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Short communication

Photosynthetic tolerances to desiccation of the co-occurring seagrasses Halophila johnsonii and Halophila decipiens

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ABSTRACT

Along the coastal lagoons of southeast Florida, *Halophila johnsonii* and *Halophila decipiens* co-occur subtidally, but only *H. johnsonii* occurs intertidally. Photosynthetic tolerances to desiccation in light and dark conditions were compared between *H. johnsonii* and *H. decipiens* using pulse amplitude-modulated (PAM) fluorescence measurements. Under light conditions (250 µmol photons $m^{-2} s^{-1}PAR$), both species exhibited near-linear decreases in effective quantum yields ($\Delta F/F'_m$) with increasing desiccation. The most rapid decline in $\Delta F/F'_m$ was observed in *H. johnsonii*. Under dark conditions, both seagrasses exhibited small decreases in maximum quantum yields (F_v/F_m) during desiccation until reaching a critical threshold of relative water content (RWC_{critical}). RWC_{critical} was significantly lower for *H. johnsonii* (0.21 ± 0.086) compared to *H. decipiens* (0.37 ± 0.15). However, the rate of decline in F_v/F_m below RWC_{critical} was greater in *H. johnsonii* than *H. decipiens*. Water loss rates, however, were not significantly different between these two species in either light or dark conditions (0.18 ± 0.047 g min⁻¹ light, 0.12 ± 0.045 g min⁻¹ light, 0.099 ± 0.044 g min⁻¹ dark for *H. johnsonii* and *H. decipiens*, respectively). Results from this study suggest that lower desiccation tolerance, at the leaf level, is not the primary factor precluding intertidal distribution of *H. decipiens*.

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1. Introduction

The seagrass *Halophila johnsonii* is an endemic species with a restricted distribution range, occurring within a 200-km segment of the coastal lagoons and bays on the east coast of Florida, between Sebastian Inlet and Biscayne Bay (Kenworthy, 1992). It is a shallow-growing seagrass, found intertidally to 3-m deep (Virnstein and Morris, 2007). Within its habitat range, *H. johnsonii* often grows intermixed subtidally with its con-specific, *Halophila decipiens*. However, *H. decipiens* has a global distribution and a wide depth range (0–85 m) but is generally not found intertidally (den Hartog, 1970).

H. johnsonii has a greater tolerance to high light than *H. decipiens*, exhibiting less photoinhibition or downregulation at high light intensities (Dawes et al., 1989; Durako et al., 2003). *H. johnsonii* also possesses UV-absorbing flavonoid pigments (UVPs) that are not present in *H. decipiens* (Durako et al., 2003; Krzysiak, 2006). It has been suggested that these UVPs may perform a 'sunscreen' function in *H. johnsonii*, increasing its tolerance to high light and UV levels (Durako et al., 2003; Kunzelman et al., 2005). However, the possible role that desiccation tolerance may play in

the vertical distribution of these two con-specifics has not been examined.

Several studies have examined physiological and morphological mechanisms for desiccation tolerance in determining the vertical distribution of con-specific and co-occurring intertidal seagrasses. Ramírez-García et al. (1998) reported that the depth distribution of Phyllospadix torreyi and P. scouleri in Baja California, Mexico, correlated with desiccation tolerances of the two species. This was also the case for Halodule uninervis and Thalassia hemprichii distribution in Taiwan (Lan et al., 2005). Although both Zostera marina and Zostera noltii grow intertidally, Z. marina has a lower tolerance to desiccation than Z. noltii due to a reduction in photosynthesis upon exposure (Leuschner et al., 1998); indicating that although one species is less tolerant, desiccation may not be the determinant factor in depth distribution. Desiccation response differences between Zostera japonica and Z. marina also could not solely account for the distribution of these two seagrasses in intertidal habitats (Shafer et al., 2007). This is in concordance with other studies that have found that variation in tolerance to desiccation among seagrasses may not correlate directly with their distribution and depth limits (Björk et al., 1999; Tanaka and Nakaoka, 2004).

The objective of this study was to determine whether differences in desiccation tolerance play a role in the observed





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upper-depth distribution limits of *H. johnsonii* and *H. decipiens.* In contrast to previous studies, desiccation was examined under both dark and light conditions to determine whether there were diel differences in species sensitivity to desiccation and air exposure.

2. Materials and methods

2.1. Material collection

Cores $(9 \text{ cm} \times 9 \text{ cm})$ of *H. johnsonii* and *H. decipiens* were collected at a 1-m deep site near Taylor Creek, FL $(27^{\circ}28.123'\text{N}, 80^{\circ}19.563'\text{W})$ in August 2007. Core samples were inserted into 9 cm^2 peat pots with ambient sediments and transported in coolers to the Center for Marine Science in Wilmington, NC and maintained in filtered seawater (salinity of 36 ± 2) in a temperature-controlled greenhouse under augmented light conditions (500 W mercury halide lamps) on a 12:12 light:dark cycle. Experiments were performed in October 2007. Two blades from the third leaf pair back from a rhizome apical were selected from plants within randomly selected peat pots for each replicate of the experiments.

2.2. Experimental set-up

The fiber optic cable of a pulse amplitude-modulated (PAM) fluorometer (mini-PAM WALZ GmbH) was positioned 5 mm above and at an angle of 90° relative to the weigh-pan of an electronic balance. For each desiccation run, leaf blades were gently blotted on a paper towel to remove excess water. The leaves were then placed adaxial side up side-by-side on the balance weigh pan, centered under the PAM fiber optic. An initial fully hydrated (wet) weight was then measured (W_w) . Simultaneous quantum yield and plant weight (W_t) measurements were obtained at 1-min intervals over the duration of the light and dark experiments. A comparative dark experimental series using 5-min measurement intervals indicated that the 1-min interval light-pulses were not actinic. Ten replicates were run for each experiment (light and dark) and PAM fluorometer settings remained constant for all experimental runs. At the end of each experimental run, leaf blades were placed in a drying oven at 60 °C for 24 h, after which time the dry weight (W_d) was measured. Relative water content (RWC) for each replicate was calculated as the following (Björk et al., 1999):

$$RWC = \frac{W_t - W_d}{W_w - W_d}$$

2.3. Photosynthetic responses to desiccation under light conditions

This experimental series was conducted with leaves exposed to 250 μ mol photons m⁻² s⁻¹ irradiance. This irradiance was determined to be just below saturation irradiance for these two species by preliminary rapid light curve measurements (data not shown). The tip of the optical fiber of a microscope illuminator was positioned at a distance of 10 cm and an angle of 60° above the weigh-pan of the balance so that the blades were fully irradiated (i.e. not shaded by the PAM optical fiber). In this experimental series, leaves were not dark-adapted, thus, effective quantum yield was calculated:

$$\frac{F'_{\rm m}-F}{F'_{\rm m}} = \frac{\Delta F}{F'_{\rm m}}$$

where $F'_{\rm m}$ is the maximum fluorescence measured after a 0.8-s pulse of light and F is the fluorescence measured under the experimental irradiance (Genty et al., 1989). Simultaneous quantum yield and plant weight (W_t) measurements were obtained every minute until $\Delta F/F'_{\rm m}$ reached zero. Light desiccation

data were fitted to a linear regression model (Sigmaplot v9.0, SPSS Inc.). Analysis of covariance (ANCOVA) was used to determine if significant (P < 0.05) differences existed between the regression slopes or intercepts for the two species (SAS).

2.4. Photosynthetic responses to desiccation in the dark

For the dark experiments, leaf blades were removed from the plant and maintained submerged in the dark for 10 min prior to commencing the experimental trials. The balance with the PAM fiber optic cable was enclosed in black plastic sheeting so that the leaves received no external illumination during the experimental period. Experiments were run until there was a consistent decline in quantum yield but no change in weight for a minimum period of 5 min.

Maximum quantum yield was calculated for the dark experimental runs:

$$\frac{F_{\rm m}-F_{\rm 0}}{F_{\rm m}}=\frac{F_{\rm v}}{F_{\rm m}}$$

where F_0 is the dark-adapted fluorescence minimum and F_m is maximal fluorescence after a 0.8-s saturating light pulse (Beer and Björk, 2000). Dark desiccation data were fitted to a hyperbolic tangent model:

$$\frac{F_{\rm v}}{F_{\rm m}} = A \tanh \frac{B \times \rm RWC}{A}$$

using a Marquardt–Levenberg non-linear regression algorithm (Sigmaplot v9.0, SPSS Inc.). Using this model, the initial rate of change in F_v/F_m (*A*), the critical RWC threshold (*A*/*B*, hereafter referred to as RWC_{critical}), and the rate of change in F_v/F_m (*B*) below RWC_{critical} were calculated. One-way ANOVAs were used to determine if these parameter estimates were significantly (P < 0.05) different between the two species (Sigmastat v3.5, SPSS Inc.).

2.5. Desiccation rate

Desiccation coefficients were calculated by fitting the change in RWC over time to an exponential decay function (Sigmaplot v9.0, SPSS Inc.):

$$RWC_t = RWC_0 e^{-kt}$$

where k is the desiccation coefficient and RWC₀ and RWC_t are the relative water contents at time 0 and time t, respectively (Tanaka and Nakaoka, 2004). Calculated desiccation coefficients were then compared between the two species using a one-way ANOVA (Sigmastat v3.5, SPSS Inc.).

3. Results

Several regression models were initially tested for fitting the light desiccation data, such as sigmoidal (Shafer et al., 2007) and exponential-decline (Björk et al., 1999) models, but the best fit (highest r^2 -values) for data from this study was provided by a linear model (Ramírez-García et al., 1998). Both species exhibited a linear trend of decrease in $\Delta F/F'_m$ with decreasing RWC (Fig. 1a and b). ANCOVA indicated that both the slopes (P < 0.001) and intercepts (P < 0.001) were significantly different between the two species. *H. johnsonii* exhibited a greater slope and had a lower intercept (Table 1). The average time it took for $\Delta F/F'_m$ to reach zero was 12.1 ± 2 min for *H. johnsonii* and 15.1 ± 2 min for *H. decipiens*.

There were no significant differences observed in the desiccation coefficients between the two species (P = 0.348) in the light

Table 1

Mean values \pm S.E. for calculated photosynthetic- and desiccation-rate parameters for *Halophila johnsonii* and *Halophila decipiens* in the light experiment

| Species | Slope | Intercept | k |
|------------------------------|---|---|---|
| H. johnsonii H. decipiens | $\begin{array}{c} 0.544 \pm 0.021 \\ 0.369 \pm 0.019 \end{array}$ | $\begin{array}{c} -0.058 \pm 0.011 \\ -0.017 \pm 0.009 \end{array}$ | $\begin{array}{c} 0.18 \pm 0.013 \\ 0.16 \pm 0.013 \end{array}$ |

Slope (rate of change in $\Delta F/F'_m \min^{-1}$), intercept ($\Delta F/F'_m$), and k (desiccation coefficient, g min⁻¹).

experiment. There were also no significant differences between the desiccation coefficients in the dark experiments (P = 0.324). However, upon comparing within each species between experiments, desiccation coefficients were significantly higher in the light than in the dark desiccation experiments for both *H. johnsonii* (P = 0.024) and *H. decipiens* (P = 0.008, Tables 1 and 2).

A hyperbolic tangent model provided a good data fit (r^2 from 0.99 to 0.92 and 0.99 to 0.90 for H. decipiens and H. johnsonii, respectively) for the two-phase change in photosynthetic responses to desiccation in the dark. For both species, photosynthetic responses (F_v/F_m) during desiccation in the dark exhibited an initial slow rate of decrease followed by a relatively rapid decline below RWC_{critical} (Fig. 1c and d). RWC_{critical} was more variable for H. decipiens compared to H. johnsonii. The rate of change in $F_v/F_m(A)$ above RWC_{critical} was not significantly different between the two species (P = 0.56). However, RWC_{critical} was significantly lower (P = 0.012) for H. johnsonii compared to H. decipiens. Below RWC_{critical}, the rate of decline in maximum quantum yield was significantly greater and more variable in H. johnsonii than H. decipiens (P = 0.03, Table 2). The average length of time each replicate was run was 23.2 \pm 4.4 min and 23.7 \pm 4.2 min for H. johnsonii and H. decipiens, respectively. This was markedly

Table 2

Mean values \pm S.E. for calculated photosynthetic- and desiccation-rate parameters for *H. johnsonii* and *H. decipiens* in the dark experiment

| Species | Α | В | RWC _{critical} | k |
|------------------------------|---|---|---|--|
| H. johnsonii H. decipiens | $\begin{array}{c} 0.741 \pm 0.009 \\ 0.728 \pm 0.017 \end{array}$ | $\begin{array}{c} 4.46 \pm 0.892 \\ 2.26 \pm 0.256 \end{array}$ | $\begin{array}{c} 0.21 \pm 0.027 \\ 0.37 \pm 0.047 \end{array}$ | $\begin{array}{c} 0.12 \pm 0.014 \\ 0.099 \pm 0.014 \end{array}$ |

A (initial rate of change of F_v/F_m , min⁻¹), B (rate of change of F_v/F_m , min⁻¹after RWC_{critical}), RWC_{critical} (A/B), and k (desiccation coefficient, g min⁻¹).

longer than the light experiments. To examine the possible reason for the hyperbolic tangent response in the dark experimental runs versus linear response in the light experiments, the time at which RWC_{critical} was reached for each replicate was noted. For *H. johnsonii* the average time was 15.8 min and 15.2 min for *H. decipiens*.

4. Discussion

Our results suggest that neither differences in desiccation tolerance nor rates of desiccation in the light, at the level of individual leaves, explain the intertidal-to-subtidal zonation of *H. johnsonii* versus the strictly subtidal zonation of *H. decipiens*. The lower rate of decline in $\Delta F/F'_m$ with decreasing RWC in our light experiments, suggests that photosynthetically, the subtidal *H. decipiens* is actually more tolerant to desiccation than *H. johnsonii*. This agrees with previous studies examining photosynthetic tolerances to desiccation among other seagrass species in relation to depth distribution (Björk et al., 1999; Shafer et al., 2007). However, several studies have shown that some seagrasses growing in more elevated sites exhibit slower desiccation rates and maintain higher photosynthetic rates at lower RWC than species in lower elevations (Leuschner et al., 1998; Ramírez-García et al., 1998).



Fig. 1. Effective ($\Delta F/F'_m$, open symbols) and maximum (F_v/F_m , closed symbols) quantum yields for *Halophila johnsonii* (a and c, squares) and *Halophila decipiens* (b and d, circles) during desiccation.

During desiccation in the dark, the RWC_{critical} for maintaining photosynthetic activity was significantly lower for H. johnsonii than H. decipiens, indicating that H. johnsonii may retain a slightly higher photosynthetic capacity than H. decipiens over a narrow RWC range (21-37%); similar to that reported for Z. japonica versus Z. marina in the light (Leuschner et al., 1998). However, the rate of decline in F_v / $F_{\rm m}$ in *H. johnsonii* below RWC_{critical} was greater than that of *H.* decipiens and the declines in F_v/F_m above RWC_{critical} were not different between the two species, which indicates that in the dark (or at night) H. decipiens may be equally tolerant to desiccation as H. johnsonii upon initial exposure to air. Ulva lactuca exhibits different responses in net photosynthetic rates when emersed in the early morning or evening (low light) versus mid-day (high light, Zou et al., 2007). In our study, differences in the photosynthetic response curves between the dark and light treatments (hyperbolic tangent versus linear) are likely an artifact of differing experimental times and light pressures. The RWC_{critical} in our dark experiments was reached at an average time comparable to the average end-time $(\Delta F/F_m'=0)$ in the light experiments. If $\Delta F/F_m'$ values had not decreased so rapidly in the light, it is possible that a hyperbolic tangent response with a RWC_{critical} could have been observed. Cells may reach a critical point of dehydration, whereby the increase in electrolyte concentrations may strongly inhibit F_v/F_m (Seddon and Cheshire, 2001), as suggested by the sharp decline at a critical point in this study.

Morphological, growth or life-history adaptations that minimize water loss, rather than physiological tolerance, may be the main mechanisms for desiccation tolerance in seagrasses (Björk et al., 1999; Seddon and Cheshire, 2001; Tanaka and Nakaoka, 2004: Shafer et al., 2007). The similarity between the two species' rates of water loss, especially under dark conditions, suggests that H. Johnsonii has no anatomical or morphological adaptations to prevent water loss upon emersion that are superior to those of *H*. decipiens. In other seagrasses, morphological and growth adaptations to desiccation include decreased leaf size (Tanaka and Nakaoka, 2004), rapid rates of leaf abscission or turn-over (Shafer et al., 2007), and flexible leaves which lay on the sediment surface (Silva et al., 2005). However, both H. decipiens and H. johnsonii have similar-sized blades and flexible petioles, although H. johnsonii leaves generally have a higher length:width ratio than H. decipiens (Virnstein and Morris, 2007). Thus, it is unlikely that H. johnsonii has more advantage over H. decipiens in these characteristics.

Additional environmental factors, including light, salinity, temperature and nutrients (Björk et al., 1999; Seddon and Cheshire, 2001), could contribute to the differing vertical distribution patterns of H. johnsonii and H. decipiens. Björk et al. (1999) hypothesized that the ability to tolerate high irradiances allows some seagrasses to occupy the uppermost intertidal zone. In addition, Lan et al. (2005) suggested that a difference in sensitivity to high UV not necessarily high irradiance alone may be a controlling factor. In this regard, Dawes et al. (1989) and Durako et al. (2003) both reported higher light tolerances for H. johnsonii compared to H. decipiens. The latter authors suggested that the presence of UV-absorbing compounds in H. johnsonii may be an additional factor allowing this species to exploit the shallowest waters without competition from the UVP-lacking H. decipiens. Although further studies are needed to examine the functional significance of these flavonoid compounds in *H. johnsonii* (Meng et al., in press), the ability of this species to tolerate high UV and PAR (Durako et al., 2003; Kunzelman et al., 2005), rather than tolerance to desiccation, may largely explain its occurrence in intertidal areas.

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