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A comparative study of alkaline phosphatase activity in two species of *Gelidium* (Gelidiales, Rhodophyta)

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A comparative study of alkaline phosphatase activity (APA) was carried out on repeat samples of representative populations of two species of *Gelidium* (Rhodophyta), *G. latifolium* and *G. sesquipedale*, using *p*-nitrophenyl phosphate (pNPP) as substrate. The APA kinetics of both species exhibited an apparent negative cooperativity for the hydrolysis of pNPP. Differences were found in phosphatase activities of the two species: *G. latifolium* showed higher APA per unit biomass and a higher proportion of the phosphate cleaved from the model substrate than *G. sesquipedale*. Temperature, pH, salinity and external phosphate all had significant effects on the APA of both species. The influence of salinity on APA was due in part to the effects of specific ions (Na^+ and Mg^{2+}). No correlation between photon irradiance and APA was observed in *G. latifolium*, but in *G. sesquipedale* maximum APA was observed at a relatively low photon irradiance. APA of the two species is discussed with respect to a possible relationship with ecophysiological conditions in nature.

Key words: Alkaline phosphatase, *Gelidium latifolium*, *Gelidium sesquipedale*, light, osmotic effects, phosphorus.

Introduction

Phosphorus is generally not considered to be a limiting nutrient in the marine environment (Goldman *et al.*, 1979; Lobban *et al.*, 1985). Increasingly, however, evidence has been reported for the role of phosphate as a regulator of primary production in a diversity of marine environments, particularly coastal waters (Rivkin & Swift, 1985; Sakshaug & Olsen, 1986; Hernández *et al.*, 1993*a*). Many algae respond to conditions of limiting inorganic phosphorus by producing non-specific alkaline phosphatases (Kuenzler & Perras, 1965; Siuda, 1984). These enzymes cleave inorganic phosphate groups from phosphomonoesters (PME), making phosphorus available to the algae (Cembella *et al.*, 1983). The origins of PME, which contribute some of the dissolved organic phosphorus compounds (DOP), are diverse (van Boekel, 1991), so the reported concentrations of PME and DOP in natural waters are variable (Chróst *et al.*, 1984; Atkinson, 1987, Veldhuis *et al.*, 1987; Hernández *et al.*, 1993*b*). Moreover, short-term pulses of DOP are probably quite common in many natural environments (Islam & Whitton, 1992) and may be quickly utilised by the algae.

The alkaline phosphatase activity (APA) of species from different groups of macroalgae has received increasing attention (Lapointe & O'Connell, 1989; Weich & Granéli, 1989; Hernández *et al.*, 1993*b*) since Walther & Fries (1976) first identified this enzyme from macroalgae. Recently, high APA in *Porphyra umbilicalis* (L.) Kützing has been related to phosphorus-limited growth (Hernández *et al.*, 1993*a*).

The aim of the present study was to estimate the APA of two species of *Gelidium*, the most important raw material for the extraction of agar in Spain. The main species growing in southern Spain were selected: *G. latifolium* in the Mediterranean and *G. sesquipedale* in the Atlantic. Along our coasts these two species live under different and well-defined conditions, with differing environmental factors such as wave action, water temperature, phosphate concentration and photon irradiance. Therefore, APA was compared in the two species, giving particular attention to the influence of environmental factors important in the field.

Materials and methods

Seaweeds and sampling sites

Gelidium sesquipedale (Clemente) Bornet *et* Thuret, a species of Atlantic distribution, was collected from the low intertidal at Punta Carnero, a cape in the area of the Straits of Gibraltar. We found the specimens throughout the year in limestone crevices of the low intertidal zone, where photon irradiance was low (maximum = 140 ± 68 μmol photon m^{-2} s^{-1}; Torres *et al.*, 1991). *Gelidium latifolium* (Greville) Thuret *et* Bornet was collected at Maro, a non-tidal rocky shore situated on the eastern coast of Málaga. This species was found from January to October. It forms dense fringes on rocks, between the lower limit of the littoral zone and the upper limit of the sublittoral zone. Maro is characterised by clearer waters and lower algal production than Punta Carnero (Aranda *et al.*, 1984; C. Jiménez, unpublished data).

After collection, plants were kept cool in darkness and transported to the laboratory in an icebox. Macrophytes were generally free of visible epiphytes and, if necessary, the epiphytes were removed carefully with forceps. Finally, to minimise any possible acclimation to laboratory conditions, plants were maintained for a short time (24 h maximum, except in the experiments on phosphorus enrichment) in frequently changed, aerated, filtered natural seawater (pH 8·2) at a constant temperature (15°C) and a photon irradiance of 150 μmol photon $m^{-1} s^{-1}$ (Sylvania F18W/GRO tube).

Alkaline phosphatase activity assay

Alkaline phosphatase is evident in the outer cortical cells or the external cell walls of these species, as was demonstrated by a histochemical stain (Hernández *et al.*, 1994*a*). Surface alkaline phosphatase was assayed colorimetrically in three replicate samples by the method of Reichardt *et al.* (1967), which uses *p*-nitrophenyl phosphate (pNPP) as substrate, as modified by Hernández *et al.* (1992). Plants were incubated in 100 ml of a reaction mixture consisting of 700 μM pNPP (Sigma), 50 mM Tris-HCl buffer, pH 8·3. The initial substrate concentration was sufficiently high to ensure that no more than 10% was hydrolysed during assay. Reagents were dissolved in filtered (0·2 μm) artificial seawater of 35 salinity (practical salinity units) (Kalle, 1945). Air was bubbled continuously during the assay to oxygenate and supply CO_2, and to stir the medium. After 45 min of incubation at 25°C 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}, formed by the hydrolysis of pNPP to phosphorus (P) and pNP.

To estimate the proportion of the phosphate liberated enzymatically that was taken up by the algae in relation to the pNP liberated, at different times (15, 30, 45, 60 min) 2·5 ml of the assay medium was sampled to determine P and APA. P was quantified as soluble reactive phosphorus (SRP) by the molybdenum blue method (Murphy & Riley, 1962). We have assumed that all the pNP from pNPP hydrolysis is released into the medium. According to previous research with glucose-6-phosphate as a substrate for alkaline phosphatase (Hernández *et al.*, unpublished data), any possible retention of pNP by the algae can be considered negligible. The non-enzymatic hydrolysis of pNPP by acid conditions can also be assumed to be negligible (McComb *et al.*, 1979). Finally the absence of a third product, acceptor phosphate (phosphoethanolamine; Wilson *et al.*, 1964), was proved by the absence of any differences between two sets of P standards, one using distilled water and the other using buffered artificial seawater as a solvent.

Apparent kinetic parameters were studied from two transformations of the Michaelis–Menten equation. The Lineweaver–Burk transformation ($1/v$ vs $1/S$) provided maximum velocity by extrapolation of the high-substrate portion of the plot. The apparent half-saturation constants (Ks) were obtained from the Eadie–Hofstee transformation (v vs v/S), since in this plot the distribution of errors is more uniform (Price & Stevens, 1982). pNPP concentrations ranged from 7×10^{-6} M to $4{\cdot}9 \times 10^{-3}$ M. Apparent cooperativity was tested from the slope of a Hill plot [$\log v/(V \max - v)/\log S$].

The temperature response of APA in both species was determined at pH 8·3 from 10 to 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 unit 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 effects of Cl^-, Na^+ and Mg^{2+} on APA were determined by replacing some ions/molecules of the artificial seawater (NaCl, $MgCl_2$ and $MgSO_4$) with mannitol, adding the necessary quantity to keep the osmotic pressure constant. The equivalence between concentration and osmotic pressure was taken from Wolf *et al.* (1982) and checked with a cryoscopic osmometer (Gonotec Osmomat 030).

To test the relationship between photon irradiance and APA, the plants were assayed in the incubation chamber under illumination with a light source (a 150 W solar lamp) in which different light intensity selective filters were inserted. The mean values obtained in the following subranges of light intensities (in μmol photon $m^{-2} s^{-1}$) were considered: 0, 0–10, 10–20, 20–50, 50–100, 100–200, 200–500, 500–1000, 1000–2000. The effect of external phosphate concentration on APA was studied by pre-incubating the plants for 5 days in 400 ml of natural seawater enriched with different quantities of Na_2HPO_4 as a source of orthophosphate. The final concentration of P added ranged from 0 to 20 μM.

Statistical analyses

For each treatment, 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). Differences of the pNP and SRP concentrations during the enzymatic assays were tested for significance by a test of equality of slopes (Zar, 1984). In all cases the null hypothesis was rejected at the 5% significance level.

Results

pNP and P concentrations during the assay

Fig. 1 shows the pNP and SRP concentrations (in μM g

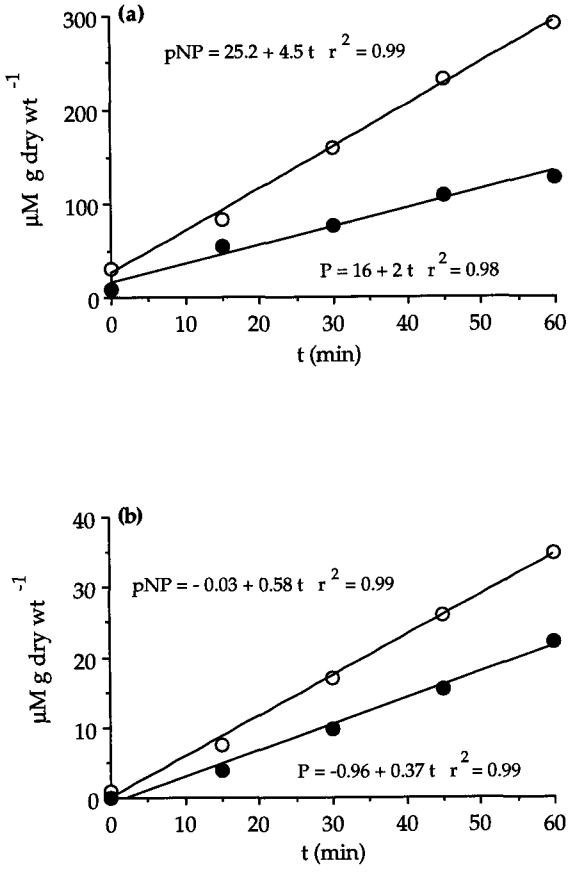

Fig. 1. Concentrations of the two products of the pNPP hydrolysis – pNP (open circles) and SRP (filled circles) (in μM g dry wt^{-1}) – measured during the assay of alkaline phosphatase activity of *Gelidium latifolium* (*a*) and *Gelidium sesquipedale* (*b*). Values are means of three replicates and SD was negligible.

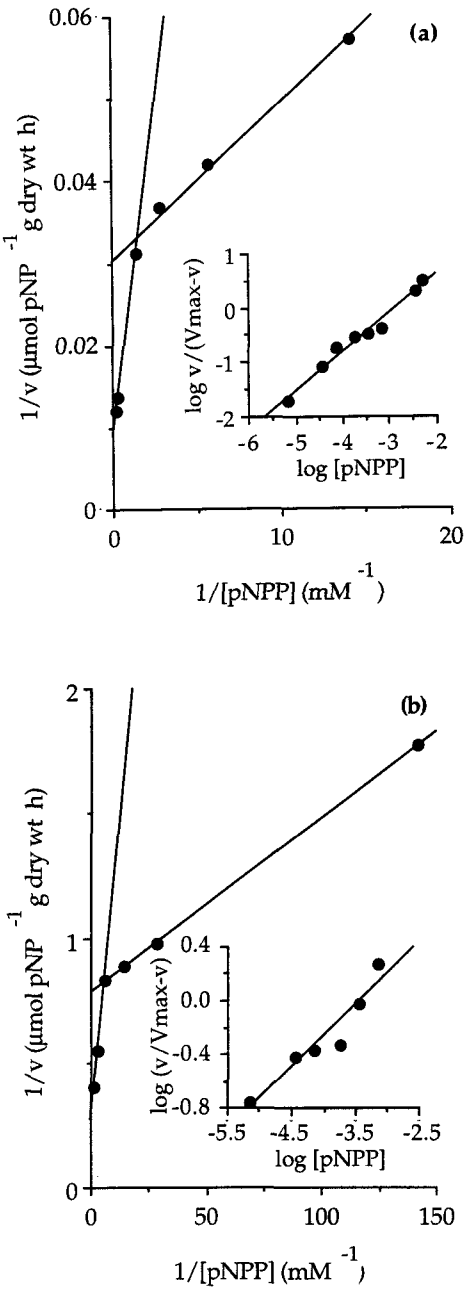

Fig. 2. Double reciprocal plot showing the dependence of the velocity of hydrolysis of pNPP on substrate concentration. An apparent negative cooperativity was suggested by the two straight lines drawn in the figure. Inset, replot of the data according to the Hill representation. Each point represents the mean of three experiments on (*a*) *Gelidium latifolium* and (*b*) *Gelidium sesquipedale*. Enzymatic activity is expressed as μmol pNP g dry wt^{-1} h^{-1} and substrate concentration as mM pNPP.

dry wt^{-1}) measured in the medium during the assay of APA. The slopes of the regression lines for both products of the enzyme reaction were significantly different. The difference between both hydrolysis products was considered to be the percentage of P taken up by the algae after pNPP cleavage, assuming that all the pNP is released into the medium. This proportion was higher in *Gelidium latifolium* (56%) than in *Gelidium sesquipedale* (35%).

Kinetic parameters of APA

The Lineweaver–Burk transformation of a Michaelis–Menten plot shows the dependence of APA on substrate concentration (Fig. 2). This non-linear reciprocal plot suggested the existence of a negative cooperativity for the hydrolysis of pNPP. Two apparent *Ks* values for pNPP can be deduced from the Eadie–Hofstee plot (Table 1) and can be interpreted in term of apparent negative cooperativity as deduced from the slope of the Hill plot, the Hill coefficient n_H. This coefficient was $n_H = 0{\cdot}72$ ($r^2 = 0{\cdot}97$, $p < 0{\cdot}001$) in *G. latifolium* and $n_H = 0{\cdot}47$ ($r^2 = 0{\cdot}91$, $p < 0{\cdot}02$) in *G. sesquipedale* (Fig. 2).

The ratio $V\max/Ks$ may be a better index of competition in PME use than is *Km* alone, as Healey (1982) stated for phosphate uptake. At low PME concentrations, similar to those observed in the sampling sites (Hernández *et al.*, 1993*b*), the apparent *Ks* value of higher affinity showed a $V\max/Ks$ ratio 2-fold higher in *G. latifolium* than in *G. sesquipedale* (Table 1).

Temperature and pH effects

Temperature showed a significant effect on APA in both species of *Gelidium* (Fig. 3). APA increased from 5 to 25°C, but decreased slightly with temperature above

Table 1. Apparent semi-saturation constants (*Ks* in μM) exhibited by alkaline phosphatase from *Gelidium latifolium* and *Gelidium sesquipedale*

Species	Ks_1	Ks_2	*V* max	*V* max / Ks_1
Gelidium latifolium	118	1748	110·8	0·93
Gelidium sesquipedale	9	385	3·8	0·43

V max (μmol pNP g dry wt^{-1} h^{-1}) was calculated from the double reciprocal plot (see text). Ks_1 and Ks_2 refer to the "high" and "low" affinity phase of the enzyme kinetic respectively. The ratio *V* max /Km was calculated using the apparent high affinity constant (Ks_1).

25°C. For this reason, data were fitted to the second-order polynomial, with optimum temperatures for APA of 30°C in *G. latifolium* and 25°C in *G. sesquipedale*. On the other hand, over the range in which a linear increase of APA with temperature was found (10–25°C), the Arrhenius plot ($\log V\max = Ea/R \times 1/T + \text{constant}$) revealed an

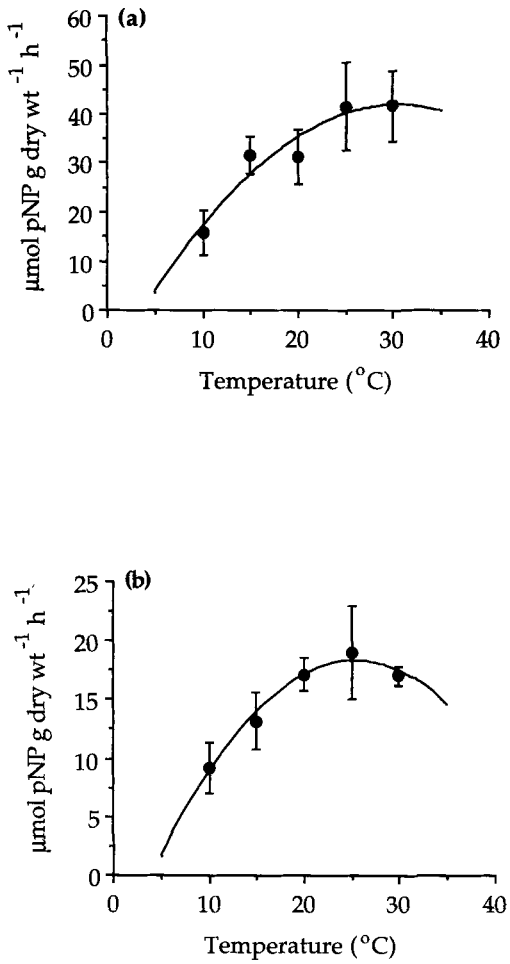

Fig. 3. Alkaline phosphatase activity as a function of temperature (pH 8·3). Data were fitted to second-order polynomials: $APA = -5{\cdot}9\,10^{-2}\,T^2 + 3{\cdot}6T - 12{\cdot}9$ ($r^2 = 0{\cdot}56, p < 0{\cdot}005$) in *Gelidium latifolium* (*a*) and $APA = -4{\cdot}0\,10^{-2}\,T^2 + 2{\cdot}03T - 7{\cdot}5$ ($r^2 = 0{\cdot}68$, $p < 0{\cdot}025$) in *Gelidium sesquipedale* (*b*). Equations are illustrated by the curves. Enzymatic activity is expressed as μmol pNP g dry wt^{-1} h^{-1}. Values are means of three replicates and bars denote SD.

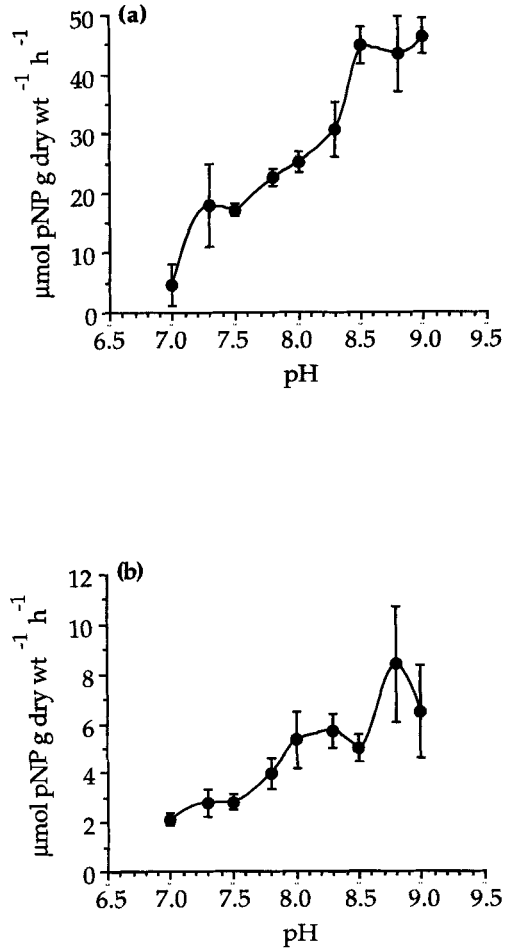

Fig. 4. pH profiles of alkaline phosphatase activity of *Gelidium latifolium* (*a*) and *Gelidium sesquipedale* (*b*) (temperature 25°C). Enzymatic activity is expressed as μmol pNP g dry wt^{-1} h^{-1}. Values are means of three replicates and bars denote SD.

activation energy (*Ea*) of 41·6 kJ mol^{-1} for *G. latifolium* and 35·8 kJ mol^{-1} for *G. sesquipedale*, giving, respectively, Q_{10} values of 1·7 and 1·6.

The rate at which *G. latifolium* and *G. sesquipedale* hydrolysed pNPP was significantly dependent on pH (Fig. 4). In the range assayed, maximum activity for *G. latifolium* (46·4 ± 8·8 μmol pNP g dry wt^{-1} h^{-1}) was found at the highest pH tested (pH 9) whereas in *G. sesquipedale* maximum APA (84·4 ± 2·3 μmol pNP g dry wt^{-1} h^{-1}) was achieved at pH 8·8.

Salinity and light effects

The effects of salinity on APA showed a similar pattern in both species (Fig. 5). Plants assayed in distilled water and low salinities exhibited very low activities. The activity increased with salinity from distilled water to a maximum APA at 43·8 salinity, which resulted in 37·4 μmol pNP g dry wt^{-1} h^{-1} for *G. latifolium* and 7·2 μmol pNP g dry wt^{-1} h^{-1} for *G. sesquipedale*. Higher salinities caused a decrease in APA.

However, increased salinity has an osmotic effect as well as an increase in the specific ion concentrations. The

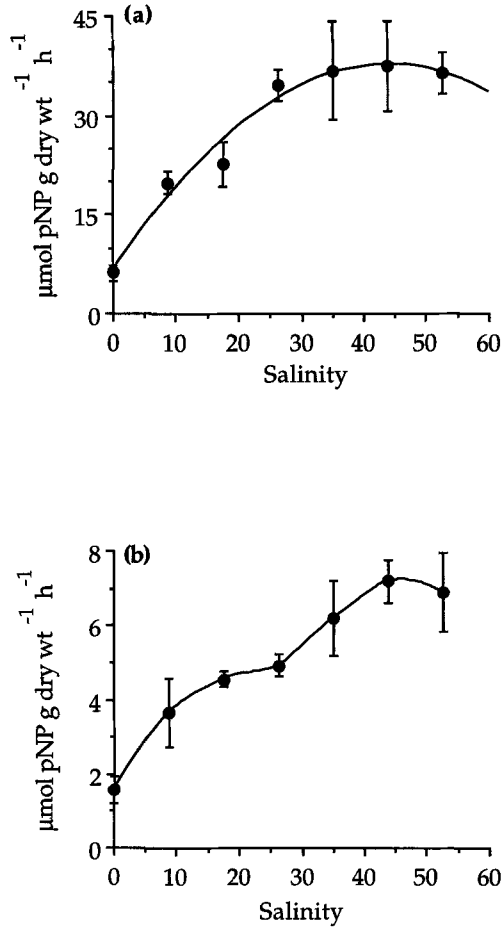

Fig. 5. Influence of salinity on alkaline phosphatase activity of *Gelidium latifolium* (*a*) and *Gelidium sesquipedale* (*b*) (pH 8·3; temperature 25°C). Enzymatic activity is expressed as µmol pNP g dry wt^{-1} h^{-1}. Values are means of three replicates and bars denote SD.

use of a non-electrolytic molecule (mannitol) permitted the two effects to be separated. A significant decrease in APA was observed in both species after the substitution of mannitol for NaCl (Table 2) compared with the control (plant assayed in standard conditions). The replacement of $MgCl_2$ did not cause any significant effects. Nonetheless, the replacement of all the magnesium compounds by mannitol led to a significant decline in APA (Table 2).

The effect of photon irradiance on APA differed in the two species (Fig. 6). In *G. latifolium*, neither a significant correlation nor any particular pattern was found between APA and photon irradiance. Thus, it seems that in this species APA is not influenced by photon irradiance level, at least in a short-term incubation. However, *G. sesquipedale* showed a more definite pattern. APA was low in the dark but showed a significant maximum (25·4 µmol pNP g dry wt^{-1} h^{-2}) at a relatively low photon irradiance (74 µmol photon m^{-2} s^{-1}). A time course of the difference between light (50–100 µmol photon m^{-2} s^{-1}) and dark showed that 15 min were enough to detect a difference in enzymatic activity (Fig. 7). Photon irradiances higher than 150 µmol photon m^{-2} s^{-1} led to a smooth decline in phosphatase activity.

Table 2. Percentage of alkaline phosphatase activity observed in *Gelidium latifolium* and *Gelidium sesquipedale* after the replacement of NaCl, $MgCl_2$ and $MgSO_4$ by mannitol in the artificial seawater used in the assay medium

Species	−NaCl, +mannitol	$-MgCl_2$, +mannitol	$-MgCl_2$, $-MgSO_4$, +mannitol
Gelidium latifolium	54·4*	103·5	55·6*
Gelidium sesquipedale	54·9*	100	62·3*

Values are referred to 100% of activity in the control (plants assayed in standard conditions). Values are means of three replicates.
*Decrease significant at the 5% level.

Effect of added phosphate on APA

When phosphate was added to the cultures up to a concentration of 20 µM, a gradual decline in APA was observed (Fig. 8). Maximum APA occurred when plants were incubated for 5 days in seawater without added

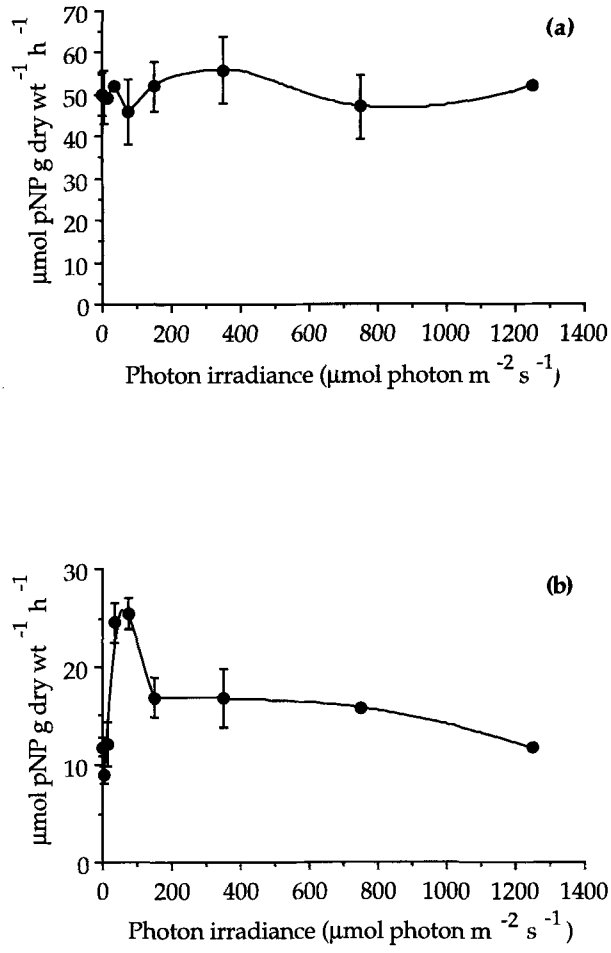

Fig. 6. Alkaline phosphatase activity as a function of photon irradiance (pH 8·3; temperature 25°C). In *Gelidium latifolium* (*a*) no relationship was found between the two variables, whereas in *Gelidium sesquipedale* (*b*) a significant maximum APA (25·4 µmol pNP g dry wt^{-1} h^{-1}) was found at 74 µmol photon m^{-2} s^{-1}. Values are means of three replicates and bars denote SD.

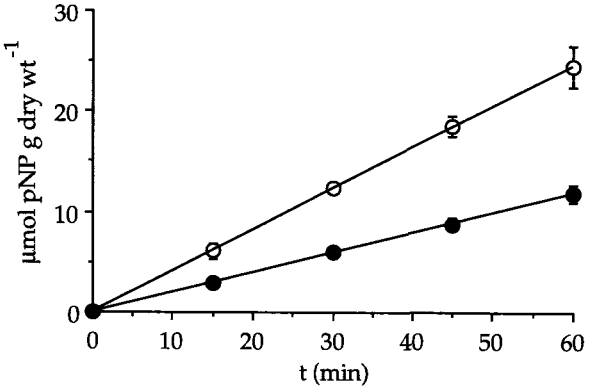

Fig. 7. *Gelidium sesquipedale.* Time course of the difference between pNP released (in µmol g dry wt^{-1}) through alkaline phosphatase activity in light (50–100 µmol photon m^{-2} s^{-1}) (open circles) and dark (filled circles). SD is shown as a bar when it exceeds the symbol size.

phosphate. The data shown in the figure were fitted to a "single exponential decay plus offset" model:

$$APA = APA_c + APA'_{max} \times e^{-bP} \quad (1)$$

where APA_c is the APA not affected by orthophosphate addition, APA'_{max} is the apparent maximum phosphatase activity (i.e. the difference between the absolute APA_{max} and APA_c), which was achieved with no addition of phosphate, b is the decay coefficient and P is the amount of orthophosphate added. The values of these parameters are shown in Table 3.

In both cases, a strong inhibition (greater than 50%) was recorded with low levels of added phosphate (less than 6 µM phosphate). However, lower phosphate additions were necessary in *G. latifolium* than in *G. sesquipedale* to repress APA (Table 3).

Discussion

Both *Gelidium latifolium* and *Gelidium sesquipedale* showed obvious alkaline phosphatase activity. The activities were always higher in *G. latifolium* than in *G. sesquipedale*, but were similar to values reported by Lapointe & O'Connell (1989) for species from inshore waters of Bermuda. However, higher phosphatase activities have been reported in other Rhodophyta (Hernández *et al.*, 1992).

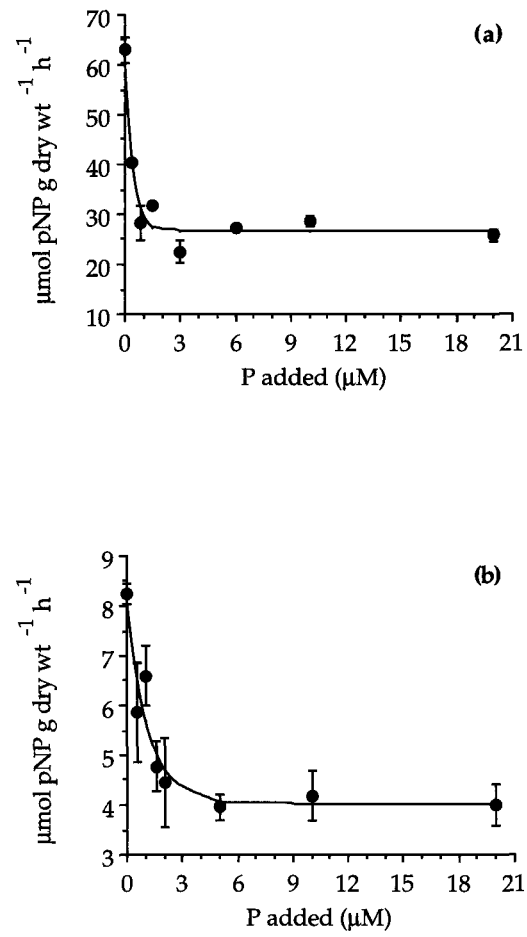

Fig. 8. Rate of hydrolysis of pNPP by alkaline phosphatase in *Gelidium latifolium* and *Gelidium sesquipedale* in media of increasing inorganic phosphate concentration (pH 8·3; temperature 25°C). Algae were incubated for 5 days in different regimes of external orthophosphate. In *G. latifolium* (*a*), final APA was 42% of the maximum rate, whereas in *G. sesquipedale* (*b*) this percentage was 49%. Enzymatic activity is expressed as µmol pNP g dry wt^{-1} h^{-1}. Values are means of three replicates and bars denote SD.

In most cases, APA of the two species of *Gelidium* varied similarity in response to changes in environmental variables. However, in some cases a different response was observed. Such responses may be explained by the ecological characteristics of the species.

Although these differences between the two species

Table 3. Values of the parameters of the "single exponential decay plus offset" model (equation 1) fitted to the effect of added phosphorus on alkaline phosphatase activity (APA)

Species	APA_c (µmol pNP g dry wt^{-1} h^{-1})	APA'_{max} (µmol pNP g dry wt^{-1} h^{-1})	b (µM^{-1})	FI (%)	P_{FI} (µM)	$P_{50\%}$ (µM)
Gelidium latifolium	26·7 (1·4)	36·4 (3·4)	2·6 (0·60)	57·7	1·7	0·75
Gelidium sesquipedale	4·01 (0·41)	4·2 (0·64)	0·91 (0·30)	51·04	5·07	4·3

Values are the mean (SE).

APA_c, APA not affected by orthophosphate addition; APA'_{max}, apparent maximum phosphatase activity (difference between the absolute APA_{max} and APA_c); b, decay coefficient; FI, percentage of final repression of APA; P_{FI}, added phosphate required to achieve 99% of the final inhibition; $P_{50\%}$, added phosphate sufficient to induce 50% inhibition.

could be genetic or environmental, we have convincing reasons to suspect that they are essentially genetic in nature. Sosa & García-Reina (1992) found the lowest genetic variability described for seaweeds in subpopulations of *Gelidium arbuscula* Bory (either gametophytic or sporophytic) from the Canary Islands. Other data suggest a relatively stable genetic composition in *Gelidium* (Freshwater & Rueness, 1994; Gorostiaga, personal communication), *G. sesquipedale* being one of the least variable species of this genus.

Non-linear reciprocal plots for APA have been reported previously in the dinoflagellate *Pyrocystis noctiluca* (Murray) Schuett (Rivkin & Swift, 1980). The behaviour of APA in *Gelidium* may result from two or more reactive sites on the same enzyme showing negative cooperativity (Cadenas, 1978), although further work is needed to demonstrate our hypothesis. In *Escherichia coli* (Migula) Castellani *et* Chalmers, a dimeric alkaline phosphatase was found to bind the substrate in a negatively cooperative fashion (Lazdunski, 1972). Similar results have been found in the mycelial alkaline phosphatase from *Neurospora crassa* Shear *et* Dodge (Palma *et al.*, 1989). This kinetic behaviour is also possible in monomeric enzymes (Cornish-Bowden & Cardenas, 1987). Sosa & García-Reina (1992, 1993) showed by electrophoresis in *Gelidium arbuscula* and *Gelidium canariensis* (Grunow) Seoane-Camba that alkaline phosphatase presented typical banding patterns that conformed to its interpretation as a diallelic, monomeric enzyme, with two bands occurring in heterozygotic sporophytes. Thalli of the two species of *Gelidium* assayed in the present work were diploid (tetrasporophytic) plants, as fertile (gametophytic) material of these species is rarely found in southern Spain (Seoane-Camba, 1965; F. Conde, personal communication). On the contrary, the foliose, haploid phase of the red alga *Porphyra umbilicalis* showed evident Michaelis–Menten type kinetics (Hernández *et al.*, 1992).

The lower of the two apparent *Ks* values observed in the two species of *Gelidium* was in the range of other *Ks* values calculated with pNPP as a substrate [from 8·7 μM for *Nostoc commune* Vaucher (Whitton *et al.*, 1990) to 320 μM for *Aphanizomenon flos-aquae* (Bornet *et* Flahault) Ralfs (Heath & Cooke, 1975)]. The ecological significance of the apparent negative cooperativity lies in the linkage of enzymatic kinetic parameters to external substrate concentrations (Levitsky & Koshland, 1976; Cembella *et al.*, 1983). *Gelidium* would achieve a constant P supply regardless of the PME concentration in the environment, as Burns & Beever (1977) hypothesised for a dual P uptake mechanism in *Neurospora crassa*. Short-term pulses of DOP are probably quite common in many natural environments (Rivkin & Swift, 1980; Islam & Whitton, 1992), including coastal waters. Potentially available substrates of the enzyme (PME) have been recorded from a nearby shore, and it was found that on some days the PME concentration was higher than that of SRP (Hernández *et al.*, 1993*b*).

The temperature optima of APA estimated for the two species of *Gelidium* (30°C in *G. latifolium* and 25°C in *G. sesquipedale*) were related to the temperature conditions in the field. In Maro, mean temperatures fluctuate between 13°C (winter) and 26·5°C (summer), whereas in Punta Carnero these fluctuations are smaller (14 to 22°C). Thus, the data suggest that temperature in the field probably reaches the optimum for APA and could even limit phosphatase activity, especially if *G. latifolium* can be exposed for short but stressing periods of time to the atmosphere. The Q_{10} found for the two species is similar to other values observed (Pettersson & Jansson, 1978; Hernández *et al.*, 1992). *G. sesquipedale* showed a lower Q_{10} than *G. latifolium*, which suggests a greater regulation of APA with temperature. This is in agreement with the position of the alga in the intertidal: *G. sesquipedale* lives in limestone crevices immersed in colder waters, which may enforce the development of enzymes optimally adapted for a narrow temperature range (Lüning, 1990).

pH is known to have a marked influence on algal APA (Siuda, 1984). A higher pH optimum was found for APA in *G. latifolium* (pH $\approx$9) than in *G. sesquipedale* (pH 8·8). In general, these values are quite close to the values reported for many cyanobacteria (Healey, 1982; Islam & Whitton, 1992), phytoplankters (Kuenzler & Perras, 1965; Pettersson & Jansson, 1978; Siuda, 1984) and macroalgae (Hernández *et al.*, 1992), although an optimum of up to pH 12 has been reported (Mahasneh *et al.*, 1990). The optimum pH levels for both species of *Gelidium* are unlikely to be encountered in their natural environment, as they are uncovered only for brief periods of time. The water probably remains at around pH 8·3, at which value APA is about 70% maximum activity. A possible explanation for the weak relationship between the pH optimum of APA and the typical pH of the natural environment from which the plants were harvested is given by Islam & Whitton (1992). These authors, on the basis of the study by Fedde & Whyte (1990) on human fibroblasts, suggest that the substrate concentration used in the assays (0·7 mM) can induce a higher pH optimum than if the assays were performed at ambient concentration (<2 μM).

The significant influence of salinity on APA in the two species of *Gelidium* has been found in other marine macrophytes growing in sites where natural fluctuations in salinity are common (Hernández *et al.*, 1994*b*). However, it is unlikely that *Gelidium* is subjected to fluctuations of salinity. The effect of salinity on APA is partly due to the increase of the ionic strength in the assay medium (Wilson *et al.*, 1964). Nevertheless, our results showed that this effect is attributable not only to the ionic strength itself, but that there was also a specific effect of particular ions, such as Na^+ and Mg^{2+}. Similar results were found by Mahasneh *et al.* (1990) in *Calothrix viguieri* Fremy. In this species the influence of NaCl on APA was not just an osmotic effect. The enhancement of APA in the presence of Mg^{2+} has been found in many alkaline phosphatases from different organisms (McComb *et al.*, 1979; Siuda, 1984; Hino, 1988), possibly because, as in

mammals (Anderson *et al.*, 1975), magnesium participates in the structure of some alkaline phosphatases. The stimulant effect of NaCl on APA may lie in the pronounced effect that both Na^+ and Cl^- could have on the membrane potential of these species, and, thus, hydrolysis and phosphate uptake (Ullrich-Eberius & Yingchol, 1974; Cembella *et al.*, 1983).

APA showed a different response to photon irradiance in the two species of *Gelidium* (Fig. 6). The lack of relationship found in *G. latifolium* was also noticed for *Porphyra umbilicalis* (Hernández *et al.*, 1992). These two species are exposed in their natural environment to a broad range of photon irradiances, which could be a selection factor for the enzyme to be independent of light intensity. However, a long period of incubation in the dark (14 h) before the assay causes higher APA in darkness compared with an assay at 100 μmol photon m^{-2} s^{-1} (Hernández, unpublished data). As APA is inversely related to internal phosphate (Wynne, 1981), a long pre-incubation in darkness may stimulate alkaline phosphatase production due to a decrease in phosphate uptake, which is an energy-dependent reaction (Healey, 1973).

Maximum APA in *G. sesquipedale* coincided with the compensation point for photosynthesis (Torres *et al.*, 1991), in a pattern similar to that observed for other variables such as photosynthetic rates, chlorophyll, phycobilins and C:N ratios. This common pattern, showing a slight photoinhibition at high irradiances, reflects the intolerance of *G. sesquipedale* to high irradiances, which is greater than that of other intertidal species in the same area (Torres *et al.*, 1991). Other studies have found different responses. Rivkin & Swift (1979) showed that enzyme activity was proportional to light intensity in *Pyrocystis noctiluca* and reported a marked reduction of APA when cells were exposed to prolonged darkness, whereas Klotz (1985) found that the alkaline phosphatase activity of *Selenastrum capricornutum* Printz was inversely related to total insolation. The irradiance optimum found in *G. sesquipedale* (within the usual range of photon irradiances observed at the collection site) and the disparity of responses of APA to light (see also Huber & Hamel, 1985; Wynne & Rhee, 1988, Weich & Granéli, 1989) support the hypothesis suggested by Klotz (1985), in the sense that temporal variations in APA noted in algae may be a factor in the co-occurrence of species in a phosphate-depleted environment, with the PME fraction made available to different species at different times of the day.

Low levels of external phosphate were sufficient to induce a repression of APA, specially in *G. latifolium* (Table 3). To compensate for the higher APA repression, this species takes up a greater proportion of the phosphate hydrolysed from PME (Fig. 1). Furthermore there is a direct relationship between the phosphate necessary to induce APA repression and the SRP concentrations at the two sampling sites, usually higher at the Atlantic site (Niell *et al.*, 1989). The inverse exponential relationship between APA and external phosphate has been found in previous studies (e.g. Veldhuis *et al.*, 1987) and may reflect an inverse relationship between APA and internal phosphate (Wynne, 1981), especially when there is a long-term effect of external phosphate upon APA. Moreover, natural populations of *Porphyra umbilicalis* exhibited a regulation of APA dependent on the internal phosphate status (Hernández *et al.*, 1993*b*), assessed by the cell quota in the sense of Droop (1983), and other results demonstrated that phosphatases are controlled by several compartments involved in phosphorus metabolism, such as particulate internal phosphorus (Gage & Gorham, 1985). Further experiments need to be done to clarify the fine control of APA by an internal compartment of phosphorus.

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