Understanding mangrove dynamics in range edge populations in South Africa

AYANDA ZIDE

A thesis submitted in fulfilment of the requirements for the degree of

MAGISTER SCIENTIAE

In the

DEPARTMENT OF BIODIVERISTY AND CONSERVATION BIOLOGY

UNIVERSITY OF THE WESTERN CAPE

Date: 25 November 2022

Supervisor: Prof. Anusha Rajkaran

Co-Supervisor: Prof. James Stephen Boatwright

ABSRACT

Mangrove distribution along the East African region occurs from Somalia to South Africa, including Madagascar and other islands. It comprises approximately 10 species, including *Avicennia marina* (Forsk.) Vierh, *Bruguiera gymnorrhiza* (L.) Lam. and *Rhizophora mucronata* Lam. that are core mangrove species and common in the region. Within the Indo-West Pacific (IWP) Province, studies have determined the genetic variation of mangrove species, but only a limited number of studies have included *B. gymnorrhiza* and *R. mucronata* populations occurring in the East African region, while none have included those occurring in South Africa. Genetic variation and environmental conditions affect plant performance and the longevity of mangrove populations. The aim of this study was to assess the genetic connectivity of two mangrove species namely *Bruguiera gymnorrhiza* and *Rhizophora mucronata*, to assess the sediment and porewater characteristics of three mangrove estuaries in the Eastern Cape using long term monitoring data and to assess the population performance of *A. marina* populations at the range edge.

First objective of the present study was to assess the genetic connectivity of these two species. Leaf samples were collected in Dar es Salaam (Tanzania), Incomati (Mozambique) and populations in South Africa as far south as Nahoon. Using a single nuclear and chloroplast region (*PAL-1* and *trnL-trnF* intergenic spacer region), results indicated no genetic polymorphisms for both species and markers and low genetic variation and low genetic differentiation among populations. Three nDNA haplotypes were found for *B. gymnorrhiza* populations and eight were found in the *R. mucronata* populations. The low genetic diversity was expected, as similar results were obtained in other studies. The second objective was to compare sediment characteristics and porewater characteristics between 2017, 2018 as well as at least one earlier date for each estuary. It was postulated that the drought conditions during the sampling period would influence the results obtained. The results showed that there have been changes in the sediment and porewater characteristics over the years. In general, the 2017 and 2018 results showed that sediment characteristics of Nahoon were significantly different from those found in Nxaxo/Ngqusi, whilst the porewater characteristics were generally similar in all the estuaries. The third objective was to determine the performance of *Avicennia marina* populations at the range edge. This achieved by measuring plant performance variables that included population structure, density, reproductive success (by measuring flower production), leaf surface area, specific leaf area, leaf C: N ratios, severity of pest infestation and signs of

disease of *A. marina* populations. The population structure results indicated that the density decreased from within the range (Mngazana – 4.17 individuals m^{-2}) to the edge of the distribution (Tyolomnqa – 0.78 m^{-2}), while the adult: seedling ratio indicated that recruitment was taking place in all estuaries but the transition from seedlings to saplings was unequal. The population density decreased from Mngazana to Tyolomnqa, which was in accordance with the size of the mangrove forest, however, the tree height and DBH did not follow a similar trend. Flowering data indicated that there is a high degree of variation with regards to the number of stalks per flower branchlet, with between 3 and 4 flowers found on each stalk, showing no specific trend in the number of stalks and flowers between the various populations. Galls were present on the leaves of all adult individuals and a black discolouration on the bark (assumed to be the consequence of fungal infection) was the highest at Tyolomnqa, with the other estuaries having a similar levels of occurrence. Growth was measured at three estuaries and the results did not show a strong trend, with the mean growth rates (cm. $year^{-1}$) being similar for the three estuaries. Understanding the genetic variation of plant populations and the performance of these populations could assist in the prediction of how these species will perform under climate driven changes, such as sea level rise and extreme weather stress, disturbances and impacts. This could help in the identification of source populations for genetic resources which may be used in restoration or rehabilitation programmes. Additionally, in determining populations which may require more conservation efforts due to low genetic diversity and poor plant performance. Thus, the information from this study could play an important role in the protection of these species as well as the conservation of the ecosystem services that they provide.

KEYWORDS

Genetic connectivity, haplotype diversity, *Bruguiera gymnorrhiza*, *Rhizophora mucronata*, plant performance, sediment condition, *Avicennia marina*

DECLARATION

I declare that this is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references'.

Signed ..... (signed by student)

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Anusha Rajkaran for all her guidance, advice, comments and continued and unwavering support throughout this journey. Anusha thank you, throughout my mangrove journey I could have not chosen a better supervisor and lecturer. I have learnt a great deal from you and for that, I will forever be indebted to you. I would also like to thank my co-supervisor, Prof James Stephen Boatwright, your guidance and advice is highly appreciated.

A debt of gratitude is also owed to those who funded this project, SAIAB, ACEP Phuhlisa Programme; University of the Western Cape – Early Career Fellowship; NRF Thuthuka Programme and Nelson Mandela Metropolitan University (NMMU) for the funding which has made this research to be possible.

I would like to thank those who helped me during my fieldwork, Dr Jacqueline Raw and Prof Anusha Rajkaran, and when doing my labwork, Dr Dimitri Veldkornet, Catherine Brown, Jabulile Malindi, Shakirah Tregonning, Christian Barnardt and Solange Akimana. To the UWC staff, Mr Lilburne Cyster, Mr Verno Gordon, Mrs Lorne Gelderbloem, Mrs Dawn Faroe and Ms Linda van Heerden. To Dr Nhlanhla Nyalungu, thanks for your advice and continued support.

I would like to give special appreciation to my friends, thank you for the encouraging words and support. Dr Asanda Mtintsilana, Elizabeth Bassey-Duke, Dr Xolane Mhlanga, Kedibone Namane, Tatenda Watungwa and Tumisho Ngobela.

Last but not the least, I would like to say Ndlovu to my parents Thumeka and Bhukhosibakhe Zide, without you none of this would be possible and the extended support from my nephew Sivuyile Nkosiyaphantsi and the rest of my family. Enkosi.

CONTENT

LIST OF FIGURES

LIST OF TABLES

Chapter 1: General Introduction

The definition of the term mangrove is twofold; (1) it could be used to describe a complex plant community fringing sheltered shores, a mangrove forest, thus describing the habitat, whilst (2) it could also be used to describe an ecological group of halophytic species which inhabit the mangrove habitat (Lugo and Snedaker, 1974). In this study, this term will be used interchangeably with some clarity if not distinguishable or when needed.

Mangrove species are facultative or obligate halophytes being able to survive in saline conditions and can grow in freshwater (Steinke, 1999; Wang *et al*., 2011). Mangroves prefer environments where the weather is humid and where there is an inflow of fresh water supplying the mangroves with nutrients and sediment (Kathiresan, and Bingham 2001). Environments which are repeatedly flooded and have well drained soils cater better for mangroves facilitating for higher growth rates and a greater diversity of species (Kathiresan, and Bingham 2001).

Mangrove distribution is categorised into two biogeographic regions namely the Indo-West Pacific (IWP) found in the Eastern hemisphere and the Atlantic Pacific region (AEP) occurring in the Western hemisphere (Duke and Schmitt, 2015). The East African region within the IWP occurs from Somalia in the North to South Africa in the South (Taylor *et al*., 2003), where Mozambique has the largest mangrove area and the largest continous mangrove stand occurs in Tanzania (Rufiji Delta) (Charrua *et al*., 2020; Monga *et al*., 2022).

These mangrove habitats are found to occur in estuaries, deltas and coastal settings (Kathiresan and Rajendran, 2005). A total of 11 species are found to occur along the East African region, including hybrid species *Ceriops somalensis* Chiov. being endemic to Somalia (Bosire *et al.* 2016) with 73 "true mangrove" and hybrid species occurring worldwide (Spalding, 2010). In general, threats to these mangroves include over exploitation, urbanisation, coastal development, natural factors such as weather disasters, pests and diseases and extreme environmental changes due to global climate changes (Gilman *et al*., 2008; Polidoro *et al*., 2010; Giri *et al*., 2011; Sandilyan and Kathiresan, 2012).

Due to the current threats to mangroves, measuring the genetic diversity of mangrove species is important for their conservation and for maintenance of the ecosystem services that they

provide. Ng *et al.* (2015) recommended that understanding the genetic variations of various mangrove species would assist in the identification of areas of conservation priority. This is supported by Giang *et al.* (2003), they noted that conducting genetic studies on populations could provide results which may be used to design strategies that would facilitate in the conservation of representative samples. Arnaud-Haond *et al*. (2006) further highlighted the need to measure the genetic composition and mating systems of edge populations to understand the environmental and demographic dynamics which shape species distribution ranges. A limited number of studies have determined the genetic variation of *R. mucronata* and *B. gymnorrhiza* of populations occurring in Tanzania, Mozambique and South Africa occurring in the East African region (Wee *et al*., 2015; Triest *et al*., 2021; Takayama *et al*., 2021). Thus, this would be a first opportunity to have a number of populations in South Africa assessed.

According to Islam *et al.* (2015) studies which provide information on the historical process and the contemporary gene flows could provide a picture on how mangroves evolved and thus be able to make predictions on how they will respond to changing ecological conditions caused by global warming (resulting sea-level rise and extreme changes in weather). Such studies could play an important role in defining conservation strategies that could be implemented.

Pautasso *et al*. (2009) states that global climate change will impact the health of plants at various levels i.e., genetic, individual, population and landscape. The genetic diversity of the populations could also influence their performance when facing stress or changes in environmental condition. A study by Guo *et al*. (2018) on a few mangrove species occurring in Indo-Malayan coast showed that their past and present plant performance was influenced by genetic diversity and the prevailing environmental conditions. This was illustrated by reviewing geological data which showed that the reduction in the effective population size of three mangrove species during past climatic events (Pleistocene glacial cycles) resulted in the loss of genetic diversity and that during a recent flooding event, the mangrove populations with lower genetic diversity had higher death rates (Guo *et al*., 2018). A range of other authors emphasised that a loss of mangroves results in lower productivity and thus loss of ecosystem services (Polidoro *et al*., 2010; Sandilyan and Kathiresan, 2012; Guo *et al*., 2018).

Osorio *et al.* (2017a) states that abiotic stress such as drought, changes in salinity can decrease the fitness of the trees making them more prone or susceptible to opportunistic pathogens which

could result in plant diseases. Plant disease and severity of pests are some factors which may compromise the health of plants (Pautasso *et al*., 2009). Gilbert *et al*. (2002) states that the impact of disease and pests is associated with population density, where susceptibility is higher at higher densities and *vice-versa*. Mangroves may provide an ideal environment for pathogenic attack due to low host diversity and high population density (Gilbert *et al*., 2002).

Environmental conditions may act on an individual which could result in changes in phenotypic variation and in the genetic variation of the population (Arnaud-Haond *et al*., 2006; Pautasso *et al*., 2010) (**Figure 1.1**). Genetic variation plays a role in plant performance, it is expected that populations with low genetic variation will have lower levels of plant performance or fitness (Kéry *et al*., 2000; Charlesworth and Willis, 2009; Engelhardt *et al*., 2014). The fitness advantage of having a higher genetic diversity may be revealed under stressful conditions such as changes in environmental conditions rather than under normal conditions (Wise *et al*., 2002; Guo *et al*., 2018). It should be noted that phenotypic variation does not necessarily indicate genetic variation but may be a result of environmental conditions. This then brings to us what actions could be taken with the information obtained from the study.

Engelhardt *et al*. (2014) suggested that for conservation of viable populations to be maximally effective, all scales of genetic diversity (from individuals to across populations and regions) should be considered. Triest (2008) recognised the importance of defining ecological significant units (ESU's) for the detection of hotspots of genetic diversity, mainly based on haplotype diversity which reflects historical seed dispersal across regions, where these units could be used as management units for conservation.According to Hardie and Hutchings (2010), in the management and conservation of range-edge populations factors such as the genetic diversity, risk to extinction, isolation to disturbance in central parts of the species range and adaptation to stressful environments should also be considered. Thus, illustrating that the results of this study may aid in understanding what management strategies would be required for the populations studied.

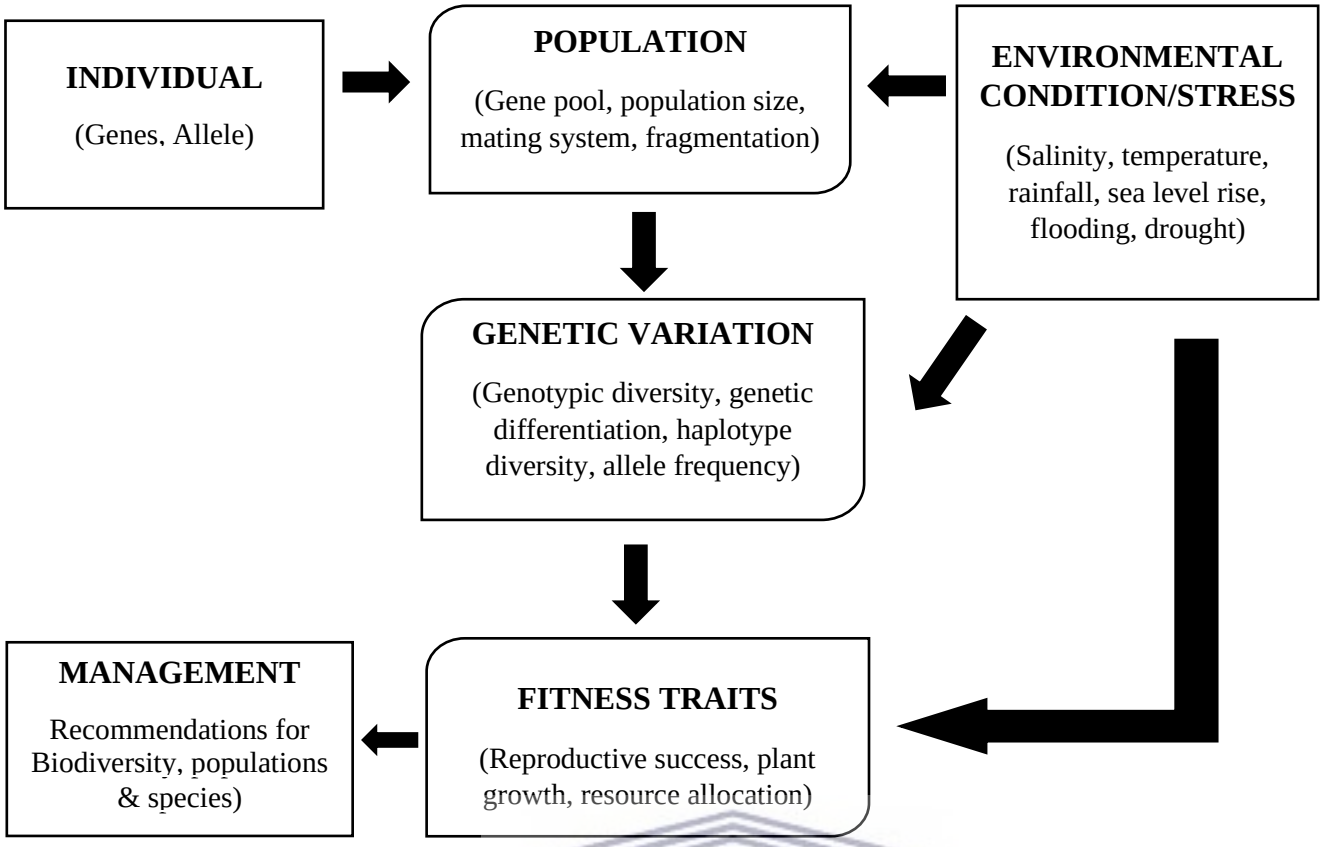


Figure 1.1: Conceptual Framework of this study.

Problem Statement

The mangrove ecosystem provides several ecosystem services and experiences several threats; some are anthropogenic in nature and others occur naturally. The mangrove environment is considered harsh and being at the range edge poses factors which may affect the performance of the mangroves which occur. To date, for the East African region only the study by Takayama *et al*. (2021) has included a single *R. mucronata* population from Beachwood (Mngeni Estuary) as part of their global study on the phylogeography of the genus *Rhizophora*. Osorio (2017b) assessed the pests and diseases *A. marina* and various authors (i.e., Rajkaran and Adams, 2012; Geldenhuys *et al*., 2016; Hoppe-Speer *et al*., 2015) have looked at the growing conditions and physical environmental of where these mangroves occur. To this end, assessing the genetic diversity of *Bruguiera gymnorrhiza* (L.) Lam and *Rhizophora mucronata* Lam. and determining the plant performance and environment conditions of *A. marina* populations for this study could play an important role in adding valuable information in our current understanding and management of this important ecosystem in the country.

Overall Aim

To conduct a literature review to determine the information gaps and thus showing the need for this study, to assess the genetic diversity of two mangrove species namely *Bruguiera gymnorrhiza* (L.) and *Rhizophora mucronata* (Lam.) from Tanzania, Mozambique and South Africa, assess the environmental conditions at three mangrove forests in the Eastern Cape and to assess the population performance of *A. marina* populations at range edge mangrove forests in the Eastern Cape of South Africa.

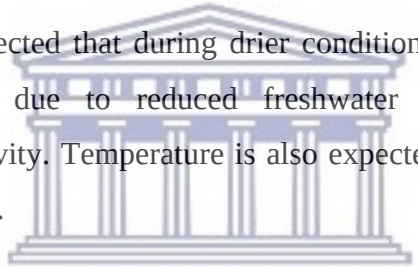
Objectives

1) Currently there is a limited number of studies which have measured the genetic variation of *Bruguiera gymnorrhiza* L. and *Rhizophora mucronata* Lam. populations occurring in the East African region (Urashi *et al*., 2013; Yan *et al*., 2016; Lo *et al*., 2014; Wee *et al*., 2015, Triest *et al*., 2021, Takayama *et al*., 2021). The objective of the study is to determine the genetic diversity and genetic connectivity of *Bruguiera gymnorrhiza* L. and *Rhizophora mucronata* Lam. populations occurring in Tanzania, Mozambique and South Africa. The following hypotheses could be made:
    a) It is expected that there will be higher gene flow between Tanzania and Mozambique due to the populations occurring along the same coastline and thus little genetic differentiation between the two regions.
    b) Due to the South African populations occurring at the distribution limit (range edge), it is expected that genetic diversity will be lower than that found in Tanzania and Mozambique (Islam *et al*., 2015).
    c) Due to the geomorphology of the South African coastline where the populations are smaller and fragmented, genetic flow within and between populations is expected to be low.

2) According to Hossain and Nuruddin (2016), mangrove forests show large variations in their sediment characterises such as salinity, pH and organic matter content. Due to the study sites at the range edge of mangrove distribution occurring in different biogeographic zones, where Mngazana occurs in the Sub-tropical region, whilst Nxaxo/Ngqusi and Nahoon occur in the warm-temperate region, it is expected that the environmental conditions will vary. This objective will describe the variation in the physical environment in terms of

sediment and pore-water characteristics of three mangrove estuaries namely; Mngazana, Nxaxo/Ngqusi and Nahoon.

a) Compare sediment characteristics and porewater characteristics between 2017, 2018 as well as at least one earlier date for each estuary.
    (i) It is expected that due to the drought conditions recorded at CAPEHERMES (dataset combination of Port St Johns station and Cape-Hermes); moisture content measured in 2017 and 2018 will be less than the earlier dates.
    (ii) Moisture content of the sediment is related to organic matter content, it is expected that it will be similar between 2017 and 2018 but lower than the previous year(s).
    (iii) It is expected that there may be a decrease in pH due to the drought, which is influenced by the organic matter present.
b) Compare porewater characteristics between 2017, 2018 and at least one earlier date for each estuary.
    (i) It is expected that during drier conditions, the salinity levels may be elevated due to reduced freshwater inputs resulting in higher conductivity. Temperature is also expected to have increased between the years.



3) To determine the plant performance of *Avicenna marina*; population density, population structure, growth rates, flower count, leaf size, leaf area, surface leaf area, C/N and severity of pest infestation and signs of disease were measured. It was postulated that *Avicennia marina* populations with higher genetic diversity (De Ryck *et al*., 2016), such as that at Mngazana Estuary would have a larger population size, greater density, higher growth rate and flower production compared to Nxaxo/Ngqusi, Kwelera, Nahoon and Tyolomnqa.

Dissertation structure

Chapter 1: General Introduction

This chapter provides a general overall view of the whole study; it intends to introduce the reader to the mangrove ecosystem linking it to genetics, environment and plant performance.

Chapter 2: Literature review

This chapter provides information on what is currently known with regards to this current study, allowing one to determine the gaps that are currently there and highlight the need for this study based on the literature that is currently available.

Chapter 3: Assessing genetic connectivity of *Bruguiera gymnorrhiza* (L.) and *Rhizophora mucronata* (Lam.) populations using the *trnL-trnF* intergenic spacer and *PAL-1* region.

This chapter provides details on haplotype diversity of *B. gymnorrhiza* and *R. mucronata*, using a single nuclear and chloroplast gene region for each species. This was achieved using the nuclear gene region *phenylalanine ammonia lyase* and chloroplast region *trnL–trnF* spacer region.

Chapter 4: Long term environmental characteristics of three mangrove estuaries in the Eastern Cape

This Chapter provides details on how changes in sediment and pore-water characteristics can be used to provide long term monitoring and used to determine its influence on plant performance.

Chapter 5: Plant performance of *Avicennia marina* at the latitudinal limit of mangrove distribution in SA

This chapter provides the first full assessment of range edge forests compared to core forest in South Africa, by measuring various plant performance variables in five mangrove forests namely the core population at Mngazana and range edge forests at Nxaxo/Ngqusi, Kwelera, Nahoon and Tyolomnqa.

Chapter 6: General discussion and conclusion

This chapter aims to provide a summary of the main findings in the study and provide recommendations for future studies and proposed management strategies for the custodians of the mangrove populations which occur in South Africa.

Chapter 2: Literature Review

Origin mangroves

To date several studies have explored and reviewed existing geological, paleontological, morphological evidence and molecular evidence to determine the possible origins of mangroves and how they have come to be distributed into two biogeographic regions. It is proposed that mangroves first evolved in the Indo-Malaysian region (IWP) due to the region having the highest mangrove diversity and through dispersal occupied various regions. This hypothesis is known as the centre-of-origin hypothesis and is based on the notion that continents have maintained their current location through time, thus not taking vicariance into consideration (Kathiresan and Bingham, 2001; McCoy and Heck, 1976).

McCoy and Heck (1976), Ricklefs and Latham (1993), Duke (1995), Ellison *et al.* (1999) and Duke and Schmitt (2015) oppose the centre-of-origin hypothesis citing the following reasons; firstly, the centre of origin does not explain why there is a marked difference in the species composition in the IWP and (Atlantic East Pacific) AEP region, secondly a comprehensive fossil record is not available and the oldest records are only found in the IWP (eastern Tethys) (thus not in accordance with the predicted dispersal routes) but are in favour of vicariance as the oldest fossils were found to occur around Tethys and elsewhere. This would then mean that mangroves did not disperse or evolve uniformly and thus have no common centre of origin. Majority of these studies suggest that the biogeography of mangroves is primarily a result of vicariance/continental drift (McCoy and Heck, 1976; Duke, 1995; Ellison *et al.*,1999; Duke and Schmitt, 2015). Alternatively, Plaziat *et al*. (2001) suggests that the current mangrove distribution is primarily a result of past climatic events. Whilst a study by Ricklefs and Latham (1993) suggests that the Southeast Asia/Malaysian region could possibly act more as a refugium than the centre of origin.

According to Duke (1995), to account for the current distribution by using the centre-of-origin hypothesis, several long-distance dispersal (LDD) events would have had to take place. Nettel and Dodd (2007) view LDD as a challenge to vicariance as the main hypothesis whilst Lo *et al*. (2014) states that these ideas are different but not mutually exclusive. This suggested a combination of dispersal and vicariance as the primary factors which have resulted in the

present distribution of mangroves. Cerón-Souza *et al*. (2015) supported this as the main determinants maybe the combination of dispersal and historical perturbations.

Presently there has been no real resolution in terms of these various ideas (Saenger, 1998; Lo *et al*., 2014). Ellison *et al*. (1999) postulates that there is still a lack of pollen evidence, thus as more evidence becomes available some resolution maybe reached. Dated molecular phylogenies may assit in solving this issue.

Species diversity

Mangrove plant species are either classified as "true mangroves" or mangrove associates; where the former describes evergreen halophytic woody plant species which are exclusive to mangrove forests, and the latter describes those which also occur in terrestrial environments. True mangrove species are distinct taxonomically from their terrestrial relatives. Mangroves have specialised physiological and morphological adaptations, such as salt regulation adaptations which exclude, excrete or accumulate salt, have aerial roots and exhibit vivipary which allows them to inhabit this environment (Tomlinson, 1986; Wang *et al*., 2011; Chakraborty, 2013; Nadia and Machado, 2014; Noor *et al*., 2015).

According to Spalding (2010) there are about 73 "true mangrove" and hybrid species found globally, a very small fraction to those which occur in the interface of freshwater and terrestrial systems (Xu *et al*., 2017). The classification of these species is not always straight forward, resulting in various studies not agreeing in-terms of what is classified as a true mangrove species, those which are associates and those which are the same species but being termed differently in different areas, thus resulting in differences in the number of species reported (Kathiresan and Bingham, 2001; Wang *et al*., 2011). The IWP has a higher species diversity, having about 63 species and hybrids, whilst the AEP region has about 19 species and hybrids (Spalding, 2010), with approximately 36-46 species in the Indo-Malay Philippine Archipelago of the IWP making it the region with the highest mangrove diversity (Polidoro *et al*., 2010).

Mangroves are found to occur in approximatelyeighteen plant families, which are not limited to those which occur in the mangrove environment (Tomlinson, 1986; Minobe *et al*., 2010; Nadia and Machado, 2014; Duke, 2017). Mangrove species are generally unrelated but share similar physiological characteristics and structural adaptations which enable them to inhabit

the mangrove environment, thus are an ecological assemblage rather than genetically related species (Sankararamasubramanian *et al*., 2012; Duke and Schmitt, 2015; Cerón-Souza *et al*., 2015). Guo *et al*. (2018) describes the adaptation of woody species to the mangrove environment as a demanding process, which may be one of the reasons why few species are able to inhabit this environment.

This study examined three species namely *A. marina* found in the Avicenniaceae and *R. mucronata* and *B. gymnorrhiza* found in the Rhizophoraceae, both these families occur in the IWP and AEP regions, dominating mangrove habitats (Hogarth, 2015; Duke, 2017).

The Avicenniaceae is one of two families which exclusively comprise of mangrove species (Duke, 2011). *Avicennia* (L.) is the only genus found to occur in this family and is the most diverse mangrove, consisting of eight mangrove species. Its growth form is either trees or shrubs occurring in tropical and subtropical coastal and estuarine habitats (Li *et al*., 2016; Raju *et al*., 2012; Schwarzbach and McDade, 2002). All species in this genus are considered true mangrove species, have specialized adaptions including pneumatophores, salt tolerance and produce viviparous (cryptoviviparous) propagules (Clarke, 1993; Schwarzbach and McDade, 2002). *Avicennia* species are sometimes referred to as pioneer species as they are generally the first occupants in most mangrove habitats, with their pneumatophores they create a suitable habitat for other species to occupy. In some sites they have been found to form pure stands and are said to play an important role in community structure (Osborne and Berjak, 1997; Naidoo *et al*., 1997). *Avicennia* species are characterised by a wide distribution range, they are the most frost tolerant mangrove genera and exhibit high salinity tolerance (Schwarzbach and McDade, 2002; Naidoo *et al*., 1997). They can tolerate the saline environment largely by excreting salt through their leaves, whilst their seeds exclude salt concentrating the salt in the parenchyma cells of the receptacle and their roots also exclude salt (Osborne and Berjak, 1997; Naidoo *et al*., 1997).

Five of these species including *A. marina* are considered to be core mangrove species (Spalding, 2010). *A. marina* also known as the grey or white mangrove for its bark (Clarke, 1993; Peer *et al*., 2018), is characterised by leaves that are simple, dorsiventrally arranged in a modified decussate (bijugate) arrangement allowing for the prevention of self-shading and have a pointed leaf apex and tip (Das, 1999; Kathiresan and Bingham, 2001; Duke and Schmitt,

2015). *A. marina* has breathing structures known as pneumatophores, parenchymatous tissue and small orange-yellow flowers (Duke, 1990; Duke and Schmitt, 2015).

The Rhizophoraceae is not exclusive to the mangrove habitat but also occurs in dry and wet forests and growth form is either trees or shrubs growing up to 50 m in height (Juncosa and Tomlinson, 1988). It consists of three monophyletic tribes including the Rhizophoreae which consists of about twenty mangrove species making it the most mangrove rich lineage (Juncosa and Tomlinson, 1988; Xu *et al*., 2017). Four genera occur within this tribe, namely; *Bruguiera*, *Rhizophora*, *Ceriops* and *Kandelia* which are exclusive to the mangrove environment (Juncosa and Tomlinson, 1988; Saenger, 2002; Lakshmi *et al*., 2002; Nadia and Machado, 2014). This tribe is characterised by species which produce viviparous propagules, vary in the type of aerial roots, have a high tannin concentration which plays a role in salt tolerance and provides resistance to herbivory (Lakshmi *et al*., 2002; Xu *et al*., 2017).

The genus *Rhizophora* (L.) has approximately six species (excluding hybrid species), species in this genus are characterised by stilt and prop roots and *R. mucronata* (Lam.) is characterised by leaves that have a pointed tip (mucronate leaf tip) and the underside generally have small reddish-brown dots termed cork warts (Juncosa and Tomlinson, 1988; Kathiresan and Bingham, 2001; Duke and Allen, 2006; Yan *et al*., 2016).

The genus *Bruguiera* (Lam.) is said to be the youngest in the tribe, with six species it differs from the other genera as it does not disperse as a seedling but rather the fruit (Saenger, 2002; Juncosa and Tomlinson, 1988). *B. gymnorrhiza*, is characterised by its knee roots which Duke (2006) describe as *"horizontal roots that occasionally form above-ground loops*", its bark is brown to grey in colour, has large leaves which are leathery and dark green in colour (Allen and Duke, 2006).

Mangrove biogeography

The Indo-West Pacific region (IWP) and the Atlantic Pacific region (AEP) accounts for about 57% and 47% of the global mangrove coverage, respectively (Spalding, 2010). The IWP and AEP are categorised based on the marked disjunction in species composition, where the former has a greater number of species than the latter as previously mentioned (Duke, 1995; Chen *et al*., 2015; Ricklefs and Latham, 1993; Duke and Schmitt, 2015), which Duke *et al*. (1998a)

estimates that IWP is four times more diverse than the AEP. Only *Acrostichum aureum* L. is found to naturally occur in both regions (Duke *et al*., 1998a; Ellison *et al*., 1999; Duke, 2006; Chen *et al*., 2015), where some studies either refer to it as a mangrove associate or a true mangrove species (Chen *et al*., 2015; Lugendo, 2016). A number of factors have been proposed to explain the distributional patterns of various mangrove species, such as intricate interactions between physiological constraints (i.e., temperature, moisture and tides), dispersal and large-scale geological and climate events (Duke, 1995).

Global coverage of Mangroves

In 2016, mangroves were estimated to have a global coverage of approximately 135 882 km^2 with about 4.3% mangrove loss over a 20-year period prior to the assessment (Spalding and Leal, 2021). Threats to mangroves include clearance for aquaculture, urbanisation (e.g., coastal development), pollution, agriculture, logging and climate change (Friess *et al*., 2019; Spalding and Leal, 2021). Even so, in some area's mangrove cover has increased, this includes range edge population which have no dispersal limitations colonizing new areas such as saltmarsh environment (Friess *et al*., 2019)

Generally, mangroves are distributed between the tropical-temperate regions between 30°N and 30°S latitude expanding into temperate regions e.g., east coast of South Africa (33°04'S) and in the North such as Japan (31°22'N), their highest occurrence is found between 5° N and 5° S latitude (Kathiresan and Bingham, 2001; Krauss *et al*., 2008; Morrisey *et al.*, 2010; Giri *et al*., 2011).

In East African region, mangroves are found to occur from Somalia to South Africa, including Madagascar and Seychelles having a cover of approximately 7,276 km^2 which accounts for about 5,4 % of the global coverage (Spalding and Leal, 2021). Eleven species are recorded to occur in this region namely; *Avicennia marina, Avicennia officinalis, Bruguiera gymnorrhiza, Ceriops tagal, Lumnitzera racemosa, Heritiera littoralis, Rhizophora mucronata, Xylocarpus granatum, Sonneratia alba, Ceriops somalensis* (which is said to be endemic to Somalia) and *Pemphis acidula* (found only in the northern regions of Mozambique, some studies classify this species as either a true mangrove or mangrove associate) (Bosire *et al.* 2016; Wang *et al.*, 2010).

In this region, *Avicennia marina*, *Rhizophora mucronata* and*Ceriops tagal* are dominant while*Bruguiera gymnorrhiza* is abundant (Kathiresan and Rajendran, 2005; Bosire *et al.*, 2016). The species diversity is relatively low when compared to other regions in the Indo West Pacific (IWP) region. This has been attributed to the possible absence of suitable habitat and the local environmental conditions (Macnae and Kalk, 1962; Ricklefs and Latham, 1993). Also because of the geographical location of this region, Polidoro *et al*. (2010) states that mangrove diversity is naturally low at the northern and southern margins of the mangrove global range. Species found to occur in the genera *Avicennia* spp., *Rhizophora* spp., *Bruguiera* spp., and *Xylocarpus* spp. are classified as core species, playing an important role in forest formation and succession enabling the widespread distribution of mangroves (Tomizawa *et al.,* 2017; Mantiquilla *et al*., 2021). Yan *et al*. (2016) states that *Rhizophora* spp. is one of the key tree species that shape the mangrove ecosystem.

Mangrove occurrence in Africa reaches its southern limit, in South Africa in the Eastern Cape Province (Adams and Rajkaran, 2021). Previously, the natural extent of mangrove was said to be at Kobonqaba Estuary (Eastern Cape) but has since been extended past the limit through transplanting activities which took place at Nahoon and Tyolomnqa estuaries, and a small natural population has been located at the Kwelera Estuary in East London (Ward and Steinke, 1982; Rajkaran and Adams, 2011; Adams and Rajkara, 2021). Of the six mangrove species found in South Africa, only three occur further south than Kosi Bay (KwaZulu-Natal) into the Eastern Cape, namely *Avicennia marina*, *Bruguiera gymnorrhiza* and *Rhizophora mucronata* (Ward and Steinke, 1982; Adams *et al*., 2004; Rajkaran and Adams, 2011; Hoppe-Speer *et al*., 2015).

Setting of mangroves (Habitat type)

Mangrove habitats are a rare global habitat, which are not uniform as they may differ significantly between continents, regions and forests (Spalding, 2010). Mangroves occur in various coastal settings such as estuaries, lagoons, deltas and coastal fringes (Kathiresan and Rajendran, 2005).

Estuaries

In South Africa an estuary is defined as "*a partially enclosed permanent water body, either continuously or periodically open to the sea on decadal time scales, extending as far as the upper limit of tidal action, salinity penetration or back-flooding under closed mouth conditions. During floods an estuary can become a river mouth with no seawater entering the formerly estuarine area or, when there is little or no fluvial input, an estuary can be isolated from the sea by a sandbar and become fresh or even hypersaline*" by the National Biodiversity Assessment of 2018 (van Niekerk *et al*., 2019). Cooper (2001) states that estuaries are dynamic due to the changes in geomorphology which may be rapid or progressive because of their location in a transitional region between land and sea, and thus experiencing the variations in the intensity of both regions. According to the National Biodiversity Assessment of 2018, the South African estuarine typology was classified into nine estuary types, including; Estuarine Lake (e.g., St Lucia), Estuarine Bay (e.g., Richard's Bay), Estuarine Lagoon, Predominantly Open (e.g., uMhlathuze), Large Temporary Closed, Small Temporary Closed, Large Fluvially Dominated (e.g., iMfolozi/uMsunduze), Small Fluvially Dominated and Arid Predominantly Closed occuring in all four biogeographical regions found in the country, namely; cool temperate, warm temprate, subtropical and tropical (van Niekerk *et al*., 2019). These estuarine types are categories based on key features and dominant physically process including, their size (areas), the percentage of time of mouth opening, geo-morphology, tidal range, salinity range, water mixing process, the stability of the sediment and the mean annual runoff (van Niekerk *et al*., 2019).

Delta

Kennish (2016) describes a delta as a "*discrete shoreline sedimentary protuberance formed where a river enters an ocean, a semi-enclosed sea, an estuary, a lake, or lagoon and supplies sediment more rapidly than it can be redistributed by basinal processes*". They are generally categorised into six types, based on the influence of wave, tide and river on the system (Bhattacharya and Giosan, 2003). The largest single mangrove forest in East African region is found to occur in the Rufiji Delta (Tanzania) where this delta is said to be a landscape with its own dynamics experiencing continuous changes in its surface (Wang *et al*., 2003). In Mozambique the largest mangrove forest is found at the Zambezi Delta (Shapiro *et al*., 2015). Spalding (2010) describes mangroves occurring on wet deltaic coasts as usually extensive,

highly developed systems with high canopies and high biomass, this study makes examples of the Meso-American Reef systems and Sundarbans Forest. According to Adams *et al*. (2004), the occurrence of extensive intertidal floodplain deltas provide habitat for complex riverine forests such as those found in estuaries like Mngazana and Mntafufu (South Africa).

Coastal Fringes

Coastal fringe mangroves are found along the shores of bays and islands occurring in small groups having single or mixed stands (Ruwa, 1993; Baldwin *et al*., 2001). Species such as *R. stylosa* have a higher affinity for this setting when compared to river dominated estuaries (Duke *et al*., 2001). In Tanzania, mangroves are the main coastal ecosystem, providing important ecosystem services (Taylor *et al*., 2003). Coastal mangroves play an important role in the protection of shorelines as they act as barriers from waves and natural disasters such as tsunamis and cyclones (Spalding, 2010; Lugendo, 2016).

Morphology

Morphological trait patterns could be a result of genetic factors or phenotypic plasticity (Bruschi *et al.*, 2014). Bruschi *et al*. (2014) states that morphological traits are related to adaptation thus could be used to determine how natural selection under variable environments could result in local adaptation.

In mangroves, morphological traits have been found to be influenced by environmental factors thus they may be less reliable indicators of taxonomic differences or genetic variation (Melville and Burchett, 2002). A study by Duke (1995) states that *A. marina* leaves on the upper canopy of a single individual can differ morphologically from those in the lower canopy attributing this to the influence of light availability. Other variables which can bring about morphological change include salinity, temperature, nutrients and inundation frequency (Duke, 1995; Lira-Medeiros *et al*., 2015). This is supported by Dahdouh-Guebas *et al.* (2004) which found morphological variations in *A. marina* leaves of mangroves growing in the landward fridge compared to the seaward fridge as the length of petiole, length of lamina and maximal width of lamina were smaller for the former. This variation was attributed to being an adaptive strategy of dealing with higher levels of salinity (above 30 psu) and drought experienced by the landward fringe when compared to the seaward fringe, this being a way to reduce

evaporation (Dahdouh-Guebas *et al.*, 2004). This study further looked at the genetic differences between the two populations, even though the study presents the results as preliminary, significant differences between the two zones was found but the study cautions that this may be a case of inbreeding effects, stochastic events, small population size and small sampling size rather than there being an ecological or physical barrier between the two zones. The author also highlights that there is no evidence of physical barrier between the two zone and proposes that the landward zone could be experiencing a higher loss of genotypes due to the stressful conditions thus the genetic differentiation found. Thus, the study shows that the morphological traits of the two zones may be a result of plasticity due to the difference in the environments experienced and highlighting that the possible genetic differences observed may be a result of the environmental differences (Dahdouh-Guebas *et al.*, 2004).

Even though morphological traits may be influenced by environmental conditions, the varieties of *A. marina* based morphological traits in Duke (1990) were confirmed with genetic variation using allozyme analyses in Duke (1998b), thus morphological traits were able to reveal the genetic variation in *A. marina*. These traits included leaf shape, bark, flower characteristics, the study identified three varieties of *A. marina* to occur in Australia. A study by Lira-Medeiros *et al*. (2015), looked at the morphological variation within populations of *A. schaueriana* and *L. racemosa*, the study found that the variation in *A. schaueriana* could be attributed to genetic variation whilst for *L. racemosa* the results were not significant. A study by Bruschi *et al*. (2014) on *Rhizophora mangle* assessed a combination of genetic variation using microsatellite markers and morphological traits. The morphological traits were correlated to the various abiotic stress levels such as salinity, exposure to pollutants and nutrient limitation experienced by the different populations rather than genetic variation which the authors attributed to the limitation of the markers that were used, as microsatellite markers are not expressed phenotypically thus may not reveal natural selection and adaptation (Bruschi *et al*., 2014). Whilst a study by Melville and Burchett (2002) showed that there was no correlation between leaf morphology and sediment characteristics in *Avicennia marina* var. *australasica*, but the study found a correlation between genetic variation using allozyme analysis and leaf morphology, thus indicating that leaf morphology may be an indicator of genetic variation. This was further supported by a study conducted by Saenger and Brooks (2008) which showed that the variations in leaf morphological characteristics were associated with genetic variation

rather than the environmental conditions for sixteen populations occurring in Australia. It should be noted that studies that used allozyme analysis are not reproducible.

Genetics, environment and plant performance

Genetics

Molecular studies and Genetic Connectivity of Mangroves in the East African region

To date, molecular methods such as microsatellite also referred to as simple sequence repeats (SSR), Amplified fragment length polymorphism (AFLP), and Random Amplification of Polymorphic DNA (RAPD), and analysis of DNA sequences (nDNA and cpDNA) have been used to determine genetic variation (genetic diversity) in mangrove species (Parani *et al*., 1998; Abeysinghe *et al.*, 2000; Maguire *et al*., 2002; Minobe *et al*., 2010; Islam *et al*., 2015). These markers, unlike morphological characters are not prone to the influence of environmental fluctuations and growth stage (Lakshmi *et al*., 2002; Dasgupta *et al.*, 2015). Ng *et al*. (2015) states that the selection of these markers is generally based on the species being analysed and its ability to reveal high genetic variation. Due to the variety of markers used, Ng *et al.* (2015) questions the practicality and constancy of comparing results obtained using different markers. This concern is supported by Yan *et al.* (2016) who states that comparative studies of population genetic parameters across species are problematic. Whilst Chiang *et al*. (2001) states that to some degree it is misleading to compare haplotype diversity among taxa which were analysed using various molecular methods at different loci.

Several molecular studies have been conducted on *A. marina*, whilst studies on *B. gymnorrhiza* and *R. mucronata* populations occurring in the IWP are low. **Table 2.1. and 2.2** below provides a summary of the results from the various studies. In summary, the studies assessed the genetic diversity and population structure over large (>400 km) and small (<50 km) geographical scales. At a larger scale, populations may experience different environmentental conditions, for example different climatic regions, occurring along different seas or oceans and different geographic regions (**Table 2.1**). Results generally showed that *B. gymnorrhiza* had low genetic diversity, which was attributed to dispersal, population size and low gene flow (Abeysinghe *et al*., 1999; Abeysinghe *et al*., 2000; Takeuchi *et al*., 2001; Lakshmi *et al*., 2002; Minobe *et al*., 2010; Islam *et al.* 2012; Urashi *et al*., 2013; Islam *et al*., 2015; Dasgupta *et al*., 2015). Contrary,

Ge *et al*. (2005) found high levels of genetic diversity along the coastline of south China and attributed the results to high gene flow and dispersal patterns.

B. gymnorrhiza in Iriomote Island (Japan) occurs at the extent of the distributional range, this may be one of the reasons for the lower genetic diversity that was found (Islam *et al*., 2012). Studies which looked at genetic structure found that populations occurring in different regions and oceans showed high differentiation between the populations (e.g., Minobe *et al*., 2010; Urashi *et al*., 2013), whilst the two studies for populations within the same region studies provided varying results of low to moderate genetic differentiation (Islam *et al*., 2012) and high genetic differentiation (Takeuchi *et al*., 2001).

For *R. mucronata* (**Table 2.2**) the studies analysed genetic variation at a large scale including different regions and oceans (or seas), showed a high differentiation between the populations and low genetic diversity within local populations (Wee *et al*., 2014; Lakshmi *et al*., 2002). This has been attributed to low genetic flow, recurrent variation of population sizes (small population size) (Lakshmi *et al*., 2002; Wee *et al*., 2014). Ng *et al.* (2015) also states that inbreeding within *R. mucronata* seems to be common. The study postulates that differentiation between populations was also a result of limited pollen and propagule dispersal.

For both *B. gymnorrhiza* and *R. mucronata*, a very limited number of studies have included populations occurring in the East African region. These studies included populations occurring in Mauritius (Mahebourg, Andilana, Ramena), Seychellles (Mahé, La Gigi, Aldabra, Mahébourg), Madagascar (Tolanaro, Morondava), Kenya (Gazi Bay, Mida Creek), Tanzania (Unguja, Zanzibar), Mozambique (Quelimane, Maputo, Pemba, Nazala, Vilanculos, Inhambane, Limpopo, Inhaca) and South Africa (Beachwood – Mngeni Estuary) (Urashi *et al*., 2013; Lo *et al*., 2014; Wee *et al*., 2015; Yan *et al*, 2016; Triest *et al*. 2021 and Takayama *et al*., 2021). This may indicate that little is known about the population structure of these two-species occurring in the study region. Whilst for *A. marina*, literature shows that three studies have included populations in South Africa. Duke *et al*. (1998b) which alloenzyme analysis to determine genetic variation in *Avicennia* spp., the second by Maguire *et al*. (2000) which showed that populations occurring at the range edge had lower levels of heterozygosity and higher levels of inbreeding, this was similar to the findings of De Ryck *et al*. (2016).

Table 2.1: Genetic diversity and population differentiation studies conducted on *B. gymnorrhiza*.

Reference	Molecular Marker	Location	Geographical scale and Description
Abeysinghe *et al*. (1999)	RAPD	Sri Lanka	Large Scale (> 200 km). Along the same coastline. Different climatic and geographic zones (Intermediate, Wet and Dry zones)
Abeysinghe *et al*. (2000)	RAPD	Sri Lanka	Large Scale (> 200 km). Along the same coastline. Different climatic and macrogeographic regions namely (Intermediate and Wet zones)
Takeuchi *et al*. (2001)	Allozyme	Japan (Iriomote Island, Okinawa Island and Amami Island)	Large Scale (about 600 km). Peripheral populations (range edge)
Lakshmi *et al*. (2002)	RAPD and RFLP	Indian coast (Pichavaram, Bhitarkanika, Muttupet, Coringa, Ratnagiri, Goa)	Large scale (>2000 km)
Ge *et al*. (2005)	Allozyme	South China	Large Scale (>1 400 km)
Minobe *et al*. (2010)	DNA sequences	Japan, Thailand, Malaysia, Indonesia, Micronesia, and India	Large Scale (three geographical regions, Pacific Ocean, Bay of Bengal and Arabian Sea)
Islam *et al*. (2012)	Microsatellite	Iriomote Island (Japan)	Small Scale (local scale)
Urashi *et al*. (2013)	DNA sequences	Australia, Indonesia, Vietnam, Malaysia, India, Madagascar	Large Scale
Islam *et al*. (2015)	Microsatellite	Iriomote Island (Japan)	Small scale (single population)
Dasgupta *et al*. (2015)	RAPD & Microsatellite	Sundarbans Islands (India)	Small Scale (local scale) (<20 km)

Table 2.2: Genetic diversity and population differentiation studies conducted on *R. mucronata*.

Reference	Molecular Marker	Location	Geographical scale and Description
Lakshmi *et al*. (2002)	RAPD and RFLP	Indian east coast (Bay of Bengal: Bhitarkanika and Corings)	Large scale (>700 km)
Inomata *et al*. (2009)	DNA sequence	Thailand (Bangkok and Surat Thani)	Large scale (>500 km)
Lo *et al*. (2014)	DNA sequence & ISSR	Global	Global study both AEP and IWP
Ng *et al*.(2015)	DNA sequence	Thailand and Malaysia	Large scale

Wee *et al*. (2015)	Microsatellite	Indian Ocean Region and the Pacific Ocean Region	Large scale (<1700 km 1st site to the furthest, three different seas)
Yan *et al.*, (2016)	Microsatellite	IWP Region	Large scale
Triest *et al*. (2021)	Microsatellite	Kenya, Tanzania, Mozambique, Seychelles, Madagascar	Large Scale (East African Region)
Takayama *et al*. (2021)	Microsatellite	Global	Global study both AEP and IWP

A review by Triest (2008) on molecular studies conducted on the same coastlines showed that these populations have high levels of gene flow. This is supported by Yan *et al*. (2016) which states that high levels of gene flow maybe exhibited by neighbouring populations along the same coastline. Though this may becorrect, but as highlighted by Wee *et al*. (2017), oceanic currents have a major influence in the genetic structure of the mangrove populations. Even though mangroves occurring in Somalia, Kenya, Tanzania, Mozambique and South Africa occur along the same coastline, a number of currents may influence the transport of mangroves in this region.

The South Equatorial Current (SEC) may transport propagules from Malaya and North-west Australia to the equator of the east coast of Africa, the Mozambique and Agulhas currents may assist propagule transport from Zanzibar to South Africa (Muir, 1933) and the transport from Zanzibar to Somalia through the East Africa Coastal Current and the Somali Current. It is postulated by De Ryck *et al*. (2016) that the SEC may create an oceanic barrier resulting in the genetic differentiation between the northern populations (Kenya and Tanzania) from those occurring in the southern regions (Mozambique and South Africa) of the East African coastline. That study examined the genetic differentiation of *A. marina* populations. Triest *et al*. (2021) were in support of the split as the study found genetic differentiation between Tanzania and Mozambique populations coinciding with the split of the SEC, thus suggesting that the SEC may form an oceanic barrier. De Ryck *et al*. (2016) states that the movement of propagules in South Africa may be further complicated due to the influence of the geomorphology of the coastline further attributed to the types of estuaries found, where some due to their functioning may restrict gene flow. This is supported by Raw *et al*. (2022) which found the connectivity of mangroves to be limited by propagule dispersal which the study attributes to the local to regional-scale coastal and estuarine dynamics this including the mouth condition, and location and size of the mouth which in some estuaries changes seasonally or subseasonal.

Central Marginal Theory/ Central - Peripheral population Hypothesis and Genetics

The range limit of a species is generally defined by the abiotic and biotic factors, and their interaction at that location (Abeli *et al*., 2014). Species occurring at the range edge/limit or periphery of their distribution are expected to have lower levels of genetic variation according to the central-peripheral population hypothesis (CPH) (Dai and Fu, 2011; Wee *et al*., 2017). At the range-edge, populations are expected to be smaller, less dense and show signs of genetic

erosion due to the environmental conditions which are less favourable (Assis *et al*., 2013; Abeli *et al*., 2014). Arnaud-Haond *et al.* (2006) states that in general studies which have compared core to peripheral populations have generally shown a lower genetic diversity and higher divergence in the latter. For example, peripheral populations of *A. marina* occurring in South Africa and South-east Asia (Maguire *et al*., 2000; Arnaud-Haond *et al*., 2006; De Ryck *et al*., 2016) and in *B. gymnorrhiza* populations occurring in the Iriomote Islands of Japan (Islam *et al*., 2015) have shown this trend. The factors cited for the observed trend/pattern included low effective population size, repeated bottlenecks, founder effects, low gene flow and high inbreeding resulting in these populations having lower levels of genetic diversity (References).

Not all plant populations exhibit this trend as they differ greatly in their dispersal abilities, life history and resilience (Dai and Fu, 2011; De Ryck *et al*., 2016). Dai and Fu (2011) also highlight that having a lower genetic diversity is not always an indicator of the CPH pattern, thus weakening the predictive power of this hypothesis. Pironon *et al*. (2015) also highlights that some studies overlook the importance of the ecological marginality of the peripheral populations which is one of the requirements for the CPH pattern and recommends identifying peripheral populations as those occurring in areas which have been recently colonized. Conditions at the range edge/limit are not uniform in all locations thus will have different outcomes, in some areas the conditions are favourable allowing the persistence of the population whilst some have areas with unfavourable conditions where the population size and density is significantly reduced resulting in lower genetic diversity, occurrence of clonal production and inbreeding (Assis *et al*., 2013).

Arnaud-Haond *et al.* (2006) proposed that through the favouring of rare genotypes due to extreme environmental conditions balancing the effect of selection and the high influx of alleles from the core populations could maintain high genetic diversity at peripheral populations, but states that in general species have not displayed these proposed hypotheses. De Ryck *et al.* (2016) argues that the arrival of high influx of new alleles from core populations may not ensure high genetic diversity due to the beneficial alleles with small coefficients being swamped by migration, thus not contributing to local adaptation.

Environment

Environmental drivers of mangroves

Mangroves live in a harsh environment, as a result this ecosystem exhibits a low species diversity when compared to other tropical forests. Duke (1998a) proposes that diversity for mangroves should not be in terms of the number of species present but should be in terms of the ability of each species to thrive in the prevailing environmental conditions. Arnaud-Haond *et al.* (2006), state that the environmental conditions at the range edge are extreme resulting in fragmented populations with low densities.

Temperature and aridity

Mangroves distribution has been correlated with temperature and aridity (Nettel and Dodd, 2007). Mangroves are generally restricted within the 20°C winter isotherm of seawater (Krauss *et al*., 2008). Quisthoudt *et al*. (2012) found that *Avicennia* and *Rhizophora* didn't exhibit common isotherms in their upper latitudinal range limits when using air temperature and sea surface temperature data, thus suggesting that it is not always the case. The decrease in air temperature and sea surface temperature with increasing latitude is generally said to be the reason for the decrease in species diversity (Clough, 1993). Krauss *et al.* (2008) agrees citing studies on populations occurring in North-eastern Asia.

Historically, the planet has gone through series of cooling (glacial) and warming (interglacial) periods which has resulted in the contraction and expansion of the distribution of certain mangrove species (Nettel and Dodd, 2007). According to Alongi (2015) with the current increase in temperatures mangroves distribution has been expanding into higher latitudes such as in Southern Africa and Australia. This expansion is being observed in subtropical and tropical regions and precipitation may also be a factor. According to Duke *et al.* (2017), in arid areas, moisture plays a bigger role to limiting mangrove extent when compared to temperature. It is expected that the increase in aridity (decrease in precipitation) in certain region such as the Caribbean and South Asia will result in the decrease in mangrove cover (Alongi, 2015).

Temperature plays an important role in the survival and growth of mangroves, thus influencing the growth rates, the primary production and species diversity found to occur in the area. This is illustrated by mangroves growing in temperate climates having lower growth rates and

biomass production than those in lower latitudes (Kathiresan and Bingham, 2001; Steven *et al*., 2006; Morrisey *et al*., 2010). According to Krauss *et al*. (2008) the physiological mechanism which restricts growth and mortality is not yet fully understood. The dispersal of mangroves is also influenced by temperature, for example *A. marina* cannot reproduce in colder climates of high latitudes thus limiting dispersal (Duke, 1995). **Table 2.3** reports on the minimum air temperature tolerance of some mangrove species.

Table 2.3: Optimal salinity and pH range of various mangrove trees and minimum air temperature tolerated.

Family	Genus	Species	Salinity (ppt)	pH	Air minimum Temperature (°C)
Avicenniaceae	*Avicennia*	*marina*	10-20[*2]	7.82, 7.55[*4]	10 (Avicennia genus, Brazil) [*5]
Combretaceae	*Lumnitzera*	*racemosa*	10-20[*2]	-	-
Lythraceae	*Sonneratia*	*alba*	1.75-17.5[*3]	-	-
Rhizophoraceae	*Bruguiera*	*gymnorrhiza*	8-26 [*6]	4.0 – 7.4 [*6]	-5 [*6]
Rhizophoraceae	*Rhizophora*	*mangle*	8-26[*1]	6.0–8.5 [*1]	0 [*1]

*1. Duke and Allen, 2006 *2. Clough, 1993 *3. Ball and Pidsley, 1995 *4. Joshi and Ghose, 2003 *5. Twilley and Day, 1999 *6. Allen and Duke, 2006, *7. Ball, 1988

Mangroves display varying tolerance to low temperatures, a study by Chen *et al*. (2017) showed that following a chilling event (temperatures ranged between 9.5 to 1.5 °C) some mangrove species occurring at various sites in China were more sensitive (e.g., *Sonneratia* spp.) whilst species such as *Avicennia marina* (considered a freeze-tolerant species), *Kandelia obovata* and *Aegiceras corniculatum* experienced minimal damage. Not only are mangroves sensitive to low temperatures but also high temperatures, where younger tissue and seedlings have been found to be more affected. Mangrove tree settling becomes impeded at air temperatures above 35 °C generally which results in a significant decline in the photosynthesis and temperatures ranging between 40 and 55 °C have been found to result in plant organ damage (Krauss *et al*., 2008; Noor *et al*., 2015).

Salinity

The mangrove environment has variable salinity ranging from hyposaline to hypersaline conditions due to its location between land and sea, freshwater input and tidal fluctuations

(Whitfield, 1992; Kathiresan and Bingham, 2001; Taylor *et al*., 2006). The salinity of sea water is generally around 35 ppt and that of permanently open estuary which can be influenced by either tides or riverine input ranges between 10-35 ppt (Whitfield, 1992; Taylor *et al*., 2006). According to Krauss *et al.* (2008), a salinity of 35 ppt equates to a percentage concentration of 86% NaCl. Literature shows that mangroves achieve optimal growth when sea level NaCl levels are between 5-75%. **Table 2.3** reports on the optimum salinity ranges that some mangroves have been found to grow in. Mangrove seedlings have been found to be more sensitive to salt stress, where their growth is said to be negatively affected in fresh water as they are halophytes and at high salinity where salinity is above 17,5 ppt (Yan *et al*., 2007). Optimal growth has been found to occur at about 9 ppt (Yan *et al*., 2007).

According to Gonçalves-Alvim *et al*. (2001) salinity may influence the primary productivity, root to shoot ratios, leaf area, internode length, leaf morphology, propagule size and tree structure. Mangroves occurring in salt stress regions have been found to have lower stature, are evergreen and have long-lived scleromorphic leaves (Gonçalves-Alvim *et al*., 2001). According to Hossain and Nuruddin (2016) at high salinity productivity and growth may be decreased as more resources will be used for maintaining water balance and ion concentration. To survive in such variable conditions, *A. marina* has specialised glands on their leaves for salt removal (Steinke, 1999), *B. gymnorrhiza* has ultra-filters in their root system, which exclude the salt from the water whilst extracting water (Kathiresan and Bingham, 2001) and *R. mucronate* exhibits salt removal by accumulating it on older leaves which are later shred off (Hoppe-Speer *et al*., 2013).

Low salinity also poses negative impacts on mangroves. Tuffers *et al*. (2001) demonstrated that the photosynthetic performance of *A. marina* occurring at Beachwood – Mngeni Estuary (salinity less than 12 ppt) was curbed as there was a reduction in carboxylation capacity and conductance which resulted in lower transpiration rates. Mangroves vary in their tolerance to the various environmental drivers (factors), the tolerance range of a species to a particular variable should not be necessarily viewed in isolation as other co-factors may also play a regulatory role. Clough (1993) states that sediment characteristics such as low moisture content, climatic factors such as low temperature and marked seasonal aridity may result in reduced tolerance of hypersaline soils whilst some species may survive at higher salinities when other environmental variables are not limiting.

Salinity ranges differ for each species, according to Duke (1995) *A. marina* has a broad estuarine range upriver from the mouth, in South African populations Ward and Steinke (1982) found this species to generally not occur in estuaries which had reduced tidal influence and low salinities. Clough (1993) states that *A. marina* and *Lumnitzera racemosa* have been found to tolerate salinities up to 90 ppt whilst *Rhizophora mangle* up to 60 ppt, these values which are significantly higher than the 35 ppt of seawater. Salinity ranges are important as they determine which species are going to be found and thus affecting interactions such as competition, predator-prey dynamics and ecosystem services (Lugo and Snedaker, 1974; Carrasco and Perissinotto, 2012; Sandilyan and Kathiresan, 2012).

Sediment

Sediment found in the mangrove environment ranges from those with a large mineral input such as your alluvial habitats to those which receive little to no allochthonous inputs of sediment such as oceanic islands (Mckee *et al*., 2007). Sediment in the mangrove environment experience occasional or prolonged periods of low oxygen which various species have morphological or physiological adaptations which allow them to persist in the environment (Kathiresan and Bingham, 2001). Mangroves prefer soils which are flooded and well-drained, and generally do not do well in stationery water (Kathiresan and Bingham, 2001). The physiochemical status of the sediment such as moisture content, organic content, pH and redox have an influence in the functioning of the mangroves, species composition and forest structure (Hossain and Nuruddin, 2016).

Moisture content

Moisture and temperature are said to be the most important factors in mangrove distribution, where conditions such as low moisture and low temperature are said to influence the occurrence of mangroves (Kathiresan and Bingham, 2001, Duke and Schmitt, 2015). Moisture in mangroves is influenced by tidal inundation, tidal flushing rates and freshwater inputs (Hoppe-Speer *et al*., 2013). High rainfall provides freshwater input and nutrient supply from rivers, and runoff thus lowering salinity levels and playing a role in plant growth (Gilman *et al*., 2008). Waterlogged soils result in anaerobic and chemically reduced conditions around the roots due

to the rapid reduction of O_2 (Naidoo *et al*., 1997). According to Naidoo *et al*. (1997) *A. marina* can survive up to 5 days in constantly waterlogged soils.

On the other hand the successful functioning of the forest may be impeded in areas which experience drought or insufficient moisture. This is supported by Duke *et al.* (2017) who suggested that the severe mangrove die back experienced at the Gulf of Carpentaria (Northern Australia) was a result of insufficient moisture in the sediment as tide levels decreased to very low levels as a result of a period of severe drought (approximately 10 to 11 months of low rainfall), high temperatures and a decrease in sea level. Moisture also plays an important role in the establishment of mangrove seedlings. Clarke and Myerscough (1991), found that even though the propagules of *Avicennia marina* var *australasia* may reach the saltmarsh environment, establishment is expected to be limited by moisture stress as the propagules would desiccate and thus not establish. Other physiochemical factors that influenced seedling establishment included high salinity levels and sediment type.

Nutrients

Nutrients supplied by terrestrial runoff play an important role in the development of mangrove forests (Chen and Twilley, 1999a). It is suggested that nutrients play an important role in the growth of mangroves, where increase in nutrient levels has been shown to increase plant growth and *vice-versa* (Lovelock *et al*., 2004; Lovelock *et al*., 2009). The disadvantage of increased nutrient supply is that those mangroves need to be adapted to environmental stressors such as drought which requires large investments in root tolerance (Lovelock *et al*., 2009)

Nutrients maybe retained within the forest because of decomposition. The amount retained is determined by the residence time of the nutrients, the amount of litter fall, rate of decomposition and tidal action. Some of the nutrients are transported to neighbouring coastal systems facilitated by tidal hydrology, thus playing an important role especially to those systems which have low productivity allowing for the coastal waters to support a greater number of aquatic communities (Gattuso *et al*., 1998; Sánchez-Andrés *et al*., 2010; Elliot and Whitfield, 2011).

Organic matter

The mangrove environment is said to be generally nutrient poor, the availability of nitrogen, phosphorus and sulphur (macronutrients) to plants is mainly obtained from organic matter (Chaudhari *et al*., 2013; Hogarth, 2015). The biogeochemical cycles of nitrogen and phosphorus largely influences organic matter dynamics because of the various processes which occur in mangrove sediment such as decomposition, mineralization and plant uptake (Chen and Twilley, 1999b).

Not only is organic matter important in nutrient cycling making it an important source of nutrients but may also play a role in improving sediment structure (Chaudhari *et al*., 2013). High organic matter content in the mangrove environment is encouraged by mangrove trees which act as a source and as a sink of allochthonous and autochthonous substances, waterlogging and low mineralization processes (Marchand, 2017; Bastakoti *et al*., 2019). A large amount of organic matter is a result of decomposed mangrove roots whilst mangrove litter and algae also act as a source (Chen and Twilley, 1999b; Bastakoti *et al*., 2019). Rajkaran and Adams (2007) found mean litter production in the Mngazana forest was 1.5 ± 0.5g m^{-2} d^{-1} and for the Nxaxo/Ngqusi, Steinke and Ward (1990) found it to be 1.24 g m^{-2} d^{-1}. Studies have shown that litter production varied between species, habitat, climatic zones, latitude and other local conditions such as seasonality (Ochieng and Erftemeijer, 2002; Rajkaran and Adams, 2007).

Redox

Mangrove sediment generally have high productivity, high organic matter and are waterlogged, thus the conditions found are generally reducing (anoxic) (Naidoo *et al*., 1997; Hogarth, 2015; Otero *et al*., 2017). According to Alongi *et al*. (2005) the high organic matter in anoxic soils facilitates higher nutrient storage in the soil. This may result in nutrient availability to mangrove roots thus facilitating in mangrove growth (Reef *et al*., 2010). Alongi (2009) states that the influence of anoxic sediment on mangrove growth may vary across species, growth stage and the duration of the anoxic state. Marchand (2017) states that the tidal zone may also play a role in redox processes that facilitate organic matter mineralization. Duration of tidal inundation plays a role in microbial transformation and exchange processes, and tidal depth plays a role in the amount of material which is stored within the forest to that which is transported to nearby coastal waters (Kristensen *et al*., 2017).

pH

According to Reef *et al.* (2010) mangroves are generally acidic. Whilst Hossain and Nuruddin (2016) studies show that mangrove sediment in tropical regions has been found to be either alkaline (ranging between 7.4-8.22) or acidic (ranging between 2.87-6.40). Joshi and Ghose (2003) assessed the performance of eight Sundarbans mangrove and mangrove associates (in Lothian Island) along the pH gradient. These species occurred in a slightly alkaline environment (sediment ranged between 7.05 to 7.89). *Avicennia marina* preferred an optimum 7.55 and 7.82, while the majority of the species preferred 7.65, these species occurred between 7.02 to 7.89. **Table 2.3** reports on the optimum pH conditions that some mangroves have been found to grow in. The pH of sediment may indirectly influence mangrove growth; due to its influence on the availability and uptake of elements, solubility of minerals and biological activity. For example at a lower pH, phosphorus is limited, limitation of phosphorus an essential element will impede microbial processes which play an important role in decomposition and thus nutrient availability (Black *et al*., 2008; Hossain and Nuruddin, 2016).

Plant Performance

Demography, fecundity and fitness

According to a review by Pironon *et al.* (2015) studies on plant performance have measured variables including population growth rate, survival, growth, fecundity and recruitment which are considered important population processes. Plant performance may be related to plant fitness, to determine overall fitness variables that are generally employed include reproductive success, growth rate, fecundity, survival, but their contribution vary, where for example fecundity maybe given preference to growth rate (Keller and Wallar, 2002; Reed and Frankham, 2003; Wise *et al*., 2003). According to Engelhardt *et al*. (2014), the influence of size and genetic diversity on plant performance will depend on population source and environmental conditions.

A study on *Brassica rapa* an herbaceous annual plant showed that when comparing populations with high genetic variation to those with low genetic variation but are similar in size, number of founders and level of inbreeding, populations with high genetic variation only displayed a fitness advantage under environmentally stressful conditions (heat stress). Under normal conditions the fitness traits measured (number of fruits and seeds, aboveground dry weight and

total seed weight) were found to be somewhat similar (Wise *et al*., 2003). This is supported by Engelhardt *et al*. (2014) on terrestrial habitats, which suggests that under environmental stress the maintenance of genetic diversity may become increasingly more important due to the reduced population persistence and evolutionary potential.

According to Wise *et al.* (2003), low levels of genetic diversity may curb the populations response to rapid environmental changes such as climate change, increase in herbivory, parasitism and disease. Pautasso *et al*. (2009) suggest that more research regardingthe role of genetic diversity on: phenological patterns, plant resistance to insect herbivory and pathogenic attack by fungi, response to climate change, growth, mortality rates and other traits.

Insect herbivory, parasitism and disease

Insect herbivory is important as it regulates and maintains the functioning of the forest ecosystem (Anderson and Lee, 1995). It may be encouraged by several factors such as tree age, leaf age, canopy cover, tidal height and nutrient enrichment (Farnsworth and Ellison, 1991). However, insect herbivory may also cause extensive damage to the mangrove trees, there are various forms of damage which can be caused by insect herbivores which include galls, holes, necrotic spots, leaf miner attack and incursions along the leaf margin (Balasubramanyan *et al*., 2010). Resh and Cardé (2009), suggest that galls are a result of complex chemical interactions that are stimulated by the gall inducing insect and that the process does not harm the host plant but only diverts plant resources. Insects form these structures so that they may receive shelter and food (Tooker and Moraes, 2008).

A study on a number of mangroves in South Africa, found *A. marina* to have a high presence of leaf galls on their leaves and to be more vulnerable to disease caused by pathogens or other microbes when compared to the other species which occur in the region. A higher incidence of tree die-back and canker where found in trees which experienced a higher occurrence of the bark beetle (which causes a hole in the bark and may result in bark bleeding) and higher pneumatophore siltation (Osorio *et al*., 2017b). According to a review by Saenger (2002), in Australia, *A. marina* was found to be more prone to interaction with *Phytophthora (Halophytophthora) operculate* (a parasitic fungus), which generally plays a role in decomposing leaf litter but may become pathogenic, attacking the roots which may result in

plant mortality when a balance between the fungi and host is lost. The attack may be enhanced by natural or anthropogenic disturbances (Saenger, 2002).

Plant resistance and resilience

Alongi (2015) states that due to the environment in which mangroves occur, most of them and associated organisms are prone to being resilient or resistant to most environmental changes. The mangrove environment may be subjected to several natural disturbances such as drought, storms, hurricanes and tsunamis which through the measurements of their performance during and post such as events have been said to be an indication of their resilience or resistance (Capdeville *et al*., 2019; Rivera-Monroy *et al*., 2019).

According to Capdeville *et al*. (2019) the return of parameters such as normalized difference vegetation index (NDVI) and the net primary productivity following a natural event has been used to determine the resilience of mangrove forests. A study on everglade mangrove forests in Florida following a hurricane event showed that the net primary productivity and forest structure was not significantly different because of the mortality and defoliation thus showing the resistance of the mangrove forests (Rivera-Monroy *et al*., 2019).

Their resistance and resilience is displayed by how they perform during and post a disturbance respectively and may be influenced by their genetic diversity. Hughes and Stachowicz (2004) found that *Zostera marina* (seagrass) with higher genotypic diversity using microsatellite markers experienced lower reduction in shoot density (number of shoots per plot) and faster rate of recoveries to "original" densities following high levels of predation by *Branta bernicla* subsp. *nigricans* (Brant geese), prior to the predation there was no difference in shoot densities according to genotypic diversity. Even though the results indicated that plants with higher genetic diversity showed resistance to the disturbance but resilience was not evident as these plants did not have a higher re-growth of shoots (shoot recovery) post disturbance. Whilst a study by Guo *et al*. (2018) found a positive correlation between genetic diversity and resilience of six mangrove species (*Avicennia marina, Aegiceras corniculatum, Ceriops tagal, Sonneratia alba, Rhizophora apiculate* and *Xylocarpus granatum*) following a climatic disturbance, populations which had lower genetic diversity experienced higher death rate following a flooding event. This study suggests that the extremely low genome-wide nucleotide diversity found in the studied 26 populations occurring along the Indo-Malayan

coast was possibly a result of historical sea level changes which lead to the effective population size being very small.

Mangrove regeneration and restoration

Mangrove structure and forest development are primarily influenced by stem mortality and recruitment (Rivera-Monroy *et al*., 2019). Seedling recruitment, growth and survival rates play an important role in the regeneration of a mangrove forest with some being indicators of restoration post disturbance (Sousa *et al*., 2003; Salmo *et al*., 2013). Salmo *et al*. (2013) suggests that tree density, biomass and Leaf Area Index (LAI) could be also considered as indicators of restoration due to their association with the age of the mangrove stand. Generally, post disturbance mangroves regenerate naturally without any intervention (Bosire *et al*., 2008). An example of this is Kobonqaba Estuary (Eastern Cape Province), which according to Mbense (2017) has shown potential for natural regeneration post experiencing a massive dieback due to a mouth closure which was a result of drought and sea storms events. Van Loon *et al*. (2016) states that the physical site conditions are vital in mangrove restoration, this includes the salinity, sediment condition and hydrology.

Mangrove restoration is generally indicated by the return of mangrove cover with the desired biomass, stand structure and productivity, occurrence of recruitment, associated biota, ecosystem function and ecosystem services (Bosire *et al*., 2008; Salmo *et al*., 2013).

Using genetic analysis, long term sediment data and plant performance as tools for conservation strategies

Ng *et al*. (2015) postulates that understanding genetic variations of various mangrove species would assist in the identification of areas of conservation priority. Due to peripheral populations tending to have lower levels of genetic variation, Dai and Fu (2011) states that it is generally expected that these populations are afforded greater conservation priority when compared to core populations. They have argued that this should not be a general rule however this decision should be species specific and based on evidence which supports the need. Thus, the above supports the need for this present study, where the genetic variation of mangroves species (*R. mucronata* and *B. gymnorrhiza*) occurring in South Africa (peripheral populations) will be determined, assisting in understanding of what best management strategies would be

required. As part of the strategies to promote resilience, McLeod and Salm (2006) recommends the establishment of baseline data which may play an important role in determining the potential impact of climate change. Adams and Human (2016) collected sediment characteristics and population structure data over some years at Lake St Lucia allowing for the documentation of how mangroves responded to changing environmental conditions. This is especially important with global warming and sea level rise, where understanding local environmental conditions and their influence on mangroves will be important.

Barbeta *et al*. (2011) advocates for long-term data on plant performance rather than "yearly" snapshots as this may provide a clearer image in longer lived species. According to Walters *et al*. (2008), quantitative data on mangroves such as tree height, basal area, stem density, biomass indicators and allometric equations may sometimes be required by decision makers to aid in their conservation. A study by Rivera-Monroy *et al*. (2019) used baseline data collected over a four-year period which included measuring productivity and density prior to the occurrence of a hurricane, to determine its impact and the recovery of the forest. Such information will also assist in which species are most likely to be influenced by climate change. Literature suggests that the impact of climate change may vary among species, where species in the genus *Rhizophora* are expected to perform better than those in the genera *Bruguiera*, *Ceriops* and *Xylocarpus* due to having higher growth and reproduction rates and more efficient dispersal capabilities (Polidoro *et al*., 2010).

Ultimately the conservation of mangroves strengthens the provision of several important ecosystem services including; land stabilization, nutrient cycling, process pollutants, they act as natural barriers against storms and cyclones thus protecting the lives and property of neighbouring communities and their environment provide nutrients to people and animals (Walters *et al*., 2008). Even though mangroves provide several ecosystem services and have been evaluated to be of high value, these forests face several threats. Threats to the mangrove ecosystem include over-exploitation, deforestation because of urban development, agriculture, aquaculture and climate change, natural factors such as diseases, pests and parasites, and natural disturbances such as cyclones and tsunamis (Giang *et al*., 2003; Gilman *et al*., 2008; Polidoro *et al*., 2010; Sandilyan and Kathiresan, 2012; Ng *et al*., 2015; Alongi, 2015; Friess *et al*.,2019).

Natural climatic events such as cyclones, flooding, drought and climate change consequences such as sea-level rise have been highlighted as some of the threats which mangrove environment face (Gilman *et al*., 2008; Alongi, 2015; Capdeville *et al*., 2019). Yang *et al.* (2014) predicted that a combination of sea level rise, substrate elevation change and sea storms may pose a threat to African estuaries occurring in the tropical regions which have a large flat intertidal areas and mangroves in the near future (years 2020, 2050 and 2100). This study therefore aims to identify range edge populations which require conservation and provide suitable recommendations for their management in South Africa, and in doing so, safeguarding the ecosystem services provided by these forests.

Management of Mangroves

The International Union for the conservation of Nature (IUCN) endorses the use of genetic analysis as a component that could be used in the conservation of biodiversity (Reed and Frankham, 2003). South Africa is a signatory to The Convention on Biological Diversity (CBD) which aims to conserve biological diversity, promote sustainable use of the components of biological diversity and ensuring fair and equitable sharing of the benefits arising out of the utilization of genetic resources. South Africa is also a signatory to the United Nations Sustainable Development Goals (SDGs), relevant to this study is SDG 14.2, which aims to "*sustainably manage and protect marine and coastal ecosystems to avoid significant adverse impacts, including by strengthening their resilience, and take action for their restoration in order to achieve healthy and productive oceans*". Nationally, various sections in the Integrated Coastal Management Act, 2008 (No 24 of 2008) speak to the management and protection of mangroves. According to the National Forest Act (Act 84 of 1998) both *B. gymnorrhiza* and *R. mucronata* are protected species. The next chapter aims to understand genetic connectivity of six *R. mucronata* and *B. gymnorrhiza* populations using molecular markers.

Chapter 3: Assessing genetic connectivity of *Bruguiera gymnorrhiza* L. and *Rhizophora mucronata* Lam. populations using the *trnL-trnF* intergenic spacer and *PAL-1* region.

Introduction

Mangrove distribution in the East African region stretches from Somalia in the north to its southern range limit in South Africa and includes Madagascar and the Seychelles (Taylor *et al*., 2003; Spalding, 2010). This region has approximately 10 mangrove species, including *Avicennia marina* (Forsk.) Vierh, *Bruguiera gymnorrhiza* L. and *Rhizophora mucronata* Lam. that are core mangrove species and common in the region (Lugendo, 2016; Tomizawa *et al.*, 2017). For this chapter, the genetic connectivity of the latter two species occurring in the region between Tanzania and South Africa will be assessed.

Tanzania hosts the largest continous mangrove forest, which occurs in the Rufiji Delta (about 480.3 km^2 of mangrove cover) (Monga *et al*., 2022; Nitibona *et al*., 2022). Mangroves in this region occur in river estuaries, bays, creeks and on gently sloping shores (Wang *et al*., 2003). The most extensive mangroves in the region occur in Mozambique, distributed along the coast from Cabo Delgado to Maputo Province, generally occurring in sheltered shorelines, deltas and estuaries prevalent in the northern and central regions of the country (Taylor *et al*., 2003; Lugendo, 2016). Mangrove populations in South Africa occur in 32 estuaries from Kosi Bay (KwaZulu-Natal Province) to Tyolomnqa (Eastern Cape Province) (10 in KwaZulu-Natal and 22 in Eastern Cape) (Adams and Rajkaran, 2021).

In comparison with other perennial woody plants species, mangrove species have been found to have low genetic diversity that has been attributed to historical changes in the conditions of their environment, such as changes in sea level in recent geological times (Xu *et al*., 2017; Mantiquilla *et al*., 2021). Studies have reported core populations of various mangrove species in the Indo-West Pacific Province (IWP) to have greater diversity than those occurring at the range limit (Mantiquilla *et al*., 2021). Using microsatellite markers, Maguire *et al*. (2000) found that range edge *Avicennia marina* populations had low genetic variation when compared to core populations, where a population in Beachwood – Mngeni Estuary (South Africa) and other range edge populations had lower heterozygosity and significant inbreeding when compared to

populations from South Australia and Papua New Guinea (core populations), which generally showed high outcrossing rates and low levels of inbreeding. Similar results were found in a study by De Ryck *et al*. (2016), which is more comparable to the current study with regards to the populations that were used, however, it should be noted that different genetic markers were used. The study found that South African populations of *A. marina* had a lower genetic diversity and higher inbreeding when compared to the core populations (Tanzania and Kenya).

The genetic connectivity of mangrove populations occurring between Tanzania and South Africa may be assessed using molecular markers (Binks *et al*., 2019; Mantiquilla *et al*., 2021). For the present study, a single chloroplast (*trnl-trnF* intergenic spacer region) and nuclear region (*PAL-1* region) were used for both *R. mucronata* and *B. gymnorrhiza*. The genetic connectivity of distant mangrove populations is majorly influenced by the dispersal of their water borne propagules, which allows for gene flow and relies on ocean currents (Tonné *et al*., 2017; Melville and Burchett, 2002). This, along with vicariance events, quaternary climatic oscillation and changes in sea level in recent geological times have played a role in the current distribution and genetic variation of mangroves (Wee *et al*., 2017; Xu *et al*., 2017).

The dispersal of mangrove propagules has been demonstrated in several studies, where evidence suggests that some mangrove species have experienced long distance dispersal (LDD) allowing for the genetic connectivity of distant populations (Lo *et al*., 2014, Pil *et al*., 2011; Takayama *et al*., 2021). According to a study by Steinke and Ward (2003), mangrove propagules from South Africa have the potential of reaching as far as South America (Brazil) and Australasia (Australia and New Zealand). This was demonstrated using plastic cards that had the same weight as *Avicennia marina* propagules (Steinke and Ward, 2003). However, factors such as (1) how long the propagules could survive, (2) the prevailing conditions while *en route* and (3) the availability of suitable habitat and environmental conditions at the receiving area play an important role in propagules being dispersed and successfully establishing in distant regions (De Ryck *et al*., 2012; Clarke, 1993; Sousa *et al*., 2003).

Propagules of *R. mucronata, B. gymnorrhiza* and *A. marina* differ in size, shape, buoyancy and dispersal strategy, thus it is expected that the genetic connectivity of distant populations of the former two species may differ from that of *A. marina* and from each other (De Ryck *et al*., 2012; Van der Stocken, 2015a; Van der Stocken and Menemenlis, 2017). Other factors that

will play a role include wind, ocean currents, water temperature, landscape structure and environmental conditions (De Ryck *et al*., 2012; Lo *et al*., 2014; Van der Stocken *et al*., 2015a; Van der Stocken *et al*., 2015b; Tonné *et al*., 2017).

Tanzania, Mozambique and South Africa occur along the same coastline. Between Tanzania and Mozambique there is no physical barrier such as a land masses, thus, high gene flow is expected (Triest, 2008; Amade *et al*., 2021). The South African landscape may limit gene flow due to mouth conditions (e.g., periods of mouth closure) and the mangrove populations are fragmented thus propagules may have difficulty in finding suitable habitat (Triest, 2008; De Ryck *et al*., 2016; Binks *et al*., 2019).

The dispersal of *R. mucronata* and *B. gymnorrhiza* propagules in the region between Tanzania and South Africa may be facilitated by three ocean currents, namely the Mozambique (Eddies) Current, the Agulhas Current and the South Equatorial Current (SEC) (Potts *et al*., 2015). The SEC connects with the Mozambique eddies and together with the water from East Madagascar current it connects with the Agulhas currents (Potts *et al*., 2015). Amade *et al*. (2021) found high gene flow between *A. marina* populations occurring along the coast of Mozambique, which the study stated may be due to the currents and eddies occurring along the Mozambique channel. It is expected that the South Equatorial Current (SEC) may have an influence in the gene flow between Tanzania and Mozambique as it splits at the border between the two countries (De Ryck *et al*., 2016). Thus, there may be population disjunction. This is supported by a study by Triest *et al*. (2021).

The present study will assess the genetic connectivity of six *R. mucronata* and *B. gymnorrhiza* populations using molecular markers. No other study has assessed the genetic connectivity of population of these two species in the East African region, albeit studies looking at larger regions, have included some populations occurring in Mozambique and South Africa. Thus, this study could provide a better understanding of the genetic connectivity of these populations and the factors that drive the populations. Measuring the genetic variation of *R. mucronata* and *B. gymnorrhiza* is important in the conservation of the genetic diversity of mangroves, which may be important in safeguarding these special habitats and maintaining the ecosystem services they currently provide. Dasgupta *et al*. (2015) stated that molecular markers could be used in the identification of priority areas for conservation. Such a study can provide information on

the historical processes and the contemporary gene flows allowing for predictions to be made on how populations may respond to ecological conditions and climate change (Islam *et al*., 2015; Guo *et al*., 2018).

Aims and Objectives

Currently there is a limited number of studies that have measured the genetic variation of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* populations occurring in the East African region (Urashi *et al*., 2013; Lo *et al*., 2014; Wee *et al*., 2015; Yan *et al*., 2016; Triest *et al*., 2021, Takayama *et al*., 2021). The objective of the present study was to determine the genetic diversity and genetic connectivity of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* populations occurring in Tanzania, Mozambique and South Africa. The following hypotheses will be tested:

a) It is expected that there will be higher gene flow between Tanzania and Mozambique and thus little genetic differentiation between the two regions.
    (i) A review by Triest (2008) on current molecular studies on mangrove trees found that genetic diversity studies of species occurring on the same coastlines, have shown high levels of gene flow.
    (ii) However, according to De Ryck *et al*. (2016) and Triest *et al*. (2021) gene flow may be limited due to the South Equatorial Current (SEC).
b) Due to the South African populations occurring at the range limit, it is expected that genetic diversity will be lower than that found in Tanzania and Mozambique (Islam *et al*., 2015).
    (i) Edge populations will have lower genetic variation when compared to the core populations (Yan *et al*., 2016; Arnaud-Haond *et al*., 2006; De Ryck *et al*., 2016, Triest, 2008). Triest (2008) suggested that these populations may be susceptible to genetic erosion.
    (ii) High genetic differentiation between Tanzania to Mozambique vs South African populations is expected (among populations) (Eckert *et al*., 2008; Geng *et al*., 2008).
    (iii) Larger northern populations, such as Richards Bay and Mngazana, will differ genetically (greater genetic variation) to those occurring in the southern regions of South Africa namely; Nxaxo/Ngqusi and Nahoon (Maguire *et al*., 2000; De Ryck *et al*., 2016)

c) Due to the geomorphology of the South African coastline, where the populations are smaller and more fragmented, genetic flow within and between populations is expected to be low.

Materials and Methods

Study Site Description

Figure 3.1 shows the mangrove distribution from Tanzania to South Africa, leaf samples were collected randomly from different plants, with a single leaf representing an individual. The number of leaves collected at Nxaxo/Ngqusi (WAVE) and Nahoon (NAH) were lower than the other collection sites due to the small population size (**Table 3.1**). Sample collection was from a single site in Tanzania and Mozambique, whilst for South Africa collections were made from four locations (populations) (**Table 3.1**).

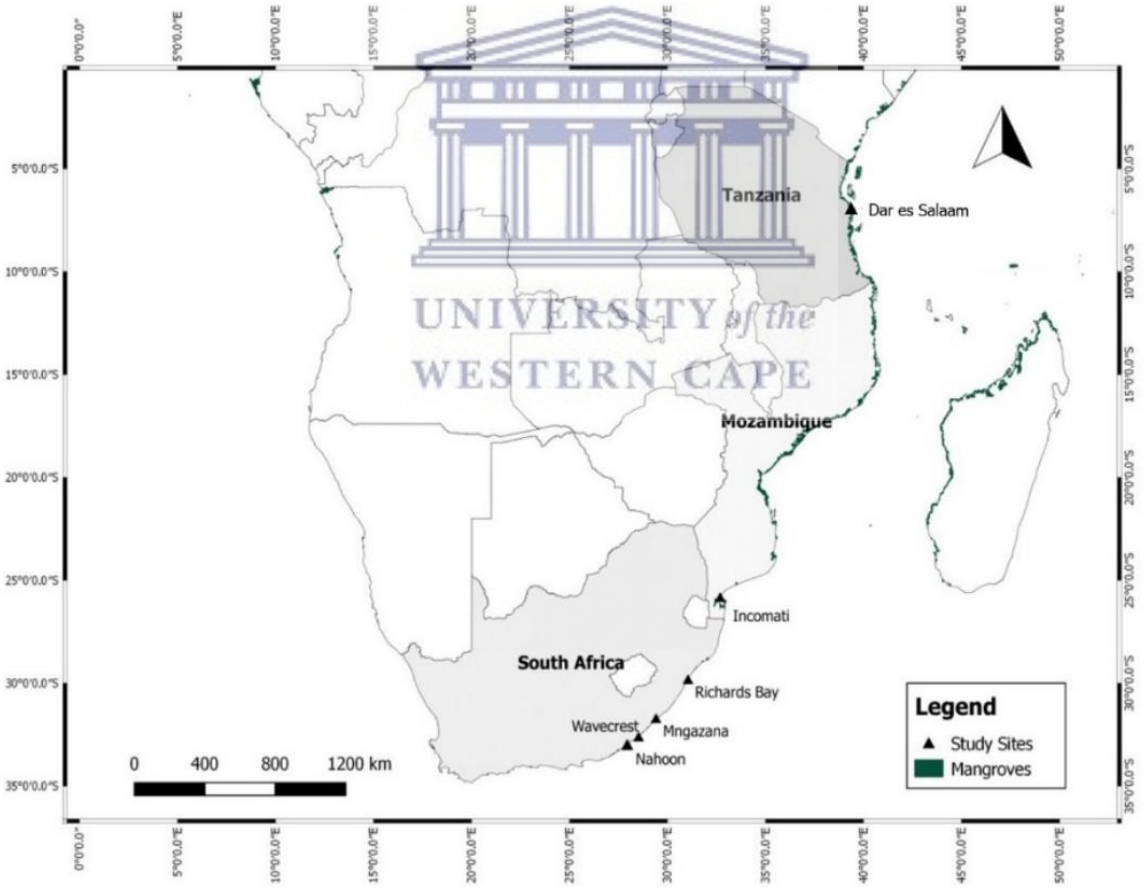


Figure 3.1: Leaf collection location and mangrove distribution in Tanzania, Mozambique and South Africa along the coast of East Africa (Mangrove distribution mapped by Giri *et al.* (2011)).

DNA extraction

DNA extraction was achieved by drying the leaf samples in silica gel. Grinding dried leaf samples into a fine powder with liquid nitrogen and extracting total DNA using a QIAGEN DNeasy Plant Mini Kit following the manufacturer's protocol.

Gel electrophoresis

A 0,8% agarose gel was prepared by adding 0.16 g of Agar to 20 ml of TBE Buffer solution (pH 8) and heating in the microwave. The solution was slightly cooled by swirling in a water bath and 4 µl of Ethidium Bromide added.

Table 3.1: Leaf sample location and number of leaves for both *Rhizophora mucronata* and *Bruguiera gymnorrhiza*.

Country	Location	Latitude	Longitude	No. of Samples	
				Rhizophora mucronata	*Bruguiera gymnorrhiza*
Tanzania	Dar es Salaam	6°44'48.48"S	39°14'34,78"E	10	10
Mozambique	Incomati	25°48'38.78"S	32°41'04.62" E	10	10
South Africa	Richards Bay	29°48'2.16"S	31° 2'34.27"E	10	10
South Africa	Mngazana	31°41'31.84"S	29°25'16.11"E	10	10
South Africa	Nxaxo/Ngqusi	32°35'1.69"S	28°31'18.74"E	6	10
South Africa	Nahoon	32°59'0.23"S	27°56'32.97"E	3	8

Selection of primers

The chloroplast marker *trnL-trnF* (**Figure 3.2**) is a non-coding region that has been found to be efficient for a wide variety of species making it suitable for use in population biology studies (Taberlet *et al*., 1991). According to Shaw *et al.* (2014) it is one of the most widely used regions in plant species, although its efficiency in revealing genetic variation can be lower when compared to other regions. Even so, in mangroves, not many gene regions have been used. This region was thus selected mostly because of its successful use in determining genetic variation in other *Bruguiera gymnorrhiza* and *Rhizophora mucronata* studies such as Minobe *et al*. (2010) and Inomata *et al*. (2009), respectively.

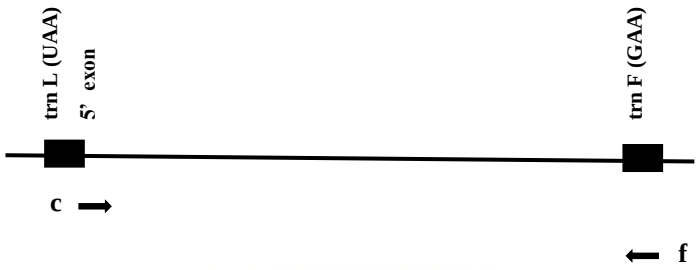


Figure 3.2: *TrnL-trnF* intergenic spacer region used in this study (Taberlet *et al*., 1991).

For the nuclear region, primers for the *phenylalanine ammonia lyase* (*PAL*) region were chosen. *PAL* plays an important role in plant development and defence, and several *PAL* enzyme copies are said to be found in the nuclear genome (Chang *et al*., 2008). In the present study, the *PAL-1* primers as described by Inomata *et al*. (2009) were used. This gene region was selected as it has been shown to be efficient in other studies, for example Inomata *et al*. (2009) used it for *Rhizophora mucronata* whilst a study by Urashi *et al*. (2013) used it for *Bruguiera gymnorrhiza.*

PCR Amplification

The polymerase chain reaction (PCR) amplification procedure followed was similar to that outlined by Inomata *et al*. (2009). PCR reactions comprised of 12,5 µl Emarald AMP GT PCR Master Mix, 0,5 µl BSA, 11,4 µl water, 0,25 µl of the reverse primer, 0,25 µl the forward primer and 1 µl DNA. Some of the DNA samples were further diluted with sterile water or cleaned using the Zymo OneStep™ PCR Inhibitor Removal Kit (Clancy *et al*., 2001). The reactions were then carried out in a BIO-RAD T100 thermal cycler where it went through initial denaturation at 95°C for 3 minutes, 35 cycles of denaturing at 95°C for 30 seconds, annealing

was conducted at 50°C for 30 seconds, polymerization at 72°C for 2 minutes and the final extension at 72°C for 7 minutes. For samples that were difficult to amplify, a temperature ramp needed to be included, this was achieved following Shaw *et al.* (2005).

Purification of PCR product using ExoSAP

The following ExoSAP procedure was used to purify the PCR products (Werle *et al*., 1994): To each PCR product 3 µl of the ExoSAP mixture containing 2.25 µl H_2O, 0.25 µl exonuclease I (Exo) and 0.50 µl shrimp alkaline phosphatase (SAP) was added. In the BIO-RAD T100 thermal cycler, the PCR product mixture was incubated at 37°C for 30 minutes, and to inactivate the enzymes the temperature was then raised to 80°C for 15 min.

Data Analysis

DNA sequence data was aligned manually using the MEGA 6 program (Tamura *et al*., 2013). To confirm the identity of the DNA sequences the nucleotide Basic Local Alignment Search Tool (BLAST) from the National Centre for Biotechnology Information (NCBI) was used.

To determine the haplotype distribution, DnaSP 6.12 was used, and alignment gaps were not considered in the analysis (Rozas *et al*., 2017). Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997) neutrality test and the fixation index or spatial variation of gene frequency Fst (Wright 1943) were computed using DnaSP 6.12 (Rozas *et al*., 2017). Tajima's D and Fu's Fs were computed using indel polymorphism multi-allelic analysis, whilst Fst was computed on haplotypes by treating gaps or indels as fifth state mutations (Rozas *et al*., 2017). Haplotype network maps were drawn using Network 5.0.1.1 (Fluxus Technologies Ltd). DNA polymorphisms (nucleotide diversity and haplotype diversity) were computed using DnaSP 6.12 (Rozas *et al*., 2017). Using Arlequin v. 3.5 (Excoffier and Lischer, 2015), the following estimates for the *PAL-1* region were calculated; population pairwise non-differentiation exact p-value, gene diversity, mean pairwise differences and nucleotide diversity. These estimates could not be provided for *trnL-trnF* as the data for this region did not have any polymorphic sites.

Results

Samples for this study were collected from six locations, although some of the samples collected could not be successfully amplified and used for analysis (**Table 3.2**). This was due

to either noisy sequences that could not be used, a PCR product could not be produced, or most likely due to interference of secondary compounds.

Table 3.2: Number and details of samples successfully sequenced from each location.

Country	Location(code)	Latitude	Longitude	No. of Samples Sequenced (*PAL-1*)		No. of Samples Sequenced (*trnL-trnF*)	
				Rhizophora mucronata	***Bruguiera gymnorrhiza***	***Rhizophora mucronata***	***Bruguiera gymnorrhiza***
Tanzania	Dar es Salaam (TAN)	-6. °44'48.48"S	39 °14'34.78"E	10	10	6	10
Mozambique	Incomati (MOZ)	25°48'38.78"S	32°41'04.62"E	9	10	10	10
South Africa	Richards Bay (RB)	29°48'2.16"S	31° 2'34.27"E	10	8	9	7
South Africa	Mngazana (MNG)	31°41'31.84"S	29°25'16.11"E	10	8	9	7
South Africa	Nxaxo/Ngqusi (WAVE)	32°35'1.69"S	28°31'18.74"E	5	8	5	7
South Africa	Nahoon (NAH)	32°59'0.23"S	27°56'32.97"E	3	7	3	6

trnL-trnF chloroplast region

For *Bruguiera gymnorrhiza*, a total of 47 sequences for the *trnL-trnF* intergenic spacer region with 933 nucleotide sites were obtained, no sites were variable (polymorphic), thus Tajima's D and Fu's Fs statistic could not be calculated. Gene flow values for each population are shown in **Table 3.4**. Haplotype (gene) diversity could not be measured. Nucleotide diversity(π) ranged between 0.00 to 0.0036 and nucleotide polymorphism(Θ) ranged between 0.00 to 0.762 showing that the nucleotide variation was low. Gene estimate values Fst and Nm were 0.06849 and 3.40, respectively, calculated using the Hudson *et al*. (1992) method.

Results for *R. mucronata* were similar, a total of 42 sequences for the *trnL-trnF* intergenic spacer region with 712 nucleotide sites were obtained, nosites were variable (polymorphic) and parsimony informative, and there were no indels. Thus, no unique haplotypes were obtained and the Tajima's D and Fu's Fs statistic could not be computed. From the data no other estimates could be computed as there were no informative sites.

Population Genetic structure and nucleotide divergence- *Bruguiera gymnorrhiza*

F_{ST} values measured on DNSP ranged between 0.000 and 0.111, which illustrated that there was low genetic differentiation, where the largest genetic differentiation occurred between Mngazana (MNG) and Tanzania (TAN), MNG and Richards Bay (RB), and MNG and

Nxaxo/Ngqusi (WAVE) (0.111) (**Table 3.3**). Nucleotide divergence between populations ranged from 0.0000 to 0.00103 (**Table 3.3**). In the analysis for pairwise comparison where gaps and missing information was excluded, nucleotide diversity was π (0.00175), whilst at individual sites nucleotide diversity was π (0.00300), which is low.

Table 3.3: Matrix of pairwise comparisons of population genetic differentiation calculated using the infinite alleles model (Fst in grey) and nucleotide divergence between populations (DXY) for the *trnL-trnF* intergenic spacer region of *Bruguiera gymnorrhiza* (BR). *Rhizophora mucronata* samples were not included as Fst could not be calculated.

Location code		TAN	MOZ	RB	MNG	WAVE	NAH	
TAN		-	0.00022	0.00022	0.00038	0.00033	0.00103	
MOZ		0.000	-	0.00011	0.00024	0,00011	0.00037	
RB		0.000	0.000	-	0.00047	0.00000	0.00037	
MNG	**FST**	0.111	0.090	0.111	-	0.00047	0.00058	**DXY**
WAVE		0.000	0.000	0.000	0.111	-	0.00055	
NAH		0.000	0.000	0.000	0.080	0.000	-	

"- " no values/null

Table 3.4: Gene flow and DNA polymorphism within each sampled *Bruguiera gymnorrhiza* (BR) and *Rhizophora mucronata* (RM) population estimated using the chloroplast DNA sequences.

Location code	Species Code	Polymorphic sites/indel/missing sites	Haplotypes (No.)	Haplotype Diversity (Hd)	k (Θ)	Nucleotide Diversity (π)
TAN	$BR_{(N=10)}$	0	1	0.000	0.000	0.0023
	$RM_{(N=6)}$	-	-	-	-	0.0015
MOZ	$BR_{(N=10)}$	2	2	0.200	0.200	0.0014
	$RM_{(N=10)}$	-	-	-	-	0.0005
RB	$BR_{(N=7)}$	0	1	0.000	0.000	0.0019
	$RM_{(N=9)}$	-	-	-	-	0.0000
MNG	$BR_{(N=7)}$	2	3	0.524	0.762	0.0040
	$RM_{(N=9)}$	-	-	-	-	0.0004
WAVE	$BR_{(N=7)}$	0	1	0.000	0.000	0.0000
	$RM_{(N=5)}$	-	-	-	-	0.0000
NAH	$BR_{(N=6)}$	1	2	0.333	0.333	0.0036

	$RM_{(N=3)}$	-	-	-	-	0.0000

"-" = Calculation could not be made

K = Theta, average number of mutations per site

N = Number of sequences/individuals

PAL-1 nuclear region - *B. gymnorrhiza*

For *B. gymnorrhiza*, a total of 51 sequences for the *PAL-1* region with 883 nucleotide sites were obtained. A total of 879 sites were invariable (monomorphic), two sites were variable (polymorphic), there were 2 indels, and one site was parsimony informative The average number of nucleotide differences in pairwise comparison was 0.281. A total of three haplotypes were found when sites with gaps were not considered ($\pi = 0.00032$, Hd = 0,275). Haplotype_1 (H_1) was common to all the mangrove populations being present in 43 sequences (84.31%), haplotype_2 (H_2) had a single sequence (1.96%) from Richards Bay (RB) and six sequences (11,76%) from Tanzania (TAN). Haplotype_3 (H_3) had a single sequence (1.96%) from Nxaxo/Ngqusi (WAVE) as shown in **Figure 3.3**. Haplotype diversity among the populations ranged from 0.29 in Nahoon to 0.82 in Tanzania measured in DNSP and the nucleotide diversity ranged from 0.00 (± 0.00) in Nahoon, Mngazana and Mozambique to 0.267 (± 0.272) in Tanzania measured in Arlequin. The results obtained did not show trends according to distribution (for example ranging from Tanzania to Nahoon and *vice versa*, the populations in between do not exhibit the expected trend. Tajima's D was 1.67676, p >0.10 (not significant), whilst Fu's Fs statistic was -0.741. Haplotype (gene) diversity was 0.275 and nucleotide diversity 0.00032. Gene estimates values Fst and Nm were 0.091 and 2.50 respectively, calculated using the Hudson *et al*. (1992) method.

Population genetic structure and nucleotide divergence - *B. gymnorrhiza*

Fst values measured in DNSP ranged between -0.095 and 0.315, which illustrated that there was a low genetic differentiation, where Nahoon and Tanzania (0.315, p-value<0.005) had the largest genetic differentiation (**Table 3.5**). Population pairwise non-differentiation exact P-value (**Table 3.6**) shows the exact test of population differentiation where a number of population pairs displayed a significant difference among populations (p-value <0.05) namely; Tanzania and Mozambique, Tanzania and Mngazana, Tanzania and Nxaxo/Ngqusi and Tanzania and Nahoon. Mean pairwise differences ranged from Nahoon, Mngazana and Mozambique (0.000 ± 0.000) to Tanzania (0.533 (± 0.482)) (**Table 3.7**). Nucleotide divergence between populations ranged from 0.0000 to 0.00082 (**Table 3.5**).

PAL-1 nuclear region - *R. mucronata*

For *R. mucronata*, a total of 47 sequences for the *PAL-1* region with 917 nucleotide sites were obtained, of these 836 were monomorphic and 47 were polymorphic, 28 indels, 35 parsimony informative sites and the average number of nucleotide differences in pairwise comparison was 7.574. A total of 8 haplotypes were found (π = 0.00841, Hd = 0.380), of these H_1 was common to all estuaries, H_2 was only found in Mngazana (MNG), Richards Bay (RB) and Tanzania (TAN), the remaining 6 were found to be "unique" in 4 estuaries; H_3 and H_4 was present in single sequence from Richards Bay (RB), H_5 had a single sequence from Tanzania(TAN) and H_6 had a 2 sequences from Tanzania (TAN), H_7 had a single sequence from Mozambique (MOZ) and H_8 a single sequence from Nxaxo/Ngqusi (WAVE) as shown in **Figure 3.4**. Haplotype diversity among the populations ranged from 0.67 in Nahoon to 0.98 in Richards Bay, measured in DNSP, and the nucleotide diversity ranged from 0.00 (± 0.00) in Nahoon, to 0.346 (± 0.191) in Tanzania measured in Arlequin (**Table 3.7**). The results obtained did not show trends according to distribution (for example ranging from Tanzania to Nahoon and *vice versa*, the populations in between do not exhibit the expected trend). The average number of differences (K, theta) ranged from 0 in Nahoon to 1.96 in Tanzania and Richards Bay (**Table 3.7**). The Tajima's D = -1.24788, p>0.10 (not significant), whilst Fu's Fs statistic was 5.959 (DNSP). Gene Estimates values Fst and Nm were 0.102 and 2.20 respectively, calculated using the Hudson *et al*. (1992) method.

Population Genetic structure and nucleotide divergence - *R. mucronata.*

FST values measured on DNSP ranged between -0.088 and 0.570, which illustrated that there was a low genetic differentiation, where Mozambique and Nxaxo/Ngqusi (0.570, p-value>0.005) had the largest genetic differentiation (**Table 3.5**). Population pairwise non-differentiation exact P-values (**Table 3.6**) showed the exact test of population differentiation where none of the populations displayed a significant difference among populations (p-value >0.05). Mean pairwise differences ranged from Nahoon (0.000 ± 0.000) to Tanzania (16.267 ± 7.931) (**Table 3.7**). Nucleotide divergence between populations ranged from 0.0000 to 0.01192 (**Table 3.5**)

Table 3.5: Matrix of pairwise comparisons of population genetic differentiation calculated using the infinite alleles model (Fst in grey) and nucleotide divergence between populations (DXY in white) for the *PAL-1* region of both *Bruguiera gymnorrhiza* (BR) and *Rhizophora mucronata* (RM).

Location code	Species code		TAN		MOZ		RB		MNG		WAVE		NAH		
			BR	RM	BR	RM	BR	RM	BR	RM	BR	RM	BR	RM	
TAN	$BR_{(N=10)}$	F_{ST}	-	-	0.00068	-	0.00065	-	0.00068	-	0.00082	-	0.00068	-	D_{XY}
	$RM_{(N=10)}$		-	-	-	0.01176	-	0.01448	-	0.01341	-	0.01192	-	0.01168	
MOZ	$BR_{(N=10)}$		0.174	-	-	-	0.00014		0.0000	-	0.00014	-	0.00000	-	
	$RM_{(N=9)}$		-	0.133	-	-	-	0.00511		0.00404	-	0.00035	-	0.00012	
RB	$BR_{(N=8)}$		0.053	-	-0.034*		-	-	0.00014	-	0.00028		0.00014	-	
	$RM_{(N=10)}$		-	0.009	-	0.042	-	-	-	0.00823	-	0.00519	-	0.00496	
MNG	$BR_{(N=8)}$		0.210	-	0.105	-	-0.082*	-	-	-	0.00014	-	0.0000	-	
	$RM_{(N=10)}$		-	-0.001*	-	0.006	-	-0.071*		-	-	0.00412	-	0.00401	
WAVE	$BR_{(N=8)}$		0.144	-	-0.049*	-	-0.095*	-	-0.029*		-	-	0.00014	-	
	$RM_{(N=8)}$		-	0.327	-	0.570	-	0.189	-	0.193	-	-	-	0.00022	
NAH	$BR_{(N=7)}$		0.315	-	0.302	-	0.045	-	-0.080*	-	0.122	-	-	-	
	$RM_{(N=3)}$			0.100	-	-0.212*	-	-0.012*	-	-0.088*	-	0.449	-	-	

N = Number of sequences

"*" Negative values

"- "no values/null

Table 3.6: Population pairwise non-differentiation exact P-value (±SD) for the PAL-1 region of both *Bruguiera gymnorrhiza* (BR) and *Rhizophora mucronata* (RM).

Location code	Species code	TAN	MOZ	RB	MNG	WAVE
MOZ	**BR$_{(N=10)}$**	0.01133 (±0.0005)	-			
	RM$_{(N=9)}$	0.33203 (± 0.0049)	-			
RB	**BR$_{(N=8)}$**	0.06797(±0.0012)	0.44193(±0.0017)	-		
	RM$_{(N=1)}$	0.71426 (±0.0051)	1.00000 (±0.0000)	-		
MNG	**BR$_{(N=8)}$**	0.01266(±0.0008)	-1.00000(±1.0000)	1.00000 (±0.0000)	-	
	RM$_{(N=10)}$	0.43717 (±0.0034)	1.00000 (±0.0000)	0.71614 (±0.0029)	-	
WAVE	**BR$_{(N=8)}$**	0.02006(±0.0008)	0.44558(±0.0021)	1.00000(±0.0000)	1.00000 (±0.0000)	-
	RM$_{(N=8)}$	0.54524 (±0.0054)	0.60917 (±0.0044)	0.84286 (±0.0030)	0.57467 (±0.0036)	-
NAH	**BR$_{(N=7)}$**	0.03352(±0.0013)	-1.00000(±1.0000)	1.00000 (±0.0000)	-1.00000(±1.0000)	1.00000 (±0.0000)
	RM$_{(N=3)}$	1.00000 (±0.0000)	1.00000 (±0.0000)	1.00000 (±0.0000)	1.00000 (±0.0000)	1.00000 (±0.0000)

RM: P value = 0.72739 ± 0.02290, 100000 Markov steps done, 10000 dememorization steps

BR: P value = 0.00028 ± 0.00015, 100000 Markov steps done

Table 3.7: Gene flow and DNA polymorphism within each sampled population for the *PAL-1* region of *Bruguiera gymnorrhiza* (BR) and *Rhizophora mucronata* (RM).

Location code	Species code	Polymorphic sites (observed indels)	Haplotypes (No.)	Haplotype Diversity (Hd)	Gene Diversity (± SD)	k (Θ)	Mean pairwise differences (± SD)	Nucleotide Diversity (π) (± SD)
TAN	**BR** (N=10)	-	-	0.82	-	-	0.533 (± 0.482)	0.267 (± 0.272)
	RM (N=10)	36 (0)	4	0.93	0.6444 (± 0.1518)	1.95636	16.267 (± 7.931)	0.346 (± 0.191)
MOZ	**BR** (N=10)	-	-	0.73	-	-	0.000 (± 0.000)	0.000 (± 0.000)
	RM(N=9)	1(0)	2	0.72	0.2222 (± 0.1662)	0.45466	0.222 (± 0.288)	0.005 (± 0.007)
RB	**BR** (N=8)	-	-	0.64	-	-	0.250 (± 0.311)	0.125 (± 0.177)
	RM (N=10)	44(0)	4	0.98	0.5333 (±0.1801)	1.95636	8.800 (± 4.438)	0.187 (± 0.107)
MNG	**BR**(N=8)	-	-	0.46	-	-	0.000 (± 0.000)	0.000 (± 0.000)
	RM(N=10)	35(0)	2	0.89	0.2000 (±0.1541)	0.42968	7.000 (± 3.594)	0.149 (± 0.086)
WAVE	**BR** (N=8)	-	-	0.75	-	-	0.205 (± 0.311)	0.125 (± 0.177)
	RM (N=5)	0	2	0.90	0.400 (±0.2373)	0.69107	0.400 (± 0.435)	0.009 (± 0.011)
NAH	**BR** (N=7)	-	-	0.29	-	-	0.000 (± 0.000)	0.000 (± 0.000)
	RM (N=3)	0	1	0.67	0.000 (± 0.000)	0	0.000 (± 0.000)	0.000 (± 0.000)

"-" = Calculation could not be made

K = Theta, average number of mutations per site

N = Number of sequences/individuals

Haplotype networks - *B. gymnorrhiza*

Three haplotypes were found in the six *B. gymnorrhiza* populations (**Figure 3.3**), the *PAL-1* haplotype network illustrates that Tanzania (TAN) and Richards Bay (RB) share a haplotype that was one nucleotide base change from the haplotype one (H_1) sequence, and the same as haplotype three (H_3), which was found only in Nxaxo/Ngqusi (WAVE). The number of haplotypes found, and the base pair changes illustrate the low levels of haplotype diversity found in this region.

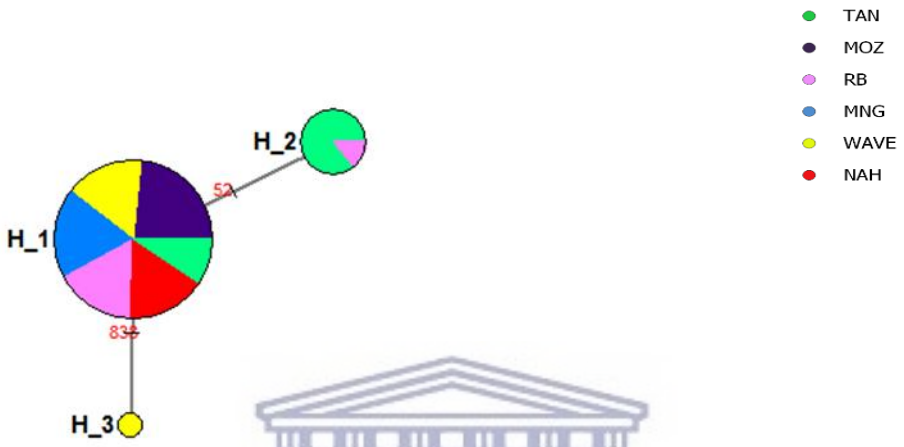


Figure 3.3: Median joining haplotype network for *PAL-1* region of *Bruguiera gymnorrhiza* collected at the six locations. In the haplotype network, the various haplotypes are represented by the circles, its size is relative to the number of samples and the various colours represent the location.

Haplotype networks– *R. mucronata*

The haplotype network for the *PAL-1* region of the six *R. mucronata* populations illustrates that eight haplotypes were found (**Figure 3.4**). H_1 occurs in various locations, H_2 occurs in Mngazana, Richards Bay and Tanzania. The other haplotypes are "unique" to four estuaries; Richards Bay (H_3 and H_4), Tanzania (H_5 and H_6), Mozambique (H_7) and Nxaxo/Ngqusi (H_8).

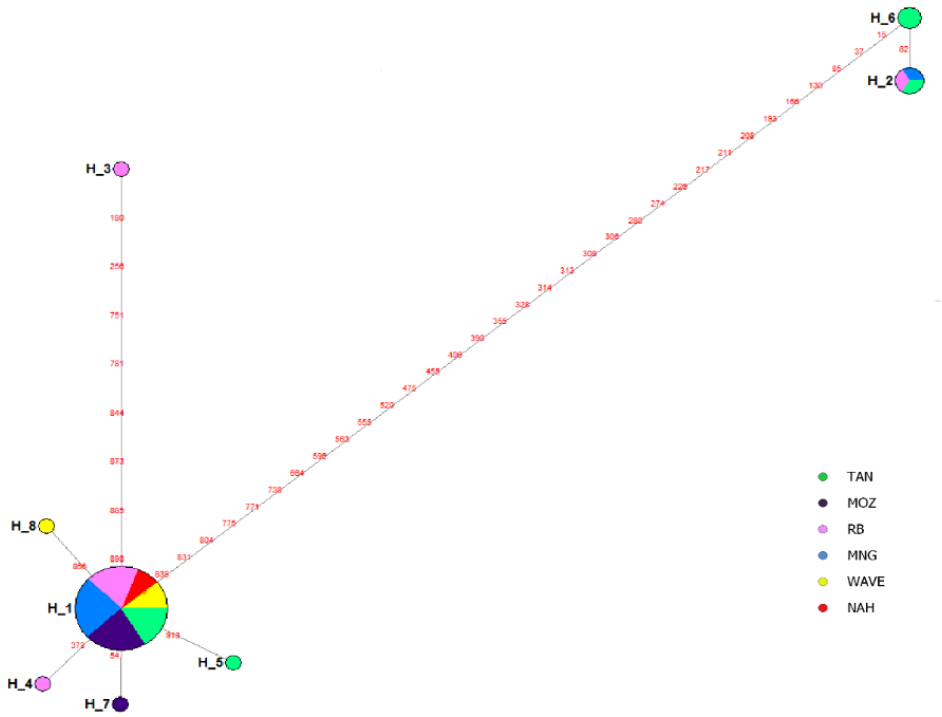


Figure 3.4: Median joining haplotype network for *PAL-1* region of *Rhizophora mucronata* collected at the six locations. In the haplotype network, the various haplotypes are represented by the circles, its size is relative to the number of samples and the various colours represent the location.

Discussion

Analysing both the maternally inherited chloroplast DNA (cpDNA) region and the biparentally inherited nuclear DNA (nDNA) region of *Bruguiera gymnorrhiza* and *Rhizophora mucronata* may provide insight into their genetic population structure and gene flow, evolutionary history, distribution and diversity, as the cpDNA provides an indication of the extent of propagule dispersal from the mother tree and nDNA provides an indication of gene flow as result of propagule and pollen dispersal (Islam *et al*., 2014). Binks *et al*. (2019) stated that the genetic connectivity of populations is important in the recovery/recruitment of populations following disturbance as other populations could provide source propagules. The findings from both the chloroplast and nuclear region of both *B. gymnorrhiza* and *R. mucronata* illustrate that there was little genetic diversity across the various sampled populations.

Little genetic variation was also observed in the chloroplast *trnL-F* intergenic spacer of *B. gymnorrhiza* populations from southwestern islands of Japan, including Okinawa and Iriomote Island (Minobe *et al*., 2010; Takeuchi *et al*., 2001). Similar results were also obtained in a study

by Islam *et al*. (2014) where *B. gymnorrhiza* populations in the Ryukyu Islands of the Japanese Archipelago had low genetic variation, limited gene flow, high inbreeding and displayed signs of recent colonization, the study also reports that previous studies (Takeuchi *et al*., 2001; Giang *et al*., 2006; Islam *et al*., 2012) had similar results.

According to Guo *et al*. (2016) low levels of genetic diversity within mangrove populations may be result of the occurrence of recurring extinction and recolonization events. Amade *et al*. (2021) stated that anthropogenic impacts that result in fragmentation and the reduction in mangrove area may further reduce genetic diversity. Leimu *et al*. (2010) defines fragmentation as the loss of suitable habitat of a species and the subsequent separation of individuals into several isolated patches separated by unsuitable habitat types. It is said to reduce the species response to climate change as with plants being dispersed into fragmented landscapes, a phenomenon known as range shifts may take place where plants occur in habitats that do not have optimal climate conditions (Leimu *et al*., 2010). With fragmented populations, the reduced genetic variation is expected to reduce the adaptive potential of species under climate change.

Species occurring in the range limit or at the periphery of the mangrove distribution where changes in sea temperature and environment are the greatest, generally have lower levels of genetic variation and may be more susceptible to climate change (Wise *et al*., 2002; Polidoro *et al*., 2010; Leimu *et al*., 2010). This is supported by Leimu *et al*. (2010) who state that species occurring in fragmented populations (in general range edge populations are fragmented) may have a reduced response to climate change due to their occurrence in habitats that do not have optimal climate conditions because of range shifts.

Several studies that used molecular markers also found low genetic diversity in a number of mangrove species (Minobe *et al*., 2010; Wee *et al*., 2014; Islam *et al*., 2015; Guo *et al*., 2017). The expectation that the genetic diversity would be lower in South African populations was not evident, thus not reflective of the influence of factors such a mouth closure and fragmentation or isolation of populations. This was contrary to what other studies in the region had found. Studies such as Maguire *et al*. (2000) and De Ryck *et al*. (2016) found the *A. marina* populations of South Africa (occurring at the range edge) to have lower genetic diversity when compared to core populations such as Tanzania and Kenya, based on microsatellite markers. However, in a review by Eckert *et al*. (2008), even though the trend of lower genetic diversity

in range-edge populations was observed in most of the studies (64.2 %), the study noted that in most cases the difference was very small. The low genetic diversity may be a result of small population size, genetic drift, abiotic or biotic factors.

In the present study, genetic connectivity between populations was evident in both the nuclear and chloroplast sequences of *B. gymnorrhiza* that showed high gene flow as the Nm value was above 1. There was also low genetic differentiation among the populations. This was also the same for the nuclear sequences of *R. mucronata*, whilst with the chloroplast sequences no estimates could be calculated as there were no informative sites. The high gene flow illustrates that there are no barriers or factors historically restricting or hindering the dispersal of propagules between these populations to an extent that genetic flow is limited. Thus, supporting the first expectation that there would be high gene flow between Tanzania and Mozambique as they occur along the same coastline with no land barriers. Even with the SEC split, findings of the present study show that propagule dispersal has not been limited. These findings are contrary to what was postulated by De Ryck *et al*. (2016) that the split in SEC may have an influence on the gene flow of *A. marina*, and to the findings of Triest *et al*. (2021) that found the split to create genetic differentiation between *R. mucronata* populations in Tanzania and Mozambique. A study by Wee *et al*. (2014) also found genetic differentiation in three *R. mucronata* populations occurring in the Malay Peninsula (Myanmar and Indonesia), which were found to correspond with the ocean currents occurring at the Malacca Strait and Andaman Sea. The Mozambique eddies were also found to have an influence in the genetic variation of *A. marina* populations in Mozambique (Amade *et al*., 2021).

No haplotypes were found in the cpDNA region of both the species, whilst the nDNA region for *B. gymnorrhiza* also showed low variation, only having three haplotypes with only a few sequences having unique haplotypes and *R. mucronata* having eight haplotypes. The distribution of the haplotypes could not be associated with the influence of the SEC and range effects. Haplotype diversity was the lowest in Nahoon and highest in Tanzania for the *PAL* region of the *B. gymnorrhiza* samples, whilst for the *R. mucronata* it was the highest in Richards Bay. The results generally showed high levels of haplotype diversity as a haplotype diversity was closer to 1, where 0 indicates no haplotype diversity. The results were expected for Nahoon and Tanzania as according to our hypothesis. Tanzania is a core population and is geographically located in a region where mangrove conditions are considered optimal for growth and regeneration while Nahoon is located at the range edge and has a small effective

population size. Richards Bay is a core population for the South African populations and three mangrove species occur, namely; *A. marina*, *B. gymnorrhiza* and *R. mucronata* (Peer *et al*., 2018). According to the study (Peer *et al*., 2018) the adult to seedling ratio of *B. gymnorrhiza* in Richards Bay was found to be 1:3 and a density of 1.5 m-2 (± 1.9 SD) and for *R. mucronata* was found to be 1:1.6 and a density of 1.2 m-2 (± 1.8 SD) during a 2014 and 2016 survey, thus illustrating that the populations of these species were similar. This may suggest that the physical conditions in Richards Bay have not had a detrimental effect on the populations reproductive success when compared to Tanzania and Mozambique. On the other hand, this may be an indication of the physical conditions of the Tanzania and Mozambique populations. Macamo *et al.* (2015) found that peri-urban mangroves at Incomati Estuary have had an increase in areas which can be categorised as "degraded" and those which are "degraded with reeds" due to forest cutting and reed invasion, while areas classified as "healthy" mangrove had dececreased.

Literature suggests that the impact of climate change may vary among species, where species in the genus *Rhizophora* are expected to perform better than those in the genera *Bruguiera*, *Ceriops* and *Xylocarpus* due to having higher growth and reproduction rates and more efficient dispersal capabilities (Polidoro *et al*., 2010). Low levels of genetic variation may also be exacerbated by anthropogenic impacts such as harvesting, aquaculture and salt pan production which are some of the threats that mangroves in the study region experience (Maguire *et al*., 2000; Rajkaran *et al*., 2004; Lugendo, 2016; Amade *et al*., 2021).

Conclusion

Maguire *et al*. (2000) states that the exploitation of mangroves, such as over-harvesting, has resulted in the loss of genetic variation in mangrove species. In South Africa, it is not only the anthropogenic activities that may be a concern regarding genetics, but also the history of these populations (founder population), the landscape and availability of genetic flow (De Ryck *et al*., 2016). According to Peer *et al*. (2018) the mangroves in South Africa have expanded since the initial assessment by Macnae in 1963, even though there may be newer populations/individuals, the results obtained from the present study suggests that their genetic variation may be low, especially due to the low numbers of *R. mucronata* and *B. gymnorrhiza* currently being found in some of the smaller populations. These results may indicate that these may not be resilient to rapid environmental shifts or extreme conditions. According to Wise *et*

al. (2002), some populations may have reduced genetic variation to the extent of not being able to respond to changes such as climate change, increase in herbivory or disease. Thus, the performance of these populations may be impaired during rapid environmental shift. This is supported by Binks *et al.* (2019) who state that the resilience and persistence of such populations are highly influenced by their genetic diversity.

Chapter 4: Long term environmental characteristics of three mangrove estuaries in the Eastern Cape

Introduction

Mangroves occur in the intertidal region where the environment is influenced by the atmospheric conditions, neighbouring sea and the terrestrial environment (Duke, 1995; Ferreira *et al*., 2010). Due to their location, they generally have hypersaline soils, experience long periods of waterlogging and have relatively high organic matter content (Otero *et al*., 2006). Kathiresan and Bingham (2001) describe the mangrove environment as one with muddy soils, variable salinity, variable temperature, strong winds, extreme tides and anaerobic soils. Ferreira *et al.* (2010) agrees that the muddy soils are generally oxygen poor and nutrient rich.

Mangroves in the Eastern Cape occur within two regions; the subtropical region which is found to occur from St Lucia until Mbashe Estuary, then in the eastern part of the warm-temperate region from Mendwana to Heuningnes (van Niekerk *et al*., 2019). Together these two regions have the highest number of estuaries and are both dominated by large and small temporary closed estuaries (van Niekerk *et al*., 2019). For **Chapter 4**, Mngazana Estuary is in the subtropical region, while Nxaxo/Ngqusi and Nahoon are in the warm temperate region. The subtropical region in South Africa experiences warm waters, the mean annual sea temperature for this region is approximately between 20-22°C due to the Agulhas current flowing southward along the east coast, typically has a summer rainfall pattern (KwaZuluNatal region), a high river discharge during summer and open estuaries characterised by high turbidity and low salinity following rainfall (Harrison, 2004; James *et al*., 2016). Estuarine mouths are predominantly maintained by river flow (Harrison, 2004; James *et al*., 2016). Whilst in the warm-temperate region, the mean annual temperature is approximately between 18-20°C also influenced by the Agulhas current, the rainfall pattern is variable (Eastern Cape region) and is said to be relatively lower than the subtropical region, tidal dominated estuaries experience elevated salinity due to having large tidal prisms and low turbidity in open systems. Estuarine mouths are mainly maintained by tidal currents (Harrison, 2004; James *et al*., 2016).

According to Clough (1993) the main drivers of mangrove growth and survival along the environmental gradient are temperature, salinity and aridity. The primary physical parameters of pore-water that play an important role in mangrove growth and their spatial distribution is

said to be salinity, pH, conductivity, redox potential and sulphide concentration (Marchand *et al*., 2004). The sediment conditions play an important role in species distribution and the growth and survival of mangrove species (Rajkaran and Adams, 2011). Some studies have shown a relationship between temperature extremes, salinity, nutrient availability and redox status of sediment in relation to primary production, growth rates and growth stature of mangroves (Feller *et al*., 2002; Morrisey *et al*., 2010). Rajkaran and Adams (2011) measured variables in the following ranges for large forests in KZN; average salinity porewater (± standard error) from 18.8 (± 2.9) to 32.6 (± 0.9) PSU, average redox sediment (± standard error) from -375.8 (± 11.9) to 282.8 (±17.1) mV, average electrical conductivity (± standard error) from 18.9 (± 1.7) to 36.9 (± 0.5) mS/cm, average pH (± standard error) from 6.2 (± 0.3) to 8.1 (± 0.1), average moisture content (± standard error) from 24.4 (± 0.7) to 68.3 (± 1.9) % and average organic content (± standard error) from 6.6 (± 1.7) to 28.9 (± 2.3) %.

In this study, we measured the pore-water and sediment characteristics of three mangrove forests in 2017 and 2018 and compared it to previous datasets to determine if major changes had taken place over a time series or if mangrove environmental conditions are stable in this part of the country.

Aims and Objectives

According to Hossain and Nuruddin (2016), mangrove forests show large variations in their sediment characterises such as salinity, pH and organic matter. Due to these estuarine systems occurring in different biogeographic zones, where Mngazana occurs in the sub-tropical region, whilst Nxaxo/Ngqusi and Nahoon occur in the warm-temperate region, it is expected that the environmental conditions will vary. The aim in this study is to describe the variation in the physical environment in terms of sediment and pore-water characteristics of three mangrove estuaries namely; Mngazana, Nxaxo/Ngqusi and Nahoon. Mangroves in Mangazana would be considered core populations whilst Nxaxo/Ngqusi and Nahoon would be range edge populations that are close to the distributional limit. This also provides important information to be taken into consideration for the next chapter on plant performance (**Chapter 5**) as the individual performance of plants is influenced by genetic diversity and environmental parameters (as fluctuating conditions lead to stress) such as moisture, rainfall, temperature play an important role (Kathiresan and Bingham, 2001; Arnaud-Haond *et al*., 2006; Butcher *et al*., 2009, Engelhardt *et al*., 2014; Anderson, 2016, Tonné *et al*., 2017). We expect that there will

be temporal variation in sediment characteristics due to changes in environmental conditions such as rainfall, disturbances (mouth conditions, drought, flooding, anthropogenic impacts etc.) which will have an influence in the performance of these three forests such as population structure, mangrove growth and thus its development (Chen and Twilley, 1999a)

The objectives of this study were to:

1. Compare sediment characteristics and porewater characteristics between 2017 and 2018 as well as at least one earlier date for each estuary.
    - It is expected that due to the drought conditions, recorded at CAPEHERMES (dataset combination of Port St Johns station and Cape-Hermes); moisture content measured in 2017 and 2018 will be less than the earlier date.
    - Moisture content of the sediment is related to organic matter content and it is expected that it will be similar between 2017 and 2018 but lower than the previous year(s).
    - It is expected that there may be a decrease in pH due to the drought, which is influenced by the organic matter present.
2. Compare porewater characteristics between 2017 and2018 as well as at least one earlier date for each estuary.
    - It is expected that during drier conditions, the salinity levels may be elevated due to reduced freshwater inputs resulting in higher conductivity. Temperature is also expected to have increased between the years.

Study site descriptions

Long term sediment data has been collected (over variable periods) from three estuaries found in the Eastern Cape, namely Mngazana, Nxaxo/Ngqusi (Wavecrest) and Nahoon between 2007 and 2018. Three mangrove species, namely; *Rhizophora mucronata*, *Avicennia marina* and *Bruguiera gymnorrhiza* occur in these estuaries (Rajkaran and Adams, 2007; Hoppe-Speer *et al*., 2015). For this present study, sediment and pore-water readings were collected from these estuaries in 2017 and 2018 and combined with any available data collected prior to this period. At Mngazana, data was included from 2007 (June and November), 2017 (July), and 2018 (October). At Nxaxo/Ngqusi, data was included from 2007 (June), 2008 (June), 2009 (June), 2010 (July), 2011 (July), 2012 (July), 2017 (June), and 2018 (October) sampling events, more datasets were included here as sampling occurred frequently and consistently. At Nahoon, data

was included from 2012 (February, June, July and November), 2017 (July), and 2018 (October) sampling events. Climate data was obtained from the South African Weather Service (SWAS), for the weather stations which occur near these three estuaries, these included data from Port St Johns, Cape-Hermes and East London (**Figure 4.1**). Data collected from Cape-Hermes and Port St Johns were combined to have a complete dataset, which could help in describing the potential weather conditions that are experienced at Mngazana.

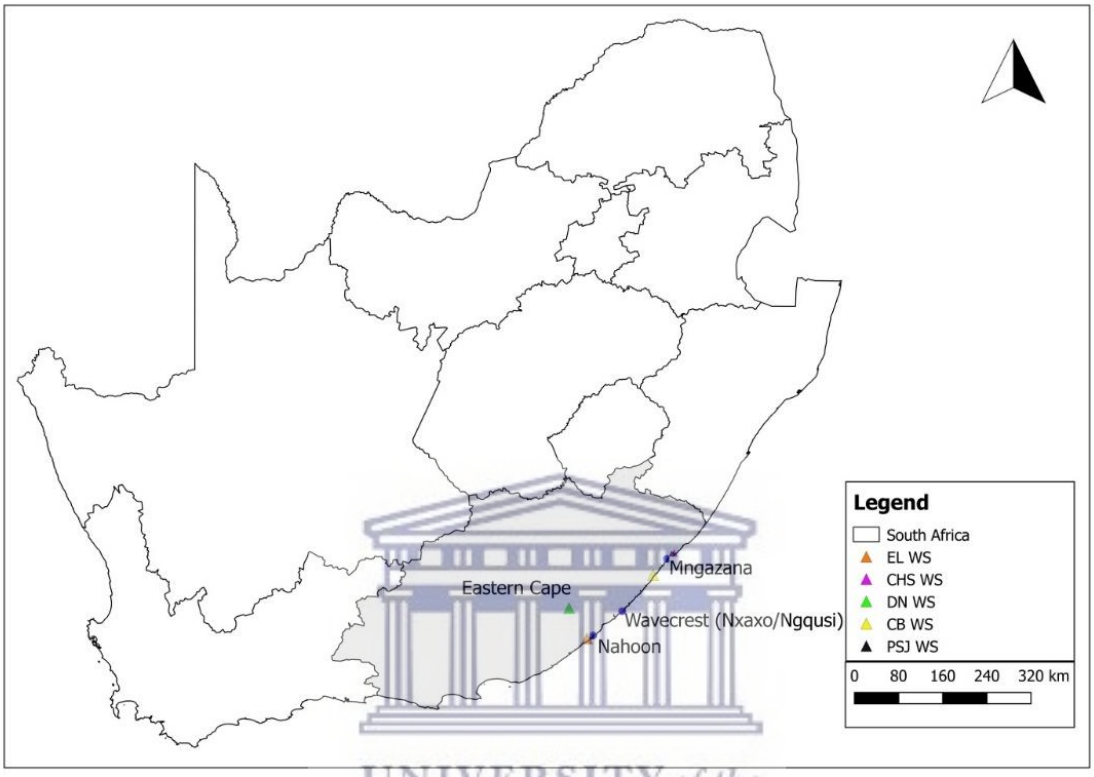


Figure 4.1: Mangrove study sites and weather stations (EL, CHS, DN, CB, PSJ) locations in the Eastern Cape, South Africa.

Table 4.1: Rainfall data for CAPEHERMES and East London stations during 2017 and 2018.

Sampling Region	Year (Month)	Total rainfall (mm) during sampling month	Total annual rainfall (mm)	Average monthly rainfall (mm)
CAPEHERMES	2017(July)	2.2	713	59.43 (±17.06)
	2018(September)	36.2	840.2	70.01 (±17.23)
East London	2017(June)	0.2	803.2	66.93 (±12.49)
	2018(September)	36.4	570	47.50 (±11.13)

"±" Standard Error

Mngazana Estuary (31°41'31.84"S; 29°25'16.11"E)

This estuary occurs in the subtropical region, near the town Port St Johns (Rajkaran *et al.,* 2004; Whitfield and Baliwe, 2013). It has a catchment area of approximately 275 km^2, the river is 35 km long and the estuary is predominately open (Rajkaran *et al.,* 2004; Whitfield and Baliwe, 2013; van Niekerk *et al*., 2019). Mangroves in this estuary cover an area of about 1,18 km^2, making it the third largest mangrove forest and largest of *R. mucronata* in South Africa (Rajkaran and Adams, 2012). These mangroves are distributed along Creek 1 and Creek 2 and the main channel (**Figure 4.2**). Mngazana Estuary occurs in a rural setting, where the mangrove forest has been harvested, for building material and firewood (Rajkaran and Adams, 2010).

Figure 4.2: The Mngazana Estuary, showing Creek 1, Creek 2 and the Main Channel (Imagery from Google Earth).

Data from two weather stations namely; Port St Johns (**PSJ, -31.6530S; 29.5070E, 26 m above sea level (asl)**) and Cape-Hermes (**CHS, -31.6350E; 29.5520S, 47 m asl**) have been merged and used to describe the rainfall and temperature at the Mngazana Estuary. Rainfall data for Cape-Hermes (CHS) was available between 1968-2010, with data from 1977 and some data in 1978 missing. Whilst for Port St Johns, data was only available for the following years; 2011,

2012, some data for 2013 and 2016-2018. The merged data is referred to as CAPEHERMES and is shown in **Figure 4.4**.

Highest monthly rainfall for this dataset was in March 1976 where total rainfall was 451.5 mm (**Figure 4.3 A**). Highest annual rainfall also occurred in the same year where total rainfall was 1 526 mm. On average, the total rainfall per year is approximately 942 (±39.68 Stardard Error (SE)) mm and the lowest annual rainfall may have occurred in 1978 with 398.1 mm, but some data was missing for that year thus this was not considered. It was therefore calculated that 2015 had the lowest annual rainfall of 400.6 mm. The rainfall data indicates that there has been a decrease in the amount of rainfall over the years (**Figure 4.3 B**). The wettest months occur between November and March (warmer months), during this period, March, on average 124 (±11.32 SE) mm has received the highest average rainfall whilst the driest months occur between June-August (winter months), with June only experiencing on average about 28 (±5.64 SE) mm of rainfall (**Figure 4.4**).

Highest monthly maximum temperature (red) (**Figure 4.5 A**) during this period was 29.6°C which occurred in January 2012 and the lowest was 18.7°C which occurred in July 1996, on average the monthly maximum temperature was 23.4 (± 0.11 SE) °C whilst the average monthly minimum temperature was 16.7 (±0.15 SE) °C, highest monthly minimum temperature (blue) during this period was 22.4°C (February 2003) and lowest was 6.5 °C (June and July 2018) (**Figure 4.5 A**). Over this period, the rainfall has decreased (significant p-value <0.05) whilst temperature appears to have increased but this was not significant (p-value >0.05) (**Figure 4.3 B and 4.5 B**).

The dataset for Cape-Hermes (CHS) and Port St Johns (PSJ) weather stations complement one another. Some data that was not available for CHS was available for PSJ and *vice-versa*, this also means one cannot determine major discrepancies between the data but due to the proximity of the sites to Mngazana, it was found suitable in this study to merge these datasets to form one dataset namely CAPEHERMES (**Figure 4.4**).

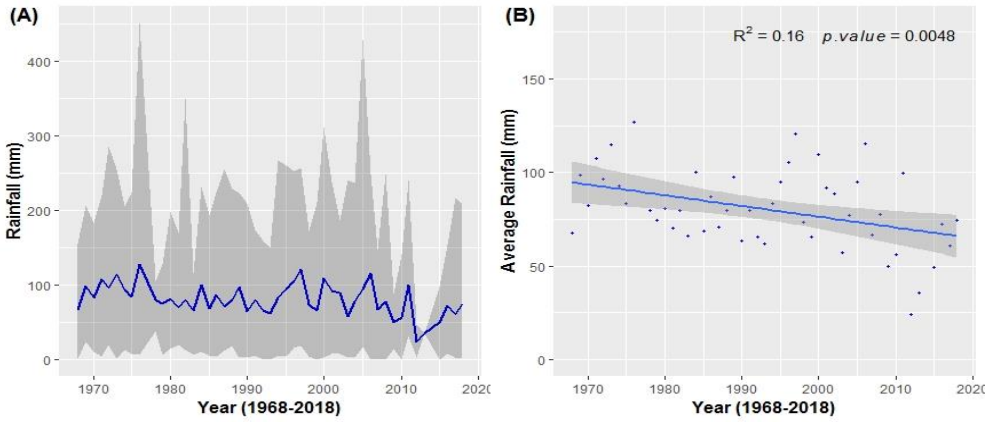


Figure 4.3: (A) Showing minimum and maximum (grey) and average monthly (blue) rainfall. (B) Data was fitted with a linear regression model showing a slight decrease in average rainfall over the period between 1968-2018.

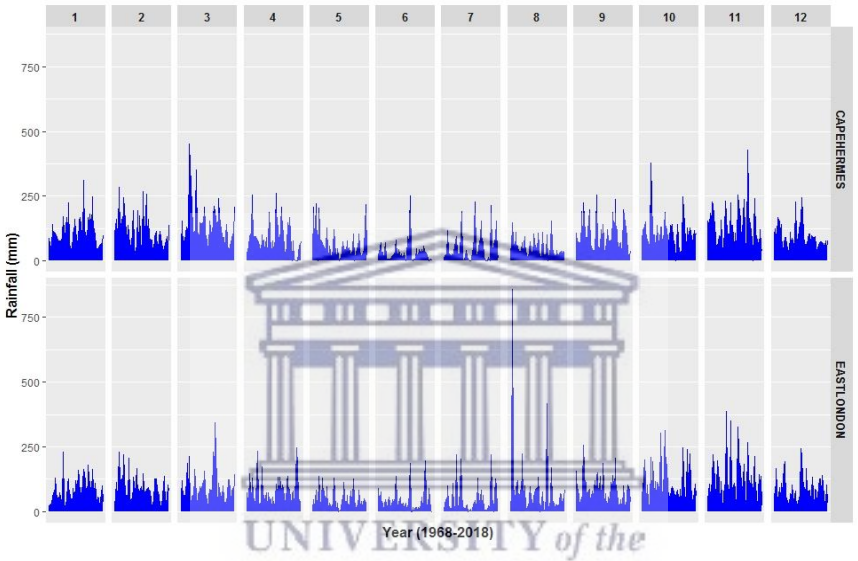


Figure 4.4: Average monthly rainfall (mm) between 1968-2018 for CAPEHERMES and East London.

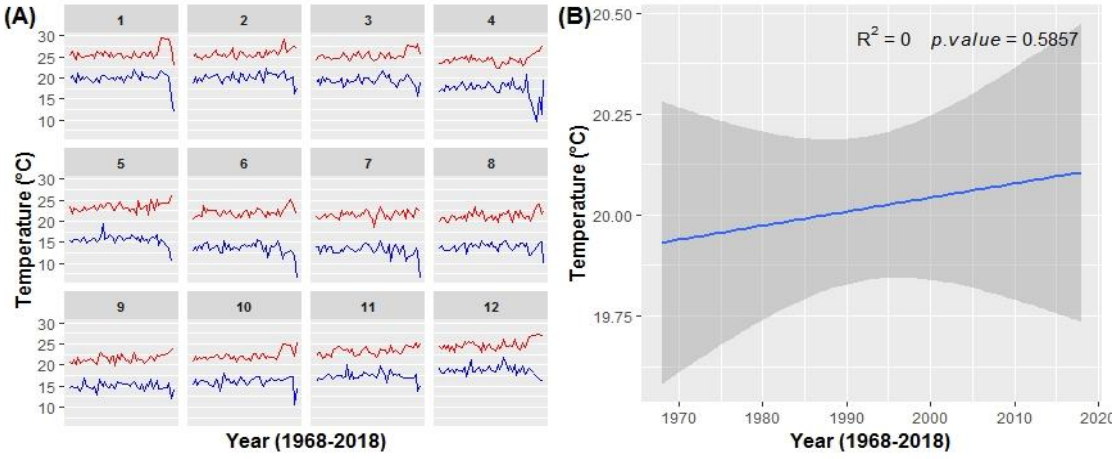


Figure 4.5: (A) Average monthly maximum (red) and minimum (blue) temperature (°C) for the period 1968-2018. (B). Linear regression model on average annual temperature (°C) data, showing an increase in temperature over the period between 1968-2018.

Wavecrest (Nxaxo- Ngqusi Estuaries, 32°35'1.69"S; 28°31'18.74"E)

The Nxaxo and Ngqusi Estuaries (**Figure 4.6**) occur in the warm temperate zone (34°52′S), situated near Centane in a rural setting (Whitfield and Baliwe, 2013). These estuaries have a common mouth and are classified as a large temporarily closed system (van Niekerk *et al*., 2019). Together they have a catchment area of approximately 134 km^2 and a tidal prism of about $11x10^6m^3$ (Strydom, 2015). Mangroves cover an area of about 0.1 km^2, where *A. marina* is dominant, having a medium density of about 1 000 to 10 000 trees per ha. Small *B. gymnorrhiza* stand and *R. mucronata* individuals are also found in this estuary (Hoppe-Speer *et al*., 2015; van Niekerk *et al*., 2019).

Figure 4.6: The Nxaxo and Ngqusi Estuaries occurring at the location Wavecrest (Imagery from Google Earth).

Data from two weather stations namely; Coffee Bay (**CB, -31.9650S; 29.1340E, 87 m asl**) and DOHNO (**DN, -32.5270S; 27.4600E, 901 m asl**) were received, however, both these stations are more than 80 kilometres from the estuaries and cannot be used in this context. East London is the next closest site at 70 km and the data is described below.

Nahoon (32°59'0.23"S; 27°56'32.97"E)

This estuary occurs in the warm temperate region and is said to be beyond the natural mangrove distribution limit (Hoppe-Speer *et al*., 2015). This micro-tidal estuary is approximately 5 km in length, is predominately open, has a catchment area of approximately 547 km ^{2} and tidal-prism is approximately 6.3x10^{5}m^3 (Cooper, 2001; Cooper, 2002; Whitfield and Baliwe, 2013). Mangroves occurring in this area are said to have been established through a transplantation event where propagules originated at Durban Bay and the experiment was conducted by Steinke, it has also been reported that there have been more planting events in this estuary but none after the year 2000 (Ward and Steinke, 1982; Hoppe-Speer *et al*., 2015). Mangrove cover is approximately 0.6 ha, dominated by *A. marina* with low density and a few *B. gymnorrhiza* and *R. mucronata* individuals are found to occur (Hoppe-Speer *et al*., 2015). Other habitats include saltmarsh vegetation, which in some areas of the estuary co-occurs with the mangroves (Geldenhuys *et al*., 2016). The estuary is in the Nahoon Estuary Nature Reserve, which is situated in an urban setting in East London in the Eastern Cape (Hoppe-Speer *et al*., 2015) (**as seen in Figure 4.7**).

Data from the East London weather stations (EL) **(WO, -33.0350S; 27.8160E, 134 m asl); (WO, -33.0330S; 27.8330E 124 m asl); (WK, -33.0330S; 27.8330E, 125 m asl)** were used to describe the rainfall and temperature experienced at Nahoon and Nxaxo/Ngqusi. While the latter is 70 km away, this represents the only dataset in this area. Rainfall data was available for the period between the year 1968 and 2018, the highest rainfall occurred during August 1970 with 858.6 mm; other high rainfall events occurred in November 1985 (385.5 mm) and 1989 (350.5 mm) which are less than half the highest rainfall event (**Figure 4.4 and Figure 4.8 A**). The highest annual rainfall occurred in 1970 with a total rainfall of 1 608 mm, on average the yearly rainfall for this region is approximately 865.23 (± 30.54 SE) mm. The lowest occurred in 2009 where the total rainfall was 550.4 mm. The wettest months occur between October and December (summer months) the highest being in November with an average of 108.53 (± 11.74) mm whilst the driest months occurred between May and July (winter months) where June had the lowest with an average of 34 (± 5.83 SE) mm (**Figure 4.4**).

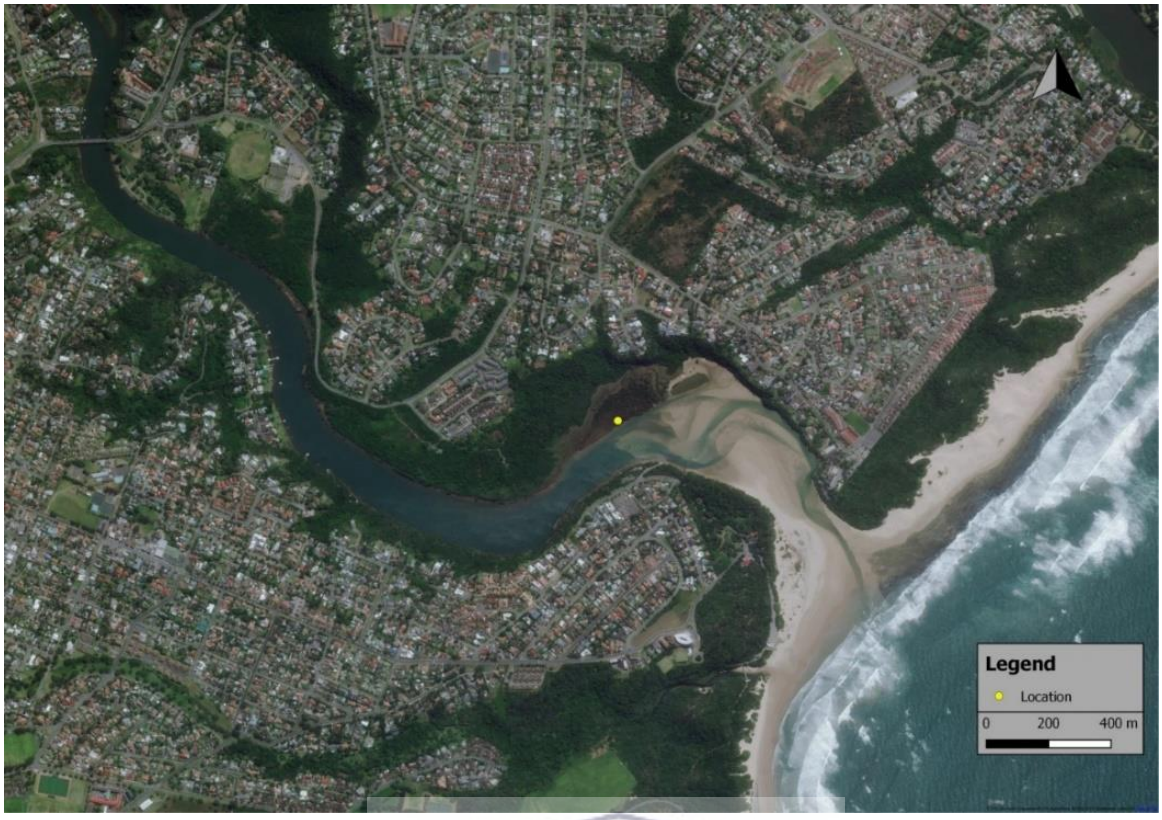


Figure 4.7: Nahoon Estuary, location of mangroves shown in yellow (Imagery from Google Earth).

Over this period, the rainfall appears to have decreased but this was not found to be significant (p-value >0.05) whilst temperature appears to have increased but this was also not significant (p-value >0.05) (**Figure 4.8 B and 4.9 B**). The highest monthly maximum temperature during this period was 27.8°C which occurred in February 2003 and the lowest was 20.25°C (February 1992). On average, the maximum temperature was 23.19(±0.06 SE) °C whilst the average monthly minimum temperature was 14.47 (±0.05 SE) °C, highest monthly minimum temperature (blue) ranged between 18.4°C (June 1968) and the lowest was 8.8 °C (July 1970) (**Figure 4.9 A**).

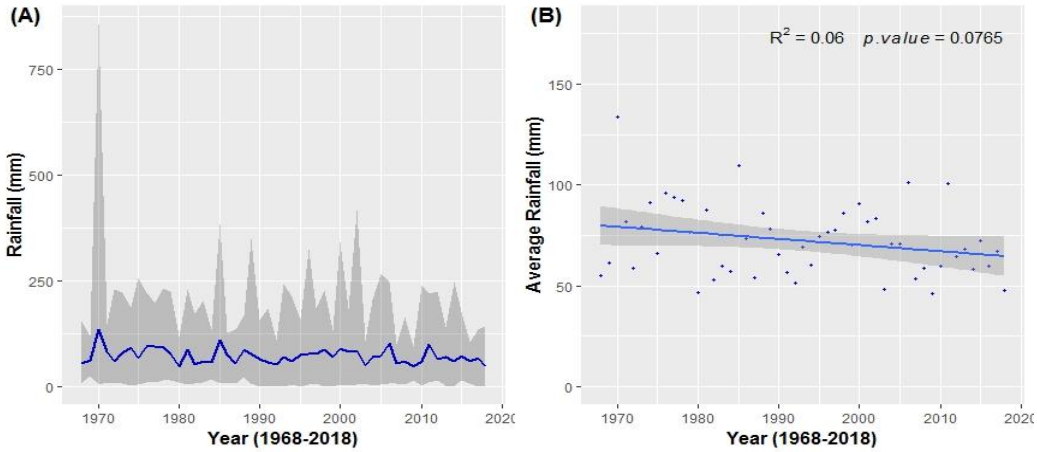


Figure 4.8: (A) Showing minimum and maximum (grey) and average monthly (blue) rainfall (mm) for the period between 1968 and 2018. (B) Data was fitted with a linear regression model showing a slight decrease in rainfall (mm).

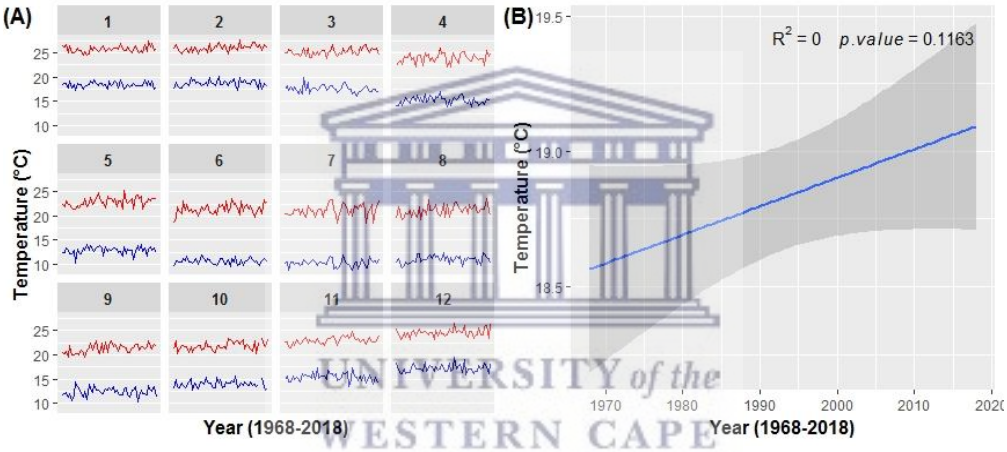


Figure 4.9: (A) Average monthly maximum (red) and minimum (blue) temperature (°C) for the period 1968-2018. (B) Linear regression model on average annual temperature (°C) data, showing an increase in temperature over the period between 1968-2018.

Description of the data used

At Mngazana, data collected in the following years; 2007 (N =8), 2017 (N =12) and 2018 (N =12) were used in this study. At Nxaxo/Ngqusi, data included 2007 (N =29), 2008 (N =29), 2009 (N =29), 2010 (N =29), 2011 (N =30), 2012 (N =30), 2017 (N =15), and 2018 (N =15). At Nahoon data included 2012 (N =24), 2017 (N =23), and 2018 (N =27). The dates prior to the sampling in 2017 and 2018 are inconsistent and were dependent on the research that was taking place at that time. The same protocols for the lab analysis were carried out to determine, sediment organiccontent, moisture content, salinity and pH for various studies conducted at

Mngazana (Rajkaran and Adams 2012), Nxaxo/Ngqusi (Hoppe-Speer and Adams, 2015; Mbense, 2017) and Nahoon (Geldenhuys *et al*., 2016) over the various years.

Materials and Methods

Sedimentary Analysis

In 2017 and 2018 sediment samples were collected in triplicate from the surface at each of the long-term growth monitoring sites that have been established prior to this study (Rajkaran and Adams 2012, Hoppe-Speer and Adams, 2015, Geldenhuys *et al*., 2016; Mbense, 2017) at the three estuaries. For the sediment collected the following properties detailed below were measured.

Redox potential and pH

Redox potential was either measured in the field (Nxaxo/Ngqusi 2017) or measured within 24 hours of collection using a redox meter (HANNA 8424 redox probe). The samples were then kept in cool conditions andreturned to the laboratory, the pH was determined by adding distilled water to 5 g of sediment to make a 50 ml solution. When the sediment had dissolved the pH reading was measured using a pH meter (HANNA 8424 pH metre, platinum–gold tipped electrode) (Geldenhuys *et al*., 2016; Mbense, 2017).

Organic and moisture content

The percentage organic and moisture content was determined by weighing out 10 g of the sample, then drying it in the oven at 100° C for 48 hours. The sample was then reweighed (Black, 1965). The loss of weight was reflected as the moisture content of the soil and converted to a percentage. The samples were then placed in an ashing oven for 5 hours at 550° C, allowed to cool and reweighed (Chambers *et al*., 2013). The following calculations were made to determine the percentage organic and moisture content:

Moisture (%) content: (wet mass-dry mass /wet mass) * 100 ……**Equation 4.1**

Organic (%) content: (initial dry mass–mass after ashing) / (initial dry mass) *100…...**Equation 4.2**

Porewater Analysis

Porewater data was collected in-situ from the holes augured during sediment collection. Porewater was allowed to pool, and then temperature, salinity, redox, oxygen (only collected in 2018) and electrical conductivity of the water was measured, with a handheld salinity, conductivity and temperature probe (model YSI Professional Plus Multimeter). The same measurements were also taken at the channel near each site.

Data Analysis

Statistical analysis were carried out using a statistical computing program R version 3.5.2 (2018-12-20) (Core Team, 2018). Packages used to run the various tests, plot the graphs and produce correlation matrices were tidyverse (Wickham and Wickham, 2017), dbplyr (Wickham and Rulz, 2018), ggplot2 (Wickham, 2011), ggpubr (Alboukadel, 2018), plotly (Sievert *et al*., 2017), ggthemes (Arnold and Arnold, 2015), ggpmisc (Aphalo, 2016), dunn.test (Dinno and Dinno, 2017), car (Fox *et al*., 2017), lattice (Sarkar *et al*., 2015) and agricolae (de Mendiburu & de Mendibutu, 2019). vegan (Dixon, 2003) and (Paradis *et al*., 2019). Shapiro-Wilk test was used to test for normality; for data which was not normally distributed a non-parametric test (Kruskal-Wallis test) was carried out, whilst for normally distributed data a One-way ANOVA (Analysis of variance) test was carried out. A Tukey HSD post hoc test was run after the ANOVA to determine significance between groups (sites or years). The Dunn's test (Bonferroni correction method) was carried out after the Kruskal-Wallis test. Correlation matrices were also generated.

Results

The following sections are divided into the sediment and then porewater characteristics. The first analyses aim to show differences between three mangrove forests only using the 2017 and 2018 datasets. Then, each forest is dealt with separately with the aim of showing differences between the 2017, 2018, and datasets collected prior.

Sediment Characteristics

Mean moisture content (%) showed no differences between the three forests ($\chi 2$ (2) =2.512, p-value >0.05). The data ranged from 41.81 (± 1.45 SE) % at Nxaxo/Ngqusi to 39.21 (± 1.84 SE) % at Nahoon (**Figure 4.10 A**). Mean organic content (%) were found to vary ($\chi 2$ (2) =10.106, p-value <0.05) between estuaries. Nahoon was found to be significantly higher than Nxaxo/Ngqusi (p-value <0.05), but similar to Mngazana (p-value>0.05). Mean organic content ranged from 9.03 (± 0.74) at Nahoon to 5.46 (± 0.73 SE) % at Nxaxo/Ngqusi (**Figure 4.10 B**). Mean sediment pH for the three estuaries were found to be significantly different (F (df = 2) =70.8, p-value <0.05), the Tukey HSD post hoc test showed that Nahoon was higher than both Mngazana and Nxaxo/Ngqusi (p-value <0.05), while Mngazana and Nxaxo/Ngqusi were found to be similar (p-value >0.05). pH data (**Figure 4.10 C**), ranged from 7.94 (± 0.05 SE) at Nahoon to 7.07 at Nxaxo/Ngqusi (± 0.07 SE). Mean sediment redox potential values were found to be significantly different (F (df = 2) =13.23, p-value<0.05). The Tukey HSD post hoc test showed that Nahoon was lower than both Mngazana and Nxaxo/Ngqusi (p-value <0.05) while Mngazana and Nxaxo/Ngqusi were found to be similar (p-value >0.05). Redox of the sediment ranged from 107.14 (± 27.55 SE) mV at Nxaxo/Ngqusi to -52.89 (± 19.10 SE) mV at Nahoon (**Figure 4.10 D**).

Sediment variables were correlated in some cases (**Figure S1**) and a strong positive relationship was found between moisture content (%) and organic content (%) (0.67). Weak negative relationship was found between pH and Moisture content (%) (-0.31), and between Redox (mV) and pH (-0.35). **See supplementary information for more details.**

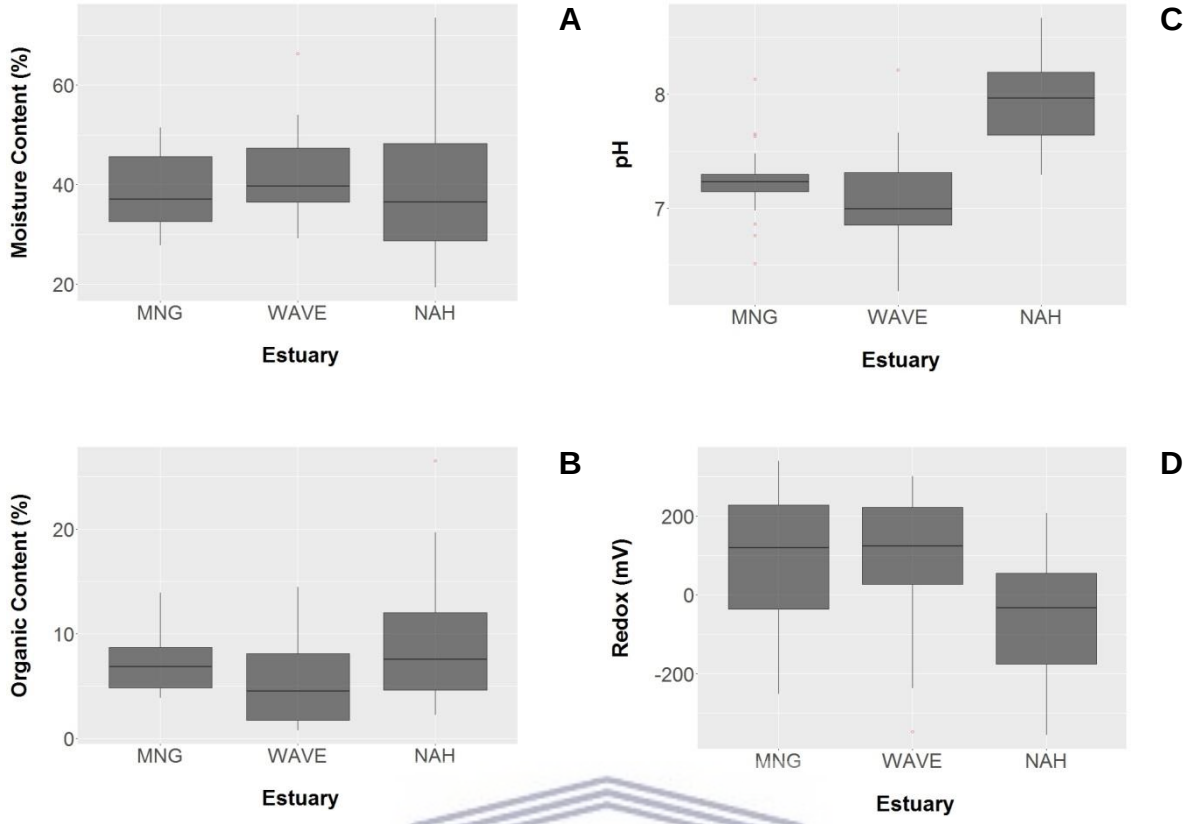


Figure 4.10: Box plots of moisture content (%) (A), organic content (%) (B), pH (C) and redox (mV) (D) measured at the three estuaries; Mngazana (MNG), Nxaxo/Ngqusi(WAVE) and Nahoon (NAH). Boxplot shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

A comparison of sediment characteristics at Mngazana mangrove forest

Sediment characteristics were collected at Mngazana in 2007, 2017 and 2018. Mean moisture content (%) were found to similar over the various years ($F_{(df = 2)}$ =0.28, p-value >0.05). Moisture content ranged from 36.85 (± 2.25 SE) in 2007 to 39.35 (± 2.47 SE) % in 2018 (**Figure 4.11 D**). Mean sediment organic content (%) values were also found to be similar between the different years ($F_{(df = 2)}$ =1.78, p-value >0.05). Mean sediment organic content ranged from 5.91 (± 0.71 SE) in 2007 to 7.78 (± 0.91 SE) % in 2018 (**Figure 4.11 C**). Mean sediment pH measured in the various years was found to be significantly different ($F_{(df = 2)}$ =4.29, p-value <0.05), the Tukey HSD post hoc test showed that pH measured in 2007 was significantly higher than 2018 (p-value <0.05). Mean sediment pH ranged from 7.16 (± 0.09 SE) in 2018 to 7.61 (± 0.14 SE) in 2007 (**Figure 4.11 A**). Mean sediment redox potential values

were found to be significantly different ($F_{(df = 2)}$ =13.35, p-value <0.05), the Tukey HSD post hoc test showed that redox measured in 2017 was higher than 2018 (p-value <0.05). Redox of the sediment ranged from -36.89(± 41.15 SE) in 2018 to 201.70 (± 29.14 SE) in 2017 (**Figure 4.11 B**). Sediment variables at Mngazana (All years) were correlated (**Figure S2**) and a strong significant relationship was found between organic content (%) and moisture content (%) (0.87). Weak negative relationships were also found between organic content (%) and Redox (-0.42), moisture content (%) and pH (-0.49) and also organic content (%) and pH (-0.43).

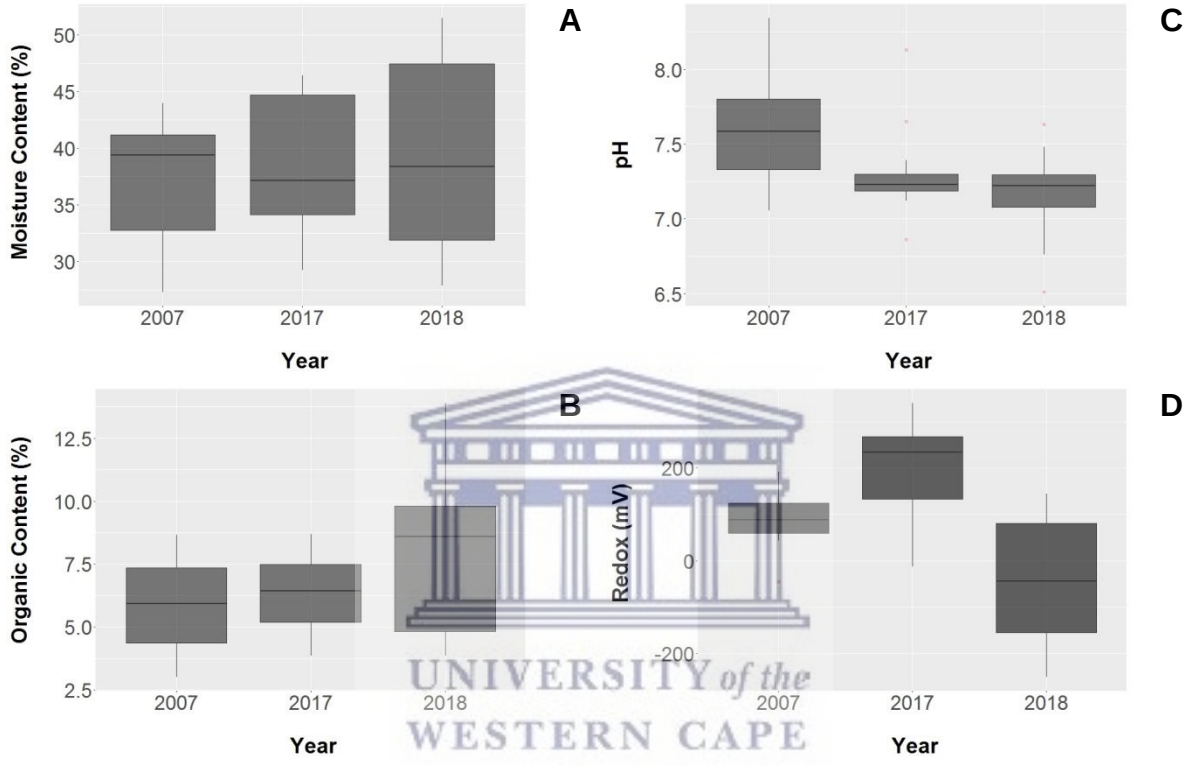


Figure 4.11: Box plots of moisture content (%) (A), organic content (%) (B), pH (C) and redox (mV) (D) at Mngazana in 2007 2017 and 2018. Boxplot shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

A comparison of sediment characteristics at Nxaxo/Ngqusi (2007- 2012, 2017 and 2018)

Mean sediment moisture content (%) measured in the various years were found to be significantly different ($\chi^2_{(7)}$ =52.375, p-value <0.05), the Dunn's test showed that moisture content measured in 2012 was higher than all the other years (p-value <0.05). Sediment moisture content (%) ranged from 37.08 (± 1.74 SE) % in 2010 to 53.89 (±1.42 SE) % in 2012 (**Figure 4.12 A**). Mean organic content (%) was also significantly different over the various

years ($\chi^2_{(7)}$ =101.01, p-value<0.05), the Dunn's test showed that Organic content measured in 2012 and 2018 values were lower than 2007, 2009, 2010, 2011 and 2017 (p-value <0.05). Organic content (%) ranged from 1.83 (± 0.14) % in 2018 to 9.08 (± 0.58 SE) % in 2017 (**Figure 4.12 B**). Mean pH values were significantly different, the Dunn's test showed that mean pH measured in 2007, 2010, 2012 was higher than that measured in 2008 and 2009 (p-value <0.05). Mean pH values measured in 2012 were also higher to those measured in 2017 and 2018 (p-value <0.05). pH values ranged from 6.61(± 0.06 SE) in 2009 to 7.47 (± 0.09 SE) in 2012 (**Figure 4.12 C**). Mean redox (mV) values were found to be significantly different ($\chi^2_{(7)}$ =101.52, p-value <0.05), the Dunn's test showed that mean redox (mV) measured in 2007 and 2008 were higher than 2009, 2010, 2011, 2012, 2018 (p-value <0.05), while measurements in 2009, 2010 and 2011 were lower than 2017 and 2018 (p-value <0.05) and redox (mV) in 2012 was lower than 2017 (p-value <0.05). Redox (mV) measured ranged from 201.95 (± 0.06 SE) mV in 2010 to 138.41 mV (± 0.08 SE) in 2008 (**Figure 4.12 D**). Sediment variables were correlated (Figure S3), no strong correlation was found between the variables.

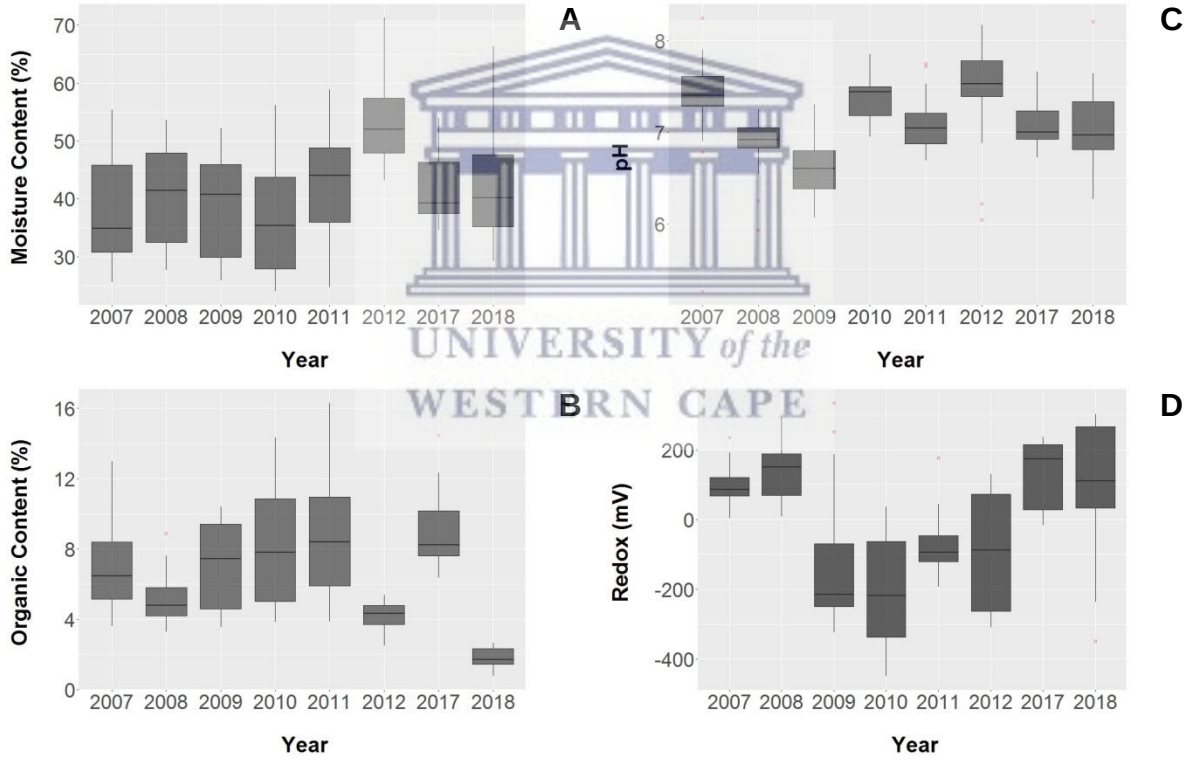


Figure 4.12: Box plots of moisture content (%) (A), organic content (%) (B), pH (C) and redox (mV) (D), measured at Nxaxo/Ngqusi in 2006 to 2012, 2017 and 2018. Boxplot shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value.

Sediment Characteristics per site - Nahoon (2012, 2017 and 2018)

Mean moisture content (%) measured in the various years were found to be significantly different ($\chi^2_{(2)} = 6.2546$, p-value <0.05), the Dunn's test showed that the moisture content (%) measured in 2012 was significantly lower than in 2017. Moisture content ranged from 31.48 (± 1.39) in 2012 to 40.41 (± 2.70) % in 2017 (**Figure 4.13 A**). Mean organic content (%) measured were also significantly different ($\chi^2_{(2)} = 32.278$, p-value <0.05), the Dunn's test showed that the organic content (%) measured in 2012 was higher than 2017 and 2018. Organic content (%) ranged from 8.98 (± 0.999) in 2017 to 18.92 (± 1.23) % in 2012 (**Figure 4.13 B**). Mean pH was found to be similar ($F_{(df = 2)} = 0.88$, p-value >0.05). pH ranged from 7.87 (±0.06) in 2018 to 8.04 (± 0.08) in 2017 (**Figure 4.13 C**). Mean redox measured in the various years were found to be significantly different ($F_{(df = 2)} = 7.298$, p-value <0.05), the Tukey HSD post hoc test showed that redox in 2017 and 2018 were higher than 2012 (p-value <0.05). Redox ranged from -166.73(± 15.29) mV in 2012 to -51.23 (± 31.90) mV in 2017 (**Figure 4.13 D**).

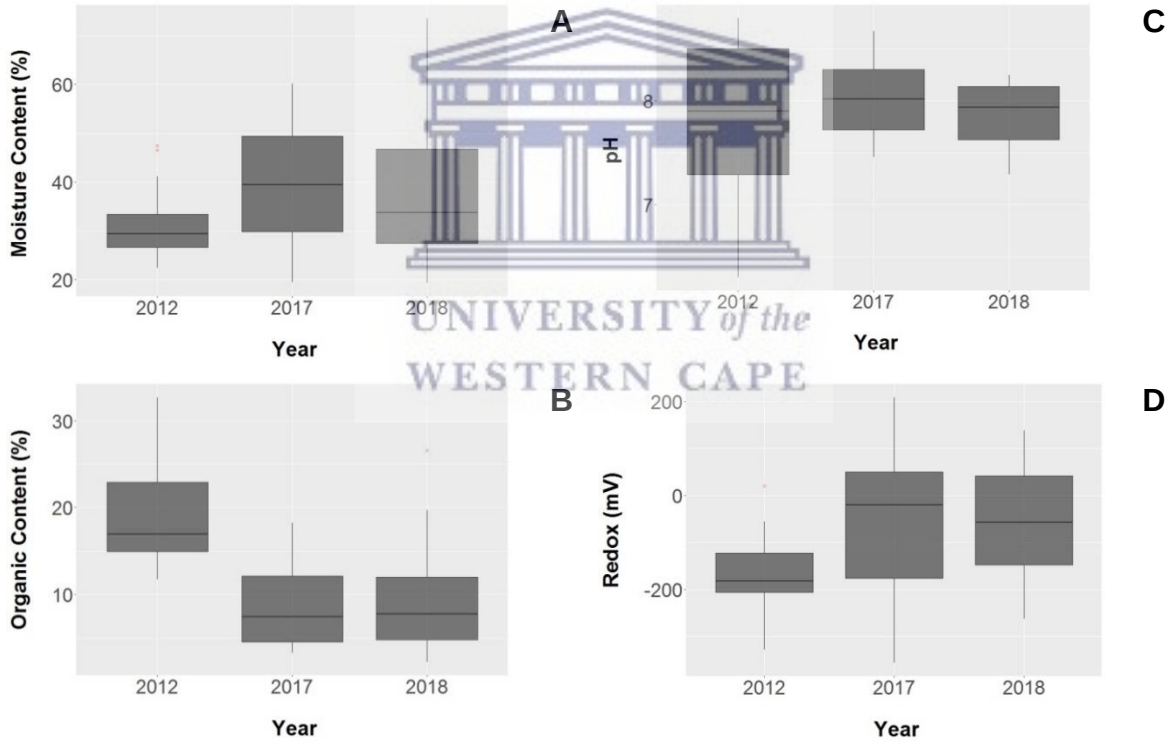


Figure 4.13: Box plots of Moisture content (%) (A), Organic Content (%) (B), pH (C) and Redox (mV) (D), collected during 2012, 2017, 2018 at Nahoon Estuary. Boxplot shows Upper whisker (greatest value excluding outliers), red circles above this represent outliers, Upper quartile (outer line at the top, represents 25% of the data that is greater than this value), Middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value.

Sediment variables were correlated (**Figure S4**), no strong relationship was found between them. Weak positive relationship was found between; Organic matter (%) and Moisture Content (%) (0.44).

Due to the sediment data not been collected for the same years at the various estuaries, a Non-metric multidimensional scaling (NMDS) analysis was run on data only collected during 2017 and 2018 using the Bray-Curtis dissimilarity matrix at 999 permutations, This was done to test if sediment characteristic collected could be separated according to estuary thus showing a difference in the sediment conditions of the three estuaries in these two years. To remove negative values in data set 370 was added to all redox values (x+ 370), the square root function was then used to transform the dataset. The results from the analysis as observed in **Figure 4.14** had a stress level of 0.042. The following tests were carried out namely; ANOISM (Analysis of similarities), PERMANOVA (Permutational multivariate analysis of variance), PERMDISP2 (through betadisper function in r). Plot shows that there is a general overlap between the three estuaries **Figure 4.14**, supported by the ANOISM (R^2= 0.094, p-value >0.05 (95% upper quantile of permutation) showing that the sediment conditions of the three estuaries were generally similar. The PERMANOVA showed a significance difference ($F_{(df = 2)}$ =8.430, R^2= 0.143, p-value <0.05), but the R^2 value displayed a very weak association between the measured variables and the estuary in which they were collected from. The PERMDISP2 showed that there is no difference in the dispersion of the three estuaries ($F_{(df = 2)}$ = 1.345, p-value >0.05) (**Figure 4.15**). Pairwise comparison shows that all estuaries were similar (p-value>0.05). The average distance to median at Mngazana was 0.068 (p-value >0.05), for Nahoon was 0.087 (p-value >0.05) and Nxaxo/Ngqusi was 0.066 (p-value >0.05), thus assumption for homogeneity of multivariate dispersions was met.

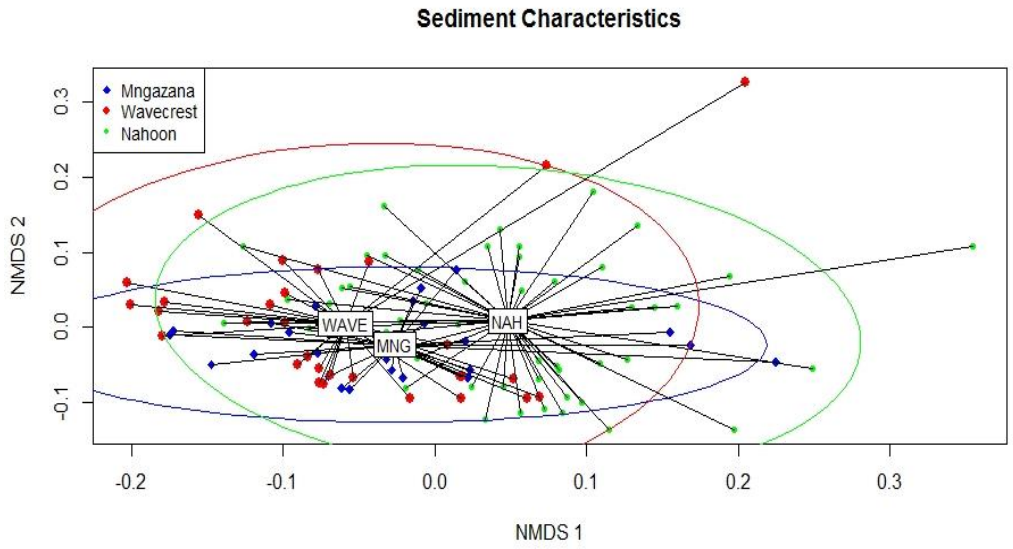


Figure 4.14: Non-metric multidimensional scaling (NMDS) plot of the sediment variables measured at the three estuaries; Mngazana (MNG), Nahoon (NAH) and Nxaxo/Ngqusi (WAVE).

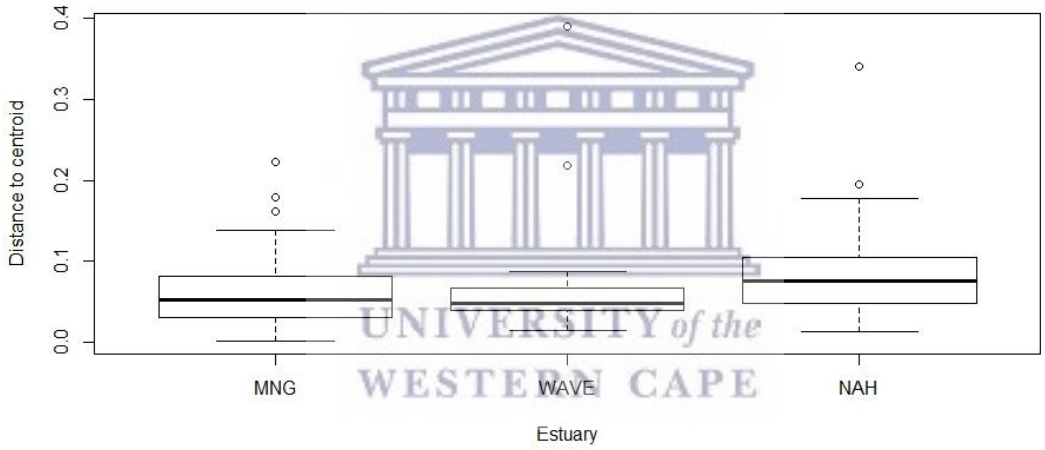


Figure 4.15: Box plot showing PERMDISP2 results of the sediment variables measured at the three estuaries; Mngazana (MNG), Nahoon (NAH) and Nxaxo/Ngqusi (WAVE).

Pore-water Characteristics

Pore-water variables measured in this study were temperature (°C), salinity (ppt), electrical conductivity (mS), dissolved oxygen (mg. L^{-1}), pH and redox (mV). Results were plotted in **Figure 4.16**. All variables did not follow a normal distribution thus a Kruskal-Wallis ($\chi^2_{(df)}$) test was carried out.

Mean temperature (°C) measured in the different estuaries was similar ($\chi^2_{(2)} = 3.358$, p-value >0.05), temperature ranged from 18.67 (± 0.31) in Nahoon to 17.80 (± 0.20) °C in

Nxaxo/Ngqusi (**Figure 4.16 A**). Mean salinity (ppt) was also significantly different, the Dunn's test showed that Mngazana and Nxaxo/Ngqusi were similar (p-value >0.05), both were significantly lower than Nahoon (p-value <0.05). Salinity ranged from 35.10 (± 0.24) at Nxaxo/Ngqusi to 37.26 (± 0.52) ppt in Nahoon (**Figure 4.16 B**). Mean electrical conductivity were found to be significantly different at the various estuaries ($\chi^2_{(2)}$ =19.359, p-value <0.05). The Dunn's test showed that Mngazana and Nxaxo/Ngqusi were similar (p-value >0.05), but both were lower than Nahoon (p-value <0.05). Electrical conductivity ranged from 53.14 (± 0.32) in Nxaxo/Ngqusi to 55.77(± 0.70) (mS) in Nahoon (**Figure 4.16 C**). Mean dissolved oxygen was similar ($\chi^2_{(2)}$ = 1.872, p-value>0.05), ranging from 0.84(± 0.08) in Nxaxo/Ngqusi to 1.07 (± 0.18) (mg. L^{-1}) in Mngazana (**Figure 4.16 D**). pH was also similar in these estuaries ($\chi^2_{(2)}$ = 10.296, p-value >0.05), ranging from 6.60 (± 0.03) in Nxaxo/Ngqusi to 6.75 (± 0.05) in Nahoon (**Figure 4.16 E**). Mean redox was similar ($\chi^2_{(2)}$ =8.887, p-value >0.05), ranging from -0.49 (± 0.08) in Nxaxo/Ngqusi to 14.81 (± 2.13) (mV) in Mngazana (**Figure 4.16 F**).

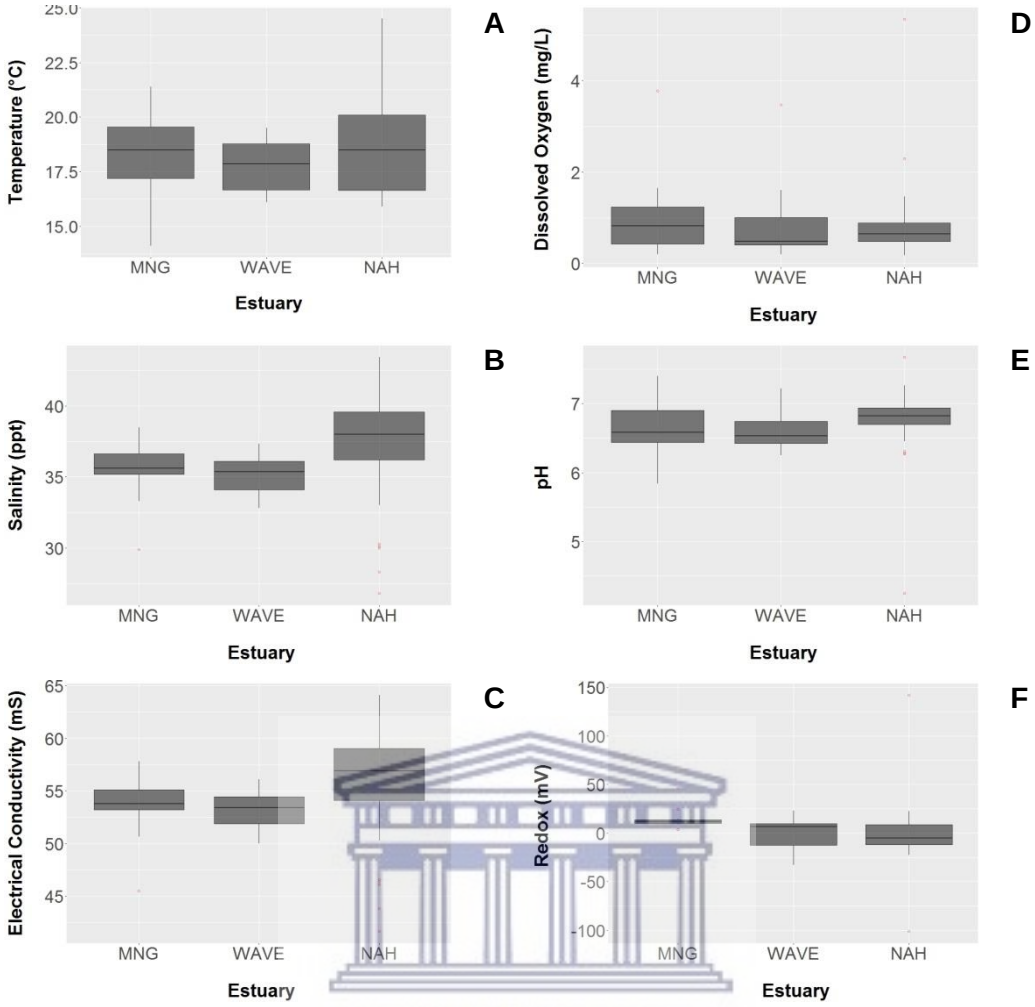


Figure 4.16: Box plots of Temperature (°C) (A) Salinity (ppt) (B), Electrical Conductivity (mS) (C), dissolved Oxygen (mg/L) (D), pH (E) and Redox (mV) (F), measured at three estuaries; Nahoon, Nxaxo/Ngqusi and Mngazana.

Pore-water Characteristics- Mngazana (2007, 2017 and 2018)

Pore-water characteristics for Mngazana collected during the year 2007, 2017 and 2018 (**Table 4.2**) of the variables measured, temperature (°C) and salinity (ppt) were recorded during the above-mentioned years, whilst electrical conductivity and pH data were only available for 2017 and 2018.

Table 4.2: Pore water variables measured at Mngazana Estuary.

Year	Season	Temperature (°C)	Salinity (ppt)	Electrical Conductivity (mS)	pH
$2007_{(N=8)}$	Winter (June) and Summer (November)	19.88 (± 1.34)	35.96 (± 0.86)	N/A	N/A
$2017_{(N=12)}$	Winter	16.79 (± 0.45)	35.92 (± 0.64)	54.16 (±0.91)	6.86 (±0.08)
$2018_{(N=11)}$	Summer	19.60 (± 0.26)	35.46 (± 0.43)	53.59 (± 0.57)	6.40 (± 0.06)

Mean temperature (**Table 4.2**) measured in the various years were significantly different ($F_{(df = 1)}$ =6.544, p-value <0.05). The Tukey HSD post hoc test showed that 2007 and 2018 were similar (adjusted p-value of 0.96, (p-value > 0.05)), but both higher than the mean value in 2017 (both combinations having an adjusted p-value of 0.01, (p-value <0.05)). Mean value was lower in 2017 as sampling was done in the winter season, whilst in 2007 sampling was done in both sampling seasons, and in 2018 was done in the slightly warmer months (October). Mean salinity were found to be similar ($F_{(df = 2)}$ = 0.196, p-value >0.05). Mean electrical conductivity measured in the two years were similar ($\chi^2_{(1)}$ = 1.5942, p-value >0.05). Whilst mean pH were significantly different ($F_{(df = 1)}$ = 20.1, p-value <0.05), the Tukey HSD post hoc test showed that 2017 was higher than 2018 (p-value < 0.05).

Pore-water variables measured at Mngazana were correlated and plotted (**Figure S6**), data showed a strong positive relationship between electrical conductivity and salinity (0.999), weak relationship between temperature and electrical conductivity (-0.43), temperature and salinity (-0.44), and between temperature and pH (-0.57).

Pore-water Characteristics- Nxaxo/Ngqusi *(2007-2012, 2017 and 2018)*

Pore-water characteristics for Nxaxo/Ngqusi collected during the following years 2007-2012, 2017 and 2018 (**Table 4.3**) and of the variables measured, temperature (°C), salinity (ppt) and electrical conductivity were recorded during the above-mentioned years, whilst pH data were only available for 2017 and 2018.

Table 4.3: Pore water variables measured at Nxaxo/Ngqusi Estuary.

Year	Season	Temperature (°C)	Salinity (ppt)	Electrical Conductivity (mS)	pH
$2007_{(N=15)}$	Winter	17.06 (± 0.35)	34.1 (± 0.44)	44.98 (± 0.81)	N/A
$2008_{(N=15)}$	Winter	16.53 (± 0.24)	30.02 (± 1.30)	38.97 (± 1.36)	N/A
$2009_{(N=15)}$	Winter	16.55 (± 0.48)	35.49 (± 0.67)	53.48 (± 1.15)	N/A
$2010_{(N=12)}$	Winter	16.26 (± 1.30)	35.48 (± 0.77)	55.61 (± 0.44)	N/A
$2011_{(N=15)}$	Winter	15.70 (± 0.23)	27.59 (± 1.44)	42.76 (± 2.04)	N/A
$2012_{(N=15)}$	Winter	14.80 (± 0.18)	33.76 (± 0.94)	41.43 (± 1.14)	N/A
$2017_{(N=15)}$	Winter	16.97 (± 0.21)	35.04 (± 0.33)	53.10 (± 0.45)	6.49 (± 0.06)
$2018_{(N=15)}$	Summer	18.64 (± 0.13)	35.15 (± 0.36)	53.18 (± 0.49)	6.71 (± 0.07)

Mean temperature measured in the various years were found to be significantly different ($\chi^2_{(7)}$ = 63.163, p-value <0.05), the Dunn's test showed that temperature measured in 2007, 2008 and 2018 was much higher than that measured in 2012 (p-value <0.05), that measured in 2018 was higher than 2008, 2009, 2010 and 2011 (p-valu e<0.05). Temperature ranged from 14.8 (± 0.35) in 2012 to 18.64 (± 0.13) ºC in 2018 (**Table 4.3**). Mean Salinity were also found to be significantly different ($\chi^2_{(7)}$ = 47.268, p-value <0.05), the Dunn's test showed that salinity measured in 2007, 2009, 2010, 2012, 2017 and 2018 was higher than 2011 (p-value <0.05), that measured in 2009, 2010, 2017 and 2018 was higher than 2008 (p-value <0.05). Salinity ranged from 27.59 (± 1.44) in 2011 to 35.49 (± 0.67) ppt in 2009 (**Table 4.3**). Mean electrical conductivity were found to be significantly different vary ($\chi^2_{(7)}$ = 84.941, p-value <0.05), the Dunn's test showed that conductivity measured in 2009, 2010, 2018 were higher than 2007 (p-value <0.05), that measured in 2009, 2010, 2017 and 2018 were higher than 2008 (p-value <0.05) and that measured in 2009, 2010, 2017 and 2018 were higher than 2011 and 2012 (p-value <0.05). Electrical conductivity ranged from 38.97 (± 1.36) in 2008 to 55.61 (± 0.44) in 2010. Mean pH values were significantly different ($\chi^2_{(1)}$ = 5.596, p-value <0.05), the Dunn's test showed that pH was higher in 2018 than in 2017.

Pore-water variables measured at Nxaxo/Ngqusi were correlated and plotted (**Figure S7**). Strong relationship between salinity and electrical conductivity (0.99), a weak negative

relationship between temperature and conductivity (-0.36) and between temperature and salinity (-0.34).

Pore-water Characteristics per Estuary – Nahoon (2012, 2017 and 2018)

Pore-water characteristics for Nahoon collected during the following years 2007-2012, 2017 and 2018 (**Table 4.4**) of the variables measured, temperature (°C), salinity (ppt) and electrical conductivity were recorded during the above-mentioned years, whilst pH data was only available for the year 2017 and 2018.

Table 4.4: Pore water variables measured at Nahoon Estuary.

Year	Season	Temperature (°C)	Salinity (ppt)	Electrical Conductivity (mS)	pH
$2012_{(N=24)}$	Winter and summer	17.94 (± 1.34)	36.83 (± 1.46)	36.88 (± 2.65)	N/A
$2017_{(N=23)}$	Winter	16.82 (± 0.16)	37.12 (± 0.74)	55.79 (±1.01)	6.92 (± 0.05)
$2018_{(N=24)}$	Summer	20.45 (± 0.29)	37.39 (± 0.75)	55.75 (± 0.99)	6.59 (± 0.11)

Mean temperature values were found to be significantly different ($\chi^2_{(2)}$ = 19.592, p-value <0.05), the Dunn's test showed that the mean values in 2012 and 2018 were similar (p-value>0.05) and both higher than 2017 (p-value <0.05). This could be explained by the season when temperature was measured, sampling in 2017 was in winter, whilst 2018 was towards the summer months, and 2012 data was sampled in both winter and summer (**Table 4.4**). Mean salinity were found to be similar ($\chi^2_{(2)}$ = 0.1200, p-value >0.05) (**Table 4.4**). Mean electrical conductivity were significantly different ($\chi^2_{(2)}$ = 32.751, p-value <0.05), the Dunn's test showed that 2012 was lower than 2017 and 2018 (p-value <0.05) whilst 2017 and 2018 were similar (p-value >0.05) (**Table 4.4**). Mean pH was significantly different ($\chi^2_{(1)}$ = 10.139, p-value <0.05), the Dunn's test showed that 2017 was higher than 2018. Pore-water variables measured at Nahoon were correlated and plotted (**Figure S8**), a strong positive relationship between electrical conductivity and salinity (0.94) was found.

Channel water properties

Physio-chemical variables namely temperature (°C), salinity (ppt), electrical conductivity (mS), dissolved oxygen (mg. L^{-1}), Redox (mV) and pH measured at the various channels at the three mangrove forests Mngazana, Nxaxo/Ngqusi and Nahoon during the 2017 and 2018 sampling events (**Table 4.5)**.

Table 4.5: Physio-chemical variables measured at channels at each estuary (2017 and 2018).

Estuary	Temperature (°C)	Salinity (ppt)	Electrical Conductivity (mS)	Dissolved oxygen (mg. L^{-1})	Redox (mV)	pH
Mngazana	19.69 (± 1.36)	35.82(± 0.64)	54.13 (± 0.86)	4.83 (± 0.94)	-42.16 (± 13.94)	7.39 (± 0.22)
Nxaxo/Ngqusi	18.86 (± 0.42)	34.01 (±0.49)	51.60 (± 0.69)	5.42 (± 0.85)	-44.32 (± 6.29)	7.76 (± 0.11)
Nahoon	20.5 (± 1.56)	34.2 (±0.43)	51.64 (± 0.35)	8.48 (± 0.66)	-71.03 (± 5.90)	7.91 (± 0.15)

Pore-water variables were correlated and plotted (**Figure S5**). The data showed that there is a strong negative relationship between Redox and pH (-0.88), weak negative relationship was also found between pH and Oxygen (-0.52). Data also showed a strong positive relationship between Electrical conductivity (mS) and Salinity (ppt) (0.91) and a weak positive relationship between Redox and Oxygen (0.41).

Non-metric multidimensional scaling (NMDS) analysis (**Figure 4.17**) was also run on porewater data collected during 2017 and 2018 using the Bray-Curtis dissimilarity matrix at 999 permutations. BcPower Transformations to Multi-normality was used to transform the data where the following power transformations were done to the porewater variable; (Salinity ^-0.0227), (Temperature ^-0.6717), (Conductivity ^0.2831) and (pH ^4.7811). The results from the analysis as observed in **Table 4.5** had a stress level of 0.021. The following tests were carried out namely; ANOISM (Analysis of similarities), PERMANOVA (Permutational multivariate analysis of variance), PERMDISP2 (through betadisper function in r). Plot shows that pore-water data for the various estuaries overlaps **Table 4.5**, this supported by the ANOISM (R^2= 0.129, p-value = 0.047 (95% upper quantile of permutation) showing that the pore-water characteristics of the three estuaries were similar. The PERMANOVA showed a significance difference ($F_{(df = 2)}$ =3.1304, R^2= 0.061, p-value <0.05), but the R^2 value displayed

a very weak association between the measured variables and the estuary in which they were collected from. The PERMDISP2 showed that there is no difference in the dispersion of the three estuaries ($F_{(df = 2)} = 0.249$, p-value >0.05), pairwise comparison shows that all estuaries were similar (p-value>0.05). The average distance to median at Mngazana was 0.091 (p-value >0.05), for Nxaxo/Ngqusi was 0.071 (p-value >0.05) and for Nahoon was 0.079 (p-value >0.05) thus assumption for homogeneity of multivariate dispersions was met.

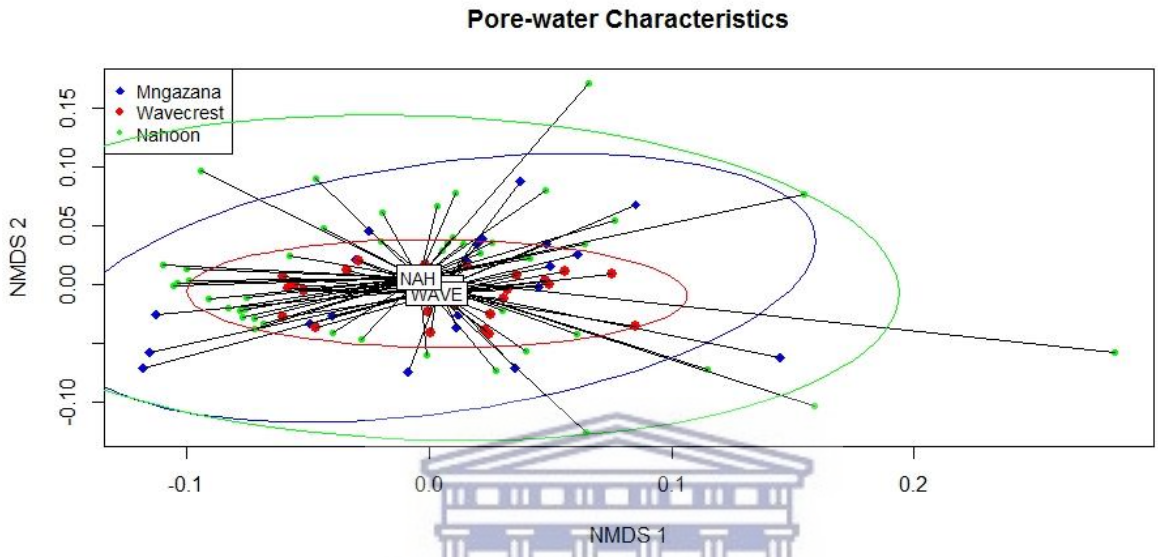


Figure 4.17: Non-metric multidimensional scaling (NMDS) plot of the pore-water variables measured at the three estuaries; Mngazana (MNG), Nahoon (NAH) and Nxaxo/Ngqusi (WAVE).

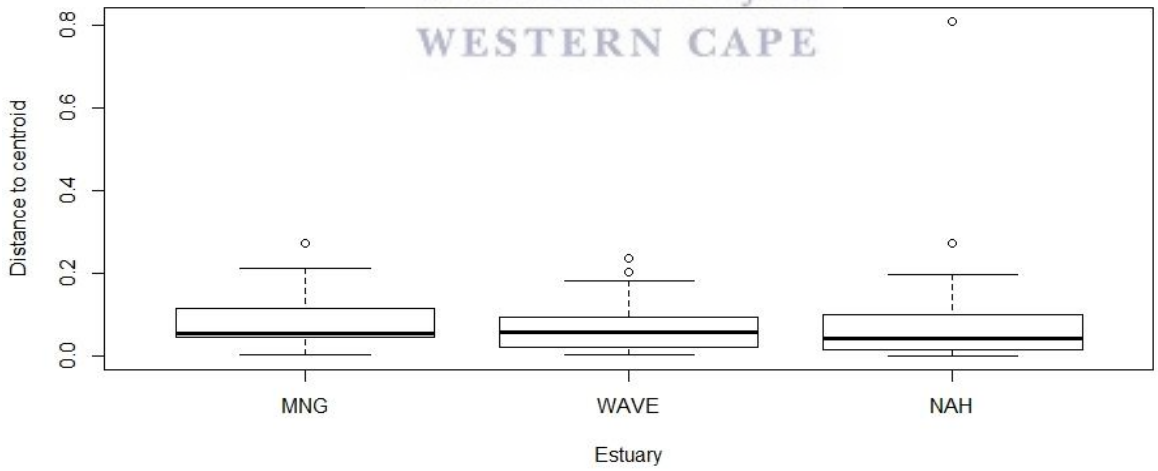


Figure 4.18: Box plot showing PERMDISP2 results of the pore-water variables measured at the three estuaries; Mngazana (MNG), Nahoon (NAH) and Nxaxo/Ngqusi (WAVE).

Discussion

The chapter provides important environmental information to be taken into consideration for **Chapter 5** as plant performance is determined by plant fitness related traits which include levels and maintenance of genetic diversity, population size, growth rates, reproductive success, dispersal, germination and establishment – all of which may be influenced by the prevailing local environmental conditions (Anderson, 2016; Butcher *et al*., 2009, Tonné *et al*., 2017).

Mangrove establishment is directly or indirectly influenced by a number of abiotic and biotic factors; the former includes temperature, light, salinity, CO_2, flooding, nutrient availability, sea level rise whilst the latter includes biota such as crabs as they consume or damage mangrove propagules, construct mounds due to burrows and alter the topography, increase sediment turnover, sediment condition (particle size) and encourage the exchange of nutrients between sediment and tidal water (Clark *et al*., 1998; Kathiresan and Bingham, 2001; Krauss *et al*., 2008).

The sediment characteristics of mangrove forests may vary due to several physical, chemical and biological dynamics (Hossain and Nuruddin, 2016). The growth and survival of mangroves is highly influenced by the physiochemical conditions of the sediment (Geldenhuys *et al*., 2016). Raw *et al.* (2019) using a structural equation model (SEM) found the mean annual rainfall and flood plain to be strong predictors of mangrove area in South African estuaries as estuaries with larger mean annual rainfall and flood plain had a larger mangrove habitat area. Raw *et al*. (2019) suggest that the addition of factors such as sediment characteristics which influence mangrove distribution could strengthen the SEM.

The occurrence of mangrove species, their structure and development are dependent on sediment variables such as salinity, organic matter, physiological position, pH and redox potential (Marchand *et al*., 2004; Hossain and Nuruddin, 2016). This study measured four variables namely; moisture content, organic content, redox and pH to determine the sediment characteristics of three mangrove forests namely; Mngazana, Nxaxo/Ngqusi and Nahoon.

Moisture content of mangroves is influenced by freshwater water inputs due to rainfall (precipitation), tidal inundation and tidal flushing rates (Hoppe-Speer *et al*., 2013). Mangrove

forests that experience low to non-freshwater inputs will experience high evaporation rates which results in high salinity levels (Alongi, 2009). Mangrove die-backs may be experienced during long periods of drought, high temperature and restricted tidal exchanges as a result of the increased stressful conditions in the mangrove environment (Hoppe-Speer *et al*., 2013; Duke *et al*., 2017). Hoppe-Speer *et al.* (2013) conducted a study in St Lucia, this study reports a moisture content less than 30% as dry, in the current study, moisture content measured in 2012 at Nahoon was approximately 32%, suggesting that it may have been experiencing a period of low freshwater input. Whilst in the same year, moisture content measured at Nxaxo/Ngqusi was approximately 54%. Hoppe-Speer *et al.* (2013) reported soils with moisture greater than 55% as waterlogged. Waterlogged soils result in low mineralization process which promotes the storing of organic matter (Marchand, 2017; Bastakoti *et al*., 2019). Due to Nxaxo/Ngqusi and Nahoon being relatively close regarding their location, the results observed illustrate that even though these estuaries may have both experienced low rainfall other factors such as tidal flooding may have influenced the moisture content of the sediment. At Nxaxo/Ngqusi, during spring high tides the forests becomes inundated, whilst during the neap high tide only the low-lying areas are inundated (Steinke and Ward, 1990) which is also the case for Nahoon. Thus, the intermediate (almost waterlogged) levels of moisture content at Nxaxo/Ngqusi may be due to tidal inundation as most sites are on the channel edge.

In the year 2017 and 2018, moisture content was similar ranging from ~42 % at Nxaxo/Ngqusi to ~39 % at Nahoon, being somewhat the "intermediate" between dry and waterlogged soils. Except for moisture content measured at Nahoon and Nxaxo/Ngqusi in 2012, the soils in these estuaries were generally "intermediate". Moisture content measured by Rajkaran and Adams (2010) in Mngazana was greater than 45% and less than 55% in non-harvested and harvested sites, this was thus similar and higher than (respectively) to what was observed in this current study.

The quantity of organic matter together with soil condition (particle size) play a role in the permeability and drainage characteristics of the soil, its redox potential, pH, salinity and nutrient availability (Clough, 1993). Organic matter provides nutrients to mangroves through the decomposition process, thus may be a good indicator of nutrient status in the sediment (Chaudhari *et al*., 2013; Hossain and Nuruddin, 2016). The availability of nutrients in sediment may play a role in the growth and productivity of the mangrove environment (Reef *et al*., 2010). According to Chen and Twilley (1999a) mangrove biomass and productivity is mainly

influenced by nutrient availability when mangroves are not subjected to physiological stress by sulphide or salinity.

Mangroves occurring at higher latitudes have been shown to have lower organic production (Hogarth, 2015). Results obtained in this study did not show a trend according to latitude. Alongi (2009) states that the accretion of organic matter may also be influenced by forest age due to the amount of dead mangrove roots that have accumulated over the years. In support of this, Marchand (2017) states that the productivity of mangroves may vary according to age, where it is generally high in forests that are older than 30 years to about 120 years, according to this study, mangrove forests reach the senescent stage at approximately 48 years of age. Rajkaran and Adams (2011) measured higher organic content (~28.9%) in Echwebeni (KwaZulu-Natal) which was the oldest mangrove forests in that study, whilst the other forest studied had similar organic content (6-10 %). This is contrary to the current study where the youngest forest at Nahoon had a similar organic content to Mngazana Estuary (oldest) and both were higher than Nxaxo/Ngqusi; even so, all three of these forests are older than 48 years. Thus, this would indicate that the variation in organic matter could be a result of other factors not primarily related to age. According to Marchand (2017) the position of the mangrove forest to the tidal zone also plays a role in the organic matter content as observed in the increase in the organic carbon content. Bastakoti *et al*. (2019) and Rajkaran and Adams (2007) suggest that tidal transport and mangrove trees may encourage high organic matter content. This is supported by Alongi (2009) who states that tidal range is one of the factors which regulate ecosystem production.

Nutrient transport from neighbouring habitats through tides and flooding will also influence the amount of available nutrients to mangroves (Hossain and Nuruddin, 2016). According to Rovai *et al*. (2018) the organic matter decomposition rates are regulated by sulphide occurrence, which is influenced by tides, where infrequent flooding may encourage sediment retention of autochthonous soil organic carbon whilst those which are frequently flooded may experience higher mineralization rates. This would then also suggest that the mouth status and period of mouth closure may play an important role. Both Nahoon and Mngazana are classified as "predominantly open" estuarine systems, whilst Nxaxo/Ngqusi as a "large temporarily closed" estuarine systems (van Niekerk *et al*., 2019).

A positive correlation between Organic Content (%) and Moisture Content (%) observed in this present study was expected, as soils with higher moisture have high organic content, higher moisture in the soil encourages higher decomposition rates by microbes of available organic matter in the water column into the soils (Pinckney *et al*. 2001, Rajkaran and Adams, 2012; Hossain and Nuruddin, 2016).

Anoxic soils are said to be encouraged by high levels of organic matter and frequent tidal flooding (Hossain and Nuruddin, 2016, Otero *et al*., 2017). They pose some challenges as mangroves must maintain internal transport of gases such as O_2 to the underground roots, in such conditions, the proportion of elements available for mangrove is altered where the availability of some elements is increased whilst some is decreased, some of these elements in their reduced state and at higher concentrations become harmful to mangroves (Joshi and Ghose, 2003; Alongi, 2009).

Nutrients available for mangrove uptake is primarily influenced by the redox status of the sediment surrounding the mangrove roots (Clough, 1993; Reef *et al*., 2010). Reducing conditions are a common feature of mangrove soils (Otero *et al*., 2017). According to Hossain and Nuruddin (2016) studies have found mangrove soils to be anaerobic (less than 100 mV). Sediment collected at the three estuaries were found to be moderately reducing, as they ranged from 107.14 mV at Nxaxo/Ngqusi to -52.89mV at Nahoon, within this range it is understood that such conditions encourage high rates of denitrification, soil nitrates are depleted, high nitrogen fixation which is largely in the form of ammonium, high rates of ammonification and iron is reduced resulting in phosphorus being released into the pore-water (Reef *et al*., 2010). Thus, anoxic soils will have an influence on the quantity of organic matter and thus nutrient availability and uptake by mangroves which may facilitate in mangrove growth (Alongi *et al*., 2005; Reef *et al*., 2010).

The change in the redox status may result in changes in pH, organic matter content and in the long run thus the ability of sediment to sequester nutrients and elements (Bastakoti *et al*., 2019). Sediment pH found in this study fell within the range 7- 8 suggesting that the various systems were neutral to slightly alkaline, this may be a result of the influence of seawater with less freshwater inputs, high biological activity and high photosynthetic activity on the sediment (Alongi *et al*., 2004; Saravanakumar *et al*., 2008; Ashok Prabu *et al*., 2008). pH values measured were similar to those measured by Rajkaran and Adams (2012) for Mngazana,

Hoppe-Speer and Adams (2015) for Nxaxo/Ngqusi and Geldenhuys *et al*. (2016) for Nahoon. These were also somewhat comparable to Marchand *et al.* (2004) where an Avicennia forest was found to range between 6.2 and 8, the second being Clark *et al.* (1998) where the range was between 5.5 and 8. Joshi and Ghose (2003) found that *A. marina* (Lothian Island, western Sundarbans) grew in variable pH conditions where its peak performance was at pH 7.82 and 7.55. Singh and Odaki (2004) states that alkaline sediment may favour the volatilization of ammonium which may lead to the loss of nitrogen, thus consequently lowering nutrient availability.

Sediment measured at Mngazana showed that pH measured in 2017 and 2018 was lower than that measured in 2007. A decrease in pH can also be linked to a high organic content due to the higher availability of microorganisms involved in decomposition and sulphate reducing conditions (Bastakoti *et al*., 2019). Other factors linked to the decrease of pH include low primary productivity, reduced salinity and temperature, increase in freshwater inputs, a flooding event and anthropogenic activities which result in sediment disturbance (Ashok Prabu *et al*., 2008; Singh and Odaki, 2004; Bastakoti *et al*., 2019). Even though the decrease in pH was found to be significant, the change was less than one. Measurements at Nxaxo/Ngqusi over the years displayed some variation where the difference was almost 1 between some years, ranging from slightly acidic to slightly alkaline sediment.

Pore-water variables measured in this study were; Salinity (ppt), pH, Conductivity (mS), Temperature (°C), Oxygen (mg. L^{-1}) and Redox potential (mV). Pore-water salinity plays an important role in mangrove forest structure (including tree height and tree density) and productivity (Twilley and Day, 1999; Tuffers *et al*., 2001; Lovelock *et al*., 2005; Rivera-Monroy, 2019). Mean salinity values for the three estuaries were around 33 to 37 ppt, this is close to that of sea water (35 ppt), which is higher than what most mangrove species require for optimal growth, but still falls within the ideal range for the growth of *A. marina* individuals (Krauss *et al.*, 2008; Hoppe-Speer *et al*., 2015).

Channel salinity values for Nxaxo/Ngqusi and Nahoon were comparable to values found in a study by Harrison (2004) for estuaries occurring in the warm-temperate region which were found to have salinities between 25-35 ppt, Strydom (2015) found the Nxaxo and Ngqusi Estuaries (Wavecrest) to range between 28-38 ppt. Whilst for subtropical regions, Harrison (2004) found the salinity to be below 20 ppt, in this study values obtained for Mngazana were

much higher. Salinity obtained in this study for Mngazana were comparable to Cotiyane *et al*. (2019), where salinity ranged between 38ppt (Winter 2015) to 20.6 ppt (Summer 2015), the study suggested that the system may be marine dominated because of insufficient river inflow. The results obtained in this current study may be an indication of lower freshwater inputs, low levels of rainfall and large tidal prism of these systems (Twilley and Day, 1999; Harrison, 2004; Alongi, 2009; Cotiyane *et al*., 2019).

Rainfall data for Mngazana (Capeharmes) had a significant decrease in rainfall whilst for Nxaxo/Ngqusi (East London) and Nahoon (East London) the decrease was not significant between 1968 and 2018, whilst between 2000 and 2018 the decreasing trend in rainfall was not significant for both. Thus, suggesting that both regions were still receiving similar amounts of freshwater inputs from rainfall over the eight-year period.

This may be somewhat contrary to the pH values of the sediment, which may have suggested that the systems were receiving sufficient freshwater inputs. Whilst pore-water pH was slightly lower than 7, thus being slightly acidic. Within the sites, pH measured in Mngazana and Nahoon was higher in 2017 than in 2018, whilst for Nxaxo/Ngqusi the inverse was true. Tidal cycles result in the regular change of the amount of water on the sediment, thus may influence the pH of porewater (Schwarzbach and McDade, 2002).

Several studies have shown how mangroves perform at various salinities, a study by Suárez and Medina (2005) used varying NaCl concentrations (0, 170, 430, 680, and 940 mol m^{-3}) treatment to demonstrate the effects of salinity on *Avicennia germinans* seedlings, results showed a decrease in leaf productivity, leaf longevity, leaf area and increased mortality rate. A study by Shiau *et al.* (2017) suggests that the growth of individuals and absorption of nutrients may be inhibited at high salinities; the study showed a decrease in nutrient uptake by *Kandelia candel* seedlings with increasing salinity, the measured salinity ranged between 0 to 35 ppt. This supported a study by Tuffers *et al*. (2001) that demonstrated a direct relationship between salinity and concentration levels of Nitrogen in *A. marina* leaves.

All sites showed a positive relationship between salinity and electrical conductivity ranging from 0.90 to 0.99, this is expected as electrical conductivity measures ion concentration which is the amount of salt in the pore water, but electrical conductivity of pore-water may be influenced by the root system of the mangroves, where oxidation on the surface of mangrove

roots can increase the electrical conductivity (Otero *et al*., 2006). In general, electrical conductivity measured within the three estuaries showed that previous years were lower than 2017 and 2018.

A study conducted by Geldenhuys *et al*. (2016) found that the growth and expansion of mangroves at Nahoon may be facilitated by salinity and temperature. Strong negative relationship between salinity and temperature was only found in Nxaxo/Ngqusi, whilst a weak relationship at Nahoon. Salinity and temperature have an influence on the amount of dissolved oxygen in porewater (Harrison, 2004; Ashok Prabu *et al*., 2008). The process of decomposition consumes oxygen resulting in the storing/accumulation of CO_2 and restoration of inorganic nutrients (Pinckney *et al*. 2001). Thus, organic content has an influence on oxygen dynamics in pore-water. Dissolved oxygen concentration levels in pore-water have an influence on the biological and chemical processes such as photosynthesis, respiration and mineralisation (Knight *et al*., 2013). Mean dissolved oxygen values for the three estuaries was low compared to what other studies have found, for example Harrison (2004) found the dissolved oxygen to be above 5 mg/L-1 for both temperate and sub-tropical regions.

Conclusion

Environment stress such as high salinity, waterlogging may influence the growth, leaf area and photosynthesis of mangroves and thus the performance of mangroves (Hogarth, 2015). The results showed that there has been changes in the sediment and porewater characteristics over the years. In general, the 2017 and 2018 results showed that sediment characteristics of Nahoon were significantly different from those found in Nxaxo/Ngqusi. Whilst the porewater characteristics were generally similar in all the estuaries. The results suggest that the soils generally had high salinity (close to seawater), were not waterlogged or dry even though they had low water fresh input and rainfall, were reducing and neutral to slightly alkaline. The results also showed that the mouth condition, tides had influence on the sediment and porewater characteristics of the various estuaries. With the understanding of the environmental conditions experienced by the range edge populations at Nahoon, Nxao/Ngqusi and Mngazana esturaies, the Chapter below assesses the plant plant performance of *Avicennia marina* populations with the inclusion of additional sites.

Chapter 5 – Plant performance of *Avicennia marina* at the latitudinal limit of mangrove distribution in South Africa

According to Anderson (2016), individual plant performance is associated with demography and evolution, which encompass the ability of the plant to exhibit fecundity, successfully germinate, flower and survive and. Factors that may play a role in plant performance include levels of heterozygosity within individual's, levels of diversity between individuals, adaptation of individuals to local environments, population level effects such as inbreeding, genetic drift, inbreeding depression, and increased extinction rate (Engelhardt *et al*., 2014). Keller and Wellar (2002) suggested that overall fitness is indicated by fecundity and viability. Several studies suggest that inbreeding and the loss of genetic variation/diversity may result in lower fitness (Kéry *et al*., 2000; Mustajärvi *et al*., 2001; Keller and Wellar, 2002; Reed and Frankham, 2003; Charlesworth and Willis, 2009). However, a review by Abeli *et al*. (2014) showed that low genetic variation does not always translate to lower fitness in plant species.

Plant performance is important as it may act as an indicator of population resilience, such information is important in the conservation of mangrove as this ecosystem provides many ecosystem services which are under threat due to global change and in particular habitat loss. Resilience is defined as the capacity of system to maintain its function during disturbance or the rate of return to its initial state post disturbance (McLeod and Salm, 2006; Capdeville *et al*., 2019). Anderson (2016), states that factors which may result in the persistence of a population include high levels of fecundity, a large population size, extensive distribution range and rapid generation times.

Abeli *et al*. (2014) determined if the occurrence of species at the range edge influenced their demographic species-specific traits, and thus the plant performance of these populations. The review organised the literature into five categories; 1) population features which included information about the population size and density, 2) demography which included population structure, growth rate and plant survival, 3) reproductive traits which included flower production and seed germination, 4) morphological traits such as leaf size and plant height and the fifth category was phenotypic plasticity which included studies where a single or multiple treatment(s) were used to measure phenotypic plasticity.

Population features such as the population size and density are good indicators of plant performance because low population size is associated with generally low genetic variation within the population. similarly, low population density is also associated with inbreeding and genetic drift (Arnaud-Hoand *et al*., 2006; Leimu *et al*., 2010). Population density may be influenced by forest age and mortality, as older forests will have larger trees and reduced density but the high loss of individuals exceeding the establishment of new individuals or slow recovery will also result in reduced density (Sousa *et al*., 2003; Alongi, 2015). A significant reduction in population size and density resulting in lower genetic variation and increased inbreeding may result in lower performance measured in other categories (Leimu *et al*., 2010; Guo *et al*., 2018).

Demography is defined as the census of mortality, reproduction, disease of a population which translates to changes in population size and population structure dynamics (Abeli *et al*., 2014; Tomizawa *et al*., 2017). Increases in climate events and disturbances such as storms, changes in temperature and other abiotic factors may influence population structure dynamics (Rivera-Monroy *et al*., 2019). Population structure is a good indicator of population dynamics, which refers to the changes in plant numbers through space and time (Picó *et al*., 2008). As part of the measures for population structure in this study, tree height was measured for each individual. Yin and Wang (2019) state that, in general, tree height has been used to determine biomass and primary productivity as it is a good measure for structural development. Population structure data can help infer if recruitment is taking place, recruitment of seedlings beingone of the most critical life-stages, playing an important role in the growth of the population, thus regulating the quality and productivity of the forest (Sousa *et al*., 2003; Barnuevo *et al*., 2017; Riascos *et al*., 2018).

Mangroves growing in temperate climates generally have lower growth rates and biomass production than those in lower latitudes (Kathiresan and Bingham, 2001; Steven *et al*., 2006; Morrisey *et al*., 2010; Naidoo, 2016). The growth of plants is said to be predominantly influenced by habitat heterogeneity such as changes in environmental conditions or resource availability (Picó *et al*., 2008). This is supported by Xiong *et al*. (2019) who states that the two main regulators of mangrove growth are; firstly, sediment condition such as anoxia, nutrient availability and salinity, these will influence the resources that are available to the mangroves. Secondly, climatic conditions; this includes rainfall, temperature and humidity which determine the prevailing environmental conditions. Thus, it would be expected that mangroves

at Mngazana in the subtropical region would have a higher growth rate than those found in Nxaxo/Ngqusi and Nahoon in the temperate region, thus a decrease from the core to range edge. In terms of resource availability, Mngazana and Nxaxo/Ngqusi are naturally occurring, so it would be expected that these sites are more favourable to mangrove growth than the planted mangrove forest at Nahoon Estuary.

The growth rate of plants may be an indicator of the plants competitive ability when related with other factors such as tree height, phenology, response to stress and response to damage (Saenger, 2002). Slower growth may reduce the competitive ability of the species with other co-occurring species which compete for the same resources for example salt marsh (Naidoo, 2016). Depending on which strategy the plants utilise for resource acquisition, storage and allocation, the increase in the above ground biomass maybe an indication that there is an abundance in available nutrients (Hayes *et al*., 2017). Plants utilise resources such as carbon, nitrogen and phosphorus to carry out three vital functions which are growth, reproduction and defence (Bazzaz *et al*., 1987; Krauss *et al*., 2008). According to Bazzaz *et al*. (1987), increasing resources allocated for defence could result in less resources being allocated for growth and reproduction.

Reproduction traits such as flower and fruit production, are generally measured in phenological studies (Clarke and Myerscough, 1991; Steinke, 1999; Almahasheer *et al.*, 2016). Higher latitudes experience shorter periods of summer thus a shorter growth period, as a result *A. marina* trees at higher latitudes are said to produce less flowers, thus showing an association between reproductive output (flowering) and latitude (Almahasheer *et al.*, 2016). This is somewhat contrary to what was observed in a study by Steinke (1999) which found that flowering in the northern estuaries occurred during the summer months whilst the higher latitudes (southern estuaries) flowering occurred throughout the year, though that study does not state whether reproduction was successful in the southern estuaries when compared to the northern estuaries. It would be thus expected that Mngazana would have a higher reproductive output then the other estuaries. But due to these being in different biogeographic regions it would be expected that their peak flowering times would be different.

Mangrove flowers, propagules, stems and leaves are sometimes subjected to herbivory by insects, crabs and mammals this has been documented in various studies (Newbery, 1980; Farnsworth and Ellison, 1991; Burrows, 2003; Sharma *et al*., 2003; Cannicci *et al*., 2008;

Menezes and Peixoto, 2009; Balasubramanyan *et al.*, 2010; Sousa and Dangremond, 2011; dos Santos *et al*., 2013; Feller *et al*., 2017). This may affect the performance and vigour of individual trees and may also improve the overall performance of the ecosystems as leaf damage could result in more leaves being abscised increasing the nutrients in the mangrove forest, this depending on the amount and nutritional value of the remaining leaf (Cannicci *et al*., 2008). Tropical regions have been found to have a higher diversity of herbivores when compared to temperate regions (Feller *et al*., 2017). Thus, it would be expected that mangroves at Mngazana may experience higher levels of herbivory than the rest of the estuaries in this study as they occur in the temperate region. Thus, it is expected that herbivory will decrease along the latitudinal gradient from the core to the range end (Mngazana to Tyolomnqa).

Phenotypic plasticity describes phenotypic variations which are a result of the prevailing environmental conditions, termed adaptive phenotypic plasticity when it confers an advantage to the individual (Arrivabene *et al*., 2014). According to Kathiresan and Bingham (2001), leaf size of mangroves may vary depending on where they occur and the prevailing environmental conditions because of variation in their genotypes or phenotypic response. A study by Arrivabene *et al*. (2014) found that *Avicennia schaueriana* occurring in Brazil exhibited varying functional traits (e.g., increase in LMA (Leaf mass per unit area) and changes in leaf area) depending on the prevailing environmental conditions. An increase in LMA is beneficial to the plant when growing in a hostile environment (e.g., drought condition) as higher LMA have higher leaf thickness and leaf density (Puglielli *et al*., 2015; Poorter *et al*., 2009). Whilst leaf size may be a good indicator of performance as a larger mean leaf area might be an indicator of greater photosynthetic efficiency (Kathiresan and Bingham, 2001). Thus, a higher LMA and larger leaf area may confer an advantage to the individual. An increase in SLA (Specific leaf area) value may illustrate the adaptive strategy of those occurring in hypersaline conditions (Naidoo *et al*., 2011). Naidoo (2016) and Feller *et al*. (2017) state that the nutritional value (nitrogen and phosphorus content) of plants may decrease when moving to higher latitudes. Thus, it would be then expected that that nitrogen content in mangrove leaves would be highest in Mngazana and lowest in Tyolomnqa, thus a decrease from the core to the range edge populations.

Depending on where mangroves occur and the prevailing environmental conditions (levels of salinity, nutrient availability, light, extent of inundation, sea-level rise), phenotypic expression may differ resulting in variable mangrove traits across their geographical distribution range

(Alongi, 2015; Saenger and West, 2018). Phenotypic plasticity is a good indicator of plant performance as it may provide a competitive advantage when found in higher levels in genotypes of important traits and may provide ways for plants to cope with changes in environmental conditions and survive in their current site or establish in new habitats (Proffitt and Travis 2010; Anderson, 2016).

Several of the discussed factors do not act in isolation, as a number of links can be observed from the various factors which is expected to influence the overall performance of the mangrove populations. The current study aims to determine the plant performance of *A. marina* at the range edge of its distribution in South Africa. These results will inform management of these forests to ensure their long-term survival and expansion. Study sites include Mngazana, Nxaxo/Ngqusi and Kwelera where mangroves occur naturally as well as Nahoon and Tyolomnqa where mangroves have been planted from different source populations.

Objectives

To determine the plant performance of *Avicennia marina;* population density, population structure, growth rates, flower count, leaf size, leaf area, surface leaf area, C/N and severity of pest infestation and signs of disease were measured. It is postulated that mangrove populations with higher genetic diversity (De Ryck *et al*., 2016), such as that at Mngazana Estuary would have a larger population size, greater density, higher growth rate and flower production compared to Nxaxo/Ngqusi, Kwelera, Nahoon and Tyolomnqa.

Study site descriptions

Along the South African coastline, *A. marina* occurs in various estuaries from Kosi Bay to Tyolomnqa, varying in population size. For this study we have selected study sites where genetic evidence (De Ryck *et al*., 2016) and where long-term growth data exists (Bolosha, 2016 (Msc, Unpublished); Mbense, 2017 (Msc, Unpublished); Geldenhuys, 2014 (Msc, Unpublished)). Study sites include Mngazana, Nxaxo/Ngqusi and Kwelera where mangroves occur naturally as well as Nahoon and Tyolomnqa where mangroves have been planted from different source populations. Along the latitudinal gradient, Mngazana (31°41'31.84"S; 29°25'16.11"E) occurs in the subtropical region while Nxaxo/Ngqusi (32°35'1.69"S; 28°31'18.74"E), Kwelera (32°54'13.19"S; 28° 3'52.63"E), Nahoon (32°59'0.23"S; 27°56'32.97"E) and Tyolomnqa (33°13'8.73"S; 27°34'51.07"E) all occur in the warm-

temperate region (**Figure 5.1**, **Table 5.1**). Genetic data and long-term data are available for Mngazana, Nxaxo/Ngqusi and Nahoon Estuary. Long term growth data was not available for Kwelera and Tyolomnqa due to the size of the populations, for this reason these two sites were not included in the determination of growth rates.

Table 5.1: A summary of the estuarine features at the Mngazana, Wavecrest, Kwelera, Nahoon and Tyolomnqa estuaries.

Estuary	**Biogeographic region (Whitfield and Baliwe, 2013)**	**Estuarine type (Whitfield and Baliwe, 2013)**	**New Estuarine type (van Niekerk *et al.*, 2019)**	**Mangrove area (Ward and Steinke 1982)**	**Mangrove area (NMU estuary habitats, 2017 - 2019)**	**Mangrove area (van Niekerk et *al.*, 2019)**
Mngazana	Subtropical	Permanently Open	Predominantly Open	150 ha	148 ha (2017)	118 ha
Wavecrest (Nxaxo/Ngqusi)	Warm-Temperate	Permanently Open	Large Temporarily Closed	14 ha	11,2 ha (2017)	10 ha
Kwelera	Warm-Temperate	Permanently Open	Predominantly Open	0.5 ha	-	<3.1 ha*
Nahoon	Warm-Temperate	Permanently Open	Predominantly Open	0.5 ha	2.61 ha (2017)	
Tyolomnqa	Warm-Temperate	Permanently Open	Large Temporarily Closed	N/A	0.6 ha (2017)	

*Individual mangrove cover was not provided for these systems in van Niekerk *et al*. (2019). However, an estimate for the total area of mangrove at Great Kei (not included here) Kwelera, Nahoon and Tyolomnqa was approximately 3.1 ha.

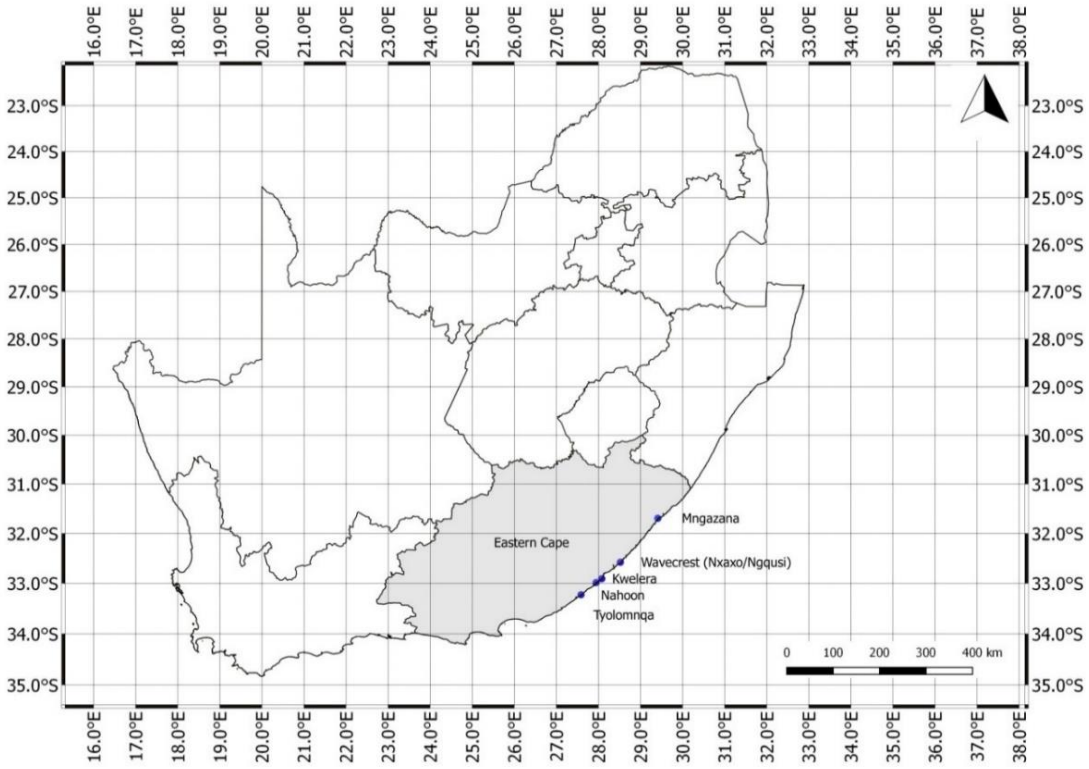


Figure 5.1: Locality map of study sites occurring in the Eastern Cape province of South Africa.

Mngazana Estuary (31°41'31.84"S; 29°25'16.11"E)

Site description has been described in **Chapter 4** (**Section 4.13**) and a figure showing location has also been provided in Chapter 4 (**Figure 4.2**).

Wavecrest (Nxaxo- Ngqusi Estuaries, 32°35'1.69"S; 28°31'18.74"E)

Site description has been described in **Chapter 4** (**Section 4.13**) and a figure showing location has also been provided in Chapter 4 (**Figure 4.6**).

Kwelera Estuary (32°54'13.19"S; 28° 3'52.63"E)

This estuary is classified as a predominately open estuary (van Niekerk *et al*., 2019) occurring in the warm temperate region and is 7 km north of East London (Whitfield and Baliwe, 2013). This micro-tidal estuary is 70 ha in size and 4.9 km in length. The estuary is flood dominated with a catchment area of approximately 391 km^2 and a tidal-prism of $5.2x10^5m^3$ (Reddering and Esterhuysen, 1987). Steinke and Ward (2003) state that mangroves occurring in this estuary may have been established naturally around the year 1967, that study also suggested that the Kobonqaba Estuary (55 km north) could act as a source population. One small mangrove stand (with *A. marina* individuals) occurs 1 km from the mouth of the estuary. More recently, *A. marina* individuals have been recorded upstream along the estuary (**Figure 5.2**) and *B. gymnorrhiza* individuals were also found. Ward and Steinke (1982) estimated that the cover was less than 0.5 ha, a study by Bolosha (2016) estimated that the mangroves have a cover of about 0.4 ha and van Niekerk *et al*. (2019) estimates that together with Tyolomnqa and Great Kei, this estuary has a mangrove cover of about 3.1 ha.

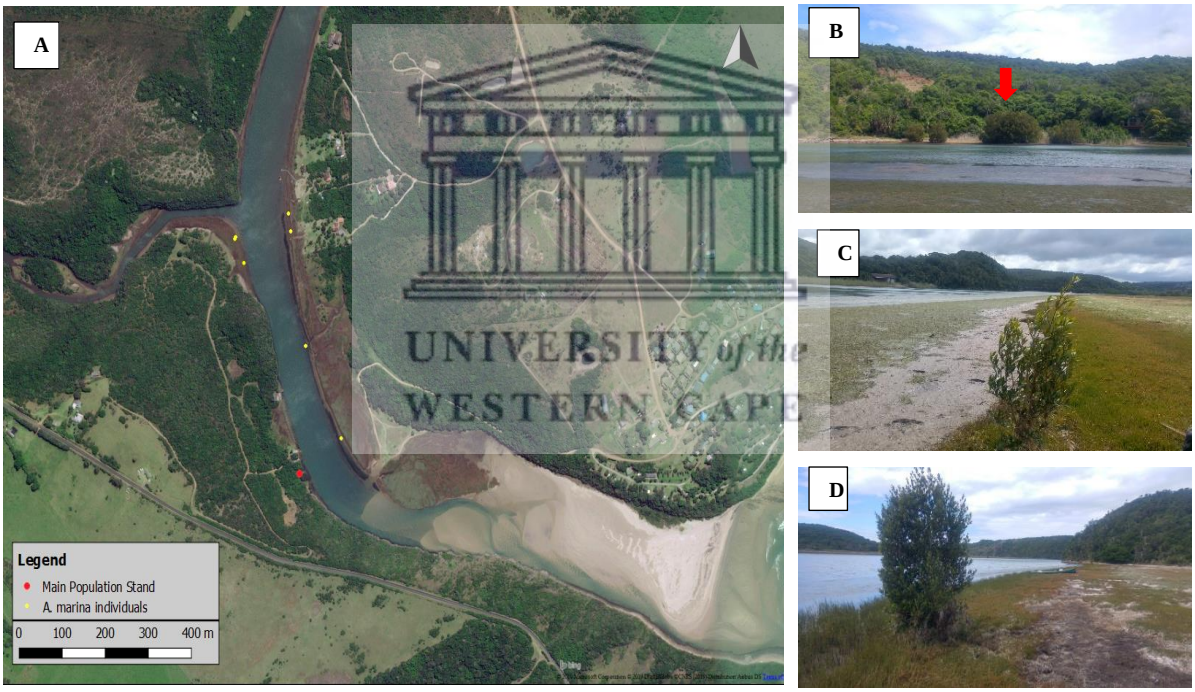


Figure 5.2: Kwelera Estuary, showing the location of *A. marina* individuals found along the estuary. The main population stand is shown in red, (A) photograph of the main population stand (Red arrow). The locality of some of the *A. marina* individuals are shown in yellow, photographs of some of these individuals shown in (B) and (C) (imagery from Goggle Earth).

Nahoon Estuary (32°59'0.23"S; 27°56'32.97"E)

Site description has been described in Chapter 4 (**Section 4.13**) and a figure showing location has also been provided in Chapter 4 (**Figure 4.7**).

Tyolomnqa Estuary (33°13'8.73"S; 27°34'51.07"E)

This estuary (**Figure 5.3**) occurs in the warm temperate region approximately 43 km south of Nahoon Estuary (East London) (Whitfield and Baliwe, 2013). It has a catchment area of 441 km^2 and is classified as a large temporarily closed estuary (van Niekerk *et al*., 2019) with a moderate tidal prism ranging between 1 to $10x10^6$ m^3 (Whitfield, 1992; James and Harrison, 2011). The intertidal area along the main channel of the estuary is limited, except at the mouth where wider intertidal areas are found (James and Harrison, 2011). The mangroves in this estuary are not natural, and were planted in the 1990s, thus making this estuary the current limit of mangroves in South Africa (Whitfield *et al*., 2016). Only *A. marina* is found to occur in this estuary and studies such as Bolosha (2016) have shown that these mangroves are expanding.

Figure 5.3: Tyolomnqa Estuary, with three small mangrove stands of varying size are found to occur along the estuary (Imagery from Goggle Earth).

Materials and Methods

Growth rate measurements

The growth rates for *A. marina* were determined at Mngazana, Nxaxo/Ngqusi and Nahoon. Previously tagged trees (cable ties with name plates) were measured; the height and diameter at breast height were determined using a meter stick and tape measure (Rajkaran and Adams, 2012) and therefore the authors could track the growth of these individuals over time. At Mngazana data was collected for the following periods 2014 - 2015 and 2017 - 2018, at Nxaxo/Ngqusi for the period 2014 - 2018, and for Nahoon 2014 - 2015 and 2017 - 2018. The plants were grouped according to the height classes (Rajkaran and Adams, 2012). Growth rates was determined by calculating the difference in the measured height and diameter at breast height between the years and averaged.

Population Structure

Within each site, two to seven quadrates ($25m^2$) were set up, the tree height and diameter at breast height (at 130 cm) was measured using a tape measure, data was then divided into three height classes (Seedling <50 cm, 50 cm=<Sapling<130 cm, Adults>=130 cm) (Adams and Human, 2016). The number of quadrats at each site were determined by the size of the estuary. Density was calculated as the number of trees per square metre (Melville and Burchett, 2002) and Adult: Seedling ratio was also calculated.

Flower, Branch and Stalk Count

To determine reproductive production between five and ten flowering trees in the same size class were selected (10 trees at Mngazana, Nxaxo/Ngqusi and Nahoon and 5 trees were randomly selected at Kwelera and Tyolomnqa). The number of trees selected was determined by population size. From each tree the number of branches off the main branch were counted, from each main branch, five branches were randomly selected and the number of stalks were counted. From five randomly selected stalks the number of flower buds were then counted (**Figure 5.4**) (adapted from Clarke and Myerscough, 1991).

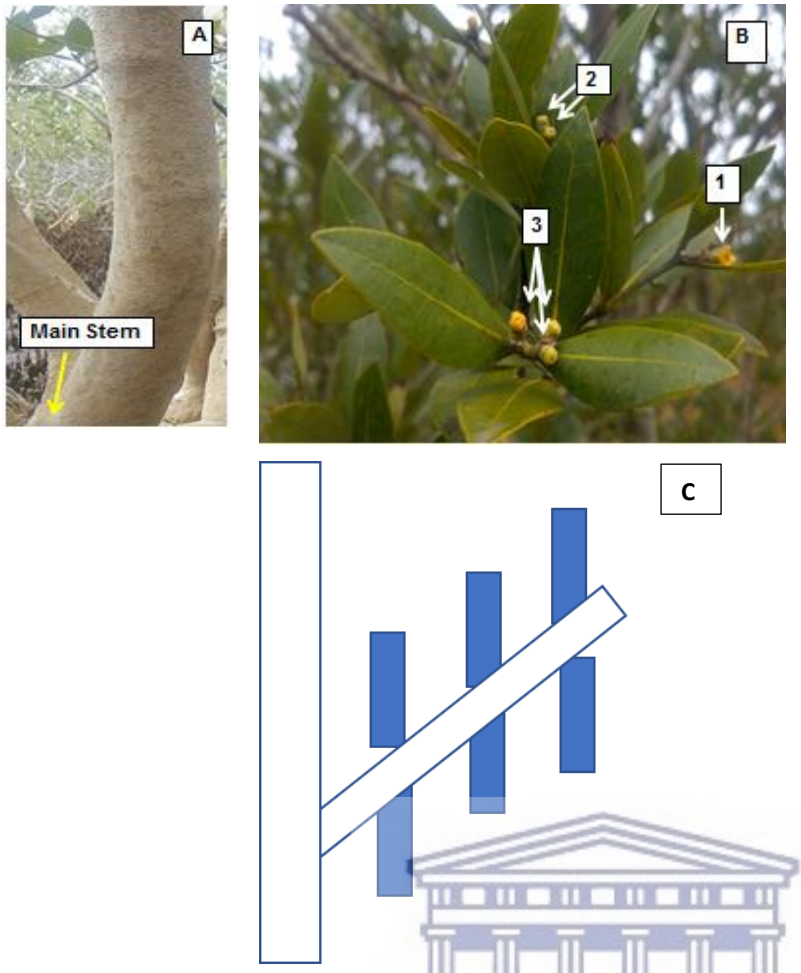


Figure 5.4: (A) Main Stem where main branches would be counted from. (B) showing flowers and buds, the numbers are indicating the number of flowers or buds in the photograph.

Stable Carbon and Nitrogen Isotope

For carbon and nitrogen analyses, 10 fully expanded leaves were collected, cleaned with deionised water within four hours of collection and transported back to the laboratory in Ziplock bags. The leaves were placed in a drying oven for 2 days at 70°C until they reached constant weight and crushed using a blender (Waring Commercial Blender). The ground material was then placed in an Eppendorf and sent to the Stable Isotope Laboratory, Mammal Research Institute at the University of Pretoria for analysis and calculations shown below. At the facility, approximately 1.1 to 1.2 mg of the plant samples was weighed into tin capsules, and some of the samples were duplicated. For Isotopic analysis, samples were combusted at 1 020°C using an elemental analyzer (Flash EA 1112 Series) coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany). Two laboratory running standards (Merck Gel: δ13C = -20.26‰, δ15N=7.89‰, C%=41.28, N%=15.29 & DL-Valine: δ13C = -10.57‰, δ15N=-

6.15‰, C%=55.50, N%=11.86) and a blank sample were run after every 11 unknown samples. Data corrections were done using the values obtained for the Merck Gel during each run. The standard deviations of the nitrogen and carbon values for the DL-Valine standard provide the ± error for the sample $\delta^{15}N$ and $\delta^{13}C$ values. These running standards were calibrated against international standards: National Institute of Standards & Technology (NIST): NIST 1557b (Bovine liver), NIST 2976 (Mussel tissue) and NIST 1547 (peach leaves). All results were referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen isotope values. Results were then expressed in delta notation using a per mille scale using the standard **Equation 5.3**:

$$\delta X\ (‰) = [(R_{sample}-R_{standard})/R_{standard}-1] \quad \text{..... Equation 5.3}$$

where X= ^{15}N or ^{13}C and R represents $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively (University of Pretoria Stable Isotope Laboratory, 2019).

Leaf morphology

At each estuary, 50 leaves were randomly collected from adult trees. Selected leaves were fully expanded and "healthy" i.e., avoiding those which had damage from herbivory or pathogenic attack. From these leaves, the leaf area was determined using Image J software 1.50i (Schneider *et al*., 2012). Leaves were cleaned in distilled water and weighed to determine fresh weight (g) within four hours of collection. The leaves were then bagged and transported to the laboratory. In the laboratory, leaves were oven dried at 80°C to determine dry mass. From these results, leaf succulence ($g.dm^{-2}$) (**Equation 5.1**) and Specific Leaf area ($cm^2\ g^{-1}$) (**Equation 5.2**) were then calculated (Wang *et al*., 2011; Iida *et al*., 2014).

$$\textbf{Leaf succulence} = \frac{fresh\ weight(g) - dry\ weight(g)}{leaf\ area(dm^2)} \quad \text{.....Equation 5.1}$$

$$\textbf{Specific Leaf area (SLA)} = \frac{leaf\ area(cm^2)}{leaf\ dry\ weight\ (g)} \quad \text{..... Equation 5.2}$$

Presence of galls and the impact of galls on leaf surface area

Within the quadrates ($25m^2$) which were set up to determine the population structure, for each tree measured, the presence of galls was recorded (see **Table 5.2** for gall description, **Figure 5.5**). A hundred leaves were randomly collected from each estuary, these were then digitally scanned and loss of surface area determined using the digital image analysis software (ImageJ, version 1.46r) with a calibrated scale bar.

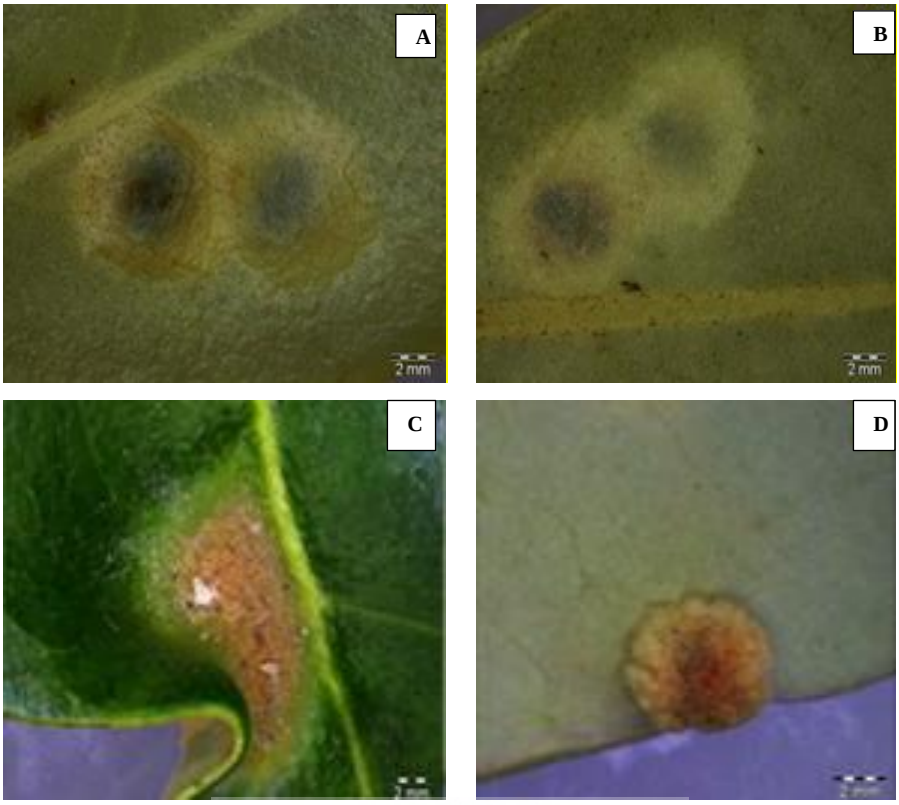


Figure 5.5: (A) Leaf of *A. marina* upper surface, (B) lower surface showing the morphology and size of type gall 1 (images taken using a light microscope), collected from Nxaxo/Ngqusi Estuary in previous study (Zide, 2013 unpublished). (C) Leaf of *A. marina* upper surface, (D) lower surface showing the morphology and size of type gall 2 (images taken using a light microscope) in previous study (Zide, 2013 unpublished).

Table 5.2: The three types of galls found to occur atthe study sites (MNG, WAVE, KLA, NAH and TYO), with brief description.

Type of Galls	Location and description	Photograph (from this study)
Type 1 gall	**Found in all study sites (MNG, WAVE, KLA, NAH and TYO).** • Gall protrudes on the upper (adaxial) surface of leaf. It has a chamber, where larvae has been found. Exit-hole on lower (abaxial) surface, indicates an empty gall, where insect has left the gall. • Fresh galls with larvae are green in colour. Empty and old galls, where there is no living larvae, are black/brown colour (on upper surface) and an exit-hole can be seen (lower surface). • Single larvae was found in each gall. It is suspected that that this type of gall is formed by a fly in Cecidomyiidae family (order Diptera), based on gall description by Sharma *et al.* (2003). • In previous study and current study, larvae found in the galls had a morphology of a fly (order Diptera) (Zide *et al*. unpublished, 2013). This is supported by Osorio *et al*. (2017b) who through personal communication with Prof. Stefan Neser and Dr Robin Adair suggest that it is a result of a midge fly from the Cecidomyiidae family (order Diptera). • Suspected fly collected but due to time constraints has not been yet identified. Further studies will have to be conducted to identify the genus and species name.	
Type 2 gall	**Found in all study sites (MNG, WAVE, KLA, NAH and TYO).** • Gall alters both the upper and lower leaf surface (**Figure 5.5 C-D**), where the lower suface protudes forming a fleshy gall which does not have a chamber. • From literature it is suspected that this galls may be formed by mites (Martin, 2018). This is supported by Osorio *et al*. (2017b) who through personal communication with Prof. Stefan Neser suggested that it is an undescribed eriophyid mite species. • This provides opportunity for further studies for the describing of the species.	
Type 3 gall	**Found only in MNG and WAVE** • Gall was generally found to be numerous and protruding on the upper surface of leaf. • Gall does not seem to have a chamber. • From this study we could not determine nor suspect what could have formed this type of gall.	

Percentage of trees showing signs of disease

Within the quadrates ($25m^2$) which were set up for the population structure, bark bleeding, bark discolouration and other disease were recorded as present or absent. According to Osorio *et al*. (2017b), stems/branches which had exit-holes and regularly accompanied by sap bleeding is a result of wood-boring beetle which the study identified as *Hypothenemus eruditus* activity. For this study, stems/branches which showed signs of sap bleeding (bark bleeding) were recorded (see **Figure 5.6**).

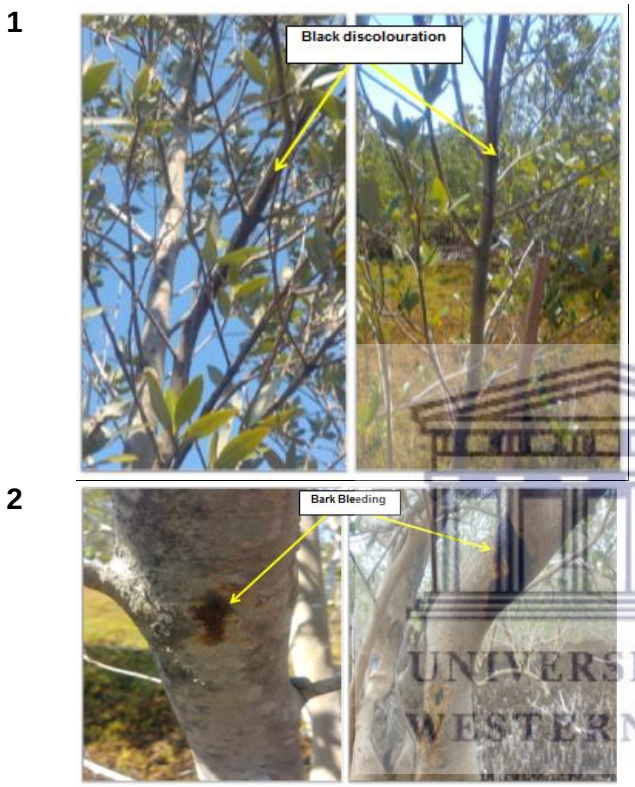


Figure 5.6: (A-B) Black discolouration (Fungi) on *A. marina* bark of adult trees and (C-D) Bark bleeding on *A. marina* adult trees, possibly caused by wood-boring bettles (Osorio *et al*., 2017b).

Data Analysis

Statistical Analysis for the data was carried out using a statistical computing program R version 3.5.2 (2018-12-20) (Core Team, 2018). Packages used to run the various tests, plot the graphs and produce correlation matrices were tidyverse (Wickham and Wickham, 2017), dbplyr (Wickham and Rulz, 2018), ggplot2 (Wickham, 2011), ggpubr (Alboukadel, 2018), plotly (Sievert *et al*., 2017), ggthemes (Arnold and Arnold, 2015), ggpmisc (Aphalo, 2016), dunn.test (Dinno and Dinno, 2017), car (Fox *et al*., 2017), lattice (Sarkar *et al*., 2015) and agricolae (de Mendiburu & de Mendibutu, 2019). Shapiro-Wilk test was used to test for normality; for data

which was not normally distributed a non-parametric test (Kruskal-Wallis test) was carried out, whilst for normally distributed data, a One-way ANOVA (Analysis of variance) test was carried out. Tukey HSD post hoc testS were run after the ANOVA to determine significance between groups (Sites). A Dunn's Test of Multiple Comparisons was carried out after the Kruskal-Wallis test, where p-values were adjusted according to the Bonferroni correction method. Significance threshold for the analysis was set at p-value = 0.05. Correlation matrices were also generated.

Results

Growth Rates

The rate at which *A. marina* individuals increase in height was measured at three mangrove estuaries namely; Mngazana, Nxaxo/Ngqusi and Nahoon, and considers data from various years. At Mngazana data was collected for the following periods 2014 - 2015 and 2017 - 2018, at Nxaxo/Ngqusi for the period 2014 - 2018, and for Nahoon 2014 -2015 and 2017-2018. The growth rate in terms of DBH was determined for each site but was only compared between the years 2017 and 2018. Mangrove trees at the various estuaries grew at similar mean rates ($\chi^2_{(2)}$ =4.56, p-value = 0.1023) in terms of height (**Figure 5.7**). The rate of increase in DBH varied ($\chi^2_{(2)}$ =19.4188, p-value <0.0001), and according to the Dunn's Test, Nxaxo/Ngqusi mangroves grew faster in terms of height than Mngazana and Nahoon (p-value<0.05), whilst Nahoon and Mngazana had similar rates (p-value >0.05) **(Figure 5.8).**

The growth rates of the different size classes at each estuary varied (MNG: $\chi^2_{(2)}$ =38.998, p-value < 0.001; WAVE: $\chi^2_{(2)}$ =12.634, p-value = 0.002; NAH: $\chi^2_{(2)}$ =29.094, p-value<0.0001) (**Table 5.3**). According to the Dunn's Test, mangrove adults at all estuaries grew faster than the saplings ((p-value <0.05). Mngazana and Nxaxo/Ngqusi adults and seedlings grew at similar rates ((p-value >0.05), whilst for Nahoon adults grew faster than seedlings ((p-value <0.05). For all estuaries seedling and sapling growth was similar ((p-value >0.05). Due to natural mortality and loss of tags (tidal action or burial) the number of inviduals in each size class was variable across the sites. All three sites had a low number of seedlings that were tracked over time. Seedlings at Nahoon had a net negative growth (0.01 ± 1. 47cm.year $^{-1}$). The highest growth rate was experienced by adult individuals which also had the highest number of individuals measured at each site.

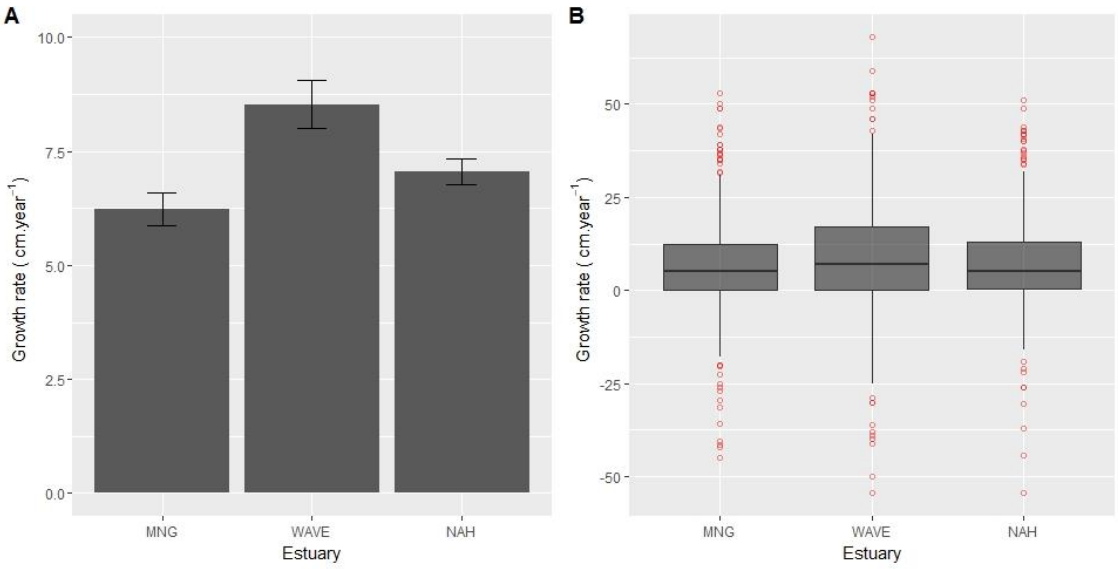


Figure 5.7: Growth rate of height calculated for Mngazana (MNG), Nxaxo/Ngqusi (WAVE) and Nahoon (NAH). (A) Shows height growth rates (cm. year $^{-1}$) with error bars. (B) Boxplot -shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

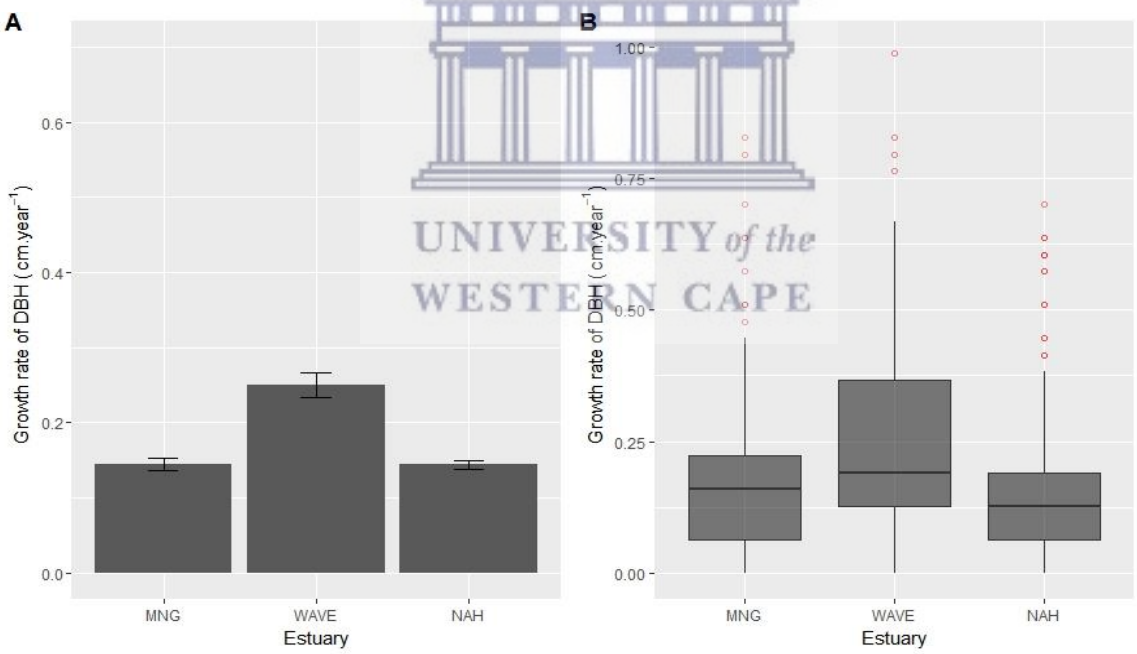


Figure 5.8: Growth rate of DBH per year calculated for Mngazana, Nxaxo/Ngqusi and Nahoon. (A) Shows data using bar graph with error bars. (B) Boxplot shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

Table 5.3: Growth rates per size class for each estuary.

Size Class	Mngazana (MNG) (cm. year $^{-1}$)	Nxaxo/Ngqusi (WAVE) (cm. year $^{-1}$)	Nahoon (NAH) (cm. year $^{-1}$)
Sd (0-50cm)	1.64 ± 2.81, N=14	6, N=1	-0.01 ± 1.47, N=26
Sap (51=130cm)	2.17 ± 0.45, N=347	2.79 ±1.10, N=59	3.76 ± 0.42, N=359
Ad (>=131 cm)	8.11 ± 0.47, N=833	9.86 ±0.74, N=391	8.26 ± 0.35, N=1143

*± SE

Population Structure

The population structure data provides an indication of whether the population is increasing, decreasing or stagnant. All estuaries displayed a reversed J-curve when a non-linear model was fitted on the data suggesting that the forests are regenerating and that recruitment is taking place (Rajkaran and Adams, 2011; Osunkoya and Creese, 1997). Mngazana, Nxaxo/Ngqusi and Nahoon showed a similar trend (**Figure 5.9 A, B and D,** respectively), where the highest number of individuals was the seedling size class and lowest was the adults displaying a high R^2 value. Whilst for Kwelera and Tyolomnqa the seedling size class had the highest individuals and the sapling had the lowest with Kwelera having a low R^2 value (**Figure 5.9 C and E,** respectively). Even though these populations showed a strong predictive power of the relationship (R^2 >0.5), the various size classes had similar number of individuals per square meter (Mngazana: $\chi^2_{(4)}$ =4.1564, p-value = 0.13; Nxaxo/Ngqusi: $\chi^2_{(2)}$ =2.724, p-value = 0.2562; Kwelera: N/A; Nahoon: $\chi^2_{(2)}$ =0.776, p-value = 0.678; Tyolomnqa: N/A). This maybe a result of having a small dataset coupled with only having three size classes. Mean density (**Figure 5.9 F**) values were found to similar ($\chi^2_{(4)}$ =9.23, p-value = 0.0557).

Mean height values at the various estuaries were different ($\chi^2_{(4)}$ = 147.68, p-value <0.0001). According to the Dunn's Test, mean height values at Mngazana was similar to Kwelera (p>0.05), but higher than the other estuaries (p<0.05). Mangroves at Kwelera were also similar to Nahoon and Tyolomnqa (p>0.05) but higher than Nxaxo/Ngqusi (p-value <0.05). (**Figure 5.10 A)**. DBH values were found to vary ($\chi^2_{(4)}$ =22.928, p-value = 0.0001). According to the Dunn's Test, DBH at Kwelera was similar to Tyolomnqa (p-value >0.05) but higher than the other estuaries ((p-value <0.05). Tyolomnqa was higher than Nxaxo/Ngqusi (p-value <0.05) but similar to the other estuaries (p-value >0.05). Mngazana, Nxaxo/Ngqusi and Nahoon were found to be similar (p-value >0.05) **(see Figure 5.10 B)**. In all sites, DBH had a positive relationship with height (p-value <0.05), with generally a strong predictive power which ranged from Kwelera (R^2 = 0.6) to Nahoon (R^2 = 0.85) (**see Figure 5.10 C)**.

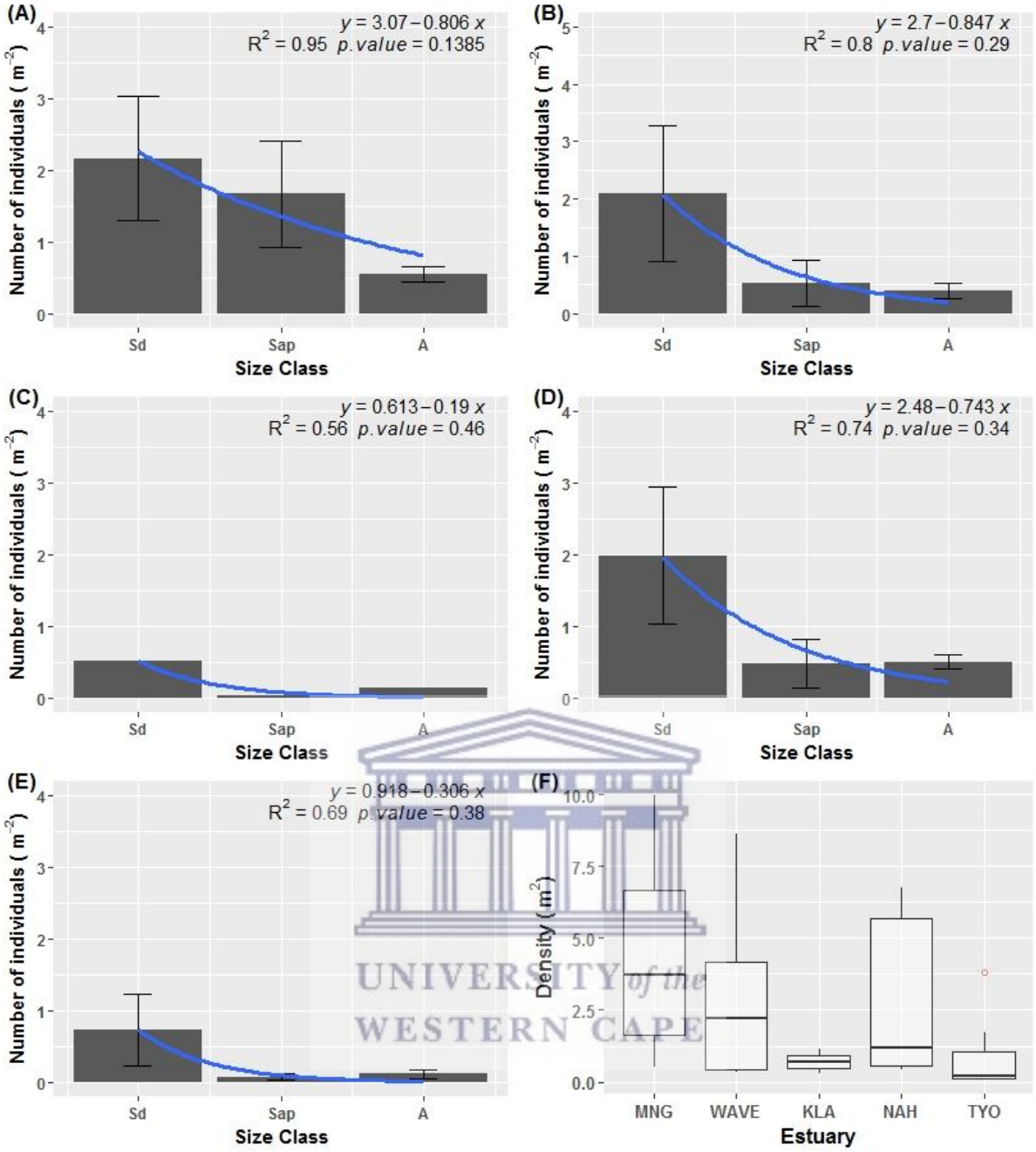


Figure 5.9: Number of individuals per square metre for each size class at A) Mngazana, B) Nxaxo/Ngqusi, C) Kwelera, D) Nahoon and E) Tyolomnqa showing standard error and fitted exponential model. F) Boxplot -shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

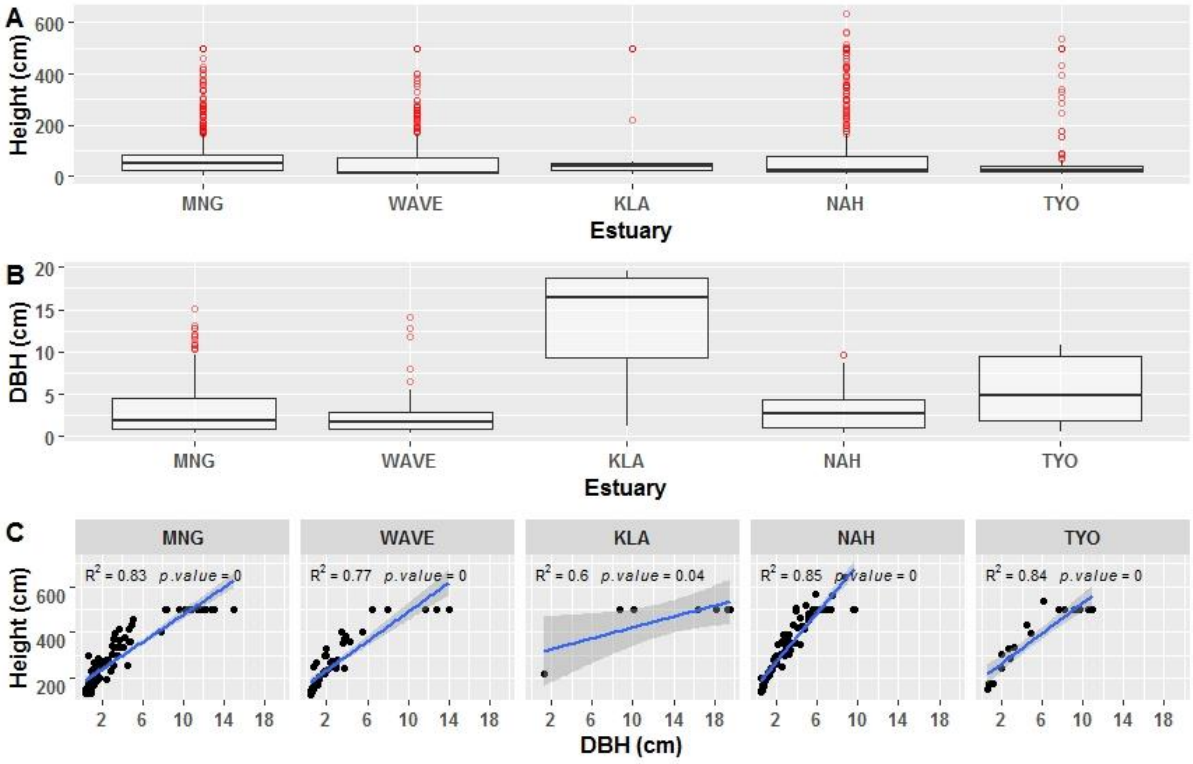


Figure 5.10: Population structure data for the three estuaries namely; Mngazana (MNG), Nxaxo/Ngqusi (WAVE) and Nahoon (NAH). (A) Boxplot of height data and (B) Boxplot of DBH data and (C) Boxplot - shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

The adult trees in the quadrats were further observed for presence and absence of flowering, propagules, bark bleeding, signs of fungal activity and the presence of galls. The whole quadrat was then observed for cattle browsing and harvesting, however, these two disturbances were only observed at Mngazana and Nxaxo/Ngqusi. **Table (5.4)** summarises the above information for all adult *A. marina* trees at all sites. Flowering was taking place at all estuaries with the highest occurrence found at Kwelera (85.71 %) and the lowest occurred at Nahoon (18.18 %), even though propagules were present at all the estuaries during this period, but not in all quadrats. Less than 1% of trees occuring in Mngazana had propagules.

The results indicate that all adult trees found at the various esturies had leaf galls, only Kwelera and Tyolomnqa did not show signs of bark bleeding (see example Figure 5.6 C-D) whilst the highest bark bleeding occurence (7.95 %) was found at Nahoon Estuary. All adult trees showed signs of fungal activity (black discolouration). Browsing was only present at Nxaxo/Ngqusi

and Mngazana, and it had been previously documente. Browsing at Nxaxo/Ngqusi (26.67 %) was slightly higher than at Mngazana (24.36 %). Harvesting was only recorded at Mngazana with just above 8% of the quadrats showing signs of harvesting. Clearing at Tyolomnqa had recently taken place outside of the quadrats and in one location only.

Table 5.4: Other measures taken from each adult within each 25 cm^2 quadrat, included the presence of flowers, propagules, occurrence of galls, fungi and bark bleeding in adult trees. The occurrence of browsing and harvesting was also recorded.

Estuary	Flowers (%)	Propagules (%)	Galls (%)	Fungi (%)	Bark Bleeding (%)	Frequency of browsing (/quadrat) (%)	Frequency of harvesting (/quadrat) (%)
Mngazana (N=272)	31.62	<1	100	72.43	5.14	24.36	8.25
Nxaxo/Ngqusi (N=60)	63.33	0	100	78.33	1.67	26.67	**N/A**
Kwelera (N=7)	85.71	0	100	85.71	0	**N/A**	**N/A**
Nahoon (N=88)	18.18	0	100	67.05	7.95	**N/A**	**N/A**
Tyolomnqa (N=21)	57.14	0	100	100	0	**N/A**	**N/A**

Flower, Branch and Stalk Count

To further quantify the ocurrence and quantity of flowers and propagules, a number of adult trees (271 to >500 cm in height) at each site were selected. Propagules were found in 30% of the trees measured at Nahoon and 10% at Mngazana while trees at other sites had no propagules. (**Table 5.5**). For the branches counted at the various trees at each estuary, the mean values were found to be similar ($F_{(df = 4)}$ = 1. 9634, p-value = 0.1217), the number of stalks ($\chi 2$ (4) =1.9548, p-value = 0.7441) and buds were also found to be similar ($F_{(df = 4)}$ = 7.9731, p-value = 0.09257) (**Table 5.5**). Positive correlation was found to occur between DBH and height (R^2 = 0.78, as also seen in the Population structure) and a weak correlation between number of Stalks and DBH (R^2 = 0.44).

Table 5.5: Various variables measured for 10 adult *A. marina* trees at each estuary to determine reproduction success and signs of disease.

Estuary	**Mean Height (cm)** ± SE	**Mean DBH** ± SE	**Propagules (%)**	**Galls (%)**	**Fungi (%)**	**Bark Bleeding (%)**	**Branches (mean** ±SE)	**Stalks (mean** ±SE)	**Buds (mean** ±SE)
Mngazana (N=10)	490.0 ± 10.00*	12.80 ± 0.94	10	100	100	20	21.3±1.99	63.22 ±12.48	4.23 ±0.24
Nxaxo/Ngqusi (N=10)	333.2 ± 21.89*	6.99 ± 2.04	0	100	100	10	19.1 ± 2.11	55.72 ± 17.61	4.47 ±0.15
Kwelera (N=5)	457.60 ± 42.40*	15.14 ± 3.44	0	100	10	10	26.4 ± 4.8	41.56 ± 15.82	4.02 ± 0.26
Nahoon (N=10)	426.05 ± 31.12*	10.16 ± 1.92	30	100	100	70	24.5 ± 1.78	42.92 ± 8.07	4.43 ±0.46
Tyolomnqa (N=5)	455.80 ± 27.45*	7.8 ± 0.88	0	100	100	0	29.0 ± 4.77	38.56 ± 10.39	3.38 ±0.18

*Includes Trees that could not be measured as they were too tall were classed as ">500".

A Non-metric multidimensional scaling (NMDS) analysis was run on the flower, branch and stalk count dataset using the Bray-Curtis dissimilarity matrix at 999 permutations; this was done to test if the data collected could be separated according to estuary. Data was transformed by taking the square root of the following variable; height, DBH, stalks and buds. The results from the analysis as observed in **Figure 5.11** had a stress level of 0.080. The following tests were carried out namely; ANOISM (Analysis of similarities), PERMANOVA (Permutational multivariate analysis of variance), PERMDISP2 (through betadisper function in r). Plot shows that there is a general overlap between Nxaxo/Ngqusi, Mngazana and Tyolomnqa whilst Kwelera and Nahoon seem to be slightly separated (**Figure 5.11)**, this was supported by the ANOISM (R^2 = 0.183, p-value <0.05 (999 permutation)) there was a difference in the dataset but R^2 was still low. The PERMANOVA showed a significance difference ($F_{(df = 4)}$ = 2.484, R^2= 0.221, p-value <0.05), but the R^2 value displayed a weak association between the measured variables and the estuary in which they were collected from. The PERMDISP2 showed that there is no difference in the dispersion of the five estuaries ($F_{(df = 4)}$ = 0.705, p-value >0.05) (**Figure 5.12**). Pairwise comparison shows that all estuaries were similar (p-value >0.05). The average distance to median at Mngazana was 0.068 (p-value >0.05), Nxaxo/Ngqusi was 0.095 (p-value >0.05), Kwelera was 0.111(p-value >0.05), Nahoon was 0.087 (p-value >0.05) and Tyolomnqa 0.087 (p-value >0.05) thus assumption for homogeneity of multivariate dispersions was met.

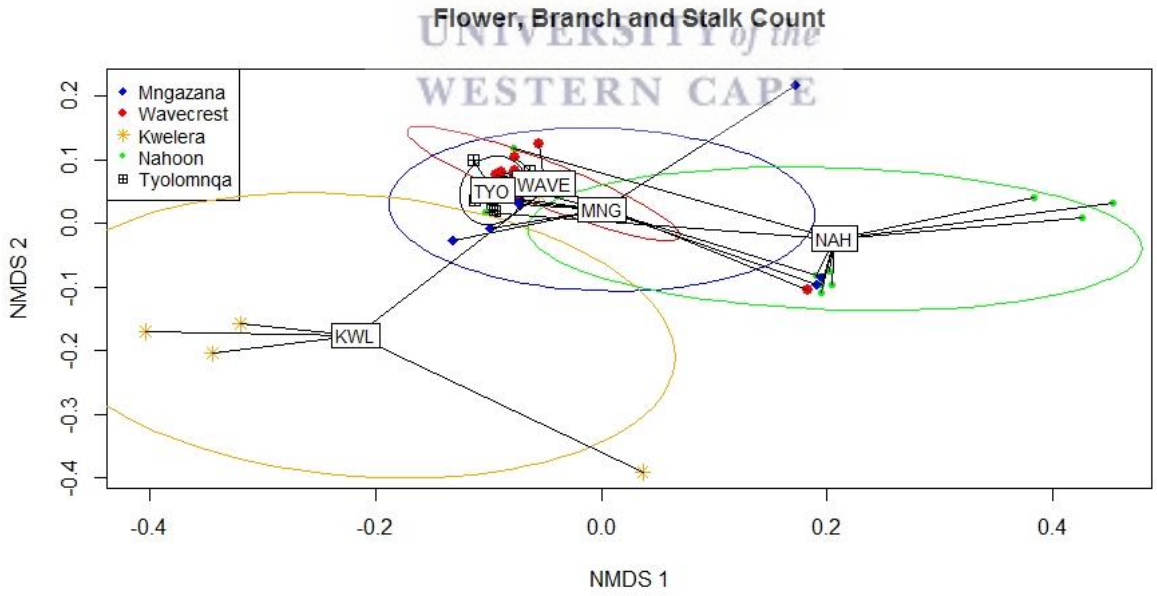


Figure 5.11: Non-metric multidimensional scaling (NMDS) plot of the Flower, Branch and Stalk Count dataset measured at the five estuaries; Mngazana (MNG), Nxaxo/Ngqusi (WAVE), Kwelera (KWL) Nahoon (NAH) Tyolomnqa (TYO).

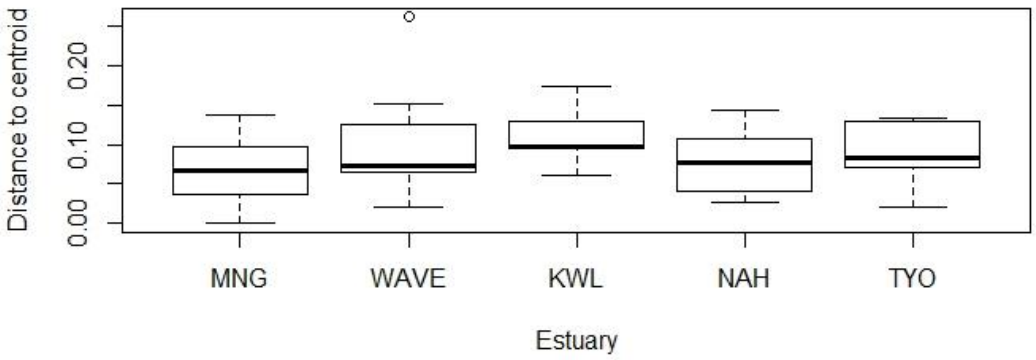


Figure 5.12: Box plot (PERMDISP2 results) of the Flower, Branch and Stalk Count dataset measured at the five estuaries; Mngazana (MNG), Nxaxo/Ngqusi (WAVE), Kwelera (KWL) Nahoon (NAH) Tyolomnqa (TYO).

Leaf morphology and composition

Specific leaf area (SLA) (**Table 5.6**) ranged from 71.76 ± 2.68 $cm^2.g^{-1}$ at Kwelera to 56.91 ± 1.81 $cm^2.g^{-1}$ at Tyolomnqa, mean values were found to differ ($\chi^2_{(4)}$ =50.049, p-value<0.0001) (**Table 5.6**). According to the Dunn's Test, mean SLA values at Kwelera and Mngazana were similar (p-value >0.05) and were higher than the rest of the estuaries (p-value <0.05). Nahoon was higher than Tyolomnqa (p-value <0.05) but were both similar to Nxaxo/Ngqusi (p-value <0.05).

Leaf succulence values ranged between 5.03 ± 0.09 $g.dm^{-2}$ at Nahoon to 4.39 ± 0.12 $g.dm^{-2}$ at Mngazana and not displaying a trend according to geographical location or size of estuary, were found to be different ($\chi^2_{(4)}$ =31.911, p-value <0.0001) (**Table 5.6**). According to the Dunn's Test, mean leaf succulence values at Nahoon, Nxaxo/Ngqusi and Tyolomnqa were similar (p>0.05), these were found to be higher than Mngazana, whilst Nahoon and Nxaxo/Ngqusi were also significantly higher than Kwelera, Kwelera and Tyolomnqa were similar (p-value >0.05).

All estuaries showed some association between leaf succulence and SLA (**Figure 5.13**), where all estuaries had an increasing leaf succulence with a decrease in SLA, predictive power of the relationship varied across Estuaries.

Table 5.6: Mean Leaf Succulence and SLA values calculated for leaves collected at each estuary.

Estuary	Ave SLA (±) SE	Ave Leaf Succulence (±) SE
Mngazana (N=50)	69.90 ± 1.61	4.39 ± 0.12
Nxaxo/Ngqusi (N=50)	59.23 ± 1.70	4.94 ± 0.12
Kwelera (N=50)	71.76 ± 2.68	4.49 ± 0.08
Nahoon (N=50)	61.65 ± 1.14	5.03 ± 0.09
Tyolomnqa (N=50)	56.91 ± 1.81	4.91 ± 0.12

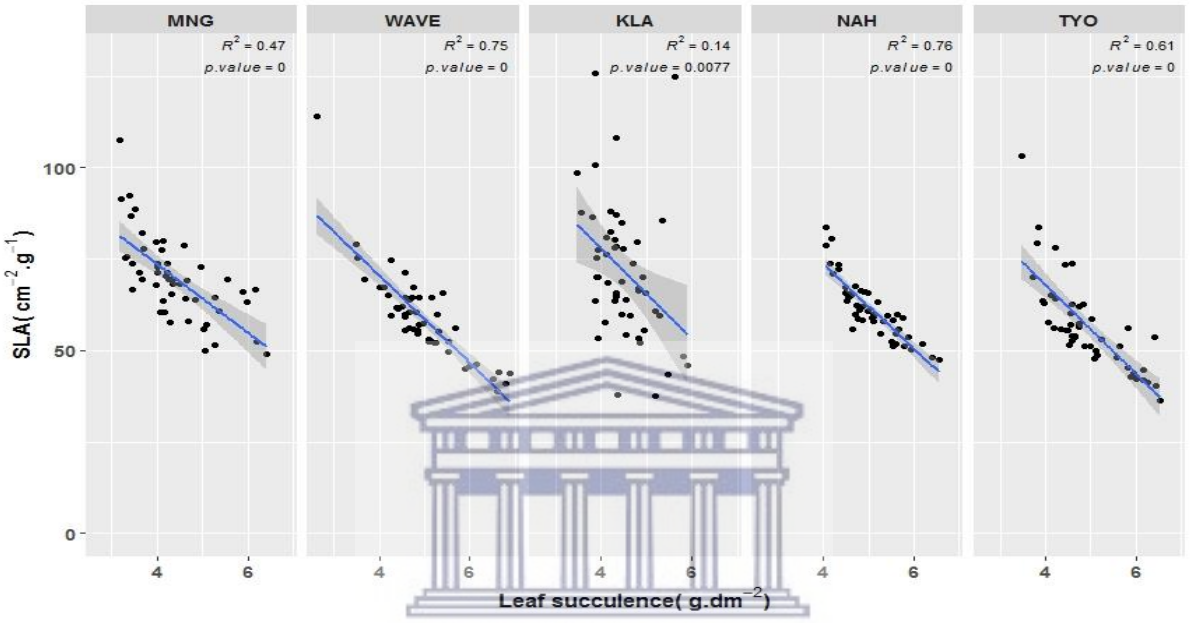


Figure 5.13: Leaf Succulence and SLA of *A. marina* leaves collected at the various estuaries.

The effect of galls on surface area

In this study, three gall types were found on the leaf surface of *A. marina* (**Table 5.2**). Only the presence of Gall type 1 and 2 were quantified to determine the area which had been altered by the galls. Results showed that the percent of surface area with galls ranged between 0.83 (± 0.13) % at Kwelera to 2.94 (± 0.41) % at Nahoon (**Figure 5.14**). The percentage of leaf area lost to galls were found to be different ($\chi^2_{(4)}$ =33.682, p-value <0.0001). According to the Dunn's Test, Nxaxo/Ngqusi was similar to Nahoon and Mngazana (p-value >0.05), Nxaxo/Ngqusi and Nahoon had a higher leaf surface damage than Tyolomnqa and Kwelera (p-value <0.05). Mngazana had a higher leaf surface damage than Kwelera (p-value <0.05) but similar to Tyolomnqa (p-value >0.05). Tyolomnqa and Kwelera were found to be similar (p-

value >0.05). None of the sites showed a relationship between Surface Area (cm^2) and Gall area (cm^2) when a linear model was fitted.

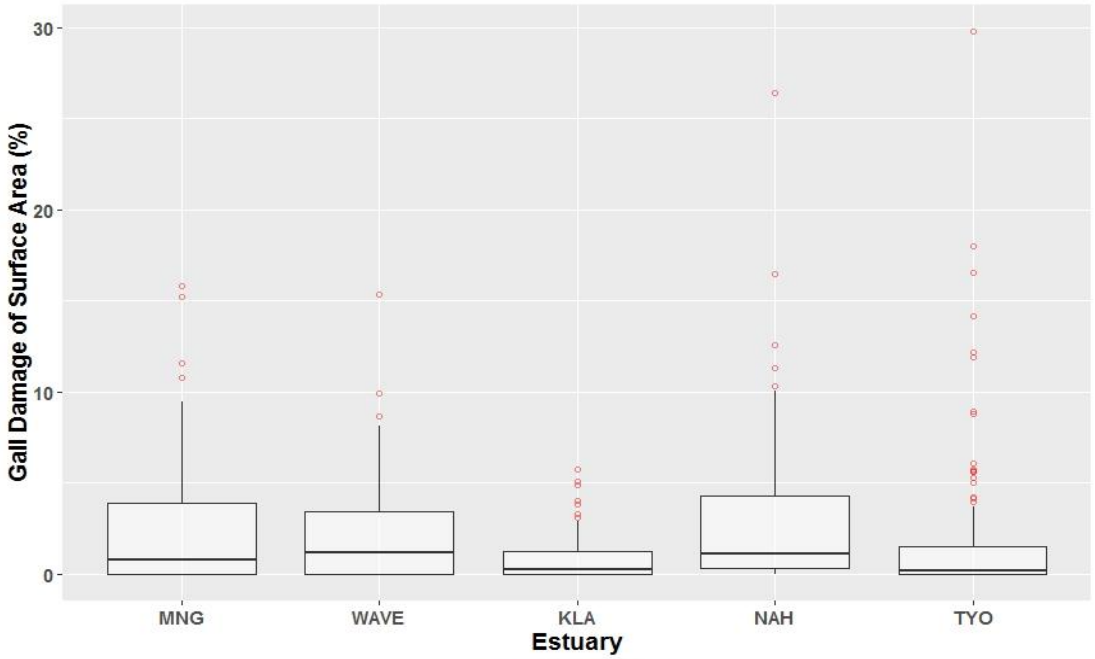


Figure 5.14: Mean percentage of area lost due to presence of type 1 gall. Boxplot -shows upper whisker (greatest value excluding outliers), red circles above this represent outliers, upper quartile (outer line at the top, represents 25% of the data that is greater than this value), middle quartile (line between the outer lines of the box, represents the median) and lower quartile (outer line at the bottom, represents 25% of the data that is less than this value).

Stable carbon and nitrogen isotope

The C/N ratio, Stable Nitrogen Isotope and Stable Carbon Isotope, Nitrogen and Carbon content in 10 healthy *A. marina* leaves was determined for each estuary. Mean C/N of the various estuaries (**Figure 5.15 A**) ranged between 23.72 ± 0.94 (Tyolomnqa) and 17.00 ± 0.53 (Kwelera), the mean C/N ratios were found to be different ($\chi 2_{(df = 4)}$ =26.3577, p-value <0.0001). According to the Dunn's Test, the ratio was significantly lower in Kwelera when compared to the other estuaries (Mngazana, Nxaxo/Ngqusi, Nahoon and Tyolomnqa (p-value <0.05). The other estuaries were all similar (p-value >0.05).

For Stable Nitrogen Isotope (δ15N), mean values were found to be different ($F_{(df = 4)}$ = 29.07, p-value <0.0001) (**Figure 5.15 B**). According to the Tukey HSD post hoc test, Kwelera was similar to Mngazana (adjusted p-value >0.05) but were both significantly higher than the other estuaries (adjusted p-value <0.05). Tyolomnqa, Nxaxo/Ngqusi and Nahoon were found to be similar to each other (adjusted p-value>0.05).

Stable Carbon Isotope (δ13C) mean values between these estuaries was found to be significant ($F_{(df = 4)}$ = 12.94, p-value <0.0001) (**Figure 5.15 C**). According to the Tukey HSD post hoc test, Kwelera was significantly lower than Nxaxo/Ngqusi and Tyolomnqa (p-value<0.05), whilst significantly similar to Mngazana and Nahoon (p-value >0.05). Nahoon and Mngazana were found to be similar (p-value >0.05). Nxaxo/Ngqusi were found to be similar to Nahoon (p-value >0.05), but lower than Tyolomnqa (p-value <0.05).

Mean Carbon content (%) values between these estuaries were found to be similar ($F_{(df = 4)}$ = 0.86, p-value = 0.495). Mean Nitrogen content (%) values were found to be different ($\chi 2_{(df = 4)}$ =26.551, p-value <0.0001). According to the Dunn's Test, Kwelera was significantly higher than the rest of the estuaries (p-value <0.05). All other estuaries had similar mean values (p-value>0.05).

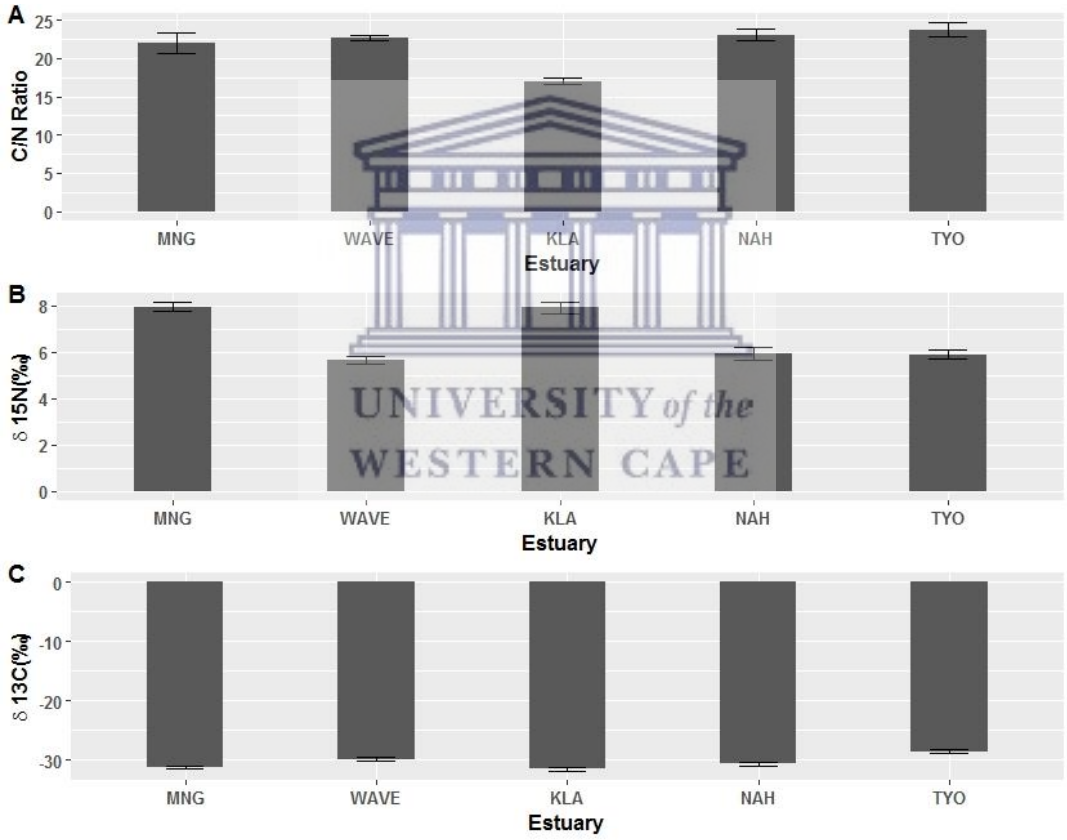


Figure 5.15: Bar plot showing the mean C/N ratio, Stable Nitrogen Isotope and Stable Carbon Isotope for leaves collected at each estuary.

Mean Carbon content (%) ranged between 40.08 ± 0.70 in leaves with Type 2 galls to 40.34 ± 0.34 in leaves with Type 1 galls, mean values were found to be similar ($F_{(df = 3)}$ =0.042, p-value

= 0.988). Whilst Nitrogen content (%) ranged between 1.75 ± 1.29 in leaves with type 3 galls to 2.168 ± 0.995 in healthy leaves (those without galls). The difference in means for Nitrogen content (%) was found to be significant ($F_{(df = 3)}$ =4.296, p-value = 0.016). According to the Tukey HSD post hoc test, healthy leaves had a higher mean Nitrogen content (%) than leaves with Type 3 galls (adjusted p-value = 0.0228).

Mean C/N values of healthy, GT1 (gall Type 1, N=10), GT2 (gall Type 2, N=5) and GT3 (gall Type 3, N=5) (**Figure 5.16 A**) ranged between 22.15 ± 1.29 in healthy leaves to 27.09 ± 0.995 in leaves with Type 3 galls (GT3). The difference in means in C/N ratio was found to be significant ($F_{(df = 3)}$ = 3.49, p-value = 0.0339). Whilst the Tukey HSD post hoc test did not find any differences in the mean values (adjusted p-value >0.05), the discord between the results is said to be a possibility and thus in this instance it wasassumed that the difference in the mean values is not significant as the post hoc test does not show any difference in the mean values (Tian *et al*., 2018).

For Stable Nitrogen Isotope (δ15N), mean values ranged between 7.45 ± 0.39 in leaves with Type 3 galls (GT3) to 7.95 ± 0. 17 in healthy leaves. The means were found to be similar ($F_{(df = 3)}$ = 0.955, p-value = 0.432). Stable Carbon Isotope (δ13C) ranged between -31.04 ± 0.31 in healthy leaves to -29.46 ± 0.39 in leaves with Type 1 galls (GT1), the difference was found to be significant ($F_{(df = 3)}$ = 4.572, p-value = 0.0129). According to the Tukey HSD post hoc test, Stable Carbon Isotope (δ13C) mean values were higher in healthy leaves than in leaves with Type 1 galls (GT1) (p-value = 0.016).

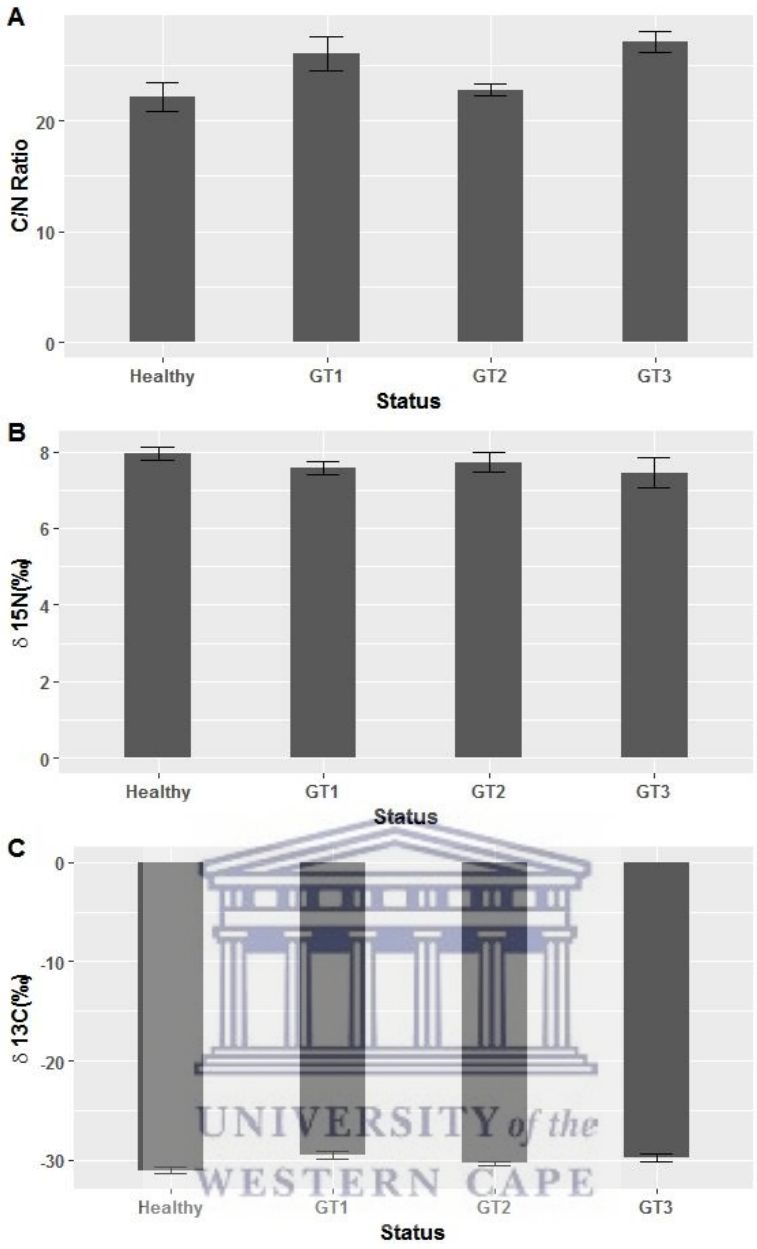


Figure 5.16: Bar plot showing the mean C/N ratio, Stable Nitrogen Isotope and Stable Carbon Isotope for leaves that were Healthy, those with Type 1 galls (GT1), Type 2 galls (GT2) and Type 3 galls (GT3) at Mngazana Estuary.

Data collected in this study was summarised (**Table 5.7**) into the following categories growth, population structure and health, leaf morphology and composition and reproduction. This was then related to information that that is currently known about the genetic diversity according to a study by De Ryck *et al*. (2016), this provides a glance at what iscurrently known about these systems occurring in the range edge and what other information would be required.

Table 5.7: Summary of biotic variables and characteristics of South African mangrove forests. Genetic data sourced from De Ryck *et al.*, (2016).

	Genetics	Growth	Population structure and health					Leaf morphology and composition				Reproduction
Estuary	**Inbreeding (F_{IS})**	**Plant growth (cm/year) ± SE**	**Density (m^2) ± SE**	**Number of seedlings per m^2**	**Seedling to sapling (ratio)**	**Presence of galls (%)**	**Bleeding Adults (%)**	**Leaf morphology (SLA)**	**Surface area damage (%)**	**Type of galls**	**C/N (Healthy leaves)**	**Average number of Flower buds**
Mngazana	0.44	H=6.24 ± 0.52 DBH= 0.14 ± 0.01	4.39 ± 1.34	2.17 ± 0.11	1:1	82.03	5.14	69.90 ± 1.61	2.43 ± 0.34	1,2 and 3	21.98 ± 1.30	4.23 ±0.24
Nxaxo/ Ngqusi	0.50	H=8.88 ± 0.95 DBH= 0.25 ± 0.02	3.01 ± 1.35	2.09 ± 0.13	4:1	85.40	1.67	59.23 ± 1.70	2.16 ± 0.28	1,2 and 3	22.66 ± 0.35	4.47 ±0.15
Kwelera	**N/A**	**N/A**	0.70 ± 0.42	0.52	13:1	71.43	0	71.76 ± 2.68	0.83 ± 0.13	1 and 2	17.00 ± 0.53	4.02 ± 0.26
Nahoon	0.64	H=7.06 ± 0.40 DBH= 0.14 ± 0.01	2.97 ± 1.10	1.99 ± 0.13	4:1	91.92	7.950	61.65 ± 1.14	2.94± 0.41	1 and 2	23.06 ± 0.74	4.43 ±0.46
Tyolomnqa	**N/A**	**N/A**	0.92 ± 0.11	0.73 ± 0.51	11:1	88.20	0	56.91 ± 1.81	2.06± 0.45	1 and 2	23.72 ± 0.94	3.38 ±0.18

Discussion

To determine plant performance, a number of variables were measured at five mangroves forests namely Mngazana, Nxaxo/Ngqusi, Kwelera, Nahoon and Tyolomnqa. It is expected that the plant performance of these estuaries will vary according to their size, age, geographical location and their expected genetic diversity. Mngazana, the largest of these mangrove forests, occurs in the subtropical region whilst Nxaxo/Ngqusi, Kwelera, Nahoon and Tyolomnqa all occur in the temperate region and vary in population size and aerial cover (van Niekerk *et al*., 2019). Amongst these populations Mngazana and Nxaxo/Ngqusi are the oldest which occur naturally, followed by Kwelera which established naturally in 1969 then Nahoon which established through transplanting activities in the same year, the youngest of these being Tyolomnqa which is said to have been established in the 1990s also through transplanting activities (Steinke and Ward, 2003; Hoppe-Speer *et al*., 2015; Ward and Steinke, 1982; Whitfield *et al*., 2016).

Recruitment and regeneration were taking place at all the study sites, shown by the reversed J-curve of the population structure (**Figure 5.6**) (Osunkoya and Creese, 1997, Rajkaran *et al*., 2009). According to Rivera-Monroy *et al.* (2019), recruitment and stem mortality are the two main factors that regulate mangrove forests and forest development. At Kwelera, Nahoon and Tyolomnqa the number of seedlings were the highest but there were fewer saplings compared to adults. Even though the J-curve was evident, the higher seedling to sapling ratio at Kwelera and Tyolomnqa may indicate that few seedlings are reaching the sapling stage thus could result in fewer individuals reaching or replacing the adult population. This may indicate instability and lower chances of sustainability of these forests (Riascos *et al*., 2018; Barnuevo *et al*., 2017; Sousa *et al*., 2003). The size of the populations could play a role in the results observed, the density of these populations were comparable, which may also indicate that other disturbances such as tide and wave action could be resulting in low survival of individuals to post seedling stage in the smaller populations (Hermansen *et al*., 2017; Faridah-Hanum *et al*., 2012).

Nxaxo/Ngqusi had a higher DBH growth rate than Mngazana and Nahoon, which may not be related to nutrient dynamics but rather population controls. Past disturbance and abiotic stress factors play an important role in growth rates of plants (Berger *et al*., 2006). Sediment characteristic dataset showed that Nahoon and Mngazana had higher organic content than Nxaxo/Ngqusi (**Chapter 4**) which could be an indicator of higher nutrients which may be used

for growth and productivity (Reef *et al*., 2010; Chen and Twilley, 1999a). Bark bleeding only observed in adult trees was higher at Mngazana and Nahoon (**Table 5.7**) when compared to Nxaxo/Ngqusi, this may have played a role in the lower DBH growth rates observed in Mngazana and Nahoon. A study by Lovelock *et al*. (2007), suggested that the lower growth rates at one of their sites may have been a result of herbivory. Thus, lower growth rates may be a result of resources being re-allocated for defence at the expense of growth. With regards to the adults of these forests, there was a significant correlation between DBH and plant height. This relationship was also found in studies by Osunkoya and Creese (1997) which did not state the possible reasons of why this was the case.

Growth of adult trees was generally found to be faster, this maybe a result of having a low number of tagged seedlings. Iida *et al*. (2014) reports that their study and other studies found the relative growth rate of taller trees to be slower than shorter trees, which was generally attributed to ageing, having higher respiration costs, more resources being allocated to reproduction, increased self –shading and a lower ratio of leaf area per unit living biomass. An increase in nutrients may result in higher growth rates of mangroves (Berger *et al*., 2006; Lovelock *et al*., 2007). Carbon Isotope (δ13C) and Nitrogen Isotope (δ15N) are involved in biogeochemical processes such as nutrient cycling (Nitrogen and Carbon cycles, respectively) (Prasad and Ramanathan, 2008). δ13C (‰) in mangroves has been found to range between -21.9 and -35.1 ‰, mean values obtained in this study fell within this range (Bouillon *et al*., 2008). δ13C (‰) is said to be an indicator of the leaf's long-term physiological activity (Bouillon *et al*., 2008). The results did not show any trend (as expected). The variation between the various sites is relatively small, which is expected as Bouillon *et al*. (2008) states that the degree of variation in δ13C (‰) in systems is less evident when compared to variation in δ15N (‰) which may vary even within the same system.

δ15N (‰) may be used as an indicator of the source of nutrients (Nitrogen) in a system (Gritcan *et al*., 2016; Duarte *et al*., 2018). The natural range for a system for δ15N (‰) is between -8 and +3 ‰ (Gritcan *et al*., 2016). Bouillon *et al*. (2008) states that high levels of δ15N (‰) are generally attributed to pollution due to urban sewerage or agriculture. Values between 10-20‰ are associated with human and animal waste inputs (Duarte *et al*., 2018). For this study mean values for δ15N were greater than 7‰ at Mngazana and Kwelera, and less than 6‰ at Nahoon, Tyolomnqa and Nxaxo/Ngqusi. These values are thus relatively higher than the natural range which may indicate that the estuaries may have a surplus of nutrients (Gritcan *et al*., 2016).

The range was similar to mean δ15N found in *A. marina* leaves at Inhaca Island, Mozambique (7.1 ± 1.2 ‰) in a study by Penha-Lopes *et al*. (2009), the high values were attributed to possible runoff water from a nearby village. Whilst in a study by Gritcan *et al*. (2016), δ15N mean value ranged between 5.2(± 0.4) - +9.9(± 0.4) ‰ in the leaves of *A. marina subsp. australasica* that study attributed the elevated levels to anthropogenic impacts such as agricultural practices and human sewage which provided additional sources of Nitrogen. Nahoon occurs in an urban setting in a nature reserve, thus the elevated levels may be a result of pollution or effluent discharge due to anthropogenic activities such development, agriculture and recreational activities (Newman and Watling, 2007; Cotiyane *et al*., 2017). The other estuaries occur in rural settings, where sites like Nxaxo/Ngqusi have nearby agricultural fields and cattle which have access to the mangroves, the animal waste could also attribute to the elevated nutrient levels found (Hoppe-Speer and Adams, 2015). These results did not display the expected trends.

High levels of C/N ratios protect mangrove leaves from herbivory as it plays role in making leaves less edible (Menezes and Peixoto, 2009). The C/N ratio values in this study did not follow any trend with regards to population size, along the latitudinal gradient, nor between planted and natural vegetation. C/N ratio values ranged between 17.00 (±0.53) and 23.72 (±0.94), which was lower than a study by Kihia *et al*. (2011) conducted in Kenya where the C/N value in *A. marina* was found to be 49.8 (± 4.7), and Camilleri (1989 (**see Table 5.8**) found it to be 30.2, but these values may differ due to mangroves occurring in different regions and not being sampled at the same time period. In this study, C/N ratio values were significantly lower in Kwelera than the other estuaries. The low levels of C/N at Kwelera may be an indication that the trees are not investing in plant defence. This estuary also had the lowest leaf surface damage when compared to the other estuaries and of the 10 measured trees, none were found to be bleeding and had lower gall infestation. This may be an indication that Kwelera is not under pressure to utilise its resources for defence but may rather use it for growth (this study has no data to support this assumption) and reproduction (when compared to the other estuaries it had a high flower occurrence and the mean flower buds were found to be similar to the larger natural populations). Thus, our results obtained do not support our expectation that at lower latitudes, there would be less herbivory (Feller *et al*., 2017; Lehndal and Ågren, 2015). According to Abeli *et al*. (2014), populations occurring at the range edge experience less interspecific competition and lower levels of predation. Thus, the position of the forest, size of estuary may have attributed in less investment in plant defence being required at Kwelera.

Davidson *et al* (2014), found that the infestation of wood-borers on *Rhizophora stylosa* individuals could be associated with its morphology, performance and fecundity. Individuals which experienced a higher incident of isopod infestation had fewer propagules and ground roots, smaller leaves and more non-foliated twigs. The results in Kwelera would suggest that the lower infestation has allowed this population to compete with the larger ones, allowing for investment of resources into reproduction etc.

The results obtained for nitrogen content did not show any trend according to their geographical location, as Kwelera was found to havesignificantly higher nitrogen content than the rest of the estuaries. Whilst a study by Tuffers *et al*. (2001) found a significant difference in the nitrogen content on leaf samples collected at Durban Bay and Beachwood – Mngeni Estuary, these estuaries occur further North of the South African coastline, the values were 3.56 (±0.30) for young and 2.88 (±0.43) old leaves collected at Durban Bay, and 2.82 (±0.29) young and 1.52 (±0.04) old leaves, respectively. Thus, the results found in this study were slightly lower than the former and within similar range to the latter (**Table 5.8**). The study attributed the low levels of nitrogen and potassium (another element measured) to the low salinities (less than 12ppt) experienced at Beachwood – Mngeni Estuary resulting in a lower photosynthetic performance. In this study low salinity may not be an attributing factor as seen in **Chapter 4**. Salinity measured at Mngazana, Nxaxo/Ngqusi and Nahoon was 35.82 (± 0.64), 34.01 (±0.49) and 34.2 (±0.43) respectively thus comparable to what was found in Durban Bay (35 ppt). Nitrogen leaf content is associated with photosynthesis forming an important part of this process, thus plays a role in its efficiency (Debez *et al*., 2006).

According to Saenger and West (2016), resources allocation in plants may be determined by measuring six leaf properties which include leaf surface area per unit dry weight (SLA) or the leaf mass per area, photosynthetic capacity, levels of nitrogen content which play an important role in proteins of photosynthetic machinery and phosphorus content. The mean SLA values for fringe (Seawater salinity site) and dwarf (Hyper salinity site) *A. marina* mangroves occurring at Richards Bay, had much higher values (>90 $cm^2.g^{-1}$) (Naidoo *et al*., 2011) when compared to what was obtained in this study. Whilst Saenger and West (2016), found the SLA value to be 50 (± 8) $cm^2.g^{-1}$ for *A. marina* leaves sampled in Australia, which was similar to this study site. In this study Kwelera and Mngazana were significantly higher than the other estuaries. Mean leaf succulence ranged between 4.39 and 5.03 $g.dm^{-2}$ which was higher than

the mean value of 2.82 g.dm^{-2} found by Wang *et al*. (2011) for *A. marina* occurring in Qinglan Bay (China). Leaf succulence were similar across the various estuaries and thus not displaying any trend according to geographical location, this may also be an indication that the various estuaries were experiencing similar environmental conditions such as water availability (rainfall amount).

Wang *et al.* (2011) states that leaf traits such as leaf succulence, SLA and Leaf nitrogen concentration on a leaf mass (LMA) have been found to play an important role in the long-term adaptive strategy of mangroves. This is further supported by Puglielli *et al*. (2015) who states that studies have found high LMA to be associated with drought adaptation. According to Burrows (2003), plants with a high LMA (Leaf mass per unit area), the inverse of SLA, are more tolerant of physical damage and are less impacted by herbivory.

Gonçalves-Alvim *et al.* (2001) states that gall abundance (in this study, this could be related to leaf surface) has been found to have a positive relationship with increasing stress gradient. According to the results obtained, Nahoon had the highest gall infestation followed by Tyolomnqa, Nxaxo/Ngqusi, Mngazana and Kwelera. Pore-water salinity (**Chapter 4**) was higher at Nahoon then Nxaxo/Ngqusi, but both the estuaries were found to be similar to Mngazana, thus salinity may not be able to explain the differences in gall infestation that was observed. Farnsworth and Ellison (1991) suggest that canopy structure may play a role on the levels of herbivory, as herbivory has been found to increase with shading, it is suggested that herbivores prefer shaded plants as it may offer protection against predators as they will be less visible and plants which receive more sunlight will have more photosynthetic resources which are associated with production of extra defence compounds.

Herbivory was generally higher at the bigger and older estuaries (Nahoon, Nxaxo/Ngqusi and Mngazana) than Tyolomnqa and Kwelera. In contrast to the results obtained by Osorio *et al*. (2017b), a higher incidence of bark bleeding was found to occur in Nahoon in comparison to Nxaxo/Ngqusi and Mngazana. Due to sampling only being carried out once, this may be an underestimation as Saenger (2002) states that a study has found that the values would be three to six times more when measured over the whole life-span of the leaves.

Conclusion

In all the study sites, anthropogenic activities were recorded, which included cattle browsing and trampling (Nxaxo/Ngqusi), harvesting (Mngazana) and pollution (Nahoon and Kwelera). Hoppe-Speer *et al*. (2015) also recorded several impacts on the Mngazana mangrove population, these included trampling by livestock and browsing, catchment degradation and sediment input and disturbance from surrounding agricultural activities. Documented pressures in mangroves in South Africa include harvesting, livestock browsing and tramping and pollution resulting in various abiotic and biotic changes (Adams and Rajkaran, 2021)

With the results obtain it is assumed that this had an influence in how resources were allocated, where Kwelera and Tyolomnqa had less investment in defence thus lower levels of C/N measured at Kwelera and comparable reproductive output. Even so, the population structure showed signs of instability and reduced sustainability suggesting that other factors maybe hindering the development of these two forests this maybe a result of harsher environmental conditions as these are populations at range edge which are also less developed than the larger forests.

The population structure results suggest that Mngazana and the other larger populations performed better than Kwelera and Tyolomnqa, but the other results would then suggest that the mangrove dynamics from these systems differ such as the size of the populations, setting and environmental conditions, which has an influence in the canopy structure, density, competition, levels of herbivory, growth, productivity and functioning of the populations.

Table 5.8: Literature values for C/N ratio, Stable Nitrogen Isotope and Stable Carbon Isotope, Nitrogen and Carbon content measured in *A. marina* and other mangrove species.

Location	Species *(part of tree)*	$\delta^{15}N$ (‰)	%N	$\delta^{13}C$ (‰)	%C	C/N	Reference
Red Sea, Saudi Arabia	*A.marina* *(leaves)*	1.72 (± 0.29)	1.89 (± 0.07)	−26.58 (± 0.13)	49.39 (± 0.94)	32.42 (± 0.9)	Duarte *et al*., 2018
southeast Australia	*A. marina* *(leaves)*	-	-	-26.7 (± 1.5*)	-	-	Kelleway *et al*., 2018
Queensland, Australia	*A. marina* *(leaves)*	-	-	- 30.5 to-25.8	-	-	Ladd and Sachs, 2013
Durban Bay, South Africa	*A. marina* *(leaves (old) – leaves (young)*	-	2.88(± 0.43) - 3.56(± 0.30)	-	-	-	Tuffers *et al*., 2001
Beachwood, South Africa	*A. marina* *(leaves (old) – leaves (young)*	-	1.52(± 0.04) - 2.82(± 0.29)	-	-	-	Tuffers *et al*., 2001
Gazi Bay, Kenya	*A. marina* *(leaf litter)*	-	-	-	-	49.8 (± 4.7)	Kihia *et al*., 2011
Inhaca Island, Mozambique	*A. marina* *(leaves)*	7.1 (± 1.2)	-	−28.8 (± 0.5)	-	-	Penha-Lopes *et al*., 2009
Queensland, Australlia	*A. marina* *(leaves)*	-	-	-	-	30.2	Camilleri, 1989
New Zealand	*A. marina sp. australasica* *(leaves)*	5.2(± 0.4) - 9.9(± 0.4)	2.0(± 0.1) - 2.2(± 0.1)	-	-	-	Gritcan *et al*., 2016
Setiu lagoon, Malaysia	*Rhizophora apiculata* *(leaves)*	3.8 (± 0.3)	-	−31.1 (± 1.1*)	-	-	Le *et al*., 2017
Florida	*Avicennia germinans*	-	1.81	-	>42 <45	24.3	Erickson *et al*., 2004
This study	*A. marina* *(leaves)*	5.7(±0.16) – 7.9(±0.18)	1.97(±0.07) – 2.71(± 0.07)	-31.5 (± 0.32) – 28.5 (± 0.36)	39.3 (± 0.53) – 40.2 (± 0.43)	17 (± 0.43) – 23.7(± 0.94)	Zide, 2022 (unpublished)

***±SD**

Chapter 6 – General Discussion and Conclusion

Mangroves in the East African region occurs from Somalia to South Africa (Taylor *et al*., 2003; Spalding, 2010). Along the coastline, a higher number of species and larger area of mangroves occurs between Kenya and Mozambique compared to South Africa (Lugendo, 2016; Giri *et al*., 2011; Bosire *et al*., 2016). This area being referred to as the core population(s), where the environmental conditions are optimal, while South Africa occurs at the range edge, where sea temperature and environmental change is the greatest, thus conditions are not optimal (Leimu *et al*., 2010; Polidoro *et al*., 2010).

The mangrove ecosystem provides several ecosystem services, making them an important ecosystem. Rajkaran and Adams in Bosire *et al*. (2016) notes that albeit the mangroves in South Africa are smaller compared to the rest of those occurring in the East African region they still provide important ecosystem services and still form an important part of biodiversity. Due to the South African populations occurring at the range-edge, this may further complicate an already complex and dynamic ecosystem thus providing an opportunity for interesting research in terms of conservation due to the several threats that this ecosystem faces. The main goal of this study was to assess the following (1) the genetic connectivity of two mangrove species namely *Bruguiera gymnorrhiza* (L.) and *Rhizophora mucronata* (Lam.) (**Chapter 3**), (2) the sediment and porewater characteristics of three mangrove estuaries in the Eastern Cape using long term monitoring data (**Chapter 4**) and (3) the population performance of *A. marina* populations at the range edge (**Chapter 5**).

The first objective was the assessment of the genetic connectivity of *Bruguiera gymnorrhiza* (L.) and *Rhizophora mucronata* (Lam.) in South Africa including a population from Tanzania and Mozambique. The first study of its kind for these two species in this region. Only a single molecular study was found to have included a South African *R. mucronata* population as part of their analysis (Takayama *et al*., 2021). The addition of a population from Tanzania and Mozambique allowed for the assessment of the core vs range edge population and the influence of ocean currents, geomorphology and other factors that may play a role.

The findings of **Chapter 3** showed that all the sampled populations had low genetic diversity. Similar results had been found in other studies of the same species and other mangrove species. There was high gene flow between populations occurring in Tanzania, Mozambique, Richards

Bay, Mngazana, Nxaxo/Ngqusi and Nahoon as the Nm value was above 1. This was somewhat unexpected as the following restrictions were expected (1) Limitation of gene flow between the core populations (Tanzania and Mozambique) and the South African populations (2) restriction in the gene flow in South African populations due to the geomorphology of their landscape. However, studies such as Triest (2008) and Yan *et al*. (2016) suggest that high gene flow can be found in neighbouring populations along the same coastline. Also showing that the above limitations did not restrict the gene flow in this area.

Mangroves maintain genetic flow between populations primarily through the dispersal of their viviparous or cryptoviviparous buoyant propagules via water influenced by wind, ocean currents allowing their wide distribution (Tomlinson, 1986; Minobe *et al*., 2010; Tomizawa *et al*., 2017; Duke, 1995), this is the only way that they could achieve long-distance dispersal (Tonné *et al*., 2017; Melville and Burchett, 2002). *Rhizophora* and *Bruguiera* spp. have the potential for longer distance dispersal and surviving longer periods in the open sea when compared to *A. marina* (Drexler, 2001; Clarke, 1993). Thus, there is potential that there may be higher gene flow in this region when compared to that of *A. marina*. Albeit the high gene flow, genetic diversity was low, this may indicate that there is not enough variety in the gene pool, thus having the introduction of propagules which are not increasing the genetic variation. This may indicate genetic drift, this expected in small populations such as Nxaxo/Ngqusi and Nahoon.

Based on the results there may be a need for further studies and investigation of other primers which may reveal more details about these populations. Having said that, the results suggest that these populations may be threatened by ecological stress or change due to the low genetic diversity. Richards Bay population may be a suitable source population for Nxaxo/Ngqusi and Nahoon which have a small population of these two species in the event of restoration programmes.

The second objective was to compare sediment and porewater characteristics of three mangrove estuaries in the Eastern Cape using long term monitoring data. Long term monitoring of sediment conditions is important as it allows for the detection of trends in temporal changes and their influence on mangrove development, growth and functioning (Lovelock *et al*., 2005). This allows for better understanding of the local conditions of the mangroves occuring in specific areas, make predictions on how environmental changes and other disturbance will

impact the population and how the populations will respond to changes or the threats in which they will encounter or currently expereience. .

The findings of **Chapter 4** showed that the environmental conditions in which the mangroves in Mngazana, Nxaxo/Ngqusi and Nahoon have been similar regarding porewater. It was expected that between 2017 and 2018, the permanently open Mngazana Estuary which has the larger mangrove area and is in the sub-tropical region would have been experiencing better environmental conditions when compared to the other two estuaries. The findings do not have any drastic changes which have occurred over the last few years (earliest dataset is from 2007 collected in Mngazana), which could suggest that the mangroves have not experienced extreme conditions which would impact their performance and the measured variables were within their range as detailed in the chapter.

The third objective was to determine the performance of *Avicennia marina* populations at the range edge. The findings of **Chapter 5** showed that recruitment/regeneration was taking place in all the estuaries. However, the ratio of seeding to sapling at Tyolomnqa and Kwelera was low (**Table 5.7**). These results suggest that many seedings do not make it to sapling stage. Recruitment and mortality (survivorship) are important for mangrove structure, forest development and population growth (Rivera-Monroy *et al*., 2019; Bosire *et al*., 2008). Thus, this could be a measure of quality of the habitat, which it would be expected that the larger populations and older forests would be of a better quality than those which are smaller and isolated. It is recommended that there should be active management of the anthropogenic impacts in and around the mangrove areas, i.e., the prevention of livestock from entering the forests and possible inclusion of walking pathways to limit the amount of trampling which occurs.

Also, edge effects and environmental conditions could play a role in population structure, as populations beyond Kobonqaba Estuary (Eastern Cape) which is located just south of Nxaxo/Ngqusi have been previously described in literature as occurring past the natural distribution range (Ward and Steinke, 1982; Rajkaran and Adams, 2011; Saintilan *et al*., 2014; Hoppe-Speer *et al*., 2015; Bolosha, 2016). Both Tyolomnqa and Kwelera have a small population size (less than 170 individuals) (Bolosha, 2016). Tyolomnqa is not a natural population and occurs past the distribution limit. Thus, the environmental conditions, habitat

quality, population density, fragmentation may be some of the factors which are limiting recruitment. In the event of extensive diebacks or loss of mangrove area e.g., due to natural hazards such as the drought and sea storms events that occurred in Kobonqaba (Mbense, 2017), flooding due to a cyclone which hit Mozambique in 1984 resulting in the loss of *A. marina* and B. *gymnorrhiza* at St Lucia (Forbes and Cyrus, 1992) or anthropogenic activities e.g., port development at Durban Bay (Peer *et al*., 2018) these populations are at a higher risk of not being able to naturally regenerate. Wise *et al*. (2003) states that studies argue that the environmental and random demographic conditions may play a more important role in resulting in the extinction of small populations than genetic factors. A number of these performance variables were only collected over one sampling period. It would be recommended that long term monitoring data be taken especially for Tyolomnqa and Kwelera as these populations are small and occur at the limit of the mangrove distribution, making them important in the expansion of the mangrove area in the country and more vulnerable to change.

This present study only assessed the genetic connectivity of *B. gymnorrhiza* and *R. mucronata* using a single primer for the nuclear and chloroplast regions. While for the plant performance variables, only a single sampling event had been carried out, thus the findings of the study may be limited and require additional research. Having said this, this study presents baseline information which future studies could build on and could be used for comparison. Regarding the genetic connectivity of the *B. gymnorrhiza* and *R. mucronata*, more primers could be used and the collecting of plant performance data over a longer period which could also factor in different seasons when collections are made.

This current study presents evidence that the range edge population require conservation priority not only because of their location but also their performance and genetic diversity. Thus, there us a need for more effort and implementation of strategies to protect especially the smaller mangrove forests. However, the larger mangrove forests also require safeguarding as they could act as source populations of propagules for the smaller forests. This study could thus be used by custodians of these mangroves in their decision making and the development of strategies in the management of mangroves in the country.

References

Abeli, T., Gentili, R., Mondoni, A., Orsenigo, S., & Rossi, G. (2014). Effects of marginality on plant population performance. *Journal of Biogeography*, *41*(2), 239-249.

Abeysinghe, P. D., Triest, L., De Greef, B., Koedam, N., & Hettiarachi, S. (1999). Genetic differentiation between *Bruguiera gymnorrhiza* and *B. sexangula* in Sri Lanka. *Hydrobiologia*, *413*, 11-16.

Abeysinghe, P. D., Triest, L., De Greef, B., Koedam, N., & Hettiarachi, S. (2000). Genetic and geographic variation of the mangrove tree *Bruguiera* in Sri Lanka. *Aquatic Botany*, *67*(2), 131-141.

Adams, J. B., & Human, L. R. D. (2016). Investigation into the mortality of mangroves at St. Lucia Estuary. *South African Journal of Botany*, *107*, 121-128.

Adams, J. B., Colloty, B. M., & Bate, G. C. (2004). The distribution and state of mangroves along the coast of Transkei, Eastern Cape Province, South Africa. *Wetlands Ecology and Management*, *12*(5), 531-541.

Adams, J. B., & Rajkaran, A. (2021). Changes in mangroves at their southernmost African distribution limit. *Estuarine, Coastal and Shelf Science*, *248*, 107158.

Alboukadel, K. (2018). ggpubr:"ggplot2" based publication ready plots. *R package version 0.4. 0*.

Allen, J. A., & Duke, N. C. (2006). *Bruguiera gymnorrhiza* (large-leafed mangrove). *Elevitch, CR Species Profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR), Hōlualoa, Hawai'i*.

Almahasheer, H., Duarte, C. M., & Irigoien, X. (2016). Phenology and Growth dynamics of *Avicennia marina* in the Central Red Sea. *Scientific reports*, 6(1), 1-9.

Alongi, D. (2009). The energetics of mangrove forests. *Springer Science & Business Media*.

Alongi, D. M. (2015). The impact of climate change on mangrove forests. *Current Climate Change Reports*, *1*(1), 30-39.

Alongi, D. M., Clough, B. F., & Robertson, A. I. (2005). Nutrient-use efficiency in arid-zone forests of the mangroves *Rhizophora stylosa* and *Avicennia marina*. *Aquatic botany*, *82*(2), 121-131.

Alongi, D. M., Wattayakorn, G., Boyle, S., Tirendi, F., Payn, C., & Dixon, P. (2004). Influence of roots and climate on mineral and trace element storage and flux in tropical mangrove soils. *Biogeochemistry*, *69*(1), 105-123.

Amade, F. M., Oosthuizen, C. J., & Chirwa, P. W. (2021). Genetic diversity and contemporary population genetic structure of *Avicennia marina* from Mozambique. *Aquatic Botany*, *171*, 103374.

Anderson, C., & Lee, S. Y. (1995). Defoliation of the mangrove Avicennia marina in Hong Kong: cause and consequences. *Biotropica*, 218-226.

Anderson, J. T. (2016). Plant fitness in a rapidly changing world. *New Phytologist*, *210*(1), 81-87.

Aphalo, P. J. (2016). ggpmisc: An R package.

Arnaud-Haond, S., Teixeira, S., Massa, S. I., Billot, C., Saenger, P., Coupland, G., Duarte, C.M. and Serrao, E.A., (2006). Genetic structure at range edge: low diversity and high inbreeding in Southeast Asian mangrove (Avicennia marina) populations. *Molecular Ecology*, *15*(12), 3515-3525.

Arnold, J. B., & Arnold, M. J. B. (2015). Package 'ggthemes'.

Arrivabene, H. P., Souza, I., Có, W. L. O., Rodella, R. A., Wunderlin, D. A., & Milanez, C. R. (2014). Functional traits of selected mangrove species in Brazil as biological indicators of different environmental conditions. *Science of The Total Environment*, *476*, 496-504.

Ashok Prabu, V., Rajkumar, M., & Perumal, P. (2008). Seasonal variations in physico-chemical characteristics of Pichavaram mangroves, southeast coast of India.

Assis, J., Coelho, N. C., Alberto, F., Valero, M., Raimondi, P., Reed, D., & Serrão, E. A. (2013). High and distinct range-edge genetic diversity despite local bottlenecks. *PLoS One*, *8*(7), e68646.

Balasubramanyan, K., Srinvasan, M. and. Kathresan, K. (2010). Insects. Centre of Advanced Study in Marine Biology, Annamalai University

Baldwin, A., Egnotovich, M., Ford, M., & Platt, W. (2001). Regeneration in fringe mangrove forests damaged by Hurricane Andrew. *Plant Ecology*, *157*(2), 151-164.

Ball, M. C. (1988). Ecophysiology of mangroves. *Trees*, *2*(3), 129-142.

Ball, M. C., & Pidsley, S. M. (1995). Growth responses to salinity in relation to distribution of two mangrove species, Sonneratia alba and S. lanceolata, in northern Australia. *Functional Ecology*, 77-85.

Barbeta, A., Penuelas, J., Ogaya, R., & Jump, A. S. (2011). Reduced tree health and seedling production in fragmented Fagus sylvatica forest patches in the Montseny Mountains (NE Spain). *Forest Ecology and Management*, *261*(11), 2029-2037.

Barnuevo, A., Asaeda, T., Sanjaya, K., Kanesaka, Y., & Fortes, M. (2017). Drawbacks of mangrove rehabilitation schemes: Lessons learned from the large-scale mangrove plantations. *Estuarine, Coastal and Shelf Science*, *198*, 432-437.

Bastakoti, U., Robertson, J., Marchand, C., & Alfaro, A. C. (2019). Mangrove removal: Effects on trace metal concentrations in temperate estuarine sediments. *Marine Chemistry*, *216*, 103688.

Bazzaz, F. A., Chiariello, N. R., Coley, P. D., & Pitelka, L. F. (1987). Allocating resources to reproduction and defense. *BioScience*, *37*(1), 58-67.

Berger, U., Adams, M., Grimm, V., & Hildenbrandt, H. (2006). Modelling secondary succession of neotropical mangroves: causes and consequences of growth reduction in pioneer species. *Perspectives in Plant Ecology, Evolution and Systematics*, *7*(4), 243-252.

Bhattacharya, J. P., & Giosan, L. (2003). Wave-influenced deltas: Geomorphological implications for facies reconstruction. *Sedimentology*, *50*(1), 187-210.

Binks, R. M., Byrne, M., McMahon, K., Pitt, G., Murray, K., & Evans, R. D. (2019). Habitat discontinuities form strong barriers to gene flow among mangrove populations, despite the capacity for long-distance dispersal. *Diversity and Distributions*, *25*(2), 298-309.

Black, C. B. (1965). Methods of sediment analysis. *American Society of Agronomy Inc., Madison, Wisconsin* (1000 pp.).

Black, F.J., Gallon, C., & Flegal, A. R. (2008). Sediment Retention and Release.

Bolosha, U. (2016). *Revising the distribution of mangrove forests in South Africa and changes in growth of mangrove species along a latitudinal gradient* (M. Sc. Thesis, Rhodes University, South Africa: 139 pp).

Bosire, J. O., Dahdouh-Guebas, F., Walton, M., Crona, B. I., Lewis Iii, R. R., Field, C., Kairo, J.G. & Koedam, N. (2008). Functionality of restored mangroves: a review. *Aquatic botany*, *89*(2), 251-259.

Bosire, J. O., Mangora, M. M., Bandeira, S. O., Rajkaran, A., Ratsimbazafy, R., Appadoo, C., & Kairo, J. G. (2016). *Mangroves of the Western Indian Ocean: status and management*.

Bouillon, S., Connolly, R. M., & Lee, S. Y. (2008). Organic matter exchange and cycling in mangrove ecosystems: recent insights from stable isotope studies. *Journal of sea research*, *59*(1-2), 44-58.

Bruschi, P., Angeletti, C., González, O., Adele Signorini, M., & Bagnoli, F. (2014). Genetic and morphological variation of *Rhizophora mangle* (red mangrove) along the northern Pacific coast of Nicaragua. *Nordic Journal of Botany*, *32*(3), 320-329.

Burrows, D. W. (2003). *The role of insect leaf herbivory on the mangroves Avicennia marina and Rhizophora stylosa* (Doctoral dissertation, James Cook University).

Butcher, P. A., McDonald, M. W., & Bell, J. C. (2009). Congruence between environmental parameters, morphology and genetic structure in Australia's most widely distributed eucalypt, Eucalyptus camaldulensis. Tree Genetics & Genomes, *5*(1), 189.

Camilleri, J. (1989). Leaf choice by crustaceans in a mangrove forest in Queensland. *Marine Biology*, *102*(4), 453-459.

Cannicci, S., Burrows, D., Fratini, S., Smith III, T. J., Offenberg, J., & Dahdouh-Guebas, F. (2008). Faunal impact on vegetation structure and ecosystem function in mangrove forests: a review. *Aquatic botany*, *89*(2), 186-200.

Capdeville, C., Abdallah, K., Walcker, R., Rols, J. L., Fromard, F., & Leflaive, J. (2019). Contrasted resistance and resilience of two mangrove forests after exposure to long-term and short-term anthropic disturbances. *Marine environmental research*, *146*, 12-23.

Carrasco, N. K., & Perissinotto, R. (2012). Development of a halotolerant community in the St. Lucia Estuary (South Africa) during a hypersaline phase. *PloS one*, *7*(1), e29927.

Cerón-Souza, I., Gonzalez, E. G., Schwarzbach, A. E., Salas-Leiva, D. E., Rivera-Ocasio, E., Toro-Perea, N., Bermingham, E. & McMillan, W. O. (2015). Contrasting demographic history and gene flow patterns of two mangrove species on either side of the Central American Isthmus. *Ecology and Evolution*, *5*(16), 3486-3499.

Chakraborty, S. K. (2013). Interactions of environmental variables determining the biodiversity of coastal-mangrove ecosystem of West Bengal, India. *Development*, *25*, 27.

Chambers, L. G., Osborne, T. Z., & Reddy, K. R. (2013). Effect of salinity-altering pulsing events on soil organic carbon loss along an intertidal wetland gradient: a laboratory experiment. *Biogeochemistry*, *115*(1-3), 363-383.

Chang, A., Lim, M. H., Lee, S. W., Robb, E. J., & Nazar, R. N. (2008). Tomato *phenylalanine ammonia-lyase* gene family, highly redundant but strongly underutilized. *Journal of Biological Chemistry*, *283*(48), 33591-33601.

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature reviews genetics*, *10*(11), 783.

Charrua, A. B., Bandeira, S. O., Catarino, S., Cabral, P., & Romeiras, M. M. (2020). Assessment of the vulnerability of coastal mangrove ecosystems in Mozambique. *Ocean & Coastal Management*, *189*, 105145.

Chaudhari, P. R., Ahire, D. V., Ahire, V. D., Chkravarty, M., & Maity, S. (2013). Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications*, *3*(2), 1-8.

Chen, R., & Twilley, R. R. (1999a). Patterns of mangrove forest structure and soil nutrient dynamics along the Shark River Estuary, Florida. *Estuaries*, *22*(4), 955-970.

Chen, R., & Twilley, R. R. (1999b). A simulation model of organic matter and nutrient accumulation in mangrove wetland soils. *Biogeochemistry*, *44*(1), 93-118.

Chen, Y., Hou, Y., Guo, Z., Wang, W., Zhong, C., Zhou, R., & Shi, S. (2015). Applications of multiple nuclear genes to the molecular phylogeny, population genetics and hybrid identification in the mangrove genus *Rhizophora*. *PLoS One*, *10*(12), e0145058.

Chen, L., Wang, W., Li, Q. Q., Zhang, Y., Yang, S., Osland, M. J., Huang, J., & Peng, C. (2017). Mangrove species' responses to winter air temperature extremes in China. *Ecosphere*, *8*(6), e01865.

Chiang, T. Y., Chiang, Y. C., Chen, Y. J., Chou, C. H., Havanond, S., Hong, T. N., & Huang, S. (2001). Phylogeography of *Kandelia candel* in East Asiatic mangroves based on nucleotide variation of chloroplast and mitochondrial DNAs. *Molecular Ecology*, *10*(11), 2697-2710.

Clark, M. W., McConchie, D., Lewis, D. W., & Saenger, P. (1998). Redox stratification and heavy metal partitioning in Avicennia-dominated mangrove sediments: a geochemical model. *Chemical Geology*, *149*(3-4), 147-171.

Clarke, P. J., & Myerscough, P. J. (1991). Floral biology and reproductive phenology of Avicennia marina in south-eastern Australia. *Australian Journal of Botany*, *39*(3), 283-293.

Clarke, P.J. (1993). Dispersal of grey mangrove (*Avicennia marina*) propagules in southeastern Australia. *Aquatic Botany 45*, 195–204.

Clancy, C. M. R. (2001). OneStep™ PCR Inhibitor Removal Kit Protocol. Zymoresearch.

Clough, B. F. (1993). Constraints on the growth, propagation and utilization of mangroves in arid regions. In Towards the rational use of high salinity tolerant plants. *Springer*, 341-352.

Cooper, J. A. G. (2001). Geomorphological variability among microtidal estuaries from the wave-dominated South African coast. *Geomorphology*, *40*(1-2), 99-122.

Cooper, J. A. G. (2002). The role of extreme floods in estuary-coastal behaviour: contrasts between river-and tide-dominated microtidal estuaries. *Sedimentary Geology*, *150*(1-2), 123-137.

Core Team, R. (2018). R: A language and environment for statistical computing. Version 3.5. 2. *R Foundation for Statistical Computing, Vienna, Austria*.

Cotiyane, P., Adams, J. B., & Rajkaran, A. (2019). Relating microalgal response to nutrient status in a mangrove-dominated estuary. *Hydrobiologia*, *843*(1), 183-199.

Cotiyane, P., Adams, J., & Rajkaran, A. (2017). Key factors that drive phytoplankton biomass and community composition in the urbanised Nahoon Estuary, South Africa. *African Journal of Aquatic Science*, *42*(3), 245-257.

Dahdouh-Guebas, F., De Bondt, R., Abeysinghe, P. D., Kairo, J. G., Cannicci, S., Triest, L., & Koedam, N. (2004). Comparative study of the disjunct zonation pattern of the grey mangrove Avicennia marina (Forsk.) Vierh. in Gazi Bay (Kenya). *Bulletin of Marine Science*, 74(2), 237-252.

Dai, Q., & Fu, J. (2011). When central populations exhibit more genetic diversity than peripheral populations: A simulation study. *Chinese Science Bulletin*, *56*(24), 2531-2540.

Das, S. (1999). An adaptive feature of some mangroves of Sundarbans, West Bengal. *Journal of Plant Biology*, *42*(2), 109-116.

Dasgupta, N., Nandy, P., Sengupta, C., & Das, S. (2015). RAPD and ISSR marker mediated genetic polymorphism of two mangroves *Bruguiera gymnorrhiza* and *Heritiera fomes* from Indian Sundarbans in relation to their sustainability. *Physiology and Molecular Biology of Plants*, *21*(3), 375-384.

Davidson, T. M., De Rivera, C. E., & Hsieh, H. L. (2014). Damage and alteration of mangroves inhabited by a marine wood-borer. *Marine Ecology Progress Series*, *516*, 177-185.

de Mendiburu, F., & de Mendiburu, M. F. (2019). Package 'agricolae'. *R Package, version*, *1*(3).

De Ryck, D. J., Koedam, N., Van der Stocken, T., van der Ven, R. M., Adams, J., & Triest, L. (2016). Dispersal limitation of the mangrove Avicennia marina at its South African range limit in strong contrast to connectivity in its core East African region. *Marine Ecology Progress Series*, *545*, 123-134.

De Ryck, D. J., Robert, E. M., Schmitz, N., Van der Stocken, T., Di Nitto, D., Dahdouh-Guebas, F., & Koedam, N. (2012). Size does matter, but not only size: Two alternative dispersal strategies for viviparous mangrove propagules. *Aquatic Botany*, *103*, 66-73.

Debez, A., Saadaoui, D., Ramani, B., Ouerghi, Z., Koyro, H. W., Huchzermeyer, B., & Abdelly, C. (2006). Leaf H+-ATPase activity and photosynthetic capacity of Cakile maritima under increasing salinity. *Environmental and Experimental Botany*, *57*(3), 285-295.

Dinno, A., & Dinno, M. A. (2017). Package 'dunn. test'. *CRAN Repos*, *10*, 1-7.

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of vegetation science*, *14*(6), 927-930.

dos Santos, R. D. C. O., Fernandes, M. E. B., & Martins, M. B. (2013). Are the species of the Genus Avicennia L. (Acanthaceae) a "Superhost" plants of gall-inducing Arthropods in mangrove forests? *Herbivory*, 17.

Drexler, J. Z. (2001). Maximum longevities of *Rhizophora* apiculata and R. mucronata propagules. *Pacific Science*, *55*(1), 17-22.

Duarte, C. M., Delgado-Huertas, A., Anton, A., Carrillo-de-Albornoz, P., López-Sandoval, D. C., Agustí, S., & Garcias-Bonet, N. (2018). Stable Isotope (δ13C, δ15N, δ18O, δD) composition and nutrient concentration of red sea primary producers.

Duke, N. C. (1990). Phenological trends with latitude in the mangrove tree *Avicennia marina*. *The Journal of Ecology*, 113-133.

Duke, N. C. (1995). Genetic diversity, distributional barriers and rafting continents—more thoughts on the evolution of mangroves. *Hydrobiologia*, *295*(1-3), 167-181.

Duke, N. C. (2006). Mangrove taxonomy, biogeography and evolution—An Indo West Pacific perspective of implications for conservation and management. *Perm. Agric. Resour*, *77*, 641-666.

Duke, N. C. (2001). Gap creation and regenerative processes driving diversity and structure of mangrove ecosystems. *Wetlands Ecology and Management*, *9*(3), 267-279.

Duke, N. C. (2011). Mangroves. In Encyclopaedia of Modern Coral Reefs. *Springer*, 655-663.

Duke, N. C. (2017). Mangrove Floristics and Biogeography Revisited: Further Deductions from Biodiversity Hot Spots, Ancestral Discontinuities, and Common Evolutionary Processes. *In Mangrove Ecosystems: A Global Biogeographic Perspective* (pp. 17-53). Springer, Cham.

Duke, N. C., & Allen, J. A. (2006). Atlantic-East Pacific red mangroves: *Rhizophora mangle, R. samoensis, R. racemosa, R. X harrisonii*. Permanent Agriculture Resources (PAR).

Duke, N. C., & Schmitt, K. (2015). Mangroves: unusual forests at the seas edge. *Tropical forestry handbook*, 1-24.

Duke, N. C., Benzie, J. A., Goodall, J. A., & Ballment, E. R. (1998b). Genetic structure and evolution of species in the mangrove genus Avicennia (Avicenniaceae) in the Indo-West Pacific. *Evolution*, *52*(6), 1612-1626.

Duke, N. C., Kovacs, J. M., Griffiths, A. D., Preece, L., Hill, D. J., Van Oosterzee, P. Van Oosterzee, Mackenzie, J. Morning, H. S. & Burrows, D. (2017). Large-scale dieback of mangroves in Australia's Gulf of Carpentaria: a severe ecosystem response, coincidental with an unusually extreme weather event. *Marine and Freshwater Research*, *68*(10), 1816-1829.

Duke, N., Ball, M., & Ellison, J. (1998a). Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology & Biogeography Letters*, *7*(1), 27-47.

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular ecology*, *17*(5), 1170-1188.

Elliott, M., & Whitfield, A. K. (2011). Challenging paradigms in estuarine ecology and management. *Estuarine, Coastal and Shelf Science*, *94*(4), 306-314.

Ellison, A. M., Farnsworth, E. J., & Merkt, R. E. (1999). Origins of mangrove ecosystems and the mangrove biodiversity anomaly. *Global Ecology and Biogeography*, *8*(2), 95-115.

Engelhardt, K. A., Lloyd, M. W., & Neel, M. C. (2014). Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. *Biological Conservation*, *179*, 6-16.

Erickson, A. A., Bell, S. S., & Dawes, C. J. (2004). Does mangrove leaf chemistry help explain crab herbivory patterns?. *Biotropica*, *36*(3), 333-343.

Excoffier, L., & Lischer, H. (2015). Arlequin ver 3.5.2 user manual; An integrated software package for population genetics data analysis. *Swiss Institute of Bioinformatics*.

Faridah-Hanum, I., Kudus, K. A., & Saari, N. S. (2012). Plant diversity and biomass of Marudu bay mangroves in Malaysia. *Pakistan Journal of Botany*, *44*(Suppl 2), 151-156.

Farnsworth, E. J., & Ellison, A. M. (1991). Patterns of herbivory in Belizean mangrove swamps. *Biotropica*, 555-567.

Feller, I. C., Ball, M. C., Ellis, J. T., Lovelock, C. E., & Reef, R. (2017). Interactive effects of climate and nutrient enrichment on patterns of herbivory by different feeding guilds in mangrove forests. *Global Ecology and Biogeography*, *26*(11), 1326-1338.

Feller, I. C., McKee, K. L., Whigham, D. F., & O'neill, J. P. (2002). Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry*, *62*(2), 145-175.

Ferreira, T. O., Otero, X. L., de Souza Junior, V. S., Vidal-Torrado, P., Macías, F., & Firme, L. P. (2010). Spatial patterns of soil attributes and components in a mangrove system in Southeast Brazil (São Paulo). *Journal of Soils and Sediments*, *10*(6), 995-1006.

Forbes, A. T., & Cyrus, D. P. (1992). Impact of a major cyclone on a southeast African estuarine lake system. *Netherlands Journal of Sea Research*, *30*, 265-272.

Fox, J., Friendly, G. G., Graves, S., Heiberger, R., Monette, G., Nilsson, H., Ripley, B., Weisberg, S., Fox, M.J., & Suggests, M. A. S. S. (2007). The car package. *R Foundation for Statistical Computing*, *1109*, 1431.

Friess, D. A., Rogers, K., Lovelock, C. E., Krauss, K. W., Hamilton, S. E., Lee, S. Y., Lucas, R., Primavera, J., Rajkaran, A., & Shi, S. (2019). The state of the world's mangrove forests: past, present, and future. *Annual Review of Environment and Resources*, *44*, 89-115.

Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*(2), 915-925.

Gattuso, J. P., Frankignoulle, M., & Wollast, R. (1998). Carbon and carbonate metabolism in coastal aquatic ecosystems. *Annual Review of Ecology and Systematics*, *29*(1), 405-434.

Ge, J. P., Cai, B., Ping, W., Song, G., Ling, H., & Lin, P. (2005). Mating system and population genetic structure of *Bruguiera gymnorrhiza* (Rhizophoraceae), a viviparous mangrove species in China. *Journal of experimental marine biology and ecology*, *326*(1), 48-55.

Geldenhuys, C. (2014). *Mangrove and salt marsh dynamics at Nahoon Estuary, Eastern Cape: a planted mangrove forest* (MSc Thesis. Botany Department, Rhodes University).

Geldenhuys, C., Cotiyane, P., & Rajkaran, A. (2016). Understanding the creek dynamics and environmental characteristics that determine the distribution of mangrove and salt marsh communities at Nahoon Estuary. *South African journal of botany*, *107*, 137-147.

Geng Q, Lian C, Goto S, Tao J, Kimura M, Islam MS, Hogetsu T (2008) Mating system, pollen and propagule dispersal, and spatial genetic structure in a high-density population of the mangrove tree *Kandelia candel*. Mol Ecol 17:4724–4739

Giang LH, Geada GL, Hong PN, Tuan MS, Lien NTH, Ikeda S, Harada K (2006) Genetic variation of two mangrove species in *Kandelia* (Rhizophoraceae) in Vietnam and surrounding area revealed by microsatellite markers. Int J Plant Sci 167:291–298

Giang, L. H., Hong, P. N., Tuan, M. S., & Harada, cph (2003). Genetic variation of *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae) in Vietnam revealed by microsatellite and AFLP *markers*. *Genes & genetic systems*, *78*(6), 399-407.

Gilbert, G. S., Mejía-Chang, M., & Rojas, E. (2002). Fungal diversity and plant disease in mangrove forests: salt excretion as a possible defense mechanism. *Oecologia*, *132*(2), 278-285.

Gilman, E. L., Ellison, J., Duke, N. C., & Field, C. (2008). Threats to mangroves from climate change and adaptation options: a review. *Aquatic botany*, *89*(2), 237-250.

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J, Duke N (2011). Status and distribution of mangrove forests of the world using earth observation satellite data (version 1.3, updated by UNEP-WCMC). Global Ecology and Biogeography 20: 154-159. doi: 10.1111/j.1466-8238.2010.00584.x . Data URL: http://data.unep-wcmc.org/datasets/4

Gonçalves-Alvim, S. J., Vaz dos Santos, M. C., & Fernandes, G. W. (2001). Leaf gall abundance on *Avicennia germinans* (Avicenniaceae) along an interstitial salinity gradient. *Biotropica*, 33(1), 69-77.

Gritcan, I., Duxbury, M., Leuzinger, S., & Alfaro, A. C. (2016). Leaf stable isotope and nutrient status of temperate mangroves as ecological indicators to assess anthropogenic activity and recovery from eutrophication. *Frontiers in plant science*, *7*, 1922.

Guo, Z., Huang, Y., Chen, Y., Duke, N. C., Zhong, C., & Shi, S. (2016). Genetic discontinuities in a dominant mangrove *Rhizophora apiculata* (Rhizophoraceae) in the Indo-Malesian region. *Journal of Biogeography*, *43*(9), 1856-1868.

Guo, W., Wu, H., Zhang, Z., Yang, C., Hu, L., Shi, X., Jian, S., Shi, S. & Huang, Y. (2017). Comparative analysis of transcriptomes in Rhizophoraceae provides insights into the origin and adaptive evolution of mangrove plants in intertidal environments. Frontiers in plant science, 8, 795.

Guo, Z., Li, X., He, Z., Yang, Y., Wang, W., Zhong, C., Greenberg, A.J., Wu C., Duke N.C., & Shi, S. (2018). Extremely low genetic diversity across mangrove taxa reflects past sea level changes and hints at poor future responses. *Global change biology*, *24*(4), 1741-1748.

Hardie, D. C., & Hutchings, J. A. (2010). Evolutionary ecology at the extremes of species' ranges. *Environmental Reviews*, *18*(NA), 1-20.

Harrison, T. D. (2004). Physico-chemical characteristics of South African estuaries in relation to the zoogeography of the region. *Estuarine, Coastal and Shelf Science*, *61*(1), 73-87.

Hayes, M. A., Jesse, A., Tabet, B., Reef, R., Keuskamp, J. A., & Lovelock, C. E. (2017). The contrasting effects of nutrient enrichment on growth, biomass allocation and decomposition of plant tissue in coastal wetlands. *Plant and soil*, *416*(1-2), 193-204.

Hermansen, T. D., Minchinton, T. E., & Ayre, D. J. (2017). Habitat fragmentation leads to reduced pollinator visitation, fruit production and recruitment in urban mangrove forests. *Oecologia*, *185*(2), 221-231.

Hogarth, P. J. (2015). *The biology of mangroves and seagrasses*. Oxford University Press.

Hoppe-Speer, S. C. L., Adams, J. B., & Rajkaran, A. (2013). Response of mangroves to drought and non-tidal conditions in St Lucia Estuary, South Africa. *African Journal of Aquatic Science*, *38*(2), 153-162.

Hoppe-Speer, S. C., & Adams, J. B. (2015). Cattle browsing impacts on stunted Avicennia marina mangrove trees. *Aquatic Botany*, *121*, 9-15.

Hoppe-Speer, S. C., Adams, J. B., & Rajkaran, A. (2015). Mangrove expansion and population structure at a planted site, East London, South Africa. *Southern Forests: a Journal of Forest Science*, *77*(2), 131-139.

Hossain, M. D., & Nuruddin, A. A. (2016). Soil and mangrove: A review. *Journal of Environmental Science and Technology*, *9*(2), 198.

Hudson, R. R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence data. *Genetics*, *132*(2), 583-589.

Hughes, A. R., & Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences*, *101*(24), 8998-9002.

Iida, Y., Kohyama, T. S., Swenson, N. G., Su, S. H., Chen, C. T., Chiang, J. M., & Sun, I. F. (2014). Linking functional traits and demographic rates in a subtropical tree community: the importance of size dependency. *Journal of Ecology*, *102*(3), 641-650.

Inomata, N., Wang, X. R., Changtragoon, S., & Szmidt, A. E. (2009). Levels and patterns of DNA variation in two sympatric mangrove species, *Rhizophora apiculata* and *R. mucronata* from Thailand. *Genes & genetic systems*, *84*(4), 277-286.

Islam, M. S., Lian, C., Kameyama, N., & Hogetsu, T. (2012). Analyses of genetic population structure of two ecologically important mangrove tree species, *Bruguiera gymnorrhiza* and *Kandelia obovata* from different river basins of Iriomote Island of the Ryukyu Archipelago, Japan.

Islam, M. S., Lian, C., Kameyama, N., & Hogetsu, T. (2014). Low genetic diversity and limited gene flow in a dominant mangrove tree species (*Rhizophora stylosa*) at its northern biogeographical limit across the chain of three Sakishima islands of the Japanese archipelago as revealed by chloroplast and nuclear SSR analysis. *Plant systematics and evolution*, *300*(5), 1123-1136.

Islam, M. S., Lian, C., Kameyama, N., & Hogetsu, T. (2015). Analysis of the mating system, reproductive characteristics, and spatial genetic structure in a natural mangrove tree (*Bruguiera gymnorrhiza*) population at its northern biogeographic limit in the southern Japanese archipelago. *Journal of Forest Research*, *20*(2), 293-300.

James, N. C., & Harrison, T. D. (2011). A preliminary survey of the estuaries on the southeast coast of South Africa, Old Woman's–Tyolomnqa, with particular reference to the fish fauna. *Transactions of the Royal Society of South Africa*, *66*(2), 59-77.

James, N. C., Whitfield, A. K., & Harrison, T. D. (2016). Grey mullet (Mugilidae) as possible indicators of global warming in South African estuaries and coastal waters. *Marine environmental research*, *122*, 188-195.

Joshi, H., & Ghose, M. (2003). Forest structure and species distribution along soil salinity and pH gradient in mangrove swamps of the Sundarbans. *Tropical Ecology*, *44*(2), 195-204.

Juncosa, A. M., & Tomlinson, P. B. (1988). A historical and taxonomic synopsis of Rhizophoraceae and Anisophylleaceae. *Annals of the Missouri Botanical Garden*, 1278-1295.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in marine biology*, *40*, 81-251.

Kathiresan, K., & Rajendran, N. (2005). Mangrove ecosystems of the Indian Ocean region.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in ecology & evolution*, *17*(5), 230-241.

Kelleway, J. J., Mazumder, D., Baldock, J. A., & Saintilan, N. (2018). Carbon isotope fractionation in the mangrove Avicennia marina has implications for food web and blue carbon research. *Estuarine, Coastal and Shelf Science*, *205*, 68-74.

Kennish, M. J. (2016). *Encyclopedia of Estuaries (Encyclopedia of Earth Sciences Series)*. Springer.

Kéry, M., Matthies, D., & Spillmann, H. H. (2000). Reduced fecundity and offspring performance in small populations of the declining grassland plants Primula veris and Gentiana lutea. *Journal of Ecology*, *88*(1), 17-30.

Kihia, C. M., Mathooko, J. M., Ruwa, R. K., & Shivoga, W. A. (2011). Influence of human disturbance on patterns of leaf herbivory at Gazi Bay mangrove forest, Kenya. *African Journal of Aquatic Science*, *36*(3), 235-241.

Knight, J. M., Griffin, L., Dale, P. E., & Sheaves, M. (2013). Short-term dissolved oxygen patterns in sub-tropical mangroves. *Estuarine, Coastal and Shelf Science*, *131*, 290-296.

Krauss, K. W., Lovelock, C. E., McKee, K. L., López-Hoffman, L., Ewe, S. M., & Sousa, W. P. (2008). Environmental drivers in mangrove establishment and early development: a review. *Aquatic botany*, *89*(2), 105-127.

Kristensen, E., Connolly, R., Otero, X., Marchand, C., Ferreira, T., Rivera-Monroy, V. (2017). Biogeochemical Cycles: Global Approaches and Perspectives. *Mangrove ecosystems: a global biogeographic perspective*, 163-209. 10.1007/978-3-319-62206-4_6

Ladd, S. N., & Sachs, J. P. (2013). Positive correlation between salinity and n-alkane δ13C values in the mangrove Avicennia marina. *Organic geochemistry*, *64*, 1-8.

Lakshmi, M., Parani, M., & Parida, A. (2002). Molecular marker assisted intra-specific variation and species relationships in the Indian mangrove tribe Rhizophoreae. *Aquatic Botany*, *74*(3), 201-217.

Le, Q. D., Haron, N. A., Tanaka, K., Ishida, A., Sano, Y., Dung, L. V., & Shirai, K. (2017). Quantitative contribution of primary food sources for a mangrove food web in Setiu lagoon from East coast of Peninsular Malaysia, stable isotopic (δ13C and δ15N) approach. *Regional Studies in Marine Science*, *9*, 174-179.

Lehndal, L., & Ågren, J. (2015). Herbivory differentially affects plant fitness in three populations of the perennial herb Lythrum salicaria along a latitudinal gradient. *PLoS One*, *10*(9), e0135939.

Leimu, R., Vergeer, P., Angeloni, F., & Ouborg, N. J. (2010). Habitat fragmentation, climate change, and inbreeding in plants. *Annals of the New York Academy of Sciences*, *1195*(1), 84-98.

Li, X., Duke, N. C., Yang, Y., Huang, L., Zhu, Y., Zhang, Z., Zhou., R., Zhong, C., Haung, Y., & Shi, S. (2016). Re-evaluation of phylogenetic relationships among species of the mangrove genus Avicennia from Indo-West Pacific based on multilocus analyses. *PLoS One*, *11*(10), e0164453.

Lira-Medeiros, C. F., Cardoso, M. A., Fernandes, R. A., & Ferreira, P. C. G. (2015). Analysis of genetic diversity of two mangrove species with morphological alterations in a natural environment. *Diversity*, *7*(2), 105-117.

Lo, E. Y., Duke, N. C., & Sun, M. (2014). Phylogeographic pattern of *Rhizophora* (Rhizophoraceae) reveals the importance of both vicariance and long-distance oceanic dispersal to modern mangrove distribution. *BMC evolutionary biology*, *14*(1), 83.

Lovelock, C. E., Ball, M. C., Martin, K. C., & C. Feller, I. (2009). Nutrient enrichment increases mortality of mangroves. *PloS one*, *4*(5), e5600.

Lovelock, C. E., Feller, I. C., Ellis, J., Schwarz, A. M., Hancock, N., Nichols, P., & Sorrell, B. (2007). Mangrove growth in New Zealand estuaries: the role of nutrient enrichment at sites with contrasting rates of sedimentation. *Oecologia*, *153*(3), 633-641.

Lovelock, C. E., Feller, I. C., McKee, K. L., & Thompson, R. C. (2005). Variation in mangrove forest structure and sediment characteristics in Bocas del Toro, Panama. *Caribbean Journal of Science*.

Lovelock, C. E., Feller, I. C., McKee, K. L., Engelbrecht, B. M., & Ball, M. C. (2004). The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama. *Functional Ecology*, *18*(1), 25-33.

Lugendo, B. (2016). Mangroves, salt marshes and seagrass beds. *Regional State of the Coast Report*, 52-68.

Lugo, A. E., & Snedaker, S. C. (1974). The ecology of mangroves. *Annual review of ecology and systematics*, *5*(1), 39-64.

Macnae, W. (1963). Mangrove swamps in south Africa. *Journal of Ecology*, *51*(1), 1-25.

Macnae, W., & Kalk, M. (1962). The Ecology of the Mangrove Swamps at Inhaca Island, Moξambique. *The Journal of Ecology*, 19-34.

Macamo, C. C., Balidy, H., Bandeira, S. O., & Kairo, J. G. (2015). Mangrove transformation in the Incomati Estuary, Maputo Bay, Mozambique. *Western Indian Ocean Journal of Marine Science*, *14*(1&2), 11-22.

Maguire, T. L., Peakall, R., & Saenger, P. (2002). Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh (Avicenniaceae) detected by AFLPs and SSRs. *Theoretical and applied Genetics*, *104*(2), 388-398.

Maguire, T.L., Saenger, P., Baverstock, P. and Henry, R., 2000. Microsatellite analysis of genetic structure in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae). Molecular Ecology, 9(11), 1853-1862.

Mantiquilla, J. A., Shiao, M. S., Shih, H. C., Chen, W. H., & Chiang, Y. C. (2021). A review on the genetic structure of ecologically and economically important mangrove species in the Indo-West Pacific. *Ecological Genetics and Genomics*, *18*, 100078.

Marchand, C. (2017). Soil carbon stocks and burial rates along a mangrove forest chronosequence (French Guiana). Forest Ecology and Management, 384, 92-99.

Marchand, C., Baltzer, F., Lallier-Vergès, E., & Albéric, P. (2004). Pore-water chemistry in mangrove sediments: relationship with species composition and developmental stages (French Guiana). *Marine Geology*, *208*(2-4), 361-381.

Martin, N. A. (2017 revised 2018). Mangrove erineum mite - Acalitus avicenniae. Interesting Insects and other Invertebrates. New Zealand Arthropod Factsheet Series Number 71. http://nzacfactsheets.landcareresearch.co.nz/Index.html. Date Accessed; February 2019. ISSN 1179-643X.

Mbense, S. (2017). *The growth and recovery of mangroves at three South African study sites* (MSc thesis, Nelson Mandela Metropolitan University).

McCoy, E. D., & Heck Jr, K. L. (1976). Biogeography of corals, seagrasses, and mangroves: an alternative to the center of origin concept. *Systematic Zoology*, *25*(3), 201-210.

McKee, K. L., Cahoon, D. R., & Feller, I. C. (2007). Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. *Global Ecology and Biogeography*, *16*(5), 545-556.

McLeod, E., & Salm, R. V. (2006). *Managing mangroves for resilience to climate change*. World Conservation Union (IUCN).

Melville, F., & Burchett, M. (2002). Genetic variation in *Avicennia marina* in three estuaries of Sydney (Australia) and implications for rehabilitation and management. *Marine Pollution Bulletin*, *44*(6), 469-479.

Menezes, L. F. T. D., & Peixoto, A. L. (2009). Leaf damage in a mangrove swamp at Sepetiba Bay, Rio de Janeiro, Brazil. *Brazilian Journal of Botany*, *32*, 715-724.

Minobe, S., Fukui, S., Saiki, R., Kajita, T., Changtragoon, S., Ab Shukor, N. A., Latiff, A., Ramesh, B.R., Koizumi, O., & Yamazaki, T. (2010). Highly differentiated population structure of a Mangrove species, *Bruguiera gymnorhiza* (Rhizophoraceae) revealed by one nuclear GapCp and one chloroplast intergenic spacer trnF–trnL. *Conservation Genetics*, *11*(1), 301-310.

Monga, E., Mangora, M. M., & Trettin, C. C. (2022). Impact of mangrove planting on forest biomass carbon and other structural attributes in the Rufiji Delta, Tanzania. *Global Ecology and Conservation*, *35*, e02100.

Morrisey, D. J., Swales, A., Dittmann, S., Morrison, M. A., Lovelock, C. E., & Beard, C. M. (2010). The ecology and management of temperate mangroves. *Oceanography and marine biology: an annual review*, *48*, 43-160.

Muir, J. (1933). The beach drift of South Africa. *Veld & Flora*, *18*(1), 5.

Mustajärvi, K., Siikamäki, P., Rytkönen, S., & Lammi, A. (2001). Consequences of plant population size and density for plant–pollinator interactions and plant performance. *Journal of Ecology*, *89*(1), 80-87.

Nadia, T. L., & Machado, I. C. (2014). Wind pollination and propagule formation in *Rhizophora mangle* L. (Rhizophoraceae): resource or pollination limitation?. *Anais da Academia Brasileira de Ciências*, *86*(1), 229-238.

Naidoo, G. (2016). The mangroves of South Africa: An ecophysiological review. *South African Journal of Botany*, *107*, 101-113.

Naidoo, G., Hiralal, O., & Naidoo, Y. (2011). Hypersalinity effects on leaf ultrastructure and physiology in the mangrove *Avicennia marina*. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *206*(9), 814-820.

Naidoo, G., Rogalla, H., & Von Willert, D. J. (1997). Gas exchange responses of a mangrove species, Avicennia marina, to waterlogged and drained conditions. In *Asia-Pacific Conference on Science and Management of Coastal Environment* (pp. 39-47). Springer, Dordrecht.

Nettel, A., & Dodd, R. S. (2007). Drifting propagules and receding swamps: genetic footprints of mangrove recolonization and dispersal along tropical coasts. *Evolution: International Journal of Organic Evolution*, *61*(4), 958-971.

Newbery, D. M. (1980). Infestation of the coccid, Icerya seychellarum (Westw.), on the mangrove *Avicennia marina* (Forsk.) Vierh. on Aldabra Atoll, with special reference to tree age. *Oecologia*, *45*(3), 325-330.

Newman, B. K., & Watling, R. J. (2007). Definition of baseline metal concentrations for assessing metal enrichment of sediment from the south-eastern Cape coastline of South Africa. *Water Sa*, *33*(5).

Ntibona, L. N., Shalli, M. S., & Mangora, M. M. (2022). Incentives and disincentives of mangrove conservation on local livelihoods in the Rufiji Delta, Tanzania. *Trees, Forests and People*, *10*, 100326.

Ng, W. L., Onishi, Y., Inomata, N., Teshima, K. M., Chan, H. T., Baba, S., Changtragoon, S., Siregar, I. Z., & Szmidt, A. E. (2015). Closely related and sympatric but not all the same: genetic variation of Indo-West Pacific *Rhizophora* mangroves across the Malay Peninsula. *Conservation Genetics*, *16*(1), 137-150.

Noor, T., Batool, N., Mazhar, R., & Ilyas, N. (2015). Effects of siltation, temperature and salinity on mangrove plants. *European Academic Research*, *2*(11), 14172-14179.

Ochieng, C. A., & Erftemeijer, P. L. (2002). Phenology, litterfall and nutrient resorption in Avicennia marina (Forssk.) Vierh in Gazi Bay, Kenya. *Trees,* 16(2-3), 167-171.

Osborne, D. J., & Berjak, P. (1997). The making of mangroves: the remarkable pioneering role played by seeds of Avicennia marina. *Endeavour*, *21*(4), 143-147.

Osorio, J. A., Crous, C. J., De Beer, Z. W., Wingfield, M. J., & Roux, J. (2017a). Endophytic Botryosphaeriaceae, including five new species, associated with mangrove trees in South Africa. *Fungal biology*, *121*(4), 361-393.

Osorio, J. A., Crous, C. J., Wingfield, M. J., De Beer, Z. W., & Roux, J. (2017). An assessment of mangrove diseases and pests in South Africa. *Forestry: An International Journal of Forest Research*, *90*(3), 343-358.

Osunkoya, O., & Creese, R. G. (1997). Population structure, spatial pattern and seedling establishment of the grey mangrove, Avicennia marina var. australasica, in New Zealand. *Australian journal of botany*, *45*(4), 707-725.

Otero, X. L., Ferreira, T. O., Vidal-Torrado, P., & Macías, F. (2006). Spatial variation in pore water geochemistry in a mangrove system (Pai Matos island, Cananeia-Brazil). *Applied Geochemistry*, *21*(12), 2171-2186.

Otero, X. L., Méndez, A., Nóbrega, G. N., Ferreira, T. O., Meléndez, W., & Macías, F. (2017). High heterogeneity in soil composition and quality in different mangrove forests of Venezuela. *Environmental monitoring and assessment*, *189*(10), 1-20.

Parani, M., Lakshmi, M., Senthilkumar, P., Ram, N., & Parida, A. (1998). Molecular phylogeny of mangroves V. Analysis of genome relationships in mangrove species using RAPD and RFLP markers. *Theoretical and Applied Genetics*, *97*(4), 617-625.

Pautasso, M. (2009). Geographical genetics and the conservation of forest trees. *Perspectives in Plant Ecology, Evolution and Systematics*, *11*(3), 157-189.

Pautasso, M., Dehnen-Schmutz, K., Holdenrieder, O., Pietravalle, S., Salama, N., Jeger, M. J., Lange, E., & Hehl-Lange, S. (2010). Plant health and global change–some implications for landscape management. *Biological Reviews*, *85*(4), 729-755.

Peer, N., Rajkaran, A., Miranda, N. A. F., Taylor, R. H., Newman, B., Porri, F., Raw, J.L, Mbense, S.P., Adams, J.B., & Perissinotto, R. (2018). Latitudinal gradients and poleward expansion of mangrove ecosystems in South Africa: 50 years after Macnae's first assessment. *African Journal of Marine Science*, *40*(2), 101-120.

Penha-Lopes, G., Bouillon, S., Mangion, P., Macia, A., & Paula, J. (2009). Population structure, density and food sources of Terebralia palustris (Potamididae: Gastropoda) in a low intertidal Avicennia marina mangrove stand (Inhaca Island, Mozambique). *Estuarine, Coastal and Shelf Science*, *84*(3), 318-325.

Picó, F. X., Rodrigo, A., & Retana, J. (2008). Plant demography. In *Encyclopedia of Ecology, Five-Volume Set* (pp. 2811-2817).

Pil, M. W., Boeger, M. R., Muschner, V. C., Pie, M. R., Ostrensky, A., & Boeger, W. A. (2011). Postglacial north–south expansion of populations of *Rhizophora mangle* (Rhizophoraceae) along the Brazilian coast revealed by microsatellite analysis. *American Journal of Botany*, *98*(6), 1031-1039.

Pinckney, J. L., Paerl, H. W., Tester, P., & Richardson, T. L. (2001). The role of nutrient loading and eutrophication in estuarine ecology. *Environmental health perspectives*, *109*(suppl 5), 699-706.

Pironon, S., Villellas, J., Morris, W. F., Doak, D. F., & García, M. B. (2015). Do geographic, climatic or historical ranges differentiate the performance of central versus peripheral populations?. *Global Ecology and Biogeography*, *24*(6), 611-620.

Plaziat, J. C., Cavagnetto, C., Koeniguer, J. C., & Baltzer, F. (2001). History and biogeography of the mangrove ecosystem, based on a critical reassessment of the paleontological record. *wetlands ecology and management*, *9*(3), 161-180.

Polidoro, B. A., Carpenter, K. E., Collins, L., Duke, N. C., Ellison, A. M., Ellison, J. C., Farnsworth, E.J., Fernando, E.S., Kathiresan, K., Koedam, N.E., Livingstone, S.R., Miyagi, T., Moore., G.E., Nam, V.N., Ong, J.E., Primavera, J.H., Salmo, S. G. Salmo, III, Sanciangco, J.C., Sukarjo, S., Wang, Y., & Yong, J. W. H. (2010). The loss of species: mangrove extinction risk and geographic areas of global concern. *PloS one*, *5*(4), e10095.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New phytologist*, *182*(3), 565-588.

Potts, W. M., Götz, A., & James, N. (2015). Review of the projected impacts of climate change on coastal fishes in southern Africa. *Reviews in fish biology and fisheries*, *25*(4), 603-630.

Prasad, M. B. K., & Ramanathan, A. L. (2008). Sedimentary nutrient dynamics in a tropical estuarine mangrove ecosystem. *Estuarine, Coastal and Shelf Science*, *80*(1), 60-66.

Proffitt, C. E., & Travis, S. E. (2010). Red mangrove seedling survival, growth, and reproduction: Effects of environment and maternal genotype. *Estuaries and Coasts*, *33*(4), 890-901.

Puglielli, G., Crescente, M. F., Frattaroli, A. R., & Gratani, L. (2015). Leaf mass per area (LMA) as a possible predictor of adaptive strategies in two species of Sesleria (Poaceae):

analysis of morphological, anatomical and physiological leaf traits. In *Annales Botanici Fennici* (Vol. 52, No. 1–2, pp. 135-144). Finnish Zoological and Botanical Publishing Board.

Quisthoudt, K., Schmitz, N., Randin, C. F., Dahdouh-Guebas, F., Robert, E. M., & Koedam, N. (2012). Temperature variation among mangrove latitudinal range limits worldwide. *Trees*, *26*(6), 1919-1931.

Rajkaran, A. & Adams, J. (2016). Mangroves of South Africa. In: Bosire J. O., Mangora M. M., Bandeira S., Rajkaran A., Ratsimbazafy R., Appadoo C. and Kairo J. G. (eds.). Mangroves of the Western Indian Ocean: Status and Management. *WIOMSA*, Zanzibar Town, 51-73.

Rajkaran, A., & Adams, J. (2011). Mangrove Forests of Northern KwaZulu-Natal: Sediment Conditions and Population Structure of the Largest Mangrove Forests in South Africa. *Western Indian Ocean Journal of Marine Science*, *10*(1), 25-38.

Rajkaran, A., & Adams, J. (2012). The effects of environmental variables on mortality and growth of mangroves at Mngazana Estuary, Eastern Cape, South Africa. *Wetlands ecology and management*, *20*(4), 297-312.

Rajkaran, A., & Adams, J. B. (2007). Mangrove litter production and organic carbon pools in the Mngazana Estuary, South Africa. *African Journal of Aquatic Science*, *32*(1), 17-25.

Rajkaran, A., & Adams, J. B. (2010). The implications of harvesting on the population structure and sediment characteristics of the mangroves at Mngazana Estuary, Eastern Cape, South Africa. *Wetlands ecology and management*, *18*(1), 79-89.

Rajkaran, A., Adams, J. B., & du Preez, D. R. (2004). A method for monitoring mangrove harvesting at the Mngazana estuary, South Africa. *African Journal of Aquatic Science*, *29*(1), 57-65.

Rajkaran, A., Adams, J., & Taylor, R. (2009). Current population structure of mangroves from Mlalazi to Mtamvuna estuaries in Kwa-Zulu Natal, South Africa. *Southern Forests*, *71*, 287-296.

Raju, A. J. S., Rao, P. V. S., Kumar, R., & Mohan, S. R. (2012). Pollination biology of the crypto-viviparous Avicennia species (Avicenniaceae). *Journal of Threatened Taxa*, *4*(15), 3377-3389

Raw, J. L., Godbold, J. A., Van Niekerk, L., & Adams, J. B. (2019). Drivers of mangrove distribution at the high-energy, wave-dominated, southern African range limit. *Estuarine, Coastal and Shelf Science*, *226*, 106296.

Reddering, JSV & Esterhuysen, K. (1987). The effects of river floods on sediment dispersal in small estuaries: a case study from East London. *South African Journal of Geology*, *90*(4), 458-470.

Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation biology*, *17*(1), 230-237.

Reef, R., Feller, I. C., & Lovelock, C. E. (2010). Nutrition of mangroves. *Tree Physiology*, *30*(9), 1148-1160.

Resh, V. H., & Cardé, R. T. (Eds.). (2009). *Encyclopedia of insects*. Access Online via Elsevier.

Riascos, J. M., Cantera, J. R., & Blanco-Libreros, J. F. (2018). Growth and mortality of mangrove seedlings in the wettest Neotropical mangrove forests during ENSO: implications for vulnerability to climate change. *Aquatic botany*, *147*, 34-42.

Ricklefs, R. E., & Latham, R. E. (1993). Global patterns of diversity in mangrove floras. *Species diversity in ecological communities: historical and geographical perspectives. University of Chicago Press, Chicago*, 215-229.

Rivera-Monroy, V. H., Danielson, T. M., Castañeda-Moya, E., Marx, B. D., Travieso, R., Zhao, X., Gaiser, E. E. & Farfan, L. M. (2019). Long-term demography and stem productivity of Everglades mangrove forests (Florida, USA): Resistance to hurricane disturbance. *Forest Ecology and Management*, *440*, 79-91.

Rovai, A. S., Twilley, R. R., Castañeda-Moya, E., Riul, P., Cifuentes-Jara, M., Manrow-Villalobos, M., Horta, P. A., Simonassi, J. C., Fonseca, A. L., P & Pagliosa, P. R. (2018). Global controls on carbon storage in mangrove soils. *Nature Climate Change*, *8*(6), 534-538.Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular biology and evolution*, *34*(12), 3299-3302.

Ruwa, R. K. (1993). Zonation and distribution of creek and fringe mangroves in the semi-arid Kenyan coast. In *Towards the rational use of high salinity tolerant plants* (pp. 97-105). Springer, Dordrecht.

Saenger, P. (1998). Mangrove vegetation: an evolutionary perspective. *Marine and Freshwater Research*, *49*(4), 277-286.

Saenger, P. (2002). *Mangrove ecology, silviculture and conservation*. Springer Science & Business Media.

Saenger, P., & Brooks, L. (2008). Phenotypic leaf variation in Avicennia marina in tropical Australia: can discrete subpopulations be recognised in the field?. *Australian Journal of Botany*, *56*(6), 487-492.

Saenger, P., & West, P. W. (2016). Determinants of some leaf characteristics of Australian mangroves. *Botanical journal of the Linnean Society*, *180*(4), 530-541.

Saenger, P., & West, P. W. (2018). Phenotypic variation of the mangrove species *Avicennia marina* (Forssk.) Vierh. from seven provenances around Australia. *Aquatic botany*, *149*, 28-32.

Saintilan, N., Wilson, N. C., Rogers, K., Rajkaran, A., & Krauss, K. W. (2014). Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global change biology*, *20*(1), 147-157.

Salmo, S. G., Lovelock, C., & Duke, N. C. (2013). Vegetation and soil characteristics as indicators of restoration trajectories in restored mangroves. *Hydrobiologia*, *720*(1), 1-18.

Sánchez-Andrés, R., Sánchez-Carrillo, S., Alatorre, L. C., Cirujano, S., & Álvarez-Cobelas, M. (2010). Litterfall dynamics and nutrient decomposition of arid mangroves in the Gulf of California: Their role sustaining ecosystem heterotrophy. *Estuarine, Coastal and Shelf Science*, *89*(3), 191-199.

Sandilyan, S., & Kathiresan, K. (2012). Mangrove conservation: a global perspective. *Biodiversity and Conservation, 21*(14), 3523-3542.

Sankararamasubramanian, H. M., Selvam, V., & Parida, A. (2012). Biotechnological tools for the conservation and enhancement of coastal ecosystem. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 82(2), 357-362.

Saravanakumar, A., Rajkumar, M., Serebiah, J. S., & Thivakaran, G. A. (2008). Seasonal variations in physico-chemical characteristics of water, sediment and soil texture in arid zone mangroves of Kachchh-Gujarat. *J. Environ. Biol*, *29*(5), 725-732.

Sarkar, D., Sarkar, M. D., & KernSmooth, S. (2015). Package 'lattice'. *Version 0.20*, *33*.

Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. (2012), "NIH Image to ImageJ: 25 years of image analysis". *Nature methods*, 9(7), 671-675.

Schwarzbach, A. E., & McDade, L. A. (2002). Phylogenetic relationships of the mangrove family Avicenniaceae based on chloroplast and nuclear ribosomal DNA sequences. *Systematic Botany*, *27*(1), 84-98.

Shapiro, A. C., Trettin, C. C., Küchly, H., Alavinapanah, S., & Bandeira, S. (2015). The mangroves of the Zambezi Delta: Increase in extent observed via satellite from 1994 to 2013. *Remote Sensing*, *7*(12), 16504-16518.

Sharma, R. M., Joshi, P. V., & Shindikar, M. (2003). First report on plant galls (zoocecidia) from mangrove swamps of Vikhroli, Maharashtra. *Zoos' Print Journal*, *18*(10), 1217-1219.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E., & Small, R. L. (2005). The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American journal of botany*, *92*(1), 142-166.

Shaw, J., Shafer, H. L., Leonard, O. R., Kovach, M. J., Schorr, M., & Morris, A. B. (2014). Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. *American Journal of Botany*, *101*(11), 1987-2004.

Shiau, Y. J., Lee, S. C., Chen, T. H., Tian, G., & Chiu, C. Y. (2017). Water salinity effects on growth and nitrogen assimilation rate of mangrove (*Kandelia candel*) seedlings. *Aquatic Botany*, *137*, 50-55.

Sievert, C., Parmer, C., Hocking, T., Chamberlain, S., Ram, K., Corvellec, M., ... & Sievert, M. C. (2017). Package 'plotly'.

Singh, V. P., & Odaki, K. (2004). *Mangrove ecosystem: structure and function*. Scientific Publishers.

Sousa, W. P., & Dangremond, E. M. (2011). Trophic interactions in coastal and estuarine mangrove forest ecosystems.

Sousa, W. P., Kennedy, P. G., & Mitchell, B. J. (2003). Propagule size and predispersal damage by insects affect establishment and early growth of mangrove seedlings. *Oecologia*, *135*(4), 564-575.

Spalding, M. (2010). *World atlas of mangroves*. Routledge.

Spalding, M., & Leal, M. (2021). The state of the world's mangroves 2021. Global Mangrove Alliance.

Steinke, T. D. (1999). Mangroves in South African estuaries. *Estuaries of South Africa*, 119-140.

Steinke, T. D., & Ward, C. J. (1990). Litter production by mangroves. III. Wavecrest (Transkei) with predictions for other Transkei estuaries. *South African Journal of Botany*, *56*(5), 514-519.

Steinke, T. D., & Ward, C. J. (2003). Use of plastic drift cards as indicators of possible dispersal of propagules of the mangrove Avicennia marina by ocean currents. *African Journal of Marine Science*, *25*, 169-176.

Stevens, P. W., Fox, S. L., & Montague, C. L. (2006). The interplay between mangroves and saltmarshes at the transition between temperate and subtropical climate in Florida. *Wetlands Ecology and management*, *14*(5), 435-444.

Strydom, N. A. (2015). Patterns in larval fish diversity, abundance, and distribution in temperate South African estuaries. *Estuaries and coasts*, *38*(1), 268-284.

Suárez, N., & Medina, E. (2005). Salinity effect on plant growth and leaf demography of the mangrove, Avicennia germinans L. *Trees*, *19*(6), 722.

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant molecular biology*, *17*(5), 1105-1109.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*(3), 585-595.

Takayama, K., Tateishi, Y., & Kajita, T. (2021). Global phylogeography of a pantropical mangrove genus *Rhizophora*. *Scientific reports*, *11*(1), 1-13.

Takeuchi, T., Sugaya, T., Kanazashi, A., Yoshimaru, H., & Katsuta, M. (2001). Genetic diversity of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Southwest Islands, Japan. *Journal of Forest Research*, *6*(3), 157-162.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, *30*(12), 2725-2729.

Taylor, M., Ravilious, C. and Green, E.P. (2003). Mangroves of East Africa V4. 0. *UNEP World Conservation Monitoring Centre (UNEP-WCMC). URL: http://data. unep-wcmc. org/datasets/7*.

Taylor, R., Adams, J. B., & Haldorsen, S. (2006). Primary habitats of the St Lucia Estuarine System, South Africa, and their responses to mouth management. *African Journal of Aquatic Science*, *31*(1), 31-41.

Tian, C. H. E. N., Manfei, X. U., Justin, T. U., Hongyue, W. A. N. G., & Xiaohui, N. I. U. (2018). Relationship between Omnibus and Post-hoc Tests: An Investigation of performance of the F test in ANOVA. *Shanghai archives of psychiatry*, *30*(1), 60.

Tomizawa, Y., Tsuda, Y., Saleh, M.N., Wee, A.K., Takayama, K., Yamamoto, T., Yllano, O.B., Salmo III, S.G., Sungkaew, S., Adjie, B. and Ardli, E. (2017). Genetic Structure and Population Demographic History of a Widespread Mangrove Plant Xylocarpus granatum (Meliaceae) across the Indo-West Pacific Region. *Forests*, *8*(12), p.480.

Tomlinson, P. B. (1986). The botany of mangroves. Cambridge University Press.

Tonné, N., Beeckman, H., Robert, E. M., & Koedam, N. (2017). Towards an unknown fate: The floating behaviour of recently abscised propagules from wide ranging Rhizophoraceae mangrove species. *Aquatic botany*, *140*, 23-33.

Tooker, J. F., & De Moraes, C. M. (2008). Gall insects and indirect plant defenses: A case of active manipulation?. *Plant signaling & behavior*, *3*(7), 503-504.

Triest, L. (2008). Molecular ecology and biogeography of mangrove trees towards conceptual insights on gene flow and barriers: A review. *Aquatic Botany*, *89*(2), 138-154.

Triest, L., Van der Stocken, T., De Ryck, D., Kochzius, M., Lorent, S., Ngeve, M., Ratsimbazafy, H. A., Sierens, T., van der Ven, R. & Koedam, N. (2021). Expansion of the

mangrove species *Rhizophora mucronata* in the Western Indian Ocean launched contrasting genetic patterns. *Scientific reports*, *11*(1), 1-16.

Tuffers, A., Naidoo, G., & Von Willert, D. J. (2001). Low salinities adversely affect photosynthetic performance of the mangrove, Avicennia marina. *Wetlands Ecology and Management*, *9*(3), 235-242.

Twilley, R. R., & Day, J. W. (1999). The productivity and nutrient cycling of mangrove ecosystem. *Ecosistemas de manglar en América Tropical. Instituto de Ecología, AC México, UICN/ORMA, Costa Rica, NOAA/NMFS, Silver Spring MD, EUA. p*, 127-151.

Tyagi, A. P. (2003). Location and interseasonal variation in flowering, propagule setting and propagule size in mangroves species of the family Rhizophoraceae. *Wetlands Ecology and Management*, *11*(3), 167-174.

University of Pretoria Stable Isotope Laboratory, 2019.

Urashi, C., Teshima, K. M., Minobe, S., Koizumi, O., & Inomata, N. (2013). Inferences of evolutionary history of a widely distributed mangrove species, *Bruguiera gymnorrhiza*, in the Indo-West Pacific region. *Ecology and evolution*, *3*(7), 2251-2261.

Van der Stocken, T., & Menemenlis, D. (2017). Modelling mangrove propagule dispersal trajectories using high-resolution estimates of ocean surface winds and currents. *Biotropica*, *49*(4), 472-481.

Van der Stocken, T., De Ryck, D. J., Vanschoenwinkel, B., Deboelpaep, E., Bouma, T. J., Dahdouh-Guebas, F., & Koedam, N. (2015b). Impact of landscape structure on propagule dispersal in mangrove forests. *Marine Ecology Progress Series*, *524*, 95-106.

Van der Stocken, T., Vanschoenwinkel, B., De Ryck, D. J., Bouma, T. J., Dahdouh-Guebas, F., & Koedam, N. (2015a). Interaction between water and wind as a driver of passive dispersal in mangroves. *PLoS One*, *10*(3), e0121593.

Van Loon, A. F., Te Brake, B., Van Huijgevoort, M. H., & Dijksma, R. (2016). Hydrological classification, a practical tool for mangrove restoration. *PloS one*, *11*(3), e0150302.

van Niekerk, L. (2019). NBA 2018 Ecosystem Classification for South African estuaries and micro-systems.

Walters B.B., Rönnbäck P., Kovacs J.M, Crona B. , Hussain S.A , Badola R., Primavera J.H, , Barbier E.,Dahdouh-Guebas F. (2008) Ethnobiology, socio-economics and management of mangrove forests: A review. *Aquatic Botany*, 89[2], 220-236pp

Wang, L., Mu, M., Li, X., Lin, P., & Wang, W. (2010). Differentiation between true mangroves and mangrove associates based on leaf traits and salt contents. *Journal of Plant Ecology*, *4*(4), 292-301.

Wang, W., You, S., Wang, Y., Huang, L., & Wang, M. (2011). Influence of frost on nutrient resorption during leaf senescence in a mangrove at its latitudinal limit of distribution. *Plant and Soil*, *342*(1), 105-115.

Wang, Y., Bonynge, G., Nugranad, J., Traber, M., Ngusaru, A., Tobey, J.,Bowen, R., & Makota, V. (2003). Remote sensing of mangrove change along the Tanzania coast. *Marine Geodesy*, *26*(1-2), 35-48.

Ward, C. J., & Steinke, T. D. (1982). A note on the distribution and approximate areas of mangroves in South Africa. *South African Journal of Botany*, *1*(3), 51-53.

Wee, A. K., Takayama, K., Asakawa, T., Thompson, B., Onrizal, Sungkaew, S., Tung, N. X., Nazre, M., Soe, K. K., Tan, H.T.W., Watano, Y., Baba, S., Kajita, T., & Webb, E. L. (2014). Oceanic currents, not land masses, maintain the genetic structure of the mangrove *Rhizophora mucronata* Lam. (Rhizophoraceae) in Southeast Asia. *Journal of biogeography*, *41*(5), 954-964.

Wee, A. K., Takayama, K., Chua, J. L., Asakawa, T., Meenakshisundaram, S. H., Adjie, B., Ardli, E. R., Sungkaew, S., Malekal, N. B., Tung, N.X., Salmo III, S. G., Yllano, O. B., Saleh, M.N., Soe, K. K., Tateishi, Y., Watano, Y., Baba, S., Webb, E. L., & Kajita, T. (2015). Genetic differentiation and phylogeography of partially sympatric species complex *Rhizophora mucronata* Lam. and *R. stylosa* Griff. using SSR markers. *BMC evolutionary biology*, *15*(1),1.

Wee, A. K., Teo, J. X. H., Chua, J. L., Takayama, K., Asakawa, T., Meenakshisundaram, S. H., Onrizal, Adjie, B., Ardli, E.R., Sungkaew, S., Suleiman, M., Tung, N. X., Salmo III, S. G., Yllano, O. B., Sasleh, M.N., Soe, K. K., Tateishi, Y., Watano, Y., Tsuda, Y., Kajita, T., & Webb, E. L. (2017). Vicariance and oceanic barriers drive contemporary genetic structure of widespread mangrove species Sonneratia alba J. Sm in the Indo-West Pacific. *Forests*, *8*(12), 483.

Werle, E., Schneider, C., Renner, M., Völker, M., & Fiehn, W. (1994). Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucleic acids research*, *22*(20), 4354.

Whitfield, A. K. (1992). A characterization of southern African estuarine systems. *Southern African Journal of Aquatic Science*, *18*(1-2), 89-103.

Whitfield, A. K., & Baliwe, N. G. (2013). *A century of science in South African estuaries: Bibliography and review of research trends* (p. 289). South African Institute for Aquatic Biodiversity (SAIAB).

Whitfield, A. K., James, N. C., Lamberth, S. J., Adams, J. B., Perissinotto, R., Rajkaran, A., & Bornman, T. G. (2016). The role of pioneers as indicators of biogeographic range expansion caused by global change in southern African coastal waters. *Estuarine, Coastal and Shelf Science*, *172*, 138-153.

Wickham, H. (2011). ggplot2. *Wiley interdisciplinary reviews: computational statistics*, *3*(2), 180-185.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., & Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1686.

Wickham, H., & Ruiz, E. (2018). dbplyr: A'dplyr'Back End for Databases. *R package version*, *1*(1).

Wise, C. A., Ranker, T. A., & Linhart, Y. B. (2002). Modeling problems in conservation genetics with Brassica rapa: Genetic variation and fitness in plants under mild, stable conditions. *Conservation biology*, *16*(6), 1542-1554.

Wise, C. A., Ranker, T. A., & Linhart, Y. B. (2002). Modeling problems in conservation genetics with Brassica rapa: Genetic variation and fitness in plants under mild, stable conditions. *Conservation biology*, *16*(6), 1542-1554.

Wright, S. (1943). Isolation by distance. *Genetics*, *28*(2), 114.

Xiong, Y., Cakir, R., Phan, S. M., Ola, A., Krauss, K. W., & Lovelock, C. E. (2019). Global patterns of tree stem growth and stand aboveground wood production in mangrove forests. *Forest Ecology and Management*, *444*, 382-392.

Xu, S., He, Z., Zhang, Z., Guo, Z., Guo, W., Lyu, H., Li, J., Yang, M., Du, Z., Haung, Y., Zhou, R., Zhong, C., Boufford, D. E., Lerdau, M., Wu, C., Duke, N. C. & Shi, S. (2017). The origin, diversification and adaptation of a major mangrove clade (Rhizophoreae) revealed by whole-genome sequencing. *National Science Review*, *4*(5), 721-734.

Yan, Y. B., Duke, N. C., & Sun, M. (2016). Comparative analysis of the pattern of population genetic diversity in three Indo-West Pacific *Rhizophora* mangrove species. *Frontiers in plant science*, *7*, 1434

Yan, Z., Wang, W., & Tang, D. (2007). Effect of different time of salt stress on growth and some physiological processes of Avicennia marina seedlings. *Marine Biology*, *152*(3), 581.

Yang, S.C., Riddin, T., Adams, J.B. and Shih, S.S. (2014). Predicting the spatial distribution of mangroves in a South African estuary in response to sea level rise, substrate elevation change and a sea storm event. *Journal of coastal conservation*, 18(4), pp.459-469.

Yin, D., & Wang, L. (2019). Individual mangrove tree measurement using UAV-based LiDAR data: Possibilities and challenges. *Remote sensing of environment*, *223*, 34-49.