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Salinity variation effects on photosynthetic responses of the mangrove species *Rhizophora mangle* L. growing in natural habitats

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Abstract

This study evaluated the photosynthetic efficiency in *Rhizophora mangle* plants in two mangrove forests, the highest salinity (HS) area, and the lower salinity (LS) area. The CO_2 assimilation rate (P_N), stomatal conductance, leaf transpiration, intrinsic water-use efficiency, and chlorophyll *a* fluorescence L-band, IP-phase, and performance index were higher in the LS area. The instantaneous water-use efficiency, initial fluorescence, maximum fluorescence, and J-step were higher in the HS area. The plants growing in the HS area exhibited greater efficiency in electron transfer between the oxygen-evolving complex and the acceptor side of the PSII. The plants growing in the LS area exhibited greater efficiency of the reduction of the final acceptors of the PSI, an important strategy to the stress conditions. The results show that a greater variation in salinity in the LS area had the effect on P_N and long-term changes in the rainfall regime may alter the vegetation community of mangrove forests.

Additional key words: energy flow; gas exchange; halophytes; JIP test; salt tolerance; tropical region.

Introduction

The mangrove ecosystem forms a unique community distributed along the coastal lines and tidal plains, growing in varied salinity conditions, from fresh water to salinity above that of seawater (Takemura *et al*. 2000, Sobrado 2005, Naidoo 2010, Chen and Ye 2014). According to Giri *et al*. (2010), all over the world, mangrove forests occupy 137,760 km^2, and are among the most productive ecosystems. One of the most important ecological characteristics of mangroves is their role in protecting the coast line from erosion caused by waves and storms (Alongi 2014, Barbier 2016). In addition, they are considered important components of detrital food chains, habitats, and feed vivarium for a great diversity of invertebrates and fish species (Takemura *et al*. 2000, Nagelkerken and van der Velde 2004, Duke *et al*. 2005).

The operation of mangroves is governed by environmental forces that exert a strong influence on carbon balance and in the growth form of mangrove species (Barr *et al*. 2009), with salinity being the main stressor and regulator of their development and productivity (Medina *et al*. 1990, Sobrado and Ball 1999). Zhu *et al*. (2012) reported that in the mangrove plant of the family Rhizophoraceae, *Bruguiera gymnorrhiza* (L.) Lam., the expression of the protein Rubisco was regulated by the salt concentration. Several field studies carried out under controlled conditions showed that, in general, the variation of salinity reduced the photosynthetic rate (P_N) in *Rhizophora mangle* L. (Hao *et al*. 2009, Bompy *et al*. 2014), *Rhizophora mucronata* Lam. (Hoppe-Speer *et al*. 2011), *B. gymnorrhiza* (Zhu *et al*. 2012), as well as in *Excoecaria agalocha* L. (Chen and Ye 2014), in parallel with the rate of leaf transpiration (E) (Chen and Ye 2014) and stomatal conductance (g_s) (Hao *et al*. 2009, Chen and Ye 2014). Experiments with different NaCl concentrations demonstrate that the maximum photochemical efficiency of PSII (F_v/F_m) is negatively affected by increased salinity in the mangrove species *Avicennia*

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Abbreviations: ABS/RC – absorption flow of the antenna chlorophyll by reaction center; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DI_0/RC – dissipation flow by RC; E – transpiration rate; F_m – maximal fluorescence yield of PSII; F_0 – minimal fluorescence yield of PSII; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; IP-phase – indicates the efficiency of electron transport around the PSI to reduce the final acceptors of the electron transport chain; J-step – expresses the efficiency with which a trapped exciton can move an electron into the electron transport chain from Q_A^- to the intersystem electron acceptors; K-band – relative variable fluorescence at 300 μs; L-band – relative variable fluorescence at 100 μs; OEC – oxygen-evolving complex; P_N – net photosynthetic rate; PI_{TOTAL} – total performance index; Q_{leaf} – luminous intensity incident on the leaf surface; RC – reaction center; T_{leaf} – leaf temperature; WUE_{ins} – instantaneous water-use efficiency (= P_N/E); WUE_{int} – intrinsic water-use efficiency (= P_N/g_s).
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officinalis L., *Heritiera fomes* Buch.-Ham., *Excoecaria agallocha* L. (Panda *et al*. 2006), *A. marina* (Naidoo *et al*. 2011), and *Rhizophora mucronata* Lam. (Hoppe-Speer *et al*. 2011). The activity of PSII is restricted by a variety of environmental stresses, *e.g*., high concentrations of NaCl inhibit the fixation of CO_2 with the resulting generation of reactive oxygen species (ROS) (Murata *et al.* 2007). In addition, changes in salinity may result in inhibition of photosynthesis (or photoinhibition), decline in CO_2 fixation, and cause cellular damage, since the increase in exposure to NaCl stress caused a decline in the expression levels of the antioxidant genes *Catl1* (catalase) and *Fer1* (ferritin), as reported by Jithesh *et al*. (2006) for the mangrove species *A. marina*. This in turn inhibits protein synthesis. However, for most mangrove species, growth is stimulated by moderate concentrations of salt (≈ 8.7 to 17.5 psu), whereas high concentrations (above 35 psu) or the absence of salt inhibits it (Ball 1988, Takemura *et al*. 2000, Parida *et al*. 2004).

The conditions of constant salinity tend to be physiologically less demanding than floating salt exposure, due to the high energy cost (Krauss *et al*. 2008) required by the mechanisms of osmotic and ionic adjustments. Soil salinity fluctuations occur due to tidal flooding throughout the day, precipitation throughout the year, and temperature leading to evaporation of soil surface water (Hutchings and Saenger 1987, Dassanayake *et al*. 2009, Lovelock *et al*. 2017). However, to cope with the variations of salinity, which vary from place to place, plants evolved a sophisticated signaling as well as metabolic response processes to salt, including the adjustment of photosynthesis and mechanisms of salt exclusion (Tuteja 2007), in addition to morphological and physiological mechanisms for water absorption under low soil hydric potential (Naidoo *et al*. 2002). These physiological adjustments reflect in greater plant development and water-use efficiency (WUE). In *R. mangle*, the higher WUE values improved salt tolerance, however, it also decreased its photosynthetic capacity, which can reflect on the ecophysiological performance under increased salinity (Lin and Sternberg 1992, Hao *et al*. 2009). Evaluating these processes elucidates the tolerance of mangrove species in response to salinity, considering that studies in different saline conditions have already indicated that these plants suffered alterations in their photosynthetic performances (Sobrado and Ball 1999, Lovelock and Feller 2003, Li *et al*. 2008, Naidoo *et al*. 2011, Dangremond *et al*. 2015).

Rhizophora mangle grows in eastern Pacific and Atlantic salt coastal areas (Duke 1992), although it develops well in freshwater (Hao *et al*. 2009). The species stands out in the Brazilian Coast Line (Schaeffer-Novelli *et al*. 1990) as well as along the coast line of the state of Espírito Santo (Brazil) (Silva *et al*. 2005, Petri *et al*. 2011) in areas under marine and freshwater influence. This species excludes salt by ultrafiltration, which occurs in the membranes of the root cells (Scholander 1968). Considering its ecological role in the ecosystem, it is necessary to study the physiological strategies presented by *R. mangle* to maintain growth and development in an extreme environment, characterized by daily variation in salinity from the daily tide fluctuation, high temperatures, luminosity, and precipitation in order to understand better the ecophysiology of mangroves. Such information about the species' specific responses to salinity would be useful to understand the factors influencing the dominance of mangroves of *R. mangle* along the coast of Espírito Santo.

This study discusses the following question: How does the variation in salinity change the photosynthetic processes of *R. mangle* growing in field conditions? To answer this question, we evaluated photosynthetic efficiency by the technique of analysis of chlorophyll (Chl) *a* fluorescence, gas exchanges, and Chl content in *Rhizophora mangle* plants growing in similar flood conditions and distinct regarding salinity in the São Mateus River estuary. This *in situ* study, involving electron transport and CO_2 assimilation processes in different salinity can allow a better understanding of the physiological mechanisms of the halophyte species under the perspectives of alterations in the rainfall regime in a tropical region.

Materials and methods

Study area: The study was carried out in Conceição da Barra (18°35'36"S; 39°43'56"W), the north region of the state of Espírito Santo, Brazil, in two fringe forests in the mangrove of the São Mateus River located in the lower and upper estuary (Fig. 1). The HS area is located 2.8 km from the mouth of the river, and has higher salinity (18.8–36.5 psu). The LS area, which is situated 7.5 km upstream from the mouth, has lower salinity (9.4–25.7 psu). Both collection points are located in areas with similar flooding conditions, with salinity being, in this study, the variable parameter to be analyzed. The region, where the study areas are inserted, within a tropical region, is characterized by a dry period (April to September) and a rainy season (October to March). In addition, an annual average precipitation of 1,311 mm is identified, with annual water deficit of 70 mm, distributed over the period from April to August and in February (Nobrega *et al*. 2008).

Plant material and sampling: For each study area, the data were obtained from 12 young plants of *R. mangle* (samples), approximately 2 m high, randomly selected within the forests, and sampled during the low water period of the syzygy tides. All measurements were made in five leaves localized at the second branch (from the apex to the base) that were completely expanded, randomly chosen, in total 60 leaf analyses per area. All measurements were performed in the morning (between 8:00 and 10:00 h). For the identification of each sample in the areas of lower salinity (LS) and high salinity (HS), the following terminologies were defined: Rh1, Rh2, Rh3, and Rh4 refer to the first (beginning of April 2016), second (September 2016), third (March 2017), and fourth (September 2017) sampling, respectively.

Measurements of Chl *a* fluorescence were made with a *Handy-PEA* portable fluorometer (*Hanstech Instruments Ltd.*, King's Lynn, Norkfolk, UK) in leaves previously adapted to the dark (30 min), using appropriate leaf clips

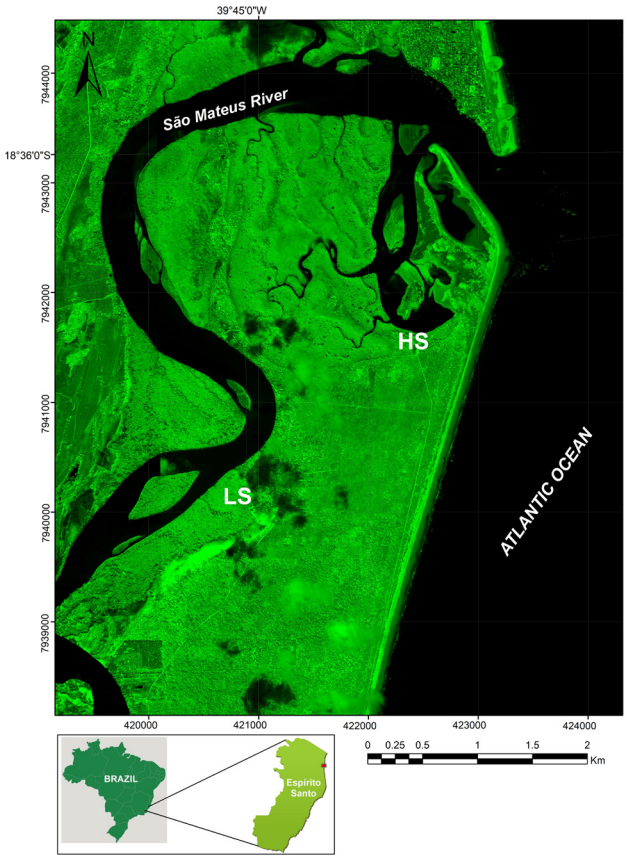

Fig. 1. Geographic location of the municipality of Conceição da Barra and of the mangrove located in the São Mateus River estuary with the areas of study called HS – high salinity and LS – low salinity. Designed by Helia Farías Espinoza, MSc.

(*Hansatech Instruments Ltd.*). Then, the leaves were subjected to a saturated light pulse of 3,000 µmol(photon) m^{-2} s^{-1} (650 nm) and the kinetics of rapid fluorescence (F_0 to F_m) was recorded from 10 µs to 1 s. The initial fluorescence intensity at 50 µ (F_0), 100 µs, 300 µs, 2 ms (F_J), 30 ms (F_I), and maximum fluorescence (F_m) were recorded and employed to obtain the parameters of the JIP test. The parameters of the selected JIP test were the specific energy flows per active reaction center: absorption flow of the antenna chlorophyll by reaction center (RC) (ABS/RC) and dissipation flow by RC (DI_0/RC). This assessment is important because it provides information about energy flows based on the Theory of Energy Flow in Biomembranes (Strasser *et al*. 2004). The performance indexes for energy conservation in the reduction of the final acceptors of the PSI (PI_{TOTAL}) were also analyzed. In addition, the parameters from the JIP test were used to calculate the L-band (V_{OK}), K-band (V_{OJ}), J-step, and IP-phase. L-band: $V_{OK} = (F_{100\mu s} – F_0)/(F_{300\mu s} – F_0)$ – variable fluorescence at 100 µs (transients normalized between F_0 and F_K) (Strasser and Stirbet 2001); K-band: $V_{OJ} = V_{OK}$ $[(F_{300\mu s} – F_0)/(F_{2ms} – F_0)]$ – variable fluorescence at 300 µs (transients normalized between F_0 and F_J) (Srivastava *et al*. 1997); J-step = $(F_{2ms} – F_0)/(F_m – F_0)$ (Strasser *et al*. 2004); and IP-phase: $\Delta V_{IP} = (F_m – F_{30ms})/(F_m – F_0)$ (Oukarroum *et al*. 2009).

Measurements of gas exchange and Chl index: The net rate of carbon assimilation (P_N [µmol m^{-2} s^{-1}]), stomatal conductance (g_s [mol m^{-2} s^{-1}]), intercellular CO_2 concentration (C_i [µmol m^{-2} s^{-1}]), leaf transpiration rate (E [mmol m^{-2} s^{-1}]), leaf temperature (T [°C]), and luminous intensity incident on the leaf surface (Q_{leaf} [µmol m^{-2} s^{-1}]) were estimated on the same leaves used to measure Chl *a* fluorescence, using the *LCi* portable system (*ADC*, *BioScientific Ltd.*, Hoddesdon, England). The estimation of water-use efficiency (WUE [µmol(CO_2) $mmol^{-1}(H_2O)$]) was calculated and determined as intrinsic water-use efficiency – WUE_{int} (P_N/g_s) (Sobrado 2005) and instantaneous water-use efficiency – WUE_{ins} (P_N/E) (Krauss *et al*. 2006).

Chl *a* and *b* indexes were obtained using a portable chlorophyll meter (*ClorofiLOG*, *model CFL 1030*, Falker, Brazil). Three measurements were made on each leaf blade, in different areas. Then, the mean of the three readings per leaf was calculated and the ratio of Chl *a*/*b* was obtained.

Salinity: Interstitial water salinity was determined from three replicates, according to Zamprogno *et al*. (2016). Likewise, the salinity of the river water at the margins of the mangrove forests was obtained. Measurements of the water electrical conductivity were taken with a portable meter model *HQ40D* (*HACH Company*, USA) and their values were transformed into salinity and the data were expressed as practical salinity unit (psu). The average monthly values (from April 2016 to September 2017), and the minimum and maximum salinity values for each study area were obtained.

Statistical analysis: The salinity values, gas-exchange parameters, and Chl index were statistically analyzed by *Kruskal-Wallis* analysis of variance (*ANOVA*), to determine differences between the areas (LS *vs*. HS) and the samplings. Differences between the medians were also evaluated using *Kruskal-Wallis* analysis, setting the value of $p<0.05$ as statistically significant.

Results

Salinity: Salinity differed between the studied areas in all samples and was higher in HS (Table 1). However, the comparison between the samples revealed lower salinity values in Rh3 in the two studied areas (18.8 and 9.44 psu for HS and LS, respectively). In HS, the highest salinity value (36.5 psu) was recorded during Rh2, while for the LS area the highest values were sampled in Rh2 and Rh4 (25.5 and 26.01 psu, respectively). In HS area, intermediate salinity values were obtained during Rh1 and Rh4 (30.8 and 31.7 psu, respectively), while in the LS area, intermediate salinity was observed in Rh1 (17.8 psu).

Gas exchange and Chl index: The CO_2 assimilation rate (P_N) and stomatal conductance (g_s) differed between areas only in Rh2, with higher values obtained in LS (Fig. 2*A*,*B*). Higher values of P_N and g_s occurred in Rh3 and Rh4. On the

Table 1. Monthly salinity data monitored between April 2016 and September 2017 in the low salinity (LS) and high salinity (HS) areas. Data are presented as minimum, maximum, and mean values expressed as practical salinity unit (psu) (n = 4). Significant differences (p<0.05) between the areas are highlighted by (*), data *in bold* correspond to the months of sampling for fluorescence and gas-exchange measurements.

	LS/2016			HS/2016			LS/2017			HS/2017		
Months/Sampling	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
January	-	-	-	-	-	-	5.4	15.2	10.6	8.2	27.0	14.6
February	-	-	-	-	-	-	7.7	15.6	12.2	18.4	23.6	21.9*
March/Rh3	-	-	-	-	-	-	**7.3**	**11.3**	**9.4**	**17.7**	**20.0**	**18.8***
April/Rh1	**17.2**	**18.2**	**17.8**	**30.3**	**31.3**	**30.8***	10.1	16.2	13.2	21.2	24.6	23.0*
May	18.4	18.9	18.6	30.1	31.2	30.7*	8.0	9.2	8.5	17.8	19.8	18.5*
June	17.8	18.5	18.1	28.6	28.9	28.8*	9.9	11.5	10.8	17.3	23.6	19.4*
July	12.5	17.8	15.3	23.3	28.2	26.8*	15.1	22.1	20.0	25.7	33.5	30.0*
August	24.4	24.9	24.6	27.4	32.4	29.7*	21.3	26.4	24.9	26.7	32.8	29.8*
September/Rh2–Rh4	**23.9**	**27.0**	**25.5**	**34.5**	**38.6**	**36.5***	**24.6**	**26.6**	**25.7**	**29.0**	**37.0**	**31.7***
October	22.8	28.5	25.3	32.3	36.4	34.2*	-	-	-	-	-	-
November	3.2	6.8	5.1	9.5	13.7	12.0*	-	-	-	-	-	-
December	1.6	5.9	3.8	11.6	15.7	13.3*	-	-	-	-	-	-

other hand, the g_s values obtained in Rh1 in the LS area did not differ significantly from Rh2, Rh3, and Rh4 (Fig. 2*B*). Leaf transpiration (E) differed between the areas in all samples (Fig. 2*C*) and, similarly to the results obtained for P_N and g_s, were higher in LS mainly for Rh3 and Rh4. For the HS area, the highest E values occurred in Rh3 and Rh2. The internal CO_2 concentration (C_i) differed between the Rh3 and Rh4 samples, being higher in the HS (Fig. 2*D*). Comparisons between samplings differed only in LS, where the highest values were recorded in Rh1 and Rh2.

The leaf temperature (T_{leaf}), as well as E, differed significantly between the areas in all samples, with the highest

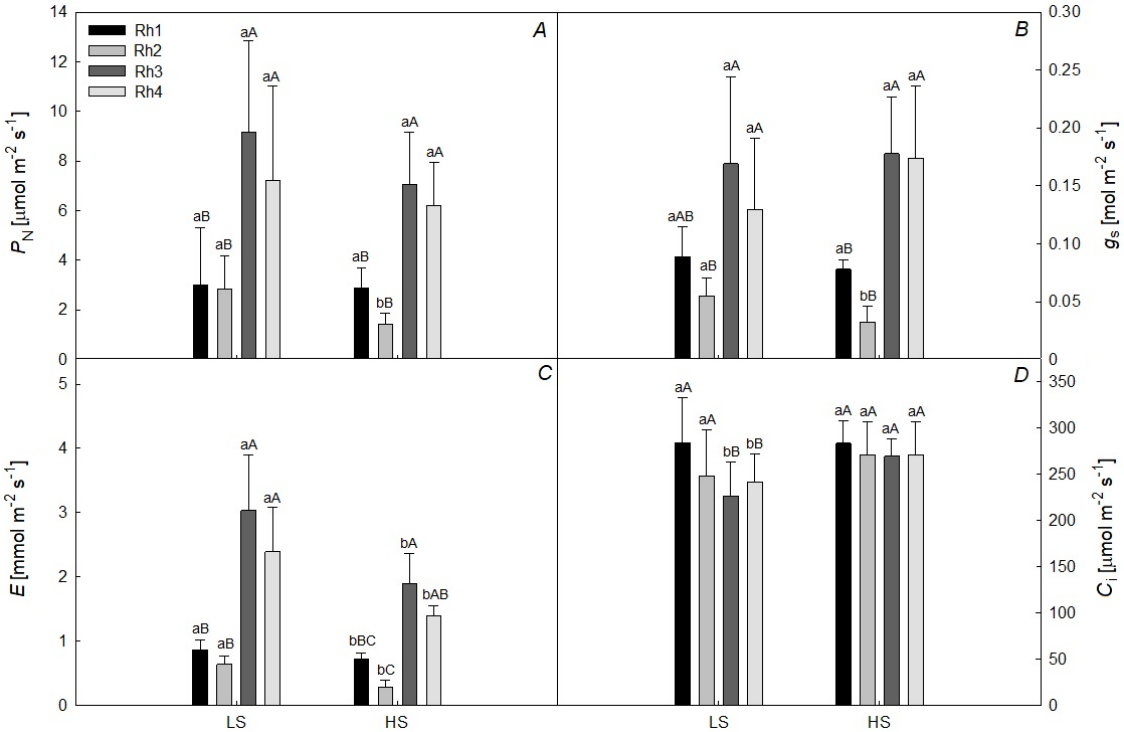

Fig. 2. Mean parameters (± standard deviation) of gas exchanges (n = 12) obtained from *Rhizophora mangle* plants in the LS (lower salinity) and HS (higher salinity) areas in April 2016 (Rh1), September 2016 (Rh2), March 2017 (Rh3), and September 2017 (Rh4). (*A*) CO_2 assimilation (P_N), (*B*) stomatal conductance (g_s), (*C*) transpiration (E), and (*D*) internal CO_2 concentration (C_i). Means differ significantly by the *Kruskal-Wallis* test, p<0.05. *Lowercase letters* indicate comparisons between different areas (HS and LS). *Uppercase letters* report comparisons among samplings in the same area.

values obtained at LS (Fig. 3*A*). The incident light intensity on the leaf surface (Q_{leaf}) was higher in LS and differed between the areas in Rh3 and Rh4 (Fig. 3*B*). Comparisons between samples distinguished a higher Q_{leaf} value in Rh3 and Rh4 and lower value in Rh1 in both areas. Rh2 did not differ between samplings in HS, but was lower in the samples of LS. The intrinsic water-use efficiency (P_N/g_s) also differed between the areas in Rh3 and Rh4, remaining higher in LS (Fig. 3*C*). Comparisons between samples showed differences only in LS, registering higher values of P_N/g_s in Rh3 and Rh4 and lower values in Rh1. On the other hand, the values of instantaneous water-use efficiency (P_N/E) were different between the areas in Rh3 and Rh4, as well as in P_N/g_s, but were higher in HS (Fig. 3*D*). The samplings did not differ in both areas.

The Chl index differed between the areas only in Rh1 and was higher in the LS area (Fig. 4*A*). In HS, the highest value of Chl *a* was obtained in Rh2, and in the LS area, the highest values were observed in Rh1 and Rh2. The Chl *b* index differed between the areas in Rh1 and Rh3, with higher values reported in the LS and HS areas, respectively (Fig. 4*B*). In HS, the comparison between samplings revealed higher values of Chl *b* in Rh2 and lower values in Rh1 and Rh3. In LS, the higher values of Chl *b* index were verified in Rh1 and Rh2. The total Chl (*a*+*b*) differed between Rh1 and Rh3 and was higher in LS and HS, respectively (Fig. 4*C*). In the HS area, the highest value of Chl (*a*+*b*) was observed in Rh2, while the lowest value was obtained in Rh1. In the LS area, the highest value of total Chl was recorded in Rh1, while the lowest value was found in Rh3. The Chl *a*/*b* ratio was distinguished between the areas in Rh1, with the highest value obtained in the HS area and in Rh3 in the LS area (Fig. 4*D*). In the LS area, the Chl *a*/*b* ratio was higher in the Rh3 sampling and differed from the other areas. No difference in the Chl *a*/*b* ratio was observed between collections in the HS area.

Chl *a* fluorescence: The L-band did not differ between the areas except Rh1 (Fig. 5*A*). All the other samples exhibited higher L-band values in the LS area. Comparisons between the samples showed higher L-band value in the HS area in Rh1 and Rh3, respectively, and lower value in Rh2. In the LS area, the highest L-band value was observed in Rh3. The K-band, similarly to the L-band, did not differ between the HS and LS areas only in Rh1 (Fig. 5*B*). The highest values of the K-band were verified in Rh2 and Rh3 in LS and in Rh4 in HS. In the HS area, the highest K-band values were observed in Rh1 and Rh3 and the lowest value in Rh4. In LS, the highest K-band value occurred in Rh1, Rh2, and Rh3. J-step values differed between the areas in Rh1 and Rh3 and was shown to be higher in HS (Fig. 5*C*,*D*). Comparisons between samplings showed lower J-step in HS in Rh3. In LS, the highest J-step value occurred in Rh2 and Rh4. The IP-phase did not differ between the areas only in Rh2. However, IP-phase values were higher in Rh1, Rh3, and Rh4 in the LS area (Fig. 5*E*).

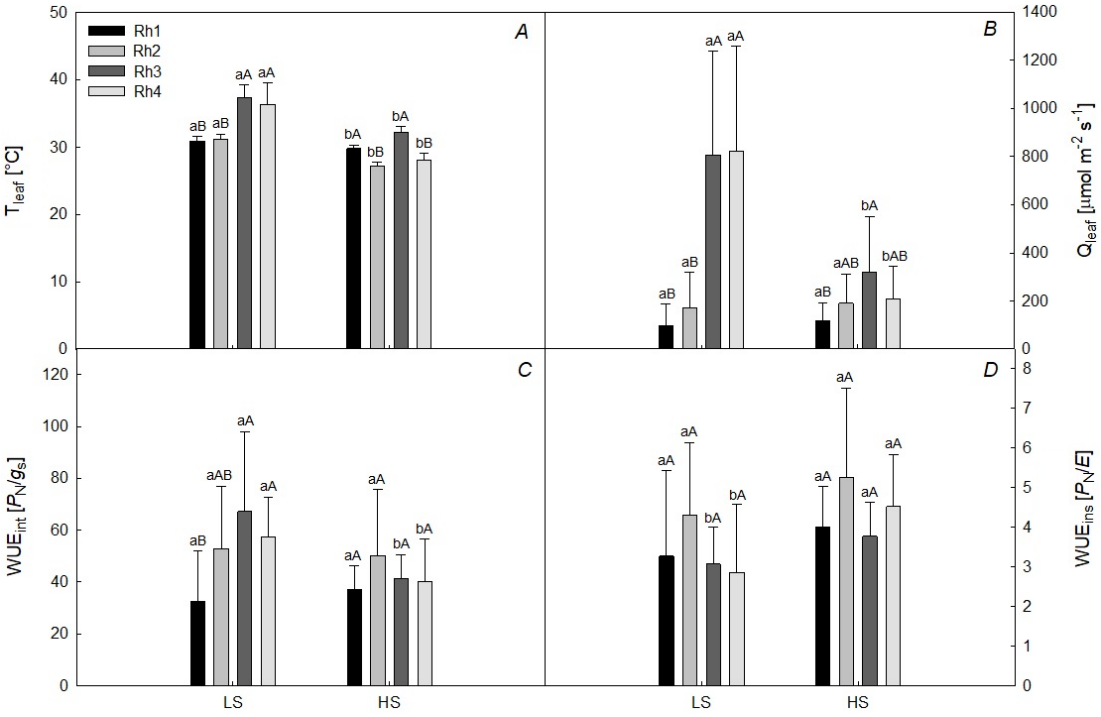

Fig. 3. Mean parameters (± standard deviation) of gas exchanges and water use (n = 12) obtained from *Rhizophora mangle* plants in the LS (lower salinity) and HS (higher salinity) areas in April 2016 (Rh1), September 2016 (Rh2), March 2017 (Rh3), and September 2017 (Rh4). (*A*) leaf temperature (T_{leaf}), (*B*) luminous intensity incident on the leaf surface (Q_{leaf}), (*C*) intrinsic water-use efficiency (WUE_{int}), (*D*) instantaneous water-use efficiency (WUE_{ins}). WUE is expressed in µmol(CO_2) mmol^{-1}(H_2O). Means differ significantly by the *Kruskal-Wallis* test, p<0.05. *Lowercase letters* indicate comparisons between different areas (HS and LS). *Uppercase letters* report comparisons among samplings in the same area.

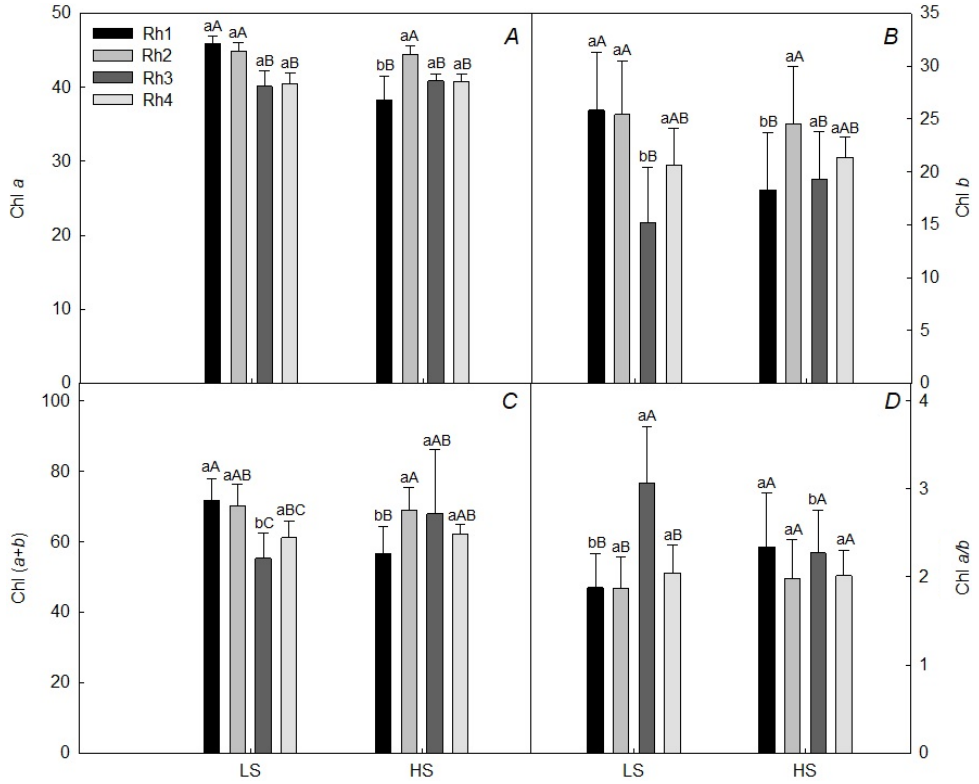

Fig. 4. Mean parameters (± standard deviation) of chlorophyll indexes (n = 12) obtained from *Rhizophora mangle* plants in the LS (lower salinity) and HS (higher salinity) areas in April 2016 (Rh1), September 2016 (Rh2), March 2017 (Rh3), and September 2017 (Rh4). (*A*) chlorophyll (Chl) *a*, (*B*) chlorophyll *b*, (*C*) total chlorophyll (*a*+*b*), and (*D*) chlorophyll *a*/*b* ratio. Means differ significantly by the *Kruskal-Wallis* test, $p<0.05$. *Lowercase letters* indicate comparisons between different areas (HS and LS). *Uppercase letters* report comparisons among samplings in the same area.

Comparisons between sampling recorded higher values of IP-phase in the HS area in Rh3 and lower values in Rh4. In the LS area, higher IP-phase values were reported in Rh1 and Rh3, while lower IP-phase values were observed in Rh2 and Rh4.

The initial fluorescence (F_0) was higher in HS, except for Rh2, which did not differ between the areas (Fig. 6*A*). In HS, when F_0 was compared among the samplings, it was higher in Rh2 and lower in Rh4. In the LS area, F_0 increased in Rh2 and decreased in Rh4. Maximum fluorescence (F_m) did not differ between the areas only in Rh1 (Fig. 6*B*). However, in all other samples, F_m values were higher in the HS area. Regarding the variations between samples, F_m values were higher and lower in Rh2 and Rh4, respectively, in the HS area. In the LS area, the highest F_m values were observed in the year of 2016 (Rh1 and Rh2) and the lowest values in 2017 (Rh3 and Rh4). The F_v/F_m ratio did not differ between the areas only in Rh2 (Fig. 6*C*), with higher F_v/F_m values observed in the LS area in Rh1 and Rh3, and in the HS area in Rh4. Comparisons between samples showed differences only in LS, in which the highest value was found in Rh1. PI_{TOTAL} did not differ between the areas only in Rh2 (Fig. 6*D*). However, in Rh1, Rh3, and Rh4 the value of PI_{TOTAL} was higher in the LS area compared to HS. In LS, the highest PI_{TOTAL} value was recorded in Rh1 and Rh3, and in the HS area in Rh3.

Values of ABS/RC did not differ between the areas only in Rh1. However, the value of ABS/RC was higher in Rh2 and Rh3 for the LS area and in Rh4 for the HS area (Fig. 7*A*). Comparisons between samplings identified higher values of ABS/RC for LS in Rh3, followed by Rh2. In the HS area, higher values of ABS/RC were observed in Rh1 and Rh3, followed by Rh2. Finally, DI_0/RC values did not differ between the areas only in Rh4. However, DI_0/RC values were higher in Rh1 in the HS area, and in Rh2 and Rh3 in the LS area (Fig. 7*B*). Comparisons between samples showed differences only in the LS area, with lower values of DI_0/RC observed in Rh1, and, from this sample, increases in DI_0/RC values were observed until the Rh3 sampling.

Discussion

Gas exchange and water use are influenced by salinity: The growth of most mangrove species is stimulated by moderate concentrations of salt. However, high salinity prevents their full development (Takemura *et al*. 2000, Parida *et al*. 2004). Due to the effects caused by soil water salinity fluctuations, mangrove species normally exhibit low P_N and g_s when compared to other C_3 species (Ball 1988). These responses are more accentuated as salt concentrations increase (Martin *et al*. 2010, Reef and Lovelock 2015). In this study, reduced values of P_N and g_s (Fig. 2*A*,*B*) were observed only in the Rh2 sampling in the

area of higher salinity (HS) when comparing the areas by collection. According to Sobrado (2004), a lower g_s limits the influx of CO_2, influencing the assimilation rates, given that stomatal conductance is very sensitive to changes in air humidity and the salinity of interstitial water. In this study, the Rh2 sampling occurred when the highest salinities in the HS area were registered (Table 1).

Throughout this study, a higher CO_2 assimilation was recorded in the salinities between 18 and 31 psu for HS and between 9 and 26 psu in LS (Fig. 2*A*, Table 1). The maintenance of photosynthetic activity in HS plants was supported by an increase in the expression of genes involved in the synthesis of ATP (*psbA*), Rubisco activation (*RCA*), ROS sequestration (*FSD3*), GABA synthesis (*GAD*), and vacuolar Na^+ sequestration (*NHX1*) (Lopes *et al*., unpublished). Similar results were observed by Zhang and Shi (2013) relating the importance of membrane transporters, including NHX1, to salt tolerance in plants. López-Hoffman *et al*. (2006) reported higher CO_2 assimilation in seedlings of *R. mangle*, growing in field conditions under higher salinity (25 psu), compared to the area of lower salinity (below 10 psu). Higher photosynthetic performance under a high salt content is characteristic for species of the Rhizophoraceae family. Several studies reported better development of *R. mangle* in salinity conditions up to 30 psu (Krauss and Allen 2003, Parida *et al*. 2003).

Our results confirmed these influences of salinity on the assimilation of CO_2 in *R. mangle*, since there was a significant difference between the samples that identified that the smallest values of P_N (Fig. 2*A*) occurred under intermediary or high salinities (in samplings Rh1 and Rh2, respectively, in both areas; Table 1). However, during the Rh4 sampling, when intermediate salinity occurred in the HS area and high salinity in the LS area, CO_2 assimilation values comparable to those obtained for Rh3 (Fig. 2*A*) were measured when salinity was low for both areas. These results, especially those obtained for the area of lower salinity, can be explained by the increase of luminous incidence, since greater luminosity can improve photosynthesis. Dangremond *et al*. (2015) observed an increase in CO_2 assimilation in *R. mangle* seedlings with

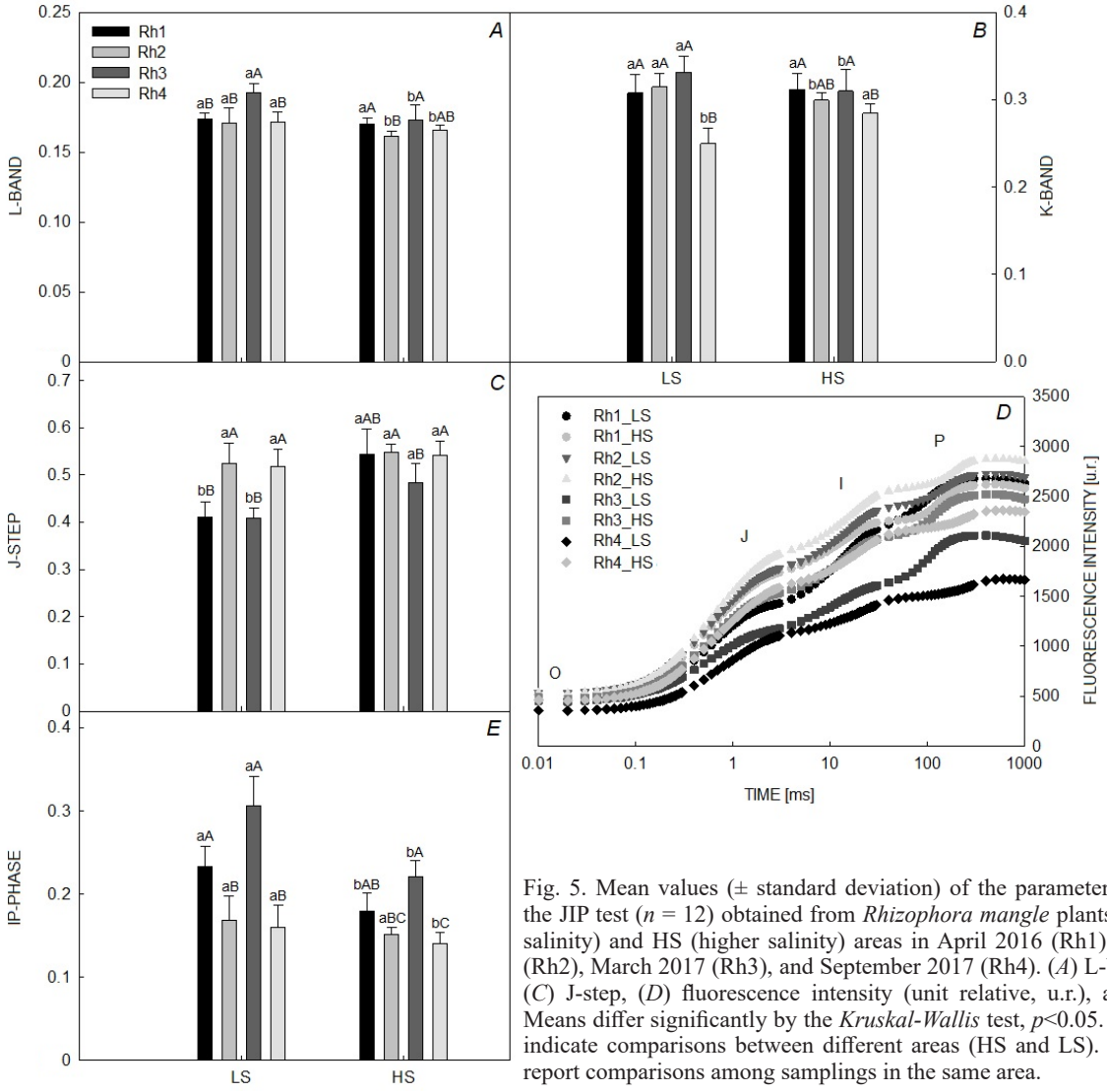

Fig. 5. Mean values (± standard deviation) of the parameters calculated from the JIP test (n = 12) obtained from *Rhizophora mangle* plants in the LS (lower salinity) and HS (higher salinity) areas in April 2016 (Rh1), September 2016 (Rh2), March 2017 (Rh3), and September 2017 (Rh4). (*A*) L-band, (*B*) K-band, (*C*) J-step, (*D*) fluorescence intensity (unit relative, u.r.), and (*E*) IP-phase. Means differ significantly by the *Kruskal-Wallis* test, $p<0.05$. *Lowercase letters* indicate comparisons between different areas (HS and LS). *Uppercase letters* report comparisons among samplings in the same area.

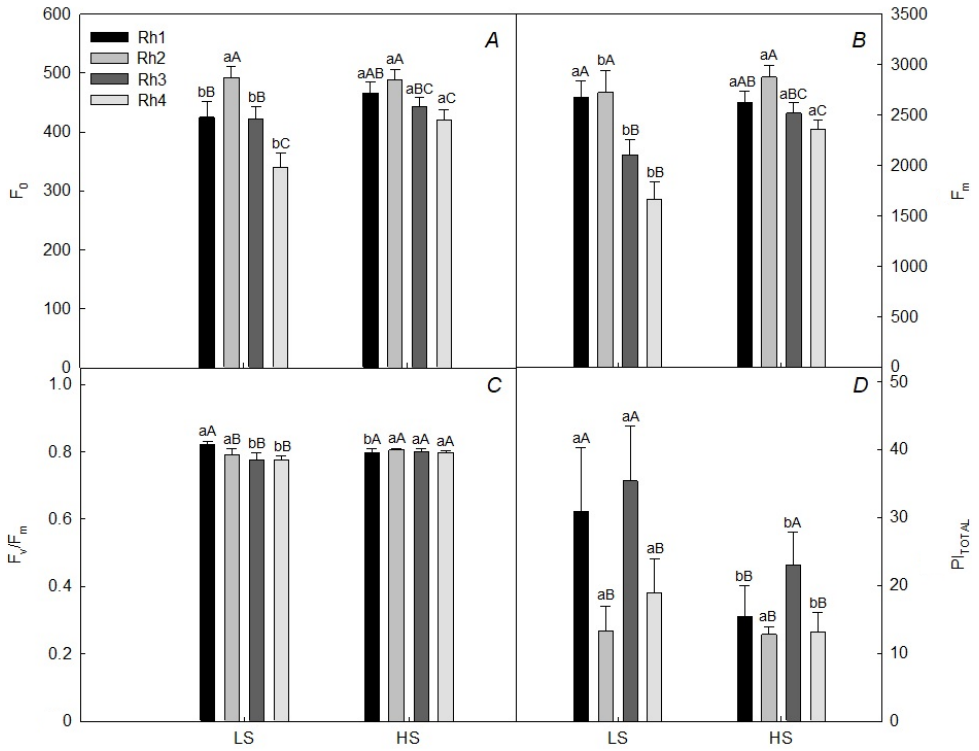

Fig. 6. Mean parameters (± standard deviation) of the JIP test (n = 12) obtained from *Rhizophora mangle* plants at the LS (lower salinity) and HS (higher salinity) areas in April 2016 (Rh1), September 2016 (Rh2), March 2017 (Rh3), and September 2017 (Rh4). (*A*) initial fluorescence intensity (F_0), (*B*) maximum fluorescence intensity (F_m), (*C*) maximum photochemical efficiency of the PSII (F_v/F_m), and (*D*) performance index for energy conservation in the reduction of the final acceptors of the PSI (PI_{TOTAL}). Means differ significantly by the *Kruskal-Wallis* test, $p<0.05$. *Lowercase letters* indicate comparisons between different areas (HS and LS). *Uppercase letters* report comparisons among samplings in the same area.

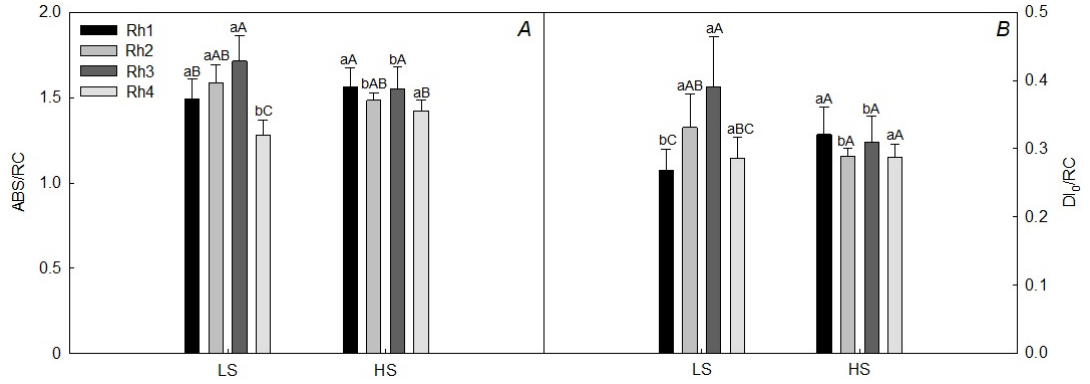

Fig. 7. Mean parameters (± standard deviation) of the JIP test describing the specific energy flows by reaction center (n = 12) obtained from *Rhizophora mangle* plants at the LS (lower salinity) and HS (higher salinity) areas in April 2016 (Rh1), September 2016 (Rh2), March 2017 (Rh1), and September 2016 (Rh2). (*A*) Absorption (ABS/RC) and (*B*) heat dissipation (DI_0/RC). Means differ significantly by the *Kruskal-Wallis* test, $p<0.05$. *Lowercase letters* indicate comparisons between different areas (HS and LS). *Uppercase letters* report comparisons among samplings in the same area.

increase of salinity and luminosity (maximum salinity of 35 psu under PPDF of 1,200 µmol m^{-2} s^{-1}) during a 12-week period. According to the authors, mangrove species commonly saturate the photosynthetic rate at low luminous intensities and direct exposure to sunlight can result in excessive excitation energy and increased leaf temperature. In this study, plants growing in the LS area, from samplings Rh3 and Rh4 (Fig. 3*B*), were subjected to greater exposure to sunlight due to the fall of larger canopy trees caused by high velocity winds, reflecting in an increased leaf transpiration rate (Fig. 2*C*). According to Larcher (2000), greater leaf transpiration is a protective mechanism against

temperature elevation. In the study by Dangremond *et al*. (2015), the effect of salinity and light on the photosynthetic processes of *R. mangle* seedlings did not influence the final height, biomass, or growth rate, but resulted in lower mortality under high luminosity at moderate salinity. On the other hand, López-Hoffman *et al*. (2006) observed that under lower luminosity, the survival rates of *R. mangle* seedlings were higher at higher salinity (25 psu). Therefore, the *Rhizophora* genus can inhabit places with high luminosity (Kitao *et al*. 2003) as long as the salinity content is moderate.

The lowest level of luminosity in the HS area, due to the greater canopy coverage, was not harmful to the plants, as we observed from the assimilation values recorded for both areas in the last two samplings (Rh3 and Rh4). However, although the LS area presented favorable conditions for the full development of its individuals (higher luminosity and moderate salinity), this did not result in better means of carbon assimilation rates (Fig. 2*A*). However, this fact may be indicative that individuals in the HS present a series of physiological adjustments, which allows them to minimize the effects of salinity and develop, even at the expense of higher energy expenditure.

WUE_{int} (P_N/g_s) did not differ in the plants of the HS area since, for all samplings, similar values were obtained (Fig. 3*C*). Comparisons between areas showed better water use for plants in LS in the last two samples, Rh3 and Rh4. However, in Rh2, when salinity was high for both areas (Table 1), WUE_{int} values were similar, indicating the influence of high salinity for plants regardless of the growth area. Soares *et al*. (2015) also did not observe differences in the internal water use comparing forests with different salinity for *R. mangle* plants. On the other hand, they recorded differences in isotope signatures (C^{14}), which evaluate the long-term water-use efficiency in the forest with the highest salinity. The invariability of WUE_{ins} among the four samplings in both areas suggests maintenance of the water-use efficiency in both areas, which happens regardless of salinity, indicating the adaptation of individuals to the local conditions. However, differences between the areas showed better water use for the plants in the HS area in the last two samples (Fig. 3*D*). It is important to emphasize the occurrence of higher luminosity in LS (as mentioned above), which resulted in higher temperature and leaf transpiration, without evident increases in carbon assimilation. Therefore, the plants of the LS area showed greater restriction of water use than that in plants of HS (Fig. 3*C*). However, in Rh2, although no statistical difference was verified between the areas in WUE_{int}, the lowest values of temperature and leaf transpiration in HS may be indicative of water restriction, since the luminous radiations between the areas were similar. However, in the LS area the assimilation was higher (Fig. 3*C*). Limitations of water use due to the higher cost of water acquisition with increased salinity contributes to the reduction of photosynthetic electron transport and, consequently, of subsequent steps of carbon reduction. Chen and Ye (2014) observed that seedlings of *Excoecaria agallocha* L., a nonviviparous mangrove species, are not able to maintain high efficiency in water use (P_N/E) under high salinity conditions (25 psu), despite the marked reduction of leaf transpiration.

Chl indices were not interfered by salinity: Generally, the increase in salinity is accompanied by a reduction in Chl *a* concentration, as a consequence of chronic photoinhibition. Therefore, reduction of photosynthetic capacity occurs (Naidoo *et al*. 2002), contrary to what was evidenced in the *R. mangle* plants in this study. Difference in the Chl *a* index between the areas occurred only in Rh1 in the LS area, for which the higher Chl *a* index was verified (Fig. 4*A*). However, the higher Chl *a* index was not followed by the increase in CO_2 assimilation, which was similar between the areas (Fig. 2*A*), as well as in Rh2 in the LS area, for which, despite the higher assimilation rate, no difference in Chl *a* content was observed.

In the HS area, when comparing the differences between the samplings, we also observed a higher Chl *a* index in the period of higher salinity (Rh2), compared to samplings with lower salinity (Fig 3, Table 1), without interference in carbon assimilation. Goussi *et al*. (2018) reported increases in Chl *a* content in the halophyte *Thellungiella salsuginea* (Pall.) O.E. Schulz with the increment of salinity from 200 to 400 mM. However, the increase in Chl *a* content was negatively correlated with growth. In *Kandelia candel* (L.) Druce seedlings, high salinity resulted in a higher concentration of Chl *a* (Wang *et al*. 2015). The results obtained in the LS area indicate that the samplings that showed higher P_N also exhibited the lowest Chl *a* index (Fig. 3*A*). However, in Rh3, when there was greater assimilation in the LS area, the period was of low salinity (Table 1).

The total Chl (*a*+*b*) is related to the improvement of the efficiency in the transfer rate of luminous energy and the production of chemical energy. As a result, increases in the CO_2 assimilation rate occurred. However, this fact was not observed between the areas, since there was no difference between the rates of P_N, even with a higher Chl (*a*+*b*) occurring in the Rh1 sampling for the LS area and in Rh3 for HS. The reduction of the pigment content in the plants in both areas, represented by the lower total Chl index, may be a consequence of the destruction of the protein-pigment complexes of the thylakoids in the photosynthetic reaction centers (RCs) (Chang *et al*. 2012) and reduction of active reaction centers. Finally, the Chl *a*/*b* ratio is considered an index of the capacity of light-harvesting complexes (LHC), which are regulated by the activity of the Chl cycle (Das *et al*. 2002, Tanaka and Tanaka 2007). The smallest Chl *a*/*b* ratio obtained in plants of the area of lower salinity (LS) is more related to the increase of Chls associated with LHC than that with the reaction centers, which is evidenced by the highest value of Chl *b* (Lambers *et al.* 2008), since Chl *b* is essential for the elaboration and operation of most LHCs (Chen 2014). The increase of Chls associated with LHC along with the reduction of active reaction centers decreases the transfer of energy and increases its dissipation in the form of heat or fluorescence (Kalaji *et al*. 2011) as a photoprotection mechanism. Although no significant difference was obtained for the assimilation rate between the studied areas, we noted that

these factors harmed the photosynthetic processes of the plants growing under higher salinity in both areas.

Photosystem activities were distinct between the areas of higher and lower salinity: The area of lower salinity (LS) exhibited lower connectivity energy among the PSII units (higher L-band value) in all samplings, when compared with the plants of the HS area (Fig. 5*A*). Analysis by sampling showed lower L-band in Rh2 in the HS area, for which the highest salinity values were recorded (36.5 psu). These results suggest that the species presents mechanisms to tolerate high salinity with better utilization of electron excitation energy and better stability of the photosynthetic system. The tolerance of this species to develop under high and/or floating salinities is reinforced by the result obtained in the LS area among the samples, given that in Rh3, when the lowest salinity value was recorded in the area, the individuals presented the lowest connectivity energy when comparing the results obtained in this area under conditions of higher salinity (26 psu). These results are indicative of maintenance of the stability of the RCs of PSII, since this constitutes part of the adaptation strategy to saline stress (Goussi *et al*. 2018).

K-band was also higher in the LS area (Fig. 5*B*), when compared to HS. The K-band reflects the disturbances in the oxygen-evolving complex (OEC), coinciding with the limitation of the donor side of the PSII (Strasser *et al*. 2004, Oukarroum *et al*. 2007, Yusuf *et al*. 2010). Consequently, the highest values of K-band obtained in LS suggest lower efficiency in electron transfer between the donor and acceptor sides of the PSII, due to inhibition in the electron transfer velocity of the OEC to the acceptor side of the PSII (Srivastava *et al*. 1997, Strasser 1997, Tomek *et al*. 2001). Comparisons between samples showed that in Rh2 in the area of higher salinity (HS), when the highest significant salinity value was observed (Table 1), there was no difference in the value of K-band between the collections, indicating that the stability of the OEC was maintained even under higher salinity. However, for the LS area, the effect of salinity on the stability of the OEC was not observed among the samples Rh1, Rh2, and Rh3, since they did not present differences in the values of the K-band and, consequently, of the stability of the OEC with the variation of salinity. However, the Rh4 sampling, although the salinity values did not differ from those obtained for Rh2 in the LS area, showed higher luminous radiation and this, associated with the effect of higher salinity of the season, may have favored electron transport between the OEC and tyrosine (Guha *et al*. 2013), reflecting in the stability of the OEC.

The highest J-step values observed in the HS area in Rh1 and Rh3 (Fig. 5*C*,*D*), when the levels of salinity for both areas were intermediate and low (Table 1), respectively, evidenced the effect of the more saline environment, since in this condition, there was greater accumulation of Q_A in the reduced state, and lower efficiency in the transport of electrons beyond the Q_A^-. A similar result was observed among the samplings in both areas when higher electron transport efficiency on the acceptor side of the PSII was recorded on occasions when salinity was between 18 and 9 psu (Table 1). Therefore, as it is a facultative halophyte, *R. mangle* showed greater efficiency in electron transport between quinones Q_A and Q_B in salinities considered in this study as intermediate and low, presenting the optimum condition for this stage of photosynthetic electron transport.

In addition, the highest IP-phase values obtained in plants growing in LS (Fig. 5*E*) suggest higher PSI activity in the plants in the area. This aspect is maintained when comparing the samples and areas, and the value of the IP-phase was always higher in LS. The increase in the activity of the PSI is an important strategy to the stress conditions, favoring the formation of the proton gradient and the synthesis of ATP, avoiding the excessive formation of $NADPH_2$ and, consequently, the formation of reactive oxygen species (ROS) (Huang *et al*. 2012). This physiological mechanism allows the maintenance of photosynthetic activity in an environment of greater salinity, accentuating the activity of the PSII to the detriment of the activity of the PSI. This analysis is supported when salinity is considered, which, in the LS area, the plants are under milder conditions and in a lesser amplitude in the variation of salinity (between 9 and 26 psu, with a mean of 19 psu), responding to the dry and rainy seasons, differently than what can be observed in HS, where salinity was higher and with lower amplitude between the extreme levels, *i.e*., between 18 and 36 psu, with an average of 30 psu.

Higher F_0 values obtained in HS (Fig. 6*A*) suggest damage to the PSII reaction centers (photochemical reaction inactivation) or interruption of the antenna excitation energy transfer to the reaction centers in an efficient manner. The Rh2 sampling, for which the highest salinity values were recorded in both areas, F_0 did not differ between the areas, indicating lower efficiency of electron transfer to the reaction centers with saline stress, or reduced electron transfer of the Q_A^- to Q_B occurring due to electron flow inactivation (Mathur *et al*. 2011). Consequently, there is a higher energy expenditure for individuals, resulting in damage to the D1 protein, interference in electron transport (Aro *et al*. 1993), and photoinhibition (Zlatev and Yordanov 2004). For other species, such as *Avicennia officinalis* L. and *Heritiera fomes* Buch Ham, higher F_0 values were obtained under the influence of higher salinity (Panda *et al*. 2006). However, in the HS area, although salinity did not differ statistically (30.8 and 31.7 psu, respectively, in Rh1 and Rh4), F_0 was significantly lower in the last sampling. This result may be due to the longer exposure time of plants to high salinity in Rh1 (data not presented), both in the dry and rainy periods (due to rainfall scarcity in previous years).

The lowest values of F_m recorded in LS (Fig. 6*B*) are indicative of accumulation of inactive reaction centers of the PSII, hindering electron transfer to reduce Q_A^- (Kalaji *et al*. 2011), resulting in the accumulation of P_{680}^+. The relationship of salinity between the samplings indicates accumulation of active reaction centers (RCs) of the PSII with the increase of salinity (Rh2) for the plants growing in the HS area (Fig. 6*B*). In the LS area, records of active and prone RCs for electron transport were higher in the samplings with intermediate and high salinity (Rh1 and

Rh2, respectively), but, although the salinity recorded for Rh4 did not differ from the salinity of Rh2, higher luminous radiation was observed in Rh4, which might act as an inhibitor of RCs activation. However, the highest F_0 values were not recorded concomitantly with the records of lower F_m in the plants, a fact that could lead to chronic photoinhibition and damage to the D1 protein (Aro *et al*. 1993, Zlatev and Yordanov 2004), indicating that the possible damage caused to electron transport in *R. mangle* plants growing in natural environments was reversible.

The maximum quantum yield values of the PSII (represented by the F_v/F_m ratio values) were altered between the areas (Fig. 6*C*). The lowest values observed for F_v/F_m in the LS area (Rh3 and Rh4) indicate the occurrence of electron flow retardation between Q_A^- and Q_B (Oukarroum *et al*. 2015), resulting from damage and inactivation of active RCs associated with PSII (Basu *et al*. 1998). Changes in the F_v/F_m ratio are attributed to changes in the efficiency of nonphotochemical quenching, and values between 0.75 and 0.85 were attributed to healthy plants (Bolhàr-Nordenkampf and Öquist 1993, Hunt 2003). The F_v/F_m values obtained in this study were still within the range of physiologically healthy mangrove plants (Panda *et al*. 2006, Hoppe-Speer *et al*. 2011), although the maximum quantum yield of the PSII was higher in the plants of the area with higher salinity (Fig. 6*C*). In the LS area, the highest photochemical efficiency occurred in the sampling with intermediate salinity (17.8 psu in Rh1), but they were less efficient under low and high salinity conditions. This result suggests that plants, despite growing in lower salinity, were more susceptible to the effects of salinity due to its variation throughout the seasons and concerning its daily amplitudes.

Higher values of the ABS/RC ratio observed in the two areas may be due to the increase of inactive RCs and antenna size (Yusuf *et al*. 2010), reducing the energy flow. These results indicate that salinity compromised the relationship between the light-harvesting complexes (ABS) and the active RCs of the PSII (Oukarroum *et al*. 2015) in both areas. According to Gomes *et al*. (2012), the ratio of active RCs is related to the energy connectivity between the units of the PSII (L-band). The highest values of the ABS/RC ratio recorded in the LS area, due to the increase in the antenna size of inactive RCs, decreased the stability of the RCs of the PSII, an aspect corroborated by the results referring to the L-band in Rh3, for which the highest values were verified (Fig. 5*A*). The reduction of the antenna size in HS (Rh2 and Rh3) and in LS (Rh4) indicate that active RCs increased, being transformed into Q_A^- reducers, or that the size of the antennas that provide energy for active RCs decreased, improving the passage of energy for active RCs (Redillas *et al*. 2011). In addition, the presence of the K-band may indicate the functional size of the PSII antenna (Yusuf *et al*. 2010) and, in this study, the most negative K-band recorded in the Rh2 sampling in the HS area (Fig. 5*B*) may suggest that there was a decrease in the antenna size and improvement in the passage of energy to active RCs, even under greater salinity.

Higher values of DI_0/RC in plants growing in the LS area in Rh2 and Rh3 (Fig. 7*B*) may be attributed to the protection mechanism of the energy absorption efficiency of the PSII and excessive absorbed energy (Zushi and Matsuzoe 2017). These results may be an indicator of the ability to prevent damage to the photosynthetic apparatus (Öquist *et al*. 1992). However, this increase in DI_0/RC may also be associated with the accumulation of inactive RCs (Kalaji *et al*. 2011) that did not capture the energy absorbed in excess, resulting in increases in nonphotochemical dissipation (heat) and reduction of efficient electron transport. The DI_0/RC values were constant among the samplings in the HS area. However, differences between samplings in the LS area indicated lower DI_0/RC in Rh1, suggesting greater efficiency of electrons transportation in PSII, since active RCs were verified in the same period. However, in this area, there was a lower flow of heat dissipation energy (DI_0/RC) without increasing CO_2 assimilation comparing to the HS area in the same period. This result suggests that salinity is compromising both the photochemical portion and the chemical stage of the Calvin cycle in the individuals sampled in the LS area.

The PI_{TOTAL} parameter, by measuring the photochemical performance from the photons absorbed by the PSII to the reduction of the final acceptors of the PSI (Oukarroum *et al*. 2015), is considered one of the most sensitive parameters to measure the quantum efficiency of the PSII (Smit *et al*. 2009). In addition, it is closely related to the final result of the plant's productive activity, such as growth or survival under stress conditions (Yusuf *et al*. 2010). The results obtained in this study showed that the plants in the LS area (Fig. 6*D*) presented higher PI_{TOTAL}, although they presented decreased active RCs, photochemical activity, and higher values of the K- and L-bands (Fig. 5*A*,*B*). In this case, the increase in the performance index (which measures the vitality of the plant) represents an increment of the efficiency of the reduction of the final acceptors of PSI, corroborating the result of the IP-phase (Fig. 5*E*), showing that in the plants of the area of lower salinity was greater activity of PSI. Comparisons among samplings showed better performances for the plants evaluated in low or intermediate salinity in both areas. In contrast, a lower PI_{TOTAL} was recorded in the highest salinity samples, a factor that may lead to inefficiency in the formation of ATP and NAPH as final acceptors in the photochemical phase of photosynthesis, since the slow activity of reducing the final acceptors of PSI is considered a consequence of a lower requirement of reducers, possibly linked to the decrease in CO_2 assimilation.

The results showed that, although the plants of the LS area were growing in lower salinity, the greater saline variation along the sampling also resulted in photochemical stress, impairing electron transport on the acceptor side of the PSII. Consequently, if the salinity pattern is altered, *R. mangle* plants may experience the effects of increased salinity in the São Mateus River estuary, resulting in changes in the productivity and structure of the adult forest. Finally, the results obtained in this study contribute to the understanding of the photosynthetic behavior of *R. mangle* in situations of salinity similar or close to sea-water, with small amplitudes of variation between the minimum and maximum concentrations, as well as its

operation under similar conditions, climatic and oceanographic, but with lower salinity and below that of the seawater, with greater amplitude of variation between the minimum and maximum values. Our study contributes to evaluation of the tolerance behavior of this species in different environmental situations, which are subject throughout their global distribution and infer physiological responses in occasions of elevation of the mean relative sea level and climate change.

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