
THE UNIVERSITY *of* EDINBURGH

# Physiological ecology of Malaysian mangroves in response to sea level rise

Siti Mariam Muhammad Nor

Thesis submitted in fulfilment of
the requirements for the degree of
Doctor of Philosophy
to the
The University of Edinburgh
2017

Declaration:

I declare that this thesis has been composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification.

Siti Mariam Muhammad Nor

November 2017

# Acknowledgement

Firstly, I would like to express special appreciation to my main supervisors Dr. Gail Jackson for the motivation and supervised me with kind and patience. Special thanks also to all my Co-supervisors Prof Maurizio Mencuccini and Dr.Yann Salmon for invaluable guidance and assisting me in statistical analysis. I would like to thanks Prof. Patrick Meir for helping in design the flooding experiment. Special thanks goes to Prof. Mark Huxham (Edinburgh Napier University) who always helping and providing invaluable guidance in chapter belowground. Many thanks to all of you for your constant help, support, patience and guiding me throughout four years PhD study. Without this help I would not able to get the thesis finish.

This work would not be possible without help from many people. I would like to acknowledge all people helping me during fieldwork in Malaysia, helping me set up the experiment plots, counting and measuring the trees dbh within the plots, collecting ingrowth cores every three months and sorted out the roots in the lab. These peoples are Safuan Mustafa, Mohd Fadli, Mr Mohd Razali Salam (UMT laboratory technician), boatman (Mr Pok Mid) and soil digger (Mr Mustapa). I would like to thanks to Alban-Mazars Simmon and Symone Duddy undergraduate student from Edinburgh Napier University who helped me collecting the data during final fieldwork in February 2016. I will remember we had great time measuring the dbh during the high tide.

I would also say thanks for people assisting me in flooding experiment. Many thanks to Dr. Alan Gray, plant ecologist at Centre for Ecology and Hydrology (CEH) Edinburgh who helps me set up the glasshouse with tropical temperature, adequate lighting, and equipment for manipulation experiments. Many thanks to CEH technical staff who make regular maintenance of the glasshouse to ensure the experiment going well for almost three months.

I would like to thanks people who helped me during flooding experiment. Mr Hanif a volunteer from School of Biological Sciences University of Edinburgh who help me to measure the stem diameter, plants height also counting the plant leaves for two months. Undergraduate student of Ecology and Environmental Sciences helping me

during plant harvesting in December 2015. Apologies not to mention all name here, but I really enjoyed to meet all of you and we had chit chatting about mangrove topics while doing work.

Many thanks to my friend in the Crew attic, Pippa, Claudia, Hemant, Elsa who advise, provide helps and support each other. My pleasure to thanks friend in the Crew Annex, Isla, Philippe and William who has very kind and nice to me. Thanks to Malaysian friend in Geosciences Wan Shafrina, who helps me with statistical thing and willing to lend your ear during my difficult time. Many thanks to other Malaysian friend Nor Amalina, Akmal and Norlizan who always give support during my critical time. My friend in University of Surrey, Maisarah who give shoulder to cried on, my UMT friend Norasmah, Nur Fariza, Norzalipah and Norliawati who has motivated me to finish PhD.

Special thanks to my parents; especially my mum Suwaibah Abdul Rahman for unwavering love and keep prays for me, my sisters and brother who always support my journey, my late dad Muhammad Nor Salleh who has strongly encourage me to pursue my PhD.

I would like to say million thanks to my lovely husband Sayuti Husain for your love, support, sacrifice, patience, your care and concern, your strength to be with me during my happy and difficult time. Many thanks for your help during the fieldwork, dig the soil, climbing trees collect the ingrowth core and I will always remember you sleep on a tree when I am doing my work. Thanks again for being a great husband and wonderful dad to our kids. Special appreciation to my little girls Huda Insyiraah and Husna who keep patience with me when am doing my work at the office particularly during weekend. I am sorry not able to accompany and play with you. I promise to bring both of you to the playground just after I submit this thesis.

Last but not least thanks to my sponsor Malaysian Ministry of higher education for the three years funding. Also thanks to School of Geoscience providing fieldwork and write up funding to support my stay in final year. Finally thanks to everyone who had providing help and contribution to my study.

*Dedicated to my husband*

*Sayuti Husain*

*My little girls*

*Huda Insyiraah & Husna*

*My parents*

# Abstract

Mangroves are threatened by rising sea levels as a consequence of climate change or locally altered coastal hydrology. Prolonged submergence resulting from increased flooding regimes may negatively influence mangrove growth and survival, particularly at the seedling stage. Seedlings from two dominant Malaysian species, *Bruguiera gymnorrhiza* and *Rhizophora apiculata*, were exposed to differing durations of flooding in order to simulate a range of sea level rise conditions. This experiment was conducted in the glasshouse for 11 weeks with four flooding treatments: 6 hrs, 18 hrs, 24 hrs and 24 hrs (stagnant). Survival, growth, xylem anatomy and a suite of ecophysiological responses were monitored to quantify and understand plant response to varying degrees of inundation. The ecophysiological measurements comprised leaf chlorophyll content, chlorophyll fluorescence, maximum photosynthetic rate (Amax) and stomatal conductance ($g_s$). Leaf carbohydrate reserves (non-structural carbohydrates, starch and sucrose) and leaf area were also quantified at the end of the experiment, after week 11 (chapter 3).

Mangrove seedling survival was 100% under all flooding treatments. Longer flooding significantly increased the seedlings' height and stem diameter but resulted in fewer leaves produced under long submergence. Morphological adaptations were observed during flooding treatment (i.e. lenticels structure on seedlings stem and adventitious root). Leaf physiological properties (chlorophyll content and chlorophyll fluorescence) exhibited significantly higher values under long submergence, although they showed period of stress during flooding experiment. Maximum photosynthesis rate and stomatal conductance remained higher during the early part of the flooding experiment and lower toward the end of flooding period. Flooding resulted in higher accumulation of total non-structural carbohydrates in *B. gymnorrhiza* than *R. apiculata* although the effect was not significant. Plant leaf area was significantly higher under the 24 hour (stagnant) treatment particularly in *B. gymnorrhiza* seedlings, implying the seedlings expanded their leaf area under long submergence. Overall, most of the response variables were not affected by the flooding treatment and in fact seedlings showed increased growth under high flooding.

Seedlings exposed to longer submergence times may suffer from oxygen deficiency, particularly where submergence times approach 24 hrs (as simulated by the 24 hr 'stagnant' treatment). To examine how flooding treatments affected the plant stem, xylem anatomy was quantified in both mangrove species. Small segments of the plant apex (3 cm from the shoot tip) and plant stem (12-15 cm from the shoot tip) were examined using light and electron microscopy. Xylem vessel diameter, cell wall thickness, vessel density and vessel hydraulic diameter were quantified. Vessel diameter varied between plant sections and species. Vessel density and lumen area were significantly higher at the plant apex ($p < 0.05$, $p < 0.001$ respectively), but vessel hydraulic diameter was significantly higher at plant mid stem ($p < 0.001$). There were surprisingly few effects on vessel metrics in response to the flooding treatment: at the

extreme treatment of 24 hrs flooding, there was marginally, but not significantly, less cell wall thickening in one species (*R. apiculata*); vessel lumen area increased to a small extent ($p > 0.05$), but vessel density did not vary among treatments. Overall, the data demonstrate significant differences in xylem anatomy associated with location on the plant (apex or stem), but very minor effects of flooding, at least following the 11 week experimental treatment (chapter 4).

This study also investigated the carbon dynamics of a mangrove forest site in Malaysia. Standing stock was quantified along with above- and belowground production at a site on the east coast of the Kelantan delta, Malaysian peninsular during 2014-2016. The above- and below-ground standing biomass of mangrove trees were quantified: although above ground biomass is larger (276.54 t ha-1), the allocation below ground was significant (20.81 t $ha^{-1}$), the two contributing to a total biomass of 297.35 t $ha^{-1}$. Aboveground productivity was 4.81 t $ha^{-1}$ $y^{-1}$ and belowground productivity was 12.70 t $ha^{-1}$ $y^{-1}$, peaking seasonally during the monsoon period in March and December 2015. These values are among the highest recorded for mangrove forests globally and the data also suggested a rapid turnover time for roots, of approximately 19 months. Thus, although standing biomass is higher above ground, more productivity is allocated below ground. Fifty-five percent of the root biomass was found in the top 30 cm and 78% of the roots, in all soil layers, consisted of fine roots (< 3 mm diameter), making the fine root component a particularly important carbon pool in this ecosystem. A positive relationship was found between fine root biomass, sediment carbon and nitrogen content. Soil temperature, salinity and dissolved oxygen were also investigated in relation to belowground production (chapter 5).

This study provides evidence that the seedlings of at least these two mangrove species can survive under flooded conditions. This may have occurred here because of high oxygen levels in the experimental conditions, suggesting that negative effects of enhanced flooding in the field could arise from oxygen starvation rather than direct effects of flooding. In addition, it showed very rapid belowground biomass accumulation and turnover in a natural mangrove forest.

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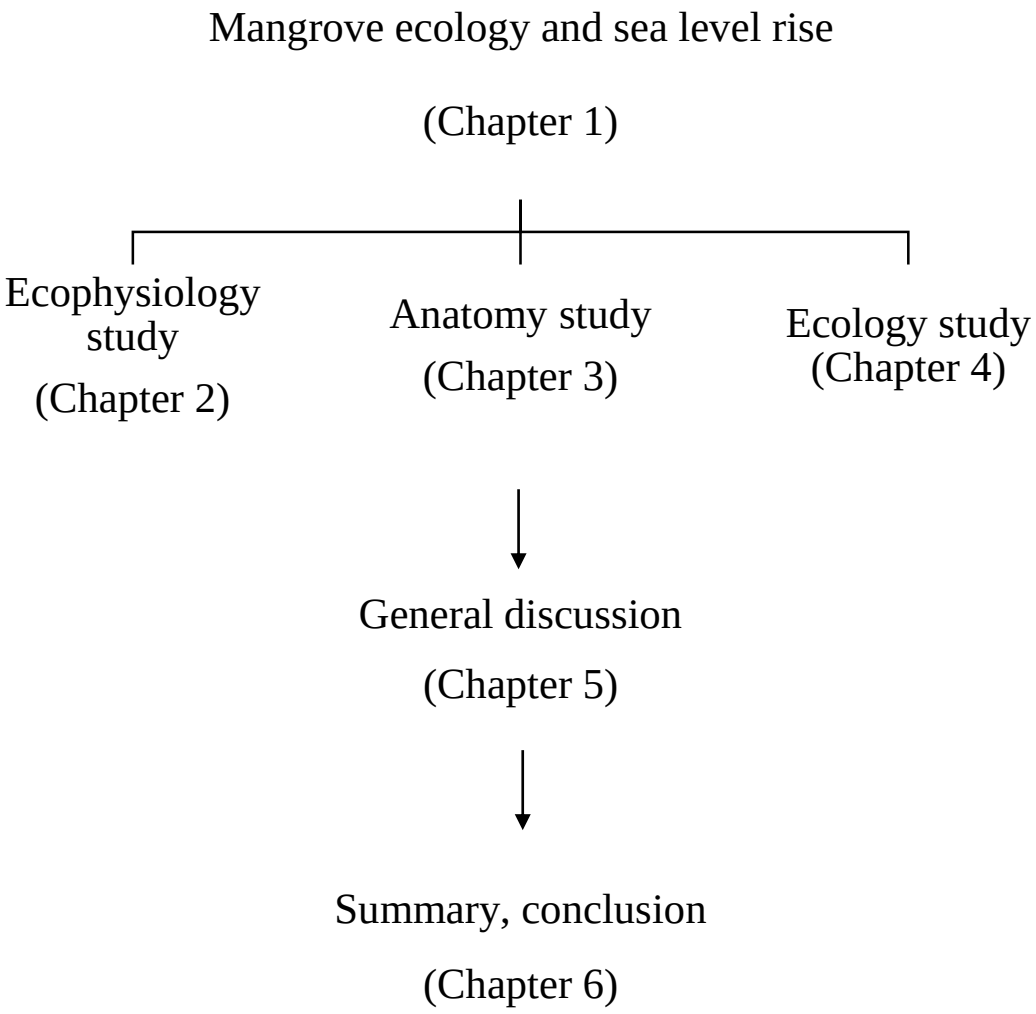
# Thesis overview

This study consist of six chapters. Chapter 1 is the introduction of the thesis and it provides an overview of mangrove ecology and the impact of sea level rise to the mangrove community. This chapter also explain the significance of the study, research objectives and hypothesis. Chapter 2 is the general background of the study in terms of ecology, physiology and anatomy. Initially this chapter started with questions of how sea level rise can occur and the impact of sea level rise to a coastal area and mangrove plants. The discussion goes further with the impact of flooding or long submergence in this case as a response to sea level rise and looking at the importance of ecophysiology and anatomy in mangrove species from previous studies. Chapter 3 investigates the ecophysiological response of mangrove seedlings to different flooding treatments which consist of manipulation of flooding to simulate four flooding levels which involve changing the duration of root submergence on mangrove seedlings. The information includes the ecophysiological responses of the seedlings, in term of seedlings survival, growth, leaf level physiology and leaf carbohydrate reserves. Chapter 4 is looking at the impact of the submergence on the xylem anatomy with emphasis on the water conducting cells in contrasting submergence, to determine the characteristic of water conducting cells under these flooding treatments. Chapter 5 reports a field experiment, examining the biomass accumulation of adult trees of *Avicennia alba* at the Malaysian peninsular. This study focussed on the above to belowground productivity between standing stock and production and root turnover. The study demonstrate how mangrove forest, at least this species responds to the sea level rise by accreting the sediment through root production but does not store the carbon as showed by rapid turnover. Chapter 6 is the final chapter, consisting the summary and conclusion of the study. Synthesize and concluding the findings and provide future research direction. The overview of the study can be find in figure below:


Mangrove ecology and sea level rise
(Chapter 1)
Ecophysiology study
(Chapter 2)
Anatomy study
(Chapter 3)
Ecology study
(Chapter 4)
General discussion
(Chapter 5)
Summary, conclusion
(Chapter 6)

# Chapter 1: Introduction

## 1.1 Mangrove ecology and climate change

Mangroves constitute a group of plant communities which can be found in tropical and subtropical regions (Tomlinson, 1986), along the coastlines. This plant community grows in intertidal areas subjected to daily tides and varying degrees of anoxia (Alongi, 2008). Specialized root systems are one important adaptation allowing mangroves to cater for waterlogged conditions. Mangroves must also tolerate high and fluctuating levels of salinity. This is particularly the case after tidal inundation periods and therefore, they have developed a series of physiological and morphological adaptations such as salt tolerance mechanisms which enable them to survive in saline water. The mechanisms for salt exclusion and salt secretion can be found within their plant tissues particularly in their leaves and root systems. Salt may be removed through salt glands, for example, in the production of white crystal formations on the leaves of *Avicennia* species, while ultrafilters within the root systems of some genera remove salt when extracting water from the soil.

Mangroves bring many benefits to humans. In ecological terms, they support numerous ecosystem services for example coastal protection as a wind and wave breaker (Lokman and Sulong, 2001), flood protection, nutrient and organic matter processing, sediment control and fisheries habitat provision (Polidoro et al. 2010). In addition, mangroves provide a range of provisioning services for coastal communities. Mangrove wood has been harvested commonly for charcoal production. Furniture, boat construction materials and fish traps also can be derived from the wood. Recent

evidence has demonstrated that mangroves are highly efficient carbon sinks (Chmura et al. 2003). Mangroves of the Indo-pacific are among the most carbon dense, with 1023 t C $ha^{-1}$ in the organic carbon in more than 1 m soil depth (Donato et al. 2011) and mangrove forests world-wide may sequester up to 24 Tg C $y^{-1}$ (Alongi, 2014). Because of this, mangroves are globally important ecosystems for their role in countering climate change through carbon sequestration.

Despite these values, mangroves are threatened nowadays for many reasons. Deterioration of mangrove forests is mainly due to human activities in coastal areas. In Southeast Asia, most mangrove forest has been converted for aquaculture practices such as shrimp and fish pond and oyster culture over 30 years ago (Valiela et al. 2001). More than 100 000 ha of mangroves were lost between 2000 and 2012 and 30 % of the mangrove conversion was caused by aquaculture (Richard and Fries, 2016). Due to the increasing demand for land uses, oil palm plantations have emerged as a new driver for accelerating mangrove destruction in Malaysia and Indonesia recently, because of increasing population and global demand in food industry (Richard and Fries, 2016). Moreover, mangroves have been cleared for urban development for example human settlement and agriculture. Chen et al. (2009) reported that urban aquaculture waste-water discharge, oil pollution, biological invasion, insect outbreak and the influence of water transportation remain serious threats to Chinese mangroves.

In recent years, there has been growing concern on the impacts of global climate change on mangrove ecosystems, particularly the impacts of sea level rise, temperature and elevated carbon dioxide (Krauss et al. 2013; McKee et al. 2007; Saintilan et al.

2014 & Langley et al. 2009). Most studies suggest that sea level rise poses the greatest threat to mangrove ecosystems (Lovelock and Ellison 2007; McLeod and Salm 2006) due to the prolonged flooding and saltwater intrusion through rivers and estuaries. This phenomenon may damage most of the freshwater species and the coastal habitats such as mangroves and salt marsh, which have other important biogeochemical and ecological roles (Ove and John, 2010).

## 1.2 Sea level rise and their impact to the coastal area

Sea level can be defined as position of sea surface relative to the land (IPCC 2013). Sea level can rise up and exceed and submerge the land due to climate and non-climate factors, for example land subsidence or uplift while climate may affect sea level through fluctuations in ocean temperature and melting of ice (IPCC 2007). The history of sea level rise can be inferred from the fossils specimen such as coral, sedimentary and archaeological materials which reflect the environmental condition in the past (IPCC 2013). However, tide gauge measurements have been used to record the sea level rise since the late $19^{th}$ century. Recent technology such as high precision satellite altimeter is also available (Nicholls and Cazenave 2010). Under climate change scenario, sea level will rise up by 60 cm by 2100 (IPCC 2007). Mean rising sea level since $19^{th}$ century is $1.7 \pm 0.3$ mm $year^{-1}$ and it accelerated up to $3.3 \pm 0.4$ mm $year^{-1}$ from 1993 to 2009 (Abalian et al. 2009) due to glaciers melting and ocean warming (IPCC 2007). If there are dramatic changes in glaciers melting, sea level is anticipated to rise by up 80 cm by 2100 (Pfeffer et al. 2008). Most low lying areas will be completely submerged, particularly in the case of small Islands where frequent flooding and long submergence will occur (Nicholls and Cazenave 2010) (fig 1).


Fig 1. World map of future sea level rise. Source: (Nicholls and Cazenave, 2010)

Coastal areas are the most vulnerable to the rising sea level where submergence and frequent flooding will occur. Coastal cities are expected to be highly affected for example Jakarta, Bangkok, Ho Chi Minh, Maldives, New York and others (FitzGerald et al. 2008; Hallgate et al. 2013). The submergence of these cities may cause chaotic scenarios where human populations may lose their home as well as suffer from socio economic distrubances. Urban developments will suffer, for example resort and jetty construction, which may causing declination of this area as well as river damming (Lovelock et al. 2015). The effect of damming construction may reduce the sediment delivery to estuarine area which may alter surface elevation at mangrove areas. In Southeast Asia, recent finding showed that mangrove areas are mainly affected by land use changes due to the clearance of mangrove forest and establishment of oil palm plantations (Richard and Fries 2016). For all these factors, the wetland area may become squeezed due to changes in the landward boundary and sea level rise

(Schleupner, 2008) (Figure 2). Therefore the coastal communities may experience landward migration as a result of the intensification pressures and consequently wetland loss of the area, (Torio and Chmura 2013).

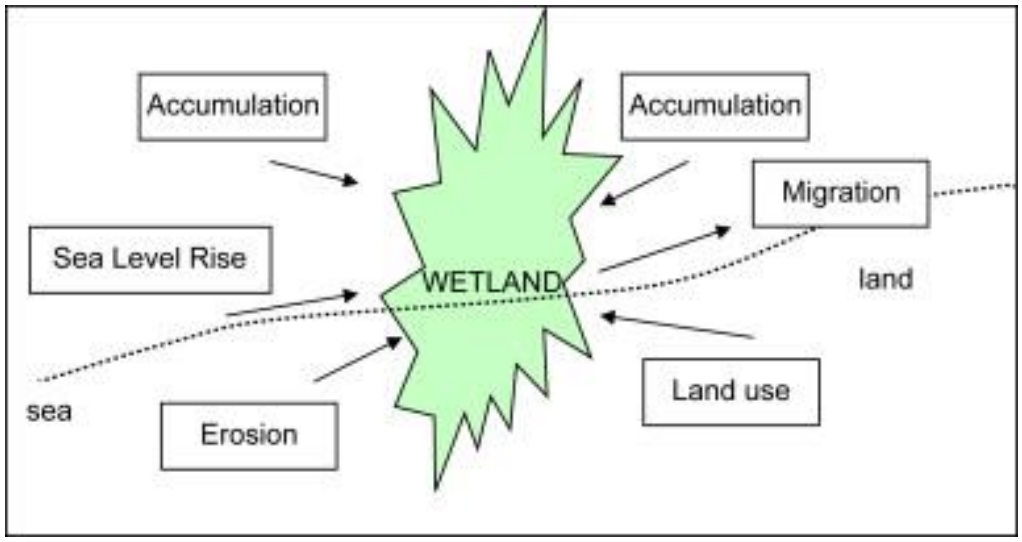



Figure 2. Factors that influencing coastal squeeze. Source: Scleupner (2008)

1.3 Impact of sea level rise in mangrove forest

Sea level rise also affects directly the vegetation of coastal communities along the shorelines particularly the mangrove ecosystems (Godoy et al. 2015). Intensification of hurricanes caused by global climate warming (Mousavi et al. 2011) can increase the coastal flooding and lead to rising sea level in mangroves forest. This scenario may become more severe due to coastal alterations in hydrology caused by human activities at coastal areas for example port and harbour work (Nicholls and Cazenave 2010).

In Southeast Asia, devastated mangrove areas, influenced by climate change, may suffer river damming (Milliman and Farnsworth 2013), resulting with a low sediment

supply to the mangrove forest (Lovelock et al. 2015). The situation may worsen if the rate of sediment accretion is less than the rate of sea level rise; it will cause the submergence of mangrove ecosystems in any future rising sea level (Ellison 2000). In Malaysia and Indonesia, destruction of mangrove forest are due to the land use changes where mangrove forest were cleared for the oil palm plantation to meet the demand for the resources globally (Richard and Fries 2016).

## 1.4 Response of mangroves seedlings to the flooding

Mangroves normally experience waterlogged phases on a daily basis through tidal inundation. Usually mangroves received semi-diurnal tide cycles of approximately 6 hours inundation twice per day during the diurnal cycle. During the spring tides, most of the mangrove forests being inundated are on the landward area whilst seaward mangrove species are inundated at the maximum tides level. Hydroperiod in mangroves i.e. flooding frequency, duration and depth are normally dependent on tidal cycles as well as upland runoff, groundwater flow and evapotranspiration (Twilley and Chen 1998). However with the accelerating sea level rise, the inundation of mangroves may become more severe, increasing salinity and sediment erosion (Ellison 2000) which may impair mangrove growth and ecophysiology.

Mangrove seedlings are the most vulnerable plant stage in relation to the rising sea level. This is because seedlings are the immature plant stage and exposed to complete submergence (Lu et al. 2013; Mongora et al. 20). Additionally, the increasing depth

and duration of submergence which may exert deleterious effects on the mangrove plants due to accelerating sea level rise (Lu et al. 2013). However, mangroves trees are relatively less affected than the seedlings under prolonged submergence due to their well-develop adaptation characteristic, but they are prone to hurricanes; there are documented examples from the Caribbean region (Cahoon et al. 2003) and the Dominican Republic (Sherman et al. 2001).

Over the past decade, research into the impact of climate change in mangrove ecosystems has emphasized the impact of sea level rise on mangrove communities. Several studies have demonstrated that increasing water level and saltwater intrusion become major factors which contribute to damage of most of the wetland species in the Lousiana Gulf Coast (e.g. Pezeshki et al. 1990; Allen et al. 1996). It was found that following flooding and a salinity increase, photosynthesis rates decreased and leaf damage occurred in most of the *Taxodium distichum* seedlings (Pezeshki et al. 1990). Allen *et al*. (1996) had further observed how flooding and salinity causes various responses in bald cypress seedlings. Physiological processes such as ion exclusion presumably play a role in plant resistance to both stresses.

In one case of mangrove species, Rasheed et al. (2013) have compared the physiological response of the two mangroves species; *Bruguiera parviflora* and *Avicennia marina* to a simulated sea level rise. Longer inundation and deep treatment negatively affected both the $A_{max}$ (Maximum photosynthetic rates) and $A_{400}$ (photosynthetic rates at ambient) of *B. parviflora* rather than in *A. marina*. This result suggests that *A. marina* is less affected by flooding treatment. Another study has

demonstrated that similar factors (increasing depth and duration of inundation) have reduced biomass accumulation, photosynthetic rate, leaf electron transport and water use efficiency (Lu et al. 2013).

## 1.5 Adaptation of mangroves plant under submergence

Waterlogged, submerged or saturated soils help to explain the fate of plants subjected to the flooding (Liu, 2016). During the flooding period, mangrove soil experiences oxygen deficiency (hypoxia or anoxia) due to the lack of plant aeration (Kozlowski et al. 1991). Soil flooding is a growth limiting factor for most of the waterlogged plants. This occur since gas-filled soil pores, are replaced with water under flooding (Kozlowski et al. 1991). The oxygen diffusion is much slower within water as compared to air, and therefore oxygen supply is limited under soil flooding. The oxygen consumption by other microorganism inhabiting the soil impedes the movement of oxygen to the roots in the flooding soil (Ayi et al. 2016).

In a study comparing flooded and unflooded soil, it was reported that soil flooding has affected several other abiotic factors; for example reduced soil redox potential, increased pH of acidic soil and decreased rate of decomposition of organic matter (Kozlowski et al. 1991). This is due to the presence of anaerobic organisms in the flooded soil and impacts on metabolism processes causing denitrification and reduction of Mn, Fe and S (Kozlowski et al. 1991). Plants under submergence also suffer from a deficiency of oxygen and an energy deficit due to reduced respiration (Mommer et al. 2006). If the absence of oxygen continues, the root eventually will die

due to the lack of ATP to perform other important processes for example absorbing nutrients in the soil.

The plants therefore develop morphological adaptations to meet the growth requirement as a response to depleted oxygen conditions. Mangrove species develop lenticels in the stem to allow gas exchange with the surrounding environment, so that substantial gas may enter via the stem and supply oxygen to the internal space. The lenticels are common in the Rhizophoraceae, where numerous lenticels occur in the root system of *Rhizophora* and knee root of *Bruguiera* species (Tomlinson 2016). Adventitious roots constitute another flooded plant adaptation (Sauter 2013) to help the plant gain oxygen during submergence.

Another common adaptation of waterlogged plants is the formation of aerenchyma as this creates large air fill cavities in the plant tissue to allow gas diffusion within inside the plant organ and transport from above-ground to below-ground (Fig 2). Aerenchyma can be found mostly in root cortex (Purnobasuki and Suzuki 2004) but in mangroves there is very limited information on the location of this tissue in the other plant parts.

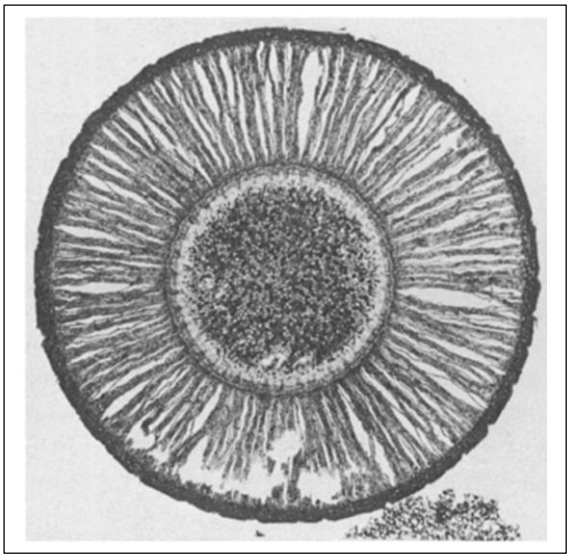


Figure 2. Aerenchyma at the root cortex in *B. gymnorrhiza*

Source: Tomlinson (2016)

1.6 Water transporting tissues in plant

Xylem is the main water conducting tissue in plants. There are several water conducting cell-types involve in the water transport system. Tracheids are elongated cells, long distance water conducting elements, which can be found in all gymnosperms (Mauseth 1988). In contrast, vessel elements are responsible for transporting water in angiosperm (Fig 3). Mangroves have shown themselves able to adjust xylem structure in response to environmental stress. The thick walls contain lignin which helps the vessel to resist the tensions that develop with high water flow. More wall thickness provides increased mechanical strength (Xiao et al. 2009). In order to describe water transport in plant, the concept of plant hydraulic conductivity is considerably important. Hydraulic conductivity defines how much water can pass

through the plant stem in response to a gradient of water potential. Most of xylem studies are describing the importance of hydraulic function as a response of mangroves to the salinity gradient (Verheyden et al. 2005; Schmitz et al. 2006, Sobrado 2007 & Robert et al. 2009). This is because the saline water has caused water deficit in the xylem tissue and this condition causes stress for many mangrove species. Therefore most of mangrove species have developed anatomy adaptations in order to adjust under stress, for example the formation of small diameter vessels with high density (Verheyden et al. 2005, Robert et al. 2009) as an alternative mechanism for water flow along the plants profile (Sobrado 2007).

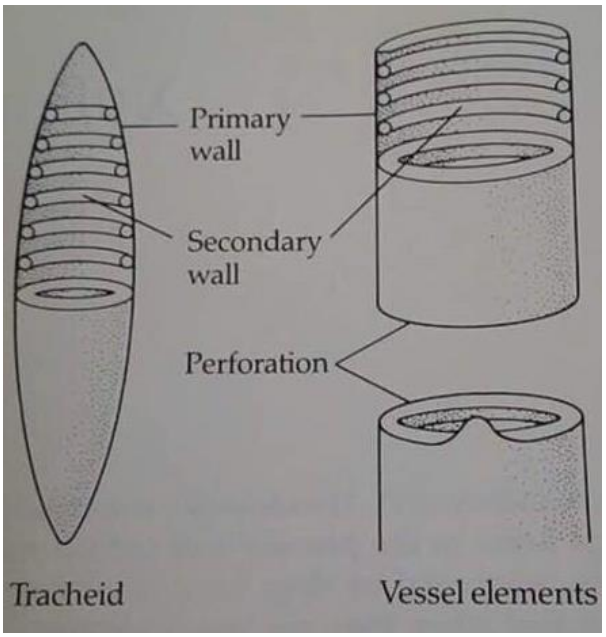



Figure 3. Differences between tracheid and vessel elements. Source (Mauseth, 1988)

As a response to flooding, previous studies have emphasized the role of aerenchyma in plants (Kozlowski 1984, Perata et al. 2011). This tissue development is triggered by ethylene production when the plant is in anaerobic conditions within waterlogged soil

(Kozlowski 1997). Aerenchyma enables gas exchange and also gas storage during submergence, helping mangroves to adapt under waterlogged conditions (Purnobasuki and Suzuki 2004). Another study in mangroves has reported the increase in mechanical strength under submergence (Xiao et al. 2009). Information on mangrove vessel characteristics remains scarce and only limited to the wood and bark anatomy as a function of flooding (Yanez-Espinosa et al. 2001).

## 1.7 Carbon dynamics in mangrove ecosystem

Mangroves can play a role in climate change mitigation through the provision and enhancement of a powerful natural carbon sink. Previous studies have recognized vegetated coastal ecosystems such as mangroves, seagrass and salt marshes as 'blue carbon' (Nelleman et al. 2009) because their highly carbon rich soils act as efficient carbon sinks (Chmura et al. 2003 & Bouillon et al. 2008). Mangroves sequester carbon from the atmosphere and store it in biomass or as organic matter in the soil. The accumulation of carbon in mangrove plants can be measured through biomass deposition within plants organs such as stem, branches, leaves and root biomass. Mangrove may store massive amounts of carbon belowground. Castaneda-Moya et al. (2013) have shown that under permanent flooding, numerous dead root was found in the deeper mangrove soils suggesting very slow decomposition of roots because of depleted oxygen. This condition helps mangroves to store more carbon belowground as well as reducing emission of carbon to the atmosphere, and contributes to the ecosystem service provided by mangroves in relation to mitigation of climate change.

Carbon storage is partitioned between sediments, living aboveground biomass (leaves, stems and branches) and belowground (roots) included within non-living biomass for example litter and dead wood (McLeod et al. 2011). However, recent studies showed that more carbon is stored in the belowground plant organ than in leaves or stems (Alongi, 2009, Donato et al. 2011). The indo-pacific region is one of the most rich stores of organic soil carbon containing an average of 1023 MgC/ha of carbon at more than 3 m deep soil. Moreover, the accumulation of carbon belowground is also contributed by mangrove roots (McKee et al 2007). This belowground process is expected, in the long-term, to cause sediment elevation (Cahoon et al. 2006; McKee 2011) helping to keep pace with sea level rise (Kraus et al. 2013).

However, carbon burial can be affected by several physical factors such as hydroperiod, salinity, nutrient status and suspended sediment supply (McLeod et al. 2011). This situation is related to global climate change factors particularly the rising sea level, salinity and temperature which are likely to affect carbon burial in mangroves sediment. Cahoon et al. (2003) has documented the increased loss of mangrove peat, comparing to the reduced carbon sink and storage associated with tree mortality due to Hurricanes in Honduras. Cahoon (2006) further demonstrated the impact of storms may influence soil elevation through surface and subsurface processes. Nevertheless, the rising sea level may slow down the decomposition of organic matter due to the anoxia conditions which therefore allows the carbon to remain stored in the intertidal sediments (McLeod et al. 2011) thus resulting in the formation of peat soils (Middleton and Mckee 2001). To date, rather little information

is available from mangrove forest on the likely impact of carbon storage under future sea level rise (McLeod et al. 2011).

Recent studies suggest that some mangroves are able to keep pace with sea level rise by building the soil vertically (e.g. McKee et al. 2007). This process is particularly likely for the mangroves that receive large amount of sediment from river system (Lovelock et al. 2015). For mangrove systems in oceanic settings which depend on their autochthonous resources (i.e. Caribbean mangrove forest), studies show mangroves are able to persist under rising sea level with the help of organic material belowground (Krauss et al. 2013). Studies exploring recent paleo-ecology also show that under future climate settings, mangroves may migrate further landward particularly mangroves in higher latitude (Christensen et al. 2007). This situation provides the chance for mangrove seedlings to grow and colonize the land when there is a capacity for mangroves to adapt to climate change.

## 1.8 Significance of the study

Although numerous studies have been conducted to investigate the effects of climate change factors, particularly flooding and salinity, on mangrove seedlings, knowledge of the ecophysiology of mangroves seedlings remains scarce. Therefore, this study will fill the knowledge gap on the effects of different durations of flooding on ecophysiological properties of mangrove seedlings of *Bruguiera gymnorrhiza* and *Rhizophora apiculata*. The information from this study will contribute important knowledge on the response of seedlings to simulated sea level rise in terms of growth, ecology, physiology and anatomy, which may prove useful for mangrove forest

management. In addition, the results from the field study, showing unusually high levels of root production and turnover, demonstrate the high levels of variation in these factors between mangroves.

## 1.9 Research questions, hypothesis and objectives

In general, this study will address the respond of mangrove seedlings to the sea level rise as represented by different flooding treatments; 6 hrs, 18 hrs, 24 hrs and 24 hrs (stagnant). After being confronted with these treatments, the seedlings will be examined for their water conducting properties where examination of xylem under the scanning electron microscope will be required. The field study will investigate biomass accumulation of mangrove trees, especially the allocation between above and belowground plant organs, in order to relate to the carbon storage of the mangrove trees as a response to sea level rise. The specific questions that have been addressed are as follows:

Question 1: How do mangrove seedlings of *Bruguiera gymnorrhiza* and *Rhizophora apiculata* respond to different flooding treatments? (Reported in chapter 3)

Hypothesis:

1. Mangrove seedlings of longer submergence in 24 hours and 24 hours (stagnant) treatment are the most affected to the flooding stress by showing slower growth, reductions in photosynthetic activity and have the lowest carbon reserves.

2. Mangrove seedlings under 6 hours flooding treatment are less affected to the flooding stress, showing an increase in seedling growth, maximum photosynthesis activity and highest carbon reserves.

3. Mangrove seedlings of 18 hours treatment show moderate response to the flooding stress compare to 6 hours, 24 hours and 24 hours (stagnant treatment).

Objective 1:

To determine seedling growth (stem diameter and seedlings height, number of leaves) under different flooding treatments.

Objective 2:

To evaluate leaf-level physiology: photosynthesis, chlorophyll content and chlorophyll fluorescence under different flooding treatments.

Objective 3:

To evaluate carbon reserves in the plant leaves under different flooding treatments.

Question 2: How do water conducting cells of mangrove seedlings of *Bruguiera gymnorrhiza* and *Rhizophora apiculata* adjust under contrasting flooding treatments? (Reported in chapter 4).

Hypothesis:

1. Mangrove seedlings under longer submergence (24 hours & 24 hours stagnant) treatment will have wider vessel size and higher vessel density than seedlings under the 6 hours treatment due to the depletion of oxygen under flooding stress.

2. Mangrove seedlings under longer submergence will have thinner vessel cell walls than seedlings under the 6 hours treatment.

3 Mangrove seedlings under longer submergence will have lower hydraulic conductivity than seedlings under the 6 hours treatment.

Objective:

To determine water conducting characteristics of mangrove seedlings *Bruguiera gymnorrhiza* and *Rhizophora apiculata* under contrasting flooding treatments using light microscope and scanning electron microscopy.

Question 3:

What are the relationships between aboveground and belowground productivity and standing stocks in a natural mangrove forest? (Reported in chapter 5).

Hypothesis:

Belowground standing stock and production will be higher than aboveground standing stock and production

Objective 1:

To estimate above to belowground productivity of mangrove tree *Avicennia alba* at the Kelantan delta, Malaysian Peninsular.

Objective 2:

To determine biomass allocation between above and belowground of an adult mangrove species, *Avicennia alba*, at the Kelantan delta, Malaysian Peninsular.

# Chapter 2: Physiological ecology of mangrove seedlings *B. gymnorrhiza* and *R. apiculata* in response to different flooding treatments

## 2.1 Abstract

Mangrove seedlings were subjected to submergence treatments to investigate the effect on seedlings ecophysiological properties under projected sea level rise in mangrove forest. The seedlings were exposed to different durations of flooding treatment and the experiment was conducted in the glasshouse for 11 weeks to follow the response of ecophysiological characteristics of the seedlings in response to the different flooding and root submergence treatments. During the flooding experiment, several physiological measurements were carried out; stem diameter, height and number of leaves, leaf chlorophyll content, leaf chlorophyll fluorescence, photosynthesis (Amax) and stomata conductance ($g_s$). Leaf carbon reserves and leaf area were quantified after week 11. Mangrove seedling survival was 100% under different flooding treatments. Plant growth i.e. stem diameter, height and leaf number exhibited no significant effect of the flooding treatments. However, seedlings stem diameter, height and leaves number were significantly different between flooding weeks ($p < 0.001$) and varied between species ($p < 0.01$). Stem diameter was higher in *R. apiculata* seedlings but plant height was higher in *B. gymnorrhiza* seedlings ($p < 0.001$). Both seedlings showed reduced stem elongation under the 24 hours (stagnant) flooding treatment although this was not significant ($p > 0.05$). Leaf greenness was significantly affected by the flooding treatments ($p < 0.001$) and showed the highest chlorophyll content in 24 hours of flooding treatments whilst lowest was in 6 hours

flooding treatments. Chlorophyll content also varied between species ($p < 0.001$), being significantly higher in *B. gymnorrhiza* than in *R. apiculata* seedlings. There was significant changes in leaf greenness and flooding weeks ($p < 0.001$) as the plants exhibited high chlorophyll content in the end of the flooding week but lowest during the initial part of the flooding week. Leaf chlorophyll fluorescence (fv/fm) exhibited significant differences between flooding treatments, species, flooding week and flooding time ($p < 0.001$) respectively. The value of fv/fm was higher in *R. apiculata* seedlings. Fv/fm was highest in the 24 hours (stagnant) treatment and lowest in the 6 hours treatments. Fv/fm was higher in the morning of flooding than in the afternoon. Plant leaf area varied across flooding treatments and species ($p < 0.05$). *B. gymnorrhiza* had larger leaf area than *R. apiculata* and the leaf area was higher in 24 hours (stagnant) flooding treatment. Photosynthesis did not show any significant difference between flooding treatments ($p > 0.05$) but changes were observed across flooding week ($p < 0.001$) and species ($p < 0.05$). *R. apiculata* had a higher photosynthesis rate than *B. gymnorrhiza* seedlings and photosynthesis was lower in week 2. Stomata conductance was not significantly affected by the flooding treatment or species ($p > 0.05$) but varied between flooding week ($p < 0.05$). Flooding treatment resulted in an increase in non-structural carbohydrate content in both species. Leaf carbon reserve was not affected by the flooding treatment but varied between species, particularly for starch ($p < 0.05$). Sucrose was barely affected by flooding treatments ($p = 0.053$).

## 2.2 Introduction

Mangrove trees live submerged for several hours daily during tidal inundation. They depend on physiological and morphological traits that enable them to survive in this harsh environment. High tides cause total submergence while ebb tides leave seedlings exposed. There may be a substantial amount of dissolved oxygen during high tides, derived from adjacent ecosystems, whilst ebb tides are dramatically depleted in dissolved oxygen in the water column (Mattone and Sheaves 2017). Mangrove plants therefore are believed to cope with different concentrations of dissolved oxygen every day due to the tidal cycle. Under future sea level rise, increase precipitation, frequent flooding (Ellison and Farnsworth 1997), long submergence and saltwater intrusion (Neubauer and Craft 2009) are anticipated to occur and this situation may negatively affect the mangrove plants (Lu et al. 2013). Mangrove seedlings are believed to be the most vulnerable to submergence when exposed to prolonged and full submergence (Mangora et al. 2014).

Flooding causes low oxygen conditions in the soil (Ponnamperuma 1972). This is because the diffusion of oxygen in the water column is slower than the atmosphere (Mommer et al. 2006) as a result of high oxygen consumption by other soil microorganism in the water (Ponnamperuma 1972, 1984). Plants have different strategies to counter anoxia under submergence (Pedersen et al. 2017), for example by increasing root porosity especially via the formation of aerenchyma (Armstrong 1979; Colmer 2003). The aerenchyma, which is caused by ethylene production as a result of oxygen deficiency (Jackson and Armstrong 1999), provides opportunity for roots to

develop an internal gas space (Kozlowski, 1984). This aerenchyma tissue has been reported in many wetland species, containing a large gas space which enable gas conducting tissue from the shoot (Evans 2004; Colmer and Flowers, 2008). The presence of numerous lenticels and extensive aerenchyma provide root ventilation (McKee, 1993) to enable oxygen diffusion within internal plant organ.

Sediment flooding also causes an energy deficit to the plants (Voesenek et al. 2016; Mustroph et al. 2014). The plants under submergence are unable to produce ATP in the absence of oxygen (Mommer et al. 2006). Anaerobic respiration with the accumulation of non-structural carbohydrates provides an alternative for the plants, to survive for the long period under anoxia condition (Gibbs and Greenway 2003). Non-structural carbohydrate content (NSC) is important to the plants under environmental stress, particularly the flooding stress (Panda and Sarkar 2014). Carbon reserves in the plants may help the plants under stress to support plant metabolism. Plants under flooding showed shoot elongation to avoid oxygen starvation under submergence by remaining in contact with the atmosphere, thus enhancing oxygen diffusion to the plants (Bailey-Serres and Voesenek 2008). Therefore, growing taller can be an important plant trait under flooding.

Sediment flooding reduces the photosynthesis rate of many plants species (Chen 2005). Low oxygen under submergence has been attributed to stomata closure (Mommer et al. 2006). Alongi (2009) listed the responses of mangrove species to waterlogged conditions, for example decreases in cytokinin export from the roots,

accumulation of abscissic acid in the leaves, rapid leaf senescence and shedding and increased foliar sodium. In addition, an accumulation of soluble phytotoxins (for example $Fe^{2+}$, $Mn^{2+}$, $H_2S$, $CO_2$ and $CH_4$) and deficiency of essential nutrients particularly nitrates and phosphates have been recorded (Yousef and Saenger 1996). Most of the plants can survive under short-term flooding but subsequently die under long-term flooding, due to their tolerance of the submerged condition. However, the tolerance of mangrove plants is species-specific. Some of the species are intolerant of flooding and occupy more of the landward zone, where infrequent inundation take place. Species with high tolerance can survive under high saline water, up to 35 ppt for example, in *Avicennia marina* in Kenya mangrove (Hoppe-Speer et al. 2011). Flood-tolerant species may develop certain mechanisms to enable them survive under waterlogging stress (He et al. 2007).

In mangrove, several studies have shown different responses of mangrove seedlings to the flooding. Ellison and Farnsworth (1997) examined the influence of rising sea level on anatomy, physiology and growth on *Rhizophora mangle* in USA. They found that, although the seedling growth under a simulated future water level performed well at the early stages, there was a big drop towards the end of experiment, shown by greater reduction in growth, net photosynthesis and stomata conductance of the species. Hopper-Speer et al. (2011) demonstrated that *Rhizophora mucronata* Lam grows best under moderate inundation (3-9 hours), when maximum photosynthetic performance and high stomata conductance were recorded. Luzhen et al. (2005) showed that prolonged exposure, of 8 to 12 hours to waterlogging leads to the accumulation of absicic acid in mature *Kandelia candel*. Previously, Ye et al. (2003) observed that

*Kandelia candel* is more tolerant than *Bruguiera gymnorrhiza* as indicated by significant changes in biomass ratios and increasing chlorophyll contents, after exposure to 8 or 12 weeks under tidal immersion. There was no substantial change in leaf and stem properties, which implies the stability of water transport and mechanical support during 4 hours of tidal immersion, however such plant might adversely affected by longer waterlogging (Xiao et al. 2009). He et al. (2007) have reported seedlings of *Avicennia marina* exhibited high survival rates in all treatments suggesting this species is the most well adapted to high flooding. Salt marsh wetland species showed reduced productivity as a results of increasing submergence and salinity stress (Janousek and Mayo 2013).

In summary, most studies demonstrated that mangroves can adapt to flooding, however the response is species-specific (Hoppe Speer et al. 2008; Lu et al. 2013). Most of the studies examining the impact of sea level rise on mangroves emphasized the complete submergence of the mangrove seedlings. Very little investigation on root submergence was carried out in mangrove species. Therefore, this study aims to investigate the response of mangrove seedlings to the prolonged submergence by varying the duration of flooding treatments to simulate future sea level rise in mangrove forest. Therefore, the objectives of this study are:

1) To determine plant growth and survival of mangrove seedlings of two species *B. gymnorrhiza* and *R. apiculata* under different flooding treatments.

2) To determine leaf physiological responses (that is, leaf area, leaf chlorophyll content, leaf chlorophyll fluorescence, photosynthesis and stomata conductance) of

mangrove seedlings of *B. gymnorrhiza* and *R. apiculata* under different flooding treatments.

3) To determine non-structural carbohydrate contents of mangrove seedlings of *B. gymnorrhiza* and *R. apiculata* under different flooding treatments.

## 2.3 Method and materials

### 2.3.1 Plant Material

Mature propagules of two mangroves species; *Bruguiera gymnorrhiza* (L.) Lam. And *Rhizophora apiculata* Blume were collected from Matang Mangroves Forest in Perak Malaysia in March 2015. Both of these species are dominant mangrove species in Malaysia, growing along the Malaysian peninsular shorelines. *R. apiculata* grows in the soft muddy soil of mangrove forest whilst *B. gymnorrhiza* is located progressively inland. The propagules were transported to Edinburgh University within three days. The propagules were covered with wet wool to keep them wet during the travelling period. As soon as the propagules were received, they were brought to the Greenhouse of the School of Biological Sciences, University of Edinburgh. Propagules of *B. gymnorrhiza* and *R. apiculata* were planted individually in small pots (10 cm x 15 cm) using a loam-peaty soil (John Innes No.2). The plants were sprouted in temperature-controlled growth rooms with a temperature of $27^0$C. The plants were raised for almost six months from the end of March to the end of September 2015. None of the plants grew for the first two months after sowing, but they were kept well-watered twice a day and they started to respond early June 2015. The plants were then planted into

bigger pots, and irrigated with saline water, about 15 ppt once every two days topped up with tap water twice a day. The saline water was derived from a dilution using aquarium salt (Instant Ocean, Ohio USA) and tap water. The saline water was checked with a refractometer to maintain salinity throughout the flooding experiment within the range of 15-17 ppt.

### 2.3.2 Experimental design and flooding hours

The flooding experiment was carried out at tropical glasshouse located at Centre for Ecology and Hydrology (CEH), Edinburgh. The treatments were arranged in a randomized block design with a 3 x 3 factorial arrangement and four replicates per treatment per species per block.

On the experimental bench, there were three blocks. Each block was illuminated with a blue and red light, which switched on during daytime and off during night time (Fig 2.1). Since the experiment was conducted during the winter season, the light was kept automatically switched on during daytime to maintain a photoperiod representing a tropical country. In the glasshouse room, the maximum photosynthetic light was between 1000 and 1200 μmol photon $m^{-2}$ $s^{-1}$.

In each block, there were 32 plants randomly assigned among treatments (altogether 96 plants within three block experiment). Each plant was placed individually in each pot on the bench. The pots were placed in a bucket and the distance between each bucket was approximately 10 cm. The bucket functioned as a container for flooding treatments. To do this, each bucket was drilled to make a hole and a stopper was used to cover the hole. During the flooding experiment, the plants were watered and drained

manually (Fig 2.2). There were four types of flooding treatments; 6 hrs flooding (as normal tidal inundation hours in mangrove forest), 18 hrs, 24 hrs and 24 hrs (stagnant) to simulate future sea level rise in mangrove forest. The difference between 24 hrs and 24 hrs (stagnant) treatment is that 24 hrs (stagnant) was never drained to mimic the extreme prolonged submergence under the influence of future sea level rise. Each treatment bucket was differentiated using four different tags colours; green (6 hrs), yellow (18 hrs), blue (24 hrs) and red (24 hrs stagnant). This experiment was simulating the projected sea level rise of low lying area of the mangroves forest for example the riverine and deltaic mangroves setting where the frequent and longer duration of submergence are expected to happen in the future.

In order to do this, the plants of the 6 hrs treatment were watered at 1000 and kept flooded for six hours; the bucket was drained at 1600 hours. The plants remained drained until the next day (implying that the low tide occurred at this time). For plants with 18 hrs flooding, the plants were irrigated with saline water starting from 1600 and were drained at 1000 on the next day. For 24 hrs flooding, plants were submerged at 1600 and were drained at 1600 the following days. For 24 hrs (stagnant) flooding, plants were irrigated from the experiment started and were kept submerged throughout the flooding experiment (11 weeks) but the saline water was topped up when the water level full within the bucket due to evaporation. There was algae accumulation within the 24 hrs (stagnant) treatment bucket and algae were removed whenever possible, since the stagnant water encouraged the algal growth within the buckets.

Figure 2.1 Mangrove seedlings on the experimental benches

(a) (b)

Figure 2.2 *Bruguiera gymnorrhiza* (a) and *Rhizophora apiculata* (b) seedlings under root submergence

### 2.3.3 Response variables and plant measurements

Several response variables were measured as follows:

1) Plant growth (stem diameter, height and number of leaves).

For plant growth measurement, dbh was measured approximately 10 cm above the roots, while the stem height was measured from the top of the plants to the base of the plants. The measurements were taken once every two weeks during daytime. Stem diameter was measured using callipers and stem height was measured using a simple tape measure. The number of leaves was counted every two weeks whilst senescent leaves were observed throughout experiment.

2) Leaf level physiology

i) Chlorophyll content

Measurement of chlorophyll content and chlorophyll fluorescence took place every week in all the plants. Measurements were done in two sessions, before and after flooding treatments. Chlorophyll content was measured using a portable chlorophyll meter SPAD-502 (Konica-Minolta, Japan). SPAD measures the relative amount of chlorophyll present by measuring the absorbance of the leaf transmittance of red and infrared wavelengths (Ling et al. 2011). This measurement was done using a pair of leaves for each individual plant. On each leaf, three readings were obtained from random spots on the leaf, in order to get the representative value of the leaf greenness, a surrogate for chlorophyll. This gave six readings for each individual plant.

ii) Chlorophyll fluorescence

Measurements of dark-adapted fluorescence (fv/fm) can indicate plant stress under experimental treatment. Therefore, chlorophyll fluorescence measurement were carried out to obtain the response of plants under flooding treatment, using a portable florescence chlorophyll photometer (Handy-Pea, Hansatech, Norfolk, UK). Similar to measurements of chlorophyll content, these measurements were done twice a day, i.e., morning and afternoon for all plants. For plants under 18 hrs and 24 hrs flooding treatment, measurements were done in the morning, when the plants were submerged (considered as after flooding) and afternoon measurement (approximately 1600-1630) to be considered as before flooding started. Prior to the measurement, the plant was kept for 15 minutes to allow dark adaptation. The maximum quantum efficiency of photosystem II (fv/fm) was measured. The data obtained from Pea Plus software were exported to Excel for statistical analysis.

iii) Photosynthesis (Amax), stomata conductance ($g_s$)

Maximum net photosynthesis (Amax) was measured using a portable photosynthetic systems Li-6400 XT (Li-Cor, Inc., Lincoln, NE) for selected plants (each treatment per species per block). Two plants with fully expanded leaves were selected from each treatment and each species was measured once every two weeks from 1100 till 1300, taking the advantage of relatively bright ambient light.

iv) Non-structural carbohydrate content (NSC)

During plant harvesting at the end of the experiment, leaf samples were collected and immediately put under microwave for 20 seconds to stop enzyme activity (Sevanto et al. 2013). The leaf samples were then oven dried at $60^0$C for 72 hours and ground to

fine powder (Galiano et al. 2011) using grinder machine (Mixer Mill MM200, Retsch, Germany). NSC were analysed following the procedures described by Sevanto et al. (2013). All NSC values are expressed as percent dry matter.

v) Leaf area

Following plant harvesting, fresh plant leaves were collected for leaf area measurement. A pair of leaves from each plant was collected and kept in a bag. The leaf samples were then scanned using a scanner (UPSON) and were traced for the leaf area using the software Image-J.

2.3.4 Environmental variables measurement

The environmental measurement was recorded twice during flooding treatment. The dissolved oxygen (mg/l) was measured using portable multiprobe (YSI Inc.). The probe was inserted into the plants' buckets of each treatments and remained for 1 minute to equilibrate. This measurement was done during daytime as it was difficult to obtain the reading during night time due to the restricted access to the glasshouse area. The salinity was measured with a refractometer (ppt) by collecting the water sample from the plants' buckets.

### 2.3.5 Statistical analysis

All the data were analysed using Minitab 17.0 statistical package. The effects of treatments (flooding duration, species, week of measurement and block) were determined for the following response variables; stem dbh, stem height, number of leaves, chlorophyll content, chlorophyll fluorescence, photosynthesis, leaf area and carbohydrate content. A partially nested general linear model was employed. Block and plant ID were employed as random factors, flooding treatment, species and week of measurement were employed as fixed factors. Species, flooding treatment and block were nested within plant ID. For some variables (i.e., chlorophyll content and fluorescence), time of day was also employed as a fixed factor. Interaction terms were included for all the fixed factors plus block. Block was never significant and was eliminated from the final model. The data were either log or square root transformed to meet the ANOVA requirement.

## 2.4 Results

### 2.4.1 Environmental parameters during flooding experiment

Dissolved oxygen was significantly higher under the 24 hour (stagnant) treatment ($p < 0.001$) (Fig 2.3), compared to 6 and 18 hours flooding. The 24-hour treatment exhibited the lowest levels of dissolved oxygen, albeit the effect was not significant. Salinity exhibited significantly higher levels under 24-hour treatment and lowest in 6-hour treatment but this was only marginally significant ($p < 0.028$).

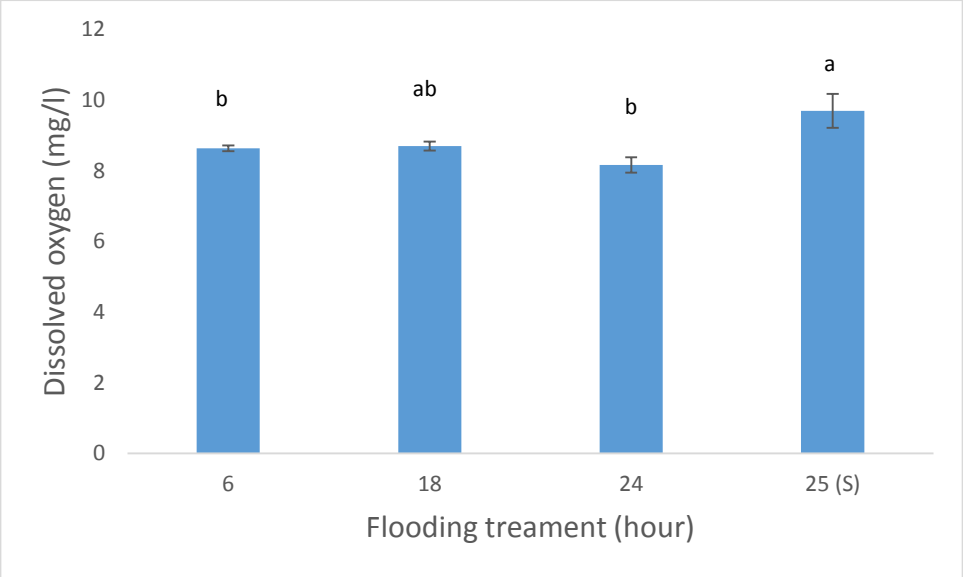



Fig 2.3. Dissolved oxygen measurement based on flooding treatment (Mean ±SE) n= 24

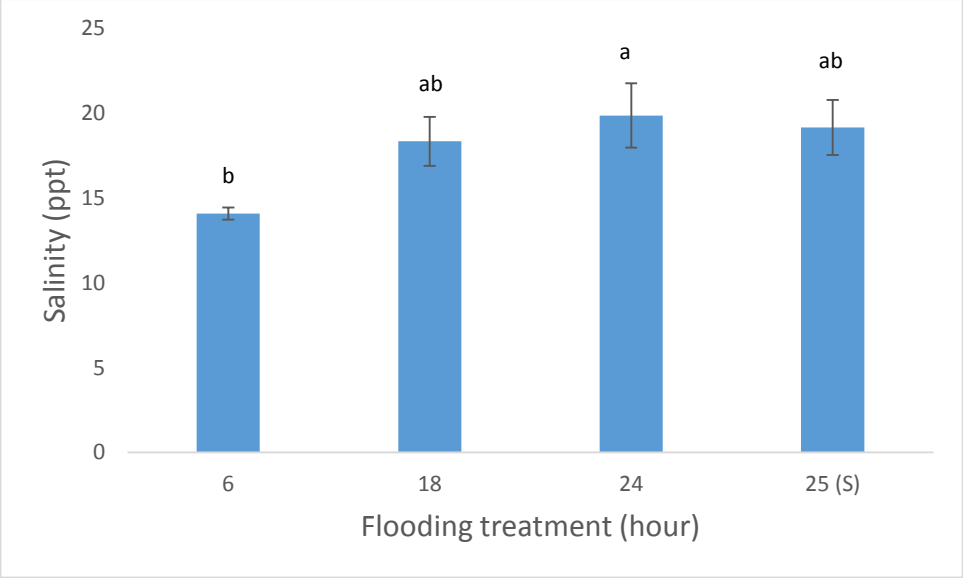



Fig 2.4. Salinity measurement based on flooding treatment (Mean ±SE) Treatment 6 hour, n= 23; Treatment 18 hour, n= 20; Treatment 24 hour, n= 24 & Treatment 24 hour (stagnant), n=24

Salinity was the lowest in 6 hrs and highest in 24 hrs treatment (Fig 2.4). In the mangrove forest, the salinity of a brackish water is approximately 15 to 20 ppt. During the experiment, salinity was maintained within the range to provide a realistic environment for mangrove seedlings to grow. It was observed that the highest salinity value was associated with the lowest dissolved oxygen content in block 2 (Table 2.1). Glasshouse temperature was maintained between $24^0$C to $27^0$C throughout flooding experiment.

Table 2.1. Environmental parameters during flooding experiment in the glasshouse. (Mean ±SE) n=3. Different letters show significant different.

| Parameters | Block 1 | Block 2 | Block 3 |
|---|---|---|---|
| Salinity (ppt) | 16.7 ± 1.0 a | 19.5 ± 1.5 a | 17.4 ± 1.3 a |
| Dissolved oxygen (mg/l) | 9.23 ± 0.3 a | 8.19 ± 0.1 b | 8.97 ± 0.3 ab |

### 2.4.2 Plant growth

#### a) Plant stem diameter

Stem diameter was significantly bigger in *B. gymnorrhiza* than *R. apiculata* seedlings ($p < 0.003$) (Fig 2.5; Fig 2.6). However, *R. apiculata* showed higher diameter growth than *B. gymnorrhiza* seedlings (Fig 2.7 and 2.8). *R. apiculata* seedlings showed the smallest DBH growth in 24-hours (stagnant) treatment and highest in 24-hours. *B. gymnorrhiza* seedlings showed a similar range of growth rates for all treatments, with the 6-hour treatment exhibiting the lowest DBH growth rate. However, both species showed an increase in stem diameter over the course of the 11 weeks experiment ($p < 0.001$). The interaction between flooding treatments and flooding weeks on stem diameter was not significant ($p > 0.180$) (Appendix 1).

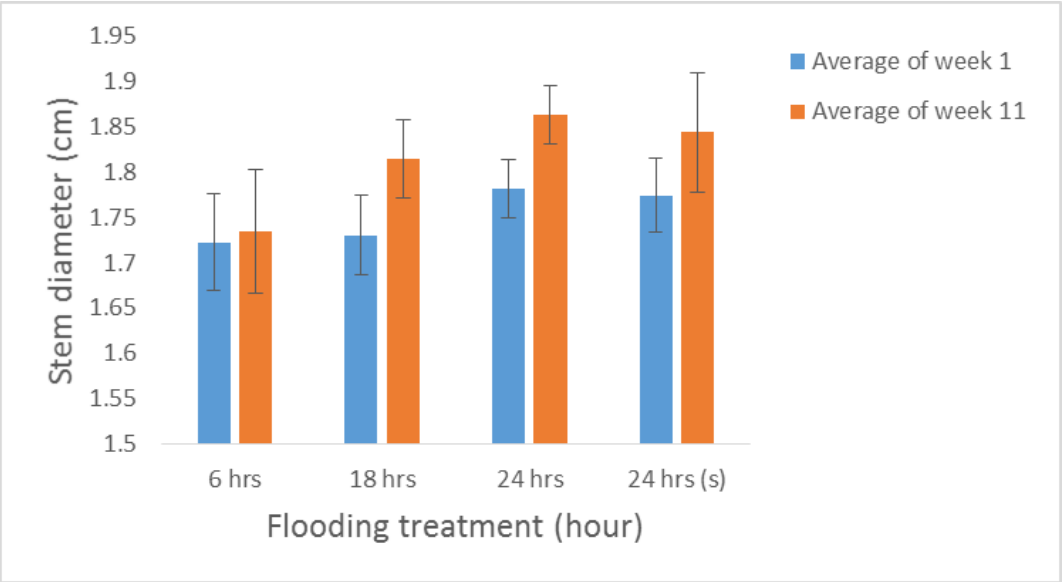



Fig 2.5. *B. gymnorrhiza* stem diameter under different flooding treatments. The initial and final measurements are shown (mean ± SE) n=12

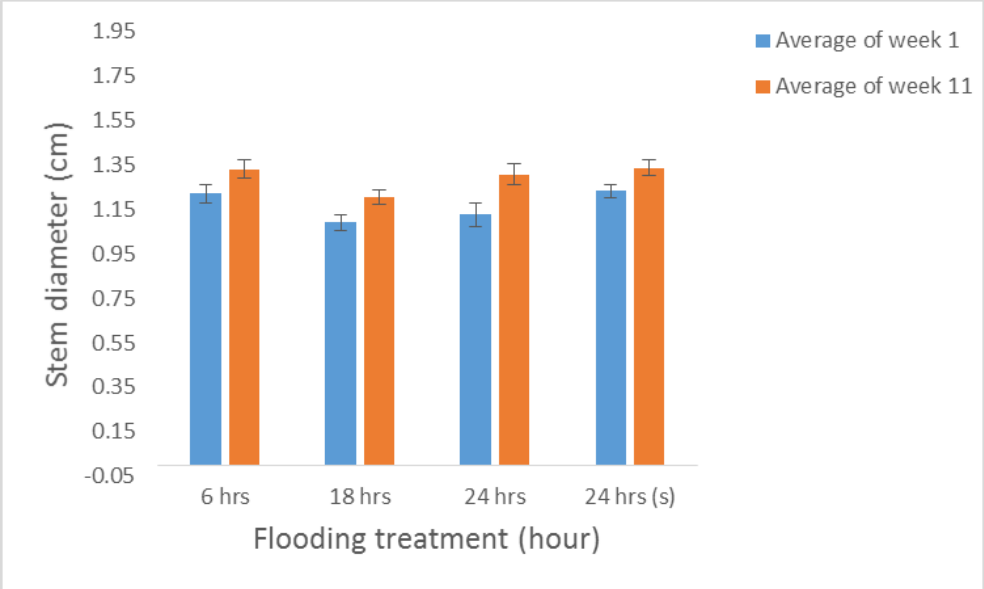



Fig 2.6. *R. apiculata* seedlings diameter under different duration of flooding treatments. The initial and final measurements are shown (mean ± SE) n=12

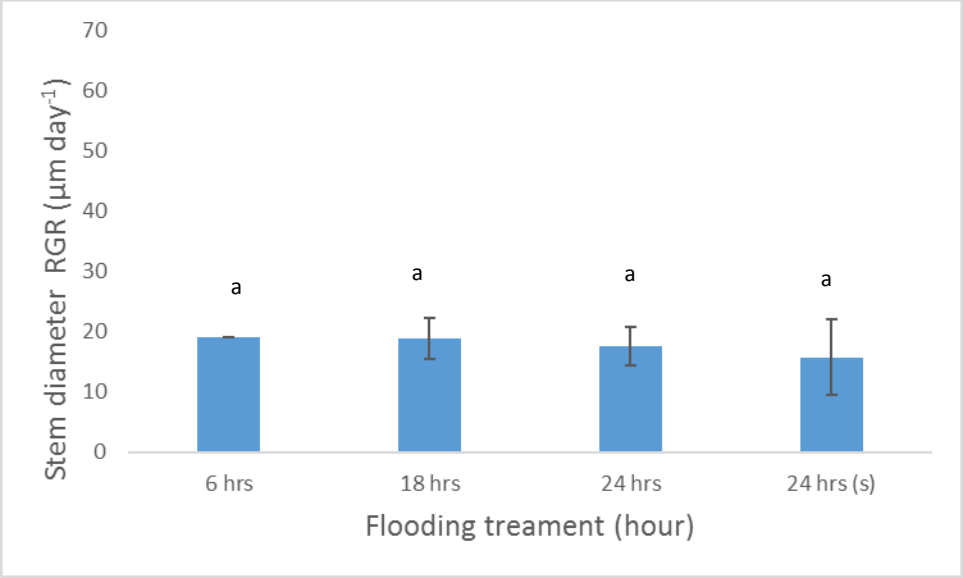



Fig 2.7. Relative growth rate of *B. gymnorrhiza* stem diameter under different flooding treatments n=12

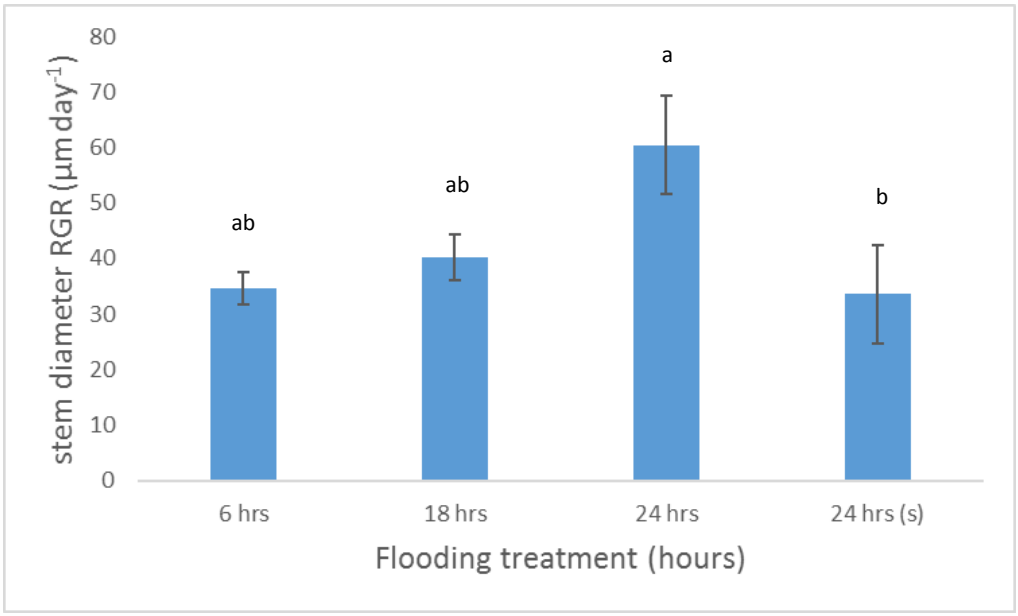



Fig 2.8. Relative growth rate of *R. apiculata* stem diameter under different flooding treatments n=12

b) Plant height

*R. apiculata* was significantly taller than *B. gymnorrhiza* ($p < 0.001$) (Fig 2.9 & 2.10). However, no significance difference was found among flooding treatments ($p > 0.127$). Both species exhibited an increase in height over the course of the 11 weeks of the experiment ($p < 0.001$). Both species show a trend to increase growth with flooding (Fig 2.11; 2.12). The maximum height growth rate was recorded at 42 µm $day^{-1}$ for *B. gymnorrhiza* seedlings under 24-hour treatment and at 24 µm $day^{-1}$ in *R. apiculata* seedlings, with the lowest values for the 18-hours treatment.

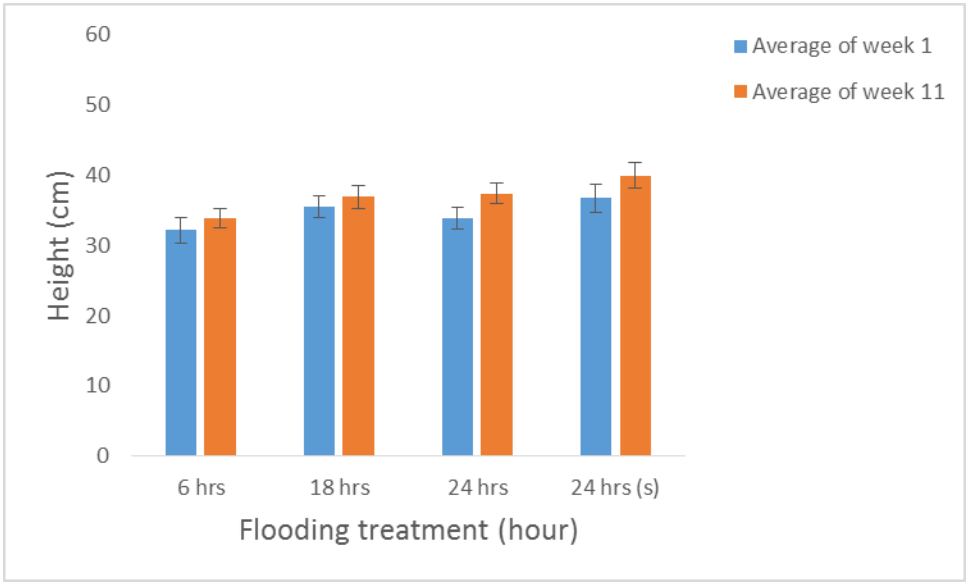



Fig 2.9. *B. gymnorrhiza* seedlings height under different duration of flooding treatments. The initial and final measurements are shown.

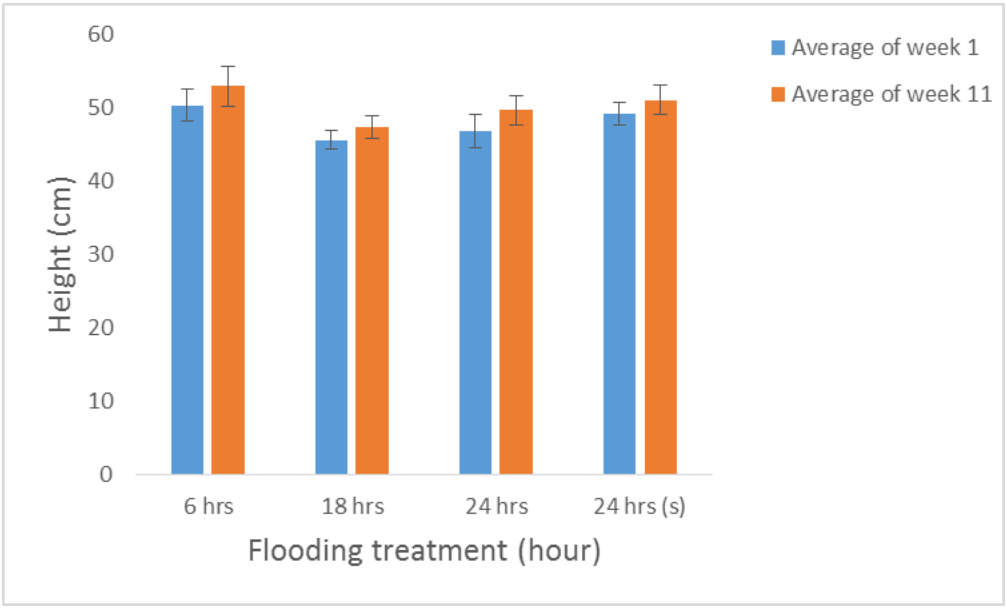



Fig 2.10. *R. apiculata* seedlings height under different duration of flooding treatments. The initial and final measurements are shown.

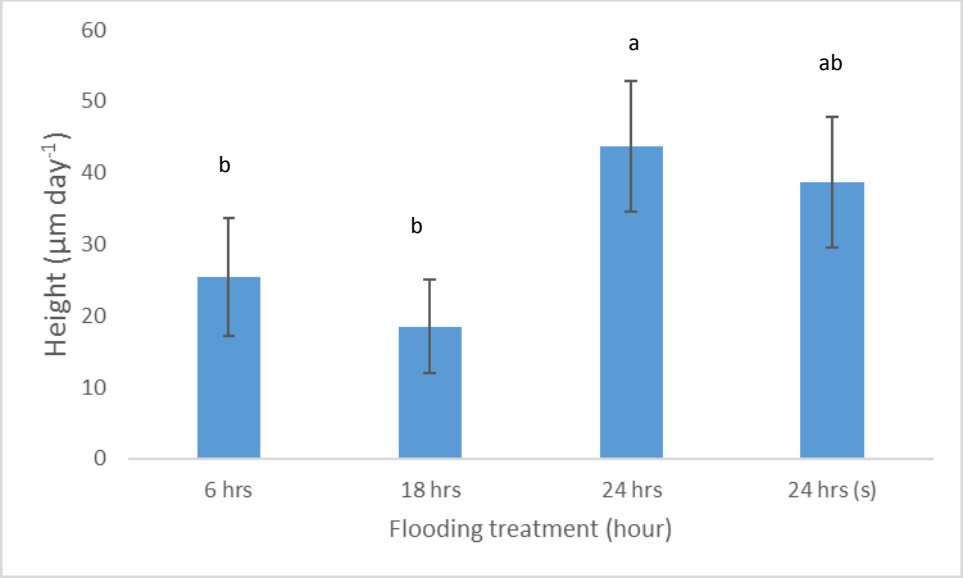



Fig 2.11 Relative growth rate of *B. gymnorrhiza* stem height under different flooding treatments n=12

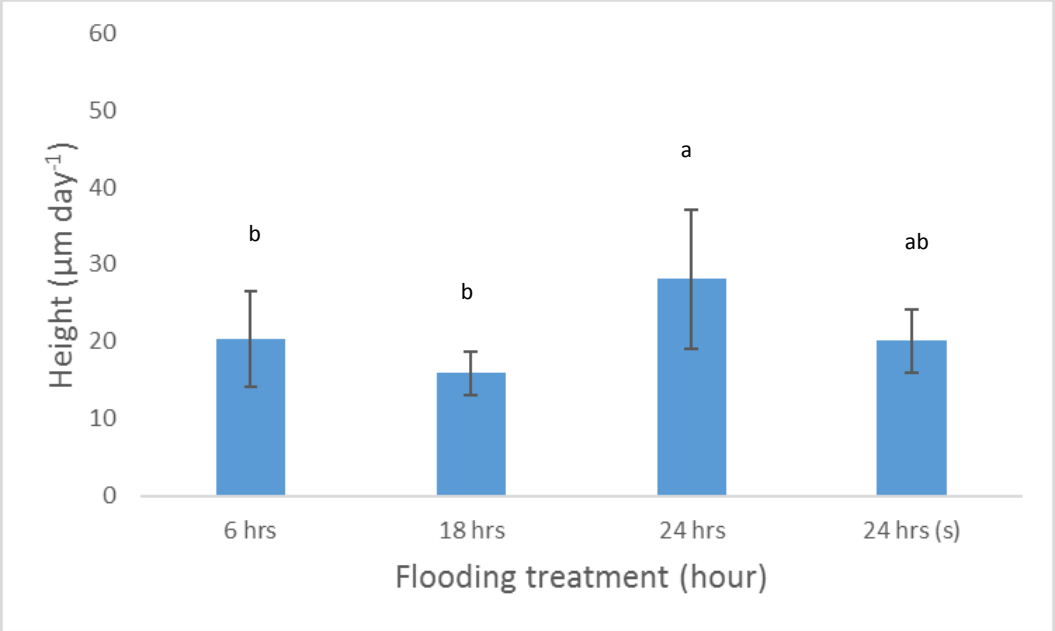



Fig 2.12 Relative growth rate of *R. apiculata* stem height under different flooding treatments n=12

c) Number of leaves

*B. gymnorrhiza* seedlings had significantly more leaves than *R. apiculata* seedlings ($p < 0.001$) (fig 2.13; 2.14). No significant different among treatments was recorded for leaf number ($p > 0.501$). Leaf number increased with flooding week ($p < 0.001$). During the first week of the experiment, *B. gymnorrhiza* and *R. apiculata* produced approximately 7 and 6 leaves respectively, whilst the final number of leaves was 19 and 12 for *B. gymnorrhiza* and *R. apiculata* respectively, over the course of experiment. Increase in leaf number was marginally higher in *B. gymnorrhiza* than *R. apiculata* under 18 hour flooding treatment, while *R. apiculata* seedlings tended to produce more leaves under 6-hours flooding ($p < 0.076$). In contrast, 24-hours and 24-hours (stagnant) flooding treatments showed lower leaf growth rates in *B. gymnorrhiza* and *R. apiculata* seedlings, respectively (Fig 2.15; 2.16)

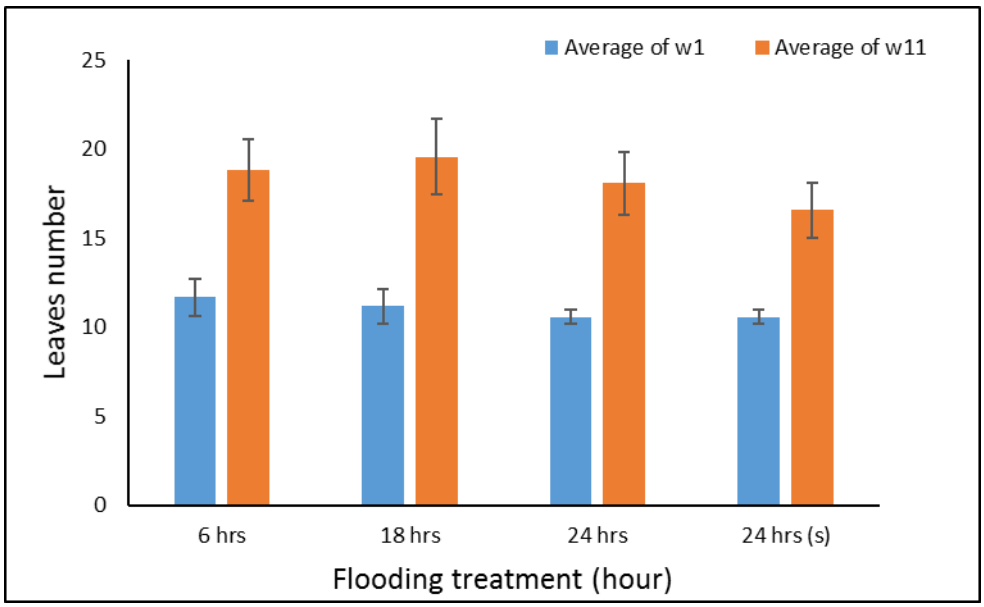



Fig 2.13 *B. gymnorrhiza* seedlings: number of leaves under different duration of flooding treatments. The initial and final measurements are shown (mean ± SE) n=12

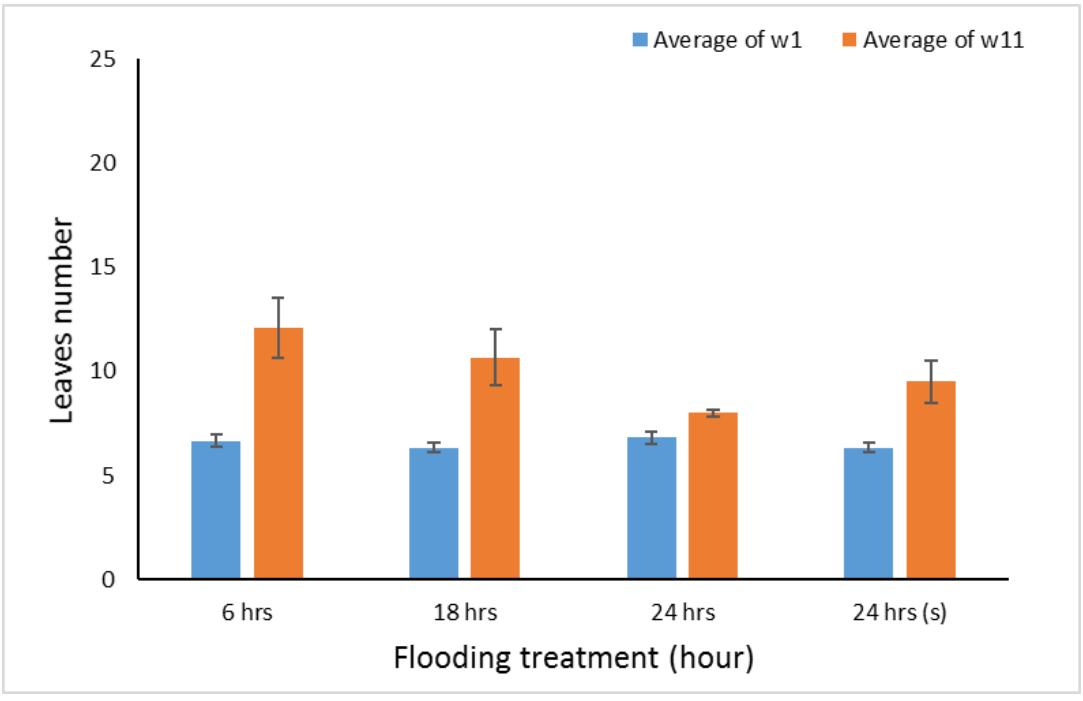



Fig 2.14. *R. apiculata* seedlings: number of leaves under different duration of flooding treatments. The initial and final measurements are shown (mean ± SE) n=12

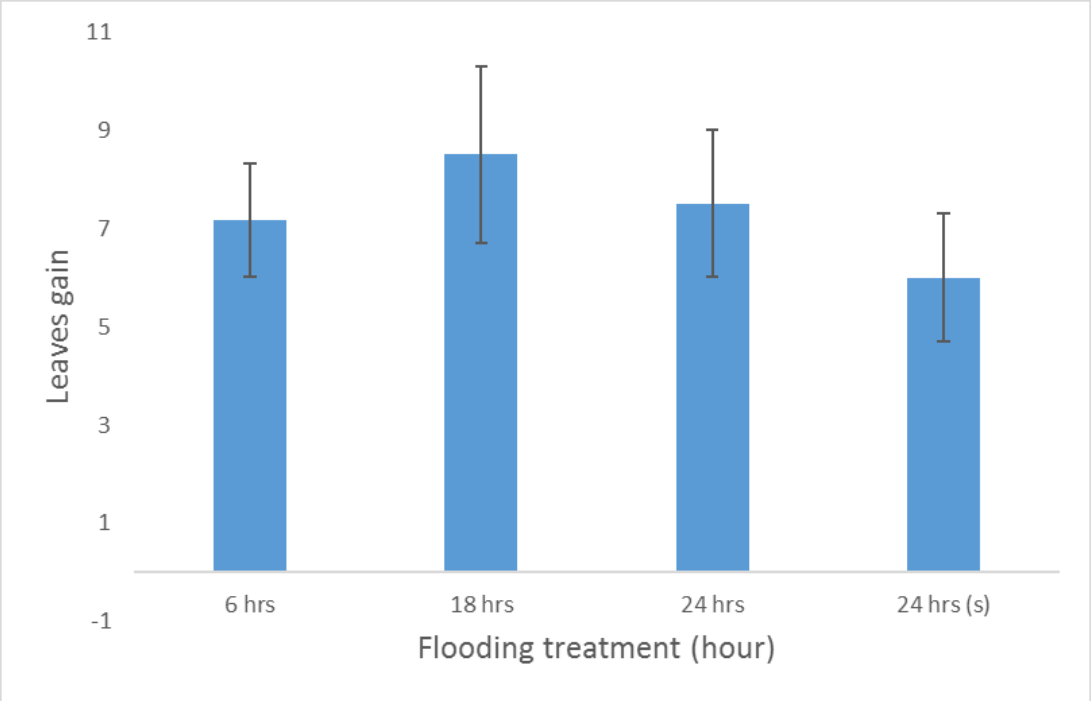



Fig 2.15 Gain in leaves of *B. gymnorrhiza* seedlings over the course of experiment n=12

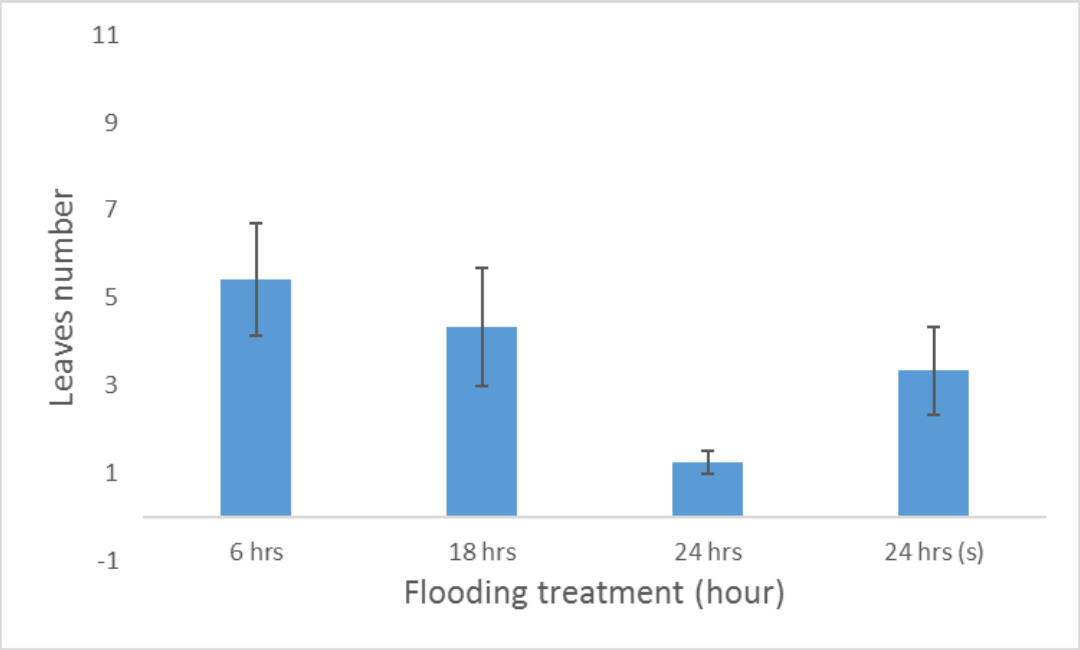



Fig 2.16 Gain in leaves of *R. apiculata* seedlings over the course of experiment n=12

### 2.4.3 Chlorophyll content

Chlorophyll content as indicated by SPAD readings was significantly affected by flooding treatments ($p < 0.001$). Seedling in the longest submergence of 24-hours and 24-hours (stagnant) treatments had the highest chlorophyll content in both *B. gymnorrhiza* and *R. apiculata* over the course of flooding experiment (fig 2.17). In contrast, seedlings with the 6-hours treatment had the lowest chlorophyll content throughout the flooding experiment for both species (Fig 2.18). There was also a significant difference among flooding weeks ($p < 0.001$) for both species. Across species and treatment, chlorophyll dropped by 25-30% between the start of the experiment and the third week, before recovering on the fifth week to its initial value. A slight increase of chlorophyll content was observed between fifth week and the end of the experiment.

Chlorophyll content was significantly higher in *B. gymnorrhiza* seedlings than *R. apiculata* seedlings ($p < 0.001$). No significant effect was recorded for both species between measurements done in the morning and in the afternoon ($p > 0.150$).

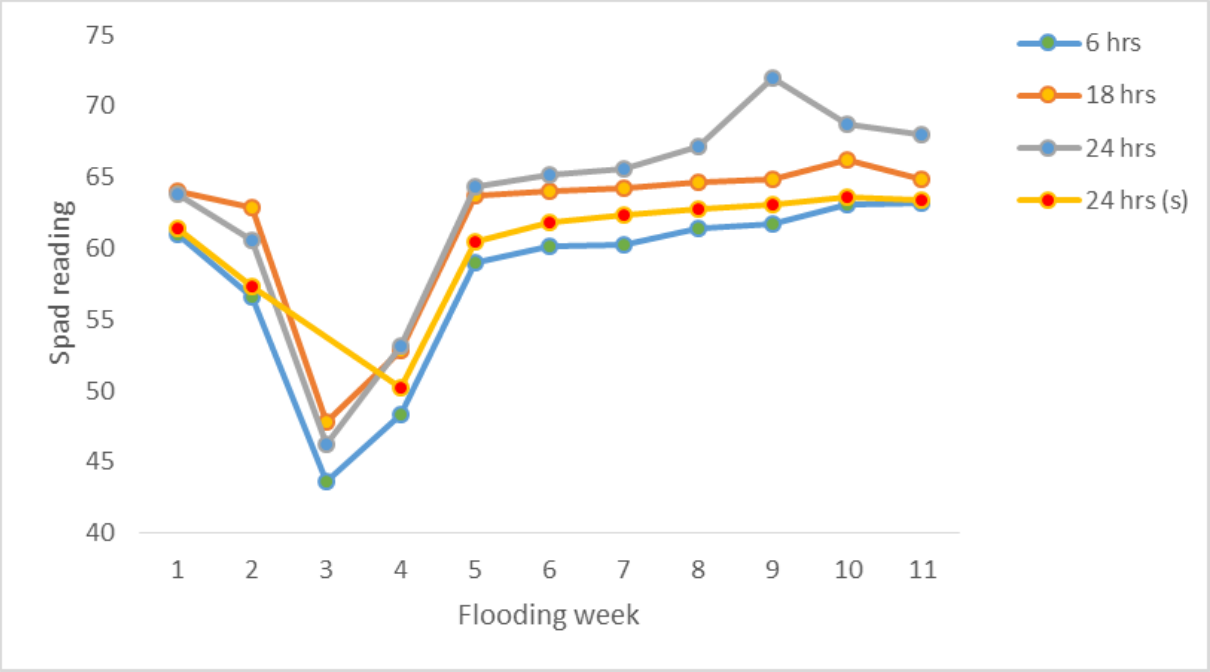



Fig 2.17. Chlorophyll content in *B. gymnorrhiza* seedlings under different durations of flooding treatments over the course flooding experiment. n=12

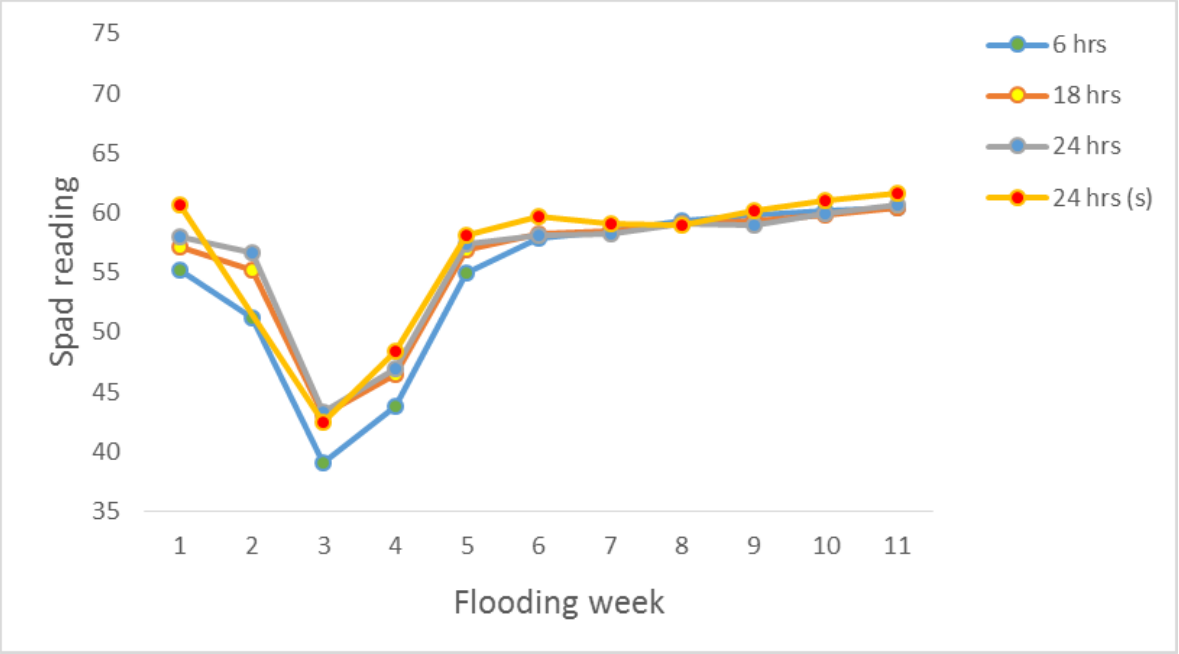



Fig 2.18 Chlorophyll content in *R. apiculata* seedlings under different durations of flooding treatments over the course of flooding experiment. (Mean ± SE) n=12

### 2.4.4 Chlorophyll fluorescence (fv/fm)

Chlorophyll fluorescence values remained between 0.7 and 0.85 over the course of the flooding experiment. Overall, readings in *R. apiculata* seedlings were significantly higher ($p < 0.001$) and exhibited more variation in fv/fm over time than *B. gymnorrhiza* seedlings. In *B. gymnorrhiza*, fv/fm was significantly higher in the 24 hours, 24 hours (stagnant) and 18 hours flooding treatments than in the 6 hours treatment both in morning and afternoon measurements (Fig 2.19; Fig 2.20).

*R. apiculata* seedlings under all treatments (except the 24 hours flooding) had significantly higher fv/fm in the morning measurements than in the afternoon (fig 2.21), whilst seedlings of 6 hours and 24-hours treatment showed lowest value in fv/fm in the afternoon measurements (Fig 2.2). The value of fv/fm of *R. apiculata* seedlings was significantly higher than in *B. gymnorrhiza* seedlings both in morning and afternoon measurements ($p < 0.001$). Fv/fm was significantly affected by the flooding weeks ($p < 0.001$). Fv/fm in *B. gymnorrhiza* seedlings under 18-hours and 24-hours (stagnant) decreased from week 1 to week 3 whilst 6 hours and 24 hours flooding treatments decreased for both and afternoon measurements. The values of plants of both species kept fluctuating in the following weeks and declined again in final week of flooding experiment.

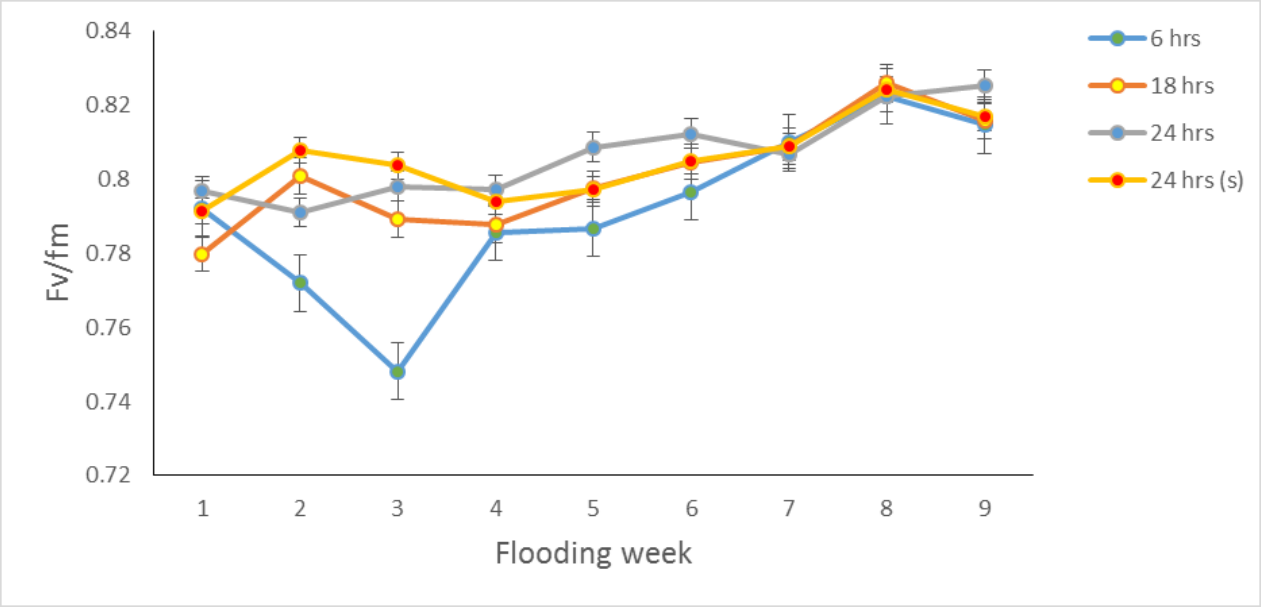



Fig 2.19 Chlorophyll fluorescence of *B. gymnorrhiza* seedlings in the morning under different durations of flooding treatments across 11 weeks flooding experiment. (Mean ± SE) n=12

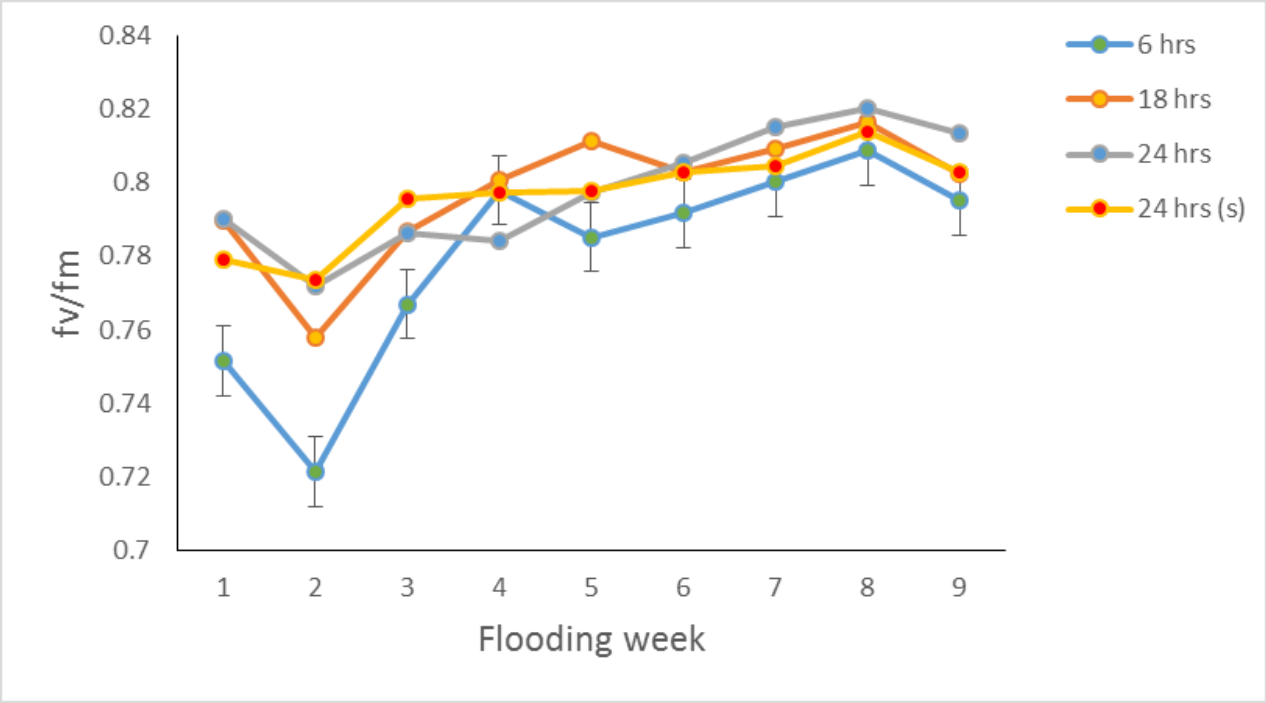



Fig 2.20 Chlorophyll fluorescence of *B. gymnorrhiza* seedlings in the afternoon under different durations of flooding treatments across 9 weeks flooding experiment. (Mean ± SE) n=12

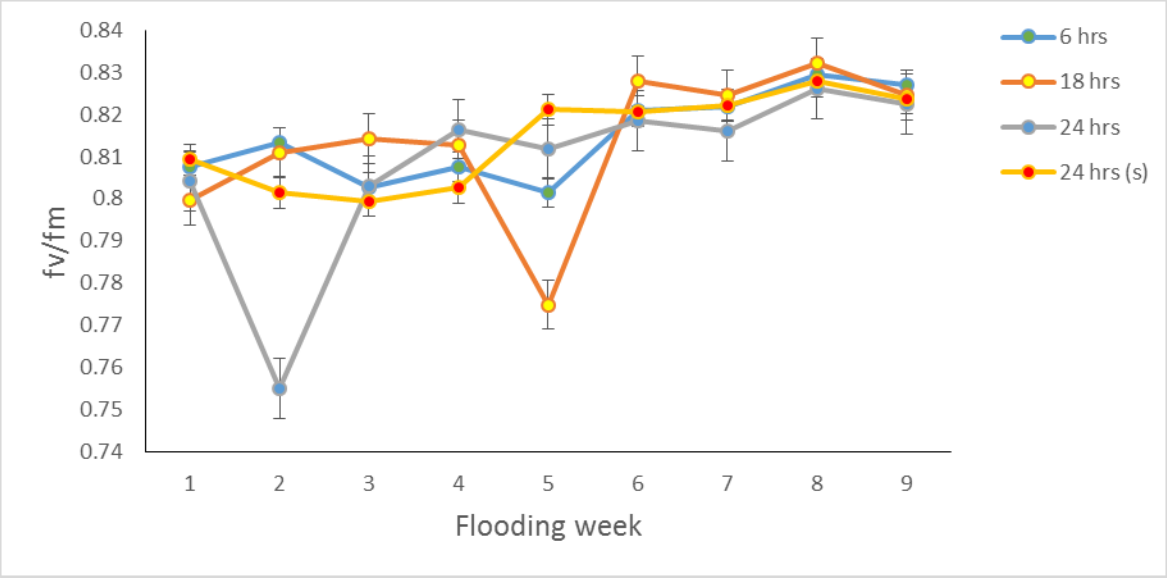



Fig 2.21 Chlorophyll fluorescence of *R. apiculata* seedlings in the morning under different durations of flooding treatments across 9 weeks flooding experiment. (Mean ± SE) n=12

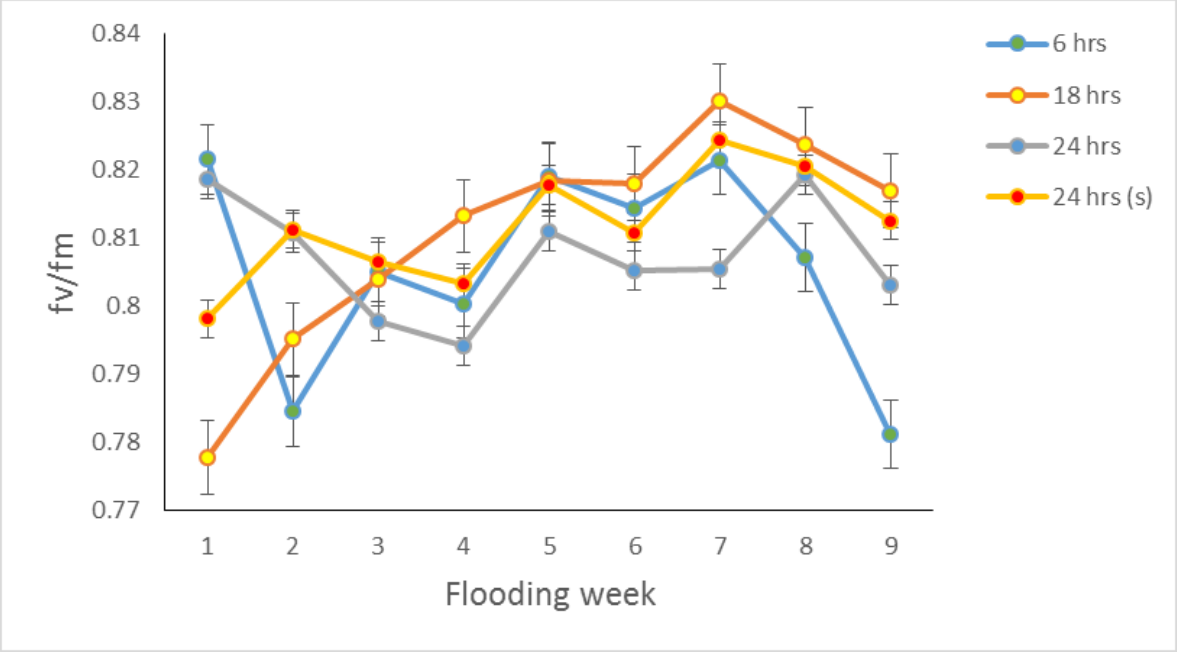



Fig 2.22 Chlorophyll fluorescence of *R. apiculata* seedlings in the afternoon under different durations of flooding treatments across 9 weeks flooding experiment. (Mean ± SE) n=12

### 2.4.5 Photosynthesis

#### a) Maximum net assimilation (Amax)

Net assimilation rate was not affected by the flooding treatments ($p > 0.111$). There was a significance difference in net assimilation among flooding weeks ($p < 0.001$). In *R. apiculata* seedlings, 6 hours flooding treatment was the highest during the initial flooding week but decreased in the following week. *B. gymnorrhiza* seedlings showed slightly higher photosynthesis rates than *R. apiculata* in week 2, but there was a reduction in the following week for seedlings of all treatments and slightly increased between week 3 and week 5. There was a reduction in photosynthesis during final measurement week of all treatments except for 24-hour flooding treatment. Photosynthesis rate was significantly higher in *R. apiculata* than *B. gymnorrhiza* seedlings ($p < 0.02$) (Appendix 2). The two species showed opposite trends in photosynthesis rates over time ($p < 0.05$) (Fig 2.23; Fig 2.24).

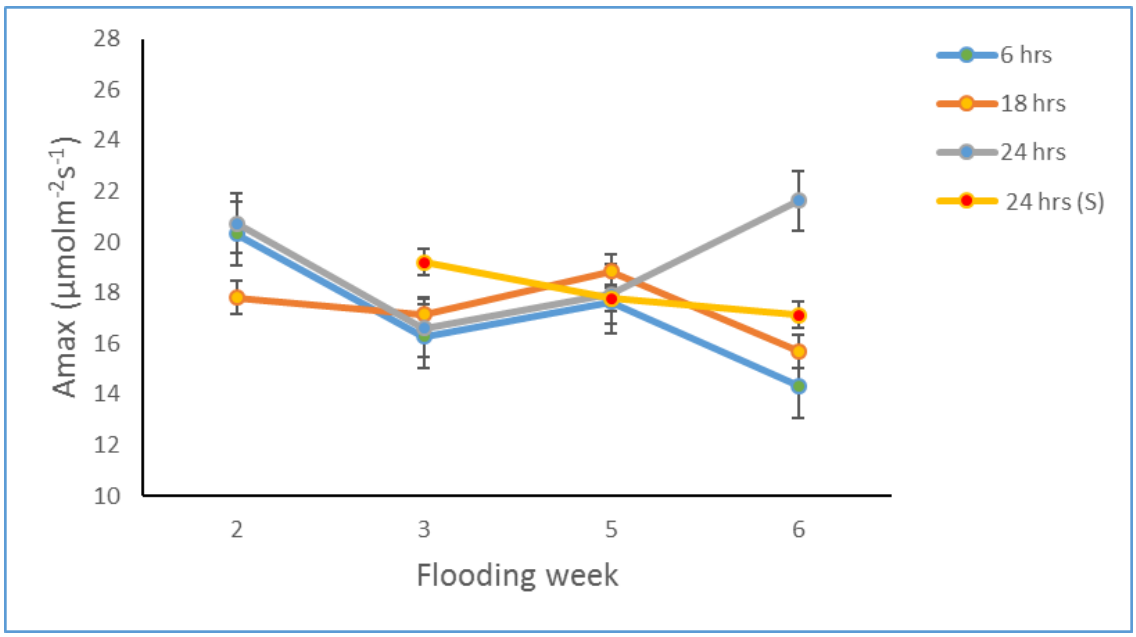



Fig 2.23 Photosynthesis Amax of *B. gymnorrhiza* seedlings under different duration of flooding treatments. (Mean ± SE) n=4

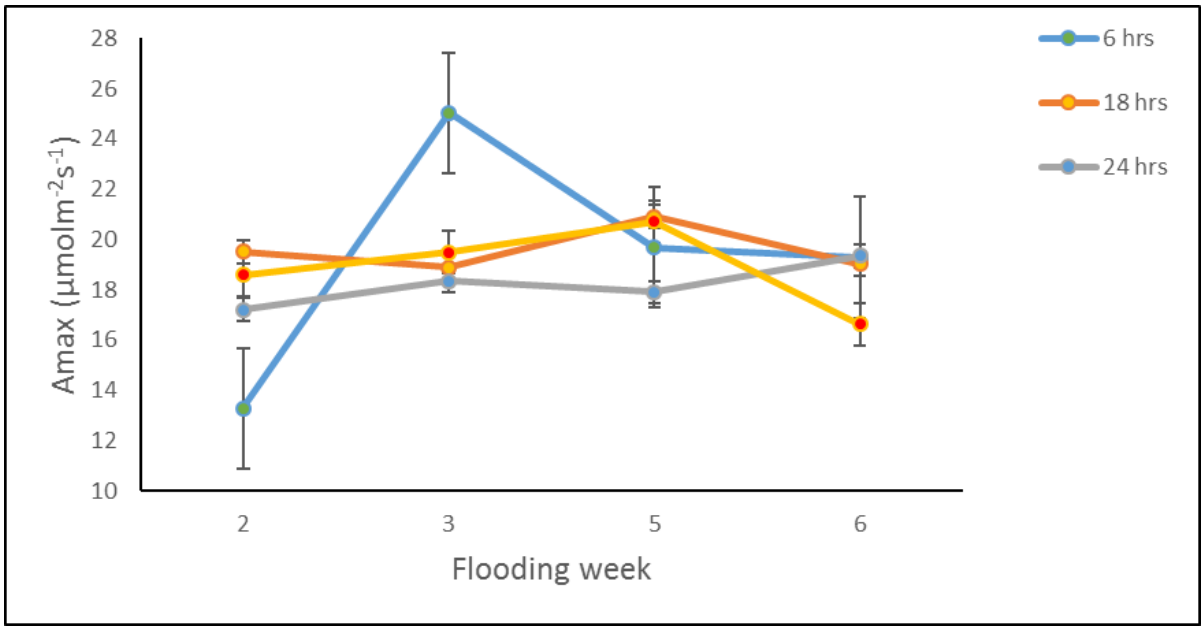



Fig 2.24 Photosynthesis Amax of *R. apiculata* seedlings under different duration of flooding treatments. (Mean ± SE) n=4

b) Stomatal conductance ($g_s$)

Stomata conductance was not affected by the flooding treatments ($p > 0.363$). However, the conductance showed a significant difference among flooding weeks ($p < 0.001$). Plants showed high stomata conductance for the first two weeks of flooding but $g_s$ was significantly decreased in week 6 for both species ($p < 0.01$) (Fig 2.25). Stomatal conductance was slightly higher in *R. apiculata* than *B. gymnorrhiza* seedlings but the difference was not significant ($p > 0.165$) Fig 2.26)

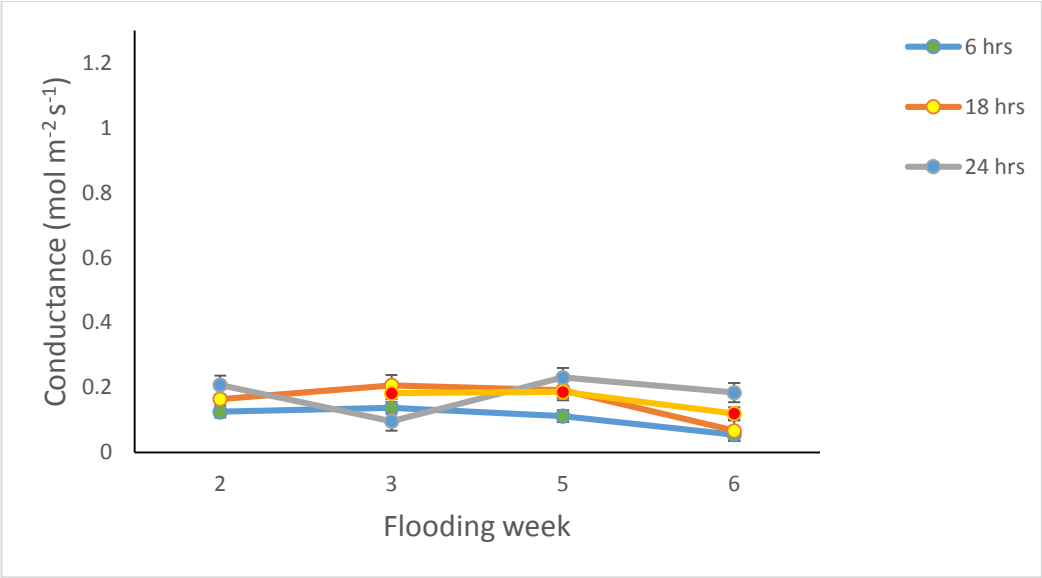



Fig 2.25 Stomata conductance of *B. gymnorrhiza* seedlings under different durations of flooding treatments. (Mean ± SE) n=4

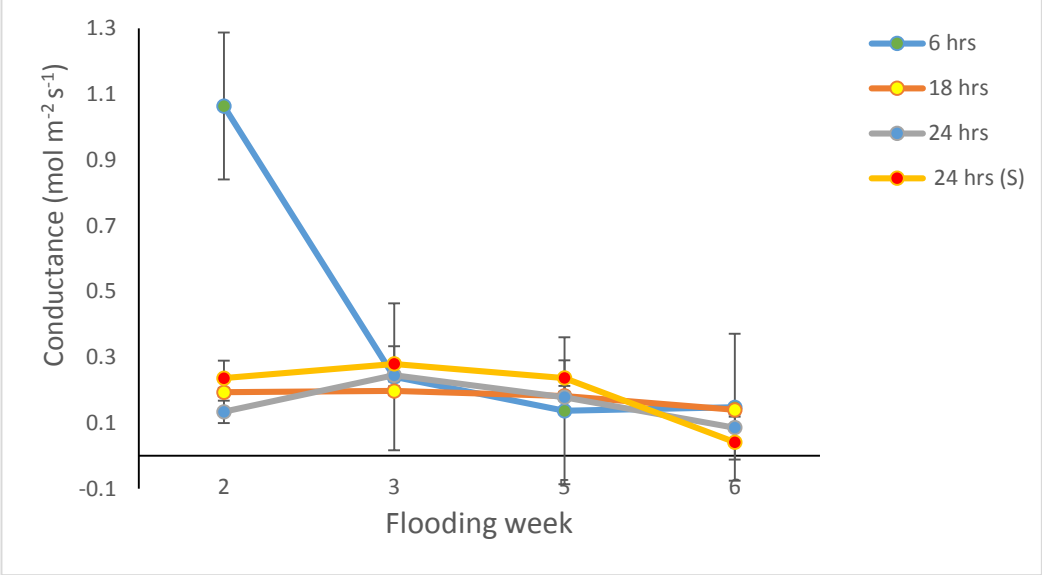



Fig 2.26 Stomata conductance of *R. apiculata* seedlings under different durations of flooding treatments. (Mean ± SE) n=4

### 2.4.6 Non-structural carbohydrate content (NSC)

a) Total non-structural carbohydrate

There was no significant difference among treatments for total non-structural carbohydrate ($p > 0.05$). No difference in NSC among treatments was recorded in *R. apiculata* seedlings (Fig 2.27). *B. gymnorrhiza* seedlings had significantly higher non-structural carbohydrate levels than *R. apiculata* seedlings ($p < 0.038$).

b) Starch

Starch content was not significantly different among treatments ($p > 0.567$). However it was observed that starch content increased with the duration of the flooding treatments in *B. gymnorrhiza* whilst little starch was produced in any treatments in *R. apiculata* seedlings. The amount of starch tended to be higher ($p < 0.070$) in *B. gymnorrhiza* seedlings than *R. apiculata* seedlings, although the difference was not significant. In contrast, *R. apiculata* seedlings had almost no starch (Fig 2.28).

c) Sucrose

Overall, the amount of sucrose tended to be higher in *R. apiculata* than *B. gymnorrhiza* seedlings but the difference was not significant ($p > 0.086$). Sucrose varied among treatments, as it was higher in 24-hrs and lowest in 24-hours (stagnant) treatment in *R. apiculata*. In *B. gymnorrhiza* seedlings, sucrose only accumulated under 6-hours treatments. It was observed that the sucrose content was almost depleted in other treatments in *B. gymnorrhiza* seedlings.

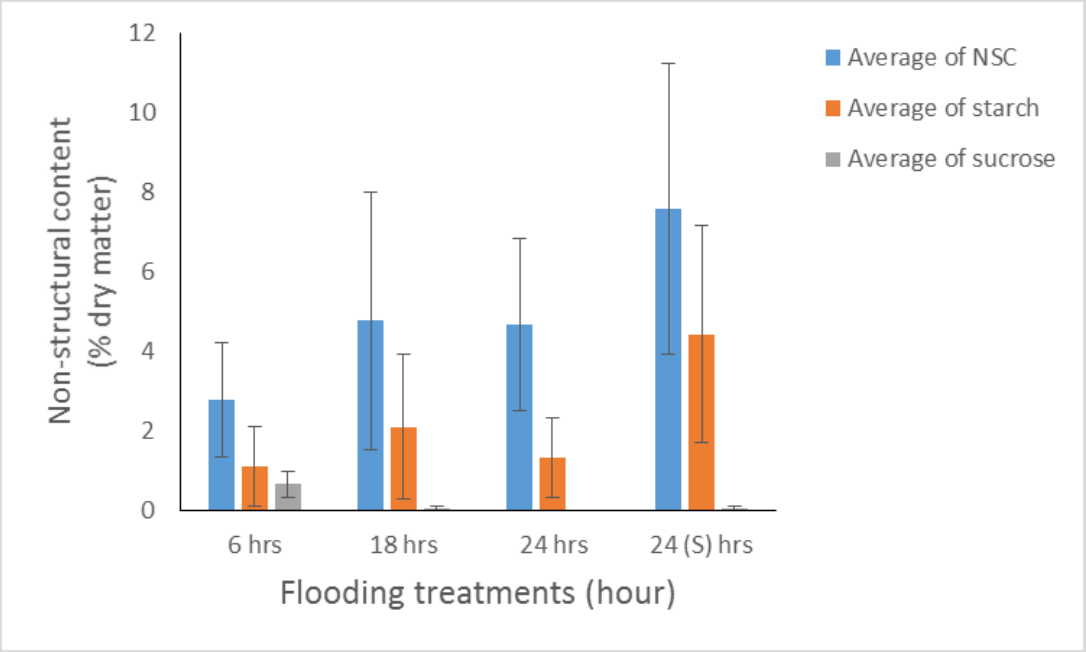



Fig 2.27 Non-structural carbohydrate in *B. gymnorrhiza* seedlings under different duration of flooding treatments. (Mean ± SE) n=3

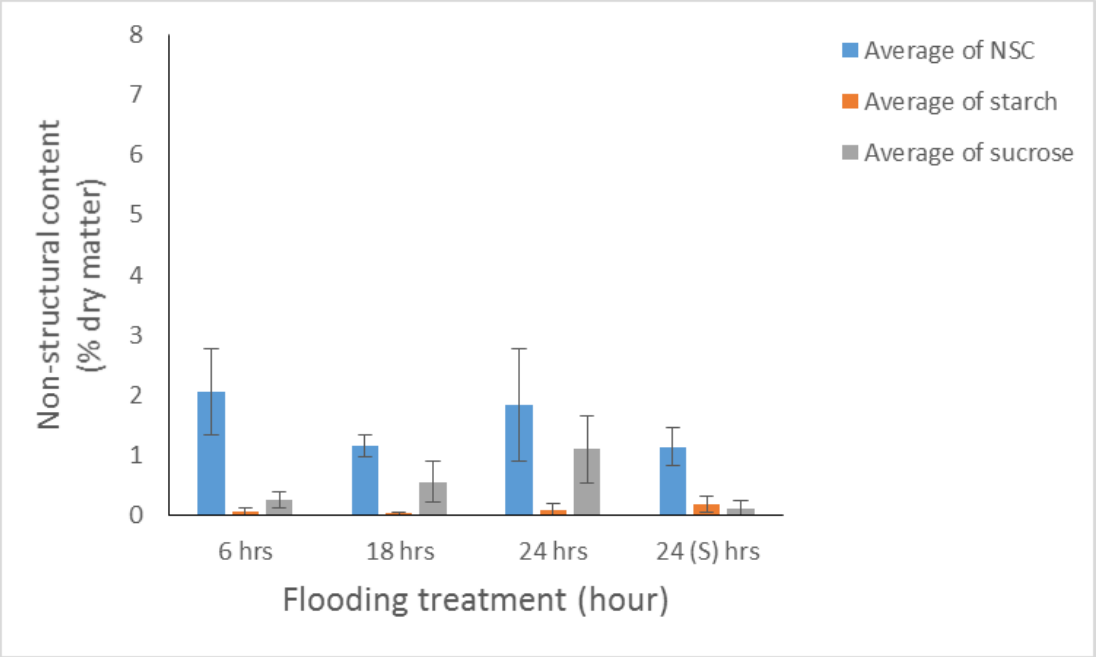



Fig 2.28 Non-structural carbohydrate in *R .apiculata* seedlings under different duration of flooding treatments. (Mean ± SE) n=3

2.4.7 Leaf area

There was a significant difference in leaf area among flooding treatments ($p < 0.024$) (Table 2.2). Leaf area was lowest in the 24-hours treatment in *B. gymnorrhiza*. However, leaf area in *R. apiculata* was significantly lower than *B.gymnorrhiza* ($p < 0.027$) (Fig 2.29).

Table 2.2 Plant leaf area under different flooding treatments, species and block (Mean ± SE).

| Block | Block 1 | | Block 2 | | Block 3 | |
|---|---|---|---|---|---|---|
| species | Bg | Ra | Bg | Ra | Bg | Ra |
| 6 hrs | 41.22 ± 4.5 | 39.8 ± 3.97 | 48.35 ± 3.56 | 41.63 ± 2.76 | 46.08 ± 5.46 | 40.70 ± 2.9 |
| 18 hrs | 43.11 ± 2.67 | 38.08 ± 6.19 | 47.02 ± 6.46 | 43.53 ± 3.37 | 40.96 ± 4.87 | 36.95 ± 3.67 |
| 24 hrs | 36.69 ± 0.9 | 40.94 ± 6.96 | 28.24 ± 4.27 | 40. 60 ± 3.31 | 45.04 ± 4.63 | 33.09 ± 2.87 |
| 24 hrs (stagnant) | 41.69 ± 3.6 | 41.96 ± 4.63 | 49.3 ± 4.58 | 40.31 ± 2.5 | 56.92 ± 2.10 | 39.66 ± 2.54 |

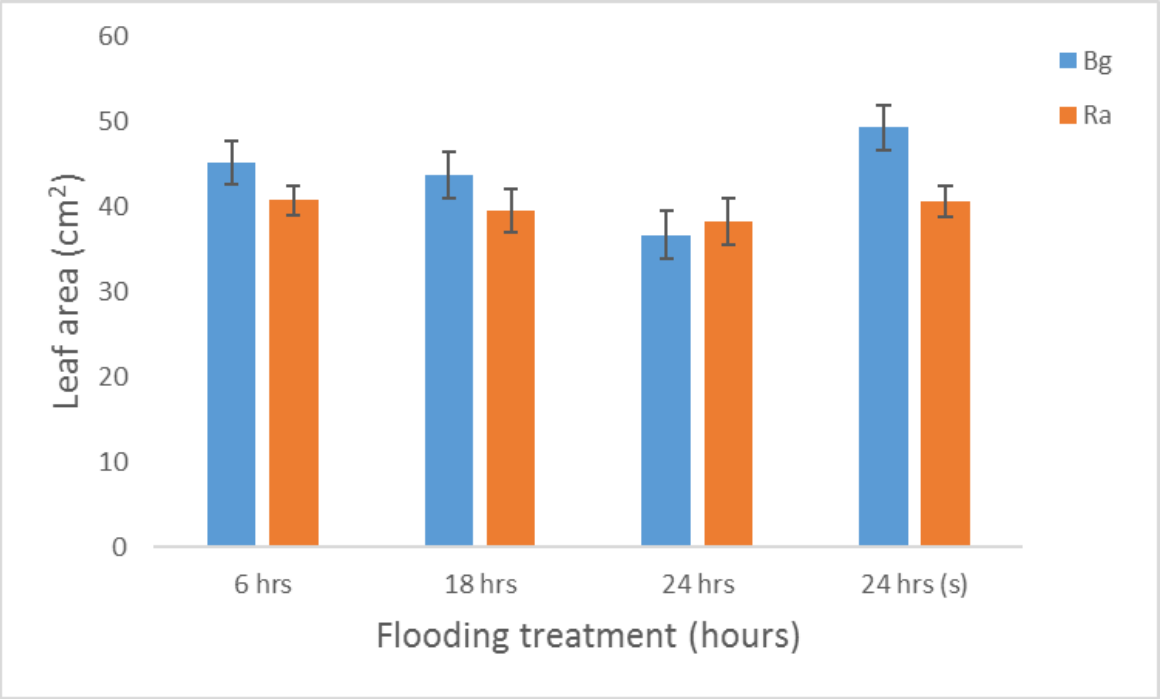



Fig 2.29 Leaf area of *B. gymnorrhiza* and *R. apiculata* seedlings under different durations of flooding treatments (Mean ± SE) n=12

2.4.8 Morphological observation

a) Lenticels

Lenticel on the stems were observed for both seedlings and adventitious roots were produced approximately during week 2 of flooding. Plants under 18-hour flooding were the first plants to produce adventitious root. Numerous lenticels were observed in *R. apiculata* seedling stems distributed densely within their hypocotyl region (Fig 2.3).


Fig 2.3 lenticels structure in *R. apiculata* hypocotyl

b) Adventitious root

An abundance of adventitious root were observed in *B. gymnorrhiza* seedlings. This roots were formed outside the root structure (Fig 2.31).

Fig 2.31 Adventitious root in *B. gymnorrhiza* seedlings

c) Leaf necrosis

In this study, plants under 24 hours (stagnant) exhibited leaf necrosis in both species (Fig 2.32).

(a) (b)

Fig 2.32 Leaf 'burn' necrosis and leaf fall (a) *B. gymnorrhiza* seedlings (b) *R. apiculata* seedlings during submergence.

## 2.5 Discussion

### 2.5.1 Influence of prolonged root submergence on mangrove seedlings growth and survival

This study demonstrated that all plants survived throughout the flooding experiment. Both species were relatively unaffected by the flooding treatments, in fact they exhibited increased growth and maintained the highest leaf physiological properties in the 24-hours treatments.

Overall, both seedlings grew well under flooding treatment particularly the long period of submergence; (that is 24 hours and 24 hours (stagnant) treatment). The environmental data surprisingly provided the highest level of dissolved oxygen under 24 hour (stagnant) treatment. This means the plants were not affected by the depleted levels of oxygen. In fact, the stagnant treatment enhanced the oxygen concentration for the plants to grow. The highest dissolved oxygen created an artefact for the flooding experiment and might be derived from photosynthetic algae or bacteria inhabiting the buckets during flooding experiment. Normally, under sufficient light and temperature, stagnant water may encourage bacterial growth. The highest dissolved oxygen may be a reason for the high leaf photosynthetic activity (such as chlorophyll content and chlorophyll fluorescence) and the high plant leaf area under 24 hours (stagnant) treatment.

### 2.5.2 Influence of prolonged root submergence on leaf physiological properties and leaf carbon reserves

The characteristics of high flooding tolerance of the seedlings can be seen from plant growth and the leaf physiology responses. In this study, the plant growth was greatest under long submergence. The plants showed growth in stem diameter and significantly increased their height under the 24 hour and 24 hour (stagnant) treatment. Shoot elongation was the most important trait of the waterlogging plant to allow contact with the atmosphere, as a mechanism to gain oxygen to satisfy their metabolism (Colmer and Voosenek 2009). The height growth was also observed in He et al. (2007), which may explain the shift of increased biomass above the water surface (Hoppe-Speer et al. 2011). Reduced seedling growth was also recorded under the 6-hour treatment particularly in *B. gymnorrhiza*. Plants might allocate substantial biomass to stem diameter in order to survive under long submergence. However, there was a lower leaf number under long submergence in this study which is similar to finding from Hoppe-Speer et al. (2011) as they found lower leaf gain of *Rhizophora mucronata* under continuous and no inundation flooding treatment. Pezeshki et al. (1989; 1997) also found leaf growth was inhibited under long flooding suggesting plants under long submergence produced fewer leaves. The reduced leaf number under flooding stress can be related to the leaf abscission observed during flooding experiment, showing the response of the stress (Omami 2005). Furthermore, the plant leaves also showed necrosis particularly for plants under 24 hour (stagnant) treatments which might be a reason for leaf fall. Necrosis is a symptom for plants that are unable to take up nutrients due to the submergence (Chen et al. 2005).

Maximum photosynthesis rates were not affected by the flooding treatments. Seedlings maintained higher photosynthesis under long submergence as supported by higher stomata conductance during flooding experiment. Additionally, there was no damage to the photosynthetic apparatus (high fv/fm), although chlorophyll content showed a reduction during the initial flooding weeks. Plant chlorophyll fluorescence also exhibited a period of stress for both species during flooding experiment. The seedlings showed resilience to the flooding as they quickly recovered from the stress period, as the chlorophyll content increased rapidly in the following week and maintained higher chlorophyll content towards the end of the experiment. Plant chlorophyll fluorescence also showed a similar trend, as it decreased during the early period of flooding, although it slightly fluctuated from week 1 until week 6 particularly for *R. apiculata*. Chlorophyll fluorescence is sensitive to the changes caused by abiotic stress (Panda 2008). The results from statistical analysis also demonstrated that chlorophyll content and chlorophyll fluorescence were significantly higher under long submergence, which is consistent with the observation that the seedlings maintained optimal photosynthetic activity under the stress. This trend is also similar to the acclimation model proposed by Harera (2013), whereby there is an early inhibitory phase in the seedlings under flooding stress, but this is followed by an acclimation progressively as the experiment develops. This is a mechanism which develops frequently in most flood tolerant species. In this study, higher chlorophyll may have increased the photosynthesis under long submergence. This is supported by increased growth of the seedlings under long submergence. However, Ye et al. (2003) has argued the higher leaf greenness may be causing an increased photosynthesis but this does not necessarily support the growth rate. This is because the stomatal resistance may be

limited under anoxia condition. However, the anoxia did not happen in this study since 24-hour (stagnant) treatment showed increased dissolved oxygen levels. Higher chlorophyll content was also recorded for the plants under inundation (Ellison and Farnsworth 1997). This finding is in contrast to a study on the response of *R. mucronata* to varying salinities and inundation, which demonstrated lowest chlorophyll fluorescence under long submergence (Hoppe-Speer et al. 2011), implying that the salinity stress exert a stronger negative influence to the mangrove seedlings than flooding gradient.

*R. apiculata* seedlings were found to have higher carbon assimilation than *B. gymnorrhiza* although not much difference was recorded among flooding treatment. A previous study has shown that mangrove plants under canopy submergence are dying due to plant leaves being under water and causing stomata closure (Luzhen et al. 2005) thus preventing the photosynthesis under low light (Lu et al. 2013; Ye et al. 2016). This is because submerged plants receive low light intensity under water (Mangora et al. 2017) since the light penetration into the water may be only due to the scatter to the surrounding or emitted back to the surface of water (Ayi et al. 2016). Therefore, this finding supports the significant decrease in photosynthesis under whole-plant submergence (Luzhen et al. 2005; Ye et al. 2003). However, root submergence in this present study clearly indicated the plant leaves remained above the water and in contact with the atmosphere, so that the leaves received sufficient light for carbon assimilation, and this may explain why they maintained higher photosynthesis under submergence, consistent with their chlorophyll content and chlorophyll fluorescence. In addition, plant leaf area was also highest under 24 hour (stagnant) treatment

particularly for *B. gymnorrhiza* seedlings. This finding was not due to the fact that *B. gymnorrhiza* itself had bigger leaves than *R. apiculata* but the statistical analysis has confirmed the flooding treatments affected the increase in leaf area under long submergence. The plants may expanded their leaf area to gain more light absorption to support photosynthesis as an adjustment under flooding stress.

During the initial stages of the flooding experiment, plants exhibited higher photosynthesis but this remarkably declined towards final experiment week. The findings of this present study are similar to the work of Youssef and Saenger (1998), as photosynthesis decreased during waterlogging treatment. The reduction in photosynthesis might be associated with the reduction of stomata conductance. Plants under oxygen deficiency triggered a changing hormone balance in plant which promoted stomata closure under long submergence (Harera 2013). Both chlorophyll content and chlorophyll fluorescence notably decreased toward the end of flooding week which might also be causing lower photosynthesis rate than in the earlier flooding weeks.

Plants accumulate non-structural carbohydrates to survive under environmental stress (Panda and Sarkar 2014). In this study, *B. gymnorrhiza* accumulated higher total non-structural carbohydrates and the highest accumulation was recorded under the 24-hour (stagnant) treatment. Long submergence may be causing injury to the plants where flooding prevents either the root nutrient absorption (Chen et al. 2005) or causes oxygen deficiency which may disturb metabolism. Therefore, the plants need substantial amounts of soluble sugar to counter these problems to support their internal metabolism under flooding. This study provided ample evidence that seedlings consumed soluble sugar to adjust the plant metabolism, as shown by greater

accumulation of total non-structural content in *B. gymnorrhiza* seedlings whilst a very small amount of starch was recorded for *R. apiculata* seedlings, suggesting that most of carbon reserve in the plant was consumed in metabolism processes. Rosa et al. (2009) described how plants adjust their metabolic regulation through gene expression to help the plant cope with the stress. It was observed that photosynthesis was significantly higher in *R. apiculata* than *B. gymnorrhiza* seedlings. However NSC accumulation was greater in *B. gymnorrhiza* than *R. apiculata* seedlings. This situation indicates that *B. gymnorrhiza* did not consume the carbon assimilation product to regulate metabolism under stress. In fact, the NSC pool increased under submergence at least for this species. In contrast, very small amount of NSC and starch were produced in *R. apiculata*, implying this species might have consumed the carbohydrate content for their metabolic adjustment during submergence. During flooding, *R. apiculata* seedlings showed more leaf necrosis leading to the leaf fall, particularly in the plants under 24 hours (stagnant) treatment. This situation may have contributed to the decrease of NSC pool when the main photosynthesis sources are removed (Li et al. 2002), resulting very small amount of NSC in *R. apiculata*. Greater amounts of sucrose were also observed in *R. apiculata* than NSC or starch.

### 2.5.3 The impact of prolonged root submergence

In this study most of the response variables were maintained at high levels under 24-hour (stagnant) treatment, indicating the plants were well adapted to the long submergence. Both species were not affected by the flooding treatment, hence they are likely to be unaffected by sea level rise in the future. However, *B. gymnorrhiza* was

shown to be affected by the flooding treatment as several variables were lower under the 6 hour treatment; implying that this species is rather sensitive to the frequent tidal changes. This is because plants exposed to the frequent changes of flooding period may experience different environments (Panda et al. 2008), that is higher oxygen levels during high tides and lower oxygen during low tides (Mattone and Sheaves 2017). For the species inhabiting further inland, the frequent changes provide a difficult situation to their physiological process and this might explain the vulnerability of *B. gymnorrhiza* to the frequent changes of flooding.

Mangroves plants showed high flooding tolerance possibly due to their development of morphological adaptations. In this study, morphological changes were also observed in the seedlings before and during flooding experiment. Based on my observations, numerous lenticels were distributed on *R. apiculata* seedling stems during their propagule stage, increasing considerably the initial formation of lenticels while attached to the mother trees. The lenticels structure in their stem likely permitted oxygen entry to their internal structure. Our study indicates that the formation of lenticel on the seedlings bark particularly in *R. apiculata*, probably helps the seedlings to get sufficient oxygen supply during submergence. Gill and Tomlinson (1977) demonstrated that an abundance of lenticel in surface area of aerial root for *Rhizophora mangle* provides the main pathway for oxygen diffusion to the substrate. Additionally, adventitious roots were developed in both *B. gymnorrhiza* and *R. apiculata* seedlings as early as week two of flooding experiment. It was observed the development of adventitious roots developed slightly above the main root structure. This is similar to Steffens and Rasmussen's (2016) observation that adventitious roots were produced

from outer root tissue. The role of adventitious roots is to help in gas exchange and also to enable water and nutrient uptake during flooding (Steffens and Rasmussen 2016). Moreover, the roots are generally were equipped with aerenchyma structure within their internal tissue (Colmer 2003) to allow oxygen diffusion.

The findings from this study provide evidence that mangrove seedlings will survive in the face of future sea level rise, as shown by their high flooding tolerance under long submergence.

## 2.6 Conclusions

All seedlings survived and grew well under flooding treatment. The response of mangrove seedlings to flooding treatments demonstrated that plants can grow significantly taller under long submergence, can increase in stem diameter with few leaves numbers and can develop significantly higher leaf area under long submergence. In both species, leaf photosynthetic activity was rather high, and significantly higher under long submergence and lowest under 6 hour treatment. The plants showed decreases in chlorophyll content during the early period of the flooding experiment but showed recovery in the following weeks. Chlorophyll fluorescence showed a stress period during flooding experiment. Photosynthesis rate (Amax) and stomata conductance ($g_s$) were not affected by the flooding treatment but showed the lowest photosynthesis towards the end of flooding period. Total non-structural carbohydrates were higher in *B. gymnorrhiza* than *R. apiculata* seedlings and higher under 24 hour (stagnant) in *B. gymnorrhiza* seedlings.

Overall, mangrove seedlings showed high flooding tolerance, as demonstrated by the small effects observed under the experimental flooding treatment. *R. apiculata* was less affected by the long submergence but *B. gymnorrhiza* was more sensitive to the frequent changes in flooding period. Under root submergence, mangrove seedlings will survive under future sea level rise, given that their high tolerance to the flooding treatment can be maintained by adequate levels of leaf physiological properties.

# Chapter 3: Xylem anatomy of mangrove seedlings *Bruguiera gymnorrhiza* and *Rhizophora apiculata* under contrasting flooding treatments

## 3.1 Abstract

Mangroves are vulnerable to sea level rise resulting in sediment oxygen deficiency through prolonged seawater submergence. Prolonged submergence may adversely affects mangrove plants, particularly the seedlings when they have been submerged during tidal inundation. Therefore, this study was carried out to investigate the response of contrasting flooding treatments; 6 hours and 24 hours (stagnant) treatments on the xylem water-conducting cells of mangrove seedlings of *B. gymnorrhiza* and *R. apiculata*. To quantify this stress, several vessel characteristics were measured; vessel: diameter (VD), wall thickness (VWT), density (Vp), lumen area (VLA) and hydraulic diameter ($D_h$). Small segments of seedling stem were analysed approximately 3 cm and 12-15 cm from the shoot tip, represented plant apex and plant mid-stem respectively. The VD, VWT, Vp, $D_h$ were analysed using both light microscope and scanning electron microscope. The VD showed no significant difference under contrasting flooding treatments, however it varied between plant sections and species. Vessel diameter and $D_h$ were significantly wider at plant mid stem ($p < 0.000$) but not varied between species. More cell wall thickening was recorded in *R. apiculata* than *B. gymnorrhiza* seedlings ($p < 0.021$).Vessel density was significantly higher at the plant apex ($p < 0.001$), however this was independent of contrasting treatments. *R. apiculata* consists of a higher number of vessels than *B. gymnorrhiza*, almost significantly more at ($p > 0.051$). Plant apex had a larger vessel lumen area ($p < 0.001$) and 24 hours (stagnant) following flooding treatment but, it was not statistically significant ($p > 0.649$). Vessel hydraulic diameter was significantly higher at plant mid stem than plant apex ($p < 0.001$) and slightly larger in *B. gymnorrhiza* than *R. apiculata* seedlings. Vessel characteristics between plant sections indicate more significant differences than flooding treatments, suggesting longer submergence does not affect

the water conducting cells in the xylem structure; indicating submergence has a little effect on plant hydraulics.

## 3.2 Introduction

Mangroves communities thrive under flooding of brackish water from fresh water outlets to tidal inundations for several hours per day. During submergence, oxygen in the surrounding soil is depleted, therefore plants depend on aeration systems to maintain an oxygen supply to the within-plant organs. These aeration systems also assist in oxygenation of the surrounding rhizosphere, benefiting other mangrove species (Youssef and Saenger 1996). Consequently, mangroves are reliant on this adaptation to cope with oxygen deficiency on a daily basis. However, under projections of sea level rise due to climate change (Lovelock et al. 2015), mangroves are expected to face prolonged seawater submergence (Lu et al. 2013). It is assumed that prolonged seawater submergence would adversely affect the mangroves due to hypoxic conditions. Earlier studies conclude that mangrove seedlings are the most vulnerable to the long submergence (Lu et al. 2013; Luzhen et al. 2005). This is postulated to be due to their abundance on the forest floor, where a large volume of whole plant immersion occurs and consequently causes mangrove dieback due to the unstable submergence occurring during critical, and seedling maturation. Hence, an accelerated sea level rise has the potential to determine future regeneration of mangrove communities.

Most of the studies to date have examined the impact of sea level rise on mangrove seedlings in terms of ecology, physiology, and anatomy (Krauss et al. 2014; Ellison and Farnsworth 1997; He et al. 2007; Luzhen et al. 2005, Ye et al. 2003, Xiao et al. 2009). Of these studies, very little information on water conducting cells under submergence were described. Xylem vessels are important in conducting water throughout plant organs (Zimmerman 1983) and to ensure the diffusion of soluble minerals such as $Na^{+}$, $Cl^{-}$, $Mg^{2+,}$ $Ca^{2+}$ and $SO_4^{2-}$ (Santini 2012). Vessel diameter and vessel density are both believed to be the most significant anatomical features for the movement of water in plants; a wider vessel diameter may increase the efficiency of water conduction but increased vessel numbers indicate the hydraulic safety (Zimmerman 1983). Terrestrial plants normally possess a larger vessel diameter than aquatic plants (Tomlinson 2016). I contrast to this, mangrove species with high wood density such as *Avicennia marina* have been found to possess a large vessel diameter in Western Australia suggesting the vessel size reflects the tree growth (Santini 2012). However, smaller vessel diameters have been recorded under saline conditions in Kenya as a response to the saline water indicating numerous vessels provide alternative for water flow (de Joelle 2010).

Hydraulic conductivity is another vessel parameter that can provide information on how much water is conducted along vessels, from the plant roots to the plant apex. Hydraulic conductivity is associated with vessel diameter and vessel density (Choat et al. 2007). Past studies have shown that hydraulic conductivity is negatively affected in response to salinity, as a result of a small conduit diameter (Verheyden et al. 2005; Sobrado 2007; Roberts et al. 2009) and low nutrient concentration (P and N) (Lovelock et al. 2006). In contrast, sedimentation has been shown to increase hydraulic

conductivity as evidenced by large vessel diameter, as reported in Kenya mangroves (Okello et al. 2017).

Numerous previous studies have debated the impact of xylem vessels under saline conditions (Verheyden et al. 2005; Schmitz et al. 2006; Robert et al. 2009 & Sobrado 2007). Under high salt concentration, mangrove plants encounter limited freshwater availability and are adapted in ways to reduce the high salt concentration so that they can uptake enough saline water to ensure optimum freshwater for transpiration. One adaptation known to cope under saline conditions is possession of small vessel diameter with high vessel density (Sobrado 2007; Verheyden et al. 2005; Robert et al. 2009). Numerous vessel numbers provide alternatives for water flow along the plant profile (Sobrado 2007).

Under flooding, several studies showed aerenchyma formation in the cortex tissue under long submergence (Purnobasuki and Suzuki 2004), but not much work has been done on water transporting tissue. From existing literature, xylem properties showed an increased in parenchyma tissue and the pith (Xiao et al. 2009) reflecting the increasing of mechanical strength under submergence. However, tangential vessel diameter, stem vessel wall thickness and fibre wall thickness decreased with extended periods of waterlogging (Xiao et al. 2010). The effect of soil flooding was observed in bark thickness and consequently altered other xylem and phloem tissue; for instance, the abundance of xylem rays, enlarged ray cells, numerous resin ducts and more phloem parenchyma cells (Yammamoto 1987). In terms of vessel cells, vessel diameter tends to be abundant with larger diameter, simple perforation and less wall thickening (Mauseth 1988) but this was reported for temperate species.

To date, the information on vessel characteristics of mangrove species in response to an increased submergence time remains scarce. Information is limited to the effect of flooding on wood and bark anatomy (Yanez-Espinosa et al. 2001) in Mexican mangrove. The findings from this study show that small vessel diameter (at least for four mangrove species *Avicennia germinans*, *Laguncularia racemosa*, *Annona glabra* and *Rhizophora mangle*), multiple vessel grouping, and taller wood rays are strongly associated with the flooded zone. There was no significant information on vessel characteristics indicated at different stem height.

Therefore, the findings illustrated by this study begin to bridge an information gap between the effects of prolonged submergence time on the plant anatomy, which could be crucial for further studies on the ecology and conservation of mangrove species. This present study aims to examine the characteristics of vessel cells in response to submergence to gain better understanding of the effect of sea level rise on mangrove seedlings in relation to water conducting cells.

The objectives of the study is to determine vessel characteristics under contrasting flooding treatments (6 hrs and 24 hrs stagnant treatment) in the plant apex and plant mid-stem of mangrove seedlings *Bruguiera gymnorrhiza* and *Rhizophora apiculata*. This study address the following hypothesis:

i) Conduit diameter may increase under 24 hrs (stagnant) flooding treatment to allow water flowing efficiently in excessive flooding,

ii) Conduit diameter increases with increasing distance from the terminal shoot.

## 3.3 Method and materials

### 3.3.1 Plant Material

Seven-month-old plants of *Brugueira gymnorrhiza* and *Rhizophora apiculata* were maintained in 96 pots filled with peaty soils in a tropical glasshouse under natural sunlight and photoperiod. The pots were placed on the bench within three experimental blocks. The arrangement of the seedlings followed a randomized block design. The temperature in the glasshouse was maintained between $23^0$C to $25^0$C. Salinity of the water was checked using a refractometer every week and adjusted to reach similar range of the salinity, 17ppt, throughout the experiment. The solutions were adjusted manually using Aquarium salt (Instant Ocean, Ohio USA). To avoid evaporation, tap water was added to the plants once every two days. In October 2015, the flooding experiment was started and all the plants were inundated to different flooding durations. There were four types of flooding treatments; six hours flooding (as is normal in mangroves forest), 18 hours flooding mimicking moderate flooding, 24 hours treatment with draining simulating future sea level rise and 24 hours (stagnant) flooding treatments representing extreme flooding stress to the mangrove plants.

### 3.3.2 Anatomical Observation

Vessel characteristics under contrasting flooding treatment were observed using both light microscope and scanning electron microscope. The stem disc segments were obtained from similar mangroves seedling for observation under both microscopes. Vessel hydraulic diameter, vessel density and vessel lumen area were observed under the light microscope whilst vessel diameter and vessel cell wall were examined using

a scanning electron microscope. The different observations were carried out using both microscopes because vessel cell wall is relatively difficult to observe and measure under the light microscope and only can be elucidated clearly under the scanning electron microscope. Therefore four seedlings of each species and treatment (that is four seedlings from 6 hrs and 24 hrs stagnant treatment) have been observed under light and scanning electron microscope for *B. gymnorhhiza* and *R. apiculata* seedlings respectively)

3.3.3 Using the light microscope

In December 2015, stem discs of seedlings were cut into 3 cm disc sections and preserved with 50% ethanol. Each of the individual plants was cut every 3 cm from the plant apex down to the roots. There are about 15-20 segments altogether for individual plants. For each species, approximately 6-8 segments were used in the xylem conduit diameter measurement. A transverse section was cut using rotary microtome (Leica RM2245; Leica Biosystems, Nussloch, Germany) set to cut at 10-15 μm thick. Sections were stained with a solution of safranine and AstraBlue (1 and 0.5 % in distilled water) and slides permanently fixed with Eukitt (BIOptica, Milan, Italy). Slides were observed under light microscope (Nikon Eclipse80i; Nikon Tokyo, Japan) at 40 x magnification and a digital image were acquires via connected camera. The image were observed using Roxas software (von Arx and Dietz 2005) for semi-automatic estimation of anatomical traits.

i) Weighted hydraulic diameter:

$D_h = \sum D^5 / \sum D^4$ D=diameter

ii) Lumen area:

= Vessel density x $(\pi/4)$*(vessel diameter)$^2$

3.3.4 Using scanning electron microscope

In order to observe the sample for use under the scanning electron microscope, the samples were rinsed with 100% alcohol and allowed 24 hours for drying. Stem segments of individual plants were immersed in liquid nitrogen for approximately 3 minutes to make sure the plant xylem structure was well maintained. At the final stage, the samples were cut manually in transverse sections using a razor blade and were attached to the sticky pads of the planchette for scanning electron microscope observation. The quantification of xylem cells were made using image photography under the Scanning Electron Microscope (Carl Zeiss SIGMA, Germany).

Water transport of the plants was quantified using two seedlings segments of 3 cm and 12-15 cm from shoot tip of plant apex and plant mid stem respectively. Of these segments, the xylem cells were measure for the vessel diameter and vessel cell wall thickness. Within the xylem area, photographs were made of the vessel which was located very close to the cambium (outermost stem part) consisting of the youngest xylem, which are likely to be related to environmental conditions according to Madrid et al. (2014), with new vessels developed as a response to the flooding treatment. High magnification was used to get a suitable image for vessel diameter (10- 20 μm) and

vessel wall thickness measurement (3-10 µm); lower magnification was used for the overall sample image (approximate 100-200 µm).

### 3.3.5 Statistical analysis

The manipulation experiment consisted of three factors, two species (*Bruguiera gymnorrhiza* and *Rhizophora apiculata*), two flooding treatments (6 hrs and 24 hrs stagnant) and two plant sections (plant apex and plant mid stem). Data on vessel diameter, vessel density, cell wall thickness and lumen area were analysed with an analysis of variance using the general linear model (GLM) procedure of Minitab 17.0 statistical software. As fixed effects, we entered flooding treatment, species and plant sections (nested with plant number) into the model, while the random effects was the plant number. Visual inspection of residual plots revealed weighted hydraulic diameter and vessel lumen area to be non-normally distributed. Therefore, both response variables were logged and square root transformed to meet ANOVA assumptions. Tukey Post-Hoc test were performed to find out differences between treatment, species and plant sections. The graphical results were carried out using Excel (Microsoft Inc.).

## 3.4 Result

Xylem anatomical characteristic under flooding treatment can be found in Table 3.1. Variability in vessel features i.e. vessel diameter, vessel density, vessel cell wall thickness showed a considerable effect in both *Bruguiera gymnorrhiza* and *Rhizophora apiculata* seedlings.

Table 3.1 Xylem anatomy characteristic of *Bruguiera gymnorrhiza* and *Rhizophora apiculata* under contrasting flooding treatments. (Mean ± SE) n=4

| Plant section | Plant apex | | Plant mid stem | |
|---|---|---|---|---|
| Treatment | 6 hrs | 24 hrs (stagnant) | 6 hrs | 24 hrs (stagnant) |
| *Bruguiera gymnorrhiza* | | | | |
| Vessel diameter (µm) | 21.82 ± 3.97 | 25.0 ± 3.77 | 32.19 ± 5.32 | 33.38 ± 4.76 |
| Vessel cell wall thickness (µm) | 1.69 ± 0.33 | 1.43 ± 0.29 | 1.59 ± 0.3 | 1.60 ± 0.37 |
| Vessel density ($mm^2$) | 245.76 ± 20.83 | 376.77 ± 65.27 | 99.21 ± 12.83 | 101.35 ± 10.24 |
| Hydraulic diameter (Dh) | 26.37 ± 0.27 | 20.46 ± 2.7 | 34.67 ± 0.44 | 35.24 ± 2.52 |
| Vessel fraction lumen area (mm x $MM^{-2}$) | 1.9 x $10^5$ ± 0.43 | 2.73 x $10^5$ ± 0.46 | 0.67 x $10^5$ ± 0.21 | 0.52 x $10^5$ ± 0.18 |
| Conduit wall reinforcement $(t/b/)^2$ | 0.68 ± 0.17 | 0.61 ± 0.42 | 0.26 ± 0.04 | 0.25 ± 0.1 |
| Potential specific hydraulic conductivity (Ks) | 4.88 ± 1.2 | 3.71 ± 1.1 | 4.80 ± 1.19 | 3.11 ± 1.50 |
| *Rhizophora apiculata* | | | | |
| Vessel diameter (µm) | 23.26 ± 2.86 | 19.03 ± 3.12 | 33.77 ± 5.48 | 34.10 ± 5.63 |
| Vessel cell wall thickness (µm) | 2.06 ± 0.45 | 1.8 ± 0.33 | 2.02 ± 0.36 | 1.7 ± 0.53 |
| Vessel density ($mm^2$) | 567.78 ± 196.03 | 722.64 ± 362.69 | 99.40 ± 8.47 | 160.62 ± 17.36 |
| Hydraulic diameter, Dh (µm) | 19.96 ± 2.08 | 20.80 ± 3.35 | 33 ± 0.42 | 32.70 ± 0.88 |
| Vessel fraction lumen area (mm x $MM^{-2}$) | 3.7 x $10^5$ ± 1.46 | 6.38 x $10^5$ ± 4.46 | 0.53 x $10^5$ ± 0.16 | 1.13 x $10^5$ ± 0.32 |
| Conduit wall reinforcement $(t/b/)^2$ | 0.87 ± 0.27 | 0.99 ± 0.24 | 0.51 ± 0.24 | 0.23 ± 0.04 |
| Potential specific hydraulic conductivity (Ks) | 4.0 ± 1.77 | 3.1 ± 1.27 | 5.4 ± 2.7 | 4.6 ± 1.59 |

3.4.1 Vessel diameter

Vessel diameter was not affected by the flooding treatments ($p > 0.898$) for either *B. gymnorrhiza* or *R. apiculata* seedlings (Fig 3.1; Fig 3.2.). Although not significant, *R. apiculata* seedlings showed lowest vessel diameter at the apex (Table 4.1) whilst there was no difference in vessel diameter between species and treatments for plant mid stem. However, vessel diameter exhibited significant differences between plant sections ($p < 0.001$) (Table 3.2).

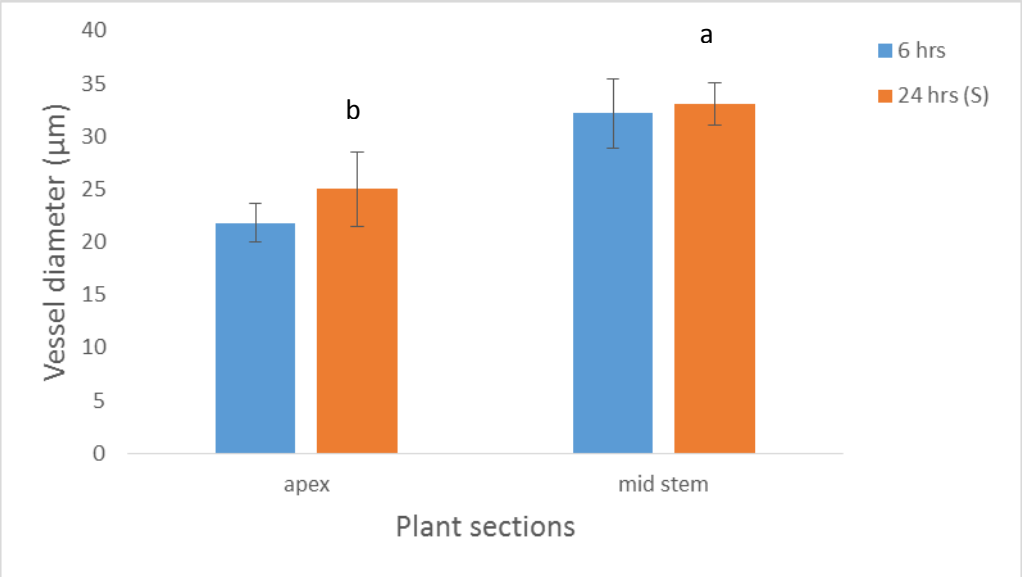



Figure 3.1. Vessel diameter of *Bruguiera gymnorrhiza* seedlings between plant apex and mid stem of 6 hrs and 24 hrs (stagnant) treatment. The bars are the mean (+ SE) n=4

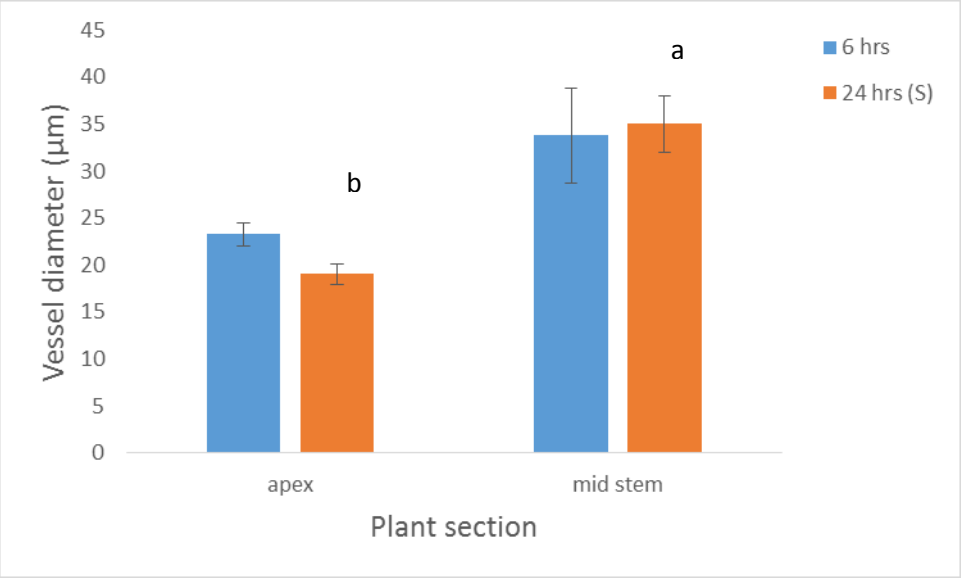



Figure 3.2 Vessel diameter of *Rhizophora apiculata* seedlings of plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are the mean (+ SE) n=4

### 3.4.2 Vessel size

Vessel diameter class was calculated for each species and flooding treatment. Overall, *B. gymnorrhiza* under 6 hrs and 24 hrs (stagnant) flooding treatment mainly consisted of 20-40 µm diameters (Fig 3.3 & Fig 3.4), whilst *R. apiculata* seedlings has produced wider vessels than *B. gymnorrhiza* seedlings (Fig 3.5). Under 6 hrs flooding treatment, the vessel diameters of *R. apiculata* seedlings were mostly under 20-30 µm, but under 24 hrs (stagnant) of flooding treatment the vessel diameter was narrower than the 6 hrs flooding treatment, with the majority of vessel diameters between 10-20 µm (Fig 3.6).

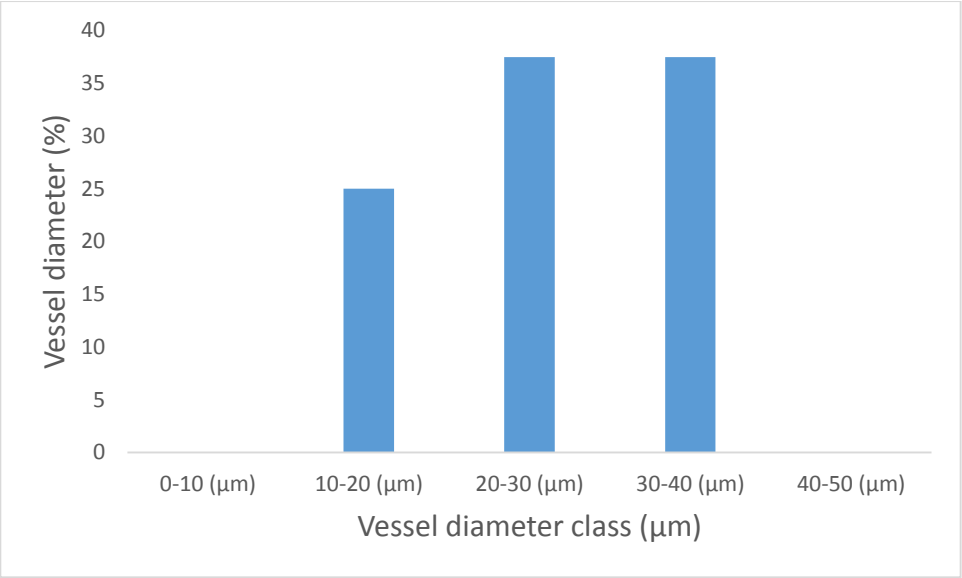



Fig 3.3 Percentage of vessel size according to 10 µm diameter classes of *B. gymnorrhiza* seedlings of 6 hrs flooding treatment.

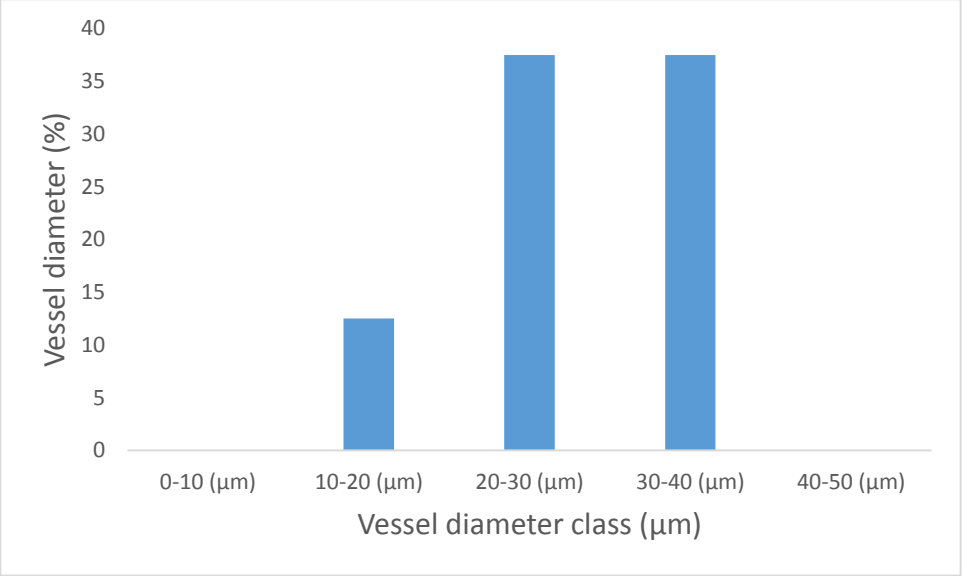



Fig 3.4 Percentage of vessel size according to 10 µm diameter classes of *B. gymnorrhiza* seedlings of 24 hrs (stagnant) flooding treatment.

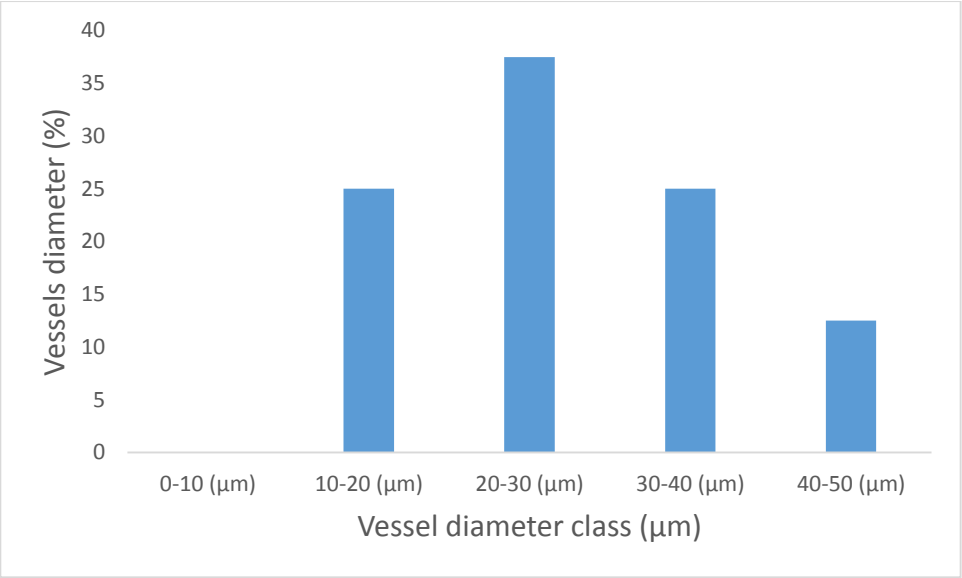



Fig 3.5 Percentage of vessel size according to 10 µm diameter classes of *R. apiculata* seedlings of 6 hrs flooding treatment.

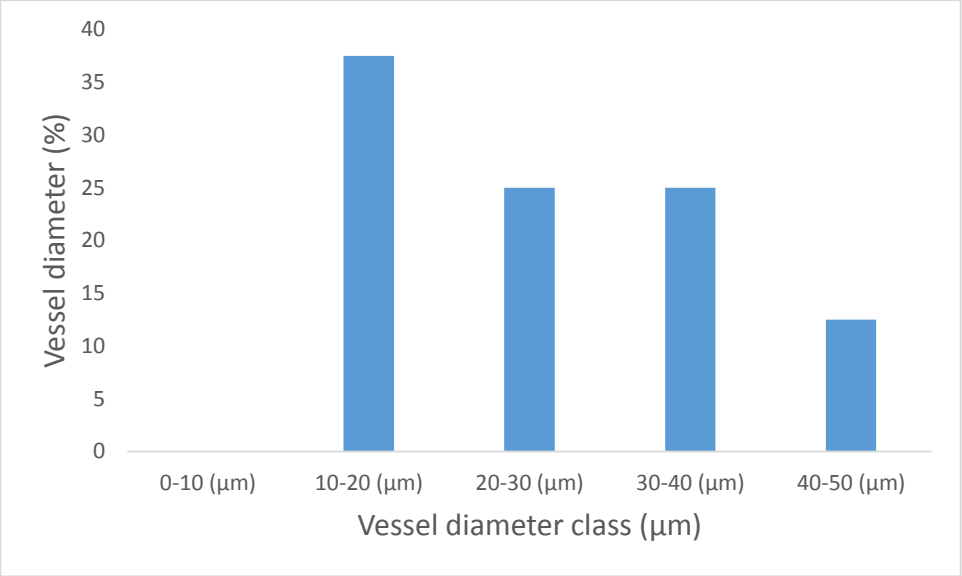



Fig 3.6 Percentage of vessel size according to 10 µm diameter classes of *R. apiculata* seedlings of 24 hrs (stagnant) flooding treatment.

### 3.4.3 Vessel cell wall thickness

Vessel cell wall thickness varied significantly between species ($p < 0.021$) (Table 4.1). *Rhizophora apiculata* seedlings had higher cell wall thickness compared to *Bruguiera gymnorrhiza* seedlings (Fig 3.7; Fig 3.8). It was shown that vessel cell wall thickness was almost significantly different between flooding treatments with thinner cell walls in the 24 hrs (stagnant) treatment than in the 6 hrs treatments ($p > 0.070$). However, cell wall thickness did not vary between plant sections for both species ($p > 0.745$) (Table 4.2).

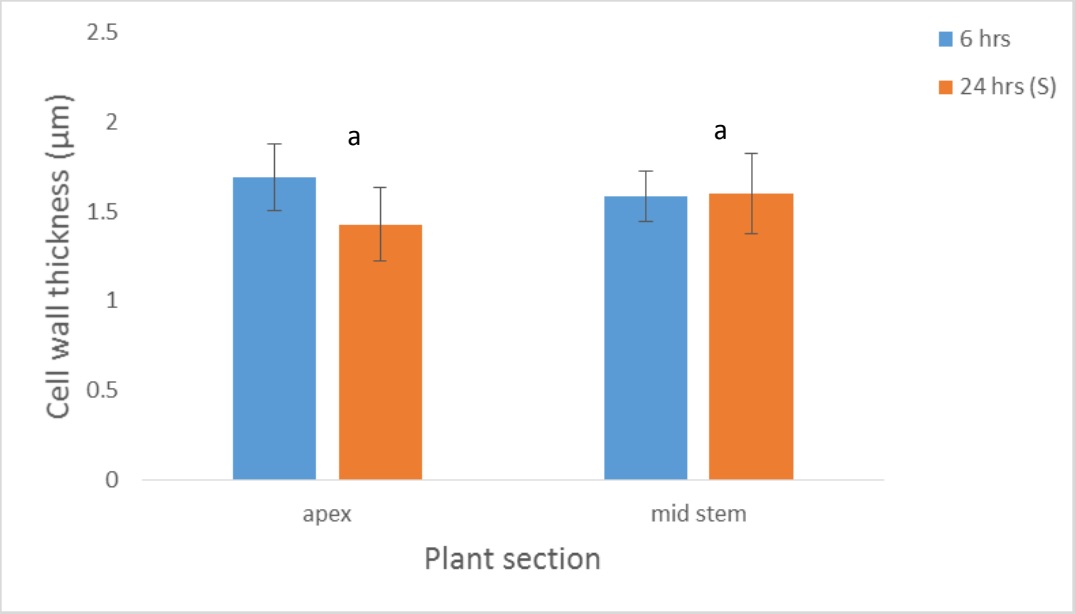



Figure 3.7 Vessel cell wall thickness between plant section of *Bruguiera gymnorrhiza* seedlings in plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are the mean (+ SE) n=4

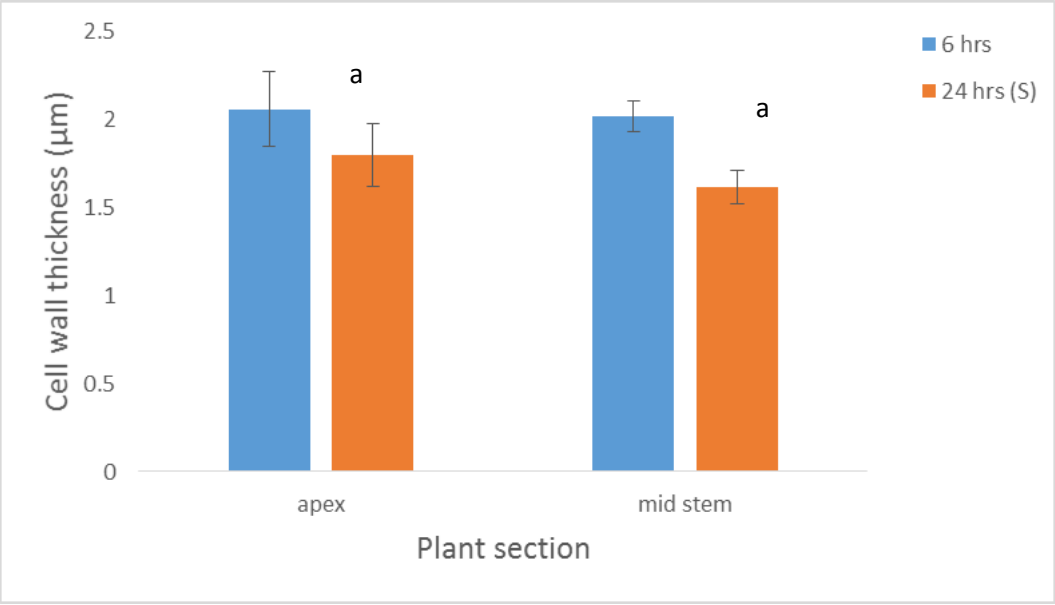



Figure 3.8 Vessel cell wall thickness between plant section of *Rhizophora apiculata* seedlings in plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are the mean (+ SE) n=4

### 3.4.4 Vessel density

There was a significance difference in vessel density between plant sections ($p < 0.001$) (Table 3.2). Vessel number was significantly higher at the plant apex, almost five and three times higher than plant mid stem in both species (Fig 3.9; Fig 3.10). *Rhizophora apiculata* seedlings exhibited higher vessel density than *Bruguiera gymnorrhiza* seedlings but almost significant ($p < 0.051$). However, vessel density did not vary between flooding treatment for both species ($p > 0.196$) although slightly higher in 24 hrs (stagnant) treatment particularly in both plant apex for both species.

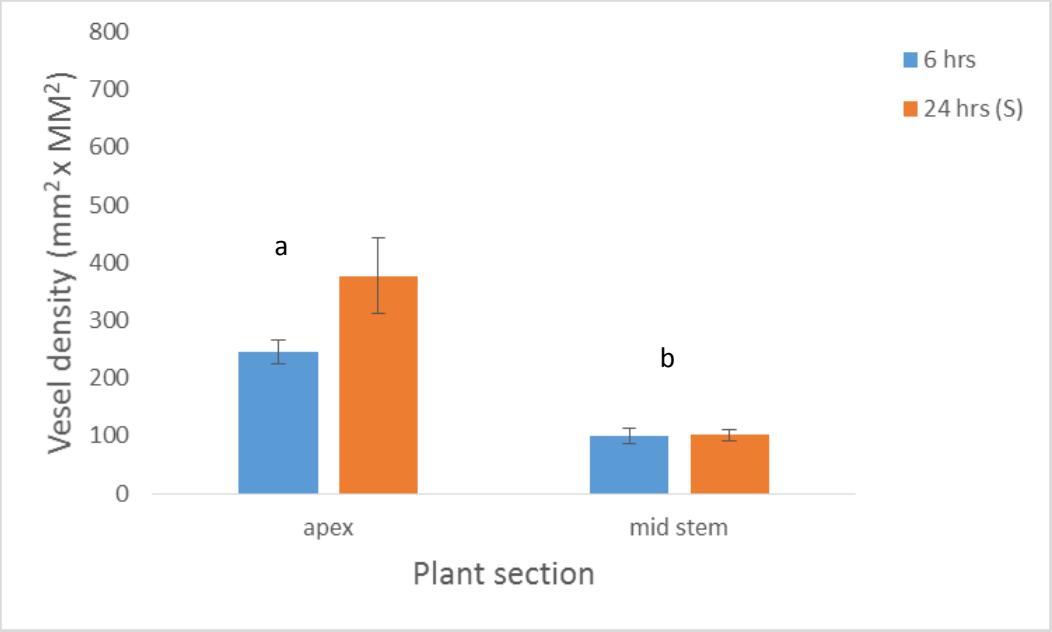



Figure 3.9 Vesel density of *Bruguiera gymnorrhiza* at the plant apex and mid stem between species of 6 hrs and 24 hrs (stagnant) treatment. The bars are (Mean + SE) n=4

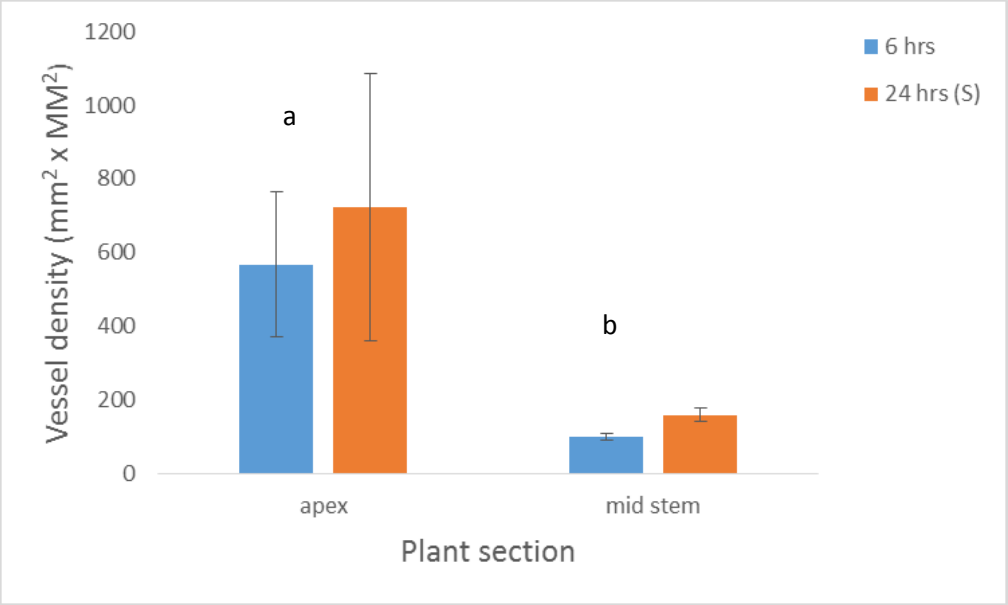



Figure 3.10 Vessel density of *Rhizophora apiculata* at the plant apex and mid stem between species of 6 hrs and 24 hrs (stagnant) treatment. The bars are (Mean + SE) n=4

### 3.4.5 Hydraulic diameter ($D_h$)

Vessel hydraulic diameter was significantly different between plant sections ($p < 0.001$) (Table 4.2). There was a strong relationship of $D_H$ along the plant profile for all cases indicating an increasing in $D_H$ from the plant shoot to the plant root (Fig 3.11). Hydraulic diameter was significantly bigger at the mid stem compared to the plant apex for both species (Fig 3.12; Fig 3.13). *Bruguiera gymnorrhiza* seedlings showed slightly larger hydraulic diameter than *Rhizophora apiculata* seedlings although not significant ($p > 0.143$). However, hydraulic diameter not vary between flooding treatments for both species ($p > 0.822$).

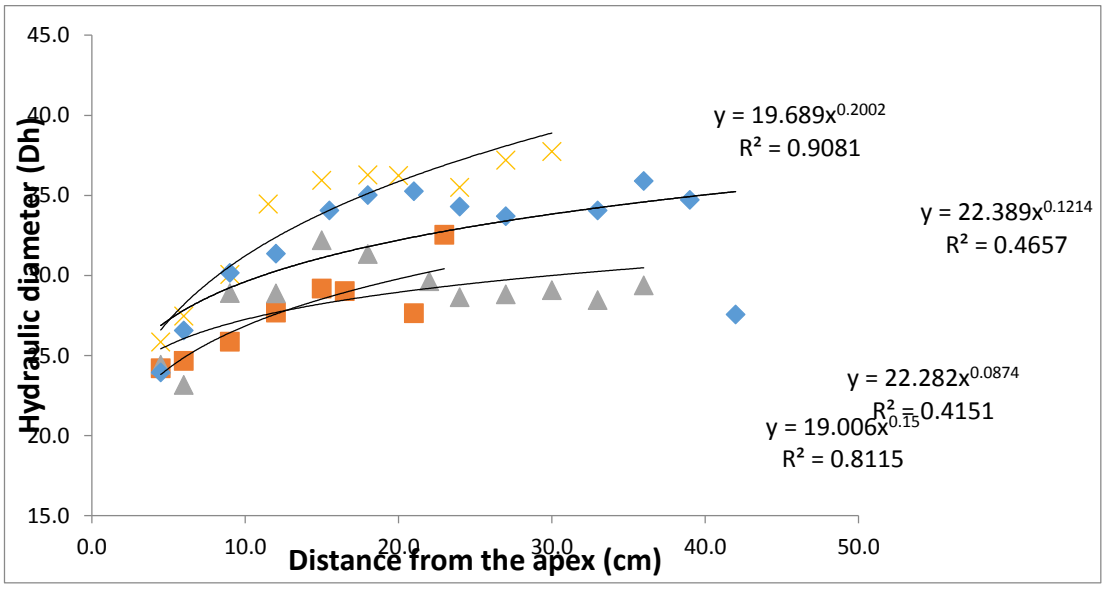



Fig 3.11 Hydraulic diameter along the plant profile

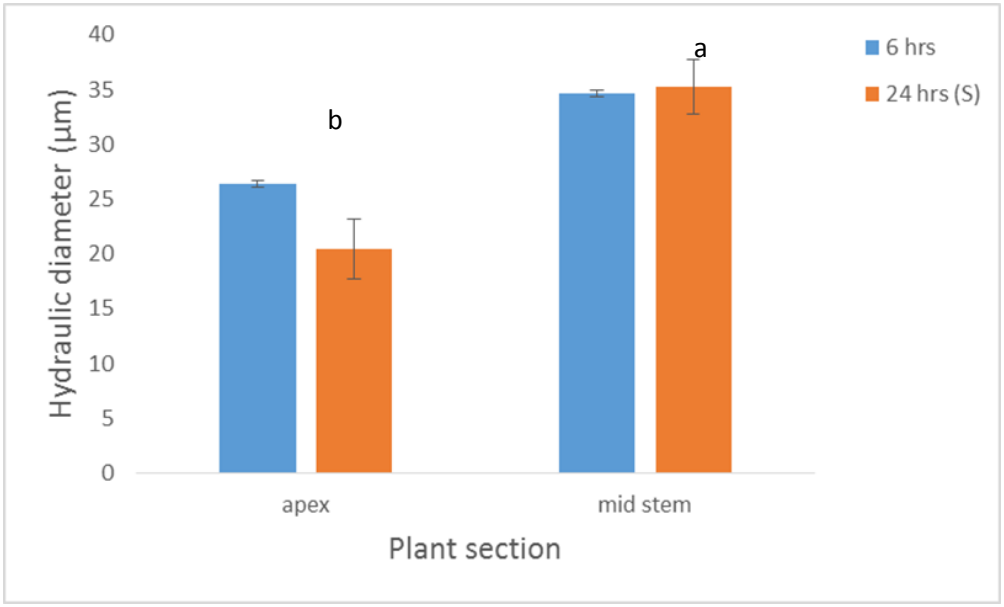



Figure 3.12 Hydraulic diameter of *Bruguiera gymnorrhiza* seedlings at the plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are (Mean + SE) n=4

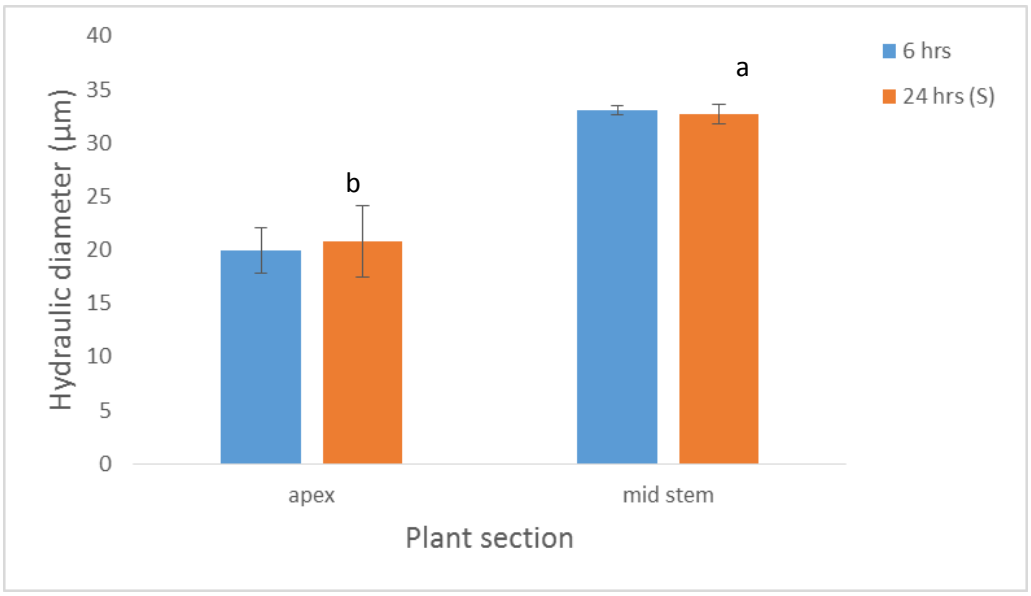



Figure 3.13 Hydraulic diameter of *Rhizophora apiculata* seedlings at the plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are (Mean + SE) n=4

### 3.4.6 Lumen area

There were significant differences in lumen area between species (Table 3.2). *B. gymnorrhiza* seedlings were significantly higher than *R. apiculata* seedlings ($p < 0.001$) (Fig 3.14; Fig 3.15). Between plant sections, the lumen area of the plant mid stem was significantly higher than that of the plant apex ($p < 0.003$). However lumen area was not affected by the flooding treatment ($p > 0.182$).

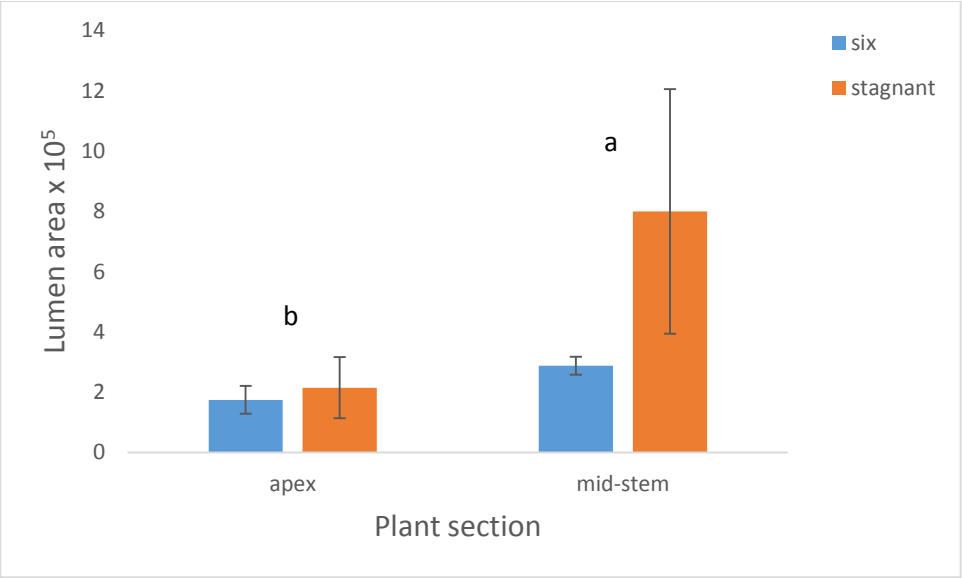



Figure 3.14 Lumen area of *B. gymnorrhiza* seedlings at the plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are (Mean + SE) n=4

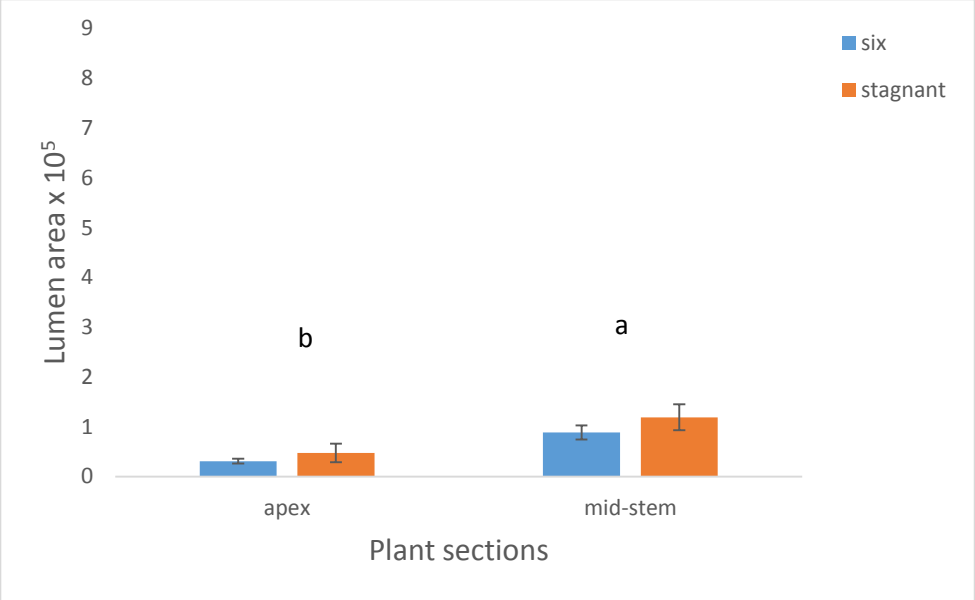



Figure 3.15 Lumen area of *R. apiculata* seedlings at the plant apex and mid stem between 6 hrs and 24 hrs (stagnant) treatment. The bars are (Mean + SE) n=4

### 3.4.7 Conduit wall reinforcement (t/b)

Flooding treatment did not affected the conduit wall thickness ($p > 0.269$). However, plant apex was significantly higher than plant mid stem for the conduit wall thickness ($p < 0.01$) (Table 3.2). Between species, wall thickness of *R. apiculata* was slightly higher than *B. gymnorrhiza* but not significant ($p > 0.107$) (Fig 3.16; Fig 3.17)

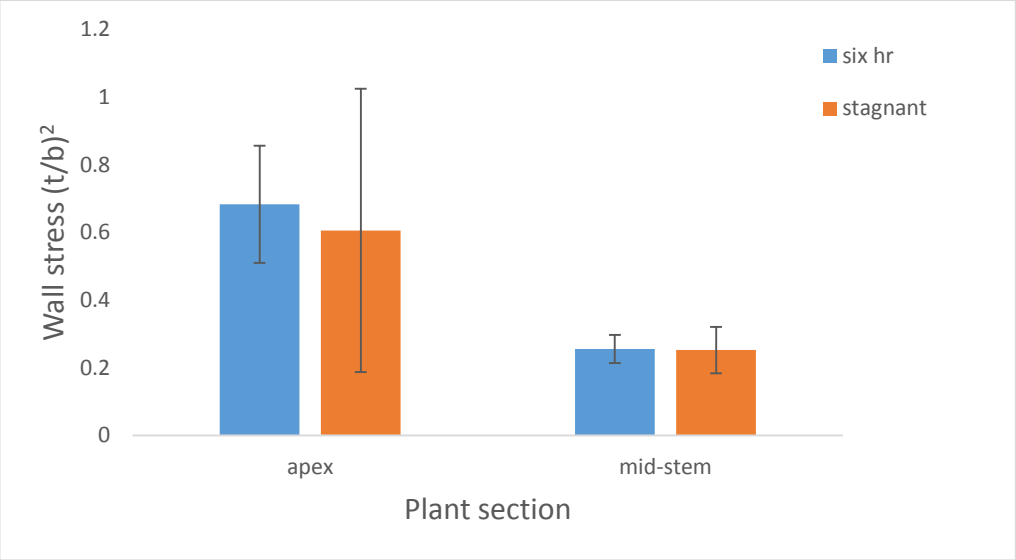



Figure 3.16 Wall thickness in *B. gymnorrhiza* seedlings

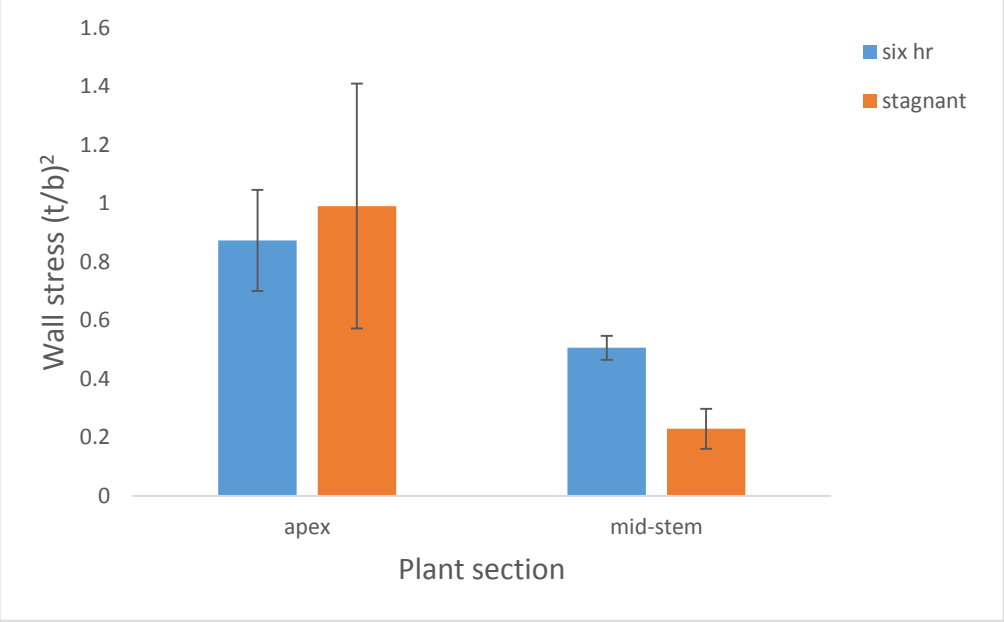



Figure 3.17 Wall thickness in *R. apiculata* seedling

3.4.8 Potential specific hydraulic conductivity (Ks)

There was a significance difference between plant sections in potential specific hydraulic conductivity. Both species showed Ks to be higher in the mid stem than the apex (Fig 3.18; Fig 3.19). However Ks was not affected by the flooding treatment. *B. gymnorrhiza* exhibited slightly higher Ks than *R. apiculata*, approaching statistical significance ($p > 0.051$).

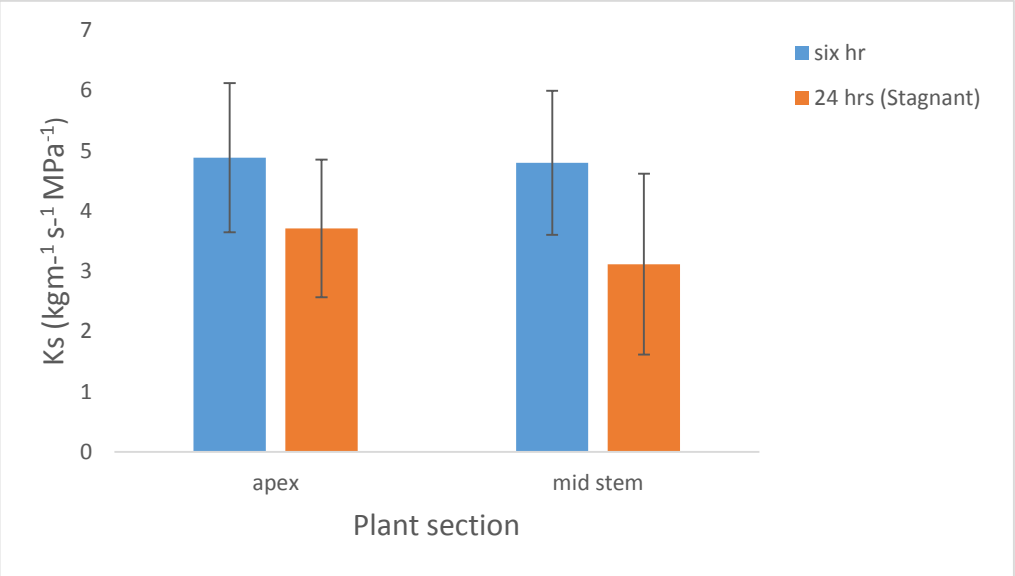



Figure 3.18 Potential hydraulic conductivity $K_s$ in *B. gymnorrhiza*

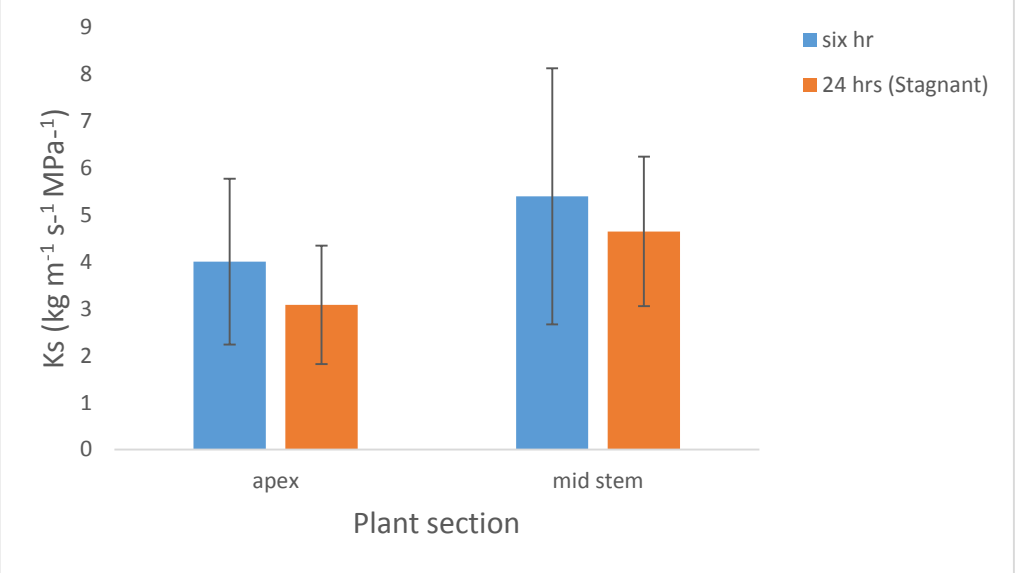



Figure 3.19 Potential hydraulic conductivity $K_s$ in *R. apiculata*

Table 3.2. Summary results of three way ANOVA of response variables for both mangrove seedlings *B. gymnorrhiza* and *R. apiculata* under contrasting flooding treatments. Statistical analysis using General linear model, repeated measurement of ANOVA on the effects of different flooding treatments (6 hrs and 24 hrs stagnant treatment) on plant segments (plant apex and plant mid stem).

| Source of variations | Vessel diameter (μm) | | Cell wall Thickness (μm) | | Vessel density | | Hydraulic diameter (μm) | | Lumen area mm x $10^5$ | | Conduit wall thickness $(t/b)^2$ | | Potential specific hydraulic conductivity, Ks | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| value | F | P | F | P | F | P | F | P | F | P | F | P | F | P |
| Species | 0.10 | 0.760 | 6.02 | 0.021 | 4.37 | 0.047 | 2.28 | 0.143 | 0.44 | 0.515 | 2.79 | 0.107 | 4.17 | 0.052 |
| Plant sections | 27.91 | 0.000 | 0.11 | 0.745 | 60.14 | 0.000 | 47.62 | 0.000 | 18.28 | 0.000 | 11.58 | 0.02 | 80.38 | 0.000 |
| Treatments | 0.00 | 0.968 | 3.55 | 0.071 | 2.13 | 0.157 | 1.02 | 0.322 | 0.21 | 0.649 | 1.28 | 0.269 | 0.08 | 0.777 |
| Species*plant sections | 0.64 | 0.430 | 0.34 | 0.564 | 0.20 | 0.661 | 0.41 | 0.527 | 0.00 | 0.957 | 0.67 | 0.422 | 0.67 | 0.422 |
| Species*treatment | 0.85 | 0.364 | 0.70 | 0.409 | 0.03 | 0.857 | 1.11 | 0.302 | 0.16 | 0.697 | 0.06 | 0.816 | 0.29 | 0.597 |
| Plant sections*treatment | 0.40 | 0.534 | 0.08 | 0.778 | 0.09 | 0.763 | 0.99 | 0.328 | 0.04 | 0.841 | 0.06 | 0.812 | 1.35 | 0.256 |

## 3.5 Discussion

### 3.5.1 Effect of flooding treatment on vessel characteristics

In this study, there was no significance difference between flooding treatments on the vessel characteristics after 6 hrs and 24 hrs (stagnant) of flooding. This finding suggesting plant hydraulics were not affected by the flooding treatment. Theoretically, it was hypothesized that submergence causes oxygen deficiency to the plant. To some extent, the experiment in the glasshouse was flawed due to the artefact of the presence of photosynthetic algae and bacteria in the 24 hours (stagnant) treatment. Therefore, the plants under 24 hrs (stagnant) treatment were induced by high dissolved oxygen as compared to other treatment, which may provide a reason the lack of an effect between flooding treatment. However, there was an effect between plant sections and species in response to the flooding treatment.

### 3.5.2 Response flooding to the plant sections

Measurements under the scanning electron microscopy and light microscope have shown that vessel diameter and weighted hydraulic diameter ($D_h$) varied between plant sections but did not differ between flooding treatment and species. Vessel diameter and weighted hydraulic diameter was significantly larger at mid stem. These results reflecting their vessel size along the plant structure as wider vessel diameter was found at plant root than the shoots. Similarly, McElrone et al. (2004) found wider vessel diameter within deeper plant roots than the plant stem for temperate tree species.

This present study demonstrated a rather small vessel diameter, ranging from 22 to 25 μm and 32 to 34 μm for the plants apex and mid stem respectively, for both *B. gymnorrhiza* and *R. apiculata* seedlings. Yanez-Espinosa et al. (2001) have reported the vessel size for the adult mangrove species in Mexico under flooding was within the range 73μm to 93μm, rather small for adult trees. Xiao et al. (2009) also reported decreased tangential diameter for the vessels under long submergence. Terrestrial plants usually have wider vessels, approximately 150-200 μm diameter, but aquatic plant are comprised of small diameter, less than 100 μm (Tomlinson 2016).

Very little information on the effect of the flooding in mangrove is available in the literature, the only work by Panshin (1932) and Jansonnius (1950) has demonstrated evidence that mangrove vessels consist of narrow and high density vessels in response to flooding. Almost no study in mangrove species has elucidated the effect of anoxia due to flooding to the mangrove vessel. Since this study has deviated from the oxygen deficiency hypothesis due to the existence of the artefact in the experiment, future work should address this issue of oxygen deficiency and its effect on the mangrove vessels.

In mangrove forest, a few studies have examined vessel characteristics under a salinity influence. The vessels are narrow and densely distributed (Schmitz et al. 2006, Robert et al. 2009; Verheyden et al. 2005; Sobrado 2007). The high density and narrow vessels can be related to the high tension created by saline water, which mangrove plants must overcome (Reef and Lovelock 2015). The narrow and high density implies a safety mechanism to the mangrove plants under salinity conditions to avoid cavitation in the vessels under saline soil. This is because frequent high tensions in the xylem can increases the chance for cavitation, but the formation of numerous vessel may provide

safety mechanisms with alternative pathways allowing water conductivity under salinity (Tomlinson 2016) and flooding. In response to the contrasting temperature, narrow vessel diameter occurs at lowest temperatures, an adaption likely to avoid embolism due to freezing (Madrid et al. 2014). In the high sedimentation, vessels are wider as an adjustment under hypoxia since high sedimentation may block the water flow (Okello et al. 2017). In comparing mangrove species with terrestrial relatives, Joelle (2010) has found that the vessel diameter of terrestrial species are wider than those in mangroves species.

Between species, vessel diameter in *Rhizophora apiculata* seedlings was slightly wider than *Bruguiera gymnorrhiza*. This trait may reflecting the position in their natural environment where *Rhizophora apiculata* is more seaward species than *Bruguiera gymnorrhiza* according to their zonation in the Malaysia mangrove forest, suggesting plants under frequent flooding may possess bigger vessel to conduct water efficiently.

Vessel wall thickness varied between species with more thickening in *Rhizophora apiculata* seedlings than in *Bruguiera gymnorrhiza* seedlings. In a study to compare *Rhizophora* genera between mangrove and inland species, mangrove species have thicker cell walls than inland species reflecting the role of cell wall in mechanical strength, particularly under flooding (Barnett and Bonham 2004; Beck 2010). Similarly, vessel cell walls were thicker under long submergence (Xiao et al. 2010) suggesting this trait is an important character to provide mechanical strength to the plant in response to long-term inundation (Carlquist 2002). However, in this study the stagnant treatment has reduced cell wall thickness under 24 hrs (stagnant) treatment possibly because the prolong submergence decreased in the mechanical strength.

Similar finding was also reported in Yamamoto et al. (1995) for of *Fraxinus mandshurica* seedlings under root submergence.

In this study, vessel density was significantly higher in *R. apiculata* seedlings in the plant apex. This finding may imply the highly requirement on water conduction in the terminal shoots (Petit et al. 2016). A study on the effect of salinity on mangrove species also demonstrated that *Rhizophora mucronata* seedlings consist of narrow and higher vessel density reflecting their adaptation toward cavitation under saline conditions (Verheyden et al. 2005, Robert et al. 2009). Both findings exhibited a higher vessel density within the same genus; possibly explaining their adaptation toward prolonged flooding since in the Malaysian mangrove forest, most of *Rhizophora* genera suffer frequent inundation. Numerous of vessels are in contact with each other provide an alternative routes for water to flow (Baas 1983; Zimmermann 1983) from roots to the shoots.

Vessel lumen area was significantly higher at the plant mid stem and was observed to increase under stagnant treatment but this was not statistically significant. Wider lumen area might have an association with hydraulic conductivity. This agrees with Schuldt et al. (2013) where the increment of vessel size may increases the hydraulic conductivity. Therefore the combination of larger lumen area and high potential hydraulic conductivity may increase the water flow within the stem.

Overall the plant apex exhibited evidence of a stress-response, with a high conduit wall thickness at the plant apex. This is because the greater wall reinforcement at the plant shoots implies the highest water stress within this structure. The plants may increase

their wall strength to prevent conduit collapse (Venturas et al. 2017) under long submergence.

### 3.5.3 Response to flooding treatment in *B. gymnorrhiza*

As an obvious anatomical adjustment under flooding, seedlings of *B. gymnorrhiza* exhibited wider vessel diameter and hydraulic diameter at plant mid stem than at the plant apex. Similarly, this result concurs with Sobrado (2007) as bigger vessel diameter at the base of the plant rather than at the plant shoot implies the vessel conducting water increased in size from the plant apex. The advantages of wider vessel diameter might be the increased the water conducting efficiency (Sobrado 2007), benefiting *B. gymnorrhiza* particularly under 24 hrs (stagnant) treatment.

### 3.5.4 Response to flooding treatment in *R. apiculata*

Under flooding treatment, *R. apiculata* apparently invested in higher vessel density, more thickening of the cell wall and higher vessel lumen area than *B. gymnorrhiza*. This trait is important for this species while conducting water, particularly when exposed to 24 hrs (stagnant) treatment. The plants may suffer from long submergence and therefore this species adjusted the vessel characteristics to survive under extreme flooding treatment. In the mangrove forest, *R. apiculata* lives in the soft muddy soil and is exposed to frequent inundation. So that the high investment in wider lumen area, high vessel density and less wall thickening may be characteristics of plants under frequent submergence to allow the high water flow efficiently under oxygen shortage.

### 3.5.5 Vessel adjustment in both flooding treatment

Both species showed increases in all vessel characteristics (except vessel cell wall) under stagnant treatment than under the 6 hrs treatment. This situation raises the question of whether the increased in vessel characteristics is due to the enhancement of dissolved oxygen which was evidently contributed by photosynthetic bacteria under the 24 hrs (stagnant) treatment. Further study is needed to clarify this. The overall analysis of xylem anatomy has shown the effect between plant section and species rather than the effect of flooding treatment, given flooding is not a limitation to the water availability in plants.

## 3.6 Conclusion

Vessels characteristic of the two mangrove species are not affected by the contrasting flooding treatments, but differences do occur between plants sections. Variation of xylem vessels also were observed between *B. gymnorrhiza* and *R. apiculata* seedlings. Vessel diameter, vessel hydraulic diameter and vessel lumen area were significantly higher in mid stem and were observed to be wider in *B. gymnorrhiza*. Vessel density was significantly higher at the plant apex and abundance of vessel numbers was higher in *R. apiculata* seedlings. More cell wall thickening was found in *R. apiculata* than in *B. gymnorrhiza* seedlings. Wall reinforcement was significantly higher at the plant apex and the potential hydraulic conductivity was higher at the plant mid stem. The presence of photosynthetic bacteria contributed to the artefact in the flooding experiment; it increased the dissolved oxygen under in the 24 hrs (stagnant) treatment.

Future work should be concerned with this issue, when designing flooding experiments under glasshouse manipulation.

# Chapter 5: Above and Belowground Productivity, Root Turnover and Standing Stocks of Mangroves in the Kelantan Delta, Malaysia.

## 4.1 Abstract

Mangroves often allocate a relatively large proportion of their total biomass production to their roots, and the belowground biomass of these forests contributes towards globally significant carbon sinks. However, little information is available on root production in mangroves due to the difficulties in carrying out measurements belowground, particularly during regular flooding. In this study, we examined fine and coarse root production in the east coast of the Malaysian Peninsula. Ingrowth cores were used over the course of 17 months. In September 2014, twenty cores were randomly placed in each of five plots. Three cores were collected from each plot (fifteen cores in total), once every three months. Each core was divided into five 10 cm layers and root dry mass was recorded. Standing root biomass was also measured at the time of final collection using an additional 15 cores. There was a seasonal pattern in root production, which peaked in March and December 2015, after and during the monsoon season. Root biomass in the cores peaked at 33.23 ± 6.3 t $ha^{-1}$ and 21.46 ± 7.3 t $ha^{-1}$ in March and December respectively. Standing root biomass in the forest was 20.81 ± 2.8 t $ha^{-1}$. After 17 months, the final root biomass in the cores was 14% less than the standing root biomass. This data suggests surprisingly rapid growth rates and turnover for mangrove roots. Total root biomass significantly increased with root depth and 78% of the roots, in all soil layers, consisted of fine roots (< 3 mm diameter). A significantly positive relationship was found between fine root biomass and

sediment carbon and nitrogen content. Soil temperature, salinity and dissolved oxygen were also investigated in relation to belowground production. The results are discussed with consideration to the significance of monsoon rainfall for mangrove ecology.

Keywords: Root stock, root production, allocation aboveground, allocation belowground, monsoon season, rapid root turnover.

## 4.2 Introduction

Mangroves are very productive ecosystems (Tomlinson 1986; Alongi 2012). As transitional habitats, positioned between the land and the sea, mangroves often receive substantial amounts of organic matter and nutrients from terrestrial and marine sources during tidal exchange and river discharge. Retention of this organic matter and associated nutrients helps promote high primary productivity in the mangrove forest (Kumara et al. 2010).

Mangroves are considered to be important organic carbon reservoirs in coastal ecosystems. Carbon is derived from the photosynthesis carried out by mangrove trees, benthic algal communities on the aboveground roots and forest floor (Alongi 2014) and from freshwater and oceanic inputs from adjacent systems (Jannerjahn and Ittekkot 2002). As one of the most carbon dense ecosystems, mangroves can store exceptionally large amounts of carbon, particularly in extensive belowground deposits (Donato et al. 2011; Gress et al. 2016).

Studies of mangrove productivity focus mainly on aboveground biomass using litter fall and stem diameter measurements (Gong and Ong 1990; Robertson and Alongi

1995; Sukarjo et al. 2013; Mitra et al. 2011). The litter fall data helps to quantify total productivity and illustrates the sources of organic matter available for secondary consumption (e.g. by crabs), burial or export to the sea. Studies of stem diameter provide information concerning biomass accumulation in the tree trunk. Allometric equations have been developed to help determine the allocation of biomass between plant components (Komiyama et al. 2005).

In recent years, research on belowground production and carbon storage have received growing attention given the significant role in climate change mitigation and sequestration into mangrove carbon sinks (Donato et al. 2011). Previous studies have demonstrated the contribution of mangrove roots and other organic material in below ground processes, for example soil formation. Mangroves have specialized root systems, including aerial roots, which allow respiration during submergence. These complex aboveground features can lower the tidal water velocities, thus allowing particle deposition in mangrove soil (Krauss et al. 2003; Kumara et al. 2010). Because of this and the rapid expansion of roots belowground, mangroves are able to build land vertically. For example, in Caribbean mangroves, refractory roots and other organic materials (i.e. benthic mat algae, leaf litter, and woody debris) are responsible for soil formation (McKee et al. 2007). The land building process is important to ensure mangroves keep pace with rising sea levels (McKee 2011). Surface elevation however can be inhibited or reversed by natural disturbances such as hurricanes and storms which can cause soil elevation loss (Cahoon et al. 2003; Barr et al. 2012; Cahoon 2006) and soil compaction under subsurface processes (McKee, 2011). Similarly, human disturbances may contribute to rapid surface elevation loss (Lang'at et al. 2014; Lovelock et al. 2015).

Mangroves have been shown to respond to soil nutrient enrichment. In depleted nutrient settings, mangroves may allocate 40-60% of their biomass to belowground production (Komiyama et al. 1987). This condition has been recognized as a strategy for plants to manage their resources efficiently under nutrient stress (Castaneda-Moya et al. 2011). Several studies have explored biomass allocation patterns in mangrove forests, particularly under nutrient deficiency. In Floridian mangroves, soil phosphorus is always limiting, which results in stunted mangrove forests. Riverine mangroves, growing in more productive sites, tend to allocate proportionately more biomass to aboveground whilst nutrient limited scrub communities show greatest biomass allocation to belowground (Castaneda-Moya et al. 2013). Mangroves of Micronesia also show greater root biomass associated with relatively low soil phosphorus (Cormier et al. 2000). This finding is contrary to a study of a karst lagoon in Mexico with high salinity, where greater root biomass and production was found with higher soil phosphorus (Adame et al. 2014). Under long tidal submergence and limited nutrients, high root biomass but lower root production and root turnover were recorded (Castaneda-Moya et al. 2011), perhaps because tidal submergence limits the root production.

A number of other environmental factors are likely to influence root production; tidal range, rainfall, salinity or soil temperature (Komiyama et al. 1987; Saintilan 1997; Paungparn et al. 2016). In general, root production is greater at locations with less frequent tidal inundation. For instance, in Thailand, greater root biomass was found in the *Rhizophora* zone, located further inland (Komiyama et al. 1987; Paungparn et al. 2016). Seasonality in mangrove root production was also observed where the highest productivity was recorded during the wet and early cool dry season (Paungparn et al.

2016). This suggests that root productivity is associated with increased rainfall and thus reduced salinity of porewater. Terrestrial forests have also shown similar findings, as seasonal root production in rubber trees correlates directly with rainfall (Maeght 2015). However, root production in Thai mangroves was inversely related to soil temperature, suggesting increasing soil temperature might promote root death and the accumulation of root necromass (Paungparn et al. 2016).

Biomass allocation varies between mangrove species and tree stand. Fast growing species such as Avicennia marina allocate proportionally more biomass belowground under optimum environmental conditions, while Rhizophora mucronata invests more aboveground (Lang'at 2013). In Gazi Bay, Kenya, the highest belowground biomass was recorded in replanted mangrove forests rather than natural stands. Sonneratia alba showed the highest root biomass in comparison to Avicennia marina and Rhizophora mucronata, perhaps due to its exposed position at the seaward fringe, where investment in roots is needed to anchor the trees against wave impacts (Tamooh et al. 2008). There may also be complementarity between different roots architectures; an experimental study at the same site demonstrated that mixed mangrove stands show greater proportional belowground productivity than monospecific ones (Lang'at et al. 2012).

As previously stated, there is growing interest in quantifying carbon storage in mangrove forests. Most studies show mangroves are efficient carbon sinks, with the largest carbon stock (more than 90%) consisting of organic carbon in the soil (Donato et al. 2011; Adame et al. 2015; Sanders et al. 2017). This finding is consistent across mangrove forest settings such as estuarine and oceanic mangroves of the Indo Pacific (Donato et al. 2011), different mangrove zonation (Kauffman et al. 2011), and natural or restored mangrove forests (Nam et al. 2016; Sahu et al. 2016). Despite the newly

discovered importance of belowground carbon storage in mangroves, and hence the belowground processes that control it, relatively little is known about belowground productivity in mangrove forests and how it relates to aboveground productivity. This study examines belowground productivity in a Malaysian forest and explores the influence of a range of environmental variables on root production. It also investigates the relationship between above and belowground growth rates.

## 4.3 Method and materials

### 4.3.1 Study site

This study was conducted on the Kelantan Delta (6012’ 46.8” N 1020 10’43.0” E), in the state of Kelantan, on the east coast of the Malaysian Peninsula (Fig. 4.1). This area consists of 17 small islands (Satyanarayana et al. 2010) with an estimated total deltaic area of 1200 ha (Shamsudin and Nasir 2005). This area experiences the monsoon from November to March which causes strong currents and brings flooding to adjacent settlements.

The annual rainfall in 2013, 2014 and 2015 was 2235 mm, 2999 mm and 2065 mm, respectively (Malaysian Meteorological Department, 2016); with the highest and lowest spring tides being 1.7 m and 1.4 m (Malaysian Hydrographic National Centre, 2018).

The Kelantan delta consists of distributaries channel fed by the Kelantan river flowing to the South China Sea. It receives run-off due to seasonal rainfall and offshore currents which contribute to the coastal morphology and hydrographical condition

(Mohd-Suffian et al. 2004). The forest is composed of five dominant species; *Avicennia alba*, *Bruguiera gymnorrhiza*, *Nypa fruticans*, *Rhizophora mucronata* and *Sonneratia caseolaris* (Satyanarayana et al. 2010).

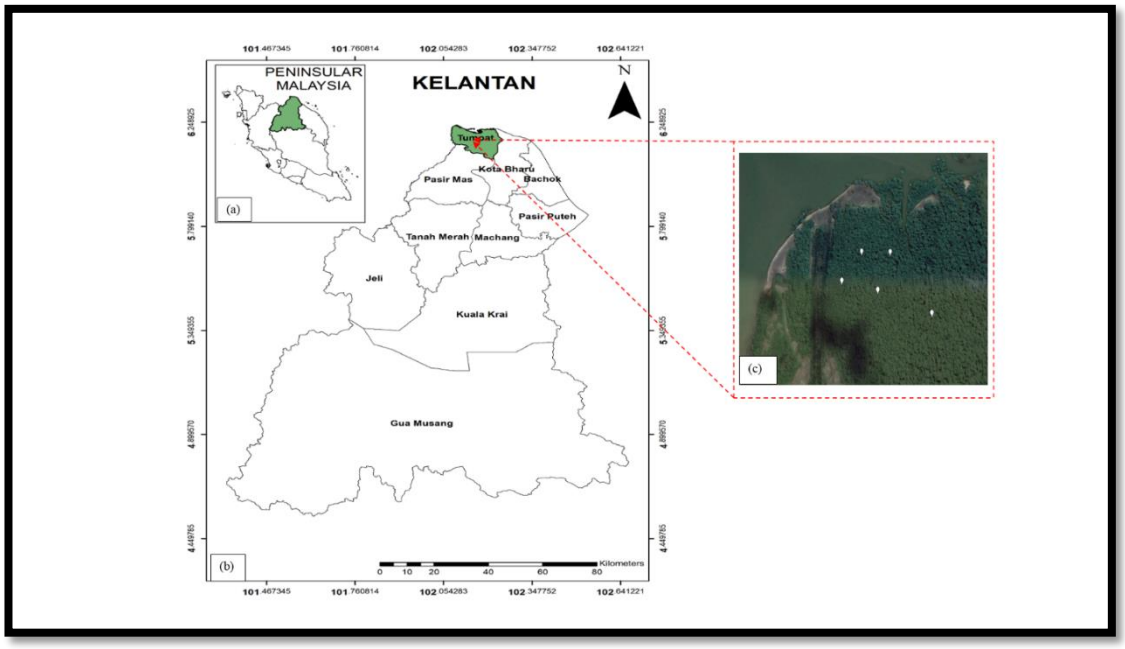



Figure 4.1. The location of the study site in (a) the Malaysian Peninsula (b) the district of study site at Kelantan Delta, and (c) The *Avicennia alba* stand in the mangrove forest, the locations of the five study plots are shown by white circles.

### 4.3.2 Sampling plots

The experiment was set up in a natural stand of *Avicennia alba* in the Kelantan Delta in September 2014. Five plots of 10 m x 10 m (0.05 ha in total) were set up in the mangrove forest (Fig. 4.1c). The plots were chosen randomly to be representative of the area of *A. alba* in the stand. All experimental plots were inundated at high tide.

### 4.3.3 Above ground monitoring

In September 2014 all the *A. alba* trees were tagged and height and diameter at breast height (DBH) recorded. DBH of the tagged trees was recorded at the end of the study in February 2016. Aboveground biomass was estimated using DBH in the allometric equation developed by Komiyama et al. (2005) for mangrove forests of Southeast Asia:

Aboveground biomass (kg $ha^{-1}$) = 0.251 x ρ x DBH (cm) $^{2.46}$

Where ρ (wood density, g $cm^3$) = 0.560

Aboveground biomass was estimated at the beginning and end of the study (a period of 17 months) and scaled to produce an annual productivity value.

### 4.3.4 Ingrowth core installation

A total of 100 ingrowth cores (50 cm depth x 15 cm diameter) were placed between 1 and 2 m from major tree trunks, within the five plots, with twenty cores per plot. They were made out of plastic mesh (sub-mesh size 1 cm x 1 cm) inserted vertically to 50 cm depth. To install the cores, a 50 cm deep hole was dug and all the soil removed. All roots found within the soil were removed and chopped into small pieces and then returned to the soil within the core, which was then placed within the hole. This procedure was carried out to ensure representative nutrient conditions in the ingrowth cores, since simply removing roots would remove an important source of nutrients (McKee 2001), while leaving them uncut would have made distinguishing new root growth difficult.

### 4.3.5 Ingrowth core collection

Three ingrowth cores per plot were collected every three months throughout the study period, i.e. 15 cores in total were collected in December 2014, March 2015, June 2015, September 2015, December 2015 and February 2016. The cores were brought to the laboratory and divided into five layers; 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm. The roots were washed from each layer using mesh sieves to remove the attached soil particles and debris. They were then rinsed several times until they were clear and free from other materials. Finally they were soaked in water and the living roots separated from the dead roots by hand. The live roots were sorted into two size categories; fine roots (< 3 mm diameter) and coarse roots (> 3 mm diameter). Very few dead roots were found, therefore these are not included in the analyses. All roots were oven dried for approximately 24 hours at 800C until constant weight.

In February 2016 the root standing stock was assessed by collecting three additional cores from each of the five plots (15 cores in total). Cores were 40 cm deep and 4 cm in diameter, and were collected between 5 to 10 m from major tree trunks.

### 4.3.6 Environmental parameters

In February 2016 a range of environmental parameters were measured in order to examine the association between belowground production and environmental conditions.

i) Soil nutrient analysis

One soil core (15 cm diameter x 50 cm height) was collected from each of the five plots. Each core was separated into five layers (0-10 cm), (10-20 cm), (20-30 cm), (30-40 cm) and (40-50 cm), and each section was analysed separately. The soil was oven dried at 800C for 72 hours and brought back to Edinburgh University. Soil was analysed for total phosphorus, total carbon, total nitrogen and the C:N ratio was calculated. 10 mg of soil from each layer was weighed for the C and N analysis and the samples measured using an elemental analyzer (NC 2500, CE instruments Ltd United Kingdom). Pseudo-total P was determined using an Aqua Regia digestion. 20 g of finely ground soil was dried overnight at 105°C. From this, a 5 g subsample was taken and ashed at 430°C overnight. Then 0.5g of ashed soil was dissolved in a 5:1 (v/v) mixture of HCl and HNO3 (respectively) whilst heated to 100°C in a water bath. The sample was evaporated to dryness then re-dissolved with 1ml of 1:1 HCl and filtered through a Whatman 4 filter paper into a 50 ml volumetric flask, then made up to 50 ml with deionised water. The concentration of P was then measured using an Auto Analyzer Applications III (Bran & Luebbe, Germany) using the molybdenate blue procedure outlined in Stewart (1974).

ii) Soil physico-chemical analysis

Pore-water samples were collected at four random locations within each plot during low tide for the determination of salinity, dissolved oxygen and soil temperature. Salinity was examined using a refractometer (Kern optics ORA 1SA, United Kingdom) whilst dissolved oxygen and soil temperature were recorded using a

portable multiprobe Pro2030 (YSI Inc., Ohio USA). The multiprobe was inserted to a depth of 30 cm and allowed to settle for two to three minutes prior to measurements.

### 4.3.7 Above and belowground parameter

In order to describe the relationship between above and belowground productivity, several parameters were calculated as follows:

i) Aboveground standing stock and production

Stem DBH data was incorporated into the allometric equation described above, following Komiyama et al. (2005), to derive initial (September 2014) aboveground biomass (dry weight) in t $ha^{-1}$ and final aboveground biomass in t $ha^{-1}$ (February 2016). The difference in biomass between these dates was used to calculate annual aboveground production (t $ha^{-1}year^{-1}$).

ii) Belowground standing stock and production

Roots were weighed and the units converted to $gm^{-2}$ to allow comparison with other studies. The surface area of cores, used to calculate root production, was 176.74 $cm^2$ whereas the surface area of the cores, used to calculate standing stock, was 12.56 $cm^2$. These values were scaled and converted to t $ha^{-1}$ for standing stock and t $ha^{-1}$ $year^{-1}$ for root production.

Annual root production was calculated by taking the mean of each of the 6 three-month root biomass totals and converting them to annual production in t $ha^{-1}$ $year^{-1}$.

iii) Root: shoot ratio of aboveground and belowground standing stock and production

Root: shoot ratios were calculated in order to determine allocation to above and belowground components for both standing stock and production.

iv) Root turnover

Root turnover was calculated following Gill and Jackson (2000), by dividing annual root production by root standing stock.

$$\text{Root Turnover (yr}^{-1}) = \frac{\text{Annual belowground production (t ha}^{-1}\text{ yr}^{-1})}{\text{Maximum belowground standing stock (t ha}^{-1})}$$

Studies from around the world reporting similar research to that described here were analysed and are summarized in Tables 4.4 , 4.5, 4.6 and figure 4.5.

4.3.8 Statistical analysis

Differences of fine, coarse and total root biomass and soil depth among the months of collection were performed using one-way ANOVAs. Differences in aboveground biomass between months were determined by one-way ANOVA. Log or square root transformations were applied to meet ANOVA requirements for non-normal data. Post hoc Tukey tests were performed to find significant differences between month of collection and soil depth. Pearson correlations were performed to find relationships

between root and aboveground biomass among environmental variables, including soil nutrients (carbon, nitrogen, C: N ratio and total phosphorus), soil temperature, salinity and dissolved oxygen. Statistical analysis was performed using Minitab 17.

## 4.4 Results

### 4.4.1 Forest structure

Forest characteristics are shown in Table 4.1. There were no significant differences in any parameters between the plots, therefore data has been combined.

Table 4.1. *Avicennia alba* forest structure in the Kelantan Delta. Mean ± SE.

| Forest characteristics | September 2014 | February 2016 |
|---|---|---|
| Tree density (stems $ha^{-1}$) | 1200 ± 0.52 | 1200 ± 0.52 |
| Average DBH (cm) | 17.58 ± 1.04 | 17.82 ± 1.04 |
| Height (m) | 14.13 ± 0.62 | - |
| Basal area ($m^2$ $ha^{-1}$) | 210.96 | 213.84 |

### 4.4.2 Environmental parameters

Physico-chemical parameters of the mangrove forest did not vary across the plots ($p > 0.05$) and data is therefore combined (Table 4.2).

The total amount of phosphorus, carbon, nitrogen and the C: N ratio did not vary significantly with soil depth. However although there were no statistically significant differences, there was a tendency for the nitrogen and carbon content to increase with depth. Phosphorous content and the C: N ratio remained consistent with depth.

Table 4.2. Environmental variables. Data recorded in February 2016 (n = 4).

| Environmental data | Mean ± SE |
|---|---|
| Pore-water salinity (ppt) | 12.08 ± 0.88 |
| Pore-water dissolved oxygen (mg/l) | 4.45 ± 0.96 |
| Pore-water soil temperature ($^0$C) | 27.94 ± 0.08 |
| Total soil phosphorus (% of mass) | 0.12 ± 0.01 |
| Soil carbon (% of mass) | 2.45 ± 0.18 |
| Soil nitrogen (% of mass) | 0.04 ± 0.01 |
| Soil C:N (% of mass) | 81.57 ± 9.53 |

4.4.3 Belowground standing biomass and production

In February 2016, the mean root standing stock across all five plots was 20.81 t $ha^{-1}$ (Table 4.3). The root biomass was 1225 gm-2 ± 123.8 and 856 gm-2 ± 153.46 for fine and coarse roots respectively. 59 % of the total root biomass was therefore fine roots.

Total root production was significantly different across the months of collection ($p < 0.001$), ranging from 665 ± 96.4 $gm^{-2}$ to 3322 $gm^{-2}$ ± 626.82 (Figure 4.2). The highest root production was in March 2015, 180 days after the experimental setup. In terms of root category, fine and coarse root production also varied significantly between the months of collection ($p < 0.001$).The highest fine root production was in March 2015, and lowest in December 2014. Maximum coarse root growth was recorded in December 2015, 15 months after cores were set up and ranged from 598 ± 85.75 $gm^{-2}$ to 2785 ± 468.9 $gm^{-2}$. In general, fine roots were the main contributor (78% on average) of total root production.

A steep decline in root production was seen in June and September 2015. These are the driest months with minimal rainfall. In fact, there was no record for coarse root production in September 2015. Root production increased again in December 2015 but decreased slightly in February 2016.The average root productivity is 12.7 t $ha^{-1}$ $year^{-1}$.

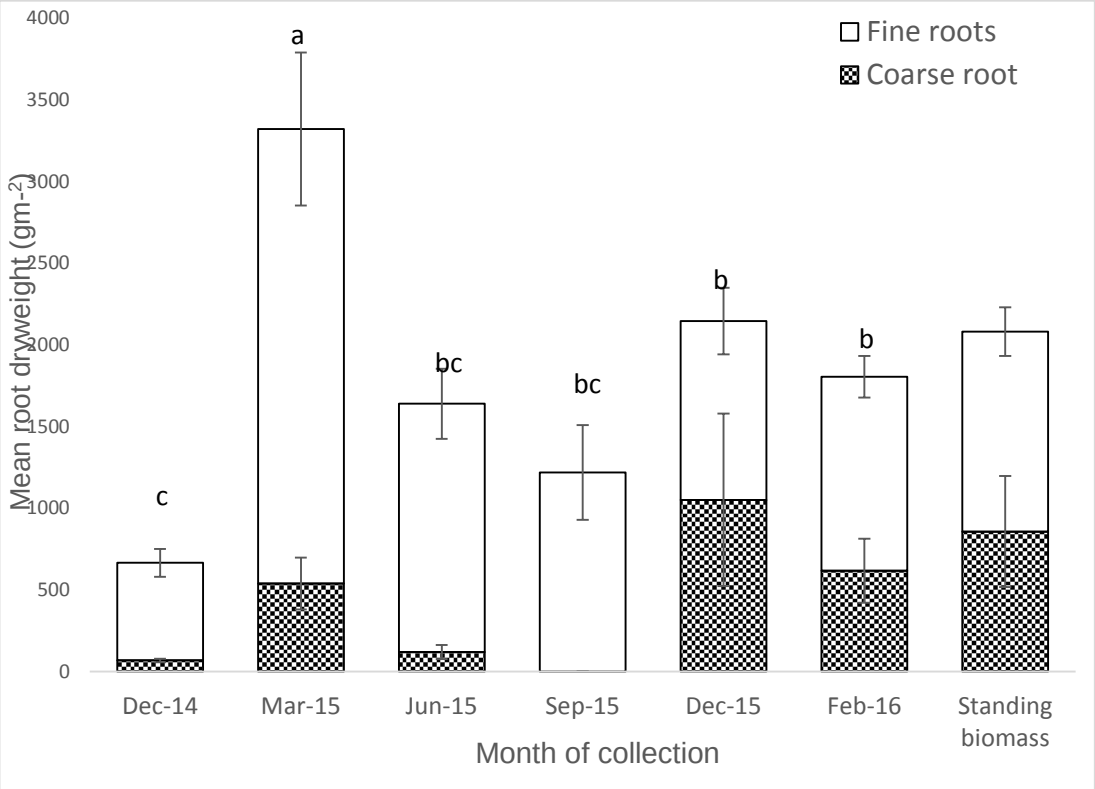



Figure 4.2. Root biomass from in-growth cores retrieved at three-month intervals used to derive root production (mean ± SE). Standing root biomass was sampled in February 2016. Bars sharing the same letters indicate no significant difference among total root biomass ($p<0.05$).

4.4.4 Root depth

Total root stock varied significantly with soil depth ($p < 0.015$). Most of the roots were found below 10 cm in the soil profile (Figure 4.3). Fine root biomass was significantly higher lower down the soil profile ($p < 0.001$), however, there was no significant difference in coarse root biomass between soil layers. 61% of total root biomass was found in the 20 to 40 cm horizon.

Root production (total roots, fine roots and coarse roots) did not vary significantly with soil depth (Figure 4.4). In terms of composition of roots in each soil layer, fine root biomass increased with increasing depth and represented 78 % of total root production. In contrast, coarse root production showed a decreasing trend with increasing soil depth.

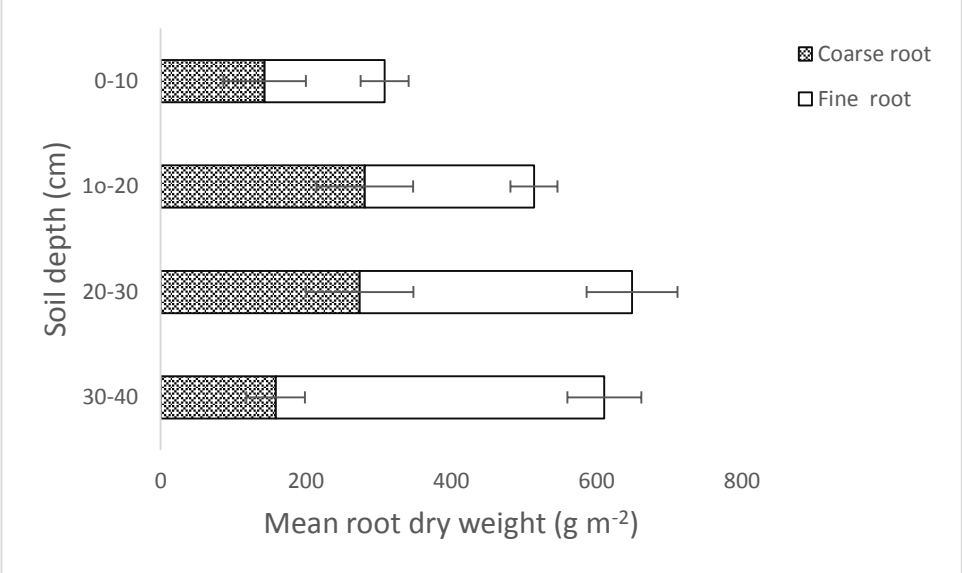



Fig 4.3. Standing root biomass (root stock) according to soil depth. Bars sharing the same letters indicate no significant difference among soil depth ($p < 0.05$). Mean ± SE

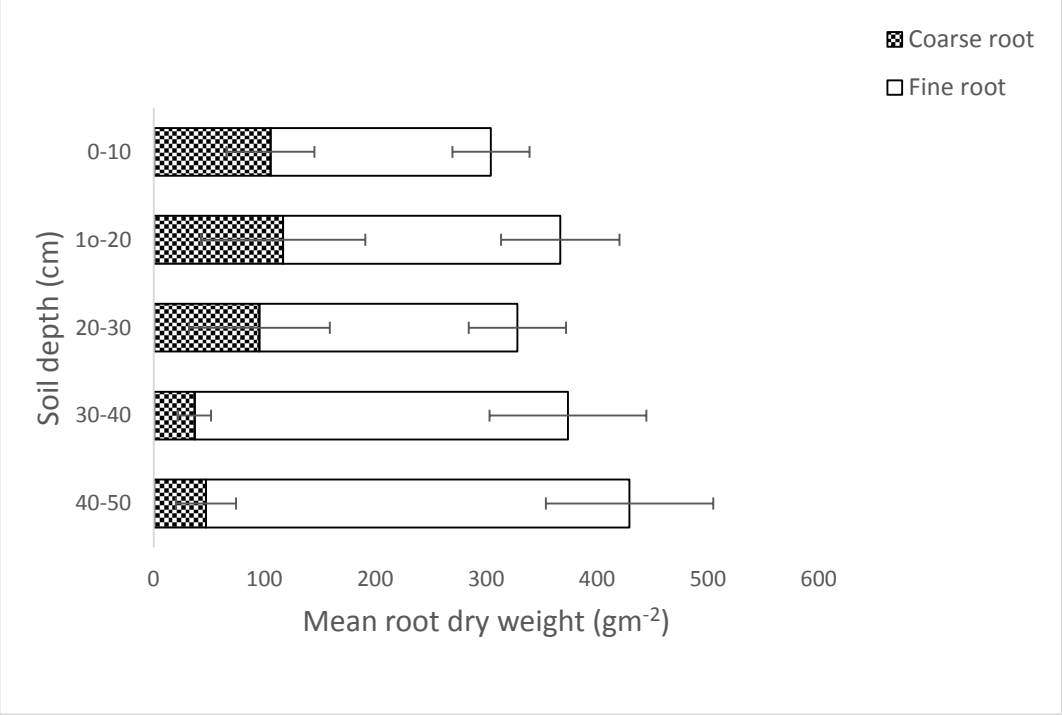



Fig 4.4 Total root production according to soil depth, over the course of 17 months. Mean ± SE.

4.4.5 Aboveground standing stock and production rate

The initial and final aboveground biomasses were 270 t $ha^{-1}$ and 277 t $ha^{-1}$ respectively, thus providing an aboveground production increment of 4.8 t $ha^{-1}$ $year^{-1.}$

4.4.6 Above and belowground allocation of biomass and production

The standing stock root to shoot ratio was surprisingly low at 0.075 (Table 4.3). However, over the course of 17 months, the ratio of below to above ground production was 2.65, thereby greatly favouring allocation to roots. Hence, 93% of standing stock was allocated aboveground and 7% belowground, in comparison with above and

below ground production allocation figures of 27% and 73% respectively (Table 4.3). Similar work close to this study were reported in Table 4.4, 4.5 and 4.6.

Table 4.3 Summary of above and belowground parameters.

| Above and below ground parameter | Standing stock | Production |
|---|---|---|
| Aboveground | 276.54 (t $ha^{-1}$) | 4.8 (t $ha^{-1}yr^{-1}$) |
| Belowground | 20.81 (t $ha^{-1}$) | 12.7 (t $ha^{-1}yr^{-1}$) |
| Fine root biomass | 12.25 (t $ha^{-1}$) (59%) | 9.88 (t $ha^{-1}yr^{-1}$) (78%) |
| Coarse root biomass | 8.56 (t $ha^{-1}$) (41%) | 2.81 (t $ha^{-1}yr^{-1}$) (22%) |
| Based on soil horizon | 39.5% total roots in the 0-20 cm soil horizon. 60.5% total roots in the 20-40 cm soil horizon. | 55.4% total roots in the 0-30 cm soil horizon. 44.6% total roots in the 30-50 cm soil horizon. |
| Total root turnover | 0.61 ($yr^{-1}$) | |
| Fine root turnover | 0.81 ($yr^{-1}$) | |
| Coarse root turnover | 0.33 ($yr^{-1}$) | |
| Root:shoot | 0.075 | 2.65 |
| Aboveground allocation | 93% | 27% |
| Belowground allocation | 7% | 73% |

Table 4.4 Comparison of aboveground production in mangrove forest of different regions

| Forest type/setting | Dominant species | Tree height (m) | Aboveground biomass (t $ha^{-1)}$ | Aboveground production (t $ha^{-1}$ $year^{-1}$) | Country | Reference |
|---|---|---|---|---|---|---|
| | *Rhizophora* | 3.5 | 240 | 6.77 | Sri Lanka | Amarasinghe and Balasubramaniam (1992) |
| | *Rhizophora and Avicennia* | 3.5 | 172 | 5.62 | Sri Lanka | Amarasinghe and Balasubramaniam (1992) |
| | *Rhizophora* | 3.5 | | 4.33 | Sri Lanka | Amarasinghe and Balasubramaniam (1992) |
| | *Avicennia* | 3.5 | 193 | 1.40 | Sri Lanka | Amarasinghe and Balasubramaniam (1992) |
| | *Rhizophora apiculata* | 21.0 | | 12.38 | Malaysia | Ong *et al.* (1995) |
| Basin/landward | *Avicennia marina* | 5.1 | 14.5 | 4.69 | Kenya | Lang'at (2013) |
| Scrub | *Ceriops tagal* | 2.4 | 11.8 | 1.97 | Kenya | Lang'at (2013) |
| Basin/interior | *Rhizophora mucronata* | 5.4 | 125.7 | 11.73 | Kenya | Lang'at (2013) |
| Fringe | *Sonneratia alba* | 6.1 | 112.9 | 5.93 | Kenya | Lang'at (2013) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | *Avicennia alba* | 11.3 | 169.9 | 8.1 | Thailand | Paungparn et al. (2016) |
| Delta | *Avicennia alba* | 14.13 | 277 | 4.8 | Eastern Malaysian Peninsular | This study (2017) |

Table 4.5 Comparison of belowground production in mangrove forest of different regions

| Forest type/setting | Dominant species | Belowground Biomass (t $ha^{-1}$) | Belowground production (t $ha^{-1}$ $yr^{-1}$) | Country | Reference |
|---|---|---|---|---|---|
| Island | *Sonneratia* | 38.5 | - | Halmahera Island, Indonesia | Komiyama et al. (1988) |
| Fringe | *Rhizophora* | | 2.65 | Rotatan Island, Honduras | Cahoon et al.(2003) |
| Basin | *Avicennia* | | 3.02 | Rotatan Island, Honduras | Cahoon et al.(2003) |
| Fringe | *R. mangle* | | 3.52 | Florida, US | Sanchez (2005) |
| Basin | *Rhizophora, Avicennia germinans and Laguncularia* | | 3.14 | Florida, US | Sanchez (2005) |
| Basin | *Avicennia germinans* | | 3.78 | Florida, US | Sanchez (2005) |
| Scrub | *R. mangle* | | 3.07 | Florida, US | Sanchez (2005) |
| | *R. apiculata, A. alba, Xylocarpus granatum* | - | 11.02 | Eastern Thailand | Komiyama (2006) |

| | | | | | |
|---|---|---|---|---|---|
| Basin | *Rhizophora and Avicennia* | | 5.25 | Twins cays, Belize | McKee et al. (2007a) |
| Fringe | *Rhizophora* | | 3.94 | Twins cays, Belize | McKee et al. (2007a) |
| Transition | *Rhizophora* | | 0.82 | Twins cays, Belize | McKee et al. (2007a) |
| | *Sonneratia* | 75 | | Gazi Bay, Kenya | Tamooh et al. (2008) |
| Riverine | *R. mangle*, *Laguncularia racemosa* and *Ceriops erectus* | | 4.65 | Shark River, Florida | Castaneda-Moya et al. (2011) |
| Riverine | *Rhizophora, Laguncularia* and *Aegiceras* | | 6.43 | Shark River, Florida | Castaneda-Moya et al. (2011) |
| Riverine | *Rhizophora, Laguncularia, Aegiceras* | | 4.69 | Shark River, Florida | Castaneda-Moya et al. (2011) |
| Scrub | *Rhizophora* | | 5.61 | Taylor River, Florida | Castaneda-Moya et al. (2011) |
| Scrub | *Rhizophora* | | 4.07 | Taylor River, Florida | Castaneda-Moya et al. (2011) |
| Fringe | *Rhizophora* and *Ceriops* | | 4.85 | Taylor River, Florida | Castaneda-Moya et al. (2011) |

| | | | | | |
|---|---|---|---|---|---|
| Basin/ landward | *Avicennia marina* | 6.03 | 3.66 | Gazi Bay, Kenya | Lang'at (2013) |
| Scrub | *Ceriops tagal* | 0.64 | 0.65 | Gazi Bay, Kenya | Lang'at (2013) |
| Basin/ interior | *Rhizophora mucronata* | | 2.54 | Gazi Bay, Kenya | Lang'at (2013) |
| Fringe | *Sonneratia alba* | 5.16 | 5.16 | Gazi Bay, Kenya | Lang'at (2013) |
| | | 9.47-30.40 | 0.46-1.85 | Celestun lagoon, Mexico | Adame et al. (2014) |
| Yela River, soil fertility gradient | Mix mangrove species | 4.48-26.41 | 45.88-118.66 $gm^{-2}$ | Micronesia | Cormier et al. (2015) |
| | *Avicennia alba* | 68.4 | 3.40 | Trat River, Thailand | Paungparn et al. (2016) |
| | | 2.43-18.69 | 571-2838 $gm^{-2}$ | Dongzhai Bay, China | Xiong et al. (2017) |
| Delta | *Avicennia alba* | 20.81 | 12.7 | Kelantan delta, Eastern Malaysian Peninsular | This study (2017) |

Table 4.6. Comparison of root: shoot ratio in mangrove forest of different regions

| Study site | Species | Root:shoot for biomass | Root:shoot for production | References |
|---|---|---|---|---|
| Indonesia | *Sonneratia* | 0.23 | | Komiyama et al. (1988) |
| | *Bruguiera* | 0.29-0.44 | | |
| | *Rhizophora* | 0.53-0.67 | | |
| Japan | *Bruguiera* | 1.38 | | Komiyama et al. (1989) |
| | *Rhizophora* | 1.39 | | |
| Thailand | *Ceriops tagal* | 1.05 | | Komiyama et al. (1989) |
| Greenhouse | *Rhizophora mangle* | 0.38 | | Pezeshki et al. (1990) |
| | *Avicennia germinans* | 0.42 | | |
| Queensland | *Avicennia marina* | 0.58 | | Mackey (1993) |
| Malaysia | *Rhizophora apiculata* | 0.05 | | Ong et al. (1995) |

| | | | |
|---|---|---|---|
| Greenhouse | *Rhizophora mangle* | 0.1 | McKee (1995b) |
| | *Laguncularia racemosa* | 0.4-1.5 | |
| | *Avicennia germinans* | 0.2-0.5 | |
| Australia | *Avicennia marina* | 4.1 | Saintilan (1997a) |
| | *Avicennia corniculatum* | 1.9 | |
| Queensland | *Avicennia marina* | 0.4-3.1 | Saintilan (1997b) |
| | *Avicennia corniculatum* | 0.4-1.4 | |
| | *Rhizophora stylosa* | 1.2-1.7 | |
| Japan | *Rhizophora stylosa* | 0.44 | Matsui (1998) |
| Australia | *Rhziophora* | 0.42 | |
| | *Ceriops* | 0.42 | |
| Dominican Republic | *Rhizophora mangle* | | Sherman et al. (2003) |
| | *Laguncularia racemosa* | < 0.5 | |

| | | | | |
|---|---|---|---|---|
| | *Avicennia germinans* | | | |
| Florida/Greenhouse | *Avicennia germinans* | | > 0.5-1 | Sanchez (2005) |
| | *Rhizophora mangle* | | > 0.5-1 | |
| Shark River, Florida | *Rhizophora mangle*, *Laguncularia racemosa* and *Ceriops erectus* | | | Castaneda-Moya et al. (2011) |
| Shark River, Florida | *Rhizophora*, *Laguncularia* and *Aegiceras* | | | Castaneda-Moya et al. (2011) |
| Shark River, Florida | *Rhizophora*, *laguncularia*, *Aegiceras* | | | Castaneda-Moya et al. (2011) |
| Taylor River, Florida | *Rhizophora* | | | Castaneda-Moya et al. (2011) |
| Taylor River, Florida | *Rhizophora* | | | Castaneda-Moya et al. (2011) |
| Taylor River, Florida | *Rhizophora* and *Ceriops* | 0.2-1.1 | | Castaneda-Moya et al. (2011)<br>Lang'at (2012) |
| Gazi Bay, Kenya | *Avicennia marina* | | 3.66 | Lang'at (2013) |

| | | | | |
|---|---|---|---|---|
| Gazi Bay, Kenya | *Ceriops tagal* | | 0.65 | Lang'at (2013) |
| Gazi Bay, Kenya | *Rhizophora mucronata* | | 2.54 | Lang'at (2013) |
| Gazi Bay, Kenya | *Sonneratia alba* | | 5.16 | Lang'at (2013) |
| Yela, Kosrae Micronesia | | 0.074 | | Cormier et al. (2015) |
| Kelantan delta, Malaysian Peninsular | *Avicennia alba* | 0.075 | 2.65 | This study (2017) |

## 4.5 Discussion

### 4.5.1 Dynamics of root stock and root production

Rapid root production occurred following the installation of the ingrowth cores in September 2014. This peaked in March 2015 and then again in December 2015, coinciding with the monsoon season. This suggests a strong seasonal pattern in root production on the east coast of the Malaysian peninsular. In this region, the northeast monsoon brings heavy rainfall, usually from November to March every year. Paungparn et al. (2016) also reported high mangrove root production after the rainfall season in Thailand. Terrestrial forests may also show a similar pattern, for example belowground production of the rubber tree (*Havea brasiliensis*) exhibited seasonal root production which was highly correlated with rainfall (Maeght et al. 2015). Heavy rainfall reduces the salinity of porewater in mangrove systems which favours root growth and stimulates high root production (Cormier et al. 2015). The mean salinity in this study was 12.08 ± 1 ppt (Table 4.2), which is considered brackish water, providing ideal conditions for optimum mangrove production.

Biomass in the ingrowth cores were similar to the root standing stock within six months of core installation, suggesting very rapid root growth. However, over subsequent months there was a reduction in biomass, indicating rapid root turnover. Turnover rates calculated across the whole experiment, for total, fine and coarse root biomass, were 0.61, 0.81 and 0.33 respectively (Table 4.3). Root turnover rates in this study decreased with increasing root size, as also found by Castaneda-Moya et al. (2011) in Florida mangrove forest.

Estimated annual root production was 12.7 t $ha^{-1}year^{-1}$. This represents the highest rate of root production reported from a mangrove forest, although it is close to the figure (11.02 t $ha^{-1}year^{-1}$) from another study in Eastern Thailand (Komiyama et al. 2006). Most other estimates of root productivity are much lower; for example 3.02 t $ha^{-1}$ year-1, 3.78 t $ha^{-1}$ $year^{-1}$, 3.66 t $ha^{-1}year^{-1}$ and 3.4 t $ha^{-1}year^{-1}$ in Honduras (Cahoon et al. 2006), Florida (Castaneda-Moya et al. 2011), Kenya (Langat, 2013), Thailand (Paungparn et al. 2016) respectively (Table 4.5). Explanations for this large productivity and fast turnover rate may lie in the environmental setting of the Kelantan Delta forest. This is a physically sheltered site with high levels of soil oxygen and low salinity, with copious freshwater input, showing a highly seasonal pattern. Investment in roots for structural strength, for example to resist wave buffeting in very muddy soils, is not necessary here. Comparably, the high salinity conditions known to encourage high root: shoot ratios in *Avicennia* species elsewhere, do not apply here. The very high turnover rates of fine roots may be driven by seasonal growth to obtain nutrients such as nitrogen and phosphorus. Additionally the use of ingrowth cores may have introduced an artifact contributing to the high rates of root production. Empty space within the soil core may have permitted excessive lateral root growth. The macerated roots, which were in the soil prior to core installation, were returned post installation and may have provided nutrients (McKee 2001) stimulating root growth.

In this study, fine roots were the main component of total root stock, providing 59% of the standing root biomass. In terms of root productivity, fine roots accounted for 78% of total root production. This figure is similar to 62-75% in Honduran mangroves (Cahoon et al. 2003). This has been explained by the primary role of fine roots in water and soil nutrient acquisition (Sanchez 2005) particularly during early root growth.

However, in contrast in Florida and Mexico Castaneda-Moya et al. (2011) and Adame et al. (2014) found a higher fraction of total root biomass was represented by coarse roots. Less coarse root biomass was found in this study, reflecting very rapid root turnover in this mangrove system, promoting a major fine root contribution to the belowground components.

The root distribution showed that roots are more abundant lower down the soil profile. However, root growth is slightly higher in the top 30 cm (55.4%) than in deeper soil layers (44.6% between 30-50 cm). This finding was similar to Castaneda-Moya et al. (2011), who observed that root biomass decreased with soil depth in a Florida mangrove forest. This might be explained by the higher concentration of soil nutrient near the soil surface (Castaneda-Moya et al. 2011).

The root standing stock found in this study (20.81 t $ha^{-1}$) was amongst the lowest reported from the literature for mature forests (Table 4.5). This may be due to the positioning of the cores relatively far away from the tree trunks approximately 50 m away from tree base. This would have led to an underestimation of root, particularly coarse root biomass. Further studies of root biomass should pay attention to this issue. In contrast, the aboveground biomass in this study was 277 t $ha^{-1}$ which is among the highest in comparison to the literature, although the highest aboveground biomass of 619 t $ha^{-1}$ was recorded in Australian mangroves (Alongi 2009). Combining these values gave a very low root: shoot ratio, which would suggest that the forest at this site invests an unusually low proportion of its productivity into its roots.

### 4.5.2 Root turnover

Annual total root turnover was 0.61 $yr^{-1}$, however fine roots turned over more than twice as quickly as coarse roots (0.81 $yr^{-1}$ in comparison with 0.31 $yr^{-1}$) (Table 3). This rate of fine root turnover is the highest in comparison with other mangrove regions. It is followed by the riverine mangroves of Florida (0.6 $yr^{-1}$), Mexican mangroves (0.4 $yr^{-1}$), Thai mangroves (0.1 $yr^{-1}$) and finally Micronesian mangroves (0.05 $yr^{-1}$) (Fig 4.5).

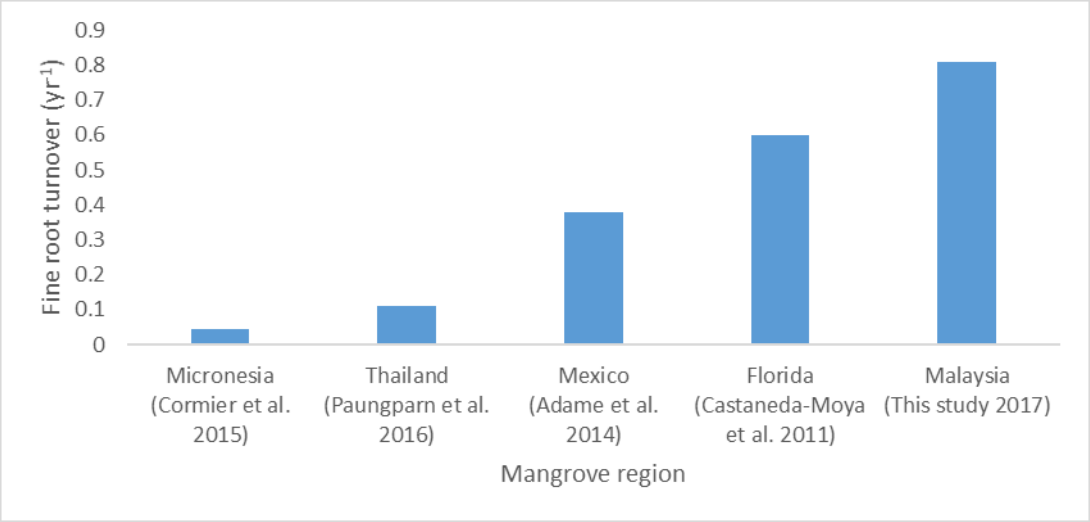



Fig 4.5 Annual fine root turnover for different global regions.

### 4.5.3 Aboveground production

Aboveground biomass measured in the present study is high study (277 t $ha^{-1}$), but comparable with results from other studies (Table 4.4.). In the present study, the average stem diameter is 17 ± 1 cm which represents a young stand. The aboveground biomass measured here is consistent with a study conducted 30 years ago on a more mature stand in the Malaysian peninsular, which found aboveground biomass to be

twice as high (500 t $ha^{-1}$ and a mean DBH of 50 cm) as in the present study (Putz and Chan 1986). Aboveground biomass of mature mangrove forests is generally greater at lower latitudes, which can be explained by the variation in temperature (Komiyama et al. 2008).

Annual aboveground production of *Avicennia alba* in this study (4.8 t $ha^{-1}$ $year^{-1}$) is similar to that of *Avicennia marina* in Kenya (4.69 t $ha^{-1}$ $year^{-1}$) (Lang'at 2013), but lower than aboveground production of the same species in Thailand (8.0 t $ha^{-1}$ $year^{-1}$) (Paungparn et al. 2015). Other aboveground studies in mangrove forest in Sri Lanka also showed low production (1.40 t $ha^{-1}$ $year^{-1}$) (Amarasinghe and Balasubramaniam 1992) as compared with this study (Table 4.4).

### 4.5.4 Correlation between environmental data and roots data

In this study, root production responded positively to soil nitrogen. Previous studies have shown than root production in mangroves might be more dependent on the available phosphorus (P), for example in the Floridian mangroves, (Castaneda-Moya et al. 2011; Adame et al. 2014; Poret et al. 2015). However, root production shows contrasting responses to soil P in other studies, as it has been found to increase with soil P in Celestun Lagoon, Mexico (Adame et al. 2014), while it increases with P deficiency within the Everglades (Florida, USA) (Castaneda-Moya et al. 2011).

The present study showed that, salinity tended to be an important environmental factor determining root production. This can be seen in the maximum root production data of the ingrowth cores recorded during the monsoon season in March (2015) and December (2015). This is because heavy rainfall during the monsoon season may

reduce seawater salinity, therefore stimulating root growth. This finding is similar to the study of Thai mangroves which also had high root production during the monsoon season (Paungparn 2016).

### 4.5.5 Biomass allocation to below to aboveground production

Mangroves growing on soil with poor nutrient content allocate most of their resources to grow belowground biomass as a strategy to optimize the limited resources (Castaneda-Moya et al 2013). In this study, root: shoot ratio for standing stock was 0.075, similar to that measured by Cormier et al. (2015) in the mangroves of Micronesia (Table 6). However, both root: shoot ratio values from the present study and Cormier et al. (2015) are much lower than that of 0.4 to 4.1 root: shoot ratio reported from other mangrove forests (Saintilan a and b 1997) (Table 4.6.). These results reflect higher biomass investment aboveground in a productive deltaic mangrove forest and are consistent with the higher allocation of biomass aboveground also observed in a productive riverine mangrove forest (Castaneda-Moya et al. 2013).

The root: shoot ratio recorded for the root production study using in-growth cores was 2.65, which is considerably higher than the ratio found through the standing stock investigation (0.075). Additionally, this result might partially be a consequence of the experimental design. Adding the chopped, dead root back to the ingrowth cores provides substantial nutrients and thus may have accelerated root growth, particularly within empty space or less compact soil in the ingrowth cores. This study site might have more dissolved oxygen in the soil porewater, which would stimulate root production.

## Conclusion

In this study, productive riverine mangrove forest allocated large amount of biomass to the above ground components particularly in the tree stems for standing stock. Whilst for productivity a high proportion of biomass was partitioned belowground, which could partly have been due to the returning of chopped roots to the soil which may have accelerated root growth. Salinity was the important environmental factor affecting root production since the root production peaked during the monsoon season as the heavy rainfall reduced the salinity of seawater.

## Acknowledgements

This study was funded by Malaysian Ministry of Higher Education for the first author. We are also grateful that the final fieldwork was supported by Centenary Agroforestry Fund School of Geosciences University of Edinburgh. YS was supported by NERC (RA0929 to M. Mencuccini) and the Academy of Finland (1284701). SJD and AMS were funded by Edinburgh Napier University.

# Chapter 5: General discussion

## 5.1 General finding

Being located in the intertidal zone, mangrove forest is one of the most vulnerable ecosystems in relation to the rising sea level (Lovelock et al. 2015). Most studies of projected sea level rise show a rise of approximately 60 cm in 2100 (IPCC 2007 ) due to melting of ice sheets and increasing activity of the ocean thermals (Church et al. 2013). When sea level increases, coastal areas may be flooded and most of coastal cities of the world will suffer flooding or be submerged (Kirwan and Megoniga 2013). This condition also poses a great threat to the mangrove forest particularly for oceanic and low lying mangroves (i.e in the Caribbean) which have limited mineral sedimentation, and river dominated mangroves which have changes in hydrology runoff (Ellison 2015) and relatively low biomass accumulation belowground (McKee 2011). Sea level rise also causes saltwater intrusion in mangrove forest (Saintilan et al. 2018) which may destroy mangrove communities and alter biogeochemical process in the estuarine ecosystem (Ove and John 2010), sediment erosion and flooding stress (Ellison 2015).

A recent study demonstrated that mangroves are able to persist under sea level rise through organic matter accumulation in the mangrove soil which is expected to contribute to soil volume expansion (McKee et al. 2007). Alternatively, the coastal squeeze phenomenon (see Chapter 1) may cause landward migration of mangrove species but much depends on the geographical setting (Gilman et al. 2008, Faraco et al. 2010) or the availability of the remaining forest. Otherwise, mangroves are liable to collapse under rising sea level if there is a continuous reduction of sediment inputs

to the estuarine area either due to urban development at the coastal area (Lovelock et al. 2015; Duncan et al. 2017) or the rate of rising sea level exceeding the vertical accretion of soil (Ellison 2015).

Under sea level rise, seedlings are expected to be more affected than adult trees. This is because the seedlings may be submerged completely and remain for long periods under the water (Mangora et al. 2014), restricting the gaseous exchange and the light intensity (Ashford and Allaway 1995). Therefore this study was carried out to examine the effect of prolonged submergence during sea level rise. Previous studies have reported canopy submergence on mangrove seedlings under simulated sea level rise (Lu et al. 2013, Mongora et al. 2014, Chen et al. 2005 & Xiao et al. 2009). However there is limited knowledge of prolonged flooding at root submergence particularly using the common Malaysian species, *Bruguiera gymnorrhiza* and *Rhizophora apiculata*. This information is important for Malaysian mangroves as Malaysia may experience the rate of sea level rise 1.7 ± 3.1 mm/year (Awang and Hamid, 2013). Therefore this study is important to gain information on the effect of projection sea level rise for Malaysian mangrove species. In order to examine the effect of accelerated sea level rise on mangrove seedlings, this study has quantified the ecophysiology of mangrove seedlings *Bruguiera gymnorrhiza* and *Rhizophora apiculata* in response to different durations of submergence (Chapter 2) whilst the effect of submergence on water conducting cells of both species were investigated in Chapter 3. In mangrove forest, above and belowground measurement were carried out to quantify the mangrove's biomass accumulation rate (and the carbon stock) of Malaysian mangrove deltaic forest as important mangrove ecosystem service which contribute to mitigate the rising sea level (Chapter 4).

In chapter 3, I investigated the survival of mangrove seedlings to maintain functioning under projection sea level rise through a manipulative flooding experiment. The results of the flooding experiment indicated root submergence has only a modest effect on the ecophysiology of mangrove seedlings though it showed some species-specific responses. All seedlings survived under prolong root submergence. For plant growth, both seedlings *Bruguiera gymnorrhiza* and *Rhizophora apiculata* grow taller under long submergence (24 hrs and 24 hrs stagnant treatment). For dbh, only *R. apiculata* seedlings were affected by the longest submergence (24 hrs stagnant treatment) due to smallest stem diameter whilst both species continued producing leaves although under extreme submergence.

Maximum net photosynthesis (Amax) and stomata conductance also showed species specific response. Seedlings *B. gymnorrhiza* showed lowest Amax and stomata conductance to 6 hrs flooding treatment compared with the long submergence treatments (24 hrs and 24 hrs stagnant). In contrast, Amax and stomata conductance for *R. apiculata* seedlings were reduced under permanent flooding (24 hrs stagnant treatment). Although Amax and stomata conductance showed species-specific responses to the flooding treatment, the photosynthesis apparatus indicated sensitivity to the shortest flooding treatment (6 hrs). Both seedlings showed highest chlorophyll content under 24 hrs and 24 hrs stagnant treatment but were sensitive to the shortest duration of flooding treatment (6 hrs treatment). Chlorophyll fluorescence (fv/fm) for both seedlings fluctuated, although the value of fv/fm was highest under long submergence and lowest under the shortest flooding treatment (6 hrs treatment) for both species. However, very low values of fv/fm (below 0.7) were seldom recorded, indicating that the photosystems were not damaged to any great extent. Leaf carbon

reserves also demonstrated species-specific responses and did not vary between treatments. Carbon reserves were significantly higher in *B. gymnorrhiza* seedlings than in *R. apiculata* seedlings indicating different energy requirement between both seedlings. Leaf area was the lowest under the 24 hrs flooding treatment for both species but significantly wider in *B. gymnorrhiza* seedlings than in *R. apiculata* seedlings.

Other studies of mangrove physiology show mangroves seedlings to be more sensitive under simulated tidal flooding particularly under canopy submergence (Lu et al 2013; Xiao et al 2009; He et al. 2007; Chen et al. 2005; Ye et al. 2003, & Ellison and Farnsworth 1997). The finding of these studies reported mangrove seedlings dead after several days of flooding, a reduction in growth, low biomass, and slow root decomposition. Although most flooding studies reported negative effects on mangrove seedlings, this current study show a modest effect might be due to the root submergence which allows the canopy of the seedlings to remain in contact with air. Therefore the carbon uptake was not limited and the continuous and rather high rates photosynthesis were not affected. Moreover, there was little effect on chlorophyll fluorescence (fv/fm) and leaf greenness under submergence. Carbon reserves were also showed less affected under submergence, and the relatively high content of carbon reserves indicate that the seedlings are not using much of their carbon reserves to counteract the stress, for example for other metabolic processes to maintain functioning under submergence.

All mangrove seedlings survived throughout 11 weeks of the flooding experiment. As mentioned earlier, this condition is probably due to the unexpected increased dissolved oxygen in the plant pot particularly under the 24 hrs (stagnant) treatment. The presence of algae and/or photosynthesising bacteria might be the main cause for a higher

dissolved oxygen within the plant pot. Any future flooding study should address this problem. In addition, the survival of the seedlings to the simulated sea level rise might be due to the rapid adaptation of both species of mangrove seedlings as they developed adventitious root as early as two weeks after flooding treatments. This is because the oxygen can be supplied through the adventitious root from water and transported to other plant structures to counteract waterlogged condition (Ayi et al. 2016). Lenticels were also observed in *R. apiculata* seedlings during early stages of flooding. These openings are important to allow gas diffusion through seedling's stems particularly during submergence (Yusof and Saenger 1996).

Overall, this ecophysiological study of mangrove seedlings in response to simulated flooding showed species-specific responses. *R. apiculata* seedlings were most affected by the permanent flooding whilst *B. gymnorrhiza* seedlings was most sensitive to the shortest flooding treatment. For example *B. gymnorrhiza* seedlings had lowest Amax, stomatal conductance, chlorophyll content and fv/fm in the 6 hrs treatment whilst *R. apiculata* seedlings had the lowest performance under long submergence for instance stem diameter, Amax and stomata conductance, and they used more carbon reserves under long submergence (24 hrs treatment). This differences implies tolerance limitations to submergence related to the different zonation of both species in the Malaysian mangrove forest.

The plants under flooding stress might be expected to 'suffocate' from oxygen deficiency. Therefore the effect of contrasting flooding treatments on mangrove seedlings *B. gymnorrhiza* and *R. apiculata* were examined for their water conducting cells in chapter three. However, the seedlings showed surprisingly few effects on vessel metrics in xylem structure as a response to flooding. The hypothesis that

mangrove seedling suffered from submergence and therefore develop smaller vessel diameters under longest submergence periods was not supported. Seedlings showed more profound effects relating to vessel characteristic between locations on stem plant seedling; plant apex and plant mid stem rather than the effect of vessel size. The vessel cell wall thickening also showed differences between species rather than treatments. Evidently, long submergence does not affect water conducting cells in the xylem, thus there is little effect on plant hydraulics. A previous study has reported that flooding stress strongly affected vessel arrangement and height of rays rather than the vessel size due to the adaptation to anoxic condition (Espinosa et al. 2001). Additionally, the aerenchyma is developed under submergence to allow oxygen diffusion from the surrounding air to the internal plant organs (Purnobasuki and Suzuki 2004). This current study also demonstrated the formation of aerenchyma for both seedlings as an adaptation to the prolong submergence. Several studies reported the xylem structure to be most affected by the salinity (Verheyden et al. 2005; Schmitz et al. 2006; Sobrado 2007 & Robert et al. 2009). Saline mangroves contribute to the small vessel diameter and high vessel density to provide alternatives for water flow along plant structures from the roots to the shoots.

In the mangrove forest, the quantification of biomass accumulation between above and belowground was carried out. The results revealed riverine mangrove forest at Kelantan delta show the highest belowground production among other mangroves globally, suggesting a most productive mangrove forest of the entire tropical region. The results also showed root production to be equal to root standing stock within 12 months of the study indicating rapid root growth and turnover rate in this study site. The findings imply rapid root turnover, contributing to the mangrove soil volume

expansion. This characteristic is important for mangroves as land builders and allows the mangroves to keep pace with sea level rise. The current study also demonstrated the above ground biomass as among the highest recorded in mangrove forest globally. The mangrove trees at this study site allocated more biomass accumulation in the tree trunk which is consistent with the study of riverine mangrove forest in Florida (Castaneda-Moya et al. 2013) reflecting the fact that the productive mangrove forest accumulated more biomass in the tree trunk rather than belowground. Another study showed that massive allocation belowground is a mechanism to maximize resources under nutrient stress (Castaneda-Moya et al. 2011). In this study, the highest biomass accumulation in both aboveground and belowground can confirm the perseverance of carbon stock as one of important ecosystem service provided by mangrove forest. Two important findings in this study showed that high biomass accumulation (hence the carbon stock) in aboveground may contribute to reduce carbon emission from the atmosphere whilst rapid root turnover rate belowground lead to the mangrove soil volume expansion in order to mitigate sea level rise.

## 5.2 Implication of the study: Mangrove management in Malaysia

In Malaysia, mangrove forest is managed under jurisdiction of the State forestry department under the Forestry Act 1984. However not all mangrove forest have been protected and gazetted as mangrove forest reserves. In Malaysia, most of mangrove forest has been destroyed mainly for economic purposes for example charcoal production to support local livelihoods although Matang Mangrove forest, which located in the state of Perak (west coast of Peninsular Malaysia), is held up as one of

the best systems of mangrove management in the world, and has practicing sustainable wood production since 1902 (Goesssens et al. 2014). A recent study showed how Malaysian mangroves are now threatened by oil palm planting (Richard and Fries 2012). This activity has destroyed massive area of mangrove forest of Peninsular and East Malaysia (Sabah and Sarawak). This destruction of the mangrove area has ignored the important ecosystem service provided by mangroves: for example as a coastline protector, nursery breeding area and carbon sink to mitigate climate change.

Therefore the findings of this study have implications for mangrove management in Malaysia. Due to the resilience of mangrove seedlings *Bruguiera gymnorrhiza* and *Rhizophora apiculata* to the prolong submergence, this species can be recommended as the potential species for replanting. Additionally, the finding from field experiment at Kelantan delta also confirm that mangrove forest demonstrates important ecosystem services through high biomass accumulation and rapid root turnover for mitigation of climate change and protection against sea level rise. Therefore, mangrove forest should be protected from land conversion and urban development at coastal area to maintain their functioning.

## 5.3 Limitation and suggestions for future study

This study has several limitations particularly relating to the flooding experiment. The stagnant treatment inadvertently allowed growth of photosynthetic organisms within the plant pots. The presence of algae provide excessive oxygen to the plant which therefore might be the main reason for the high dissolved oxygen within the stagnant pots. This higher dissolved oxygen has offset some of the negative effects that the treatments might otherwise have caused. Therefore the effect of severe flooding cannot

be seen properly. In the future flooding study, the existence of algae within the plants pots should be dealt with immediately to avoid the similar effect which can distract the actual effect of flooding stress.

For xylem anatomical study, replication of each plant treatment should be added and also there is a need to emphasize development of the aerenchyma tissues. These oxygen diffusion pathways are crucial.

In studies of the aboveground and belowground biomass, the root sorting should be done in more detail particularly for dead roots. The results might be misleading if careful steps are not made to distinguish among different root types.

# Chapter 6: Summary and conclusion

## 6.1 Summary

Mangrove plants are resilient and are well adapted to the stresses of life in changeable intertidal environments. However, predicted rates of sea level rise suggest large impacts on some mangrove forests, with seedlings particularly vulnerable. This study has examined the responses of mangrove seedlings to experimental flooding regimes and explored their adaptation under prolonged root submergence. Mangroves often suffer from hypoxia under long submergence in the field. Among the flooding treatments that were applied here, it was hypothesized that the 24 hrs (stagnant) treatment would exert a strong negative effect on the seedlings due to hypoxia. However, the effects of hypoxia did not occur here. Instead, oxygen levels stayed high even in the stagnant treatment. This was probably due to colonisation of the pots by photosynthetic bacteria and algae, which kept the water oxygenated. Although the 24 hrs treatment showed the lowest dissolved oxygen and highest salinity throughout flooding experiment, these conditions were not severe enough to cause plant death or significant morbidity.

Seedling survival was 100% under all the flooding treatments. The data demonstrated that seedlings showed significant shoot elongation under longer flooding treatments. Plants under long submergence also increased in stem diameter where much biomass was allocated to the stem. However, fewer leaves were produced under long submergence, possibly due to leaf abscission as a consequence of nutrient deficiency shown by ‘burn’ marks. Leaf level physiology provides further evidence that

mangrove seedlings showed high flooding tolerance. The seedlings demonstrated resilience to the flooding treatments as they quickly recovered from the longer submergence. This was exhibited by a period of stress in photosynthetic apparatus; chlorophyll content and chlorophyll fluorescence both declined but then recovered quickly and maintained high performance through the flooding experiment. Maximum photosynthesis rate and stomatal conductance were not affected by the flooding treatments, in fact a wider leaf area in *B. gymnorrhiza* seedlings supported relatively high photosynthesis during long submergence. Leaf carbohydrate reserves were also not affected by the flooding treatments; reserves accumulated higher in *B. gymnorrhiza* than *R. apiculata* seedlings.

Water conducting cells were not affected by the flooding treatments. Wider vessel diameter was observed for both flooding treatment implying little effect on flooding treatment on plant hydraulics. More cell wall thickening in *R. apiculata* resulted in greater mechanical strength for the plants under long submergence. In Malaysian mangrove forests, *R. apiculata* occupy a frequent inundation zone and soft muddy soil where anaerobic conditions occur during submergence. Thus thick cell walls function to support the plant under flooding.

In the mangrove forest, *Avicennia alba* stands showed rapid belowground biomass accumulation which clearly peaked in the monsoon season. This data suggests the production of belowground biomass is associated with the rainfall. Although aboveground biomass was higher than belowground, the rapid production in belowground biomass provided estimates of belowground productivity that are among the highest for any mangrove forest. This productivity did not translate into a large standing biomass, but rather implied a rapid root turnover which was recorded for six

months. The dynamic production of belowground biomass therefore has implications for the carbon pool/cycling in this mangrove forest. In contrast to other forests, it may be that mangrove forests in Peninsular Malaysia do not store large amounts of carbon belowground, possibly because of the relatively well-aerated mineral rich sediments in which they grow.

## 6.2 Conclusions

The response of mangrove seedlings to sea level rise was investigated in this study by exposing the mangrove seedlings of *B. gymnorrhiza* and *R. apiculata* to varying flooding treatments with different frequencies of root submergence. The mangrove seedlings under all treatments exhibited 100% survival. In terms of plant ecophysiology, mangrove seedlings exhibited high plasticity with high flooding tolerance and resilience. *B. gymnorrhiza* and *R. apiculata* seedlings showed different adjustments under varying flooding treatment. Water conducting cells were not affected by the two contrasting flooding treatments but did differ depending on location within the plant. Water conductivity was highest at plant mid stem. Greater wall reinforcement at the plant apex implied higher water stress at the plant shoot than plant mid stem. In the mangrove forest, relatively low belowground biomass contrasted with a high below-ground productivity and hence rapid root turnover. This pattern contrasts with the high root:shoot ratios (and perhaps lower root turnover) recorded in other mangrove forests, and may reflect the good environmental conditions, including high levels of soil oxygen, found at this site.

## 6.3 Future recommendation

This present study found high flooding tolerance of seedlings to the prolonged root submergence as shown by several growth, physiology and anatomy variables. Plant metabolic studies are needed in the future in order to gain a better understanding of the plasticity of mangrove seedlings under conditions of increased flooding such as may occur under accelerated sea level.

The important of aerenchyma tissue along the plant profile (from shoot tip to the root) in the plant aeration system should be prioritized in future flooding studies to explore the actual effect of long submergence.

Belowground productivity remains a poorly understood element of mangrove ecology. The factors that influence root productivity and turnover, including environmental settings such as pore water salinity and oxygen, deserve further study. How root productivity interacts with carbon storage and forest responses to sea level change are important topics for future work.

# Appendix 1

Results of repeated measurement ANOVA on the effects of different flooding treatment (6 hour, 18 hour, 24 jour and 24 hour (stagnant), species (*B. gymnorrhiza* and *R. apiculata*) on the relative growth rate of leaf number and the increase in shoot length under flooding treatment.

| | RGR Stem dbh | | RGR Stem height | | RGR Leaves number | |
|---|---|---|---|---|---|---|
| | F | P | F | P | F | P |
| Species | 10.99 | 0.001 | 0.60 | 0.441 | 16.59 | 0.000 |
| Treatment | 0.90 | 0.446 | 3.74 | 0.015 | | |
| Species*Treatment | 0.73 | 0.535 | 0.68 | 0.567 | | |

Spad and fvfm

Results of repeated measurement ANOVA on the effects of different flooding treatment (6 hour, 18 hour, 24 jour and 24 hour (stagnant), species (*B. gymnorrhiza* and *R. apiculata*), flooding week (week 1, week 11) and time (morning, afternoon) on the chlorophyll content, chlorophyll fluorescence under flooding treatment.

| | Chlorophyll content | | Chlorophyll fluorescence | |
|---|---|---|---|---|
| | F | P | F | P |
| Species | 1469.87 | 0.000 | 140.57 | 0.000 |
| Treatment | 12.20 | 0.000 | 6.93 | 0.000 |
| Flooding week | 67.80 | 0.000 | 47.53 | 0.000 |
| Time | 2.07 | 0.150 | 17.75 | 0.000 |
| Species*Treatment | 93.16 | 0.000 | 13.55 | 0.000 |
| Species*Time | 3.19 | 0.074 | 2.77 | 0.005 |
| Treatment*Flooding week | 2.12 | 0.000 | 2.37 | 0.000 |
| Flooding week*Time | 12.81 | 0.000 | 3.37 | 0.001 |

# Appendix 2

Photosynthesis Amax and stomata conductance

Results of repeated measurement ANOVA on the effects of different flooding treatment (6 hour, 18 hour, 24 jour and 24 hour (stagnant), species (*B. gymnorrhiza* and *R. apiculata*) and flooding week (week 1, week 11) photosynthesis rate and stomata conductance under flooding treatment.

| | Photosynthesis Amax | | Stomata conductance | |
|---|---|---|---|---|
| | F | P | F | P |
| Species | 10.58 | 0.002 | 1.96 | 0.165 |
| Treatment | 2.07 | 0.111 | 1.08 | 0.363 |
| Flooding week | 8.10 | 0.000 | 12.12 | 0.000 |
| Species*Flooding week | 3.42 | 0.021 | 4.24 | 0.008 |
| Treatment*Flooding week | 2.31 | 0.024 | | |

Total non-structural carbohydrate

Results of repeated measurement ANOVA on the effects of different flooding treatment (6 hour, 18 hour, 24 jour and 24 hour (stagnant), species (*B. gymnorrhiza* and *R. apiculata*) total non-structural carbohydrate and plant leaf area under flooding treatment.

| | Total Nsc | | Starch | | Sucrose | | Leaf area | |
|---|---|---|---|---|---|---|---|---|
| | F | P | F | P | F | P | F | P |
| Species | 5.13 | 0.038 | 4.22 | 0.070 | 3.36 | 0.086 | 5.06 | 0.027 |
| Treatment | 0.11 | 0.954 | 0.72 | 0.567 | 1.50 | 0.257 | 3.30 | 0.024 |
| Species*Treatment | 0.53 | 0.669 | 0.38 | 0.767 | 2.28 | 0.119 | | |

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