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Desiccation and Phosphate Uptake by Intertidal Fucoid Algae in Relation to Zonation

By CATRIONA L. HURD* and M. J. DRING

Division of Environmental and Evolutionary Biology, School of Biology and Biochemistry, Queen's University, Belfast BT7 1NN, Northern Ireland, UK

Removal of phosphate from ambient sea-water (c. 1 μg-at PO_4 l^{-1}) by four species of fucoid algae subjected to different drying treatments was followed over 2- or 3-h periods. For *Fucus spiralis*, *Ascophyllum nodosum* and *F. serratus*, the rate of uptake of phosphate decreased with increasing loss of water from the thalli, and at least some plants of all three species showed a net loss of phosphate from the thalli over the first 2 h in water following severe desiccation. In *Pelvetia canaliculata*, however, there was no significant effect of desiccation on the mean rate of phosphate uptake, and no plants released phosphate, even after extreme desiccation (up to 85% of volatile water lost). The time courses of phosphate uptake over 3 h following severe desiccation showed that both the rate and the extent of recovery of uptake after resubmersion increased with the height on the shore at which each species is typically found. *P. canaliculata* showed no effect of desiccation even in the first 30 min after resubmersion; some plants of *F. spiralis* lost phosphate during the first 30 min but recovered to the undesiccated rate of uptake after 1·5 h; no plants of *A. nodosum* showed net uptake within 30 min of resubmersion; whereas all plants of *F. serratus* lost large amounts of phosphate in the same period. These results suggest that the sensitivity to desiccation of the nutrient uptake mechanism in different species could contribute to the typical pattern of zonation of these species on rocky shores in N.W. Europe. Phosphate uptake was not enhanced by desiccation in any of the species studied, even in plants of *F. spiralis* which had been incubated in sea-water enriched with nitrate for 24 h prior to the drying treatments.

Intertidal fucoid algae can be divided into two groups with respect to their ability to take up phosphate over a tidal cycle and, within both these groups, the uptake rates of each species are directly correlated with their height in the littoral zonation (Hurd & Dring, 1990). The *Fucus* species have uptake rates that allow them to remove relatively large amounts of phosphate from ambient sea-water, while *Pelvetia canaliculata* and *Ascophyllum nodosum* have lower uptake rates and remove correspondingly less phosphate over a tidal cycle. The uptake measurements on which these conclusions are based were conducted with fully hydrated plants (Hurd & Dring, 1990) but it was recognized that, in their natural habitat, each species would be subjected to different degrees of desiccation and that this may affect phosphate uptake.

Desiccation stress has been shown to "prune back" the upper limits of some fucoid species on the shore through direct damage to the thallus (Schonbeck & Norton, 1978) and to affect differentially the extent of recovery of photosynthesis in different species following severe drying (Dring & Brown, 1982). If severe dehydration affects phosphate uptake in a similar manner, the recovery of phosphate uptake after periods of desiccation may also be critical in determining the zonation of fucoid algae on the shore. However, desiccation appears to enhance the uptake of nitrate and ammonia in four intertidal species on the west coast of Canada (*Pelvetiopsis limitata*, *Fucus distichus*, *Enteromorpha intestinalis* and *Gigartina*

*Present address: Department of Oceanography, University of British Columbia, 6270 University Boulevard, Vancouver, B.C., Canada V6T 1W5.

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papillata; Thomas, Turpin & Harrison, 1987). The percentage desiccation required to produce the maximum enhancement was found to be related to the position occupied by each species on the shore, although the degree of enhancement did not correlate with shore height.

The aims of this work were to investigate the effects of desiccation on phosphate uptake by the complete range of fucoid algae found on north-west European shores, to determine whether phosphate uptake was enhanced following desiccation and to establish the pattern of recovery of uptake after severe desiccation.

MATERIALS AND METHODS

Collection site and preconditioning

Plants of *Pelvetia canaliculata* (L.) Dcne. et Thuret, *Fucus spiralis* L., *Ascophyllum nodosum* (L.) Le Jolis and *Fucus serratus* L. were collected in August and September 1990 from Port Kelly, Co Down, a semi-exposed rocky shore on the east coast of Northern Ireland (Irish grid ref. J628467) at 3·4, 3·1, 2·26 and 0·93 m above chart datum, respectively. Plants were collected between 07.00 and 09.30 h on the morning before an experiment and were transported to the laboratory within 10 min of collection. Plants were cleaned of epiphytes by rinsing them under running sea-water and then wiping the thallus with tissue. They were then cut to the required size using a scalpel blade and placed in aerated, filtered (0·45 µm GF/C) sea-water containing 0·8 µg-at l^{-1} phosphate in darkness at 12°C for 22–26 h (Hurd & Dring, 1990).

Desiccation chamber

Plants were desiccated under controlled conditions of irradiance, temperature, air movement and humidity. In order to vary the degree of desiccation, plants were suspended in a desiccation chamber for different periods of time. The desiccation chamber was based on the design of Thomas & Turpin (1980). Each chamber consisted of a 10-l clear perspex tank, fitted with a clear perspex lid that was sealed to the tank with vaseline during the experiments. One litre of the desiccant silica gel was placed in the bottom of the chambers. The silica gel was reactivated between experiments by placing it overnight in an oven at 100°C. A Micronel 12 v fan (62 mm × 62 mm) placed in each sealed chamber provided air circulation. Five thin perspex strips were positioned across the top of each chamber and one plant was suspended from each strip with nylon line. A maximum of five plants could be fitted into the chamber at any one time. In one chamber, wet tissues replaced the silica gel to provide a "humid chamber". The relative humidity in the chambers was measured using a humidity meter (ELE International Limited) and was 40–50% in the desiccation chamber and 100% in the humid chamber. Irradiance and temperature during all desiccation treatments were 170 µmol m^{-2} s^{-1} and 12°C, respectively.

Measurement of phosphate uptake

Phosphate uptake was measured using the 5-l flask method, which is described in detail by Hurd & Dring (1990). Experiments were carried out in 5-l Pyrex beakers containing 5 l of filtered sea-water maintained at a temperature of 12°C, an irradiance of 170 µmol m^{-2} s^{-1} and with water motion provided by magnetic stirring bars. Phosphate was added to each beaker to give an initial concentration of c. 1·0 µg-at l^{-1}. In each experiment, 20–30 g wet weight of plant material was used. For *Pelvetia canaliculata* and *Fucus spiralis*, whole plants were used but this was not possible for *Ascophyllum nodosum* and *F. serratus* because the desiccation chamber was only 13 cm high. Therefore, apical sections of *F. serratus* measuring about 12 cm were used, while the entire thallus of *A. nodosum* was cut into 12-cm sections.

Experimental procedure

On the morning of the experiment, plants were removed from the preconditioning sea-water, weighed, and placed in either a desiccation chamber or a humid chamber, or returned to the preconditioning treatment. After between 30 min and 5 h in the pre-experimental conditions, the plants were reweighed and placed in the 5-l flask apparatus. Water samples were removed at the start of the experiment and then every 30 min for 3 h. The phosphate concentration of each sample was estimated immediately using the method of Murphy & Riley (1962).

Calculations

The uptake rate was calculated as described by Hurd & Dring (1990). The air dry weight of five plants of each species was measured by suspending the plants in the desiccation chamber

under experimental conditions for 24 h. The volatile water content of each plant was calculated as

$$\text{volatile water} = \frac{\text{fresh weight} - \text{air dry weight}}{\text{fresh weight}}$$

The degree of desiccation was expressed as the % volatile water lost from the plant and was calculated using the following equation

$$\%\ \text{volatile water lost} = \frac{(\text{fresh weight} - \text{dried weight}) \times 100}{\text{fresh weight} \times \text{volatile water}}$$

where volatile water is the mean volatile water for each species as a proportion of fresh weight.

RESULTS

Mean rates of phosphate uptake over 2 h

The mean rate of phosphate uptake over the first 2 h of the experiment was calculated for each plant and plotted against % volatile water lost by that plant (Fig. 1). For *F. spiralis*, *A. nodosum* and *F. serratus*, there was a significant negative correlation ($P < 0{\cdot}001$) between uptake rate and % volatile water lost. After severe desiccation, negative uptake rates were recorded for at least some plants of all three of these species, indicating that phosphate was released from the thalli. In contrast, there was no correlation between uptake rate and % volatile water lost for *P. canaliculata* and, even at the highest levels of desiccation, no plants were observed to lose phosphate from the thalli (Fig. 1).

Time course of phosphate uptake over 3 h

On the basis of the % volatile water lost, each plant was placed in one of the following desiccation groups: preconditioned only, humid (3–8%), low (9–20%), medium (21–55%) and severe desiccation (59–86%). The mean uptake rate of the plants in each group was calculated for every 30-min time interval. In four of the desiccation groups (preconditioned, humid, low and medium desiccation), there were no significant differences over the 3-h experiment among the uptake rates for different time intervals in

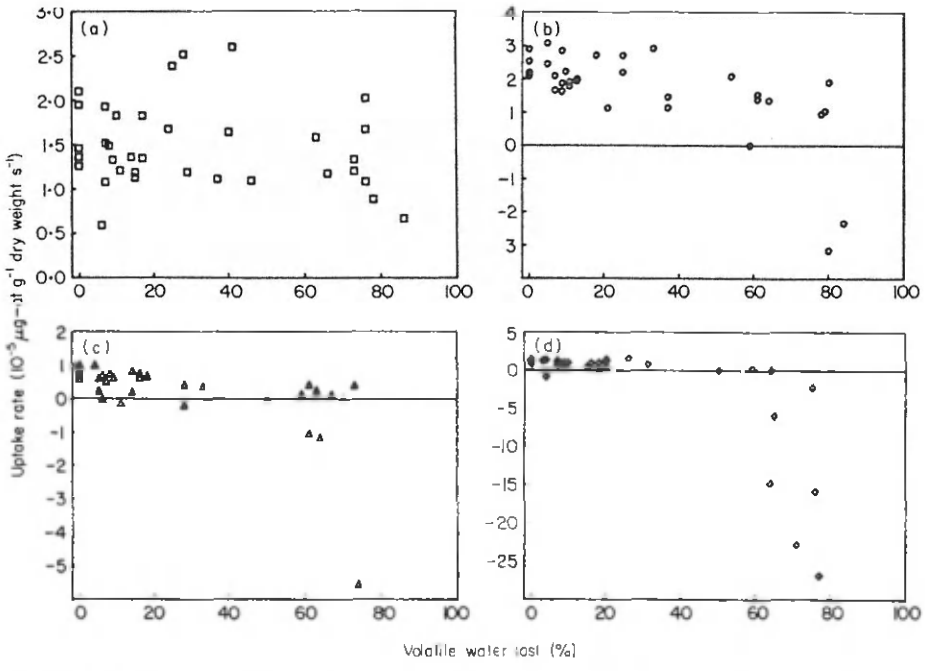

FIG. 1. Mean rate of phosphate uptake by four species of fucoid algae over a 2-h period following different degrees of desiccation. (a) *Pelvetia canaliculata*, (b) *Fucus spiralis*, (c) *Ascophyllum nodosum*, (d) *Fucus serratus*. Each point represents the result obtained for a single plant. Correlation coefficients for each species ($N = 29$–35) are: *Pelvetia*, $r = 0{\cdot}15$; *Fucus spiralis*, $r = -0{\cdot}66$; *Ascophyllum*, $r = -0{\cdot}58$; *F. serratus*, $r = -0{\cdot}60$.

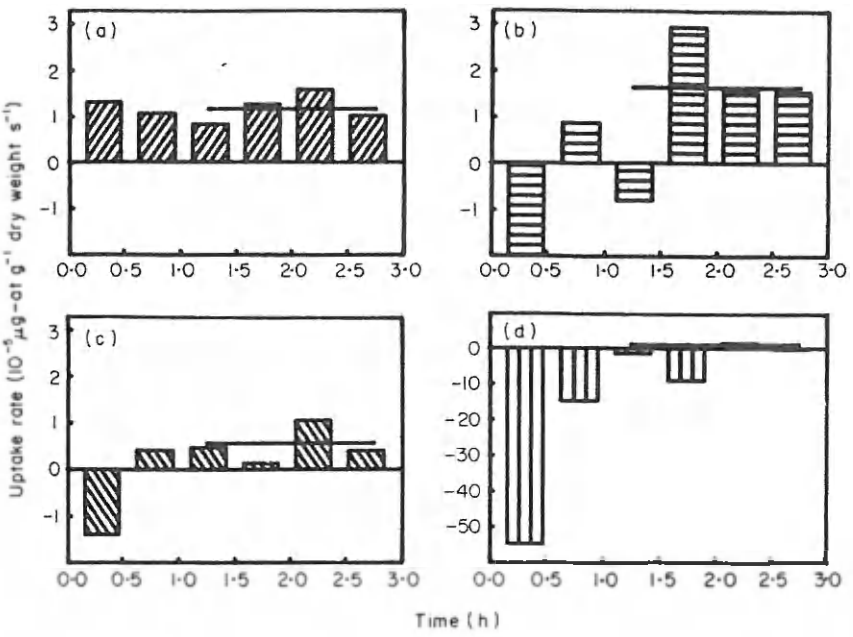

FIG. 2. Variation in the rate of phosphate uptake with time over 3 h in four species of fucoid algae which had been subjected to severe desiccation. (a) *Pelvetia canaliculata*, (b) *Fucus spiralis*, (c) *Ascophyllum nodosum*, (d) *Fucus serratus*. At the start of the uptake experiment, all plants had lost 59–86% of their volatile water, and eight or nine such plants of each species were investigated. The horizontal line covering the last four time periods for each species represents the mean rate of uptake by undesiccated plants of the same species.

any of the species, largely because of the high variability recorded among the replicate plants exposed to each treatment. In the severe desiccation group, however, different responses to desiccation were recorded for the different species (Fig. 2).

Pelvetia canaliculata maintained a positive uptake rate throughout the experiment, and there were no significant variations with time over the 3 h. The uptake rate of severely desiccated *P. canaliculata* was similar to that of fully hydrated plants (Fig. 2). The mean uptake rate recorded for *F. spiralis* over the first 30-min interval was negative, although this rate was not significantly different (at $P = 0{\cdot}05$) from the means for later time periods. Nevertheless, some plants in the population leaked phosphate on their return to water after severe drying. The mean uptake rate of *F. spiralis* from 1·5 h onwards was similar to that of fully hydrated plants.

The mean uptake rate of severely desiccated plants of *A. nodosum* during the first 30 min after return to water was also negative and, unlike the comparable value for *F. spiralis*, was significantly lower than the mean uptake rate over the remaining 2·5 h of the experiment. During this latter period, *A. nodosum* exhibited a positive uptake rate which was high enough to compensate for the phosphate lost over the first 30 min so that there was no net loss of phosphate over the complete experiment. Severely desiccated plants of *F. serratus* released 20–30 times more phosphate than the other species during the first 30 min after submersion, and continued to release phosphate over the next 1·5 h (Fig. 2). A positive uptake rate was recorded in only one 30-min period (2–2·5 h) and, over the full 3-h experiment, there was a substantial net loss of phosphate.

Effect of nitrate supply on response of *F. spiralis* to desiccation

The effects of desiccation on phosphate uptake by *F. spiralis* were also studied in plants which had been preconditioned in seawater enriched with 880 µg-at l^{-1} KNO_3 (equivalent to the nitrate concentration in f/2

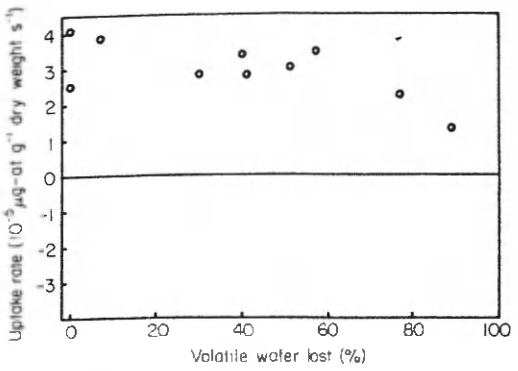

FIG. 3. Mean rate of phosphate uptake by *Fucus spiralis* over a 2-h period following different degrees of desiccation of plants which had been preconditioned for about 24 h in sea-water enriched with nitrate. Each point represents the result obtained for a single plant. The correlation coefficient ($r = -0{\cdot}68$) is significant at $P = 0{\cdot}05$.

medium; McLachlan, 1973). The uptake rates of these plants were similar to those of *F. spiralis* plants preconditioned in ambient sea-water (NO_3 concentration = 0–0·2 µg-at NO_3-N l^{-1}) and a similar negative correlation was observed between % volatile water lost and phosphate uptake rate (Fig. 3). However, no plants lost phosphate or showed zero uptake over 2 h, as was observed for some plants which had not been pretreated with nitrate [Fig. 1(b)].

DISCUSSION

Unlike most higher plants, seaweeds possess no anatomical features, such as stomata or waxy cuticules, that enable them to reduce the rate of water loss (Levitt, 1980). The *Fucus* species, *P. canaliculata* and *A. nodosum* all lose water from their thalli at similar rates (Kristensen, 1968; Schonbeck & Norton, 1979) and the rate of water loss is only slightly slower than evaporation from free sea-water (Schonbeck & Norton, 1979). Jones & Norton (1979) concluded that the rate of evaporation from the fronds of fucoid algae was controlled primarily by environmental factors. Fucoid algae must, therefore, be described as desiccation tolerators rather than desiccation avoiders (Rugg & Norton, 1987), and must be able to decrease or repair the damage caused by desiccation stress (Levitt, 1980).

With respect to phosphate uptake, all the species studied here show some degree of desiccation tolerance because, after losing up to 50% of their volatile water, most of the plants tested showed a positive uptake rate over 2 h (Fig. 1). The degree of tolerance increases with increasing shore height. *Pelvetia canaliculata* appears to be the most desiccation tolerant of all the species studied as it showed no decrease in the uptake rate measured over 2 h, even after severe desiccation (Fig. 1). Even during the first 30 min after resubmersion, the uptake rates of plants that had been severely desiccated was as high as those of fully hydrated plants (Fig. 2). *Fucus spiralis*, *A. nodosum* and *F. serratus* all showed a decrease in uptake rate with increasing loss of volatile water and, after severe desiccation, phosphate leaked from the thalli of at least some plants. The rate of loss of phosphate of these three species immediately after resubmersion was related to their position on the shore: only two plants out of nine replicates of *F. spiralis* released phosphate after severe desiccation (59–83% volatile water lost), whereas all plants of *A. nodosum* either released phosphate or showed zero uptake over the first 30 min. All plants of *F. serratus* lost phosphate at rates 20–30 times greater than those of the other species.

Release of inorganic and organic substances after desiccation has been previously recorded for intertidal algae. On the west coast of Canada, natural populations of *Gracilaria pacifica* are found growing on the high and low shore and also subtidally. The degree of desiccation required to give a negative uptake rate was found to correlate with the positions of the plants on the shore: high shore plants showed no negative uptake after losing 50% of their total water content, while subtidal plants leaked nitrate after only 10% water loss (Thomas, Harrison & Turpin, 1987). Organic carbon may also be released after severe desiccation. Moebus, Johnson & Sieburth (1974) showed that when

A. nodosum lost over 70% of its water, 1–2% of its total dry matter was lost as organic carbon.

The release of both inorganic and organic substances from plants following desiccation may indicate that the cell membrane has been disrupted (Levitt, 1980). The degree to which a plant can minimize this disruption, or the rate at which it can repair the damage, may be critical in determining the maximum height at which intertidal fucoid algae can grow on the shore. Since *P. canaliculata* released no phosphate after severe desiccation, this species is clearly able to resist or immediately repair the effects of desiccation on membrane integrity. Both *F. spiralis* and *A. nodosum* released phosphate for the first 30 min after resubmergence, but the uptake rate of both species recovered so that the mean uptake rate over the last 2 h was similar to that of hydrated plants. These species appear, therefore, to suffer damage to the cell membrane but repair is effected relatively quickly. *Fucus serratus*, on the other hand, seems to be so severely damaged by prolonged desiccation that repair and recovery is not possible within 3 h.

A similar interpretation could be applied to a set of comparable results obtained in a study of the effects of desiccation on photosynthesis in brown algae (Dring & Brown, 1982). Full recovery of photosynthesis was observed in *Pelvetia* after 96% desiccation; most plants of *F. spiralis* recovered fully after 80% desiccation, although some plants recovered to only 60% of the predesiccated photosynthetic rate; and *F. serratus* failed to recover fully after losing only 60% of its tissue water. It is possible that the rate and extent of recovery of photosynthesis after desiccation is also related to the extent of membrane damage and the rate of its repair.

If more than 20% of the bound water is lost through dehydration, the bilayer structure of a typical cell membrane collapses and is replaced by a hexagonal structure in which the hydrophobic tails of the lipid molecules point outwards (Simon, 1978). The membrane proteins, which are usually found within the bilayer, may be displaced during this hexagonal phase. Upon rehydration, the membrane may reform the bilayer within 10 s but complications, such as membrane components being pushed aside by the rapid influx of water, may lead to a delay in reforming the bilayer. During this delay, ions could leak out of the cell and, if the damage caused by incoming water is severe, this leakage could result in the death of the cell (Simon, 1978). If cell membranes of fucoid algae undergo this transition to the hexagonal phase following dehydration, variations in the degree of damage caused upon rehydration, and the speed at which the injury is repaired, could account for the differential recovery of both phosphate uptake and photosynthesis in different species.

Phosphate uptake was not enhanced by desiccation in any of the intertidal fucoid algae studied here, including plants of *F. spiralis* which had been preconditioned in sea-water containing saturating levels of nitrate (Fig. 3). These observations contrast with those of Thomas, Turpin & Harrison (1987) who found that nitrate and ammonia uptake by *Pelvetiopsis limitata*, *F. distichus*, *Enteromorpha intestinalis* and *Gigartina papillata* were increased following desiccation, and that the degree of desiccation that produced the maximum enhancement was correlated with the position of the species on the shore.

Since two of the species studied by Thomas, Turpin & Harrison (1987; *P. limitata*, *F. distichus*) are similar to species used in the present investigation, it seems unlikely that the absence of enhancement can be attributed purely to differences between species. However, the uptake experiments of Thomas, Turpin & Harrison (1987) were conducted over short periods (10–30 min) at high, and possibly saturating, concentrations of nitrate and ammonia (30 μg-at l^{-1}, compared with ambient levels of < 1 μg-at l^{-1}), whereas the current work utilized phosphate concentrations that were close to the maximum concentrations that the plants would experience in the sea (c. 1 μg-at l^{-1}) and measured uptake over

longer time periods. It is possible, therefore, that Thomas, Turpin & Harrison (1987) observed the enhancement of short-term, "luxury" uptake following desiccation, whereas longer-term uptake of nutrients from concentrations more typical of natural sea-water shows the type of response to desiccation reported here. It is also possible that, as suggested by Thomas, Turpin & Harrison (1987), enhancement of nutrient uptake by desiccation occurs only after repeated periods of drying, such as plants would experience during successive low tides in hot weather. The influence of these factors on the response of nutrient uptake to desiccation remains to be investigated.

The uptake of phosphate was also enhanced by desiccation in *F. distichus*, provided that the plants had been incubated in sea-water enriched with all nutrients except phosphate for 24 h prior to the drying treatments; without such pretreatment, drying inhibited phosphate uptake (Thomas & Turpin, 1980). This observation again suggests that it is short-term, luxury uptake of nutrients which is enhanced by desiccation, since luxury uptake would occur only in plants which had previously been starved of the nutrient concerned. The apparent absence of desiccation-enhanced phosphate uptake in *F. spiralis* which had been pretreated in a high nitrate concentration (Fig. 3) could, therefore, be due to an inability to detect luxury uptake in experiments at ambient phosphate concentrations (cf. 30-min experiment at 30 μg-at l^{-1}; Thomas & Turpin, 1980). Enhancement of nutrient uptake by desiccation may be of rather limited significance to plants in natural conditions, and certainly seems to be less important in fucoids than the damaging effects of desiccation on the nutrient uptake rates of low shore species.

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