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Light-dependent phosphate uptake of a submersed macrophyte *Myriophyllum spicatum* L.

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ABSTRACT

The uptake kinetics of phosphate (Pi) by *Myriophyllum spicatum* was determined from adsorption and absorption under light and dark conditions. Pi uptake was light dependent and showed saturation following the Michaelis–Menten relation (in light: $V = 16.91 \times [Pi](1.335 + [Pi])$, $R^2 = 0.90$, $p < 0.001$; in the dark: $V = 5.13 \times [Pi](0.351 + [Pi])$, $R^2 = 0.77$, $p < 0.001$). Around 77% of the loss of Pi in the water column was absorbed into the tissue of *M. spicatum*, and only 23% was adsorbed on the surface of the plant shoots. Our study shows that *M. spicatum* shoots have a much higher affinity (in light: 3.9 $\mu mol\, g^{-1}\, dw\, h^{-1}\, \mu M^{-1}$; in the dark: 3.7 $\mu mol\, g^{-1}\, dw\, h^{-1}\, \mu M^{-1}$) and V_{max} (maximum uptake rate, shoot light) for Pi uptake than many other aquatic macrophytes (in light: 0.002–0.23 $\mu mol\, g^{-1}\, dw\, h^{-1}\, \mu M^{-1}$; in the dark: 0.002–0.19 $\mu mol\, g^{-1}\, dw\, h^{-1}\, \mu M^{-1}$), which may provide a competitive advantage over other macrophytes across a wide range of Pi concentrations.

1. Introduction

Submersed macrophytes are important for ecosystems to function, especially in shallow lakes. The community composition of macrophytes is influenced by the availability of certain resources such as phosphorus, nitrogen, and light (Jupp and Spence, 1977; Orians and Jones, 2001; Cronin and Lodge, 2003). Phosphorus overloading causes eutrophication in freshwater lakes (Schindler, 1977; Zhou et al., 2001) and is associated with excess algal biomass, resulting in decreased water clarity and undesirable ecological changes (Newton and Jarrell, 1999).

The Eurasian water-milfoil *Myriophyllum spicatum* L. is a cosmopolitan submersed macrophyte species. This plant has been considered a weed in North America for its expansive growth since its unintended introduction in the late 1800s (Aiken et al., 1979; Pieterse and Murphy, 1993). Due to its high growth rate, *M. spicatum* can rapidly occupy eutrophicated waters, forming dense beds, and thereby suppressing the growth of other macrophytes (Blackburn and Weldon, 1967; Madsen et al., 1991; Madsen, 1994; Boylen et al., 1999). Some researchers have suggested that the fast spread of other invasive aquatic plants, such as the *Elodea* genus, is encouraged by eutrophication (Grime, 1988; Madsen, 1998). *M. spicatum* is native to China but has increased in abundance due to ongoing eutrophication, and has replaced *Potamogeton maackianus* A. Benn. and *Potamogeton malaianus* Miq. as a dominant species in many lakes along the Yangtze River (Jin, 2003; Jin et al., 2005). Water column concentrations of phosphorus are high in *M. spicatum* habitats, being 0.202 mg P L^{-1} on average and reaching 1.45 mg P L^{-1} (Wu et al., 2006; Wang et al., 2007). In contrast, in our field observations, these *Potamogeton* species thrive in phosphorous concentrations less than 0.2 mg L^{-1}, usually within 0.02–0.15 mg L^{-1}.

Many rooted submersed macrophytes can absorb phosphate (Pi) from both the water column and the sediment through shoots and roots, respectively (Bristow and Whitcombe, 1971; Thursby and Harlin, 1984; Madsen and Cedergreen, 2002; Gras et al., 2003; Angelstein and Schubert, 2008). *M. spicatum* can also absorb Pi through both tissues (Bristow and Whitcombe, 1971; Bole and Allan, 1978), under high loading of phosphorus of both water column and sediment (Carignan and Kalf, 1980). Phosphorus is a limiting nutrient for the growth of many macrophytes; therefore, it important to explore the mechanism of Pi utilization of *M. spicatum*. Light availability in the water column is another factor affecting the growth and competition of submersed macrophytes (Jupp and Spence, 1977; Orians and Jones, 2001; Cronin and Lodge, 2003). In some seagrasses, Pi uptake declines in darkness, and shows strong dependence on light (Brix and Lyngby, 1985; Gras et al., 2003; Angelstein and Schubert, 2008), suggesting that it is coupled with photosynthesis (Angelstein and Schubert, 2008). A similar light dependency of NH_4^+ and NO_3^- uptake and salt absorption was

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found by Lee and Dunton (1999) and Ingold (1936), respectively. External Pi enrichment was also found to affect concentrations of cations (e.g. K^+, Ca^{2+} and Mg^{2+}) in tissues and carbohydrate metabolism in aquatic plants (Müller et al., 2004; Steinbachová-Vojtíšková et al., 2006).

In this study, we aimed to measure the uptake properties of Pi by *M. spicatum*, and evaluate plant dominance in relation to Pi utilization strategies in eutrophic lakes. *M. spicatum* was incubated in solutions of varying Pi concentrations under light and dark conditions. The adsorption and absorption of Pi by *M. spicatum* shoots were examined. The effects of light and phosphorus concentration on carbohydrate and cation metabolism of the plant were also evaluated.

The following hypotheses were tested: (1) Pi uptake in *M. spicatum* increases with Pi supply. (2) This Pi uptake in *M. spicatum* responds to light. (3) Increased Pi supply decreases formation of reserve carbohydrates in *M. spicatum*. (4) Along with reduced light supply, increased Pi supply causes significant changes in cation metabolism.

2. Materials and methods

The experiments were carried out at Donghu Experimental Station of Lake Ecosystems (30°33′N, 114°23′E) in August of 2007. *M. spicatum* was collected from Bao'an Lake and pre-cultured for two years in aquaria (diameter: 88 cm, depth: 70 cm) with tap water (NO_3^--N: 1.06 mg L^{-1}, NH_4^+-N: undetectable, and PO_4^{3-}-P: 0.015 mg L^{-1}) and sediment (TN: 3.64 mg g^{-1}, TP: 1.2 mg g^{-1}) collected from Lake Donghu.

2.1. Experiment I

The first experiment was conducted to examine the uptake kinetics of Pi in the shoots of *M. spicatum* during 2 h incubation. The experimental treatments included 2 light levels (light and complete darkness) and 10 PO_4^{3-}-P concentrations (0, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, and 6.4 mg L^{-1}), using four replicates. Phosphorus was added (as $H_2PO_4^-$) to provide the range of concentrations. Experiments were conducted according to a factorial design. Prior to the start of Experiment I, 80 apical shoots (25 cm length, 2.26 ± 0.24 g fresh weight, FW) of *M. spicatum* were collected from the aquaria and incubated in a diluted Pi-free Hoagland solution (×0.05) for 24 h, with an irradiance just above the shoots of 89 μmol m^{-2} s^{-1} PAR (light source: visible-light filament lamp), measuring with a sensor (LiCor LI-1400) and a diurnal cycle of 16 h of light and 8 h of dark at 25 °C. Shoots were separately placed in 80 transparent glass tubes (50 mL) which contained the 10 different treatment solutions and were subjected to the 2 lighting conditions [i.e., light (89 μmol m^{-2} s^{-1}) or dark conditions] for 2 h. Subsequently, the treated shoots were collected, rinsed thrice with distilled water and oven-dried at 80 °C to constant weight.

2.2. Experiment II

The second experiment was conducted to examine Pi adsorption and absorption of in *M. spicatum* shoots during 2 h incubation. Based on Experiment I, only concentration treatments 0, 0.64, and 6.4 mg L^{-1} were used in this experiment. Experimental treatments still employed the same light and dark conditions, the factorial design, and four replicates. The water quality in the incubation system is shown in Table 1. Again apical tips of *M. spicatum* (25 cm, without roots, 2.10 ± 0.19 g) were selected and prepared for incubation. Before this 2 h incubation, 24 colorimetric tubes (50 mL) with the same specifications as those in Experiment I were washed, dried, and cooled at room temperature and then weighed. A 62 mL treatment solution was added into each tube, and then the entire culture system without plants was weighed. After 2 h incubation, each plant was harvested separately and carefully drained for 3–5 min using a funnel, and the entire culture system without plants was weighed again. The difference in weight of an incubation system before and after incubation was less than 2 g, averaged 1.347 g, and had a volume of about 2.175 mL ($p < 0.05$). The plants were subsequently washed twice in two cups of 100 mL distilled water, respectively. Finally, both aliquots of the 100 mL rinsed water were pooled and analyzed for Pi. The plant shoots were immediately dried to constant weight at 80 °C, and subsequently ground for dry sample analysis.

Table 1
Water quality parameters of the medium used in the three treatments of Experiment II. Presented are means ± SE.

	Pi (mg L^{-1})		
	0	0.64	6.4
pH	8.13 ± 0	5.77 ± 0.042	5.72 ± 0.091
TIC (mg L^{-1})	0.41	0.74 ± 0.16	0.49 ± 0.036
PO_4^{3-}-P (mg L^{-1})	0.012	0.65 ± 0.020	6.45 ± 0.055
K^+ (mg L^{-1})	11.73	12.54	19.81
Mg^{2+} (mg L^{-1})		2.43	
Ca^{2+} (mg L^{-1})		8.02	
NH_4^+-N (mg L^{-1})		Undetected	
NO_3^--N (mg L^{-1})		1.36–1.50	

To determine Pi and concentrations of biochemicals in *M. spicatum* in both experiments, the dry shoots were ground into powder with mortar and pestle. Non-structural carbohydrate in the plant shoots was extracted following the method described by Cao et al. (2007). Soluble carbohydrate (SC) and starch were measured by the anthrone method (Yemm and Willis, 1954) and the I_2–KI reagent method (Dirk et al., 1999), respectively. Total phosphorus (P) in the plant shoots was measured by the phosphomolybdenum blue method after the plant material was digested in H_2SO_4–H_2O_2 (Allen, 1989). Concentrations of K^+, Ca^{2+} and Mg^{2+} in the treatment solutions were measured by ion chromatography (DX-100, USA), and Pi and P concentrations were measured following the methods described by Golterman (1969) and Ebina et al. (1983), respectively.

2.3. Data processing and statistical analysis

The uptake kinetics of Pi was quantified using the Michaelis–Menten equation:

$$V = V_{max}\frac{S}{K_m + S}$$

where V is the uptake rate, μg g^{-1} FW h^{-1}, S is the concentration of Pi, mg L^{-1}, V_{max} is the maximum uptake rate, μg g^{-1} FW h^{-1}, and K_m is the concentration when $V = 1/2V_{max}$, mg L^{-1}.

Pi affinity (α) is the initial linear slope of the kinetic curve. Absorption of Pi was estimated as the difference between the adsorbed Pi and the loss of Pi in the solutions. The amount of Pi adsorbed by the shoot of *M. spicatum* was calculated from the pooled volume of rinse water multiplied by the concentration of Pi in the rinse water. To compare the uptake kinetics of Pi among macrophytes, values for affinity, V_{max}, and K_m for other aquatic macrophytes in literature were also compiled (Table 2).

All data were tested for normality or transformed using the expression $\ln(x+1)$ or $\mathrm{sqrt}(x+1)$, before performing multiple comparisons of post hoc test (Tukey test with Bonferroni correction) at a significance level of 0.025. Also independent-sample *t*-test was performed the comparison between only two groups, e.g. comparison of the parameters between two light treatments. Two-way analysis of variance ANOVA and correlation analysis were then conducted. Statistical analysis was carried out with SPSS 17.0. *N*th order formation kinetics of *M. spicatum* Pi uptake was

Table 2
Kinetics parameters of submersed macrophytes cited from literature and the present study, Pi, V_{max} (μmol g^{-1} h^{-1}), K_m (μM), and linear affinity (α; modified from Gras et al., 2003).

Species	Incubation time	Michaelis–Menten kinetics			Linear affinity kinetics		
		V_{max}[a]	K_m (μM)	Pi (μM)	α(μmol g^{-1} dw h^{-1} μM^{-1})	Pi (μM)	Ref.
Seagrasses							
Thalassia testudinum	Leaf light (600 min)	1.9	11.93	0.5–25	0.23	0–2	I
	Leaf dark (720 min)	2.1	12.43	0.5–25	0.19	0–2	I
	Root light (600 min)	0.57	3.75	0.5–25	0.10	0–2	I
	Root dark (720 min)	0.38	4.05	0.5–25	0.09	0–2	I
Thalassia hemprichii	Leaf (600 min)[b]	2.63	11.23	5–70	0.15	0–10	II
Zostera noltii	Excised leaf (5 min)	7.0	10.0	2.5–25	0.52[c]	2.5–5.4	III
	Leaf whole plant (5 min)	43.0	12.1	1.9–30	1.54[c]	1.9–5.8	III
	Excised leaf (180 min)[d]	0.9	7.0	2.5–25	0.14[c]	2.5–5.4	III
	Leaf whole plant (180 min)[d]	3.0	7.1	1.9–30	0.15[c]	1.9–5.8	III
Ruppia maritima	Leaf × Pi root (900–1080 min)	14.1	9.2	2.5–20	–	–	IV
	Leaf + Pi root (900–1080 min)	9.7	8.1	2.5–20	–	–	IV
	Root + Pi leaf (900–1080 min)	4.6	3.1	2.5–20	–	–	IV
Submersed freshwater macrophytes							
Hydrilla verticillata	Shoot dark (780 min)[e]	7.5[f]	–	0.16–101.3	0.002–0.021[g]	0.16–45.5	V
Myriophyllum spicatum	Shoot light (120 min)	5.5[f]	43.1	0–206.5	3.86	0.65–10.3	[j]
	Shoot light (120 min)[h]	4.5[f]	29.7	0–206.5	2.96[i]	0.65–20.7	[j]
	Shoot dark (120 min)	1.7[f]	11.3	0–206.5	3.66	0.65–10.3	[j]
	Shoot dark (120 min)[h]	2.0[f]	13.8	0–206.5	2.02[i]	0.65–20.7	[j]
Emergent freshwater plants							
Canna indica	Seedlings (12/12 h, 4 weeks)	22	157	3–240	–	–	VI
Schoenoplectus validus	Seedlings (12/12 h, 4 weeks)	18	60	3–240	–	–	VI

References: I. Gras et al. (2003); II. Stapel et al. (1996); III. Pérez-Lloréns and Niell (1995); IV. Thursby and Harlin (1984); Wang et al. (2008).
[a] The unit of V_{max}: μmol g^{-1} dw h^{-1}.
[b] Maximum incubation time.
[c] Values are the linear slopes calculated by using initial Pi concentrations and associated uptake rates.
[d] Kinetic parameters generated by Edwards and Walker model (Pérez-Lloréns and Niell, 1995).
[e] Biosorption kinetics parameters calculated by the Power Function model (Wang et al., 2008).
[f] The unit of V_{max} was transformed from μmol g^{-1} FW h^{-1} to μmol g^{-1} dw h^{-1} by a coefficient of 10.
[g] Values are the linear slopes calculated by using initial Pi concentrations and associated uptake rates.
[h] Kinetic parameters generated by one-order and three-order curve-fit kinetic model giving the best fit (the largest R^2 among all fitting models) in light and dark conditions, respectively.
[i] R^2 and p-level for P affinity or α were 0.94 and <0.001, and 0.74 and <0.05 in light and dark conditions, respectively.
[j] This study; VI. Zhang et al. (2009).

studied using curve-fitting software (TableCurve 2D v5.01). The Michaelis–Menten model was calculated by nonlinear regression using SPSS 17.0.

3. Results

3.1. Experiment I

Pi uptake rates of *M. spicatum* shoots followed the Michaelis–Menten equation, along the range of Pi concentrations 0–6.4 mg L^{-1}. The V_{max} value was found to be 16.91 and 5.13 μg g^{-1} FW h^{-1} in light and dark conditions, respectively, and the K_m value 1.335 and 0.351 mg L^{-1} (Fig. 1a). Maximum Pi uptake rates were found at S = 6.4 mg L^{-1} in Experiments I and II (Fig. 1b). In Experiment II, maximum Pi uptake rates of the plant shoots were 7.81 and 5.37 μg g^{-1} FW h^{-1} in light and dark, respectively. The maximum Pi-uptake rates of two light treatments in Experiment II were in the range of these values in Experiment I, respectively, and no significant difference between each value in both experiments (p = 0.479 > 0.025 in the light, p = 0.224 > 0.025 in the dark Fig. 1b). Pi affinity (α) of the plant shoots was 3.86 μmol g^{-1} dw h^{-1} μM^{-1} in the light and 3.66 μmol g^{-1} dw h^{-1} μM^{-1} in the dark (Table 3).

The contents of total phosphorus in *M. spicatum* shoots in the light were slightly higher than those in the dark (p > 0.05, Fig. 2a). No significant difference in tissue P was found as a function of P concentration (p > 0.025, Fig. 2b). A t-test showed no significant

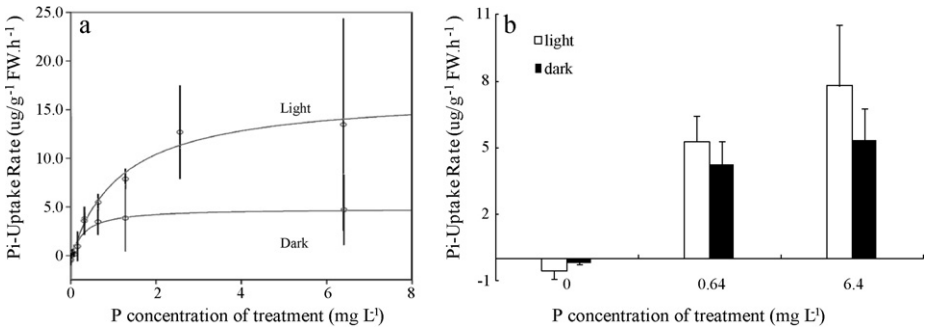

Fig. 1. (a) Pi-uptake kinetics of *M. spicatum* along an external Pi gradient under light and dark conditions in Experiment I; the curves represent the best fits, according to the Michaelis–Menten model. The uptake kinetics parameters (V_{max} and K_m) of different treatments were as follows: V_{max} = 16.91 ± 1.48 μg g^{-1} FW h^{-1}, K_m = 1.335 ± 0.285 mg L^{-1} (R^2 = 0.90) (in the light); V_{max} = 5.13 ± 0.53 μg g^{-1} (FW) h^{-1}, K_m = 0.351 ± 0.116 mg L^{-1} (R^2 = 0.77) (in the dark). The uptake rate was calculated through the loss of Pi in the incubation solution at the end of experiments. (b) Pi-uptake rates of *M. spicatum* at different external Pi concentrations in Experiment II. Data represent means + SE (n = 4).

Table 3
Parameters for Michaelis–Menten kinetics: V_{max} and K_m, with asymptotic standard errors of the parameters in parentheses.

Experiment	Pi range (μM)	V_{max} (μg g^{-1} FW h^{-1})	K_m (mg L^{-1})	α (μmol g^{-1} dw h^{-1} μM^{-1})	R^2	p-Level
Uptake kinetic model						
Shoot light	0–206.5	16.91 ± 1.48	1.335 ± 0.285		0.90	<0.001
Shoot dark	0–206.5	5.13 ± 0.53	0.351 ± 0.116		0.77	<0.001
Affinity model						
Shoot light	0.65–10.3			3.86 ± 0.34	0.93	<0.01
Shoot dark	0.65–10.3			3.66 ± .0.73	0.91	<0.01

Data presented for Experiment I. Pi-affinity was calculated from the slope of linear equation at low Pi range (0.65–10.3 μM).

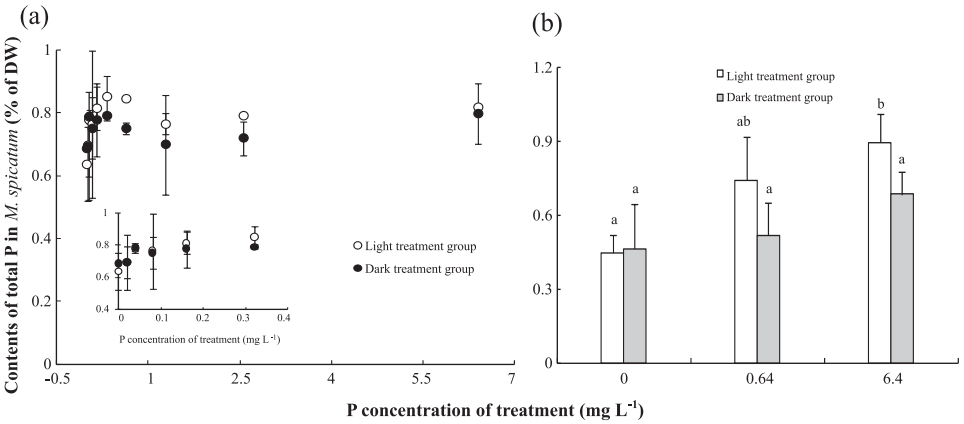

Fig. 2. Changes in contents of total P in tissues of *M. spicatum* treated by different P concentrations under light and dark conditions: (a) in Experiment I and (b) in Experiment II. "% Of dw" in figures represents the mass faction of P content in the plants (dry weight) at the end of the experiment. Different letters indicate significant difference ($p < 0.05$) among groups. Data represent means ± SE in Experiment I and means + SE in Experiment II, respectively ($n = 4$).

difference ($p > 0.05$) in TP contents between the light and dark treatments, except for those with a Pi concentration of 6.4 mg L^{-1}, and the contents of TP was 23.5% higher in the light than in the dark (Fig. 2b). Plant TP content in the light treatment was slightly higher (4.32%) than in the dark treatment (Fig. 2a), resulting in an increase of about 0.106 mg P per shoot. The concentrations of K^+ and Mg^{2+} in the culture solutions were significantly lower in the light than in the dark ($p < 0.001$, $t = 5.288$ and 3.780, respectively). No significant difference was found in Ca^{2+} concentrations between the two light levels (Fig. 3, $p = 0.341$). The contents of soluble carbohydrate (SC) and starch ranged from 15.63 to 39.81 mg g^{-1} dw, and 3.02–119.2 mg g^{-1} dw, respectively (Fig. 4a and b). The difference between two light treatments was not significant ($p = 0.610$ and 0.778 for SC and starch, respectively, Fig. 4a and b). The starch/SC ratio was significantly depressed in the dark, compared with the light ($p < 0.001$, Fig. 4c).

3.2. Experiment II

Around 77% of the lost Pi was absorbed into the tissue of *M. spicatum*, while 23% of the Pi was adsorbed on the plant surface (Table 4). The amounts of both the absorbed-Pi and adsorbed-Pi were much greater in the light treatment than in the dark treatment (Table 4).

The two-way ANOVA results show that light availability was the primary factor affecting Pi uptake, starch/SC ratio, and K^+ and Mg^{2+} levels in *M. spicatum* ($p < 0.001$, Table 5). External Pi concentrations also significantly affected Pi uptake and K^+ content of the plant ($p < 0.001$, Table 5). Interaction of light availability and the external Pi concentrations significantly affected Pi uptake ($p < 0.001$) and content of Ca^{2+} ($p < 0.05$) in the plant (Table 5). Contents of P, Starch and SC in plant were not significantly affected by Pi treatment and light conditions ($p > 0.05$, Table 5). P content in *M. spicatum* was negatively correlated with starch and SC content (correlation coefficient = −0.416, $p < 0.01$; correlation coefficient = −0.264, $p < 0.05$,

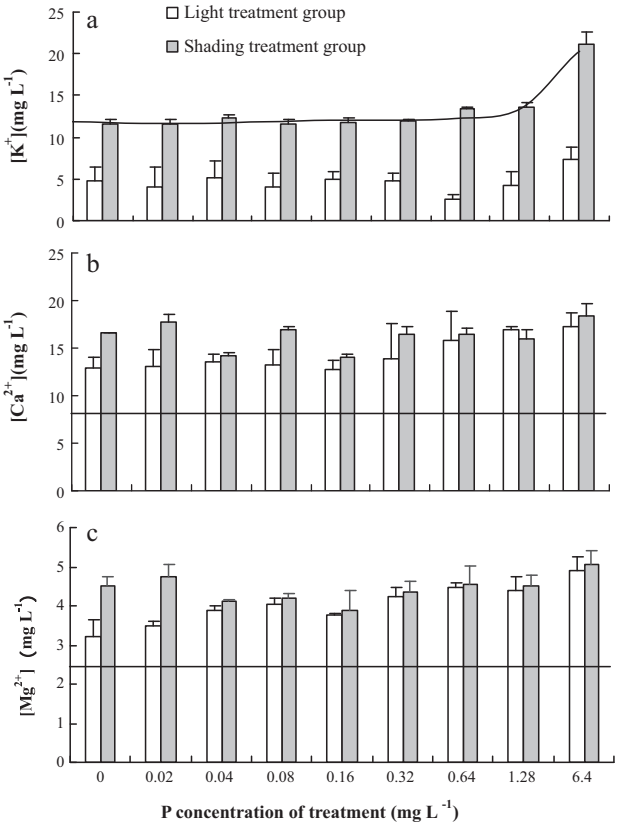

Fig. 3. Changes in concentrations of K^+, Ca^{2+} and Mg^{2+} in the treatment solutions for *M. spicatum* in Experiment I. The lines running across the bar graphs indicated the background value of K^+, Ca^{2+} and Mg^{2+} in incubation solutions. Data represent means + SE ($n = 4$).

Table 4
Absorption and adsorption of Pi by shoots of *Myriophyllum spicatum* treated by different Pi-concentration for 2 h in Experiment II.

Pi-treatments ($mg\,L^{-1}$)	Pi addition in culture system (mg)	Pi amount unabsorbed into the plants ± SE (mg)	Pi amount by plant adsorption ± SE (mg)	Pi amount by plant absorption (mg)	Absorption: adsorption (absorption %)
Light condition					
0	0	0.0050 ± 0.0035	0.0033 ± 0.0022	−0.0050	
0.64	0.040	0.0227 ± 0.0062	0.0050 ± 0.0027	0.0173	3.5 (78.5)
6.4	0.40	0.36 ± 0.015	0.011 ± 0.0010	0.04	3.6 (78.4)
Dark condition					
0	0	0.0061 ± 0.0013	0.0054 ± 0.0012	−0.0061	
0.64	0.040	0.0233 ± 0.0023	0.0029 ± 0.00087	0.0167	5.8 (85.2)
6.4	0.40	0.37 ± 0.0087	0.0088 ± 0.0021	0.03	3.4 (77.3)

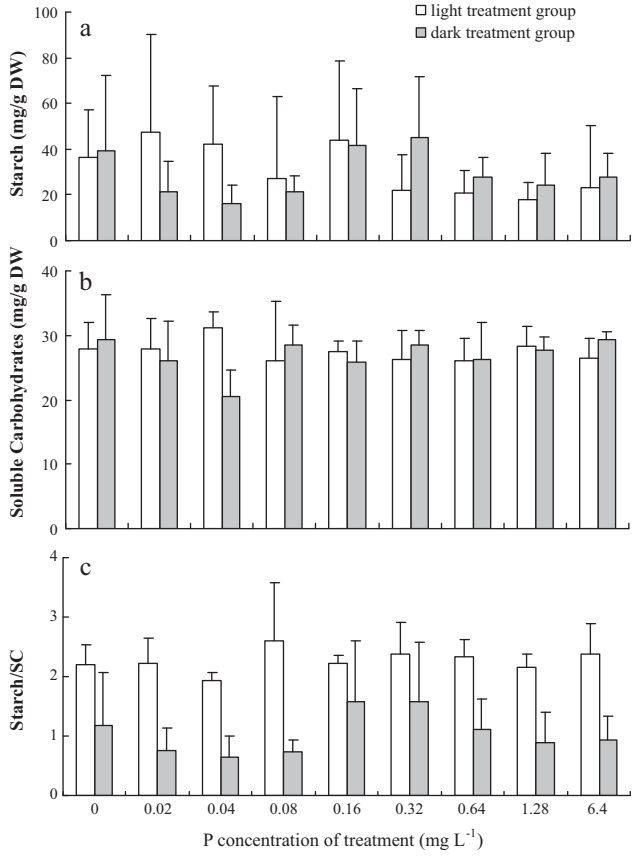

Fig. 4. Starch contents (a), soluble carbohydrates (SC) contents (b) and starch/SC ratio (c) in tissues of *M. spicatum* exposed to different Pi concentrations under light and dark conditions at the end of Experiment I. Data represent means + SE ($n = 4$).

respectively). Starch content was closely correlated with SC content in *M. spicatum* (correlation coefficient = 0.641, $p < 0.01$) and was negatively correlated with Ca^{2+} concentration in solution (correlation coefficient = −0.267, $p < 0.05$, Table 6). Three cations correlated negatively with the starch/SC ratio ($p < 0.05$, Table 6).

Table 5
Two-way ANOVA analysis on variance of Pi uptake rate and contents of P, starch, SC, K^+, Mg^{2+} and Ca^{2+} of *Myriophyllum spicatum* treated with ten levels of Pi availability and two levels of light conditions in Experiment I.

	N	df	Light	Pi	Pi × light
Uptake rate	80	9	45.20^{***}	49.09^{***}	7.37^{***}
P content	80	9	0.66^{ns}	1.37^{ns}	0.20^{ns}
Starch	72	8	0.53^{ns}	0.054^{ns}	0.99^{ns}
SC content	72	8	0.25^{ns}	0.26^{ns}	1.37^{ns}
Starch/SC	72	8	26.8^{***}	0.34^{ns}	0.27^{ns}
K^+	72	8	388.51^{***}	5.41^{***}	2.49^{*}
Mg^{2+}	72	8	11.66^{***}	0.23^{ns}	0.16^{ns}
Ca^{2+}	72	8	1.42^{ns}	1.84^{ns}	2.36^{*}

$^{ns}p > 0.05$.
$^{*}p < 0.05$.
$^{***}p < 0.001$.

4. Discussion

The present study shows that Pi uptake by shoots of *M. spicatum* was enhanced in light and followed the Michaelis–Menten kinetics model. Our results indicate that Pi uptake by shoots of *M. spicatum* was coupled to photosynthesis, which is consistent with observations in *Zostera marina* and *Zostera noltii* (McRoy and Barsdate, 1970; McRoy et al., 1972; Brix and Lyngby, 1985). Pi uptake by the leaves of these plants increased under lit conditions and decreased in the dark. This result was in accord with the hypothesis regarding the coupled relationship between photosynthesis and Pi uptake. Pi is important for adenosine triphosphate (ATP) recycling and the transfer of energy within plant cells (Brooks, 1986). Photosynthesis generates the energy necessary for Pi uptake, which in turn facilitates the transport of photosynthate and energy between photosynthetic and non-photosynthetic tissues. Therefore, a positive relationship between photosynthesis and Pi uptake may make canopy-forming macrophytes (e.g. *M. spicatum*) have priority to light as well as Pi utilization over deeper, bottom-dwelling species.

The values of V_{max} and K_m of *M. spicatum* were higher than those of the seagrasses *Thalassia testudinum*, *Thalassia hemprichii*, and *Z. noltii* were (Table 2). Generally, *M. spicatum* is taken to inhabit eutrophied lakes (Smith and Barko, 1990), where the con-

Table 6
Correlation analysis among variance of Pi uptake rate and contents of P, starch, SC, K^+, Mg^{2+} and Ca^{2+} of *Myriophyllum spicatum* treated with ten levels of Pi availability and two levels of light conditions in Experiment I.

	Uptake rate	TP content	Starch	SC content	Starch/SC	K^+	Mg^{2+}
P content	0.257	1					
Starch	−0.068	-0.416^{**}	1				
SC content	−0.053	-0.264^{*}	0.641^{**}	1			
Starch/SC	0.211	0.096	0.039	−0.091	1		
K^+	−0.052	−0.157	0.121	0.03	-0.603^{**}	1	
Mg^{2+}	0.372^{**}	0.098	0.022	0.074	-0.312^{*}	0.617^{**}	1
Ca^{2+}	0.307^{*}	0.219	-0.267^{*}	−0.049	-0.294^{*}	0.203	0.622^{**}

$^{*}p < 0.05$.
$^{**}p < 0.01$.

centration of P is often higher than 0.02 mg/L in the water column (Vollenweider, 1976), generally much higher than concentrations in seawater (Valiela, 1984). The higher value of V_{max} implies that *M. spicatum* would have an advantage at high concentrations of Pi, while the higher value of K_m suggests its inefficiency in acquiring Pi at low concentrations. The V_{max} value of *M. spicatum* was lower than that of *Hydrilla verticillata* (Table 2). The latter species has been reported to prefer mesotrophic sediment (Chambers, 1987; Mony et al., 2007). The relatively high value of V_{max} in *H. verticillata* is expected, as this species rapidly proliferates in newly available habitat.

The process by which *M. spicatum* removes Pi from the surrounding water includes two steps (Pérez-Lloréns and Niell, 1995): a rapid adsorption of Pi on the shoot (Epstein, 1972; Clarkson, 1974) and the absorption of Pi into the plant tissues (Raven, 1974; Ullrich-Eberius et al., 1981). A similar biphasic accumulation process of ammonium uptake was found by Short and McRoy (1984). In this study, around 77% of the Pi lost from the water column was attributed to absorption, and 23% to adsorption by *M. spicatum*. However, for *H. verticillata*, adsorption of Pi accounted for approximately 6–9% of the lost Pi in the water column (Wang et al., 2008), which is far less than that for *M. spicatum* (23%). A plausible explanation for the difference of Pi adsorption between two aquatic plants is that *M. spicatum* has a much higher affinity to Pi than *H. verticillata* (Table 2). These ecophysiological characteristics of *M. spicatum* may make it favorable to adsorb relatively more Pi to its surface and may provide a competitive advantage over other macrophytes over a large range of Pi concentrations in freshwater.

A rapid uptake of Pi by *M. spicatum* in the light treatment was associated with a decline in the content of K^+ and a slight increase in the content of Mg^{2+} and Ca^{2+} in the treatment solution. These results are consistent with those for *Ricinus communis*, *Zea mays*, and *Calamagrostis villosa* seedlings when they were cultured in high concentrations of nitrate and ammonia rather than in phosphorus (Vale et al., 1988; Peuke et al., 1994; Gloser and Gloser, 2000). Spalding and Goldsmith (1993) found that a light-induced increase in K^+ permeability of cell membrane was gained via ATP-activated channels, resulting in increased cytosolic concentration of K^+. The results suggested that light could drive ATP-coupled K^+ pumps to promote the Pi uptake of *M. spicatum*. This potential mechanism in Pi sequestration could be different from its response to darkness.

The decreasing starch/soluble sugars ratio was found in darkness, which indicates increased metabolic activity of the tissue grown under dark condition. This situation may prompt Pi uptake at the expense of starch accumulation under dark condition, and high Pi conditions of 0.02–0.16 mg L^{-1}. Under light condition, the starch contents of *M. spicatum* markedly declined in the high phosphorus treatment. The result was consistent with the short-term experimental results in *Glycine max* by Fredeen et al. (1989). It indicates that high P could inhibit starch accumulation in water-milfoil shoots.

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