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Mangrove Seedling Net Photosynthesis, Growth, and Survivorship are Interactively Affected by Salinity and Light[1]

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ABSTRACT

We hypothesized that salinity and light interactively affect mangroves, such that net photosynthesis, growth, and survivorship rates increase more with increase in light availability at low than high salinity. Using greenhouse and field experiments, we determined that net photosynthesis, growth rates, and size increased more with light at low than high salinity. At high salinity, the ratio of leaf respiration to assimilation increased fourfold, suggesting that salinity may have contributed to declines in net photosynthesis. Stomatal conductance, leaf-level transpiration, and internal CO_2 concentrations were lower at high salinity. Ratios of root mass to leaf mass were higher at high salinity. Stomatal limitations and increased respiratory costs may explain why at high salinity, the seedlings did not respond to increased light availability with increased net photosynthesis. Increased root mass relative to leaf mass suggests that at high salinity, either water or nutrient limitations may have prevented the seedlings from increasing growth with increasing light availability. At both low- and high-salinity zones in the field, seedling survivorship increased with light availability, and the effect of light was stronger at low salinity. However, at low light, survivorship was higher at high than low salinity, indicating that there may be a trade-off between survivorship and growth. The interactive effects observed in the greenhouse were robust in the field, despite the presence of other factors in the field such as inundation and nutrient gradients and herbivory. This study provides a robust test of the hypothesis that salinity and light interactively effect mangrove seedling performance.

RESUMEN

Presentamos la hipótesis de que la salinidad y la luz afectan de forma interactiva a los mangles, de tal forma que la fotosíntesis neta, el crecimiento, y la sobrevivencia se incrementa con el aumento de disponibilidad de luz a salinidad baja que a salinidad alta. Observamos que con experimentos de invernadero y de campo nuestra hipótesis fue correcta con respecto a la fotosíntesis neta, tasa de crecimiento y tamaño; todos estos factores se incrementaron más con luz a baja salinidad. En alta salinidad, la tasa de respiración de la hoja con respecto a la asimilación aumentó cuatro veces, sugiriendo que la salinidad pudo haber contribuido a descensos en la fotosíntesis neta. La conductividad estomática, la transpiración foliar, y las concentraciones internas de CO_2 fueron más bajos a salinidad alta. Las limitaciones estomáticas y el aumento de los costos en la respiración, pueden explicar por qué las plántulas en salinidad alta no respondieron a la disponibilidad de luz con un incremento en la fotosíntesis neta. El aumento en la tasa raíz/hoja en salinidad alta sugiere que ya sean las limitaciones de agua o los nutrientes evitaron que las plántulas crecieron más al aumentar la disponibilidad de luz. Tanto en la zona de alta como de baja salinidad, la sobrevivencia de las plántulas aumentó con la disponibilidad de luz pero el efecto lumínico fue mayor a baja salinidad. Sin embargo, a baja luz, la sobrevivencia fue mayor a alta salinidad que en baja, indicando la existencia de un compromiso entre sobrevivencia y crecimiento. Los efectos interactivos observados en el invernadero fueron robustos en el campo, a pesar de la presencia de otros factores tales como inundación, gradientes de nutrientes y herbivoría. Este estudio provee de una prueba robusta de la hipótesis de que la salinidad y la luz afectan interactivamente el desempeño de las plántulas.

Key words: ecophysiology; gas exchange; salinity tolerance; Venezuela.

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MANGROVE FORESTS ARE STRONGLY STRUCTURED BY GRADIENTS of soil salinity, waterlogging, and nutrient availability (Thom 1967, Clarke & Hannon 1970, Boto 1982, Hutchings & Saenger 1987, McKee 1995). Superimposed upon these gradients is a light gradient caused by treefall gaps and the edge effects of mangrove forests growing along waterways (Smith 1992, Feller & McKee 1999). In this study, we examine the interactive effects of salinity and light gradients on *Rhizophora mangle* seedlings. Previous studies have shown that salinity and light interactively affect mangrove seedling growth and survivorship (Ball 2002) and growth and gas exchange (Krauss & Allen 2003). Using a combination of greenhouse and field experiments, and a robust experimental design, we assess how functional responses to environmental conditions are reflected in the net photosynthesis, growth, and survivorship of mangrove seedlings.

At low salinity, mangrove seedlings respond to increased light by increasing mass, but at high-salinity, seedling size does not increase with increase in light availability (Ball 2002, Hoffman 2003). High salinity limits water uptake in mangroves (Clough 1984), causing low leaf intercellular CO_2 concentrations (Farquhar *et al.* 1982, Andrews & Muller 1985, Ball 1986), decreased photosynthetic rates (Ball & Farquhar 1984, Lin & Sternberg 1992, Sobrado 1999b), and reduced net photosynthesis (Ball 1988b, Pezeshki *et al.* 1990). At the whole-plant level, high salinity, typically above 50 percent seawater (SW), causes depressed growth rates (Burchett *et al.* 1984, Clough 1984, Smith 1992, Ball 1996) and increased allocation to root mass relative to leaf mass (Ball 1988b). It may be that at high salinity, water limitations at the leaf and whole-plant levels prevent mangrove seedlings from responding to increased light with increased carbon gain and growth. Photosynthesis at high light is limited by stomatal conductance (Lambers *et al.* 1998). Water limitations at high salinity would be intensified by the combination of high salinity and high light.

In this study, we examine the effects of salinity and light on the carbon gain, growth, and survivorship of *R. mangle* seedlings. We hypothesize that: (1) at the leaf level, net carbon gain increases more with light at low salinity than at high salinity; and (2) growth rates, size, and survivorship increase more with light availability at low than high salinity. Further goals are (1) to gain insight into the mechanisms of salinity–light interactions; (2) to determine whether the photosynthetic and respiratory responses of leaves are consistent with overall patterns of plant growth and biomass allocation; and (3) whether the salinity–light effects observed in the greenhouse are robust under field conditions where factors such as herbivory, nutrients, and flooding also influence mangrove seedling performance (Ellison & Farnsworth 1996, Feller *et al.* 1999, Sousa *et al.* 2003).

Methods

PLANT MATERIAL.—We conducted one greenhouse experiment and two field experiments. Mature *R. mangle* propagules for all experiments were collected from the same seven trees at our field sites in Venezuela. Propagules for the greenhouse experiment were collected in August 1998. Propagules for the first and second field experiments were collected in May 1999 and June 2000, respectively.

GREENHOUSE EXPERIMENT.—The greenhouse design was fully factorial with two complete, replicate blocks in two different greenhouses at Stanford University, Stanford, CA, USA. There were three salinity levels (20, 70, and 167% of full SW), and four light levels (5, 12, 25, and 50% PAR, photosynthetically active radiation) giving 12 treatment combinations. The treatment levels reflected the natural range of conditions in the field, where annual soil salinity levels vary from 0 to 14 and 100 to 200 percent SW in the low- and high-salinity sites, respectively (Narváez 1998, Hoffman 2003). The greenhouses were whitewashed to filter 50 percent of outside light. And 50, 75, and 90 percent light filtration shade cloth tents were used for the 25, 12, and 5 percent PAR treatments, respectively. Instant Ocean® aquarium salt (Aquarium Systems, Inc., Mentor, OH, USA) was used for the salinity treatments.

The mangrove seedlings were grown in individual pots placed in 946 liter Rubbermaid® stock tanks (Rubbermaid Corporation, Fairlawn, OH, USA), 12 tanks per greenhouse. Within a greenhouse, each tank represented a unique salinity and light treatment. Due to limited space, a set-up similar to Ellison and Farnsworth (1997) was used. Side-by-side tanks were plumbed in a recirculating series, sharing saltwater, but differing 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 pump timing was changed to simulate tidal progression. This design simulated tides that occur in mangrove swamps, avoiding permanently inundating the plants. The potential confounding effects of sharing water were minimized by weekly readjusting the salinity levels, and periodically cleaning the tanks and changing the water. At no point was it suspected that the water sharing compromised the experiment's integrity; nonetheless, the connected tanks were accounted for in the statistical analysis (see below).

In the greenhouse experimental setup, we used large volume pots of 66 cm lengths of 15.3 cm diameter. PVC drainpipe were filled with a 1:1 mixture of sand and potting soil. The plants were fertilized monthly with 0.8 L of 100 percent N:P:K 20:20:20 fertilizer (0.67 g NPK/mo). Day and nighttime temperatures were 32 ± 3°C and 29 ± 3°C , respectively, and relative humidity was 70 ± 5 percent, conditions comparable to the field (Narváez 1998).

Prior to the experiment, 1500 propagules were cultivated hydroponically in a 20 percent SW solution for 3 mo. On 23 November 1998, 312 similarly sized seedlings were planted into the tanks (13 per tank). The fresh mass of the transplanted seedlings was weighed prior to transplanting. Upon transplanting, the water in the tanks was at 20 percent SW, and was held at this level for 1 week. To prevent "osmotic shock," salinity was gradually increased, 25 percent of full treatment per week, over 3 weeks. The final treatments were imposed on 22 December 1998. Initially, each tank had 13 plants; some plants died in 2 weeks after transplanting (leaving between 8 and 13 plants per tank). The seedlings that died were not replaced. No plants died after the experiment began on 22 December, therefore the seedling mortality was not related to the treatments, but rather to transplanting.

The greenhouse harvest began on 25 September 1999, 276 days after the experiment began. The harvested plants were separated into roots, stems, branches, and leaves. The leaf area of a subset of fresh leaves was measured with the LI-3100 meter (LiCor, Lincoln, Nebraska, USA). All plant material was then dried at 80°C for 4 d and then weighed. Using dry weights, we calculated the following parameters for each plant: final mass, root mass ratio (ratio of root to whole-plant mass), leaf mass ratio (LMR; ratio of total leaf to whole-plant mass), specific leaf area (SLA, ratio of leaf area to leaf mass), leaf area ratio (LAR; ratio of total leaf area to whole-plant mass), root/leaf ratio (ratio of root to leaf mass), support mass ratio (ratio of sum of root, hypocotyl, stem, and branch mass to whole-plant mass), and relative growth rate (RGR).

RGR was calculated according to West *et al.* (1920) by comparing a seedling's estimated dry mass at the start of the experiment with its measured final dry mass. At the beginning of the experiment, 20 seedlings similar in size to the transplanted seedlings were used to estimate the relationship between seedling fresh and dry weight. During the course of the experiment, we collected and weighed all the dead leaves from each plant. For the RGR calculation, the leaf mass of a plant lost during the experiment was added to the final harvest mass (Anten & Ackerly 2001).

Leaf gas exchange was measured in only four treatments, 20 and 167 percent SW, and 5 and 25 percent PAR, combined. Measurements were taken on four plants per treatment per greenhouse. For each plant, the measurements were taken on 3 different days between September 15 and 17, 1999, and on two young, fully expanded leaves. We use the average of these measurements. All measurements were made when the tanks were at "low" tide. A portable photosynthesis system with an attached light source (LI-6400, LiCor) was used. The chamber was set to 27°C and the reference CO_2 to 380 ppm. Light-saturated net assimilation rate A_{sat} (μmol/m^2/sec) was measured between 0900 h and 1130 h with the light source at 1200 μmol/m^2/sec. Dark respiration R_{dark} (μmol/m^2/sec) was measured prior to the sunrise. A_{sat} and R_{dark} were measured on the same leaves.

Incident light was estimated on 3 August and 17 September 1999. Every hour a quantum sensor, attached to a data logger (LI-190 and LI-1000, LiCor), was placed near a representative exposed leaf and oriented at the same angle. Photon flux values were integrated to estimate daily total incident light per unit leaf area. A simplified estimate of daily net photosynthesis per unit leaf area was calculated following Hirose *et al.* (1997). A nonrectangular hyperbola was used to characterize the light response of net leaf photosynthesis (P_L, μmol/m^2 sec; Marshall & Biscoe 1980)

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

where R_{dark} is the measured respiration rate, A_{max} is A_{sat} plus R_{dark}, and I_L is the measured incident photon flux. The apparent quantum yield (ϕ) was 0.04 (Ehleringer & Björkman 1977), and the curvature factor (θ) was 0.8 of quantum yield, an average value of a range of species (Anten & Hirose 2001). The light response at different times throughout the day, based on the measured light levels, was integrated for a 24-h total of net photosynthesis (Hirose & Werger 1987).

Because during the gas exchange measurements, the photosynthesis meter's leaf chamber was set to a constant temperature and reference CO_2 concentration, there were no treatment differences in vapor pressure deficits (VPD) or leaf temperatures during the measurements (grand means, 1.94 KPa and 28.6°C, respectively; all $P > 0.1$). Under normal ambient greenhouse conditions (*i.e.*, when the leaves were not in the LiCor chamber), the different light availability levels could have caused treatment differences in leaf temperature, which may have affected photosynthetic rates.

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, a conservative ANOVA design was used. Within a salinity level, the 5 and 12 percent PAR tanks were connected by pumps, as were the 25 and 50 percent PAR tanks. For ANOVA, the 5 and 12 percent PAR tanks were labeled low light, and the 25 and 50 percent PAR tanks were labeled high light, giving six treatments (2 light × 3 salinity). While interpretations are based on this conservative ANOVA, the figures report 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 only four treatments were studied, not including 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 used in the ANOVA were tested for normality; no data transformations were necessary. These analyses were performed with Data Desk Version 6.1 (Data Description Inc., 1997; Ithaca, NY, USA).

FIELD EXPERIMENT.—The field experiments were conducted in the Río Limón mangroves of Lake Maracaibo, Venezuela (10°96′70″N, 71°73′24″W). In May 1999, we located 12 low- salinity (near the mouth of a freshwater river) and 12 high-salinity plots (in a forest of stunted *Avicennia germinans*). Several months later, the high-salinity plots were completely destroyed by a seasonal creek that overran its banks. In June 2000, we restarted the experiment, using the same low-salinity plots, and more appropriately located high-salinity sites. However, in July 2000, all the low-salinity seedlings were eaten in an unusual caterpillar blight. Therefore, we report low salinity results from the first year, and high salinity results from the second year.

Each plot was cleared of vegetation and debris but not of *A. germinans* pneumatophores. At high salinity, high intertidal, light levels less than 10 percent are rare (Hoffman 2003). Therefore, shade cloth was placed over three high-salinity plots to test seedling responses to less than 10 percent PAR. For balanced design, shade cloth was also used at three low-salinity plots.

Between May 7–21, 1999 and August 15–19, 2001, under uniform, cloudy skies, four hemispherical canopy photos were taken per plot. A Minolta X-700 camera (Konica-Minolta Corporation, Ramsey, NJ, USA) with a Sigma 8 mm F4 fisheye lens (Sigma Corporation, Tokyo, Japan) mounted on a leveled tripod, and Kodak Velvia film (Eastman Kodak Company, Rochester, NY, USA) were used. The photos were analyzed with 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.

The same methodology was used for both years. Fifty uniformly sized propagules were planted into the plots, with the base 5 cm into the soil. Two weeks later, the plots were thinned to

25 seedlings. The first year, the propagules were planted in May 1999 and harvested in March 2000, a period of 9 mo. The second year, the propagules were planted in June 2000 and harvested in August 2001, a period of 13 mo. Between 8 and 17 seedlings per plot survived until the time of harvest. The plants were harvested using water to wash away the soil, allowing differentiation of the roots from other belowground material. They were separated into roots, stems, branches, and leaves, and dried at 70°C for 5 d.

For data analysis, a mean response from all surviving individuals in a plot was obtained. Because the results from the low- and high-salinity sites were obtained from two different years, in experiments of different lengths of time, they cannot be directly compared. For each salinity level, we report the regression relationship between light availability and final mass (g), root ratio (g/g), root/leaf ratio (g/g), LMR (g/g), and SLA (m^2/g). Because the second year experiment (high-salinity data) was 4 mo longer, the plants were larger, and the *y*-intercept was greater. Thus, the only parameter for comparison is the slope of the relationship between the functional characteristics and light at each salinity level. In addition, we calculated the monthly survivorship rate of the plants within a plot, 1 − ((log(number of survivors) − log(original number))/number of months). Because we calculated a monthly rate, accounting for the different time lengths of the two experiments, we were able to use ANOVA to analyze the effects of salinity on light on survivorship. All analyses of the field data were performed using Data Desk.

RESULTS

GREENHOUSE EXPERIMENT.—Light saturated photosynthetic rates (A_{sat}) increased with increase in light and decreased with salinity (Table 1; Fig. 1A). The absolute values of dark respiration (R_{dark}) were similar across both treatments (Fig. 1B); however, the ratio of R_{dark} to A_{sat} increased considerably with salinity (Table 1; Fig. 1C). The plants had lower stomatal conductance, transpiration rates, and internal CO_2 concentrations at high salinity than at low salinity (Table 1; Figs. 1D–F). There were interactive effects of salinity and light on net photosynthesis per unit leaf area; net photosynthesis increased more with increase in light at low salinity than high salinity (Fig. 2).

We observed interactive effects of salinity and light on plant size and growth rates (Fig. 3; Table 2). Biomass and RGR increased with increase in light availability at low and intermediate salinities, but did not increase with light at high salinity. Furthermore, the increase in growth and size was greater at low salinity than intermediate salinity (Table 2; Fig. 3). For example, the difference in biomass between plants from the lowest and highest light treatments was fourfold at low salinity, 1.5-fold at intermediate salinity, and undetectable at high salinity. At low light, allocation to roots was higher at high salinity, and there were interactive effects of light and salinity: root allocation increased with increase in light availability at low and intermediate salinities, but not at high salinity (Table 2;

TABLE 1. *Results of ANOVAs of the effects of salinity and light on gas exchange characteristics of greenhouse seedlings grown in four treatments, 20 and 167 percent seawater (SW) and 5 and 25 percent PAR, combined.*

	df	Sums of squares	*F*-ratio	*p*	Sums of squares	*F*-ratio	*p*
		A_{sat} ($\mu mol/m^2/sec$)			R_{dark} ($\mu mol/m^2/sec$)		
Light	1	1.2E + 01	8.3E + 00	0.06	1.2E + 01	8.3E + 00	0.37
Sal	1	4.7E + 01	3.2E + 01	0.01	4.7E + 01	3.2E + 01	0.76
Light × Sal	1	8.8E − 01	5.9E − 01	0.50	8.8E − 01	5.9E − 01	0.75
Greenhouse	1	2.4E + 00	1.6E + 00	0.29	2.4E + 00	1.6E + 00	0.27
Error	3	4.5E + 00			4.5E + 00		
Total	7	6.8E + 01			6.8E + 01		
		Internal CO_2 ($\mu mol/mol$)			Transpiration ($mmol/m^2/sec$)		
Light	1	4.0E + 03	1.4E + 01	0.03	1.2E + 01	8.3E + 00	0.60
Sal	1	2.5E + 03	8.8E + 00	0.06	4.7E + 01	3.2E + 01	0.01
Light × Sal	1	5.5E + 02	1.9E + 00	0.26	8.8E − 01	5.9E − 01	0.70
Greenhouse	1	1.1E + 02	3.8E − 01	0.58	2.4E + 00	1.6E + 00	0.32
Error	3	8.7E + 02			4.5E + 00		
Total	7	8.1E + 03			6.8E + 01		
		Stomatal Condition ($\mu mol/m^2/sec$)			R_{dark}/A_{sat}		
Light	1	1.2E + 01	8.3E + 00	0.42	1.2E + 01	8.3E + 00	0.15
Sal	1	4.7E + 01	3.2E + 01	0.04	4.7E + 01	3.2E + 01	0.02
Light × Sal	1	8.8E − 01	5.9E − 01	0.25	8.8E − 01	5.9E − 01	0.27
Greenhouse	1	2.4E + 00	1.6E + 00	0.69	2.4E + 00	1.6E + 00	0.27
Error	3	4.5E + 00			4.5E + 00		
Total	7	6.8E + 01			6.8E + 01		

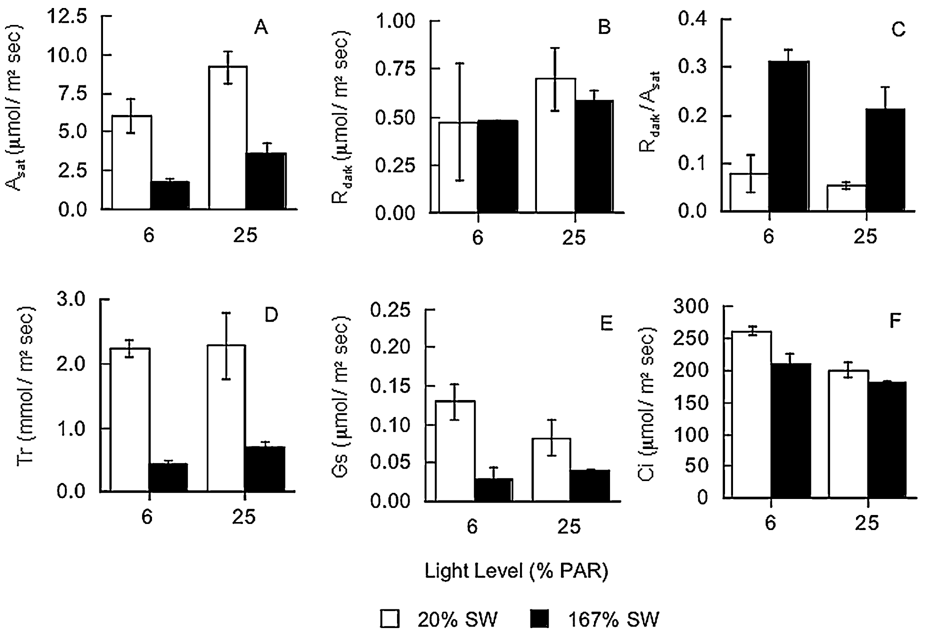

FIGURE 1. Effects of salinity (expressed as % seawater, SW) and light on greenhouse seedling gas exchange. Values are means of the two tank means (±SE). A_{sat} is light saturated photosynthesis, R_{dark} is dark respiration, Tr is leaf-level transpiration, Gs is stomatal conductance, and Ci is the intercellular CO_2 concentration.

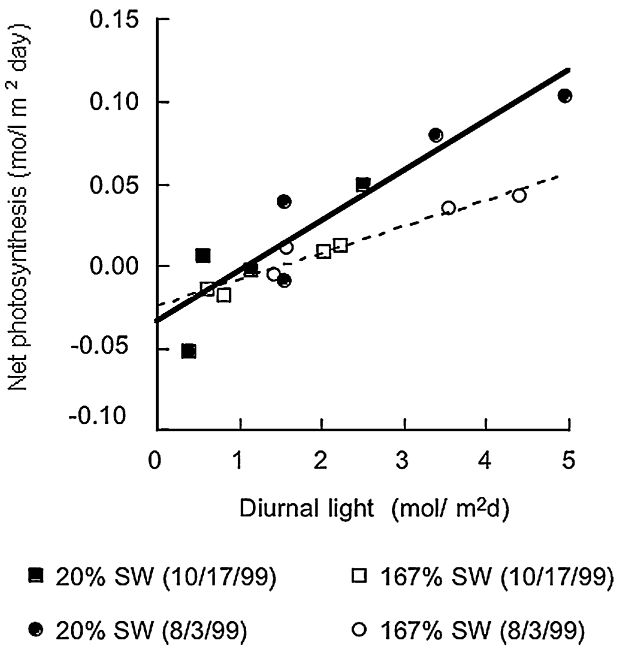

FIGURE 2. The relationship between calculated net photosynthesis per unit leaf area of greenhouse seedlings (estimated from photosynthetic parameters) and measured diurnal light. The values are tank means. ANCOVA with light as covariate and salinity as fixed factor revealed: Light $P \leq 0.001$, Salinity $P = 0.546$ and Light × Salinity $P = 0.043$. The significant interaction indicates that leaf-level carbon gain increased more with light at low salinity than at high salinity. The solid and dashed lines are the linear relationships at low and high salinity, respectively. There were no differences between days for net photosynthesis at a given salinity (all $P > 0.15$), so data are grouped.

Fig. 4A). Root/leaf ratios were highest at high salinity (Fig. 4B). Allocation to support mass (roots, hypocotyl, stem, and branches) was greatest at high salinity (Fig. 4C). There was a significant light by salinity interaction in leaf mass allocation. At low light, plants in all salinity treatments had similar LMRs. LMRs increased with light availability at low and intermediate salinities, but decreased with increase in light at high salinity (Table 2; Fig. 4D). SLA was lower at high salinity, and at all salinity levels, SLA decreased with increase in light availability (Fig. 4E). LAR at high salinity was lower than at low and intermediate salinities, and LAR generally decreased with increase in light (Table 2; Fig. 4F).

Field experiment.—Final plant mass increased less with increase in light availability at the high-salinity sites than the low-salinity sites as indicated by the differences in the slope (Table 3; Fig. 5A). The longer growth period at high salinity could lead to higher slopes for measures of growth, due to cumulative growth; however, as high-salinity slopes were lower, we do not think that the duration of the experiments can explain the differences. The slopes of the relationships between root and LMRs and light availability were greater at low salinity than high salinity (Table 3; Figs. 5B and C). The relationship between root/leaf ratio and light availability was almost flat at low salinity and positive at high salinity (Table 3; Fig. 5D). At both the low- and high-salinity sites, SLA decreased with light availability (Table 3; Fig. 5E). At both low and high salinities, the probability of seedling survivorship increased with light level, and the effect of light was stronger at low salinity. At low light levels, seedling survivorship rates were higher at high salinity. Both the main effects of salinity and GSF, and their interactive effects were significant (Fig. 6; all $P < 0.01$).

TABLE 2. *Results of ANOVAs of the effects of salinity, light, and greenhouse on biomass allocation patterns and growth analysis parameters. 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 connections (see main text). RGR is the relative growth rate.*

	df	Sums of squares	*F*-ratio	*p*	Sums of squares	*F*-ratio	*p*
		Final mass (g)			RGR (g/g/day)		
Light	1	5.6E+02	3.6E+01	<0.01	5.6E + 02	3.6E + 01	<0.01
Sal	2	9.1E+02	2.9E+01	<0.01	9.1E + 02	2.9E + 01	<0.01
Light × Sal	2	5.0E+02	1.6E+01	<0.01	5.0E + 02	1.6E + 01	<0.01
Greenhouse	1	7.8E−01	4.9E−02	0.83	7.8E − 01	4.9E − 02	0.36
Error	17	2.7E+02			2.7E + 02		
Total	23	2.2E+03			2.2E + 03		
		Root mass ratio (g/g)			Root/leaf ratio (g/g)		
Light	1	3.3E − 03	5.9E + 00	0.03	5.6E + 02	3.6E + 01	0.20
Sal	2	4.2E − 03	3.8E + 00	0.04	9.1E + 02	2.9E + 01	0.00
Light × Sal	2	4.4E − 03	4.0E + 00	0.04	5.0E + 02	1.6E + 01	0.31
Greenhouse	1	2.6E − 04	4.6E − 01	0.51	7.8E − 01	4.9E − 02	0.21
Error	17	9.4E − 03			2.7E + 02		
Total	23	2.1E − 02			2.2E + 03		
		Leaf mass ratio (g/g)			Specific leaf area (m^2/g)		
Light	1	5.6E + 02	3.6E + 01	0.50	5.6E + 02	3.6E + 01	<0.01
Sal	2	9.1E + 02	2.9E + 01	0.00	9.1E + 02	2.9E + 01	<0.01
Light × Sal	2	5.0E + 02	1.6E + 01	0.07	5.0E + 02	1.6E + 01	0.50
Greenhouse	1	7.8E − 01	4.9E − 02	0.08	7.8E − 01	4.9E −02	0.64
Error	17	2.7E + 02			2.7E + 02		
Total	23	2.2E + 03			2.2E + 03		
		Leaf area ratio (m^2/g)					
Light	1	5.6E + 02	3.6E + 01	0.05			
Sal	2	9.1E + 02	2.9E + 01	<0.01			
Light × Sal	2	5.0E + 02	1.6E + 01	0.16			
Greenhouse	1	7.8E − 01	4.9E−02	0.15			
Error	17	2.7E + 02					
Total	23	2.2E + 03					

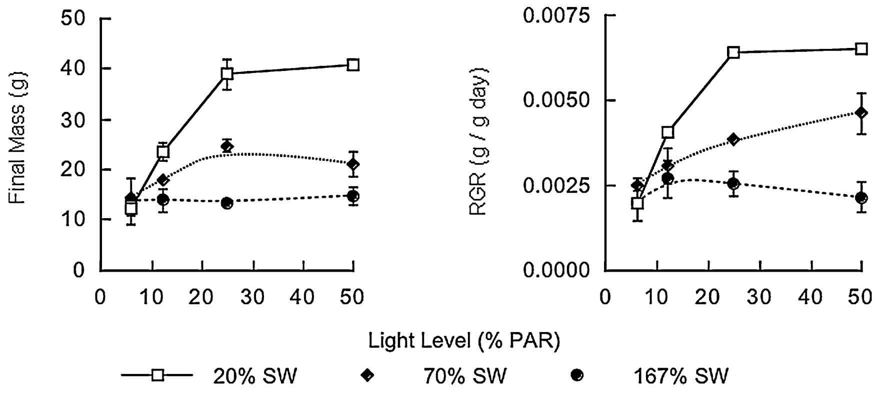

FIGURE 3. Effects of salinity and light on the final harvest biomass and the relative growth rate (RGR) of greenhouse *R. mangle* seedlings. RGR compares size at final harvest and size at the beginning of the experiment. Values are the means of the tank means (±SE, $N = 2$).

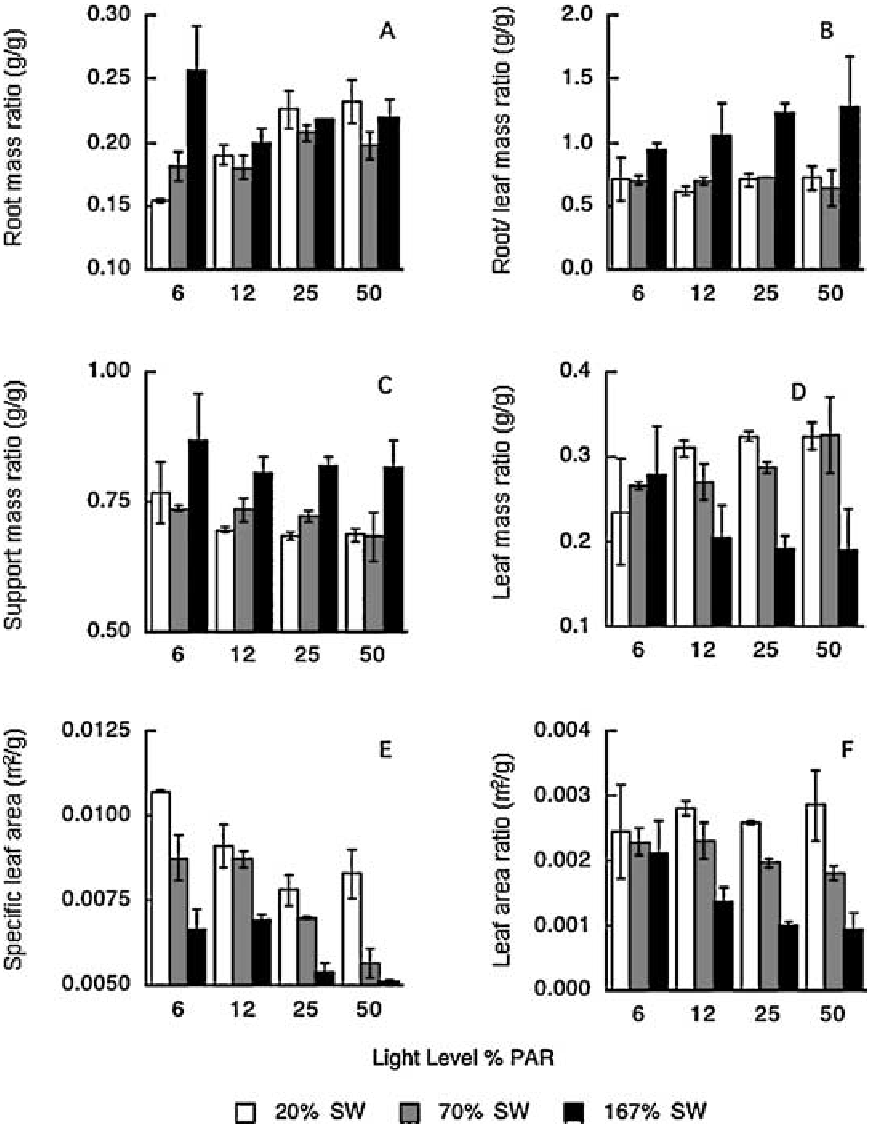

FIGURE 4. Effects of salinity and light on the biomass allocation of greenhouse *R. mangle* seedlings at the time of final harvest. Values are the means of the tank means (±SE, $N = 2$).

TABLE 3. *Regression statistics at low and high salinity, years one and two, respectively, for the relationships between GSF and field experiment seedling growth parameters. GSF (Global Site Factor) is a measure of light availability.*

	Low salinity Year 1			High salinity Year 2		
	R^2	m	p	R^2	m	p
Final mass (g)	0.66	38.771	<0.01	0.58	6.311	<0.01
Root mass ratio (g/g)	0.60	0.138	<0.01	0.63	0.052	<0.01
Root/leaf ratio (g/g)	0.10	0.095	0.38	0.54	1.143	0.02
Leaf mass ratio (g/g)	0.60	0.297	<0.01	0.08	−0.037	0.43
Leaf area ratio (m²/g)	0.39	0.002	0.05	0.28	−0.001	0.12
SLA (m²/g)	0.31	−0.005	0.10	0.75	−0.003	<0.01

DISCUSSION

LEAF-LEVEL RESPONSES TO SALINITY AND LIGHT.—The results of the greenhouse experiment are consistent with our first hypothesis; net photosynthesis per unit leaf area increased more with light at low salinity than at high salinity. Light-saturated photosynthesis, stomatal conductance, leaf-level transpiration, and internal CO_2 concentrations were lower at high salinity. This suggests that at high salinity, net photosynthesis may be constrained by leaf-level water limitations, which may have prevented the seedlings from enhancing net photosynthesis in response to increased light. Studies of mangroves report that reductions in stomatal conductance are in part responsible for reductions in light-saturated photosynthesis (Ball & Farquhar 1984, Sobrado 1999a,b, Naidoo *et al.* 2002, Parida *et al.* 2004).

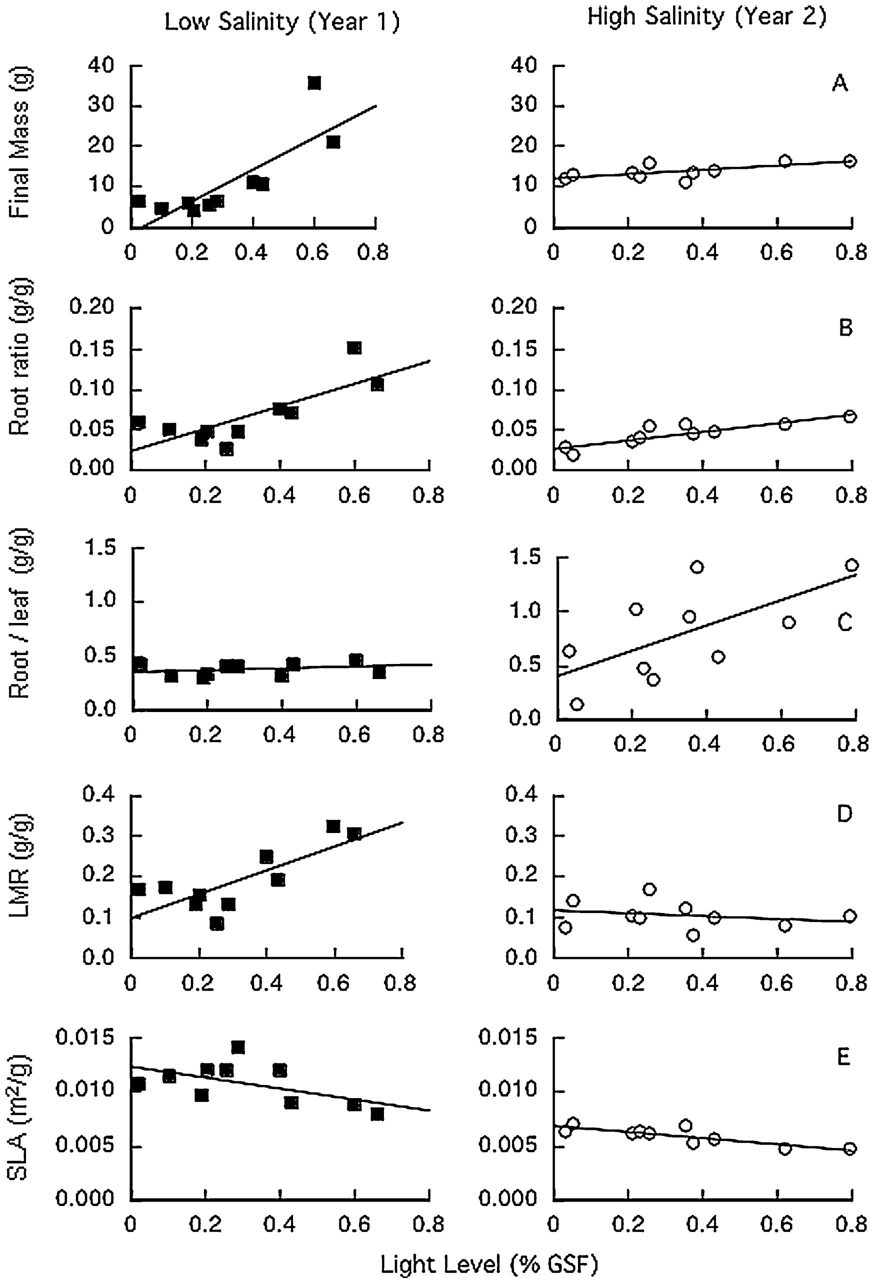

FIGURE 5. Regressions at low and high salinity, years one and two, respectively, for the relationships between light availability (% GSF) and field experiment seedling growth parameters. Graphs are paired horizontally, and the letters refer to paired graphs.

An additional reason for lower net photosynthesis at high salinity is that rates of dark respiration relative to assimilation were higher. Assimilation and dark respiration are generally positively correlated (Hirose & Werger 1987). R_{dark} averages about 7 percent of light-saturated photosynthesis in most plants (Givnish 1988, Anten & Hirose 2001). Here the R_{dark}/A_{sat} ratios were in this range at low salinity, but at high salinity, they increased to 28 and 19.5 percent at 6 and 25 percent PAR, respectively. This suggests that there are considerable additional respiratory costs at high levels of salinity, as we also observed in *A. germinans* (Hoffman 2003).

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 salinity than at high salinity. Many studies report lower seedling mass and growth rates at higher salinity levels, above 50 percent SW (*e.g.*, Downton 1982, Clough 1984, Ball & Pidsley 1995). Ball (2002) demonstrates that salinity and light interactively affect mangrove seedling mass, although due to low sample size the results are not significant in the field data. Using a robust statistical design, our study provides similar results supporting the conclusion

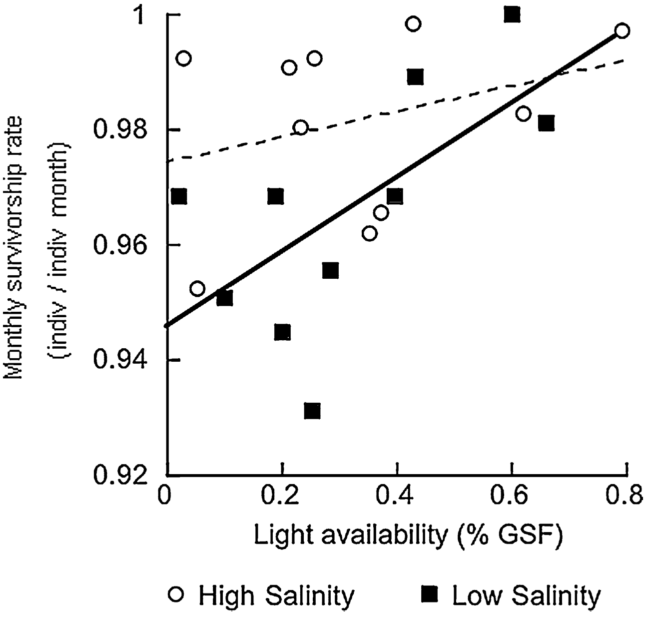

FIGURE 6. Monthly survivorship rates of seedlings in the field experiment at the low- and high-salinity zones. The solid line is the relationship at low salinity, and the dotted line the relationship at high salinity. Both the main effects of salinity and GSF, and their interactive effects were significant (all $P < 0.01$).

that salinity and light interactively affect seedlings, extending the pattern observed in Australia by Ball (2002) to a Neotropical mangrove, and including a consideration of gas exchange.

At high salinity, the *R. mangle* seedlings allocated more mass to roots than to leaves. Ball (1988) also reports increased allocation to root mass at high salinity. Allocation to roots at the expense of leaves is associated with higher requirements for water and nutrients (Van den Boogaard *et al.* 1996). A recent study indicates that nitrogen uptake in mangrove seedlings is not inhibited by high salinity (Kao *et al.* 2001), but an increase in nitrogen availability increases photosynthetic rates and leaf biomass. This finding is supported by Lovelock and Feller (2003), who reported that nitrogen fertilization increased photosynthetic rates in hypersaline mangrove forests. It has been long recognized that high-salinity levels create water deficits for mangroves (Scholander 1968, Clough 1984). It may be that increased water and/or nutrient requirements at high salinity prevented the seedlings from increasing growth rates and size in response to increased light availability—explaining the observed interactive effects of light and salinity. This is consistent with our leaf-level gas exchange results that indicate that water limitations and increased respiratory costs prevent seedlings from responding to increased light by increasing net photosynthesis.

In the field experiment, the monthly rate of seedling survivorship increased more with increase in light availability at low salinity than at high salinity. However, at low light levels, survivorship rates were higher at high salinity than low salinity. In contrast, growth rates and whole-plant mass are higher at low salinity than at high salinity. We speculate that there may be a physiological trade-off between growth and survivorship; it may be that at high-salinity, seedlings invest more energy and resources in survivorship at the expense of growth, whereas, at low-salinity, seedlings invest more in growth than in survivorship. Ball (2002), in a study of Australian Rhizophoraceae species, found similar results; seedling survival increased with irradiance, and there were no salinity effects on survival at high light. Ball (2002) proposes two explanations: that there is more herbivory under closed canopies (*e.g.*, Osborne & Smith 1990) and that mortality in the shade could be due to below-ground competition with surrounding adults (*e.g.*, Passioura *et al.* 1992).

IMPLICATIONS FOR FOREST STRUCTURE AND DYNAMICS.—At our field sites in the Río Limón mangrove system of Lake Maracaibo in western Venezuela, *R. mangle* and *A. germinans* are the most common species (Narváez 1998). In a comparison of the results of this study with other work on *A. germinans*, it appears that of the two species, *R. mangle* is the less salt tolerant, exhibiting optimal performance at lower salinity levels than *A. germinans*. At intermediate salinity levels (between 20 and 70 percent SW), *A. germinans* seedlings increase growth in response to increased light, while *R. mangle* seedlings do not (Hoffman 2003). Other studies also report that mangroves in the Rhizophoraceae have optimal performance at salinity levels below 30 percent SW (Clough 1984, Krauss & Allen 2003, Parida *et al.* 2004).

Our experimental observations are consistent with the natural species distribution along tidal gradients in our field sites; adults, juveniles, and seedlings of both species are present in low-salinity zones, although *R. mangle* is more abundant. In hypersaline areas, where the forest canopy is more open and light levels are higher, *A. germinans* of all sizes are present, but only *R. mangle* seedlings can be found (Narváez 1998, Hoffman 2003). This suggests that while *R. mangle* propagules can disperse into the area and establish and survive as seedlings, their growth rates are not high enough to allow them to transition to juveniles and adults.

The species differences we detected are generally consistent with other observations of Neotropical mangroves in Mexico, Florida, and Venezuela (Lopez-Portillo & Ezcurra 1989, Lin & Sternberg 1992a,b, Sobrado 1999a,b, Sobrado 2000). Furthermore, our results are in accordance with a study of mangrove species distributions over a range of salinity and drought conditions in Venezuela, which reports that *A. germinans*, not *R. mangle*, is most common in high salinity, open sites that experience drought stress during the dry season (Medina & Francisco 1997).

Mangrove forests are very complex ecosystems. In addition to light and salinity gradients, mangrove forests are structured by numerous abiotic and biotic factors, such as tidal gradients, which cause gradients in nutrient availability and waterlogging, drought stress, herbivory, and patterns of propagule dispersal (Rabinowitz 1978, Smith 1992, Medina & Francisco 1997, Feller *et al.* 1999, Lovelock *et al.* 2004). A comprehensive understanding of mangrove forest dynamics should consider all of these factors and their interactive effects (see Smith 1992, Clarke & Allaway 1993, Ellison & Farnsworth 1996, Chen & Twilley 1998, Ball 2002, Krauss & Allen 2003). For example, in the second year field experiment, shortly after transplanting, caterpillars consumed all the low salinity *R. mangle* seedlings, but the high-salinity seedlings were not affected, illustrating the complex, interactive nature of these factors (see Sousa *et al.* 2003). 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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