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Alkaline phosphatase activity in marine macrophytes: histochemical localization in some widespread species in southern Spain

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Abstract Alkaline phosphatase activity (APA) was determined in 44 species of marine macrophytes collected throughout 1991 and 1992 along the southern coast of Spain. Activity varied between 0.83 µmol paranitrophenol (pNP) released g^{-1} dry wt h^{-1} in *Ulva rigida* var. *gigantea* and 238.8 µmol pNP g^{-1} dry wt h^{-1} in *Bangia fuscopurpurea*. Using a histochemical method, APA sites were located in five of these species: *Corallina elongata* Ellis et Soland, *Gelidium latifolium* (Grev.) Thur. et Born., *G. sesquipedale* (Clem.) Born. et Thur., *Porphyra umbilicalis* (L.) Kützing and *Zostera noltii* Hornem. Enzymatic activity was found in the outer part of the thallus, either on the cell wall or in the cortical cells. In the marine phanerogam *Z. noltii* activity was also located in the vascular bundle. The results suggest that APA is present in many, if not all, marine macrophytes from southern Spain, and plays a relevant role in the utilization of dissolved organic phosphorus compounds.

Introduction

Alkaline phosphatase is a non-specific phosphomonoesterase that hydrolyzes phosphate from organic phosphomonoesters (PME), a fraction that in lakes and coastal waters may constitute between 10 and 70% of the dissolved organic phosphorus pool (Taft et al. 1977; Kobori and Taga 1979; Veldhuis et al. 1987).

There has been increasing evidence recently that phosphate is the factor limiting algal growth in a diversity of marine environments, particularly in coastal waters (Sakshaug and Olsen 1986; Veldhuis et al. 1987; Wheeler and Björnsäter 1992), and a consequent increasing interest in the study of alkaline phosphatase activity (APA) in benthic macroalgae from coastal and inshore waters (Atkinson 1987; Lapointe and O'Connell 1989; Weich and Granéli 1989; Hernández et al. 1993).

Previous studies on microalgae and bacteria have demonstrated that APA is common in the outer surface of cells (Kuenzler and Perras 1965; Cembella et al. 1983; Islam and Whitton 1992), either in the cell wall (Matagne et al. 1976; Doonan and Jensen 1977) or at the plasmalemma (Kuenzler and Perras 1965; Patni et al. 1974; Rivkin and Swift 1980). In some cases, low activity has been detected in cell organelles such as the chloroplast or mitochondria (Aaronson and Patni 1976). Other studies have confirmed the existence of extracellular APA (Grainger et al. 1989; Lubián et al. 1992). However, it is difficult to distinguish whether the enzyme is actually excreted, or whether the activity is a result of cell degradation (Cembella et al. 1983).

Histochemical studies to locate APA in cells have been carried out by several workers. Brandes and Elston (1956) localized alkaline phosphatase on the cell wall of *Chlorella vulgaris*; Wynne (1977) found APA mainly inside the cells of *Peridinium cinctum*, while Islam and Whitton (1992) recorded it over the whole surface of the tapered trichomes of *Calothrix* D764. In studies on multicellular algae, histochemical techniques have only been employed by Gibson and Whitton (1987), who used staining to reveal enzymatic activity in the cell wall and hairs of some species of Chaetophorales. However, as far as we are aware, there are no previous records of the histochemical localization of APA in marine macrophytes.

During the present study, alkaline phosphatase activity was recorded for a large number of species of benthic macrophytes from the southern coast of Spain. The aim of the

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study was to screen the presence of alkaline phosphatase in marine macrophytes and, using a histochemical method, to locate the sites of activity in five selected widespread species.

Materials and methods

Sampling sites

Plants were collected throughout 1991 and 1992 from four different places along the southern coast of Spain (provinces of Málaga and Cádiz, Fig. 1): Punta Carnero is a cape in the Straits of Gibraltar; Palmones River estuary is a eutrophic, shallow estuary (0.9 m maximum depth), characterized by great energy input and high production (Pérez-Llorens and Niell 1989). The estuary is situated in Algeciras Bay, near the Straits of Gibraltar; Lagos and Maro are Mediterranean rocky shores on the eastern coast of Málaga, characterized by clear waters and lower production than the Atlantic site (Punta Carnero).

After collection, the plants were kept cool in darkness and transported to the laboratory in an icebox. Epiphytes were carefully removed and the plants were maintained (12 h max.) in renewed cultures of aerated, filtered, natural seawater (pH 8.2) at a constant temperature of 15 °C and a photon-flux density of 150 µmol photon m^{-2} s^{-1} (Sylvania, F 20W/D-RS).

Measurement of alkaline phosphatase activity

Alkaline phosphatase activity was assayed 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. Macrophytes (0.25 to 0.5 g wet wt) were incubated in a medium containing 50 ml of 10^{-3} *M* 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 phosphate concentration and 35‰ S (Kalle 1945 as cited in Riley and Skirrow 1945, p. 601). Air was bubbled continuously during the assay to oxygenate and stir the medium. After 45 min incubation at 25 °C and 200 µmol photon m^{-2} s^{-1}, the absorbance was read at 410 nm against a blank (buffer and substrate solution without plant) in a Beckman DU-7 spectrophotometer. Activity is reported as µmol paranitrophenol (pNP) released g^{-1} dry wt h^{-1}.

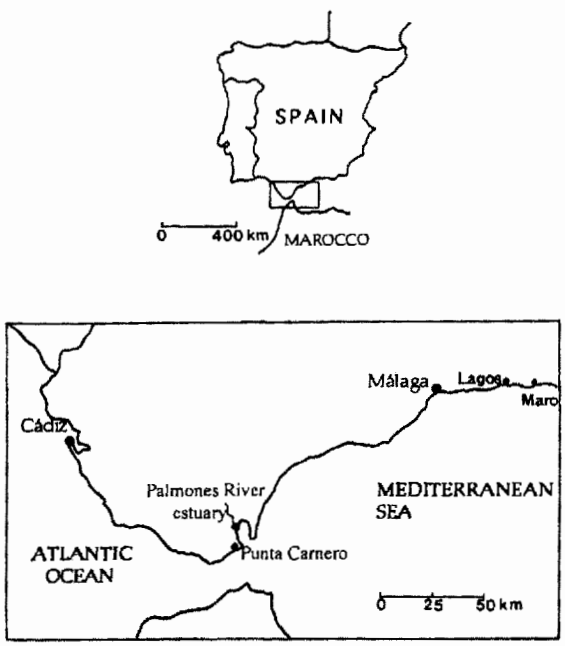

Fig. 1 Sampling sites

Statistics

The significance of differences in APA among locations or taxonomical divisions was tested by a non-parametric ANOVA test (Kruskal and Wallis 1952) at the 5% significance level. Multiple post-hoc comparisons were performed by a non-parametric Tukey test, using Dunn's (1964) correction to estimate the standard error (SE). Comparison between two means was performed by a Student's *t*-test (Fisher 1925).

Histochemical localization of alkaline phosphatase activity

APA in the plants was located histochemically using a modification of the method proposed by Gomori (1939), which uses β-glycerophosphate as a substrate. Thalli were cut in a cryostate (Reichert-Jung 2800 Frigocut) at −25 °C. The sections (10 µm wide) were mounted on clean glass slides coated with a 0.1% (wt/vol) solution of poly-L-lysine (Sigma). The slides were then immersed in the assay medium. This medium was composed of 10 ml β-glycerophosphate (3% wt/vol), 10 ml barbital (5% wt/vol), 15 ml anhydrous $CaCl_2$ (2% wt/vol), 10 ml $MgSO_4$ (2% wt/vol) and 5 ml solvent medium. All the solutions were made up using 0.5 *M* NaCl as the solvent medium. After 85 min incubation at 29 °C, and a rinse in running water (1 min), the sites of the alkaline phosphatase activity were determined by incubating the sections as follows: 5 min in $CO(NO_3)_2$ (0.5% wt/vol), 3 min rinse in running water, 2 min in $(NH_4)_2S$ (0.5% v/v), and a final rinse of 10 min in running water. APA sites were indicated by a black or brownish black precipitate of cobalt sulfide. Finally, the sections were mounted in Aquatex jelly (Merck) for light microscopy observation. The stained sections were compared against a blank (sections incubated in a medium of assay without substrate).

Results

Alkaline phosphatase activity was detected in all marine macrophytes assayed (44 species), including the three divisions of benthic macroalgae (Chlorophyta, Rhodophyta and Phaeophyta) and marine phanerogams (Table 1). Activity varied widely among species, from 0.83 µmol pNP g^{-1} dry wt h^{-1} in *Ulva rigida* var. *gigantea* to 238.8 µmol pNP g^{-1} dry wt h^{-1} in *Bangia fuscopurpurea*. Most species (72%) displayed an APA of <20 µmol pNP g^{-1} dry wt h^{-1} (Fig. 2), with most activities generally lying between 4 and 6 µmol pNP g^{-1} dry wt h^{-1}. A few species (23%) displayed activities of >60 µmol pNP g^{-1} dry wt h^{-1} (Fig. 2).

Mean APA was highest in the Phaeophyta and lowest in the marine phanerogams (Table 2). A non-parametric ANOVA test revealed significant differences among means ($p = 0.016$). However, at the 5% significance level, the post-hoc comparison test failed to detect differences between any pair of means, although the APA of the Phaeophyta differed from that of the Chlorophyta and marine phanerogams at the 10% level.

APA was highest in species collected at Maro and lowest in species from Palmones River (Table 3). A non-parametric ANOVA test revealed that difference in activities

Table 1 Alkaline phosphatase activity (μmol pNP g^{-1} dry wt h^{-1} ± SD; means of 3 replicates) of benthic marine macrophytes collected from coast of Málaga and Cádiz. Assays were performed at pH 8.3 and 25 °C (*pNP* paranitrophenol)

Division, Order, Species	Estuarine	Mediterranean		Atlantic
		Lagos	Maro	
Chlorophyta				
Cladophorales				
Chaetomorpha aerea				51.7 ± 2.0
Codiales				
Codium adhaerens				5.8 ± 1.3
C. decorticatum				11.4 ± 1.0
Siphonocladales				
Valonia utricularis				15.0 ± 0.05
Ulvales				
Enteromorpha linza			77.5 ± 6.3	
Ulva gigantea	0.8 ± 0.10			
U. rigida			6.4 ± 0.9	8.6 ± 0.4
Rhodophyta				
Bangiales				
Bangia fuscopurpurea			238.8 ± 13.3	96.2 ± 1.5
Porphyra umbilicalis		187.6 ± 33.4		22.8 ± 0.8
Ceramiales				
Ceramium rubrum				50.8 ± 0.9
Bostrychia scorpioides	5.1 ± 0.1			
Laurencia pinnatifida			8.1 ± 0.5	4.6 ± 0.02
Pterosiphonia complanata				168.9 ± 15.04
Cryptonemiales				
Peyssonnelia coriacea				4.6 ± 0.4
Amphiroa rigida				3.8 ± 0.06
Corallina elongata			12.8 ± 3.4	
Jania rubens			7.5 ± 0.2	
Gigartinales				
Gigartina acicularis		27.9 ± 4.6		
Gracilaria bursa-pastoris	1.4 ± 0.5			
Gigartinales				
Schizymenia dubyi			17.4 ± 0.5	
Plocamium cartilagineum			18.9 ± 0.03	10.1 ± 0.05
Gymnogongrus norvegicus				4.5 ± 0.7
Schottera nicaensis				22.01 ± 0.06
Rissoella verruculosa		2.8 ± 0.3	5.3 ± 0.1	
Nemaliales				
Asparagopsis armata				41.8 ± 3.7
A. armata (phase *Falkenbergia rufolanosa*)			17.7 ± 6.5	
Gelidium latifolium			30.07 ± 4.06	
G. pusillum				19.2 ± 2.7
G. sesquipedale				5.3 ± 0.4
Pterocladia capillacea			11.0 ± 0.6	
Nemalion helminthoides			32.7 ± 0.7	
Phaeophyta				
Dictyotales				
Dictyopteris membranacea				31.7 ± 4.6
Dilophus ligulatus			200.6 ± 4.6	
Padina pavonia				137.6 ± 15.2
Fucales				
Cystoseira compressa		23.7 ± 2.8		18.6 ± 0.2
C. mediterranea			131.8 ± 7.8	
C. tamariscifolia				27.0 ± 0.5
Fucus spiralis				20.3 ± 2.8
Scyctosiphonales				
Colpomenia sinuosa				22.8 ± 4.4
Sphacelariales				
Cladostephus verticillatus				25.4 ± 2.2
Halopteris filicina		141.6 ± 11.9		
Marine phanerogams (seagrasses)				
Posidonia oceanica			5.9 ± 0.3	
Zostera marina (leaf)			4.6 ± 0.02	
Z. noltii (leaf)	10.04 ± 0.04			
Z. noltii (stem)	1.6 ± 0.01			
Z. noltii (root)	1.75 ± 0.01			

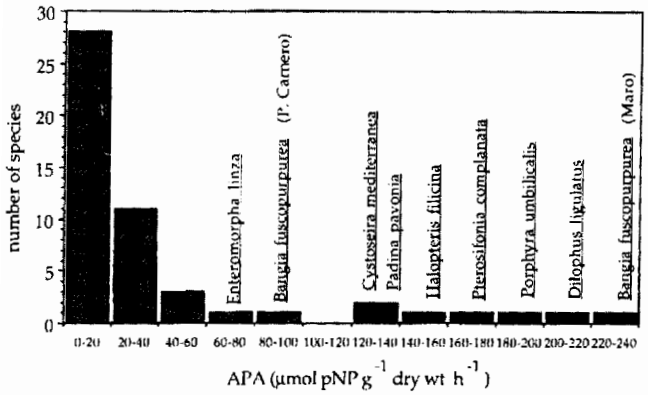

Fig. 2 Frequency distribution of alkaline phosphatase activity in marine macrophytes assayed, showing skewed distribution towards low values of enzymatic activity (*pNP* paranitrophenol)

Table 2 Mean alkaline phosphatase activity (*APA*; µmol pNP g^{-1} dry wt h^{-1}) in three divisions of macroalgae and marine phanerogams (seagrasses) (*n* number of species assayed in each division; *pNP* paranitrophenol)

Division	(*n*)	APA ± SE
Chlorophyta	(8)	22.1 ± 9.7
Rhodophyta	(29)	37.2 ± 11.1
Phaeophyta	(11)	71.01 ± 20.3
Marine phanerogams	(3)	6.9 ± 1.6

Table 3 Mean alkaline phosphatase activity (*APA*, µmol pNP g^{-1} dry wt h^{-1}) in species from four sampling sites (*n* number of species assayed from each location; *pNP* paranitrophenol)

Location	(*n*)	APA ± SE
Punta Carnero	(25)	33.2 ± 8.4
Palmones	(4)	4.3 ± 2.1
Lagos	(5)	76.7 ± 36.9
Maro	(17)	48.7 ± 17.6

between the sampling sites was almost significant ($p = 0.054$). However, when the two Mediterranean locations (Lagos and Maro) were combined and compared to the Atlantic site (Punta Carnero) a significant difference among means emerged ($p = 0.032$). A post-hoc comparison showed that the APA of the species from the Palmones River estuary was significantly lower than that of species from the Punta Carnero or the Mediterranean locations. Moreover, in all those cases where one and the same species was sampled from different sampling sites (with the exception of *Ulva rigida*), the activities of Punta Carnero (Atlantic) individuals were significantly lower than those of individuals from Lagos or Maro (Mediterranean).

Figs. 3 and 4 show the sites of APA in the five species selected for histochemical assays. For all these species, a stained section was compared with a section incubated in the absence of substrate. In *Corallina elongata* (Fig. 3A, B), the APA was located in the monolayer of cortical cells (epithallial cells). Phycobiliproteins were found mainly in a lower cell layer (vegetative initial cells), but these cells lost their characteristic red colour after the assay, probably because of pigment thermolability (incubations were performed at 29 °C). In *Gelidium latifolium* (Fig. 3C, D), APA was located specifically in the outer region of smaller cells (cortex). However, in *G. sesquipedale* (Fig. 3E, F) the APA was clearly revealed on the thick external cell wall, whereas the cortex displayed no difference in APA compared to the control. *Porphyra umbilicalis* also exhibited APA on the thick external cell wall (Fig. 3G, H) but, in contrast to *G. sesquipedale*, activity was apparent only on one side of the alga. Finally, in *Zostera noltii* (Fig. 4) the reaction was somewhat masked by the green colour of the chlorophylls in the epidermal cells. However, two characteristics were determined. First, as in *P. umbilicalis*, APA occurred mainly on one side of the leaves (Fig. 4A); and second, the activity was confined specifically to two sites: the epidermal cells (Fig. 4C) and the vascular bundle (Fig. 4D).

Discussion and conclusions

Alkaline phosphatase in marine macrophytes

There are but few APA data reported in the literature with which to compare the APA data obtained in the present study. Lapointe and O'Connell (1989) measured APA in eight species of macroalgae from inshore waters of Bermuda. The activities they recorded (converted to the same units as used in the present study) ranged from 21.5 µmol pNP g^{-1} dry wt h^{-1} in *Cladophora prolifera* to ≈1 µmol pNP g^{-1} dry wt h^{-1} for *Codium taylori*. Although Lapointe and O'Connell incubated the algae at 18 °C, the APA values they found all fell within the first interval of APA values recorded in our study (0 to 20 µmol pNP g^{-1} dry wt h^{-1}, Fig. 2).

For microalgae, APA is usually expressed as µ*M* substrate released per unit time (e.g. Pick 1987; Heath and Francko 1988) or in units normalized by some other biomass indicator such as chlorophyll, cells, or mg protein (e.g. Burkholder and Wetzel 1990; Van Boekel and Veldhuis 1990; Lubián et al. 1992), making a comparison with the results of the present work impossible. Exceptions are the studies of Whitton et al. (1990), who reported a cell-bound phosphomonoesterase activity value of ≈510 µmol pNP g^{-1} dry wt h^{-1} for *Nostoc commune* at pH 8.3, and Grainger et al. (1989), who reported a cell-bound APA of ≈3.75 mmol pNP g^{-1} dry wt h^{-1} for the cyanobacterium *Calothrix parietina*, also at pH 8.3. A maximum activity of 28 mmol pNP g^{-1} dry wt h^{-1} (at pH 12.2) was found for *C. viguieri* (Mahasneh et al. 1990), and recently, Whitton et al. (1991) reported cell-bound APA values between 2.91 and 5.24 mmol pNP g^{-1} dry wt h^{-1} for several cyanobacterial strains (at pH 10.3). All these previous studies in microalgae were carried out at 32 °C and a lower initial sub-

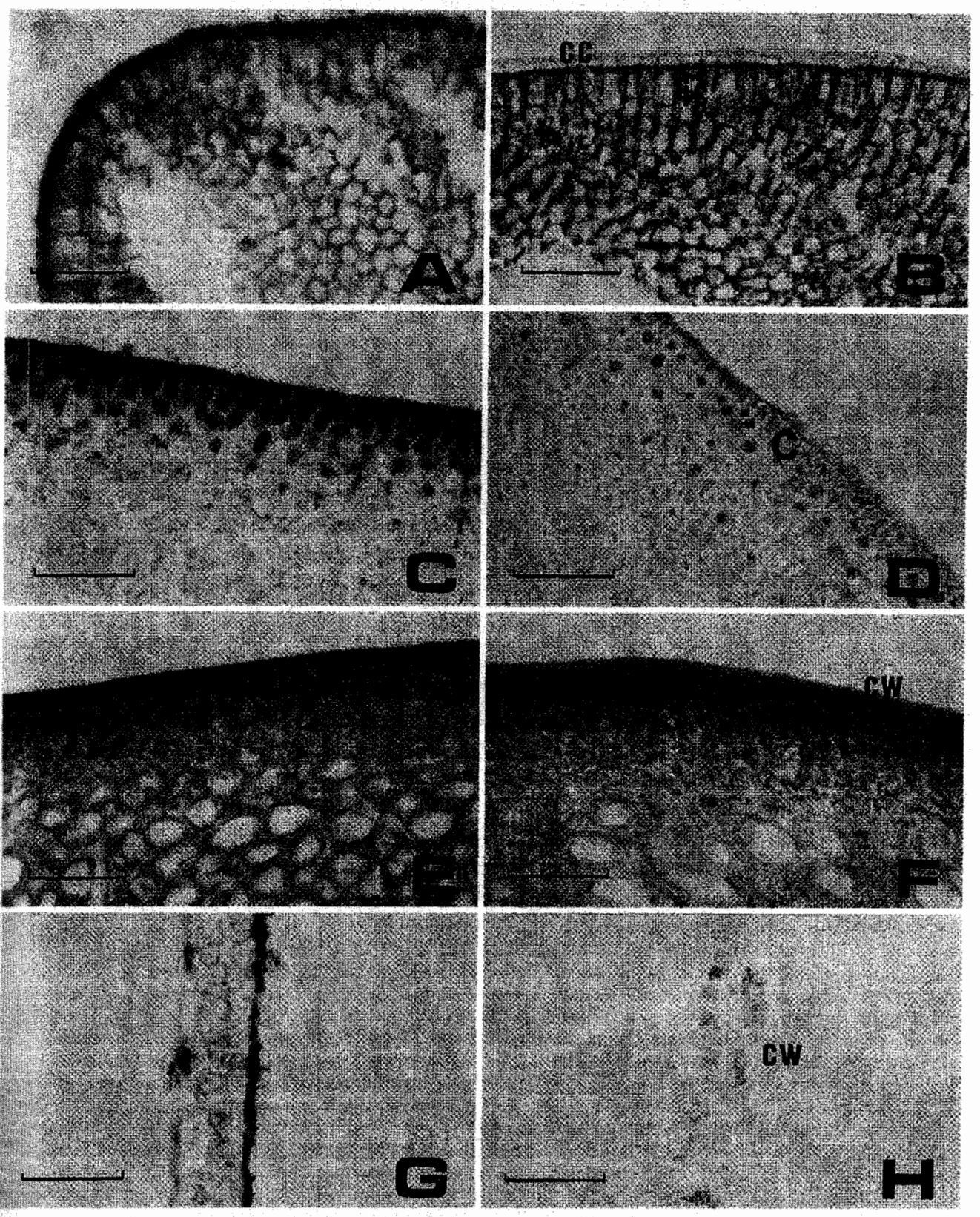

Fig. 3 Gomori staining, revealing localization of alkaline phosphatase activity (black, brownish-black precipitates) on thallus. **A, B** *Corallina elongata*, positive reaction and control, respectively; **C, D** *Gelidium latifolium*, positive reaction and control, respectively; **E, F** *Gelidium sesquipedale*, positive reaction and control, respectively; **G, H** *Porphyra umbilicalis*, positive reaction and control, respectively. (Scale bars = 50 μm) (*c* cortex; *cc* cortical cells; *ic* initial cells; *cw* cell wall))

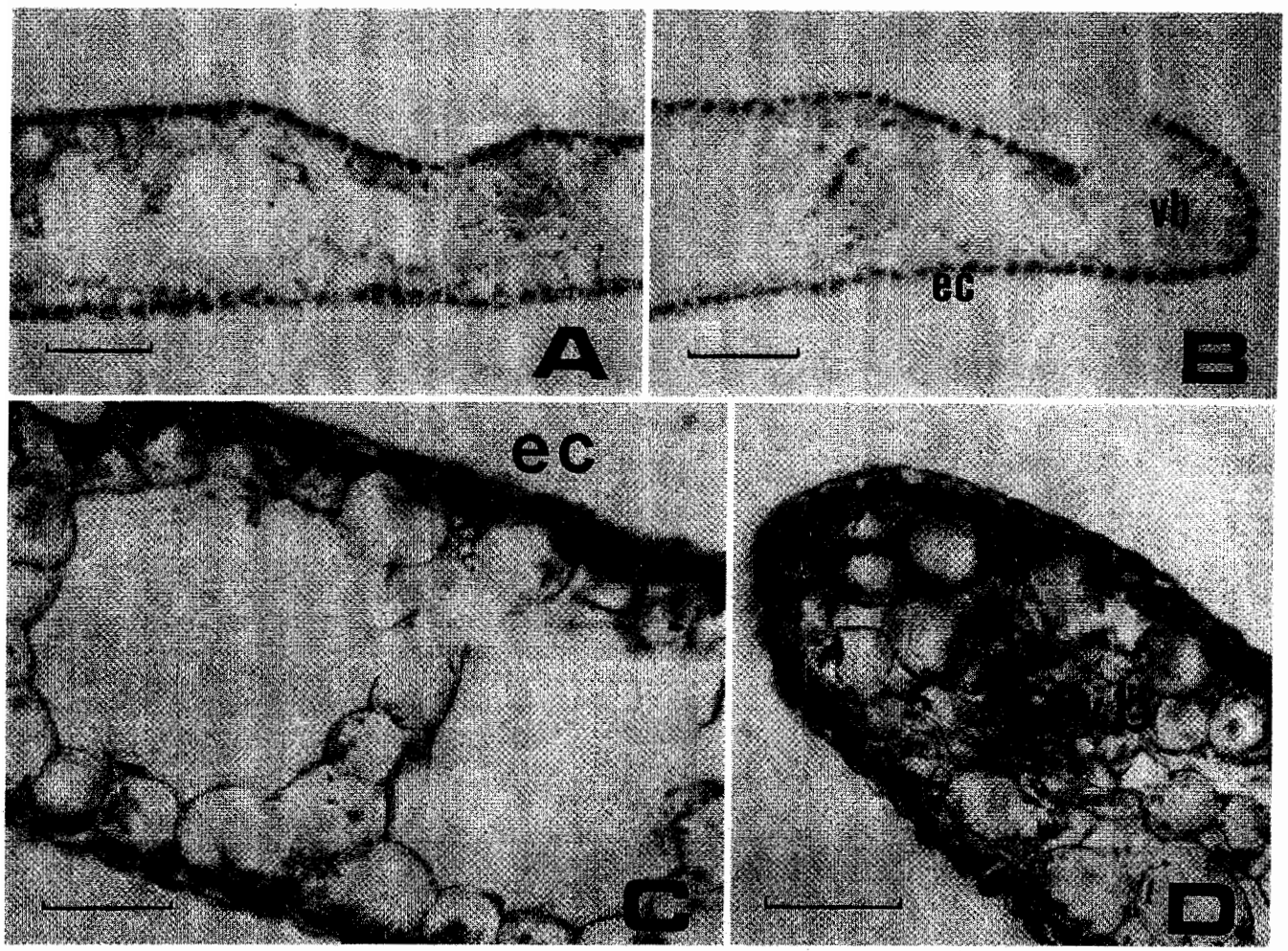

Fig. 4 *Zostera noltii*. Gomori staining, revealing localization of alkaline phosphatase activity (black, brownish-black precipitates) on leaves. **A** Positive reaction; **B** control; **C** detail of a section showing main positive reaction in epidermal cells of one side of a leaf; **D** detail of positive reaction in a vascular bundle. (Scale bars = 100 µm in **A, B** and 50 µm in **C, D**) (*ec* epidermal cells; *vb* vascular bundle))

strate concentration (250 µ*M* pNPP vs 500 µ*M* in our study). However, it seems clear that the APA values recorded for microalgae are undoubtly higher than those reported for macroalgae. This difference may have arisen from the high phosphate demand of the planktonic species, with a consequent higher PME turnover rate. Differences in the surface:volume ratio have also been proposed to explain disparities in APA (Aaronson and Patni 1976). In the present study, most of the highest APA values were recorded in leafy or branched species with a high surface: volume ratio (i.e., *Enteromorpha linza*, *Porphyra umbilicalis*, *Dilophus ligulatus*, Fig. 2). Similarly, Wallentinus (1984) found that phosphate uptake rates were faster in filamentous species such as *Ceramium tenuicorne* than in species with more fleshy thalli.

APA was found in all the marine benthic macrophytes examined in the present study. This suggests that this enzyme plays an important role in phosphorus metabolism (Wetzel 1981; Boavida and Heath 1986; Pick 1987). However, the activities shown in Table 1 were not constant over time. It has been well documented that APA changes seasonally (Wynne 1977; Hernández et al. 1993) and is influenced by external or/and internal phosphate concentrations (Rhee 1973; Healey 1982; Gage and Gorham 1985; Hernández et al. 1992), with higher APA values during periods of phosphorus deficiency.

Nevertheless, some conclusions can be drawn from the data in Table 1. The highest APA was recorded for the Phaeophyta, whereas the lowest activity was found in the marine phanerogams. Although the non-parametric ANOVA test was significant, no differences were detected between any pair of means. However, at the 10% significance level, the APA level in the Phaeophyta was significantly different from that in the Chlorophyta and the marine phanerogams. This simply reflects the fact that Type II errors are more likely to occur in multiple-comparison testing than with an ANOVA (Zar 1984).

The lowest APA was found in the marine phanerogams. In seagrasses, phosphate may be assimilated not only through the leaves but also through the roots (Denny 1972; Penhale and Thayer 1980), which still retain many functional characteristics of terrestrial plants (Tomlinson 1980). Phosphorus in the interstitial water may be enough

to meet seagrass growth for up to ≈3 yr (Patriquin 1972). We do not have data on the sediments of Maro; however, the soluble reactive phosphorus (SRP) content of the interstitial water (0 to 5 cm depth) of the Palmones River estuary was high (mean 15 μM; Clavero et al. 1991), whilst the mean PME concentration in the water was 0.3 μM throughout the year (Hernández et al. 1994). All these factors may contribute to limit the necessity for high APA in seagrasses. Moreover, the total phosphorus content is generally higher in seagrasses than in macroalgae, as demonstrated by Duarte (1992) in an extensive study. The inverse relationship between APA and phosphorus content has been demonstrated in other studies (e.g. Wynne 1981; Gage and Gorham 1985; Hernández et al. 1993).

The species collected in the Palmones River estuary, which is a eutrophic estuary chracterized by a great energy input and consequent high production (Pérez-Llorens and Niell 1989), displayed the lowest activity. At this site the mean SRP throughout the year was 3.89 μM (SE = 1.13) (Clavero et al. 1992), a perceptibly higher value than the phosphate concentrations observed at the rest of the sampling sites. Furthermore, the mean APA in the species collected from the Atlantic location (Punta Carnero), was lower than the mean APA in species collected from the Mediterranean sampling sites (Lagos and Maro). This is most clearly demonstrated by comparing the activity of species collected in Punta Carnero with data for the same species from a Mediterranean site (Table 1). The APA differences were inversely related to environmental nutrient supplies, especially NO_3^- and SRP, which are usually higher at Punta Carnero (Torres 1986; Niell et al. 1989; Hernández et al. 1993). Lower APA levels in species from localities with constantly higher phosphate concentrations suggest an inverse relationship between APA and external phosphate concentration, as described in other studies (Healey 1973; Smith and Kalff 1981; Jansson et al. 1988).

Finally, we failed to relate APA levels to vertical distribution, zonation or intertidal location of the species, but this possibility should be tested in future studies.

Localization of enzyme activity

Hernández et al. (1992) found that APA in *Porphyra umbilicalis* is significantly influenced by salinity. Distilled water and low salinities strongly inhibit the activity of this enzyme. For histochemical purposes, the use of a 0.5 M NaCl solution as a solvent medium allows sections to be incubated in salinity conditions close to those of natural environment, thus obviating the possible influence of deviations from natural conditions.

In the four macroalgae assayed, APA was stained on the outer part of the thallus. In *Gelidium sesquipedale* and *Porphyra umbilicalis* (Fig. 3E, G) activity was clearly identified on the external cell wall, as has been shown for *Chlorella vulgaris* (Brandes and Elston 1956). In *Corallina elongata* and *Gelidium latifolium* (Fig. 3A, C) activity was in the cortical cells. Unfortunately, it was not possible to distinguish whether the site of the activity was on the cell wall, the plasmalemma, or even in the whole cell as in *Peridinium cintum* (Wynne 1977). However, the external location on the thallus suggests that alkaline phosphatase hydrolyzes the external PMEs, utilizing them as an additional source of phosphate.

APA was revealed at two sites in the leaves of *Zostera noltii*: in the epidermal cells and the vascular bundle (Fig. 4). The APA located in the vascular bundle may reflect the unspecific phosphomonoesterase activity of substrates translocated through the leaves. Although phosphorus is transferred to the xylem stream mainly as phosphate (Bieleski and Ferguson 1985), phosphatase is present in small amounts in the bleeding sap of vascular plants (Bieleski 1973); this is possibly related to hydrolysis of esters such as phosphatyl choline (Maizel et al. 1956), which is the main component of the organic phosphorus fraction of the sap of vascular plants.

In the two species that grow parallel to the substrate, especially at low tide, APA was stained mainly on one surface of the blades (*Porphyra umbilicalis*) or on one side of the leaves (*Zostera noltii*) only. This suggests some degree of asymmetry arising from different rates of enzyme synthesis by the upper and lower surfaces. Leaves of submerged aquatic plants such as *Elodea canadensis*, *E. nuttallii*, *Egeria densa* and *Potamogeton lucens* exhibit a polar reaction in light (Helder and Boerma 1973). Upon illumination, the medium on the lower leaf surface is acidified whereas the upper surface becomes more alkaline (Elzenga and Prins 1989). The rate of synthesis of alkaline phosphatase could differ between the upper and lower surface, being higher on the surface exposed to light. In *Porphyra umbilicalis* and *Z. noltii*, APA is largely dependent on pH, with maximum APA being achieved at pH 8.8 (Hernández et al. 1992; Hernández et al. 1994). This pH may easily be attained during direct exposure to sunlight, when active photosynthesis occurs.

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References

Aaronson S, Patni NJ (1976) The role of surface and extracellular phosphatase in the phosphorus requirement of *Ochromonas*. Limnol Oceanogr 21:838–845

Atkinson MJ (1987) Alkaline phosphatase activity of coral reef benthos. Coral Reefs 6:59–62

Bieleski RL (1973) Phosphate pools, phosphate transport and phosphate availability. A Rev Pl Physiol 24:225–252

Bieleski RL, Ferguson IB (1985) Physiology and metabolism of phosphate and its compounds. In: Läuchli A, Bieleski RL (eds) Inorganic plant nutrition. Encyclopedia of plant physiology. New series. Vol XV-A. Springer Verlag, Berlin, pp 442–449

Boavida MJ, Heath RT (1986) Phosphatase activity on *Chlamydomonas acidophila* Negoro (Volvocales, Chlorophyceae). Phycologia 25:400–404

Brandes D, Elston RN (1956) An electron microscopical study of the histochemical localization of alkaline phosphatase in the cell wall of *Chlorella vulgaris*. Nature, Lond 77: 274–275

Burkholder JM, Wetzel RG (1990) Epiphytic alkaline phosphatase on natural and artificial plants in an oligotrophic lake: Re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. Limnol Oceanogr 35: 736–747

Cembella AD, Antia NJ, Harrison PJ (1983) The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: Part 1. CRC critical Rev Microbiol 10: 317–391

Clavero V, Fernández JA, Niell FX (1992) Bioturbation by *Nereis* sp. and its effects on the phosphate flux across the sediment–water interface in the Palmones river estuary. Hydrobiologia 235/236: 387–392

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

Denny P (1972) Sites of nutrient absorption in aquatic macrophytes. J Ecol 60: 819–829

Doonan BB, Jensen TE (1977) Ultrastructural localization of alkaline phosphatase in the blue-green bacterium *Plectonema boryanum*. J Bact 132: 967–973

Duarte CM (1992) Nutrient concentration of aquatic plants: patterns across species. Limnol Oceanogr 37: 882–889

Dunn OJ (1964) Multiple contrasts using rank sums. Technometrics 6: 241–252

Elzenga JTM, Prins HBA (1989) Light-induced polar pH changes in leaves of *Elodea canadensis*. I. Effects of carbon concentration and light intensity. Pl Physiol 91: 62–67

Fisher RA (1925) Application of Student's distribution. Metron 5: 90–124 (Cited from Zar 1984)

Gage MA, Gorham E (1985) Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. Freshwat Biol 15: 227–233

Gibson MT, Whitton BA (1987) Hairs, phosphatase activity and environmental chemistry in *Stigeoclonium*, *Chaetophora* and *Draparnaldia* (Chaetophorales). Br phycol J 22: 11–22

Gomori G (1939) Microtechnical determination of phosphatase in tissue section. Proc Soc exp Biol Med 42: 23–29

Grainger SLJ, Peat A, Tiwari DN, Whitton BA (1989) Phosphomonoesterase activity of the cyanobacterium (blue-green alga) *Calothrix parietina*. Microbios (Cambridge, UK) 59: 7–17

Healey FP (1973) Characteristics of phosphorus deficiency in *Anabaena*. J Phycol 9: 383–394

Healey FP (1982) Phosphate. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell, Oxford, pp 105–124

Heath RT, Francko DA (1988) Comparison of phosphorus dynamic in two Oklahoma reservoirs and a natural lake varying in abiogenic turbidity. Can J Fish aquat Sciences 45: 1480–1486

Helder RJ, Boerma J (1973) Exchange and polar transport of rubidium ions across the leaves of *Potamogeton*. Acta bot neerl 22: 686–693

Hernández I, Fernández JA, Niell FX (1993) Influence of phosphorus status on the seasonal variation of alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. J exp mar Biol Ecol 173: 181–196

Hernández I, Niell FX, García JA (1992) Alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. J exp mar Biol Ecol 159: 1–13

Hernández I, Pérez-Llorens JL, Fernández JA, Niell FX (1994) Alkaline phosphatase activity in *Zostera noltii* Hornem. and its contribution to the release of phosphate in the Palmones river estuary. Estuar cstl Shelf Sci (in press)

Islam MR, Whitton BA (1992) Phosphorus content and phosphatase activity on the deepwater rice-field cyanobacterium (blue-green alga) *Calothrix* D764 (Cambridge, UK). Microbios 69: 7–16

Jansson M, Olsson H, Pettersson K (1988) Phosphatases: origin, characteristics and function in lakes. Hydrobiologia 170: 157–175

Kobori H, Taga N (1979) Phosphatase activity and its role in the mineralization of organic phosphorus in coastal sea water. J exp mar Biol Ecol 36: 23–29

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion analysis of variance. J Am statist Ass 47: 583–621

Kuenzler EJ, Perras JP (1965) Phosphatases of marine algae. Biol Bull mar biol Lab, Woods Hole 128: 271–284

Lapointe BE, O'Connell J (1989) Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: eutrophication of a confined, phosphorus-limited marine ecosystem. Estuar cstl, Shelf Sci 28: 347–360

Lubián LM, Blasco J, Establier R (1992) A comparative study of acid and alkaline phosphatase activities in several strains of *Nannochloris* (Clorophyceae) and *Nannochloropsis* (Eustismatophyceae). Br phycol J 27: 119–130

Mahasneh IA, Grainger SLJ, Whitton BA (1990) Influence of salinity on hair formation and phosphatase activities of the blue-green alga (Cyanobacterium) *Calothrix vignieri* D253. Br phycol J 25: 25–32

Maizel JV, Benson AA, Tolbert NE (1956) Identification of phosphoryl-choline as an important constituent of plant sap. Pl Physiol 31: 407–408

Matagne RF, Loppes R, Deltour R (1976) Phosphatases of *Chlamydomonas reinhardtii*: Biochemical and cytochemical approach with specific mutants. J Bact 126: 937–950

Niell FX, Espejo M, Fernández JA, Algarra P (1989) Performances and use of energy in intertidal systems related to random disturbances and thermal stress. Scientia mar 53: 293–299

Patni NJ, Billmire E, Aaronson S (1974) Isolation of the *Ochromonas danica* plasma membrane and identification of several membrane enzymes. Biochim biophys Acta 373: 347–355

Patriquin DG (1972) The origin of nitrogen and phosphorus for the growth of marine angiosperm. Mar Biol 15: 35–46

Penhale PA, Thayer GN (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-Llorens JL, Niell FX (1989) Emergence and submergence effects on the distributional pattern and exchange of phosphorus in the seagrass *Zostera noltii* Hornem. Scientia mar 53: 497–503

Pick FR (1987) Interpretations of alkaline phosphatase activity in Lake Ontario. Can J Fish aquat Sciences 44: 2087–2094

Reichardt W, Overbeck J, Steubing L (1967) Free dissolved enzymes in lake waters. Nature, Lond 216: 1345–1347

Rhee GY (1973) A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. J Phycol 9: 495–506

Riley JP, Skirrow G (eds) Chemical oceanography. Vol 2. 1975. Academic Press, London

Rivkin RB, Swift E (1980) Characterization of alkaline phosphatase and organic phosphorus utilization in the oceanic dinoflagellate *Pyrocystis noctiluca*. Mar Biol 61: 1–8

Sakshaug E, Olsen Y (1986) Nutrients status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. Can J Fish aquat Sciences 43: 389–396

Smith REH, Kalff J (1981) The effect of phosphorus limitation on algal growth rates: evidence from alkaline phosphatase. Can J Fish aquat Science 38: 1421–1427

Taft JL, Loftus ME, Taylor WR (1977) Phosphate uptake from phosphomonoesters by phytoplankton in the Chesepeake Bay. Limnol Oceanogr 22: 1012–1021

Tomlinson J (1980) Leaf morphology and anatomy in seagrasses. In: Phillips RC, McRoy PC (eds) Handbook of seagrasses biology: an ecosystem perspective. STPM Press, Grassland. pp 247–260

Torres M (1986) Diseño de un modelo de producción de base fenológica para *Caulacanthus ustulatus* (Maertens) Kützing. (Sphaerococcaceae, Rhodophyta). MS thesis. Universidad de Málaga

Van Boekel WHM, Veldhuis MJW (1990) Regulation of alkaline phosphatase synthesis in *Phaeocystis* sp. Mar Ecol Prog Ser 61: 281–289

Veldhuis MJW, Venekamp LAH, Ietswaart T (1987) Availability of phosphorus sources for blooms of *Phaeocystis pouchetii* (Haptophyceae) in the North Sea: impact of the river Rhine. Neth J Sea Res 21:219–229

Wallentinus I (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar Biol 80: 215–225

Weich RG, Granéli E (1989) Extracellular alkaline phosphatase activity in *Ulva lactuca* L. J exp mar Biol Ecol 129: 33–44

Wetzel RG (1981) Longterm dissolved and particulate alkaline phosphatase activity in a hardwater lake in relation to lake stability and phosphorus enrichments. Verh int Verein Limnol 21: 369–381

Wheeler AP, Björnsäter BR (1992) Seasonal fluctuations in tissue nitrogen, phosphorus and N:P for five macroalgal species common to the Pacific northwest coast. J Phycol 28: 1–6

Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21: 85–98

Whitton BA, Potts M, Simon JW, Grainger SLJ (1990) Phosphatase activity of the blue-green alga (cyanobacterium) *Nostoc commune* UTEX 584. Phycologia 29: 139–145

Wynne D (1977) Alterations in activity of phosphatases during the *Peridinium* bloom in Lake Kinneret. Physiologia Pl 40: 219–224

Wynne D (1981) The role of phosphatases in the metabolism of *Peridinium cintum* from lake Kinneret. Hydrobiologia 83: 93–99

Zar JH (1984) Biostatistical analysis. 2nd edn. Prentice-Hall, Englewood Cliffs, New Jersey