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## Biotic and abiotic uptake of phosphorus by periphyton in a subtropical freshwater wetland

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### Abstract

Phosphorus (P) demand in extremely oligotrophic shallow water wetlands often exceeds supply and maintains water chemistry in a P-limiting condition. Phosphorus uptake by the calcareous periphyton community in the oligotrophic Florida Everglades was examined. Phosphorus removal from solution was used to obtain uptake parameters for epipelton, epiphyton, and metaphyton. Nutrient ratios (C:N:P) were higher in epiphyton compared to metaphyton or epipelton but all periphyton types were P-limited. Michaelis–Menten kinetic experiments resulted in  $K_m$  values ranging from 8.5 to 16.4  $\mu\text{M}$  and  $V_{\max}$  values ranging from 0.24 to 0.74  $\mu\text{mol g}^{-1}$  dry weight  $\text{min}^{-1}$ , in the order epiphyton, metaphyton, epipelton. Removal of inorganic P ( $\text{P}_i$ ) as  $\text{KH}_2\text{PO}_4$  and dissolved organic P ( $\text{P}_o$ ) as ATP from solution was best described by a first-order equation with rate constants ranging between 0.02 and 0.17  $\text{min}^{-1}$ . The values of  $K_m$  were greater than ambient dissolved reactive P (DRP) concentrations ( $<0.2 \mu\text{M}$ ); therefore  $V_{\max}$  is not reached under normal field conditions. The hydrolysis of  $\text{P}_o$  as ATP was rapid, being  $>0.67 \mu\text{mol g}^{-1}$  DW  $\text{min}^{-1}$ . After hydrolysis  $\text{P}_o$  uptake was similar to  $\text{P}_i$  uptake. It was hypothesized that P adsorption with  $\text{CaCO}_3$  in the periphytic matrix would add to the removal of P from solution. Using  $\text{H}_3^{32}\text{PO}_4$  and  $[^{32}\text{P}]\text{ATP}$  and a 0.01 M HCl extraction technique, P incorporated by epipelton was partitioned into biotic and abiotic compartments. The biotic compartment contained  $>83\%$  of the incorporated P after 12 h incubations. Biological demand exceeds abiotic adsorption in this P-limited system but adsorption mechanisms are responsible for a portion ( $<15\%$ ) of water column P removal.

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**Keywords:** Periphyton; Phosphorus; Uptake kinetics;  $^{32}\text{P}$  radioisotopes; Everglades

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## 1. Introduction

Phosphorus (P) is an essential nutrient frequently limiting the productivity of freshwater ecosystems. In highly oligotrophic wetlands, such as the interior of the Florida Everglades, total P (TP) is often below  $10 \mu\text{g l}^{-1}$  and dissolved reactive P (DRP) concentrations are typically at or below colorimetric detection limits ( $\sim 0.1 \mu\text{M}$ ; McCormick and O'Dell, 1996). Several studies have shown that P most often limits the productivity of pristine Everglades (Vymazal et al., 1994; Craft et al., 1995; McCormick et al., 1996). Despite low water P concentrations extensive mats of periphyton are common in sparsely vegetated, open water, Everglades marshes (Gleason and Spackman, 1974; McCormick et al., 2001).

Periphyton communities, the attached floral and faunal microorganisms that grow on submerged surfaces (Wetzel, 1983), of the Everglades are composed of complex assemblages of cyanobacteria, eubacteria, diatoms and eukaryotic algae (McCormick and O'Dell, 1996). High rates of periphyton productivity have been measured in several wetlands (Goldsborough and Robinson, 1996), particularly in subtropical and tropical systems (Zedler, 1980; Browder et al., 1982; Rejmánková and Komárková, 2000). In wet marshes of the Everglades, periphyton can account for a majority of the biomass ( $\sim 40\text{--}800 \text{ g m}^{-2}$ ; see Buzzelli et al., 2000). The presence of highly productive periphyton communities in P-limited systems has been linked to the increased uptake efficiency and rapid recycling of nutrients due to the close association of autotrophic and heterotrophic microbial components (Sand-Jensen, 1983; Wetzel, 1996). Periphyton productivity and nutrient cycling influences many of the biological and physicochemical aspects of these environments (Carlton and Wetzel, 1988; Hansson, 1989; McCormick et al., 1997).

Periphyton has a high affinity for P and responds more rapidly to P inputs relative to other wetland components (McCormick et al., 2001; Noe et al., 2002) and thus is important in the uptake and storage of P (Wetzel, 1999; McCormick and Scinto, 1999; McCormick et al., 2001). Previous research suggests periphytic uptake of P depends on numerous factors including water column P concentration, periphyton P content, forms of P available, and the growth stage and thickness of the mat (Horner et al., 1983; Sand-Jensen, 1983; Cotner and Wetzel, 1992). Algal P content in the range of  $1.1\text{--}2.8 \text{ mg g}^{-1}$  (dry weight (DW)) is considered necessary for normal growth (Müller, 1983). However, Grimshaw et al. (1993) observed prolific periphyton growth in the oligotrophic Everglades with periphyton P contents  $< 0.01 \text{ mg g}^{-1}$ . Hillebrand and Sommer (1999) found the optimal C:N:P stoichiometric ratio for maximal benthic microalgal growth rate was 119:17:1, slightly higher than the Redfield ratio of 106:16:1. They further suggest that a N:P ratio  $< 13$  and a C:N ratio  $> 10$  shows periphyton to be N-limited while a N:P ratio  $> 22$  and a C:P ratio  $> 180$  determines P-limitation (Hillebrand and Sommer, 1999).

Algae and bacteria are able to utilize dissolved organic P (DOP) in addition to inorganic P ( $\text{P}_i$ ). Few studies have measured DOP uptake by periphyton, but several have measured DOP utilization by algal and bacterial components of plankton (Ammerman and Azam, 1985; Bentzen et al., 1992; Cotner and Wetzel, 1992). Organic P mineralization rates are influenced by the C:P ratio of organic matter undergoing decomposition and the rate of phosphatase enzyme production (Newman and Reddy, 1993; Reddy et al., 1999; Wetzel, 1999).

The photosynthetic activity of periphyton coupled with high dissolved mineral content in Everglades water creates environmental conditions (high pH, low CO<sub>2</sub> partial pressure) favorable to the precipitation of CaCO<sub>3</sub> (Gleason and Spackman, 1974). Dissolved inorganic P can be co-precipitated or adsorbed to this CaCO<sub>3</sub> (Otsuki and Wetzel, 1972). Abiotic adsorption of P was suspected to account for P not recovered (48–72%) in addition experiments in epipelton-dominated marshes in Belize (Rejmánková and Komárková, 2000) and has been suggested as an effective mechanism for the removal of P from agriculturally enriched Everglades water (Grimshaw et al., 1993). To our knowledge quantification of abiotic uptake in periphyton has not been studied.

The objective of this study was to obtain a mechanistic understanding of P uptake by periphyton by: (i) comparing P<sub>i</sub> uptake kinetics between periphyton types, i.e. epipelton, epiphyton, and metaphyton; (ii) determination of the P<sub>i</sub> and P<sub>o</sub> uptake kinetics of epipelton; and (iii) assessing the relative magnitude of biotic and abiotic uptake mechanisms by epipelton. Uptake was determined in a series of laboratory and field studies using a solution depletion technique. Partitioning of periphyton-incorporated P by biotic and abiotic mechanisms was evaluated using <sup>32</sup>P and a simple extraction procedure.

## 2. Methods

### 2.1. Study site

The original Everglades has been fragmented into a series of hydrologic units: the Everglades Agricultural Area (EAA), three Water Conservation Areas (WCAs), and Everglades National Park (ENP). Water Conservation Area 2 is the smallest of the three WCAs (547 km<sup>2</sup>). Despite being impacted by nutrient enriched water derived from the EAA (Belanger et al., 1989; McCormick et al., 1996) the interior of WCA2 retains characteristics of unimpacted Everglades such as low P concentrations, low macrophyte biomass and characteristically high periphyton biomass (McCormick et al., 2001). Periphyton commonly covers the peat soil (epipelton), the submerged portions of macrophytes (epiphyton), and forms floating mats often in association with purple bladderwort (*Utricularia purpurea* Walt.) (metaphyton) (sensu the nomenclature of Stevenson et al., 1996; and McCormick et al., 1998). Field experiments and sample collection were conducted at an interior site (26°17.17' N, 80°24.73' W ± 30 m) of WCA2A.

### 2.2. Periphyton collection and characterization

Periphyton, used in laboratory experiments, was collected from the field on 18 October 1994 and 8 June 1995. Epipelton, associated with the surficial layers of detrital material at the peat soil surface (aka. microflora of detritus; sensu Wetzel, 1996) was collected with a 1 mm mesh screen. Metaphyton floating in the water column was gently collected by hand. Epiphyton grew in thick (up to 3 cm) encasements on sawgrass culms, particularly those that were senescent. Epiphyton was collected by harvesting senescent culms from which the epiphyton was removed by scraping with a scalpel. Periphyton was stored in site water under lights (cool-white fluorescent; photosynthetic photon flux density, ppfd = 95 μmol m<sup>-2</sup> s<sup>-1</sup>

at bench top) on 16 h light and 8 h dark cycle until used in uptake experiments (<7 days). Phosphorus uptake was considered a function of the periphyton community including the activities of all heterotrophic and autotrophic components. No taxonomic characterization was conducted. However, the periphyton taxonomy of this area was previously characterized by McCormick and O'Dell (1996) and McCormick et al. (1996, 1998).

Periphyton dry weight was determined after oven drying at 70 °C to constant weight (72 h) followed by combustion in a muffle furnace at 450 °C for 12 h to determine ash content. Ashing at 450 °C, as opposed to 500 °C (APHA, 1992) avoids disruption of carbonate material. This procedure has been used for Everglades periphyton (Craft et al., 1995) and has been shown to efficiently remove organic carbon from soils (Nelson and Sommers, 1996). Subsamples were digested with perchloric acid and the digestate was analyzed colorimetrically for total P (APHA, 1992). Total carbon (TC) and nitrogen (TN) were determined on oven-dried samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ; Nelson and Sommers, 1996). Total inorganic C (TIC) was determined on ashed material (as above) using the CNS analyzer. Total organic C (TOC) was determined by difference (Craft et al., 1995).

### 2.3. Periphyton P uptake kinetics

Epipelon, metaphyton, and epiphyton were incubated in the laboratory by placing 2 g periphyton with 200 ml site water to achieve a 1:100 wet weight to solution ratio. Inorganic P as  $\text{KH}_2\text{PO}_4$  was added to bring the initial solution concentrations ( $C_0$ ) to 3.5  $\mu\text{M}$  (4.7–6.0  $\mu\text{mol g}^{-1}$  DW when normalized for periphyton mass). Triplicate vessels of each periphytic type and a control without periphyton were incubated in a light booth at 27 °C and  $\text{ppfd} = 95 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Solution aliquots (10 ml) were collected at 0, 5, 15, 30, and 45 min after P additions, filtered (0.2  $\mu\text{m}$ ), and analyzed colorimetrically for DRP (APHA, 1992). Dissolved reactive P remaining in solution, normalized for periphyton dry mass and decreasing volume, was plotted against time. Phosphorus removal from the water column ( $\mu\text{mol P g}^{-1} \text{DW min}^{-1}$ ) was described by the first-order equation,  $C = C_0 \times \exp^{-kt} + b$ ; where  $C$  is P mass in solution normalized for periphyton dry weight ( $\mu\text{mol g}^{-1}$  DW) at any time ( $t$ ),  $C_0$  the initial solution P mass ( $\mu\text{mol g}^{-1}$  DW),  $k$  the first-order rate constant ( $\text{min}^{-1}$ ), and  $b$  is the asymptote or the dry weight normalized mass in solution at which P influx to periphyton equals P efflux. Coefficients describing P removal were obtained using non-linear, least-squares regression.

To determine uptake kinetic parameters each of the periphyton types were incubated at 15 initial DRP (as  $\text{KH}_2\text{PO}_4$ ) concentrations (0.2–40.5  $\mu\text{M}$ ) for 15–90 min (based on the apparently linear portions of the preceding depletion curves). The experimental protocol was as above, but with each vessel being sampled only once. Coefficients of uptake rates, normalized for periphyton dry weights, and substrate concentration were fit to the Michaelis–Menten equation with non-linear, least-squares regression.

### 2.4. Inorganic and organic P uptake by epipelon

We focused additional experiments on the epipelon because of its prevalence (a nearly continuous covering of the soil surface), its association with detrital material, and because

of a suspected role in P sequestration in the soil. We conducted field experiments, in June 1995, to compare the hydrolysis and uptake of a labile organic P ( $P_o$ ) containing substrate, adenosine triphosphate (ATP), to  $P_i$  uptake. Initially, filtered water ( $0.2 \mu\text{m}$ ) was distributed ( $0.90 \text{ l}$ ) into polypropylene mason jars fit into floating racks. These vessels were open to the atmosphere but were submerged such that the liquid portion was in the water column to help maintain ambient temperatures. Approximately  $9 \text{ g}$  of wet weight epipelton was added to each vessel to provide a nominal  $1:100$  wet weight to water ratio. Phosphorus ( $P_i$  as  $\text{KH}_2\text{PO}_4$ ) was added to achieve five initial concentrations (four replications per concentration). Initial concentrations were: ambient ( $0.1 \mu\text{M}$ ),  $0.9$ ,  $1.7$ ,  $3.3$ , and  $6.6 \mu\text{M}$  ( $0.2$ ,  $1.5$ ,  $2.7$ ,  $5.6$ , and  $10.6 \mu\text{mol DRP g}^{-1} \text{ DW}$  when normalized for epipelton mass). Water samples were collected at  $5$ ,  $15$ ,  $30$ ,  $45$ , and  $60 \text{ min}$  after P addition and were filtered ( $0.2 \mu\text{m}$ ), stored in ice water, and transported to the laboratory. After incubation the epipelton was collected by gravity filtration (nominal  $0.7 \mu\text{m}$  porosity, Whatman #41) and dried. Uptake calculations were normalized for volume and epipelton dry weight. A similar experiment was then conducted where  $0.90 \text{ l}$  of filtered water was augmented with ATP to achieve total dissolved P (TDP) concentrations of ambient ( $0.6 \mu\text{M}$ ),  $2.2$ ,  $3.8$ ,  $7.0 \mu\text{M}$  ( $0.9$ ,  $3.3$ ,  $5.5$ , and  $10.1 \mu\text{mol TDP g}^{-1} \text{ DW}$ ). Water samples were collected from each vessel before P addition and at  $15$ ,  $30$ ,  $60$ ,  $120$ , and  $240 \text{ min}$ , filtered, and analyzed for DRP and TDP. The concentration of dissolved  $P_o$  was determined by difference. Epipelton was collected after incubation as above. A control of unfiltered water augmented with ATP to bring the initial TP concentration to  $3.8 \mu\text{M}$  was included. First-order kinetics were fit to DRP in the  $P_i$  experiment and to TDP concentrations in the  $P_o$  uptake incubations as above. In all experiments site water or solution samples were analyzed colorimetrically for DRP, and/or digested by persulfate oxidation then analyzed colorimetrically for TDP (APHA, 1992).

### 2.5. Biotic versus abiotic P uptake by epipelton

Abiotic adsorption of P to the  $\text{CaCO}_3$  matrix of periphyton may be partially responsible for water column P removal especially during active photosynthesis. We therefore conducted experiments where  $P_i$  and  $P_o$  uptake by epipelton was separated into biotic and abiotic processes. We tested the effects of light, solution P concentration, and incubation time on the relative magnitude of biotic and abiotic uptake. Uptake was partitioned into biotic and abiotic by using an acid extraction. An optimal extractant would dissolve  $\text{CaCO}_3\text{-P}$  (abiotic), releasing DRP to solution, but would not cause the release of biotically bound  $P_o$ . Release of  $P_o$  is evident as the difference in TDP (after digestion) and DRP in the extracted solution. Preliminary research showed extractions using  $0.01 \text{ M HCl}$  in a  $1:100$  wet weight epipelton to solution ratio for  $2 \text{ h}$  ( $\sim\text{pH} = 2.5\text{--}3.0$ ) removed abiotically bound  $P_i$  without causing severe cell damage or hydrolysis of soluble  $P_o$ . Extracted solutions had  $\text{TDP} = \text{DRP}$  as analyzed by *t*-tests ( $P > 0.05$ ). Periphyton samples, with and without extraction were examined by scanning electron microscopy (SEM) (S-4000, Hitachi, Tokyo, Japan) to confirm  $\text{CaCO}_3$  removal and cellular integrity.

In  $16$  total incubations (four replicates each) epipelton was added to  $50 \text{ ml}$  filtered ( $0.2 \mu\text{m}$ ) field-collected water, to maintain a  $1:100$  wet weight to water ratio.  $^{32}\text{P}$  as  $\text{H}_3\text{PO}_4$  in  $0.02 \text{ M HCl}$  ( $P_o$

an activity of  $111 \text{ TBq mmol}^{-1}$ . Additions of 0.39–1.16 MBq were made to each vessel. The  $^{31}\text{P}$  was maintained at ambient ( $0.13 \mu\text{M}$ ) or was increased by addition of unlabelled  $\text{KH}_2\text{PO}_4$  to  $3.33 \mu\text{M}$  in half the  $\text{P}_i$  treatments (four each). Similarly,  $\text{P}_o$  treatments contained nominally ambient concentrations ( $0.32 \mu\text{M}$ ; the addition of  $^{32}\text{P}$ ATP increased the  $^{31}\text{P}$  concentration in the ambient  $\text{P}_o$  treatments to  $0.42 \pm 0.03 \mu\text{M}$ , mean  $\pm$  S.D.) or were increased by addition of unlabelled ATP to  $3.62 \pm 0.03 \mu\text{M}$  (including  $^{31}\text{P}$  added with the  $^{32}\text{P}$ ATP). Two sets of  $\text{P}_i$  and two sets of  $\text{P}_o$  incubations of each concentration were incubated under lights ( $\text{ppfd} = 95 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) while two sets of each were incubated in the dark. Of these, one set each was analyzed after incubations of 1 and 12 h for  $\text{P}_i$  treatments and at 4 and 12 h for the  $\text{P}_o$  incubations. Distilled water blanks provided control activities.

After incubation, vessel contents were filtered ( $0.2 \mu\text{m}$ ) and  $100 \mu\text{l}$  of the filtrate was added to 10 ml of scintillation cocktail (Scinti-Verse I, Fisher Scientific). Radioactivity was determined by liquid scintillation counting (LSC; LS3801, Beckman Instruments Inc.). The epipelton was washed into 50 ml of 0.01 M HCl and extracted for 2 h, followed by filtration ( $0.2 \mu\text{m}$ ) and counting. The initial activity was partitioned into that which remained in the water, was abiotically bound (released during extraction), or was biotically incorporated (by digestion of epipelton after extraction as above). All fractions were normalized to recovered activity.

## 2.6. Statistics

Unless noted, data in text, figures, and tables, are presented as means  $\pm$  S.D. Parameters describing first-order uptake or Michaelis–Menten kinetics were obtained by non-linear, least-squares regressions and are presented as means  $\pm$  S.E. Differences in parameter estimates were evaluated with an *F*-test and pseudo-posthoc analyses based on the principle of conditional error (Milliken and Johnson, 1984). Three-way ANOVAs were conducted individually for  $\text{P}_i$  and  $\text{P}_o$  uptake experiments. ANOVAs were determined using time, light, and initial  $^{31}\text{P}$  concentration as factors. All model fits and statistics were performed on the SPSS 10.1 for Windows statistical package (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Periphyton chemical characterization

Total P contents of epipelton were higher than for metaphyton or epiphyton (Table 1). Metaphyton chemical characteristics were generally intermediate to epipelton and epiphyton, suggesting a continuity of types. Ash contents indicate that epiphyton was more encrusted with  $\text{CaCO}_3$  than epipelton or metaphyton. Epiphyton TP contents were approximately 40% that of epipelton, but when normalized for differences in organic matter content (TOC), the TP was about 70% that of epipelton. Total C and TOC increased inversely to decreasing ash content. The TN content was between 11 and 23 mg TN  $\text{g}^{-1}$ . Like P, TN content followed the order epipelton > metaphyton > epiphyton. Total N:TP molar ratio ranged 152–231, the ratio being higher for epiphyton than for metaphyton of epipelton. Total N increased proportionally to an increase in TOC content and was between 9 and 13% (g/g) of TOC

Table 1

Selected chemical characteristics (dry weight basis) of epipelton, metaphyton, and epiphyton used in kinetic uptake experiments and of epipelton used in field comparisons of inorganic P and organic P ( $P_i$  and  $P_o$ , respectively) uptake, and in biotic and abiotic partitioning experiments

Chemical component	Kinetic determinations			$P_i$ and $P_o$	
	Epipelton	Metaphyton	Epiphyton	Uptake epipelton	Partitioning epipelton
Total P ( $\text{mg kg}^{-1}$ )	289	189	104	272	228
Total C ( $\text{g kg}^{-1}$ )	298	247	218	282	272
Total inorganic C ( $\text{g kg}^{-1}$ )	96	104	105	96	96
Total organic C ( $\text{g kg}^{-1}$ )	202	143	113	187	177
Total N ( $\text{g kg}^{-1}$ )	20	13	11	23	22
Ash content ( $\text{g kg}^{-1}$ )	434	541	619	472	472
TOC:TN:TP (mol:mol)	1806:153:1	1954:152:1	2770:231:1	1777:187:1	2007:214:1

for all samples. Total P increased with increasing TC and TOC and was inversely related to TIC and ash content.

### 3.2. Periphyton P uptake kinetics

Comparisons of P uptake by epipelton, metaphyton and epiphyton showed that water column P reduction was most rapid in the order epiphyton > metaphyton > epipelton (Fig. 1a). During the first 45 min of the incubation, solution DRP in epiphyton treatments was reduced to  $0.19 \pm 0.01 \mu\text{M}$ , whereas metaphyton ( $1.26 \pm 0.34 \mu\text{M}$ ) and epipelton ( $2.21 \pm 0.16 \mu\text{M}$ ) treatments contained significantly greater P ( $P < 0.05$ ; not shown). The reduction of P in solution when normalized for periphyton dry weight followed first-order kinetics for all three periphyton types ( $P < 0.001$ ). The fit equations were used to produce uptake rate constants ( $k$ ) and to predict the expected P concentration (dry weight normalized) at which continued uptake ceases (asymptotic intercept,  $b$ ; Fig. 1a and Table 2). The rate constants were in the order of epiphyton > metaphyton = epipelton with epiphyton having significantly higher rates ( $P < 0.001$ ) than both metaphyton and epipelton (Table 2). Results of an  $F$ -test showed that uptake rates between metaphyton and epipelton were not statistically different ( $P > 0.05$ ). Although ranging  $0.47$ – $1.73 \mu\text{mol g}^{-1} \text{DW}$ , the predicted asymptotic P concentrations were not statistically different for the periphyton types (Table 2).

Uptake rates, determined at 15 P concentrations, varied within periphyton types with rates ranging in: epiphyton =  $0.039$ – $0.623 \mu\text{mol P g}^{-1} \text{DW min}^{-1}$ , metaphyton =  $0.028$ – $0.397 \mu\text{mol P g}^{-1} \text{DW min}^{-1}$ , and epipelton =  $0.017$ – $0.195 \mu\text{mol P g}^{-1} \text{DW min}^{-1}$ . At ambient levels ( $0.2 \mu\text{M P}$ ) the change in solution concentration was minimal and remained within the analytical variability for DRP ( $0.1 \mu\text{M}$ ). Thus uptake was not determined at ambient concentration. For all periphyton types, initial uptake rate was dependent on initial P in solution, and was in the order epiphyton > metaphyton > epipelton, with epiphyton generally being two to three times higher than the other two types. Similarly, maximum uptake rates ( $V_{\text{max}}$ ) estimated by fitting data to the Michaelis–Menten equation by non-linear, least-squares regression (Fig. 2, all fits  $P < 0.001$ ) were in the order epiphyton > metaphyton > epipelton (Table 3). The Michaelis constants ( $K_m$ ) were in

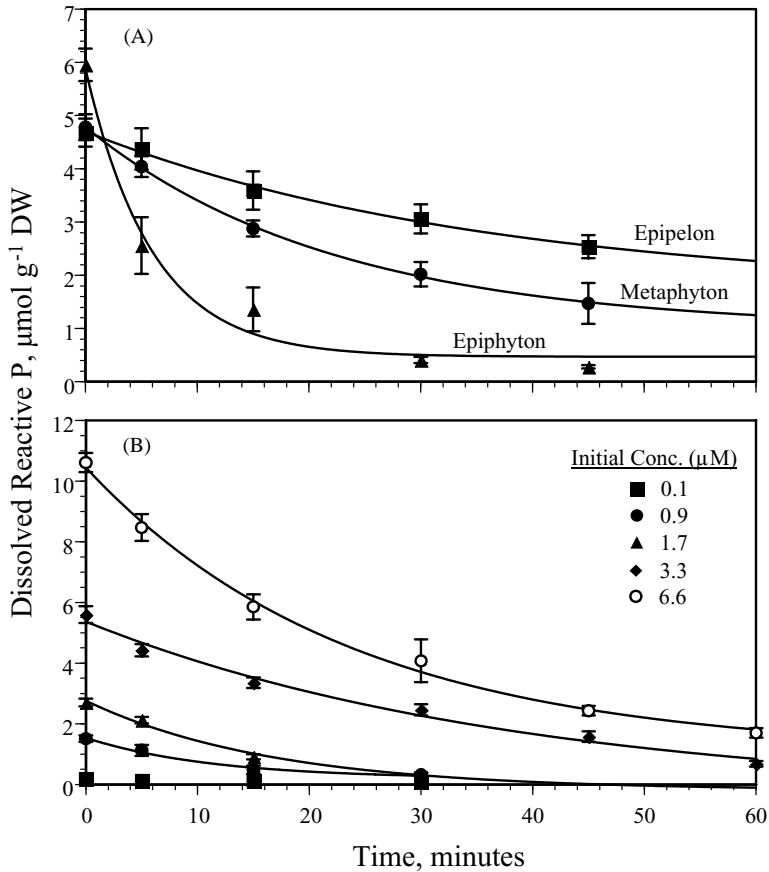


Fig. 1. Removal from solution of dissolved reactive P (DRP) (mean  $\pm$  S.D.,  $n = 3$ ), normalized for solution volume and periphyton dry weight, with data fit to curves describing first-order kinetics. (A) Laboratory conditions with epipelton, metaphyton, and epiphyton. (B) Field incubations using epipelton at several initial DRP concentrations ( $n = 4$ ).

the order epipelton > metaphyton > epiphyton, but were similar for epipelton and metaphyton (Table 3). An  $F$ -test showed that the three curves were significantly different from each other regarding estimated values of  $V_{\text{max}}$  ( $P = 0.039$ ) and  $K_m$  ( $P = 0.038$ ) but pseudo-posthoc tests (also based on the principle of conditional error) were not able to differentiate individual comparisons between periphyton types for either parameter.

### 3.3. Inorganic and organic P uptake by epipelton

Experiments, conducted in the field, showed rapid removal of P (as  $\text{KH}_2\text{PO}_4$ ) from solution at concentrations above ambient (Fig. 1b). At ambient P levels no detectable change in concentration was observed. Ambient concentrations were reached in 30–45 min when

Table 2

Solution initial P concentration (mean  $\pm$  S.D.) and iteratively estimated curve parameters (mean  $\pm$  S.E.) and  $r^2$  of the exponential P removal curves. Fitted curves are presented in Figs. 1 and 3, and have the form  $C = C_0 \times \exp^{-kt} + b$

Experiment	Initial P concentration <sup>a</sup> ( $C_0$ , $\mu\text{mol g}^{-1}$ DW)	Removal rate constant ( $k$ , $\text{min}^{-1}$ )	Asymptotic intercept ( $b$ , $\mu\text{mol g}^{-1}$ DW)	$r^{2b}$
Kinetic determinations				
Epiphyton	$6.0 \pm 0.3$	$0.17 \pm 0.02$ a	$0.47 \pm 0.17$ a	0.97
Metaphyton	$4.8 \pm 0.2$	$0.05 \pm <0.01$ b	$1.00 \pm 0.34$ a	0.97
Epipelton	$4.7 \pm 0.3$	$0.03 \pm <0.02$ b	$1.73 \pm 0.97$ a	0.91
Epipelton $P_i$ uptake				
	$1.5 \pm 0.1$	$0.08 \pm 0.01$ a	$0.17 \pm 0.04$ a	0.96
	$2.7 \pm 0.1$	$0.07 \pm <0.01$ a	$0.10 \pm 0.05$ a	0.99
	$5.6 \pm 0.3$	$0.03 \pm <0.01$ b	$-0.37 \pm 0.53$ a	0.98
	$10.6 \pm 0.3$	$0.04 \pm <0.01$ c	$1.07 \pm 0.37$ b	0.98
Epipelton $P_o$ uptake				
	$3.3 \pm 0.4$	$0.09 \pm 0.02$ a	$0.98 \pm 0.11$ a	0.84
	$5.5 \pm 0.2$	$0.04 \pm <0.01$ b	$0.70 \pm 0.11$ a	0.97
	$10.1 \pm 0.4$	$0.02 \pm <0.01$ c	$0.43 \pm 0.33$ a	0.97

Parameter estimates followed by similar letters within an experiment were not significantly different ( $P < 0.05$ ).

<sup>a</sup> Initial concentration (normalized for periphyton dry weight) of dissolved reactive P for kinetic determinations and epipelton  $P_i$  uptake and total dissolved P for epipelton  $P_o$  uptake.

<sup>b</sup> All curve fitting was significant at  $P < 0.001$ .

the initial solution P was below  $1.7 \mu\text{M}$ . First-order rate constants were obtained for P uptake curves fit for initial concentrations (normalized for epipelton dry weight) greater than ambient (all fits  $P < 0.001$ ; Fig. 1b and Table 2). The first-order rate constants were in the range of  $0.03$ – $0.08 \text{ min}^{-1}$ , and were generally lower at highest concentrations. Initial concentration

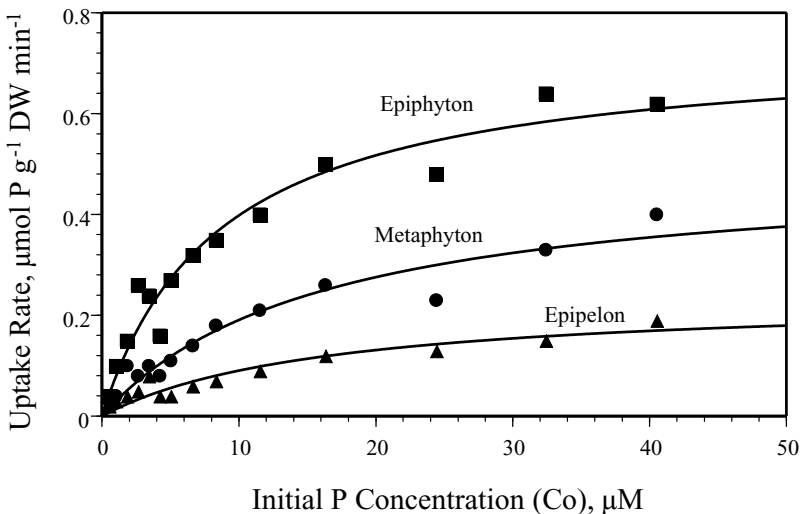


Fig. 2. Uptake rate ( $V_{\text{max}}$ ) vs. initial dissolved reactive P (DRP) concentration for epipelton, metaphyton, and epiphyton. Data was iteratively fit to Michaelis–Menten kinetics. All curves  $r^2 = 0.89$ – $0.94$  ( $P < 0.001$ ).

Table 3  
Michaelis–Menten parameters for inorganic phosphorus ( $P_i$ ) uptake by three periphyton types

Periphyton type	Initial P range <sup>a</sup> ( $\mu\text{M}$ )	$V_{\text{max}}$ ( $\mu\text{mol g}^{-1} \text{DW min}^{-1}$ )	$K_m$ ( $\mu\text{M}$ )	$r^2$ <sup>a</sup>
Epiphyton	0.2–40.5	$0.74 \pm 0.05$	$8.5 \pm 1.6$	0.94
Metaphyton	0.2–40.5	$0.50 \pm 0.06$	$16.1 \pm 4.1$	0.93
Epipelon	0.2–40.5	$0.24 \pm 0.04$	$16.4 \pm 5.2$	0.89

Parameters are mean  $\pm$  S.E. resulting from fit curves.

<sup>a</sup> All curve fitting was significant at  $P < 0.001$ .

had a significant effect on the uptake rate constant ( $P < 0.001$ ). The predicted asymptotic concentrations of P remaining in solution were not significantly different ( $P > 0.05$ ) for initial concentrations =  $5.6 \mu\text{mol g}^{-1} \text{DW}$ . A significantly greater mass of P (expressed  $\text{g}^{-1} \text{DW}$ ) was predicted to remain in solution for the highest initial concentration ( $10.6 \mu\text{mol g}^{-1} \text{DW}$ ).

In the  $P_o$  uptake experiment, changes in P concentrations showed that the added ATP (initially  $P_o$ ) was hydrolyzed to DRP (minimal  $P_o$ ) within 15 min for all initial concentrations (Fig. 3). This rapid hydrolysis occurred in all epipelon treatments and in a water-only control ( $C_0 = 3.4 \mu\text{M}$ ). Once hydrolyzed the P concentration in the epipelon-containing treatments decreased rapidly while that in the control remained relatively constant (Fig. 3). Dissolved  $P_o$  was calculated as the difference between TDP (by digestion) and DRP. Analytical variability occasionally produced negative concentrations of  $P_o$ , which were interpreted as complete enzymatic hydrolysis of  $P_o$  to DRP. Phosphorus concentrations returned to ambient P levels within 120 min for all epipelon-containing treatments. The reduction in TDP in solution followed first-order kinetics ( $P < 0.001$ ) for all initial concentrations greater than ambient and produced rate constants that decreased with increasing initial TDP concentration (Table 2). An *F*-test showed rate constants to differ with initial concentration ( $P < 0.05$ ) but that the asymptotic P remaining in solution did not differ by concentration (Table 2).

#### 3.4. Biotic versus abiotic P uptake by epipelon

Visual inspection of epipelon under SEM showed filaments encrusted with  $\text{CaCO}_3$ . These crystals were absent in epipelon treated with 0.01 M HCl (Fig. 4). Presumably, dissolution of this  $\text{CaCO}_3$  causes release of abiotically bound P. Filaments remained intact after acid extraction suggesting the method did not cause cell damage and release of cellular constituents. Preliminary evaluation using reagent grade  $\text{CaCO}_3$ ,  $\text{KH}_2\text{PO}_4$ , glucose-6-phosphate (G6P), and ATP showed that this extraction completely dissolved  $\text{CaCO}_3$  releasing  $\text{KH}_2\text{PO}_4$  to solution but did not hydrolyze appreciable quantities of the added  $P_o$  models G6P and ATP (data not shown; Scinto, 1997).

We determined the relative proportion of biotic and abiotic mechanisms to total P uptake by quantifying the  $^{32}\text{P}$  activity remaining in the water, in extracted solutions, or in digests of epipelon. Relative activity of each fraction was determined as a percentage of the total activity recovered which averaged  $89.65 \pm 15.37\%$  for all incubations. Biotic activity dominated the uptake and partitioning of  $P_i$  and  $P_o$  under all conditions.

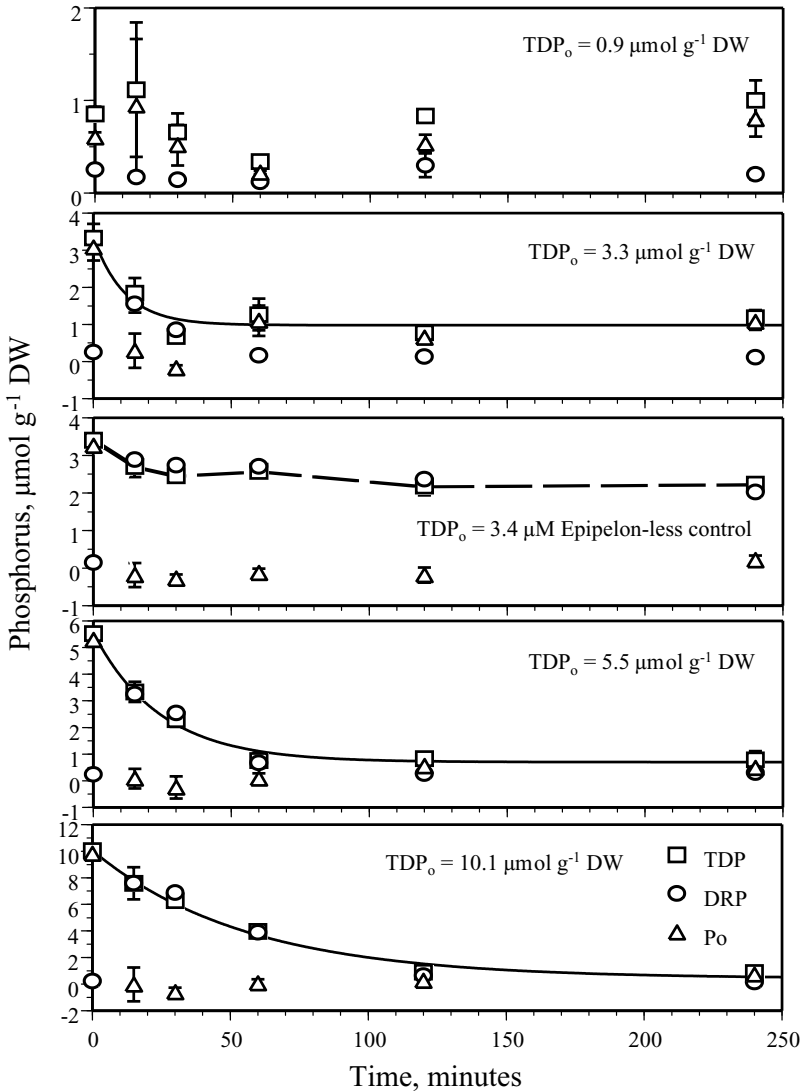


Fig. 3. Concentrations of total dissolved P (TDP), dissolved reactive P (DRP), and organic P ( $P_o$ ) (by difference), normalized for volume and dry weight (DW), of epipelton during field  $P_o$  uptake experiments. Unfiltered site water was spiked to 0.9 (ambient), 3.3, 5.5, and 10.1  $\mu\text{mol TP g}^{-1}$  DW epipelton with ATP. Unfiltered water controls devoid of epipelton were run at  $C_0 = 3.4 \mu\text{M}$ . First-order kinetic curves were fit to the TDP data for initial concentrations above ambient. Points indicate mean solution concentration ( $\pm$ S.D.,  $n = 4$ ).

Incubation time significantly affected the  $^{32}\text{P}_i$  activity in all three compartments (Table 4). There was a movement of the activity from the water and abiotic fractions to the biotic with time (Fig. 5). This transfer was not affected by light condition (non-significant main effect in all three fractions). At ambient concentrations the activity in the water fraction was

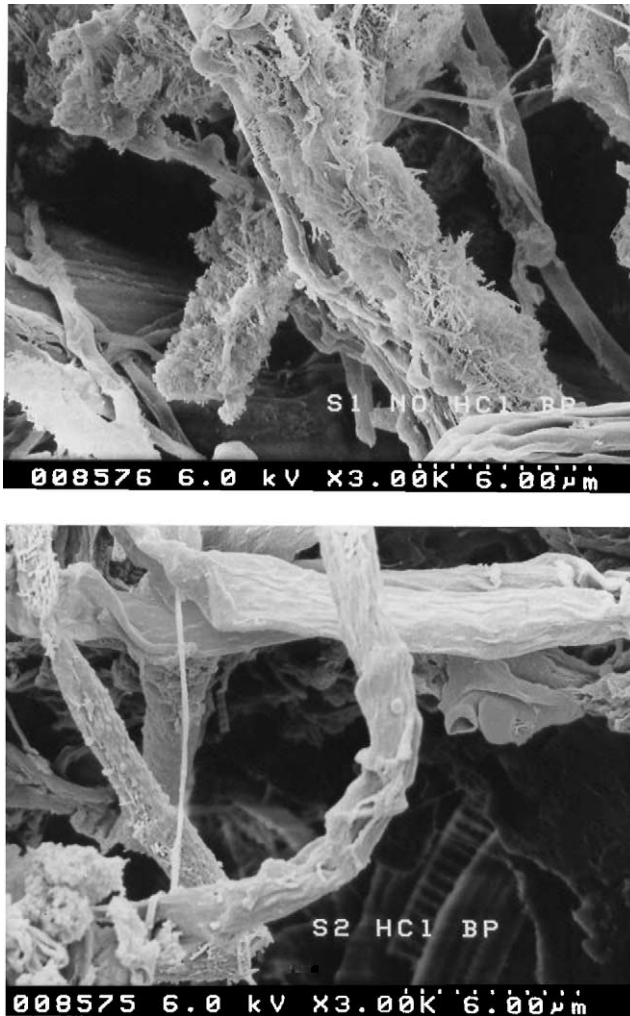


Fig. 4. Scanning electron micrograph of typical filament from epipelton showing  $\text{CaCO}_3$  encrustation (upper) and a typical filament after 0.01 M HCl extraction (lower). Note lack of encrustation after 2 h treatment with dilute (pH = 2.5–3.0) acid.

reduced to its minimum by the 1 h initial sampling and did not significantly change through the duration of the incubation ( $P = 0.263$ ). Conversely, in the enriched incubations the water activity was high at 1 h but moved into the biotic fraction by 12 h. Abiotic uptake was responsible for a larger percentage of uptake at ambient concentrations than at enriched but accounted for less than 14% of the added activity under all conditions. By the end of the incubations >83% of the added  $^{32}\text{P}_i$  activity was in the biotic fraction.

The partitioning of  $^{32}\text{P}_o$  activity appeared similar to that of the  $\text{P}_i$  with uptake dominated by biotic mechanisms under all conditions (Fig. 6). Again, the significant effect of time

Table 4

Results of ANOVA comparing effects of time, light, and initial inorganic  $^{31}\text{P}_i$  ( $\text{KH}_2\text{PO}_4$ ) concentration on the partitioning of  $^{32}\text{P}$  (carrier-free  $\text{H}_3\text{PO}_4$ ) activity in water, abiotic, and biotic fractions during uptake by epipelton

Source of variation	d.f.	MS	<i>P</i>
<b>Water</b>			
Time	1	1177.0	<0.001
Light	1	1.7	0.742
Concentration (conc.)	1	1.8	0.732
Time $\times$ conc. <sup>a</sup>	1	794.9	<0.001
Error	22	15.3	
<b>Abiotic</b>			
Time	1	317.0	<0.001
Light	1	77.5	0.056
Concentration (conc.)	1	1226.1	<0.001
Error	22	19.1	
<b>Biotic</b>			
Time	1	1717.4	<0.001
Light	1	2.5	0.654
Concentration (conc.)	1	1.0	0.773
Time $\times$ conc.	1	349.0	<0.001
Error	22	12.2	

<sup>a</sup> Note: Only significant interactions are presented.

was to move P from the water and abiotic fractions into the biotic (Table 5). Slightly less activity was removed from the water and more P was abiotically bound under light than in dark conditions. Partitioning was affected by initial P concentrations: relatively greater abiotic uptake occurred under ambient concentrations while more activity partitioned into the biotic fraction with spiked initial concentrations. However, numerous significant interactions complicated these comparisons (Table 5).

## 4. Discussion

### 4.1. Characterization of periphyton

The chemical characteristics of periphyton in this study were similar to those previously reported for oligotrophic Everglades wetlands. The TP contents of our periphyton, ranging 104–289 mg kg<sup>-1</sup> DW are consistent with similar values reported for interior Everglades periphyton (Swift and Nicholas, 1987; McCormick et al., 2001). This TP is less than that reported in areas impacted by P-laden canal discharges (Grimshaw et al., 1993; McCormick et al., 1996; Vymazal et al., 1994). The N:P ratio ranged from 152 to 231:1 (mol:mol), with the ratio generally higher in epiphyton than in epipelton. These high N:P ratios were similar to those previously reported for the Everglades (Grimshaw et al., 1993; McCormick et al., 2001) and substantiate the P-limited nature of pristine Everglades periphyton (cf. Hillebrand and Sommer, 1999). Ash content ranged 43–62% (dry weight) and was higher in epiphyton

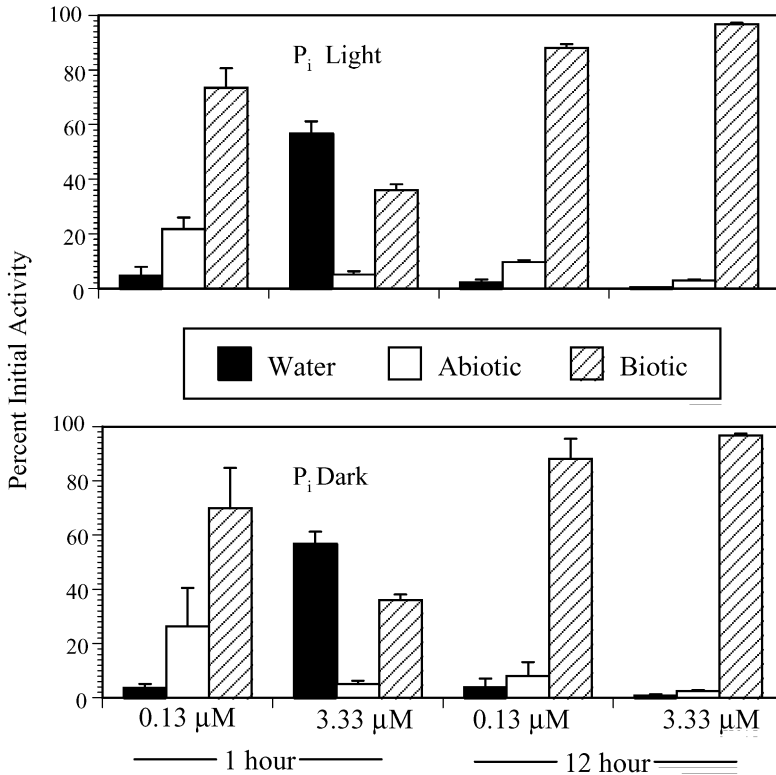


Fig. 5. Partitioning of added  $^{32}\text{P}$  between solution and epipelton. Epipelton was incubated in  $0.2 \mu\text{M}$  filtered site water to which  $^{32}\text{P}$  was added as carrier-free  $\text{H}_3\text{PO}_4$  for either 1 or 12 h. Spiked treatments involved additions of  $3.20 \mu\text{M}$   $^{31}\text{P}$  as  $\text{KH}_2\text{PO}_4$  to bring  $C_0 = 3.33 \mu\text{M}$ . Concurrent sample sets were incubated under light and dark conditions. After the uptake experiments epipelton was collected by filtration and extracted with  $0.01 \text{M}$   $\text{HCl}$  for 2 h. The activity released during extraction was considered abiotically complexed. The activity not extracted (by difference) represents biotic incorporation. Bars represent mean  $\pm$  S.D.,  $n = 4$ .

then in metaphyton followed by epipelton. These ash contents were similar to those of periphyton studied in calcium carbonate waters of the Everglades (48–81%; Browder et al., 1982; Vymazal et al., 1994) and of Belize marshes (Rejmánková and Komárková, 2000). Variation in the chemical composition of the three periphyton types reflects their position in the water column. Higher TP and TOC contents in the epipelton are due to this material lying on the peat soil surface. The epipelton can obtain P from overlying and interstitial water (Hansson, 1989). Interstitial water has higher equilibrium DRP concentrations than overlying water in the oligotrophic Everglades (Koch and Reddy, 1992; Scinto, 1997). The epipelton is also associated with the detrital layer and thus receives material inputs settling from the water column. Epiphyton obtains P from the water column and possibly also from the macrophyte substrate as was shown in a hardwater, P-limited lake where as much as 24% of the epiphyton P was derived from the macrophyte (Moeller et al., 1988), and at least

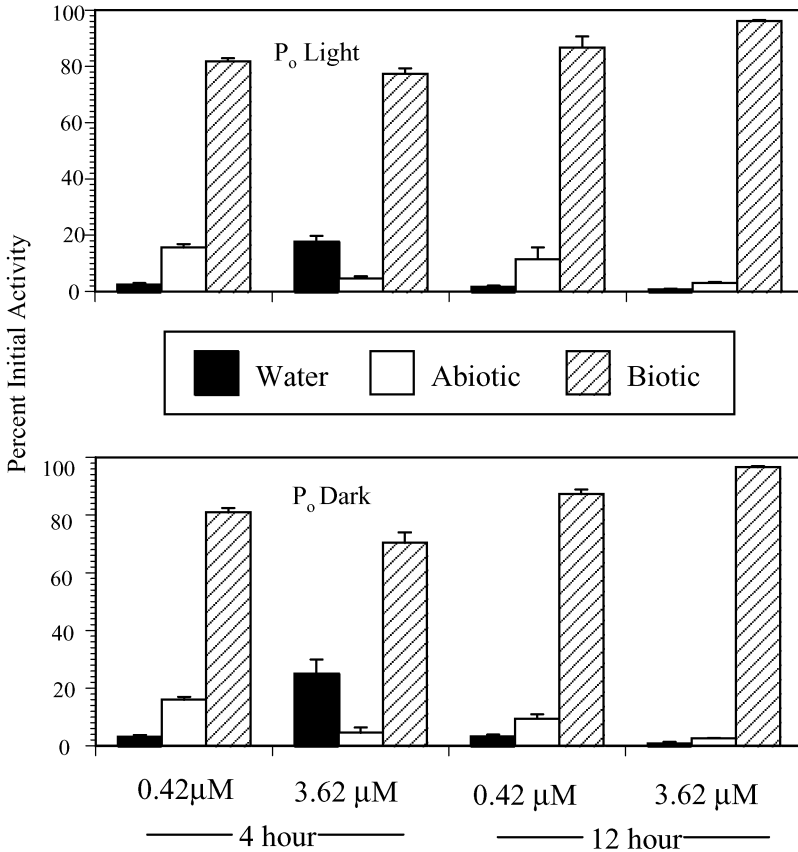


Fig. 6. Partitioning of added  $^{32}\text{P}$  between solution and epipelton incubated in  $0.2\ \mu\text{M}$  filtered site water to which  $^{32}\text{P}$  was added as adenosine 5'-( $\gamma$ - $^{32}\text{P}$ ) triphosphate ( $111\ \text{TBq}\ \text{mM}^{-1}$ ) for either 4 or 12 h. Ambient concentrations ( $0.32\ \mu\text{M}$ ) were increased to  $0.42 \pm 0.03\ \mu\text{M}$  by the addition of [ $^{32}\text{P}$ ]ATP (nominally ambient). Spiked treatments were increased by addition of unlabelled ATP to  $3.62 \pm 0.03\ \mu\text{M}$  (including  $^{31}\text{P}$  added with the [ $^{32}\text{P}$ ]ATP). Concurrent sample sets were incubated under light and dark conditions. After the uptake experiments the epipelton was extracted with  $0.01\ \text{M}$  HCl for 2 h. The activity released during extraction was considered abiotically complexed. The activity not extracted (by difference) represents biotic incorporation. Bars represent mean  $\pm$  S.D.,  $n = 4$ .

partially reduced epiphytic P-stress (Burkholder and Wetzel, 1990). In these lake studies the epiphyton was on the order of 2 mm thick (Burkholder and Wetzel, 1990), however, in the present study the epiphyton developed thick encrustations on the macrophyte ( $\sim 3\ \text{cm}$ ), contained the lowest P concentrations, and was the most P-limited (N:P 231:1 mol:mol) of the three types. This may suggest that this community becomes increasingly dependent on the water column for P as the adnate proportion of the epiphytes becomes relatively reduced in thick encrustations. Total P in all periphyton forms was inversely related to the TIC content and increased with TOC suggesting that most of the P was associated with the biotic fraction.

Table 5

Results of ANOVA comparing effects of time, light, and initial organic  $^{31}\text{P}_o$  (adenosine 5'-triphosphate) concentration on the partitioning of  $^{32}\text{P}$  (adenosine 5'-( $\gamma$ - $^{32}\text{P}$ ) triphosphate) activity in water, abiotic, and biotic fractions during uptake by epipelton

Source of variation	d.f.	MS	<i>P</i>
<b>Water</b>			
Time	1	827.1	<0.001
Light	1	113.3	<0.001
Concentration (conc.)	1	2.7	0.511
Time $\times$ conc.	1	1005.1	<0.001
Light $\times$ conc.	1	52.5	0.007
Time $\times$ light $\times$ conc.	1	6.0	0.006
Error	22		
<b>Abiotic</b>			
Time	1	205.2	<0.001
Light	1	52.5	0.049
Concentration (conc.)	1	1708.0	<0.001
Error	22	12.2	
<b>Biotic</b>			
Time	1	879.6	<0.001
Light	1	10.8	0.140
Concentration (conc.)	1	165.2	<0.001
Time $\times$ light	1	21.1	0.044
Time $\times$ conc.	1	486.9	<0.001
Error	22	4.6	

#### 4.2. Periphyton uptake kinetics

Everglades periphyton responds more rapidly to P additions than most other ecosystem components and is important in maintaining low water column P concentrations (McCormick et al., 2001; Noe et al., 2002). We compared estimates of  $K_m$  and  $V_{max}$  for epipelton, epiphyton, and metaphyton obtained by observing P removal from solution. The values of  $K_m$  have been used in algal competition studies where a lower  $K_m$  is interpreted as a measure of a species advantage for limited P (Grover, 1989). In our study the predicted  $K_m$  was lowest for epiphyton suggesting that it had a higher affinity for low DRP concentrations than did metaphyton or epipelton. Maximum uptake rates ( $V_{max}$ ) were highest for the epiphyton, which predicts a higher capacity to incorporate P when P is present in high concentrations. However, under ambient conditions, P uptake will not approach  $V_{max}$ , as ambient TP concentrations are typically below 0.32  $\mu\text{M}$  and DRP is often below detection limits (McCormick and O'Dell, 1996). Turnover times, calculated as the reciprocal of the first-order constant ( $k$ ) ranged 5.4–55.3 min and were shortest for epiphyton > metaphyton > epipelton. The  $V_{max}/K_m$  quotient was 0.09 for epiphyton, 0.03 for metaphyton, and 0.2 for epipelton which according to Hwang et al. (1998) shows that epiphyton would have a competitive advantage over metaphyton or epipelton in severely P-limited water. Hwang et al. (1998) summarized several Michaelis–Menten type kinetic studies, using periphytic algae, which showed a wide range in  $V_{max}$  of 62–5736  $\mu\text{g P g}^{-1} \text{DW h}^{-1}$  and  $K_m$  values between 0.5 and 251.1  $\mu\text{g}$

$\text{P l}^{-1}$ . Our  $V_{\max}$  estimates were within the range of those summarized (epiphyton = 1376, metaphyton = 930, and epipelton =  $446 \mu\text{g P g}^{-1} \text{DW h}^{-1}$ ) but our  $K_m$  values were higher than those reported (epiphyton = 264, metaphyton = 499, and epipelton =  $508 \mu\text{g P l}^{-1}$ ). The kinetic parameters coincide with the P-limitation of the periphyton types with epiphyton, the most P-limited, having greater uptake rates than metaphyton or epipelton. Epipelton and metaphyton had similar N:P ratios and non-statistically different uptake rates.

#### 4.3. Inorganic and organic P uptake by epipelton

We expected the high P demand by periphyton in the P-limited WCA2A to result in rapid depletion of  $\text{P}_i$  from solution. Further we expected rapid hydrolysis rates of DOP (Bentzen et al., 1992; Cotner and Wetzel, 1992) with subsequent uptake of the liberated P. Phosphorus as ATP was used to determine the hydrolysis and uptake rate of  $\text{P}_o$  by epipelton. Bentzen et al. (1992) found that ATP was an effective substrate for tracing  $\text{P}_o$  dynamics in phytoplankton. Our added ATP was rapidly hydrolyzed to DRP within the first sampling of 15 min therefore the actual hydrolysis rate could not be determined. However, based on the maximum ATP addition of  $10.1 \mu\text{mol P g}^{-1} \text{DW}$  and a 15 min time step, the calculated hydrolysis rates were  $>0.673 \mu\text{mol P g}^{-1} \text{DW min}^{-1}$ . Hydrolysis can occur at the cell surface or in the water if phosphatase is free in solution (Bentzen et al., 1992). Rapid hydrolysis of ATP in the periphytonless control (unfiltered water) without subsequent P uptake suggests that there was an abundance of free phosphatase enzyme present. Once hydrolyzed, the uptake of  $\text{P}_o$  was similar to  $\text{P}_i$  as suggested by comparable first-order uptake rate constants. Likewise, Bentzen et al. (1992), found that ATP and  $\text{PO}_4^{3-}$  uptake by phytoplankton were analogous, the later being slightly greater.

#### 4.4. Biotic versus abiotic P uptake by epipelton

Rates measured by P removal from solution estimate uptake by periphyton without differentiating the mechanisms involved. An abiotic interaction, mediated by periphytic activity may occur between  $\text{Ca}^{2+}$ ,  $\text{CaCO}_3$ , and  $\text{PO}_4^{3-}$ , and this interaction may be partially responsible for removing P from solution. To test this hypothesis, epipelton was incubated in solutions containing either  $^{32}\text{P}_i$  as  $\text{H}_3\text{PO}_4$  or  $[^{32}\text{P}]\text{ATP}$ . In general, the partitioning of  $\text{P}_i$  and  $\text{P}_o$  was similar. Under all conditions biotic uptake was much higher than abiotic uptake. Rapid uptake removed almost all water  $^{32}\text{P}$  activity within 1 h ( $\text{P}_i$ ) or 4 h ( $\text{P}_o$ ) at ambient concentrations and within 12 h at spiked concentrations, suggesting that even at spiked concentrations (high mass loading) the P uptake capacity of epipelton was not exceeded. The abiotic P fraction decreased with time signifying that some of the P initially adsorbed to  $\text{CaCO}_3$  remained available for longer term biotic uptake. By the end of the incubations the biotic fraction contained  $>83\%$  of added P showing that biological demand for P exceeds abiotic adsorption of P to  $\text{CaCO}_3$ . It therefore seems unlikely that adsorption alone would account for P not recovered in Belize addition studies (Rejmánková and Komárková, 2000). The lack of strong light effects suggests that the observed abiotic retention was due to adsorption to the periphytic  $\text{CaCO}_3$  rather than to co-precipitation during active photosynthetically-induced calcification. The conditions during which these partitioning

experiments were conducted however, may not have encouraged co-precipitation. Conditions that favor periphytic  $\text{CaCO}_3$  encrustation and subsequent P adsorption are: high calcium concentrations, high alkalinity, high pH, low partial pressure of  $\text{CO}_2$ , high levels of  $\text{CaCO}_3$  saturation, and high temperatures (Otsuki and Wetzel, 1972). Diaz et al. (1994) observed rapid precipitation of P as Ca–P in Everglades water when pH was manipulated to  $>8.0$ . Our experiments suggest that abiotic mechanisms account for a small ( $\sim 14\%$ ) proportion of periphyton P retention in the oligotrophic northern Florida Everglades. Whereas we did not determine the long-term stability of this abiotic P fraction it is possible that this  $\text{CaCO}_3$ -associated P is deposited in the soil when the periphyton degrades, thus increasing the areal retention of P (Reddy et al., 1993).

## 5. Conclusions

Our study suggests that the periphyton of the interior of WCA2 exists in a condition of severe P-limitation. Differences in chemical contents of epipelton, epiphyton, and metaphyton are due to their position in the water column and the nutrient sources available to them. Phosphorus uptake was most rapid for epiphyton followed metaphyton and epipelton. However, ambient DRP levels in the interior of WCA2 are always below  $K_m$  and therefore uptake rates will always be  $<V_{\max}$  with the biological uptake capacity of periphyton greatly exceeding typical P supply. The rapid hydrolysis rates of labile  $\text{P}_o$  further attests to P-limitation. Once hydrolyzed, the uptake of  $\text{P}_o$  is similar to  $\text{P}_i$ . Therefore, active and abundant periphyton aides in maintaining low water column P. Partitioning experiments show that there are two mechanisms involved in removing P from solution. Most P is incorporated into the biota but a fraction (14%) was retained by abiotic adsorption.

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