ELSEVIER

Journal of Experimental Marine Biology and Ecology, 241 (1999) 1–14

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

Testing the allometric scaling of alkaline phosphatase activity to surface/volume ratio in benthic marine macrophytes

Ignacio Hernández[a,*], Jesús R. Andría[a], Martin Christmas[b], Brian A. Whitton[b]

[a]*Departamento de Ecología, Universidad de Cádiz, Facultad de Ciencias del Mar, 11510 Puerto Real, Cádiz, Spain*

[b]*Department of Biological Sciences, University of Durham, Durham DH1 3LE, UK*

Received 29 September 1998; received in revised form 11 March 1999; accepted 26 April 1999

Abstract

Alkaline phosphatase activity (APA), tissue P, N and C were determined for 23 species of benthic marine macrophytes (22 macroalgae, 1 seagrass). In addition, estimates of the surface area/volume ratio (SA:V) of these species were derived from experimental work and from the literature. The tissue nutrient content (especially P) does not explain differences in APA among the species of marine macrophytes. On the contrary, a regression analysis suggested that size-specific variation in APA can be attributed in part to changes in relative surface area among benthic marine macrophytes. The slope of the logarithmically transformed equation relating APA and SA:V was 0.48 (SE = 0.16). The slope increased to 0.65 (SE = 0.09) when three ''anomalous'' species, showing high SA:V ratio but negligible APA were not taken into account. The relationship between APA and SA:V suggests an allometric scaling of APA in benthic marine macrophytes. Along with other indicators, the equation might help to indicate to what extent a population of benthic marine macrophytes is limited by phosphorus. The results agree with previous reports on allometric relationships between nutrient uptake (N and P) and SA:V within narrow size-ranges in macroalgae. They also support the hypothesis that there is a general coupling between ecophysiological and morphological properties in aquatic photosynthetic organisms.

Keywords: Alkaline phosphatase; Allometry; Marine macrophytes; Phosphorus

*Corresponding author. Tel.: +34-956-470-835; fax: +34-956-470-811.
E-mail address: ignacio.hernandez@uca.es (I. Hernández)

0022-0981/99/$ – see front matter
PII: S0022-0981(99)00070-2

1. Introduction

Size and morphology are essential factors of the ecology of macroalgae (Littler and Littler, 1980; Dudgeon et al., 1995). Photosynthesis, nutrient uptake and growth in marine macrophytes are all known to be strongly size-dependent (Agustí et al., 1994; Hein et al., 1995; Nielsen et al., 1996). Algae with a higher surface area:volume ratio (SA:V) usually achieve higher rates of photosynthesis, have higher specific growth rates and a faster transport of nutrient uptake per unit biomass. The SA:V may be of great interest in ecophysiological studies, as it relates the exchange potential (energy-light absorption- and matter-nutrient assimilation-) of a given photosynthetic organism to the biomass present (Sournia, 1982).

Phosphorus is an essential nutrient for macroalgal metabolism. It plays a significant role in most cellular processes, especially those involved in generating and transforming metabolic energy. Many studies in coastal zones from different water masses suggest that this nutrient may limit macroalgal primary production, at least during some part of the year (Wheeler and Björnsäter, 1992; Delgado et al., 1994; Flores-Moya et al., 1997; Hernández et al., 1997; Lapointe, 1997).

Macroalgae obtain much of their phosphorus from the aquatic environment as dissolved orthophosphate (Pi) or from phosphomonoesters (PME), the latter forming a variable fraction of the dissolved organic phosphorus pool (Cembella et al., 1984). Phosphate from this fraction is available after enzymatic hydrolysis by a phosphatase. The optimum pH of the enzyme in benthic marine macrophytes is in the alkaline range (Hernández et al., 1999); therefore the enzyme is termed an alkaline phosphatase. Due to the possibility of using this source of phosphate, the enzyme plays an essential role in the phosphorus supply during Pi depletion. An inverse relationship between ‘‘surface’’ alkaline phosphatase activity (APA) and internal phosphorus content has been shown for a number of algae, including the intertidal *Porphyra umbilicalis* (Hernández et al., 1993). High phosphatase activity has therefore been considered as an indication of phosphorus limitation of productivity (Lapointe, 1989).

Previous studies have suggested that phosphate uptake is correlated with size or the calculated SA:V ratio in both benthic macroalgae (Odum et al., 1958) and microalgae (Smith and Kalff, 1982; Wen et al., 1997). Changes in SA:V ratio influence the exchange of solutes across the macroalgae surface, as uptake is controlled in part by the surface area available for nutrient uptake. For instance, Pi is taken up by active transport, presumably by ATP-mediated Pi permeases at the plasmalemma (Cembella et al., 1984).

Studies have shown that APA varies markedly among marine macrophytes (e.g. Lapointe et al., 1992; Hernández et al., 1994). The enzymatic activity at the species level is influenced by multiple factors, either biological or environmental (reviewed by Hernández et al., 1999). Among these, internal phosphorus is generally considered a key factor controlling APA; however, the few studies where APA and tissue P have been measured in macroalgae (e.g. Lapointe et al., 1992) show no relationship between the two variables among different species. In addition, in some previous studies, high APA values have been recorded in leafy or branched species with a high SA:V ratio (Hernández et al., 1994). As far as we are aware, the study of the influence of relative size on the differences in APA among marine macroalgae has not been attempted.

The aim of the present study was to test if variations in APA among benthic marine macrophytes (mostly macroalgae) of different morphologies, growth strategies and life forms can be attributed in part to variations in SA:V. We tested the hypothesis of allometric scaling of APA to SA:V ratio, as different studies suggest that the enzyme is located at the external part of the algae (Flynn et al., 1986; Hernández et al., 1994; González-Gil et al., 1998); thus we expected that species with high SA:V ratios would have a selective competitive advantage for PME enzymatic hydrolysis.

2. Material and methods

2.1. Field sampling

Fronds of different species of benthic marine macrophyte (22 macroalgae and a seagrass) with differing growth strategies and morphologies were collected from the upper and mid-littoral zone at two sites: Tyne Sands (southeast Scotland) in June and September 1996, and El Chato (Cádiz, southern Spain) in November 1996. The following were selected from Tyne Sands: *Cladophora rupestris* (L.) Kütz., *Ulva rigida* C. Agardh, *Enteromorpha intestinalis* (L.) J. Agardh, *Ralfsia verrucosa* (Aresch.) J. Agardh, *Fucus spiralis* L., *Pelvetia canaliculata* Dcne. et Thur., *Porphyra* sp., *Dumontia contorta* (S. G. Gmel.) Ruprecht, *Chondrus crispus* Stackh., *Ceramium nodulosum* (Lightfoot) Ducluzeau, *Polysiphonia fucoides* (Huds.) Grev., and *Zostera marina* (L.). Two other non wholly identified macroalgae from Tyne Sands were included, a hairy, branched green algae (possibly a *Cladophora* species) and a finely branched red algae (possibly a *Polysiphonia* species) while *Codium fragile* (Sur.) Hariot, *Dictyota dichotoma* (Huds.) Lamour., *Halopteris scoparia* (L.) Sauv., *Cladostephus spongiosus* (Huds.) C. Agardh, *Cystoseira tamariscifolia* (Huds.) Papenf., *Chondria coerulescens* J. Agardh, *Pterocladia capillacea* (S.G. Gmel.) Born. ex Born. et Thur., *Halarachnion ligulatum* (Woodw.) Kütz., and *Sphaerococcus coronopifolius* Stackh. were collected from El Chato. *H. ligulatum* grows subtidally but many detached healthy plants were found in the intertidal.

The plants were rinsed of sediments and debris and immediately transferred to the laboratory in an ice chest. After careful removal of any visible epiphytes with a forceps, the plants were kept (12 h maximum) in aerated culture at 18°C under continuous light (100 μmol photon m^{-2} s^{-1}) in filtered (Whatman GF-F) seawater from the sample site.

2.2. Alkaline phosphatase analysis

The APA was assayed in 3–4 replicate samples using the artificial substrate p-nitrophenyl phosphate (pNPP). This compound is cleaved by enzymatic hydrolysis rendering the coloured compound p-nytrophenol (pNP) and Pi. The procedure followed a modification of the method proposed by Reichardt et al. (1967) and adapted for marine macrophytes (Hernández and Whitton, 1996). The assay medium consisted of 50 mL of 500 μM pNPP (which enables maximum velocity of the enzymatic reaction) and 500

μM HEPPS-NaOH buffer, pH 8.3, dissolved in P-free artificial seawater of 33‰ salinity (Woelkerling et al., 1983). The samples were incubated for 1 h. with constant gentle shaking under standard conditions of temperature (20°C). After the incubation, the absorbance of the assay medium was read at 410 nm. A control of the absorbance (assay medium without plant incubated under the same assay conditions) was subtracted from each measurement. Enzymatic activity was expressed as μmol pNP released g dry weight^{-1} h^{-1}.

2.3. Tissue nutrient analysis

Tissue P was measured in samples of ground tissue by the persulfate digestion method (APHA, 1992). Other subsamples of plant tissue were used to determine C and N concentration using a CNHS elemental analyzer (Carlo Erba CA 1108).

2.4. Estimation of surface area/volume ratios

Plant SA:V ratio data (expressed as cm^2 cm^{-3}) were obtained from three different sources. When available, we used a large data-set of values already published (Hein et al., 1995). The surface of other species from Tyne Sands was determined using a scanner and related computer software (Delta Scan©). In still other species from El Chato, surface values were estimated from a calibration curve using portion of thalli of known surface (thalli approximated to geometric figures as circle or cylinder) and dyed with malachite green. The thalli were placed for 2 min in 50 ml of seawater and the absorbance of malachite green was read at 660 nm. The plant volumes were estimated from densities. Plant densities were estimated by the water displacement of a portion of thallus of known fresh weight in a thin graduated cylinder. Then, the density value was used to calculate the volume of plants of known surface (estimated from the calibration curve). Therefore, we did not used the same portion of thallus to calculate surface and volume.

2.5. Biostatistical analyses

The relationship between APA and SA:V was evaluated by least-squares linear regression (model I). Both data were logarithmically transformed to obtain a better homogeneity of variances (homoscedasticity) (Draper and Smith, 1966). Although geometric mean linear regression may be recommended when the two variates are subject to error of measurement (e.g., Nielsen and Sand-Jensen, 1990), model I has also been used if the average variance in the estimation of the independent variable is small (Wen et al., 1997). The use of any of these two approaches is still a matter of controversy and definitive recommendations are difficult to make (Sokal and Rohlf, 1981). In any case, the slopes by these two regression analysis are rather similar and do not affect the conclusions of our work. Differences between two means were tested by a two-tailed Student's *t*-test. Correlations between variables were tested by the Pearson correlation coefficient (Zar, 1984). Comparison between the slope of the linear

regression of APA vs. SA:V and a hypothesized value of 0.75 was tested using Student's *t* statistic. In all cases the significance was set at 0.05 probability.

3. Results

The values of APA, SA:V, P and N:P ratio of the species used in the assays are listed in Table 1. Data from any species that was collected and assayed twice, either from different locations on the shore (*C. crispus*) or in the two sampling days at Tyne Sands (*C. rupestris, E. intestinalis, R. verrucosa, F. spiralis, C. crispus*, and *Z. noltii*) were pooled, as differences between means evidenced discrepant results. The pooled APA data from Tyne Sands or El Chato evidenced no significant differences between sampling sites ($P > 0.5$). Table 1 shows that mean APA ranged from less than 0.3 μmol pNP g dry wt^{-1} h^{-1} in *U. rigida* to 44 μmol pNP g dry wt^{-1} h^{-1} in *P. fucoides*. In some species (*U. rigida, Porphyra* sp., *H. ligulatum*) or occasions (*E. intestinalis* in June), the low activity may be regarded as negligible.

The APA of benthic marine macroalgae was usually well correlated with their tissue P,

Table 1
Alkaline phosphatase activity (APA; μmol pNP g dry weight^{-1} h^{-1}), surface area/volume ratio (SA:V; cm^{-1}), tissue P (mg g dry weight^{-1}) and N:P atomic ratio of the benthic marine plants assayed[a]

Species	APA±SD	SA:V	P±SD	N:P±SD
Cladophora rupestris (C)	12.0±1.6 (8)	670	2.0±0.16	44.7±3.4
Ulva rigida (C)	0.28±0.28 (4)	167	1.6±0.14	21.6±2.2
Enteromorpha intestinalis (C)	1.0±0.65 (7)	529	1.7±0.69	17.7±3.6
Unidentified green algae (C)	9.54±1.8 (4)	1118	3.5±0.87	21.5±6.0
Codium fragile (C)	0.60±0.25 (4)	9	1.9±0.03	29.8±1.5
Rafsia verrucosa (P)	2.7±1.9 (7)	36	1.6±0.29	27.4±4.2
Fucus spiralis (P)	1.4±0.66 (8)	34	1.4±0.08	20.3±4.6
Pelvetia canaliculata (P)	0.72±0.09 (4)	17	1.0±0.06	16.6±0.4
Dictyota dichotoma (P)	0.72±0.28 (4)	40	0.88±0.03	27.7±0.15
Halopteris scoparia (P)	34.8±7.0 (4)	850	1.4±0.09	29.7±3.1
Cladostephus spongiosus (P)	1.9±0.68 (4)	21	3.2±0.18	n. d.
Cystoseira tamariscifolia (P)	3.8±0.88 (4)	32	2.8±0.15	17.4±0.7
Porphyra sp. (R)	0.56±0.18 (4)	628	3.2±0.07	20.4±0.64
Dumontia contorta (R)	3.0±0.95 (8)	34	2.5±0.13	25.4±0.87
Chondrus crispus (R)	1.1±0.6 (16)	75	1.9±0.34	21.9±6.0
Ceramium nodulosum (R)	7.5±0.7 (4)	191	2.4±0.13	34.6±1.5
Polysiphonia fucoides (R)	44.2±7.4 (4)	1600	3.5±0.27	n.d.
Unidentified red algae (R)	6.2±1.6 (4)	1100	3.0±0.60	21.5±3.7
Chondria coerulescens (R)	1.3±0.20 (4)	21	2.4±0.11	n. d.
Pterocladia capillacea (R)	5.9±0.05 (3)	100	6.5±0.25	11.7±0.15
Halarachnion ligulatum (R)	0.42±0.11 (4)	35	2.8±0.64	23.3±5.1
Sphaerococcus coronopifolius (R)	5.4±2.08 (4)	100	2.0±0.64	14.8±1.6
Zostera marina (SG)	6.7±0.38 (8)	43	5.6±0.88	14.4±3.6

[a] The number of replication is similar for APA, tissue P and N:P ratio and is given between brackets. n.d., non-determined. C, Chlorophyta; P, Phaeophyta; R, Rhodophyta; SG, Seagrass.

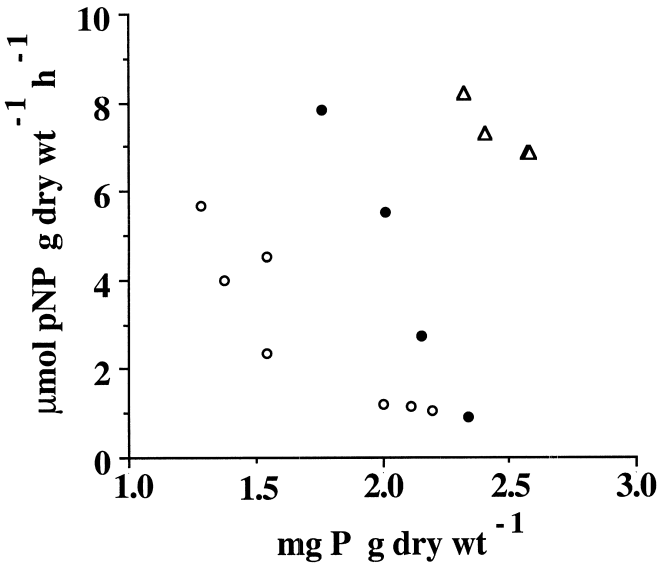

Fig. 1. Relationship between alkaline phosphatase activity and tissue P in three selected species of benthic macroalgae: *Ceramium nodulosum* (△), *Sphaerococcus coronopifolius* (●) and *Ralfsia verrucosa* (○).

as shown in Fig. 1 for three representative species. However, when pooling all the values of APA and tissue P of the species from the two sampling sites, the two variables are not correlated ($P > 0.5$; Fig. 2). The absence of correlation was also observed when the data were analysed separately each sampling day (data not shown). A similar conclusion can be achieved when APA of the different species was plotted against N:P ratio ($P > 0.1$; Fig. 3) or C:P ratio ($P > 0.5$; data not shown). Again, no significant correlation was evident.

However, when plotting APA versus SA:V ratio (both on logarithmic scale), a significant positive relationship was evident (Fig. 4). Plants with low SA:V ratio tended to have lower APA than plants with high SA:V ratio. APA increased non-linearly with increasing SA:V ratio, as described by the regression equation (standard errors in parenthesis): $\log \text{APA} = -0.57\ (\pm 0.35) + 0.48\ (\pm 0.16) \log \text{SA:V}$

$R^2 = 0.29, n = 23, P < 0.01$

The regression equation showed that 29% of the variance in APA was attributable to SA:V. However, the relationship between APA and SA:V improves if the three species with high SA:V but low APA (*E. intestinalis, U. rigida* and *Porphyra* sp.) are not taken into account (see discussion). In that case the regression equation was:

$\log \text{APA} = -0.81\ (\pm 0.22) + 0.68\ (\pm 0.11) \log \text{SA:V}$

$R^2 = 0.68, n = 20, P < 0.001$

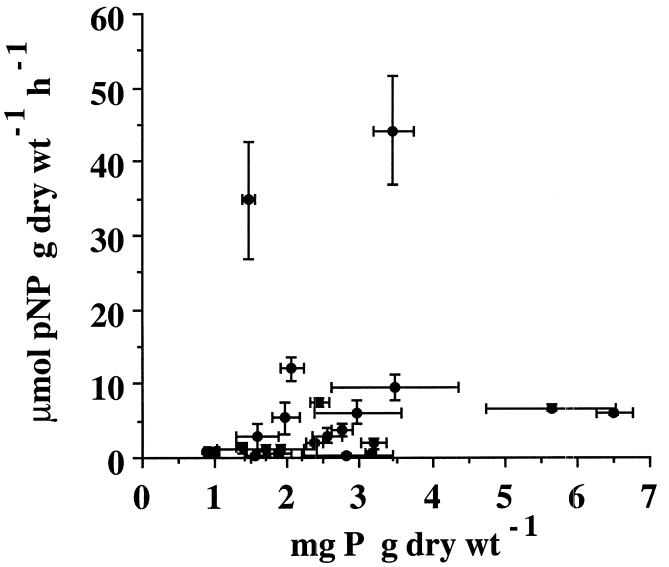

Fig. 2. Alkaline phosphatase activity of benthic marine macrophytes according to their tissue P. Data are means of 3–16 separate plants and bars represent SD.

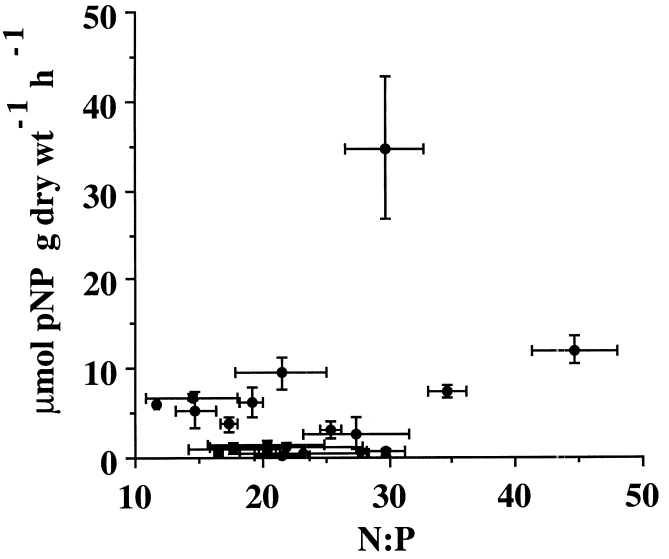

Fig. 3. Alkaline phosphatase activity of benthic marine macrophytes according to their tissue N:P atomic ratio. Data are means of 3–16 separate plants and bars represent SD.

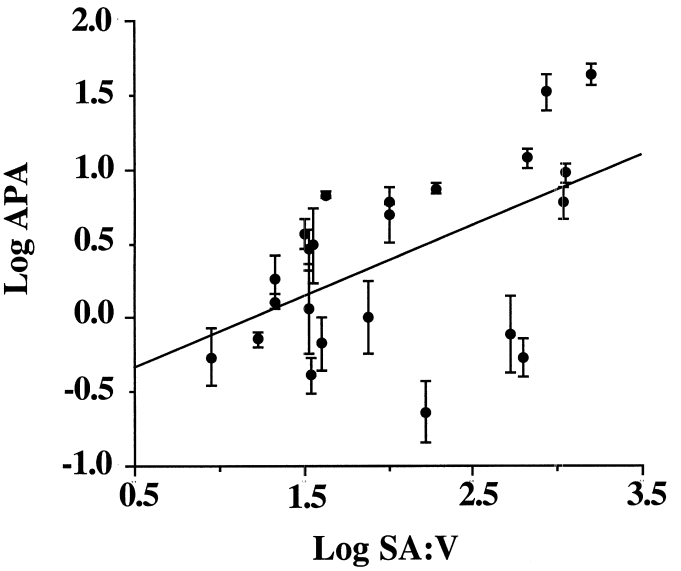

Fig. 4. Regression analysis of alkaline phosphatase activity (APA) with relative surface area (SA:V) in benthic marine macrophytes. Values of APA are means of 3–16 replicates and bars represent SD.

This new equation indicates that APA is scaled approximately to the 0.68 power of SA:V ratio. The slope of the regression equation was not statistically different from 0.75 (*t*-test, $p > 0.5$), as was expected from the general allometry. The SA:V accounted for 68% (SE = 0.33) of the variance in APA.

4. Discussion

4.1. Low APA values in some macroalgal species

Several macroalgal species showed relatively low APA values (≈less than 1 μmol pNP g dry wt^{-1} h^{-1}). Some of these species have a low SA:V ratio, which may be, in part, responsible for the low APA observed. However, three of the benthic macroalgae tested showed low APA but high SA:V ratio (*Enteromorpha intestinalis, Ulva rigida, Porphyra* sp.; Table 1) and therefore, other factors seems to affect APA in these species. The low activity in the two Ulvales agrees with the relatively low APA values found in other species of these genera (Lapointe et al., 1992; Delgado and Lapointe, 1994; Hernández et al., 1994). *Ulva* and *Enteromorpha* are fast-growing, opportunistic algae which show high Vmax:Km ratios for nutrient uptake compared to other macroalgae (Wallentinus, 1984; Morand and Briand, 1996), which give them the advantage of nutrient uptake at low concentration. This may decrease the need of utilization for PME by alkaline phosphatase as an additional source of phosphorus.

The relatively high cell quota for phosphorus found in this study for *Porphyra* sp.

(3.15 mg P g dry wt^{-1}) may be high enough to repress APA, so that only constitutive or negligible activity is produced, as found for *P. umbilicalis* (Hernández et al., 1993) and phytoplankton (Gage and Gorham, 1985). However, *Porphyra* sp. from Tyne Sands might exhibit APA under lower tissue P content. At least, an inverse correlation between APA and internal P content has been demonstrated in *P. umbilicalis* from the Mediterranean; this species showed high APA activity when the tissue P content was lowest (Hernández et al., 1993).

The cause of the low APA values of some species which evidenced high SA:V ratios may not be easily addressed and further studies are needed two answer this question. The N:P ratio found in *Ulva, Enteromorpha* and *Porphyra* are well below mean values reported for macroalgae (Atkinson and Smith, 1984; Duarte, 1992) suggesting that these species were not P-limited. However, a fast growth rate, typical of species with high SA:V ratio, associated with a high thallus P content, may create a high P demand for growth, and hence the need to supplement inorganic P uptake with PME utilization through APA if Pi is low. In spite of that, differences between the actual tissue P and the minimum tissue P concentration necessary to sustain growth may help to explain the low APA values in these species. High reserves of internal nutrients (which imply low APA) may support growth rates for several days, even under low external nutrient supply (Pedersen and Borun, 1996). In addition, we can not ruled out the possibility of higher APA values in *Ulva, Enteromorpha* and *Porphyra*. A high APA value has been measured in *E. linza* from a Mediterranean shore (78 μmol pNP g dry wt^{-1} h^{-1}; Hernández et al., 1994). Furthermore, marked changes of APA of *P. umbilicalis* have been found in a weeks time or less (Hernández et al., 1993).

Chapman and Craigie (1977) suggested that growth of the kelp *Laminaria longicruris* can proceed for long periods (months) of low external nutrient availability based on stored N pools. If a concomitant high tissue P pool is assumed, low APA would be expected. Although growing in a nutrient-richer environment, we have measured negligible APA in *L. digitata* from the North Sea (I. Hernández, unpublished data).

4.2. Size-dependence of APA

The present study has shown that differences in APA among benthic marine macrophytes can be attributed in part to size-specific differences in relative surface area. Phosphate, like other inorganic nutrients and resources has to be transported across the plant surface to become available for metabolism. This nutrient can be taken up directly or after enzymatic hydrolysis from PME. Alkaline phosphatase is known to be located in the cell surface, as shown for phytoplanktonic cells (e.g., Flynn et al., 1986; Dyhrman and Palenik, 1997). Histochemical staining in macroalgae and seagrasses has also revealed external localization of the enzyme (Gibson and Whitton, 1987; Hernández et al., 1994). The influence of SA:V ratio on APA was previously suggested by Hernández et al. (1994) to explain the high APA values in leafy or branched macroalgal species from southern Spain. Also, Delgado and Lapointe (1994) found higher APA values in fleshy versus calcareous macroalgae in a tropical marine environment. Besides, when comparisons of units of enzymatic activity are possible, it seems clear that APA values recorded from cyanobacteria (with high SA:V ratio) are undoubtedly higher (values of

several mmol pNP released g dry wt^{-1} h^{-1}: e.g., Mahasneh et al., 1990; Whitton et al., 1991) than those reported for marine macrophytes. Conversely, maximum APA values in underground organs or leaves of aquatic rooted angiosperms are in the order of 100 nmol pNP g dry wt^{-1} h^{-1} (Kufel, 1982), suggesting a tendency for higher APA with higher SA:V ratio along a larger range of sizes among aquatic photosynthetic organisms. However, caution is needed in the interpretation of APA values in aquatic rooted angiosperms, as the sediments can have high phosphate availability and APA may be low also because of root uptake of Pi.

Our results seems to agree with previously reported allometric relationship between Pi uptake and SA:V ratio within narrow size-ranges: phytoplankton (Smith and Kalff, 1982; Wen et al., 1997) or marine benthic algae (Odum et al., 1958; Wallentinus, 1984) and support the statement claimed by others (e.g., Hein et al., 1995) of a general coupling between physiological and morphological properties in algae. The importance of the SA:V ratio has been pointed out in other physiological processes among algae, as shown by nitrogen uptake rates (Hein et al., 1995) or growth rates across photosynthetic organisms (Nielsen et al., 1996).

Changed in SA:V ratio explained 29% of the variation in APA. This percentage increased to 68% if species with high SA:V but low APA were not considered in the regression analysis. In this case, our findings suggest that APA may supports the ''surface rule'', according to which ''the metabolic rate per unit weight decreases with increasing size, but is constant per unit surface'' (von Bertalanffy, 1951). The slope of the allometric relationship (0.68) is close to that expected from the ''3/4 law'' governing the size dependence of metabolic rates in animals (Peters, 1983). The slope of the equation relating APA to SA:V (without the three ''anomalous'' data) is similar to other values reported for allometric scaling of maximum velocity of nitrate and ammonium uptake (0.66 and 0.61, respectively) and SA:V across a large size range of micro- and macroalgae (Hein et al., 1995). Nielsen and Sand-Jensen (1990) estimated previously a slope of 0.66 between growth and SA:V (both in logarithmic scale) in aquatic photosynthetic organisms.

However, it must be clear from Fig. 4 that, considering all species, the percentage of the variation in APA explained by SA:V ratio is, although highly significant, relatively small. Thus, the allometric relation between APA and SA:V must be treated with caution. Firstly, both APA and SA:V are subject to experimental errors. Secondly, substantial remaining variation can be assigned to species-specific variation in APA, as maximum APA may vary considerably, about 10-fold, within a single species (Hernández et al., 1993; Delgado et al., 1996), depending on several biological and physicochemical factors such as tissue P (Hernández et al., 1999). However, the allometric relationship found here suggests that differences of SA:V ratio among marine macrophytes should be taken into account when comparing phosphatase activities among marine macrophytes. In this context, when maximum APA values for a single species are discussed, the enzymatic activity should be compared with other published values in closely related (e.g., similar algal size) species. Additionally, the tissue P content also may help to discuss the relative APA values in terms of a possible P limitation.

Finally, the importance of algal size in the estimation of APA also draw attention to

the necessity of removing epiphytes from the plants. These epiphytes generally exhibit high SA:V ratio and thus may overestimate the APA values of a single species.

4.3. Ecological implications

It is known empirically that small organisms tend to be metabolically more active than large ones. Relationships between physiological variables and size (or SA:V) are usually allometric. Examples among micro- and macroalgae are the relationship between N and P acquisition and size (Odum et al., 1958; Smith and Kalff, 1982; Rosemberg and Ramus, 1984; Hein et al., 1995; Wen et al. 1997). The allometric relationship between APA and SA:V ratio suggested in our study supports these previous findings. In addition, small photosynthetic organisms seems to exhibit higher efficiency of photon capture than large ones (Agustí et al., 1994). The main consequence of the higher resource acquisition is that maximum growth rates of different aquatic photosynthetic organisms seems to decrease with increasing organism size (Littler and Littler, 1980; Nielsen and Sand-Jensen, 1990).

The relationship between APA and SA:V suggests that hairs, fine branches and other morphological features that increase the macrophyte surface relative to its volume may enhance the P uptake (from PME) from the external environment. At least in cyanobacteria and *Chaetophoraceae* (Chlorophyta) the presence of hairs are directly correlated to APA (Gibson and Whitton, 1987; Whitton, 1988). According to the maximum APA values (per dry wt) reported for cyanobacteria and rooted angiosperms compared to marine macrophytes (see above), small aquatic photosynthetic organisms may be favoured under low external P availability. Although this may be true for direct Pi acquisition (Smith and Kalff, 1982; Wen et al., 1997), more work is needed to reach such a conclusion regarding APA. The competitive superiority in PME utilization in nature depends also on the external substrate available or the enzymatic affinity for natural substrates (Cembella et al., 1984), which are difficult to measure under field conditions (Hernández et al., 1999).

Another ecological implication of the allometric relationship described in our work is to provide additional insights to what extent a selected species or population of marine macrophyte shows P limitation. The comparison of observed APA values with the predicted values for an approximate SA:V ratio may support the conclusions drawn from other strongest indicators of P limitation, as tissue P (Hernández et al., 1993) or atomic N:P ratios (Duarte, 1992).

Finally, there have been some preliminary efforts in using APA of benthic marine macrophytes as a tool for environmental monitoring purposes in coastal ecosystems (Whitton and Hernández, 1996). If marine macrophytes are used to test the phosphorus status of the environment, that is, to show a reproducible response of APA to P stress, the selected species should fulfil some of the following conditions. According to the results found here they should preferably be finely branched (high SA:V ratio) and show a strong response in APA to the internal P concentration. Additionally they should be widespread (in order to use the same species in different coastal ecosystems) and easy to identify. There are some genera whose species fulfil at least some of these conditions

(*Bangia, Gelidium, Polysiphonia, Cladophora, Stypocaulon*), and therefore future research on this field should focus on these or similar macroalgal species.

5. Conclusions

Although there seems to be obvious exceptions (macroalgae with negligible APA values despite high SA:V ratios), differences in APA among benthic marine macrophytes can partially be explained by size-specific changes in SA:V and not by differences in their tissue P. Comparisons of reported APA values in other aquatic photosynthetic organisms (cyanobacteria and rooted angiosperms) suggest that small algae can hydrolise PME at a faster rate than large aquatic photosynthetic organisms. Along with other indicators (tissue P or N:P ratio), the allometric equation may help to confirm to what extent a selected species or population of benthic marine macrophyte is P limited. This study may also help future efforts in using APA in monitoring programs in the coastal ecosystem.

Acknowledgements

This study was supported by project HB1995-0166 of the Acciones Integradas Programme (British Council and Ministerio Español de Educación y Cultura). The authors thank an anonimous referee for critical comments on an earlier version of the manuscript.

References

Agustí, S., Enríquez, S., Frost-Christensen, H., Sand-Jensen, K., Duarte, C.M., 1994. Light harvesting among photosynthetic organisms. Funct. Ecol. 8, 273–279.

APHA (American Public Health Association), 1992. Standard methods for the examination of water and wastewater, 18th edition. American Public Health Association, Washington, D.C.

Atkinson, M.J., Smith, S.V., 1984. C:N:P ratios of benthic marine plants. Limnol. Oceanogr. 28, 568–574.

von Bertalanffy, L., 1951. Metabolic types and growth types. Am. Nat. 85, 111–117.

Cembella, A.D., Antia, N.J., Harrison, P.J., 1984. The utilization of inorganic and organic phosphorous compounds as nutrients by eukariotic microalgae: a multidisciplinary perspective: part 1. CRC Crit. Rev. Microbiol. 10, 317–391.

Chapman, A.R.O., Craigie, J.S., 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40, 197–205.

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

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

Delgado, O., Rodríguez-Prieto, C., Gacia, E., Ballesteros, E., 1996. Lack of severe nutrient limitation in *Caulerpa taxifolia* (Vahl) C. Agardh, an introduced seaweed spreading over the oligotrophic northwestern Mediterranean. Bot. Mar. 39, 61–67.

Draper, N.R., Smith, H., 1966. Applied Regression Analysis, John Wiley & Sons, Chichester.

Duarte, C.M., 1992. Nutrient concentration of aquatic plants: Patterns across species. Limnol. Oceanogr. 37, 882–889.

Dudgeon, S.R., Kübler, J.E., Vadas, R.L., Davidson, I.R., 1995. Physiological responses to environmental variation in intertidal red algae: does thallus morphology matter? Mar. Ecol. Prog. Ser. 117, 193–206.

Dyhrman, S.T., Palenik, B.P., 1997. The identification and purification of a cell-surface alkaline phosphatase from the dinoflagellate *Prorocentrum minimum* (Dinophyceae). J. Phycol. 33, 602–612.

Flores-Moya, A., Altamirano, M., Cordero, M., González, M.E., Pérez, M.G., 1997. Phosphorus-limited growth in the seasonal winter red alga *Porphyra leucosticta* Thuret in le Jolis. Bot. Mar. 40, 187–191.

Flynn, K.J., Opik, H., Syret, P.J., 1986. Localization of alkaline phosphatase and 5′-nucleotidase activities of the diatom *Phaeodactylum tricornutum*. J. Gen. Microbiol. 132, 289–298.

Gage, M.A., Gorham, E., 1985. Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. Freshwater Biol. 15, 227–233.

Gibson, M.T., Whitton, B.A., 1987. Hairs, phosphatase activity and environmental chemistry in *Stigeoclonium, Chaetophora* and *Draparnaldia* (Chaetophorales). Br. Phycol. J. 22, 11–22.

González-Gil, S., Keafer, B.A., Jovine, R.V.M., Aguilera, A., Lu, S., Anderson, D.M., 1998. Detection and quantification of alkaline phosphatase in single cells of phosphorus-starved marine phytoplankton. Mar. Ecol. Prog. Ser. 164, 21–35.

Hein, M., Pedersen, M.F., Sand-Jensen, K., 1995. Size-dependent nitrogen uptake in micro- and macroalgae. Mar. Ecol. Prog. Ser. 118, 247–253.

Hernández, I., Christmas, M., Yelloly, J.M., Whitton, B.A., 1997. Factors affecting surface alkaline phosphatase activity in the brown alga *Fucus spiralis* at a North Sea intertidal site (Tyne Sands, Scotland). J. Phycol. 33, 569–575.

Hernández, I., Fernández, J.A., Niell, F.X., 1993. Influence of phosphorus status on the seasonal variation of alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. J. Exp. Mar. Biol. Ecol. 173, 181–196.

Hernández, I., Niell, F.X., Fernández, J.A., 1994. Alkaline phosphatase activity in marine macrophytes. Study of its localization in some widespread species in southern Spain. Mar. Biol. 120, 501–509.

Hernández, I., Niell, F.X., Whitton, B.A., 1999. Phosphatase activity of benthic marine algae. An overview. In: Whitton, B.A., Hernández, I. (Eds.), Phosphatases in the Environment, Kluwer Academic Publishers, Dordrecht (in press).

Hernández, I., Whitton, B.A., 1996. Retention of p-nitrophenol and 4-methylumbelliferone by marine macroalgae and implications for measurement of alkaline phosphatase activity. J. Phycol. 32, 819–825.

Kufel, L., 1982. Phosphorus and alkaline phosphatase activity in some aquatic macrophytes. Bull. Acad. Pol. Sci. 30, 1–12.

Lapointe, B.E., 1989. Macroalgal production and nutrient relations in oligotrophic areas of Florida Bay. Bull. Mar. Sci. 44, 312–323.

Lapointe, B.E., 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnol. Oceanogr. 42, 1119–1131.

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

Littler, M.M., Littler, D.S., 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. Am. Nat. 116, 25–44.

Mahasneh, I.A., Grainger, S.L.J., Whitton, B.A., 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.

Morand, P., Briand, X., 1996. Excessive growth of macroalgae: a symptom of environmental disturbance. Bot. Mar. 39, 491–516.

Nielsen, S.L., Enríquez, S., Duarte, C.M., Sand-Jensen, K., 1996. Scaling maximum growth rates across photosynthetic organisms. Funct. Ecol. 10, 167–175.

Nielsen, S.L., Sand-Jensen, K., 1990. Allometric scaling of maximal photosynthetic growth rate to surface/volume ratio. Limnol. Oceanogr. 35, 177–181.

Odum, E.P., Kuenzler, E.J., Blunt, M.X., 1958. Uptake of ^{32}P and primary productivity in marine benthic algae. Limnol. Oceanogr. 3, 340–345.

Pedersen, M.F., Borun, J., 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar. Ecol. Prog. Ser. 142, 261–272.

Peters, R.H., 1983. The Ecological Implications of Body Size, Cambridge University Press, Cambridge.
Reichardt, W., Overbeck, J., Steubing, L., 1967. Free dissolved enzymes in lake waters. Nature 216, 1345–1347.
Rosemberg, G., Ramus, J., 1984. Uptake of inorganic nitrogen and seaweed surface area:volume ratios. Aquat. Bot. 19, 65–72.
Smith, R.E., Kalff, J., 1982. Size dependent phosphorus uptake kinetics and cell quotas in phytoplankton. J. Phycol. 18, 275–284.
Sokal, R.S., Rohlf, F.J., 1981. Biometry, 2nd ed, W.H. Freeman and Company, San Francisco.
Sournia, A., 1982. Form and function in marine phytoplankton. Biol. Rev. 57, 347–394.
Wallentinus, I., 1984. Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar. Biol. 80, 215–225.
Wen, Y.H., Vézina, A., Peters, R.H., 1997. Allometric scaling of compartmental fluxes of phosphorus in freshwater algae. Limnol. Oceanogr. 42, 45–56.
Wheeler, P.A., Björnsäter, B.O., 1992. seasonal fluctuations in tissue nitrogen, phosphorus, and N:P for five macroalgal species common to the Pacific northwest coast. J. Phycol. 28, 1–6.
Whitton, B.A., 1988. Hairs in eukaryotic algae. In: Round, F.E. (Ed.), Algae and the aquatic environment, Biopress Ltd, London, pp. 446–470.
Whitton, B.A., Grainger, S.L.J., Hawley, G.R.W., Simon, J.W., 1991. Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb. Ecol. 21, 85–98.
Whitton, B.A., Hernández, I., 1996. Algal phosphatase activity as indicator of environmental phosphorus status. VII Int. Conf. Ass. Appl. Algol., J. Appl. Phycol. 8, 463.
Woelkerling, W.J., Spencer, K.G., West, J.A., 1983. Studies on selected corallinaceae (Rhodophyta) and other macroalgae in a defined marine culture medium. J. Exp. Mar. Biol. Ecol. 67, 61–77.
Zar, J.H., 1984. Biostatistical Analysis, Prentice-Hall, Englewood Cliffs, NJ.