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A Comparison of Internal and External Supply of Nutrients to Algal Mats in Two First Magnitude Springs in Florida

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ABSTRACT

We measured nutrient sources available to *Lyngbya* and *Vaucheria* algal mats in two springs in Florida, Ichetucknee and Manatee, to evaluate whether the algal growth was supported by internal nutrient recycling of nitrogen (N) and phosphorus (P) or external inputs from spring boils. The mats had maximum depths of 40 to 60 cm, interstitial waters had levels of nitrate and soluble reactive phosphorus (SRP) near the analytical detection limit, and ammonium concentrations were as high as 17 mg L^{-1}. We estimated both internal and external nutrient fluxes to actively growing portions of algal mats and compared them to measurements of algal net nutrient uptake. Several lines of evidence suggest that algal N and P needs are primarily met by external nutrient inputs from spring boils: (1) the daily mass flux of SRP and N-NO_3^- from spring boils exceeds daily net algal nutrient uptake by 1 to 3 orders of magnitude; (2) the amount of N-NH_4^+ diffusing from the interior of mats to the water column comprised <1% of daily net algal N uptake; (3) advection of nutrients from the mats to the water column was <12% of daily net algal N uptake and <0.05% of daily net algal P uptake; and (4) N-NO_3^- concentrations in spring waters were observed to decline as they passed over the algal mats, yielding rates of N loss very similar to estimated N uptake by the algae.

Key words: algal biomass, Florida, macroalgae, nutrient recycling.

INTRODUCTION

More than 700 freshwater springs in northern Florida are fed by the Floridan Aquifer (Copeland 2003). The largest of these springs have discharges at their boils (i.e., the region where groundwater is vented in mass to the surface) >2.8 m^3 s^{-1} and are classified as first-magnitude springs. Prior to wide-scale development in Florida springs and their watersheds, the submerged aquatic vegetation (SAV) community was dominated by vascular plant species, primarily *Sagittaria kurziana* (Brown et al. 2008). However, long-term observations at many of the larger springs show increasing abundance of nonindigenous aquatic vascular plants such as *Hydrilla verti-*

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cillata, *Myriophyllum spicatum*, and *Eichhornia crassipes*, along with several taxa of nuisance algae (Brown et al. 2008). In a recent survey of 60 first- and second-magnitude springs in Florida (boil flow between 0.28 and 2.8 $m^3 s^{-1}$), the most commonly observed algal taxa were filamentous mat-forming cyanobacteria of the genus *Lyngbya* and the green alga *Vaucheria* (Stevenson et al. 2004).

Replacement of SAV with nuisance algae has negatively affected the environment and human enjoyment of first-magnitude springs throughout Florida (Brown et al. 2008). When algal mats senesce and die they cause localized depletion of oxygen and the release of ammonia and hydrogen sulfide, which degrades downstream water quality. The spread of macroalgae in Florida has resulted in reductions in fish and crab harvests, lost tourism, and increased removal costs (Bonn and Bell 2003). More than 70 biologically active compounds have been isolated from *Lyngbya*, many of which are toxic and/or carcinogenic to humans (Osborne et al. 2001). *Lyngbya* are a source of lyngbyatoxin and aplysiatoxin, which produce a condition known as "swimmer's itch" (Mynderse et al. 1977, Cardellina et al. 1979). *Lyngbya wollei* can produce a variety of paralytic shellfish poisons (e.g., saxitoxin) and other toxins capable of producing dermatitis in humans (Carmichael et al. 1997, Onodera et al. 1997, Stewart et al. 2006) and deaths in domestic and wild animals that consume algal mats (Hamill 2001, Gugger et al. 2005).

Several researchers have hypothesized that the shift from native SAV to macroalgae in Florida springs (Brown et al. 2008, Cowell and Dawes 2008) and elsewhere in the United States (Vis et al. 2008) was driven by landuse change in springsheds, specifically increased loading of organic matter and dissolved nutrients from agriculture and urbanization. For example in Ichetucknee Springs, mean annual nitrate ($N\text{-}NO_3^-$) concentration increased from 0.35 mg L^{-1} in 1975 to 0.70 mg L^{-1} in 2001 (Scott et al. 2004), and in the spring-fed Rainbow River $N\text{-}NO_3^-$ increased 15-fold between 1957 and 2006 (Cowell and Dawes 2008). A recent evaluation of water quality in 13 first-magnitude springs showed that mean $N\text{-}NO_3^-$ levels increased from 0.05 mg L^{-1} to 0.9 mg L^{-1} between 1970 and 2002 (Scott et al. 2004). However, Stevenson et al. (2004) evaluated the relationship between nitrogen (N) and phosphorus (P) concentrations and algal cover in 60 Florida springs and found only a weak relationship between *Lyngbya wollei* abundance and P concentrations in spring water and sediments, and no relationship between *Lyngbya wollei* abundance and N concentrations of water or sediment. For *Vaucheria*, there was a weak relationship between abundance and water column N, but not P.

Based on these findings, Stevenson et al. (2004) concluded that there was no simple cause and effect relationship between the chemistry of aquifer water and algal abundance, and they hypothesized that additional biological, physical, or chemical factors are working in concert with nutrient loading to cause increases in algal abundance. For example, invasive fish species (e.g., Asian grass carp) changes in disturbance regimes brought on by declines in grazing pressure by native mullets, or physical removal of SAV in swimming areas may have helped bring about the SAV-algal regime shift in Florida springs.

One possible mechanism for the persistence and growth of macroalgae is regeneration of nutrients within thick algal mats that form seasonally in most first-magnitude springs. As mats grow in size they slowly accumulate N and P in living and dead tissues and bottom sediments. For example, *Lyngbya* can attain biomass densities of 13.8 kg m^{-2} (Beer et al. 1986). As the mats thicken, the interior portions die due to lack of sunlight, and microbial decomposition of algal tissues releases N and P into the overlying water column through diffusion and advective transport. Thus, once *Lyngbya* and *Vaucheria* are given a toehold in a spring due to physical disturbance or some other factor, internal nutrient cycling may help the mats persist and grow.

Several studies have investigated internal nutrient recycling within algal mats and underlying sediments in freshwater and marine environments. In two coastal embayments of Sweden, benthic efflux of inorganic nutrients supplied a majority of the N and P requirements for initial macroalgal growth in the late spring and early summer (Sundback et al. 2003). *In situ* regeneration of nutrients was measured and found to be an important factor supporting algal mats in the Peel Harvey estuary in Australia (Hodgkin and Birch 1986). These and other studies (McGlathery et al. 1997, Krause-Jensen et al. 1999, Stimson and Larned 2000, Trimmer et al. 2000) demonstrate the potential for internal nutrient regeneration from algal mats and underlying sediments to release algal populations from complete dependence on external nutrient sources; however, no similar studies have been conducted in Florida springs.

The major objective of our study was to quantify nutrient supply to macroalgae in Florida springs and determine the potential for large algal mats to internally regenerate nutrients to sustain algal growth. Our specific objectives were to: (1) measure nutrient fluxes from spring boils, (2) estimate the amount of internally regenerated nutrients supplying actively growing portions of algal mats, and (3) compare net nutrient uptake of N and P by algal biomass to nutrient fluxes and pools in two first-magnitude springs. To accomplish the first objective we used boil discharge measurements at Manatee and Ichetucknee springs along with periodic measurements of water chemistry to compute external nutrient fluxes. For the second objective we performed field surveys to quantify and map algal biomass and nutrient pools in these springs. In addition, we periodically deployed interstitial water samplers to measure dissolved N and P profiles, which were used to estimate internal nutrient pools. The nutrient pool measurements were then coupled with published rates of diffusive and advective nutrient fluxes out of the mats to directly estimate the supply of internally regenerated nutrients available to actively growing algae. Finally, using *in situ* growth and net nutrient uptake rates of spring algae, we then compared rates of external and internal nutrient supply to algal nutrient uptake. Our mass balance approach provides a first approximation of the relative importance of internal and external sources of nutrients for the growth and persistence of large algal mats in first-magnitude springs in Florida and is relevant to ongoing spring protection programs.

MATERIALS AND METHODS

Study Sites

Our study was conducted at two first-magnitude springs in northern Florida: Ichetucknee Springs Blue Hole and Manatee Springs (Figure 1a). Measurements were limited to the spring boils and adjacent spring runs. Blue Hole is located within Ichetucknee Springs State Park. At Ichetucknee, the spring pool and run studied was 50 m in length and terminated at the confluence with the Head Spring run (Figure 1b). Discharge from the boil ranged from 6.0 to 6.9 m^3 s^{-1} between May 2005 and May 2006. The spring had a no-swim fence across its entire width 20 m downstream from the boil. This fence divided the swimming area from the rest of the spring run to the river, obstructed the overall current flow, and was covered in submerged aquatic vegetation. The dominant alga in Ichetucknee Blue Hole was *Lyngbya wollei*.

Manatee Springs is located within Manatee Springs State Park. The total spring length from the boil to the Suwannee River is 370 m and the average width of the run is 22 m (Figure 1b). The length of the spring pool and run we surveyed was 135 m and terminated at a zone of mixing between the spring water and the highly tannic Suwannee River. Discharge from the boil ranged from 5.7 to 7.8 m^3 s^{-1} between May 2005 and May 2006. The dominant alga in Manatee Spring was *Vaucheria* sp.

Algal Abundance Measurements

Surveys of the two springs were conduct approximately monthly beginning May 2005 and ending April 2006 (n = 12). The surveys were conducted by establishing nine transects running perpendicular to the spring run and evenly spaced along the length of the observed run (135 and 50 m for Manatee and Ichetucknee, respectively). The spacing between the transects was dependant on the size of the spring; for Ichetucknee Blue Hole the spacing was 5 m, and in the larger Manatee Spring the spacing was 15 m. In each spring the first transect crossed the boil and had nine sampling points along its width, yielding 81 observations in each spring on each sampling date. The length of each transect was measured using a tape measure, and the location of each transect point determined using a GPS unit.

At each of the 81 sampling points we measured water depth (by sounding rod or electronic depth sounder), substrate type, and macroalgae species and mat thickness (measured with a sounding rod). Five primary categories were used for visual classification of substrate types: sand, fine sediment, wood, and rock. At a subset of points, samples of algal tissue were also collected.

Field data were entered into ESRI ArcMap version 9.1 to map the location and occurrence of algal mats and other morphological characteristics of the springs. Data were converted into raster format using a cell size of 0.177 m for Ichetucknee Spring and 0.333 m for Manatee Spring. Shapefiles used in the data analyses were projected as Albers Conical Equal Area using North American Datum 83 (NAD-83). Spring perimeters were defined from digital orthophoto quadrangles and entered into the GIS database as polygon Shapefiles, which served as a mask for spatial interpolation analyses.

Kriging was used to create GIS layers of algal mat extent and thickness using the Geostatistical Analyst extension in Arc-

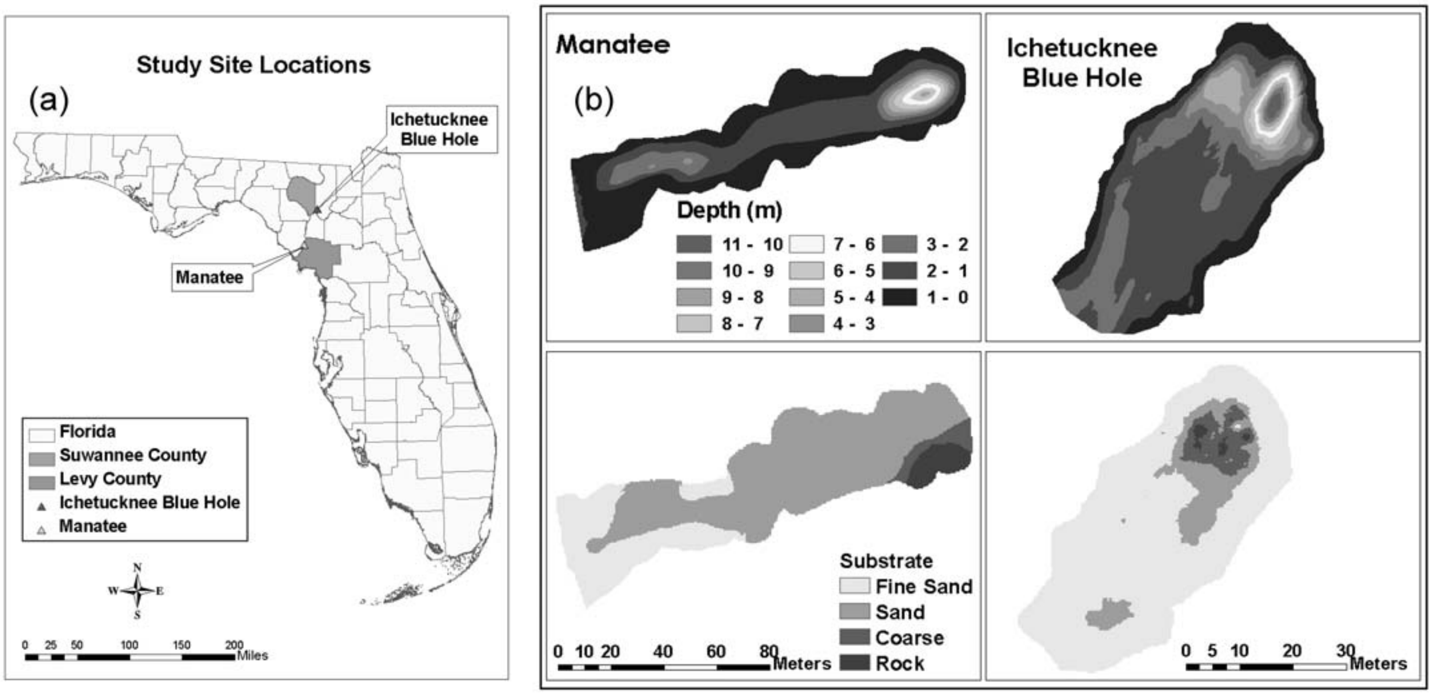

Figure 1. Study sites (a) and bathymetry and substrate composition of Manatee and Ichetucknee springs (b). The direction of water flow is from right to left in diagrams.

Map. Anisotropy was checked and the lag size was adjusted to 0.9. The search neighborhood included five neighbors and was restricted to at least two. The angle of shape was 43.5°, the major semi axis 20°, and the minor 10°. After the surfaces were generated they were exported as raster files, and the raster calculator was used to clip the surfaces to the spring perimeter and compute algal mat area and gross volume.

Samples of algal tissues for mass and elemental analysis (approximately 10 g) were rinsed in the field with spring water to remove coarse sediments. In the lab, samples were placed into containers of deionized water and picked clean of macroinvertebrates using a dissecting scope and forceps. For algae volume and macroalgae ash-free dry mass (AFDM), a pinch of algae was placed into a volumetric cylinder filled with water. Algae were suspended to allow them to fill their natural volume, and the volume they filled (not the volume displaced by the algae) was recorded. Next, the algae were patted dry, weighed for fresh mass, and oven dried at 105 C for later dry mass measurement on an analytical balance. These measurements allowed us to determine the relationship between macroalgal mat thickness and macroalgae mass per surface area; information needed to compute total carbon (C), N, and P content of the *in situ* mats. Using gross algal mat volume and estimates of natural volume described above, the fresh mass of algal tissue in the mats was computed. Next, using the relationship between fresh mass and dry mass, we computed the dry mass of algae in the mats. The mean and standard error of the ratio of dry mass to fresh mass at Ichetucknee and Manatee springs were 0.19 ± 0.01 and 0.12 ± 0.01, respectively. The mean and standard error of the dry mass of algae per unit volume were 4.7 ± 0.27 and 3.0 ± 0.39 mg dry mass/cm^3, for Ichetucknee and Manatee springs, respectively. Finally, we multiplied the elemental composition of replicate algal samples (measured in mg C, N, or P per gram dry mass; methods described below) by dry mass per unit volume and then by the total volume of algal mats obtained by GIS analysis to estimate the total content of C, N, and P in the algal mats. These computations were repeated for each spring on each sampling date.

Interstitial Water Collections

An interstitial water sampler (multisampler) was deployed in a *Vaucheria* sp. mat in Manatee Springs in April 2006. The sampler was an adaptation of the sediment sampler of Martin et al. (2003) and consisted of a 1.5-m tall PVC pipe with small holes drilled every 10 cm. Tygon tubing (0.25 in ID) was glued to the inner surface of each hole (inside the multisampler) and run through its entire length. Two layers of 500-µm nylon screen were glued to the outside of each hole in the form of a blister to help filter particulates and keep the tube from being blocked.

The multisampler was inserted vertically through the thickest part of the algal mat, pushed to a depth of approximately 10 to 20 cm into the sediment, and left in place for one week to allow equilibration. Researchers in a canoe then used a syringe to draw water samples. To purge stagnant water from the tubing, two tube-volumes of water were withdrawn from each tube and discarded before water samples were collected. The multisampler allowed samples to be drawn from discrete layers in the sediments upward through algal mats and into spring waters above the mats.

Parameters measured in the samples were: total Kjeldahl nitrogen (TKN), total phosphorus (TP), soluble reactive phosphorus (SRP), N-NO_3^-, N-NH_4^+, and dissolved organic carbon (DOC). Total nitrogen (TN) was computed as the sum of TKN plus N-NO_3^-. Chemical assays are described in the next section. In computing the quantity of N and P in interstitial waters within the Vaucheria mats, we assumed that 50%, by volume, of the mat was algae and 50% was extracellular water.

Chemical Sampling and Laboratory Analyses

Water samples were collected directly over the boil on each algal survey date. In addition, during September 2005, water samples were collected at each of the 81 survey points to access spatial variation in N-NO_3^- concentrations along the spring run. Filtered water samples were prepared using a filter holder and syringe to pass water through a 0.45-µm polycarbonate membrane. Filtered aliquots were collected for SRP, DOC, N-NH_4^+, and N-NO_3^-. Unfiltered samples were collected concurrently for TKN and TP. Samples for TKN, TP, DOC and N-NH_4^+, N-NO_3^- were acidified to pH 2 with concentrated H_2SO_4. All samples were placed into clean, high-density polyethylene bottles or glass vials (DOC samples), transported on ice, and stored at 4 C until analyzed. Holding times were 30 days for N-NO_3^-, SRP, DOC, TKN, and TP, and 3 days for N-NH_4^+.

Soluble reactive phosphorus, N-NH_4^+, and N-NO_3- in monthly samples were measured on a flow-injection analyzer using EPA Methods 365.1, 350.1, and 353.2 respectively. For intensive sampling of the springs during September 2005, N-NO_3^- was analyzed by ion-chromatography on a Dionex ion chromatograph; DOC was measured on a Shimadzu 5050 TOC analyzer. Total Kjeldahl nitrogen was determined by H_2SO_4, and Kjeldahl salt digestion and flow-injection determination of ammonium (EPA Method 351.2). Total nitrogen was computed as the sum of TKN plus N-NO_3^-. Total phosphorus was measured as SRP on Technicon Autoanalyzer after digestion with H_2SO_4 and potassium persulfate (EPA Method 365.1). Carbon and nitrogen content of lyophilized algal tissues were determined on a Thermo Flash EA 1112 elemental analyzer. Phosphorus content of dried algal tissues was measured on combusted (550 C) and acid digested (6N HCl) samples as SRP on the Technicon Autoanalyzer (Andersen 1976).

Spring Discharge Data

Spring discharge data from May 2005 to May 2006 were obtained from the U.S. Geological Survey Flows and Levels website (http://waterdata.usgs.gov/fl/nwis). Flow data were only available from May 2005 through September 2005 at Ichetucknee Blue Hole, so we used the average instantaneous discharge for this period (6.3 m^3 s^{-1}) for flux computations in October 2005 through May 2006. At Manatee Spring, flow data were available up to March 2006. For April 2006 mass flux calculations we used the average flow between May 2005 and March 2006 (6.7 m^3 s^{-1}).

RESULTS AND DISCUSSION

Algal Surveys in Manatee and Ichetucknee Springs

Both springs had relatively simple bathymetry consisting of a boil ranging in depth from 10 to 11 m and a shallow, 1 to 3 m deep spring run (Figure 1b). Bottom composition at Manatee consisted primarily of sand and fine sand with a small area of coarse gravel and rock adjacent to the boil (Figure 1b). Finer sand covered the majority of Ichetucknee Blue Hole with some coarser bottom materials occurring in the nearby boil.

Maps of algal thickness are presented for dates with maximum algal biomass in Ichetucknee and Manatee springs and provide a general picture of algal distribution in these springs throughout the study period (Figure 2). Locations of the swim fence obstructing flow at Ichetucknee and the swim line at Manatee are indicated. *Vaucheria* sp. mats covered a majority of the bottom of Manatee Spring in August 2005, while *Lyngbya wollei* mats at Ichetucknee covered <50% of the bottom. The thickest (40-50 cm) and most extensive algal mats at Ichetucknee formed in the lee of the swim fence where water velocity was relatively low. At Manatee Spring, in contrast, a semi-continuous mat formed along nearly the entire length of the run with peak thickness of 50 to 60 cm (Figure 2).

The area of spring bottom covered by algae and the mean thickness of algal mats in Manatee and Ichetucknee springs varied considerably during the study period (Figure 3). At Manatee Spring, *Vaucheria* cover varied from near zero in the late spring and early summer of 2005 to a peak of 3500 m^2 in late winter 2006. Over the course of the study, mean thickness varied from 0.03 to 0.18 m, and gross volume ranged from near 0 to 518 m^3. At Manatee spring, we believe that strong seasonal patterns of Vaucheria cover and thickness resulted from phenological drivers such as solar insolation.

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

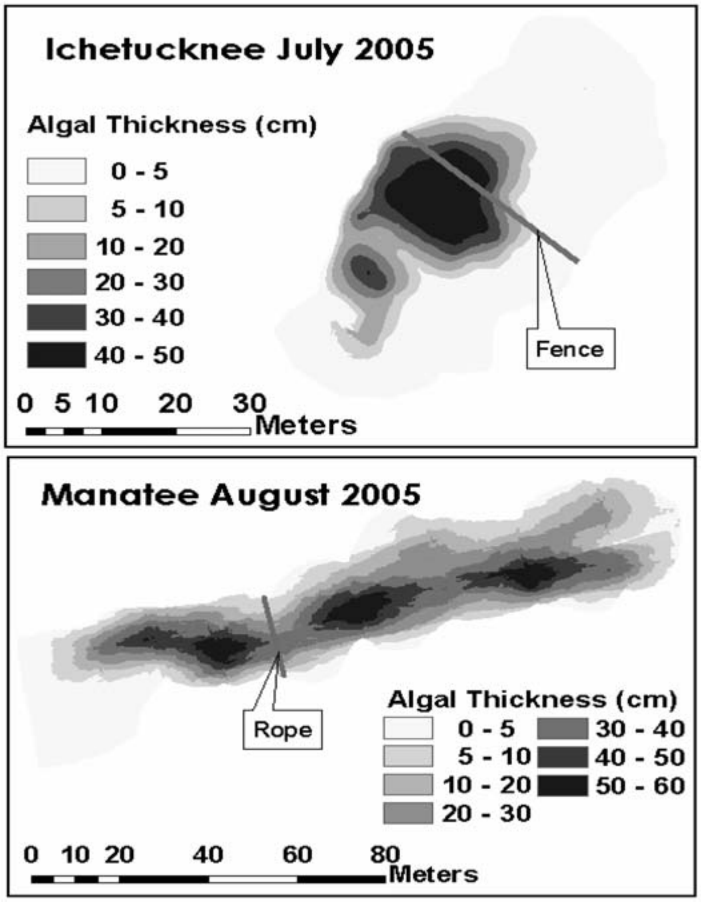

Figure 2. Algal mat extent and thickness for months with maximum algal biomass at Manatee and Ichetucknee springs. Location of swim fence and rope are indicated.

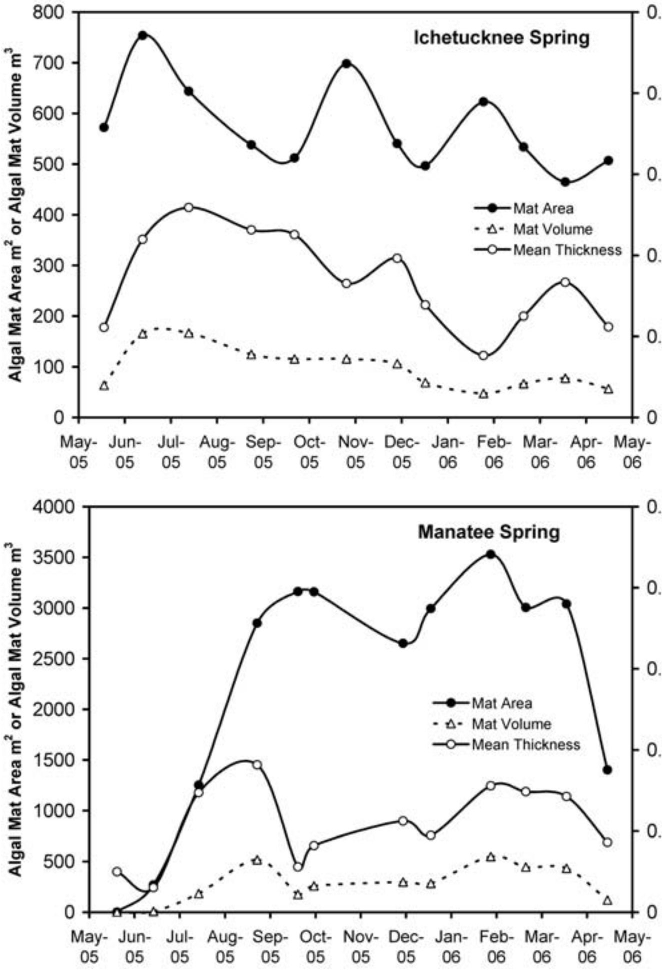

Figure 3. Patterns of algal mat area, thickness, and volume at Ichetucknee and Manatee springs.

Table 1. Dissolved nutrient concentrations in Ichetucknee and Manatee springs. Concentrations are averages for the period of May 2005 through April 2006 (± standard error) and are expressed in µg of element per liter.

	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	Dissolved Organic Nitrogen	Soluble Reactive Phosphorus	Dissolved Organic Phosphorus
Ichetucknee	794 ± 23	15 ± 4.9	152 ± 32	37 ± 1.8	9.6 ± 2.3
Manatee	1799 ± 40	19 ± 7.2	236 ± 64	20 ± 1.4	7.6 ± 1.5

Spring-Boil Chemistry

Nitrate was the primary form of nitrogen in boil water from both Ichetucknee and Manatee springs, with mean concentrations of 794 and 1799 µg N L^{-1}, respectively (Table 1). Dissolved organic nitrogen was the next most abundant nitrogen species, with mean concentrations of 152 and 236 µg N L^{-1}, respectively. Ammonium concentrations in both spring boils were at or near the detection limit. Soluble reactive phosphorus was the most abundant form of phosphorus, with mean concentrations of 37 and 20 µg P L^{-1}, respectively. Dissolved organic phosphorus in boil water at both springs was <10 µg P L^{-1}.

During September 2005, the mean and standard error of nitrate concentrations for 81 locations in both Ichetucknee and Manatee springs were 770 ± 17 and 2149 ± 59 µg N L^{-1}, respectively. These samples were collected from a kayak at the water surface. There was statistically significant inverse relationship between $N\text{-}NO_3^-$ concentrations and distance from the boil (Figure 4). These trends were evaluated with Kendall tau and t-statistic tests to confirm that the inverse relations were valid and that the slope of the regression was significantly different from zero at the 0.05 level. The slopes of these relationships were 0.39 and 0.15 µg N L^{-1} m^{-1} for Ichetucknee and Manatee springs, respectively. Similar decreases in nitrate have been observed along spring runs (Albertin 2009) and spring-fed rivers (Brabandere et al. 2007) in Florida and have been attributed to algal uptake.

Spring Boil Fluxes and Nutrient Content of Algal Mats

The C, N, and P content of the algal mats in Ichetucknee and Manatee springs were summarized (Tables 2 and 3) along with dissolved nutrient fluxes computed from spring discharge and TOC, TN, and TP measurements. At Ichetucknee, mean C, N, and P flux in boil water were 436 kg-C d^1, 515 kg-N d^{-1}, 24 kg-P d^{-1}. At Manatee Spring, mean fluxes of C, N, and P were 864 kg-C d^{-1}, 1124 kg-N d^{-1}, 16 kg-P d^{-1}. For both springs, the amount of C, N, and P contained in algal biomass was typically in the range of 100 to 300 kg C, 10 to 150 kg N and <5 kg P. Owing to greater algal volume at Manatee, C mass in algal mats was, on average, 50% greater than at Ichetucknee (average percent C of algal tissues were 39 and 40% at Ichetucknee and Manatee springs, respectively). Mean mass of algal N at the springs was fairly similar (29.5 and 31.3 kg for Ichetucknee and Manatee, respectively) because of higher N content of algae at Ichetucknee (6.4 vs 4.9% N), which made up for lower algal volume. Mean mass of P in algal biomass at Manatee Spring was more than double that in Ichetucknee owing to greater mat volume and overall higher P content (0.52 vs. 0.38% P).

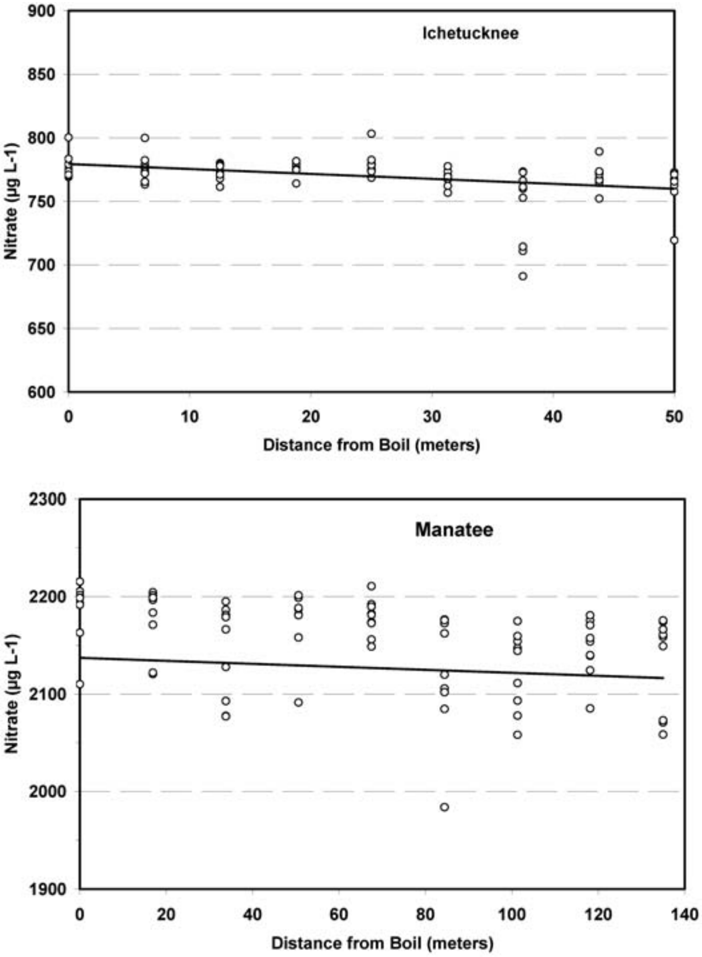

Figure 4. Variability of $N\text{-}NO_3^-$ concentrations along the springs runs at Ichetucknee and Manatee springs during September 2005. Surface samples were collected at 81 sites within each spring along transects running from the boil and then down stream. Regression lines describe the relationship between $N\text{-}NO_3^-$ concentration and distance from the boil. The slopes of the lines were significantly different from zero at the 0.05 level.

Interstitial Water Chemistry

Strong nutrient gradients were observed in the *Vaucheria* sp. mats in Manatee Spring (Figure 5). In the water column above the mat, nitrate dominated the dissolved N pool (1.5 mg L^{-1}). At a depth of 10 to 20 cm within the mat (position above the sediment-water interface of 30 to 40 cm; Figure 4a), nitrate concentrations declined rapidly to the detection limit while ammonium increased to 17 mg L^{-1}. Similar ammonium patterns have been observed previously in estuarine macroalgal mats and have been attributed to anoxia and the

Table 2. Carbon, N, and P in algal biomass and C, N, and P flux from boil water at Ichetucknee Spring. For C flux from the boil, only organic carbon forms are considered.

Date	Organic C Mass in Mat (kg-C)	Organic C flux from Boil (kg-C d^{-1})	Nitrogen Mass in Mat (kg-N)	Nitrogen Flux from Boil (kg-N d^{-1})	Phosphorus Mass in Mat (kg-P)	Phosphorus Flux from Boil (kg-P d^{-1})
May	141	478	25.1	536	1.11	27.0
June	320	421	51.3	500	2.78	24.0
July	253	419	39.0	484	2.68	24.0
August	268	433	42.2	470	3.04	27.0
September	207	429	34.3	510	2.64	23.0
October	248	436	36.5	482	3.38	15.0
November	221	436	38.2	514	2.20	15.0
December	166	436	28.0	558	1.30	28.0
January	60.8	436	10.5	567	0.61	23.0
February	82.8	436	14.5	547	0.64	29.0
March	119	436	19.1	445	0.79	24.0
April	100	436	16.5	564	0.98	26.0
Average	182	436	29.5	515	1.85	24.0

coupling of nitrification and denitrification (Krause-Jensen et al. 1999), suggesting that the biogeochemistry of the center of the Manatee mats was dominated by heterotrophic metabolism. Furthermore, the ammonium levels in the mats are well above thresholds of ammonium toxicity observed for phytoplankton and algae (Dugdale et al. 2007 and references cited therein) and, coupled with low light levels, suggests little active algal growth in areas of the mat with high ammonium concentrations.

The ammonium profiles at Manatee also indicate that ammonium was diffusing out of the mat into the overlying water column where it could have been assimilated by actively growing algae. Within the mat, dissolved organic N ranged from <0.5 to about 2 mg L^{-1}. Concentrations of ammonium and TKN were slightly lower in the sediments below the mat than the peak concentrations observed within the mat, indicating that diffusion would transport these chemical species out of the mat and into the sediments. Concentrations of SRP were uniformly at the detection limit within the water column and mat; there was a small increase in SRP in the deepest sediment sample (Figure 4b). In contrast, TP, primarily in the form of dissolved organic P, increased within the mat; concentrations ranged from 2 to 3 mg L^{-1}. Similarly high concentrations of TP were observed in the sediments.

Sources of Nutrient to Vaucheria and Lyngbya Mats

The algal mats in this study were seasonally abundant and covered high percentages of the bottoms of Manatee and Ichetucknee springs in north Florida. Elemental analysis of algal tissues and interstitial waters demonstrated that large *Vaucheria* and *Lyngbya* mats within these springs contained biologically significant quantities of N and P that could potentially be mineralized and thereby supply actively growing algae with macronutrients. In addition, the interstitial waters within the mats contained very high concentrations of N-NH_4^+ and potentially bioavailable dissolved organic phosphorus.

In our mass balance approach to assessing internal and external nutrient supply, we sought to compare net N and P uptake by actively growing algae to both external and internal nutrient fluxes. The spring boil was assumed to be the

Table 3. Carbon, N, and P in algal biomass and C, N, and P flux from boil water at Manatee Spring. For C flux from the boil, only organic carbon forms are considered.

Date	Organic C Mass in Mat (kg-C)	Organic C Flux from Boil (kg-C d^{-1})	Nitrogen Mass in Mat (kg-N)	Nitrogen Flux from Boil (kg-N d^{-1})	Phosphorus Mass in Mat (kg-P)	Phosphorus flux from Boil (kg-P d^{-1})
May	0.06	1156	0.01	1516	0.00	19
June	22.4	734	2.68	921	0.20	12
July	203	734	25.0	1062	2.85	11
August	444	998	50.6	1208	6.42	19
September	149	1013	16.3	1396	1.11	19
October	299	815	37.6	1008	5.53	15
November	319	749	36.7	955	4.79	14
December	318	895	35.0	1191	3.66	22
January	347	705	45.0	908	5.62	12
February	415	914	45.4	1232	4.90	15
March	592	793	61.7	1008	8.83	15
April	157	864	19.2	1083	2.04	15
Average	272	864	31.3	1124	3.83	16

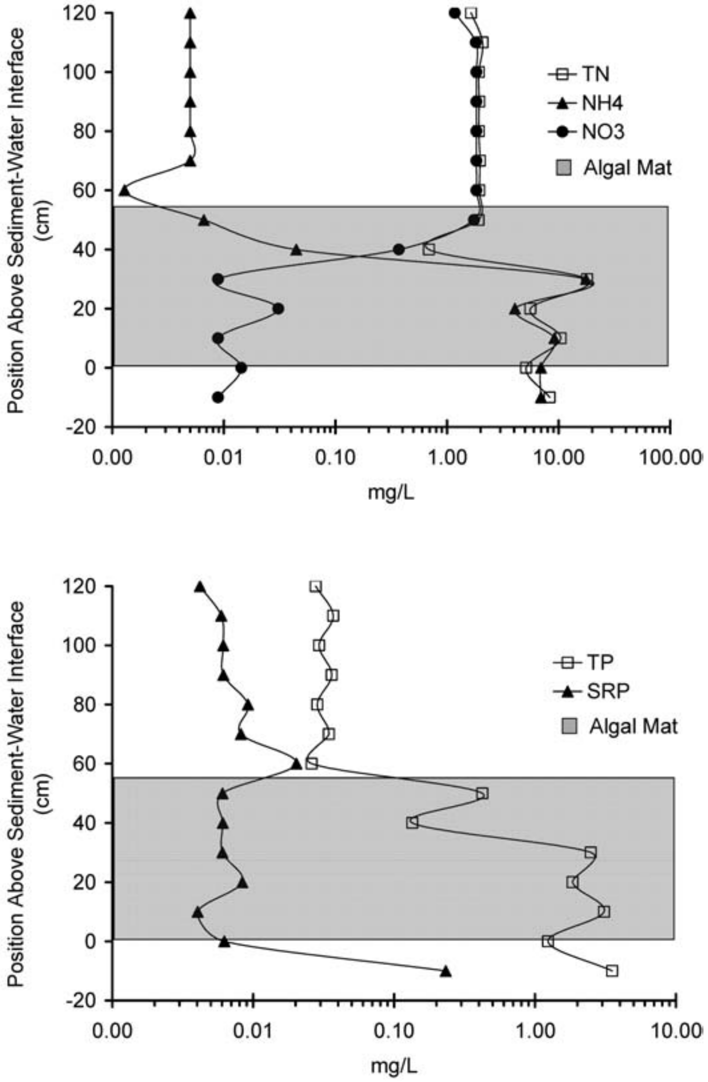

Figure 5. Profiles of N and P species in Manatee Spring on 19 April 2006. Samples were collected using duplicate multisamplers (the duplicate profiles were very similar, so only one is presented here). The approximate location of the water column, algal mat, and sediments are shown to the right of each figure. The zero point of the y-axis is the sediment-water interface.

dominant external nutrient input. For internal nutrient sources we quantified the size of the available nutrient pools (contained in algal biomass and interstitial waters) and used recently published measurements of diffusion and advection rates to estimate the mass of internally regenerated nutrients available to actively growing portions of algal mats at Manatee Spring.

Net nutrient uptake by mat algae was estimated by three methods. In the first approach we computed the average daily rate of N and P accumulation in algal biomass between June and August 2005 when maximum algal growth rates were observed (Figure 3; Table 3). This approach yielded an N uptake rate of 3.2 kg d^{-1} and P uptake of 0.4 kg d^{-1} for total algal biomass in the spring (Table 4).

In a second approach to estimating net nutrient uptake, we used published uptake rates for various macroalgae spe-

TABLE 4. ALGAL NUTRIENT CONTENT AND ESTIMATED INTERNAL REGENERATION OF NUTRIENTS FROM THE *VAUCHERIA* MAT IN MANATEE SPRINGS DURING APRIL 2006. ALSO INCLUDED ARE ESTIMATES OF NET NUTRIENT UPTAKE BY ALGAL BIOMASS COMPUTED BY DIRECT OBSERVATIONS OF MAT NUTRIENT ACCUMULATION (TABLE 3) AND WATER COLUMN NUTRIENT DEPLETION (FIGURE 4; SEE TEXT FOR EXPLANATIONS ON COMPUTATIONS). ESTIMATED INTERNAL REGENERATION OF NUTRIENTS ARE BASED UPON DIFFUSIVE AND ADVECTIVE FLUXES OF NH_4-N AND SRP MEASURED IN MANATEE SPRING MATS BY ALBERTIN (2009).

	External	Internal Pools		Internal Regeneration		Algal Net Nutrient Uptake		
Element	Element Flux From Spring Boil (kg d^{-1})	Algal Biomass (kg)	Interstitial Waters of Algal Mat (kg)	Diffusive Flux (kg d^{-1})	Advective Flux (kg d^{-1})	From In-Situ Nutrient Accumulation (kg d^{-1})	From Published Bioassays (kg d^{-1})	From Observed Nutrient Depletion (kg d^{-1})
Nitrogen	1083	19.2	0.42	7.1×10^{-3}	0.38	3.2	5.1	6.8
Phosphorus	15	2.04	0.10	0	3.6×10^{-4}	0.4	0.4	—

cies measured in cultures and reported by Wallentinus (1984). In this computation we used the average of maximum nutrient uptake rates measured for 57 algal species: N uptake = 988 µg-N/g dry weight per day and P uptake = 82.6 µg-P/g dry weight per day. Using these average values we computed N uptake of 5.1 kg d^{-1} and P uptake of 0.4 kg d^{-1} for the algal biomass in Manatee Spring during April.

In the third approach we used observed $N\text{-}NO_3$ depletion to compute daily uptake for N. Using the slope of the graphed line (Figure 4) for Manatee spring (units of $\mu g\ N\ L^{-1}\ m^{-1}$), and multiplying by the length of the algal cover (135 m) and daily boil discharge ($L\ s^{-1}$), and assuming that $N\text{-}NO_3^-$ uptake is restricted to daylight hours ($s\ d^{1}$), we computed an N uptake rate of 6.8 kg N d^{-1} (i.e., $0.15\ \mu g\ N\ L^{-1}\ m^{-1} \times 135\ m \times 7815\ L\ s^{-1} \times 43200\ s\ d^{-1} \times 10^{9}\ kg\ \mu g^{-1} = 6.8\ kg\ N\ d^{-1}$).

All three approaches produced similar daily N and P uptake rates for the *Vaucheria* mats in Manatee (Table 4). Algal N uptake ranged between 3.2 and 6.8 kg d^{-1}, which exceeded the mass of $N\text{-}NH_4^+$ in the interstitial waters of the mat by about 10-fold. In contrast, the amount of N flux in boil discharge per day (i.e., external N supply) was 200 to 300 times greater than net N uptake by the algal mat. Based on our calculations, the entire N content of the algal mats during April 2006 (19.2 kg N) equals about 5 days of algal N uptake. Estimated P uptake was 0.4 kg d^{-1}, 4-fold greater than the mass of P in interstitial waters (Table 4). Daily boil flux of P exceeded daily P uptake by only a factor of 6, but P content of algal tissues equaled about 5 days of algal P uptake. For P, interstitial waters contained about 25% of daily P uptake, although the major form of P within the interstitial was organic P, which would require more energy expenditure for algal assimilation compared to PO_4^{3-}.

Internal regeneration of nutrients was estimated using diffusion and advection rates computed for the Manatee mat during April 2006 by Albertin (2009). In this companion study, Albertin used profiles (Figure 5) along with Fick's law to estimate diffusion of inorganic nutrients out of the mat. Tracer studies were also performed to measure horizontal advective movement of water in the same mats. Computations of diffusion and advection assumed an average mat porosity of 0.5 and water temperature of 18 C. Using computed diffusion rates in Albertin (2009) and a mat surface area of 1403 m^2 (Figure 3) we estimate that diffusion of ammonium out of the Manatee mat (and hence the potential flux of N to actively growing algae from internal nutrient regeneration) was 0.0071 kg N d^{-1} during April 2006, which was <1% of daily net N uptake by the mat (Table 5). Concentration gradients of SRP were such that there was no diffusion of SRP out of the mat.

Albertin (2009) modeled horizontal advective water movement in the Manatee mat as flow through porous media using NaCl tracers and Darcy's Law and arrived at a specific discharge of $3.7 \times 10^{7}\ m\ s^{-1}$. Vertical advective water movement in the mat was certainly much less than the horizontal movement, but we conservatively assumed that the ratio of vertical to horizontal movement was 0.5 to put an upper limit on the rate of nutrient advection out of the mat. Using this assumption we estimate that advection of ammonium and SRP could equal 0.38 kg N d^{-1} and 3.6×10^{-4} kg P d^{-1}, respectively, during April 2006 (Table 5), which represents about 12 and 0.09% of measured net N and P uptake per day. Even with this over-estimate of advective N supply, the majority of N and P being used by actively growing algae in the Manatee mat clearly must be directly supplied by water from the spring boil. While N and P mass in the mats was high on an areal basis, the sheer volume of water flow from the boils results in a much greater potential rate of external nutrient delivery to actively growing algae on the surface of the mats. How much of the N demand of *Lyngbya* is supplied by N-fixation is an open question, but we hypothesize the contribution is low because it is unlikely that algal N-limitation can occur with such high inputs of $N\text{-}NO_3^-$ from the spring boil.

While our study suggests that external nutrient inputs likely supply the majority of N and P to algae in the first-magnitude springs we studied, it is uncertain whether the same holds true for second- and third-magnitude springs (i.e., flows <0.28 $m^3\ s^{1}$) in Florida. With lower boil flows, shallower water depths, and relatively greater volumes of algal mat relative to spring volume, algal productivity might be more tightly coupled to internal nutrient cycling in smaller springs. In addition, some first magnitude springs, such as Weeki Wachee, have enormous accumulations of algae with thickness of >3 m (A. Albertin, pers. observ.). In such cases, internal regeneration of N and P mass from decaying algal tissues may take on greater importance.

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