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Periphyton responses to eutrophication in the Florida Everglades: Cross-system patterns of structural and compositional change

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Abstract

We examined periphyton along transects in five Everglades marshes and related compositional and functional aspects to phosphorus (P) gradients caused by enriched inflows. Results were compared to those of a P-addition experiment in a pristine Everglades marsh. While the water total P (TP) concentration was not related to P load in the marshes or experiment, the concentration of TP in periphyton was strongly correlated with the distance from the P source. Increased P concentration in periphyton was associated with a loss of biomass, particularly of the calcifying mat-forming matrix, regardless of the growth form of the periphyton (epiphytic, floating, or epilithic). Diatom species composition was also strongly related to P availability, but the TP optima of many species varied among marshes. Enriched periphyton communities were found 14 km downstream of P inputs to one marsh that has been receiving enhanced P loads for decades, where other studies using different biotic indicators show negligible change in the same marsh. Although recovery trajectories are unknown, periphyton indicators should serve as excellent metrics for the progression or amelioration of P-related effects in the Everglades.

Water column total phosphorus (TP) concentration has been shown to be a poor metric of eutrophication in P-limited shallow wetlands and ponds (Gaiser et al. 2004). In all but the most hypereutrophic settings, the majority of P is in the biota (Hudson et al. 2000), which, in shallow water, are mainly benthic rather than planktonic. However, water column measures are still used to assess P transport in wetlands and, together with sediment P accretion rates, provide the input for loading models used to calculate nutrient assimilation efficiency (Richardson and Qian 1999). More sensitive indices of enrichment based on benthic algal community attributes have been developed for some wetlands (Hill et al. 2000; Stevenson 2001), but though they provide a useful protocol for creating benthic algal metrics, their performance in systems outside those for which they were developed has not been evaluated.

The Everglades of South Florida contain an expansive wetland mosaic, many parts of which have been subject to rapid eutrophication by anthropogenically enhanced P inputs from canals draining the surrounding agricultural landscape (Noe et al. 2001). While marsh water column P concentrations are uncorrelated with P loading from canals (Smith and McCormick 2001; Childers et al. 2003), rapid and significant ecological changes have been detected downstream of sources of P enrichment (McCormick et al. 1996; Cooper et al. 1999; Pan et al. 2000). Experiments have shown that this ecosystem sequesters added P rapidly while changing ecologically, meaning that significant ecosystem changes occur as a result of slight increases in P load but without detectable downstream transport of P in the water column (Gaiser et al. 2005). Biotic indicators of enrichment are, therefore, extremely insightful in this system.

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Benthic algal communities has been advocated as a valuable tool for monitoring P enrichment in the Everglades and elsewhere (McCormick and Stevenson 1998; Stevenson 2001). In the Everglades, periphyton mats containing a mixture of benthic algae, bacteria, fungi, and detritus are prolific and ubiquitous. Steps have been taken to build models to interpret levels of enrichment from periphyton community attributes (McCormick and Stevenson 1998), but the models were generated with data collected from a limited area of the Everglades (water conservation area 2A) that has received considerably more scientific attention because of its long history of P enrichment. Although periphyton is widespread in the larger system, the subbasins within the landscape differ substantially in topography, bedrock substrate, hydrology, water quality, and enrichment history, which creates a spatially diverse environmental template that drives a rich mosaic of habitat types (Gleason and Stone 1994). These among-basin differences in geochemistry and history result in a variety of periphyton community types distributed throughout the Everglades that may respond differently to increased P loading because of different natural starting points. Because periphyton is such an important component of these systems, the characterization of ''ambient'' or unenriched periphyton communities for the entire mosaic of Everglades wetland communities is critical to providing a solid empirical basis for periphyton-based indicators of P enrichment.

The purpose of this study was to (1) evaluate periphyton response to P availability along enrichment gradients that exist from canal inputs to the interior of five of the largest Everglades subbasins, (2) compare these responses among different naturally occurring periphyton assemblages, and (3) create basin-specific periphyton-based metrics of P enrichment. In addition, to determine the underlying causes for relationships between P availability and periphyton community structure and composition, we compared the P-related patterns in our survey to responses measured in a long-term, large-scale experimental P-addition study in the Everglades National Park (ENP). By combining a multibasin gradient analysis with the P-addition experiment, we examined generalities in the response of periphyton to enhanced P and characterized unique responses for managing P inputs on a basin-specific basis.

Methods

Transects—Transects were established in five separate freshwater marsh areas representative of varying environmental conditions within the region. Each transect originated at a canal or flow structure (belonging to the South Florida Water Management District) and extended away from the canal into the interior of the marsh. Four transects included sites originally sampled for vegetation and soils by Doren et al. (1997) in water conservation areas 1 (WCA-1, the Loxahatchee Wildlife Refuge), 2A (WCA-2A), and 3A (WCA-3A) and the Shark River Slough (SRS), and an additional transect was established for this study in the Taylor Slough basin (TS) (Fig. 1). We extended the lengths of the transects sampled by Doren et al. (1997) to ensure that each transect originated in marshes that were close to the major canals that drained agricultural areas, where suspected nutrient-induced ecological changes were obvious, and extended well into natural communities without apparent nutrient-induced changes. A 30-km transect was established in WCA-1, extending east to west across the marsh from the S-6 structure to the L-40 canal. Transect WCA-2A extended approximately 12 km southwest of the S-10C structure, while transect WCA-3A extended 16 km north of the S-12C structure. Transect SRS, located within the ENP, extended 16 km south of the S-12C gate structure on the C-4 canal, and transect TS, also within the ENP, extended 8 km southwest of the S-332 inflow structure on the C-111 canal (Fig. 1).

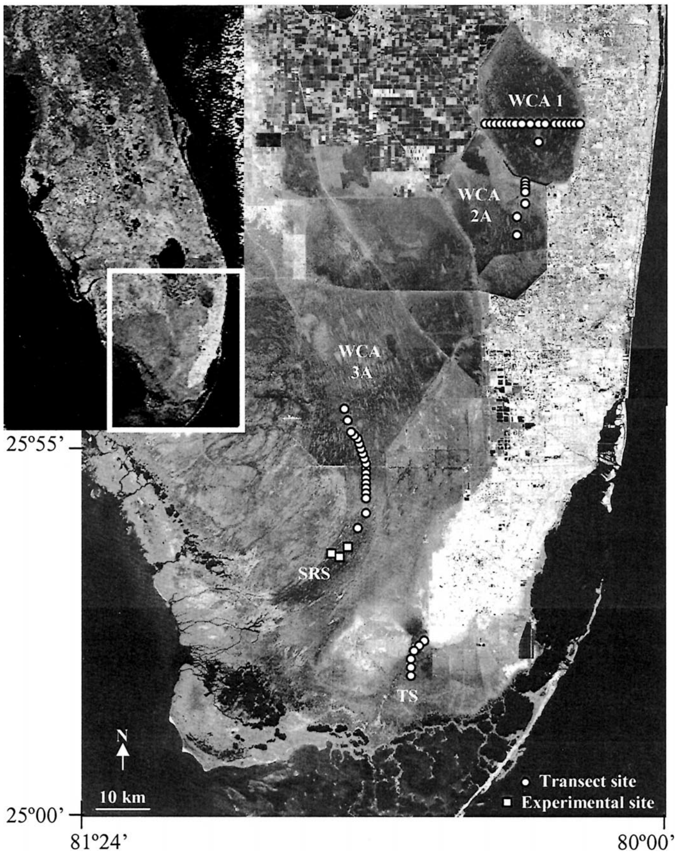

Fig. 1. Satellite photo of Florida with inset of the Everglades showing the locations of the sampling transects in water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS), as well as the sites of the three experimental P-dosing facilities in the SRS.

Although water availability in Everglades marshes is both naturally variable and dependent on water management for civil needs, the subtropical climate of South Florida imposes a seasonal periodicity in surface-water availability. This is typically manifested in a ''dry'' season during the cooler winter months (November–April) and a ''wet'' season during the warm summer months (May–October). To account for the effects of this seasonal variation on periphyton community structure, we planned to sample during these two seasons. However, the years of our sampling (1999–2000) included a prolonged dry season that extended into summer, followed by late-fall tropical storms that provided for an uncharacteristically wet winter. Therefore, we conducted our sampling to include the driest and wettest periods, which, for these years, were opposite the long-term pattern. To avoid the wet/dry season confusion, we designated the sampling episodes ''summer'' (April–June 1999) and ''winter'' (January–February 2000). All transects were sampled during these periods, except for the TS transect, for which the second (winter) sampling took place in October 1999. In the summer sampling period, site locations along transects followed a geometric model where sites were established 0, 0.5, 1, 2, 4,. . .km from the canal to the final interior site, 30, 12, 16, 16, and 8 km from the canal for WCA-1, WCA-2A, WCA-3A, SRS, and TS, respectively (Fig. 1). A more complete sampling took place during the winter period, where we followed a linear model with sites established 0, 1, 2, 3, 4, 5,. . .km from the flow structures to the same interiormost site.

Sampling sites were accessed primarily by helicopter during the summer and primarily by airboat during the winter using a Global Positioning System programmed with predetermined coordinates. Because Everglades marshes typically contain a mosaic of several plant community types, we decided a priori to restrict periphyton sampling to open-water or wet prairie habitats where periphyton is typically most abundant. Wet prairie marshes in the Everglades have been characterized as having peat-based soils, abundant periphyton, and a macrophyte community dominated by *Utricularia purpurea*, *Eleocharis cellulosa*, *Panicum hemitomon*, and *Sagittaria lancifolia* (Gunderson 1994). As part of a larger study, other parameters (e.g., vegetation, soils, water quality, consumers) were sampled within additional community types at each site; the methods and results of these studies are presented elsewhere (*see* Childers et al. 2003). For periphyton collections, we located the nearest open-water or wet prairie habitat to each site coordinate and randomly located three 1-m^2 sampling plots in which to conduct our sampling.

Within each 1-m^2 sampling plot, we measured the water depth and pH and took triplicate water samples for TP analysis. Because dissolved P is typically present in concentrations below instrumental detection limits in this system (Gaiser et al. 2004), we chose not to include it as metric in this study. To estimate the aerial cover of periphyton in the sampling plot, we visually estimated the percentage of coverage by substrate type in the following categories: calcitic metaphyton (calcareous periphyton mat floating on the water surface), emergent epiphyton (periphyton growing attached to submerged stems of emergent vegetation), calcitic epilithon (calcareous mat attached to sediment or rock), submerged epiphyton (periphyton on fully submerged macrophytes), filamentous green aggregates (floating or submerged aggregations of filamentous green algae), and flocculent detritus (disaggregated periphyton on the soil surface). A more precise estimate of the cover of the floating mat was measured by taking a photograph of the quadrat using a digital camera (Nikon Coolpix, 3 mp). Images were later downloaded to a computer, and the percentage of the quadrat containing periphyton was measured using a color-calibrated image analysis system with Image Pro 4.0 analysis software.

To estimate the standing crop biomass of calcitic metaphyton, epilithon, submerged epiphyton, filamentous green algae, and flocculent detritus, we removed 15 small cores of periphyton using a 4.2-cm^2, 10-cm-long coring device. Because periphyton mats are relatively cohesive, this coring device allowed us to remove a consistent volume of material from the different substrate types. Cores from each substrate type were composited into a single sample for each quadrat. To estimate the standing crop biomass of emergent epiphyton, we collected the submersed portion of the dominant emergent macrophyte from each quadrat. Three mature stems were clipped at the sediment–water interface and at the water surface and combined into a single sample. All samples were frozen until they could be analyzed.

In the laboratory, periphyton samples were thawed and examined under a dissecting microscope, where extraneous plant material and animals were manually separated from the periphyton. Epiphytic material was gently scraped from the submersed stems of emergent macrophytes using a plastic blade and was teased apart manually with forceps from submersed plants. The remaining periphyton was homogenized and diluted to a measured volume with distilled water. Measured aliquots were then removed for dry-weight, ash-free dry mass (AFDM), chlorophyll *a* (Chl *a*), TP, and compositional analyses. Two subsamples were dried to constant weight at 100°C (~2 d) and weighed. One dried subsample was subsequently combusted in a muffle furnace at 500°C for the estimation of mineral mass (mainly calcium carbonate). AFDM was calculated as the difference between the dry and mineral mass, and its proportion relative to the dry mass was expressed as organic carbon content. The second dried subsample was analyzed for TP in the same manner as water samples, by colorimetric analysis after dry combustion (Solorzano and Sharp 1980; U.S. Environmental Protection Agency 1983). The TP content of periphyton was expressed on a dry-weight basis because the organically incorporated P was not separable from that bound to calcite. Chl *a* was measured from a subsample collected on a glass microfiber filter. Filters were frozen, and Chl *a* was extracted in 90% acetone that was added between 48 and 96 h prior to fluorometric analysis. Biomass estimates in μg cm^{-2} of substrate were expanded to g m^{-2} using the percentage of cover of each periphyton type. Epiphytic periphyton biomass was expanded to the entire 1-m^2 plot by multiplying per stem estimates by the number of stems per plot.

A final subsample was retained for diatom compositional analysis. First, frozen subsamples were thawed and cleaned of calcite and organic matter using a combination of strong acids and chemical oxidizers (Hasle and Fryxell 1970). A subsample of neutralized material was then placed on a coverslip and permanently fixed to a glass microslide using Naphrax mounting medium. At least 500 diatom valves were counted and identified on random, measured transects on a compound light microscope at ×1,000. We used standard and regional references to identify diatoms; difficult species were given numeric designations. Diatom samples and permanent slides are archived in a curated collection in the microscopy laboratory at Florida International University and are available from the authors upon request.

Experiment—We conducted our transect survey in conjunction with a P-addition experiment to determine the levels of P that elicited changes in natural Everglades communities and compared responses on several temporal and spatial scales. The experiment was conducted in the ENP using a flow-through flume design to continuously deliver P at three concentrations above background while maintaining natural water depth and flow. Dosing in this manner delivers P continually to a flowing water system at a given concentration, mimicking the way nutrients are actually delivered from canals into the marshes.

Three replicate flow-through experimental flumes (A, B, and C) were constructed in oligotrophic, unenriched marshes of the SRS in the ENP. In this region of the Everglades, natural water column concentrations of soluble reactive P are typically below detection limits ($<2\ \mu g\ L^{-1}$, $<0.06\ \mu mol\ L^{-1}$), and TP is usually $<10\ \mu g\ L^{-1}$ ($<0.32\ \mu mol\ L^{-1}$) (McCormick et al. 1996). The flumes are located in peat-based wet prairie marshes that tend to contain deeper, faster-flowing water than surrounding short-hydroperiod marshes; however, the wet prairie marshes are still subject to stagnating flows during the dry season (November–May) and drying during drought years.

Each of the experimental flumes contained four 100-m-long × 3-m-wide channels that enclosed areas of natural marsh and were open at both ends. Channels were positioned parallel to the predominant flow of water, and an automated system delivered $NaH_2PO_4 + Na_2HPO_4$ (pH = 7) at concentrations of 0, 5, 15, and 30 $\mu g\ L^{-1}$ ($\sim$0, 0.16, 0.48, and 0.97 $\mu mol\ L^{-1}$, respectively) above ambient to a 10-m-long mixing box at the head of each channel. Concentrations in the mixing box were kept constant by continuously adjusting P input according to velocity and stage as measured by a pressure transducer and acoustic Doppler flow sensor at the head of each channel. Channels were separated by heavy-gauge plastic walls that were driven into the sediments and attached at the top to rollers on the edge of floating walkways that extended the length of the channels. The shading effect of walls was minimized, because the plastic lengthened and shortened on the rollers as the water depth changed. An untreated, unwalled area adjacent to each flume was established as a reference area to account for any wall effects or leakage and was sampled in the same manner as the experimental channels. For further details about the experimental setup, *see* Childers et al. (2001) and Noe et al. (2002).

P dosing occurred when velocities exceeded 2 mm s^{-1} from October 1998 to March 2003. These data were collected in March 1999 and February 2000 to coincide with the sampling schedule for the transect study. We measured the TP in the water and the following aspects of the periphyton community: aerial cover, dry-weight biomass, ash-free dry biomass, Chl *a*, TP, and species composition. Samples were taken from 1-m^2 quadrats located 5, 18.5, 36.5, and 66.5 m from the mixing box at the head of each channel. Field and laboratory methods for biomass quantification and diatom enumeration were identical to the protocols followed for the transect study described above.

Data analysis—The mass of P delivered to each wetland in the calendar year 1999 was calculated using water flow and TP data available from the South Florida Water Management District corporate environmental database (DBHYDRO; http://www.sfwmd.gov/org/ema/dbhydro/index.html). The database was queried for daily water TP and flow through each of the inflow structures (S-6 and L-40-1, S-10C, S-12CN, S-12CS, and S-332C for WCA-1, WCA-2A, WCA-3A, SRS, and TS, respectively). Daily water TP was unavailable for some structures, so monthly averages of available TP concentration and volumetric flow data were taken and divided by the number of days in the month to determine the average daily TP load (kg d^{-1}). The annual load was then calculated by summing the daily values. For the experiment, the TP load was calculated from continuous velocity, stage, and P-input recordings. P dosing was controlled by velocity and stage, so the P load was directly calculable from input recordings. Daily loads were summed for the entire duration of dosing prior to sampling.

To examine spatial patterns relative to P gradients within each marsh and experimental flume channel, we first fit a negative exponential, asymptotic decay model ($TP = a + \exp(-b_1 \times \text{distance} + b_0)$, where a = asymptote, b_1 = slope, and b_0 = initial concentration) to the relationship of water and periphyton TP content (averaged among seasons) with distance from P source. The asymptotic point in the model relating periphyton TP to distance was used to delineate ‘‘enriched’’ from ‘‘ambient’’ sites for subsequent analyses; periphyton attributes between these two site types were compared within each marsh using multiple means comparison. Similarly, significant departures from ambient conditions in each plot in each experimental channel were determined by comparison to paired control plots. Relationships between periphyton biomass and TP content were explored within and among marshes using linear regression. TP data were log transformed prior to analysis to fit a normal distribution.

We used a combination of ordination and weighted-average regression to determine the pattern in the distribution of diatom species among and within wetland basins. Species by site data matrices were established for the entire data set and for each transect separately, and species present in $<1\%$ of samples were removed prior to analysis. Species relative abundances were then 4th root transformed prior to analysis, because this provided the most even distribution of the percentage data. To ordinate sites based on the relative abundance of diatom species, we used nonmetric multidimensional scaling (NMDS) (Kruskal and Wish 1978), employing the Bray–Curtis dissimilarity metric to determine site distances for 1–10 dimensions. A final ordination was retained that contained the number of dimensions after which no appreciable decrease of stress occurred. Biplots were then created using site scores for each dimension. The significance of patterns observed in the biplots was determined using analysis of similarity (ANOSIM, using PRIMER software) (Clarke and Gorley 2001), which examines the difference within and among sample categories. In this analysis, samples were grouped by marsh, season, or enrichment level. The reported Global R values increase from 0 to 1 with increasing among-sample dissimilarity. We report significant dissimilarity when the probability of a sample pair originating from the same community is <0.001. To determine the effects of linear variables on composition, we fitted vectors to the plane explaining the most variation in site–site distances in the two-dimensional NMDS. Because axes in NMDS are arbitrary, we rotated them according to the maximum variation (vector) in TP, the variable of greatest interest in this study. We further examined the relationship of compositional similarity to TP concentration in periphyton by relating site–site dissimilarities in diatom composition, using the same distance matrix as above, to paired site–site differences in periphyton TP within each marsh.

We then used weighted-averaging regression and calibration to determine the strength of the relationship of species composition to P availability. We first quantified the effects of P on the variation in abundance of individual diatom taxa by fitting a quadratic regression model of the abundance of each species against periphyton TP content (abundance of species $I = b_1 P + b_2 P^2$). While the quadratic model may not have been the best choice for all taxa, the assumption that species optima fall within the sampled gradient where their abundance is highest is the same assumption employed in the calibration component of our TP prediction model. For calibration, we used a weighted-averaging approach that defines the optimum or the mode where abundances are greatest and a tolerance that defines the breadth of appearance along a gradient. The value of an environmental variable can then be calculated for a sample from an unknown environment, using the average of the optima of the species present, weighted by their abundances and possibly tolerances. Using the computer program C2 (Juggins 2003), we estimated the P optimum and tolerance for each species as the average periphyton TP among sites in which the taxon occurred. Because taxa had unequal occurrences, we used the number of occurrences to adjust the tolerance assigned to each taxon. Species with narrow distributions along the P gradient were weighted more heavily than species with broadly dispersed or erratic distributions when tolerances were incorporated into the model. We used classical regression to eliminate shrinkage in the range of inferred values because it resulted in the most evenly distributed residuals. TP optima and tolerances were calculated for each taxon using a complete data set including all five transects and then separately for each transect and the experimental data set, which resulted in seven ''calibration'' data sets. Relationships among optima derived from the different data sets were determined by linear regression. We then estimated the TP at each site along each transect by randomly selecting samples from (1) the same calibration data set (with replacement), and (2) another calibration data set (or ''test'' set) derived from a different marsh or from the experiment. The resulting estimates of periphyton TP for each sample were then plotted against observed values, and the regression coefficient and bootstrapped root mean square error of prediction (RMSE) were calculated for each model.

In addition to the multispecies approach to TP prediction, we identified individual taxa that provide particularly strong inferences of TP. To be selected as a TP indicator, taxa were required to have (1) an occurrence in $>5\%$ of samples; (2) a mean abundance when present of $>1\%$; (3) a strong relationship to TP, identified by regression; and (4) a weighted-average estimated tolerance less than the mean tolerance for all species.

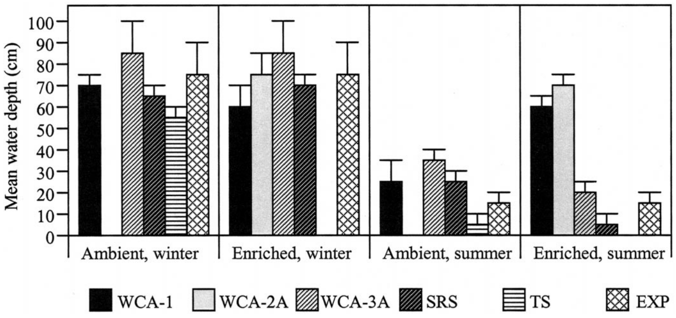

Fig. 2. Mean water depth during each season and site category (enriched or ambient) along transects in water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS) and the experimental (EXP) sites in the SRS. Error bars represent the standard deviation among sites within each category. Designation of sites to ambient or enriched categories is provided in Table 1.

Results

Seasonal and spatial patterns at unenriched sites—Water depth varied significantly between seasons in all marshes (Fig. 2), though less so in WCA-1 and WCA-2A, where flood control structures allowed sites near canals to maintain higher water levels than interior sites during dry periods. The average pH was lower in WCA-1 (5.8) and WCA-3A (6.4) than in the other wetlands (7.3, 7.0, and 7.1 for WCA-2A, SRS, and TS, respectively).

Water depth and pH influenced the structural form of periphyton in the unenriched interior of each marsh. Periphyton in the interior of WCA-1 contained little calcite (90% organic) and was primarily associated with submersed vegetation (including, abundantly, *U. purpurea*) and the flocculent detritus. WCA-3A also had significant epiphytic periphyton communities, primarily on *Eleocharis elongata* and *Nymphaea odorata.* The organic content of periphyton mats decreased southward through the system, with shallow areas of the SRS and TS having the highest proportion of calcite-rich mats (only 22% and 20% organic, respectively). These mats were found either floating on the surface, often associated with *U. purpurea* (particularly in the SRS) or growing directly on the limerock surface (the dominant form in the TS). The experimental control sites in the SRS were dominated by a floating, calcitic mat, similar to that of the nearby 16-km station in the SRS (Fig. 3; Table 1). Dry-weight biomass was highest in these southern sites, with the calcitic periphyton mats comparable to the organic-rich mats of the northern basins (Table 1).

P-related trends—Flow rates and TP concentration of water flowing into WCA-1, WCA-2A, WCA-3A, SRS, and TS averaged 5,730, 7,590, 1,275, 15,150, and 6,450 liters s^{-1} and 109, 89, 11.1, 11.1, and 8.7 $\mu g\ L^{-1}$, respectively, yielding annual TP inputs of 25,150, 18,270, 320, 3,190, and 1,290 kg yr^{-1} into each of these basins, respectively. If this input were evenly distributed throughout the basins that differ in size (572, 101, 2,036, and 5,200 km^2 for WCA-1, WCA-2A, WCA-3A, and ENP), then the equivalent aerial

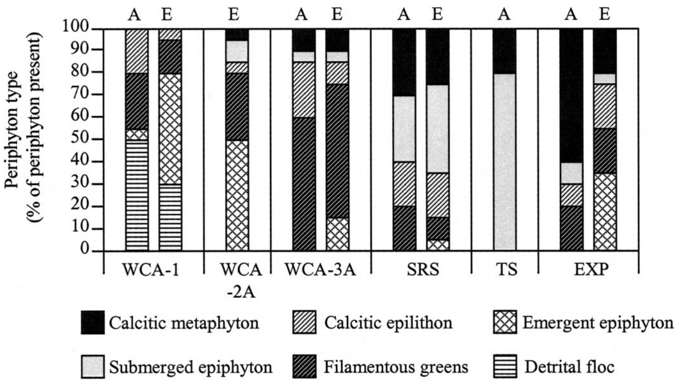

Fig. 3. Periphyton growth forms in (A) ambient and (E) enriched sites along transects in water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS) and the experimental sites in the SRS. Designation of sites as ambient or enriched categories is provided in Table 1. The proportion of each type relative to all periphyton present was averaged among sites and seasons.

input rates would be 3.3, 18, 0.015, and 0.086 g m^{-2} yr^{-1}. In the experimental flumes, a total of 1.1, 3.2, and 5.4 kg of P were delivered to channels receiving 5, 15, and 30 μg of P L^{-1} by February 2000 (14 months of dosing). The average annual input was 0.6, 1.7, and 3.3 kg of P to channels receiving 5, 15, and 30 μg of P L^{-1}, which, if delivered evenly and conservatively over the 300 m^2 of marsh in each channel, amounted to an average annual aerial input of 2, 6, and 10 g m^{-2} yr^{-1}, respectively. This P load was delivered to water ranging in depth from 0.34 to 1.7 m (average = 0.71 m) at velocities ranging from 0 to 18 mm s^{-1} (average = 4.5 mm s^{-1}).

The water column TP concentrations were greater at the site nearest to the canal inputs, and they decreased thereafter to asymptotic levels (5–11 μg L^{-1}) in WCA-1, WCA-3A, and SRS (Fig. 4a,b). Water TP was highly elevated at sites 0–3 km from the canal input at WCA-2A (mean = 148 μg L^{-1}). No TP elevation was detected along the TS transect, although values were highest at the 4-km site. In the experimental flumes, water TP at the 5-m site was greater than the control in the 15 and 30 μg L^{-1} treatments. Water TP concentration in the remaining portion of all channels did not differ from control concentrations. There were no seasonal differences in water column TP concentrations within marshes.

TP concentrations in periphyton were elevated significantly further downstream of P inputs than were water TP concentrations (Fig. 4c,d). Periphyton TP reached asymptotic levels of 423, 187, and 99 μg of P g^{-1} in WCA-1, WCA-3A, and SRS at 3, 1, and 1 km downstream of the sources, respectively. Although periphyton TP also declined exponentially in WCA-2A, interior values remained high (500 μg of P g^{-1}), so all sites in this basin were categorized as ‘‘enriched.’’ Conversely, concentrations in the calcareous mats of the TS were low throughout (mean = 124 μg of P g^{-1}) and unrelated to distance, so all sites in this marsh were treated as ‘‘ambient.’’ In the experiment, the periphyton TP concentration was significantly elevated relative to the controls at sites 5 m downstream of the input in all treatment channels. Further downstream, sites were significantly enriched relative to the controls at 18 and 37 m downstream of the 15- and 30-μg L^{-1} treatments, respectively. Periphyton TP concentrations were significantly lower in the summer than in the winter in SRS, WCA-3A, and WCA-2A.

The periphyton dry-weight biomass was significantly higher in the summer than in the winter for all transects, except for the TS transect, and was negatively related to periphyton TP content across all sites (Fig. 5a). The negative relationship of periphyton biomass to TP was weak but significant for each transect and the experimental flumes during both seasons, except for SRS and WCA-2A during the winter. The AFDM of periphyton (in g m^{-2}) was also negatively correlated to TP content across all sites ($R^2 = 0.21$, $p < 0.001$). Chl *a*, however, was significantly higher at enriched sites than in interior sites (0.3–0.6 vs. 0.1–0.2 g m^{-2}, respectively). The organic content of mats, measured as the ratio of AFDM to dry mass, was higher in the winter than

Table 1. The mean (±SE) of water total phosphorus (TP) and periphyton cover, TP content, dry mass, organic content, and Chl *a* at enriched (E) and unenriched (A) transect sites in WCA-1, WCA-2A, WCA-3A, Shark River Slough (SRS), and Taylor Slough (TS) and experimental plots (EXP) in SRS. Sites were defined as ambient or enriched based on the change-point in periphyton TP content; the range of distances downstream from canal discharge for each type is provided.

				Periphyton				
Site	Type	Distance (km)	Water TP (μg L^{-1})	Cover (%)	TP content (μg g^{-1})	Dry mass (g m^{-2})	Organic (%)	Chl *a* (g m^{-2})
WCA-1	A	3–15	25 (7)	86 (9)	430 (110)	163 (89)	90 (7)	0.1 (0.1)
WCA-1	E	0–2	14 (3)	54 (32)	904 (409)	148 (91)	90 (10)	0.5 (1.4)
WCA-2A	E	0–14	151 (47)	52 (26)	2,242 (2,208)	119 (120)	90 (30)	0.6 (0.9)
WCA-3A	A	1–16	13 (12)	74 (13)	193 (61)	88 (38)	71 (2)	0.2 (0.1)
WCA-3A	E	0–0.5	12 (13)	59 (26)	328 (94)	83 (57)	87 (7)	0.1 (0.1)
SRS	A	2–16	8 (6)	81 (12)	100 (48)	975 (955)	22 (27)	0.1 (0.1)
SRS	E	0–1	6 (3)	69 (30)	415 (275)	270 (198)	60 (32)	0.3 (0.2)
TS	A	0–8	5 (3)	73 (25)	97 (67)	1,137 (620)	20 (4)	0.3 (0.4)
EXP	A	*	11 (11)	82 (52)	178 (101)	124 (32)	42 (28)	0.1 (0.1)
EXP	E	*	17 (12)	57 (30)	458 (103)	58 (98)	17 (32)	0.3 (0.2)

* Experimental sites were categorized by significant difference relative to paired control plots.

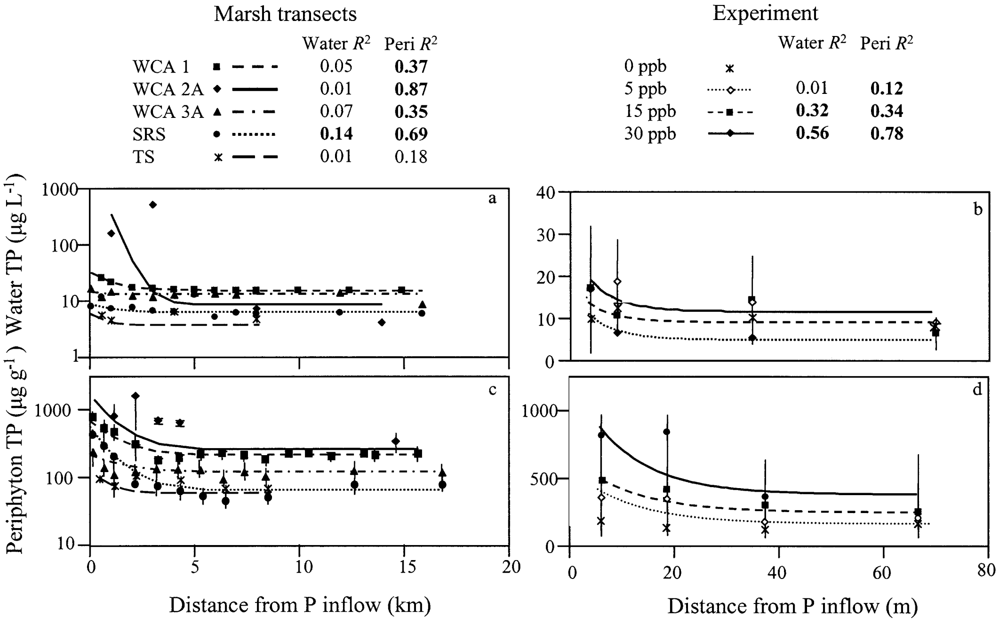

Fig. 4. Negative exponential curves fitting the contrastingly weak and strong relationships of (a, b) water TP and (c, d) periphyton TP content, respectively, to distance from P inflow along the (a, c) transects in water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS) and the (b, d) experimental sites in the SRS. Error bars show the standard deviations among sample dates, and significant R^2 values for the models are presented in bold.

in the summer for SRS and WCA-3A and was positively correlated to mat TP content (Fig. 5b). This relationship was strongest within the SRS and the experimental channels (R^2 = 0.75 and 0.69, respectively) and was weaker for WCA-3A, WCA-1, WCA-2A, and TS (R^2 = 0.28, 0.01, 0.10, and 0.60, respectively). The most highly organic periphyton communities were found close to input sources and were dominated by epiphytic, filamentous green algal aggregates (Table 1; Fig. 3).

Compositional trends—A total of 129 taxa representing 35 genera were collected from the transects and experimental dosing facility. Genera represented by the most species were *Achnanthes* (7), *Amphora* (6), *Encyonema* (10), *Eunotia* (8), *Fragilaria* (5), *Gomphonema* (10), *Navicula* (6), *Nitzschia* (11), and *Pinnularia* (8). Sixty taxa were unidentifiable after a considerable literature review and were assigned numeric designations. The most abundant taxa (present in >50% samples and >5% mean abundance) were *Mastogloia smithii, M. smithii* var. *lacustris, Encyonema evergladianum, E.* ftsp02, *Brachysira neoexilis, Fragilaria synegrotesca,* and *Nitzschia amphibia.* Seventy-nine taxa were considered rare (present in <1% of samples and <0.1% mean abundance). The 50 nonrare taxa (Table 2) were incorporated into further analyses of distribution patterns.

Although the NMDS showed differences in community composition among transects, the stress values were somewhat low (0.19), indicating that the ordination may not fully represent the dissimilarities among sample sites. However, the ordination clearly separated stations in WCA-1 from the remainder of the transects (Fig. 6). The ANOSIM test confirmed the impression from the NMDS and found a significant difference between the composition at WCA-1 and the remaining marshes (Global R = 0.58). Seasonal differences were not detected in the combined data sets, but slight differences among seasons were detected in WCA-3A and the TS (Global R = 0.14 and 0.13, respectively). Enriched sites were compositionally distinct from unenriched sites in WCA-1, WCA-3A, SRS, and the experiment (Global R = 0.42, 0.54, 0.56, and 0.68, respectively, $p < 0.001$). This is also shown in the NMDS where the compositional dissimilarity among sites was highly correlated with the TP content in the periphyton among sites (Vector R^2 = 0.59). Within each marsh, the compositional dissimilarity increased linearly with the logarithm of the difference in periphyton TP content (Fig. 7).

The effects of mat TP content on species composition were quantified for each transect by relating the diatom-inferred TP content for each sample (derived from the weighted-averaging TP optima for the species in that sample) to the measured TP content at the site, with errors generated via a bootstrapped subset of samples. The regression coefficient and bootstrapped RMSE of prediction of periphyton TP (in units of μg of P g^{-1}) from diatom species composition were 0.63 (220), 0.69 (160), 0.59 (140), 0.81 (240), 0.54 (140), 0.68 (160), and 0.57 (150) for the experimental data set, all transects combined, WCA-1, WCA-2A, WCA-3A, SRS, and TS, respectively.

Although strong relationships between species composition and periphyton TP content existed within each transect and the experiment, optima calculated for the same species differed considerably among transects and the experimental data sets. This was true even though compositional overlap among all transects and the experimental data sets was high

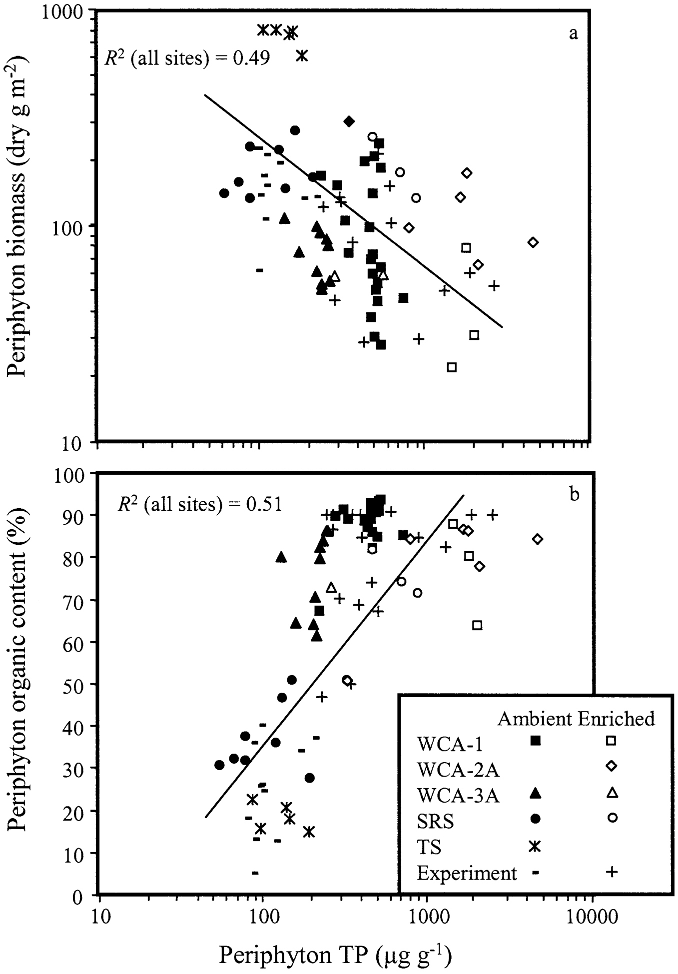

Fig. 5. Relationship of (a) periphyton dry-weight biomass and (b) percentage of organic content to periphyton TP content among sites in water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS) and the experimental sites in the SRS. Sites are designated as enriched or ambient following the designations provided in Table 1.

(mean among-marsh Bray–Curtis dissimilarity = 35%), except for WCA-1, which was compositionally very different from all other data sets (mean Bray–Curtis dissimilarity to other marshes = 75%). The lack of a substantial overlap in the species composition for WCA-1 to other sites precluded us from testing predictions of TP from optima derived from other data sets.

Because of this instability in species optima along the TP transects both in the natural system and the experiment, predictions of mat TP for a particular sample using optima derived from a different sample set are not as strong as predictions derived from the same sample set. Using the experimental data set to predict TP content from species composition in the natural system produced good predictions for the SRS and TS, where species composition and optima were most similar to those in the experiment (R^2 = 0.70 and 0.67; RMSE = 160 and 200 μg of TP g^{-1}, respectively; Fig. 8). However, the experimental calibration set worked poorly to predict the periphyton TP at stations along the WCA-2A and WCA-3A transects (R^2 = 0.40 and 0.25; RMSE = 1,700 and 160 μg of TP g^{-1}, respectively). Errors were mostly to due to the underestimation of TP, particularly at the lower end of the TP gradient.

Taxa identified as significant indicators of P availability were divided into three groups according to their weighted-averaging TP optima (in μg of P g^{-1}): low P taxa (100–300), medium P taxa (300–500), and high P taxa (>800). Low P indicator taxa included *B. neoexilis* var. 02, *M. smithii, E. evergladianum, E.* ftsp02, *E. microcephala,* and *F. synegrotesca.* Medium P indicator taxa included *Eunotia flexuosa* and *Navicula cryptotenella.* High P indicators were *Eunotia incisa, F.* ctsp02, *Rhopalodia gibba, Amphora veneta, N. amphibia, Gomphonema parvulum,* and *G.* egsp01. The relationships of the abundance of these species to the periphyton TP content, showing the fit of a quadratic function, are provided in Fig. 9.

Discussion

A large proportion of the organic biomass across the wide spectrum of habitats sampled in the Everglades was composed of periphyton. When compared to living and dead plant and consumer biomass from the same study locations and dates, summarized by Childers et al. (2003), periphyton made up an average of 50%, 80%, 40%, 50%, and 80% of total biotic biomass in unenriched slough sites in WCA-1, WCA-2A, WCA-3A, SRS, and TS, respectively. Periphyton is, therefore, the primary biomass producer in Everglades wet prairies, in most cases exceeding the production of their macrophyte substrates. Therefore, periphyton-based metrics of P enrichment should have the capacity to be employed ubiquitously throughout the Everglades.

While most of the measured periphyton parameters could be used to measure exposure to an enriched P load, some showed consistent patterns among the different marshes, while other responses were basin-specific. The biomass and TP content of periphyton mats responded predictably to P gradients in the flumes and in the experiment, while species responses differed somewhat among basins, depending on the initial composition. Such inconsistencies in response and a lack of testing in other systems preclude us from developing a multimetric periphyton index of enrichment. We are cautious about combining response variables to develop a univariate metric of ecosystem condition from periphyton attributes for the same reasons as McCormick and Stevenson (1998): the high utility and simplicity of such indices encourage their rapid incorporation into management, so the models must be rigorously tested in other areas before they are presented for use in management. Rather, we advocate using the means and ranges presented in Table 1 and the species response models as system-specific guides to measuring enrichment in this system and a base for which to compare our results to other systems outside the Everglades.

Periphyton TP content was a consistent indicator of P enrichment in all the sampled basins. Periphyton from areas directly downstream of accelerated P inputs to WCA-1 and WCA-2A was highly enriched relative to sites near inputs to WCA-3A, SRS, and TS, which receive lower loads. In

Table 2. The 50 most common diatom taxa found in the Everglades transects listed by weighted-average periphyton phosphorus (TP) optima (opt) and tolerances (tol), shown with mean relative abundance (Rel. abd.) (discounting absences), total number of occurrences (occ) (of 197 samples), and presence in WCA-1 (1), WCA-2A (2), WCA-3A (3), Shark River Slough (4), and Taylor Slough (5), with the site of maximum abundance given in bold. Taxa not identifiable to species were given alphanumeric codes that are linked to archived photographs, descriptions, and specimens available from the Algal Herbarium of the Southeast Environmental Research Center, Florida International University, Miami.

Diatom taxon	TP opt	TP (tol)	Rel. abd.	No. occ	Sites
Brachysira neoexilis L.-Bert. forma 02	100	(56)	0.9	(51)	3,**4**,5
Encyonema sjsp01	102	(38)	2.1	(40)	1,**3**,4,5
Caponea caribbea Podz.	108	(43)	0.3	(5)	**4**
Mastogloia smithii Thwaites ex W. Sm.	140	(96)	26.2	(135)	2,3,4,**5**
Encyonema ftsp04	146	(132)	1.0	(24)	**1**,3,4,5
M. smithii var. *lacustris* Grun.	158	(97)	4.5	(61)	**1**,3,4,5
Encyonema evergladianum Krammer	169	(116)	18.7	(147)	1,2,**3**,4,5
Nitzschia serpentiraphe L.-Bert.	174	(176)	1.3	(104)	1,3,4,**5**
Diploneis oblongella (Naeg. ex Kütz.) Ross	175	(151)	0.6	(25)	4,**5**
Encyonema ftsp01	190	(134)	2.8	(132)	1,2,**3**,4,5
Encyonema silesiacum var. *elegans* Kr.	195	(90)	1.3	(66)	1,**3**,4,5
Navicula radiosa Kütz. var. 01	195	(174)	0.9	(72)	2,3,**4**,5
Encyonema ftsp02	207	(123)	5.2	(149)	**1**,2,3,4,5
B. neoexilis L.-Bert. var. 01	225	(194)	4.0	(138)	1,3,**4**,5
Brachysira brebissonii Ross in Hartley	231	(147)	11.2	(63)	**1**,4
Mastogloia lanceolata Thwaites ex W. Sm.	231	(49)	1.3	(5)	**2**
Gomphonema affine Kütz. var. 01	247	(207)	0.9	(99)	**1**,2,3,4,5
Encyonopsis egsp01	248	(187)	0.8	(15)	3,**4**,5
Navicula subtillisima Cleve	251	(175)	0.8	(23)	1,2,**3**,4,5
Nitzschia palea var. *debilis* (Kütz.) Grun.	254	(359)	1.8	(122)	**1**,2,3,4,5
Encyonopsis microcephala (Grun.) Kr.	266	(194)	3.0	(116)	**1**,2,3,4,5
Fragilaria synegrotesca L.-Bert.	270	(202)	13.5	(149)	1,**2**,3,4,5
Achnanthes caledonica L.-Bert.	277	(221)	1.9	(86)	1,2,3,4,**5**
Fragilaria nanana L.-Bert.	308	(268)	1.4	(64)	**1**,2,3,4,5
Nitzschia palea (Kütz.) W. Sm.	313	(228)	1.3	(18)	1,**2**,4
Cyclotella meneghiniana Kütz.	313	(325)	0.6	(73)	**1**,2,3,4,5
Sellaphora laevissima (Kütz.) Kr.	324	(314)	0.5	(38)	2,3,4,**5**
Eunotia egsp01	327	(248)	0.8	(46)	1,3,**4**,5
Eunotia flexuosa Bréb ex Kütz.	344	(249)	1.0	(68)	**1**,3,4,5
Navicula cryptotenella L.-Bert.	354	(333)	0.9	(68)	2,3,**4**,5
Nitzschia amphibiodes Hust.	357	(322)	1.8	(19)	**2**,3,4,5
Pinnularia streptoraphe Cl. var. 01	370	(415)	0.9	(10)	1,**2**,4
Gomphonema clavatum Ehr. var. 01	370	(401)	1.2	(38)	1,2,3,4,**5**
Gomphonema sdsp01	375	(391)	0.9	(86)	**1**,2,3,4,5
Frustulia rhomboides var. *crassinerva* (Bréb.) Ross	414	(280)	6.5	(15)	**1**
Nitzschia serpentiraphe L.-Bert. var. 01	462	(438)	3.1	(23)	1,**2**,3,4,5
Eunotia rabenhortii Grun.	467	(280)	0.8	(12)	**1**
Eunotia naegelii Migula	469	(325)	1.5	(28)	**1**,3,4,5
Diploneis parma Cl. Var. 01	507	(665)	1.0	(63)	**2**,3,4,5
Pinnularia gibba Ehr. var. 03	510	(346)	1.6	(11)	**1**
Eunotia monodon Ehr. var. 01	614	(301)	0.9	(17)	1,3,**4**,5
Eunotia incisa W. Sm. ex Greg. var. 01	679	(387)	1.2	(32)	**1**,2,3,4,5
Fragilaria ctsp01	731	(838)	0.7	(20)	**1**,2,3,4,5
Fragilaria ctsp02	877	(779)	0.8	(29)	1,**2**,3,4,5
Rhopalodia gibba (Ehr.) O. Müll.	964	(839)	0.9	(16)	**1**,2,3,5
Nitzschia amphibia var. *frauenfeldii* (Grun.) L.-Bert.	1,560	(1,964)	1.6	(6)	**1**,2,3
Amphora veneta Kütz.	1,730	(625)	5.1	(1)	**2**
N. amphibia Grun.	1,928	(1,462)	6.0	(12)	1,**2**,3,4,5
Gomphonema parvulum Kütz. var. 01	3,304	(2,984)	7.5	(37)	**1**,2
Gomphonema egsp01	5,046	(3,927)	7.3	(9)	1,**2**

the experiment, periphyton TP concentrations at the head of the channels also increased exponentially with the P load, and together, these findings confirm that periphyton TP concentration is one of the best indicators of P enrichment throughout Everglades marshes. Havens et al. (1999) also found periphyton TP concentrations significantly elevated relative to the water column in experimental P-enrichment mesocosms in Lake Okeechobee, Florida. They found periphyton P concentrations to be a more sensitive estimate of P availability than standard water column or soil measure-

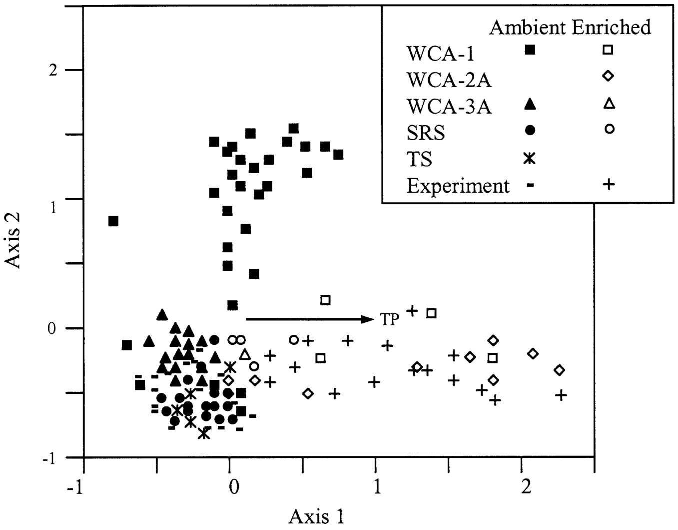

Fig. 6. Nonmetric multidimensional scaling biplot showing site scores based on diatom compositional similarity among sites in water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS) and the experimental sites in the SRS. Sites are designated as enriched or ambient following designations in Table 1. The vector representing the plane of least-squared deviation of TP with compositional dissimilarity is given ($R^2 = 0.59$).

ments, because they respond on timescales appropriate for early detection and management. Because the Everglades is so naturally depleted in P, any anthropogenically derived P entering the system is quickly removed by the active microbial community in the periphyton mats and detritus (Noe et al. 2003; Gaiser et al. 2004). Loading rates based on water column or soil concentrations will be underestimated, except in cases where the P-loading rates exceed the capacity for biotic uptake. Soils and water can take years to respond even to large increases in P-input concentration because of the rapid incorporation and downstream spiraling in the biota. By the time either parameter is detectably elevated in P, most ecosystem components have been significantly altered. This explains why the water column TP was only significantly correlated with distance from the inputs in WCA-2A, which has been enriched for decades and is already substantially altered ecologically (Childers et al. 2003). Soil concentrations in this marsh are also enriched, whereas they are not enriched in any other part of the system (Childers et al. 2003).

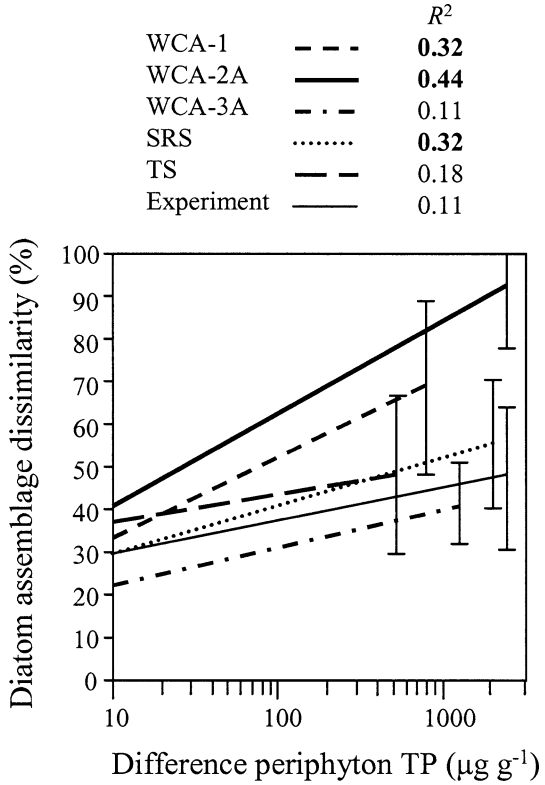

Fig. 7. Positive relationships of paired sample–sample differences in diatom composition and TP content within water conservation areas (WCA) 1, 2A, and 3A, Shark River Slough (SRS), and Taylor Slough (TS) and the experimental sites in the SRS. Error bars represent 95% confidence intervals for the entire relationship, and the correlations of the linear relationship of compositional dissimilarity (measured with the Bray–Curtis metric) with the logarithm of the TP difference are provided (significance indicated in bold).

Ambient concentrations of P in periphyton at unenriched sites differed among the marshes, due, in part, to differences in the contribution of calcite to the dry mass in which P was quantified. Background periphyton TP concentrations ranged from 370 to 480, 150 to 260, 60 to 190, and 50 to 170 μg of TP g^{-1} in WCA-1, WCA-3A, SRS, and TS, respectively, while the proportion of dry mass that was composed of calcite in these mats ranged in concert from 0% to 20%, 10% to 40%, 50% to 80%, and 70% to 90%, respectively. Because organic matter can sequester larger quantities of P than can be adsorbed to calcium carbonate, P concentrations in the carbonate-rich periphyton of the southern Everglades are artificially, or comparatively, deflated. If the proportion of P adsorbed to calcite were consistent throughout the marsh, we could calculate organic P by difference. However, though calcite can bind considerably less P than an equal mass of organic molecules, the binding capacity is variable and is influenced by a large suite of variables (Scinto and Reddy 2003). One obvious solution is to measure P on only the organic fraction after acidification to remove calcite and adsorbed P. However, estimates relying on this extraction procedure have been questioned because considerable interstitial and organically incorporated P can be liberated in the acidification process and incorrectly attributed to the calcite fraction (Scinto and Reddy 2003). Therefore, we would advocate that the detection of elevated TP in periphyton be based on basin-specific ambient ranges or that those ranges apply only to periphyton communities having a similar background organic content. For example, WCA-2A lies on calcitic bedrock and, at most interior sites, contains a floating or benthic mat containing up to 80% calcite, similar to mats in the SRS or TS. Applying ambient criteria for those marshes to WCA-2A suggests that concentrations of 450 μg of TP g^{-1} found at even the interiormost sites are significantly enriched relative to mats of a similar organic content in the SRS and TS (mean = 100 μg of TP g^{-1}).

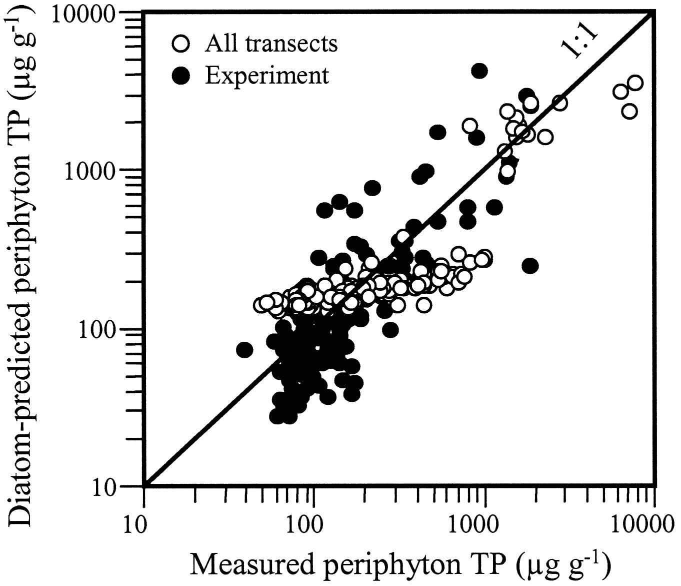

Fig. 8. Relationship of diatom-based predictions of periphyton TP content to actual measured values. Predictions were based on diatom TP optima derived from the combined transect (R^2 = 0.78) or experimental (R^2 = 0.67) data sets and tested with a random subset of the same data set.

A related and conspicuous consequence of increased P availability to Everglades periphyton communities is the disintegration of calcitic, mat-forming aggregations. While the negative relationship between periphyton biomass and TP content was most marked in the P-addition experiment, weaker relationships existed within each of the transects. This biomass loss is attributable, in part, to the loss of the calcite fraction of the periphyton. Metabolic processes that result in decreased water pH could contribute to calcite dissolution, in addition to the loss of bicarbonate-utilizing algae. Therefore, the organic content of the periphyton is a reliable indicator of P load, as long as it is measured relative to background conditions in the same marsh. Periphyton in WCA-1 is naturally highly organic, as this wetland is underlaid by silica sand rather than limestone as in the other basins. Therefore, although dry-weight biomass is weakly correlated with the P load in this basin, this metric is not as useful as in the calcareous marshes where highly organic periphyton communities are found only in enriched settings.

The decline of calcareous floating periphyton mat communities with increased P availability has been shown in another enrichment experiment in WCA-2A (Chiang et al. 2000; Pan et al. 2000), but whether other types of periphyton communities (i.e., epilithic, epiphytic, noncalcareous) behaved similarly was unknown. While, in our study, Chl *a* content was typically much higher in enriched than in ambient settings, ash-free dry-weight biomass declined with increasing periphyton P content within all the marshes and in the experiment. The negative relationship is therefore not implying that algal productivity is paradoxically decreasing with the addition of the limiting nutrient. Rather, production by some algae (mainly filamentous chlorophytes) is increasing while the original mat matrix must be losing considerable quantities of detrital and/or bacterial biomass, in addition to cyanobacteria and their calcite precipitate. The mechanism of the loss of the calcifying periphyton matrix appears to be an unusual characteristic of subtropical, calcareous wetlands subject to P enrichment that needs to be examined further. Rejmánková (2001) found a similar collapse of cyanobacterial communities in fertilized plots in a wetland in Belize, Central America. It is possible that green algal communities replacing the cyanobacterial–diatom community are capable of more rapid, and possibly luxury, consumption of P, thereby contributing to the biomass–TP relationship. Although biomass estimates are inherently more variable than TP measures and therefore should not be used alone to detect P enrichment, the repeated appearance of a negative trend when expecting a positive one shows that biomass should be monitored in conjunction with other parameters, given its importance to functional aspects of the aquatic community.

Environmental predictions based on compositional data often provide stronger estimates than single structural or functional parameters, partly because of their multidimensionality but also because of the tendency for species to have unique physiological requirements, especially with regard to limited resources in a system. Therefore, many investigators have advocated the type of multispecies environmental calibration models presented in this study for both monitoring current (Cooper et al. 1999; Slate and Stevenson 2000) and reconstructing past environmental change (Birks et al. 1990). Diatom-based P-calibration models have been developed for the Everglades but exclusively from WCA-2A (Cooper et al. 1999; Pan et al. 2000), where relationships of species to P availability are very strong and predictable. While there is no doubt of the utility of both experimental and spatially derived models in predicting P enrichment from a compositional analysis of a periphyton sample from this basin, data from the present study call into question the general applicability of these models to other Everglades basins. Weighted-averaging calibration models assume that optima and tolerances are consistent among the calibration and prediction data sets, an assumption that is typically robust when calibration sets are large enough (spanning a long environmental gradient with significant taxonomic turnover) and if the species are at equilibrium with the environmental variable in question.

We found that while each basin contained a predictable diatom community associated with P enrichment, the among-basin inconsistencies in P optima and tolerances of these taxa precluded the application of diatom-based calibration models for the strict quantification of P in basins outside those included in the calibration set. The among-basin variation in P optima and tolerances can be attributed to differences in the length of time that communities have been exposed to enhanced P. This is exemplified when comparing P optima derived from the experimental calibration set to those of the transects. Optima calculated for SRS and WCA-2A are higher than the experimental data would predict, suggesting that taxa are occurring in the natural environment at P concentrations above levels that limit their abundance when newly exposed to P in the experiment. While the P gradient in WCA-2A exceeded the range in the

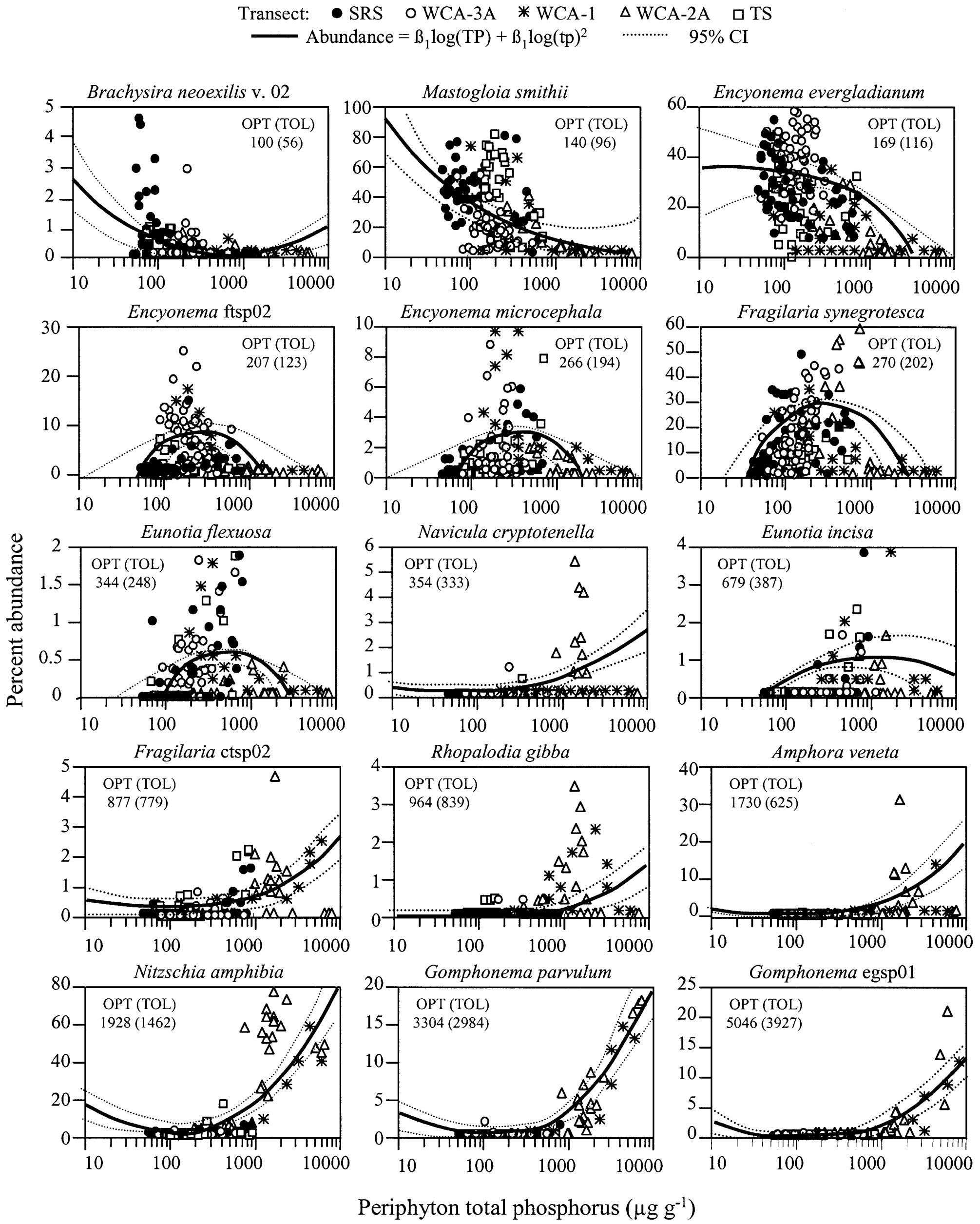

Fig. 9. Relationships of select diatom indicator taxa to periphyton TP content, modeled using a quadratic function that has been fitted to data from all sites and all transects. Weighted-average optima and tolerances for these taxa are also provided, and sites are coded by transect.

experiment, leading to an inflation of estimated optima at the gradient extremes, the range and distribution of P in the SRS transects was similar to the experiment and would not result in such mathematical over- or underestimations. Rather, it appears that even where there is considerable overlap in both the expression of the environmental gradient and taxonomic composition, optima can vary among data sets and can result in biased estimates of P when applied arbitrarily.

Variation in environmental optima, whether resulting from temporal lags in species responses to environment or to the modification of one environmental response by other environmental parameters, should not be assumed to be negligible in calibration studies, particularly those that derive "training sets" (i.e., Birks et al. 1990) from experimental data.

Quantitative estimations of diatom optima along experimental or spatial P gradients varied among data sets, but some species could be considered reliable indicators of unenriched conditions and others of enriched settings. Several species showed predictable trends in abundance that were consistent among sites. Of these, *A. veneta, G. parvulum,* and *N. amphibia* have been identified elsewhere as eutrophic indicators, while *M. smithii, E. evergladianum,* and *F. sy-*

negrotesca have been associated with unenriched sites (Raschke 1993; Cooper et al. 1999; Slate and Stevenson 2000). While absolute abundances of these species cannot be used as a quantitative estimate of P content because of high among-site variability, tracking trends in their abundances at particular sites can provide strong evidence for enrichment, particularly when combined in a multivariate approach with other less common species. The relative abundance of diatom taxa therefore offers another metric for measuring the magnitude of low-level P enrichment. This is an important alternative to metrics that are based on the full algal community, as P enrichment at most of the sites in this study did not cause water to be driven beyond the Redfield ratio (16 : 1, N : P), making traditional algal metrics that depend on shifts to N-fixing cyanobacteria unapplicable.

Because periphyton differs compositionally and structurally from basin to basin within the Everglades, the metrics that we present provide useful indications of enrichment when compared to baseline, unaffected conditions in the same marsh. Parameters that provide the most reliable indication of a P-loading history are periphyton TP concentration, the organic content of the mats, and species composition, although it is best to take a marsh-specific approach in monitoring change because the marshes have different natural starting points. Another periphyton metric not evaluated in this study but shown to provide a good indication of enrichment in WCA-2A is alkaline phosphatase activity, which decreases as the supply of dissolved P increases relative to demand (Newman et al. 2003). Together, these parameters can provide an early indication of the progression or management-related abatement of P-related effects in Everglades marshes. Overall, periphyton indicates the onset of enriched conditions significantly further downstream of soil and plant indicators in both the experiment and the transects (Childers et al. 2003; Gaiser et al. 2004). Childers et al. (2003) detected enriched conditions in soil and macrophyte communities up to 1, 4, 0.5, and 1 km downstream of inputs into WCA-1, WCA-2A, WCA-3A, and SRS, respectively, while periphyton indicated effects at 2, 14, 5, and 2 km downstream in the same marshes. Further, in comparing their data to that from the same transect sampled by Doren et al. (1997), Childers et al. (2003) found a doubling of soil P and an expansion of cattail downstream from canal inputs to WCA-1 and WCA-2A between 1989 and 1999, while no significant decadal changes in these parameters were detected in WCA-3A or SRS. Rashke (1993) related diatom diversity to sediment P concentrations in a 1990 survey along transects in the SRS and found P-related effects up to 1.6 km downstream of the P source, which is similar to our findings of change 1–2 km from the same input. This may be evidence that a reduction in P loading in protected areas of the ENP is having a beneficial effect or is at least halting the progression of eutrophication in downstream areas of the SRS.

Regular sampling of these transects, to verify decadal-scale environmental effects or improvements, will continue to be an important tool for long-term management and restoration of the Everglades. Although efforts are under way to reduce P loading in parts of the Everglades system, projections are that even low-level enhancements (5 μg L^{-1} above ambient) will continue to cause the progression of P-related ecosystem change at a rate of 10–1,000 m per decade (Gaiser et al. 2005). Dong et al. (2002) predict that periphyton recovery trajectories will be different and much slower than enrichment effects. Because of its ability to sequester P from the water column rapidly, periphyton-based P treatment of wetlands has been proposed to reduce P loading in protected marshes (Kadlec and Knight 1996). Our study shows, however, that this mechanism of treatment is likely to be effective only if the periphyton is subjected to very low-level P enhancements; otherwise, P-associated biomass loss and compositional changes reduce the aerial uptake capacity, and the amount of land required for efficient P removal will be vast. There is now ample evidence that periphyton-based metrics are perhaps the most reliable indicators of the onset of P enrichment in the Everglades, and the application of these models in large-scale restoration efforts should serve as a working model for other threatened wetlands.

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