Ecophysiological Responses of Viviparous Mangrove

*Rhizophora stylosa* Seedlings to Simulated Sea-Level Rise

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ABSTRACT

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As the only forests situated at the transition between land and sea, mangrove forests constitute one of the first ecosystems vulnerable to rising sea level. When sea level rises, plants are exposed to increased salinity, as well as tidal flooding. The responses of mangrove forests to changing sea levels depend on the synergistic effects of tidal flooding and salinity on plants, especially plants at the seedling stage. Therefore, the aims of this paper were to assess the ability of viviparous mangrove *Rhizophora stylosa* Griff. seedlings to withstand tidal flooding given different seawater salinities, as well as to investigate the effects of tidal flooding and salinity on plant growth. To accomplish this, a mesocosm experiment was initiated to examine the synergistic effects of tidal flooding and salinity on the growth and physiology of *R. stylosa* samples subjected to seven tidal flooding times (0, 2, 4, 6, 8, 10, and 12 h simulated semidiurnal tide, twice daily) and two levels of water salinity (15 and 30 PSU [practical salinity units]) over the course of 100 days. Throughout the experimental period, *R. stylosa* seedlings exhibited the traits of flood-tolerant plants, including such mechanisms as high stem growth rate and leaf assimilation rate, as well as efficient utilization of carbohydrate reserves stored in hypocotyls of seedlings. The growth and physiological responses of *R. stylosa* seedlings were significantly affected by both salinity and flooding time. However, elevated salinity had less effect on growth and metabolism than simulated sea-level rise by the increase of flooding time. From these results, it can be concluded that *R. stylosa* at higher salinity exhibits competitive dominance and thus good adaptation of seedlings to future predicted rise in sea level, which could presumably result in niche shifting.

**ADDITIONAL INDEX WORDS:** Tidal inundation, salt stress, photosynthesis, alcohol dehydrogenase (ADH) activity, leaf gas exchange.

INTRODUCTION

The potential effects of global climate change on coastal ecosystems have attracted considerable attention. As the only forests situated at the transition between the terrestrial environment and hydrosphere, mangrove forests will be one of the first ecosystems affected by rising sea levels accompanying global climate change (Krauss et al., 2014; Semeniuk, 1994). The accurate prediction of mangrove responses to sea-level rise would enable strategic mangrove planting in order to minimize and offset anticipated mangrove losses (Gilman, 2006).

As sea levels rise, plants are exposed to elevated salinity as well as increased tidal flooding (Knight et al., 2008). Meanwhile, tidal flooding and salinity have been recognized as two major factors influencing mangrove recruitment, early growth, and zonation (Knight et al., 2008; Lugo and Snedaker, 1974; Piou et al., 2006). When tidal flooding is combined with elevated salinity, the effects on seedling tolerance may influence the rapidity with which coastal forests are eliminated during a period of rising seas (Krauss et al., 2014).

The synergistic effects of tidal flooding and salinity on mangroves have been studied in a few species (Naidoo, 1985; Yáñez-Espinosa, Terrazas, and López-Mata, 2001). To some extent, the response of mangroves to sea-level rise depends on the growth and physiological reactions of seedlings (Krauss et al., 2008). Some previous studies have reported on the ecophysiological responses (including leaf gas exchanges and leaf or root oxidation activities that reflect plant tolerance to environmental stress) of mangrove seedlings to different salinities or tidal flooding separately, either in the field or under controlled laboratory conditions (Chen et al., 2013; Chen, Wang, and Lin, 2005; Ellison and Farnsworth, 1997; Lu et al., 2013; McKee, 1993). Mangroves are equipped with a suite of adaptations that facilitate growth in chronically flooded habitats and, hence, can respond to multiple tidal flooding levels with both gradual physiological and structural adjustments that can be quantified (Chen et al., 2013; Ellison and Farnsworth, 1997; Youssef and Saenger, 1996). Simultaneously, some previous studies were conducted at optimal salinities for mangrove growth (Krauss et al., 2006). Nevertheless, the halophytic nature of mangroves creates difficulties for scientists attempting to investigate the effects of altered hydroperiod on these coastal systems, and salinity at any concentration may confound interpretation of the synergistic effects of flooding on mangroves (Krauss et al., 2006). The importance of increased salinity in the context of tidal flooding...
is extremely difficult to determine by field manipulation or transplant experiments (Maricic et al., 2006). Thus, a mesocosm experiment under controlled tidal conditions would be helpful to examine the synergistic effects of tidal flooding and salinity on mangrove seedlings.

The red viviparous mangrove Rhizophora stylosa Griff. is probably the best-known mangrove plant because of its distinctive root system and widespread distribution. It is commonly found close to the seaward edge of mangrove forests where its roots are submerged during high tides. In the present study, the potential effects of sea-level rise on the growth and ecophysiology of this species were examined. The aim was to assess the ability of R. stylosa seedlings to withstand tidal flooding given different seawater salinities. To accomplish this, a mesocosm experiment was initiated to examine the combined effects of tidal inundation and salinity on the growth and physiology of R. stylosa samples subjected to seven flooding times (0, 2, 4, 6, 8, 10, and 12 h) and two levels of water salinity (15 and 30) during a 100-day experimental period.

METHODS

The natural habitats of R. stylosa forests and seedlings are shown in the Appendix. The design and arrangement of artificial tidal tanks simulating the semidiurnal tide are shown in Figure 1 and were designed according to our previous studies (Chen et al., 2013; Chen, Wang, and Lin, 2004, 2005).

Mature R. stylosa hypocotyls of similar size and fresh weight were harvested from the Dongzhaiang Nature Reserve of Hainan Province, China (19°54′ N, 110°20′ E) for cultivation. Twenty hypocotyls were randomly selected to estimate initial water content. Every hypocotyl was weighed, and initial biomass was calculated according to water content. Hypocotyls were randomly planted into sand-filled pots, each with a diameter of 25 cm and a depth of 25 cm, with one hypocotyl per pot. Seedlings were subjected to two levels of water salinity (15 and 30) and seven tidal-flooding treatments (0, 2, 4, 6, 8, 10, and 12 h per tidal cycle) with two cycles per day. Total flooding time for each treatment was equal to the time it took to fill each tank, typically 2 hours, plus the actual treatment time, as noted above. Thus, a 12-hour flooding treatment was equal to 2 hours for filling and 10 hours of flooding. The two water salinity levels were determined according to the field survey where 15 PSU and 30 PSU were typical of the average upper- and lower-limit salinities, respectively, in the middle intertidal zone of Dongzhaiang, the hypocotyl collecting sites in the present study. Every three pots planted with R. stylosa seedlings were put into the bottom of an artificial tidal tank. Three sets of artificial tidal tanks for each flooding time and salinity served as three independent replicates. Artificial seawater with salinity of 15 PSU or 30 PSU was prepared by mixing tap water and seawater and was used for cultivation. Tap water was added daily to compensate for evaporation losses, and the seawater was renewed weekly. Water salinity was monitored about every two days with a conductivity meter (Orion Two-Electrode Conductivity Cells, Model 115A, Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) and adjusted to the target values. All seedlings were grown in a greenhouse in Xiamen University, Fujian Province, China, at an air temperature of 26°C–32°C and an average relative humidity of 80% for 100 days. Photosynthetic photon flux density within the greenhouse attained maximum levels between 700 and 1000 μmol m⁻² s⁻¹.

Measurement of Growth and Biomass Parameters

After 100 days of cultivation, each seedling had two to three pairs of leaves, and the second leaf blades had matured. Then all seedlings were harvested. Stem length, mature leaf area, and total leaf area of each seedling were measured. Leaf area was measured with an LI-3100 Area Meter (LI-COR, Lincoln, Nebraska, U.S.A.). The harvested seedlings were briefly rinsed in freshwater and then divided into roots, stem, leaves, and hypocotyl. Each part of the seedling was weighed before and after oven drying (80°C). The total biomass in the present study did not include the hypocotyl.

The initial dry mass of each hypocotyl was calculated by multiplying fresh weight and water content. The loss of dry weight of the hypocotyl during the 100-day culture was determined by comparing the initial dry mass of the hypocotyl at planting and that upon harvesting.

Leaf Gas Exchange Measurements

Gas exchange parameters of mature leaves were measured using a CIRAS-1 Portable Photosynthesis System (PP Systems International, Inc., Amesbury, Massachusetts, U.S.A.). All measurements were taken when the tidal tanks were at “low” tide (empty). Measurements were taken between 1000 and 1200 in natural sunlight ranging in intensity from 800 to 1000 μmol m⁻² s⁻¹ and in temperature ranging from 29°C to 32°C, with a reference CO₂ concentration of 380 μmol mol⁻¹. Three to five fully expanded mature leaves for each replicate were chosen for the measurements of A (assimilation rate), gs (stomatal conductance), and C₅ (intercellular CO₂ concentration) and then averaged as one replicate.

Light-response curves were generated for mature leaves using the same portable photosynthesis system equipped with an artificial red-blue light-emitting diode (LED) light source. The measurements were carried out at 10 levels of illumination intensity (1400, 1200, 1000, 800, 600, 400, 200, 100, 50, and 0 μmol photons m⁻² s⁻¹) with the same red-blue LED light source in sequence from highest to lowest values at 2–4 minute intervals. The photosynthetic indexes of the seedlings subjected to the 12-hour treatment were not measured for the small leaf area.
was defined as (SPSS Inc., Chicago, Illinois, U.S.A.). Statistical significance employed to determine the potential source of the difference.

In the 15 PSU salinity treatments, total biomass (not salinity treatments by univariate analysis of variance. If the differences among tidal-flooding treatments and between parameters, and physiological parameters were analyzed for calculated. Data on all growth parameters, leaf gas exchange parameters, and root physiological characteristics of *Rhizophora stylosa* seedlings.

**Physiological Analysis**

Fresh roots of each replicate were collected and measured for root oxidation activity and alcohol dehydrogenase (ADH) activity, according to Chen, Wang, and Lin (2005). Root oxidation activity was expressed as the quantity of α-naphthylamine oxidized by a given amount of root in a given time (μg, α-naphthylamine h⁻¹ mgFW⁻¹ [milligram Fresh Weight]). Root ADH activity was expressed as units per mg protein. Protein was determined using Coomassie Brilliant Blue G-250 (Sigma-Aldrich Co., LLC, Steinheim, Germany) (Bradford, 1976), using bovine serum albumin as a standard.

**Data Analysis**

Mean and standard error (SE) of three replicates were calculated. Data on all growth parameters, leaf gas exchange parameters, and physiological parameters were analyzed for the differences among tidal-flooding treatments and between salinity treatments by univariate analysis of variance. If the difference was significant at \( p \leq 0.05 \), a post hoc test was employed to determine the potential source of the difference. All statistical analyses were performed with SPSS, version 16.0 (SPSS Inc., Chicago, Illinois, U.S.A.). Statistical significance was defined as \( p < 0.05 \).

**RESULTS**

All viviparous hypocotyls rooted and had at least two pairs of leaves at the time of harvest, even those submerged in water for the long 12-hour treatment, indicating minor effects of water salinity and tidal flooding on germination. In the 15 PSU salinity treatments, total biomass (not including hypocotyl) decreased gradually with the increase in flooding duration from 0 hours to 8 hours and then decreased sharply during the 10 hour and 12 hour treatments. In contrast, total biomass in 30 PSU salinity treatments remained nearly constant from 2 hours to 6 hours of treatment, subsequently decreasing sharply. In the midflooding treatments of 2–6 hours, the growth of leaf and stem of *R. stylosa* seedlings at salinity 15 PSU was significantly lower than that at salinity 30 PSU, resulting in a significant decrease in total biomass (Figure 2; Table 1). Flooding with saline water restricted the growth of leaves and roots of seedlings. Flooding suppressed leaf expansion by decreasing both the mature and total leaf area (Figure 3; Table 1). Water salinity had no effect on leaf area, except for the control in high salinity. In 15 PSU and 30 PSU salinity treatments, the average leaf area during the 12-hour treatments was decreased by 79.1% and 60.6%, respectively. Stem biomass, stem height, and total biomass of *R. stylosa* were all greater in high-salinity than in low-salinity treatments (Figures 2 and 3; Table 1). Compared with flooding time, water salinity had fewer pronounced effects on the growth of *R. stylosa* seedlings. Tidal flooding and salinity each had significant effects on biomass allocation of seedlings (Tables 1 and 2). With increased flooding time, more dry matter was allocated to stem and roots but less to leaf. This trend was independent of water salinity.

After 100 days of culture, water salinity and tidal flooding had significant combined effects on the loss of hypocotyl dry mass (Tables 1 and 3). With increased flooding time, loss of hypocotyl dry mass increased, peaking at 8 hours of flooding in 15 salinity treatments and at 4–6 hours of flooding in 30 PSU salinity treatments, then subsequently decreasing.

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Table 1. Results of univariate ANOVA tests (given as F-values) showing the effects of water salinity, flooding time, and their combined effects on seedling growth, leaf gas exchange characteristics, and root physiological characteristics of *Rhizophora stylosa* seedlings.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water Salinity (S)</th>
<th>Flooding Time (T)</th>
<th>S x T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root biomass</td>
<td>49.401***</td>
<td>34.279***</td>
<td>4.743**</td>
</tr>
<tr>
<td>Stem biomass</td>
<td>1.363</td>
<td>19.379***</td>
<td>2.617*</td>
</tr>
<tr>
<td>Leaf biomass</td>
<td>0.161</td>
<td>84.514***</td>
<td>16.442***</td>
</tr>
<tr>
<td>Total biomass</td>
<td>48.883***</td>
<td>95.753***</td>
<td>16.049***</td>
</tr>
<tr>
<td>Total leaf area</td>
<td>1.583</td>
<td>90.807***</td>
<td>20.257***</td>
</tr>
<tr>
<td>Mature leaf area</td>
<td>0.038</td>
<td>8.656***</td>
<td>0.032</td>
</tr>
<tr>
<td>Stem length</td>
<td>44.382***</td>
<td>74.182***</td>
<td>8.366***</td>
</tr>
<tr>
<td>Root biomass partitioning</td>
<td>16.206***</td>
<td>33.362***</td>
<td>8.106***</td>
</tr>
<tr>
<td>Stem biomass partitioning</td>
<td>82.957***</td>
<td>97.763***</td>
<td>2.151</td>
</tr>
<tr>
<td>Leaf biomass partitioning</td>
<td>7.716***</td>
<td>46.162***</td>
<td>2.663*</td>
</tr>
<tr>
<td>Loss of hypocotyl weight</td>
<td>8.247**</td>
<td>19.202***</td>
<td>2.892*</td>
</tr>
<tr>
<td>A</td>
<td>11.737***</td>
<td>14.155***</td>
<td>0.734</td>
</tr>
<tr>
<td>gᵢ</td>
<td>2.058</td>
<td>30.596***</td>
<td>0.663</td>
</tr>
<tr>
<td>Cᵢ</td>
<td>2.393</td>
<td>4.069**</td>
<td>1.247</td>
</tr>
<tr>
<td>Root oxidase activity</td>
<td>15.511***</td>
<td>16.635***</td>
<td>1.209</td>
</tr>
<tr>
<td>Root ADH activity</td>
<td>0.195</td>
<td>12.612***</td>
<td>14.147***</td>
</tr>
</tbody>
</table>

ANOVA = analysis of variance, A = assimilation rates, gᵢ = stomatal conductance, Cᵢ = intercellular CO₂ concentration, ADH = alcohol dehydrogenase. F-values marked with asterisks are significant at * \( p < 0.05 \); ** \( p < 0.01 \); and *** \( p < 0.001 \).
Leaf Gas Exchange Characteristics

Prolonged flooding time and high salinity significantly inhibited leaf assimilation rates of *Rhizophora stylosa* seedlings (Figure 4, Table 1). In low-salinity treatments, A maintained higher levels under 0 hours and 2 hours of flooding, at 14.0 μmol m⁻² s⁻¹ and 14.9 μmol m⁻² s⁻¹, respectively. Then A decreased gradually with flooding of 4 to 10 hours. In high salinity, A increased during the initial 2 hours and then reached a plateau at 2 and 4 hours, subsequently decreasing sharply. The longest flooding time (12 hours) induced a strong decrease in leaf assimilation rates, from 12.2 to 2.2 μmol m⁻² s⁻¹, and a similar decline in A was found for salinity. In flooding treatments, gs and Cᵢ both declined with an increase of flooding time greater than 2 hours; however, the difference between the two salinity treatments was negligible (Table 1).

Figure 5 reveals important photosynthetic characteristics of light-response curves of *R. stylosa* seedlings influenced by tidal flooding, saline, and their combined effects. The light-response curves of mature leaves were expressed by A in different illumination ranges from 0–1400 μmol m⁻² s⁻¹. In short-term flooding at salinity 15 PSU, A was much higher, and the light saturation point occurred at 800–1000 μmol m⁻² s⁻¹ of illumination. These results were greatly reduced by long-term flooding times of 8 hours and 10 hours (Figure 5A). The shapes of photosynthetic light-response curves for treatments of 2, 4, and 6 hours at salinity 30 PSU were similar to those of the shorter flooding treatments at salinity 15 PSU, but these gradually decreased with an increase of flooding time >6 hours (Figure 5B).

### Physiological Parameters

Tidal flooding had significant effects on root oxidase activity (Figure 6). Under both salinities, root oxidase activity was greater when the flooding time was less than 4 hours and then fell significantly when flooding time was greater than 4 hours. Under the 15 PSU salinity treatments, maximum root oxidase activity was observed in the 4-hour treatment at 19.6 μg h⁻¹ gFW⁻¹, approximately three times higher than the value in the 12-hour treatment. Root oxidase activity was lower and less variable under higher salinity and decreased with the increase in flooding time.

Under salinity 15 PSU, the capacity for alcohol fermentation, as measured by root ADH activity, increased greatly from 3.68 U (unit) mgPr⁻¹ at 0 hours to 8.07 U mgPr⁻¹ at 6 hours (Figure 6). When flooding time was longer than 6 hours, root ADH activity declined sharply with increased flooding time. Root ADH activity in the 6-hour treatment was 5.4 and 6.4 times higher than that in the 10-hour and 12-hour treatments, respectively. Under salinity 30 PSU, no significant change was observed in root ADH activity between 0-hour and 4-hour flooding treatments. However, when flooding time exceeded 4 hours, root ADH activity decreased and remained constant until the 12-hour treatment. Therefore, similar to root oxidase activity, root ADH activity also showed less variability under higher salinity. In contrast to water salinity, tidal flooding time had greater effects on root ADH activity (Table 1).

### Table 2. Effects of flooding time and water salinity on biomass allocation of *Rhizophora stylosa* seedlings. Values include standard errors of the mean (n = 3).

<table>
<thead>
<tr>
<th>Flooding Time (h)</th>
<th>% Leaf Biomass</th>
<th>% Stem Biomass</th>
<th>% Root Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salinity 15 PSU</td>
<td>Salinity 30 PSU</td>
<td>Salinity 15 PSU</td>
</tr>
<tr>
<td>0</td>
<td>41.6 ± 3.2</td>
<td>31.6 ± 5.6</td>
<td>24.6 ± 1.9</td>
</tr>
<tr>
<td>2</td>
<td>29.1 ± 1.9</td>
<td>33.2 ± 0.6</td>
<td>27.5 ± 1.9</td>
</tr>
<tr>
<td>4</td>
<td>27.8 ± 3.3</td>
<td>29.1 ± 2.5</td>
<td>31.9 ± 0.6</td>
</tr>
<tr>
<td>6</td>
<td>23.7 ± 2.0</td>
<td>22.6 ± 0.9</td>
<td>47.0 ± 6.0</td>
</tr>
<tr>
<td>8</td>
<td>22.9 ± 5.3</td>
<td>18.4 ± 2.7</td>
<td>53.0 ± 5.8</td>
</tr>
<tr>
<td>10</td>
<td>20.2 ± 1.0</td>
<td>17.1 ± 2.1</td>
<td>54.2 ± 3.6</td>
</tr>
<tr>
<td>12</td>
<td>9.2 ± 2.1</td>
<td>12.9 ± 5.0</td>
<td>36.1 ± 2.9</td>
</tr>
</tbody>
</table>

### Table 3. Loss of dry weight of *Rhizophora stylosa* hypocotyls during a 100-day culture under various flooding times with two water salinities. Values include standard errors of the mean (n = 3).

<table>
<thead>
<tr>
<th>Loss of Dry Weight (g/individual)</th>
<th>Flooding Time (h)</th>
<th>Salinity 15 PSU</th>
<th>Salinity 30 PSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.76 ± 0.13</td>
<td>2.06 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.00 ± 0.05</td>
<td>2.16 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.96 ± 0.10</td>
<td>2.32 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.18 ± 0.12</td>
<td>2.30 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.60 ± 0.05</td>
<td>2.18 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.59 ± 0.22</td>
<td>1.86 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.14 ± 0.16</td>
<td>1.53 ± 0.15</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Effects of flooding time and water salinity on the leaf area and stem length of *Rhizophora stylosa* seedlings: (A) mature leaf area; (B) total leaf area; (C) growth of stem length. Vertical bars indicate standard errors of the mean (n = 3).
DISCUSSION

It is known that tidal flooding and/or salinity tolerance results in mangrove species–specific niches along intertidal zones under the process of sea-level rise (Knight et al., 2008; Lugo and Snedaker, 1974). Although the effects of tidal flooding and salinity on mangrove plants have been widely tested separately, the synergistic effects of these two factors has been rarely documented, which limits our knowledge on mangrove niches shifting in future sea-level–rise scenarios. Controlled experiments were used in the present study to test the tolerance of mangrove seedlings grown in different tidal flooding times when fully covered by artificial seawater.

Tidal Flooding and Salt Tolerance of R. stylosa Seedlings

Viviparous species in the Rhizophora genus are tolerant to both tidal flooding and water salinity (Krauss et al., 2008; Lovelock et al., 2016). The viviparous hypocotyl contains an abundance of carbohydrates, which fuels the initial outgrowth of shoots and roots (Lin and Sternberg, 1995; Rabinowitz, 1978). After the outgrowth of shoots and roots, carbohydrates for seedling growth either originate from the autonomous photosynthesis of leaves or hypocotyl epidermis or are stored in the hypocotyl (Smith and Snedaker, 2000). The physiological nature of seedlings during early growth reflects not only environmental tolerance but also population maintenance in the field.

The change in dry weight of the hypocotyl during seedling growth is an indicator of the utilization of carbohydrate reserve stored in the hypocotyl (Wang et al., 2011). In this study, a significant loss of hypocotyl dry weight occurred after the 100-day culture, and both water salinity and flooding time had significant effects on this loss and on the utilization of carbohydrate reserves stored in hypocotyl (Table 1). Normal flooding promotes the utilization of nutrients stored in hypocotyl, whereas either the absence of flooding or flooding longer than 8 hours under salinity 15 and longer than 6 hours under salinity 30 PSU is sufficient to inhibit this utilization. Changes in the utilization of carbohydrates stored in the hypocotyl were similar to changes in the photosynthetic rate of individual seedlings, as determined by multiplying net photosynthetic rate and leaf area per seedling, 

\[ r_{\text{low salinity}} = 0.6362, \quad p < 0.05; \quad r_{\text{high salinity}} = 0.9263, \quad p < 0.01.\]

A higher photosynthetic rate implied more effective utilization of this carbohydrate reserve. Additionally, after 100 days of cultivation, biomass increase was negative because the total biomass was signifi-

Figure 4. (A) Assimilation rates \( A \), (B) stomatal conductance \( g_s \), and (C) intercellular \( CO_2 \) concentration \( C_i \) of the leaves of Rhizophora stylosa seedlings cultivated under different flooding times with saline treatments. Vertical bars indicate standard errors of the mean \( (n = 3) \).

Figure 5. Light-response curves of mature leaves of Rhizophora stylosa seedlings subjected to different flooding times under salinities of (A) 15 and (B) 30. Carbon assimilation rate \( A \) is reported on a per–leaf area basis. Vertical bars indicate standard errors of the mean \( (n = 3) \).

Figure 6. Effects of flooding time and water salinity on root physiological traits of Rhizophora stylosa seedlings: (A) root activity and (B) root ADH activity. Vertical bars indicate standard errors of the mean \( (n = 3) \).
cantly lower than the loss of dry weight of the hypocotyl upon harvesting, as observed in all treatments (Figure 2; Table 3).

Photosynthesis of leaves produced nutrients for further seedling growth. In the long-term flooding treatments, light-response curves showed significant inhibition of photosynthesis, indicating inhibitory effects of flooding on carbon assimilation rates (Figure 4). Net assimilation rate (A) measured at a fixed illumination confirmed this trend (Figure 5). Similar results have been found in other mangrove species, such as Avicennia germinans, Laguncularia racemosa, and R. mangle (Pezeshki et al., 1989); A. marina (Lu et al., 2013); R. mangle (Ellison and Farnsworth, 1997); and Kandelia obovata (Chen, Wang, and Lin, 2005). R. stylosa leaves exposed to high salinity had lower A compared with leaves exposed to low salinity, especially when no flooding treatment was involved. Lower A at high salinity and 0-hour flooding could be attributed to salt stress and acid-sulfide soil caused by saline drainage; the inhibited water transport leads to stomatal closure, which has been demonstrated in some other studies (Ellison and Farnsworth, 1997; McKee, 1993).

Furthermore, tidal flooding with saline seawater may result in long periods of oxygen deprivation, as well as accumulation of phytotoxins (Ponnamperuma, 1984). Mangroves are especially adapted to root oxygen deficiency through two processes: O₂ release from the roots and the enzymatic pathway of peroxidase in the roots (Chiu and Chou, 1993). The transportation of O₂ from the above-ground portion to roots has been proven in many mangroves (Chiu and Chou, 1993; Tomlinson, 1986; Youssef and Saenger, 1996). In general, morphological adaptations of mangroves, e.g., various forms of aerial roots, lenticels, and well-developed internal ventilation systems, could ensure sufficient oxygen transport to below-ground roots to oxidize the rhizosphere (Tomlinson, 1986). Morphological adaptations, which mainly appear as the development of aerenchyma, are considered important traits that enable plants to tolerate soil anaerobiosis (Blom and Voesenek, 1996; Insuausti et al., 2001). Consequently, the small size of mangrove seedlings results in limited access to air caused by partial or total submergence, and absence of aerial roots and undeveloped internal ventilation systems result in low efficiency of oxygen transport (McKee, 1993). Under these conditions, anaerobic metabolism would be inevitable.

In addition to anatomical adaptations, mangroves are also adapted metabolically to tidal flooding. Alcohol fermentation is the main metabolic pathway for generating adenosine triphosphate in the roots of mangroves, and ADH is a measure of the potential for anaerobic respiratory flux, which acts as an important factor in tidal flooding tolerance (Chen, Wang, and Lin, 2005). In this study, increasing ADH activity at low salinity implied that (1) anaerobic metabolism had been activated, (2) the enzymatic pathway was included in the oxidation process, and (3) metabolic adaptation may be involved in the tolerance of R. stylosa to flooding (Figure 6), similar to the results of another mangrove, K. obovata (Chen, Wang, and Lin, 2005).

However, ADH activity at high salinity did not show the characteristic increase with flooding, and it even decreased when flooding time was longer than 2 hours (Figure 6). This trend was also found in the low-intertidal salt marsh species Spartina anglica (Maricle et al., 2006). These results indicate that R. stylosa has lower aerobic demand and is more resistant to flooding compared with seedlings at low salinity. This can be explained by the well-developed parenchyma in roots, as found in other mangrove species (Xiao, Wang, and Chen, 2010). McKee (1996) and Youssef and Saenger (1996) also found that root porosity, an index of the development of parenchyma, was only marginally affected by different edaphic conditions and water depths in R. mangle.

In this study, most responses of R. stylosa were affected by both elevated salinity and prolonged flooding time. Among them but independent of water salinity, stem biomass, leaf biomass, total leaf area, mature leaf area, gₛ, Cᵢ, and root ADH activity were significantly affected by flooding time (Table 1). These results suggest that elevated salinity has less effect on growth and metabolism than increased flooding time, as might be seen during times of rising sea levels. Thus, the difference in mangrove seedling survival may be more attributable to inundation tolerance than saline tolerance.

**Growth Patterns and Niche Shifting of R. stylosa in Future Sea-Level Rise Scenarios**

Mangroves are naturally colonized along the intertidal zones from seaward edge to landward edge. As a pioneer species, R. stylosa usually dominates the seaward edge having long flooding time and high salinity (Tomlinson, 1986). Scenarios of future sea-level rise are expected to increase the flooding time and/or seawater salinity (IPCC, 2013). The growth responses of R. stylosa seedlings to likely future changes will help in predicting its niche shifting after sea-level rise.

In this study, long flooding time and high salinity resulted in smaller leaf size with lower total leaf area and smaller leaf biomass compared with these parameters during short-term flooding and in low-saline water (Figures 2 and 3). The adverse effects of flooding on the growth of leaves have been reported in both flood-tolerant and flood-intolerant plants (Kozlowski, 1997). This was consistent with other mangrove species tested in a similar mesocosm, including viviparous K. obovata (Chen, Wang, and Lin, 2004) and A. marina (Lu et al., 2013), and nonviviparous Sonneratia spp. (Chen et al., 2013). Inhibited formation of new leaves, slowed expansion of leaves, and the induction of leaf abscission resulted in leaves subjected to growth inhibition (Kozlowski, 1997). Similarly, flooding depressed the growth of roots, as reported in many mangrove species (Krauss et al., 14). This resulted in a 64.1% and 50.3% decrease in root biomass in low and high salinities, respectively, during the 12-hour treatment (Figure 2).

In contrast to the inhibitory effects of flooding on the growth of roots and leaves, flooding promoted stem growth and elongation, as observed under both low and high salinities (Figures 2 and 3). Simultaneously, more dry mass was allocated to the stem (Table 2). The elongation of stems is a plastic response commonly observed in flood-tolerant plants that contributes to the maintenance of a diffusion pathway for oxygen transport from air-exposed organs to submerged roots (Chen et al., 2013; Insuausti et al., 2001). For mangrove seedlings, survival rates in flooding conditions are usually influenced by fluctuations in water level. Survival also depends on the ability of seedlings to elongate sharply and protrude.
above the water level (Lovelock et al., 2016). Increased stem length and biomass of *R. stylosa* seedlings indicated good tolerance to flooding. The use of atmospheric oxygen to maintain aerobic respiration in root tissues after stems protrude above the water level is essential as a survival strategy during extended flooding (Laan and Blom, 1990).

In field conditions, seawater salinity usually ranges from 15 to 25 within the natural distribution area of *R. stylosa*, which is also considered a highly saline-tolerant species (Wang and Wang, 2007). In future scenarios of rising sea levels, water salinity is predicted to correspondingly increase. The present data also suggests an inhibitory effect of salinity on root and leaf growth but a promotive effect on stem elongation and biomass accumulation, which resulted in an increase of total biomass with increasing salinity (Figure 2), suggesting good saline tolerance of *R. stylosa* seedlings.

Flooding with water salinity caused a change in allocation of photosynthetic products within plants. When the flooding time was shorter than 10 hours, the carbohydrate partition favored the stem at the expense of root and leaf (Table 2). In other words, the promotion of stem growth by flooding is at the cost of leaf and root growth. This can be explained as an adaptive adaptation to flooding, furthering stem protrusion above the water level for oxygen transport to submerged roots (Insausti et al., 2001). Likewise, this adaptation was lost and the growth of stem and leaf severely restricted by flooding during the 12-hour treatment. Similarly, in high-saline conditions, more biomass was allocated to stems but less to roots. Taken together, these results suggested high salinity and flood tolerance of such pioneer mangrove species as *R. stylosa*. In Dongzhaihang, *R. stylosa* was scattered in the mangrove forests where water salinity was 15, and it turned into the dominant species where water salinity was 30 PSU. This means that *R. stylosa* has a competitive advantage in high-saline habitats. This might be the reason for the better growth of *R. stylosa* in high salinity, as shown in our present experiment.

**CONCLUSIONS**

The results in this study suggest that the growth and physiological responses of *R. stylosa* seedlings are significantly affected by both salinity and flooding time, indicating its high adaption to prolonged flooding and increased salinity in future sea-level rise scenarios. As sea level rises, some individuals of *R. stylosa* on the seaward edge would still survive in increased flooding and saline conditions through the use of carbohydrate fuels stored in hypocotyls, and stem growth would be promoted. However, these same conditions would instigate a simultaneous landward shift of mangroves. Thus, the niche for this species is expected to expand in future sea-level scenarios. In further studies simulating rising sea levels, the combined effect of tidal flooding and increasing salinity should be considered for their impacts on mangrove tolerance and for predicting possible niche shifting.

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**LITERATURE CITED**


Appendix Figure. Photographs of *Rhizophora stylosa* adults and seedlings (A) and forests (B) in natural habitats of Dongzhaiang Nature Reserve, China.