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*Full Length Research Paper*

# A study of the effects of salinity stress in the red mangrove, *Rhizophora mangle* L.

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**The mangrove habitat exhibits many unique physical features, one of the most important of which is a salinity gradient. Photosynthetic rates, as measured by leaf stomatal conductance and leaf chlorophyll fluorescence induction, were tested as indicators of salinity stress in seedlings of the red mangrove, *Rhizophora mangle,* grown under five different salinity levels: 0, 15, 30, 45, and 60 parts per thousand. Photosynthetic gas exchange (measured by stomatal conductance), as well as the light reaction of photosynthesis (measured by chlorophyll fluorescence) were found to decrease as salinity increased. The use of leaf stomatal conductance and chlorophyll fluorescence as a measure of photosynthesis allowed a rapid and reliable quantification of the known stressor, salinity, in seedlings of *R. mangle*. These non-destructive *in-vivo* techniques were found to be rapid and reliable for monitoring photosynthetic stress, an important physiological parameter determining survival and growth of mangrove plants. These techniques should be considered in forestry management and mangrove restoration projects to assess plant condition.**

**Key words:** chlorophyll fluorescence, photosynthesis, *Rhizophora*, stomatal conductance, salinity.

## INTRODUCTION

Various species of mangroves form the dominant woody vegetation in the intertidal zones of tropical and subtropical coastlines around the world. The mangrove habitat exhibits many unique physical features, an important one of which is a salinity gradient from freshwater to seawater (Odum et al., 1982). This salinity gradient has long been recognized as a potential stressor and an important factor that regulates physiological processes such as growth, height, survival, and zonation patterns in mangroves (Lin and Sternberg**,** 1993). Mangroves may be found occurring naturally along a gradient of salinity, from riverine forests with salinities close to or at zero parts per thousand (ppt), to fringe forests with typical salinities around 35 ppt, and even in hypersaline areas where the salinity may reach 70 ppt (Hutchings and Saenger**,** 1987). Although mangroves are found growing over a wide range of salinities, some species have been found to grow ideally in low salinity of approximately two ppt (Lin and Sternberg**,** 1992) while other species tolerate much higher salinities before exhibiting signs of stress (Sten and Voigt**,** 1959). Photosynthetic rates are good indicators of physiological stress levels in all plants, including mangroves (Salisbury and Ross**,** 1978**;** Lichtenthaler**,** 1996). Mangroves growing in areas of high salinity showed increasing stress as measured by decreased carbon dioxide assimilation, stomatal conductance, and growth rates (Clarke and Hannon**,** 1967). In another study, Teas recalculated data collected by Bowman in 1917 and found that evapotranspiration, measured by stomatal conductance, by red mangrove (*R. mangle*) seedlings ceased at salinities above 65 ppt (Saenger et al.**,** 1983). Changes in photosynthetic rate and stomatal conductance with changes in salinity and humidity were also measured by Ball and Farquhar (1984). They found these two measures of carbon dioxide assimilation decreased with increasing salinity. These studies all indicate the importance of high salinity stress on reduced photosynthesis and growth in mangrove plants, particularly seedlings. Photosynthetic gas exchange through the leaf stomata can be determined using stomatal resistance to water vapor loss and is a sensitive indicator of the physiologi-cal state of the plant. As a plant becomes stressed the stomatal resistance often increases, and water vapor conductance decreases (Ball and Farquhar**,** 1984).

Another useful and rapid measure of photosynthesis is the induction of chlorophyll fluorescence (Kautsky effect), a sensitive indicator of photosynthetic energy conversion that occurs during the light reaction (Schreiber et al., 1995) . Chlorophyll fluorescence can be modified by any factor affecting the light reaction pathway of photosynthesis, including many environment-tal stressors (Lichtenthaler, 1996).

In this study the physiological state of the photosynthetic apparatus was determined by measuring stomatal conductance and the chlorophyll fluorescence yield in seedlings of the red mangrove, *R. mangle* subject to five different salinity levels: 0, 15, 30, 45, and 60 ppt. The null hypothesis ($H_0$) was that both stomatal conductance and the chlorophyll fluorescence yield are not statistically different across all salinity levels in these sensitive early life-stages of the red mangrove. These two rapid and non-destructive, non- intrusive techniques lend themselves well to rapid field assessments of plant condition and could be used for forestry management and/or mangrove restoration projects where salinity is a concern.

## MATERIALS AND METHODS

This study was conducted following modified methods of Snedaker et al. (1996) in an insulated, constant-environment room (air temperature 25±2°C, relative humidity 65±5%, 200±20 µmol photons $m^{-1}$ $s^{-1}$ provided by Vitalite™ fluorescent PAR lamps on a 12:12 h light-dark cycle) to simulate the understory environment in established mangrove swamps (Cheeseman et al., 1997). Sixty seedlings of nursery grown *R. mangle*, approximately 1 to 1.5 years old, were planted in 1 L plastic pots filled with sieved beach sand and allowed to acclimate for one month. During the one month acclimation period, commercially available, soluble 20:20:20 N:P:K fertilizer with trace minerals was added to each pot at a rate of 30 ml once weekly. After the pre-treatment acclimation period, salinity in the pots was adjusted to 0, 15, 30, 45, and 60 ppt by the successive addition of solutions created by adding Biosalt™ SeaCrystals to de-ionized water. Salinity was measured every 2 weeks using a refractometer (Cambridge Instruments Inc, Cat. No. 10419) and adjusted as necessary. The rhizosphere was kept >75% saturated during acclimation and the experimental manipulation periods by monitoring a water-level indicator, external to the pot.

One month after salinity levels were adjusted to experimental treatments the plant responses were measured over a 24 h period. Stomatal conductance measurements were made using an AP4 Porometer (Delta-T Devices Ltd) between 9am-2pm when photosynthesis tends be high. Chlorophyll fluorescence yield measurements were taken with an SF-10 Fluorometer (Richard Brackner Research Ltd.) at night to maximize the yield ratio (Fv/Fm).

Induction of chlorophyll fluorescence provides valuable information on the efficiency of light conversion by photosystem II (P680). Leaf samples need to be kept in the dark long enough to cause complete re-oxidation of the P680 reaction centers and to permit relaxation of any fluorescent quenching associated with a pH gradient across the thylakoid membrane in the light, this time being 20-60 min for mangroves (Bjorkman et al., 1988). In the standard fluorescence induction curve this is represented as $F_0$, the oxidized state during which all reaction centers are open, and $F_{max}$ represents the reduced state when all reaction centers are closed and fluorescence is at a maximum. Fluorescence yield (Fv/Fm) was calculated as the variable fluorescence (Fv = $F_{max} - F_0$) divided by the maximum fluorescence intensity ($F_{max}$).

All porometer and fluorometer measurements were taken on the uppermost four leaves of each seedling and averaged to standardize with-in plant variations. Mean stomatal conductance and chlorophyll fluorescence yield of the four leaves on each plant were analyzed by one-way fixed factor ANOVA. Where significant results were obtained on the main effect, post- hoc analysis using Tukey's Honestly Significant Difference (HSD) test was used to determine treatments causing the significant effect.

## RESULTS

Leaf stomatal conductance rates decreased significantly ($F_{4, 54}$=21.579, P<0.001) with increasing salinity (Figure 1). Conductance lies between 0.35-0.5 cm/sec for *R. mangle* at low salinities less than 20 ppt. This decreased to 0.15 cm/sec in plants at 30 ppt, which is close to mean seawater (35 ppt). Very low conductance rates, 0.015 - 0.04 cm/sec, were measured in plants at salinities greater than 40 ppt (Figure 1). All salinity levels were found to significantly differ from each other, based on Tukey's HSD post-hoc analysis (Figure 1).

Fluorescence yield (Fv/Fm) decreased significantly ($F_{4,59}$=4.053, P<0.01) with increasing salinity (Figure 2). Fluorescence yield decreased from 0 ppt (Fv/Fm = 0.83) to 45 ppt (0.74) and then increased again at 60 ppt (0.76) salinity. Adjacent salinity levels were not found to be significantly different from each other, based on Tukey's HSD post-hoc analysis, with the exception of 30 ppt compared to 45 ppt (Figure 2).

## DISCUSSION

Growth of many halophytic species is maximal/optimal under relatively low salinities (Flowers et al., 1986). Generally mangroves are facultative halophytes and may survive and grow well in freshwater conditions. However, there are reports indicating the importance of salt for some mangroves, as well as evidence that different species exhibit different tolerances and salinity optima (Pezeshki et al., 1989).

The Avicenniacaeae appear to be more tolerant to salinity stress than the Rhizophoraceae (Hutchings and Saenger, 1987). For instance, *Avicennia marina* trees seem to grow well up to 75% seawater, with a hypothesized growth inhibition at higher salinities due to high sodium chloride (NaCl) concentrations in the tissues (Clough, 1984). However, seedlings of *A. marina* exhibited various growth responses under different salinity regimes, with highest growth recorded at 50% seawater (17 ppt). Lowest overall growth was found in seedlings of *A. marina* raised under 0 ppt salinity, which was even lower than growth of seedlings raised in 100% seawater (35 ppt) (Downtown, 1982; Ghowail et al., 1993). These studies suggest that even within a species, salt tolerance

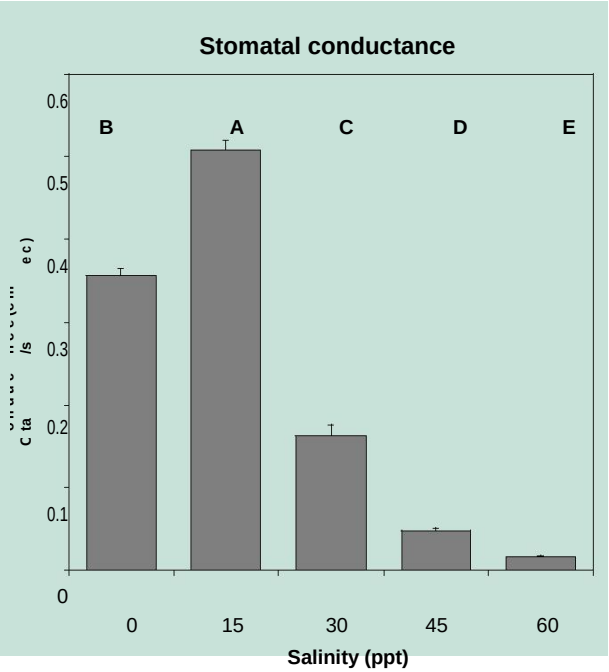


**Figure 1.** Average stomatal conductance (±1 SE; N=12) of *R. mangle* seedlings grown under 5 different salinities. Tukey's HSD treatment groups are indicated by different letters.

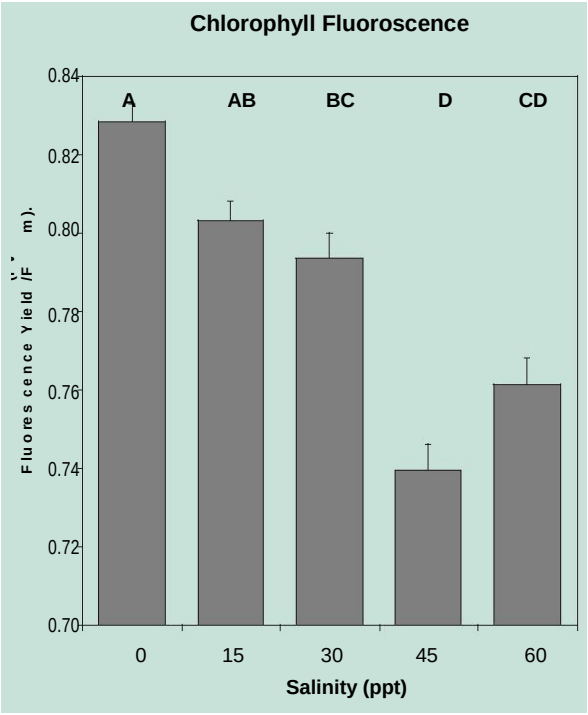


**Figure 2.** Average chlorophyll fluorescence (±1 SE; N=12) of *R. mangle* seedlings grown under 5 different salinities. Tukey's HSD treatment groups are indicated by different letters.

changes depending on life stage, with seedlings being potentially more sensitive to salt stress than mature trees.

In comparison, salinity of 15 ppt was found to be optimal for growth of seedlings of *Rhizophora apiculata* (Kathiresan and Thangamara**,** 1990). *Rhizophora stylosa* was found to exhibit poor growth at salinities greater than 25-50% seawater. Water stress may be the main effect of high salinity on growth of the Rhizophoraceae (Clough**,** 1984). However, *R. stylosa* appears to be less tolerant of high salinity than *R. mangle*. The red mangrove was shown to be tolerant of salinities up to 35 ppt, from the initial propagule stage to the early seedling stage, with no observed adverse effects on growth (Sten and Voigt, 1959). A fluctuating salinity regime on the other hand had a significant negative effect on photosynthesis (as measured by leaf stomatal conductance) and plant growth rates in *R. mangle* relative to constant salinities with the same mean (Lin and Sternberg**,** 1993).

It is apparent that different species of mangroves exhibit different tolerances to salinity stress and the ability to cope with salinity stress may change over the course of development. One of the major problems associated with the use of growth as a parameter to measure salinity stress is the necessity for long-term studies to achieve results. The use of non-invasive, *in-vivo* techniques for monitoring stress by looking at physiological parameters allows a more rapid quantifica-tion of short-term acute effects, as well as long-term chronic effects of salinity stress in mangroves.

It has been observed that increasing salinity is often accompanied by a decrease in turgor of the leaves (Ball and Munns**,** 1992), a factor that was also noted in this study in plants in the 45 and 60 ppt treatments. High salinities associated with intertidal mangrove habitats impose two potential restrictions on the photosynthetic rate of mangrove leaves: high leaf water deficits (i.e., a loss of turgor) and low stomatal conductance rates (Bjorkman et al.**,** 1988). Leaf stomatal conductance rates in mangroves have been found to be lower than for non-halophytic $C_3$ plants (Hutchings and Saenger**,** 1987). Bjorkman et al*.* (1988) proposed that low stomatal conductance is a requisite for a low ratio of transpiration to C fixation (i.e., high water use efficiency), which may be required for the maintenance of a physiologically acceptable salt/carbon balance within the leaves (And-rews and Muller**,** 1985). Rates of photosynthetic $CO_2$ fixation were found to decline with increasing salinity and this was attributed to stomatal limitations on $CO_2$ uptake (Ball and Farquhar**,** 1984; Bjorkman et al.**,** 1988).

The use of chlorophyll fluorescence is a more recent technological advance and allows rapid assessments of light reaction kinetics associated with PSII (Schreiber et al.**,** 1995). These kinetics can be related to $CO_2$ uptake and $O_2$ production (Schreiber et al.**,** 1995), therefore, this technique provides rapid assessment of plant photosyn-thesis. All Fv/Fm values that were observed in our study indicated that leaves were photosynthetically active (Cheeseman et al.**,** 1997). Ratios near 0.83 are indicative

of healthy photosystem function, whereas ratios less than 0.75 were associated with unhealthy trees (Duke et al., 2001).

In other studies using chlorophyll fluorescence, the quantum yield of PSII photochemistry in the dark-adapted state (Fv/Fm) was significantly higher in *Lumnitzera racemosa* seedlings grown in both 7.5 and 15 ppt compared to those at 0 and 30 ppt salinities (Fan et al., 1999). The non-photochemical fluorescence quench-ing (qN) of seedlings grown in 30 ppt salinity increased indicating that the reduction in Fv/Fm was due to increased heat dissipation, whereas the photochemical quenching (qP) was lower at 0 ppt reflecting the higher capacity of P680 reaction centers (Fan et al.,1999). These results help explain the better growth and physiological performance of seedlings of *L. racemosa* when grown at intermediate salinities. Other species, *Avicennia marina* and *Bruguiera gymnorrhiza*, were found to have higher Fv/Fm and electron transport rate (ETR) at a high salinity site of 35 ppt than at 12 ppt, the low salinity site (Naidoo et al., 2002). Photochemical (qP) and non-photochemical quenching (qN) were corres- pondingly lower in plants at the high salinity site. A possible mechanism for these observed reductions in Fv/Fm is a salinity-induced potassium deficiency causing loss of photosystem II (P680) function through depletion of the atrazine-binding polypeptide (Ball et al., 1987).

The results of this study are in accordance with these previously published findings, in that photosynthetic gas exchange rates (measured by stomatal conductance), as well as photosynthetic light reaction performance (measured by chlorophyll fluorescence) decreased as salinity stress increased. The use of leaf stomatal conductance and chlorophyll fluorescence as a measure of photosynthesis allowed a rapid and reliable quantification of a known stressor, salinity, in seedlings of *R. mangle*. It is suggested that these techniques can be applied to rapidly assess the health of mangrove plants in forestry, nursery, and restoration activities.

**REFERENCES**


Andrews TJ, Muller GJ, (1985). Photosynthetic gas exchange by the mangrove, *Rhizophora stylosa* in its natural environment. Photosynthetica. 14: 40-47.

Ball MC, Farquhar GD, (1984). Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long term salinity and humidity conditions. Plant Physiol. 74: 1-6.

Ball MC, Chow WS, Anderson JM, (1987). Salinity-induced potassium deficiency causes loss of functional photosystem II in leaves of the grey mangrove, *Avicennia marina*, through depletion of the atrazine-binding polypeptide. Aust. J. Plant Physiol. 14: 351-61.

Ball MC, Munns R, (1992). Plant responses to salinity under elevated atmospheric conditions of $CO_2$. Aust. J. Bot. 40: 515-525.

Bjorkman O, Demming B, Andrews TJ, (1988). Mangrove photosynthesis: Response to high-irradiance stress. Aust. J. Plant Physiol. 15: 43-61.

Cheeseman JM, Herendeen LB, Cheeseman AT, Clough BF, (1997). Photosynthesis and photoprotection in mangroves under field conditions. Plant Cell Environ. 20: 579-588.

Clark LD, Hannon NJ, (1967). The mangrove swamp and saltmarsh communities of the Sydney district, III. Plant growth in relation to salinity and waterlogging. J. Ecol. 58: 351-369.

Clough BF, (1984). Growth and salt balance of the mangroves *Avicennia marina* and *Rhizophora stylosa* in relation to salinity. Aust. J. Plant Physiol. 11: 419-430.

Downtown WJS, (1982). Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity. Aust. J. Plant Physiol. 9: 519-528.

Duke NC, Roelfsema C, Tracy D, Godson L, (2001). Preliminary investigation into the dieback of mangroves in the Mackay region. Report to the Queensland Fisheries Service. QDPI, Brisbane, Australia. p. 81.

Fan KC, Sheu BH, Chang CT (1999). Effects of salinity on the growth of the mangrove *Lumnitzera racemosa* seedlings. Quart. J. Chinese Forestry. 32: 299-312.

Flowers TJ, Hajibagheri MA, Clipson NJW, (1986). Halophytes. Quart. Rev. Biol. 61: 313-337.

Ghowail SI, Abdel-Monem AM, El-Ghamry WM, Saber NE, (1993). Preliminary studies on the effects of different salinity levels on germination, growth and anatomy of the mangrove (*Avicennia marina*). In: Leith H and Al Massom A (eds) Towards the Rational use of High Salinity Tolerant Plants, Kluwer Academic Publishers, The Netherlands, pp 237-244.

Hutchings P, Saenger P, (1987). Ecology of Mangroves. University of Queensland Press, St Lucia, Australia. 388p.

Kathiresan K, Thangama TS, (1990). A note on the effect of salinity and pH on growth of *Rhizophora* seedlings. The Indian Forester. 116: 243-244.

Lichtenthaler HK, (1996). Vegetation stress: an introduction to the stress concept in plants. J. Plant Physiol. 148: 4-14.

Lin G, Sternberg L, (1992). Effect of growth form, salinity, nutrient and sulfide on photosynthesis, carbon isotope discrimination and growth of red mangrove (*Rhizophora mangle*). Aust. J. Plant Physiol. 19: 509-517.

Lin G, Sternberg L, (1993). Effects of salinity fluctuation on photosynthetic gas exchange and plant growth of the red mangrove (*Rhizophora mangle*). Journal of Experimental Botany. 44: 9-16.

Naidoo G, Tuffers AV, Willert DJ, (2002). Changes in gas exchange and chlorophyll fluorescence characteristics of two mangroves and a mangrove associate in response to salinity in the natural environment. Trees-Structure and Function. 16: 140-146.

Odum WE, McIvor CC, Smith III TJ, (1982). The Ecology of Mangroves of South Florida. National Coastal Ecosystems Team, Fish and Wildlife Service, US Department of the Interior. 144p.

Pezeshki SR, deLaune RD, Patrick Jr. WH, (1989). Differential response of selected mangroves to soil flooding and salinity: gas exchange and biomass partitioning. Can. J. Forest Res. 20: 869-874.

Saenger P, Hegerl EJ, Davie JDS, (1983). Global Status of Mangrove Ecosystems. In: Anon (ed) Working Group on Mangrove Ecosystems of the IUCN Commission on Ecology, IUCN, Gland, Switzerland, p. 88.

Salisbury FB, Ross CW, (1978). Plant Physiology. Wadsworth Publishing Co. Inc., Belmont, CA. 422p.

Schreiber U, Bilger W, Neubauer C, (1995). Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis In: Schulze ED and Caldwell MM (eds) Ecophysiology of photosynthesis, Springer-Verlag, Berlin, pp 49-70.

Snedaker SC, Biber PD, Araújo RJ, Smith SM, Raineri KD, Dubart KM, (1996). Comparative oil/orimulsion spill assessment program: Task 4b – Mangroves. University of Miami, FL. 36p.

Sten WL, Voigt GK, (1959). Effect of salt concentration on growth of red mangrove in culture. Biol. Gazette. 9: 36-39.