REGULATION OF MACROALGAL GROWTH AND ACCRUAL BY NUTRIENTS IN FLORIDA SPRINGS

DEP CONTRACT NUMBER S0183
FINAL REPORT

Submitted to the Florida Department of Environmental Protection

December 5, 2006

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Task 1 – Gather and thoroughly analyze existing data

Stevenson, Pinowska, and others gathered considerable data in past FDEP-funded surveys of Florida springs and small scale experiments. During these surveys, macroalgal composition and abundance, water chemistry, and diatom species composition were determined at 60 spring sites in 28 different springs. Filamentous macroalgae were observed in all springs, except Cypress Springs, and it covered an average of approximately half of the bottom of spring sites. Twenty-four different filamentous macroalgal taxa were observed. The most common were the cyanobacterium *Lyngbya wollei* and a xanthophyte *Vaucheria*. No relationship between nutrient concentrations and *Lyngbya* % cover in springs was observed, but a relationship between *Vaucheria* % cover and total nitrogen concentration in springs was observed.

Diatom indicators of nutrient concentrations can be more precise and accurate indicators of bioavailable nutrient concentrations than the one-time sampling of nutrient concentrations in streams and wetlands (Stevenson 2006). Diatom indicators of nutrient concentrations were developed using several methods, but responses of diatoms were complicated by many other factors, such as the great variability in conductivity among springs. Thus, little variation in *Lyngbya* or *Vaucheria* % cover in springs could be explained by diatom indicators of nutrient concentrations.

Powerpoint presentations of the analyses of past results are included in the files submitted that accompany this report. These presentations were given at national and international scientific meetings. The basic results of past work have been presented in past reports to FDEP.

The results of the first spring surveys of macroalgae and nutrients lead to a more broad research approach to evaluate relationships between macroalgae and nutrients in Florida springs. First, additional information has been gathered about spring conditions that can be used to complement the information gathered in the first surveys, including existing and new. Existing data such as flow conditions, weather, and land use were gathered. New data was collected by revisiting sites from the earlier survey (Task 7 of this study) and with new observations along nutrient gradients in springs to control for variation in extraneous factors among springs (Tasks 2.2 and 7 of this study). In addition, season variation, effects of disturbance, and internal nutrient cycling within thick macroalgal mats were evaluated as causes of complexity in macroalgal-nutrient relationships (Tasks 2.1, 2.3, and 3 of this study). Controlled experiments were also conducted at 3 scales to characterize macroalgal-nutrient relationships: in test tube microcosms and donut shaped circular streams in the laboratory and in larger recirculating streams located in a greenhouse (Tasks 4 and 5 of this study). Results of this work are introduced below and will be analyzed more thoroughly through a synthesis report that will be delivered in May 2007 through a separate grant with FDEP.

Task 2 – Field Surveys and In-Channel Experiments along Nutrient Gradients in Springs

Task 2.1 Seasonal Variability and Spring Conditions

Ichetucknee Blue Hole spring (a site with *Lyngbya wollei* mats) and Manatee spring (a site with *Vaucheria* mats) were selected for monitoring of seasonal variability of macroalgae and spring

conditions. Manatee spring was affected by severe flooding from the Suwannee River that had a drastic impact on the filamentous algal mats. Ichetucknee Blue Hole was not affected by flooding and filamentous algal mats were more persistent.

Methods

In May 2005 Aga Pinowska trained a crew from UF (Andrea Albertin and Martin Andersen) to conduct the RPHA (Rapid Periphyton and Habitat Assessment) (Stevenson and Bahls, 1999) and to collect algae samples. In addition, initial sampling was conducted. The following samples were collected at each site: macroalgae samples for AFDM, macroalgae samples for %N, %P, %C, epiphyte samples for AFDM, macroalgae samples for ID confirmations, water samples. Water DO, pH, temperature and conductivity were also measured. Collection of samples was concluded in April 2006. Field work and initial processing and preserving of sample, water sample analyses and macroalgae sample analyses for %N, %P, %C were conducted by UF. Data entry from RPHAs into Excel spreadsheets, macroalgae IDs, processing of preserved samples were conducted by MSU. All data from RPHAs were entered into Excel spreadsheets and macroalgae IDs were confirmed or corrected microscopically in the lab. Macroalgae and epiphyte samples for AFDM were processed. Plant surface areas from which epiphyte samples were collected were measured using National Institute of Health Image J software.

TKN flux from the boil was calculated using water samples analyzed monthly for TKN and flow data obtained from the USGS Surface Water Daily Data for Florida website (http://waterdata.usgs.gov/fl/nwis/sw). The flow data (in cubic feet per second) was converted to L/s and then calculated with the mg/L of TKN from the boil and converted into kg/day, representing the spring boil flux.

Based on the algal data collected during monthly field sampling, algal surface area for Manatee and Ichetucknee Blue Hole Springs was interpolated using ordinary kriging techniques in the Geostatistical Analyst extension within ArcView 9.1. Kriging the algal thickness data created a surface representing the algal mat thickness for both sites for every month. This thickness map was then reclassified so that the algal cover area could be calculated utilizing the Zonal Statistics function of Spatial Analyst within ArcView 9.1. The algal mat area was then used to calculate the amount of N concentrated in the algal biomass of the mat.

The algae samples collected each month were calculated for volume and analyzed for %N. The algal area in cm^2 was multiplied by the average algal thickness (cm) for each month. The algal dry weight was multiplied by the lab determined sample volume to give results in g/cm^3. The algal area volume in cm^3 was multiplied by the dry weight volume thus leaving the results in grams. This is multiplied by the %N and converted to kg to provide the algal biomass of N for a given month in kg.

Results

In Figure 2.1.1 TKN flux from the boil at Ichetucknee Blue Hole is compared to the total amount of nitrogen contained in the algal mats each month. The general trend is that the daily flux of TKN into the spring from the groundwater source is three times greater than the amount that could be released from the algal mat itself.

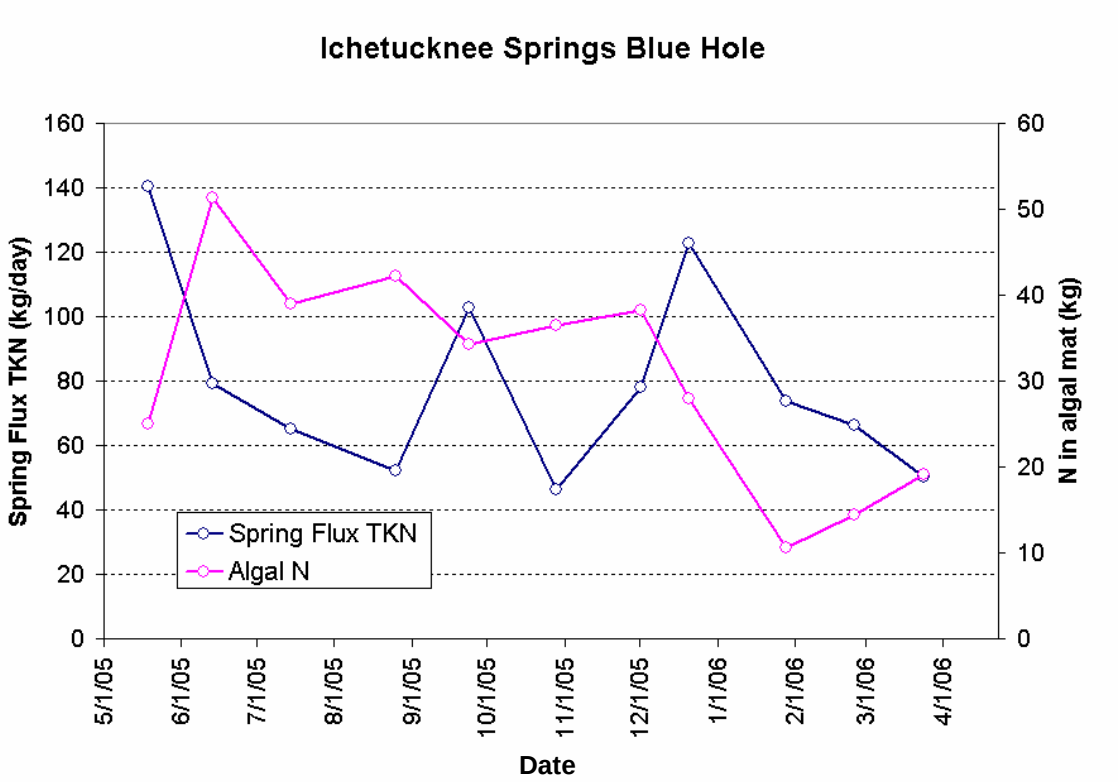


Figure 2.1.1. TKN flux in spring water and algal mat N as a function during 1 year period.

The algal mat surface mapped in ArcView 9.1 illustrates algal mat thickness along a reach of spring. The dark blue area represents the greatest thickness of the mat and the area of the mat with the lighter green coloration represents thinner portions of the mat. Figure 2.1.2 shows algal mat thickness at Manatee Springs in August, the summer month with the greatest average mat thickness. Figure 2.1.3 shows algal mat thickness and cover in January, the winter month of greatest average mat thickness. August mat thickness was greater than January mat thickness, in which the mat was becoming thinner overall (Figure 2.1.2 and 2.1.3).

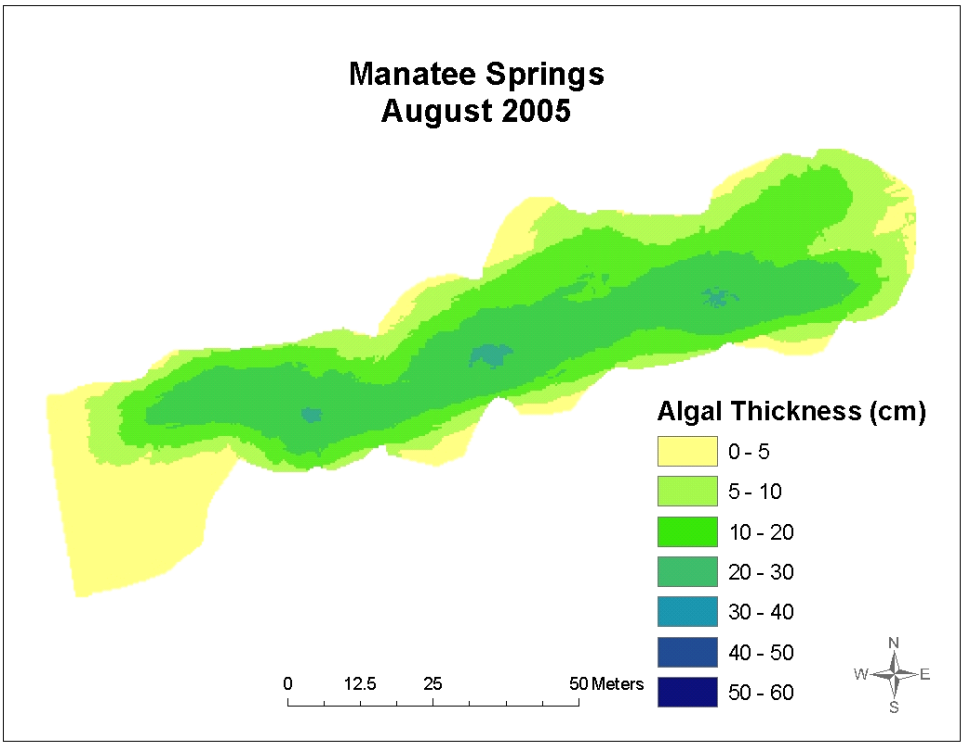


Figure 2.1.2. Map of algal mat thickness in Manatee Springs During August 2005

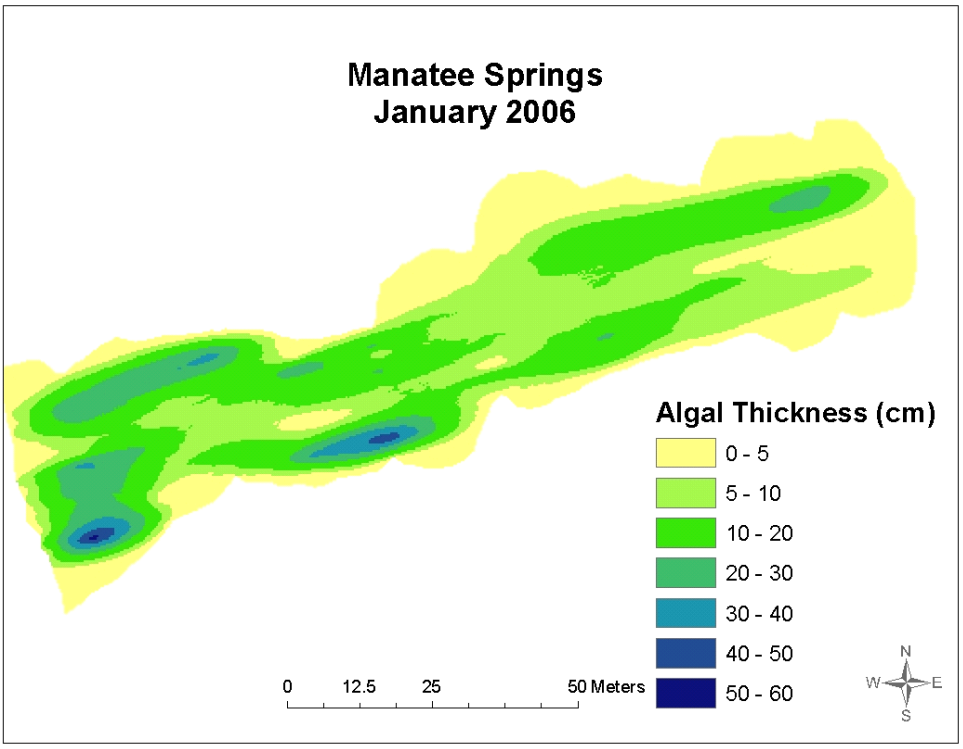
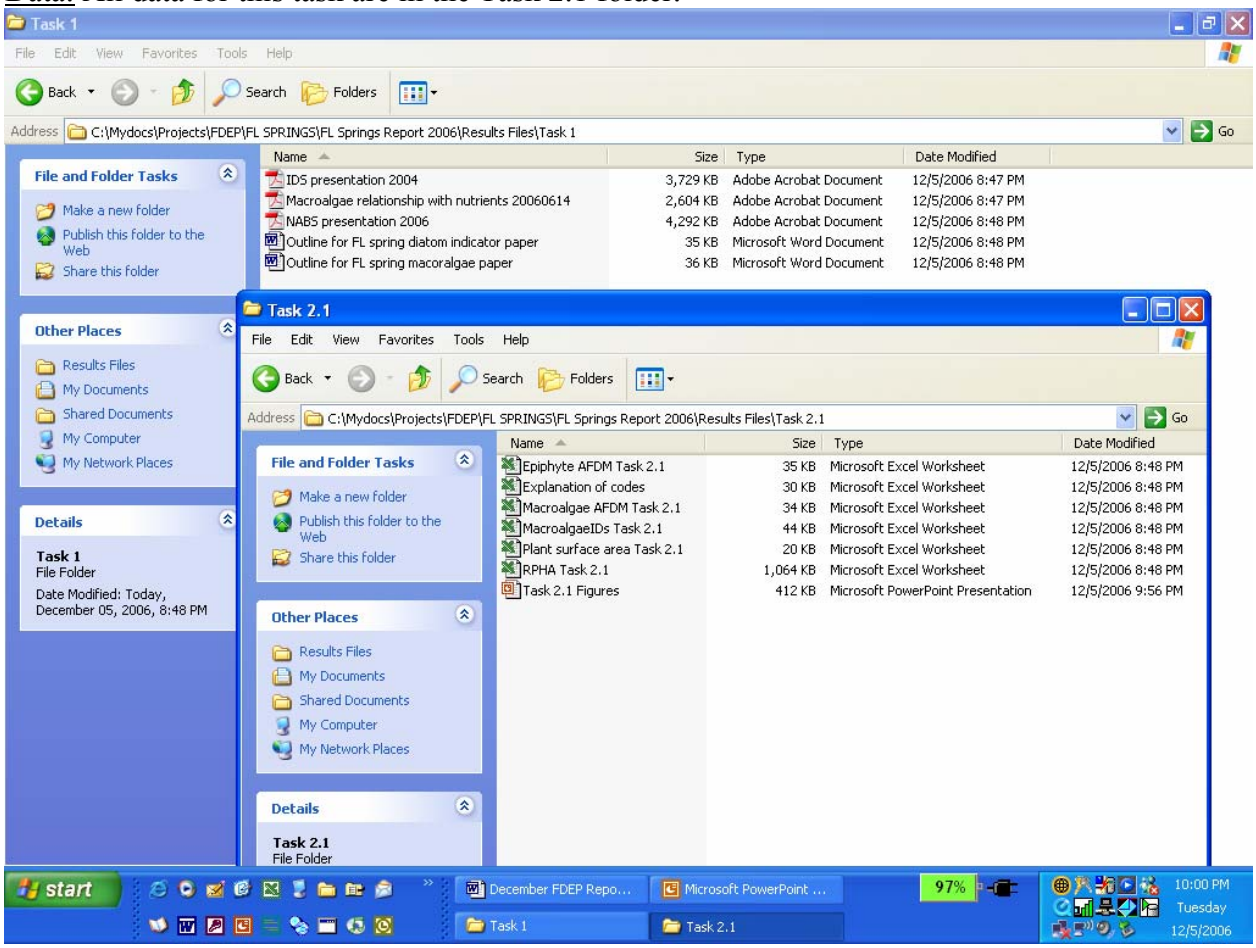


Figure 2.1.3. Map of algal mat thickness in Manatee Springs During January 2006.

Data: All data for this task are in the Task 2.1 folder.



Task 2.2 Nutrient Depletion and Spring Conductivity & Trophic Status (Gradient study)

Nutrient and isotopic analysis of water, algae and sediments along spring gradients may provide information on: 1) thresholds in algal responses to nutrients without complexity of between-spring variation, 2) nutrient uptake rates of algae, 3) nutrient status (N vs P limitation) of algae and 4) the presence of local sources of nutrients. Gradient studies were conducted in four springs: Weeki Wachee, Rainbow, Wakulla and Silver River. Sampling was conducted at six or more sites in each spring in order to see if there were differences in water nutrient concentrations or algal biomass/nutrient/isotopic composition from the boil down the spring run.

Methods

RPHA surveys were conducted at 27 sites in the four springs. At each site, macroalgae samples were collected to determine AFDM, chlorophyll a, %N, %P, %C, and for ID confirmations. Sediment samples were collected for %N, %P, %C, available N and P. Epiphyte samples were collected for chlorophyll a and AFDM. Water samples were collected for nutrient analyses. Algae and sediment samples were collected for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis. *Lyngbya* and

Vaucheria were sampled when these species were present, as well as any other dominant species of macroalgae.

Water DO, pH, temperature and conductivity were also measured in the field. Field work and initial sample processing was conducted by MSU and UF. Water samples for nutrient analyses were sent to DEP. Data entry from RPHA, macroalgae IDs, and processing of preserved samples were conducted by MSU. All data from RPHA were entered into Excel spreadsheets and field identifications of macroalgae were evaluated microscopically. Macroalgae and epiphyte samples for AFDM and chlorophyll a were processed. Plant surface areas from which epiphyte samples were measured using National Institute of Health Image J software.

Sediments were stored frozen until they were dried in an oven at 60ºC for 5 days, ground in a ball mill, and passed through a sieve. Algal samples were picked clean of invertebrates and freeze dried. Carbon and nitrogen content were measured by high temperature combustion on a Thermo-electron model 1112 elemental analyzer. Percent phosphorus was measured colorimetrically as soluble-reactive phosphorous following acid digestion. Carbon and nitrogen stable isotopes were measured on a Thermo-Finnigan isotope ratio mass spectrometer. Prior to elemental and isotopic analysis, sediment samples were acid fumigated with HCl to remove inorganic carbon (Harris et al. 2001).

Results

Nitrate concentration at the boil of Wakulla Spring in January 2006 was 0.75 mg/L and dropped to 0.58 mg/L at the last sampling site 6 miles down from the spring head. Average mat thickness was the highest at the spring head and was over 8 cm (Figure 2.1.1). Percent algal mat cover was the highest within the first mile of the spring run and was over 70%. The slightly lower percentage of algal mat cover at the boil area is due to the fact that the beach area is regularly cleaned of algae by people and the very deep part of the boil is probably too deep for the colonization by mat forming algae. Results for all four springs will be presented in the synthesis report.

Isotopic analyses of algae and sediment revealed a substantial range of values in the stable isotopic composition of algae and sediment (Figure 2.2.2). For algae, $\delta^{13}C$ ranged from -10 to -45 permil and $\delta^{15}N$ ranged from +8 to -8 permil. This wide range of isotopic composition was surprising. Very low $\delta^{13}C$-algae values may be indicative of strong respiration inputs of CO_2 which would produce dissolved inorganic carbon with $\delta^{13}C$ of -20 permil which, when fractionated during carbon uptake by algae, can produce algal tissues of -45 permil. Sediments typically had higher $\delta^{13}C$, -30 to -20 permil and higher $\delta^{15}N$, +1 to +14 permil than algae. For both algae and sediments, $\delta^{15}N$ values above +5 permil may be indicative of the anthropogenic inputs of N from fertilizers and animal wastes given that the $\delta^{15}N$ of nitrate in many Florida springs measured during our study was greater than +20 permil (Figure 2.2.3). Elemental data for spring algae and sediments are contained in Excel spreadsheets submitted to DEP. These data represent samples from many springs, and will be used to evaluate different sources of nutrients to springs, even spatial variation in sources within spring runs.

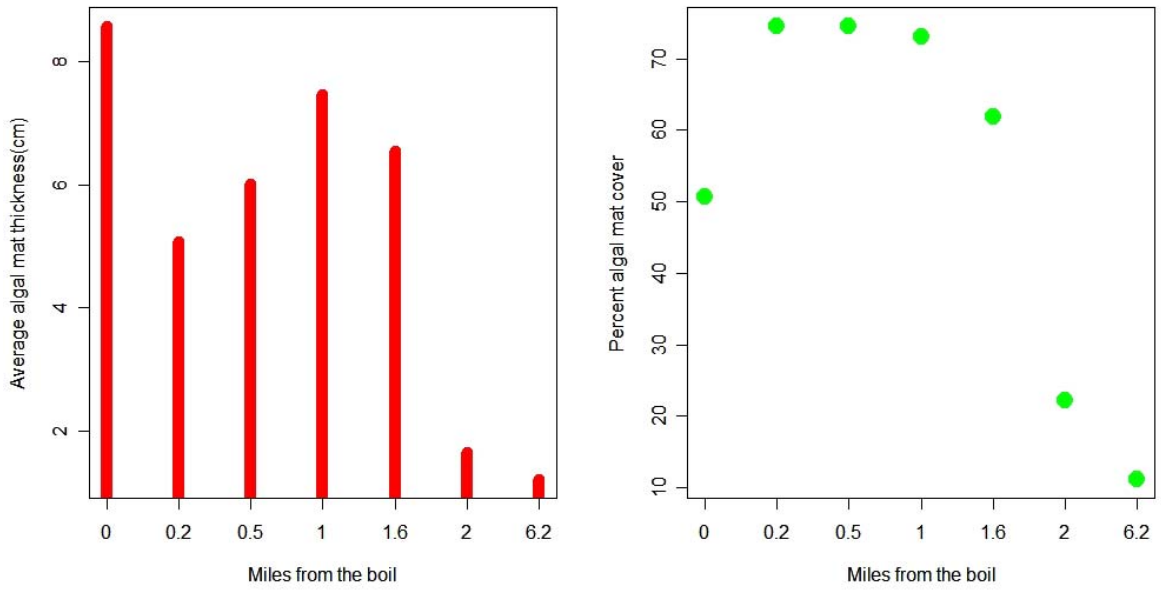


Figure 2.2.1. Average algal mat thickness and percentage algal mat cover at 7 sampling sites along Wakulla River in January 2006.

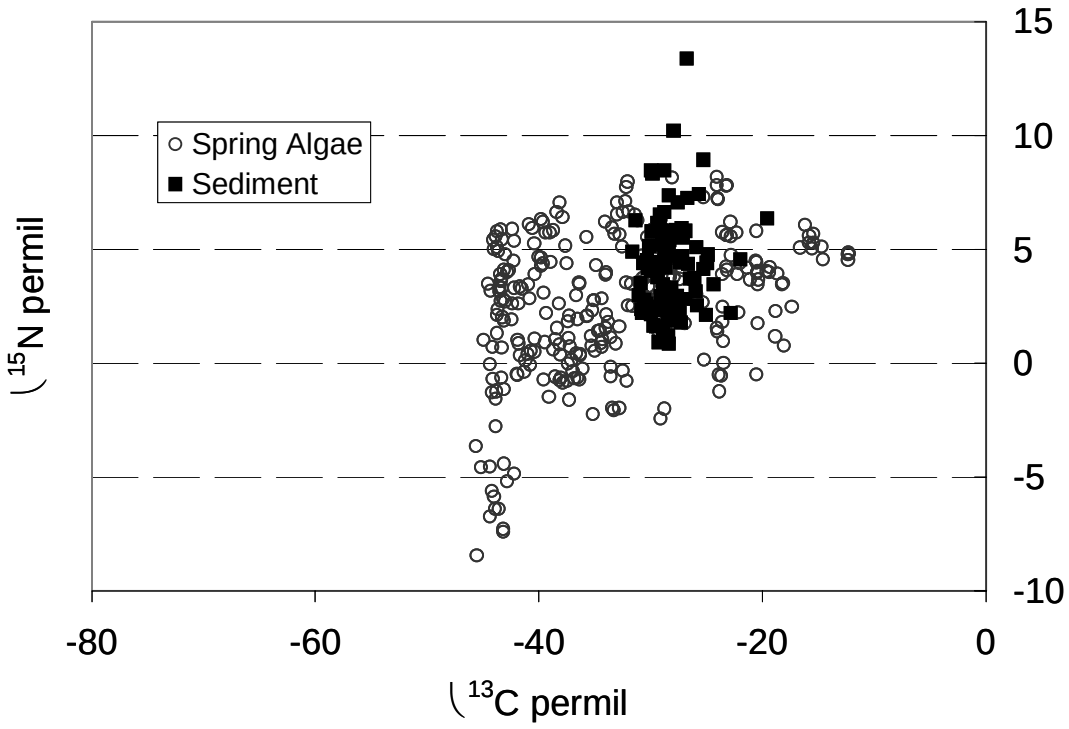


Figure 2.2.2. Stable isotope composition of algae and sediments measured in multiple Florida springs during 2006.

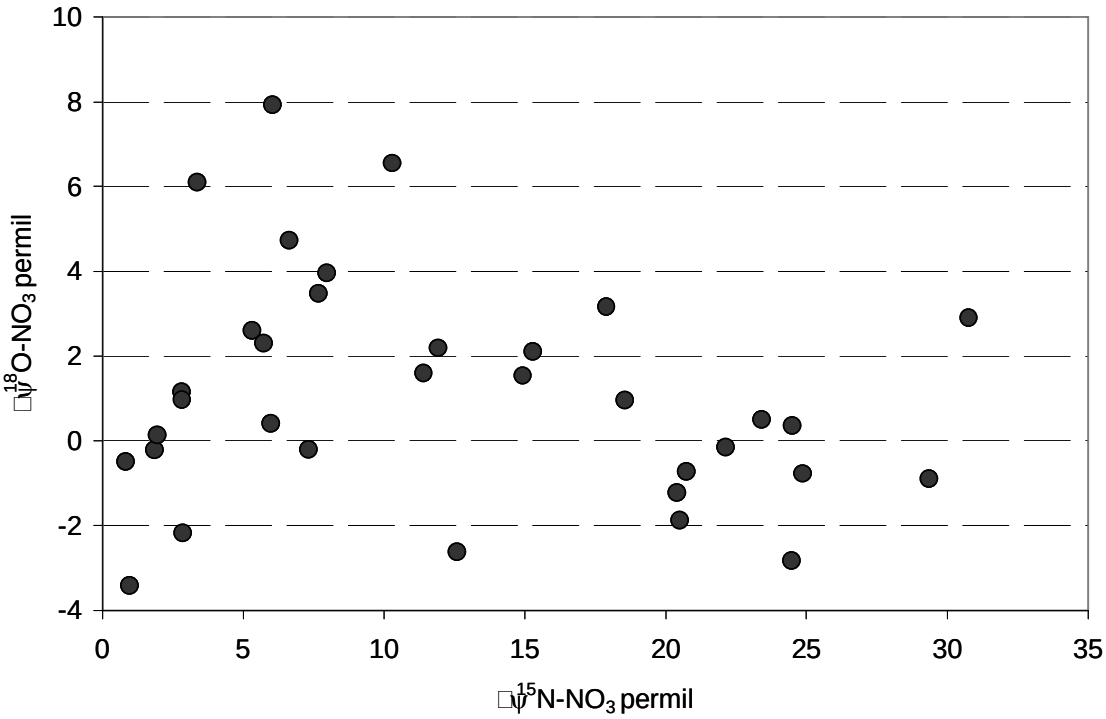
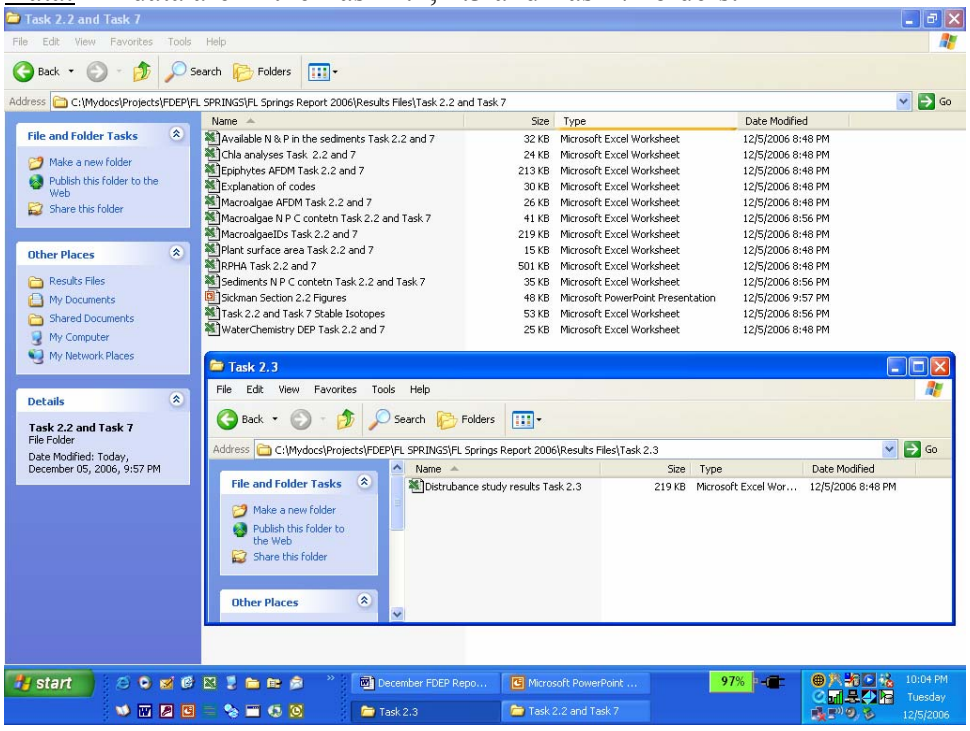


Figure 2.2.3. Dual isotope composition of nitrate measured in multiple Florida springs during 2006.

Data: All data are in the Task 2.2, 2.3 and Task 7 folders.



Task 2.3 Disturbance and Time (Disturbance study)

Disturbance due to visitation by large grazers (manatees and fish), people walking on the spring bottom and flooding can play an important role in the dynamics of algal mats. This study was conducted to monitor the regrowth of algal mats after disturbance.

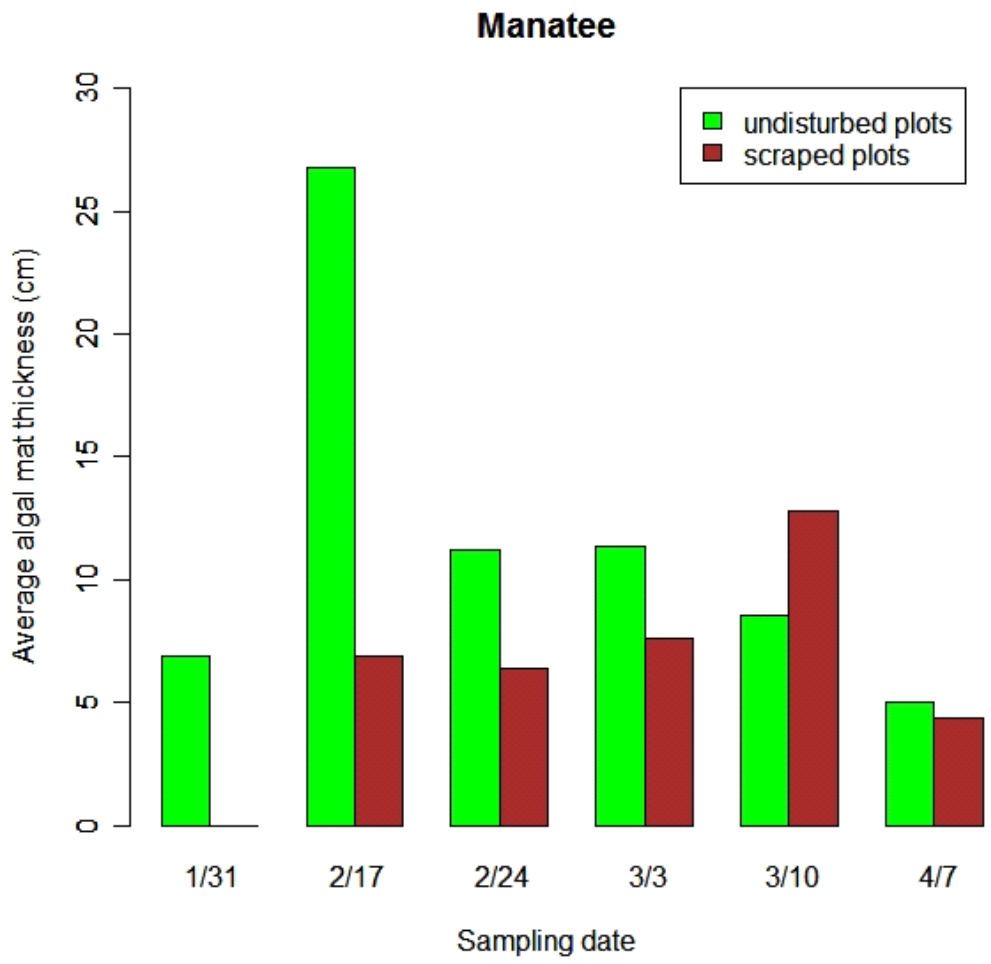


Figure. 2.3.1. Average thickness of *Vaucheria* mats at Manatee Spring on the plots that were scraped on January 31 and on control plots.

Methods

Blue Hole spring and Manatee spring were selected for the disturbance study. The disturbance sites were set up in areas with pre-existing algal mats and at the locations that have restricted access. At Ichetucknee's Blue Hole the algal mats were comprised of *Lyngbya*. At Manatee Springs the dominant algal species was *Vaucheria*. The study was conducted from February to

May 2006. Three sites were selected within each spring. Each site consisted of 2 experimental square plots (50cm x 50cm) delineated by PVC poles driven into the sediments. At each site initial (pre-disturbed) mat thickness was measured. After algal mat thickness was measured, one plot within each site was disturbed. All visible algae were removed down to the sediments using a rake. The recolonization of disturbed areas and algal growth on the undisturbed plots was initially monitored every 2 weeks, initially, and less frequently later depending on observed recolonization rates. On each observation date, mat thickness was measured on each plot, plus water depth, water DO, pH, conductivity and temperature.

Results

Algal mats at Manatee springs (formed by *Vaucheria*) recolonized scraped plots within 6 weeks (Figure 2.3.1). This was probably due to high growth rates and high colonization rate by *Vaucheria* mat fragments drifting along the spring run. The boil area of Manatee spring is highly affected by swimmers and SCUBA divers and mat fragments drifting along the spring run were often observed. The detaching and drifting of *Vaucheria* mat fragments could also be a natural phenomenon and a part of this species ecology. Algal mats at Ichetucknee Blue Hole formed by *Lyngbya* did not fully recover within the 5 months of this study.

Task 3 – Assessment of Nutrient Cycling in Mats

Task 3 Assessment of Nutrient Cycling and Nutrient Profiles in Algal Mats and Adjacent Sediments

Nutrient re-supply from senescing algal biomass and spring sediments could help explain how large algal mats are sustained in springs with relatively low nutrient concentrations. To study these processes, multisamplers, devices that allow sampling of sediment and algal mat interstitial waters, were deployed at Weeki Wachee, Manatee and Silver Glen Springs.

Methods

The multisampler design was adapted from Martin et al. (2003) and consists of a 1.5 m tall PVC pipe with small holes drilled every 10 cm (Figure 3.1). A plastic tube (1/4 inch in diameter) is glued to the inner surface of each hole (inside the multisampler) and run through its entire length. A nylon screen is glued to the outside of each hole to help filter particulates and keep the tube from being blocked.

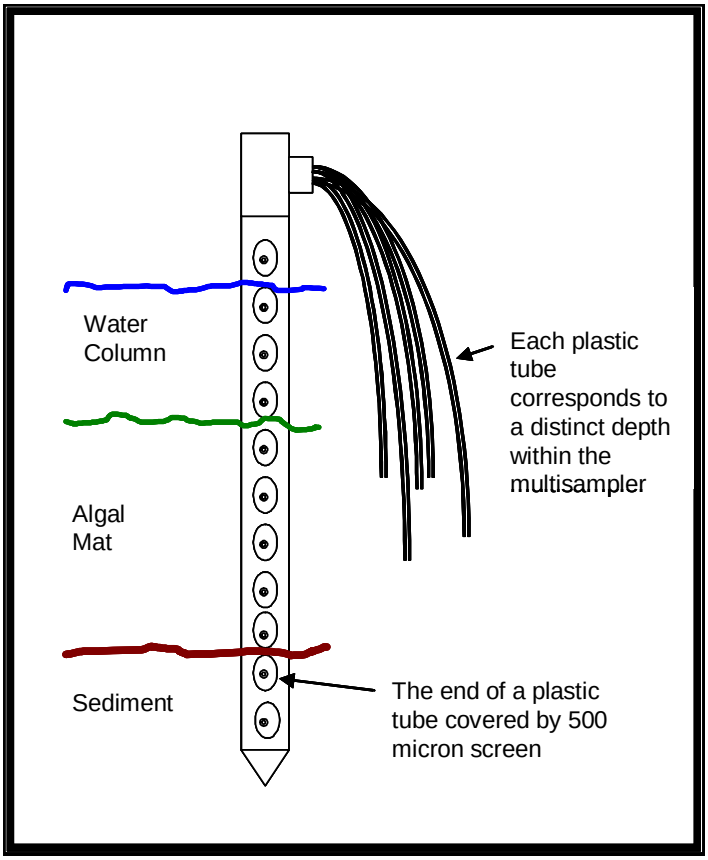


Figure 3.1. Schematic diagram of a multisampler.

The multisamplers are inserted vertically through the thickest part of the algal mat and pushed to a depth of approximately 20 cm into the sediment. They are left standing for one week to allow for equilibration. Water samples are then drawn from each tube, which corresponds to a discrete depth in the multisampler, using a syringe while sitting in a boat or canoe. This technique allows for samples to be drawn from sediments upwards through algal mats and into spring waters above the mats, all in a vertical column.

Mutisamplers were deployed twice at Weeki Wachee and Manatee Springs (April and August 2006) and once at Silver Glen Springs (September 2006). Weeki Wachee and Manatee Springs were chosen because Weeki Wachee had the thickest *Lyngbya* mats and Manatee Springs the thickest *Vaucheria* mats observed during surveys conducted in January 2006. Silver Glen was added as a comparison site to Weeki Wachee because it also has thick *Lyngbya* mats, but the water chemistry varies between the two sites. Duplicate multisamplers are deployed at each site, near the boil area, where the algal mats are thickest.

Parameters being measured from samples collected at each depth in the multisampler are: TKN, TP, SRP, NO_3, NH_4, DOC and trace metals. Additionally, algae and sediment samples are taken from the algae/sediment interface, in the outer (surface) portion of the mat and in the deeper portions. They will be analyzed for % C, N and P and $\delta^{13}C$ and $\delta^{15}N$ isotope analysis.

In order to estimate diffusion rates of nutrients into and out of thick algal mats and sediment, a dye tracer study was conducted in August 2006 at Weeki Wachee, Manatee and Silver Glen Springs using multisamplers. Two liters of a lightly colored Rhodamine FWT solution with an additional conservative tracer (NaCl) were injected into the sediment, algal mat and water column directly adjacent to one multisampler at each site. A syringe was then used to draw a sample of water from 5 ports (tubes) of the multisampler at the following times after injection was completed: 5 minutes, 15 minutes, 30 minutes and 45 minutes. The samples were then analyzed using a fluorometer (to test for the Rhodamine tracer) and a conductivity meter (to test for the NaCl tacer).

Analysis

Mat nutrient profiles (from water samples collected using the multisampler) and algae tissue samples will be used to assess the nutrient status of the interior and exterior portions if the mat as well as at the mat/sediment interface. Non-linear regression will be used to analyze data obtained with the multisamplers. Results obtained from the $\delta^{13}C$ and $\delta^{15}N$ isotope analysis will be used to determine sources of carbon and nitrogen in the algae and sediments.

Results

Preliminary results show strong vertical nutrient gradients from the sediment/algal interface upwards through algal mats and into spring waters above the mats at Weeki Wachee, Manatee and Silver Glen Springs. At both Manatee Springs (a *Vaucheria* sp. site) and Silver Glen Springs (a *Lyngbya wollei* site) TKN values were greatest at the sediment /algal mat interface, declined upwards through the algal mat and were lowest in the water column (Figures 3.2 and 3.3). The nutrient gradient was different at Weeki Wachee (a *Lyngbya wollei* site). TKN values were

highest at the center of the algal mat, but still lowest in the water column above the mat (Figure 3.4).

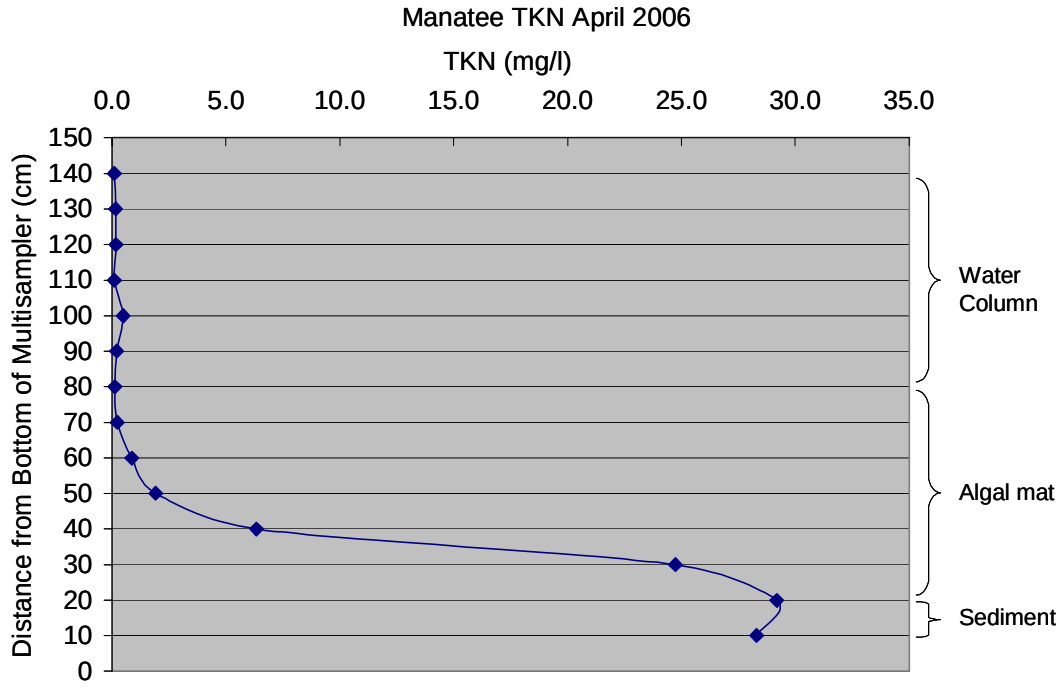


Figure 3.2. Vertical Total Kjeldahl Nitrogen profile of a *Vaucheria* sp. mat in Manatee Springs, September 2006.

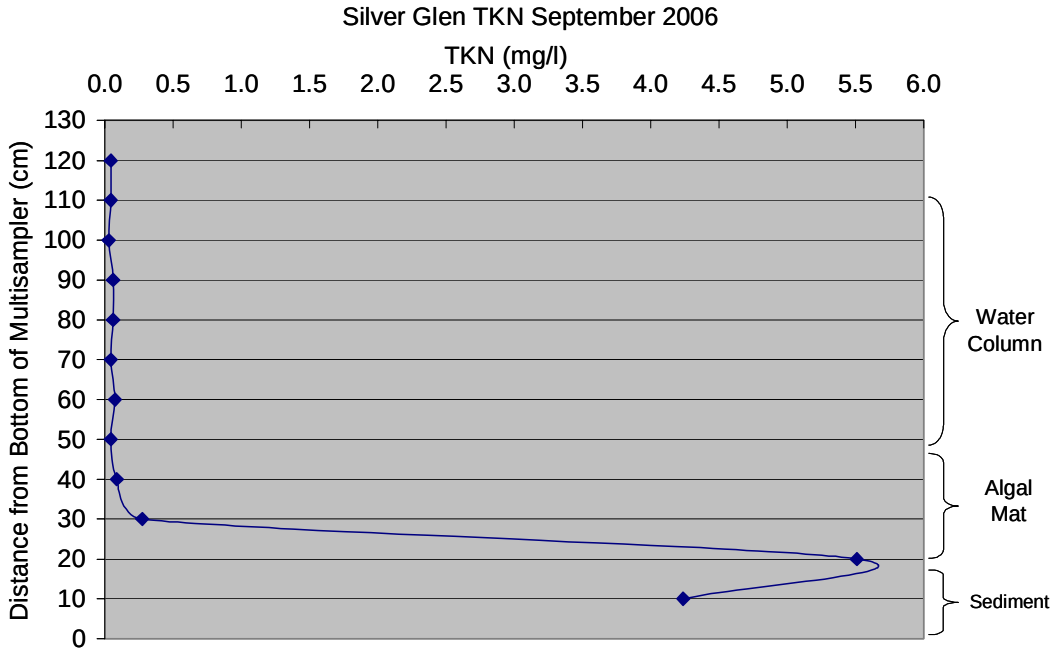


Figure 3.3. Vertical Total Kjeldahl Nitrogen profile of a *Lyngbya wollei* mat in Silver Glen Springs, September 2006.

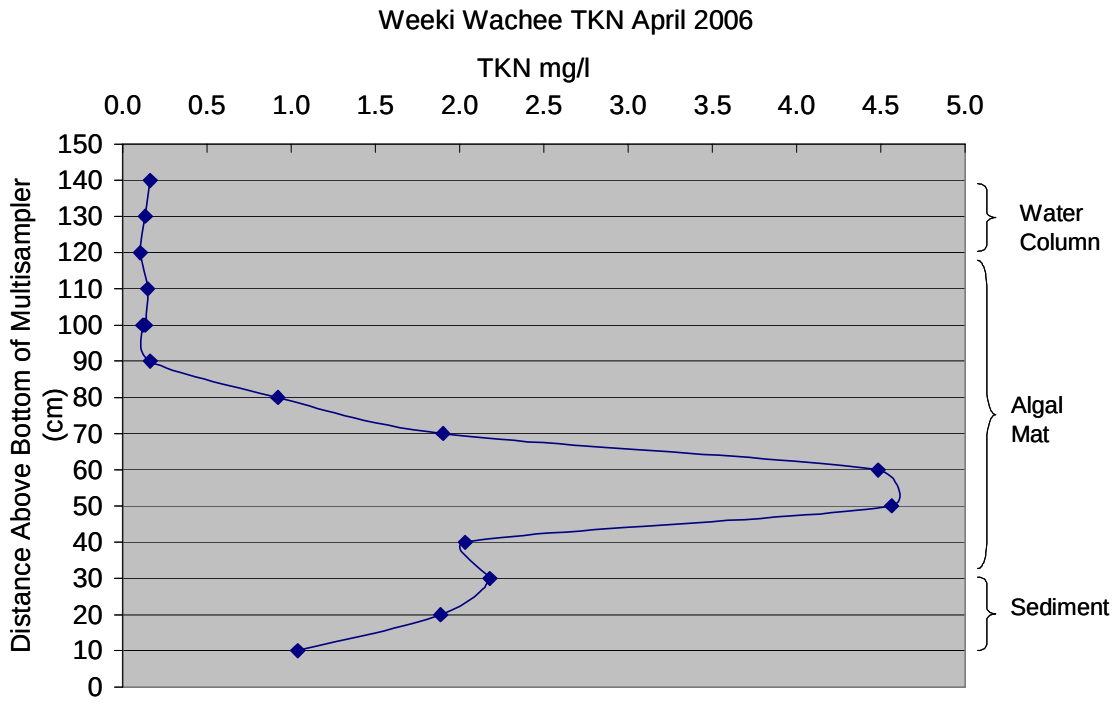
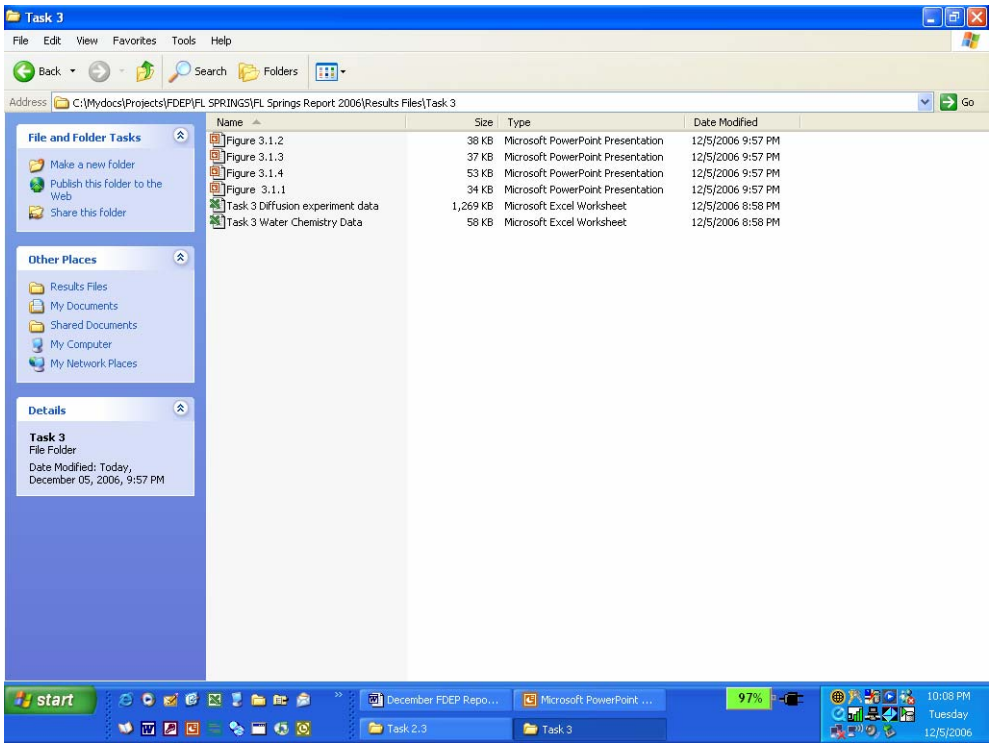


Figure 3.4 Vertical Total Kjeldahl Nitrogen profile of a *Lyngbya wollei* mat in Weeki Wachee Springs, April 2006.

Data: All data for Task 3 are in this folder.



Task 4 – Laboratory Experiments on Vaucheria and Lyngbya

All experimental work and data entry were conducted at MSU. Algae for experiments were collected by UF, MSU and DEP. All experimental work and data analysis were completed.

Methods

Two experimental systems were developed for growing filamentous algae. The large microcosm system consisting of Plexiglas cylindrical raceways was placed on a flat platform and a rotary shaker table (60RPM) (Fig. 4.1). This system allowed experimentation on algal mats of different sizes. Each experimental unit was filled with 100ml of growth medium. The fluorescent growth lights provided 150 µmol/m^2/s of light. Media were changed everyday. Experiments were conducted in the temperature controlled growth room with the mean temperature of 22.5ºC. Fresh and dry algal mass were measured to monitor algal growth.

The small microcosm system consisted of 1.5ml microcentrifuge tubes, each holding 1ml of growth medium (Fig. 4.0). Racks of tubes were placed on the rotary shaker (60RPM). Racks with microcentrifuge tubes were covered with neutral density filters. Light inside the tube was measured to be 25 µmol/m^2/s of light. This system allowed experiments to be conducted on individual algal filaments. Growth media were changed every 48 hours. Experiments were conducted in the temperature controlled growth room with the mean temperature of 22.5ºC. The length and thickness of algal filaments was measured using a digital video camera system and National Institute of Health Image J software. The change in length and biovolume of algal filaments were used to monitor algal growth. Growth medium Z8 was modified to simulate median chemistry of macronutrients of Florida springs water (referred to as medium Z8FL).

Figure 4.0. Microcosms in microcentrifuge tubes.

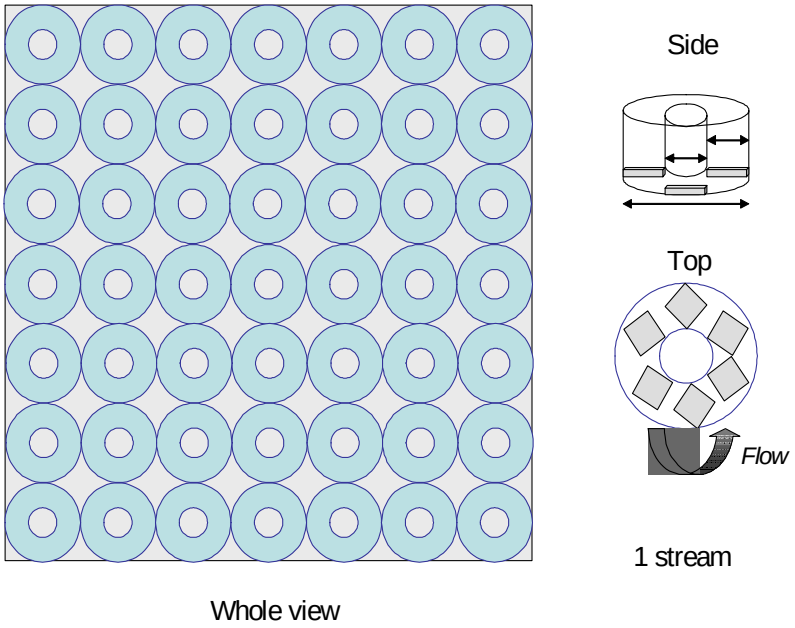


Figure 4.1. Laboratory microcosms. Cylindrical raceways placed on a flat platform and a shaker table.

Results: Large microcosm experiments

Algal relative growth rate (RGR) was calculated using the following formula:

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

In large microcosm experiments, increases in nitrate concentration resulted in increased *Lyngbya* growth rates with the largest increase in growth rate observed for the small mats (initial biomass 0.01g) growing in nitrate concentrations of 500µg/L and higher. The largest increase in *Lyngbya* growth rates was observed when phosphate concentration was 60µg/L and above for the small algal mats (initial biomass 0.01g). For larger algal mat,s the increase in growth rates was smaller even at the highest phosphate concentration) (Figures 4.2-4.3).

A large increase in the growth rates of *Vaucheria* in algal mats of all sizes was also observed for nitrate concentrations greater than or equal to 500µg/L. Increases in phosphate concentration above 30 µg/L affected small and medium size mats of *Vaucheria* and did not have any effect on the growth rates of large mats (initial biomass 0.2g) (Figures 4.4-4.5).

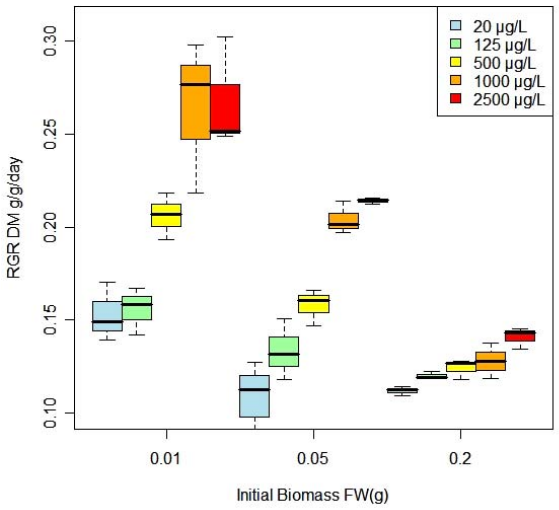


Figure 4.2. RGR of *Lyngbya* incubated in large microcosms in a factorial experiment with two factors: nitrate concentration (5 levels) and initial mat biomass (3 levels). RGR was calculated based on dry mass (DM).

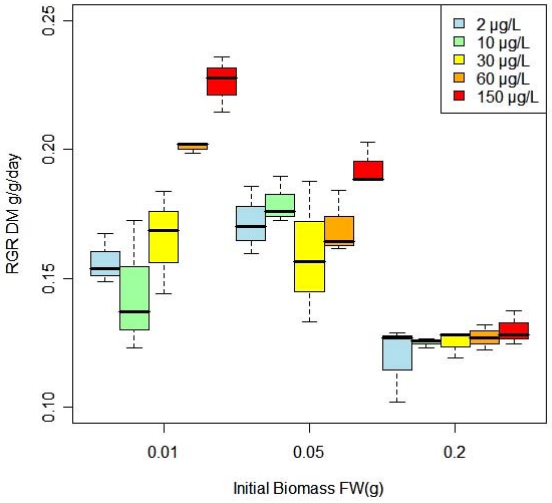


Figure 4.3. RGR of *Lyngbya* incubated in large microcosms in a factorial experiment with two factors: phosphate concentration (5 levels) and initial mat biomass (3 levels). RGR was calculated based on dry mass (DM).

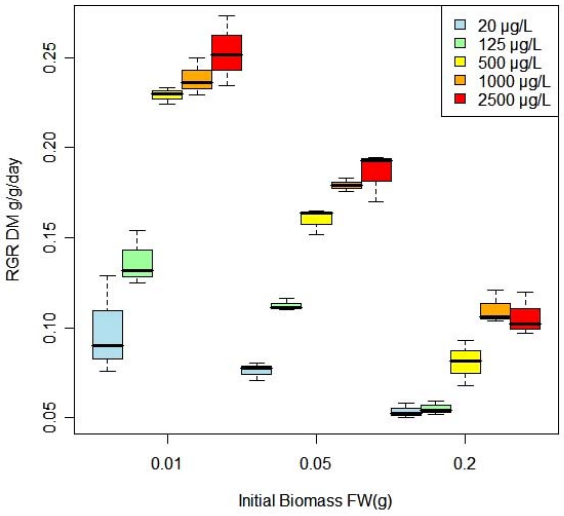


Figure 4.4. RGR of *Vaucheria* incubated in large microcosms in a factorial experiment with two factors: nitrate concentration (5 levels) and initial mat biomass (3 levels). RGR was calculated based on dry mass (DM).

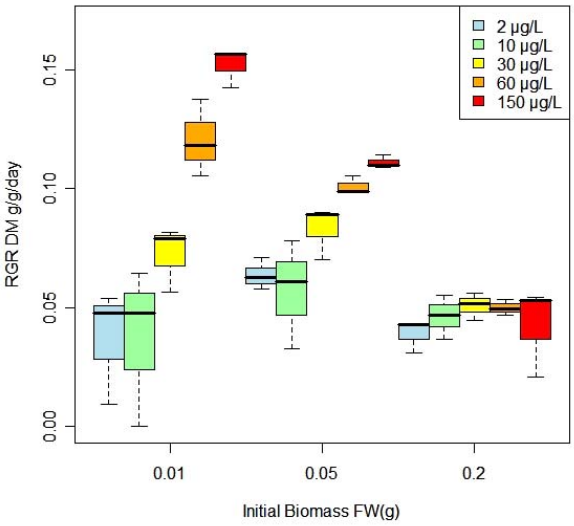


Figure 4.5 RGR of *Vaucheria* incubated in large microcosms in a factorial experiment with two factors: phosphate concentration (5 levels) and initial mat biomass (3 levels). RGR was calculated based on dry mass (DM).

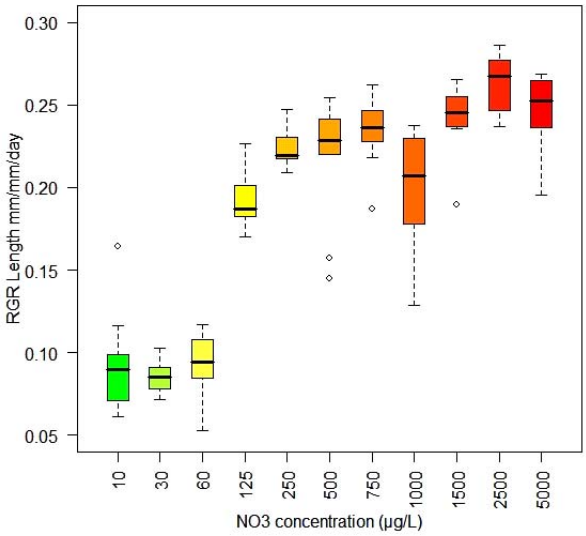


Figure 4.6. RGR of *Lyngbya* incubated in small microcosms in 11 different nitrate concentrations. RGR was calculated based on algal filament length.

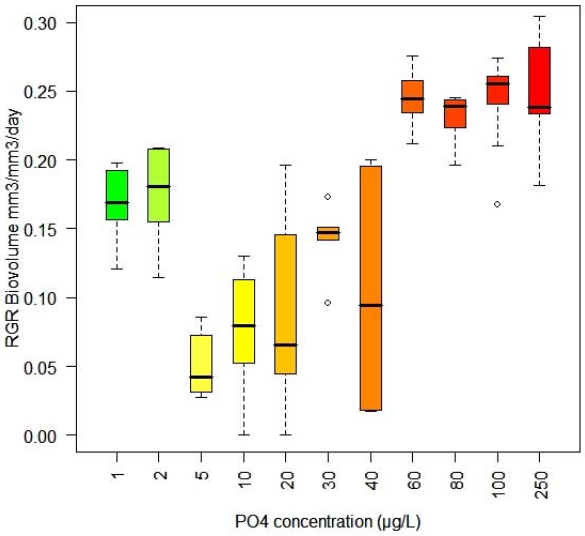


Figure 4.7. RGR of *Lyngbya* incubated in small microcosms in 11 different phosphate concentrations. RGR was calculated based on algal filament length.

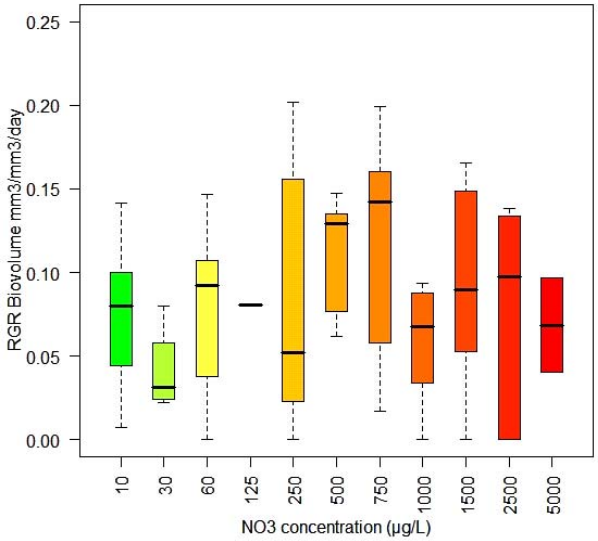


Figure 4.8 RGR of *Vaucheria* incubated in small microcosms in 11 different nitrate concentrations. RGR was calculated based on algal filament length.

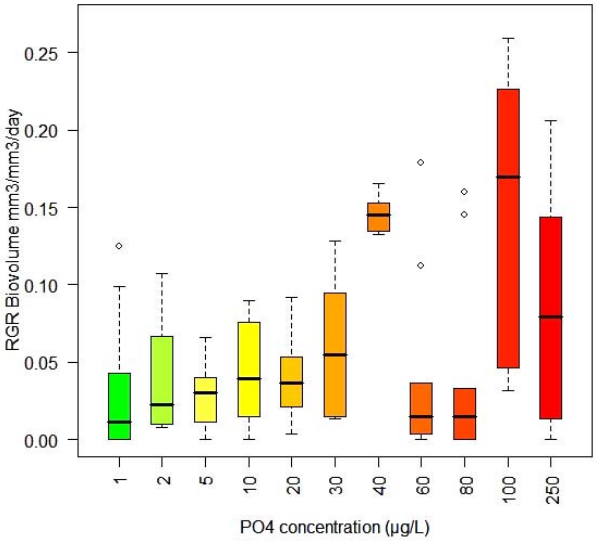
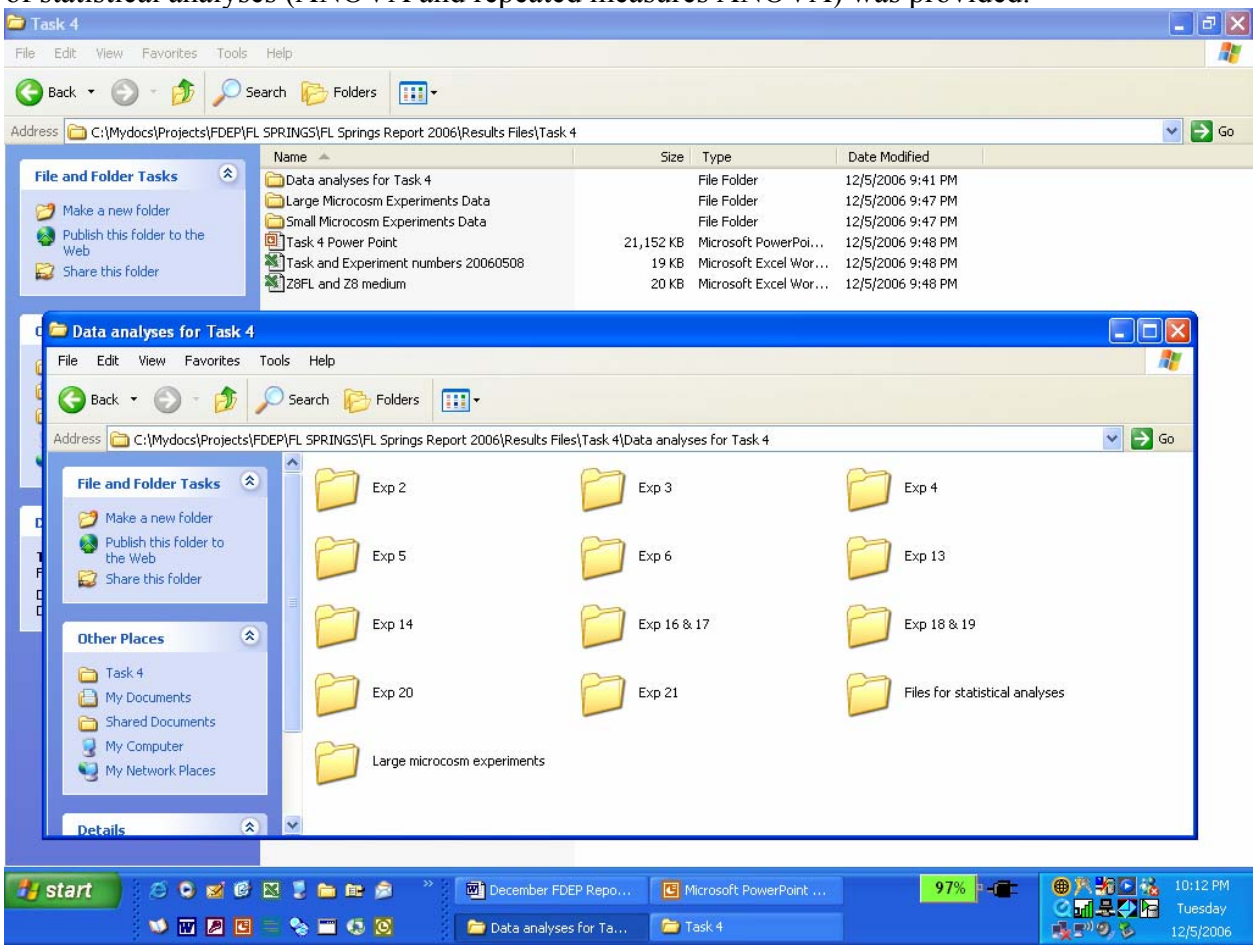


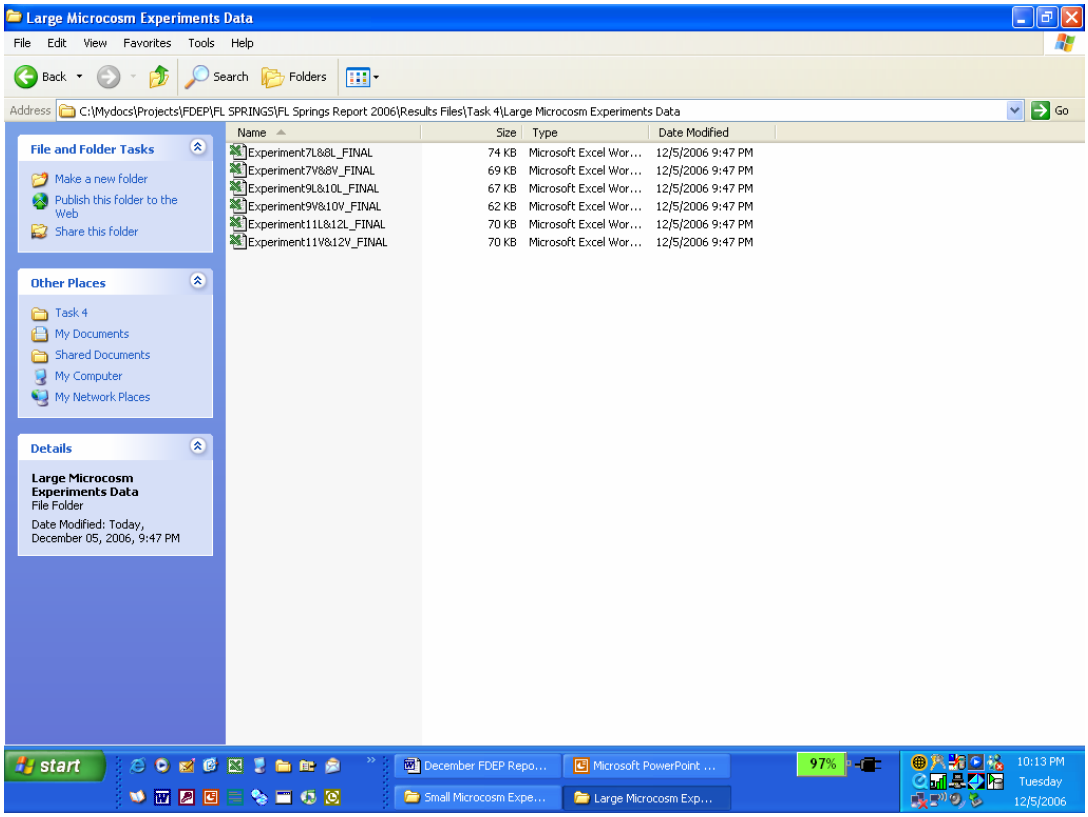
Figure 4.9. RGR of *Vaucheria* incubated in small microcosms in 11 different phosphate concentrations. RGR was calculated based on algal filament length.

Results: Small microcosm experiments

In small microcosm experiment growth of *Lyngbya* increased when nitrate concentration was 125µg/L and higher and phosphate concentration was 60µg/L and higher. No such trend was observed for *Vaucheria* (Figures 4.6-4.9).

Data: All data are in the Task 4 folder. The results are in the "Large Microcosm Experiments Data" folder and "Small Microcosm Experiments Data folder". The "jpgs" of original graphs and program codes for data analysis from R and SAS software are included in the "Data analyses for Task 4" folder and are grouped by the experiment. A binder with printouts of graphs and results of statistical analyses (ANOVA and repeated measures ANOVA) was provided.

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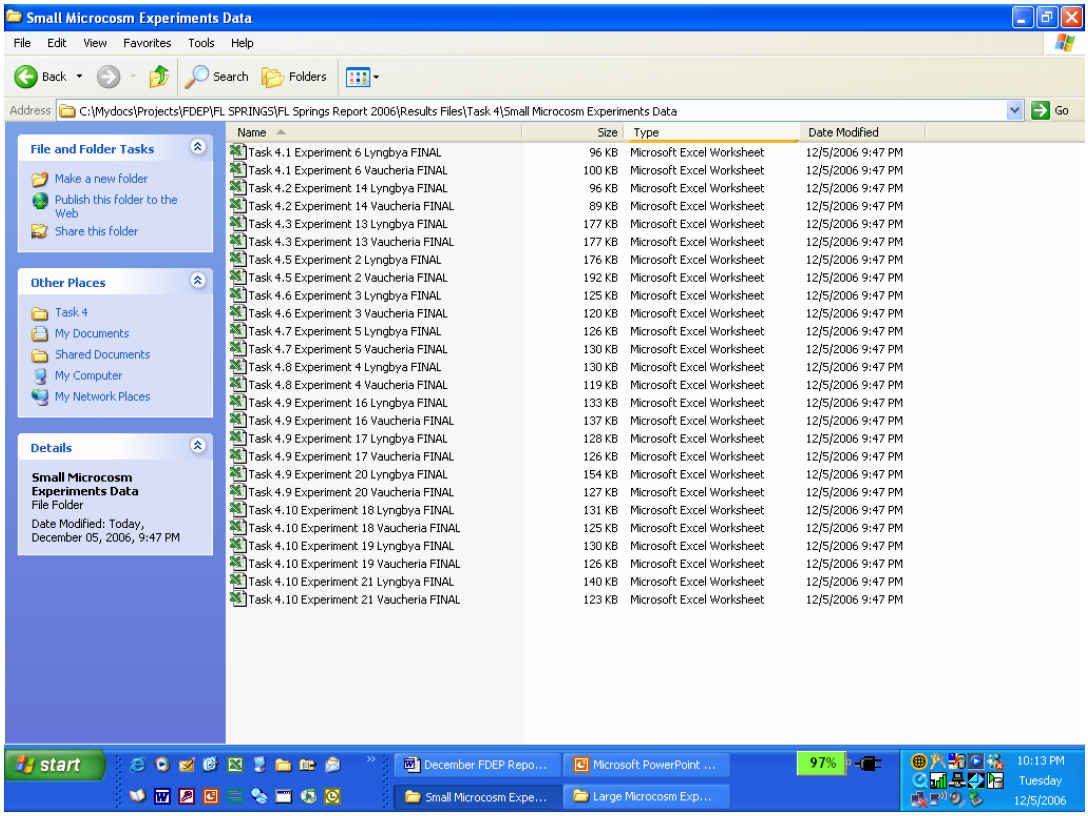
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Task 5 – Stream-side Experiments on Vaucheria and Lyngbya

A new mesocosm system was constructed in the University of Florida greenhouses for use in experiments to determine factors regulating macroalgal growth. These mesocosms are the same design employed by Stevenson at experimental stream facilities at the University of Louisville and the University of Michigan Biological Station. This system was different than earlier models because the mesocosms could not be placed near a stream or spring and could not have a constant supply of spring water. Thus, new spring water medium had to be added to the mesocosms (Figure 5.1).

A series of mesocosm experiments were attempted during 2006 at the University of Florida to assess the effects of N and P additions on macroalgae and growth in the mesocosms when using water transported to the UofF greenhouses from springs. The first two experiments in the series were designed to examine the interactive effects of N and P additions (Task 5.1). These first experiments failed owing to the development of micronutrient limitation within a few days of initiation of the experiments. Micronutrient limitation occurred due to the recirculation design of the mesocosms (i.e. micronutrient demand exceeded supply as algal biomass increased) and because Fe and other trace elements were depleted when experimental waters from the Floridian Aquifer were oxygenated by the aeration with water circulation systems.

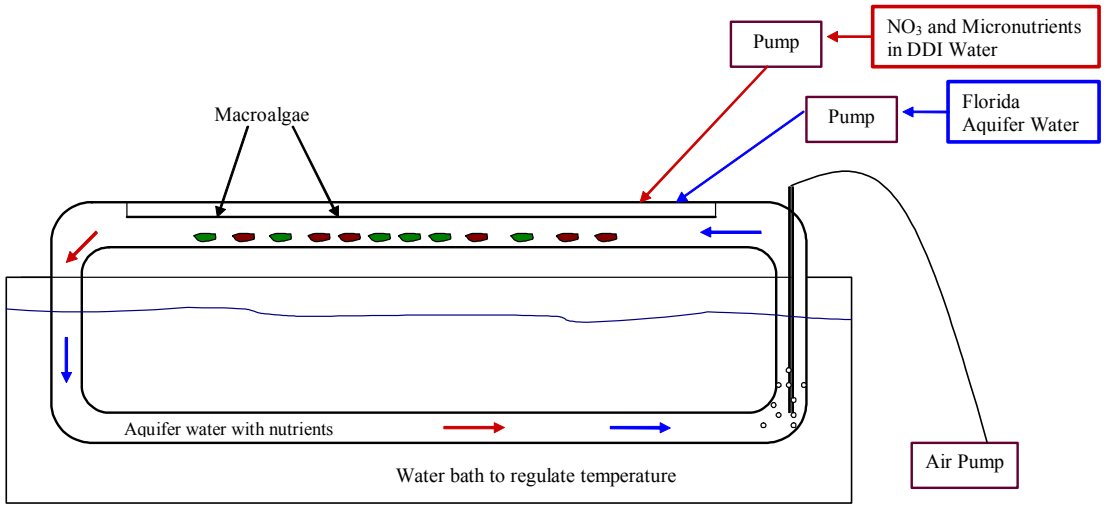


Figure 5.1. Cross-sectional view of a single stream channel. Water is lifted by air bubbles at one end of stream and flows over substrata in top of pipe, which has been cut out to expose open channel.

In the next two experiments (Tasks 5.2 and amended Task 5.3), a micro-nutrient media was added to all streams and eliminated these problems, allowing us to examine the effects of macro-nutrient inputs on algal growth. Two experiments using a range of nitrate concentrations were successfully completed. The first examined a wide range of NO3 levels. Potential threshold concentrations observed in the first NO3 experiment were retested in the second NO3 experiment. Two additional experiments (Tasks 5.4 and 5.5) were cancelled due to time constraints.

Task 5.2 Recirculating stream experiment: Nitrate Gradient 1

Methods

The mesocosm system consisted of 20 recirculating streams in which both *Lyngbya wollei* and *Vaucheria* spp. were grown in each stream channel on unglazed 1" square ceramic tiles. A schematic view of a single stream channel is shown in Figure 5.1. Each recirculating stream is made of 2" PVC pipe and is 48" long and 36" tall. The upper horizontal section of the stream channel was cut in half length-wise and the tiles with algae were placed inside. Florida aquifer water and a nutrient solution of nitrate plus micronutrients were continuously added to each stream using two peristaltic pumps. Circulation was maintained with an air pump, which produced bubbles that lifted the water in each stream channel, causing it to circulate. All 20 streams were placed in a water bath (concrete blocks covered with pond lining) to regulate stream channel temperature. Current velocity in each stream was maintained at approximately 25 cm/s using a valve that controlled the amount of air pumped in.

Floridian Aquifer water was obtained on the UF campus from a 350 ft deep well near the Law School. Numerous wells on campus were tested and this one was found to have the lowest N and P concentrations (NO_3<MDL, SRP= 0.009 mg/l). Every 3 to 4 days, 350 gallons of water were pumped from the well into a tank and transported to the greenhouse by truck in order to maintain water flow in the 20 stream channels. Although using water from a low nutrient concentration spring would have been ideal, it was logistically infeasible given time constraints.

This experiment tested a wide range of nitrate concentrations and ran for 28 days. It consisted of 7 treatments of varying target nitrate additions (in the form of $NaNO_3$) to the aquifer water: (1) only aquifer water (control), (2) only a micronutrient solution, (3) 0.5 µg/l NO_3 + micronutrients, (4) 5 µg/l NO_3 + micronutrients, 50µg/l NO_3 + micronutrients, 500 µg/l NO_3 + micronutrients and 5000 µg/l NO_3 + micronutrients. Each treatment was randomly assigned to three stream channels except for the control, which was assigned to two channels. The nutrient treatments were pumped into the channels for three days before the algae were added. Due to the recirculating design of the experiment, additions of micronutrients were necessary to prevent the development of micro-nutrient limitation.

The *Vaucheria* used in the experiment was collected at Alexander Springs and the *Lyngbya* was collected at Ichetucknee Head Springs. Small fragments of algae were allowed to "drip dry" and weighed. The initial fresh mass for both species was approximately 1g. Each fragment was then attached to an unglazed 1" square white ceramic tile with fishing line. Eight tiles each of *Vaucheria* and *Lyngbya* were randomly placed in each stream channel.

Temperature, conductivity, pH and dissolved oxygen were measured every 4-5 days in every stream channel. Light measurements were taken on three days to characterize light conditions in the greenhouse. Water chemistry (TKN, TP, SRP, NO_3, NH_4, DOC and trace metals**)** was measured 5 times during the experiment, on Days 0, 7, 14, 21 and 28. Two tiles per species in each stream were destructively harvested on Days 7, 14, 21 and 28. The algae were weighed, freeze-dried, weighed again, ground and analyzed for %C, %N and TP.

Results

At the end of this experiment, an increase in biomass for *Lyngbya wollei* was observed in Treatments 6 and 7 (Target concentrations of 500 and 5000µg/l NO_3; actual concentrations of 310 and 4144 µg/l NO_3, respectively). *Vaucheria* sp. showed an increase in biomass in Treatment 6. This was used as an approximate number around which to retest a narrower NO_3 concentration range during the second nitrate gradient experiment (Task 5.3).

Task 5.3 Recirculating stream experiment: Nitrate Gradient 2

The recirculating stream experiment consisted of 7 treatments of varying target nitrate additions ($NaNO_3$) to the aquifer water: (1) aquifer water + micronutrient solution, (2) 25µg/l NO_3 + micronutrients, (3) 50µg/l NO_3 + micronutrients, (4) 150µg/l NO_3 + micronutrients, (5) 250µg/l NO_3 + micronutrients, (6) 500µg/l NO_3 + micronutrients and (7) 750µg/l NO_3 + micronutrients.

Methods

The same methods were used as described in the previous experiment (Task 5.2) and the same parameters listed were measured with the same frequency as described above.

Results

Preliminary results show greater biomass accumulation for *Lyngbya wollei* in Treatments 4, 5, 6 and 7 as compared to Treatments 1, 2 and 3; potential threshold values may exist at concentrations above 181 µg/l NO_3 (Figure 5.3.1). Preliminary results for *Vauchera* sp. show greater biomass accumulation at lower nitrate concentrations (Treatments 2, 3, 4 and 5) than in highest nitrate concentration treatments (Treatments 6 and 7)(Figure 5.3.2).

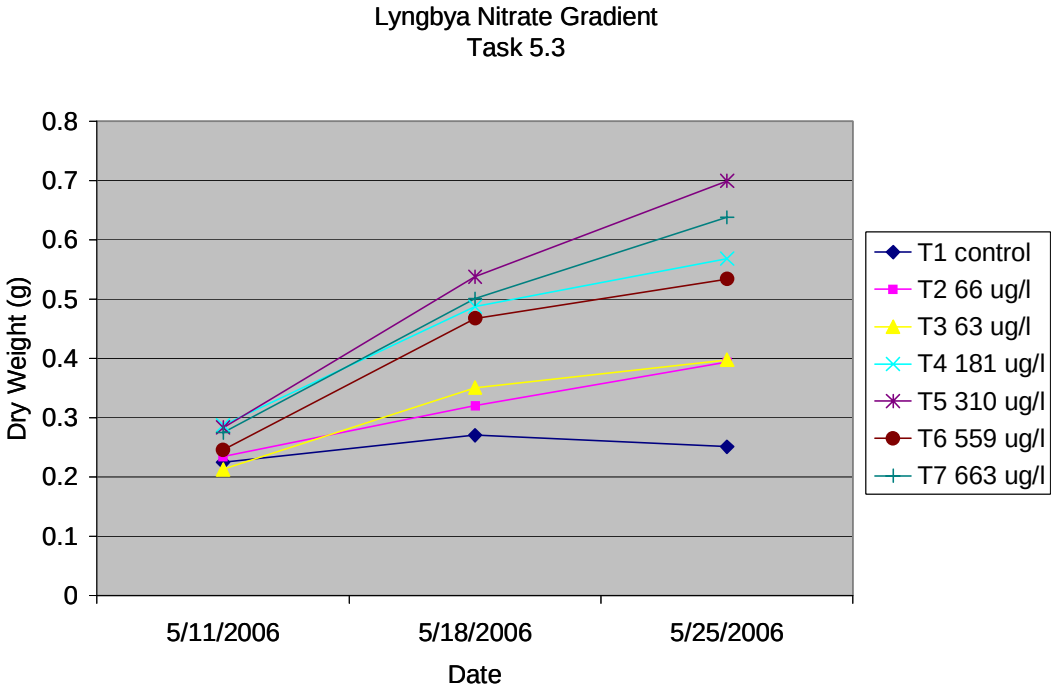


Figure 5.3.1. *Lyngbya wollei* biomass accumulation in response to varying nitrate concentrations in recirculating stream channels.

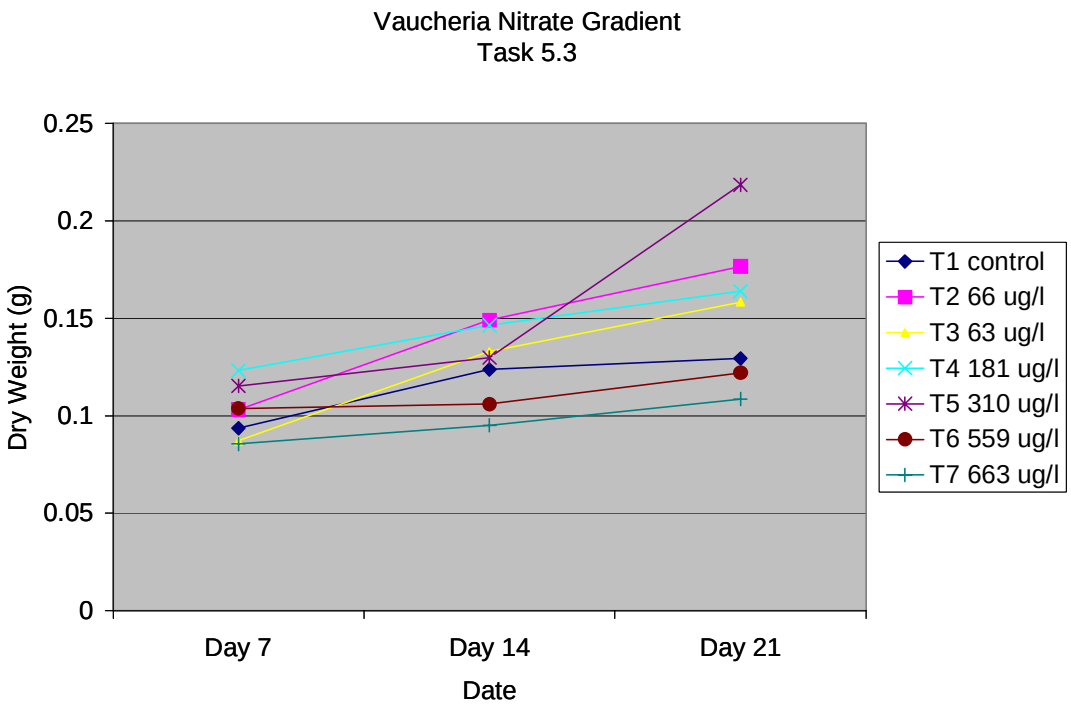
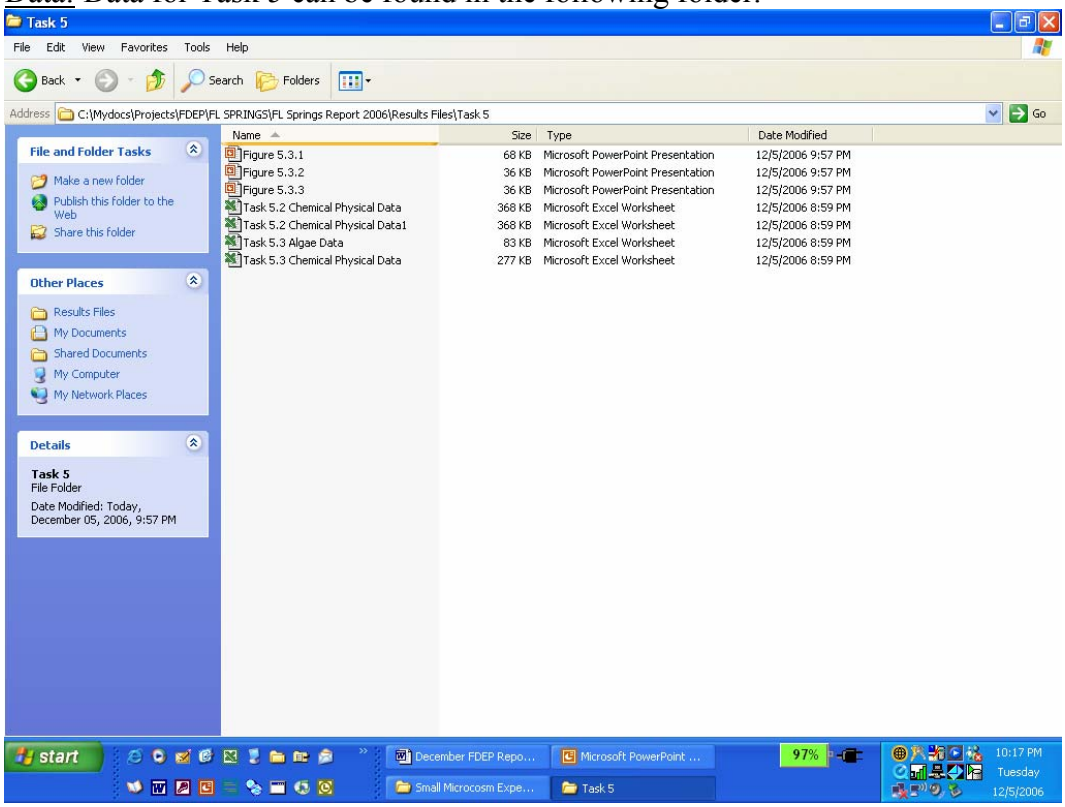


Figure 5.3.2. *Vaucheria* sp. biomass accumulation in response to varying nitrate concentrations in recirculating stream channels.

Data: Data for Task 5 can be found in the following folder.



Task 6 – Analysis of Genetic Differences Among Lyngbya and Vaucheria Populations.

160 samples was collected for *Lyngbya* DNA analyses and a total of 40 *Lyngbya* samples was collected for microscopic analyses. Samples were sent to Hans Pearl at University of North Carolina at Chapel Hill. These samples could not be analyzed before the end of the contract period.

Task 7 – Rapid Survey of Florida Springs to Refine Chemical Characterizations and Relation to Lyngbya abundance

Since nitrogen and phosphorus concentrations in the water column of springs and macroalgae abundance revealed few relations, additional environmental variables were collected to reanalyze the factors affecting the abundance of *Lyngbya*.

Variations in the composition of water, algae and sediments within larger population of Florida springs may provide information on factors controlling algal abundance and in particular, *Lyngbya* distribution. Furthermore, such data may reveal variations in trophic status of springs in relationship to landuse patterns and the presence of local sources of nutrients.

Methods

Sixty-two sites was sampled. Twenty seven of these were sampled as a part of Task 2.2. Forty-eight sites were sampled during previous surveys and 14 sites were new. At each site a full RPHA was conducted or a quick survey to determine whether macroalgae abundance had changed in comparison with the previous sampling. If the abundance had changed a quick RPHA was conducted. We also specifically looked for presence of *Lyngbya* at the sites where it was not found during the 2003 surveys. If *Lyngbya* was rare at the site, it might have been missed by the RPHA survey. Surveying the whole site (not just area covered by transects) exposed presence of small mats of *Lyngbya* at some of the sites where it was not recorded before.

1. The following samples were collected at each site: macroalgae samples for AFDM, macroalgae samples for chlorophyll a, macroalgae samples for %N, %P, %C and sediment samples for %N, %P, %C, sediment samples for available N and P, epiphyte samples for chlorophyll a and AFDM if epiphytes samples were not collected from that site before, algae samples for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis, sediment samples below algal mats and from the exposed river bottom $\delta^{13}C$ and $\delta^{15}N$ isotope analysis, macroalgae samples for ID confirmations, and water samples for water chemistry analyses. Water DO, pH, temperature and conductivity were also measured. Field work and initial sample processing was conducted by MSU and UF. Water samples for nutrient analyses were sent to DEP. Data entry from RPHA, macroalgae IDs, processing of preserved samples were conducted by MSU. All data from RPHA were entered into Excel spreadsheets and field identifications of macroalgae ID were evaluated. Macroalgae and epiphyte samples for AFDM and chlorophyll a were processed. Plant surface areas from which epiphyte samples were measured using National Institute of Health Image J software.

MSU purchased a dissolved CO2 analyzer from Mettler Toledo. This analyzer was developed for fermentation processes in the food industry; however the Mettler Toledo representative stated that it could be applied for the kind of field work conducted for the project. MSU received the

instrument and made all the necessary changes to adapt it for field work. We tested the analyzer in the field while sampling at Jackson Blue springs. The instrument was not rigorous enough for the type of sampling conducted. The instrument was returned to Mettler Toledo.

Results

Nitrogen, phosphorus and carbon content in algal mats were different between species. Additionally differences among sites were observed for both species (see example for *Lyngbya* in Figure 7.1).

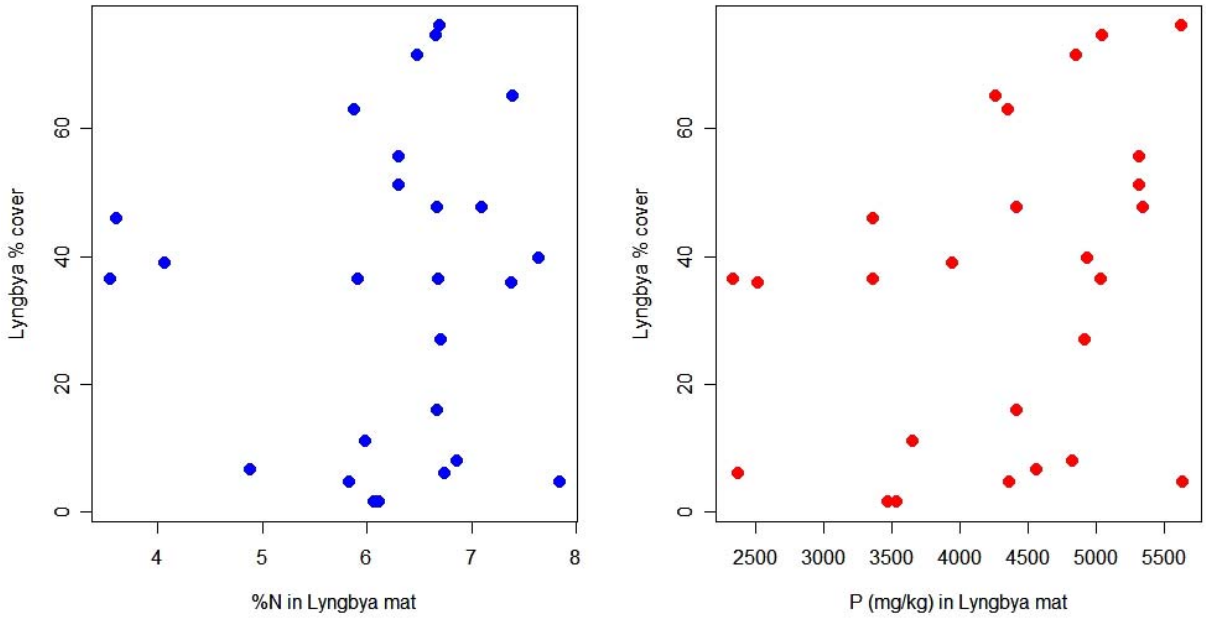


Figure 7.1. Lyngbya % cover as a function of Lyngbya mat nitrogen and phosphorus content for the data and samples collected in January 2006.

Analysis of nitrogen and phosphorus content in the sediments indicated that sediments collected from under algal mats had higher nitrogen and phosphorus content as compared to sediments from the open areas without macroalgal cover. Isotopic analysis of algae and sediment revealed a substantial range of values in the stable isotopic composition of algae and sediment (Figures 2.2.2 and 2.2.3). These data are discussed above under Task 2.2.

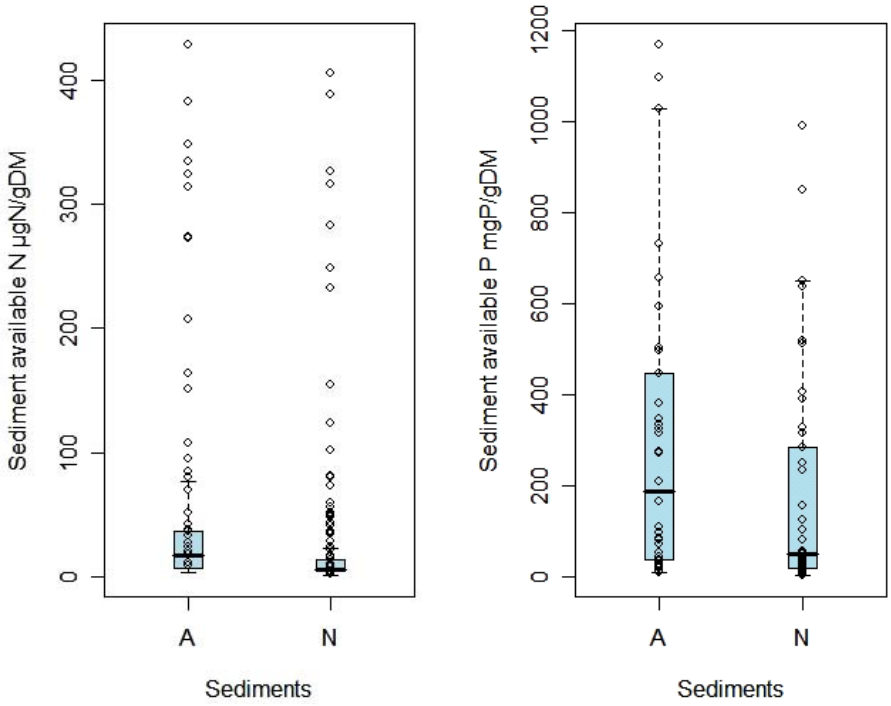
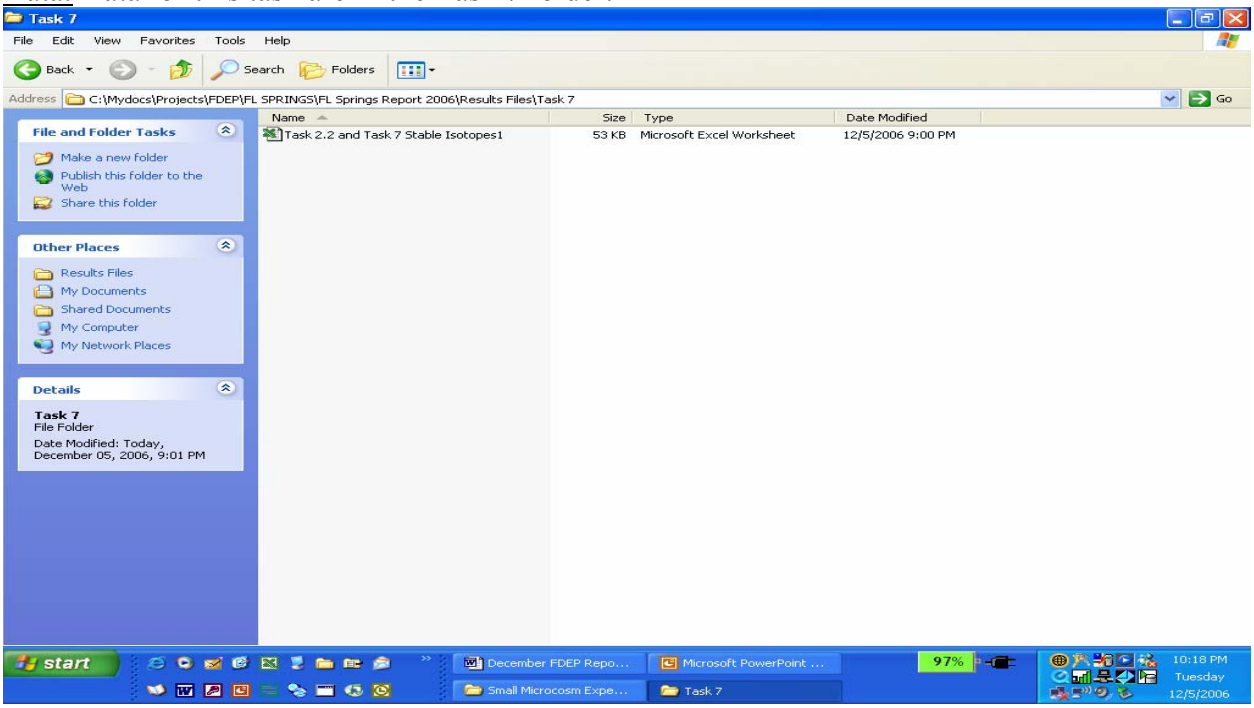


Figure 7.2. Nitrogen and phosphorus content in the sediments collected from under algal mats (A) and from the open areas without the mats (N).

Data: Data for this task are in the Task 7 folder.



References

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Stevenson, R.J. 2006. Refining diatom indicators for valued ecological attributes and development of water quality criteria. In: Ognjanova-Rumenova, N. And K. Manoylov, eds. Advances in Phycological Studies. Pp. 365-383. Pensoft Publishers. Moscow, Russia.

Stevenson, R. J. and L. L. Bahls. 1999. Periphyton protocols. In: M. T. Barbour, J. Gerritsen, & B. D. Snyder, eds. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Second Edition. EPA 841-B-99-002 United States Environmental Protection Agency, Washington. pp 6-1 to 6-22.