

**Vulnerability to future environmental conditions and population genetics of the
seagrass, *Zostera capensis***

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University of Stellenbosch



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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Date: 24/11/2015

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Abstract

Climate and anthropogenic-associated environmental changes are pressuring coastal ecosystems and their services. Genetic diversity is one aspect providing resilience for species to persist through environmental perturbations and maintain functional roles in ecosystems. Seagrasses are a poorly studied yet vital component of coastal systems, serving as spawning grounds and nurseries for ecologically and commercially important species. As such, this thesis studied the distribution determinants of the seagrass, *Zostera capensis*, in order to assess the potential impact of environmental change on its distribution in South Africa. Further, I investigated the population genetics of *Z. capensis* in southern Africa.

Generalised additive modelling was applied in chapter one to quantify the relative importance of likely determinants of the contemporary distribution of *Z. capensis*, then to project its potential future distribution to examine its vulnerability to projected South African environmental conditions. Estuarine mouth state and precipitation were major determinants of contemporary *Z. capensis* distribution. My model projected the loss of significant areas (30%) of suitable habitat for *Z. capensis* near current range limits, placing the species at increased risk of contraction into a diminishing range of only a few estuaries on the south-eastern coast. These results align with projections of diminishing ranges for other seagrasses globally and raise concerns about future declines in extent and quality of seagrass habitat, particularly the negative effects on coastal biodiversity, commercially important fish populations with seagrass-dependant juvenile stages and trophic transfers to adjacent marine ecosystems. I recommend that marine spatial and biodiversity management planning be guided by assessing the on-going status of such species through monitoring, while also targeting seagrass protection in southerly sites to optimize future resilience.

Chapter two aimed to understand genetic diversity, gene flow and population structure of *Z. capensis* populations in southern African using microsatellite markers designed for a sister species, ITS and matK regions. Although many seagrasses reproduce largely vegetatively, species such as

Zostera marina display high levels of clonal diversity, significant isolation by distance and strong population structure at both regional and fine scales. As *Z. capensis* is found across a broad range of temperature and environmental conditions, and across South African bioregions, I expected to find similar trends to those observed in *Z. marina*. Sequences from the matK region confirmed the species identification of all samples and showed little to no variation across all populations. ITS was excluded as multiple fragments of similar size were amplified by this marker. The nine microsatellites markers were unsuccessful at amplifying *Z. capensis*, and due to time and financial constraints, new microsatellite markers could not be designed. The lack of observed variation suggests a high level of connectivity, gene flow, clonal reproduction or selection, but is more likely due to insufficient marker resolution. In this region genetic structuring of estuarine species often corresponds with the three main biogeographic regions and physical barriers to gene flow.

Future research using higher resolution markers, such as next-generation sequencing, may reveal more about the vulnerability, resilience and adaptations of *Z. capensis*, with implications for its future.

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List of Abbreviations

In order of appearance in text

Convention on Biological Diversity	CBD
National Biodiversity Assessment	NBA
maturaseK	maK
rDNA–internal transcribed spacer locus	ITS
International Union for Conservation of Nature	IUCN
Intergovernmental Panel on Climate Change	IPCC
Department of Environmental Affairs (DEA)	DEA
Nelson Mandela Metropolitan University	NMMU
Generalised Additive Model	GAM
Akaike Information Criterion	AIC
Hadley Centre’s coupled Atmospheric and Oceanic Global Environmental Model version 2	HADGEM2-AO
Coupled Model Inter-comparison Project phase 5	CMIP5
Representative Concentration Pathways	RCP
Geographic Information System	GIS
World Geodetic System	WGS
Random Amplified Polymorphic DNA	RAPD
Isolation-By-Distance	IBD
Kenyan Marine Fisheries Research Institute	KMFRI
Central Analytical Facility of Stellenbosch	CAF
Analysis of Molecular Variance	AMOVA
Next-Generation Sequencing	NGS
Single Nucleotide Polymorphism	SNP

Chapter 1

General Introduction

1.1 Background

With global change being an inevitable feature in the future of our planet (Steffen *et al.* 2006), it is important to study its potential impacts on vital resources, such as exploited species, biological and genetic diversity, and essential ecosystems services. These aspects have been emphasised by the Convention on Biological Diversity (CBD), as their three main objectives are the conservation of biological diversity, to promote the sustainable use of the components of biological diversity and to ensure the fair and equitable sharing of the benefits arising out of the utilization of genetic resources (www.cbd.int). The management of biodiversity is becoming increasingly important, as with the growth in the human population comes an escalation in the diversity and intensity of anthropogenic environmental stressors (Goudie 2013). The human influence on nature is such that this the current era has been termed the “anthropocene” (Crutzen & Stoermer 2000). Historically, habitat loss and over-exploitation were focused on as the main human impacts to the environment, yet pollution, invasive species and climate change have also been recognised as human-associated impacts (Jackson 2001; Crain *et al.* 2008; Mead *et al.* 2013). The effect of humans on the environment is not limited to terrestrial systems, but marine systems also face mounting pressures (McCauley *et al.* 2015).

Despite the fact that terrestrial defaunation has been occurring for tens of thousands of years, marine defaunation, which emerged only hundreds of years ago, is rapidly increasing in pace and severity, profoundly impacting functioning and provisioning of services in every ocean (McCauley *et al.* 2015). Coastal ecosystems are increasingly dominated by human impacts such as development, pollution and agricultural run-off, with marine resources disproportionately relied upon and overused (Weinstein *et al.* 2012). One of the major exploiters of the oceans’ resources is the fisheries industry, with fish catches increasing drastically in the past two decades (Hilborn *et al.* 2003; Houde & Rutherford 2013). Notably, commercially exploited fisheries have since begun to plateau and even decrease in more recent years, not as a result of decreased demand, but due to the

depletion of stocks (Hilborn *et al.* 2003; Houde & Rutherford 2013). The loss of biodiversity caused by the fishery industry and other human activities has been linked to an increase in the rate of resource collapse and a marked decrease in recovery potential, stability and water quality (Worm *et al.* 2006). This then forms a negative feedback loop which further impairs the ocean's capacity to provide food and ecosystem services.

Estuarine areas are highly important as spawning grounds and nurseries for many marine and freshwater species, including commercially important fishery species (Blaber & Cyrus 2000; Beck *et al.* 2001; Vasconcelos & Reis-Santos 2007; Whitfield & Cowley 2010). This function is partly due to seagrasses which, as keystone species, form the habitat in which many of these species live at some stage in their life-history (Beckley 1983; Beck *et al.* 2001; Green & Short 2003). As such, the persistence of seagrass through global changes will be of vital importance both ecologically and economically in many regions. In this context, the concept of resilience is of particular importance and can be defined as “the capacity of a system to maintain functioning, structure, and feedbacks in the face of disturbance” (Folke *et al.* 2004). Resilience can be divided into three components, the first being the amount of change a system can experience and still maintain the same functioning (i.e. resistance). The second component is the capacity for post-disturbance recovery (often solely referred to as resilience), and the third component is the degree to which a system can adapt to new conditions (Bernhardt & Leslie 2011; Hodgson *et al.* 2015; Nimmo *et al.* 2015). If a population is not able to maintain the same level of functioning during environmental change, recover from disturbances or adapt to new conditions, it faces the risk of collapse and local extinction.

In marine environments, where connectivity is generally thought to be higher, species level extinctions are rarely reported compared to local population level extinctions (Roberts and Hawkins 1999; Short *et al.* 2011). However, the cumulative effect of anthropogenic impacts, climate change and local population level extinctions will ultimately lead to more concern for species level extinctions. During the recent National Biodiversity Assessment (NBA), 79% of South Africa's

estuarine area was classified as threatened and 72% of estuaries in protected areas (65 900 ha) are in a poor condition. Despite the importance and fragility of estuarine systems, 83% of South Africa's estuarine area is without adequate protection (Van Niekerk *et al.* 2012). Estuaries face many potential threats, including habitat modification, exploitation of coastal resources, pollution, urbanisation and climate change (Mead *et al.* 2013). These pressures are compounded by the effects of invasive species and desalination, imperilling estuarine diversity in South Africa (Mead *et al.* 2013). Although estuaries are geologically transient in nature and exhibit fluctuating conditions, the potential for *in situ* speciation in estuarine populations is high. This is because estuaries present distinct selective regimes and can restrict gene flow (Bilton *et al.* 2002), resulting in physiologically adapted populations representing sibling or cryptic taxa which are divergent from their marine counterparts (Beheregaray & Sunnucks 2001; Phair *et al.* 2015; von der Heyden *et al.* 2015). Yet estuaries are highly important environments, supporting biodiversity, harbouring endangered species and providing vital ecosystem services.

Biodiversity, abundance and species richness assessments are not always a sufficient evaluation when examining the conservation potential of an area. A molecular approach in the management of coastal systems is imperative as genetic information describes many aspects of populations and species such as genetic diversity, gene flow, connectivity and evolutionary history (von der Heyden 2009; Beger *et al.* 2014). Further, these features provide insight into climate tolerance, behavioural traits, dispersal ability, probable range limits and evolutionary potential (Beger *et al.* 2014). The identification of genetic breaks along the coast line and evolutionary distinct lineages is indispensable, as genetically distinct populations warrant protection in order to preserve genetic potential (Rocha *et al.* 2007). Knowledge of the direction and scale of connectivity among populations is particularly pertinent to effective marine conservation (Olds *et al.* 2012). Intraspecific genetic diversity is the foundation for biodiversity, and its conservation has been recognised by the IUCN and emphasised in the CBD (Laikre *et al.* 2009). Research supporting the importance of preserving genetic diversity to sustain species and ecosystems continues to build (Whitham and

Bailey 2006, Beger *et al.* 2014). Nevertheless, genetic diversity remains largely unmonitored while ecosystem and species level diversity have received the bulk of the attention (Laikre 2010).

Genetic diversity is tightly linked to the concept of evolutionary resilience, which is the magnitude of environmental disturbance that can be withstood and recovered from before ecosystem functions are lost and there is a shift from one stable state to another, due to genetic diversity, connectivity and adaptive ability (Van Oppen & Gates 2006; Sgrò *et al.* 2011). A retrospective view as well as insight into the future evolutionary potential of a population or species can be gained by investigating demographic history under different anthropogenic disturbances through the study of genetic diversity, population structure and connectivity (Procaccini *et al.* 2007). For example, high allele and genotype diversity in seagrasses has been linked to an increased ability to withstand environmental perturbations (resistance) as well as increased post-disturbance recovery (resilience) (Ehlers *et al.* 2008; Hughes *et al.* 2008; Massa *et al.* 2013). Similarly, allele diversity and heterozygosity are also positively associated with the maintenance of ecosystem services in various seagrasses (Massa *et al.* 2013).

Globally, seagrasses have experienced declines in response to a combination of impacts including global warming, increased turbidity, major storm events, invasive organisms, anthropogenic influences such as coastal development, damming and pollution, and importantly, disease (Green & Short 2003; Orth, Carruthers *et al.* 2006; Short *et al.* 2007; Waycott *et al.* 2009). Estimates indicate that between 1879 and 2006 about 29% of the world's seagrass area has been lost (Waycott *et al.* 2009). Further, a review of over 100 studies has shown that most tropical and many subtropical seagrasses are already at their maximum thermal limits, although the negative effect of increasing sea surface temperatures is somewhat mitigated by increased photosynthesis (Koch *et al.* 2013) due to ocean acidification (resulting from increasing atmospheric CO₂). The extent to which this interaction may occur is still unknown.

In South Africa, *Zostera capensis* is the most widespread and dominant seagrass species, also referred to as eelgrass, occurring in estuaries from the southern west coast to the northern east coast (Green & Short 2003). This species is also listed as being present further along the tropical east African coast in Mozambique, Tanzania and Kenya (Green and Short 2003; Fig. 1.1). However, there is some uncertainty among seagrass experts in the region regarding if and where *Z. capensis* is present, specifically in northern Mozambique and Tanzania (S.O. Bandeira & L. Nordlund pers. comm.). Although it is able to reproduce both sexually and vegetatively, and its 2-2.5 mm seeds are thought to be able to form a seed bank, very little is known about its flowering biology (Waycott *et al.* 2014). Further, the flowering of *Z. capensis* has been observed under controlled laboratory conditions at 18 and 24 °C (McMillan 1980), yet research surrounding its reproductive strategies is currently scarce. *Zostera capensis* beds are often small compared to other seagrass species (Green & Short 2003), but they support a thriving fishing industry including economically important species in South Africa, such as various kob, stumpnose, mullet and kingfish species, among many others (Hemminga & Duarte 2000; Lamberth & Turpie 2003; Pillay *et al.* 2010). In 2002 it was estimated that estuarine and estuarine-dependent fisheries in South Africa were worth R1,251 billion (Lamberth & Turpie 2003). Despite their importance and the observed global decline of seagrasses (Orth, Carruthers *et al.* 2006; Waycott *et al.* 2009), as of yet there has been no effort to assess the potential future of *Z. capensis* in South Africa.



Figure 1.1 The range of *Zostera capensis*, extending from the west coast of South Africa to the southern coast of Kenya, based on IUCN data (www.iucnredlist.org).

Due to the pressures facing coastal ecosystems such as estuaries, the future persistence of key species like seagrasses is uncertain. Therefore, the first chapter of my thesis will examine the factors driving the distribution of the southern African seagrass, *Z. capensis*, in order to model its potential future distribution. This will provide insight into the persistence of this environmentally and economically important species under current climate scenarios and projected environmental change.

As intraspecific genetic diversity is the foundation for biodiversity and can provide a wealth of insight into both the demographic history as well as the evolutionary potential of a species, my second chapter will investigate the population genetic structure of *Z. capensis* along the south and east African coast. Examining population genetics is an important step in gaining a better

understanding of the conservation potential for this seagrass in South Africa. This chapter may be useful in identifying evolutionary significant units or management units to assist in the management of the seagrass, as well as detecting populations which are perhaps too genetically depauperate for effective conservation measures.

1.2 Research Question/Objectives

This thesis will address two main research questions. The first question examines the vulnerability of *Z. capensis* to future climate and environmental conditions and how this vulnerability might result in changes in the future distribution and persistence of seagrass populations in South Africa. The second question seeks to characterise the genetic structure and gene flow of *Z. capensis* populations in South Africa with reference to a Kenyan population of *Z. capensis*.

Chapter 2:

Contemporary drivers of distribution and future declines of the seagrass *Zostera capensis*

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2.1 Introduction

Threats to coastal systems

In the era of the anthropocene, human associated pressures and climate change are expected to have far reaching effects on species and their distributions (Pernetta *et al.* 1994; Crutzen & Stoermer 2000). Coastal systems worldwide will be exposed to increased risk of habitat degradation in the future, due to on-going climate change coupled with direct human pressures (Nicholls *et al.* 2007). One of the main consequences of climate change is the alteration of ocean temperatures, which is often greatest near the surface (IPCC 2014). The upper 75 m of the ocean has warmed by 0.11 C° per decade over the last 30 years, with this trend being projected to increase further in the future (IPCC 2014). A global increase in sea level has also been recorded over the past two decades, with levels rising by 0.32 m per decade on average (IPCC 2014). Along with predicted warming and acidification of oceans, and other human-induced pressures, sea level is expected to rise (IPCC 2014). Consequently, it is essential to assess the potential response of coastal ecosystems and their associated organisms to a suite of environmental shifts associated with climate change.

Seagrasses predominantly inhabit the interface between terrestrial and aquatic environments. Due to the fluctuating nature of their habitat (Newman *et al.* 2002), seagrasses are thought to be particularly vulnerable to climate change effects such as changing temperatures, sea level rise and greater frequency of severe storms (Green & Short 2003). A recent study on the vulnerability of the Mediterranean seagrass, *Zostera noltii*, to climate change projected a ± 900 km poleward shift in the suitable habitat of this species, and a retreat of its equatorward populations by the end of the 21st century (Valle *et al.* 2014). Additionally, rising sea levels are expected to precipitate a landward migration of the species as a result of new suitable habitat becoming available (Valle *et al.* 2014), but whether this will compensate for habitat loss is unquantified. These complex distributional changes in seagrass communities present unique challenges for effective conservation management and planning.

The distribution of seagrasses is generally regulated by abiotic factors such as light availability, substratum type, physical exposure, inorganic carbon, nutrients, temperature, salinity, oxygen and sulphide levels, and biotic factors including grazing and competition for space, light and nutrients (Greve & Binzer 2004). Eutrophication in coastal systems threatens the persistence of seagrasses as elevated nutrient loads favour algal growth, which reduces the available light and can result in smothering of the seagrass (Hemminga & Duarte 2000). However, biotic interactions between seagrass and fouling communities are not fully understood, so the extent of the effect of this in the future is uncertain. High nutrient loads, and the resultant light reduction, can also be linked to increased sedimentation, associated with human activities upriver such as agriculture, aquaculture and development (Hemminga & Duarte 2000). Yet, the relationship between seagrasses and epiphytic algae is an example of an interaction which can be both beneficial and detrimental to the seagrass. While seagrass acts as a substrate for the epiphytic algae, the seagrass may become overwhelmed by algal fouling and suffer from a significant reduction in photosynthesis due to the shading effect (Fong *et al.* 2000). Yet seagrass beds may also benefit from the presence of algae as algae reduce water movement and desiccation, and when algae die, decomposing matter can become a source of nutrients (Fong *et al.* 2000).

Importance of seagrass

Seagrass plays a key role in coastal systems as an ecosystem engineer and keystone species (Beckley 1983; Siebert & Branch 2006), functioning as spawning grounds and nurseries for many species, including commercially important invertebrate and fish species (Beck *et al.* 2001; Vasconcelos & Reis-Santos 2007; Whitfield & Cowley 2010; Bertelli & Unsworth 2014; Blandon *et al.* 2014; Jackson *et al.* 2015). They provide both food and shelter from potential marine predators to the juvenile stages of many of these species (Adams 1976). In addition, seagrasses are known for their coastal protection and erosion control, with their complex rhizome network binding sediments and thus enhancing nutrient retention and water quality, as well as reducing erosion of

the benthos (Orth 1976; Green & Short 2003; Barbier *et al.* 2011; Lucas *et al.* 2012). Their capacity to attenuate wave forces and diminish the effects of storm surges is strongest in long-lived, stable seagrass beds with high biomass (Ondiviela *et al.* 2014). It has also been suggested that seagrass beds play an important role in nutrient cycling (Green & Short 2003) and in maintaining trophic function and overall productivity in shallow-water coastal zones (Adams 1976).

In 1997 it was estimated that seagrasses contributed 5.3 trillion USD to the global economy based on its provision of ecosystem services such as climate regulation, erosion control, nutrient cycling, refuge provision, food production, raw materials, genetic resources and recreational and cultural significance. When reassessed in 2011, this estimate increased to 6.8 trillion USD which, in terms of marine ecosystems, is only topped in value by coral reefs (9.9 trillion USD) (Costanza *et al.* 2014). Given the value of seagrasses both ecologically and economically, it is imperative that the vulnerability of seagrass to future climate change scenarios is assessed.

Seagrass declines

While seagrasses occur in most of the world's seas and oceans, a marked decline has recently been noted in their cover (Orth, Carruthers *et al.* 2006; Waycott *et al.* 2009). Estimates indicate that between 1879 and 2006 about 29% of the world's seagrass area has been lost (Waycott *et al.* 2009). Seagrass has been described as an indicator species, providing early warning of environmental changes, with decreasing seagrass cover signalling the loss of important ecosystem services which they provide (Bricker *et al.* 2003; Orth, Carruthers *et al.* 2006). These declines are largely due to a combination of impacts including global warming, increased turbidity, eutrophication, major storm events, invasive organisms, anthropogenic influences such as coastal development, damming and pollution, and importantly, disease (Green & Short 2003; Orth, Carruthers *et al.* 2006; Short *et al.* 2007).

One of the major declines occurred in the early 1930's on both sides of the North Atlantic Ocean due to the so-called 'eelgrass wasting disease' and resulted in almost 90% reduction of cover (Short

et al. 1988). The reduced functionality, production, cover and biomass of seagrass beds disrupted coastal and near-shore environments. The near elimination of seagrass was associated with the collapse of many of its residents, notably fishery species, water fowl populations (Orth, Carruthers *et al.* 2006) and the first historical extinction of a marine gastropod from an ocean basin (Carlton *et al.* 1991). This loss also led to changes of sediment distribution, water current patterns, coastal food chains and other habitats in close proximity to seagrass such as salt marshes and mangroves (Stevens 1939; Orth, Carruthers *et al.* 2006). Similar large-scale seagrass losses have been experienced elsewhere (Cambridge *et al.* 1986; Marbá *et al.* 1996) and seagrasses and their decline are well documented in Europe, North America and Australia (Cambridge *et al.* 1986; Marbá *et al.* 1996; Olsen *et al.* 2004; Orth, Carruthers *et al.* 2006; Waycott *et al.* 2009; Coyer *et al.* 2013). Major gaps in information exist in Africa, South America and the Indo-Pacific (Waycott *et al.* 2009). Due to the decline of seagrass systems, the current distribution is uncertain for many species and this uncertainty is exacerbated by the skewed distribution of studies towards developed countries. Although ‘eelgrass wasting disease’ has had profound ecological and economic impacts since its initial rise in the 1930’s, its cause has yet to be conclusively determined (Den Hartog 1987).

Climate and environmental change

In addition to the predicted increase in sea surface temperatures and sea level rise, other climate change effects may further threaten the persistence of seagrass beds. These include an increase in the frequency and intensity of extreme events such as floods and severe storms, which are known to erode coastal habitats, as well as run-off and pollution (Van Niekerk *et al.* 2012; IPCC 2014). In South Africa, it is expected that climate change will have varying local effects on the different coastal regions and estuary types, resulting in ‘hotspots’ of hydrological change (Schulze *et al.* 2005). Rainfall and storm frequency and intensity are expected to increase on the east coast, while decreasing on the west coast (Lumsden *et al.* 2009). Both the winter rainfall region of the Western Cape Province and the bimodal rainfall zone of the southern Cape will experience an increased

frequency and intensity of floods and droughts (Schulze *et al.* 2005). This is likely to result in a reshaping of the available estuarine habitat and a general shift to more open mouth conditions in estuaries (Whitfield 1992; DEA (Department of Environmental Affairs) 2013; Valle *et al.* 2014). The potential loss of seagrass coverage is of particular concern, as a high proportion of South Africa's inhabitants are highly dependent on coastal resources for subsistence, which extends to the national fishery industry as a vital component of the nation's economy (Lamberth & Turpie 2003; Theron 2007). The persistence of seagrasses and the full range of their ecosystem services are tied to time-sensitive and actionable decisions from conservation planning and management.

Zostera capensis and South African estuaries

Zostera capensis is a seagrass species with a wide geographic distribution from the west coast of South Africa to southern Kenya (Green and Short 2003; Fig. 1.1). This is rare amongst seagrasses as this range encompasses cool-temperate, sub-tropical and tropical environments. The South African coastal environment is unique in that it experiences two contrasting temperature regimes in currents; the cold Benguela Upwelling System on the west and warm Indian Ocean on the east, which mix at the south coast (Nelson & Hutchings 1983). While the east coast has 245 densely distributed estuaries along the coastline, the west displays the opposite pattern with only 13 estuaries (Van Niekerk *et al.* 2012; bgis.sanbi.org). Recent assessments indicate that *Z. capensis* occurs in 48 out of the 270 estuaries surveyed (Prepared by J.B. Adams of NMMU for NBA 2012).

Zostera capensis is found more often in permanently open estuaries where there is tidal exchange, and the more marine salinities of the lower estuarine reaches, specifically 15-35‰ salinity (Howard-Williams & Liptrot 1980; Adams & Bate 1994). This seagrass is less likely to recover from, and more sensitive to, changes in salinity compared to other submerged macrophytes such as co-occurring non-native *Ruppia cirrhosa* (Adams & Bate 1994). Estuaries with lower turbidity levels, and therefore increased light availability, are favoured by *Z. capensis* (Day 1951; Weisser & Howard-Williams 1982). However, Warne (1994, Msc thesis) indicated that the morphological and

photosynthetic response of *Z. capensis* suggests that it is more shade-tolerant relative to *Ruppia cirrhosa*. Further, these two species have been observed to partition themselves vertically along the shore and during dry periods, when water clarity is improved, *Z. capensis* can be found in deeper waters (Howard-Williams & Allanson 1981). *Zostera capensis* also exhibits a high degree of desiccation tolerance due to a sheath which protects the basal meristem, enabling the species to withstand extended periods of exposure (Gessner 1971).

In the latest NBA, 79% of South Africa's estuarine area was classified as threatened and 72% of estuaries in protected areas (65 900 ha) are thought to be in a poor condition (Van Niekerk *et al.* 2012). The IUCN has rated *Z. capensis* as 'vulnerable', largely due to its fragmented distribution (Short *et al.* 2010). Yet estuaries can produce a naturally disjunct distribution and *Z. capensis* exists in a wide range of physicochemical and climatic conditions along the coastline, suggesting a high level of phenotypic plasticity. This combination of the ability to maintain a fragmented range across a wide range of climatic conditions could indicate inherent resilience to climate change associated impacts. On the other hand, the post-Miocene establishment and long term stability since that time, of cold west coast and warm east coast conditions set up by the Benguela and Agulhas current respectively, could imply long term local adaptation, and thus low resilience. Therefore, the question presents itself; is *Z. capensis* vulnerable to environmental change?

2.2 Aims

This chapter aimed to assess the vulnerability and potential future distribution of *Z. capensis* in the face of climate change and anticipated habitat alterations. The first objective is to assess the factors driving the contemporary distribution of *Z. capensis*. The second objective is to model suitable habitat available for *Z. capensis* in the future, and thereby investigate its physical vulnerability to projected environmental change. The combined approaches of understanding contemporary drivers of *Z. capensis* distribution, in conjunction with models of projected future change, will aid strategic planning to ultimately preserve *Z. capensis* habitats along with associated ecosystem services.

2.3 Hypothesis

As the range of *Z. capensis* covers temperate to tropical conditions, I hypothesize that temperature will not be a significant determinant of its contemporary distribution. Secondly, due to seagrasses requiring calm, sheltered conditions, I hypothesize that estuarine mouth state (degree of opening to the sea), precipitation and turbidity will play an important role in determining the current distribution of *Z. capensis*. As salinity is known to be important to seagrasses, I expect this to play an important role in its distribution. More specifically, I expect that *Z. capensis* will favour estuaries with open-mouth conditions (greater marine influence), higher precipitation, lower turbidity and more marine salinities. Lastly, I hypothesize that the projected suitable habitat, and therefore *Z. capensis* distribution, will be reduced compared to its current suitable habitat, with 2070 displaying a greater reduction than 2050. This may be due to the changes in precipitation expected for South Africa in the coming decades, based on similar projections that have been made for other seagrass species globally.

2.4 Methods

Bioclimatic envelope modelling

This study combined occurrence records for *Z. capensis* from the Nelson Mandela Metropolitan University (NMMU) botanical database (Prepared by J.B. Adams of NMMU for NBA 2012) as available in January 2015, and available relevant environmental data from multiple sources (Russell 1996; Harrison 2004; Lin *et al.* 2004; Hijmans *et al.* 2005; Wepener 2007; Van Niekerk *et al.* 2012; Whitfield & Baliwe 2013) in order to fit distribution models. While the botanical database contained 270 estuaries, only 106 of these were included in this chapter due to availability of environmental data. Of the 106 estuaries included in this chapter, *Z. capensis* was listed as present in 36. Environmental variables and their sources, which are included in both the initial saturated model and the refined model are listed in Table 2.1.

The classification of estuary mouth state, also referred to as estuary type or classification, used in this study follows that of Whitfield (1992), including permanently open estuaries, temporarily open/closed estuaries and estuarine bays. It should be acknowledged that the temporarily open/closed classification encompasses a broad range of estuaries. For example, some of these estuaries may only be open 15% of the time, while others may be open for 80% of the time. This leaves a large amount of important variation unaccounted for and could affect the outcome of the analyses. Estuarine condition was rated excellent (negligible human impact), good (no major negative human impact), fair (noticeable ecological degradation) or poor (major ecological degradation), based on the degree to which human activities have changed the functioning or viability of the system as defined by Whitfield and Baliwe (2013). This assessment of estuarine condition was based on expert opinion of the estuary and its surrounding catchment. Another environmental variable warranting further explanation is flow change. The threat of flow change was rated as low, medium or high and each estuary was characterised during the most recent NBA (Van Niekerk *et al.* 2012) by the perceived threat to a modification of river inflow to the estuary,

based on expert opinion and the hydrological model developed by the Department of Water Affairs (DEA; de Jager *et al.* 2004).

Table 2.1 Environmental variables and their sources used in the saturated and refined models

Source	Environmental variables	Saturated model	Refined model
Whitfield and Baliwe (2013)	Mouth State	✓	✓
	Biogeographic Region	✓	
	Estuary Condition	✓	✓
Harrison (2004)	Secchi Disk Depth (m)	✓	
	Water Temperature (°C)	✓	
Supplemented by:	Salinity (PSS)	✓	
Lin <i>et al.</i> (2004), Wepener (2007), Russell (1996)	Dissolved Oxygen (mg l ⁻¹)	✓	
	Turbidity (NTU)	✓	✓
NBA 2011:	Estuarine Habitat (km ²)	✓	✓
Van Niekerk <i>et al.</i> (2012)	Flow Change	✓	✓
	Pollution	✓	
	Habitat Loss	✓	
	Mining	✓	
	Artificial Breaching	✓	
	Fishing	✓	
	Bait Collection	✓	
Last of The Wild Project v2:	Human Impact	✓	
Sanderson <i>et al.</i> (2002)			
WorldClim.org:	Winter Precipitation	✓	✓
Hijmans <i>et al.</i> (2005)	Summer Precipitation	✓	✓
	Maximum Temperature	✓	
	Minimum Temperature	✓	✓

In order to better understand future climate scenarios and their potential impact on the distribution of *Z. capensis*, bioclimatic envelope modelling was carried out by utilising generalised additive models (GAMs). Current climate data such as summer and winter precipitation, and minimum and maximum air temperatures, were obtained from WorldClim (Hijmans *et al.* 2005) at a spatial

resolution of 30 arc-seconds (1 km) and used together with existing environmental data (Table 2.1) to model the bioclimatic envelope and fit GAMs for *Z. capensis* in South Africa. GAMs have become increasingly popular as they are a versatile method for species modelling (Segurado & Araujo 2004). This is particularly due to the fact that the functional form of the relationship between the predictors and the response is determined by the data itself rather than being restricted to specific parametric forms (Guisan *et al.* 2002). They are frequently used when a model contains multiple independent variables. For this study GAMs were built using the ‘mgcv’ package (Wood 2011) in the statistical environment R (R Core Development Team 2008), by means of Rstudio (“RStudio” 2012) Version 0.98.1102. Default smoothing splines were used for non-parametric variables and the dimension k was set to ten (see supplementary information for all R scripts). Backward selection of variables was carried out using the Akaike Information Criterion (AIC; see Burnham and Anderson, 2002) and p-values to create the best fitting refined model (Wood 2011). As GAMs do not have the same properties as models based on ordinary least squares regression, a pseudo- R^2 was used as an indication of model performance. This was calculated as $1 - (\text{residual deviance} / \text{null deviance})$, referred to as explained deviance (Swartzman 1992).

Projection of future distribution

Future climate scenarios were obtained from WorldClim (Hijmans *et al.* 2005) at a spatial resolution of 30 arc-seconds (1 km). The Hadley Centre’s coupled Atmospheric and Oceanic Global Environmental Model version 2 (HADGEM2-AO) as described by Lee *et al.* (2010) from the Coupled Model Inter-comparison Project phase 5 (CMIP5) was selected, as it is considered to be a leading climate model, and features improvements to previous models in terms of sea surface temperature projections (Collins *et al.* 2008; Martin *et al.* 2011). The model made use of the Representative Concentration Pathways (RCPs) 2.6 and 8.5 in order to assess the best and worst case future climate scenarios (van Vuuren *et al.* 2011). Future scenarios were established for the remaining environmental variables included in the refined model (Table 2.1) by ‘downgrading’ all

environmental conditions. For example, all estuaries currently rated to be in ‘good’ condition by the NBA (Van Niekerk *et al.* 2012) were set to ‘fair’ condition in the future scenarios. Future scenarios were based on a general decline of conditions according to the predicted change in rainfall patterns across South Africa, as well as an increase in storm frequency, intensity and wave action. These future scenarios were established separately for each coast as the west, south and east coasts are all affected by climate change in different ways (Lumsden *et al.* 2009). Further, each estuary type was taken into account separately as permanently open and temporarily open/closed estuaries, as well as estuarine bays, are all expected to be effected in different ways (Whitfield 1992; Scavia *et al.* 2002). See supplementary information for future scenarios, environmental/bioclimate data, *Z. capensis* presence/absence and estuary lists. These future scenarios are by no means exhaustive and they only serve to illustrate the vulnerability of this seagrass to change, not to make a precise prediction of the future distribution of this species.

Predictive GAMs produced worst and best case projected distributions of *Z. capensis* in 2050 and 2070. The GAM approach assigns probability values for the occurrence of the modelled species, ranging from zero to one. These were transformed into presence and absence records using a cut-off threshold of 0.33, defined by the current recorded prevalence of *Z. capensis*. A prevalence-dependent cut-off threshold is appropriate as it maximizes the agreement between observed and predicted distributions (Cramer 2003; Jiménez-Valverde & Lobo 2007). This approach for identification of cut-offs is also useful due to its simplicity and effectiveness in relation to more complex approaches (for discussion see Liu *et al.* 2005b and Franklin, 2010). Predicted presence and absence of *Z. capensis* in South African estuaries was mapped in Quantum GIS (QGIS) version 2.4 using the WGS 84 projection (Quantum GIS 2014). Note that the worst and best case scenarios produced the same projected distributions and therefore only one map was produced for each time period. The proportion of *Z. capensis* occurrences were calculated for each future scenario as well as for its current distribution.

2.5 Results

Bioclimatic envelope Modelling

After running the saturated model and performing backward selection of variables using p-values and AIC as a selection tool, mouth state, summer and winter precipitation, size of the estuarine habitat, minimum temperature, flow change, turbidity and estuary condition were selected for the refined model (Table 2.1). AIC values indicated that models including these eight variables were the most parsimonious. The probability of occurrence of *Z. capensis* in South African estuaries can be significantly explained by mouth state ($p < 0.001$), summer precipitation ($p < 0.01$) and winter precipitation ($p < 0.01$). According to the pseudo- R^2 , the refined model explains 64.9% of the variance observed in the data.

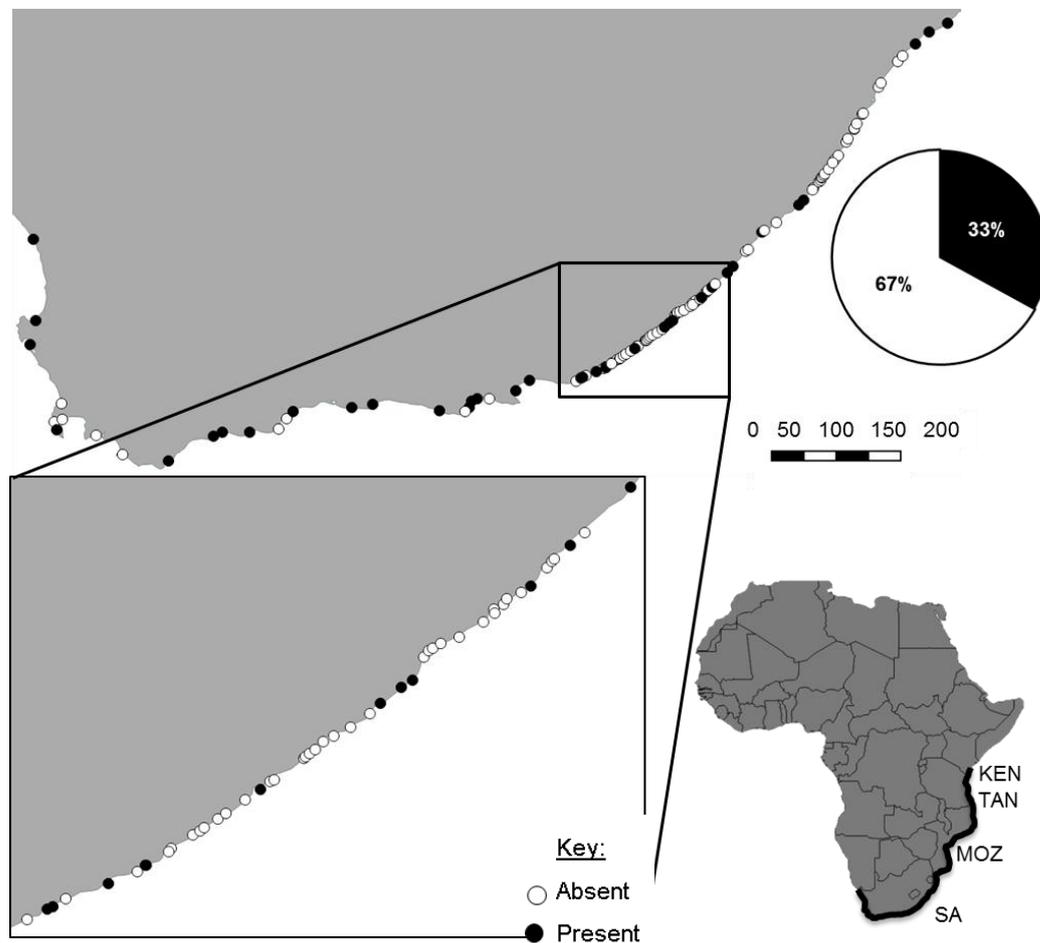


Figure 2.1 The current distribution of *Zostera capensis* in South African estuaries, with an inset of the African continent highlighting the range of *Z. capensis* (South Africa, Mozambique, Tanzania and southern Kenya). Black dots represent estuaries where *Z. capensis* is present and white dots, absent. The enlarged inset displays the clustered estuaries on the south-east coast. The percentage occurrence is displayed in the pie chart.

Projection of future distribution

Under future climate scenarios, suitable habitat for *Z. capensis* will decrease from 33% of South African estuaries (Fig. 2.1) to 27% in 2050 and 23% in 2070 (Fig. 2.2). The models also indicated a general shift in the suitable habitat, and therefore distribution of *Z. capensis*, from being well-spread along the South Africa coastline, to mainly occupying estuaries on the south-east coast (Fig. 2.2). Further declines from 2050 to 2070 are projected to include the loss of *Z. capensis* in the estuaries at the western and eastern extremes of its 2050 distribution. By 2070, the projected *Z. capensis* distribution is limited to permanently open estuaries currently in ‘good’ condition with minimal threat of flow change. The size of these estuaries, in terms of estuarine habitat, varied greatly with

areas as small as 0.072 km² and as large as 13.995 km². Summer and winter precipitation levels in these estuaries varied with summer precipitation ranging 106–194 mm and winter precipitation ranging 62–105 mm. Minimum temperature in these estuaries ranged from 7.7 to 10.9 °C.

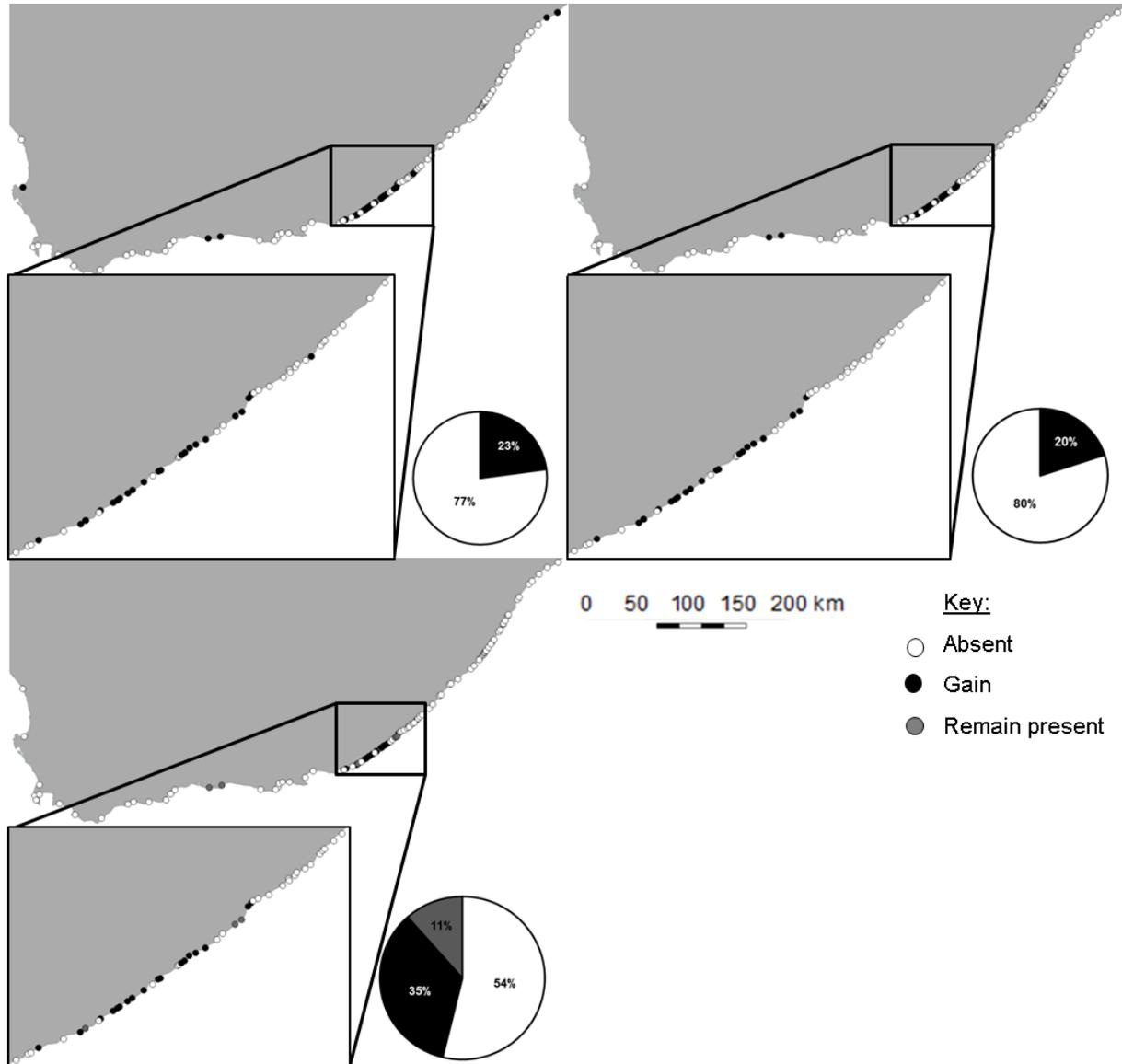


Figure 2.2 The projected distribution of *Z. capensis* in 2050 (top left) and 2070 (top right), with black dots representing estuaries where *Z. capensis* is present and white dots, absent. The enlarged inset displays the clustered estuaries on the south-east coast. The percentage occurrence is displayed in the pie chart. The projected distribution change in the form of absence (white dots) and gain (black dots) of *Z. capensis* in South African estuaries by 2070 (bottom left). Estuaries where *Z. capensis* remained present from the current distribution to the projected 2070 distribution are represented by grey dots. The percentage of each of these categories is displayed in the pie chart.

2.6 Discussion

Seagrasses worldwide are under threat from various pressures, including climate and anthropogenic impacts, yet despite their vulnerability and their vital role in coastal systems, they remain understudied (Green & Short 2003). The results of this study indicate that the southern African seagrass, *Z. capensis*, is likely to follow the declining trend recorded for many seagrasses globally (Orth, Carruthers *et al.* 2006; Waycott *et al.* 2009). Along with severe range contraction, *Z. capensis* projections point to the increasing risk of local extinction of many of its populations over the next few decades, exacerbating its vulnerability to disturbance and disease (Fig. 2.2). Targeted interventions may be required for on-going conservation of this valuable resource and the key ecosystem services it provides in the functioning of coastal ecosystems.

What is driving the contemporary distribution of Zostera capensis?

Water temperature is generally recognized as a key determinant of seagrass distribution and abundance globally (Bjork *et al.* 2008; Valle *et al.* 2014). However, *Z. capensis* can be found in an unusually broad range of temperatures and as such, bioclimatic envelope modelling suggests that temperature alone does not have a significant influence on its distribution at the spatial scale of the South African coastline. This is in contrast to the predictions made for its sister species, *Zostera noltii*, which was found to respond negatively to projected sea surface temperature increase and sea level rise (Valle *et al.* 2014). The two main abiotic factors that appear to drive the contemporary distribution of *Z. capensis* are the mouth state of the estuary and the amount of summer and winter precipitation. Unsurprisingly, *Z. capensis* appears to favour permanently open estuaries over estuaries which are closed for a greater period and estuarine bays, which may be more heavily developed. The higher and more consistent rainfall experienced on the east coast also appears to be preferred by the seagrass, as shown by the model, over the drier and more variable conditions experienced on the west coast. Mouth state itself is controlled by an interaction between many factors (Mead *et al.* 2013) and similarly, it has been found to be the primary driver of estuarine fish

community composition (James & Van Niekerk 2011). Although the model did not identify salinity as a key driver of *Z. capensis* distribution, the emergence of mouth state as a significant determinant of seagrass distribution could reflect the preference of this seagrass for marine salinities (Mead *et al.* 2013). The reason for this may be that recorded salinities do not accurately represent the estuaries, as in reality, conditions undergo daily, seasonal and sporadic fluctuations. This is further supported by the preference shown by *Z. capensis* in the model for permanently open over temporarily open/closed estuaries, which can be more turbid (Whitfield & Bate 2007), inhibiting seagrass persistence (Greve & Binzer 2004). River inflow, largely influenced by rainfall, along with oceanic wave action are two of the most important factors controlling mouth closure (Whitfield 1992) and would therefore both indirectly effect the distribution of *Z. capensis*.

Both summer and winter precipitation levels were significant predictors of seagrass distribution possibly because winter precipitation dominates the west coast and summer precipitation, and more consistent rainfall, typifies the east coast (Tibane & Vermeulen 2013). In South Africa, rainfall is expected to decrease on the west coast while increasing on the east coast (Lumsden *et al.* 2009). On either coast, if the dominant rain season experiences increased levels of precipitation, associated with increased storm frequency, intensity, wave exposure and disturbance (Lumsden *et al.* 2009), the prevalence of *Z. capensis* would be negatively impacted. This is especially so on the east coast where subtropical and tropical conditions result in high levels of rainfall (Day 1981). However, increased precipitation may also lead to an increase in open mouth conditions. Similarly, if either coast experiences a decrease in precipitation over the dominant rain season, *Z. capensis* prevalence would be negatively impacted. The west coast experiences comparatively drier summer months and therefore, these estuaries are more vulnerable to closure, and seagrass beds more vulnerable to prolonged exposure (Lumsden *et al.* 2009; James & Van Niekerk 2011). Prolonged periods of mouth closure, which can be linked to development as well as reduced inflow, is also likely to increase eutrophication, resulting in increased algal fouling and smothering (Nunes & Adams 2014). However, these anticipated local extinctions may not be permanent, as it has been seen that

seagrasses are capable of re-expansion after dying back during times of adverse conditions (Short *et al.* 2011).

Other abiotic factors such as river flow, wave action and estuary morphology likely also influence the distribution of *Z. capensis*, along with biotic variables which are extremely poorly understood, and thus not included in the model. As previously discussed, variables such as the estuarine mouth state are strongly linked to river flow and wave action, and should at least partially account for their impact. At present, substrate is not thought to play a role in the distribution of *Z. capensis* (Hanekom & Baird 1988). In terms of biotic interactions, seagrasses are primarily affected by competition, herbivory and through interactions with seagrass epiphyte communities (Greve & Binzer 2004; Hughes *et al.* 2004). While herbivores such as urchins, fishes and dugongs feed directly on seagrass, epiphytic algae compete for light and nutrients (Greve & Binzer 2004). As such, eutrophication, which promotes algal growth, poses a major threat to seagrass persistence. Microalgae grazers and epiphyte grazers provide the essential service of decreasing microalgae and epiphyte biomass in seagrass communities (Hughes *et al.* 2004). However, an important point to remember is that the relative importance of biotic and abiotic processes in regulating organismal distribution is scale-dependent, with distribution at largest spatial scales being determined primarily by abiotic processes, whereas finer, localised distribution patterns are likely influenced by biotic interactions (Westerbom *et al.* 2008). In the context of this study, the abiotic variables used should be adequate to model shifts and vulnerability of *Z. capensis* in response to predicted environmental change, given that the model aims to project potential seagrass distributional shifts over a large spatial scale.

The future for Zostera capensis in the face of climate change

Bioclimatic modelling for 2050 and 2070 shows that *Z. capensis* could lose significant areas of its western and eastern range limits in response to projected environmental change. More specifically, under the future climate scenarios, *Z. capensis* may lose 18% of its current suitable habitat by 2050

and 30% by 2070. This corresponds with predicted losses for seagrasses globally, especially in developing regions such as Asia, east and west Africa, and the Caribbean, although the extent of loss expected for these areas is as yet unknown (Duarte 2002). Furthermore, if conditions worsen, *Z. capensis* may follow the same trajectory as the Mediterranean seagrass, *Posidonia oceanica*, which is expected to be functionally extinct by 2050 (Jordà *et al.* 2012). Notably, the losses projected for *Z. capensis* point to local population level extinctions, and not simply a range shift. This is because, in contrast to other seagrasses that may be able to shift their distribution, the unique conditions experienced along the South African coastline do not allow for shifting of coastal species to more northerly or southerly latitudes. South African coastal species, similar to their Australian counterparts, occupy a precarious position, lacking north-south coastlines that allow for latitudinal range shifts during climate extremes (Grant & Bowen 2006). This is particularly true on the west coast where estuaries become more distant from each other towards the equator, making successful dispersal and colonisation of seagrasses unlikely. The projected overall decrease in precipitation on the west coast may contribute to local extinctions of *Z. capensis* populations. Conversely, the projected increase in summer precipitation, and associated shift to less saline conditions, on the upper east coast may also negatively impact the persistence of the seagrass, resulting in the projected reduced suitable habitat, and therefore distribution, to the south-eastern coast. It is reasonable to assume that further north along the east African coast *Z. capensis* may experience similar losses, especially as seagrasses in tropical conditions are already at their maximum thermal limits (Koch *et al.* 2013).

As *Z. capensis* could be largely limited to estuaries along the south-eastern coast, this area might serve as a focus for targeted conservation management in the near future, especially as seagrass may have to contend with increasing development in this area. This geographic clustering of populations at the south-east coast may lead to an increase in connectivity between seagrass-populated estuaries in comparison to its current disjunct distribution. This may thus aid in the persistence of the *Z. capensis* (Almany *et al.* 2009). Conversely, this area currently coincides with high levels of human

development which is likely to increase in the future, and could thus further negatively impact the persistence of this species. It has been emphasised that sea level rise will have the most severe impact on seagrass populations due to a reshaping of the available estuarine habitat (Valle *et al.* 2014). Sea level rise was not included in this model due to its complexity, as a shifted coastline would make estuarine conditions problematic to predict, yet may well contribute an additional threat to South African seagrasses. Due to the key role of seagrass in overall ecosystem functioning, a steep decline in a habitat availability and quality can be expected to threaten species that are dependent on seagrass, including economically important fishes and invertebrates (Green & Short 2003). Consequently, the recruitment success of larvae from commercial species might be substantially reduced. Thus, it is vital to consistently monitor the state of seagrass communities in South Africa.

Many human associated pressures, such as coastal development, damming and habitat alteration, could threaten seagrass beyond levels predicted in this model and impact their habitat quality and resilience. However, this model does illustrate the sensitivity of this seagrass to changing climate and human associated conditions. Future research on the influence of eutrophication and sea level rise on estuaries, as well as the potential influence of gene flow and local adaptation on seagrass persistence, would be beneficial for seagrass ecosystem management planning. Regardless of its wide temperature range, *Z. capensis* still appears to be vulnerable to climate change and human induced pressures. Overall, it is not a few specific factors, but instead, the cumulative effect of multiple changes in the environment over time that will influence the persistence of this seagrass into the future. The downward trend predicted for *Z. capensis* appears to be shared by other seagrasses globally (Collier *et al.* 2011; Rasheed & Unsworth 2011; Valle *et al.* 2014), potentially to the detriment of coastal linked economies and biodiversity. Thus, it is highly recommended that marine spatial plans and biodiversity management planning should carefully consider not just the present state, but also the potential future of such vital species when developing coastal policies and plans.

Chapter 3

Population genetics of *Zostera capensis* in South Africa

3.1 Introduction

Seagrass communities

Seagrasses are a distinctive feature of many subarctic, estuarine, temperate and tropical sub-tidal coastal areas (Den Hartog 1970). Seagrasses are angiosperms, capable of producing flowers and seed, though their aquatic nature results in distinct differences in seed dispersal relative to their terrestrial relatives. While all seagrasses are capable of both asexual and sexual reproduction, vegetative reproduction is thought to often dominate the maintenance and expansion of beds (Tomlinson 1974; Phillips *et al.* 1983). Different seagrass species vary greatly in their reproductive strategies and the proportion of asexual/sexual reproduction can differ between populations of the same species according to their proximity to the species range edge (Phillips *et al.* 1983). The seed output, size, buoyancy, dormancy, survival and dispersal all vary greatly among seagrass species (Orth, Harwell *et al.* 2006). Seagrasses grow submerged in calm shallow waters and in many cases cover extensive areas, often being referred to as seagrass meadows or beds (Green & Short 2003). Seagrass forms a fundamental part of a complex ecosystem, supporting considerable biodiversity and a high level of productivity. For example, a North American study found that as much as 64% of total primary production in the estuarine study system was provided by seagrasses (Richard 1973). As such, seagrass ecosystems represent one of the richest coastal habitats and are vital in the maintenance of an array of ecologically and commercially important marine, freshwater and estuarine organisms from various trophic levels (Orth, Carruthers *et al.* 2006).

Seagrass can be defined as an 'autogenic' ecosystem engineer, increasing structural complexity in their environment by virtue of the presence of their extensive network of roots and rhizomes, as well as their flattened blade-like leaves which can grow up to 125 cm in length. In addition, seagrass beds can form dense aggregations; for example in Mozambique some beds have been found as dense as 4561 shoots per m² (Green & Short 2003). The complex web of interactions associated with seagrass beds has both direct and indirect affects within seagrass communities

(Siebert & Branch 2006). The modified environment provided through their complex above and below ground structures provides a variety of niches in the water column, on the plant surface and both on and within the sediment (Green & Short 2003). Seagrass-dependent species range from epiphytic algae to large aquatic herbivores, such as manatee, dugong and the critically endangered green sea turtle which feed directly upon the seagrass (Kitting *et al.* 1984; Green & Short 2003). Some residents move freely in and out of seagrass beds while others may be restricted during certain life stages or they may even be obligate residents, found nowhere else (Green & Short 2003). Residents may utilise seagrass beds for habitat, shelter, dietary or reproductive requirements (Green & Short 2003). Many seagrass residents represent endangered or threatened species, such as the dugong, manatee, horseshoe crab, green turtle and various grouper fishes and seahorses (Walter & Gillett 1998).

South African Oceanography and Biogeography

The unique oceanography of the South African coast line drives the complexity of the biogeographic patterns seen in marine and estuarine species. South Africa is the only country globally that experiences two contrasting temperature regimes in the currents along the two coasts, which mix at a transition zone on the south coast. The cold Atlantic Ocean and Benguela Upwelling System are found on the west coast and the warm Indian Ocean and Agulhas Current on the east coast (Nelson & Hutchings 1983; Lutjeharms & Van Ballegooyen 1988). Patterns of biodiversity are determined largely by oceanographic elements such as currents, the associated sea temperatures and features of the continental shelf. The South African marine coastal environment is distinguished by very high species richness due to its long coastline and variable conditions (Awad *et al.* 2002). In fact, South Africa is recognised as a biodiversity hotspot and the number of marine species in South Africa was estimated to be at least 12 900 in 2010 with a high level of endemism at 33% (Awad *et al.* 2002; Griffiths *et al.* 2010). Further, it is estimated that there are still many undescribed taxa, particularly benthic organisms, algae and fungi (Griffiths *et al.* 2010). In estuarine

fish species, this richness tends to show a gradient with higher species richness in estuaries on the warmer eastern coast and lower on the cooler western coast (Harrison 2002).

Numerous studies have examined the biogeographic patterns of species distributions along the South African coast and have recognised between two and five broad regions, with some slight variation in the naming and region boundaries (Stephenson & Stephenson 1972; Brown & Jarman 1978; Bustamante & Branch 1996; Bolton & Anderson 1997; Turpie *et al.* 2000; Bolton *et al.* 2004). There are three general temperature delimited marine bioregions (Ridgway *et al.* 1998; Evans *et al.* 2004; Teske *et al.* 2006, 2007; Edkins *et al.* 2007): the cool-temperate West Coast extending from the mouth of the Orange river to Cape Agulhas, characterised by the cold Atlantic waters, low rainfall and high evaporation; the warm-temperate South Coast from Cape Agulhas to Port St Johns, defined by minimum winter temperatures of 12–14° C and variable rainfall; and subtropical east coast from Port St Johns to Mozambique, distinguished by the warm Indian Ocean waters with temperatures above 16° C and high summer rainfall (Stephenson & Stephenson 1972; Day 1981). While these bioregions were delineated for rocky-shore biota, similar regions have been classified for estuarine organisms (Stephenson & Stephenson 1972; Day 1981; Harrison 2002) based mainly on water temperature, rainfall and river flow.

A more recent assessment of South African marine biodiversity has led to the biogeographic delineation of the coast into six near-shore regions (Driver *et al.* 2012; Sink *et al.* 2012). The cool-temperate Namaqua Bioregion is found on the west coast until Cape Columbine, from where the South-western Cape Bioregion begins and extends till Cape Point. The warm-temperate Agulhas Bioregion extends from Cape point along the south coast to the Mbashe River. The subtropical Natal Bioregion on the east coast merges in the far north at Cape Vidal into the tropical Delagoa Bioregion, which extends northward into Mozambique (Fig. 3.1). It is important to note that these regions are by no means absolute for every taxon and that a variety of localised habitats exist within

each bioregion (Griffiths *et al.* 2010). Many species inhabit several regions and some have been found to exhibit region-specific adaptations (Teske *et al.* 2009).

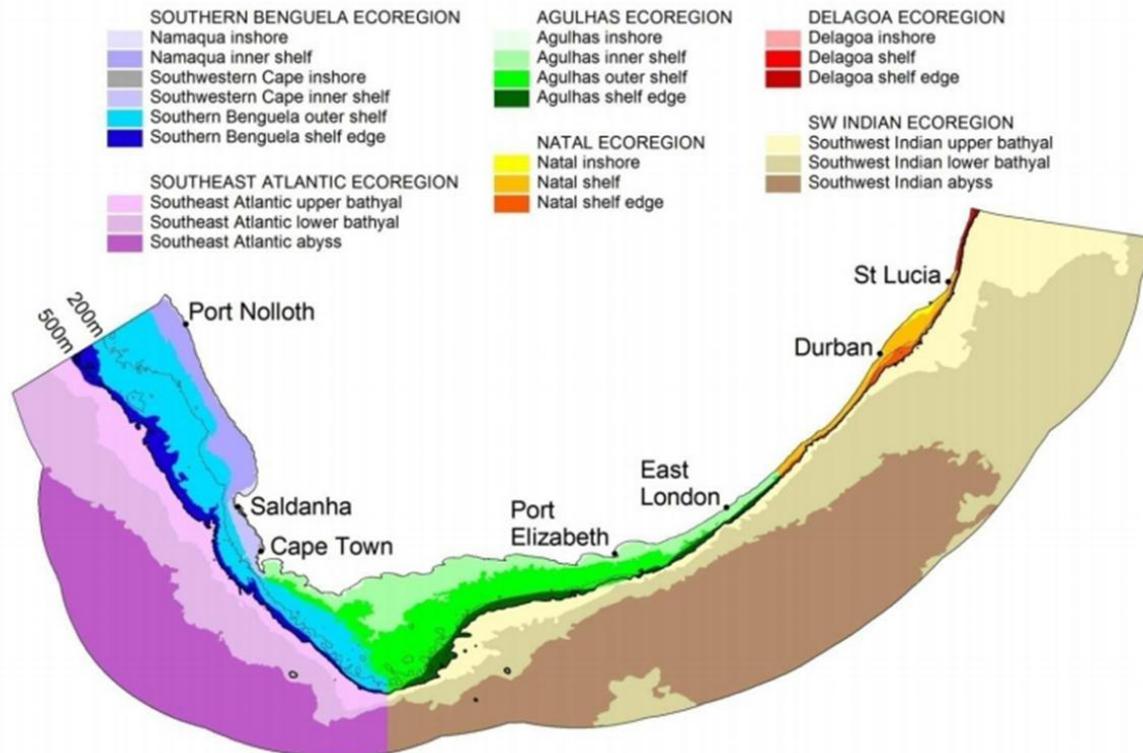


Figure 3.1 South Africa's coastal and marine inshore and offshore ecoregions (National Biodiversity Assessment – marine component; Sink *et al.* 2012).

Despite the high level of connectivity often expected in marine environments, many marine species show population structuring along the South African coast line (von der Heyden 2009; Teske *et al.* 2011; Wright *et al.* 2015), and the same has been shown for various estuarine species (Teske *et al.* 2006, 2007). Estuaries generally restrict gene flow and impose distinct selective regimes (Bilton *et al.* 2002). As such, the genetic structure of some estuarine species has been associated with the three classical temperature delimited bioregions (Teske *et al.* 2006, 2007; Edkins *et al.* 2007; Maake *et al.* 2013; Phair *et al.* 2015). For instance, invertebrates with both active and passive dispersal have been found to exhibit genetic structure and differentiation along these bioregions, including mudprawns, isopods, cumaceans (Teske *et al.* 2006) and shrimp (Teske *et al.* 2007).

However, this is not the case for all species in this region. For example, species richness and composition of seaweed populations along the South African coast correlate with temperature, yet do not reflect the previously mentioned bioregions (Bolton *et al.* 2004). Two distinct tropical groups exist to the north while the southern east coast flora is indistinct and represents an overlap between the tropical northern and temperate southern elements (Bolton *et al.* 2004). The salt marsh species, *Salicornia meyeriana*, shows differentiation along the South African coast into distinct clades corresponding with both geographic distance and ecological profiles (Slenzka *et al.* 2013).

East African Biogeography

Population genetic studies, and more specifically those on aquatic plants, are lacking along the East African coast. Biogeographic and phylogeographic studies that have been conducted tend to focus on coastal fishes and crabs as well as reef organisms. Organisms on these reefs in the western Indian Ocean commonly show widespread structuring off the East African coast and greater connectivity amongst the southeast African reefs (Ridgway & Sampayo 2007). One study in southern Mozambique was conducted on the seagrass *Thalassodendron ciliatum*, which grows in a rocky and a sandy habitat in two distinct forms (Bandeira & Nilsson 2001). However, the study found that these forms did not differ genetically when examining Randomly Amplified Polymorphic DNA (RAPD). This seagrass was shown to be highly genetically diverse, indicating the possibility of frequent sexual reproduction or immigration in these populations. Most of the genetic variation was detected within populations rather than between populations, indicating lack of population structure, with no significant correlation between geographic and genetic distance. Population structure has also been studied within the mangrove habitat along the East African coast. The mangrove crab, *Perisesarma guttatum*, was examined on the East African coast and two clades were discovered with populations in southern Mozambique differentiated from those in northern Mozambique, Tanzania and Kenya (Silva *et al.* 2010). This indicates a break between southern Mozambique and the northern populations of crab inhabiting the mangroves. These crabs are reliant

on mangroves during part of their larval stage but dispersal of newly hatched larvae is expected to be high as a result of ocean currents (Flores *et al.* 2002). Consequently, there was no population structure within each clade.

Zostera capensis in southern and eastern Africa

Although there are 60 described species of seagrasses globally, this remains an understudied taxonomic group in Africa (Green & Short 2003). On the South African coast four seagrass species have been described, whilst the sub-tropical East African coast displays much higher diversity and overlap with tropical species of the Indo-Pacific, with 13 described species (Short *et al.* 2007). Seagrasses exhibit low diversity with three independent lineages (Hydrocharitaceae, Cymodoceaceae complex, and Zosteraceae) having evolved from one monocotyledonous flowering plant (Les *et al.* 1997). Zosteraceae is a temperate seagrass family consisting of four genera, *Phyllospadix*, *Zostera*, *Nanozostera* and *Heterozostera* (Coyer *et al.* 2013). Using both molecular and ecological approaches, seagrasses have been studied almost globally, with *Zostera* investigated in Spain (Diekmann *et al.* 2005), New Zealand (Jones *et al.* 2008), Japan (Kato *et al.* 2003), Australia (Les *et al.* 2002), Europe and North America (Olsen *et al.* 2004); *Heterozostera* in Australia, Chile and North America (Les *et al.* 2002; Tanaka *et al.* 2003; Coyer *et al.* 2013); *Phyllospadix* and *Nanozostera* in various regions (Coyer *et al.* 2013).

Several molecular markers have been used to study phylogenetic and phylogeographic aspects of the seagrass family Zosteraceae. Plastid markers such as chloroplast DNA maturaseK and nuclear markers such as rDNA–internal transcribed spacer locus (ITS) are common in plant studies and have been used to study seagrass phylogenies (Olsen *et al.* 2004). It is useful to have both a plastid and a nuclear marker as plastid markers are known to mutate relatively fast when compared to nuclear markers, resolving recent differentiation, while the slower mutating nuclear marker displays less recent differentiation. Microsatellite loci are often more species specific and are commonly used as molecular markers in population genetics studies. Although microsatellite markers have

been successfully used to study *Z. marina* (Olsen *et al.* 2004; Coyer, Diekmann *et al.* 2004; Becheler *et al.* 2010; Muñiz-Salazar *et al.* 2005), the five specific microsatellite loci utilised were unable to successfully cross-amplify sister species *Zostera noltii* and *Zostera japonica* (Reusch 2000). Nine microsatellite markers later designed for *Z. noltii* have seen successful cross-amplification in *Z. japonica* and partial success in *Z. marina* (Coyer, Diekmann *et al.* 2004).

Zostera capensis is a temperate species of seagrass belonging to the family Zosteraceae. It has a wide geographic distribution from the west coast of South Africa to southern Kenya (Short *et al.* 2010; Fig. 1.1), which is unique amongst seagrasses as this range encompasses cool-temperate, subtropical and tropical environments. Throughout its range, *Z. capensis* is highly fragmented as it is confined to areas with low water movement, such as lagoons, estuaries and intertidal flats (Green & Short 2003). The fragmentation of *Z. capensis* and threats to seagrasses in general have led to its classification as ‘vulnerable’ on the IUCN red list of threatened species (www.iucnredlist.org). However, only about 13% of seagrass habitat in South Africa can be found in protected areas (Van Niekerk *et al.* 2012) and anthropogenic pressures outside of South Africa have not been quantified. As a result, the actual distribution of *Z. capensis*, particularly along the east African coast, is currently uncertain.

Seagrass communities specifically are particularly impacted by the disturbance caused by the increase in sea storms as a result of climate change (Mead *et al.* 2013). Further, overfishing in seagrass communities leads to a trophic cascade; the removal of top level predators causes mesopredator release, which then eliminates the grazers that reduce seagrass epiphyte loads (Mead *et al.* 2013). *Zostera capensis* has also experienced population declines, with reports suggesting that some populations have been reduced by around 50% in Mozambique due to bivalve harvesting (Green & Short 2003). It is expected that this decline will continue until the point of local extinction with consequent social-economic impacts in the area (Green & Short 2003). In addition, many *Z. capensis* populations are highly fouled by epiphytic algae (Källén *et al.* 2012). About 38% of

seagrass cover in Langebaan Lagoon has been lost since 1960 and in some areas, only 2% of the historical cover remains (Pillay *et al.* 2010). Areas worst affected by the decline of seagrass cover have experienced a reduction in invertebrate species richness by up to 50% and the localised extinction of resident invertebrates (Pillay *et al.* 2010). For example, *Siphonaria compressa*, an estuarine species of limpet, has specialized to live on *Z. capensis* blades in the mid to upper intertidal (Herbert 1999). This limpet is South Africa's most endangered marine invertebrate and is now restricted to two localities on the west coast, namely Langebaan Lagoon and Knysna estuary (Herbert 1999; Mead *et al.* 2013).

Interestingly, in Langebaan Lagoon in South Africa, *Z. capensis* appears to exhibit two morphotypes, one short and stunted on muddy tidal flats which experience prolonged aerial exposure and the other is longer with a higher biomass on sand flats that experience shorter exposure (D. Pillay, pers. comm.). A similar situation was found in *Z. marina* in northern Europe's Wadden Sea where adaptive divergence was found to be taking place (Oetjen *et al.* 2009). This functional selection has been linked to genes involved in osmoregulation and reproductive processes, suggesting different osmotic stress conditions and life history strategies in different environments, specifically on tidal flats and permanently submerged habitat (Oetjen *et al.* 2009). However, it is still unclear what is driving the two morphotypes in Langebaan Lagoon.

Numerous molecular studies have been conducted on the widespread *Zostera marina*, at both local and regional scales (Becheler *et al.* 2010), while more restricted species such as *Z. capensis* remain unstudied from a molecular perspective. When studied using microsatellite markers, *Z. marina* has been found to exhibit strong population structure at a regional scale and low levels of gene flow along the Pacific coast of Baja California and the Gulf of California. Significant Isolation-By-Distance (IBD) was found in the Gulf of California but not along the Pacific coast of Baja California possibly due to shifting currents during El Niño Southern Oscillation at the time (Muñiz-Salazar *et al.* 2005). Strong population structure and genetic differentiation has also been found in

Brittany, France, at a fine-grained local scale, despite a dispersal potential of over 100 m (Harwell & Orth 2002; Becheler *et al.* 2010). This study utilised both microsatellite and ITS markers, and revealed a mosaic of clones with distinct origins in space and time (Becheler *et al.* 2010). A large-scale study in the North Atlantic, make use of microsatellite, ITS and matK markers, detected significant genetic differentiation between populations and strong IBD was found at some locations such as the central Baltic Sea. At other locations IBD was not present, suggesting that dispersal and gene flow is restricted by environmental factors (Olsen *et al.* 2004).

Phylogeographic trends in *Z. marina*, the most widespread temperate seagrass, may give some indication of the genetic patterns to be expected in *Z. capensis*. Given that *Z. marina* typically has low levels of gene flow and strong population structure, *Z. capensis* is unlikely to display high levels of connectivity in the disjunct populations found in South Africa. The relative isolation of populations may promote and retain genetic diversity, however as *Z. capensis* has been experiencing a widespread decline, populations are likely to be small, increasing the risk of local extinction, loss of genetic diversity and reducing the ability to recruit back into the system. Despite the importance of the uniquely distributed and threatened *Z. capensis* in community structuring and as an ecosystems service provider, it still lacks molecular investigation. This will be the first study to examine the genetic diversity and population structure of seagrass along the South African and east African coast.

3.2 Aims

Using chloroplast DNA maturase K gene (*matK*), nuclear rDNA–internal transcribed spacer (ITS-1) locus and nine microsatellite markers, this chapter aimed to understand genetic diversity levels, gene flow and population structure of *Z. capensis* populations in southern African and give insight into the pertinent evolutionary forces. With the use of these molecular tools, distinct evolutionary lineages and genetic diversity can be determined. This information can be used for identifying evolutionary significant units and management units by the prioritisation of estuaries harbouring particular genetic potential to aid conservation management planning. Genetic diversity and uniqueness data can also be used to assess the vulnerability of South Africa's estuaries using *Z. capensis* as an indicator species. Due to the declining cover of seagrass and the potential ramifications in seagrass communities, the surrounding environment and fishery industries, the results of this chapter are imperative for informing coastal and estuary spatial biodiversity planning.

3.3 Hypothesis

I hypothesised that *Z. capensis* will exhibit strong population structuring with higher genetic diversity expected between populations than within. Further, I expected low levels of gene flow in *Z. capensis* as it is found in disjunct populations along the South African coast, and although little is known about its dispersal and reproduction, the harsh South African marine environment would not be conducive to its survival during conveyance between estuaries. I also expected that the phylogeography of *Z. capensis* will reflect the three broad biogeographic regions (cool-temperate West Coast, warm-temperate South Coast and subtropical East Coast), as found in other estuarine species.

3.4 Methods

Collections

Samples were collected from nine estuaries along the South African coast and one location in southern Kenya. The sampling localities along the South African coastline include the Olifants, Berg, Langebaan estuaries on the west coast; the Breede, Kynsna and Swartkops estuaries on the south coast; and Nahoon, Mngazana and Richards Bay estuaries on the east coast (Fig. 3.2; Table 3.3). Researchers in Mozambique, Tanzania and Kenya were contacted to establish where *Z. capensis* is currently found. Samples from Shimoni, southern Kenya, were collected by Dr Nina Wambiji from the Kenyan Marine Fisheries Research Institute (KMFRI) following the described protocol and transported to the University of Stellenbosch. Due to a combination of factors (for example, the lack of resources on the east African coast and the unavailability of willing collaborators) no other samples were obtained from the east African coast. At each location three samples were collected from five beds at two separate sites, at least 1 km apart, for a total of 30 cuttings. Each sample was blotted dry and cleaned of debris before being placed in a labelled paper envelope. The envelopes from each location were then be sealed in an airtight plastic bag containing silica gel crystals in order to remove excess moisture from the samples, preventing contamination of the plant tissue by mould. It should be noted that the samples from Kenya were not stored in silica gel and thus were moldy when they arrived.

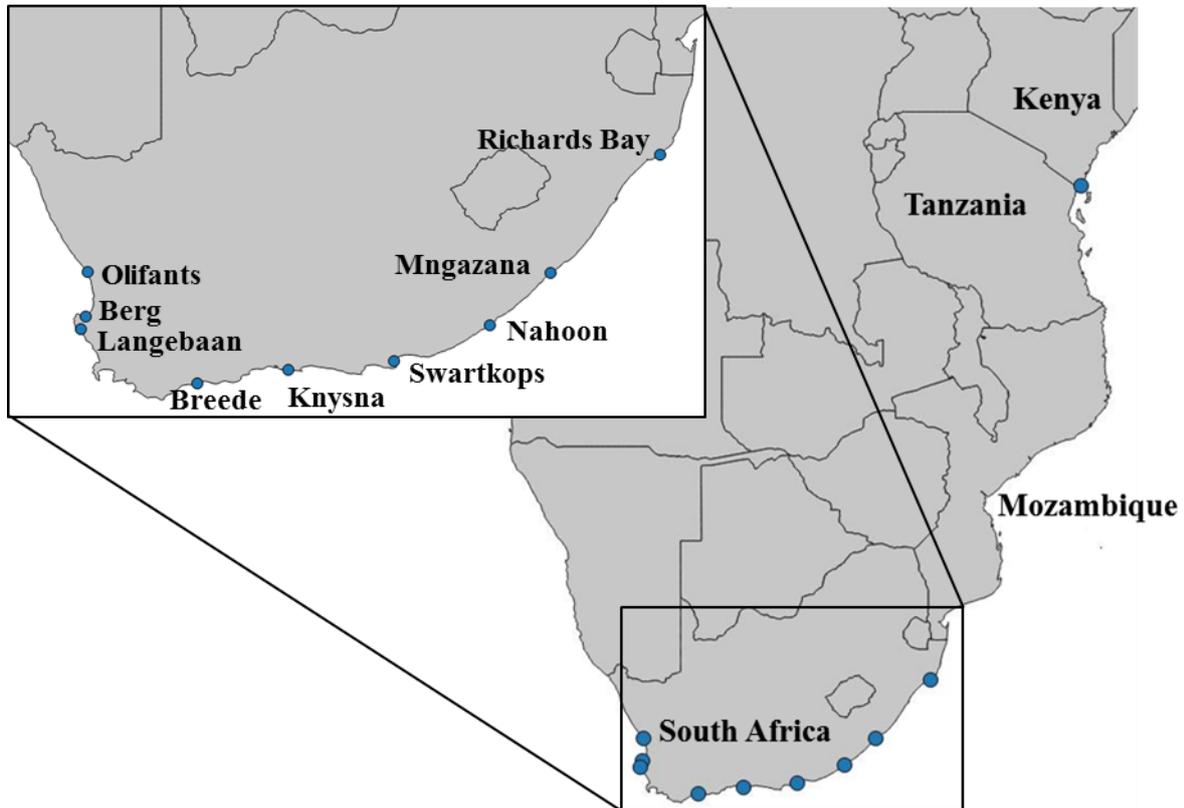


Figure 3.2 Sampling locations at estuaries along the South African and east African coasts.

Laboratory protocols

Dried plant material was lysed using Zymo tissue lysis kit BashingBeads™ in a tissue lyser machine at the Central Analytical Facility of Stellenbosch (CAF). Genomic DNA was then extracted from the samples using the Qiagen DNeasy plant mini kit following standard protocol. The chloroplast DNA maturase K intron (matK) and the nuclear rDNA–internal transcribed spacer locus 1 (ITS; White *et al.* 1990) were amplified according to the conditions set out in Table 3.1. The matK and ITS markers PCR products were run on a 1% agarose gel to check for quality and fragment size. Samples which exhibited multiple bands were extracted from the gel and purified using a BioFlux gel extraction kit, following standard protocol. Sequencing was carried out at CAF using a BigDye Terminator V3.1 sequencing kit (Applied Biosystems). I encountered some difficulties in getting the matK marker to amplify all 30 samples from each location. As a result, I used GENEIOUS V8.0 software package (Drummond *et al.* 2010) to design specific primers for *Z. capensis* using sequences from the successfully amplified samples. While this did not result in all

30 samples from each location being successfully amplified, it still enabled me to obtain sequences from a greater number of individuals per location.

Although the *matK* region is often used for phylogenetic analyses, its use was continued here for population genetic analyses as it has the highest substitution rate among plastids (Olmstead & Palmer 1994) and there have been some cases in which this marker has shown variation in widespread populations (Kado *et al.* 2004; Olsen *et al.* 2004; Talbot *et al.* 2006; Patreze and Tsai 2010).

Table 3.1 Primer name, sequence and PCR conditions for each locus

Fragment	Locus	Primer (5'-3')	PCR conditions
Species specific			94°C 2'
Chloroplast	<i>matK-F</i>	CTTAGTTCAAATTCTACAATCCTGG	40 x (94°C 20", 45°C 20", 72°C 1')
maturase K	<i>matK-R</i>	GAATTCTATATGGTKGAGACC	
intron			
Nuclear rDNA–			94°C 2'
internal	<i>ITS5-F</i>	GGAAGTAAAAGTCGTAACAAGG	40x (94°C 20", 48°C 20", 72°C 1')
transcribed	<i>ITS2-R</i>	GCTGCGTTCTTCATCGATGC	
spacer locus I			72°C 5'

Nine microsatellite markers, which were designed for sister species *Z. noltii*, were identified from the literature and tested for use in *Z. capensis* (Table 3.2). Cross-amplification has been successfully carried out with all nine of these markers in *Z. japonica* and with four of these markers in *Z. marina* (Coyer, Diekmann *et al.* 2004; Coyer, Reusch *et al.* 2004; Diekmann *et al.* 2005). All nine of these markers were tested on samples from each of the nine locations. In attempting to optimise the conditions for these primers, conditions were varied as follows. The amount of genomic DNA applied was increased from 1 µl to 3 µl and 1 in 100, 1 in 20 and 1 in 10 dilutions of the genomic DNA were utilised. Following this, I adjusted the amount of Mg²⁺ added to the PCR reaction from 1 µl to 2.5 µl. I also increased the number of PCR cycles from 35 to 40 and used a gradient PCR machine to test annealing temperatures between 50°C and 60°C. All PCR products were run on 1%

agarose gel to check for quality and fragment size. A nanodrop test was used to check for the presence of PCR inhibitors as well as the concentration of the genomic DNA. Concentration of the genomic DNA was confirmed by Qubit analysis at CAF Stellenbosch.

Table 3.2 Nine microsatellite markers, their primer sequences and suggested PCR conditions (Coyer, Reusch *et al.* 2004)

Locus	Primer (5'-3')	PCR conditions
ZnB1	F: TTGACAAAAGTAGGTGGAGTTGA	
	R: GGCAGAGAAGAGCAGTACACG	
ZnB3	F: CTTTCGTTTCGTTTCATAAAGCA	
	R: CCCAATTCTCATCGACAACC	
ZnB8	F: AGATGGCGGAAATCAAACC	
	R: CCTGTCACTTGCCACTTGTC	
ZnD6	F: GACCACGTGCAATTCTGAAA	
	R: TTCCCTGCACTTGTAGACCC	94°C 3'
ZnE7	F: AAACAGGAACGCGAAGAAGA	35 x (94°C 40", 57.5°C 40", 72°C 3')
	R: TGGTGACCTTTCTCATTGGA	72°C 10'
ZnF8	F: GCCACGACAATGTGAACAAC	
	R: CGTGATGAATGAAAGAGCGA	
ZnF11	F: AGACGCAGACTCGGACTCAT	
	R: GAAGATGCATTATTCATTCACCC	
ZnH8	F: TTCGACGACAGACAGAAACG	
	R: AGAGAGGAAGACGGTGACGA	
ZnH10	F: TCTGCCGGTGTGTGAAACT	
	R: CGTCGTTTTAAATTGCCTCTT	

Complications

Even though PCR products were gel extracted and purified, the ITS marker still displayed poor sequence quality across all locations. This could be attributed to the fact that the ITS region is a multi-copy region and therefore can produce multiple similarly sized fragments which are difficult to resolve using agarose gel electrophoresis (El Tai *et al.* 2000; Álvarez & Wendel 2003; Li *et al.* 2011). They were thus excluded from further analysis as they were likely to be unreliable.

Unfortunately, in addition to this, none of the microsatellite markers reliably amplified *Z. capensis* and after multiple attempts to optimise conditions, these primers were discarded.

Data analysis

The matK sequences were aligned and edited using GENEIOUS V8.0 software package (Drummond *et al.* 2010). Sequences were submitted to the Basic Local Alignment Search Tool (BLAST) on the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm the species identity. DNAsp was used to create input file for ARLEQUIN 3.5 (Excoffier & Lischer 2010), which calculated nucleotide (π) and haplotype (h) diversity as well as pairwise F_{st} measures to investigate population structure. An Analysis of Molecular Variance (AMOVA) was carried out using ARLEQUIN 3.5 (Excoffier & Lischer 2010) in order to estimate the population differentiation.

3.5 Results

A 369 bp fragment of the matK region was obtained from a total of 149 South African *Z. capensis* samples. At each location, 12-25 samples were sequenced, excepting Kenya, where three samples were sequenced, because of the poor quality material that was received (Table 3.3). According to the BLAST search of the matK region sequences, all of the samples, including those from Kenya, were positively identified as *Z. capensis*. The matK region of the chloroplast DNA was not variable among *Z. capensis* populations, with a resultant nucleotide diversity of $\pi = 0$ and haplotype diversity of $h = 0$. However, one polymorphic locus was identified in a single individual from the Berg estuary. As expected, the population level AMOVA displayed no population level differentiation ($F_{st} = 0.013$, $P > 0.05$). No structure was found between *Z. capensis* populations, with all pairwise F_{st} values being insignificant.

Table 3.3 The number of maturaseK sequences obtained from each South African sampling location

Locality	Number of sequences
	matK marker (chloroplast)
West coast	
Olifants	12
Berg	13
Langebaan	14
South coast	
Breede	20
Knysna	17
Swartkops	12
East coast	
Nahoon	25
Mngazana	22
Richards Bay	14
Kenya	3

3.6 Discussion

Although there does not appear to be any variation in the matK region of *Z. capensis* among populations in South Africa, the analysis of the matK region did enable the verification of the species identity of all samples as *Z. capensis*. This was particularly important for the Kenyan samples, being at the northern extreme of the species range and with the east African *Z. capensis* distribution not being well described. Further, some east African seagrasses are difficult to distinguish from *Z. capensis*. The confirmation of the presence of this species in southern Kenya, and the lack of variation between the Kenyan and South African samples, suggest that this species is likely to also be present in the intervening coastline, where experts are currently uncertain of its presence (S.O. Bandeira & L. Nordlund pers. comm.). The lack of observed variation may result from high levels of gene flow and connectivity via the long distance dispersal of propagules, as seen in the tropical ‘turtle grass’, *Thalassia testudinum* (van Dijk *et al.* 2009). However, this is unlikely as the estuarine environments in which *Z. capensis* are found tend to restrict gene flow and impose distinct selective conditions (Bilton *et al.* 2002). This is well illustrated in Jones *et al.* (2008) where the estuarine eelgrass, *Zostera muelleri*, displayed high levels of genetic differentiation between populations in New Zealand at both coarse and fine spatial scales. The lack of observed variation may also result from selection to environmental conditions, as matK has been linked to developmental regulation mechanisms and photosynthesis (Barthet & Hilu 2007). The absence of genetic diversity could also indicate a high level of clonal reproduction and thus inbreeding, as observed in an Australian species of seagrass, *Amphibolis antarctica*, commonly known as ‘sea nymph’ or ‘wire weed’ (Waycott *et al.* 1996). Nevertheless, the lack of variation observed here is more likely to be a result of a low resolution marker.

Although the matK marker is often utilised in a phylogenetic context, it has also been used to study population genetics and has shown variation at large spatial scales (Table 3.4). For example,

examination of the maidenstears, *Silene vulgaris*, across the Northern hemisphere revealed nine variable sites in the matK region (Houliston & Olson 2006) and the willow, *Salix melanopsis*, has shown seven variable sites in the matK region across four north-western American states (Carstens & Richards 2007). The cornicabra tree, *Periploca laevigata*, has shown a similar level of matK variation at a smaller scale, with five variable sites resulting in two haplotypes across the Canary Islands (García-Verdugo *et al.* 2015). In terms of coastal systems, a study on mangroves in Vietnam found one species with three geographically distinct lineages and a second species with two distantly separate geographic lineages (Kado *et al.* 2004). Variation in the matK region has also been seen in seagrasses, with two *Z. marina* haplotypes found in Japanese populations (Talbot *et al.* 2006) and four variable matK sites in North Atlantic populations (Olsen *et al.* 2004).

Table 3.4 Examples of variation in the matK region in different species and at different spatial scales

Source	Species	Scale	Variation
Houliston & Olson 2006	Maidenstears, <i>Silene vulgaris</i>	Northern hemisphere	Nine variable sites
Carstens & Richards 2007	Willow, <i>Salix melanopsis</i>	Continental	Seven variable sites
García-Verdugo <i>et al.</i> 2015	cornicabra tree, <i>Periploca laevigata</i>	Regional	Five variable sites
Kado <i>et al.</i> 2004	Mangroves, <i>Vicennia marina</i> , <i>Kandelia candel</i> and <i>Lumnitzera racemosa</i>	National	Two to three geographically distinct lineages
Talbot <i>et al.</i> 2006	Seagrass, <i>Z. marina</i>	National	Two Haplotypes
Olsen <i>et al.</i> 2004	Seagrass, <i>Z. marina</i>	Regional	Four variable sites

Conversely, microsatellite markers are capable of showing variation at a finer spatial scale (Selkoe & Toonen 2006). Microsatellite markers have proven more successful than allozyme or RAPD markers at observing diversity in clonal organisms (Arnaud-Haond *et al.* 2005). Yet not all microsatellites are equally valuable in analysing the population genetics of clonal organisms. A

study on the optimisation of markers for clonal organisms found that tri- and hepta-nucleotide microsatellite markers revealed a specific population to have extremely low clonal diversity, yet with dinucleotide microsatellite markers the same population emerges as one of the most genetically diverse (Arnaud-Haond *et al.* 2005). Yet techniques with even greater resolution do exist, and these may prove the most beneficial to future research on clonal species such as *Z. capensis*.

The best solution to the question of resolution when studying such species may lie in Next-Generation Sequencing (NGS). NGS has recently come to the fore as a tool for studying the molecular ecology of non-model organisms (Ekblom & Galindo 2011; Puritz *et al.* 2012). It enables the detection of genetic diversity by scanning the genome for Single Nucleotide Polymorphisms (SNPs), which are useful for analysing both neutral (Morin *et al.* 2004) and adaptive variation (Ekblom & Galindo 2011; Willette *et al.* 2014). NGS has multiple uses in the realms of genomics, transcriptomic and epigenomics, including the study of gene regulation, expression, transcriptome characterisation, development of molecular markers, nucleotide profiling and genome assembly (Ekblom and Galindo 2011). Although several different technologies fall under the umbrella of NGS, they all generate unprecedented amounts of sequence data with similar general features and can be used to address ecological and evolutionary questions (Metzker 2010). Unfortunately, due to time and financial constraints, I could not use NGS in this MSc nor could I design specific microsatellite markers which may have revealed variation in *Z. capensis*.

Even with high resolution markers, relative to matK, other seagrass species display varying levels of genetic diversity within and between populations. For example, under RAPD analysis *Thalassodendron ciliatum* exhibits high genetic diversity among populations in southern Mozambique, suggesting a high level of sexual reproduction (Bandeira & Nilsson 2001). Yet *Thalassia testudinum* in Mexico shows little to no variation in populations up to 350 km apart and a

high degree of connectivity in populations up to 1350 km apart, when investigated using six microsatellite markers (van Dijk *et al.* 2009).

Although to date there has been no other work on the phylogeography of South African seagrasses, and very little on the phylogeography of marine or estuarine flora in general, the population genetics of many other species has been investigated in this region. These studies have led to the identification of various genetic breaks along the coastline, some coinciding with biogeography. For instance, divergence of caridean shrimp *Palaemon peringueyi* has been found along the three main biogeographic regions due to passive dispersal of larvae in currents (Teske *et al.* 2007). Other genetic discontinuities correspond to physical barriers to gene flow, reflecting the importance of population genetic studies for conservation planning (von der Heyden 2009). For example, the estuarine roundherring *Gilchristella aestuaria* (Norton 2006) and estuarine isopod *Exosphaeroma hylecoetes* (Teske *et al.* 2006) show a genetic break or limited gene flow around Cape Point. Other breaks in gene flow have been revealed by estuarine species at Cape Agulhas in the crustaceans *Upogebia africana* and *E. hylecoetes*, between Knysna and Port Alfred in the cumacean *Iphinoe truncata*, and between Kenton on Sea and Haga Haga in the estuarine prawn, *Upogebia africana* (Teske *et al.* 2006). Lastly, in the estuarine prawn *Callianassa kraussi* (Teske *et al.* 2009) and the coral *Pocillopora verrucosa* (Ridgway *et al.* 2008), genetic differentiation has been found between the subtropical north-eastern coast of South Africa and the tropical southern coast of Mozambique. Additionally, this region displays a shift in the species composition of macroalgae (Bolton *et al.* 2004).

Although no variation was found in *Z. capensis* in the matK region, it is still likely that population structure may be found in this species when applying a molecular marker with a greater resolution. Given the wide range of conditions that its current distribution covers, and the manner in which estuaries restrict gene flow, a high level of population differentiation can be expected from this species, as evidenced by other seagrass species. This differentiation may be similar to what has

been found in other estuarine species, reflecting the three main biogeographic regions (Teske *et al.* 2007), genetic breaks common to estuarine species (Norton 2006; Teske *et al.* 2006, 2009), or simply isolation by distance. I recommend that this work on unravelling the vulnerability, resilience and adaptation of *Z. capensis* be continued in southern Africa to determine the future potential for this keystone species and its ecosystem services.

Chapter 4

General Conclusion

This MSc thesis investigated the drivers of the contemporary distribution of *Z. capensis*, as well as assessing the vulnerability of this seagrass to future environmental conditions. Further, this study made a first attempt at characterising the genetic diversity and population structure of *Z. capensis*. From chapter two, I can conclude that *Z. capensis* is likely to be highly vulnerable to projected future environmental conditions. According to projections, this seagrass is expected to experience both a reduction and a shift in its distribution by 2050, worsening by 2070. Although predictive modelling cannot produce conclusive results, this method nevertheless illustrates the sensitivity of this seagrass to changing climate and human associated conditions. By 2070 *Z. capensis* may possibly only be found in estuaries on the south-east coast due to unfavourable conditions elsewhere, providing a focal point for future conservation, especially in the light of intense human development in this area. These declines in seagrass presence may have negative feedback effects on the estuarine systems for which they are a keystone species (Beckley 1983). The ecosystems services that seagrasses provide, such as increasing nutrient retention, wave attenuation and serving as a nursery and spawning ground for commercially important species, are also likely to be negatively impacted (Green & Short 2003; Orth, Carruthers *et al.* 2006). Changes in conditions that would be most detrimental to the persistence of this seagrass include a shift in estuary mouth state to more permanently closed mouth conditions and a change in rainfall patterns. As expected, due to the broad range of conditions covered by the contemporary distribution of this species, temperature was not found to play a significant role. These findings are in support of the hypotheses for this chapter.

The management of this species could be greatly enhanced if future research were to include the effect of sea-level rise, eutrophication and biotic interactions, such as fouling, in distribution models. Although no such information is available at this time, mouth state could be more accurately represented as a percentage rather than as a classification ‘type’, in order to capture the variability in period for which the estuaries are open to the sea. More information on the dispersal, pollination and ratio of sexual and vegetative reproduction of this species would also be highly

beneficial for ecological studies as well as distribution modelling studies. More intense surveys of *Z. capensis* including all of South Africa's estuaries may also assist researchers and conservation managers to form a more accurate picture of the current and potential future state of this species. This species may be useful as an indicator of estuarine ecosystem functioning, with long-term monitoring efforts to track the occurrence and abundance of this seagrass in estuaries across South Africa.

The results from the molecular research conducted in chapter three do not support the proposed hypotheses. I expected to find a high level of population structure and a low level of gene flow between *Z. capensis* populations, however the matK region exhibited no variation between populations. Unfortunately the microsatellite markers, which were expected to provide better resolution compared to matK, did not amplify *Z. capensis*. I expected that these nine microsatellite markers had a high probability of successfully amplifying *Z. capensis* as they were designed for *Z. noltii* and cross-amplified *Z. japonica*, which are the two species most closely related to *Z. capensis* according to nuclear and chloroplast evidence (Coyer *et al.* 2013). However, I was able to confirm the identity of the Kenyan samples as *Z. capensis*. This is important because its presence in Kenya together with the lack of variation between Kenyan and South African samples, suggests that it also exists on the intervening coastline, where experts are currently uncertain of its distribution and where it can be difficult to distinguish from other morphologically similar seagrasses.

Future research on this species is essential in order to address the questions posed in this thesis. Is *Z. capensis* largely genetically homogenous, or would a higher resolution markers reveal genetic structure among populations? For instance, genetic variation in this seagrass may be recovered using microsatellites as in Muñiz-Salazar *et al.* (2005) and Jahnke *et al.* (2015) or using NGS as in Smith *et al.* (2013).

As the pressures facing coastal systems from climate change and human influence are expected to increase in the next few decades, the management and preservation of coastal species such as

seagrasses in a deteriorating environment become ever more relevant (Duarte 2002). Based on the combined results of chapter two and three, I recommend that future management efforts for the preservation of *Z. capensis* be focused on the south-east coast as this may serve as a fragile but final refuge for the seagrass. Without any apparent genetic variation, based on the matK region alone, populations outside of this area are not likely to be able to withstand or recover from the anticipated environmental changes. Such an extreme bottleneck may have dire consequences for *Z. capensis* persistence in southern Africa.

Despite being globally recognised as a foundation species performing numerous important ecosystem services including binding sediment, increasing nutrient retention, nutrient cycling, carbon sequestration and habitat provision, seagrass habitats are declining around the world (Green & Short 2003). Resilience, which can be considered the ability to recover from disturbance without switching to an alternate stable state (Folke *et al.* 2004), is paramount to the preservation and persistence of seagrass ecosystems. A crucial component of resilience is genetic diversity, which contributes to our understanding of not just population structure but also connectivity and reproductive strategies (Jahnke *et al.* 2015). For example, experimentally increased genotypic diversity of *Z. marina* has led to enhanced biomass and associated biodiversity despite near-lethal water temperatures caused by intense warming in European waters (Reusch *et al.* 2005). This is suggested to aid in ecosystem recovery following disturbances such as climatic extremes (Reusch *et al.* 2005; Procaccini *et al.* 2007). Therefore, in light of the projected decrease and shift in suitable habitat revealed in chapter two, future research on the genetic diversity of *Z. capensis* is essential if management efforts are to mitigate this potential loss.

Supplementary Information

R scripts with annotations for Generalised Additive Models (GAMs)

```
#Load the following libraries
```

```
library(raster)
```

```
library(rgdal)
```

```
library(xlsx)
```

```
library(rJava)
```

```
library(xlsxjars)
```

```
#Data preparation:
```

```
#Add the following raster value sets to the environment from path "~/bio" and name them  
#“winterprec” #etc. These prj.adf files were obtained from WorldClim.org and Last of The Wild
```

```
#Project v2.
```

```
winterprec<-raster("bio/bio_13/prj.adf")
```

```
summerprec<-raster("bio/bio_17/prj.adf")
```

```
maxtemp<-raster("bio/bio_5/prj.adf")
```

```
mintemp<-raster("bio/bio_6/prj.adf")
```

```
HumanImpact<-raster("hii_global_geo_grid/hii_global_geo_grid/hii_v2geo/prj.adf")
```

```
#Create mydata from the Estuary_variables.csv file
```

#If your excel file contains multiple sheets, first save each sheet as a separate .csv file, or import the
#.xlsx file using the “sheet” argument.

```
mydata <- read.csv("Estuary_variables.csv", 1)
```

#Extracting data from raster at point localities one variable at a time, and name them
#“winterprecDat” #etc.

```
winterprecDat <- extract (x=winterprec, y=cbind(mydata$x,mydata$y))
```

```
summerprecDat <- extract (x=summerprec, y=cbind(mydata$x,mydata$y))
```

```
maxtempDat <- extract (x=maxtemp, y=cbind(mydata$x,mydata$y))
```

```
mintempDat <- extract (x=mintemp, y=cbind(mydata$x,mydata$y))
```

```
HIDat <- extract (x=HumanImpact, y=cbind(mydata$x,mydata$y))
```

#Adding the columns (eg. HI with the HumanImpact data) to mydata etc.

```
mydata$HI<- cbind (HIDat)
```

```
mydata$winterprec<- cbind (winterprecDat)
```

```
mydata$summerprec<- cbind (summerprecDat)
```

```
mydata$maxtemp<- cbind (maxtempDat)
```

```
mydata$mintemp<- cbind (mintempDat)
```

#WorldClim temp data needs to be transformed to degrees Celsius by dividing by 10

```
#This is done within mydata creating columns called “maxtemp” and “mintemp”
```

```
mydata$maxtemp<- cbind ((maxtempDat)/10)
```

```
mydata$mintemp<- cbind ((mintempDat)/10)
```

```
#Use columns 6-34 of mydata to create GAMdata (this cuts out the unwanted coordinate info)
```

```
GAMdata <- cbind (mydata[6:34])
```

```
#Running the GAM:
```

```
#Load the library
```

```
library(mgcv)
```

```
#Run the function gam to create file GAM using ZosteraCapensis (presence/absence data) as the  
#dependant variable (indicated by ~), and several predictive independent variables seperated by  
#“+”.Specify family=dependent data type (binomial), and specify the source of the data =filename  
#containing the variables (GAMdata). Further arguments can be added to this.
```

```
#Can also add in an interaction effect between variables if they may be linked eg.
```

```
#+MouthState*Salinity
```

```
GAM<-
```

```
gam(ZosteraCapensis~MouthState+ClimateZone+Condition+Depth+Temperature+Salinity+Dissol  
vedOxygen+Turbidity+FlowChange+Polution+HabitatLoss+Mining+ArtificialBreaching+Fishing+
```

```
BaitCollection+      HI+winterprec+summerprec+maxtemp+mintemp,      family=binomial,
data=GAMdata)
```

```
#See the summarised output
```

```
summary(GAM)
```

```
#Check significance (p-values) of variables
```

```
anova(GAM)
```

```
#Check AIC score
```

```
AIC(GAM)
```

```
#Backwards selection of variables depending on significance (p-values) and AIC score to obtain
```

```
#fitted GAM, called "GAMreduced".
```

```
GAMreduced          <-          gam
(ZosteraCapensis~EstuarineHabitat+Condition+MouthState+Turbidity+winterprec+FlowChange+s
ummerprec+mintemp, family=binomial, data=GAMdata)
```

```
summary (GAMreduced)
```

```
#fitted GAM with smoothing factors non-parametric variables and interactions (*) of collinear variables
```

```
GAMreducedsmooth <- gam  
(ZosteraCapensis~s(EstuarineHabitat,k=10)+Condition*FlowChange+MouthState+s(Turbidity,k=10)+s(winterprec,k=10)+s(summerprec,k=10)+s(mintemp,k=10), family=binomial, data=GAMdata)  
  
summary (GAMreducedsmooth)
```

```
#Check significance (p-values) of variables
```

```
anova(GAMreducedsmooth)
```

```
#Check AIC score
```

```
AIC(GAMreducedsmooth)
```

```
#Preparing for predictive gam
```

```
#Add the new data files with estimated future conditions, containing identical variable names (eg.
```

```
#MouthState, Turbidity etc.).
```

```
futurescenario50_26 <- read.csv("Estuary_variables_future1.csv", 1)
```

```
futurescenario50_85 <- read.csv("Estuary_variables_future1.csv", 1)
```

```
futurescenario70_26 <- read.csv("Estuary_variables_future2.csv", 1)
```

```
futurescenario70_85 <- read.csv("Estuary_variables_future2.csv", 1)
```

```
#Add the following raster value sets to the environment for the future scenarios (obtained from  
#WorldClim.org)
```

```
winterprec_future70_85<-raster("future//bio//2070//85//hd85bi7013.tif")
```

```
summerprec_future70_85<-raster("future//bio//2070//85//hd85bi7017.tif")
```

```
mintemp_future70_85<-raster("future//bio//2070//85//hd85bi706.tif")
```

```
winterprec_future70_26<-raster("future//bio//2070//26//hd26bi7013.tif")
```

```
summerprec_future70_26<-raster("future//bio//2070//26//hd26bi7017.tif")
```

```
mintemp_future70_26<-raster("future//bio//2070//26//hd26bi706.tif")
```

```
winterprec_future50_85<-raster("future//bio//2050//85//hd85bi5013.tif")
```

```
summerprec_future50_85<-raster("future//bio//2050//85//hd85bi5017.tif")
```

```
mintemp_future50_85<-raster("future//bio//2050//85//hd85bi506.tif")
```

```
winterprec_future50_26<-raster("future//bio//2050//26//hd26bi5013.tif")
```

```
summerprec_future50_26<-raster("future//bio//2050//26//hd26bi5017.tif")
```

```
mintemp_future50_26<-raster("future//bio//2050//26//hd26bi506.tif")
```

```
#Extracting data from raster at point localities one by one
```

```
winterprecDat_future70_85      <-      extract      (x=winterprec,  
y=cbind(futurescenario70_85$x,futurescenario70_85$y))  
  
summerprecDat_future70_85      <-      extract      (x=summerprec,  
y=cbind(futurescenario70_85$x,futurescenario70_85$y))  
  
mintempDat_future70_85        <-      extract      (x=mintemp,  
y=cbind(futurescenario70_85$x,futurescenario70_85$y))  
  
winterprecDat_future70_26      <-      extract      (x=winterprec,  
y=cbind(futurescenario70_26$x,futurescenario70_26$y))  
  
summerprecDat_future70_26      <-      extract      (x=summerprec,  
y=cbind(futurescenario70_26$x,futurescenario70_26$y))  
  
mintempDat_future70_26        <-      extract      (x=mintemp,  
y=cbind(futurescenario70_26$x,futurescenario70_26$y))  
  
winterprecDat_future50_85      <-      extract      (x=winterprec,  
y=cbind(futurescenario50_85$x,futurescenario50_85$y))  
  
summerprecDat_future50_85      <-      extract      (x=summerprec,  
y=cbind(futurescenario50_85$x,futurescenario50_85$y))  
  
mintempDat_future50_85        <-      extract      (x=mintemp,  
y=cbind(futurescenario50_85$x,futurescenario50_85$y))
```

```
winterprecDat_future50_26      <-      extract      (x=winterprec,  
y=cbind(futurescenario50_26$x,futurescenario50_26$y))  
  
summerprecDat_future50_26     <-      extract      (x=summerprec,  
y=cbind(futurescenario50_26$x,futurescenario50_26$y))  
  
mintempDat_future50_26       <-      extract      (x=mintemp,  
y=cbind(futurescenario50_26$x,futurescenario50_26$y))
```

#Adding the extracted data to futurescenario70_85 etc.

```
futurescenario70_85$winterprec<- cbind (winterprecDat_future)
```

```
futurescenario70_85$summerprec<- cbind (summerprecDat_future)
```

```
futurescenario70_85$mintemp<- cbind (mintempDat_future)
```

```
futurescenario70_26$winterprec<- cbind (winterprecDat_future70_26)
```

```
futurescenario70_26$summerprec<- cbind (summerprecDat_future70_26)
```

```
futurescenario70_26$mintemp<- cbind (mintempDat_future70_26)
```

```
futurescenario50_85$winterprec<- cbind (winterprecDat_future50_85)
```

```
futurescenario50_85$summerprec<- cbind (summerprecDat_future50_85)
```

```
futurescenario50_85$mintemp<- cbind (mintempDat_future50_85)
```

```
futurescenario50_26$winterprec<- cbind (winterprecDat_future50_26)
```

```
futurescenario50_26$summerprec<- cbind (summerprecDat_future50_26)
```

```
futurescenario50_26$mintemp<- cbind (mintempDat_future50_26)
```

```
#WorldClim temp data needs to be transformed to degrees C by dividing by 10 as before.
```

```
futurescenario70_85$mintemp<- cbind ((mintempDat)/10)
```

```
futurescenario70_26$mintemp<- cbind ((mintempDat)/10)
```

```
futurescenario50_85$mintemp<- cbind ((mintempDat)/10)
```

```
futurescenario50_26$mintemp<- cbind ((mintempDat)/10)
```

```
#Trim columns of futurescenario70_85 to create GAMdatafuture70_85 (this cuts out the unwanted  
#variables and coordinate info)
```

```
GAMdatafuture70_85 <- cbind (futurescenario70_85[6:14])
```

```
GAMdatafuture70_26 <- cbind (futurescenario70_26[6:14])
```

```
GAMdatafuture50_85 <- cbind (futurescenario50_85[6:14])
```

```
GAMdatafuture50_26 <- cbind (futurescenario50_26[6:14])
```

```
#Predictive gam:
```

```
#Create “pred70_85” etc. using function “predict”, dataset, type and se.fit as specified.
```

```
pred70_85<-predict(GAMreduced, GAMdatafuture70_85, type="response", se.fit=F)
```

```
pred70_26<-predict(GAMreduced, GAMdatafuture70_26, type="response", se.fit=F)
```

```
pred50_85<-predict(GAMreduced, GAMdatafuture50_85, type="response", se.fit=F)
```

```
pred50_26<-predict(GAMreduced, GAMdatafuture50_26, type="response", se.fit=F)
```

```
#Predictive gam with smoothing functions:
```

```
pred70_85smooth<-predict(GAMreducedsmooth, GAMdatafuture70_85, type="response", se.fit=F)
```

```
pred70_26smooth<-predict(GAMreducedsmooth, GAMdatafuture70_26, type="response", se.fit=F)
```

```
pred50_85smooth<-predict(GAMreducedsmooth, GAMdatafuture50_85, type="response", se.fit=F)
```

```
pred50_26smooth<-predict(GAMreducedsmooth, GAMdatafuture50_26, type="response", se.fit=F)
```

```
#Create csv files from the outputs of the GAM with smoothing functions, containing presence
```

```
#likelihoods at each location.
```

```
write.csv(pred70_85smooth, file = "pred70_85.csv", na="")
```

```
write.csv(pred70_26smooth, file = "pred70_26.csv", na="")
```

```
write.csv(pred50_85smooth, file = "pred50_85.csv", na="")
```

```
write.csv(pred50_26smooth, file = "pred50_26.csv", na="")
```

Estuary variables**Table S1.** Presence/absence of *Z. capensis* in each surveyed estuary (Prepared by J.B. Adams of NMMU for NBA 2012) and modelled estuary (2050 and 2070).

System	Current	2050	2070
Langebaan	Present	Absent	Absent
Diep	Absent	Absent	Absent
Wildevool	Absent	Absent	Absent
Krom	Present	Absent	Absent
Sand	Absent	Absent	Absent
Olifants	Present	Absent	Absent
Berg	Present	Present	Absent
Palmiet	Absent	Absent	Absent
Uilkraals	Absent	Absent	Absent
Knysna	Present	Present	Present
Blinde	Absent	Absent	Absent
Hartenbos	Present	Absent	Absent
Tsitsikamma	Present	Absent	Absent
Seekoei	Present	Absent	Absent
Kabeljous	Present	Absent	Absent
Van Stadens	Absent	Absent	Absent
Boknes	Absent	Absent	Absent
Kasuka	Absent	Present	Present
Riet	Absent	Present	Present
Wes-Kleinemonde	Present	Present	Present
Oos Kleinemonde	Present	Present	Present
Old Woman's	Absent	Present	Present
Mpekweni	Absent	Present	Present
Mtati	Absent	Present	Present
Mgwalana	Absent	Present	Present
Bira	Absent	Present	Present
Gqutywa	Absent	Present	Present
Mtana	Absent	Present	Present
Ngqinisa	Absent	Present	Present
Kiwane	Absent	Present	Present
Ross' Creek	Absent	Absent	Absent
Ncera	Absent	Absent	Absent
Mlele	Absent	Present	Present

Mcantsi	Absent	Present	Present
Gxulu	Absent	Present	Present
Goda	Absent	Present	Present
Hickmans	Absent	Present	Present
Cintsa	Absent	Present	Present
Cefane	Absent	Present	Present
Kwenxura	Absent	Absent	Absent
Nyara	Absent	Absent	Absent
Haga-Haga	Absent	Absent	Absent
Morgan	Absent	Absent	Absent
Gxara	Absent	Absent	Absent
Ngogwane	Absent	Absent	Absent
Qolora	Absent	Absent	Absent
Cebe	Absent	Absent	Absent
Zalu	Absent	Absent	Absent
Ngqwara	Absent	Absent	Absent
Heuningnes	Present	Absent	Absent
Bree	Present	Absent	Absent
Duiwenhoks	Present	Absent	Absent
Goukou	Present	Absent	Absent
Gourits	Absent	Absent	Absent
Keurbooms	Present	Present	Present
Kromme	Absent	Absent	Absent
Gamtoos	Present	Absent	Absent
Swartkops	Present	Absent	Absent
Sundays	Present	Absent	Absent
Bushmans	Present	Absent	Absent
Kariega	Present	Absent	Absent
Kowie	Present	Absent	Absent
Great Fish	Absent	Absent	Absent
Keiskamma	Present	Absent	Absent
Buffalo	Absent	Absent	Absent
Nahoon	Present	Absent	Absent
Gqunube	Present	Present	Present
Kwelera	Present	Present	Present
Great Kei	Absent	Absent	Absent
Kobonqaba	Absent	Absent	Absent
Ngqusi/Inxaxo	Present	Present	Absent
Qora	Present	Absent	Absent
Shixini	Absent	Absent	Absent

Mbashe	Present	Absent	Absent
Xora	Present	Absent	Absent
Mtata	Absent	Absent	Absent
Mdumbi	Absent	Absent	Absent
Richard's Bay	Present	Present	Absent
Mtentwana	Absent	Absent	Absent
Kandandlovu	Absent	Absent	Absent
Mpenjati	Absent	Absent	Absent
Umhlangankulu	Absent	Absent	Absent
Kaba	Absent	Absent	Absent
Mbizana	Absent	Absent	Absent
Bilanhlolo	Absent	Absent	Absent
Mhlangeni	Absent	Absent	Absent
Mtentweni	Absent	Absent	Absent
Mhlangamkulu	Absent	Absent	Absent
Intshambili	Absent	Absent	Absent
Fafa	Absent	Absent	Absent
Sezela	Absent	Absent	Absent
Mpambanyoni	Absent	Absent	Absent
Mahlongwa	Absent	Absent	Absent
Little Manzimtoti	Absent	Absent	Absent
Manzimtoti	Absent	Absent	Absent
Mhlanga	Absent	Absent	Absent
Mdloti	Absent	Absent	Absent
Mdlotane	Absent	Absent	Absent
Zinkwasi	Absent	Absent	Absent
Mngazana	Present	Absent	Absent
Mngazi	Absent	Absent	Absent
Mntafufu	Absent	Absent	Absent
Msikaba	Present	Absent	Absent
Mtentu	Present	Absent	Absent
Mzamba	Absent	Absent	Absent
Mzinkulu	Absent	Absent	Absent
Mkomazi	Absent	Absent	Absent
Matigulu/Nyoni	Present	Absent	Absent
Mlalazi	Present	Present	Absent

Table S2. Current estuary conditions used for fitting GAMs.

System	y	x	Zostera Capensis	Mouth state	Degree of opening	Climate zone	Condition	Depth	Temperature	Salinity
Langebaan	-33.0836	18.03663	Present	0.75	Estuarine embayment	Cool temperate	Good			
Diep	-33.8903	18.48345	Absent	0.5	Temporarily open/closed	Cool temperate	Poor	0.77	16.86	21.53
Wildevool	-34.1278	18.34528	Absent	0.5	Temporarily open/closed	Cool temperate	Poor	0.45	19.75	2.28
Krom	-34.2311	18.37972	Present	0.5	Temporarily open/closed	Cool temperate	Excellent	0.53	19.25	9.08
Sand	-34.1058	18.47371	Absent	0.5	Temporarily open/closed	Cool temperate	Fair	1.23	20.78	11.32
Olifants	-31.689	18.18861	Present	1	Permanently Open	Cool temperate	Fair	1.48	13.95	17.85
Berg	-32.7711	18.15202	Present	1	Permanently Open	Cool temperate	Fair	2.73	16.42	20
Palmiet	-34.3404	18.99583	Absent	1	Permanently Open	Cool temperate	Good	1.83	14.97	17.77
Uilkraals	-34.6064	19.40917	Absent	1	Permanently Open	Cool temperate	Good	0.83	17.33	15.43
Knysna	-34.0792	23.05628	Present	0.75	Estuarine Bay	Warm temperate	Good	2.45	19.25	28.55
Blinde	-34.2073	22.01306	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.77	20.85	12.33
Hartenbos	-34.1186	22.12417	Present	0.5	Temporarily open/closed	Warm temperate	Fair	1.35	20.19	17.64
Tsitsikamma	-34.135	24.43833	Present	0.5	Temporarily open/closed	Warm temperate	Fair	0.65	17.73	0.63
Seekoei	-34.0864	24.90833	Present	0.5	Temporarily open/closed	Warm temperate	Fair	1.08	19.26	6.13
Kabeljous	-34.0047	24.93694	Present	0.5	Temporarily open/closed	Warm temperate	Good	1	17.65	16.45
Van Stadens	-33.9694	25.22139	Absent	0.5	Temporarily open/closed	Warm temperate	Fair	2.2	20.69	14.55
Boknes	-33.7267	26.58694	Absent	0.5	Temporarily open/closed	Warm temperate	Fair	1.8	21.12	16.67
Kasuka	-33.6547	26.73556	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.53	26.11	24.84
Riet	-33.5611	27.01472	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.67	23.98	20.02
Wes-Kleinmond	-33.5411	27.0475	Present	0.5	Temporarily open/closed	Warm temperate	Good	1.92	23.2	18.58
Oos Kleinmond	-33.5392	27.04861	Present	0.5	Temporarily open/closed	Warm temperate	Good	1.23	23	15.13
Old Woman's	-33.4828	27.14806	Absent	0.5	Temporarily open/closed	Warm temperate	Good	2.3	17.42	25.87
Mpekweni	-33.4381	27.23083	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.74	18.14	19.57

Mtati	-33.4222	27.26	Absent	0.5	Temporarily open/closed	Warm temperate	Good	2.2	17.52	19.47
Mgwalana	-33.4131	27.27444	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.04	19.42	27.99
Bira	-33.3825	27.32611	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.7	19.99	29.76
Gqutywa	-33.3625	27.35861	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.98	20.6	49.34
Mtana	-33.3183	27.43278	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.9	19.28	29.4
Ngqinisa	-33.2533	27.52972	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.37	17.2	31.53
Kiwane	-33.2492	27.54389	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.95	18.45	20.16
Ross' Creek	-33.1764	27.65782	Absent	0.5	Temporarily open/closed	Warm temperate	Fair	0.57	18.94	6.58
Ncera	-33.17	27.66614	Absent	0.5	Temporarily open/closed	Warm temperate	Fair	1.22	20.46	33.66
Mlele	-33.1597	27.68083	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.07	19.77	15.3
Mcantsi	-33.1456	27.70306	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.03	23.03	14.1
Gxulu	-33.1178	27.73194	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.37	22.06	29.64
Goda	-33.1	27.775	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.97	21	32.55
Hickmans	-33.0697	27.83944	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.6	21.98	18.83
Cintsa	-32.8308	28.11639	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.38	23.78	31.13
Cefane	-32.8083	28.13639	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.93	23.5	28.9
Kwexura	-32.7992	28.15056	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.8	19.7	29.1
Nyara	-32.7839	28.18222	Absent	0.5	Temporarily open/closed	Warm temperate	Excellent	0.55	19.03	21.77
Haga-Haga	-32.7617	28.25417	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.35	21.33	25.8
Morgan	-32.7075	28.34556	Absent	0.5	Temporarily open/closed	Warm temperate	Good	0.57	21.98	8.63
Gxara	-32.6667	28.38333	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.3	21.37	18.97
Ngogwane	-32.6489	28.42167	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.47	22.62	20.58
Qolora	-32.6303	28.43306	Absent	0.5	Temporarily open/closed	Warm temperate	Good	3.4	21.28	16.52
Cebe	-32.5228	28.585	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.2	24.22	25.67
Zalu	-32.5025	28.60333	Absent	0.5	Temporarily open/closed	Warm temperate	Good	1.1	20.58	20.62
Ngqwara	-32.4922	28.61417	Absent	0.5	Temporarily open/closed	Warm temperate	Excellent	0.9	21.9	25.58

Heuningnes	-34.7139	20.12028	Present	1	Permanently Open	Warm temperate	Fair	1.63	14.85	18.17
Bree	-34.409	20.84806	Present	1	Permanently Open	Warm temperate	Fair	2.52	19.06	10.71
Duiwenhoks	-34.365	20.9991	Present	1	Permanently Open	Warm temperate	Good	2.68	15.88	31.63
Goukou	-34.3772	21.42278	Present	1	Permanently Open	Warm temperate	Fair	1.48	17.26	23.33
Gourits	-34.3411	21.88778	Absent	1	Permanently Open	Warm temperate	Fair	2.1	17.41	33.64
Keurbooms	-34.0381	23.38667	Present	1	Permanently Open	Warm temperate	Good	1.63	21.36	27.19
Kromme	-34.1408	24.84333	Absent	1	Permanently Open	Warm temperate	Fair	2.39	18.84	30.23
Gamtoos	-33.9694	25.03194	Present	1	Permanently Open	Warm temperate	Fair	1.59	19.16	19.8
Swartkops	-33.865	25.63333	Present	1	Permanently Open	Warm temperate	Fair	2.22	18.58	30.21
Sundays	-33.7219	25.84917	Present	1	Permanently Open	Warm temperate	Fair	2.41	20.45	18.11
Bushmans	-33.6912	26.66333	Present	1	Permanently Open	Warm temperate	Fair	2.44	21.05	32.28
Kariega	-33.6819	26.6875	Present	1	Permanently Open	Warm temperate	Fair	2.39	22.95	31.59
Kowie	-33.6031	26.90278	Present	1	Permanently Open	Warm temperate	Fair	2.75	21.48	30.04
Great Fish	-33.4936	27.13722	Absent	1	Permanently Open	Warm temperate	Good	1.92	21.55	12.6
Keiskamma	-33.2822	27.4925	Present	1	Permanently Open	Warm temperate	Fair	2.01	18.41	22.09
Buffalo	-33.0222	27.91416	Absent	1	Permanently Open	Warm temperate	Fair	3.43	18.17	31.17
Nahoon	-32.9861	27.95222	Present	1	Permanently Open	Warm temperate	Fair	2.32	19.41	32.61
Gqunube	-32.9331	28.03306	Present	1	Permanently Open	Warm temperate	Good	1.68	19.98	32.81
Kwelera	-32.9072	28.07639	Present	1	Permanently Open	Warm temperate	Good	1.56	21.01	32.12
Great Kei	-32.6786	28.3875	Absent	1	Permanently Open	Warm temperate	Good	1.78	22.17	4.43
Kobonqaba	-32.6069	28.49028	Absent	1	Permanently Open	Warm temperate	Good	1.53	20.38	28.36
Ngqusi/Inxaxo	-32.5847	28.52556	Present	1	Permanently Open	Warm temperate	Good	2.14	18.06	28.03
Qora	-32.4472	28.6725	Present	1	Permanently Open	Warm temperate	Excellent	1.03	17.02	23.38
Shixini	-32.4028	28.7275	Absent	1	Permanently Open	Warm temperate	Good	1.03	18.84	30.86
Mbashe	-32.2486	28.90083	Present	1	Permanently Open	Warm temperate	Good	2.75	20.24	14.64
Xora	-32.1576	28.99667	Present	1	Permanently Open	Warm temperate	Good	2.1	22.43	27.84

Mtata	-31.9522	29.1825	Absent	1	Permanently Open	Warm temperate	Fair	3.08	21.4	12.59
Mdumbi	-31.9306	29.21611	Absent	1	Permanently Open	Warm temperate	Good	2.08	21.54	27.76
Richard's Bay	-28.8111	32.08806	Present	0.75	Estuarine Bay	Subtropical	Poor	2	25.9	34.5
Mtentwana	-31.0892	30.18556	Absent	0.5	Temporarily open/closed	Subtropical	Good	1.8	28.05	14.2
Kandandlovu	-30.9967	30.26861	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1	24.48	11.38
Mpenjati	-30.9725	30.28389	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.77	22.22	10.1
Umhlangankulu	-30.9444	30.30278	Absent	0.5	Temporarily open/closed	Subtropical	Fair	2	23.37	4.05
Kaba	-30.9353	30.30833	Absent	0.5	Temporarily open/closed	Subtropical	Fair	0.85	26.73	12.08
Mbizana	-30.9076	30.33389	Absent	0.5	Temporarily open/closed	Subtropical	Fair	2.13	24.7	3.49
Bilanhlolo	-30.8892	30.34944	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.37	26.37	10.53
Mhlangeni	-30.8183	30.40611	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.07	27.03	13.6
Mtentweni	-30.7092	30.48167	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.5	25.57	10.02
Mhlangamkulu	-30.6881	30.49861	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.68	22.9	0.5
Intshambili	-30.6367	30.53694	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.15	25.4	3.17
Fafa	-30.4547	30.65361	Absent	0.5	Temporarily open/closed	Subtropical	Fair	0.73	28.16	2.34
Sezela	-30.4139	30.6775	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.8	25.7	4.27
Mpambanyoni	-30.2789	30.76083	Absent	0.5	Temporarily open/closed	Subtropical	Poor	0.68	24.14	4.46
Mahlongwa	-30.2683	30.765	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.35	26.77	2.3
Little Manzimtoti	-30.0772	30.8725	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.23	20.83	1.45
Manzimtoti	-30.0594	30.88333	Absent	0.5	Temporarily open/closed	Subtropical	Poor	1.05	21.83	1.7
Mhlanga	-29.7039	31.10083	Absent	0.5	Temporarily open/closed	Subtropical	Fair	0.45	20.67	9.57
Mdloti	-29.6519	31.12861	Absent	0.5	Temporarily open/closed	Subtropical	Fair	2.33	21.83	0.1
Mdlotane	-29.3519	31.36533	Absent	0.5	Temporarily open/closed	Subtropical	Fair	2.93	28.07	0.23
Zinkwasi	-29.2792	31.44073	Absent	0.5	Temporarily open/closed	Subtropical	Fair	1.3	27.73	12.25
Mngazana	-31.695	29.42333	Present	1	Permanently Open	Subtropical	Good	2.33	23.48	28.48
Mngazi	-31.6738	29.45725	Absent	1	Permanently Open	Subtropical	Good	2.08	24.1	19.25

Mntafufu	-31.5636	29.63203	Absent	1	Permanently Open	Subtropical	Good	3.18	24.76	20.3
Msikaba	-31.3203	29.96556	Present	1	Permanently Open	Subtropical	Excellent	4.08	25.84	17
Mtentu	-31.2481	30.04694	Present	1	Permanently Open	Subtropical	Excellent	3.85	25.6	18.9
Mzamba	-31.1083	30.17528	Absent	1	Permanently Open	Subtropical	Good	2.3	26.75	23.93
Mzimkulu	-30.7397	30.45889	Absent	1	Permanently Open	Subtropical	Poor	1.06	23.53	14.08
Mkomazi	-30.2008	30.80306	Absent	1	Permanently Open	Subtropical	Poor	0.85	24.43	3.44
Matigulu/Nyoni	-29.11	31.61917	Present	1	Permanently Open	Subtropical	Fair	1.12	23.54	3
Mlalazi	-28.9407	31.81611	Present	1	Permanently Open	Subtropical	Good	1.72	26.98	9.2

Table S2. Current estuary conditions used for fitting GAMs (continued).

System	Turbidity	Flow change	Pollution	Habitat loss	Mining	Artificial breaching	Fishing	Bait collection	Estuarine habitat	Dissolved oxygen
Langebaan									834.71	
Diep	14.25	2	h	h	n	y	l	y		5.69
Wildevool	6.67	2	h	m	n	n	l	y	230.87	8.68
Krom	6	1	l	l	n	n	n	n		4.92
Sand	20	2	h	m	n	y	m	y	155.48	7.9
Olifants	30.8	2	m	m	n	n	vh	y	1353.66	8.73
Berg	13.6	2	h	m	n	n	vh	y	6799	6.76
Palmiet	4	2	l	m	n	n	l	y	33	8.43
Uilkraals	5.33	2	m	m	n	n	m	y	104.7	8.49
Knysna	5.075	1	m	l	n	n	h	y	1865.95	7.2
Blinde	7.67	1	m	l	n	n	l	y	0.5	5.46
Hartenbos	3	2	h	m	n	y	l	y	40.59	7.05
Tsitsikamma	34.6	2	m	l	n	n	l	y	6.5	8.45
Seekoei	9.4	2	m	h	n	n	l	y	132.22	10.59
Kabeljous	2.5	1	m	m	n	n	l	y	117.94	5.45
Van Stadens	0	1	l	l	n	n	l	y	24.2	6.07
Boknes	1.8	1	l	l	n	n	l	y	20	7.93
Kasuka	0.8	1	l	l	n	n	l	y	20.7	4.41
Riet	1.2	1	l	l	n	n	l	y	73.06	4.51
Wes-Kleinmond	1.1	1	m	l	n	n	l	y	47.8	4.92
Oos Kleinmond	3.7	1	m	l	n	n	l	y	52.12	6.37
Old Woman's	2	1	m	m	n	n	l	y	25.12	6.68

Mpekweni	8.4	1	m	l	n	n	m	y	141.41	8.65
Mtati	8.4	1	l	l	n	n	l	y	286.35	7.56
Mgwalana	28.2	1	l	l	n	n	m	y	226.72	9.5
Bira	14.14	1	l	l	n	n	l	y	163.54	8.14
Gqutywa	15.75	1	l	l	n	n	l	y	51.64	5.82
Mtana	9.5	1	m	l	n	n	l	y	15.69	7.6
Ngqinisa	15	1	m	m	n	n	l	y	12.67	8.81
Kiwane	6.25	1	l	l	n	n	l	y	18.8	11.01
Ross' Creek	20	1	m	l	n	n	l	y	1.3	6.62
Ncera	2.4	1	m	l	n	n	l	y	28.4	7.7
Mlele	16	1	m	l	n	n	l	y	3.6	7.51
Mcantsi	6.67	1	m	m	n	n	l	y	9	6.96
Gxulu	3.17	1	l	l	n	n	l	y	48.5	6.41
Goda	5.33	1	l	l	n	n	l	y	17.2	6.09
Hickmans	3.67	1	m	l	n	n	l	y	4.3	6.42
Cintsa	3.5	1	m	l	n	n	l	y	29.3	5.53
Cefane	6	1	l	l	n	n	l	y	82.7	5
Kwexura	35.67	1	l	l	n	n	l	y	29.1	6.98
Nyara	94.5	1	l	l	n	n	l	y	17.1	3.5
Haga-Haga	23	1	l	l	n	n	l	y	3.4	8.01
Morgan	100.33	1	m	l	n	n	l	y	24	8.37
Gxara	16.5	1	l	l	n	n	l	y	23.9	5.71
Ngogwane	0.33	1	l	l	n	n	l	y	9.12	5.64
Qolora	4.33	1	l	l	n	n	l	y	22.9	4.52
Cebe	11.33	1	l	l	n	n	l	y	16.53	6.74
Zalu	4.33	1	l	l	n	n	l	y	12.36	6.25

Ngqwara	1	1	l	l	n	n	l	y	19.36	6.41
Heuningnes	25	2	m	m	n	y	m	y	518.05	7.49
Bree"	7.4	2	l	l	n	n	h	y	1564.6	7.36
Duiwenhoks	5.75	2	l	l	n	n	h	y	203.07	6.58
Goukou	2.75	2	m	m	n	n	h	y	154.76	6.17
Gourits	10.75	2	m	m	n	n	h	y	112.58	6.28
Keurbooms	0.63	1	l	l	n	n	l	y	674.74	5.23
Kromme	1.3	3	h	m	n	n	h	y	408.22	6.53
Gamtoos	11.4	2	m	m	n	n	h	y	501.25	8.08
Swartkops	1.5	1	h	h	n	n	h	y	499	6.25
Sundays	23.9	2	h	m	n	n	h	y	485.7	7.61
Bushmans	14.5	1	m	m	n	n	l	y	340.9	6.91
Kariega	4.7	2	l	m	n	n	l	y	84.1	6.59
Kowie	6.9	2	m	m	n	n	l	y	118.63	7.02
Great Fish	73	2	m	m	n	n	h	y	365.68	7.88
Keiskamma	43.38	1	m	m	n	n	h	y	743.86	6.77
Buffalo	10	3	h	l	n	n	l	y	98	7.85
Nahoon	5.8	2	h	m	n	n	h	y	57.7	8.45
Gqunube	15.75	1	m	l	n	n	m	y	53.4	6.78
Kwelera	15.8	1	l	l	n	n	h	y	50.1	6.82
Great Kei	1300	1	m	l	n	n	h	y	222.4	7.1
Kobonqaba	5.5	1	l	l	n	n	l	y	26.4	7.05
Ngqusi/Inxaxo	7.14	1	l	m	n	n	l	y	159.48	6.55
Qora	83.33	1	l	l	n	n	l	y	89.63	7.94
Shixini	14.33	1	l	l	n	n	l	y	22.1	7.83
Mbashe	163	1	m	l	n	n	h	y	131.95	7.36

Xora	17.67	1	l	l	n	n	l	y	150.85	6.7
Mtata	100.2	2	h	m	n	n	h	y	168.79	7.62
Mdumbi	8	1	l	m	n	n	h	y	76.07	7.32
Richard's Bay	7.56	1	m	h	n	n	h	y	2044	37.65
Mtentwana	11	1	m	h	n	n	n	y	11.43	7.43
Kandandlovu	8	1	m	m	n	n	l	y	5.3	4.3
Mpenjati	8	1	m	m	y	y	l	y	33.1	5.62
Umhlangankulu	9	1	m	m	n	y	l	y	15.8	5.94
Kaba	9.5	1	m	m	n	n	l	y	14.65	7.36
Mbizana	5	1	m	m	y		l	y	28.4	6.15
Bilanhlole	5	1	m	m	n	y	l	y	16.6	6.05
Mhlangeni	13	1	m	m	n	y	l	y	15.6	6.94
Mtentweni	4.33	1	m	m	n	n	l	y	18.48	5.39
Mhlangankulu	9.5	1	m	m	n	n	l	y		4.7
Intshambili	9.33	1	l	m	n	n	l	y	10.45	5.02
Fafa	8.67	1	m	m	y	y	l	y	51	6.83
Sezela	18.67	1	h	m	n	y			28	3.11
Mpambanyoni	18.33	1	m	m	y		l	y	12.57	6.91
Mahlongwa	10	1	m	m	y	y	m	y	13.9	6.1
Little Manzimtoti	26.33	2	h	h	n	y	n	n	10	2.31
Manzimtoti	49.33	1	h	h	y	y	n	n	21.17	5.24
Mhlanga	36.67	2	h	m	n	y	l	y	100.1	4.22
Mdloti	8.33	1	h	h	y	y	l	y	58.1	2.93
Mdlotane	10.67	1	l	m	n	n	l	y	25.42	1.74
Zinkwasi	58.25	1	m	h	y	y	l	y	71.16	4.4
Mngazana	12.5	1	m	m	n	n	h	y	224.85	5.9

Mngazi	86.2	1	m	l	n	n	l	y	17.1	5.9
Mntafufu	19.5	1	l	m	n	n	h	y	24.07	6.86
Msikaba	10.25	1	m	n	n	n	h	y	15.13	7.33
Mtentu	4.5	1	l	l	n	n	h	y	52.93	7.43
Mzamba	13.33	1	m	l	n	n	h	y	70.94	7.01
Mzimkulu	12	2	m	m	y	y	h	y	117.9	7.06
Mkomazi	591.25	1	m	m	y	y	h	y	74.7	6.5
Matigulu/Nyoni	55.71	1	l	l	n	n	h	y	127	5.21
Mlalazi	19.6	1	m	m	n	y	h	y	238.771	6.66

Table S3. Future scenarios (estuary variables) established for 2050 and 2070 for use in the refined GAM.

			Future scenario 2050					Future scenario 2070				
System	latitude	longitude	Mouth State	Condition	Turbidity	Estuarine Habitat	Flow Change	Mouth State	Condition	Turbidity	Estuarine Habitat	Flow Change
Langebaan	33°5'16"S	18°01'42"E	0.5	Fair		626.0325		0.5	Fair		208.6775	
Diep	33°53'25"S	18°29'00"E	0.25	Poor	17.8125		3	0.25	Poor	24.9375		3
Wildevool	34°07'40"S	18°20'43"E	0.25	Poor	8.3375	173.1525	3	0.25	Poor	11.6725	57.7175	3
Krom	34°13'52"S	18°22'47"E	0.25	Good	7.5		2	0.25	Good	10.5		2
Sand	34°06'21"S	18°28'36"E	0.25	Poor	25	116.61	3	0.25	Poor	35	38.87	3
Olifants	31°42'03"S	18°11'19"E	0.5	Poor	38.5	1015.245	3	0.5	Poor	53.9	338.415	3
Berg	32°46'10"S	18°08'44"E	0.5	Poor	17	5099.25	3	0.5	Poor	23.8	1699.75	3
Palmiet	34°20'37"S	18°59'45"E	0.75	Fair	5	24.75	3	0.75	Fair	7	8.25	3
Uilkraals	34°36'23"S	19°24'33"E	0.75	Fair	6.6625	78.525	3	0.75	Fair	9.3275	26.175	3
Knysna	34°04'38"S	23°03'33"E	0.75	Fair	6.34375	1399.463	2	0.75	Fair	8.88125	466.4875	2
Blinde	34°12'34"S	22°00'47"E	0.25	Fair	9.5875	0.375	2	0.25	Fair	13.4225	0.125	2
Hartenbos	34°07'07"S	22°07'27"E	0.25	Poor	3.75	30.4425	3	0.25	Poor	5.25	10.1475	3
Tsitsikamma	34°08'06"S	24°26'18"E	0.25	Poor	43.25	4.875	3	0.25	Poor	60.55	1.625	3

Seekoei	34°05'11"S	24°54'30"E	0.25	Poor	11.75	99.165	3	0.25	Poor	16.45	33.055	3
Kabeljous	34°00'17"S	24°56'13"E	0.25	Fair	3.125	88.455	2	0.25	Fair	4.375	29.485	2
Van Stadens	33°58'10"S	25°13'17"E	0.25	Poor	0	18.15	2	0.25	Poor	0	6.05	2
Boknes	33°43'36"S	26°35'13"E	1	Poor	2.25	15	2	1	Poor	3.15	5	2
Kasuka	33°39'17"S	26°44'08"E	1	Fair	1	15.525	2	1	Fair	1.4	5.175	2
Riet	33°33'40"S	27°00'53"E	1	Fair	1.5	54.795	2	1	Fair	2.1	18.265	2
Wes-Kleinmond	33°32'28"S	27°02'51"E	1	Fair	1.375	35.85	2	1	Fair	1.925	11.95	2
Oos Kleinmond	33°32'21"S	27°02'55"E	1	Fair	4.625	39.09	2	1	Fair	6.475	13.03	2
Old Woman's	33°28'58"S	27°08'53"E	1	Fair	2.5	18.84	2	1	Fair	3.5	6.28	2
Mpekweni	33°26'17"S	27°13'51"E	1	Fair	10.5	106.0575	2	1	Fair	14.7	35.3525	2
Mtati	33°25'20"S	27°15'36"E	1	Fair	10.5	214.7625	2	1	Fair	14.7	71.5875	2
Mgwalana	33°24'47"S	27°16'28"E	1	Fair	35.25	170.04	2	1	Fair	49.35	56.68	2
Bira	33°22'57"S	27°19'34"E	1	Fair	17.675	122.655	2	1	Fair	24.745	40.885	2
Gqutywa	33°21'45"S	27°21'31"E	1	Fair	19.6875	38.73	2	1	Fair	27.5625	12.91	2
Mtana	33°19'06"S	27°25'58"E	1	Fair	11.875	11.7675	2	1	Fair	16.625	3.9225	2
Ngqinisa	33°15'12"S	27°31'47"E	1	Fair	18.75	9.5025	2	1	Fair	26.25	3.1675	2

Kiwane	33°14'57"S	27°32'38"E	1	Fair	7.8125	14.1	2	1	Fair	10.9375	4.7	2
Ross' Creek	33°10'35"S	27°39'31"E	1	Poor	25	0.975	2	1	Poor	35	0.325	2
Ncera	33°10'12"S	27°40'11"E	1	Poor	3	21.3	2	1	Poor	4.2	7.1	2
Mlele	33°09'35"S	27°40'51"E	1	Fair	20	2.7	2	1	Fair	28	0.9	2
Mcantsi	33°08'44"S	27°42'11"E	1	Fair	8.3375	6.75	2	1	Fair	11.6725	2.25	2
Gxulu	33°07'04"S	27°43'55"E	1	Fair	3.9625	36.375	2	1	Fair	5.5475	12.125	2
Goda	33°06'00"S	27°46'30"E	1	Fair	6.6625	12.9	2	1	Fair	9.3275	4.3	2
Hickmans	33°04'11"S	27°50'22"E	1	Fair	4.5875	3.225	2	1	Fair	6.4225	1.075	2
Cintsa	32°49'51"S	28°06'59"E	1	Fair	4.375	21.975	2	1	Fair	6.125	7.325	2
Cefane	32°48'30"S	28°08'11"E	1	Fair	7.5	62.025	2	1	Fair	10.5	20.675	2
Kwenxura	32°47'57"S	28°09'02"E	1	Fair	44.5875	21.825	2	1	Fair	62.4225	7.275	2
Nyara	32°47'02"S	28°10'56"E	1	Good	118.125	12.825	2	1	Good	165.375	4.275	2
Haga-Haga	32°45'42"S	28°15'15"E	1	Fair	28.75	2.55	2	1	Fair	40.25	0.85	2
Morgan	32°42'27"S	28°20'44"E	1	Fair	125.4125	18	2	1	Fair	175.5775	6	2
Gxara	32°40'00"S	28°23'00"E	1	Fair	20.625	17.925	2	1	Fair	28.875	5.975	2
Ngogwane	32°38'56"S	28°25'18"E	1	Fair	0.4125	6.84	2	1	Fair	0.5775	2.28	2
Qolora	32°37'49"S	28°25'59"E	1	Fair	5.4125	17.175	2	1	Fair	7.5775	5.725	2

Cebe	32°31'22"S	28°35'06"E	1	Fair	14.1625	12.3975	2	1	Fair	19.8275	4.1325	2
Zalu	32°30'09"S	28°36'12"E	1	Fair	5.4125	9.27	2	1	Fair	7.5775	3.09	2
Ngqwara	32°29'32"S	28°36'51"E	1	Good	1.25	14.52	2	1	Good	1.75	4.84	2
Heuningnes	34°42'50"S	20°07'13"E	0.75	Poor	31.25	388.5375	3	0.75	Poor	43.75	129.5125	3
Bree	34°24'26"S	20°50'53"E	0.75	Poor	9.25	1173.45	3	0.75	Poor	12.95	391.15	3
Duiwenhoks	34°21'54"S	21°00'5.0"S	0.75	Fair	7.1875	152.3025	3	0.75	Fair	10.0625	50.7675	3
Goukou	34°22'38"S	21°25'22"E	0.75	Poor	3.4375	116.07	3	0.75	Poor	4.8125	38.69	3
Gourits	34°20'37"S	21°53'16"E	0.75	Poor	13.4375	84.435	3	0.75	Poor	18.8125	28.145	3
Keurbooms	34°02'17"S	23°23'12"E	0.75	Fair	0.7875	506.055	2	0.75	Fair	1.1025	168.685	2
Kromme	34°08'27"S	24°50'36"E	0.75	Poor	1.625	306.165	3	0.75	Poor	2.275	102.055	3
Gamtoos	33°58'10"S	25°01'55"E	0.75	Poor	14.25	375.9375	3	0.75	Poor	19.95	125.3125	3
Swartkops	33°51'54"S	25°38'00"E	0.75	Poor	1.875	374.25	2	0.75	Poor	2.625	124.75	2
Sundays	33°43'19"S	25°50'57"E	0.75	Poor	29.875	364.275	3	0.75	Poor	41.825	121.425	3
Bushmans	33°41'41"S	26°39'48"E	1	Poor	18.125	255.675	2	1	Poor	25.375	85.225	2
Kariega	33°40'55"S	26°41'15"E	1	Poor	5.875	63.075	3	1	Poor	8.225	21.025	3
Kowie	33°36'11"S	26°54'10"E	1	Poor	8.625	88.9725	3	1	Poor	12.075	29.6575	3
Great Fish	33°39'37"S	27°08'10"E	1	Fair	91.25	274.26	3	1	Fair	127.75	91.42	3

Keiskamma	33°16'56"S	27°29'33"E	1	Poor	54.225	557.895	2	1	Poor	75.915	185.965	2
Buffalo	33°01'42"S	27°54'57"E	1	Poor	12.5	73.5	3	1	Poor	17.5	24.5	3
Nahoon	32°59'10"S	27°57'08"E	1	Poor	7.25	43.275	3	1	Poor	10.15	14.425	3
Gqunube	32°55'59"S	28°01'59"E	1	Fair	19.6875	40.05	2	1	Fair	27.5625	13.35	2
Kwelera	32°54'26"S	28°04'35"E	1	Fair	19.75	37.575	2	1	Fair	27.65	12.525	2
Great Kei	32°40'43"S	28°23'15"E	1	Fair	1625	166.8	2	1	Fair	2275	55.6	2
Kobonqaba	32°36'25"S	28°29'25"E	1	Fair	6.875	19.8	2	1	Fair	9.625	6.6	2
Ngqusi/Inxaxo	32°35'05"S	28°31'32"E	1	Fair	8.925	119.61	2	1	Fair	12.495	39.87	2
Qora	32°26'50"S	28°40'21"E	1	Good	104.1625	67.2225	2	1	Good	145.8275	22.4075	2
Shixini	32°24'10"S	28°43'39"E	1	Fair	17.9125	16.575	2	1	Fair	25.0775	5.525	2
Mbashe	32°14'55"S	28°54'03"E	1	Fair	203.75	98.9625	2	1	Fair	285.25	32.9875	2
Xora	32°09'30"S	28°59'48"E	1	Fair	22.0875	113.1375	2	1	Fair	30.9225	37.7125	2
Mtata	31°57'08"S	29°10'57"E	1	Poor	125.25	126.5925	3	1	Poor	175.35	42.1975	3
Mdumbi	31°55'50"S	29°12'58"E	1	Fair	10	57.0525	2	1	Fair	14	19.0175	2
Richard's Bay	28°48'40"S	32°05'17"E	1	Poor	9.45	1533	2	1	Poor	13.23	511	2
Mtentwana	31°05'21"S	30°11'08"E	1	Fair	13.75	8.5725	2	1	Fair	19.25	2.8575	2

Kandandlovu	30°59'48"S	30°16'07"E	1	Poor	10	3.975	2	1	Poor	14	1.325	2
Mpenjati	30°58'21"S	30°17'02"E	1	Poor	10	24.825	2	1	Poor	14	8.275	2
Umhlangankulu	30°56'40"S	30°18'10"E	1	Poor	11.25	11.85	2	1	Poor	15.75	3.95	2
Kaba	30°56'07"S	30°18'30"E	1	Poor	11.875	10.9875	2	1	Poor	16.625	3.6625	2
Mbizana	30°54'34"S	30°20'02"E	1	Poor	6.25	21.3	2	1	Poor	8.75	7.1	2
Bilanhlolo	30°53'21"S	30°20'58"E	1	Poor	6.25	12.45	2	1	Poor	8.75	4.15	2
Mhlangeni	30°49'06"S	30°24'22"E	1	Poor	16.25	11.7	2	1	Poor	22.75	3.9	2
Mtentweni	30°42'33"S	30°28'54"E	1	Poor	5.4125	13.86	2	1	Poor	7.5775	4.62	2
Mhlangankulu	30°41'17"S	30°29'55"E	1	Poor	11.875		2	1	Poor	16.625		2
Intshambili	30°38'12"S	30°32'13"E	1	Poor	11.6625	7.8375	2	1	Poor	16.3275	2.6125	2
Fafa	30°27'17"S	30°39'13"E	1	Poor	10.8375	38.25	2	1	Poor	15.1725	12.75	2
Sezela	30°24'50"S	30°40'39"E	1	Poor	23.3375	21	2	1	Poor	32.6725	7	2
Mpambanyoni	30°16'44"S	30°45'39"E	1	Poor	22.9125	9.4275	2	1	Poor	32.0775	3.1425	2
Mahlongwa	30°16'06"S	30°45'54"E	1	Poor	12.5	10.425	2	1	Poor	17.5	3.475	2
Little Manzimtoti	30°04'38"S	30°52'21"E	1	Poor	32.9125	7.5	3	1	Poor	46.0775	2.5	3

Manzimtoti	30°03'34"S	30°53'00"E	1	Poor	61.6625	15.8775	2	1	Poor	86.3275	5.2925	2
Mhlanga	29°42'14"S	31°06'03"E	1	Poor	45.8375	75.075	3	1	Poor	64.1725	25.025	3
Mdloti	29°39'07"S	31°07'43"E	1	Poor	10.4125	43.575	2	1	Poor	14.5775	14.525	2
Mdlotane	29°21'07"S	31°22'26"E	1	Poor	13.3375	19.065	2	1	Poor	18.6725	6.355	2
Zinkwasi	29°16'45"S	31°26'35"E	1	Poor	72.8125	53.37	2	1	Poor	101.9375	17.79	2
Mngazana	31°41'29"S	29°25'24"E	1	Fair	15.625	168.6375	2	1	Fair	21.875	56.2125	2
Mngazi	31°40'32"S	29°27'40"E	1	Fair	107.75	12.825	2	1	Fair	150.85	4.275	2
Mntafufu	31°33'49"S	29°38'10"E	1	Fair	24.375	18.0525	2	1	Fair	34.125	6.0175	2
Msikaba	31°19'13"S	29°57'56"E	1	Good	12.8125	11.3475	2	1	Good	17.9375	3.7825	2
Mtentu	31°14'53"S	30°02'49"E	1	Good	5.625	39.6975	2	1	Good	7.875	13.2325	2
Mzamba	31°06'30"S	30°10'31"E	1	Fair	16.6625	53.205	2	1	Fair	23.3275	17.735	2
Mzimkulu	30°44'23"S	30°27'32"E	1	Poor	15	88.425	3	1	Poor	21	29.475	3
Mkomazi	30°12'03"S	30°48'11"E	1	Poor	739.0625	56.025	2	1	Poor	1034.688	18.675	2
Matigulu/Nyoni	29°06'36"S	31°37'09"E	1	Poor	69.6375	95.25	2	1	Poor	97.4925	31.75	2
Mlalazi	28°56'42"S	31°48'58"E	1	Fair	24.5	179.0783	2	1	Fair	34.3	59.69275	2

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