NUTRIENT DYNAMICS IN FLORIDA SPRINGS AND RELATIONSHIPS TO ALGAL BLOOMS

By

ANDREA RUTH ALBERTIN

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2009

To my husband, Francisco, for all of your loving support

ACKNOWLEDGMENTS

I thank my advisors, Dr. James Sickman and Dr. Mark Clark for all of their support and encouragement throughout these four years. This has been an incredibly enriching experience. I would also like to thank the rest of my committee members, Drs. H. Popenoe, M. Brenner and E. Phlips for all of their input and support. The results of this study are a collaborative effort among people in numerous institutions and departments within UF. I thank all of you: Martin Anderson, Delores Lucero, Aga Pinowska, Mi Youn Ahn, R. Jan Stevenson, Scott Fulbright, Kathleen McKee, Sylvia Lang, Kevin Ratkus, Ed Dunne, Jordan Mayor, Kathy Curtis, Jason Curtis, Alicia Peon, Cynthia Gomez-Martin, Leonardo Martinez, Antonio de la Pena, Jenny Saqui, Pio Saqui, Dina Liebowitz, Larry Korhnak, Matt Cohen, Andy Ogram, Rupesh Bhodia, Dakshina Kadiyala, Jaya Das, Jango Badha, Eric Ostmark, Martin Sandquist, Alyson Dagang, Solomon Haile, Yu Wang, Gavin Wilson, Yubao Cao, Cheryl Combs, Rhiannon Pollard, Kelly Jacoby, Grace Crummer, Ted Schuur, Melissa Martin, Haryun Kim, Hiral Gohil, Moshe Dorin, Abid Al-Agely, Robert Compitello, Todd Osbourne, Kanika Inglett, Patrick Inglett, Tae-Goo Oh. I thank the Florida Department of Environmental Protection, particularly Russ Frydenborg and Denise Miller for funding and for their support of the project, as well as the numerous park rangers that helped me while I was doing my field work in springs within the Florida State Parks system. Finally, I would like to deeply thank my family, my husband Chico, my mother Verena, my sisters, Helena and Christina and my brothers-in-law, Mark and Stefan for all of their unwavering support throughout these four years.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

NUTRIENT DYNAMICS IN FLORIDA SPRINGS AND RELATIONSHIPS TO ALGAL BLOOMS

By

Andrea Ruth Albertin

August 2009

Chair: Mark W. Clark
Cochair: James O. Sickman
Major: Soil and Water Science

Increased abundance of filamentous algae has been observed in many of Florida's karst springs over the past 50 years and has been associated with increased ambient nitrate concentrations. However, no quantitative relationship exists between nitrate concentrations and algal biomass. Studies were conducted to assess nutrient dynamics in Florida springs, particularly the effects of increased nitrate levels on the growth of *Lyngbya wollei*, and *Vaucheria* sp., the two most common mat-forming algal species found in these springs. Threshold values of nitrate for algal growth were studied in two recirculating stream experiments. The stable isotopes of algae and spring sediments ($\delta^{15}N$ and $\delta^{13}C$) as well as nitrate ($\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3) and dissolved organic carbon ($\delta^{13}C$) in spring water were assessed regionally, at multiple boil sites throughout North central Florida and the Panhandle and along four spring river runs. Additionally, seasonal variation in stable isotope composition of algae was measured over the course of one year at two springs. In the final study, nutrient cycling within algal mats and in adjacent sediments was assessed using interstitial water samplers and advective and diffusive flow through mats was estimated. Results indicate that *Lyngbya wollei*and *Vaucheria* sp. growth is stimulated by nitrate additions despite very low phosphorus conditions.

Multiple factors are likely affecting stable isotopic values in algae, but results point to relatively distinct species-specific $\delta^{13}C$ compositions, which may be indicative of an algae's relative uptake of and degree of preference for CO_2 (aq) vs. HCO_3^- as a carbon source. Unlike $\delta^{13}C$, algal $\delta^{15}N$ values did not show strong species-specific signatures. Finally, thick algal mats contain relatively large amounts of nutrients, particularly NH_4^+ and organic phosphorus, and diffusion of nutrients occurs out of algal mats into the sediment as well as into the overlying water column.

CHAPTER 1
INTRODUCTION

More than 700 karst springs are found in the state of Florida and their discharge comes from underlying aquifer systems. These aquifers are recharged by seepage from the surface and by sinking streams and sinkholes, making the groundwater that feeds springs particularly susceptible to human activities and land use within a spring recharge basin. Nitrate levels have increased in most springs over the last 50 years, while P concentrations have remained relatively low (Scott *et al.*, 2004). Increased nuisance growth of algae observed in springs throughout the state has been associated with increased concentrations of nitrate in the water (Florida Springs Task Force, 2000) although N:P ratios and algal growth potential assays indicate that P alone or both N and P in combination limit algal growth (Stevenson *et al.*, 2007; Pinowska *et al.*, 2009). Additionally, extensive surveys conducted at springs throughout the state indicate that nutrient supply rates alone do not control the distribution of algae (Pinowska *et al.*, 2009).

The Florida Springs Research Initiative was created by the state in 1999 in order to develop management strategies and establish nutrient criteria to reduce the adverse effects of increased nutrient loading in springs. This study forms part of a larger project conducted in collaboration with Drs. A. Pinowska and R.J. Stevenson from Michigan State University, funded by the Florida Department of Environmental Protection as part of the Springs Initiative. A primary goal of the project was to help establish water quality targets, mainly nitrate concentration levels, for Florida springs.

The overall objective of my research was to assess nutrient dynamics in Florida springs, particularly the effects of increased nitrate levels on the growth of *Lyngbya wollei*, and *Vaucheria* sp., the two most common nuisance algae found in these springs. Four studies were conducted. In the first study (discussed in Chapter 2), two recirculating stream experiments were

conducted to test the effect of nitrate additions on the growth of *Lyngbya wollei* and *Vaucheria* sp. under conditions of low phosphorus, conditions found in many springs throughout the state. Additionally, I sought to obtain threshold values for nitrate stimulation of algal growth that could be used in nutrient criteria establishment. I used stable isotope analysis in two studies to determine nitrate and carbon sources to benthic algal mats and possible factors controlling algal abundance, discussed in Chapters 3 and 4. The stable isotopes of nitrate ($\delta^{15}N$ and $\delta^{18}O$) and dissolved inorganic carbon ($\delta^{13}C$-DIC) in spring water were measured at multiple headwater springs throughout north central Florida. Algae and spring sediments ($\delta^{15}N$ and $\delta^{13}C$) were measured regionally, at multiple boil sites throughout north central Florida and the Panhandle and along four spring-fed river runs, the Weeki Wachee, Rainbow, Silver and Wakulla Rivers. Finally, seasonal variation in stable isotope composition of algae was measured over the course of one year at two springs, Manatee and Ichetucknee Blue Hole. In the fourth study, discussed in Chapter 5, the primary objective was to determine the potential for thick *Lyngbya wollei* and *Vaucheria* sp. mats to regenerate nutrients to sustain algal growth. Interstitial water samples were used to obtain nutrient profiles within algal mats and I estimated advective and diffusive movement of dissolved nutrients out of large algal mats.

CHAPTER 2
GROWTH RESPONSEOF LYNGBYA WOLLEI TO NITRATE ADDITIONS UNDER CONDITIONS OF LOW PHOSPHORUS

Introduction

Eutrophication is a severe problem in aquatic ecosystems world-wide and often results in the undesirable proliferation of algae. Solutions usually involve prescriptive reductions in nutrient loading from point and non-point pollution sources, however, much controversy exists over the total and relative amounts of nutrient reduction required to reverse eutrophication effects (Schindler *et al*., 2008; Howarth & Paerl 2008; Lewis and Wurtsbaugh 2008).

In freshwater systems, nitrogen and phosphorus are the two most important nutrients determining algal growth (Elser *et al.*, 2007; Borchardt, 1996), and phosphorus has traditionally been considered more likely to limit primary production than nitrogen (Hutchinson 1957). In a long-term whole-lake study, Schindler *et al*. (2008) found that P additions, with no N additions, maintained eutrophic conditions, with no reduction in phytoplankton biomass. They additionally suggest that reducing N can favor N-fixing cyanobacteria and therefore, mitigation efforts in freshwater systems and possibly estuaries, should focus on P reduction. However, in a large meta-analysis study of nitrogen and phosphorus bioassay experiments, Francoeur (2001) found that nitrogen was as likely to limit algal biomass growth as phosphorus in lotic environments. Similar results were found by Dodds & Welch (2000). Howarth & Paerl (2008) argue that both N and P mitigation efforts are required to control coastal eutrophication and Lewis & Wurtsbaugh (2008) propose a new N+P control paradigm of nutrient limitation in freshwater systems to replace the original P paradigm. They state, though, that reducing phosphorus loads is still likely to be the most practical management tool for controlling phytoplankton abundance.

In many of Florida's karst springs, N:P ratios and algal growth potential assays indicate that P alone or both N and P in combination (i.e., co-limitation) limit algal growth (Stevenson *et*

al., 2007; Pinowska *et al.*, 2009). Yet, increases in filamentous algae in Florida springs are often attributed to N rather than P since N concentrations have been increasing in many springs, while P concentrations have remained relatively stable and low (Florida Springs Task Force, 2000; Scott *et al.*, 2004). Strong (2004) found significant increases in mean nitrate-N concentrations over the last 100 years in a population of 109 springs (from 0.43 to 1.13 mg L^{-1}), but no significant changes in orthophosphate were found in a population of 35 springs where values ranged from 0.046 to 0.096 mg L^{-1}. Nitrate-N increased in the spring-fed Rainbow River between 1957 and 2006 from 0.08 mg N L^{-1} to 1.22 mg N L^{-1} (Cowell & Dawes, 2008) and nitrate concentrations of several springs in the Suwannee River Basin have increased in the last 40 years (from 0.1 mg N L^{-1} to more than 5 mg N L^{-1}) (Hornsby & Ceryak, 1999 cited in Katz *et al.*, 1999). Phosphorus concentrations are thought to remain low in most springs due to the rapid adsorption of phosphorus by calcitic soils and by the limestone matrix of the aquifer (Rhue, Harris & Nair, 2006; Cohen, 2008). Potential sources of nitrate to groundwater and springs in Florida include inorganic fertilizers, confined animal feeding operations, sewage effluent and atmospheric deposition (Bacchus & Barile, 2005; Katz *et al.*, 1999).

Lyngbya wollei (Farlow ex Gomont) Speziale and Dyck, and *Vaucheria* sp. De Candolle are the two most common filamentous algae found in Florida springs, forming large benthic or floating algal mats (often more than 1 m thick and 2+ meters wide) (Stevenson *et al.*, 2004; Stevenson *et al.*, 2007). Algal mats can be detrimental to spring ecology by out-competing native submerged aquatic vegetation (Doyle & Smart, 1998) and algal decay can cause oxygen depletion in the water column (Anderson, Gilbert & Burkholder, 2002). Dense algal mats also interfere with the recreational use of springs in Florida (Cowell & Botts, 1994), particularly *L. wollei* mats, which can cause an allergic reaction known as “swimmer’s itch” due to the

production of lyngbyatoxin and aplysiatoxin (Mynderse *et al.*, 1977; Cardellina, Marner & Moore, 1979).

Although most of the observed increase in algal biomass in Florida springs is anecdotal, a recent study by Quinlan *et al.* (2008) of Silver Springs, one of the largest springs in Florida, found that the epiphytic and algal mat biomass is higher today than that reported in 1957 (Odum, 1957a). At the same time, nitrate concentrations in the water had doubled, from 0.50 mg to 1.1 mg N L^{-1}(Phelps, 2004 cited by Quinlan *et al.*, 2008). Reference studies by Odum (1957b) and Whitford (1957) also provide estimates of earlier algal species composition and biomass from which to draw comparisons, adding weight to the argument that algal biomass has increased in many springs. However, it is unclear how increasing nitrogen inputs to Florida springs can cause eutrophication when algal and springwater stoichiometry suggests P limitation.

Liebig's Law of the Minimum states that growth is controlled not by the total supply of nutrients, but by the nutrient in scarcest supply (Hooker, 1917). This principle of nutrient control of primary production has been validated in many studies which tested algal growth response to altered N and/or P supply (e.g. Francoeur, 2001; Elser *et al.*, 2007). But few experiments have addressed the question of whether additions of a non-limiting nutrient can cause increased algal growth when another nutrient is found in limiting amounts, e.g., can algal growth be stimulated by additions of nitrogen under conditions of apparent phosphorus limitation?

The main objective of my study was to determine if nitrate additions could stimulate growth of *Lyngbya wollei* and *Vaucheria* sp. under conditions of apparent phosphorus limitation, simulating what is believed to have occurred in many Florida springs during the 20th Century. In doing so, I also sought to establish threshold values for nitrate stimulation of algal growth that could be used as nutrient criteria for Florida's springs. Additionally, I investigated the influence

of varying nitrate concentrations on algal molar C:N:P ratios under conditions of low phosphorus. However, due to confounding results with *Vaucheria* growth data, only experimental results for *L. wollei* will be presented and discussed. I used a series of recirculating stream channels (Rier & Stevenson, 2006; Mulholland *et al.*, 1991), operated under controlled laboratory conditions to test two specific hypotheses in regard to algal growth in Florida springs: (1) *L. wollei* growth can increase with additions of nitrate under conditions of apparent phosphorus limitation (i.e., less than the Redfield N:P ratio of 16:1) and (2) threshold values of nitrate concentration for algal growth exist for *L. wollei*.

Methods

Study Site and Experimental Setup

Two experiments were carried out in a climate controlled greenhouse on the University of Florida campus in Gainesville, Florida between March 21 and May 25, 2006. Both studies were conducted in 20 recirculating stream channels. Each channel consisted of a closed loop made of 5-cm-diameter PVC pipe, 122 cm long and 91 cm tall. The upper horizontal section of the stream channel was cut in half length-wise to provide a channel for the algal cultures to grow in ambient sunlight (Figure 2-1).

Groundwater from the Floridan Aquifer and a nutrient solution of nitrate plus micronutrients were continuously added to the recirculating streams using two peristaltic pumps. The groundwater was obtained on the University of Florida campus from a 350-ft well and had low nutrient concentrations ($NO_2^- + NO_3^- < 1$ μg N L^{-1}, soluble reactive phosphorus = 9 μg P L^{-1}); there are only a few natural springs in Florida with lower N and P concentrations (Stevenson *et al.*, 2004). Although using water from a low-nutrient-concentration spring would have been ideal, it was logistically infeasible given time and transportation constraints (the nearest spring was over 3 hours away by car). Every 3 to 4 days, 350 gallons of well water were pumped into a

plastic tank and trucked to the greenhouse. The anoxic well water was aerated for 24 hours to remove hydrogen sulfide (H_2S) before the water was added into the stream channels; high levels of H_2S are harmful to algae. During the oxygenation process nearly all of the ambient iron in the water was precipitated, therefore iron and other micronutrients were included in the experimental nutrient additions to reach ambient levels in natural springs and avoid micronutrient limitation. Earlier studies of Florida springs indicate that micronutrient limitations is not likely *in situ* (Stevenson *et al.*, 2004)

Before nutrient dosing occurred in Experiments 1 and 2, the stream channels were sterilized by soaking in a 5% bleach solution for approximately 24 hours, rinsed and soaked in tap water for 48 hours and then soaked with water from the Floridan Aquifer for 4 days. All the tubing used in the experiment was also soaked for 4 days in water from the aquifer prior to nutrient dosing.

Continuous water flow in each channel was maintained with an air pump which produced bubbles that lifted the water (e.g. an air-lift), causing it to circulate. Current velocity was maintained at approximately 25 cm s^{-1} using a valve in each stream channel that controlled the amount of air that was pumped into the channel. This flow rate is a good approximation of the water velocity found in many first magnitude springs in Florida. A small hole (0.7 cm) was drilled at the end of each stream channel to allow excess water to flow out. There was a complete turnover of water in each channel every eight hours, with an injection rate of 15.1 ml min^{-1}. To help regulate stream channel water temperature, the channels were operated in a large pool of water made up of concrete blocks covered with pond lining which was filled with 0.5 m of water.

Nutrient Dosing

Experiment 1 was run for 28 days and consisted of 7 treatments: 2 treatments with no nitrate additions (Control A and Control B) and 5 treatments of varying nitrate additions (from 1

to 5000 μg N L^{-1}) in the form of $NaNO_3$ (Table 2-1). All but one of the control treatments (Control A) received a micronutrient supplement (FLZ8) to prevent micronutrient limitations during the course of the experiment (Table 2-2). The micronutrient solution was continuously pumped into each channel at a rate of 0.198 ml min^{-1}. The supplement was adapted from the Z8 medium (Kotai, 1972) to reflect median water chemistry in Florida springs based on extensive field surveys in 2003 (Stevenson, Pinowska & Wang, 2004). The only P the algae received came from the well water and any P that was released from the algal tissues themselves and subsequently assimilated. All treatments were randomly assigned to three stream channels except for the control treatment with no micronutrient additions, which was randomly assigned to two stream channels.

Based on the results from Experiment 1, a narrower range of nitrate levels was tested in Experiment 2. Experiment 2 was run for 21 days and consisted of 7 treatments: 1 control treatment with no nitrate additions and 6 treatments of nitrate additions (in the form of $NaNO_3$) ranging from 25 to 750 μg N L^{-1} (Table 2-1). All seven treatments received the FLZ8 micronutrient supplement used in Experiment 1 (Table 2-2) at the same injection rate (0.198 ml min^{-1}) and P was not added to any treatment. The six treatments receiving nitrate additions were randomly assigned to three stream channels and the control treatment was randomly assigned to two stream channels.

Algae Collection and Sample Preparation

The *Vaucheria* sp. used in Experiments 1 and 2 was collected in the boil area of Alexander Springs in the Ocala National Forest (latitude 29.08128, longitude 81.57563). The *Lyngbya wollei* for Experiment 1 was collected in the boil area of Ichetucknee Head Springs (latitude 29.98408, longitude 82.76184) and in the boil area of Alexander Springs for Experiment 2.

Small fragments of the algal mat of each species were gently patted with 0.35 μm Nitex bolting cloth (TETKO, Inc., Elmsford, NY, U.S.A.) to remove excess moisture and then weighed. The fresh mass of each fragment was 1g (± 0.1 g). Each weighed fragment was then attached to an unglazed 2.5 cm^2 white ceramic tile with one small rubber band, making sure that the majority of filament ends were loose and free-floating. The tiles were previously soaked for 2 weeks in deionized water. Six tiles each of *Vaucheria* sp. and *L. wollei* were randomly placed in every stream channel for Experiment 1. Nine tiles per species were placed in each stream channel for Experiment 2.

During both experiments, all 20 streams were covered with a gray plastic screen placed four inches above the channels to reduce incident light levels by approximately 50%, to prevent photo-inhibition and better represent field conditions. With the screen in place, light levels reaching the algae at 12:00 pm varied from as low as 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during cloudy days to as high as 850 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during sunny days. Light measurements were taken using a Licor Li-192 underwater quantum sensor (LI-COR, Inc. Lincoln, NE, U.S.A.).

Water Sampling and Analysis

During Experiments 1 and 2, temperature, conductivity, pH, and dissolved oxygen (DO) were measured every three days in every stream channel using a YSI 556 Multi-probe System (YSI Incorporated, Yellow Springs, OH, U.S.A.). For Experiment 1 (four weeks duration), the pH ranged from 6.3 to 8.8, the DO from 8.3 to12 mg L^{-1}, the conductivity range was 292 to 357 $\mu S\ cm^{-1}$ and the temperature range was 19.6 to 22.4 ºC. During Experiment 2 (3 weeks duration), the pH range was 7.0 to 8.6 and the DO ranged from 3.5 to 9.9 mg L^{-1}. The conductivity range was from 228 to 323 $\mu S\ cm^{-1}$ and temperature ranged from 19.1 to 21.6 ºC. Oxygen concentrations, particularly for Experiment 1, were often higher than what is found in the upwelling areas of many springs, but within the range of concentrations found in lower reaches

of spring-fed rivers, where DO concentrations range from 0.9 to 10 mg L^{-1} and algae is found in high abundance (Stevenson *et al.*, 2004). These relatively high DO levels were unavoidable since the stream channels were aerated in order to maintain circulation. All other parameters were always within the range of values found in Florida springs.

Temperature was measured throughout the course of the experiments in two stream channels every 15 minutes with a HOBO H8 Outdoor/industrial 4-channel external data logger (Onset Computer Corporation, Bourne, MA, U.S.A.). The average temperature was 21.0 ºC during Experiment 1 and 20.5 ºC during Experiment 2.

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 (TKN), total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate (NO_3), ammonium (NH_4), and dissolved organic carbon (DOC). Samples were filtered through a 0.45 µm polycarbonate membrane using a filter holder and syringe. Filtered aliquots were collected for SRP, DOC, NH_4^+, and NO_3^-. Unfiltered samples were collected for TKN and TP. Samples for TKN, TP, DOC, NH_4^+, NO_3^- were acidified to pH 2 with concentrated H_2SO_4. All samples were transported on ice and stored at 4°C until analyzed except for SRP samples, which were stored frozen. The holding time was 28 days for NO_3^-, NH_4^+, SRP, DOC, TKN, and TP.

Soluble reactive phosphorus, NH_4^+, and NO_3^- were measured on a Bran+Luebbe Auto Analyzer 3 (Bran+Luebbe, Norderstedt, Germany) using EPA Methods 365.1, 350.1 and 353.2 respectively. DOC was measured in a Shimadzu 5050 TOC analyzer (Shimadzu Corporation, Kyoto, Japan). Total Kjeldahl nitrogen was determined by H_2SO_4 and Kjeldahl salt digestion and flow-injection determination of ammonium (EPA Method 351.2). Total phosphorus was

measured as SRP on a Bran+Luebbe Auto Analyzer 3 after digestion with H_2SO_4 and potassium persulfate (EPA Method 365.1).

Algae Sampling and Analysis

During Experiment 1, one tile per species in each stream was harvested on Days 7 and 14 and two tiles per species were harvested on Days 21 and 28. During Experiment 2, three tiles per species per stream were harvested on Days 7, 14 and 21. Each algal fragment was removed from its tile, gently patted with Nitex bolting cloth to remove excess moisture, weighed for fresh mass and stored frozen. The fragments were subsequently freeze-dried at -91°C under a 35 mTorr vacuum, weighed for dry mass and ground and homogenized in a ball grinder. Percent nitrogen and carbon of the dried algal tissue were measured by high temperature combustion using a Flash EA 1112 Nitrogen/Carbon Analyzer with MAS 200 R Autosampler (Thermo Fisher Scientific Inc, Waltham, MA, U.S.A.). Phosphorus content of dried algal tissues was measured on combusted (550°C) and acid digested (6N HCl) samples as SRP (Anderson, 1976) on a Technicon Autoanalyzer (Technicon Instruments Corporation Wilmington, MA, U.S.A.).

Statistical Analysis

Lyngbya wollei and *Vaucheria* sp. growth data were analyzed separately. Nitrate concentration effects on algal growth were expressed in terms of relative growth rate (RGR), which was calculated as follows (Hunt, 1990):

RGR = ln (final dry mass) - ln (initial dry mass)/# of days (2-1)

Treatment effects on algal relative growth rate over time were analyzed using a repeated measures ANOVA with SAS statistical software. Pair-wise comparisons were analyzed using least squares means.

To determine threshold values of nitrate leading to increased algal growth, the relative growth rate data from Experiments 1 and 2 were combined. Treatment 1 of Experiment 1

(Control A) was excluded from this analysis, however, because it was the only treatment of the two experiments that did not receive the FLZ8 micronutrient addition. The data were analyzed using a four-parameter logistics model in the drc (dose response curve) package of the R statistical software package (Ritz & Streibig, 2005). This model is appropriate for data that has an asymmetric dose response, where the variance is not homogeneous, and the data are not normally distributed.

Molar nutrient ratios were calculated from total nitrogen, total carbon and total phosphorus content of algal tissue. Nitrate concentration effects on algal C:N, C:P and N:P ratios were analyzed with a repeated measures ANOVA using SAS.

Results

Water Chemistry

In Experiment 1, nitrate concentrations on Day 0 were similar to target treatment values, ranging from 7 μg L^{-1} to 5798 μg L^{-1} NO_3 (Table 2-3). Nitrate decreased significantly in all treatments throughout the course of the experiment. Soluble reactive phosphorus ranged from 5 μg L^{-1} to 9 μg L^{-1} on Day 0 and by Day 7, the range was 2 to 3 μg L^{-1} where it remained until Day 28 in all treatments. Total phosphorus ranged from 12 to 16 μg L^{-1} on Day 0 to 4 to 6 μg L^{-1} on Day 28. The DIN:TP ratio was relatively low in Treatments 1 to 5 (Control to 50μg L^{-1}), ranging from 0.9 to 4.5. It increased dramatically in Treatments 6 and 7 (500 and 5000 μg L^{-1}), ranging from 28 to 1081. Dissolved organic carbon increased in all treatments by Day 21 and then dropped by Day 28 to values similar to initial concentrations.

Nitrate concentrations on Day 0 ranged from 5 μg L^{-1} in the control to 766 μg L^{-1} and were similar to target treatment values during Experiment 2 (Table 2-4). Nitrate concentrations decreased in all treatments with time, with the greatest decline occurring between Day 0 and Day 7. As in Experiment 1, SRP and TP values were low in all treatments on all days since no

phosphate was supplemented. SRP ranged from 2 μg L^{-1} to 9 μg L^{-1} on Day 0 and was reduced by Day 7 in all treatments to 2 to 3 μg L^{-1}. Total P ranged from 8 to 36 μg L^{-1} on Day 0 to 3 to 4 μg L^{-1} on Day 21. The DIN:TP ratio increased in all but one treatment (Treatment 4, 150 μg L^{-1}) from Day 0 to Day 21. The highest ratios (above 100) were obtained in the higher nitrate treatments (500 and 750 μg L^{-1} NO_3). Dissolved organic carbon levels during Experiment 2 increased in all treatments from Day 0 to Day 21.

Algal Relative Growth Rate

The growth response of *Lyngbya wollei* to nitrate additions for both experiments is shown in Figure 2-2 and treatment vs. time effects are shown in Table 2-5. Relative growth rate (g/g/day) of *L. wollei* in Experiment 1 decreased significantly ($p<0.05$) in all treatments from Day 0 to Day 28. Growth rate was positively affected by nitrate concentration ($p<0.05$). The highest average growth rates on Day 7 and 28 were observed in Treatment 7 (5000 μg L^{-1} target concentration), with rates of 0.143 and 0.083 g/g/day respectively. Treatment 1 (Control A, which had no micronutrient additions) had the lowest average growth rates, with rates of 0.113 and 0.036 g/g/day on Days 7 and 28. Significant differences in growth rates ($p<0.05$) were found between Treatment 1 and all other treatments.

In Experiment 2, the relative growth rate of *L. wollei* was significantly affected by time ($p<0.05$). The control and two lowest nitrate treatments had consistently lower RGR than the high nitrate treatments (p-value = 0.08) (Table 2-5). Relative growth rate (g/g/day) of *L. wollei* decreased significantly ($p<0.05$) in all Treatments from Day 0 to Day 21 (Figure 2-3). Treatment 7, which had the highest nitrate additions, had the highest growth rate on Day 7 (0.188 g/g/day) while Treatment 5 (250 μg L^{-1}) had the highest growth rate on Day 21 (0.105 g/g/day). The control **(**Treatment 1) showed the lowest average growth rates on Days 7 and 21, with rates of 0.153 and 0.059 g/g/day, respectively.

The growth response of *Vaucheria* sp. to nitrate additions for both experiments is shown in Figure 2-3 and treatment vs. time effects are shown in Table 2-6. For Experiment 1, relative growth rate data for *Vaucheria* sp. were log transformed to meet the assumption of normality. Growth rate was significantly affected by nitrate concentration and time ($p<0.05$) (Table 2-6). All treatments in Experiment 1 had higher relative growth rates on Day 28 than on Day 7 and the peak in growth rate occurred on Day 21 for all treatments except for Treatment 3 (0.005 mg/L), which had continually increasing growth rates from Day 7 to 28 (Figure 2-3, top). Treatment 7 (5.0 mg/L) had the highest relative growth rate on Days 7 and 28 (0.040 and 0.063 g/g/day, respectively). Both control treatments (Treatment 1 and 2) had the negative growth rate on Day 7 and Treatment 1 had the lowest growth rate on Day 28 **(**0.021 g/g/day).

In Experiment 2, *Vaucheria* sp. relative growth rate was not significantly affected by nitrate concentration or time (Table 2-8) and growth rates were low overall (Figure 2-3, bottom). Treatment 4 had the highest average relative growth rate on Day 7 (0.066 g/g/day) and Treatment 5 (0.025 mg/L) had the highest average relative growth rate on Day 21 (0.047 g/g/day). The highest nitrate concentration treatment, Treatment 7 (5 mg/L), had the lowest average growth rates on Days 7 and 21 (0.015 and 0.005 g/g/day respectively). Relative growth rate decreased in all but two treatments (Treatments 2 and 3) from Day 7 to Day 21.

C:N:P Ratios of Algal Tissue

Lyngbya wollei C:N, C:P and N:P ratios were significantly affected by both nitrate concentration and time ($p<0.05$) in both Experiments 1 and 2 (Table 2-5). During Experiment 1, C:N ratios increased in all treatments from Day 0 to Day 28, with the greatest increase (approximately two-fold) in Treatments 2 through 5 (Table 2-6). C:P ratios also increased in all treatments over time, with Treatments 5, 6 and 7, rising from about 200 to over 400. N:P ratios decreased in the lower

nitrate concentration treatments (Treatments 1 to 4) over time, increased slightly in Treatment 5 and almost doubled in Treatments 6 and 7 (from 29 on Day 0 to 52 and 57 on Day 21).

For *L. wollei* in Experiment 2, all nutrient ratios increased between Day 0 and Day 21 except for the N:P ratio of the control (Treatment 1), which decreased slightly from 19 on Day 0 to 18 on Day 21 (Table 2-6). The largest increase in C:N ratio was approximately two-fold and found in Treatments 1, 2 and 3, which received the lowest nitrate concentrations. C:P ratios increased 2 to 4 times in Treatments 1 to 7 from initial to final experiment days. Treatment 4 showed the greatest increase, from a value of 140 on Day 0 to 576 on Day 21. The N:P ratio of the control decreased, but initial N:P ratios of 19 increased to a range of 24 to 48 on Day 21 in all other treatments.

In Experiment 1, *Vaucheria* sp. C:N, C:P and N:P ratios were significantly affected by both nitrate concentration and time ($p<0.05$) (Table 2-6). All C:N and C:P ratios increased from Day 0 to Day 28. The highest C:N ratios (19 and 20) were obtained on Day 28 in Treatments 4 and 5 (Table 2-8). C:P ratios increased by approximately 2-fold in the lower nitrate concentration treatments (Treatments 1 to 4) and by approximately 4-fold in Treatments 6 and 7; the initial C:P ratio of 163 was increased to 606 and 672 respectively on Day 28. The N:P molar ratio decreased in Treatment 1 from Day 0 to Day 28 (from 18 to 17) but increased considerably in Treatments 6 and 7, from 18 to 45 and 51.

In Experiment 2, *Vaucheria* sp. C:N, C:P and N:P ratios were not significantly affected by nitrate concentration, but they were significantly affected by time (Table 2-6). The C:N ratio of *Vaucheria* sp. increased approximately two-fold in all treatments, from the initial ratio of 8 to either 15 or 16 (Table 2-8). C:P ratios increased by 2 to 3-fold in all treatments between initial

and final days, with the highest ratios ranging from 441 to 459 in Treatments 4 through 7. N:P ratio increased from 18 on Day 0 to a range of 20 to 30 on Day 21.

Lyngbya wollei Growth Response to Nitrate Additions

In order to determine threshold nitrate values for *Lyngbya. wollei* growth, a 4-parameter logistics model was used. The dose response curve for *L. wollei* is shown in Figure 2-4 and the parameter estimates of the model are listed in Table 2-9. The lower limit of response (lowest relative growth rate) was 0.088 g/g/day and the upper limit of response was 0.127 g/g/day. The nitrate concentration resulting in 50% growth saturation, the ED_{50}, was 41.5 μg NO_3 L^{-1}(Table 2-10). The ED_{10} (the nitrate concentration at which the growth response was 10% saturated) was 15.6 μg NO_3 L^{-1}. The ED_{90} (concentration at which the growth response was 90% saturated), was 110 μg NO_3 L^{-1}. No dose response curve (drc) models were found that effectively described *Vaucheria* sp. relative growth rate.

Discussion

Algal Response to Nitrogen Additions under Apparent Phosphorus Limitation

Unlike many experiments conducted on the effect of nutrient amendments on algal growth (Rier & Stevenson, 2006; Stelzer & Lamberti, 2001; Francoeur, 2001; Luttenton & Lowe, 2006), this study tested the effects of supplementing only one nutrient, nitrogen, while maintaining phosphorus under what would traditionally be defined as limiting conditions. Despite no addition of phosphorus, both *Lyngbya wollei* showed positive relative growth rates throughout the course of both experiments. The growth rates, however, became increasingly lower by the end of each experiment (the maximum average growth rate for *L. wollei* at Day 28 of Experiment 1 was 0.083 g/g/day and 0.105 g/g/day at Day 21 of Experiment 2). Although low, these values are within the range of values reported in the literature for *L. wollei* (converted to

relative growth rates in Pinowska *et al.* 2009), which range from 0.052 (Yin, Carmichael & Evans, 1997) to 1.47 (Speziale, Turner & Dyck, 1991). In *L. wollei* single filament experiments where both N and P amendments were added, Pinowska *et al*. (2009) obtained maximum relative growth rates of 0.4, more than twice the maximum growth rate obtained in either Experiments 1 or 2. Additionally, they obtained higher growth rates at low P and high N concentrations than at low N and high P concentrations. Relative growth rate values for *Vaucheria* sp. were inconsistent and are difficult to explain. During Experiment 1, growth rates were higher on Day 28 than Day 7, with highest growth rates occurring in the highest nutrient concentration treatments (5 and 0.5 mg L^{-1} NO_3) (Figure 2-3), while during Experiment 2, growth rates were lower in all treatments on Day 21 than Day 7 (Figure 2-3), and the lowest NO_3 concentration treatments (the control and 0.25 mg L^{-1}) showed the highest growth rates.

Despite low growth rates and confounding data for *Vaucheria* sp., the question still remains as to how the algae were able to grow for three to four weeks with only nitrate additions under conditions of extremely low phosphorus (SRP concentrations by Day 7 in all treatments of both experiments ranged from 2 to 4 μg L^{-1} and TP ranged from 15 to 3 μg L^{-1}, Tables 2-3 and 2-4). This appears to contradict Liebig's Law of the Minimum, which states that growth is controlled by the essential nutrient in shortest supply, which was phosphorus. The application of Liebig's Law to algal ecology is grounded in the concept of ecological stoichiometry. The Redfield Ratio of 106C:16N:1P is commonly used to assess nutrient limitations in freshwater systems, although it was originally developed for oceanic phytoplankton (Redfield, 1958) and both ambient and nutrient cell ratios are often used to predict which nutrient may potentially limit growth.

When using ambient ratios to predict nutrient limitations, disagreement exists about which nutrient forms are more indicative of availability (e.g. DIN:SRP vs TN:TP) (Lohman, Jones & Baysinger, 1991; Dodds, 2003). Inorganic forms of nitrogen (NO_2/NO_3 and NH_4) are readily available for uptake, as are some dissolved organic forms, like urea (Berman & Chava, 1999). Large organic N molecules may be available to varying degrees and particulate N is only available once transformed into inorganic forms by bacteria (Lewis & Wurtsbaugh, 2008). With phosphorus, SRP is highly available and dissolved organic phosphorus (DOP) becomes bio-available when alkaline and acid phosphatases are excreted which enzymatically cleave phosphate groups off organic molecules (Paerl, 1982). Particulate phosphorus (that which is not part of living tissue) is available to varying degrees, either unavailable (e.g. metallic precipitates) or potentially available (e.g., adsorbed P on clay or silt) (Lewis & Wurtsbaugh, 2008). Therefore, using TN:TP can overestimate readily available nutrients, while using DIN:SRP can underestimate nutrient availability (Lohman *et al.*, 1991). Morris & Lewis (1988) found that the DIN:TP ratio best predicted nutrient limitation for phytoplankton because it incorporates both external and intracellular nutrient sources, indicating that particulate P is more available that particulate N. They classified lakes with a DIN:TP ratio (by weight) of <0.6 as N-limited and lakes with a ratio > 4 as P-limited, while those with ratios between 0.6 and 4 were considered to be under intermediate limitation.

In both experiments of my study, ambient ratios indicate intermediate limitation in all but one of the nitrate treatments at or below 50μg L^{-1}, with DIN:TP ranging from 1.1 to 4.7 (Tables 2-3 and 2-4). Dodds (2003) found that at low DIN and SRP concentrations, both N and P can limit growth, and this is likely the case in my low nutrient treatments. Treatments with concentrations above 50μg L^{-1} suggest P limitation, with DIN:TP ratios from 11 to 1080.

Additionally, the DIN:TP ratio in the water decreased in all treatments on Days 7 and 14, as nutrients were being drawn down during the days of the highest growth. DIN:TP then increased in the final day of every treatment, further pointing toward P-limiting conditions. Nitrate levels in the highest concentration treatments (Treatment 7 of Experiment 1 and Treatments 6 and 7 of Experiment 2) remained near target concentrations, indicating saturated N concentrations. It is therefore surprising that growth rates were greatest in the higher concentration treatments, where the DIN:TP ratios were extremely high, especially at the end of the study.

Examination of molar cell ratios in both experiments also points to apparent P-limitation when compared to the Redfield benchmark of 106:16:1, particularly in the treatments with nitrate concentrations above 50µg L^{-1}. I obtained N:P ratios of up to 57:1 and C:P ratios of up to 630:1 for *L. wollei* and N:P ratios of up to 51:1 and C:P ratios of up to 672:1 for *Vaucheria* sp. by weeks three and four of the experiments suggesting depleted internal P supplies. However, the optimal stoichiometric ratio for *L. wollei* and *Vaucheria* sp. in Florida springs is not known and this can deviate from the Redfield ratio benchmark due to both species-specific and environmental factors (Duarte *et al.,* 1992; Borchardt, 1996). Hillebrand & Sommer (1999) found that benthic algae from the Baltic Sea had an optimal stoichiometric ratio of 119C:17N:1P, while a review of published data by Kahlert (1998) proposed an optimum stoichiometric ratio for freshwater periphyton of 158:18:1. Townsend *et al.* (2008) found that the optimal ratio for the freshwater algae *Spyrogyra fluviatilis* was much higher, at 1800:87:1. They attributed the higher carbon content of the algae to more cellular structural requirements, particularly the thallus, which is not found in phytoplankton. Therefore, the optimal cell ratios for *L. wollei* and *Vaucheria* sp. may be much higher than the Redfield ratio and needs to be further investigated.

Although both ambient and cell nutrient ratios point to likely P-limitation, both *L. wollei* and *Vaucheria* sp. growth was stimulated by nitrate additions and therefore it is doubtful that it was truly under limiting P conditions as defined by Liebig's Law of the Minimum. Positive growth rates in all treatments at the end of both experiments (including the control which received no N or P supplements) may be due to several factors. First, the algae likely relied on luxury P supplies stored in their cells to supplement low phosphorus concentrations, which helps explain higher growth rates for *L. wollei* in both experiments and *Vaucheria* sp. (in Experiment 2 only) during the initial weeks of the experiment as compared to the end, when, as shown by the high N:P and C:P ratios, nutrient stores were most likely exhausted. The growth response curve fitted for *L. wollei* (Figure 2-4) did not follow the typical "inverted J" form of Michaelis-Menten, Monod or Droop nutrient uptake models (Droop, 1974; Borchardt, 1996) in which initial growth rates are approximately zero. Instead, the growth rate of *L. wollei* was best described by an S-shaped, logistic curve in which rates, even at the lowest nitrate concentrations, were above zero; e.g., the algae were never completely depleted of nutrients and presumably came in with a luxury store of P.

Phosphorus uptake in algae can be 5 to 50 times greater than physiological requirements (Cembella, Antia & Harrison, 1984), which does not occur to the same degree with nitrate ions (Reynolds, 1993). As previously mentioned, Pinowska *et al.* (2009) found that *L. wollei* growth rates were more negatively affected by a lack of N than P, indicating in part the algae's ability to better store P than N in its cells. Nutrient molar ratios of the initial *Lyngbya* mats (Day 0) showed that the algae were initially more P-limited in Experiment 1 (N:P = 29:1) than in Experiment 2 (N:P = 19) (Table 2-7) and this may have accounted for the slightly higher growth rates, particularly during the initial weeks of Experiment 2 as compared to Experiment 1 (Figure

2-2). Decreasing growth rates, however, may have also been due (at least in part) to self-shading and reduction of nutrient diffusion into internal portions of the algal fragments as patches of algae increased in biomass. In addition, senescing algae, diatoms and bacteria from biofilm that grew in the stream channels, particularly those with higher nutrient concentrations, may have been sources of both N and P during the experiments. Dissolved organic carbon concentrations increased in 6 of 7 treatments in Experiment 1 and in all treatments of Experiment 2 (Tables 2-3 and 2-4). Recycled nutrients were likely immediately taken up by algae (and diatoms, bacteria, etc.) to help maintain growth; high nutrient demand and turnover rates resulted in very low nutrient concentrations (Dodds, 2003).

Finally, the algae in this study may have required lower nutrient concentrations to grow than algae under natural conditions which can be more physiologically stressful. Algae were grown under relatively high light levels (not near-limiting for growth) and temperature conditions remained constant and were similar to conditions found in the springs. Algae growing under less than optimal light conditions (the light:nutrient hypothesis (Sterner *et al.*, 1997)) may require more nutrients to grow (Dickman, Vanni & Horgan, 2006; Hessen *et al.,* 2002; Borchardt, 1996), although studies testing this hypothesis in benthic ecosystems have shown mixed results (Cross *et al.,* 2005; Leonardos & Geider, 2004; Hill & Fanta, 2008).

Nutrient Criteria for Florida Springs

Although many studies have been conducted on algal growth response to nutrient amendments, relatively little information is available on actual threshold concentrations by which to establish nutrient criteria (Francoeur, 2001; Stevenson *et al.*, 2008). The threshold values generated by the logistics model for *Lyngbya wollei* (Figure 2-4 and Tables 2-9 and 2-10), with 10% growth rate saturation at 15.6 μg L^{-1} (ED_{10}), 50% saturation at 41.5 μg L^{-1} (ED_{50}) and 90% saturation at 110.0 μg L^{-1} (ED_{90}) are similar to threshold, or breakpoint values reported in

the literature. As mentioned previously, no dose response model was found to describe *Vaucheria* sp. growth. Grimm & Fisher (1986) suggested that periphyton growth in an Arizona stream was N-limited at concentrations below 55µg L^{-1} NO_3-N and results from Lohman *et al.* (1991) indicate that periphyton growth is N-limited in an Ozark stream at concentrations as high as 100µg L^{-1}. In a large meta-analysis of benthic algae in temperate streams, Dodds, Smith & Lohman (2002) found that above 40µg L^{-1} total N, mean chlorophyll values were considerably higher.

Using the threshold values generated by my model to set nutrient criteria for springs would be difficult since these values are very low when compared to nitrate concentrations found in many Florida springs. Results from a comprehensive survey of 63 spring sites throughout Florida conducted in 2006 (Pinowska *et al.*, 2007) show that only eight sites had values below 110µg L^{-1}, only one site had concentrations below 41.5µg L^{-1} and none of the sites had NO_3/NO_2 concentrations below 15.6 µg L^{-1}. NO_3/NO_2 concentrations from 1907-1979 for 87 springs range from below detection limit to 5000µg L^{-1}, with an arithmetic mean of 430µg L^{-1} (Strong, 2004).

As mentioned previously, in many of Florida's springs, N:P ratios and algal growth potential assays indicate P alone or both N and P limit algal growth (Stevenson *et al.*, 2007). Yet, results from this experiment show that under laboratory conditions, *Lyngbya wollei* can grow if given N even if P is in very low supply and that higher NO_3 concentrations result in higher growth rates. Therefore, reductions in N concentrations should reduce algal growth rates in spring systems where this nutrient is found in excess supply. However, algal biomass would likely continue to accumulate, albeit at a slower rate, and nutrient reduction programs may not show quick results. The situation is further complicated because high concentrations of dissolved inorganic N and P are stored within thick *L. wollei* mats (>1m) (Sickman *et al.*, 2009) which are

potentially available for uptake by the algae. Additionally, even though *L. wollei* cannot store N in its cells as it can P, growth continued even in the control treatments of-my experiments, where NO_3 concentrations ranged from 2-10μg L^{-1}.

Finally, biomass accumulation in benthic algae is heavily dependent on factors other than nutrients, such as light, disturbance, grazing and the presence of submerged aquatic vegetation (e.g. Doyle & Smart, 1998; Lohman *et al.*, 1991; Luttenton & Baisden, 2006; Borchardt; 1996). Pinowska *et al*. (2009) did not find a direct relationship between nutrient concentrations and algal abundance in Florida springs and other factors either alone or in combination with nutrients must be affecting algal growth. Studies are needed on how biotic and abiotic factors interact in controlling algae abundance in order to establish management plans to reduce *L. wollei* biomass. The ability of *L. wollei* to grow under surprisingly low nutrient conditions, even when water column and tissue stoichiometry suggest that they should not be growing, makes the task of establishing nutrient criteria in Florida springs more difficult.

Table 2-1. Target nitrate concentrations for Experiments 1 and 2.

Treatment	Target nitrate concentrations ($\mu g\ L^{-1}$)	
	Experiment 1	Experiment 2
1	0	0
2	0	25
3	1	50
4	5	150
5	50	250
6	500	500
7	5000	750

Table 2-2. FLZ8 micronutrient concentrations. The micronutrient solution was continuously pumped into each stream channel at a rate of 0.198 ml min^{-1}. Each stream channel contained 7 L of water at a given time, and water was pumped in continuously at a rate of 15 ml min^{-1}.

Micronutrients	Concentration (µg/L)
$FeCl_3 \cdot 6H2O$	231
EDTA-Na_2	306
$ZnSO_4 \cdot 7H_2O$	7
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	22
$Co(NO_3)_2 \cdot 6H_2O$	4
$VOSO_4 \cdot 6H_2O$	1
$Al_2(SO_4)_3K_2SO_4 \cdot 2H_2O$	12
$NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$	5
$Cd(NO_3)_2 \cdot 4H_2O$	4
$Cr(NO_3)_3 \cdot 7H_2O$	1
$Na_2WO_4 \cdot 2H_2O$	1
KBr	3
KI	2
$Cu(SO_4) \cdot 5H_2O$	3
H_3BO_3	78
$MnSO_4 \cdot H_2O$	42

Table 2-3. Average stream water nutrient concentrations (µg L^{-1}) and DIN/TP ratio for Experiment 1. N refers to the number of stream channels per treatment.

Treatment	Target NO_3 Concentrations	Experiment Day	n	N-NO_3	N-NH_4	TKN	TP	SRP	DIN/TP Ratio	DOC
1	Control A	0	2	10	16	203	12	15	2.2	1335
	0	7	2	2	12	205	13	4	1.1	2290
		14	2	2	16	184	15	3	1.2	1654
		21	2	8	5	166	8	2	1.6	1820
		28	2	8	5	207	7	4	1.9	1941
2	Control B	0	3	7	17	272	12	9	2.0	1854
	0	7	3	2	7	347	11	3	0.8	2443
		14	3	2	8	200	10	2	1.0	2132
		21	3	8	10	176	4	2	4.5	2308
		28	3	8	5	159	5	3	2.6	1756
3	0.5	0	3	7	28	242	12	7	2.9	1953
		7	3	2	17	266	9	3	2.1	2393
		14	3	2	8	215	11	3	0.9	2160
		21	3	8	5	206	7	3	1.9	2349
		28	3	8	5	197	5	3	2.6	2087
4	5	0	3	16	14	301	13	7	2.3	1867
		7	3	2	12	367	11	2	1.3	2406
		14	3	4	10	262	10	2	1.4	2188
		21	3	8	3	206	7	2	1.6	2078
		28	3	8	5	236	4	3	3.3	1855

Table 2-3. Continued.

Treatment	Target NO_3 Concentrations	Experiment Day	n	N-NO_3	N-NH_4	TKN	TP	SRP	DIN/TP Ratio	DOC
5	50	0	3	61	11	418	16	8	4.5	1731
		7	3	4	6	341	9	2	1.1	2373
		14	3	2	10	316	10	2	1.2	2364
		21	3	8	4	181	7	3	1.7	2043
		28	3	8	5	207	6	2	2.2	1922
6	500	0	3	507	13	350	14	7	37	1779
		7	3	315	11	453	9	2	36	2242
		14	3	250	34	301	10	3	28	2279
		21	3	330	9	295	5	2	68	2212
		28	3	226	5	270	6	3	39	1876
7	5000	0	3	5798	22	486	15	5	388	1815
		7	3	4395	9	380	8	2	551	2405
		14	3	3462	24	387	9	2	387	2178
		21	3	4320	5	330	4	2	1081	2262
		28	3	4207	5	324	5	3	842	1838

Table 2-4. Average streamwater nutrient concentrations (μg L^{-1}) for Experiment 2. N refers to the number of stream channels per treatment.

Treatment	Target NO_3 Concentrations	Experiment Day	n	NO_3	NH_4	TKN	TP	SRP	DIN/TP Ratio	DOC
1	Control	0	2	5	10	149	11	2	1.4	1656
		7	2	5	4	299	10	4	0.9	2432
		14	2	8	9	277	4	4	4.3	2615
		21	2	5	6	226	3	2	3.7	2368
2	25	0	3	37	9	274	13	3	3.5	2177
		7	3	5	5	303	4	3	2.5	2413
		14	3	5	8	338	7	2	1.9	2803
		21	3	5	8	308	3	1	4.3	2482
3	50	0	3	45	7	230	11	3	4.7	2079
		7	3	5	2	308	4	2	1.8	2631
		14	3	7	8	328	4	2	3.8	2784
		21	3	71	8	245	3	2	26.3	2465
4	150	0	3	186	7	274	11	3	17.5	2291
		7	3	9	4	299	4	2	3.3	2579
		14	3	62	6	287	4	2	17.0	2607
		21	3	37	10	230	3	1	15.7	2437
5	250	0	3	376	7	358	36	2	10.6	2195
		7	3	18	5	274	4	2	5.8	2547
		14	3	66	14	367	3	2	26.7	2689
		21	3	237	12	303	3	2	83.0	2632

Table 2-4. Continued

Treatment	Target NO_3 Concentrations	Experiment Day	n	NO_3	NH_4	TKN	TP	SRP	DIN/TP Ratio	DOC
6	500	0	3	659	8	319	8	3	83.4	2404
		7	3	292	5	352	5	2	59.4	2572
		14	3	367	11	435	7	5	54.0	2635
		21	3	522	11	367	3	2	177.7	2514
7	750	0	3	766	9	284	8	9	96.9	2104
		7	3	559	5	342	6	2	94.0	2503
		14	3	622	10	401	6	3	105.3	2666
		21	3	743	14	347	4	2	189.3	2440

Table 2-5. Repeated measures analysis of variance for *Lyngbya wollei* relative growth rates and nutrient molar ratios in Experiments 1 and 2. Significant P values ($p<0.05$) are shown in bold.

Experiment	Variable	Source of variation (between groups)	P	Source of Variation (within groups)	P	Source of Variation (within groups)	P
1	Relative Growth Rate	NO_3 Concentration (N)	**<0.0001**	Time	**<0.0001**	Time x N	0.7403
	C:N		**<0.0001**		**<0.0001**		**0.0275**
	C:P		**<0.0001**		**<0.0001**		**<0.0001**
	N:P		**<0.0001**		0.3242		**<0.0001**
2	Relative Growth Rate	NO_3 Concentration (N)	0.0778	Time	**<0.0001**	Time x N	0.9287
	C:N		**0.0015**		**<0.0001**		**<0.0001**
	C:P		**0.0155**		**<0.0001**		**0.0005**
	N:P		**<0.0001**		**<0.0001**		**0.0001**

Table 2-6. Repeated measures analysis of variance for *Vaucheria* sp. relative growth rates and nutrient molar ratios in Experiments 1 and 2. Significant P values ($p<0.05$) are shown in bold.

Experiment	Variable	Source of variation (between groups)	P	Source of Variation (within groups)	P	Source of Variation (within groups)	P
1	Relative Growth Rate	$N0_3$ Concentration (N)	**0.0008**	Time	**0.0043**	Time x N	0.1484
	C:N		**<0.0001**		**<0.0001**		**0.0221**
	C:P		**<0.0001**		**<0.0001**		**<0.0001**
	N:P		**<0.0001**		**0.0014**		**<0.0001**
2	Relative Growth Rate	NO_3 Concentration (N)	0.2733	Time	0.3969	Time x N	0.5047
	C:N		0.6131		**<0.0001**		0.2804
	C:P		0.6841		**<0.0001**		0.2042
	N:P		0.2108		**0.0034**		0.5987

Table 2-7. Initial and final C:N, C:P and N:P molar ratios of *Lyngbya wollei* in Experiments 1 and 2. Target treatment concentrations (NO_3 µg L^{-1}) are shown below treatment numbers.

Experiment	Molar Ratio	Day	Treatment number and nitrate concentration (µg L^{-1})						
			1	2	3	4	5	6	7
			Control A	Control B	0.5	5	50	500	5000
1	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

			Treatment number and nitrate concentration (µg L^{-1})						
			1	2	3	4	5	6	7
			Control	25	50	150	250	500	750
2	C:N	0	7	7	7	7	7	7	7
		21	15	16	13	12	11	11	11
	C:P	0	140	140	140	140	140	140	140
		21	268	372	408	576	510	532	528
	N:P	0	19	19	19	19	19	19	19
		21	18	24	32	48	46	48	47

Table 2-8. Initial and final C:N, C:P and N:P molar ratios of *Vaucheria* sp. in Experiments 1 and 2. Target treatment concentrations (NO_3 µg L^{-1}) are shown below treatment numbers.

Experiment	Molar Ratio	Day	Treatment number and nitrate concentration (µ L^{-1})						
			1	2	3	4	5	6	7
			Control A	Control B	0.0005	0.005	0.05	0.50	5.00
1	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

			Treatment number and nitrate concentration (µ L^{-1})						
			1	2	3	4	5	6	7
			Control	0.025	0.05	0.15	0.25	0.50	0.75
2	C:N	0	8	8	8	8	8	8	8
		21	16	15	16	16	15	16	15
	C:P	0	142	142	142	142	142	142	142
		21	318	363	390	449	446	459	441
	N:P	0	18	18	18	18	18	18	18
		21	20	24	24	28	29	29	30

Table 2-9. Parameter estimates of the dose response curve (model) for *Lyngbya wollei*. Relative growth rate (RGR) data from Experiments 1 and 2 were combined.

Parameter (intercept)	Parameter description	Estimate	Standard error	t-value	p-value
b	Slope	2.245	0.836	2.685	0.0078
c	Maximum RGR	0.127	0.003	46.831	1.02E-115
d	Minimum RGR	0.088	0.004	23.942	4.00E-63
e	ED50	41.54	8.39	4.95	1.47E-06
Power	Power	0.964	0.453	2.129	0.0344

Heterogeneity adjustment: variance is a power of the mean

Table 2-10. Estimated effect doses (ED) of NO_3 for *Lyngbya wollei.*

Effect Dose (ED)	NO_3 dose ($\mu g\ L^{-1}$)	Standard error
10	15.6	6.47
50	41.5	8.39
90	110	46.3

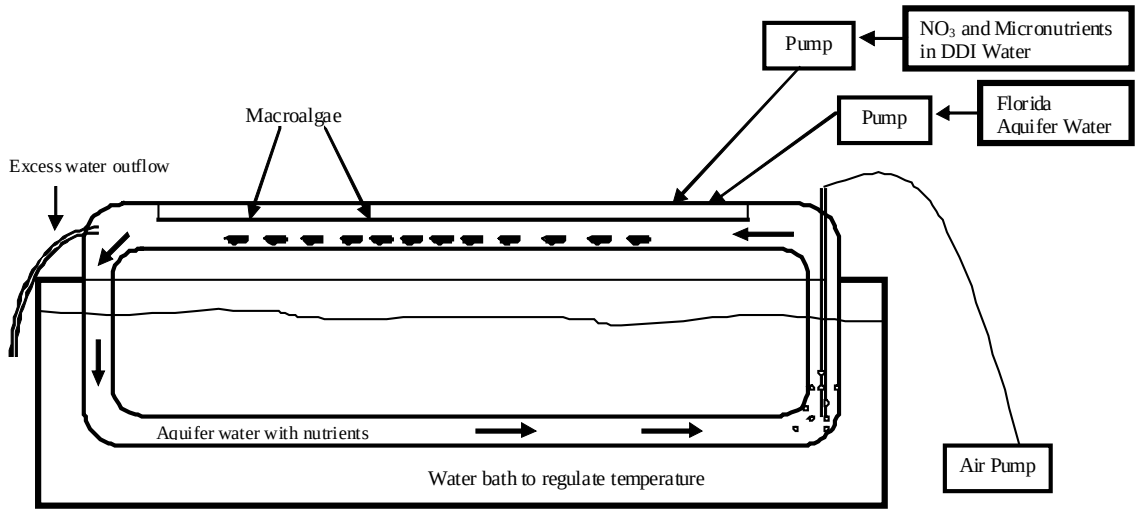
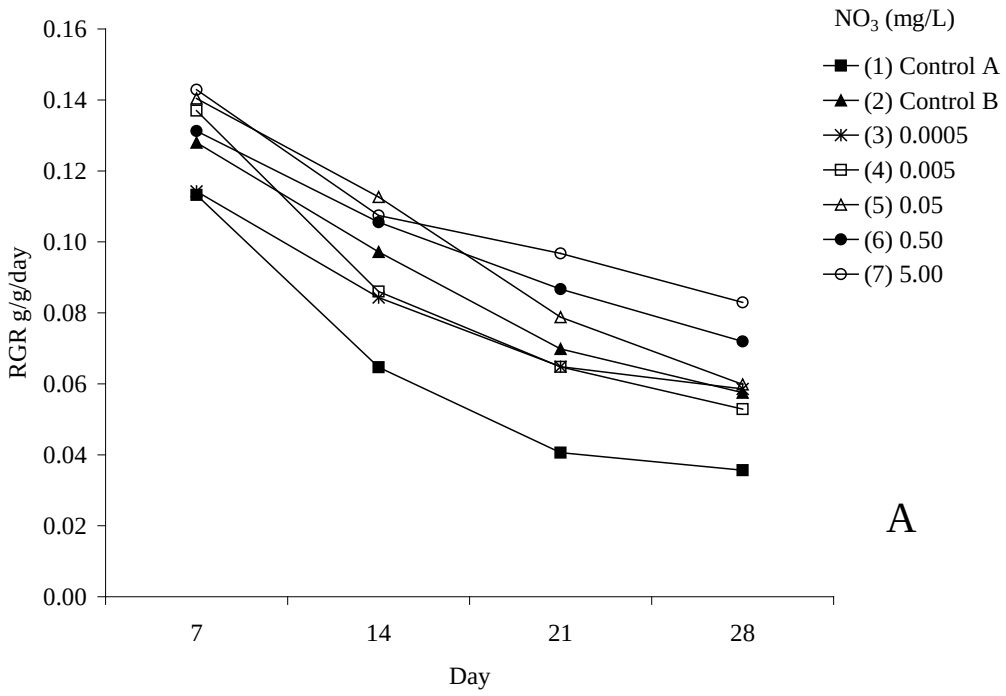


Figure 2-1. Cross-sectional view of a single stream channel.



B

Figure 2-2. *Lyngbya wollei* relative growth rates (RGR) under different nitrate concentrations during Experiments 1 and 2. A) Experiment 1. B) Experiment 2.

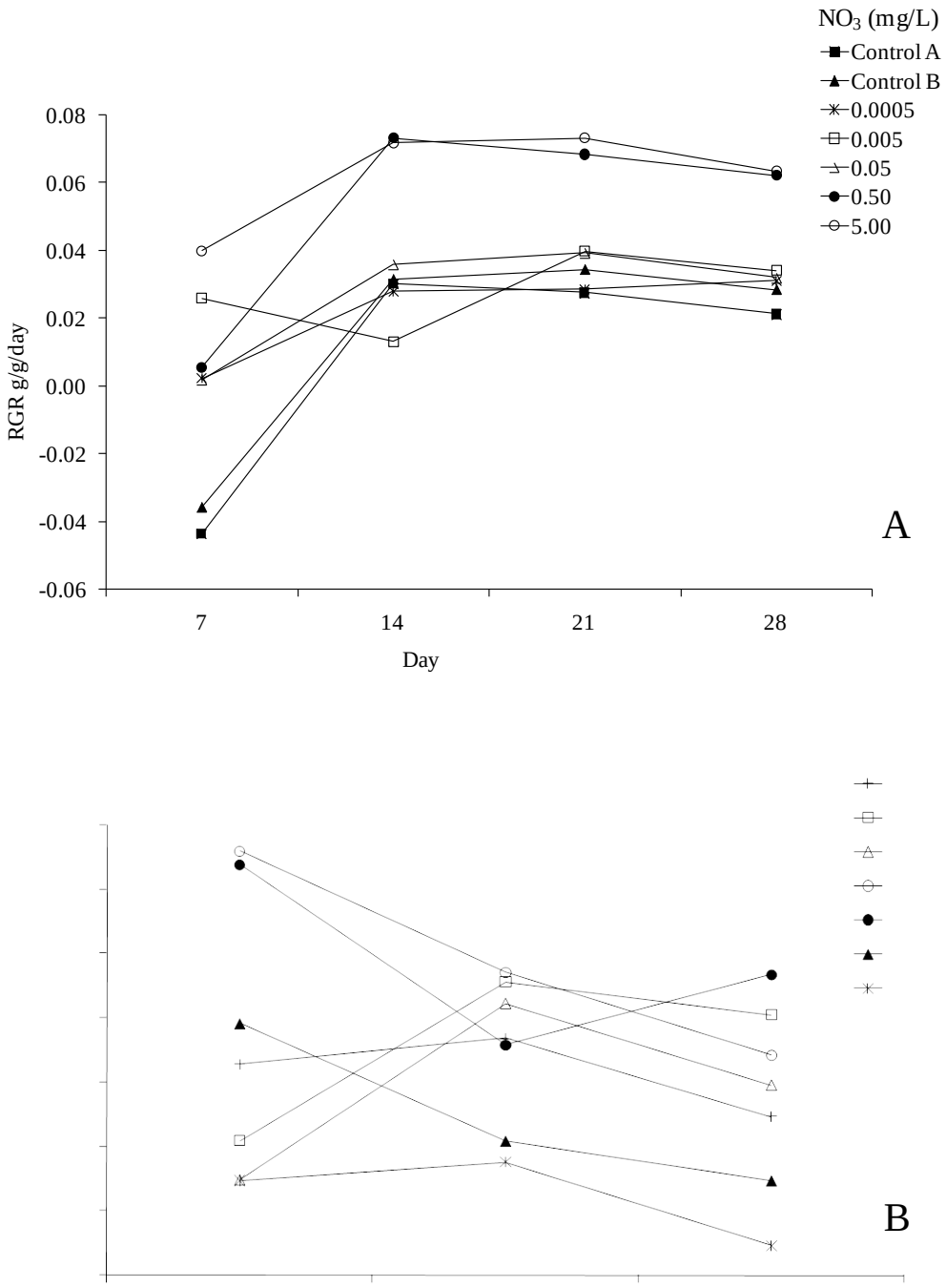


Figure 2-3. *Vaucheria* sp. relative growth rates (RGR) under different nitrate concentrations during Experiments 1 and 2. A) Experiment 1. B) Experiment 2.

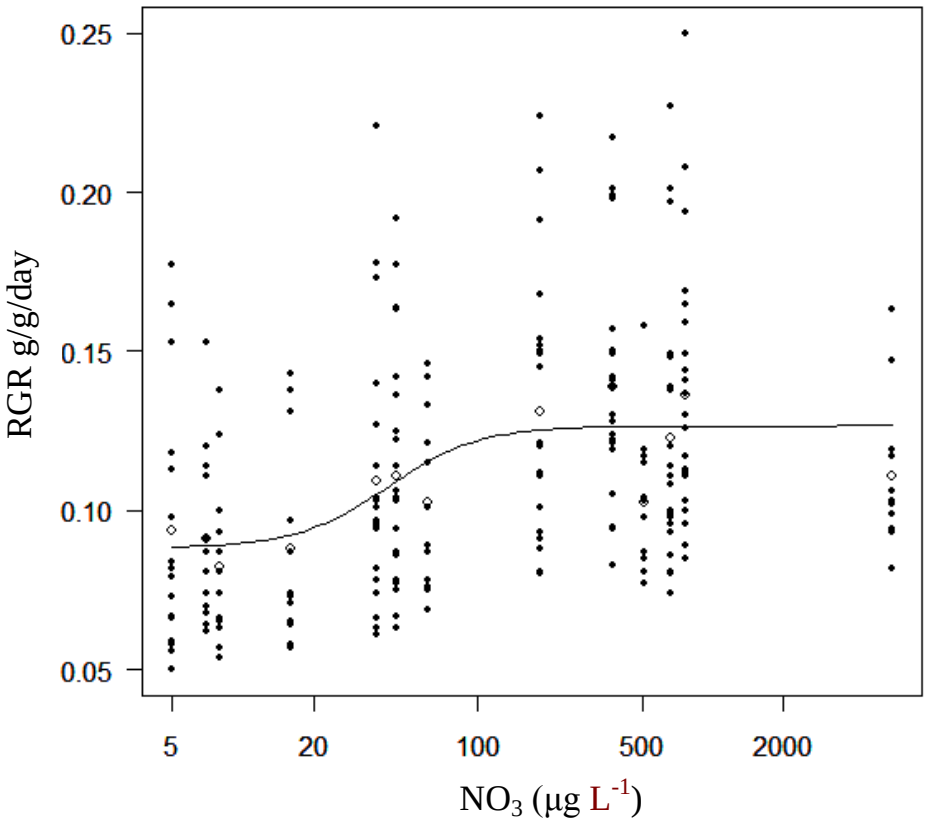


Figure 2-4. *Lyngbya wollei* relative growth rate dose response curve. Relative growth rates (RGR) for Experiments 1 and 2 are combined. Treatment means are shown as open diamonds. The lower limit of response (lowest relative growth rate) is 0.088 g/g/day and the upper limit of response is 0.127 g/g/day. The nitrate concentration resulting in 50% growth saturation, the ED_{50}, is 41.5 µg NO_3 L^{-1}. The ED_{10} (the nitrate concentration at which the growth response was 10% saturated) is 15.6 µg NO_3 L^{-1} and the ED_{90} (concentration at which the growth response is 90% saturated), is 110 µg NO_3 L^{-1}.

CHAPTER 3
δ^{15}N STABLE ISOTOPE COMPOSITION OF ALGAE, SEDIMENT AND NITRATE IN FLORIDA SPRINGS

Introduction

Increasing human populations and land-use change in Florida have led to increases in nitrate in the Floridan Aquifer (de Brabandere, Frazer & Montoy,. 2007; Munch *et al.* 2006; Katz, Bohlke & Hornsby, 2001), which extends throughout the entire state of Florida and parts of Georgia, South Carolina, Mississippi and Alabama (Cohen, 2008). The aquifer is particularly susceptible to land-use activities due to its karst topography, which provides a direct conduit between the surface application of nitrogen and the aquifer (Bacchus & Barille, 2005; Katz, 2004). Florida's calcitic soils and the limestone matrix of the aquifer adsorb phosphorus (Rhue, Harris & Nair., 2006; Cohen, 2008), but highly mobile nitrate anions have no such exchange capacity (Panno *et al*., 2001).

Nitrogen levels in many Florida karst springs have been steadily increasing over the last 50 years from background concentrations of less than 0.1mg/L to concentrations as high as 5 mg/L, while phosphorus levels have remained relatively stable (Hornsby and Ceryak, 1999 in Katz *et al*. 1999; Scott *et al*. 2004; Strong, 2004). At the same time, an increase in filamentous algae has been observed in many springs, particularly *Lyngbya wollei* and *Vaucheria* sp., the two most common mat-forming species found in Florida's springs (Stevenson *et al*., 2004). Increases in algae are often attributed to increases in nitrate concentrations in spring water, although no direct link has been found between nitrate concentrations and algal abundance (Pinowska *et al*., 2009, Stevenson *et al*., 2004). However, micro and mesocosm experiments indicate that reducing nitrogen loads to springs where concentrations are high could reduce algal growth rate and therefore, biomass accumulation (Stevenson *et al*. 2008; Chapter 2, this document).

Sources of nitrogen to groundwater and springs include inorganic fertilizers, confined animal feedlot operations, sewage effluent and atmospheric deposition (Bacchus & Barile, 2005; Katz *et al.,* 1999). Tracing these sources in Florida's springs is challenging, however, because spring systems reflect groundwater quality vertically, spatially and temporally (Katz, 2004). Stable isotope analysis ($\delta^{15}N$) can be a useful tool to elucidate sources of N in systems, but many natural and anthropogenic sources have overlapping $\delta^{15}N$ values and therefore distinguishing between sources of nitrogen can be difficult (Kendall, 1998; Einsiedl & Mayer, 2006; Derse *et al*., 2007). Interpretation is further complicated due to the mixing of multiple N sources as well as fractionation processes, such as denitrification and assimilation of nitrogen by primary producers (Kendall, 1998; de Brabandere, Frazer & Montoya, 2007). The denitrification process produces isotopically lighter N_2 and N_2O gases, leaving behind ^{15}N-enriched residual nitrate. The $\delta^{15}N$ signature of the residual N can be similar to that of animal and septic waste (Panno *et al*,. 2001). Potential fractionation during assimilation of NH_4 and NO_3 by algae can range from -27 to 0 ‰ (Fogel & Cifuentes, 1993 in Kendall, 1998). Nitrate concentrations in the water column can also affect algal $\delta^{15}N$, since under limiting nitrogen conditions, most of the nitrogen would be assimilated. Algal $\delta^{15}N$ would therefore reflect source $\delta^{15}N$ since little or no isotopic fractionation would occur (Umezawa *et al.*, 2007).

The dual isotope analysis of nitrate in water ($\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3) is used to further differentiate sources of nitrate when $\delta^{15}N$ ranges overlap (Fukada, Hiscock, & Dennis, 2004, Dahnke *et al.* 2008; Pellerin *et al.* 2009; Wankel *et al.* 2009). For example, $\delta^{18}O$ can be used to separate NO_3 fertilizer from soil nitrogen and NH_4 in fertilizer and rain. Additionally, both the $\delta^{18}O$ and $\delta^{15}N$ of the residual nitrate increase systematically as a result of denitrification. The enrichment of $\delta^{18}O$ relative to $\delta^{15}N$ is close to 1:2 (producing a line with a slope of

approximately 0.5, with $\delta^{15}N$ on the x-axis) and this may allow isotopically distinct sources to be identified even when significant denitrification has occurred (Böttcher *et al.*, 1990; Kendall, 1998).

In this study, I used both the dual isotopic analysis of spring water and $\delta^{15}N$ analysis of filamentous mat-forming algae and spring sediments to help determine nutrient sources to benthic algal mats and identify factors controlling algal abundance. Specifically, I assessed nitrate sources to spring water using the dual-isotopic analysis of nitrate ($\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3) and determined the $\delta^{15}N$ of algae and spring sediments at two scales, (1) regionally, at multiple spring sites throughout Central Florida and the Panhandle and (2) along four spring–fed river runs to assess spatial variability. I also evaluated the $\delta^{15}N$ of algae over the course of one year at two springs to examine seasonal variability. Additionally, I assessed the relationships between $\delta^{15}N$ indicators of nutrient availability and sources through correlation analysis.

Methods

Study Sites

Stable isotope analysis ($\delta^{15}N$) was conducted on filamentous algae and sediments in Florida springs on three scales: (1) regionally, (2) along spring river-run gradients (starting at the spring boil of four separate springs and sampling progressively further downstream) and (3) on a monthly basis during the course of one year at two springs. For the regional study, 63 spring sites throughout the Panhandle and north central Florida were sampled in 2006. The spring sites included the boil area as well as sites downstream. The complete list of sites and locations sampled is found in Appendix A. Additionally, the boil area of 17 springs was sampled in the summer of 2005, January 2006 and summer of 2008 for the dual-isotopic analysis of nitrate ($\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3) in spring water. However, not all springs were sampled all three years

(Table 1). Four spring river runs were sampled in January 2006 for the gradient study: Silver River, Rainbow River, Wakulla River and the Weeki Wachee River. The sites sampled along each river run are listed in Table 2 and site codes correspond to the codes listed in Appendix 1. Finally, the seasonal study was conducted monthly from April 2005 to March 2006 at Manatee Springs and Ichetucknee Blue Hole Spring. Only the boil areas were sampled.

Algae, Sediment and Water Sample Collection

Algae and sediment samples were collected either within the boil area of each spring, or for sites located downstream from the boil, samples were collected along a 100 m section of the river run. Samples were primarily collected by snorkeling, but had to be collected from a canoe at some locations due to the presence of alligators. At each site, a composite sample of each of the most common algal species was collected, shaken in the water to remove any loosely attached debris and placed into 1 gallon Ziploc bags (SC Johnson, Racine, WI, USA) filled with site water. Additionally, a small algal sample (approx 0.5-1 g fresh mass) was collected and placed in a scintillation vial to confirm the accuracy of field identification. Samples were then transported to the laboratory on ice.

Separate sediment samples were collected from the exposed spring bottom and from under algal mats by coring with a 2.5 cm diameter syringe to a depth of approximately 3 to 10 cm, depending on the composition of the spring bottom. The open end of the syringe was then sealed with a spatula to bring the sample to the surface. When sampling from a canoe, an Eckman Dredge was used to collect the sediment. Whenever possible, at least three samples each of exposed and covered sediment were collected and combined to form a composite sample. Samples were transported in plastic containers to the laboratory on ice.

Water samples were collected at each site either from a kayak or from the shore depending on the location of the boil, at a depth of 0.5 m from the surface. If the site was located along the

river run, the sample was collected at the start (upstream end) of the 100-m section. Samples were filtered in the field either through 0.45-µm cartridge filters (Millipore model number-GWSC04550, Millipore, Billerica, MA, U.S.A.) using a peristaltic pump or through 0.45-µm polycarbonate membranes (Whatman Inc., Florham Park, NJ, U.S.A.) using a filter holder and syringe. Filtered aliquots were collected for δ^{15}N-NO_3 and δ^{18}O-NO_3, SRP, DOC, NH_4^+, and NO_3^-. Unfiltered samples were collected for TKN and TP. Samples for TKN, TP, DOC, NH_4^+, NO_3^- were acidified to pH 2 with concentrated H_2SO_4. All samples were transported on ice and stored at 4°C until analyzed except for δ^{15}N-NO_3 and δ^{18}O-NO_3 and SRP samples, which were stored frozen. The holding time was 28 days for NO_3^-, NH_4^+, SRP, DOC, TKN, and TP. The holding time for the isotope samples was 28 days to three years (all samples were analyzed in 2008). Additionally, temperature, conductivity, pH, and dissolved oxygen (DO) were measured at each site using a YSI 556 Multi-probe System (YSI Incorporated, Yellow Springs, OH, U.S.A.). Measurements were taken directly above the boil or if the site was located along the river run, at the start of the 100-m section.

Algae, Sediment and Water Sample Processing and Analysis

Algae samples were picked clean of invertebrates and debris within 24 hours of field collection, stored frozen and later lyophilized at -91°C under a 35-mTorr vacuum. Once dry, they were again picked clean of any debris initially missed and ground and homogenized in a ball mill. The samples collected and processed to determine δ^{15}N of algae and sediments were also used to determine percent C, N and P. Algae samples that were placed in scintillation vials for species verification were preserved using M3 solution and sent to the Center for Water Sciences at Michigan State University, where they were identified.

Macroinvertebrates were removed from sediment samples which were then homogenized by stirring, also within 24 hours of field collection. Sediments were stored frozen and

subsequently dried in an oven at 60°C for 5 days. They were then passed through a sieve to remove coarse debris (e.g, twigs, leaves, whole mollusk shells) and ground and homogenized in a ball mill.

Nitrogen isotopic composition of algae and sediments was measured on a Thermo Finnigan Delta-Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.) at the University of Florida using an elemental analyzer inlet system and continuous flow of He. The International Atomic Energy Association standard for N1 was included in each run. Nitrogen isotope values are reported in δ notation relative to atmospheric air. Percent nitrogen and carbon of the dried algal tissue and sediments were measured by high temperature combustion using a Flash EA 1112 Nitrogen/Carbon Analyzer with MAS 200 R Autosampler (Thermo Fisher Scientific Inc, Waltham, MA, U.S.A.). Phosphorus content of dried algal tissues and sediment was measured on combusted (550°C) and acid digested (6N HCl) samples as SRP (Anderson, 1976) on a Technicon Autoanalyzer (Technicon Instruments Corporation Wilmington, MA, U.S.A.). At Michigan State University, sediments were analyzed for % water content, dry mass (DM), ash free dry mass (AFDM) (Eaton et al. 1995), available phosphorus (PO_4^-) and available nitrogen (NH_4^+, NO_2/NO_3^-) following the extraction of 1g of wet sediment with Truog's reagent and KCl (Allen 1989).

All samples for the dual-isotope analysis of nitrate ($\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$) in spring water were analyzed at the University of Florida using the bacterial denitrifier method (Sigman *et al.,* 2001; Casciotti *et al.,* 2002) in which nitrate is converted to N_2O by the denitrifying bacteria *Pseudomonas aureofasciens*. The $\delta^{15}N$ and $\delta^{18}O$ of the N_2O produced were then measured on a Thermo Finnigan Delta-Plus XP isotope ratio mass spectrometer at the University of Florida using continuous flow of He. The International Atomic Energy Association standard

for N3 was included in each run. Nitrogen isotope values are reported in δ notation relative to atmospheric air, oxygen isotope values are reported in δ notation relative to the standard VSMOW.

Soluble reactive phosphorus, NH_4^+, and NO_3^- were measured on a Bran+Luebbe Auto Analyzer 3 (Bran+Luebbe, Norderstedt, Germany) using EPA Methods 365.1, 350.1 and 353.2, respectively. Total Kjeldahl nitrogen was determined by H_2SO_4 and Kjeldahl salt digestion and flow-injection determination of ammonium (EPA Method 351.2). Total phosphorus was measured as SRP on a Bran+Luebbe Auto Analyzer 3 after digestion with H_2SO_4 and potassium persulfate (EPA Method 365.1).

Rapid Habitat and Periphyton Assessment (RHPA)

At each of the spring sites, a modified Rapid Habitat and Periphyton Assessment (RHPA) (Stevenson and Bahls 1999) was conducted. Although not directly a part of this study, data obtained were used in a correlation analysis with the algal and sediment isotope data. The RHPA consisted of establishing 7 to 9 transects across the spring run at each site, positioned approximately 10 m apart. Nine observation points were designated for each transect, for a total of either 63 or 81 points per site. At each point, current velocity was estimated, the substratum type was characterized, macrophytes and algae were identified and the thickness of the algal mat and stream depth were measured. Additionally, any bank conditions (binding roots, canalized, or incised) were documented and for every second transect, the buffer composition (trees, shrubs, herbs, or bare) was evaluated and the canopy cover was measured with a spherical convex crown densitometer.

Statistical Analysis

Spearman correlations were used to determine relationships between algal and sediment stable isotope composition and indicators of nutrient availability and sources, as well as other

environmental variables collected during the RHPA. Variables correlated to algal and sediment $\delta^{15}N$ include: (1) site water physical-chemical parameters from the 2006 survey plus data from a survey conducted in 2003 for FDEP by A. Pinowska and R.J. Stevenson (Michgan State University), (2) algal and sediment C:N:P molar ratios, as well as bioavailable N and P of the sediments, (3) average site depth and current velocity, (4) average site canopy cover, (5) site buffer zone characteristics, (6) diatom water quality and trophic-state indicators developed by Stevenson *et al.* (2008). (6) land use characteristics and LDIs. Land use characteristics for each site and LDI (Landscape Development Intensity) indexes were calculated from data provided by the Florida Department of Environmental Protection (FDEP) by A. Pinowska. A subset of the regional study sites (34) were used in the correlation analysis. Only samples from the spring boil areas were used, in order to avoid autocorrelation among multiple sites along spring runs.

Results

Isotopic Analysis of Spring Water Nitrate ($\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3)

Nitrate $\delta^{15}N$ and $\delta^{18}O$ values of the 17 springs analyzed are shown in Figure 3-1 and listed in Table 3-1 by year. $\delta^{15}N$ ranged from 3 to 20 ‰ and δ18O ranged from 3 to 15 ‰. The highest $\delta^{15}N$ and $\delta^{18}O$ values per site (both $\delta^{15}N$ and $\delta^{18}O \geq 10$ ‰) were found at Troy , Wekiwa, Volusia, Lafayette, Little River and Wakulla Springs, all sampled in 2008. The lowest values (both $\delta^{15}N$ and $\delta^{18}O \leq 5$ ‰) were found at Ichetucknee Head, Jackson Blue, Ichetucknee Blue Hole, Madison Blue and Rainbow Springs, all sampled in 2005. A positive relationship was found between $\delta^{15}N$ and $\delta^{18}O$ ($r^2 = 0.71$), with most sites falling along a line with slope of 0.64.

Algae and Sediment $\delta^{15}N$

The relationship between the $\delta^{15}N$ of algae and sediments and the $\delta^{15}N$-NO_3 of water from 10 headwater springs sampled in 2006 is shown in Figure 3-2. The springs sampled were: Fanning, Guaranto, Lafayette Blue, Little Fanning, Little River, Madison Blue, Rainbow, Silver

River, Troy and Wakulla. No distinct relationship was found between algal and sediment $\delta^{15}N$ and the $\delta^{15}N$-NO_3 in water. If a trendline is drawn through the algal data, the r^2 value is 0.34. The range of algal $\delta^{15}N$ values was substantial, from -8 to +6 ‰, and was not species-specific, while the range in $\delta^{15}N$-NO_3 was narrower, from 4 to 10 ‰. The lowest $\delta^{15}N$ values of algal tissue were found for *Vaucheria* sp. at Little River Springs (-7 ‰) and Troy Springs (-6 ‰), while the highest values were found for *Spirogyra* sp. at multiple sites along the Wakulla Springs river run (7 and 8 ‰). No distinct relationship was found between sediment $\delta^{15}N$ and the $\delta^{15}N$-NO_3 in water. Unlike algae, sediment $\delta^{15}N$ was never negative and ranged from 0 to 10 ‰. Exposed sediment showed a wider range in values than sediment under algal mats. No relationship was found between the $\delta^{15}N$ of algae, sediments and spring water NO_3 and NO_3 concentrations in the sites sampled in 2006 (Figure 3-3).

Correlations among Algal Stable Isotope Signatures, Water Quality and Environmental Variables

Significant Spearman correlations ($p<0.001$) between algal tissue $\delta^{15}N$ and sediment $\delta^{15}N$ and variables relating to indicators of nutrient availability and sources are listed in Table 3-2. To avoid Type 1 errors in this analysis, I set the p level at 0.001 to account for the relatively large number of correlations that were made. For algal $\delta^{15}N$, significant positive and negative correlations were found between numerous variables. The strongest associations were found with average water concentrations of total N and NO_2/NO_3-N (negative correlations) of the 3 major sampling events (Fall and Spring 2003 and Winter 2006). These relationships are not surprising because when both total N and NO_2/NO_3-N concentrations are high, more isotopically light N ($\delta^{14}N$) is available to the algae for uptake, resulting in isotopically lighter algal tissues (lower $\delta^{15}N$ values).

For sediment collected beneath an algal mat, the strongest correlation with $\delta^{15}N$ was the $\delta^{15}N$ of the exposed sediment (positive). $\delta^{13}C$ and C:N analysis of algal tissues indicate that the majority of the organic matter in the sediment (both under algal mats and in exposed sediment) comes from vascular plants (see Chapter 4 of this document) and therefore, the strong correlation between the $\delta^{15}N$ of both types of sediment samples is likely because the organic matter is of similar origin. For exposed sediment $\delta^{15}N$, (sediment with no algal mat cover), the strongest correlation was with the percent C of sediment under algae (negative), which may be an indicator of denitrification occurring in the sediment. During denitrification, carbon is used as a source of energy (so less C remains in the sediment) and the residual N is enriched (higher amount of $\delta^{15}N$).

Stable Isotopic Variation along Longitudinal Gradients

The sites sampled at each of the four spring river runs and their distances from the spring boil are listed in Table 3-3. The site codes are the same ones used (and therefore the same locations) as in the regional study.

Dual Isotope Analysis of Spring Water Nitrate along Longitudinal Gradients

Each of the four river runs sampled in the gradient study had distinct dual isotopic signatures of spring water nitrate ($\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$) (Figure 3-4). $\delta^{15}N\text{-}NO_3$ ranged from 4 to 10 ‰, depending on the river run and the $\delta^{18}O\text{-}NO_3$ ranged from 5 to 7 ‰. The variability among sites within each river run was remarkably small (maximum of 1 ‰ for both $\delta^{15}N$ and $\delta^{18}O$), despite sampling distances of up to 9 km between different sites.

Silver Springs River Run

Three algal species, *Lyngbya wollei*, *Vaucheria* sp. and *Spirogyra* sp. were found along the Silver Springs River run. The $\delta^{15}N$ of algae, and nitrate of spring water in relation to distance from the boil is shown in Figure 3-5. Algal $\delta^{15}N$ values ranged from 2 to 6 ‰. *L. wollei* showed

an increasing trend in $\delta^{15}N$ values from the boil until the fifth site (from 2 to 6 ‰), with a decline in the last site to 4 ‰. *Vaucheria* sp. $\delta^{15}N$ showed an increasing trend with increasing distance from the boil area, from 3 to 6 ‰. The $\delta^{15}N\text{-}NO_3$ of spring water showed almost no variability, remaining around 7 ‰ at each site (total length of 7.2 km).

Weeki Wachee Springs River Run

Seven species of algae were found along the Weeki Wachee river run: *Vaucheria* sp., *Spirogyra* sp., *Lyngbya wollei, Hydrodictyon sp., Cladophora glomerata, Aphanothece* sp. and *Calaglossa* sp. (Figure 3-5). Algal $\delta^{15}N$ varied from site to site, with values ranging from -1 to 5 ‰. *Lyngbya wollei* had the narrowest range of values along the river run, (1 to 2 ‰). The $\delta^{15}N\text{-}NO_3$ of spring water remained between 6 and 7 ‰ throughout the river run (total length of 8 km).

Rainbow Springs River Run

Stable isotope composition ($\delta^{15}N$) of the algae and water nitrate along the Rainbow Springs river run are shown in Figure 3-5. $\delta^{15}N$ values ranged from -2 to 9 ‰, with exposed sediment samples having the highest $\delta^{15}N$ values. *Lyngbya wollei* $\delta^{15}N$ had a narrow, but increasing trend as distance from the boil increased (-2 to 0 ‰). The $\delta^{15}N\text{-}NO_3$ of spring water remained at 4 ‰ throughout the run (total length of 7.7 km).

Wakulla Springs River Run

Five species of algae were found along the Wakulla Springs river run: *Compsopogon* sp., *Vaucheria* sp., *Spirogyra* sp., *Lyngbya wollei* and *Enteromorpha* sp. $\delta^{15}N$ of algae and nitrate along the river run gradient are shown in Figure 3-5. Algal $\delta^{15}N$ values showed relatively little within-species variation from site to site. The $\delta^{15}N\text{-}NO_3$ of spring water remained between 9 and 10 ‰ throughout the run (total length of 9.8 km).

Seasonal Variability in Algal $\delta^{15}N$

I observed seasonal variation in $\delta^{15}N$ of *Vaucheria* sp. at Manatee springs, with a relatively wide range of values (-8 to 1 ‰) (Figure 3-6). The lowest values were obtained in July 2005 and February 2006 and the highest value was obtained in May 2008. At Ichetucknee Blue Hole, $\delta^{15}N$ values of *Vaucheria* sp. fluctuated more than those of *Lyngbya wollei* throughout the course of the year (Figure 3-6). *L. wollei* values were confined to a range of -1 to +1 ‰, while *Vaucheria* sp. values ranged from -1.3 to 3.5 ‰.

Discussion

Dual Isotope Analysis of Spring Water Nitrate ($\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3)

Nitrate in groundwater is derived from both natural and anthropogenic sources. The majority of terrestrial materials have $\delta^{15}N$ values between -20 and +30 ‰ and normal ranges of $\delta^{15}N$ of groundwater nitrate can generally be attributed to the following sources: (1) inorganic fertilizer (-7 to +7 ‰), (2) cultivated and natural soils (-3 to +14 ‰), (3) atmospheric deposition (NH_4 and NO_3 in rain, -7 to +8 ‰) and (4) animal and septic waste (+2 to +25 ‰) (Einseidl & Mayer, 2006; Kendall, 1998; Aravena & Robertson, 1998; Kreitler, 1979). The $\delta^{18}O$ values of nitrate of these same sources fall within the following ranges (1) inorganic fertilizer (15 to 25 ‰), (2) cultivated and natural soils (-5 to 15 ‰), (3) atmospheric deposition (NH_4 and NO_3 in rain, 20 to 80 ‰) and (4) animal and septic waste (-5 to +15 ‰) (Einseidl & Mayer, 2006; Kendall, 1998; Durka *et al.*, 1994). Therefore, when isotopic ranges of N sources overlap, the dual isotope analysis of nitrate can be used to differentiate between sources. However, the utility of this method depends on being able to identify the importance of denitrification processes in the particular system studied because the residual nitrate of denitrification has a similar isotopic signature as that of animal waste (Fukada, 2004).

If one assumes that variations in nitrate isotopes measured in the springs are the result of mixing of different sources rather than denitrification, then inferences can be made about the dominant sources of nitrate to the springs. Five springs sampled in 2008, Troy, Wekiwa, Volusia, Lafayette and Little River had both $\delta^{15}N$ and $\delta^{18}O$ between 10 and 20 ‰, which strongly indicates organic N sources, such as manure or septic waste (Table 3-1, Figure 3-1) (Kendall, 1998, Panno, 2001). The remaining springs (which also include those mentioned above, but sampled in previous years) had both $\delta^{15}N$ and $\delta^{18}O$ values between 3 and 9 ‰ , which indicate an inorganic N-source, such as NH_4 from either fertilizer and/or rain or soil nitrogen, but which also fall within the lower range of organic N sources (Kendall, 1998). Katz (2008) found that inorganic fertilizers were major sources of nitrogen at Ichetucknee Head Springs and Blue Hole, which is consistent with signatures found in my study. Additionally, Katz (2004) obtained a $\delta^{15}N$ value for Wakulla Springs of 8 ‰ (similar to my study, 9 ‰ in 2005 and 2006), which he attributed to both inorganic and organic sources based on N mass balance calculations for the spring by Chelette *et al*. (2002, in Katz, 2004). The most important sources of N identified (in decreasing importance) were: treated wastewater effluent, atmospheric deposition, wastewater residuals, fertilizers and on-site waste-disposal systems.

Isotope values are higher for both $\delta^{15}N$ and $\delta^{18}O$ in samples taken in 2008 versus those taken in 2005 for several springs. Troy, Lafayette, and Little River show relatively large increases in values (3 to 13 ‰ in both $\delta^{15}N$ and $\delta^{18}O$), while Ichetucknee Head Spring and Manatee Springs increased to a lesser extent (1 to 3 ‰ in both). Several possible explanations need to be considered to explain these results. First, the 2005 samples were stored frozen for three years before being analyzed and holding time may have affected the water samples (all samples for the dual isotope analysis of nitrate were analyzed in the summer of 2008). However,

six other springs that were sampled across multiple years did not show this trend (Fanning, Guaranato, Ichetucknee Blue Hole, Rainbow, Silver River and Wakulla). Additionally, the $\delta^{15}N$-NO_3 values obtained for Troy Springs in 2005 and 2006 (7 ‰) and Manatee Springs (6 ‰) are the same values listed by Katz (2004, derived from Bölke (2002)) for both springs. Therefore, holding time does not appear to have influenced my results.

Another possible explanation would be that in 2005, sampling may have occurred when the aquifer was in a condition of low flow, while in 2008 sampling may have occurred during conditions of higher flow or recharge. Water sampled in 2008 could have had a shorter residence time, passing more quickly through preferential flows into the aquifer and therefore reflected more heavily local source values, while water discharged in 2005 may have been older and a reflection of mixing of multiple sources across a larger area of the springshed. Katz (2004) indicates that there is a substantial increase in water contributions from local flow systems like sinkholes during conditions of high recharge. Using dye tracer studies, Wilson & Skiles (1988, in Katz, 2004), showed that water can move through conduit systems to springs in as little as days to weeks, although groundwater discharged from Suwannee River Basin springs has average residence times of 10 to 20 years (Katz, Hornsby & Böhlke, 2001).

Additionally, samples could have included more atmospheric nitrate deposition in 2008, which has relatively high $\delta^{18}O$ values, than those obtained in 2005. Einsiedl & Mayer (2006) found that during recharge conditions in a karst aquifer in southern Germany, $\delta^{18}O$ values were up to 25 ‰ higher than during conditions of low flow in the aquifer, which they attributed to atmospheric deposition. However, atmospheric deposition measured in the Bradford Forest of North central Florida was lower in 2008 than in 2005 (http://nadp.sws.uiuc.edu). During the spring and summer of 2005, wet deposition (NO_3 + NH_4) was 3.88 and 4.54 kg/ha respectively,

while in spring 2008, wet deposition was 1.36 kg/ha. Data for summer 2008 are not available yet. Therefore, although recharge vs. low flow conditions in the aquifer does not appear to be a factor in explaining higher isotope values in 2008 vs 2005, it cannot be ruled out due to a lag time between aquifer recharge and spring discharge.

As mentioned previously, both $\delta^{18}O$ to $\delta^{15}N$ are enriched during the denitrification process and isotopic values are similar to those obtained with animal waste, complicating source identification. Katz (2004) used several lines of evidence to support the supposition that denitrification in Florida karstic systems is negligible: (1) spring waters are aerobic and contain low concentrations of DOC (i.e., redox levels are above those required for denitrification and there is little substrate to support heterotrophic respiration) and (2) ratios of N_2:Ar gases dissolved in spring waters were consistent with atmospheric equilibration during groundwater recharge. Excessive N_2:Ar, ratio for example, would indicate an additional source of N_2 gas in the aquifer, e.g., denitrification input. However, Katz does not rule out the possibility of denitrification occurring during the past within the aquifer system.

Although no measurements of the N_2:Ar ratio were done in this study to determine the importance of denitrification, the 0.64 slope of the trend line shown in Figure 3-1 raises the possibility that most of the springs I sampled could have had a common nitrate source and that variations in nitrate isotopic composition were driven by denitrification in the aquifer system (a slope of approximately 0.5 would be expected as a result of denitrification). While boil water had low, but measurable oxygen levels (1-2 mg L^{-1}), previous studies have shown that discharging groundwater in most springs is a mixture of matrix and conduit water sources, and each travels along different flowpaths and has different residence times (Martin & Dean, 2001). It is possible that denitrification is occurring in matrix flows, which have longer residence time, or at

microsites within the conduit flow, but the anoxic character of the water is lost when mixing with oxygenated waters takes place. Denitrification does not rely on carbon alone as an electron donor and was shown to occur with ferrous iron, pyrite (H_2S) and organic matter as possible electron donors in a karst aquifer in France (Pauwels, Foucher & Kloppmann, 2000) and likely occurs with H_2S and DOC in a karst aquifer in Germany (Einseidl & Mayer, 2006; Einsiedl, Maloszewski & Stichler, 2005).

If the trend line in Figure 3-1 is extrapolated to the x-axis intercept, one could infer that the common nitrate source to the springs has a $\delta^{15}N\text{-}NO_3^-$ value of approximately -3 ‰, which is similar to $\delta^{15}N$ values typical for ammonia-based fertilizers or ammonium and nitrate in precipitation (Kendall, 1998). In the majority of N budgets calculated for springsheds, inorganic fertilizers are the largest anthropogenic source of nitrates (based on mass load) (Cohen, 2008). However, the amount of nitrogen that actually reaches the Upper Floridan Aquifer is unaccounted for since load reduction in the soil matrix due to biological uptake, for example, is unknown and likely highly variable across the landscape (Cohen, 2008).

Not knowing the importance of denitrification in matrix flows of the aquifer makes interpretation of the results of this study difficult. Strong variation in the $\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$ within sites across multiple years as well as between springs underlines the complexity of spring systems, which integrate surface derived inputs across wide areas and multiple time scales. Short residence times occurring through preferential flows provide little potential for N-source fractionation, while longer residence times through matrix flows allow for source transformation and subsequent fractionation, and can result in the loss of the isotopic signature of the original nitrogen source. Additionally, Cohen (2008) stresses that biological nitrogen fixation (BNF) is usually not taken into account in watershed-scale nitrogen budgets although it can

represent up to 28% of the total budget (Van Breemen *et al*., 2002, in Cohen, 2008) and occurs in Florida at high but variable rates in the understory of long-leaf pine savannas (Hiers *et al*., 2003 in Cohen, 2008). The isotopic signature of BNF is from -3 to 1 ‰, similar to that of the atmosphere (0 ‰) (Kendall, 1998) and can decrease the $\delta^{15}N\text{-}NO_3$ signal of a more enriched source through mixing.

$\delta^{15}N$ of Algae and Sediment

A poor positive relationship ($r^2 = 0.34$) was found between the $\delta^{15}N$ of algae and the $\delta^{15}N\text{-}NO_3$ in spring water discharged from the boil, the primary source of N to the algae (Figure 3-3). Algal $\delta^{15}N$ was always lower than that of the source water, likely due to fractionation during algal uptake, during which more ^{14}N than ^{15}N is assimilated resulting in an isotopically lighter signature than that of the source (Fry, 2002). The poor relationship is likely due to different fractionation factors for algae of different species as well as algae of the same species but from different locations. De Brabandere, Frazer & Montoya (2007) found that fractionation in periphyton attached to macrophytes in the spring-fed Chassahowitzka and Homossassa rivers varied from 0.7 to 2.5 ‰ and state that fractionation reported in the literature for free-floating algae in the water column ranges from 2.5 to 10 ‰ (multiple sources listed therein). Fogel and Cifuentes (1993) state that fractionation values of up to -27‰ have been recorded for algae growing in culture.

For algal $\delta^{15}N$, there was a strong negative correlation to water column total N and NO_2/NO_3-N concentrations (Table 2-2) and strong positive correlations to P availability indices, i.e., $\delta^{15}N$ was high when N was in short supply, but P was available. If N is in short supply compared to P it would suggest N limitation and therefore more complete assimilation of water column nitrate and less isotopic fractionation. In contrast, when N supplies are relatively greater than P demand, then algal cells can be more discriminating in the isotope form of their nitrate

source (^{14}N vs. ^{15}N), thereby producing greater fractionation and isotopically light algal tissues. However, when nitrate concentrations in the water column were plotted against the $\delta^{15}N$ of algae, sediments and spring water at 10 spring sites, no direct relationship was found (Figure 3-3). This disparity in results is difficult to explain but may be due to sample size. A larger data set was used to calculate the Spearman correlations (63 spring sites), whereas only 10 sites were analyzed for the data shown in Figures 3-2 and 3-3, since $\delta^{15}N\text{-}NO_3$ was only available for these sites.

Unlike algae, the $\delta^{15}N$ of sediment (exposed or beneath an algal mat) was never negative and no relationship was found with either the $\delta^{15}N\text{-}NO_3$ in spring water (Figure 3-2) or with NO_3 concentrations in spring water (Figure 3-3). Sediment $\delta^{15}N$ is a reflection of the organic matter $\delta^{15}N$ (both autochthonous and that of terrestrial origin) as well as diagenic processes, such as denitrification (which leave enriched residual $\delta^{15}N$) (Brenner *et al.*, 1999; Finlay, 2001) and the lack of negative values may point to denitrification as an important process occurring in sediments. The $\delta^{15}N$ of algae was not strongly correlated to $\delta^{15}N$ of sediment under algal mats, which would be expected if the source material under sediments was primarily algae and little or no transformation/fractionation had occurred.

For both algae and sediments, $\delta^{15}N$ values above 5 ‰ may be indicative of N from soils (both natural and fertilized soil) as well as animal and/or septic waste. However, as mentioned previously, denitrification can also lead to high $\delta^{15}N$ values of residual N in sediments (Kendall 1998, Fry et al. 2003). $\delta^{15}N$ values of -8 to +4 ‰ can indicate inorganic fertilizers as a source of nitrogen, but theses values can overlap with values of other sources, including soil N (fertilized or natural), N from precipitation and nitrogen fixation. *Lyngbya wollei* has the ability to fix atmospheric N_2 (Phlips, 1992) and this process would result in a $\delta^{15}N$ of 0 to +2 ‰ (Kendall,

1998). Again, fractionation during the uptake of NH_4 and NO_3 is an additional factor that complicates source definition based on bulk $\delta^{15}N$ values.

$\delta^{15}N$ Gradients in Spring-Fed River Runs

De Brabandere, Frazer & Montoya (2007) found that the $\delta^{15}N$ of epiphytic periphyton, macrophytes and dissolved nitrate increased as nitrate concentrations decreased downstream. Decreasing concentrations were attributed to biological uptake of N with little to no nitrate inputs from surface or ground water. This resulted in a smaller nitrogen pool and therefore less source discrimination or isotopic fractionation during assimilation. In Toda *et al.* (2002), the isotopic signature of the nitrogen source, rather than a decrease in fractionation, was more important in determining the isotopic signature of periphyton. The $\delta^{15}N$ of epilithic periphyton (predominantly attached algae) increased with increasing total N concentrations downstream. Concentration increases were ascribed to increased N loading rates from several sewage treatment plants and livestock operations downstream. These sources have a relatively high $\delta^{15}N$ signature which was reflected in the enriched $\delta^{15}N$ of the periphyton.

We did not find such distinct patterns between nitrate concentrations and/or the $\delta^{15}N$-NO_3 and algal $\delta^{15}N$ in my study. Results from the four river runs sampled illustrate the difficulty in interpreting $\delta^{15}N$ values of different algal species in many Florida Springs (Figure 5). Neither the nitrate-$\delta^{15}N$ and $\delta^{18}O$ nor the nitrogen water chemistry varied within the same river along a longitudinal gradient (Figure 3-4 and Table 3-3), yet some algal species showed relatively large isotopic variability while others did not. For example, the $\delta^{15}N$ of *Spirogyra* sp. in the Weeki Wachee River increased from -1 to 4 ‰ within a 0.6 km distance, while the $\delta^{15}N$ of *L. wollei* did not vary along 8 km of the same river run. Differences in species-specific fractionation during assimilation can help explain differences in the $\delta^{15}N$ of different species at the same site. The effect of environmental factors (e.g. light and current velocity) and physiological factors (e.g.

growth rate) on fractionation during the uptake of N need to be better understood to explain species-specific $\delta^{15}N$ variability at different sites despite same source $\delta^{15}N$-NO_3 values.

Seasonal Variation in Algal $\delta^{15}N$

The $\delta^{15}N$ of *Vaucheria* sp. at Manatee Springs varied substantially (from -8 to +1), but values were predominantly below -4 ‰ for the majority of the year, which indicates inorganic NH_4 fertilizer and/or N from rain as probable sources (Figure 3-6). Although monthly $\delta^{15}N$-NO_3 of spring water is not available for Manatee, I obtained $\delta^{15}N$-NO_3 values of 6 and 7 ‰ in August 2005 and April 2008, respectively, and Katz (2004) lists a value of 6 ‰. These values also indicate NH_4 fertilizer, N from rain and soil nitrogen as important sources, which corresponds with values found in the algae samples. Lower $\delta^{15}N$ values are expected for algae than for N-NO_3 in the source water due to fractionation during algal uptake.

The average algal mat area and mat thickness for Manatee and Ichetucknee Blue Hole were calculated by Sickman *et al.* (2009). When the $\delta^{15}N$ of *Vaucheria* sp. is compared to mean thickness of the algal mat both Manatee and Blue Hole (both variables were sampled on the same day), the most enriched $\delta^{15}N$ values correspond to months in which the average algal mat thickness was lowest, while the lowest $\delta^{15}N$ signatures were obtained during months when the average algal mat was thickest (average thickness ranged from < 1 cm to 20 cm.) These values indicate a change in the source of N for the algae corresponding to algal mat thickness. When the mat is thickest, the algae may be relying on N that has been recycled within the mat itself due to decreased diffusion of N into the inner portions of the mats (Stevenson & Glover, 1993). Checkley & Miller (1989, in Fogel and Cifuentes, 1993) found lighter $\delta^{15}N$ signatures for autotrophs that were taking up isotopically light NH_4 regenerated from decomposing zooplankton. A similar processes may be occurring within *Vaucheria* mats, where the algae is taking up isotopically lighter N from decomposing algae as well as other biota within the mat.

Unlike *Vaucheria* sp., the $\delta^{15}N$ values for *Lyngbya wollei* at Ichetucknee Blue Hole were relatively constant (Figure 3-6). The range in isotopic signature of *L. wollei* was typical of inorganic fertilizers, soil N and/or nitrogen fixation (-1 to +1 ‰), of which this species is capable (Phlips, 1992). The lack of variability in $\delta^{15}N$ values throughout the year, however, despite high variability in *Vaucheria* sp. $\delta^{15}N$, points to nitrogen-fixation as a likely source of N for *L. wollei*.

Conclusion

In conclusion, the stable isotope measurements of algae, sediments and nitrate provided information on potential sources of N to spring algae and sediments, however, owing to the complexity of biogeochemical cycling of N in these systems many questions remain unanswered. Assuming that nitrate isotope composition is indicative of N sources, some springs may be receiving inputs from animal operations (high $\delta^{15}N\text{-}NO_3$) while others show signs of fertilizer pollution or inputs from atmospheric deposition. However, the tight correlation between O and N isotopes of nitrate in most springs, might suggest a uniform nitrate source to most Florida springs; isotopic variation of nitrate, instead, is being produced by denitrification in the aquifer. Finally, studies need to be conducted on physiological and environmental factors affecting within and between-species variability in fractionation during algal uptake of nitrogen.

Table 3-1. Stable isotopes of nitrate ($\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$) from 17 Florida springs sampled in 2005, 2006 and 2008. Water samples were collected directly above the boil of each spring, at a depth of 0.5 m.

Spring	Year sampled	$\delta^{15}N\text{-}NO_3$	$\delta^{18}O\text{-}NO_3$
Fanning	2005	8.0	7.4
	2006	7.9	5.5
	2008	7.7	5.7
Guranato	2006	5.1	8.0
	2008	5.4	7.2
Ichetucknee Head	2005	3.5	2.9
	2008	3.9	6.3
Ichetucknee Blue Hole	2005	4.4	3.9
	2008	4.2	7.2
Jackson Blue	2005	2.9	4.9
Lafayette Blue	2005	8.0	7.5
	2006	9.3	9.3
	2008	13.3	11.4
Little Fanning	2006	7.9	5.4
Little River	2006	5.7	7.8
	2008	11.0	11.1
Madison Blue	2005	3.6	3.0
	2006	4.2	6.4
Manatee	2005	5.7	5.1
	2008	6.6	7.0
Rainbow	2005	3.9	3.3
	2006	4.0	6.0
	2008	4.2	5.8
Silver River	2005	6.8	6.2
	2006	7.4	7.4
	2008	7.6	7.4
Troy	2005	7.1	6.5
	2006	7.2	10.1
	2008	20.2	15.3
Volusia Blue	2008	14.5	10.8
Wakulla	2005	8.8	8.2
	2006	9.2	5.5
	2008	9.7	5.1
Wekiwa	2008	15.9	13.9
Weeki Wachee	2008	6.2	4.6

Table 3-2. Significant Spearman correlations ($p \leq 0.001$) between algal $\delta^{15}N$ and sediment $\delta^{15}N$ and indicators of nutrient availability and nutrient sources. Only variables collected in spring boil areas were analyzed for a total of 34 sites. There were often multiple algal species per site. $\delta^{15}N$ of algae and sediments was correlated to two water chemistry data bases: (1) water chemistry from the 2006 survey and (2) water chemistry from an average of the 2003 and 2006 surveys.

	Variables	Correlation coefficient	n
$\delta^{15}N$ of algal tissue and	Total Kjeldahl N of springwater (2006)	0.427	56
	Sediment under algae C:N molar ratio	-0.442	50
	Average NO_2/NO_3-N of springwater (2003 + 2006)	-0.586	47
	Total N of springwater (2003 + 2006)	-0.598	47
$\delta^{15}N$ of sediment under algae and	Exposed sediment $\delta^{15}N$	0.513	48
	Total Kjeldahl N of spring water (2006)	0.465	52
	Sediment under algae $\delta^{13}C$	0.453	49
	Sediment under algae %C	-0.453	51
	Sediment under algae C:N	-0.468	50
$\delta^{15}N$ exposed sediment and	Sediment under algae $\delta^{15}N$	0.513	48
	Total P in springwater (2006)	0.443	53
	Sediment under algae %C	-0.575	37

Table 3-3. Spring river run longitudinal study site numbers, site codes and their distance from the spring boil (km).

Spring Run	Site Number	Site Code	Distance from boil
Silver Springs	1	SLV-01	0.0
	2	SLV-02	0.5
	3	SLV-03	1.3
	4	SLV-04	3.2
	5	SLV-05	5.3
	7	SLV-07	7.2
Rainbow Springs	1	RAI-01	0.0
	2	RAI-05	1.3
	3	RAI-02	1.6
	4	RAI-06	3.5
	5	RAI-03	5.2
	6	RAI-07	7.6
	7	RAI-04	7.7
Wakulla Springs	1	WAK-01	0.0
	2	WAK-04	0.5
	3	WAK-05	1.0
	4	WAK-02	1.6
	5	WAK-06	2.6
	6	WAK-03	3.2
	8	WAK-08	9.8
Weeki Wachee Springs	1	WEK-01	0.0
	2	WEK-02	0.2
	3	WEK-03	0.6
	4	WEK-04	1.8
	5	WEK-05	3.1
	6	WEK-06	4.0
	7	WEK-07	8.1

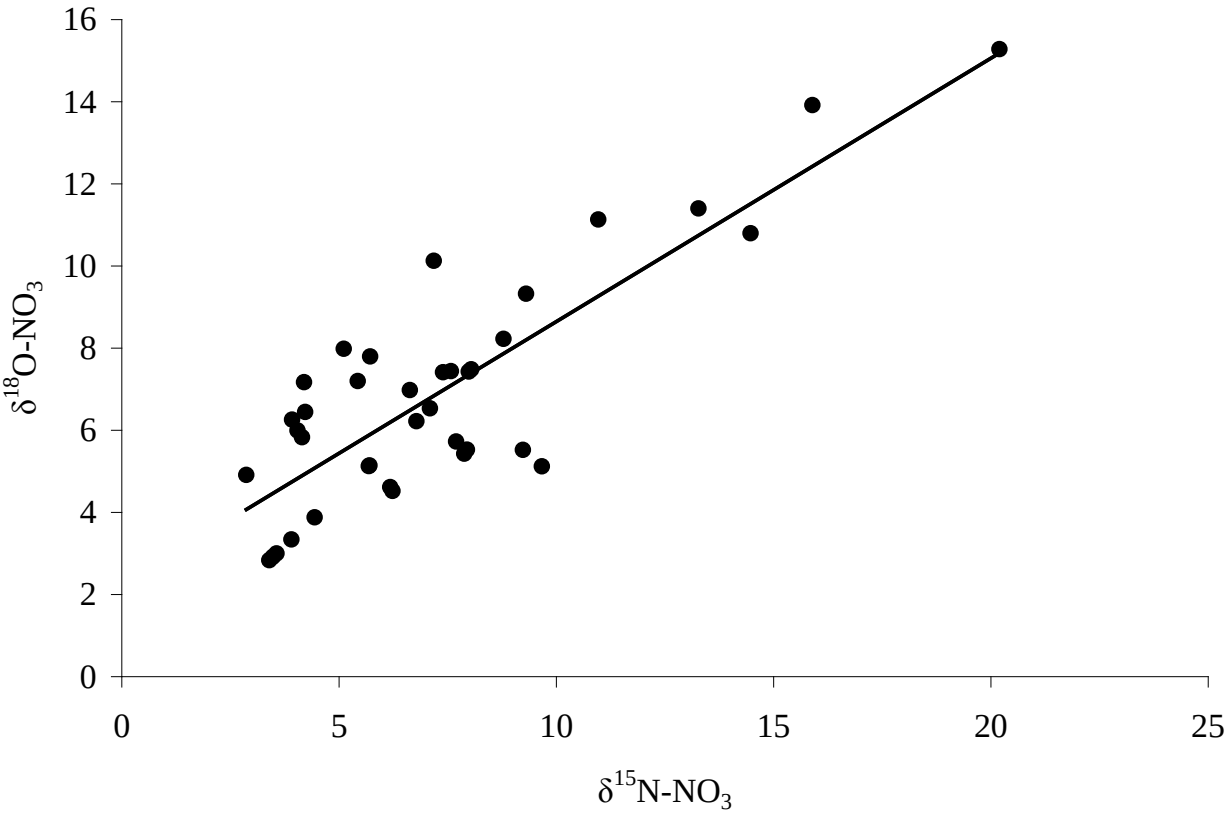


Figure 3-1. Stable isotopes of nitrate (δ^{15}N-NO_3 and δ^{18}O-NO_3) from 17 Florida springs sampled in 2005, 2006 and 2008. Water samples were collected directly above the boil of each spring, at a depth of 0.5 m. $R^2 = 0.71$ and the slope of the line is 0.64.

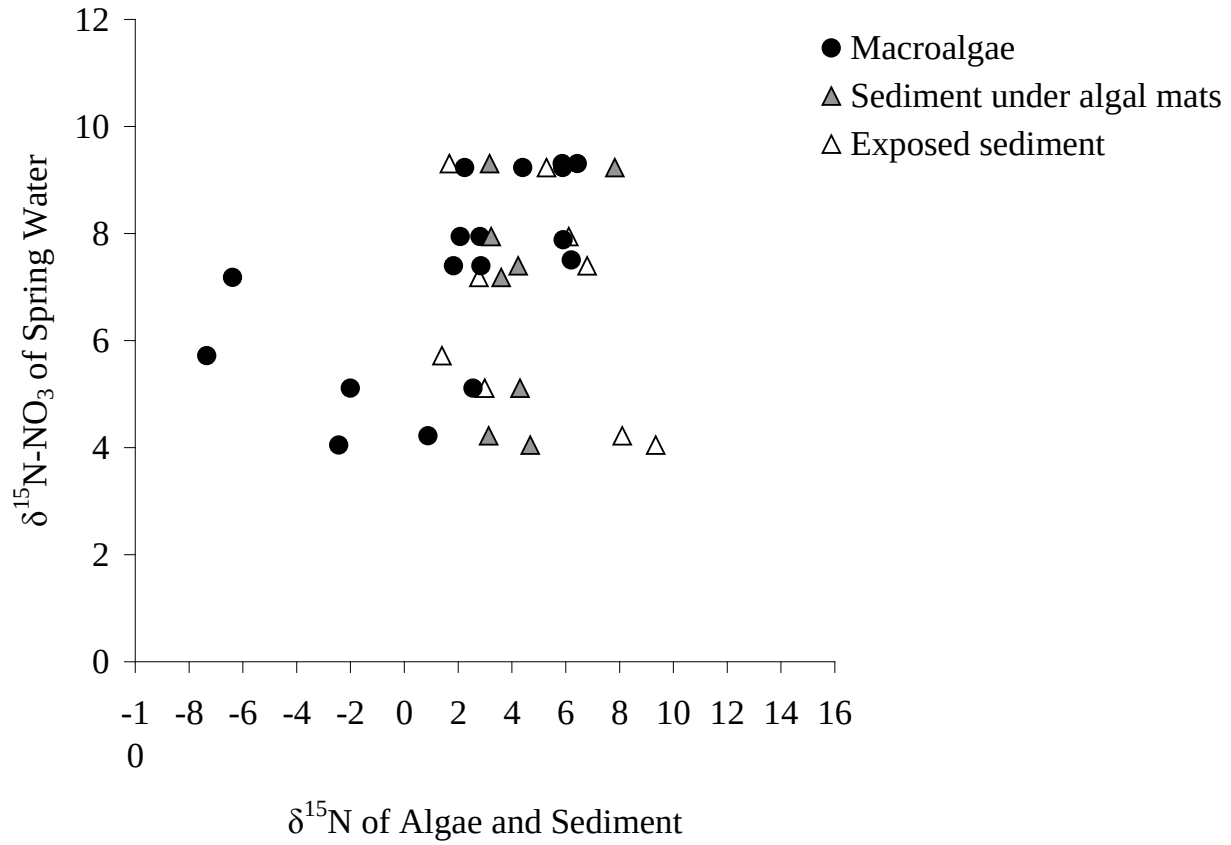


Figure 3-2. Stable isotope composition ($\delta^{15}N$) of algae and sediment and the $\delta^{15}N\text{-}NO_3$ of springwater from 10 headwater springs sampled in 2006.

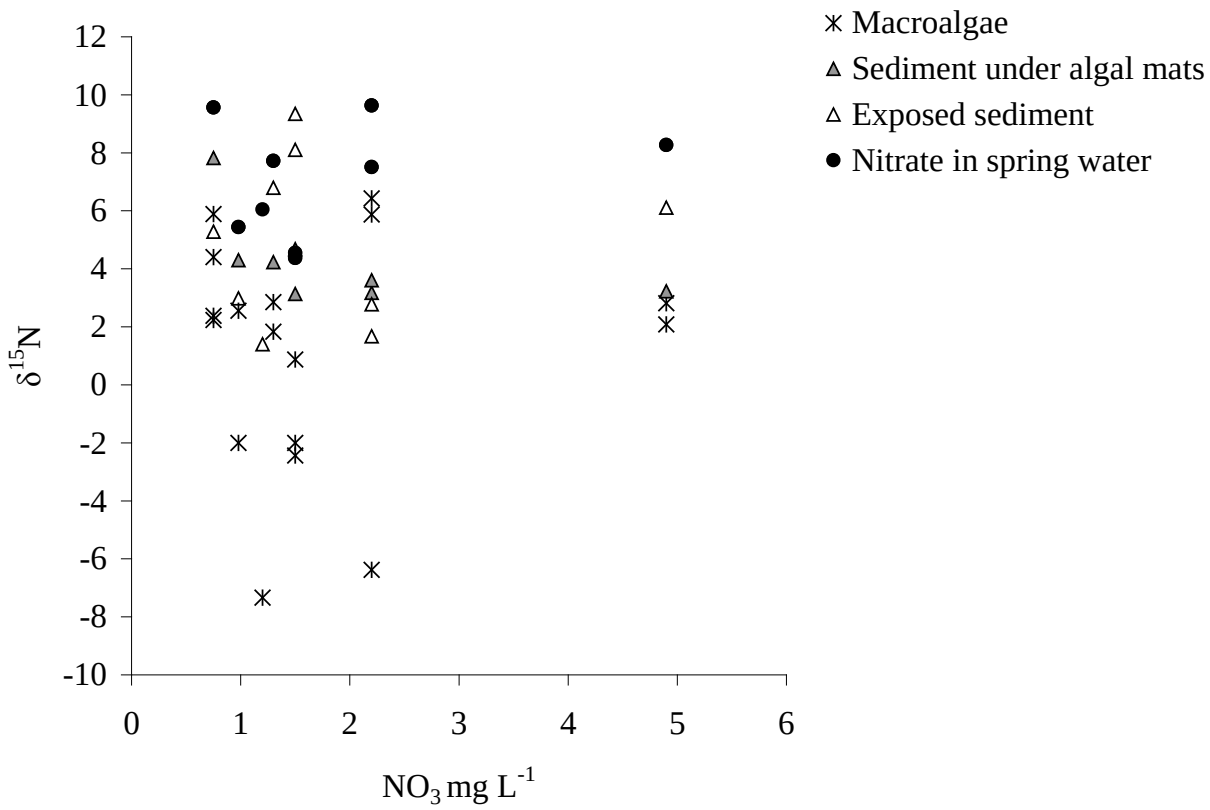


Figure 3-3. Relationship between the $\delta^{15}N$ values of algae, sediments and nitrate in NO_3 of spring water and nitrate concentrations (mg L^{-1}) of 10 headwater springs sampled in 2006.

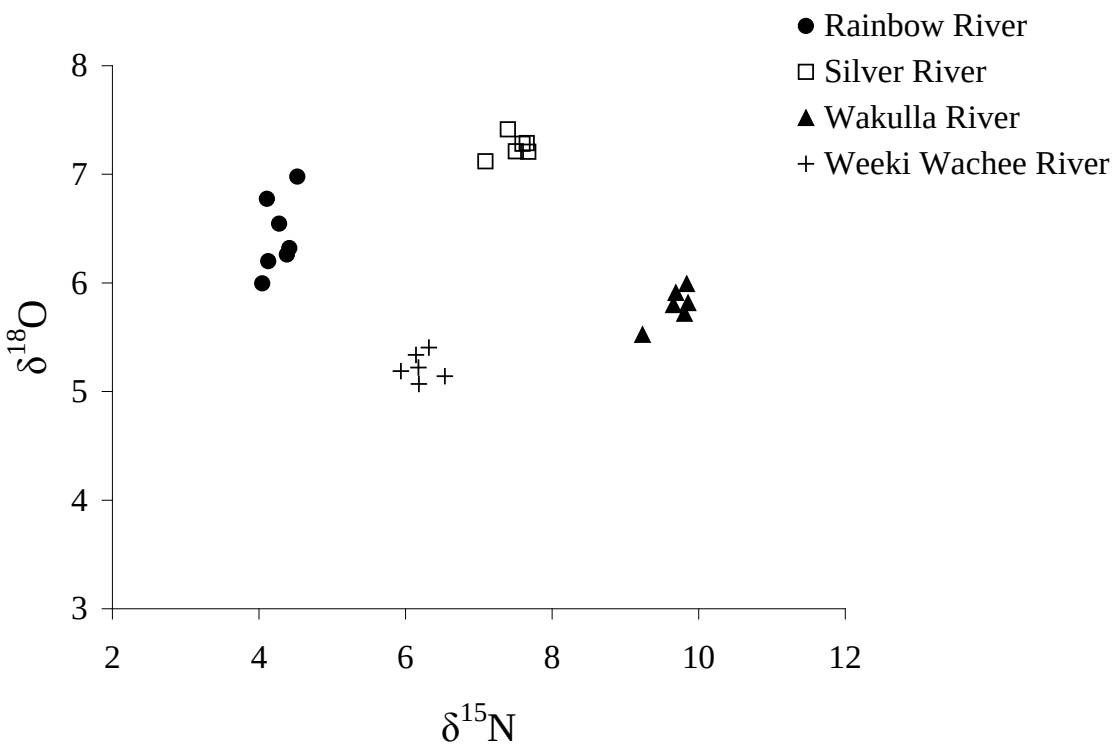


Figure 3-4. Stable isotope composition of nitrate in spring water from the Rainbow, Silver, Wakulla and Weeki Wachee River runs sampled in 2006. Samples were taken starting at the boil area of each site and ending 7.2 to 9.8 km downstream, depending on the site.

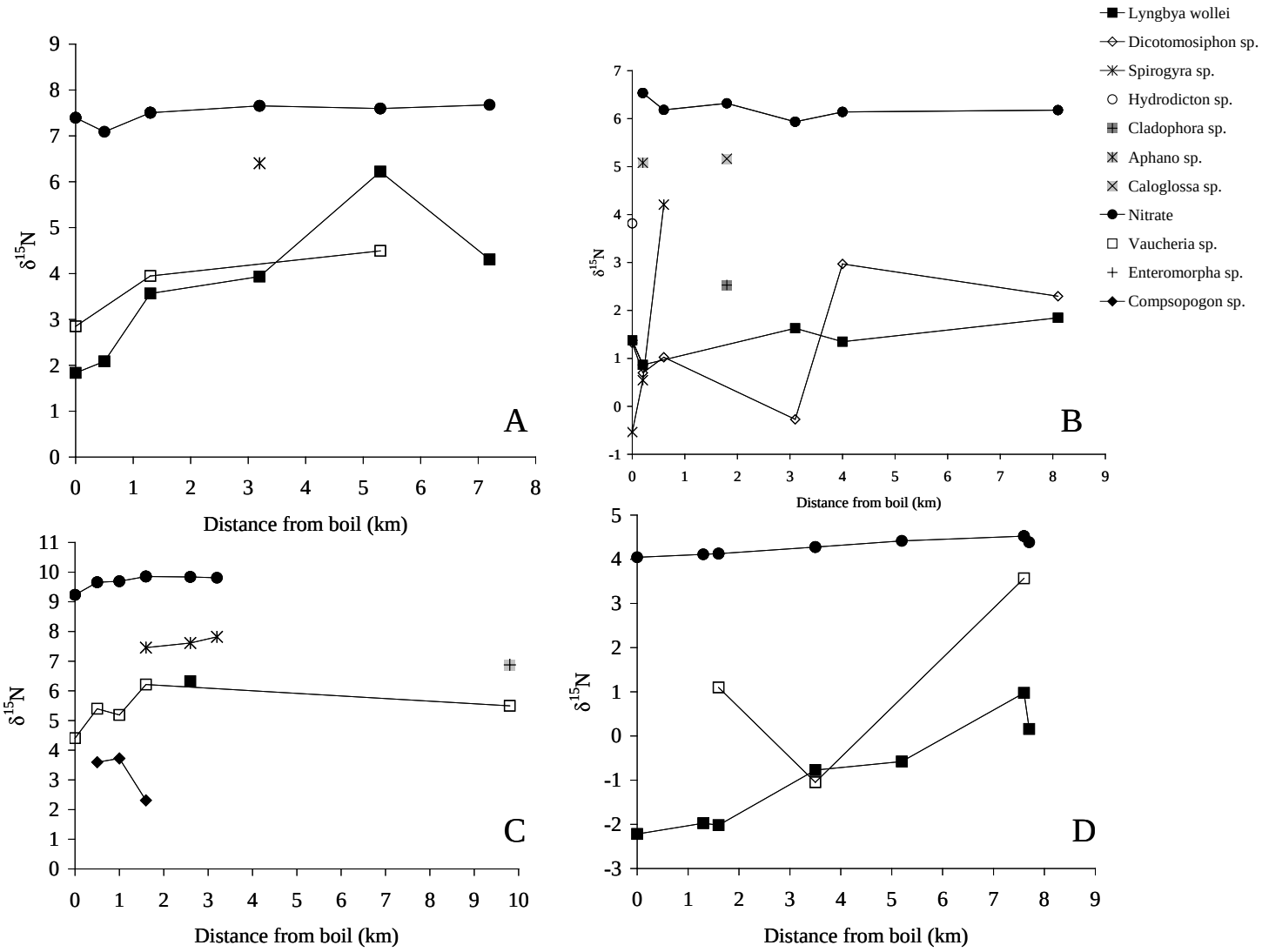


Figure 3-5. Stable isotope composition of algae and $\delta^{15}N\text{-}NO_3$ of spring water measured along four spring river runs in January 2006. A) Silver River. B) Weeki Wachee River. C) Wakulla River. D) Rainbow River.

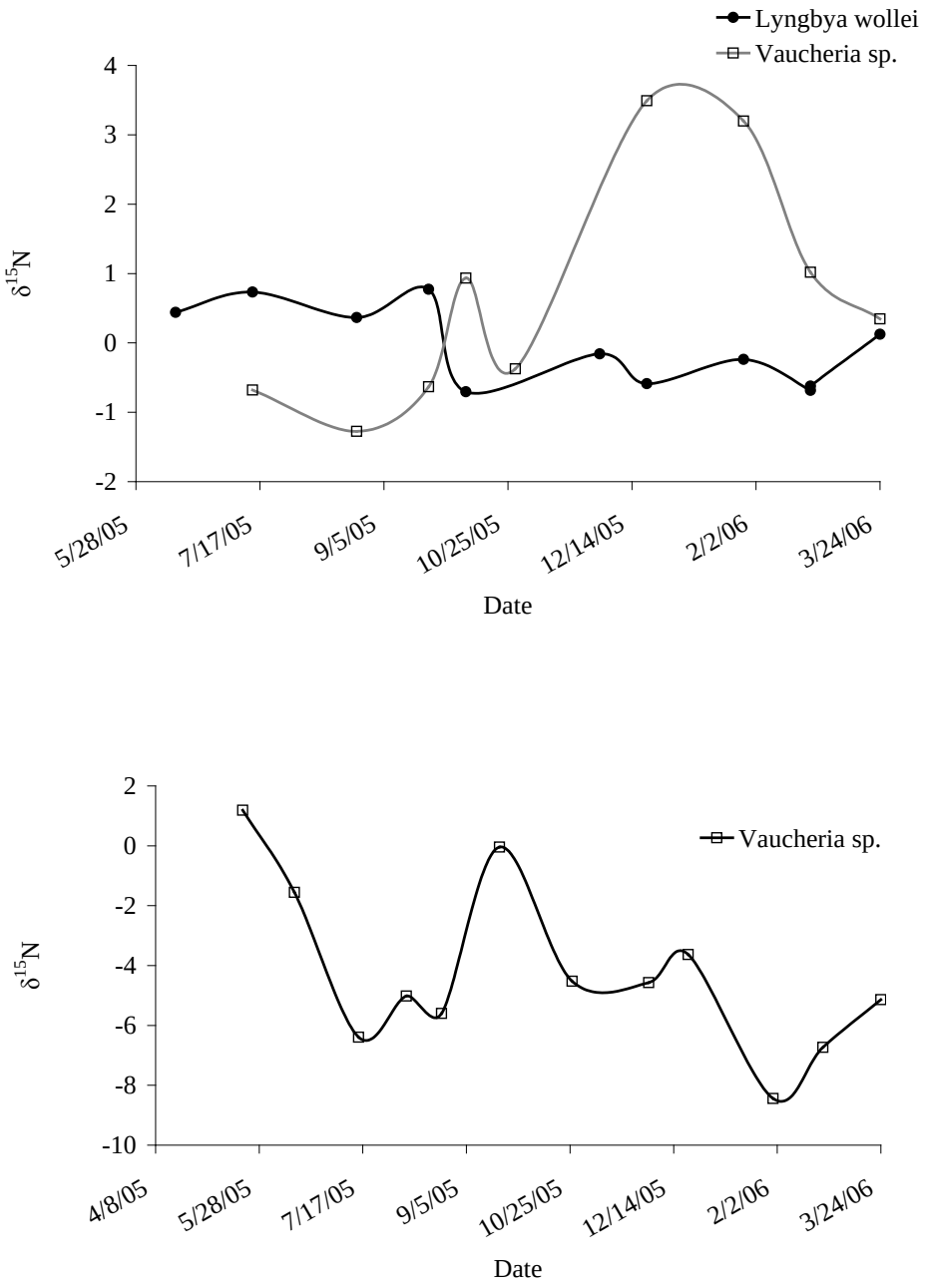


Figure 3-6. $\delta^{15}N$ composition of algae at Ichetucknee Blue Hole and Manatee Springs from May 2005 to March 2006. A) $\delta^{13}C$ of *Lyngbya wollei* and *Vaucheria* sp. at Ichetucknee Blue Hole. B) $\delta^{13}C$ of *Vaucheria* sp. at Manatee Springs.

CHAPTER 4
$\delta^{13}C$ STABLE ISOTOPE COMPOSITION OF ALGAE, SEDIMENT AND DISSOLVED INORGANIC CARBON IN FLORIDA SPRINGS

Introduction

Increases in the abundance of floating and benthic algal mats have been observed in many of Florida's karst springs during the last 50 years, particularly of *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck, and *Vaucheria* sp. De Candolle, the two most widely distributed mat-forming species (Odum, 1957; Whitford, 1956; Quinlan *et al*., 2008; Stevenson *et al.* 2008). These nuisance algal mats can out-compete native submerged aquatic vegetation (Doyle & Smart, 1998), greatly altering the ecology of spring systems. Additionally, increased algal biomass detrimentally affects the recreational use of springs in Florida (Florida Springs Task Force, 2000; Cowell & Botts, 1994). Although nutrient availability is thought to affect algal abundance (Florida Springs Task Force, 2000; Stevenson *et al*. 2007), a direct cause-effect relationship has not been clearly shown and multiple factors are likely influencing both biomass accrual and distribution in springs. The stable isotope signature of carbon ($\delta^{13}C$) in algae and dissolved inorganic carbon (the primary carbon source of algae in springs) can be used to gain a better understanding of factors affecting algal growth and distribution.

In aquatic systems, two primary factors affect the $\delta^{13}C$ composition of algae: i) the $\delta^{13}C$ of various forms of dissolved inorganic carbon (DIC) used in photosynthesis and ii) fractionation of C isotopes during algal uptake (i.e., preferential use of ^{12}C over ^{13}C) (Fry, 2006; Finlay 2004). In Florida springs, DIC sources include atmospheric CO_2 charged in precipitation, CO_2 produced by heterotrophic respiration in soils as water percolates into the Floridan Aquifer and DIC produced by weathering of carbonate minerals in the aquifer. Most algae have the ability to use both CO_2 and HCO_3^- as a source of carbon during photosynthesis and the relative abundance of these DIC species in spring waters depends on the pH and geochemical character of the water. Previous

studies have found differences of 7 to 10 ‰ in $\delta^{13}C$, between HCO^{-}_{3} and CO_2 (aq) (Finlay 2004). For example, algal reliance on dissolved CO_2, with relatively low $\delta^{13}C$ values, caused by strong respiratory production of CO_2, could produce algal tissue of -45 ‰ (Fry 2006). Additionally, both light regime and current velocity can affect DIC source discrimination by algae. Increased insolation results in increasing rates of photosynthesis, resulting in higher carbon demand which in turn can lead to less source discrimination and higher $\delta^{13}C$ values in algae (Hill and Middleton, 2006). However, under conditions of higher current velocity, a system can be replenished by isotopically lighter CO_2 (aq), resulting in lower algal $\delta^{13}C$ values (Finlay, 1999).

The $\delta^{13}C$ of organic matter in spring sediments is affected by the $\delta^{13}C$ of source materials (terrestrial vs aquatic), the $\delta^{13}C$ of the DIC taken up during aquatic photosynthesis (Brenner, 1999), and post depositional processes such as decomposition and methanogenesis. The $\delta^{13}C$ composition of aquatic primary producers is often more variable than that of terrestrial autotrophs (Finlay, 2004); terrestrial C_3 plants generally produce detrital materials ranging from -34 to -22 ‰ $\delta^{13}C$ (Rounick and Winterbourne 1986, Finlay, 2004) while $\delta^{13}C$ values in algae are more variable, with ranges of -3 to -46 ‰ (Raven *et al*. 2002, Fry, 2006). Finlay (2001) found that the particulate terrestrial detritus of temperate streams has a constrained mean value of -28.2 +/- 0.2 ‰, most likely because the $\delta^{13}C$ of the detritus in these systems was integrated through space and time. Diagenesis occurring in the sediment once the organic matter has been deposited also affects $\delta^{13}C$ values (Brenner 1999). For example, microbial decomposition of the organic matter may select for isotopically lighter carbon (^{12}C) over ^{13}C, resulting in enriched residual carbon.

In Florida springs, the $\delta^{13}C$ stable isotope of carbon of algae and spring sediments as well as $\delta^{13}C$ of the dissolved inorganic carbon (DIC) of spring water were measured at multiple scales

to help determine carbon sources to benthic algal mats and factors controlling algal abundance. The specific objectives of this study were to: (1) determine the $\delta^{13}C$ of DIC and total DIC concentrations at multiple headwater springs throughout the state, (2) determine the $\delta^{13}C$ composition of algae and spring sediments through surveys at a regional scale and along four spring river runs, (3) evaluate the $\delta^{13}C$ composition of filamentous mat-forming algae over the course of one year at two springs, and (4) assess the relationships between the $\delta^{13}C$ of algae, carbon sources and indicators of nutrient availability at the regional scale .

Methods

Study Sites

$\delta^{13}C$ analysis was conducted on mat-forming algae and sediments in Florida springs on three scales: (1) regionally, (2) along a longitudinal gradient (starting at the spring boil and sampling 8 to 10 km progressively further downstream) of the spring-fed Silver, Rainbow, Wakulla and the Weeki Wachee Rivers and (3) on a monthly basis in the boil areas of Manatee and Ichetucknee Blue Hole Springs during the course of one year. The same sites were sampled as for the $\delta^{5}N$ analysis of algae and sediments, which is described in Chapter 3. The complete list of sites and locations sampled for the regional, gradient and seasonal studies is found in Appendix A. Specific sites sampled for the longitudinal gradient study are listed in Table 3-3 and site codes correspond to those listed in Appendix A.

Additional sampling trips were conducted in April and August of 2008 to 18 headwater springs in order to collect samples for dissolved inorganic carbon (DIC) analysis of spring water and $\delta^{13}C$ analysis of algae. The sites sampled are listed in Table 4-2 and site codes correspond to those listed in Appendix A.

Algae, Sediment and Water Sample Collection

The same samples that were collected for the $\delta^{15}N$ analysis of algae and sediment (described in Chapter 3) were also used for $\delta^{13}C$ analysis of algae and sediment. Water samples for chemical/physical parameters (TKN, TP, NH_4^+, NO_x^-, SRP, DOC, temperature, pH, conductivity and dissolved oxygen) used in a correlation analysis with the $\delta^{15}N$ of algae and sediment (also described in Chapter 3) were used in a correlation analysis with $\delta^{13}C$ of algae and sediment, described in the statistical analysis section below. Detailed descriptions of sampling methodology can be found in the methods section of Chapter 3. This includes samples from the 2006 regional study, the 2006 longitudinal gradient study and the seasonal study conducted in 2005 and 2006.

Algae, Sediment and Water Sample Processing and Analysis

Algae samples were picked clean of invertebrates and debris within 24 hours of field collection, stored frozen and later lyophilized at -91°C under a 35-mTorr vacuum. Once dry, they were again picked clean of any debris initially missed and ground and homogenized in a ball mill. The samples collected and processed to determine $\delta^{13}C$ of algae and sediments were also used to determine percent C, N and P. Algae samples that were placed in scintillation vials for species verification were preserved using M3 solution and sent to the Center for Water Sciences at Michigan State University, where they were identified.

Macroinvertebrates were removed from sediment samples which were then homogenized by stirring, also within 24 hours of field collection. Sediments were stored frozen and subsequently dried in an oven at 60°C for 5 days. They were then passed through a sieve to remove coarse debris (e.g, twigs, leaves, whole mollusk shells) and ground and homogenized in a ball mill. Prior to $\delta^{13}C$ isotopic analysis, sediment samples were acid fumigated with HCl to remove inorganic carbon (Harris *et al.*, 2001). A subset of algae samples collected in 2006 was

also acid fumigated to see whether or not calcium carbonate was deposited on the algal tissue and affected $\delta^{13}C$ values.

Carbon isotopic composition of algae and sediments was measured on a Thermo Finnigan Delta-Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.) at the University of Florida using an elemental analyzer inlet system and continuous flow of He. The International Atomic Energy Association standard for sucrose was included in each run and $\delta^{13}C$ isotope abundances are reported in δ notation relative to international standards (Vienna PeeDee Belemnite for C).

Percent carbon and nitrogen of the dried algal tissue and sediments were measured by high temperature combustion using a Flash EA 1112 Nitrogen/Carbon Analyzer with MAS 200 R Autosampler (Thermo Fisher Scientific Inc, Waltham, MA, U.S.A.). Phosphorus content of dried algal tissues and sediment was measured on combusted (550°C) and acid digested (6N HCl) samples as SRP (Anderson, 1976) on a Technicon Autoanalyzer (Technicon Instruments Corporation Wilmington, MA, U.S.A.). At Michigan State University, sediments were analyzed for % water content, dry mass (DM), ash free dry mass (AFDM) (Eaton *et al.*, 1995), available phosphorus (PO_4^-) and available nitrogen (NH_4^+, NO_2/NO_3^-) following the extraction of 1g of wet sediment with Truog's reagent and KCl (Allen, 1989).

Soluble reactive phosphorus, NH_4^+, and NO_3^- were measured on a Bran+Luebbe Auto Analyzer 3 (Bran+Luebbe, Norderstedt, Germany) using EPA Methods 365.1, 350.1 and 353.2, respectively. Total Kjeldahl nitrogen was determined by H_2SO_4 and Kjeldahl salt digestion and flow-injection determination of ammonium (EPA Method 351.2). Total phosphorus was measured as SRP on a Bran+Luebbe Auto Analyzer 3 after digestion with H_2SO_4 and potassium persulfate (EPA Method 365.1).

Sample Collection and Processing for Total DIC, δ^{13}C-DIC and δ^{13}C of Algae

Water samples for DIC analysis were collected in April and August of 2008 either from a kayak or from the shore above the spring boil depending on the location of the boil. Glass vials (30ml) containing 4 to 6 mg of blue copper sulfate crystals were used for sample collection (US EPA, 2006). The cap was unscrewed while underwater (approximately 0.3 to 0.5 m below the surface), the sample vial was completely filled (leaving no headspace) and then the cap was screwed back on while still underwater, making sure that the sample had no contact with the atmosphere to avoid CO_2 exchange (US EPA, 2006). Samples were transported on ice to the laboratory where they were stored in a refrigerator at 4°C until analysis. For reasons that are still unknown, many of the water samples collected in April 2008 froze in the refrigerator and burst. A subsequent sampling trip was then rescheduled in August to revisit sites where the samples had been lost as well as to resample several sites to compare DIC variability in samples that had not burst, which is why some, but not all sites, have two samples (April and August) (Table 4-2).

A composite sample of each of the dominant algal species was also collected at each site by snorkeling, shaken in the water to remove any loosely attached debris and placed into 1 gallon Ziploc bags (SC Johnson, Racine, WI, USA) filled with site water. Samples were then transported to the laboratory on ice. The algal samples were processed as described above. An additional step was taken, however. A subsample was taken from each site and washed in a solution of HCl (pH = 2). This was done in order to compare δ^{13}C values of acidified vs. unacidified samples to see if $CaCO_3$ potentially deposited on the algal tissues during drying affected the δ^{13}C value. Additionally, a YSI 556 Multi-probe System (YSI Incorporated, Yellow Springs, OH, U.S.A.) was used to measure temperature, conductivity, pH, and dissolved oxygen at each site above the spring boil.

All samples were analyzed at the University of Florida. Total DIC was measured on an AutoMate Automatic Acidification System Coupled with a UIC 5011 Coulometer and the $\delta^{13}C$ of DIC and algae was measured on a Delta-Plus XP Isotope Ratio Mass Spectrometer.

Statistical Analysis

Spearman correlations were used to determine relationships between algal and sediment $\delta^{13}C$ composition and indicators of nutrient availability and sources, as well as other environmental variables collected during the Rapid Habitat Periphyton Analysis, which is described in the Methods section of Chapter 3. Variables correlated to algal and sediment $\delta^{13}C$ are the same as those listed in Chapter 3 and include: (1) site water physical-chemical parameters, (2) algal and sediment C:N:P molar ratios, as well as bioavailable N and P of the sediments, (3) average site depth and current velocity, (4) average site canopy cover, (5) site buffer zone characteristics, (6) land use characteristics and LDIs. Land use characteristics for each site and LDI (Landscape Development Intensity) indexes were calculated from data provided by the Florida Department of Environmental Protection (FDEP) by A. Pinowska. A subset of the regional study sites (34) was used in the correlation analysis. Only samples from the spring boil areas were used, in order to avoid autocorrelation among multiple sites along spring runs. Samples collected in April and August of 2008 for DIC analysis were not included in the correlation analysis.

Results

Analysis of Algae and Sediment Stable Isotopes and C:N Molar Ratios

$\delta^{15}N$ values of algae and sediment obtained for analysis in Chapter 2 were plotted against $\delta^{13}C$ values of the same samples (Figure 4-1). Algae and sediment in the 63 springs sampled for the 2006 regional study revealed a substantial range of values in stable isotopic composition. For algae, $\delta^{13}C$ ranged from -12 to -44 ‰. The highest $\delta^{13}C$ values (-12 ‰) were found for

Spirogyra sp. collected from the boil areas of Juniper and Alexander Springs in the Ocala National Forest. The lowest $\delta^{13}C$ values (-44 ‰) were obtained for *Vaucheria* sp. from Wakulla, Guaranto, Volusia and Troy Springs. $\delta^{15}N$ values ranged from +8 to -7 ‰. The highest values (+8 ‰) were found in *Spirogyra* sp. collected along the Wakulla Springs river run and in *Lyngbya wollei* samples collected near the boil area of Alexander Springs. The lowest $\delta^{15}N$ value (-7 ‰) was found in *Vaucheria* sp. from Little River Springs.

Isotope values were less variable for sediments than for algae (Figure 4-1) and all but two sediment samples fell within the $\delta^{13}C$ and $\delta^{15}N$ ranges found for algae. Exposed sediment refers to sediment samples collected from an area with no overlying benthic algal mat. $\delta^{13}C$ values for exposed sediment ranged from -20 to -32 ‰, with the most enriched value found at Ponce de Leon Springs and the lowest value found along the Weeki Wachee and Silver River spring runs. $\delta^{15}N$ values for exposed sediment ranged from 0 to 10 ‰. The highest value was obtained at both Rainbow and Wekiwa Springs and the lowest value (0 ‰) was found along the Weeki Wachee Springs river run. Sediment samples collected from underneath algal mats had a narrower range of $\delta^{13}C$ values, from -25 to -32 ‰. The most enriched values (-25 ‰) were found in Pitt, Juniper and Silver Glen Springs, and the lowest value was found at Ichetucknee Springs. $\delta^{15}N$ values for sediment under algal mats showed a similar range as that of exposed sediment (0 to 8 ‰).

The relationships between the $\delta^{13}C$ of algae and sediment and their respective C:N molar ratios are shown in Figure 4-2. Sediment C:N ratios (both exposed and under algal mats) showed a wide range of values, from 6 to 112, with both the highest and lowest values for exposed sediment, while algal C:N range was much lower, from 6 to 18. There was no apparent relationship between $\delta^{13}C$ of algae and sediment and C:N ratio.

Variation in Isotope Signatures among Algal Species

The three most common species found during the regional study, *Lyngbya wollei*, *Vaucheria* sp. and *Spirogyra* sp., had relatively distinct $\delta^{13}C$ isotopic compositions (Figure 4-3). *Vaucheria* sp. had both the lowest $\delta^{13}C$ and $\delta^{15}N$ values of the three species. $\delta^{13}C$ ranged from -44 to -33 ‰, with the lowest values found at Wakulla, Guaranto, Volusia and Troy Springs. $\delta^{15}N$ values ranged from -7 ‰ (Little River Springs) to +6 ‰ (Wakulla and Weeki Wachee Springs). *Spirogyra* sp. had both the highest $\delta^{13}C$ and $\delta^{15}N$ values. $\delta^{13}C$ ranged from -38 to -12 ‰, with the most enriched values found at Wakulla Springs. $\delta^{15}N$ values ranged from -1 (Weeki Wachee Springs) to +8 ‰ (Wakulla Springs). For *L. wollei*, $\delta^{13}C$ values ranged from -40 (Williford Springs) to -21 ‰ (Juniper Springs) and $\delta^{15}N$ values ranged from -2 (Rainbow Springs) to +7 ‰ (Alexander Springs).

Correlations among Algal Stable Isotope Signatures, Water Quality and Environmental Variables

Significant correlations ($p<0.001$) between algal tissue $\delta^{13}C$ and variables relating to indicators of nutrient availability and sources are listed in Table 4-1. To avoid Type 1 errors in this analysis, I set the p level at 0.001 to account for the relatively large number of correlations that were made. For both algal $\delta^{13}C$, significant positive and negative correlations were found between numerous variables. The strongest correlations were found with the pH of water (positive) followed by the C:P ratio of exposed sediment (negative).

Significant correlations ($p<0.001$) among sediment $\delta^{13}C$ and variables relating to indicators of nutrient availability and sources are shown in Table 4-1. For sediment collected beneath an algal mat, the strongest relationships to $\delta^{13}C$ were the C:N ratio of sediment under algae and the average concentration of orthophosphate in the water. This average includes data from 2003 (Fall and Spring) and the 2006 winter sampling event. The $\delta^{13}C$ of exposed sediment (sediment with

no algal mat on top of it), was most strongly related (negatively) to average conductivity (2003 + 2006) and the diatom indicator of sulfate concentrations in spring water. The strongest positive correlations were with an indirect measure of low P conditions (an indicator developed by R.J. Stevenson representing the percent of diatoms that grow under low phosphorus conditions) and dissolved oxygen.

Relationships between Total Dissolved Inorganic Carbon, pH and the $\delta^{13}C$ of Dissolved Inorganic Carbon and Algae

pH was positively correlated to algal $\delta^{13}C$ (Table 4-1) and species-specific relationships are shown in Figure 4-4. The r^2 values of the relationship between the $\delta^{13}C$ of *Vaucheria* sp., *Lyngbya wollei* and *Spirogyra* sp. and pH at 63 spring sites sampled in 2006 were 0.48, 0.40 and 0.41, respectively (Figure 4-4). For samples collected in 2008, algal $\delta^{13}C$ showed a stronger relationship to pH (positive) ($r^2 = 0.76$) and total DIC (negative) ($r^2 = 0.70$) than to the $\delta^{13}C$ of dissolved inorganic carbon (DIC) (positive) ($r^2 = 0.50$) (Figure 4-5). Species-specific distributions relating to total DIC were also discernible. *Vaucheria* sp.was generally found in springs with higher total DIC concentrations, while *Spirogyra* sp. was found in all but one case, in springs with low total DIC (Figure 4-5, A). *Lyngbya wollei* was more ubiquitous, but tended to be present in sites with lower total DIC. Since algal $\delta^{13}C$ was also related to the $\delta^{13}C$ of DIC and pH, species-specific tendencies were also found with these variables. The $\delta^{13}C$ of DIC also showed a stronger relationship to total DIC (negative) ($r^2 = 0.519$) than pH (positive) ($r^2 = 0.34$), while the r^2 value of total DIC vs pH was 0.812 (negative), the strongest relationship of all (Figure 4-6).

Variation in $\delta^{13}C$ of Algae and Sediment along Longitudinal Gradients

The sites sampled at each of the four spring river runs and their distance from the spring boil are listed in Table 3-3 of Chapter 3 (this document). The site codes are the same ones used

(and therefore the same locations) in the regional study and exact locations are listed in Appendix A.

Rainbow Springs River Run

$\delta^{13}C$ values of the algae and the sediment from the Rainbow Springs river run are shown in Figure 4-7. Two algal species were found, *Lyngbya wollei*, which showed a wide range of values along the river run (-34 to -24 ‰) and *Vaucheria* sp., with a much narrower range (-38 to -36 ‰). Sediment samples were generally more enriched than algal samples (-28 to -24 ‰), and the exposed sediment had the highest $\delta^{13}C$ values.

Weeki Wachee Springs River Run

Seven species of algae were found along the Weeki Wachee river run: *Vaucheria* sp., *Spirogyra* sp., *Lyngbya wollei, Hydrodictyon sp., Cladophora glomerata., Aphanothece* sp.balls and *Calaglossa* sp. (Figure 4-7). Algal species and sediment samples were separated out fairly well based on their $\delta^{13}C$ values, except for *Spirogyra* sp. which showed a sharp increase in values within the first km, from -37 to -21 ‰. The range of *Dichotomosiphon* sp. $\delta^{13}C$ (-44 to -42 ‰) was very similar to values seen at other sites for both *Vaucheria* sp. and *Compsopogon* sp. (Figure 4-7). $\delta^{13}C$ values did not vary widely for sediment samples (-31 to -26 ‰).

Wakulla Springs River Run

The $\delta^{13}C$ values of algae and sediments for the Wakulla Springs river run are shown in Figure 4-7. Five species of algae were found along the run: *Compsopogon* sp., *Vaucheria* sp., *Spirogyra* sp., *Lyngbya wollei* and *Enteromorpha* sp. *Compsopogon* sp. and *Vaucheria* sp. had very similar, low ranges in $\delta^{13}C$ (between -45 and -40 ‰) that did not vary much along the river run, while values for *Sprirogyra* were much more enriched and increased sharply with a 1.5 km distance,from -32 to -23. The sediment samples showed little variation along the run, from (-31 to -27 ‰).

Silver Springs River Run

Three algal species, *Lyngbya wollei*, *Vaucheria* sp. and *Spirogyra* sp. were found along the Silver Springs River run (Figure 4-7). The three algal species and the sediment samples were found to have relatively distinct $\delta^{13}C$ isotopic compositions. Algal $\delta^{13}C$ values ranged from -41 to -31 ‰, with *Vaucheria* sp. having the lowest values (-41 to -39 ‰). Sediment $\delta^{13}C$ values were more enriched, ranging from -31 to -25 ‰. $\delta^{13}C$ values were lowest at the spring boil for the algae and sediment sampled under a benthic algal mat and the values for sediment collected under an algal mats more closely followed the pattern of *L. wollei* and *Vaucheria* sp. than exposed sediment.

Seasonal Variation in Algal $\delta^{13}C$

Vaucheria sp.was the dominant algae found at Manatee Springs. $\delta^{13}C$ values decreased sharply (8 ‰) from May to June 2005 (from -36 to -44) and then remained relatively stable until March 2006, fluctuating between -43 and -46 ‰ (Figure 4-8). At Ichetucknee Blue Hole, $\delta^{13}C$ values varied seasonally in both *Lyngbya wollei* and *Vaucheria* sp. (Figure 4-8). *Vaucheria* sp. values ranged from -45 to -40 ‰ and *L. wollei* $\delta^{13}C$ ranged from -40 to -34 ‰.

Discussion

Dissolved Inorganic Carbon in Florida Springs

Dissolved inorganic carbon (DIC), composed of CO_2 and HCO^-_3 to varying degrees, is the primary carbon source available to algae for photosynthesis in Florida Springs. Therefore, the $\delta^{13}C$ of algae is directly affected by spring water DIC. The $\delta^{13}C$ of the DIC at 18 headwater springs sampled in my study ranged from -13 to -6 ‰ (Table 4-2, Figure 4-6). The more negative values are typical of groundwater values found by Deines *et al.* (1974) representing carbonate dissolution in a closed-system in Pennsylvania (-12 to -14 ‰), where half of the DIC comes from carbonate dissolution and the remaining half was contributed by CO_2 produced

through soil respiration. Carbonate rock generally has a $\delta^{13}C$ value of 0 to -2 ‰ (Deines *et al.*, 1974) and in Florida, calcite $\delta^{13}C$ from a limestone sediment core in Biscayne Bay ranged from +0.33 to -3.5 ‰ with most values between 0 and -1 per mil. Soil CO_2, on the other hand, has very negative $\delta^{13}C$ values of approximately -22 ‰, reflecting the $\delta^{13}C$ of the predominant source of organic matter, i.e. terrestrial vegetation (Deines *et al.*, 1974; Doctor *et al*., 2008). More negative $\delta^{13}C$-DIC values (-15 to -20 ‰) would indicate water from an open system where soil CO_2 has stronger influence, but CO_2 exchange with the atmosphere also occurs, which increases $\delta^{13}C$ values as atmospheric CO_2 has a value of approximately -8 ‰ (Doctor *et al*. 2008). Therefore, Florida springs with more enriched isotopic values may be indicative of a greater contribution from carbonate dissolution and atmospheric CO_2 than soil CO_2. Marfia *et al.* (2004) found a similar range of total DIC (5.4 to 112.9 mg C L^{-1}) and $\delta^{13}C$-DIC (-7.4 to17.4 ‰) in groundwater in a karst aquifer in Belize as those found in my study and they attributed these values to both open and closed system carbonate dissolution.

I found an inverse relationship between total DIC and $\delta^{13}C$-DIC ($r^2 = 0.51$) (Figure 4-6, A). At high total DIC (25 to 50 mg $C^{-1}L$), the most negative $\delta^{13}C$ values were obtained (-10 to -12 ‰). Marfia *et al*. (2004) found the opposite trend between total DIC and $\delta^{13}C$-DIC in groundwater: increasing total DIC with increasing $\delta^{13}C$-DIC, which they attributed to closed-system carbonate dissolution. However, they found that at low total DIC, surface waters generally had more enriched $\delta^{13}C$-DIC values than groundwater (the same trend I found in my study). They suggested several possibilities for this such as degassing of CO_2 and preferential uptake by plants of ^{12}C vs ^{13}C of DIC for photosynthesis, both resulting in an isotopically more enriched substrate and lower total DIC concentrations. Doctor *et al.* (2008) also found that enriched $\delta^{13}C$ values of DIC coupled with decreasing DIC concentration were influenced by

degassing of CO_2 in a headwater stream in Vermont, as did Waldron, Scott & Soulsby (2007) in a headwater sub-catchment of the Dee River in Scotland.

The values I found for $\delta^{13}C$ of DIC as well as total concentrations in Florida springs could therefore indicate that similar processes are occurring and that the aquifer is a mix between open and closed carbonate dissolution systems. However, since the water samples were collected above the spring boil, preferential uptake by primary producers of the isotopically lighter carbon has not occurred at this point. High total DIC and low $\delta^{13}C$ could be indicative of greater influence from CO_2 from the soil and the atmosphere, e.g. under recharge conditions, and more enriched $\delta^{13}C$ values coupled with low DIC concentrations could be indicative of degassing of CO_2 as the water comes up out of the spring boil, leaving more enriched HCO_3 and less total DIC. The strong negative relationship found between total DIC values and pH ($r^2 = 0.81$) (Figure 4-6, C) also supports this as the lowest pH values (7.6 to 8) correspond to the highest total DIC concentrations (27 to 50 mg C L^{-1}) and at these pH levels, there is more CO_2 (approximately 10%) than at pH above 8.2 where almost all of the DIC is in the form of HCO^-_3. The poor relationship between $\delta^{13}C$-DIC and pH ($r^2 = 0.34$) (Figure 4-6, D) may be because at the lowest pH levels (7.6), the majority of the DIC in the water is still in the form of HCO^-_3 so the scatter in $\delta^{13}C$ may reflect the mix of HCO^-_3 (the predominant form) and CO_2 ions as well as a mix of sources of the CO_2 ions (i.e. atmospheric vs. soil).

Katz (2004) states that many of the springs in Florida discharge water that comes largely from the shallow portions of the Floridan Aquifer, rather than deeper portions, adding weight to the argument that the range in isotope and total concentration values for DIC represents both open and closed system carbonate dissolution. Katz based his findings on several chemical characteristics, such as high dissolved oxygen levels in groundwater discharged and low

dissolved solids in the water. At both Rainbow and Silver Springs, (Faulkner, 1973, cited in Katz, 2004) found that groundwater flowing toward the springs was comprised of 92% shallow and 8 % deep water from the aquifer. However, this does not occur at all springs or at all times. Osmond *et al*. (1971, cited in Katz 2004) found that at base flow, water discharged at Wakulla Springs was comprised of 35% shallow and 65% deeper water.

Factors Affecting $\delta^{13}C$ Values in Algae

Multiple factors are likely affecting $\delta^{13}C$ values in algae, however, results from the regional and gradient studies point to relatively distinct species-specific $\delta^{13}C$ compositions. *Spirogyra* sp. consistently had the most enriched $\delta^{13}C$ values, up to -12 ‰, while *Vaucheria* sp., *Dichotomosiphon* sp. and *Compsopogon* sp. consistently had the lowest values, commonly ranging from -45 to -38 ‰ (Figures 4-4 and 4-7). Species-specificicity in the $\delta^{13}C$ of algae is likely due to several reasons. A primary one may be an algal species' relative uptake of and/or degree of preference for CO_2 (aq) vs. HCO^-_3 ion as a carbon source. The proportion of each of these sources assimilated will cause shifts in $\delta^{13}C$, as HCO^-_3 ions tends to have higher $\delta^{13}C$ values than CO_2 (aq) (Fogel & Cifuentes, 1993; Finlay, 2004). However, enriched $\delta^{13}C$ does not necessarily mean that algae are taking up the isotopically heavier carbon from HCO^-_3 since there may simply not be as much light CO_2 available and therefore less isotope source discrimination would result.

The pH of spring water can also cause shifts in the CO_2 (aq)- HCO_3^- equilibrium, with HCO_3^- prevalent as pH rises within the range of pH observed in Florida springs (ca. 6.0 to 8.6). The most enriched values for *Spirogyra* sp. (-12 ‰), *Lyngbya* wollei (-20 ‰) and *Vaucheria* sp. (-32 ‰) came from springs where pH values were above 8 (Figure 4-4). At this pH virtually all of the DIC is in the form of HCO_3^-. The most negative $\delta^{13}C$ values (below -35 ‰) for algae found during the 2006 regional study came from sites with pH below 6.5 (Figure 4-4) and at this

pH, approximately 50% of the DIC is composed of CO_2. If concentrations are not limiting, CO_2 uptake does not require energy expenditure by the algae, while HCO^-_3 assimilation is actively pumped into algal cells (Fogel & Cifuentes,1993) When DIC concentrations are low, however, DIC (CO_2 as well) is actively transported into algae and the majority of this DIC doesn't leave the algal cell until it is fixed through RuBP carboxylase in photosynthesis, resulting in relatively little fractionation (-5 ‰) and in turn in more enriched algal $\delta^{13}C$ (Fogel & Cifuentes,1993).

Highly depleted $\delta^{13}C$ values likely indicate strong respiration inputs as well because respired CO_2 (approximately -20 ‰) is further fractionated by approximately -20 ‰ when assimilated by algae during photosynthesis, resulting in $\delta^{13}C$ values as low as -45 ‰ (Fry, 2006). I measured values as low as -46 ‰ for *Vaucheria* sp. at Manatee Springs during the seasonal study. (Note: during respiration, heterotrophic bacteria selectively respire $^{12}CO_2$ over $^{13}CO_2$ which reduces the $\delta^{13}C$ of CO_2 (aq)) The strong positive correlation of algal $\delta^{13}C$ to the pH of spring water (Table 4-1, Figure 4-5, C) may also be due in part to high respiration rates which result in low $\delta^{13}C$ of CO_2 as well as increased CO_2 in the water column, which in turn can lower the pH of the water through its effect on carbonate equilibrium.

When the $\delta^{13}C$ of algae was plotted against Total DIC concentrations, pH and the $\delta^{13}C$ of DIC (Figure 4-5), the strongest relationship was found with pH ($r^2 = 0.76$), then total DIC ($r^2 = 0.70$) and finally $\delta^{13}C$ of DIC ($r^2 = 0.50$), which was surprising, as I expected the strongest correlation to be with the $\delta^{13}C$-DIC, a direct reflection of the isotopic composition of the carbon source. However, multiple factors can affect isotopic fractionation during DIC uptake, including light and current velocity and this may help explain the weaker relationship with the $\delta^{13}C$-DIC. Species-specific patterns were also observed in relation to DIC and pH (Figure 4-5). *Vaucheria* sp. was generally found in springs with lower pH and higher total DIC concentrations, while

Spirogyra sp. was found in springs with higher pH and low total DIC (Figure 4-5, A). *Lyngbya wollei* was more ubiquitous, but tended to be present in sites with lower total DIC and higher pH. Since algal $\delta^{13}C$ was also related to the $\delta^{13}C$ of DIC, species-specific tendencies were also found with this variable. These findings support the argument that some species are better adapted to or show preference for HCO^-_3 uptake vs. CO_2 uptake. The relationship between algae, total DIC concentrations and pH merits further investigation and may be an important factor in determining algal distribution in Florida Springs.

Several studies have shown that light regime also affects algal $\delta^{13}C$ values. Cornelisen *et al.* (2007) observed higher $\delta^{13}C$ values in *Ulva pertusa* under saturating light conditions and they offered two possible explanations: (1) higher irradiance leads to increased photosynthesis rates and the algae need to actively take up HCO^-_3 to meet carbon demand and/or (2) increased irradiance results in more efficient CO_2 fixation inside algal cells regardless of the original carbon source and increased retention of heavier carbon (^{13}C) inside algal cells, leads to more enriched values. Kubler &Raven (1995 in Cornelisen, 2007) found that higher light conditions not only increased carbon demand, but also provided the energy required to assimilate HCO^-_3, which is actively pumped in and has more enriched $\delta^{13}C$ than CO_2. Wiencke & Fischer (1990) found differences of 20 ‰ in the algae *Desmaretsia antarctica*, which they attributed to changes in light regime. I did not measure the effects of light regime or current velocity on $\delta^{13}C$ of the algae, but these would be important considerations in future investigations to better understand $\delta^{13}C$ composition of spring algae. Increased velocity can reduce $\delta^{13}C$ values as the algae can be replenished with isotopically lighter CO_2 and HCO_3 (Finlay, 1999).

Another potentially important finding from the stable isotope measurements and correlation analysis was the strong negative correlation between algal $\delta^{13}C$ and diatom indicators

of P availability in spring boil water (i.e., % high P individuals in springs and Florida Springs Nutrient Index (Table 4-1). There is evidence to suggest that algal taxa switch between CO_2 (aq) and HCO_3^- carbon sources as a function of the availability of limiting nutrients (R. Jan Stevenson, unpublished data). When P availability is low, algae may primarily utilize HCO_3^- ion as a carbon source during photosynthesis; when P availability is high, algae may utilize greater amounts of CO_2 (aq). Given the differences in $\delta^{13}C$ of these DIC species previously discussed, increasing inputs of P to springs would increase the assimilation of isotopically lighter CO_2 (aq) (relative to HCO_3^-) and produce algal tissues with lower $\delta^{13}C$. Beardall *et al.* (1982 in Fogel & Cifuentes,1993) found this same trend in phytoplankton under N limited conditions, which resulted in the activation of the system for concentrating HCO_3 in phytoplankton. As N limitation increased, amounts of carboxylating enzymes decreased, which are required in RuBP carboxylase activation. They suggested that the increased CO_2 concentrations within the cell (from pumped HCO^-_3) needed for RuBP carboxylase activation outweigh the energy required to assimilate HCO_3.

Seasonal and Longitudinal Variation in Algal $\delta^{13}C$

At Manatee Springs, there was a rapid decline in *Vaucheria* sp. $\delta^{13}C$ from April to June 2005 (from -36 to -44 ‰) and then values remained relatively stable until March 2006 (Figure 4-8). The rapid decline in $\delta^{13}C$ coincides with a time of rapid biomass increase at the spring (Sickman *et al*., 2009). Although rapid biomass accrual can result in enriched $\delta^{13}C$ due to increased carbon demand and less source discrimination (Hill and Middleton, 2006), DIC is most likely not a limiting nutrient in this case. Instead, this rapid decline lends strength to the argument that high respiration rates (and the assimilation of respired CO_2) results in very low $\delta^{13}C$. At Ichetucknee Blue Hole, high respiration rates of primary producers may also account, in

large part, for the low isotopic signatures found in both *Lyngbya wollei* and *Vaucheria* sp. as the boil area of Blue Hole is completely covered by the macrophyte *Vallisneria americana*.

Along longitudinal gradients, *Compsopogon*, *Vaucheria* and *Dicotomosiphon* sp. consistently showed the lowest $\delta^{13}C$ values with little variability along 8 to 10 km of the four river runs (Figure 4-7). These species likely have similar uptake capacities of CO_2 vs. HCO^-_3. Additionally, microsite variability along the river run appears to have affected these species' isotopic composition less than *Lyngbya wollei* and *Spirogyra* sp. These two species showed more variability in $\delta^{13}C$ from site to site within each river run, with changes of up to 10 ‰, which may have been due to factors such a current velocity and irradiance.

Factors Affecting $\delta^{13}C$ Values in Spring Sediments

Sediment $\delta^{13}C$ had a relatively well-constrained $\delta^{13}C$ range, regardless of whether or not the source was from exposed sediments or from sediment collected from under an algal mat. More than 90% of the samples fell within the range of -30 to -25 ‰, which was higher than the majority of algal samples (Figure 4-1). This pattern was also consistent in the four river runs sampled (Figure 4-7). This range is similar to that reported by Finlay (2001) of -28.2 +/- 0.2 ‰ for particulate terrestrial detritus of temperate streams. The typical range for $\delta^{13}C$ of terrestrial C_3 plants is -34 to -22 ‰ (Rounick & Winterbourne, 1986) and for C_4 plants, $\delta^{13}C$ values are approximately -12 to -13 ‰. Therefore, the sediment carbon isotope values could suggest a mixture of both terrestrial organic matter and algal material. However, sediment $\delta^{13}C$ values of -30 to -25 ‰ could also result from post-depositional processing of isotopically depleted algal tissues (30 ‰) in sediments since decomposition/respiration/methanogenesis all favor loss of ^{12}C over ^{13}C leaving the residual substrate enriched in ^{13}C.

When the $\delta^{13}C$ values of algae and sediment were plotted against C:N molar ratios, the majority of sediment samples had C:N ratios above 10 and up to 112, while all of the algal

samples had C:N ratios between 5 and 18 (Figure 4-2). C:N ratio is used to determine differences in origin of organic matter in sediments because algal C:N generally has a value in the range of 5 to 8 while vascular plants generally exhibit ratios from 20 to 80, largely due to recalcitrant support tissue (Meyers & Ishiwatari, 1993; Vreca & Muri, 2006). Therefore, the majority of the organic matter in my sediment samples likely came from vascular plants. In a study conducted in Lake Panasoffkee, FL, a shallow lake dominated by macrophytes, Brenner *et al*. (2006) found the highest C:N ratios (25 to 30) as well as low $\delta^{13}C$ values (an average of -27 ‰) in emergent vegetation, which they attributed to recalcitrant structural tissue (e.g. lignin and cellulose) and unlimited CO_2 availability from the atmosphere, respectively. Phytoplankton mean C:N was 6.5. The relatively higher range in C:N found in algal samples in my study (up to 17) may reflect more cellular structural requirements, such as the thallus, which isn't found in phytoplankton (Townsend *et al.*, 2008) as well as possible N-limitation at some spring sites (Borchardt, 1996). The variable that was most strongly correlated to the $\delta^{13}C$ of sediment under algal mats was its C:N molar ratio (-0.576) (Table 4-1), again pointing to vascular plants (not necessarily of terrestrial origin, however) as the primary component of the organic matter in spring sediments of the sites sampled and shows a similar trend as that found by Brenner *et al*. (2006).

In summary, I found a very strong relationship between carbon isotope composition, pH and total DIC. This relationship likely results from differential use of HCO^-_3 and CO_2 (aq) during photosynthesis as these DIC species have disparate $\delta^{13}C$ values and their relative usage can set the $\delta^{13}C$ of the algae. Additionally, under high total DIC conditions, more isotopically light CO_2 and HCO^-_3 are available to the algae for assimilation, resulting in more negative $\delta^{13}C$ values. Factors controlling the relative usage of these DIC species during algal photosynthesis include water column pH, respiration rates of primary producers and perhaps the availability of P. The

effects of current velocity and irradiance on isotopic composition, which were not analyzed in this study need to be considered in future studies to better understand within-species isotopic variability. The relatively strong species-specific relationship between algae, total DIC concentrations and pH may be an important factor in determining algal distribution in Florida Springs and merits further investigation.

Table 4-1. Significant Spearman correlations ($p \leq 0.001$) between algal $\delta^{13}C$ and sediment $\delta^{13}C$ and indicators of nutrient availability and nutrient sources. Only variables collected in spring boil areas were analyzed for a total of 34 sites. There were often multiple algal species per site. $\delta^{13}C$ of algae and sediments was correlated to two water chemistry data bases: (1) water chemistry from the 2006 survey and (2) water chemistry from an average of the 2003 and 2006 surveys.

	Variables	Correlation coefficient	n
Algal tissue $\delta^{13}C$ vs.	pH (2006)	0.683	54
	Sediment under algae $\delta^{13}C$	0.475	52
	Dissolved oxygen	0.466	50
	Diatom indicator: Species richness	-0.453	51
	NO_2/NO_3-N of springwater (2006)	-0.463	56
	Exposed sediment %C	-0.468	51
	Sediment under algae C:N molar ratio	-0.468	50
	Diatom indicator: % high P individuals in springs	-0.470	51
	Diatom indicator: Florida Springs Nutrient Index	-0.477	47
	Exposed sediment C:P molar ratio	-0.487	51
$\delta^{13}C$ of sediment under algae vs.	Ortho-phosphate in spring water (2003+2006)	0.535	43
	Total P in spring water (2003+2006)	0.520	51
	Dissolved oxygen (2006)	0.496	46
	Sediment under algae C:P molar ratio	-0.444	51
	Sediment under algae C:N molar ratio	-0.576	51
$\delta^{13}C$ of exposed sediment vs	Diatom indicator: % low P individuals	0.613	37
	Dissolved oxygen (2006)	0.607	36
	Algal N:P molar ratio	0.475	43
	Total P in spring water (2006)	-0.499	42
	Diatom indicator: Florida Springs TP Index	-0.541	51
	Diatom indicator: Strontium	-0.550	34
	Ortho-phosphate in spring water (2006)	-0.564	41
	Total P in spring water (2003+2006)	-0.572	33
	Average of buffer width	-0.621	39
	Diatom indicator: Sulfate	-0.699	51
	Conductivity (2003 + 2006)	-0.754	37

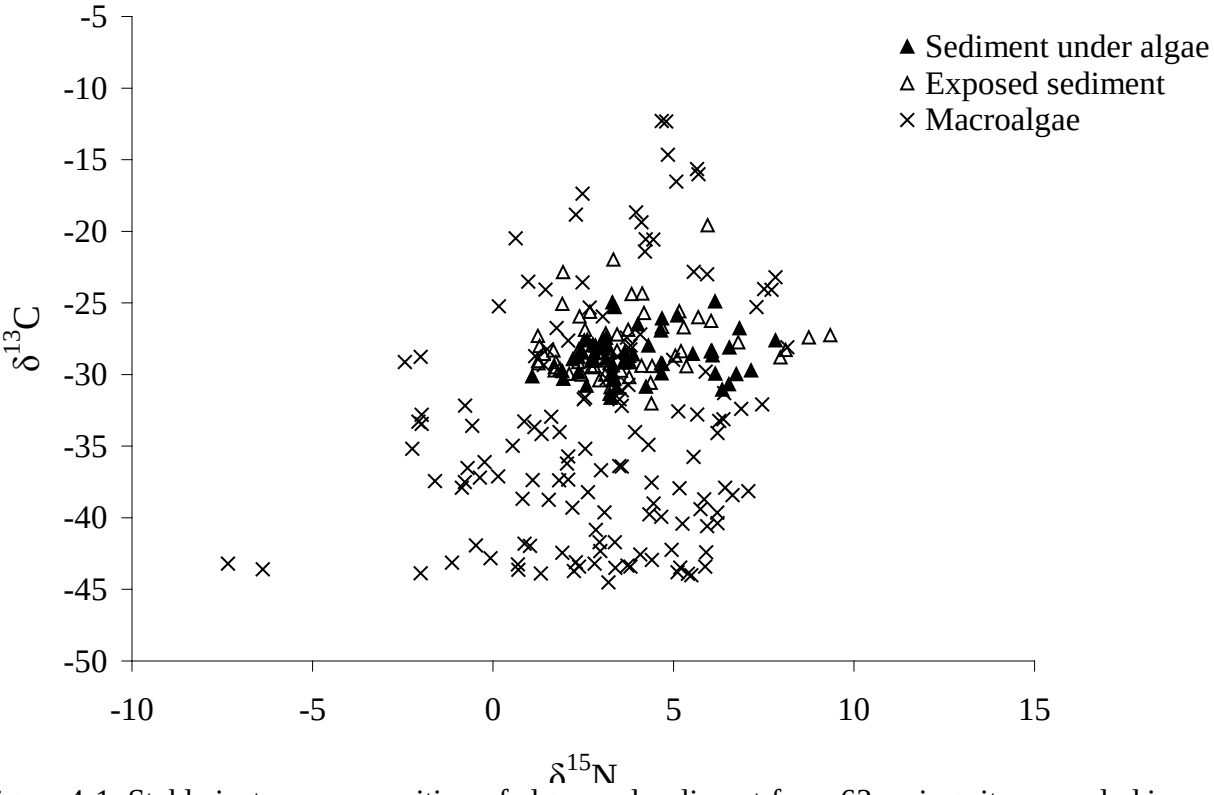


Figure 4-1. Stable isotope composition of algae and sediment from 63 spring sites sampled in 2006.

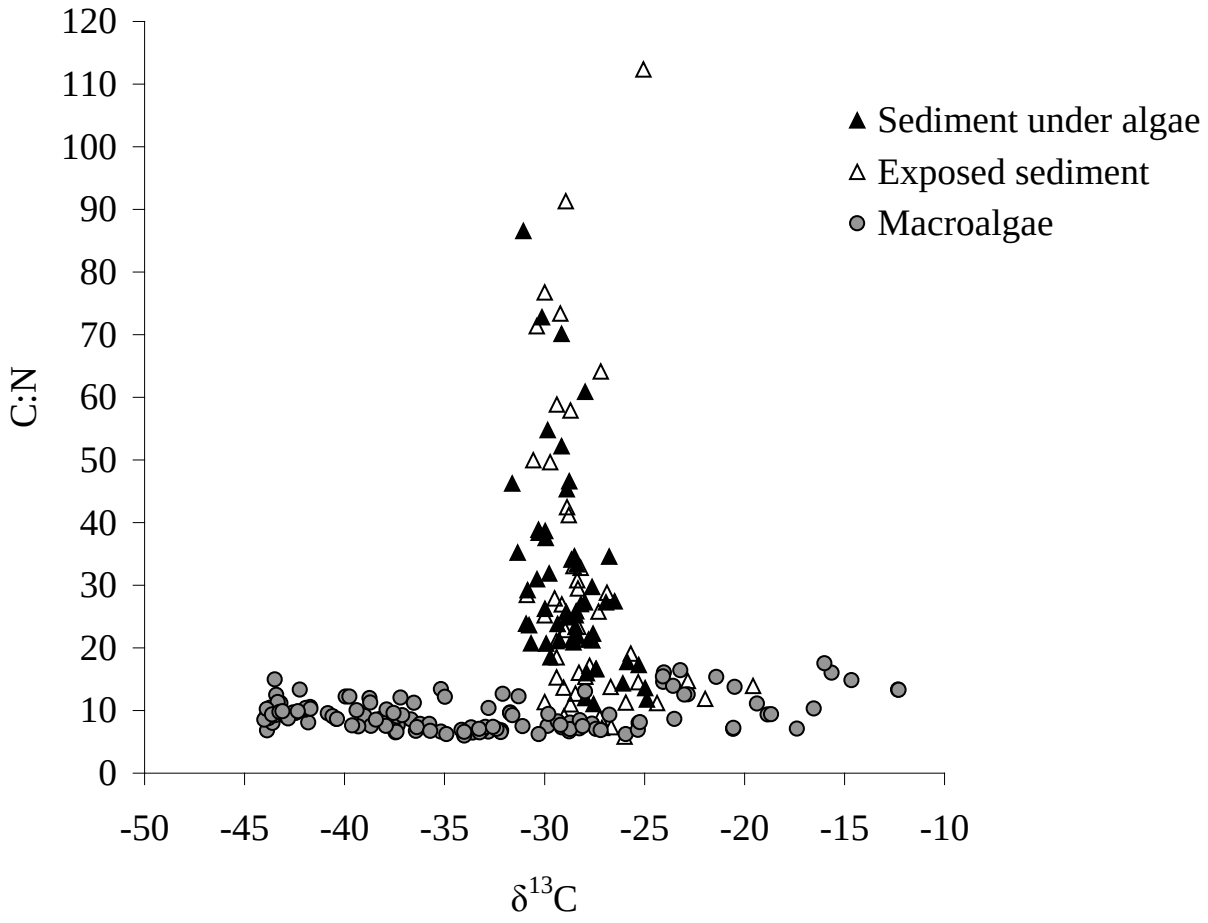


Figure 4-2.The relationship between the $\delta^{13}C$ and C:N molar ratio of algae and sediment from 63 spring sites sampled in 2006.

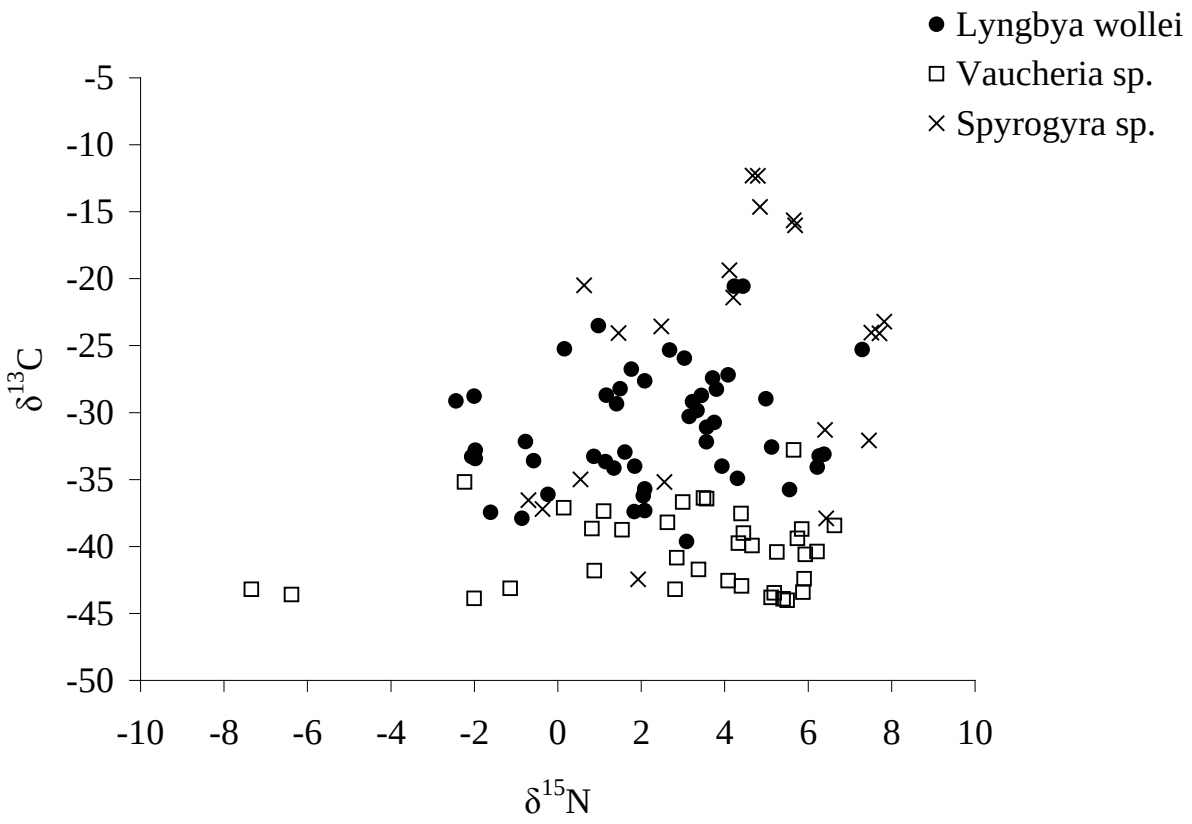


Figure 4-3. Stable isotope composition of *Lyngbya wollei*, *Vaucheria* sp. and *Spirogyra* sp. from 61 spring sites, 2006.

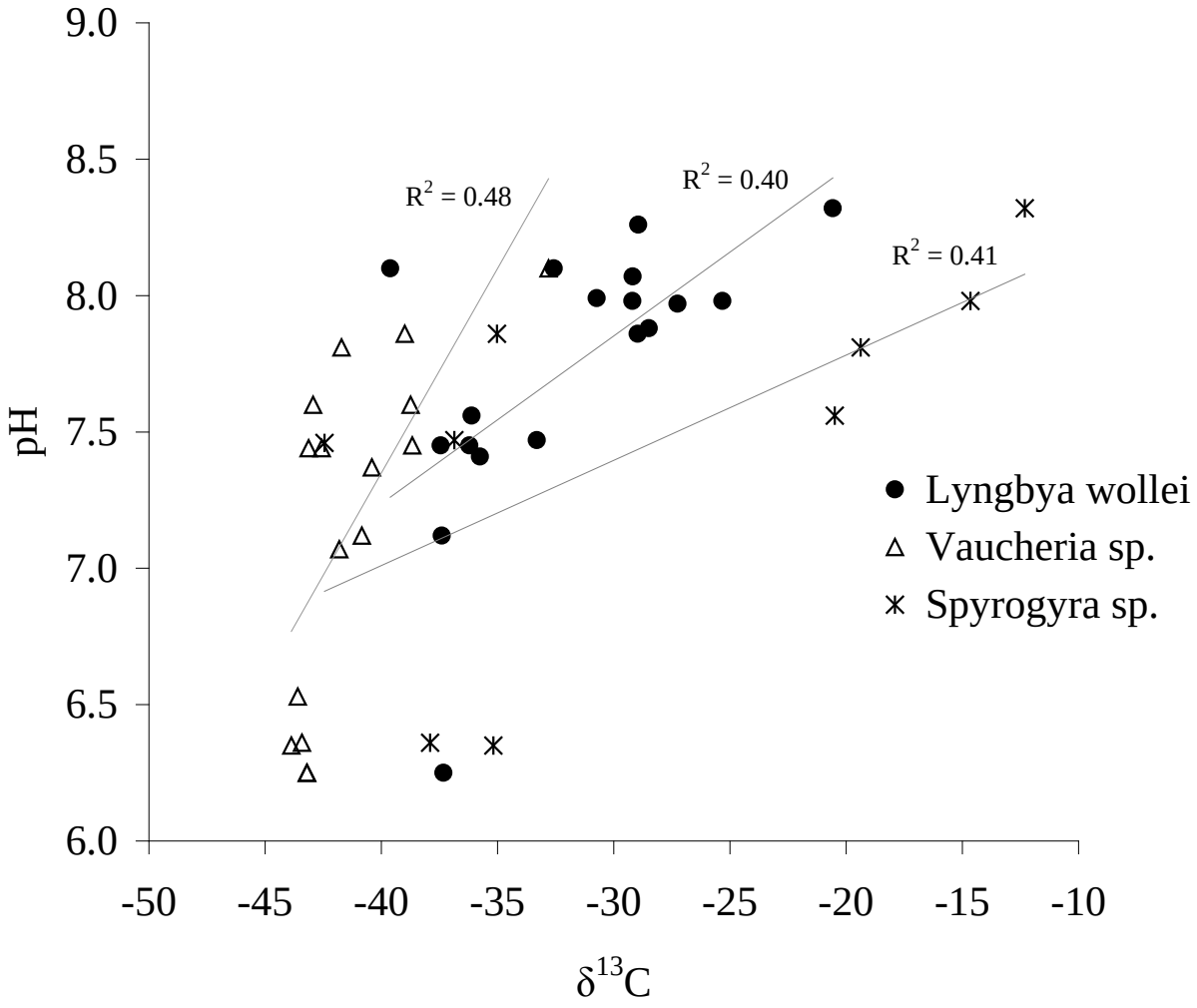


Figure 4-4. The relationship between the $\delta^{13}C$ of the three dominant algal species and spring water pH at boil areas sampled in 2006. Each trend line is for a particular species: *Vaucheria* sp., $r^2 = 0.48$, *Lyngbya wollei*, $r^2 = 0.40$ and *Spirogyra* sp., $r^2 = 0.41$.

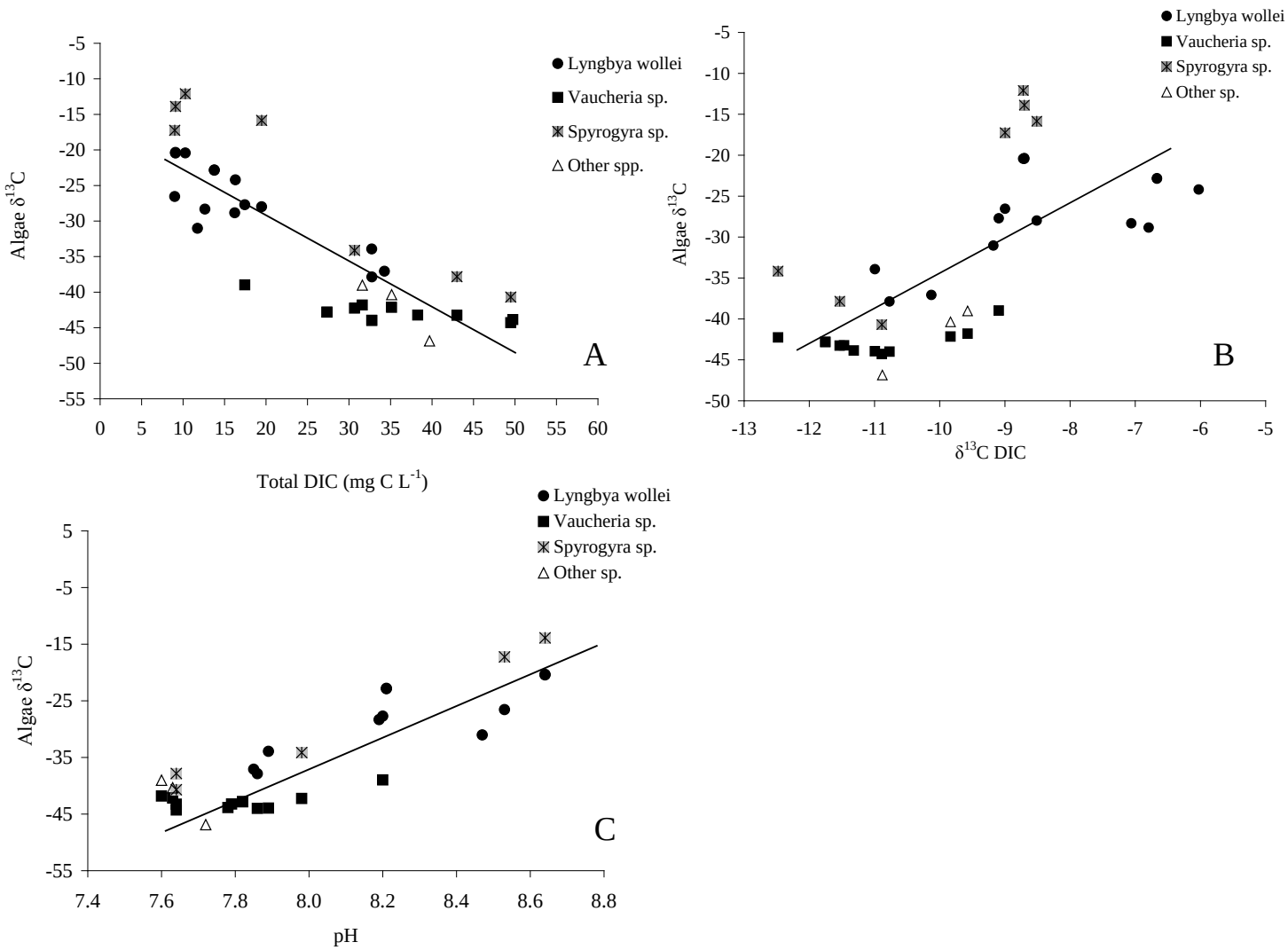


Figure 4-5. Relationships between the $\delta^{13}C$ of algae and total DIC, $\delta^{13}C$ of DIC and pH of headwater springs sampled in April and August 2008. A) total DIC (mg C L^{-1}) vs. the $\delta^{13}C$ of algae ($r^2 = 0.70$). B) $\delta^{13}C$ of DIC vs. the $\delta^{13}C$ of algae ($r^2 = 0.50$). C) pH and the $\delta^{13}C$ of algae ($r^2 = 0.76$).

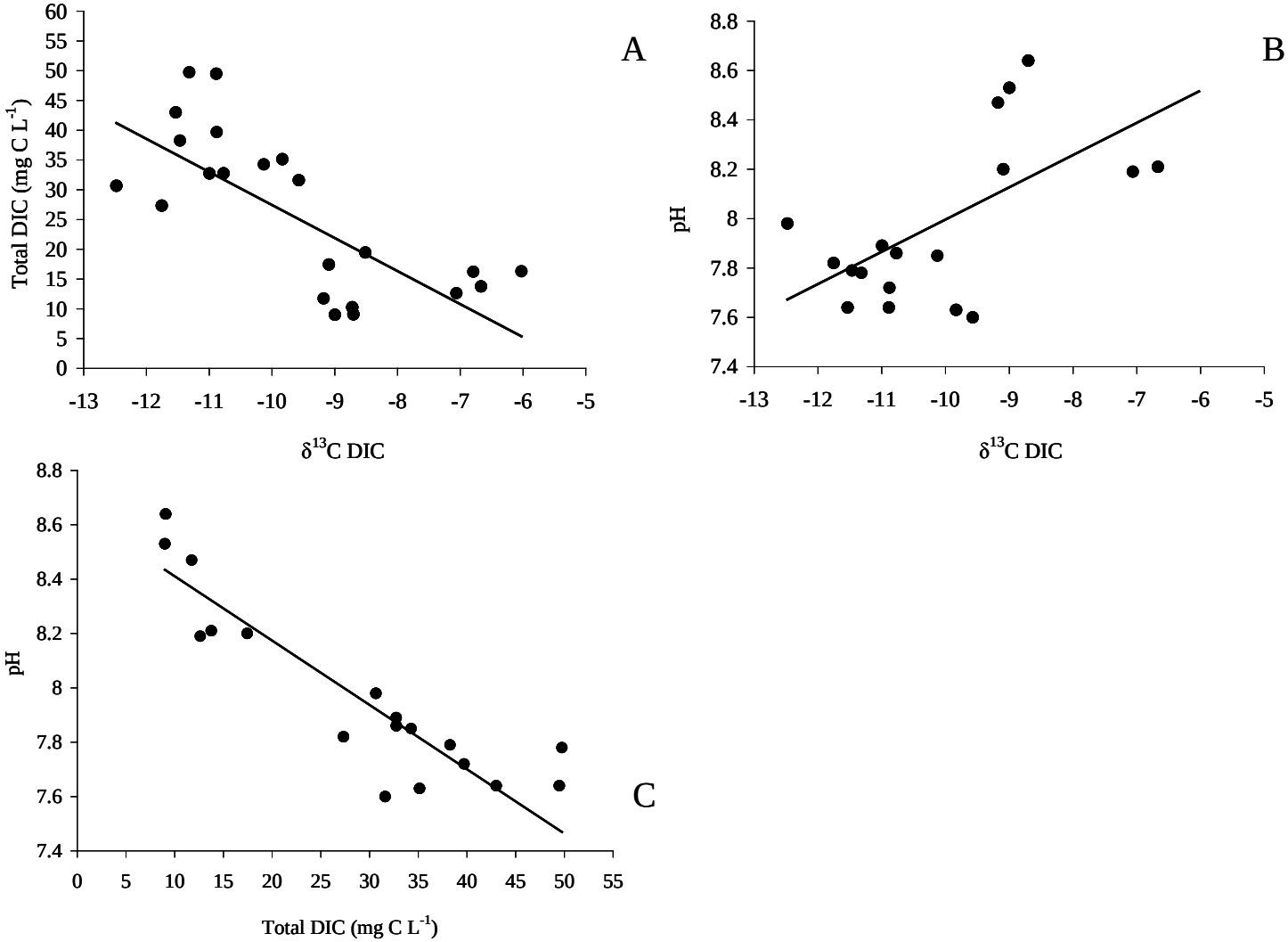


Figure 4-6. Relationships between spring water total DIC (mg C L^{-1}), $\delta^{13}C$ of DIC and pH of headwater springs sampled in April and August 2008. A) $\delta^{13}C$ of DIC vs. total DIC (mg C L^{-1}) ($r^2 = 0.52$). B) $\delta^{13}C$ of DIC vs. pH ($r^2 = 0.34$). C) Total DIC vs. pH ($r^2 = 0.81$).

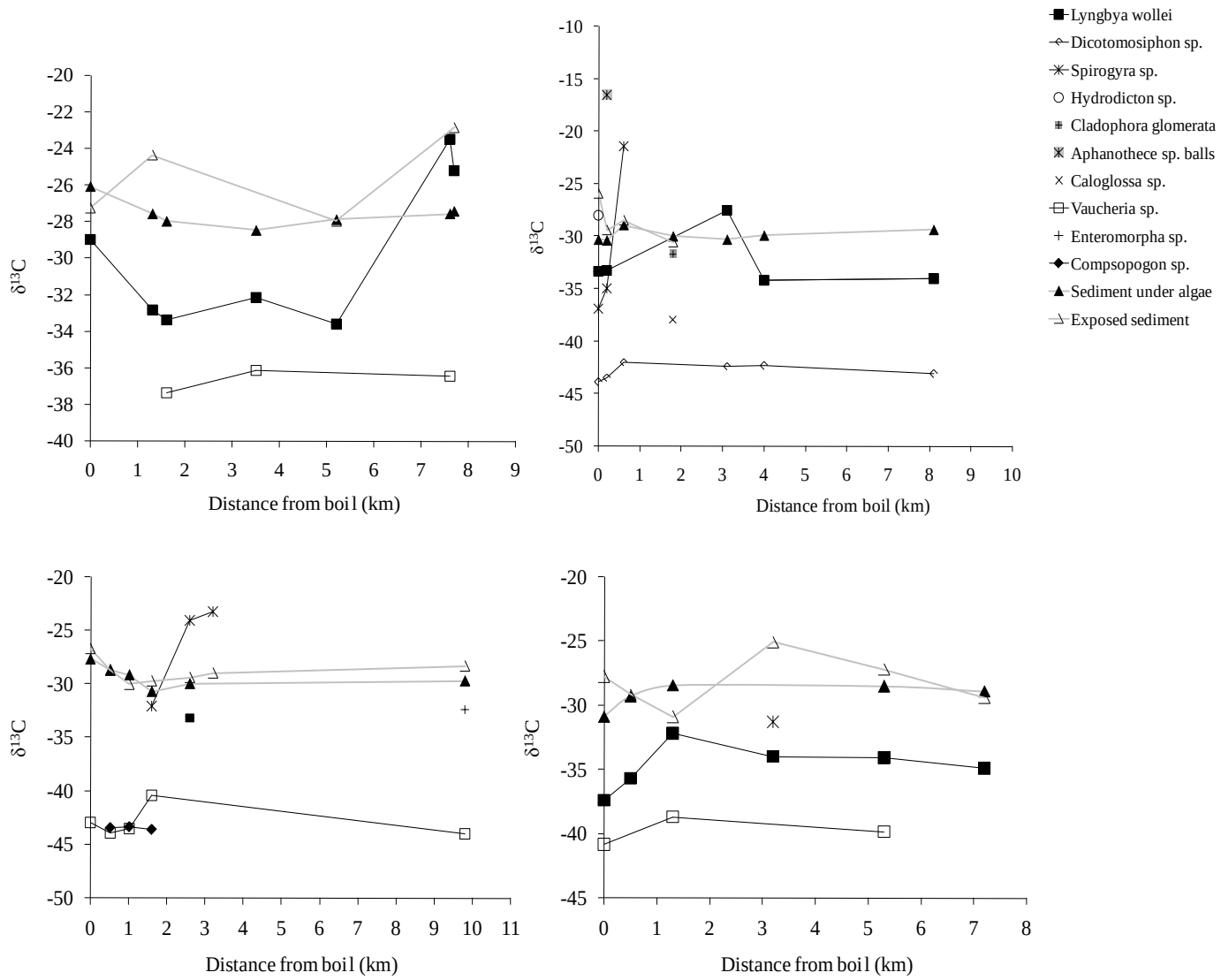


Figure 4-7. $\delta^{13}C$ composition of algae and sediment measured along four spring river runs in January 2006. A) Rainbow River. B) Weeki Wachee River. C) Wakulla River. D) Silver River.

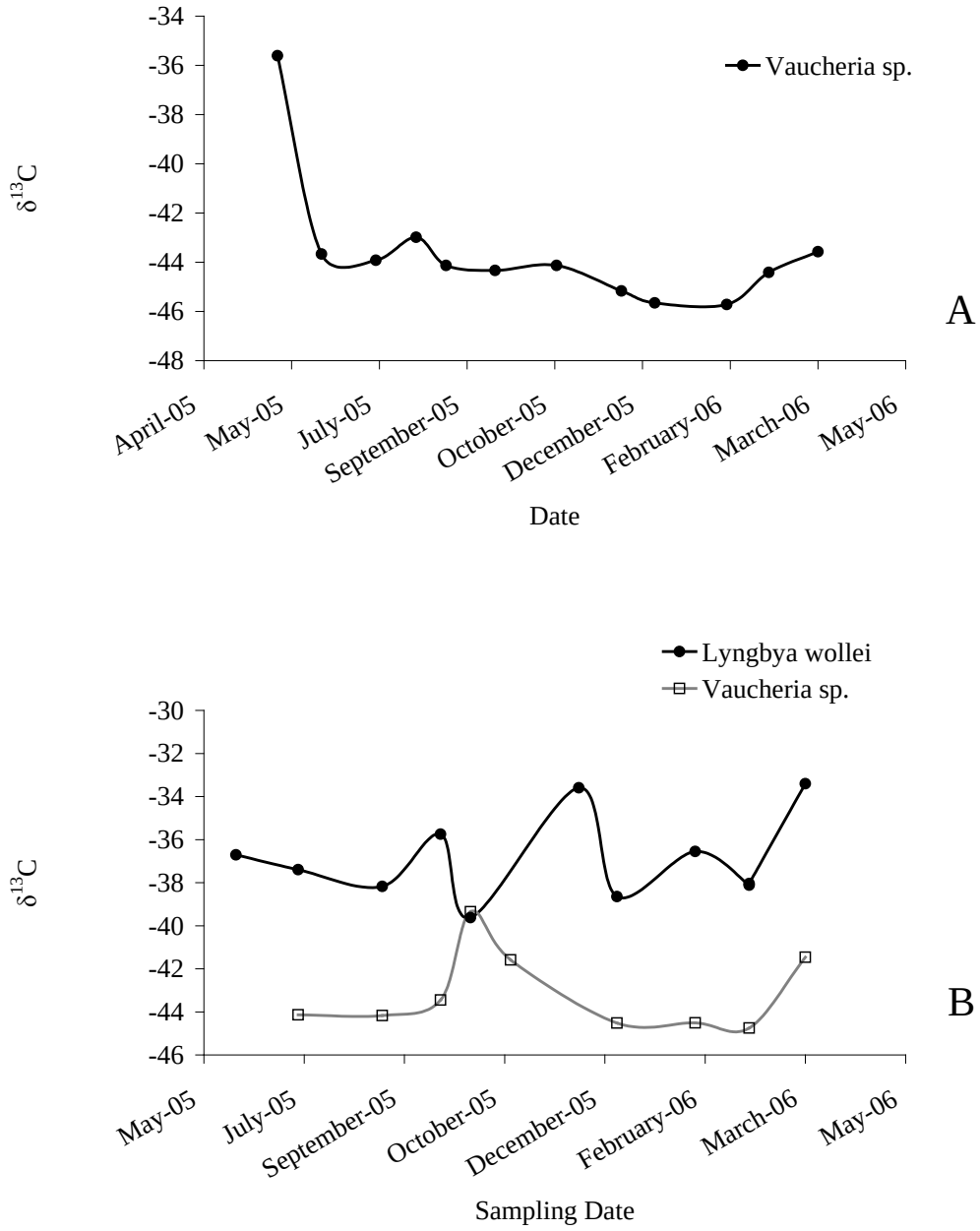


Figure 4-8. $\delta^{13}C$ composition of algae at Manatee Springs and Ichetucknee Blue Hole from May 2005 to March 2006. A) $\delta^{13}C$ of *Vaucheria* sp. at Manatee Springs. B) $\delta^{13}C$ of *Lyngbya wollei* and *Vaucheria* sp. at Ichetucknee Blue Hole.

CHAPTER 5
NUTRIENT PROFILES OF ALGAL MATS IN FLORIDA SPRINGS

Introduction

The filamentous algae *Lyngbya wollei* and *Vaucheria* sp. form thick benthic mats in many of Florida's karst springs (Stevenson *et al*., 2007). Despite the high biomass accrual of algae that has occurred over the past 50 years (Florida Springs Task Force, 2000; Quinlan *et al*. 2008), relatively little is known about the physiology of benthic and floating algal mats and factors that allow such thick mats to occur in many springs with relatively low ambient nutrient concentrations.

Spring nutrient supply rates alone do not control the distribution of algae in Florida springs (Pinowska *et al*., 2009). At Silver Glen Springs, for example, persistent *Lyngbya wollei* mats more than 1 m thick occur under ambient NO_x concentrations of 0.05 mg L^{-1} and orthophosphate of 0.03 mg L^{-1}. One possible explanation may be that the algae are relying at least in part on the regeneration of nutrients within the mat itself as it grows in size and inner portions begin to senesce due to lack of light and/or nutrients. Benthic efflux of nutrients or nutrient regeneration within mats has been shown to supply algae with nutrients necessary for growth, rather than having to rely solely on direct supply from the water column (Sündback *et al.*, 2003; Trimmer *et al*., 2000; Tyler, McGlathey & Anderson, 2003; McGlathery *et al*. 1997). *Lyngbya* sp. mats have been observed to attain high biomass densities, and this may allow for nutrient entrainment and recycling within mats . Beer, Spencer & Bowes (1986) calculated fresh weight densities of up to 13.8 kg/m^2 (equivalent to 1.14 kg dry weight (DW)) for *L. birgei* in the Southeastern U.S. and Cowell (1990) recorded biomass densities of 1.25 kg DW/ m^2 for *L. wollei* in King's Bay, FL.

The major objective of my study was to determine the potential for large algal mats to regenerate nutrients to sustain algal growth. Specific objectives of this study were to 1)

characterize nutrient profiles of large *Lyngbya wollei* and *Vaucheria* sp. mats, the two most common species of algae found in Florida Springs and 2) estimate advective and diffusive movement of dissolved nutrients out of large algal mats. To accomplish this, I analyzed mat internal dissolved nutrients using interstitial water samplers known as multisamplers at Weeki Wachee, Manatee and Silver Glen Springs during spring and summer 2006. I also analyzed the isotopic composition of algal tissue and underlying sediment ($\delta^{15}N$ and $\delta^{13}C$) and measured NaCl movement out of algal mats.

Methods

Sampling Locations

Nutrient profiles within algal mats as well as advective flow through mats were measured in the boil areas of Manatee, Weeki Wachee and Silver Glen Springs (Figure 5-1). These sites were chosen because Weeki Wachee had the thickest *Lyngbya wollei* mats and Manatee Springs the thickest *Vaucheria sp*. mats observed during surveys conducted in January 2006. Silver Glen was added as a comparison site to Weeki Wachee because it also has thick *L. wollei* mats, but the water chemistry varied between the two locations.

Algal Mat Nutrient Profiles

Interstitial water samplers, or multisamplers (Martin *et al*., 2003), were used to assess nutrient profiles within algal mats. Samplers were deployed twice at Weeki Wachee and Manatee Springs (April and August 2006) and once at Silver Glen Springs (September 2006). Sampling was conducted at different times of the year in order to observe nutrient profiles during different stages of algal growth cycles and environmental conditions. Duplicate samplers were deployed at each site, near the boil area, where the algal mats were thickest. They were generally 2 to 3 meters apart.

The samplers used in my study were an adaptation of the sediment sampler of Martin *et al*., (2003) and consisted of a 1.5-m-tall PVC pipe with small holes drilled every 10 cm (Figure 5-2). Tygon tubing (0.25 inch ID) was glued to the inner surface of each hole (inside the multisampler) and run through its entire length. Two layers of 500-µm nylon screen were glued to the outside of each hole in the form of a blister to help filter particulates and keep the tube from being blocked.

The multisamplers were inserted vertically through the thickest part of the algal mat and pushed to a depth of approximately 10-20 cm into the sediment. They were left in place for one week to allow for equilibration. Water samples were drawn from each tube using a syringe while sitting in a canoe. To purge stagnant water from the tubing, one tube-volume of water was withdrawn from each tube (15-30 ml, depending on the length of each tube) and discarded before water samples were collected. The multisampler allowed samples to be drawn from the sediments upwards through algal mats and into spring waters above the mats, all in a vertical column.

Parameters measured from samples collected at each depth in the multisampler were: TKN, TP, SRP, NO_3, DOC and trace metals for all sampling dates and sites. NH_4, was measured only during the April 2006 sampling event at Manatee and Weeki Wachee Springs. Additionally, algae and sediment samples were taken from the algae/sediment interface, in the upper (surface) portion of the mat as well as in the deeper portions to be analyzed for % C, N and P as well as $\delta^{13}C$ and $\delta^{15}N$ isotope analysis.

Chemical Sampling and Laboratory Analyses

Water samples were filtered through a 0.45-µm polycarbonate membrane using a filter holder and syringe. Filtered aliquots were collected for soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), NH_4^+, and NO_3^-. Unfiltered samples were collected

concurrently for total Kjeldahl nitrogen (TKN) and total phosphorus (TP). Samples for TKN, TP, dissolved organic carbon (DOC) and NH_4^+, NO_3^- were acidified to pH 2 with concentrated H_2SO_4. All samples were transported on ice and stored at 4°C until analyzed except for SRP samples, which were stored frozen. Holding times were 28 days for NO_3^-, SRP, DOC, TKN and TP, and NH_4^+.

Soluble reactive phosphorus, NH_4^+, and NO_3^-, were measured on a flow-injection analyzer using EPA methods (EPA Methods 365.1, 350.1 and 353.2 respectively). Total Kjeldahl nitrogen was determined by H_2SO_4 and Kjeldahl salt digestion and flow-injection determination of ammonium (EPA Method 351.2). Total nitrogen (TN) was computed as the sum of TKN plus NO_3^-. Total phosphorus was measured as SRP on a Technicon Autoanalyzer after digestion with H_2SO_4 and potassium persulfate (EPA Method 365.1). Carbon and nitrogen content of lyophilized algal tissues were determined on a Thermo Flash EA 1112. Phosphorus content of dried algal tissues was measured on combusted (550°C) and acid digested (6N HCl) samples as SRP on the Technicon Autoanalyzer (Anderson, 1976).

Prior to $\delta^{13}C$ isotopic analysis, sediment samples were acid fumigated with HCl to remove inorganic carbon (Harris et al. 2001). Samples used for sediment $\delta^{15}N$ analysis did not undergo acid fumigation. Carbon and nitrogen stable isotopic composition of algae and sediments was measured on a Thermo Finnigan Delta-Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.) at the University of Florida using an elemental analyzer inlet system and continuous flow of He. The International Atomic Energy Association standards for sucrose and N1 were included in each run. $\delta^{13}C$ isotope abundances are reported in δ notation relative to international standards (Vienna Pee Dee Belemnite for C and atmospheric air for N).

Nutrient Diffusion Out of Algal Mats

Ammonium and phosphate diffusion out of algal mats was calculated using the nutrient profiles obtained with the multisamplers in April 2006. One profile from Weeki Wachee Springs (*Lyngbya wollei*) and one profile from Manatee Springs (*Vaucheria* sp.) were used. Diffusive flux was calculated using Fick's Law:

$$J = \varphi D(dC/dX) \qquad (5\text{-}1)$$

Where:

J = flux; mass diffusing across unit area per unit time (i.e., $mg/m^2/day$)
D = Diffusion coefficient (ie. 10^{-6} cm^2/sec)
φ = soil porosity or algal mat porosity in this case
dC = change in concentration (mg/ L)
dX = distance between the changes in concentration that are being considered (cm)

The diffusion coefficients used were the following (Li & Gregory, 1974):

(1) NH^{4+} : 16.8 x 10^{-6} cm^2/sec at 18°C and 19.8 x 10^{-6} cm^2/sec at 25°C
(2) H_2PO^{4-} : 7.15 x 10^{-6} cm^2/sec at 18°C and 8.46 x 10^{-6} cm^2/sec at 25°C

Flux was calculated using two different estimates of algal mat porosity to obtain a range of values: 0.5 and 0.8. Porosities of 0.63 to 0.86 were calculated for shallow, muddy estuarine sediment in Florida Bay from depths of 20 cm to 0 (sediment/water column interface), respectively(Ullman & Aller, 1982). Porosities of 0.5 are typical of an uncompacted soil of medium to fine texture (Brady & Weil, 2002) and I assumed that 50% of the algal mat, by volume, was algae and 50% was extracellular water. NO_3 flux was not calculated because there were only trace concentrations within the algal mat.

Algal Mat Tracer Study

In order 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. Two liters of NaCl tracer solution were injected along the edge of

replicate multisamplers facing the boil (i.e., what I interpreted to be the "upstream" side of the sampler). To inject the tracers I used a 2-meter-long rod onto which a length of 0.25 in Tygon tubing was attached. Tracer was forced into the tubing at a consistent rate while the end of the tubing was moved up and down along the entire length of the installed multisamplers. In the case of Manatee spring, tracer was also injected in the water column above the mat. Water column injection was done because the experiment had to be conducted in a backwater area in the spring owing to a lack of algal mats in swifter flowing portions of the spring. A different syringe was then used to draw a sample of water from each sampler port within the mat. Samples were collected at the following times: 5 minutes, 15 minutes, 30 minutes and 60 minutes after injection. The samples were then analyzed using a conductivity meter to quantify NaCl tracer.

Results

Interstitial Nutrient Profiles in Large Algal Mats

Multisampler deployments were conducted in both *Vaucheria* and *Lyngbya* mats at Weeki Wachee, Manatee and Silver Glen springs, however, due to natural variations in algal growth patterns, my observations in *Vaucheria* mats (Manatee spring) are limited to senescing rather that actively growing algae (Table 5-1). I observed complex patterns and strong gradients of algal nutrients within all algal mats at all three springs (Figures 5-3 to 5-7). Even on the same day, there could be substantial variability in nutrient profiles between the two replicate multisamplers. Therefore, in this section I will describe the broad patterns of nutrient concentrations observed on each date rather than try to describe and interpret every observable variation.

Manatee Springs

Nutrient concentrations and gradients in *Vaucheria* mats at Manatee were the highest of the three springs studied; this may be related to the fact that the mat was senescing. TKN and TP concentrations increased by 2-3 orders of magnitude from the overlying water column moving

into the mat interior. Peak TKN values were found within algal mats and were 18 to 28 mg L^{-1} on April 19, 2006 and 6 to 9 mg L^{-1} on August 24, 2006 (Figures 5-3 and 5-4). The mats studied in April 2006 were 60-80 cm deep, while those studied in August were thinner, 20-50 cm, which may account for the lower TKN and TP values observed in August deployments. There were abrupt changes in nitrate (decreasing), ammonium (increasing) and Fe (increasing) within the mat, suggesting low redox conditions, however, the depth of the oxic-anoxic transition was near the bottom of the algal mat rather than near the surface. Within the sediment, TKN and ammonium concentrations declined slightly. TP concentrations followed the same general pattern as TKN, however peak concentrations (0.7 to 3.8 mg L^{-1}) were observed within the sediments rather than the bottom of the algal mat. Based on the difference between SRP and TP, the vast majority of the TP observed in mat interstitial waters and sediments was organic P; for TKN, the majority was contributed by organic N, however, there was still a substantial amount of inorganic N in the form of ammonium. One of the most noteworthy observations from the multisampler deployments at Manatee was the extremely high Fe concentrations observed within the senescing algal mat. Concentration peaks were 38 to 50 mg L^{-1} in April 2006 and 5.8 to 8.1 mg L^{-1} in August 2006, and on both dates Fe concentrations were greater than TKN levels on a weight:weight basis. The concentration of Fe generally peaked at the very bottom of the mat and then declined slightly within the surficial sediments.

Weeki Wachee

At Weeki Wachee on both deployment dates, TKN and TP values within the *Lyngbya* mats varied by 1-2 orders of magnitude over the multisampler profile which encompassed the water column, algal mat and about 20-30 cm of sediment material (Figures 5-5 and 5-6). Peak TKN values were found within the algal mat and ranged between 2 to 4.5 mg L^{-1} with the majority of N contributed by ammonium and organic nitrogen. At a point 10-30 cm within the algal mat

(measured from the surface), there were strong gradients in both nitrate (decreasing) and ammonium (increasing), suggesting that this was a zone of rapidly declining dissolved oxygen, as was observed in the Manatee profiles. Increasing Fe concentration within this same region reinforces the inference for anoxic conditions. Concentrations of TKN and ammonium (measured in April only) declined below the sediment-algal interface. Profiles of TP and SRP were generally similar to patterns for TKN, however the gradients of P were more modest; peak TP levels were ca. 0.2 to 0.4 mg L^{-1} with the majority contributed by organic P.

Silver Glen

Multisampler deployments in *Lyngbya* mats at Silver Glen were conducted only on September 5, 2006. The replicate mats ranged in thickness from about 30 to 70 cm. Concentrations and gradients of nutrient were highest in the thicker algal mat (Figure 5-7). Peak TKN and TP were generally found near the bottom of the algal mats and values ranged from 5.5 to 39 mg-N L-1 and 0.46 to 10.4 mg-P L-1 for the two replicate samplers, respectively. While ammonium was not measured during the multisampler deployments, I observed no strong gradients in nitrate concentrations, which suggests that redox levels were higher than those required for nitrate reduction within the mats. For both TKN and TP the majority of N and P were contributed by organic N and P.

Stable Isotope Composition and C:N:P Ratios in Algal Mat Profiles

The isotopic profile data ($\delta^{13}C$ and $\delta^{15}N$ of algal tissue and sediment) as well as C:N:P molar tissue ratios and interstitial water chemistry for the algal mat profiles at Weeki Wachee, Silver Glen and Manatee Springs are shown in Table 5-2. The isotopic composition of the Weeki Wachee Springs *Lyngbya wollei* profile are shown in Figure 5-8. $\delta^{13}C$ values of algae ranged from -21 to -31 ‰. The most enriched $\delta^{13}C$ was in the middle of the algal mat, at a depth of 70 cm in the multisampler, and the lowest values were found in the sediment/algal mixture (depths

10 and 50) and at the surface of the mat (depth 30). The most enriched $\delta^{15}N$ was found in the mixture of *Lyngbya*/sediment (+4 ‰) and the lowest values were toward the top of the mat, but not at its surface (+1 ‰). The isotopic composition of the Silver Glen Springs *Lyngbya wollei* profile is shown in Figure 5-9. $\delta^{13}C$ of the algae showed very little variation through the profile, ranging from -20 to -17 ‰. The sediment sample was the least enriched and had a value of -29 ‰. The $\delta^{15}N$ values of the algal profile ranged from 0 to 3 ‰, with decreasing value moving upwards through the algal mat to the surface. The $\delta^{15}N$ value for the sediment was lower, at -4 ‰. A profile of the algal mat was not shown for Manatee Springs because of the small sample size (two sediment samples collected in April 2006 were analyzed for $\delta^{13}C$ and $\delta^{15}N$, and only two algal tissue samples from August 2006 were analyzed).

Very little phosphorus was found in the decomposing *Lyngbya*/sediment samples taken at Weeki Wachee Springs, as is evident by the high N:P and C:P ratios (72 and 1052, respectively (Table 5-2). For the algal samples, C:N, N:P and C:P ratios all decreased moving from the lower (deeper) portions of the mat towards the surface. The C:N ratio of the sample taken at the top of the mat (multisampler depth 130 cm) is close to what would be expected from the Redfield Ratio (C:N:P of 106:16:1). The N:P and C:P ratios show strong phosphorus limitation. The C:N ratios at Silver Glen Springs did not vary widely throughout the profile, ranging from 7 to 8 (Table 5-2). The N:P and C:P ratios of the sediment were very low, 2 and 15, respectively. Algal tissue showed strong P-limitation throughout the profile except for the sample taken at the depth corresponding to 30 cm in the multisampler. The two sediment samples taken at Manatee Springs in April 2006 had very different molar ratios for N:P and C:P (Table 5-2). Algal tissue molar ratios for samples taken in August 2006 did not vary from each other.

Diffusive Flux out of Algal Mats

At both Weeki Wachee and Manatee Springs, there was diffusive flux of NH_4 out of the algal mat and down into the sediments as well as into the overlying water column (Table 5-4), with the highest flux out of the *Vaucheria* mat and into the water column (5 to 9.5 $mg/m^2/day$ depending on the porosity of the algal mat) . Flux of PO_4 was much lower. The highest flux was out of the *Lyngbya wollei* mat at Weeki Wachee, 0.03 to 0.05 $mg/m^2/day$, depending on porosity of the mat.

NaCl Tracer Experiments in Large Algal Mats

Salt tracer concentration was measured in units of $\mu S\ cm^{-1}$ (i.e., conductivity units) and corrected for background conductivity of spring water. Concentrations measured at each of the multisampler ports took from 15 to 30 minutes to reach peak levels before they began to slowly decline. The rate of decrease of NaCl tracer expressed as the amount of change (percent) since the peak concentration was reached, was computed for each multisampler port and plotted against port elevation above the bottom (Figure 5-10). Tracer decrease is likely proportional to the rate of advective water movement through the mat and my observations suggest that it varied as a function of the distance of the multisampler port above the spring bottom (Figure 5-10).

Average advective flow through the mat at Weeki Wachee was at least 10x greater than at Silver Glen, with Manatee flows falling in between. Advective flow was generally greatest at the top of the mat, followed by the bottom of the mat; flow within the middle of the mats was lowest. Higher apparent advective flow at the bottom of the mats could be indicative of vertical water movement out of sandy bottom sediments or binding of Na^+ and Cl^- ions to exchange sites in the sediments.

The two sampling points at Manatee spring that are denoted with an * are locations above the algal mat (all of the other samples are within the mat). As previously noted, the Manatee mat

used to install the multisamplers was in a zone of very low water velocity. Interestingly, the rate of tracer decline at these two points is less than that measured within the Weeki Wachee mat and only slightly greater than the rate within the interior of the Manatee mat.

While it is difficult to translate the tracer data into actual water velocities, I 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. I assumed that the tracer was evenly mixed within a rectangular column of water with dimensions of 0.5 m long, 0.5 m wide and with a height equal to the thickness of the algal mat (in meters). Next, I assumed that water moved through this control volume, in a horizontal direction only through the upstream face of the control volume; this face had an area of 0.5 m x the mat thickness (height). Next I computed the average fractional rate of decrease of tracer within the control volume per second and multiplied this rate by the total volume of the control volume - the product is the volume of water exchanged in the control volume per second. Finally I divided the volume of water exchanged per second by the cross-sectional area of control volume (0.5 m x the mat thickness) - this quotient is analogous to specific discharge through a porous medium such as soil (i.e., units = $m\ s^{-1}$).

Mean values for specific 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 (Table 5-10). Maximum rates determined for regions near the top of the mats were about 2x greater than the mean values shown in Table 5-10. For comparison, hydraulic conductivity through silty sand ranges from ca. 10^{-3} to 10^{-7} $m\ s^{-1}$.

Discussion

Elemental analysis of interstitial waters demonstrated that large *Vaucheria* and *Lyngbya* mats within these springs contain biologically significant quantities of N and and potentially

bioavailable dissolved organic phosphorus that could supply actively growing algae. High dissolved nutrient levels could mean that algal nutrient demand is less than the rate of nutrient production within the mat. In other stream systems of North America, nutrient levels in algal mats (thinner mats) reach very low levels owing to uptake by algae (R. Jan Stevenson, unpublished data). But, nutrient exchange between the inner parts of the mat and the upper, actively growing layers could be transport-limited and driven primarily by diffusion rather that advective water fluxes. Middle portions of the mat exhibited the highest nutrient concentrations and lowest advective flow. Additionally, N:P ratios of *Lyngbya wollei* tissue at both Silver Glen and Weeki Wachee Springs indicate phosphorus limitation, and therefore much of the organic phosphorus in interstitial waters may not be available to this species. *Vaucheria* sp. mat tissue concentrations did not indicate P-limitation. Despite high nutrient concentrations in interstitial water, Sickman *et al*. (2009) showed that the total mass of C, N and P contained within these same algal mats (both in algal biomass and interstitial waters) represents only a few hours of flux of organic C, N (predominantly NO_3^-) and P from the spring boils. While C, N and P mass in the mats was high on an areal basis, the volume of groundwater flow from the boils may result in a greater potential rate of external nutrient delivery to actively growing algae on the surface of the mats (Sickman *et al*., 2009).

L. wollei mats are persistent at Weeki Wachee and Silver Glen Springs, while *Vaucheria* sp. mats at Manatee Springs undergo sloughing and almost completely disappear at certain times of the year, although not at regular intervals (A. Albertin, personal observation). Higgins, Hecky & Guilford (2008) found that in thick *Cladophora glomerata* mats (a green algae) of Lake Erie, sloughing occurred at both nutrient-depleted and nutrient-enriched sites and they hypothesized that self-shading, once the algal mats attained a certain size and density, was responsible. This

may be occurring at Manatee Springs. Additionally, seasonal flooding of the Suwanee River, which is located approximately 300 m from the boil, caused incursions of tannins into the spring run, which attenuated light in portions of the run. After flooding events, algal die-off was observed. Self-shading may not be as much of a problem in *Lyngbya wollei* mats because this species can absorb light across the entire visible spectrum due to the presence of phycobilins, chlorophyll *a* and carotenoid pigments (Speziale *et al*., 1991). Speziale *et al*. (1991) found that maximum photosynthesis occurred within the subsurface layers of *L. wollei* mats in the southeastern U.S. and that surface layers of floating mats were photoinhibited at high irrandiances.

While my study discovered potential sources of nutrients to large algal mats in Florida springs, I was unable to definitively determine the primary source of N and P to actively growing *Lyngbya* and *Vaucheria* mats. The strongest evidence for internal nutrient cycling is simply the fact that high nutrient levels exist in the mats as compared to other locations where algae completely draw down internal nutrient sources and are reliant on external nutrient supplies. From a mass balance perspective, external nutrient supply from groundwater is much larger than the standing stock of dissolved nutrients within the mats (Sickman *et al*. 2009). However, this mass balance approach is static and does not take into consideration the turnover time of nutrients within the mat. More information is needed to definitively determine whether, and to what degree, actively growing algae are using internally regenerated N and P. Dissolved oxygen measurements and metal speciation analysis within the mats and underlying sediments would allow for better understanding of biogeochemical processes taking place within large mats. Diurnal variations in nutrient uptake within mats need also be considered. In future studies, isotopic tracers (e.g., ^{15}N-labelled ammonium and nitrate or ^{32}P- labeled phosphate) could be

released within large mats and used to quantify potential rates of internal nutrient uptake. Finally, at sites like Weeki Wachee and Silver Glen Springs, where thick mats persist for months to years without completely dying back, I hypothesize that large and diverse populations of heterotrophic microbes may make up a significant portion of the biomass in these mats. To my knowledge, there have been few or no studies of microbial ecology or biogeochemistry in algal mats of Florida springs. Taken together, these proposed studies could provide insights into rates of decomposition and internal nutrient loading and be used in modeling simulations of algal mats.

Table 5-1. Dates of multisampler deployment, and the make-up and condition of algal mats in three Florida springs.

Site/Date	Dominant Algal Species	Condition of Mat on Surface
Weeki Wachee		
4-19-2006	*Lyngbya wollei*	Growing
8-23-2006	*Lyngbya wollei*	Growing
Manatee		
4-19-2006	*Vaucheria* spp.	Senescing
8-24-2006	*Vaucheria* spp.	Senescing
Silver Glen		
9-5-2006	*Lyngbya wollei*	Growing

Table 5-2. Stable isotopic composition (δ13C and δ15N), molar nutrient ratio profiles and water chemistry of three algal mats found in Florida Springs, 2006. Multisampler depth is in cm and water chemistry concentrations are in mg/L.

										Water Chemistry			
Sampling Date	Spring	Multi-sampler No.	Depth in Sampler	Sample type	$\delta^{15}N$	$\delta^{13}C$	C:N	N:P	C:P	NO_x	TKN	TP	SRP
8/23/2006	Weeki Wachee	1	10	Lyngbya/ Sediment	4	-32	15	72	1052	0.013	0.526	0.061	0.007
8/23/2006		1	50	Sediment	4	-33	16	65	1030	0.011	1.500	0.081	0.011
8/23/2006		1	70	Lyngbya	2	-21	13	73	971	0.012	1.616	0.090	0.015
8/23/2006		1	90	Lyngbya	1	-25	12	70	819	0.012	3.278	0.296	0.046
8/23/2006		1	110	Lyngbya	1	-26	12	61	719	0.013	1.238	0.127	0.019
8/23/2006		1	130	Lyngbya	3	-31	8	39	318	0.072	0.220	0.010	0.001
9/5/2006	Silver Glen	2	20	Lyngbya/ Sediment	-4	-29	8	2	15	0.042	23.735	4.848	0.869
9/5/2006		2	30	Lyngbya	3	-20	8	34	267	0.046	39.501	10.442	0.882
9/5/2006		2	40	Lyngbya	2	-17	8	18	156	0.027	13.972	2.473	0.381
9/5/2006		2	60	Lyngbya	2	-20	7	38	272	0.022	0.088	0.028	0.004
9/5/2006		2	90	Lyngbya	0	-17	8	39	308	0.021	0.074	0.026	0.003
4/19/2006	Manatee	1	10	Vaucheria/ Sediment	-5	-32	22	30	651	0.009	8.311	3.534	0.233
4/19/2006		2	10	Vaucheria/ Sediment	2	-32	20	2	38	0.020	28.295	3.845	0.004
8/24/2006		1	30	Vaucheria	-1	-22	10	13	132	0.162	0.598	0.053	0.003
8/24/2006		1	40	Vaucheria	5	-26	10	12	126	0.177	0.191	0.030	0.003

Table 5-3. Specific discharges through algal mats at three springs.

Spring	Specific Discharge ($m\ s^{-1}$)
Weeki Wachee	9.0×10^{-7}
Manatee	3.7×10^{-7}
Silver Glen	4.0×10^{-8}

Table 5-4. Diffusion flux out of a large *Lyngbya wollei* mat (Weeki Wachee Springs) and out of a large, senescing *Vaucheria* sp. mat (Manatee Springs) in April 2006. Flux is calculated for two temperatures (18°C and 25°C) and for two porosities (0.5 and 0.8). Negative flux indicates diffusion from the mat to the sediment and positive flux indicates diffusion upwards to the water column. MS= multisampler number and it refers to profiles shown in Figures 5-3 (Manatee) and 5-5 (Weeki Wachee). Depth in MS refers to distance from the bottom of the multisampler).

									Porosity= 0.5		Porosity = 0.8	
Site	MS Profile	Nutrient	Diffuison coefficient 18°C $10^{-6}cm^2/sec$	Diffuison Coefficient 18°C $10^{-6}cm^2/sec$	Start (Depth in MS)	End (Depth in MS)	Total distance (cm)	Concentration gradient (ug/cm^3 or mg/L)	Flux at 18°C $mg/m^2/day$	Flux at 25°C $mg/m^2/day$	Flux at 18°C $mg/m^2/day$	Flux at 25°C $mg/m^2/day$
Weeki Wachee	1	NH_4^+	16.8	19.8	10	60	50	-2.83	-0.41	-0.48	-0.66	-0.77
Weeki Wachee	1	NH_4^+	16.8	19.8	60	90	30	3.46	0.84	0.99	1.34	1.58
Weeki Wachee	1	PO_4^-	7.15	8.46	10	60	50	-0.23	-0.01	-0.02	-0.02	-0.03
Weeki Wachee	1	PO_4^-	7.15	8.46	60	90	30	0.27	0.03	0.03	0.04	0.05
Manatee	2	NH_4^+	16.8	19.8	10	20	10	-3.53	-2.57	-3.02	-4.10	-4.84
Manatee	2	NH_4^+	16.8	19.8	20	60	40	27.94	5.07	5.97	8.11	9.56
Manatee	2	PO_4^-	7.15	8.46	10	60	50	-0.01	0.00	0.00	0.00	0.00

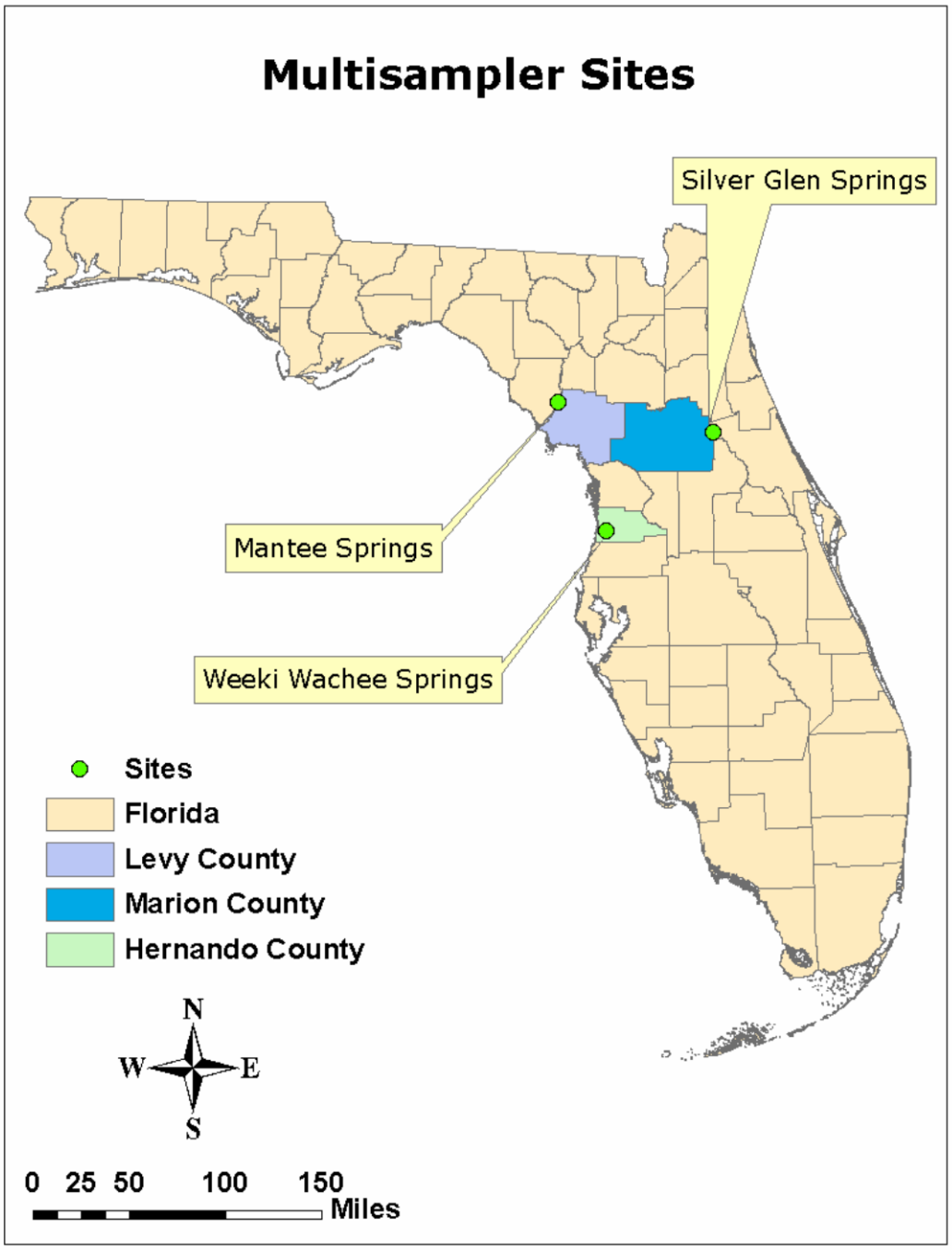


Figure 5-1. Location of multisampler deployments and investigations of nutrient profiles and movement within algal mats. Map made by Martin Anderson.

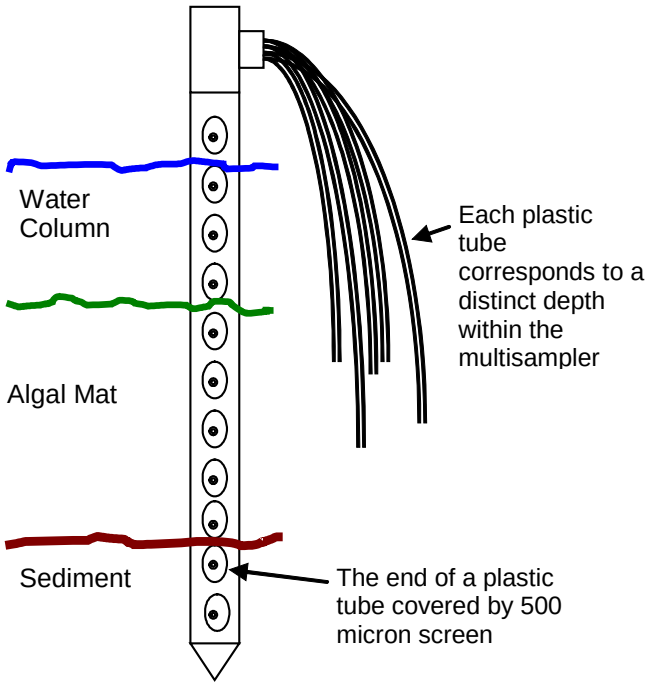


Figure 5-2. Multisampler device used to collect water column, algal mat and sediment interstitial waters.

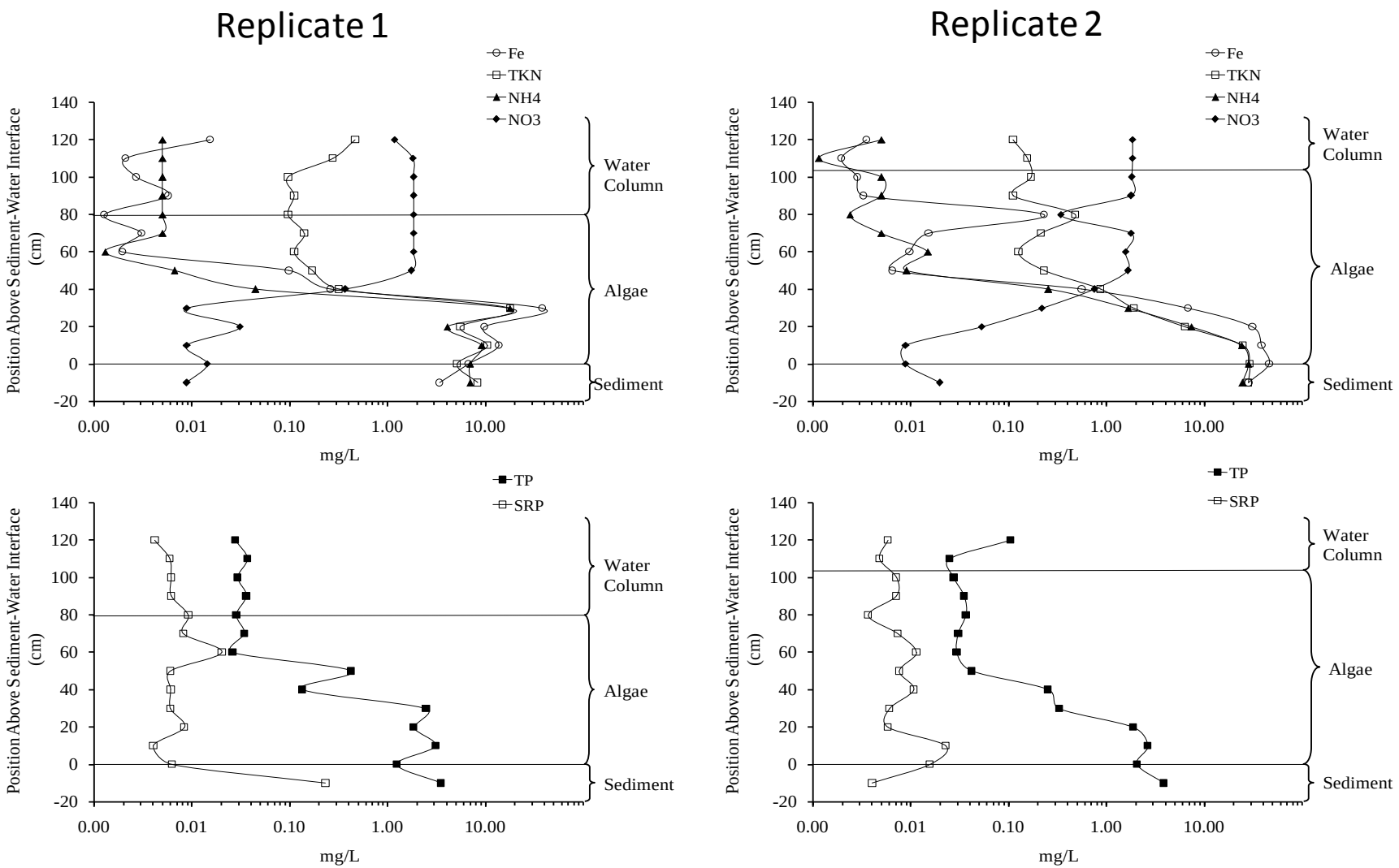


Figure 5-3. Chemical profiles measured by multisamplers at Manatee Springs on April 19, 2006. At each spring, two replicate samplers were simultaneously installed. Approximate position of sediment, algae and water column are indicated along the right side of each figure. The x-axes are logarithmic.

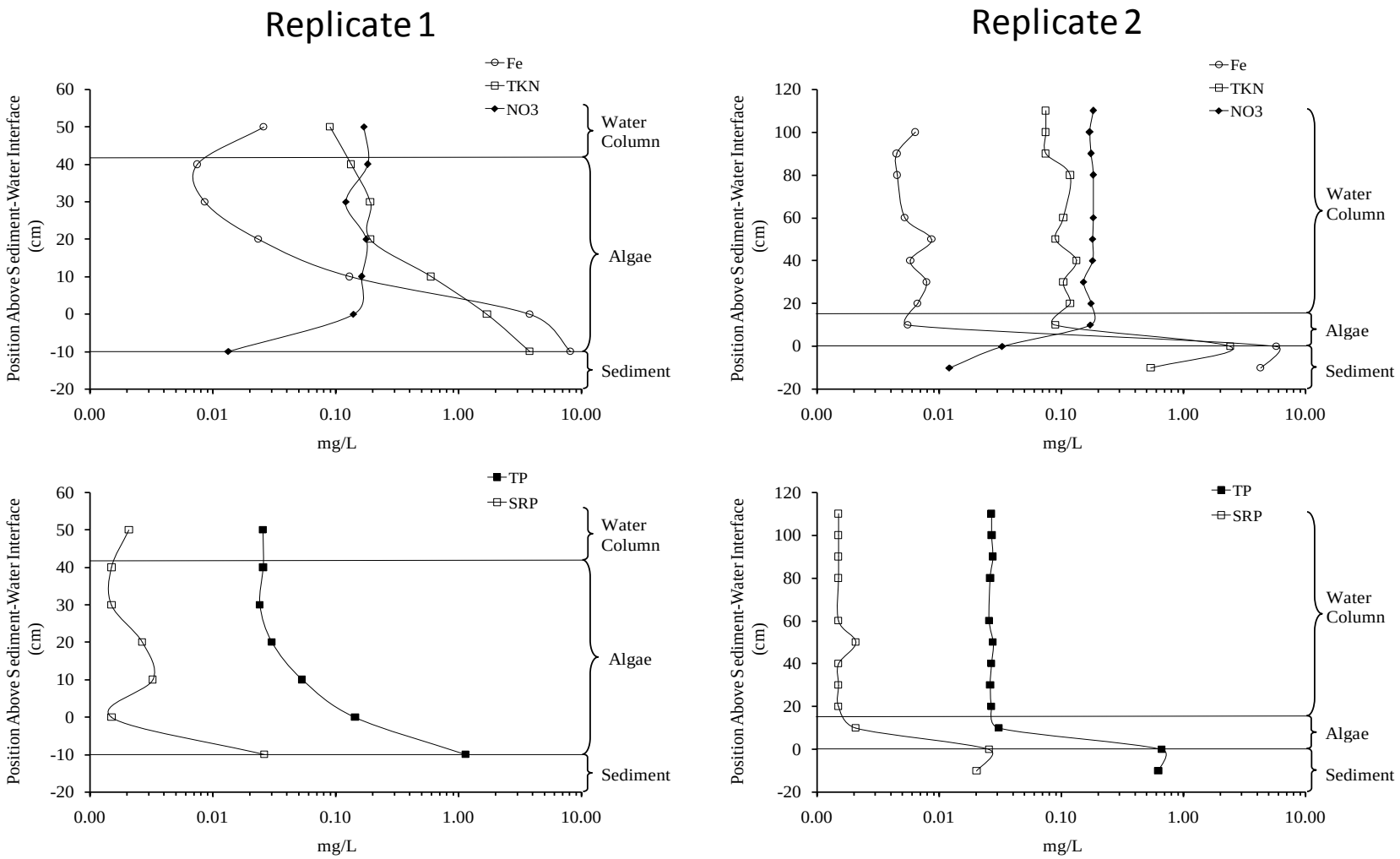


Figure 5-4. Chemical profiles measured by multisamplers at Manatee Springs on August 24, 2006. At each spring, two replicate samplers were simultaneously installed. Approximate position of sediment, algae and water column are indicated along the right side of each figure. The x-axes are logarithmic.

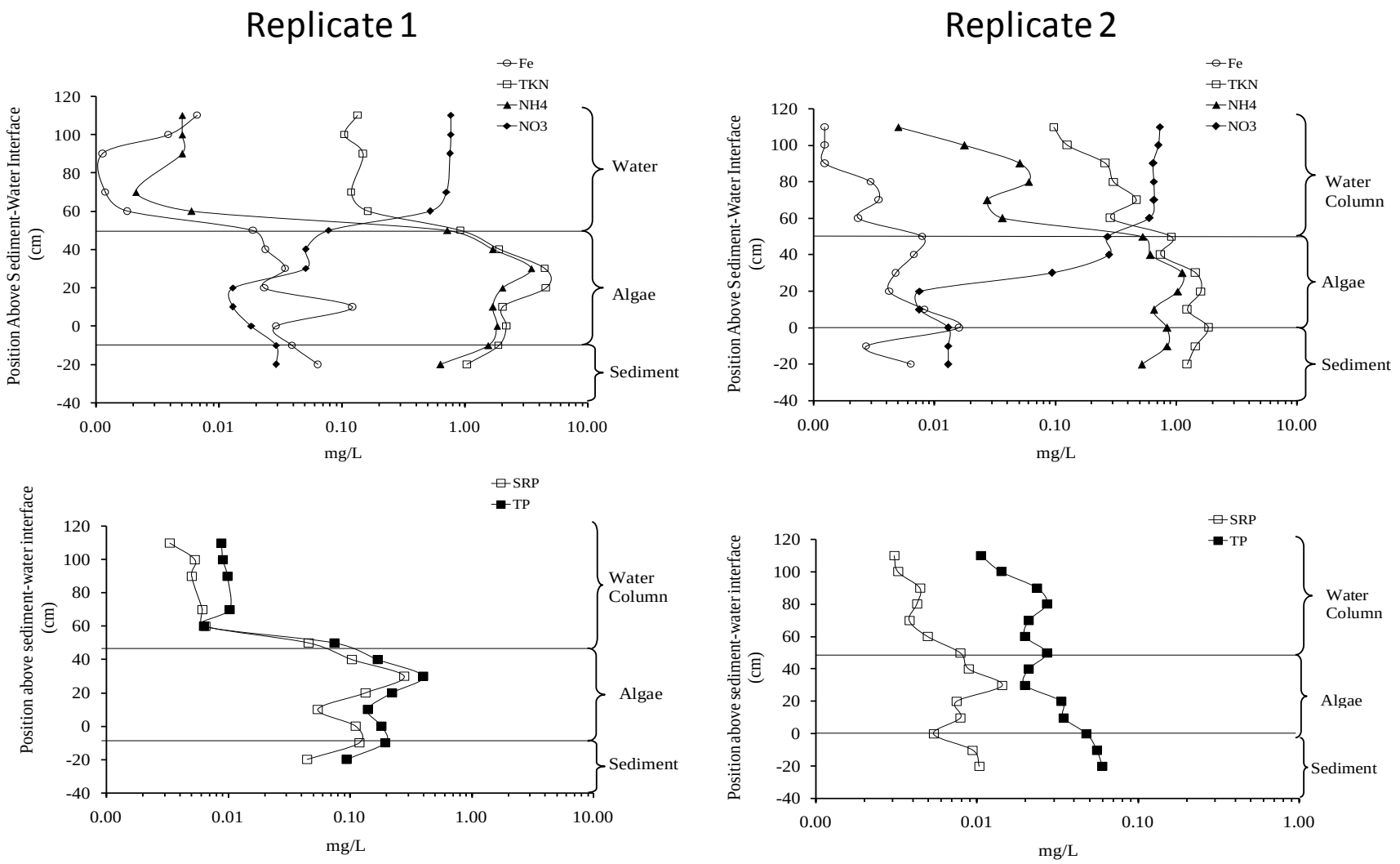


Figure 5-5. Chemical profiles measured by multisamplers at Weeki Wachee Springs on April 19, 2006. At each spring, two replicate samplers were simultaneously installed. Approximate position of sediment, algae and water column are indicated along the right side of each figure. The x-axes are logarithmic.

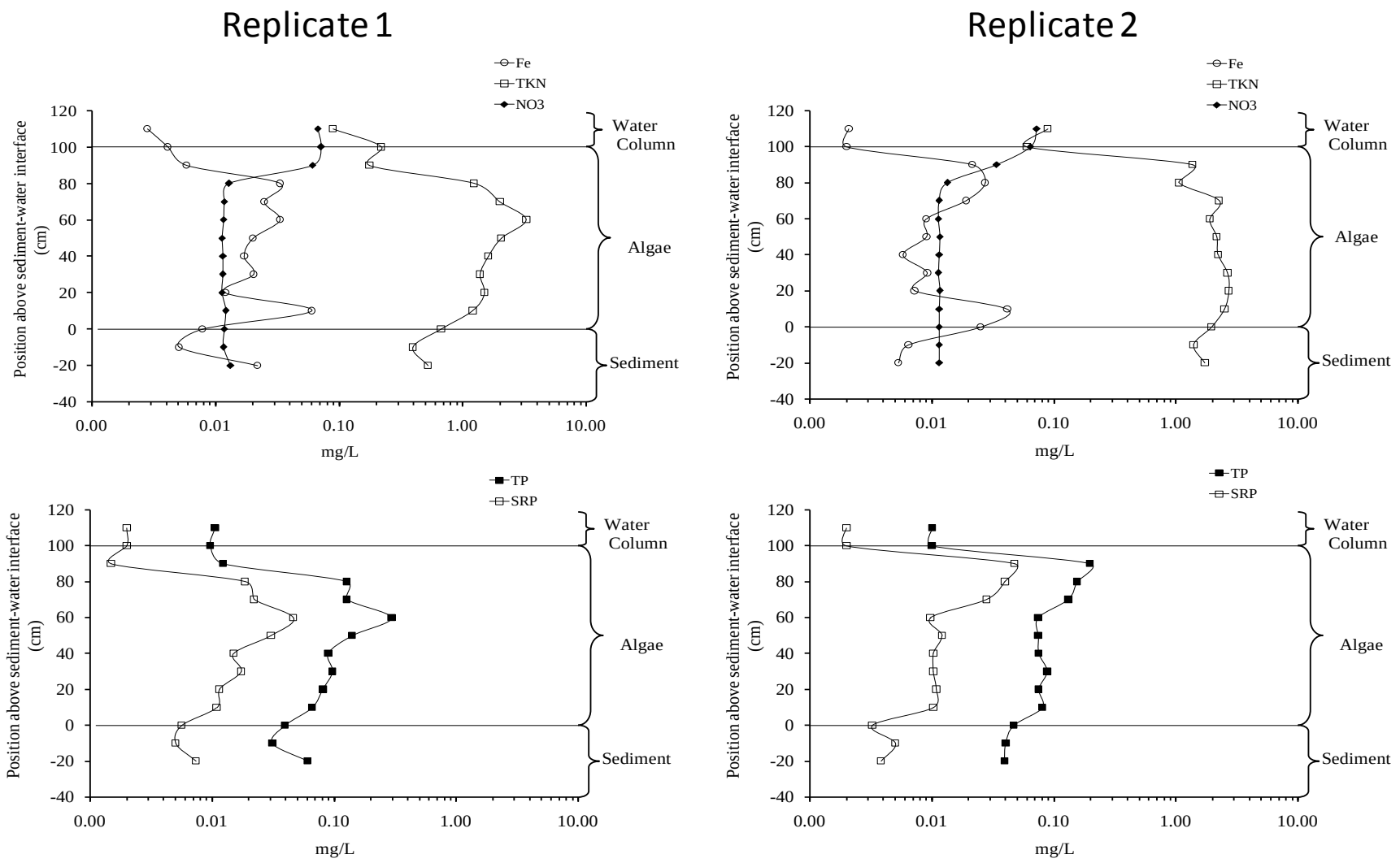


Figure 5-6. Chemical profiles measured by multisamplers at Weeki Wachee on August 23, 2006. At each spring, two replicate samplers were simultaneously installed. Approximate locations of sediment, algae and water column are indicated along the right side of each figure. The x-axes are logarithmic.

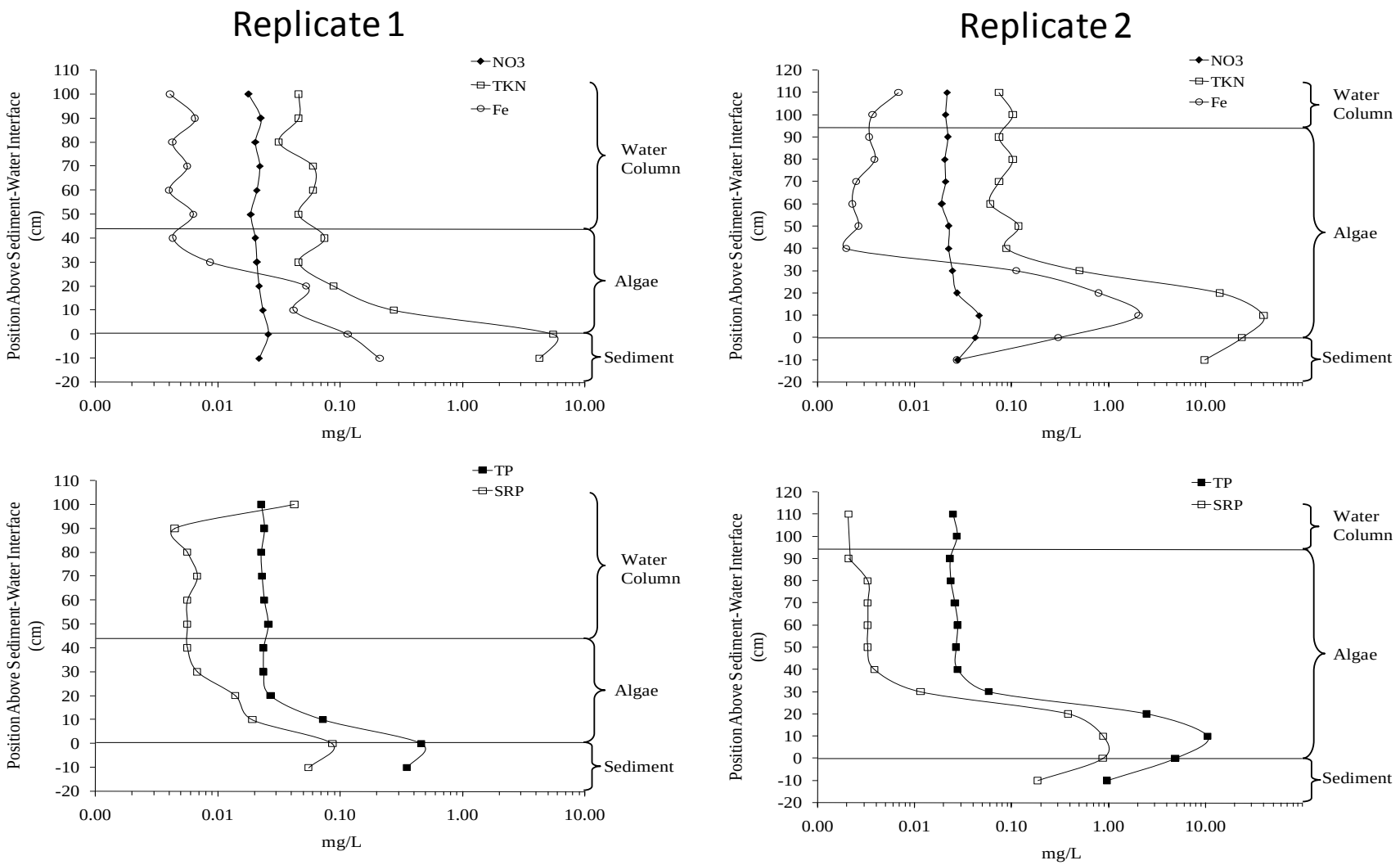


Figure 5-7. Chemical profiles measured by multisamplers at Silver Glen Springs September 5, 2006. At each spring, two replicate samplers were simultaneously installed. Approximate locations of sediment, algae and water column are indicated along the right side of each figure. The x-axes are logarithmic.

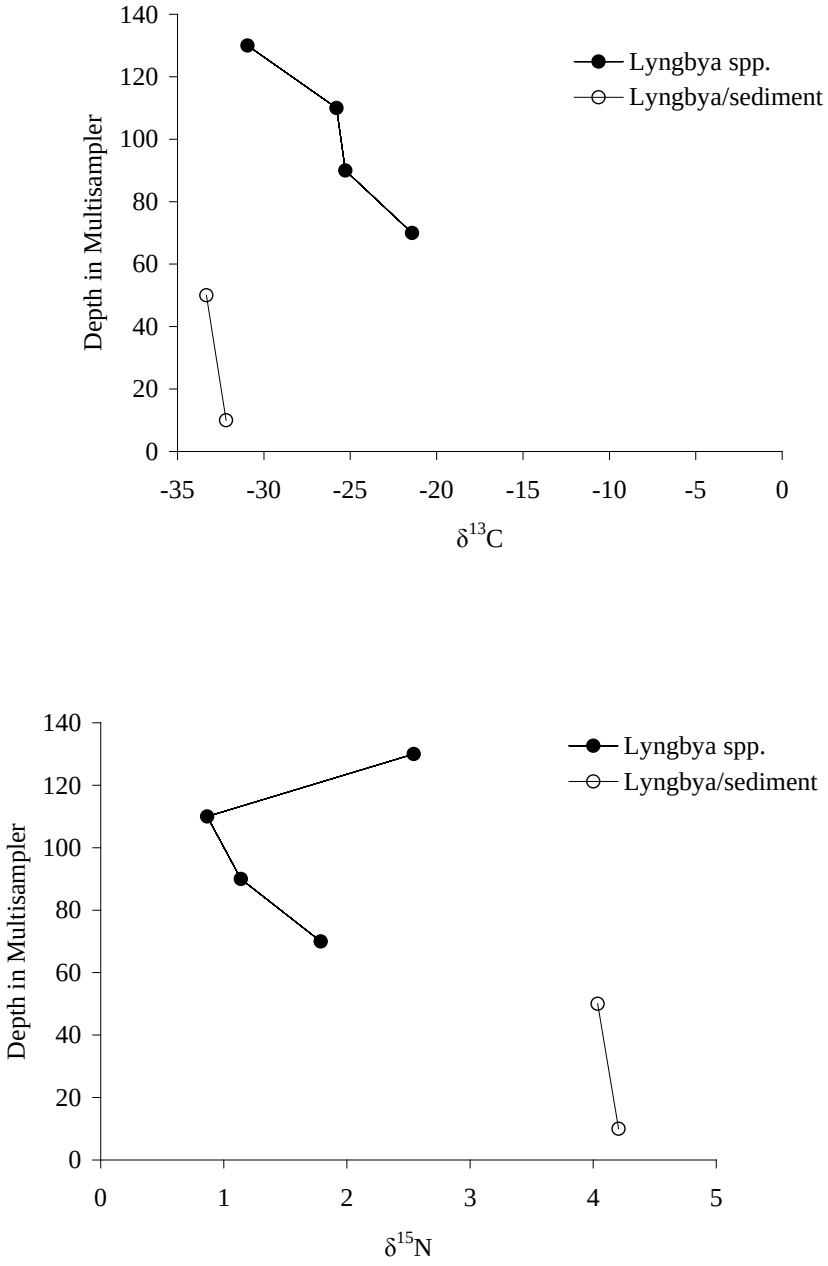


Figure 5-8. Stable isotope profile of algae and sediment surrounding Multisampler 1 at Weeki Wachee Springs, August 23, 2006. A) $\delta^{13}C$ vs. the depth in the multisampler (cm). B) $\delta^{15}N$ vs. the depth in the multisampler (cm). *Lyngbya*/sediment is so labeled because of the large amount of decomposing algae in the sediment sample.

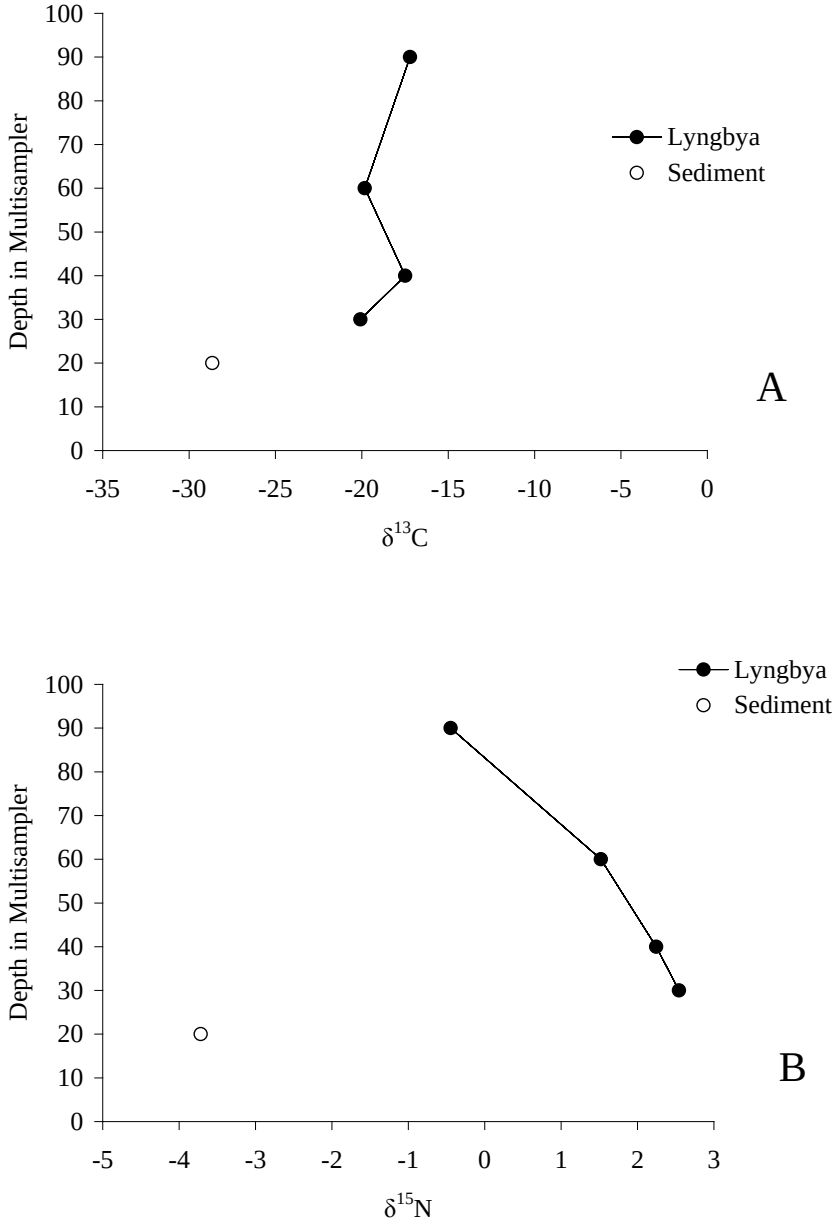


Figure 5-9**.** Stable isotope profile of *Lyngbya wollei* and sediment surrounding Multisampler 2 at Silver Glen Springs, September 2, 2006. A) $\delta^{13}C$ vs. the depth in the multisampler (cm). B) $\delta^{15}N$ vs. the depth in the multisampler (cm).

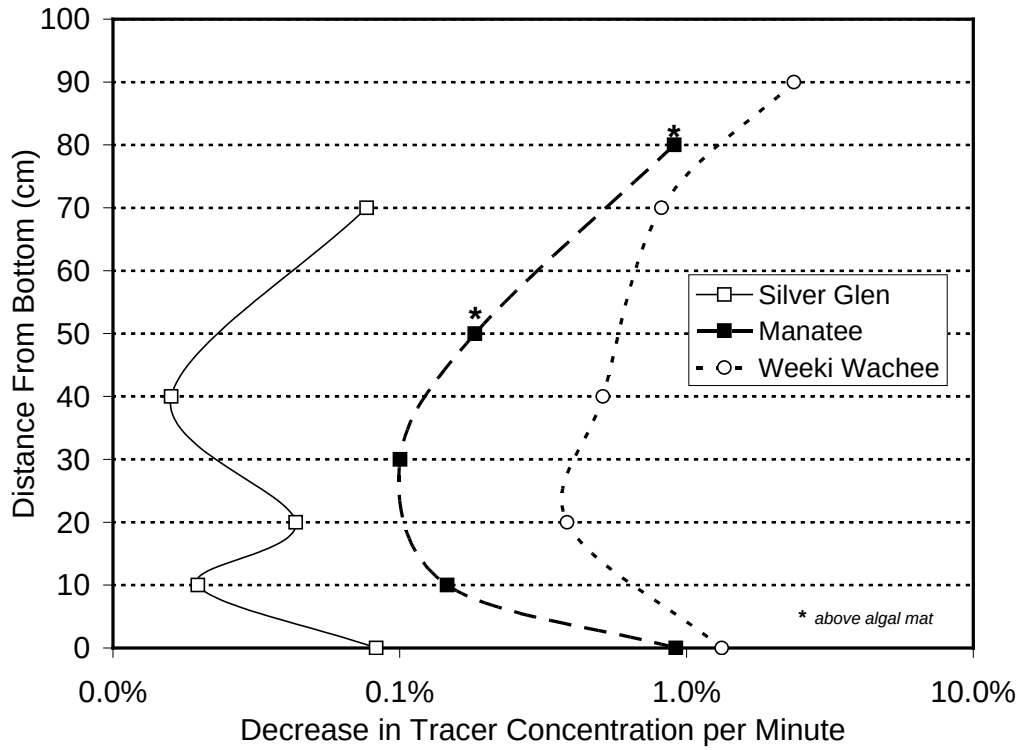


Figure 5-10. Profiles of tracer dilution at three springs. The rate of decrease of NaCl tracer (measured in units of conductivity) expressed as percent change from peak concentration are plotted on the x-axis.

CHAPTER 6
CONCLUSION

There is no simple cause and effect relationship between nutrient concentrations and algal abundance in Florida Springs. Results from extensive field surveys conducted throughout North central Florida and the Panhandle indicate that the abundance of *Lyngbya wollei*, one of the most abundant filamentous algae found in springs, was not directly related to nitrate or phosphorus concentrations in spring water (Stevenson *et al*. 2007; Pinowska *et al*. 2009). Multiple factors, both biotic and abiotic, are likely affecting algae in springs.

Despite these complex relationships, these studies shed light on factors affecting algal growth. I found that under laboratory conditions, *Lyngbya wollei* growth is stimulated by additions of nitrogen, even if phosphorus is in very low supply. The stoichiometry of algal tissue at the end of the experiments suggested strong P-limitation, N:P ratios of up to 57:1 and C:P ratios of up to 630:1. However, growth rates for *L.wollei* remained positive, indicating that it was never under truly limiting P conditions and implies that the optimal stoichiometric ratio for *L. wollei* in Florida springs likely deviates from the Redfield ratio benchmark. Higher growth rates were obtained in my experiments at higher nutrient concentrations and therefore, reductions in N concentrations should reduce algal growth rates in spring systems although algal biomass would continue to accumulate. The threshold value for *L.wollei* growth produced by logistics model, 50% growth saturation at 41.5 μg L^{-1} and 90% saturation at 110.0 μg L^{-1} are low when compared to nitrate concentrations found in many Florida springs, making the task of establishing nutrient criteria in Florida springs difficult.

The dual isotope analysis of nitrate ($\delta^{15}N$ and $\delta^{18}O$) indicates that organic nitrogen sources, such as manure or septic waste are likely important sources of N at springs such as Troy, Wekiwa, Volusia, Lafayette and Little River. At springs such as Ichetucknee Head Springs,

Ichetucknee Blue Hole, Rainbow, Jackson and Madison Blue Springs, inorganic N sources, such as NH_4 from either fertilizer and/or rain or soil nitrogen are likely important sources. However, I did find variability in $\delta^{15}N$ and $\delta^{18}O$ of nitrate at some springs across multiple years and therefore seasonal sampling is crucial. Additionally, the importance of denitrification in and above the Floridan Aquifer needs to be established in order to better interpret isotope signatures; the tight correlation between O and N isotopes of nitrate in most springs, might suggest a uniform nitrate source, such as inorganic fertilizers, rather than heavy inputs from organic N sources.

Algal $\delta^{15}N$ signatures were not species-specific and varied across sites despite little variation in $\delta^{15}N$ and $\delta^{18}O$ of nitrate in spring water at those same sites. This variability is likely due to environmental and physiological factors affecting fractionation during algal uptake of N, which need to be understood in order to better interpret results. In contrast, relatively strong species-specific $\delta^{13}C$ values were found across spring sites as well as a strong relationship between algal $\delta^{13}C$ values, pH and total DIC. This relationship likely results from differential use of HCO_3^- and CO_2 (aq) during photosynthesis as these DIC species have disparate $\delta^{13}C$ values. Species-specific tendencies found with total DIC concentrations and pH may be important in determining algal distribution in springs and need to be further investigated.

Finally, I found that high nutrient concentrations exist within thick *Lyngbya wollei* and *Vaucheria* sp. mats, although I was unable to determine whether or not this was a primary source of N and P to actively growing algae. More information, such as dissolved oxygen measurements and studies on the microbial ecology within thick algal mats and in underlying sediments would allow for a better understanding of ongoing biogeochemical processes.

APPENDIX
LOCATIONS OF SAMPLING SITES

Spring	Spring code	Site name	Site code	Boil	Latitude	Longitude
Alexander	ALE	Head	ALE-01	Yes	29.08128	-81.57563
		Downstream	ALE-02	No	29.08231	-81.57754
Chassahowitzka	CHA	Blue holes	CHA-01	Yes	28.71617	-82.57502
		Dock	CHA-02	Yes	28.71558	-82.57630
		Brown spring	CHA-03	Yes	28.71721	-82.57586
Cypress	CYP	Head	CYP-01	Yes	30.65855	-85.68430
Fanning	FAN	Head	FAN-01	Yes	29.58757	-82.93541
Gainer	GAI	Pipe	GAI-01	Yes	30.42736	-85.54827
		Side boil	GAI-02	Yes	30.42884	-85.54854
		Morten Spring	GAI-03	Yes	30.42875	-85.54649
Guaranto	GUR	Head	GUR-01	Yes	29.77973	-82.94001
Ichetucknee	ICH	Head	ICH-01	Yes	29.98408	-82.76184
		Blue Hole	ICH-02	Yes	29.98068	-82.75866
		Below Blue Hole	ICH-03	No	29.98007	-82.75895
		Mission spring	ICH-04	Yes	29.97628	-82.75783
		Devils Ear	ICH-05	Yes	29.97388	-82.75996
		Mill Pond	ICH-06	Yes	29.96658	-82.76005
		Before bridge	ICH-07	No	29.95495	-82.78507
		Coffee spring	ICH-08	Yes	29.95937	-82.77526
Indian	IND	Head	IND-01	Yes	30.25077	-84.32203
Jackson Blue	JAC	Head	JAC-01	Yes	30.79037	-85.13998
		Boat ramp	JAC-02	No	30.78249	-85.16022
		Rock cliff	JAC-04	Yes	30.79023	-85.14290
Juniper	JUN	Head	JUN-01	Yes	29.18365	-81.71201
		Fern Hammock	JUN-02	Yes	29.18364	-81.70801
		After bridge on route 19	JUN-04	No	29.21283	-81.65431
Lafayette Blue	LAF	Head	LAF-01	Yes	30.12592	-83.22617
Little River	LTR	Head	LTR-01	Yes	29.99642	-82.96675
Madison Blue	MAD	Head	MAD-01	Yes	30.48056	-83.24439
Manatee	MNT	Head	MNT-01	Yes	29.48952	-82.97692
Pitt	PIT	Head	PIT-01	Yes	30.43288	-85.54616
Ponce de Leon	PON	Head	PON-01	Yes	30.72090	-85.93071
Rainbow Spring	RAI	Head	RAI-01	Yes	29.10223	-82.43741
		KP Hole	RAI-02	Yes	29.09294	-82.42848
		Before tubers sign	RAI-03	No	29.06305	-82.42788
		Before bridge	RAI-04	No	29.05223	-82.44700
			RAI-05	No	29.09275	-82.43133
			RAI-06	No	29.07650	-82.42760
		After bridge	RAI-07	No	29.05407	-82.44717

Spring	Spring code	Site name	Site code	Boil	Latitude	Longitude
Silver Glen	SGL	Head	SGL-01	Yes	29.24603	-81.64345
		Natural Well	SGL-02	Yes	29.24583	-81.64385
		Trial in the woods	SGL-03	Yes	29.24400	-81.6463
Silver River	SLV	Head	SLV-01	Yes	29.21619	-82.05252
		Second pool	SLV-02	Yes	29.21584	-82.04987
		Birds of prey	SLV-03	No	29.21561	-82.04112
		Old swimming area	SLV-04	No	29.20500	-82.02902
		Cabbage palm	SLV-05	No	29.20211	-82.01127
			SLV-07	No	29.20715	-81.99660
Troy	TRY	Head	TRY-01	Yes	30.00598	-82.99756
Volusia Blue	VOL	Head	VOL-01	Yes	28.94758	-81.33969
		Downstream from stairs	VOL-02	No	28.94679	-81.33921
Wakulla	WAK	Head	WAK-01	Yes	30.23533	-84.30287
		Turnaround	WAK-02	No	30.23318	-84.28870
		Bird colony	WAK-03	No	30.22507	-84.27470
			WAK-04	No	30.23650	-84.29831
			WAK-05	No	30.23439	-84.29505
			WAK-06	No	30.22836	-84.28001
		Upstream from bridge on 98	WAK-08	No	30.18037	-84.24817
Washington Blue	WGT	Head	WGT-01	Yes	30.45279	-85.53044
			WGT-02	Yes		
Weeki Wachee	WEK	Head	WEK-01	Yes	28.51747	-82.57349
		Boat dock	WEK-02	No	28.51901	-82.57361
		WMA	WEK-03	No	28.52481	-82.59583
		Roger's Park	WEK-04	No	28.53057	-82.62407
			WEK-05	No	28.51874	-82.57739
			WEK-06	No	28.52216	-82.58459
			WEK-07	No	28.51860	-82.59268
Wekiwa	WKW	Head	WKW-01	No	28.71193	-81.46037
		Canoe launch	WKW-02	No	28.71269	-82.45948
Williford	WIL	Head	WIL-01	Yes	30.43966	-85.54763

LIST OF REFERENCES

Allen S.E. (1989) *Chemical analysis of ecological materials*, Blackwell Scientific, Oxford.

Anderson D.M., Glibert P.M. & Burkholder J.M. (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries*, **25**, 704-726.

Anderson J.M. (1976) An ignition mehtod for the determination of total phosphorus in lake sediments. *Water Research*, **10**, 329-331.

Aravena R.& Robertson W.D. (1998) Use of multiple isotope tracers to evaluate denitrification in ground water: Study of nitrate from a large-flux septic system plume. *Ground Water*, **36**, 975-982.

Bacchus S.T. & Barile PJ. (2005) Discriminating sources and flowpaths of anthropogenic nitrogen discharges to Florida springs, streams and lakes. *Environmental & Engineering Geoscience*, **11**, 347-369.

Beardall J., Griffiths H. & Raven J.A. (1982) Carbon isotope discrimination and the CO_2 accumulating mechanism in *Chlorella emersonii*. *Journal of Experimental Botany*, **33**, 729-737.

Beer S., Spencer W. & Bowes G. (1986) Photosynthesis and growth of the filamentous blue-green alga *Lyngbya birgei* in relation to its environment. *Journal of Aquatic Plant Management*, **24**, 61-65.

Berman T. & Chava S. (1999) Algal growth on organic compounds as nitrogen sources. *Journal of Plankton Research*, **21**, 1423-1437.

Bohlke J.K. (2002) Groundwater recharge and agricultural contamination. *Hydrogeology Journal*, **10**, 153-179.

Borchardt M.A. (1996) Nutrients. In: *Algal Ecology* (Eds. R.J. Stevenson, M.L. Bothwell & R.L.Lowe) , pp. 184-227. Academic Press, San Diego.

Bottcher J., Strebel O., Voerkelius S. & Schmidt H.L. (1990) Using isotope fractionation of nitrate nitrogen and nitrate oxygen for evaluation of microbial denitrification in a sandy aquifer. *Journal of Hydrology*, **114**, 413-424.

Brady N.C. & Weil R.R. (2002) *The Nature and Properties of Soil*, Prentice Hall, Upper Saddle River, NJ.

Brenner M., Whitmore T.J., Curtis J.H., Hodell D.A.& Schelske C.L. (1999) Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) signatures of sedimented organic matter as indicators of historic lake trophic state. *Journal of Paleolimnology*, **22**, 205-221.

Brenner M., Hodell D.A., Leyden B.W., Curtis J.H., Kenney W.F., Gu B.H. & Newman J.M. (2006) Mechanisms for organic matter and phosphorus burial in sediments of a shallow, subtropical, macrophyte-dominated lake. *Journal of Paleolimnology*, **35**, 129-148.

Cardellina J.H., Marner F.J. & Moore R.E. (1979) Seaweed dermatitis - Structure of Lyngbyatoxin-A. *Science*, **204**, 193-195.

Casciotti K.L., Sigman D.M., Hastings M.G., Bohlke J.K. & Hilkert A. (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Analytical Chemistry*, **74**, 4905-4912.

Cembella A.D., Antia N.J.& Harrison P.J. (1984) The utilization of inorganic and organic phosphorus-compounds as nutrients by eukaryotic microalgae - A multidisciplinary perspective .1. *Crc Critical Reviews in Microbiology*, **10**, 317-391.

Chambers R.M. & Odum W.E. (1990) Porewater oxidation, dissolved phosphate and the iron curtain - Iron-phosphorus relations in tidal fresh-water marshes. *Biogeochemistry*, **10**, 37-52.

Checkley D.M. & Miller C.A. (1989) Nitrogen isotope fractionation by oceanic zooplankton. *Deep-Sea Research Part A-Oceanographic Research Papers*, **36**, 1449-1456.

Chelette A.R., Pratt T.R. & Katz B.G. (2002) *Nitrate loading as an indicator of nonpoint source pollution in the lower St.Marks-Wakulla Rivers watershed*. Water Resources Special Report 02-1. Northwest Florida Water Management District, Havana, FL.

Cohen M.J. (2008) Springshed nutrient loading, transport and transformations. In: *Summary and Synthesis of the available literature on the effects of nutrients on spring organisms and systems* (Ed. M.T. Brown), pp. 53-134. Florida Department of Environmental Protection, Tallahassee, FL.

Cornelisen C.D., Wing S.R., Clark K.L., Bowman M.H., Frew R.D. & Hurd C.L. (2007) Patterns in the $\delta^{13}C$ and $\delta^{15}N$ signature of *Ulva pertusa*: Interaction between physical gradients and nutrient source pools. *Limnology and Oceanography*, **52**, 820-832.

Cowell B. (1990) Population dynamics and factors regulating the distribution of *Lyngbya* at Crystal River, FL. In: An evaluation of factors contributing to the growth of *Lyngbya* sp. in Kings Bay/Crystal River, Florida, Final Report (Ed. F. Romie), Florida Department of Environmental Protection Regulation, Brookesville, FL.

Cowell B.C. & Botts P.S. (1994) Factors influencing the distribution, abundance and growth of *Lyngbya wollei* in Central Florida. *Aquatic Botany*, **49**, 1-17.

Cowell B.C. & Dawes C.J. (2008) Seasonal comparisons of the phytoplankton and decline in water transparency in the spring-fed Rainbow River, Florida. *Journal of Freshwater Ecology*, **23**, 169-177.

Cross W.F., Benstead J.P., Frost P.C. & Thomas S.A. (2005) Ecological stoichiometry in freshwater benthic systems: Recent progress and perspectives. *Freshwater Biology*, **50**, 1895-1912.

Dahnke K., Bahlmann E. & Emeis K. (2008) A nitrate sink in estuaries? An assessment by means of stable nitrate isotopes in the Elbe estuary. *Limnology and Oceanography*, **53**, 1504-1511.

De Brabandere L., Frazer T.K. & Montoya J.P. (2007) Stable nitrogen isotope ratios of macrophytes and associated periphyton along a nitrate gradient in two subtropical, spring-fed streams. *Freshwater Biology*, **52**, 1564-1575.

Deines P., Langmuir D. & Harmon R.S. (1974) Stable carbon isotope ratios and existence of a gas-phase in evolution of carbonate ground waters. *Geochimica et Cosmochimica Acta*, **38** , 1147-1164.

Deines P. (2004) Carbon isotope effects in carbonate systems. *Geochimica et Cosmochimica Acta*, **68** , 2659-2679.

Derse E., Knee K.L., Wankel S.D., Kendall C., Berg C.J.& Paytan A. (2007) Identifying sources of nitrogen to Hanalei Bay, Kauai, utilizing the nitrogen isotope signature of macroalgae. *Environmental Science & Technology*, **41**, 5217-5223.

Dickman E.M., Vanni M.J.& Horgan M.J. (2006) Interactive effects of light and nutrients on phytoplankton stoichiometry. *Oecologia* , **149**, 676-689.

Doctor D.H., Alexander E.C., Petric M., Kogovsek J., Urbanc J., Lojen S. & Stichler W. (2006) Quantification of karst aquifer discharge components during storm events through end-member mixing analysis using natural chemistry and stable isotopes as tracers. *Hydrogeology Journal*, **14**, 1171-1191.

Doctor D.H., Kendall C., Sebestyen S.D., Shanley J.B., Ote N. & Boyer E.W. (2008) Carbon isotope fractionation of dissolved inorganic carbon (DIC) due to outgassing of carbon dioxide from a headwater stream. *Hydrological Processes*, **22**, 2410-2423.

Dodds W.K. & Welch E.B. (2000) Establishing nutrient criteria in streams. *Journal of the North American Benthological Society*, **19**, 186-196.

Dodds W.K., Smith V.H. & Lohman K. (2002) Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**, 865-874.

Dodds W.K. (2003) Misuse of inorganic N and soluble reactive P concentrations to indicate nutrient status of surface waters. *Journal of the North American Benthological Society*, **22**, 171-181.

Doyle R.D. & Smart R.M. (1998) Competitive reduction of noxious *Lyngbya wollei* mats by rooted aquatic plants. *Aquatic Botany*, **61**, 17-32.

Droop M.R. (1974) Nutrient status of algal cells in continuous culture. *Journal of the Marine Biological Association of the United Kingdom*, **54**, 825-855.

Duarte CM. (1992) Nutrient concentration of aquatic plants - Patterns across species. *Limnology and Oceanography*, **37**, 882-889.

Durka W., Schulze E.D., Gebauer G. & Voerkelius S. (1994) Effects of forest decline on uptake and leaching of deposited nitrate determined from $\delta^{15}N$ and $\delta^{18}O$ measurements. *Nature*, **372** , 765-767.

Eaton A.D., Clesceri L.S. & Greenberg A.E. (1995) *Standard methods for the examination of water and wastewater*, American Public Health Association, Washington D.C.

Einsiedl F., Maloszewski P. & Stichler W. (2005) Estimation of denitrification potential in a karst aquifer using the $\delta^{15}N$ and $\delta^{18}O$ of NO_3. *Biogeochemistry*, **72**, 67-86.

Einsiedl F. & Mayer B. (2006) Hydrodynamic and microbial processes controlling nitrate in a fissured-porous karst aquifer of the Franconian Alb, Southern Germany. *Environmental Science & Technology*, **40**, 6697-6702.

Elser J.J., Bracken M.E.S., Cleland E.E., Gruner D.S., Harpole W.S., Hillebrand H., Ngai J.T., Seabloom E.W., Shurin J.B. & Smith J.E. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, **10**, 1135-1142.

Faulkner G.L. (1973) *Geohydrology of the cross-Florida Barge Canal area with special reference to the Ocala vicinity*. Water Resources Investigation,73-1, US Geological Survey.

Finlay J.C., Power M.E. & Cabana G. (1999) Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. *Limnology and Oceanography*, **44**, 1198-1203.

Finlay J.C. (2001) Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. *Ecology*, **82**, 1052-1064.

Finlay J.C. (2004) Patterns and controls of lotic algal stable carbon isotope ratios. *Limnology and Oceanography*, **49**, 850-861.

Florida Springs Task Force (2000) Florida's Springs: Strategies for protection and restoration. Florida Department of Environmental Protection, Tallahassee, Florida

Fogel M.L. & Cifuentes L.A. (1993) Isotope fractionation during primary production. In: *Organic Geochemistry* (Eds M.H. Engel & S.A. Macko), pp. 73-98, Plenum Press, New York.

Francoeur S.N. (2001) Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society*, **20**, 358-368.

Fry B. (2006) *Stable Isotope Ecology*, Springer Science+Business Media, L.L.C., New York.

Fukada T., Hiscock K.M. & Dennis P.F. (2004) A dual-isotope approach to the nitrogen hydrochemistry of an urban aquifer. *Applied Geochemistry*, **19**, 709-719.

Gervais F. & Riebesell U. (2001) Effect of phosphorus limitation on elemental composition and stable carbon isotope fractionation in a marine diatom growing under different CO_2 concentrations. *Limnology and Oceanography*, **46**, 497-504.

Grimm N.B. & Fisher S.G. Nitrogen limitation in a Sonoran Desert stream. *Journal of the North American Benthological Society* 5, 2-15. 1986.

Harris D., Horwath W.R. & van Kessel C. (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal*, **65**, 1853-1856.

Hessen D.O., Agren G.I., Anderson T.R., Elser J.J. & De Ruiter P.C. (2004) Carbon sequestration in ecosystems: The role of stoichiometry. *Ecology*, **85**, 1179-1192.

Hiers J.K., Mitchell R.J., Boring L.R., Hendricks J.J. & Wyatt R. (2003) Legumes native to longleaf pine savannas exhibit capacity for high N_2-fixation rates and negligible impacts due to timing of fire. *New Phytologist*, **157**, 327-338.

Higgins S.N., Hecky R.E. & Guilford S.J. (2008) The collapse of benthic macroalgal blooms in response to self-shading. *Freshwater Biology*, **53**, 2557-2572.

Hill W.R. & Middleton R.G. (2006) Changes in carbon stable isotope ratios during periphyton development. *Limnology and Oceanography*, **51**, 2360-2369.

Hill W.R. & Fanta S.E. (2008) Phosphorus and light colimit periphyton growth at subsaturating irradiances. *Freshwater Biology*, **53**, 215-225.

Hillebrand H. & Sommer U. (1999) The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. *Limnology and Oceanography*, **44**, 440-446.

Hooker H.D. (1917) Liebig's law of the minimum in relation to general biological problems. *Science*, **46**, 197-204.

Hornsby H.D. & Ceryak R. (1999) *Springs of the Suwanee River Basin in Florida*. Suwanee River Water Management District Annual Report WR-96-02. Live Oak, Florida.

Howarth R, Paerl HW. (2008) Coastal and marine eutrophication: control of both phosphorus and nitrogen is necessary. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, E103.

Hunt R. (2008) *Basic growth analysis*, Unwin Hyman Ltd., London.

Hutchinson G.E. (1957) *A Treatise on Limnology*, John Wiley and Sons, New York.

Kahlert M. (1998) C : N : P ratios of freshwater benthic algae. *Arch.Hydrobiol.Spec.Issue Adv.Limnol*, **51**, 105-114.

Katz B.G., Hornsby H.D., Bohlke J.F. & Mokray M.F. (1999) *Sources and Chronology of Nitrate Contamination in Spring Waters, Suwannee River Basin, Florida* -Resources Investigations Report 99-4252, U.S. Geological Survey, Tallahassee.

Katz B.G., Bohlke J.K. & Hornsby H.D. (2001) Timescales for nitrate contamination of spring waters, northern Florida, USA. *Chemical Geology*, **179**, 167-186.

Katz B.G. (2004) Sources of nitrate contamination and age of water in large karstic springs of Florida. *Environmental Geology* , **46**, 689-706.

Katz B.G. & Griffin D.W. (2008) Using chemical and microbiological indicators to track the impacts from the land application of treated municipal wastewater and other sources on groundwater quality in a karstic springs basin. *Environmental Geology*, **55**, 801-821.

Kendall C. (1998) Tracing Nitrogen Sources and Cycling in Catchments. In: *Isotope Tracers in Catchment Hydrology*, 519-576, Elsevier, Lausanne.

Kotai J. (1972) *Instructions for preparation of modified nutrient solution Z8 for algae*. Norwegian Institute for Water Research Publication B-11/69, Norwegian Institute for Water Research, Blindern, Oslo.

Kreitler C.W. (1979) Nitrogen-Isotope Ratio Studies of Soils and Groundwater Nitrate from Alluvial Fan Aquifers in Texas. *Journal of Hydrology*, **42**, 147-170.

Leonardos N. & Geider R.J. (2004) Effects of nitrate: phosphate supply ratio and irradiance on the C : N : P stoichiometry of Chaetoceros muelleri. *European Journal of Phycology*, **39**, 173-180.

Lewis W.M. & Wurtsbaugh W.A. (2008) Control of lacustrine phytoplankton by nutrients: erosion of the phosphorus paradigm. *International Review of Hydrobiology*, **93**, 446-465.

Li Y.H. & Gregory S. (1974) Diffusion of ions in sea-water and in deep-sea sediments. *Geochimica et Cosmochimica Acta*, **38**, 703-714.

Lohman K., Jones J.R. & Baysinger D.C. (1991) Experimental evidence for nitrogen limitation in a northern Ozark stream. *Journal of the North American Benthological Society*, **10**, 14-23.

Luttenton M.R. & Lowe R.L. (2006) Response of a lentic periphyton community to nutrient enrichment at low N : P ratios. *Journal of Phycology*, **42**, 1007-1015.

Luttenton M.R. & Baisden C. (2006) The relationships among disturbance, substratum size and periphyton community structure. *Hydrobiologia*, **561**, 111-117.

Marfia A.M., Krishnamurthy R.V., Atekwana E.A. & Panton W.F. (2004) Isotopic and geochemical evolution of ground and surface waters in a karst dominated geological setting: A case study from Belize, Central America. *Applied Geochemistry*, **19**, 937-946.

Martin J.B. & Dean R.W. (2001) Exchange of water between conduits and matrix in the Floridan aquifer. *Chemical Geology*, **179**, 145-165.

McGlathery K.J., Krause-Jensen D., Rysgaard S. & Christensen P.B. (1997) Patterns of ammonium uptake within dense mats of the filamentous macroalga *Chaetomorpha linum*. *Aquatic Botany*, **59**, 99-115.

Meyers P.A. & Ishiwatari R. (1993) The early diagenesis of organic mater in lacustrine sediments. In: *Organic geochemistry*, pp. 185-209, Plenum Press, New York.

Morris D.P. & Lewis W.M. (1988) Phytoplankton nutrient limitation in Colorado mountain lakes. *Freshwater Biology*, **20**, 315-327.

Mulholland P.J., Steinman A.D., Palumbo A.V., Elwood J.W. & Kirschtel D.B. (1991) Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology*, **72**, 966-982.

Mynderse J.S., Moore R.E., Kashiwagi M. & Norton T.R. (1977) Anti-leukemia activity in Oscillatoriaceae: Isolation of Debromoaplysiatoxin from *Lyngbya*. *Science*, **196**, 538-540.

National Amospheric Deposition Program. NADP/NTN Monitoring Location FL03 Bradford Forest. http://nadp.sws.uiuc.edu. Last accessed: May 25, 2009.

Odum H.T. (1957) Primary production measurements in eleven florida springs and a marine turtle-grass community. *Limnology and Oceanography*, **2**, 85-97.

Odum H.T. (1957) Trophic structure and productivity of Silver Springs, Florida. *Ecological Monographs*, **27**, 55-112.

Osmond J.K., Buie B.F., Rydell H.S., Kaufman M.I. & Wallick E.I. (1971) Uranium and tritium as natural tracers in the Floridan aquifer. Florida Water Resources Research Center Publication No. 14, University of Florida, Gainesvile, Florida

Paerl H.W. (1982) Factors limiting productivity of fresh-water ecosystems. *Advances in Microbial Ecology*, **6**, 75-110.

Panno S.V., Hackley K.C., Hwang H.H. & Kelly W.R. (2001) Determination of the sources of nitrate contamination in karst springs using isotopic and chemical indicators. *Chemical Geology*, **179**, 113-128.

Pauwels H., Foucher J.C. & Kloppmann W. (2000) Denitrification and mixing in a schist aquifer: influence on water chemistry and isotopes. *Chemical Geology*, **168**, 307-324.

Pellerin B.A., Downing B.D., Kendall C., Dahlgren R.A., Kraus T.E.C., Saraceno J., Spencer R.G.M. & Bergamaschi B.A. (2009) Assessing the sources and magnitude of diurnal nitrate variability in the San Joaquin River (California) with an in situ optical nitrate sensor and dual nitrate isotopes. *Freshwater Biology*, **54**, 376-387.

Phelps G.G. Chemistry of groundwater in the Silver Springs Basin, Florida, with an emphasis on nitrate. U.S. Geological Survey Scientific Investigation Report 2004-5144,U.S.Geological Survey.

Phlips E.J., Ihnat J. & Conroy M. (1992) Nitrogen fixation by the benthic fresh-water cyanobacterium *Lyngbya wollei*. *Hydrobiologia*, **234**, 59-64.

Pinowska A., Stevenson R.J., Albertin A.R., Sickman J.O. & Anderson M.W. Differential effect of nitrate, ammonia and phosphate on the growth of mat forming cyanobacterium *Lyngbya wollei* (Oscillatoriales, Cyanophyta) from Florida Springs. *Journal of Phycology*, accepted.

Pinowska A., Stevenson R.J., Sickman J.O., Albertin A. & Anderson M. (2007) Integrated interpretation of Survey for determining nutrient thresholds for macroalgae in Florida springs, Florida Department of Environmental Protection Tallahassee, Florida.

Quinlan E.L., Phlips E.J., Donnelly K.A., Jett C.H., Sleszynski P. & Keller S. (2008) Primary producers and nutrient loading in Silver Springs, FL, USA. *Aquatic Botany*, **88**, 247-255.

Redfield A.C. (1958) The Biological Control of Chemical Factors in the Environment. *American Scientist*, **46**, 205-221.

Reynolds C.S. (1993) *The Ecology of Freshwater Phytoplankton*, Cambridge University Press, Cambridge.

Rhue R.D., Harris W.G. & Nair V.D. (2006) A retardation-based model for phosphorus transport in sandy soil. *Soil Science*, **171**, 293-304.

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

Ritz C. & Streibig J.C. (2005) Bioassay analysis using R. *Journal of Statistical Software*, **12**, 1-22.

Rounick J.S. & Winterbourn M.J. (1986) Stable carbon isotopes and carbon flow in ecosystems. *Bioscience*, **36**, 171-177.

Schindler D.W., Hecky R.E., Findlay D.L., Stainton M.P., Parker B.R., Paterson M.J, Beaty K.G., Lyng M. & Kasian S.E.M. (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11254-11258.

Scott T.M., Means G.H., Meegan R.P., Means R.C., Upchurch S.B., Copeland R.E., Jones J., Roberts T., Willet A.(2004) *Springs of Florida Bulletin No. 66*. Florida Geological Survey, Tallahassee, Florida.

Sickman J.O., Anderson M.W., Albertin A.R., Pinowska A., Stevenson R.J. Carbon, nitrogen and phosphorus content of algal mats and groundwater flows in two first magnitude springs in Florida. *Journal of Aquatic Plant Management*, accepted.

Sigman D.M., Casciotti K.L., Andreani M., Barford C., Galanter M.& Bohlke J.K. (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Analytical Chemistry*, **73**, 4145-4153.

Silva S.R., Kendall C., Wilkison D.H., Ziegler A.C., Chang C.C.Y. & Avanzino R.J. (2000) A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. *Journal of Hydrology*, **228**, 22-36.

Speziale B.J., Turner E.G. & Dyck L.A. (1991) Physiological characteristics of vertically-stratified *Lyngbya wollei* mats. *Lake Reservoir Management*, **7**, 107-114.

Speziale B.J., Turner E.G. & Dyck L.A. (1991) Physiological characteristics of vertically-stratified *Lyngbya wollei* mats. *Lake and Reservoir Management*, **1**, 107-114.

Stelzer R.S.& Lamberti G.A. (2001) Effects of N : P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnology and Oceanography*, **46**, 356-367.

Sterner R.W., Elser J.J., Fee E.J., Guildford S.J. & Chrzanowski T.H. (1997) The light : nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *American Naturalist*, **150**, 663-684.

Stevenson R.J. & Glover R. (1993) Effects of Algal Density and Current on Ion-Transport Through Periphyton Communities. *Limnology and Oceanography*, **38**, 1276-1281.

Stevenson R.J., Pinowska A. & Wang Y. (2004) Ecological Condition of Algae and Nutrients in Florida Springs. Florida Department of Environmental Protection, Tallahasse, Florida.

Stevenson R.J., Pinowska A., Albertin A. & Sickman J.O. (2007) Ecological condition of algae and nutrients in Florida springs: The Sythesis Report. Florida Department of Environmental Protection, Tallahassee, Florida.

Stevenson R.J., Hill B.H., Herlihy A.T., Yuan L.L. & Norton S.B. (2008) Algae-P relationships, thresholds, and frequency distributions guide nutrient criterion development. *Journal of the North American Benthological Society*, **27**, 783-799.

Strong W.A. (2004) Temporal water chemistry trends within individual springs and within a population of Florida Springs. MSc. Thesis, University of Florida.

Sündback K., Miles A., Hulth S., Pihl L., Engstrom P., Selander E. & Svenson A. (2003) Importance of benthic nutrient regeneration during initiation of macroalgal blooms in shallow bays. *Marine Ecology-Progress Series*, **246**, 115-126.

Toda H., Uemura Y., Okino T., Kawanishi T. & Kawashima H. (2002) Use of nitrogen stable isotope ratio of periphyton for monitoring nitrogen sources in a river system. *Water Science and Technology*, **46**, 431-435.

Townsend S.A., Schult J.H., Douglas M.M. & Skinner S. (2008) Does the Redfield ratio infer nutrient limitation in the macroalga Spirogyra fluviatilis? *Freshwater Biology*, **53**, 509-520.

Trimmer M., Nedwell D.B., Sivyer D.B. & Malcolm S.J. (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.

Tyler A.C., McGlathery K.J.& Anderson I.C. (2003) Benthic algae control sediment-water column fluxes of organic and inorganic nitrogen compounds in a temperate lagoon. *Limnology and Oceanography*, **48**, 2125-2137.

U.S.Environmental Protection Agency (2006) Great River Ecosystems Field Operations Manual, EPA/620/R-06/002. Environmental Monitoring and Assessment Program; Great River Ecosystems, U.S.Environmantal Protection Agency Washington D.C.

Ullman W.J. & Aller R.C. (1982) Diffusion coefficients in nearshore marine-sediments. *Limnology and Oceanography*, **27**, 552-556.

Umezawa Y., Miyajima T., Tanaka Y. & Koike I. (2007) Variation in internal delta $\delta^{15}N$ and $\delta^{13}C$ distributions and their bulk values in the brown macroalga *Padina australis* growing in subtropical oligotrophic waters. *Journal of Phycology*, **43**, 437-448.

Van Breemen N., Boyer E.W., Goodale C.L., Jaworski N.A., Paustian K., Seitzinger S.P., Lajtha K., Mayer B., Van Dam D., Howarth R.W., Nadelhoffer K.J., Eve M. & Billen G. (2002) Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern U.S.A. *Biogeochemistry*, **57**, 267-293.

Vreca P. & Muri G. (2006) Changes in accumulation of organic matter and stable carbon and nitrogen isotopes in sediments of two Slovenian mountain lakes (Lake Ledvica and Lake Planina), induced by eutrophication changes. *Limnology and Oceanography*, **51**, 781-790.

Waldron S., Scott E.M.& Soulsby C. (2007) Stable isotope analysis reveals lower-order river dissolved inorganic carbon pools are highly dynamic. *Environmental Science & Technology*, **41**, 6156-6162.

Wankel S.D., Kendall C. & Paytan A. (2009) Using nitrate dual isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) as a tool for exploring sources and cycling of nitrate in an estuarine system: Elkhorn Slough, California. *Journal of Geophysical Research-Biogeosciences*, **114**.

Whitford L.A. (1956) The communities of algae in the springs and spring streams of Florida. *Ecology*, **37**, 433-442.

Wilson W.L. & Skiles W.C. (1988) Aquifer characterization by quantitative dye tracing at Ginnie Spring, Northern Florida. *Proceedings of the second conference on environmental problems in karst terrains and their solutions*, pp. 121-141, The Association of Groundwater Scientists and Engineers Dublin, OH.

Yin Q.Q., Carmichael W.W. & Evans W.R. (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.

BIOGRAPHICAL SKETCH

Andrea Albertin was born and lived in Costa Rica until she was 7 years old. She and her family, her parents and two sisters, then moved to the United States, Panama, Pakistan and back to the United States again in 1991 after she graduated from high school. She studied biology with an emphasis in botany at the College of William and Mary in Virginia and then moved back to Costa Rica in 1996 to pursue practical training in tropical plant taxonomy. She returned to the United States in 1999 to study agroforestry and received a master's degree from the School of Forest Conservation and Resources at the University of Florida. She subsequently worked in Costa Rica for three years for the Organization for Tropical Studies. She returned to the University of Florida in 2005 and received her Ph.D. from the Soil and Water Science Department in the summer of 2009. She and her husband, Francisco, have a beautiful baby boy, Matthias.