The response of the red mangrove *Rhizophora mucronata* Lam. to changes in salinity, inundation and light: predictions for future climate change

By

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SUMMARY

Mangrove forests are subjected to many environmental factors which influence species distribution, zonation patterns as well as succession. Important driving factors in these forests are salinity, water level fluctuations and available light. This study investigated the response of red mangrove (*Rhizophora mucronata* Lam.) seedlings to these factors in controlled laboratory experiments. Increase in salinity and prolonged inundation within estuaries are predicted impacts resulting from sea level rise due to climate change. The study investigated the effect of five salinity treatments (0, 8, 18, 35 and 45 ppt) with a semi-diurnal tidal cycle on seedling growth. In a separate experiment the effect of different inundation treatments: no inundation, 3, 6, 9 hour tidal cycles and continuous inundation (24 h) were investigated. Both morphological and physiological responses of *R. mucronata* seedlings were measured. There was a decrease in growth (plant height, biomass and leaf production) with increasing salinity. Seedlings in the seawater, hypersaline and no inundation treatments showed symptoms of stress, having increased leaf necrosis ('burn marks'). The highest growth occurred in the low salinity (8 ppt) treatment, but the highest photosynthetic performance and stomatal conductance occurred in the freshwater treatment (0 ppt). The typical response of stem elongation with increasing inundation was observed in the 24 hr inundation treatment.

In the light and salinity combination study there were ten different treatments of five different light treatments (unshaded, 20%, 50%, 80% and 90% shade) combined with two salinity concentrations (18 and 35 ppt). In this study the seedling growth: plant height, biomass, leaf surface area and leaf production were higher in the moderate salinity (18 ppt) treatments compared to the seawater (35 ppt) treatments. Biomass in the 35 ppt experiment decreased with increasing shade as well as in the unshaded treatments. Photosynthetic performance and stomatal conductance were lower for the unshaded treatment in both 18 and 35 ppt salinity compared to all other treatments with the same salinity. This suggests that *R. mucronata* more shade than sun tolerant, but overall it can be concluded that the species has a broad tolerance range. The results may be relevant in mangrove rehabilitation and predicting responses to climate change. This is important as mangrove ecosystems may adapt to changing sea levels and in order to restore areas it will be necessary to choose the mangrove species which will grow best. The results may also help to increase the protection of existing mangrove habitats.

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1. GENERAL INTRODUCTION

The importance of mangroves and their response to climate change has recently been highlighted as important as these habitats are fast disappearing due to natural and human impacts (Nicholls *et al.,* 2007). Natural impacts include sea level rise and an increase in salinity occurring when tides push up further into the estuaries as well as the intertidal zone. This is also coupled with longer inundation cycles and changes in the intertidal region. The sea level rise was predicted to be between 10 to 20 cm in the last 100 years (Ye *et al.,* 2004) and an increased sea level was predicted to range between 0.05 to 0.11 m in the 21st century (Nicholls *et. al.,* 2007). This will have negative effects on low lying areas. Extensive modeling done by McFadden *et al.* (2007) suggests that as much as 33-44% of mangrove ecosystems may be lost between 2000 to 2080 due to an increase of between 36 and 72 cm in sea level. This however, would be site specific. This would apply, for example, to the Atlantic and Gulf of Mexico coast of North and Central America as well as the Caribbean and most small islands (Nicholls & Lowe, 2004). It is therefore necessary to study the impacts that these changes have on plant growth. The red mangrove *Rhizophora mucronata* Lam. is found worldwide from East Africa and India through Asia as well as Indonesia to the western Pacific, wet tropical regions of Australia (Duke, 2006) and into Mozambique and South Africa, where it is distributed from Kosi Bay in KwaZulu-Natal to the southern limit in Pondoland (Macnae, 1963; Steinke, 1995). However, some individuals of *R. mucronata* have been found further south at Wavecrest in the Nxaxo river mouth, Transkei (Adams *et al.,* 2004). High densities of this species have been recorded in Mngazana and the Mntafufu estuaries along the Transkei coast (Adams *et al.,* 2004). Because of its limited distribution, this is an important species that must be protected together with its associated ecosystems. The aim of this study was to determine the growth responses of the *R. mucronata* mangrove seedlings to different inundation periods, salinity concentrations and light conditions in order to predict the responses to climate change. Growth experiments were conducted in a controlled greenhouse environment where natural conditions were simulated as far as possible. This species was chosen for the study as it typically occurs in the lower intertidal zone, fringing rivers and lagoons (Duke, 2006). A typical feature of this mangrove is the large prop roots, used for anchorage as well as gas exchange. The results from this research can be used to predict the response of mangroves to climate change. Knowledge of the ecophysiological tolerances of the mangrove will also assist decision makers in rehabilitating mangrove areas. This is an important habitat to conserve as mangroves provide many

necessary services such as buffers against storms and tsunamis, as well important nurseries for biota.

The following hypotheses were tested in this study.

1. *Rhizophora mucronata* seedling relative growth rate (RGR) and size is significantly reduced by increased salinity (>50 % seawater). There will be an increase in biomass allocation to root mass relative to shoot mass in response to high salinity (>50 % seawater).

2. *R. mucronata* seedling relative growth rate (RGR) and size will be significantly reduced both by prolonged inundation (24 h at 50% seawater) and no inundation (0 h at 50% seawater). Prolonged inundation will stimulate stem elongation.

3. *R. mucronata* relative growth rate (RGR) and size will be significantly reduced under low light (>90 % shade, 16.8 µmol m^{-2} s^{-1}) and high salinity (>50% seawater) conditions.

1. LITERATURE REVIEW

2.1. The socio-economic importance of mangrove ecosystems

Globally the weather patterns are changing fast. This is due to natural and anthropogenic activities that have contributed to an already increasing sea surface temperature. It was reported in the last Intergovernmental Panel on Climate Change (IPCC) Report that, global temperatures will increase and there will be a significant increase in sea levels, which have been predicted to increase from 0.05 to 0.11 m in the 21th century alone (IPCC- Nicholls *et al.,* 2007). With climate change there will be other impacts, for example the magnitude and frequency of tropical storms, such as hurricanes and tsunamis, are predicted to increase. This will be devastating to many coastal areas the social, economical and environment of many coastal areas (Nicholls & Lowe, 2004). Nicholls & Lowe (2004) also warned that global warming is not the only threat to the environment, but the rapid increase of the human population is also a threat. In addition, they report that many of the world's coastal regions are prime residential areas for holiday makers and are inhabited by as much as 23% of the world's population. The vulnerability of these areas to sea level rise is apparent and numerous studies have been done, to find possible solutions for protecting the environment, human property and human life by protecting the natural vegetation of the coastlines, e.g. Kumar (2006).

Estuaries, especially in tropical and subtropical regions, are constantly changing environments that are particularly vulnerable to climate and sea-level change (Woodroffe, 2000). This will be detrimental to species in low lying areas, which due to sea level rise will have to cope with longer inundation periods and higher salinity concentrations in an uncertain future. Mangrove ecosystems are included in these regions and provide irreplaceable protection services to communities, who live there as they act as physical barriers against strong tides and storms (Dahdouh-Guebas *et al*., 2005). Vermaat & Thampanya (2006) suggested that mangroves mitigate tsunami damage as they found that land and property destruction was lower behind mangrove forests after such an event. *Rhizophora* spp. are examples of these species that form barriers as they are often the species fringing the coast and estuaries. Mangrove forests are the most threatened forests in the world (Valiela *et al.*, 2001) as more than 50% of these habitats have been destroyed, mostly due to anthropogenic activity, in the 20th century alone (Dahdouh-Guebas *et al.*, 2005). They also emphasized that forests are still being lost at a rate of up to 20% per annum. With the tsunami along the Asian coastlines in 2004, the importance of

mangrove ecosystems have become clearer and much research has been done to re-establish them in the areas where they were lost. Mangrove forests are known to be very resilient. However, natural regeneration will not counteract the destruction caused by logging timber (Blanchard & Prado, 1995), or the clearing of mangroves forests for shrimp and rice farming in Thailand and China (Woodroffe, 2000). Management of mangrove harvesting is necessary to reduce biodiversity loss (Rajkaran, *et al*., 2007). Coastal developments will limit the possible landward migration of mangroves caused by sea level rise (Parkinson, 1989).

Mangroves provide many environmental services. They produce large amounts of litter fall, which forms one of the most important links within the food webs, creating a unique habitat for other mangrove dependant biota (Barnes & Hughes, 2004). Mangrove habitats are also important breeding and nursery grounds for many different fish species which have economic importance to the fishery industry (Sheridan & Hays, 2003). These ecosystems play an important role in nutrient export into the ocean and also in the carbon cycle, as nutrient fluxes drive many microorganism activities in the ocean and contribute to the ocean's primary production (Duarte, *et al*., 2005).

2.2. The distribution of mangroves

Mangroves are also referred to as mangals. They can be defined as woody plants found as trees, but also as shrubs that occur in specific habitats and rarely somewhere else (Biber, 2006). They are halophytic plants with highly specialized flowering parts (angiosperms), and thus have terrestrial ancestry (Tomlinson, 1994). Mangroves are able to survive the harsh land and sea interface and are almost exclusively found within the intertidal to supratidal zones in suptropical to tropical areas (Ahmed and Abdel-Hamid, 2007). Previously it was believed that they occurred only in high rainfall areas, but it was found that they may occur in deserts as well (Singh & Odaki, 2004). This is because they have adapted physiologically to saline habitats which represent similar stresses to those found in arid environments (Balls & Sobrado, 1999).

The intertidal area has typical tidal fluctuations which are subjected to strong water currents and storm events, as well as a complex physico-chemical environment (Mauseth, 2003). For this reason these species are highly adapted to these environmental conditions. Periodic inundation by seawater and the changing of the tides create variable salinity conditions to which the plants have adapted. Soil characteristics, such as nutrients, sediment type, pH, redox potential and

salinity, together with biotic interactions have been shown in numerous studies to be important factors influencing the distribution of specific species of mangrove (Zomlefer, *et al.,* 2006). Species in different geographical areas may have different requirements as these ecosystems are extremely complex.

2.2.1 Tidal gradient and species zonation, response to light

The distribution of mangroves is greatly influenced not only by the coastal geomorphology, but also by geographical latitudes, wave action, aridity, salinity, nutrient inputs, soil quality (He *et al.,* 2007) and animal activity (Steinke, 1999). The succession of mangroves depends on the species growth potential, species competition, predation and natural dispersal of propagules (Steinke, 1999). Mangroves are "restricted to elevations between mean sea level and highest tides" (Duke, 2006) and, as sea levels rise, these communities will have to migrate landwards. However, the successful re-establishment and recruitment depends greatly on available space. The natural formation of forest depends on the intensity and frequency of disturbance (Proffitt, *et al.*, 2006) such as tree fall due to storms or after heavy flood events. Understanding these ecosystems and their structural characteristics and physiological responses to environmental changes will enable more successful regeneration of the forests (Ward *et al.,* 2006). The relationships between different life forms and the physico-chemical factors are very complex and there is much that still needs to be understood.

Kathiresan & Bingham, (2001) identified different types of mangrove forests, which can be summarized as:

1) "Overwash mangrove forest" (formed by tidal washings and thus creating small mangrove islands);
2) "Fringing mangrove forests" (mangals that are influenced by tidal inundation and occur in sheltered regions);
3) "Riverine mangrove forest" (mangals that occur along rivers and creeks. They are also influenced by daily tidal inundation);
4) "Basin mangrove forest" (mostly stunted trees that are found in the interior part of a swamp);
5) "Hammock mangrove forests" (are also stunted trees that occur on higher elevation); and
6) "Scrub mangrove forest" (typically dwarfed trees along coastal fringes).

Salinity is considered to be one of the most important driving forces for mangrove zonation patterns (Suarez and Medina, 2008). However, other factors such as light also influence the zonation patterns of mangroves. Light changes within the forest, especially under the canopies (Russel *et al.,* 1989) and in response to temporal disturbances such as storms, tree fall and sea level rise which influence the edges of the shores (Kathiresan & Bingham, 2001).

The photosynthetically-active radiation (PAR) is the solar radiant energy used by plants, ranging between 400 and 700 nm for fixing carbon. Maximum PAR or direct sunlight is around 2000 µmol m^{-2} s^{-1} (Demmig-Adams *et al.,* 2006). When plants are adapted to grow in shady conditions and are suddenly exposed to increased or high light, then a process called 'photoinhibition' would expected to be the immediate response to the light changes. Photoinhibition can be defined as the "light-dependent loss of photosynthetic efficiency, normally occurring under conditions of light harvesting antennae absorbing more excitation energy than can be dissipated by photochemistry of photosynthesis" (Rama Das, 2004). Excessive light is damaging to the photosynthetic activity especially to the photosystem II. The damage due to excessive light will negatively affect plant growth (Demmig-Adams, *et al.,* 2006).

The shade adapted plants on the forest floor are able to grow in these low light conditions, where up to 0.5% of irradiance is only available for photosynthesis (Critchley, 1988). Many studies have been done to investigate how different light conditions affects photosynthesis, and how plants adapt morphologically by having different leaf characteristic that develop with different light saturations. According to Smith and Smith (2001) immediate response to reduced light levels (PAR below light saturation) is a reduction in the rate of net photosynthesis per unit of leaf area. However, plants that are exposed to low light over longer periods will adapt by shifting their biochemistry, physiology and morphology. Morphological changes in plants as a response to long term low light would be increased leaf surface area, thinner leaves, as well as a greater leaf production compared to those grown in high light conditions. Table 1 indicates the general adaptations for sun and shade plants (Givnish, 1988). Increased leaf surface area would be due to a compensation for limited light availability. The shift from root biomass production would be expected i.e. with increasing shade there will be a decrease in the ratio of root biomass (g) to leaf area (cm^2) (Smith and Smith, 2001).

Table 1: Characteristic differences between plants adapted or acclimated to sunny versus shady extremes (Givnish, 1988)

Trait	Sun	Shade
Leaf –level		
Photosynthetic light response		
Light-saturated rate	High	Low
Compensation irradiance	High	Low
Saturation irradiance	High	Low
Morphology		
Leaf mass	High	Low
Leaf thickness	High	Low
Stomatal size	Small	Large
Stomatal density	High	Low
Canopy level		
Leaf area-index	High to Low	Low
Plant level		
Fractional allocation to leaves	Low	High
Fractional allocation to roots	High	Low

Within South Africa, the most common mangrove is *Avicennia marina* (Forssk.) Vierh., which is found to be the most widespread along the coastline and is usually a pioneer species among the mangroves, where it can be found to occur in dense monospecific stands. *A. marina* can establish rapidly on newly formed mudbanks and has a wide tolerance range (Steinke, 1999). Another common mangrove species along the South African coastline is *Bruguiera gymnorrhiza* (L.) Lam but it is not considered as a pioneer mangrove (Steinke, 1999), but rather prefers to grow in the high intertidal region with less frequent inundation than *A. marina*. In the vulnerable seedling stage *B. gymnorrhiza* also prefers shaded conditions. *B. gymnorrhiza* however, will out compete *A. marina* at times of mouth closure as this high inundation will affect the roots of *A. marina* negatively *Rhizophora mucronata* is found to be less common than *A. marina* and *B. gymnorrhiza* in South Africa. *R. mucronata* are more restricted in their distribution, where it occurs along channels and fringing river habitats (Steinke, 1999).

2.2.2. Germination and Propagule establishment

The dispersal and early growth patterns of seedlings helps determine species distribution and abundance within mangrove forests (Clarke *et al.,* 2001). Mangrove reproduction differs from other plants as the investment in seeds is extremely high and unusual. The seeds also known as 'propagules', have a vivipary adaptation germinating while still attached to the parent tree. The means of dispersal is predominantly by water or seedlings may establish right under the parent plant. When seedlings are 'mature' (of a certain colour and size) they drop from the flower (Tomlinson, 1994). According to Rabinowitz (1978) ecological sorting at the early life-history stage has important influences on seedling distribution and, as a consequence, the differentiation within a mature mangrove forest. These would also include the tidal sorting of propagules, where the heavier propagule producing species occur close to the water fringes and those having light propagules are found to be more landward (Rabinowitz 1978). The heavier propagules are long and cigar shaped (hypocotyls) as seen in *Bruguiera* and *Rhizophora* species and fall spear like into the mud where they root (Branch & Branch, 1995). This reduces the chances of being swept out to sea by the incoming tides. In contrast, *Avicennia* species have round seeds that are carried around by the water before they root and establish in a new area (Delgado *et al*., 2001). This makes it a successful pioneer species. A study by Delgado *et al*. (2001) on *Laguncularia racemosa*; *Avicennia germinans*; and *A. bicolor* showed that propagule buoyancy and water movement affected distribution. The *Avicennia* spp. were most dominant in the upper intertidal zone. *Rhizophora mucronata* has large and propagules that are stay afloat for awhile (several months) before rooting. The floating period is influenced by salinity as well as light for initial seedling establishment (Hogarth, 1999). He also stated that large propagules travel only short distances from parent plant.

2.2.3. Sediment characteristics

Mangrove distribution is largely dependent on the sediment availability where climate change may affect the geomorphology of areas (Field, 1995). Mangroves grow in a variety of sediments which include sand, mud, peat and coral rock. However, the preferred sediment is the muddy soil typically found in deltas, lagoons, bays and estuaries (Singh & Odaki, 2004). These can be characterized into different sediment habitats, described by Chapman (1940) in Singh & Odaki (2004). The different sediment habitats are:

1) “The muddy substratum” (which can be found close to the mouth area of rivers or estuaries);

2) “The rocky substratum” (found at rocky outcrops or coral reefs where mangroves may have established);
3) “Sandy substratum” (sandy soils such as sand shoals, sand bars, and sand cays found to be close to the ocean) and
4) “Peaty substratum” (These are found at sheltered coasts where the river flow has little influence on the system).

Duke (2006) also suggested that mangroves to some extent create their own physico-chemical conditions of the sediment and are also responsible for stabilizing it. Studies done by various authors have contributed to the understanding of the important relationship between the mangrove roots and the sediment it grows in. For example, Lacerda *et al.*, (1993, 2001) found that some mangrove genera such as *Avicennia* and *Rhizophora* spp. influenced the soil redox potential by the physiological activities of the roots.

2.2.4. Temperature

The northern limits for mangrove distribution are around the 16 ºC January isotherm while the southernmost limit (Atlantic and Indian Ocean) is the 16 ºC July isotherm (Duke *et al.* 2006). Mangroves are typically found in areas that have 20 ºC and greater atmospheric temperature and where the seasonal temperature fluctuation is not more than 5 ºC (Singh & Odaki, 2004). Due to the biogeographical range of mangroves, their distribution is largely controlled by temperature. Duke (2006) suggests that they may tolerate low temperatures (up to 10 ºC in Australia), but are particularly sensitive to frost.

Seedlings are most vulnerable to low temperatures. Sea temperature plays an important part in the distribution of the mangrove species (Tomlinson, 1994). Mangroves can grow in sea surface temperatures of 24 ºC and optimal growth is known to be between 28-32 ºC (Singh & Odaki, 2004) with a relative atmospheric humidity above 60%. However, an increase in temperature will influence the species distribution as mangroves are typically sensitive to extreme temperatures (Field, 1995). Temperatures between 38 to 40 ºC will reduce the photosynthetic capacity within the mangrove leaves (Kathiresan & Bingham, 2001). This was shown in a study

by Steinke & Naidoo (1991) for *Avicennia marina* where photosynthesis decreased with increasing temperature up to 35 ºC. This had negative effects on the overall growth of the plants. Most mangroves have an optimal ambient temperature of 25 ºC.

Maintaining high water use efficiency and lower leaf temperatures increases carbon fixation and thus competitive advantage (Hogarth, 1999). With global warming and the resulting sea level rise, it is expected that mangrove habitats will shift more towards the head of the estuary (Parkinson, 1989). However, with increasing human population and developments the landward expansion of mangroves will be prevented, leading to habitat loss (Kjerfve & Macintosh, 1997).

2.2.5. Salinity, Rainfall and Freshwater runoff

With increasing global temperature it is predicted that there will be reduced rainfall and freshwater runoff. This will result in increased salinity and increased seawater-sulfate concentrations which will result in decreased mangrove production in those areas (Snedaker, 1982). In general, saline environments create two problems for terrestrial plants: osmotic regulation and toxicity. With increased salinity plants have to cope with osmosis. This is the immediate effect (Smith & Smith, 2001). Plants have to absorb inorganic ions to counteract the osmotic gradient, but an excess of these become toxic and have negative effects on plant growth, reproduction and survival (Mehlig, 2006).

Halophytes such as mangroves are able to flourish in saline environments. Desert plants, which have evolved to grow in low moisture and high saline environments, have made similar adaptations to harsh environmental conditions (Duke 2006). Studies by Kathiresan *et al.,* (1996) have shown that mangroves produce more biomass in lower salinities (5-18 ppt), which resemble arid conditions even if water is plentiful. The saline conditions result in responses in plants, as a change in salinity will cause a change in the plant's photosynthesis, photosynthetic pigment content, transpiration rate as well as in the enzyme activity (Falqueto *et al*., 2008). Naidoo (1987) stated that with increasing salinity there may be a decrease in growth as plants have to adjust to the increasing osmotic potential. This has be seen in numerous salinity experiments showing that at high salinity (>18 and 25 ppt) mangroves invested energy in water balance maintenance through ion accumulation within the tissues (Suarez & Medina, 2008) and consequently produce less biomass (Clough, 1984; Naidoo, 1985; Li *et al.,* 2008). Depending

on the species (Table 2) they may grow in a wide range of salinity conditions from freshwater to seawater (35 ppt). Different species have different tolerance ranges (Suarez & Medina, 2005).

Mangrove forests have much higher biomass production in regions of low salinity and where rainwater runoff provides the available nutrients (Kathiresan & Bingham, 2001). These plants have the ability to cope with high salinities due to their specialized adaptations: by maintaining high osmotic potential, allowing high salinity conditions within the root tissue, elimination of salt by salt secreting glands and by limiting, or even excluding, salt uptake into the roots. In some systems where hypersaline conditions occur, for example in Pakistan in the Indus Delta (Aziz & Khan, 2001b), they are able to survive due to their saline exclusion abilities. Kathiresan & Thangam (1990), suggest that fluctuations in salinity within a system seem to have more profound effects than continuous hypersalinity, where adult plants have higher tolerance ranges compared to seedlings. This is because younger plants are generally more sensitive to salt and grow best in low salinities and become more salt tolerant as they grow into trees (Schmitz *et al.* 2006). Schmitz *et al.,* (2006) also suggest that seedlings of *Rhizophora mucronata* grow well in high salinity such as 30 ppt, but there are differences, even within the same genus, as well as for different ages. For example, *R. apiculata* seedlings prefer to grow in lower salinities of 15 ppt. *Rhizophora* has adapted to salt accumulation (3 ppt) within the plant tissues. This is up to 100 times higher than that of other terrestrial plants. It achieves this by having special salt glands on the leaves where it can excrete saline fluids of up to 40 ppt. *Avicennia* species are known to exclude up to 90 % of salt from the root surface, which may increase if the salinity concentration of the surrounding water increases (Branch & Branch, 1995). It is suggested that this exclusion is achieved by creating a negative hydrostatic pressure within the plant providing enough negative osmosis pressure within the roots to allow water to enter, while all other ions are excluded (Moon *et al.,* 1986 in Hogarth, 1999). Despite these adaptations salinity at high concentrations and for prolonged periods will affect mangroves as seen in studies by Naidoo (1985) where both *Bruguiera gymnorrhiza* and *Rhizophora mangle* had negative growth rate (Koch & Snedaker, 1997).

Table 2: Documented salinity ranges for various mangrove species (adapted from Riddin & Adams, 2007).

Species	Optimum range (ppt)	Effects of salinity change	Reference
Avicennia marina	5 - 35	0 ppt for 6 months results in reduced growth; > 20 ppt delayed germination and reduced growth; > 35 ppt caused stunting	Downton (1982), Ball and Farquhar (1984), Burchett *et al*. (1984), Clough (1984), Naidoo (1987)
Bruguiera gymnorrhiza	≥ 10	> 35 ppt reduced seed growth and germination and caused senescence	Ward (1976), Steinke and Charles (1986), Naidoo (1990)
Ceriops tagal	5-16	> 30 ppt for 5.5 months reduced growth and seed germination; > 42 ppt for 5.5 months no seedling growth	Smith (1988)
Rhizophora mucronata	17.5	Reduced growth at salinity > 17.6 ppt	Khan & Aziz (2004)

The general response of plants to high salinity would be reduced growth, such as reduced expansion rate of the leaf area and leaf production rate (Lugo *et al.,* 2007). Excess salt is dealt with by depositing it in the leaves which are then shed. *Bruguiera gymnorrhiza* and *Rhizophora mucronata* are also salt excluders that accumulate salt in the more mature leaves, which are shed to rid salt from the plant (Steinke, 1999). *Avicennia* also has salt glands and excretes salt through leaves. However, these are only formed in highly saline conditions. Leaf shedding and leaf production have important physiological consequences to the plant as they have an effect on the total leaf area. This in turn again influences carbon fixation as well as the nutrient uptake of the whole plant (Suarez & Medina, 2005).

Mangroves have to cope with strong external salt gradient and do so by regulating ion and water uptake (Ball *et al.* 1997). " To maintain water uptake, mangroves not only have to restrict water loss by having conservative morphological and physiological adaptations, but also they need to maintain sufficiently low water potentials" (Krauss, *et al*., 2008).

Sodium and chloride ions are higher in mangrove tissues than in other plants and this is known to inhibit some important enzymes. Within mangroves the enzymes are more resistant to inhibition and are largely protected by other solutes in the tissues. Previous studies showed that different species of mangroves have different salinity tolerance ranges and most species require some salt for optimal growth and grow poorly in freshwater alone (Pezeshki, *et al*., 1989).

Optimal salinities vary for different species and the optimal salinity range reported for *Avicennia marina* has been different for different geographical regions i.e. between 10 to 50% seawater and for *Rhizophora mangle* it may be 25% and 100% seawater, whereas *R. mucronata* is normally found in low salinities (< 35 ppt, Smith & Lee, 1999). However, "mangrove species vary more in range of their tolerance than in salinity for optimal growth" (Hogarth, 1999). Suarez & Medina (2005) showed that *Avicennia geminans* seedlings were significantly affected by salinity and had a high salt tolerance compared to other similar species. They suggested that this may be due to the expense of low relative growth rates (RGR) and possible effective water-use efficiency. This is because salinity tolerance comes at a high price as saline conditions make water less available to the plants than in non halophytic conditions (Aziz & Khan, 2001a). Mangroves cope with less available water by increasing their root biomass to compensate for water uptake. *Rhizophora mangle* had a shift of biomass allocation from the leaves to the roots with the root mass increasing with increasing salinity (Lopez-Hoffman *et al.,* 2006 and 2007).

Even though water supply is in excess, the plants cannot readily take it up due to the high saline concentrations and osmotic potential. However, to cope with this problem, mangroves have additional adaptations to use water more efficiently. For example, with high soil salinity, stomatal conductance will be reduced to conserve water from transpiring. This will negatively affect the uptake of carbon dioxide and thus carbon fixation, which in turn reduces plant growth (Jayatissa *et al.,* 2008). It is also known that small leaved mangroves, for example *Avicennia,* cope better in higher salinities than most large and broad leaved species (Hogarth, 1999).

Lopez-Hoffman *et al*., (2006) also suggested "that there are physiological trade-offs between growth and survivorship, it may be that at high salinity seedlings invest more energy and resources in survivorship at the expense of growth, whereas at low-salinity, seedlings invest more in growth than in survivorship." A study on adult *Rhizophora mucronata* trees by Schmitz *et al*., (2006) determined that the trees responded to high salinity by increasing their vessel density to facilitate increased and better water transport in hypersaline environments.

Mangroves are very successful in stressful intertidal areas by coping with saline concentrations and waterlogging, as mentioned before. This however, comes with a cost such as reduced leaf area and photosynthesis. These may explain why there are many dwarf *Avicennia* plants found

in stressful habitats such as those described by Aziz & Khan (2001); Naidoo (2006) and Ahmed & Abdel-Hamid (2007).

The importance of freshwater supply to mangrove ecosystems has been emphasized in previous studies from Kitheka (1998); Schwendenmann *et al*., (2006) and Mazda & Ikeda (2006), where freshwater runoff and groundwater were found to sustain large areas of mangrove vegetation. Freshwater input not only dilutes the saline waters, but also provides essential nutrients and minerals for the plants (Schwendenmann *et al*., 2006). However, "the physical behaviour and hydraulic mechanism of groundwater are suspected to depend on the topography of a particular region" (Mazda & Ikeda, 2006).
Mangrove trees are dependent on the available groundwater supplies and an intrusion of seawater into the groundwater system would result in an exclusion of mangrove forest in these affected areas (Kitheka, 1998).

2.2.6. Inundation

Watson (1928) in Tomlinson (1994) was one of the pioneer scientists who described the importance of tidal flooding for mangroves and classified this according to different inundation classes (Table 4).

Table 3: Watson's inundation classes for mangrove habitats

Inundation class	Flooded by	Height above Datum line (feet)		Times flooded per month	
		From	To	From	To
1	All high tides	0	8	56	62
2	Medium high tides	8	11	45	59
3	Normal high tides	11	13	20	45
4	Spring high tides	13	15	2	20
5	Abnormal (equinoctial tides)	15	-	-	2

Tomlinson (1994) has said that this classification may be too simplified as mangrove habitats are complex and interconnected ecosystems, where each area is unique. However, the inundation classes can be used as a guideline for mangrove distribution along waterways.

All terrestrial plants that are actively photosynthesizing and growing need enough water and rapid gas exchange with the surrounding environment. This exchange also occurs between roots and the sediment (Smith & Smith, 2001). Prolonged waterlogged soil conditions provide problems for any plant in maintaining effective respiration as the pores between sediment particles become filled with water and oxygen diffusion is limited. This reduces soil redox potential and gaseous exchange (Pezeshki *et al.,* 1997).

Mangroves are adapted to periodic waterlogging, but different species respond differently to the period of inundation (Table 3) (Luzhen *et al.*, 2005). Prolonged inundation can cause mangrove die-back. This occurred in the Kosi Estuary in KwaZulu Natal, South Africa when the mouth closed and water level increased. Large areas experienced mass die offs of mangroves (Branch & Branch, 1995). Mouth closure in such systems is normally a rare event, but may result from heavy storms; however freshwater abstraction in the upper reaches may also pose a threat. Table 4 indicates that a water level increase to 50cm caused die-back of three species.

Table 4: Documented water levels for various mangrove species (adapted from Riddin & Adams, 2007).

Species	**Optimum range**	**Influence of water level change**	**Reference**
Bruguiera gymnorrhiza	0-75 cm		Ward (1976)
Avicennia marina		Die-back from 50 cm increase for 154 days	Breen and Hill (1969)
Ceriops tagal		Die-back from 50 cm increase for 154 days	Breen and Hill (1969)
Rhizophora mucronata		Die-back from 50 cm increase for 154 days	Breen and Hill (1969)

Mangroves are particularly sensitive to soils that have low oxygen concentrations. These can become completely anoxic over time, except for the first top centimeters of the surface (Krauss *et al*., 2008). With the low oxygen concentrations, plants will have to shift from aerobic respiration to anaerobic respiration. Anaerobic soil conditions also pose another problem, as they inhibit the uptake and transport of ions within plants and thus largely reduce the concentrations of nitrogen, phosphorus and potassium in the leaves. This affects biomass accumulation in the plant as a whole (Pezeshki *et al.,* 1997). This occurs in the following stages; firstly oxygen depleted soils have a shift in aerobic to anaerobic bacterial activity. This condition or oxygen reduction process is easily measured as redox potential. According to Hogarth (1999) anaerobic bacteria convert nitrate to nitrogen. At a later stage irons are transformed from Fe3+

(ferric) to Fe2+ (ferrous) irons. The ferric salts are insoluble while ferrous irons are more soluble. Therefore these soluble irons and inorganic phosphates are easily released and, although they can be used by plants, in large amounts they may become toxic**.** In addition, with very low redox potentials within the soil, sulphate is reduced to toxic sulphide. This is very important as plants are sensitive to both this and the conversion of carbon dioxide to methane (Boto, 1984). Both these reactions cause the mud to have a characteristically bad odour.

Oxygen is needed for respiration. Mangroves have evolved to cope with these low oxygen conditions in the soil by having modified roots, known also as 'aerial roots', which differ in morphology between species (Mauseth, 2003). In *Rhizophora* species the roots characteristically diverge from the main stem and may extend over some distance before penetrating the mud again. These roots are known as stilt or prop roots (Branch & Branch, 1995). They provide support to the plant as well as aeration. In flooded conditions, when oxygen concentrations are low, the cells within the roots are stimulated to form interconnected gas-filled chambers, known as 'aerenchyma'. During low tide, oxygen enters small pores (lenticels) on the roots and is passed down to the submerged roots in the sediment (Smith & Smith, 2001).

In *Rhizophora* species the roots within the sediments are mostly made up of aerenchyma tissue. These air spaces provide rapid diffusion of oxygen from the lenticels to the rest of the submerged root system (Lovelock, *et al*., 2006b). Depending on the sediment conditions the air spaces differ. In sand for example, the soil is well drained and oxygenated. The air spaces in sand prevent it from becoming much less anoxic than muddy sediments. Hogarth (1999) suggests that in waterlogged soils the roots need a higher proportion of air spaces for the increased demand of air movement. Different forms of roots exist in other mangrove species. For example, *Bruguiera* spp. have many "shallow horizontal roots" (Duke, 2006) that emerge from the sediment and these make loops before they penetrate the soil again. These are also known as "knee roots".

Avicennia species have many "horizontal roots that radiate outwards" (McKee, 2001). They have vertical roots that may grow up to about 30 cm long, emerging vertically from the sediment. They are also known as 'pneumatophores', and contain many lenticels that provide air movement to the roots. These are hydrophobic, and, through respiration during high tide, carbon dioxide dissolves into the surrounding water reducing the pressure within the roots. Oxygen that was used for respiration will be replaced when the lenticels open during low tide,

when exposed to the atmosphere. It is suggested that the gas transport provides oxygen even to the surrounding soil. The increasing anoxic condition results in the plants being stimulated to increase the number of pneumatophores to cope with the demand for oxygen (McKee, 2001).

Adaptation to both saline and anoxic conditions is rare. The extremes together are avoided as can be seen in Australian mangrove species that are distributed according to certain tolerance ranges of salinity and waterlogging. This means that species that are adapted to high salinity do not occur in waterlogged habitats and vice versa (Krauss *et al,* 2008).

2.2.7. Nutrients

For plants to photosynthesize and grow, sufficient amounts of nutrients are required. Nitrogen and phosphorus, converted to inorganic nitrate and phosphate, are among the most important sources. Inorganic nutrients are added to the system by rain water run-off and others are of terrestrial and oceanic origin. In the past it was believed that mangrove trees created their own mud. It is true, however, that they create a complex relationship between the microorganisms. They are responsible for trapping sediments, which again hold the organic and inorganic nutrients that they need for growth (Schwendenmann *et al.,* 2006). They also reported that freshwater run off and ground water flow though the mangrove forests are important for nutrient cycling. Tidal cycles and rainfall also influence the nutrient cycles (Kitheka, 1998; Mazda & Ikeda, 2006).

Bacteria play a vital role in fixing atmospheric nitrogen for many plants and there may be similar relationships in mangrove systems. Species that have some nitrogen-fixing ability are *Rhizophora mangle* and *Avicennia germinans* (Hogarth, 1999). The most important bacterial activity is the breakdown of byproducts into forms that are available to the plants. As mentioned before, the soil is mostly anoxic producing ammonia which diffuses to the upper more aerobic zone. There it is then broken down further by aerobic bacteria into nitrate, which may be taken up by the roots of the mangroves. This available nitrate depends on the amount of available ammonia, and mostly on the availability of aerobic bacteria. Inundation therefore plays a vital role in nutrient availability (Ewel *et al*., 1998). Animal activities, such as crab holes, influence oxidation of the soil as do leakages from roots to the surrounding soil (Kathiresan and Bingham, 2001). Crab activity also contributes to the cycling and availability of nutrients in these forests (Ruwa, 1990).

Phosphorous settles within the sediment as ferric phosphate, where anaerobic bacteria reduce ferric phosphate to ferrous phosphate. The ferrous phosphates are known to be soluble and may therefore be lost to the soil, and thus the plants**.** However, this greatly depends on the soil characteristics and porosity**.** For example, fine clayed soils have better water holding capacity, trapping the phosphate in the soil. This is noticeable as plants have increased growth in these soils. Nutrients may limit mangrove growth as more sediment is deposited in the lower shore so will it also trap more phosphates. The lower waters within the intertidal zone are inundated with water for longer periods and more frequently than the high water mark, resulting in higher concentrations of phosphates (Boto & Wellington, 1984).

3. MATERIAL & METHODS

3.1. Propagule collection prior to the study

Mature propagules of the species *Rhizophora mucronata* were collected from the Mngazana Estuary (31º 42'S, 29º25'E) located south of Port St. Johns on the Wild Coast of the Eastern Cape Province in South Africa. Five propagules were planted in each plastic pot containing mangrove mud collected in the same area. All prepared pots (2 * n = 25 for the salinity and inundation study and n=50 for the salinity-shade study) were kept in a greenhouse with air temperature of 27-35 ºC and under natural light to allow seedling establishment. Propagules were allowed to germinate and grow for ± 3 months prior to the experiments. Then all established seedlings within the pots were moved from the Nelson Mandela Metropolitan University's greenhouse to Bayworld's Research Laboratory facilities.

3.2. Tank-set up in Research Laboratory at Bayworld Oceanarium

The idea for a simulated tidal tank set-up was similar to that used by Luzhen *et al.* (2005) although the tanks and their additional systems were altered for the purposes of this study. The research laboratory was restructured similarly to greenhouse conditions, by providing natural light (transparent corrugated roofing and large windows) and a continuously controlled ambient temperature using a manual extractor fan (Xpelair model 90012 AW) and monitored with a data logger.

Within the Research Laboratory at Bayworld Oceanarium, two custom made tanks were set up next to each other. Thick glass (10 mm) was used to provide support for large volumes of water. The dimensions of each tank were length 2.64 m, width 0.52 m and height 0.60 m. These were separated into five equal sized compartments (0.52 x 0.6 x 0.52 m) each containing 162.24 liters of water. Each tank was placed on a galvanized steel stand 0.6 m from the floor (Plate 1, A). Each compartment contained an inlet and an outlet pipe, a siphon on outlet pipe, thermostats, air tubes and air stones to create water circulation and provide aeration. All compartments also contained crates for pots to stand on. The volume of the crates and pots with the seedlings was taken into account when calculations were done for programming the tidal cycles of each compartment / treatment.

3.3. Apparatus for the simulation of natural estuarine tidal inundation and salinity variation

3.3.1 Experimental design

A three part flow-through and recycling system was designed to simulate natural tidal cycles. This experimental design was modified by Dylan Bailey. The system consisted of four 1000 liter storage batch containers, a custom-built programmable unidirectional liquid multiplexer that consisted of four input and twelve output points and two large five-compartment tanks (See Figure 1). Fresh seawater was pumped from the sea and fed from the Bayworld Aquariums to the storage containers, when full then the water was pumped into the system. Three water sources of varying salinity (0, 35 and hypersaline, 45 ppt) continuously supplied the tanks. All incoming water was treated beforehand. The freshwater was filtered through two activated carbon filters. The seawater went through a reticulation process where incoming water was treated with chlorine, then mechanically filtered with hydro-anthracite and dechlorinated with activated carbon filtering. The freshwater and seawater supplies were configured as a flow-through system because the water passed through the tanks only once and went to waste thereafter. However, the hypersaline water supply was recirculated between the treatment and 1000 liter storage batch container. The batch was replaced twice during the 14 week study period. The hypersaline water was made using ready to use aquarium salt (aQuality Research grade Synthetic Reef Salt, Cape Town, South Africa).

Each storage batch container had its own water pump (Eden 140, 3000 litre/hour, 3 m head). The water flow of the pumped water was throttled back to 500 litre/hour. This was calibrated by testing the water flow using a 5 litre jug and a stopwatch. The delivery capacity was then adjusted using the flow control valve on each pump. This was a simple, but important calibration process which determined the amount of water pumped to the tanks and used in the programming of the tidal flow system of each treatment.

The water supply of each storage batch container was fed into a four input by twelve output multiplexer.

3.3.2 The multiplexer

The components of the multiplexor are shown in Figure 2. It consisted of a rotating delivery pipe made of PVC piping, which routed the mixtures to one of twelve receiving funnels. Each funnel was connected to the pipe running to the corresponding tank. The four lines coming from the storage batch containers into the multiplexer were used to mix the water. Only ten of the twelve multiplexer outputs were used for the experiment. Each lead to the tank representing a certain treatment (five compartments were used for salinity and inundation treatments respectively).

The multiplexor provided the treatment water based on the following calculation; each 36 sec at 500 litre/hour provided 5 litres of mixed treatment water. Each pump turned on sequentially delivering five litres for each part depending on the requirement of the salinity concentration in each treatment (Details included in Appendix I). The rotating arm of the multiplexer was connected to a motor and encoder which were in turn connected to a Programmic Logic Controller (PLC) (Allen-Bradley Micrologix-1000) which determined the mixing process and was programmed according to the needs of the experiment. The system had an electricity backup UPS (UPScom model 4CZ1400) (uninterruptible power supply) in case of power interruptions, with a 1kVa capacity that allowed for one complete 12 hour cycle to run before becoming depleted.

3.3.3 Programmed Logic Controller

Water supply was programmed on the PLC that ran as a time based sequencer, running in 15 min intervals, also known as steps. For example, to make up a 12 hour tidal cycle there were 48 steps in total. For each step the programme had the compartment number, representing the different treatments, as well as the number of parts that were required to make up these treatments, from each of the three sources coming from the 1000 liter storage batch containers. For tidal simulation each compartment had a siphon pipe which allowed the tanks to drain to the low water mark (LWM) (low tide just covered the bottom of the tank with 5 cm of water) when the compartments were overfilled. The simulated high tide caused all seedlings to be flooded with only the upper foliage exposed. Overfilling the compartments was used to start the siphoning that created a low tide. To create a tidal change from low to high tide the programme was able to allow the pumping of a certain already mixed batch into the required compartment until the HWM was reached. Tidal change was initiated by pumping just enough of the same mixture into the treatment to start the siphon automatically, draining the water and resulting in a

low tide again. The drainage pipes or outlets of the siphon were set to either of two drains, one leading to waste, resulting in a flow-through system, and the other drained one of the hypersaline storage batch containers which was used for recirculation (See Figure 3). Salinity treatment concentrations of 0, 8, 18, 35 and 45 ppt were chosen, where 18 ppt represented the control and 35 ppt was undiluted seawater while 45 ppt represented the hypersaline concentration. These treatments all had a semi-diurnal tidal cycle. Different tidal cycles were used for the inundation experiment. The no inundation treatment (0 h) consisted of standing water as did the continuous inundation treatment (24 h). The other three treatments were 3, 6 and 9 h tidal cycles. These treatments all had the same salinity concentration of 18 ppt.

3.4. Light and Salinity experimental tank and shade cloth set up

In the basic design for the shade cloth set-up, as shown in Figure 4 and Plate 1 B, two cables supported the different pieces of shade cloth that were fastened with cable-ties on to the cables to secure the position over the treatments. For the light-salinity combination experiments, two salinity concentrations were chosen, 18 ppt (50% seawater) and 35 ppt (100% seawater). These were combined with five different light conditions; unshaded, 20%, 50%, 80% and 90% shade. Hence there were in total ten different treatments. The light available to the plants was reported as the percentage grade of shade cloth as sold by the manufacturer Cape Shade. The mean light conditions were measured on four occasions during the study period using a LI-COR Quantum/Radiometer/Photometer (Model LI-189, USA). The mean light measurements for the different light /shade treatments were:

unshaded ~ 843.7 ± 67.30 µmol m^{-2} s^{-1}
20% shade ~ 450.5 ± 33.82 µmol m^{-2} s^{-1}
50% shade ~ 281.17 ± 18.95 µmol m^{-2} s^{-1}
80% shade ~ 131.5 ± 6.48 µmol m^{-2} s^{-1}
90% shade ~ 16.59 ± 1.0 µmol m^{-2} s^{-1}

Shade cloth with the above specifications were chosen as Rajkaran (2009, unpublished data) has shown that light under the adult *Rhizophora mucronata* mangrove stands in the Mngazana Estuary ranged between 182 - 901 µmol m^{-2} s^{-1}, measured over two summer and one winter period. The available literature (Appendix 6) was also consulted to see what light ranges were used in other controlled light experiments with mangroves

A.

B.

PLATE 1: A. The salinity and inundation experimental set up; B. The light-salinity experimental set up, using shade cloth.

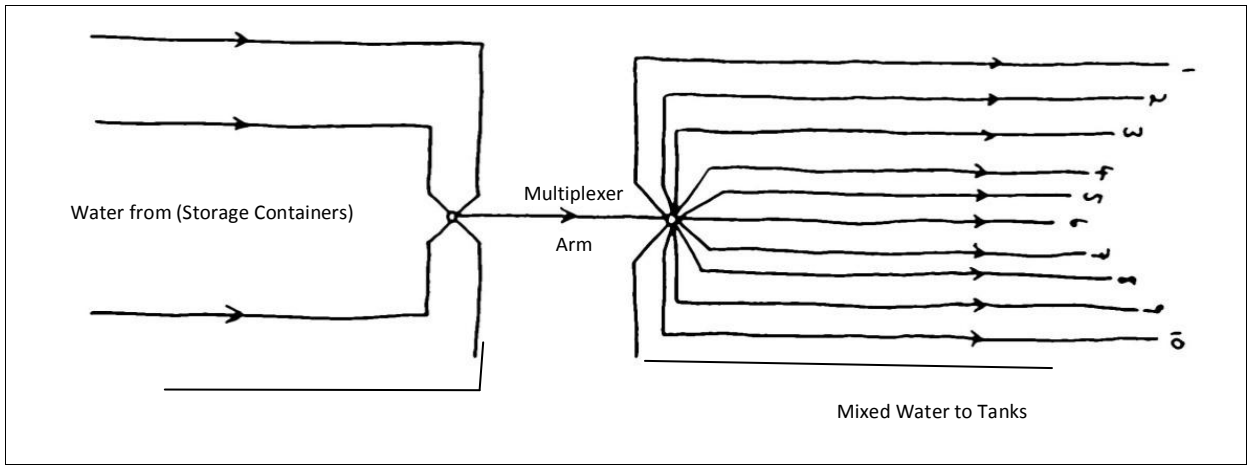

Figure 1: The basic design of the multiplexer following the lines of 4 input points.

Figure 2: The multiplexer with its rotary arm and the receiving funnels.

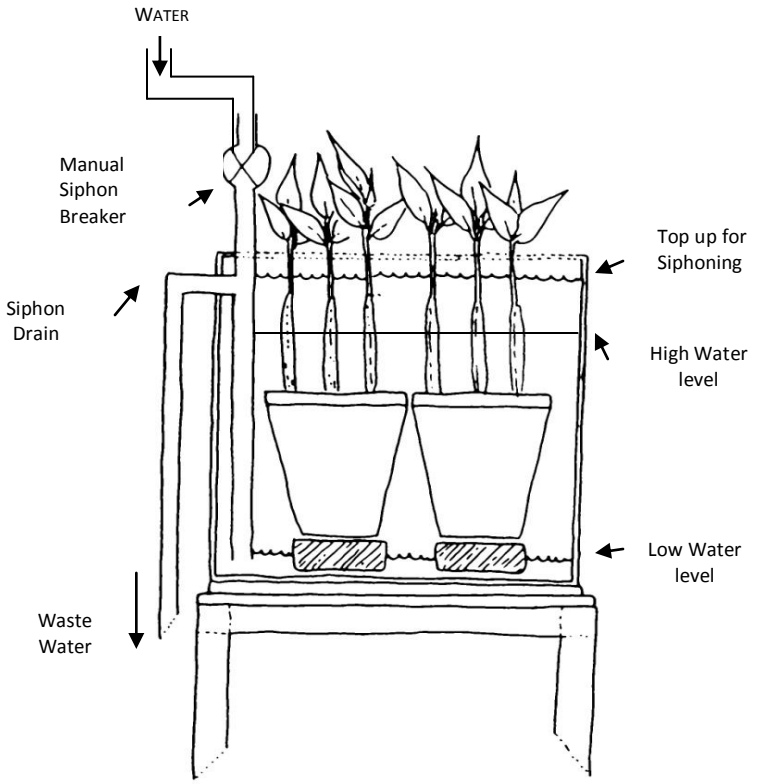

Figure 3: Pots in treatments indicating the high and low water.

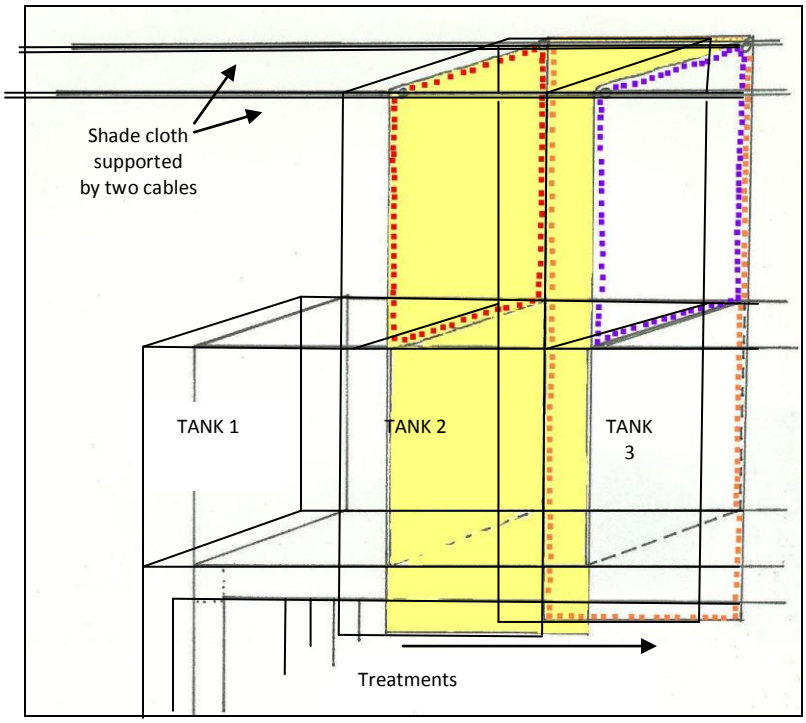

Figure 4: The basic design for the shade cloth experiment.

3.5. Measurements for salinity, inundation and light-salinity experiments

There were five seedlings in each pot and each salinity and inundation treatment had five replicate pots. Five weeks were used to acclimatize the different pots to the different salinity concentrations. This was done with care to avoid osmotic shock. These pots were then placed into the representative treatment compartments (25 pots represented the replicates for each treatment) for two weeks to acclimatize to the tidal cycles. The two experiments, salinity and inundation, ran parallel for a 14 week study period.

Measurements included the relative growth rate (RGR) in which the plant height and number of leaves of each seedling within all the pots were measured and any morphological changes noted. Leaf surface area was measured additionally for the light - salinity experiment. Leaf stomatal conductance and leaf chlorophyll fluorescence (Fv/Fm) were measured using a Leaf Porometer (Decagon Device, US/Canada) and a Hansatech Plant Efficiency Analyser (Hansatech Instruments, Norfolk, England). Measurements were done at the start of each experiment and every two weeks thereafter for a total of 14 weeks. Jayatissa *et al.* (2008) also used a 14 week study duration. Each seedling was tagged with a number and these were secured to the stem of the seedling with plastic cable ties. One mature leaf of each seedling was chosen and tagged with a thin piece of thread. In both cases care was taken not to break the leaves or secure the materials too tightly so that growth would not be inhibited. Stomatal conductance and fluorescence measurements were made on the abaxial surface of fully expanded, mature leaves of the same age. The same tagged leaves were measured every 2nd week. When the plants were harvested leaf water content was measured by first recording the wet weight (WW) of the leaves and then drying these at 60 °C for a week. After the dry weight (DW) was measured leaf water content was determined as: WW-DW / Number of leaves. The root : shoot ratio was determined by dividing the DW of roots with the DW of shoots for each treatment.

The objective was to determine if plants were stressed by different salinity concentrations as well as by different inundation periods. Before measuring fluorescence with the Hansatech Plant Efficiency Analyser, the seedlings were dark-adapted for 30 minutes. This was done by closing the shutterplates and placing the leafclips on tagged leaves then setting the stopwatch for 30 min. After dark adaptation the sensor unit was placed over the leafclip and the shutterplate opened. Then the leaf was illuminated and measured with the sensor unit. The unit

automatically calculated the Fv/Fm parameters which represent the “quantum yield or efficiency of photochemistry in PS II” (Björkman & Demmig, 1987).

Sediment characteristics (soil pH and redox potential or ORP) were measured using a HANNA redox/pH meter (HANNA Instruments) and a platinum-gold tipped electrode at the start and end of the study. Sediment electrical conductivity and salinity were measured at the end of the study. For sediment conductivity, 250 g of sediment of each replicate seedling pot was measured out and transferred into separate labeled plastic beakers. Distilled water (100 ml) was added to create a paste until no more water was left on top of the sediment. The mixture was left standing for an hour then filtered with a vacuum filter using Whatman, Schleicher& Schnell Ø110 mm filter paper. The conductivity of the solution was then measured using a CyberScan, Hand-held Conductivity/TDS/Temperature meter (CON ID/100/200).

Dry weight (DW) of the seedlings was measured after the completion of the study. Fourteen seedlings were harvested at the start of the experiment to use as the initial biomass. The seedlings were removed from the pots, the roots separated from the mud and each seedling placed in a labeled plastic bag. These were transported to the laboratory at the University where the process continued. Each seedling was separated into leaves, stems, hypocotyls and roots, and weighed separately. Wet weight was measured using an electrical scale (College B502, Mettler Toledo) and the seedlings were then placed in separate, labeled glass beakers. These were oven dried at 60 °C for a week until completely dry. Thereafter the seedlings were weighed to obtain the dry biomass.

3.6. Statistical Analysis

Pots within the treatments were randomly moved every 2nd week to achieve a random block design. Data were checked for normality and when data were found to be normal an One-way ANOVA was run in conjunction with a *Post hoc* Tukey HSD test, was used to analyze the physiological responses and determine significant difference of the different treatments using STATISTICA Version 8 (2008). For the data that was not normal, non-parametric, Kruskal-Wallis ANOVA tests were done to determine the significant differences within treatments.

4. The response of *Rhizophora mucronata* Lam. to salinity and inundation

4.1. RESULTS

4.1.1. Seedling height

There was a decrease in seedling height with increasing salinity concentrations (Figure 5). Stem elongation was highest for the low salinity, 8 ppt treatment, although the different replicate seedlings showed variable responses in height. Stem elongation was lowest for the hypersaline treatment (45 ppt), although the height values of 0, 18 and 35 ppt treatments were not significantly different to that of the 45 ppt treatment ($F = 4.38$, $p > 0.05$, $n = 25$). Seedling height for the 8 ppt treatment was significantly higher compared to the 35 ppt treatment ($F = 4.38$, $p < 0.05$; $n = 25$) and 45 ppt ($F = 4.38$, $p < 0.05$; $n = 25$) treatments but were not significantly different compared to the 0 and 18 ppt treatments.

The 24 h treatment that represented continuous inundation had the greatest increase in height which was significantly different to the no inundation ($F = 5.66$, $p < 0.05$; $n = 25$) and 3 h ($F = 5.66$, $p < 0.05$; $n = 25$) treatment (Figure 6). Stimulation of stem elongation is a typical response to prolonged inundation. The difference between maximum stem elongation in the 24 h treatment and minimum stem elongation in the no inundation treatment was 6.21 mm per week. The seedlings exposed to the 3 h inundation treatment were the healthiest of all the treatments (Plate 2, C). The average weekly increase in height was similar (approximately 7 mm) for the 3, 6 and 9 h treatments. The results for the seedlings exposed to the 9 h treatment were more variable than the 3 and 6 h treatments. Stem elongation was reduced in the no inundation treatment and was significantly lower ($F = 5.66$, $p < 0.05$; $n = 25$) than all other treatments.

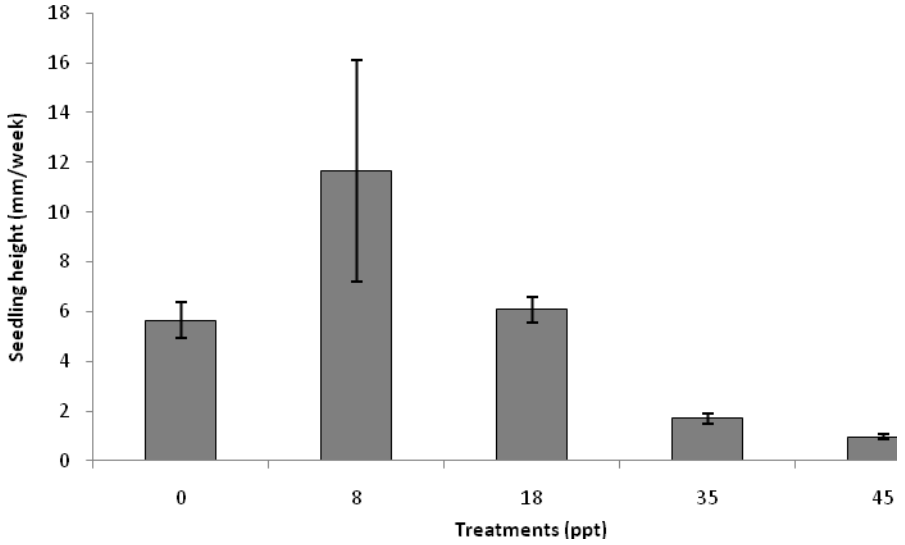

Figure 5: The effect of different salinity concentrations on *Rhizophora mucronata* height over the 14 week treatment period (Bars = SE).

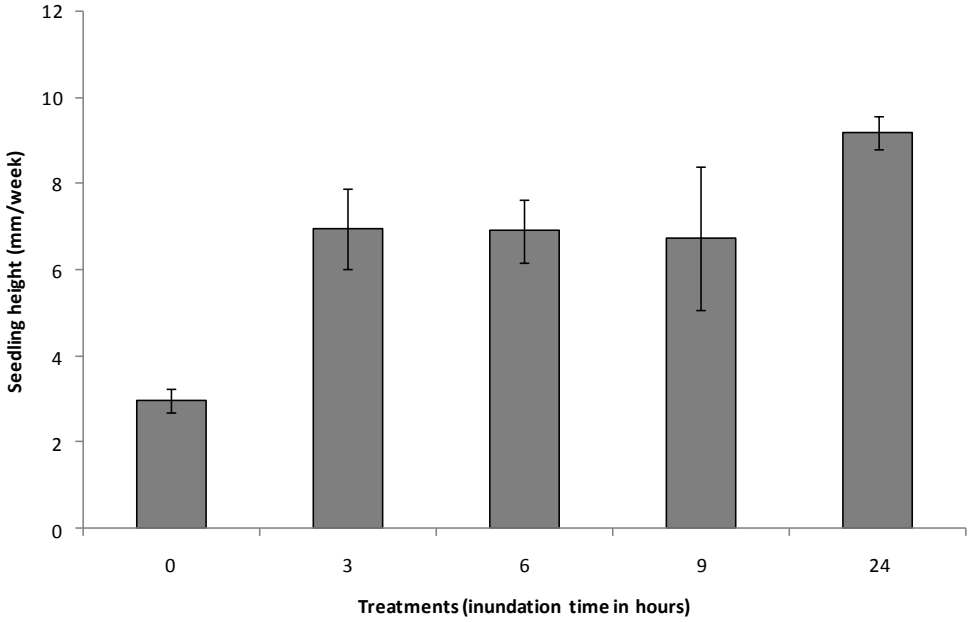

Figure 6: The effect of different inundation treatments on *Rhizophora mucronata* seedling height over the 14 week treatment period (Bars = SE).

4.1.2. Leaf gain and leaf loss

Morphological changes in the plants were noted for the different salinity and inundation treatments. Seedlings for the high salinity treatments (35 and 45 ppt) had greater salt secretion from leaves (Plate 2, A) as well as thicker and more rigid leaves. All replicate seedlings experienced leaf necrosis (Plate 2, B) in the 45 ppt treatment and plant height, leaf area and number of leaves produced were lower than for the other treatments. Seedlings exposed to the inundation treatments (9 h and 24 h) shed their leaves. The no inundation treatment resulted in morphological changes similar to the hypersaline treatment as there was a decrease in plant height, leaf area and number of new leaves, but leaf thickness and rigidity increased.

As expected, leaf gain increased with a decrease in salinity, which was also significantly lower for the 45 ppt treatment compared to all other treatments ($F = 22.74$, $p < 0.05$; $n = 25$, Figure 7). Leaf production in freshwater was similar to the 8 ppt treatment but was significantly higher than the 35 ppt ($F = 22.74$, $p < 0.05$; $n = 25$) treatment. Leaf gain at 35 ppt was significantly lower compared to the 8 and 45 ppt treatments ($F = 22.74$, $p < 0.05$; $n = 25$).

No significant difference was found in leaf loss in the salinity experiment ($F = 2.49$, $p > 0.05$, $n = 25$) for the different treatments (Figure 7). Leaf loss in the 8 ppt treatment was probably due to the production of new leaves whereas leaf loss in the 45 ppt treatment was related to stress.

Leaf gain was lowest for the 0 and 24 h inundation treatments (Figure 8) and was significantly lower for the 24 h inundated treatment compared with the no inundation treatment ($F = 3.55$, $p < 0.05$, $n = 25$). The 9 h inundation treatment produced significantly more leaves than the 24 h treatment ($F = 3.55$, $p < 0.05$, $n = 25$). However they did not seem to be much difference between the responses to the 3 - 9 h inundation treatments. The seedlings in the 3, 6 and 9 h treatments produced on average 3.5 new leaves over the experimental period. Moderate inundation (3, 6 and 9 h) similar to natural conditions promoted the production of new leaves, whereas the stress treatments (0 and 24 h) reduced leaf production. Leaf production was similar for the favorable salinity and inundation treatments.

Leaf shedding also occurred in the continuous inundation treatment and was highest at the end of the 14 week treatment period (Figure 8), with the 24 h inundation treatment having significantly higher ($F = 32.79$, $p < 0.05$, $n = 25$) leaf loss compared to the 0 and 9 h inundation treatments.

A:

B:

C:

PLATE 2: A. Salt crystals on leaves due to salt secretion; B. Leaf necrosis and C. Healthy seedlings of the 3 h treatment.

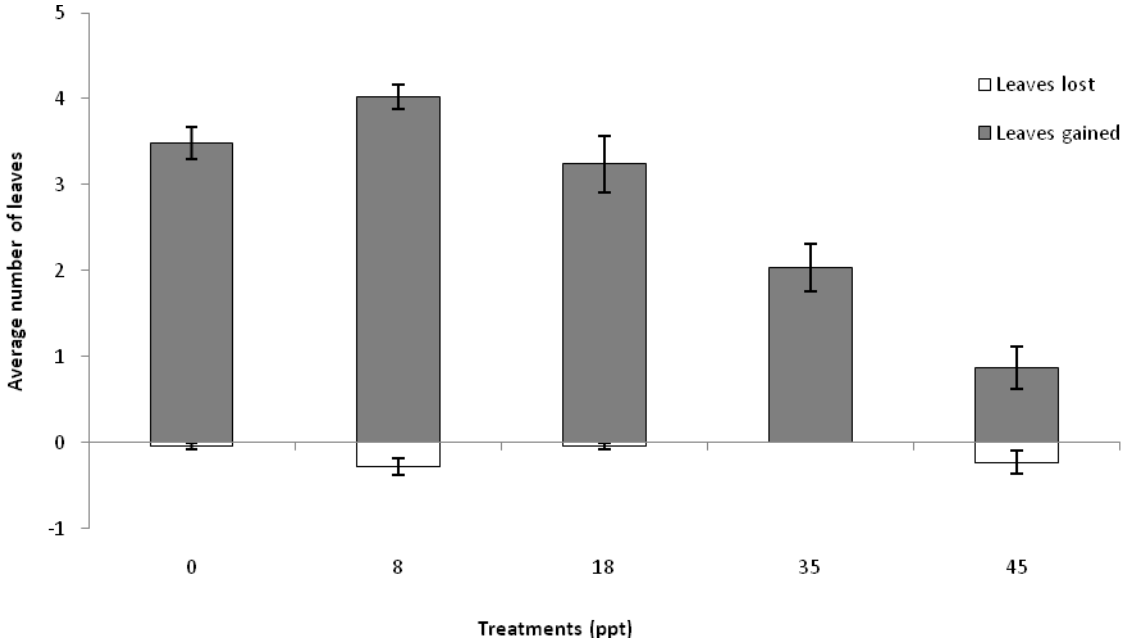

Figure 7: Average number of new leaves and leaves shed over 14 week treatment period for the salinity experiment (Bars = SE).

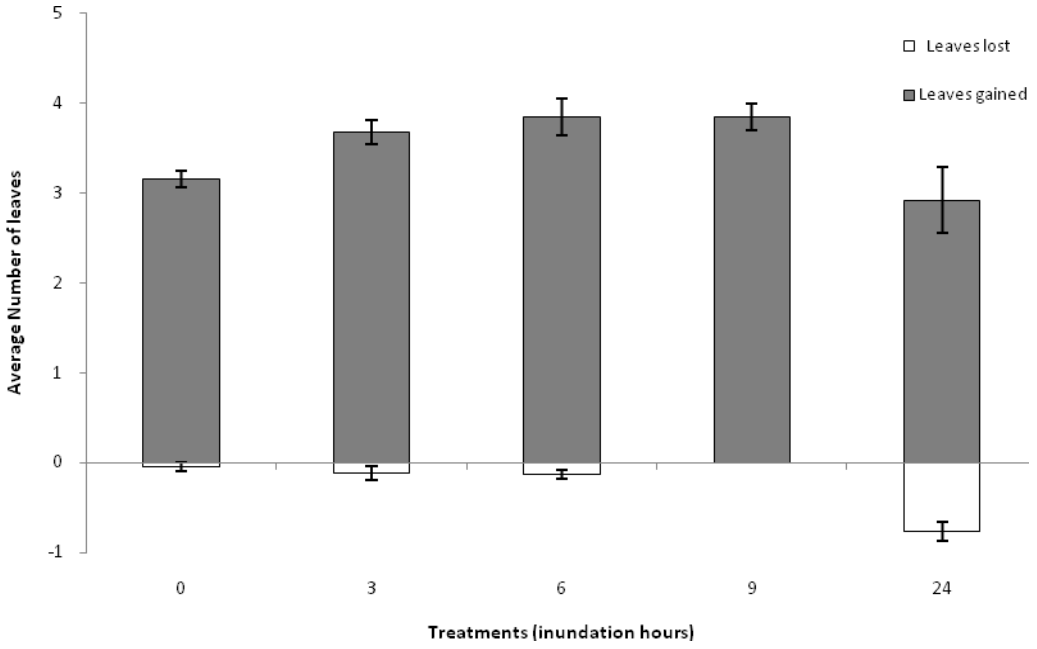

Figure 8: Average number of new leaves and shed leaves over 14 week treatment period for the inundation experiment (Bars = SE).

4.1.3. Leaf water content

For the salinity and the inundation experiments the water content showed no significant difference ($F = 0.84$ and $F = 2.70$, $p > 0.05$, $n = 25$) for the different treatments (Figure 9). However, the leaf water content was higher in the inundation treatments compared to the salinity treatments. The freshwater treatment had the lowest leaf water content and the hypersaline treatment (45 ppt) had the highest leaf water content recordings. However, this 45 ppt treatment also showed variable results. The highest leaf water content was observed in the no inundation treatment as well as the continuous inundation treatment (Figure 10).

4.1.4. Biomass partitioning

Seedling biomass decreased with an increase in salinity from 8 to 45 ppt (Figure 11). Highest root and leaf biomass (DW) were recorded for the low salinity treatment, where leaf DW was significantly higher in the 8 ppt treatment compared to the 45 ppt treatment ($F = 3.53$, $p < 0.05$, $n = 25$). Root biomass was mostly higher in each treatment compared to the leaf biomass. For the hypersaline treatment (45 ppt) the root and leaf biomass were almost equal.

Highest root and leaf biomass (DW) were recorded in the 9 h inundation treatment (Figure 12), where root biomass exceeded the leaf biomass. Lowest root biomass occurred in the continuous inundation treatment (24 h). The leaf biomass exceeded the root biomass for this treatment. All other treatments showed similar allocation of resources to stem, shoot and root biomass. No significant differences ($F = 0.83$, $p > 0.05$, $n = 25$) were found in the salinity experiment between the ratio of roots to shoot (Table 5). This was also true for the inundation experiment where no significant differences ($F = 2.29$, $p > 0.05$, $n = 25$) were found. Biomass was the highest for the propagules. However, these were considered not to have changed over the 14 week study period. The stem, root and leaf biomass are indicators of where biomass allocation was the highest for each treatment. As previously stated, the 8 ppt treatment had the most growth in stems, root and leaves while 0 ppt had higher stem, leaves and root allocation than 18, 35 and 45 ppt treatments.
For the inundation treatments the 3 h treatment had the highest root and leaf biomass. The continuous and no inundation treatments had the lowest recordings of root:shoot. No significant differences ($F = 2.29$, $p > 0.05$, $n = 25$) were found between the inundation treatments.

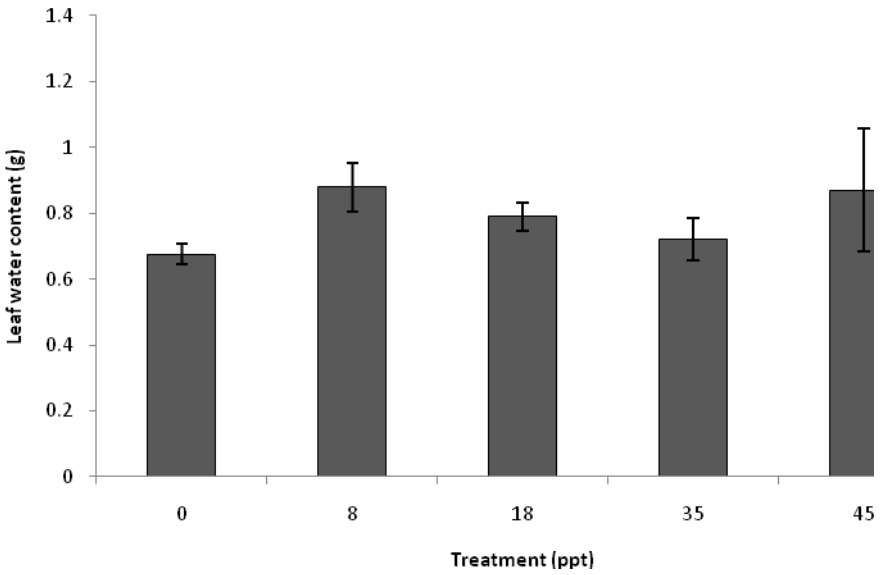

Figure 9: Leaf water content over 14 week treatment period for the salinity experiment (Bars = SE, n = 25).

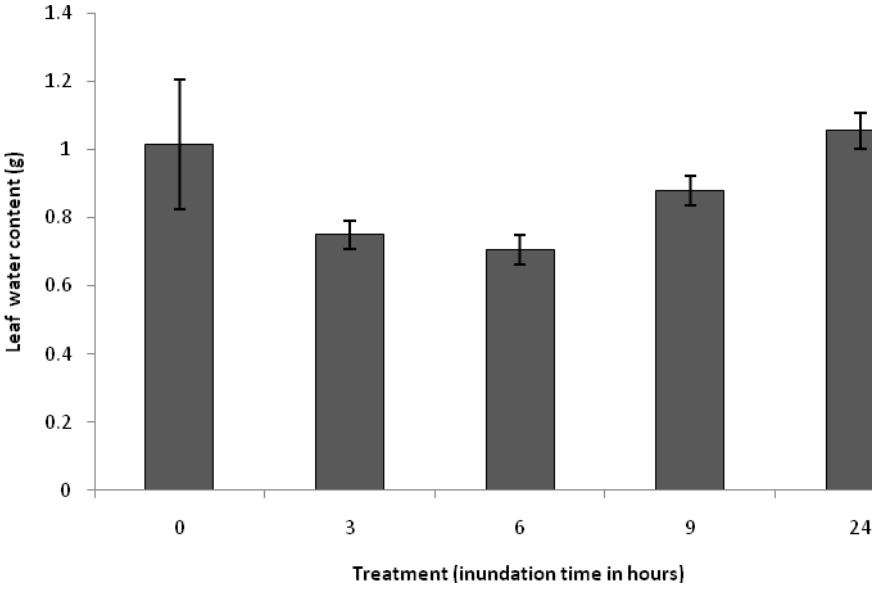

Figure 10: Leaf water content over 14 week treatment period for the inundation experiment (Bars = SE, n = 25).

Table 5: The root: shoot ratio of the different salinity and inundation treatments. (Shoot = leaves and stems, excluding propagules)

Salinity (ppt)	Root : Shoot	Inundation (hrs)	Root : Shoot
0	0.87 ± 0.10	0	0.55 ± 0.05
8	0.78 ± 0.15	3	1.01 ± 0.28
18	0.73 ± 0.07	6	0.74 ± 0.10
35	0.71 ± 0.01	9	0.93 ± 0.12
45	0.63 ± 0.07	24	0.49 ± 0.03

± = SE, n = 5

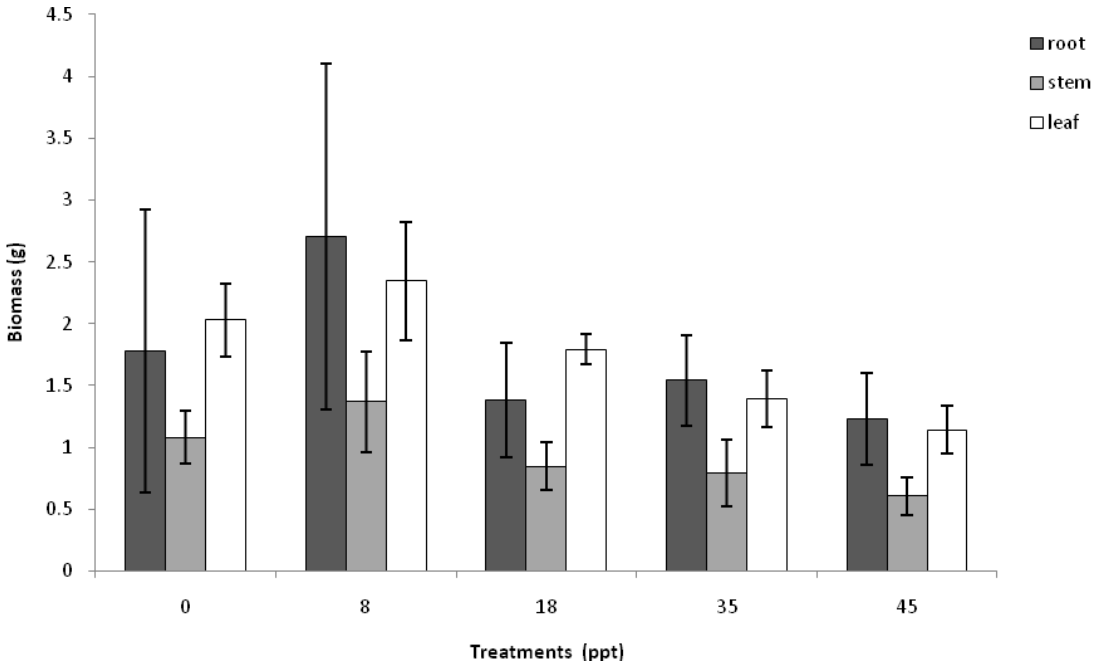

Figure 11: The effect of different salinity treatments on *Rhizophora mucronata* seedling biomass over the 14 week treatment period (Bars = SE, n = 25).

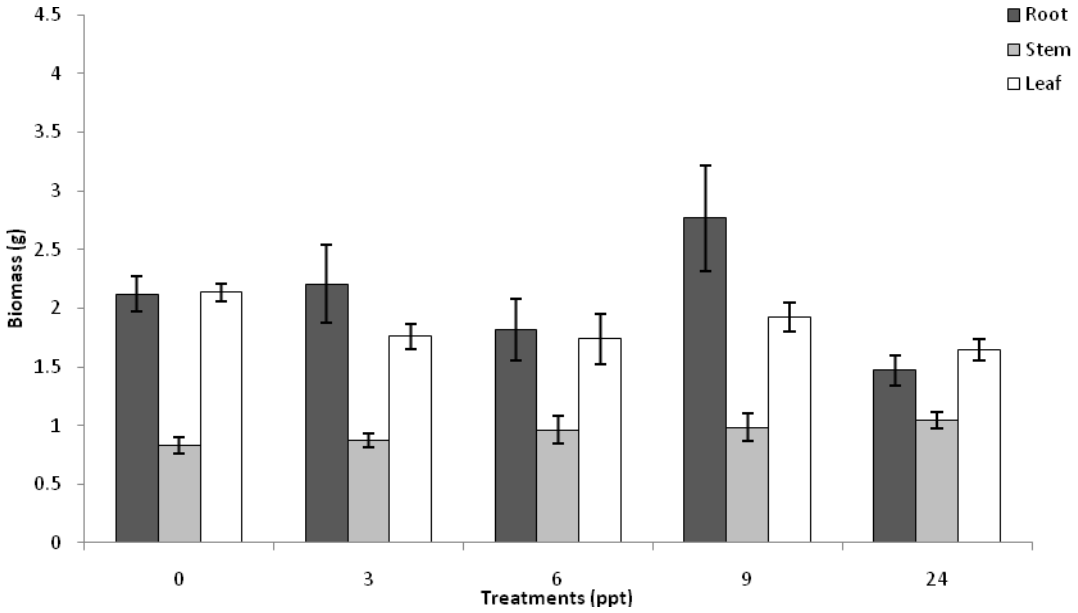

Figure 12: The effect of different inundation treatments on *Rhizophora mucronata* seedling biomass over the 14 week treatment period (Bars = SE, n = 25).

4.1.5 Photosynthetic performance (Fv/Fm)

The highest Fv/Fm ratios that were recorded were within the unstressed range of 0.7 to 0.8 for most treatments throughout the 14 week period (Figure 13). Only the 45 ppt treatment showed a decrease in the Fv/Fm ratio to 0.6 at 12 – 14 weeks. The Fv/Fm readings for the seedlings in the 45 ppt treatment were significantly lower ($F = 5.94$, $p < 0.05$, $n = 25$) compared to all other treatments at two weeks. The drop in the Fv/Fm ratio occurred after the eighth week due to the simultaneous shedding of the lower leaves of the seedlings in the 45 ppt treatment. Unfortunately most tagged leaves were the lower senescent ones. Despite this, the seedlings appeared healthy even though the lower leaves had been lost. The 0 ppt treatment had the highest photosynthetic performance compared to the other salinity treatments. At six weeks there was a decrease in photosynthetic performance for the 8 and 18 ppt treatments.

Photosynthetic performance was variable for most of the inundation treatments (Figure 14). The no inundation and continuous inundation treatments had the largest variability in Fv/Fm recordings. The 3 h inundation treatment had the highest Fv/Fm readings and showed a consistent increase in photosynthetic performance over the 14 week study period. The treatments of 3, 6 and 9 h had similar Fv/Fm values. Seedlings that were continuously inundated had the lowest Fv/Fm readings. Similar to the high salinity treatment, leaf shedding occurred in the continuous inundation treatment after 8 weeks, resulting in significant differences between treatments in weeks 12-14, where 24 h was significantly lower ($F = 7.83$, $p < 0.05$, $n = 25$) compared to all other treatments (0, 3, 6, and 9 h) . There was simultaneous shedding of the lower leaves. Unfortunately, most tagged leaves were the lower senescent leaves. Despite this, the seedlings looked healthy.

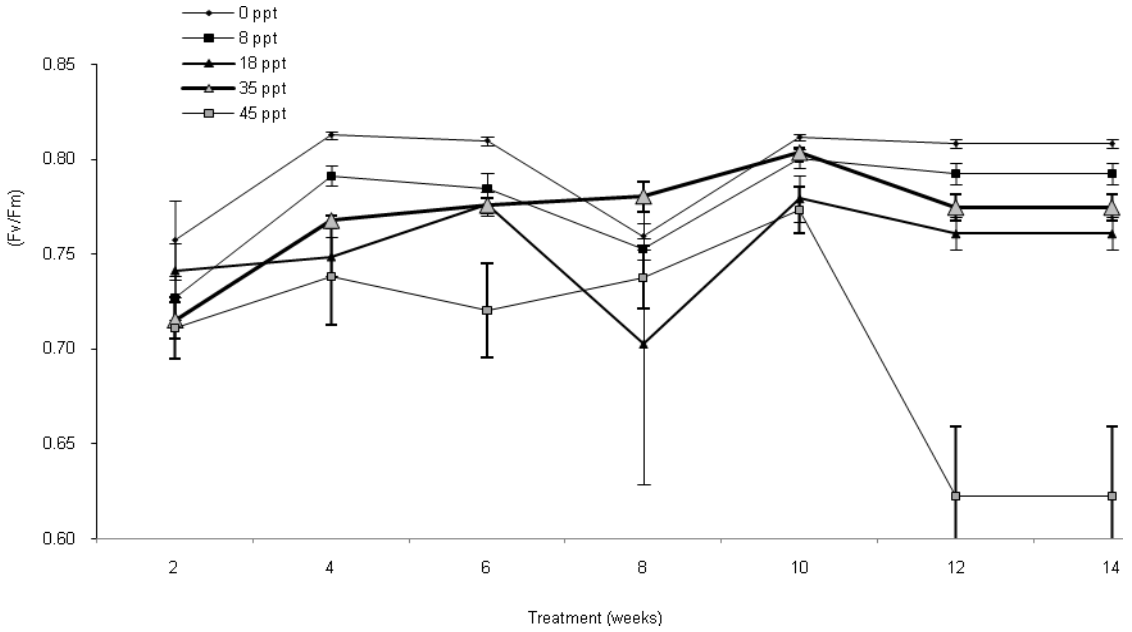

Figure 13: The effect of different salinity concentrations on the Fv/Fm values for *Rhizophora mucronata* seedlings over the 14 week treatment period (Bars = SE, n = 25) with 18 ppt being the control.

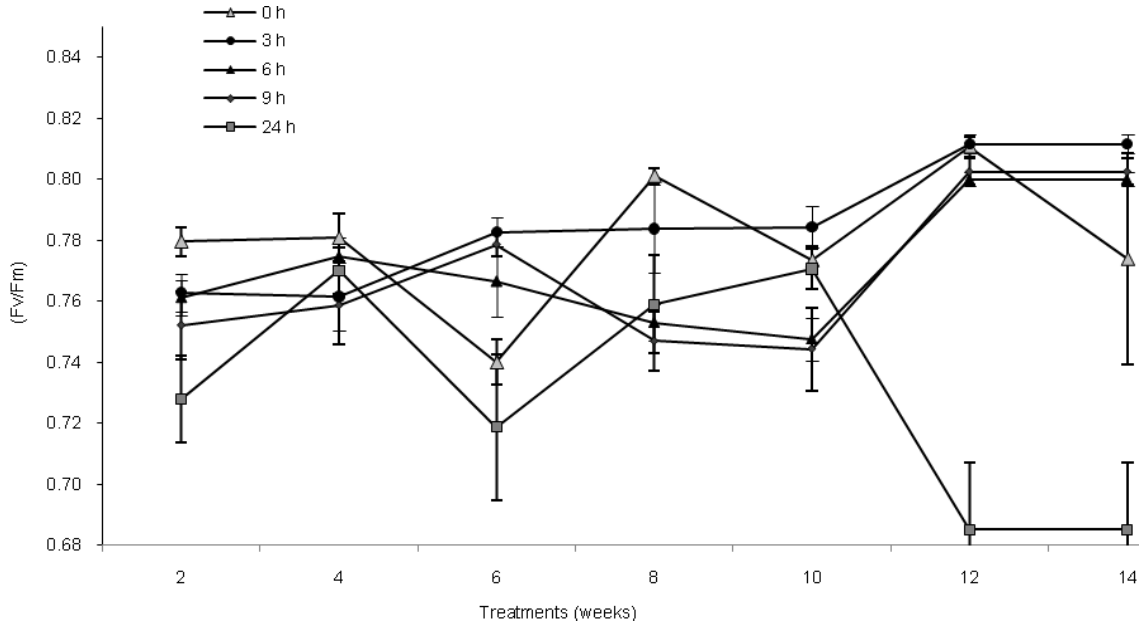

Figure 14: The effect of different inundation treatments on the Fv/Fm values for *Rhizophora mucronata* seedlings over the 14 week treatment period (Bars = SE, n = 25) with 6 h as the control**.**

4.1.6 Stomatal conductance

Stomatal conductance increased with a decrease in salinity (Figure 15), resulting in greater variability of readings with reduced salinity and less variability in higher salinity treatments. Stomatal conductance was highest for the 0 ppt treatment and lowest for the 45 ppt treatment, where the 0 ppt treatment had significantly higher ($F = 7.41$, $p < 0.05$, $n = 25$) stomatal conductance than all the other treatments. In weeks 12 to 14 the 35 and 45 ppt treatments had significantly lower ($F = 13.21$, $p < 0.05$, $n = 25$) stomatal conductance compared to all other treatments. Over the 14 week period stomatal conductance remained consistently low for the 35 and 45 ppt treatments.

The lowest recordings of stomatal conductance were for the 24 h treatment which was found to be significantly lower than the 3 and 6 h treatment ($F = 8.39$, $p < 0.05$, $n = 25$) for weeks four to twelve (Figure 16). Stomatal conductance for the 24 h treatment was less variable compared to the other treatments. The 6 h inundation treatment had the highest stomatal conductance up to week twelve, whereas the 3 and 9 h treatments exceeded the 6 h treatment in weeks 12-14. The moderate inundation treatments (3, 6, and 9 h) had significantly higher stomatal conductance ($F = 8.39$, $p < 05$, $n = 25$) compared to the no inundation treatment, however results were variable for the different weeks.

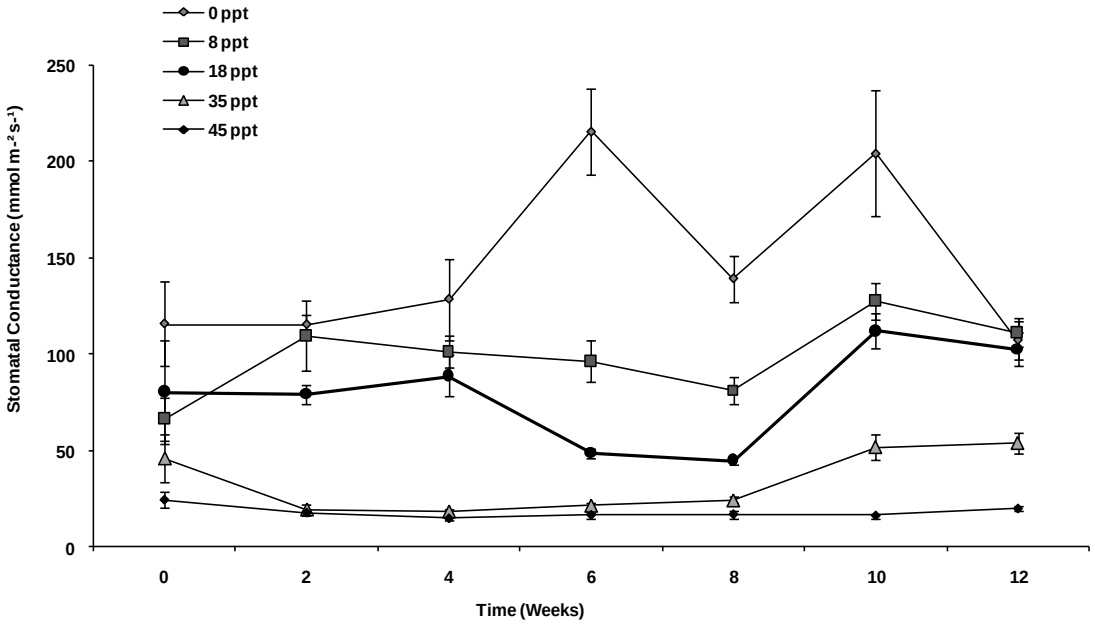

Figure 15: The effect of different salinity concentrations on the stomatal conductance for *Rhizophora mucronata* seedlings over the 14 week treatment period (Bars = SE, n = 25) with 18 ppt as the control.

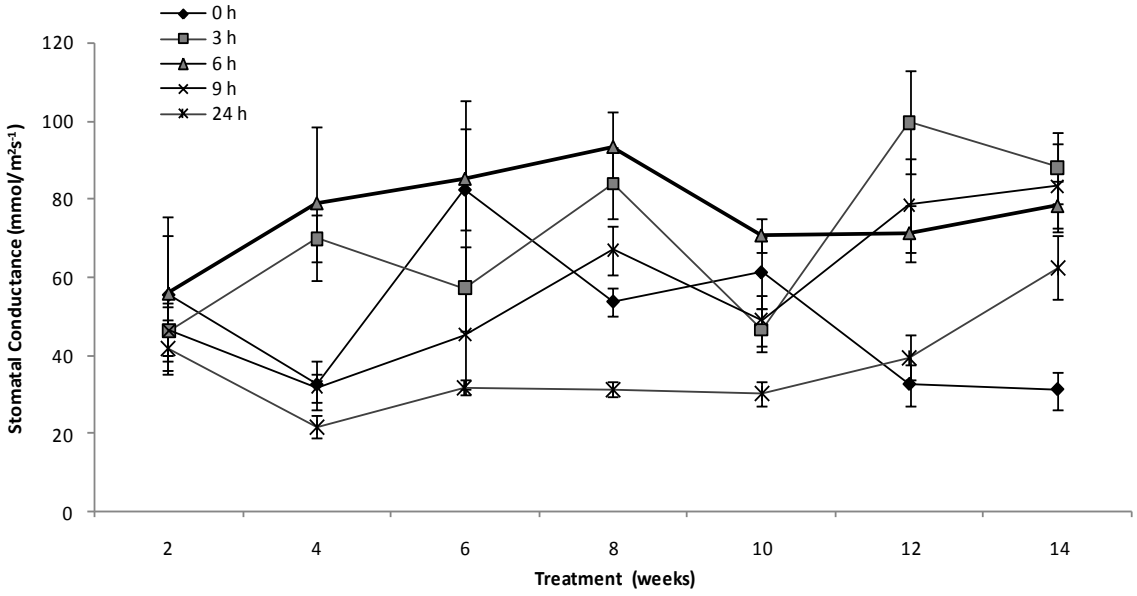

Figure 16: The effect of different inundation treatments on the stomatal conductance for *Rhizophora mucronata* seedlings over the 14 week treatment period (Bars = SE, n = 25) with 6 h as the control.

4.1.7. Physical soil parameters

Soil redox potential was the lowest for the 45 ppt treatment (Table 6) as well as for the 6 h and 24 h inundation treatment however there were no significant differences ($F = 1.78$, $p > 0.05$, $n = 25$).This may be due to some animal activity in the 24 h treatment (Plate 3, A). The redox potential of the no inundation treatment was significantly higher ($F = 4.38$, $p < 0.05$, $n = 25$) compared to the 6 and 24 h inundation treatments. The soil salinity increased with increasing salinity treatment. Electrical conductivity and soil salinity was significantly higher ($F = 98.16$ and $F = 221.5$, $p < 0.05$, $n = 25$) in the 35 and 45 ppt treatments compared to the 0 and 8 ppt treatments. The difference between the maximum and minimum soil salinity within the salinity treatments was 12.7 ppt or 21.3 mS. The electrical conductivity and salinity was significantly higher ($F = 329.33$ and $F = 340$, $p < 0.05$, $n = 25$) in the no inundation (0 h) treatment compared to the 24h treatment. Salt crystals formed on the surface of the soil (Plate 3, B).

Table 6: Soil parameters for the salinity and inundation experiments. Measurement had five replicates for each treatment except for inundation where n = 3

	Salinity treatments (ppt)				
Variable	0	8	18	35	45
Redox (mV)	-219.7 ± 67	-323.4 ± 24	-218.5 ± 63	-304.8 ± 13	-340.3 ± 12
Electrical cond. (mS)	2.43 ± 0.3	7.24 ± 1.2	11.3 ± 0.1	12.29 ± 0.3	23.73 ± 0.9
Salinity (ppt)	1.55 ± 0.09	3.95 ± 0.9	5.62 ± 0.09	12.29 ± 0.1	14.24 ± 0.5

	Inundation treatments				
	0 h	3 h	6 h	9 h	24 h
Redox (mV)	-289.3 ± 10	-317.9 ± 17	-354.5 ± 2.4	-328.5 ±7	-351.3 ± 2
Electrical cond. (mS)	57.89 ± 2.1	13.22 ± 0.7	12.05 ± 0.3	12.46 ± 0.9	10.57 ± 0.3
Salinity (ppt)	28.9 ± 1.07	6.64 ± 0.3	6.02 ± 0.1	6.15 ± 0.4	5.25 ± 0.1

A.

B.

PLATE 3: A. Animal activity within the 24 h treatment; B. Salt crystals formed on the soil surface of the no inundation treatment.

5. DISCUSSION

5.1 Seedling growth response

The most important driving factors of mangrove distribution and survival are the response to soil and water column salinity (Krauss *et al.*, 2008; Krauss & Allen, 2003; and Li, 2008) and the response to inundation and water level fluctuations (Xiao *et al*., 2009). Mangroves are known to be facultative halophytes but usually also grow well in freshwater. However, a study on the effects of salinity on *Rhizophora mucronata* seedlings by Khan and Aziz (2004) showed that this species had optimum growth at 17.5 ppt (50% seawater). Within the same study, a decrease in growth was observed as salinity increased up to 100% seawater. Another study by Jayatissa *et al.* (2008) found that *R. mucronata* saplings flourished at 26 ppt. They used seedlings with their first leaves unfolded and of similar height and study period (3 months) compared to this experiment. This study showed that *R. mucronata* displayed maximum growth at low (8 ppt) to moderate salinity (18 ppt), with a less than optimum growth in freshwater. This was similar to the Khan and Aziz (2004) study which attributed the results to the high affordability of water uptake and loss through increased evapotranspiration rates (Naidoo, 1987). Mangroves are known to use the NaCl as a source of nutrients which, depending on the species and its specific tolerance range, occur in different concentrations within the plant tissue (Jayatissa *et al.,* 2008). Naidoo's (1987) study on *Avicennia marina* reported that the nutrients did not affect tissue water potential and that salinity was the determining factor of osmotic potentials within plants. Within the current study, the ability of the seedlings of *R. mucronata* to cope with high salt and continuous inundation was better than expected, especially at this vulnerable seedling stage. However, NaCl will become toxic at high concentration and inhibit growth. This was observed in the seawater and hypersaline treatments in this experiment where significantly less growth was observed.

Adaptations to different salinity concentrations depend on the species as well as the growth stage (Krauss, 2008). Numerous studies conducted on the genera Rhizophoraceae have shown that within the genus there are different tolerance ranges. Biber (2006) observed that *Rhizophora apiculata, R. stylosa* and *R. mangle* all had optimal growth at 15 ppt which is similar to the *R. mucronata* tolerance range as found in this study (8 -18 ppt) and by Khan and Aziz (2004) (17.5 ppt). Tolerance to high salinity comes at a high price, with physiological tradeoffs as growth is reduced due to energy invested in tolerance mechanisms (Kathiresan & Bingham, 2001). This is a general response to increased salinity (Ghoulam *et al*. 2002). This could be

seen in this study where *R. mucronata* seedlings within the high salinity (35 – 45 ppt) range had reduced growth with significantly less plant height, dry weight biomass and leaf production. This response suggests a shift from plant growth to survival (Jayatissa *et al.,* 2008).

Jayatissa *et al.* (2008) showed that *R. mucronata* occurred in both landward and riverine fringe sites but seedlings differed in root : shoot biomass allocation. They reported that this species can be considered to be restricted to ecosystems that have riverine influences. In the hypersaline lagoons (40 ppt) in Puerto Rico, Lugo *et al*. (2007) reported that tree height was observed to be proportional to salinity resulting in stunted trees (<1.5 m) of *R. mangle*. Naidoo (1987) and Naidoo, (2006) reported stunted growth (<1.5 m) of *Avicennia marina* in stressful environments in South Africa. However, nothing has been reported on *R. mucronata* as yet. Lovelock *et al.* (2006a) studied *R. mangle* on the barrier islands of Belize. They suggested that tree height is mainly determined by the effect of reduced hydraulic conductivity within the plants and thus, as a result nutrient deficiency is the driver for stunted trees. Trees of less than 1.5 m are found more landwards compared to the taller trees (4-7 m) at the water's edge at the river fringes. Along arid coastlines Lugo *et al*. (2007) observed that much taller trees (up to 10 m) grew near the water's edge or ocean fringe and suggested that this may be due to lower porewater salinity because of continuous tidal flushing. They indicated that freshwater discharge and groundwater input were very important for these large mangrove trees and that those that received freshwater during the year grew the tallest.

This study showed that continuous inundation resulted in a significant increase in seedling height. Seedlings in the no inundation treatment had significantly lower seedling height compared to the other treatments. However, the significantly higher soil salinity (28.9 ppt) in the no inundation treatment (water column was 18 ppt) may have had a negative effect on plant growth. Plants that are usually adapted to arid conditions are also said to be able to survive saline conditions, and *vice versa*, where physiological adaptation plays a major role in the plant's survival (Atreya *et al*., 2009). In this study, dry arid conditions would be represented by the no inundation treatment, where evaporation from the soil left it more saline. In this treatment plants showed symptoms of drought (such as desiccation of leaves) and in particular salt stress, such as high accumulation of salt within leaf tissue, as seen by the excess salt secretion on the leaves. This is an adaptation to cope with the increasing osmotic pressure at high salinity. The high soil salinity was due to the high evaporation rate and salt deposition in the surface layers of the pot (Plate 3, B).

Within the intertidal zone seedlings are influenced by tidal inundation. This study showed that seedlings in the moderate inundation (3 to 9 h) treatments had maximum photosynthetic performance and high stomatal conductance. However seedling height was greatest in the continuous inundation treatment. This stem elongation response is usually a shift in biomass accumulation from roots to shoots, as plants adapt to these stressful conditions. It can also be due to decreasing Eh (redox potential) within the soil as a result of the high water level and continual flooded conditions (Pezeshki *et al*., 1997). However, soil redox potential of the pots was anoxic and this was similar for all inundated treatments. It was therefore suggested that water level was the determining factor for stem elongation. This was also shown in the He *et al.* (2007) study, where stem elongation was a response to flooding by 30 cm water. The elongation of stems or petioles is a response to prolonged inundation and to waterlogged soils, typically found in wetland plants (Jackson & Drew, 1984 in Adams *et al.,* 1994). The plants grow rapidly to increase the plant biomass over the water surface.

Photosynthetic performance and stomatal conductance were the lowest for the continuous inundation treatment, indicating that these conditions were stressful to the seedlings. *Rhizophora mangle* was recognized as a pioneer species in flooded areas (He *et al.,* 2007) where it was able to cope and adapt to long flooding duration which was observed to be 50 to 70% of the year in the North American mangrove forests. In an eight month study conducted by Ellison and Farnsworth (2006), within the Belizean barrier reef lagoon in three different regions, it was reported that *R. mangle* flourished in the lower intertidal area, where it had increased height compared to those in the upper intertidal area. They also mentioned that leaf production and biomass accumulation increased with inundation. In this study *R. mucronata* had highest dry weight biomass accumulation and leaf production in the moderate inundation cycles (3, 6 and 9 h treatments) which is similar to the tidal conditions it would experience in the intertidal habitat of South African estuaries. Ellison and Farnsworth (1997) reported that seedlings (*R. mangle*) showed a rapid increase in height in the higher inundation areas for the first year. However, over a longer period of time (after 2.5 years), the growth of the seedlings that were exposed to moderate inundation in the mid-water levels exceeded those in the low-water areas. They therefore concluded that the response to inundation was a rapid ecological response rather than the previously thought physiological adaptation.

The results from this study showed that plants exposed to the high salinity and continuous inundation treatments showed symptoms of stress such as leaf shedding, excessive salt secretion, reduced leaf production and leaf necrosis. 'Burn marks' are symptoms recognized as necrosis (Omami, 2005). Similar symptoms occur when the plants experience nutrient deficiencies. If excess salt is present, uptake of water is generally limited and therefore nutrients are limited restricting plant growth (Grattan and Grieve, 1999). Leaf shedding is one of the coping-mechanisms of halophytes in high salinity concentrations (Krauss *et al*., 2008). They report that species such as *Avicennia marina* translocate excess salt and accumulate it in the lower mature leaves. These are then shed to rid the plant of excess salt. Ye *et al.* (2005) found that plants with high salt tolerance also secreted high concentrations of salt from the leaves. Salt saturation within plant tissues was kept fairly constant but this osmoregulation came at a high cost to growth.

Pezeshki *et al*. (1989 & 1997) found that leaf growth of *R. mangle* was significantly inhibited when plants experienced flood stress. It was also noted in this study that *R. mucronata* seedlings increased leaf thickness with significantly higher leaf loss and lower leaf gain in the continuous inundation treatment compared to the other treatments. However, low leaf production also occurred in the no inundation treatment. This result was different to the study by Ellison and Farnsworth (1997) on *R. mangle* which found that increased inundation resulted in an increase in leaf production. In their study, inundation was achieved by flooding plants for 16 cm above the rim of the pot, which was also the median height from the soil to cotyledonary scar of the seedling. This treatment was compared with a low inundation treatment where the water level was below the pots.

A shift in root : shoot dry weight was recorded in treatments of continuous inundation and no inundation. Seedlings in these stressful treatments had the lowest root: shoot biomass compared to the moderate inundation treatments. The seedlings in the continuous inundation treatment had the highest root : shoot measurements. In the salinity experiment there was a decrease in root : shoot dry weight with increasing salinity concentrations. It was suggested that high salinity reduced growth and biomass accumulation in halophytes as a typical response is a stunted growth form (Lugo *et al*., 2007). In this study on *Rhizophora mucronata* seedlings a tidal cycle or inundation period of 3 h, produced the highest root biomass.

5.2. Physiological responses to salinity and inundation

In this study measurements of the morphological characteristics such as seedling height, leaf production and biomass allocation showed that seedlings at low salinity (8 ppt) had the highest growth response. This was different to the physiological results of the same treatments, where seedlings in freshwater (0 ppt) displayed optimal photosynthetic performance and lowest stomatal resistance compared with the 8 ppt seedlings. It can be deducted that freshwater poses no salt stress on the plant and thus the increase in the photosynthetic performance and stomatal conductance. However, at low salinity concentrations (8 ppt) the available NaCl provides some nutrients to the plants thus contributing positively to growth. Photosynthetic performance and stomatal conductance were the highest for seedlings in the moderate inundated treatments, these results were similar to the growth measurements within those treatments. Several studies show similar results to this study where photosynthetic performance decreases with increasing salinity; this is also true for stomatal conductance. Biber (2006) showed similar responses to salinity in *Rhizophora mangle*. A study by Pezeshki *et al.* (1997) using the same species, showed a decrease in photosynthetic performance with a decrease in soil redox in response to an increase in inundation. However, comparing this to field studies conducted by Falqueto *et al*. (2008) the opposite was reported for *R. mangle.* Photosynthetic performance decreased in the rainy season when salinity was diluted. This might be because in field experiments other environmental factors would have had an influence on the results.

5.3. Relevance of results for mangrove rehabilitation and prediction of responses to climate change

Other mangrove species found in South Africa such as *Avicennia marina* and *Bruguiera gymnorrhiza* have different tolerance ranges to salinity and inundation. *A. marina* was more tolerant to salt (can grow in soil salinity of up to 65 ppt) (Smith 1992 in Hogarth, 1999) than *R. mucronata*, while *B gymnorrhiza* was less tolerant to salinity than the other two mangrove species (Jayatissa *et al*., 2008). Within South Africa these few key mangrove species can be recognized for rehabilitation of areas, when their tolerance range and ability to survive is taken into account. *Avicennia marina* which is a pioneer species can colonize newly formed mudbanks with tidal changes and can also tolerate high water levels but only for short periods. This mangrove is sensitive to long periods of inundation as the pneumatophores are flooded (Steinke, 1999), *Bruguiera gymnorrhiza* prefers to grow in the high intertidal region, with drier

soils and less frequent inundation such as spring tides. It is also a very shade tolerant species. *Rhizophora mucronata* are more restricted in their distribution and where occur along channels and fringing river habitats, close to the waters edge (Steinke, 1999). It is important to understand the tolerance ranges of the different species as there may be successional changes of species in response to climate change. Ecogeomorphological aspects will be influenced by climate change such as sea level rise (Nicholls, 2004), changes in the intensity and frequency of sea storms (Nicholls *et al.,* 2007) freshwater input changes, sedimentation as well as nutrient input (Kitheka, 1998; Schwendenmann *et al.,* 2006). Mangrove forests act as a natural buffer protecting coasts from climate change effects such as erosion. Management plans for these ecosystems therefore should achieve a balance between maintaining a healthy system with all its services and surplus of resources and for the protection of human settlements. This is particularly important because coastal and estuarine systems are constantly changing ecosystems and sensitive to human impact (Branch & Branch, 1995). Increased sea level, predicted to range between 0.05 to 0.11 m in the 21st century (Nicholls *et. al.,* 2007), will have negative effects on low lying areas. Mangrove ecosystems may adapt to changing sea level rise as they are capable of growing and expanding towards the land where new intertidal areas are created. The genus *Rhizophora* is very suitable for this as they have the typical prop roots that create the much needed support for these mangroves to grow in highly muddy and tidal areas and also provide the much needed gaseous exchange (Steinke, 1999). Plants that are able to grow within an estuary have to be able to adapt to a changing environment. In this study *Rhizophora mucronata* seedlings were found to be resilient to environmental change and well adapted to different salinity and inundation conditions. This species exhibited response patterns typical of halophytes with broad tolerance ranges. Biber's (2006) study on *R. mangle L.* found that this species was tolerant of high salinity (35 ppt) at the seedling stage. He also suggested that species within the same genus, as well as different genera, had different tolerant ranges and that the ability to cope with high salinity changed at different growth stages. This study showed that *R. mucronata* adapted well in high salinity and flourished under moderate salinity and inundation conditions (50% seawater and 3-9 h inundation). This makes it a suitable species to grow in mid water intertidal areas. This species is also fast growing, especially at the seedling stage, therefore biomass accumulation is rapid. Jayatissa *et al*., (2008) found that at low salinity (3 - 5 ppt), *R. mucronata* had a shoot height of 1.64 cm per week. This was over a three month treatment period. Their seedling height recordings were higher then what was found in this study, where the shoot height had an average of 0.12 cm ± 0.04 per week in low salinity (8 ppt) for the 3.5 month treatment period.

6. The response of *Rhizophora mucronata* Lam. to light and salinity

6.1. RESULTS

6.1.1. Seedling height

There was no significant difference (F = 1.13, p > 0.05, n = 0.05) in seedling height between the different light treatments for the 18 ppt and 35 ppt salinity treatments (Figure 17). The shade treatments did not influence growth. However salinity did influence growth as overall seedling growth was higher in the 18 ppt (50% seawater) shade treatments compared to the 35 ppt (100% seawater) shade treatments. The 90% shade treatment had the highest seedling height for both salinity experiments 18 ppt (1.09 ± 0.28 mm/week) and 35 ppt (0.83 ± 0.08 mm/week). Lowest recordings were found in the full sun treatments for both 18 ppt (0.69 ± 0.11 mm/week) and 35 ppt (0.61 ± 0.17 mm/week).

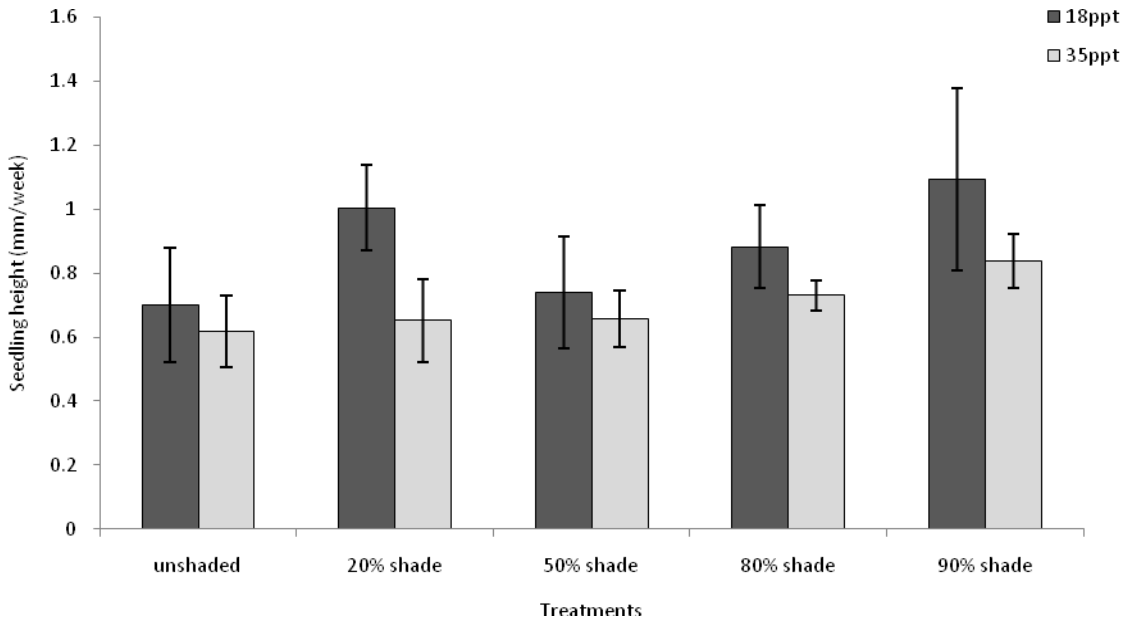

Figure 17: The effect of salinity (18 and 35 ppt) and light (unshaded and shade treatments, 20% to 90%) on the growth (seedling height) of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light in the unshaded treatment was 800 ± 200 µmol m^{-2} s^{-1}.

6.1.2. Leaf surface area

For 18 ppt the leaf surface area (cm^2) of seedlings increased from the 20% to the 90% shade treatment. A similar response was recorded for the 35 ppt salinity treatment (Figure 18). The leaf surface area for the 90% shade - 18 ppt treatment was significantly higher compared to all other light treatments ($F= 3.44$, $p < 0.05$, $n = 25$). No significant differences ($F = 3.44$, $p > 0.05$, $n = 25$) were found for the different light treatments for 35 ppt due to the variable results (Figure 19). Overall, leaf surface area was more variable for the 35 ppt treatments (20, 50 and 80% shade) compared with the 18 ppt treatments.

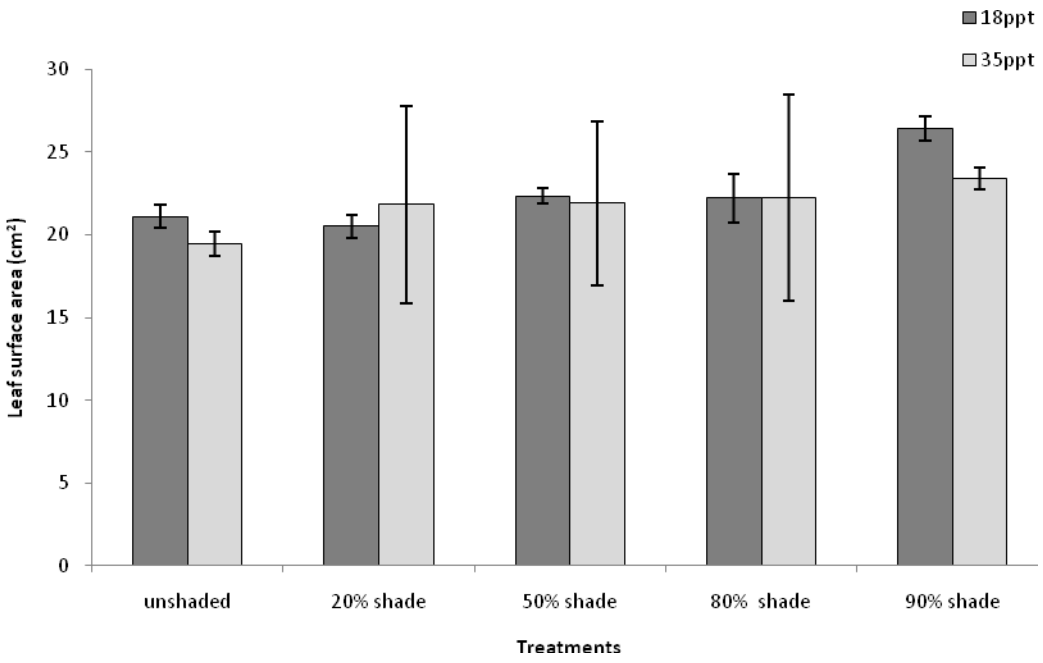

Figure 18: The effect of salinity (18 and 35 ppt) and light (Unshaded and shade treatments 20% to 90%) on the leaf surface area (cm2) of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light in the unshaded treatment was 800 ± 200 µmol m^{-2} s^{-1}.

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6.1.3. Leaf gain and leaf loss

Leaf loss was significantly higher ($F = 3.81$, $p < 0.05$, $n = 25$) than leaf production in all the light treatments for both salinity treatments (18 and 35 ppt). The exceptions were the 80% shade-18 ppt and 50% shade-35 ppt treatment. Leaf loss was significantly lower ($F = 3.81$, $p < 0.05$, $n = 25$) for the 35 ppt treatments compared with the 18 ppt treatments except for the 80% shade-18 ppt treatment (Figure 19). In the 18 ppt salinity treatments leaf production was less variable than leaf loss. No significant differences ($F = 1.53$, $p > 0.05$, $n = 25$) were found in leaf gain in both salinity treatments (18 and 35 ppt). However for leaf loss the 80% shade -18 ppt treatment was significantly lower ($F = 3.81$, $p < 0.05$, $n = 25$) than the 90% shade-18 ppt treatment. No significant differences ($F = 3.81$, $p > 0.05$, $n = 25$) were recorded for the leaf gain within the different shade treatments in the 18 ppt salinity. For leaf loss however, the 50% shade-35 ppt treatment, was significant lower ($F = 3.81$, $p < 0.05$, $n = 25$) than the 90% shade - 35 ppt treatment. Besides the differences between salinity treatments; the results were variable with no clear pattern.

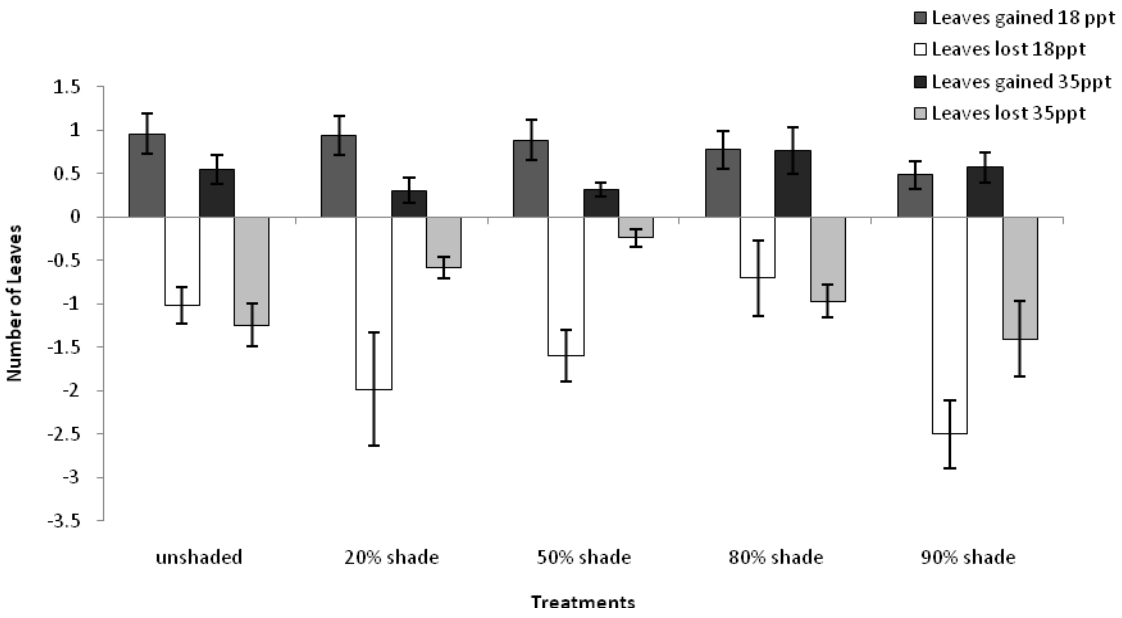


Figure 19: The effect of salinity (18 and 35 ppt) and light (unshaded and shade treatments 20% to 90%) on the leaf gain and leaf loss of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light in the unshaded treatment was 800 ± 200 µmol m^{-2} s^{-1}.

6.1.4. Leaf water content

Leaf water content was similar for all treatments (18 ppt) as no significant differences were found ($F = 1.53$, $p > 0.05$, $n = 25$). However, leaf water content was the highest (0.87 ± 0.11 g) in the 90%-18 ppt treatment (Figure 20). The 50%-18 ppt treatment had the lowest (0.65 ± 0.04 g) recordings. There were no significant differences ($F = 1.53$, $p > 0.05$, $n = 25$) between leaf water content for the 18 ppt and the 35 ppt treatments.

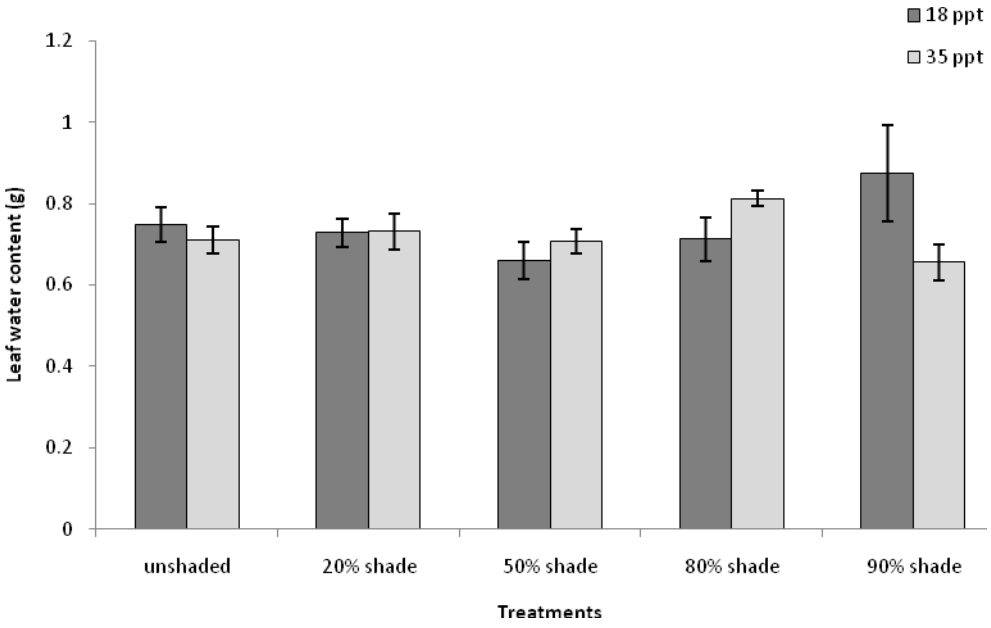


Figure 20: The effect of salinity (18 ppt) and light (unshaded and shade treatments, 20% to 90%) on the water content of the leaves of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light in the unshaded treatment was PAR 800 ± 200 µmol m^{-2} s^{-1}.

6.1.5. Biomass partitioning

No significant differences ($F = 1.04$, $p > 0.05$, $n = 25$) were found in the root to shoot biomass in the different light treatments for both salinity experiments (Table 7). There was a shift in root biomass to shoot biomass with increasing light. The unshaded treatment had a low root to shoot biomass in the seawater experiment. In the 18 ppt experiment the highest root to shoot biomass was recorded in the 50% shade treatment.

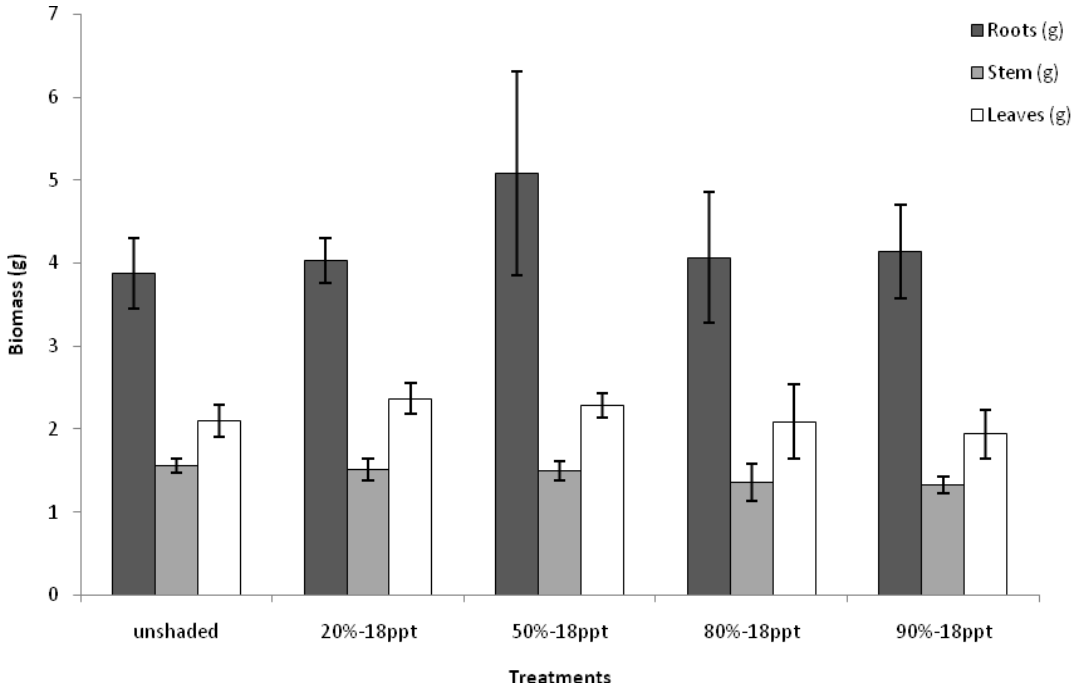
Table 7: The root : shoot ratio of the different light and salinity treatments (Shoot = leaves and stems, excluding propagules)

	Salinity	
Light treatments	**18 ppt Root : Shoot**	**35 ppt Root : Shoot**
Unshaded (869.4 µmol m^{-2} s^{-1})	1.06 ± 0.09	1.19 ± 0.10
20% shade (483.9 µmol m^{-2} s^{-1})	1.05 ± 0.05	1.41 ± 0.18
50% shade (309.2 µmol m^{-2} s^{-1})	1.30 ± 0.21	1.29 ± 0.05
80% shade (140.2 µmol m^{-2} s^{-1})	1.18 ± 0.04	1.25 ± 0.10
90% shade (16.8 µmol m^{-2} s^{-1})	1.26 ± 0.09	1.12 ± 0.06

± = SE, n = 5

Root biomass was higher than stem and leaf biomass for all treatments at both salinities (18 ppt and 35 ppt) (Figure 22). The highest overall biomass (leaves, stem and roots) was recorded for the 50% shade -18 ppt treatment (Figure 21, A). The unshaded treatment for the 18 ppt experiment had the lowest biomass accumulation compared with all the other light treatments at the same salinity. The 90% shade -35 ppt treatment had the overall lowest biomass accumulation in both salinity experiments. Root biomass decreased linearly from the 20% to 90% shade treatment in the 35 ppt experiment (Figure 21, B). However, no significant differences ($F = 0.60$, $p > 0.05$, $n = 25$) were found between the different light treatments for both the 18 ppt and 35 ppt experiments. There were also no significant differences ($F = 0.60$, $p > 0.05$, $n = 25$) in biomass accumulation between the two salinity experiments.

A.



B.

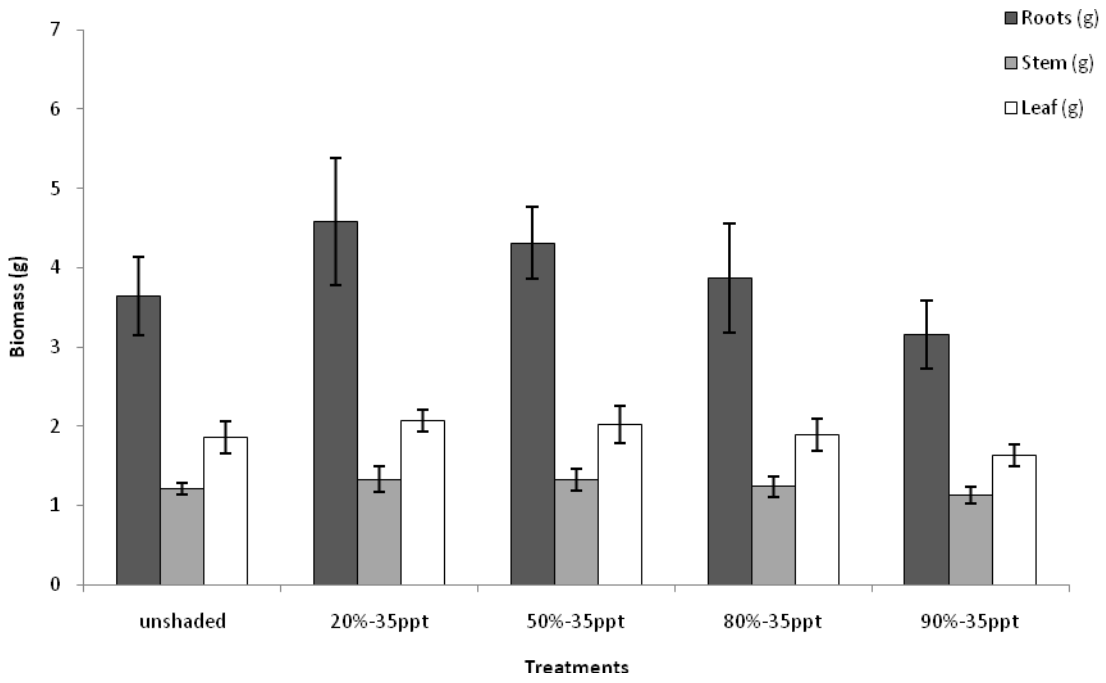


Figure 21: The effect of salinity (A =18 ppt and B = 35 ppt) and light (unshaded and shade treatments 20 to 90%) on the biomass of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light in the unshaded treatment was 800 ± 200 µmol m^{-2} s^{-1}.

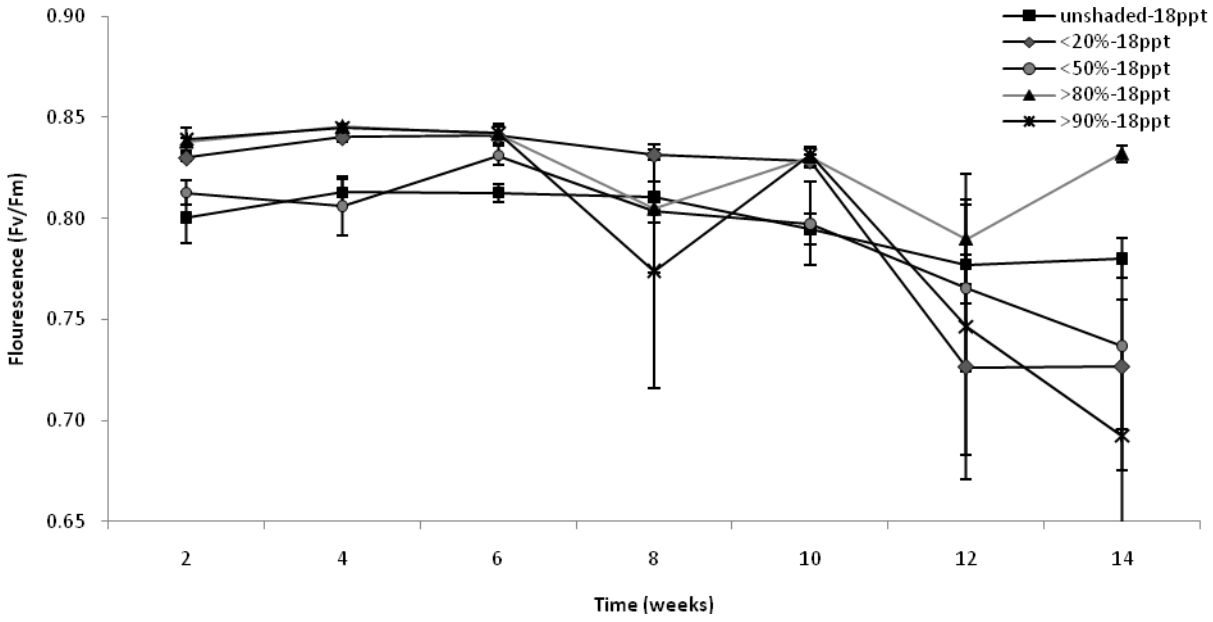
6.1.6. Photosynthetic performance (Fv/Fm)

From weeks 10 to 14 there was a decrease in photosynthetic performance for all the treatments in the 18 ppt experiment (Figure 22, A). The exception was the 80% shade - 18 ppt treatment that showed an increase in Fv/Fm from week 12 to 14 (Figure 22). However, no significant difference ($F = 3.05$, $p > 0.05$, 0.05, $n = 25$) was found for 18 ppt seedlings when comparing the different light treatments, this may be due to the variable Fv/Fm readings. Seedlings in the unshaded - 35 ppt - treatment had significantly lower ($F = 4.72$, $p < 0.05$, $n = 25$) Fv/Fm readings compared to all other treatments (Figure 22, B). There was a significant decrease ($F = 4.72$, $p < 0.05$, $n = 25$) in photosynthetic performance from week 8 to 14. All other light treatments had similar Fv/Fm values and they were not significantly different ($F = 4.72$, $p > 0.05$, $n = 25$). When comparing the two different salinity treatments (18 and 35 ppt) with the corresponding light treatments (20, 50, 80 and 90% shade) Fv/Fm values were significantly higher ($F = 3.05$, $p < 0.05$, $n = 25$) for the unshaded - 18 ppt treatment compared with the 20% and 90% shade -35 ppt treatments.

6.1.7. Stomatal conductance

No significant differences ($F = 3.14$, $p > 0.05$, $n = 25$) were found in the 18ppt experiment. However, the stomatal conductance of seedlings in the 50% shade – 18 ppt treatment had the lowest ($F = 3.14$, $p > 0.05$, $n = 25$) compared to the unshaded, 80% and 90% shade - 18 ppt treatments, resulting in the lowest stomatal conductance compared to all other treatments in the 18 ppt. Stomatal conductance showed high variability within all the treatments (Figure 23, A). As stomatal conductance showed high variability, few significant differences ($F = 3.14$, $p>0.05$, $n = 25$) within the light treatments were found. The 35 ppt (Figure 23, B) exceptions were in unshaded - 18 ppt and 50% shade treatments which were significantly lower ($F = 3.14$, $p< 0.05$, $n=25$) than 80% shade treatment in the 18 ppt experiment in weeks 12-14 (Figure 23, A). The unshaded - 35 ppt treatment had a constant decrease in stomatal conductance throughout the study period. The 20% shade -35 ppt treatments has similar results to the unshaded - 35 ppt treatment ($F = 3.14$, $p > 0.05$, $n = 25$. When comparing the two different salinity treatments (18 and 35 ppt) and their successive shade treatments (unshaded, 20, 50, 80 and 90%) there were significantly lower stomatal conductance ($F = 3.14$, $p < 0.05$, $n = 25$) recordings found in 50 and 80% shade -18 ppt compared with unshaded, 50% and 90% shade - 35 ppt treatments.

A.



B.

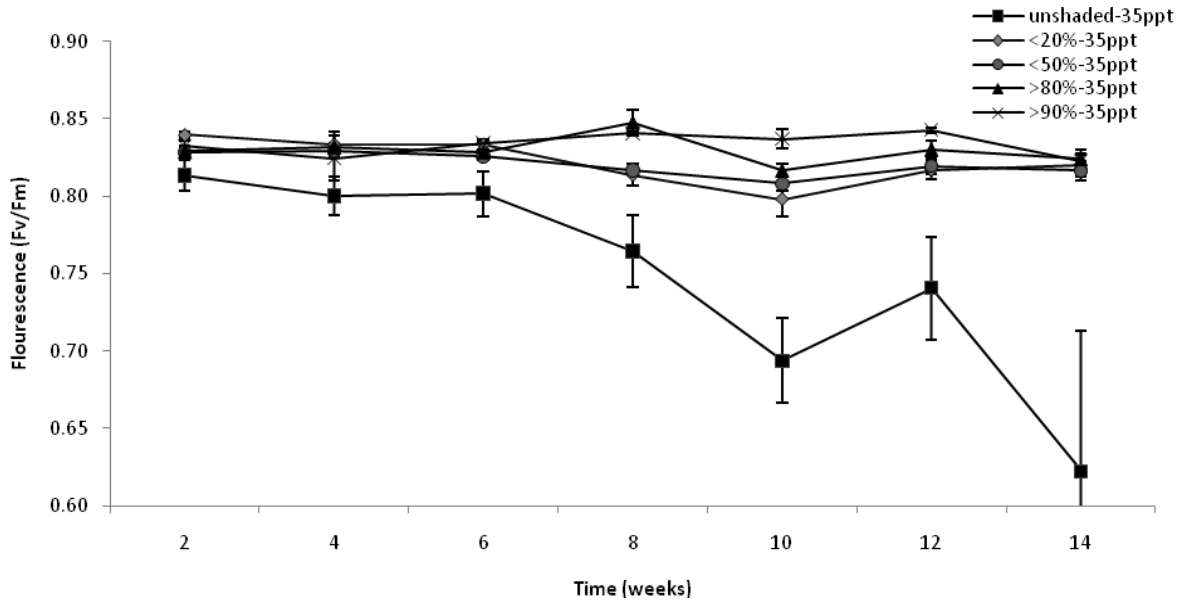
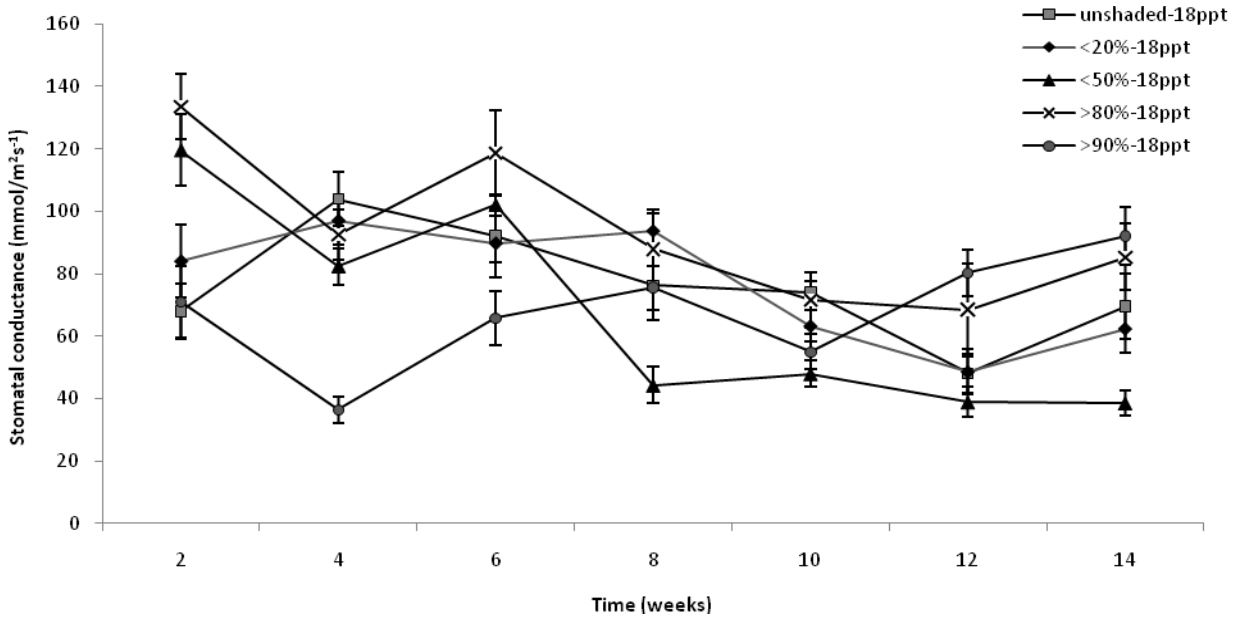


Figure 22: The effect of salinity (A = 18ppt and B = 35 ppt) and light (unshaded and shade treatments, 20% to 90%) on the photosynthetic performance (Fv/Fm) of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light in the unshaded treatment was 800 ± 200 µmol m^{-2} s^{-1}.

A.



B.

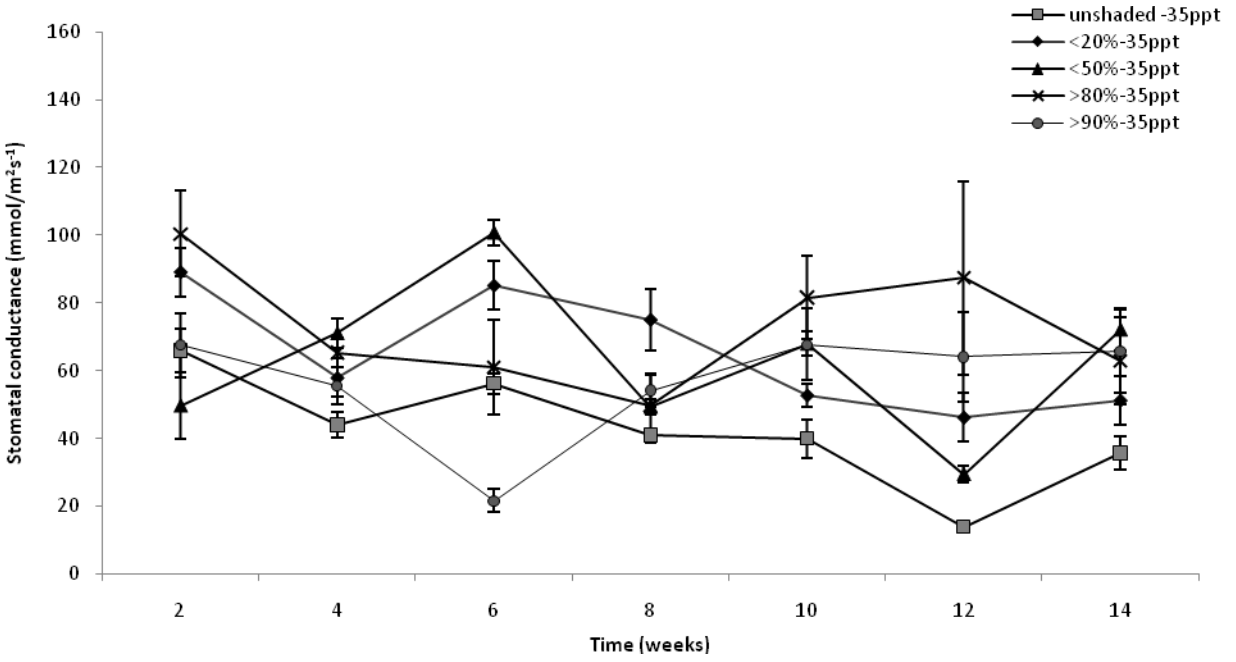


Figure 23: The effect of salinity (A = 18 ppt and B = 35 ppt) and light (unshaded and shade treatments 20% to 90%) on the stomatal conductance of *Rhizophora mucronata* seedlings over the14 week treatment period (Bars = SE, n = 25). Maximum light for the unshaded treatment was 800 ± 200 µmol m^{-2} s^{-1}.

6.1.8. Physical soil parameters

Soil conditions were measured in the pots on conclusion of the treatment period. Soil redox potential was significantly lower ($F = 27.21$, $p < 0.05$, $n = 25$) in the 20% shade-18 ppt treatment compared to the 80% and 90% shade-18 ppt treatments (Table 8). In the 35 ppt experiment the unshaded treatments had significantly lower ($F = 27.21$, $p < 0.05$, $n = 25$) soil redox potential compared to the 50%, 80% and the 90% shade treatments. The unshaded treatment-35 ppt was also significantly higher ($F = 27.21$, $p < 0.05$, $n = 25$) compared to all the light treatments in the 18 ppt experiment. The soil electrical conductivity was found to be significantly lower ($F = 12.8$ and $F = 1.3$, $p < 0.05$, $n = 25$) in the unshaded treatment in the 35 ppt experiment compared to the 50, 80 and 90% shade-18ppt treatments. Soil pH was found to be significantly lower ($F = 5.25$, $p < 0.05$, $n = 25$) in the 20% shade-18 ppt compared to the 80% and 90% shade-18 ppt. No significant differences ($F = 5.25$, $p > 0.05$, $n = 25$) of the soil pH were found in the 35 ppt experiment. When comparing the 18 ppt and 35 ppt experiments then pH for 50% shade-18 ppt was significantly higher ($F = 5.25$, $p < 0.05$, $n = 25$) compared to 50%, 80% and 90% shade-35 ppt treatments.

Table 8: Soil parameters for the light and salinity experiment. Measurement had five replicates for each treatment

Variable		18 ppt treatments				
	F	Unshaded	20%	50%	80%	90%
Redox (mV)	27.2	-130.1 ± 14.07	-105.9 ± 2.27	-168.1 ± 7.40	-152 ± 10.05	-138 ± 4.68
Electrical cond. (mS)	12.8	23.1 ± 0.91	18.8 ± 1.14	17.6 ± 0.93	15.9 ± 1.13	13.7 ± 1.30
Salinity (ppt)		11.4 ± 5.10	9.1 ± 4.07	8.3 ± 3.71	7.7 ± 3.46	6.6 ± 2.96
pH	5.25	7.5 ± 0.10	7.4 ± 0.07	7.6 ± 0.01	7.5 ± 0.13	7.5 ± 0.14
		35 ppt treatments				
Redox (mV)	27.2	-222 ± 7.41	-249.2 ± 14.30	-144 ±7.52	-123.5 ± 8.32	-119.6 ± 4.32
Electrical cond. (mS)	12.8	24.2 ± 1.20	21.3 ± 0.21	23.1 ± 0.63	22.3 ± 0.54	21 ± 0.97
Salinity (ppt)		12.1 ± 5.43	11 ± 4.94	11.5 ± 5.17	11.1 ± 4.98	10.6 ± 4.75
pH	5.25	7.5 ± 0.08	7.5 ± 0.1	7.1 ± 0.06	7 ± 0.04	7.1 ± 0.03

7. DISCUSSION

Mangrove forests are strongly influenced by salinity, inundation, nutrients and soil redox (Lopez-Hoffman *et al.,* 2006). Suarez and Medina (2008) have suggested that salinity is the most important factor contributing to mangrove zonation patterns. However, these are influenced by factors such as light, which would be one of the main influences in structuring mangrove forests. Light changes within forests in response to temporal disturbances such as storms, tree fall and sea level rise which influence the edges of the shores. Mangrove forests differ from other terrestrial forests in that there is usually one dominant genus and the trees may occur in monospecific stands. This is either because of the harsh environmental conditions, or the competitive advantages of a particular species (Duke, 2006). Some mangrove species are known to be pioneer species, and are able to be sun - and shade – tolerant, for example *Avicennia marina* (Steinke, 1999). This is because they have different distribution patterns, i.e. individual plants survive and flourish even in the open areas which are exposed to high solar irradiance, while others, such as seedlings and saplings have grown in close proximity and flourished under low light shaded canopies (Smith & Lee, 1999). This reduction of light under canopies demands special morphological adaptations such as larger leaf size as well as some anatomical adjustments such as chlorophyll content and extra light harvesting pigments (Osmond *et al.,* 1999). Plants that grow under the forest canopy are able to make the most of the low light and adjust to sudden 'sunflecks' that may pass over them. Smith and Smith (2001) explained 'sunflecks' as the direct light that passes through openings in the forest canopies and these can be as much as 70 to 80% of the only light that reaches the forest floor.

Another factor that influences mangrove succession and zonation patterns is the geomorphology of the area i.e. the stability of the specific intertidal zone or coastline, where regeneration takes place after a disturbance such as a sea storm (Chen & Twilley, 1998). Studies on forest gap dynamics have helped to understand present vegetation patterns of mangroves (such as the Everglades National Park, along the Shark River Estuary). These studies used the FORMAN model to predict the vegetation patterns. "FORMAN was developed as a tool to investigate the development of mangrove wetlands in relation to their soil characteristics" (Chen & Twilley, 1998). They also emphasized the importance of the complex interactions between the biotic and abiotic factors and the influence on the mangrove forest dynamics. In this study on the red mangrove, *Rhizophora mucronata*, the two important drivers (light and salinity) were isolated from other possible environmental factors. In order to

understand regeneration and recruitment it is important to determine responses of this species to shade and saline conditions. These factors influence species succession and zonation within an area. Ball and Critchley (1982) in Krauss *et al.* (2008) suggested that mangroves are adapted to low light and that they have achieved photosynthetic saturation points at 40% irradiance and are even able to survive in less and that irradiance is often too much and in excess for the plants to use. Björkman *et al.* (1988) have said that often too much light results in reduced growth as a result of photoinhibition and photoprotection.

7.1 Seedling growth response

In this study there was an increase in seedling height with increasing shade, where the 90% shaded (16.8 µmol m^{-2} s^{-1}) treatments in both the 18 and 35 ppt treatments had the highest seedling height. Therefore this study showed that the species *R. mucronata* was shade tolerant particularly at the seedling stage. This was different to a study by Farnsworth and Ellison (1996) in which *Rhizophora mangle* plants were taller (49.6 cm ± 3.9) in the unshaded treatments compared to the shaded treatments (40.9 cm ± 6.4). Farnsworth and Ellison (1996) measured the leaves of *Rhizophora mangle* plants and its flexibility to different light conditions in the field. The light gradient that they measured in field was between 400 µmol m^{-2} s^{-1} in the shade and 2300 µmol m^{-2} s^{-1} (unshaded) where *R. mangle* was growing along the low intertidal areas. In their study the leaf surface area and water content increased with increasing shade. The increase in leaf surface area was a response to coping with shade, where plants have to compensate for the low light conditions by maximising photosynthesis in order to grow and survive. Similar to our study was that of Jayatissa *et al.* (2008) who also used *R. mucronata* seedlings and reported a reduction in leaf size and lower leaf water content with increasing salinity. This was an osmotic response to high salinity. In this study the seawater treatment also had the highest leaf loss and this increased with increasing shade.

Highest overall biomass (leaves, stem and roots) of *R. mucronata* was recorded for the 50% shade (309.2 µmol m^{-2} s^{-1}) treatment in the moderate salinity (18 ppt) experiment. In this study *R. mucronata* seedlings showed a high shade tolerance range (from maximum light in the unshaded treatment (869.4 µmol m^{-2} s^{-1}) to 90% shade (16.8 µmol m^{-2} s^{-1}) in moderate salinity conditions while having a reduced biomass accumulation with increased shade in high salinity (35 ppt) conditions. A study conducted by Krauss & Allen (2003) on a similar species, *R. mangle*, seedlings showed that there was an increase in root biomass in more unshaded

environments (750 – 850 µmol m^{-2} s^{-1}), which was different to the increase in height and leaf area in the shaded treatments (80% shade). They showed that biomass allocation in shaded conditions was greater in the leaves and less in the roots. This was similar to this study where root biomass decreased from 20% shade (483.9 µmol m^{-2} s^{-1}) to 90% shade (16.8 µmol m^{-2} s^{-1}) in the high salinity (35 ppt) experiment. This was also found in a study done by Lopez-Hoffman *et al.* (2006) on *R. mangle* seedlings. They reported that these seedlings responded to different light and salinity gradients by having an increase in biomass accumulation and RGR with increasing light (max. light was 1200 µmol m^{-2} s^{-1}) at low salinity (20% seawater) and moderate salinity (70% seawater). There was no increase in biomass with increased light at high salinity (167% of full seawater).

7.2. Physiological responses to light and salinity

The (Fv/Fm) and stomatal conductance of the seedlings in the moderate (18 ppt) salinity experiment decreased slightly within the shade treatment after two months but was variable with no significant differences between treatments. However, for the unshaded treatment in the 35 ppt experiment, the Fv/Fm and stomatal conductance were significantly lower compared to all the other light treatments. The salt stress, in combination with the increased irradiance resulted in a decrease in photosynthetic performance and an increase in water conservation. This in turn affected the seedling growth and biomass accumulation. Mangroves such as *R. mucronata* have adapted to different environmental gradients by adjusting their leaf morphology, photosynthetic performance, plant growth, and biomass accumulation in response to available resources and changing environmental conditions. This was also found in studies done by Farnsworth and Ellison (1996) which reported that another species of the same genus, *R. mangle* had similar responses of these plants to their changing environmental conditions. However, they included nutrient uptake response, which was not included in this study, but is an important factor to consider as nutrients are coupled with carbon fixation which influences biomass gain (Lovelock, *et al.*, 2006a). Snedaker (1982) in Smith & Lee, (1999) as well as in studies done by Farnsworth and Ellison (1996) have identified *Rhizophora mangle*, L. as a species with a wide light tolerance range to both sun and shade. However, in this study the species *R. mucronata* has shown to be more shade-tolerant and moderate light (between 20% shade = 483.9 µmol m^{-2} s^{-1} to 80% shade = 140 µmol m^{-2} s^{-1}) was found to be optimal for this species. *Rhizophora* seedlings even survived in low light (90% shade = 16.8 µmol m^{-2} s^{-1}), and

may be more shade-tolerant than previously reported (Sousa *et al*. in Duke, 2006). Duke (2006) also observed that *R. stylosa* seedlings flourished under closed canopies of *Avicennia marina* and that the genera *Bruguiera* are highly shade tolerant. He considers them to be the "shade specialists". Steinke (1999) as well as Branch and Branch (1995) reported that *Bruguiera gymnorrhiza* seedlings seems to grow better in shade and often become established under parent trees, whereas *A. marina* prefers to colonize areas with abundant light and doesn't grow well in shaded conditions. Although Steinke (1999) as well as Branch and Branch (1995) did not mention the requirements of *R. mucronata* their requirements are probably intermediate between the other two species.

'Sunflecks' are difficult to recreate in greenhouse conditions and have not been included in this study. However they play an important part in the vegetation below the forest canopy and possible field studies can be useful in this regard. Knowledge of the tolerance ranges of light and salinity are fundamental to establishing whether *R. mucronata* is responding to high light by photoinhibition or low light and high salinity by being photoinactive (Osmond *et al.*, 1999).

8. GENERAL CONCLUSION

It can be concluded that *Rhizophora mucronata* prefers moderate salinity, inundation and light, therefore the hypotheses (1, 2 and 3) tested, which suggested that seedling relative growth rate (RGR) and size was significantly reduced by increased salinity (>50 % seawater), prolonged inundation and no inundation; and low light in high salinity (100% seawater), can be accepted. Hypothesis 2, which stated that prolonged inundation will stimulate stem elongation can also be accepted. However the shift in biomass allocation from shoot to root with increasing salinity, inundation and decreasing light has to be rejected as no significant differences were found for these growth parameters.

The tsunami that hit South East Asia on December 26th, 2004, has highlighted the importance of mangroves as natural barriers to protect residential areas and human life against such catastrophes and ocean surges. Therefore, when planning, rehabilitation or reconstruction areas where mangroves have been previously lost, it is important to have a well developed scientific plan in place, to select the most suitable species that are capable of surviving the harsh hydrological and environment conditions (Jayatissa *et al*., 2008). In the past many attempts in restoring mangrove forests in the past were unsuccessful due to poor planning as these habitats are complex ecosystems with many interlinking factors. Therefore, a good understanding of the mangrove species tolerance ranges, and thus the proposed planting sites, are the key to success in reestablishing mangrove forests. This study contributes to this information. In South Africa the rivers along the KwaZulu-Natal and eastern coast that contain mangroves forests are permanently open to the ocean. These trees require the connection to the sea as well as the freshwater inflow from the catchment. The ocean currents and tidal cycles, as well as geomorphology of the river banks, are important and keep the ecosystems balanced and production to a maximum (Macnae, 1963).

Different mangrove species share similar mechanisms and adaptations to their environment. The success and survival of seedlings are dependent to a large extent on factors such as salinity, temperature, periods of inundation, physico-chemical characteristics of the sediment and also competition with other species of the same genus. Biotic interactions (negative and positive) with the animals in the same habitat are also important. The human impacts contribute a large portion to the distribution of mangroves in the world. The effects of climate change such as sea level rise, increased sea surface temperatures and storms, will threaten mangrove

habitats and these ecosystems with their important environmental services will need comprehensive management plans and conservation practices to conserve them. The data from this study on the tolerance ranges of *R. mucronata* seedlings can contribute to these plans.

9. Recommendations and future research

Soil and water nutrients should be taken into account for further studies as nutrient limitation will influence mangrove growth and distribution as shown in numerous studies such as those by Mwashote *et al.* (2002); Marchard *et al.* (2004); Lovelock *et al*. (2006a); and Feller *et al*. (2007). Nutrient availability influences role in plant growth and biomass accumulation, but was not dealt with in this study as the medium that was used to grow seedlings was obtained from the same sites where the propagules were collected and no additional nutrients were added. Another recommendation would be to upgrade the research laboratories so as to have a more controlled environment. Even though temperatures were similar there was still variability in ambient temperature (25°C ± 8) and water temperature (19°C ± 6) between the storage containers. The current study was successful in measuring the effect of salinity and inundation on growth, fluorescence and stomatal conductance of *R. mucronata* seedlings. However the duration of the light and salinity experiment (14 weeks) may have been too short to fully reveal the slower responses of growth to light. Light studies with a longer duration (for example in Lopez-Hoffmann *et al.* 2006 and 2007, the study period was 39 weeks) would be needed as the response to different light conditions may produce differences in the biomass, shoot to root allocation, leaf succulence and photosynthetic performance but only after an extended time period. The tidal tank set-up developed in this project was successful and has great potential to be used in future research studies on the ecophysiological tolerances of coastal plants. This type of research is necessary in order to understand the responses of these plants to climate change.

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APPENDIX 1

Table 1: PLC Sequencer program for mixing salinity and inundation treatments (36 sec at 500 l/h = 5 l per part)

A:Time (h)	B: Tank nr.	Treatments ppt (salinity) inundation (time)	C: Time x15min	D: nr of 45 ppt Parts	E: nr of 0 ppt parts	F: nr of 35 ppt parts	G: Total Parts	H: Fill Time (min)	I: Total Litres
7h00 / 19h00	**9**	**9 ind**	**1**	**0**	**0**	**30**	**30**	**18**	**150**
7h30 / 19h30	**8**	**6 ind**	**3**	**0**	**0**	**30**	**30**	**18**	**150**
8h00 / 20h00	**7**	**3 ind**	**5**	**0**	**0**	**30**	**30**	**18**	**150**
8h30 / 20h30	**1**	**0 ppt**	**7**	**0**	**30**	**0**	**30**	**18**	**150**
9h00 / 21h00	**2**	**8 ppt**	**9**	**0**	**20**	**10**	**30**	**18**	**150**
9h30 / 21h00	**3**	**18 ppt**	**11**	**0**	**15**	**15**	**30**	**18**	**150**
10h00 / 22h00	**4**	**35 ppt**	**13**	**0**	**0**	**30**	**30**	**18**	**150**
10h30 / 22h30	**5**	**45 ppt**	**15**	***30**	**0**	**0**	**30**	**18**	**150**
11h00 / 11h30	**7**	**0 ind**	**17**	**0**	**0**	**8**	**8**	**4.8**	**40**
12h30 / 00h30	**8**	**6 ind**	**23**	**0**	**0**	**8**	**8**	**4.8**	**40**
13h30 / 01h30	**1**	**0ppt**	**27**	**0**	**8**	**0**	**8**	**4.8**	**40**
14h00 / 02h00	**2**	**8 ppt**	**29**	**0**	**6**	**2**	**8**	**4.8**	**40**
14h30 / 02h30	**3**	**18 ppt**	**31**	**0**	**4**	**4**	**8**	**4.8**	**40**
15h00 / 03h00	**4**	**35 ppt**	**33**	**0**	**0**	**8**	**8**	**4.8**	**40**
15h30 / 03h30	**5**	**45 ppt**	**35**	****8**	**0**	**0**	**8**	**4.8**	**40**
16h00 / 04h00	**9**	**9 ind**	**37**	**0**	**0**	**8**	**8**	**4.8**	**40**
16h30 / 04h30	**6**	**0 ind**	**39**	**0**	**0**	***30**	**30**	**18**	**150**
17h00 /05h00	**10**	**24 ind**	**41**	**0**	**0**	***30**	**30**	**18**	**150**

A = Hourly time for particular step

B = Tank or compartment number

C = Time intervals per 15 min since start of program cycle. Cycle is 48 times 15 min equals 12 hours

D = Number of parts for 45 ppt. Total amount pumped equals the number of parts times 5 litres

E = Number of parts for 0 ppt. Total amount pumped equals the number of parts times 5 litres

F = Number of parts for 35 ppt. Total amount pumped equals the number of parts times 5 litres

G = Total number of parts added together

H = Total time it takes to mix all parts

I = Total number of litres pumped for all parts

* 30 parts fills the compartment to high water mark (HWM)

**8 parts tops up the compartment to start siphon to bring water level down to low water mark (LWM)

***Only did water change for continuous and no inundation treatments

APPENDIX 2

SALINITY STATISTICAL OUTPUT

Tukey HSD test; variable ? End-Start (mm) (Stats Codes Sal_ RGR Plant height deviation of beginning and end results)
Approximate Probabilities for Post Hoc Tests

Error: Between MS = 3500.1, df = 20.000

	Plant Height	0	8	18	35	45
1	0		0.263986	0.999884	0.653584	0.500257
2	8	0.263986		0.331238	0.018885	0.010847
3	18	0.999884	0.331238		0.562895	0.414983
4	35	0.653584	0.018885	0.562895		0.999027
5	45	0.500257	0.010847	0.414983	0.999027	

	SS	Degr. of	MS	F	p
Plant Height	61420.7	4	15355.2	4.38704	0.01043
Error	70002.6	20	3500.1		

Tukey HSD test; variable R/S (Root_ shoot of all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .04689, df = 20.000

	Root:Shoot ratio	0	8	18	35	45
1	0		0.967469	0.853316	0.779812	0.426858
2	8	0.967469		0.996055	0.985185	0.795816
3	18	0.853316	0.996055		0.999885	0.940567
4	35	0.779812	0.985185	0.999885		0.972757
5	45	0.426858	0.795816	0.940567	0.972757	

	SS	Degr. of	MS	F	p
Root:Shoot ratio	0.15729	4	0.03932	0.8387	0.516790
Error	0.93777	20	0.04689		

Tukey HSD test; variable DW leaves_Sal (Stats Codes Sal DW_Biomass)
Approximate Probabilities for Post Hoc Tests

Error: Between MS = .47148, df = 20.000

	DW Leaves	0	8	18	35	45
1	0		0.723698	0.999068	0.956977	0.176205
2	8	0.723698		0.854137	0.331353	0.014401
3	18	0.999068	0.854137		0.877645	0.111529
4	35	0.956977	0.331353	0.877645		0.488394
5	45	0.176205	0.014401	0.111529	0.488394	

	SS	Degr. of	MS	F	p
DW Leaves	6.6697	4	1.6674	3.5366	0.024439
Error	9.4297	20	0.4715		

Tukey HSD test; variable Roots DW (Stats Codes Sal DW_Biomass)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 3.3979, df = 20.000

	DW Roots	0	8	18	35	45
1	0		0.941271	0.998474	0.936275	0.475637
2	8	0.941271		0.835141	0.560821	0.151309
3	18	0.998474	0.835141		0.987967	0.646373
4	35	0.936275	0.560821	0.987967		0.89515
5	45	0.475637	0.151309	0.646373	0.89515	

	SS	Degr. of	MS	F	p
DW Roots	22.0195	4	5.5049	1.62007	0.208229
Error	67.9584	20	3.3979		

Tukey HSD test; variable Stem DW (Stats Codes Sal DW_Biomass)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .38903, df = 20.000

	DW Stem	0	8	18	35	45
1	0		0.763337	0.989379	1	0.458967
2	8	0.763337		0.951991	0.779584	0.063689
3	18	0.989379	0.951991		0.991746	0.233426
4	35	1	0.779584	0.991746		0.441915
5	45	0.458967	0.063689	0.233426	0.441915	

	SS	Degr. of	MS	F	p
DW Stem	3.48778	4	0.87194	2.24135	0.100874
Error	7.78052	20	0.38903		

Tukey HSD test; variable #leaves end-start (Stats Codes_ Sal_Number of leaves)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .34020, df = 20.000

	Leaves gain	0	8	18	35	45
1	0		0.59612	0.96459	0.021813	0.000135
2	8	0.59612		0.252802	0.000926	0.000132
3	18	0.96459	0.252802		0.083229	0.00015
4	35	0.021813	0.000926	0.083229		0.011433
5	45	0.000135	0.000132	0.00015	0.011433	

	SS	Degr. of	MS	F	p
Leaves gain	30.9454	4	7.7364	22.7406	0
Error	6.804	20	0.3402		

Multiple Comparisons p values (2-tailed); lost leaves (Stats Codes Sal_Number of leaves)

Independent (grouping) variable

Kruskal-Wallis test: H (4, N= 25) =9.661114 p =.0465

Leaves lost	0	8	18	35	45
0		0.781297	1	1	1
8	0.781297		0.781297	0.254648	1
18	1	0.781297		1	1
35	1	0.254648	1		0.980817
45	1	1	1	0.980817	

	SS	Degr. of	MS	F	p
Leaves lost	0.3244	4	0.0811	2.49538	0.075539
Error	0.65	20	0.0325		

Tukey HSD test; variable Var2 (leaf succ_sal)
Approximate Probabilities for Post Hoc Tests

Error: Between MS = 2.3306, df = 20.000

	Leaf water content	**0**	**8**	**18**	**35**	**45**
1	0		0.586664	0.918668	0.997056	0.625008
2	8	0.586664		0.964518	0.782063	0.999996
3	18	0.918668	0.964518		0.987241	0.975454
4	35	0.997056	0.782063	0.987241		0.814554
5	45	0.625008	0.999996	0.975454	0.814554	

	SS	Degr. of	MS	F	p
Leaf water content	0.16193	4	0.04048	0.8483	0.511277
Error	0.95444	20	0.04772		

	Fv/Fm	**0**	**8**	**18**	**35**	**45**
1	0		0.971567	0.38732	0.696175	0.000135
2	8	0.971567		0.743814	0.957533	0.000145
3	18	0.38732	0.743814		0.98334	0.000394
4	35	0.696175	0.957533	0.98334		0.000202
5	45	0.000135	0.000145	0.000394	0.000202	

	Test	Value	F	Effect	Error	p
Fv/Fm	Wilks	0.197273	5.944	8	38	0.00006

Tukey HSD test; variable 7.000000 (Stats Codes Sal_Stomatal conductance)
Approximate Probabilities for Post Hoc Tests

Error: Between MS = 281.43, df = 20.000

	Stomatal conductance	**0**	**8**	**18**	**35**	**45**
1	0		0.99561	0.99328	0.000681	0.000132
2	8	0.99561		0.931031	0.000367	0.000132
3	18	0.99328	0.931031		0.001556	0.000132
4	35	0.000681	0.000367	0.001556		0.031205
5	45	0.000132	0.000132	0.000132	0.031205	

	Test	Value	F	Effect	Error	p
weeks 12-14 **Stomatal conductance**	Wilks	0.069906	13.2154	8	38	0
weeks 4-12 **Stomatal conductance**	Wilks	0.012548	7.4101	20	54.01587	0

APPENDIX 3

INUNDATION STATISTICAL OUTPUT

Tukey HSD test Approximate Probabilities for Post Hoc Tests
Error: Between MS = 746.91, df = 20.000

	Plant Height	No	3H	6H	9H	24H
1	No		0.04986	0.054402	0.069968	0.001345
2	3H	0.04986		0.999999	0.999817	0.471907
3	6H	0.054402	0.999999		0.999943	0.447508
4	9H	0.069968	0.999817	0.999943		0.379277
5	24H	0.001345	0.471907	0.447508	0.379277	

	SS	Degr. of	MS	F	p
Plant Height	16917.3	4	4229.3	5.6624	0.003245
Error	14938.1	20	746.9		

Tukey HSD test; variable DW leaves Approximate Probabilities for Post Hoc Tests

Error: Between MS = .09633, df = 20.000

	DW leaves	No	3H	6H	9H	24H
1	No		0.3461	0.291642	0.809495	0.132619
2	3H	0.3461		0.999958	0.922624	0.97587
3	6H	0.291642	0.999958		0.881031	0.989404
4	9H	0.809495	0.922624	0.881031		0.634154
5	24H	0.132619	0.97587	0.989404	0.634154	

Tukey HSD test; variable DW Roots Approximate Probabilities for Post Hoc Tests

Error: Between MS = .47384, df = 20.000

	DW roots	No	3H	6H	9H	24H
1	No		0.999643	0.955473	0.582085	0.576253
2	3H	0.999643		0.896017	0.701488	0.458451
3	6H	0.955473	0.896017		0.227039	0.926824
4	9H	0.582085	0.701488	0.227039		0.050971
5	24H	0.576253	0.458451	0.926824	0.050971	

	Degr. of	DW leaves SS	DW leaves MS	DW leaves F	DW leaves p
Leaves	4	0.73949	0.18487	1.9192	0.146529

Tukey HSD test; variable DW Stem Approximate Probabilities for Post Hoc Tests
Error: Between MS = .05045, df = 20.000

	DW stem	No	3H	6H	9H	24H
1	No		0.998038	0.871938	0.804795	0.551729
2	3H	0.998038		0.964624	0.928974	0.732771
3	6H	0.871938	0.964624		0.999899	0.976167
4	9H	0.804795	0.928974	0.999899		0.991815
5	24H	0.551729	0.732771	0.976167	0.991815	

	SS	Degr. of	MS	F	p
DW stem	0.1555	4	0.03888	0.7705	0.557042
Error	1.00907	20	0.05045		

Tukey HSD test; variable Leaves Gained Approximate Probabilities for Post Hoc Tests

Error: Between MS = .25250, df = 20.000

	Leaves gain	No	3H	6H	9H	24H
1	No		0.493006	0.230787	0.230787	0.948516
2	3H	0.493006		0.982616	0.982616	0.167687
3	6H	0.230787	0.982616		1	0.061015
4	9H	0.230787	0.982616	1		0.061015
5	24H	0.948516	0.167687	0.061015	0.061015	

	SS	Degr. of	MS	F	p
Leaves gain	3.5886	4	0.8972	3.553	0.024024
Error	5.05	20	0.2525		

Multiple Comparisons p values (2-tailed); Leaves lost (Stats Codes Ind_Number of leaves)

Independent (grouping) variable: leaves lost

Kruskal-Wallis test: H (4, N= 25) =16.02693 p =.0030

Leaves lost	No	3H	6H	9H	24H
No		1	1	1	0.042726
3H	1		1	1	0.151979
6H	1	1		1	0.334316
9H	1	1	1		0.008687
24H	0.042726	0.151979	0.334316	0.008687	

	SS	Degr. of	MS	F	p
Leaves lost	2.9126	4	0.72815	32.79955	0
Error	0.444	20	0.0222		

Tukey HSD test; Approximate Probabilities for Post Hoc Tests

Error: Between MS = 2.7288, df = 20.000

	Leaf water content	No	3H	6H	9H	24H
1	No		0.478476	0.447679	0.83967	0.635661
2	3H	0.478476		0.999998	0.967811	0.998897
3	6H	0.447679	0.999998		0.956987	0.997692
4	9H	0.83967	0.967811	0.956987		0.9957
5	24H	0.635661	0.998897	0.997692	0.9957	

	SS	Degr. of	MS	F	p
Leaf water content	0.48163	4	0.12041	2.7061	0.059664
Error	0.88991	20	0.0445		

Tukey HSD test; Approximate Probabilities for Post Hoc Tests

From week 6

Error: Between MS = .00060, df = 20.000

	Fv/Fm	No	3H	6H	9H	24H
1	No		0.999997	0.958823	0.984201	0.000132
2	3H	0.999997		0.94411	0.976062	0.000132
3	6H	0.958823	0.94411		0.999859	0.000133
4	9H	0.984201	0.976062	0.999859		0.000133
5	24H	0.000132	0.000132	0.000133	0.000133	

	Test	Wilks	Value	F	Effect	Error	p
	Fv/Fm		0.142506	7.83	8	38	0.000004

Tukey HSD test; Approximate Probabilities for Post Hoc Tests
Weeks 12-14

Error: Between MS = 123.40, df = 20.000

	Stomatal conductance		3H	6H	9H	24H
1	No		0.000383	0.000142	0.999984	0.572939
2	3H	0.000383		0.697908	0.000329	0.000138
3	6H	0.000142	0.697908		0.00014	0.000132
4	9H	0.999984	0.000329	0.00014		0.627863
5	24H	0.572939	0.000138	0.000132	0.627863	

	Test	Wilks	Value	F	Effect	Error	p
	Stomatal conductance		0.020757	8.3953	16	52.57348	0

Tukey HSD test; variable R/S (Root_ shoot of all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .11286, df = 20.000

	Root:Shoot ratio	No	3H	6H	9H	24H
1	No		0.240073	0.902715	0.411772	0.998044
2	3H	0.240073		0.710842	0.995870	0.142741
3	6H	0.902715	0.710842		0.891787	0.762912
4	9H	0.411772	0.995870	0.891787		0.265318
5	24H	0.998044	0.142741	0.762912	0.265318	

	SS	Degr. of	MS	F	p
Root:Shoot ratio	1.03570	4	0.25892	2.2943	0.094933
Error	2.25710	20	0.11286		

APPENDIX 4

LIGHT AND SALINITY STATISTICAL OUTPUT

Tukey HSD test; variable Total/13 weeks (cm) (Stats Codes for RGR of shade and salinity experiment 2009)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .11218, df = 40.000

Plant height (mm)		FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.906833	1.000000	0.996830	0.697909	0.999996	1.000000	1.000000	1.000000	0.999655
2	20_18	0.906833		0.959097	0.999874	0.999992	0.716622	0.808438	0.816276	0.949882	0.998440
3	50_18	1.000000	0.959097		0.999561	0.808438	0.999878	0.999992	0.999994	1.000000	0.999981
4	80_18	0.996830	0.999874	0.999561		0.991209	0.959476	0.983351	0.984861	0.999283	1.000000
5	90_18	0.697909	0.999992	0.808438	0.991209		0.449028	0.552571	0.562513	0.785581	0.967832
6	FS_35	0.999996	0.716622	0.999878	0.959476	0.449028		1.000000	1.000000	0.999935	0.987954
7	20_35	1.000000	0.808438	0.999992	0.983351	0.552571	1.000000		1.000000	0.999997	0.996432
8	50_35	1.000000	0.816276	0.999994	0.984861	0.562513	1.000000	1.000000		0.999998	0.996882
9	80_35	1.000000	0.949882	1.000000	0.999283	0.785581	0.999935	0.999997	0.999998		0.999959
10	90_35	0.999655	0.998440	0.999981	1.000000	0.967832	0.987954	0.996432	0.996882	0.999959	

Univariate Tests of Significance for Total/13 weeks (cm) (Stats Codes for RGR of shade and salinity experiment 2009)

Sigma-restricted parameterization

Effective hypothesis decomposition

	SS	Degr. of	MS	F	p
Plant height (mm)	1.14539	9	0.12727	1.1345	0.362129
Error	4.48707	40	0.11218		

Tukey HSD test; variable rep (Leaf surface area codes Shade_sal)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 4.9885, df = 40.000

	Leaf surface area	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.999992	0.996375	0.998416	0.017660	0.973793	0.999953	0.999883	0.998065	0.819038
2	20_18	0.999992		0.949646	0.967551	0.005624	0.998938	0.994581	0.991527	0.963952	0.566000
3	50_18	0.996375	0.949646		1.000000	0.146837	0.576300	0.999997	0.999999	1.000000	0.998752
4	80_18	0.998416	0.967551	1.000000		0.121584	0.634774	1.000000	1.000000	1.000000	0.997073
5	90_18	0.017660	0.005624	0.146837	0.121584		0.000715	0.064846	0.074555	0.127007	0.531682
6	FS_35	0.973793	0.998938	0.576300	0.634774	0.000715		0.801703	0.769023	0.621507	0.168737
7	20_35	0.999953	0.994581	0.999997	1.000000	0.064846	0.801703		1.000000	1.000000	0.978305
8	50_35	0.999883	0.991527	0.999999	1.000000	0.074555	0.769023	1.000000		1.000000	0.984875
9	80_35	0.998065	0.963952	1.000000	1.000000	0.127007	0.621507	1.000000	1.000000		0.997570
10	90_35	0.819038	0.566000	0.998752	0.997073	0.531682	0.168737	0.978305	0.984875	0.997570	

	SS	Degr. of	MS	F	p
Leaf surface area	154.71	9	17.19	3.446	0.003165
Error	199.54	40	4.99		

Tukey HSD test; variable Rep L_ gained (Leaf loss and gain codes Shade-Sal)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .19331, df = 40.000

	Leaf gain	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		1.000000	1.000000	0.999583	0.787726	0.889629	0.381718	0.410308	0.999425	0.923104
2	20_18	1.000000		1.000000	0.999835	0.825519	0.915440	0.424886	0.454649	0.999762	0.943323
3	50_18	1.000000	1.000000		0.999994	0.907310	0.964168	0.547511	0.579077	0.999989	0.978802
4	80_18	0.999583	0.999835	0.999994		0.988148	0.997889	0.798872	0.823801	1.000000	0.999218
5	90_18	0.787726	0.825519	0.907310	0.988148		1.000000	0.999676	0.999827	0.990179	0.999999
6	FS_35	0.889629	0.915440	0.964168	0.997889	1.000000		0.996826	0.997955	0.998382	1.000000
7	20_35	0.381718	0.424886	0.547511	0.798872	0.999676	0.996826		1.000000	0.813314	0.993063
8	50_35	0.410308	0.454649	0.579077	0.823801	0.999827	0.997955	1.000000		0.837310	0.995239
9	80_35	0.999425	0.999762	0.999989	1.000000	0.990179	0.998382	0.813314	0.837310		0.999425
10	90_35	0.923104	0.943323	0.978802	0.999218	0.999999	1.000000	0.993063	0.995239	0.999425	

	SS	Degr. of	MS	F	p
Leaf gain	2.67454	9	0.29717	1.5373	0.168374
Error	7.73227	40	0.19331		

Tukey HSD test; variable Rep L_lost (Leaf loss and gain codes Shade-Sal)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .60086, df = 40.000

	Leaf loss	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.626965	0.970914	0.999712	0.106217	0.999983	0.996191	0.847794	1.000000	0.998523
2	20_18	0.626965		0.998523	0.249636	0.985891	0.880475	0.154985	0.030182	0.559895	0.970914
3	50_18	0.970914	0.998523		0.718397	0.705225	0.999175	0.559895	0.182724	0.951389	0.999994
4	80_18	0.999712	0.249636	0.718397		0.022378	0.982619	1.000000	0.993709	0.999932	0.913595
5	90_18	0.106217	0.985891	0.705225	0.022378		0.264895	0.011550	0.001614	0.084421	0.445812
6	FS_35	0.999983	0.880475	0.999175	0.982619	0.264895		0.937889	0.577810	0.999905	0.999999
7	20_35	0.996191	0.154985	0.559895	1.000000	0.011550	0.937889		0.999390	0.998523	0.807279
8	50_35	0.847794	0.030182	0.182724	0.993709	0.001614	0.577810	0.999390		0.891251	0.372075
9	80_35	1.000000	0.559895	0.951389	0.999932	0.084421	0.999905	0.998523	0.891251		0.996191
10	90_35	0.998523	0.970914	0.999994	0.913595	0.445812	0.999999	0.807279	0.372075	0.996191	

	SS	Degr. of	MS	F	p
Leaf loss	20.64162	9	2.29351	3.8171	0.001503
Error	24.03434	40	0.60086		

Tukey HSD test; variable Leaves (g) (DW Shade_sal codes)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .29666, df = 40.000

	DW leaves	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.998531	0.999911	1.000000	0.999982	0.999319	1.000000	1.000000	1.000000	0.934620
2	20_18	0.998531		1.000000	0.998279	0.961285	0.887651	0.996732	0.989091	0.986453	0.516793
3	50_18	0.999911	1.000000		0.999887	0.989959	0.956114	0.999712	0.998334	0.997728	0.665938
4	80_18	1.000000	0.998279	0.999887		0.999987	0.999433	1.000000	1.000000	1.000000	0.939020
5	90_18	0.999982	0.961285	0.989959	0.999987		1.000000	0.999997	1.000000	1.000000	0.995833
6	FS_35	0.999319	0.887651	0.956114	0.999433	1.000000		0.999755	0.999978	0.999988	0.999700
7	20_35	1.000000	0.996732	0.999712	1.000000	0.999997	0.999755		1.000000	1.000000	0.955451
8	50_35	1.000000	0.989091	0.998334	1.000000	1.000000	0.999978	1.000000		1.000000	0.980735
9	80_35	1.000000	0.986453	0.997728	1.000000	1.000000	0.999988	1.000000	1.000000		0.984202
10	90_35	0.934620	0.516793	0.665938	0.939020	0.995833	0.999700	0.955451	0.980735	0.984202	

	SS	Degr. of	MS	F	p
DW leaves	1.9416	9	0.2157	0.7272	0.681357
Error	11.8662	40	0.2967		

Tukey HSD test; variable Stem (g) (DW Shade_sal codes)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .09012, df = 40.000

	DW stem	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		1.000000	0.999999	0.988768	0.974406	0.716194	0.971344	0.967290	0.947728	0.465491
2	20_18	1.000000		1.000000	0.998074	0.993890	0.836832	0.992862	0.991446	0.983710	0.607324
3	50_18	0.999999	1.000000		0.999333	0.997391	0.881586	0.996851	0.996093	0.991743	0.672634
4	80_18	0.988768	0.998074	0.999333		1.000000	0.998263	1.000000	1.000000	1.000000	0.970433
5	90_18	0.974406	0.993890	0.997391	1.000000		0.999597	1.000000	1.000000	1.000000	0.986603
6	FS_35	0.716194	0.836832	0.881586	0.998263	0.999597		0.999688	0.999773	0.999944	0.999996
7	20_35	0.971344	0.992862	0.996851	1.000000	1.000000	0.999688		1.000000	1.000000	0.988321
8	50_35	0.967290	0.991446	0.996093	1.000000	1.000000	0.999773	1.000000		1.000000	0.990148
9	80_35	0.947728	0.983710	0.991743	1.000000	1.000000	0.999944	1.000000	1.000000		0.995213
10	90_35	0.465491	0.607324	0.672634	0.970433	0.986603	0.999996	0.988321	0.990148	0.995213	

	SS	Degr. of	MS	F	p
DW stem	0.79289	9	0.08810	0.978	0.472855
Error	3.60479	40	0.09012		

Tukey HSD test; variable Roots (g) (DW Shade_sal codes)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 2.2299, df = 40.000

	DW roots		FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1		FS_18		1.000000	0.951653	1.000000	1.000000	1.000000	0.998915	0.999981	0.999999	0.998803
2		20_18	1.000000		0.980337	1.000000	1.000000	0.999992	0.999865	1.000000	1.000000	0.994411
3		50_18	0.951653	0.980337		0.984360	0.990557	0.871962	0.999936	0.997827	0.993852	0.574407
4		80_18	1.000000	1.000000	0.984360		1.000000	0.999984	0.999922	1.000000	1.000000	0.992573
5		90_18	1.000000	1.000000	0.990557	1.000000		0.999939	0.999978	1.000000	1.000000	0.987388
6		FS_35	1.000000	0.999992	0.871962	0.999984	0.999939		0.990805	0.999333	0.999855	0.999951
7		20_35	0.998915	0.999865	0.999936	0.999922	0.999978	0.990805		1.000000	0.999993	0.879197
8		50_35	0.999981	1.000000	0.997827	1.000000	1.000000	0.999333	1.000000		1.000000	0.964061
9		80_35	0.999999	1.000000	0.993852	1.000000	1.000000	0.999855	0.999993	1.000000		0.981730
10		90_35	0.998803	0.994411	0.574407	0.992573	0.987388	0.999951	0.879197	0.964061	0.981730	

		SS	Degr. of	MS	F	p
	DW roots	12.1034	9	1.3448	0.6031	0.786739
Error		89.1970	40	2.2299		

Tukey HSD test; variable 7 (Stats Codes Flourecence of shade and salinity experiment 2009)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .00886, df = 40.000

weeks 12-14

	Fv/Fm		FS_35	20_35	50_35	80_35	90_35	FS_35	20_35	50_35	80_35	90_35
1		FS_18		0.995522	0.999163	0.996619	0.895497	0.227535	0.999597	0.999815	0.999034	0.999278
2		20_18	0.995522		1.000000	0.747178	0.999890	0.760892	0.855042	0.881051	0.817704	0.831191
3		50_18	0.999163	1.000000		0.840962	0.999034	0.653281	0.922080	0.939510	0.895497	0.905297
4		80_18	0.996619	0.747178	0.840962		0.386737	0.032266	1.000000	1.000000	1.000000	1.000000
5		90_18	0.895497	0.999890	0.999034	0.386737		0.971046	0.514614	0.554203	0.464947	0.482068
6		FS_35	0.227535	0.760892	0.653281	0.032266	0.971046		0.053822	0.062255	0.044524	0.047580
7		20_35	0.999597	0.855042	0.922080	1.000000	0.514614	0.053822		1.000000	1.000000	1.000000
8		50_35	0.999815	0.881051	0.939510	1.000000	0.554203	0.062255	1.000000		1.000000	1.000000
9		80_35	0.999034	0.817704	0.895497	1.000000	0.464947	0.044524	1.000000	1.000000		1.000000
10		90_35	0.999278	0.831191	0.905297	1.000000	0.482068	0.047580	1.000000	1.000000	1.000000	

		Test	Value	F	Effect	Error	p
	Fv/Fm	Wilks	0.021728	3.05	63	197.5981	0.000000

	Fv/Fm		FS_35	20_35	50_35	80_35	90_35
1		FS_35		0.048651	0.032472	0.000637	0.001430
2		20_35	0.048651		0.999674	0.281793	0.496009
3		50_35	0.032472	0.999674		0.374465	0.612777
4		80_35	0.000637	0.281793	0.374465		0.993401
5		90_35	0.001430	0.496009	0.612777	0.993401	

		Test	Value	F	Effect	Error	p
	Fv/Fm	Wilks	0.065813	4.72	16	52.57348	0.000009

Tukey HSD test; variable 6th (Stats Codes for Stomatal conductance of shade and salinity experiment 2009)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 722.39, df = 40.000

weeks 12-14
Stomatal conductance

		FS_35	20_35	50_35	80_35	90_35	FS_35	20_35	50_35	80_35	90_35
1	FS_18		1.000000	0.999932	0.967686	0.673818	0.599283	1.000000	0.982180	0.402634	0.993913
2	20_18	1.000000		0.999881	0.973550	0.697244	0.574854	1.000000	0.977785	0.425205	0.995477
3	50_18	0.999932	0.999881		0.767343	0.337150	0.895393	0.999990	0.999899	0.154634	0.893028
4	80_18	0.967686	0.973550	0.767343		0.999460	0.069735	0.945201	0.410918	0.980653	1.000000
5	90_18	0.673818	0.697244	0.337150	0.999460		0.011871	0.603468	0.114808	0.999991	0.993482
6	FS_35	0.599283	0.574854	0.895393	0.069735	0.011871		0.669752	0.995301	0.003641	0.125600
7	20_35	1.000000	1.000000	0.999990	0.945201	0.603468	0.669752		0.991328	0.340342	0.986828
8	50_35	0.982180	0.977785	0.999899	0.410918	0.114808	0.995301	0.991328		0.042750	0.577955
9	80_35	0.402634	0.425205	0.154634	0.980653	0.999991	0.003641	0.340342	0.042750		0.928600
10	90_35	0.993913	0.995477	0.893028	1.000000	0.993482	0.125600	0.986828	0.577955	0.928600	

weeks 12-14
Stomatal conductance

Test	Value	F	Effect	Error	p
Wilks	0.335539	3.1475	18	78	0.000236

weeks 2-8

Stomatal conductance

		FS_35	20_35	50_35	80_35	90_35	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.997915	0.285682	0.940023	0.000158	0.000158	0.000270	0.013524	0.002029	0.000200
2	20_18	0.997915		0.783513	0.999952	0.000158	0.000164	0.001764	0.104135	0.019729	0.000819
3	50_18	0.285682	0.783513		0.968889	0.000273	0.002173	0.148568	0.941754	0.602933	0.079664
4	80_18	0.940023	0.999952	0.968889		0.000159	0.000197	0.007504	0.288988	0.071842	0.003372
5	90_18	0.000158	0.000158	0.000273	0.000159		0.996181	0.289643	0.007531	0.045495	0.450914
6	FS_35	0.000158	0.000164	0.002173	0.000197	0.996181		0.823210	0.074893	0.297942	0.934628
7	20_35	0.000270	0.001764	0.148568	0.007504	0.289643	0.823210		0.865661	0.996681	1.000000
8	50_35	0.013524	0.104135	0.941754	0.288988	0.007531	0.074893	0.865661		0.999536	0.714180
9	80_35	0.002029	0.019729	0.602933	0.071842	0.045495	0.297942	0.996681	0.999536		0.975605
10	90_35	0.000200	0.000819	0.079664	0.003372	0.450914	0.934628	1.000000	0.714180	0.975605	

weeks 2-8

Stomatal conductance

Test	Value	F	Effect	Error	p
Wilks	0.054032	7.0946	27	111.6219	0.000000

Tukey HSD test; variable WW-DW (Leaf water content codes)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .01424, df = 40.000

	Leaf water content	FS_35	20_35	50_35	80_35	90_35	FS_35	20_35	50_35	80_35	90_35
1	FS_18		1.000000	0.973065	0.999980	0.805368	0.999964	1.000000	0.999923	0.997152	0.963492
2	20_18	1.000000		0.994865	1.000000	0.657267	1.000000	1.000000	1.000000	0.981952	0.992044
3	50_18	0.973065	0.994865		0.999347	0.158344	0.999562	0.993064	0.999756	0.590847	1.000000
4	80_18	0.999980	1.000000	0.999347		0.517260	1.000000	1.000000	1.000000	0.944585	0.998768
5	90_18	0.805368	0.657267	0.158344	0.517260		0.494881	0.682089	0.465012	0.997891	0.140591
6	FS_35	0.999964	1.000000	0.999562	1.000000	0.494881		1.000000	1.000000	0.935540	0.999159
7	20_35	1.000000	1.000000	0.993064	1.000000	0.682089	1.000000		0.999999	0.985858	0.989515
8	50_35	0.999923	1.000000	0.999756	1.000000	0.465012	1.000000	0.999999		0.921780	0.999503
9	80_35	0.997152	0.981952	0.590847	0.944585	0.997891	0.935540	0.985858	0.921780		0.553045
10	90_35	0.963492	0.992044	1.000000	0.998768	0.140591	0.999159	0.989515	0.999503	0.553045	

	SS	Degr. of	MS	F	p
Leaf water content	0.19670	9	0.02186	1.535	0.169182
Error	0.56957	40	0.01424		

Tukey HSD test; variable R/S (Root_ shoot of all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .06195, df = 40.000

	Root:Shoot	FS_35	20_35	50_35	80_35	90_35	FS_35	20_35	50_35	80_35	90_35
1	FS_18		1.000000	0.887345	0.998713	0.955213	0.998470	0.465441	0.892454	0.972869	0.999997
2	20_18	1.000000		0.847429	0.996887	0.932047	0.996371	0.407963	0.853507	0.956224	0.999979
3	50_18	0.887345	0.847429		0.999205	1.000000	0.999344	0.999238	1.000000	0.999999	0.979654
4	80_18	0.998713	0.996887	0.999205		0.999962	1.000000	0.905219	0.999312	0.999994	0.999995
5	90_18	0.955213	0.932047	1.000000	0.999962		0.999972	0.993771	1.000000	1.000000	0.995810
6	FS_35	0.998470	0.996371	0.999344	1.000000	0.999972		0.911229	0.999435	0.999996	0.999993
7	20_35	0.465441	0.407963	0.999238	0.905219	0.993771	0.911229		0.999121	0.987501	0.706761
8	50_35	0.892454	0.853507	1.000000	0.999312	1.000000	0.999435	0.999121		0.999999	0.981192
9	80_35	0.972869	0.956224	0.999999	0.999994	1.000000	0.999996	0.987501	0.999999		0.998273
10	90_35	0.999997	0.999979	0.979654	0.999995	0.995810	0.999993	0.706761	0.981192	0.998273	

	SS	Degr. of	MS	F	p
Root:Shoot	0.58242	9	0.06471	1.045	0.423124
Error	2.47788	40	0.06195		

APPENDIX 5

SOIL CHARACTERISTICS STATISTICAL OUTPUT

SALINITY EXPERIMENT

Tukey HSD test; variable Redox (mV) (Stats code_physico chem all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 9474.3, df = 20.000

Redox		0	8	18	35	45
1	0		0.464675	1.000000	0.645331	0.320341
2	8	0.464675		0.454123	0.998080	0.998691
3	18	1.000000	0.454123		0.634188	0.311695
4	35	0.645331	0.998080	0.634188		0.976988
5	45	0.320341	0.998691	0.311695	0.976988	

	SS	Degr. of	MS	F	p
Redox	67723	4	16931	1.7870	0.171069
Error	189485	20	9474		

Multiple Comparisons p values (2-tailed); Econduc (mS) (Stats code_physico chem all)

Independent (grouping) variable: Treatments

Kruskal-Wallis test: H (4, N= 25) =23.07692 p =.0001

Electrical conductivity	0	8	18	35	45
0		1.000000	0.316864	0.012707	0.000173
8	1.000000		1.000000	0.316864	0.012707
18	0.316864	1.000000		1.000000	0.316864
35	0.012707	0.316864	1.000000		1.000000
45	0.000173	0.012707	0.316864	1.000000	

Salinity (ppt)	0	8	18	35	45
0		1.000000	1.000000	0.041454	0.001473
8	1.000000		1.000000	0.319720	0.012947
18	1.000000	1.000000		1.000000	0.118103
35	0.041454	0.319720	1.000000		1.000000
45	0.001473	0.012947	0.118103	1.000000	

	SS	Degr. of	MS	F	p
Treatments	522.011	4	130.503	98.1639	0.000000
Error	23.930	18	1.329		

INUNDATION EXPERIMENT

Tukey HSD test; variable Redox (Stats code_physico chem all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 568.06, df = 11.000

Redox		No	3H	6H	9H	24H
1	No		0.540548	0.037744	0.265397	0.028246
2	3H	0.540548		0.464198	0.980874	0.380544
3	6H	0.037744	0.464198		0.765824	0.999810
4	9H	0.265397	0.980874	0.765824		0.674541
5	24H	0.028246	0.380544	0.999810	0.674541	

	SS	Degr. of	MS	F	p
Redox	9973	4	2493	4.389	0.023061
Error	6249	11	568		

Multiple Comparisons p values (2-tailed); Econduc (mS) (Stats code_physico chem all)

Kruskal-Wallis test: H (4, N= 25) =18.01846 p =.0012

Electrical conductivity	No	3H	6H	9H	24H
No		0.781297	0.227716	0.112432	0.000308
3H	0.781297		1.000000	1.000000	0.161218
6H	0.227716	1.000000		1.000000	0.586853
9H	0.112432	1.000000	1.000000		1.000000
24H	0.000308	0.161218	0.586853	1.000000	

	SS	Degr. of	MS	F	p
Electrical conductivity	8415.67	4	2103.92	329.332	0.00
Error	127.77	20	6.39		

Salinity (ppt)	No	3H	6H	9H	24H
No		1.000000	0.215203	0.052246	0.000447
3H	1.000000		1.000000	1.000000	0.119518
6H	0.215203	1.000000		1.000000	0.745658
9H	0.052246	1.000000	1.000000		1.000000
24H	0.000447	0.119518	0.745658	1.000000	

	SS	Degr. of	MS	F	p
Salinity (ppt)	2095.477	4	523.869	340.504	0.00
Error	30.770	20	1.539		

LIGHT AND SALINITY EXPERIMENT

Tukey HSD test; variable Redox (Stats code_physico chem all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 392.47, df = 40.000

	Redox		FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1		FS_18		0.648789	0.105660	0.764783	0.999749	0.000158	0.000158	0.981066	0.999938	0.997512
2		20_18	0.648789		0.000641	0.021757	0.270282	0.000158	0.000158	0.103075	0.919663	0.982479
3		50_18	0.105660	0.000641		0.951410	0.352417	0.003830	0.000160	0.656006	0.029508	0.013067
4		80_18	0.764783	0.021757	0.951410		0.980140	0.000216	0.000158	0.999722	0.427689	0.260496
5		90_18	0.999749	0.270282	0.352417	0.980140		0.000159	0.000158	0.999973	0.974763	0.898230
6		FS_35	0.000158	0.000158	0.003830	0.000216	0.000159		0.488998	0.000164	0.000158	0.000158
7		20_35	0.000158	0.000158	0.000160	0.000158	0.000158	0.488998		0.000158	0.000158	0.000158
8		50_35	0.981066	0.103075	0.656006	0.999722	0.999973	0.000164	0.000158		0.821772	0.640504
9		80_35	0.999938	0.919663	0.029508	0.427689	0.974763	0.000158	0.000158	0.821772		0.999999
10		90_35	0.997512	0.982479	0.013067	0.260496	0.898230	0.000158	0.000158	0.640504	0.999999	

		SS	Degr. of	MS	F	p
	Redox	96146	9	10683	27.219	0.000000
Error		15699	40	392		

Tukey HSD test; variable Econduc (mS) (Stats code_physico chem all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = 4.5357, df = 39.000

	Electrical conductivity	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.064949	0.006492	0.000276	0.000150	0.999133	0.930714	1.000000	0.999842	0.849624
2	20_18	0.064949		0.996231	0.505861	0.018986	0.016299	0.681866	0.070621	0.228938	0.807802
3	50_18	0.006492	0.996231		0.955021	0.155182	0.001621	0.185556	0.007168	0.031864	0.276932
4	80_18	0.000276	0.505861	0.955021		0.848247	0.000178	0.008484	0.000291	0.000992	0.015275
5	90_18	0.000150	0.018986	0.155182	0.848247		0.000150	0.000212	0.000150	0.000153	0.000274
6	FS_35	0.999133	0.016299	0.001621	0.000178	0.000150		0.592316	0.998744	0.950105	0.460534
7	20_35	0.930714	0.681866	0.185556	0.008484	0.000212	0.592316		0.940516	0.998651	1.000000
8	50_35	1.000000	0.070621	0.007168	0.000291	0.000150	0.998744	0.940516		0.999906	0.865623
9	80_35	0.999842	0.228938	0.031864	0.000992	0.000153	0.950105	0.998651	0.999906		0.991412
10	90_35	0.849624	0.807802	0.276932	0.015275	0.000274	0.460534	1.000000	0.865623	0.991412	

	SS	Degr. of	MS	F	p
Electrical conductivity	525.89	9	58.43	12.883	0.000000
Error	176.89	39	4.54		

Tukey HSD test; variable pH (Stats code_physico chem all)

Approximate Probabilities for Post Hoc Tests

Error: Between MS = .03796, df = 40.0

pH		FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
1	FS_18		0.999999	0.994654	1.000000	0.999977	1.000000	1.000000	0.210537	0.038135	0.071787
2	20_18	0.999999		0.962138	1.000000	0.998563	1.000000	0.999948	0.360702	0.080395	0.142554
3	50_18	0.994654	0.962138		0.981523	0.999983	0.979638	0.999287	0.025129	0.003064	0.006383
4	80_18	1.000000	1.000000	0.981523		0.999668	1.000000	0.999996	0.291253	0.059218	0.107779
5	90_18	0.999977	0.998563	0.999983	0.999668		0.999595	1.000000	0.077432	0.010980	0.022115
6	FS_35	1.000000	1.000000	0.979638	1.000000	0.999595		0.999994	0.299452	0.061564	0.111699
7	20_35	1.000000	0.999948	0.999287	0.999996	1.000000	0.999994		0.137780	0.022115	0.043106
8	50_35	0.210537	0.360702	0.025129	0.291253	0.077432	0.299452	0.137780		0.998780	0.999960
9	80_35	0.038135	0.080395	0.003064	0.059218	0.010980	0.061564	0.022115	0.998780		1.000000
10	90_35	0.071787	0.142554	0.006383	0.107779	0.022115	0.111699	0.043106	0.999960	1.000000	

	SS	Degr. of	MS	F	p
Treatment	1.794	9	0.199	5.25	0.000101
Error	1.518	40	0.038		

Salinity (ppt)	FS_18	20_18	50_18	80_18	90_18	FS_35	20_35	50_35	80_35	90_35
FS_18		1.000000	0.456817	0.170067	0.016156	1.000000	1.000000	1.000000	1.000000	1.000000
20_18	1.000000		1.000000	1.000000	1.000000	0.332200	1.000000	1.000000	1.000000	1.000000
50_18	0.456817	1.000000		1.000000	1.000000	0.045649	1.000000	0.415653	1.000000	1.000000
80_18	0.170067	1.000000	1.000000		1.000000	0.013679	0.743681	0.153241	0.456817	1.000000
90_18	0.016156	1.000000	1.000000	1.000000		0.000825	0.096467	0.014262	0.053205	0.301289
FS_35	1.000000	0.332200	0.045649	0.013679	0.000825		1.000000	1.000000	1.000000	1.000000
20_35	1.000000	1.000000	1.000000	0.743681	0.096467	1.000000		1.000000	1.000000	1.000000
50_35	1.000000	1.000000	0.415653	0.153241	0.014262	1.000000	1.000000		1.000000	1.000000
80_35	1.000000	1.000000	1.000000	0.456817	0.053205	1.000000	1.000000	1.000000		1.000000
90_35	1.000000	1.000000	1.000000	1.000000	0.301289	1.000000	1.000000	1.000000	1.000000	

	SS	Degr. of	MS	F	p
Salinity (ppt)	1969.170	9	218.797	1.37616	0.231176
Error	6359.617	40	158.990		

APPENDIX 6

References for light treatments used in mangrove studies

Photosynthetic photonflux density (PPFD)	References
Maximum light 800 µmol m^{-2} s^{-1} Using 80% neutral-density shade cloth	Allen & Krauss (2006)
Unshaded maximum levels between 700 and 800 µmol m^{-2} s^{-1} Shaded maximum levels around 100 µmol m^{-2} s^{-1} using 80% shade cloth	Allen *et al.* (2006)
Field experiment Full sun = 2300 µmol m^{-2} s^{-1} under canopy maximum light 394 ± 14 PAR	Farnsworth & Ellison (1996)
Unshaded maximum levels between 750 and 850 µmol m^{-2} s^{-1} Shaded maximum levels around 125 µmol m^{-2} s^{-1} using 80% shade cloth	Krauss & Allen (2003)
Light source 1200 µmol m^{-2} s^{-1} Treatments were 5, 12, 25 and 50% PAR natural light	López-Hoffmann *et al.* (2006)
Maximum light in greenhouse 900 - 1000 µmol m^{-2} s^{-1} Treatments were 6, 50, 75, 90 % light filtration shade cloth	López-Hoffmann *et al.* (2007)
Maximum natural light 800 -1000 µmol m^{-2} s^{-1}	Luzhen *et al.* (2005)
High light = 1550 - 2000 µmol m^{-2} s^{-1} Medium light = 260 - 340 µmol m^{-2} s^{-1} Low light = 46 - 60 µmol m^{-2} s^{-1}	Smith & Lee (1999)
Maximum levels of PAR 1550 ± µmol m^{-2} s^{-1}	Suárez & Medina (2005)