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Short-term phosphate uptake kinetics in *Zostera noltii* Hornem: a comparison between excised leaves and sediment-rooted plants

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Abstract

Short-term phosphate uptake by excised leaves of *Zostera noltii* Hornem. as well as by leaves of sediment-rooted plants were characterized and compared in a kinetic framework. Time courses of phosphate disappearance were measured over a wide range of initial substrate concentrations. Phosphate uptake determined by this perturbation method did not follow Michaelis-Menten kinetics. Both excised leaves and sediment-rooted plants exhibited a biphasic uptake pattern as a function of phosphate concentration. However, rooted plants showed higher uptake rates and accumulated higher amounts of phosphate than excised leaves. The results point out the importance of the structural and functional coupling between shoots and underground parts during the nutrient foliar uptake processes. Our study also indicates that *Zostera noltii* leaves function as a phosphate sink in the water column.

A second objective of this work is to compare the perturbation and the multiple flask methods in determining the uptake kinetic parameters. The obtained results support that both methods provide valuable and complementary information in determining the uptake rates.

Introduction

Seagrasses are important primary producers in coastal marine and estuarine ecosystems (McRoy & McMillan, 1977). Their production is the basis of detrital food chains and other secondary production (Short, 1987), providing habitat and nursery areas for many aquatic organisms (Kikuchi, 1980). No satisfactory explanations about the functioning of the seagrass production mechanisms, and their associated ecosystem, are possible without some considerations of the nutrient supply for growth, since considerable nutrient uptake is required to maintain plant development at the high primary production rates observed in seagrass beds (McRoy & McMillan, 1977).

Zostera noltii Hornem., as other submerged aquatic angiosperms, is capable of taking up phosphate from the surrounding water by the leaves as well as by the root-rhizomes from the interstitial water of the sediment. Presumably the sediment is the principal source of phosphate, but the relative concentrations of phosphate in the water and sediment determine the main site for uptake (McRoy & Barsdate, 1970; Patriquin, 1972; McRoy & McMillan, 1977; Carignan & Kalff, 1979; Penhale & Thayer, 1980; Carignan, 1982; Thursby & Harlin, 1984; Brix & Lyngby, 1985; Granéli & Solander, 1988; Pérez-Lloréns *et al.*, 1993). Under the eutrophic conditions in Palmones river estuary (SW Spain), where large populations of *Z. noltii* occur, these plants will therefore probably be able to cover its phosphate requirements largely from the overlying water via the leaves if foliar uptake is a significant source of phosphate.

Despite of the literature cited above, there is a paucity of quantitative information concerning the nutrient uptake kinetics by seagrasses. Only a limited number of papers deal with nitrogen uptake kinetics (McRoy & Goering, 1974; Iizumi & Hattori, 1982; Thursby & Harlin, 1982, 1984; Short & McRoy, 1984; Zimmerman *et al.*, 1987; Hemminga *et al.*, 1991),

while no previous studies are available for phosphate short-term uptake kinetics.

The present investigation was undertaken to characterize the short-term phosphate uptake (≤3 h) by excised leaves *versus* sediment-rooted plants of *Zostera noltii* in a kinetic framework. The comparison of the kinetic parameters obtained in both approaches, might give an idea of the structural and functional coupling between leaves and underground parts during the foliar uptake of this nutrient. One might expect a priori that cutting the leaves would alter the uptake rates due to a wounding responses or to interruption of the transport system or elimination of active sink regions (young rhizomes and roots). In such case, the physiological measurements reported in the literature obtained from detached plant parts, should be taken with care when they are extrapolated to the whole plant or ecosystem.

A second objective of this work is to compare two different methods (perturbation or batch *versus* multiple flask method) in determining the uptake rates based in the disappearance of the nutrient from the culture medium.

Material and methods

Sampling site and harvesting

Plant material was collected in June 1988 from a small (*ca* 3.75 km^2) and shallow (mean depth 2 m) estuary (Palmones) located in the Algeciras Bay, Southern Spain (36 °11′N, 5 °27′S). Phosphate concentrations in water and sediment are highly variable (0.5–25 μM and <50 μM respectively) depending upon tide and wastewater discharge (Clavero *et al.*, 1991). The intertidal mudflats are covered by *Zostera noltii* beds, remaining exposed at low tide.

Two harvesting techniques were used depending on the experiment. For the incubations performed with excised leaves, green healthy shoots free of visible epiphytes were collected. In experiments to study of phosphate uptake kinetics by leaves of sediment-rooted plants, clear PVC cylinders (n = 12) were employed by pushing the open-bottomed cylinders (length 35 cm, diameter 14 cm) into the sediment 10 cm and sealing the bottoms with a plastic rubber after pulling.

Since uptake determinations on whole plants are preferable for ecological measurements (Harrison & Druehl, 1982), the 'corer methodology' was used instead of the two-compartment chamber method (*i.e.* Pérez-Lloréns *et al.*, 1993), because uprooting a plant could result in stress which may affect the nutrient uptake processes; in addition, it is extremely difficult to approximate the sediment-like conditions surrounding the rhizosphere (Penhale & Thayer, 1980).The use of control cylinders without plants is necessary to check the exchange of phosphate between sediment and overlying water.

Detached leaves and enclosures were kept cool and transported to the laboratory within 3 hours of collection. There, plant material was further washed to remove attached remaining algae and other organisms. Senescent leaves in the enclosures were removed, leaving an enclosed standing crop of 1.65±0.2 g DW in each cylinder.

Three days before starting the experiments, all shoots within the control cylinders were gently removed, leaving the underground parts in the undisturbed sediment. Corers with just bare sediment are not suited to serve as controls because the absence of living and dead underground plant parts results in a sediment layer with different characteristics compared to vegetated sediment, being important in relation with nutrient exchange rates (Caffrey & Kemp, 1990).

Pre-experimental treatment

Excised leaves and sediment-rooted plants were subjected to the same pre-experimental treatment.

Plant material was preincubated in phosphate-deficient natural seawater before any experimental work. The seawater was stripped of phosphate (<0.05 μM) by incubating 1 g DW of *Ulva* sp. per litre for 24 h at 15 °C at 120 $\mu E\ m^{-2}\ s^{-1}$. The seawater was filtered (Whatman GF/C) and enriched with *f*/2 concentrations of nitrogen, trace metals and vitamins (Guillard & Ryther, 1962). Temperature was kept at 15 °C and continuous photon flux density (2 π collector LiCor LI 193 SA) of 120 $\mu E\ m^{-2}\ s^{-1}$ (saturating for photosynthesis of *Z. noltii*, Jiménez *et al.*, 1987) was provided by Sylvania® VHO Daylight fluorescent tubes. Mixing was provided by aeration.

Experimental design

A series of duplicate 1-litre flasks (containing excised leaves) and 3-litre PVC cylinders (with sediment-rooted plants) filled with enriched seawater (see above), were employed in the incubations. The plant biomass to water volume ratio was 1.5 to 1.8 g DW

l^{-1}. Curves of phosphate disappearance for a range of initial substrate additions (2 to 30 μM) were performed in each container. Water samples (1 ml) were collected at 0, 5, 10, 20, 30, 60, 120 and 180 minutes after phosphate addition (perturbation or batch method). Uptake rates in the first 5 minutes after the actual substrate addition, were calculated to demonstrate the importance of the initial 5 minute period.

Experiments were conducted under the same conditions of temperature, photon flux density and mixing as in the pre-experimental treatment. Since experiments were short, phosphate determinations were made shortly after the samples were taken. Nutrient analyses were carried out on an Auto-Analyzer Technicon AAII® according to Fernández *et al.* (1985).

Incubation methods, as well as the kinetic terminology, have been employed following the recommendations of Harrison & Druehl (1982) and Harrison *et al.* (1989).

Estimation of uptake kinetic parameters

Phosphate uptake rate *versus* substrate curves were obtained from the phosphate-time courses (3 h) performed over a wide range of initial substrate concentrations in the two categories of experiments conducted. Uptake rates were estimated for each sampling time periods (V^{0--5}, V^{5--10}, V^{10--20}, V^{20--30}, V^{30--60}, $V^{60--120}$ and $V^{120--180}$) and related to the phosphate concentrations at the beginning of these time intervals (C_0) according to the expression

$$V = \frac{C_0 - C_1}{tW}$$

where V = uptake rate (μmol P g^{-1} DW h^{-1}), C_0 and C_1 = phosphate content at the beginning and end of time period (μmol), t = time of uptake (h), W = dry weight (g) determined by drying the seagrass leaves to a constant weight on aluminium foil trays in a oven at 60 °C.

In addition, uptake rates calculated from the first 5 minutes after the actual substrate addition (V^{0--5}), were plotted *versus* the range of initial phosphate concentrations (multiple flask method). In this case uptake could be modelled by a Michaelis-Menten type expression (Edwards & Walker, 1983 equation 13.41):

$$V^{0-5} = V^{0-5}_{\max} \frac{S - \mathrm{CP}}{S + Ks}$$

where $V^{0--5}{}_{\max}$ and Ks are constants representing maximum uptake rate and the value of S where $V^{0--5} = 0.5\ V^{0--5}{}_{\max}$ respectively; CP is the compensation point for phosphate (*i.e.* the minimum phosphate concentration required for phosphate uptake to take place). The degree of nutrient affinity at low concentrations (*i.e.* $S<Ks$) is in direct proportion to the magnitude of the initial slope (α) of the hyperbolic function; this is given as a derivative of the equation of the curve, with respect to S, as S approaches zero (*i.e.* $\alpha = V^{0--5}{}_{\max}/Ks$, Parslow *et al.*, 1985). The kinetic parameters Ks, $V^{0--5}{}_{\max}$ and CP were obtained by a direct fit of the data to the Edwards & Walker (1983) equation using a computerized, iterative, nonlinear, last-squares regression program (Elsevier-Biosoft Enzfitter program).

Saturation point (S_{sat}) could be defined in the same way as the saturation onset parameter (E_k) for light (see Kirk, 1983), *i.e.* the phosphate concentration corresponding to the point of intersection between the extrapolated linear part of the uptake-substrate curve and the horizontal line at $V^{0--5}{}_{\max}$.

Results

Time courses of phosphate disappearance, performed over a wide range of initial substrate concentrations, were conducted with detached leaves of *Zostera noltii* (Fig. 1, a–f), sediment- rooted plants (Fig. 2, a–f) and control cylinders containing sediment without shoots. In excised leaves and sediment-rooted plants, the disappearance of phosphate from the medium was not linear with time, and, consequently, the uptake rate (*i.e.* dS/dt) decreased with increasing incubation time. This reflects a 'first order kinetics' or concentration dependent, rate-unsaturated transport, indicating that hyperbolic models (Michaelis-Menten type) do not apply to these short-term uptake data when they are calculated as the slopes (dS/dt) from successive intervals of the S vs t curves (*i.e.* perturbation or batch mode).

Since control cylinders showed no significant changes in phosphate concentrations of the overlying water during the time course of the short-term incubations (Fig. 3), we assumed the phosphate decrease in water during the incubations with sediment-rooted plants, was due to the uptake by shoots.

Uptake by *Zostera noltii* exhibited a biphasic pattern as a function of phosphate concentration. This pattern was qualitatively similar in detached leaves (Fig. 1, a'–f') and in sediment-rooted plants (Fig. 2, a'–f'): The first phase was characterized by a rapid phosphate uptake rate proceeding in the first 5 minutes

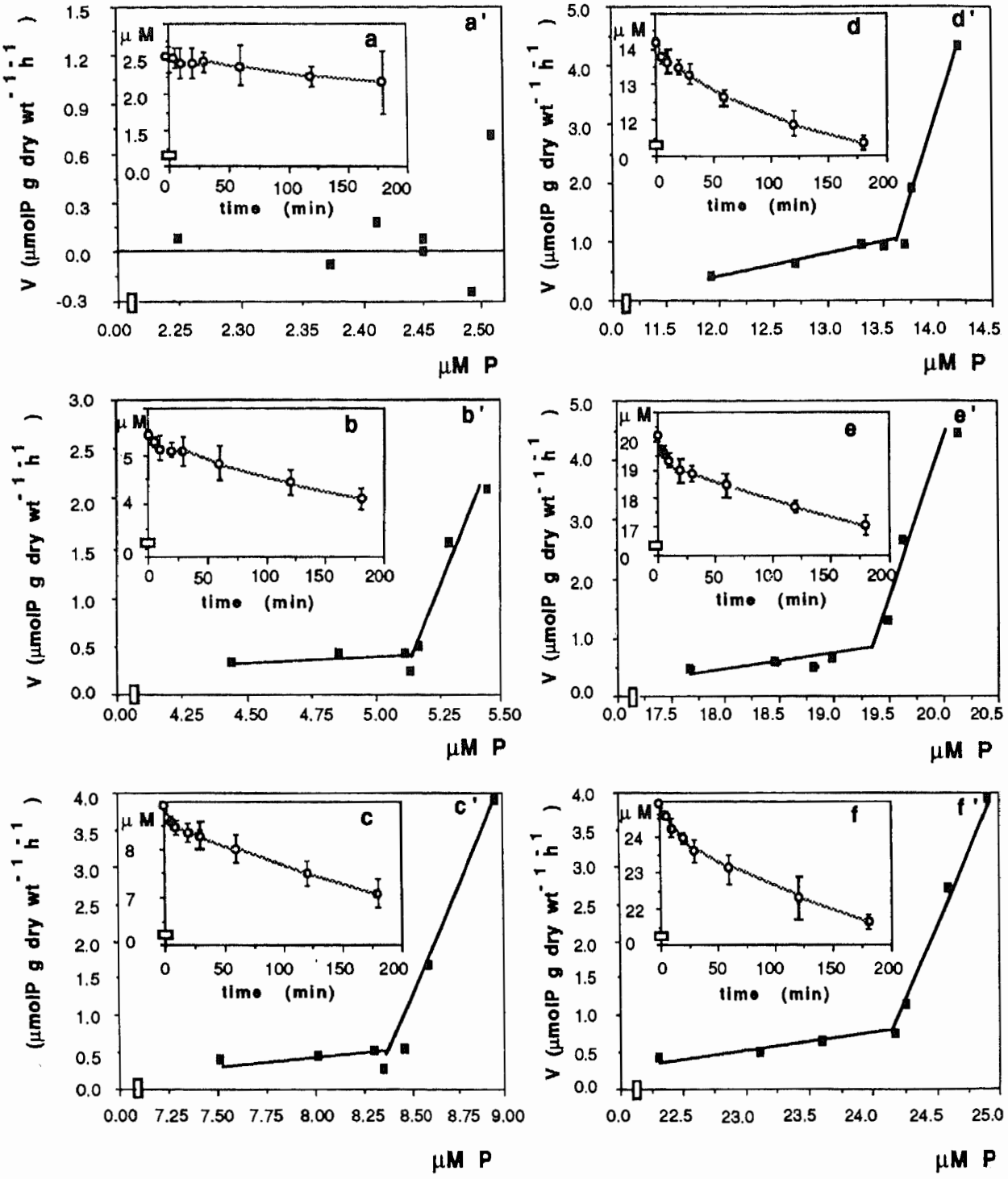

Fig. 1. (*a–f, inset graphs*). Six phosphate disappearance curves were measured in excised leaves of *Zostera noltii* (perturbation method). Initial concentrations of phosphate disappearance curves ranged from 2.5 µM to 24.9 µM. Error bars represent±SD ($n=2$). (a'–f', *main graphs*). Mean phosphate uptake rates, computed from the successive time intervals after phosphate additions (inset graphs), as a function of mean phosphate concentrations. Curves were fitted visually.

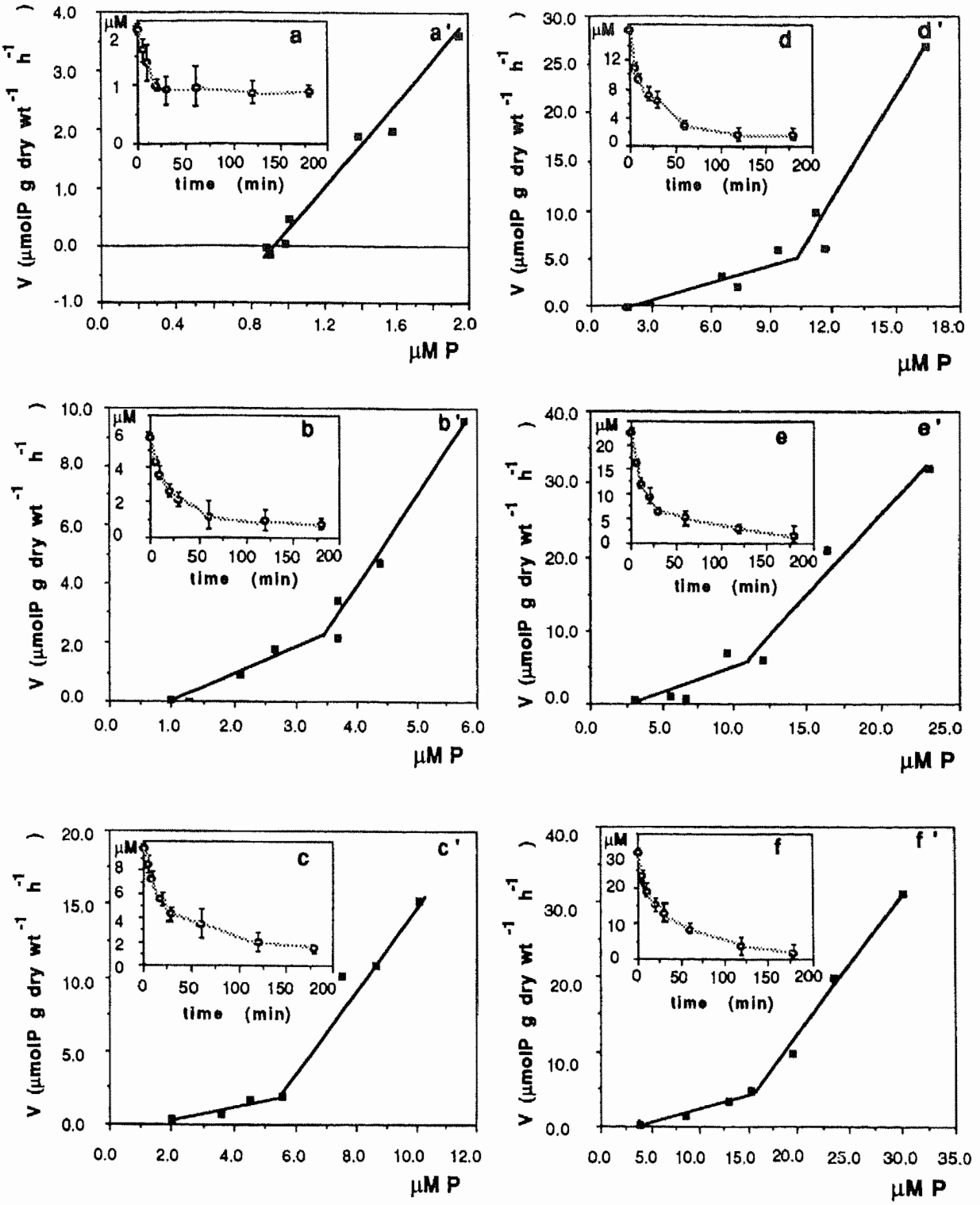

Fig. 2. (*a–f, inset graphs*). Six phosphate disappearance curves were measured in sediment-rooted plants of *Zostera noltii* (perturbation method). Initial concentrations of phosphate disappearance curves ranged from 1.9 µM to 30.0 µM. Error bars represent±SD ($n=2$). (a'–f', *main graphs*). Mean phosphate uptake rates, computed from the successive time intervals after phosphate additions (inset graphs), as a function of mean phosphate concentrations. Curves were fitted visually.

Table 1. Different kinetic parameters for phosphate uptake by excised leaves and sediment-rooted plants of *Zostera noltii*. Data calculated from curves in Figures 1 and 2 respectively (perturbation or batch method). $[P]_i$ = initial phosphate concentrations; $([P]_i)_{t=T}$ = Percentage of initial phosphate incorporated when the change in the kinetic phases occurs; $([P]_i)_{t=180}$ = Percentage of the initial phosphate incorporated at the end of the incubations; T = time when the change in kinetic phases occurs; V^{0--5}, V^{20--30}, V^{0--180} = Uptake rates calculated for the time intervals denoted by superscript; CP = compensation point for phosphate.

$[P]_i$ (μM)	$([P]_i)_{t=T}$ (%)	$([P]_i)_{t=180}$ (%)	T (min)	V^{0-5}	V^{20-30}	V^{0-180}	CP (μM)
				(μmolP gDW^{-1} h^{-1})			
Excised leaves							
2.5	–	12.8	20	0.6	–	0.1	2.2
5.4	5.7	24.9	20	2.1	0.4	0.5	nr
8.9	6.5	20.7	20	3.9	0.4	0.6	nr
14.2	4.6	20.0	20	4.4	0.7	0.7	nr
20.1	5.5	15.4	20	4.5	0.6	0.8	nr
24.9	3.1	13.1	20	4.0	0.5	0.7	nr
Sediment-rooted plants							
1.9	–	53.8	–	3.5	0.5	0.3	0.9
5.8	36.3	86.6	20	9.5	2.0	0.9	1.0
10.0	44.3	86.1	30	15.3	2.5	2.5	1.3
16.4	33.0	89.6	10	28.7	5.9	2.3	1.6
23.0	43.5	91.9	20	32.1	6.2	2.8	1.9
30.0	41.7	92.3	20	32.0	5.8	2.8	2.1

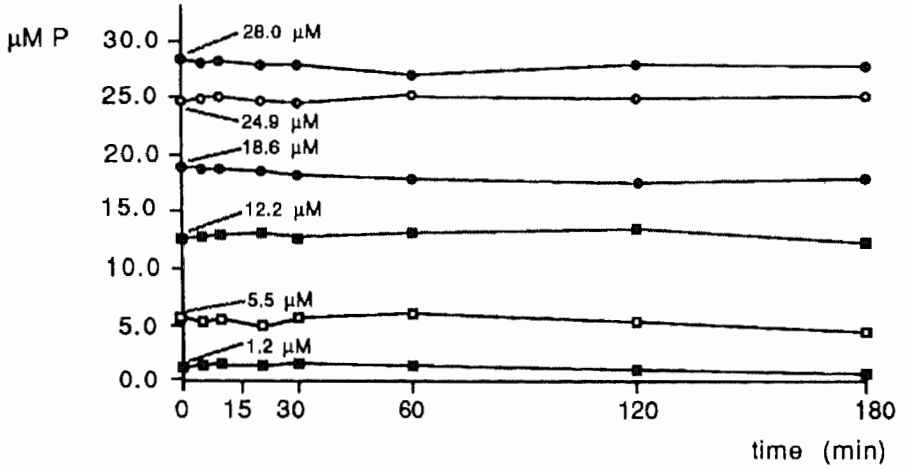

Fig. 3. Temporal changes in phosphate concentrations in the overlying water of the control enclosures. The six different initial phosphate concentrations were similar to those used in the incubations with sediment-rooted plants.

(after nutrient addition), dropping quickly to a lower, nearly constant uptake rate from the first 20 minutes up to the end of the incubations (second phase).

Although excised leaves and sediment-rooted plants showed a similar qualitative uptake pattern, the differences in relation to the kinetic parameters were obvious. The results of kinetic analysis of all phosphate depletion experiments are summarized in Table 1. Whole plants showed higher uptake rates (V^{0--5}, V^{0--180}, V^{20--30} (as representative of the

Table 2. Kinetic parameters for phosphate uptake by excised leaves and sediment-rooted plants of *Zostera noltii*, estimated from the fitting of the Edwards & Walker model to the 5 min data points of the six initial phosphate additions (multiple flask method, Fig. 4).

Kinetic parameters	Excised leaves	Rooted plants
V_{max}^{0-5} (μmol g DW^{-1} h^{-1})	7.0	43.0
Ks(μM)	10.0	12.1
CP(μM)	2.5	2.6
S_{sat} (μM)	11.1	15.2
α (= V_{max}^{0-5} / Ks)	0.7	1.1

second phase of the uptake curves)) accumulating higher amounts of phosphate over the range of initial substrate additions. Thus, an average of 39.8% of the available phosphate was taken up by leaves of rooted-sediment plants during the first 20 min. (first phase, denoted as $([P]_i)_{t=T}$, while detached leaves accumulated only an average of 5.1%. Independently of the size of the initial phosphate addition, rooted-sediment plants achieved the compensation point (CP) for this nutrient within 3 h of incubation, ranging from 0.9 μM to 2.1 μM. The higher the initial phosphate concentrations were, the higher the CP achieved. (Table 1). Excised leaves only reached the CP (2.2 μM) at the lowest phosphate addition. At the end of the incubations, sediment-rooted plants incorporated 83.4%, on average, of the available phosphate (denoted as $([P]_i)_{t=180}$), while excised leaves only 17.8%.

Figure 4 (a, b) shows the uptake *vs* substrate curves calculated from the 5 min time (V^{0-5}) of the six phosphate addition (multiple flask method) in incubation with detached leaves and sediment-rooted plants. Uptake curves, calculated in this way, followed saturation kinetics, with saturation occurring at 11.1 and 15.2 μM, respectively. Edwards & Walker's (1983) model showed a good fit to the experimental data (χ^2, $p<0.1$). CP and Ks were similar in excised leaves (2.5 μM and 10.0 μM) and in sediment-rooted plants (2.6 μM and 12.1 μM), however, the sediment-rooted plants reached higher values of $V^{0-5}{}_{max}$ (43 μmol P g DW^{-1} h^{-1}) and a (1.1 1 g DW^{-1} h^{-1}) than excised leaves (7 and 0.7, respectively). Results of kinetic analysis are summarized in Table 2.

Discussion

Patterns of phosphate uptake

According to Harrison *et al.* (1989), a time course of the disappearance of the nutrient must be run to observe how the pattern of uptake rate changes with time. It will provide valuable information about what method (perturbation or multiple flask) should be used in determining the uptake rates. Moreover, the use of a series of perturbations at different phosphate concentrations, as was done in this study, with short sampling intervals, provides accurate estimations of different kinetic parameters.

Detached leaves and sediment-rooted plants showed qualitatively similar relationships between phosphate uptake and phosphate concentration, although the kinetic patterns were highly dependent on the method used in these estimations. Thus, the relationships were hyperbolic (Michaelis-Menten type) when the multiple flask method was used, while biphasic (non-saturable) kinetics were obtained from the perturbation or batch method. The main reason why the typical hyperbolic V vs S curves are not obtained from the latter approximation is the non-linear disappearance of the phosphate from the medium (see Harrison *et al.*, 1989). It implies that initial phosphate uptake is high, but short-lived (10 to 20 min) and decreased with increasing time. This transient enhanced uptake rate following a pulse of the nutrient is greatly affected by the prehistory or preconditioning of plant material (D'Elia & DeBoer, 1978; Lefebvre & Glass, 1982; Harrison & Druehl, 1982; Thomas & Harrison, 1985), and it could be expected, since seagrass leaves were phosphate-starved before starting the experiments. In addition, uptake rates determined with the perturbation method are affected by the nutrient past history of the plants. It results in reduced uptake rate later in the experiment due to the accumulation of nutrient by the plants during exposure to the initial high concentration (D'Elia & DeBoer, 1978; Topinka, 1978; Harrison & Druehl, 1982; Fujita, 1985).

Non-linear nutrient disappearance, or biphasic V vs S curves, have been reported in phytoplankton (Conway *et al.*, 1976; McCarthy & Goldman, 1979; Goldman *et al.*, 1981; Goldman & Gilbert, 1982, Parslow *et al.*, 1984b, c; Harrison *et al.*, 1989; Cochlan & Harrison, 1991), procaryotic nannoplankton (Lehman & Sandgren, 1982) and seaweeds (Harrison & Druehl, 1982; Fujita, 1985; Thomas & Harrison, 1985, 1987). However, little work has been devoted to seagrasses.

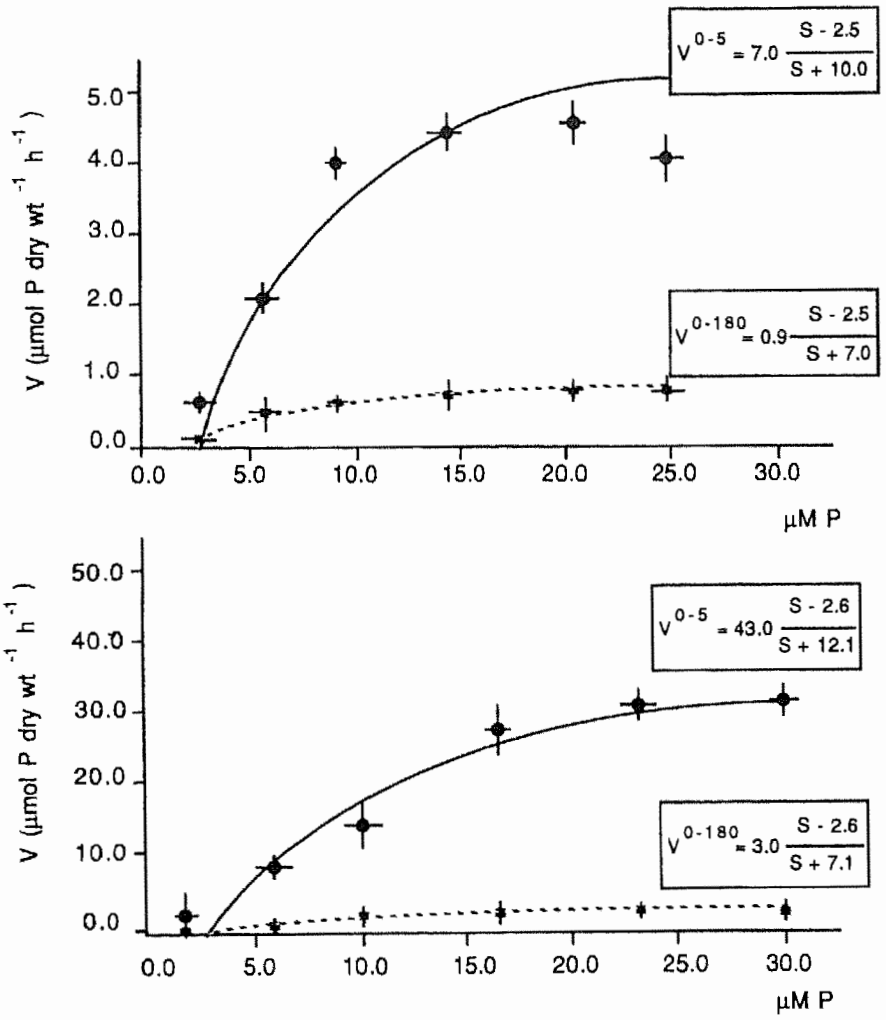

Fig. 4. Uptake *versus* substrate curve calculated for the 5 min (solid line) or the 180 min (dotted line) data points of the six phosphate disappearance curves measured with: a) excised leaves of *Zostera noltii* presented in Fig. 2 (a–f). b) sediment-rooted plants presented in Fig. 3 (a–f). Points are the mean uptake rates plotted against average phosphate concentrations. Error bars represent±SE ($n = 2$). Lines represent the fit of the Edwards & Walker expression.

Only Short & McRoy (1984) reported a biphasic accumulation of NH_4^+ by leaves of *Zostera marina* (L.). They found that leaves rapidly accumulated ammonium by adsorption in the first 15 min followed by accumulation at a slower constant rate by absorption for the next 2 h.

Our study was not directed at elucidating the specific biochemical mechanisms whereby phosphate is incorporated by the leaves of *Zostera noltii*, but some interesting ideas could emerge from the kinetic patterns observed (Figs 1 and 2). In phytoplankton studies (Conway *et al.*, 1976; Harrison *et al.*, 1989), the initial rapid uptake of nutrients has been termed *Vs* (surge uptake), representing a maximal uptake rate that declines as an internal pool is filled due to feedback inhibition. Although it could be expected that 'simple' algae exhibit different physiological mechanisms to cope their nutrient demands than complex rooted phanerogams, the kinetic patterns observed are similar. According to Short & McRoy (1984), the enhanced uptake rates in *Z. marina*, accounting for the first minutes, could be explained by two coupled processes in nutrient uptake: adsorption and absorption. Adsorption refers to the movement of nutrients into the apoplast (Epstein, 1972; Clarkson, 1974). The leaf anatomy in seagrasses (Tomlinson, 1980) appears to be adapted for rapid uptake of nutrients into water-filled spaces on the cell wall and eventually into the plant tissue. Further,

the adsorbed nutrients could move (absorbed) into an internal cell pool rather than remaining at the cell surface. This absorption or uptake of phosphate through the plasmalema is active, against electrochemical gradient (Raven, 1974b; Ulrich-Eberius *et al.*, 1981), and coupled to the plant metabolism. The suggestion that seagrass leaves act as a sponge or sink for nutrient uptake (Short & McRoy, 1984; Granéli & Solander, 1988; Hemminga *et al.*, 1991) is really true for the phosphate uptake by the leaves of *Zostera noltii*.

The second phase of the biphasic *V* vs *S* curve, at lower phosphate concentrations, is characterized by a slight slope. Short & McRoy (1984) pointed out that this phase represented the actual absorption process during nitrogen uptake by leaves of *Zostera marina*. The change from the first phase, with an initial rapid or surge uptake, to the second phase, with a decreased uptake, could be explained on the basis of a feedback inhibition and/or a regulation of the phosphate efflux. Lefebvre & Glass (1982), working on barley roots, pointed out that internal concentration of phosphorus may serve as the feedback signal modulating its own uptake. In the same way, hyperbolic models could describe in accurate way the second phase in nitrogen uptake kinetics by phytoplankton (Conway *et al.*, 1976; Harrison *et al.*, 1989). This phase was termed *Vi*, representing the uptake rate when the internal pool is full and the cell quota is increasing slowly towards a maximal value.

A second mechanism to reduce the net uptake of phosphate is regulating the P-efflux. Previous studies (Bieleski, 1973; Bieleski & Ferguson, 1983; Elliot *et al.*, 1984; Cogliatti & Santa Maria, 1990) have shown phosphate uptake is not a one-way movement into plant cells (influx) but is accompanied by an efflux such that net phosphate uptake can be very low at times. This studies pointed out that the balance between phosphate influx and efflux is critical to plant growth. Considering the important role of phosphorus in the primary production and, thus, in the nutrient dynamics in the seagrass ecosystems, further studies are needed to determine the influx/efflux kinetics of phosphate as well as biochemical mechanisms for storage and utilization of this nutrient.

Effects of cutting on foliar phosphate uptake

Harrison & Druehl (1982) investigated different methods to determine nutrient uptake by seaweeds, suggested that measurements on whole thalli are preferable for ecological measurements. It becomes really important in studies involving seagrasses, since structural and functional leaf-root interactions exist (Thursby & Harlin, 1982, 1984; Brix & Lyngby, 1985; Pérez-Lloréns, *et al.* 1993). Our results indicate that cutting the leaves resulted in a reduction of the phosphate net uptake rates, as well as in the amount of the nutrient accumulated after 3 h incubations (Table 2). This response could be expected, since higher phosphate release from interrupted vascular system, would be higher in detached leaves. This findings have also been reported in leaves of the seagrass *Zostera marina* (McRoy & Barsdate, 1970).

The results indicate the presumed dominance of basipetal translocation of phosphorus in *Zostera noltii* during the incubations. Predominance of shoot-to-root translocation has been shown in *Z. marina* for nitrogen and heavy metals (Faraday & Churchil, 1979; Brinkhuis *et al.*, 1980; Iizumi & Hattori, 1982; Thursby & Harlin, 1982; Brix & Lyngby, 1985), although the magnitude of this transport will be dependent on the relative phosphate concentrations in the interstitial and in the overlaying water (McRoy & Barsdate, 1970; Carignan, 1982).

Ecological implications

Several studies (Penhale & Thayer, 1980; Iizumi & Hattori, 1982; Short & Short, 1983; Short & McRoy, 1984; Brix & Lyngby, 1985; Zimmerman *et al.*, 1987; Hemminga *et al.*, 1991) have emphazised the importance of dissolved nutrients (phosphorus and nitrogen) in the water column on uptake and growth of *Zostera marina*. Short & McRoy (1984) pointed out that the large surface area and concentration-dependent uptake mechanism enable eelgrass to take full advantage of transient water masses containing high water concentrations, proposing that eelgrass leaves function as a sponge or sink for ammonium.

Long-term studies with the sediment as the only source of phosphate pointed out that submerged macrophytes with root: shoot biomass ratios (RSR) of 0.015 to 0.24 were able to derive enough phosphate from sediment to cope normal growth requirements (Granéli & Solander, 1988). Moreover, McRoy & Barsdate (1970) and Carignan (1982) stated that the relative importance of the roots and shoots to the nutrition of an aquatic plant depends on the ion concentrations in the sediment and surrounding water. In this way, the root (including rhizome): shoot ratio (RSR) of *Zostera noltii* populations growing in Palmones river estuary varies seasonally, being always <1 (Pérez-Lloréns, & Niell, 1993).

This estuary is eutrophic, with phosphate concentrations in water ranging from 0.5 to 25 μM, depending on tide and wastewater discharge (Clavero *et al.*, 1991). Pérez-Lloréns & Niell (1989) in a preliminary study, concluded that in such conditions, these plants would probably be able to cover their shoot phosphorus requirements largely from the surrounding water during the major part of the growing season, making it 'not necessary' to develop large belowground biomass. Further, Pérez-Lloréns (1991), using the Carignan (1982) model, estimated in 55% (average yearly basis) the relative importance of underground parts in phosphate uptake by *Zostera noltii*. However, the Carignan model is simple and nonspecific, and a much better estimation of phosphorus requirements, in relation to phosphorus water supply, could be made from the specific uptake kinetics obtained. Thus, using (1) the relationship for phosphate uptake by sediment-rooted plants calculated for a 180 min incubation period (V^{0--180}) (Fig. 4b); 2) an average water column phosphate concentration of 3.5 μM (Clavero *et al.*, 1991) (maintained by a water flow through the seagrass bed); (3) a foliar production rate of 3.6 10^{-4} g g^{-1} h^{-1} for this especies (Vermaat *et al.*, 1987); and (4) a foliar phosphorus requirement of 0.09 μmol P g^{-1} h^{-1} (for a mean shoot phosphorus content of 0.75% (Pérez-Lloréns & Niell, 1993)), the water column would supply 2.8 times the phosphorus requirement of leaves. In addition, assuming a production rate of belowground parts similar to that reported for *Z. marina* (1.3 10^{-6} g g^{-1} h^{-1}, Jacobs, 1979) and a requirement of 0.04 μmol P g^{-1} h^{-1} (for a mean underground phosphorus content of 0.65% (Pérez-Lloréns & Niell, 1993)), the whole plant could obtain 1.9 times the phosphorus requirement from the water column. Increased accumulation could take place at higher phosphate concentrations or at shorter incubation times (Fig. 4b), suggesting that *Zostera noltii* leaves function as a phosphorus sink in the water column. Further detailed studies on phosphate uptake by underground parts should be also undertaken, because the relative importance in supplying phosphorus increases when the plants occur exposed to the air at low tide or when the phosphate concentration in the water column is low, as was pointed out by Pérez-Lloréns *et al.* (1993) in *Z. noltii*. These authors found that 70% of the phosphate taken up by roots and rhizomes of this plant was translocated to shoots when phosphate concentration in water surrounding the leaves was low (0.7 μM).

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References

Bieleski, R. L., 1973. Phosphate pools, phosphate transport, and phosphate availability. Ann. Rev. Pl. Physiol. 24: 225–252.

Bieleski, R. L. & B. Ferguson, 1983. Physiology and metabolism of phosphate and its compounds. In Lauchli, A. & R. L. Bieleski (eds), Encyclopedia of Plant Physiology. Springer. New York. (15A): 423–449.

Brinkhuis, B. H., W. F. Penello & A. C. Churchill, 1980. Cadmium and manganese flux in eelgrass *Zostera marina* II. Metal uptake by leaf and root-rhizome tissues. Mar. Biol. 58: 187–196.

Brix, H. & J. E. Lyngby, 1985. Uptake and translocation of phosphorus in eelgrass (*Zostera marina* L.). Mar. Biol. 90: 111–116.

Carignan, R. & J. Kalff, 1979. Quantification of the sediment phosphorus available to aquatic macrophytes. J. Fish. Res. Bd Can. 36: 1002–1005.

Carignan, R., 1982. An empirical model to estimate the relative importance of roots in phosphorus uptake by aquatic macrophytes. Can. J. Fish. aquat. Sci. 39: 243–247.

Clarkson, D. T., 1974. Ion transport and cell structure in plants. McGraw Hill, New York.

Clavero, V., F. F. X. Niell & J. A. Fernández, 1991. Effects of *Nereis diversicolor* O. F. Muller abundance on the dissolved phosphate exchange between sediment and overlying water in Palmones river estuary (Southern Spain). Estuar. coast. Shelf Sci. 33: 193–202.

Cochlan, W. P. & P. J. Harrison, 1991. Uptake of nitrate, ammonium and urea by nitrogen-starved cultures of *Micromonas pusilla* (Prasinophyceae): transient responses. J. Phycol. 27: 673–679.

Cogliatti, D. H. & G. E. Santa Maria, 1990. Influx and efflux of phosphorus in roots of wheat plants in non-growth-limiting concentrations of phosphorus. J. exp. Bot. 41: 601–607.

Conway, H. L., P. J. Harrison & C. O. Davis, 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. Mar. Biol. 35: 187–199.

D'Elia, C. F. & J. A. DeBoer, 1978. Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. J. Phycol. 14: 266–272.

Edwards, G. & D. A. Walker, 1983. C3, C4: mechanisms, cellular and environmental regulation of photosynthesis. BSP. Oxford.

Elliott, G. C., J. Lynch & A. Lauchli, 1984. Influx and efflux of P in roots of intact maize plants. Pl Physiol. 76: 336–341.

Epstein, E., 1972. Mineral nutrition of plants: Principles and perspectives. Willey, New York.

Faraday, W. E. & A. C. Churchill, 1979. Uptake of cadmium by the eelgrass *Zostera marina*. Mar. Biol. 53: 293–298.

Fernández, J. A., F. X. Niell & J. Lucena, 1985. A rapid and sensitive automated determination of phosphate in natural waters. Limnol. Oceanogr. 30: 227–230.

Fujita, R. M., 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J. exp. mar. Biol. Ecol. 92: 283–301.

Goldman, J. C., C. D. Taylor & P. M. Glibert, 1981. Nonlinear time-course uptake of carbon and ammonium by marine phytoplankton. Mar. Ecol. Prog. Ser. 6: 137–148.

Goldman, J. C. & P. M. Gilbert, 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. Limnol. Oceanogr. 27: 814–827.

Granéli, W. & D. Solander, 1988. Influence of aquatic macrophytes on phosphorus cycling in lakes. Hydrobiologia 170: 245–266.

Guillard, R. R. L. & J. H. Rhyter, 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* (Hustedt) and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8: 229–239.

Harrison, P. J. & D. L. Druehl, 1982. Nutrient uptake and growth in the Laminariales and other macrophytes: a consideration of methods. In Srivastava, L. M. (ed.) Synthetic and Degradative Processes in Marine Macrophytes. Walter de Gruyter, Berlin: 99–120.

Harrison, P. J., J. S. Parslow & H. L. Conway, 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar. Ecol. Prog. Ser. 52: 301–312.

Hemminga, M. A., P. G. Harrison & F. van Lent, 1991. The balance of nutrient losses and gains in seagrass medows. Mar. Ecol. Progr. Ser. 71: 85–96.

Iizumi, H. & A. Hattori, 1982. Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. III. The kinetics of nitrogen uptake. Aquat. Bot. 12: 245–256.

Jacobs, R. P. W. M., 1979. Distribution and aspects of the production and biomass of eelgrass, *Zostera marina* L., at Roscoff, France. Aquat. Bot. 7: 151–172.

Jiménez, C., F. X. Niell & P. Algarra, 1987. Photosynthetic adaptation of *Zostera noltii* Hornem. Aquat. Bot. 29: 217–226.

Kikuchi, T., 1980. Faunal relationships in temperate seagrass beds. In Phillips, R. C. & C. P. McRoy (eds), Handbook of Seagrass Biology: An Ecosystem Perspective. Garland STPM Press, New York: 153–172.

Kirk, J. T. O., 1983. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press.

Lefebvre, D. D. & A. D. M. Glass, 1982. Regulation of phosphate influx in barley roots: effects of phosphate deprivation and reduction of influx with provision of orthophosphate. Physiol. Plant. 54: 199–206.

Lehman, J. T. & C. D. Sandgren, 1982. Phosphorus dynamics of the procaryotic nannoplankton in a Michigan lake. Limnol. Oceanogr. 27: 828–838.

McCarthy, J. J. & J. C. Goldman, 1979. Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. Science 203: 670–672.

McRoy, C. P. & R. J. Barsdate, 1970. Phosphate absorption in eelgrass. Limnol. Oceanogr. 15: 6–13.

McRoy, C. P. & J. J. Goering, 1974. Nutrient transfer between the seagrass *Zostera marina* and its epiphytes. Nature 258: 173–174.

McRoy, C. P. & C. McMillan, 1977. Production ecology and physiology of seagrasses. In McRoy, C. P. & C. Helfferich (eds), Seagrass Ecosystems: A Scientific Perspective. Marcel Dekker, N.Y.: 53–88.

Parslow, J. S., P. J. Harrison & P. A. Thompson, 1984b. Development of rapid ammonium uptake during starvation of batch and chemostat cultures of the marine diatom *Thalassiosira pseudonana*. Mar. Biol. 83: 43–50.

Parslow, J. S., P. J. Harrison & P. A. Thompson, 1984c. Saturated uptake kinetics: transient response of the marine diatom *Thalassiosira pseudonana* to ammonium, nitrate, silicate or phosphate starvation. Mar. Biol. 83: 51–59.

Parslow, J. S., P. J. Harrison & P. A. Thompson, 1985b. Interpreting rapid changes in uptake kinetics in the marine diatom *Thalassiosira pseudonana* (Hustedt). J. exp. mar. Biol. Ecol. 91: 53–64.

Patriquin, D., 1972. The origin of nitrogen and phosphorus for growth of the marine angiosperm *Thalassia testudinum*. Mar. Biol. 15: 35–46.

Penhale, P. A. & G. N. Thayer, 1980. Uptake and transfer of carbon and phosphorus by eelgrass (*Zostera marina* L.) and its epiphytes. J. exp. mar. Biol. Ecol. 42: 113–123.

Pérez-Lloréns, J. L., 1991. Estimaciones de biomasa y contenido interno de nutrientes, ecofisiología de incorporación de carbono y fosfato en *Zostera noltii* Hornem. PhD Thesis. University of Málaga.

Pérez-Lloréns, J. L. & F. X. Niell, 1989. Emergence and submergence effects on the distributional pattern and exchange of phosphorus in the seagrass *Zostera noltii* Hornem. Scient. Mar. 53: 497–503.

Pérez-Lloréns, J. L., P. de Visscher, P. H. Nienhuis & F. X. Niell, 1993. Light-dependent uptake, translocation and foliar release of phosphorus by the intertidal seagrass *Zostera noltii* Hornem. J. exp. mar. Biol. Ecol. 166: 165–174.

Pérez-Lloréns, J. L. & F. X. Niell, 1993. Seasonal dynamics of biomass and nutrient content in the intertidal seagrass *Zostera noltii* Hornem. from Palmones river estuary, Spain. Aquat. Bot. 46: 49–66.

Raven, J. A., 1974b. Energetics of active phosphate influx in *Hydrodictyon africanum*. J. exp. Bot. 25: 221–229.

Short, F. T. & C. Short, 1983. The seagrass filter: Secondary and tertiary purification of coastal waters. Est. Res. Conference Abstract. Estuaries 6: 291.

Short, F. T. & C. P. McRoy, 1984. Nitrogen uptake by leaves and roots of the seagrass *Zostera marina* L. Bot. Mar. 17: 547–555.

Short, F. T., 1987. Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. Aquat. Bot. 27: 41–57.

Thomas, T. E. & P. J. Harrison, 1985. Effect of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata* (Rodophyta). Mar. Biol. 85: 269–278.

Thomas, T. E. & P. J. Harrison, 1987. Rapid ammonium uptake and nitrogen interactions in five intertidal seaweeds grown underfield conditions. J. exp. mar. Biol. Ecol. 107: 1–8.

Thursby, G. B. & M. M. Harlin, 1982. Leaf-root interactions in the uptake of ammonia by *Zostera marina* L. Mar. Biol. 72: 109–112.

Thursby, G. B. & M. M. Harlin, 1984. Interaction of leaves and roots of *Ruppia maritima* in the uptake of phosphate, ammonia and nitrate. Mar. Biol. 83: 61–67.

Tomlinson, P. B., 1980. Leaf morphology and anatomy in seagrasses. In Phillips, R. C. & C. P. McRoy (eds), Handbook of Seagrass Biology: An Ecosystem Perspective. Garland STPM Press, New York.

Topinka, J. A., 1978. Nitrogen uptake by *Fucus spiralis* (Phaeophyceae). J. Phycol. 14: 241–247.

Ullrich-Eberius, C. I., A. Novacky, E. Fisher & U. Luttge, 1981. Relationship between energy-dependent phosphate uptake and electrical membrane potential in *Lemna gibba* Gl. Pl. Physiol. 67: 797–801.

Vermaat, J. E., M. J. M. Hootsmans & P. H. Nienhuis, 1987. Seasonal dynamics and leaf growth of *Zostera noltii* Hornem., a perennial intertidal seagrass. Aquat. Bot. 28: 287–299.

Zimmerman, R. C., R. D. Smith & R. S. Alberte, 1987. Is growth of eelgrass nitrogen limited?. A numerical simulation of the effects of light and nitrogen on the growth dynamics of *Zostera marina*. Mar. Ecol. Prog. Ser. 41: 167–176.