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Influence of phosphorus status on the seasonal variation of alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing

I. Hernández [a], J. A. Fernández [b] and F. X. Niell [c]

[a] *Department of Biology, Division of Ecology, Faculty of Marine Sciences, University of Cádiz, Cádiz, Spain;* [b] *Department of Plant Physiology,* [c] *Department of Ecology, Faculty of Sciences, University of Málaga, Campus Universitario de Teatinos, Málaga, Spain*

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Abstract: The influence of internal and external compartments of phosphorus on the alkaline phosphatase activity (APA) of *Porphyra umbilicalis* during the presence of its gametophytic phase in southern Spain was investigated. The compartments of internal soluble phosphorus were significantly correlated with the percentage cover of *Porphyra* on the sampling area. APA was inversely correlated with total internal phosphorus and particulate phosphorus. A dynamic model (resembling the cell quota model of Droop) was proposed to relate the enzymatic activity to the internal phosphorus content. According to this model, a maximum APA of 98.5 μmol *p*-nitrophenol (pNP) released g·dry $wt^{-1}\cdot h^{-1}$ and a minimum APA of 12.05 μmol pNP g·dry $wt^{-1}\cdot h^{-1}$ during the gametophytic cycle were estimated. Similarly, a maximum total phosphorus content of 4.2 mg P g·dry wt^{-1} and a theoretical minimum total phosphorus content of 0.51 mg P g·dry wt^{-1} throughout the gametophytic phase were estimated. The latter value might be close to the subsistence quota for phosphorus; the minimum quota for life.

Key words: Alkaline phosphatase activity; Cell quota; Internal phosphorus; *Porphyra umbilicalis*

Introduction

Available phosphorus in the water has been reported as the most important factor that controls alkaline phosphatase activity (Healey, 1973; Cembella et al., 1983). Although there are some exceptions (Stevens & Parr, 1977; Hino, 1988), a large number of studies on phytoplanktonic communities has revealed an inverse relationship between alkaline phosphatase activity (APA) and external concentrations of orthophosphate (Fitzgerald & Nelson, 1966; Smith & Kalff, 1981; Cembella et al., 1984). The inverse relationship has been found in cultures of cyanobacteria (Reichardt, 1971; Healey, 1973) and microalgae (Rhee, 1973; Keenan & Auer, 1979) and in many meso-eutrophic lakes (Berman, 1970; Heath & Cooke, 1975; Healey & Hendzel, 1980; Pettersson, 1980; Jansson et al., 1988). In all cases, low activities were associated with high external concentrations of orthophosphate, as phosphate inhibits alkaline phos-

Correspondence address: I. Hernádez, Department of Biology, Division of Ecology, Faculty of Marine Sciences, University of Cádiz, 11510 Puerto Real, Cádiz, Spain.

phatase activity (Berman, 1969; Davies & Smith, 1988). On the contrary, high levels of APA were observed when external phosphate was depleted.

Moreover, inverse relationships between APA and internal phosphorus content have been described in communities and phytoplanktonic species (Kuenzler & Perras, 1965; Taft et al., 1977; Healey & Hendzel, 1979; Pettersson, 1980; Huber & Kidby, 1985) as in the chlorophyceae *Scenedesmus* (Rhee, 1973), dinophyceae (Wynne, 1981; Elgavish et al., 1982) or diatoms (Møller et al., 1975). Specifically, Gage & Gorham, (1985) found an inverse relationship between APA and the internal content of particulate phosphorus. The phosphorus deficiency is generally revealed as an increase in APA (Kuenzler & Perras, 1965; Bone, 1971; Rhee, 1973; Wynne, 1981; Elgavish et al., 1982), due to a de novo synthesis of the enzyme (Garen & Levinthal, 1960; Lien & Knutsen, 1973).

In spite of all these works, the scarce previous references about APA in marine macroalgae (e.g. Lapointe & O'Connell, 1989) do not precisely identify the internal or external compartments of phosphorus that regulate APA. It is unknown if there are significant changes of the activity during the algal life cycle and the variables that control these changes, which have only been discussed partially for phytoplanktonic communities, specially in freshwater ecosystems.

In the marine environment, orthophosphate does not undergo fluctuations which are as great as in the eutrophic lakes. However, increasing evidence has been reported recently for phosphate being the limiting factor for the algal growth in a diversity of marine environments, particularly in coastal waters (Perry, 1976; Taft et al., 1977; Huber et al., 1985; Sakshaug & Olsen, 1986; Veldhuis et al., 1987; Wheeler & Björnsäter, 1992). This fact justifies the increasing concern for the study of APA in benthic macroalga from coastal and inshore waters (Lapointe & O'Connell, 1989; Weich & Granéli, 1989; Hernández et al., 1992)

The aim of this work was to determine the compartment of phosphate that controls alkaline phosphatase activity in a marine macroalga, the rhodophyta *Porphyra umbilicalis* (L) Kützing, during the gametophytic phase of its life cycle. Two reasons led us to select *Porphyra* for this work. (1) This species was studied in a previous project (Hernández et al., 1992), and it was observed that the enzyme was well adapted to the physical and chemical environment of the organism. (2) This species presented an ephemeral short seasonal presence, which was appropiate for a study of these characteristics.

Materials and Methods

SAMPLING AREA AND SAMPLING METHODS

The study was carried out from December 1991 to April 1992, the season when *P. umbilicalis* is present along the coast of southern Spain. This species presents a gametophytic, haploid phase (the leafy phase), during the cold season. Under particular

environmental conditions, at the end of this period, blades become sexual, producing male gametes which fertilize females on blades. The resulting zygotospores are then liberated (Guiry, 1990). These spores develop into filaments known as the Conchocelis phase (Drew, 1956).

Porphyra was collected in Lagos (Málaga, southern Spain) from the supralittoral zone of flat rocks. An area of 1.5 m long × 0.5 m wide was selected, from which plants were collected throughout the gametophytic phase. Seawater and rock temperature were directly measured in situ. Percentage cover of the sampling area by *Porphyra* was determined by a modification of the linear transect method (Hernández et al., 1993). The cover of the sampling area began in the middle of December, reaching maximum percentage cover (80%) in the first week of February. From this week the percentage cover decreased, with the complete disappearance of the gametophytic form in the middle of March (Hernández, 1992; Hernández et al., 1993). Carpospores were observed in the last 2 weeks of the gametophytic cycle.

Samples of seawater and blades were collected weekly and always at the same hour (11.30 to 12.00, solar time). Whole plants of similar size were carefully pulled up from the holdfast. Immediately after collection, three replicates of blades were rinsed in filtered seawater (0.2 μm) and used for the determination of APA. Other plants were collected and wrapped in aluminium foil. These plants and the samples of seawater were immersed in liquid nitrogen and transported to the laboratory. In each sampling, the plants were examined by light microscopy in search of epiphytes, but in no case were they observed.

ASSAY OF ALKALINE PHOSPHATASE ACTIVITY

Immediately after collection, APA was determined in triplicate using the method of Reichardt et al. (1967), as modified by Hernández et al. (1992). This method uses *p*-nitrophenyl phosphate (pNPP) as a dissolved organic substrate. The assay medium contained 50 ml of 10^{-3} M pNPP mixed with an equal volume of 0.1 M Tris-HCl buffer, pH 8.3. Both reagents were dissolved in filtered (0.2 μm) artificial seawater of 35% salinity (Kalle, 1945). During the APA assays, plants were incubated in transparent plastic bottles at seawater temperature. The bottles were placed in a net and immersed slightly in the seawater to stir the medium and keep the temperature constant. A blank (assay medium without plant) was also incubated. After 45 min of incubation an aliquot of the assay medium was placed into capped plastic test tubes and immersed in liquid nitrogen. Once in the laboratory, the samples were quickly defrosted and the absorbance read at 410 nm in a Beckman DU-7 Spectrophotometer. Enzymatic activity was expressed as μmol of paranitrophenol (pNP) released g·dry wt^{-1}·h^{-1}. For a better estimation of the real APA in nature, the values of APA were expressed at the temperature of the sampling rock. For that purpose, the enzymatic activity was corrected for temperature with a Q_{10} value of APA (1.78) for *P. umbilicalis*, calculated by Hernández et al. (1992) at the same study site. The enzymatic activities

were not absolute, since they depended on the concentration of substrate. The initial pNPP concentration was close to the one that produced the maximum reaction velocity (Hernández et al., 1992), which allowed to using the activities comparatively (Mc Comb et al., 1992).

DETERMINATION OF PHOSPHORUS COMPOUNDS

Internal phosphorus fractions

The internal phosphorus fractions were determined in triplicate by the molybdate ascorbic acid method (Murphy & Riley, 1962) as modified by the USEPA (1971) in the manner outlined in Fig. 1. Plants were defrosted and dried (24 h, 70 °C). Then the material was ground and homogenized. Internal soluble reactive phosphorus (SRP) was measured dissolving the dry plants in 40 ml of water. The mixture was filtered through a Whatman GF-C filter and the content of phosphorus in the filtrate determined. Internal total soluble phosphorus (TSP) was quantified after acid digestion

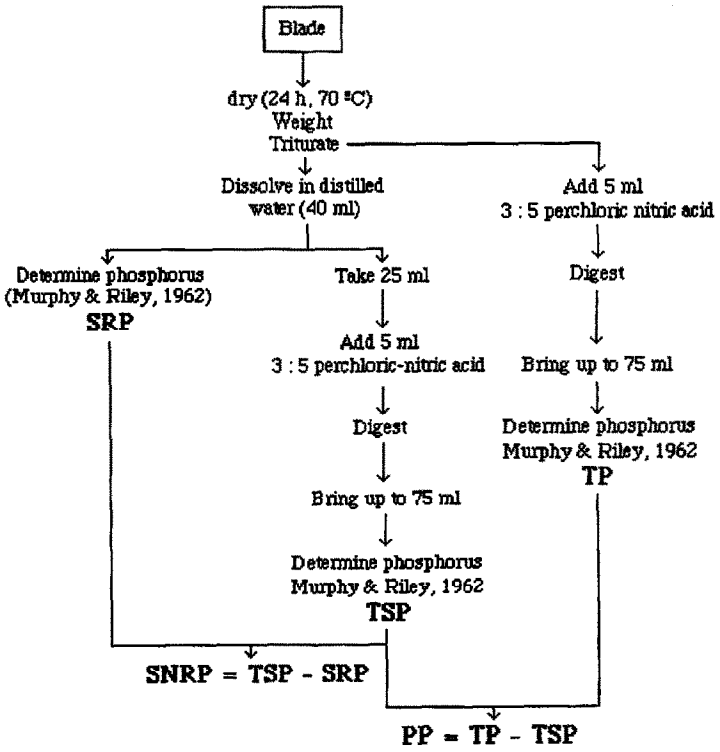

Fig. 1. Diagram of the procedures followed for the determination of the internal compartments of phosphorus. SRP = soluble reactive phosphorus; SNRP = soluble non-reactive phosphorus; TSP = total soluble phosphorus; TP = total phosphorus; PP = particulate phosphorus.

(Sommer & Nelson, 1972). 25 ml of the filtrate were digested with 5 ml of a mixture of perchloric-nitric acid (3:5 v/v). A standard dilution series of phosphate was also digested. The internal soluble non-reactive phosphorus (SNRP) was determined as the difference between TSP and SRP. Total internal phosphorus (TP) was also measured by an acid digestion. 5 ml of the perchloric-nitric mixture was added to 100 mg of ground dry plants. Particulate phosphorus (PP) was quantified as the difference between TP and TSP. In all cases, phosphorus compounds were expressed as mg P g·dry wt^{-1} of *P. umbilicalis*.

External phosphorus fractions

External soluble reactive phosphorus (ESRP) was determined by the Malachite green method (Fernández et al., 1985). This method showed more sensitivity than the Murphy & Riley (1962) in cases of low concentration of ESRP (Fernández et al., 1985). Phosphomonoesters (PME) were detected as the increase in SRP following incubation with calf intestinal mucosa alkaline phosphatase (Francko & Heath, 1979). Concentration was measured by comparison with a standard dilution series of glucose-6-phosphate.

STATISTICAL ANALYSES

Differences among means were tested by a single factor analysis of variance (ANOVA), at the 5% level of significance. Multiple post hoc comparisons were performed by the Tukey-Kramer test (Zar, 1984). Simple linear correlations among variables were tested by the simple correlation coefficient of Pearson (Pearson, 1920). The null hypothesis (absence of correlation) was rejected at the 5% level of significance.

RESULTS

The concentration of internal soluble phosphorus compounds in *Porphyra* during the gametophytic phase is shown in Fig. 2. The variation of SRP content (maximum value of 0.71, on February 7, 1992, and minimum of 0.27 mg P g·dry wt^{-1}, on December 24, 1991) and the internal TSP (maximum value of 1.5 mg P g·dry wt^{-1}, on February 4, 1992, and minimum of 0.60 mg P g·dry wt^{-1}, on December 24, 1991) were significant seasonally ($p = 0.005$). In both cases, the internal phosphorus content was maximum at the time of maximum cover (first week of February; Hernandez, 1992; Hernández et al., 1993), and minimum during the initial and final days of the presence of *Porphyra* on the sampling area. Both SRP and TSP were directly correlated with the percentage of the sampling area covered by the macroalga (Table I). As a consequence, the pattern of the internal SNRP was similar to the former compartments. The SNRP values varied between 0.32 mg P g·dry wt^{-1} (on December 24, 1991) and 0.96 mg P g·dry wt^{-1} (on February 4, 1992) and were also correlated with the

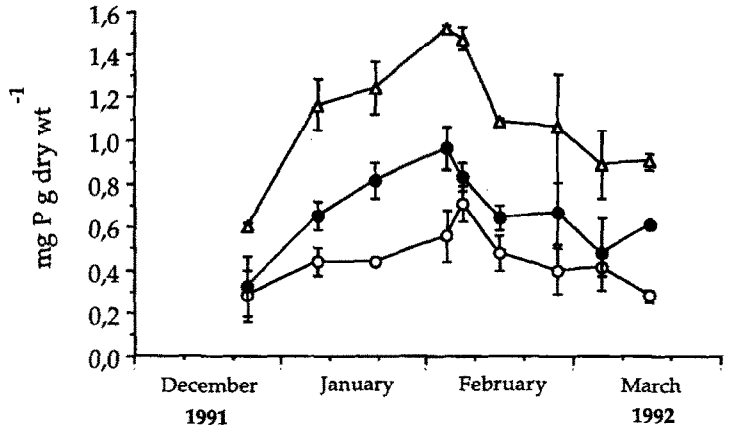

Fig. 2. Variation of the internal soluble fractions of phosphorus during the gametophytic phase of *P. umbilicalis*. ○, Soluble reactive phosphorus; ●, soluble non-reactive phosphorus; △, total soluble phosphorus. Phosphorus expressed in mg P g·dry wt^{-1}. Values are mean of three replicates and bars denote SD. Data from December 19, 1991 were lost.

percentage cover (Table I). The percentage of each internal soluble fraction with regard to total phosphorus is shown in table II.

TP, however, showed a different pattern (Fig. 3). Maximum TP content occurred at the beginning of the gametophytic phase (4.03 mg P g·dry wt^{-1}; on December 19, 1991), and fell drastically at the end of the presence of the blades on the sampling area. The internal TP varied significantly during the sampling period ($p = 0.001$). Although one sample for the PP determination was lost, the rest of the data showed a similar pat-

TABLE I

Correlation between external and internal variables measured during the gametophytic phase of *P. umbilicalis*. % COV = percentage cover of the macroalga on the sampling area. SRP = internal soluble reactive phosphorus. SNRP = internal soluble non-reactive phosphorus. TSP = total internal soluble phosphorus. TP = total internal phosphorus. PP = internal particulate phosphorus. ESRP = external soluble reactive phosphorus. PME = phosphomonoesters. APA = alkaline phosphatase activity. * = Significant at the 5% level. ** = Significant at the 1% level.

	% COV	SRP	SNRP	TSP	TP	PP	ESRP	PME	APA
% COV	1.0	0.90**	0.74**	0.88**	0.45	−0.07	−0.20	0.16	−0.007
SRP		1.0	0.46*	0.78**	0.37	−0.14	−0.50	−0.16	−0.33
NSRP			1.0	0.92**	0.02	−0.50	−0.5	−0.37	0.08
TSP				1.0	0.15	−0.41	−0.46	−0.02	−0.03
TP					1.0	0.84**	−0.06	−0.30	−0.84**
PP						1.0	0.19	−0.26	−0.76**
ESRP							1.0	0.25	0.38
PME								1.0	0.11
APA									1.0

TABLE II

Percentages of the internal fractions of phosphorus with regard to the total internal phosphorus during the gametophytic phase of *P. umbilicalis*. SRP = soluble reactive phosphorus. SNRP = soluble nonreactive phosphorus. TSP = total soluble phosphorus. PP = particulate phosphorus.

Date	SRP	SNRP	TSP	PP
24.12.91	9.0	10.4	19.4	80.6
8.1.92	18.5	30.7	49.2	50.8
20.1.92	17.3	32.7	50.0	50.0
4.2.92	19.2	33.2	52.4	47.6
7.2.92	26.7	29.1	55.8	44.2
14.2.92	15.9	20.0	35.9	64.1
25.2.92	17.2	28.7	45.9	54.1
6.3.92	18.2	21.0	39.2	60.8
16.3.92	18.7	41.9	60.0	39.4
Mean	17.8	27.5	45.4	54.6

tern for PP and TP was observed (Fig. 3). The PP content fluctuated between 0.59 mg P g·dry wt^{-1} (on March 16, 1992) and 2.5 mg P g·dry wt^{-1} (on December 24, 1991) and represented the maximum percentage of the total internal phosphorus throughout the sampling period (mean 55%, Table II).

Figure 4 shows the alkaline phosphatase activity of *P. umbilicalis* throughout the gametophytic phase. APA changed significantly ($p = 0.001$) during the sampling period. Maximum activities were significantly different to the minimum. As is shown in the figure, the minimum activity (12.4 μmol pNP g·dry wt^{-1} h^{-1}; on December 19, 1991) occurred at the beginning of the presence of Porphyra on the sampling area. There was

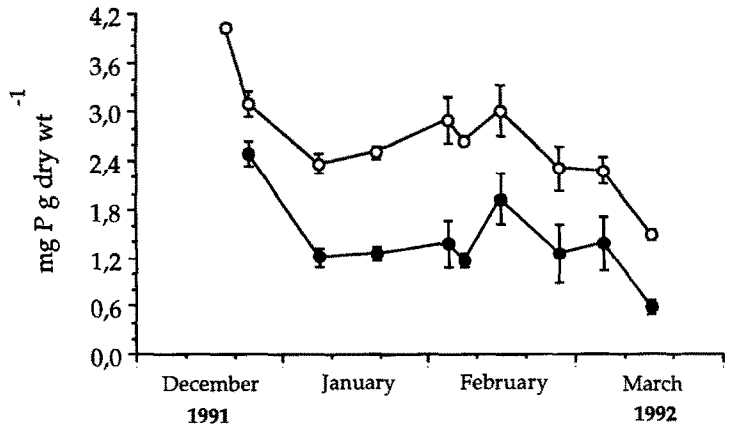

Fig. 3. Internal particulate phosphorus content (●) and total phosphorus content (○) during the gametophytic phase of *P. umbilicalis*. Phosphorus expressed in mg P g·dry wt^{-1}. Values are mean of three replicates and bars denote SD.

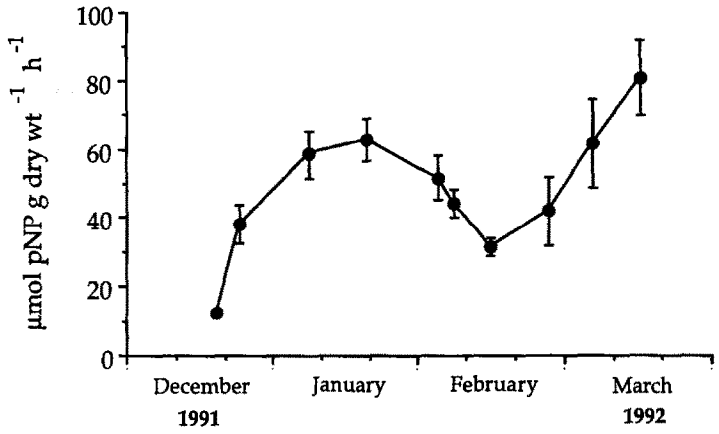

Fig. 4. Alkaline phosphatase activity during the gametophytic phase of *P. umbilicalis*. The activity was refered to the temperature on the sampling rock in each sampling day. Enzymatic activity expressed as µmol pNP g·dry wt $^{-1}$·h $^{-1}$. Values are mean of three replicates and bars denote SD.

an increase at the end of the cycle, when the maximum APA was measured (81.1 µmol pNP g·dry wt $^{-1}$·h $^{-1}$; on January 20, 1992). APA was inversely correlated with the internal TP (Table I). Moreover, as the main percentage of the total internal phosphorus was in the internal PP compartment (Table II), an inverse correlation between internal PP and APA was found (Table I).

The linear correlations shown in Table I were calculated as a previous step with the aim of identifying what compartment of phosphorus regulated APA. However, these correlations, a priori, did not presuppose the final model. Once the influence of TP as the main regulator of APA was proved, this regulation was explained in a broader conceptual frame.

Considering TP as the cell quota (Q) for phosphorus – according to the Droop definition (1968); the weight of internal nutrient per unit biomass – a relationship between APA and the inverse of the cell quota (C) was found. Figure 5 shows the variation of APA as a function of the inverse of the cell quota for phosphorus along the presence of the gametophytic phase of the macroalga. *P. umbilicalis* exhibited the minimum APA when the cell quota was maximum. This APA value was considered to be constitutive (i.e. the APA which is independent of the P status of the cells). Maximum APA values were found when the cell quota was minimum. In order to compute the values of cell quota and APA, data were fitted to a non-linear saturation model [that resembles the cell quota model proposed by Droop (1983)], in which APA depends on the nutrient status for phosphorus:

$$APA = APA_c + APA_{max}\,[1 - (C_m/C)] \quad (1)$$

Where "APA_c" and "APA_{max}" are the minimum and maximum alkaline phosphatase activity, respectively; "C" is the inverse of the cell quota for phosphorus and C_m is the

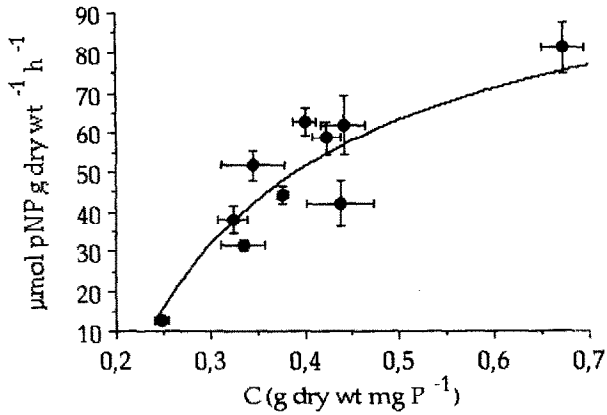

Fig. 5. Alkaline phosphatase activity versus "*C*", the inverse of the cell quote for phosphorus. The values were fitted to a non linear saturation model, whose equation is showed in the text. Enzymatic activity expressed as µmol pNP g·dry wt^{-1}·h^{-1} and cell quota as mg P g·dry wt^{-1}. Values are mean of three replications and bars denote SE.

minimum value of the inverse of the cell quota for phosphorus. Thus, the parameters obtained from the fitting were 12.05 µmol pNP g·dry wt^{-1}·h^{-1} (SE = 6.65) for APAc, 98.5 µmol pNP g·dry wt^{-1}·h^{-1} (SE = 10.35) for APAmax and 0.24 (SE = 0.04) for C_m; that is, the maximum cell quota estimated was 4.2 mg P g·dry wt^{-1}. According to the model, the major variable affecting APA in *Porphyra* throughout the gametophytic phase was the cell status with regard to phosphorus. Such variable determined a seasonal variation of more than 75% in APA.

As it is shown in Eqn. (1), maximum values of APA occurred at the time of maximum C_m:*C* ratio. With the computed figure for APA_{max}, a hypothetic value of maximum *C* can be estimated. Its inverse is the minimum cell quota during the cycle (0.51 mg P g·dry wt^{-1}). This value might be close to the subsistence quota (K_q) for phosphorus; i. e. the minimum quota necessary for life (Droop, 1983).

The external fractions of phosphorus determined in this work are shown in Fig. 6. ESRP was usually low during the sampling period (maximum 0.65 µM). Phosphomonoesters varied from 0 to 0.88 µM and were generally lower than ESRP concentration. APA did not correlate with either ESRP or PME.

Discussion

A great number of works have pointed out the existence of an inverse relationship between phytoplanktonic APA and external concentrations of phosphate. This relationship is based on an increase of the biosynthesis of alkaline phosphatase when the external concentration is lower than a critical value. The magnitude of the response

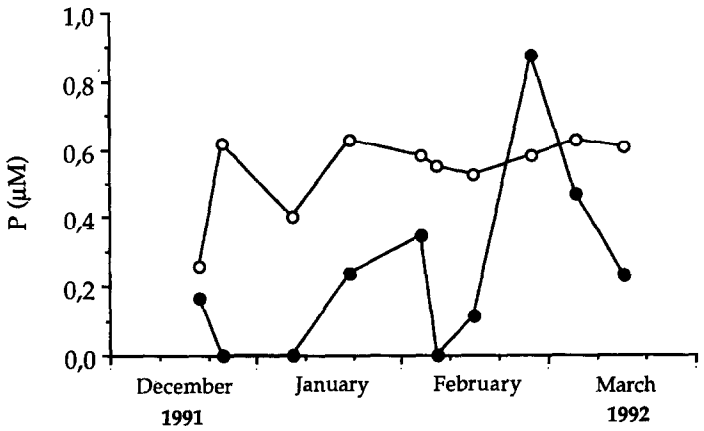

Fig. 6. External fractions of phosphorus during the presence of the gametophytic phase of *P. umbilicalis* on the sampling area. ○, Soluble reactive phosphorus; ●, phosphomonoesters. Values are mean of three replicates and are expressed in μM.

depends on the species, PME availability and degree of limitation of the cells (Cembella et al., 1983). Inversely, there is an inhibition of the activity when phosphate concentration is high (Kuenzler & Perras, 1965; Fitzgerald & Nelson, 1966; Reichardt et al., 1967; Healey, 1973; Pettersson, 1980; Davies & Smith, 1988).

However, as several researchers have pointed out (Healey, 1973; Inhlenfeld & Gibson, 1975; Weich & Granéli, 1989) an increase in the external phosphate leads to an increase in the internal content of phosphorus. As a result, APA reflects the internal content of phosphorus in phytoplanktonic organisms and benthic macroalgae.

In this work, a significant correlation between APA and internal TP was found. An inverse relationship between internal phosphorus and APA has been reported previously (Rhee, 1973; Møller et al., 1975; Fitzgerald & Nelson, 1966; Taft et al., 1977; Wynne, 1981; Elgavish et al., 1982; Huber & Kidby, 1985). These relationships have been usually described without identifying the internal compartment that controls the activity. As an exception, Gage & Gorham (1985) found an inverse correlation between APA and internal PP, whereas Elgavish et al. (1982) demonstrated that APA was inversely correlated with the quantity of storage of polyphosphates in *Peridinium, Cosmarium* and *Pediastrum*.

The inverse correlation between APA and TP found in *Porphyra* raises the question whether the total internal phosphorus regulates the activity or if there is a specific compartment responsible for the observed changes in APA. It was found that the profiles of internal soluble phosphorus were well correlated with the percentage cover of the rock surface and might reflect the nutritional status of the population. However, an inverse linear correlation between PP and APA was observed. According to Gage & Gorham (1985), the particulate phosphorus is the internal compartment that controls APA. They found an exponential inverse relationship between these variables. It

could thus be concluded that this compartment is the main regulator of the activity. However this is not surprising, taking into account the fact that PP constitutes the major percentage of total internal phosphorus (Table II).

The Droop model (Droop, 1983), established the concept of cell quota. The cell quota model has been successfully applied for phosphorus (as the limiting nutrient) in phytoplanktonic populations (Jones et al., 1978; Droop, 1983). We used this concept to formulate a new model to explain the relationship between APA and TP.

The model describing the relationship between APA and C; the inverse of the cell quota for phosphorus [Eqn. (1)], in contrast with the linear correlation, predicted a maximum value of APA when $1/Q$ was maximum. Similarly, the minimum APA occurred when $1/Q$ was minimum. Thus, the theoretical minimum value of TP (0.51 mg P g·dry wt^{-1}) may be calculated from the maximum APA estimated throughout the sampling period. This value of TP might be close to the subsistence quota for phosphorus (K_q). According to the Droop (1983) definition of K_q as the minimum quota necessary for life, the minimum value of TP estimated throughout the cycle: (1) occurs at the end of the gametophytic phase of *Porphyra*, when blades are disappearing; i. e. the plants are dying (Fig. 3); (2) it is related to the SNRP fraction, as the decrease in TP content between two successive sampling days corresponded to an increase in the proportion of SNRP in the tissue content (Fig. 7A) and a decrease in the proportion of PP in the tissue content (Fig. 7B). Rhee (1973) showed that in *Scenedesmus* P-limited cells, the intracellular growth-rate limiting pool (K_q) was related to polyphosphate (a SNRP constituent).

On the other hand, the minimum value estimated for $1/Q$ (0.24 mg P g·dry wt^{-1}) allowed the estimation of a maximum cell quota of 4.2 mg P g·dry wt^{-1}. This is in the range of TP values found by Wheeler & Björnsäter (1992) for *Porphyra* sp. over a complete seasonal cycle in the Pacific northwest coast (minimum 3.7 mg P g·dry wt^{-1}, maximum 8.6 mg P g·dry wt^{-1} throughout the cycle).

Therefore, the model proposed in this work accurately formulates the inverse relationship observed between APA and internal phosphorus. In a dynamic approach, the model considers the nutritional status, in terms of phosphorus, as the main regulator of APA.

APA varied significantly during the gametophytic phase of *Porphyra*, as shown in Fig. 4. These changes have been related to the fact that the growth of *Porphyra* is limited by P, according to the seasonal variation of the internal C:N:P ratio (Hernández et al., 1993). However, the significant increase of APA at the end of the seasonal cycle of the gametophytic form may be linked to the development of the carpospores. At this time, blades produced carpogonia and spermatangia. The fertilized carpogonia divide into carpospores which germinate to become the Conchocelis phase. The final increase in APA could provide phosphate to the carpospores at the moment of their germination. Berman (1970) pointed out that an increase in APA parallel with a decrease in internal phosphorus during the final stage of an algal population implied the release of a high quantity of phosphate to the extracellular environment. The increase in APA at

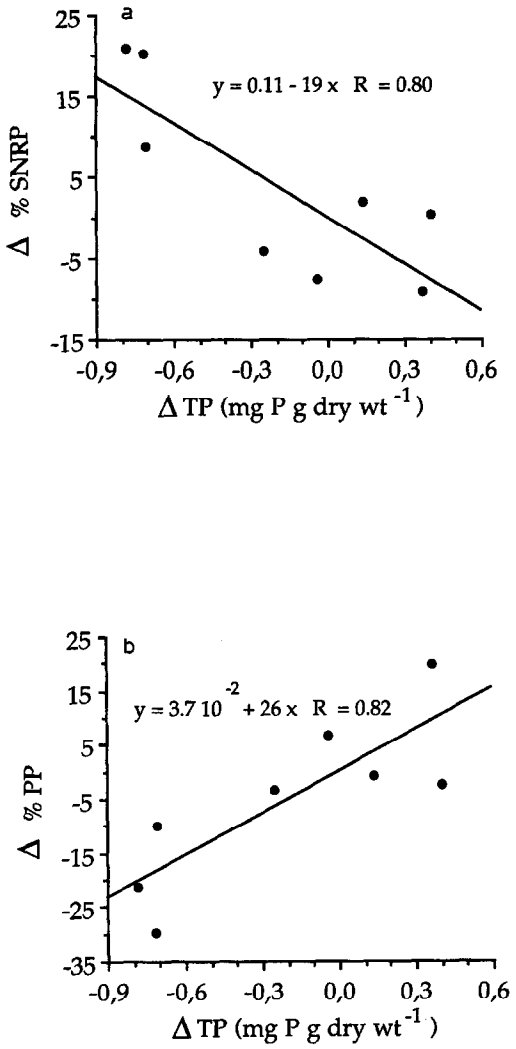

Fig. 7. (a). Correlation between the changes in TP content between two successive sampling days and the changes in the percentage of SNRP in the tissue content. (b) Correlation between the changes in TP content between two successive sampling days and the changes in the proportion of PP in the tissue content. Both correlations were significant ($p < 0.02$). TP = total internal phosphorus; SNRP = internal soluble non reactive phosphorus; PP = internal particulate phosphorus.

the end of a phytoplanktonic bloom has been linked to the ageing of the cells (Grusky & Aaronson, 1969; Wynne, 1977). Wynne (1981) observed an increase in APA at the end of a bloom of *Peridinium*, just when the population tended to undergo senescence with the formation of cysts. Similarly, APA has been implicated in the encystation of *Acanthamoeba castellanii* (Martin & Byers, 1976).

The absence of correlation between ESRP and APA (Table I), is explained by the absence of large changes in ESRP during the sampling period. In the same way, no big variations in the potentially available substrates of the enzyme (PME) were observed. On some days, the PME concentration was higher than the ESRP (Fig. 6), which shows the potential importance of PME as a source of orthophosphate. The same result was observed by De Jonge & Postma (1974) and Whitte & Paine (1980). However, a correlation between APA and PME was not found. The absence of correlation between PME and APA might be due to the low PME concentration, which makes the precise measurement of PME difficult. Furthermore, the concentration of both fractions of external phosphorus (determined in samples taken directly from the seawater) may be different in the water in contact with the algae, as in some days (mainly due to the wind direction), *Porphyra* received occasional seawater splashes. Thus, the PME and ESRP determined in the seawater may sometimes have been different from the layer of seawater around the plant. This fact makes more difficult to establish a reliable relationship between external fractions of phosphorus and APA like those described for eutrophic lakes (Heath & Cooke, 1975; Boavida & Heath, 1988). Finally, it should be taken into account that the affinities of *Porphyra* alkaline phosphatase and calf intestinal mucose alkaline phosphatase for the PME may be different (Wetzel, pers. comm.). In future studies, we will try to purify the *Porphyra* alkaline phosphatase in order to check this.

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