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Alkaline Phosphatase Activity in *Zostera noltii* Hornem. and its Contribution to the Release of Phosphate in the Palmones River Estuary

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Alkaline phosphatase activity (APA) was studied in *Zostera noltii* Hornem., a sea-grass collected in the Palmones river estuary (southern Spain). The higher activity was found in the leaves, with minor contributions in the stem and the underground parts of the plant. The enzymatic activity showed a two-phase kinetic versus substrate concentration between 5 μM and 25 mM. The influence of some environmental factors important in nature (temperature, pH, salinity, photon irradiance and external phosphate) on the enzymatic activity is discussed. Over an ecophysiological range of these factors, maximum APA was found at 30 °C (22·6 μmol pNP released g dry wt^{-1} h^{-1}), pH 8·8 (35·6 μmol pNP g dry wt^{-1} h^{-1}) and salinity 43·8 (27·8 μmol pNP g dry wt^{-1} h^{-1}). With regard to light, APA and phosphate uptake in shoots were light-saturated and showed similar values for maximum velocity and half-saturation constant. In the range of phosphate concentration tested (0–20 μM), APA was independent of the external phosphate concentration. Finally, as *Z. noltii* incorporated only 16% of the phosphate hydrolysed from the model phosphomonoester used in the assay, the significance of *Z. noltii* population in the enzymatic release of phosphate to the estuary was estimated. A minimum of 8·4 nM Pi liberated per day and a maximum of 99·8 nM Pi day^{-1} was found.

Introduction

Many organisms that inhabit the aquatic ecosystems may use dissolved organic phosphorus (DOP) compounds as a source of orthophosphate. These compounds are used after enzymatic hydrolysis by a cell-surface alkaline phosphatase (Cembella *et al.*, 1983). Phosphomonoesters (PME) are considered to be the main DOP fraction capable of being used by the aquatic organisms (Boavida & Health, 1988). According to several workers (Berman, 1970; Taft *et al.*, 1977; Kobori & Taga, 1979; Veldhuis *et al.*, 1987),

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this fraction constitutes between 10 and 70% of DOP. Alkaline phosphatase activity (APA) has been described commonly in many planktonic species, either bacteria (Kobori & Taga, 1979; Chan & Dean, 1987), cyanobacteria (Healey, 1982; Whitton, 1992) or many species of unicellular algae (Siuda & Chróst, 1987; Wynne & Rhee, 1988; Van Boekel, 1991; Lubián *et al.*, 1992). Generally high phosphatase activity has been considered as an indication of phosphorus deficiency (Boavida & Heath, 1986; Pick, 1987). With regard to macroalgae, enzymatic activity has been reported in small freshwater species of the Chaeophorales (Gibson & Whitton, 1987) and in marine macroalgae such as *Ulva lactuca* (Weich & Granéli, 1989) or *Porphyra umbilicalis* (Hernández *et al.*, 1992). Kufel (1982) reported APA in homogenized extracts of some freshwater aquatic macrophytes, such as the common red *Phragmites australis* or the water lily *Nuphar luteum*. However, the latter work did not indicate whether the enzymatic activity was related to the cleavage and phosphate transfer reactions in metabolic pathways within the cell or if the activity came from a cell-surface alkaline phosphatase with the ecophysiological significance found in phytoplanktonic organisms.

As far as we are aware, APA in a sea-grass has never been reported. The study of APA in *Zostera noltii* Hornem. is of great interest, as this study is dealing with a sea-grass. According to Den Hartog (1970), the sea-grasses must have evolved directly from terrestrial ancestors. Alkaline phosphatase activity in this species might have resulted as a consequence of an adaptation to a new habitat with a more diluted nutrient concentration (particularly phosphorus). Alkaline phosphatase activity constitutes an additional mechanism of getting phosphorus that could keep this species in a competition level according to the new environment.

The aim of this work was to study alkaline phosphatase activity of *Z. noltii* and investigate the dependence of the enzymatic activity on some environmental factors important in nature (temperature, pH, salinity, light and soluble reactive phosphorus). These selected factors are of interest as they vary daily on the sampling site (Pérez-Llorens, 1991). Finally, a third goal was to estimate the significance of APA in the release of phosphate to the estuary.

Material and methods

Collection and pre-treatment

Zostera noltii was collected from the Palmones river estuary, southern Spain (36°11'N, 5°27'S), an eutrophic, shallow estuary (0·9 m at maximal depth) located in the Algeciras Bay, near the Straits of Gibraltar. The estuary is characterized by a great energy input with a high production (Pérez-Llorens & Niell, 1989). Tidal movements have a maximum amplitude of 1 m, causing extensive areas of mud to emerge daily at low tide (Clavero *et al.*, 1991) and then causing the beds of *Z. noltii* to emerge for several hours daily. The collected plants belonged to a large-leaved morphotype (Pérez-Llorens & Niell, 1993*a*), which are out of the water about 5–15% of the time. As a consequence, these plants are exposed to a broad range of temperatures and photon irradiances. The salinity of the water ranges from 20 to 35 (practical salinity scale), except in the case of heavy rainfall, in which values close to 5 have been measured (López-Figueroa, 1984). Soluble reactive phosphorus (SRP) varies between 0·5 and 11 μM throughout the year (Clavero, 1992). For further details about the localization of the estuary and benthic

infauna see Clavero *et al.* (1991). The biomass of *Z. noltii* displays regular (unimodal) seasonal fluctuations throughout the year, with maximum biomass in summer and minimum during the winter (Pérez-Llorens & Niell, 1993*b*).

After collection, plants were kept cool and transported to the laboratory in an icebox within 2 h of collection; they were gently scraped to remove all loosely attached material. Before any experimental work, plants were maintained (5 days maximum) in renewed cultures of aerated, filtered natural seawater (pH 8·2) at constant temperature (15 °C) and photon irradiance of 150 μmol photons m^{-2} s^{-1} (solar LFD 18 W tube). The orthophosphate (Pi) concentration of the cultures was kept constant at 1 μM, similar to the mean phosphate concentration in the field (Clavero, 1991).

Alkaline phosphatase activity assay

Alkaline phosphatase activity was determined in three replicate samples by the method of Reichardt *et al.* (1967), as modified by Hernández *et al.* (1992). This method uses *p*-nitrophenyl phosphate (pNPP) as organic substrate. Shoots (cluster of leaves attached to a small stem, the aerial parts) (0·25 g wet wt) were incubated in a medium containing 50 ml of 1 mM pNPP and 50 ml of 0·1 M Tris–HCl buffer, pH 8·3. Both reagents were dissolved in filtered (0·2 μm) artificial seawater of negligible Pi concentration and 35 salinity (Kalle, 1945). The Mg^{2+} concentration in the artificial seawater (55 mM) was high enough to ensure a fully active enzyme. Air was bubbled continuously during the assay to oxygenate and stir the medium. After 45 min of incubation at 25 °C and 200 μmol photons m^{-2} s^{-1}, the absorbance was read at 410 nm against a blank (buffer and substrate solution without plants) in a Beckman DU-7 spectrophotometer. Activity is reported as μmol paranitrophenol (pNP) released g dry wt^{-1} h^{-1}. Alkaline phosphatase activity was expressed per unit of dry weight rather than per unit of surface area as Figure 1 includes activity in the roots–rhizomes. The underground parts of *Z. noltii* include hairs and thin roots, so is not possible to express APA per unit of surface area. However, the ratio surface:dry wt is constant at different parts of the shoots (Hernández, unpubl. data), being probably higher at the underground parts (higher surface:volume ratio, especially in the thinnest and smallest roots).

Experimental set-up

To determine kinetic parameters, pNPP concentrations ranging from $5\ 10^{-6}$ to $2{\cdot}5\ 10^{-2}$ M were used. The Eadie transformation (*v vs. v/S*) (Eadie, 1942) of the Michaelis–Menten equation was used to determine the kinetic constants of the enzyme.

The temperature response of APA was determined at pH 8·3 within a range between 10 and 30 °C. Temperature-dependent changes in pH from 8·3 were compensated for by the addition of Tris or HCl. Parameters concerning the influence of temperature on the enzymatic reaction were calculated from the Arrhenius equation (Price & Stevens, 1982). The effect of pH on enzymatic activity was tested using different Tris–HCl buffers at 0·25-pH intervals between 7 and 9. Different salinities of the assay medium were obtained by making up solutions of artificial seawater from 0 salinity (distilled water) to 52·5, varying quantities of all reagents in the artificial seawater. The effect of external phosphate concentration on APA was studied by pre-incubating the plants for 5 days in 400 ml natural seawater enriched with different quantities of K_2HPO_4 as a source of orthophosphate. The final concentration of Pi added ranged from 0 to 20 μM.

The influence of photon irradiance on APA and phosphate (Pi) uptake was tested by illuminating the incubation chamber with a light source (a 150 W halogen lamp) in which different light intensity selective filters were inserted. For both APA and Pi uptake, the mean values obtained in the following subranges of photon irradiance (in μmol photons m^{-2} s^{-1}) were considered: 0, 0–10, 10–20, 20–50, 50–100, 100–200, 200–500, 500–1000, 1000–2000. Phosphate uptake rates were estimated in artificial seawater enriched with 15 μM of Pi at a constant temperature of 25 °C. After 10 min incubation, at each selected photon irradiance 1 ml of water sample was removed with a syringe and phosphate concentration measured (Murphy & Riley, 1962). Uptake rates were calculated directly from the depletion data and normalized to dry weight. Five replicates were used in all cases. The values for different parameters concerning the light influence on both processes were obtained with an enzfitter (Elsevier-Biosoft) computer program.

To assay the enzymatic activity in different parts of *Z. noltii*, plants were previously cut into five different sections (Pérez-Llorens & Niell, 1989): leaf tip (the oldest leaf part; LT) leaf centre (LC), leaf base (LB), stem (S) and underground parts (roots–rhizome; R). To test the influence of the age of the leaves on the enzymatic activity, APA was assayed separately in internal leaves (the youngest) and external (oldest) leaves.

To estimate the proportion of the phosphate enzymatically liberated that was taken up by *Z. noltii* in relation to the pNP liberated (pNPP hydrolysed), at different times (15, 30, 45 and 60 min), 2·5 ml of the assay medium were sampled to determine Pi and APA; Pi was quantified as SRP by the molybdenum blue method (Murphy & Riley, 1962). In this condition, the non-enzymatic hydrolysis of pNPP by acid conditions can be assumed to be negligible (McComb *et al.*, 1964). Moreover, the yellow colour of the pNP liberated did not interfere with the Murphy and Riley method, as the colour vanished totally after the acidification caused by the molybdate reagent. Finally, the absence of a third product, acceptor phosphate (phosphoethanolamine; Wilson *et al.*, 1964) during the assay was proved by the non-existence of any differences in absorbance between two sets of Pi standards (between 0 and 35 μM phosphate), one using distilled water and other using buffered artificial seawater as a solvent (Hernández, 1992).

The velocity of phosphate release from PME was calculated from the Michaelis–Menten equation, using values of kinetics parameters from the high-affinity phase of *Z. noltii* alkaline phosphatase and substrate concentration observed in the samples of freshly collected estuarine water. Phosphomonoesters were estimated essentially as by Francko and Heath (1979) as the increase in SRP following incubation with calf intestinal mucosa alkaline phosphatase. To 2·5 ml of filtered (0·2 μm) water samples was added 0·05 ml of a solution containing 0·7 mg calf intestinal mucosa enzyme (Sigma) per ml of 0·1 mM Tris–HCl, 0·01 M $MgCl_2$, pH 9. Samples were incubated for 24 h at 37 °C. Concentration was determined by comparison with a standard dilution series of glucose-6-phosphate.

Statistical analyses

Differences among means were tested by a single factor analysis of variance (ANOVA). Multiple *post hoc* comparisons were performed by the Tukey–Kramer test (Zar, 1984). The comparison of two means was performed by a Student's *t*-test (Fisher, 1925). Differences of the pNP and SRP concentrations during the enzymatic assays were tested for significance by a test to compare two slopes (Zar, 1984). In all cases the null hypothesis was rejected for the 5% significance level.

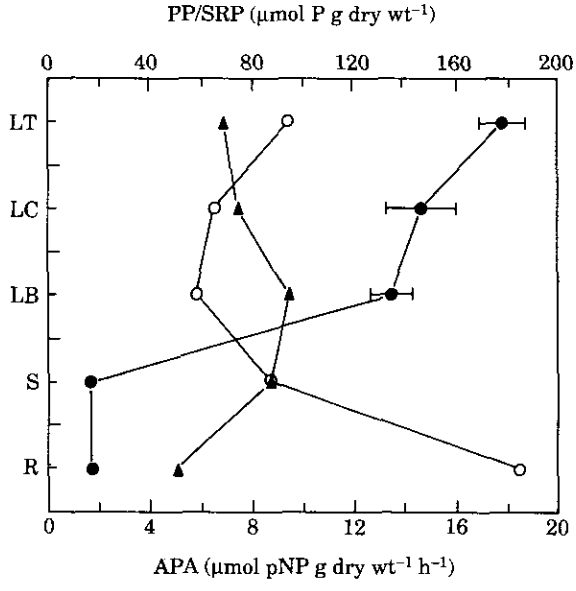

Figure 1. Comparative values of APA (●) internal PP (○) and SRP (▲) contents in different sections of plants of *Zostera noltii*. LT, Leaf tip; LC, leaf centre; LB, leaf base; S, stem; R, roots–rhizome. Values of APA are the mean of three replications and bars denote SD. Values of PP and SRP are simplified from Pérez-Llorens and Niell (1989), with permission of the authors.

Results

Figure 1 shows APA in the different sections considered in *Z. noltii*. As a whole, APA was higher in leaves than in stems and underground parts. In leaves, APA was maximum in the leaf tip (17·9 μmol pNP g dry wt^{-1} h^{-1}) and decreased to the leaf base. The differences in APA among plant sections were significant ($P<0·001$). Alkaline phosphatase activity in the leaves was significantly higher than the activity in stems and roots–rhizomes. Besides, in leaves, APA in the leaf tip was significantly higher than APA in the leaf centre and leaf base. However, although APA was higher in the internal leaves than in the external ones (Figure 2), these differences were not significant.

Figure 3 shows the pNP and SRP concentrations (in μM g dry wt^{-1}) measured in the medium during the assay of APA. The slopes of the regression lines for both end products of the enzyme reaction were significantly different. The difference between the quantities liberated to the medium can be considered as the percentage of phosphorus which is taken up by the plants after the hydrolysis of pNPP. This percentage was low, as only 16% of the phosphate hydrolysed through APA was taken up by the sea-grass.

Figure 4 shows the Eadie transformation of Michaelis–Menten plot, illustrating the dependence of APA on substrate concentration. The Eadie plot revealed the existence of a two-phase kinetic between 5 μM and 25 mM pNPP. Both phases followed Michaelis–Menten-type kinetics. The transition between the two phases was *c.* 0·25 mM. The half-saturation constant ($Km \pm$ SE) and the maximum velocity ($V_{max} \pm$ SE) of the high-affinity phase were $12·1 \pm 5·6$ μM and $3·5 \pm 0·66$ μmol pNP g dry wt^{-1} h^{-1}, respectively, whereas for the low-affinity phase $Km = 5·2 \pm 1·2$ mM and $V_{max} = 94·1 \pm 10·8$ μmol pNP g dry wt^{-1} h^{-1}.

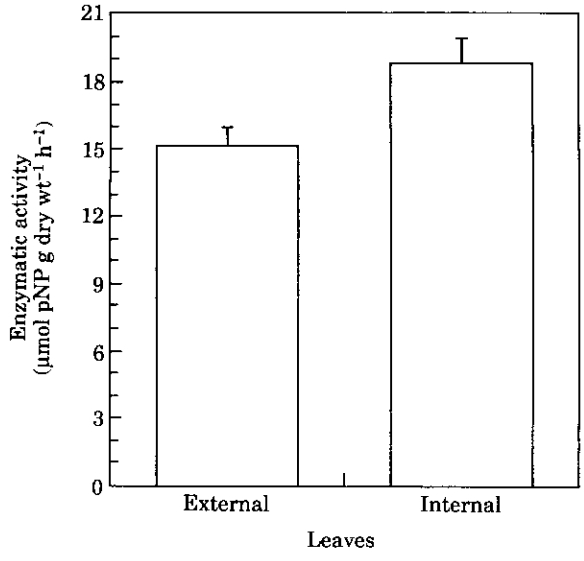

Figure 2. Comparative values of APA in young (internal) leaves and adult (external) leaves of *Zostera noltii*. A Student's *t*-test did not demonstrate significant differences between both kinds of leaves. Columns are the mean of three replicates and bars denote SD.

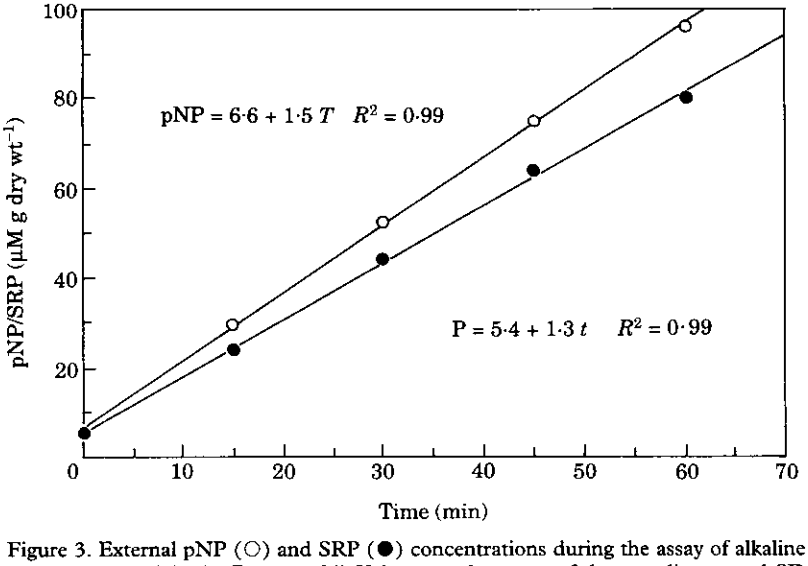

Figure 3. External pNP (○) and SRP (●) concentrations during the assay of alkaline phosphatase activity in *Zostera noltii*. Values are the mean of three replicates and SD was negligible.

Figure 5 shows the variation of APA as a function of temperature in a range in keeping with natural conditions. The activity increased linearly in the range analysed (i.e. no inhibition was observed at temperatures up to 30 °C) with a maximum activity of 22·6 μmol pNP g dry wt^{-1} h^{-1} at 30 °C. The regression line fitted (R^2=0·77) was

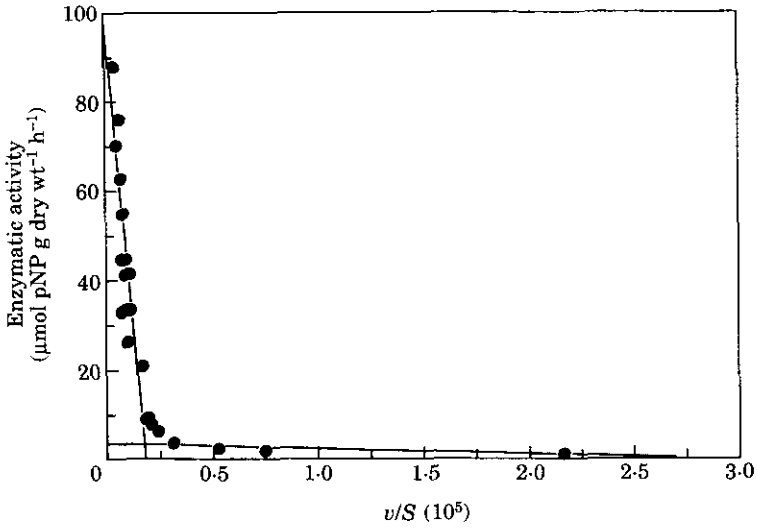

Figure 4. Eadie plot relating the dependence of the velocity (v) of hydrolysis of pNPP on substrate (S) concentration. A two-phase kinetic was evident by the two straight lines drawn in the figure. The two slopes give the values for *Km* in both phases and the two y-axis intercepts, both values for V_{max}. Substrate concentration expressed as μM pNPP.

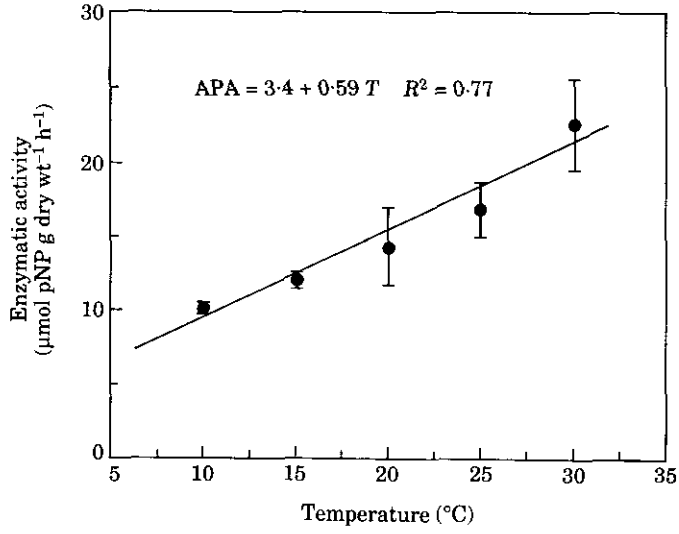

Figure 5. Rate of hydrolysis of pNPP by alkaline phosphatase of *Zostera noltii* as a function of temperature (pH=8·3). In the range assayed, APA was related linearly with temperature. Values are the mean of three replicates and bars denote SD.

APA=3·4+0·59 T, with 2·4 and 0·08 being the standard errors of the y-intercept and the slope, respectively. The Arrhenius plot (Log $V_{max}=Ea/R \times 1/T+$constant) revealed an activation energy (Ea) of 26·9 kJ mol^{-1}. That gives a Q_{10} value of 1·4.

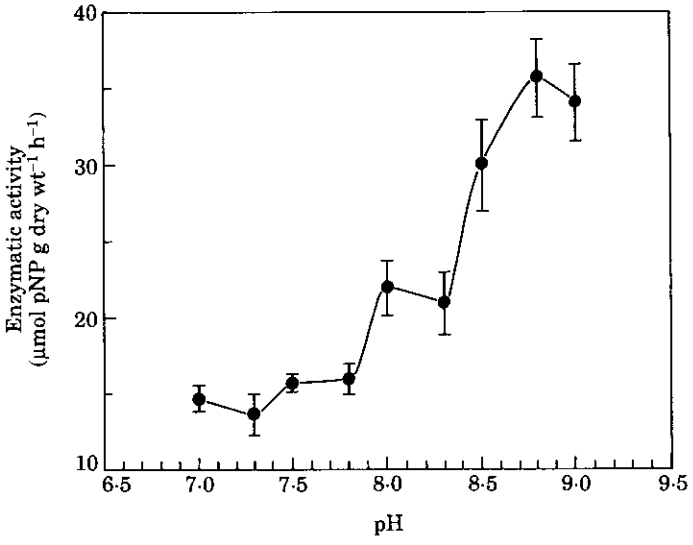

Figure 6. Rate of hydrolysis of pNPP by alkaline phosphatase of *Zostera noltii* as a function of pH (temperature = 25 °C). Maximum APA was 35·6 μmol pNP g dry wt^{-1} h^{-1} at pH 8·8. Values are the mean of three replicates and bars denote SD.

The rate at which *Z. noltii* hydrolysed pNPP was significantly dependent on pH (Figure 6). Maximum activity (35·6 μmol pNP g dry wt^{-1} h^{-1}) occurred at pH 8·8, but activity was found in the whole range of pH assayed (7–9).

Salinity also influenced APA significantly (Figure 7). Maximum activity occurred at salinity 43·8 and resulted in 27·8 μmol pNP g dry wt^{-1} h^{-1}. On the contrary, plants assayed in distilled water and low salinities showed the lowest activities.

The influence of photon irradiance on APA is shown in Figure 8(a). *Zostera noltii* exhibited the minimum APA in dark. The activity increased with photon irradiance until reaching a maximum activity of 7·6 μmol pNP g dry wt^{-1} h^{-1} at 132 μmol photons m^{-2} s^{-1}. An additional increase in light intensity did not change the activity perceptibly. For this reason, data were fitted to a saturation curve:

$$APA = APA_{max} \times \frac{I}{I_{0\cdot5} + I} \quad (1)$$

giving a value of $I_{0\cdot5}$ (half-saturation constant for photon irradiance) of 19·1 μmol photons m^{-2} s^{-1} and a maximum APA (APA_{max}) of 7·9 μmol pNP g dry wt^{-1} h^{-1}. This pattern was similar to the influence of photon irradiance on Pi uptake by leaves of *Z. noltii* [Figure 8(b)], that was also fitted to a Michaelis–Menten model [just replacing APA by velocity of Pi uptake in equation (1)]. In this case, V_{max} was found to be 9·8 μmol Pi g dry wt^{-1} h^{-1} and $I_{0\cdot5}$ was 27 μmol photons m^{-2} s^{-1}.

Alkaline phosphatase activity of *Z. noltii* was not affected by the orthophosphate concentration in the water, at least in the range of SRP tested, according to the range of SRP found throughout the year. The pre-incubation (5 days) of the plants in different regimes of external phosphate concentration did not affect the enzymatic activity (Figure 9).

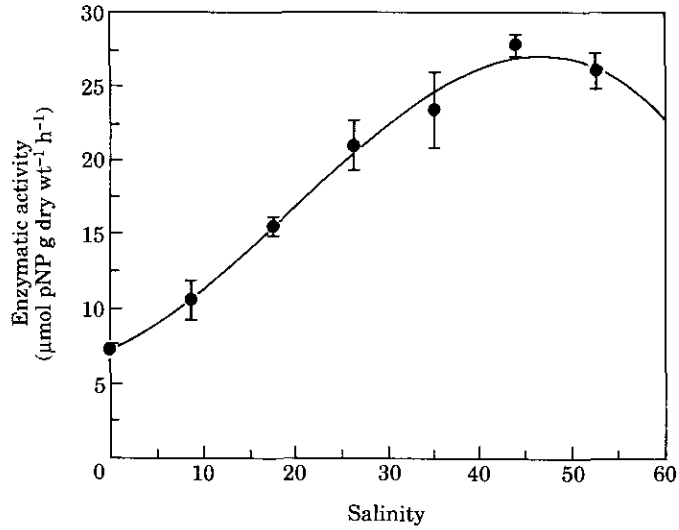

Figure 7. Rate of hydrolysis of pNPP by alkaline phosphatase of *Zostera noltii* (pH=8·3; temperature=25 °C) in media of assay prepared at different salinities. Maximum APA was 27·8 μmol pNP g dry wt^{-1} h^{-1} at salinity 43·8. Values are the mean of three replicates and bars denote SD.

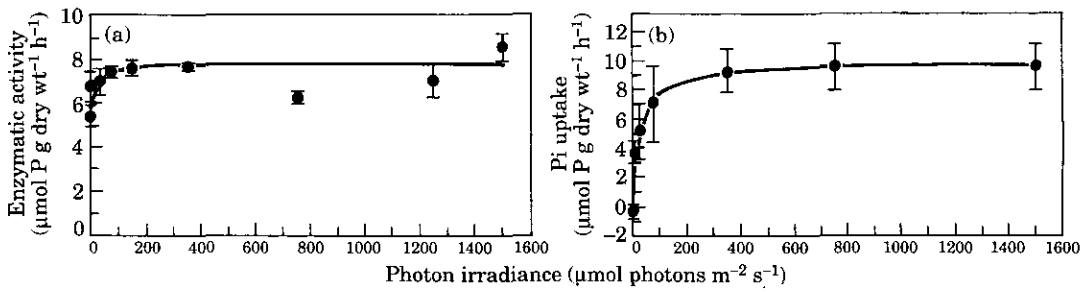

Figure 8. (a) Rate of hydrolysis of pNPP by alkaline phosphatase in shoots of *Zostera noltii* (pH=8·3; temperature=25 °C) as a function of photon irradiance. Maximum APA estimated was 7·9 μmol pNP g dry wt^{-1} h^{-1}. Values are the mean of three replicates and bars denote SD. (b) Phosphate uptake in shoots of *Zostera noltii* (pH=8·3; temperature=25 °C) as a function of photon irradiance. Maximum Pi uptake estimated was 9·8 μmol P g dry wt^{-1} h^{-1}. Values are the mean of five replicates and bars denote SD. In both figures a saturation curve was fitted to the data.

The mean PME value in the water throughout the sampling period was 0·28 μM (SD=0·2, n=10) (data not shown). Differences in PME in the water column were not observed. Thus, the velocity of release of phosphate from PME through the hydrolytic action of *Z. noltii* phosphatase can be estimated. We used the kinetic parameters of the high-affinity phase of APA in the aerial parts (shoots) of the plant (as the mean PME concentration is lower than *Km*). Assuming that only 16% of the phosphate released from PME is taken up by the sea-grass, the daily contribution of *Z. noltii* to the release of phosphate to the estuary through enzymatic hydrolysis of PME can be calculated as follows

$$V_{rel}(\text{nmol Pi } l^{-1} \text{ day}^{-1}) = 1{\cdot}3 \times \frac{\text{PME}}{12{\cdot}1 + \text{PME}} \times 0{\cdot}84 \times \frac{24 \times AB \times AE \times PC \times 1000}{VE} \quad (2)$$

where 1·3 (in μmol pNP g dry wt^{-1} h^{-1}) and 12·1 (in μM) are the V_{max} and the *Km*, respectively, of the high-affinity phase of APA in the aerial parts; PME is given in μM; *AB* is the aerial biomass (in g dry wt m^{-2}); *AE* is the area of the estuary (approximately $5{\cdot}2 \times 10^6$ m^2; *PC* is the coverage of *Z. noltii* in the estuary; and *VE* is the mean volume of the estuary ($7{\cdot}9 \times 10^6$ m^3).

The data are shown in Table 1. These contributions ranged from 8·4 nM Pi day^{-1} in winter with a coverage of 20% to 99·8 nM P day^{-1} in summer with a coverage of 50%.

Discussion

The turions of *Z. noltii* showed alkaline phosphatase activity essentially at the leaves, with minor activities at the stems and underground parts. Pérez-Llorens and Niell (1989) studied the internal phosphorus pools in the communities of this sea-grass in the Palmones river estuary. The pattern for particulate phosphorus content (PP) and internal SRP reported in their work was related to the pattern for APA found in the present work (Figure 1). In leaves, APA was inversely correlated with internal SRP content but directly correlated with the internal PP content. However, in the underground parts the profile for APA was clearly inverse to the PP content. Therefore, APA seems to be controlled by different compartments of phosphorus depending on the part of the thallus.

Alkaline phosphatase in *Z. noltii* demonstrated a two-phase kinetic. Although APA usually shows Michaelian-type kinetic versus pNPP (e.g. Flynn *et al.*, 1986; Wynne & Rhee, 1988), a three-phasic APA system was found earlier by Rivkin and Swift (1980) in the dinoflagellate *Pyrocystis noctiluca*. So far, we have not been able to resolve isoenzymes of alkaline phosphatase of *Z. noltii*, and this is being studied in a present research. Thus, we have two main hypotheses to explain the two-phase nature of APA in *Z. noltii*. First, it might result from two isoenzymes of high and low affinity, which operate under conditions of low and high PME concentration. The kinetic behaviour also might result from two or more reactive sites on the same enzyme, showing negative cooperativity (Cadenas, 1978). In *Z. noltii*, the Hill coefficient of cooperativity, which can be determined easily by the Hill procedure (Hill, 1913), was 0·73.

The ecological significance of the two-phase kinetic lies in the linkage of enzymatic kinetic parameters to the external substrate concentration in the most bioenergetically favourable way (Levitzky & Koshland, 1976; Cembella *et al.*, 1983) so that *Z. noltii* would achieve a constant phosphorus supply (from PME) regardless of the PME concentration in the environment, as Burns and Beever (1977) hypothesized for Pi uptake two-phase kinetic in *Neurospora crassa*. Short-term pulses of DOP are probably quite common in many natural environments (Rivkin & Swift, 1980; Islam & Whitton, 1992). In Palmones river estuary, such pulses have been observed in low tide and wastewater discharge.

The *Km* of the high-affinity phase was similar to other *Km* values reported for alkaline phosphatase with pNPP as a substrate, either in phytoplanktonic species (Wynne & Rhee, 1988) or macroalgae (Hernández *et al.*, 1992).

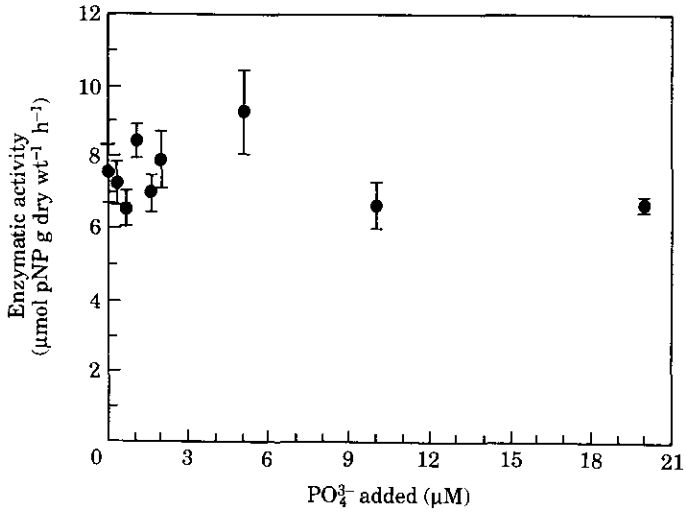

Figure 9. Rate of hydrolysis of pNPP by alkaline phosphatase of *Zostera noltii* (pH=8·3; temperature=25 °C) in media of increasing inorganic phosphate concentration. Plants were incubated for 5 days in different regimes of external orthophosphate. No relationship was evident between the two variables. Values are the mean of three replicates and bars denote SD.

TABLE 1. Velocity of enzymatic release of phosphate from PME in the Palmones river estuary[a], calculated using kinetic parameters of the high-affinity phase of the aerial parts of *Zostera noltii* alkaline phosphatase[b]. The estimates are based on the use of an artificial substrate (*p*-nitrophenyl phosphate). All data in nmol Pi released l^{-1} day^{-1}. It was assumed that only 16% of the phosphate released from PME is taken up by the sea-grass

Aerial biomass (g dry wt m^{-2})	Percentage cover	
	20%	50%
40 (winter)	8·4	38·0
190 (summer)	39·9	99·8

[a]Estuary data: area=5·2 10^6 m^2. Mean depth=1·5 m. Mean volume=7·9 10^6 m^3.
[b]Kinetic parameters: Km=12·1 μM. V_{max}=3·5 μmol pNP g dry wt^{-1} h^{-1}.

Alkaline phosphatase activity seemed to be well adapted for the physical and chemical changes that *Z. noltii* undergoes in the environment. Due to the tidal movements in the estuary *Z. noltii* is exposed for several hours daily. During this time the plants are soaked in a layer of water and the effect of desiccation is reduced by clumping. Thus, *Z. noltii* is in the presence of PME within a range of pH, salinity, photon irradiance and temperature. This is manifested by the relative independence of APA versus temperature. In this range (according to the probable values experienced by *Z. noltii* in its natural environment) the Q_{10} value showed that less than 50% of APA is reduced or increased when temperature varies 10 °C. This low value of Q_{10} suggests a strong regulation of APA with temperature, as occurs typically in many enzymes from intertidal species such as anemones and winkles, which have values of Q_{10} about 1 (i.e. rates do not change significantly with temperature) (Price & Stevens, 1982). The Q_{10} value found for APA

in *Z. noltii*, is lower than most of the values reported previously (Reichardt *et al.*, 1967; Pettersson & Jansson, 1978; Healey & Herdzel, 1979; Hernández *et al.*, 1992) but similar to the Q_{10} value proposed by Pick (1987) for the algal APA in Lake Ontario.

On the other hand, within the range assayed, maximum APA was observed at pH 8·8 (Figure 6). This pH value can be attained in the periplasmic space in the case of emergence, when direct sunlight exposure and high photosynthetic rate cause a OH^- disposal (Lucas & Berry, 1985). That means a maximum extra phosphorus supply in cases of high rates of carbon fixation. The pH optimum found for APA in *Z. noltii* is similar to that found in *Porphyra umbilicalis* (Hernández *et al.*, 1992). In several cyanobacteria, APA is optimal between pH 8 and 10 (Healey, 1982 and references cited therein) but higher optima have been reported (Flynn *et al.*, 1986; Gibson & Whitton, 1987; Mahasneh *et al.*, 1990).

Salinity also influenced APA (Figure 7). The results suggest that *Z. noltii* obtains higher extra phosphorus under seawater flooding rather than brackish waters. However, in the latter case low but measurable activity was found. A similar pattern for APA versus salinity was found in *P. umbilicalis* (Hernández *et al.*, 1992). The increase of APA with salinity may be attributable to a higher ionic strength (Wilson *et al.*, 1964) or a specific ion, a fact that should be tested in future research. However, we have present evidence that the influence of salinity on APA is not just an osmotic effect but Na^+ could be directly involved (Hernández, 1992). The fact that APA is dependent on salinity could be of ecological significance in cases of direct solar radiation and high temperature. A higher phosphorus supply from PME may balance states of low nutrient concentration as stress could force an additional demand of nutrients (Hernández *et al.*, 1992).

A similar pattern for APA and Pi uptake versus photon irradiance was found, with values for $I_{0·5}$ and V_{max} surprisingly similar (Figure 8). The stimulation of APA by light suggests a dependence on metabolic energy from photophosphorylation (Weich & Granéli, 1989). A higher activity in light rather than dark has been pointed out by some other researchers as Rivkin and Swift (1979) in *Pyrocystis noctiluca* and Patni *et al.* (1977) in *Chlamydomonas reinhartdii*. These authors suggested that this performance is internally controlled and linked to an oxidative metabolism. Furthermore, Weich and Granéli (1989) found in *Ulva lactuca* that external APA and Pi uptake were strongly stimulated in light, suggesting a dependence of energy metabolism on phosphate availability, and postulating that both processes may occur as an energy loss.

Alkaline phosphatase activity usually decreases after Pi addition (Jansson *et al.*, 1981; Pick 1987; Van Boekel & Veldhuis, 1990). An increase in APA under conditions of phosphorus deficiency has been generally observed (Kuenzler & Perras, 1965; Wynne, 1981; Elgavish *et al.*, 1982). However, in *Z. noltii* APA seems to be dependent on internal phosphorus content (Figure 1) rather than external phosphate (Figure 9). Alkaline phosphatase was not affected at all up to 20 μM of the external SRP concentration. We have not tested if higher, unrealistic external SRP concentrations cause any loss of activity by decrease in enzyme synthesis. However, these results are in agreement with Stevens and Parr (1977), Huber and Kidby (1985) and Hino (1988), who found that algal APA was independent on the external concentration of Pi in lake water. Furthermore, in some species, especially Chrysophytes and Cryptomonads, APA was not easily inhibited by additions of orthophosphate (Kuenzler, 1965; Aaronson & Patni, 1976; Stewart & Wetzel, 1982).

Zostera noltii releases a certain amount of phosphate to the estuary through APA. We assumed that the kinetic parameters of hydrolysis of natural PME were comparable with

those calculated for pNPP as a substrate. This substrate is reliable for the investigation of some algal phosphatases (Flint & Hopton, 1977; Rivkin & Swift, 1980) and has been used to compare the rate of hydrolysis of PME with the total phosphate demand of the plankton (Boavida & Heath, 1988). However, the calf intestinal enzyme used to estimate PME may differ in substrate specificity from that of *Z. noltii*. Also, minor differences are possible between the actual amount of PME susceptible to be used by *Z. noltii* and the concentration quantified. Both questions should be answered in a future study.

The rates of enzymatic release of phosphate from PME shown in Table 1 should be taken into account in future models for a better understanding of the dynamics of phosphate in the estuary. The idea of extrapolating the velocity of phosphorus release from PME obtained in the laboratory to the situation in the Palmones river estuary is just an attempt, and we are aware of the limitations. Further research will study: (1) fluctuations of APA throughout the year that probably undergo *Z. noltii*, as observed in algal communities (Hernández *et al.*, 1993), (2) PME dynamics, as substrate concentration depends upon tide and wastewater discharge, (3) the influence of temperature, pH, salinity and light on the overall process, as shown by the data presented here, and (4) the influence of other algal species of the whole plant community, especially *Ulva rotundata*, that may increase the total Pi liberated from PME hydrolysis.

The estimates presented here did not result in important contributions to the total SRP measured in the estuary. However, when compared with other processes affecting the phosphorus dynamics, these results are similar to the estimates given by Pérez-Llorens (1991) to the daily contribution which represents the Pi liberated through the leaves of *Z. noltii* in the same sampling area (minimum 2 nM Pi day^{-1} in winter with a coverage of 20% and maximum of 30 nM Pi day^{-1} in summer with a coverage of 50%) and higher than the values found for this species occurring in the Dutch estuary of Oosterschelde (Pérez-Llorens *et al.*, 1993). These authors reported a minimum Pi released through leaves of 0·2 nM day^{-1} (winter, 20% coverage) and a maximum of 9·2 nM day^{-1} (summer, 60% coverage). Still, the relevance of phosphorus release from APA is lower compared with the flux of phosphorus from *Z. noltii* to the detritus compartment (48–119 nm day^{-1}; Pérez-Llorens & Niell, 1993*a*) and the flux of dissolved phosphorus between sediment and overlying water in Palmones river estuary (137–323 nM day^{-1}; Clavero *et al.*, 1991).

Conclusion

The sea-grass *Z. noltii*, showed APA essentially in the aerial parts, in a pattern that seems to be controlled by the internal SRP pool. The enzymatic activity showed a two-phase kinetic versus pNPP concentration, which may allow the enzyme to have relatively constant activity in the presence of fluctuations in substrate concentration. Alkaline phosphatase activity was modulated by temperature, pH, salinity and light. Future studies on the dynamics and turnover of phosphate in the estuary should take into account the contribution of *Z. noltii* to the release of phosphate through APA.

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