The phylogeography, biomass allocation and phenology of *Salicornia tegetaria* (S. Steffen, Mucina & G. Kadereit) Piirainen & G. Kadereit, an endemic salt marsh species in South Africa

by

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

MAGISTER SCIENTIAE

in the

Department of Biodiversity and Conservation Biology

at the

UNIVERSITY OF THE WESTERN CAPE

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AUGUST 2018

Declaration

I declare that *The phylogeography, biomass allocation and phenology of* Salicornia tegetaria *(S. Steffen, Mucina & G. Kadereit) Piirainen & G. Kadereit, an endemic salt marsh species in South Africa* is my own work, that it has not been submitted 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 by complete references.

Catherine Eileen Brown

09 August 2018

C. Brown

Signature

Abstract

Salicornia tegetaria is an endemic salt marsh macrophyte that is widely distributed in estuaries along the South African coast. The aims of the study were to understand the phylogeography of the species, compare the biomass allocation in two regions and to determine phenological patterns of *S. tegetaria* between the warm and cool temperate biogeographical regions. The phylogeography of *S. tegetaria* was studied using the non-coding chloroplast DNA region *rpS16* and nuclear rDNA *ITS* region. Five samples each were collected from eighteen estuaries stretching from Orange River in the Northern Cape to Mngazana Estuary in the Eastern Cape. Above- and belowground biomass was collected and physico-chemical conditions measured at Olifants, Berg and Langebaan Estuaries in the cool temperate, and Heuningnes, Nahoon and Kwelera Estuaries in the warm temperate biogeographical regions. The growth and flowering phenology of *S. tegetaria* in relation to environmental conditions was investigated in the cool temperate Langebaan Estuarine Embayment and compared to findings in the warm temperate, permanently open Kowie Estuary. The physico-chemical gradient found between the cool and warm temperate biogeographical regions may be useful to study climate change effects on plant species. The comparison of similar habitats in each region may provide insight into how different climate regimes may affect biomass allocation and phenology.

Eleven haplotypes were recovered from *ITS* DNA sequences with one haplotype found exclusively between Olifants and Breede Estuaries and a dominant haplotype found at Berg Estuary and between Goukou and Mngazana. Eight haplotypes were recovered from *rpS16* DNA sequences, one of which was dominant, comprising 76 % of the sequences. The remaining haplotypes from *ITS* and *rpS16* sequences were single rare haplotypes predominantly in the warm temperate region. A high amount of gene flow and an increase in genetic divergence from west to east was found illustrating hydrochory. *Salicornia tegetaria* had an average aboveground biomass of 1.31 ± 0.06 kg.m^{-2} and belowground biomass of 3.66 ± 0.21 kg.m^{-2} in the six estuaries sampled with a root/shoot ratio of 3.77 ± 0.32 in the cool temperate and 3.03 ± 0.41 in the warm temperate region. Sediment moisture, organic content and electrical conductivity was significantly higher in the cool temperate region whereas pH and porewater temperature was significantly higher in the warm temperate region. The belowground biomass and root/shoot ratio of *S. tegetaria* was the lowest at Heuningnes Estuary in the warm temperate region, whereas belowground biomass was similar at the remaining estuaries sampled. The root/shoot ratio was correlated to sediment pH which was strongly correlated to biogeographical region and salinity. In the cool temperate Langebaan Estuarine Embayment salt marsh vegetation was dense and increased significantly during the winter rainfall period. *Salicornia tegetaria* had a peak flowering time from November to January 2017, with a maximum of 77.24 ± 28.16 inflorescences per m^2, and peak seeding time from February to March 2017, with a maximum of 2 952.51 ± 599.84 seeds per m^2. Flowering and seeding times occurred later in the year in the warm temperate region with similar reproductive output.

The phylogeography of *S. tegetaria* shows that there is connectivity between estuaries that allows for the exchange of genetic material and that more haplotype diversity exists than previously thought. There are differences in the genetic diversity and phenology of *S. tegetaria* along the warm and cool temperate biogeographical gradient. The higher root/shoot ratio in the cool temperate estuaries indicate that the environmental conditions are more stressful and that sediment pH and salinity are determining factors in biomass allocation. The longer and earlier flowering times in the cool temperate Langebaan Estuarine Embayment also indicate more stressful environmental conditions. This study provides baseline information on a salt marsh species for which there is a paucity of data that is widely distributed in an ecosystem that will be impacted by climate change. Considering genetic diversity is important in conservation management. Estuaries should be protected to conserve the maximum amount of haplotypes to ensure the resilience of these ecosystems.

Keywords

rpS16, ITS, chloroplast DNA, nuclear DNA, halophyte, glasswort, *Sarcocornia*, salinity, pH, sea-level, climate change

Acknowledgements

I would like to thank my supervisor Dr. Anusha Rajkaran for just being awesome, for her guidance, advice and making this project possible. I am grateful for the opportunities provided to take part at the South African Marine Science Symposium, the Nelson Mandela University estuarine management workshop, the Diversity of the Indo-Pacific Network molecular tools workshop and the South African Association of Botanists Conference.

Also thank you to Dr. Adriaan Engelbrecht and Dr. Stephen Boatwright for technical advice and the use of laboratory equipment and to Dr. Dimitri Veldkornet for providing the basis of this study and mentorship.

I gratefully acknowledge data provided by Nelson Mandela University and the South African Weather Service.

Thanks to all my field assistants, Jabu Malindi, Luther Adams and Eric Van Onselen, who made it feel like more like fun than work.

Thank you to Cape Nature, the DAFF and SANPARKS for granting sampling permits and to Pierre Nel and Debbie Winterton at SANPARKS for their assistance.

Thank you to Lorne Gelderbloem for support in organising field trips and the University of the Western Cape for the opportunity to study and for use of a research vehicle. Also thank you Linda van Heerden for help with endless admin and general morale.

Funding for this project is gratefully acknowledged from the South African National Research Foundation (Thuthuka) and the Early Career Fellowship provided by the University of the Western Cape. Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard.

To my family and friends for cheering me on and up and my partner for immeasurable help and support, keeping me fed and hydrated and helping me wash muddy roots till late at night.

A special thanks to my mom and dad for everything.

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Chapter 1. General introduction

South Africa has more than 300 estuaries unequally distributed along 3 400 km of coastline. Approximately 11 400 ha of intertidal and supratidal salt marsh exists in South African estuaries with Langebaan containing the largest portion of salt marsh habitat (approximately 32 %) and the highest diversity of species (O'Callaghan, 1994; Van Niekerk and Turpie, 2012). The salt marsh community consists of xerohalophytes dispersed along an elevation gradient according to the species tolerance to drought and salinity (Adam, 1993). Grasses such as *Spartina* (L.) Roth species and *Sporobulus virginicus* (L.) Kunth exclude salt uptake through the roots and can withstand prolonged submergence (Mucina *et al.*, 2006). Succulence is a widespread strategy of salt marsh species to reduce salt content in tissues (Mucina *et al*., 2006). Low growing succulents such as *Salicornia* L. species, *Bassia diffusa* (Thunb.) Kuntze and *Triglochin* L. species form a mosaic of colour in the lower and middle intertidal zone and prostrate succulents such as *Disphyma crassifolium* (L.) L.Bolus and *Salicornia pillansii* (Moss) Piirainen & Kadereit are found in the supratidal zone (Adams *et al*., 2016).

Globally salt marshes offer coastal protection from storms and erosion as the vegetation reduces the velocity, height and duration of waves during surges by slowing down water movement, stabilising sediment and increasing the intertidal height (Stumpf, 1983; King and Lester, 1995; Massel *et al*., 1999; Fagherazzi *et al*., 2013). Salt marshes sequester millions of tons of carbon per year (Connor and Chmura, 2000; Chmura *et al*., 2003; Barbier *et al*., 2011; Chmura, 2013) as the salt marsh sediments can store large amounts of carbon, have a high rate of belowground production (Chmura, 2013) and anoxic sediments often have a slower rate of decay (Ponnamperuma,1972). Salt marshes are also important to fisheries as they provide a protective habitat for nekton such as shrimp and shellfish (Boesch and Turner, 1984; Bell, 1997; Meynecke *et al*., 2008). In South Africa the value of estuarine habitats for important fish and invertebrate nurseries have been estimated at R1 – 5 million per year with a recreational value of R10 – 20 million per year (Van Niekerk and Turpie, 2012). The total fishery and nursery values of estuarine and estuarine-dependent fisheries was estimated at R1.251 billion in 2002 (Lamberth and Turpie, 2003). These habitats also provide feeding and breeding areas for many important bird species. Six estuaries are currently listed as Ramsar sites and twenty are classified as Important Bird Areas (Van Niekerk and Turpie, 2012).

Estimates of the above- and belowground biomass allocation of salt marsh vegetation could provide insight into the current state of salt marshes (Turner *et al*., 2004; Turner, 2011) and aid the management of these ecosystems as well as improve estimates of their ecosystem services with regard to carbon sequestration (Costanza *et al*., 2014). Salt marshes adapt to sea level rise by trapping sediment and biologically by increasing belowground biomass (Larsen and Harvey, 2010; Marani *et al*., 2013; Bornman *et al*., 2016). During tidal flooding the aboveground shoots of wetland plants slow down water velocities, mineral sediment settles out and organic matter is added to the soil surface (Reed, 1989; Morgan *et al*., 2009). Lower intertidal marshes are inundated for longer, but also experience stronger water turbulence (Reed, 1989; Olff *et al*., 1997). Belowground, roots grow and decay, adding organic matter below the surface creating a feedback loop in which vegetation stabilises their relative elevation (Temmerman *et al*., 2003; McKee *et al*., 2007; Marion *et al*., 2009; Fagherazzi *et al*., 2012). It has been observed that belowground biomass comprise a large portion of the salt marsh primary productivity, contributing to the detritus of food webs in coastal waters (Boesch and Turner, 1984). Changing temperatures will cause a shift in plant phenology which also affects above- and belowground growth allocation and thus accretion and dispersal (Crosby *et al*., 2015). Comparing the phenology of a plant species between the warm temperate and cool temperate biogeographical regions may also provide insights to the shifts that could occur with changing climatic conditions.

Major shifts in climate are expected over the next century (Stocker *et al*., 2013). This requires adaptive and mitigation strategies in which the studies of vulnerable systems will identify their climate-related weaknesses (Tol, 2007; Wigand *et al*., 2015). Estuaries will be affected by hydrological changes, sea level rise, increased temperatures and increased storm frequency and intensity with ecogeomorphic consequences (Van Niekerk and Turpie, 2012). In South Africa the subtropical and cool temperate estuaries will be most impacted both structurally and functionally whereas warm temperate estuaries will be more vulnerable to temperature shifts that determine species ranges (Van Niekerk and Turpie, 2012). Globally wetlands have become more valuable as carbon and methane sinks that need to be conserved (Bartlett *et al*., 1987; Chmura *et al*., 2003; Adams *et al*., 2012; Ouyang and Lee, 2014). Increased inundation due to sea level rise could lead to the loss of salt marshes (FitzGerald *et al*., 2008) unless salt marshes keep pace by increasing their elevation by sediment deposition and belowground growth or by landward migration. Salt marshes have adapted to sea-level fluctuations for thousands of years (Redfield, 1972; McKee *et al*., 2007; Kirwan and Megonigal, 2013; Kirwan *et al*., 2016) but may be impeded by topography and human development adjacent to salt marshes in South Africa (Chmura, 2013; Bornman *et al*., 2016). The rate of eustatic sea-level rise on the South African coast is 0.24 mm.y^{-1} along the west coast, 3.55 mm.y^{-1} along the east coast and 1.57 mm.y^{-1} along the southern coast (Mather *et al*., 2009). At present, studies in the northern Atlantic indicate that salt marsh accretion is not keeping pace with the current rate of sea level rise at a local level and negative accretion rates have already been measured for low marshes occurring where the tipping point has already been reached (Crosby *et al*., 2016). A short-term study at the Swartkops Estuary in the Eastern Cape showed that salt marshes in South Africa may be able to increase in elevation at a rate that keeps pace with sea level rise (Bornman *et al*., 2016).

An important conservation objective is ensuring resilience in a system by conserving genetic variation (Gunderson, 2000; Hughes and Stachowicz, 2004). Genetic variation is required to respond to evolutionary processes occurring over a short time period as can be expected with local and global environmental changes. If conditions change, species or populations with higher genetic diversity are more likely to persist (Gunderson, 2000;

Hughes and Stachowicz, 2004). To maximise the resilience of ecosystems, conservation should plan to retain the maximum amount of different alleles, learn as much as possible about the relationship between the ecosystem services and their genetic diversity and include this in management plans that consider the whole seascape (Granek *et al*., 2010; Beger *et al*., 2014; von der Heyden *et al*., 2014). Phylogeography may be able to discriminate between historic and recurrent gene flow events that have influenced the genetic structure of species by interpreting the genealogical relationships between haplotypes and their patterns of geographic distribution (Schaal *et al*., 1998). Understanding the genetic structure and haplotype diversity of estuarine species may aid in quantifying the genetic importance and resilience of salt marsh macrophytes, identifying important areas for protection and may provide insights into their origin, spread and the level of connectivity between estuaries (Friess *et al*., 2012; Van Niekerk and Turpie, 2012).

Salicornia tegetaria is a salt marsh species that is endemic to southern Africa, widely distributed in the lower to middle intertidal zone of estuaries along the coast. The global distribution of this species stretches from Namibia to Mozambique, from the cool temperate west coast, the warm temperate southern and east coast, and subtropical and tropical east coast of southern Africa (Steffen *et al*., 2010). It is a succulent halophyte characterised by mat-forming growth that generally forms monospecific stands (Kadereit *et al.*, 2006). The major lineages of Salicornioideae, a subfamily of Chenopodiaceae, were present by the Middle Miocene. These succulent hygrohalophytes with articulated stems and reduced leaves evolved in coastal habitats and were adapted to colonise inland saline environments as the climate cooled and dried after the Eocene, making it the oldest lineage of hygrohalophytes in the angiosperms presently known (Kadereit *et al*., 2012). It is found in Eurasia and North, West and East Africa (ca. 35 spp.), where it most likely originated in Asia and the Mediterranean Basin, North America and Hawaii (9 spp.), Central and South America (8 spp.), South Africa (ca. 20 spp.) and Australia (ca. 47 spp.). Ancestors of the *Salicornia/Sarcocornia* clade diverged during the early Miocene and the clade dispersed from Eurasia to Africa in the middle-late Miocene (Steffen *et al*., 2015; Piirainen *et al*., 2017). The estimated time the ancestor of *Salicornia tegetaria* arrived in South Africa was around 13.8-5.5 Mya (Piirainen *et al*., 2017). High phenotypic plasticity due to the variability of the environment, polyploidy and frequent hybridisation cause difficulties in determining the phylogeny of *Salicornia* species (Kadereit *et al*., 2006) which has been an ongoing process from 1840 (Moquin, 1840) until present (Piirainen *et al.,* 2017*)* with two recent name changes of the species. Taxonomic treatments of *Salicornia tegetaria* include O'Callaghan (1992) Steffen *et al.* (2009), (2010), Steffen *et al.,* (2015) and Piirainen *et al.* (2017).

The first contemporary study of *S. tegetaria* in South Africa was done in 1994 which investigated the species tolerance range to salinity and inundation (Adams and Bate, 1994). During this time *S. tegetaria* in South Africa was considered to be the same species as *Sarcocornia perennis* (Miller) A.J. Scott found in Europe. Davy *et al.* (2006) published a review of the ecology, biology, phenology, community characteristics and environmental conditions of *S. perennis* in the British Isles, which included the South African then named *Sarcocornia perennis*. *Salicornia tegetaria* has also been included in broader studies of salt marsh vegetation in South Africa in both published studies (Adams *et al.*, 2016; Geldenhuys *et al.*, 2016) and dissertations (Vromans, 2011; Veldkornet, 2012; 2015; Geldenhuys, 2014) relating to zonation, physico-chemical variables, morphology and phenology.

Overall aim

The overall aim of this study is to compare the genetic structure and functioning of *Salicornia tegetaria* between two biogeographical regions. I test whether there are differences in genetic structure, biomass allocation and phenology of *S. tegetaria* between the cool temperate and warm temperate regions at the landscape, regional and local scale. At the landscape scale I assess whether the populations of this species display genetic structure and diversity between 18 estuaries, four in the cool temperate, 13 in the warm temperate and one in the subtropical biogeographical region. At the regional scale I test if the allocation of resources of *S. tegetaria* are affected by the different climatic regimes found in the two biogeographical regions or by local physico-chemical variables that can be affected by climate change. Then finally, the timing of reproductive phases between the two biogeographical regions are compared.

Objectives

Figure 1 illustrates the objectives of this study. Objective 1 is to determine if the populations of *Salicornia tegetaria* show distinct genetic structure and if the structure reflects biogeographical disjuncts and answers the following questions:

1. Are the populations of *S. tegetaria* equally connected and were they so in the past?
2. Is there an association between the haplotypes found in *S. tegetaria* and the geographic locations at which they were sampled?
3. Is there genetic structure between populations of *S. tegetaria* either due to disjunct or historical processes?
4. If genetic structure between populations of *S. tegetaria* is found, is there a pattern, gradient, differentiated patches or is it continuous in space suggesting isolation by distance?
5. Where is the area of highest genetic differentiation, denoting restricted gene flow?
6. Can these patterns be related to geographic selection and demographic processes?

The second objective is to determine if there is a difference in the biomass and biomass allocation of *S. tegetaria* between the two temperate biogeographical regions. Here it is assumed that differences in rainfall and temperature between the warm and cool temperate biogeographical regions may affect the functioning of this estuarine macrophyte.

1. Are there differences in the physico-chemical conditions between the warm temperate and cool temperate biogeographical regions and estuaries?
2. What are the optimum growth conditions of *S. tegetaria*? Does temperature, precipitation, salinity, pH, sediment moisture, organic content, redox potential or

porewater depth affect the above- or belowground biomass of *S. tegetaria* between biogeographical regions and estuaries differently?

3. Are there differences in the re-allocation of resources between biogeographical regions and estuaries?
4. If so, do climatic variations between the warm temperate and cool temperate biogeographical regions result in a difference in the root/shoot ratio?
5. Do higher temperatures or precipitation result in lower root/shoot ratios?
6. Can changes to the root/shoot ratio be related to salinity, pH, sediment moisture, organic content, redox potential or porewater depth?
7. Is there an increase in plant height at higher salinity in response to turgor pressure?

The third objective is to determine if there is a difference in the phenology of *S. tegetaria* between the two temperate biogeographical regions.

1. Is there a difference in the growth, reproductive timing and seed production of *S. tegetaria* between the warm and cool temperate biogeographical regions?
2. If the phenology of *S. tegetaria* is different between biogeographical regions, are the differences in temperature and precipitation between the two regions affecting the timing of reproductive phases?

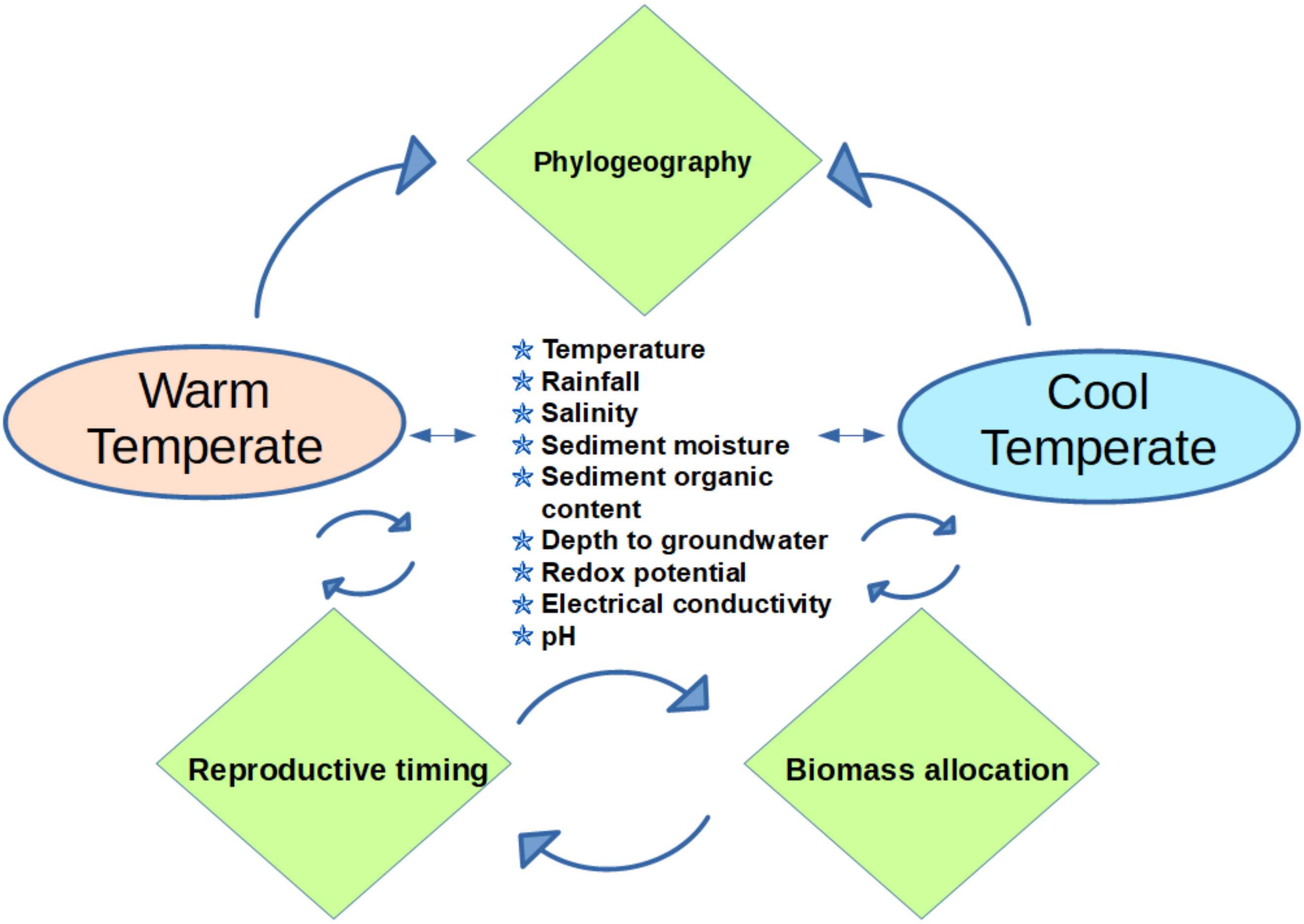


Figure 1: Outlay of objectives.

Layout of the thesis

Chapter 1 provides the rationale for the study and identifies the questions that I ask in this thesis, it also provides an outlay of how I plan to answer these questions.
Chapter 2 is a literature review and includes the general introduction to estuaries and the environment where the research will be conducted. The thesis is then divided into three sections where I test three hypotheses concerning *S. tegetaria*.
Chapter 3 concerns the phylogeography of *S. tegetaria* in which I use DNA collected at 18 estuaries to determine genetic and haplotype diversity and possible population structure.
Chapter 4 is dedicated to the biomass allocation of *S. tegetaria* and environmental variables found between the different types of estuaries in the different biogeographical regions.
Chapter 5 aims to understand the phenology of *S. tegetaria* at one estuary in the cool temperate region for a year and compares it to available literature from the warm temperate region.
Chapter 6 provides a general discussion of the findings in this study and whether the objectives have been achieved and the research questions answered (Figure 2).

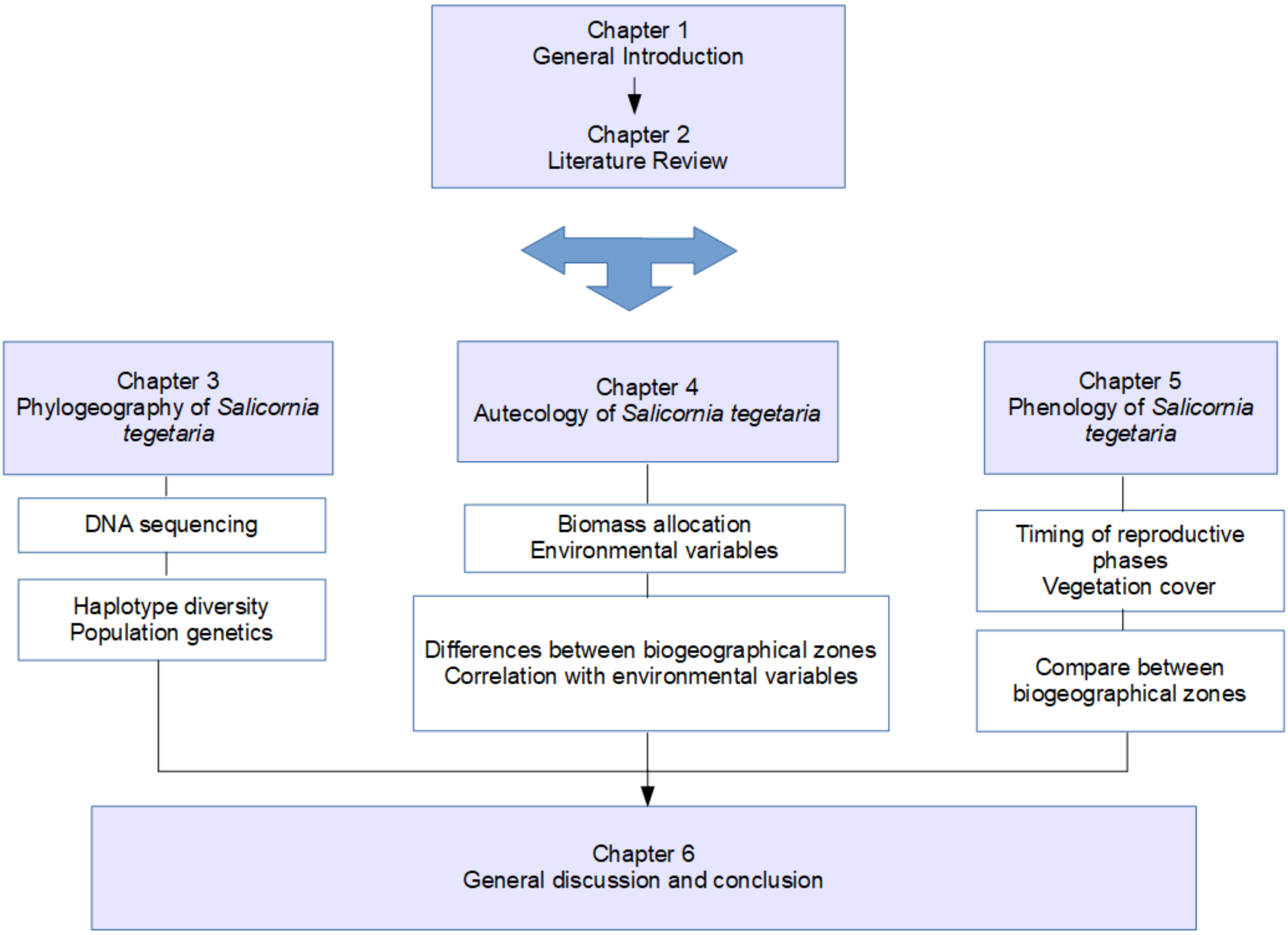


Figure 2: A flow diagram illustrating the thesis layout.

Chapter 2. Literature review

Estuarine and coastal ecosystems (ECE's) are an interconnected mosaic made up of a variation of marshes, mangroves, nearshore coral reef systems, seagrass beds, kelp forests, sand beaches and dunes. ECE's comprise only 4 % of the Earth's total land area and act as an interface between the coast, land and watershed (Moberg and Rönnbäck, 2003; Barbier *et al*., 2011; Costanza *et al*., 2014). Coral reef ecosystems found in tropical climates such as the Caribbean and Indo-Pacific region are high in biodiversity (von der Heyden *et al*., 2014). Seaweed is found in shallow coastal rocky habitats that are sheltered up to 3 km from the seashore where they can form dense stands, remaining permanently under water, but exposed to tidal fluctuations. They are found in temperate and Arctic regions and cooler water tropical habitats (Bolton, 2010). Algal beds can also be found in the intertidal zones where rocky platforms form tidal pools that are permanently or intermittently submerged (Mucina *et al*., 2006). Mangroves, marshes and seagrass beds are found along the tidal gradient in estuaries forming an entire connected habitat (Johnston *et al*., 2002; Mucina *et al*., 2006). ECE's are interconnected and interdependent making them more valuable as a whole (Moberg and Rönnbäck, 2003). The temporal and environmental variability found along these habitats may contribute to the regulation of nekton migration, nursery habitats and food webs (Mumby *et al*., 2004; Rountree and Able, 2007; Meynecke *et al*., 2008). For example small juvenile coral reef fish shelter in seagrass beds and migrate to mangroves at an intermediate size before moving to their adult habitat, increasing their survival rate and influencing community structure (Mumby *et al*., 2004). Herbivorous fishes in turn maintain the resilience of coral reefs by preventing algal overgrowth (McClanahan, 1995). Salt marshes also provide a protective habitat for nekton such as shrimp and shellfish (Boesch and Turner, 1984; Bell, 1997; Meynecke *et al*., 2008) and provide feeding and breeding habitats for many important bird species (Van Niekerk and Turpie, 2012). Estuarine habitats can further benefit the neighbouring ecosystems by enriching primary production with an efflux of detritus and nutrients (Van Niekerk and Turpie, 2012).

The definition of an estuary has been modified over the years. Day (1980) defines an estuary as "*a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage*", incorporating Pritchard's (1967) definition that covers estuaries in the northern hemisphere, and also includes temporarily open/closed estuaries mostly found in South Africa and Australia. Potter *et al*. (2010) defines an estuary as "*a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible"*, thereby including hypersaline systems.Whitfield (2005) points out that definitions that mention land drainage refer to, but do not distinguish between freshwater received by rivers or groundwater seepage. Furthermore, systems around the world, including Langebaan in South Africa, only receive freshwater through groundwater aquifers and contain estuarine biota. Langebaan can thus be treated as an estuarine embayment and is incorporated in the most recent definition "*a semi-enclosed coastal body of water which is connected to the sea either permanently or periodically, has a salinity that is different from that of the adjacent open ocean due to freshwater inputs, and includes a characteristic biota*" (Elliott and Whitfield, 2011).

Estuaries are ephemeral with lifetimes of thousands to ten thousands of years, occurring as sea level fluctuates between glacial and interglacial periods (Schubel and Hirschberg, 1978). Present estuaries were formed from flooded river valleys during sea flooding, bar depositions that formed lagoons, fjords and during periods of tectonic activity (Pritchard, 1967; De Wit, 1999). After formation these systems continue to age as they are gradually filled with sediment (Hume and Swales, 2003). The flooding of present day estuaries occurred during the last interglacial period of the Holocene as sea level increased by 120 m approximately 12 000 – 6 500 years ago (Milliman and Emery, 1968; Peltier and Fairbanks, 2006; Kemp *et al*., 2011). Categories of estuaries can range from those formed by marine processes; with course sediments, indented behind a barrier and those formed by river processes; with fine sediments, protruding into a basin (Day, 1989). Globally most estuaries are drowned river valley estuaries which are usually V-shaped with a longitudinal seaward gradient, increasing in cross-section toward the mouth (Day, 1989). The depth and width vary greatly depending on the regional setting, climate and type of rock found. Fjords formed at high latitudes where carved by retreating ice-sheets and formed new glacial valleys, usually where a previous river valley occurred. Fjord estuaries have a U-shape, with a uniform width and a shallow sill near the mouth. Coastal lagoons formed in river valleys with low relief, usually along microtidal coasts. These systems experience small river discharges where a barrier formed and encloses the shallow lagoon or embayment. Estuaries on tectonically active coasts were formed through faulting, graben formation, landslides or volcanic eruptions (Pritchard, 1967; Day, 1989; Perillo, 1995).

Estuaries can be wave-dominated or tide-dominated depending on the geological deposition that has influenced their morphology (Dalrymple *et al*., 1992). Tides can range between < 2 m to > 6 m in an estuary. The rising tide moves along the estuary towards the head where it is reflected and returns to the sea (Wolanski and Elliott, 2015) influencing physiographic characteristics by the deposition of sand which determines the position and size of the intertidal area. Microtidal estuaries have a tidal range < 2 m mostly at inlets, dominated by wind and wave action with depositions forming flood deltas and bars. The most common estuaries are mesotidal, dominated by tidal currents, with a tidal range between 2 and 4 m, forming tidal deltas, salt marshes and tidal flats. Macrotidal estuaries are broad-mouthed, funnel-shaped, dominated by tidal currents, with a tidal range of > 4 m and have extensive tidal flats and salt marshes (Hayes, 1975).

Circulation in an estuary is induced by riverine inflow, tidal currents, the differences in the density of freshwater and salt water, temperature, wind, storms and oceanic events such as an upwelling. Tides are diurnal and semi-diurnal with slightly different periods, resulting in a spring-neap tide cycle of 28 days and a high tide that shifts about 50 minutes a day (Allanson and Baird, 2008). The degree to which hydrodynamics has an effect on the fluxes of dissolved matter (such as oxygen, nutrients, pollution and salt) and particulate matter (such as sediment, detritus and plankton) depends on the residence time of a parcel of water in an estuary (Balls, 1994). In a well-flushed estuary dissolved oxygen is introduced regularly and sediment does not accumulate (Uncles *et al*., 1998; Uncles *et al*., 2006). The residence time varies depending on the position in the estuary, increasing from the outer reaches towards the upper reaches. Stagnation zones can form due to stratification in which the residence time increases. The residence time is also higher in intertidal wetlands that can store water for longer periods (Deleersnijder *et al*., 2001; Wolanski and Elliott, 2015). Mixing determines the extent of longitudinal and vertical gradients of salinity, temperature and the concentration of suspended sediment along the water column which is formed when less dense freshwater creates a layer above the denser sea water and warmer water floats over colder water (Wolanski and Elliott, 2015). Estuaries can be broadly classified based on circulation and stratification from highly stratified, partially stratified to well-mixed estuaries as the tidal range increases, freshwater input is reduced and the bathymetry of the estuary varies (Day, 1989).

The South African Coastline

The South African continental coastline is approximately 3 400 km in length from the Orange River mouth on the west coast, bordered by the Atlantic Ocean, to Kozi Bay on the east coast, bordered by the Indian Ocean (Van Niekerk and Turpie, 2012). Africa has a number of shallow continental features, including the broad and gradual sloping Agulhas Bank at the southern tip of Africa. Two ocean currents are important influences on South African coastal waters and coastal climate on either side of the Agulhas Bank, the Agulhas Current in the Indian Ocean and the Benguela Current in the Atlantic Ocean (Van Niekerk and Turpie, 2012). The warm Agulhas Current flows within 31 km of the east coast close to the continental shelf from the equatorial zone. The continental shelf broadens from north to south, deflecting the Agulhas Current away from the coast which cools as it flows poleward (Beckley and Van Balleygooyen, 1992; Bryden *et al*., 2005). Average annual sea surface temperatures vary from approximately 22 ºC in KwaZulu-Natal to 19 ºC in the Eastern Cape (Lutjeharms *et al*., 2000). The Agulhas Retroflection and return current meanders northward as it is strongly influenced by the Agulhas Plateau (Lutjeharms and de Ruijter, 1996; Leeuwen *et al*., 2000; Penven *et al*., 2001). Annually three to five Natal pulses (cyclonic circulation in the nearshore region) propagate southwestward (Gründlingh, 1979; Leeuwen *et al*., 2000), causing the Agulhas Current to meander onshore, then offshore and back with a moderate weakening of its transport (Bryden *et al*., 2005). When a Natal Pulse enters the Agulhas Retroflection, warm Agulhas rings form and move across the South Atlantic region (Leeuwen *et al*., 2000). The Agulhas Undercurrent flows northward carrying intermediate water and North Atlantic deep water below the southwestward flowing Agulhas Current. As the Agulhas Current meanders offshore, the undercurrent strengthens and penetrates upward and surfaces, with northward flowing water throughout the water column (Beal, 2009; Bryden *et al*., 2005) (Figure 3).

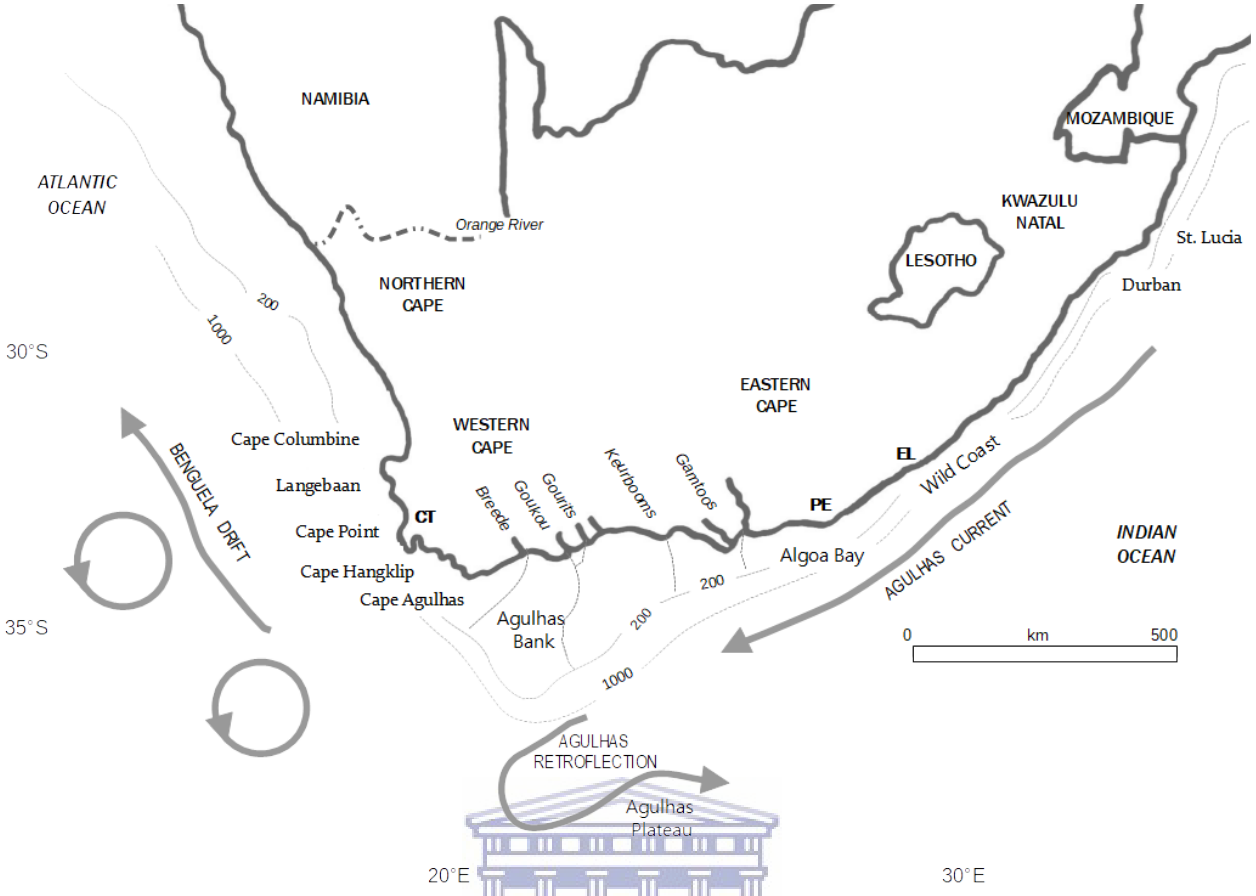


Figure 3: Map of South Africa showing main locations referred to in the text, ocean currents, bathymetry (200, 1000 m contours), extended position of rivers during lower sea-level (Breede, Goukou, Gourits, Keurbooms, Gamtoos), Cape Town (CT), Port Elizabeth (PE) and East London (EL) (adapted from Dingle and Rogers, 1972; Ramsay and Cooper, 2002 and Casal *et al*., 2009).

The cold waters of the Benguela Current flows north along the west coast, trapped by warm waters to the north and south, with average annual surface temperatures of 13 ºC to 15 ºC (Lutjeharms and Meeuwis, 1987). The Benguela is one of the world's major upwelling centres with a high level of productivity and biodiversity of zooplankton, fish, birds and marine mammals (Demarcq *et al*., 2003; Shannon and O'Toole, 2003). Upwelling occurs during summer due to off-shore winds and results in an increase in nutrients in surface waters of the pelagic zone (Lutjeharms *et al*., 2001; Shannon and O'Toole, 2003). Upwelling cells along the South African west coast have been identified at Cape Columbine, Cape Peninsula and the southernmost upwelling on the Agulhas Bank, west of Cape Agulhas (Shannon *et al*., 1983; Lutjeharms and Meeuwis, 1987). On the southern coast water temperature is variable due to the mixing of the warm and cold sea currents (Swart and Largier, 1987). The eastern boundary of the Benguela system and western boundary of the Agulhas system is located at the western Agulhas Bank (Largier *et al*., 1992). Stratification and circulation on the inner shelf is driven by coast-parallel upwelling winds, whereas the outer shelf is dominated by oceanic forcing (Largier *et al*., 1992). As the shelf narrows at Cape Point, a front and jet flows toward the west coast (Largier *et al*., 1992). Over the eastern Agulhas Bank warm surface water and cold central water is forced onto the continental shelf (Swart and Largier, 1987). Inshore of the cold ridge the subsurface water flows westward and the mean surface flow may be negligible or eastward (Shannon and Chapman, 1983). There is a zone of divergence of near-surface water on the axis of the Agulhas Bank as the eastern Agulhas Bank water flows eastward and the western Agulhas Bank water flows westwards (Shannon and Chapman, 1983; Largier *et al*., 1992) (Figure 3).

The two ocean currents have a major influence on the climate of South Africa (Walker, 1990; Mason, 1995). The cold upwelling waters of the Benguela Current are responsible for the low rainfall on the west coast (Nicholson and Entekhabi, 1987) and the offshore distance of the Agulhas Current influences the summer rainfall on the south-east coast (Jury *et al*., 1993). The South African coastline can be divided into different climatic (Van Niekerk and Turpie, 2012) and biogeographical zones (Harrison, 2004). Rainfall decreases from east to west, with higher run-off in the wetter subtropical climate (Conradie, 2012). Rainfall in South Africa as in other subtropical countries has a high amount of variability with a wetter eastern and drier western region. There is a high degree of seasonality, with summer rainfall in the north, winter rainfall in the south and a more uniformly distributed rainfall throughout the year in the narrow southern coastal belt and adjacent areas (Allanson and Baird, 2008). There are three marine biogeographical regions identified along the coast of South Africa, the cool temperate west coast, warm temperate south coast and the subtropical east coast (Van Niekerk and Turpie, 2012). Transition zones are found between the cool and warm temperate regions approximately between Cape Point and Cape Agulhas, and between the warm temperate and subtropical regions between Algoa Bay and the central Wild Coast (Bolton *et* al., 2004; Teske *et al*., 2011). The cool temperate region with cooler coastal waters occurs from the Orange River mouth on the west coast to Cape Columbine. This region changes from a hot arid desert climate with a mean annual temperature greater than 18 ºC and precipitation of less than 300 mm per annum to a cold arid desert climate with an annual mean temperature less than 18 ºC and an increase in precipitation towards Cape Columbine (Conradie, 2012). From Cape Columbine to Cape Agulhas dry summers and 400 – 700 mm per annum of winter rainfall can be found. Further along the south coast in an eastward direction, there is a gradual change from cool, arid conditions with precipitation spread more evenly throughout the year to a warm, humid climate with warm summers. Further east from Algoa Bay the climate becomes warmer and fully humid to the KwaZulu-Natal coast which is subtropical with a summer rainfall of more than 1000 mm per annum (Conradie, 2012).

The variable climate and contrasting rainfall patterns influences the catchment size of estuaries, which are the largest in the cool temperate region (Van Niekerk and Turpie, 2012). The physico-chemical characteristics of estuaries correspond to the variations in climate and oceanographic conditions of the three biogeographical regions of South Africa (Harrison, 2004). Temperatures in estuaries have a similar pattern to coastal waters where temperature increases down the cool temperate west coast, along the warm temperate south coast and up the subtropical east coast. Estuaries in the temperate region have high salinity and low turbidity due to low rainfall with high seawater input and evaporation in summer, whereas subtropical estuaries have low salinity and high turbidity due to high rainfall (Harrison, 2004).

Historical Changes of the South African Coastline

During the Quaternary (2.5 Mya – present), the cyclic variations of the orbit and rotation of the Earth caused large-scale temperature changes (Bintanja *et al*., 2005). During the periods of cooler climates the polar ice caps accumulated large volumes of water from the sea which resulted in lower sea-levels. During warmer periods the ice caps retreated and sea-levels were higher (Shackleton and Opdyke, 1973; Bintanja *et al*., 2005). Glacial and interglacial periods brought about periodic alterations to the South African coastline (Butzer and Helgren, 1972; Hendey and Volman, 1986; Van Andel, 1989). From the deep sea isotope records spanning the last 5.3 Myr, sea-level fluctuations had a lower amplitude of between 45 m and 75 m, becoming increasingly large (Lisiecki and Raymo, 2005). From Marine Isotope Stage 16 (MIS 16) (678 to 621 ka), periodic sea-level fluctuations have increased to 120 m in amplitude (Brintanja *et al*., 2005) with sea-levels lower than 75 m during MIS 14, MIS 12, MIS 10, MIS 8, MIS 6, MIS 4 and most recently MIS 2 (36.5 to 13.9 ka), when the sea-level was between -75 m and -130 m (Clark *et al*., 2009; Compton, 2011). Compared to areas with steeper continental slopes, small vertical shifts in sea-level height would cause rapid changes to the coastline on shallow shelves (Fisher *et al*., 2010; Compton, 2011). The area of the Agulhas Bank rapidly expanded when sea-levels were below 75 m and greatly expanded during the last glacial maximum when the sea-level was 120 m lower than at present (Dingle and Rogers, 1972; Van Andel, 1989). During glacial terminations the sea-level rose rapidly, flooding the exposed habitat and relocating estuaries (Clark *et al*., 2009; Compton, 2011; Cawthra *et al*., 2014). During Pleistocene sea-level fluctuations coastal habitats were repeatedly fragmented, exposing or flooding large areas of suitable habitat for estuarine macrophytes on the continental shelf around Cape Agulhas, Cape Hangklip and Cape Point (Fisher *et al*., 2010; Compton, 2011; Cawthra *et al*., 2014) (Figure 3).

Phylogeography of the South African Coast

Phylogeography studies the genetic distribution of the same or closely related species and can be used to determine the historical and genetic processes that gave rise to the diversity of morphology, adaptation and ecology of plants over millions of years. It combines population genetics, which studies the inter- and intraspecific genetic variability of populations with phylogenetics, which studies the evolutionary relationships of species and higher taxa, and biogeography, adding the aspect of intraspecific genealogy within spatial and temporal dimensions (Schaal *et al*., 1998; Avise, 2000; Kreft and Jetz, 2010). Phylogeography usually involves more than one population distributed across a landscape where barriers to genetic exchange such as dispersal occur or were important in the past between some populations (Hamrick, 1982).

The Atlantic/Indian Ocean transition zone along the coast of South Africa is characterised by phylogeographic patterns linked to the biogeographic regions of marine organisms

(Harrison, 2004; Teske *et al*., 2011). In this zone, the distribution of many of the genetic lineages of coastal and estuarine zoological species have distribution patterns and population dynamics driven by dispersal abilities, the effects of currents, water temperatures, historical hydrodynamics and past climatic conditions which were reviewed in Teske *et al.* (2011). Although the phylogeographic patterns along the Atlantic/Indian Ocean transition zone are inconsistent, many studies have found distinct phylogenetic groups which can be divided into at least four biogeographical regions: the cool temperate west coast, the warm temperate south coast and the subtropical and tropical east coast. Between the temperate regions, a south-west transitional zone occurs between Cape Point and Cape Agulhas. The warm temperate and subtropical regions have a transition zone between Algoa Bay and the Wild Coast, and a disjunct between the subtropical and tropical regions is found at St. Lucia (Teske *et al*., 2011).

The estuaries of South Africa are geographically separated with habitats that shift according to the influence of tidal and freshwater as well as terrestrial climatic conditions resulting in estuarine species with distinct eco-physiological boundaries (Bilton *et al*., 2002). On geological time scales estuaries are short-lived in their individual locations and are consequently assumed to be relatively low in species and genetic diversity (Dingle and Rogers, 1972; Whitfield, 1994; Attrill, 2002; Bilton *et al*., 2002). The extent of the geographic separation of estuaries, stressful environmental conditions, the dispersal mechanisms of species and historical processes along the South African coastline may influence genetic flow and patterns of divergence (Bilton *et al.,* 2002).

The Estuarine Habitat

According to the National Biodiversity Assessment (Van Niekerk and Turpie, 2012), there are nearly 300 functional estuaries in South Africa. Whitfield and Baliwe (2013) include 280 estuaries with another 108 small systems identified that could have some limited estuarine function. The variation between spring and neap tide is large in South African estuaries and seasonal changes in freshwater runoff, winds, waves and insolation cause large changes to circulation altering the water column structure (Allanson and Baird, 2008). For example, heavy rainfall causes scouring of the mouth and low rainfall or abstraction causes hypersalinity in the upper reaches (Reddering, 1987; Bornman *et al*., 2002). In South Africa the runoff from catchments into the estuaries is low and outflow into the sea is minimal compared to estuaries in the northern hemisphere (Allanson and Baird, 2008).

Whitfield (1992) distinguished between five types of estuaries in South Africa based on physical characteristics including tidal prism, size, mouth state, mixing processes and salinity characteristics. Estuaries that are permanently open to the sea usually have large catchments, perennial river inflow and a moderate tidal prism. Mixing is driven by tidal and riverine currents with salinity of 10 - >35 (salinity is reported as ppt, no units are provided as per the journal of Estuarine, Coastal and Shelf Science). Estuaries that are closed from the sea during low river flow periods have smaller catchments and tidal prisms. During the closed phase there is no tidal influence and salinity is usually low, though hypersaline conditions can occur during drought due to evaporation. River mouths have large catchment areas and are river dominated with a small tidal prism. Estuarine lakes are separated from the sea by varying degrees resulting in a small tidal prism and variable salinity that also depend on the amount of freshwater input, and evaporation. Estuarine bays are permanently connected to the sea with large tidal prisms and salinity greater than 25 in the lower reaches. Whitfield (2005) proposed a sixth type of estuary: Langebaan Lagoon as an estuarine embayment. The source of freshwater to the embayment is groundwater, resulting in the occurrence of typical estuarine biota.

Estuaries are made up of diverse habitat types: the open water in the estuary channel, sand, mudflats, rocks, algae and plant communities that are subject to freshwater and seawater fluctuations, elevation and salinity (Day, 1980). Estuarine plant communities include submerged macrophytes in the sublittoral zone, salt marsh and/or mangroves in the intertidal zone that are flooded periodically and supratidal terraces that are only flooded during high water levels such as spring tides. In the freshwater and brackish zones of estuaries reeds and sedges dominate (Van Niekerk and Turpie, 2012). River flow influences the salinity and water temperature of the water channel which affects the community composition of plankton and phytoplankton that occur from freshwater to marine environments and provide food to many species including many fishes that spend some of their life cycle in estuaries, birds and mammals (Van Niekerk and Turpie, 2012).

Low lying coastal areas that are protected from wave action are fronted by mud flats that form through the deposition of fine sediments brought in by tidal action (Boorman, 1999). River flow also supplies sediment to the coast, influencing beach morphodynamics and the maintenance of unconsolidated sediment habitats in the subtidal environment. Fluvial fans are formed by mixed mud and sand banks deposited by rivers creating spawning sites for fishes (Bennett, 1993). Subtidal sand, mudflats and rock are habitats to benthic microalgae. Intertidal and subtidal macroalgae can be free floating or attached including filamentous mat forming algae, such as *Enteromorpha* (L.) Nees and *Cladophora* Kütz species. Marine species can also be washed into the estuary such as species of the genera *Codium* Stackhouse, *Caulerpa* (Forsskål) J.V. Lamouroux, *Gracilaria* (Greville) and *Polysiphonia* (Greville) (Adams *et al*., 1999; Van Niekerk and Turpie, 2012). Seagrasses start to colonize soft muddy substrates below the level of the mean high water neap tide. Submerged macrophytes are rooted to the bottom sediment and are completely submerged, for example, *Stukenia pectinata* (L.) Böerner which is found in salinity of less than 10 and *Ruppia cirrhosa* (Petagna) Grande commonly found in temporarily open/closed estuaries (TOCE's). Submerged macrophytes may also be exposed on the low tide such as *Zostera capensis* Setch., found in the intertidal zones of permanently open Cape estuaries (Van Niekerk and Turpie, 2012). Pioneer species such as *Spartina* and *Salicornia* can also be found here (Boorman, 1999).

Salt marshes and mangrove forests form where low-energy shorelines along continental margins are protected from waves such as in bays and estuaries, and behind barrier-island systems and consist of a complex of vegetated and non-vegetated habitats, including the intertidal flats, creeks and vegetated marsh, marsh pools and ponds, and subtidal creeks (Rountree and Able, 2007). Alluvial sediment is deposited to form the sandy to muddy substrates of salt marshes and mangroves that are flooded by seawater daily according to the tide. The seeds of vegetation, tolerant of submersion by seawater, from seed banks or brought in by the tide can become established when enough sediment accretion has occurred for the tide to cover the sediment for less than 50 % of the time. Plants intercept and trap more sediment, increasing the rate of accretion, and increase organic matter accumulation by the prevention of re-suspension of litter and root growth, thereby stabilising the sediment surface (Stumpf, 1983; Boorman, 1999). Sediment supply determines the process of salt marsh development and tidal wetland stability is dependent on channel sediment fluxes (Reed, 1989; Cahoon and Reed, 1995). As the level increases more species occur in a sequence from lower to upper levels in positions dependent on species response to salinity and inundation (Boorman, 1999). The macrophytes found in estuaries, mainly grasses and herbs, have a characteristic zonation pattern affected by physical processes (elevation, salinity, tidal inundation and nutrient availability) (Mucina *et al*., 2003; Rountree and Able, 2007), as well as biological interactions such as competition (Dormann *et al*., 2000), facilitation (Bertness and Hacker, 1994; Friess *et al*., 2012) and grazing (Silliman *et al*., 2005).

The salinity of the water channel determines the salinity of sediment as it may be inundated daily (Taylor *et al*., 2006) and tidal flooding creates a gradient of environmental factors, from higher to lower environmental stress. Environmental stress occurs at lower elevation due to seawater flooding (Mucina *et al.,* 2006). As elevation increases, hypersalinity and drought become more important, resulting in vertical zonation of vegetation, sometimes with only a few centimetres in elevation change (Mucina *et al.,* 2006). The intertidal area is formed below the mean high water at spring tides with the lower intertidal salt marsh flooded with seawater at a higher frequency and longer period than the upper intertidal salt marsh (Mucina *et al.,* 2006). In permanently open estuaries in tropical and subtropical climates, mangroves are found in the intertidal zone, including the most widespread species *Avicennia marina* (Forssk.) Vierh. In temperate climates the intertidal zone is dominated by a few salt marsh plants, including *Afrocornia* species (Piirainen & Kadereit), *Salicornia* spp., *Triglochin* spp., *Bassia diffusa, Limonium* Mill. species and *Juncus kraussii* Hochst. (Van Niekerk and Turpie, 2012). The supratidal salt marsh occurs above the mean high water spring tide and a common species found is *Salicornia pillansii* (Van Niekerk and Turpie, 2012). Reeds, sedges and rushes such as *Phragmites australis* (Cav.) Steud., *Schoenoplectus scirpoides* (Schrad.) Browning and *Bolboschoenus maritimus* (L.) Palla are common species found in freshwater and brackish zones of estuaries (Van Niekerk and Turpie, 2012). There is approximately 90 800 ha of estuarine habitat in South Africa, of which 4 310 ha comprise intertidal salt marsh (Van Niekerk and Turpie, 2012) (Figure 4).

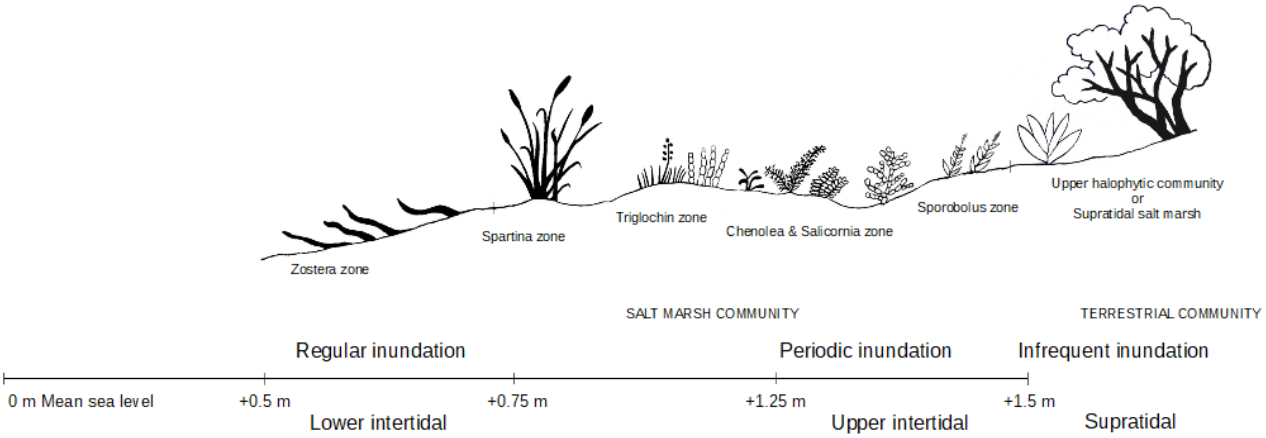


Figure 4: Typical salt marsh vegetation zonation in relation to mean sea-level (adapted from Lubke *et al*., 1997 and Adams and Ngesi, 2002, redrawn by C. E. Brown).

The Structure and Functioning of a Salt Marsh

Seawater is composed of many elements such as sodium (Na^+), chloride (Cl^-), ferrous ions (Fe^{2+}), manganese (Mn^{2+}), sulphate (SO_4^{2-}), sulfide (S^{2-}) and boron (B) and many chemical reactions occur in the salt marsh sediment. Increased inundation reduces the available oxygen, redox potential and the solubility of ions in the sediment depending on the mineral composition, microbial activity and pH of the soil (Ponnamperuma, 1972). The concentrations of nutrients such as nitrogen depend on the input and leaching of seawater, the capacity of soils to absorb nutrients and mineralisation in the soil (Rozema *et al*., 1985). There is usually a high carbonate content in the sediment giving it a pH of about 6-8 which changes the concentrations of ions in soils. The solubility or insolubility of ions can cause injury to macrophytes due to excessive uptake or deficiency (Ponnamperuma, 1972). Accumulations of many of the chemicals found in the low redox potential soils of the intertidal marsh may be harmful to plants (Rozema *et al.*, 1985). The redox potential and chemical composition of the soil is patchy, with anoxic conditions increasing with depth, depending on the sand content (Ponnamperuma, 1972; Rozema *et al.*, 1985). The sediment of the intertidal area usually has a dark colour due to the precipitation of iron(II) sulphide (FeS) and a distinctive smell of hydrogen sulphide (H_2S), with a brown/red precipitate forming around the roots as they release oxygen and oxidise ferrous iron and iron(III) oxide (Fe_2O_3). The stress factors that limit salt marsh vegetation growth are interrelated and the effect on vegetation will usually not be dominated by one effect alone (Rozema *et al.*, 1985).

Inundation and sediment moisture may affect the sediment redox potential which affects the nutrient and oxygen availability to roots in the sediment (Brümmer, 1974; Husson, 2013). There may be interacting effects between organic matter, salinity, redox potential and pH which may influence nutrient availability in the sediment and plant biomass. Plants are the main source of organic matter and retain moisture in the sediment (Jobbagy and Jackson, 2000; Gómez-Plaza *et al*., 2001; Noe and Zedler, 2001b; Bai *et al*., 2005;

Palomo and Niell, 2009). Sediment organic matter serves as an electron reservoir, lowering the redox potential of the soil and maintaining a pH buffer (Husson, 2013). The rate of organic matter decomposition increases under oxidizing conditions providing humic acids to the sediment releasing nutrients within the sediment matrix (Shaver and Billings, 1975; Bell and Sultan, 1999; Lovelock *et al*., 2007; Angiolini *et al.*, 2013). Salinity and pH also affect the decomposition rate of organic matter, whereas organic matter may ameliorate the effects of high pH and salinity (Benner *et al*., 1985; Rao and Pathak, 1996; Craft, 2007). Studies have found sediment organic matter is positively correlated to salinity, total nitrogen and flooding, and negatively correlated to pH (Molina *et al*., 2003; Angiolini *et al*., 2013), which could increase primary production (Houle, 2008).

Along the elevation gradient, species from the lower intertidal area have higher growth rates than upper intertidal species reflecting different strategies to reduce stress (Rozema *et al.*, 1985). Halophytes possess more than one mechanism for survival in high salinity environments, depending on their anatomy, morphology and physiology. Mechanisms to regulate the concentrations of salt uptake include highly selective potassium uptake to increase K/Na ratios in the plant and salt excluding membranes in roots. Mechanisms to dilute salt concentrations adapted by plants are increased succulence and continuous growth, for example *Salicornia* species have a high water content (Albert, 1975; Flowers and Colmer, 2015). Mechanisms for the extrusion of salt are also widespread such as salt glands or vesicular bladder hair, translocation of salt and the shedding of salt saturated organs. Furthermore, halophytes adjust their water potential through rapid salt accumulation under osmotic stress (Albert, 1975).

Biomass Allocation

Studies into the belowground biomass of salt marsh macrophytes commenced in the 1970's as it was realised that salt marsh species generally have equal or higher belowground biomass than aboveground biomass and should be included in primary productivity studies. The methods used to collect the roots and results obtained were variable, but illustrated the importance of belowground biomass in salt marsh systems (De La Cruz and Hackney, 1977; Groenendijk and Vink-Lievaart, 1987). Quantifying the allocation of biomass between shoots, stems and roots can be an important indicator of estuarine resilience. For example, significantly lower belowground biomass has been found at sites classified as impaired. There is a direct relationship between the accumulation of organic matter and accretion which has implications for the health of estuaries under climate change (Turner *et al.*, 2004). Production can be a better indicator of stress than the identification of salinity or flooding gradients (Guo and Pennings, 2012). Root profiles and root/shoot ratios are variable and related to the species being studied (Gallagher and Plumley, 1979) as well as abiotic factors. These include seasonal variation, atmospheric CO_2, salinity and nutrient concentrations in the sediment and changing water levels. These may be correlated to other factors, directly or indirectly affecting salt marsh biomass allocation (Lenssen *et al*., 1995; Adam, 2002; Minden and Kleyer, 2011). Salinity and flooding, which is strongly correlated with inundation frequency and therefore elevation, are the main factors that cause stress to salt marsh species (Adams, 1963;

Rozema *et al*., 1985; Lenssen *et al*., 1995; Egan and Ungar, 2001; Silvestri *et al*., 2005). Soil salinity gradually increases with soil elevation where evaporation periods become longer reaching a maximum just above mean high sea level and then decreases beyond the mean high sea level where there is less frequent tidal flooding, thereby reducing salt input (Silvestri *et al*., 2005). An increase in belowground allocation is characteristic of some species in salt marshes where there is limited nutrient availability and during times of stress where the salinity, moisture content and tidal inundation is exceeded or reduced to beyond the optimal limits of a species (Lee and Dunton, 2000; Silvestri *et al*., 2005). Some studies have found an increase in root/shoot ratios with an increase in salinity (Blits and Gallagher, 1991; Saintilan, 1997), whereas others have found that increased salinity reduced growth (Lenssen *et al*., 1995; Janousek and Mayo, 2013). For example, the root/shoot ratio of mangrove species were found to increase logarithmically as salinity increased linearly (Saintilan, 1997) which could be a response to a conservative water use strategy in a water stressed environments (Ball, 1998). In the upper reaches where aboveground biomass was highest, ratios were less than 0.5, increased to between 0.9 and 1.5 in the more saline estuary mouth and increased to 3.5 in hypersaline environments due to a decrease in aboveground biomass.

There are interactive effects between salinity and inundation on biomass production and allocation (Janousek and Mayo, 2013). Tidal inundation, soil type, conductivity and topography influence oxygen availability, reducing root growth, photosynthesis, germination and seedling growth (Ungar, 1987a; Shumway and Bertness, 1992; Pezeshki, 2001; Silvestri *et al*., 2005). As the redox potential decreases due to waterlogging, the productivity of plants decreases, even those adapted to anaerobic respiration (Mendelssohn *et al*., 1981). Reduced oxygen availability changes the soil chemistry which can become toxic, releasing reduction products such as H_2S, (Ponnamperuma, 1972). Flood tolerance of the belowground portion of plants is an important factor in determining plant growth and zonation aboveground, corresponding to elevation where species have varying aerobic demands and tolerances to sulphide (Maricle *et al*., 2006). Increased tidal inundation can cause lower light availability and an increased availability of nutrients or toxins under low redox potentials (Lamers *et al*., 1998; Darby and Turner, 2008), resulting in shallower root growth or rooting at the nodes in waterlogged soils (Blom and Voesenek, 1996; Colmer and Voesenek, 2009). Plants may have variable characteristic soil elevations, tidal inundation, duration and frequency between marshes but do, however, show consistency in elevation with relation to each other and the level of stress, measured as entropy, in the environment, suggesting that distribution is more related to the space-time variability of processes rather than single edaphic factors (Silvestri *et al*., 2005).

Nutrients affect biomass allocation in salt marshes with important implications for estuarine health. Higher nutrient loads can decrease soil strength, causing cracks that lead to creek-bank collapse and salt marsh loss (Turner, 2011; Deegan *et al*., 2012) due to a decreases in belowground biomass combined with an increase in mycorrhizal activity and decomposition (Morris and Bradley, 1999; Wigand *et al*., 2009). Nitrogen is the main limiting nutrient in the aboveground production of salt marshes (Valiela *et al*., 1976), and when supplied in treatments, directly relate to an increase in aboveground biomass

(Valiela *et al*., 1976; Poluektov and Topazh, 2005; Darby and Turner, 2008) but may not have an effect at high salinity (Naidoo, 1987). Increased nitrogen (N) has either resulted in a reduction in belowground biomass (Valiela *et al*., 1976) or had no effect (Darby and Turner, 2008), whereas increased amounts of phosphorus (P) or iron (Fe) may decrease belowground biomass, reducing the root/shoot ratio by 50 %, possibly as the need for roots to forage is reduced by P-limited soil microbes (Darby and Turner, 2008).

Drought is also an important variable in South Africa. As precipitation decreases, root mortality may increase, root turnover may increase while root diameter may decrease (Meier and Leuschner, 2008). Macrophytes in the salt marsh environment have adopted xeromorphic features such as reduced stem and leaf surfaces, waxy cuticles and hairiness to decrease transpiration and salt intake (Rozema *et al.*, 1985). With climate change other variables such as temperature, CO_2 and sea level rise will become more important. Feedback exists between sediment supply and sea-level rise that may affect carbon storage. It has been found that carbon accumulation increases in response to acceleration in sea-level rise but that above- and belowground biomass could decrease at higher elevations (Mudd *et al*., 2009). Increases in salinity and flooding due to sea-level rise may increase the physiological stress of salt marsh (Janousek and Mayo, 2013).

Reproduction and Dispersal

The phenology of plants is the cyclic biological events that occur throughout the year, usually seasonally, such as growing, flowering, fruiting, seeding and senescence. The adaptability of species to changing environmental cues may be an important determinant for survival as the climate changes (Bradley *et al*., 1999; Peñuelas and Filella, 2001; Sparks and Menzel, 2002). Reproductive allocation is influenced by environmental conditions, life-history traits and genotype (Bazzaz *et al*., 1987). The timing of reproductive allocation depends on relative growth rate, competition, access to pollinators and use of reserves stored during the vegetative phase to maximise seed set (Bazzaz *et al.*, 1987). Clonal propagation is advantageous in stressful environments as exhibited by most salt marshes and horizontal growth is favoured in which rooting at nodes create distinct plants (Bazzaz *et al.*, 1987).

Peaks in the aboveground biomass of some species occur during the growing season during high summer temperatures, but not in others (Walther, 2003; Charles and Dukes, 2009; Gedan and Bertness, 2010). The effects of temperature are unique to each species as their optimum growth temperature varies, for example, a decrease in root/shoot ratio was reported for grasses as temperature increased toward the growth optimum of each species after which the ratio increased as the temperature exceeded the growth optimum (Davidson, 1969). Studies on belowground productivity show that the translocation of belowground biomass may play an important role in the seasonal growth patterns and reproduction of salt marsh species, especially those that produce rhizomes (Schubauer and Hopkinson, 1984). For example, most belowground production of *Spartina alterniflora* Loisel. occurs between flowering and senescence and is influenced by the length of the growing season and length of flowering time. Crosby *et al.* (2015) found that as flowering of *S. alterniflora* commenced, aboveground production ceased and belowground allocation increased. Other studies have found that the belowground biomass of *Spartina alterniflora, Spartina cynosuroides* (L.) Roth and *Spartina patens* (Aiton) Muhl was translocated to aboveground biomass at the beginning of the growing season. As photosynthesis increased during summer, total plant biomass reached a seasonal peak (Valiela *et al*., 1976; Hackney and De La Cruz, 1986; Connor and Chmura, 2000). A reduction in belowground biomass followed with inflorescence production and increased during early winter during complete shoot senescence (Hackney and De La Cruz, 1986). Two similar peaks in biomass were found in a *Juncus roemerianus* (Scheele) dominated marsh (De La Cruz and Hackney, 1977). Schubauer and Hopkinson (1984) found the highest peak in winter due to an increased contribution of rhizomes to the storage of carbohydrates for both *S. alterniflora* and *S. cynosuroides* with the second smaller peak in autumn. The same patterns are not found for all species or at all locations (e.g. Gallagher and Plumley, 1979; Roman and Daiber, 1984; Connor and Chmura, 2000). For example, the same pattern of seasonal growth was not found for *S. patens* and *Plantago maritima* (L.), indicating a lower rate of aboveground primary production with less belowground storage reserved for spring growth (Connor and Chmura, 2000). Seasonal patterns have been reported for perennial *Salicornia* spp. (Curcó *et al*., 2002; Scarton *et al*., 2002; Palomo and Niell, 2009). The above- and belowground biomass of *Salicornia fruticosa* (L.) Piirainen & Kadereit reached maximum biomass in the late summer and early autumn in Italy where the salt marsh is regularly inundated and receives relatively high rainfall throughout the year (Scarton *et al*., 2002), whereas it was low during spring and summer and high in autumn and winter in Spain where estuaries are inundated irregularly and salinity is high in summer (Curcó *et al.*, 2002). The belowground biomass of *Salicornia perennis* subsp. *alpini* (Lag.) Castrov also showed seasonal variation which was lower in summer and higher in winter (Palomo and Niell, 2009).

Spring phenophases such as first flowering dates, time of budbreak, seedling growth and biomass allocation may be influenced by threshold temperatures and photoperiod, for example first flowering may occur sooner as the climate becomes warmer (Fitter and Fitter, 2002; Sparks and Menzel, 2002) and seedlings produce more culms and rhizomes with shorter photoperiods (Seneca and Broome, 1972; Seneca and Blum, 1984). Flowering can also be induced by stress such as low nutrient availability or sub-optimal photoperiod (Wada and Takeno, 2010; Shimakawa *et al*., 2012). The phenology of salt marsh genus *Spartina* has been well studied globally. Along the U.S. Atlantic coast *S. alterniflora* occurs along a latitudinal gradient. Flowering and senescence occurs earlier from north to south including when grown from seed or transplanted to a greenhouse regardless of alterations to environmental conditions, indicating a genetic basis for phenotypic variation (Somers and Grant, 1981; Crosby *et al*., 2015). Another study found that the flowering period of *S. alterniflora* determined the diversity of pollen and rates of seed set and germination (Fang *et al*., 2004a). Flowering time is important in determining pollination donors and receptors, and seed production (Somers and Grant, 1981), for example in wind pollinated species, cross-pollination occurs if flowering times occur simultaneously in subhabitats and self-incompatible species with different flowering times could reduce the number of viable seeds produced (Somers and Grant, 1981; Fang *et al*., 2004b). Species with an ocean-dispersal-based strategy tend to produce high quantities of offspring (Gaines and Bertness, 1992) and a large reproductive output has been found in aquatic plants such as seagrass (Silberhorn *et al*., 1996), mangroves (Clarke, 1992) and *Spartina* spp. (Callaway and Josselyn, 1992). Seed production is variable between species and at different locations. *Spartina* hybrids had a lower flowering intensity compared to native plants, but some plants had higher seed and pollen production and early seedling growth than native plants (Ayres *et al*., 2008). *Phragmites* seed sets and seed weight production was related to different climatic conditions, becoming restricted to the north in Britain due to late flowering (McKee and Richards, 1996).

The two main dispersal strategies of the larvae or propagules of estuarine species are retention within the estuary or export into shelf waters with return migration or drift. Intermediate strategies are also employed and depend on the species and characteristics of the estuary (Bilton *et al*., 2002). Both strategies have advantages. In the case of macrophytes, the production and dispersal of propagules are important in the establishment and maintenance of populations and seed banks ensure that macrophytes can recolonise habitats where local extinctions have occurred (Van der Valk and Davis, 1978; Ungar, 1987b; Mucina *et al*., 2006; Whitfield *et al*., 2008; Vromans, 2011), whereas export increases the chance of transport to other estuaries, increasing the exchange of genetic material and the persistence of species on a geomorphologically evolving coastline (Bilton *et al*., 2002). A study at the local scale found that the transport of salt marsh macrophyte propagules was mainly determined by tidal currents. Propagules were exchanged within the marsh, a few propagules were imported with the incoming tide and a large number, especially from lower marsh species, were exported with the ebb tide (Huiskes *et al*., 1995). It is generally assumed that clonal growth employed by many perennial estuarine halophytes results in low genetic diversity (Adam, 1993), but studies on *S. alterniflora* suggest that this is not the case (Richards *et al*., 2004; Travis *et al*., 2004; Edwards *et al*., 2005; Travis and Proffitt, 2016). Some of these species also produce large quantities of seeds which may be flushed into the sea (Van der Valk and Davis, 1978; Huiskes *et al*., 1995; Vromans, 2011). The fruits, seeds, fragments of inflorescences or seedlings of salt marsh plants can float in sea water for varying periods of time which enables them to drift with ocean currents over long distances (Nelson, 1978; Cappers, 1993; Johansson and Nilsson, 1993) or by rafting on mats of wrack (Minchinton, 2006). The drift of propagules in coastal waters are subject to stochastic processes that involve wind driven processes, currents and upwelling (Bilton *et al*., 2002), disseminule buoyancy and seed viability (Cappers, 1993; van den Broek *et al*., 2005; Friess *et al*., 2012). The buoyancy of disseminules may be increased by aerial tissues, hairs that trap air bubbles or a morphology that increases the surface/weight ratio (Dalby, 1963). Long distance dispersal of salt marsh seeds mediated by birds also occurs (Figuerola *et al*., 2003; Chang *et al*., 2005; Neff and Baldwin, 2005).

Salicornioideae

The first critical treatment of Salicornioideae which included anatomy, flower and fruit morphology was conducted by Ungern-Sternberg (1866) which contained the family

Salicornieae with 22 species. Ulbrich (1934) divided Salicornioideae into two tribes: Halopeplideae and Salicornieae and subdivided Salicornieae into two subtribes: Halostachyinae and Salicorniinae. Scott (1977) reinstated the family status to Salicorniaceae but accepted the subtribes and later Kühn *et al*. (1993) accepted the division into two tribes but abandoned the subtribes. In South Africa, Moss (1954) and Tölken (1967) recognised two genera in the South African Salicornioideae using morphological characters: *Arthrocnemum* Moq. (10 species) and *Salicornia* L. (three species). Scott (1977) split the genera into *Sarcocornia* (nine species), *Salicornia* (three species) and *Arthrocnemum* (one species). O'Callaghan (1992) recognised nine species and three varieties in the genus *Sarcocornia.*

Taxonomic revisions based on molecular phylogenetics has been a useful tool in delimiting the species of Salicornioideae, bringing about many recent changes (Kadereit *et al*., 2006; Shepherd and Wilson, 2007; Steffen *et al*., 2009; de la Fuente *et al*., 2011; Yaprak, 2012; de la Fuente *et al*., 2013). Molecular studies confirmed that the genera *Sarcocornia* and *Salicornia* are monophyletic, but paraphyletic to each other, and well-separated from *Arthrocnemum* (Kadereit *et al*., 2006; Kadereit *et al*., 2007). The relationships between *Salicornia* and *Sarcocornia* remained unclear and were grouped separately. Though they are closely related in morphology and ecology and can be distinguished from other Salicornioideae by seeds that lack a perisperm, *Sarcocornia* species can be distinguished from the genus *Salicornia* by their perennial life form and flower morphology (Kadereit *et al*., 2006). Systematic, biogeographic and phylogenetic studies of South African *Sarcocornia* identified three new species, *Sarcocornia decussate, Sarcocornia freitagi* and *Sarcocornia tegetaria*, which are endemic to southern Africa. *Sarcocornia tegetaria* represents an independent evolutionary entity similar in morphology to the diphyletic Eurasian species *Sarcocornia perennis* (Steffen *et al.,* 2009). In literature prior to 2009 the species *S. perennis* also refers to *S. tegetaria.* Scott (1977) recognised the genus *Sarcocornia,* and distinguished the species *Sarcocornia perennis* (Mill.) A. J. Scott which included southern African species. The genus *Sarcocornia* A.J. Scott (Scott, 1977) contained approximately 28 perennial, halophytic species found in coastal and inland saline habitats in the warm temperate and subtropical regions of Eurasia, North and South America, Australia and Africa. Southern Africa was identified as the centre of diversity of *Sarcocornia* with 12 recognised species (Steffen *et al.,* 2010).

Due to the paraphyly of *Sarcocornia* and the limitations of the usefulness of the morphological characteristics used to define the separation of *Sarcocornia* and *Salicornia,* Piirainen *et al.* (2017) proposed an updated genetic classification of Salicornioideae, where *Sarcocornia* and *Salicornia* are congenetic, and include four new sublineages for the phylogenetically and geographically distinct *Salicornia* s.str (subg. *Salicornia*), South African/Australian *Sarcocornia* (subg. *Afrocornia*), American *Sarcocornia* (subg. *Amerocornia*), and Mediterranean/Eurasian *Sarcocornia* (subg. *Arthrocnemoides*) (which includes *Sarcocornia perennis*). The newly proposed genus *Salicornia* (Piirainen *et al.,* 2017) consists of annual and perennial species that have a herbaceous to shrub-like habit, with decumbent to erect articulated stems with succulent cylindrical, barrel-, or clubshaped internodes and constricted nodes where opposite leaves are strongly reduced in size, visible as a small rim between segments.

The species *Sarcocornia tegetaria*, representing *S. perennis* in southern Africa, was newly described by Steffen *et al.* (2009) following morphological and phylogenetic study. The species could be distinguished from other species of *Salicornia* by their perennial, mat-forming habitat, flowers of equal size arranged in a row and seeds that lack a perisperm and have a membranous hairy testa (Steffen *et al.* 2010). Succulence is due to the cortex that surrounds the stem, formed by opposite leaves fused at the margin (Davy *et al.,* 2006). *Salicornia tegetaria* is an endemic salt marsh macrophyte found in the lower intertidal and middle tidal zone estuaries of southern Africa spanning both the Atlantic and Indian oceans. It is distributed from the west coast, including the coast off the Namib Desert near Lüderitz to Durban on the east coast, and the southern Mozambique coast and neighbouring islands (Steffen *et al.,* 2009). Morphological studies suggest that *S. tegetaria* frequently hybridises with other species in the subgenus *Afrocornia* where they occur sympatrically (Steffen *et al.,* 2009). Only a few characters in combination such as seed coat structure, presence of perisperm, growth form and inflorescence characters can be used to distinguish between species in the *Afrocornia* subgenus (Steffen *et al*., 2010). High phenotypic plasticity and polyploidy also causes difficulties in determining the phylogeny of *Salicornia* species (Kadereit *et al*., 2006). In this study, the name *Salicornia tegetaria* will be used for species previously referred to as *Sarcocornia tegetaria* and *Sarcocornia perennis* in South Africa in previous literature (Piirainen *et al.,* 2017). The subgenus *Afrocornia* refers to the genus of perennial *Sarcocornia* and the subgenus *Salicornia* refers to the annual *Salicornia* in South Africa.

Salicornia tegetaria is a woody perennial prostrate to decumbent subshrub with mats up to 20 cm high. The main branches are prostrate and rooting at the nodes with lateral branches arising in pairs from the nodes. The succulent segments are barrel-shaped to obconical. The dead cortex adheres to the stem until it disintegrates, leaving the main stem mostly bare (Steffen *et al.,* 2009). Internodes are green, turning yellow to red or brown with a cylindrical or club shape, becoming barrel-shaped as they get older of which the basal internodes may be keeled (Davy *et al.,* 2006). Inflorescences are spike-like thyrses with 2-22 fertile segments, which are thicker than non-fertile segments. Each segment bears a pair of opposite cymes with three flowers per row, the middle flower is slightly larger and separates the two lateral flowers. The flowers are embedded in the segment, forming a horizontal line at the base (Steffen *et al.,* 2009). Most *Afrocornia* flowers are protogynous (Dalby, 1963; Connor, 1984), though gynodioecous populations could be common and have been found in *S. perennis* (Davy *et al.,* 2006; Steffen *et al*., 2010). The perianth becomes cork-like when mature, adhering to the seed. Seeds are 1.3 mm long and 1.1 mm wide and covered in adjacent to erect hairs at the edge (Steffen *et al.,* 2009) (Plate 1).

Plate 1: *Salicornia tegetaria* interspersed with *Bassia diffusa* at Langebaan Estuarine Embayment. Photo A. Rajkaran.

There are no studies that have determined the dispersal strategies of *Afrocornia* species. Kadereit *et al*. (2007) summarized what is known about the dispersal of annual *Salicornia* species which are similar in seed morphology (Shepherd *et al*., 2005), though these two subgenera have different life-histories and as such there may be differences that have not been studied. The seeds of the subgenus *Salicornia* are not very buoyant and only a small number of seeds are likely dispersed over a long distance by rolling along the sediment surface with tidal currents. Most seeds are trapped by sediment depressions, algae and vegetation where the hooked hairs on the testa may anchor the seed to the substrate. The seeds are also dispersed in broken infructescences, partial infructescences or the persistent perianth which may float for several months as it is corky. Seedlings are buoyant and remain alive, remaining viable for several months (Kadereit *et al*., 2007).

Interest has increased in the commercial growth of halophytes such as *Salicornia* species that can be irrigated with seawater (Ventura *et al*., 2011a; Ventura and Sagi, 2013). As a sea vegetable *Salicornia* species could be harvested year-round if flowering is regulated (Ventura *et al*., 2011b), are convenient to prepare and are potentially high in functional ingredients such as minerals and antioxidants (Lu *et al*., 2010; Ventura *et al*., 2014). These species have more potential uses such as bioremediation and as biofuel, oil and foraging crops as they are easily cultivated (Glenn *et al*., 1998; Ventura and Sagi, 2013; Ventura *et al*., 2014). Yields and nutritional value depend on genotype, which can be improved by selective breeding, and agronomic techniques (Zerai *et al*., 2010; Ventura and Sagi, 2013). Most experiments on the yield and production of *Salicornia* species are conducted under laboratory conditions and no information is available for species in South Africa. Species in this tribe may be valuable genetic resources in future and knowledge of the genetic diversity and distribution of species will be useful in the collection and use of plant species and wild types (Zerai *et al.*, 2010).

Most salt marsh species are correlated to elevation, including *S. tegetaria* which is found at the lower to middle intertidal zone. Widespread salt marsh species are found across a broad range of physico-chemical conditions (Adams *et al*., 2016) illustrating the high degree of phenotypic plasticity displayed by these species (Silva *et al*., 2007; Adams *et al*., 2016). *Salicornia tegetaria* is widespread in the warm temperate and cool temperate biogeographical regions of South Africa and therefore large-scale processes could affect the patterns of genetic variation in this species (Vellend, 2003; Vellend and Geber, 2005). Changes in the genetic diversity of a common species may have direct implications for associated species or associated species may undergo the same processes (Vellend, 2003; Hughes and Stachowicz, 2004; Crutsinger *et al*., 2006; Johnson *et al*., 2006; Crawford *et al*., 2007; Johnson and Stinchcombe, 2007). Studying the genetic diversity of a widespread estuarine macrophyte could provide insight into the biodiversity and processes that maintain biodiversity in estuaries in general (Vellend, 2003; Hughes and Lotterhos, 2014). This topic will be covered in the next chapter.

Chapter 3. Phylogeography of *Salicornia tegetaria*

Introduction

Comparative phylogeographic analyses of regional biota aid in determining the processes such as population dynamics and biogeography that drive genetic divergence in a region (Bermingham and Moritz, 1998; Soltis *et al*., 2006). A particular genetic structure found for a single species may not occur for all species across the same land- or seascape in which case combining taxa can improve the statistical power of phylogeographic studies (e.g Kelly and Palumbi, 2010). Comparisons of multiple taxa in an area with a complex phylogeography, for example the macrophytes of the Mediterranean Basin, which is a biodiversity hotspot, can also provide information about different glaciation, environmental, paleoclimatic and evolutionary patterns not found in other temperate regions (Feliner, 2014). Phylogeography is also useful in making conservation decisions by identifying species with low genetic diversity or isolated populations which aid the determination of which species to conserve and to incorporate genetic information into spatial conservation planning (Rao and Hodgkin, 2002; Escudero *et al*., 2003). Areas of genetic diversity, distinctness or connectivity can be identified, prioritised and be incorporated into standard conservation practices to more fully represent processes regulating biodiversity (Escudero *et al*., 2003; von der Heyden *et al*., 2008; Beger *et al*., 2014).

There is a paucity of data relating to the genetic structure of estuarine macrophytes along the South African coast. These are expected to have a pattern reflecting their dynamic habitats which are subject to terrestrial and marine processes. There are only two studies describing the phylogeography of macrophytes at the species level in South Africa. Genetic structure has been described for *Salicornia meyeriana* Moss (Slenzka *et al*., 2013) and *Juncus kraussii* (Potts *et al*., 2016). Potts *et al*. (2016) found a break between the Knysna and Groot Estuaries for *J. kraussii* using the cpDNA *rpS16* intron and Slenzka *et al.* (2013) found geographically distinct lineages of *S. meyeriana* between the west and south coast between Heuningnes and Knysna Estuary using the rDNA *ETS* region. The phylogeography of *Salicornia tegetaria* has not been studied, but a PhD thesis (Veldkornet, 2015) revealed possible genetic structure in this species between the warm and cool temperate biogeographical regions along the South African coast. The study included a screening process for primers that may be useful in the intra-specific phylogeographic analysis of estuarine macrophytes (Veldkornet, 2015).

In this study I used the cpDNA *rpS16* intron and nuclear ribosomal internal transcribed spacers (*ITS*) to study the phylogeography of *S. tegetaria.* Nuclear ribosomal internal transcribed spacers have been widely used to study plant phylogeny and with a higher mutation rate may provide greater levels of divergence than cpDNA (Small *et al*., 2004). Chloroplast DNA (cpDNA) is contained in approximately 120 genes that code for transfer and ribosomal RNAs and the polypeptides involved in photosynthesis and protein synthesis (Avise, 2009). The cpDNA *rpS16* intron is a variable noncoding spacer that is unlikely to be under selective constraint, making it useful for the inference of phylogeographic patterns and the population history of plants (Shaw *et al*., 2005).

The genetic diversity and genetic structure of flowering plants is regulated by reproductive mode and gene flow. Gene flow occurs via disseminules, such as seeds, fruit or seedlings and pollen dispersal. Most cpDNA does not recombine as it is maternally transmitted through seed dispersal (though paternal or biparental inheritance has been found in some groups e.g. Gymnosperms) (Harris and Ingram, 1991; Mogensen, 1996). The nuclear DNA of plants is transferred biparentally through pollen and seed dispersal (Corriveau and Coleman, 1988). The extent of disseminule dispersal and survival can therefore be determined by analysis of cpDNA where long-distance seed dispersal events leave genetic signatures for a time by influencing the genetic differentiation of populations (Le Corre *et al*., 1997).

Many of the studies on the phylogeography of marine organisms on the coast of South Africa that used mtDNA genetic markers exhibited a genetically homogeneous distribution as listed in Teske *et al.* (2014). By combining mtDNA and nuclear DNA it was shown that genetic homogeneity may be due to the matrilineal inheritance of mtDNA (Teske *et al*., 2014). Studies of plants on the other hand found that genetic differentiation was much higher in cpDNA than that of nuclear markers, further supporting a multilocus approach to inferring population evolution (e.g. McCauley, 1994). The aim of this study was to determine the population genetic structure of *S. tegetaria* along the temperate coast of southern Africa using a combination of nuclear and chloroplast DNA sequences.

I expect that the genetic diversity of the species will be low as they are found in a stressful environment that favours clonal reproduction and a high degree of plasticity (Escudero *et al.,* 2003; Richards *et al*., 2010; Adams *et al.,* 2016). I also hypothesise that populations of *S. tegetaria* are well connected as they are widely distributed along the South African coast, but that the species may have been separated historically due to the ephemeral nature of estuaries (Schubel and Hirschberg, 1978). I expect that a pattern of genetic differentiation may reflect the movement of disseminules that could be related to oceanic currents (Huiskes *et al.*, 1995).

Study Site Description

Salicornia tegetaria leaves were collected from eighteen estuaries: four cool temperate estuaries, thirteen warm temperate estuaries and one subtropical estuary (Figure 5). Along the west coast samples were collected from Orange River, Olifants, Berg and Langebaan, on the south coast at Goukou, Klein Brak, Groot Brak, Gouritsmond, Knysna, Keurbooms, Heuningnes and Breede, and along the east coast at Nahoon, Keiskamma, Tyolomnqa, Kwelera, Kobonqaba and Mngazana (Table 1).

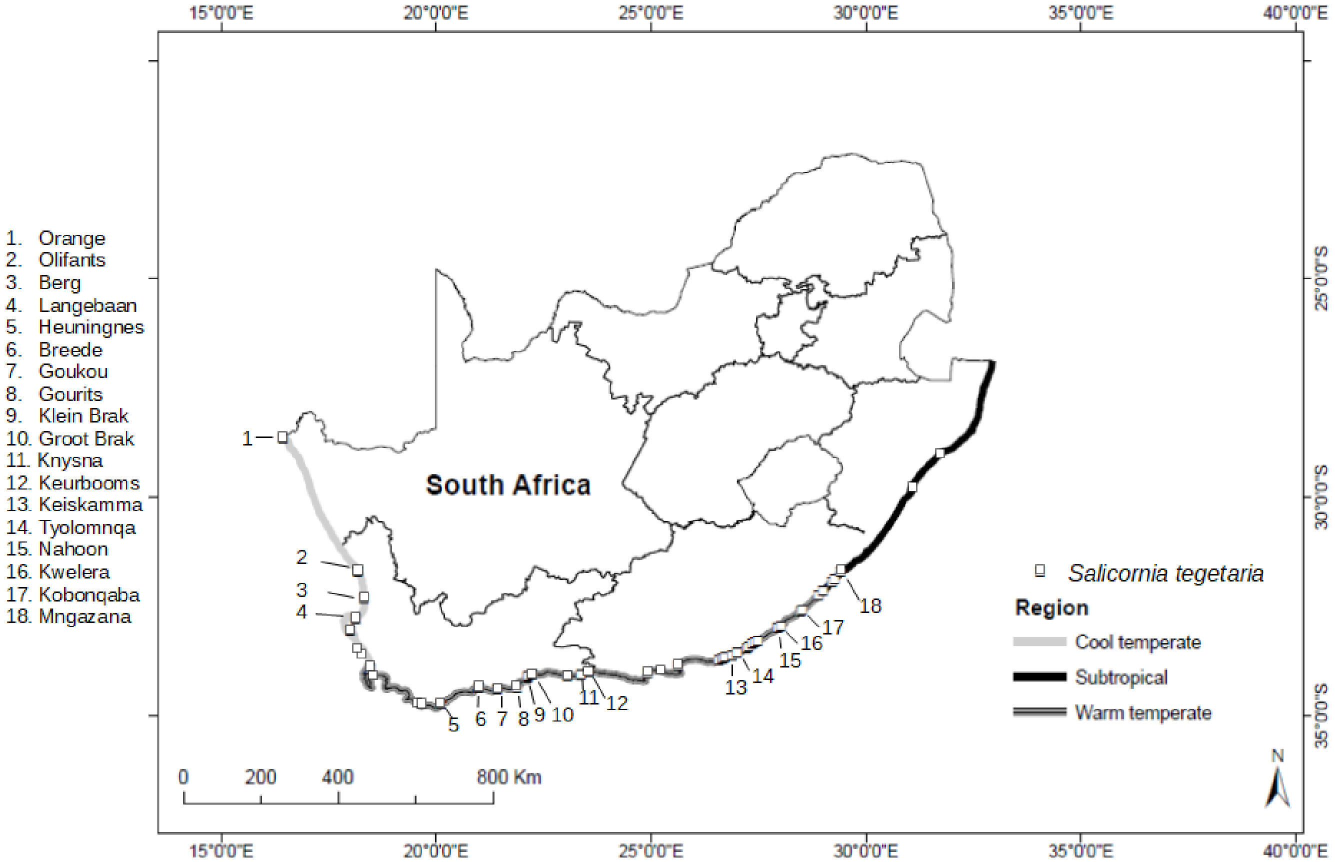


Figure 5: Distribution of *S. tegetaria* in South Africa (white squares) along the coastline of South Africa with study sites indicated from Orange River Estuary in the west to Mngazana Estuary in the east.

Materials and Methods

DNA extraction and sequencing

Five leaves of *S. tegetaria* were collected from each of the eighteen estuaries, giving a total of sixty samples, and stored in silica gel until dry. DNA was extracted from 20 mg of dried tissue using a DNeasy Mini Plant Kit (Qiagen, Hilden, Germany) and visualised on 0.8% agarose gels. The DNA sequences were amplified by Polymerase Chain Reactions (PCR) using a Tecne TC-3000x thermal cycler. Primers used were *ITS4* (forward): TCC TCC GCT TAT TGA TAT GC (White *et al*., 1990), *ITS5m* (reverse): GGA AGG AG AAG TCG TAA CAA GG (Sang *et al*., 1995), *rpS16F* (forward): AAA CGA TGT GGT ARA AAG CAA C and *rpS16R* (reverse): AAC ATC WAT TGC AAS GAT TCG ATA (W = T, U, or A) (Shaw *et al*., 2005). Reactions consisted of 25 µl, made up with 1 µl of template DNA, 12.5 µl 2X EmeraldAmp® PCR Master Mix, 0.5 µl BSA, 1 µl of each primer and sterile distilled H_2O. The PCR protocol consisted of a denaturing step at 94 ºC for 2 minutes followed by 40 cycles at 94 ºC for 1.45 minutes, 55 ºC for 30 seconds, 72 ºC for 2 minutes and 72 ºC for 6 minutes. PCR products were visualised on 0.8 % agarose gels to test for quality and to ensure that there was no contamination in blank controls. PCR products were each cleaned with 0.25 µl Exonuclease I, 0.50 µl of Shrimp Alkaline Phosphate and 2.25 µl sterile distilled H_2O (Werle *et al*., 1994). Sequencing was performed by Macrogen (Seoul, Korea).

Table 1: GPS coordinates of sampling locations, the type of estuary, biogeographical zone in which they are located and the condition of estuaries according to Van Niekerk and Turpie (2012). CT = cool temperate, WT = warm temperate, ST = sub-tropical; RM = river mouth, POE = permanently open estuary, TOCE = temporarily open/closed estuary, EE = estuarine embayment, EB = estuarine bay.

Estuary	Region	Classification	Condition	Mixed or monospecific stands	x_coordinates (E)	y_coordinates (S)	Nr. collec-ted	Nr. sequen-ced
Orange	CT	RM	Fair	Single plants	16.449578	-28.630307	5	5
Olifants	CT	POE	Good	Monospecific	18.187659	-31.702122	5	5
Berg	CT	POE	Fair	Monospecific	18.143885	-32.769749	5	4
Langebaan	CT	EE	Good	Monospecific	18.005668	-33.048156	5	2
Heuningnes	WT	POE	Fair	Monospecific	20.119246	-34.71479	5	5
Breede	WT	POE	Excellent	Mixed	20.845332	-34.407434	5	5
Goukou	WT	POE	Fair	Mixed	21.423527	-34.378352	5	3
Gourits	WT	POE	Fair	Monospecific	21.885904	-34.345341	5	5
Klein Brak	WT	TOCE*	Fair	Mixed	22.148586	-34.09293	5	4
Groot Brak	WT	TOCE**	Fair	Mixed	22.239292	-34.057254	5	4
Knysna	WT	EB	Good	Monospecific	23.061453	-34.082706	5	5
Keurbooms	WT	POE	Good	Monospecific	23.378187	-34.04985	5	3
Keiskamma	WT	POE	Fair	Mixed	27.491233	-33.28148	5	5
Tyoloqmna	WT	POE	Good	Monospecific	27.583421	-33.225772	5	4
Nahoon	WT	POE	Fair	Monospecific	27.951704	-32.986438	5	5
Kwelera	WT	POE	Good	Monospecific	28.077002	-32.90736	5	5
Kobonqaba	WT	POE	Good	Monospecific	28.490359	-32.607836	5	5
Mngazana	ST	POE	Good	Monospecific	29.422861	-31.692177	5	4

*historically closed 10 % of the time, **more likely to close during Jan/Feb and June, recently more frequent

Sequence alignment

Sequences were aligned by eye using MEGA 4.0 (Tamura *et al*., 2011). The identity of *Salicornia tegetaria* was confirmed by a BLAST search (Altschul *et al*., 1990; http://www.ncbi.nlm.nih.gov/). Sequences were tested for recombination with RDP v.4 (Martin *et al*., 2015).

Haplotype network

Population genetics use phylogenetic networks that group haplotypes because there are fewer characters available for analysis at the intra-species scale and because populations and haplotypes do not usually have the same evolutionary history due to homoplasy, recombination between genes and coalescence or lineage sorting (Woolley *et al*., 2008).

The phylogeny of *Salicornia tegetaria* was reconstructed with a statistical parsimony network in TCSv1.21 (Clement *et al*., 2002) which can incorporate ambiguities and reticulated relationships. All haplotypes were included in the *ITS* and *rpS16* network at 95 % confidence limit. Haplotype diversity (*h*, the probability that two haplotypes chosen in a sample at random are different) and genetic diversity (π, the mean number of differences between all pairs of haplotypes) (Nei and Li, 1979; Nei and Tajima, 1981) for all samples were calculated in DnaSP v5 (Librado and Rozas, 2009).

Spatial and genetic structure

To detect population genetic structure an analysis of pairwise DNA haplotype divergence (Φ-statistics) was performed in Arlequin v.3.5.2.2 to test for significance under the null hypothesis of panmixia with 10 000 permutations (Excoffier and Lischer, 2010). Sequences were divided into two groups according to the *ITS* haplotype network (group 1 = Olifants, Berg, Langebaan, Heuningnes, Breede and group 2 = Goukou, Gourits, Klein Brak, Groot Brak, Knysna, Keurbooms, Keiskamma, Tyolomnqa, Nahoon, Kwelera, Kobonqaba and Mngazana). Pairwise calculations of Φ_{ST}, an analogues of F_{ST}, consider haplotype frequency and the extent of differentiation among haplotypes and assumes that the mutation rate is negligible compared to the migration rate. To test for isolation by distance a Mantel test (Manly, 2006) was performed with 1 000 replicates in Alleles In Space 1.0 (AIS) (Miller, 2005).

Demographic patterns

To infer the demographic history of *S. tegetaria*, the mismatch distribution frequencies of nucleotide differences between pairs of haplotypes observed for all samples combined were compared to the expected frequencies under a model of exponential growth or mutation-drift equilibrium. DnaSP v5 (Librado and Rozas, 2009) was used to detect if range expansion or contraction has occurred in the historic past of *S. tegetaria* (Harpending, 1994). Under a model of population growth a unimodal distribution is expected and a multimodal or ragged distribution is expected under equilibrium (Rogers and Harpending, 1992). As the mismatch distribution is a conservative test, Fu's F_S and Tajima's D value were also calculated in Arlequin v.3.5.2.2 with 10 000 simulations as they have been shown to be more powerful (Ramos-Onsins and Rozas, 2002). Tajima's and Fu's tests test the selective neutrality of polymorphisms and are both based on the infinite-site model without recombination, appropriate for short DNA sequences (Tajima, 1989; Fu, 1997). Large negative F*s* and D values are expected when there is population expansion (Ramos-Onsins and Rozas, 2002; Excoffier *et al*., 2009). A graphical representation of patterns of genetic distance between estuaries along the coast was chosen to visualise the genetic diversity across the landscape instead of calculating the pairwise F_{ST} for each estuary due to the small number of samples per estuary. A three dimensional surface plot of the spatial distribution of *ITS* and *rpS16* variation was visualised in AIS 1.0 with default settings (Miller, 2005) using the midpoint of edges from Delaunay triangulation (Brouns *et al*., 2003) and the inverse distance-weighted interpolation of genetic distances represented as surface height overlaid on a grid of the sampled landscape. Peaks represent large genetic distances for which the average of inter-individual genetic distance from the same GPS coordinates have been used for calculations. AIS was used as knowledge of populations are not required *a priori* and is useful in the analysis of the genetic data of a few individuals collected from many locations (Miller, 2005).

Results

Sequence alignment

ITS samples from Orange River Mouth formed a single haplotype with a 2 % divergence from the dominant haplotype by 8 consistent point mutations and *rpS16 se*quences from Orange River Mouth formed two separate haplotypes with 5.5 % sequence divergence and 6 and 7 mutational steps. When the sequences were compared to the NCBI database with BLAST, the sequences from Orange River mouth were more similar to *Sarcocornia mossiana* (Toelken) A.J. Scott (1978) (DQ340165.1) and *Sarcocornia terminalis* (Toelken) A.J. Scott (1978) (DQ340174.1) and were removed from further analysis.

A total of 73 *ITS* sequences and 82 *rpS16* sequences of *Salicornia tegetaria* were successfully sequenced from seventeen estuaries. After indeterminate bases from the ends of sequences were removed, the length of *ITS* sequences were 592 bp and *rpS16* sequence lengths were 852 bp.

Haplotype network

The genetic diversity was low for both nuclear and chloroplast gene regions. The 82 chloroplast gene sequences had 839 monomorphic sites, 11 variable sites, 2 indels and only 2 parsimony informative sites. Out of the 8 haplotypes recovered (π = 0.00059, h = 0.34), one was dominant (76 %), with a second haplotype (11.5 %) that diverged 0.1 % from the dominant haplotype with one point mutation. The remaining six haplotypes consisted of one sequence each with a sequence divergence between 0.1-0.6 % from the dominant haplotype and all occur in the warm temperate zone. The *ITS* sequences had 570 monomorphic sites, 19 variable sites, 3 indels and 7 parsimony informative sites. Eleven haplotypes were recovered for the 73 *ITS* sequences (π = 0.0019, h = 0.59). Two of the haplotypes were dominant with 0.17 % sequence divergence between the two. Most of the sequences belonged to one haplotype found from Berg to Mngazana Estuaries (59 %) and 26 % of sequences belonged to the second dominant haplotype found exclusively from Breede to Olifants Estuaries (Figure 6). The remaining haplotypes consist of one or two sequences, representing 19 % of the samples with a sequence divergence between 0.17-1.35 % from the dominant haplotype. Both gene regions were characterised by an ancestral dominant haplotype that gave rise to derived haplotypes (Donnelly and Tavarè, 1986; Crandall and Templeton, 1993). One of the *ITS* haplotypes at Berg Estuary had the same base pair arrangement as the dominant haplotype and a shared *rpS16* haplotype.

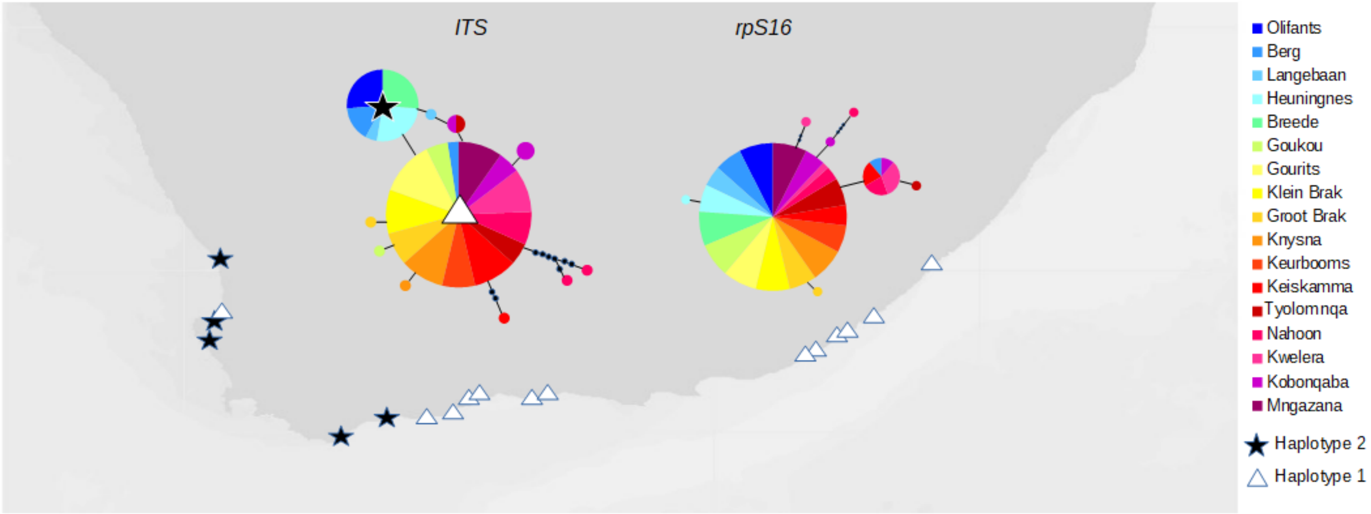
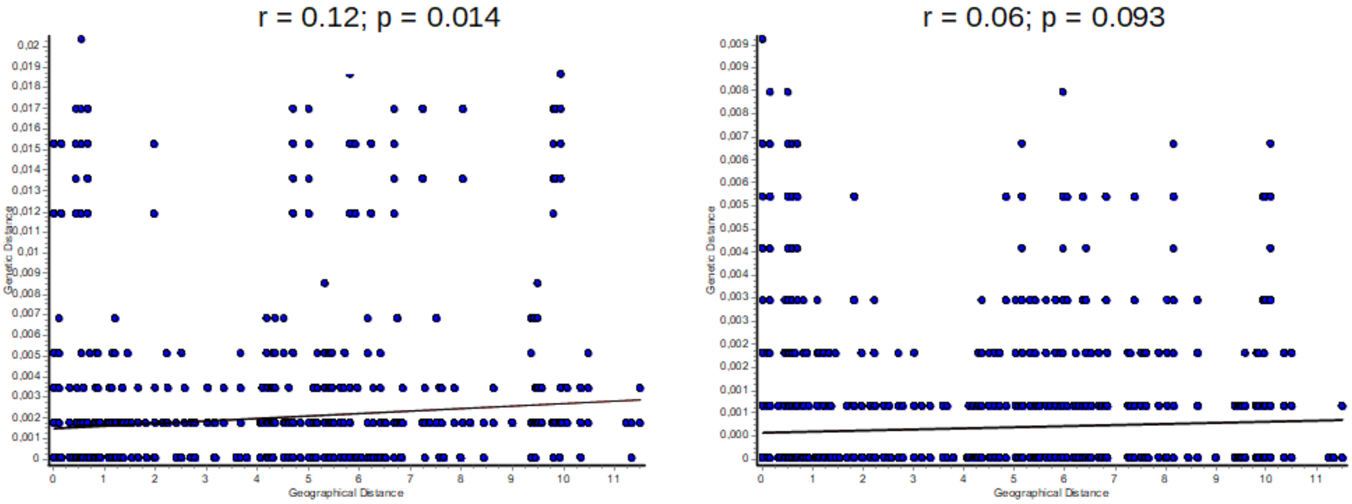


Figure 6: Haplotype network for *ITS* and *rpS16*. Estuaries sampled are identified by colour, circle sizes represent haplotype frequency, branch segments represent single base changes or indels. Small black circles represent putative intermediate haplotypes. The location of the two dominant *ITS* haplotype locations are shown on the map of the South African coast.

Spatial and genetic structure

When estuaries were considered as populations the results of a global AMOVA analysis indicated a high degree of gene flow with 59.40 % of variation within estuaries and 40.60 % of variation among estuaries for *ITS* sequences (Φ_{ST} = 0.38; p <0.0001). The global AMOVA analysis of *rpS16* sequences showed 87.59 % of variation within estuaries and 12.41 % of variation among estuaries (Φ_{ST} = 0.12; p = 0.0073). Samples were further divided into groups according to the *ITS* haplotype network. *ITS* haplotype diversity (54.35 %) could be accounted for by grouping the estuaries between the west and south coast (p <0.0001), with 4.44 % of variation among estuaries within groups (p = 0.025) and 41.21 % within estuaries (p = <0.0001). There was less variation among estuaries within groups and a lower Φ_{ST} using *rpS16* markers than *ITS* markers. A Mantel test of *ITS* had a very low but significant (p<0.05) isolation by distance signal, whereas there was no isolation by distance for *rpS16* (*ITS: r* = 0.12, p = 0.014; *rpS16*: *r* = 0.06, p = 0.093) (Figure 7). Results of the AMOVA are given in appendix Tables 1A & 2A.



ITS *rpS16*

Figure 7: Results of the Mantel tests for *ITS* and *rpS16* sequences. Geographical distance is shown on the x-axis and genetic distance on the y-axis.

Demographic patterns

The observed mismatch distribution analysis of *ITS* and *rpS16* data showed a ragged distribution indicating that *S. tegetaria* populations are old and have not undergone major historical extinction and recolonization events (appendix Figure 1A). The Tajima's D and Fu's Fs statistic rejected the null hypothesis of constant size. The Tajima D value was -2.15 (p = 0.0005) and Fu's Fs was -5.16 (p = 0.009) for *ITS* sequences and the Tajima D value was -2.07 (p = 0.0008) and Fu's Fs was -5.40 (p = 0.002) for *rpS16*. Genetic landscape shapes displayed a pattern of genetic diversity/divergence that increases along the coastline from west to east. The *ITS* and *rpS16* landscape shapes had similar shapes and only *ITS* is shown in Figure 8.

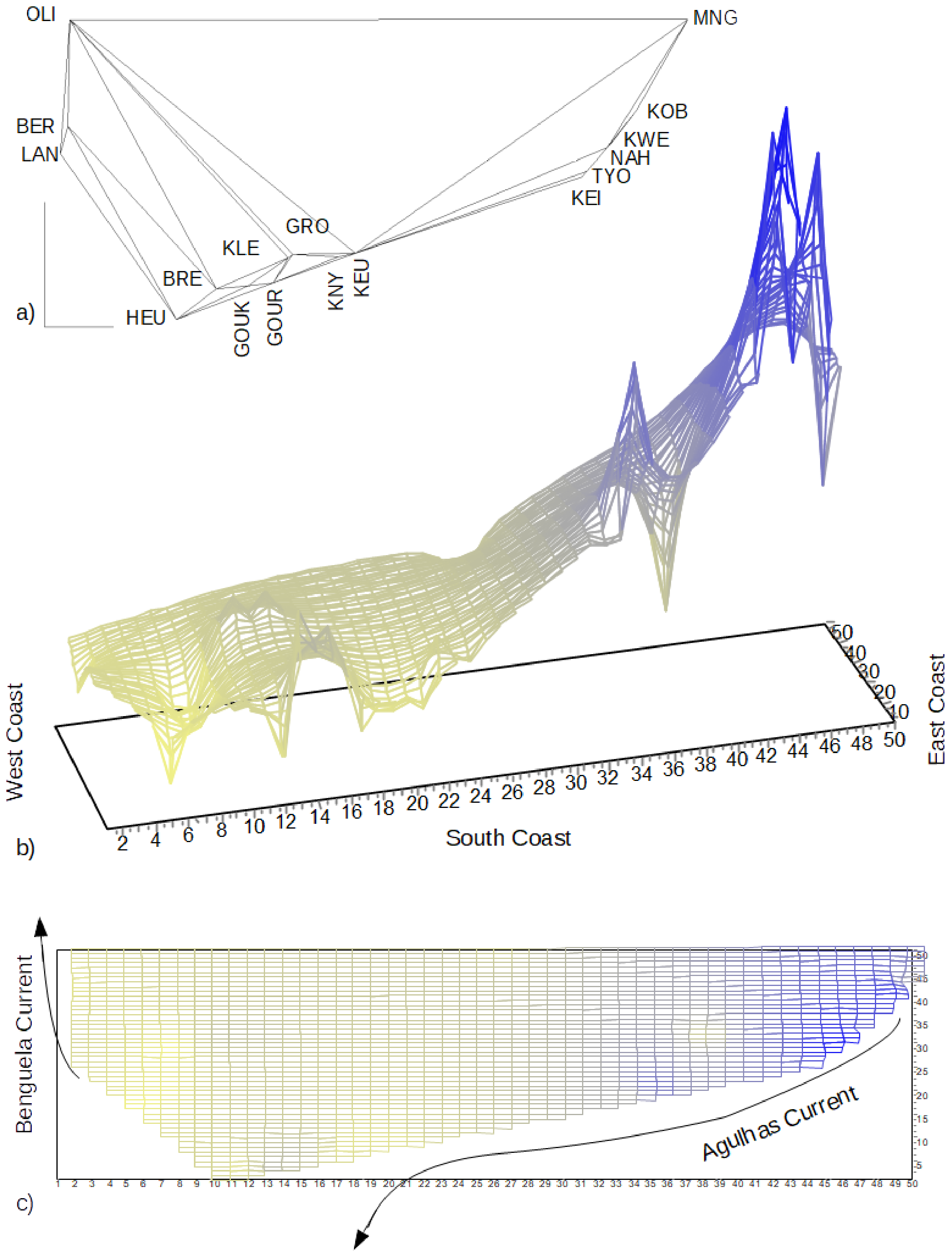


Figure 8: Connectivity network (the midpoint of edges from Delaunay triangulation) a) used for calculations of genetic diversity across the landscape, viewed at different angles in b) and c), between sample locations in relation to the direction of ocean currents. Estuaries are abbreviated as follows: OLI=Olifants, BER=Berg, LAN=Langebaan, HEU=Heuningnes, BRE=Breede, GOUK=Goukou, GOUR=Gourits, KLE=Klein Brak, GRO=Groot Brak, KNY=Knysna, KEU=Keurbooms, KEI=Keiskamma, TYO=Tyolumnqa, NAH=Nahoon, KWE=Kwelera, KOB=Kobonqaba and MNG=Mngazana.

Discussion

The objective of this study was to determine the phylogeography of *S. tegetaria* and therefore make inferences about the biogeographical history of the species. Weak genetic structure was found in this study with a break in the warm temperate region between Breede and Goukou for nuclear DNA haplotypes adding to the growing molecular evidence of similar historical vicariance events for estuarine macrophytes along the south coast. A unique haplotype occurs between Olifants and Breede Estuary, which is not found further east. The divergence is shallow with one mutational point separating these two haplotypes, suggesting that there was a geographic barrier recently or for a short duration during which the polymorphism became fixed (Graham and Fine, 2008). The glacial and interglacial periods that occur at about 100 000 year cycles repeatedly changed the South African coastline (Fisher *et al*., 2010). Four major glaciation events occurred during the Pleistocene during which the sea-level was about 120 m lower than at present (including the last glacial maximum about 18 000 years ago), exposing large areas of the continental shelf (Van Andel, 1989; Compton, 2011). During glacial maxima the coastal plain on the Agulhas Bank was widened from 50 km to 200 km south of where the Breede Estuary is located today and coastal rivers extended up to 100 km seaward as sea-levels fluctuated (Van Andel, 1989). The shoreline was rocky with cliffs west of Cape Agulhas and flat terrain to the east (Van Andel 1989). Bathymetric surveys and sedimentological data suggests that the Breede River flowed in a south westerly direction and that the estuary was located south east of Cape Agulhas (Dingle and Rogers, 1972). The Gourits, Keurbooms and Gamtoos Rivers flowed southwards and were possibly separated from the Breede Estuary by the topography of the Agulhas Bank and divergent ocean currents (Dingle and Rogers, 1972; Shannon and Chapman, 1983; Largier *et al*., 1992) (Figure 3). The river systems were located in an area that experienced a great extent of expansions and contractions during glacial and interglacial periods and the estuaries would have relocated over large distances which may account for the genetic structure seen in Figure 6. Disjuncts on the south coast were also described for *J. kraussii* and *S. meyeriana* where the continental shelf is shallow and glacial/interglacial cycles exposed large areas. *Salicornia meyeriana* had geographically distinct lineages that overlap with the disjunct of *J. kraussii* (further east between Knysna and Groot Estuary) and *S. tegetaria*. Comparison of these three species suggest that the historical processes that caused this were similar and occurred at a fine scale on the Agulhas Bank.

A small amount of genetic variation of rDNA (1-8 point mutations and 2 deletions) and cpDNA (1-5 point mutations and 3 deletions) nucleotide sequences of *S. tegetaria* were found within and between estuaries in the warm and cool temperate biogeographical regions of South Africa. Along the Atlantic and Gulf coasts of North America, the chloroplast DNA haplotype diversity of *Spartina alterniflora* ranged between h = 0.11 and h = 0.83 and between h = 0.31 and h = 0.59 for nuclear DNA (Blum *et al*., 2007). By using a measurement of clonal diversity instead, Hughes and Lotterhos (2014) and Richards *et al*. (2004), found high diversity for *Spartina alterniflora* which was highly variable among salt marshes. These studies suggest that genetic diversity of clonal plants may be higher than previously thought and may occur on a finer scale which may be correlated with environmental conditions (Hughes and Lotterhos, 2014), influencing the salt marsh community structure and function (Travis and Proffitt, 2016). Interactions between clones for example may have an effect on reproductive effort (Travis and Proffitt, 2016). Calculating the clonal diversity of *S. tegetaria* populations at a finer scale may provide more insights into *S. tegetaria* population dynamics.

The low Φ_{ST} values reflect the homogeneous genetic structure of *S. tegetaria* throughout the study area, which could indicate a high amount of gene flow among estuaries. *Salicornia tegetaria* have salt tolerant seeds that are adapted to spread along the coastline with small hairs that trap air, making them light enough to float in the water column where dispersal is determined by the direction and velocity of tidal currents (Dalby, 1963; Huiskes *et al.*, 1995). Long distance dispersal is likely due to seeds travelling with birds, either intestinally or by adherence (Figuerola *et al*., 2003; Chang *et al*., 2005; Neff and Baldwin, 2005). The seeds of *Salicornia* have survived long distances with the colonization of Africa from Eurasia at least twice in its history (Piirainen *et al*., 2017) and was proposed as the mechanism by which *S. meyeriana* spread to Africa (Slenzka *et al*., 2013). The haplotype networks show connectivity between estuaries and biogeographical regions. A link between west and east coast is evident from *ITS* and *rpS16* haplotypes at Berg Estuary (Figure 6).

Genetic diversity is influenced by mutation rate, gene flow and the genetic effective population size (Avise, 2009). The low genetic diversity of the *rpS16* intron could be due to lower mutation rates or due to a lower effective population size. It is assumed that the chloroplast DNA of *S. tegetaria* has a smaller effective population size than nuclear DNA due to their different mode of inheritance and a lower amount of gene flow for cpDNA than rDNA is expected. It is not known if the cpDNA of *S. tegetaria* is inherited paternally, maternally or biparentally, but is assumed to be maternally inherited as are most angiosperms (Mogensen, 1996). The higher $\Phi_{ST} = 0.41$ ($p < 0.0001$) of *ITS* haplotypes compared to $\Phi_{ST} = 0.12$ ($p = 0.0073$) of *rpS16* haplotypes could suggest that seed-mediated gene flow (which transmits cpDNA and nDNA) is more successful than pollen-mediated gene flow (which only transmits nDNA). The highly reduced flowers suggest wind pollination where there is reduced gene flow with distance. *Salicornia tegetaria* displays clonal growth and outcrossing. The progeny of clonal plants are identical, but can produce highly diverse offspring from the clonal plants when outcrossing occurs (Stebbins, 1950).

When considering the phylogeography of plants, it is necessary to consider reticulate evolution (Linder and Rieseberg, 2004). Hybridisation and different ploidy levels are known to occur in species of the subgenus *Afrocornia* (O'Callaghan, 1992; Steffen *et al.,* 2010; Steffen *et al.,* 2015) and may be important in the evolution of *Sarcocornia* in South Africa (Steffen, 2006 cited in Steffen *et al.,* 2010). For example 9 % of *Sarcocornia* samples studied by Steffen *et al.* (2010) were identified as putative hybrids including *S. tegetaria x capensis*, *S. tegetaria x decumbens*, *S. tegetaria x natalensis*, *S. tegetaria x pilansii* and *S. tegetaria x littorea. Salicornia tegetaria* was the parent in 61 % of the samples with a supposed history of hybridisation. The haploid, uniparentally inherited *rpS16* intron reveals one half of the parentage of *S. tegetaria* and could identify them as belonging to one of the two clades of the parents without revealing the hybrid ancestry if introgression and fixation have not occurred (Small *et al*., 2004).

The significantly negative Tajima's D and Fu's F_S reflect an excess of rare polymorphisms in *S. tegetaria* which may be due to positive selection or a recent increase in population size (Kreitman, 2000). Both *ITS* and *rpS16* loci had negative Tajima's D and Fu's F_S values, supporting the latter explanation of population size increase (Purugganan and Suddith, 1999; Kreitman, 2000; Chiang *et al*., 2004). The high h and low π (h > 0.5 and π < 0.5 %) of the *ITS* DNA region is characterised by one or two dominant haplotypes embedded in a cluster of haplotypes that differ by a single or few mutations from the dominant haplotype (Grant and Bowen, 1998) due to the retention of new mutations under expansion (Avise, 2000; Rogers and Harpending, 1992). Single rare *ITS* haplotypes were predominantly found in warm temperate estuaries including Goukou, Groot Brak, Knysna, Keiskamma, Nahoon, Tyolomnqa and Kobonqaba and one in the cool temperate at Langebaan (Figure 6). Single *rpS16* haplotypes were found in the warm temperate region at Heuningnes, Groot Brak, Tyolomnqa, Nahoon, Kwelera and Kobonqaba. These estuaries may be important genetic repositories.

A pattern of increased genetic differentiation along the coast can be seen in Figure 8. An increase in the fixation of polymorphisms of species in the warm temperate and subtropical regions may occur when species have smaller effective population sizes, barriers to dispersal, experience founder effects (Mindell and Thacker, 1996) or have longer independent evolutionary histories (Hackett and Rosenberg, 1990) as well as historical climatic shifts (Soltis *et al*., 1997; Taberlet, 1998) in which increases in stable temperatures, precipitation and habitat could possibly promote speciation and divergence over time (Martin and McKay, 2004). The very low but significant isolation by distance signal of nuclear DNA (Figure 7) suggests that pollen and seed dispersal is more likely to occur over short distances. The r-value was calculated using direct distances. It is possible that the r-value would increase if distance along the coast were used instead (e.g. Islam *et al*., 2014). There was no spatial autocorrelation found for cpDNA suggesting that seeds can travel larger distances though studies have shown that the dispersal capability of Chenopodiaceae are low e.g. *Salicornia europa* L. remain buoyant for 1.5-2 hours (max 24 h), making long distance dispersal less likely. The seeds may also have travelled on broken infructescences, as seedlings (Kadereit *et al*., 2007) or on mats of wrack which can travel 6.5-15.9 km during one ebb or flood tide (Minchinton, 2006). Seeds of *S. europa* have been found present in wrack and successfully germinated (Minchinton, 2006). The direction of increased genetic differentiation suggests that dispersal has occurred in the same direction as the Agulhas Current. The Agulhas Current flows close to the continental shelf except during upwelling events in summer (Bryden *et al*., 2005; Lutjeharms and de Ruijter, 1996). The timing of reproduction may therefore be an important determinant of successful disseminule dispersal which may become limited between the west and south coast near the Agulhas Bank as the current changes position. Patterns of genetic differentiation of marine and estuarine species that disperse with currents along the South African coast have been variable. For example, a decrease in invertebrate genetic diversity was found from the west of Cape Agulhas to the east coast due to recent range expansion in the east (Evans *et al*., 2004), whereas an increase in genetic diversity was found in a coastal crab, mudprawn and fishes from the west coast to the south and east coast (von der Heyden *et al*., 2008; Teske *et al*., 2009, 2014). It is possible that similar processes could shape the genetic diversity along the coast in some cases for macrophytes and marine organisms.

Genetic delimitation of *Afrocornia* subspecies may be useful in identification. The decumbent *Salicornia* plants of about 6 cm height found at Orange River Mouth were similar in morphology to *S. tegetaria* leading to the misidentification and collection of genetic material. Though the morphology was similar, they only occurred as single plants and did not form the mats found at other estuaries, but may have been newly established plants. The differences in genetic sequences collected at Orange River Mouth were visually determined to be unique compared to sequences from the rest of the estuaries for both nuclear and chloroplast regions and shared a 99 % similarity with *S. terminales* and *S. mossiana*. *Salicornia terminales* samples were collected at Kamieskroon, Namaqualand in the Northern Cape and the genetic material of *S. mossiana* was collected at Dwarskerbos in the Western Cape (Kadereit *et al.,* 2006). *Salicornia mossiana* is described as an erect shrub, up to 50 cm high, endemic to the Western Cape mainly occurring inland where coastal plains were cut off from the sea during the Pleistocene and on elevated supratidal terraces of large rivers. *Salicornia terminalis,* described as a shrub up to 100 cm high, found along the riverbanks of Namaqualand, can be considered an eco-morphotype of *S. pillansii,* which was seen in the supratidal habitat of Orange Estuary. *ETS* sequencing placed *S. terminalis* in an unresolved clade with *S. natalensis* and *S. pillansii* making it difficult to differentiate using molecular data (Steffen *et al.,* 2010).

Conclusion

In this chapter the phylogeography of *S. tegetaria* was explored. It is shown that there is a dominant chloroplast DNA haplotype along the South African coast while two different nuclear DNA haplotypes were found with a disjunct between Breede and Goukou Estuary. These estuaries are situated on the edge of the continental shelf that forms part of the Agulhas Bank and the split most likely resulted from historical sea-level changes. The results also indicate that there is gene flow between the populations of *S. tegetaria*, most likely in the direction of the oceanic currents and via long distance dispersal, and that rare haplotypes are present in many of the estuaries sampled. This suggests that there is clonal diversity along the coast in which genotypes may have variable functional traits that could affect community structure and genetically diverse seed banks. This could increase the resilience of the ecosystem following disturbance (Edwards *et al*., 2005; Proffitt *et al*., 2005; Crutsinger *et al*., 2006; Hughes, 2014). The genetic diversity of clonal species such as *S. tegetaria* depend on environmental conditions, intraspecific competition, clonal versus sexual reproduction and the degree of outcrossing (Davis *et al*., 2004; Travis and Hester, 2005; Hughes and Lotteros, 2014; Travis and Proffitt, 2016). Phenotypic plasticity is a trait that enables salt marsh species to tolerate the wide range of ecological conditions found in estuaries (Callaway *et al*., 2003). It has also been shown that the growth rate and reproductive timing of *Salicornia* species in relation to environmental heterogeneity may in part be due to genetic differentiation (Harper and White, 1974; Jefferies *et al*., 1981; Silva *et al*., 2007). Patterns of genetic differentiation of species that display phenotypic plasticity such as *S. tegetaria* may be due to variation in environmental conditions as can be found in the varying climatic regions along the southern African coast (Adam, 1993; Van Niekerk and Turpie, 2012). As yet, differences in the growth and reproduction of salt marsh species have not been compared in the different biogeographical regions of South Africa. In the next two chapters the biomass allocation and phenology of *S. tegetaria* will be investigated and compared between the warm and cool temperate biogeographical regions to determine if there are differences in response to the varying abiotic factors. Biomass allocation is an important mechanism by which plants respond to resource based and non-resource based environmental stress (Bazzaz *et al.*, 1987; Minden *et al*., 2012). In Chapter 4 the biomass of vegetative growth above- and belowground are compared in six estuaries spanning the two biogeographical regions. The time at which *S. tegetaria* seeds are released is an important determinant in the dispersal of seeds on the west coast and further along on the south east coast as they rely on oceanic currents that are seasonally variable, which has implications for the genetic diversity of the species. In Chapter 5 the phenology of *S. tegetaria* is investigated.

Chapter 4. Measuring Variation in the Biomass of *Salicornia tegetaria* along a Latitudinal Range.

Introduction

Plants produce biomass through the accumulation of carbon and nutrients during the processes of photosynthesis and cellular growth. Biomass is determined by fresh or oven-dried biomass which is the product of CO_2 assimilation by aboveground plant organs minus the respiratory loss of the whole plant (Brouwer, 1962; Amthor, 2000). The biomass production of salt marsh species is primarily affected by elevation (Mudd *et al.*, 2009; Ouyang *et al*., 2017), tidal range, freshwater input (Hsiao *et al*., 1976; Valiela *et al*., 1978a; Charles and Dukes, 2009; Ouyang *et al.*, 2017), groundwater level (Armstrong *et al*., 1985; Minden and Kleyer, 2011), salinity (Naidoo, 1987; Saintilan, 1997; Minden and Kleyer, 2011), redox potential (Mendelssohn *et al*., 1981), seasonal variation (Pennings and Callaway, 1992; Crosby *et al.*, 2015) including photoperiod (Ventura and Sagi, 2013) and temperature (Charles and Dukes, 2009; Gedan and Bertness, 2010; Baldwin *et al*., 2014), nutrient availability (Valiela *et al*., 1976; Morris and Bradley, 1999; Lee and Dunton, 2000; Lovelock *et al*., 2007; Darby and Turner, 2008; Palomo and Niell, 2009), soil texture (Troyo-Diéguez *et al*., 1994), atmospheric CO_2 (Curtis *et al*., 1990; Ziska *et al*., 2004), species interactions (Castellanos *et al*., 1994; Dormann *et al*., 2000) as well as the interaction between environmental conditions (Naidoo, 1987; Pennings and Callaway, 1992; Lenssen *et al.*, 1995; Minden and Kleyer, 2011).

Biomass is divided into aboveground biomass (AGB) and belowground biomass (BGB). The aboveground biomass includes all living vegetation above the soil including stems, bark, foliage and seeds (Kajimoto *et al*., 1999; Marchiori *et al*., 2016) while the belowground biomass includes all living biomass below the soil including roots, rhizomes and bulbs (Ravindranath and Ostwald, 2008). The growth of roots can be altered by short-term environmental signals such as the availability of oxygen, water and nutrients (Shahzad and Amtmann, 2017) and the ratio of BGB/AGB represents the allocation of biomass to plant organs to capture limiting resources (Brouwer, 1962; Kajimoto *et al.*, 1999), which is restrained by a salt-waterlogging gradient in halophytic salt marsh species (Minden *et al*., 2012). The belowground biomass accounts for more than half of the total plant biomass in salt marsh systems (Schubauer and Hopkinson, 1984; Ouyang *et al.*, 2017). The most important factors that influence biomass allocation in intertidal salt marshes are groundwater level, salinity and nutrient availability (Minden and Kleyer, 2011; Minden *et al*., 2012). Responses to these factors can vary widely for halophytes, for example a study found that there was a greater allocation to shoots resulting in a 33 % decrease in the BGB/AGB ratio of the succulent shrub *Salicornia virginica* (L.) with a moderate increase in salinity (± 20), while it caused a drastic reduction in growth with a 90 % increase in the BGB/AGB ratio of the sedge *Scirpus robustus* (Pursh.) (Pearcy and Ustin, 1984). Increases in root biomass may ameliorate a decrease in porewater nutrients or freshwater water availability (Schubauer and Hopkinson, 1984; Colmer and Voesenek, 2009; Minden and Kleyer, 2011). For example, higher BGB/AGB ratios of perennial

Salicornia spp. have been found in hypersaline conditions, whereas biomass production was highest at a salinity similar to that of sea water (Mahall and Park, 1976a; Ibañez *et al*., 1999; Curcó *et al*., 2002; Pont *et al*., 2002; Scarton *et al*., 2002; Redondo-Gómez *et al*., 2006; Guo and Pennings, 2012). Extensive studies describing the AGB, BGB and biomass production of salt marsh macrophytes, especially *Spartina* spp. have been conducted globally, though physico-chemical variables are lacking and only broad inferences can be made. These include estuaries along the cold temperate to warm temperate Atlantic coast, the tropical coast of the Gulf of Mexico with some studies of Chenopodiaceae species in Mediterranean climates (see Table 2 for examples).

The BGB/AGB ratios of the growth form of the same species may be very different. For example *Sparina alterniflora* occurs as distinct height forms related to the mean high tide. Tall *S. alterniflora* (1-3 m high) are found along the creeks and bay fronts, becoming smaller further inland. Short *S. alterniflora* (less than 0.5 m high) are found in backmarshes (Valiela *et al*., 1978b; Niering and Scott Warren, 1980; Gross *et al*., 1991). A study found that tall *S. alterniflora* had a lower BGB/AGB ratio (4.25) compared to short *S. alterniflora* (10.74) (Roman and Daiber, 1984). Furthermore, the tall growth form showed significant seasonal variability, whereas the short growth form did not (Gross *et al.*, 1991). It was hypothesised that disturbance at creekbank sites results in a higher mortality rate with younger populations and less competition for nutrients compared to backmarsh sites, where competition for nutrients is higher, resulting in an increase in allocation to roots, root senescence and production (Smart, 1986 cited in Gross *et al.*, 1991). The short *S. alterniflora* macrophytes would thus be better able to compete for resources by maintaining a higher root biomass throughout the year (Gross *et al.*, 1991). Variation has also been found between the same species at different locations. *Spartina cynosuroides* in estuaries in Georgia with a similar latitude but higher salinity had lower ratios and higher production than in Mississippi (Schubauer and Hopkinson, 1984; Hackney and De La Cruz, 1986). Latitudinal variation in the root/shoot ratio of macrophytes have been studied on the Atlantic coast. Gross *et al.* (1991) found that *S. alterniflora* had similar live root/shoot ratios in three warm-temperate estuaries and three cold-temperate estuaries, but that the portion of dead belowground biomass was lower in warm temperate estuaries, possibly due to an increase in the decomposition rate. The higher root:shoot ratios found in the Netherlands and Nova Scotia, seen in Table 2, suggest that seasonal freezing may have an effect on the root:shoot ratio in colder climates where the aboveground biomass dies back completely during winter. The belowground primary production of Chenopodiaceae in semi-arid Mediterranean salt marshes (Curcó *et al*., 2002) was found to be lower than in temperate salt marshes (Gallagher and Plumley, 1979; Groenendijk and Vink-Lievaart, 1987). Furthermore, Mediterranean-type salt marshes dominated by evergreen succulent Chenopodiaceae species may or may not have marked seasonal changes in biomass (Curcó *et al.*, 2002; Palomo and Niell, 2009), whereas seasonal fluctuations are found in temperate Atlantic estuaries dominated by grasses (Schubauer and Hopkinson, 1984).

Table 2: A global assessment of aboveground biomass (AGB), belowground biomass (BGB) and biomass allocation ratios found for various salt marsh species. Average values were calculated from multiple sampling dates during the growth period of each species.

Area studied	**Conditions**	**Species**	**AGB ($kg.m^{-2}$)**	**BGB ($kg.m^{-2}$)**	**BGB/AGB Ratio**	**Literature**
California	Mediterranean	*Sarcocornia pacifica*	16.11	10.0	0.62	Mahall and Park (1976a)
Mississippi, similar latitude to Georgia	Gulf Coast, Sub-tropical, short mild winters. Lower salinity than in Georgia	*Juncus roemerianus*	2.0	11.05	5.53	De La Cruz and Hackney (1977)
Mississippi, similar latitude to Georgia	Gulf Coast, Sub-tropical, short mild winters. Lower salinity than in Georgia	*Spartina cynosuroides*	2.96	7.7	2.6	Hackney and De La Cruz (1986)
Blackbird Creek, 60km upstream from Delaware Bay	Atlantic Salinity 10-15	*Spartina alterniflora* *Spartina patens* *Phragmites australis*	2.29 1.36 1.25	9.65 14.2 7.80	4.21 10.44 6.24	Roman and Daiber (1984)
Georgia, similar latitude to Mississippi	South Atlantic, temperate/ tropical. Higher salinity than in Mississippi	*Spartina alterniflora* *Spartina cynosuroides*	7.0 28	43 55	6.14 1.96	Schubauer and Hopkinson (1984)
Oosterschelde Estuary, Netherlands	Temperate, cold winters	*Spartina anglica* *Elymus pycnanthus* *Halimione potulacoides* *Triglocan maritima*	0.58 0.45 0.86 0.27	12.6 7.76 13.34 14.24	21.74 17.10 15.47 52.74	Groenendijk and Vink-Lievaart (1987)
Along a latitudinal gradient from Georgia to Nova Scotia	Atlantic, warm to cool temperate with cold winters	*Spartina alterniflora* South *Spartina alterniflora* North	0.46 0.32	0.83 0.85	1.82 2.62	Gross *et al*. (1991)
San Francisco Bay	Pacific, Temperate	*Spartina alterniflora* *Spartina foliosa*	0.86 0.18	1.99 0.97	2.32 0.55	Callaway and Josselyn (1992)
Odiel, Spain	Mediterranean	*Sarcocornia perennis*	0.53	16.65	3.12	Castellanos *et al*. (1994)
Dipper Harbor, Bay of Fundy, Nova Scotia	Atlantic, cold winters	*Spartina alterniflora* *Spartina patens* *Plantago maritima*	0.27 0.18 0.15	72.1 27.8 18.3	26.89 15.35 11.89	Connor and Chmura (2000)
Po Delta, Italy	Atlantic – Mediterranean transition zone	*Sarcocornia fruticosa* *Phragmites australis*	0.68 0.46	28.29 32.51	4.17 7.15	Scarton *et al*. (2002)
Ebre Delta, Mediterranean	Mediterranean	*Sarcocornia fruticosa*	28.24	0.94	0.33	Curcó *et al*. (2002)
Palmones River Estuary, South of Spain	Mediterranean, Industrial and densely populated area	*Sarcocornia perennis* subsp. *alpini*	3.40	2.33	0.69	Palomo and Niell (2009)

Salt marshes can be sources or sinks of organic matter and nutrients (Elliott and Whitfield, 2011). Exchanges of organic matter and mineral nutrients between the terrestrial environment, salt marshes and the sea are driven by tidal flow, groundwater and precipitation (Valiela *et al*., 1978a). Inorganic nutrients that enter estuaries are converted to organic matter and then released as organic or inorganic nutrients (Valiela *et al*., 1978a), increasing productivity in the marine zone (Turpie, 2007) or are sequestered in the sediment (Chmura *et al*., 2003). The capacity of a salt marsh to sequester carbon can be linked to the allocation of biomass, decomposition and primary productivity which in turn can be linked to temperature, precipitation, sea-level, nutrients and sediment type as well as biological factors, such as species composition (Mcleod *et al.*, 2011). Chenopodiaceae species may be important carbon sinks (Palomo and Niell, 2009). While this study did not measure the export of biomass out of the system, sediment organic matter can provide some indication of whether study sites are storing or exporting plant biomass (Palomo and Niell, 2009; Waters, 2011; Chmura, 2013; Ouyang *et al*., 2017).

Succulent halophytes such as *Salicornia tegetaria* adjust their internal osmotic potential by producing organic solutes that are osmotically active and by taking up water from the surrounding environment that has a higher salinity, accumulating more ions which are sequestrated in the vacuoles (Jefferies, 1981; Cheeseman, 1988; Naidoo and Raghunanan, 1990; Flowers and Colmer, 2015). When salinity becomes too high, the high energy cost of maintaining a low water potential may result in a reduction in growth (Ownbey and Mahall, 1983). A greenhouse experiment of the growth responses, water potential and ion accumulation of *Salicornia natalensis* (Bunge ex Ung.-Sternb.) A.J. Scott subsp. *natalensis*, which is also widespread in South African estuaries (Steffen *et al.,* 2010), illustrates the mechanisms involved under different but constant salinity (Naidoo and Rughunanan, 1990). Naidoo and Rughunanan (1990) showed that both the above- and belowground biomass of *S. natalensis* increases with increasing salinity, primarily due to ion uptake and a concomitant increase in water content, reaching a peak at salinity of 21.6. Turgor pressure in the cells stimulate growth (further increasing the photosynthetic area) (Pearcy and Ustin, 1984; Redondo-Gómez *et al*., 2006) which then lowers ion concentrations in the cell. Water contributes significantly to the increase in biomass and an increase in stem diameter. Na^+ and Cl^- ions contribute significantly to the ion content which occurs at a higher concentration in the shoots than the external environment. At peak biomass production the BGB/AGB ratio is at its lowest. At higher salinity the total dry mass (which includes ion content) decreases due to a decrease in ion uptake and a decrease in K^+ concentrations (which is required for growth) (Flowers and Colmer, 2015), especially in the shoots. At salinity higher than 21.6 the osmotic potential remains low and tugor is maintained as carbon resources are re-allocated from growth to osmotic adjustment and cellular maintenance resulting in an increase in the BGB/AGB ratio (Amthor, 2000). This is a common pattern found in other succulent halophytes such as *Disphyma australe* Soland., *Sueda maritima* (L.) Dumort., *Sueda fruticosa* (L.) Forssk., *Triglochin maritimum* L., *Spergularia marina* (L.) Besser, *Aster tripolium* L. and *Salicornia rubra* Nels. (Neales and Sharkey, 1981; Khan *et al*., 2000, 2001; reviewed in Flowers and Colmer, 2008). A study of *S. fruticosa* shows that when salinity reaches toxic levels (Na^+ can no longer be sequestered), apical meristems die off and photosynthetic area is reduced (Redondo-Gómez *et al*., 2006).

Salicornia natalensis and *S. tegetaria* co-occur in South Africa. *Salicornia natalensis* is found in estuaries and inland locations such as salt pans as well as along tidal rivers and freshwater wetlands. The estuaries where the species are found are usually less saline due to a higher freshwater influx (Steffen *et al.,* 2010). A greenhouse experiment with *S. tegetaria* showed that it is more sensitive to submerged conditions than high salinity. Succulence also increased as salinity increased, but depended on the level of submergence. Growth and turgor decreased when salinity was higher than 35 (Adams and Bate, 1994). Stem elongation was highest when plants were grown in freshwater and damp conditions but when salinity increased to 35, submerged plants were able to grow better. The authors point out that in the field, environmental conditions are fluctuating and that *S. tegetaria* has been found at a wide range of salinity (12-42) and O'Callaghan (1992) has found *S. tegetaria* growing at Langebaan where salinity in the intertidal zone is highly variable, ranging from 15 to 140. *Salicornia tegetaria* is adapted to inundation and a wide range of physico-chemical conditions in the intertidal salt marsh environment (Tölken, 1967; Adams *et al*., 2016). Mean physico-chemical variables that have been recorded in field studies are summarised in Table 3.

Table 3: Physico-chemical conditions of *Salicornia tegetaria* found in other studies. PW = porewater, S = sediment, W = warm temperate, C = cool temperate

Estuary	**PW Salinity**	**PW EC (mScm^{-1})**	**PW Temp (ºC)**	**PW pH**	**PW depth (cm)**	**S Redox (mV)**	**S EC (mS.cm^{-1})**	**S Salinity**	**S Moisture (%)**	**S Organic (%)**	**S pH**	**Literature**
Nahoon (W)	29.4	-	20	6.4	-	-106.5	41.4	-	36.0	3.6	-	Geldenhuys *et al*. (2016)
11 Estuaries (W & C)	-	33.1	-	-	46.4	3.0	42.4	-	20.9	3.3	7.6	Adams *et al*. (2016)
East Kleinmonde (W)	-	-	-	-	9.7	-74.9	42.3	22.4	18.7	2.6	7.1	Vromans (2011)
Kowie (W)	-	-	-	-	27.7	90.1	37.0	22.1	-	-	7.6	Vromans (2011)

Objectives and hypotheses

The objective of this chapter is to determine if there are differences in the biomass allocation of *S. tegetaria* between the warm and cool temperate biogeographical regions of South Africa and whether physical drivers of biomass allocation are similar in each region. In this study permanently open estuaries have been chosen as study sites as they contain a larger area of salt marshes with stable conditions compared to TOCE's (Whitfield *et al.*, 2008; Vromans, 2011; Van Niekerk and Turpie, 2012). Biomass allocation may be driven by the different climatic regimes found in the warm and cool temperate regions. The warm temperate region has higher rainfall and milder temperatures compared to the cool temperate region in which estuaries have higher salinity and increased evaporation in summer (Harrison, 2004; Van Niekerk and Turpie, 2012). These differences may affect the growth of *S. tegetaria* (Riehl and Ungar, 1982; Pennings and Callaway, 1992; Sanchez *et al.*, 1998) as soil moisture and soil salinity codetermine soil water potential (Hsiao *et al.*, 1976; McGraw and Ungar, 1981; Riehl and Ungar, 1982). Temperature may also influence growth and production. The effects of temperature are unique to each species as their optimum growth temperature varies. A decrease in BGB/AGB ratio at the growth optimum is expected after which the ratio would increase as the temperature exceeds the growth optimum (Davidson, 1969). The optimum temperature for growth of *S. tegetaria i*s not known.

Salicornia tegetaria is found in the lower to middle intertidal area which is strongly correlated to elevation which determines the physico-chemical conditions found. The physico-chemical variables are therefore expected to be similar in all estuaries (Adams *et al.*, 2016). Even so, *S. tegetaria* displays a high degree of plasticity and grows in a wide range of physico-chemical conditions (Adams *et al.*, 2016) and it is expected that differences between the physico-chemical conditions measured may vary between the two regions which may influence the resource allocation of *S. tegetaria*. I hypothesise that plants will increase in stem height at a higher salinity due to turgor induced growth aboveground with an accumulation of ions (Naidoo and Rughunanan, 1990; Adams and Bate, 1994). A lower BGB/AGB ratio is expected at lower salinity (Adams and Bate, 1994). Higher BGB/AGB ratios are expected when the threshold salinity is reached at which aboveground biomass decreases and osmotic stress belowground increases. The physiological effects of pH has not been investigated for *Salicornia* species to my knowledge, but most halophytic plants grow at an optimum pH of 6.0-6.5 which is linked to the proton pumping activity in the cell membranes of roots in saline conditions (Braun *et al.*, 1986). I expect that pH levels will be lower at higher salinity (Al-Busaidi and Cookson, 2003; Angiolini *et al.*, 2013).

Sediment salinity could become higher due to evaporation (Angiolini *et al.*, 2013) and both sediment moisture and salinity is influenced by the water level of the adjacent water channel by which it is inundated regularly (Adams *et al.*, 1999). I hypothesise that total plant biomass may be higher in sediment with a higher moisture content which could be related to freshwater influxes, evaporation and the frequency of tidal flushing (Zedler *et al.*, 1999). The BGB/AGB ratio may increase under lower sediment moisture conditions or as the depth to groundwater increases, though these conditions are expected to be stable at the elevation that *S. tegetaria* grows (Bornman *et al*., 2002).

Study Site Descriptions

The study was conducted in estuaries with large intertidal areas containing monospecific stands of *Salicornia tegetaria*. Olifants, Berg and Langebaan were chosen in the cool temperate biogeographical region, and Heuningnes, Nahoon and Kwelera in the warm temperate biogeographical region (Figure 5). Olifants and Berg on the west coast and Nahoon and Kwelera on the east coast are permanently open estuaries whereas Heuningnes is a temporarily open/closed estuary that is kept artificially open and Langebaan is an estuarine embayment. Olifants, Berg, Langebaan and Heuningnes fall within the semi-arid Mediterranean climate with winter rainfall resulting in lower salinity during winter and hot, dry summers with day temperatures above 30 ºC. Nahoon and Kwelera fall within the warm temperate biogeographical region where the rainfall pattern is highly variable throughout the year and is usually slightly higher during autumn (March) and spring (October/November) and at a minimum in winter (June) (Schulze, 1965; Heydorn and Tinley, 1980; Jury and Levey, 1993). The temperature in the warm temperate region is mild ranging between 10-22 °C in winter and summer (Lubke and De Moor, 1998) (Figure 9). Olifants and Berg maintain their open state through river flow as their catchments reach beyond the arid coastal zone (Allanson and Baird, 2008). Estuaries on the southwest coast have smaller catchments that receive less rainfall. In warm temperate estuaries tidal currents maintain the open state of Nahoon and Kwelera (Allanson and Baird, 2008) (Table 4). Olifants, Berg and Heuningnes are important nursery systems that are high in biodiversity and together with Nahoon and Kwelera provide habitat for juveniles of the economically important *Argyrosomus japonicus* (kob) (Van Niekerk and Turpie, 2012).

Most of the ecosystem types in the cool temperate biogeographical region are rated critically endangered in the National Biodiversity Assessment (Van Niekerk and Turpie, 2012). In the warm temperate region 68 % of the habitat is least threatened and 32 % is critically endangered. Olifants, Berg and Kwelera have been identified as requiring partial protection. Nahoon, Heuningnes and Langebaan form part of Nature Reserves (Van Niekerk and Turpie, 2012).

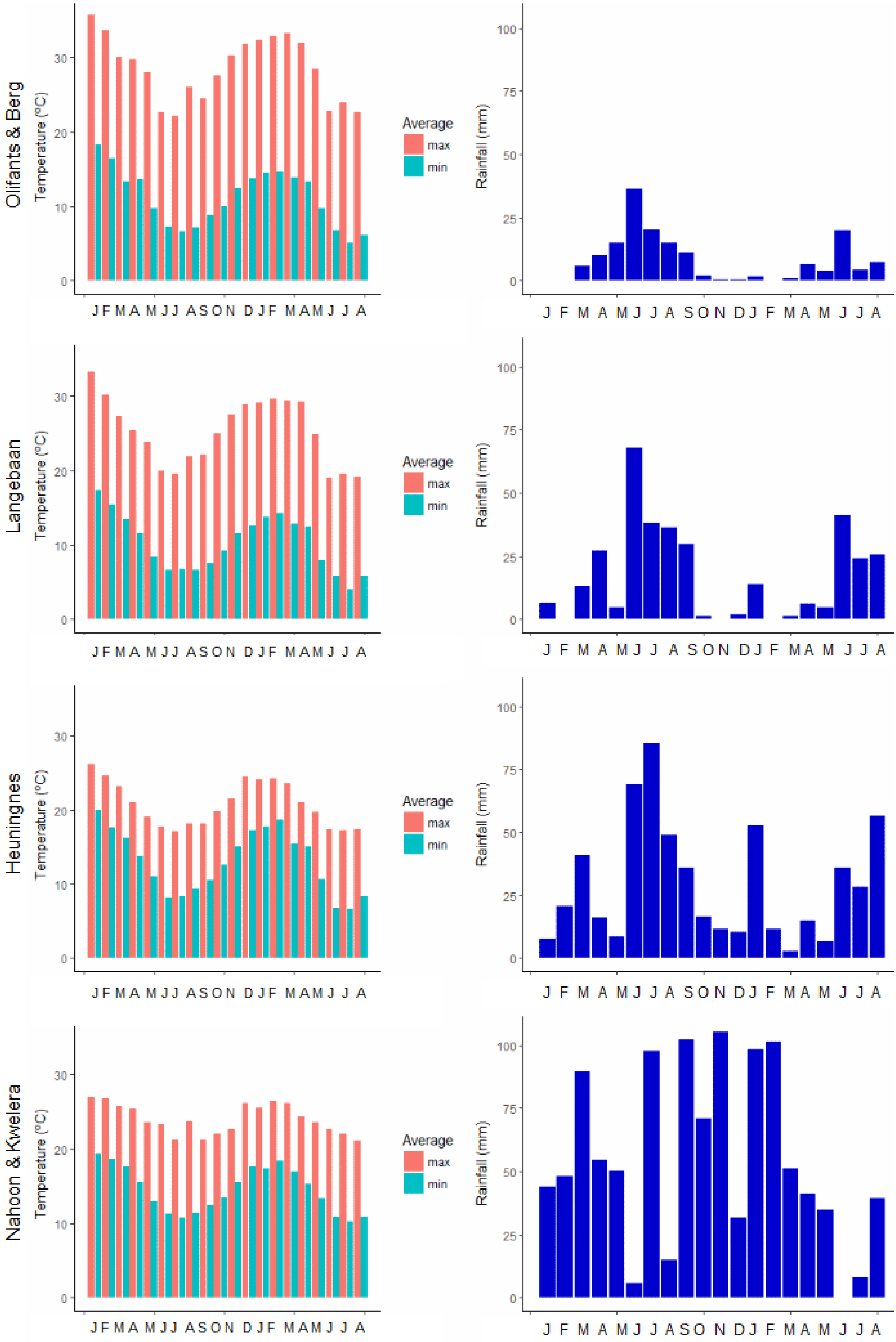


Figure 9: Daily maximum and minimum temperature and precipitation (Jan 2016 – Aug 2017) at Olifants and Berg Estuaries, Langebaan, Heuningnes Estuary, and Nahoon and Kwelera Estuary.

Table 4. Health index of estuaries (from Van Niekerk and Turpie, 2012). Langebaan was not included in the report.

Estuary	**Total Estuarine Habitat**	**Intertidal Salt Marsh**	**Water Quality**	**Physical Habitat**	**Estuary Health State**	**Ecological Category**
Olifants	974	83.9	Fair	Good	Fair	Moderately modified
Berg	6799	1677	Fair	Fair	Fair	Largely modified
Langebaan	834.7	123.3	-	-	-	-
Heuningnes	518.1	5.53	Fair	Fair	Fair	Largely modified
Nahoon	57.7	2.8	Poor	Good	Fair	Moderately modified
Kwelera	50.1	9.3	Good	Fair	Good	Largely natural with few changes

Olifants

Olifants/Doring system is one of the 20 largest catchments in South Africa, approximately 250 km long in the arid region of South Africa, contributing 2.8 % of the MAR of the country (Van Niekerk and Turpie, 2012). The estuary is 36 km long, extending to Lutzville causeway that acts as a barrier to further water flow. The estuary widens to 400 m toward the lower reaches with large areas of salt marsh on either side and a blocked channel forms a 2 km long backwater area 2 km from the mouth (Lamberth *et al.*, 2008). It is under flow modification pressure and the total freshwater to the system has been reduced by 34 % with two large dams in the catchment (Lamberth *et al.*, 2008; Van Niekerk and Turpie, 2012). Due to flow reductions the system remains marine-dominated for most of the year, becoming freshwater-rich during winter rainfall events. There is a waste water treatment works discharging into the river upstream from the estuary at Lutzville and pollution loading into the catchment from agriculture (Van Niekerk and Turpie, 2012). Olifants is part of Important Bird Areas sites and is utilised by non-breeding flocks of the African Black Oystercatcher (Van Niekerk and Turpie, 2012). Sampling was done along the blocked channel in the lower reaches of the estuary. The lower intertidal zone was waterlogged and standing water was present. Grazing was observed by sheep. *Bassia diffusa* and *Triglochin bulbosa* L. were present in the lower intertidal. Flowering and seeding inflorescences of *S. tegetaria* were observed in June 2016 (Plate 2).

Plate 2: Salt marsh at Olifants Estuary composed of mixed stands of *Salicornia tegetaria* and *Triglochin bulbosa* and a well filling with water (bottom right). Photos A. Rajkaran.

Berg

The Berg Estuary is a river-dominated estuary with extensive floodplains. The catchment lies in the Western Cape and receives winter rainfall. The estuary is approximately 65 km long, 3-5 m deep on average and 100-200 m wide at the channelised mouth, becoming narrower and shallower upstream. It falls within the Important Bird Areas (IBA), providing habitat to abundant Greater and Lesser Flamingos, and breeding colonies of Caspian Terns (Van Niekerk and Turpie, 2012). The estuary is under flow modification pressure with three major dams and numerous smaller farm dams in the catchment. The Berg River receives a natural annual runoff of 931 Mm^3 with flow reductions of 30 % (AEC, 2010; Van Niekerk and Turpie, 2012). Salinity and nutrient inputs vary with flow conditions. Seawater penetrates up to 40 km into the estuary during low-flow summer conditions and during winter inflow salt water is pushed back to within 10 km of the mouth. Upwelling at sea occurs during summer introducing water with lower oxygen levels and cooler temperatures into the lower reaches. The health of the estuary is also affected by siltation due to erosion in the catchment, development, overfishing and deterioration of water quality due to agriculture, residential and industrial pollution. An estimated 130 000 m^3 of industrial water flows into the estuary per day (Van Niekerk and Turpie, 2012). Nutrient inputs from the catchment have increased with an increase in pollution, for example total nitrogen concentration at the head of the estuary has increased from 300 ug.l^{-1} before 1980 to 2 000 ug.l^{-1} in 2005. *Zostera capensis* is found in the subtidal lower estuary, followed by *Spartina maritima* at lower intertidal elevations, and *Bassia diffusa, Triglochin* spp., *Cotula* L. species and *Salicornia tegetaria* intertidal salt marsh at higher elevations. The supratidal salt marsh is dominated by *Salicornia pillansii.* During sampling in June 2016 *Salicornia* *tegetaria* was flowering. Standing water was present in the lower intertidal zone. Three transects were designated from the supratidal edge to the edge of the creek bank in the lower intertidal marsh adjacent to housing development (Plate 3).

Plate 3: Patches of *Bassia diffusa, Triglochin bulbosa* and *Salicornia tegetaria* at Berg Estuary. Photos A. Rajkaran.

Langebaan

Langebaan was originally classified as a lagoon by (Day, 1959) due to the absence of an inflowing river resulting in a lack of a salinity gradient characteristic of estuaries. However, the system is much deeper and larger than coastal lagoons (14.5 km in length, 4.0 km wide and an average depth of 1-2 km) and receives freshwater inflow from groundwater and contains estuary-associated biota with well developed salt marshes in the south (Flemming, 1977). Whitfield (2005) proposed classifying Langebaan as a new type of estuary, a coastal embayment and the classification is employed here. The Langebaan Embayment is situated 100 km north of Cape Town and controlled by the National Parks Board. The Potsberg Peninsula forms a long arm, protecting the embayment from the Atlantic Ocean, resulting in calm, tidal conditions suitable to salt marsh formation. Farming is not suitable due to the sandy nutrient poor soil, and the farms surrounding the embayment are now a part of the West Coast National Park and an important site for birds (Underhill, 1987; Hanekom *et al.*, 2009). Langebaan falls within the Mediterranean climate and is semi-arid with a mean annual precipitation between 253.1 – 269.9 mm (Flemming, 1977). Due to the lack of low freshwater inflow, sediment from surface runoff is very low and the embayment lacks open mud flats. The area with the most mud content is the southern part of the lagoon with a mud content over 10 %, with a clay-mineral fraction less than 2 %, the rest being silt (Flemming, 1977). Beach ridges separate the salt marsh from the embayment, consisting of quartz sand and mud, which increases as it merges with the underlying marsh muds. The groundwater seepage occurs at Oostewal and Geelbek where the area is dominated by *Phragmites australis* (Shannon and Stander, 1977; O'Callaghan, 1994). The rich diversity of invertebrate and thus bird species in the embayment is sustained by salt marsh plants as plankton entering the bay is consumed by mussel beds in the channels. The nutrient rich waters from the Atlantic are absorbed by salt marshes during the growing season and released after decomposition by bacteria (Christie, 1981) to be returned to the system as guano (Bosman and Hockey, 1986). The study site chosen at Langebaan was Bottelary. During sampling in August 2016 *S. tegetaria* was not flowering. Grazing by ostriches was observed. Other species found in the salt marsh community was *Salicornia meyeriana* (Moss) Piirainen & Kadereit and *Bassia diffusa. Sporobulus virginicus* and *Limonium scabrum* (Thunb.) Kuntze where also present on raised tussocks (Plate 4)

Plate 4: Bottelary at Langebaan Estuarine Embayment with a mosaic of *Salicornia* spp. and *Bassia diffusa* (top left). Photos C. E. Brown.

Heuningnes

Heuningnes is near the southern tip of Africa, 15 km north of Cape Agulhas, with large flood plains fringed with salt marsh and fertile plains for agricultural use and mobile dune fields driven by wind that often blocked the river mouth, flooding the low-lying farmland during the rainfall season (Lubke and Hertling, 2001). The Heuningnes catchment lies within the eastern boundary of the Mediterranean climate with winter rainfall and hot, dry summers with a mean annual precipitation of 400 mm. Heuningnes Estuary was a temporarily open/closed estuary that was artificially permanently opened by stabilising sand dunes with *Ammophila arenaria* L. and indigenous dune plant seeds to eventually form a brushwood barrier that created the large flood tide delta (Lubke and Hertling, 2001). Most of the catchment consists of farmland, with an irrigation dam, road bridge, weir and pipe culverts. Zoetendalsvlei acts as a reservoir with runoff to the sea occurring during overflow events. Due to the flat topography, sedimentation rates are low and there is not much sediment transport from runoff. The estuary extends 12 km across the flat coastal plain, with strong tidal activity in the lower 2 km. The water in the inlet channel is clear with high salinity. An influx of sand from the sea into the estuary is favoured by the weak river discharge and low elevation of the estuary relative to the sea. Salt marshes near the mouth occur on sandy substrates dominated by *Limonium* spp., *Salicornia meyeriana* with *Afrocornia* spp. Terrestrial vegetation encroach onto this area due to a restriction of tidal interaction. Further upstream, areas that are flooded more often, consisting of *Salicornia tegetaria, Sarcocornia decumbens,* a mixed zone with *Bassia diffusa* and *Sueda maritima,* followed by *Limonium scabrum* and *Juncus kraussii* are situated on mud (Bickerton and Pierce, 1984). The estuary is a Ramsar site and the lower reaches of the estuary fall within the De Mond Nature Reserve. The salt marsh close to the mouth was sampled in this study. A clear zonation pattern was found with a narrow portion of *S. tegetaria* and *Bassia diffusa. S. tegetaria* inflorescences were in flower in May 2016 (Plate 5).

Plate 5: A clear zonation pattern was visible at Heuningnes Estuary with *Salicornia tegetaria* in the foreground and *Bassia diffusa* in the background (top left). Photos A. Rajkaran.

Nahoon

Nahoon Estuary which is formed from a drowned river valley is 5 km long with an average depth of 2.24 m. Nahoon is a marine dominated estuary surrounded by suburban development with a tidal influence up until Abbotsford Bridge which forms a barrier to further tidal flow. Nahoon Dam and dams in the sub-systems modify the flow into Nahoon Estuary. Sandstone outcrops occur on the eastern bank of the mouth and marine sediments are deposited in the lower part of the estuary forming a wide portion where the tidal flats have formed (Wiseman *et al.*, 1993). The Nahoon catchment area which is 547 km^2 falls within the bimodal summer rainfall region with the highest rainfall in October to December and a lower peak from January to March, and possible flooding during spring tides or storms. The catchment receives 719 mm mean annual precipitation and has a temperate to warm and humid climate (Reddering, 1987). Large monospecific stands of *S. tegetaria* were found in the lower intertidal marsh during sampling (Plate 6).

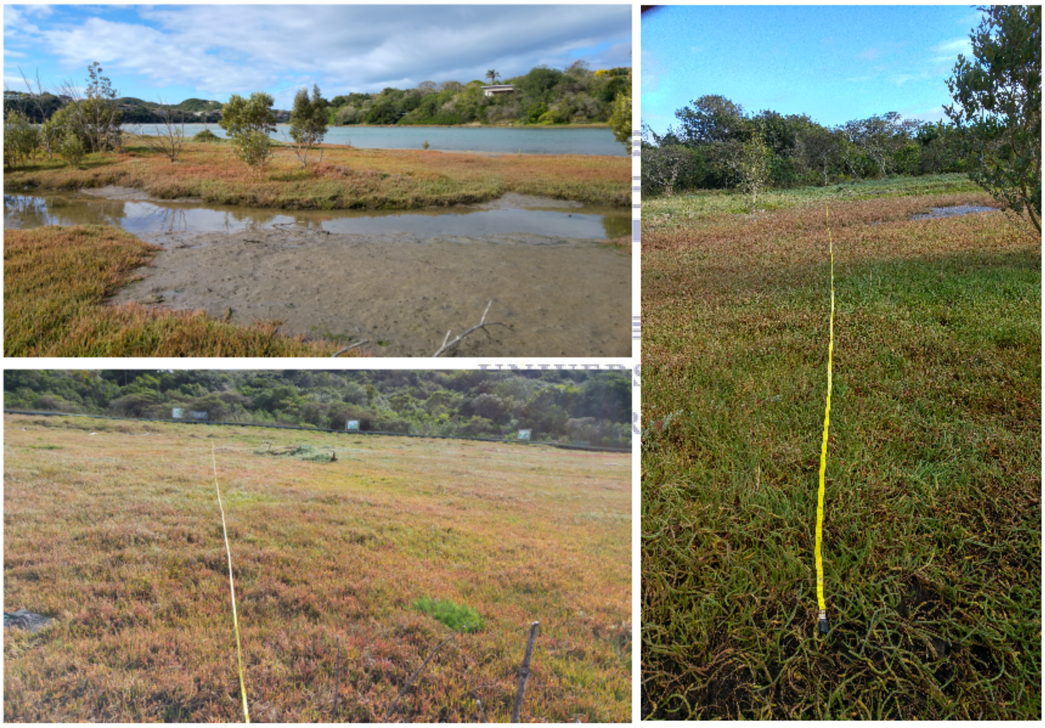


Plate 6: Salt marsh and mangrove species co-occur at Nahoon Estuary. *Salicornia tegetaria* is seen in the foreground and *Avicennia marina* in the background. Small patches of *Triglochin bulbosa* and *Bassia diffusa* are also visible (lower left). Photos A. Rajkaran.

Kwelera

The Kwelera catchment has an area of 391 km^2 and receives 824 mm mean annual precipitation. There are no major dams in the catchment and agricultural activity consists of diversified farming (Reddering, 1987). It lies on the same stretch of coast as Nahoon Estuary with the same climatic conditions, similar shapes, sizes tidal inlet configurations and bedrock characteristics (Reddering, 1987). The flood-tide wich dominates the estuary is 1.26 m deep and 4.9 km long, where a natural rapid prevents further tidal flow. Intertidal flats occur adjacent to the north bank with well developed stands of *Z. capensis* which trap suspended clay that form mud banks. The Kwelera Estuary has poorly developed intertidal flats but are well developed near the flood-tidal delta becoming narrower further upstream (Reddering, 1987). An island occurs in the channel which originated as point bars during river floods. Sampling locations were chosen on the intertidal flats between the island and the flood-tidal delta (Plate 7).

Plate 7: Kwelera Estuary with *Salicornia tegetaria* and *Bassia diffusa* in the foreground. Photo A. Rajkaran.

Materials and Methods

Sampling was undertaken during the winter of 2016 (May – August) and the summer of 2017 (November – February). Three quadrats containing 100 % *S. tegetaria* cover were chosen at the lower, middle and upper part along three permanent transects in the lower intertidal zone of each estuary. The three permanent transects were demarcated with wooden stakes (30 cm in length) and their GPS coordinates recorded. Measuring tapes were used to locate three sampling quadrats between the stakes. Three sediment samples were collected at each quadrat. Sediment cores were collected at the surface and at 50 cm depth. Sediments were analysed for redox potential *in situ* with a HANNA redox/pH meter by placing the probe into the sediment at each quadrat. Sediment pH, moisture content, organic matter content and electrical conductivity were measured in the laboratory. Sediment pH and redox potential was measured over one season only in summer. To measure pH, 50 ml of distilled water was added to 5 g of sediment and thoroughly mixed and measured with a HANNA redox/pH meter. To measure electrical conductivity, 250 g of sediment was air dried and enough distilled water added to create a paste which was filtered through Whatman No. 40 filter paper with a Buchman filter and measured with a YSI Conductance Meter (Model 35). The moisture and organic content was measured using the same 10 g of sediment sample. The samples were placed in crucibles and dried at 65 ºC for 48 hours. The difference in weight before and after drying was used to calculate the % of moisture content (Black *et al.*, 1965). Particle size was not measured as a number of other studies have conducted this type of analysis. Those studies are cited and discussed in the discussion.

Moisture content was calculated using the formula:

$$\frac{wet\,mass - dry\,mass}{wet\,mass} \text{ x } 100\ \%$$

The previously dried samples were ashed at 550 ºC in an ashing oven for 5 hours and the difference in weight before and after ashing was used to calculate the % of organic content (Briggs, 1977; Heiri *et al.*, 2001).

Organic content was calculated using the formula:

$$\frac{dry\,mass - mass\,after\,ashing}{dry\,mass}\ x\ 100\ \%$$

Porewater was measured as water filled the wells in which biomass and sediment were collected. In each augured well, porewater temperature, salinity and pH were measured using a YSI Professional Plus multimeter. Porewater depth was measured as the level to which water filled the wells. Temperature and rainfall data was provided by the South African Weather Service (SAWS). Daily maximum and minimum temperature and precipitation at Olifants and Berg Estuaries were measured at Vredenburg, 44 km and 237 km away respectively. Data for Heuningnes Estuary was sampled at Struisbaai, a distance of 33 km, and data from East London was used for Nahoon Estuary (12.4 km) and Kwelera Estuary (30.9 km) (Figure 5).

Plant height was measured for ten *S. tegetaria* stems and the aboveground biomass was harvested by clipping plants at the sediment surface within three 0.15 m x 0.15 m quadrats at each transect. All the standing live biomass within the quadrats were placed in plastic bags and transported to the University of the Western Cape (UWC) for processing. Samples were rinsed with water and any attached litter removed by hand. The aboveground biomass was dried in an oven for 48 hours at 60 °C and weighed (Schubauer and Hopkinson, 1984; Gross *et al.*, 1991).

Belowground biomass was collected by removing the sediment containing the roots to a depth of 10 cm within the three quadrats after the aboveground biomass was collected. Most of the roots were found up to a depth of 10 cm (Curcó *et al.*, 2002; Palomo and Niell, 2009). The sediment was placed in plastic bags and transported to UWC for processing. The belowground biomass was washed by hand with water to remove the bulk of sediment using a 1 mm sieve to trap fine roots. A second wash with 10 g Sodium polyphosphate in water removed the rest of the sediment. The water was passed through a 1 mm sieve until all the roots were recovered at each washing. The roots were dried for 48 hours at 60 °C and weighed (Hopkinson and Dunn, 1984).

The root/shoot ratio was calculated as $\frac{Belowground\ biomass\ dry\ weight}{Aboveground\ biomass\ dry\ weight}$

Statistical analysis

Statistical analyses were performed using R Statistical Software (R Development Core Team, 2005) and the packages Dplyr, ggbiplot, Hmisc, FSA, rcompanion and FactomineR. Data was tested for normality using Shapiro-Wilk normality test and non-parametric tests were used where data was not distributed normally. Sediment samples analysed at 0 cm and at 50 cm were not significantly different and pooled for further analyses. The number of replicates (N) was 106 for biomass, sediment electrical conductivity, moisture and organic content; 53 for sediment pH and redox and 89 for porewater measurements. Values are reported as means (±SE).

The statistical analyses were divided into three categories. Firstly, all data was analysed together at the landscape scale using a principal component analysis (PCA) to identify the major patterns of covariance between climatic, physico-chemical and biomass variables. The sediment redox potential and sediment pH was measured over one season and was not included in the PCA. Spearman's correlation tests were used to perform correlation tests including sediment pH and redox potential, which was excluded from the PCA.

Secondly, non-parametric Wilcoxon rank sum tests were used to test the variance of biological and physico-chemical variables between biogeographical regions and between winter and summer. A PCA was performed at the regional scale in the cool temperate and warm temperate biogeographical regions, respectively. Spearman's correlations were tested in each region including sediment pH and redox potential.

Thirdly, data for each estuary was analysed. A Kruskal Wallis non-parametric analysis of variance (ANOVA) was used to test the variance of biological variables (BGB, AGB and BGB/AGB ratio) and physico-chemical variables between estuaries. Porewater depth was not included as measurements were not taken deeper than 100 m. A Kruskal-Wallis multiple comparison post-hoc test was performed and p-values were adjusted with the Benjamini-Hochberg method. Spearman's correlations were also tested between physico-chemical and biomass variables for each estuary. Significance was determined at $p < 0.05$.

Results

Sediment moisture ranged between 18.4 – 72.1 % in the six estuaries sampled with a mean of 51.6 ± 1.9 % in the cool temperate and 30.1 ± 0.9 % in the warm temperate region. Sediment organic content ranged between 1.11 – 38.8 % with an average of 13.6 ± 0.9 % in the cool temperate and 7.0 ± 0.9 % in the warm temperate biogeographical region. Sediment redox potential ranged between -296.8 and 439.6 mV during the study and sediment pH was 6.7 ± 0.1 in the cool temperate and 8.1 ± 0.1 in the warm temperate biogeographical region. The electrical conductivity of the sediment was found to be between 20.1 and 93.6 mS.cm^{-1} with a mean of 49.1 ± 2.2 mS.cm^{-1} in the cool temperate and 36.1 ± 1.1 mS.cm^{-1} in the warm temperate region (Figure 10). Porewater temperature was 16.4 ± 0.4 ºC in the cool temperate region and 17.9 ± 0.5 ºC in the warm temperate region and porewater salinity was 36.8 ± 1.4 in the cool temperate and 37.1 ± 1.6 in the warm temperate region. Porewater pH was 7.07 ± 0.1 in the cool temperate and 7.66 ± 0.1 in the warm temperate region (Figure 11). The sediment and porewater measurements in each estuary and biogeographical region are summarised in appendix Table 3A & 4A.

Sediment moisture content, sediment organic content and sediment electrical conductivity was significantly lower in the warm temperate than the cool temperate region ($W = 2547$, $p < 0.0001$; $W = 2335$, $p < 0.0001$ and $W = 2222$, $p < 0.0001$), whereas sediment pH, porewater temperature and porewater pH was significantly higher ($W = 42$, $p < 0.0001$; $W = 747$, $p = 0.049$ and $W = 632$, $p = 0.0036$). Porewater salinity and sediment redox potential was not significantly different between the two biogeographical regions. Significant seasonal variation was only found for porewater variables. Water level, porewater pH and temperature was higher in summer ($W = 949$, $p = 0.0016$; $W = 392$, $p < 0.0001$ and $W = 56$, $p < 0.0001$) and porewater salinity was lower in summer ($W = 1439$, $p < 0.0001$) in both biogeographical regions (Figure 10 & 11).

Figure 10 and 11 show the multiple comparison of physico-chemical variables between estuaries. Overall there was a high amount of variability between estuaries that was not related to biogeographical region. Olifants and Berg had significantly higher sediment moisture content. Langebaan and Nahoon had a more similar sediment moisture content and Heuningnes had a sediment moisture content similar to Kwelera. A similar, but not as significant pattern, can be seen in the sediment organic content. The redox potential of the sediment was lower at Olifants and slightly higher at Heuningnes, but were not significantly so. Berg Estuary had significantly higher sediment electrical conductivity and Heuningnes lower sediment electrical conductivity than other estuaries. Heuningnes also had significantly higher porewater pH than found at the other estuaries. The porewater salinity did not show any significant differences between estuaries. Statistical data of the Kruskal-Wallis ANOVA and multiple comparison tests are provided in appendix Table 6A – 15A.

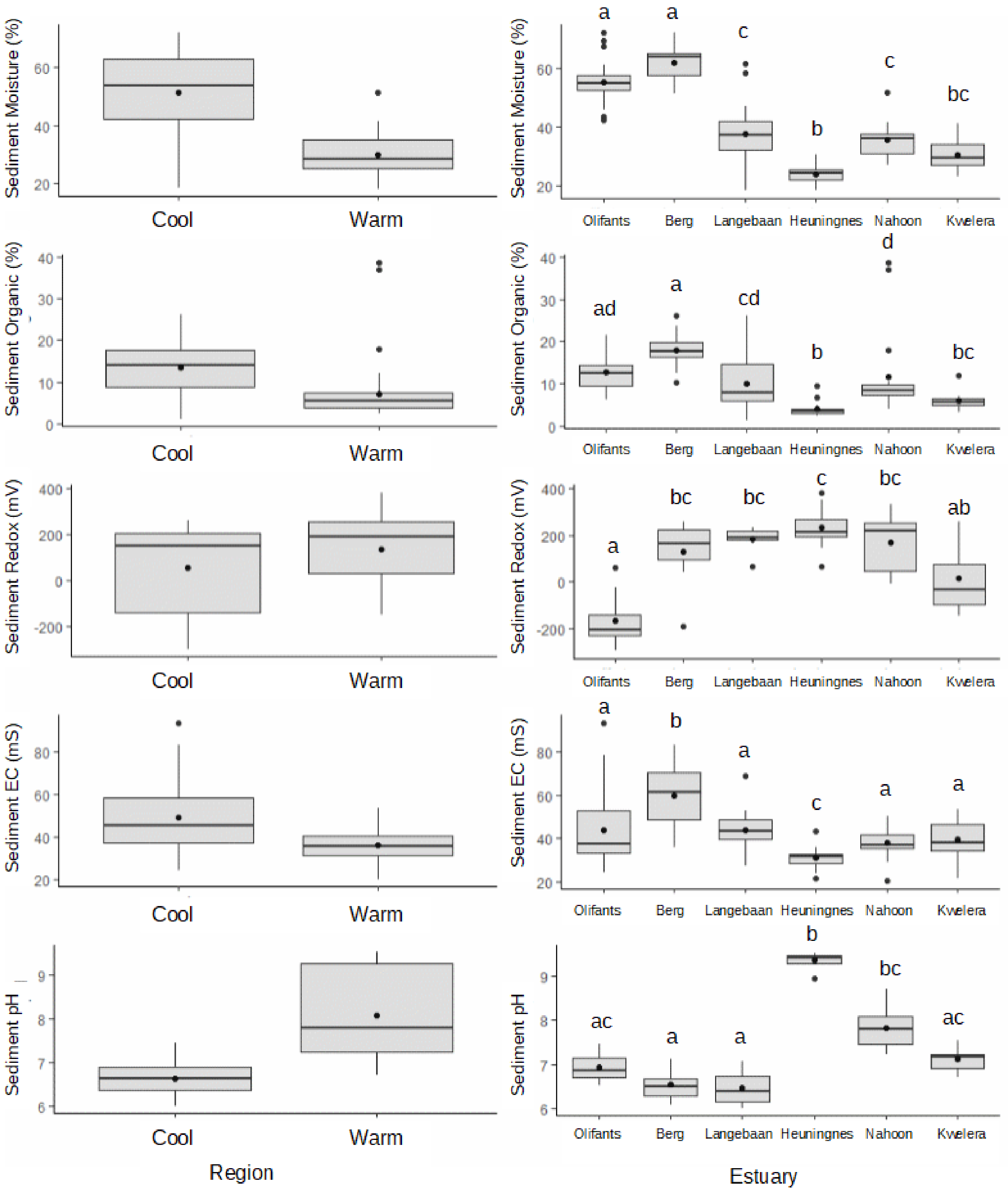


Figure 10: Boxplots of sediment variables between biogeographical regions and estuaries. The line indicates the median and points inside the boxes show the mean. Outliers are shown as points outside the boxes. Estuaries sharing a letter are not significantly different (Kruskal-Wallis multiple comparison test).

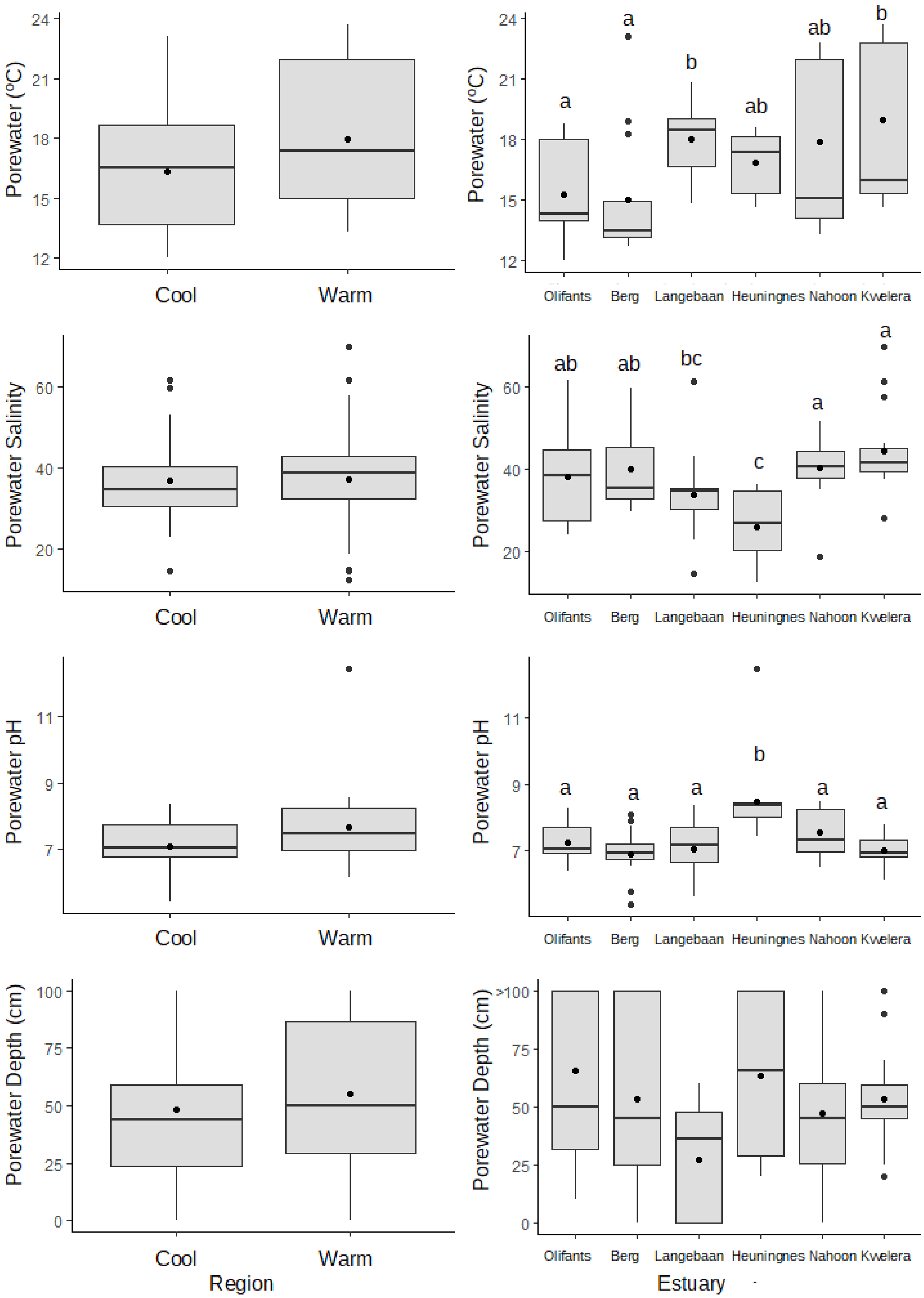


Figure 11: Boxplots of porewater variables between biogeographical regions and estuaries. The line indicates the median and points inside the boxes show the mean. Outliers are shown as points outside the boxes. Estuaries sharing a letter are not significantly different (Kruskal-Wallis multiple comparison test).

T-tests of biomass at the regional scale

The AGB and stem height was not significantly different between biogeographic regions whereas BGB and BGB/AGB ratio was significantly higher in the cool temperate region (W = 1973, p = 0.0016 and W = 1919, p = 0.0047) (Figure 12). A summary of the biomass found during the study period is provided in appendix Table 5A.

Analysis of variance of biomass between estuaries

The stem height, above- and belowground biomass and allocation ratio was significantly different between estuaries ($p < 0.0001$), but showed no pattern of significance in relation to the two biogeographical regions. *Salicornia tegetaria* plants at Olifants, Berg, Nahoon and Kwelera Estuaries had similar stem heights and both Berg and Nahoon had significantly higher stem heights than found at Langebaan and Heuningnes. The AGB at Berg, Heuningnes, Nahoon and Kwelera were not significantly different. The AGB at Olifants Estuary was significantly higher than at Langebaan, Heuningnes and Kwelera. Langebaan had the lowest AGB of 0.86 ± 0.09 kg.m^{-2} and the lowest recovered in winter 2016 (0.59 ± 0.08 kg.m^{-2}), but the AGB at Langebaan was not significantly different from Heuningnes or Kwelera. While washing the roots from the different estuaries it was evident that *S. tegetaria* in Heuningnes Estuary grew in a much sandier, coarse grained sediment compared to the other estuaries, with visibly less roots. Heuningnes had significantly lower BGB than found at the other estuaries (1.51 ± 0.24 kg.m^{-2}), whereas the BGB was not significantly different between the other estuaries. The root/shoot ratio was significantly lower at Heuningnes (1.36) than at the other five estuaries (Figure 12). Statistical data of the multiple comparison tests are provided in appendix Table 16A – 19A.

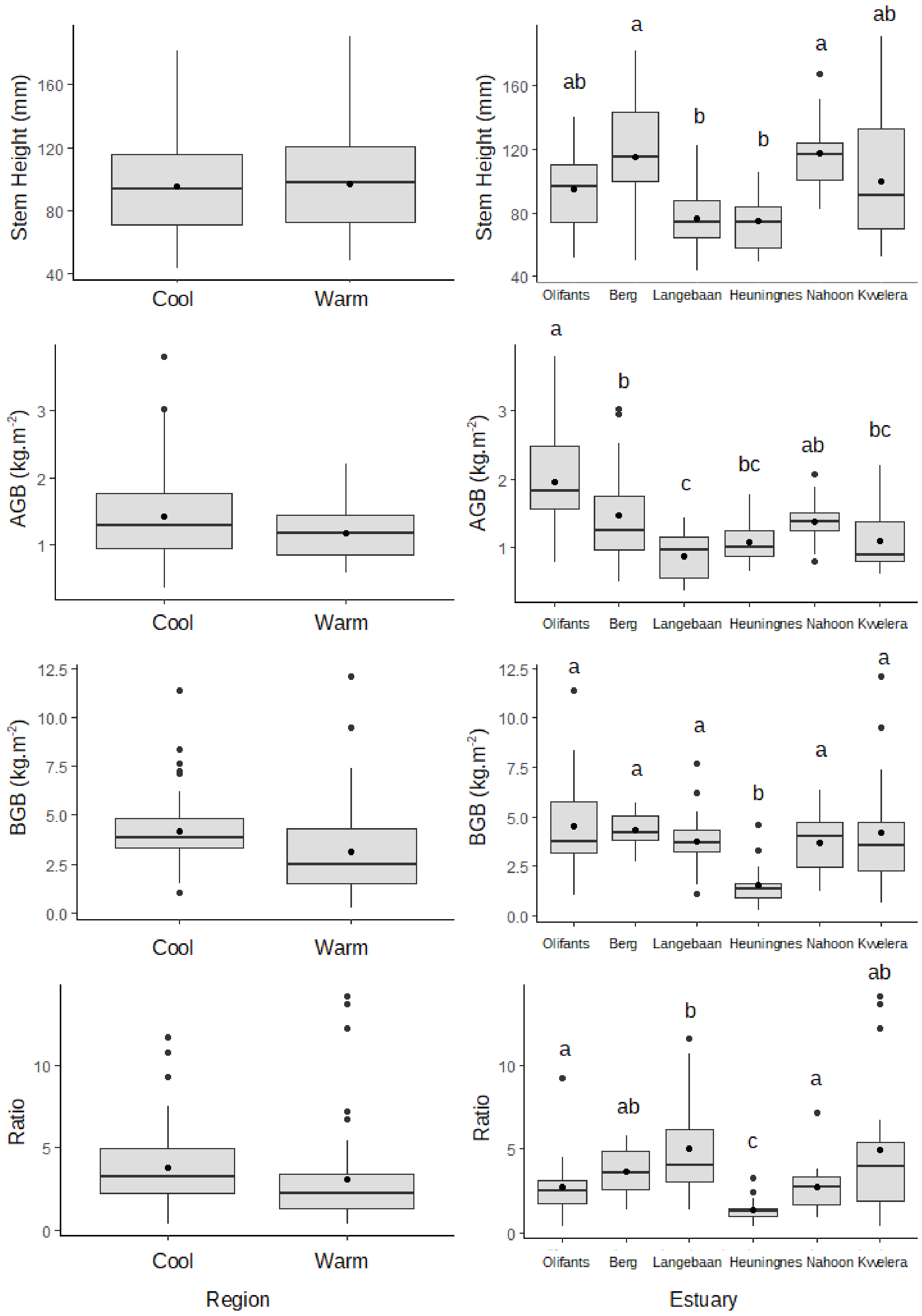


Figure 12: Boxplots of biomass variables between biogeographical regions. The line indicates the median and points inside the boxes show the mean. Outliers are shown as points outside the boxes. Estuaries sharing a letter are not significantly different (Kruskal-Wallis multiple comparison test).

Principal Component Analysis at the landscape scale

The PCA was constrained by the regional climatic and local environmental conditions to determine the drivers of biomass allocation and their covariance. The first two axes of the PCA explained 54.05 % of the variance (Figure 13). The first PC was most strongly related to sediment moisture and temperature followed by sediment EC, sediment organic content and precipitation. The second PC only explained 19.42 % of the variation and was correlated to porewater salinity and porewater pH. An increase in salinity with a resultant decrease in pH was found. The ordination placed warm temperate individuals situated on the left and cool temperate individuals on the right of the plot (Figure 14). Both warm and cool temperate individuals were towards the centre of the plot, but the cool temperate individuals were more scattered. The minimum daily temperature was negatively correlated and the maximum daily temperature positively correlated to the first principal component. On average, the cool temperate region had a maximum of 27.10 ± 0.32 ºC and minimum of 10.74 ± 0.19 ºC and the warm temperate had a maximum of 22.81 ± 0.23 ºC and minimum of 14.09 ± 0.23 ºC during the study period (Figure 9). Eigenvalues and contributions of variables to the PCA are given in appendix Table 20A.

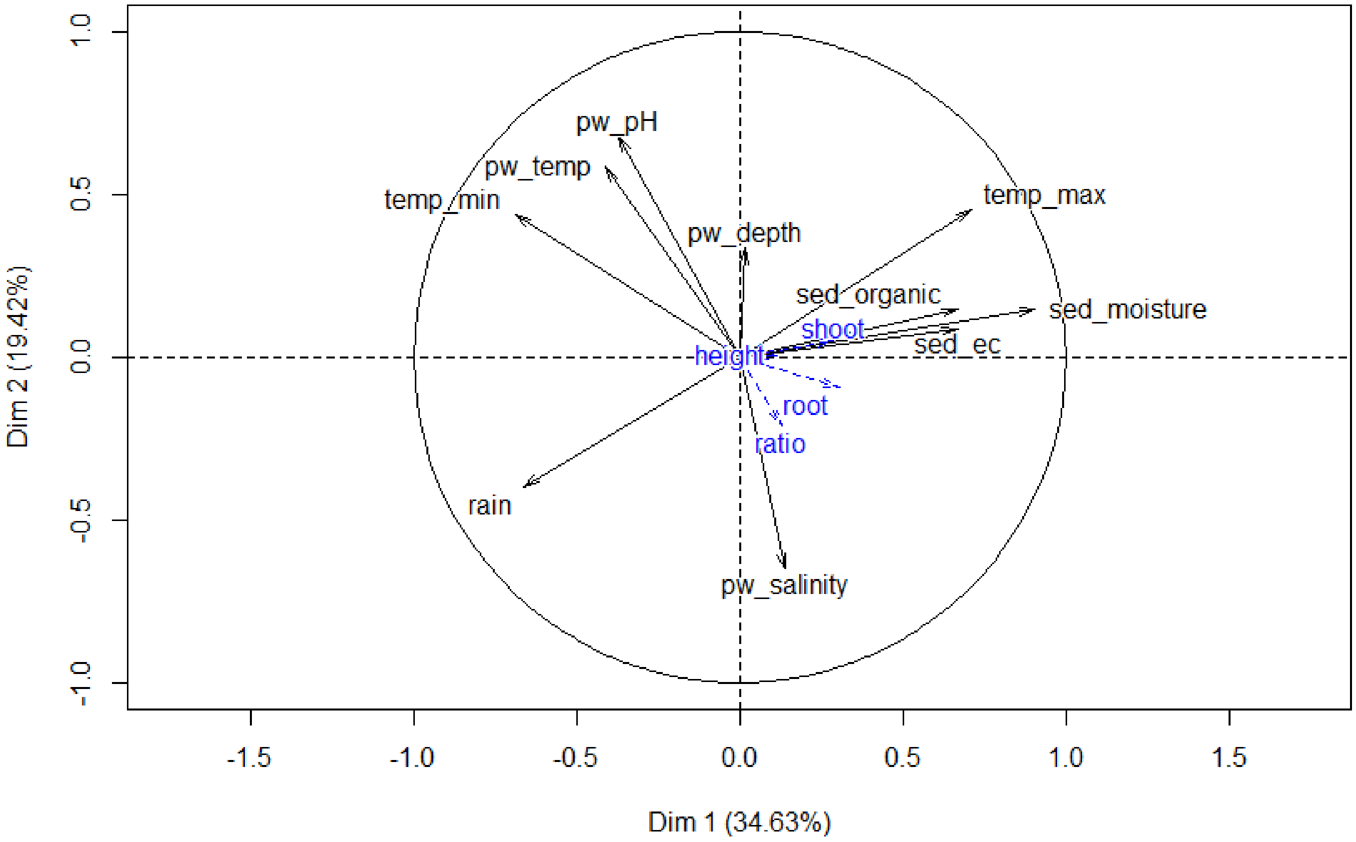


Figure 13: PCA variables factor map of the six estuaries sampled.

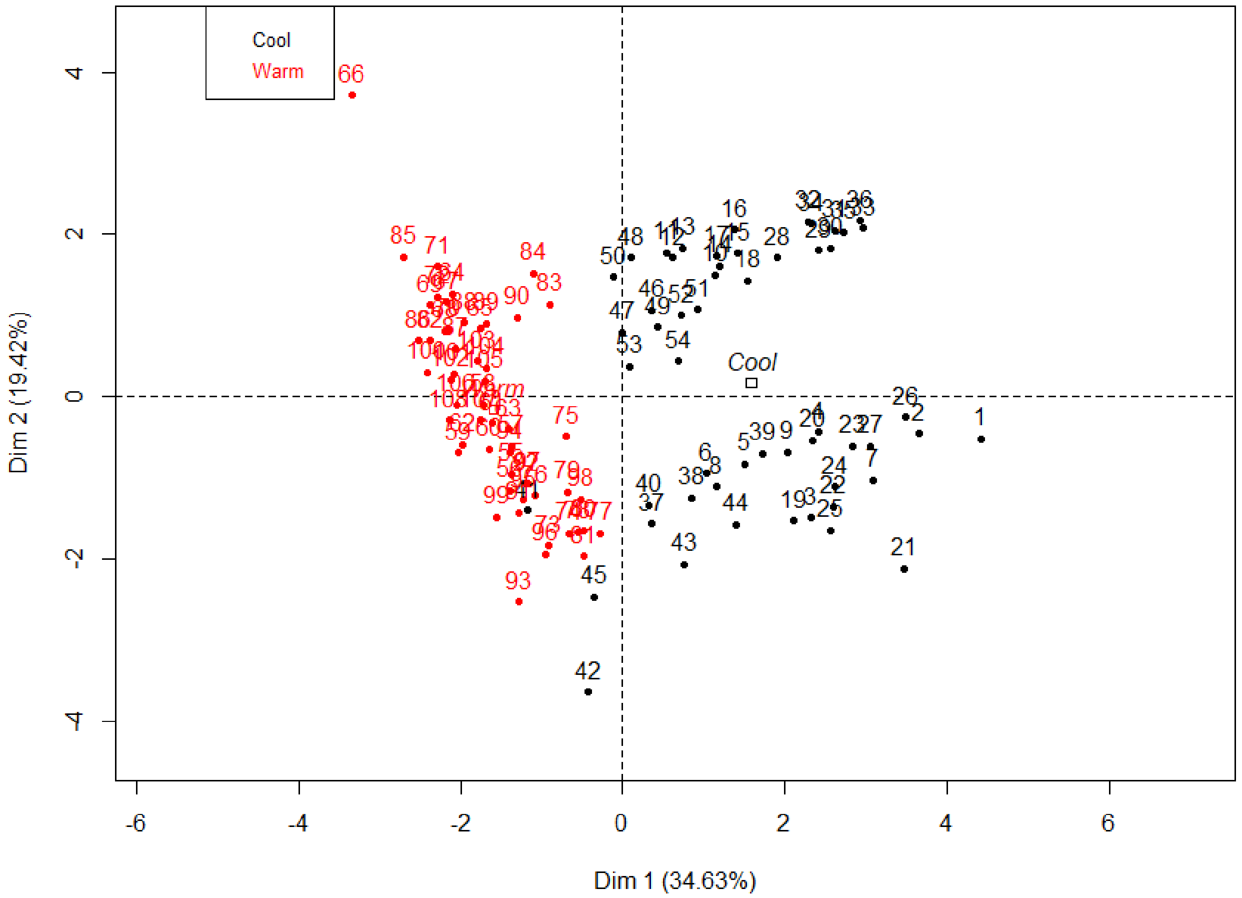


Figure 14: PCA individuals factor map demarcating the warm temperate (red) and cool temperate (black) biogeographical regions.

Correlation Tests at the Landscape Scale

Correlations including sediment pH and sediment redox potential are shown in Table 5. The biomass variables were only weakly correlated to climatic conditions and the BGB/AGB ratio was not affected by climate. Total plant growth was weakly correlated to maximum air temperature (AGB: $r = 0.31$, $p = 0.0010$; BGB: $r = 0.35$, $p = 0.0002$; stem height: $r = 0.24$, $p = 0.013$) and AGB to rainfall ($r = -0.21$, $p = 0.028$).

The maximum temperature was positively correlated to sediment moisture, sediment organic matter and sediment EC, and negatively correlated to rainfall, sediment redox potential and sediment pH. The minimum temperature was negatively correlated to sediment moisture, sediment organic matter and sediment EC, and positively correlated to precipitation, sediment pH, porewater temperature and porewater pH. Precipitation was negatively correlated to sediment moisture, sediment organic matter and sediment EC, and positively correlated to porewater temperature and porewater salinity. The cool temperate region was characterised by higher sediment moisture content, higher maximum daily temperatures, lower minimum temperatures and higher above- and belowground biomass with a higher allocation to BGB though the PCA had a low predictive value and no strong correlations ($r > 0.60$) were found between physico-chemical or climatic variables and biomass. There was a strong positive correlation between sediment moisture content and sediment organic matter and strong negative correlation between sediment moisture content and sediment pH. Sediment pH was moderately negatively correlated to sediment electrical conductivity, porewater salinity, porewater pH and porewater depth, and porewater salinity was moderately negatively correlated to porewater depth (Table 5).

Sediment moisture content was only weakly positively correlated to biomass variables (AGB: $r = 0.30$, $p = 0.002$; BGB: $r = 0.27$, $p = 0.006$), whereas sediment pH had a moderate correlation to belowground biomass (BGB: $r = -0.57$, $p = < 0.0001$; BGB/AGB ratio: $r = -0.51$, $p < 0.0001$). Sediment pH was also strongly correlated to temperature. The rest of the variables were only weakly correlated to biomass which included sediment organic content (BGB) and sediment electrical conductivity (BGB), porewater temperature (AGB), porewater salinity (BGB and BGB/AGB ratio) and porewater pH (AGB, BGB and BGB/AGB ratio). At the landscape scale sediment pH had the strongest correlation to belowground biomass and biomass allocation of all the variables measured. Sediment pH was 8.1 ± 0.1 in the warm temperate and 6.7 ± 0.1 in the cool temperate region. The correlations suggest that sediment moisture promoted total plant growth and that porewater salinity and sediment pH was related to a re-allocation of biomass.

Principle Component Analysis and Correlations at the Regional Scale

The PCA's in Figure 15 explained 69.92 % of the variation in the cool temperate region and 58.97 % in the warm temperate region. Both the first axes were strongly correlated to temperature and precipitation. In the warm temperate region, characterised by higher and more frequent precipitation than found in the cool temperate region, stem height, BGB and the BGB/AGB ratio was positively correlated to the maximum temperature and precipitation. Rainfall decreased in the cool temperate region during the growing season which affected the AGB, but not the BGB, decreasing the BGB/AGB ratio. The BGB/AGB ratio was influenced more by AGB in the cool temperate region and BGB in the warm temperate region (Table 6 & 7).

In the cool temperate region, the maximum temperature was positively correlated to minimum temperature and negatively to rainfall. The minimum temperature was also very strongly negatively correlated to rainfall. The maximum and minimum temperature was positively correlated to porewater pH and porewater depth. The minimum temperature positively correlated to porewater temperature and negatively correlated to porewater salinity. Rainfall was negatively correlated to porewater depth and sediment moisture content. Sediment moisture content was positively correlated to sediment organic matter content and electrical conductivity, and negatively correlated to sediment pH. Sediment organic matter was also negatively correlated to sediment pH. Sediment pH and porewater temperature was negatively correlated to porewater salinity. Porewater pH was positively correlated to porewater temperature and negatively correlated to porewater salinity (Table 6).

In the warm temperate region, the maximum temperature had a positive correlation to minimum temperature and a positive correlation to rainfall. The maximum temperature was positively correlated to sediment moisture content and electrical conductivity, and negatively correlated to sediment pH and porewater depth. The minimum temperature was positively correlated to porewater temperature and negatively correlated to porewater depth. Rainfall was positively correlated to sediment moisture content, electrical conductivity and porewater salinity, and negatively correlated to sediment and porewater pH. Sediment moisture was negatively correlated to sediment pH, and sediment pH was negatively correlated to sediment electrical conductivity and porewater salinity (Table 7).

There were more correlations between the physico-chemical conditions and biomass variables in the warm temperate region compared to the cool temperate region. Sediment moisture, sediment redox, sediment pH, sediment electrical conductivity, porewater salinity and porewater pH were correlated to biomass variables. A higher sediment moisture content resulted in an increase of AGB and plant height, but did not affect the BGB and BGB/AGB ratio. Sediment EC and porewater salinity was positively correlated to BGB and to a lesser extent plant height which resulted in an increase in the BGB/AGB ratio as salinity increased. The sediment redox potential was weakly positively correlated to BGB with an increase in the BGB/AGB ratio. The sediment redox potential was negatively correlated to sediment moisture and positively correlated porewater pH. Sediment and porewater pH was correlated to biomass variables in both biogeographical regions. BGB was lower when pH was higher, and in the warm temperate estuaries stem height also decreased when pH was higher, though the correlation was not as strong as was found for BGB (Table 6 & 7). Eigenvalues and contributions of variables to the PCA are provided in appendix Tables 21A and 22A.

Correlations at the Local Scale

At Olifants, Langebaan and Heuningnes sediment pH was negatively correlated to BGB and at Langebaan and Heuningnes negatively correlated to AGB. In Olifants, Langebaan, Nahoon and Kwelera the AGB was higher in quadrats where the porewater depth was shallower. The correlation was strongest at Langebaan where porewater was often found at the surface and did not exceed 60 cm during sampling times. At Berg the water level exceeded 100 cm in 6 quadrats during summer and at Heuningnes all 9 quadrats exceeded 100 cm in winter. In Kwelera, the BGB/AGB ratio was higher during winter sampling and resulted in a negative correlation to porewater temperature. At Heuningnes the AGB was higher in quadrats with higher sediment organic content (Table 8). Correlations between all variables for each estuary are given in appendix Table 23A.

Table 5: Correlations of physico-chemical variables and biomass variables at the landscape scale. Spearman's correlation coefficient rho = 0.00-0.19 "very weak", 0.20-0.39 "weak", 0.40-0.59 "moderate", 0.60-0.79 "strong", 0.80-1.0 "very strong". Correlations with biomass variables are in red. Abbreviations are as follows: SM = soil moisture, SOM = soil organic matter, Rx = redox potential, S-pH = sediment pH, SEC = sediment electrical conductivity, Pw ºC = porewater temperature, Pw S = porewater salinity, P-pH = porewater pH, WL = porewater depth. Significant values are given as *p < 0.05, **p < 0.001, ***p < 0.0001.

	Height	AGB	BGB	Ratio	Max	Min	Rain	SM%	SOM	Rx	S-pH	SEC	Pw ºC	PwS
Height	-													
AGB	0.48***	-												
BGB			-											
Ratio	-0.25*	-0.47***	0.71***	-										
Max	0.24*	0.31**	0.35**		-									
Min						-								
Rain		-0.21*			-0.55***	0.53***	-							
SM%		0.30*	0.27*		0.72***	-0.45***	-0.53***	-						
SOM			0.22*		0.45***	-0.26*	-0.25*	0.70***	-					
Rx					-0.39*			-0.38*		-				
S-pH			-0.57***	-0.51**	-0.75***	0.64***		-0.67***	-0.33*		-			
SEC			0.19*		0.49***	-0.28*	-0.31**	0.57***	0.37**		-0.45**	-		
Pw ºC		-0.24*				0.63***	0.31*	-0.31*		-0.37*			-	
Pw S			0.29*	0.24*			0.35**				-0.47*	0.26*	-0.25*	-
P-pH	-0.21*		-0.25*	-0.25*		0.45***		-0.35**		0.33*	0.47*	-0.23*	0.27*	-0.43***
W L											-0.43*			

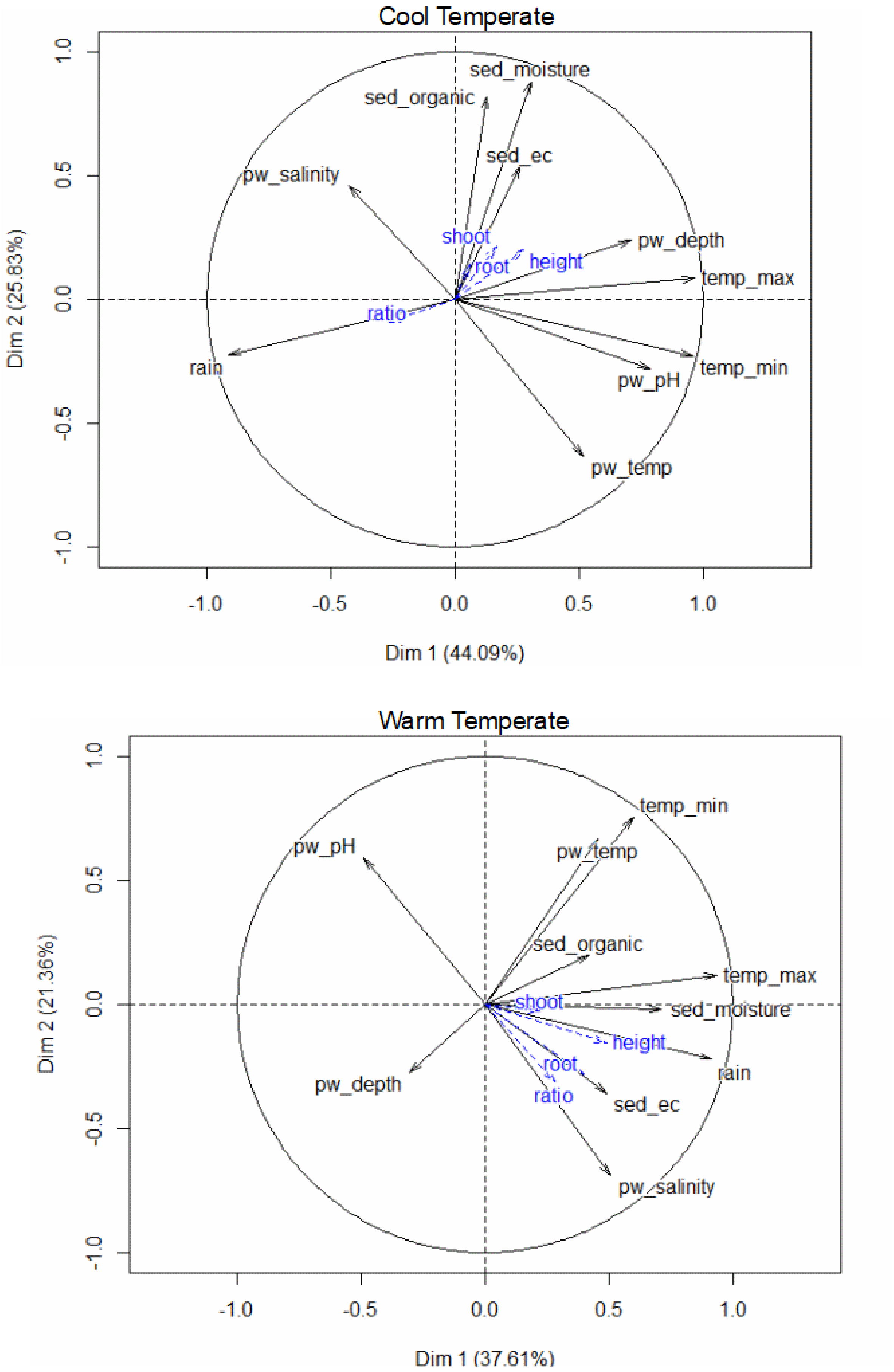


Figure 15: PCA variables factor map of the cool temperate and warm temperate region.

Table 6: Correlations of physico-chemical variables and biomass variables in the cool temperate biogeographical region. Spearman's correlation coefficient rho = 0.00-0.19 "very weak", 0.20-0.39 "weak", 0.40-0.59 "moderate", 0.60-0.79 "strong", 0.80-1.0 "very strong". Correlations with biomass variables are in red. Abbreviations are as follows: SM = soil moisture, SOM = soil organic matter, Rx = redox potential, S-pH = sediment pH, SEC = sediment electrical conductivity, Pw ºC = porewater temperature, Pw S = porewater salinity, P-pH = porewater pH, WL = porewater depth. Significant values are given as *p < 0.05, **p < 0.001, ***p < 0.0001.

	Height	AGB	BGB	Ratio	Max	Min	Rain	SM%	SOM	Rx	S-pH	SEC	Pw ºC	PwS
Height	-													
AGB	0.45**	-												
BGB			-											
Ratio	-0.41**	-0.67***	0.36*	-										
Max	0.36**	0.29*		-0.35*	-									
Min					0.92***	-								
Rain	-0.40**	-0.37*		0.40**	-0.98***	-0.83***	-							
SM%					0.37*		-0.48**	-						
SOM								0.84***	-					
Rx										-				
S-pH			-0.42**					-0.50***	-0.54***		-			
SEC								0.44**	0.34*	0.29*	-0.36*	-		
Pw ºC		-0.39**			0.37*	0.66***		-0.37*	-0.29*		0.33*		-	
Pw S					-0.32*	-0.47**					-0.44**		-0.45**	-
P-pH				-0.29*	0.67***	0.76***	-0.58***			0.32*			0.56***	-0.42**
W L			0.28*		0.72***	0.70***	-0.70***							

Table 7: Correlations of physico-chemical variables and biomass variables in the warm temperate biogeographical region. Spearman's correlation coefficient rho = 0.00-0.19 "very weak", 0.20-0.39 "weak", 0.40-0.59 "moderate", 0.60-0.79 "strong", 0.80-1.0 "very strong". Correlations with biomass variables are in red. Abbreviations are as follows: SM = soil moisture, SOM = soil organic matter, Rx = redox potential, S-pH = sediment pH, SEC = sediment electrical conductivity, Pw ºC = porewater temperature, Pw S = porewater salinity, P-pH = porewater pH, WL = porewater depth. Significant values are given as *p < 0.05, **p < 0.001, ***p < 0.0001.

	Height	AGB	BGB	Ratio	Max	Min	Rain	SM%	SOM	Rx	S-pH	SEC	Pw ºC	PwS	P-pH
Height	-														
AGB	0.67***	-													
BGB			-												
Ratio		-0.40**	0.91***	-											
Max	0.40**		0.43**	0.36**	-										
Min					0.67***	-									
Rain	0.50**		0.47**	0.35**	0.82***	0.34*	-								
SM%	0.46**	0.28*			0.60***	0.33*	0.64***	-							
SOM					0.29*		0.36*	0.29*	-						
Rx			0.32*	0.39**				-0.29*		-					
S-pH	-0.38**		-0.59***	-0.50***	-0.56***		-0.82***	-0.46**			-				
SEC	0.27*		0.34*	0.30*	0.45**		0.43**				-0.45**	-			
Pw ºC					0.36**	0.80***	0.34*		0.29*				-		
Pw S	0.29*		0.47**	0.47**	0.39**		0.64***				-0.78***	0.37**		-	
P-pH	-0.32*				-0.31*		-0.52***	-0.37*		0.38**	0.61***	-0.34*		-0.51**	-
W L					-0.49**	-0.40**						-0.28*			

Table 8: Correlations of physico-chemical variables to AGB, BGB and the BGB/AGB ratio in each estuary

Estuary	Variable 1	Variable 2	r	p
Olifants	BGB	Sediment pH	-0.54	0.022
	AGB	Porewater Depth	-0.56	0.013
Berg	AGB	Sediment Moisture	-0.55	0.018
	Ratio	Sediment Moisture	0.49	0.039
Langebaan	AGB	Sediment Redox	0.73	0.001
	AGB	Sediment pH	-0.63	0.005
	BGB	Sediment pH	-0.50	0.035
	AGB	Porewater Depth	0.77	<0.0001
	Ratio	Porewater Depth	-0.65	0.004
Heuningnes	AGB	Sediment Organic	0.52	0.027
	AGB	Sediment pH	-0.53	0.025
	BGB	Sediment pH	-0.53	0.025
Nahoon	AGB	Porewater Depth	-0.51	0.032
Kwelera	BGB	Sediment Redox	0.71	0.001
	Ratio	Sediment Redox	0.70	0.001
	BGB	Porewater Temperature	-0.51	0.031
	Ratio	Porewater Temperature	-0.54	0.022

Discussion

Salt marsh vegetation distribution at the local scale is governed by a gradient of physico-chemical conditions that change with elevation (Adams *et al*., 2016). Stress-tolerant plants are adapted to persist in the lower intertidal environment, which is characterised by high salinity, regular tidal inundation and low productivity (Pennings and Callaway, 1992; Bertness and Hacker, 1994; Guo and Pennings, 2012). Other factors also determine physico-chemical conditions and plant growth such as clay content in the sediment, which determines drainage and influences the nutrient, water and oxygen availability (Olff *et al.*, 1997; Bai *et al*., 2005), and groundwater tables that further determine the oxygen status and thus redox potential of the sediment (Armstrong *et al*., 1985). It has been found that biomass production (which is estimated from standing biomass) and biomass allocation may be better predictors of plant stress than salinity or inundation gradients (Brouwer, 1962; Guo and Pennings, 2012; Minden *et al*., 2012). This is because there is a shift in allocation to the plant organs that are needed to acquire limiting resources (Brouwer, 1962). This study measured the standing aboveground and belowground biomass of *Salicornia tegetaria* in six estuaries in South Africa located in the warm temperate and cool temperate biogeographical regions to determine if there are differences in the allocation of resources between regions and whether drivers of biomass allocation could be identified.

Biomass allocation and the standing biomass of macrophytes have not been studied in estuaries in South Africa. The root/shoot ratio of *S. tegetaria* (3.40) was lower on average than reported for S*partina* species (most values were between 2.32 and 10.74) (Roman and Daiber, 1984; Schubauer and Hopkinson, 1984; Hackney and De La Cruz, 1986; Gross *et al*., 1991; Callaway and Josselyn, 1992), *Phragmites australis* (2.58-6.24) (Roman and Daiber, 1984; Scarton *et al*., 2002) and *Juncus roemerianus* (5.53) (De La Cruz and Hackney, 1977). The aboveground biomass of *S. tegetaria* (1.31 kg.m^{-2}) was within the range reported for other *Salicornia* species (0.53-3.40 kg.m^{-2}) (Mahall and Park, 1976a; Castellanos *et* al., 1994; Curco *et* al., 2002; Scarton *et* al., 2002; Palomo and Niell, 2009). The high aboveground biomass and low root/shoot ratio of *S. perennis* ssp. a*lpini* (0.68) reported by Palomo and Niell (2009) was likely due to the high level of eutrophication of the system studied in Spain. The belowground biomass found in the current work was higher (3.66 kg.m^{-2}) than that found for *Salicornia* species in Mediterranean climates (0.94-3.25 kg.m^{-2}) (Table 2). In this study the cool temperate region was characterised by higher sediment moisture content, higher maximum daily temperatures, lower minimum temperatures but similar belowground biomass and root/shoot ratios. Comparisons between different geographic regions have shown a similar root/shoot ratio with latitudinal variation in *S. alterniflora* (Gross, 1991), whereas the root/shoot ratio of *S. patens* increased from Delaware (10.44) (Roman and Daiber, 1984) northward to Nova Scotia (15.35) (Connor and Chmura, 2000).

The physico-chemical variables measured were similar to those found in other estuaries in South Africa (Table 3). Most comparable are the findings of Adams *et al.* (2016) that measured physico-chemical variables in 11 estuaries in both the warm and cool temperate biogeographical regions. Adams *et al.* (2016) found similar average groundwater depth, sediment EC and sediment pH compared to this study. The sediment moisture and organic content found in this study was higher than found by Vromans (2011), Adams *et al*. (2016) and Geldenhuys *et al*. (2016), but lower than in Knysna Estuary (Muir, 2000 cited in Vromans, 2011). The particle size was not measured in this study but is an important determinant of the physico-chemical variables that were measured during sampling. At Langebaan, previous studies found that the sediment at Oosterwal adjacent to Bottelary had a lower clay and silt content (96 % sand and 4 % silt & clay) than sites further south of the embayment (e.g. 35 % sand and 65 % silt and clay at Geelbek and 60.47 % silt and clay at Schrywershoek) (Reaper, 1995) (A map of Langebaan is presented in Figure 17 in Chapter 5). During this study the roots at Bottelary were condensed in the top layer (± 2-3 cm) of sediment with a coarse sandy layer continuing beneath. This is probably because there is no river input and sediment input is of marine origin (Flemming, 1977). Geldenhuys (2014) found that the sediment at Nahoon is primarily sand particles throughout the salt marsh and mangrove habitats with 50 % sand, 35 % clay and 15 % silt in the lower salt marsh. The sand particle size distribution in Kwelera Estuary is similar to Nahoon Estuary. Both were found to have coarser grained marine sand on the flood-tidal delta reaching 50 %, and decreasing in size further up the estuary with mud dominating in the middle reaches (Reddering, 1987). The sandy nature of the sediment at Heuningnes was visible to the eye, most likely due to the influx of sand from the sea. Bickerton (1984) reported that the salt marshes near the mouth occur on sandy substrates which was also observed during sampling. The sediment at the historically river-dominated Olifants and Berg estuaries was finer than at the other estuaries. Bornman *et al*. (2002) found that the top layer of sediments at the lower intertidal zone of Olifants Estuary had a clay/silt loam texture. Sediment moisture and organic content was higher in these estuaries. The higher surface tension of soils with higher organic and clay/silt content result in a higher water and nutrient holding capacity than sandy soils (Barko and Smart, 1986; Gómez-Plaza *et al*., 2001; Bai *et al*., 2005). The resultant lower sediment redox potential and pH values (which were negatively correlated to sediment moisture in this study) may have slowed down the decomposition of organic matter, which was significantly higher in the cool temperate biogeographical region (Husson, 2013; Ouyang *et al*., 2017). Furthermore, organic matter buffers the pH in the soil, maintaining a neutral to acidic environment (Costa *et al*., 2003; Husson, 2013). In the cool temperate region the sediment pH was significantly lower than in the warm temperate region and sediment pH was negatively correlated to organic matter content (Figure 10; Table 5). Sediment and porewater pH was negatively correlated to sediment and porewater salinity. A decrease in pH with an increase in salinity (Redondo-Gómez *et al*., 2007; Arslan and Demir, 2013) and a decrease in pH with an increase in soil moisture (Rogel *et al*., 2000) has been reported in other salt marsh studies.

The correlations in the biogeographical regions and individual estuaries may indicate what factors are limiting in the environment and promote growth in each system. There were no strong correlations between climatic and biological variables in either region, and the moderate to weak correlations found were expected in accord with the growth season. With an increase in temperature, there was an increase in stem height in both regions, an increase in AGB in the cool temperate region and an increase in BGB in the warm temperate region. This resulted in a decrease in the AGB:BGB ratio in the cool temperate and an increase in the AGB:BGB ratio in the warm temperate region during the growing season. In the cool temperate region, rainfall had a negative correlation to AGB and stem height, most likely because rainfall occurs in winter, whereas stem height and BGB increased with increased precipitation in the warm temperate region during the growth season. These correlations may have been stronger or weaker in the cool temperate region due to drought conditions in the Western Cape during the study period, for example a higher rainfall in winter could have resulted in a stronger negative correlation to AGB or stem height, or might have had some correlation to BGB. A lower rainfall could also have reduced growth the following season, or indirectly affected biological variables by, for example, influencing sediment salinity or pH. With the drought conditions during this study, rainfall was only correlated to soil moisture and porewater pH (Table 6).

Soil moisture content affects the growth rate of plants: when soil moisture is not available to the plant, more energy is used to move water from the soil to the roots and the weight or the development of organs may decrease (Kramer, 1944). A lower sediment moisture content can also result in an increase in salinity or EC which causes osmotic stress and reduces the moisture availability in the sediment (Kramer, 1944; Keiffer and Ungar, 2002). This could explain the positive correlation of sediment moisture to above- and belowground biomass and stem height found. At Berg Estuary soil moisture had a negative impact on the aboveground biomass which was unexpected. There may have been an interactive effect between more than one variable that may explain the result. For example the sediment moisture content at Berg Estuary was negatively correlated to sediment pH, which may have had an impact on aboveground biomass (Table 6 & 8). Furthermore, the sediment EC was significantly higher at Berg Estuary which may have resulted in an environment where water and nutrients could not be absorbed sufficiently.

Sediment redox potentials were positively correlated to belowground biomass and the root/shoot ratio. The belowground plant organs were more sensitive to the stressful conditions than aboveground (Table 7 & 8) which has been found in other studies in submerged conditions (Sekhon *et al*., 1993). The effect is only seen at the regional and local scale analyses as the redox potential is widely variable in time and space (Husson, 2013). Following submergence, the redox potential in the sediment becomes lower as oxygen is depleted in the sediment by roots, microorganisms and soil reductants that are formed when oxidised compounds are used as electron acceptors and carbon dioxide accumulates (Pezeshki and DeLaune, 2012). At +350 mV the root medium becomes oxygen-deficient which can reduce the rate of photosynthesis, cause energy deficiency in the roots and reduce water and nutrient uptake (Pezeshki and DeLaune, 2012). The positive correlation between belowground biomass and porewater depth in the cool temperate region and negative correlation between porewater depth and aboveground biomass at Olifants and Nahoon Estuary suggests that with an increase in belowground biomass there may be a decrease in aboveground biomass as resources were allocated to roots to forage deeper for water, oxygen or nutrients (Williams *et al*., 1999; Bornman *et al*., 2002; Markesteijn and Poorter, 2009).

The increase in aboveground biomass found with higher sediment organic content could be due an increase in the availability of nutrients released during mineralisation (van Wijck *et al*., 1992; Husson, 2013). At Heuningnes Estuary organic matter possibly decomposed at a faster rate, which could explain the correlation to aboveground biomass reported in this study (Angiolini *et al.*, 2013; Husson, 2013). Heuningnes is a sandy estuary and the resulting high availability of oxygen and low salinity that increase organic matter decomposition may have had a positive effect on aboveground growth (Valiela *et al*., 1976; Poluektov and Topazh, 2005; Darby and Turner, 2008; Husson, 2013; Ouyang *et al.*, 2017). This is supported by the significantly lower belowground biomass recorded. Other salt marsh species including *S. alterniflora*, *S. patens* and *S. perennis* ssp. *alpini* have shown a decrease in belowground biomass with higher nutrient availability (Valiela *et al*., 1976; Gross *et al*., 1991; Palomo and Niell, 2009).

The positive correlations between porewater and sediment salinity and stem height, belowground biomass and the root/shoot ratio could be an indication that salinity changed the water potential of the rhizosphere creating a water stressed environment. An increase in root growth would enhance the absorption of water and ions needed for osmotic adjustment (Naidoo and Rughunanan, 1990). An increase in stem height suggests that the plants also absorbed more water and ions to adjust water potential in the cells (Naidoo and

Rughunanan, 1990). Root/shoot ratios have been found to increase with increased salinity which increases osmotic stress to the plants (Mahall and Park, 1976b; Lenssen *et al*., 1995; Saintilan, 1997; Van Zandt *et al*., 2003) and the higher ratios found in the cool temperate region correspond to the higher sediment salinity found.

Geldenhuys (2014) found that sediment pH was an important determinant in salt marsh species distribution at Nahoon Estuary. Studies have shown that nutrients become more soluble at lower pH levels, promoting plant growth as has been found in other plants, including glycophytes (Ponnamperuma, 1972; Peterson, 2009; Husson, 2013). The belowground biomass of *S. tegetaria* was affected by sediment pH more strongly than above- and belowground biomass was affected by porewater pH. The pH level is important in the rhizosphere as roots require a positive membrane potential and a transmembrane pH gradient for the transport of ions as H^+-translocating ATPases drives the transmembrane flux of other ions. H^{+-}translocating activity in saline conditions was found to be optimum at pH 6.2 in the halophyte *Atriplex nummularia* (Lindl.) (Braun *et al.*, 1986). Whereas glycophytes show an increase in the root/shoot ratio at higher pH (Peterson, 2009), *S. tegetaria* showed a decrease in the root/shoot ratio.

The correlations between the root/shoot ratio of *S. tegetaria* and sediment pH and porewater salinity (which had a strong negative correlation) in the warm temperate region suggest a possible relationship between an increase in stem elongation at higher salinity and the role of pH in ion flux. The root/shoot ratio and stem height increased as salinity increased and decreased as pH increased. The positive relationship between the root/shoot ratio and salinity suggests that osmotic stress became higher as salinity increased due to the accumulation of ions in the rhizosphere. Resources may have been re-allocated belowground to facilitate the uptake of water and ions in the shoots to regulate their water potential in saline conditions (Flowers and Colmer, 2015). Both the increase in belowground biomass and lower pH in the sediment would have aided ion fluxes at higher salinity. The optimum pH of *S. tegetaria* is not known and the pH at which plants grow best in the natural environment do not necessarily reflect the optimum pH for plant growth (Peterson, 2009) which is further complicated by salinity (Braun *et al.*, 1986) and waterlogging (Adams and Bate, 1994).

The amount of variability explained at the landscape scale and regional scale was weak to moderate as these included many different estuarine systems. At the local scale the strength of the correlations improved as the amount of variability was reduced. The lack of strong correlations found at the landscape scale between biomass and other physico-chemical variables, climatic conditions or the axes of the PCA further suggests that the variation that was detected did not have a strong effect on the biomass of *S. tegetaria* and that biomass production is fairly stable in permanently open estuaries with large salt marsh areas. This study did not measure porewater nutrient content which may have aided the interpretation of these results. It is expected that nutrients would have affected aboveground biomass which was variable between some estuaries (Darby and Turner, 2008). Furthermore, sediment particle analysis may have improved the predictive ability of the PCA as particle size was visibly different at Heuningnes and therefore would be expected to affect the belowground biomass.

Conclusion

The results from this study provide an indication of the environmental factors that promote growth in this species and which physico-chemical variables may influence a re-allocation of resources (Figure 13; Table 5). Higher root/shoot ratios may indicate a more stressful environment which was influenced by processes above- and belowground. In the cool temperate region aboveground processes had a stronger influence on the root/shoot ratio, whereas belowground processes affected the ratio more strongly in the warm temperate region. Moisture content in the sediment improved aboveground growth and stem height, salinity and sediment redox potential influenced belowground growth and pH affected growth above- and belowground. This concurs with many studies that have identified salinity and waterlogging as the main environmental factors that cause stress to macrophytes in the lower salt marsh habitat (e.g. Minden *et al*., 2012; Janousek and Mayo, 2013). The results from this chapter have shown that the belowground biomass was similar at all estuaries except at Heuningnes, whereas the aboveground biomass and stem height lacked a pattern between the individual warm and cool temperate estuaries suggesting that variables that influenced plant growth aboveground were different between estuaries, but that belowground growth was not affected significantly by physico-chemical variables or the different climatic regimes found between the warm and cool temperate biogeographical regions. Future studies, including the sub-tropical estuaries on the east coast and the biomass of other salt marsh species may reveal more information.

Growth, survival and reproduction are constrained by limited resources whereby an increase in resources to one process results in the decrease in resources to another process (Obeso, 2002). In this chapter the allocation of resources of vegetative growth were measured in the vicarious environment of lower intertidal salt marshes in response to environmental conditions and differing climatic regimes. A re-allocation of resources also occurs when plants produce reproductive organs and propagules which are timed by environmental cues (Petanidou *et al*., 1995; Luterbacher *et al*., 2007) and it has been shown that the production of inflorescences and seeds can result in a re-allocation of above- or belowground biomass (Hackney and De La Cruz, 1986; Obeso, 2002; Crosby *et al*., 2015). In the next chapter the reproductive timing and output of *S. tegetaria* is compared on a monthly basis between the warm and cool temperate biogeographical regions.

Chapter 5 – Phenology of *Salicornia tegetaria* at Langebaan Estuarine Embayment

Introduction

There is a trade-off between the allocation of resources to reproduction and growth (Bazzaz *et al.,* 1987). In general 1-60 % of the total biomass of a plant is allocated to reproductive structures and new inflorescences that require a high amount of nitrogen and phosphorus (Bazzaz *et al.,* 1987). These structures are important for attraction, reproduction, dispersal and genetic recombination. Vegetative propagules are allocated 0-30 % of total plant biomass and change from sinks to sources of photosynthate that are important in local dispersal and horizontal growth (Bazzaz *et al.,* 1987). The life history of a plant as well as interspecific competition will also have an influence on the allocation of resources to reproduction. In a densely populated habitat, resource allocation to growth would be more important than reproductive allocation compared to an open habitat and annual species require a higher reproductive output than perennial species to ensure survival (Bazzaz *et al.,* 1987). The highly variable environmental conditions found in estuaries influence the reproduction of estuarine macrophytes that are highly plastic to maximise fitness (Chapin *et al*., 1987; Ungar, 1987b; Marani *et al*., 2006; Richards *et al*., 2010), for example plants that alternate between clonal growth or sexual reproduction under different scenarios (Lieffers and Shay, 1981) or different species that employ different growth periods in the same environment (Zedler *et al*., 1999).

Salt marsh macrophytes usually germinate in spring, relying on environmental cues for reproductive timing such as photoperiod (Lombardi *et al*., 1997), increases or decreases in temperature (McKee and Richards, 1996; Ekstam and Forsby, 1999) or precipitation (Lombardi *et al*., 1997; Noe and Zedler, 2001a; Dreyer *et al*., 2006). Salinity which is reduced during high rainfall (Noe and Zedler, 2001b; Bornman, 2002) also plays an important role in salt marsh phenology and growth, especially germination, as high salinity reduces the water potential of the growth medium hindering the absorption of water of imbibing seeds (Lombardi *et al*., 1997; Redondo *et al*., 2004; Deegan *et al*., 2005). Experiments have shown that the flowering of genotypes in the tribe Salicornieae, is induced by the shortening day length during late summer (York *et al*., 2000; Fu and Zhao, 2003; Ventura *et al*., 2011b) but that salinity did not have an effect on the timing of flowering (Ventura *et al*., 2011b). In field experiments, *Salicornia* species which are annuals, germinate in winter (February) in Britain, and in spring (May) in inland North America, in relation to reduced salinity (Ungar *et al*., 1979; Davy *et al*., 2001). Flowering in Britain occurs during summer and autumn with seed set occurring in autumn (between September – November). The seeds remain dormant until late winter (February) (Davy *et al*., 2001). Perennial *Salicornia* species, such as *Salicornia perennis* in Britain, remain green during winter and flower in late summer and autumn (from August to September). The seeds ripen mainly in autumn (October) and have been released by late autumn (November) (Davy *et al*., 2006). In the Mediterranean, *S. perennis* also flowers from late summer to autumn (August to October) (Fernández-Illescas *et al*., 2010). In South Africa, most *Afrocornia* species flower in summer and the flowering and fruiting period of *S. tegetaria* is from summer to winter (December to July) (Table 9; Figure 16) (Steffen *et al.,* 2010).

The growth and flowering phenology of salt marsh macrophytes, which included *S. tegetaria*, was compared in the warm temperate biogeographical region between a TOCE at Kleinmonde Estuary and a POE at Kowie Estuary by Vromans (2011). Temperature, sediment redox potential and salinity were important determinants of macrophyte phenology in the POE. *Salicornia tegetaria* reproduced vegetatively as the seeds that were produced did not germinate and seasonal re-growth ensured a high cover abundance. *Salicornia tegetaria* produced more seeds in the TOCE than in the POE despite significantly lower plant cover. Over a two year period peak flowering occurred between February and March, and March and April in the TOCE and peak seeding occurred in May and June. In the POE peak flowering occurred in February and March, and from January to April and peak seeding occurred in June and May (Vromans, 2011).

The aim of this study is to describe the phenology of *S. tegetaria* in an estuary in the cool temperate biogeographical region and to compare findings to the study on the same species in the warm temperate region at Kowie Estuary by Vromans (2011). The objective of this kind of study is to determine if the differences in climate in these two regions have affected phenology and infer the response of the species to further climatic perturbations. Growth and reproduction was measured over the period of a year and reproductive output calculated. Temperature and rainfall data as well as porewater temperature, pH, salinity and groundwater level were used in a principal component analysis to determine the effects of environmental conditions on biological responses. Findings of similar species around the world are also compared to the findings in this study. Furthermore, the study of *Salicornia* species such as *S. tegetaria* in South Africa provide baseline information under field conditions that may be used to promote seed, vegetable or oil production in future.

Table 9. Description of *Afrocornia* species in South Africa from Steffen *et al*. (2010).

Species	Description	Inflorescences
S. tegetaria	Found in Cape estuaries, intertidal, prostrate to decumbent subshrub, mat-forming, rooting at nodes, segments barrel-shaped to obconical	3 flowers per cyme.
S. capensis	Found in Cape estuaries, supratidal, shrub, decumbent, creeping clonal, irregular branching, branches are thin, tough and long. Segments very regular, cylindrical, keeled, elongated	Inflorescences terminal and lateral. 3 flowers per cyme.
S. decumbens	Found in Cape estuaries, mat-forming decumbent subshrub, segments obconical to cylindrical, keeled	3-7 flowers per cyme, perianth corky when fruiting.
S. littorea	Found in Cape estuaries, coastal cliffs, Erect shrub, segments cylindrical, distinctly keeled	3 flowers per cyme.
S. natalensis ssp. *natalensis*	Found in Cape estuaries, prostrate herb to subshrub, segments are obconical to cylindrical, keeled, soft	Flowers covered by subtending bract. 3 flowers per cyme.
S. natalensis ssp. *affinis*	Found in Cape estuaries, prostrate to decumbent subshrub, segments obconical to cylindrical, keeled, corky when old	Flowers covered by subtending bract. 3 flowers per cyme.
S. pillansii	Found in Cape estuaries, supratidal, erect low shrub, segments cylindrical to obconical	3 flowers per cyme.
S. mossambicensis	Found in subtropical estuaries, prostrate – decumbent, mat-forming, segments barrel-shaped to obconical	3 flowers per cyme.
S. xerophila	Inland, shrub, segments cylindrical to barrel-shaped	3 flowers per cyme.
S. dunensis	Endemic to Namib Desert, decumbent creeping subshrub, segments short, corky	3 flowers per cyme.
S. mossiana	Inland, shrub, segments short, keeled	3 flowers per cyme.
S. freitagi	Inland, short-lived perennial herb, segments obconical, not keeled	3 flowers per cyme.
S. decussata	Inland, shrub, segments boat-shaped, laterally compressed	3 flowers per cyme.

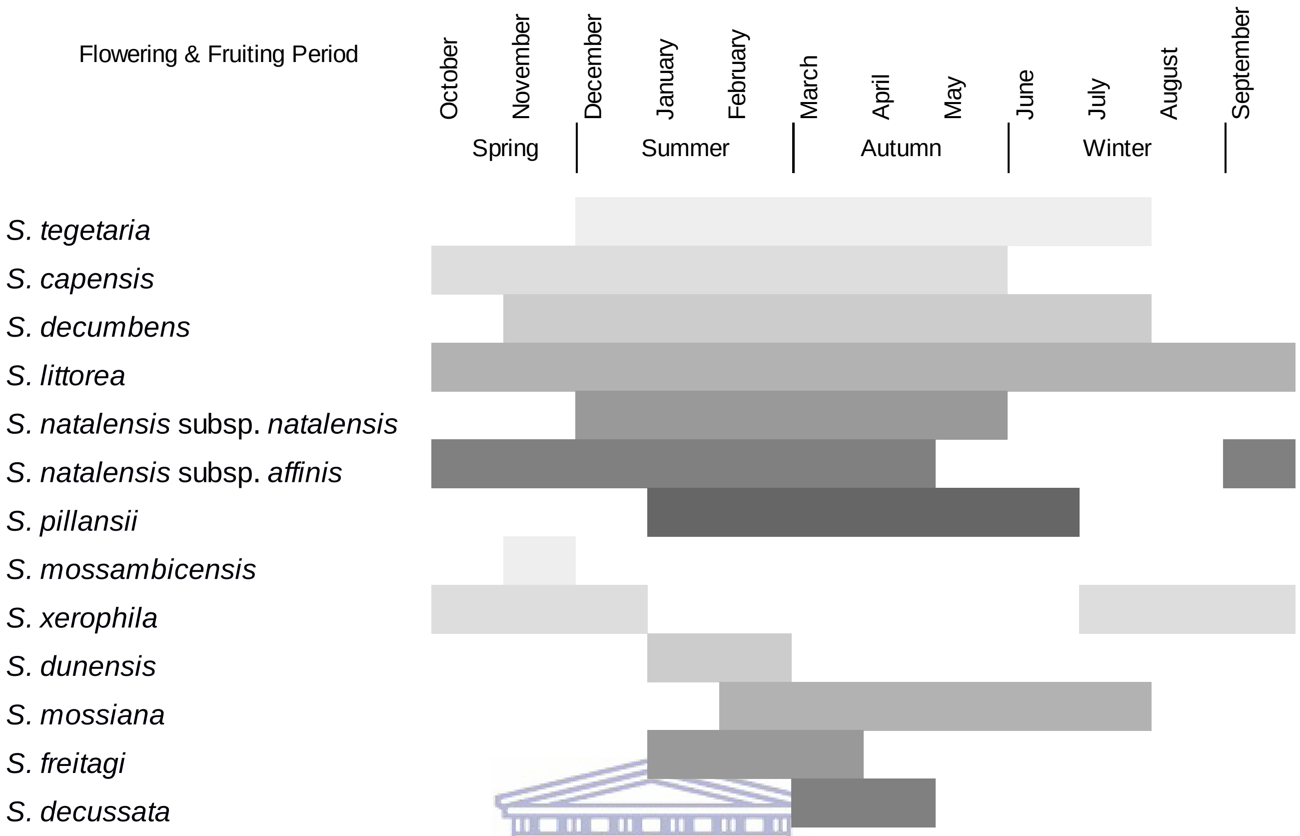


Figure 16. Flowering and fruiting times of *Afrocornia* species in South Africa (Steffen *et al*., 2010).

Study Site Description

The study site was located at Bottelary in the Langebaan Estuarine Embayment where the tidal flow is semidiurnal and water is partially exchanged with Saldanha Bay (Shannon and Stander, 1977). Freshwater inflow is low and consequently so is sediment supply, therefore the embayment lacks open mud flats (Flemming, 1977). Beach ridges separate the salt marsh from the embayment and merges with the underlying marsh muds. Groundwater seepage occurs at Oostewal and Geelbek where the area is dominated by *Phragmites australis* (Shannon and Stander, 1977; O'Callaghan, 1994) (Figure 17). Langebaan falls in the Mediterranean climatic zone in the cool temperate biogeographical region with winter rainfall and summer drought. The area of Langebaan receives approximately 260 mm of rainfall throughout the year, half of which falls during May – July (Flemming, 1977), and temperatures ranging from a winter night time low of 5 °C and summer high of 34 °C (Day, 1959). During the study period, the area received a total of 150.8 mm precipitation, most of the rainfall occurred from June – August and the average temperature ranged between 12-22 °C (Figure 18). Kowie Estuary is 70 km long and has a catchment area of 769 km^2 (Heinecken and Grindley, 1982). River flow can be widely variable and erratic with frequent floods and droughts (Cowley *et al*., 2004). In the warm temperate biogeographical region where Kowie Estuary is situated, the rainfall pattern is highly variable. In general, approximately 500 mm of rainfall occurs throughout the year and is usually slightly higher during autumn (March) and spring (October/November), and at a minimum in winter (June) (Heydorn, 1980; Jury and Levey, 1993). The temperature in the warm temperate region is mild ranging between 10-22 °C in winter and summer (Lubke and De Moor, 1998).

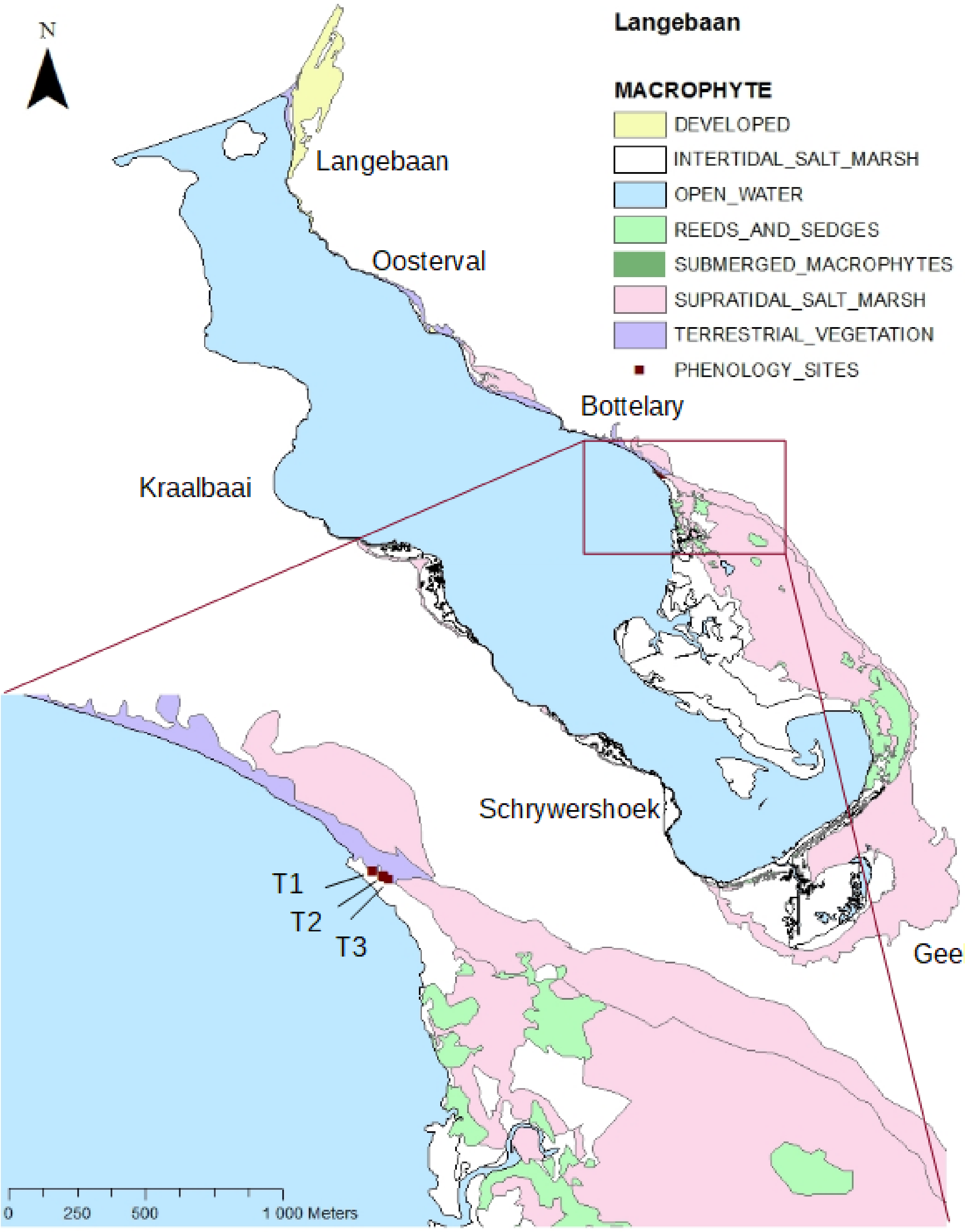


Figure 17: Map of Langebaan with the study area enlarged. Transects are demarcated as points: T1=16 m, T2=25 m, T3=30 m, T4=40 m.

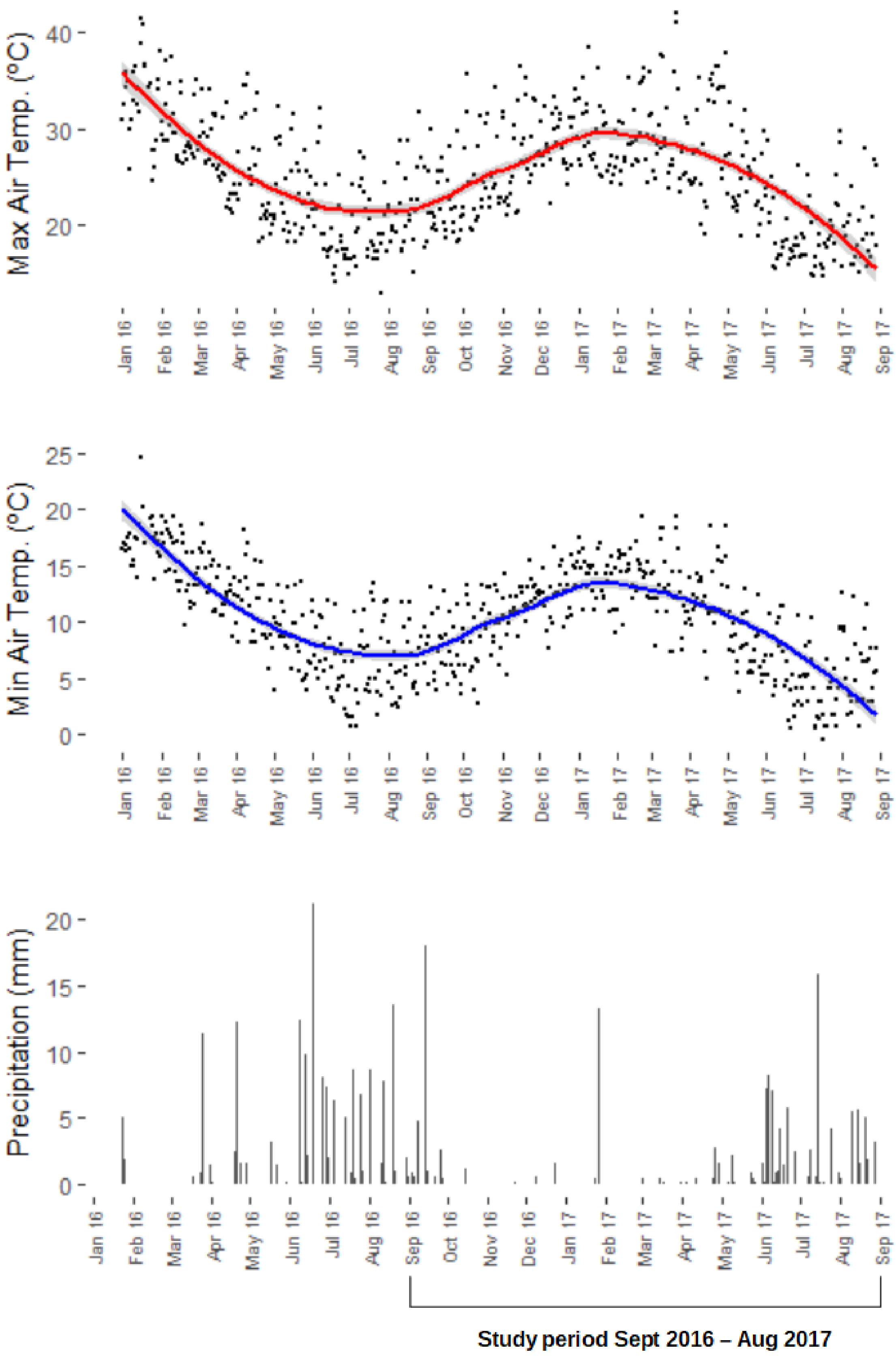


Figure 18: Average daily temperature and daily precipitation from January 2016 to August 2017 with study period demarcated. Temperature data has been smoothed to aid the visualisation of a pattern using the loess method shown as red for maximum temperature and blue for minimum temperature. The confidence interval around smoothed line is shown by the grey area. Climate data provided by SAWS.

Materials and Methods

The phenology and plant height of *Salicornia tegetaria* was recorded in Langebaan at monthly intervals from September 2016 to August 2017. Four permanent transects were demarcated with stakes at Bottelary, Langebaan. During an initial survey of Langebaan possible study sites were identified. Bottelary was chosen as it has large patches of *S. tegetaria*. Periodic trips to the other sites confirmed that flowering times at Bottelary were representative of the whole area. A total of 68 quadrats of 0.5 m x 0.5 m were allocated along four transects. An area of 0.25 m^2 was chosen as the salt marsh species are small with a high stem density and a larger quadrat would increase data inaccuracies. The methods used in this study are adapted from Vromans (2011) to make comparison possible as it is the only comprehensive study on the phenology of *S. tegetaria* globally. The percentage of vegetation cover within each 0.25 m^2 quadrat was determined by visual estimation in each quadrat and plant height measured for five of *S. tegetaria* plants. Due to their small size, the number of inflorescence were counted in a 15 cm x 15 cm quadrat within the 0.5 m x 0.5 m quadrats. The percentage of flowering, fruiting and seeding phase of the inflorescences were estimated visually in the 0.25 m^2 quadrats. The density of inflorescences per m^2 was calculated as the product of the number of inflorescences counted per 0.25 m^2 extrapolated to 1 m^2 and the proportion of vegetation cover per m^2. Fruiting and seeding phase was combined as they developed together. Thirty inflorescences were collected from outside the quadrats to count the number of seeds on each, and was used to calculate the average number of seeds per m^2 as the product of the number of inflorescences per m^2, number of seeds per inflorescence and the proportion of inflorescences in the fruiting and seeding phase (Plate 8). Results from Langebaan, which is situated in the cold temperate zone, was compared to results of a previous study at Kowie Estuary in the warm temperate zone (Vromans, 2011). The summarised data (without standard errors) was provided and permission to use the data in this format was attained from the Department of Botany of Nelson Mandela University. Three porewater wells were made during each trip to measure porewater temperature, salinity and pH using a YSI Professional Plus multimeter.

Statistical analysis

Values are presented as means (±SE) (n = 63). Data was tested for normality using Shapiro-Wilk normality test and non-parametric dependent t-tests were used to test for significant differences between months for stem height, vegetation cover and the production of flowers and seeds per m^2. An ordination of *S. tegetaria* data was obtained using Principal Components Analysis (PCA) constrained by environmental data where water was obtained for the months of September – November 2016, January – April 2017 and June – August 2017. The maximum and minimum temperatures had similar correlations and were thus analysed together as the average temperature.

Plate 8: Inflorescences in a) flowering and b) fruiting/seeding phase of *S. tegetaria.* Flowers and fruit/seed indicated with arrows. Photo C.E. Brown.

Results

Figure 19 shows the physico-chemical variable measurements taken during nine of the twelve months. Groundwater was too deep to measure physico-chemical variables in December 2016, May 2017 and June 2017, and only one well filled with water in October 2016 and August 2017. Porewater temperature was highest from January to April and lowest from June to August. Porewater salinity was the highest in January (59.06 ± 1.74) and lowest in July (28.20 ± 4.50) with an average of 41.17 ± 1.90. Porewater pH ranged between 4.58 and 7.85 ± 0.12 with a mean of 7.06 ± 0.12. The total amount of rainfall during the study period was 150.8 mm which mostly occurred during September 2016 and June – August 2017 (Figure 18).

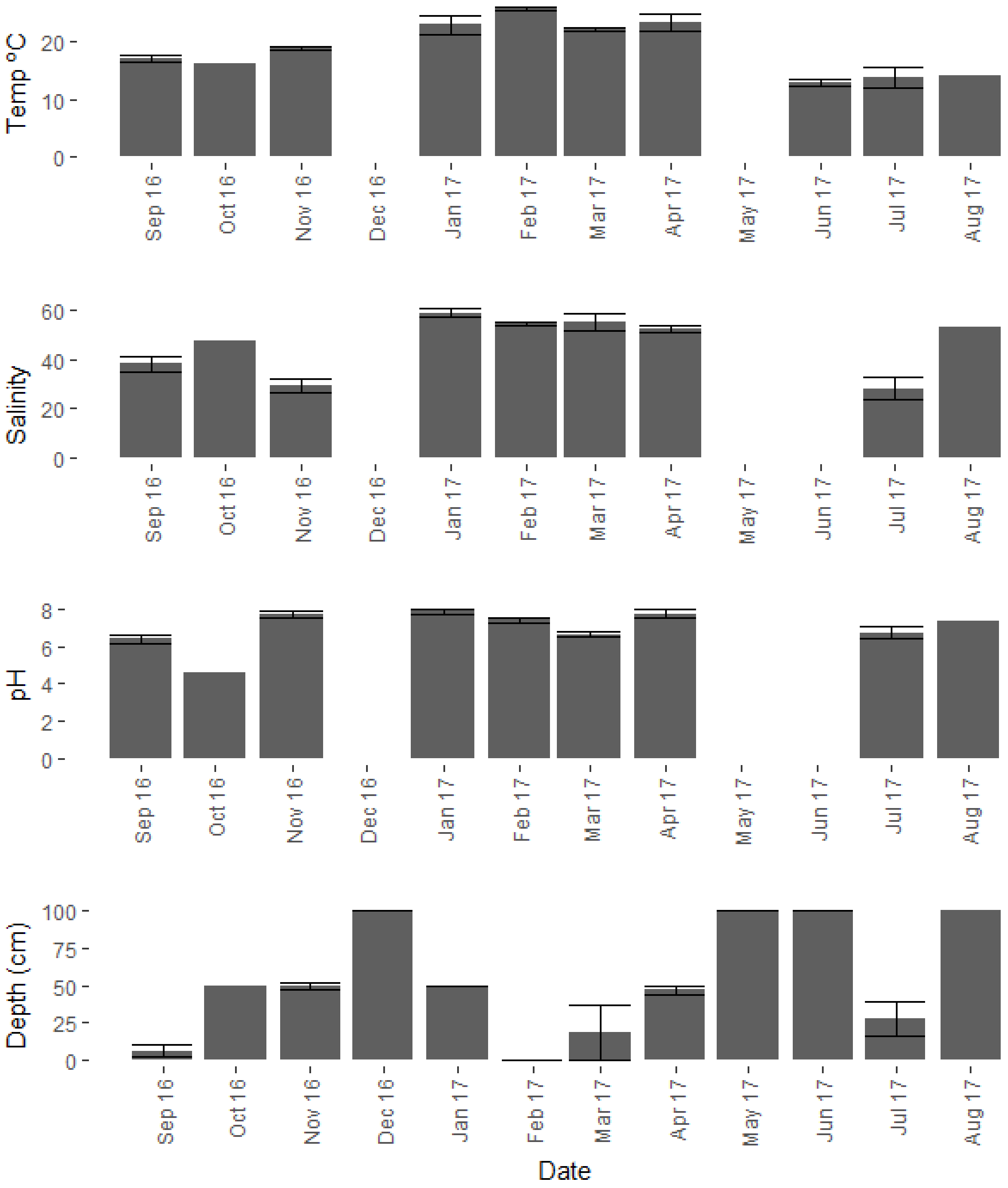


Figure 19: Physico-chemical variables measured during the study period, including porewater temperature, salinity, pH and groundwater level.

The plant species found within the quadrats were *Triglochin bulbosa, Bassia diffusa, Salicornia meyeriana, Salicornia capensis* (Moss) Piirainen & Kadereit and *Salicornia tegetaria.* Patches of bare ground and patches covered with macroalgae were also observed. Seedlings of the annual *Salicornia meyeriana* were recorded from May 2017 and had increased to about 1-3 cm in height by the end of the study period. The live vegetation cover of *S. tegetaria* remained stable throughout the study period and ranged from a minimum of 61.44 ± 4.29 % (January 2017) to a maximum of 77.38 ± 4.09 % (September 2016) with a mean of 67.64 ± 1.21 %. The percentage of dead vegetation in the quadrats ranged between 1.11 ± 0.67 % (August 2017) to 26.17 ± 2.30 % (January 2017) and the average plant height was 65.40 ± 0.76 mm (Figure 20). Vegetation cover decreased significantly from October 2016 to November 2016, and December 2016 to January 2017 as temperature increased during the summer and increased significantly from May 2017 to June 2017 during the rainfall period in winter. At Kowie Estuary, the vegetation cover of *S. tegetaria* significantly increased from April to May 2009, a month earlier than found at Langebaan, but did not change significantly during the rest of the study though seasonal growth was observed and vegetation cover peaked in autumn and summer (Vromans, 2011) (Figure 20). Statistical data of dependent t-tests are given in appendix Table 24A.

Flowering occurred from November 2016 to May 2017 at Langebaan, though one flower was observed in October 2016 signalling the start of the flowering period. Fruiting and seeding were observed from January 2017 to June 2017. Peak flowering (November – January 2017) and seeding (January – April 2017) time occurred during the high temperature and low rainfall period in summer and autumn. Flowering increased significantly in November 2016 and decreased from February 2017 to March 2017. Seed production increased significantly in January to March 2017 and decreased in April 2017 (Figure 21). A maximum of 77.24 ± 28.16 inflorescences per m^2 were observed in flowering phase in Langebaan in December 2016. Most inflorescences had 5 fertile segments with 6 flowers and the average number of seed per inflorescence was calculated as 31 ± 1.52. In comparison the species at Kowie Estuary flowered from January to June (Figure 21). The study at Kowie Estuary was conducted over two years (2009 – 2010) during which time two flower and fruit/seed production peaks were observed. The number of inflorescences in flowering phase reached a maximum of 200 ± 122 inflorescences per m^2 in June 2009 and a maximum of 69 ± 50 inflorescences per m^2 the following year in February 2010. Inflorescences had 19 fertile segments with 6 flowers and the average number of seed per inflorescence was calculated as 116 (Vromans, 2011). In Langebaan, fruiting and seeding peaked at 2 952.51 ± 599.84 seeds per m^2 in February compared to Kowie Estuary with 16 958 ± 10 772 seeds per m^2 in June 2009 and 1 587 ± 1 275 seeds per m^2 in May 2010 the following year (Vromans, 2011).

The first two dimensions of the PCA explained 67.59 % of the variance (Figure 22). The first axis of the PCA was strongly correlated to temperature which increased with an increase in pH and salinity. The second axis was positively correlated to rainfall which resulted in an increase in salinity and a decrease in pH and water level (Table 10). Flowering and seeding occurred in conjunction with an increase in temperature, pH and salinity. The percentage of dead vegetation cover increased with an increase in salinity, the percentage of live vegetation cover increased with a decrease in temperature and plant height decreased with an increase in pH. Flowering commenced in conjunction with an increase in pH and seed production with an increase in salinity.

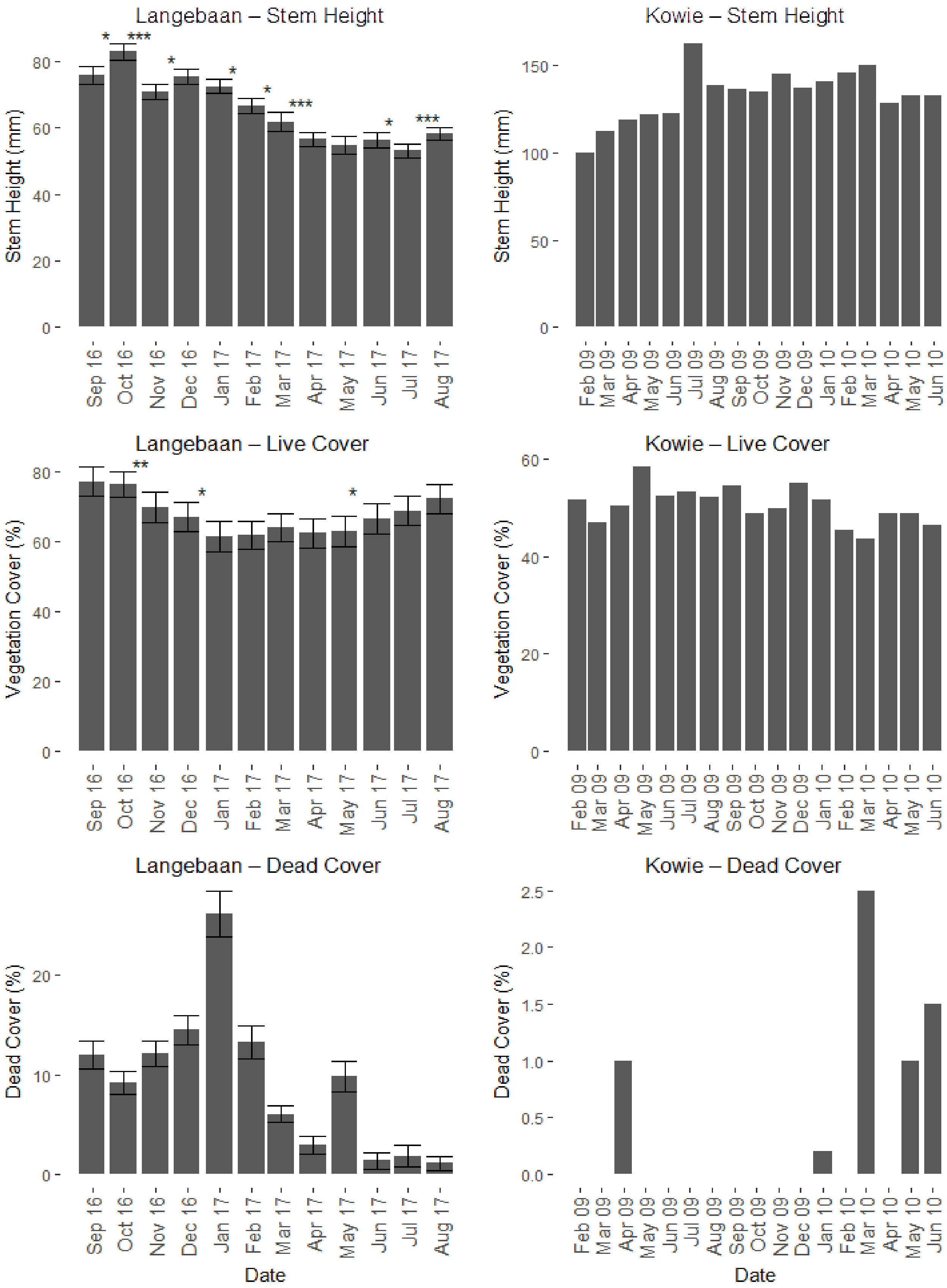


Figure 20: Means (±SE) of plant height, % vegetation cover, % dead cover of *S. tegetaria* at Langebaan (Sept 2016 – Aug 2017) are shown on the left and compared to means reported at Kowie Estuary (Feb 2009 – June 2010) on the right. Dependent t-tests of monthly plant height and cover between months are indicated as: *p<0.05, **p<0.005, ***p<0.0001.

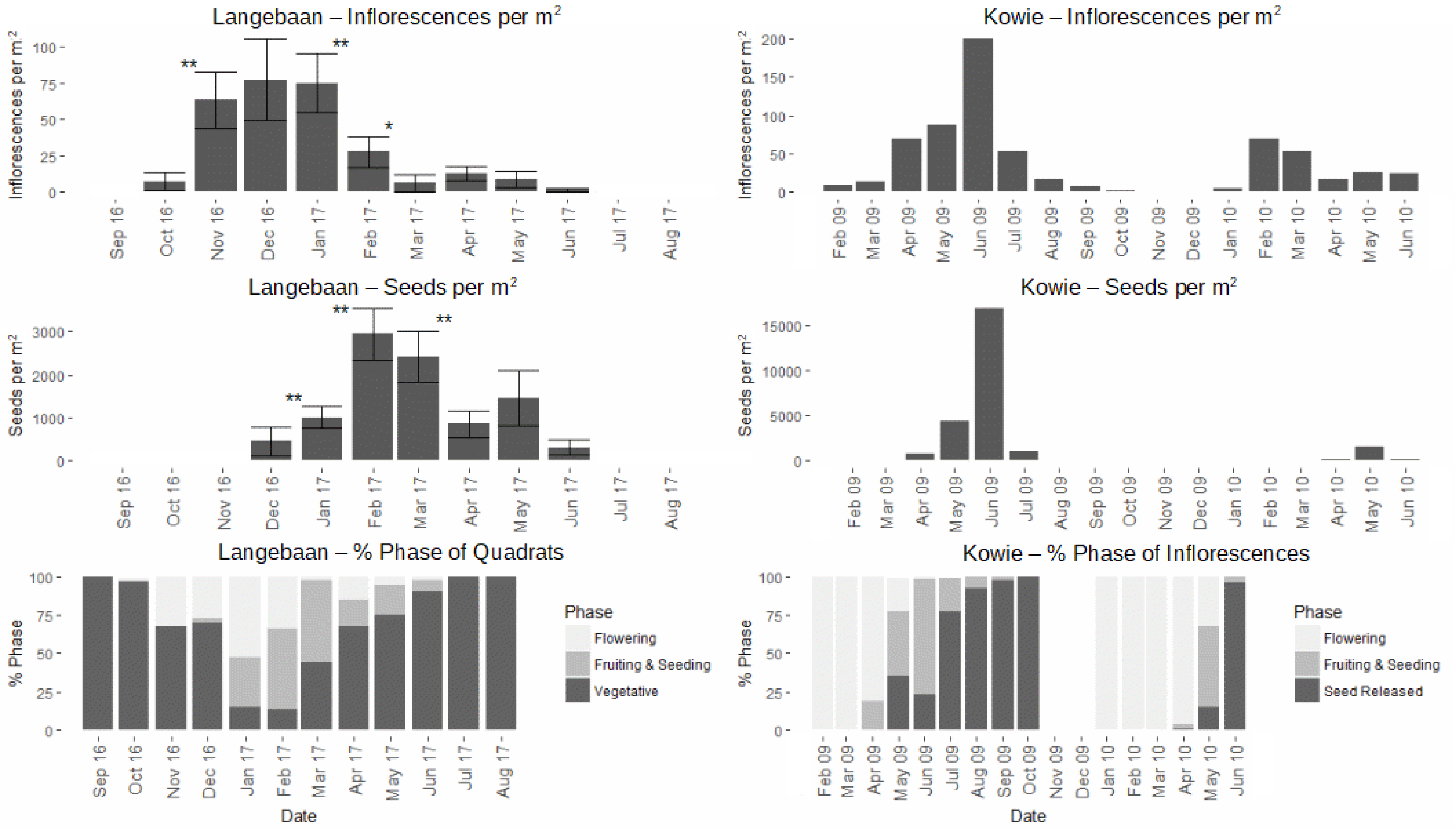


Figure 21: Means (±SE) of the number of flowers per m^2, number of seeds per m^2 of *S. tegetaria* and % of quadrats that contained flowering or seeding vegetative only individuals at Langebaan during the study period (Sept 2016 – Aug 2017) are shown on the left and compared to means reported at Kowie Estuary (Feb 2009 – June 2010) on the right. Dependent t-tests of monthly flower and seed production between months are indicated as: *p<0.05, **p<0.005, ***p<0.0001.

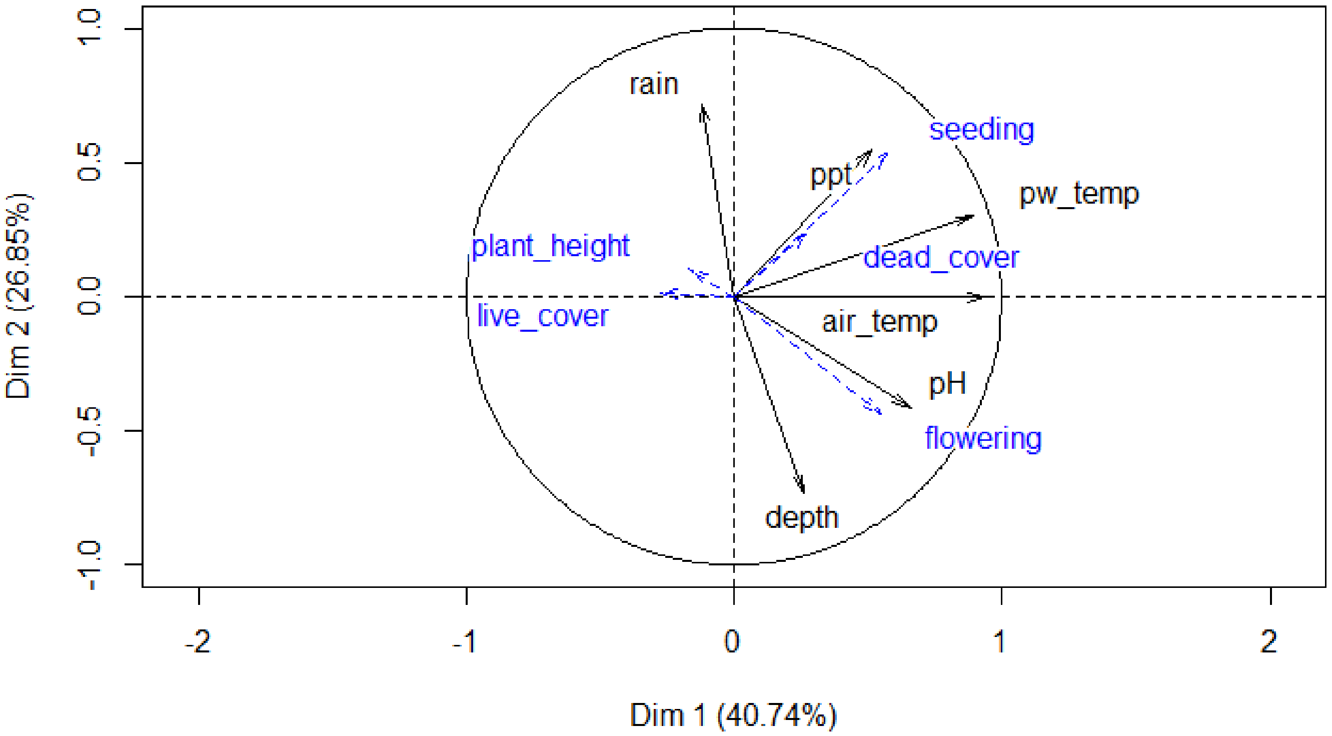


Figure 22: PCA analysis constrained by environmental variables.

Table 10: The eigenvalues and correlation of variables in the determination of principal components and correlations of phenological variables to the dimensions.

PC	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigenvalue	2.44	1.61	0.85	0.81	0.19	0.10

Environmental Variable	Correlation of variable to dimension				
	PC 1	PC 2	PC 3	PC 4	PC 5
Porewater Temperature	0.89	0.30	-0.087	-0.22	0.10
Salinity	0.51	0.54	-0.063	0.63	-0.20
pH	0.66	-0.41	0.51	-0.29	-0.24
Water Level	0.26	-0.73	0.28	0.53	0.18
Air Temperature	0.93	-0.0027	-0.23	-0.081	0.15
Rainfall	-0.12	0.72	0.67	-0.0062	0.16
Biological Correlations	Correlation of biological variables to principal components				
Flowering	0.55	-0.44	0.18	-0.24	0.34
Seeding	0.57	0.53	-0.21	-0.053	-0.039
% Vegetation Cover	-0.28	0.015	-0.29	-0.23	0.13
% Dead Cover	0.27	0.24	0.22	-0.17	0.31
Plant Height	-0.17	0.10	-0.21	-0.13	0.25

The reproductive timing found in the cool temperate region in this study was earlier than reported in the warm temperate region by Vromans (2011). Flowering was reported from December to July by Steffen *et al*. (2010) and from January to June by Vromans (2011) at Kowie Estuary. At Langebaan flowering was observed from October to June. The fruiting and seeding period was from April to September at Kowie Estuary (Vromans, 2010) and from December to June at Langebaan (Figure 23).

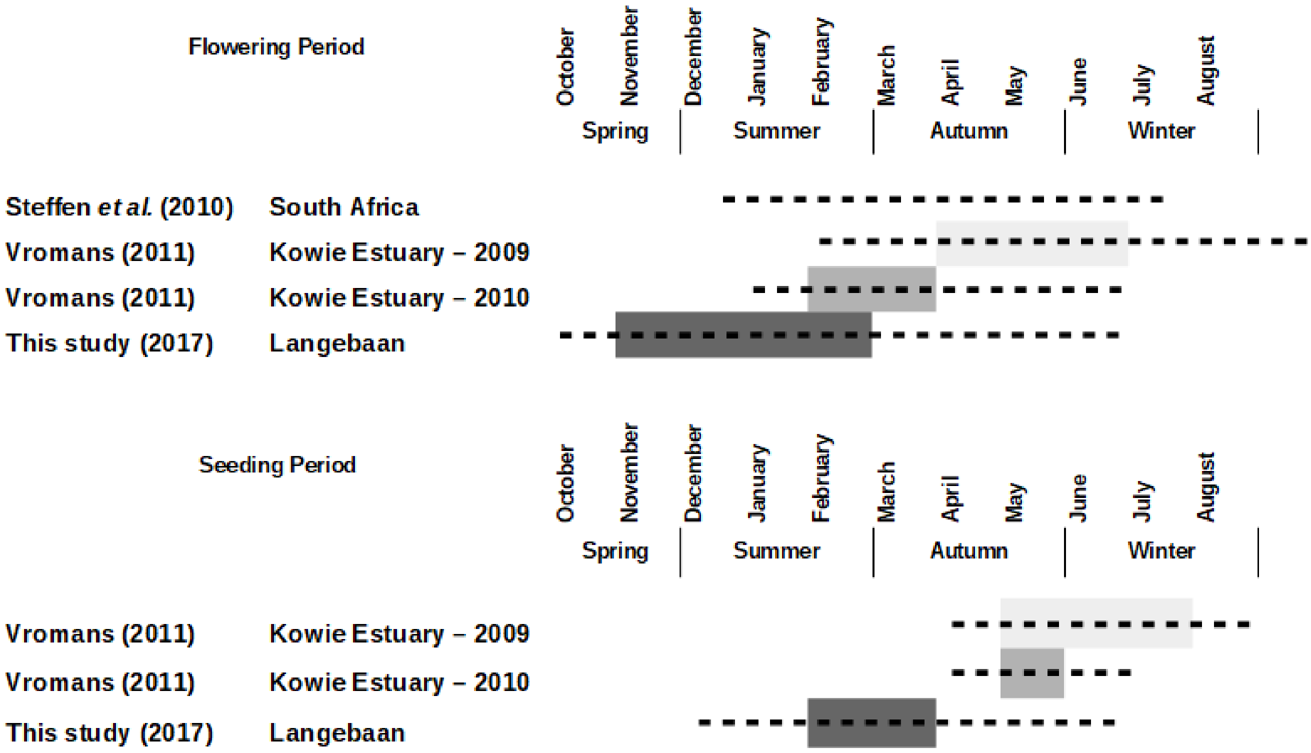


Figure 23: Comparison of reproductive timing reported for *S. tegetaria* in Steffen *et al.* (2010) and Vromans (2011) and the current study. Reproductive times are shown as dashed lines with peak flowering and seeding times highlighted in grey.

Discussion

A seasonal pattern of growth and reproductive timing of *Salicornia tegetaria* was observed at Langebaan Embayment where vegetative growth decreased and sexual reproduction increased during the higher temperatures of summer. Spring peaks in the growth of *S. tegetaria* in South Africa and *S. perennis* in Britain have also been reported by Adams and Bate (1994) and Davy *et al.* (2006). The more significant seasonal pattern in vegetation cover seen at Langebaan could be due to the more pronounced seasonal variation between winter and summer compared to Kowie Estuary in the warm temperate region, though the percentage of vegetation cover was not strongly correlated to the first or second axis of the PCA (Figure 22; Table 10). Also, the drought experienced in the Western Cape at the time of the study may have intensified seasonal patterns in the estuarine embayment.

At the Kowie Estuary, the stem height of *S. tegetaria* increased significantly from June to July 2009 which was attributed to an increase in rainfall. In this study stem height was variable and did not have a visible seasonal pattern or strong correlations to the axes of the PCA. The stem height of *S. tegetaria* growing at Kowie was higher (100-160 mm) compared to plants at Langebaan (53-83 mm). It could be that the much lower amount of rainfall in the cool temperate region (151 mm during the study period) compared to Kowie Estuary (approximately 540 mm during 2010) (Vromans 2011) resulted in stressful conditions for growth at Langebaan. Increased precipitation may also have reduced the salinity at Kowie Estuary which may have resulted in increased growth (Noe and Zedler, 2001b; Charles and Dukes, 2009), whereas the higher salinity at Langebaan may have resulted in reduced growth. Reduced growth of *S. tegetaria* at salinity above 35 ppt has been observed in greenhouse experiments (Adams and Bate, 1994). It has also been found that increased precipitation accelerates decomposition in salt marshes, which may have provided nutrients that resulted in an increase in water and ion uptake and an increase in stem height at Kowie Estuary (Naidoo and Rughunanan, 1990; van Wijck *et al*., 1992; Charles and Dukes, 2009; Husson, 2013). Kowie may receive additional pulses of nutrient input from occasional freshwater floods which may also reduce salinity (Baird *et al.,* 1988). The growth response as seen in this study and at Kowie Estuary further illustrates the plasticity of *S. tegetaria* and other *Salicornia* species (Naidoo and Rughunanan, 1990; Adams and Bate, 1994; Redondo-Gómez *et al*., 2006; Silva *et al*., 2007).

Early flowering can be an indication of stressful conditions (Wada and Takeno, 2010; Shimakawa *et al*., 2012). It has been found that the timing of the flowering of *Spartina* and *Salicornia* species was induced by shortening photoperiod and/or increasing temperatures influenced by the latitude of the species and that salinity did not have a significant effect on the timing (Seneca and Blum, 1984; Crosby *et al*., 2015; Somers and Grant, 1981; Ventura *et al*., 2011b). A similar result was found at Langebaan where the increase in inflorescences of *S. tegetaria* was not correlated to salinity. In this study flowering was correlated to an increase in pH. This could have been due to the low pH (4.58) found in October 2016 before the number of inflorescences increased significantly and may therefore not have been a direct attributing factor in the flowering phenology of *S. tegetaria*. The relation of pH and the flowering period of salt marsh macrophytes has not been studied to my knowledge, but may prove significant in future studies.

The seed production of *S. tegetaria* coincided with higher salinity (Figure 22). Salt marsh macrophytes may time their growth to avoid the period of highest salinity when seeds germinate. For example, in Britain, where the salinity is lower in winter (Davy, 2001), the flowering of *S. perennis* occurs in summer and autumn (August – September) and seeds are released in autumn (October) (Davy, 2006). Whereas the above-mentioned studies did not find a relation to flower induction and salinity, other studies have found that the flowering and seed production of salt marsh macrophytes in Mediterranean climates were influenced by salinity (Ungar, 1987a; Laegdsgaard, 2006). For example in a greenhouse experiment, flowering of *Salicornia quinqueflora* (Bunge ex Ung.-Sternb.) A.J. Scott was initiated by high salinity in summer (Clarke and Hannon, 1970). Similar results were found at Kowie Estuary where flower and seed production occurred when salinity was highest (from January to July), and flower and seed production was completed when salinity was lower (Vromans, 2011). No seeds of *S. tegetaria* germinated at Kowie Estuary or at Langebaan (Vromans, 2011) and vegetation cover remained stable in both salt marshes. This suggests that the salt marsh studied at Langebaan was well established with dense cover of clonal species which favoured vegetative growth, especially since *S. tegetaria* formed monospecific stands where the germination of seeds would be reduced (Ungar, 1978; Shumway and Bertness, 1992; Baldwin *et al*., 1996; Redondo *et al*., 2004; Javier *et al*., 2005; Davy *et al*., 2006). Perennial species such as *S. tegetaria* do not require a persistent seed bank or a high percentage of germination success as they can rely on vegetative propagation (Ungar, 1987b).

The highly reduced flowers of *S. tegetaria* suggest that the species is wind pollinated. The flowering time and duration of flowering of plants in close proximity would have been important in determining pollination donors, pollination receptors and seed production (Somers and Grant, 1981). Flowering and seeding occurred earlier in the year in Langebaan than that reported in the warm temperate Kowie Estuary. However, earlier and longer flowering times did not result in a conclusive difference in flower production when the two estuaries were compared as flower and seed production was similar between Langebaan and Kowie Estuary during one year and much higher at the Kowie Estuary during another year (Figure 21). The maximum temperature was higher and minimum temperatures lower at Langebaan compared to the more mild temperatures in the warm temperate Kowie which may have influenced earlier flowering. Fernández-Illescas *et al*. (2010) measured the inflorescences, flower and seed production in a Mediterranean salt marsh for *S. perennis.* It produced 1 826 ± 415 inflorescences per m^2 and 80 016 seeds per m^2, higher that found at Kowie or Langebaan. The inflorescences had 7 fertile segments with 6 flowers and the number of seeds per inflorescence was calculated as 44 similar to *S. tegetaria* in this study. Physico-chemical variables were not measured making comparison difficult and the methods used to count inflorescences in that study was different to the current work and Vromans (2011), which may account for the large differences reported.

The variation in reproductive effort observed as well as the seed production per reproductive shoot at Kowie Estuary compared to Langebaan could be due to environmental conditions that increase growth. For example increased nutrients, will increase reproductive effort (Jefferies and Perkins, 1977) and environmental conditions that reduce growth, for example high salinity (Ungar, 1987a, 1987b; Blits and Gallagher, 1991) or low soil temperatures (Jefferies *et al*., 1983), reduce reproductive effort. Alternative strategies are employed depending on the species and environment, for example, experiments have shown that moderate increases in salinity can stimulate increased sexual reproductive effort even though biomass is reduced, with a sharp decrease in seed production at high salinity (Van Zandt *et al*., 2003). The allocation of resources also depends on the unpredictability of the environment. Plants with a lower mortality rate in a more favourable environment (Jefferies *et al*., 1981; Jerling and

Liljelund, 1984) or high density populations that inhibit seedling recruitment (Jefferies *et al*., 1981) could have lower reproductive effort. The salinity at Kowie Estuary was lower compared to Langebaan and was related to an increase in the number of inflorescences (Vromans, 2011). The differences in stem height could also have attributed to the differences in seeds per inflorescence found between Langebaan and Kowie Estuary. The number of seeds per inflorescence were similar in the Mediterranean which has a similar climate as found at Langebaan (Fernandez-Illescas, 2010), whereas Kowie Estuary had a higher annual rainfall which influenced plant height (Vromans, 2011). An increase in sexual reproductive effort with increasing ramet size have been found in small densely populated clonal plants that grow horizontally, whereas vegetative reproductive effort was independent of size (Hartnett, 1990). *Salicornia tegetaria* displays clonal growth which produces identical plants, and outcrossing, which can produce highly diverse offspring (Stebbins, 1950). Future studies may benefit from germination experiments to determine seed viability and the importance of sexual reproduction under less stable conditions.

The PCA only explained 67.59 % of the variance suggesting that other factors also contributed to the phenology of *S. tegetaria*. Measurements at more frequent intervals may have included more of the spatio-temporal variability that may not have been captured during monthly sampling (Noe and Zedler, 2001b). Other possible variables that could have had an effect on the phenology of *S. tegetaria* include porewater nutrient concentrations (Jefferies and Perkins), plant density (Jefferies *et al*., 1981) and inundation period (Adams and Bate, 1994). It is also important to note that the interpretation of the results from this study should take into account that observations were only made during one year in drought conditions. It may be found that the phenology of the species may vary when measured for longer, as was found at Kowie Estuary.

Conclusion

The results from this chapter show that there are differences in the phenology of *S. tegetaria* between the cool temperate biogeographical region at Langebaan Embayment and the warm temperate biogeographical region at Kowie Estuary. The climate in these two regions vary widely. The cool temperate region is characterised by an arid Mediterranean winter rainfall climate with higher maximum temperatures and lower minimum temperatures than found in the warm temperate region. In the warm temperate region precipitation is higher and more frequent with a slightly bimodial distribution during autumn and spring (Van Niekerk and Turpie, 2012). Climate plays an important role in the phenology of plants, determining their timing of growth and reproduction (Luterbacher *et al*., 2007). At Langebaan live vegetation cover increased during the winter rainfall period and decreased in summer, whereas vegetation cover peaked in autumn and summer at Kowie. Differences in precipitation and freshwater flooding may have influenced the size of plants in the two estuaries and may have resulted in a lower production of seeds per reproductive shoot at Langebaan. A reduction in precipitation and freshwater to estuaries in the cool temperate region may result in lower seed production and smaller seed banks. This in turn could affect the dispersal of genetic material and genetic diversity in the region (Bilton *et al*., 2002). Flowering and seed production also occurred earlier at Langebaan than found at Kowie which may have implications for belowground biomass production and salt marsh elevation gain if there are environmental changes in future (Crosby *et al.*, 2015).

Chapter 6: General discussion and conclusion

There are interactions between the resource allocation, phenology and the genetic diversity of plant species. Differences in the growth and allocation of resources, dependent on the availability of resources and the abiotic environment, may affect the reproductive output of plants (Bazzaz *et al.*, 1987). Species such as *S. tegetaria* that carry phenotypic plasticity may display a wide range of responses, including the allocation of biomass and phenology, to the variable conditions found in estuaries and biogeographical regions which could have a genetic basis or result in selective traits (Adam, 1993; Silva *et al*., 2007). The production of inflorescences and propagules can further result in a re-allocation of above- or belowground biomass (Hackney and De La Cruz, 1986; Petanidou *et al.*, 1995; Obeso, 2002; Luterbacher *et al.*, 2007; Crosby *et al*., 2015). The dispersal mechanisms and reproductive timing of *S. tegetaria* are further important determinants of the genetic diversity of the species (Bilton *et al*., 2002). Salt marshes are of high conservation value as habitats that support high levels of biodiversity, are important to fisheries and have a high recreational value. Furthermore, the resilience of coastal ecosystems are interlinked with the resilience of estuaries (Van Niekerk and Turpie, 2012). This thesis presented the phylogeography, autecology and phenology of *Salicornia tegetaria* in South African estuaries spanning two biogeographical regions. New insights have been gained into the population dynamics of the species, factors that affect the growth and allocation of resources between two biogeographical regions as well as the reproductive effort and timing in a cool temperate estuarine embayment.

The first objective of the study was to determine if the populations of *Salicornia tegetaria* show distinct genetic structure that reflect biogeographical disjuncts and asked whether populations are equally connected between estuaries or show a pattern of genetic differentiation. It was found that *S. tegetaria* had some genetic structuring that could be due to the geomorphological evolution of the South African coastline during the glacial and interglacial cycles that occur every 100 000 years. The location of estuaries shifted during these events whereby habitat availability changed and became fragmented periodically. This study contributes to the phylogeography of estuarine macrophytes in South Africa and the hypothesis that similar historical events may have influenced the genetic structure of macrophytes on the south coast. Similar results were found in *Juncus kraussii* (Potts *et al*., 2016) and *Salicornia meyeriana* (Slenzka *et al*., 2013). A shallow genetic structure was evident between the west and south coast with a disjunct between Breede and Goukou. Unique single rare haplotypes were identified mostly in the warm temperate region suggesting recent range expansion. The genetic and haplotype diversity, Fu's F_S and Tajima's D statistic further supported this finding. The rare haplotypes of *S. tegetaria* were found at Langebaan, Heuningnes, Goukou, Groot Brak, Knysna, Keiskamma, Nahoon, Kwelera, Tyolomnqa and Kobonqaba, representing permanently open and temporarily open/closed estuaries, an estuarine bay and an estuarine embayment. These estuaries may prove to be important reservoirs of genetic diversity of associated species (Hughes and Stachowicz; Crutsinger *et al*., 2006; Johnson *et al*., 2006; Crawford *et al*., 2007;

Johnson and Stinchcombe, 2007). Of these estuaries Langebaan, Heuningnes, Nahoon and Keiskamma have some protection in nature reserves. The salt marshes at Langebaan form part of the West Coast National Park and are under full protection. Heuningnes Estuary falls within the De Mond Forest Reserve in the lower reaches and is bordered by farmlands upstream. Nahoon Estuary is surrounded by suburban development and the salt marshes fall within the East London Coastal Nature Reserve and Keiskamma Estuary falls within the Hamburg Nature Reserve. Langebaan and Heuningnes have been identified as estuaries with important salt marsh macrophyte populations, including *Salicornia* species (Van Niekerk and Turpie, 2012). Future studies may find similar patterns for other macrophytes where areas of high genetic diversity are an important source to the genetic pool of species, ensuring resilience to the ecosystem and maintaining biodiversity (Taberlet, 1998; Redding and Mooers, 2006; Beger *et al*., 2014).

The migration of species is important in maintaining biodiversity during climate change (Pompe *et al.*, 2008) and the connectivity between estuaries will be important in maintaining the biodiversity of coastal wetland species (Bilton *et al*., 2002). The haplotype network and Φ_{ST} values show that there is connectivity between estuaries that should ensure resilience in these ecosystems. Results from this study suggest that *S. tegetaria* disperses via hydrochory which is influenced by oceanic currents as well as long distance possibly avian dispersal (Dalby, 1963; Huiskes *et al*., 1995; Slenzka *et al*., 2013). The area of highest genetic differentiation was found on the east coast forming a gradient from east to west with a low isolation by distance signal. It is hypothesised that dispersal in this species has occurred in the direction of the Agulhas Current on the south and south-east coast. It was found in Chapter 5 that *S. tegetaria* seeds are released from January to June on the west coast, and April to September further along on the south east coast. The timing therefore avoids the summer upwelling events making it more probable that seeds can be dispersed west along the coast. Climate change may have an effect on dispersal as it is predicted that the Agulhas Current may move further from the shore more frequently (Lutjeharms and de Ruijter, (1996). The distribution of *S. tegetaria* continues to Mozambique and surrounding islands (Steffen *et al.,* 2009). The latitudinal increase in genetic diversity suggest that a more pronounced genetic structure may be found with further sampling along the east coast in the subtropical and tropical biogeographical regions. It is important to note that the sampling size of DNA sequence analysis of each estuary was relatively small due to the breadth of sampling undertook, which may result in a less robust interpretation of the results at the population level. Even so, the study does aid the understanding of the dynamics of this species.

The second objective was to determine if there is a difference in the functioning of *S. tegetaria* between the two temperate biogeographical regions. It was hypothesised that differences in rainfall and temperature between the warm and cool temperate biogeographical regions may result in a difference in biomass allocation driven by varying abiotic factors. *Salicornia tegetaria* was found to grow in a wide range of physico-chemical conditions though significant differences between the two regions were observed. Sediment moisture content, sediment organic content and sediment electrical conductivity were significantly lower in the warm temperate region, and sediment pH, porewater temperature and porewater pH were significantly higher in the warm temperate region. The study provided an indication of the environmental factors that promote the growth of *S. tegetaria* and which physico-chemical variables caused a re-allocation of resources. The aboveground biomass of *Salicornia tegetaria* grew better in sediments with a higher moisture and organic content, and resource re-allocation belowground occurred in relation to pH and porewater salinity. At the local scale resource allocation was influenced by the sediment redox potential, moisture content, porewater depth and porewater temperature. The correlations at the local scale were stronger as variability found between estuaries was reduced.

Climatic variation and the physico-chemical measurements taken between the warm and cool temperate biogeographical regions during this study did not have a strong effect on total biomass and the standing above-and belowground biomass of *S. tegetaria* was fairly stable in the estuaries studied. The variability found in the aboveground biomass and stem height between estuaries was not significantly different between the warm and cool temperate region. Heuningnes had significantly lower belowground biomass and root/shoot ratios than the other estuaries, whereas the belowground biomass was not significantly different between the remaining estuaries. Heuningnes also had the lowest salinity and highest pH, which may have influenced belowground growth. Lower salinity in the rhizosphere may have lowered the water potential in the soil and plants required less belowground biomass for the absorption of water (Flowers and Colmer, 2015). At lower salinity the rate of organic matter decay also increases, providing nutrients that promote the re-allocation of biomass aboveground (Darby and Turner, 2008; Ouyang *et al.*, 2017). Furthermore, the study showed that lower pH values were related to higher belowground growth possibly as a response to the pH requirements of the roots in transporting ions under saline conditions (Braun *et al.*, 1986).

The different sediment sources and quantities of sediment delivered to the estuaries may have implications on belowground biomass production during climate change (Reed, 1989; Van Niekerk and Turpie, 2012). It has been calculated that sea level is increasing by approximately 0.42 mm per year off the west coast, 1.57 mm per year on the southern coast and 3.55 mm per year along the east coast (Mather *et al.*, 2009). Langebaan has no river input to supply sediment to the system, but did have a high root/shoot ratio, whereas Heuningnes receives mainly marine input and had lower belowground biomass than the other estuaries. An increase in sea storms may cause in increase in wave height and an increase in marine sediment along areas of sediment-rich coastline which could affect the biomass of salt marsh macrophytes near the mouth of Heuningnes Estuary (Van Niekerk and Turpie, 2012). Salt marshes are classified as blue carbon ecosystems that sequester carbon within the sediment over the long term, and above-and belowground biomass over the short term, at a higher rate than terrestrial ecosystems (Chmura *et al.*, 2003; Mcleod *et al.*, 2011). The accumulation of organic matter (OM) is linked to the production of aboveground biomass import or export and the production and decomposition of belowground biomass. Measuring the belowground biomass and organic matter in salt marshes will aid estimates of the amount of carbon they store (Ouyang *et al.*, 2017) which in turn affects the response of salt marshes to sea-level rise that depends on sediment supply and accretion (Lovelock *et al.*, 2015). The total carbon (TC) content of standing *S. perennis* subsp. *alpini* was calculated as 403 ± 19 $mg.g^{-1}$ belowground and approximately 722 $mg.g^{-1}$ aboveground. The carbon concentrations in the sediments were calculated as 12.8 ± 0.2 kg $TC.m^{-2}$ in spring and 16.5 ± 0.3 kg $TC.m^{-2}$ in winter (Palomo and Niell, 2009). The carbon content of organic matter in salt marsh sediments varies depending on the species present but on average has been calculated globally at 0.039 ± 0.003 $g.cm^{-3}$ (Chmura *et al*., 2003; Wang *et al.*, 2003). Using the quadratic relationship reported by Craft *et al.* (1991):

$$\% \text{ organic carbon} = (0.04)\ \text{OM} + (0.0025)\ \text{OM}^2,$$

the percentage of organic carbon present in organic matter was calculated as 0.40 % in the warm temperate region and more than double at 1.01 % in the cool temperate region, making these estuaries valuable as carbon reservoirs and in carbon trading (Van Niekerk and Turpie, 2012). The high aboveground biomass found at Olifants and Berg Estuary are also potentially providing an important habitat to nekton and a source of nutrients and detritus to surrounding ecosystems, improving the productivity of the coastal environment (Van Niekerk and Turpie, 2012). The health state of the estuaries that were studied were rated as Fair/Good in the National Biodiversity Assessment (Van Niekerk and Turpie, 2012). The biomass of urban estuaries may be more severely affected by pollution and an excess of nutrients. In a eutrophic environment, belowground biomass would be expected to be much lower. The BGB/AGB ratio of *S. perennis* in a eutrophic system was 0.16, much lower than found in estuaries that are in a good condition (Palomo and Niell, 2009). The values reported in this study may be a useful indicator in comparison with salt marshes that are suspected to be in a poor condition.

The third objective was to determine if there is a difference in the phenology of *S. tegetaria* between the two temperate biogeographical regions by comparing the growth, reproductive timing and seed production between two estuaries found in the warm and cool temperate regions, respectively. Phenology could be an important indicator of shifts in changing climatic conditions, and differences in the phenology of a species found in two different climate regimes may provide insights into the effects that environmental changes may have (Luterbacher *et al.*, 2007). The vegetative growth had a more seasonal pattern at Langebaan compared to Kowie Estuary. At Langebaan live vegetation cover increased during the winter rainfall period and decreased in summer. The timing of flowering and seeding occurred earlier in the year at Langebaan for a longer period. The earlier reproductive phases could be due to the higher maximum and lower minimum temperatures found in the cool temperate region compared to the warm temperate region or an indication of stressful conditions (Seneca and Blum, 1984; Wada and Takeno, 2010; Shimakawa *et al.*, 2012; Crosby *et al.*, 2015). At Kowie Estuary stem height was influenced by precipitation and vegetation cover by salinity. The differing precipitation and freshwater runoff between these two estuaries may have influenced the size of plants with a lower production of seeds per reproductive shoot. Flower and seed production was similar one year and lower during another year compared to results at Kowie Estuary. This study only recorded the reproductive output over one year, and as shown at Kowie Estuary over two years the reproductive output could be quite variable. The results from this study therefore should be interpreted with caution. It may be found that results over the one year of sampling could have been affected by drought conditions in the Western Cape during the study period. Continued monitoring of the phenology of *S. tegetaria* at Langebaan may prove useful in determining the effects of climatic variation. Whereas flower production was related to salinity at Kowie, salinity was more predictive of seed production in Langebaan. Climate change could alter the reproduction of *S. tegetaria* as it has been predicted that precipitation will be reduced with a decrease in freshwater runoff in the cool temperate region (Van Niekerk and Turpie, 2012) which may result in lower seed production and smaller seed banks. The resilience of this species could also be affected as seed production and dispersal could affect the genetic diversity in the region (Bilton *et al*., 2002). Earlier flowering and seed production at Langebaan than found at Kowie may also have implications for biomass allocation (e.g. Crosby *et al.*, 2015).

To date very little research has been done on the phylogeography of macrophytes found in the estuaries of South Africa. The results of the DNA sequence analyses of *S. tegetaria* provides the first understanding of the population dynamics of this species and adds to the knowledge of the dynamics of estuarine macrophyte species. *Salicornia tegetaria* is a common species in the lower intertidal salt marshes of South Africa and therefore may be a useful indicator of underlying processes that occur in the salt marsh environment as it has been found that parallel processes may affect both the genetic diversity of a common species/population and the species diversity within the community (Vellend, 2005; Vellend and Geber, 2005). The study also provides new insights into the relationships of the phenology and biomass allocation of *S. tegetaria* in relation to multiple environmental variables and climatic conditions. These relationships may prove to be important determinants of genetic diversity and ecosystem resilience in the lower intertidal salt marsh habitat with climate change as for example phenotypic variability may be related to the diversity of *S. tegetaria* populations and belowground biomass production to the adaptation of this species to elevation (Vellend and Geber, 2005; McKee *et al.*, 2007). The root/shoot ratios reported here agree with the general consensus that salt marsh species store a higher amount of carbon belowground than terrestrial macrophytes (Schubauer and Hopkinson, 1984). This study represents the first quantitative estimation of the standing biomass and therefore short-term carbon storage of a salt marsh species in South Africa which can be included in global carbon budgets and the evaluation of salt marsh ecosystem services (Costanza *et al.*, 2014; Ouyang and Lee, 2014).

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Appendix

Table 1A: AMOVA of *ITS4* gene region of *S. tegetaria* from 17 estuaries.

Source of variation	df	Sum of squares	% variation	Φ statistic	*P*
All populations					
Among pop Within pop	16 56	21.12 18.82	40.60 59.40	Φ_{ST} 0.41	<0.0001
(Olifants – Breede) + (Goukou – Mngazana)					
Among groups Among pop Within pop	1 15 56	13.76 7.36 18.82	54.35 % 4.44 41.21	Φ_{CT} 0.54 Φ_{SC} 0.097 Φ_{ST} 0.54	<0.0001 0.025 <0.0001

Table 2A: AMOVA of *rpS16* gene region of *S. tegetaria* from 17 estuaries

Source of variation	df	Sum of squares	% variation	Φ statistic	*P*
All populations					
Among pop Within pop	16 65	5.97 14.40	12.41 87.59	Φ_{ST} 0.12	0.0073
(Olifants – Breede) + (Goukou – Mngazana)					
Among groups Among pop Within pop	1 15 65	0.35 5.62 14.40	-0.27 12.55 87.72	Φ_{CT} -0.0027 Φ_{SC} 0.13 Φ_{ST} 0.12	0.39 0.010 0.0065

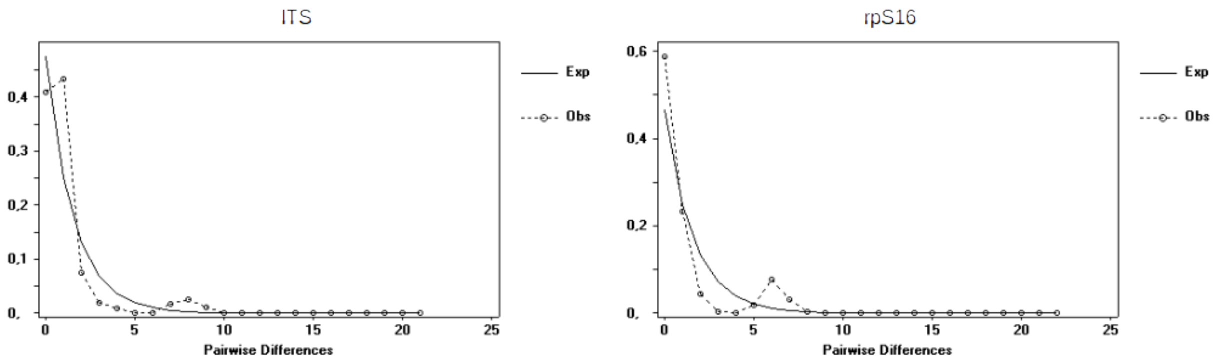

Figure 1A: Mismatch distribution frequencies of nucleotide differences between pairs of haplotypes observed for *ITS* and *rpS16* sequences.

Table 3A: Sediment characteristics at each estuary and in each biogeographical region.

Estuary	Sediment Moisture (%)			Sediment Organic (%)			Sediment Redox (mV)			Sediment Electrical Conductivity (m.S)			Sediment pH		
	Min	**Max**	**Ave ± SE**	**Min**	**Max**	**Ave ± SE**	**Min**	**Max**	**Ave ± SE**	**Min**	**Max**	**Ave ± SE**	**Min**	**Max**	**Ave ± SE**
Olifants	42.5	72.0	55.2 ± 2.0	6.1	21.4	12.8 ± 1.1	-296.8	225.1	-165.3 ± 28.6	23.9	93.6	43.8 ± 4.4	5.53	7.69	6.9 ± 0.1
Berg	51.5	72.1	62.0 ± 1.5	10.3	26.2	18.0 ± 0.9	-189.9	258.4	128.9 ± 32.5	35.6	83.0	59.9 ± 3.2	5.50	7.10	6.6 ± 0.1
Langebaan	18.5	61.5	37.8 ± 2.7	1.11	26.3	9.9 ± 1.7	-124.6	231.7	183.9 ± 11.8	27.3	69.0	44.0 ± 2.2	6.02	7.94	6.5 ± 0.1
Heuningnes	18.4	30.8	24.1 ± 0.7	2.42	9.35	3.9 ± 0.4	31.79	382.7	232.0 ± 23.1	21.6	43.1	30.8 ± 1.1	7.56	9.53	9.4 ± 0.0
Nahoon	27.2	51.6	35.6 ± 1.4	3.97	38.84	11.7 ± 2.4	-58.21	331.5	168.1 ± 29.2	20.1	50.1	38.1 ± 1.7	6.84	8.72	7.8 ± 0.1
Kwelera	23.3	41.2	30.6 ± 1.2	3.10	11.96	5.8 ± 0.4	-147.2	439.6	13.9 ± 35.6	21.3	53.3	39.4 ± 2.1	6.72	8.60	7.1 ± 0.1

Biogeographical Region	Sediment Moisture % Ave ± SE	Sediment Organic % Ave ± SE	Sediment Redox Ave ± SE	Sediment Electrical Conductivity m.S Ave ± SE	Sediment pH Ave ± SE
Cool Temperate	51.6 ± 1.9	13.6 ± 0.9	57.4 ± 25.3	49.1 ± 2.2	6.7 ± 0.1
Warm Temperate	30.1 ± 0.9	7.0 ± 0.9	138.0 ± 20.8	36.1 ± 1.1	8.1 ± 0.1

Table 4A: Porewater characteristics in winter and summer at each estuary and in each biogeographical region in total.

Estuary/Region	Season	Temperature (°C)			Salinity			pH			Salinity		
		Min	Max	Ave ± SE	Min	Max	Ave ± SE	Min	Max	Ave ± SE	Min	Max	Ave ± SE
Olifants	Winter	12.0	14.8	13.76 ± 0.31	27.9	61.5	42.71 ± 3.58	6.39	7.14	6.89 ± 0.09	10	>100	32.83 ± 6.13
	Summer	18.0	18.9	18.35 ± 0.12	23.9	40.2	29.15 ± 2.48	6.98	8.29	7.96 ± 0.10	25	>100	44.50 ± 5.61
Berg	Winter	12.7	13.8	13.31 ± 0.11	29.6	59.7	41.24 ± 3.76	5.38	7.00	6.57 ± 0.20	10	50	30.00 ± 5.07
	Summer	17.5	23.1	20.10 ± 0.87	30.7	39.3	35.75 ± 1.06	7.45	8.08	7.92 ± 0.06	0	>100	28.67 ± 8.60
Langebaan	Winter	14.8	19.6	17.06 ± 0.56	31.1	61.3	38.33 ± 3.07	5.60	7.31	6.40 ± 0.22	0	30	5.87 ± 3.94
	Summer	17.8	20.8	18.92 ± 0.29	14.6	40.4	29.27 ± 2.58	7.14	8.36	7.71 ± 0.15	42	60	49.11 ± 2.13
Heuningnes	Winter	14.6	16.2	15.13 ± 0.14	26.7	42.0	33.97 ± 1.21	7.29	8.44	8.01 ± 0.12	>100	>100	>100
	Summer	17.4	18.6	17.99 ± 0.16	12.4	31.6	20.33 ± 2.02	7.90	12.5	8.79 ± 0.47	20	31	27.11 ± 1.35
Nahoon	Winter	13.3	16.9	14.14 ± 0.22	37.6	51.4	42.52 ± 1.72	6.51	7.32	6.97 ± 0.10	0	>100	20.63 ± 4.12
	Summer	19.7	22.8	22.11 ± 0.16	18.7	46.8	38.04 ± 3.18	7.79	8.47	8.26 ± 0.07	40	>100	55.71 ± 3.66
Kwelera	Winter	14.6	16.0	15.41 ± 0.17	37.6	69.7	49.11 ± 3.69	6.95	7.79	7.31 ± 0.10	20	90	50.11 ± 7.21
	Summer	20.1	23.7	22.93 ± 0.24	27.9	43.0	38.48 ± 1.63	6.14	7.54	6.69 ± 0.10	40	>100	51.50 ± 2.20
Cool Temperate	16.37 ± 0.37				36.79 ± 1.41			7.07 ± 0.10			30.65 ± 2.80		
Warm Temperate	17.93 ± 0.47				37.06 ± 1.62			7.66 ± 0.14			40.54 ± 2.56		

Table 5A: Mean ± SE of above- and belowground biomass and allocation ratio in winter and summer at each estuary and in each biogeographical region in total.

Estuary	**Season**	**Plant height (mm)**			**Aboveground biomass (kg.m^{-2})**			**Belowground biomass (kg.m^{-2})**			**Root/shoot ratio**		
		Min	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**
Olifants	Winter	51.5	131.4	90.04 ± 0.3	0.98	3.79	2.26 ± 0.3	1.00	11.38	4.86 ± 1.1	0.34	4.47	2.40 ± 0.5
	Summer	55.5	139.8	99.36 ± 0.2	0.77	2.52	1.66 ± 0.2	2.21	8.34	4.17 ± 0.6	1.20	9.27	2.99 ± 0.8
Berg	Winter	552	148.1	105.69 ± 0.3	0.77	2.95	1.59 ± 0.3	3.55	5.66	4.45 ± 0.3	1.52	5.75	3.45 ± 0.5
	Summer	49.8	181.6	124.48 ± 0.2	0.50	3.02	1.35 ± 0.2	2.70	5.64	4.24 ± 0.3	1.34	5.82	3.77 ± 0.5
Langebaan	Winter	62.8	112.5	76.60 ± 0.1	0.36	1.05	0.59 ± 0.1	1.06	6.22	3.46 ± 0.5	2.66	11.64	6.36 ± 1.1
	Summer	43.4	122.1	76.58 ± 0.1	0.73	1.43	1.14 ± 0.1	1.61	7.66	4.06 ± 0.6	1.35	5.52	3.66 ± 0.5
Heuningnes	Winter	48.8	105.1	79.60 ± 0.1	0.71	1.76	1.17 ± 0.1	0.23	4.57	1.57 ± 0.5	0.33	3.27	1.26 ± 0.3
	Summer	54.1	102.5	69.22 ± 0.1	0.64	1.26	0.98 ± 0.1	0.62	2.47	1.45 ± 0.2	0.96	2.38	1.47 ± 0.1
Nahoon	Winter	81.9	150.9	119.59 ± 0.1	0.80	2.06	1.42 ± 0.1	1.22	6.33	3.21 ± 0.6	0.89	7.20	2.44 ± 0.6
	Summer	87.3	166.7	115.46 ± 0.1	0.96	1.54	1.33 ± 0.1	2.40	5.78	4.07 ± 0.4	1.61	3.83	3.08 ± 0.2
Kwelera	Winter	55.3	141.6	96.08 ± 0.1	0.60	1.82	0.91 ± 0.1	0.63	12.10	5.76 ± 1.1	0.35	14.17	7.43 ± 1.6
	Summer	52.3	190.3	103.77 ± 0.2	0.68	2.19	1.26 ± 0.2	1.57	4.14	2.62 ± 0.3	0.75	5.20	2.50 ± 0.5
Biogeographic region		**Plant height (mm)**			**Aboveground biomass kg.m^{-2}**			**Belowground biomass (kg.m^{-2})**			**Root/shoot ratio**		
		Min	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**
Cool-temperate		43.4	181.6	95.5 ± 4.44	0.36	3.79	1.43 ± 0.11	1.00	11.38	4.20 ± 0.25	0.33	11.64	3.77 ± 0.32
Warm-temperate		48.8	190.3	97.3 ± 4.47	0.60	2.19	1.18 ± 0.05	0.23	12.10	3.11 ± 0.31	0.33	14.17	3.03 ± 0.41

Table 6A: Kruskal-Wallis one way ANOVA by ranks of physico-chemical variables, plant height, above- and belowground biomass and allocation between estuaries.

Variable	Df	Chi-squared	P-value
height	5	29	<0.0001
shoot	5	34	<0.0001
root	5	31.14	<0.0001
ratio	5	34.70	<0.0001
Sediment Moisture	5	81.10	<0.0001
Sediment Organic	5	61.75	<0.0001
Sediment EC	5	45.05	<0.0001
Sediment pH	5	41.67	<0.0001
Sediment Redox	5	23.75	0.00024
Porewater Temperature	5	20.46	0.0010
Porewater Salinity	5	30.25	<0.0001
Porewater pH	5	31.02	<0.0001

Table 7A: Kruskal-Wallis multiple comparisons of mean ranks of sediment moisture content. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	7.6221918	2.494041e-14	3.741062e-13
2	be - kw	5.5943118	2.214986e-08	1.107493e-07
3	he - kw	-1.9182024	5.508535e-02	7.511638e-02
4	be - la	4.0496282	5.129907e-05	1.282477e-04
5	he - la	-3.5725636	3.535036e-04	7.575078e-04
6	kw - la	-1.6029547	1.089447e-01	1.361808e-01
7	be - na	4.3206877	1.555437e-05	4.666311e-05
8	he - na	-3.3015041	9.616792e-04	1.602799e-03
9	kw - na	-1.3357956	1.816161e-01	2.095570e-01
10	la - na	0.2710595	7.863453e-01	7.863453e-01
11	be - ol	0.9042550	3.658602e-01	3.919931e-01
12	he - ol	-6.6082592	3.888654e-11	2.916490e-10
13	kw - ol	-4.6244584	3.755785e-06	1.408419e-05
14	la - ol	-3.0871021	2.021181e-03	3.031772e-03
15	na - ol	-3.3542612	7.957720e-04	1.492072e-03

Table 8A: Kruskal-Wallis multiple comparisons of mean ranks of sediment organic content. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	6.9391220	3.945444e-12	5.918166e-11
2	be - kw	5.3507136	8.760809e-08	6.570607e-07
3	he - kw	-1.5884084	1.121940e-01	1.294546e-01
4	be - la	3.5996695	3.186219e-04	7.965547e-04
5	he - la	-3.3394524	8.394372e-04	1.798794e-03
6	kw - la	-1.7510441	7.993832e-02	1.090068e-01
7	be - na	3.0833305	2.046977e-03	3.838082e-03
8	he - na	-3.7559428	1.726901e-04	6.475880e-04
9	kw - na	-2.1903904	2.849593e-02	4.749322e-02
10	la - na	-0.4645426	6.422591e-01	6.422591e-01
11	be - ol	1.6236988	1.044401e-01	1.305501e-01
12	he - ol	-5.2155745	1.832484e-07	9.162420e-07
13	kw - ol	-3.6500221	2.622178e-04	7.866533e-04
14	la - ol	-1.9241742	5.433275e-02	8.149912e-02
15	na - ol	-1.4392162	1.500893e-01	1.608099e-01

Table 9A: Kruskal-Wallis multiple comparisons of mean ranks of sediment electrical conductivity. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	6.5505143	5.733925e-11	8.600887e-10
2	be - kw	3.6078381	3.087590e-04	1.157846e-03
3	he - kw	-2.9426762	3.253885e-03	6.972611e-03
4	be - la	2.5196333	1.174772e-02	1.957953e-02
5	he - la	-4.0308811	5.556815e-05	4.167612e-04
6	kw - la	-1.0882049	2.765047e-01	3.770518e-01
7	be - na	3.8978487	9.705102e-05	4.852551e-04
8	he - na	-2.6526656	7.985893e-03	1.497355e-02
9	kw - na	0.2900106	7.718081e-01	8.269373e-01
10	la - na	1.3782154	1.681368e-01	2.522052e-01
11	be - ol	3.4109502	6.473691e-04	1.942107e-03
12	he - ol	-3.1395641	1.691994e-03	4.229984e-03
13	kw - ol	-0.1968879	8.439152e-01	8.439152e-01
14	la - ol	0.8913169	3.727592e-01	4.659490e-01
15	na - ol	-0.4868985	6.263303e-01	7.226888e-01

Table 10A: Kruskal-Wallis multiple comparisons of mean ranks of sediment pH. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	-5.0212917	5.132514e-07	3.849385e-06
2	be - kw	-2.0604085	3.935951e-02	6.559918e-02
3	he - kw	2.9608833	3.067582e-03	7.668955e-03
4	be - la	0.1678851	8.666736e-01	8.666736e-01
5	he - la	5.1891769	2.112257e-07	3.168386e-06
6	kw - la	2.2282936	2.586094e-02	5.541631e-02
7	be - na	-3.6324238	2.807715e-04	1.052893e-03
8	he - na	1.3888679	1.648729e-01	2.060911e-01
9	kw - na	-1.5720153	1.159470e-01	1.739205e-01
10	la - na	-3.8003089	1.445158e-04	7.225790e-04
11	be - ol	-1.3399965	1.802465e-01	2.079767e-01
12	he - ol	3.5313720	4.134099e-04	1.240230e-03
13	kw - ol	0.6588933	5.099643e-01	5.463903e-01
14	la - ol	-1.5028690	1.328728e-01	1.811902e-01
15	na - ol	2.1839722	2.896429e-02	5.430804e-02

Table 11A: Kruskal-Wallis multiple comparisons of mean ranks of sediment redox potential. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	-1.40413021	1.602801e-01	0.2671334656
2	be - kw	1.23624508	2.163675e-01	0.3245512204
3	he - kw	2.64037528	8.281427e-03	0.0248442800
4	be - la	-0.53417997	5.932170e-01	0.6355896697
5	he - la	0.86995024	3.843276e-01	0.5240830895
6	kw - la	-1.77042505	7.665636e-02	0.1642636295
7	be - na	-0.54944226	5.827020e-01	0.6723484409
8	he - na	0.85468795	3.927239e-01	0.4909049016
9	kw - na	-1.78568733	7.414990e-02	0.1853747443
10	la - na	-0.01526228	9.878229e-01	0.9878229313
11	be - ol	2.93540671	3.331108e-03	0.0124916535
12	he - ol	4.29761310	1.726472e-05	0.0002589708
13	kw - ol	1.73607282	8.255096e-02	0.1547830492
14	la - ol	3.45363740	5.530805e-04	0.0027654024
15	na - ol	3.46844399	5.234816e-04	0.0039261122

Table 12A: Kruskal-Wallis multiple comparisons of mean ranks of porewater temperature. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	-2.1266423	0.0334498179	0.083624545
2	be - kw	-3.4943975	0.0004751330	0.007126995
3	he - kw	-1.3941499	0.1632723299	0.272120550
4	be - la	-3.3920081	0.0006938238	0.005203679
5	he - la	-1.2599487	0.2076878706	0.311531806
6	kw - la	0.1578659	0.8745625012	0.937031251
7	be - na	-2.2632538	0.0236200464	0.070860139
8	he - na	-0.1448984	0.8847910514	0.884791051
9	kw - na	1.2447920	0.2132082021	0.290738457
10	la - na	1.1086072	0.2675996700	0.334499588
11	be - ol	-0.4939067	0.6213720880	0.716967794
12	he - ol	1.6060189	0.1082697541	0.203005789
13	kw - ol	2.9596050	0.0030803376	0.015401688
14	la - ol	2.8509605	0.0043587390	0.016345271
15	na - ol	1.7426305	0.0813981819	0.174424676

Table 13A: Kruskal-Wallis multiple comparisons of mean ranks of porewater salinity. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	2.85096455	4.358683e-03	1.634506e-02
2	be - kw	-1.60052337	1.094825e-01	1.824709e-01
3	he - kw	-4.77294108	1.815549e-06	2.723324e-05
4	be - la	1.39023005	1.644590e-01	2.466885e-01
5	he - la	-1.67637508	9.366475e-02	1.756214e-01
6	kw - la	3.28679847	1.013333e-03	5.066666e-03
7	be - na	-1.03005307	3.029851e-01	3.495982e-01
8	he - na	-4.11644082	3.847681e-05	2.885761e-04
9	kw - na	0.59063302	5.547663e-01	5.943925e-01
10	la - na	-2.62310713	8.713187e-03	2.178297e-02
11	be - ol	0.07191126	9.426725e-01	9.426725e-01
12	he - ol	-2.77516343	5.517397e-03	1.655219e-02
13	kw - ol	1.67739974	9.346434e-02	2.002807e-01
14	la - ol	-1.31145521	1.897040e-01	2.586873e-01
15	na - ol	1.10585420	2.687896e-01	3.359870e-01

Table 14A: Kruskal-Wallis multiple comparisons of mean ranks of porewater pH. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	-4.5199839	6.184434e-06	4.638325e-05
2	be - kw	-0.3037156	7.613446e-01	7.613446e-01
3	he - kw	4.6184585	3.866014e-06	5.799021e-05
4	be - la	-0.8179387	4.133922e-01	5.167402e-01
5	he - la	4.1354184	3.543084e-05	1.771542e-04
6	kw - la	-0.5627317	5.736176e-01	6.618664e-01
7	be - na	-2.1946117	2.819147e-02	7.047866e-02
8	he - na	2.4664297	1.364675e-02	4.094025e-02
9	kw - na	-2.0761209	3.788277e-02	8.117736e-02
10	la - na	-1.5593166	1.189215e-01	2.229777e-01
11	be - ol	-1.0390204	2.987953e-01	4.481929e-01
12	he - ol	3.4247602	6.153422e-04	2.307533e-03
13	kw - ol	-0.8213155	4.114666e-01	5.610908e-01
14	la - ol	-0.3202511	7.487780e-01	8.022621e-01
15	na - ol	1.0993880	2.715989e-01	4.526648e-01

Table 15A: Kruskal-Wallis multiple comparisons of mean ranks of porewater depth. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	-0.8321304	0.405335322	0.60800298
2	be - kw	-0.6281243	0.529922537	0.72262164
3	he - kw	0.2040062	0.838348676	0.83834868
4	be - la	1.8843728	0.059514566	0.22317962
5	he - la	2.7165032	0.006597555	0.04948166
6	kw - la	2.5124971	0.011988010	0.05994005
7	be - na	0.2174276	0.827875100	0.88700904
8	he - na	1.0495581	0.293921344	0.55110252
9	kw - na	0.8455519	0.397802769	0.66300462
10	la - na	-1.6669452	0.095525308	0.28657592
11	be - ol	-1.2374585	0.215916915	0.46267910
12	he - ol	-0.4053281	0.685236389	0.79065737
13	kw - ol	-0.6093342	0.542302926	0.67787866
14	la - ol	-3.1218313	0.001797299	0.02695949
15	na - ol	-1.4548861	0.145700790	0.36425198

Table 16A: Kruskal-Wallis multiple comparisons of mean ranks of stem height. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	3.6078553	3.087386e-04	0.0015436929
2	be - kw	1.4128106	1.577114e-01	0.1971392776
3	he - kw	-2.1950447	2.816039e-02	0.0844811721
4	be - la	3.3843598	7.134443e-04	0.0026754162
5	he - la	-0.2234955	8.231499e-01	0.8819463376
6	kw - la	1.9715492	4.866109e-02	0.0811018094
7	be - na	-0.5667206	5.709040e-01	0.6587353841
8	he - na	-4.1745759	2.985416e-05	0.0004478124
9	kw - na	-1.9795312	4.775623e-02	0.0895429259
10	la - na	-3.9510805	7.779916e-05	0.0005834937
11	be - ol	1.5804322	1.140079e-01	0.1554653513
12	he - ol	-2.0274231	4.261916e-02	0.0913267716
13	kw - ol	0.1676216	8.668810e-01	0.8668809804
14	la - ol	-1.8039277	7.124265e-02	0.1068639740
15	na - ol	2.1471528	3.178112e-02	0.0794527923

Table 17A: Kruskal-Wallis multiple comparisons of mean ranks of AGB. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	1.52731357	1.266831e-01	1.727496e-01
2	be - kw	1.58585172	1.127730e-01	1.691594e-01
3	he - kw	0.05853815	9.533200e-01	9.533200e-01
4	be - la	2.71138071	6.700365e-03	2.010109e-02
5	he - la	1.18406714	2.363865e-01	2.954831e-01
6	kw - la	1.12552899	2.603649e-01	3.004211e-01
7	be - na	-0.53748666	5.909315e-01	6.331409e-01
8	he - na	-2.06480023	3.894191e-02	7.301607e-02
9	kw - na	-2.12333838	3.372551e-02	7.226894e-02
10	la - na	-3.24886737	1.158655e-03	4.344956e-03
11	be - ol	-2.42933326	1.512662e-02	3.781655e-02
12	he - ol	-3.95664682	7.600918e-05	3.800459e-04
13	kw - ol	-4.01518498	5.939919e-05	4.454939e-04
14	la - ol	-5.14071396	2.736964e-07	4.105447e-06
15	na - ol	-1.89184660	5.851142e-02	9.751904e-02

Table 18A: Kruskal-Wallis multiple comparisons of mean ranks of BGB. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	5.08985519	3.583371e-07	5.375056e-06
2	be - kw	1.29574463	1.950635e-01	3.657440e-01
3	he - kw	-3.79411056	1.481736e-04	5.556512e-04
4	be - la	1.38088596	1.673140e-01	4.182851e-01
5	he - la	-3.70896923	2.081047e-04	6.243141e-04
6	kw - la	0.08514133	9.321490e-01	9.987311e-01
7	be - na	1.29574463	1.950635e-01	4.179932e-01
8	he - na	-3.79411056	1.481736e-04	7.408682e-04
9	kw - na	0.00000000	1.000000e+00	1.000000e+00
10	la - na	-0.08514133	9.321490e-01	1.000000e+00
11	be - ol	0.81948531	4.125096e-01	6.875160e-01
12	he - ol	-4.27036988	1.951491e-05	1.463618e-04
13	kw - ol	-0.47625932	6.338896e-01	7.923621e-01
14	la - ol	-0.56140065	5.745244e-01	8.617867e-01
15	na - ol	-0.47625932	6.338896e-01	8.643950e-01

Table 19A: Kruskal-Wallis multiple comparisons of mean ranks of AGB/BGB ratios. Estuaries are abbreviated as follows: ol = Olifants, be = Berg, la = Langebaan, he = Heuningnes, na = Nahoon and kw = Kwelera.

	Comparison	Z	P.unadj	P.adj
1	be - he	4.2570057	2.071831e-05	1.553873e-04
2	be - kw	0.1064251	9.152450e-01	9.152450e-01
3	he - kw	-4.1505806	3.316330e-05	1.658165e-04
4	be - la -	0.9791113	3.275250e-01	3.779134e-01
5	he - la	-5.2361170	1.639900e-07	2.459850e-06
6	kw - la	-1.0855365	2.776841e-01	3.471052e-01
7	be - na	1.5059158	1.320888e-01	1.981332e-01
8	he - na	-2.7510899	5.939733e-03	1.781920e-02
9	kw - na	1.3994906	1.616659e-01	2.204535e-01
10	la - na	2.4850271	1.295416e-02	3.238539e-02
11	be - ol	1.9422589	5.210577e-02	9.769832e-02
12	he - ol	-2.3147469	2.062679e-02	4.420026e-02
13	kw - ol	1.8358337	6.638225e-02	1.106371e-01
14	la - ol	2.9213702	3.484955e-03	1.306858e-02
15	na - ol	0.4363431	6.625878e-01	7.099155e-01

Table 20A: The eigenvalues of principal components, contributions of variables in the determination of principal components and correlations of biomass variables to the dimensions at the landscape scale.

Principal Component	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Eigenvalue	3.46	1.94	1.37	1.02	0.65	0.60	0.40	0.31	0.13	0.11

Environmental Variable	Contribution of variable to dimension				
	PC 1	PC 2	PC 3	PC 4	PC 5
Maximum Temperature	0.71	0.45	0.21	-0.13	0.11
Minimum Temperature	-0.69	0.44	0.44	-0.10	0.08
Rainfall	-0.66	-0.40	0.53	-0.14	-0.004
Sediment Moisture	0.91	0.15	0.15	-0.10	-0.12
Sediment EC	0.67	0.09	0.26	0.06	0.60
Sediment Organic	0.67	0.15	0.33	-0.16	-0.45
Porewater salinity	0.14	-0.65	0.56	0.27	0.08
Water Level	0.02	0.34	0.25	0.86	-0.18
Porewater Temperature	-0.41	0.58	0.47	-0.28	-0.04
Porewater pH	-0.37	0.68	-0.23	0.20	0.12
Biological Correlations	Correlation of biological variables to principal components				
Belowground Biomass	0.31	-0.09	0.23	0.07	-0.01
Aboveground Biomass	0.29	0.05	-0.04	0.05	-0.01
Ratio	0.13	-0.21	0.18	-0.10	0.06
Stem Height	0.12	0.002	0.29	-0.08	0.04

Table 21A: The eigenvalues of principal components, contributions of variables in the determination of principal components and correlations of biomass variables to the dimensions in the cool temperate region.

Principal Component	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Eigenvalue	4.41	2.58	0.94	0.81	0.46	0.42	0.28	0.08	0.02	0.00

Environmental Variable	Contribution of variable to dimension				
	PC 1	PC 2	PC 3	PC 4	PC 5
Maximum Temperature	0.96	0.084	0.01	-0.19	-0.01
Minimum Temperature	0.96	-0.23	0.01	-0.04	0.08
Rainfall	-0.91	-0.22	-0.01	0.25	0.05
Sediment Moisture	0.30	0.88	-0.28	0.01	0.04
Sediment EC	0.26	0.54	0.36	0.68	-0.20
Sediment Organic	0.12	0.82	-0.42	0.11	0.26
Porewater salinity	-0.43	0.46	0.64	-0.14	0.37
Water Level	0.71	0.24	0.39	-0.24	-0.07
Porewater Temperature	0.52	-0.63	-0.04	0.32	0.44
Porewater pH	0.79	-0.28	0.03	0.26	-0.05
Biological Correlations	Correlation of biological variables to principal components				
Belowground Biomass	0.06	0.14	0.001	-0.25	-0.01
Aboveground Biomass	0.17	0.21	0.03	-0.37	-0.38
Ratio	-0.27	-0.09	-0.24	0.29	0.29
Stem Height	0.27	0.20	-0.07	-0.15	-0.14

Table 22A: The eigenvalues of principal components, contributions of variables in the determination of principal components and correlations of biomass variables to the dimensions in the warm temperate region.

Principal Component	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Eigenvalue	3.76	2.14	1.39	0.83	0.74	0.46	0.43	0.14	0.10	0.01

Environmental Variable	Contribution of variable to dimension				
	PC 1	PC 2	PC 3	PC 4	PC 5
Maximum Temperature	0.93	0.12	-0.22	0.05	-0.03
Minimum Temperature	0.59	0.75	-0.09	-0.13	0.12
Rainfall	0.91	-0.22	0.17	-0.03	0.04
Sediment Moisture	0.71	-0.02	0.19	-0.12	-0.55
Sediment EC	0.49	-0.36	-0.50	0.10	0.45
Sediment Organic	0.42	0.20	0.47	0.69	-0.04
Porewater salinity	0.50	-0.69	0.20	0.14	0.20
Water Level	-0.31	-0.27	0.80	-0.21	0.24
Porewater Temperature	0.45	0.67	0.35	-0.30	0.31
Porewater pH	-0.49	0.59	0.00	0.38	0.13
Biological Correlations	Correlation of biological variables to principal components				
Belowground Biomass	0.40	-0.28	-0.01	0.10	0.18
Aboveground Biomass	0.22	-0.03	-0.04	0.08	-0.11
Ratio	0.28	-0.31	-0.05	0.06	0.25
Stem Height	0.49	-0.16	-0.03	0.02	-0.16

Table 23A: Spearman's correlations for individual estuaries, n = number of observations, r = correlation coefficient, p = p-value.

OLIFANTS

Variable 1	Variable 2	n	r	p
shoot	ratio	18	-0.56	0.0155
root	ratio	18	0.67	0.0023
sed_moisture	sed_organic	18	0.86	<0.0001
root	sed_pH	18	-0.54	0.0218
sed_organic	sed_pH	18	-0.63	0.0053
sed_moisture	sed_ec	18	0.83	<0.0001
sed_organic	sed_ec	18	0.74	0.0004
sed_pH	sed_ec	18	-0.57	0.0127
sed_moisture	pw_temp	18	-0.50	0.0336
sed_redox	pw_temp	18	-0.52	0.0286
sed_ec	pw_temp	18	-0.68	0.0019
sed_organic	pw_salinity	18	0.50	0.0358
sed_pH	pw_salinity	18	-0.72	0.0007
sed_ec	pw_salinity	18	0.52	0.027
pw_temp	pw_salinity	18	-0.50	0.0327
sed_pH	pw_pH	18	0.49	0.0378
sed_ec	pw_pH	18	-0.57	0.0138
pw_temp	pw_pH	18	0.73	0.0006
pw_salinity	pw_pH	18	-0.48	0.0447
shoot	pw_depth	18	-0.57	0.0126
ratio	pw_depth	18	0.49	0.0388
sed_organic	pw_depth	18	0.50	0.0355

BERG

Variable 1	Variable 2	n	r	p
shoot	ratio	18	-0.89	<0.0001
shoot	sed_moisture	18	-0.55	0.0182
ratio	sed_moisture	18	0.49	0.0392
sed_moisture	sed_organic	18	0.66	0.0030
sed_organic	sed_redox	18	-0.51	0.0297
sed_moisture	sed_pH	18	-0.56	0.0148
sed_organic	sed_pH	18	-0.51	0.0319
sed_redox	sed_pH	18	0.72	0.0009
sed_redox	sed_ec	18	0.56	0.0150
sed_ec	pw_temp	18	0.69	0.0016
sed_redox	pw_salinity	18	-0.49	0.0400
sed_pH	pw_salinity	18	-0.69	0.0014
sed_redox	pw_pH	18	0.62	0.0057
sed_pH	pw_pH	18	0.56	0.0155
sed_ec	pw_pH	18	0.74	0.0004
pw_temp	pw_pH	18	0.72	0.0007
pw_salinity	pw_pH	18	-0.48	0.0426
sed_redox	pw_depth	18	0.81	<0.0001
sed_ec	pw_depth	18	0.56	0.0159
pw_pH	pw_depth	18	0.48	0.0416

LANGEBAAN

Variable 1	Variable 2	n	r	p
shoot	ratio	18	-0.59	0.0103
root	ratio	18	0.52	0.0259
sed_moisture	sed_organic	18	0.88	<0.0001
shoot	sed_redox	18	0.73	0.0006
shoot	sed_pH	18	-0.63	0.0049
root	sed_pH	18	-0.50	0.0345
sed_moisture	sed_pH	18	-0.47	0.0467
sed_redox	sed_pH	18	-0.50	0.0338
sed_redox	pw_temp	18	0.54	0.0206
pw_temp	pw_salinity	18	-0.51	0.0314
sed_redox	pw_pH	18	0.68	0.002
sed_ec	pw_pH	18	0.49	0.0402
pw_temp	pw_pH	18	0.61	0.0069
shoot	pw_depth	18	0.77	0.0002
ratio	pw_depth	18	-0.65	0.0038
sed_redox	pw_depth	18	0.84	<0.0001
sed_pH	pw_depth	18	-0.57	0.0133
pw_pH	pw_depth	18	0.74	0.0004

HEUNINGNES

Variable 1	Variable 2	n	r	p
shoot	root	18	0.50	0.0351
root	ratio	18	0.93	<0.0001
shoot	sed_organic	18	0.52	0.0270
shoot	sed_pH	18	-0.53	0.0248
root	sed_pH	18	-0.53	0.0247
sed_redox	pw_temp	18	0.55	0.0192
sed_pH	pw_temp	18	0.70	0.0013
sed_redox	pw_salinity	18	-0.60	0.0086
sed_pH	pw_salinity	18	-0.78	0.0001
pw_temp	pw_salinity	18	-0.76	0.0003
sed_redox	pw_pH	18	0.54	0.0215
pw_salinity	pw_pH	18	-0.51	0.0303
sed_redox	pw_depth	18	-0.63	0.0051
sed_pH	pw_depth	18	-0.81	<0.0001
pw_temp	pw_depth	18	-0.94	<0.0001
pw_salinity	pw_depth	18	0.81	<0.0001

NAHOON

Variable 1	Variable 2	n	r	p
root	ratio	18	0.83	<0.0001
sed_redox	sed_ec	18	-0.58	0.0122
sed_redox	pw_temp	18	0.54	0.0202
sed_pH	pw_temp	18	0.58	0.0114
sed_ec	pw_temp	18	-0.62	0.0057
sed_pH	pw_salinity	18	-0.66	0.0031
sed_redox	pw_pH	18	0.55	0.0174
sed_pH	pw_pH	18	0.63	0.0053
sed_ec	pw_pH	18	-0.47	0.0468
pw_temp	pw_pH	18	0.93	0.0000
shoot	pw_depth	18	-0.51	0.0316
sed_moisture	pw_depth	18	0.48	0.0415
sed_ec	pw_depth	18	-0.67	0.0022
pw_temp	pw_depth	18	0.61	0.0077

KWELERA

Variable 1	Variable 2	n	r	p
shoot	root	18	-0.57	0.0141
shoot	ratio	18	-0.66	0.0028
root	ratio	18	0.96	<0.0001
sed_moisture	sed_organic	18	0.82	<0.0001
root	sed_redox	18	0.70	0.0011
ratio	sed_redox	18	0.70	0.0013
sed_moisture	sed_redox	18	-0.62	0.0061
root	pw_temp	18	-0.51	0.0313
ratio	pw_temp	18	-0.54	0.0220
sed_moisture	pw_temp	18	0.50	0.0363
sed_redox	pw_temp	18	-0.68	0.0018
sed_moisture	pw_salinity	18	-0.51	0.0296
pw_temp	pw_salinity	18	-0.52	0.0278
pw_temp	pw_pH	18	-0.66	0.0031
sed_pH	pw_depth	18	-0.53	0.0243

Table 24A: Dependent t-test of monthly plant height, cover, flower and seed production

Month		Height		Live Cover		Flowers (p.m^2)		Seeds (p,m^2)	
		V	p	V	p	V	p	V	p
September	October	436	0.0032	548	0.23	0	0.37	0	-
October	November	1441	<0.0001	759	0.0021	0	0.00014	0	-
November	December	525	0.026	629	0.12	119	0.81	0	0.37
December	January	939	0.25	755	0.0074	166	0.27	46	0.0011
January	February	1132	0.0025	426	0.30	417	0.00099	165	0.00058
February	March	597	0.0040	488	0.15	122	0.0057	542	0.41
March	April	1164	0.001	585	0.83	9	0.12	419	0.00013
April	May	968	0.098	663	0.81	47	0.25	83	0.93
May	June	625	0.16	286	0.015	14	0.11	95	0.053
June	July	1066	0.0055	385	0.198	0	1	15	0.059
July	August	279	<0.0001	462	0.53	0	-	0	-