

Photosynthetic tolerances to desiccation of tropical intertidal seagrasses

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ABSTRACT: The aim of the present study was to investigate whether tropical intertidal seagrasses were better adapted to tolerate desiccation than subtidally growing seagrasses. To do this, the photosynthetic performance of 8 seagrass species, growing from the upper intertidal to the shallow subtidal in Zanzibar, East Africa, was studied during the event of air exposure and the subsequent rehydration. Photosynthetic efficiencies were measured by pulse amplitude modulated (PAM) fluorometry as effective electron quantum yields of photosystem II (Y) since it had recently been shown that this measure parallels rates of O₂ evolution for several species under a defined irradiance. Contrary to our expectations, it was found that the shallow intertidal species were in general more sensitive to desiccation than the deeper species. This was expressed both as a faster decline in Y at decreasing water contents and as an inability to regain full photosynthetic rates during rehydration following even mild desiccation, as compared with the deeper-growing species. One exception was the subtidally growing *Syringodium isoetifolium*, which was very sensitive to desiccation. The 2 species which grow highest up in the intertidal zone, *Halophila ovalis* and *Halodule wrightii*, may not desiccate much *in situ* during low tide because the leaves lie flat on the moist sand and, for the latter species, overlap one another so as to minimise water loss. Thus, it seems that desiccation tolerance is not a trait which determines the vertical zonation of tropical seagrasses. Rather, it is hypothesised that the ability to tolerate high irradiances, as well as to benefit from high nutrient inputs from the shore, allows the shallow species to occupy the uppermost intertidal zone.

KEY WORDS: Desiccation · PAM fluorometry · Photosynthesis · Seagrass · Tropical

INTRODUCTION

Marine angiosperms, or seagrasses, are generally thought of as plants which grow submerged in seawater. However, several species grow in intertidal habitats where they are exposed to air for part of the day. This has been reported for temperate species of *Zostera* (Bulthuis 1995, Vermaat & Verhagen 1996, Ramage & Schiel 1998). On the physiological level, it was shown that both *Z. noltii* and *Z. marina* could maintain high rates of photosynthesis in air provided that their leaves were fully hydrated (Leuschner & Rees 1993), but that such rates were negatively affected by water loss in a linear fashion (Leuschner et

al. 1998). In addition, more tropical species can grow in the intertidal, where they often endure extreme conditions of high irradiance and a dry, desiccating, atmosphere (Jupp et al. 1996). Although emergence has generally been viewed as adverse for seagrass development (Yabe et al. 1995, Stapel et al. 1997), the physiological responses which underlie the potential of such tropical seagrasses to grow in the intertidal have not been studied before.

Unlike for seagrasses, physiological responses to air exposure have been studied extensively for intertidally growing marine macroalgae (see e.g. a recent review by Davison & Pearson 1996). Regarding desiccation, it was shown that species growing in the upper intertidal zone could maintain their photosynthetic rates at relatively low water contents of the thalli and/or that they possessed the ability to regain high rates of photo-

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synthesis shortly after re-submersion (Dring & Brown 1982, Brown 1987, Einav et al. 1995). On the ecological level, it has long been considered that these and similar physiological responses (cf. Chapman 1966) reflect the different species' ability to occupy various niches along the vertical gradient of the intertidal zone. This finally results in the typical algal zonation pattern found in many intertidal areas.

Given the paucity of eco-physiological studies of tropical intertidal seagrasses, we set out to test the desiccation tolerances of several species from Zanzibar, East Africa. The present study is a continuation of previous ones initiated by Björk et al. (1997), who concluded that shallow-growing species in general possessed an efficient way of acquiring inorganic carbon from the seawater, including catalysed extracellular HCO_3^- to CO_2 conversions and possibly also direct HCO_3^- uptake. Based on this, it was suggested that those species growing highest up in the intertidal zone were best adapted to utilise the abundant irradiance found there. In another study, it was shown that the 2 species growing highest up (*Halophila ovalis* and *Halodule wrightii*) were resistant to high irradiances as indicated by the fact that down-regulation of photosynthesis during a mid-day low tide was minimal (Beer & Björk 2000). All measurements in both studies were carried out in water on fully hydrated plants, and no consideration was given to their hydration status during emergence. Here, we report on the photosynthetic performance of 8 seagrass species from Zanzibar during desiccation and following rehydration. This was done in order to investigate basic processes in response to emergence *per se*, but also in an attempt to find a physiological cause for their marked vertical zonation pattern within a tropical intertidal ecosystem.

MATERIALS AND METHODS

Study area. This study was performed at the Institute of Marine Sciences, University of Dar-Es-Salaam, Zanzibar, Tanzania. Seagrasses were collected from the Mbweni Beach area located ca 5 km south of Zanzibar Town. The area is a large sand flat which is mostly exposed at low tide; the vertical tidal difference is up to 4 m. The first seagrass species to emerge with the outgoing tide is *Halophila ovalis*. The second highest growing species is *Halodule wrightii*, followed by *Cymodocea rotundata* and *Thalassia hemprichii*, which only become exposed to air around spring tides. *C. serrulata* grows in the upper subtidal and *Syringodium isoetifolium*, *Thalassodendron ciliatum* and

Enhalus acoroides grow in deeper water. The vertical distribution of these species is depicted in Fig. 1.

Whole plants were collected at low tide, and kept in aerated seawater in dim light for a maximum of 3 h before experimentation. All experiments were carried out in the laboratory of the Institute of Marine Sciences, Zanzibar.

Experimental set-up. The electron quantum yield of photosystem II (Y) was measured with a pulse amplitude modulated (PAM) fluorometer (the Diving-PAM, Waltz). This parameter was measured during steady-state photosynthesis in the light using the saturating-pulse method (Schreiber & Bilger 1993) as $(F_m' - F_t)/F_m'$, where F_m' designates maximal fluorescence during an 0.8 s saturating light pulse, and F_t is the steady state fluorescence at a given irradiance.

For desiccation experiments, a leaf of a specific seagrass was placed on a balance, with the main optical light guide of the PAM mounted ca 8 mm from, and at a 60° angle to, the leaf surface. At that time, the fully hydrated weight (HW) was also recorded. The leaf was irradiated with a photosynthetic photon flux (PPF) of $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at a temperature of 28°C . Y was then recorded every minute together with the concomitant weight (CW) of the leaf. After the termination of each experiment, the dry weight of the leaf (DW) was determined after drying at 60° for 24 h, and its relative water content at each fluorescence measurement during the experiments was calculated as $(\text{CW} - \text{DW})/(\text{HW} - \text{DW})$.

For the rehydration experiments, leaves were dried, under the same environment as in the desiccation experiments, to various pre-determined water contents ranging between 15 and 60%. Each leaf was then

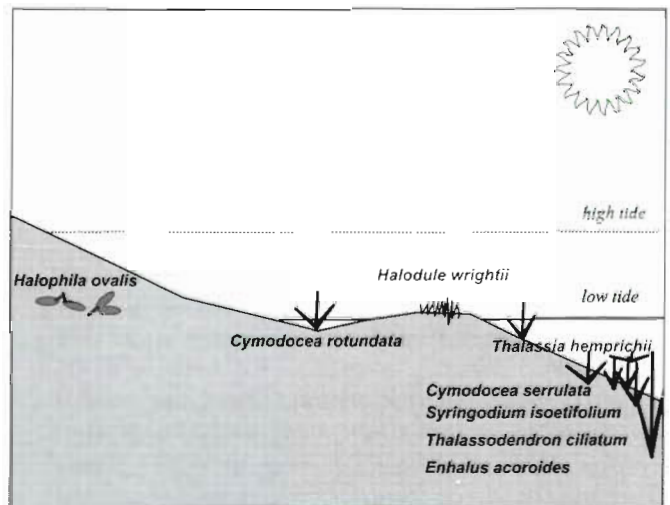


Fig. 1. Distribution of 8 seagrass species along the intertidal and shallow subtidal depth gradient of Mbweni Beach, Zanzibar

mounted on the light cable of the diving-PAM with the 'leaf distance clip' (Walz, Germany), which was then submerged in 28°C seawater at a PPF of 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Y was measured every minute until stable values were obtained.

RESULTS

The results of the desiccation and rehydration experiments for the upper, intertidally growing seagrasses are given in Fig. 2. *Halophila ovalis*, the uppermost

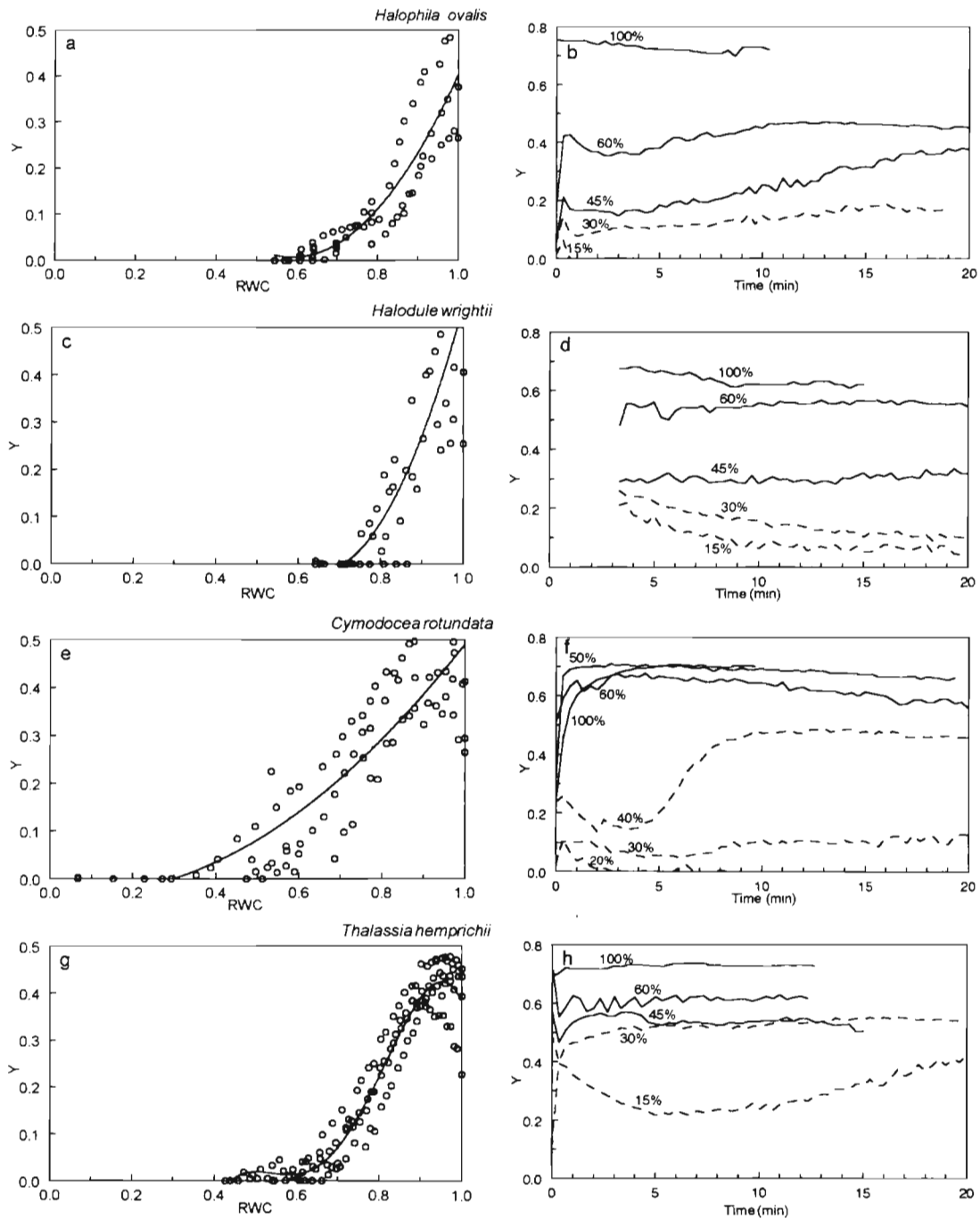


Fig. 2. (a,b) *Halophila ovalis*; (c,d) *Halodule wrightii*; (e,f) *Cymodocea rotundata*; (g,h) *Thalassia hemprichii*. Electron quantum yields of photosystem II (Y) as a function of relative water contents (RWC) of the leaves (left panel, 3 to 5 replicate leaves) during emergence, and (b,d,f,h) after re-submergence following desiccation to predetermined water contents for various shallow-growing intertidal seagrasses

growing species, was very sensitive to desiccation as indicated by a drop in Y to half its original value after losing only 10% of its water (Fig. 2a), and by not being able to regain the original activity after losing 40% (i.e. containing 60%) water (Fig. 2b). Similar responses were obtained for *Halodule wrightii* (Fig. 2c,d), but this plant showed a slightly higher tolerance to desiccation in that it was able to regain a higher Y following a 40% water loss. Both *Cymodocea rotundata* and *Thalassia hemprichii*, growing in the lower intertidal zone, were more tolerant to desiccation. This is reflected in 50% of the Y remaining at a lower water content during desiccation (75 and 80%, respectively; Fig. 2e,g), and by the ability to restore close to original Y values after substantial water losses. Within this group, *Thalassia hemprichii* showed a remarkable capacity to regain close to its original Y even after losing 85% water.

Cymodocea serrulata, an upper subtidal species, was relatively resistant to the effect of desiccation during emergence, but could not regain its original Y after losing 55% water (Fig. 3a,b). The subtidal species *Thalassodendron ciliatum* and *Enhalus acoroides* showed even higher tolerances to desiccation as they were able to regain full Y s after desiccating to 45% of their original water contents (Fig. 3d,f). One difference between these 2 species was that *E. acoroides* was able to regain part of its photosynthetic activity after a water loss of 85% during re-submergence, while *T. ciliatum* seemed to be irreversibly damaged by this desiccation level. *Syringodium isoetifolium* was the only subtidal species which was very sensitive to desiccation. This is evidenced in a 50% drop in Y at less than 10% water loss, and the inability to regain close to a full Y after only a 40% water loss.

A different measure of desiccation resistance is the time it takes for the leaves to lose water when exposed to the air. The time it took for leaves to lose 50% of their original water content was very similar for all species except *Enhalus acoroides* and *Syringodium isoetifolium*, which became desiccated at a

much slower rate (Table 1). This is probably due to their leaves being much thicker than the leaves of the other species.

DISCUSSION

The various ways in which physiological desiccation tolerances of intertidal plants can be appreciated include measurements of photosynthetic rates during desiccation (Beer & Eshel 1983, Leuschner et al. 1998) and following re-submersion (Beer & Kautsky 1992). These are measured either CO_2 (in air) or O_2 (in water) exchange. In this work, PAM fluorometry was used to measure Y (the effective quantum yield of photosystem II) of seagrasses being affected by desiccation. This was done since it had earlier been shown that rates of photosynthetic electron transport (ETR) based on measurements of Y were positively correlated with rates of photosynthetic O_2 evolution in a number of species (Beer et al. 1998), including *Halophila ovalis* and *Halodule wrightii* (Beer & Björk 2000). Because the ETR is principally derived from multiplying Y with the irradiance level (Beer et al. 1998, Beer & Björk 2000), it follows that Y is proportional to O_2 evolution at a constant irradiance. One advantage of PAM fluorometry over gas exchange measurements is that the plants need not be confined to enclosures. Thus, Y could here be measured repetitively while simultaneously estimating water contents by simply leaving a leaf on a balance throughout the time course of measurements.

Using Y as a measure of the photosynthetic ability, we found no correlation between the desiccation tolerance of 8 tropical seagrass species and their vertical distribution along a depth gradient ranging from the upper intertidal to the shallow subtidal. For example, *Halophila ovalis* and *Halodule wrightii* growing in the upper intertidal were among the least tolerant species while *Thalassodendron ciliatum* and *Enhalus acoroides* from the subtidal were among the most tolerant ones. There is only 1 other investigation in which correlations between desiccation responses and growth habitat of seagrasses has been indicated. Leuschner et al. (1998) found that the temperate *Zostera nolitti*, which grew on more elevated sites in the intertidal of the German Wadden Sea than did *Z. marina*, maintained high photosynthetic rates at lower leaf water contents than did the latter. However, no comparison was made between the species regarding their capacity to regain photosynthetic activity during re-submersion. Our results are unlike those for marine macroalgae, where a positive correlation between physiological adaptations to emergence and vertical zonation is often present. In particular the capacity to regain high rates of gas exchange upon re-submersion following desiccation was

Table 1. Time taken for leaves of the various seagrass species to lose 50% of their original water content (calculated as $(CW-DW)/(HW-DW)$, see 'Experimental set-up'). The experiment was carried out in the laboratory at 26°C and 75% relative humidity. Data are average of 5 replicates \pm SD

Seagrass	Time (min) \pm SD
<i>Cymodocea rotundata</i>	10 \pm 3
<i>Cymodocea serrulata</i>	11 \pm 4
<i>Thalassodendron ciliatum</i>	11 \pm 4
<i>Halodule wrightii</i>	14 \pm 2
<i>Halophila ovalis</i>	14 \pm 3
<i>Enhalus acoroides</i>	37 \pm 5
<i>Syringodium isoetifolium</i>	45 \pm 3

found to correlate with the ability of temperate algae to grow at an elevated position in the intertidal (e.g. Dring & Brown 1982). For example *Fucus spiralis*, growing in the upper intertidal, was able to tolerate desiccation better than other *Fucus* species growing lower down

(Beer & Kautsky 1992). For the seagrasses studied here, as well as 3 species of *Fucus* (cf. Beer & Kautsky 1992), the rates of water loss did not indicate that species of the upper intertidal possessed any special means to reduce water loss from the leaves.

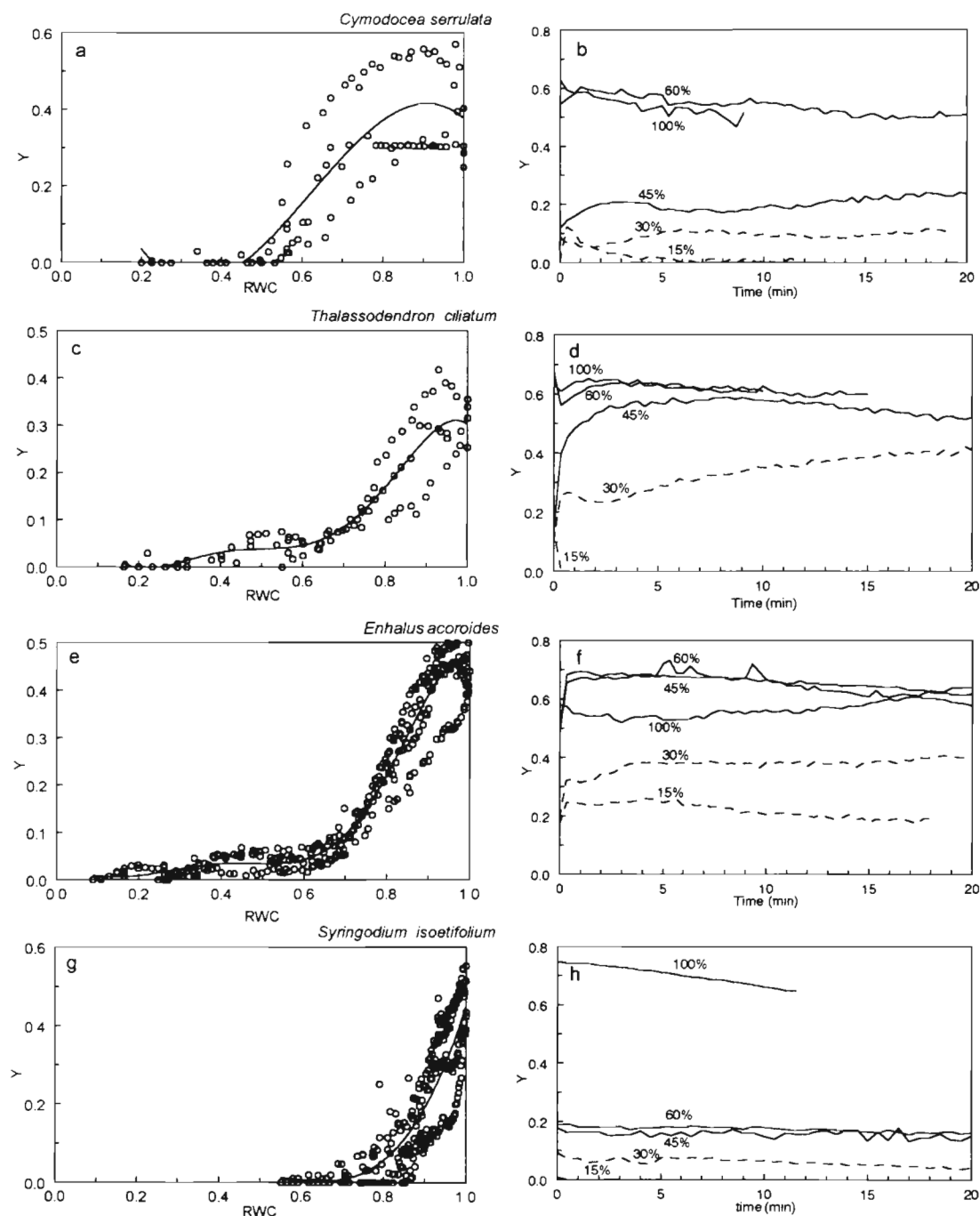


Fig. 3. (a,b) *Cymodocea serrulata*; (c,d) *Thalassodendron ciliatum*; (e,f) *Enhalus acoroides*; (g,h) *Syringodium isoetifolium*. Electron quantum yields of photosystem II (Y) as a function of relative water contents (RWC) of the leaves (a,c,e,g, 3 to 5 replicate leaves) during emergence, and (b,d,f,h) after re-submergence following desiccation to predetermined water contents for various deeper-growing intertidal and subtidal seagrasses

The results of this study indicate that even though seagrasses do grow in the tropical intertidal, physiological adaptations to prevent or endure water loss are not traits which determine these plants' ability to grow in this habitat, and are therefore not a cause for the vertical zonation pattern. Given that the 2 uppermost growing species showed the highest sensitivity to desiccation, one may ask how they can survive air exposure at all during low tides? We observed that these plants, although growing under emergent conditions for several hours a day, may actually not desiccate much in the field. For *Halophila ovalis*, this is because the leaves are supported by thin and flexible petioles which allow them to bend down so as to lie flat against the sand when the water retreats during low tide. This sand is always kept moist by pore-bound seawater and by water seepage from higher elevations on the shore. In addition to being thin and flexible, the often densely growing leaves of *Halodule wrightii* also cover one another during low tide so as to reduce evaporation from those leaves not lying directly on the moist sand. Thus, it seems that it is the ability of these seagrasses to 'avoid' excessive water loss (rather than coping with it on the physiological level) that enables them to evade the otherwise detrimental effects of desiccation. In contrast, it is suggested that the deeper-growing species which have larger and more erect leaves would be affected more directly by the desiccating atmosphere if they were to be exposed to air. Indeed, those parts of the leaves of *Cymodocea rotundata* and *Thalassia hemprichii* which became air-exposed during extremely low tides died.

If there is no physiological adaptation to desiccation, what is it then that allows the thin-leaved *Halophila ovalis* and *Halodule wrightii* to grow preferentially in the upper intertidal? In a previous study it was found that these 2 species could utilise inorganic carbon very efficiently (Björk et al. 1997). This notion was supported by the observation that they were well adapted to tolerate the high irradiances found *in situ* as indicated by the low extent of photosynthetic down-regulation during the high ambient mid-day irradiances (Beer & Björk 2000). In addition, at the site where this investigation was conducted, these plants may have benefited more than other species from the nutrient-rich runoff water seeping through the sand of the upper intertidal. This is because they are smaller, and thus have a shallower root system than the larger species, which would limit their nutrient supply if they grew farther away from the shore.

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