SCI. MAR., 60 (2-3): 297-306 SCIENTIA MARINA 1996

Alkaline phosphatase activity of the red alga *Corallina elongata* Ellis et Solander*

I. HERNÁNDEZ[1†], F.X. NIELL[2] and J.A. FERNÁNDEZ[3]

[1]Departamento de Ecología, Universidad de Cádiz, Facultad de Ciencias del Mar, 11510 Puerto Real (Cádiz), Spain.
[2]Departamento de Ecología. Universidad de Málaga, Facultad de Ciencias, 29071 Málaga, Spain.
[3]Departamento de Biología Vegetal, Universidad de Málaga, Facultad de Ciencias, 29071 Málaga, Spain.

SUMMARY: Alkaline phosphatase activity (APA) was investigated in a representative population of *Corallina elongata*, a common calcareous red alga in the Mediterranean Sea, using p-nitrophenyl phosphate (pNPP) as a model substrate. A total of 66 % of the phosphate cleaved from the substrate was immediately taken up by the algae, which may suggest a connection between APA and phosphate uptake. APA kinetic exhibited an apparent negative cooperativity for the hydrolysis of pNPP. APA sites were located by a histochemical method. The staining technique showed that phosphatase activity was located on the monolayer of cortical cells (epithallial cells). The influence of factors important in nature on enzymatic activity was tested. In a range according with natural conditions, temperature, pH, salinity and photon irradiance influenced APA significantly. However, external phosphate showed little effect. The relationship between APA and the physical and chemical environment of the algae is discussed.

Key words: Alkaline phosphatase, benthic algae, *Corallina elongata*, dissolved organic phosphorus, histochemical stain.

RESUMEN: UN ESTUDIO SOBRE LA ACTIVIDAD FOSFATASA ALCALINA EN LA RODOFÍCEA *CORALLINA ELONGATA* ELLIS ET SOLANDER. – Se ha estudiado la actividad fosfatasa alcalina (APA) en una población representativa de *Corallina elongata*, una rodofícea ampliamente representada en el Mar Mediterraneo, utilizando como sustrato modelo el *p*-nitrofenil fosfato (pNPP). Un 66 % del fosfato hidrolizado del sustrato se incorporó inmediatamente tras la hidrólisis enzimática, lo que sugiere un acoplamiento entre la APA y la incorporación de fosfato. La cinética de la APA frente al pNPP mostró una cooperatividad negativa aparente. Los lugares de actividad se estudiaron por un método de tinción histoquímica. La técnica utilizada localizó la actividad en la monocapa de células corticales (epitalo). Se investigó el efecto de algunas variables ambientales en la actividad enzimática. En un rango acorde con las condiciones naturales en que se encontró la especie, la temperatura, el pH, la salinidad, la irradiancia fotónica y la concentración de fósforo externo influenciaron significativamente la actividad enzimática. Por ello se discute la relación entre la APA y el ambiente fisicoquímico del macrófito.

Palabras clave: Fosfatasa alcalina, alga béntica, *Corallina elongata*, fósforo orgánico disuelto, tinción histoquímica.

INTRODUCTION

Seaweeds are able to use phosphate from phosphomonoesters (PME); compounds of the dissolved organic phosphorus (DOP) pool. The utilization of such compounds by marine algae is possible through the action of alkaline phosphatase. This enzyme cleaves phosphate from PME, so that inorganic phosphate (Pi) becomes available to the algae (Siuda, 1984). High alkaline phosphatase activity (APA) is generally believed to indicate phosphorus deficiency (Cembella *et al.*, 1983). Some previous studies suggest that phytoplankton and macroalgae from the oligotrophic Mediterranean Sea can be limited by the availability of phosphorus, especially during the

*Received 29 June 1994. Accepted 25 July 1995.
†To whom correspondence should be addressed.

summer (Delgado *et al.*, 1994; Thingstad and Rassoulzadegan, 1995). In addition, most of the P can exist as a DOP compounds (Banoub and Williams, 1972; Thingstad and Rassoulzadegan, 1995). These two facts indicate that DOP may serve as an important extra P-supply to the algal community, particularly during periods of P limitation.

Since Walther and Fries (1976) identified the enzymatic activity from multicellular algae, an increasing number of investigations on APA of macroalgae have been carried out (e. g. Weich and Graneli, 1989; Lapointe *et al.*, 1992; Hernandez *et al.*, 1995). *Corallina elongata*, a heavily calcified Rhodophyta, has been extensively studied in relation to N metabolism (Lopez-Figueroa, 1993; Vergara and Niell, 1993), but as far as we are aware there have been no previous reports of studies on P metabolism, specifically on phosphatase activity. The aim of the present investigation was to study and locate the enzymatic activity in *C elongata*, with special attention to the response of the enzymatic activity to external factors such as temperature, pH, salinity, photon irradiance and orthophosphate concentration.

MATERIAL AND METHODS

Collection site and preconditioning

Plants of *Corallina elongata* were collected on a rocky shore in Maro (Málaga, southern Spain). This species was found throughout the year on wave-exposed rocks at the midlittoral zone. Maro is characterized by clear waters, corresponding to type I-II of the Jerlov classification of coastal waters (Jiménez, 1985).

After collection, plants were kept cool in darkness and transported to the laboratory in an icebox. Macrophytes were generally free of visible epiphytes but, if necessary, the epiphytes were carefully removed with forceps. To minimize any possible acclimatation to laboratory conditions, plants were maintained for a short time (24 h maximum, except in the experiments on P-enrichment) in renewed cultures of aerated, filtered natural seawater (pH 8.2) at constant temperature (15 oC) and photon irradiance of 150 µmol photon m^{-1} s^{-1} (solar LFD 18 W tube). The orthophosphate concentration of the cultures was kept constant at 0.2 µM, similar to the mean phosphate concentration in the field (Hernández *et al.*, 1993).

Measurement of alkaline phosphatase activity

Alkaline phosphatase activity was measured in three replicate samples by a modification of the colorimetric method of Reichardt *et al.* (1967) which uses *p*-nitrophenyl phosphate (pNPP) as an artificial substrate. The method was modified as proposed by Hernández *et al.* (1992). Plants were assayed in a reaction mixture consisting of 100 ml of 700 µM pNPP, 50 mM Tris-HCl buffer, pH 8.3. The initial substrate concentration was sufficiently high to ensure that no more than 10% was hydrolyzed during the assay. Reagents were dissolved in filtered (0.2 µm) artificial seawater of negligible Pi concentration and 35 salinity (practical salinity units) (Kalle, 1945). Air was bubbled continuously during the assay to oxygenate and stir the medium. After 45 min of incubation at 25°C and 150 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^{-1} dry wt $\cdot$ h^{-1}, formed by the hydrolysis of pNPP to P and pNP. Absorbance units were converted to concentration using a different molar extinction coefficient for each pH value (Hernández, 1992):

$$E_{pH} = \frac{16{,}925}{1 + e^{18.4 - 2.6\,pH}} \qquad (1)$$

Where 16,925 M^{-1} cm^{-1} (SE 72.2) is the estimated maximum molar absorption coefficient of pNP in seawater at 410 nm.

To determine the proportion of the phosphate enzymatically liberated 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 were sampled to determine Pi (as soluble reactive phosphorus; SRP) (Murphy and Riley, 1962) and APA. We have assumed that all the pNP from pNPP hydrolysis is released into the medium. According to previous research with glucose-6-P as a substrate for alkaline phosphatase (Hernández *et al.*, 1996) any possible retention of pNP by the algae was considered negligible. Both end-products can be measured in the reaction mixture without any interference, as demonstrated previously (Hernández *et al.*, 1994).

To determine kinetic parameters, APA was assayed at different pNPP concentrations ranging from 7.10^{-6} to $3.5.10^{-3}$ M. The Michaelis-Menten equation

was rearranged according to two transformations. The Lineweaver-Burk plot (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 and Stevens, 1982). Apparent cooperativity was tested from the slope of a Hill plot [Log v/(Vmax-v)/Log S].

To quantify the effect of temperature on APA, different plants were assayed at 5°C intervals between 10 and 30°C. Temperature-dependent changes in pH 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 and Stevens, 1982). The influence of pH on activity was tested using a range of 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 effects of Na^+ and Mg^{2+} on APA were determined replacing some ions/molecules of the artificial seawater (NaCl, $MgCl_2$ and $MgSO_4$) by mannitol, adding the necessary quantity to keep the osmotic pressure constant. The equivalence between concentration and osmotic pressure were taken from Wolf *et al.* (1982) and checked with a cryoscopic osmometer (Osmomat 030).

To test the relationship between photon irradiance and APA, the plants were assayed under illumination of the incubation chamber 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. To quantify any possible regulating effect of external orthophosphate on APA, algae were pre-incubated for 5 days in 400 ml of seawater enriched with different amount of inorganic phosphate (as Na_2HPO_4). Final concentration of Pi added ranged from 0 to 20 µM. Seawater of the cultures was renewed every12 hours.

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). A more powerful *a posteriori* test (sum of squares, simultaneous test procedure; Fry, 1993) was used when comparing groups of means. 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.

Staining

The histochemical localization of APA in *Corallina elongata* was tested by microscopy using ß-glycerophosphate as a substrate (Gomori, 1939). Thalli were cut in a cryostate (Reichert-Jung 2800 Frigocut) at -25°C. The sections (10 µm width) were mounted on clean glass slides coated with a 0.1% (wt/v) solution of poly-L-lysine (Sigma). The slides were then immersed in the assay medium. This medium was composed of 10 ml ß-glycerophosphate (3% wt/vol), 10 ml barbital (5% wt/vol), 15 ml of anhydrous $CaCl_2$ (2% wt/vol), 10 ml of $MgSO_4$ (2% wt/vol) and 5 ml of 0.5 M NaCl. 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 APA were stained by incubating the sections as follows: 5 min in $Co(NO_3)_2$ (0.5% wt/vol), 3 min wash 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 precipitate of cobalt sulfide. Finally, the sections were mounted in Aquatex® jelly (Merck). The stained sections were compared against a blank (sections incubated in a medium of assay without substrate).

RESULTS

During the assay of APA in *Corallina elongata*, the concentration of SRP and pNP increased in the incubation chamber (Fig. 1). However, the slopes of the regression lines for both end products of the enzyme reaction were significantly different ($p < 0.001$). The difference between pNP and SRP was considered to be phosphorus taken up by the algae after hydrolysis of the substrate, assuming that all the pNP is released into the medium. This percentage was relatively high, as 66% of the phosphate cleaved by APA was taken up by the algae during the assay.

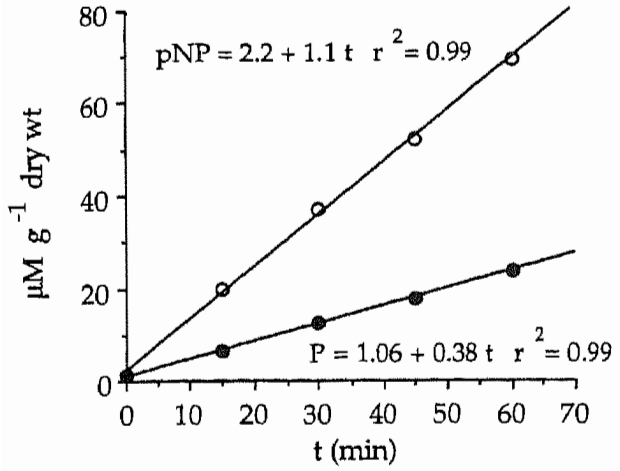

FIG. 1. – Concentrations of the two products of the pNPP hydrolysis: pNP (open circles) and SRP (solid circles) (in µM . g^{-1} dry wt) measured during the assay of alkaline phosphatase activity of *Corallina elongata*. Values are the mean of three replicates and SD was negligible.

The Lineweaver-Burk transformation of Michaelis-Menten plot, showing the dependence of APA on substrate concentration, is presented in Fig. 2. This non lineal reciprocal plot suggested the existence of a negative cooperativity for the hydrolysis of pNPP, with maximum velocity of 17.8 µmol pNP g^{-1} dry wt $\cdot h^{-1}$. Two apparent Ks values were deduced from the Eadie-Hofstee plot, one having a high affinity (Ks_1 = 40 µM) and one of low affinity (Ks_2 = 948 µM). These Ks values 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 $n_H = 0.61$ ($r^2 = 0.98$, $p < 0.001$)

The temperature dependence of APA (Fig. 3) showed maximum activity at the highest temperature tested (16.04 ± 2.2 µmol pNP g^{-1} dry wt $\cdot h^{-1}$ 30°C). Fitting a first-order polynomial to the data generated the equation APA = 0.52 T - 0.84 (r^2 = 0.85; $p < 0.01$), with 1.6 and 0.08 being the SE of the *y* intercept and the slope respectively. The Arrhenius plot of the data showed an activation energy (Ea) of 40.3 kJ mol^{-1}. The Q_{10} of the reaction was 1.7.

Fig. 4 shows the pH dependence of APA of *Corallina elongata*. The activity was significantly higher at pH > 8.3, compared with the activity at any other lower pH value. Within this pH range (8.3 - 9), maximum activity (23.6 ± 3.5 µmol pNP g^{-1} dry wt $\cdot h^{-1}$), was observed at pH 8.8.

Salinity influenced significantly APA (Fig. 5). The activity was significantly higher at salinities above 36 compared with lower salinities. However, only a slight increase in activity was observed with

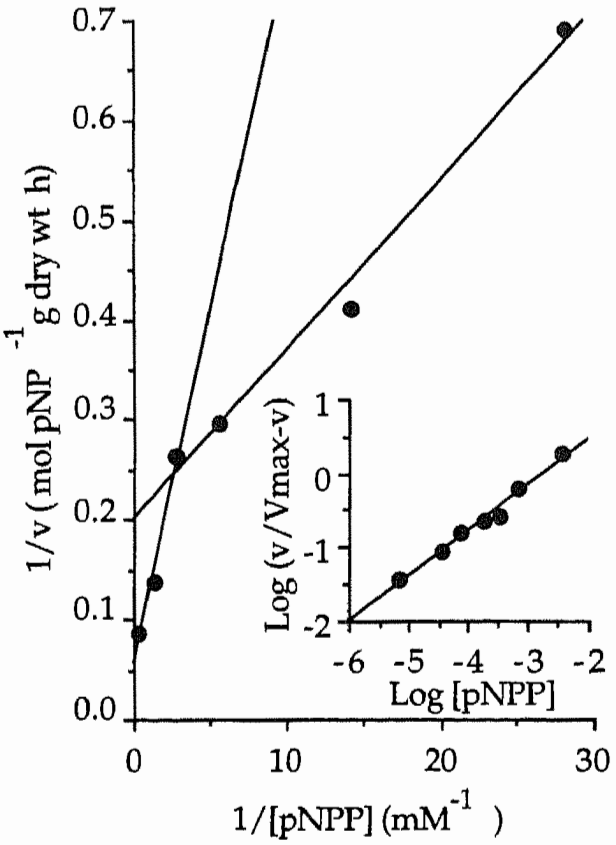

FIG. 2. – Double reciprocal plot relating the dependence of the velocity of hydrolysis of pNPP on substrate concentration. An apparent negative cooperativity was suggested by the two straight lines drawn in the figure. Inset, replot of the data according to the Hill representation. Each point represents the mean of three experiments. Enzymatic activity is expressed as µmol pNP g^{-1} dry wt $\cdot h^{-1}$ and substrate concentration as mM pNPP.

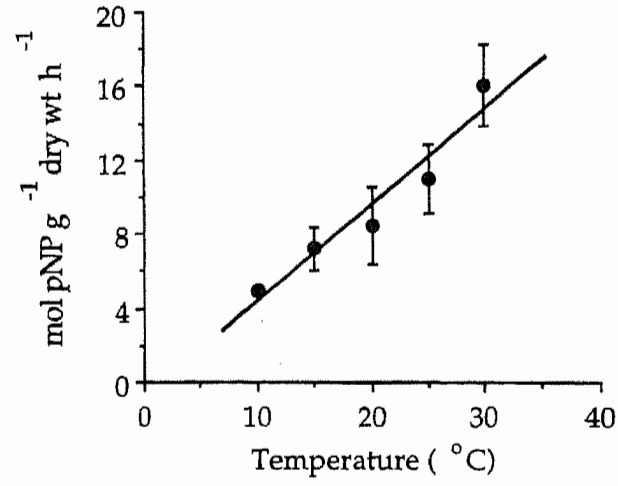

FIG. 3. – Alkaline phosphatase activity as a function of temperature. Data were fitted to a first order polynomial (see text). Enzymatic activity is expressed as µmol pNP g^{-1} dry wt $\cdot h^{-1}$. Values are the mean of three replicates and bars denote SD.

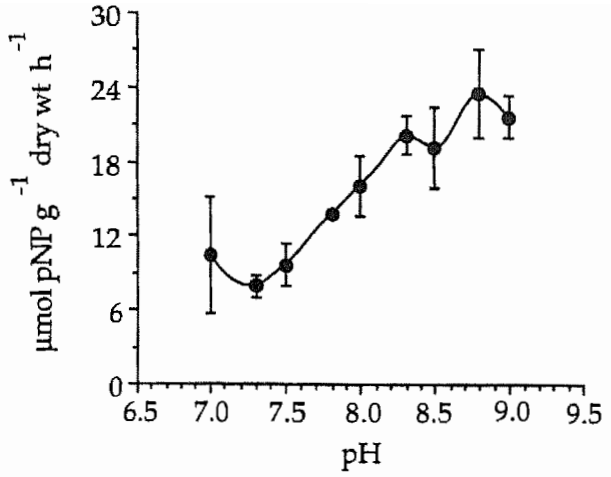

FIG. 4. – pH profiles of alkaline phosphatase activity of *Corallina elongata* (temperature 25°C). Enzymatic activity is expressed as µmol pNP g^{-1} dry wt · h^{-1}. Values are the mean of three replicates and bars denote SD.

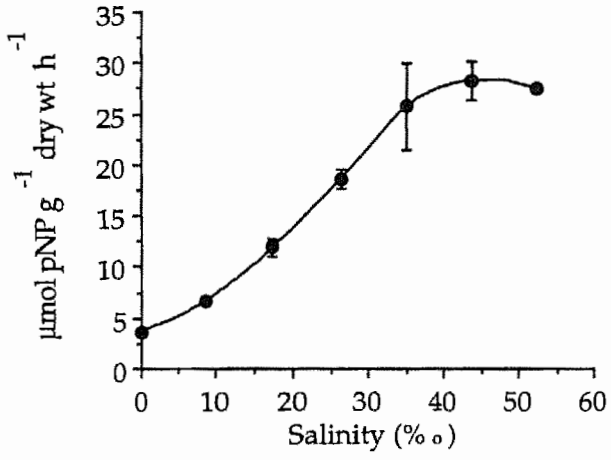

FIG. 5. – Influence of salinity on alkaline phosphatase activity of *Corallina elongata* (pH 8.3, temperature 25°C). Enzymatic activity is expressed as µmol pNP g^{-1} dry wt · h^{-1}. Values are the mean of three replicates and bars denote SD.

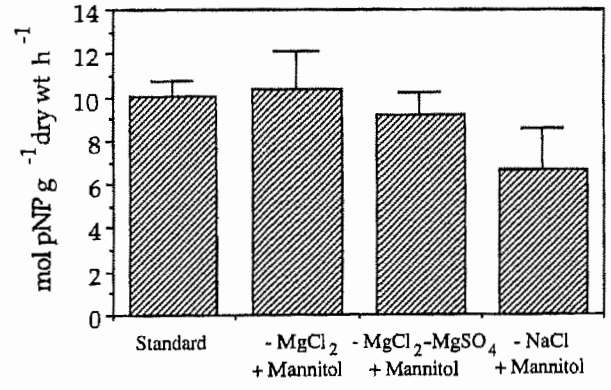

FIG. 6. – Percentage of alkaline phosphatase activity observed in *Corallina elongata* after the replacement of $MgCl_2$, all the Mg^{2+} compounds and NaCl by mannitol in the artificial seawater used in the assay medium. Values are compared to the activity in the control (algae assayed in standard conditions). Values are the mean of three replicates and bars detote SD.

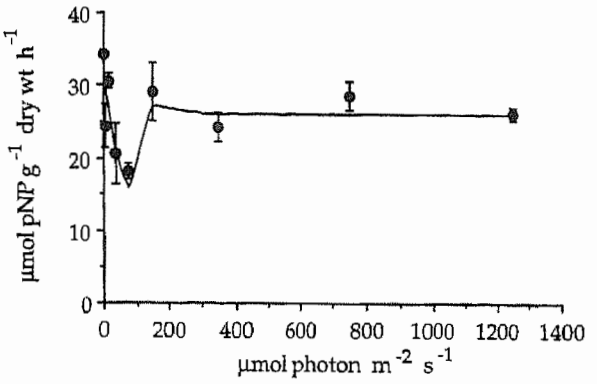

FIG. 7. – Alkaline phosphatase activity of *Corallina elongata* as a function of photon irradiance (pH 8.3, temperature 25°C). Maximum activity (34.3 µmol pNP g^{-1} dry wt · h^{-1}) was found in darkness whereas the minimum activity was achieved between 50-100 µmol photon m^2 s^{-1}. Values are the mean of 3-6 replicates in the range of photon irradiances specified in the text. Bars denote SD.

increasing salinities above 36. Maximum activity (23.8 µmol pNP g^{-1} dry wt · h^{-1}) was observed in plants incubated at 43.8 salinity

The increase in salinity implies an osmotic effect as well as an increase in the specific ionic concentrations. The use of a non-electrolytic molecule (mannitol) permitted the two effects to be separated. The data shown in Figure 6 point out that salinity itself is responsible of a high percentage of the influence of salinity on APA, as the replacement of all Mg^{2+} compounds of the artificial seawater ($MgCl_2$ and $MgSO_4$) by mannitol did not cause any effect on APA compared to the control (plant assayed in standard conditions). With regard to NaCl, the effect of a substitution of mannitol for NaCl caused a decrease of APA close to the significance level ($0.1 < p < 0.05$).

Corallina elongata showed a significant variation of APA with incident photon irradiance (Fig. 7). Maximum activity (34.3 µmol pNP g^{-1} dry wt · h^{-1}) was achieved in darkness whereas the minimum activity (18.2 µmol pNP g^{-1} dry wt · h^{-1}) was observed at relatively low irradiance (50 - 100 µmol photon m^{-2} s^{-1}). On the contrary, plants assayed at irradiances above 150 µmol photon m^{-2} s^{-1} exhibited similar phosphatase activity (≈ 26 µmol pNP g^{-1} dry wt · h^{-1}).

The relationship between APA and external phosphate concentration is presented in Fig. 8. In contrast with other studies in phytoplankters (e. g. Pick, 1987) or macroalgae (Delgado and Lapointe, 1994), *C. elongata* was poorly related to the external orthophosphate concentration. Similar to the effects of phosphate addition on APA in other macroalgae

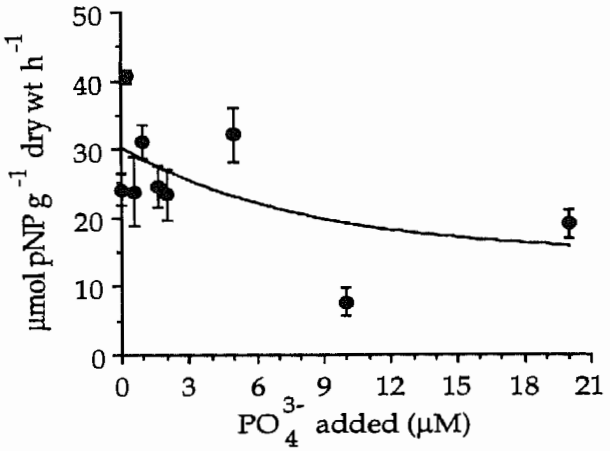

FIG. 8. – Alkaline phosphatase activity in *Corallina elongata* in media of increasing inorganic concentration (pH 8.3, temperature 25°C). Final activity was 48% of the maximum rate. Enzymatic activity is expressed as µmol pNP g^{-1} dry wt $\cdot h^{-1}$. Values are the mean of three replicates and bars denote SD.

(Hernández *et al.*, 1995) data were fitted to a "single exponential decay plus offset" model:

$$APA = APA_c + APA'_{max} * e^{-bPi} \quad (2)$$

where APA_c (14.4 µmol pNP g^{-1} dry wt $\cdot h^{-1}$; SE = 9) is the APA not affected by orthophosphate addition, APA´max (15.9 µmol pNP g^{-1} dry wt $\cdot h^{-1}$; SE = 9) 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 (0.12 μM^{-1}; SE = 0.38) and Pi is the amount of orthophosphate added. Under this model, *Corallina elongata* showed a smooth decrease in APA with increasing phosphate, as for 50% inhibition to occur, high phosphate addition (25.6 µM) was required.

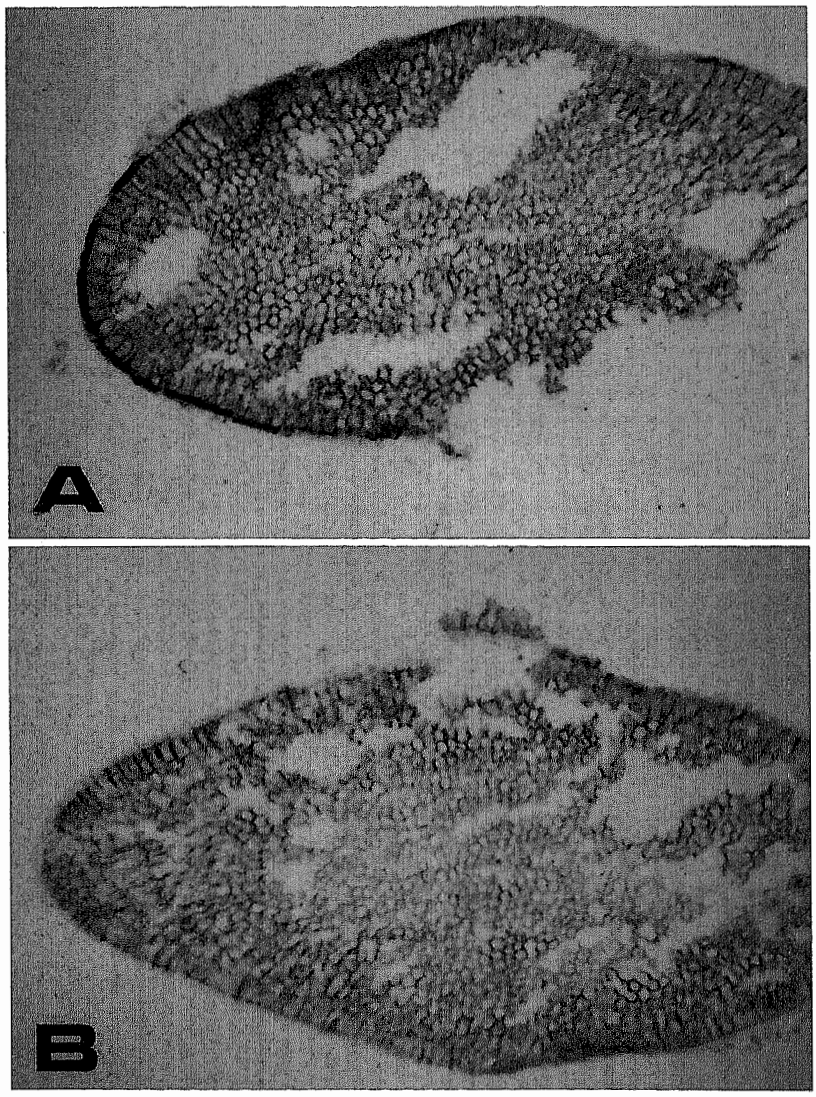

FIG. 9. – *Corallina elongata*. Gomori staining revealing localization of alkaline phosphatase activity (black precipitates) on thallus. A. Positive reaction showing the enzymatic activity in the monolayer of cortical cells. B. Control.

The histochemical staining for APA in *C. elongata* is shown in Figure 9. The staining section is compared against a blank consisting of sections incubated in the absence of substrate. An obvious localization of activity on the monolayer of cortical cells (epithallial cells) was observed, whereas there was negligible APA associated with any other part of the thallus (vegetative initial cells and medullar cells).That suggests that hydrolysis of PME may probably take place at the outer surface of the cortical cells.

DISCUSSION

The APA of *Corallina elongata* can be compared with other values reported in different studies of phosphatase activity in macroalgae. Lapointe and O´Connell (1989) assayed APA in several macroalgae from Bermuda's inshore waters. These activities (measured at 18°C and seawater pH) ranged from ≈ 1 µmol pNP g^{-1} dry wt · h^{-1} in *Codium taylori* to ≈ 21.5 µmol pNP g^{-1} dry wt · h^{-1}, reported for *Cladophora prolifera*. Similarly, Lapointe *et al.*, (1992) assayed APA in an extensive number of macroalgae collected from siliciclastic and carbonate-rich tropical coastal waters. These activities varied between 0.65 µmol pNP g^{-1} dry wt · h^{-1} for *Bryopsis pennata* and 113 µmol pNP g^{-1} dry wt · h^{-1} for *Dictyota dicotoma*. The "standard" activity of *C. elongata* found in the present study (≈ 18 µmol pNP g^{-1} dry wt · h^{-1}) fits within all these values. Similar activities were found in *Gelidium latifolium* (Hernández *et al.*, 1995). However higher APA values were found during the gametophytic cycle of the red alga *Porphyra umbilicalis* (Hernández *et al.*, 1993).

Furthermore, an estimate of a 22% of ash free content per dry wt unit in *C. elongata* (Perez-Llorens, unpublished data) gives APA values of ≈ 78 µmol pNP g^{-1} ash free dy wt · h^{-1}. This enzymatic activity is slightly higher than APA values reported by Delgado and Lapointe (1994) in calcareous macroalgae. These authors found activities up to 42.3 µmol pNP g^{-1} ash free dy wt · h^{-1} in summer populations of *Halimeda opuntia*.

APA in phytoplanktonic species usually shows Michaelian type kinetic versus pNPP (e. g. Wynne and Rhee, 1988). However, a non lineal reciprocal plot for APA was found earlier by Rivkin and Swift (1980) in the dinoflagellate *Pyrocystis noctiluca*. The nature of APA found in *Corallina elongata* has also been found in two species of the red alga *Gelidium* (Hernández *et al.*, 1995). So far, we have not been able to resolve isoenzymes of alkaline phosphatase of *C. elongata*, thus several explanations may account for the behaviour of APA. First, it might result from two isoenzymes of high and low affinity, which operate under conditions of low and high PME concentration. However the most possible explanation may result from the existence of two or more reactive sites on the same enzyme, showing negative cooperativity (Cádenas, 1978). In *Escherichia coli*, 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* (Palma *et al.*, 1989). Finally, this kinetic may be also attributable to an heterotrophic effect between Pi and pNPP. As 34% of the Pi released from pNPP was not taken up by the algae, (Fig. 1), the more pNPP in the assay medium, the more phosphate concentration. If phosphate binds to a site distinct from the active site, the relationship between P and substrate is allosteric (Price and Stevens, 1982), then causing a consequent change in the kinetic properties of the enzyme. However, further work is needed to demonstrate these hypothesis.

The apparent lower Ks found in *Corallina elongata* was similar to Km values reported for alkaline phosphatase with pNPP as a substrate, either in phytoplanktonic species (e. g. Whitton *et al.*, 1990) or macroalgae (Hernández *et al.*, 1992). The ecological significance of the negative cooperativity lies in the linkage of enzymatic kinetic parameters to external substrate concentration (Levitzky and Koshland, 1976; Cembella *et al.*, 1983), which might allow rapid uptake in the presence of transient high DOP concentration. Short-term pulses of DOP are probably quite common in many natural environments (Islam and Whitton, 1992). Besides, potentially available substrates of the enzyme (PME) have been recorded in a nearby shore, where some days the PME concentration was higher than SRP (Hernández *et al.*, 1993).

A high percentage (66%) of the phosphate cleaved from pNPP was immediately taken up by the algae. That may suggest a connection between APA and phosphate uptake, although further studies are needed to demonstrate this hypothesis. The possibility of the enzyme not only cleaving but also immediately transfering the phosphate to an acceptor in the cell was early suggested by Kuenzler and Perras (1965).

Although *Corallina elongata* lives in the littoral zone, the algae can be exposed to the atmosphere for short but stressing periods of time, especially during periods of calm or neap tides. An increase of APA with temperature over the range assayed was observed, without loss of activity due to thermal stress. Thus, the occurrence of stressing exposure to direct solar radiation may force the existence of an enzymic machinery acclimated to the environmental temperatures (mean temperatures fluctuate between 13 and 26.5 °C ; data from the Oceanographic Institute of Málaga). In *Gelidium sesquipedale*, a red algae growing in colder waters, APA declined with temperatures higher than 25°C (Hernández *et al.*, 1995). The Q_{10} found in *C. elongata* (1.7) suggests a strong regulation of APA with temperature, as occurs typically in many enzymes from intertidal species such as anemones and winkles (Price and Stevens, 1982). The Q_{10} is similar to other values recorded previously in algae (Pettersson and Jansson, 1978; Hernández *et al.*, 1992).

Within the range assayed, maximum APA in laboratory assays was observed at pH 8.8 (Fig. 4). This pH value can be commonly attained in the seawater immediately outside the cortical cells during calcification (Digby, 1977), particularly during sunlight exposure and high photosynthetic rate. That means a maximum extra phosphorus supply in cases of high rates of carbon fixation. The pH optimum found for APA in *Corallina elongata* is similar to the optimum observed for APA in other phytoplanktonic species (Pettersson and Jansson, 1978) or red algae (Hernández *et al.*, 1992), although pH optima for APA in microalgae greater than 9 have been reported (Rivkin and Swift, 1980).

The significant influence of salinity on APA in *Corallina elongata* has been found in other red algae growing in sites where natural fluctuations of salinity may occur (Hernández *et al.*, 1992). Thus, a higher activity with high salinity may balance states of nutrient requirement from the energy cost in osmotic adjustment. However, it is unlikely that *C. elongata* is subjected to marked fluctuations of salinity. The influence of salinity on APA is essentially due to the increase of the ionic strength in the assay medium (Wilson *et al.*, 1964; Pettersson, 1979). However the results suggest that NaCl (and possibly Na^+) itself may be directly involved in this effect. Similar results were found by Mahasneh *et al.* (1990) in *Calothrix viguieri*. In this species the influence of NaCl on APA was not just an osmotic effect. 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 and Yingchol, 1974).

Maximum APA was found in darkness. Thus, alkaline phosphatase may balance the P requirements when the direct uptake of orthophosphate is reduced under conditions of limiting reducing power. In other macroalgae APA showed a different response. In *Ulva lactuca* APA was stimulated in light whereas inhibition was detected in darkness (Weich and Graneli, 1989). Also *Gelidium sesquipedale* showed maximum APA at 70-100 µmol photon $m^{-2}s^{-1}$ (Hernández *et al.*, 1995), the photon irradiance where *Corallina elongata* demonstrated the minimum activity. The differences in temporal variations in APA noted in algae may be a factor in the co-ocurrence of species in a phosphate-poor environment, with the PME fraction made available to different species at different times of the day (Klotz, 1985).

Compared to many other studies where an inverse hyperbolic relationship between APA and Pi concentrations was observed (Van Boekel and Veldhuis, 1990; Hernández *et al.*, 1995) APA of *Corallina elongata* was poorly suppressed by external phosphate concentration, as high, unrealistic Pi concentration were necessary to detect a 50% suppression of enzymatic activity. Also Delgado and Lapointe (1994) found a marked decrease of phosphatase activity in a set of P-enrichment experiments performed in calcareous macroalgae. However, a weak relationship between APA and ambient Pi has been noted previously (Klotz, 1985), and has been linked to internal P reserves or to rapid turnover of ambient Pi (Wynne 1977). It is also known that in some species, especially chrysophytes and cryptomonads, APA is not easily inhibited by additions of orthophosphate (Aaronson and Patni, 1976; Stewart and Wetzel, 1982). Inhibitory effects of Pi on coralline calcification have been reported (Brown *et al.* 1977). As Lobban *et al.* (1985) suggest, this fact implies that the growth of coralline could be inhibited in phosphate polluted coastal waters, where DOP concentration is high. In that case, the existence of a significant APA in the algae could lead to an increase of the limiting growth.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Dr I. Núñez de Castro for his helpful advice about enzyme kine-

tics and Dr. J. L. Peréz-Llorens for providing data of ash free content in plants of *Corallina elongata*. This research was supported by the scientific group nº 4062 of the "Junta de Andalucía" and is a contribution to the CICYT research project AMB 93-1211. I. Hernández held a FPI fellowship from the Spanish Ministry of Education and Science.

REFERENCES

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

Banoub, M.W. and P.J.B. Williams. – 1972. Measurements of microbial activity and organic material in the western Mediterranean sea. *Deep Sea Res.*, 19: 433-443.

Brown, V., S.C. Ducker and K.S. Rowan. – 1977. The effect of orthophosphate concentrations on the growth of the coralline algae (Rhodophyta). *Phycologia* 16: 125-131.

Cádenas, E. – 1978. *Enzimas alostéricas*. H. Blume ed., Madrid.

Cembella, A.D., N.J. Antia and P.J. Harrison. – 1983. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: part 1. *CRC Crit. Rev. Microbiol.*, 10: 317-391.

Delgado, O. and B.E. Lapointe. – 1994. Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. *Coral Reefs*, 13: 151-159.

Delgado, O., E. Ballesteros and M. Vidal. – 1994. Seasonal variation in tissue Nitrogen and Phosphorus of *Cystoseirea mediterranea* Sauvageau (Fucales, Phaeophyceae) in the northwestern Mediterranean Sea. *Bot. Mar.*, 37: 1-9.

Digby, P.S.B. – 1977. Growth and calcification in the coralline algae, *Clathromorphum circumscriptum* and *Corallina officinalis*, and the significance of pH in relation to precipitation. *J. Mar. Biol. Assoc. U. K.*, 57: 1095-1099

Fry, J.C. – 1993. *Biological data analysis: A practical approach*. Oxford University Press, Oxford.

Gomori, G. – 1939. Microtechnical determination of phosphatase in tissue section. *Proc. Soc. Exp. Biol. Med.*, 42: 23-29.

Hernández, I. – 1992. *Aportaciones metodológicas al conocimiento funcional y significado ecofisiológico de la actividad fosfatasa alcalina en macrófitos marinos y microorganismos acuáticos*. Ph. Thesis. Univ. Málaga.

Hernández, I., J.A. Fernández and F.X. Niell. – 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., J.A. Fernández and F.X. Niell. – 1995. A comparative study of alkaline phosphatase activity of two species of *Gelidium* (Gelidiales, Rhodophyta). *Eur. J. Phycol.*, 30: 69-77.

Hernández, I., S.-J. Hwang and R.T. Heath. – 1996. Measurements of phosphomonoesterase activity with a radiolabelled glucose-6-phosphate. Role in the phosphorus requirement of phytoplankton and bacterioplankton in a temperate mesotrophic lake. *Arch. Hydrobiol.*, (In press).

Hernández, I., F.X. Niell and J.A. Fernández. – 1992. Alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. *J. Exp. Mar. Biol. Ecol.*, 159: 1-13.

Hernández, I., J.L. Perez-Llorens, J.A. Fernández and F.X. Niell. – 1994. Alkaline phosphatase activity in *Zostera noltii* Hornem. and its contribution to the release of phosphate in the Palmones river estuary. *Estuar. Coast. Shelf Sci.* 39: 461-476.

Islam, M.R. and B.A. Whitton. – 1992. Phosphorus content and phosphatase activity of the deepwater rice-field cyanobacterium (blue-green alga) *Calothrix* D764. *Microbios*, 69: 7-16.

Jimenez, C. – 1985. *Adaptación fotosintética divergente en dos especies del género* Zostera *(L)*. M. Sci. Thesis, Univ. Málaga.

Kalle, K. – 1945. In J. P. Riley and G. Skirrow (eds.): *Chemical ,Oceanography, Volume II*, 1975. Academic Press, London.

Klotz, R.L. – 1985. Influence of light on the alkaline phosphatase activity of *Selenastrum capricornutum* (Chlorophyceae) in streams. *Can. J. Fish. Aquat. Sci.*, 42 : 384-388.

Kuenzler, E.J. and J.P. Perras. – 1965. Phosphatases of marine algae. *Biol. Bull.*, 128: 271-284.

Lapointe, B.E. and J. O'connell. – 1989. Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: Eutrophication of a confined, phosphorus-limited marine ecosystem. *Est. Coast. Shelf Sci.*, 28: 347-360.

Lapointe, B.E., M. M. Littler and D. S. Littler. – 1992. Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. *Estuaries*, 15: 75-82.

Lazdunski, M. – 1972. Flip-flop mechanisms and half-site enzymes. In: B. L. Horecker and E. R. Stadtman (eds.): *Current Topics in Cellular Regulation, Vol. VI*, pp. 267-309. Academic Press, Inc., New York.

Levitzky, A. and D.E. Koshland. – 1976. The role of negative cooperativity and half-of-the-sites reactivity in enzyme regulation. In: B.L. Horecker and E.R. Stadtman (eds.): *Current Topics in Cellular Regulation, Vol. X*, pp. 1-40. Academic Press, Inc., New York.

Lobban, C.S., P.J. Harrison and M. Duncan. – 1985. *The physiological ecology of seaweeds*. Cambridge University Press. London.

Lopez-Figueroa, F. – 1993. Photoregulation of Nitrogen metabolism and protein accumulation in the red alga *Corallina elongata* Ellis et Soland. *Z. Naturforsch.* 48: 788-794.

Mahasneh, I.A., S.L.J. Grainger and B.A. Whitton. – 1990. Influence of salinity on hair formation and phosphatase activities of the blue-green alga (Cyanobacterium) *Calothrix viguieri* D253. *Br. Phycol. J.*, 25: 25-32.

Murphy, J. and J.P. Riley. – 1962. Determination of phosphate in natural waters. *Anal. Chim. Acta*, 27: 31-36.

Palma, M.S., S.W. Han and A. Rossi. – 1989. Dissociation and catalytic activity of phosphate-repressible alkaline phosphatase from *Neurospora crassa*. *Phytochem.* 28: 3281-3284.

Pettersson, K. – 1979. Enzymatic determination of orthophosphate in natural waters. *Int. Revue. Ges. Hydrobiol.*, 64: 585-607.

Pettersson, K. and M. Jansson. – 1978. Determination of phosphatase activity in lake water: a study of methods. *Verh. Internat. Verein. Limnol.*, 29: 1226-1230.

Pick, F.R. – 1987. Interpretations of alkaline phosphatase activity in Lake Ontario. *Can. J. Fish. Aquat. Sci.*, 44: 2087-2094.

Price, N.C. and L. Stevens. – 1982. *Fundamentals of enzymology*. Oxford University Press, Oxford.

Reichardt, W., J. Overbeck and L. Steubing. – 1967. Free dissolved enzymes in lake waters. *Nature*, 216: 1345-1347.

Rivkin, R.B. and E. Swift. – 1980. Characterization of alkaline phosphatase and organic phosphorous utilization in the oceanic dinoflagellate *Pyrocystis noctiluca*. *Mar. Biol.*, 61: 1-8.

Siuda, W. – 1984. Phosphatases and their role in organic phosphorus transformation in natural waters. A review. *Pol. Arch. Hydrobiol.*, 31: 207-233.

Stewart, A.J. and R.G. Wetzel. – 1982. Phytoplankton contribution to alkaline phosphatase activity. *Arch. Hydrobiol.*, 93: 265-271.

Thingstad, T.F. and F. Rassoulzadegan. – 1995. Nutrient limitations, microbial food webs, and biological C-pumps. Suggested interactions in a P-limited Mediterranean. *Mar. Ecol. Prog. Ser.*, 117: 299-306.

Ullrich-Eberius, C.I. and Y. Yingchol. – 1974. Phosphate uptake and its pH-dependence in halophytic and glycophytic algae and higher plants. *Oecologia* 17: 17-26.

Van Boekel, W.H.M. and M.J.W. Veldhuis. – 1990. Regulation of alkaline phosphatase synthesis in *Phaeocystis* sp. *Mar. Ecol. Prog. Ser.*, 61: 281-289.

Vergara, J.J. and F.X. Niell. – 1993. Effects of nitrate availability and irradiance on internal nitrogen constituents in *Corallina elongata* (Rhodophyta). *J. Phycol.* 29: 285-293

Walther, C. and L. Fries. – 1976. Extracellular alkaline phosphatase in multicellular algae and their utilization of glycerophosphate. *Physiol. Plant.*, 36: 118-122.

Weich, R.G. and E. Granéli. – 1989. Extracellular alkaline phosphatase activity in *Ulva lactuca* L. *J. Exp. Mar. Biol. Ecol.*, 129: 33-44.

Whitton, B.A., M. Potts, J.W. Simon and S.L.J. Grainger. – 1990. Phosphatase activity of the blue-green alga (Cyanobacterium) *Nostoc commune* UTEX 584. *Phycologia*, 29: 139-145.

Wilson, I.B., J. Dayan and K. Cyr. – 1964. Some properties of alkaline phosphatase from *Escherichia coli*. *J. Biol. Chem.*, 239: 4182-4185.

Wolf, A.V., M.G. Brown and P.G. Prentiss. – 1982. Concentrative properties of aqueous solutions: conversion tables. In: R. C.

Weast (ed.): *Handbook of Chemistry and Physics* 63rd edition. CRC Press, Inc. Boca Ratón, Florida.
Wynne, D. – 1977. Alterations in activity of phosphatases during the *Peridinium* bloom in Lake Kinneret. *Physiol. Plant.*, 40: 219-224.
Wynne, D. and G.-Y. Rhee. – 1988. Changes in alkaline phosphatase activity and phosphate uptake in P-limited phytoplankton, induced by light intensity and spectral quality. *Hydrobiologia*, 160: 173-178.
Zar, J.H. – 1984. *Biostatistical analysis*. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Scient. ed.: E. Ballesteros