ECOLOGICAL CONDITION OF ALGAE AND NUTRIENTS IN FLORIDA SPRINGS

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EXECUTIVE SUMMARY

Nuisance growths of macroalgae have been identified as a problem for the recreational use of and aquatic life support in Florida springs. Nuisance algal growths have been associated with human activity and increases in nutrients in the springs. Development of nutrient criteria and the tools to implement the criteria will help protect springs and other water bodies of the state. However, prior to this study, no systematic studies of macroalgal occurrence and their relationships to nutrients in Florida springs of this scope are available.

A survey of filamentous macroalgae was conducted at 60 sites in 28 springs of north and central Florida, with the following results:

- Macroalgae were found at all sites and covered over half of the bottoms of Florida springs.
- *Vaucheria* and a noxious cyanobacterium, *Lyngbya majuscula*, were the two most common of a great diversity of algae at the sites.
- The percent of the bottom of springs covered by *Vaucheria* was related to total nitrogen concentrations in springs, but *Lyngbya* occurrence was not constrained by the low nutrients in the ranges studied.

Experiments, conducted using a variety of techniques to determine whether the nitrogen or phosphorus supply limited the growth of algae in springs, had the following results:

- Laboratory bioassays using water from the springs indicated that algae were limited by P at 56% of spring sites, by N at 19%, and by both N and P at 22%.
- In situ bioassays with nutrient-diffusing substrata embedded in sediments were not conclusive because of low colonization rates and the loss of many substrata.
- Outdoor mesocosm studies in recirculating streams indicated *Vaucheria* growth rates were related to nitrate concentrations, but not phosphorus concentrations, and *Lyngbya* growth rates were not limited by the range of nutrients tested.
- Outdoor mesocosm studies in small tubs indicated that conductivity affected the response of *Vaucheria* to nutrients, but did not affect *Lyngbya*.

Changes in microalgae on plants, macroalgae and sandy spring bottoms were surveyed to develop algal indicators of nutrient conditions in springs. These indicators will complement the measurement of nutrients in springs to characterize nutrient conditions.

- When variability in conductivity was reduced by eliminating high conductivity sites from analyses, the importance of nutrient regulation of diatom species composition became evident.
- Variation in nitrogen and phosphorus concentrations among spring sites was not correlated as in most studies, so different diatom indicators of nitrogen and phosphorus conditions were developed.
- Existing diatom indicators of nutrient conditions were weakly correlated to P conditions in springs, but not to N; but newly developed indicators show value.

Regulation of nitrogen may control macroalgal growths in Florida springs, but more evidence is required for development of specific nutrient criteria.

CHAPTER 1. INTRODUCTION

Nuisance growths of macroalgae have been identified as a problem in Florida springs, affecting both recreational use and support of aquatic life (Florida Springs Task Force, 2000). These springs have great economic importance for the state (Bonn and Bell, 2003) and represent unique resources for the support of recreation and biodiversity Florida Springs Task Force, 2000). Nuisance algal growths have been observed in many springs and have been associated with increases in human activity and nutrients, particularly nitrate. Development of nutrient criteria and the tools to implement the criteria will help protect springs and other water bodies of the state (USEPA 1999). However, no previous systematic studies of macroalgal occurrence and their relationships to nutrients in Florida springs of this scope are available.

Three separate lines of research were developed to determine the following:

1. Nutrient and algal conditions;
2. The relationships between nutrients and algae; and
3. Monitoring and management tools to protect and restore the springs.

First, a large number of sites in Florida springs were surveyed to determine the diversity of the algae; the factors affecting algal diversity; the range of nutrient conditions; and the relationships between the extent of algal growth and nutrients. Springs were sampled and surveyed during both the spring and the fall to determine whether conditions differed during the two seasons. The relationships among the macroalgal cover of spring bottoms, the thickness of macroalgae, and nutrient concentrations were the main focus. Results of these surveys are in Chapter 2.

In many studies, nitrogen and phosphorus co-vary; so the nutrients that limit algal growth and would affect algae if reduced had to be identified. While the magnitude of correlations provides a hint about which factors most affect biology in ecosystems, experiments were necessary to show cause-effect relationships and determine whether management of nitrogen or phosphorus would be more effective (e.g., Pan et al., 2000). Therefore, experiments were conducted to determine the ranges of nitrogen and phosphorus that regulate algal growth in springs. Due to the variations in size and growth forms of algae in springs and the lack of research available on macroalgae in mesocosms, three different experimental approaches were used:

1. Recirculating streams in an outdoor setting: Spring water was recirculated in the streams, with varying amounts of nutrients added.
2. Algal growth rates were observed in small tubs in which conductivity and nutrients were manipulated.
3. Small experimental devices were placed in streams. These devices slowly leaked nutrients through a fine-mesh screen upon which algae could grow in as natural setting a setting as possible.

The results of these experiments can be found in Chapters 3-5.

Assessing and monitoring nutrient conditions in streams and springs is challenging because nutrient concentrations vary with algal metabolism during the day, with weather-related runoff events that rinse nutrients into springs, and with natural weekly variations in algal accrual and die-off cycles (USEPA 1999). Diatoms are microscopic algae that are often the dominant photosynthetic organisms in aquatic habitats. They are an important base of the food web and are very sensitive to changes in nutrient concentrations. Because there are so many different species of diatoms, and the species have different sensitivities to nutrients, diatoms can be sensitive and precise indicators of nutrient conditions. In addition, because they live for extended periods of time in springs, diatoms provide a temporally integrated indication of nutrient conditions in springs that may more accurately and precisely characterize the nutrient regime of a water body than the sampling and measurement of nutrient chemistry (Stevenson and Smol, 2003). Thus, studies of diatoms and other microalgae in streams and their relationships to nutrients were conducted to better determine the effects of nutrients on algae and to develop indicators of nutrient conditions in springs. Results of this study can be found in Chapter 6.

This research was intensive, but the study's timeline was foreshortened by the state. Thus, the strategy for success was to collect as much information during surveys, experiments, and laboratory work as possible, use all viable experimental approaches, analyze the most promising data that resulted from this work, and present that data in this report. Sufficient time was not available to analyze and present the results of all the data that were collected. However, those data have been organized in electronic format and re provided with this report to the Florida Department of Environmental Protection. The files in which data are stored are presented in Chapter 7.

CHAPTER 2. ASSESSMENT OF MACROALGAL BIOMASS AND TAXONOMIC COMPOSITION IN FLORIDA SPRINGS AS A FUNCTION OF NUTRIENT CONCENTRATIONS

Introduction

Florida Springs are an important and unique resource used by many for recreational activities, such as swimming, boating, and sight-seeing (Florida Springs Task Force, 2000). Concerns have developed that the occurrence and biomass of macroalgae in the springs have increased during the last decade (Southwest Florida Water Management District, 2003). In the last fifty years many of the springs experienced drastic changes in their watershed and in the area directly surrounding springs. An increase in nutrients, especially nitrate, was observed in some springs (Rosenau et al., 1977). That the elevated nutrients associated with increased land use have stimulated the occurrence of nuisance algal blooms in Florida springs is cause for concern.

Very few published studies document the occurrence of algae in Florida springs. Odum (1957) reported the presence of small *Spirogyra* sp., *Oedogonium* sp. and *Rhizoclonium* sp. mats in Silver Springs. Their biomass was very low compared to the rest of the primary producers in Silver Springs; consequently, macroalgal biomass was not included in the estimates of system productivity in Odum's study. A list of algae from Florida springs compiled by Whitford (1956) includes macroalgae taxa such as *Vaucheria* sp. and *Cladophora* sp., but mat thickness and spatial extend of cover were not recorded. *Dichotomosiphon tuberosus* was found in Ichetucknee, Turtle, Manatee, Poe and Silver Springs by Davis and Gworek (1972).

Very few published studies document relationships between macroalgae and nutrients. Relationships between *Cladophora* and nutrients have been described, but in streams in Montana, Michigan, and Kentucky (Dodds et al., 1997, Stevenson et al., accepted). However, these relationships may not apply to the types of algae in Florida springs and to the physical-chemical conditions in Florida springs.

The objectives of this study were to document the types of macroalgae occurring in Florida springs, the spatial extent and thickness of algal mats in springs, and the relationships among the amount of macroalgae, nutrients, and other environmental factors. A survey approach was used to observe and collect macroalgae at a large number of sites in a large number of springs. This approach was believed to be the best for characterizing the status of conditions in Florida springs and determining the relationships between the macroalgal biomass and nutrients. This study was the first systematic study of benthic algae in a large number of Florida springs that incorporated macroalgal percent cover and macroalgal mat thickness during more than one season.

Methods

Study sites

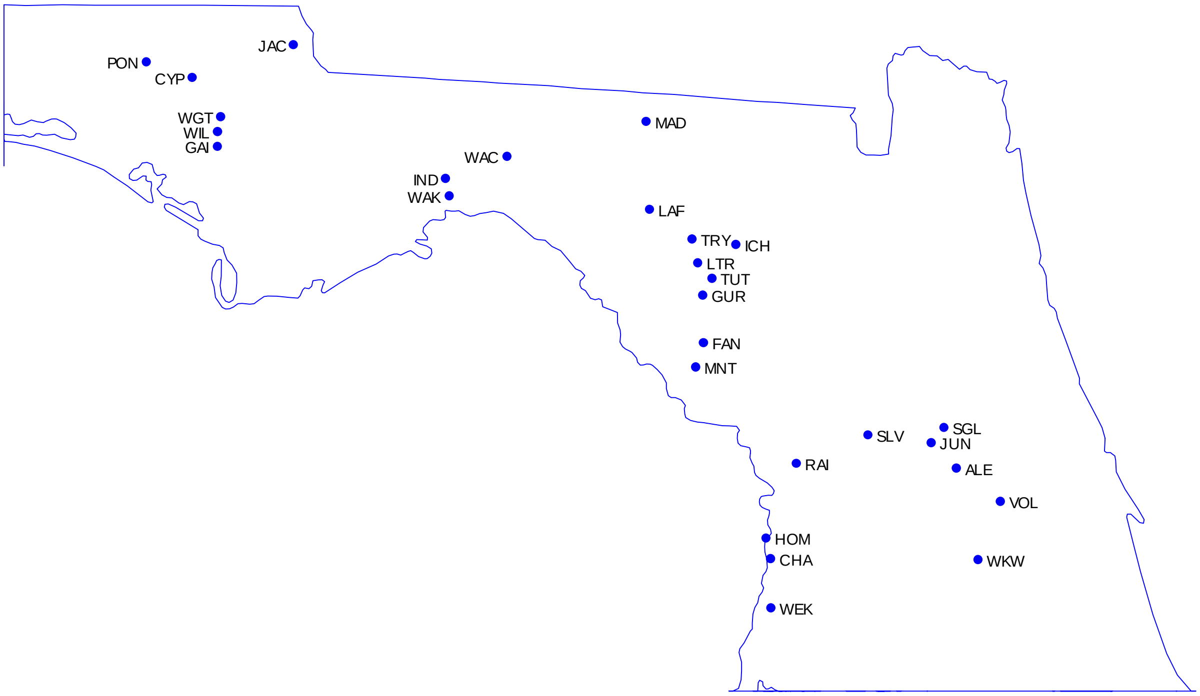
Twenty eight springs, mostly of first and second magnitude and located in northern and central Florida, were selected for this study (Table 2.1, Figure 2.1).

Table 2.1
Study sites, spring and site codes, sampling dates, and latitude and longitude of transect 1 for each site.

Spring	Spring code	Site name	Site code	Latitude	Longitude	Date sampled spring	Date sampled fall
Alexander	ALE	Head	ALE-01	29.08128	81.57563	3/28/2003	10/29/2003
		Downstream	ALE-02	29.08231	81.57754	3/28/2003	10/29/2003
Chassahowitzka	CHA	Blue holes	CHA-01	28.71617	82.57502	4/1/2003	11/2/2003
		Dock	CHA-02	28.71558	82.57630	4/1/2003	11/2/2003
		Brown spring	CHA-03	28.71721	82.57586	4/1/2003	11/2/2003
Cypress	CYP	Head	CYP-01	30.65855	85.68430		9/24/2003
Fanning	FAN	Head	FAN-01	29.58757	82.93541		10/2/2003
Gainer	GAI	Pipe	GAI-01	30.42736	85.54827	4/26/2003	9/25/2003
		Side boil	GAI-02	30.42884	85.54854	4/26/2003	9/25/2003
Guranato	GUR	Head	GUR-01	29.77973	82.94001		10/2/2003
Homosassa	HOM	After bridge	HOM-01	28.79961	82.85905	4/2/2003	11/4/2003
Ichetucknee	ICH	Head	ICH-01	29.98408	82.76184	3/16/2003	11/22/2003
		Blue Hole	ICH-02	29.98068	82.75866	4/20/2003	11/22/2003
		Below Blue Hole	ICH-03	29.98007	82.75895	3/17/2003	11/22/2003
		Mission spring	ICH-04	29.97628	82.75783	4/23/2003	11/9/2003
		Devils Ear	ICH-05	29.97388	82.75996	4/23/2003	11/9/2003
		Mill Pond	ICH-06	29.96658	82.76005	4/23/2003	11/9/2003
		Before bridge	ICH-07	29.95495	82.78507	4/24/2003	11/8/2003
		Coffee spring	ICH-08	29.95937	82.77526	4/21/2003	11/8/2003
Indian	IND	Head	IND-01	30.25077	84.32203		9/26/2003
Jackson Blue	JAC	Head	JAC-01	30.79037	85.13998	4/19/2003	9/24/2003
		Boat ramp	JAC-02	30.78249	85.16022	4/19/2003	9/23/2003
		Arrowhead camp ground	JAC-03	30.75609	85.18680	4/18/2003	9/23/2003
Juniper	JUN	Head	JUN-01	29.18365	81.71201	3/27/2003	10/31/2003
		Fern Hammock	JUN-02	29.18364	81.70801	3/27/2003	10/31/2003
		River fork	JUN-03	29.18519	81.70726	3/27/2003	10/31/2003
		After bridge on route 19	JUN-04	29.21283	81.65431	3/26/2003	10/31/2003
Lafayette Blue	LAF	Head	LAF-01	30.12592	83.22617		9/29/2003
Little River	LTR	Head	LTR-01	29.99642	82.96675		9/28/2003
Madison Blue	MAD	Head	MAD-01	30.48056	83.24439		9/29/2003
Manatee	MNT	Head	MNT-01	29.48952	82.97692		10/3/2003
Ponce de Leon	PON	Head	PON-01	30.72090	85.93071	4/26/2003	9/22/2003
Rainbow Spring	RAI	Head	RAI-01	29.10223	82.43741	4/4/2003	11/12/2003
		KP Hole	RAI-02	29.09294	82.42848	4/4/2003	11/12/2003
		Before tubers sign	RAI-03	29.06305	82.42788	4/4/2003	11/12/2003
		Before bridge	RAI-04	29.05223	82.44700	4/4/2003	11/12/2003
Silver Glen	SGL	Head	SGL-01	29.24603	81.64345	3/26/2003	10/30/2003
Silver River	SLV	Head	SLV-01	29.21619	82.05252	4/3/2003	11/13/2003
		Second pool	SLV-02	29.21584	82.04987	4/3/2003	11/13/2003
		Birds of prey	SLV-03	29.21561	82.04112	4/3/2003	11/13/2003
		Old swimming area	SLV-04	29.20500	82.02902	4/3/2003	11/13/2003
		Cabbage palm	SLV-05	29.20211	82.01127	4/3/2003	11/12/2003
Troy	TRY	Head	TRY-01	30.00598	82.99756		9/29/2003
Turtle	TUT	Head	TUT-01	29.84742	82.89041		9/30/2003

Spring	Spring code	Site name	Site code	Latitude	Longitude	Date sampled spring	Date sampled fall
Volusia Blue	VOL	Head	VOL-01	28.94758	81.33969	3/29/2003	11/20/2003
		Downstream from stairs	VOL-02	28.94679	81.33921	3/29/2003	11/20/2003
Wacissa	WAC	Head RR	WAC-01	30.33979	83.99244	4/7/2003	9/27/2003
		Minnow	WAC-02	30.33020	83.98776	4/7/2003	9/27/2003
		Big Blue	WAC-03	30.32770	83.98484	4/7/2003	9/27/2003
Wakulla	WAK	Head	WAK-01	30.23533	84.30287	4/8/2003	10/1/2003
		Turnaround	WAK-02	30.23318	84.28870	4/8/2003	10/1/2003
		Bird colony	WAK-03	30.22507	84.27470	4/8/2003	10/1/2003
Washington Blue	WGT	Head	WGT-01	30.45279	85.53044		9/26/2003
Weeki Wachee	WEK	Head	WEK-01	28.51747	82.57349	3/24/2003	11/5/2003
		Boat dock	WEK-02	28.51901	82.57361	3/24/2003	11/5/2003
		WMA	WEK-03	28.52481	82.59583	3/24/2003	11/4/2003
		Roger's Park	WEK-04	28.53057	82.62407	3/24/2003	11/5/2003
Wekiwa	WKW	Head	WKW-01	28.71193	81.46037	3/30/2004	11/21/2003
		Canoe launch	WKW-02	28.71269	82.45948	3/30/2004	11/21/2003
Willford	WIL	Head	WIL-01	30.43966	85.54763		9/25/2003

Figure 2.1
Location of springs selected for the study. For spring codes, refer to Table 2.1.



This project began in March 2003. Samples were collected twice: 1) in the spring of 2003 (March and April), and 2) in the fall of 2003 (September to November). The second sampling event was moved from summer to fall since many of the studied sites are highly affected by human recreational activity during the summer (Bonn and Bell, 2003) and that activity was believed to reduce macroalgal cover and biomass. In the spring, 48 sites within 16 springs were sampled, and in the fall, 60 sites within 28 springs were sampled (Table 2.1). Originally, 53 sites were selected for both sampling events, but due to flooding along Suwannee River in the spring of 2003, 5 springs could not be sampled. The spring sampling showed few springs with low TP concentrations. Therefore, during the second sampling period (fall), the originally selected 53 sites were sampled, along with 7 additional sites that were selected specifically for their low TP concentrations. A description of the springs selected for this study can be found in Rosenau et al. (1977) and the Florida Springs Task Force Report (2000). Maps of sampled sites provided by DEP are in Appendix A.1.

Rapid Habitat and Periphyton Assessment (RHPA)

At each of the study sites a modified Rapid Habitat and Periphyton Assessment (Stevenson and Bahls, 1999) was conducted. Sites were sampled by wading, snorkeling, or making observations from a canoe or boat, depending upon the depth of the spring run and site accessibility. At each site, researchers designated 9 transects going across the spring run and positioned about 10 m apart. At most sites the first transect crossed the most upstream, boil area and the following transects were located downstream from the most upstream boil. Nine observation points were designated for each transect, resulting in a total of 81 observation points at each site. The length and buffer width were measured for each transect. Buffer width was defined within a 20 m range, unless more than 20 m was visible, in which case it was treated as 50 m wide. Any bank conditions (binding roots, canalized or incised) were documented. 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 densiometer). At each point, the stream depth was measured, current velocity was estimated, and the substratum type was characterized. The presence and taxonomic identity of macrophytes (Dressler, 1991; Ramey, 1995) and macroalgae and the thickness of the macroalgal mat were noted. The thickness of macroalgal mats was recorded on an ordinal scale: 0.5 for mat thickness <0.5 cm, 2 for mat thickness 0.5-2 cm, 5 for mat thickness 2-5 cm, 20 for mat thickness 5-20 cm, 50 for mat thickness 20-50 cm and 100 cm for mat thickness >50 cm. For other benthic algae, epiphyte thickness and epipelon thickness were estimated and also recorded on an ordinal scale: 0.5 for slimy biofilm, 1 for biofilm that tracks with a finger, 2 for biofilm 0.5-1 mm thick, 3 for biofilm 1-5 mm thick, 4 for biofilm 5-20 mm thick, and 5 for biofilm >20 mm thick. If the biofilm was formed by macroalgae and was more than 20 mm thick, it was recorded as macroalgae. At each site the RPHA was repeated at one randomly selected transect to characterize sampling error. However, it was impossible to go back exactly to the original points sampled, so the very patchy distribution of plants and macroalgae between the original and repeated transect assessment was characterized. Global positioning system (GPS) coordinates were recorded in the middle of transect 1 and transect 9.

The original RHPA was developed for sites that were wadeable and in which the stream bottom could be viewed easily. However, most of the study sites in this project were very deep, with 65% of sampled points more than 1 m deep and an average depth in excess of 1 m at most sites. Thus, the original sampling methods had to be modified for deeper water. Measurement of flow with a flow meter was not possible, because the centers of the channel at almost all sites were too deep to conduct measurements with the instrument. Current velocity was estimated on a scale: 0 (below 5 cm/s), 1 (5-15 cm/s), 2 (16-50 cm/s) and 3 (>50 cm/s). Measurement of current velocity with a current meter at each observation point was impractical.

The percent cover or percent presence of plants, macroalgae, different substrata types, etc., was based on results from the RHPA assessment and was calculated as the percent of sampled points where particular plant macroalgae or substrata were observed. Usually, at any one point more, than one plant, macroalga or substratum type was observed.

The Landuse Development Intensity Index (LDI) was provided by FDEP. Spring discharge was based on USGS data (Rosenau et al., 1977). The distance from boil was measured by using the tracking tool along the river run in Microsoft Streets & Trips 2000.

Algae samples

Macroalgae

From each transect, a macroalgae sample was collected to confirm the accuracy of field identification (198 samples in the spring and 463 samples in the fall).

At each site, a composite macroalgal sample was also collected. This was a qualitative sample consisting of pinches taken from each transect. Forty-seven samples were collected in the spring and 60 samples (including 2 samples collected for QAQC) were collected during the fall. A subsample was taken for algal mat total nitrogen and total phosphorus analyses (analyzed by FDEP). For macroalgae chlorophyll *a* analyses, a small pinch was patted dry, weighed for fresh mass (FM) and frozen. For macroalgae volume and macroalgae ash free dry mass (AFDM), a pinch of algae was placed into a volumetric cylinder filled with water. Macroalgae were suspended to allow them to fill their natural volume, and the volume they filled (not the volume displaced by the macroalgae) was recorded. This measurement allowed the relationship between macroalgae mat thickness and macroalgae mass per surface area (1ml of volume used by macroalgae equals 1 cm thick macroalgal mat per cm^2) to be calculated. Then the macroalgae were patted dry, weighed for FM, and frozen for AFDM analyses.

The dominant macroalgal taxa were identified under the dissecting scope (up to 100x magnification). A microscope (up to 400x) was used if identification was not possible under the dissecting scope.

Epiphytes

At each transect (if macrophytes were present), a mid-stem section of a plant was collected for a composite epiphyte sample. The original proposed collection of tips and mid-stem sections could not be executed at all sites due to the difficulty of sampling in deep water. Originally, SCUBA was planned to be used for sampling in deep water, however, this was not possible due to time limitations (in the trial it took a full working day to sample one site while using SCUBA) and personnel restrictions (volunteers were required to assist with SCUBA). In addition, sampled aquatic plants exhibited different growth patterns. *Saggitaria* sp. and *Vallisneria* sp. (the most common taxa in this study) have the oldest sections of the plant at the tip and youngest at the base, which is not the case for other plant taxa. To correct for this difference in growth patterns, only mid-stem sections were collected. Epiphytes were brushed off and rinsed from plant fragments. Forty-five samples were collected during the spring and 61 (including 3 repeated samples for QAQC) samples were collected during the fall. A subsample was taken for chlorophyll *a* analysis and frozen immediately. Plants from which algae were removed were frozen for plant surface analysis. Macrophytes were spread flat on a white sheet of paper and scanned with a flatbed scanner. The plant surface area was calculated using the scanned image and Image-J software (Rasband, 2003).

Epipelon

At each transect, if sediments were exposed to microalgal colonization, a sediment core (5.2 cm in diameter and 0.3 cm deep) was collected. A composite quantitative epipelon sample consisted of all microalgae collected with cores at the site. Algae were removed from sediments by swirling and rinsing the sediments with distilled water (Stevenson and Stoermer, 1983). This swirl-pour procedure was repeated 10 times to remove most algae from sediments. Forty-six samples were collected during the spring and 21 samples were collected in the fall. The latter were collected from 6 sites sampled in the spring, 12 sites not sampled in the spring and 3 repeated samples for QAQC. A subsample was taken for chlorophyll *a* analysis and frozen immediately.

Chlorophyll a and AFDM

Chlorophyll *a* analyses were conducted within 4 weeks of sample collection. Chlorophyll *a* was extracted with 90% ethanol and analyzed on a Turner Designs TD-700 fluorometer (Clesceri et al., 1998). Samples for AFDM were preserved with M3 and were processed following Clesceri et al. (1998).

Water analyses

Water temperature, pH, conductivity and dissolved oxygen (DO) were measured at each site at transect 1 and 9 with YSI or Hydrolab. Water clarity (light extinction coefficient with PAR measured at the surface, at 0.5m and at 1m) was measured using a LICOR LI-250. From the most upstream transect, water samples were collected to analyze for alkalinity, ammonia nitrogen, calcium, chloride, iron, magnesium, Kjeldahl nitrogen, nitrates, orthophosphate, potassium, silica, sodium, strontium and sulfate. Samples were analyzed by FDEP. When the concentration of a compound was below the detection limit of the laboratory method, the method detection limit is reported (Table 2.2). Algal growth potential and algal limiting nutrient bioassays were conducted by FDEP on water samples collected from 15 sites in the spring and 12 sites in the fall.

Table 2.2
Detection limits of water chemical analyses. When concentration of a compound was below detection limit of the laboratory method, the method detection limit is reported.

Compound	Detection limit (mg/L)
Alkalinity	5
Ammonia-N	0.010
Calcium	0.050
Chloride	0.200
Iron	0.010
Magnesium	0.028
N_KJEL_TOT	0.060
NO2NO3-N	0.004
O-Phosphate-P	0.004
Potassium	0.025
Silica	0.300
Sodium	0.150
Strontium	7
Sulfate	0.200
Total-P	0.004

Soft epiphytic algae

A subsample from a composite epiphyte sample was preserved with M3 for taxonomic algal analyses (Clesceri et al., 1998). Subsamples were collected by homogenizing samples on a magnetic stirrer, then collecting aliquots of each sample with a pipette for each subsample. Multiple aliquots were collected to reduce error due to patchiness. Algae were enumerated in a Palmer-Maloney counting chamber (Wildco®) under 400x. The count was continued until at least 300 live algal units were counted. Both live and dead diatoms were counted, but dead diatoms did not contribute to the 300 live units count. Each diatom cell was counted as one unit with the exception of diatoms that formed chains; then a chain was counted as a unit. Algae were identified to the lowest possible taxonomic level (Dillard, 1989; Komarek, 2003; Sheath, 2003; Whitford and Schumacher, 1984; Komarek and Anagnostidis, 1999; Prescott, 1982; Starmach, 1963).

Diatoms

A subsample was taken for diatom analyses from each macroalgae, epiphyte and epipelon sample. Diatom subsamples were collected by homogenizing samples on a magnetic stirrer and collecting aliquots of each sample with a pipette for diatom subsample. Multiple aliquots were collected to reduce error due to patchiness. Diatoms were identified under 1000x after acid-cleaning and mounting in Naphrax® on microscope slides. Data analyses of diatom samples are presented in Chapter 6.

Statistical analyses

Pearson correlations between measured environmental variables were calculated using SYSTAT 10 software. Repeated measures ANOVA on DM, AFDM, Chla and Phoephytin data was conducted using SAS/STAT.

Multivariate ordination analysis was employed to determine the effect of environmental variables on the percent cover of macroalgae at the studied sites. Analysis was conducted with CANOCO v.4 for Windows. Species data were log-transformed and species scores were divided by the standard deviation. Detrended correspondence analysis (DCA) was used for indirect gradient analysis (only species data), and environmental variables were used as supplementary environmental data to calculate axes correlations with environmental variables (Leps and Smilauer, 2003). Since species distributions followed unimodal patterns (based on the maximum spread between sites and species distributions), canonical correspondence analysis (CCA) was used for direct gradient analysis (species and environmental variables) (Leps and Smilauer, 2003).

Results

Rapid Habitat Assessment (RHA)

Sites varied in their buffer width and the character of the bank. Data describing the buffers at the study sites are in Appendix A.2, and data describing the types of banks are in project Access database (from spring sampling in tblRHA_Canopy_Buffer_TLgth_spring2003 and from fall sampling in tblRHA_Canopy_Buffer_TLgth_fall2003). Most of the study sites were deep and wading was not possible (Appendix A.3). Sites at the head of the spring had at least one boil, but in a few cases, it was not possible to sample directly over the boil. For a few springs, a different number of boils was observed in the spring and in the fall. Also, a few sites had high grazer density, which could have affected the epiphyte and epipelon biomass. Wide ranges of current velocity were observed; however, the average current velocity was low (Appendix A.3). Sand was the dominant substratum and was found at all sites (Appendix A.4).

Chemical and physical water parameters

A wide range of conductivity (113 - 3872 µS/cm) and dissolved oxygen (0.27-9.98 mg/L) was observed. However, water temperature (20.06-23.93°C) and pH (7.13-8.33) were relatively similar (Appendix A.5). Water chemistry data are in Appendix A.6.

Macroalgae

Taxonomic composition

Macroalgae were found at 59 out of 60 sampled sites and were present at 45% of all points sampled in Florida springs. The only spring in which no macroalgae were found was Cypress Spring. Troy Spring had macroalgae present but mat thickness was very low; thus, it was not possible to collect enough macroalgae for all of the analyses. In all, 24 taxa of macroalgae were found (Table 2.3). The most commonly observed taxa were *Lyngbya majuscula* and *Vaucheria* sp. (Figure 2.2). Thick algal mats were formed by *Lyngbya majuscula*, *Vaucheria* sp., *Compsopogon* sp., and *Dichotomosiphon* sp.

Table 2.3
Macroalgal taxa found in Florida Springs and codes for macroalgae used in this study.

Taxa	Algae code
Cyanophyta:	
Lyngbya majuscula	L
Oscillatoria sp.	O
Lyngbya aestuarii	Ls
Aphanothece sp. balls	Ap
Phormidium sp.	Ph
Rhodophyta:	
Polysiphonia subtilissima	Po
Caloglossa sp.	Cg
Compsopogon sp.	Cm
Audouionella sp.	U
Batrachospermum sp.	Br
Bacillariophyta:	B
Pleurosira leavis	
Terpsinoe musica	
Aulacosira sp.	
Xanthophyceae:	
Vaucheria sp.	V
Chlorophyta:	
Spirogyra sp.	S
Cladophora cf *glomerata*	C
Rhizoclonium hieroglyphicum	Rh
Dichotomosiphon sp.	D
Hydrodiction sp.	Hd
Enteromorpha sp.	E
Chaetomorpha sp.	Ch
Stigeoclonium sp.	
Oedogonium sp.	Od
Schizomeris sp.	

Figure 2.2
Proportion of macroalgal taxa found at 59 sampling sites during spring and fall sampling based on results of PRHA assessment (8676 points).

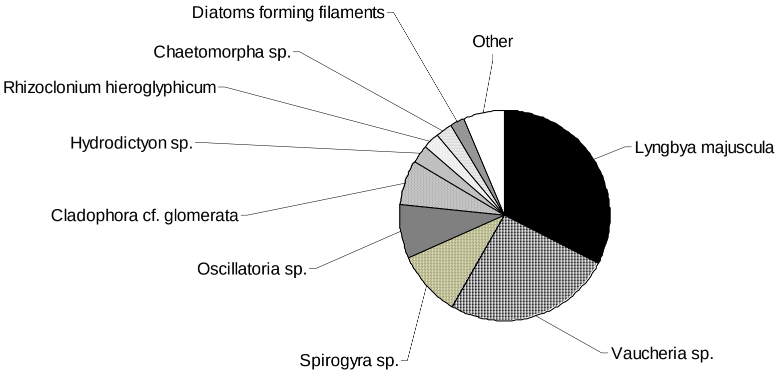


Figure 2.3
Percent macroalgal cover at 59 sites in the fall.

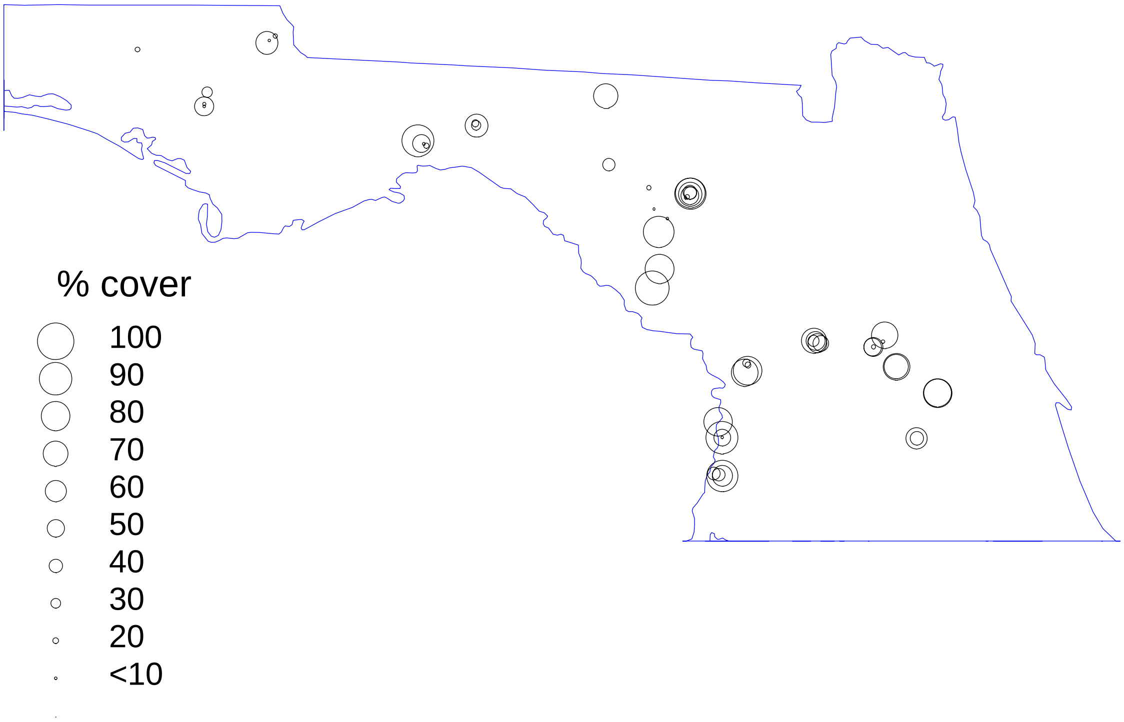


Table 2.4
Pearson correlation matrix for macroalgae and environmental variables in the fall ($p<0.05$ in bold).

	Average cover thickness	Percent cover	DM	AFDM	Chl a	Pheophytin	*Lyngbya majuscula* percent cover	*Vaucheria* sp. percent cover
ALKALINITY	0.131	0.185	0.057	0.045	0.127	-0.050	0.012	**0.465**
AMMONIAN	-0.094	-0.080	-0.092	-0.074	-0.064	-0.006	-0.102	-0.104
CALCIUM	0.206	0.243	0.153	0.207	0.157	0.032	0.091	**0.478**
CHLORIDE	0.030	0.029	0.028	0.086	0.092	0.088	-0.071	-0.014
IRON	-0.122	-0.092	-0.133	-0.192	-0.183	0.005	**-0.299**	0.195
N_KJEL_TOT	-0.055	0.135	**0.356**	-0.029	0.196	0.111	-0.240	**0.300**
NO2NO3N	0.023	0.057	0.031	-0.064	-0.082	-0.010	-0.114	**0.375**
OPHOSPHATE	-0.031	0.225	0.062	-0.021	0.082	0.191	0.094	0.130
SILICA	0.038	0.088	0.096	0.069	0.052	0.038	0.089	-0.165
SODIUM	0.028	0.029	0.028	0.085	0.085	0.090	-0.073	-0.023
SULFATE	0.119	0.135	0.178	**0.263**	0.073	0.148	0.143	0.045
TOTALP	-0.033	0.221	-0.001	-0.019	0.056	0.153	0.093	0.152
TOTALN	0.019	0.065	0.053	-0.065	-0.069	-0.003	-0.128	**0.391**
MAGNESIUM	0.024	0.040	0.051	0.084	0.056	0.087	-0.041	-0.009
STRONTIUM	0.158	0.209	0.202	**0.328**	0.203	0.165	0.229	0.071
LATITUDE	-0.249	**-0.346**	-0.093	**-0.286**	**-0.382**	0.042	**-0.361**	-0.182
LONGITUDE	-0.144	**-0.375**	-0.174	-0.240	**-0.297**	-0.104	**-0.368**	-0.212
PH	0.002	-0.076	-0.017	0.082	-0.019	-0.025	0.149	**-0.533**
CONDUCTIVITY	0.067	0.053	0.056	0.118	0.109	0.095	-0.048	0.051
DO	-0.144	**-0.293**	-0.175	-0.085	-0.213	0.108	0.135	**-0.328**
TEMPERATURE	0.234	**0.367**	0.106	**0.285**	**0.315**	-0.001	**0.341**	0.140
CANOPY	-0.187	-0.081	-0.077	-0.211	-0.083	-0.112	-0.138	0.070
BUFFER	**-0.270**	**-0.271**	**-0.342**	**-0.258**	-0.099	0.016	-0.176	-0.191
Transect Length	0.042	-0.033	0.018	0.096	-0.094	-0.040	-0.038	-0.135
TEES	-0.137	-0.011	-0.249	-0.100	-0.093	0.159	0.063	-0.147
SHRUBS	-0.062	0.016	-0.239	-0.100	0.031	0.034	-0.091	-0.202
HERBS	**0.306**	0.120	0.205	**0.356**	**0.305**	0.009	**0.340**	-0.008
BARE	**0.279**	**0.288**	**0.356**	0.207	0.119	-0.134	0.167	**0.357**
LDI	0.149	0.104	0.097	0.123	0.215	0.043	0.091	**0.475**
DISCHARGE	0.044	0.028	0.052	0.117	0.019	**0.296**	**0.395**	-0.028
MAT TN	0.097	0.092	0.008	0.07	0.08	0.024	0.157	-0.126
MAT TP	-0.021	0.016	-0.068	-0.043	0.027	0.008	0.06	-0.124
MAT TN/TP	0.15	-0.092	0.008	0.102	-0.018	-0.12	-0.044	-0.116

Figure 2.4
Average macroalgal mat thickness at 59 sites in the fall.

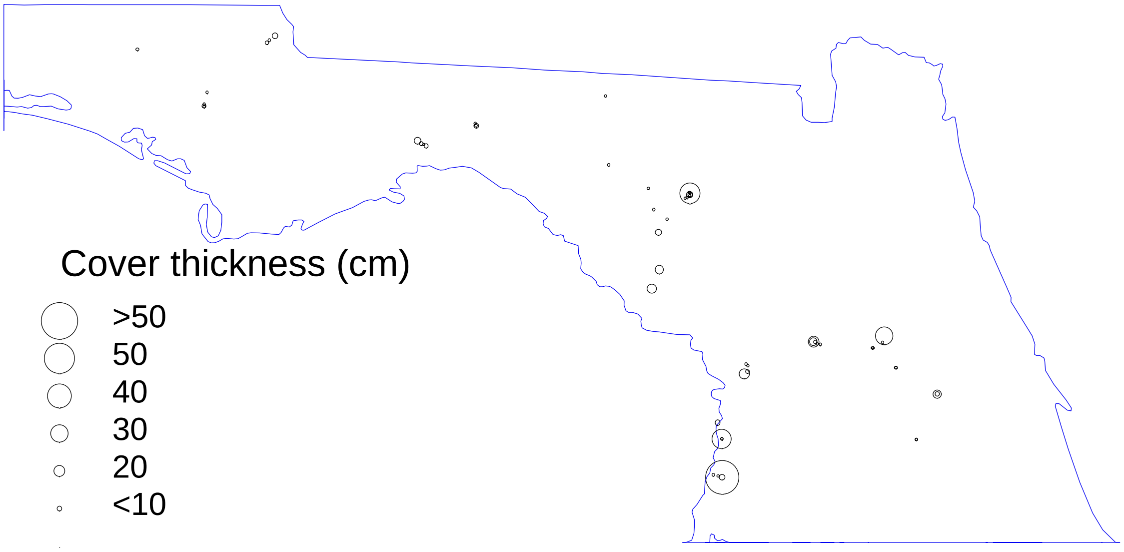


Figure 2.5
Lyngbya majuscula percent cover in the fall.

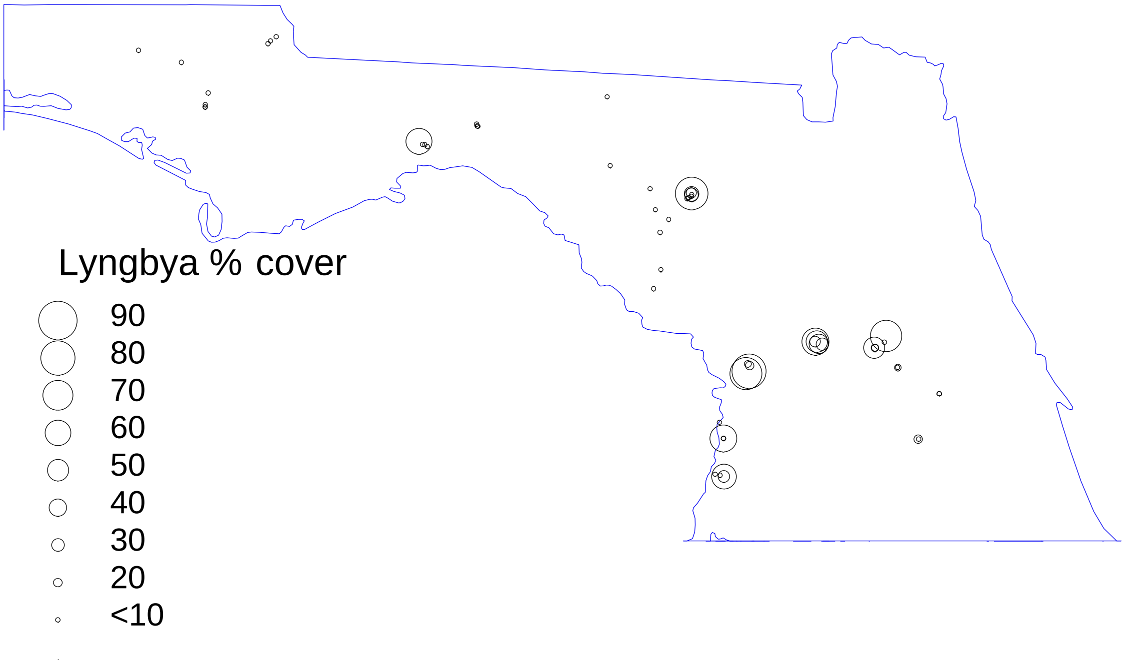


Figure 2.6
Vaucheria sp. percent cover in the fall.

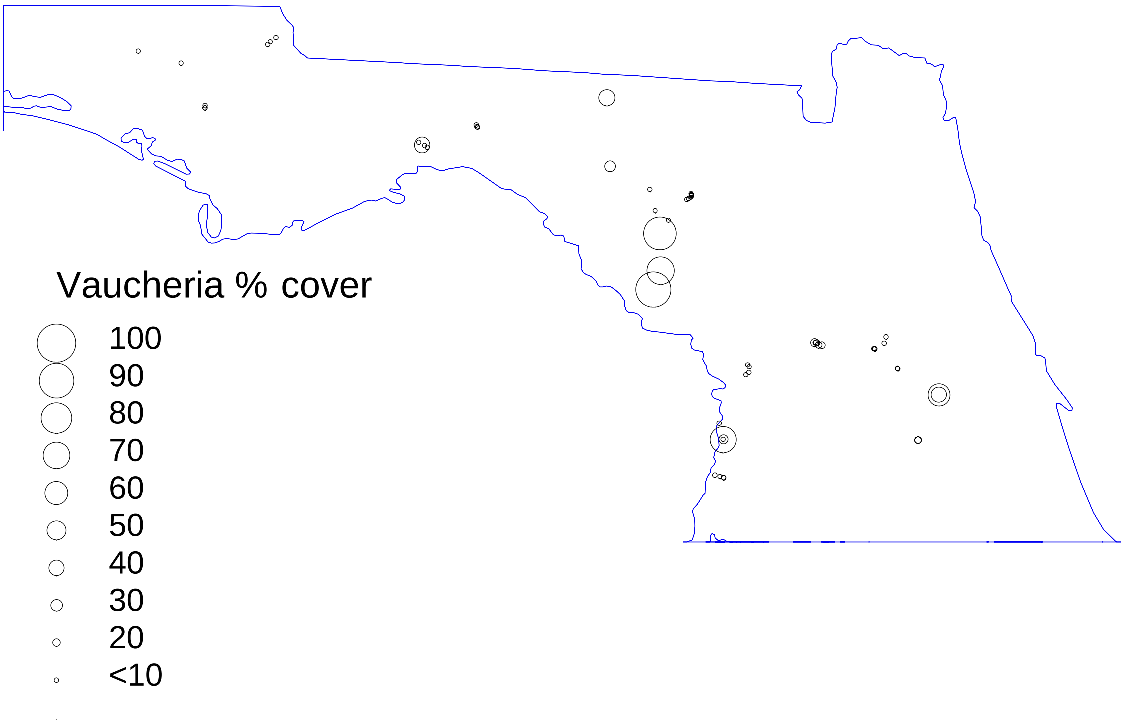


Figure 2.7
Macroalgae dry mass (DM) and ash free dry mass (AFDM) at 59 sites in the fall.

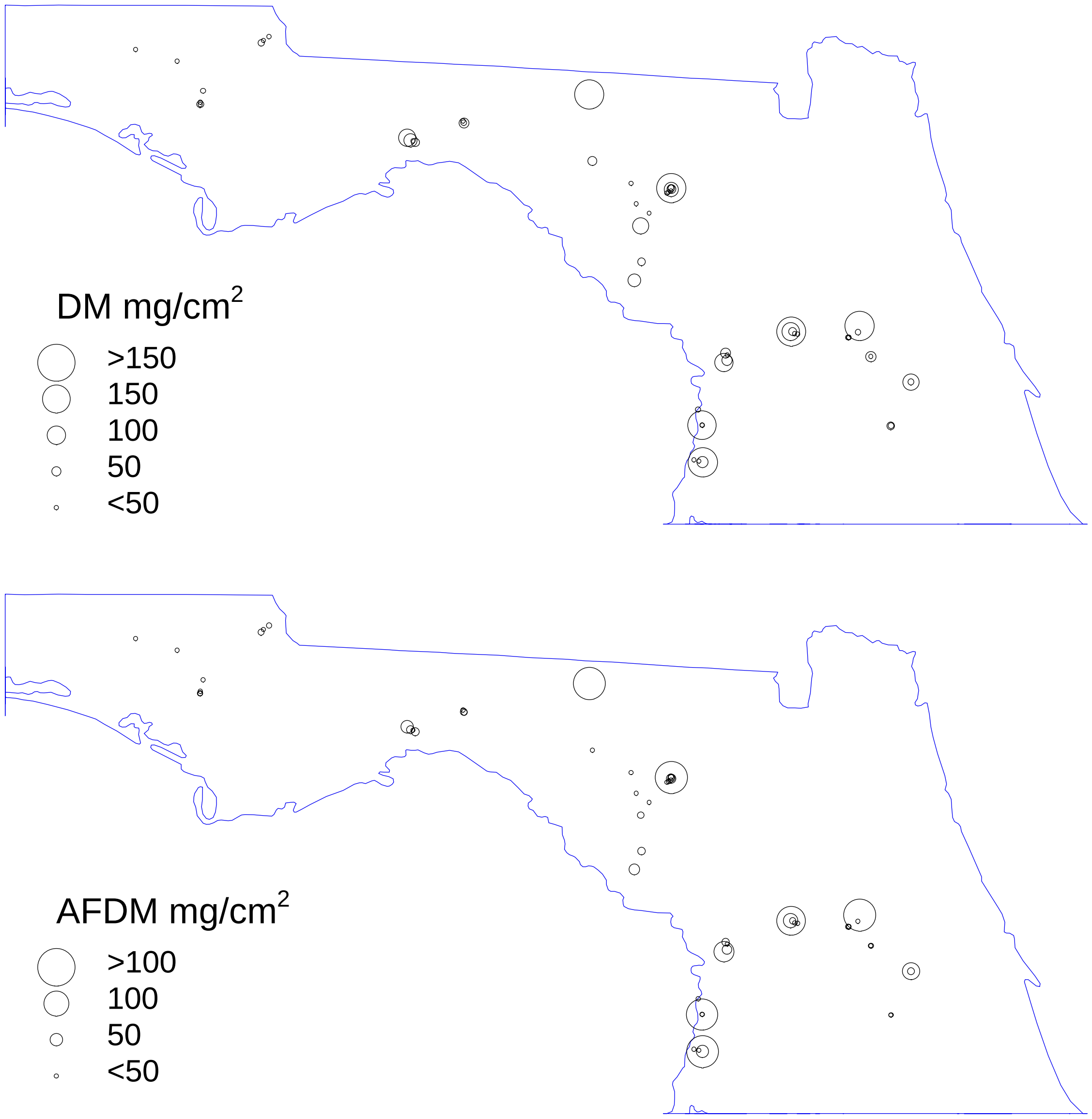
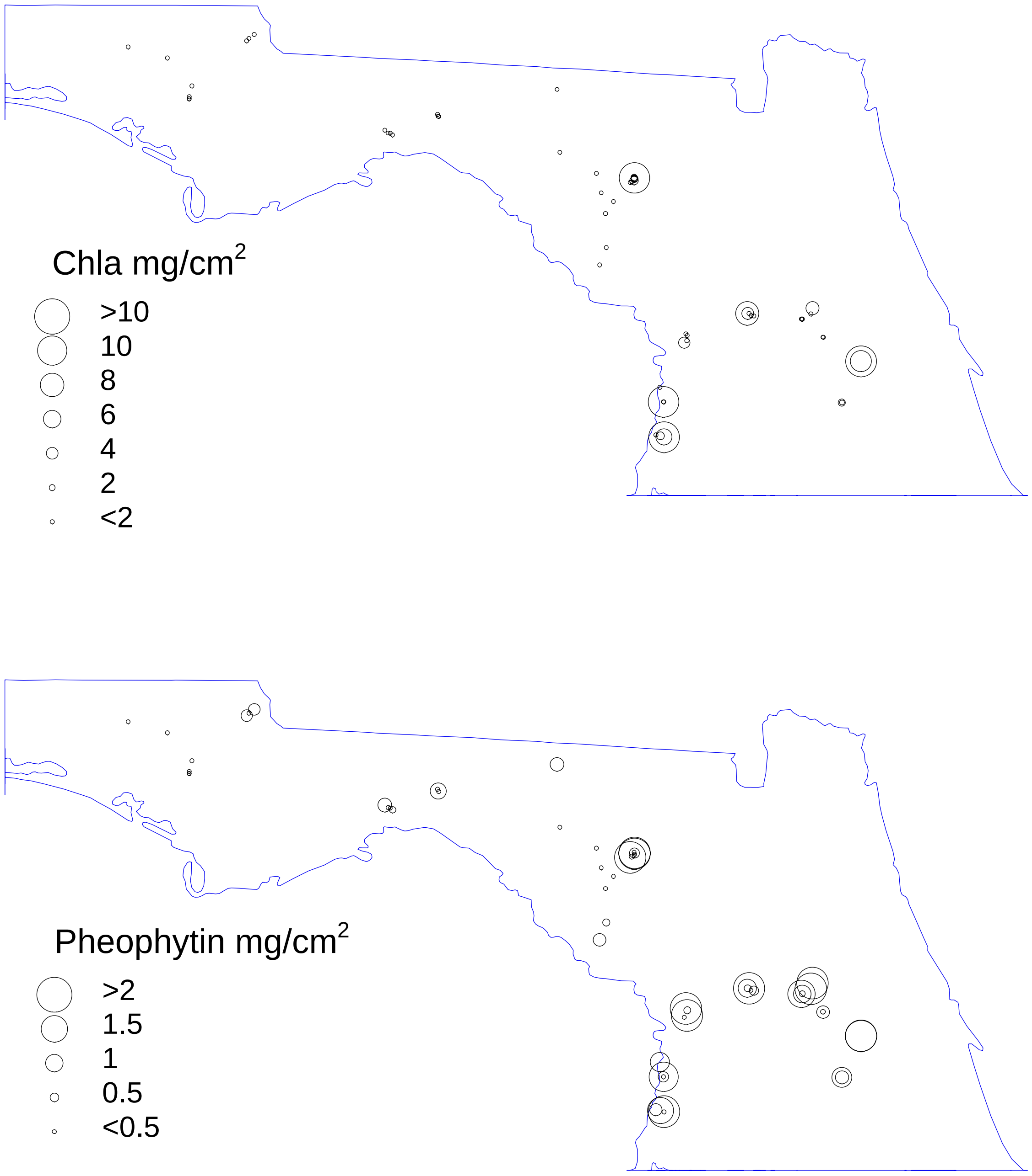


Figure 2.8
Macroalgae chlorophyll *a* and pheophytin at 59 sites in the fall.



Percent cover and biomass

The percentage of macroalgae cover varied from 0% in Cypress Spring to 94% in Manatee Spring and was higher for southern sites than for northern sites (Figure 2.3, Table 2.4, and Appendix A.7).

The average macroalgae mat thickness varied from 0 cm for Cypress Spring to 55 cm at the head of Weeki Wachee Spring (Figure 2.4, Appendix A.7). The *Lyngbya majuscula* percent cover was the highest in the southern portion of our study area (Table 2.4), while the *Vaucheria* sp. percent cover was the highest in Manatee Spring and Guranato Spring (Figure 2.5 and 2.6). Other macroalgal taxa were also common, but their percent cover was lower than that of *Lyngbya majuscula* and *Vaucheria* sp. (Appendix A.8). Macroalgae aerial AFDM and chlorophyll *a* were also higher in the south (Table 2.4, Figure 2.7 and Figure 2.8, Appendix A.9).

Macroalgae, water TN and TP, and environmental variables

The water in southern springs was characterized by higher TP (Figure 2.9, Table 2.5). There was no relation between macroalgae percent cover and macroalgal mat thickness, and TN and TP (Figure 2.10, Figure 2.11, and Table 2.4). There was no relation between *Lyngbya majuscula* percent cover and TN and TP (Figure 2.12, Table 2.4). However, *Vaucheria* sp. percent cover was positively correlated with TN and was not correlated with TP (Figure 2.13, Table 2.4). A threshold in percent cover of *Vaucheria* was indicated near 0.6 mg TN/L (Figure 2.13). Below 0.6 mg TN/L, *Vaucheria* accrual was constrained to about 10% cover or less. The proportion of sites with high *Vaucheria* cover (> 10%) decreased with both TP and TN (Figure 2.13), but these relationships were not evaluated with probability statistics due to time constraints. *Vaucheria* sp. percent cover was positively correlated with alkalinity (Table 2.4). Mat TN and TP content was not correlated with the macroalgae percent cover and mat thickness for all taxa combined or for *Lyngbya* and *Vaucheria* percent cover (Table 2.4). Water alkalinity was not significantly correlated with latitude (Pearson correlation p= 0.686) or longitude (Pearson correlation p= 0.199) (Figure 2.14).

Similarities in the macroalgal assemblages between sites were identified using DCA of macroalgal percent cover data. Axis 1 explained 14.4% and axis 2 explained 11% of the variance in the species data (Figure 2.15). The first axis grouped sites based on conductivity. High conductivity sites HOM-01 and CHA-03, characterized by *Chaetomorpha* sp. and *Enteromorpha* sp., grouped on the right side of the first axis. Sites with lower conductivity grouped on the left side of the first axis. The grouping along the second axis can be partially explained by nutrients and alkalinity. Higher nutrient sites grouped with *Lyngbya majuscula*, *Vaucheria* sp. and *Dichotomosiphon* sp., and grouped opposite to low nutrient sites characterized by *Cladophora* sp., *Hydrodictyon* sp. and *Spirogyra* sp.

Figure 2.9
Water total phosphorus and total nitrogen in spring water at 60 sites in the fall.

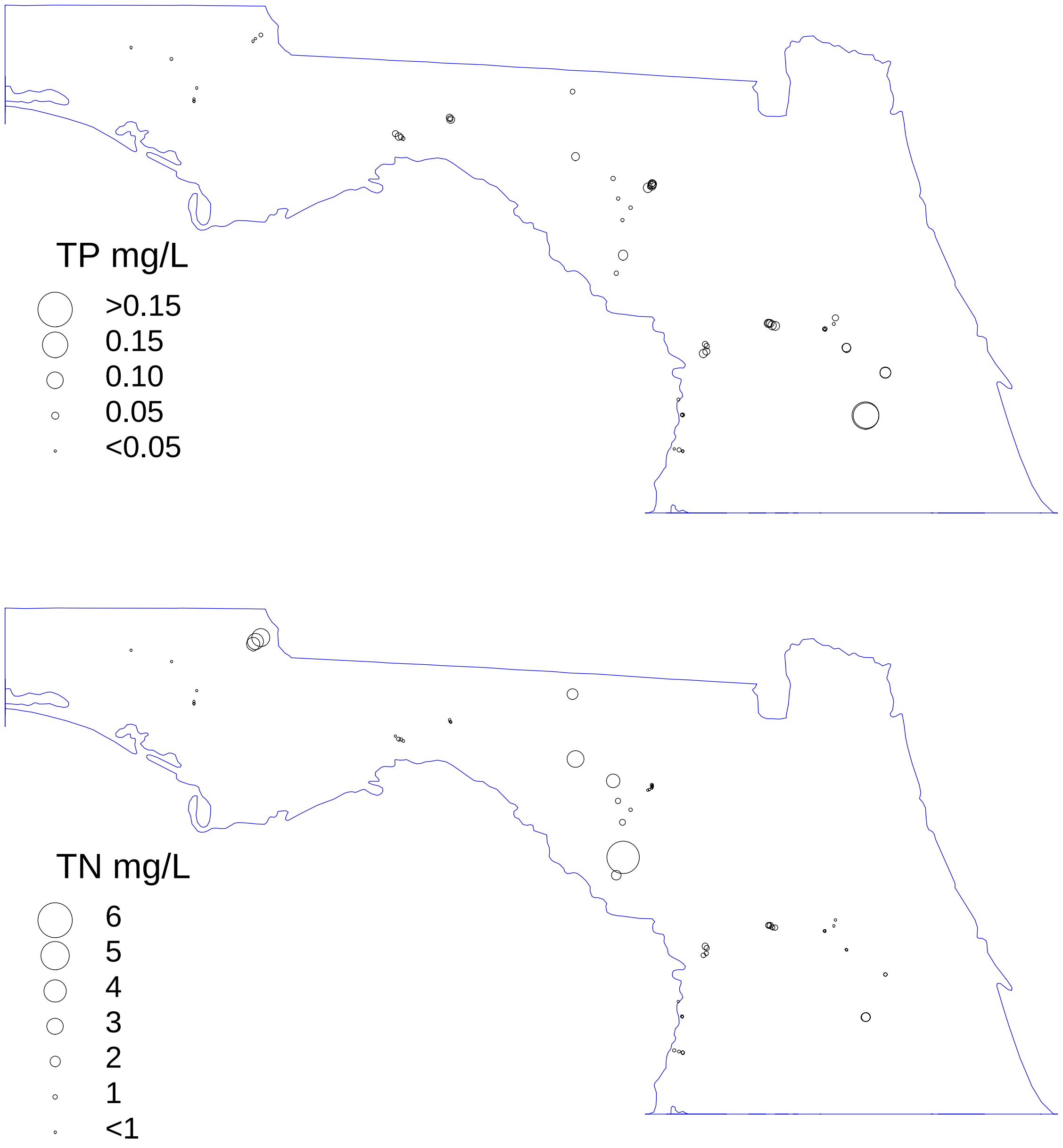


Table 2.5
Pearson correlation matrix for Total P and Total N in the water and environmental variables in the fall ($p<0.05$ in bold).

	TOTALP	TOTALN
ALKALINITY	0.197	**0.414**
AMMONIAN	-0.143	**0.307**
CALCIUM	0.139	**0.393**
CHLORIDE	-0.034	-0.166
IRON	-0.001	-0.014
N_KJEL_TOT	0.220	0.115
NO2NO3N	0.173	**0.998**
PHOSPHATE	**0.894**	0.154
SILICA	**0.288**	**-0.310**
SODIUM	-0.033	-0.169
SULFATE	0.096	-0.137
TOTAL P		0.186
TOTAL N	0.186	
MAGNESIUM	0.050	-0.155
STRONTIUM	0.139	-0.201
LATITUDE	**-0.286**	0.113
LONGITUDE	**0.452**	-0.059
PH	**-0.320**	**-0.466**
CONDUCTIVITY	-0.017	-0.096
DO	**-0.339**	0.004
TEMPERATURE	**0.300**	0.067
CANOPY	-0.109	-0.143
BUFFER	0.093	**-0.307**
Transect Length	-0.062	**0.325**
TREES	0.236	-0.178
SHRUBS	-0.018	-0.193
HERBS	-0.036	-0.119
BARE	-0.020	**0.268**
LDI	0.065	0.168
DISCHARGE	0.119	0.135

Figure 2.10
Macroalgae percent cover (PCCOVER) as a function of water TN and TP for all sites (scatter plots) and grouped into 3 categories: 0-25%, 25-50% and over 50% (bar plots) in the fall.

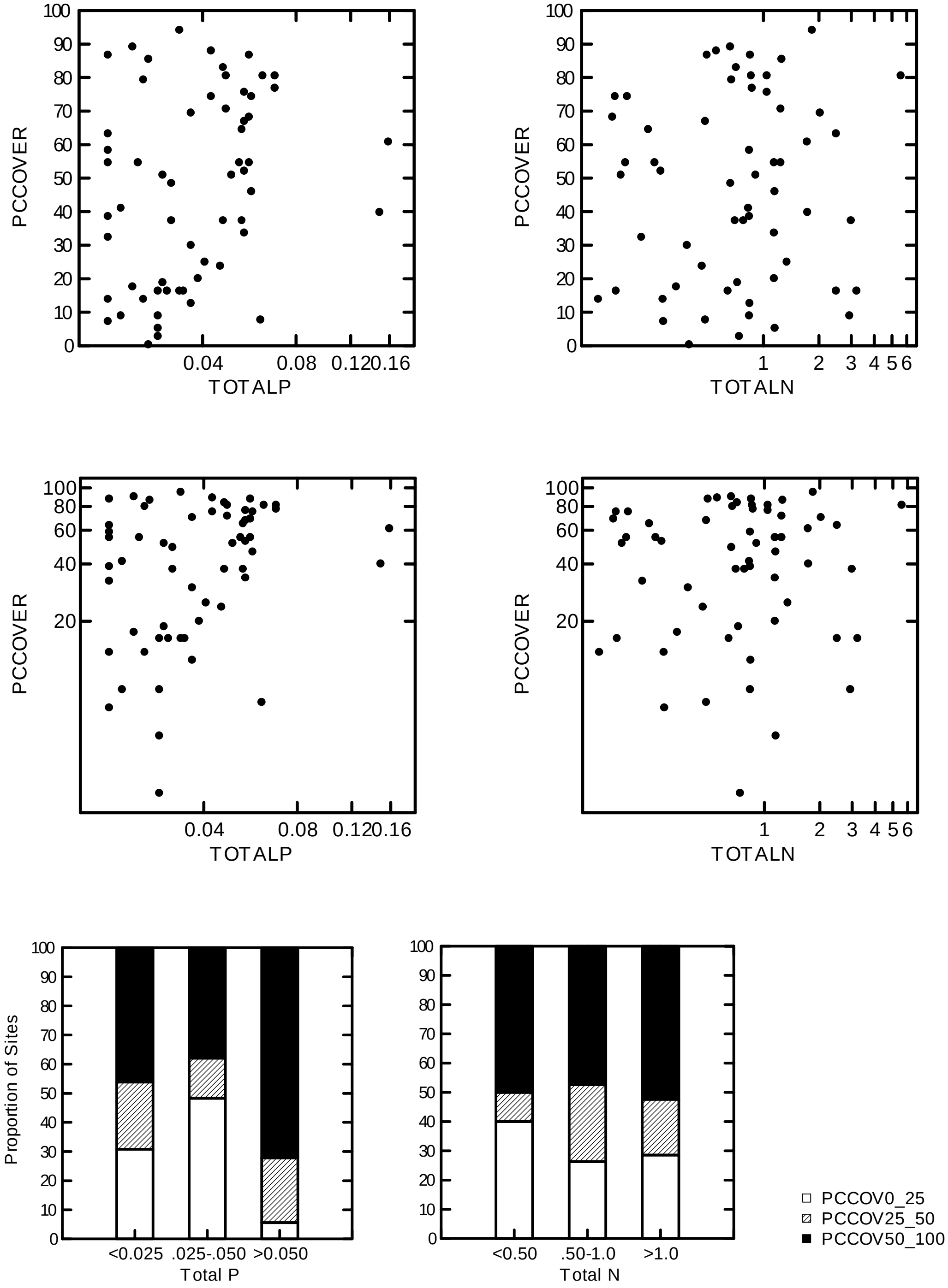


Figure 2.11
Macroalgae average cover thickness (AVGCOVTHK) as a function of water TN and TP for all sites (scatter plots) and grouped into 3 categories: 0-3 cm, 3-8 cm and over 8 cm (bar plots) in the fall.

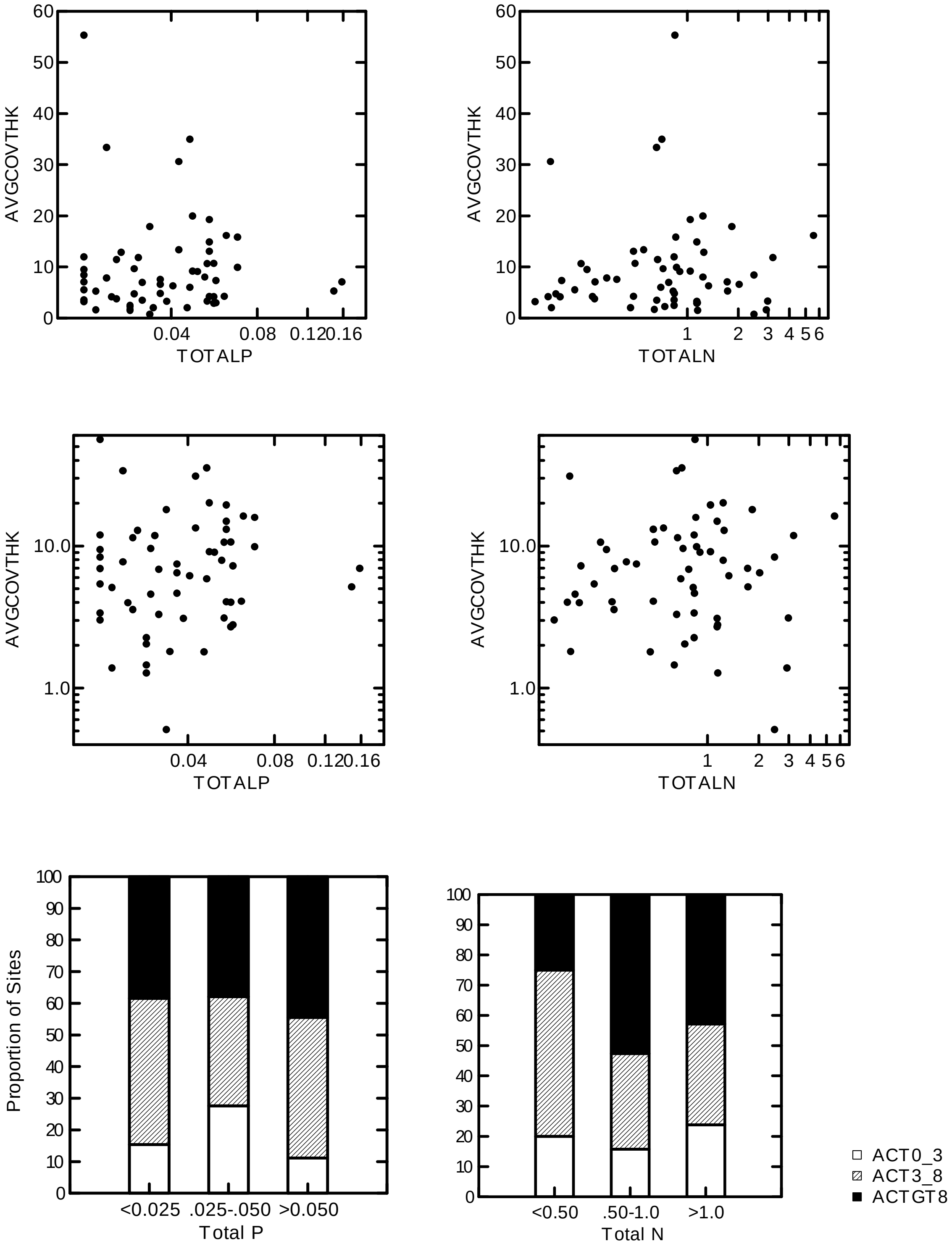


Figure 2.12
Lyngbya majuscula percent cover as a function of water TN and TP for all sites (scatter plots) and grouped into 3 categories: 0%, 0-10% and over 10% (bar plots).

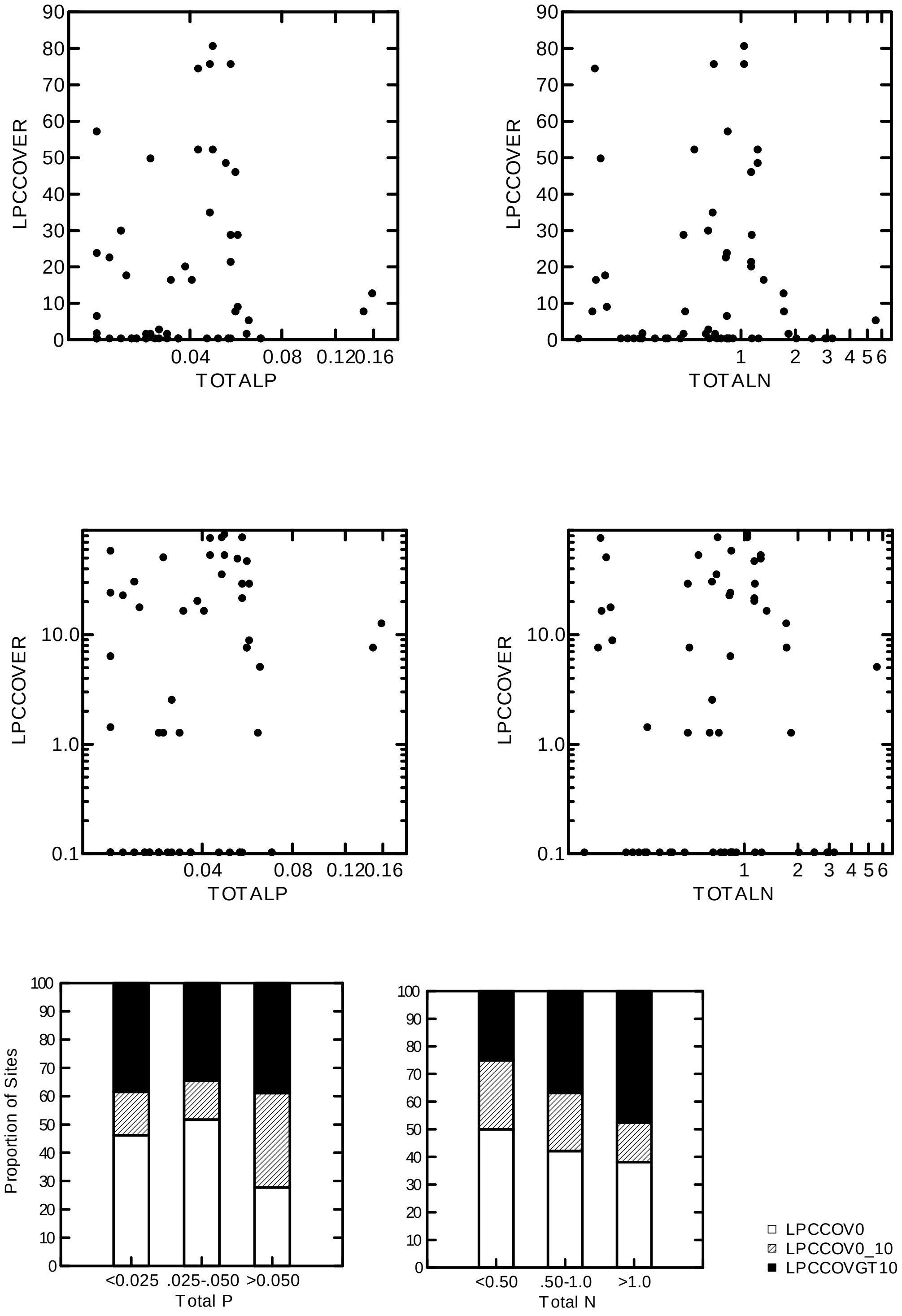


Figure 2.13
Vaucheria sp. percent cover as a function of water TN and TP for all sites (scatter plots) and grouped into 3 categories: 0%, 0-10% and over 10% (bar plots).

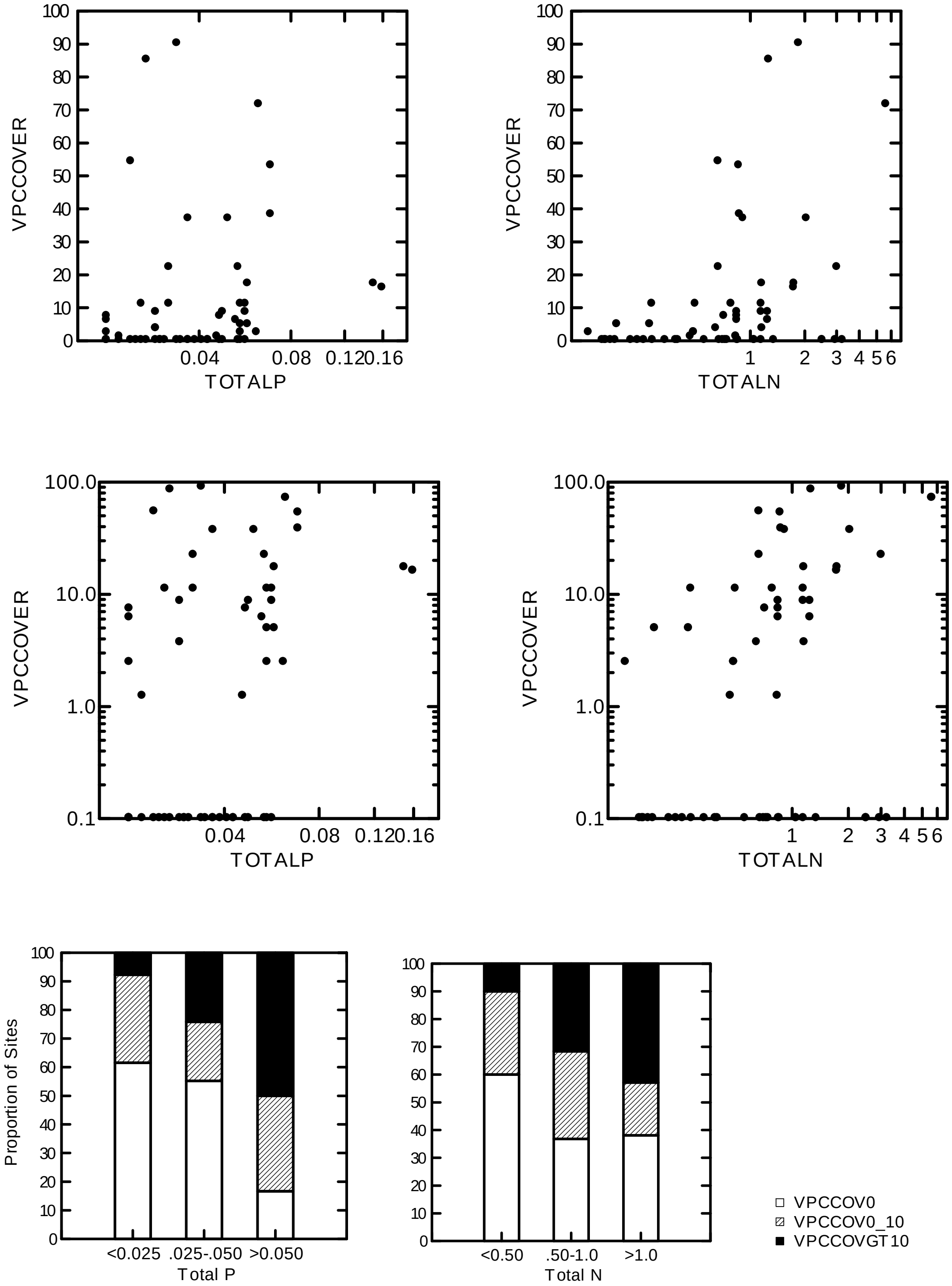


Figure 2.14
Alkalinity of spring water at 60 sites in the fall.

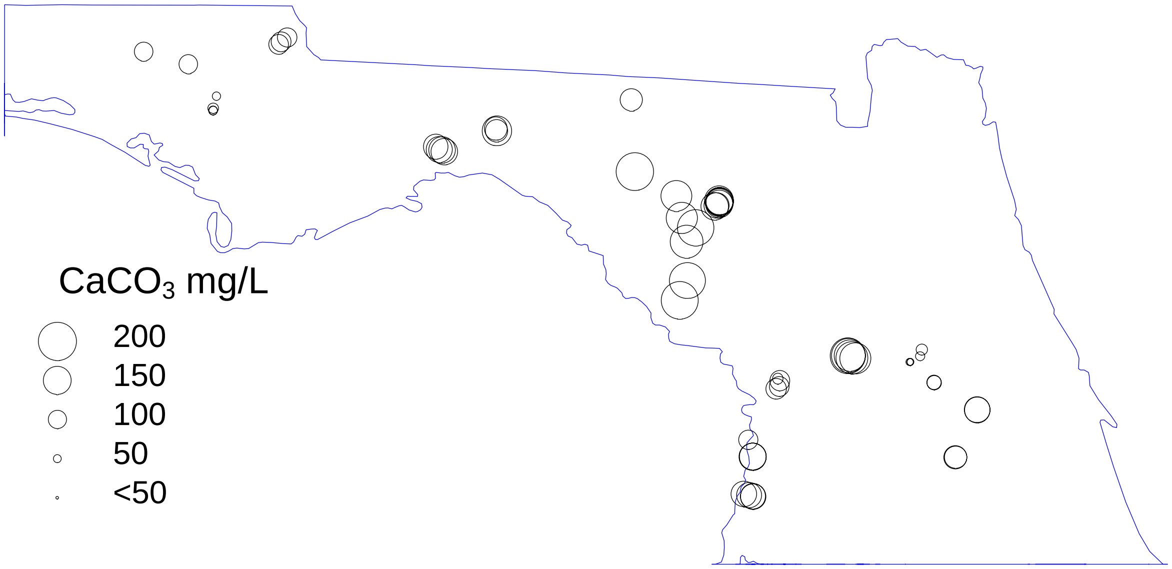


Figure 2.15
Ordination diagram based on DCA of macroalgal percent cover (60 sites and 20 species of macroalgae) displaying 25% of variance. Eigenvalues for the first three axes are 0.363, 0.277 and .0173. First and second axis were well correlated with environmental variables (r=0.872 for the first axis and r=0.911 for the second axis).

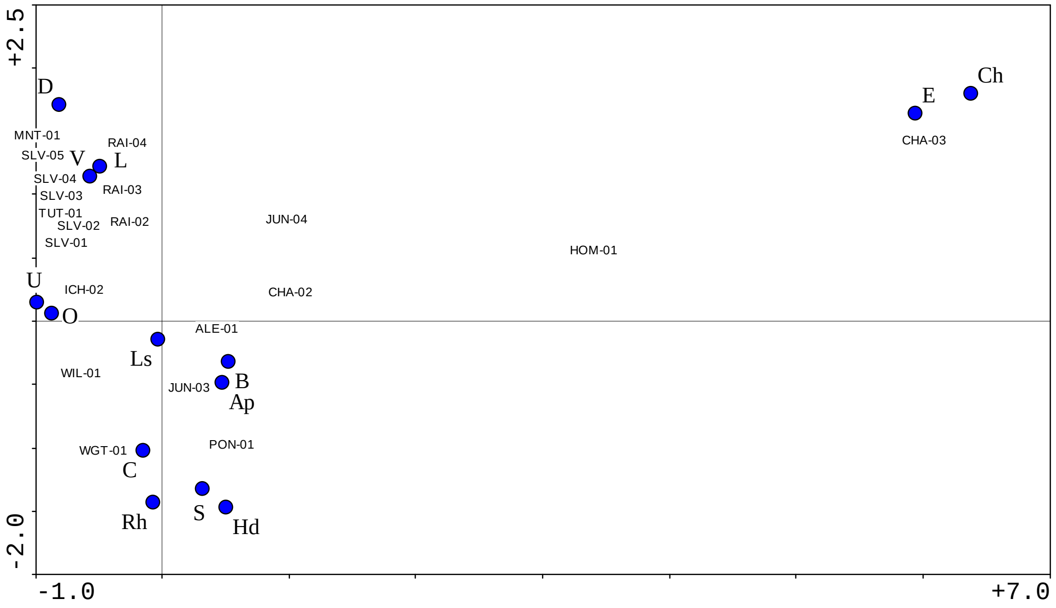


Figure 2.16
Correlation triplot based on CCA of the macroalgal percent cover (20 macroalgae species, 60 sites and 16 environmental variables). Number of sites and species presented were restricted by species weight and site fit. Eigenvalues of the first three axes are 0.319, 0.222 and 0.201. The sum of all canonical eigenvalues is 1.418. Significance of all canonical axes together was tested by Monte Carlo permutation test F-ratio=2.109, p=0.005.

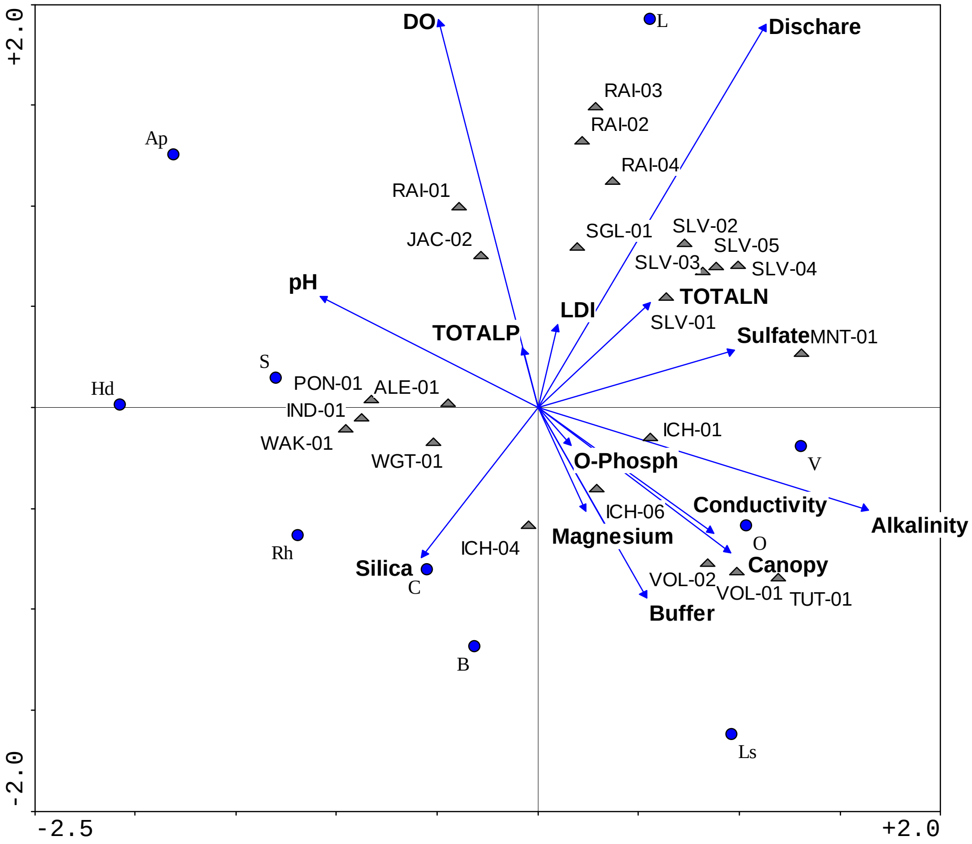


Figure 2.17
Correlation biplot based on CCA of the macroalgae percent cover (all data as on Figure 1.*2, only species and environmental variables are shown).

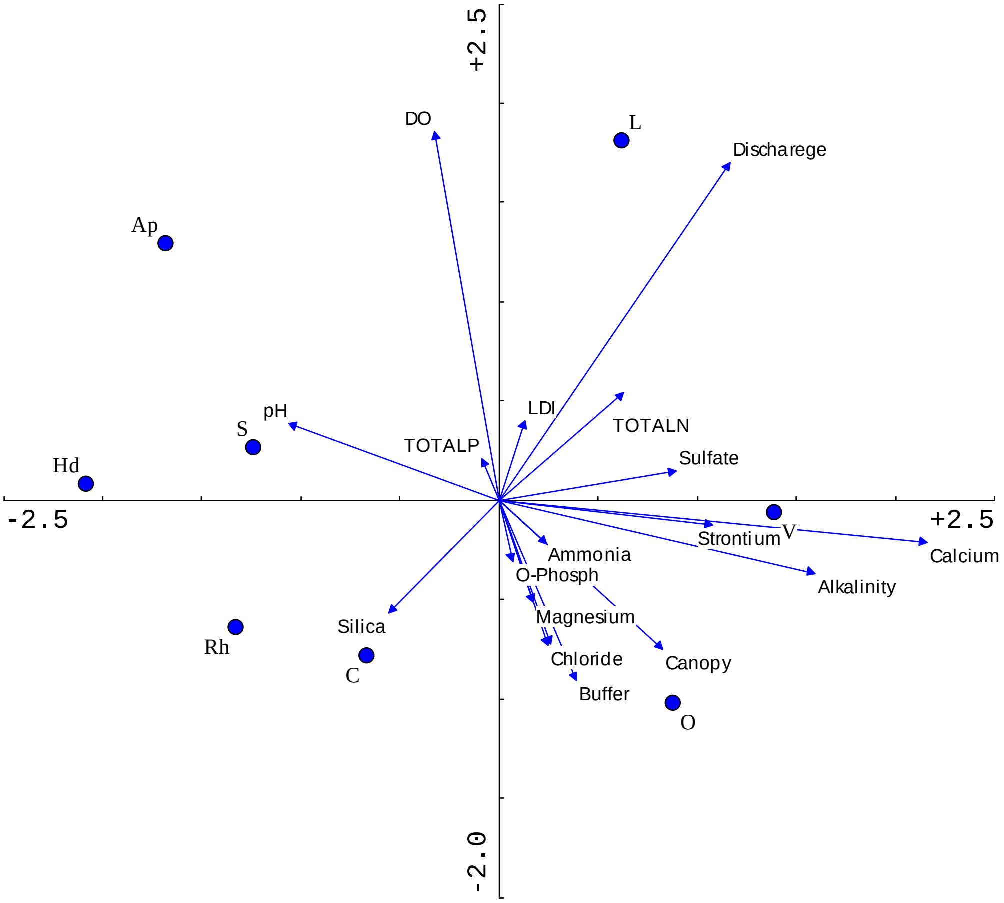


Figure 2.18
Relation between macroalgae mat thickness and distance from the boil. Macroalgae thickness was measured as up to 0.5cm, 0.5 – 2 cm indicated as 2 cm, 2 -5 cm indicated as 5 cm, 5-20 cm indicated as 20 cm, 20-50 cm indicated as 50 cm and over 50 cm indicated as 100 cm.

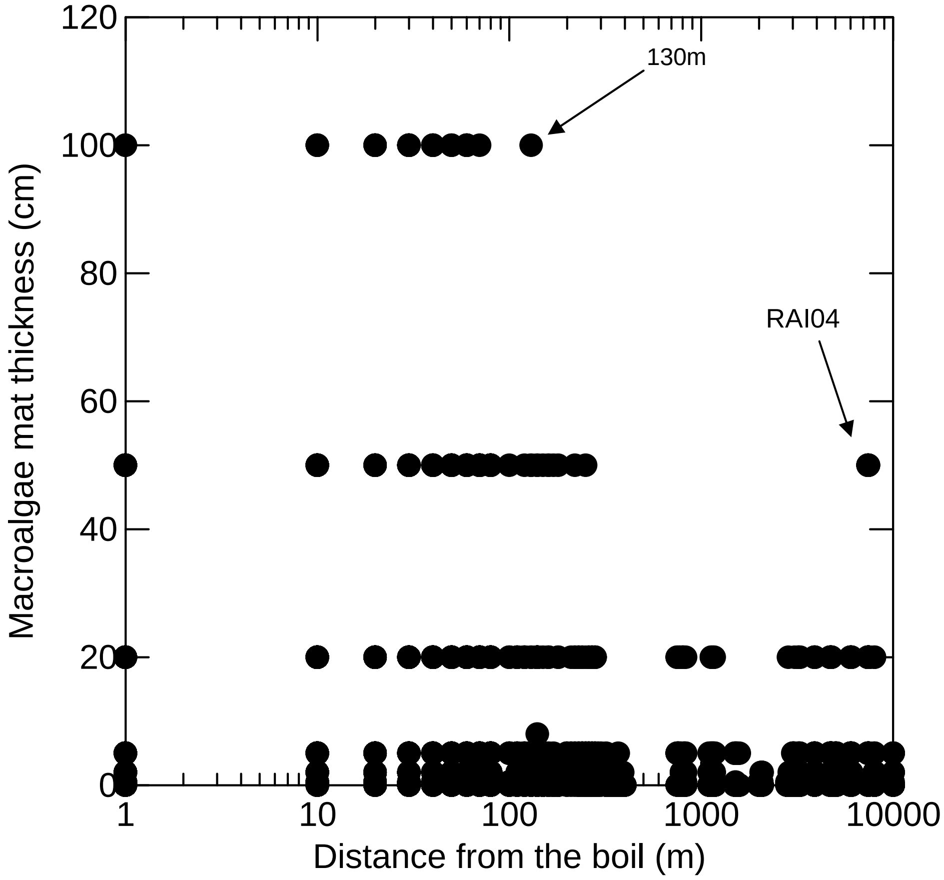


Figure 2.19
Relation between macroalgae mat thickness and water depth. Macroalgae thickness was measured as up to 0.5 cm, 0.5 – 2 cm indicated as 2 cm, 2 -5 cm indicated as 5 cm, 5-20 cm indicated as 20 cm, 20-50 cm indicated as 50 cm and over 50 cm indicated as 100 cm.

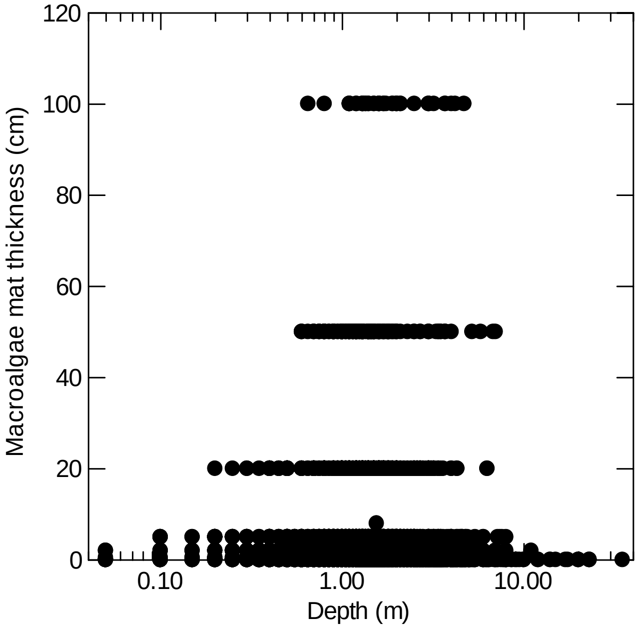
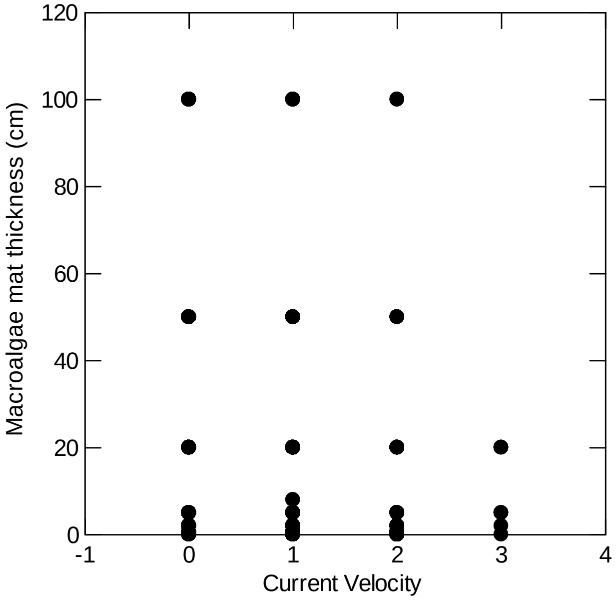


Figure 2.20
Relation between macroalgae mat thickness and estimated current velocity (0 – current velocity less than 5 cm/s, 1 – current velocity 5-15 cm/s, 2- current velocity 16-50 cm/s, 3 – current velocity above 50 cm/s). Macroalgae thickness was measured as up to 0.5 cm, 0.5 – 2 cm indicated as 2 cm, 2 -5 cm indicated as 5 cm, 5-20 cm indicated as 20 cm, 20-50 cm indicated as 50 cm and over 50 cm indicated as 100 cm.



Since high conductivity sites (HOM-01 and CHA-03) and taxa (*Enteromorpha* sp. and *Chaetomorpha* sp.) affected grouping in DCA, these sites and taxa were removed from CCA analysis. The first CCA axis grouped samples according to nutrient levels, alkalinity and pH (Figure 2.16), while the second axis grouped samples according to spring discharge, DO, silica and magnesium. *Lyngbya majuscula* was associated with high nutrient and high discharge sites, and *Vaucheria* sp. was associated with high alkalinity sites. Diatoms were associated with high silica sites, and *Hydrodictyon* sp. and *Spirogyra* sp. were associated with high pH sites (Figure 2.17).

Macroalgae mat thickness, water depth, distance from the boil and current velocity

Analysis of macroalgae cover thickness based on RPHA data collected for 4851 points in the fall indicated that the thickest macroalgal mats (over 50 cm thick) were found only within 130 m from the boil and mats 20 to 50 cm thick were found within 250 m from the boil (Figure 2.18). The only site that had a mat 20 to 50 cm thick occurring far from the boil was RAI-04. This site is affected by an old phosphate mine pit called Blue Cove. Thicker macroalgal mats were found in water between 1 and 10 m deep (Figure 2.19). Current velocity did not affect macroalgal mat thickness (Figure 2.20).

Macroalgal mat TN and TP

Macroalgae mat TN and TP content was the highest for macroalgae from Ichetucknee Springs (Figure 2.21, Appendix A.10). Mat TN and TP content was positively correlated with water phosphate and was not affected by water TN and TP (Table 2.6). The molar TN:TP ratio in mats was greater than the 16:1 Redfield ratio for most of the study sites ((Borchardt, 1996), Figure 2.22). Only 6 sites (WEK-03, WKW-01, VOL-01, MNT-01, ICH-07 and ICH-06) had a TN/TP ratio below 16:1, indicating the phosphate limitation of macroalgal growth if nutrient concentrations were low enough to regulate algal growth.

Algal Limiting Nutrients (ALN) and Algal Growth Potential (AGP) bioassays

Phosphorus was the limiting nutrient at 15 and nitrogen at 6 out of 27 sites in the ALN bioassay (Appendix A.11). Five sites were co-limited by both phosphorus and nitrogen. Algal growth potential was highest for the water sample from WKW-01 and lowest for the water sample from MNT-01 (Appendix A.11).

Epiphytes

Epiphyte DM, AFDM, and chlorophyll *a* varied among study sites (Figure 2.23, Appendix A.9) and were as high as 7 mg/cm2, 6 mg/cm^2, and 40 µg/cm^2, respectively. Epiphyte AFDM and chlorophyll *a* were not correlated with any of the environmental variables (Figure 2.24, Table 2.7). DM was correlated with chloride, iron, sodium, magnesium, sulfate and conductivity (Table 2.7). Epiphyte thickness estimated in RHPA varied from slimy to over 5 mm (Appendix A.12). Excluding diatoms, 62 taxa of soft epiphytic algae were identified (Appendix A.13; pictures of dominant taxa can be found in Appendix A.14). Epiphytic algal cell density per unit plant leaf surface area can be found in Appendix A.15.

Table 2.6
Pearson correlation matrix for macroalgal mat TN and TP content and environmental variables. Mat TN and TP measured in FM mg/Kg (p<0.05 in bold).

	Mat TN	Mat TP	Mat molar TN/TP
ALKALINITY	0.187	0.174	-0.250
AMMONIAN	-0.109	-0.112	0.097
CALCIUM	0.062	0.044	-0.242
CHLORIDE	-0.142	-0.141	0.017
IRON	-0.154	-0.127	0.030
N_KJEL_TOT	-0.202	-0.161	-0.238
NO2NO3N	-0.155	-0.145	0.096
PHOSPHATE	**0.284**	**0.384**	**-0.420**
SILICA	0.210	0.244	-0.150
SODIUM	-0.141	-0.140	0.016
SULFATE	-0.150	-0.130	-0.115
TOTAL P	0.108	0.251	**-0.440**
TOTAL N	-0.166	-0.154	0.080
MAGNESIUM	-0.138	-0.126	-0.036
STRONTIUM	-0.135	-0.123	-0.195
LATITUDE	0.170	0.098	0.254
LONGITUDE	-0.112	-0.151	**0.385**
PH	0.059	0.053	**0.301**
CONDUCTIVITY	-0.133	-0.132	-0.007
DO	-0.213	-0.209	**0.282**
TEMPERATURE	-0.180	-0.112	-0.150
CANOPY	**0.320**	**0.334**	0.110
BUFFER	**0.405**	**0.380**	-0.130
Transect Length	-0.222	-0.235	0.176
TEES	0.078	0.070	0.071
SHRUBS	**0.351**	**0.320**	-0.078
HERBS	0.169	0.181	0.097
BARE	-0.224	-0.188	-0.120
LDI	-0.133	-0.099	-0.084
DISCHARGE	-0.193	-0.180	-0.031

Table 2.7
Pearson correlation matrix for epiphyte DM, AFDM (mg/cm^2), epiphyte chlorophyll a and pheophytin (µg/cm^2) per leaf surface area and environmental variables in the fall ($p<0.05$ in bold).

	DM	AFDM	Chl *a*	Pheophytin
ALKALINITY	-0.077	-0.170	0.078	0.017
AMMONIAN	**0.324**	0.102	-0.056	-0.074
CALCIUM	0.055	-0.115	0.052	-0.026
CHLORIDE	**0.435**	0.194	-0.055	-0.065
IRON	**0.369**	0.185	-0.103	0.015
N_KJEL_TOT	**0.286**	0.139	-0.058	-0.054
NO2NO3N	-0.017	-0.095	-0.072	-0.072
PHOSPHATE	-0.062	0.040	0.201	-0.040
SILICA	0.167	0.162	0.181	0.176
SODIUM	**0.434**	0.196	-0.053	-0.063
SULFATE	**0.301**	0.118	-0.045	-0.052
TOTAL P	-0.024	0.058	0.080	-0.050
TOTAL N	0.001	-0.085	-0.076	-0.075
MAGNESIUM	**0.406**	0.170	-0.060	-0.041
STRONTIUM	0.198	0.069	-0.018	-0.054
LATITUDE	-0.006	-0.071	0.079	0.191
LONGITUDE	0.073	0.168	0.105	-0.067
PH	0.071	0.145	0.042	-0.013
CONDUCTIVITY	**0.407**	0.156	-0.065	-0.083
DO	0.169	0.076	-0.128	-0.071
TEMPERATURE	0.117	0.035	-0.077	**-0.260**
CANOPY	-0.222	-0.039	0.073	-0.157
BUFFER	0.085	0.022	0.204	0.006
Transect Length	**0.314**	0.092	-0.056	0.092
TEES	0.164	0.100	0.072	0.037
SHRUBS	0.152	0.235	0.234	0.065
HERBS	0.109	0.151	0.157	0.196
BARE	-0.212	-0.199	-0.146	-0.055
LDI	0.080	-0.072	-0.106	-0.187
DISCHARGE	-0.046	-0.114	-0.076	0.003

Table 2.8
Pearson correlation matrix for epipelon DM, AFDM (mg/cm^{2}), epipelon chlorophyll a and pheophytin (µg/cm^{2}) and environmental variables for spring and fall data combined ($p<0.05$ in bold).

	DM	AFDM	Chl *a*	Pheophytin
ALKALINITY	-0.026	**0.269**	-0.06	0.045
AMMONIAN	-0.026	0.026	0.064	0.01
CALCIUM	-0.11	0.147	-0.091	-0.002
CHLORIDE	0.055	-0.047	0.047	0.019
IRON	0.085	0.076	-0.095	-0.067
N_KJEL_TOT	-0.013	-0.121	0.146	0.097
NO2NO3N	-0.127	-0.052	0.011	-0.031
PHOSPHATE	-0.113	-0.103	0.065	-0.099
SILICA	**0.325**	0.339	-0.163	-0.019
SODIUM	0.053	-0.052	0.033	-0.001
SULFATE	-0.026	-0.033	-0.063	-0.042
TOTAL P	-0.068	-0.035	0.08	-0.091
TOTAL N	-0.127	-0.059	0.02	-0.025
MAGNESIUM	0.098	0.039	0.006	0.012
STRONTIUM	-0.053	-0.027	-0.036	-0.047
LATITUDE	-0.019	0.158	**-0.305**	-0.149
LONGITUDE	-0.02	-0.123	0.146	0.017
PH	0.078	-0.059	0.043	-0.035
CONDUCTIVITY	0.041	-0.024	0.045	0.026
DO	0.235	0.255	0.129	0.113
TEMPERATURE	-0.045	-0.094	**0.297**	0.144
CANOPY	-0.252	**-0.259**	-0.231	-0.137
BUFFER	0.045	**0.284**	-0.112	0.019
Transect Length	0.172	0.234	0.089	0.094
TREES	**0.385**	**0.453**	0.120	0.097
SHRUBS	-0.027	-0.136	0.057	-0.042
HERBS	0.043	-0.150	**0.277**	**0.260**
BARE	-0.185	-0.237	0.135	0.123
LDI	-0.014	0.031	**0.394**	**0.495**
DISCHARGE	0.222	**0.398**	0.186	-0.002

Figure 2.21
Macroalgal mat TN and TP content in FM mg/Kg.

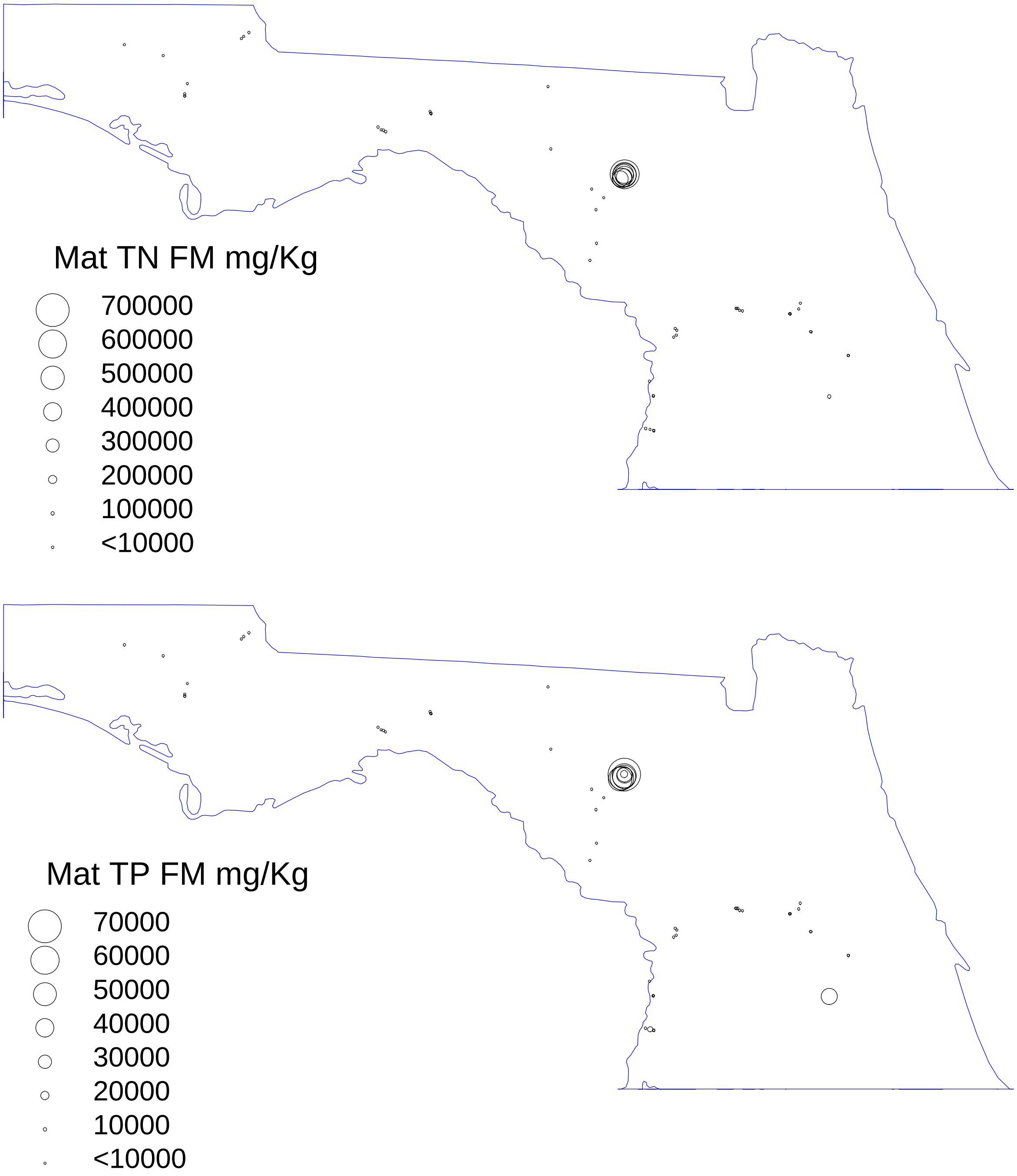


Figure 2.22
Macroalgal mat molar TN/TP ratio.

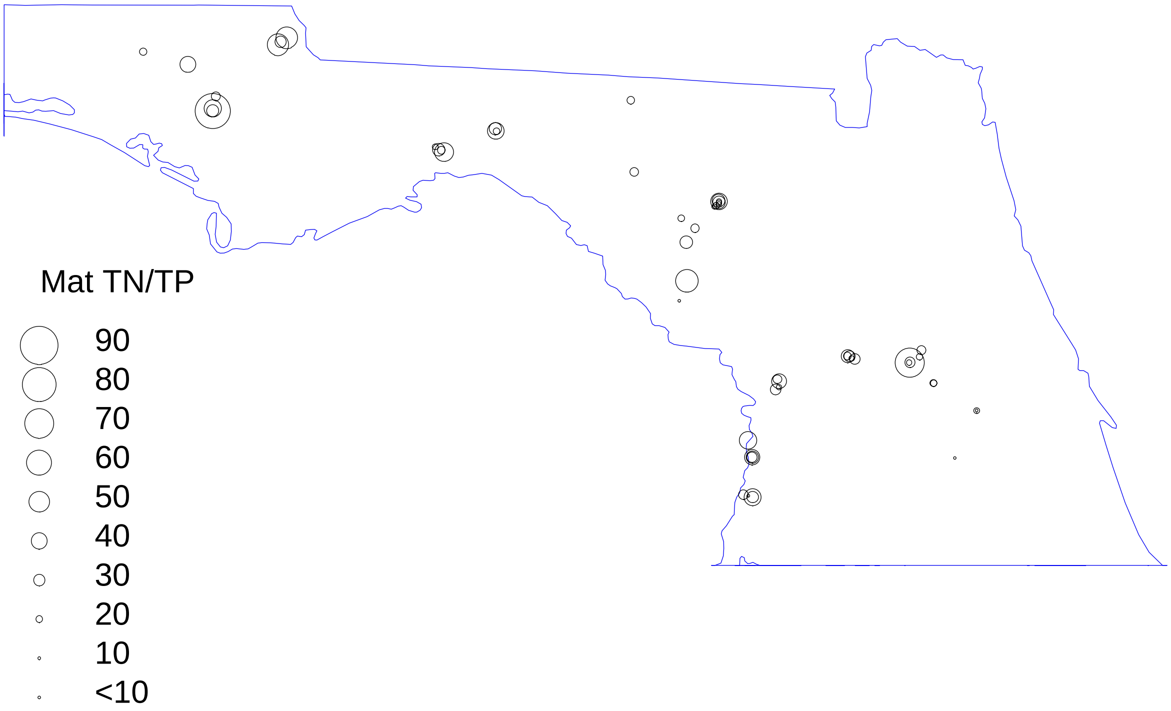
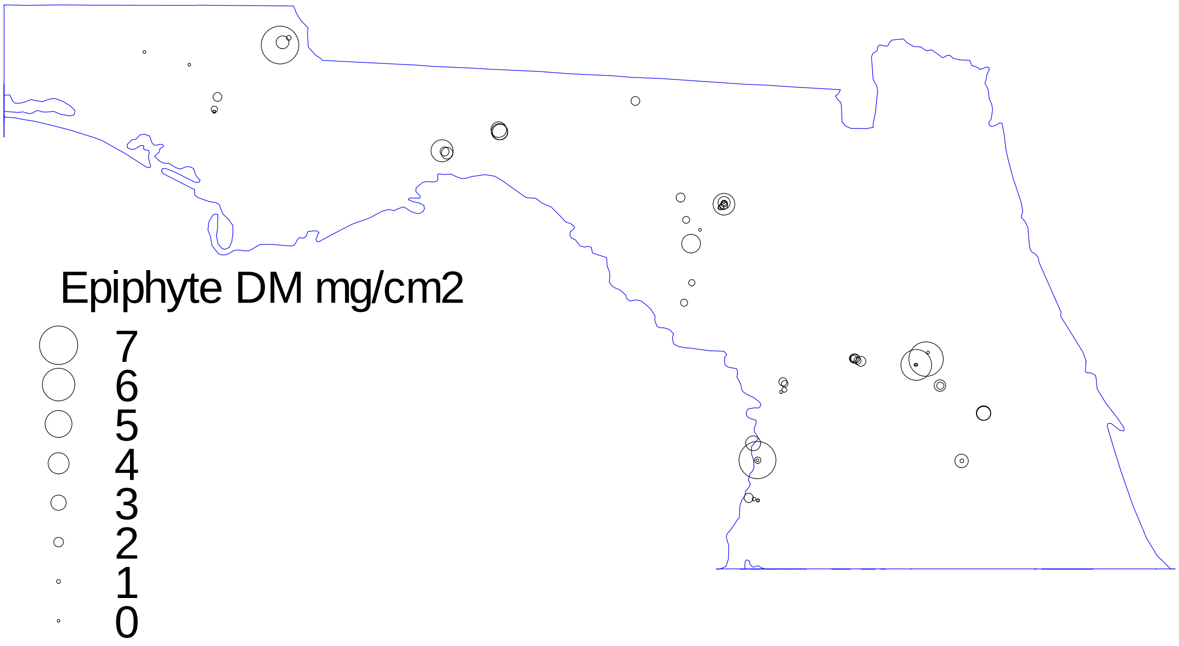


Figure 2.23
Epiphyte DM and AFDM expressed per surface of leaf surface are at 58 sites in the fall.



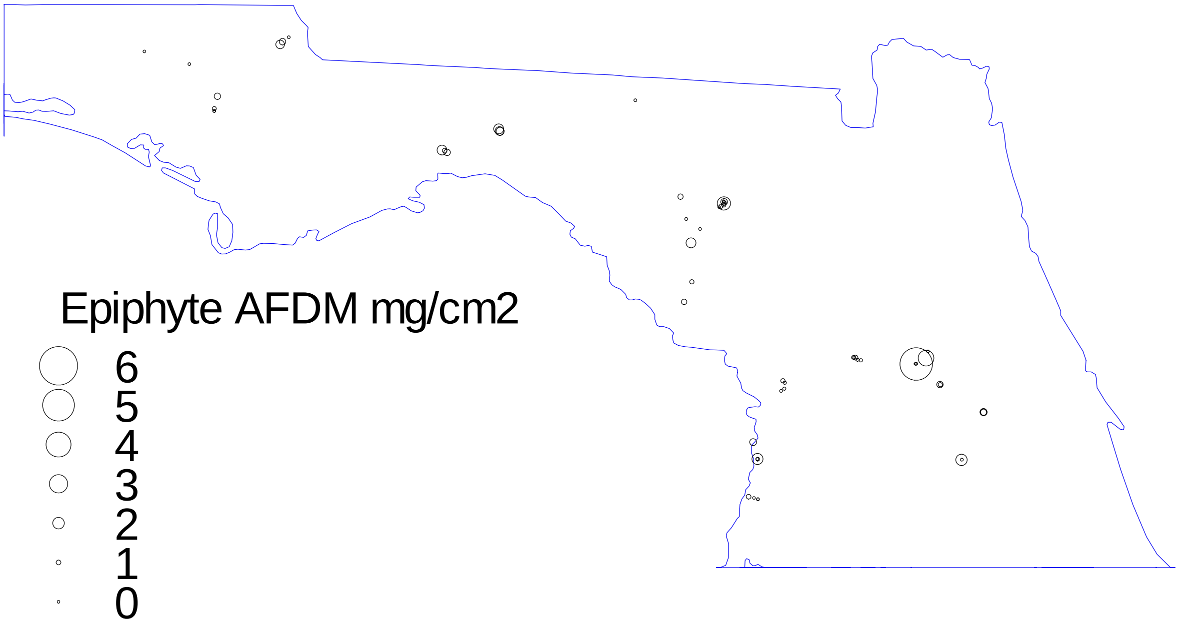


Figure 2.24
Epiphyte chlorophyll a and pheophytin expressed per surface of leaf surface are at 58 sites in the fall.

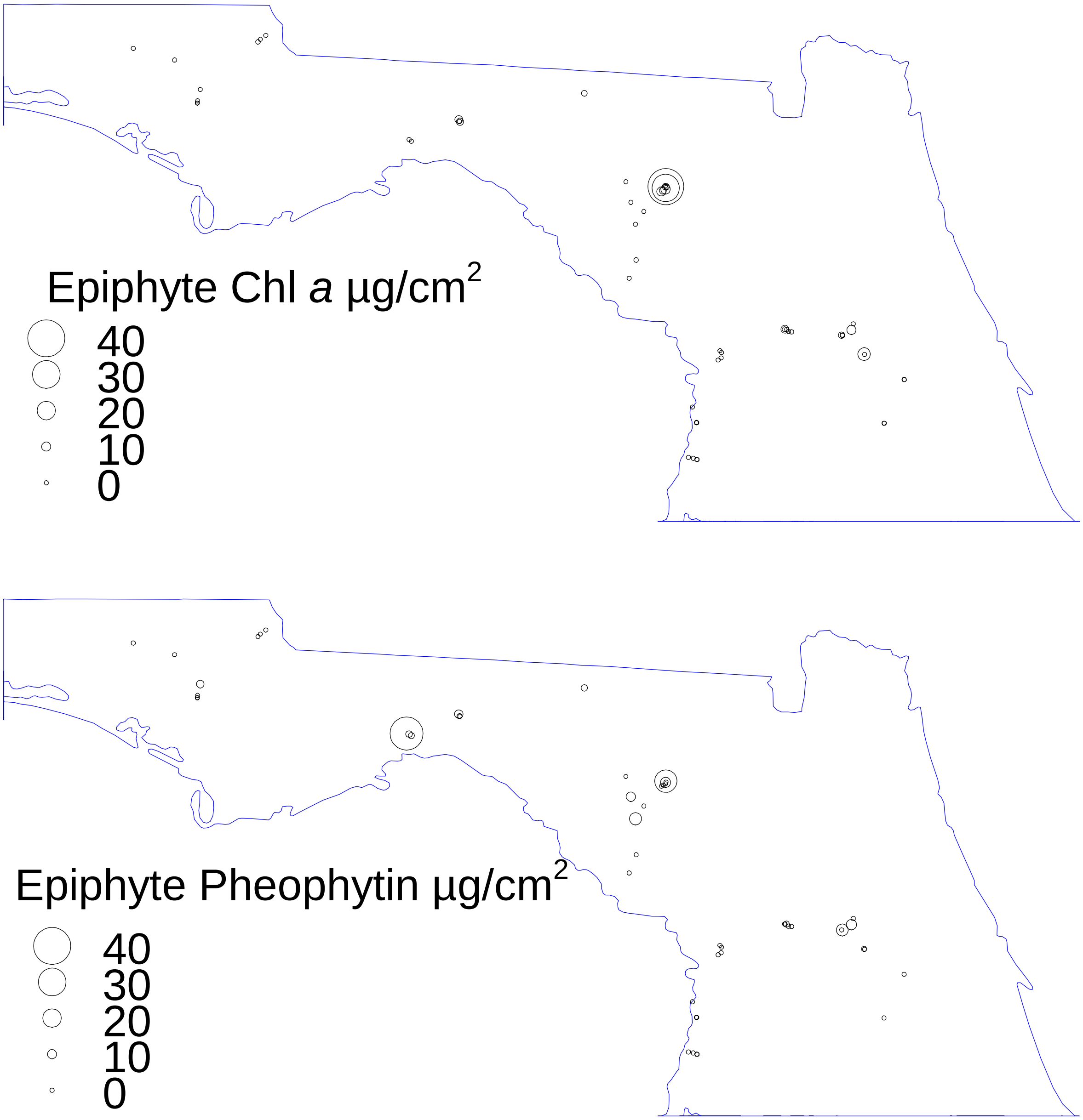


Figure 2.25
Epipelon DM and AFDM expressed per bottom surface area at 59 sites (based on spring and fall sampling).

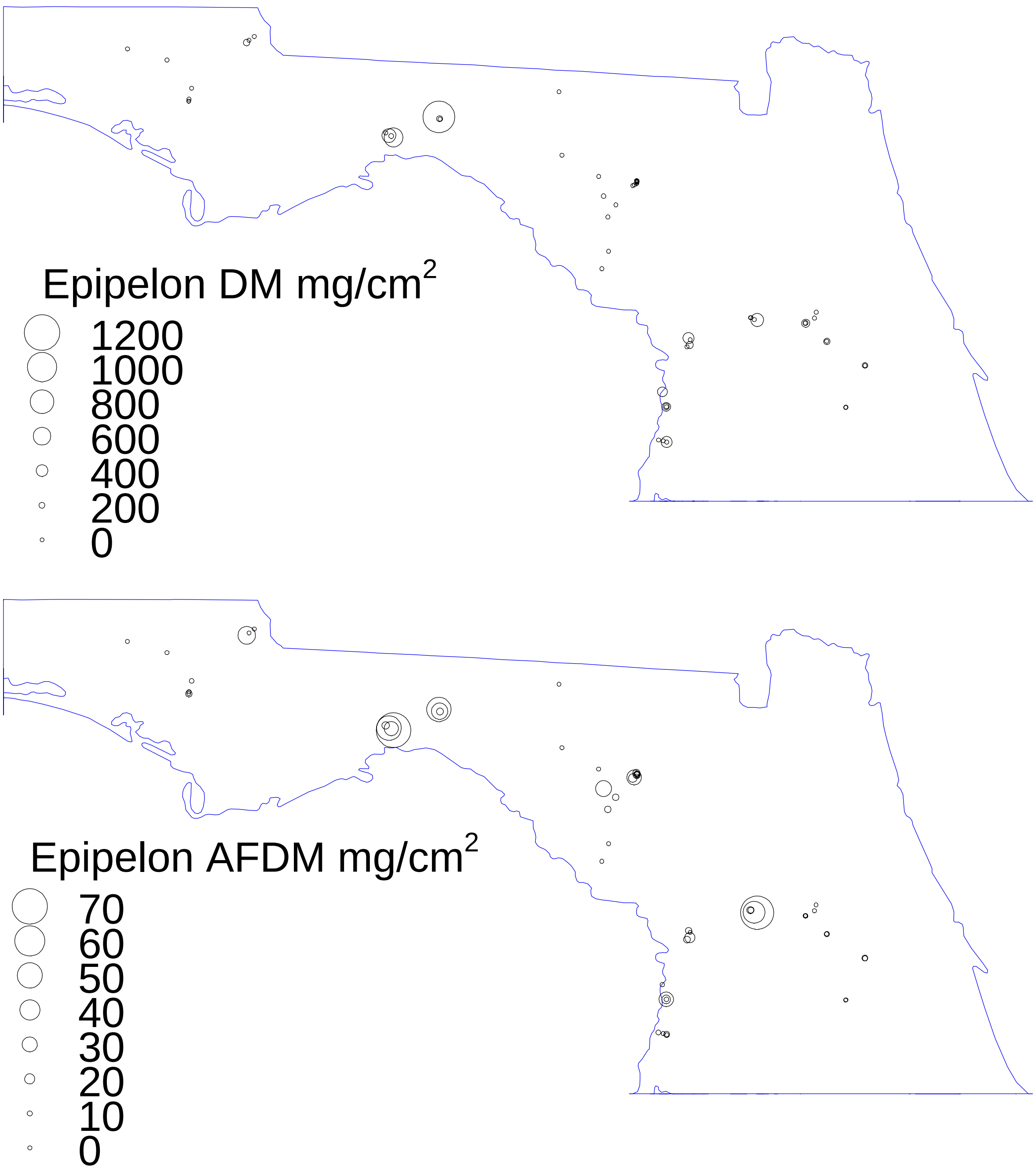


Figure 2.26

Epipelon chlorophyll a and pheophytin expressed per bottom surface area at 59 sites (based on spring and fall sampling).

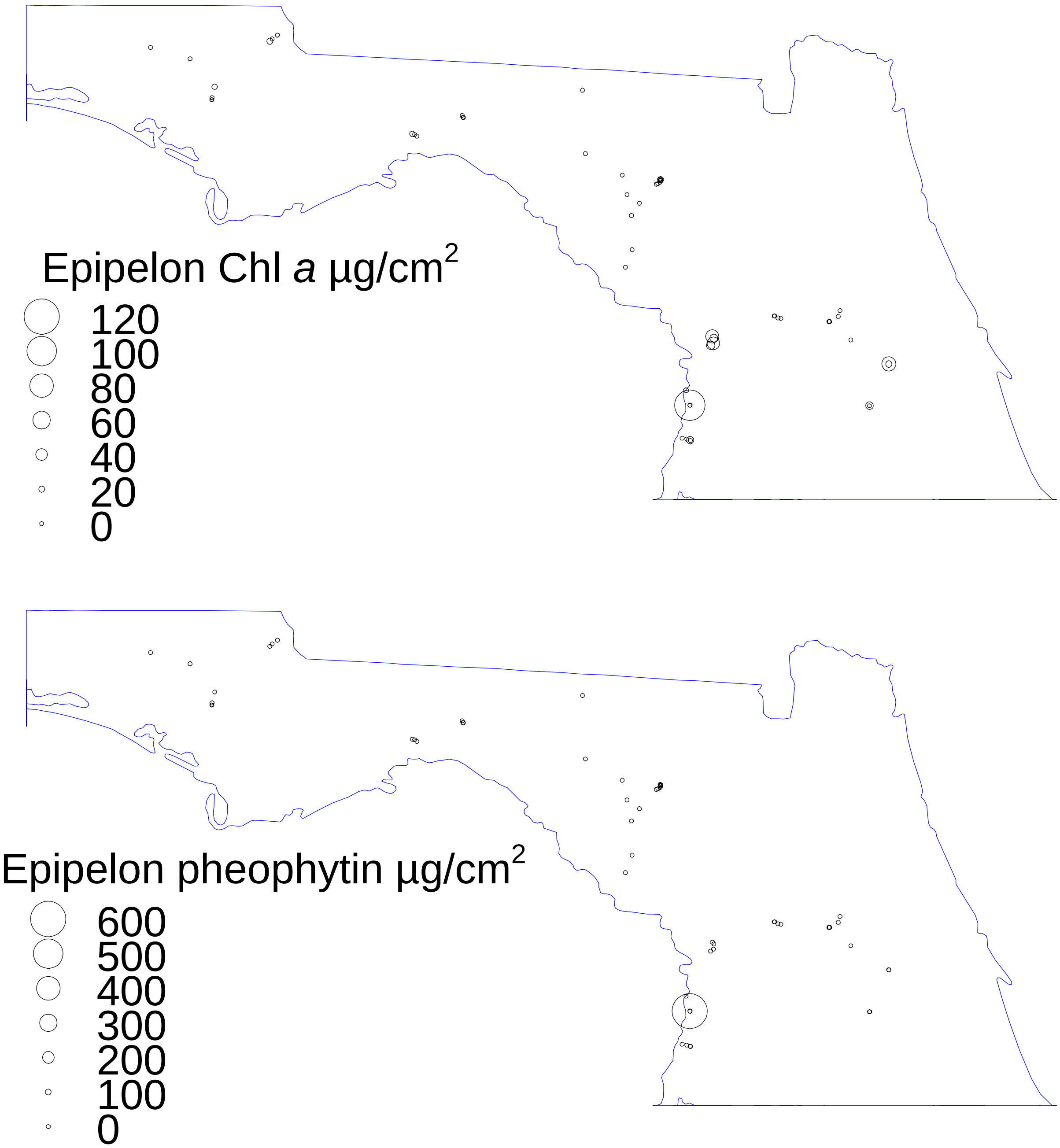


Table 2.9
Aquatic plant taxa found in Florida Springs and codes for aquatic plants used in this study.

Taxa	Aquatic plant code
Atternantherea philoxeeroides	A
Ceratophyllum sp.	Ce
Chara sp.	Ch
Cladium jamaicense	Cj
Cobamba sp.	Co
Crinum americanum	Sl
Cycuta sp.	Ct
Egeria densa	Ed
Eichhornia crassiper	W
Eleocharis sp.	Sg
Hydrilla sp.	H
Hydrocotil sp.	Hy
Hygrophila polysperma	Hp
Lemna sp.	Le
Ludwigiabsp.	Lu
moss	Mo
Myriophyllum sp.	M
Najas guadalupensis	N
Najas sp.	N2
Nuphar sp.	Nu
Nymphoides sp.	Y
Panicum repens	Tr
Pistia stratiotes	Pi
Pontedaria sp.	Pn
Potamogeton pectinatus	Pp
Potamogeton pusillus	Pu
Potamogeton spp.	Ps
Riccia sp.	Rc
Rorippa sp.	R
Sagittaria kurziana	Sk
Sagittaria latifolia	St
Salvinia minima	Sm
Scirpus sp.	Br
Typha sp.	T
Utricularia sp.	U
Vallisneria sp.	Val
Zizania sp.	Z

Epipelon

Epipelon AFDM was correlated with large springs with high discharge (like SLV, WAK and WAC), indicating that a higher flow can support a larger biomass and greater deposition of organic material into the sediments. It was also positively correlated with tree cover (Figure 2.25, Table 2.8, and Appendix A.9). Epipelon chlorophyll *a* and pheophytin were positively correlated with the Land Development Intensity index (LDI) (Figure 2.26). Epipelon thickness estimated in RHPA varied from slimy to over 5 mm (Appendix A.12).

Aquatic plants

In IND-01 and LAF-01, no aquatic plants were found. The remaining sites had aquatic plants present, with the highest aquatic plant cover at ICH-04 (Appendix A.16). A total of 37 taxa of aquatic plants were found (Table 2.9). The most common were *Vallisneria* sp. and *Sagittaria kurziana* (Appendix A.17). At many sites both were present, and when sampling was conducted from a boat, there was a potential for confusion of the two.

Seasonal changes

AFDM and pheophytin measurements did not differ between spring and fall (repeated measures ANOVA for time effect AFDM p=0.8641, pheophytin p=0.4579). DM and chlorophyll *a* measurements differed between spring and fall (repeated measures ANOVA for time effect DM p=0.0033, Chl a p=0.0170) (results of analysis in Stats/AFDM_ChlaRMANOVA.doc).

Discussion

Macroalgae were found at almost all of the studied sites and on average, covered about half of the bottom area of the spring reaches studied. *Lyngbya* and *Vaucheria* were the most commonly observed macroalgae. Many of the macroalgal taxa observed in this study were also reported in previous studies (Davis and Gworek, 1972; Odum, 1957; Whitford, 1956). However, a large *Lyngbya* taxon, which we identified as *L. majuscula*, was not reported in any of these past studies. This indicates that it was not common or was not present at these sites in the past.

Nutrients were not the most important factors affecting geographic distribution of different macroalgal taxa. Conductivity, alkalinity, pH, and factors related to the size of springs were indicated by ordination statistics as the most important determinants. This is one of the first surveys of macroalgal biodiversity and relationships to environmental variables. Although regulation of diatom biodiversity by conductivity, alkalinity, and pH is well known (e.g., Pan et al., 1996), the regulation of macroalgae had not been documented before this study.

Overall, nutrient concentrations, whether soluble or total phosphorus or nitrogen, were not directly related to the total algal mat cover or cover by *Lyngbya majuscula*. However, *Vaucheria* cover was clearly related to NO_2-NO_3, total N concentrations and two indicators of human disturbance: % bare area of the riparian zone along the reach and LDI. The threshold in *Vaucheria* cover response at 0.6 mg TN/L was similar to observed responses in *Cladophora* in streams (Dodds et al., 1997; Stevenson et al., accepted). The response of *Vaucheria* to TP is not as certain, although a few sites had low TP concentrations. Of those sites with low TP (<0.025 mg/L), less than 10% of the sites had a *Vaucheria* cover greater than 10% of the spring bottom.

Lyngbya majuscula is possibly an exotic species in a phase of expansion. It is a nitrogen fixer and is not affected by low nitrogen levels (Lundgren et al., 2003). It also grows well in low light levels (Rossi et al., 1997). It formed thick mats only in the proximity to the boil, which could indicate that very thick mats of *Lyngbya majuscula* are limited by carbon, and that CO_2-saturated water discharged from the boil facilitated the development of thick mats. Alternatively, unmeasured nutrients, such as micronutrients, may have been more available near the boil and depleted rapidly downstream. Southern sites had a higher macroalgal percent cover than northern sites.

The taxonomic status of *Lyngbya majuscula* is not clear. It was suggested that it is originally a marine species that can adapt to fresh water (Shannon et al., 1992). It was also described by (Speziale and Dyck, 1992) as a new species, *Lyngbya wollei.* Recent molecular work by (Joyner, 2004) indicated that *Lyngbya wollei* is not related to *Lyngbya majuscula.* It is possible that there are many species within what is termed *Lyngbya majuscule.* This would also explain why there were no strong relationships between the *Lyngbya* percent cover and the environmental variables in our study. The percent cover of *Vaucheria* was correlated with TN, and in Manatee Spring, *Vaucheria* covered the bottom completely.

Cowell and Botts (1994), in the field survey of aquatic plants and macroalgae in Kings Bay/Crystal River estuarine system, also did not find a relation between water nutrients and *Lyngbya* biomass. Cowell and Botts suggested that a downstream drift of the *Lyngbya* mat might contribute to error and might result in the underestimation of the true algal biomass. They also performed a series of laboratory experiments and did not find a direct relation between nutrients and growth of *Lyngbya.* In their study, the addition of calcium to the growth media stimulated the growth of *Lyngbya,* but only in low and medium phosphate and nitrate concentrations.

The results of this survey of macroalgae in Florida springs do not provide the information needed to determine conclusively whether anthropogenic increases in nutrients in spring waters have caused higher algal biomass than naturally occurs. No quantitatively and systematically collected data is available that can be used to characterize the algal biomass and nutrient concentrations in Florida springs before humans altered these ecosystems. Modern human activity was likely preceded by disturbance by Native Americans. Native American activity dates back many years. Although there are records of increasing nitrogen levels (Rosenau et al., 1977) and increasing phosphorus concentrations could be suspected, it is not known with absolute certainty that they caused an increase in algal biomass.

The results of this study do indicate that regulating nutrients could constrain macroalgal cover, but more work is necessary before such an investment is made. Control of nitrogen could constrain nuisance growths of macroalgae, either directly or indirectly. The positive correlation between *Vaucheria* abundance in springs and TN concentrations indicate that TN regulates its abundance. Although this correlation between *Vaucheria* and TN does not prove causation, evidence from experimental streams in Chapter 3 does indicate that there is a cause-effect relationship between N and *Vaucheria* growth rates. However ,TN:TP molar ratios were usually greater than the 16:1 N:P in the macroalgae, indicating P would be limiting if nutrients were low enough. The N supply may limit the growth of algae in streams, although this has not been documented widely. N may be limiting even when water column N:P ratios are greater than 16:1. P recycling in algal mats is considered to be high because of the luxury consumption of P by algae, and is probably more efficient than N recycling. Benthic algae may not be able to hold onto N as effectively as P. N:P ratios in benthic diatoms in experiments decrease as biomass increases during colonization, even though N and P concentrations remained the same in the water during that period (Humphrey and Stevenson 1992). N:P ratios in periphyton of Kentucky and Michigan streams were less than 16:1 despite higher ratios in overlying waters (Stevenson et al., unpublished data).

Even though, in this study, *Lyngbya* biomass and growth (Chapter 3) did not show a relationship to nutrients, the control of nutrients could constrain proliferations of this important nuisance alga for several reasons. First, researchers may not have been able to measure *Lyngbya* responses precisely enough. However, results indicate *Lyngbya* is adapted well to growth in low nutrient conditions. Second, *Lyngbya* proliferations could be controlled indirectly by reducing the proliferation of other benthic macroalgae that do respond to nutrient reductions. *Lyngbya* has no direct method to hold onto surfaces, except by becoming entangled with rough surfaces or objects attached to the bottom. Normally, *Lyngbya* was found attached to *Vaucheria* and aquatic plants. Proliferation of both *Vaucheria* and aquatic plants could be constrained by nutrient regulation. Cowell ant Botts (1994) suggested that *Lyngbya* colonizes new areas after a disturbance (prescribed removal of *Hydrilla* or after hurricane damage to beds of aquatic plants). Possibly the remains of aquatic plants form a good attachment point for *Lyngbya*, and since *Lyngbya* grows fast and shades the remains of aquatic plants, the macrophyte beds cannot recover.

Many factors affecting algal cover were not considered for the analyses because there was insufficient information. Disturbance from flooding may affect macroalgae. Some sites never flood and some are flooded regularly. Sites along Suwannee River are flooded regularly with dark water that limits light availability for macroalgae and can result in macroalgal biomass depletion. Direct disturbance by human recreation in the springs may open space for macroalgal growth during periods when human activity levels are low. Human activity may have also reduced grazing by fish and manatees. All of these factors could have profound impacts on macroalgal accrual on substrata. They should be considered as factors that may affect nuisance growths of macroalgae and development of nutrient-response relationships.

Macroalgal mats of the size observed in the Florida springs must have unique nutrient dynamics inside the mat. The response of these mats to water nutrients must also be different from the response of microalgal biofilms. Macroalgal mats were attached to the bottom and to aquatic plants. Usually at least part of the mat was attached directly to the bottom and was in contact with the sediment. *Vaucheria* mats develop rhizoids that grow directly into the sediment. This indicates that also nutrients from sediments could be very important to macroalgal growth (as has been observed for aquatic plants) and could contribute to the lack of relationships between macroalgal biomass and water nutrients.

Overall, this study indicates that macroalgae are very common and diverse in Florida springs. Only a few taxa (*L. majuscula, Vaucheria* sp., *Dichotomosiphon* sp. and *Compsopogon* sp.) formed very thick mats. *Lyngbya majuscula* could be a new species that was recently introduced in the springs or a species that was historically present, but not sufficiently common to be listed. The apparent adaptation of this taxon to low nutrient concentrations indicates its control would be difficult. However, regulation of nuisance growths of *Vaucheria*, and indirectly *Lyngbya*, may be possible if low levels of nutrients constrain the growth of *Vaucheria*.

CHAPTER 3. EXPERIMENTAL CONFIRMATION OF LIMITING NUTRIENTS USING EXPERIMENTAL STREAMS

Introduction

Enrichment of surface waters with nutrients can result in increased growth of benthic algae (Stockner and Shortreed, 1978; Biggs, 2000). Nitrogen and phosphorus are the two main nutrients affecting benthic algal growth (Borchardt, 1996). Nitrate and ammonia are the bioavailable forms of nitrogen. Bioavailable phosphorus comes in the form of phosphates. Bioavailable forms of nutrients are rapidly taken up by autotrophic and heterotrophic microbes and plants in surface waters and transformed into organic form. Particulate and dissolved organic N and P can be recycled to bioavailable forms by microbial and photochemical processes. All of these forms of nutrients originate from both natural and anthropogenic sources. Important natural sources include nitrogen-fixing bacteria in soils and water and dissolution of minerals, such as the phosphate-rich rocks found in some regions of Central Florida. Important anthropogenic sources include septic tanks, waste water treatment plants, and fertilizers. Abandoned phosphate pits, which can be found along spring runs (e.g., Ichetucknee and Rainbow River), may also leak phosphate after rains and high water levels.

Distinguishing effects of N versus P with correlations between the algal biomass and nutrient concentrations observed in field surveys of multiple sites is complicated. First, N and P concentrations covary among sites (Dodds and Welch, 2000; Stelzer and Lambertii, 2002; Stevenson, 1996), because many human activities produce both (Arbuckle and Downing, 2001). Second, bioavailable nutrients are consumed by benthic algae and can be depleted as algal biomass increases. TN and TP are more commonly related to the benthic algal biomass in streams than to dissolved inorganic nutrients, but this is likely the result of the sloughing of benthic algae and the accrual of suspended particulate N and P. Thus, TN and TP are imperfect indicators of bioavailable N and P in field surveys. The level of human activities in a watershed that generates nutrients, estimated loading rates of nutrients, and diatom indicators of nutrients can also be used to indicate the bioavailable nutrients in streams.

Experiments provide a valuable complement to field surveys by documenting the independent and interactive effects of contaminants and natural environmental factors (Pan, et al. 2000). Although the researchers would argue that the results of experiments should not take precedence over the results of field surveys to establish environmental criteria (Stevenson, et al. 2004), experiments do indicate the range of nutrient concentrations. Without the clear effects of nutrients on *Lyngbya* and only the effects of N on *Vaucheria* in field surveys (Chapter 2), experiments could confirm the relative sensitivity of these two macroalgae to nutrients and the likely range of nutrient concentrations that would affect their growth.

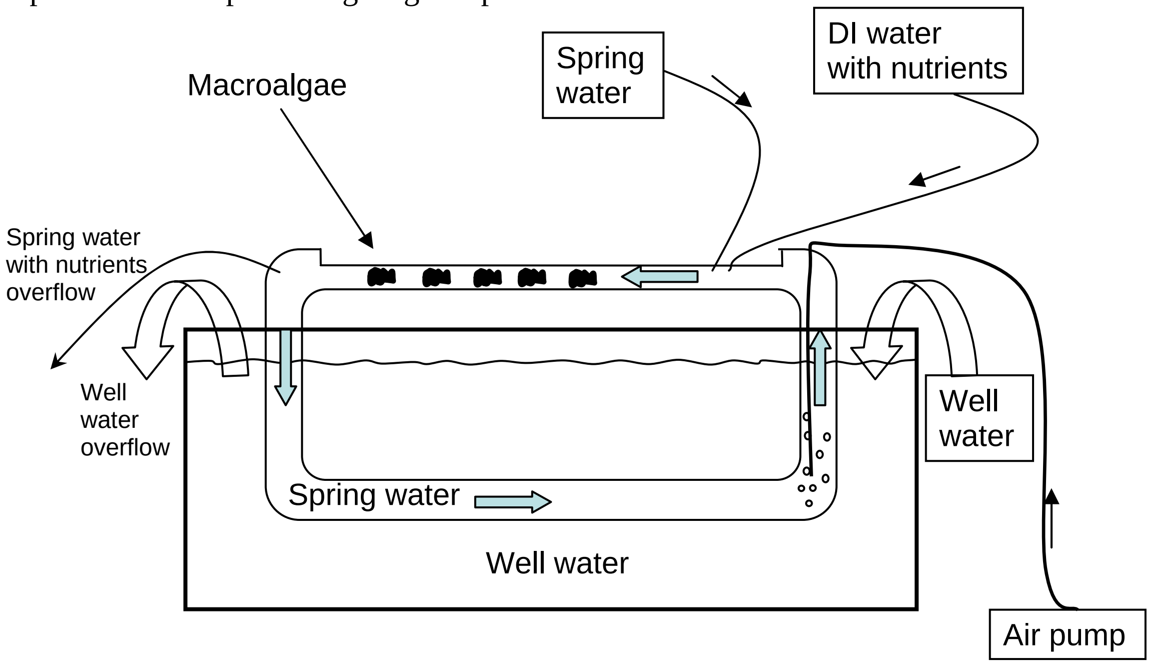
The objective of this study was to investigate the effect of an experimentally manipulated range of nitrate and phosphate concentrations on the growth of the macroalgae *Lyngbya majuscula* and *Vaucheria*. These two taxa were selected for study because they were the two most common algae in springs and the most likely to cause problems. The researchers independently manipulated nitrate and phosphate concentration in two experiments. They created a range of phosphate concentrations in artificial streams with elevated nitrate concentrations to determine the concentrations of phosphate that constrain growth of the two macroalgae. Then the researchers created a range of nitrate concentrations with elevated phosphate to determine the nitrate concentrations that constrain growth.

Methods

Experimental setup

Experiments were conducted in Silver River State Park (N 29.20020 W -82.03518) in Ocala, Florida, in November and December 2003. Running experimental streams required access to electricity, well or stream water, access to spring water low in N and P, and a secure site with sufficient exposure to sunlight. Experimental streams (ES) were built from 2" PVC, were 1 m long and 30 cm wide, and were filled with from Juniper Spring (Figure 3.1).

Figure 3.1
Experimental setup showing single experimental stream.



Juniper Spring water was judged to have the lowest nutrient concentrations in the region. An air pump produced bubbles at the bottom of the upstream vertical tube that lifted and circulated water in the ES. Current velocity was regulated at 25 cm/s (equivalent to medium-high current velocity observed in the field) with air flow.

Fresh spring water used in the experiments was pumped into a large tank from Juniper Spring in Ocala Forest and transported three times a week to Silver River State Park. Juniper Spring has a low dissolved solids concentration compared with most springs in Florida, and its mean nutrient concentrations, based on measurements from 1984 to 2000, were 30 µg SRP/L, 30 µg TP/L and 80 µg NO_x/L (St. Johns River Water Management District, 2002). On March 27, 2003, water from Juniper Spring had 29 µg SRP/L, 60 µg TP /L, 58 µg NO_x/L and 120 µg TN/L.

A peristaltic pump continuously delivered spring water through large tubing and distilled water with dissolved nutrients through small tubing to the ES at the target rate of 12 mL/min for spring water and 0.68 mL/min for water with nutrients. Excess water in the streams flowed through an opening at the downstream end of each experimental stream. The lower 2/3 of the experimental streams were submerged in well water in a pool to reduce the high diurnal variability in water temperatures that could result in exposed streams and to better simulate the relatively stable temperatures of springs receiving groundwater.

Before the experiments were started, the ES's were power washed with a hose and well water. The large peristaltic pump tubing and experimental streams were then cleaned with a 5% bleach solution for several hours, rinsed with well water for at least 48 hours, and rinsed with spring water for at least 48 hours. The small peristaltic pump tubing was cleaned with 5% sulfuric acid and rinsed with distilled water for at least 96 hours.

Experimental streams were covered with a screen made of 6 mil clear vinyl to protect them from rain and dust, and to shade them slightly (35% light reduction). The ES were located in an area exposed to direct sun from app. 8:00am to 11:30am and from 1:00pm to 6:00pm. The area was shaded by trees from 11:30am to 1:00pm.

Nutrient dosing to the ES started 24 hours before algae were introduced. Algae were added to experimental streams on day 0. The phosphate experiment (21 streams) was conducted first and was finished on day 8. The nitrate experiment (20 streams) was conducted second and was finished on day 10. Based on the growth rates observed in the phosphate experiment, the researchers decided to extend the duration of this experiment. Every 2-3 days actual flow rates were tested from each piece of peristaltic pump tubing. Pump tubing that did not meet expected flows was replaced.

Nutrient dosing

Two experiments, nitrate additions and phosphate additions, were conducted. Sodium nitrate was employed as a source of nitrogen and potassium phosphate, as a source of phosphorus (Table 3.1). A gradient of either nitrate or phosphate conditions, ranging from 0 to saturating concentrations, was established among experiment channels. In the nitrate dosing experiment, nitrate was varied among channels (0, 0.005, 0.025, 0.1, 0.5, 1.0, and 5.0 mg N/L) and phosphate was added in all streams to attain a target concentration of 0.240 mg P /L, which was expected to saturate P demand. Mass-based Redfield ratios were less than 7 for all treatments except the 5 mg N/L treatment. In the phosphate experiment phosphate concentrations were varied among channels (0, 0.003, 0.009, 0.027, 0.060, 0.120, and 0.240) and nitrate was added to the saturating level, which was targeted at 1 mg N/L. Here the mass-based Redfield ratios were more than 7 for all treatments, except the 0.240 mg P/L. Also, due to an error, treatment 0 mg P/L had no nitrate added.

Table 3.1
Target concentration of P and N in the experimental streams and in the holding bottles from which nutrients were being dosed.

Nitrate dosing			Phosphate dosing		
Target addition of N mg/L in ES	Concentration of N mg/L in dosing water	n	Target addition of P mg/L in ES	Concentration of P mg/L in dosing water	n
0.000	0.000	3	0.000	0.000	3
0.005	0.094	3	0.003	0.056	3
0.025	0.469	3	0.009	0.169	3
0.100	1.876	2	0.027	0.507	3
0.500	9.380	3	0.060	1.126	3
1.000	18.760	3	0.120	2.251	3
5.000	93.800	3	0.240	4.502	3

Chemical and physical analyses of water and algae

In each stream, the researchers measured the temperature, pH, dissolved oxygen and conductivity every 1-2 days. On day 1, day 5 and the last day of the experiment, samples were collected from each stream for water chemistry analyses (alkalinity, ammonia, calcium, chloride, iron, magnesium, Kjeldahl N, nitrates, phosphate, silica, sodium, sulfate and TP). Samples for nitrogen and phosphorus content in *L. majuscula* and *Vaucheria* sp. were collected in triplicate at the beginning of the experiment from the original algal mat and from one sample from each stream at the end of the experiment. Changes in algae TN and TP content are good indicators to confirm nutrient availability and measure physiological response to nutrient manipulations. Water nutrient analyses and algal mat nitrogen and phosphorus content were analyzed by FDEP. Algal mat Kjeldahl N and TP content are expressed per unit of macroalgae fresh mass. When the compound concentration was below the detection limit, the method detection limit was reported (for detection limits, refer to Table 2.2).

Algae

The experiment was conducted on two taxa of macroalgae: *Lyngbya majuscula* collected from Juniper Spring and *Vaucheria* sp. collected from Alexander Spring. Fragments of algal mats were gently patted dry between Nitex screens and weighed. The initial fresh mass of individual macroalgae fragments was 0.200±0.010g for both algal taxa. Algae were attached to clean (soaked in distilled water) 1”x1” unglazed tiles with clear thread and placed in the experimental streams. Four tiles with *L. majuscula* and four tiles with *Vaucheria* sp. were placed in each stream. One of each was used for algae N and P content analyses, and the remaining three of each were used to determine growth rates with measures of fresh mass (FM) and ash free dry mass (AFDM)). The biomass of three nested replicates within each stream was combined and constituted one composite biomass per one stream (one experimental unit). In addition, at the beginning of experiment 10, algal samples from each taxon were weighed for FM and frozen to be processed for AFDM to measure initial FM and initial AFDM for algae placed in the streams. A lgae were weighed for FM and frozen at the end of the experiment. In the laboratory, all frozen samples were processed for AFDM (Clesceri et al., 1998). Algae relative growth rate was calculated as follows (Hunt, 1990):

$$RGR=[\ln(\text{final mass})-\ln(\text{initial mass})]/\#\text{ of days}$$

Statistical analyses

The experiments had a factorial design with 7 nutrient levels (plus initial mat nutrient content) and 2 taxa (*L. majuscula* and *Vaucheria* sp.). (The number of replicates for each treatment is listed in Table 3.1). The biomass of 3 nested replicates within each stream was combined by summation and thereby constituted one composite initial and composite final biomass assessment per stream (1 experimental unit). ANOVA (glm model) was used to analyze data. Differences were considered significant if $p<0.05$ (SAS, 2000). Tukey's or LS means test was used to test differences between means. All graphs show means and standard error (SE).

Results

Nitrate dosing experiment

Nutrient dosing and nutrient concentration in the ES water

The average actual flow rate of spring water from large tubing was 10.631 ml/min (SD=0.689, SE=0.089). For small tubing with distilled water with nutrients, the average flow rate was 0.621 ml/min (SD=0.032, SE=0.004). Nitrate and phosphate dosing resulted in the expected nitrate concentration gradient and high TP concentration in all treatments (Table 3.2). With control NO_x averaging 0.075 mg N/L on day 1, nutrient additions resulted in NO_x-N concentrations varying from 0.075 to 5.467 mg/L among treatments and TN varying from 0.135 to 5.527 mg/L. Ammonia concentrations were mostly below the detection limit of 0.010 mg/L. NO_x concentrations in the recirculating streams decreased about 0.05 mg/L in most treatments during the experiment, despite constant loading rates. This was likely due to increases in algal biomass and nutrient uptake by algae during the experiment.

ES water temperature, pH, conductivity and DO

Water temperature averaged 17.0°C (SD=2.1, SE=0.21, min=13.3, max=19.1). There were no differences between experimental streams (ANOVA, F=0.01, p=1). The pH averaged 7.77 (SD=0.098, SE=0.010, min=7.58, max=7.94), and there were no differences between experimental streams (ANOVA, F=0.47, p=0.967). DO was saturating and averaged 9.61 mg/L (SD=0.7, SE=0.07, min=8.2, max=10.72), and there were no differences between experimental streams (ANOVA, F=0.12, p=1). Conductivity was higher in high nitrate dosing treatments (0.5, 1 and 5 mg N/L; Table 3.3) than the control treatment.

Table 3.2
Average nutrient concentrations in the stream water in nitrate dosing experiment

Target N dosing mg/L	Day	TN mg/L	TP mg/L	Ammonia N mg/L	Kjeldahl N mg/L	NOx mg/L	Calcium mg/L	Chloride mg/L	Iron mg/L	Magnesium mg/L	Sodium mg/L	Sulfate mg/L
0.000	1	0.135	0.257	0.010	0.060	0.075	11.600	4.333	0.010	4.200	2.577	5.733
0.000	5	0.107	0.267	0.010	0.060	0.047	12.133	4.267	0.010	4.167	2.440	5.333
0.000	10	0.081	0.260	0.012	0.060	0.021	12.667	4.167	0.010	4.233	2.480	5.033
0.005	1	0.156	0.263	0.010	0.080	0.076	11.733	4.233	0.010	4.167	2.630	5.433
0.005	5	0.117	0.267	0.010	0.060	0.057	12.567	4.733	0.010	4.300	2.530	7.733
0.005	10	0.087	0.267	0.010	0.060	0.027	12.733	4.200	0.010	4.233	2.487	5.200
0.025	1	0.149	0.257	0.010	0.060	0.089	11.867	4.233	0.010	4.167	2.577	5.233
0.025	5	0.128	0.267	0.010	0.060	0.068	12.167	4.333	0.010	4.167	2.470	5.633
0.025	10	0.102	0.273	0.010	0.060	0.042	12.700	4.200	0.010	4.233	2.507	5.100
0.100	1	0.270	0.275	0.010	0.095	0.175	11.950	4.200	0.010	4.250	2.785	5.250
0.100	5	0.205	0.275	0.010	0.060	0.145	12.400	5.250	0.010	4.300	2.635	11.550
0.100	10	0.175	0.290	0.011	0.060	0.115	12.500	4.200	0.010	4.200	2.630	5.050
0.500	1	0.717	0.277	0.010	0.060	0.657	11.533	4.133	0.010	4.100	3.570	5.200
0.500	5	0.643	0.273	0.010	0.060	0.583	12.533	4.200	0.010	4.267	3.393	5.133
0.500	10	0.617	0.277	0.011	0.060	0.557	12.733	4.167	0.011	4.233	3.393	5.033
1.000	1	1.260	0.267	0.010	0.060	1.200	11.500	4.200	0.010	4.133	4.480	5.300
1.000	5	1.233	0.273	0.010	0.100	1.133	12.500	4.267	0.010	4.267	4.317	5.100
1.000	10	1.193	0.270	0.011	0.060	1.133	12.833	4.200	0.010	4.333	4.327	5.000
5.000	1	5.527	0.260	0.010	0.060	5.467	11.800	4.200	0.010	4.200	11.980	5.233
5.000	5	5.860	0.273	0.010	0.060	5.800	12.533	5.700	0.010	4.300	11.833	14.567
5.000	10	5.893	0.293	0.013	0.060	5.833	12.600	4.167	0.010	4.233	12.167	5.067

Table 3.3
Conductivity (µS/cm) in nitrate dosing experiment (ANOVA, F=411.6, p<0.0001). Means sharing the same superscript letter did not differ (Tukey's test).

Nitrate dosing Mg/L	Mean	SD	SE	MIN	MAX
0.000	107.26^{a}	0.79	0.21	105	108
0.005	107.60^{a}	0.63	0.16	106	108
0.025	107.33^{a}	1.05	0.27	104	108
0.100	107.50^{a}	1.08	0.34	105	108
0.500	111.60^{b}	0.91	0.24	110	114
1.000	116.00^{c}	0.53	0.14	115	117
5.000	150.60^{d}	7.48	1.93	136	162

Table 3.4

Results of ANOVA for mat Kjeldahl Nitrogen and TP content at the end of nitrate dosing experiment for *Lyngbya majuscula* and *Vaucheria* sp. Data were log (x+1) transformed to meet the assumption of normality and variance homogeneity.

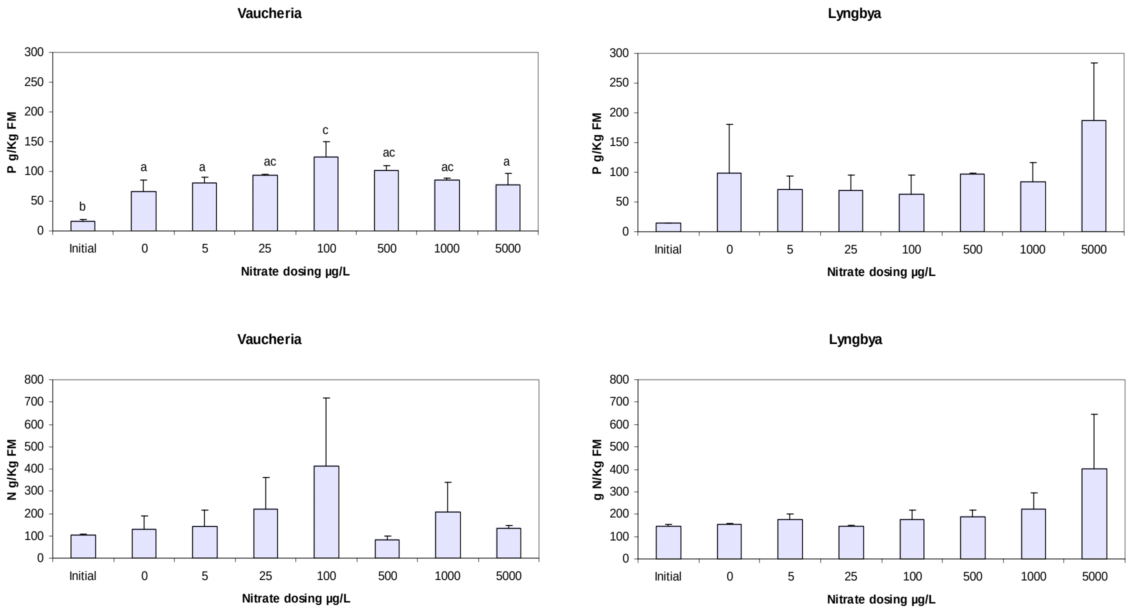
Kjeldahl Nitrogen

Source of Variation	DF	SS	MS	F	p
Nitrate	6	1.32	0.22	0.46	0.832
Algae	1	0.78	0.78	1.61	0.217
Nitrate*Algae	6	1.44	0.24	0.50	0.803

TP

Source of Variation	DF	SS	MS	F	p
Nitrate	6	1.07	0.18	0.37	0.891
Algae	1	0.30	0.30	0.61	0.444
Nitrate*Algae	6	1.93	0.32	0.66	0.680

Figure 3.2
Algal mat TP and Kjeldahl Nitrogen content in nitrate dosing experiment. Bars sharing the same letter do not differ (LSmeans test).



Nitrogen and phosphorus content in algal mats

The water nitrate concentration gradient did not affect the final mat Kjeldahl Nitrogen and TP content, and there were no differences between two studied taxa (Figure 3.2, Table 3.4). The Kjeldahl Nitrogen content in the mat at the end of the experiment did not differ from the initial content for both taxa (ANOVA, F=0.68, p=0.780). TP in the mat of *Lyngbya* at the end of experiment also did not differ from the initial content (ANOVA, F=1.93, p=0.140). However, the final mat TP content in the *Vaucheria* sp. mat was higher than in the initial mat (ANOVA, F=5.54, p=0.005), indicating a possible P limitation in the mat collected from the field. The molar mat TN/TP ratio at the end of the experiment for both taxa did not differ from the initial ratio and was not affected by the nitrate concentration gradient (Table 3.5). All TN/TP ratios, except the initial ratio in the *Lyngbya majuscula* mat, were below 16, indicating a nitrogen limitation even in the highest nitrate dosing treatment.

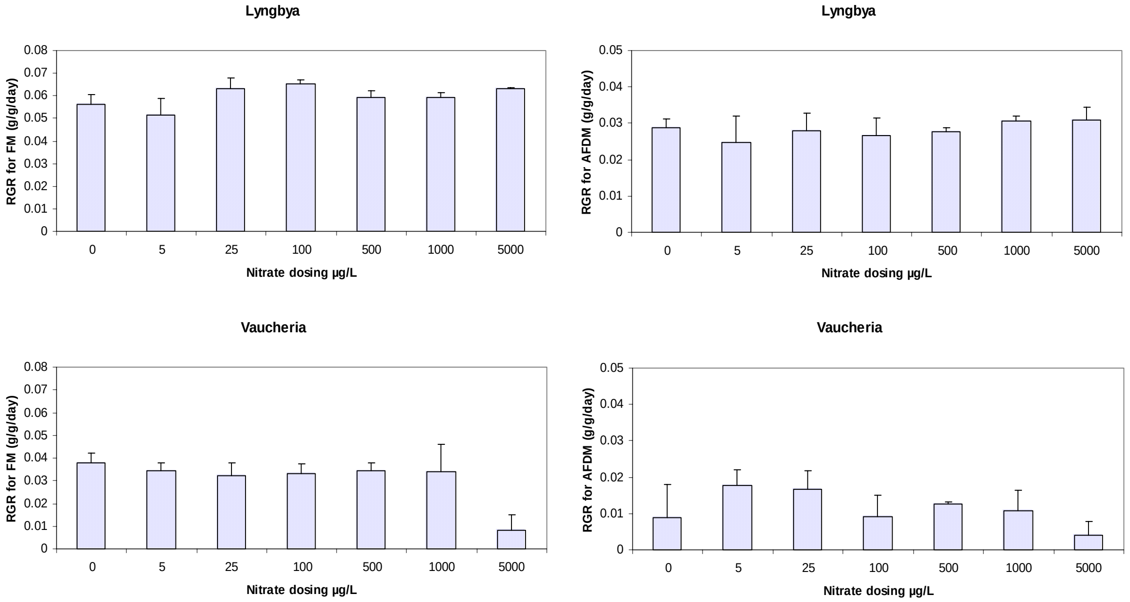
Table 3.5
Mat molar TN/TP ratio in nitrate dosing experiment in the algal mat collected from the field (Initial) and algal mat at the end of experiment from different nitrate dosing concentrations. Mean ±SE. (ANOVA for Lyngbya sp. F=2.36, p=0.081; ANOVA for Vaucheria sp. F=1, p=0.477).

Treatment (mg/L)	*Lyngbya* sp.	*Vaucheria* sp.
Initial	22.66±0.5	14.3±1.6
0	10.7±8.9	5.4±3.7
0.005	8.2±4.5	4.5±2.8
0.025	8.3±5.1	5.0±3.2
0.100	9.6±6.6	6.5±4.1
0.500	4.3±0.6	1.9±0.5
1.000	8.2±3.2	5.5±3.7
5.000	4.3±0.4	5.2±2.5

Macroalgae growth rates

Macroalgal growth rates were not affected by increased concentrations of nitrate (RGR based on FM for: *L. majuscula* ANOVA, F=1.21, p=0.359 and *Vaucheria* sp. ANOVA, F=2.43. p=0.085; and for AFDM for *L. majuscula* ANOVA, F=0.29, p=0.930 and *Vaucheria* sp. ANOVA, F=1.09. p=0.419) (Figure 3.3). *Lyngbya majuscula* had a growth rate averaging 0.059 g FM/g FM/d and 0.028 g AFDM/g AFDM/d. *Vaucheria* sp. had a growth rate averaging 0.030 g FM/g FM/d and 0.010 g AFDM/g AFDM/d. *Lyngbya majuscula* had a significantly higher growth rate than *Vaucheria* sp (based on FM, ANOVA for Algae effect, F=92.12, p<0.0001 and AFDM, ANOVA for Algae effect, F=35.19, p<0.0001).

Figure 3.3
Algal relative growth rate (RGR) in nitrate dosing experiment based on fresh mass and ash free dry mass.



Phosphate dosing experiment

Nutrient dosing and nutrient concentration in the ES water

The average actual flow rate of spring water from the large tubing was 11.386 ml/min (SD=0.653, SE=0.069), and the flow rate for small tubing with distilled water with nutrients was 0.603 ml/min (SD=0.077, SE=0.009). Phosphate and nitrate dosing resulted in the expected TP concentration gradient and high nitrogen concentration in all treatments (Table 3.6). There was an error in phosphate dosing in 240µg/L treatment on days 1, 2 and 3 that resulted in a low TP concentration on day 1 (Table 3.6). With TP averaging 0.021 mg P/L in the control treatment during the whole experiment, phosphate additions resulted in TP concentrations varying from 0.021 to 0.268 mg/L among treatments. TP and NO_x concentrations in the recirculating streams did not decrease during the experiment, as NO_x did in the nitrate experiment.

ES water temperature, pH, conductivity and DO

Water temperature averaged 18.3°C (SD=2.1, SE=0.16, min=13.8, max=23.0), and there were no differences between experimental streams (ANOVA, F=0.02,p=1). The pH averaged 7.72 (SD=0.166, SE=0.012, min=7.31, max=8.15), and there were no differences between experimental streams (ANOVA, F=0.15, p=1). Conductivity averaged 114.1 µS/cm (SD=2.93, SE=0.21, min=109, max=120), and there were no differences between experimental streams (ANOVA, F=0.78, p=0.730). DO was saturating and averaged 9.59 mg/L (SD=0.573, SE=0.044, min=8.60, max=10.96), and there were no differences between experimental streams (ANOVA, F=0.35, p=0.996).

Table 3.6
Average nutrient concentrations in the stream water in phosphate dosing experiment.

Target P dosing mg/L	Day	TN mg/L	TP mg/L	Ammonia N mg/L	Kjeldahl N mg/L	NOx mg/L	Calcium mg/L	Chloride mg/L	Iron mg/L	Magnesium mg/L	Sodium mg/L	Sulfate mg/L
0.000	1	0.136	0.025	0.010	0.100	0.036	13.733	4.367	0.010	4.567	2.633	5.467
0.000	5	0.110	0.016	0.010	0.080	0.030	13.633	4.567	0.010	4.433	2.663	6.100
0.000	8	0.093	0.023	0.010	0.060	0.033	13.267	4.500	0.010	4.500	2.640	5.567
0.003	1	1.020	0.028	0.010	0.080	0.940	12.567	4.100	0.010	4.167	4.090	5.267
0.003	5	1.110	0.018	0.010	0.080	1.030	12.600	4.400	0.010	4.167	4.163	7.200
0.003	8	1.073	0.021	0.010	0.060	1.013	12.300	4.267	0.010	4.200	4.207	5.300
0.009	1	1.050	0.033	0.010	0.100	0.950	12.700	4.100	0.010	4.233	4.113	5.233
0.009	5	1.133	0.023	0.010	0.100	1.033	12.200	4.733	0.010	4.100	4.103	15.667
0.009	8	1.043	0.031	0.010	0.060	0.983	12.400	4.300	0.010	4.233	4.240	5.300
0.027	1	1.240	0.058	0.010	0.080	1.160	12.733	4.033	0.010	4.200	4.527	5.167
0.027	5	1.180	0.043	0.010	0.100	1.080	12.800	4.700	0.010	4.267	4.237	14.767
0.027	8	1.110	0.038	0.010	0.060	1.050	12.467	4.300	0.010	4.233	4.283	5.300
0.060	1	1.030	0.078	0.010	0.100	0.930	12.867	4.100	0.010	4.333	4.400	5.233
0.060	5	1.140	0.073	0.010	0.100	1.040	12.500	4.567	0.010	4.167	4.130	10.267
0.060	8	1.213	0.077	0.010	0.060	1.153	12.267	4.233	0.010	4.167	4.470	5.267
0.120	1	1.077	0.140	0.010	0.100	0.977	12.667	4.100	0.010	4.233	4.167	5.300
0.120	5	1.167	0.143	0.010	0.100	1.067	12.267	4.633	0.012	4.133	4.210	12.533
0.120	8	1.093	0.143	0.010	0.060	1.033	12.400	4.233	0.010	4.200	4.280	5.267
0.240	1	1.010	0.048	0.010	0.080	0.930	12.767	4.167	0.010	4.200	4.213	5.233
0.240	5	1.143	0.250	0.010	0.100	1.043	12.367	4.433	0.010	4.167	4.183	8.567
0.240	8	1.335	0.287	0.010	0.065	1.270	12.000	4.267	0.010	4.100	4.573	5.133

Table 3.7

Results of ANOVA for mat Kjeldahl Nitrogen and TP content at the end of phosphate dosing experiment for *Lyngbya majuscula* and *Vaucheria* sp. Data were log(x+1) transformed to meet the assumption of normality and homogeneity of variance.

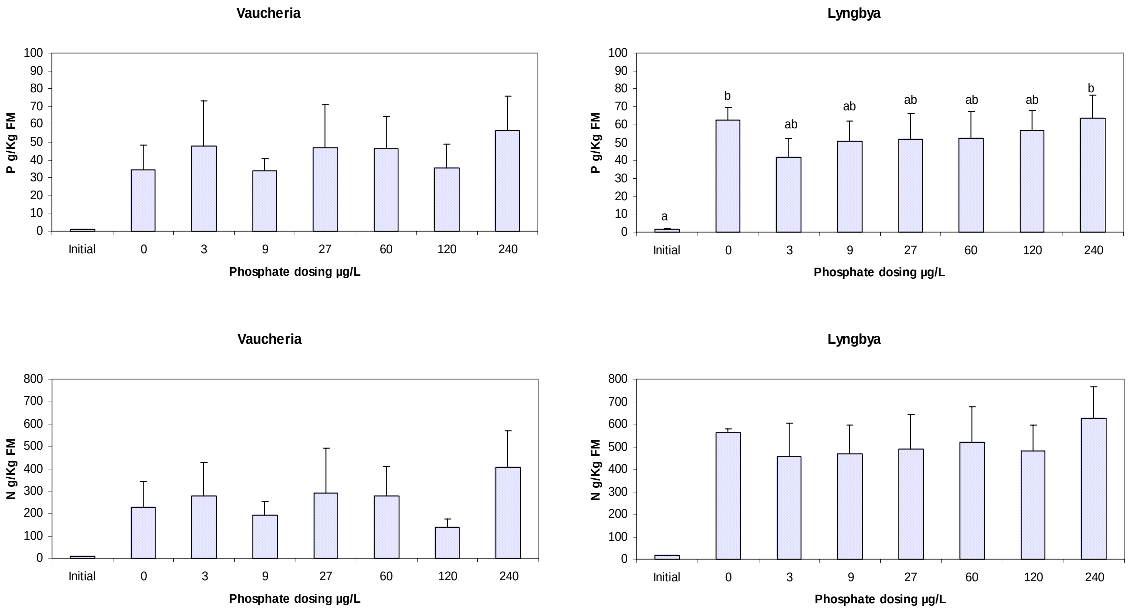
Kjeldahl Nitrogen

Source of Variation	DF	SS	MS	F	p
Phosphate	6	1.14	0.19	0.41	0.867
Algae	1	8.56	8.56	18.38	**0.000**
Phosphate *Algae	6	0.52	0.09	0.18	0.979

TP

Source of Variation	DF	SS	MS	F	p
Phosphate	6	0.41	0.07	0.21	0.971
Algae	1	1.31	1.31	3.93	0.057
Phosphate *Algae	6	0.51	0.08	0.25	0.954

Figure 3.4
Algal mat TP and Kjeldahl Nitrogen content in phosphate dosing experiment.



Nitrogen and phosphorus content in algal mats

The water phosphate concentration gradient did not affect final mat Kjeldahl Nitrogen and TP content (Figure 3.4, Table 3.7). At the end of the experiment, *Lyngbya majuscula* had a higher Kjeldahl Nitrogen content than *Vaucheria* sp. (ANOVA for Algae effect F=18.38, p=0.000). For TP, such a difference was not observed (ANOVA for Algae effect F=3.93, p=0.057). The Kjeldahl Nitrogen content in the mat at the end of the experiment did not differ from the initial content for both taxa (ANOVA, F=0.68, p=0.780). The TP content in the *Lyngbya* mat at the end of the experiment was higher than it was initially (ANOVA, F=3.09, p=0.029), indicating a possible P limitation in the mat collected from the field. The final TP content in the *Vaucheria* sp. mat did not differ from the initial content (ANOVA, F=0.96, p=0.489).

The molar mat TN/TP ratio at the end of the experiment for both taxa did not differ from the initial ratio and was not affected by the phosphate concentration gradient (Table 3.8). All TN/TP ratios in the *Lyngbya majuscula* mat were higher than 16, indicating a phosphorus limitation even in the high phosphate treatment. All TN/TP ratios in *Vaucheria* sp. mat were below 16, indicating a nitrogen limitation.

Table 3.8
Mat molar TN/TP ratio in phosphate dosing experiment in the algal mat collected from the field (Initial) and algal mat at the end of experiment from different phosphate dosing concentrations (in mg/L). Mean ±SE. (ANOVA for Lyngbya sp. F=0.85, p=0.561; ANOVA for Vaucheria sp. F=1.22, p=0.349).

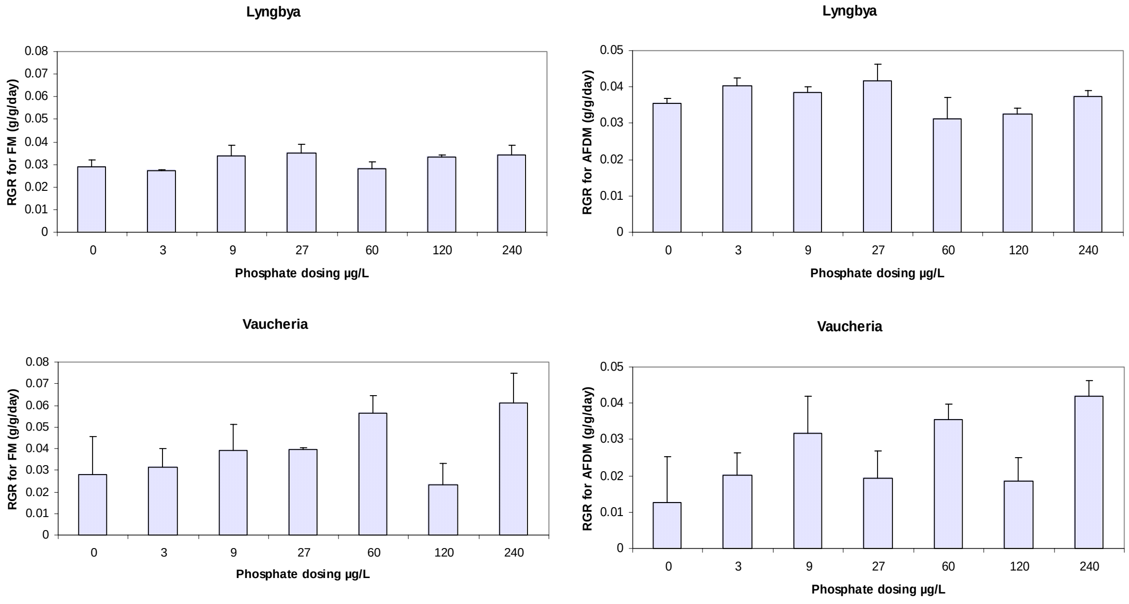
Treatment	*Lyngbya* sp.	*Vaucheria* sp.
Initial	22.3±1.1	12.8±0.1
0	20.2±1.5	13.5±1.6
0.003	23.4±1.7	12.5±0.9
0.009	19.9±1.1	12.1±1.1
0.027	20.7±1.2	10.9±2.7
0.060	21.8±0.9	13.1±1.2
0.120	19.1±2.8	9.2±0.9
0.240	21.5±0.9	14.9±2.2

Macroalgae growth rates

Macroalgal growth rates were not affected by the increased concentrations of phosphate (RGR based on FM for: *L. majuscula* ANOVA, F=1.01, p=0.456 and *Vaucheria* sp. ANOVA, F=1.60. p=0.218; and for AFDM for *L. majuscula* ANOVA, F=1.45, p=0.264 and *Vaucheria* sp. ANOVA, F=1.89. p=0.154) (Figure 3.5). *Lyngbya majuscula* had a growth rate averaging 0.032 g FM/g FM/d and 0.037 g AFDM/g AFDM/d. *Vaucheria* sp. had a growth rate averaging 0.040g FM/g FM/d and about 0.025 g AFDM/g AFDM/d.

Lyngbya majuscula had a significantly higher growth rate than *Vaucheria* sp based on AFDM (ANOVA for Algae effect, F=11.68, p=0.002), but for FM the two taxa did not differ in their RGR (ANOVA for Algae effect, F=3.53.19, p=0.071).

Figure 3.5
Algal relative growth rate (RGR) in phosphate dosing experiment based on fresh mass and ash free dry mass.



Discussion

No increase in macroalgal growth was observed with increased nutrient concentrations. The lack of macroalgal growth response to nutrients could be due to the short duration of experiments, artifacts of the experimental environment, growth limitation by microelements (like calcium or iron), unfavorable light conditions or the ability of these algae to efficiently utilize the relatively low nutrient concentrations in Juniper Spring water.

The short duration of experiments can be a problem if algae have not had time to respond or have not become acclimated to environmental conditions. Both macroalgae did have measurable growth rates, so they likely did have time to respond. The growth rates were relatively slow compared to the growth rates of diatoms in other experiments, but within the range of rates observed in other studies (Stevenson, 1984; Rier et al., accepted). Algal responses to experimental treatments have been observed in other, short-term experiments within this short duration, which has the advantage of reducing artifacts due to removal from natural conditions and confinement in an experimental setting. Macroalgal responses in 1-week experiments were observed in the conductivity experiment (Chapter 3, this report). Algae can store luxury quantities of nutrients in their cells, and can use these to support growth during periods of low nutrient availability. The *Vaucheria* from Alexander Spring and *Lyngbya* from Juniper Spring may have had luxury quantities of nutrients when taken from their natural settings; however, these settings were chosen because of their relatively low nutrient supplies. In addition, exposure to elevated P concentrations in both experiments resulted in a significant increase in the P content of algal cells relative to their initial P content. The same was true for N in the P experiment, but not in the N experiment. Therefore, acclimation to experimental treatments was probably not an issue.

Artifacts of the experimental environment may have constrained algal responses to nutrients. Spring water close to the boil had high quantities of dissolved carbon dioxide that “bubbles” out to the atmosphere as soon as water reaches the surface due to a decrease in pressure as water emerges from the ground. Since the water used in the experiments was transported, the dissolved CO_2 concentration had to have been much lower than that observed in the springs, where the thickest algal mats were observed only close to boils. Nutrient uptake by algae in experimental settings may constrain growth rates. Although N and P were added, the many micronutrients that algae require were not added. However, the macroalgae did grow, indicating that any limitation, if present, was not severe. The growth rates were low, but this was probably due to the binding of masses of macroalgae rather than limitations due to a low micronutrient supply. Masses of macroalgae were bound in tight balls, and growth patterns indicated that the surface algae were the only algae that grew. Algae deep in the bound masses probably had little exposure to light and nutrients.

Lyngbya majuscula growth might have been restrained by very low iron concentration in the experimental stream water, which was below 0.01 mg/L. The optimum iron concentration for the growth of *L. majuscula* is 1.4-6.1 mg/L (Gross and Martin, 1996). Also, the calcium concentration in Juniper Spring water was low compared to other springs and in this study averaged 12.5 mg/L. *Lyngbya majuscula* (*L. wollei*) had higher growth rates when calcium was added to the growth media (Cowell and Botts, 1994). *Lyngbya majuscula* (*L. wollei*) is a nitrogen fixer, and optimum conditions for that process are low light and low oxygen concentration (Philips et al., 1992), which is the opposite of the conditions in the experimental streams in this study. The accrual of thick mats of *L. majuscula* could facilitate nitrogen fixation by the filaments located deeper in the mat. The experimental algal mats were very small, and may have had good oxygen penetration in the mat.

Algae in this experiment were exposed to relatively high light intensity. *Vaucheria bursata* growth in laboratory conditions was saturated at very low levels (20-50 µmol/m^2/s) (Leukart and Hanelt, 1995). *L. majuscula's* ability to fix nitrogen was reduced when exposed to light.

The water used in the experiment, from Juniper Spring, the spring in the Silver River area with the lowest P, had higher TP than expected. So target concentrations from even control and low P treatments were higher than planned. Little is known about the nutrient concentrations that constrain the growth of *Vaucheria* and *Lyngbya,* however, there is considerable evidence that phosphate availability in streams with 0.030 mg TP/L is sufficient to support nuisance growths of *Cladophora* (Dodds et al., 1997; Stevenson et al., accepted). *Cladophora* growth seems to be constrained in streams with less 0.600 mg TN/L (Dodds et al. 1997). These nutrient concentrations constrain relatively dense growths of *Cladophora*, not the thin layer of macroalgae exposed on the surface of the bound masses in the experiment. Rapid nutrient uptake and slow mixing in dense mats of algae cause competition for nutrients in periphyton, which increases in intensity with the thickness of algal mats and algal density on substrata (Stevenson and Glover 1993; Dodds, 1991). The 0.020 mg TP/L and 0.100 mg TN/L found in Juniper Spring water should surely constrain the growth of dense mats of macroalgae, but perhaps not the relatively thin, active outer layer of bound masses used in the experiment. The accrual of thick benthic masses of macroalgae in Florida springs may be constrained by naturally low nutrient concentrations, but that hypothesis was not tested in the experiments that were conducted.

The results of this study and previous studies on *Lyngbya majuscula* (*L. wollei)* (Cowell and Botts, 1994) suggest that growth of this macroalga is probably controlled by a combination of factors: concentration of macro and micronutrients, flow, dissolved oxygen and CO2 concentrations, and light availability. Less is known about what affects the growth of *Vaucheria* sp.

CHAPTER 4. MACROALGAL GROWTH IN HIGH AND LOW CONDUCTIVITY SPRING WATER

Introduction

Many factors may regulate the ability of algae to respond to nutrients. Temperature, light, pH, grazers, and conductivity are important factors. Conductivity is one of the most important variables regulating the changes of algal species composition in aquatic ecosystems (Pan et al., 1999; Chapter 6, this report). In the field survey, the researchers observed that sites with high conductivity often had thicker algal mats and a higher percentage of the spring bottom was covered by macroalgae. Great inter-specific differences in species abilities to utilize nutrients and even differences among populations within species were expected (Carpenter and Guillard, 1971).

To test the hypothesis that water conductivity and algal growth rates are positively correlated, the growth rates were compared of algae in mesocosms using water from 3 springs with similar nutrient concentrations but differing water conductivity. In addition, the hypotheses that growth rates of species and populations within species differ were tested by comparing growth rates of a *Vaucheria* population and two *Lyngbya majuscula* populations.

Methods

Experimental setup

This experiment had 6 treatments with 3 replicates. A factorial design was used, with two factors: water conductivity (low, medium and high) and water nutrients (no nutrients added and nutrient enrichment with addition of 240 µg P/L as phosphate and 1000µg N/L as nitrate). Three kinds of algae were grown in each mesocosm: *L. majuscula* from 2 springs and *Vaucheria* sp. Eighteen Glad® containers were used as mesocosms. Each held 1.5 L of water. Water was mixed with aeratorsin each mesocosm to generate current. The mesocosms were placed in a water bath to moderate diurnal variability in water temperatures. The water bath was a small swimming pool filled with well water and heated with 3 aquarium heaters. This pool was covered with a screen (as described in Chapter 3). Water with low conductivity (110µS/cm) was collected from Juniper Spring, water with medium conductivity (1100 µS/cm) was collected from Alexander Spring, and water with high conductivity (2000 µS/cm) was collected from Silver Glen Spring. It was decided not to amend low conductivity water with salts so as to reflect the conditions of natural springs as much as possible. Water in mesocosms was replaced with new water every two days. The water temperature, conductivity, pH and DO also were measured every two days. Fresh water was collected from the springs three times during the course of the experiment. This experiment started on day 0 and ended on day 7.

Algae

The experiment was conducted on two taxa of macroalgae. *Lyngbya majuscula* was collected from Juniper Spring and from Silver Glen Spring, and *Vaucheria* sp. was collected from Alexander Spring. Algae were prepared and attached to tiles as described in the previous experiments (Chapter 3). On day 0 algae were introduced to the mesocosms. Three tiles with *L. majuscula* from Juniper Spring, three tiles with *L. majuscula* from Silver Glen Spring and two tiles with *Vaucheria* sp. from Alexander Spring were placed in each mesocosm. One tile with *L.*

majuscula from each of two springs was used for N and P content analyses and the remaining tiles were used for FM and AFDM analyses. Two tiles of each kind were treated as nested replicates, and the FM mass from each mesocosm was summed up. In addition, at the beginning of the experiment, 10 algal samples from each taxon were weighed for FM and frozen to be processed for AFDM to aid in calculating the initial AFDM from the initial FM for algae placed in the mesocosms. At the end of experiment, algae were weighed for FM and frozen. In the laboratory, all frozen samples were processed for AFDM (Clesceri et al., 1998). Biomass-specific growth rates of each algal taxon were calculated as in Chapter 3.

Chemical and physical analyses of water and algae

The temperature, pH, dissolved oxygen and conductivity were measured in each mesocosm every two days. On day 1, day 5 and the last day of the experiment (day 7) samples were collected from each mesocosm for water analyses (alkalinity, ammonia, calcium, chloride, iron, magnesium, Kjeldahl N, nitrates, phosphate, silica, sodium, sulfate and TP). Samples for nitrogen and phosphorus content in *L. majuscula* from Juniper Spring and *L. majuscula* from Silver Glen Spring were collected in triplicate at the beginning of the experiment from the original algal mat and one sample from each mesocosm was collected at the end of the experiment. Samples for nitrogen and phosphorus in *Vaucheria* sp. were not collected due to funding restrictions. Samples were analyzed as described in Chapter 3.

Statistical analyses

Biomasses for the two pseudoreplicates from each mesocosm were summed on respective dates and constituted one true replicate used for statistical analyses. ANOVA (glm model) was used to analyze data. Differences were considered significant for $p<0.05$ (SAS, 2000). The least squares (LS) means test was used to compare individual means. All graphs show means and standard error (SE).

Results

Nutrient concentration in mesocosm water

Nutrient addition resulted in high TP, TN and nitrate concentrations (Table 4.1). Juniper Spring water (low conductivity) had the lowest concentration of calcium, chloride, magnesium, sodium and sulfate. Alexander Spring water (medium conductivity) had higher concentrations of these salts, and Silver Glen Spring water (high conductivity) had the highest concentrations of these salts (Table 4.1).

Table 4.1
Average nutrient concentrations in the conductivity experiment.

Spring water from	Nutrients	Day	TN mg/L	TP mg/L	Ammonia N mg/L	Kjeldahl N mg/L	NOx mg/L	Calcium mg/L	Chloride mg/L	Iron mg/L	Magnesium mg/L	Sodium mg/L	Sulfate mg/L
Juniper	0	1	0.127	0.041	0.010	0.060	0.067	13.467	4.633	0.010	4.667	2.690	5.567
Juniper	0	5	0.072	0.024	0.010	0.068	0.004	13.467	5.233	0.010	4.233	2.800	5.867
Juniper	0	7	0.082	0.028	0.011	0.077	0.005	13.933	5.833	0.010	4.333	3.050	6.300
Juniper	1	1	1.163	0.260	0.010	0.063	1.100	13.667	4.633	0.010	4.700	4.473	5.567
Juniper	1	5	0.976	0.227	0.012	0.080	0.897	13.567	5.500	0.010	4.267	4.650	5.867
Juniper	1	7	0.917	0.240	0.011	0.077	0.840	13.433	6.233	0.010	4.200	4.653	6.200
Alexander	0	1	0.175	0.056	0.010	0.137	0.038	49.300	270.000	0.010	22.367	148.333	69.000
Alexander	0	5	0.124	0.033	0.010	0.120	0.004	48.233	270.000	0.010	19.933	136.000	68.000
Alexander	0	7	0.191	0.041	0.010	0.187	0.004	48.800	276.667	0.010	20.033	133.333	71.333
Alexander	1	1	1.183	0.270	0.010	0.183	1.000	49.367	273.333	0.010	22.433	149.667	68.667
Alexander	1	5	0.943	0.237	0.022	0.183	0.760	48.233	263.333	0.010	19.967	138.000	68.000
Alexander	1	7	1.060	0.260	0.010	0.180	0.880	48.300	280.000	0.010	19.767	133.333	70.333
Silver Glen	0	1	0.160	0.037	0.010	0.130	0.030	73.200	443.333	0.010	35.933	255.000	166.667
Silver Glen	0	5	0.137	0.024	0.015	0.133	0.004	73.533	466.667	0.010	33.367	244.667	166.667
Silver Glen	0	7	0.188	0.033	0.010	0.183	0.005	73.633	476.667	0.010	33.233	242.000	173.333
Silver Glen	1	1	1.193	0.250	0.010	0.160	1.033	72.800	443.333	0.010	35.667	254.667	166.667
Silver Glen	1	5	0.880	0.220	0.012	0.160	0.720	74.400	473.333	0.010	33.833	248.667	173.333
Silver Glen	1	7	0.983	0.243	0.010	0.183	0.800	74.867	483.333	0.010	33.533	244.333	173.333

Water conductivity, temperature, pH and DO

The water temperature averaged 17.9°C (SD=2.4, SE=0.29, min=14.0, max=21.4), and there were no differences between mesocosms (ANOVA, F=0.02, p=0.999). The pH averaged 8.31 (SD=0.16, SE=0.019, min=7.99, max=8.60), and there were no differences between mesocosms (ANOVA, F=0.01, p=1). DO was close to saturating and averaged 7.43 (SD=0.69, SE=0.082, min=6.06, max=8.44), and there were no differences between mesocosms (ANOVA, F=0.72, p=0.610). Water conductivity had a steep gradient between treatments (Table 4.2). Addition of nutrients slightly increased water conductivity in the low conductivity treatment (Juniper Spring water).

Table 4.2
Water conductivity (µS/cm) in mesocosms in the conductivity experiment (ANOVA, F=12293.3, p<0.0001)

Spring water from	Nutrients	Mean	SD	SE	MIN	MAX
Juniper	0	118.91	4.87	1.41	114	130
	1	128.50	6.45	1.86	122	143
Alexander	0	1156.83	19.17	5.53	1138	1197
	1	1168.83	12.14	3.51	1150	1191
Silver Glen	0	1903.42	40.26	11.62	1852	1961
	1	1924.58	39.82	11.50	1871	1976

Nitrogen and phosphorus content in algal mats

The final algal mat Kjeldahl Nitrogen and TP content, for *Lyngbya majuscula* from Juniper Spring and *L. majuscula* from Silver Glen Spring, was not affected by water conductivity and by the addition of nutrients. It neither differed from the initial content nor between the two strains of *L. majuscula* (Figure 4.1, ANOVA for Kjeldahl N, F=0.28, p=0.989; ANOVA for TP, F=1.94, p=0.070). The molar mat TN/TP ratio was not affected by the experimental treatment but differed between the two strains of *Lyngbya* (Table 4.3). All TN/TP ratios were above 16 (Redfield ratio) indicating phosphorus limitation even in the treatments with nutrients (Table 4.4).

Table 4.3
Results of ANOVA for mat TN/TP ratio in conductivity experiment for *Lyngbya majuscula* from Silver Glen and Juniper Springs. Data were 1/(x+1) transformed to meet the assumption of normality and variance homogeneity.

TN/TP					
Source of Variation	DF	SS	MS	F	p
Treatment	6	0.00034	0.00006	2.26	0.066
Algae	1	0.00293	0.00293	116.14	**<0.0001**
Treatment*Algae	6	0.00011	0.00002	0.75	0.618

Table 4.4
Mat molar TN/TP ratio in conductivity experiment in the algal mat collected from the field (Initial) and algal mat at the end of experiment for two strains of *Lyngbya*. Mean ±SE. (ANOVA for Silver Glen *Lyngbya* F=1.61, p=0.216; ANOVA for Juniper *Lyngbya* F=1.45, p=0.263).

Treatment	Silver Glen *Lyngbya*	Juniper *Lyngbya*
Initial	36.7±1.1	23.1±1.0
Alexander	32.3±5.1	19.1±2.1
Alexander with nutrients	29.2±1.9	22.3±0.9
Juniper	31.3±1.4	19.4±1.6
Juniper with nutrients	27.8±1.0	18.7±1.3
Silver Glen	31.5±1.7	20.5±0.6
Silver Glen with nutrients	28.3±0.3	19.9±0.1

Macroalgae growth rates

Algae growth rates based on FM and AFDM differed among taxa (Tables 4.5 and 4.6). *Lyngbya majuscula* from Juniper Spring had a higher FM growth rate than *L. majuscula* from Silver Glen Spring, but AFDM growth rates did not differ (LS means p<0.05; Figure 4.2). Both *Lyngbya majuscula* populations had a higher growth rates than *Vaucheria* (LS means p<0.05).

The source of spring water or conductivity affected the FM and AFDM growth of *Vaucheria* sp. (Table 4.7). *Vaucheria* sp. grew fastest in Alexander Spring water, which had intermediate conductivity levels (LS means p<0.05). The addition of nutrients did stimulate *Vaucheria* growth, especially in the moderate conductivity waters of Alexander Spring. Nutrients and spring water conductivity had no effect on the growth of either strain of *Lyngbya*.

Figure 4.1
Algal mat TP and Kjeldahl Nitrogen content in the conductivity experiment. Algae grown in water from different springs without (0) and with (1) addition of nutrients.

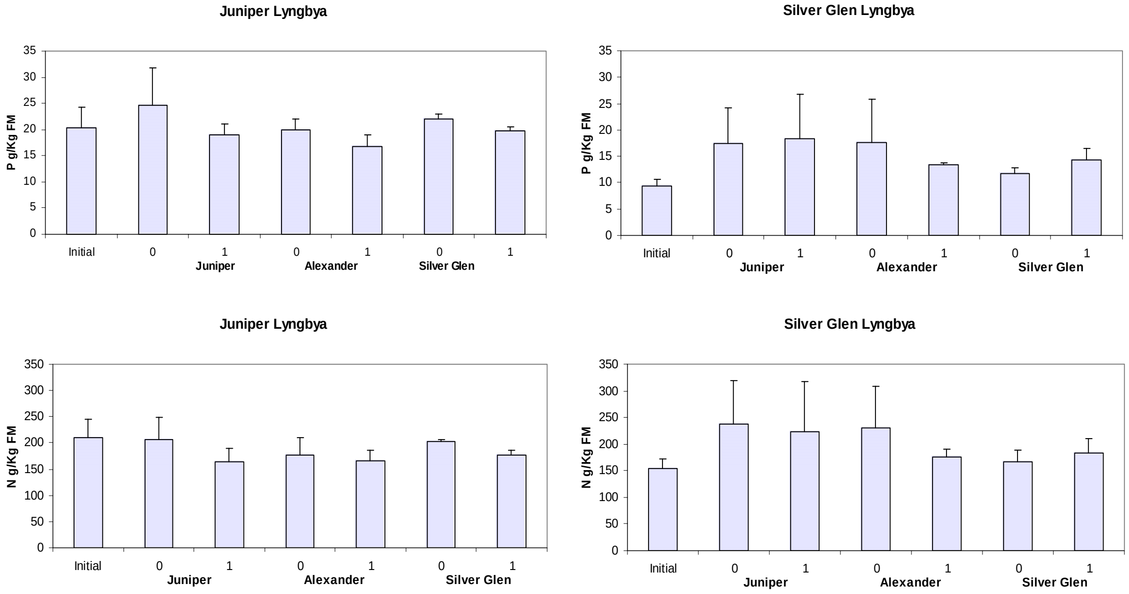


Table 4.5
Results of ANOVA for algae RGR based on algal FM in the conductivity experiment.

Source of Variation	DF	SS	MS	F	p
Conductivity (Spring)	2	0.00	0.00	6.13	**0.005**
Nutrients	1	0.00	0.00	2.47	0.125
Algae	2	0.05	0.03	80.81	**<.0001**
Conductivity*Nutrients	2	0.00	0.00	1.57	0.221
Conductivity*Algae	4	0.00	0.00	1.79	0.151
Nutrients*Algae	2	0.00	0.00	4.05	0.026
Conductivity*Nutrients*Algae	4	0.00	0.00	1.04	0.403

Table 4.6
Results of ANOVA for algae RGR based on algal AFDM in the conductivity experiment.

Source of Variation	DF	SS	MS	F	p
Conductivity (Spring)	2	0.00	0.00	6.59	**0.004**
Nutrients	1	0.00	0.00	2.06	0.160
Algae	2	0.01	0.01	25.87	**<.0001**
Conductivity*Nutrients	2	0.00	0.00	2.06	0.143
Conductivity*Algae	4	0.00	0.00	2.54	0.057
Nutrients*Algae	2	0.00	0.00	0.10	0.902
Conductivity*Nutrients*Algae	4	0.00	0.00	1.16	0.345

Table 4.7
Results of ANOVA for RGR of *Vaucheria* sp. based on FM and AFDM.

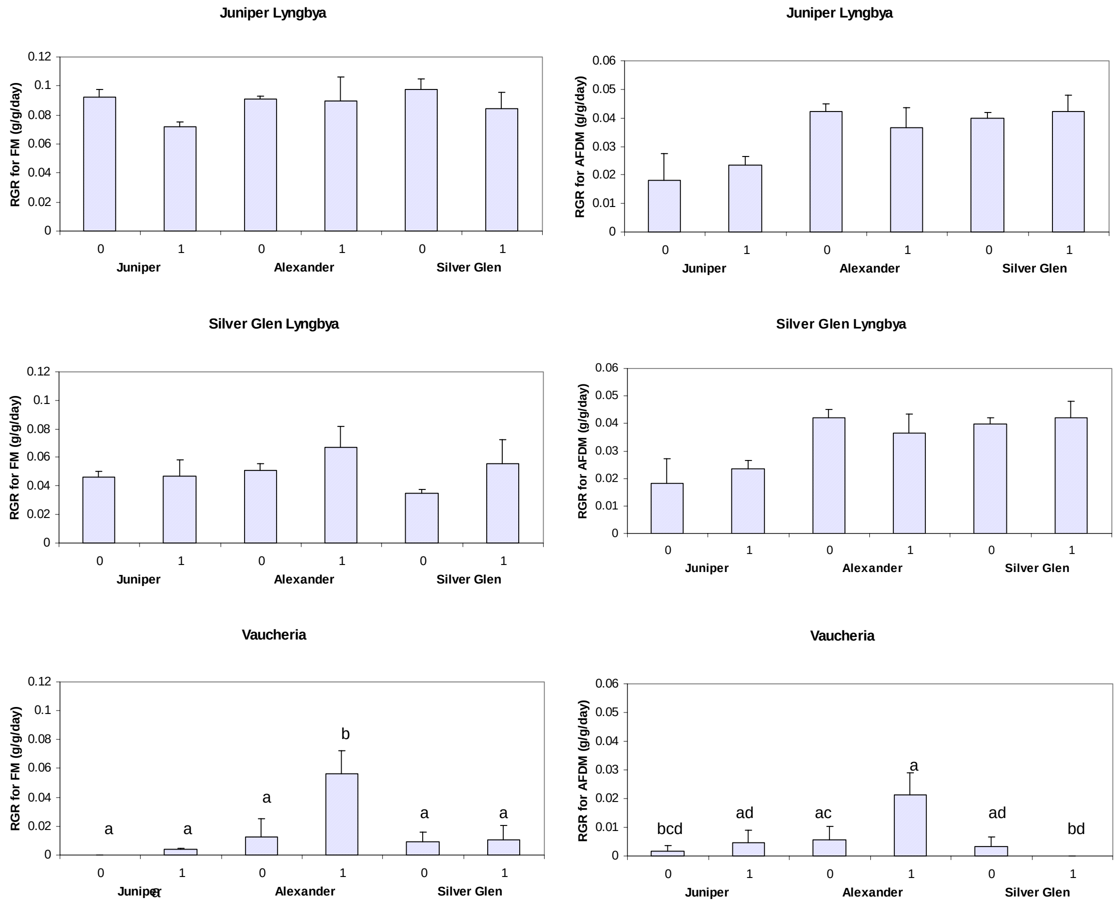
FM

Source of Variation	DF	SS	MS	F	p
Spring	2	0.0055	0.0027	6.55	**0.012**
Nutrients	1	0.0023	0.0023	5.38	**0.039**
Spring*Nutrients	2	0.0018	0.0009	2.17	0.157

AFDM

Source of Variation	DF	SS	MS	F	p
Spring	2	0.0021	0.0011	5.02	**0.026**
Nutrients	1	0.0001	0.0001	0.26	0.620
Spring*Nutrients	2	0.0011	0.0006	2.66	0.111

Figure 4.2
Algal relative growth rate (RGR) in the conductivity experiment based on fresh mass and ash free dry mass. Algae grown in water from different springs without (0) and with (1) addition of nutrients.



Discussion

This study was designed to address the role of conductivity on macroalgal growth. Since natural spring water was used, other factors in addition to conductivity were manipulated. Micronutrients as well as toxic salts may vary among springs. However, all salt concentrations measured increased in concentration with increases in conductivity among springs. If it is assumed that responses of macroalgae to spring waters were due to differences in conductivity and associated salts alone, then *Vaucheria* sp. grew best at intermediate conductivity levels. Salinity and conductivity can affect the distribution of *Vaucheria* sp. in streams (Moreno et al., 2001). In chapter 2 a positive correlation was reported between alkalinity and the distribution of *Vaucheria* sp. Freshwater species of *Vaucheria* sp. are known to adapt to different salinity levels (Schneider et al., 1996).

Nutrient enrichment had no effect on the *Lyngbya* growth rate, as in Chapter 2 (this report). Thus, these results confirm that *Lyngbya* is adapted to grow in very low nutrient concentrations. *Vaucheria* growth rates were stimulated by nutrient concentrations when higher conductivity conditions were created in the conductivity experiment than in recirculating streams. This result differs from the results obtained in the recirculating stream experiment, which used low conductivity water from Juniper Spring. The response of *Vaucheria* to nutrients in the conductivity experiment indicates that *Vaucheria* proliferations in springs with conductivity similar to Alexander Spring may be managed by reductions in nutrient concentrations.

The differences in the growth rates of two populations of *Lyngbya majuscula* indicated that genetically different *Lyngbya* taxa may be present. Thus, a distinction may not have been made between two different taxa, or phenotypes of populations may vary among springs. Both of these factors have implications for future work and management of *Lyngbya*. *Lyngbya majuscula* is known to adapt well to different environments (Burja et al., 2002; Rossi et al., 1997); it also requires iron for growth (Gross and Martin, 1996). In cultures, *L. majuscula* grows well in low light. In nutrient rich media its growth is carbon-limited (Burja et al., 2002). Also, the taxonomic position of *Lyngbya majuscula* inhabiting fresh waters of Florida is not clear, possibly because it represents more than one species (Joyner, 2004; Shannon et al., 1992; Speziale and Dyck, 1992).

CHAPTER 5. NUTRIENT DIFFUSING SUBSTRATA ASSESSMENT OF NUTRIENT LIMIATION OF MICROALGAE

Introduction

Many approaches exist for the experimental assessment of nutrient limitation. Laboratory assays, field mesocosms, and nutrient diffusing substrata (NDS) are three such approaches. NDS is an established method of assessing potential nutrient limitations of microalgae in field settings (Fairchild and Lowe, 1984; Fairchild et al., 1985; Fairchild et al., 1989; Francoeur et al., 1999; Marks and Lowe, 1993; Mosisch et al., 2001; Pillsbury et al., 2002; Pringle, 1990; Snyder et al., 2002). For this report, researchers investigated whether nitrogen and/or phosphorus limits periphyton growth in 16 springs in northern and central Florida. Nutrient diffusing substrata were exposed to algal colonization for three weeks and periphyton chlorophyll *a* was measured. This study complemented the other methods, discussed in Chapters 1 and 2, that were used to assess the nutrients regulating algal growth in Florida springs.

Methods

NDS were prepared from clear polystyrene snap-cap vials (13 dr, 46 ml I.D. x H: 30x53mm) with a 21 mm diameter hole cut out in the cap and a Nitex screen (30 µm mesh) spread over the rim and held in place by the cut out cap. The Nitex screen served as a colonization surface for periphyton. The Nitex screen approach was modified from Biggs et al. (1998) and also Francoeur et al. (1999). Each vial was filled with a 25 ml agar (BactoAgar™) and nutrient solution. There was a 3 cm clearance between solidified agar and the Nitex screen, which provided space for algal growth with exclusion of potential grazers. Potassium phosphate monobasic (KH_2PO_4) was used as a source of phosphorus and sodium nitrate ($NaNO_3$) was used as a source of nitrogen. Four treatments were used: (C) control (2% agar), (N) nitrate enriched (2% agar, 0.5 M $NaNO_3$), (P) phosphate enriched (2% agar and 0.5 M KH_2PO_4) and (NP) nitrate and phosphate combined (2% agar, 0.5 M $NaNO_3$ and 0.5 M KH_2PO_4). Vials filled with agar were refrigerated prior to placement in the field. At the site, vials were filled with site water, and the opening was covered with the Nitex screen pulled tightly over the rim and secured with snap-cap. Four vials (C, N, P and NP) were glued with a craft hot glue gun inside a Glad® sandwich box (16.5x16.5x5cm deep, 739 ml volume). Each sandwich box was filled with sand collected at the site and the box was placed on the bottom of the spring. The upper 3 cm of each vial was positioned above the sand and was exposed to light. Six boxes containing 4 NDS (C, N, P and NP) were deployed at each site. When the initial NDS were deployed, they were placed in a sunny location close to the boil at the depth of approximately 1 m; however, most of the boxes placed in such a location were missing or were damaged when the sites were revisited 7 days later. Consequently, boxes were placed in areas not easily accessible to people and hidden from view. NDS were left in the field for 3 weeks. In the initial trials NDS were left in the field for 2 weeks. However, there was very little algal colonization after 2 weeks and an additional week of exposure was added. In all, 96 boxes (384 NDS) were placed at16 sites. Although 278 NDS were recovered, only 148 NDS were not damaged and remained exposed to the water column. Consequently, representative data from NDS recovered in good condition consisting of 3 replicates (at least 3 NDS recovered from each treatment) were collected from only 7 sites (GAI-02, ICH-01, JUN-02, RAI-01, SLV-01, VOL-01, WAC-02). See Table 5.1 for more complete statistics on recovery.

Table 5.1. Number of NDS recovered from each site; number of NDS recovered in good condition and their exposure to light.

Spring	Site code	NDS recovered	NDS recovered in good condition	Light exposure
Alexander	ALE-01	13	8	sunny/shade
Chassahowitzka	CHA-01	18	0	shade
Gainer	GAI-02	24	20	sunny/shade
Homosassa	HOM-01	20	8	sunny/shade
Ichetucknee	ICH-01	24	16	sunny/shade
Jackson Blue	JAC-01	12	0	sunny
Juniper/Fern Hammock	JUN-02	20	16	shade
Ponce de Leon	PON-01	16	0	shade
Rainbow Spring	RAI-01	24	24	sunny
Silver Glen	SGL-01	8	0	sunny
Silver River	SLV-01	16	12	sunny/shade
Volusia Blue	VOL-01	20	20	shade
Wacissa	WAC-02	20	20	sunny/shade
Wakkulla	WAK-01	16	0	sunny
Weeki Wachee	WEK-01	16	0	sunny
Wekiwa	WKW-01	11	4	shade
Total recovered		278	148	

Nitex screens were removed from the recovered substrata and discs colonized by algae were cut out and frozen immediately. In the laboratory, algae were rinsed off from the Nitex discs with DI water, combined to form 3 replicates of each treatment per site (if there were enough samples), and analyzed fluorometrically for chlorophyll *a* following extraction in 90% ethanol (Clesceri et al., 1998).

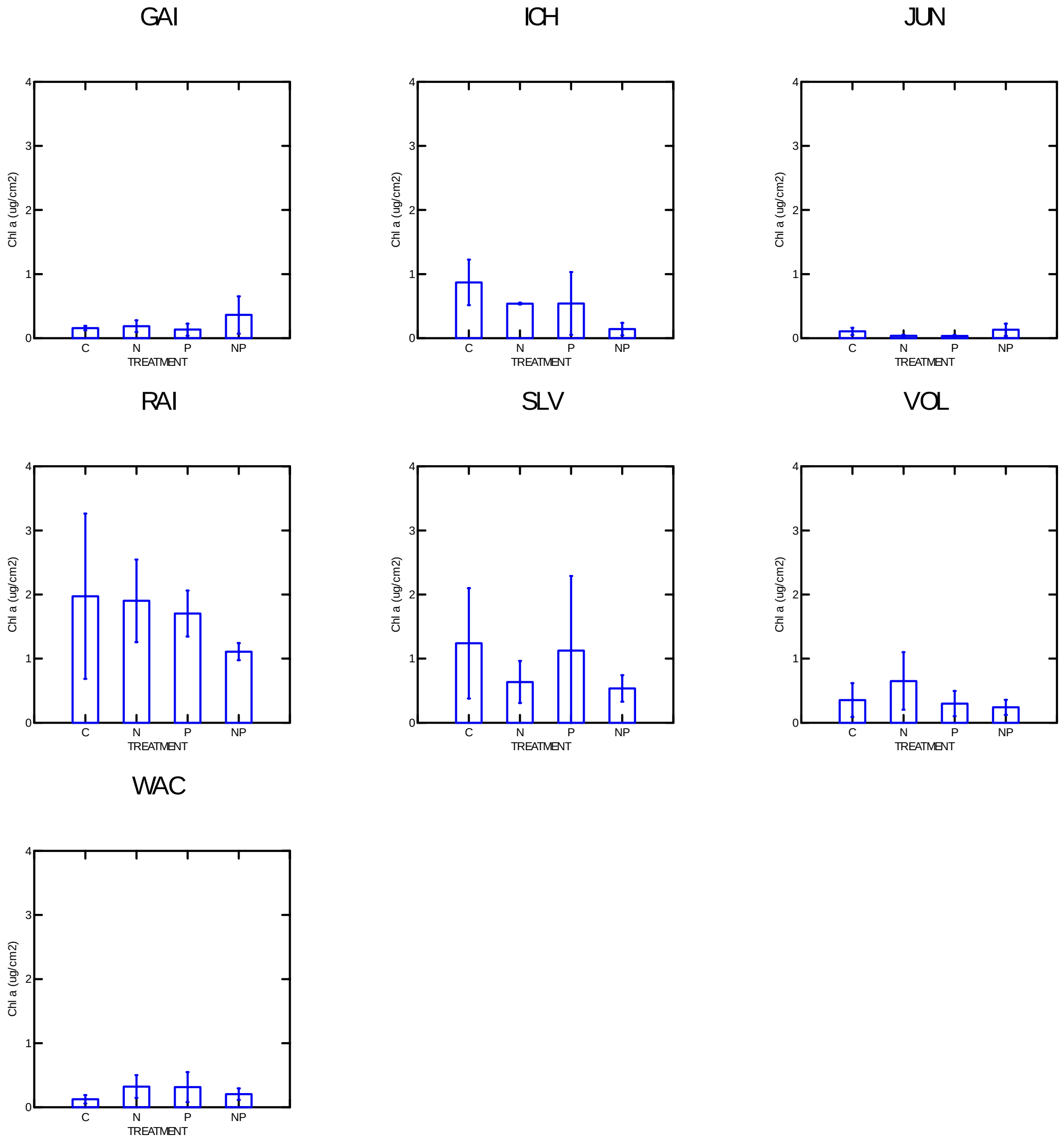
Results

The algal biomass on nutrient diffusing substrata varied among sites, but nutrient treatment effects were not significant (Table 5.2, Figure 5.1). The algal biomass was usually very low in all treatments, ≤ 1 µg chl a/cm^2.

Table 5.2. Results of ANOVA for nutrient diffusing substrata experiment for 7 sites (GAI-02, ICH-01, JUN-02, RAI-01, SLV-01, VOL-01, WAC-02).

Source	SS	df	MS	F	P
Nutrient treatment	1.031	3	0.344	1.039	0.382
Site	21.953	6	3.659	11.062	**0.000**
Nutrient treatment * Site	2.758	18	0.153	0.463	0.963
Error	18.522	56	0.331		

Figure 5.1. Algal biomass on nutrient diffusing substrata measured as chl a (Mean ±SE).



Discussion

Nutrient diffusing substrata are an excellent tool in assessing limiting nutrients directly at the study site, but they are easily damaged. The recovery rate of NDS was very poor. Substrata were damaged by humans and animals and were covered with macroalgal mats, plants, and sediments. This indicates that springs are very dynamic environments with a lot of disturbance and with many factors affecting algal growth and distribution. Although a small grazer effect on the substrata was reduced by allowing algae to colonize under Nitex, sites did have different exposures to light and current velocity. Although the effects of light and current velocity may have masked the effects of nutrient enrichment, the low algal biomasses obtained indicated that another factor may be important. With grazing minimized and saturating nutrient concentrations, high densities of algae should have accumulated during a 3-week period (e.g., Humphrey and Stevenson, 1992; Rier et al., accepted). The low colonization rates may have been due to the unique nature of springs, which likely have very low densities of suspended algae because they arise from groundwater. Suspended algae in most shallow streams with short residence time accumulate from the downstream transport of benthic algae (Stevenson and Peterson, 1993). Suspended algae provide the initial colonists for community development on artificial substrata (Stevenson, 1983). Low suspended algal densities would limit immigration, growth, and community development on any artificial substratum. Use of artificial substrata in springs may require either inoculation with algae by special means or much longer exposure times.

NUTRIENT DIFFUSING SUBSTRATA (NDS) AND MACROALGAE

Nutrient diffusing substrata were modified to expose macroalgae to increased nitrogen and phosphorus levels. The snap-cap vials nutrient diffusing substrata described above were modified by attaching Glad round 125ml cups with a hole cut in the bottom and a lid replaced by nylon mesh. A weighed sample of macroalgae was placed in the cup. In the initial 5-day trials macroalgae were found to be doing well and some growth was observed. In addition to nitrate and phosphate treatments, calcium and calcium carbonate treatments were added. There were 6 treatments (C, N, P, NP, NPCa and $NPCaCO_3$). Agar nutrient solutions were prepared as described above. The NPCa treatment was prepared by adding $CaHPO_4$ (0.1M) and keeping N and P concentrations as in the NP treatment. The $NPCaCO_3$ treatment was prepared by adding $CaCO_3$ (0.1M) to solution, as in the NP treatment. There were 6 replicates of each treatment (36 modified NDS per site). Approximately 1 g fresh mass of macroalgae was placed in each cup. Prepared substrata were filled with site water and pressed into the bottom at the study site. Modified NDS were placed at 3 sites with restricted access (no swimming area): JUN-02 (cups with *Lyngbya majuscula* collected from JUN-01), WAK-01 (cups with *Vaucheria* sp. collected at the site) and WEK-01 (cups filled with *L. majuscula* collected at the site). A total of 108 substrata were left in the field for two weeks. After two weeks at JUN-01, the substrata were damaged (they appeared to have been chopped by an alligator), ripped out of the bottom, or covered by sediments. Less than half of the substrata placed were recovered. At WAK-01, the substrata were completely covered with *Vaucheria* sp., which had grown into the containers. At WEK-01, the substrata were covered with a *L. majuscula* mat or missing.

CHAPTER 6. DIATOM INDICATORS OF NUTRIENT CONDITIONS IN FLORIDA SPRINGS

Introduction

Using the species composition of diatoms can be sensitive, precise, and therefore, useful indicators of biological and nutrient conditions in streams (Pan et al., 1996; Chetelat et al., 1999; Wang et al., in press). Diatom indicators of nutrient conditions may be even more sensitive than chlorophyll and biomass measurements and more precise than a one-time measurement of nutrients (Stevenson and Bahls, 1999; Stevenson and Smol, 2003). Diatoms should be better indicators of biological responses to nutrient enrichment than macroinvertebrates and fish, because they respond directly to changes in nutrient concentration. Although the latter hypothesis has not been rigorously tested, that rationale and past results with diatom indicators have been used to support the potential value of this study.

Florida springs water varies greatly in solute concentrations, and water age and origin among sites (Rosenau et al. 1977; Katz et al. 1999, 2001). Conductivity is recognized as one of the major factors affecting diatom assemblages (Pan et al. 1996). It is expected that differentiating the effects of nutrients from conductivity will be more challenging for nutrient indicator development in Florida springs than in other regions.

The overall objective of this study was to develop diatom indicators of nutrient conditions for Florida springs. To do this, first the relationships were determined between nutrients and other environmental variables in Florida springs. Second, the relationship were determined between nutrients and diatom assemblages in Florida springs. Finally, indicator species were determined for different nutrient conditions in the springs and multispecies indicators of these nutrient conditions were tested.

Materials and Methods

Study sites

Twenty-eight springs located in northern and central Florida were selected for this study. Samples were collected twice: first in the spring of 2003 (March and April) and second in the fall of 2003 (September to November). In the spring 48 sites within 16 springs were sampled, and in the fall 60 sites within 28 springs were sampled. (For a description of study sites, refer to Chapter 2.)

Diatom sampling

A detailed description of sampling protocol can be found in Chapter 2. A composite quantitative sample of epiphytes was made with algae brushed off and rinsed from the mid-section of plant fragments of known surface area collected at the site. Diatom subsamples were collected by homogenizing samples on a magnetic stirrer, and collecting aliquots of each sample with a pipette for diatom subsamples. Multiple aliquots were collected to reduce error due to patchiness.

Diatoms were also sampled from the surface of macroalgae. Pinches of macroalgae were collected from 9 transects at each site to produce one composite macroalgal sample per site. Since it was impossible to homogenize macroalgae with a blender or Biospec biohomogenizer, a subsample for diatom analysis was taken by grouping pinches of different taxa of macroalgae into separate piles and cutting out 1/4 of each pile with a razor blade. Diatoms were separated from macroalgae by oxidation and digestion of organic material with nitric acid, leaving just the glass cell walls of diatoms for identification. Only macroalgal diatom subsample DM was determined. The surface area of macroalgae was not determined.

A composite quantitative epipelon sample consisted of multiple sediment cores (approximately 0.3 cm deep and 5.2 cm diameter) that were collected at each transect. Algae were collected through swirling and rinsing the sample 10 times with distilled water (Stevenson and Stoermer, 1983). Diatom subsamples were collected by homogenizing samples on a magnetic stirrer, and collecting aliquots of each sample with a pipette. Multiple aliquots were collected to reduce error due to patchiness.

Diatoms were digested with boiling nitric acid and by adding a catalyst, potassium dichromate. Diatoms were mounted in Naphrax® on microscope slides and identified to the lowest possible level, which was usually species. Identification of diatoms was primarily based on Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b); Patrick and Reimer (1966, 1975) were used as supplemental sources of information.

Water analyses

At each site, on transect 1 and 9 water temperature, pH, conductivity and dissolved oxygen (DO) were measured. From the most upstream transect water samples were collected for assays of alkalinity, ammonia nitrogen, calcium, chloride, iron, magnesium, Kjeldahl nitrogen (KN), nitrates, orthophosphate, silica, sodium, strontium and sulfate. Water chemistry samples were analyzed by FDEP. (For a description of water sample analyses, refer to Chapter 2.)

Data Analysis

Only data from research during the spring of the year were used in analyses for this chapter, because fall samples were still being counted at the time that data analysis began. The relationships among environmental variables were analyzed first with Pearson correlation analyses to determine the covariation among environmental attributes. After initial exploration, a new data set was formed by removing high conductivity sites (> 2SD + mean), because conductivity is an important factor regulating diatom community structure. Sites with highly unusual species composition can greatly reduce precision when they are used in a dataset to develop indicators.

Canonical correspondence analyses (CCA) were executed with the full dataset and then with a reduced dataset. For the reduced dataset, sites with unusual chemistry were removed to assess the relationship between diatom species composition and environmental variables and the scale at which diatom responses to nutrients could be observed. Epiphytes were analyzed first as an example for epipelon and epi-macroalgae. Nutrients and potassium were square-root transformed; the other environmental variables were natural-log transformed.

Species composition of diatom assemblages on plants was used for testing nutrient indices. It was decided to limit analysis and presentation of data to one habitat and during one season because of time constraints. Analysis of plants was chosen, because past experience indicated that epiphytic diatoms are more sensitive to changes in nutrients than diatoms in other habitats (Wang, unpublished data). Nutrient indicator values were extracted from the literature, past analysis of Florida streams data, and recent results from the Mid-Atlantic Integrated Assessment (MAIA) conducted by the Environmental Monitoring and Assessment Program of the USEPA. Nutrient indices (NI) were calculated as a weighted average of species nutrient indicator values (θ_i) based on species relative abundance in samples (p_i). Thus, $NI_i = \Sigma (p_i\theta_i)/\Sigma p_i$ for $i=1,S$, where S is the number of species for which a nutrient indicator value is known. The correlations between values of nutrient indices and nutrient concentrations were evaluated by Pearson correlation.

In addition to use of old autecological characterizations of taxa, new characterizations were developed with the epiphytic algal data sampled during the spring. Standard weighted average regression techniques (ter Braak and van Dam, 1989) were used to develop and test indicators of TN and TP using the CALIBRATE® program (Juggins and ter Braak, 1992).

Species tolerances to low nutrient conditions and requirements for high nutrients were further analyzed with indicator species analysis (ISA) (Dufrene and Legendre 1997). TP was categorized according to its concentration range in the sampling sites. $TP < 20$ μg/L was assigned as 1, $20 \leq TP < 30$ μg/L was assigned as 2, $30 \leq TP < 40$ μg/L was assigned as 3, and $TP \geq 40$ μg/L was assigned as 4. A TP category of 5 was created for a few sites that had very high TP concentrations. TN was also categorized according to its concentration and range of sites sampled. One was assigned to $TN < 0.5$ mg/L, 2 was assigned to $0.5 \leq TN < 1.0$ mg/L, 3 was assigned to $1.0 \leq TN < 1.5$ mg/L, and 4 was assigned to $TN \geq 1.5$ mg/L. Indicator species analysis was conducted with datasets that included all sites and with sites with unusual chemistry removed, as with most other analyses, to isolate the complicating factor, conductivity, from nutrients. Species were selected as indicator species if their $p < 0.1$ in the ISA. TN or TP categories were assigned according to results of ISA.

Results

When all sites were included in the analysis, N and P concentrations were not correlated. TN was positively correlated with NOx, alkalinity, and Ca and negatively correlated with Mg, Si, and S (Table 6.1). KN was positively correlated with NH_4-N, Cl, Na, K, and conductivity. NOx was positively highly correlated with TN and alkalinity and negatively correlated with Si, Na, Sr, S, and pH. NH_4-N was positively correlated with KN and negatively correlated with PO_4 and TP. TP was positively correlated with PO_4, Cl, Mg, Si, Na, Sr, S, temperature, conductivity, and K but was negatively correlated with DO and NH_4. PO_4 was also negatively correlated with DO, pH, and NH_4 but was positively correlated with TP, Si, and S.

Alkalinity was highly and positively correlated with Ca, but negatively correlated with pH (Table 6.1). Both Sr and conductivity were highly correlated with cations, Cl, and S. The pH data were negatively correlated with cations, S, and conductivity, but positively correlated with DO.

Table 6.1.
Correlations among nutrients and environmental variables at all sites during spring. Only correlations with P<0.05 are recorded. * represents 0.01< p < 0.05, ** represents 0.01 < p < 0.001, *** represents p < 0.001.

		Alk	NH4	Ca	Cl	Mg	TN	Kj-N	NOx	PO4	TP	K	Si	Na	Sr	S	DO	pH	Temp
NH4	R																		
	P																		
Ca	R	0.82																	
	P	***																	
Cl	r			0.42															
	p			**															
Mg	r			0.52	0.90														
	p			***	***														
TN	r	0.40		0.30		-0.33													
	p	**		*		*													
Kjet-N	r		0.51		0.49	0.32													
	p		***		***	*													
NOx	r	0.44				-0.37	0.99												
	p	**				**	***												
PO4	r		-0.40																
	p		**																
TP	r		-0.34		0.35	0.45				0.86									
	p		*		*	**				***									
K	r			0.36	0.94	0.89		0.41			0.30								
	p			*	***	***		**			*								
Si	r	0.41		0.34		0.44	-0.31		-0.29	0.49	0.47								
	p	**		*		**	*		*	***	***								
Na	r			0.44	1.00	0.92		0.47	-0.31		0.36	0.93							
	p			**	***	***		***	*		*	***							
Sr	r			0.61	0.75	0.80			-0.29		0.43	0.65	0.34	0.77					
	p			***	***	***			*		**	***	*	***					
S	r			0.55	0.78	0.88	-0.31		-0.35	0.31	0.51	0.71	0.40	0.80	0.96				

		Alk	NH4	Ca	Cl	Mg	TN	Kj-N	NOx	PO4	TP	K	Si	Na	Sr	S	DO	pH	Temp
	p			***	***	***	*		*	*	***	***	**	***	***				
DO	r	-0.32								-0.59	-0.48		-0.47						
	p	*								***	***		**						
pH	r	-0.76		-0.71		-0.41			-0.30	-0.32			-0.32	-0.30	-0.35	-0.39	0.38		
	p	***		***		**			*	*			*	*	*	**	*		
Temp	r				0.47	0.31		0.31			0.31	0.36		0.44	0.51	0.47			
	p				**	*		*			*	*		**	***	**			
Cond	r			0.63	0.94	0.91		0.48			0.33	0.93		0.94	0.83	0.84		-0.41	0.35
	p			***	***	***		***			*	***		***	***	***		**	*

Four ordination axes explained 29.9% of the variance in species composition of epiphytic diatoms with CCA when all sites were included in the analysis (Table 6.2). The first and all canonical axes were statistically significant (F = 4.2, p = 0.005; F = 2.3, p = 0.005). The four axes explained 62.9% of species-environment variance. The first CCA axis was highly correlated with Cl and conductivity (r = 0.82, $p < 0.001$; r = 0.75, $p < 0.001$); the second CCA axis was positively correlated with Sr (r = 0.62, $p < 0.001$) and negatively correlated with alkalinity (r = -0.32, p = 0.03) (Table 6.3). A CCA biplot indicated that most sites clustered around the mid-point of the CCA with a few, high-conductivity sites strongly affecting the analysis (Figure 6.1). A table with species scores on the CCA axes can be found in Appendix B (Table A).

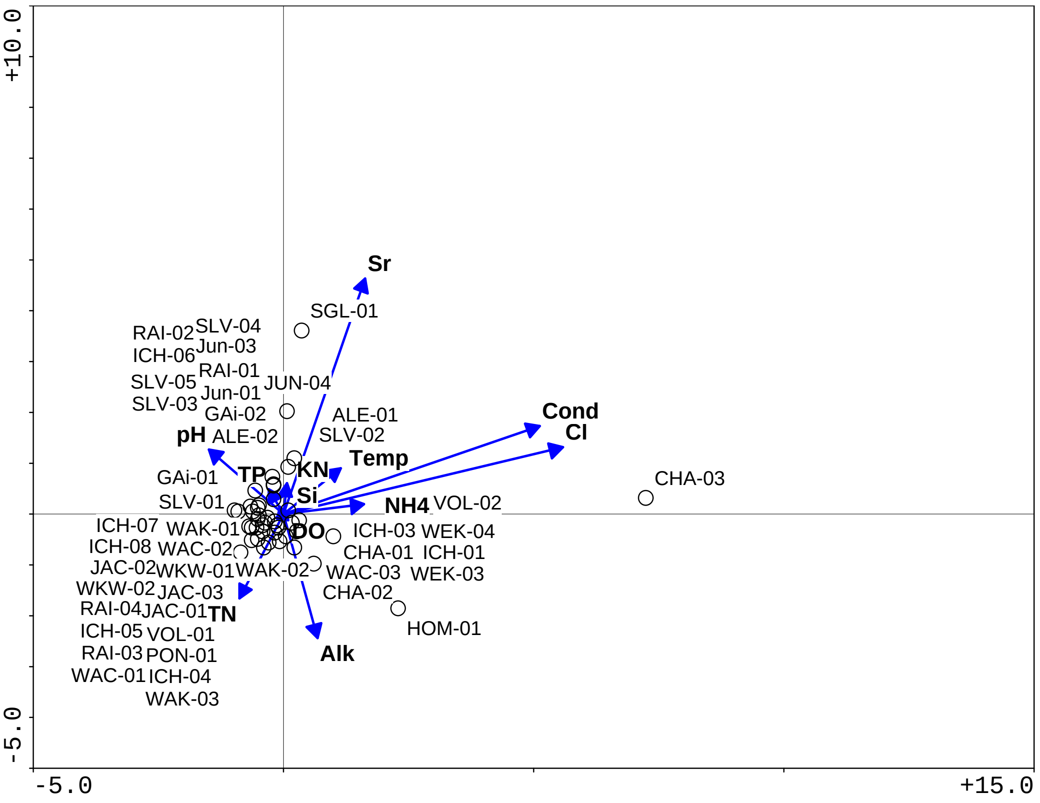
Table 6. 2.
The percentages of variance explained in the CCA. The CCA was analyzed with epiphytic diatoms and environmental variables and data from all sites during spring.

Axes	1	2	3	4	Total inertia
Eigenvalues (λ)	0.604	0.396	0.285	0.230	5.063
Species-environment correlation	0.953	0.865	0.93	0.849	
Cumulative % variance of:					
Species data	11.9	19.8	25.4	29.9	
Species-enviroment relationship	25.1	41.6	53.4	62.9	
Test of significance of first canonical axis	F=4.2, p=0.005				
Test of significance of all canonical axes	F=2.3, p=0.005				
Sum of all unconstrained λ					5.063
Sum of all canonical λ					2.406

Table 6.3.
The correlation between CCA and environmental variables on the first two axes. Epiphytic diatom data and environmental variables from all sites collected during the spring were used. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.

Environmental variables	CCA1	CCA2
Alkalinity		-0.32^{*}
NH_4-N		
Cl	0.82^{***}	
TN		
Kjeldahl N		
PO_4		
TP		
Si		
Sr		0.62^{***}
DO		
pH		
Temperature		
Conductivity	0.75^{***}	

Figure 6.1.
A CCA biplot of the first 2 axes. The CCA was analyzed with epiphytic diatoms and environmental variables.



After removing high conductivity sites (ALE-01, ALE-02, CHA-03, HOM-01, JUN-04, SGL-01, VOL-01, and VOL-02), a second CCA was executed (Figure 6.2). Four ordination axes explained 29.3% of the variance in epiphytic diatom assemblage with CCA (Table 6.4). The first and all canonical axes were statistically significant (F = 2.62, p = 0.01; F = 1.83, p = 0.005). Four axes explained 59.9% of species-environment variance. The first CCA axis was positively correlated with Cl (p = 0.79, r < 0.001), but negatively correlated with NH4-N and TN (r = -0.38, p = 0.008; r = -0.39, p = 0.009, respectively). The second CCA axis was negatively correlated with TP and Si (r = -0.32, p = 0.06; r = -0.32, p = 0.06), but positively correlated with NH4-N, Cl, TN, KN, and DO (r = 0.72, p < 0.001; r = 0.38, p = 0.02; r = 0.48, p = 0.006; r = 0.80, p < 0.001; r = 0.55, p < 0.00; respectively) (Table 6.5). A table with species scores on the CCA axes can be found in the Appendix B (Table A).

Figure 6.2.
A CCA biplot of the first 2 axes. The CCA analysis was conducted using epiphytic diatoms and environmental variables. High conductivity sites were removed from this analysis.

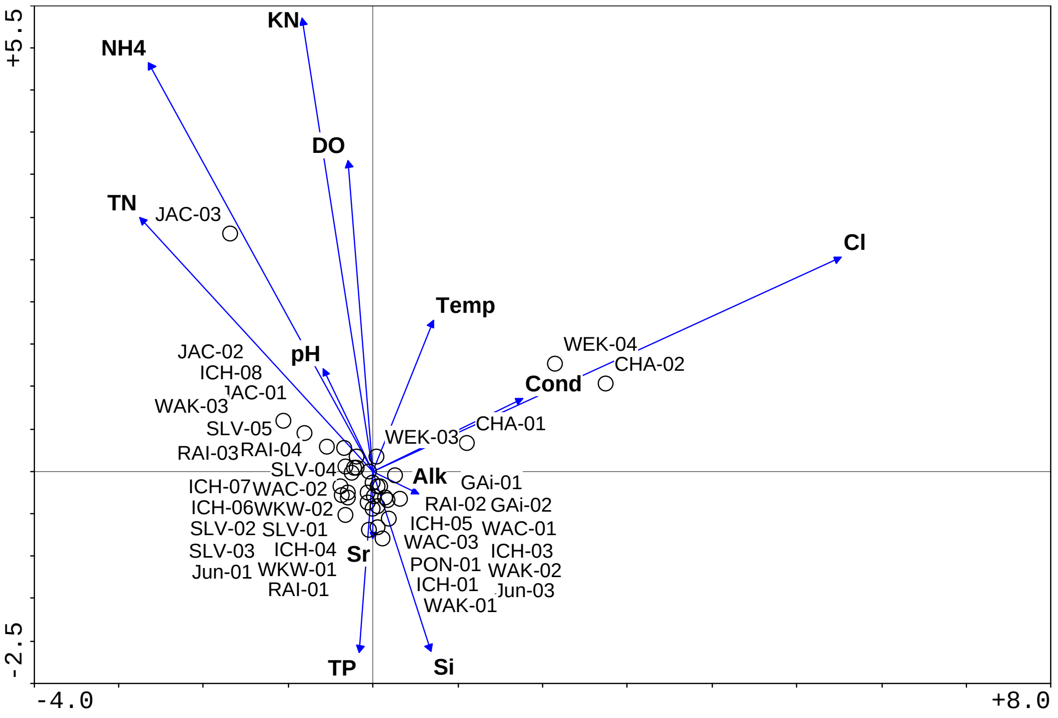


Table 6.4.
The variance explained by CCA axes. The CCA analysis was conducted using epiphytic diatoms and environmental variables. High conductivity sites were removed from this analysis.

Axes	1	2	3	4	Total inertia
Eigenvalues (λ)	0.351	0.293	0.205	0.155	3.428
Species-environment correlation	0.936	0.933	0.893	0.85	
Cumulative % variance of:					
Species data	10.2	18.8	24.8	29.3	
Species-enviroment relationship	21	38.4	50.7	59.9	
Test of significance of first canonical axis	F=2.62, p=0.01				
Test of significance of all canonical axes	F=1.83, p=0.005				
Sum of all unconstrained λ					3.428
Sum of all canonical λ					1.675

Table 6.5.
The correlation between CCA on the first two axes and environmental variables. Epiphytic diatom data and environmental variables collected during the spring were used. High conductivity sites were excluded from this analysis. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.

Environmental variables	CCA1	CCA2
Alkalinity		
NH_4-N	-0.38^{*}	0.72^{***}
Cl	0.79^{***}	0.38^{*}
TN	-0.39*	0.48^{**}
Kjeldahl N		0.80^{***}
TP		-0.32
Si		-0.32
Sr		
DO		0.59^{*}
pH		
Temperature		
Conductivity		

Conductivity was the dominant environmental constraint on species composition of diatoms on macroalgae and in epipelon, but nutrients were also important. Four ordination axes explained between 23.2 and 30.1 % of the variation in diatom species composition among sites, either with or without including high conductivity sites (Tables B, C, D and E in the Appendix B). Four axes explained between 52.1 and 60.4% of species-environment variance. Significant relationships between diatom species composition on macroalgae and nutrients were only observed after removing high conductivity sites from the dataset (Figures A and B in the Appendix B). Relationships between epipelic diatom species composition and nutrients were evident with both the full dataset and dataset without high conductivity sites, but relationships with nutrients in the later dataset were more important (Figures C and D in the Appendix B). In all cases, TN or NO_x were correlated to diatom species composition in different directions than TP or SRP. Table with species scores on the CCA axes can be found in the Appendix B (Tables G and J).

Diatom indicators of nutrient conditions were complexly correlated to nutrient concentrations, and not correlated as predicted (Table 6.6). The MAIA trophic index was correlated to more measures of nutrient concentrations than other indicators. The FL_{avg} and FL_{new} trophic status indicators were correlated to 3 of 8 nutrient measurements. Most of these indicators were negatively correlated with N measurements, except that the FL TP indicator was positively correlated with TN and NOx. All indicators were positively correlated with P measurements in springs.

Diatom inference models of TP and TN developed with weighted averaging using diatoms on plants were highly correlated to TP and TN concentrations ($0.59 < r^2 < 0.71$) before testing with jackknifing, but were not highly correlated afterwards ($0.15 < r^2 < 0.26$). Models for TP had higher r^2 values than models for TN before jackknifing, but the opposite was true after jackknifing (Figure 6.3). Little difference in r^2 values was observed between models using datasets with all sites and without high conductivity sites. Indicator values for diatom taxa are provided in Tables K and L. Indicator values for epiphytes using the dataset with all sites are provided because these metrics with all sites performed as well as metrics based on the dataset without high conductivity sites.

Table 6.6.
Correlations between nutrient concentrations and previously developed diatom indicators of nutrient conditions using species composition of diatoms on plants in Florida Springs. Only correlations with P<0.05 are indicated.

		Ammonia	TN	Kje-N	TNs	NOx	PO_4	TP	TPs
FL TP	Pearson Correlation		0.355			0.345			
	Sig. (2-tailed)		0.020			0.023			
FL TN	Pearson Correlation								
	Sig. (2-tailed)								
MAI	Pearson Correlation	0.555		-0.481			0.392	0.361	0.348
	Sig. (2-tailed)	0.000		0.001			0.009	0.016	0.021
Slate SRP	Pearson Correlation								
	Sig. (2-tailed)								
Slate TP	Pearson Correlation								0.306
	Sig. (2-tailed)								0.044
WHITMORE	Pearson Correlation	0.435							
	Sig. (2-tailed)	0.003							
VD N	Pearson Correlation			-0.450					
	Sig. (2-tailed)			0.002					
VD Salinity	Pearson Correlation								
	Sig. (2-tailed)								
VD Troph	Pearson Correlation								
	Sig. (2-tailed)								
FL avg	Pearson Correlation			-0.362			0.312		0.304
	Sig. (2-tailed)			0.016			0.039		0.045
FL new	Pearson Correlation		-0.399	-0.349		-0.377			
	Sig. (2-tailed)		0.007	0.020		0.012			

ISA showed lists of indicator species with respect to different groups of nutrients. The results from ISA generally supported the observations from CCA. Under low conductivity conditions, species such as *Achnanthes exigua*, *Nitzschia inconspicua*, *Navicula arvensis*, *Nitzschia subacicularis*, *Navicula viridula* var. *rostrata*, *Fragilaria brevistriata*, *Sellophora pupula* preferred a high TP environment. Species such as *Cymbella microcephala* and *Fragilaria construens* preferred low TP conditions (Table 6.7). Under low conductivity conditions, *Achanthes minutissima*, *Cymbella affinis*, *Melosira varians*, *Cymbella microcephala*, *Fragilaria construens*, and *Fragilaria capucina* were grouped as category 4 in TN preference, while *Nitzschia dissipata* was grouped as category 1 in TN preference (Table 6.8). Under high conductivity conditions, species such as *Navicula tenelloides*, *Bacillaria paradoxa*, *Fragilaria brevistriata*, *Navicula gregaria*, *Fragilaria capucina*, *Gomphonema clevei*, *Navicula sanctaecrucis*, *Synedra fasciculata*, *Nitzschia gracilis*, and *Gomphonema mexicana* were categorized as group 3 or 4 of TP and group 1 or 2 of TN; thus they had a high TP and low TN preferences. In addition, species such as *Navicula menusculus*, *Nitzschia recta*, *Fragilaria pinnata*, *Eunotia subarcutoides*, *Gomphonema angustum*, *Gomphonema dichotumus*, and *Navicula contenta* preferred both category 1 of both TP and TN (Table 6.9).

Figure 6.3.
Plots of observed nutrient concentrations (TN mg/L; TP µg/L) vs. weighted average inferred nutrient concentrations. A and C included all sites; B and D excluded high conductivity sites.

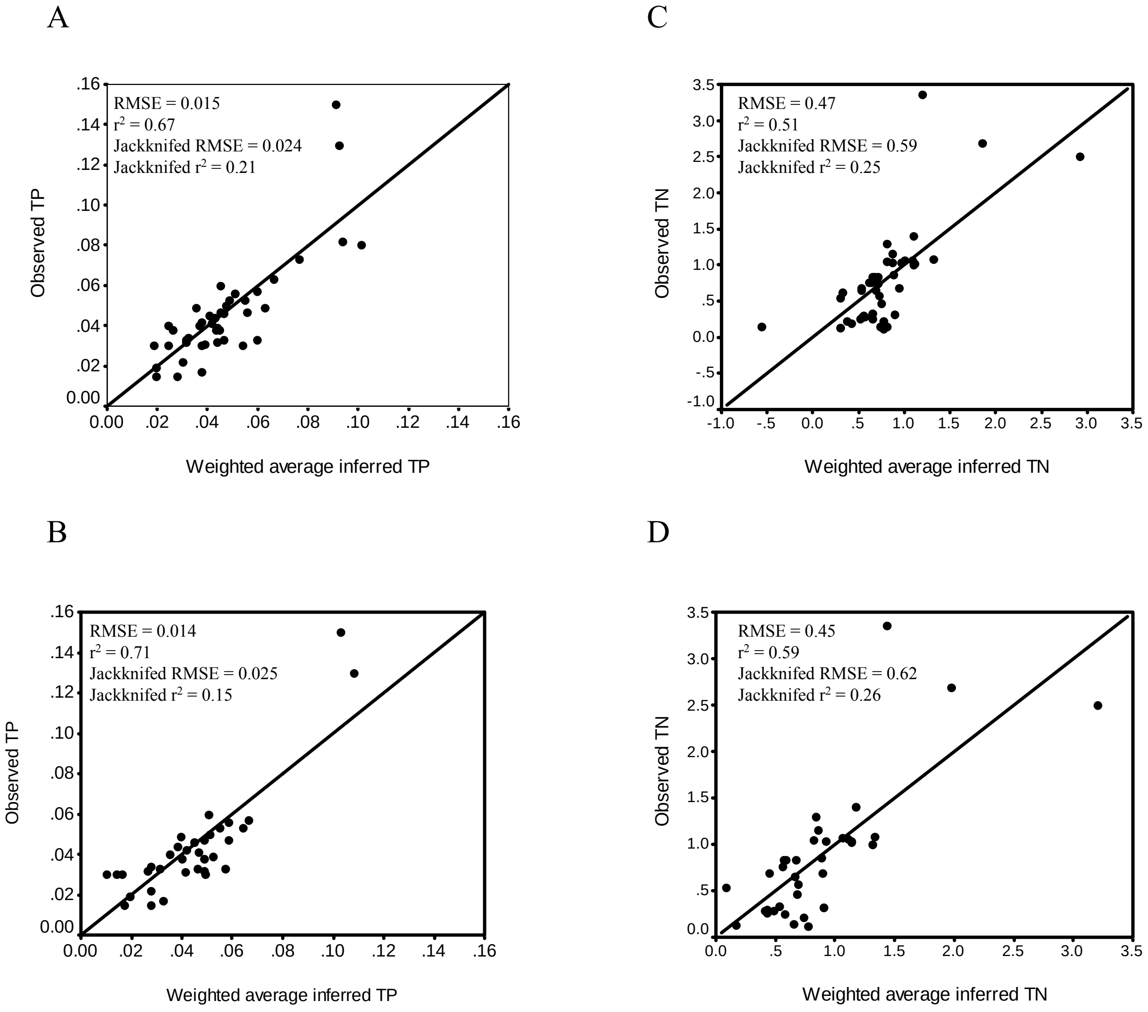


Table 6.7.
Species TP preference in different environmental conditions assessed by indicator species analysis.

All sites				Selected sites			
Species	IV	p	Group	Species	IV	p	Group
NAtendes	74.1	<0.001	3	ACexigua	82	<0.001	5
ACdelica	44.5	0.040	3	NIincons	100	0.001	5
ROcurvat	38.3	0.068	3	NAarvens	87.2	0.002	5
PTlanceo	35.9	0.071	3	NIsubaci	91	0.004	5
NIspp	41	0.092	3	GOparvul	54.8	0.008	4
BAparado	92.4	<0.001	4	NAvirros	60.7	0.008	5
FRbrevis	83.9	<0.001	4	FRcapmes	56.7	0.011	4
NAgregar	98.6	<0.001	4	CAbacill	47.6	0.026	3
FRcapuci	78	0.005	4	NArhyger	54.5	0.026	4
GOclevei	48.7	0.010	4	AMveneta	54.4	0.038	3
NAsancru	50	0.012	4	FRconstu	55.9	0.044	1
SYfascic	50	0.012	4	CMaffins	51.3	0.046	1
NIgracil	62.1	0.018	4	FRbrevis	53.3	0.046	5
GOmexica	59.1	0.021	4	SFpupula	43.6	0.047	5
AMinarie	47.9	0.022	4	CCneodim	62.7	0.048	3
FRellipt	65.9	0.030	4	ACclevei	49.9	0.054	3
PTlanfre	46.4	0.041	4	PTlanros	53.7	0.055	5
EPadnata	37.4	0.049	4	GOclevei	50	0.059	5
GOgracil	43.4	0.087	4	NAgoeppe	50	0.059	5
NAcapita	31.6	0.092	4	NAingenu	50	0.059	5
				NAmolest	50	0.059	5
				NIgracil	44.2	0.064	4
				SYulna	52.3	0.072	2
				AUcrenul	44.1	0.078	4
				ACminuti	53.2	0.082	1
				GOpumilu	46.9	0.092	2

Table 6.8.
Species TN preference in different environmental conditions assessed by indicator species analysis.

TN (High cond)				TN without outliers			
Species	IV	p	Group	Species	IV	p	Group
AMlibyca	74.7	<0.001	2	ACminuti	66.6	0.005	4
NAcapita	72.5	<0.001	2	CMaffins	61.1	0.005	4
EPadnata	75	0.001	1	ACclevei	63	0.006	3
NIspp	74.7	0.002	1	FRbrevis	62	0.006	3
ACdelica	67.7	0.004	2	CCplacen	53.4	0.008	2
GOaffine	50	0.013	1	MEvarian	59.1	0.011	4
RPbrebis	50	0.013	1	CMmicroc	58.1	0.012	4
GOclevei	48.7	0.016	2	FRconstu	59.9	0.012	4
NAsancru	50	0.016	2	FRcapuci	61.5	0.015	4
SYfascic	50	0.016	2	NArecens	42.2	0.042	2
FRcapuci	73.7	0.021	1	NIsubaci	36.4	0.043	3
AMinarie	47.9	0.023	2	NIdissip	41.7	0.049	1
NAtendes	43.8	0.032	2	ACrupdes	52.7	0.065	3
PTlanfre	46.4	0.037	1	NAphylle	27.8	0.070	3
NAgregar	45.9	0.056	2	NAarvens	23	0.076	3
PTlanceo	35.9	0.072	1	SYulna	56.1	0.079	2
ROcurvat	38.3	0.082	1	CAbacill	33.4	0.083	3
				NIgracil	39.7	0.089	1

Table 6.9.
Species with preference for low NP conditions assessed by indicator species analysis.

Species	IV	p	Group
NAmenscl	95.9	0.001	1
NIrecta	64.4	0.007	1
FRpinnat	82.1	0.058	1
EUsubarc	33.3	0.065	1
GOangust	33.3	0.065	1
GOdichot	33.3	0.065	1
GOspp	33.3	0.065	1
NAconten	33.3	0.065	1
CMaffins	49	0.075	1
FRbidens	30.8	0.084	1
PTlanceo	31.5	0.084	1
CMsilesi	30.2	0.086	1
PTlanros	72.5	0.099	1

Discussion

The lack of correlation among TN, TP, and conductivity in Florida springs is unusual. After removing high conductivity sites, we found that TN and TP were negatively correlated among most sites in FL springs. In most studies, TN and TP are positively correlated, and both are usually correlated with conductivity or Cl and human disturbance in watersheds (e.g., Johnson et al., 1997; Herlihy et al., 1998; Stevenson et al., accepted). In low conductivity waters, increases in conductivity can be related to landscape disturbance (Bolstad & Swank, 1997) and can be used as indicators of increased nutrient supply (Biggs, 1988). Variation in water chemistry among Florida springs represents a special case of nutrient relations in aquatic systems and likely reflects the diversity in sources of water and contamination among spring systems in Florida.

The magnitude of correlations between diatom species composition and environmental variables in Florida springs was similar to values observed in other studies. CCA analyses commonly explain between 20 and 40% of variation in species composition among streams and lakes in a region (Hall and Smol, 1992; Pan et al., 1996). CCA analyses may explain less variation in diatom species composition when great physical-chemical diversity occurs among sites, such as wetlands (Stevenson et al., 1999).

Conductivity explained more variation in diatom species composition among Florida springs than nutrients did. Conductivity, pH, or other factors indicating ionic/osmotic gradients are commonly a dominant attribute regulating variation in diatom species composition among streams, wetlands, and lakes (Hall and Smol, 1992; Pan et al. 1996; Stevenson et al., 1999). Florida springs were unusual, because the variation in conductivity was great and the effects of other environmental variables on species composition were masked unless the variation in conductivity was accounted for. A simple approach was used in this study to account for conductivity related variables. A limited number of sites with the highest conductivity were removed from the dataset. Results suggested that nutrients become important variables affecting species composition if the range in conductivity was constrained.

CCA analyses indicated that diatoms responded differently to TN and TP variation among Florida spring sites. Indicator species analyses also indicated that different species were relatively more abundant in high N and high P environments, as well as low NP conditions. From ISA, in general, high TP species belonged to the low TN species group and low TP species belonged to the high TN group. In high conductivity sites, species listed in the table belonged to the low TN and high TP group. Another group of species preferred both low TN and low TP conditions. A similar pattern can be observed from the CCA. Although it is known that different species occur in low nutrient conditions than in high nutrient conditions, usually high nutrient conditions include both high N and P. Few cases exist in which we can distinguish diatoms that thrive in low N/high P conditions and high N/low P conditions.

Existing diatom indicators of nutrient conditions need to be refined before application to Florida spring systems. Existing diatom metrics indicating nutrient conditions were unpredictably correlated to N and P concentrations in springs. Most existing trophic status metrics were positively correlated to TP, but unpredictably correlated to TN. Diatom indicators of nutrient conditions from other ecosystems were developed in conditions where N and P concentrations were positively correlated, and usually where P is hypothesized to be the most limiting factor (Stevenson, unpublished data and reports). If diatom taxa respond differently to independent N and P enrichment, as indicated by CCA, then, with the existing metrics, a more positive response would be expected to the TP concentration than to TN.

Refined diatom indicators of nutrient conditions based on autecological information developed with data from diatoms and environmental conditions in Florida springs show promise for application, but they should be tested more completely. Weighted average regression models developed separately to indicate TN or TP were relatively precise before testing by jackknifing. Correlations with r^2 greater than 0.5 usually indicate a good fit (Pan et al. 1996). However, poor correlations between diatom-inferred and measured nutrient concentrations ($r^2 < 0.26$) after jackknifing suggested caution should be exercised with use. This reduction in goodness of fit of nutrient models after jackknifing is common for nutrient models (see Pan et al., 1996, for discussion). In addition, the issue of whether N or P models should be used to imply trophic status should be explored. Thus, the N and P regulation of productivity in Florida springs and streams should be better understood. If springs or streams are P limited, then the TP model should be applicable. However, high TN may also be a problem.

It is also recommended that weighted average models be used to compare the trophic status between reference and test sites and not be used to infer specific nutrient concentrations unless calibrated more thoroughly to conditions in those systems. Weighted average models are transferable among systems when the same nutrients regulate species composition, however, they may be biased and over- or underestimate actual nutrient concentrations. In a study of transferability of nutrient indicators from streams to wetlands, Wang et al. (in preparation) found that the rank of trophic status was correctly identified, but the actual nutrient concentrations were underestimated.

In conclusion, nutrient indicators developed in this study show significant promise. Further analysis of the data collected and comparison to existing data on Florida streams is warranted. Use of categorical autecological characterizations of taxa is recommended, like those used by van Dam et al. (1994), which will enable the ranking of sites. Indicator models using categorical autecological information can be calibrated to nutrient concentrations to infer specific nutrient concentrations if complements to nutrient measurements are needed.

CHAPTER 7. DATA DESCRIPTION

Data sets can be found in Appendix C. Data are organized in folder with corresponding chapter number. Data for each chapter can be found in the Excel files and in the Access databases. Excel files include the original data and the final data after calculations were performed. Access databases show calculations for the data sets. Summary of all data files can be found in Table 7.1.

Chapter 2

The original data from RPHA and field measurements can be found in RPHA_field_data_fall and in RPHA_field_data_spring Excel files. These two datasets were imported in parts to Access database - Florida Springs 20040531 for further analyses. Descriptions of codes and abbreviations used can be found in the Metadata for Chapter 2 Excel file. All tables in the Florida Sprigs 20040531 database can be linked by Site_code or Long_site_code.

Epiphytes soft counts data can be found in Access database - Epiphytes_soft_counts. Final results for epiphytes soft counts can be found in Epiphytes_Cells_mm2 Excel file.

Chapter 3 and 4

The original data and results of calculations for nitrate and phosphate dosing experiments can be found in Nitrate_and_Phosphate_dosing_experiments Excel file. The original data and results of calculations for nitrate and phosphate dosing experiment can be found in Conductivity_experiments. Calculations for this data sets can be found in Access database Experimental_streams. Description of codes and abbreviations used can be found in the Metadata for Chapter 3 and 4 Excel file.

Chapter 5

The original data and results of calculations for NDS can be found in NDS_data Excel file. Calculations for NDS data can be found in NDS_chla Access database.

Chapter 6

Results of diatom counts for all samples from spring and fall are in the Diatom_counts Excel file. Description of codes for diatom taxa can be found in Diatom_taxa_codes Excel file. Images of common diatom taxa (contributing at least 5% to any of the diatom counts) can be found in a PowerPoint file Florida Springs Common Diatoms.

Table 7.1
Summary of data description.

Chapter	Data description	Excel	Access
2	- data sheet used in the field	Datasheet fall 2003	
2	- RPHA - Chemical analyses of water and algal mats	RPHA_field_data_fall RPHA_field_data_spring	Florida Springs 20040531
2	- Epiphytes soft counts	Epiphytes_Cells_mm2	Epiphytes_soft_counts
3	- Nitrate dosing experiment - Phosphate dosing experiment	Nitrate_and_Phosphate_dosing_experiments	Experimental_streams
4	- Conductivity experiment	Conductivity_experiments	Experimental_streams
5	- NDS	NDS_data	NDS_chla
6	- Diatom counts	Diatom_counts Diatom_taxa_codes	

CHAPTER 8. REFERENCES

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CHAPTER 9. APPENDICES

APPENDIX A. Additional tables for Chapter 2.

APPENDIX B. Additional tables and figures for Chapter 6

APPENDIX C. Project original data and calculations