995. Are evolutionary tradeoffs evident in responses of benthic diatoms to nutrients? Pages 71-81 *in* D. Marino, editor. Proceedings of the 13th International Diatom Symposium 1994.
Stevenson, R. J., Y. Pan, and P. Vaithiyanathan. 2002. Ecological assessment and indicator development in wetlands: the case of algae in the Everglades, USA. Verhandlungen Internationale Vereinigung für Theoretische und Andgewandte Limnologie 28:1248-1252.
Stevenson, R. J., and A. Pinowska. 2007. Diatom indicators of ecological conditions in Florida springs. Florida Department of Environmental Protection, Tallahassee, Florida.
Stevenson, R. J., A. Pinowska, and Y.-K. Wang. 2004. Ecological condiiton of algae and nutrients in Florida springs. Florida Department of Environmental Protection, Tallahassee, FL.
Stevenson, R. J., S. T. Rier, C. M. Riseng, R. E. Schultz, and M. J. Wiley. 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia 561:140-165.
Stimson, J. and S.T. Larned. 2000. Nitrogen efflux from the sediments of a subtropical bay and the potential contribution to macroalgal nutrient requirements. Journal of Experimental Marine Biology and Ecology 252:159-180.
Sundbäck, K., A. Miles, S. Hulth, L. Pihl, P. Engström, E. Selander, A. Svenson. 2003. Importance of benthic nutrient regeneration during initiation of macroalgal blooms in shallow bays. Marine Ecology Progress Series 246:115-126.
Trimmer, M., D.B. Nedwell, D.B. Sivyer, and S.J. Malcolm. 2000. Seasonal organic mineralisation and denitrification in intertidal sediments and their relationship to the abundance of *Enteromorpha* sp. and *Ulva* sp. Marine Ecology Progress Series 203:67-80.
USEPA. 2000. Nutrient criteria technical guidance manual: rivers and streams. EPA-822-B-00-002, United States Environmental Protection Agency, Washington, D.C.
Watkinson. A., O'Neil, J.M., Dennison, W.C. 2005. Ecophysiology of the marine cyanobacterium *Lyngbya majuscula* (Osciallatoriacea). Harmful Algae 4:697-715.

White, D. S., and S. P. Hendricks. 2000. Lotic macrophytes and surface-subsurface exchange processes. Pages 363-379 *in* J. J. Jones and P. J. Mulholland, editors. Streams and Ground Waters. Academic Press.

Yin, Q. Q., W. W. Carmichael, and W. R. Evans. 1997. Factors influencing growth and toxin production by cultures of the freshwater cyanobacterium *Lyngbya wollei* Farlow ex Gomont. Journal of Applied Phycology 9:55-63.

Appendix 1. Study sites, spring names and site codes, sampling dates, and latitude and longitude of transect 1 for each site.

Spring	Spring code	Site name	Site code	Boil	Latitude	Longitude	Date sampled spring 2003	Date sampled fall 2006	Date sampled 2006
Alexander	ALE	Head	ALE-01	Yes	29.08128	81.57563	3/28/2003	10/29/2003	2/2/2006
		Downstream	ALE-02	No	29.08231	81.57754	3/28/2003	10/29/2003	2/2/2006
Chassahowitzka	CHA	Blue holes	CHA-01	Yes	28.71617	82.57502	4/1/2003	11/2/2003	1/21/2006
		Dock	CHA-02	Yes	28.71558	82.57630	4/1/2003	11/2/2003	1/21/2006
		Brown spring	CHA-03	Yes	28.71721	82.57586	4/1/2003	11/2/2003	1/21/2006
Cypress	CYP	Head	CYP-01	Yes	30.65855	85.68430		9/24/2003	1/13/2006
Fanning	FAN	Head	FAN-01	Yes	29.58757	82.93541		10/2/2003	7/26/2006
Gainer	GAI	Pipe	GAI-01	Yes	30.42736	85.54827	4/26/2003	9/25/2003	1/12/2006
		Side boil	GAI-02	Yes	30.42884	85.54854	4/26/2003	9/25/2003	1/12/2006
		Morten Spring	GAI-03	Yes	30.42875	85.54649			1/12/2006
Guranato	GUR	Head	GUR-01	Yes	29.77973	82.94001		10/2/2003	7/26/2006
Homosassa	HOM	After bridge	HOM-01	Yes	28.79961	82.85905	4/2/2003	11/4/2003	
Ichetucknee	ICH	Head	ICH-01	Yes	29.98408	82.76184	3/16/2003	11/22/2003	1/29/2006
		Blue Hole	ICH-02	Yes	29.98068	82.75866	4/20/2003	11/22/2003	1/29/2006
		Below Blue Hole	ICH-03	No	29.98007	82.75895	3/17/2003	11/22/2003	1/29/2006
		Mission spring	ICH-04	Yes	29.97628	82.75783	4/23/2003	11/9/2003	1/29/2006
		Devils Ear	ICH-05	Yes	29.97388	82.75996	4/23/2003	11/9/2003	1/29/2006
		Mill Pond	ICH-06	Yes	29.96658	82.76005	4/23/2003	11/9/2003	1/29/2006
		Before bridge	ICH-07	No	29.95495	82.78507	4/24/2003	11/8/2003	1/29/2006
		Coffee spring	ICH-08	Yes	29.95937	82.77526	4/21/2003	11/8/2003	1/29/2006
Indian	IND	Head	IND-01	Yes	30.25077	84.32203		9/26/2003	1/16/2006
Jackson Blue	JAC	Head	JAC-01	Yes	30.79037	85.13998	4/19/2003	9/24/2003	1/14/2006
		Boat ramp	JAC-02	No	30.78249	85.16022	4/19/2003	9/23/2003	1/14/2006
		Arrowhead camp ground	JAC-03	No	30.75609	85.18680	4/18/2003	9/23/2003	
		Rock cliff	JAC-04	Yes	30.79023	85.14290			1/14/2006
Juniper	JUN	Head	JUN-01	Yes	29.18365	81.71201	3/27/2003	10/31/2003	2/2/2006
		Fern Hammock	JUN-02	Yes	29.18364	81.70801	3/27/2003	10/31/2003	2/2/2006

Spring	Spring code	Site name	Site code	Boil	Latitude	Longitude	Date sampled spring 2003	Date sampled fall 2006	Date sampled 2006
		River fork	JUN-03	No	29.18519	81.70726	3/27/2003	10/31/2003	
		After bridge on route 19	JUN-04	No	29.21283	81.65431	3/26/2003	10/31/2003	
Lafayette Blue	LAF	Head	LAF-01	Yes	30.12592	83.22617		9/29/2003	7/26/2006
Little River	LTR	Head	LTR-01	Yes	29.99642	82.96675		9/28/2003	7/26/2006
Madison Blue	MAD	Head	MAD-01	Yes	30.48056	83.24439		9/29/2003	7/26/2006
Manatee	MNT	Head	MNT-01	Yes	29.48952	82.97692		10/3/2003	1/31/2006
Pitt	PIT	Head	PIT-01	Yes	30.43288	85.54616			1/12/2006
Ponce de Leon	PON	Head	PON-01	Yes	30.72090	85.93071	4/26/2003	9/22/2003	1/10/2006
Rainbow Spring	RAI	Head	RAI-01	Yes	29.10223	82.43741	4/4/2003	11/12/2003	1/19/2006
		KP Hole	RAI-02	Yes	29.09294	82.42848	4/4/2003	11/12/2003	1/19/2006
		Before tubers sign	RAI-03	No	29.06305	82.42788	4/4/2003	11/12/2003	1/19/2006
		Before bridge	RAI-04	No	29.05223	82.44700	4/4/2003	11/12/2003	1/19/2006
			RAI-05	No	29.09275	82.43133			1/19/2006
			RAI-06	No	29.07650	82.4276			1/19/2006
		After bridge	RAI-07	No	29.05407	82.44717			1/19/2006
Silver Glen	SGL	Head	SGL-01	Yes	29.24603	81.64345	3/26/2003	10/30/2003	2/01/2006
		Natural Well	SGL-02	Yes	29.24583	81.64385			2/01/2006
		Trial in the woods	SGL-03	Yes	29.24400	81.6463			2/01/2006
Silver River	SLV	Head	SLV-01	Yes	29.21619	82.05252	4/3/2003	11/13/2003	1/23/2006
		Second pool	SLV-02	Yes	29.21584	82.04987	4/3/2003	11/13/2003	1/23/2006
		Birds of prey	SLV-03	No	29.21561	82.04112	4/3/2003	11/13/2003	1/23/2006
		Old swimming area	SLV-04	No	29.20500	82.02902	4/3/2003	11/13/2003	1/23/2006
		Cabbage palm	SLV-05	No	29.20211	82.01127	4/3/2003	11/12/2003	1/23/2006
Troy	TRY	Head	TRY-01	Yes	30.00598	82.99756		9/29/2003	7/26/2006
Turtle	TUT	Head	TUT-01	Yes	29.84742	82.89041		9/30/2003	

Spring	Spring code	Site name	Site code	Boil	Latitude	Longitude	Date sampled spring 2003	Date sampled fall 2006	Date sampled 2006
Volusia Blue	VOL	Head	VOL-01	Yes	28.94758	81.33969	3/29/2003	11/20/2003	1/30/2006
		Downstream from stairs	VOL-02	No	28.94679	81.33921	3/29/2003	11/20/2003	1/30/2006
Wacissa	WAC	Head RR	WAC-01	Yes	30.33979	83.99244	4/7/2003	9/27/2003	
		Minnow	WAC-02	No	30.33020	83.98776	4/7/2003	9/27/2003	
		Big Blue	WAC-03	Yes	30.32770	83.98484	4/7/2003	9/27/2003	
Wakulla	WAK	Head	WAK-01	Yes	30.23533	84.30287	4/8/2003	10/1/2003	1/17/2006
		Turnaround	WAK-02	No	30.23318	84.28870	4/8/2003	10/1/2003	1/17/2006
		Bird colony	WAK-03	No	30.22507	84.27470	4/8/2003	10/1/2003	1/17/2006
			WAK-04	No	30.23650	84.29831			1/17/2006
			WAK-05	No	30.23439	84.29505			1/17/2006
			WAK-06	No	30.22836	84.28001			1/17/2006
		Upstream from bridge on 98	WAK-08	No	30.18037	84.24817			1/16/2006
Washington Blue	WGT	Head	WGT-01	Yes	30.45279	85.53044		9/26/2003	1/13/2006
			WGT-02	Yes					1/13/2006
Weeki Wachee	WEK	Head	WEK-01	Yes	28.51747	82.57349	3/24/2003	11/5/2003	1/25/2006
		Boat dock	WEK-02	No	28.51901	82.57361	3/24/2003	11/5/2003	1/25/2006
		WMA	WEK-03	No	28.52481	82.59583	3/24/2003	11/4/2003	1/26/2006
		Roger's Park	WEK-04	No	28.53057	82.62407	3/24/2003	11/5/2003	1/26/2006
Wekiwa	WKW	Head	WKW-01	No	28.71193	81.46037	3/30/2004	11/21/2003	1/26/2006
		Canoe launch	WKW-02	No	28.71269	82.45948	3/30/2004	11/21/2003	1/26/2006
Willford	WIL	Head	WIL-01	Yes	30.43966	85.54763		9/25/2003	1/15/2006