

The effects of air exposure on the desiccation rate and photosynthetic activity of *Thalassia hemprichii* and *Enhalus acoroides*

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Abstract The effects of air exposure on the desiccation rate and photosynthetic activity of *Thalassia hemprichii* and *Enhalus acoroides* were studied using chlorophyll fluorescence. The desiccation rate of *T. hemprichii* was higher than that of *E. acoroides*, while the critical threshold of relative water content ($RWC_{critical}$) showed opposite trend. Neither the desiccation rate nor $RWC_{critical}$ of *T. hemprichii* was significantly affected by temperature, while the desiccation rate of *E. acoroides* was affected significantly. For both species, the effective photochemical efficiency of PSII decreased gradually above the $RWC_{critical}$, while declined rapidly below the $RWC_{critical}$. After initial air exposure, the relative maximum electron transport rate of both seagrasses increased gradually to a maximum and then decreased to initial value near the $RWC_{critical}$. Therefore, short-term air exposure above the $RWC_{critical}$ is beneficial for both seagrasses, and the $RWC_{critical}$ should be considered when

examining the response of seagrass photosynthesis to air exposure and selecting transplantation sites.

Introduction

Intertidal seagrass meadows are an important ecological and economic resource in the coastal zone (Unsworth and Cullen 2010) and are periodically exposed to air during low tide. Tidal exposure modifies light levels, temperature, and the duration of emersion and immersion (Short et al. 2001; Massa et al. 2009; Van Der Heide et al. 2010; Unsworth et al. 2012). Seagrasses are sensitive to desiccation and temperature stress, both of which tend to reduce photosynthetic gains (Leuschner et al. 1998; Clavier et al. 2011). They are also subjected to varying concentrations of inorganic carbon such as gaseous CO_2 during emersion, and dissolved CO_2 , bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) during immersion (Ouisse et al. 2011). However, the duration of air exposure is the main factor affecting seagrasses biomass accumulation (Stapel et al. 1997; Tanaka and Nakaoka 2004; Rasheed and Unsworth 2011; Nicastro et al. 2012).

Interspecific differences in desiccation tolerance have been hypothesized to explain zonation patterns of seagrasses (Shafer et al. 2007). The zonation patterns of *Zostera noltii* and *Zostera marina* were attributed to the differences in their photosynthetic abilities when leaf-water content was low (Leuschner et al. 1998). The vertical distribution of *Halodule uninervis* and *T. hemprichii* in Taiwan was correlated with the desiccation tolerance of the two species (Lan et al. 2005). However, the responses of photosynthetic activity to desiccation at the level of the individual leaf were insufficient to explain the observed zonation patterns of *Zostera japonica* and *Z. marina* in Padilla Bay,

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Washington (Shafer et al. 2007). Moreover, tropical seagrass species inhabiting the uppermost part of the intertidal bed also did not necessarily have the highest physiological desiccation tolerance (Björk et al. 1999). These various findings indicate that the exact relationship between desiccation tolerance and zonation patterns of seagrasses is currently still unclear (Tanaka and Nakaoka 2004; Shafer et al. 2007).

A mixed seagrass meadow with an area of about 200 ha lies in the southern shallow waters of Xincun Bay, Hainan Island, China. *T. hemprichii* and *E. acoroides* are the dominant species (Huang et al. 2006). Along the lagoon of Xincun Bay, *T. hemprichii* meadows extend from high intertidal to partial low intertidal (vertical gradient), while *E. acoroides* meadows extend from middle intertidal to low intertidal. *T. hemprichii* and *E. acoroides* are co-occurring between middle intertidal and partial low intertidal (Huang et al. 2006; Jiang et al. 2013). In recent years, *E. acoroides* has gradually become the dominant species in middle intertidal, with a trend of extending to high intertidal. In high intertidal, the emersion period for seagrass in Xincun Bay is about 3 h in each spring tide, while in low intertidal, seagrass exposed to the air for only short period of time (<1 h) during spring tides (Jiang et al. 2013). In this study, we aim to address two main questions: (1) Does emergence stress influence the distribution of these two tropical seagrass species in Xincun Bay? (2) Do the desiccation rate and photosynthetic activity of these two species show species-specific responses to temperature variation during the tidal exposure?

Habitat demands of the seagrasses, such as sediment type, air, and hydrodynamic exposure, are required when selecting suitable transplantation sites for seagrasses (Van Katwijk and Wijgergangs 2004; Bos and Van Katwijk 2007; Van Katwijk et al. 2009). Seagrasses transplanted in the region of the shore above the normal upper limit may grow more slowly and die earlier than those transplanted in the normal range (Tanaka and Nakaoka 2004). Additionally, periods of air exposure, as well as periods proceeding and following exposure, may provide critical windows for sufficient light to allow for positive net photosynthesis (Pollard and Greenway 1993; Beer and Rehnberg 1997; Silva et al. 2005). The critical threshold of relative water content ($RWC_{critical}$) of seagrasses may be a key factor in determining the photosystem II (PSII) quantum efficiency (Kahn and Durako 2009). However, information on the relationship between the $RWC_{critical}$ and overall photosynthetic capacity of seagrasses (as indicated by the electron transport rate derived from rapid light curves (RLCs) of chlorophyll fluorescence) is not currently available. The importance of the $RWC_{critical}$ as a parameter to be considered in site selection of seagrass transplantation is also not currently known.

Consequently, the objectives of this study are to examine the effects of air exposure on desiccation rate, effective photochemical efficiency of PSII, and rapid light curve of tropical seagrasses *T. hemprichii* and *E. acoroides* during tidal exposure. Especially, in order to demonstrate the $RWC_{critical}$ as a very relevant parameter to improve site selection for seagrass transplantation, the relationship between the $RWC_{critical}$ and overall photosynthetic capacity was emphatically examined. To the best of our knowledge, this is the first study on the relationship between the $RWC_{critical}$ and overall photosynthetic capacity.

Materials and methods

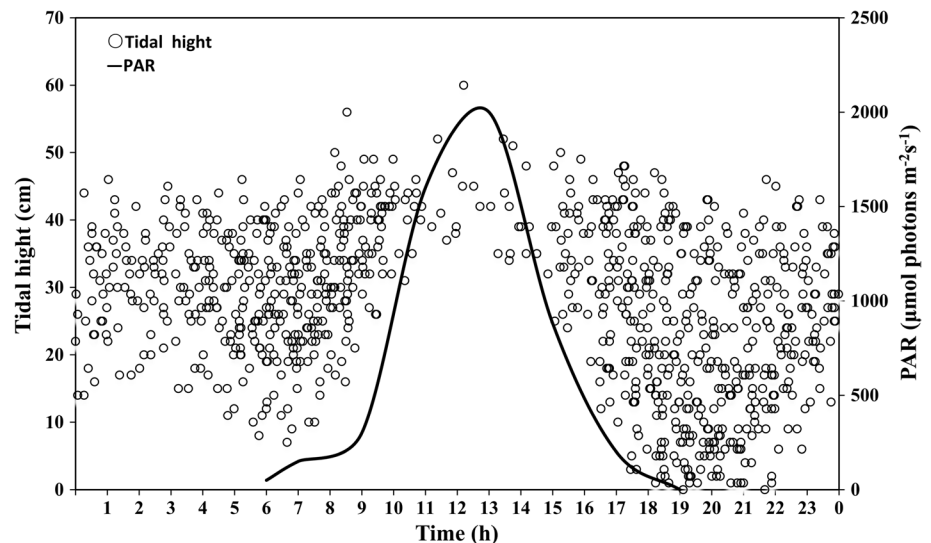
Sediment cores (20 cm in diameter) containing *T. hemprichii* and *E. acoroides* plants were collected at low tide from the mid-intertidal zone in Xincun Bay (18°24'34"N–18°24'42"N, 109°57'42"E–109°57'58"E), which located in the southeastern area of Hainan Island, Southern China. The samples were then transported in coolers to laboratory and cultured with in situ seawater under saturating light conditions (ca. 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) on a 12:12 light/dark cycle. The seawater temperature was controlled at 25 °C. They were maintained in this condition for 1 week prior to the start of the experiments.

Pulse-amplitude-modulated fluorescence (Mini-PAM) was used to determine the stress during emersion of seagrasses. Desiccation induced cellular dehydration, enhancing the concentration of electrolytes within the cell. This changed membrane-bound structures including the thylakoid (Wiltens et al. 1978; Kim and Garbary 2007). Chlorophyll α fluorescence is a sensitive indicator of structural damage to the thylakoid membrane, since chlorophyll protein complexes are contained within this membrane (Schreiber and Bilger 1987; Kim and Garbary 2007). This noninvasive technique yields information about the physiological state of the regulatory processes of in vivo photosynthetic energy allocation and rapidly quantifies changes in photosynthetic efficiency (Ralph and Gademann 2005).

Experimental design

We carried out two experiments. One was to examine the effects of air exposure on desiccation rate and effective photochemical efficiency of PSII ($\Delta F/F_m'$) using seagrass leaf sections and the other was to examine the effect of air exposure on rapid light curve using the whole plant with cores of sediments. The two experiments were both under ambient light conditions (approximately 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) with relative humidity of 55 %. According to the statistical analysis of low tide when seagrasses

Fig. 1 Statistical analysis of the exact timing and tidal height of low tide when seagrasses can emerge in Xincun Bay during 2008–2013, with corresponding irradiance



can emerge in Xincun Bay during 2008–2013 (Fig. 1), the exact timing of the low tide in the daytime most commonly occurs between 6:00 and 9:30 am or between 4:00 and 7:00 pm; at these times, intertidal exposure to irradiance is expected to be relatively low. Thus, the level of light irradiance used in the experiment may largely reflect irradiance levels during daytime intertidal exposure in Xincun Bay. Furthermore, low tide may occasionally occur around noon, when intertidal exposure irradiance would be high; this would induce photoinhibition and significantly affect the photosynthetic response of the seagrasses to air exposure; however, this does not fall within the scope of the present experiment.

Experiment 1: effects of air exposure on desiccation rate and effective photochemical efficiency of PSII

Before the experiment, the middle of the blades on the shoots was cut off (Tanaka and Nakaoka 2004). This process was repeated for five replicate leaf sections of each species. Dehydration responses were evaluated by first gently blotting 3–4 cm leaf sections of *T. hemprichii* and *E. acoroides* to remove excess water. Samples were then placed on a balance, and the fully hydrated wet weight (W_w) was recorded. The tip of the microfiber optics was placed at a distance of 5 mm and an angle of 60° to the samples using the leaf distance clip. Simultaneous $\Delta F/F_m'$ and plant weight (W_t) measurements were obtained at 5-min intervals over the duration of the experiment. At the end of each experimental run, leaf blades were placed in a drying oven at 60°C for 24 h, and then the dry weight (W_d) was measured. In this study, we exposed seagrass leaves to two temperatures: 32 and 24°C , which represent the average winter and summer temperatures, respectively.

Experiment 2: effect of air exposure on rapid light curve

Cores of sediments containing plants were cultured in a tank with in situ seawater under saturated light conditions (approximately $250\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$) at 25°C . About 1 week later, seawater was pumped out of the tank, simulating in situ emersion under low tide. We used a Mini-PAM to measure rapid light curves at 10-min intervals. The air temperature was 24°C during the experiment.

Plant analysis

Relative water content and desiccation rate

Relative water content (RWC) of each replicate was calculated as follows (Björk et al. 1999):

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

Desiccation coefficients were calculated by fitting the change in RWC over time to an exponential decay function (Statistica 6.0):

$$\text{RWC}_t = \text{RWC}_0 e^{-kt}$$

where k is the desiccation coefficient, and RWC_0 and RWC_t are the relative water content at time 0 and time t , respectively (Tanaka and Nakaoka 2004; Kahn and Durako 2009). The length of drying time to achieve the specified levels of RWC (25, 50, and 75 %) was also compared.

Chlorophyll fluorescence

$\Delta F/F_m'$ was measured under ambient light conditions (approximately $250\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$) without dark adaptation using a PAM fluorometer (Mini-PAM, Waltz

GmbH) according to the procedures described in Björk et al. (1999). $\Delta F/F_m'$ was calculated as below:

$\frac{F_m' - F}{F_m'} = \frac{\Delta F}{F_m'}$, where F_m' is the maximum fluorescence measured during a 0.8-s saturating light pulse, and F is the steady-state fluorescence at a given irradiance (Genty et al. 1989). Simultaneous $\Delta F/F_m'$ and plant weight (W_t) measurements were obtained continuously until there was a consistent decline in $\Delta F/F_m'$ but no change in weight for a minimum period of 5 min. Using a Marquardt–Levenberg nonlinear regression algorithm (Statistica 6.0), light desiccation data were fitted to a hyperbolic tangent model (Kahn and Durako 2009):

$$\frac{F_m' - F}{F_m'} = A \tanh \frac{B \times \text{RWC}}{A}$$

Using this model, the initial rate of change in $\Delta F/F_m'$ (A), the critical RWC threshold (A/B, hereafter referred to as $\text{RWC}_{\text{critical}}$, and the rate of change in $\Delta F/F_m'$ (B) below the $\text{RWC}_{\text{critical}}$ were calculated (Kahn and Durako 2009). $\text{RWC}_{\text{critical}}$ was the critical RWC threshold for photosynthetic damage during air exposure, below which tissue physiological injuries and $\Delta F/F_m'$ decrease more rapidly (Taiz and Zeiger 1998; Lawlor and Cornic 2002; Kahn and Durako 2009).

Pulse-amplitude-modulated fluorometry could accurately assess the photosynthetic characteristics of these plants and reflect the trend of photosynthetic response of seagrasses to experimental treatment (Kahn and Durako 2008; Howarth and Durako 2013). Some seagrass species also showed that photosynthetic electron transport rates could be quantitatively related with the rates of O_2 evolution (Ralph et al. 1998; Beer and Björk 2000). A Mini-PAM fluorometer was used to generate RLCs. RLCs were initiated by attaching a dark leaf clip to the middle part of the second innermost leaf of every shoot to reduce within-shoot variability. Eight consecutive light levels (88, 179, 328, 512, 729, 1,121, 1,563, and 2,400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) were applied at 10-s intervals. $\Delta F/F_m'$ measurement was taken using a saturation pulse of 0.8 s before the actinic light was applied (~quasi-darkness), and at the end of each 10-s irradiance step, resulting in nine $\Delta F/F_m'$ measurements (Ralph and Gademann, 2005). All measurements were conducted between 10:00 and 11:30 am. Leaf absorbance was not directly measured. Without knowledge of the actual amount of light being absorbed, fluorescence measurements can be used as an approximation of electron transport rates (Ralph et al. 1998). Thus, the RLCs used a relative measure of electron transport rates (rETR) (Schwarz et al. 2000; Beer et al. 2001), which was calculated from the following equation:

$$\text{rETR} = \Delta F/F_m' \times \text{PAR} \times 0.5 \times 0.84$$

We used the instrument default AF value (AF = 0.84) to calculate rETR, as recommended by Beer et al. (2001). The relative maximum electron transport rate (rETR_{max}), representing photosynthetic capacity, was derived by fitting the RLCs to the double exponential decay function (Platt et al. 1980), using a least-squares nonlinear curve-fitting algorithm:

$$\text{rETR} = \text{Ps} \left(1 - e^{-(\alpha \text{PAR}/\text{Ps})} \right) e^{-(\beta \text{PAR}/\text{Ps})}$$

Ps was a scaling factor, which was in turn used to calculate rETR_{max} , α (the initial slope of the light-limited relationship), and β (down-regulation).

$$\text{rETR}_{\text{max}} = \text{Ps} [\alpha / (\alpha + \beta)] [\beta / (\alpha + \beta)]^{(\beta/\alpha)}$$

The minimum saturating irradiance (E_k) was calculated by dividing the rETR_{max} by the initial slope. All calculations were performed in Statistica 6.0 (StatSoft, Tulsa, OK, USA).

Statistical analysis

Means and standard errors of all variables were calculated. One-way ANOVA was used to determine whether the parameter estimates were significantly different ($p < 0.05$) (Statistica 6.0). Treatment means were compared and separated by least significant difference (LSD).

Results

Effects of air exposure on desiccation rate and effective photochemical efficiency of PSII

The time course of the changes in RWC and $\Delta F/F_m'$ of the two tropical seagrasses at 24 and 32 °C are shown in Fig. 2. Both *T. hemprichii* and *E. acoroides* lost water in a linear manner initially and then reached a constant RWC. The time for *T. hemprichii* leaves to reach the same RWCs (75, 50, and 25 %) at 24 °C was almost all identical to those at 32 °C, while the time for *E. acoroides* to lose 25, 50, and 75 % of their original water content at 24 °C was all significantly higher than those at 32 °C (Table 1). Furthermore, the time to reach the same RWCs and $\Delta F/F_m'$ for *T. hemprichii* were both shorter than those for *E. acoroides* at both temperatures. $\Delta F/F_m'$ of *T. hemprichii* at 24 and 32 °C approached zero at 215 and 140 min, respectively, while $\Delta F/F_m'$ of *E. acoroides* approximated zero at 210 and 170 min, respectively.

The responses of $\Delta F/F_m'$ to desiccation in leaf sections of *T. hemprichii* and *E. acoroides* at 24 and 32 °C are shown in Fig. 3 and Table 2. For *T. hemprichii*, there was no significant difference in either the desiccation rate ($p = 0.274$)

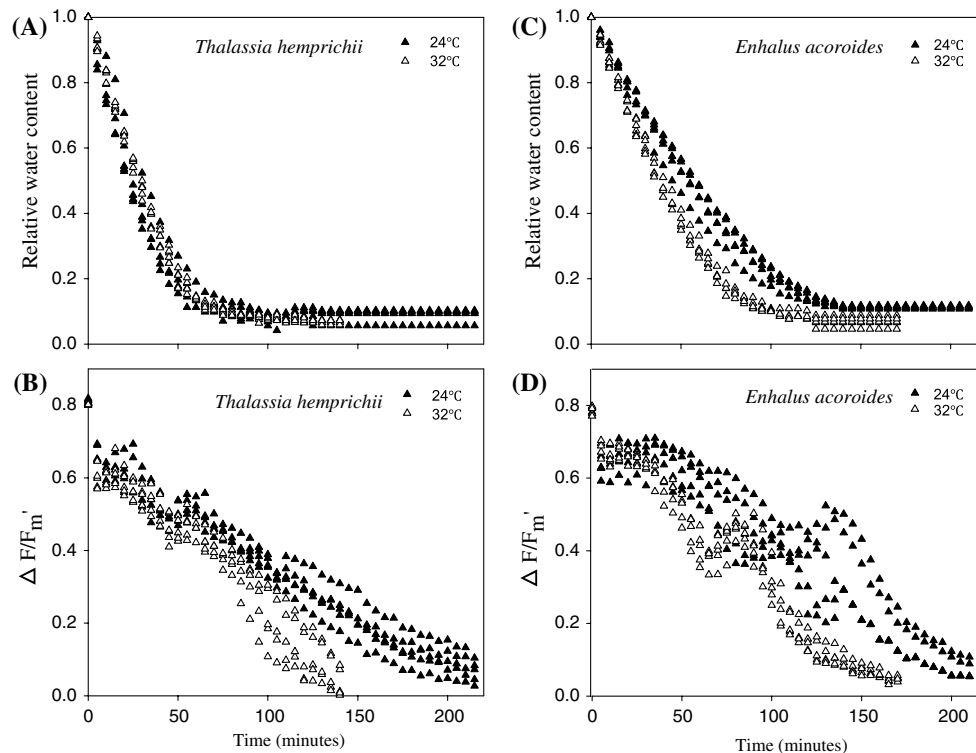


Fig. 2 Changes in the relative water content and effective quantum yields ($\Delta F/F'_m$) of *T. hemprichii* (a, b) and *E. acoroides* (c, d) during periods of desiccation at 24 and 32 °C. Data points are from five repeat measurements

Table 1 The time at which 25, 50, and 75 % dehydration occurs in *T. hemprichii* and *E. acoroides*, and corresponding effective quantum yields ($\Delta F/F'_m$) at 24 °C and 32 °C

Species	Temperature (°C)	25 %		50 %		75 %	
		Time (min)	$\Delta F/F'_m$	Time (min)	$\Delta F/F'_m$	Time (min)	$\Delta F/F'_m$
<i>T. hemprichii</i>	24	12 ± 2 ^a	0.625 ± 0.03 ^a	23 ± 3 ^a	0.613 ± 0.06 ^b	45 ± 2 ^a	0.488 ± 0.02 ^b
	32	12 ± 2 ^a	0.615 ± 0.04 ^a	27 ± 2 ^a	0.546 ± 0.03 ^a	45 ± 3 ^a	0.463 ± 0.04 ^b
<i>E. acoroides</i>	24	25 ± 2 ^b	0.656 ± 0.03 ^{ab}	55 ± 3 ^c	0.612 ± 0.04 ^b	95 ± 4 ^c	0.444 ± 0.05 ^b
	32	17 ± 2 ^a	0.673 ± 0.02 ^b	40 ± 2 ^b	0.571 ± 0.03 ^{ab}	65 ± 3 ^b	0.380 ± 0.02 ^a

Different superscript letters indicate significant difference ($p < 0.05$) among means. Error bars represent SE ($n = 5$)

or the $RWC_{critical}$ ($p = 0.632$) between the two temperatures, while for *E. acoroides*, a significant difference in the desiccation rate ($p < 0.05$) was only observed between the two temperatures. Although the $RWC_{critical}$ of both species showed no significant difference between the two temperatures, the $RWC_{critical}$ of both species was relatively higher at 24 than at 32 °C. The desiccation rate of *T. hemprichii* was higher than that of *E. acoroides* at both temperatures, while the $RWC_{critical}$ showed a reverse trend. $\Delta F/F'_m$ of both species displayed an initial slow rate of decline followed by a relatively rapid decrease below the $RWC_{critical}$. At both temperatures, the $RWC_{critical}$ of *T. hemprichii* was reached within 45–50 min, while the $RWC_{critical}$ of *E. acoroides* was reached after 80 min at 24 °C and 55 min at 32 °C. The rate

of change in $\Delta F/F'_m$ (A) above the $RWC_{critical}$ was lower for *T. hemprichii* than *E. acoroides* at both temperatures, while the opposite trend was observed below the $RWC_{critical}$ (Fig. 3; Table 2).

Effect of air exposure on rapid light curve

Rapid light curves and their derived parameters are shown in Figs. 4 and 5, respectively. rETR of both *T. hemprichii* (Fig. 4a) and *E. acoroides* (Fig. 4b) with different rose linearly with PAR, and then reached a maximum, following by some decline. When the tissue-water content varied from 21 to 83 %, rETR of *T. hemprichii* were all higher than that in 100 % tissue-water content at the same irradiance

Fig. 3 Effective quantum yields ($\Delta F/F_m'$) responses to dehydration in leaf sections of *T. hemprichii* (a, b) and *Enhalus acoroides* (c, d) during periods of desiccation at 24 and 32 °C. The arrows show the critical threshold of relative water content ($RWC_{critical}$) and the parameters of the nonlinear regression can be seen in Table 2. Data points are from five repeat measurements

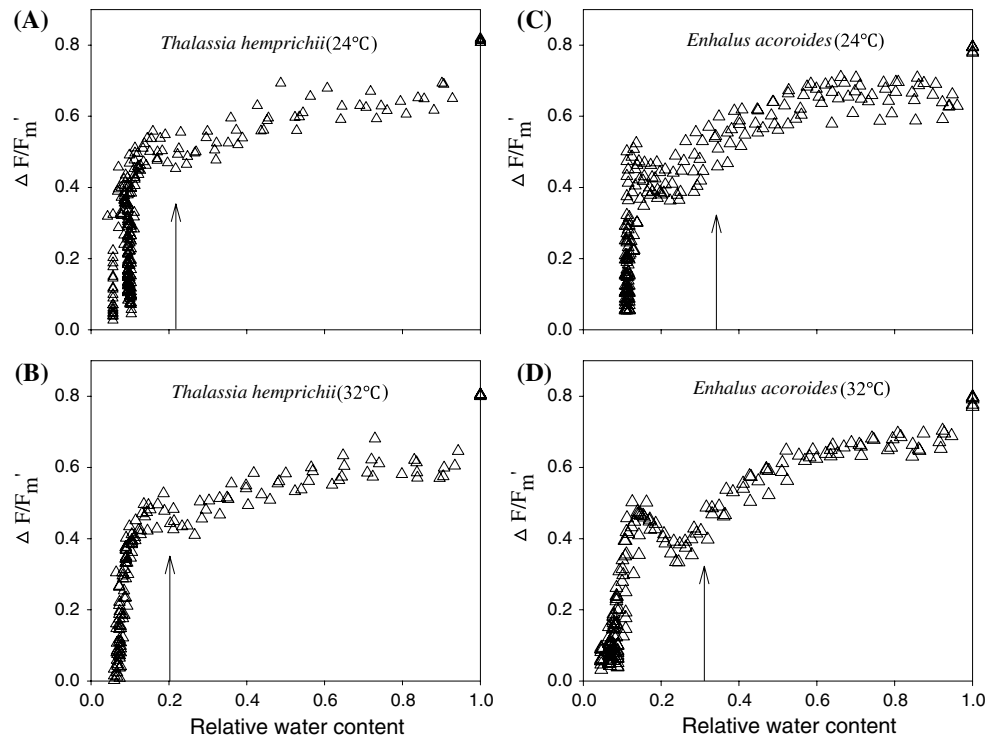


Table 2 Mean values of calculated photosynthetic and desiccation rate parameters for *T. hemprichii* and *E. acoroides* at 24 and 32 °C: initial rate of change in $\Delta F/F_m'$, \min^{-1} (A), rate of change in

$\Delta F/F_m'$, \min^{-1} after $RWC_{critical}$ (B), $RWC_{critical}$ (A/B), and desiccation coefficient (k) (\min^{-1})

Species	Temperature (°C)	A	B	$RWC_{critical}$	k
<i>T. hemprichii</i>	24	0.652 ± 0.014^b	3.067 ± 0.508^b	0.217 ± 0.034^a	0.028 ± 0.004^c
	32	0.607 ± 0.031^a	3.056 ± 0.374^b	0.202 ± 0.033^a	0.027 ± 0.001^c
<i>E. acoroides</i>	24	0.689 ± 0.031^c	2.086 ± 0.405^a	0.341 ± 0.069^b	0.014 ± 0.001^a
	32	0.674 ± 0.015^{bc}	2.197 ± 0.286^a	0.312 ± 0.053^b	0.020 ± 0.001^b

Different superscript letters indicate significant difference ($p < 0.05$) among means. Error bars represent SE ($n = 5$)

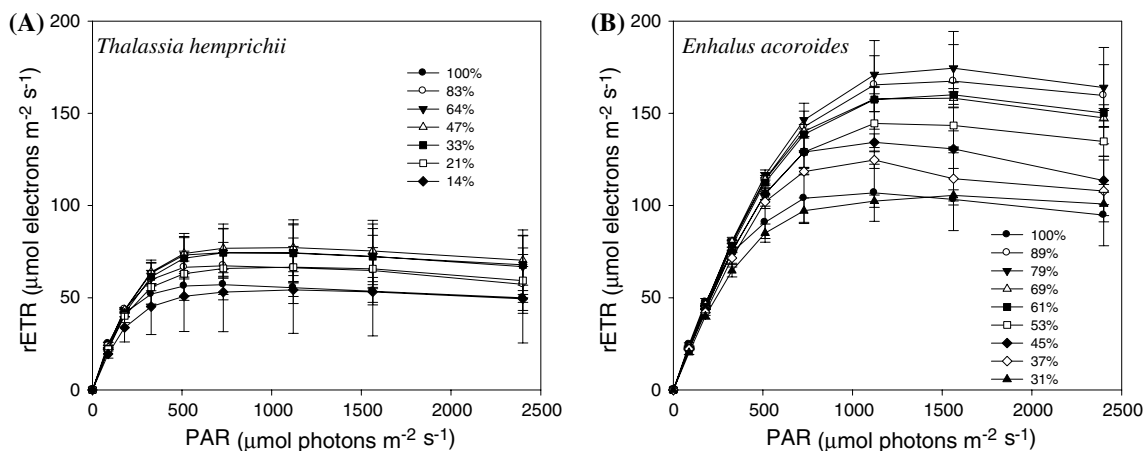


Fig. 4 Rapid light curves at the different water content for *T. hemprichii* and *E. acoroides* fitting with a double exponential decay function using a least-squares nonlinear curve-fitting algorithm (“Chlorophyll fluorescence” section). Error bars represent SE ($n = 3$)

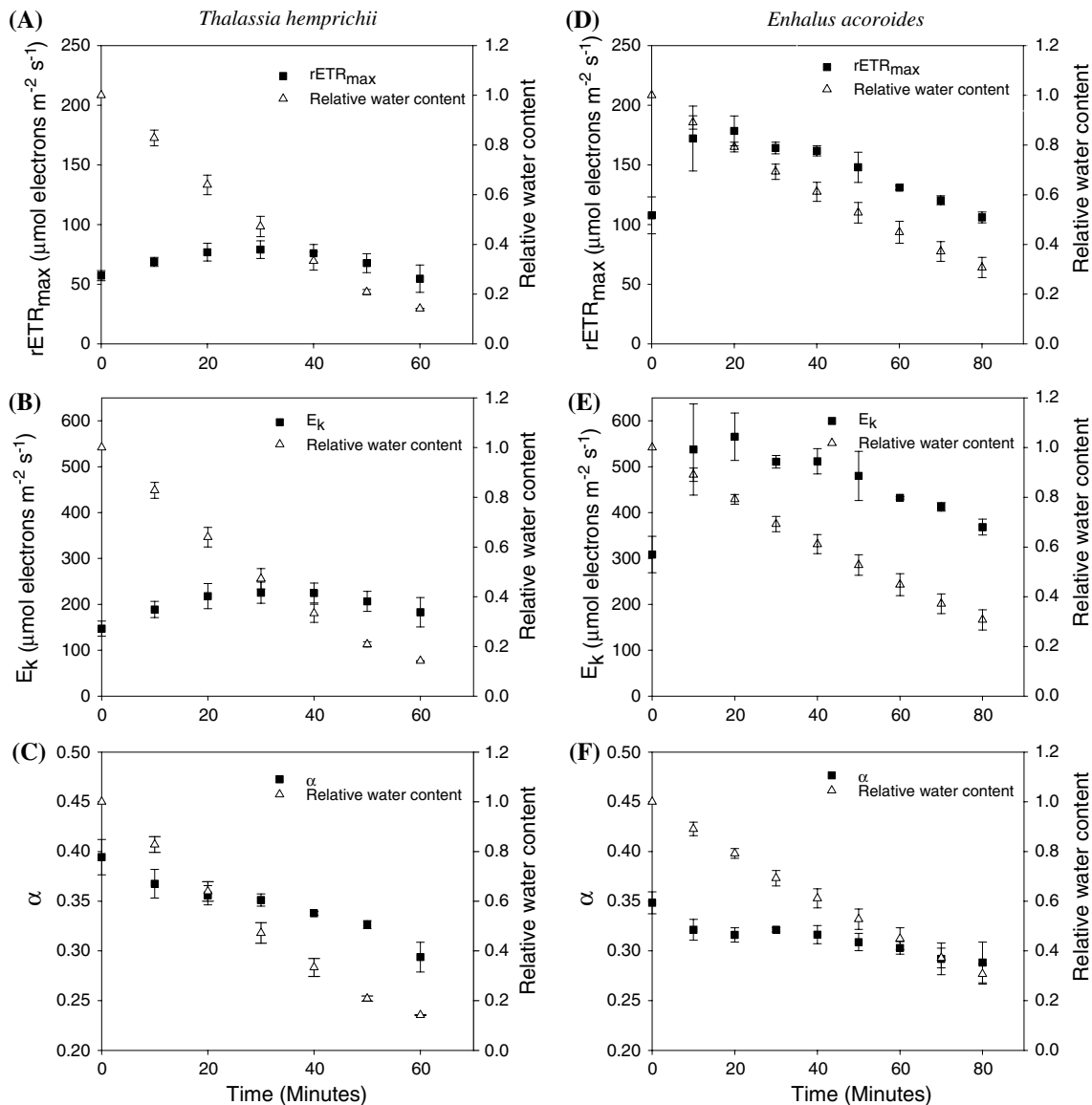


Fig. 5 Variations in the relative maximum electron transport rate ($rETR_{max}$), minimum light saturating light (E_k), and initial slope of rapid light curve (α) of *T. hemprichii* (a–c) and *E. acoroides* (d–f) under air exposure. Error bars represent SE ($n = 3$)

beyond $328 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. $rETR$ of *E. acoroides* showed a similar trend when the tissue-water content varied from 37 to 89 %. When the above data were fit to the double exponential decay function, the $rETR_{max}$ of *T. hemprichii* rose as soon as desiccation began and peaked when the RWC dropped to 53 % at approximately 30 min, then declined with further desiccation, and returned to the initial value near the $RWC_{critical}$ at approximately 50 min (Fig. 5a). A similar trend was observed for E_k (Fig. 5b). Similarly, the $rETR_{max}$ of emerged *E. acoroides* increased immediately after desiccation began, but quickly reached a maximum value when approximately 20 % of tissue water had been lost (at approximately 20 min). Following that, the $rETR_{max}$ of *E. acoroides* started to decline and then

returned to the initial value near the $RWC_{critical}$ at around 80 min (Fig. 5d). $rETR_{max}$ and E_k of submerged *T. hemprichii* were both lower than those of *E. acoroides* and greater enhancement of $rETR_{max}$ and E_k (Fig. 5e) during desiccation for *E. acoroides* was observed. However, α (the initial slope of the light-limited relationship) of *T. hemprichii* and *E. acoroides* both dropped gradually once desiccation started (Fig. 5c, f).

Discussion

In this study, *E. acoroides* lost water more slowly and exhibited a slower rate of reduction in $\Delta F/F_m'$ than

T. hemprichii at both 24 and 32 °C. This indicates that *E. acoroides* is more desiccation-resistant than *T. hemprichii*. The greater resistance of *E. acoroides* to desiccation does not agree with the vertical distribution of seagrasses observed in Xincun Bay. These findings are in agreement with Björk et al. (1999) who similarly found that *E. acoroides* had the highest desiccation resistance out of seven Indo-Pacific seagrass species, owing to the thick waxy leaves preventing water loss. Nevertheless, the morphological traits of *E. acoroides* may in reality make them more vulnerable to exposure-related loss than other intertidal species, because of the thick strap-like blades causing part of the blade base to stay “proud” above the substrate rather than lying flat on the surface (Unsworth et al. 2012). Furthermore, the $RWC_{critical}$ of *E. acoroides* was markedly higher than that of *T. hemprichii* at both temperatures, indicating that *E. acoroides* might retain a slightly lower photosynthetic capacity than *T. hemprichii* over a narrow RWC range. This was supported by the finding that *T. hemprichii* had a capacity to recover close to its original photosynthetic ability following a loss of 85 % of its initial water content (Björk et al. 1999).

It has been found that long and frequent tidal exposure could result in desiccation and temperature stress in seagrasses (Erftemeijer and Herman 1994; Stapel et al. 1997). In the present study, temperature did not significantly affect the desiccation rate and $RWC_{critical}$ of *T. hemprichii*, but significantly affected the desiccation rate of *E. acoroides*. This demonstrates that *T. hemprichii* is less sensitive to the effect of temperature change. It is likely because *T. hemprichii* inhabits the higher intertidal zone, and therefore exposed to longer and more frequent air exposure, which allowed the species to acclimate to higher temperatures. The temperature ranges for the present experiment might not exceed the critical temperature threshold at which a significant effect would be observed in the desiccation rate of *T. hemprichii*. The high-temperature condition did considerably enhance the desiccation rate of *E. acoroides*; so, temperature could not be excluded as a contributing factor to exposure-related stress of *E. acoroides* (Unsworth et al. 2012). Significant dieback of *E. acoroides* may occur under short-term desiccation stress at high temperatures, which is similar to that exposure to desiccation at high temperatures caused a sudden dieback of *Amphibolis antarctica* and *Posidonia australis* along 95 km of coast in Spencer Gulf, South Australia (Seddon and Cheshire 2001).

$\Delta F/F_m'$ is an index of photosynthetic efficiency under ambient light conditions, while RLCs reveal information about light acclimation and overall photosynthetic capacity (French and Moore 2003). In this study, $\Delta F/F_m'$ and α of both *T. hemprichii* and *E. acoroides* decreased gradually during the period of air exposure. This implied that the

efficiency of light harvesting, energy conversion in photosynthesis, and PSII activity were all reduced in response to air exposure. However, initial air exposure increased the E_k of *T. hemprichii* and *E. acoroides*, suggesting an enhancement of light tolerance in both species. This was further supported by the observation that β (down-regulation, derived from rapid light curve) of both seagrasses increased immediately after desiccation commenced (data not shown). Under desiccated conditions, cyclic electron flow around PSI can induce thermal dissipation by generating a pH gradient, protecting the photosynthetic apparatus against photodestruction and excess ATP (Heber and Walker 1992; Munekage et al. 2002; Gao et al. 2011). Additionally, both species displayed an initial increase in the $rETR_{max}$ in response to desiccation, representing the ability to transfer more electrons and a greater energy investment in the biochemical machinery for CO₂ fixation. The initial enhancement of $rETR_{max}$ in *E. acoroides* was higher than that in *T. hemprichii*, indicating *E. acoroides* had a higher tolerance to high irradiance. Besides increased light, the initial enhancement in photosynthetic capacity could also be attributed to the increased CO₂ diffusion from air into the leaves induced by the loss of the thin water layer (Ji and Tanaka 2002). Gross community production of *Z. noltii* measured as CO₂ uptake was always higher in air than in water (Silva et al. 2005), and the aboveground biomass and area of a seagrass meadow were positively correlated with the number of hours of daylight tidal exposure (Rasheed and Unsworth 2011). All these findings indicate that air exposure may enhance photosynthesis and growth of seagrass as long as the leaf-water content remains sufficiently enough during the periods of desiccation (Leuschner et al. 1998). Several studies have demonstrated an enhancement of photosynthesis in seagrasses under conditions of CO₂ enrichment in seawater (Zimmerman et al. 1997; Jiang et al. 2010; Alexandre et al. 2012). This trend leads to the question: does the rising level of atmospheric CO₂ also stimulate photosynthesis and growth of emerged seagrass? It should also be noted, however, that extended periods of desiccation, particularly when combined with other environmental factors, such as heat and UV stress, can significantly reduce carbon gains (Leuschner et al. 1998; Clavier et al. 2011; Rasheed and Unsworth 2011). For example, Nicastrò et al. (2012) found that above a depth of −0.5 m, *Z. muelleri* exhibited a decrease in biomass with increasing elevation, presumably as a result of desiccation stress. Specifically, the assimilation of CO₂ by some seagrass shoots embedded in small seawater pools at low tide might result in the extinguishment of CO₂. This might make CO₂ as a limiting factor.

In the present study, the $rETR_{max}$ of both *T. hemprichii* and *E. acoroides* increased gradually to a maximum following emergence and then decreased gradually to the

initial value near the $RWC_{critical}$. Additionally, the decline of $\Delta F/F_m'$ below the $RWC_{critical}$ was much more rapid than that above the $RWC_{critical}$. This suggested dehydration caused an increase in the concentration of electrolytes in cells (Seddon and Cheshire 2001) below the $RWC_{critical}$, which might significantly inhibit $\Delta F/F_m'$. Hence, the $RWC_{critical}$ is a key parameter to be considered in the change trend of seagrass photosynthetic response to air exposure. Since the duration of low tide exposure is one of the crucial factors determining the success of seagrass transplantation (Thangaradjou and Kannan 2008), the success rate of seagrass transplantation may be greater at transplantation sites at which the time of emergence is less than the time required to reach the $RWC_{critical}$. However, high transplant mortality at shallow sites observed by Tanaka and Nakaoka (2004) and Li et al. (2013) was probably at least partially due to high hydrodynamic (wave and current) stresses and/or desiccation and chlorosis of leaves caused by tidal exposure. Thus, to determine the suitable locations for seagrass transplantation, a habitat suitability map should be created based on a GIS model using the duration of exposure to air and other factors as model parameters (Van Katwijk et al. 2009). Furthermore, as sea level rises, reduced tidal range will occur when the increase in low tide is greater than the increase in high tide in some specific geomorphological locations. Reduced tidal range would impact intertidal seagrasses in those locations by decreasing the amount of air exposure (Short and Neckles 1999). If the reduced tidal range results in shorter desiccation periods above the $RWC_{critical}$, sea-level rise may favor the growth and expansion of seagrass distribution shoreward (Short and Neckles 1999). Thus, the $RWC_{critical}$ of different kinds of seagrasses and the frequency and length of exposure at low tide should be considered in the prediction model of the effects of sea-level rise on seagrass distribution.

Actually, desiccation rates in natural seagrass populations definitely diverge from laboratory estimations, since other factors such as changing light, temperature, relative humidity and wind, and the degree of clumping of shoots under natural conditions must affect desiccation rate and physiological response. However, for better practical application in seagrass transplantation, the $RWC_{critical}$ value for a specific seagrass can also be determined from field dehydration responses with control of consistent light intensity using neutral density screens (this is just for avoiding photoinhibition induced by natural high light, since photoinhibition of photosynthesis in seagrasses during the experiment might significantly reduce $\Delta F/F_m'$ (Ralph 1999; Sharon et al. 2011), making it not suitable to make quantitative comparisons along time). Firstly, the exact timing of low tide (emersion) most commonly occurs in the daytime for a specific seagrass should be identified by statistical analysis of low tide, as well as corresponding light

intensity. And the light saturation point of photosynthesis for the specific seagrass should also be measured through photosynthesis–irradiance curves using oxygen evolution (Torquemada et al. 2005). Secondly, leaves should be put on sediment surface of original location when seagrass is exposed at the chosen low water spring tides, and simultaneous $\Delta F/F_m'$, leaf weight, the corresponding light intensity, relative humidity and wind speed should be measured at several minutes intervals. According to the changing light intensity, a number of neutral density screens should be adjusted promptly above the leaves for consistent saturating light intensity during the experiment. Thirdly, rapid light curve of in situ seagrass shoots should also be measured at the same time at several minutes intervals, to verify whether the $RWC_{critical}$ value is the threshold for photosynthetic damage during air exposure. Before each rapid light curve measurement, the actual amount of light being absorbed by the seagrass leaves should be determined by the fluorometer PAR quantum sensor (Beer and Björk 2000). The use of this specific absorption factor instead of the instrument's default value of 0.84 would allow the calculation of absolute electron transport rates (Silva and Santos 2003). Then, the second and third experiments should be performed under different seasons for calculating the average $RWC_{critical}$ value of different temperature ranges. Finally, seagrass transplant site should be determined according to the local tide, the average $RWC_{critical}$ value, and other parameters, and seagrass transplant should be carried out to further affirm the validity of the $RWC_{critical}$ value in improving site selection.

Conclusion

Our results indicate that *E. acoroides* was more desiccation-resistant than *T. hemprichii*, while *T. hemprichii* showed higher photosynthetic capacity over a narrow RWC range and greater adaptability to high temperatures. Desiccation significantly reduced $\Delta F/F_m'$ and photosynthetic efficiency (α) of both seagrasses. Short-term desiccation above the $RWC_{critical}$ appeared beneficial for *T. hemprichii* and *E. acoroides*, since it enhanced the photosynthetic capacity and high-light tolerance for both seagrasses. The $RWC_{critical}$ is a key parameter to be considered in the site selection of seagrass transplantation, in the examination of the response of seagrass photosynthesis to air exposure, and in the prediction of the effects of reduced tidal range caused by sea-level rise on seagrasses.

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