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JOBOS BAY MANGROVES REVISITED: GAS EXCHANGE, SALINITY, AND NUTRIENT RELATIONS

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ABSTRACT

We report results of an ecophysiological characterization of three mangrove species (*Avicennia germinans, Laguncularia racemosa,* and *Rhizophora mangle*) and a mangrove associated species (*Thespesia populnea*) co-occurring at the Jobos Bay National Estuarine Research Reserve in Aguirre, Puerto Rico. The study was conducted in the summer of 2009 and included environmental measurements during a two-week period, sampling for leaf dimensional analysis, and determinations of leaf sap osmolality, gas exchange, and elemental composition (C, N, S, P, Na, K, Al, Ca, Mg, Mn, and Fe). Adult leaves of *Thespesia* were the largest (35 cm^2) and lightest (0.23 g), and *Rhizophora* had the heaviest (0.61 g) and second largest leaves (31 cm^2). The smallest leaf areas were recorded for *Laguncularia* (18 cm^2) and *Avicennia* (15 cm^2). The leaf area/weight ratio was high in *Thespesia* (149 cm^2/g), whereas mangrove species ranged from 52 to 60 cm^2/g. Mangrove leaves were rather succulent. The largest average values were measured in *L. racemosa* (42 mg/cm^2), while values of *Thespesia* were about one half of those of mangrove leaves. *Avicennia* leaves had the highest concentrations of N, S, P, K, Mg, and Mn, whereas *Laguncularia* showed the highest concentrations of Al, Ca, and percent of Ash. Concentrations of N and P, and those of Ca and Al, were positively correlated for all species. Leaf sap osmolality (mmol/kg) followed the sequence *Avicennia* (3140), *Laguncularia* (1769), *Rhizophora* (1696), *Thespesia* (1273). *Rhizophora* had consistently higher photosythetic rates and stomatal conductances but *Laguncularia* had the highest values of water use efficiency. Comparison with previous ecophysiological analyses in the same area, indicate more stressful conditions during the present study, as evidenced by the lower values of photosynthesis and leaf conductances.

Keywords: elemental composition, mangroves, osmolality, photosynthesis, salinity.

INTRODUCTION

The mangrove forests at Jobos Bay in Puerto Rico constitute a preferred area for ecological studies as they are legally protected as a national estuarine research reserve (Field 2002). The ecophysiological characterization of the mangrove species in the reserve was conducted during the late 90s in a study that analyzed the performance of three mangrove species along gradients of interstitial soil salinity including leaf gas exchange and leaf properties (nutrients and osmolality), and the analysis of nutrient cycling within fringe mangrove communities (Lugo et al. 2007). The present study reanalyzed the salinity and nutrient relations, in conjunction with an assessment of the relationships between leaf properties and photosynthesis of mangrove tree species. The study was carried out within the framework of a training course for high school students (see Acknowledgments).

The objective was to identify differences between mangrove species based on leaf morphology, leaf sap osmolality, and nutrient concentration, together with measurements of gas exchange. Also, we studied a transect including mangrove species and a presumably non-halophytic tree growing on soils at the interface between the back of mangrove communities and the beach, not flooded by seawater but exposed to salt spray. This study included leaf dimensions, osmolality and ionic composition of leaf sap, element concentration, and photosynthetic capacity. The results will be contrasted with a previous study conducted during a strong dry season several years before (Lugo et al. 2007).

MATERIALS AND METHODS

Measurements were carried out in the area of Camino de los Indios within the Jobos Bay National Estuarine Research Reserve (JOBANERR, Aguirre, Puerto Rico) (Fig. 1) during the 2nd and 3rd weeks of July 2009. Trees selected for analysis grew as part of the fringe mangrove vegetation dominated by *Rhizophora mangle* L. with *Laguncularia racemosa* (L.) Gaertn. f. as secondary species (A site in Fig. 1), and trees of *Avicennia germinan*s (L.) L. bordering the interior lagoon (B site in Fig. 1). For the sake of comparison with a presumably non-halophytic tree, specimens of *Thespesia populnea* (L.) Sol. ex Correa were also sampled during the 3rd week. These trees were found on sandy soils, beyond the reach of tides, behind the mangrove fringe.

From now on the species will be designated only by the genus name. The trees selected were growing approximately within the same area studied in 1998 by Lugo et al. (2007).

Environmental Measurements

Climate parameters (rainfall, temperature, relative humidity, and photosyntetically active radiation) were recorded at 15 minutes intervals by the automatic meteorological station of JOBANERR.Temperature and humidity, and air CO_2 concentrations were also measured outside and below the canopy of a mangrove fringe using a Vaisala MI70 recorder with Humidicap and Carbocap sensors.

Interstitial and surface water was sampled to measure salinity *in situ* (refractometer), osmolality in the JOBANERR laboratory (Wescor osmometer), and ion concentrations at the International Institute of Tropical Forestry laboratory in Río Piedras, using Inductively Coupled Plasma spectrometry (ICP).

Piezometers were located about 30 cm depth within areas rooted by *Rhizophora* and *Avicennia* to determine potential changes in salt concentration of interstitial water. Samples were also taken from piezometers located in an area devoid of mangrove roots at the bay near the coastline.

Leaf Properties

Healthy leaves of different age, differentiated by their position in the shoot as young, adult, and old, were collected into plastic bags, and stored in dark coolers for transportation to the laboratory, where leaf area and fresh mass were measured upon arrival. The most practical procedure to determine leaf area is the use of an optical planimeter, if it is not available leaf area can be easily determined drawing the leaf on paper and then cutting and weighing the shape.

Paper shapes of known area are used to calibrate the weight-area relationship. If it is necessary to measure leaf area in the field without severing the leaf, the use of allometric relationships is appropriate. During the field course 50 adult leaves per mangrove species were collected, and their maximum dimensions of length and width were measured. The product length x width was regressed against leaf area measured using the drawing technique.

Leaves were ovendried at 65º C for 24 hours, and weighted again (dry mass). With these values we calculated the specific leaf area (SLA) (leaf area/leaf dry mass), the leaf water content (fresh mass – dry mass) and succulence (leaf water content/leaf area).

Leaf sub-samples were stored in plastic syringes and frozen in dry ice. After 24 hours syringes were thawed in the lab counter and squeezed with a hand press to extract leaf sap, on which osmolality and ionic composition were measured using the methods mentioned above.

Samples of adult leaves were dried in a ventilated oven at 65º C, grinded, and ashed at 490º C. Ashes were dissolved in HCl 1N. Concentrations of Na, Mg, Al, P, S, K, Ca, Mn, and Fe were measured by ICP. The S concentration measured corresponds only to the inorganic S fraction, as organic S is certainly lost during ashing as SO_2. Carbon and N were measured with a Leco elemental analyzer. All concentrations except of % Ash are expressed in mmol/kg dry mass for direct interelemental comparisons.

Gas exchange (CO_2 and H_2O) was measured with an infrared gas analyzer (LCPro, ADC). Measurements were conducted from early morning to early afternoon (ca. 8 am to 1 pm). The gas analyzer records differential of CO_2 and water vapor concentrations (vpm) between the air surrounding the leaf and the air passing through the leaf chamber at constant airflow rates. In addition, intensity of incoming radiation (μmol $m^{-2}s^{-1}$) and leaf temperature (ºC) are recorded simultaneosly. Rates of following processes are calculated with these parameters:

1. Rates of net of CO_2 uptake (photosynthesis A) or production (respiration R) (μmol $m^{-2}s^{-1}$)
2. Stomatal conductance (gs) (mmol $m^{-2}s^{-1}$) and rate of transpiration (E) (mmol $m^{-2}s^{-1}$)
3. Water use efficiency calculated either as the quotient between net photosynthesis and transpiration (A/E), or as the quotient A/gs (intrinsic water use efficiency) (μmol/mmol).

Statistical Analyses

Data were submitted to analyses of variance and regression using the JMP 8.0 statistical package (SAS).

Figure 1. Study site: Camino de los Indios, Jobos Bay, Guayama, PR. The blue line indicates the location of the wooden path within the fringe mangrove. Imagery date: 11/1/2006. Lat 17.935437º long -66.253300º elev 0 m eye alt 298 m *Main camp; A Fringe site (*R. mangle* + *L. racemosa*; B Basin site (*A. germinans*); C Bank site (*Thespesia populnea*)

RESULTS

Environmental Conditions

The climate parameters recorded during the month of July indicated rainfall only at the beginning (about 9 mm during the first 2 days of the month). Temperature ranged from a minimum average of 25 to 27º C (except in days 15th and 17th when minimum temperatures were 23º C) and maxima surpassing 30º C except in days 16 and 27. Relative humidity followed the expected opposite pattern with maxima between 70 and 90 percent in the night and minima around 54 percent, except day 21st (minimum at 40 percent). The pattern of the sum of PAR during the measuring period indicates that most days received a little more than 30 moles quanta m^{-2} day^{-1}. During cloudy days (20nd and 21st day) PAR decreased dramatically.

During sunny days maximum temperatures outside the vegetation approached 35º C and minimum temperatures were between 25 and 27º C. In the understory of a forest plot dominated by *Rhizophora,* maximum temperatures were nearly 2º C lower, but minimum temperatures were less than a 1º C lower. The relative humidity followed an opposite pattern, maximum values being recorded near the end of the night varying in three nights between 77 and 84 percent. Minimum humidities recorded about 2 hours after noon, were lower outside and varied between 56 and 50 percent.

The Vaisala CO_2 probe was set up only in the understory of the *Rhizophora* plot to evaluate levels and variability of CO_2 concentrations. Carbon dioxide concentrations in the understory measured at 1-minute intervals varied strongly throughout the measuring period. The 4-minute moving average showed minimum values of 380 ppm measured near the end of the light period, and 480 ppm measured at the end of the night period.

Leaf Characteristics

The regression lines for the product length x width were quite similar and provided a good estimation of leaf area (Fig. 2). The three species followed similar linear relationships indicating a common leaf shape. However, the slope of the regression lines increased slightly but significantly from *Rhizophora* to *Avicennia* and *Laguncularia*. In this data set, average leaf area (cm^2) was greater for *Rhizophora* (38.8 ± 6.7), and smaller for *Laguncularia* (17.6 ± 4.3) and *Avicennia* (15.5 ± 3.7).

Leaf Dry Mass and Area

The leaves of *Rhizophora* and *Avicennia* followed broadly the same weight-area relationship (Fig. 3). Leaves of *Laguncularia* showed a tendency to develop heavier leaves per unit area, whereas *Thespesia* showed the opposite tendency (Fig. 3). Discrimination of leaf groups by age and species revealed that *Rhizophora*, *Avicennia,* and *Thespesia* increased both dry mass and area from the young to the adult stage remaining similar at the old stage, whereas in *Laguncularia* both parameters continued to increase into the group of old leaves (Fig. 4A, B).

Leaf succulence remained similar for all age groups in *Avicennia* and *Thespesia*, increased slightly with age in *Rhizophora*, whereas in the case of *Laguncularia* succulence increased significantly from young to adult and old leaves (Fig. 4C). Leaf area/mass ratios of the mangrove species decreased slightly, whereas *Thespesia* increased slightly, as the leaves age (Fig. 4D).

Composition and Osmolality of Interstitial Water and Leaf Sap

Measurements of interstitial water salinity during two consecutive weeks showed that there was a small but consistent tendency for interstitial water obtained from within red mangrove roots to be more concentrated (higher values of salinity, conductivity, and osmolality) than the water samples obtained from root-free sites (Table 1). Average interstitial water from sites occupied by *Avicennia* was much more concentrated than the average of *Rhizophora* sites. A comparable site free of *Avicennia* roots was not available. In the *Avicennia* site, salinity values were lower during the 2nd week, whereas those of the *Rhizophora* site increased slightly.

The osmolality of leaf sap from the species studied confirmed that *Avicennia* is usually the species with higher leaf sap osmolality followed by *Laguncularia* and *Rhizophora* (Fig. 5). *Thespesia* showed the lowest osmolality values.

FIGURE 2. Regression of the product of length x width of mangrove leaves vs actual leaf area. *Rhizophora mangle* (red)(n=49) A cm^2 = 4.42793 + 0.62559 * (L x A); R^2 Adj: 0.94 L*aguncularia racemosa* (green)(n=50) A cm^2 = -0.73106 + 0.79375 * (L x A); R^2 Adj: 0.92 *Avicennia germinans* (black)(n=50) A cm^2 = 0.76645 + 0.66201 * (L x A); R^2 Adj: 0.93

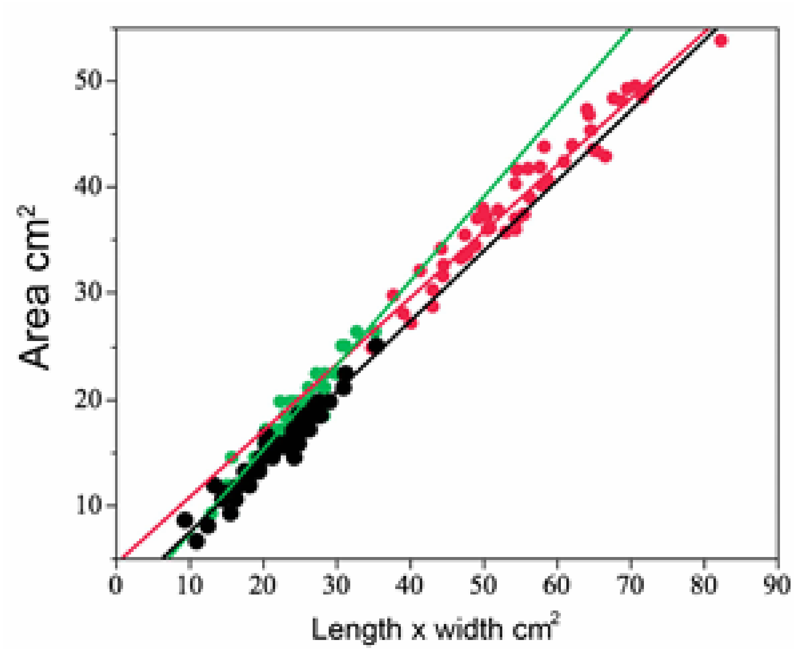


FIGURE 3. Area/mass relationship of different species from tree species at Jobos Bay. The regression equations are: Area (A cm^2) and mass (W g)
Thespesia (blue) A= 5.615 +111.890 W; R^2adj=0.87; F=927, P>F <0.0001; n= 146
Avicennia (black) A= 3.235 +43.358 W; R^2adj= 0.75; F=893, P>F < 0.0001; n=299
Rhizophora (red) A= 4.857 +41.599 W; R^2adj= 0.89; F= 2436, P>F <0.0001; n=297
Laguncularia (green) A= 7.146 +24.772 W; R^2adj= 0.68; F= 321, P>F <0.0001; n=149

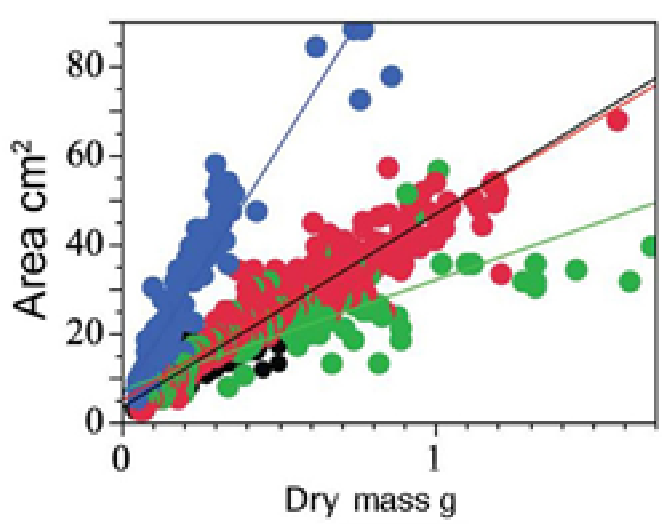


Figure 4. Comparison of means of leaf dry weight (A), area (B), succulence (C) and area/weight ratio (D) using analysis of variance, and an a posteriori test (Tukey-Cramer Honest Significant Difference). Black dots: *Avicennia*; Green dots: *Laguncularia*; Red dots: *Rhizophora*. Y: young leaves; A: adult leaves; O: old leaves. The green diamonds depict the result of the analysis of variance. Overlapping diamonds indicate that the means are statistically identical at P≤ 0.01.

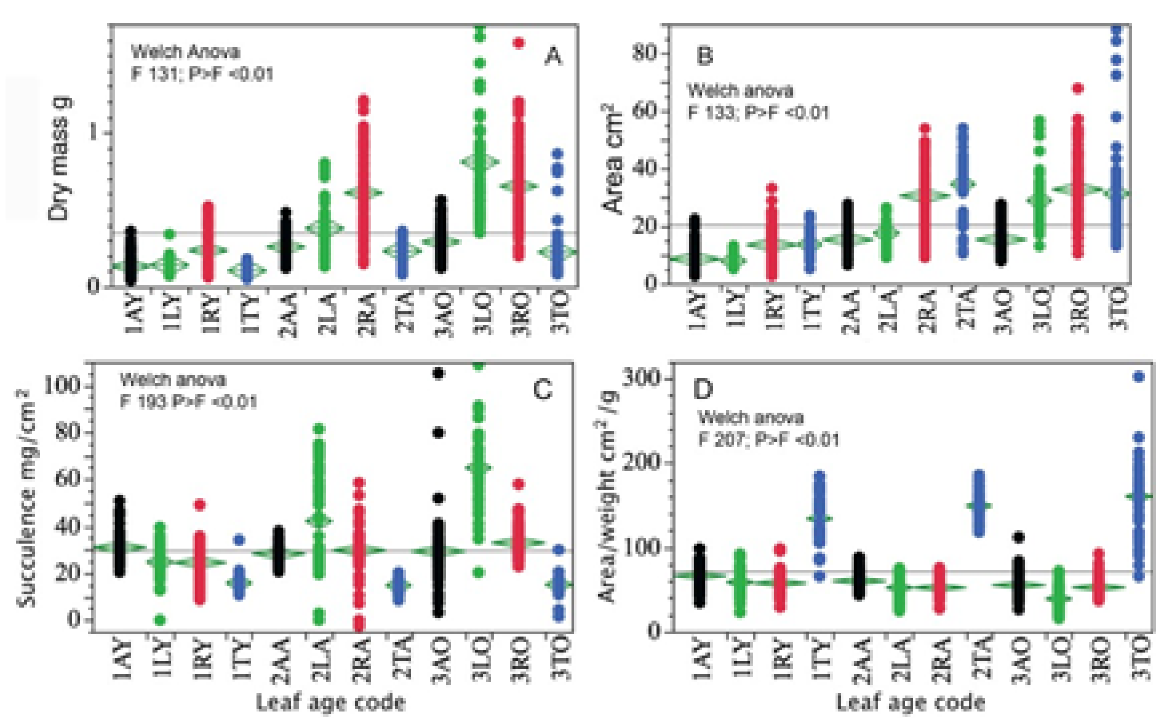


Table 1. Salinity of interstitial water collected from piezometers at 30 cm depth (mean ± standard deviation)

Site and time measurement	n	Salinity %	Conductivity µS	Osmolality mmo/kg
July 13 (11:00; 11:30; 14:00)				
Rhizophora site				
Outside roots	3	39 (2)	58 (0)	1099 (3)
Inside roots	3	43 (1)	60 (3)	1147 (15)
Avicennia site	3	92 (1)	120 (1)	2831 (38)
July 20 (11:00; 11:30; 14:00)				
Rhizophora site				
Outside roots	3	42 (1)	59 (0)	1274 (245)
Inside roots	3	55 (1)	75 (0)	1499 (4)
Avicennia site	3	59 (2)	80 (2)	1632 (48)

Analysis of the elemental composition of leaf sap from adult leaves of the mangrove species revealed interspecific differences in the nutritional requirements (Table 2). Compared to standard sea water (DOE 1997) the element concentration in leaf sap gave following ratios: K was 11, 13, and 7 times higher in *Avicennia*, *Laguncularia,* and *Rhizophora*, respectively; Ca was slightly lower in *Avicennia*, 7 times higher in *Laguncularia* and nearly 5 times higher in *Rhizophora*; Mg was 3, 2, and 1.5 times higher in *Avicennia, Laguncularia,* and *Rhizophora* respectively; Na was twice as high in *Avicennia*, 0.8 times in *Laguncularia* and *Rhizophora;* S was 4, 2, and 3 times higher in *Avicennia, Laguncularia,* and *Rhizophora* respectively.

Nutrient Concentration of Adult Leaves

Avicennia leaves stand out for their higher concentrations of N, S, and P. *Laguncularia* and *Rhizophora* leaves showed similar values of Na and S, but lower C concentration than the other two species (Table 3). *Avicennia* stands out again due to the significantly higher concentrations of K, Mg, and Mn, whereas *Laguncularia* showed the largest values of Al, Ca, and of %Ash (Table 3). As expected, the %Ash was linearly correlated with the ∑cations (%Ash= 1.66038 + 0.00429 * ∑cations; R^2= 0.95), and Na was the main ion responsible of this high correlation (%Ash= 5.28766 + 0.00473 * Na; R^2= 0.72). The usual high level of correlation between Ca and %Ash did not apply due to the reduced Ca uptake by *Avicennia.* The Ca concentrations in all species appeared to be correlated with the concentration of Al. The two extremes of the correlation are *Avicennia*, with low Ca and Al concentrations, and *Laguncularia*, with high Ca and Al concentrations.

Thespesia differed strongly from the mangrove species for its high concentrations of N and P (Table 4). In addition, *Thespesia* showed significantly lower Na and higher K concentrations within the group of species studied. These results point to the non-halophytic character of *Thespesia.*

Gas Exchange and Water Use Efficiency

We found a linear relationship between leaf conductance and net assimilation, and between transpiration and net assimilation for all the mangrove species combined (Fig. 6). The averages of photosynthesis, transpiration, and leaf conductance of adult leaves at light intensities above 900 µmol $m^{-2}s^{-1}$ were higher for *Rhizophora* compared to the leaves of the other mangrove species (Table 5). These rates were attained at leaf temperatures around 38ºC. Calculated water use efficiency (A/E) was also higher in *Rhizophora*, but intrinsic water use efficiency (A/gs) was similar for *Rhizophora* and *Laguncularia* (Fig. 7). Both ratios were lower for *Avicennia*, the species that generally occupies the most saline, and at times, drier locations in mangroves along semiarid coastlines.

DISCUSSION

Environmental conditions experienced during the measuring period exemplify the high radiation and temperature stress to which mangroves in the Caribbean are submitted during summer months. These stress factors compound the effect of interstitial water salinity derived from seawater moved diurnally by high tides. Mechanisms to tolerate this stressful environmental conditions are well known. They include adaptations of the photochemical apparatus in mangrove leaves for avoiding photoinhibition through several pathways of photochemical and non-photochemical quenching (Naidoo et al. 2002, Thomas et al. 2009); regulation of leaf temperature and evapotranspiration by high levels of leaf inclination reducing

Figure 5. Osmometry of leaf sap obtained from adult leaves. The green diamonds correspond to the visual representation of the one-way analysis of variance performed by JMP. Black dots: *Avicennia*; Green dots: *Laguncularia*; Red dots: *Rhizophora*. Y: young leaves; A: adult leaves; O: old leaves. Significance of the green diamonds as in Fig 3.

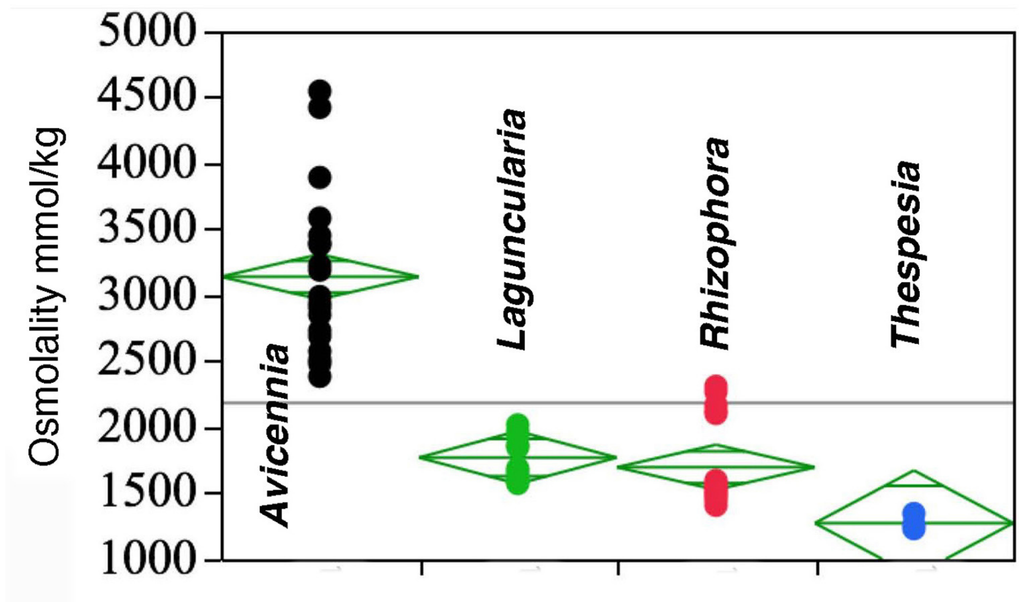


Table 2. Ion concentration (mmol/kg)(standard deviation) in insterstitial water and leaf sap extracted from adult leaves.

	n	Ca	K	Mg	Na	P	S
Standard seawater	-	10	10	53	486	-	29
Interstitial water no roots	2	16	5	41	500	0.0	38
Interstitial water with *Rhizophora* roots	2	12	4	41	509	0.0	37
Interstitial water w. *Avicennia* roots	2	45	107	107	993	0.0	95
Leaf sap Mean (standard deviation)							
Avicennia germinans	8	7 (4)	111 (28)	144 (32)	1022 (219)	4.8 (1)	124 (18)
Laguncularia racemosa	8	70 (28)	128 (15)	83 (7)	395 (28)	5.1 (2.3)	45 (8)
Rhizophora mangle	8	47 (19)	70 (14)	117 (36)	408 (193)	2.9 (0.9)	84 (47)

Table 3. Average elemental composition of adults leaves of mangroves at Jobos Bay (July 2009). Means followed by the same letter are not statistically different (Tukey-Kramer HSD test, P=0.05). N= 10

Non-metallic elements	C mol/kg	N	S	P
		--------------------mmol/kg--------------------		
Avicennia	38.5a	1040a	268a	45a
Laguncularia	32.6b	588b	173b	32b
Rhizophora	39.2a	659b	158b	37ab

Metallic elements	Na	K	Mg	Al	Ca	Mn	Fe	∑cation	%Ash
	--mmol/kg--								
Avicennia	2217a	503a	784a	3.6b	257c	3.5a	1.1a	3267a	15.4b
Laguncularia	2227a	311b	500b	13.8a	1117a	0.8c	0.8ab	3920a	19.3a
Rhizophora	1147b	322b	400b	4.8b	535b	2.5b	0.7c	2091b	11.0c

Table 4. Average element concentrations (mean ± standard deviation) in adult leaves of *Thespesia populnea* compared to the mangrove species growing nearby.

Species	n	C mol/kg	N	P
			-------mmol/kg-------	
Avicennia	9	37.2 (1.7)	1273 (87)	55.9 (4.5)
Laguncularia	4	32.0 (1.0)	544 (67)	29.2 (2.1)
Rhizophora	9	38.9 (2.8)	811 (78)	38.7 (3.9)
Thespesia	5	36.0 (0.8)	2191 (100)	98.2 (6.8)

Species	n	Na	K	Mg	Ca
		--------------------------mmol/kg --------------------------			
Avicennia	9	2073 (326)	759 (181)	684 (65)	187 (42)
Laguncularia	4	2427 (136)	262 (38)	657 (41)	1671 (132)
Rhizophora	9	1281 (600)	256 (97)	357 (135)	405 (147)
Thespesia	5	319 (33)	1263 (110)	358 (23)	1243 (57)

Figure 6. Relationship between leaf conductance (gs), transpiration (E), and CO_2 assimilation (A) recorded in leaves of mangroves in Jobos Bay, July 2009. Black dots: *Avicennia*; Green dots: *Laguncularia*; Red dots: *Rhizophora*. Y: young leaves; A: adult leaves; O: old leaves.
Significance of the green diamonds as in Fig 3. Overall regressions: A (mmol/m^2.s) = 1.2012 + 68.859 gs (mol/m^2.s); R^2adj= 0.871
A (mmol/m^2.s)= 0.882 + 3.130 E (mmol/m^2.s)

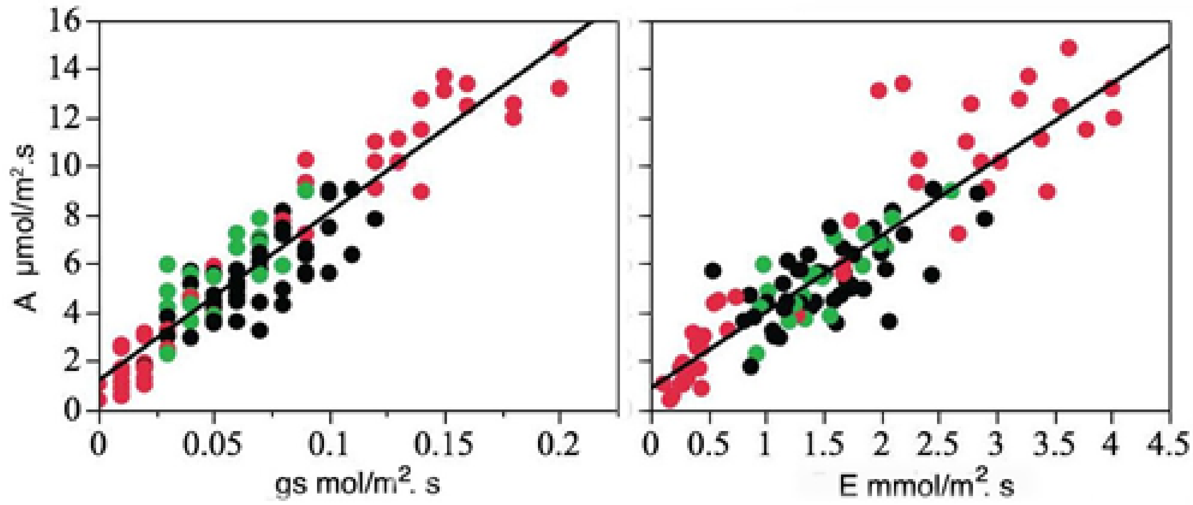


Table 5. Gas exchange parameters (95 percent confidence limits) of adult mangrove leaves exposed to light intensities ≥ 900 µmol m^{-2} s^{-1} measured in July 2009 in Jobos Bay, Aguirre, Puerto Rico.

	Rhizophora	***Laguncularia***	***Avicennia***
A (µmol m^{-2} s^{-1})	8.4 (6.7-10.1)	5.5 (4.8-6.3)	5.5 (4.7-6.3)
g_s (mmol m^{-2} s^{-1})	99 (74-119)	53 (44-61)	63 (53-73)
Intrinsic water use efficiency (µmol/mmol)	103	110	89
Q leaf (µmol m^{-2} s^{-1})	1242 (1163-1320)	1177 (1110-1243)	1253 (1181-1325)
T leaf (°C)	38.2 (37.6-38.8)	38.6 (38.1-39.2)	38.3 (38.0-38.7)

the total energy absorbed by the leaf (Ball et al. 1988); osmoregulation through the accumulation of ions in the vacuole that counteract osmotic effect of interstitial water salinity (Popp 1984, Medina and Francisco 1997, Paramita et al 2007); and organic molecules presumed to be accumulated in the cytoplasm (compatible solutes) (Popp et al. 1984; Medina et al. 1990).

The nutritional aspects associated to mangrove adaptations to salinity stress are also comparatively well known. It is generally accepted that mangrove communities, particularly mangrove fringes dominated by *Rhizophora mangle,* are frequently regulated by the availability of P, causing stunted growth and dwarfism (Feller 1995, Cheeseman and Lovelock 2004, Medina et al. 2010). However, N can also be limiting particularly in areas where water runoff and sediment supply are limited (Boto and Wellington 1983, McKee et al. 2002).

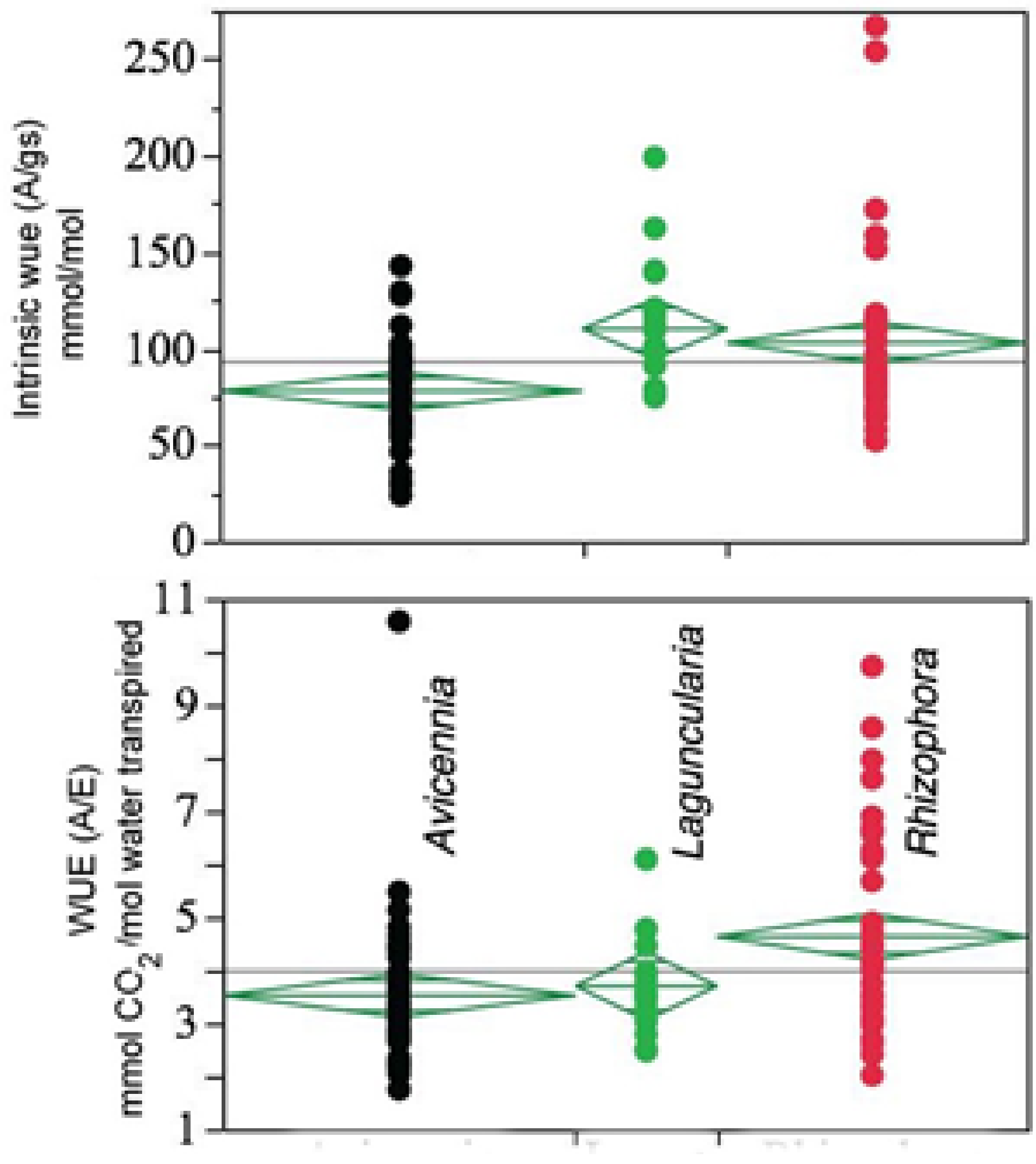
The elemental composition of mangroves has been investigated mostly with regards to the osmotic adaptations, but comparatively little is known about specific requirements of metallic elements and sulfur. The documented differences in elemental composition among mangrove species, does not have a comprehensive physiological explanation (Medina et al. 1990, 2007, Lugo et al. 2007). The recent review of Reef et al. (2010) deals mainly with N and P nutrition but the ecophysiological role of S is not mentioned. In fact, a thorough documentation of the ionome profile of mangrove species in the sense of Salt et al. (2008) is missing in the analysis of the ecophysiological relationships of these species.

Mangroves develop roots in saline soils. In the process of water and nutrient absorption from interstitial water ions such as Na and Cl are preferentially excluded resulting in increased salt concentration in the root environment (Passioura et al. 1992). As far as we know this statement has not been tested under field conditions. The process is probably more pronounced in species such as *R. mangle*, described as a salt excluder, than in *A. germinans*, a salt secreting species (Scholander 1968). Our results indicated that interstitial waters within *Rhizophora* roots had higher concentrations of Ca, Na, and S, but lower concentrations of K and Mg. Water within *Avicennia* roots showed a distinctive pattern with higher concentrations of Ca (4 times), K, Mg, and Na (≈ 2 times) than standard sea water. This pattern may be explained by evaporation in the *Avicennia* site that is not compensated by wave movement and tides as in the *Rhizophora* site.

The substantially higher concentration of Ca may also be related to the rejection of this ion by *Avicennia* roots. Results presented here must be considered preliminary, but support the assumption of accumulation of salt in the water surrounding the root system of *Rhizophora* trees. Detailed and extensive measurements of this type that account for the effect of tides in counteracting the concentration process may help improve our understanding of salt regulation in mangrove communities.

Laguncularia had the heaviest leaves per unit area among the mangrove species, whereas dry coastal forest species *Thespesia* had comparatively the lightest leaves per unit area. Adult *Rhizophora* leaves were always larger and heavier than leaves of the other species studied, in agreement with several other studies (Medina et al. 2007, Medina and Francisco 1999, Medeiros and Sampaio 2013). A gram of dry weight invested in leaves corresponds to 32 cm^2 in *Laguncularia*, about 46 cm^2 in *Avicennia* and *Rhizophora,* and 118 cm^2

Figure 7. Comparison of indices of water use-efficiency (wue): Intrinsic water use-efficiency= A/gs in mmol/mol; Water use-efficiency= A/E in mmol CO_2/ mol water transpired.



in *Thespesia*. These differences imply that investment of photosynthate and nutrients for building photosynthetic area may be relevant in determining the efficiency of organic matter production of these coastal communities.

Regarding succulence, the species studied were similar only at the young stage. At the adult and old stages *Laguncularia* leaves always had the highest, and *Thespesia* the lowest, water content per unit area. *Avicennia* and *Rhizophora* had similar succulence values at all leaf stages and, including *Thespesia,* this index did not increase from adult to old leaves. The implied mechanism of diluting excess salt in leaf tissues through parallel increased uptake of water may have implications for leaf demography in *Laguncularia.* A recent study showed that leaf life span of *L. racemosa* was 2 months shorter than that of *R. mangle* and about 6 months shorter than that of *A. schaueriana* (Medeiros and Sampaio 2013).

The consistently higher leaf sap osmolality in *Avicennia* may be associated with the higher permeability for salt in the roots of this species, and also by its occurrence in saltier sites. *Avicennia* has leaf salt glands that actively secrete salt throughout its lifetime and salt secretion rates increase with interstitial water salinity (Suárez and Medina 2008). *Laguncularia* also has leaf salt secreting glands (Sobrado 2004), but osmolality of leaf sap is much lower than that of *Avicennia* and similar to that of *Rhizophora*, a salt excluding mangrove (Scholander 1968). This may be the result of an increase in the water content per unit area induced by growth in saline environments (Biebl and Kinzel 1964), leading to similar leaf sap concentrations as in the salt excluding *Rhizophora*. The concentration of Na in leaf sap showed a pattern similar to osmolality among species, around 1000 mmol/kg in *Avicennia*, and 400 in *Laguncularia* and *Rhizophora*. But it is remarkable that *Laguncularia* and *Avicennia* have similar K concentrations, both significantly higher than those of *Rhizophora.* In addition, Ca is ten times more concentrated in *Laguncularia* compared to *Avicennia*, whereas S (as sulfate) is three times higher in *Avicennia* compared to *Laguncularia*. The physiological causes and implications of these ionic relationships are known only for the case of Ca. *Avicennia* is an "oxalate plant" sensu Kinzel (1989). The production of oxalic acid prevents the accumulation of soluble Ca in leaf sap.

Nutrient analyses of adult leaves essentially confirm results reported by Lugo et al. (2007). *Avicennia* showed the highest values for N, P, K, per dry mass and highest values of Na and lowest Ca values in leaf sap. The present paper reports also high concentrations of S and Mg compared to the other species. *Laguncularia* is the species with highest Ca concentration in contrast to the previous results. The Na/K ratios of the mangroves around the same range measured previously, varying between 7.2 in *Laguncularia* and 3.6 in *Rhizophora*. *Thespesia* trees on the other hand, stand out with much higher concentrations of N, P, K than those found in the mangrove species, and the Na/K ratio was only 0.25, a clear indication of the non-halophytic character of this species, and that it was not submitted to salt stress.

The present data set includes concentrations of Al, Mn, and Fe in adult leaves of mangroves trees. The former was always correlated with leaf Ca content, a relationship that was somewhat unexpected because of the inmmobilization of Al in soils as a consequence of pH increases brought about by presence of Ca carbonate. This is one of the research questions that should be addressed using cultivated plants.

Photosynthetic rates and indices of water use efficiencies expand previous findings in JOBANERR mangroves (Lugo et al. 2007). Photosynthesis was operating at intensities above 900 μmol m^{-2} s^{-1} and temperatures above 35º C, however, leaf conductances were below the 100 mmol $m^{-2}s^{-1}$ indicating a certaindegree of stomata closure. Results reported in this paper correspond to the middle of the summer in Puerto Rico (July), and to a certain extent contrast with previous reports from the same site that were obtained in the middle of the winter (December) (Table 6). Present data indicates that plant were under water stress, their leaf sap osmolalities were well above, and leaf conductances and photosynthetic rates were considerably below those reported from the same site by Lugo et al. (2007). Meteorological data confirm that July 2009 was a dry month (no effective rainfall) with an average temperature of 27.9º C, whereas December 2006 was humid and relatively cool (average temperature 24.4º C).

Table 6. Comparison of leaf sap osmolality and photosynthetic parameters of mangrove species in the Jobos Bay National Estuarine Research Reserve site obtained during winter (Lugo et al. 2007) and summer seasons (present paper). Temperature and rainfall data are from the Aguirre Station, Salinas, Puerto Rico (http://weather-warehouse.com/).

	Rhizophora	***Laguncularia***	***Avicennia***
SUMMER			
Leaf sap osmolality mmol kg^{-1}	1696	1769	3140
A (µmol m^{-2} s^{-1})	8.4	5.5	5.5
g_s (mmol m^{-2} s^{-1})	99	53	63
Q leaf (µmol m^{-2} s^{-1})	1242	1177	1253
T leaf (°C)	38.2	38.6	38.3
WINTER			
Leaf sap osmolality mmol kg^{-1}	1305	988	1799
A (µmol m^{-2} s^{-1})	12.7	10.7	7.9
g_s (mmol m^{-2} s^{-1})	283	241	185
T leaf (°C)	29.0	28.0	29.5
	Av T °C	Rainfall mm	
July 2009	27.9	0	
December 1986	24.4	52.3	

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