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# FACTORS AFFECTING SURFACE ALKALINE PHOSPHATASE ACTIVITY IN THE BROWN ALGA *FUCUS SPIRALIS* AT A NORTH SEA INTERTIDAL SITE (TYNE SANDS, SCOTLAND)[1]

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## ABSTRACT

*Surface alkaline phosphatase activity (APA) was measured in the tips of* Fucus spiralis *L. thalli over an approximately 1-year period, using small plants from upper littoral rock pools at a site on the east coast of Scotland. Maximum APA (4.4 μmol* p*-NP·g dry wt$^{-1}$·h$^{-1}$) occurred in April, coincident with a sharp increase in tissue N:P ratio of the tips and the probable period of most rapid growth. Enzymatic activity was not correlated with any of the physical and chemical factors (temperature, pH, external phosphate, salinity) measured in the pools or nutrient concentrations (C, N, P) in the apical part of the thallus. No obvious correlation was found between APA and the position of a plant on the shore. However, APA was significantly higher in plants emersed at low tide than in plants submerged during the whole tidal cycle. There was a significant positive correlation between APA and tissue P within a single thallus, with the highest value at the tip (which includes the meristematic zone). Changes in APA of* Fucus spiralis *appear to result from a complex interaction of biotic and environmental factors, including stress by emersion, growth period, rapid changes in tissue nutrient content and N:P ratio, and region of thallus.*

*Key index words: alga; alkaline phosphatase; decomposing seaweeds;* Fucus spiralis*; intertidal seaweed; phosphorus; tidepool*

Algae living in rock pools in the upper littoral zone are subjected to marked environmental changes during each tidal cycle. Fluctuations in salinity and temperature are likely to depend on the size of the pool and atmospheric conditions (Nybakken 1993). Marked changes in nutrient concentration can also occur within a single tidal cycle (Khoja et al. 1984). Other studies (Hanisak 1993, Yelloly and Whitton 1996) have demonstrated that sporadic deposits of algae on the shore contribute to nutrient inputs to coastal ecosystems. Among these nutrients, phosphorus has been suggested as a limiting factor for macroalgal growth (Delgado et al. 1994). In addition to inorganic phosphorus, some compounds of the dissolved organic phosphorus pool, the phosphomonoesters, can be used as a source of P after enzymatic hydrolysis (Walter and Fries 1976). Such hydrolysis due to "surface" alkaline phosphatase activity (APA) has been reported in numerous studies of macroalgae (e.g. Lapointe et al. 1992, Hernández et al. 1994). APA is generally enhanced when algae become P deficient (Gage and Gorham 1985, Lapointe et al. 1992), and therefore it has been used as an indicator of P limitation (Weich and Granéli 1989).

The phaeophyte *Fucus spiralis* L. is one of the most abundant algae (in terms of biomass) growing in rock pools of the upper and mid-littoral zone of the North Sea (Lee 1992, Yelloly and Whitton 1996) and is subjected to a variety of potentially stressful environmental conditions, such as high light or temperature (Schonbeck and Norton 1979, Madsen and Maberly 1990). On the northern Atlantic coast, populations of *F. spiralis* on semi-exposed rocky shores are among the most strongly P limited of the intertidal fucoids (Hurd and Dring 1990). Hurd et al. (1993) suggested that *F. spiralis* formed hairs as a response to increased nutrient demand. Other eukaryotic algae are known to show marked localization of APA on hairs (Whitton 1988).

A recent study in intertidal pools at Tyne Sands on the Scottish coast of the North Sea (Yelloly and Whitton 1996) showed that P concentrations vary markedly throughout the year. There are long periods near or below the detection limit (2 μg P·L$^{-1}$) and other periods with concentrations two to three orders of magnitude higher, following deposition of detached seaweeds (mostly *Laminaria*) in the supralittoral zone by storms. Previous studies on *Rivularia atra* (cyanobacterium) in rock pools of the upper intertidal zone showed periods of P limitation and marked phosphatase activity (Khoja et al. 1984, Yelloly and Whitton 1996). *Fucus spiralis,* which extends from the upper to lower intertidal zones, is also abundant in the same rock pools. Other studies (Strömgren 1986) showed that this species has periodically high growth rates, suggesting a transient high nutrient demand. Therefore it may be possible that *F. spiralis* also shows seasonal changes in APA.

The aim of the present study was to investigate the influence of seasonal and environmental variables on APA of *F. spiralis* at Tyne Sands. This was

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achieved by temporal studies in rock pools over one year, a detailed transect down the shore, and a comparison of APA in different parts of the thallus.

## MATERIALS AND METHODS

*Sampling site.* The study was carried out in a sheltered bay at Tyne Sands, East Lothian, Scotland (56°0′ N, 2°35′ W), where the estuary of the River Tyne opens to the North Sea. Details of the intertidal community, water chemistry, and cyanobacterial nitrogen fixation are given by Khoja et al. (1984) and Yelloly and Whitton (1996). The upper mid-littoral zone has numerous rock pools in which small plants of *Fucus spiralis* are abundant, often attached to the surface of the rock pool at the edge of the pool and with the thallus hanging into the pool. The pools in the zone chosen for most detailed study were covered by many, but not all, high tides. At other times the pools become isolated and the nutrient regime may be influenced by water draining from the drift seaweed and also algae and invertebrates living inside the pools (Yelloly and Whitton 1996).

Eight pools were selected: three near the supralittoral fringe (drift seaweed zone; 5.0 m height: W1–W3) and five pools in the upper littoral zone (4.5–4.7 m height: *Fucus* pools = F1–F5). A map showing the location of the pools is given in Yelloly and Whitton (1996). Pools were monitored monthly from February to December 1995, 1 h after high tide and at ca. 1200 GMT. Additional samples were also collected from the main body of seawater during the ebb tide.

Salinity was measured with an ATAGO refractometer. Temperature, conductivity, and pH were measured in the field using Wissenshaftliche Technische Werkstätten (WTW) meters using a WTW probe for the first two variables and a Russell CL06 probe for pH. Water samples were filtered (Whatman GF-F) in the field and then stored in an ice chest. Phosphorus analyses were conducted immediately on return to the laboratory, including filtrable reactive phosphorus (FRP) by the colorimetric method of Murphy and Riley (1962) and total filtrable phosphorus (TFP) determined after persulphate acid digestion according to Eisenreich et al. (1975). Filtrable organic phosphorus (FOP), the fraction including phosphomonoesters, was taken as the difference between TSP and FRP. All values given here are for the element, rather than $PO_4^{-3}$.

*Alkaline phosphatase assays.* APA of *F. spiralis* growing in pools in the upper littoral zone (F1–F5) was measured in the field using three replicates according to Hernández et al. (1993). Apical pieces (4–5 cm tips; ca. 0.3 g wet weight) of thallus, either growing inside the pools or having the terminal parts of the thalli continuously immersed in them, were selected haphazardly. The tips were cut carefully with a scalpel and rinsed for 5 min in the pool. Mature receptacles were observed in August and September, but only nonreproductive tips were selected. The apices were shaken gently to remove excess water and finally placed in small transparent glass bottles with assay medium. The medium consisted of 20 mL of 500 µM *p*-nitrophenyl phosphate (*p*-NPP) and 50 µM EPPS–NaOH buffer, pH 8.3, dissolved in P-free artificial seawater (Woelkerling et al. 1983). The samples were incubated for 1 h at the pool temperature with gentle shaking approximately every 10 min. A subsample of the medium was then placed into a capped plastic vial and immersed in liquid nitrogen. These vials were quickly defrosted in the laboratory and the absorbance read at 410 nm. In each pool, two controls were also incubated with the samples to correct the final absorbance: a sample of assay medium without plant and three replicates of buffered (pH 8.3) artificial seawater with plants of *F. spiralis.* This second control was run due to the slight absorbance (410 nm) by polyphenolics released from vacuoles after cutting the thallus (Reviers 1989), which influences APA measurements (Hernández and Whitton 1996). Enzymatic activity was expressed as µmol *p*-nitrophenol (*p*-NP) released·g dry weight$^{-1}$·h$^{-1}$. The plants for assay in the laboratory were transferred in an ice chest.

Separate studies were also made on the influence of the intertidal location of plants on their APA and the distribution of APA on the *F. spiralis* thallus. In May, APA was measured in the field using plants in a transect from the upper littoral (4.8 m height) to the lower littoral zone. Triplicate assays were made every 30–50 m and at the same sites water samples were collected for analysis of phosphorus fractions. We expected plants in the upper littoral zone to show higher activity as a consequence of the higher P stress during emersion. In November, APA was compared in different parts of the thallus. For that purpose, three whole plants of similar size (12–14 cm) were collected from the upper littoral zone. These thalli were kept for 12 h in aerated cultures of 20 L at 18° C and under continuous light (120 µmol photon·m$^{-2}$·s$^{-1}$) in filtered (Whatman GF-F) seawater collected offshore from the sampling site (FRP, 0.8 µM). The thalli were cut in five sections of approximately the same length (2–3 cm; ca. 0.06 g dry wt): tip or apex (A), apex-middle (AM), middle (M), middle-basal (MB), and basal region (B). Prior to the assays, sections were maintained for 1 h in filtered seawater to reduce the effect of polyphenolics on the absorbance measurements. Assays were carried out at 20° C for 1 h with continuous gentle shaking.

TABLE 1. *Range of temperature (°C), salinity (practical salinity scale), and pH values in waters sampled in Tyne Sands from February–December 1995. W: drift seaweed pools in supralittoral fringe. F:* Fucus *pools in upper littoral zone.*

| | Temperature | Salinity | pH |
|---|---|---|---|
| W1–W3 | 5.2–27.2 | 7–42 | 7.6–8.8 |
| F1 | 5.3–19.6 | 34–41 | 8.0–8.5 |
| F2 | 5.2–20.3 | 34–46 | 7.9–8.6 |
| F3 | 5.2–21.0 | 34–40 | 7.9–8.8 |
| F4 | 5.2–19.7 | 33–53 | 8.0–8.7 |
| F5 | 5.4–19.6 | 34–39 | 8.0–8.7 |
| Coastal seawater | 5.2–16.8 | 34–37 | 7.9–8.3 |

*Plant nutrient analysis.* The P concentration in *Fucus* tips was measured in triplicate samples of ground dry tissue by the persulphate digestion method (APHA 1992). Other subsamples of tissue were used to determine C and N concentration using a CHNS elemental analyzer (Carlo Erba CA 1108).

*Statistical analyses.* Differences between two means were assessed by a Student's *t*-test (Zar 1984). Mean APA values during the autumn and December were grouped and compared by a sum of squares simultaneous test procedure (Fry 1993). Correlation between variables was tested with a Pearson simple correlation test. Comparisons between P fractions from the pools were made using a multiple correlation test (Zar 1984). The influence of several predictor variables on APA was assessed by a multiple regression (Fry 1993). In all cases, the null hypothesis was rejected at the 5% significance level.

## RESULTS

*Physical and chemical variables.* Water in the drift seaweed pools (W1–W3) and *Fucus* pools (F1–F5) showed considerable fluctuations compared to coastal seawater (Table 1). The pattern of temperature changes was quite similar in all the pools, with the highest values in August and the lowest in February. Salinity also showed considerable variation, especially in the drift seaweed pools, where it ranged from 7‰ in spring to 42‰ in summer. In the *Fucus* pools, the highest salinities were recorded in April, when the offshore wind prevented the high tide from reaching some of the pools and warm weather caused evaporation; the effect was less evident in the largest *Fucus* pool (F5, Table 1). The pH did not show strong variations throughout the year, and coastal seawater showed little difference in any of these variables.

The presence of drift seaweed in pools W1–W3

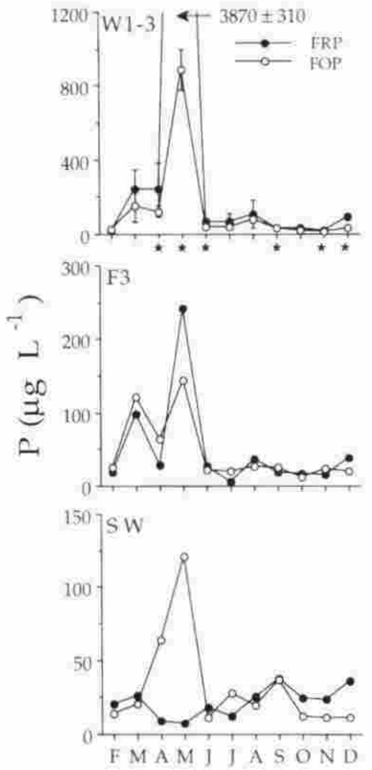

FIG. 1. Filtrable reactive phosphorus (FRP) and filtrable organic phosphorus (FOP) in water from A) the drift seaweed pools W1–W3, B) *Fucus* pool F3, and C) coastal water (SW). Asterisks indicate when major seaweed deposits were observed in the supra- and upper littoral zones.

often, but not always, led to high P concentration in the pools (Fig. 1). For instance, algae deposited in autumn showed little signs of decomposition, and therefore, high nutrients in the water were not observed. W1–W3 showed maximum FRP and FOP concentrations in May (Fig. 1A), whereas concentrations were less than 40 $\mu g \cdot L^{-1}$ for much of the rest of the year. The changes in aqueous P in one *Fucus* pool (F3) are shown in Fig. 1B. Following a high tide, water was seen to drain from the drift seaweed pools into the *Fucus* pools. Thus, FOP and FRP in all the *Fucus* pool (except F4) correlated positively with the concentrations in W1–W3 ($P < 0.05$). Overall, the *Fucus* pools showed the highest P concentration in May, but the values were much lower than the drift seaweed pools (maximum 450 $\mu g$ FRP$\cdot L^{-1}$ and 250 $\mu g$ FOP$\cdot L^{-1}$ in F1; data not shown). For all *Fucus* pools except F4, FRP and FOP followed similar seasonal patterns ($P < 0.05$). Coastal seawater also displayed high FOP in May (Fig. 1C).

Throughout the year, the mean FOP as a percentage of TFP was highest in the *Fucus* pools (57.9 ± 12.5%). The proportion of FOP in seawater was similar (50.6 ± 22.0%), but was lower (38.9 ± 13.2%) in the drift seaweed pools.

*Alkaline phosphatase activity.* The seasonal pattern of changes in APA of *F. spiralis* tips was similar in all the *Fucus* pools, so only the mean values for all months and plants are shown (Fig. 2). APA was high in winter and spring when the lowest water temperatures were observed. Maximum APA was measured in April (4.4 $\mu$mol *p*-NP$\cdot$g dry wt$^{-1} \cdot h^{-1}$); the minimum occurred in summer (ca. 1 $\mu$mol *p*-NP$\cdot$g dry wt$^{-1} \cdot h^{-1}$), when water temperature was highest (ca. 20° C). In December, APA increased significantly with respect to the period September–November ($P < 0.05$).

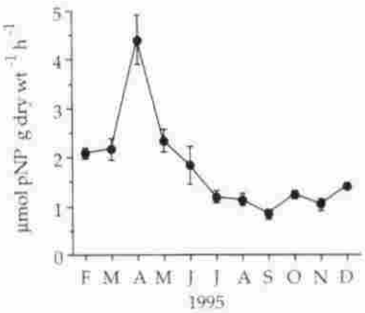

FIG. 2. Changes in alkaline phosphatase activity in tips of *Fucus spiralis* from February to December 1995. Values are the means of 15 separate plants (5 rock pools and three thalli per pool); bars represent SD.

Multiple regression showed that in none of the pools was APA predicted for any of the physical and chemical variables in the water, even after data transformations (data not shown). Pool F4 showed a highly significant correlation between APA and FOP ($P < 0.002$), but this correlation was strongly biased due to the high APA and FOP found in April. Without these data, no correlation was found.

*Elemental composition of thallus.* The C, N, and P concentrations of tips showed a similar seasonal pattern in pools F1–F5, and hence mean values were plotted. Tissue C was more or less constant throughout the year (Fig. 3A), forming 35–38% dry wt. Tissue N was maximal in winter (3% dry wt in March; Fig. 3B), decreasing during spring and summer to a minimum in August (0.8% N). From this month onward a continuous increase in tissue N was noticed. Tissue P was maximum in winter (0.25% dry wt in March; Fig. 3C), with a gradual decrease during the spring to low concentrations from June to November (ca. 0.14% P). An increase in tissue P was observed in December. No significant correlation between APA and element composition was observed. In contrast, tissue N and P showed a highly significant correlation during the study period ($r = 0.74$, $P < 0.001$, $n = 120$).

The tissue C:N:P ratio showed a clear cycle of changes during the year (Fig. 4). In this figure, the proportions of C and N may be read directly from the axes, whereas P is equal to 1. The C:N:P values ranged from 360:25:1 (March) to 730:13:1 (August).

*Effect of intertidal location on aqueous and tissue P contents and APA.* The experiment in May showed that at low tide FOP decreased with decreasing shore height (Fig. 5). Although FRP was highest in

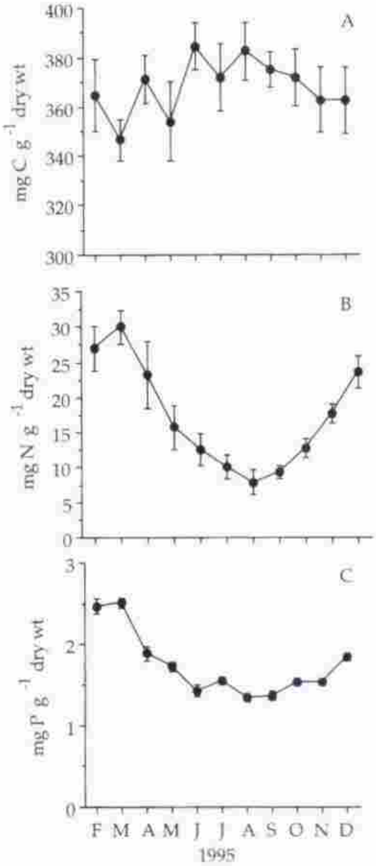

FIG. 3. Changes in tissue nutrient concentrations from February to December 1995 in tips of *Fucus spiralis*. A) carbon; B) nitrogen; C) phosphorus. Values are the means of 15 replicates and bars represent SD: see legend to Fig. 2).

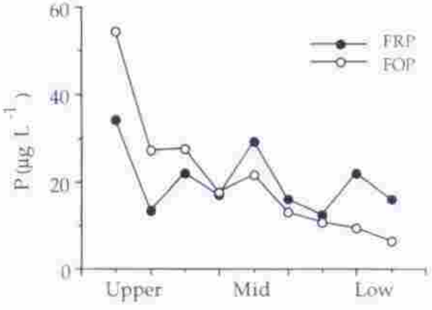

FIG. 5. Filtrable reactive phosphorus (FRP) and filtrable organic phosphorus (FOP) of water samples collected at low tide from shallow pools along a transect in the littoral zone. Values are the means of two replicates and SD was smaller than the symbol size.

the upper littoral zone, it remained relatively constant down the rest of the shore. Hence, FOP ranged from over 60% TFP in the upper littoral zone to less than 10% in the lower littoral zone.

The period of immersion during a tidal cycle influenced the total P content of *F. spiralis*. Thallus tips in the upper littoral zone had about 0.14% P but this value increased gradually to 0.25% P in the lower littoral zone (Fig. 6). However, APA did not show the expected inverse pattern, there being no inverse correlation between the two variables. To explain the difference in activity between thalli, the plants were grouped into those that were exposed at low tide (see marks in Fig. 6) and those that were growing in shallow pools along the littoral zone and therefore remained submerged during the whole tidal cycle. APA in thallus tips exposed at low tide was significantly higher than APA in tips that remained submerged during the whole tidal cycle ($P < 0.01$). Furthermore, tissue P was significantly higher in submerged than exposed thalli ($P < 0.005$) (Fig. 7).

*Enzymatic activity profile in a thallus.* APA was highest in the tips (meristematic zone) and decreased toward a constant value in the middle and basal parts of the thallus (Fig. 8). Tissue P and N concentrations (% dry wt) both showed the same trend, with the highest values in tips and a decrease toward the middle and basal parts of the thallus, whereas tissue C increased from the apex to the basal parts of the thallus (Fig. 8). There was a significant positive correlation down the thallus between APA and total P ($r = 0.9$, $P < 0.001$; Fig. 9). Furthermore, APA was positively correlated with tissue N and neg-

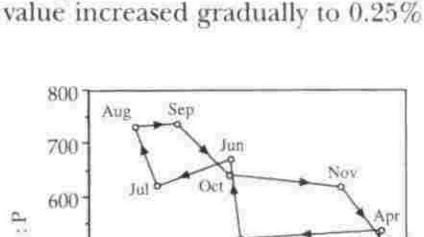

FIG. 4. Plot of C:P vs. N:P (atomic ratios) in tips of *Fucus spiralis* showing the evolution of the Redfield ratio throughout the year. Points are linked with arbitrary lines to show the time course.

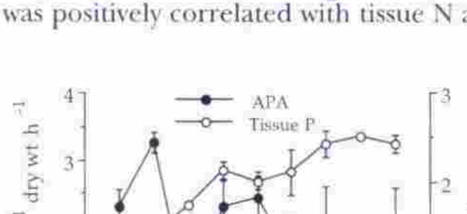

FIG. 6. Alkaline phosphatase activity (APA) and tissue P in tips of *Fucus spiralis* collected along a transect in the littoral zone. Arrows indicate plants that were submerged in sandy or rock pools even at low tide. Values are the means of 3 replicates and bars represent SD.

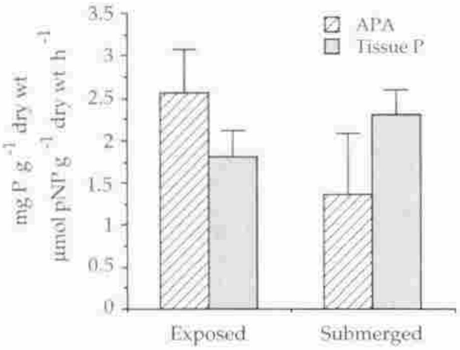

FIG. 7. Comparison of alkaline phosphatase activity (APA) and tissue P in plants of *Fucus spiralis* exposed at low tide (n = 12) and plants submerged during the whole tidal cycle (n = 15) along a transect in the littoral zone. Bars represent SD.

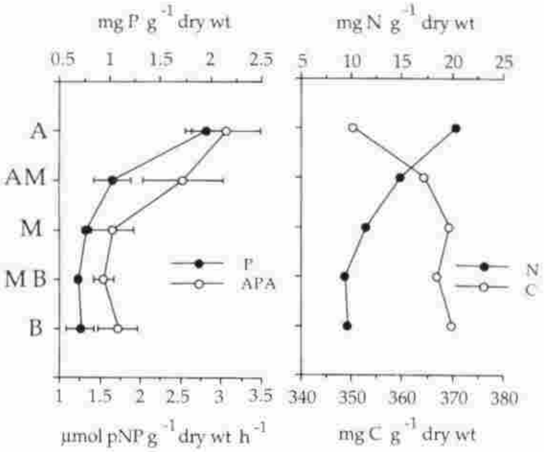

FIG. 8. Distribution of alkaline phosphatase activity (APA), tissue P, tissue N, and tissue C along thalli of *Fucus spiralis* in November 1995. Plants of similar size (12–14 cm) were divided into five sections of approximately the same length (2–3 cm). A: apex. AM: apex-middle. M: middle. MB: middle base. B: base and stipe. Values for APA and P are the means of three replicates, and bars represent SD. Values for N and C are means of three pooled sections.

atively correlated with tissue C and C:N and C:P ratios.

## DISCUSSION

Alkaline phosphatase activity was evident in the tips of *Fucus spiralis* growing in upper littoral rock pools throughout the year, but the activity showed no obvious relationship to the ambient physical and chemical conditions of the rock pools. Short-term laboratory studies have shown, however, that temperature, salinity, pH, and ambient phosphate can influence algal APA (Lubián et al. 1992, Hernández et al. 1995). External phosphate concentration in the field has also been reported as a variable influencing phytoplankton APA (Chróst et al. 1984). Some of these influences might be masked in our study, as the activity in the field was always measured under standard conditions of pH and salinity (8.3 and 34‰).

In many other algae, APA has been found to be inversely related to tissue P (e.g. Gage and Gorham 1985, Hernández et al. 1993), but this is not the case in the present study, possibly because N, rather than P, limits the growth of *F. spiralis* for most of the year. From May to November, tissue N was below the critical level of 1.7% proposed by Pedersen and Borum (1996) for *F. vesiculosus* (Fig. 3B), and during the entire summer, values were below the critical level of 1.2% suggested by Rosenberg et al. (1984) for *F. distichus.* In addition, from May to August, the increase in C:N ratio was accompanied by a decrease of the N:P ratio (Fig. 4), further supporting our suggestion of a period of strong N limitation. During most of the year, the C:N ratio was well above the mean value of 22 proposed by Atkinson and Smith (1983) for marine benthic plants, whereas N:P ratio is always lower than the mean value of 30 proposed by these authors. The only period suggesting P limitation is March–April, when the enhancement of N:P, C:N, and C:P ratios was coincident with a marked peak in APA (Fig. 2). This peak in activity also coincided with a decrease in tissue N and P contents (Fig. 3) and relatively high values of FOP in some of the *Fucus* pools (Fig. 1).

There may be an additional explanation for the higher APA from February to May and especially the high value in April. It is suggested that this is a physiological response to increased nutrient demand. April is a period of rapid increase in growth rate for *F. spiralis* and other *Fucus* species in different areas of the northern Atlantic (Schonbeck and Norton 1980, Strömgren 1986, Carlson 1991, Hurd et al. 1993, Pedersen and Borum 1996). This high metabolic demand during the growth period exceeds uptake, and therefore may result in a decrease in both tissue N and P due to a dilution effect. An increase in storage products resulting from increasing periods of photosynthesis in *Fucus* from the Scottish coasts are known from February to June (Black 1949). The onset of hair formation at this time is also suggested to be a response to high metabolic activity and increased nutrient demand for growth (Hurd et al. 1993). In contrast, the lower APA dur-

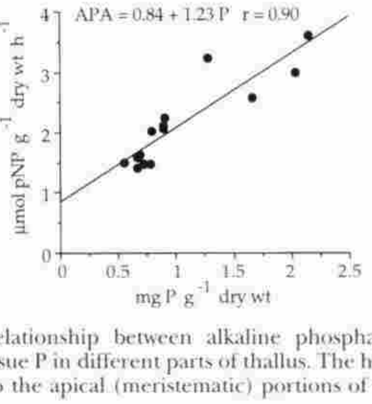

FIG. 9. Relationship between alkaline phosphatase activity (APA) and tissue P in different parts of thallus. The highest values correspond to the apical (meristematic) portions of thalli.

ing July and August may be due to a lower requirement for phosphate in the summer. Similarly, *F. spiralis* showed higher values of P uptake in winter than in summer (Hurd and Dring 1990). In other brown algae, such as *F. gardneri* and *Laminaria digitata*, nitrate reductase activity also showed seasonal changes correlated with variations in seasonal growth rate (Davidson et al. 1984, Hurd et al. 1995). Phosphorus accumulated by kelp in the sublittoral zone is cycled to *F. spiralis* as a result of storms depositing detached plants on the supralittoral and extreme upper littoral zones. The release of phosphate from the drift seaweed after a storm has not been followed in detail, but presumably temperature and desiccation are important factors; however, it is likely that *Laminaria* also contains the highest P content in spring (D. M. John, pers. comm.).

The proportion of FOP in pool water at low tide in May decreased toward the lower littoral zone, suggesting that this fraction (which includes phosphatase substrates) is being utilized by the algal community when water drains towards the sea. Due to the special topography of this intertidal zone, which has a gentle slope and sandy bottom in the littoral zone enabling some thalli to remain submerged in shallow pools even at low tide, no correlation was found between APA and the vertical distribution patterns. Similarly, Thomas et al. (1987a) found no obvious correlation between nitrate uptake kinetics and vertical distribution patterns for *F. distichus*. The significance of APA in *F. spiralis* was evident when exposed thalli were compared with submerged plants. Removal of thalli from seawater deprives them of their source of nutrients, as shown by the lower tissue P content (Fig. 7). This may stimulate alkaline phosphatase synthesis, which would partly compensate for the intermittent availability of phosphate. Hurd and Dring (1990) demonstrated that *F. spiralis* in the upper littoral zone can take up the same amount of phosphate during a 2-h submersion as other *Fucus* species (*F. vesiculosus* and *F. serratus*) that remain submerged for longer periods over a tidal cycle. Thomas and Turpin (1980) and Thomas et al. (1987b) showed that desiccation enhanced nutrient uptake in *F. distichus* and other intertidal seaweeds. Furthermore, other studies have suggested that emersion produces a general stimulation of several aspects of algal metabolism (Murphy et al. 1986, 1988).

The high APA and N and P contents of the apical region of *F. spiralis* may be compared with previous studies showing high metabolic activity in apical portions of *Fucus* species. Higher nutrient uptake rates have been reported for the apex than middle sections of the same thallus (Topinka 1978, Wallentinus 1984, Hurd and Dring 1990) and much higher nitrate reductase activity in *F. gardneri* in the apical and mid-regions than the stipe (Hurd et al. 1995), coincident with the distribution of APA in the present study. A direct relationship between APA and total P in the thallus has not been reported previously for phytoplankton or macroalgae, but contrasts with the widespread observation that the highest APA occurs when internal P is lowest (Wynne 1981, Gage and Gorham 1985, Hernández et al. 1994).

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