ECOPHYSIOLOGY

Salinity and light interactively affect neotropical mangrove seedlings at the leaf and whole plant levels

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Abstract We have studied the interactive effects of salinity and light on *Avicennia germinans* mangrove seedlings in greenhouse and field experiments. We hypothesized that net photosynthesis, growth, and survivorship rates should increase more with an increase in light availability for plants growing at low salinity than for those growing at high salinity. This hypothesis was supported by our results for net photosynthesis and growth. Net daily photosynthesis did increase more with increasing light for low-salinity plants than for high-salinity plants. Stomatal conductance, leaf-level transpiration, and internal CO_2 concentrations were lower at high than at low salinity. At high light, the ratio of leaf respiration to assimilation was 2.5 times greater at high than at low salinity. Stomatal limitations and increased respiratory costs may explain why, at high salinity, seedlings did not respond to increased light availability with increased net photosynthesis. Seedling mass and growth rates increased more with increasing light availability at low than at high salinity. Ratios of root mass to leaf mass were higher at high salinity, suggesting that either water or nutrient limitations may have limited seedling growth at high salinity in response to increasing light. The interactive effects of salinity and light on seedling size and growth rates observed in the greenhouse were robust in the field, despite the presence of other factors in the field—such as inundation, nutrient gradients, and herbivory. In the field, seedling survivorship was higher at low than at high salinity and increased with light availability. Interestingly, the positive effect of light on seedling survivorship was stronger at high salinity, indicating that growth and survivorship rates are decoupled. In general, this study demonstrates that environmental effects at the leaf-level also influence whole plant growth in mangroves.

Keywords Growth analysis · *Avicennia germinans* · Gas exchange · Ecophysiology · Venezuela

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Introduction

An outstanding challenge in plant ecology is to understand the effects of multiple environmental conditions on plant performance and how effects on specific organs are reflected in whole-plant performance (Mooney 1991; Smallwood et al. 1999; Ackerly and Monson 2003). Mangroves are ideal for investigating concepts of scaling and multiple interactions because the forests are strongly structured by environmental gradients. The tidal gradients within many mangrove swamps create contrasting low- and high-salinity areas (Clarke and

Hannon 1970; Hutchings and Saenger 1987); a light gradient caused by tree-fall gaps and edge effects of forests growing along waterways is superimposed, creating variable combinations of light and salinity (Smith 1992; Feller et al. 1999). In this study, we examined the interactive effects of salinity and light gradients on *Avicennia germinans* seedlings. Studies have shown that salinity and light interactively affect mangrove seedling growth and survivorship (Ball 2002), and growth and gas exchange (Krauss and Allen 2003). We present the first fully integrated study of leaf-level carbon gain, whole-plant growth rates and biomass accumulation, and seedling survivorship—linking experimental data from the greenhouse to that in the field. We also present new evidence that respiratory costs, relative to assimilation rates, may be a critical factor limiting growth at high salinity and high light.

The effects of salinity on mangrove growth are not independent from those of light availability. An early hypothesis was that the costs of salinity tolerance would be increases in mangroves' energy and light requirements, suggesting that high salinity levels would negatively impact growth at low light availability (Janzen 1985). However, the suppression of whole-plant growth by high salinity should be stronger at high than low light. Salinity limits water uptake by plants (Clough 1984). Low soil water potentials at high salinity require lower leaf water potentials to drive transpiration (Scholander et al. 1964). Low leaf water potentials lead to reduced stomatal conductance (Schulze 1991), causing lower leaf intracellular CO_2 concentrations (Andrews and Muller 1985), and decreased photosynthetic rates (Ball and Farquhar 1984; Lin and Sternberg 1992; Sobrado 1999b). Conservative leaf-level water use and low photosynthetic rates result in reduced carbon gain at high salinity (Ball 1988; Pezeshki et al. 1990). Thus, the negative effects of salinity on leaf-level carbon gain should be greater at high than at low light, because at high light, photosynthesis is limited by stomatal conductance (Lambers et al. 1998). At the whole-plant level, high salinity, typically above 50% seawater, causes depressed growth rates (Clough 1984; Smith 1992; Ball 1996) and increased allocation to root mass relative to leaf mass (Ball 1988). Several studies have indeed shown that the combination of high salinity and high light may be most limiting (Ball 2002, Krauss and Allen 2003; López-Hoffman et al. 2006).

We hypothesize that: (1) the negative effects of salinity on net photosynthesis will be greater at high than at low light availability, and (2) consequently, we will find similar interactive effects of light and salinity on whole-plant growth rates, size, and survivorship. If our

hypothesis is supported, it will indicate that the effects of salinity and light are consistent at the leaf and whole-plant levels. A major goal is to test whether the salinity by light effects observed in greenhouse experiments are robust under field conditions, where factors such as herbivory, nutrients, and flooding also influence mangrove seedling performance (Ellison and Farnsworth 1996; Feller et al. 1999; Minchinton and Dalby-Ball 2001; Lovelock and Feller 2003; Sousa et al. 2003a).

Materials and methods

This study involved paired greenhouse and field experiments with factorial combinations of light availability and salinity. The greenhouse experiment ran for 39 weeks and involved measurements of leaf-level instantaneous gas exchange, analysis of daily net photosynthesis (calculated from the instantaneous measurements), growth analysis, and final biomass accumulation and allocation. The field experiment was conducted for 40 weeks and provided measurements of whole-plant growth, biomass accumulation, herbivory, and seedling survival.

Collection of plant material and field experiments were conducted at Lake Maracaibo, Venezuela (N 10°96′70″ W 71°73′24). Low-salinity, low intertidal field experiment plots were located near the mouth of a freshwater river where soil salinities varied annually from 0% to 14% seawater (SW). The high-salinity, high-intertidal plots were located 0.75 km NNW, in a forest of stunted *A. germinans*, along the landward edge of the mangrove swamps. Here, soil salinity varied annually from 100% to 200% SW (López-Hoffman 2003). In other locations in Venezuela, similar hypersaline sites are prone to drought during the dry season (Medina and Francisco 1997). Of the three species of mangrove present, *Laguncularia racemosa*, *Rhizophora mangal*, and *A. germinans*, the latter is considered the most salt tolerant (Medina and Francisco 1997; Sobrado and Ball 1999). Only *A. germinans* inhabits both low- and high-salinity zones (Narváez 1998). Mature propagules of *A. germinans* for the greenhouse and field experiments were collected on August 1998 and September 2000, respectively, from the same ten trees at our low-salinity site. Propagules with signs of insect infestation were excluded.

Greenhouse experiment

The greenhouse experiment involved a fully factorial design with two complete, replicate blocks, each in a separate greenhouse at Stanford University, CA, USA.

There were three salinity levels (20, 70, and 167% of full SW) and four light levels (6, 12, 25, and 50% PAR, photosynthetically active radiation), giving 12 combinations. The treatments reflected the natural range of field conditions (see below). The greenhouses were whitewashed to filter 50% of outside light. Mid-day noon irradiance within the greenhouse on clear days in August 1999 was 900–1,000 µmol m^{-2} s^{-1} (measured using a quantum sensor and data logger; LI-190 and LI-1000, LiCor, Nebraska, USA). For the 25, 12, and 6% PAR treatments, 50, 75, and 90% light filtration shade cloths, respectively, were used. Instant Ocean aquarium salt was used for the salinity treatments. Average day and nighttime temperatures were 32±3 and 29±3°C, respectively. Average relative humidity was 70±5%, and the vapor pressure deficit (VPD) in the greenhouse was approximately 1.5 kPa.

The seedlings grew in individual pots placed in 946-l Rubbermaid stock tanks, with 12 tanks per greenhouse. Within a greenhouse, each tank was a unique salinity and light treatment. Due to limited space, a design similar to Ellison and Farnsworth (1997) was used. Side-by-side tanks were plumbed in a recirculating series; they shared water but differed in light level. Every 6 h, water was pumped from tank to tank; at any point, one tank was at "low" and one at "high" tide. Periodically, the timing was changed to simulate tidal progression. This design simulated mangrove tidal systems and avoided permanently inundating the plants. Readjusting the salinity levels weekly and periodically cleaning the tanks and changing the water minimized the potential confounding effects of sharing water. At no point was it suspected that the water-sharing compromised the experiment's integrity; nonetheless, this was accounted for in the statistical analysis (see below).

Large volume pots were constructed from 66-cm lengths of 15.3-cm-diameter PVC drainpipe, filled with a 1:1 mixture of sand and potting soil. The plants were fertilized monthly with 0.8 l of 100% N:P:K 20:20:20 fertilizer (0.67 g NPK/mo). Prior to the experiment, 1,000 propagules were cultivated in 20% SW solution for 3 months. On 23 November 1998, 312 similarly sized seedlings were planted into the tanks (1 per pot, 13 pots per tank). An additional 20 seedlings were used to determine the average initial dry mass (mean=3.3, SE±0.16 g). The salinity level in the tanks was held at 20% SW for 1 week, whereupon to prevent "osmotic shock," salinity was gradually increased over 3 weeks. The final treatments were imposed on 22 December 1998. Some seedlings died during the 2 weeks after transplanting (leaving between 11–13 per tank), but this occurred before the experiment began and they

were not replaced. Thus, mortality was related to transplanting, not final treatments; nonetheless, there were no treatment effects on survivorship (all P=0.26).

The two greenhouse harvests began 8 July and 25 September 1999, 197 days and 276 days, respectively, after the experiment began. The plants were assigned to first and second harvest groups according to height at the first harvest, such that the mean and variance of the groups were similar. The plants were separated into roots, stems, branches, and leaves. Leaf area of a subset of fresh leaves was measured using the LI-3100 meter (LiCor, Lincoln, Nebraska, USA). All plant material was dried at 80°C for 4 days and then weighed. During the course of the experiment, we collected all dead leaves from each plant. Leaf mass and area lost during the experiment was added to the final harvest data (Anten and Ackerly 2001). Growth analysis was conducted, according to West et al. (1920), to calculate relative growth rate (RGR) and to partition it into net assimilation rate (NAR) and leaf area ratio (LAR). Plants from the first and second harvests were randomly paired to obtain conservative confidence intervals on growth parameters. We report the average leaf mass ratio and specific leaf area (LMR and SLA) of the two harvests.

Leaf gas exchange was measured using a portable photosynthesis system and an attached light source (LI-6400, LiCor) in only four treatments—20% and 167% SW, and 6% and 25% PAR—combined. All gas-exchange measurements were made when the tanks were at "low" tide, on young fully expanded leaves. For all the measurements mentioned below, the chambers were set to 27°C. Under ambient greenhouse conditions, when the leaves were not in the LiCor chamber, the different light treatments could have caused treatment differences in leaf temperature, which may have affected photosynthetic rates.

Light-saturated net assimilation rate and dark respiration (A_{sat} and R_{dark}) measurements were taken from four plants per treatment per greenhouse, on 3 different days between 17 and 25 September 1999. Each measurement was made on two leaves. The reference CO_2 was set to 380 ppm. For A_{sat}, the light source was at 1,200 µmol m^{-2} s^{-1}. A_{sat} was measured between 0900 hours and 1130 hours. There were no visible signs of photodamage (i.e., leaf bleaching). R_{dark} was measured 45 min prior to sunrise; the plants had been in the dark all night. During these gas-exchange measurements, there were no treatment differences in VPD or leaf temperatures in the Licor photosynthesis meter's leaf-chamber (grand means, 1.94 kPa and 28.6°C, respectively; all P values >0.1). Ac_i measurements were first taken at 380 ppm CO_2,

followed by measurements at 300, 200, 100, and 50 ppm, CO_2. The maximum catalytic capacity of Rubisco per unit leaf area (V_{max} µmol m^{-2} s^1) was estimated with the Collatz photosynthesis model (Collatz et al. 1991). In addition, incident light was measured on 3 August and 17 October 1999. Hourly, from 0630 hours until 1930 hours, a quantum sensor (LI-190, LiCor) was placed near a representative exposed leaf and oriented at the same angle.

We calculated daily net photosynthesis following methods described by Hirose et al. (1997). We used a non-rectangular hyperbola to characterize the light response of net leaf photosynthesis (P_L, µmol m^{-2} s^1; Marshall and Biscoe 1980)

$$P_L = \frac{(A_{max} + \phi I_L) - [(A_{max} + \phi I_L)^2 - 4\phi\theta A_{max} I_L]^{0.5}}{(2\theta)} - R_{dark}.$$

This equation was used to calculate the light response at different times throughout the day, based on the measured hourly light levels from 3 August and 17 October 1999. The equation was parameterized as follows. We used the measured hourly incident light values (I_L) and the measured R_{dark} and A_{sat} values presented in Fig. 1 (A_{max} in the above equation is A_{sat} plus R_{dark}). We used previously published values for the apparent quantum yield (ϕ) and the curvature factor (θ) and assumed that these values were constant across treatments. The quantum yield (ϕ) was 0.04 (Ehleringer and Björkman 1977) and θ was 0.8 of the quantum yield, an average value of a range of species (Anten and Hirose 2001). These values were integrated for a 24-h total of net photosynthesis (Hirose and Werger 1987); the pre-dawn R_{dark} values were used for the nighttime respiration.

Greenhouse statistical analysis

To avoid pseudoreplication, a mean response from all individuals in a tank was obtained, resulting in $n=2$ tanks per treatment, one in each greenhouse. For biomass allocation and growth analysis, all 12 treatments were studied. Despite precautions to mitigate the potential effects of linking tanks, we use a conservative ANOVA design. Within a salinity level, pumps connected the 6% and 12% PAR tanks, and the 25% and 50% PAR tanks. For ANOVA, the 6% and 12% PAR tanks were labeled low light, and the 25% and 50% PAR tanks were labeled high light, giving six treatments (2 light ɜ 3 salinity). While interpretations are based on this conservative ANOVA, in the figures, we show four light levels, as results of a full 12 treatment ANOVA are quite similar. The conservative design does not apply to the gas-exchange data because the four treatments studied did not include pairs of linked tanks. For all ANOVAs, greenhouse (block) was random, salinity and light were fixed, all factors were tested relative to the residual term, and interactions with greenhouse were not included. All variables were tested for normality and transformed if necessary to meet assumptions of parametric statistics (Crawley 1993). These analyses were performed with Data Desk 6.1 (Data Description Inc., 1997; Ithaca, NY, USA).

In this study, we analyzed the effect of salinity on the ratio of R_{dark} to A_{sat} with ANOVA. Ideally, this relationship should be analyzed using ANCOVA, although many ratios are commonly analyzed with ANOVA (e.g., RGR, LAR, LMR). The ANCOVA of R_{dark} to A_{sat} did not give a significant interaction because we had only eight data points, due to our conservative tank mean approach, and the ANCOVA required three degrees of freedom rather than one for the ANOVA. Ratios are of concern when the numerator and denominator are not reported; in this case, we report both in Fig. 1. Furthermore, in López-Hoffman (2003), an ANCOVA of R_{dark} to A_{sat} with a combined data set of *Avicennia germinans* and *Rhizophora mangle* showed a significant salinity effect.

Field experiment

In the field, in each of two intertidal positions that varied dramatically in salinity, we identified 12 sites along a continuous gradient of light levels. The sites ranged from closed canopy to forest gaps of different sizes. At each site, we established at 5ɜ5-m experimental plot. One low-salinity plot was destroyed by a tree fall. Each plot was cleared of low-stature vegetation and debris but not of *A. germinans* pneumatophores. At high salinity, light levels less than 10% are rare (López-Hoffman 2003). Therefore, shade cloth was placed over three high-salinity plots to test seedling responses to less than 10% PAR. For balanced design, shade cloth was also used at three low-salinity plots of similar light levels. Between 15 and 19 August 2001, under uniform, cloudy skies, four hemispherical canopy photos were taken per plot. A Minolta X-700 camera with a Sigma 8 mm F4 Fisheye lens mounted on a leveled tripod and Kodak Velvia film were used. The photos were analyzed using HemiView 2.1 Canopy Analysis Software (Delta-T Devices Ltd, UK). The plot mean of Global Site Factor (GSF) was used as the measure of light availability. GSF is the proportion of global radiation under a plant canopy relative to that in
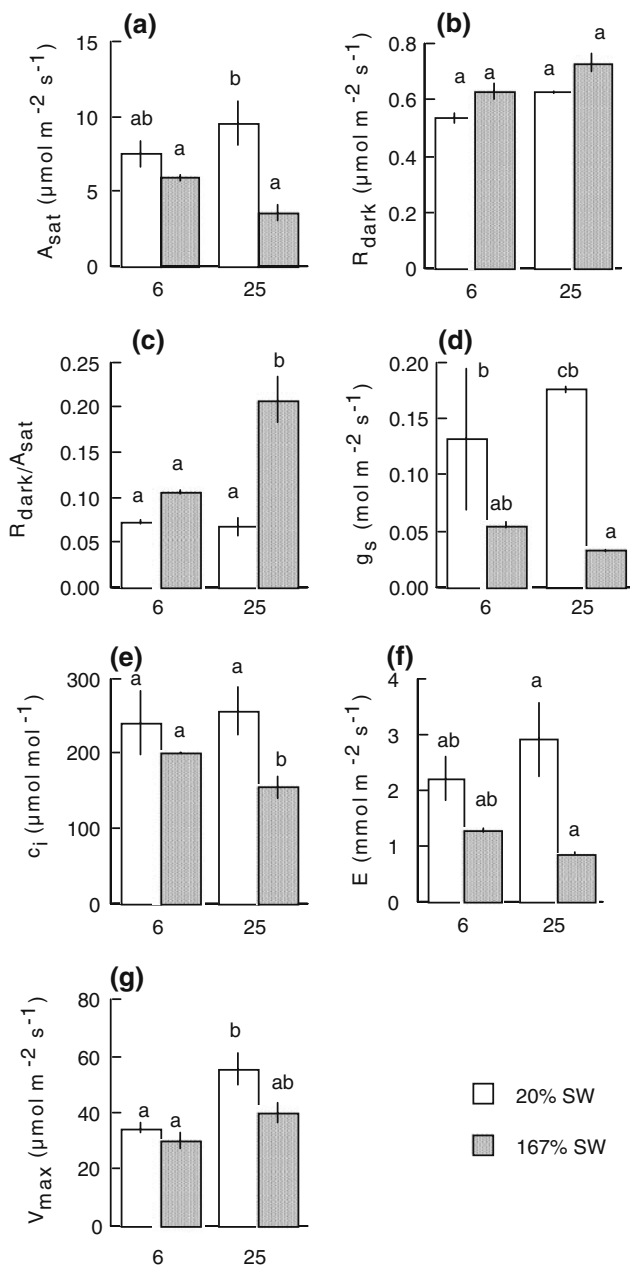
Fig. 1 Effects of salinity (% seawater) and light on the gas exchange of greenhouse seedlings. Values are means of the tank means (±SE). The *shaded bars* represent high salinity (167% SW) and the *unshaded bars* low salinity (20% SW). Treatments that share a letter are not significantly different according to Scheffe post-hoc analysis

the open; it is the sum of direct and diffuse radiation and does not include reflected radiation (Rich et al. 1999).

For 6 weeks prior to the experiment, 3,000 *A. germinans* propagules were germinated individually in 10-cm, sand-filled, plastic cups, in shallow trenches of 20% SW. Between 1 and 3 November 2000, 50 uniformly sized seedlings were transplanted into each plot. Two

weeks later, the plots were thinned to 30 seedlings. Between 8 and 23 August 2001, the plants were harvested, using water to wash away the soil, allowing differentiation of the roots from other below-ground material. Whole-plant herbivory was estimated using a method described by Dominguez et al. (1989). The plants were separated into roots, stems, branches, and leaves, and dried at 70°C for 5 days. For statistical analysis, a mean response from all surviving individuals in a plot was obtained (13–30 plants). In addition, we calculated the monthly survivorship rate of the plants within a plot [1–(log(original number)–log(number of survivors))]/number of months. The data were analyzed by means of general linear models using GLIM 3.77 (Royal Statistical Society, London, UK), where salinity was a discrete factor with two levels (low and high) and GSF (continuous) was nested within salinity (Crawley 1993). We used the post-hoc *t*-test recommended by Crawley (1993) to determine whether GSF had a different effect within each salinity zone.

Results

Greenhouse experiment

There was a significant salinity effect on light-saturated photosynthetic rates (A_{sat}; Fig. 1a; Table 1). Dark respiration (R_{dark}) did not differ between treatments (Fig. 1b; Table 1). However, there were significant interactive effects of salinity and light on the ratio of R_{dark} to A_{sat}. At low light, there was no salinity effect on R_{dark}/A_{sat}, but at high light, the ratio was higher at high salinity (Fig. 1c; Table 1). There were interactive effects of salinity and light on stomatal conductance (g_s) and leaf-level transpiration (E); both increased with light at low salinity but not at high salinity (Fig. 1d, f; Table 1). Internal CO_2 concentrations (c_i) decreased with light at high salinity, but stayed constant at low salinity (Fig. 1e; Table 1). V_{max} increased with light availability and the increase was lower, although not significantly so, at high salinity (Fig. 1g; Table 1). At high light, both salinity treatments had similar V_{max} values.

Both measured and calculated net daily photosynthesis increased with light availability, and the increase was significantly greater at low than high salinity (Fig. 2; ANCOVA salinity by light interaction $P<0.05$). Several of the daily net photosynthesis values measured at low light were negative, likely due to overcast skies on the measurement day.

There were interactive effects of salinity and light on plant size. The effect of light depended on the salinity

Table 1 ANOVA results of the greenhouse experiment. For gas exchange, only four treatments were studied—20% and 167% seawater and 5% and 25% PAR—combined. For growth and biomass allocation analyses, within a salinity level, the two lowest (6% and 12% PAR) and two highest (25% and 50%) light treatments were grouped together to control for tank linkage (see main text)

	df	A_{sat} (µmol m^{-2} s^{-1})		R_{dark} (µmol m^{-2} s^{-1})		g_s (mol m^{-2} s^{-1})	
		Sums of squares	P value	Sums of squares	P value	Sums of squares	P value
Light	1	$1.9E + 00$	0.26	$1.2E - 02$	0.65	$4.0E - 04$	0.39
Salinity	1	$1.9E + 01$	0.02	$4.1E - 02$	0.41	$1.6E - 02$	<0.01
Light∋salinity	1	$5.0E + 00$	0.11	$8.6E - 02$	0.26	$6.3E - 03$	0.03
Greenhouse	1	$1.7E + 00$	0.28	$6.1E - 02$	0.33	$1.0E - 03$	0.21
Error	3	$3.0E + 00$		$1.4E - 01$		$1.2E - 03$	
Total	7	$3.0E + 01$		$3.4E - 01$		$2.5E - 02$	

	df	E (mmol m^{-2} s^{-1})		Internal CO_2 (µmol mol^{-1})		R_{dark}/A_{sat}	
		Sums of squares	P value	Sums of squares	P value	Sums of squares	P value
Light	1	$3.9E - 02$	0.77	$1.2E + 03$	0.10	$3.2E - 03$	0.03
Salinity	1	$4.4E + 00$	0.04	$6.4E + 03$	<0.01	$1.0E - 02$	<0.01
Light∋salinity	1	$6.2E - 01$	0.30	$1.9E + 03$	0.06	$1.4E - 02$	<0.01
Greenhouse	1	$6.8E - 02$	0.71	$1.8E + 03$	0.06	$2.0E - 03$	0.06
Error	3	$1.2E + 00$		$6.3E + 02$		$6.6E - 04$	
Total	7	$6.3E + 00$		$1.2E + 04$		$3.0E - 02$	

	df	Final Mass (g)		AGR (g day^{-1})		Root/leaf (g g^{-1})	
		Sums of squares	P value	Sums of squares	P value	Sums of squares	P value
Light	1	$7.6E + 03$	<0.01	$6.8E - 02$	<0.01	$2.0E - 01$	<0.01
Salinity	2	$4.4E + 03$	<0.01	$4.0E - 02$	<0.01	$5.0E - 02$	0.02
Light∋salinity	2	$1.6E + 03$	<0.01	$1.4E - 02$	<0.01	$6.0E - 02$	≤0.01
Greenhouse	1	$2.5E + 02$	0.14	$2.5E - 03$	0.136	$2.0E - 02$	0.07
Error	17	$1.8E + 03$		$1.7E - 02$		$9.2E - 02$	
Total	23	$1.6E + 04$		$1.4E - 01$		$4.3E - 01$	

	df	Root mass ratio (g g^{-1})		Leaf mass ratio (g g^{-1})		SLA (m^2 g^{-1})	
		Sums of squares	P value	Sums of squares	P value	Sums of squares	P value
Light	1	$1.3E - 02$	<0.01	$1.7E - 02$	<0.01	$7.2E - 05$	<0.01
Salinity	2	$6.8E - 03$	<0.01	$1.5E - 03$	0.167	$4.2E - 05$	<0.01
Light∋salinity	2	$2.8E - 03$	0.02	$6.8E - 03$	0.002	$4.0E - 06$	0.07
Greenhouse	1	$2.9E - 04$	0.34	$4.0E - 03$	0.005	$0.0E + 00$	0.83
Error	17	$5.0E - 03$		$6.4E - 03$		$1.1E - 05$	
Total	23	$2.7E - 02$		$3.6E - 02$		$1.3E - 04$	

	df	RGR (g g^{-1} day^{-1})		LAR (m^2 g^{-1})		NAR (g m^{-2} day^{-1})	
		Sums of squares	P value	Sums of squares	P value	Sums of squares	P value
Light	1	$4.3E - 05$	<0.01	$1.7E - 05$	<0.01	$9.3E + 00$	<0.01
Salinity	2	$3.7E - 05$	≤0.01	$1.1E - 05$	<0.01	$6.1E - 01$	0.29
Light∋salinity	2	$5.0E - 06$	0.51	$2.0E - 06$	<0.01	$4.4E - 01$	0.40
Greenhouse	1	$1.5E - 05$	0.05	$1.0E - 06$	0.39	$1.3E + 00$	0.03
Error	17	$5.6E - 05$		$1.0E - 06$		$3.9E + 00$	
Total	23	$1.6E - 04$		$3.1E - 05$		$1.6E + 01$	

level; mass increased more with light at low and intermediate salinity than at high salinity (Fig. 3a; Table 1). There were main effects of salinity and light on RGR (which compared the first and final harvest), but no interactive effects (Fig. 3b; Table 1). Because all the plants were similar in size at the beginning of the experiment, the final mass also reflects the relative growth over the entire experiment. Therefore, there were interactive effects of salinity and light on growth rates over the course of the experiment. Root allocation was higher at high salinity; both root and root/leaf ratios increased with light at low and intermediate
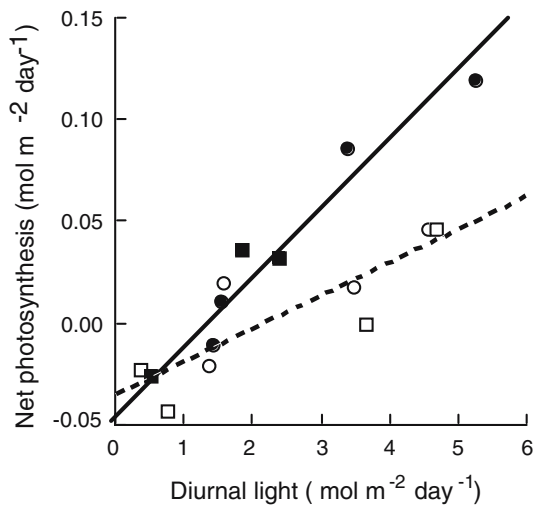
Fig. 2 The relationship between calculated daily net photosynthesis of greenhouse seedlings and measured diurnal light. The results of ANCOVA with light as covariate and salinity as fixed factor were: light $P<0.01$, salinity $P=0.44$, and light∍salinity $P<0.01$. The *shaded symbols* represent low salinity (20% SW) and the *unshaded* high salinity (167% SW). The *square symbols* represent data taken on 17 October 1999. The *circle symbols* are from 3 August 1999. The data from both days are combined because there were no significant differences (all P values >0.29). All values are tank means, and the *solid* and *dashed lines* represent the relationships at low and high salinity, respectively. The significant interaction values indicate that net photosynthesis increased more with light availability at low than at high salinity

salinity, but at high salinity, there was no change with light (Fig. 4e, f; Table 1). There was a significant light by salinity interaction in LMR; at low and intermediate salinities, LMR decreased with light, but at high salinity, there was no response to light (Fig. 4c; Table 1). SLA decreased with both light and salinity and was lowest at high salinity (Fig. 4d; Table 1).

Plants in the high-salinity treatments had the lowest LAR. At low and intermediate salinity, the plants responded to increased light with lower LAR; but, at high salinity, LAR did not change with change in light level (Fig. 4a; Table 1). In all treatments, NAR increased with light (Fig. 4b; Table 1). Figure 5 shows the relationships between RGR, NAR, and LAR. Increasing light decreased LAR, so all increases in RGR were due to NAR. At 6% and 12% PAR, as salinity increased from 20% to 70% SW, LAR decreased, so the increase in RGR was due to NAR. At 25% and 50% PAR, RGR did not increase as salinity increased from 20% to 70% SW. As salinity increased from 70% to 120% SW, declining RGR was due to both LAR and NAR at 6, 25, and 50% PAR; at 12% PAR declining RGR was mostly due to LAR.

Field experiment

Final plant mass increased less with increase in light availability (%GSF) at the high-salinity than the low-salinity sites, as indicated by differences in slope ($P<0.05$). At low light, seedling size was similar in both intertidal environments (Fig. 6a, Table 2). There were no effects of salinity and light on SLA. Overall, high-salinity plants had lower LMR, because high-salinity plants allocated relatively more to support components (stems, branches, and roots) than to leaves (Table 2). There were significant salinity effects on root mass and root/leaf ratios; high-salinity plants allocated more to roots and less to leaf mass (Table 2). There were no effects of salinity or light on the herbivory of field-grown seedlings (Table 2). Mean seedling survivorship rates were significantly higher at low than at high salinity. At both low and high salinity, seedling
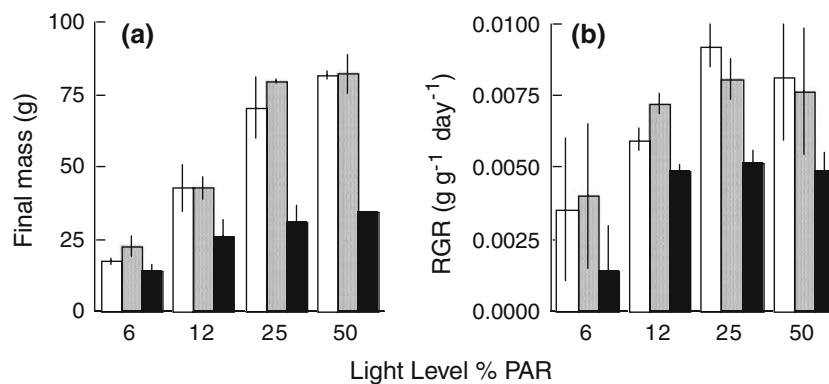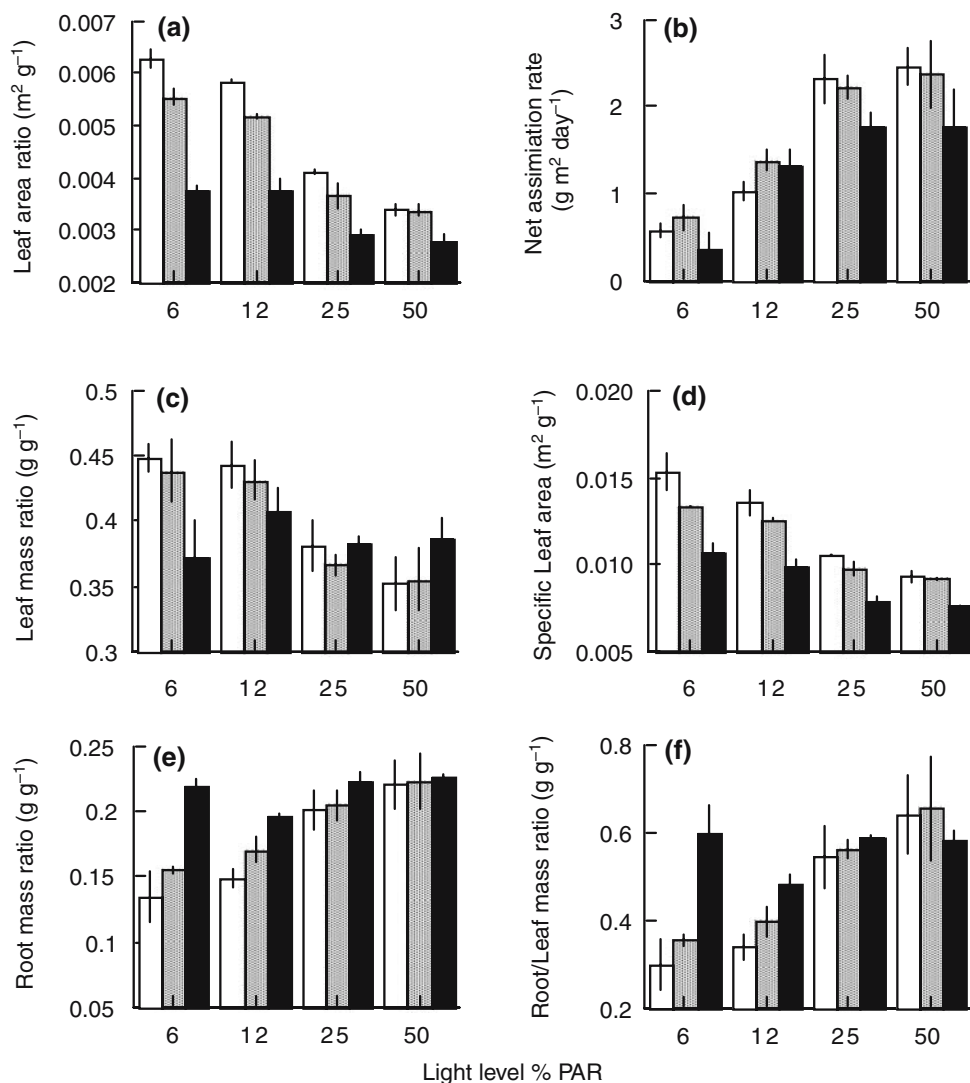

Fig. 3 Effects of salinity and light on the final harvest biomass and relative growth rates (RGRs) of greenhouse seedlings. RGR compares the first and final harvests. Because all the plants were similar in size at the beginning of the experiment, the final mass also reflects the relative growth over the entire experiment.

Values are the means of the tank means (±SE). The *unshaded bars* represent low salinity (20% SW), the *shaded bars* intermediate salinity (70% SW), and the *black bars* high salinity (167% SW)

Fig. 4 Effects of salinity and light on the growth analysis and biomass allocation of greenhouse seedlings. Values are the means of the tank means (±SE). The *unshaded bars* represent low salinity (20% SW), the *shaded bars* intermediate salinity (70% SW), and the *black bars* high salinity (167% SW)

survivorship increased with light, but the increase in survivorship with light was greater at high than low salinity (*P*<0.05; Fig. 6b; Table 2).

Discussion

Leaf-level responses to light and salinity

The results of the greenhouse experiment are consistent with our first hypothesis; net photosynthesis (both measured instantaneous and calculated daily) increased more with light at low than at high salinity. Both stomatal conductance and leaf-level transpiration increased with light at low salinity but not at high salinity. This suggests that, at high salinity, stomatal limitations may have prevented the seedlings from increasing net photosynthesis in response to increased

light; the maximum carboxylation capacity of rubisco (V_{max}) was not greatly affected by salinity. However, other studies of mangroves note that reductions in photosynthetic capacity are due to reductions in both stomatal conductance and V_{max} (Ball and Farquhar 1984; Naidoo et al. 2002; Sobrado 1999a, b).

An additional reason for lower net photosynthesis at high salinity is that rates of dark respiration relative to assimilation were higher (Fig. 2). In a study of *Avicennia marina*, absolute leaf respiration rates increased with salinity to 100% SW (Burchett et al. 1984). However, assimilation and dark respiration are generally positively correlated (Hirose and Werger 1987). In most plants, R_{dark} averages about 7% of light-saturated photosynthesis (Givnish 1988; Anten and Hirose 2001). In this study, the R_{dark}/A_{sat} ratios were in this range only at low salinity; at high salinity, they increased to 17.5%. Dark respiration in mature tissues is
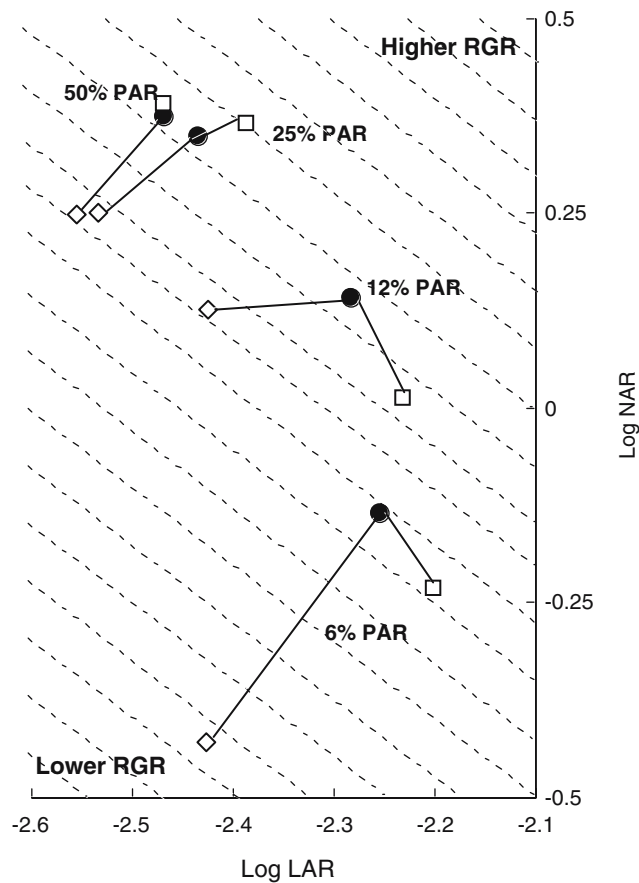
Fig. 5 Relationship between LAR, NAR, and RGR. The *solid lines* connect salinity levels within a light level. The *dashed lines* represent RGR isoclines (slope=–1). Increasing light decreased LAR, so all increases in RGR were due to NAR. At 6% and 12% PAR, as salinity increased from 20% to 70% SW, LAR decreased, so the increase in RGR was due to NAR. At 25% and 50% PAR, RGR did not increase as salinity increased from 20% to 70% SW. As salinity increased from 70% to 167% SW: at 6, 12, and 50% PAR, RGR declined due to both LAR and NAR; at 25% PAR, the decline RGR was mostly due to LAR

related to the maintenance of ion gradients across cell membranes and rates of protein turnover (Penning de Vries 1975). Assuming that protein turnover rates are roughly proportional to A_{sat} (Hirose and Werger 1987), as most leaf proteins are associated with photosynthesis, the additional increase in respiration may represent the costs of maintaining intra-cellular ion gradients at high salinity.

Whole-plant responses to light and salinity

The results of the field and greenhouse experiments were consistent with our second hypothesis that whole-plant growth rates and size increase more with light at low than at high salinity. Ball (2002) also demonstrated that salinity and light interactively affect mangrove
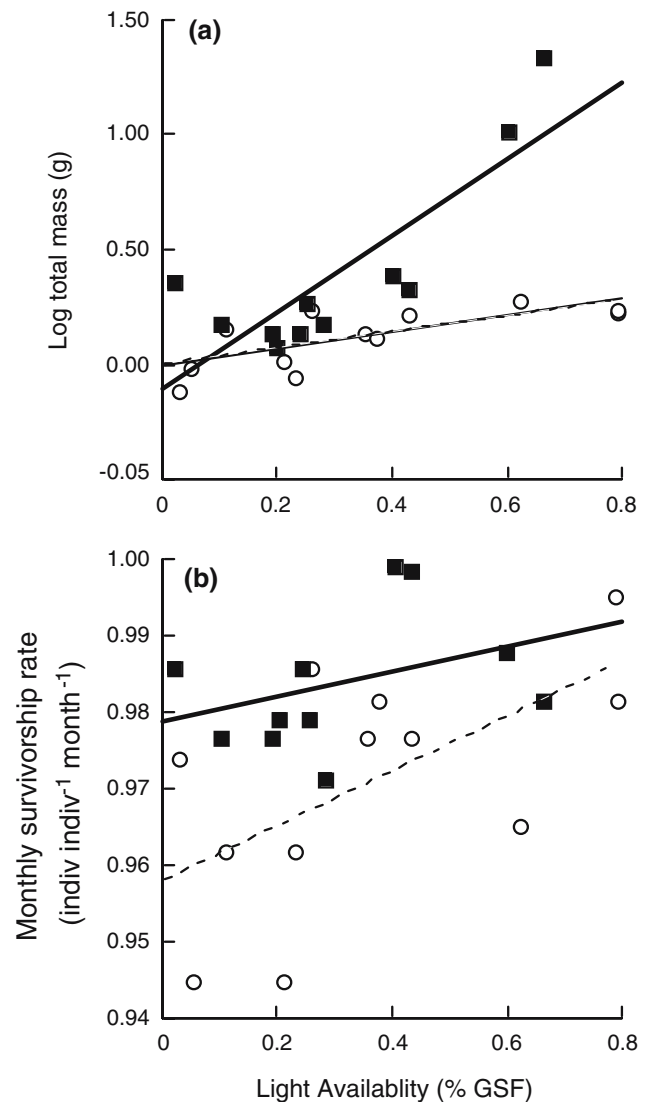

Fig. 6 The effects of light (GSF) and salinity on the total mass and survivorship of field-experiment seedlings. GSF was nested in salinity. The *solid line* and *dashed lines* represent the linear relationships at low and high salinity, respectively. The *black squares* show plot means at low salinity and the *open circles* plot means at high salinity. According to post-hoc analysis, the difference in slopes was significant ($P<0.01$) for both total mass and survivorship

seedling mass, although due to low sample size the results were not significant in the field data. Our study provides robust statistical support for this pattern and demonstrates that whole-plant responses are consistent with patterns of leaf-level gas exchange.

A. germinans seedlings adjusted their biomass allocation in response to increased light at low salinity but not at high salinity (Fig. 4). At low salinity, the seedlings exhibited the typical plant response to increased light: increased root mass and decreased leaf mass (Bouwer 1962). As light increases, increasing roots at

Table 2 General linear model results of the effects of salinity (discrete) and GSF (a measure of light availability; continuous, nested in salinity) on field-experiment seedlings. The degrees of freedom for salinity, GSF(salinity), error, and total were 1, 2, 19, and 22, respectively. To meet the assumptions of parametric statistics, the log values of total and above-ground mass, and the angular transformation of percentage herbivory were used in the analysis, but untransformed means are reported. We report the mean values at low and high salinity, a P value for the significance of the overall effect, and the slope GSF at low and high salinity

Parameter	Mean at low salinity	Mean at high salinity	P value of salinity	P value of GSF	Slope GSF at low salinity	Slope GSF at high salinity
Total mass (g)	4.350	1.380	≤0.01	≤0.01	1.672	0.366
Above-ground mass (g)	3.524	1.015	≤0.01	≤0.01	1.687	0.321
Leaf mass ratio (g g^{-1})	0.272	0.188	≤0.01	NS	0.085	–0.010
SLA (m^2 g^{-1})	0.012	0.002	NS	NS	–0.010	–0.004
Root/leaf ratio (g g^{-1})	0.812	1.582	≤0.01	NS	–0.465	0.509
Root mass Ratio (g g^{-1})	0.188	0.253	≤0.01	NS	–0.013	0.074
Support mass ratio (g g^{-1})	0.728	0.812	≤0.01	NS	–0.085	0.010
Survivorship rate (ind ind^{-1})	0.979	0.971	0.03	0.03	0.012	0.035
% Herbivory	15%	16%	NS	NS	–0.371	–1.359

the expense of leaves is associated with higher requirements for water and nutrients (vd Boogaard et al. 1996). However, at high salinity, the seedlings in our study already had high root mass and root/leaf ratios (relative to low salinity), and did not further allocate to roots in response to increased light. Because our field sites experience soil desiccation in the dry season, at high salinity, the consequence of not increasing root mass with increase in light may have been diminished water uptake, aggravating water shortages at the leaf-level and increasing stomatal closure.

Previous studies of mangroves suggest that changes in RGR with salinity are due to changes in both LAR and NAR (Ball and Pidsley 1995; Ball 2002). In this study, the relative influence of LAR versus NAR on RGR in response to increased salinity depended on the levels of salinity and light; in the treatment combinations of low and intermediate salinity and light, NAR was most important. In the high light, high-salinity treatment, declining RGR was due to both NAR and LAR. At high salinity, declines in NAR reflect the observed increases in dark respiration and decreases in rates of gas exchange.

In an early paper, Janzen (1985) wondered why mangrove forests lacked a distinctive understory. He hypothesized that the costs of salinity tolerance entail larger maintenance costs due to which plants would be unable to maintain a positive carbon balance under shaded conditions. Our results contradict this hypothesis. First, relative to photosynthesis, leaf respiratory costs only increased at high light and not at low light. Second, in both the greenhouse and field experiments, salinity under low light conditions had only a very small negative effect on biomass increment.

In the field experiment, we observed that mean seedling survivorship rates were greater at low than at high salinity. This is consistent with overall higher net photosynthesis and growth rates at low than at high salinity. Furthermore, at both low and high salinity, seedling survivorship increased with light availability. A number of studies have found that in general there is higher mangrove seedling survivorship in gaps than non-gaps (Ball 2002; Clarke and Kerrigan 2000). Ball (2002) proposes two explanations: that there is more herbivory under closed canopies (e.g., Osborne and Smith 1990; Sousa et al. 2003b; Lindquist and Carroll 2004); and that mortality in the shade could be due to below ground competition with surrounding adults (Passioura et al. 1992). In this study, low light seedling survivorship was 2% less at high salinity than at low salinity. A 2% reduction in survival means that the mortality rate doubled with salinity (i.e., it increased from 2% to 4%); this could have important demographic consequences (López-Hoffman 2003). Furthermore, in the present study it is curious that the increase in survivorship with increase in light was greater at high than at low salinity. At high light, the seedling survivorship rates at low and high salinity were quite similar.

Our results demonstrate that growth and survival can be decoupled, and that higher seedling growth rates do not necessarily indicate higher survivorship rates. In a study of tropical terrestrial trees, Kitajima (1994) showed that, in the shade, species with higher RGR have lower survivorship rates than species with lower RGR. Survival in the shade depended on morphological protections against herbivores (tougher leaves, well-established root, high wood density) that implied high construction costs, resulting in lower

carbon gain and RGR. In this study, the increase in allocation to support and root mass at high salinity may increase mangrove seedling survival at the cost of leaf mass and, thus, growth. This might explain why, at high light, high-salinity seedlings have similar survivorship rates as low-salinity seedlings, despite lower growth rates.

Mangrove forests are complex ecosystems. In addition to light and salinity gradients, they are structured by tidal gradients that cause gradients in nutrient availability, waterlogging, drought stress, herbivory, and patterns of propagule dispersal (McGuinness 1997; McKee 1995; Medina and Francisco 1997; Sousa et al. 2003b; Lovelock et al. 2004). A comprehensive understanding of mangrove forest dynamics should consider all of these factors and their interactive effects. Future studies at our field sites in western Venezuela should investigate the interactive effects of salinity, light, herbivory, and dry season soil desiccation. Nonetheless, an important outcome of this study is that the interactive effects of salinity and light observed in the field were consistent with the greenhouse results, despite the presence of complicating factors in the field. This suggests that an understanding of the interactive effects of salinity and light is essential for understanding the structure and dynamics of mangrove forests.

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