

Emergence stress and morphological constraints affect the species distribution and growth of subtropical intertidal seagrasses

Yoshiyuki Tanaka^{1,*}, Masahiro Nakaoka²

¹Ocean Research Institute, University of Tokyo, Minamidai 1-15-1, Nakano, Tokyo 164-8639, Japan

²Graduate School of Science and Technology, Chiba University, Yayoi-cho 1-33, Inage, Chiba 263-8522, Japan

ABSTRACT: The effects of emergence stress and morphological constraints on the distribution and growth of 3 subtropical seagrass species, *Cymodocea rotundata*, *C. serrulata* and *Thalassia hemprichii*, were examined in a series of transplantation experiments and a laboratory desiccation experiment. *C. rotundata* and *T. hemprichii* occur in both the intertidal and subtidal zones off Ishigaki Island, southwest Japan, whereas *C. serrulata* is found only in the subtidal zone. Within the same species, intertidal shoots for both *C. rotundata* and *T. hemprichii* are significantly smaller than subtidal shoots. A subtidal-to-intertidal transplantation experiment was conducted on 3 occasions: July and September 2001 and January 2002. In July and September, when the intertidal sites were exposed to air for a shorter period, shoot densities of the transplanted 3 species did not decrease. In contrast, the shoot densities of all 3 transplanted species decreased significantly in February, when the exposure to air was longer. The survival of *C. serrulata* (2% of the initial density) was much lower than that of *C. rotundata* (48%) and *T. hemprichii* (48%). In subtidal-to-intertidal transplants, the size of *C. rotundata* and *T. hemprichii* leaves decreased, approaching that of intertidal shoots, indicating high morphological plasticity. *In situ* observations revealed that transplanted seagrasses with large leaves were exposed to air for longer than were intertidal controls. In the desiccation experiment, *T. hemprichii* leaves had higher water contents than those of *C. rotundata* and *C. serrulata*. The morphological traits of *C. rotundata* and *T. hemprichii* and their ability to change shoot size appear to be related to the better survival of these species in the intertidal area as compared to *C. serrulata*. This is consistent with the observed variation in the species distribution in the intertidal zone.

KEY WORDS: Seagrass · Emergence · Desiccation · Morphological plasticity · Transplantation · Ishigaki Island

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INTRODUCTION

In the intertidal zone, many benthic organisms have distinct patterns of vertical distribution, including macrophytes. It is generally believed that differences in desiccation tolerance are a major factor in determining the upper limits of species distribution (Norton 1986, Davison & Pearson 1996, Abe et al. 2001). Specific zonation patterns with different upper limits have also been observed for tropical and temperate seagrasses (Mukai et al. 1987, Leuschner et al. 1998, Björk et al. 1999, De Troch et al. 2001, Seddon & Cheshire

2001, Huong et al. 2003). Some studies have investigated the relationship between emergence stress and intertidal seagrass distribution. Leuschner et al. (1998) attributed the zonation patterns of *Zostera noltii* and *Z. marina* to the difference in their photosynthetic abilities at a low leaf water content. Seddon & Cheshire (2001) showed that *Amphibolis antarctica* underwent desiccation more slowly than did *Posidonia australis*, which might be a reason for its greater abundance in the upper tidal level. Conversely, Björk et al. (1999) showed that tropical seagrass species that inhabit the uppermost part of the intertidal bed do not necessarily

*Email: y-tanaka@ori.u-tokyo.ac.jp

have the highest physiological desiccation tolerance. They suggested that morphological traits that minimize desiccation stress may be more important in determining species distribution. However, few field experiments have examined survival in response to emergence stress directly (e.g. Huong et al. 2003).

As with other plants, seagrasses show intraspecific morphological variation along environmental gradients. In deep habitats with low light availability, the leaves of *Zostera marina*, *Posidonia oceanica*, and *Cymodocea nodosa* become larger and thinner to capture light efficiently (Bintz & Nixon 2001, Olesen et al. 2002). Ultraviolet radiation causes the leaves of *Z. capricorni*, *Cymodocea serrulata*, and *Syringodium isoetifolium* to become thicker to protect the UV-sensitive organelles (Abal et al. 1994, Dawson & Dennison 1996). The leaves of *Thalassia testudinum* and *T. hemprichii* are shorter when exposed to fast currents (van Tussenbroek 1996, Tomascik et al. 1997), possibly to minimize water resistance and damage. However, little attention has been paid to the relationship between emergence stress and intraspecific morphological variation in seagrasses (Phillips 1967). Interspecies comparisons of morphological responses to emergence stress may help to explain the distributions of seagrass species in the intertidal and subtidal zones.

This study examined whether emergence stress influences the distributions of intertidal seagrass species and whether among- and within-species variation in morphological traits and plasticity is related to variation in species distribution. We focused on 3 dominant seagrasses in the tropical Indo-Pacific Ocean: *Thalassia hemprichii*, *Cymodocea rotundata*, and *C. serrulata*. Generally, *T. hemprichii* and *C. rotundata* are found higher on the intertidal gradient than is *C. serrulata* (Mukai et al. 1987, Björk et al. 1999, Y.T. & H. Kayanne, pers. obs.). We conducted a transplantation experiment at a seagrass bed off Shiraho, Ishigaki Island, where the emergence stress varies greatly within a short distance owing to the steep depth gradient. Shoot survival, changes in leaf growth and length, and the emergence conditions of seagrass shoots were recorded. In addition, a desiccation experiment was conducted in the laboratory to examine the water loss rate of the leaves of the 3 species.

MATERIALS AND METHODS

Study site. This study was conducted at a seagrass bed off Shiraho, Ishigaki Island, in the Ryukyu Islands, southwest Japan (24° 20' N, 124° 9' E, Fig. 1). The water level at this site is stable for several hours at low tide, because a well-developed coral reef crest separates the moat from the outer ocean (Kayanne et al. 1995).

The seagrass bed consists of numerous patches with a trapezoidal cross-section (Fig. 2). Patch area varies between 10 and >100 m². Usually, the tops of the patches emerge every day (intertidal), while the side surfaces of each patch never emerge (subtidal). Therefore, the duration of air exposure changes dramatically over very small spatial scales. The frequency and degree of emergence of the top surface level was estimated using a tide table (Japan Meteorological Agency 2000, 2001). *Thalassia hemprichii* and *Cymodocea rotundata* occur in both intertidal and subtidal areas, whereas *C. serrulata* occurs only in subtidal areas. The substrate conditions and other environmental factors in the intertidal and subtidal zones were similar.

In July 2001, 30 non-transplanted shoots of each species at each site (subtidal and intertidal) were tagged and punched using a hypodermic needle (total 150 shoots). The punched shoots for leaf growth measurement by the hole punch method (Dennison & Alberte 1982) were collected 16 to 21 d later, and the morphological traits (leaf length, vertical rhizome length, sheath area and sheath dry weight) and leaf growth were measured. The length of the longest leaf of a shoot was used when making comparisons.

Transplantation experiment. A transplantation experiment was conducted to test whether the survival of the 3 seagrass species varied when they were transplanted from the subtidal to the intertidal zone, and whether transplantation caused any changes in mor-

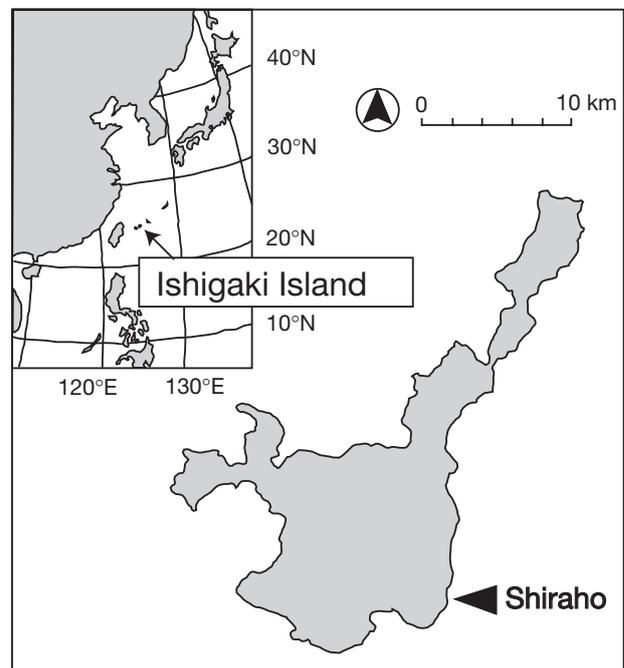


Fig. 1. Study site at Shiraho, Ishigaki Island, southwest Japan

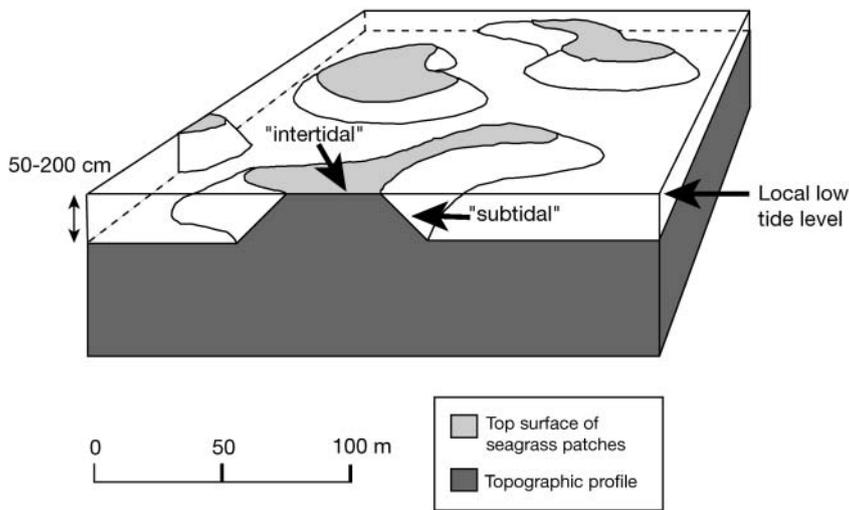


Fig. 2. Schematic presentation of the study site at Shiraho. Topographic profile of each seagrass patch was a trapezoid shape

phology. To account for seasonal changes in tidal regimes and other environmental factors, the experiment was repeated in 3 seasons: 2 July to 23 July 2001, 23 September to 18 October 2001, and 30 January to 25 February 2002. In addition to transplanting plants from the subtidal to the intertidal zone (the transplanted plots), the 3 species were transplanted within the subtidal zone to the same depth as they had been growing at previously (subtidal controls), as were the 2 species in the intertidal zone (intertidal controls). For each species and treatment, 5 replicate samples were prepared. The seagrass and associated sediment were removed using a stainless-steel corer (diameter 20 cm, height 50 cm). First, the corer was used to create a hole where the seagrass was to be transplanted. Then, using the same corer, a turf of seagrasses was dug up along with sediments to a depth of approximately 30 cm and moved gently to the prepared hole. The level of the sediment surface was carefully adjusted to that of the surrounding sediment. After transplantation, all seagrasses within 10 cm of each plot were removed and flags were set at each plot to facilitate identification of the plots. Most plots contained some untargeted species. The above and below ground parts of those species were carefully removed using scissors and by hand. The manipulation of the surrounding vegetation and untargeted species in the plots did not alter the light conditions of the targeted species because the canopies of the untargeted species were lower than those of the 3 species. Nevertheless, as possible spurious effects of transplantation, the growth rate and leaf length might have been reduced because the below sediment parts were damaged. To evaluate the actual effect of emergence, 3 types of transplantation (subti-

dal-to-intertidal, subtidal-to-subtidal, and intertidal-to-intertidal) were compared for *Thalassia hemprichii* and *Cymodocea rotundata*, and 2 types (subtidal-to-intertidal and subtidal-to-subtidal) were compared for *C. serrulata*. In these comparisons, transplantation was a common factor. Simultaneously, non-manipulated shoots were compared with transplanted controls to check for any effect of the transplantation artifact in the first experiment, conducted in July.

Live shoot density was assessed approximately once a week after transplantation. For the plots transplanted in July, the growth rate was measured using the hole-punch method (Dennison & Alberte 1982). Five shoots per plot were tagged randomly and a hole was made using a hypodermic needle (total 200 shoots). The tagged shoots were collected 20 to 22 d later, and then leaf

growth and length were measured to the nearest 0.1 mm using calipers. The length of the longest leaf of a shoot was used when making comparisons. For the plots transplanted in September and January, the growth rate was measured by marking the leaves with plastic gem clips to follow temporal changes in the growth rate. Each gem clip was 25 mm long, 8 mm wide, and weighed 100 mg. The clips gripped the leaves but did not damage them. Two shoots per plot were marked with plastic gem clips (total 80 shoots per measurement). Each clip was attached to the basal part of the youngest leaf; if the youngest leaf was too small, the second youngest leaf was marked. Growth was measured repeatedly at 3 to 4 d intervals and the clips were reset after each measurement.

The plots that were transplanted in September and January were left *in situ* to examine long-term changes in morphological traits. The length of the longest leaf of a shoot and the position of the meristem (defined here as the vertical rhizome length plus the sheath length) were measured for 5 shoots randomly selected from a plot in February 2002 (147 d after transplantation for the September plots) and May 2002 (232 d after transplantation for the September plots, 103 d for the January plots).

Emergence conditions. During the spring tides in October and February, we observed the emergence conditions of the transplanted plots and intertidal controls within 10 d after transplantation. The emergence levels of each plot were recorded every hour for 7 h, starting during the ebb tide when the water level was still higher than the mean sea level, through low tide, and finishing during the flood tide once all the plots were submerged again. For each treatment, 35 (5 plots

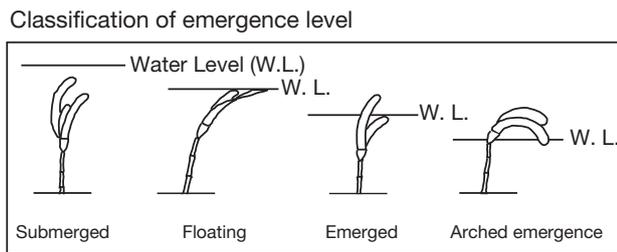


Fig. 3. Schematic presentation of emergence level classification for seagrass shoots. Each plot was categorized as one of the following 4 emergence levels: (1) submerged: no leaf reached water surface, (2) floating: at least 1 leaf reached water surface, (3) emerged: at least 1 leaf was emerged from the water, (4) arched emergence: more than half of the leaf blade was exposed to air

× 7) measurements of emergence levels were recorded as percentages. Using these data, the average emergence time of each plot was calculated for each treatment. Each plot was categorized into one of the following 4 emergence levels: submerged: no leaf reached the water surface; floating: at least 1 leaf reached the water surface; emerged: at least 1 leaf emerged above the water surface; and arched emergence: more than half of the leaf blade (in an arched position) was exposed to air (Fig. 3).

Desiccation experiment. Laboratory experiments were conducted in October 2001 and February 2002 to measure the water loss rate of seagrass leaves exposed to air while controlling both temperature and humidity (27°C, 55% in October and 24.5°C, 70% in February). Shoots of *Cymodocea rotundata*, *C. serrulata* and *Thalassia hemprichii* were collected with their below-ground parts from the subtidal zone and kept in an outdoor container with circulating seawater for a few days. Just before beginning the experiment, the second youngest leaves on the shoots were cut off at the border between the leaf and the sheath. Six leaves per species were gently wiped with a kitchen towel, weighed (IW, initial weight), and laid on corrugated cardboard, which was obtained by stripping away one side of the flat surface of ordinary cardboard, to minimize contact between the substrate and the leaf surface. The change in the weight of the leaves (W_t , weight at time t) was measured over 65 min at 5 min intervals in October and over 150 min at 10 min intervals in February. After completing the measurements, the leaves were dried at 105°C for at least 24 h and weighed (DW, dry weight). The change in the relative water content was calculated as $(W_t - DW)/(IW - DW)$ (Björk et al. 1999). Exponential curves were fitted to each water loss trace, and the desiccation coefficient was estimated using the following formula:

$$I_t = I_0 e^{-kt}$$

where I_t is the water content at time t , I_0 is the water content at time 0, and k is the desiccation coefficient.

Statistical analyses. The differences in the morphological traits (leaf length, vertical rhizome length, and sheath area) and leaf growth of the non-manipulated shoots of subtidal and intertidal sites for each species were tested using the t -test. The Welch correction was made before subjecting data with heterogeneous variance to the t -test (checked using the F -test).

Temporal changes in the shoot density in the transplanted plots and controls were represented as the ratio of the density at each time step relative to the initial density. The variation in the relative shoot density among the 3 transplantation types (transplanted plots, subtidal controls and intertidal controls) of *Thalassia hemprichii* and *Cymodocea rotundata*, and between the transplanted plots and subtidal controls of *C. serrulata* was tested using repeated-measure ANOVA. The data on relative shoot density were arcsine-transformed before conducting ANOVAs. The variation in leaf growth in July, when the hole-punch method was adopted, was tested using 1-way ANOVA, whereas data from September and January, when growth was measured repeatedly at 3 to 4 d intervals, were tested using repeated-measure ANOVA. Variations in leaf lengths in the transplanted plots and controls were tested using 1-way ANOVA for the 3 seasons. Variations in the position of the meristem of leaves in the transplanted plots and controls were tested using 1-way ANOVA for the 3 seasons. The desiccation coefficients of the 3 species were also compared using 1-way ANOVA. When ANOVA detected significant variation among transplantation types or species, post-hoc comparisons were carried out with Scheffé's F method (at a significance level of $\alpha = 0.05$). For the shoot density data, *post-hoc* comparisons were conducted only for the data recorded at the end of each experiment. Before these analyses, the heterogeneity of variance was tested using Cochran's C -test.

RESULTS

Tidal condition

The frequency and duration of emergence changed seasonally. The predicted emergence frequency was highest and the total emergence time was longest in January 2002 (Table 1). By contrast, these values were lowest and shortest in July 2001 and should have been intermediate in September 2001; however, low atmospheric pressure associated with several typhoons in September 2001 resulted in elevated sea levels and high waves. Consequently, the actual tide level in this period was higher than predicted by the tide table.

Table 1. Emergence period and frequency at local low tide level in the intertidal part of the study site

	Period	Days	Total emergence (h)	Emergence frequency (no.)	Average emergence time /emergence (h)	/day (h)
Jul (2001)	2 Jul to 23 Jul	21	83	19	4.4	4.0
Sep (2001)	23 Sep to 18 Oct	25	145	37	3.9	5.8
Jan (2002)	30 Jan to 25 Feb	26	215	43	5.0	8.3
Year (2001 to 2002)	1 Jun to 31 May	365	2187	488	4.5	6.0

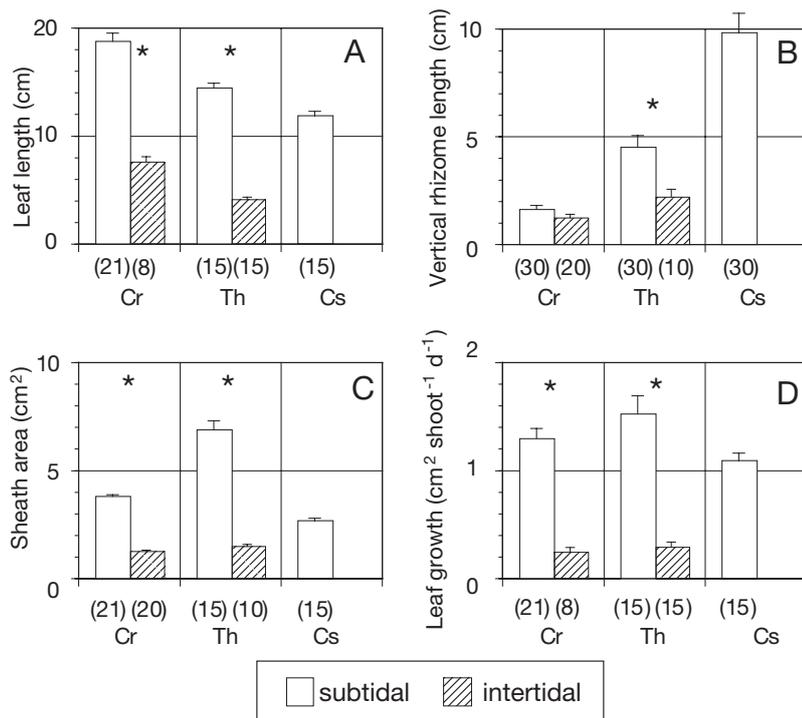


Fig. 4. *Cymodocea rotundata* (Cr), *Thalassia hemprichii* (Th) and *C. serrulata* (Cs). (A) Leaf length, (B) vertical rhizome length, (C) sheath area, and (D) leaf growth in natural conditions in July 2001 at sites with different emergence stress. Columns are means and error bars are SE of the mean. *: significant difference between the subtidal and the intertidal. The numbers in parentheses indicate sample sizes. *C. serrulata* was not observed in the intertidal

Variation in seagrass morphology and growth with tide level

Under natural conditions, the leaves of *Cymodocea rotundata* and *Thalassia hemprichii* were longer in the subtidal zone than in the intertidal zone (Fig. 4A, *t*-test; $t = 12.3$, $p < 0.001$ for *C. rotundata* and $t = 20.6$, $p < 0.001$ for *T. hemprichii*). The lengths of vertical rhizomes of *C. rotundata* were similar in the subtidal and intertidal zones, but those of *T. hemprichii* were longer in the subtidal zone (Fig. 4B, *t*-test; $t = 1.60$, $p = 0.058$ for *C. rotundata* and $t = 3.63$, $p < 0.001$ for *T.*

hemprichii). The sheath areas of *C. rotundata* and *T. hemprichii* were larger in the subtidal zone (Fig. 4C, *t*-test; $t = 24.58$, $p < 0.001$ for *C. rotundata* and $t = 12.86$, $p < 0.001$ for *T. hemprichii*). The leaf growth rates of *T. hemprichii* and *C. rotundata* were higher in the subtidal zone (Fig. 4D, *t*-test; $t = 10.53$, $p < 0.001$ for *C. rotundata* and $t = 7.11$, $p < 0.001$ for *T. hemprichii*). Sheath dry weight, which was measured only for the subtidal populations of each species, was largest for *T. hemprichii* (mean \pm SE, 211.9 ± 14.6 mg DW shoot⁻¹) followed by *C. rotundata* (52.6 ± 12.9) and *C. serrulata* (32.8 ± 5.9).

Transplantation experiment

Shoot density

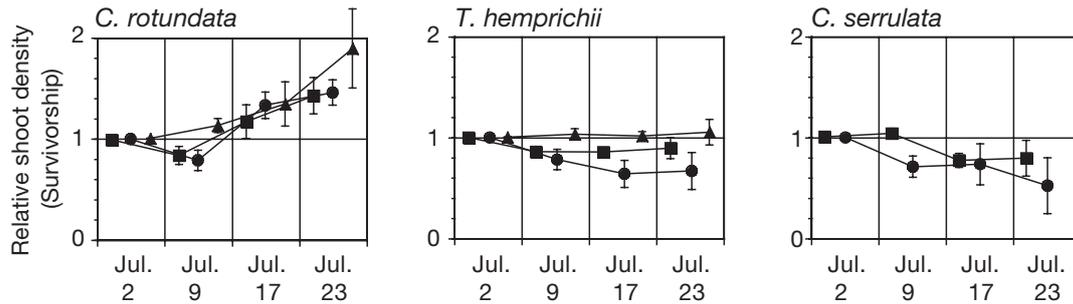
The shoot density of *Cymodocea rotundata* increased in July in all of the treatments, whereas those of *Thalassia hemprichii* and *C. serrulata* did not (Fig. 5A). The among-treatment variation was not significant for the 3 species (Table 2A). In September, the shoot densities of the transplanted and intertidal controls of *C. rotundata* increased, as did that of transplanted *C. serrulata* during the last 10 d of the experimental period. The shoot density did not change in the other treatments (Fig. 5B). Significant among-treatment variation was detected only in *C. rotundata* (Table 2B). The post-hoc comparison revealed that the intertidal controls had the greatest shoot density, followed by the transplanted plots, and then the subtidal controls.

In January, the shoot density of *Cymodocea rotundata* decreased in all of the plots (Fig. 5C). The among-treatment variation was significant (Table 2C), and the

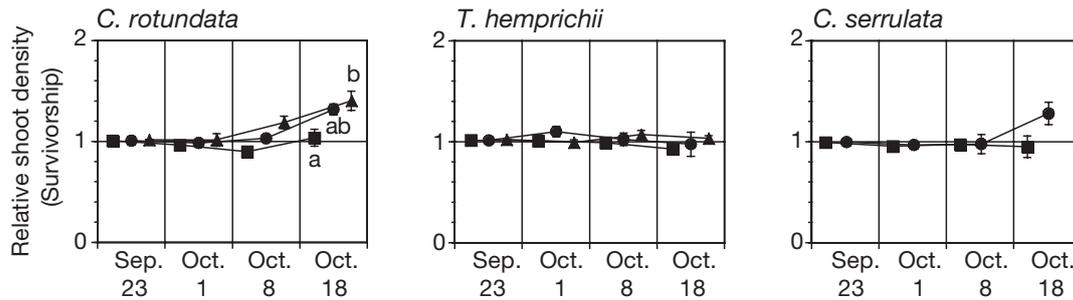
survival of the subtidal controls was higher than that of the intertidal controls and plants in the transplanted plots. The shoot density in the plots of transplanted *C. rotundata* decreased significantly to 48% of the initial density within 1 mo. The shoot density also decreased in all of the *Thalassia hemprichii* plots (Fig. 5C). Significant among-treatment variation was found (Table 2C). The variation was greater in the subtidal and intertidal controls than in the transplanted plots. The shoot den-

sity in transplanted *T. hemprichii* plots decreased to 48% of initial values (Fig. 5C). The shoot density of *C. serrulata* decreased in both treatments (Fig. 5C). Significant among-treatment variation was detected (Table 2C), with a greater decrease in density in the transplanted plots. The shoot density in the transplanted plots of *C. serrulata* decreased to 2% of the initial density, and all of the shoots died by 25 February in 4 out of 5 plots.

A: Transplanted in July 2001



B: Transplanted in Sep. 2001



C: Transplanted in Jan. 2002

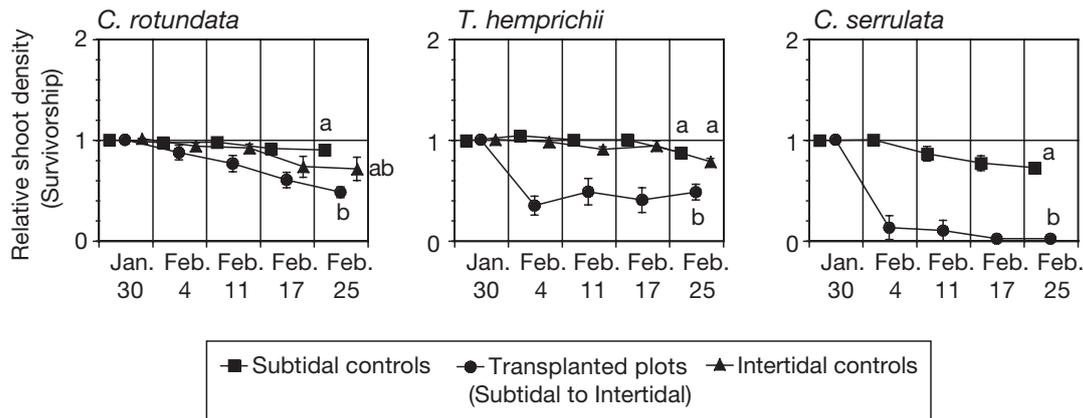


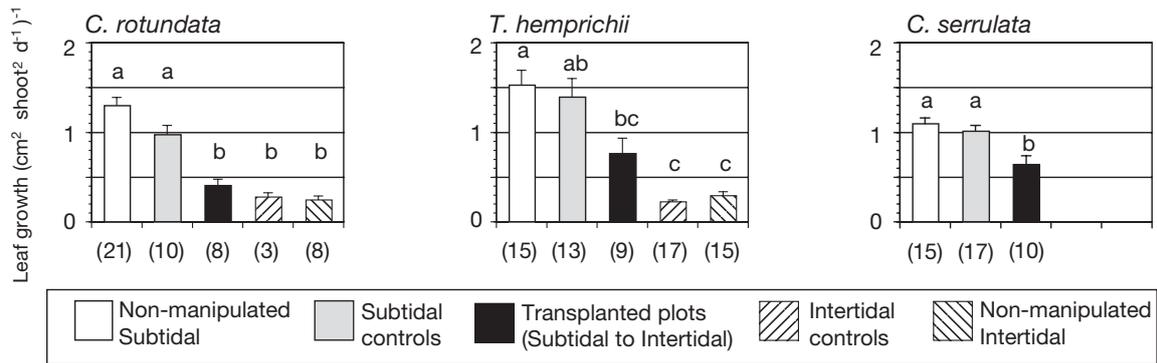
Fig. 5. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Temporal changes in survivorship at transplantation experiments. ■, ●, ▲ are the means of 5 replicate plots, and error bars are the SE of the mean. Different letters denote pairs of treatments where significant variations were detected by the post-hoc comparisons

Leaf length

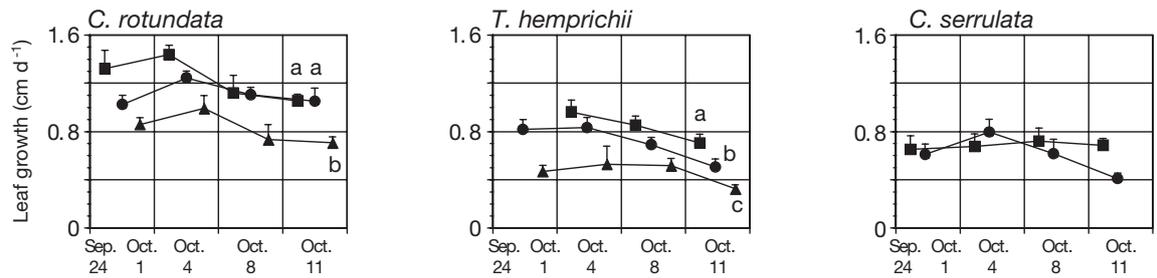
In July, the leaves in the transplanted plots were significantly shorter than those of the subtidal controls for all 3 species after 3 wk (Table 4A). The leaf length in the transplanted plots was 64% that of the subtidal controls for *Cymodocea rotundata*, 71% for *Thalassia hemprichii*, and 80% for *C. serrulata* (Fig. 7A). The leaves of the transplanted *C. rotundata* were as short as those of the intertidal controls, whereas those of *T.*

hemprichii were intermediate between the subtidal and intertidal controls. The leaf lengths of the subtidal and intertidal controls did not differ significantly from those of the corresponding non-manipulated shoots, except for subtidal *T. hemprichii* controls. In long-term observations of the September plots, leaves in the transplanted plots became significantly shorter than those in the subtidal controls, except for *C. serrulata* in May 2002, 232 d after transplantation (Fig. 7B, Table 4B). The leaf lengths of the transplanted plots of *C. rotundata* and *T.*

A: Transplanted in July 2001



B: Transplanted in Sep. 2001



C: Transplanted in Jan. 2002

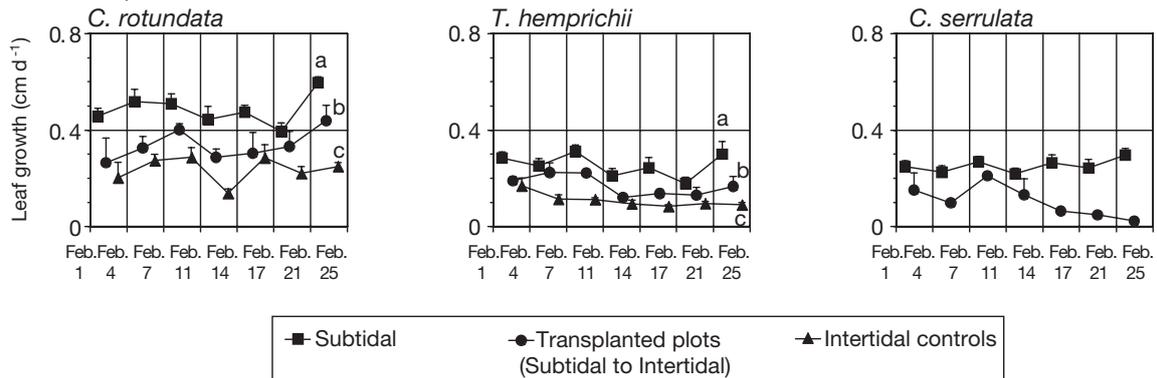


Fig. 6. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Difference in leaf growth among transplantation treatments. For July, columns are the means of replicates and error bars are the SE of the mean. The numbers in parentheses show the sample sizes. For September and January, ■, ●, ▲ are the means of replicates, and error bars are the SE of the mean. The sample size varied between 2 and 10. Different letters denote pairs of treatments where significant among-treatment variations were detected by the post-hoc comparisons

hemprichii did not differ significantly from those of the intertidal control plots. In long-term observations of the January plots, the leaves in the transplanted plots were significantly shorter than those of the subtidal controls and were similar to those of the intertidal controls for *C. rotundata* and *Thalassia hemprichii* in May 2002, 103 d after transplantation (Fig. 7C, Table 4C). For *C. serrulata*, the long-term change in the transplanted plots was not examined because all the plots died by May 2002.

Position of the meristem

In long-term observations of the September plots, the position of the meristem of the transplanted plots became significantly shorter than the subtidal controls (Fig. 8A, Table 5A). The position of the meristem of the transplanted plots of *Cymodocea rotundata* and *Thalassia hemprichii* did not differ significantly from that of the intertidal control plots. In long-term observations of the January plots, the meristem in the transplanted plots was significantly shorter than that of the subtidal controls and was similar to the intertidal controls for *C. rotundata* and *T. hemprichii* in May 2002 (Fig. 8B, Table 5B). For *C. serrulata*, the long-term change in the transplanted plots was not examined because all the plants died by May 2002.

Description of the emergence conditions

In October, the intertidal controls of *Cymodocea rotundata* and *Thalassia hemprichii* never emerged, whereas the transplanted plots of all 3 species did emerge. A greater proportion of *C. serrulata* shoots was exposed to air for longer than was the case for either *C. rotundata* or *T. hemprichii* (Fig. 9). In February, all of the plots, including the intertidal *C. rotundata* and *T. hemprichii* controls, were subjected to prolonged exposure to air. Of the transplanted plots, *T. hemprichii* and *C. serrulata* experienced longer periods of exposure to air than did *C. rotundata* (Fig. 9).

Desiccation experiments

Cymodocea rotundata and *C. serrulata* showed similar patterns of water loss in the desiccation experiments, which were conducted in 2 seasons under different conditions (27°C and 55% humidity in October, 24.5°C and 70% humidity in February) (Fig. 10). The water content of *Thalassia hemprichii* leaves was sig-

Table 3. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Result of ANOVAs comparing leaf growth in different transplantation treatments. In July, non-manipulated shoots are added and 1-way ANOVA is used for testing leaf growth during the whole period. In September and February, repeated-measure ANOVA is used because growth was measured continuously basically in 3 to 4 d intervals. Analysis was not performed for *C. serrulata* in February 2002 because the number of survived shoots were too small for the statistical test

Species	Source of variance	df	MS	F	p
A. Transplanted in July 2001					
<i>C. rotundata</i>	Treatments	4	244.79	22.87	<0.001
	Error	45	10.70		
<i>T. hemprichii</i>	Treatments	4	547.08	23.19	<0.001
	Error	64	23.59		
<i>C. serrulata</i>	Treatments	2	66.05	8.85	<0.001
	Error	39	7.47		
B. Transplanted in September 2001					
<i>C. rotundata</i>	Between subjects				
	Treatments	2	1.024	16.35	<0.001
	Error	27	0.063		
	Within subjects				
	Temporal	3	0.277	6.00	<0.01
	Treatments × temporal	6	0.032	0.69	0.66
<i>T. hemprichii</i>	Between subjects				
	Treatments	2	0.793	17.85	<0.001
	Error	25	0.044		
	Within subjects				
	Temporal	2	0.354	21.63	<0.001
	Treatments × temporal	4	0.006	0.39	0.81
<i>C. serrulata</i>	Between subjects				
	Treatments	1	0.172	4.23	0.055
	Error	18	0.041		
	Within subjects				
	Temporal	3	0.081	1.92	0.16
	Treatments × temporal	3	0.082	1.96	0.15
<i>C. serrulata</i>	Between subjects				
	Treatments	2	0.871	42.52	<0.001
	Error	27	0.020		
	Within subjects				
	Temporal	6	0.067	4.55	<0.001
	Treatments × temporal	12	0.013	0.85	0.60
<i>T. hemprichii</i>	Between subjects				
	Treatments	2	0.394	35.62	<0.001
	Error	27	0.011		
	Within subjects				
	Temporal	6	0.025	4.27	<0.001
	Treatments × temporal	12	0.007	1.20	0.29
<i>T. hemprichii</i>	Between subjects				
	Treatments	2	0.394	35.62	<0.001
	Error	27	0.011		
	Within subjects				
	Temporal	6	0.025	4.27	<0.001
	Treatments × temporal	12	0.007	1.20	0.29
<i>T. hemprichii</i>	Between subjects				
	Treatments	2	0.394	35.62	<0.001
	Error	27	0.011		
	Within subjects				
	Temporal	6	0.025	4.27	<0.001
	Treatments × temporal	12	0.007	1.20	0.29
<i>T. hemprichii</i>	Between subjects				
	Treatments	2	0.394	35.62	<0.001
	Error	27	0.011		
	Within subjects				
	Temporal	6	0.025	4.27	<0.001
	Treatments × temporal	12	0.007	1.20	0.29
<i>T. hemprichii</i>	Between subjects				
	Treatments	2	0.394	35.62	<0.001
	Error	27	0.011		
	Within subjects				
	Temporal	6	0.025	4.27	<0.001
	Treatments × temporal	12	0.007	1.20	0.29

Table 4. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Result of 1-way ANOVA comparing the leaf length among different transplantation treatments. Non-manipulated shoots are added for comparison in July

Species	Date of observation	Source of variance	df	MS	F	p
A. Transplanted in July 2001						
<i>C. rotundata</i>	Jul 2001	Treatments	4	307.5	34.4	<0.001
		Error	45	8.9		
<i>T. hemprichii</i>	Jul 2001	Treatments	4	309.0	97.4	<0.001
		Error	64	3.2		
<i>C. serrulata</i>	Jul 2001	Treatments	2	24.0	8.1	<0.01
		Error	39	3.0		
B. Transplanted in September 2001						
<i>C. rotundata</i>	Feb 2002	Treatments	2	69.1	14.2	<0.001
		Error	72	4.9		
	May 2002	Treatments	2	322.4	64.2	<0.001
		Error	72	5.0		
<i>T. hemprichii</i>	Feb 2002	Treatments	2	124.3	29.6	<0.001
		Error	72	4.2		
	May 2002	Treatments	2	167.5	23.0	<0.001
		Error	68	7.3		
<i>C. serrulata</i>	Feb 2002	Treatments	1	114.4	25.2	<0.001
		Error	38	4.5		
	May 2002	Treatments	1	4.1	1.9	0.18
		Error	35	2.2		
C. Transplanted in January 2002						
<i>C. rotundata</i>	May 2002	Treatments	2	546.5	76.7	<0.001
		Error	72	7.1		
<i>T. hemprichii</i>	May 2002	Treatments	2	353.5	63.0	<0.001
		Error	48	5.6		

Table 5. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Result of 1-way ANOVA comparing the position of meristem in different transplantation treatments

Species	Date of observation	Source of variance	df	MS	F	p
A. Transplanted in September 2001						
<i>C. rotundata</i>	Feb 2002	Treatments	2	9.62	5.25	<0.01
		Error	72	1.83		
	May 2002	Treatments	2	50.25	30.58	<0.001
		Error	72	1.64		
<i>T. hemprichii</i>	Feb 2002	Treatments	2	52.72	17.33	<0.001
		Error	72	3.04		
	May 2002	Treatments	2	54.19	82.65	<0.001
		Error	68	0.66		
<i>C. serrulata</i>	Feb 2002	Treatments	1	218.65	121.32	<0.001
		Error	38	1.80		
	May 2002	Treatments	1	79.60	19.51	<0.001
		Error	35	4.08		
B. Transplanted in January 2002						
<i>C. rotundata</i>	May 2002	Treatments	2	103.92	146.92	<0.001
		Error	72	0.71		
<i>T. hemprichii</i>	May 2002	Treatments	2	97.03	59.68	<0.001
		Error	48	1.63		

nificantly higher than those of *C. rotundata* and *C. Serrulata*. The desiccation coefficient of *T. hemprichii* leaves (0.026) was lower than those of *C. rotundata* (0.046) and *C. serrulata* (0.049) in October (1-way ANOVA, $F_{2, 15} = 48.36$, $p < 0.001$), and that of *T. hemprichii* (0.013) was lower than those of *C. rotundata* (0.020) and *C. serrulata* (0.021) in February (one-way ANOVA, $F_{2, 15} = 56.8$, $p < 0.001$). In October, *T. hemprichii* leaves lost 80% of their water in 60 min and those of *Cymodocea* species did so in 30 min. In Febru-

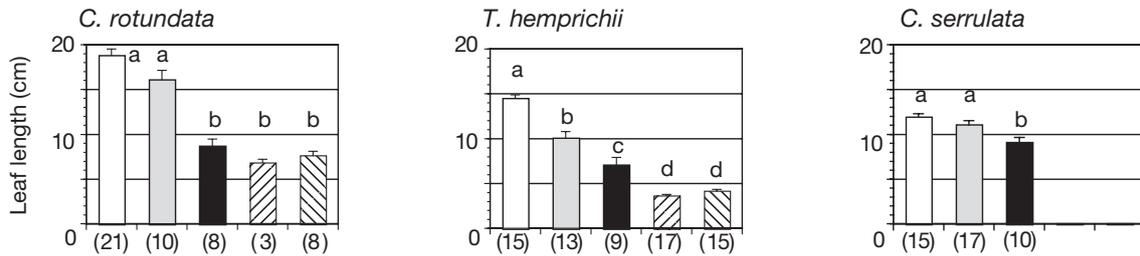
ary, it took 120 min for *T. hemprichii* leaves and 60 min for *C. rotundata* and *C. serrulata* leaves to lose 80% of their water content (Fig. 10).

DISCUSSION

We found that the 3 dominant tropical seagrass species had different tolerances to emergence stress, which is thought to be the major factor influencing the

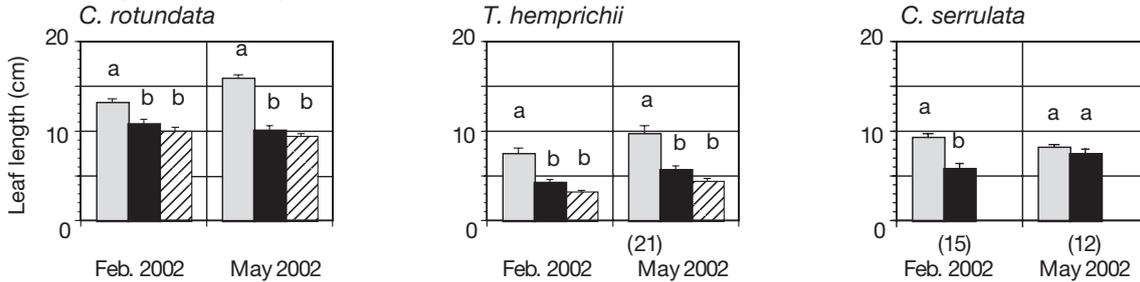
Short-term observations

A: Transplanted in July 2001



Long-term observations

B: Transplanted in Sep. 2001



C: Transplanted in Jan. 2002

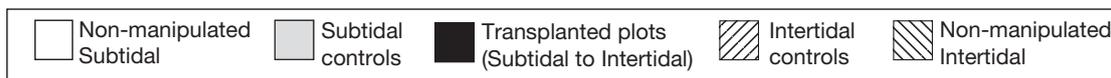
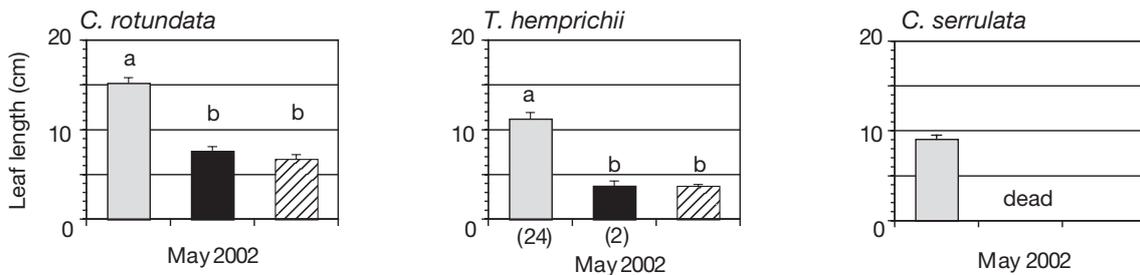


Fig. 7. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Temporal changes in leaf length in transplantation experiments. Columns are the means of replicates, and error bars are the SE of the mean. Different letters denote pairs of treatments where significant among-treatment variation was detected by the post-hoc comparison. Sample size was 25 for each treatment except those shown in parentheses

upper limit of distribution of these species in the intertidal zone. Experimentally, we showed that *Cymodocea serrulata* is the most sensitive to emergence stress (Fig. 5); this result agrees with the distribution at

Shiraho, Ishigaki Island, where *C. serrulata* was not found in the intertidal zone. This also agrees with its intertidal distribution at other sites around Ishigaki Island (Tanaka 2004), in Papua New Guinea (Mukai et

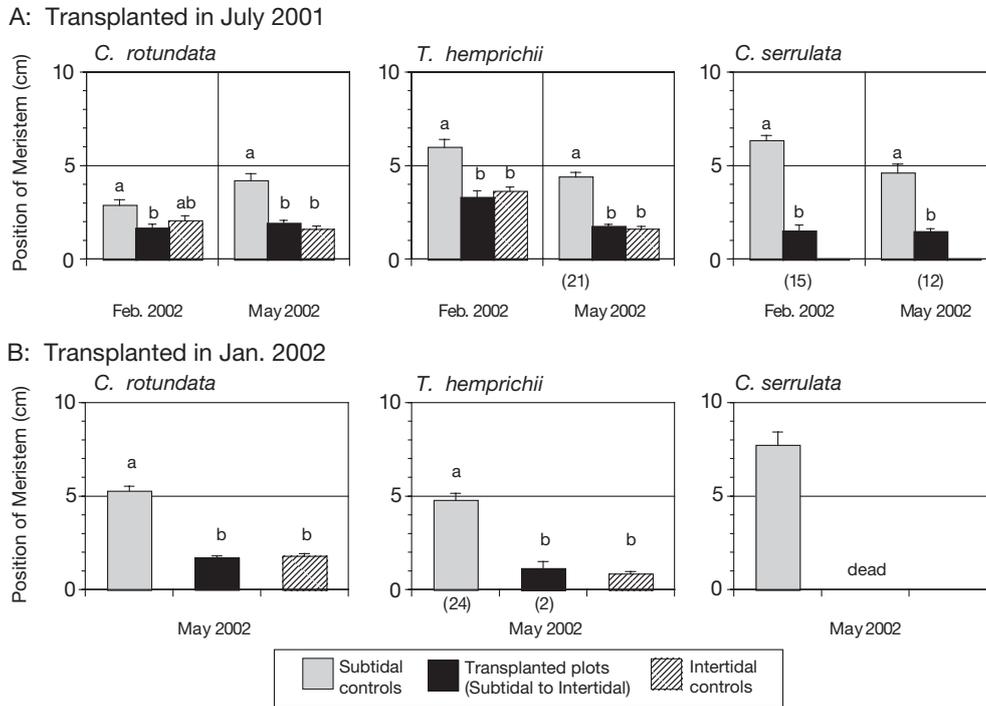


Fig. 8. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Temporal changes in position of meristem in transplantation experiments. Columns are the means of replicates, and error bars are the SE of the mean. Different letters denote pairs of treatments where significant among-treatment variation was detected by the post-hoc comparison. Sample size was 25 for each treatment except those shown in parentheses

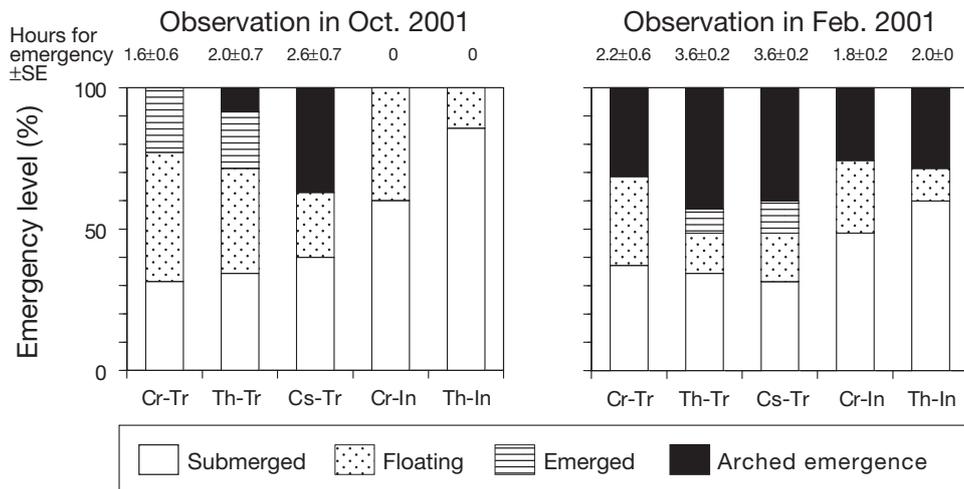


Fig. 9. Difference in emergence conditions among the transplantation treatments during daytime spring tide. Columns show the ratio of 4 emerging levels during 7 h. Hours for emergence are calculated as emerged plus arched emergence. Cr-Tr = transplanted plots of *Cymodocea rotundata*, Th-Tr = transplanted plots of *Thalassia hemprichii*, Cs-Tr = transplanted plots of *C. serrulata*, Cr-In = intertidal controls of *C. rotundata*, Th-In = intertidal controls of *T. hemprichii*

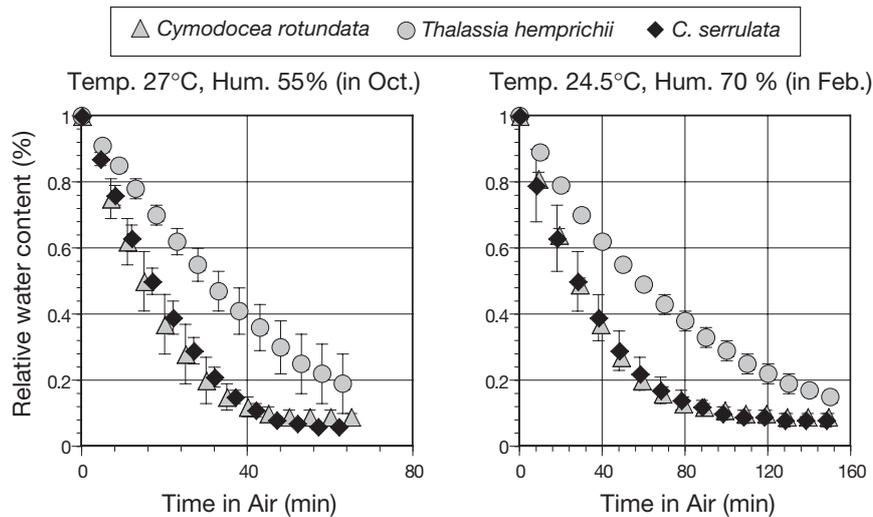


Fig. 10. *Cymodocea rotundata*, *Thalassia hemprichii* and *C. serrulata*. Change in water content of the leaves exposed to air in the desiccation experiment. Error bars are SD of the mean (6 replicates). Hum.: humidity

al. 1987), and in Tanzania (Björk et al. 1999), where *C. rotundata* and *Thalassia hemprichii* occur in shallower water than does *C. serrulata*.

This study also showed that the survival of seagrass changed seasonally with the seasonal changes in exposure to air. In July and September, when exposure was short, no decrease in shoot density was apparent. Conversely, in January, when exposure was long, the shoot density in the transplanted plots decreased significantly in all 3 species. Similar seasonal changes in seagrass abundance in relation to changes in the tide level have been reported for *Enhalus acoroides* and *Thalassia hemprichii* in Indonesia (Erftemeijer & Herman 1994, Stapel et al. 1997), and *Phyllospadix iwatanensis* in Japan (Yabe et al. 1995, 1996).

Comparing 8 tropical species of different sizes, Björk et al. (1999) showed that physiological tolerance of desiccation does not necessarily determine the upper limit of the distribution of seagrasses at intertidal sites. They found that *Cymodocea rotundata* and *C. serrulata* did not regain photosynthesis ability after losing 70% of their water, whereas *Thalassia hemprichii* showed a remarkable capacity to recover close to its original photosynthesis ability after losing 85% of its water (Björk et al. 1999). Our study also showed that *C. rotundata* and *C. serrulata* lose water much more quickly than does *T. hemprichii* (Fig. 10). These results suggest that it is highly likely that *C. rotundata* and *C. serrulata* leaves are more vulnerable to desiccation. However, this variation in physiological tolerance among the species does not coincide with the observed variation in the upper limits of distribution or the variation in survival measured in the transplantation experiment.

The observations of emergence conditions in the field showed that morphological variation among species is important in determining the upper limit of each species. When leaves are damaged, the efficiency of photosynthesis decreases. Moreover, if the meristem is lost, the shoots will die. *Cymodocea rotundata* has a short vertical rhizome (Fig. 4B) and a narrow strap-like sheath. This morphological characteristic allows the above sediment parts of this species to lie on the ground and remain in contact with the wet substrate, thus avoiding water loss when the seagrass bed is exposed. *Thalassia hemprichii* has a longer vertical rhizome and an inflexible sheath as compared to *C. rotundata*. Therefore, *T. hemprichii* is exposed to air for longer than is *C. rotundata*. However, this may be partly offset by the fact that the leaves of *T. hemprichii* have the greatest physiological tolerance to desiccation, as mentioned above. In addition, the large sheath of *T. hemprichii* may help to prevent water loss from the meristem.

Cymodocea serrulata is exposed to the air for longer than *Thalassia hemprichii* or *C. rotundata* because of its long vertical rhizome. In addition, the sheath of *C. serrulata* is narrower and lighter than those of the other 2 species and is kept away from the wet substrate by the long vertical rhizome; none of these characteristics are suitable for protecting the meristem from desiccation (Fig. 4C). Moreover, *C. serrulata* has leaves which have a low physiological tolerance to desiccation. Therefore, *C. serrulata* is the least tolerant of desiccation of the 3 species, and cannot survive in the intertidal area, as demonstrated by its extremely low survival in the transplantation experiment in February (Fig. 5C). The long vertical rhizome of *C. serrulata* is

considered advantageous for obtaining light and for avoiding physical burial at deep subtidal sites (Vermaat et al. 1996, Duarte et al. 1997). However, this morphological character is a disadvantage to survive at the intertidal site.

Thalassia hemprichii and *Cymodocea rotundata*, which are both distributed in the intertidal and subtidal zones, showed great morphological within-species variation. In natural conditions, they have shorter vertical rhizomes and leaves in the intertidal zone than in the subtidal zone (Fig. 4A). Shorter vertical rhizomes reduce the probability of the meristem emerging, and shorter leaves decrease damage to photosynthetic organs. The reduced shoot size in the intertidal zone decreases the emergence probability, as shown in the observations of emergence condition (Fig. 9). Downsizing in the intertidal zone has also been observed in *Halodule wrightii* in the United States (Phillips 1967) and in *Halophila ovalis*, *Halodule uninervis*, and *Zostera capricorni* in Australia (Dawson & Dennison 1996). Therefore, downsizing is considered one of the general responses of seagrasses to emergence stress.

In the transplantation experiment, the leaf sizes of *Thalassia hemprichii* and *Cymodocea rotundata* decreased drastically when transplanted from the subtidal zone to the intertidal zone (Fig. 7), which seems to be an adaptive trait for survival in an intertidal environment that is subjected to emergence stress. By contrast, we could not examine the degree of intraspecific variation in the leaf size plasticity of *C. serrulata*, because we lacked intertidal controls for this species. Nevertheless, a decrease in leaf size is not likely to increase survival in this species because the meristem is located above a long vertical rhizome. To increase survival during emergence, *C. serrulata* would need to produce new shoots with a shorter vertical rhizome.

In the long-term observations of the September plots (Fig. 8A), the meristems of transplanted *Cymodocea serrulata* were significantly closer to the substrate than were those in control plots, mostly owing to the production of new shoots with short vertical rhizomes. Shoot spacing along horizontal rhizomes was similar in all 3 species: 4.8, 6.9 and 5.3 cm for *C. rotundata*, *Thalassia hemprichii*, and *C. serrulata*, respectively, while the horizontal elongation rates were noticeably different: 34, 21 and 78 cm apex⁻¹ yr⁻¹, respectively (Vermaat et al. 1995). Hence, the productivity of new shoots is highest in *C. serrulata*. Moreover, elongation of the vertical rhizome is also highest in *C. serrulata* (13 cm shoot⁻¹ yr⁻¹) as compared to *C. rotundata* (1.5 cm) and *T. hemprichii* (3.0 cm) (Vermaat et al. 1995), which prevents *C. serrulata* from surviving despite the high potential productivity of new shoots. Moreover, it takes longer to change morphological traits by producing new shoots than it does to change

leaf size plastically, as observed in *C. rotundata* and *T. hemprichii*, and it is also more expensive. Hence, *C. serrulata* cannot adapt to the sudden change associated with emergence.

In conclusion, this study demonstrated that inter-species variation in tolerance to emergence stress is a significant factor influencing the upper limit of 3 mid-sized species in tropical seagrass beds. Inter-species variation in morphological traits, ability to change size plastically, and physiological tolerance of leaves to desiccation stress, including the water loss rate, are all involved in the inter-species variation in tolerance of emergence stress. Similar complex mechanisms may also explain the distribution of other seagrass species in subtropical areas. Further studies that also consider the morphological adaptation of seagrasses to emergence are important for elucidating the inter-species variation in distribution in mixed tropical seagrass meadows.

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