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RESEARCH ARTICLE

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Measurement of chlorophyll fluorescence reveals mechanisms for habitat niche separation of the intertidal seagrasses *Thalassia hemprichii* and *Halodule uninervis*

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Abstract In Taiwan, *Thalassia hemprichii* dominates the upper intertidal zone, whereas *Halodule uninervis* occupies the lower intertidal zone. We tested the hypothesis that *T. hemprichii* is better adapted to high irradiance and more resistant to air exposure than *H. uninervis*. The photosynthetic efficiency, damage, and extent of recovery were determined by measuring chlorophyll fluorescence using pulse amplitude modulated fluorometry. Both species growing in tidal pools, in response to high irradiance alone, revealed a small depression in maximal quantum yield of photosystem II (Fv/Fm) at noon. The second experiment compared the effect of air exposure alone and the combined effect of air exposure with high irradiance by interposing a shading screen on both species, growing in the intertidal zone over a diurnal cycle. Values of Fv/Fm of both the shaded and irradiated *T. hemprichii* remained high at low tide. However, *H. uninervis* exhibited a marked depression following air exposure and a synergistic depression under the combined effect. The experimental manipulations of exposure time demonstrated that the tolerance of *T. hemprichii* to the combined effect was longer and the recovery from air exposure following re-submersion was better than those of *H. uninervis*. Both species were more susceptible to the combined effect in the dry season than in the wet season. Our results suggest that air exposure is more important than high irradiance in constraining the distribution of *H. uninervis* in the upper intertidal zone. This was confirmed by transplantation experiments in which a rapid decline of *H. uninervis* was observed after transplantation into the upper intertidal zone. In the lower intertidal zone, measurements of the response of the photosynthetic electron transport rate to irradiance demonstrated that the transplanted *T. hemprichii* exhibited a sun-type response and *H. uninervis* a shade-type response.

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Introduction

Thalassia hemprichii and *Halodule uninervis* are dominant intertidal seagrasses along coasts of the Indo-Pacific (Mukai 1993). In Taiwan, *T. hemprichii* occupies the upper intertidal zone, whereas *H. uninervis* inhabits the lower intertidal zone (authors' personal observations). The reason for this distribution pattern has not been studied. A better understanding of these mechanisms might allow for more-effective management for conservation and restoration.

Interspecific competition and resource availability may be important factors in structuring intertidal seagrass communities. There is little evidence of an allelopathic effect of interference competition among seagrass species (Whittaker and Feeney 1971). Björk et al. (1997) suggested that seagrasses growing highest up in the intertidal zone are best adapted to utilize high irradiance and possess an efficient way of acquiring inorganic carbon from seawater. Tilman (1985) attributed the depth distribution of seagrasses to a balance between competition for irradiance and nutrients. In the Caribbean, *T. testudinum* and *H. wrightii* are considered to be sister species of the Indo-Pacific *T. hemprichii* and *H. uninervis*, respectively (Dawes 1998). In contrast to the vertical zonation of *T. hemprichii* and *H. uninervis* in the Indo-Pacific, *H. wrightii* occupies the intertidal zone, whereas *T. testudinum* inhabits the shallow subtidal zone (Iverson and Bittaker 1986). Powell et al. (1991) and Fourqurean et al. (1992) found that increased nutrient concentrations might stimulate the growth of *H. wrightii*. Fourqurean et al. (1995) attributed the decline of *T. testudinum* at fertilized sites to *H. wrightii* outcompeting the other for irradiance in response to nutrient enrichment.

The intertidal zone is regularly exposed to high irradiance and air that induces thermal and desiccation stresses. Intertidal seagrasses have to cope with high irradiances, high temperatures, and desiccation (Jupp et al. 1996). Seddon and Cheshire (2001) found that high temperatures alone did not significantly affect photosynthetic efficiency in the intertidal seagrasses *Amphibolis antarctica* and *Posidonia australis*. Physiological responses to air exposure or desiccation have been considered to be a main factor restricting the upper limit of some intertidal seagrasses (Adams and Bate 1994; Leuschner et al. 1998). However, Björk et al. (1999) suggested that tolerance to high irradiance rather than to desiccation is the cause for the vertical zonation of tropical intertidal seagrasses. Dawson and Dennison (1996) suggested that UV radiation might restrict the upper limit of *H. uninervis*. Moreover, Adam and Bate (1994) suggested that although the photosynthetic efficiency of *Zostera capensis* is sensitive to desiccation, a better recovery from desiccation following re-submersion allows it to occupy the shallow intertidal zone, as compared with the more-deeply growing species of *Ruppia cirrhosa*. The relative importance of high irradiance, desiccation, and extent of recovery in structuring depth distributions of tropical seagrasses in the intertidal zone has not been determined.

Chlorophyll fluorescence measurements have proven to be useful in the assessment of photosynthetic efficiency (Krause and Weis 1991). Silva and Santos (2003) suggested that when characterizing the photosynthetic behavior of intertidal seagrasses, one should consider both the diurnal fluctuations in the plant's photosynthetic activity as well as their vertical distribution frequencies. Repeated field measurements of photosynthetic efficiency of seagrasses over a diurnal cycle were made possible after the development of a submersible pulse amplitude modulated (Diving-PAM) fluorometer (Beer and Björk 2000). Hence, we applied this technique to test the hypothesis that *T. hemprichii* is better adapted to high irradiance and more resistant to air exposure than *H. uninervis*. Specifically, the aims of this study were: (1) to compare the effect of high irradiance alone on the photosynthetic efficiencies of *T. hemprichii* and *H. uninervis* over a diurnal cycle, (2) to compare the effect of air exposure alone and the combined effect of exposure with high irradiance on both species over a diurnal cycle, (3) to compare the effect of exposure time on the photosynthetic efficiencies and the extents of recovery of both species, (4) to determine whether the effect of exposure time differs between the dry and wet seasons, and finally (5) to confirm if habitat niches of both species truly diverge using transplantation experiments.

Materials and methods

Study sites

This study was conducted in intertidal seagrass beds along the coastline of Nanwan, located on the southern tip of Taiwan (21°57′N; 120°44′E). The seagrass beds are partly protected from wave action by a 5–10-m wide zone of elevated fringing reefs, and the substrata were covered by at least 10 cm of coral sand and debris. These beds covered about 0.7 ha and comprise a *Thalassia*-*Halodule* community. The coastline at Nanwan is subjected to a mixed and predominantly semidiurnal tidal type (Lin and Shao 1998). Water column salinity varied within a small range of 32.6–34.7 psu (Lin and Shao 1998). The light extinction coefficient (k) in the water column remained low, with a range of 0.59–1.26 m^{-1}.

Southern Taiwan is dominated by a tropical climate, with distinct dry and wet seasons. The maximum air temperature (about 28°C) often occurs in July, and the minimum (about 20°C) occurs in January (data from the Central Weather Bureau, 1971–2000). In the wet season from May to October, average monthly rainfall frequently exceeds 320 mm, when typhoons and south-westerly winds bring considerable quantities of rain. During the dry season of November–April, when northeasterly winds prevail, mean monthly rainfall normally does not exceed 60 mm. The northeasterly monsoonal winds, or 'downhill winds', are extremely forceful (3.5–6.1 $m\ s^{-1}$). Incident irradiance remains at around 1,500 µmol quanta $m^{-2}\ s^{-1}$ in winter and 2,000 µmol quanta $m^{-2}\ s^{-1}$ in summer.

Environmental variables

Environmental variables in the sediment and water column were measured for each habitat of *T. hemprichii* and *H. uninervis* every 3 months from July 2001 to April 2003, with the exception of the seasonal low tide exposure during daylight, which was studied four times in 2002. Environmental variables of sediment, including depth, grain size, silt/clay content, and sorting coefficient were determined in triplicate on each occasion. For the collection of sediment, PVC corers with an inner diameter of 3.5 cm were pushed 5 cm deep into the sediment at low tide. Granulometry of the sediment was determined following Buchanan and Kain (1971) and Hsieh et al. (1995).

Samples for analyses of dissolved inorganic nutrients in the water column and of sediment pore water were collected in triplicate on each occasion and immediately placed on ice in a cooler. The water samples were collected at low and high tides respectively to take account of tidal changes. In the laboratory, each water sample was filtered through pre-combusted (at 450°C for 4 h) Whatman GF/F filters. Dissolved inorganic nitrogen (DIN: $NO_3^{\ -} + NO_2^{\ -} + NH_4^{\ +}$) and dissolved inorganic phosphorus (DIP: $PO_4^{\ -3}$) were determined colorimetrically (Strickland and Parsons 1972) with a flow injection analytical method (Pai et al. 1990).

Chlorophyll fluorescence

The photosynthetic performance of both species was determined in the field under sunlight by measuring the chlorophyll fluorescence of photosystem II using Diving-PAM (Waltz, Germany). The tip of the instrument's main optical fiber was inserted into a transparent plastic tube whose other end opened on a leaf clip in the shape of a "Y" in such a way that the tip was constantly located at a distance of 1 cm and an angle of 45° to the leaves. The upper middle portion of the third-youngest leaf was measured, as there was less variation in chlorophyll fluorescence relative to older leaves which had a lower productivity, or immature leaves which had variable chlorophyll contents. The effective quantum yield, or Φ, of photosystem II was determined by the saturating-light method as $(Fm' - Fs)/Fm'$, where Fs is the actual chlorophyll fluorescence of a light-adapted leaf, and Fm' is the corresponding maximal chlorophyll fluorescence of a light-adapted leaf following a saturating flash of light. Values of Fv/Fm (the maximal quantum yield of photosystem II) were also determined after acclimating leaves in tightly wrapped aluminum foil to darkness for 20 min (determined as being sufficient time for full oxidation of reaction centers), where $Fv = Fm - Fo$, with Fo being the initial chlorophyll fluorescence after dark adaptation when all reaction centers are open, and Fm being the maximal chlorophyll fluorescence after dark adaptation when all reaction centers are closed following a saturating flash of light.

The electron transport rate (ETR, μmol electrons $m^{-2}\ s^{-1}$) was then determined by the equation $ETR = PAR \times \Phi \times 0.5 \times AF$, where underwater PAR (μmol quanta $m^{-2}\ s^{-1}$) was measured with a Li-189 light meter (LiCor, USA), the factor of 0.5 was based on the assumption that photosystem II absorbs half the quanta of PAR, and AF is the absorption factor value of the seagrass leaf. The AF values of both species were determined according to Beer et al. (1998) by placing leaves on the top of a Li-189 light meter with an artificial light source overhead at a fixed distance. Irradiance reaching the sensor through 1–7 layers of leaves was respectively recorded, and the natural logarithm-transformed values were then plotted against the number of leaf layers. AF value of each species was calculated as $[1-\exp(-\beta)]$, where β is the regression coefficient of the linear correlation.

The ETR derived from the fluorescence parameters of Diving-PAM was used as an estimate of the photosynthetic rate of seagrasses (Beer et al. 1998; Beer and Björk 2000). The relationship between the electron-transport rate and underwater irradiance (ETR-E curve) of each species is described by the hyperbolic tangent function suggested by Jassby and Platt (1976):

$$ETR = ETR_M \times \tanh\left(\frac{\alpha \times E}{ETR_M}\right)$$

where ETR is dependent on the irradiance (E) and two physiologically interpreted parameters (α and ETR_M). The parameter, α, characterizes the ascending slope of the ETR-E curve at low irradiance levels. ETR_M is the maximal ETR at saturation irradiance. The half-saturation irradiance, E_k, was determined by the equation: $E_k = ETR_M/\alpha$. A simultaneous fit of α and ETR_M to the present data was obtained using the nonlinear curve-fitting procedure of the program SigmaPlot 2000 (vs. 6.0).

Experiment 1: Effect of high irradiance alone on *Fv/Fm*

Values of Fv/Fm of seagrass leaves were monitored at intervals of 30 min in tidal pools where both species remained submerged over a diurnal cycle in July 2003. Measurements for both species were carried out on subsequent days when the skies were clear (underwater irradiance was >1,500 μmol quanta $m^{-2}\ s^{-1}$).

Experiment 2: Effect of air exposure alone and the combined effect of air exposure with high irradiance on *Fv/Fm*

Values of Fv/Fm of leaves of both species growing in the intertidal zone were monitored at intervals of 30 min from initial submersion in the morning to air exposure and then to re-submersion for a diurnal cycle in July 2003. Two treatments were respectively applied to each species: one was irradiated under sunlight and the other was shaded and received low irradiance (about 150 μmol quanta $m^{-2}\ s^{-1}$) by interposing a 90% shading screen. Measurements for both species were also carried out on subsequent days when the skies were clear (underwater irradiance was >1,500 μmol quanta $m^{-2}\ s^{-1}$).

Experiment 3: Effect of exposure time on *Fv/Fm*

Four quadrats (20×10 cm) in each habitat were randomly sampled in July 2002 (wet season) and January–February 2003 (dry season) respectively. The seagrasses (about 30–40 shoots) including sediments were then removed from each quadrat and put into a plastic container to receive treatments of 30, 60, 90, and 120 min of exposure time during 10:00–14:00, respectively. At the end of each treatment, the shoots were re-submerged again for 2 h. The value of Fv/Fm was measured for seagrasses before they were dug out, during exposure to air for different times, and at 2 h following re-submersion. Measurements for both species were carried out simultaneously on a day when the sky was clear (underwater irradiance was $>1{,}500$ μmol quanta m^{-2} s^{-1}).

Experiment 4: Transplantation experiment

To confirm if habitat niches of these two species truly diverge in the intertidal zone, five random quadrats with about 30–40 shoots from each habitat were transplanted to the other habitat in June 2003. The experiment showed a rapid decline of *H. uninervis* when transplanted into the upper intertidal zone. Therefore, only photosynthetic parameters derived from the ETR-E curve of the transplanted *T. hemprichii* into the lower intertidal zone and that of *H. uninervis* remaining in the lower intertidal zone were compared for the light requirement of each species. The value of $(Fm'-Fs)/Fm'$ of each species was monitored at intervals of 1 h from 12:00 to 19:00 when leaves received different levels of irradiance to determine the ETR-E curves. Measurements for both species were simultaneously carried out on a day when the sky was clear (underwater irradiance was $>1{,}500$ μmol quanta m^{-2} s^{-1}).

Data analysis

A *t* test was used to examine whether environmental variables differed between the two habitats. A one-way fixed ANOVA model was used to determine whether the value of Fv/Fm and extent of recovery of each species differed among exposure times (four levels). If the results of ANOVA indicated significant main effects at the 0.05 probability level, then Fisher's protected least significant difference (LSD) test was used to determine which means significantly differed. The data were tested for homogeneity of variance (Hartley's F_{max}-test; Sokal and Rohlf 1995) before the analyses.

Results

Environmental variables

The time of seasonal low tide exposure during daylight for *T. hemprichii* averaged 7.63 h, as compared to 0.75 h for *H. uninervis* (Table 1). The exposure time of *T. hemprichii* in the upper intertidal zone was about 10 times longer than that of *H. uninervis* in the lower intertidal zone (*t* test, $P<0.001$). The silt/clay content of the sediment was significantly higher in the habitat of *H. uninervis* than in that of *T. hemprichii* (*t* test, $P<0.001$). Other environmental variables in the water column and sediment, however, were not found to significantly differ between the two habitats.

Table 1 Comparisons of physical and chemical variables (mean ± SE) for the habitats of *Thalassia hemprichii* and *Halodule uninervis* during July 2001–April 2003

Variable	*Thalassia hemprichii*	*Halodule uninervis*
Exposure time during daylight* (h) ($n=4$)	7.63 ± 0.21	0.75 ± 0.65
Grain size (mm) ($n=8$)	0.30 ± 0.02	0.36 ± 0.03
Sorting coefficient ($n=8$)	1.26 ± 0.02	1.39 ± 0.05
Silt/clay content* (%) ($n=8$)	2.0 ± 0.2	2.8 ± 0.6
Interstitial DIN (μM) ($n=8$)	8.0 ± 2.5	12.2 ± 3.8
Interstitial DIP (μM) ($n=8$)	0.8 ± 0.6	0.7 ± 0.5
Water column DIN (μM) ($n=8$)	22.4 ± 4.8	24.1 ± 6.2
Water column DIP (μM) ($n=8$)	2.4 ± 1.2	2.6 ± 1.3

DIN dissolved inorganic nitrogen, *DIP* dissolved inorganic phosphorus
Asterisk indicates significance at $P<0.05$

Experiment 1: Effect of high irradiance alone on Fv/Fm

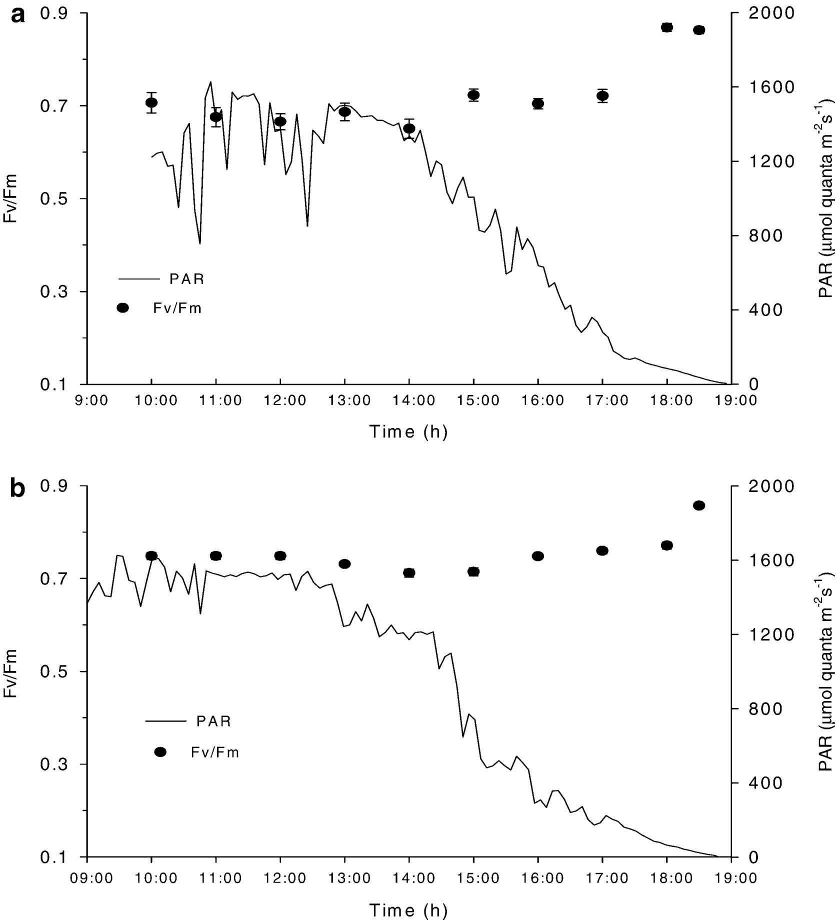
The values of Fv/Fm of *T. hemprichii* (Fig. 1a) and *H. uninervis* (Fig. 1b) growing in tidal pools remained about 0.70 during daylight when the irradiance was $>1{,}500$ μmol quanta m^{-2} s^{-1} and then reached about 0.84 at 18:00 (dusk) when underwater irradiance was <100 μmol quanta m^{-2} s^{-1}. No distinct diurnal changes in Fv/Fm were detected between the two species.

Experiment 2: Effect of air exposure alone and the combined effect of air exposure with high irradiance on Fv/Fm

In the intertidal zone, *T. hemprichii* was exposed at 10:30 and was re-submerged at 19:10. There was virtually no change in the value of Fv/Fm for the shaded *T. hemprichii* over the diurnal cycle, and it remained about 0.80 following 8 h of air exposure as compared to the initial value of 0.81 for the submerged *T. hemprichii* in the early morning (Fig. 2a). A small decrease in Fv/Fm (to about 0.65) was observed in the irradiated *T. hemprichii* following 4 h of air exposure. In the late afternoon, although *T. hemprichii* was still exposed, the Fv/Fm value recovered to about 0.70 when the irradiance was <600 μmol quanta m^{-2} s^{-1}.

Because *H. uninervis* occupies the lower intertidal zone, it remained submerged until 11:30 and was re-submerged earlier at 14:30. A marked depression in the value of Fv/Fm was observed in both the shaded and irradiated *H. uninervis* following shoot exposure to air at low tide (Fig. 2b). The depression was greater under the combined effect of air exposure and high irradiance. The value of Fv/Fm of the irradiated *H. uninervis* declined to only 0.20 following 3 h of exposure as compared to 0.60 for the shaded *H. uninervis*. The value of Fv/Fm of the shaded and irradiated *H. uninervis* began to recover following re-submersion at the flood tide, although the irradiance was still $>1{,}000$ μmol quanta m^{-2} s^{-1}.

Fig. 1 Diurnal changes in underwater photosynthetically active radiation (PAR) and *Fv*/*Fm* of **a** *Thalassia hemprichii* and **b** *Halodule uninervis* which remained submerged in tidal pools (mean ± SE, $n = 10$)



Experiment 3: Effect of exposure time on *Fv*/*Fm*

In the wet season, a small decrease in *Fv*/*Fm* was observed for *T. hemprichii* and it remained at 0.60 following 60 min of exposure time (Fig. 3a). The value of *Fv*/*Fm* was significantly lower following >90 min of exposure time (Table 2). The extent of recovery of *T. hemprichii* from air exposure following re-submersion decreased with increasing exposure time. The threshold time for complete recovery was between 90 min and 120 min.

The responses of *H. uninervis* to air exposure were more sensitive than those of *T. hemprichii* (Fig. 3b). The value of *Fv*/*Fm* of *H. uninervis* decreased from 0.60 to 0.38 following 30 min of exposure but showed complete recovery after re-submersion. Following >60 min of exposure, little recovery of *H. uninervis* from air exposure was detected (Table 2).

Both species showed a larger depression in *Fv*/*Fm* in the dry season than in the wet season in response to air exposure. The value of *Fv*/*Fm* of *T. hemprichii* decreased with increasing exposure time and rapidly declined to 0.20 after >60 min of exposure (Fig. 4a), whereas that of *H. uninervis* was completely depressed after 30 min of exposure (Fig. 4b). The threshold time for the complete recovery of *T. hemprichii* from air exposure was shortened to 60 min. However, *H. uninervis* showed little recovery under any exposure time in the dry season (Table 2).

Experiment 4: Transplantation experiment

In the upper intertidal zone, the transplanted *H. uninervis* rapidly declined. In the lower intertidal zone, the ETR-E curve (Fig. 5) of the transplanted *T. hemprichii* exhibited a sun-type response ($n = 7$, $r^2 = 0.99$) relative to a shade-type response by *H. uninervis* ($n = 7$, $r^2 = 0.92$). Greater values for $\mathrm{ETR_M}$ (223 μmol electrons $\mathrm{m^{-2}\ s^{-1}}$) and $\mathrm{E_k}$ (1,355 μmol quanta $\mathrm{m^{-2}\ s^{-1}}$) of the ETR-E curve of the transplanted *T. hemprichii* than those of *H. uninervis* ($\mathrm{ETR_M}$, 112 μmol electrons $\mathrm{m^{-2}\ s^{-1}}$; $\mathrm{E_k}$, 539 μmol quanta $\mathrm{m^{-2}\ s^{-1}}$) remaining in the lower intertidal zone indicated that the photosynthetic performance of *T. hemprichii* was more efficient at high irradiance levels. However, the higher α (0.21) of the ETR-E curve of *H. uninervis* than that (0.17) of *T. hemprichii* showed that the photosynthetic performance of *H. uninervis* was more efficient at low irradiance levels.

Discussion

Depression of photosynthetic efficiency at noon is a general phenomenon for terrestrial plants and intertidal seagrasses (Silva and Santos 2003). *Fv*/*Fm* in both seagrass species growing in tidal pools also showed a small diurnal depression (Fig. 1). However, our results

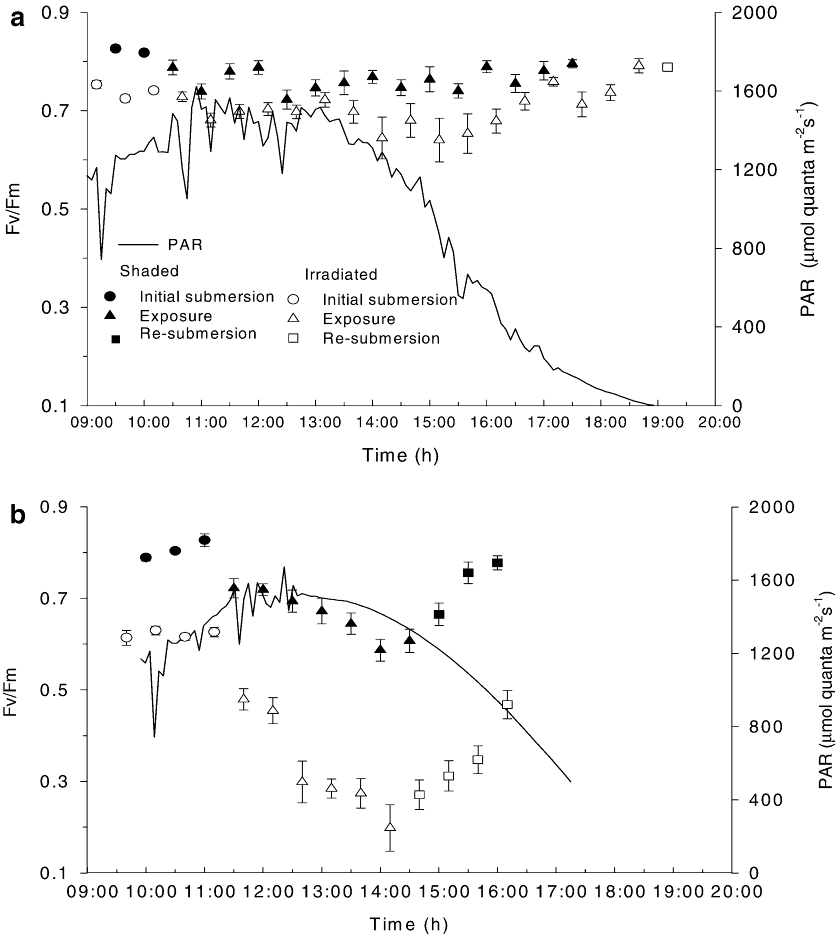


Fig. 2 Diurnal changes in underwater photosynthetically active radiation (PAR) and *Fv*/*Fm* of **a** *Thalassia hemprichii* and **b** *Halodule uninervis* from initial submersion to air exposure and then to re-submersion (mean ± SE, $n = 10$)

support *T. hemprichii* as being better adapted to tolerate not only high irradiance alone and air exposure alone, but also their combined effect compared to *H. uninervis*. This was evident by the ETR-E curve of *T. hemprichii* exhibiting a sun-type response relative to the shade-type response of *H. uninervis*. Moreover, the values of *Fv*/*Fm* of *T. hemprichii* were little affected by air exposure alone in the shading experiment conducted in the intertidal zone. Although a small decrease in *Fv*/*Fm* of *T. hemprichii* was detected under the combined effect of high irradiance and air exposure, our results showed that *T. hemprichii* could tolerate this combined effect for at least 90 min. The tolerance time to the combined effect for *H. hemprichii* was much longer and the extent of recovery from air exposure was better than that for *H. uninervis*, which assures the dominance of *T. hemprichii* in the upper intertidal zone. This may be the reason that in Indonesia, only *T. hemprichii* grows in habitats which are seasonally exposed to air during daylight (Stapel et al. 1997).

The experimental manipulations of exposure time showed that the threshold time of *T. hemprichii* for complete recovery from air exposure after re-submersion was less than 2 h in the wet season (Fig. 3) and 1 h in the dry season (Fig. 4). However, the length of the seasonal low tide during daylight for *H. hemprichii* averaged 7.63 h (Table 1). It appears that *T. hemprichii* would be unable to survive in the intertidal zone for this length of time, because the average exposure time during daylight was much longer than the determined threshold time. As a matter of fact, high irradiance levels during the exposure of *T. hemprichii* were restricted only to around 10:00–14:00. The field experiment conducted in the intertidal zone showed that *T. hemprichii* could tolerate the combined effects of air exposure and high irradiance for 4 h at irradiance levels of $> 1{,}200$ µmol m^{-2} s^{-1}. The shorter tolerance time in the experimental manipulations of exposure time as compared to the field experiment conducted in the intertidal zone possibly resulted from some loss of overlying water during the transfer of seagrasses with sediment into the plastic containers.

The ETR-E curve of *H. uninervis* exhibiting a shade-type response indicated that the photosynthetic performance was less efficient at high irradiance levels (Fig. 5). *Halodule uninervis* is known to be one of the most-sensitive species, which exhibits the largest decrease in photosynthetic efficiency in response to UV radiation (Dawson and Dennison 1996). Nevertheless, the values of *Fv*/*Fm* of *H. uninervis* were little affected in the tidal pool experiment, indicating that photosystem II was not damaged by high irradiance alone. It appears that water prevents most of the UV radiation from reaching the

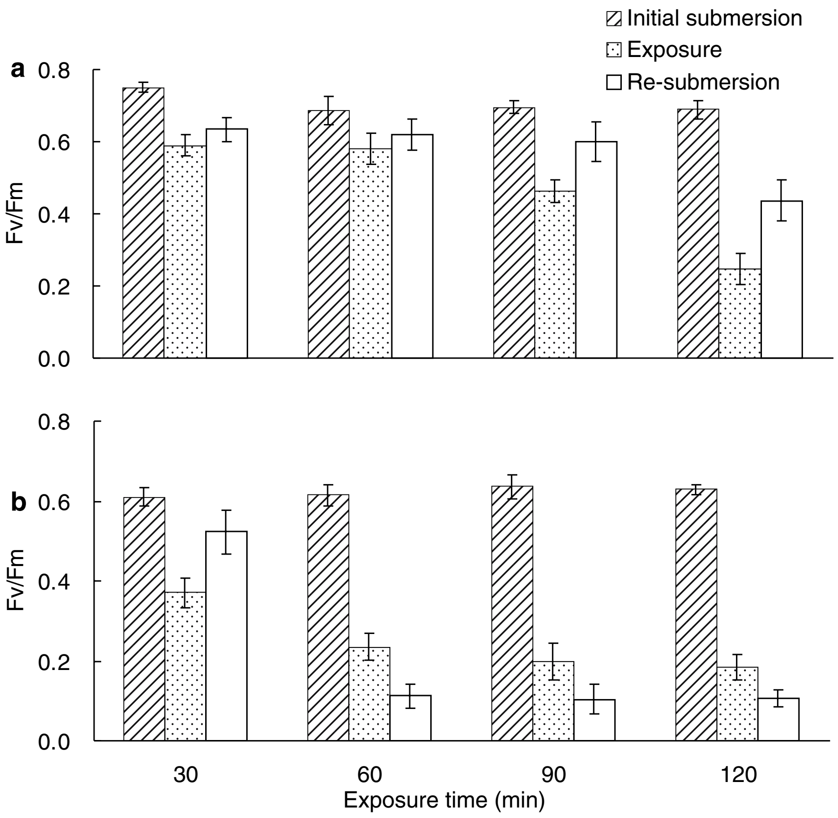


Fig. 3 Effect of exposure time on values of *Fv*/*Fm* for **a** *Thalassia hemprichii* and **b** *Halodule uninervis* and the extent of recovery 2 h after re-submersion in the wet season (mean ± SE, *n* = 9–20)

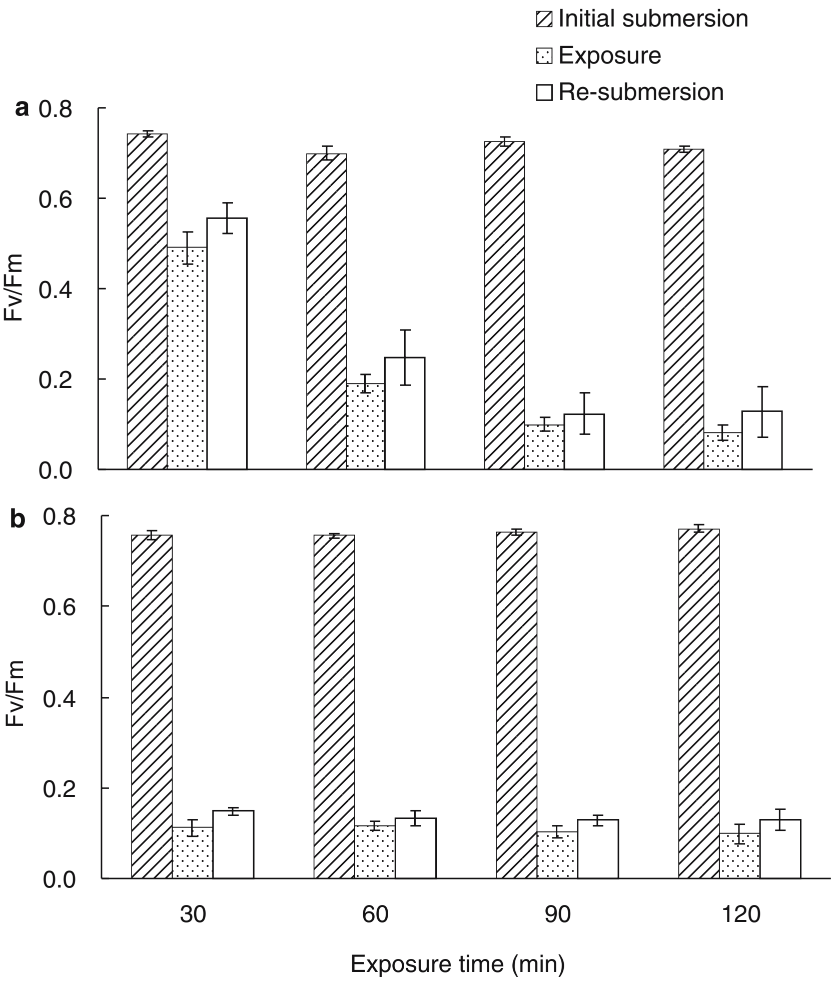
leaves. On the other hand, air exposure alone resulted in a marked depression in *Fv*/*Fm* of *H. uninervis* in the intertidal zone (Fig. 2b). The combined effect of air exposure with high irradiance further caused a synergistic effect by showing a large depression in *Fv*/*Fm*. Air exposure appears to be more important than high irradiance in constraining the distribution of *H. uninervis* in the lower intertidal zone.

The mechanism for the depth distribution of *T. hemprichii* and *H. uninervis*, however, was inconsistent with the mechanism proposed for the vertical zonation of their respective sister species of *T. testudinum* and *H. wrightii* in the Caribbean. Fourqurean et al. (1992) suggested that the availability of phosphorus in the sediment pore water controls the dominance of *T. testudinum* and *H. wrightii*. Fourqurean et al. (1995) further indicated that *T. testudinum* is outcompeted for irradiance by *H. wrightii* in response to nutrient enrichment. In Taiwan, nutrient concentrations in the water column and the sediment pore water of the habitat of *H. uninervis* were not higher than those of *T. hemprichii* (Table 1). Therefore, the upper limits of *H. uninervis* did not appear to be set by nutrient concentrations in the water column or in sediment

Table 2 One-way ANOVA results for the experiment comparing the effects of exposure time (30, 60, 90, and 120 min) to air on *Thalassia hemprichii* and *Halodule uninervis*

Season	Factor	*df*	*F*	*P*	Separation
Wet	*Thalassia hemprichii*				
	Initial submersion	3	1.35	0.26	
	Exposure	3	18.1	0.001	30 = 60 > 90 > 120
	Re-submersion	3	4.10	0.009	30 = 60 = 90 > 120
	Halodule uninervis				
	Initial submersion	3	0.27	0.85	
	Exposure	3	5.36	0.002	30 > 60 = 90 = 120
	Re-submersion	3	33.5	< 0.001	30 > 60 = 90 = 120
Dry	*Thalassia hemprichii*				
	Initial submersion	3	3.30	0.26	
	Exposure	3	70.9	0.001	30 > 60 > 90 = 120
	Re-submersion	3	13.1	0.001	30 > 60 = 90 = 120
	Halodule uninervis				
	Initial submersion	3	0.95	0.42	
	Exposure	3	0.74	0.53	
	Re-submersion	3	1.86	0.15	

Fig. 4 Effect of exposure time on values of *Fv*/*Fm* for **a** *Thalassia hemprichii* and **b** *Halodule uninervis* and the extent of recovery 2 h after re-submersion in the dry season (mean ± SE, $n = 9–20$)



pore water. It is clear that the sister species of seagrasses between the Caribbean and the Indo-Pacific do not necessarily possess the same ecological adaptation strategies. The physiological cause for the subtidal zonation of *T. testudinum* and *H. wrightii* in the Caribbean is unable to explain the intertidal zonation of their respective sister species in Taiwan.

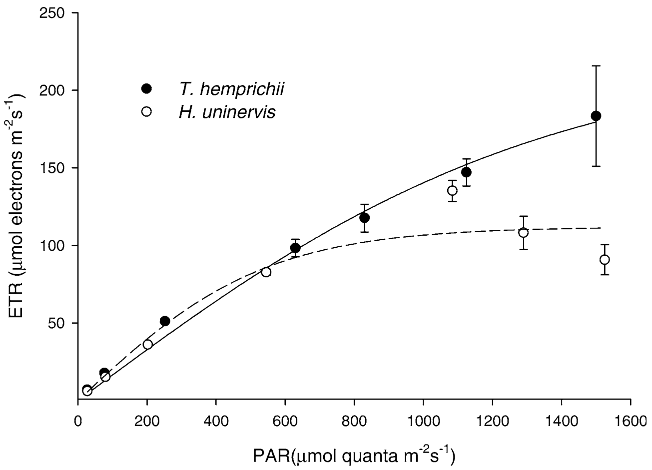


Fig. 5 ETR-E curve of *Thalassia hemprichii* 22 d after being transplanted to the habitat of *Halodule uninervis* and that of *H. uninervis* remaining in the lower intertidal zone (mean ± SE, $n = 10$)

The other possibility for the depth distributions of *T. hemprichii* and *H. uninervis* is a difference in the silt/clay content of sediment between the two habitats. The silt/clay content of the sediment was significantly higher in the habitat of *H. uninervis*, which might have resulted from the trapping of silt and clay in the lower intertidal zone carried by wave activity. This was supported by the trend of a bigger grain size and a higher sorting coefficient in the habitat of *H. uninervis*, although the difference could not be shown to be statistically significant ($P = 0.10$). *Thalassia hemprichii* appears to prefer growing in a habitat with a lower sediment silt/clay content. However, the transplantation experiments showed that the growth of *T. hemprichii* was unaffected and the new-growth leaves became even larger in sediment with a higher silt/clay content.

The experiment of air exposure time showed that both species were more susceptible to the combined effect in the dry season (winter) than in the wet season (summer). Our results support the observations of Lin and Shao (1998) of the seasonal patterns of leaf growth rate and productivity of *T. hemprichii* in southern Taiwan which are lower in winter. The authors attributed the seasonal downhill wind which specifically occurs in winter to be the main cause. It is likely that the prevalence of the downhill wind in winter may cause seagrass leaves to dry out faster and enhance water loss when they are exposed to air. In the short term, photosynthetic inhibition resulting from desiccation is reversible, but an extension of desiccation can result in irreversible damage due to cellular shrinkage (Seddon and Cheshire 2001). The tolerance time of *T. hemprichii* to the combined effect was shown to be at least 90 min in the wet season, but the value of Fv/Fm declined to only 0.20 and showed little recovery for the same period in the dry season. Leuschner et al. (1998) found that the degree of leaf water loss is a function of exposure time. They reported that leaves of *Zostera noltii* lost up to 50% of their water content after 5 h of exposure to air at low tide. The downhill wind appeared to shorten the tolerance time of *T. hemprichii* to the combined effect to less than 60 min. There is little evidence that *H. uninervis* can tolerate the combined effect and recover its capability in the dry season.

In our view, the dominance of *H. uninervis* in the lower intertidal zone most likely results from the competition for space. In the Caribbean, *H. wrightii* has been classified as a pioneer species due to its rapid growth rate and ‘guerilla-like’ branching patterns that allow for expansive lateral growth (Gallegos et al. 1994). Rose and Dawes (1999) found interspecific interactions with *H. wrightii* to be an important determinant of *T. testudinum* productivity. In this study, the transplantation experiments showed that *T. hemprichii* can grow well in the lower intertidal zone without competition for space by *H. uninervis*. However, the poor resistance to desiccation and high irradiance of *H. uninervis* restricted its depth distribution to the upper intertidal zone. This may simply be because the leaves of *H. uninervis* are much smaller and thinner than those of *T. testudinum*, and thus dry out much faster. Consequently, *H. uninervis* declined rapidly when transplanted into the upper intertidal zone.

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