

**Final Project Report:**

The Effect of Flow Rate on Phosphorus Uptake and Taxonomic Composition  
of Everglades Periphyton

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## PROJECT ABSTRACT

The Everglades Forever Act of 1994 set a goal of reducing total phosphorus concentrations to levels compatible with traditional Everglades vegetation or to a default concentration of  $10 \mu\text{gL}^{-1}$ . To achieve this goal, the Phase II of the Everglades Program is centered on identifying, demonstrating and implementing storm water treatment technologies. Supplemental technologies are anticipated to work with or in place of Storm Water Treatment Areas to meet the target for phosphorus concentration. The primary objective of this research is to assist in developing and optimizing supplementary water quality treatment applications by testing the effect of flow rate on phosphorus removal within constructed mesocosms receiving outflow water from the Storm Water Treatment Areas.

Analysis of the relationship of water velocity to taxonomic composition and phosphorus uptake in these periphyton-based mesocosms is a valuable tool for evaluating the phosphorus removal efficiency of advanced treatment technologies for Everglades Restoration. Periphyton taxonomic composition, biomass, water and tissue total phosphorus (TP) were analyzed in three slow velocity ( $0.22 \text{ cm s}^{-1}$ ) and three fast velocity ( $2.0 \text{ cm s}^{-1}$ ) mesocosms. Hydrologic loading rate, retention time and surface area were kept constant, with water velocity the single variable between treatments. There was no significant difference in taxonomic composition. Dominant eutrophic and oligotrophic indicator genera exhibited significant and consistent changes in relative abundance related to location along the treatment path, with the eutrophic taxa more

abundant in the beginning of the treatment path and oligotrophic taxa becoming more prevalent in the downstream portion of the mesocosm. There were also no significant differences in water column or tissue TP concentrations between the slow and fast treatments. However, biomass accrual was significantly greater in the fast treatment by 30%. Consequently, total TP uptake, determined by the combination of biomass accrual and tissue TP concentration, was significantly greater in the fast treatment. These results point to the importance of optimizing flow velocity through constructed treatment wetlands in the Everglades to maximize the efficiency of nutrient removal by periphyton.

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## INTRODUCTION

The Florida Everglades is a subtropical wetland ecosystem that previously encompassed more than 1.2 million ha of the southern Florida peninsula from Lake Okeechobee to Florida Bay (Davis *et al.* 1994). Within this ecosystem, hydrology is one of the most important physical driving forces creating, maintaining and shaping its ecological features (Davis and Ogden 1994b, DeAngelis 1994). Historically, this oligotrophic, phosphorus (P)-limited system was maintained by a natural hydrologic regime of periodic and seasonal inundation and dry downs and received very low nutrient inputs, primarily from atmospheric deposition and sheetflow runoff from Lake Okeechobee (Light and Dineen 1994, Vymazal and Richardson 1995).

Extensive anthropogenic alterations of the natural hydrology of central and southern Florida began more than a century ago, continuing most intensively within the past fifty years, in response to an increasing population and its demand for flood control and water to serve agricultural, commercial, and urban needs (Light and Dineen 1994, SFWMD *et al.* 1998). Major land use conversions, most notably those associated with drainage for agriculture and urban development, have transformed nearly half of the historic greater Everglades wetlands (Davis and Ogden 1994a). The extensive network of canals and levees constructed since 1950 has compartmentalized the remaining Everglades into several regions: Water Conservation Areas; the Everglades Agricultural

Area; and Everglades National Park including Florida Bay.

Water management practices have disrupted the natural annual periodicity of water levels and length of inundation, directly affecting both the abiotic and biotic components of the ecosystem (Light and Dineen 1994). Agricultural runoff and urban wastewater discharges into wetland areas and drainage canals have altered water quality and primary productivity in many areas of the marsh ecosystem (Davis 1994, Craft *et al.* 1995, Craft and Richardson 1998). Background water-column total phosphorus (TP) concentrations in less impacted interior marsh areas of the Everglades often measure less than  $10 \mu\text{g L}^{-1}$ , while drainage canal concentrations may exceed  $100 \mu\text{g L}^{-1}$  and even reach levels near  $1000 \mu\text{g L}^{-1}$  (Davis 1994, McCormick *et al.* 1996, Smith and McCormick 2001).

Increased chemical and nutrient loading, in particular P loading, have been associated with many ecological changes. These changes include: accelerated rates of soil accretion and nutrient storage (Craft and Richardson 1993); increased emergent macrophyte cover and net primary production and P uptake by macrophytes (Davis 1994, Craft *et al.* 1995); changes in periphyton community composition (Swift and Nicholas 1987, McCormick *et al.* 1996, McCormick and O'Dell 1996, Pan *et al.* 2000); loss of open-water habitats (McCormick *et al.* 1998); and shifts in community structure and assemblages of other plant and animal species, in particular a shift in macrophyte dominance from sawgrass, *Cladium jamaicense*, to cattail, *Typha domingensis* (Davis 1994, Newman *et al.* 1998, McCormick *et al.* 2001). Phosphorus (P) enrichment as well as hydrologic changes in the Everglades have resulted in the loss of the abundant

calcareous (calcium-precipitating) periphyton mats typically dominated by cyanobacteria and diatoms. These calcareous species have been replaced in many areas by assemblages dominated by eutrophic cyanobacteria and filamentous green algae (Swift and Nicholas 1987, Browder *et al.* 1994, McCormick *et al.* 1996, McCormick and O'Dell 1996, McCormick *et al.* 2001). This shift in community composition is of concern to restoration ecologists because of the essential role that the oligotrophic, calcareous assemblages play in ecosystem processes.

### *Significance and role of periphyton in the Everglades*

Originally described in 1905 (Cooke 1956), the periphyton or “aufwuchs” community is defined broadly as a complex assemblage of aquatic organisms including algae, bacteria, fungi, and protozoans, and associated invertebrate and vertebrate grazers adhering to shallow, submerged substrates or forming floating algal mats. First described by Dachnowski-Stokes in 1928, the South Florida periphyton community is one of the most widely distributed communities in the Everglades ecosystem, comprising up to fifty percent of the vegetative biomass in some areas (Wood and Maynard 1974, Browder *et al.* 1982). Over 350 documented species of algae, predominantly cyanobacteria (Cyanophyta), diatoms (Bacillariophyta) and greens (Chlorophyta) constitute several primary periphyton community types classified by a gradient of environmental factors including hydroperiod, water depth, calcite encrustation, pH, and P enrichment (Browder *et al.* 1994).

Periphyton are consumed by many aquatic organisms, and along with the detritus of aquatic macrophytes, are an integral component of the base of the Everglades ecosystem food web (Browder *et al.* 1994, Vymazal and Richardson 1995). Because algae vary greatly in their edibility, perturbations in the nutritional value of the assemblage can influence the trophic levels of the aquatic community (Browder *et al.* 1994, Lamberti 1996, McCormick and Cairns 1997, McCormick *et al.* 1998). As primary producers, periphyton can contribute substantially to marsh primary production, oxygenation of the water column, regulation of nutrient cycling (*e.g.*, nitrogen-fixing cyanobacteria, P uptake and storage) and energy flux in aquatic ecosystems (Gleason and Spackman 1974, Browder *et al.* 1982, Mulholland *et al.* 1994, McCormick *et al.* 1998). The large quantities of calcite precipitated under alkaline conditions by calcareous periphyton during photosynthesis form a calcitic mud or marl that constitutes one of the two primary soil types within the Everglades (Gleason and Spackman 1974, Browder *et al.* 1982, Browder *et al.* 1994). The precipitation of calcium by many cyanobacteria and diatoms may be correlated with the bioavailability of P and contributes to the low natural concentrations of P in the Everglades system (Swift and Nicholas 1987, Browder *et al.* 1994).

The species richness and diversity of periphyton assemblages, as well as their physiological attributes, make them ideal organisms to employ as site-specific, sensitive indicators of environmental conditions. The taxonomic composition of periphyton is sensitive to many environmental factors (Stevenson 1996a) and reflects substrate characteristics, local water chemistry, hydrologic conditions (hydroperiod, water depth,

velocity) and nutrient, especially P, conditions (Van Meter-Kasanof 1973, Gleason and Spackman 1974, Browder *et al.* 1981, 1982, Horner *et al.* 1983, Swift and Nicholas 1987, Grimshaw *et al.* 1993, Raschke 1993, Browder *et al.* 1994, Vymazal and Richardson 1995). The relatively short generation times, constant contact with the water column, and sessile nature of periphyton allow a rapid response to environmental stress and early warning of eutrophication and other negative impacts. Algal survival requires varying degrees of environmental tolerance to shifts in aquatic condition (Lowe and Pan 1996, Growns 1999). Diatoms have been used as indicators of P enrichment because of their degree of sensitivity to changes in P levels (Swift and Nicholas 1987, Raschke 1993, Pan and Stevenson 1996). Recent research conducted in the Everglades suggests that surface sediment P levels and changes in algal assemblage composition (Pan *et al.* 2000), periphyton tissue P concentrations (Gaiser 2003), and periphyton phosphatase activity (Newman *et al.* 2003b) are sensitive indicators of P enrichment and may be useful as early indicators of elevated P loading when used in conjunction with other relative indicators. Some investigators suggest that studying algal assemblages also may prove to be a cost-effective means of ecological monitoring (McCormick and Cairns 1994, 1997, McCormick and Stevenson 1998).

### *Phosphorus Removal Technologies and Restoration*

One of the primary mechanisms of P removal within a wetland system is by sedimentary deposition of organic material as peat soils (Richardson 1985, Jones and

Amador 1992, Guardo *et al.* 1995). Data showing significant accretion of macrophyte and periphyton-derived peat sediments and associated P in the Everglades system suggest that this is the primary P removal mechanism within the Water Conservation Areas (Guardo *et al.* 1995). Secondary means of removal from the water column include microbial and algal uptake and subsequent sedimentation, soil adsorption, geochemical adsorption by aluminum and iron minerals in areas with mineral soils, and coprecipitation of calcium carbonate with inorganic P salts at high pH (Richardson 1985, Guardo *et al.* 1995, McCormick and Scinto 1999).

Growing recognition of significant ecosystem changes in the Everglades resulting from altered hydrology prompted the reevaluation of regional water management practices and the implementation of a long-term and ecosystem-wide Comprehensive Everglades Restoration Plan a.k.a. CERP (<http://www.evergladesplan.org>). The documented ability of constructed or re-established wetlands to remove excess nutrients from surface waters is the basis for their use as a primary component of water quality improvement in the Everglades. The Everglades Construction Project, mandated by the 1994 Everglades Forever Act (Section 373.4592 Florida Statutes), includes the construction of approximately 16,600 ha of wetlands called Storm Water Treatment Areas (STAs) designed to use naturally occurring biological processes to filter nutrient-loaded urban and agricultural effluent before it is discharged into natural areas. In addition to the STAs, the Everglades Construction Project involves completion of several hydropattern restoration projects that will improve the volume, timing, and distribution of water entering the Everglades.

One of the primary goals of stormwater treatment technology, along with a combination of Best Management Practices (BMPs) and Advanced Treatment Technologies (ATTs), is to achieve P reductions necessary to meet legally mandated water-quality standards set by the Everglades Forever Act, most notably, the standard defining a threshold P concentration that does not lead to a system imbalance of aquatic flora or fauna and provides a net improvement in areas already impacted (interim goal of 50 ppb). The act requires the South Florida Water Management District to conduct research and monitoring programs to ensure compliance with the water quality criteria of the act and to optimize nutrient removal performance of the STAs (SFWMD 2003a).

Advanced Treatment Technology research is a critical component of the restoration program and currently involves ongoing analysis of promising biological and chemical technologies including Periphyton-based Stormwater Treatment Areas (PSTAs). Current research is addressing the feasibility of full-scale implementation of using periphyton-dominated ecological communities as a long term and predictable technology for TP reduction. Experiments to determine the effects of different hydraulic loading rates (HLRs), hydraulic retention times (HRTs) and water depths on treatment performance are in progress (Bays *et al.* 2001); however, the effects of different velocities on P uptake, periphyton taxonomic composition, and treatment performance in this system have not been evaluated. Recent test cell studies of PSTAs confirm the efficiency of these systems for significant P removal and their sensitivity to sediment nutrient concentrations and wetland hydraulic efficiency (Newman *et al.* 2003a).

### *Velocity-Periphyton Relationships*

Whitford and Schumacher (1961) were the first researchers to quantitatively correlate increased water velocity and P uptake in a number of algal taxa with an increase in diffusion gradient at or near the algal cell surface. Subsequent studies conducted in lotic systems have found that increases in low rates of flow are positively correlated with increased nutrient uptake by periphyton communities (Schumacher and Whitford 1965, Lock and John 1979, Riber and Wetzel 1987, Horner *et al.* 1990, Stevenson and Glover 1993, Borchardt 1996). Mulholland *et al.* (1994) argue that nutrient cycling characteristics of stream periphyton are linked closely to biomass and hydraulic characteristics of the system.

Current research indicates that there are several factors governing nutrient uptake and internal nutrient cycling in moving water: diffusion rates related to boundary layer thickness of both algal cells and algal mats; extent of the nutrient concentration gradient; mass transport; metabolism; and biomass accumulation as well as algal mat density (Riber and Wetzel 1987, Stevenson and Glover 1993, Borchardt 1996, Mulholland 1996, Stevenson 1996b). Algal cells are surrounded by a quiescent layer called a “boundary layer” through which nutrients must diffuse to reach the cell surface. Increases in water flow over the cell surface reduce the thickness of this layer, allowing increased nutrient diffusion (Whitford 1960, Borchardt *et al.* 1994). In moving water, the interaction of velocity and nutrient availability can be examined together through the concept of mass transport. Borchardt (1996) suggested that nutrient mass transport is the product of

discharge or flow (volume time<sup>-1</sup>) and concentration (mass volume<sup>-1</sup>), describing the mass of nutrient moving past a given point per unit time.

Whitford and Schumacher (1964) suggested that P uptake rate is influenced by the metabolic rate of a particular algal species and that individual species thus exhibit differential uptake responses based on water velocity. Results from several studies indicate that algae adopt specific adaptations to different velocities and these are reflected in the taxonomic composition of the community (McIntire 1966a, 1968, Horner *et al.* 1983, Horner *et al.* 1990). Even though Everglades water velocities are much slower than lotic systems, the species-specific difference in nutrient uptake documented in other freshwater systems should still hold true for the Everglades periphyton communities and might be reflected in taxonomic differences observed at different flow rates.

The relationship between the ecology of the Everglades periphyton communities and P enrichment suggests that periphyton may provide a reliable measure of the P bioavailability and assimilative capacity of this wetland ecosystem (McCormick and O'Dell 1996). Studying the effects of water velocity on periphyton characteristics such as species abundance and composition, biomass accumulation, and P uptake may provide data that can be used to optimize PSTA design and establish greater nutrient removal efficiency of this alternative treatment technology (Guardo *et al.* 1995). These characteristics can be considered important indicators of the success of restoration treatment strategies and management decisions (McCormick *et al.* 1998). This success can be evaluated by monitoring changes within the natural habitats and communities (*e.g.*, periphyton) that play a dominant role in Everglades ecology. One of the Everglades

restoration research needs identified by the National Research Council of the National Academy of Sciences is the determination of the effects of flow on P uptake in order to evaluate the performance of water treatment technologies. A second need is to refine the relationships between abiotic processes, e.g., velocity, and biotic responses, such as periphyton community composition (NRC 2003).

The primary purpose of this research was to help address these needs by describing and comparing the taxonomic composition of Everglades periphyton communities growing within artificial freshwater mesocosms at different flow rates.

A secondary objective of this study was to elucidate dominant algal indicator taxa for each velocity treatment. These taxa might be used to establish an indicator assemblage that can be incorporated into a multimetric standardized periphyton index for monitoring ecosystem change (McCormick and Cairns 1994, McCormick and Stevenson 1998). Such an index that includes taxonomic composition related to flow rate might provide a good estimate of P bioavailability and P uptake in periphyton-based STAs (McCormick and Stevenson 1998), facilitate improvements in their design, and optimize current water quality treatment applications within this alternative treatment technology. Optimization of water velocity through PSTAs may lead to greater biomass accrual and greater efficiency in nutrient removal, in particular, P uptake, by the periphyton communities. This optimization will contribute to achieving final post-treatment discharge concentrations of water-column TP that meet restoration goals and compliance with the amended Everglades Forever Act of 1994. These concentrations will also help reestablish and maintain the natural plant communities of the historical oligotrophic

Everglades.

This study tested the following four hypotheses:

H<sub>1</sub>: A ten-fold increase in water velocity between treatments will result in a significant difference in generic taxonomic composition of the periphyton communities in the fast treatment mesocosms compared to the slow treatment mesocosms over the course of the study.

H<sub>2</sub>: Within both the slow and fast treatment mesocosms, taxonomic composition will change from a eutrophic to a more oligotrophic assemblage along the treatment path from the inflow toward the outflow end of the mesocosm. This taxonomic change will reflect changes in nutrient levels as a result of uptake and elimination of P from the water column by periphyton.

H<sub>3</sub>: Periphyton TP levels will be significantly higher in the fast compared to the slow velocity treatment samples and in the inlet compared to the outlet samples.

H<sub>4</sub>: A ten-fold increase in water velocity between treatments will result in a statistically significant greater rate of periphyton biomass accrual in the fast treatment compared to the slow treatment.

## MATERIALS AND METHODS

### *Study Site Description*

This research was conducted within the former Everglades Nutrient Removal Project (ENRP) area, a demonstration wetland located just northwest of the northern end of the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Water Conservation Area 1) in western Palm Beach County, Florida (Figure 1). This prototype marsh system, encompassing 1,514 ha of converted cropland, has been operated by the South Florida Water Management District since 1994 and was designed to filter and remove excess nutrients from agricultural runoff and urban wastewater before it flows into the Everglades. The ENRP is now incorporated into Storm Water Treatment Area 1-W (STA-1W) which has a 75% larger effective treatment area of 2,702 ha (Figure 2).

Surface inflow comes from Everglades Agricultural Area runoff diverted from the West Palm Beach canal (C-51) into the S5A pump station and enters STA-1W. Cell 1 and the former buffer cell of the ENRP have been combined in order to accommodate increased flow into STA-1W. Water is routed both westward into Cells 5A and 5B and southward toward one of two treatment trains consisting of either Flow-way Cell 1 and Polishing Cell 3 or Flow-way Cell 2 and Polishing Cell 4 (Figure 2).

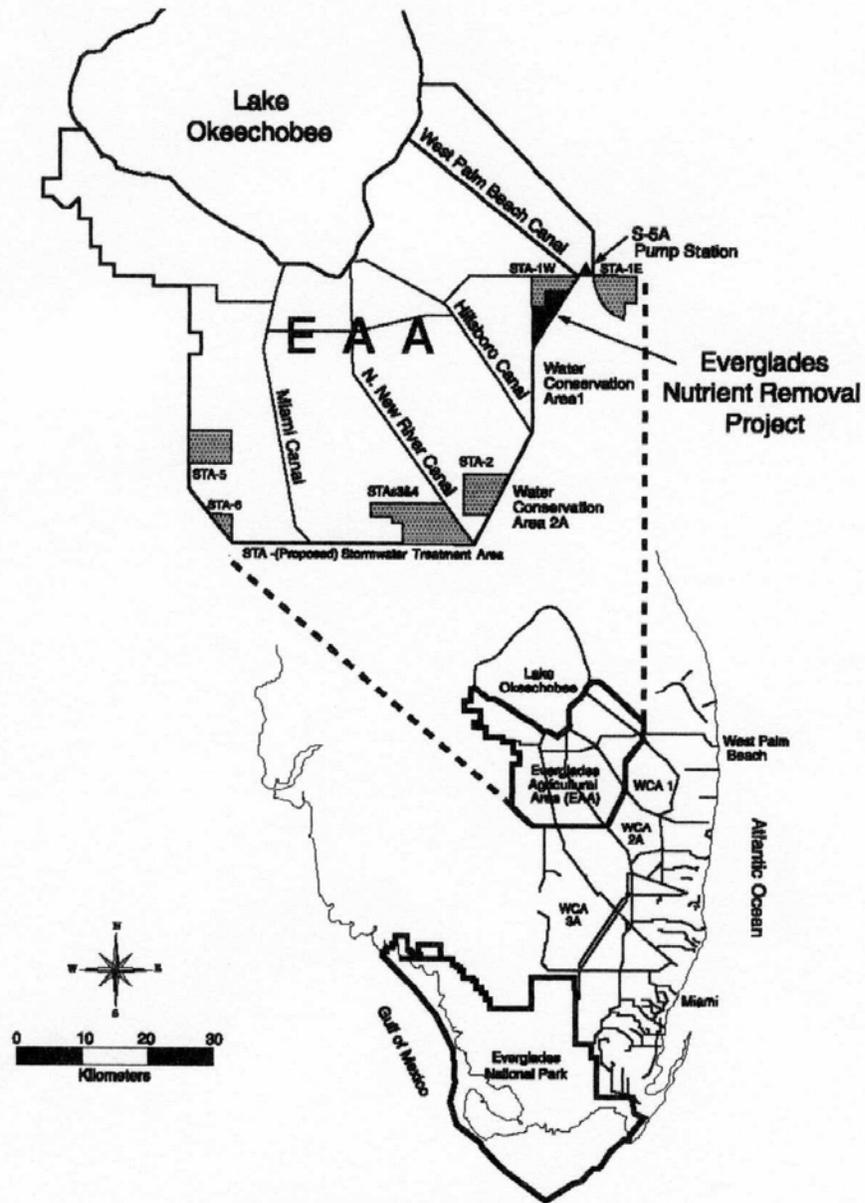


Figure 1. Location of the ENRP study site in western Palm Beach County, Florida. Courtesy: South Florida Water Management District (SFWMD).

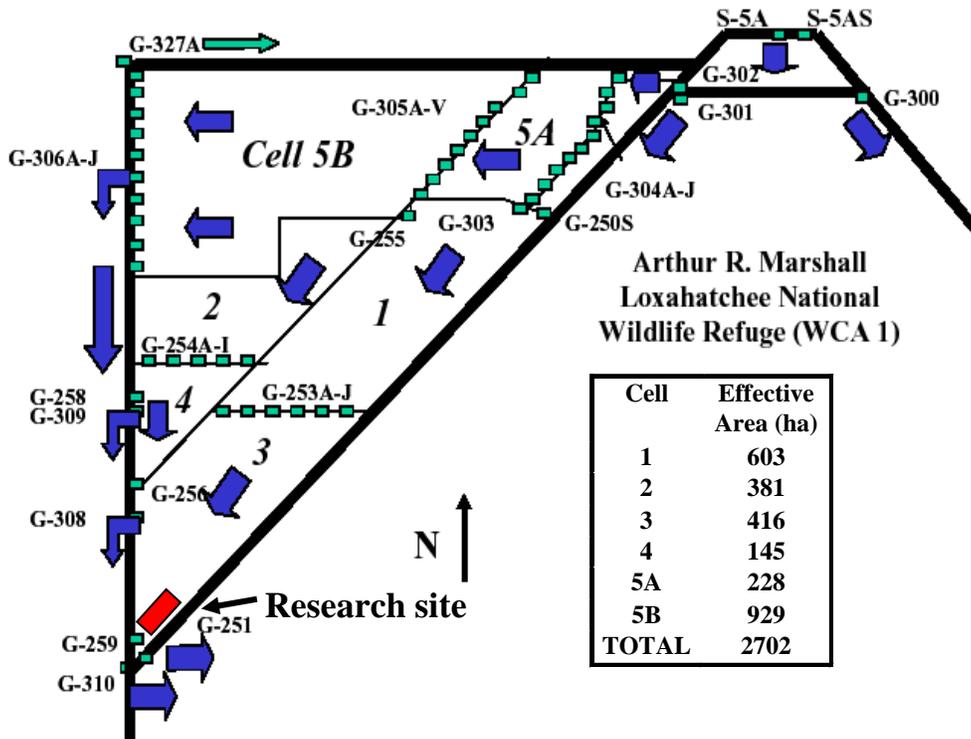


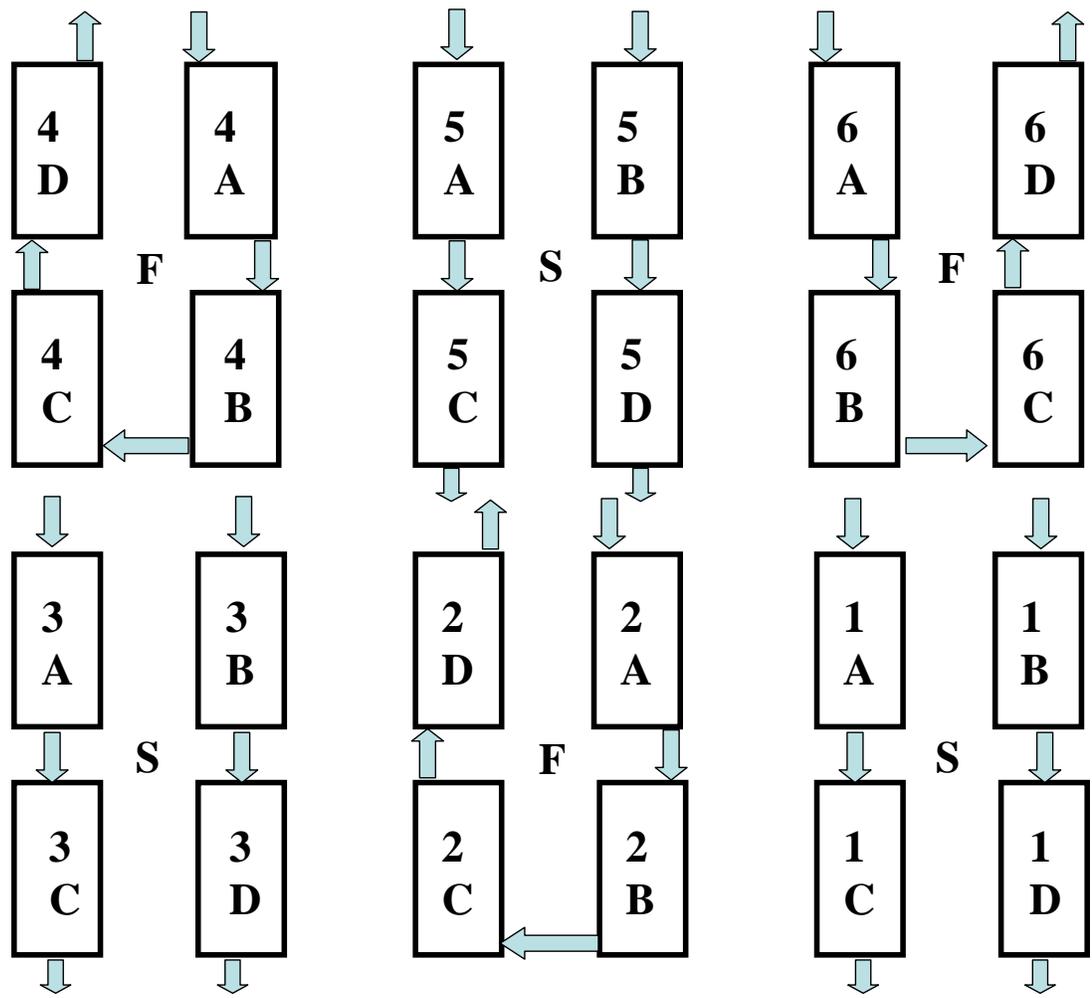
Figure 2. Schematic diagram of STA-1W and research site (not to scale). SFWMD 2003.

Cells 1 and 2, where initial P removal occurs, are naturally revegetated marshes dominated by cattail (*Typha* spp.) and significant amounts of periphyton and submerged aquatic vegetation (SAV). Polishing cell 3 is dominated by cattail but also contains a stable community of native wetland vegetation planted during construction. Polishing cell 4 is maintained as an open water SAV and periphyton cell by design. These polishing cells serve to further reduce P levels. Cell 5A is dominated by cattail and maintained as an emergent marsh and cell 5B is an SAV/periphyton-dominated cell (Goforth and Bechtel *et al.* 2003). The outflow from STA-1W is discharged via three collection canals into the L-7 borrow canal in northwestern WCA-1.

### *Mesocosm Design*

Mesocosms were installed at the outlet (south) end of the ENRP. Twenty-four experimental boxes were constructed of Plexiglas® and measured 1.22 m x 2.44 m x 0.31 m in size. A total of 6 mesocosm units of equal size were created by connecting a set of 4 boxes together via 7.6 cm diameter inlet and outlet PVC pipes to form one flow-through treatment unit. These six units were comprised of 3 replications of a slow velocity treatment and 3 replications of a fast velocity treatment (Figure 3).

The mesocosms were designed to ensure that possible confounding environmental and experimental variables were minimized or controlled during the study period. The boxes were mounted level on wooden frames and were arranged on the site in a general north-south alignment and alternated by treatment (Figure 3). This arrangement



**S = Slow velocity mesocosm (1, 3, 5)**

**F = Fast velocity mesocosm (2, 4, 6)**

Figure 3. Top view of study site mesocosm layout. Six mesocosm units, three slow velocity and three fast velocity, are comprised of four boxes (A, B, C, D) each.

minimized shading effects and ensured that an equal number of boxes per treatment received approximately the same irradiance levels. The four boxes comprising each fast treatment were “stepped down” in four levels so that water flowed by gravity from the highest (A) where the inflow was located, to the lowest box (D) where the outflow pipe was located. The two sets of boxes in the slow treatment were “stepped down” in two levels from the inlet boxes (A and B) to the outlet boxes (C and D). Each mesocosm provided a uniform bare artificial Plexiglas® surface area (32 m<sup>2</sup>) for colonization by periphyton and uniform initial pH and temperature conditions.

Opaque manifolds containing adjustable coffer dams were installed at each end of the boxes and were covered to eliminate additive incident light during the study period. Slow treatment manifolds had five inlet and five outlet holes. Fast treatment manifolds had one inlet and one outlet hole, each equal in size to the combined area of the small inlet and outlet holes, respectively, in the slow treatment mesocosms. This ensured an equal volume of water entering and exiting each box. A pump delivered outflow STA water from the outlet canal of the ENRP through a 15-cm PVC access supply pipe to the study site.

A set of 4 Plexiglas® partitions measuring approximately 20 cm high and approximately 203 cm in length was installed inside each box according to treatment design specifications. Partitions in the fast treatment boxes were positioned to create five water channels configured in a serpentine path of 11.125 m per box for a total channel treatment length of 44.5 m per mesocosm unit. Partitions in the slow treatment formed a short wide path of 2.23 m per box for the movement of water. The slow treatment was

designed to ensure that fifty percent of the water volume flowed through each of two sets of two conjoined boxes creating a total channel treatment length of approximately 4.5 m per mesocosm unit (Figure 4). The open channel area within each box measured approximately 223 cm x 119 cm x 17.4 cm and had a 461 L capacity.

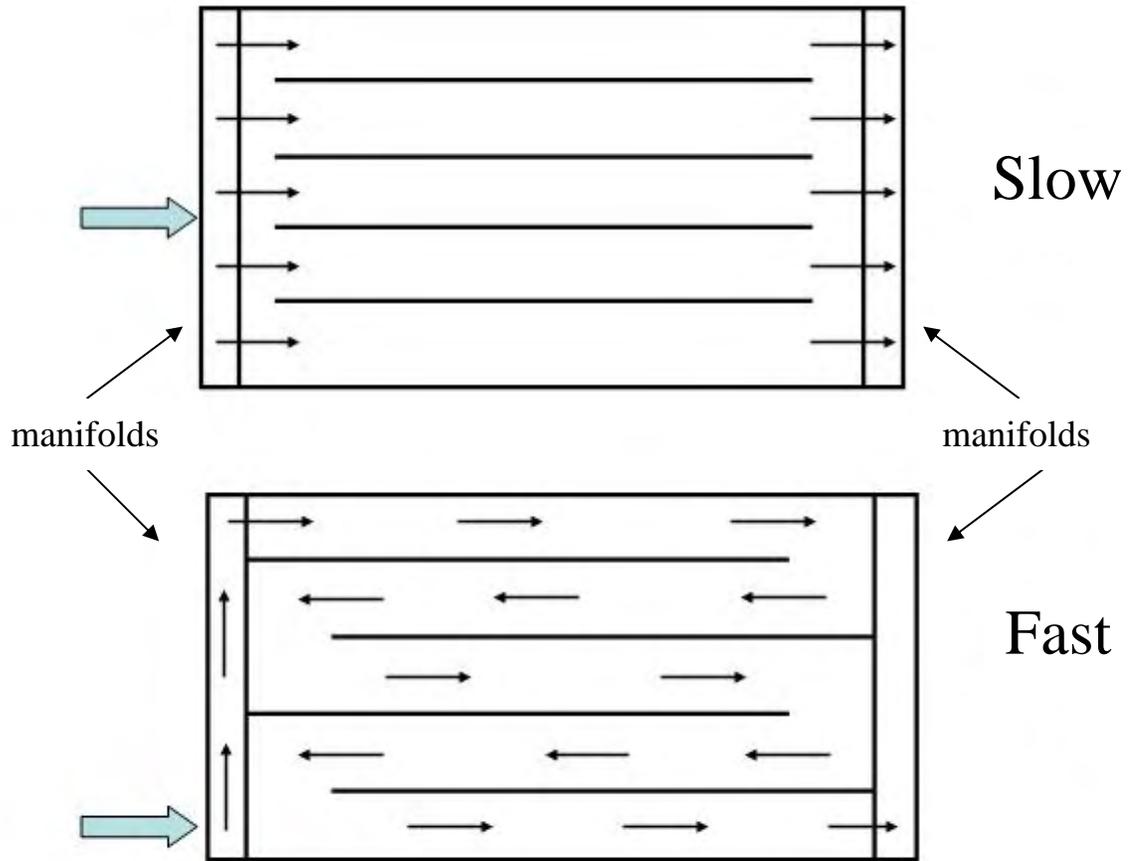


Figure 4. Top view of mesocosms and treatment design. All interior partitions were the same length (203 cm) and height (20 cm). Total path length for the slow treatment was 4.46 m (21.23 m per box) and for the fast treatment was 44.5m (11.125 m per box).

Water inlet volume was controlled in each mesocosm by a Kobold flow meter and monitored on a continuous basis. Because the slow treatment consisted of two boxes each receiving half the total incoming water supply for that mesocosm, it was necessary to monitor these flow rates weekly and balance them, when necessary, using gate valves installed on the inlet pipes.

Minor coffer dam adjustments were made as needed to maintain uniform water levels within each mesocosm. The flow meters, manifolds, adjustable coffer dams within the manifolds, and the 7.6 cm diameter inlet and outlet PVC pipes in each mesocosm unit ensured an equal volume of water entering and exiting each box. A mean water level of 16.8 cm was maintained during the study period.

At the initiation of the experiment, flow meters were set at an appropriate rate ( $3000 \text{ L h}^{-1}$ ) to achieve a channel velocity of approximately  $2.0 \text{ cm s}^{-1}$  in the fast treatment and  $0.22 \text{ cm s}^{-1}$  in the slow treatment ( $24 \text{ cm channel width} \times 17.35 \text{ cm channel depth} \times 2 \text{ cm s}^{-1} = 833 \text{ cm}^3 \text{ s}^{-1}$ ). Rates of each flow meter were monitored regularly and the meters were cleaned and adjusted as necessary to maintain desired velocities. A food-coloring dye test was performed shortly before initiation of the study to visually observe short-circuiting of flow or “dead zones” within the mesocosms. The only visible reduction in flow appeared to occur in the corners of the boxes and was most likely a result of mesocosm design. A rhodamine dye tracer test was conducted in the mesocosms on April 2, 2002 to determine flow patterns and quantitatively assess hydraulic efficiency, i.e. deviations in residence time. Results confirmed that the average residence time in the slow velocity treatment was not significantly different from the fast

velocity treatment.

Both sets of treatments received the same volume of water per unit time from the same source. Therefore, the hydraulic loading rate, the hydraulic residence time, and colonizing surface were equal between treatments and replications. This ensured that water flow rate was the single treatment variable. Outlet water from the mesocosms discharged into a main drain line and was routed to the ENRP outlet canal just upstream of the G-251 pumping station.

### *Periphyton Sampling and Processing*

Periphyton protocols for this study were established using methods consolidated from several standard references and personal communications (Sládecková 1962, Aloï 1990, Wetzel and Likens 1991, Lowe and Pan 1996, McCormick and O'Dell 1996, APHA 1998, Stevenson 1984, Stevenson and Bahls 1999, FDEP 2000, O'Dell 2001 personal communication, Phycotech, Inc. 2001 personal communication, U.S. EPA 2002). A set of 15.2 cm x 7.6 cm x 0.32 cm Plexiglas® periphytometers (uniform artificial substrate devices used for algal colonization and sampling) was installed in each of the twenty-four treatment boxes on the first day of the experiment, March 27, 2002. Each set was comprised of seven vertically-oriented periphytometers clipped side-by-side onto the partition wall of a mesocosm channel and seven corresponding samplers placed side-by-side on the bottom of the channel. This design was chosen to more effectively capture a representative algal community sample within the channel at each

periphytometer location. Periphytometer sets were installed 50 cm away from the inlet manifold hole within slow treatment boxes A and B, and 60 cm away from the inlet hole in fast treatment box A. These locations were chosen to capture inlet taxonomic composition and biomass accrual while minimizing turbulence and disturbance of the substrate from incoming water. To capture outlet taxonomic composition and biomass accrual while minimizing shading from metaphyton accumulation near outlet holes, periphytometer sets were placed 65 cm from the outlet manifold hole in the C and D boxes of the slow treatment and 280 cm from the outlet in the D box of the fast treatment.

A series of 7 sequential collections of the periphytometers was conducted over a 22-week period beginning on the seventh day after initiation of the experiment, when a biofilm was plainly visible on the Plexiglas® substrate. Further collections were conducted 14, 21, 36, 55, 78, and 150 days after initiation. Forty-eight samples, one side and one corresponding bottom periphytometer, were collected from one of the randomly chosen seven periphytometer locations in each of the 24 boxes during each of the sampling events for a total collection of 336 samples. A significant grazing event that began on day 73 precluded the use of the sixth and seventh set of samples for data analysis and shortened the length of the experiment to fifty-five days. The amphipod *Hyallela azteca*, Ram's horn snails (*Planorbella* sp.) and water scavenger beetles (*Berosus* sp.) were identified and likely consumed the majority of biomass in the mesocosms over a period of three days. Previous studies indicate that eight weeks is an adequate length of time for significant biomass accrual, stochastic colonization and development of mature periphyton structure in many communities (Cattaneo and

Amireault 1992, Lowe *et al.* 1996, APHA 1998).

Water column temperature and pH measurements (one in each of the 24 boxes at the location of the periphytometers) were taken on days 0, 7, 14, 21, 30, 36, 43, and 55 using a calibrated Corning pH meter. Samples for water column TP were collected on days 13, 29, 36, and 55. Samples for periphyton TP and dry weight biomass were collected on days 7, 14, 21, 36, and 55. A photographic log of each of the 24 boxes was maintained throughout the study, beginning on day 7, to document gross visual algal colonization, biomass accrual, metaphyton development, and biomass loss due to sloughing, export and grazing (Appendix A).

Periphytometers were transported from the field in plastic zip-lock bags on ice and were processed as soon as possible upon return to the laboratory. The surface area of algal colonization on each of the periphytometers was measured with a metric ruler and then the algae was gently and thoroughly scraped and rinsed with distilled water into 125 mL or 250 mL plastic bottles. Samples were weighed and the contents preserved in a final concentration of either 4.7-5% buffered formalin (day 7, 14, and 21 samples) or a neutralized 0.5% solution of glutaraldehyde (day 36, 55, 78, and 150 samples). The preservative was changed from formalin to glutaraldehyde to facilitate better long-term preservation of pigments and cellular integrity of the higher biomass samples. The pH measurements of randomly selected samples ranged from 6.46 to 7.65 and were satisfactory for long-term preservation. Specimens were stored refrigerated and protected from light.

Field samples were homogenized (Bio Homogenizer Model 133, Biospec

Products, Inc.) at low speed for 15 to 30 seconds to assure uniform distribution and density of algae within the samples. For taxonomic analyses, the homogenized samples were processed into composite sub-samples comprised of equal volumes of the side and corresponding bottom periphytometer samples in order to capture assemblage composition at a particular sampling location. Of these composites, a total of twelve samples, one from the A (inlet) and D (outlet) boxes of each fast treatment and a composite sample of the A&B (inlet) and C&D (outlet) boxes of each slow treatment, from each of five collection periods, were processed for a total of 60 samples. Sub-samples for both soft algae and diatom analysis were diluted as necessary with distilled water to achieve algal suspensions that produced between 10 and 20 well-distributed cells per 1000X microscopic field. These suspensions provided more optimal densities for accurate counting and identification of cells (Stevenson and Bahls 1999).

Diatom and soft-bodied algae counts were conducted separately and microscope slide mounts were processed accordingly for each type of analysis. A minimum of three semi-permanent wet mounts using the 10% Taft's syrup medium (TSM) technique were prepared for the identification of soft-bodied non-siliceous algae (Stevenson 1984). One mL or 0.5 mL aliquots of TSM/algal suspension (depending upon algal density) were pipetted onto 22mm x 22mm glass cover slips and air-dried overnight or heated gently on a slide warmer until dry. Cover slips were then rehydrated with 1.0 mL of 10% TSM and inverted onto a clean glass microscope slide. The slides were warmed slowly and allowed to cool. Cover slip edges were sealed with clear, acrylic nail polish (Wet n' Wild®). Because soft algae mounts are subject to desiccation and pigment deterioration,

microscopic enumeration and identification was conducted as soon as possible after slide preparation to assure optimal fluorescence and cellular integrity.

To produce mounts for the identification and enumeration of diatoms, organic matter was first cleared from sub-samples with either a concentrated nitric acid (Phycotech, Inc.) or concentrated sulfuric acid and potassium permanganate solution oxidation technique (E. Gaiser, personal communication). After oxidation, samples were rinsed with distilled water until pH 6.0 (equivalent to the pH of the rinse water) was achieved in all samples. One mL or 0.5 mL aliquots (depending upon diatom density) of acid-cleaned samples were pipetted onto 22mm x 22mm glass cover slips and allowed to air dry overnight or heated gently on a slide warmer until dry. Dried cover slips were inverted onto clean microscope slides containing several drops of a refractive media, Naphrax® (Northern Biological Supply, Ipswich, Great Britain). Slides were heated until the Naphrax® flash-boiled and were then cooled rapidly to reduce air bubble formation and to seal the cover slip to the slide. Excess Naphrax® was scraped from the edges of the cover slips, slides were cleaned with glass cleaner, and the cover slip edges were sealed with clear, acrylic nail polish (Wet n' Wild®). A minimum of three permanent slide mounts were prepared for each diatom sample.

### *Taxonomic Analysis*

Slide preparation and taxonomic analyses were conducted separately for diatoms and soft-bodied algae on each of the 60 sub-samples, comprising a minimum of 120 analyses.

Evaluation of genus-area curves for both diatoms and soft algae, using PC-ORD software (McCune and Mefford 1999), indicated that 60 was an adequate sub-sample size for this community data set (Figures 5 and 6). Generic richness, relative abundance, mean density, and diversity of sampled periphyton assemblages were assessed by counting and enumerating cells microscopically and computing metrics of interest using the cell counts from a known volume and dilution of sample. Microscopic analyses were conducted using a Nikon Eclipse E600 compound light microscope with a blue filter, bright field illumination, epi-fluorescence and DIC (Nomarski) optics. All slides were first scanned at both 400X and 1000X to determine and record presence of dominant genera. Representative examples of both identified and unknown algae were measured using Scion Image v. 1.62 digital imaging software that was calibrated with a standard ocular micrometer. Algae was then documented with digital (Scion Image, Kodak, Nikon) or 35mm film photography to assist with identification.

Fields of known focal area on replicate microscope slides were then examined at 1000X magnification in a systematic manner, a semi-random “W” pattern, until the desired number of algal natural units or diatom valves or frustules per slide were identified and enumerated. Natural units for coenocytic and filamentous genera were defined as segments of the thallus or filaments visible within the field of view. Cells comprising sheathed or colonial algae were counted as one natural unit. Enumeration of cyanobacterial filaments was greatly enhanced by the use of epi-fluorescence microscopy which also aided in discriminating major algal divisions from each other and from bacterial content. Only viable algae, i.e., those containing a visible protoplast, cytoplasm,

or intact valves and frustules, were counted.

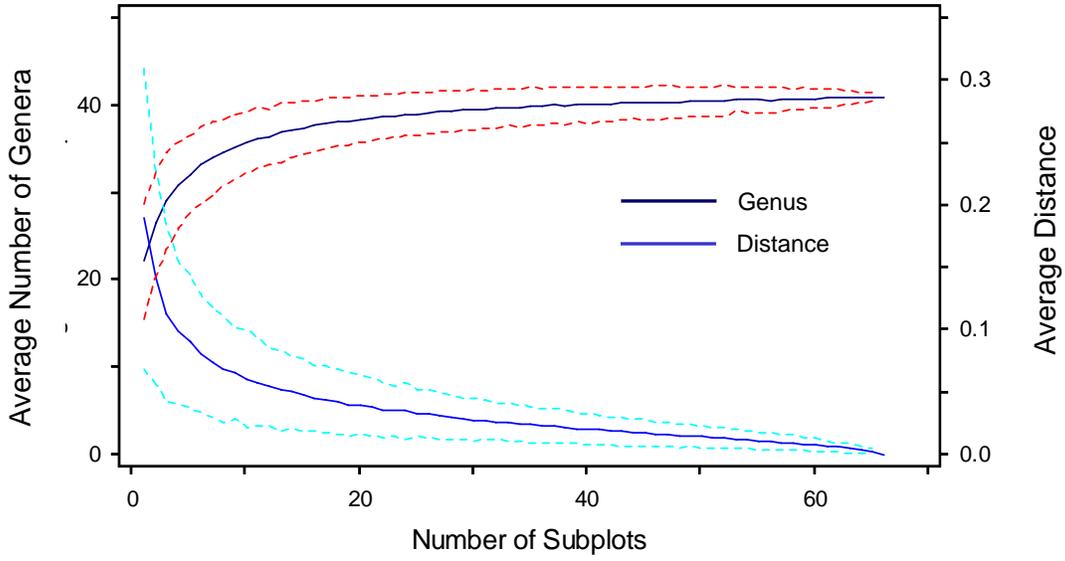


Figure 5. Diatom genus area curve (constructed with PC-ORD) for taxonomic samples.

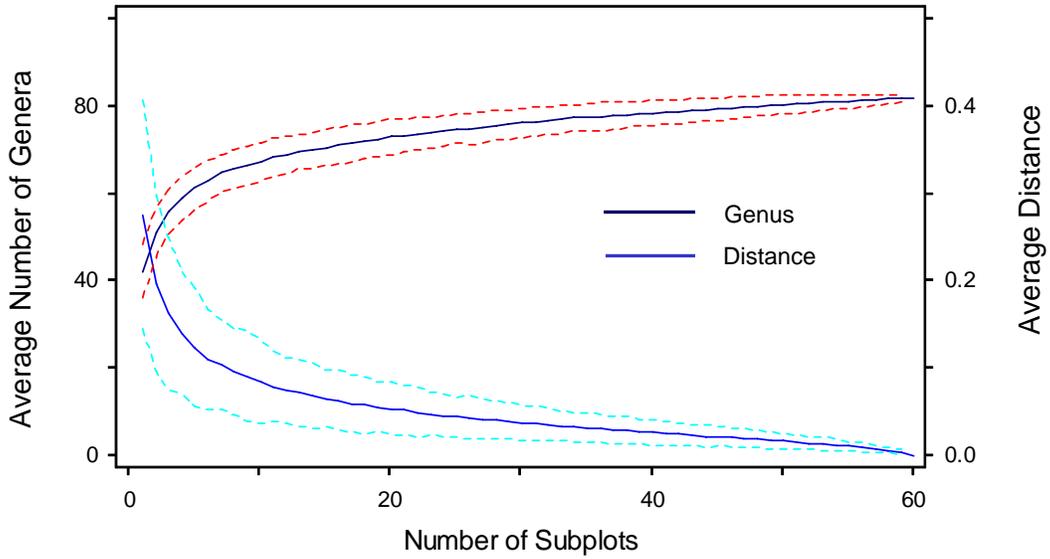


Figure 6. Soft algae genus area curve (constructed with PC-ORD) for taxonomic samples.

A minimum of 250 soft algae natural units per each of two replicate slides were counted using oil immersion (1000X) magnification for a minimum total of 500 with a mean of 652 natural units and 57 fields for all samples analyzed. Concurrently, diatom frustules in each field of soft algae slides were counted (but not identified) in order to calculate their density in each sample. Diatom identification was conducted on acid-cleaned slides. A minimum of 300 diatom valves per each of two replicate slides were counted at 1000X for a minimum total of 600 valves and a mean of 779 valves and 45 fields for all samples analyzed. Diatom frustules suffer considerable separation into their respective valves during the acid-cleaning process thus valve enumeration is standard protocol for these specimens.

Taxa were identified to the lowest practical taxonomic level (genus) using assistance from experienced consultants and standard taxonomic references (Hustedt 1930, Smith 1950, Desikachary 1959, Drouet 1963, Patrick and Reimer 1966 and 1975, Bourrelly 1968, 1970 and 1972, Drouet 1968, Collins 1970, Drouet 1973, Prescott 1973, Prescott *et al.* 1975 and 1977, Prescott 1978, Prescott *et al.* 1981 and 1982, Croasdale *et al.* 1983, Komárek 1983, Komárek and Fott 1983, Pentecost 1984, Whitford and Schumacher 1984, Anagnostidis and Komárek 1985 and 1988, Komárek and Anagnostidis 1986, Tikkanen 1986, Komárek and Hindak 1988, Comas 1989, Dillard 1989 through 1991, Komárek and Anagnostidis 1989, Anagnostidis and Komárek 1990, Round *et al.* 1990, Cox 1996, Dillard 1999, Komárek and Anagnostidis 1999, 2000, Graham and Wilcox 2000, Wilkinson *et al.* 2001, John *et al.* 2002, Suda *et al.* 2002, and Wehr and Sheath 2003).

### *Periphyton Biomass*

Dry weight, expressed as  $\text{g m}^{-2}$ , was the standard method used to estimate periphyton biomass in this study. Periphytometer biomass was analyzed on aliquots of both side and bottom samples from slow treatment boxes A (inlet) & C (outlet) and fast treatment boxes A (inlet) & D (outlet) for all collection dates except for day 36, which included analysis of all periphytometer samples.

A total of 9 mL of sample was slowly dispensed onto pre-weighed 47 mm glass fiber filters resting on a vacuum device. Filters were placed in labeled pre-weighed trays and dried in a forced air oven at 70 °C for a minimum of four hours to ensure complete drying and a constant weight. This temperature is recommended to reduce the loss of some volatile organic compounds that can occur at higher temperatures (Aloi, 1990). Dried filters were re-weighed and net dry weight biomass was determined by subtracting pre-drying weights of the clean filters from post-drying filter weights. Dry-weight measurements included the mass of both inorganic and organic matter in the sample and thus included not only algal mass, but also bacterial, invertebrate and detritus mass as well.

### *Periphyton and Water Column Total Phosphorus*

Analyses for water column TP were conducted on samples collected from both the inlet and outlet manifolds of slow and fast velocity mesocosms. Analyses for

periphyton total P were conducted on aliquots from the same periphytometer samples as those analyzed for dry weight biomass. Samples were first digested using a persulfate autoclave procedure. The P concentration in digested samples was determined using an ammonium molybdate and ascorbic acid colorimetric detection procedure (Zhou and Struve 2000).

### *Quality Assurance*

A voucher collection of all original periphyton samples and composite samples is currently being stored in the dark and under refrigeration. Acid-cleaned diatom samples are being stored in the dark at room temperature. A voucher collection of semi-permanent and permanent microscope slide specimens is being stored in commercially available slide boxes and is maintained at room temperature. Approximately 1500 photographic (digital and 35 mm print) images and a corresponding written photographic log supplement the voucher collection.

The replicate counting technique utilized for both soft algae and diatoms enabled the assessment of consistency in counting, taxon identification, and metrics due to variations in sample homogeneity, density, sample preparation and other analytical variability. It also ensured more accurate determination of the presence and relative abundance of both dominant and rare taxa. A Sørensen's similarity coefficient index (Odum 1971, Vymazal 1988, Lincoln *et al.* 1998) and Whittaker's (1952) percent community similarity (PSc) index were calculated from proportional counts of common

genera (> 1.0% relative abundance) for 24 randomly chosen replicate slides from both diatom and soft algae counts (20 % of the total number of slides counted). The Sørensen's similarity index of the slides ranged from 94.7 to 100.0 %, and the Whittaker index ranged from 79.7 % to 95.9%, both exceeding the minimum of 75.0 % recommended in standard bioassessment protocols (Stevenson and Bahls 1999).

Algal taxonomists throughout the country, including those located at the Florida Department of Environmental Protection, Phycotech, Inc., John Carroll University, Florida International University, Bowling Green University, and the South Florida Water Management District were consulted by phone, in person, or by e-mail on a regular basis, to verify taxonomic identifications. A minimum of 10% of the taxonomic samples were randomly selected and sent for review to taxonomists at Phycotech, Inc. and the Florida Department of Natural Resources to assure consistency of identification. Discrepancies in identification were reconciled in the database.

### *Statistical Analysis*

Mean generic diversity was examined separately for soft algae and diatoms using the Shannon diversity index (Shannon and Weaver 1949, Zar 1996). This index is less affected by the lack of data on rare species than the Simpson diversity index and is heavily based on species richness (Brower *et al.* 1997). After calculating diversity by day of sampling, velocity, and location of periphytometers within the mesocosm (inlets and outlets), statistical comparison of the indices was conducted using a T-test statistic.

The degree of overall or total similarity of generic composition between slow and fast, and inlet and outlet algal assemblages was compared by computing Sørensen's similarity coefficient (S) or index (%), based upon presence or absence of genera (Odum 1971, Lincoln *et al.* 1998, Vymazal 1988):  $S = 2C/(A+B)$ , where C = the number of genera common to both samples, and A and B = the number of genera seen in samples A and B, respectively. The index ranges from a lack of correspondence or similarity (0) to complete correspondence or similarity (1 or 100%, if expressed as a percentage). An SC lower limit of 0.40 or 40% can be used to distinguish between similarity ( $> 0.40$ ) and dissimilarity,  $\leq 0.40$  (Raschke 1993).

Whittaker's (1952) percent community similarity index,  $I_a$  or  $PS_c$ , was calculated using relative abundances of common genera, to compare similarity of randomly selected slide replicates used for taxonomic counts per standard periphyton bioassessment protocols (Stevenson and Bahls 1999):  $PS_c = 100 - .5 \sum_{i=1} |a_i - b_i| = \sum_{i=1} \min(a_i, b_i)$ , where:  $a_i$  = percentage of species or genera  $i$  in sample A and where  $b_i$  = percentage of species or genera  $i$  in sample B. The index ranges from a lack of commonality or similarity (0) to complete (100%) commonality or similarity.

Relative abundances for soft algae and diatoms within each sample were first determined separately from counts derived from soft algae slide mounts and acid-cleaned diatom mounts, respectively. The number of natural units, valves, or frustules counted for each taxon was divided by the total number of cells or units counted and expressed as a percentage. Changes in the proportional relative abundances of separate taxa as well as the major algal divisions were then examined and plotted as a function of velocity,

location along the treatment path within the mesocosm, and date of collection. The proportional abundance of each diatom taxa counted from the acid-cleaned specimens was calculated and applied to the total number of diatoms counted during soft algae enumeration to provide density information as well as a direct comparison of abundances between diatoms and non-siliceous algae.

Linear regression analysis was used to evaluate the difference between treatments for periphytometer biomass accrual and tissue TP. Analysis of Variance (ANOVA) was used to examine the relationships between variables (dry weight biomass, periphyton TP, pH, and temperature) and to detect statistically significant differences in the periphyton characteristics both between and within treatments (Microsoft® Excel 2002).

Non-metric Multidimensional Scaling (NMS) ordination analyses were conducted using PC-ORD version 4.14 software (McCune and Mefford 1999) to examine multidimensional variance both between and within treatments. This technique was also used to measure the correlation of several abiotic and biotic environmental factors with community generic composition. Analysis was limited to taxa that were present in at least 90% of the samples. NMS analyses were performed on a main matrix produced from taxonomic counts normalized to periphytometer surface area, sample dilution, and number of fields counted. The secondary matrix was produced from quantified variables of interest including pH, temperature, date of sampling, periphyton TP, biomass, velocity, and location of periphytometers within the mesocosm. Sørensen (Bray-Curtis) was selected as the distance measure. Scree plots and Monte Carlo tests were used for selecting dimensionality. Genus area curves for diatom and soft algae taxa were

constructed using PC-ORD software to verify adequate sample size for taxonomic analysis.

Indicator genus analysis was also conducted utilizing PC-ORD software (McCune and Mefford 1999) to elucidate groups or individual genera that were defined by environmental variables and/or experimental treatments, i.e., were indicators of the environmental condition of interest. Based upon Dufrene and Legendre's (1997) method, this analysis combines information on both the proportional abundance and proportional frequency, or faithfulness of occurrence, of a genus in a particular group. The indicator values produced for each genus, based upon standards of a perfect indicator, are tested for statistical significance using a randomization (Monte Carlo) technique. The values range from zero or no indication to 100 or perfect indication (McCune and Grace 2002).

## RESULTS

### *Water column pH and Temperature*

There was no significant difference in overall mean water column temperature between the slow ( $26.6 \pm 0.3$  °C) and fast ( $26.3 \pm 0.3$  °C) velocity treatments during the study (Figure 7). The minimum temperature recorded was 23.5 °C and the maximum was 30.0 °C with a mean for all data of  $26.4 \pm 0.1$  °C. There was, however, a significant small difference between inlet and outlet temperatures ( $p \leq 0.05$ ) with a mean inlet temperature of  $26.2 \pm 0.2$  °C. and mean outlet temperature of  $26.8 \pm 0.2$  °C. Mean slow and fast outlet temperatures were slightly higher than mean slow and fast inlet temperatures, respectively, throughout the study. The higher mean temperature in the outlet mesocosm boxes, especially the fast outlets, is most likely due to increased warming of the water column from irradiance as distance from inlet source water (i.e., treatment path length) increases.

There was a significant difference in pH ( $p \leq 0.01$ ) between the mean slow and fast velocity measurements, mean inlet and outlet measurements, and inlet and outlet measurements by velocity treatment for the study period. The pH ranged from a minimum of 6.9 to a maximum of 9.1 with an overall mean of  $7.7 \pm 0.02$ . The mean pH of the slow velocity mesocosms exceeded that of the fast velocity mesocosms on all

recording dates after day 0 (Figure 7). The overall mean pH for the slow velocity treatment and outlets was  $7.8 \pm 0.04$  and for the fast velocity treatment and inlets was  $7.6 \pm 0.04$ . An unexplained decline in pH, particularly in the fast treatment, occurred between days 30 and 36.

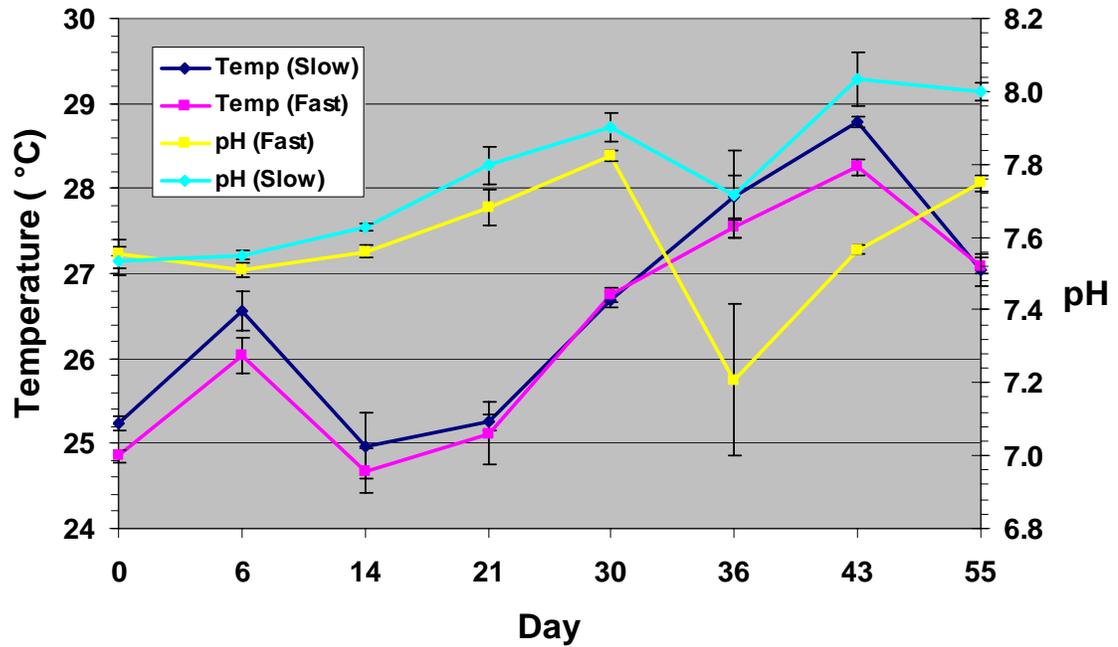


Figure 7. Mean water column pH, temperature ( $^{\circ}$  C) and standard error by day and velocity treatment (slow or fast).

#### *Dry-weight Biomass*

The final mean dry weight biomass of the fast treatment periphytometers,  $17.2 \pm 1.0 \text{ mg cm}^{-2}$ , was significantly greater ( $p \leq 0.05$ ) than the final mean biomass of the slow treatment periphytometers,  $12.1 \pm 1.1 \text{ mg cm}^{-2}$ , and was equivalent to a 29.5% greater

measured biomass per unit area than the slow treatment. As determined by linear regression, the relative growth rate of periphyton on the periphytometers was significantly greater ( $p \leq 0.001$ ) in the fast compared to the slow treatment throughout the study, with an average 30.0 % greater biomass accrual rate (Figure 8). Throughout the study, the mean dry weight biomass of the inlet periphytometers was significantly greater than outlet periphytometer biomass (Figure 9). When compared by velocity treatment, the mean dry weight biomass of the slow and fast inlet periphytometers was significantly greater than the slow and fast outlet periphytometer biomass, respectively, throughout the study except for the slow inlet vs. slow outlet on day 55 (Figure 9).

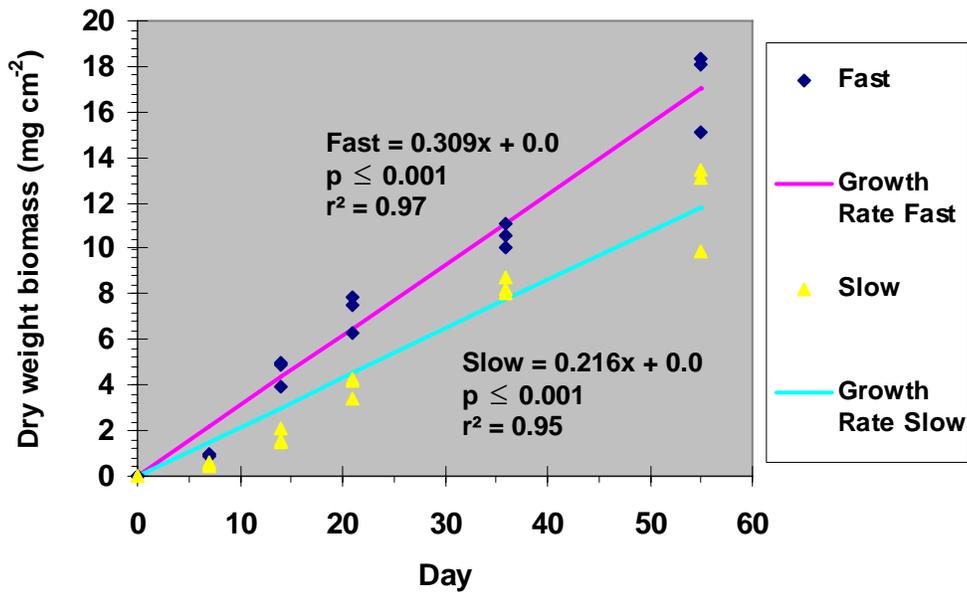


Figure 8. Periphytometer dry weight biomass accrual ( $\text{mg cm}^{-2}$ ) for days after velocity treatments began (Slow =  $0.22 \text{ cm sec}^{-1}$ , Fast =  $2.0 \text{ cm sec}^{-1}$ ).

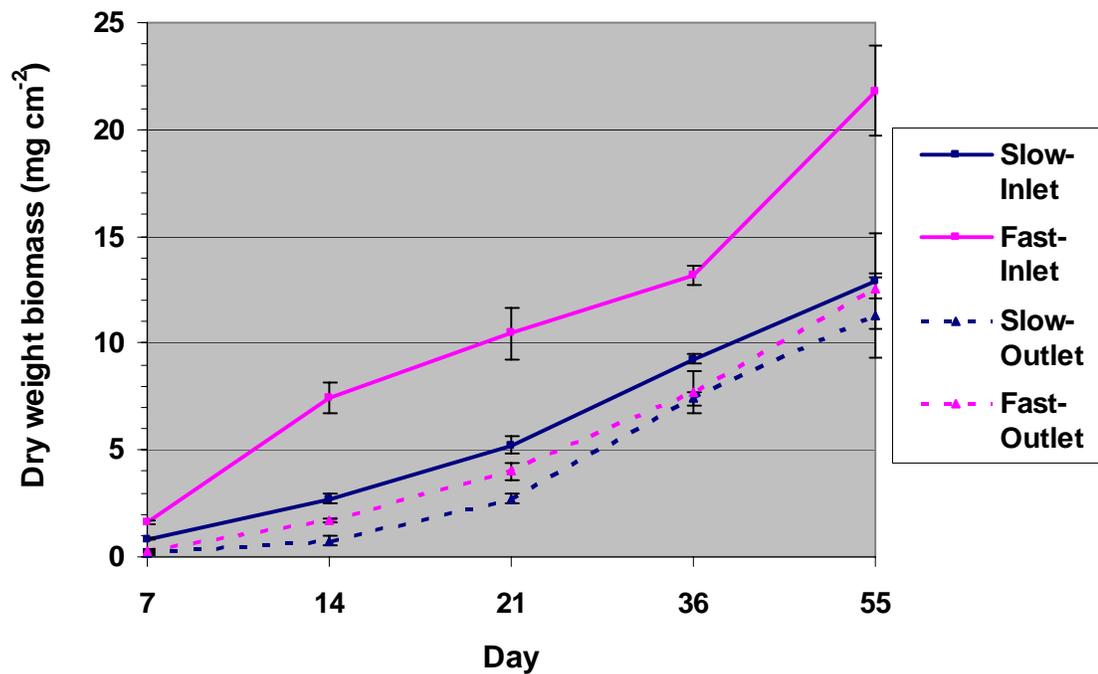


Figure 9. Mean periphytometer dry weight biomass ( $\text{mg cm}^{-2}$ ) and standard error by velocity treatment and location along treatment path (inlet or outlet).

#### *Periphyton Total Phosphorus*

There was no significant difference in overall mean periphyton TP levels between the fast and slow treatments. However, there was a significant difference between mean inlet and outlet TP concentrations on day 55, and between inlets and outlets by velocity treatment during the period from days 21 through 55 (Table 1). Mean fast outlet TP levels exceeded mean slow outlet TP levels during this period as well.

Table 1. Periphytometer mean TP concentrations ( $\mu\text{g P g}^{-1}$ ), standard errors, and p-values by velocity treatment (slow or fast), location along treatment path (inlet or outlet), and day; NS = not significant.

	Day 14	Day 21	Day 36	Day 55	Days 21-55	Mean
Slow ( $0.22 \text{ cm s}^{-1}$ )	$2,648 \pm 819$	$939 \pm 185$	$1,477 \pm 154$	$1,637 \pm 144$	$1,351 \pm 133$	$1,676 \pm 262$
Fast ( $2.0 \text{ cm s}^{-1}$ )	$2,608 \pm 624$	$1,514 \pm 327$	$1,805 \pm 81$	$1,593 \pm 109$	$1,637 \pm 111$	$1,880 \pm 201$
p-value	NS	NS	NS	NS	NS	NS
Inlets	$2,509 \pm 153$	$1,637 \pm 336$	$1,844 \pm 172$	$1,861 \pm 187$	$1,781 \pm 134$	$1,963 \pm 125$
Outlets	$2,747 \pm 963$	$816 \pm 210$	$1,338 \pm 140$	$1,370 \pm 77$	$1,208 \pm 107$	$1,593 \pm 276$
p-value	NS	NS	NS	$p \leq 0.05$	$p \leq 0.01$	NS
Slow Inlet	$2,198 \pm 37$	$1,156 \pm 277$	$1,818 \pm 348$	$1,953 \pm 256$	$1,642 \pm 193$	$1,781 \pm 160$
Slow Outlet	$3,098 \pm 1,669$	$722 \pm 392$	$1,138 \pm 78$	$1,322 \pm 129$	$1,061 \pm 150$	$1,570 \pm 458$
p-value	NS	NS	NS	NS	$p \leq 0.05$	NS
Fast Inlet	$2,820 \pm 140$	$2,118 \pm 505$	$1,870 \pm 161$	$1,769 \pm 318$	$1,919 \pm 186$	$2,144 \pm 183$
Fast Outlet	$2,397 \pm 1,313$	$911 \pm 241$	$1,740 \pm 37$	$1,418 \pm 103.7$	$1,356 \pm 143$	$1,616 \pm 329$
p-value	NS	NS	NS	NS	$p \leq 0.05$	NS
Slow Inlet	$2,198 \pm 37$	$1,156 \pm 277$	$1,818 \pm 348$	$1,953 \pm 256$	$1,642 \pm 193$	$1,781 \pm 160$
Fast Inlet	$2,820 \pm 140$	$2,118 \pm 505$	$1,870 \pm 161$	$1,769 \pm 318$	$1,919 \pm 186$	$2,144 \pm 183$
p-value	$p \leq 0.05$	NS	NS	NS	NS	NS
Slow Outlet	$3,098 \pm 1,669$	$722 \pm 393$	$1,138 \pm 78$	$1,322 \pm 129$	$1,061 \pm 150$	$1,570 \pm 458$
Fast Outlet	$2,397 \pm 1,313$	$911 \pm 241$	$1,740 \pm 37$	$1,418 \pm 104$	$1,356 \pm 143$	$1,616 \pm 329$
p-value	NS	NS	$p \leq 0.01$	NS	NS	NS

There was a decline in mean TP concentrations in all samples between days 14 and 21 followed by a general recovery and stabilization period over time. The linear regression of log transformed periphyton TP levels against log transformed dry weight biomass (Figure 10) indicates that as biomass accumulates, periphyton TP levels per dry weight decrease.

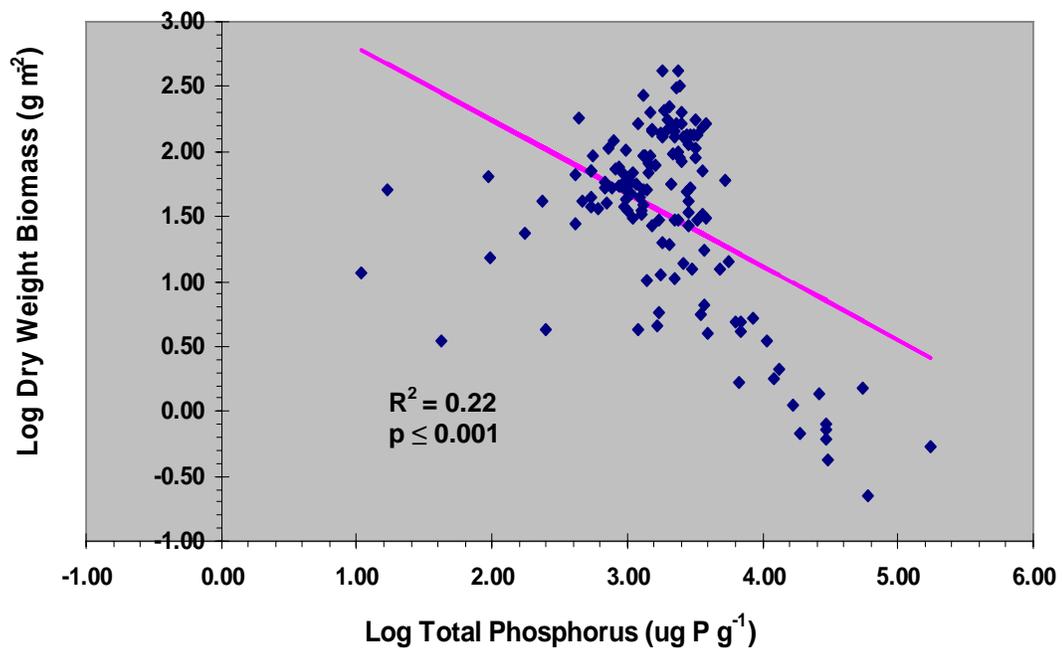


Figure 10. Linear regression of periphyton TP ( $\mu\text{g P g}^{-1}$ ) vs. dry-weight biomass ( $\text{g m}^{-2}$ )

### *Water Column Total Phosphorus*

During the period, from March 2002 through May 2002, the monthly flow-weighted mean inflow concentration of surface water TP at S5A in the northern end of STA-1W ranged from  $106 \mu\text{g L}^{-1}$  to  $110 \mu\text{g L}^{-1}$ , while the monthly flow-weighted mean outflow TP concentration at G-251 in the south end of the ENRP (where the study site is located) ranged from  $20.0 \mu\text{g L}^{-1}$  to  $21.0 \mu\text{g L}^{-1}$  (SFWMD 2002). Concentrations of TP in water supplying the mesocosms, measured at SFWMD monitoring station ENR012 located just upstream of the G251 ENR outflow pump station, ranged from  $18.0 \mu\text{gL}^{-1}$  to  $45.0 \mu\text{gL}^{-1}$  and averaged  $25.0 \pm 2.8 \mu\text{gL}^{-1}$  throughout the study (SFWMD 2003c). Mean water column TP concentrations of samples taken from mesocosm inlet manifolds ranged from  $12.0 \mu\text{gL}^{-1}$  to  $25.1 \mu\text{gL}^{-1}$  and averaged  $20.7 \pm 2.9 \mu\text{gL}^{-1}$  throughout the study (Table 2).

There was no significant difference in mean water column TP concentrations between slow and fast velocity mesocosm samples or between inlet and outlet samples through the course of the study except on day 36, when the mean TP concentration of the fast inlet samples was significantly higher than the fast outlet samples. Fast outlet samples exhibited an increasingly greater percent decrease in TP concentration compared to the fast inlet samples as the study progressed (3% on day 13 to 23% on day 55), averaging an overall 14% decrease between the two (Table 2, Figure 11).

Table 2. Mesocosm mean water column TP concentrations ( $\mu\text{g L}^{-1}$ ), standard errors, and p-values by velocity treatment (slow or fast), location along treatment path, and day; NS = not significant.

	Day 13	Day 29	Day 36	Day 55	Mean
Slow ( $0.22 \text{ cm s}^{-1}$ )	$20.4 \pm 0.3$	$12.2 \pm 1.5$	$24.6 \pm 1.5$	$25.4 \pm 0.7$	$20.7 \pm 3.0$
Fast ( $2.0 \text{ cm s}^{-1}$ )	$21.6 \pm 1.9$	$12.1 \pm 0.4$	$22.9 \pm 0.7$	$23.0 \pm 0.7$	$19.9 \pm 2.6$
p-value	NS	NS	NS	NS	NS
Inlets	$20.9 \pm 1.8$	$12.3 \pm 0.9$	$24.8 \pm 0.20$	$24.6 \pm 0.5$	$20.7 \pm 2.9$
Outlets	$21.0 \pm 0.4$	$12.1 \pm 1.0$	$24.1 \pm 2.1$	$22.8 \pm 4.2$	$20.0 \pm 2.7$
p-value	NS	NS	NS	NS	NS
Slow Inlet	$19.8 \pm 0.8$	$12.0 \pm 1.5$	$24.7 \pm 0.3$	$25.1 \pm 0.8$	$20.4 \pm 3.0$
Slow Outlet	$20.8 \pm 0.8$	$12.4 \pm 1.5$	$24.6 \pm 2.9$	27.0	$21.2 \pm 3.2$
p-value	NS	NS	NS	NS	NS
Fast Inlet	$22.0 \pm 3.8$	$12.6 \pm 1.2$	$25.0 \pm 0.2$	$24.1 \pm 0.7$	$20.9 \pm 2.8$
Fast Outlet	$21.3 \pm 0.1$	$11.7 \pm 1.7$	$20.9 \pm 1.3$	18.6	$18.1 \pm 2.2$
p-value	NS	NS	$p \leq 0.05$		NS
Slow Inlet	$19.8 \pm 0.8$	$12.0 \pm 1.5$	$24.7 \pm 0.3$	$25.1 \pm 0.8$	$20.4 \pm 3.0$
Fast Inlet	$22.0 \pm 3.8$	$12.6 \pm 1.2$	$25.0 \pm 0.2$	$24.1 \pm 0.7$	$20.9 \pm 2.8$
p-value	NS	NS	NS		NS
Slow Outlet	$20.8 \pm 0.8$	$12.4 \pm 1.5$	$24.6 \pm 2.9$	27.0	$21.2 \pm 3.2$
Fast Outlet	$21.3 \pm 0.1$	$11.7 \pm 1.7$	$20.9 \pm 1.3$	18.6	$18.1 \pm 2.2$
p-value	NS	NS	NS		NS

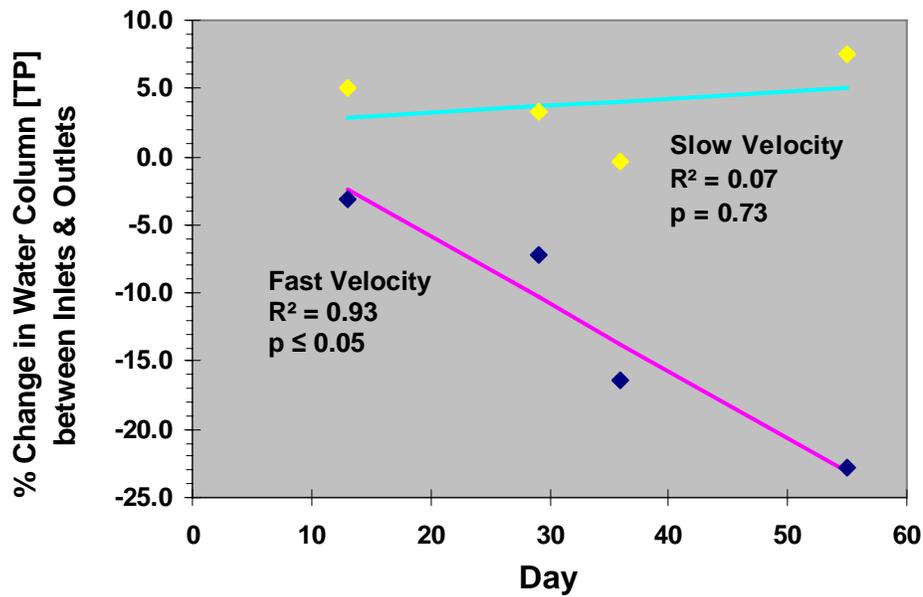


Figure 11. Linear regression of percent change in water column TP concentrations over time by velocity treatment.

#### *Taxonomic Richness, Abundance and Diversity*

A total of 117 genera were identified during taxonomic analysis. Bacillariophyta (diatoms) were represented by 40 genera and non-siliceous algae were represented by 77 genera (Appendix B). Among the algal divisions, diatoms had the highest relative abundance within both the slow and fast velocity treatment assemblages, as well as inlet and outlets assemblages, with the next highest relative abundances represented by the cyanophytes and chlorophytes, respectively (Table 3). The mean diatom relative abundance was highest in the fast velocity samples (65.38%) and conversely, the mean total soft algae relative abundance was highest in the slow velocity samples (37.72%).

Table 3. Summary of mean relative abundances (%) of algal divisions by velocity treatment (slow or fast), and location along treatment path (inlet or outlet).

	# of genera	SLOW (0.22 cm s <sup>-1</sup> )	FAST (2.0 cm s <sup>-1</sup> )	INLETS	OUTLETS
Mean Relative Abundance (%)					
Bacillariophyta	40	62.28	65.38	62.71	64.93
Soft Algae Total		37.72	34.62	37.29	35.07
Chlorophyta	36	10.74	8.09	8.76	9.86
Cyanophyta	30	26.15	25.89	27.70	24.55
Euglenophyta	5	0.13	0.12	0.15	0.10
Cryptophyta	2	0.00	0.002	0.00	0.002
Pyrrophyta	2	0.03	0.02	0.03	0.03
Chrysophyta	1	0.03	0.04	0.03	0.04
Xanthophyta	1	0.02	0.03	0.02	0.03
Misc. Unknowns		0.63	0.43	0.60	0.45

The highest total richness (112 genera) was found in the slow velocity samples (Table 4). Soft algae richness in fast velocity samples exceeded slow velocity richness on all collection days except for day 21 (Figure 12). Total soft algae richness increased from 59 genera on day 7 to a maximum richness of 65 genera at the end of the study period, day 55. In contrast, diatom richness in slow velocity samples exceeded fast velocity richness on all collection days except for day 21 (Figure 12). These changes in richness for both diatoms and soft algae on day 21 coincide with the beginning of a sharp decline in diatom relative abundance and a sharp increase in cyanobacteria relative abundance beginning on that day (Figure 13). Stevenson and Bahl (1999) noted that reduction in live diatoms can be due to the accumulation of heavy sedimentation and

Table 4. Summary of similarity and diversity indices and p-values by velocity treatment (slow or fast) and location along treatment path (inlet or outlet).

	Diatom Richness	Diatom Diversity*	Diversity p-value	Diatom SSI (%)	Soft Algae Richness	Soft Algae Diversity*	Diversity p-value	Soft Algae SSI (%)
Slow	40	0.92			72	1.33		
Fast	37	0.91	NS	96.1	70	1.34	NS	93.0
Slow Day 7	40	0.89			55	1.26		
Fast Day 7	35	0.85	≤ <b>0.01</b>	93.3	56	1.25	NS	88.3
Slow Day 14	33	0.79			52	1.28		
Fast Day 14	32	0.83	≤ <b>0.01</b>	90.6	55	1.29	NS	89.7
Slow Day 21	33	0.82			56	1.27		
Fast Day 21	34	0.87	≤ <b>0.01</b>	92.5	55	1.26	NS	86.5
Slow Day 36	36	0.93			57	1.27		
Fast Day 36	35	0.92	NS	95.8	59	1.31	≤ <b>0.01</b>	91.4
Slow Day 55	36	1.01			57	1.24		
Fast Day 55	31	0.97	≤ <b>0.01</b>	90.9	62	1.29	≤ <b>0.01</b>	87.4
Inlet	37	0.92			72	1.33		
Outlet	40	0.90	≤ <b>0.01</b>	96.1	68	1.34	NS	94.3
Slow Inlet	37	0.92			67	1.32		
Fast Inlet	37	0.92	NS	97.3	63	1.33	NS	86.2
Slow Outlet	39	0.90			63	1.34		
Fast Outlet	36	0.89	NS	93.3	66	1.33	NS	85.3
Slow Inlet	37	0.92			67	1.32		
Slow Outlet	39	0.90	≤ <b>0.05</b>	94.7	63	1.34	≤ <b>0.05</b>	87.7
Fast Inlet	37	0.92			63	1.33		
Fast Outlet	36	0.89	≤ <b>0.01</b>	95.9	66	1.33	NS	91.5

NS = not significant

\* Shannon-Weaver index, H' (Shannon and Weaver 1949)

SSI = Sørensen's Similarity Index (Odum 1971, Lincoln *et al.* 1998, Vymazal 1988)

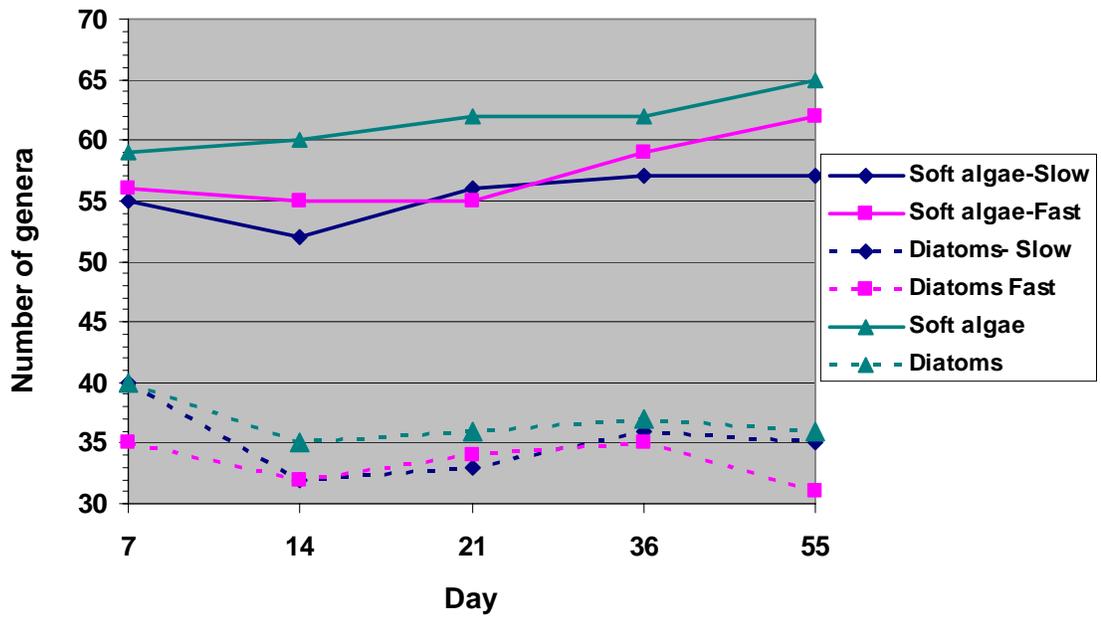


Figure 12. Overall generic richness of diatoms and soft algae by day and generic richness of slow and fast velocity samples by day.

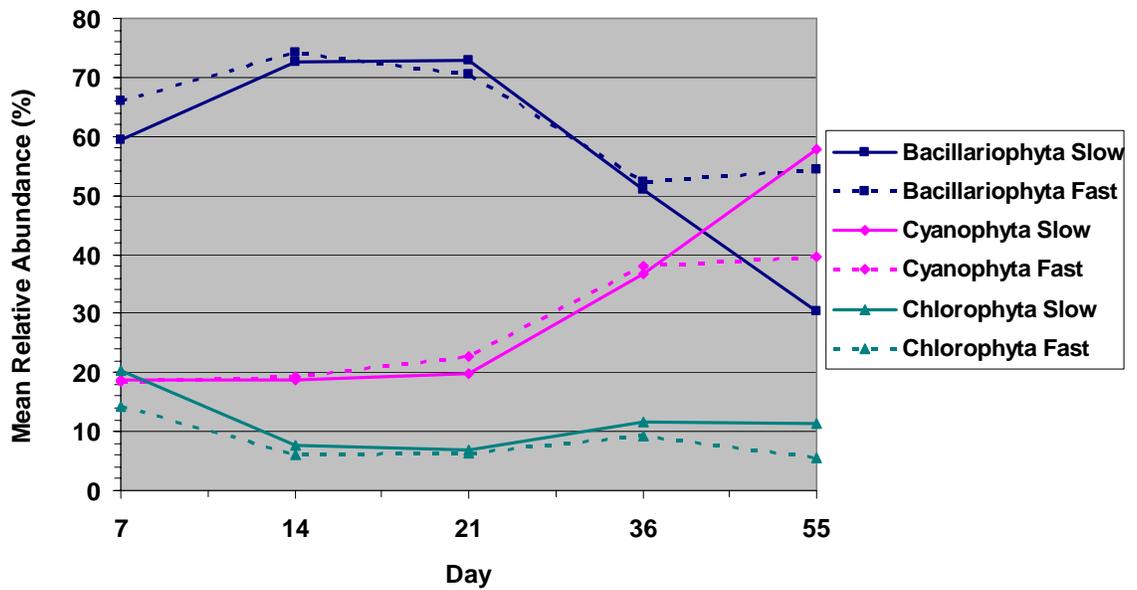


Figure 13. Mean relative abundance (%) of major algal divisions by velocity treatment (slow or fast) and day.

increased aging and senescence of cells as algal biomass accrues on substrates. Total diatom richness decreased from maximum richness of 40 genera on day 7 to 36 genera at the end of the study period.

Both diatoms and soft algae showed a very high degree of similarity (86.5 % to 96.1%) between the slow and fast velocity samples and between inlet and outlet samples (Table 4). The ten dominant (> 1.0 % relative abundance) diatom genera and nineteen dominant soft algae genera identified in the samples were the same for the slow and fast velocity samples, as well as the inlet and outlet samples (differing only in relative abundance) and accounted for 94% to 96% and 83% to 85%, respectively, of the total composition of each group (Table 5). Dominant, as well as less dominant (< 1.0 % relative abundance) genera that have been referenced in the literature as indicators of nutrient enrichment (Appendix C) are denoted in the table.

Of both the dominant and non-dominant genera listed in Table 5, 12 of 13 diatom genera and 18 of 25 soft algae genera had significant differences in mean relative abundance between either the velocity treatments, the location along the treatment path (inlet vs. outlet) or the combination of velocity treatment and inlets vs. outlets. There was no significant difference between overall diatom or soft algae taxonomic diversity between the slow and fast velocity samples (Table 4). When compared by day, there were some significant differences in diatom and soft algae diversity by velocity treatment, but different overall values were not large and there was no distinct pattern in diversity by day (Table 4).

Table 5. Mean relative abundances (% RA) and p-values for dominant and indicator genera by velocity treatment and inlet / outlet.

Diatoms	I <sup>B</sup>	SLOW	FAST	% diff.	p-value	INLETS	OUTLETS	% diff.	p-value	SLOW	SLOW	% diff.	p-value	FAST	FAST	% diff.	p-value
		0.22 cm s <sup>-1</sup>	2.0 cm s <sup>-1</sup>							INLETS	OUTLETS			INLETS	OUTLETS		
<b>&gt; 1% RA</b>																	
<i>Fragilaria/Synedra</i>	E/O†	26.2	25.0	-4.7	NS	26.1	24.6	-5.4	NS	27.0	25.4	-6.0	0.04	25.7	24.2	-6.0	NS
<i>Nitzschia</i>	E‡	23.1	25.4	9.2	NS	27.7	21.0	-24.1	0.00	26.6	19.5	-26.6	0.00	29.1	21.8	-25.0	NS
<i>Brachysira</i>	O*	18.4	16.4	-11.0	0.03	13.3	20.5	35.1	0.00	15.2	21.7	29.7	0.00	12.5	20.3	38.5	0.00
<i>Achnanthisidium</i>	E/O	9.3	7.7	-17.9	NS	7.4	10.4	28.8	0.01	6.8	11.9	-42.8	0.00	7.1	8.3	14.5	NS
<i>Encyonema</i>	O	5.5	7.5	26.9	0.00	5.4	7.7	30.7	0.00	5.0	6.0	17.0	0.01	5.5	9.6	42.9	0.00
<i>Encyonopsis</i>	O	4.1	4.4	6.8	NS	3.2	5.1	36.4	0.00	3.6	4.6	22.8	0.03	2.9	5.9	51.5	0.00
<i>Navicula</i>	E	3.0	4.4	32.5	0.00	5.4	1.9	-64.1	0.00	4.1	1.9	-54.7	0.00	6.7	2.1	-69.2	0.00
<i>Achnanthes</i>	E/O	2.5	2.1	-17.5	NS	2.6	2.1	-17.7	NS	2.7	2.4	-11.6	NS	2.3	1.9	-19.2	NS
<i>Gomphonema</i>	E	2.0	2.2	6.7	NS	2.5	1.8	-28.7	0.02	2.3	1.8	-24.5	0.03	2.5	1.8	-27.5	NS
Total		94.2	95.0			93.6	95.2			93.3	95.1			94.3	95.8		
<b>Diatoms &lt; 1% RA</b>																	
<i>Amphora</i>	E/O	0.65	0.40	-38.4	0.03	0.57	0.49	-12.9	NS	0.69	0.62	-10.2	NS	0.44	0.36	-18.8	NS
<i>Cyclotella</i>	E	0.56	0.43	-23.5	0.01	0.60	0.39	-34.2	0.00	0.67	0.45	-32.4	0.00	0.53	0.33	-36.7	0.02
<i>Rhopalodia</i>	E	0.30	0.13	-56.4	0.05	0.25	0.19	-25.7	NS	0.38	0.23	-40.7	NS	0.12	0.15	18.1	NS
<i>Mastogloia</i>	E/O	0.29	0.27	-7.0	NS	0.28	0.28	0.0	NS	0.38	0.19	-49.0	0.01	0.19	0.36	47.3	0.00
<b>Soft Algae</b>																	
<b>&gt; 1% RA</b>																	
<i>Leptolyngbya</i>	E/O	12.5	12.6	0.4	NS	13.6	11.5	-15.8	NS	14.3	10.8	-24.6	0.05	13.0	12.2	-6.2	NS
<i>Pseudanabaena</i>	E/O	12.0	11.8	-2.0	NS	11.5	12.3	6.0	NS	12.0	12.0	0.2	NS	11.0	12.5	12.0	NS
<i>Limnothrix</i>	E/O	8.7	6.4	-26.6	0.00	7.4	7.7	4.1	NS	8.8	8.6	-3.0	NS	5.9	6.8	13.2	NS
<i>Oscillatoria</i>	E/O	6.4	7.3	-12.4	NS	7.8	5.9	-23.8	0.02	6.7	6.1	-9.3	NS	8.8	5.8	-34.8	0.01
<i>Synechocystis</i>		5.6	6.1	8.1	NS	5.2	6.4	18.3	0.01	4.5	6.6	31.4	0.00	5.9	6.2	4.3	NS
<i>Spirogyra</i>	E	4.8	3.4	-30.1	0.03	4.8	3.4	-28.1	0.02	5.9	3.7	-37.2	0.02	3.6	3.1	-13.3	NS
<i>Kirchneriella</i>		4.5	3.9	-14.5	0.01	2.4	5.9	58.7	0.00	3.0	6.0	49.3	0.00	1.9	5.9	68.3	0.00
<i>Aphanocapsa</i>	O	4.0	5.0	19.7	0.02	4.6	4.5	-1.4	NS	4.3	3.8	-11.8	NS	4.9	5.2	7.3	NS
<i>Oocystaceae</i> <sup>A</sup>		3.4	2.3	-32.0	0.00	2.8	3.0	7.3	NS	3.3	3.6	6.6	NS	2.2	2.4	8.3	NS
<i>Lyngbya</i>	E	3.4	3.7	9.8	NS	4.2	2.9	-31.5	0.00	3.6	3.1	-14.7	NS	4.8	2.7	-44.3	0.00
<i>Chlamydomonas</i>	E	2.7	1.7	-35.5	NS	2.2	2.3	3.3	NS	2.5	2.9	13.0	NS	1.9	1.6	-12.3	NS
<i>Anabaena</i>	E	2.7	3.8	29.1	0.03	3.4	3.1	-8.4	NS	2.6	2.8	8.1	NS	4.2	3.4	-19.0	NS
<i>Chroococcus</i>	O	2.6	3.0	14.9	NS	2.7	2.9	7.0	NS	2.6	2.6	0.0	NS	2.9	3.2	11.3	NS
<i>Planktolingbya</i>		2.6	2.5	-1.3	NS	3.0	2.1	-29.8	NS	2.8	2.3	-16.0	NS	3.2	1.9	-41.9	NS
<i>Merismopedia</i>	O	2.6	2.6	1.0	NS	2.1	3.1	33.3	0.00	2.2	2.9	23.8	NS	1.9	3.3	41.8	0.01
<i>Monoraphidium</i>		2.3	1.9	-15.9	NS	1.9	2.3	17.4	NS	2.0	2.6	25.2	NS	1.8	2.0	7.2	NS
<i>Scenedesmus</i>	E	2.1	1.9	-9.3	NS	1.7	2.3	27.2	0.00	1.9	2.3	18.7	NS	1.5	2.3	35.8	0.00
<i>Aphanothece</i>	E/O	1.6	1.4	-11.2	NS	1.7	1.4	-18.7	NS	1.8	1.4	-25.3	NS	1.5	1.4	-10.7	NS
<i>Cylindrospermum</i>		0.1	1.2	95.6	NS	1.1	0.1	-89.8	NS	0.04	0.06	36.0	NS	2.2	0.2	-90.9	NS
Total		84.6	82.6			84.1	83.1			85.0	84.1			83.2	82.1		

Table 5 cont'd. Summary of mean relative abundances (%) and p-values for dominant and indicator genera by velocity and inlet / outlet.

Soft Algae	I	SLOW				FAST				SLOW		FAST		FAST			
		INLETS	OUTLETS	% diff.	P-value	INLETS	OUTLETS	% diff.	P-value	INLETS	OUTLETS	% diff.	P-value	INLETS	OUTLETS	% diff.	P-value
<b>&lt; 1% RA</b>																	
<i>Mougeotia</i>	O	0.66	0.70	6.3	NS	0.53	0.83	36.7	0.01	0.50	0.81	38.7	NS	0.55	0.85	34.8	NS
<i>Ankistrodesmus</i>	E	0.64	0.42	-33.9	0.01	0.52	0.54	4.1	NS	0.66	0.62	-5.9	NS	0.38	0.46	18.0	NS
<i>Chlorella</i>	E	0.60	0.59	-2.4	NS	0.71	0.48	-32.8	NS	0.63	0.58	-7.7	NS	0.80	0.38	-52.5	0.04
<i>Staurastrum</i>	E	0.39	0.42	5.9	NS	0.38	0.43	12.6	NS	0.42	0.37	-12.7	NS	0.34	0.50	32.6	0.02
<i>Closterium</i>	E	0.29	0.48	39.6	0.04	0.56	0.21	-62.4	0.00	0.37	0.22	-40.9	NS	0.76	0.21	-72.8	0.00
<i>Pediastrum</i>	E	0.10	0.13	22.5	NS	0.09	0.15	44.3	0.03	0.11	0.10	-4.1	NS	0.06	0.21	68.6	0.00

† E/O = Eutrophic and/or oligotrophic indicator

‡ E = Eutrophic indicator

\* O = Oligotrophic indicator

NS = Not significant

Significant at  $p \leq 0.05$

<sup>A</sup> Oocystaceae includes equivocal identifications of the genera *Monoraphidium*, *Quadrigula*, and *Selenastrum*

<sup>B</sup> See Appendix C for a summarized list of indicator taxa and references

Although there were some significant differences in diversity among diatoms and soft algae when comparing inlet and outlet samples, as well as inlets and outlets among velocity treatments, absolute differences in diversity values were exceedingly small and likely were not ecologically meaningful (Table 4).

### *Ordination Analysis*

Ordination analyses were conducted on the total community data set (diatoms and soft algae combined), as well as on the separate diatom and soft algae data, with similar results. The Non-Metric Multidimensional Scaling (NMS) ordination analyses indicated a stable periphyton community structure exhibiting the strongest correlations with day and biomass (Figure 14). Scree-plot analysis showed that a two-dimensional solution was best for the data, as adding more dimensions or axes yielded only slight reductions in stress. Biomass ( $r^2 = 0.57$ , axis 2) and day of sampling ( $r^2 = 0.57$ , axis 2), captured the majority of the taxonomic variance in the periphyton communities. These results are most likely a reflection of taxonomic compositional variation due to colonization and biomass accrual within the mesocosms over time.

Water column temperature exhibited the next strongest correlation ( $r^2 = 0.35$ ) with the vector being driven along axis 1 by the increasing temperatures measured as the study progressed. Though there was a significant difference in mean pH ( $p \leq 0.05$ ) between both the slow and fast velocity data, and the inlet and outlet data during the study period, ordination analysis did not yield a strong correlation with this variable. Thus, it is difficult to make inferences about the ecological significance of pH as it relates

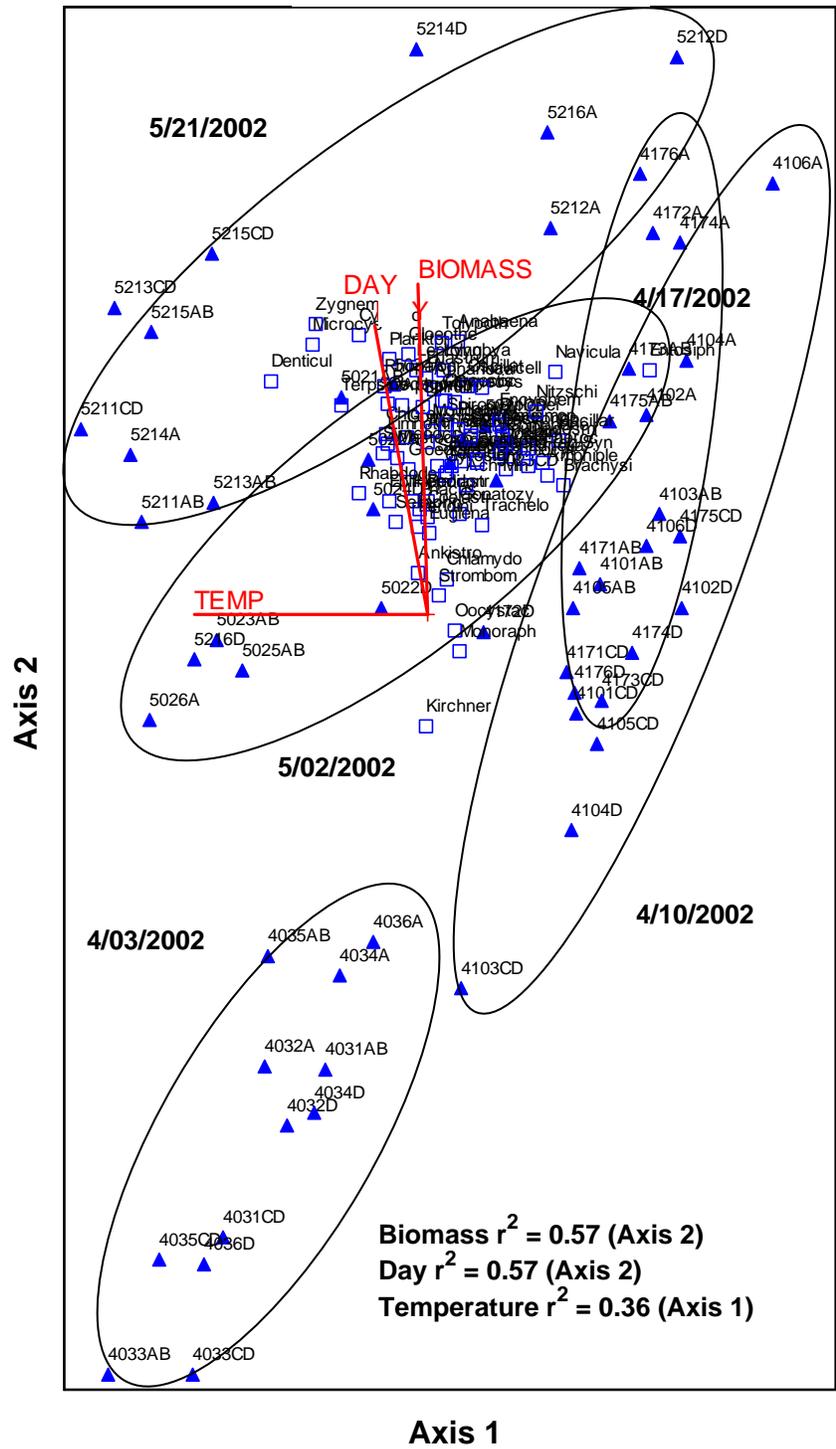


Figure 14. NMS ordination biplot of periphyton sample units and generic composition.

to taxonomic composition in this study. Because distances between the genera points on the joint plot are approximately proportional to the dissimilarities between the samples (McCune and Grace 2002), the very tight arrangement of this data set reflects the high degree of similarity substantiated by both the diversity and similarity indices.

### *Indicator Genera Analysis*

Examination of the mean relative abundances of the inlet and outlet samples in Table 5 yielded a striking pattern related to their documented sensitivity to nutrient enrichment. With the exception of two green algae genera, *Scenedesmus* and *Staurastrum*, the significant eutrophic indicator genera of both diatoms and soft algae listed in Table 5 exhibited higher mean relative abundances (mean difference of 37.8 % and 32.7%, respectively) in the inlet samples than the outlet samples. All the significant oligotrophic indicator genera exhibited the opposite pattern; with higher mean relative abundances for both diatoms and soft algae (mean difference of 34.1% and 35.0%, respectively) in the outlet samples (Figures 15 and 16). These patterns also held true when examining the mean relative abundances of the inlet and outlet samples by velocity. Of those genera considered more cosmopolitan in their pollution tolerance (Lowe 1974, VanLandingham 1982, McCormick *et al.* 2000), the *Fragilaria* and *Synedra* diatom genera and the cyanobacteria genus *Oscillatoria* exhibited the eutrophic pattern, while the diatom genus *Achnanthydium* exhibited the oligotrophic pattern. These deviations from expected patterns may be due to the fact that taxonomic analysis in this study was

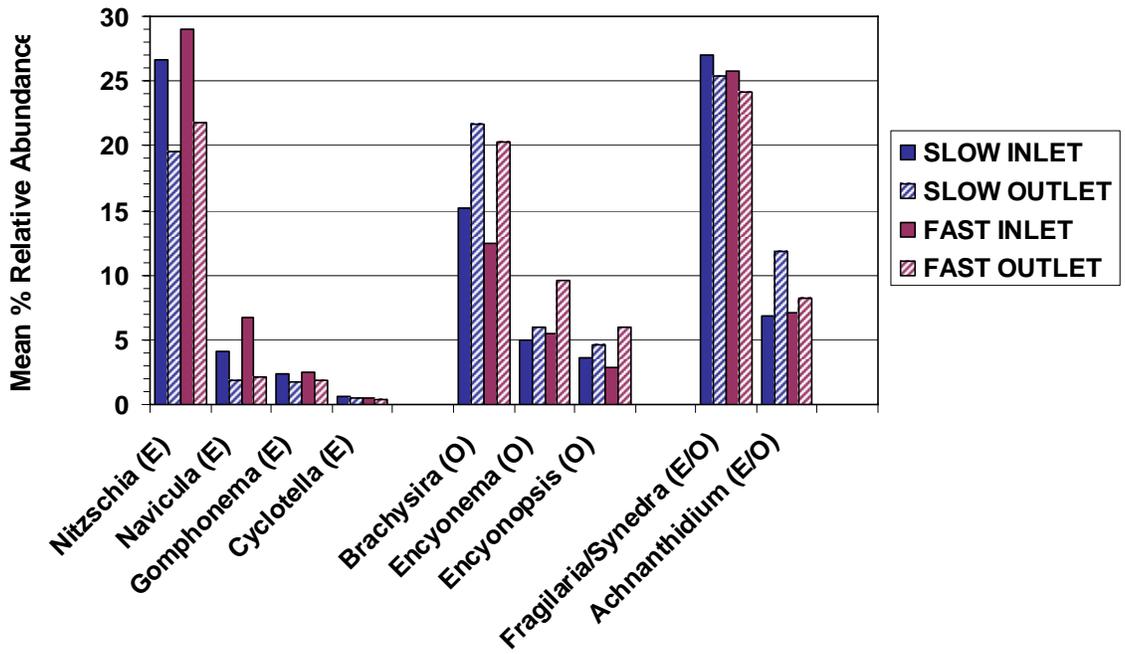
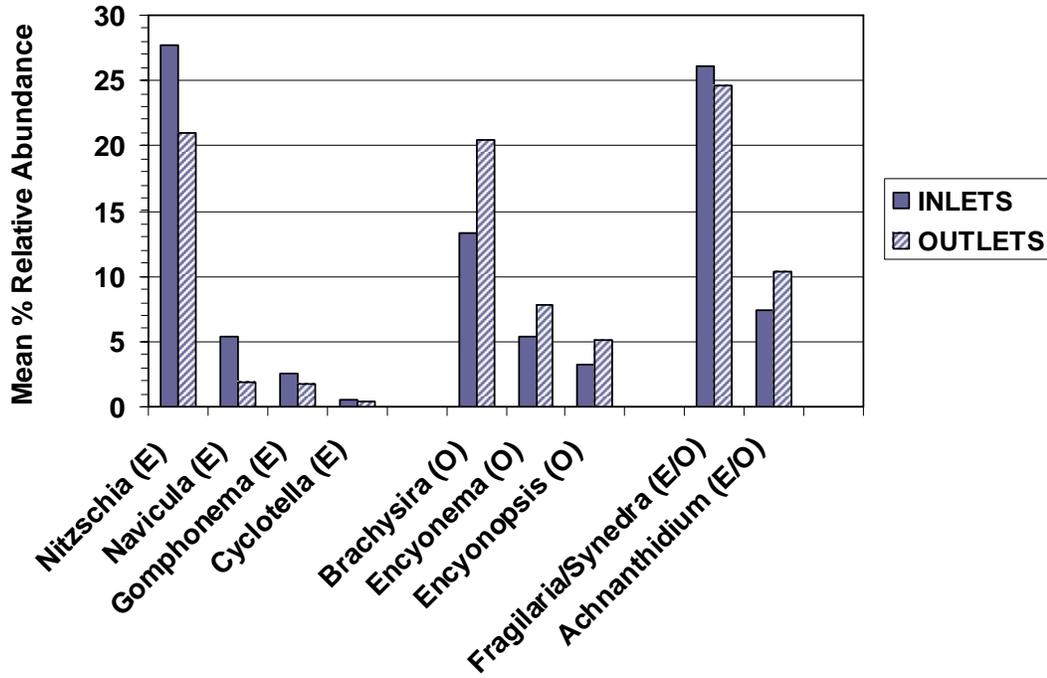


Figure 15. Mean relative abundance (%) of significant diatom indicator genera by velocity treatment and location along treatment path (inlet or outlet). E = eutrophic; O = oligotrophic; E/O = eutrophic/oligotrophic.

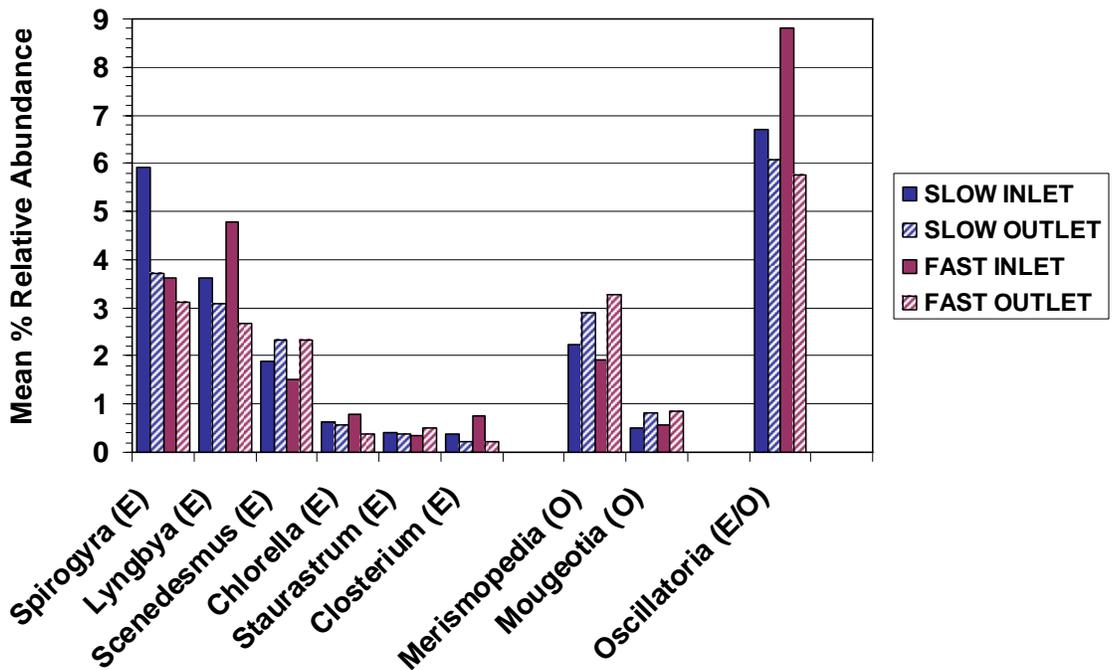
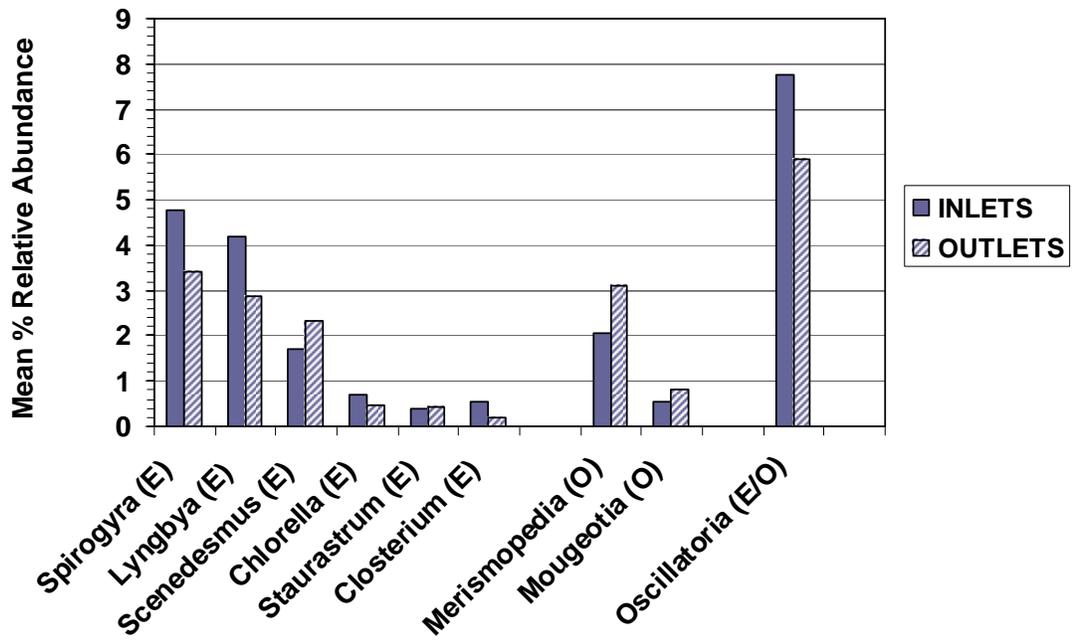


Figure 16. Mean relative abundance (%) of significant soft algae indicator genera by velocity treatment and location along treatment path (inlet or outlet). E = eutrophic; O = oligotrophic; E/O = eutrophic/oligotrophic.

conducted at the genus level and species-specific differences in P uptake cannot be detected nor interpreted. In the case of *Scenedesmus*, *Pediastrum* and *Staurastrum*, interpretation is difficult due to the low relative abundance of these genera in this assemblage. There was no apparent pattern of differences in mean relative abundances of eutrophic and oligotrophic genera between the slow and fast velocity samples.

Of the genera listed in Table 5, nine potential diatom and nine potential soft algae indicator genera related to velocity, distance along the treatment path and/or periphyton TP were elucidated by PC-ORD indicator analysis (Table 6). When examined by their trophic categorization, the inlet and outlet diatom genera exhibited the same pattern seen above. The ordination analysis yielded an inconsistent pattern in comparing the slow and fast velocity composition. Of those indicator genera cited in the literature, one diatom genus and seven filamentous soft algae genera were elucidated as significant indicators of periphyton TP concentrations (Table 6).

Table 6. PC-ORD indicator analysis results for significant genera from Table 5.

<b>Diatom genera</b>	<b>I</b>	<b>Slow</b>	<b>Fast</b>	<b>Inlets</b>	<b>Outlets</b>	<b>Tissue TP</b>
<i>Achnanthes</i>	E/O		*			
<i>Amphora</i>	E/O			*		
<i>Cyclotella</i>	E			†		
<i>Encyonema</i>	O		†		†	
<i>Encyonopsis</i>	O		*		*	
<i>Fragilaria/Synedra</i>	E/O		†	*		
<i>Gomphonema</i>	E		†	†		
<i>Navicula</i>	E		†	†		C
<i>Nitzschia</i>	E		†	*		
<b>Soft Algae genera</b>						
<i>Aphanocapsa</i>	O					B
<i>Closterium</i>	E		*			
<i>Leptolyngbya</i>	E/O					B
<i>Limnothrix</i>	E/O					B
<i>Lyngbya</i>	E					B
<i>Merismopedia</i>	O				*	
<i>Mougeotia</i>	O					B
<i>Oocystaceae</i> <sup>1</sup>		*		*		
<i>Oscillatoria</i>	E/O					C
<i>Spirogyra</i>	E					A

\*  $p \leq 0.05$

†  $p \leq 0.01$

A  $p \leq 0.05$  at  $500-999 \mu\text{g P g}^{-1}$

B  $p \leq 0.05$  at  $1000-1999 \mu\text{g P g}^{-1}$

C  $p \leq 0.05$  at  $2000-4999 \mu\text{g P g}^{-1}$

I = trophic indicator:

E = Eutrophic

O = Oligotrophic

E/O = Eutrophic and/or oligotrophic

<sup>1</sup> Oocystaceae includes equivocal identifications of the genera *Monoraphidium*, *Quadrigula*, and *Selenastrum*

## DISCUSSION AND CONCLUSIONS

There was no significant difference between overall diatom or soft algae taxonomic diversity between the slow and fast velocity samples in this study. This finding is consistent with the high degree of similarity in diversity and composition between the two velocity treatments as determined by the Sørensen's similarity index (86.5 to 96.1%). These results do not support the first hypothesis that a ten-fold increase in water velocity between treatments will result in a significant change in the algal genera taxonomic composition and dominant taxa in the periphyton communities of the fast treatment compared to the slow treatment.

There were, however, small, but significant differences in diversity observed between slow and fast velocity samples analyzed by date of collection, beginning with day 7 in the diatoms and day 36 in the soft algae (Table 4). These differences are most likely a reflection of small variations in relative abundances due to difference in algal life cycles, autogenic changes and colonization strategies that determine development of a stable community structure over time (Steinman and McIntire 1986, McCormick and Stevenson 1991, Lowe *et al.* 1996). Diatoms often attach first to bare substrates and colonize rapidly, as each diatom frustule is a potential colonist capable of immediate attachment and reproduction. Non-siliceous algae, particularly filamentous taxa, need time to reproduce by binary fission or to produce mature filaments and zoospores

(Stevenson 1990) and thus, take longer to develop into a mature community. Sixty days or less has often been cited in the literature as sufficient time for stochastic colonization and community development of periphyton communities (McIntire 1966a, Lowe *et al.* 1996, McCormick *et al.*, 1996, APHA 1998, Barbour *et al.* 1999). Other studies suggest that it may take as long as one year for community stabilization (Vymazal 2003). Such a determination about the periphyton assemblages growing within the mesocosms used in this study would require longer colonization times and subsequent taxonomic analysis. Under high velocities such as those found in lotic systems, algal species vary in their ability to take advantage of the positive effects of water movement and exhibit differential nutrient uptake responses (Whitford and Schumacher 1964). In this study, the treatment flow rates, which are within the range found for both the STAs and the natural Everglades marsh communities (Walker 1999, Ball and Schaffranek 2000), were not high enough to significantly affect algal composition to the degree observed in other studies conducted at higher velocities.

The development and abundance of metaphyton (floating algal mats) in shallow wetlands is influenced by numerous factors including irradiance, wind, precipitation, flow, photosynthetic activity, and nutrient conditions (Wetzel 1996). Metaphytic algal communities are a dominant component of the Everglades ecosystem and create shaded microhabitats that may affect local primary productivity, nutrient cycling, and assemblage development (Browder *et al.* 1994, McCormick *et al.* 2001). The variation in metaphyton distribution and abundance within the mesocosm channels in this study, as noted by visual observation and photographic documentation, was apparent but not

quantified. Determination of the shading effects of metaphyton on the taxonomic composition of the mesocosms was beyond the scope of this study.

Even though there were slight differences in the periphyton taxonomic composition between the two velocity treatment samples, there was a large significantly different response in periphyton growth. Periphyton biomass accrued at a 30.0% faster rate and resulted in a 29.5% greater measured final dry weight biomass per unit area in the fast treatment mesocosms compared to the slow treatment mesocosms. These results support the fourth hypothesis that a ten-fold increase in water velocity between treatments would result in a statistically significant greater rate of periphyton dry weight biomass accrual in the fast treatment compared to the slow treatment over the test period. These findings are consistent with numerous studies showing greater biomass accumulation at higher velocities in many periphyton communities due to the stimulatory effect of water current (Horner *et al.* 1990, Stevenson 1996b, Simmons 2001). These effects include: enhancement of metabolic processes such as photosynthesis and respiration (Whitford and Schumacher 1964, McIntire 1966b, Lock and John 1979); increased rates of nutrient utilization from mixing effects due to reduction in thickness of the relatively stagnant and nutrient depleted laminar boundary layer around the algal cell surface, steepening of the diffusion gradient and hence, increase in the rate of molecular diffusion across boundary layer (Whitford and Schumacher 1964, McIntire 1966b, Stevenson 1983, Horner and Welch 1981, Horner *et al.* 1983, Stevenson and Glover 1993, Borchardt 1996); improved utilization of substrate for attachment and colonization (McIntire 1966a); and possible higher immigration rates and colonization rates during

community development (McCormick and Stevenson 1991).

Changes in periphyton assemblages have been correlated with both water column and periphyton TP gradients, and periphyton TP has been proposed as a valuable early warning indicator of trophic changes within the Everglades (McCormick and Stevenson 1998, Gaiser 2003). Everglades periphyton TP levels reported in the literature range from  $30 \mu\text{g P g}^{-1}$  (Vymazal and Richardson 1995) in detached periphyton from oligotrophic sites to over  $4000 \mu\text{g P g}^{-1}$  in periphyton from nutrient enriched sites (Swift 1981, 1984, Swift and Nicholas 1987, McCormick and O'Dell 1996). The mean periphyton TP levels measured in this study (Table 1) are consistent with values characteristic of eutrophic Everglades conditions.

There was no significant difference in periphyton TP levels between the slow and fast treatment samples, thus, these findings do not support the third hypothesis that tissue P levels will differ significantly between the two velocity treatments. However, since the TP concentration of the periphyton was not significantly different between treatments, the greater biomass accrued in the fast treatment would contain a greater total mass of P. Thus, the greater biomass accrual in the fast treatment represents removal of substantially more P from the water column than in the slow treatment. This supports the well documented finding that, like biomass accrual, P uptake in algae is positively correlated with increased velocity up to a species-specific saturation level and flow rate (Whitford and Schumacher 1961, 1964, Lock and John 1979, Riber and Wetzel 1987, Horner *et al.* 1990).

The significant difference in periphyton TP levels between inlet and outlet

samples beginning the third week of the study may be a reflection of the significant differences observed in diatom diversity and relative abundance of dominant genera and, to a lesser extent, soft algae diversity and relative abundance differences between inlet and outlet samples. Though no shifts in taxa were documented along the treatment path as hypothesized, mean relative abundances of eutrophic indicator genera were, in most cases, significantly higher in the inlet samples than the outlet samples. On the other hand, the mean relative abundances of oligotrophic indicator genera were significantly higher in the outlet samples than the inlet samples (Figures 15 and 16). Proximity of the inlet periphytometers to the incoming water source facilitated increased mixing and availability of nutrients. The significantly higher mean concentrations of tissue P measured in the inlet samples compared to the outlet samples correspond with increased P uptake by the periphyton at the beginning of the treatment path in each mesocosm. The lower mean periphyton TP concentrations measured in the outlet samples compared to inlet samples, particularly in the fast treatment, correspond with reduced uptake and/or availability of P at the end of the treatment path. Mulholland *et al.* (1991) found that internal nutrient cycling increased and often exceeded uptake in response to reduced concentrations in flowing water and steepened concentration gradients within periphyton mat boundary layers. The reduced availability of water column TP at the end of the treatment path is most evident in the fast treatment mesocosms. The increasingly greater percent decrease in fast outlet TP concentrations compared to fast inlet TP concentrations observed as the study progressed, (3% on day 13 to 23% on day 55), is related to the longer treatment path length (44.5 m) of the fast treatment, as this pattern was not

observed in the shorter treatment path (4.45 m) of the slow velocity mesocosms (Table 2, Figure 11). This is further substantiated by the 14.6 % mean decrease between the slow outlet TP and fast outlet TP concentrations.

Autoecological studies conducted to assess environmental tolerances of algae to nutrient levels suggest that taxa exhibit specific optima for nutrient utilization (Stevenson *et al.* 2002). Oligotrophic taxa have reduced P demands and are adapted to survival under P-limited conditions, as exemplified by the historic Everglades assemblages (McCormick and Scinto 1999). This finding is consistent with the higher relative abundance of oligotrophic indicator genera observed in the outlet samples compared to the relative abundance of the same genera in the inlet samples in this study. In studies conducted within the Everglades, McCormick *et al.* (1996, 1998) and Raschke (1993) found that the net P uptake rate by periphyton was significantly higher in the eutrophic sites than oligotrophic sites, and observed a shift from oligotrophic assemblage taxa to eutrophic taxa at water column TP concentrations between  $10 \mu\text{g L}^{-1}$  and  $20 \mu\text{g L}^{-1}$ .

In addition to the established indicator genera examined in this study, taxonomic analysis yielded two potential oligotrophic indicator genera, the unicellular cyanobacteria *Synechocystis*, and a non-filamentous green algae, *Kirchneriella*. Though ecological information is lacking for these two genera, they might be worth investigating further as potential indicators for this ecosystem.

Flow weighted monthly means of post-STA water column TP supplying the mesocosms during this study ranged from  $20 \mu\text{g L}^{-1}$  to  $21 \mu\text{g L}^{-1}$  (SFWMD 2002). Measurements of inlet mesocosm water column TP concentrations taken during this

study ranged from  $9.1\mu\text{gL}^{-1}$  to  $29.5\mu\text{gL}^{-1}$  and averaged  $20.7 \pm 2.9\mu\text{g L}^{-1}$ . Given this range of TP levels and the fact that within the Everglades ecosystem, such a range reflects both oligotrophic and eutrophic conditions, it is not unexpected that this periphyton assemblage is characterized by both “clean water” and “pollution tolerant” genera. The virtual absence of the oligotrophic calcareous cyanobacteria genera *Scytonema* and *Schizothrix* within the mesocosms can be attributed to several factors: seasonality; mean water column TP concentrations; substrate; and taxonomic classification. These two dominant genera are commonly reported in late summer in marsh areas with concentrations of less than  $10\mu\text{g L}^{-1}$  (Swift and Nicholas 1987, McCormick and O’Dell 1996). Most previous studies of Everglades periphyton used Drouet’s system of filamentous cyanobacteria identification which included placing several hundred species of the Oscillatoriales order into the genus *Schizothrix* (Swift and Nicholas 1987, Vymazal 2003). Based upon the more current taxonomic scheme of Komárek and Anagnostidis used in this study, this genus was not definitively identified in the mesocosms. Lastly, the commonly identified *Schizothrix calcicola* is an aerophytic calcareous species typically growing on calcite, a substrate not utilized in this study. The high abundance of the filamentous algae genera *Oscillatoria*, *Lyngbya*, and *Spirogyra*, at the water column TP concentrations measured in the mesocosms, is consistent with other studies (Swift and Nicholas 1987, McCormick and O’Dell 1996). These findings partially support the second and third hypotheses that periphyton TP levels will be significantly different between inlet and outlet samples and will be reflected in significant changes in periphyton taxonomic composition between these samples.

A general decline in periphyton TP concentration, followed by recovery and stabilization was observed in both slow and fast velocity samples and inlet and outlet samples over the course of the experiment (Table 1). Horner *et al.* (1983) proposed that algae take up available P during the initial colonization and exponential growth phase, then deplete it until a recovery phase occurs in which either P recycling balances uptake or until uptake stabilizes. That algae can exhibit “luxury” consumption of P and store the excess of that needed for immediate growth was also proposed for Everglades periphyton communities (Swift & Nicholas 1987).

Vymazal (1988) found that elimination of nutrients, including orthophosphate, from the water column stabilized at a certain level while the biomass concentration of periphyton on several types of artificial substrates increased. Mulholland *et al.* (1994) observed lower P uptake and greater internal cycling of P in higher biomass periphyton communities. This internal cycling involves slower diffusion-controlled processes within the periphyton matrix. Biomass accumulation creates transient storage zones of stagnant water and thicker algal mats that limit the mass transfer of soluble nutrients from the water to the algae. This biomass accrual leads to an increase in internal nutrient cycling related to the size of the transient storage zones in order to meet nutrient demands. Horner *et al.* (1990) observed that areal uptake rates of soluble reactive P (SRP) of algae grown in artificial laboratory streams increased with water column SRP concentration and varied inversely with biomass accrual. Their results also suggested that diffusion and uptake rates were stimulated by increased flow rate and increased nutrient concentration up to certain levels, but then became limited by lower metabolic P demands due to algal

mat thickness, reduced light levels, CO<sub>2</sub> levels and algal cell senescence. The measured initial decline and subsequent stabilization of periphyton TP concentration in the mesocosms over time (Table 1) and the documented inverse relationship between periphyton TP levels and dry weight biomass (Figure 10) in this study are consistent with these findings.

### *Conclusions*

The results of this study suggest that analysis of the taxonomic composition of PSTA periphyton communities, in conjunction with other appropriate metrics, can be used to evaluate the efficiency and success of P removal from stormwater treatment areas. Selected periphyton indicator taxa that are sensitive to P availability can be targeted for evaluation when examining inflow and outflow assemblages. The large differences between inlet and outlet % relative abundance of both oligotrophic and eutrophic indicator genera in this small-scale study (33% to 38%) would most likely be observed to an even greater degree within larger-scale PSTA assemblages. These taxa might be included in an indicator assemblage that can be incorporated into a multimetric standardized periphyton index for monitoring changes within the PSTAs.

The observation that a ten-fold difference in velocity did not significantly alter the periphyton assemblage in the mesocosms suggests that short term fluctuations in PSTA flow rates that may occur with seasonal hydrological events, may not affect the stability of a reference community. This stability supports the argument for continued

investigation of this technology as a viable component of the ECP. Further studies are needed to examine the long-term effects of different flow rates on periphyton taxonomic composition of the PSTAs.

This study also suggests that optimization of flow through PSTAs will enhance the rate of biomass accrual, P uptake and nutrient cycling within these constructed wetlands. This increased efficiency of nutrient removal will contribute to achieving water column TP concentrations that are compliant with the requirements of the Everglades Forever Act of 1994.

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**APPENDIX A**

**PHOTOGRAPHS OF SLOW AND FAST VELOCITY  
BOXES**



**Day 7: Slow Velocity ( $0.22 \text{ cm s}^{-1}$ ) inlet box**



**Day 7: Fast Velocity ( $2.0 \text{ cm s}^{-1}$ ) inlet box**



**Day 21: Slow Velocity ( $0.22 \text{ cm s}^{-1}$ ) inlet box**



**Day 21: Fast Velocity ( $2.0 \text{ cm s}^{-1}$ ) inlet box**



**Day 57: Slow Velocity ( $0.22 \text{ cm s}^{-1}$ ) outlet box**



**Day 57: Fast Velocity ( $2.0 \text{ cm s}^{-1}$ ) outlet box**

## **APPENDIX B**

### **kMASTER LIST OF ALGAE GENERA IDENTIFIED (1000X magnification)**

<b>Diatoms (Bacillariophyta)</b>		<b>Blue-green algae (Cyanophyta)</b>	
<b>Family</b>	<b>Genus</b>	<b>Family</b>	<b>Genus</b>
Achnantheaceae	<i>Achnanthes</i>	Nostocaceae	<i>Anabaena</i>
Achnanthidiaceae	<i>Achnanthidium</i>	Merismopedioideae	<i>Aphanocapsa</i>
Amphipleuraceae	<i>Amphipleura</i>	Synechococcaceae	<i>Aphanothece</i>
Catenulacaeae	<i>Amphora</i>	Xenococcaceae	<i>Chroococcidiopsis</i>
Anomoeneidaceae	<i>Anomoeneis</i>	Chroococcaceae	<i>Chroococcus</i>
Aulacoseiraceae	<i>Aulacoseira</i>	Gomphosphaeriodeae	<i>Coelomoron</i>
Bacillariaceae	<i>Bacillaria</i>	Gomphosphaeriodeae	<i>Coelosphaerium</i>
Brachysiraceae	<i>Brachysira</i>	Nostocaceae	<i>Cylindrospermum</i>
Pinnulariaceae	<i>Caloneis</i>	Microcystaceae	<i>Eucapsis</i>
Cocconeidaceae	<i>Cocconeis</i>	Microcystaceae	<i>Gloeocapsa</i>
Coscinodiscaceae	<i>Coscinodiscus</i>	Synechococcaceae	<i>Gloeothece</i>
Stauroneidaceae	<i>Craticula</i>	Gomphosphaeriodeae	<i>Gomphosphaeria</i>
Stephanodiscaceae	<i>Cyclotella</i>	Synechococcoideae	<i>Johannesbaptista</i>
Cymbellaceae	<i>Cymbella</i>	Pseudanabaenaceae	<i>Leptolyngbya</i>
Bacillariaceae	<i>Denticula</i>	Pseudanabaenaceae	<i>Limnothrix</i>
Diadesmidaceae	<i>Diadesmis</i>	Oscillatoriaceae	<i>Lyngbya</i>
Diploneidaceae	<i>Diploneis</i>	Merismopedioideae	<i>Merismopedia</i>
Cymbellaceae	<i>Encyonema</i>	Microcystaceae	<i>Microcystis</i>
Cymbellaceae	<i>Encyonopsis</i>	Oscillatoriaceae	<i>Oscillatoria</i>
Rhopalodiaceae	<i>Epithemia</i>	Phormidiaceae	<i>Phormidium</i>
Eunotiaceae	<i>Eunotia</i>	Pseudanabaenaceae	<i>Planktolyngbya</i>
Sellaphoraceae	<i>Fallacia</i>	Pseudanabaenaceae	<i>Pseudanabaena</i>
Fragilariaceae	<i>Fragilaria</i>	Synechococcoideae	<i>Rhabdoderma</i>
Gomphonemataceae	<i>Gomphonema</i>	Synechococcoideae	<i>Rhabdogloea</i>
Pleurosigmataceae	<i>Gyrosigma</i>	Scytonemataceae	<i>Scytonema</i>
Bacillariaceae	<i>Hantzschia</i>	Gomphosphaeriodeae	<i>Snowella</i>
Naviculaceae	<i>Hippodonta</i>	Pseudanabaenaceae	<i>Spirulina</i>
Diadesmidaceae	<i>Luticola</i>	Synechococcaceae	<i>Synechococcus</i>
Mastogloioaceae	<i>Mastogloia</i>	Merismopedioideae	<i>Synechocystis</i>
Cymbellaceae	<i>Navicella</i>	Microchaetaceae	<i>Tolypothrix</i>
Naviculaceae	<i>Navicula</i>		
Neidiaceae	<i>Neidium</i>	<b>Green algae (Chlorophyta)</b>	
Bacillariaceae	<i>Nitzschia</i>	Oocystaceae	<i>Ankistrodesmus</i>
Pinnulariaceae	<i>Pinnularia</i>	Dictyosphaeriaceae	<i>Botryococcus</i>
Rhopalodiaceae	<i>Rhopalodia</i>	Oedogoniaceae	<i>Bulbochaete</i>
Sellaphoraceae	<i>Sellaphora</i>	Chariaceae	<i>Characium</i>
Stauroneidaceae	<i>Stauroneis</i>	Chlamydomonadaceae	<i>Chlamydomonas</i>
Surirellaceae	<i>Surirella</i>	Oocystaceae	<i>Chlorella</i>
Fragilariaceae	<i>Synedra</i>	Chlorococcaceae	<i>Chlorococcum</i>
Biddulphiaceae	<i>Terpsinoe</i>	Closteriaceae	<i>Closterium</i>
		Coelestraceae	<i>Coelastrum</i>
		Desmidiaceae	<i>Cosmarium</i>
		Scenedesmaceae	<i>Crucigenia</i>

<b>Green algae (Chlorophyta) cont.</b>		<b>Golden-brown algae (Chrysophycophyta)</b>	
<b>Family</b>	<b>Genus</b>	<b>Family</b>	<b>Genus</b>
Desmidiaceae	<i>Desmidium</i>	Dinobryaceae	<i>Dinobryon</i>
Elakatotrichaceae	<i>Elakatothrix</i>		
Desmidiaceae	<i>Euastrum</i>	<b>Cryptophytes (Cryptophyta)</b>	
Volvocaceae	<i>Eudorina</i>		
Gloeococcaceae	<i>Gloeococcus</i>	Campylomonadaceae	<i>Chilomonas</i>
Coccomyxaceae	<i>Gloeocystis</i>	Cryptomonadaceae	<i>Cryptomonas</i>
Peniaceae	<i>Gonatozygon</i>		
Oocystaceae	<i>Kirchneriella</i>	<b>Euglenoids (Euglenophyta)</b>	
Desmidiaceae	<i>Micrasterias</i>	Paranemaceae	<i>Entosiphon</i>
Microsporaceae	<i>Microspora</i>	Euglenaceae	<i>Euglena</i>
Oocystaceae	<i>Monoraphidium</i>	Euglenaceae	<i>Phacus</i>
Zygnemataceae	<i>Mougeotia</i>	Euglenaceae	<i>Strombomonas</i>
Oedogoniaceae	<i>Oedogonium</i>	Euglenaceae	<i>Trachelomonas</i>
Oocystaceae	<i>Oocystis</i>		
Volvocaceae	<i>Pandorina</i>	<b>Dinoflagellates (Pyrrophyta)</b>	
Hydrodictyaceae	<i>Pediastrum</i>		
Desmidiaceae	<i>Pleurotaenium</i>	Phytodiniaceae	<i>Cystodinium</i>
Oocystaceae	<i>Quadrigula</i>	Peridiniaceae	<i>Peridinium</i>
Scenedesmaceae	<i>Scenedesmus</i>		
Oocystaceae	<i>Selenastrum</i>	<b>Yellow-green algae (Xanthophyta)</b>	
Palmellaceae	<i>Sphaerocystis</i>		
Zygnemataceae	<i>Spirogyra</i>	Ophiocytaceae	<i>Ophiocytium</i>
Desmidiaceae	<i>Staurastrum</i>		
Chlorococcaceae	<i>Tetraedron</i>		
Zygnemataceae	<i>Zygnema</i>		

## **APPENDIX C**

### **LIST OF ALGAL INDICATOR TAXA COMMONLY CITED AND REFERENCES**

Indicators of P enrichment/pollution tolerance/eutrophic conditions:

- Amphipleura pellucida*<sup>6</sup>  
*Amphora veneta*<sup>10</sup>  
*Cyclotella meneghiniana*<sup>3, 11, 14</sup>  
*Encyonema silesiacum*<sup>3</sup>  
*Epithemia adnata*<sup>10</sup>  
*Eunotia flexuosa*<sup>3</sup>  
*Eunotia naegelii*<sup>3</sup>  
*Fragilaria capucina* Desm. var. *capucina*<sup>6</sup>  
*Fragilaria* spp.<sup>6, 11</sup>  
*Fragilaria* sp. 02<sup>3</sup>  
*Gomphonema intricatum* (Kütz.)<sup>6</sup>  
*Gomphonema parvulum*<sup>2, 3, 6, 8, 9, 10, 11, 12, 14</sup>  
*Mastogloia smithii*<sup>3, 13</sup>  
*Navicula confervaceae* (syn. *Diadesmis confervaceae*)<sup>14</sup>  
*Navicula cryptotenella*<sup>13</sup>  
*Navicula cryptocephala*<sup>6, 17</sup>  
*Navicula cuspidata*<sup>6, 17</sup>  
*Navicula disputans* (Patrick)<sup>14</sup>  
*Navicula minima*<sup>6, 8, 10</sup>  
*Navicula pupula*<sup>6</sup>  
*Navicula rhynchocephala* Kützing (Wallace) Patrick<sup>6, 14</sup>  
*Navicula viridula* (Kützing)<sup>6, 8</sup>  
*Navicula* spp.<sup>6</sup>  
*Nitzschia amphibia*<sup>3, 6, 8-14</sup>  
*Nitzschia confervaceae* (Kützing) Grunow<sup>10, 14</sup>  
*Nitzschia filiformis*  
*Nitzschia fonticola*<sup>6, 10</sup>  
*Nitzschia frustulum*<sup>6, 8</sup>  
*Nitzschia palea*<sup>3, 6, 8-12, 14</sup>  
*Nitzschia* spp.<sup>2, 6, 11, 17</sup>  
*Rhopalodia gibba*<sup>3, 6, 8, 9, 10</sup>  
*Synedra ulna*<sup>6, 11</sup>, other *Synedra* spp.<sup>6</sup>
- Anabaena* spp.<sup>8, 11, 14, 16</sup>  
*Lyngbya* spp.<sup>8, 11, 16</sup>  
*Microcoleus (lyngbyaceus)* spp.<sup>2, 11, 14, 16, 20</sup>  
*Oscillatoria* spp.<sup>8, 11, 16</sup>  
*Oscillatoria limosa*, *tenuis* et. al.<sup>11, 16</sup>  
*Oscillatoria amphibia*<sup>16</sup>  
*Oscillatoria princeps*<sup>9, 10, 11, 16</sup>  
*Pseudanabaena catenata*<sup>16, 19</sup>

Indicators of P enrichment/pollution tolerance/eutrophic conditions(cont.)

*Ankistrodesmus falcatus*<sup>11</sup>  
*Bulbochaete* spp.<sup>14</sup>  
*Chlamydomonas* spp.<sup>11</sup>  
*Chlorella* spp. (*vulgaris*)<sup>11</sup>  
*Closterium* spp.<sup>11</sup>  
*Cosmarium* spp.<sup>4, 8</sup>  
*Micrasterias* spp.<sup>15</sup>  
*Oedogonium* spp.<sup>14</sup>  
*Pandorina* spp.<sup>15</sup>  
*Pediastrum* spp.<sup>11</sup>  
*Scenedesmus* spp.<sup>8, 11</sup> incl. (*quadricada*)  
*Spirogyra* spp.<sup>2, 9, 10, 11</sup>  
*Staurastrum* spp.<sup>15</sup>  
*Stigeoclonium* spp.<sup>2, 11, 14</sup>

*Euglena viridis*<sup>11</sup>  
*Phacus* spp.<sup>11</sup>  
*Trachelomonas* spp.<sup>11</sup>

Indicators of low P / oligotrophic conditions/ high mineral content (CaCO<sub>3</sub>)/ hard-water, clean water spp.

*Amphora lineolata*<sup>9, 10</sup>  
*Amphora veneta*<sup>14</sup>  
*Anomoeoneis serians*<sup>7, 10, 12</sup>  
*Anomoeoneis vitrea* (syn. *Brachysira vitrea*)<sup>8, 9, 10, 12, 14</sup>  
*Brachysira neoexilis*<sup>3</sup>  
*Cymbella* spp.<sup>2</sup>  
*Cymbella lunata*<sup>7-10, 12</sup>  
*Cymbella microcephala*<sup>14</sup>  
*Cymbella minuta* v. *pseudogracilis*<sup>8, 14</sup>  
*Cymbella ruttneri*<sup>14</sup>  
*Cymbella turgida*<sup>10</sup>  
*Encyonema evergladianum*<sup>3</sup>  
*Gomphonema* cf. *affine* v. *insigne*<sup>14</sup>  
*Mastogloia smithii*<sup>2, 6, 9, 12, 13, 14</sup>  
*Synedra pahokeensis* sp. nov.<sup>14</sup>  
*Synedra rumpens*<sup>8</sup>  
*Synedra (Fragilaria) synegrotasca*<sup>3, 8, 10, 13</sup>

Indicators of low P / oligotrophic conditions/ high mineral content (CaCO<sub>3</sub>)/ hard-water, clean water spp. (cont.)

*Aphanocapsa* spp.<sup>16</sup>  
*Chroococcus turgidus* (E/O)<sup>16</sup>  
*Oscillatoria agardhii* Gomont 1892<sup>16</sup>  
*Oscillatoria anguina*<sup>16</sup>  
*Oscillatoria limnetica*<sup>9, 10, 16</sup>  
*Oscillatoria planctonica*<sup>16</sup>  
*Schizothrix calcicola*<sup>1, 2, 7, 9, 10, 14</sup>  
*Scytonema hofmannii* , *Scytonema* spp.<sup>2, 4, 7, 9, 10, 14, 16</sup>  
*Tolypothrix lanata*<sup>16</sup>  
  
*Mougeotia*<sup>2</sup>

Eutrophic and/or oligotrophic conditions; saproxenous (both clean and polluted water)

*Achnanthes* spp.<sup>6</sup>  
*Amphora* spp.<sup>6</sup>  
*Caloneis* spp.<sup>6</sup>  
*Stauroneis* spp.<sup>6</sup>

Low P , low nutrient, low mineral, ionic content; soft water

*Anomoeoneis serians*<sup>9, 14</sup>  
*Anomoeoneis serians* var. *brachysira*<sup>14</sup>  
*Anomoeoneis vitrea*<sup>14</sup>  
*Cymbella amphioxys*<sup>9, 14</sup>  
*Cymbella minuta* var. *silesiaca*<sup>14</sup>  
*Frustulia rhomboides*<sup>9 14</sup>  
*Eunotia naegeli*<sup>9, 14</sup>  
*Navicula subtilissima*<sup>14</sup>  
*Nitzschia* sp. 7 sp. nov.<sup>14</sup>  
*Pinnularia biceps*<sup>14</sup>  
*Stenopterobia intermedia*<sup>14</sup>  
*Synedra tenera*<sup>14</sup>

*Bulbochaete* spp.<sup>9</sup>  
*Mougeotia* spp.<sup>9, 14</sup>  
*Oedogonium* spp.<sup>4</sup>  
*Pleurotaenium* spp.<sup>9</sup>  
some *Spirogyra* spp.<sup>4</sup>  
Desmids<sup>14</sup>

Pollution – sensitive<sup>8</sup>

*Achnanthes exigua*  
*Achnanthes hustedti*  
*Achnanthes linearis*  
*Achnanthes microcephala*  
*Achnanthes minutissima* (syn. *Achnanthidium minutissimum*)  
*Amphora ovalis*  
*Anomoeoneis serians*  
*Anomoeoneis vitrea*  
*Cymbella lunata*  
*Cymbella (Encyonopsis) microcephala*  
*Cymbella minuta*  
*Mastogloia smithii*  
*Navicula radiosa*  
*Synedra rumpens*

P - sensitive

*Anabaena subcylindrica*<sup>8</sup>  
*Anomoeoneis vitrea*<sup>8, 10</sup>  
*Cymbella lunata*<sup>8, 10</sup>  
*Cymbella minuta*<sup>8</sup>  
*Mastogloia smithii*<sup>8</sup>  
*Synechococcus cedrorum*<sup>8</sup>

Oligosaprobic (o)/Xenosaprobic (x):low levels of nutrients and dissolved organic matter

o- *Achnanthidium minutissimum*<sup>6, 17</sup>  
o- *Caloneis bacillum*<sup>6, 17</sup>  
o- *Cocconeis placentula* (Ehr.)<sup>6</sup>  
o- *Fragilaria* spp.<sup>6</sup>  
o- *Fragilaria construens*<sup>6, 17</sup>  
o- *Navicula exigua*<sup>17</sup>  
o- *Nitzschia fonticola*<sup>6, 17</sup>  
o- *Pinnularia borealis*<sup>6</sup>, o-x *P. subcapitata*<sup>17</sup>  
o- *Synedra acus* Kutz. var. *acus*<sup>6, 17</sup>; other *Synedra* spp.<sup>6</sup>  
o- *Merismopedia* or weakly mesosaprobic; borderline eutrophic<sup>16</sup>

Poly/Mesosaprobic

*Pseudanabaena catenata* and *P. galeata*<sup>16</sup>  
*Limnothrix* (syn. *Oscillatoria redekei*)<sup>16</sup>  
*Synedra* spp.<sup>6</sup>

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