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Nutrient Limitation of Periphyton in a Spring-Fed, Coastal Stream in Florida, USA

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ABSTRACT

There is strong evidence to suggest that ground-water nitrate concentrations have increased in recent years and further increases are expected along portions of the central Gulf coast of Florida. Much of the nitrate enriched ground-water is discharged into surface waters through numerous freshwater springs that are characteristic of the area and the potential for eutrophication of their receiving waters is a legitimate concern. To test the potential effects of elevated nutrient concentrations on the periphyton community an *in situ* nutrient addition experiment was conducted in the spring-fed Chassahowitzka River, FL, USA, during the summer of 1999. Plastic tubes housing arrays of glass microscope slides were suspended in the stream. Periphyton colonizing the microscope slides was subjected to artificial increases in nitrogen, phosphorus or a combination of both. Slides from each tube were collected at 3- to 4- day intervals and the periphyton communities were measured for chlorophyll concentration. The addition of approximately 10 µg/L of phosphate above ambient concentrations significantly increased the amount of periphyton on artificial substrates relative to controls; the addition of approximately 100 µg/L of nitrate above ambient concentrations did not. The findings from this experiment implicated phosphorus, rather than nitrogen, as the nutrient that potentially limits periphyton growth in this system.

Key words: Nitrogen, Phosphorus, Bioassay, Eutrophication, Nutrient Enrichment.

INTRODUCTION

Ground-water nitrate enrichment is being documented throughout much of Florida, particularly in the north central region of the state (Hatzell 1996, Ham and Hatzell 1996, Jones et al. 1997). Because of the occurrence of large and productive aquifer systems and highly permeable karst geology, there are numerous locations and multiple pathways by which nitrate enriched ground-water can permeate and mix with the surface water environment. For example, freshwater springs, of which there are greater than 300 in the state, provide a direct conduit for ground-water to move from the aquifer to surface waters (Katz et al. 1997).

Elevated nitrate concentrations in ground-water can result from both past and present land use activities, such as commercial or residential fertilizer application (Jones et al. 1997). Because of a presumed lag-time associated with ground-water movement, it is likely that further increases in nitrate concentrations in spring discharge will occur because of past and present human population growth and land use activities in this region. As nutrient-enriched ground-water enters the surface water system via spring input there is a potential for ecological change to occur. In many instances, increases in nutrient concentrations to streams with low nutrient concentrations have been linked to changes in autotrophic community composition, vegetative biomass and an increase of nuisance species (Wright and McDonnell 1986a, 1986b). Such changes can, in turn, affect shifts in community structure and alter food web dynamics of a given system (Hershey et al. 1988, Peterson et al. 1993).

Although increased nutrient concentrations can influence periphyton abundance, the responses to nutrient additions are frequently variable. For example, nitrogen alone may stimulate periphyton growth (Stelzer and Lamberti 2001) especially when light is not a limiting factor (Lohman et al. 1991, Mosisch et al. 2001). In other cases, the addition of phosphorus, both solely (Bothwell 1985, Pan and Lowe 1994) and concurrently with nitrogen (Dodds et al. 1997, Winter and Duthie 2000), has been shown to increase periphyton abundance.

The objective of this study was to examine the potential consequences of additional nutrient inputs on periphyton abundance as measured as chlorophyll per unit area. To accomplish this, an *in situ* manipulative field experiment was conducted to determine if increasing water column nutrient concentrations of nitrogen and phosphorus would affect the abundance of periphyton growing on submerged surfaces.

MATERIALS AND METHODS

The Chassahowitzka River located at 28° 42' 54" North, 82° 34' 35" West, is a spring-fed coastal river of about 8 km in length located in southwest Citrus County, FL, USA. The land surrounding the river typically has elevations of 3 m or less and is dominated by coastal flatwoods and wetlands that transition into an extensive salt marsh complex along the rocky flat coast of the Gulf of Mexico. The climate is subtropical and Wolfe et al. (1990) provides further details on the ecology of the region.

The Chassahowitzka River is shallow with a mean depth of about 0.9 m and mean flow rates of less than 0.20 m/s. Average stream discharge calculated from measurements taken near the main spring between 1998 to 2000, ranged from 2 to 8 m^3/s. The primary substrate is approximately 54% sand,

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although various mixtures of sand, mud, and rock are common. The river is sufficiently wide that terrestrial vegetation is not a significant factor in reducing light availability for photosynthesis, as terrestrial canopy coverage only shades about 3% of the total river area. Submersed aquatic vegetation is ubiquitous and the light environment is favorable for the growth of rooted macrophytes, macroalgae and associated periphyton, as the river bottom is visible throughout most of the river. Light attenuation coefficients (K_d) though variable, are generally less than 1.5/m. Submersed aquatic vegetation occurs throughout most of the river, but declines gradually with distance downstream. Common aquatic plants include tape grass, (*Vallisneria americana* Michx.), Sago pondweed, (*Potamogeton pectinatus* L.), southern naiad, (*Najas guadalupensis* (Spreng.) Magnus), Eurasian water milfoil, (*Myriophyllum spicatum* L.), and hydrilla, (*Hydrilla verticillata* (L.f.) Royle). Filamentous macroalgae, including *Lyngbya* sp. and *Chaetomorpha* sp., are also abundant.[2]

In the summer of 1999, an *in situ* experiment was conducted to test the effects of nutrient enrichment on periphyton growth. Periphyton sampling was similar to that of Peterson et al. (1983) and allowed replicate treatments of nitrogen, phosphorus and nitrogen combined with phosphorus and control groups to be simultaneously exposed to ambient environmental conditions of stream flow and light. Eight 1.2-m long by 9.2-cm inside-diameter sections of clear acrylic tubing were attached to a sheet of Plexiglas (Figure 1) to house the glass slides. The structure was supported in the water column by a wood frame and anchored to the bottom so that it was oriented below the surface and parallel to stream flow. Within each tube, plastic baffles were placed in the upstream end to facilitate mixing of water passing through the tube. Five microscope slide holders, each holding six microscope slides, were fixed inside each tube (Figure 2).

Nutrients were added through tubes connected to remote supplies of concentrated nutrient solution near the upstream end of the experimental assembly (Figure 1). Nutrient supply containers were large enough to last several days, and the entire experiment was monitored at regular time intervals of 2 to 3 days to ensure proper function. Target nutrient enrichment concentrations were 500 µg/L for nitrate and 25 µg/L for soluble reactive phosphorus, representing an increase of approximately 100 µg/L of nitrate and 10 µg/L of soluble reactive phosphorus above ambient concentrations measured at the study location.[2] Potassium nitrate and potassium phosphate were the specific salts used in the nutrient addition and both were certified to meet American Chemical Society specifications for purity of greater than 99%.

Once the experimental assembly was in place, 6 days were allowed for colonization. Nutrient additions were initiated on the 7th day (see Peterson et al. 1983). Four days after nutrient addition, one slide from each of the five assemblies was randomly removed from each tube. This sampling methodology was repeated three more times, on the 14th, 18th and 21st day following the initial set-up. After removal, the microscope slides were placed in pre-labeled plastic bags containing a small amount of deionized water. Samples were brought to the laboratory inside coolers filled with ice for subsequent processing. The periphyton associated with the microscope slides was harvested by scraping both sides clean with a razor blade into a small container with deionized water. These contents were then filtered through a 47-mm Gelman® type A/E glass-fiber filter. Filters were stored over silica gel desiccant and frozen prior to analysis. Chlorophyll was subsequently extracted with a hot ethanol method described by Sartory and Grobbelaar (1984) and chlorophyll concentrations were determined spectrophotometrically (APHA 1989).

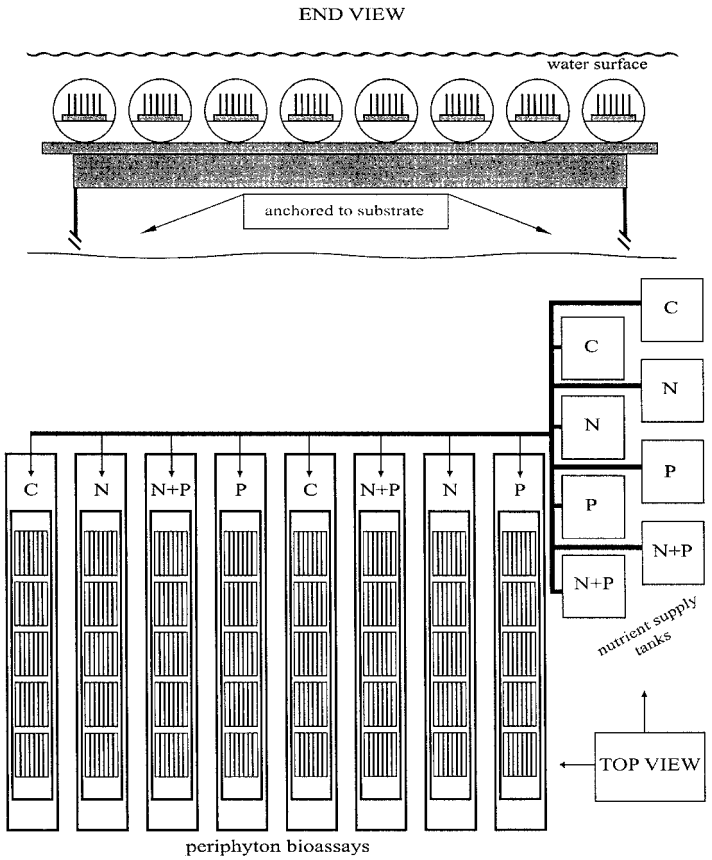

Figure 1. Schematic representation of the experimental set-up (C = control, N = nitrogen addition, P = phosphorus addition and N + P = combined nitrogen and phosphorus addition).

Ten samples from the control, nitrogen, phosphorus and phosphorus plus nitrogen treatment groups for each collection period were pooled and mean chlorophyll values calculated. Mean data were then $\log_{10}$-transformed to accommodate heteroscedasticity (Sokal and Rohlf 1981). Log_{10}-transformed data were analyzed with a repeated-measures ANOVA (PROC Mixed, SAS Institute 1996), where each tube assembly was considered a subject (random factor) and sampling interval and treatments considered as fixed factors. Various covariance structures were tested to determine which model best fit the data, with a first-order autoregressive structure resulting. Follow-up pair-wise comparisons were carried out with a least squares means procedure (SAS Institute 1996). Statements of significance imply $P < 0.05$, unless noted otherwise.

[2]Frazer, T. K., M. V. Hoyer, S. K. Notestein, J. A. Hale and D. E. Canfield, Jr. 2001. Physical, chemical and biological characteristics of five Gulf Coast rivers. Final Report. Southwest Water Management District. Brooksville, FL 357 pp.

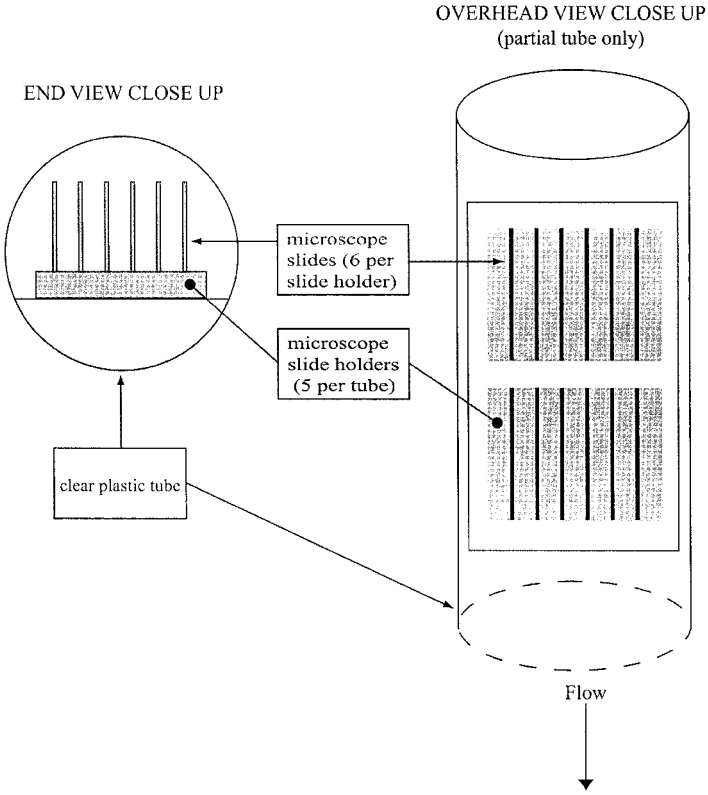

Figure 2. Enlarged view of the artificial substrates and their layout in the experiment.

RESULTS AND DISCUSSION

The experiment was conducted approximately 200 m downstream from the main spring, which serves as the origin of flow at this location. The mean depth was approximately 1 m and the mean stream velocity was about 0.11 m/s, which represents a discharge of ca. 4 m^3/s. The stream was oriented east to west and riparian vegetation was not a factor in stream shading. Submersed plants were not immediately adjacent to the study site although were prevalent nearby. The ambient concentrations of nitrate and soluble reactive phosphorus at the time of sampling were approximately 400 µg/L and 14 µg/L, respectively[2] (Table 1).

Both treatment and time effects were statistically significant (P = 0.055) and the interaction between these two effects was not significant. A minimum mean periphyton biomass of 1.2 µg chl/cm^2 was calculated from the control treatment on day 11 of the experiment and a maximum mean periphyton biomass of 5.7 µg chl/cm^2 was calculated on day 21 (Figure 3). The nitrogen treatment had a minimum mean periphyton biomass of 1.2 µg chl/cm^2 on day 11 of the experiment and a maximum mean periphyton biomass of 6.8 µg chl/cm^2 was calculated on day 21. The phosphorus treatment had a minimum mean periphyton biomass of 1.4 µg chl/cm^2 on day 11 of the experiment and a maximum mean periphyton biomass of 7.8 µg chl/cm^2 was calculated on day 21. The nitrogen plus phosphorus treatment had a minimum mean periphyton biomass of 1.4 µg chl/cm^2 on day 11 of the experiment and a maximum mean periphyton biomass of 8.2 µg chl/cm^2 was calculated day 21 (Figure 3).

TABLE 1. CHEMICAL AND PHYSICAL VARIABLES MEASURED JUST BELOW THE MAIN-SPRING IN THE CHASSAHOWITZKA RIVER. EACH VALUE IS THE MEAN OF 15 SAMPLES EXCEPT SOLUBLE REACTIVE PHOSPHORUS, WHERE N = 12.

Parameter	Mean	Std Dev
Total Nitrogen (µg/L)	464.0	84.2
Ammonium (µg/L)	15.5	12.5
Nitrate (µg/L)	397.0	71.7
Total Phosphorus (µg/L)	19.5	5.1
Soluble Reactive Phosphorus (µg/L)	13.8	2.7
Chlorophyll (µg/L)	2.5	1.4
Dissolved Oxygen (mg/L)	6.3	2.2
Total Alkalinity (mg/L as $CaCO_3$)	143.7	2.0
Specific Conductivity (mS/cm)	3.2	1.8
Color (Pt-Co units)	3.6	2.1
pH	7.89	0.1
Temperature (C)	23.6	1.1
Salinity (ppt)	1.7	1.0
Depth (m)	0.82	0.09
Flow (m/s)	0.11	0.09
Discharge (m^3/s)	4.0	2.4

The addition of phosphorus and nitrogen plus phosphorus resulted in greater periphyton biomass than that of the controls (Table 2, Figure 3). Nitrate additions alone did not result in greater periphyton biomass than that of the controls (Table 2, Figure 3). Differences among the treatments containing phosphorus, nitrogen with phosphorus and nitrogen were not significant.

The experiment was terminated 26 days after installation, before the maximum potential periphyton biomass may have been achieved, because the microscope slides began to be colonized by an epiphytic species of *Chaetomorpha*, a filamentous green alga. Strands of *Chaetomorpha* sp. began to grow long enough so as to become entangled with strands attached to neighboring microscope slides. This made quantifying the amount of material attached to any single

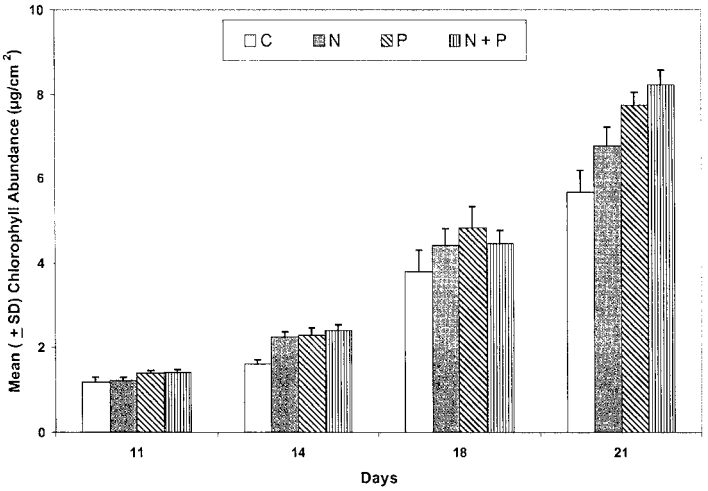

Figure 3. Mean periphyton biomass in µg chl/cm^2 by treatment type and time of collection (C = control, N = nitrogen addition, P = phosphorus addition and N + P = combined nitrogen and phosphorus addition).

Table 2. Least Squares Means comparisons for the bioassay periphyton experiment. Treatments are as follows: C = control, N = nitrogen addition, P = phosphorus addition, N+P = combined nitrogen and phosphorus addition. Time refers to the date at which samples were collected with 1 being the first and 4 being the last. Significant differences are in bold.

Treatment	Significance	Time	Significance
C vs. N	0.07	1 vs. 2	**0.0001**
C vs. N+P	**0.02**	1 vs. 3	**0.0001**
C vs. P	**0.02**	1 vs. 4	**0.0001**
N vs. N+P	0.26	2 vs. 3	**0.0001**
N vs. P	0.33	2 vs. 4	**0.0001**
N+P vs. P	0.85	3 vs. 4	**0.0001**

microscope slide uncertain and would have confounded the determination of the effects of nutrient addition.

In the Chassahowitzka River, we were able to increase periphyton abundance, as measured by the amount of chlorophyll per unit area, by adding phosphorus; suggesting that phosphorus was the primary limiting nutrient for periphyton during the course of this experiment. Nitrogen addition treatments did not exhibit a different response from controls. However, the nitrogen addition treatments were not different than those treatments where only P was added either. This latter observation suggests that the addition of nitrogen might also produce an increase in periphyton in this system. Nevertheless, phosphorus appears to be the primary nutrient limiting periphyton growth. The ambient nitrogen to phosphorus ratio in the stream, which was approximately 24:1 by weight, further supports the potential for phosphorus limitation (Goldman et al. 1979).

Urban development within the Chassahowitzka River watershed is expected to continue and as a consequence, further increases in nutrient loading (nitrate in particular) are likely to occur. However, our findings suggest that increased delivery of nitrate-nitrogen may not significantly alter the abundance of periphyton in this river. Concomitant increases in phosphorus, on the other hand, will likely stimulate the growth of periphyton. Water resource managers are compelled to consider the implications of these suggestions, particularly as they relate to nutrient remediation and reduction strategies.

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