Vulnerability, Resilience and Adaptation: the Future for the Seagrass, *Zostera capensis*

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DECLARATION

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Abstract:

The seagrass *Zostera capensis* forms a vital component of southern African estuarine systems as it provides critical ecosystem services which support biodiversity, estuary functioning and economically important fishery industries. This intertidal seagrass is restricted to estuaries and sheltered bays, and appears to rely chiefly on vegetative reproduction, limiting its dispersal capacity along the often-harsh coastlines of southern Africa. As such, these isolated and highly clonal populations are likely to be more vulnerable to the impacts of global change, the effects of which are likely to cascade through the ecosystem. South African estuaries are both highly threatened and poorly protected, and little is known about the standing of the southern-east African coastline in this regard, increasing the urgency of assessing the status of this keystone estuarine species. A genomic approach can provide a cost-effective, comprehensive characterisation of evolutionary history and potential, and can be applied to evaluate vulnerability, resilience and adaptive potential. As such, the ezRAD method was employed to obtain SNP data and examine both the neutral and putatively adaptive genomic variation and differentiation of 12 *Z. capensis* populations across its range. Anthropogenic drivers of genomic variation were investigated, and a spatial planning approach was utilised to evaluate regions that protect genomic diversity and evolutionary resilience. Results showed that every meadow had a high degree of clonality and low genomic diversity; this in combination with the lack of effective protection and negative feedback between environmental pressures and genomic diversity, increase the vulnerability of this species to further declines and even local extinctions. However, variation at putatively adaptive loci indicate local adaptation to temperature and precipitation regimes, which could confer some level of resilience to future environmental change. Although loci under selection are shared across sites, differences in their observed frequencies differentiate sites into a west coast and an east coast cluster. The formation of these clusters may have occurred as far back as the last glacial maximum where ensemble models project a loss of habitat between the two clusters, as well as a stable area of suitable habitat on the western-south coast, in terms of sea surface temperature, which may have served as a refugial area. In order to increase the representativeness of marine protected areas and the persistence of species therein, it is critical that conservation planning take measures of genomic variability into account. In this regard current and proposed MPAs based solely on

habitat are far from sufficient, and their shortcomings are compounded by discordance with the distribution and intensity of environmental pressures. However, by including any one measure of genomic diversity, distinctness or adaptive potential, conservation managers may sufficiently represent the evolutionary processes behind the patterns of variation, while simplifying the conservation prioritisation procedure.

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Table of Contents

	Page:
Declaration	2
Abstract	3
Acknowledgements	5
List of figures	7
List of tables	9
General introduction	11
Data chapters-	
Chapter 1: Assessment of the population genomic structure and diversity of *Zostera capensis*	28
Chapter 2: Adaptive potential and resilience of *Zostera capensis*	46
Chapter 3: Impact of environmental condition on genomic diversity	74
Chapter 4: Applying genomic data to conservation	89
Chapter 5: General conclusion	109
References	113
Supplementary Materials	161

List of figures

Figure caption	Page
Figure I. South Africa's coastal and marine inshore and offshore ecoregions (National Biodiversity Assessment – marine component; from Sink et al. 2012)	16
Figure II. The Western Indian Ocean, defined by the east African coast and the Saya de Malha, Nazareth and Cargados Carajos banks of the Mascarene Plateau. The principal currents that define the region are coded by the circled letters A–E. A) South Equatorial Current, B) eddies and the Comoros Gyre, C) East Africa Coastal Current, D) Somali Current, E) Agulhas Current. Bathymetric contours were selected to illustrate the main plateau and bank features, at 60, 200 and 1000 m depth (Obura 2012).	19
Figure III. The range of *Zostera capensis*, extending from the west coast of South Africa to the southern coast of Kenya	22
Figure IV. Schematic depicting the flow of information and interconnectivity of the thesis chapters.	27
Figure 1.1 Sampling locations at estuaries along the South African and east African coasts with an inset indicating the two sites in Langebaan Lagoon.	33
Figure 1.2 Clustering analysis of the twelve sites estimated in BAPS for only neutral loci, with all twelve sites falling into one cluster.	40
Figure 1.3 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons among the 12 sampling sites, for the subset of SNPs contained in the simulated neutral dataset.	40
Figure 2.1 Clustering analysis of the twelve sites estimated in BAPS for the complete dataset, with the twelve sites grouped into two clusters.	57
Figure 2.2 A Venn diagram illustrating the overlap between outlier loci identified using the four different approaches. Only three loci were identified by all four programs.	58
Figure 2.3 The frequency of outlier loci across sampling sites, as identified in chapter 1 by A) all four approaches (only three loci; identified by Lositan, BayeScan, BayeScEnv and PCAdapt), B) at least two approaches (10 loci), C) PCAdapt utilising the non-simulated dataset (32 loci).	59
Figure 2.4 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons among the 12 sampling sites for the complete dataset. Sites grouping with cluster one and two are indicated by the red and green bar, respectively, in the legend.	60
Figure 2.5 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons of outlier loci among the 12 sampling sites. Sites grouping with cluster one and two are indicated by the red and green bar, respectively, in the legend.	60
Figure 2.6 Ensemble model projections of probability of habitat suitability for the present-day (top) and the LGM (bottom), with surveyed estuaries represented by blue circles.	62
Figure 2.7 Projected changes in suitable habitat, in terms of SST, from the LGM to present with the probability of occurrence graphically represented along the x and y axes.	63

Figure 2.8 Pearson's correlation scatter plot of the association between distance from the range core and measures genomic diversity; number of SNPs, nucleotide diversity, number of private SNPs and allelic richness (from left to right). 65

Figure 3.1 Locality maps adapted from the NBA 2011 indicating the level of infrastructure development, flow modification, pollution, fishing effort and habitat degradation in each estuary as well as the overall threat status by biogeographic region. 81

Figure 3.2 Diagram illustrating the impacts of each of the environmental stressors retained by the GLM on seagrass biomass or cover and seagrass genomic diversity. 85

Figure 4.1 Location of formally protected and partially protected estuaries in South Africa (from Van Niekerk et al. 2012). 90

Figure 4.2 A flow of the implementation of genomic data in Marxan for spatial conservation prioritisation. 97

Figure 4.3 The spatial patterns of selected conservation priority areas across all scenarios with high to low planning unit selection frequency represented by dark to light blue. B = baseline, AR = allelic richness, H = heterozygosity, N = nucleotide diversity, S = SNPs, PS = private SNPs, AV = adaptive variation, ALL=combined (also see Table 4.2). 100

Figure 4.4 nMDS plot displaying dissimilarities among scenarios. 101

Figure 4.5 Spatial patterns of selected priority conservation areas derived from conserving habitat as well as diversity measures, with units selected by more than one scenario in blue and those selected only by the scenario based on AR in yellow, on H in orange, on N in green and on S in purple. 102

Figure 4.6 Change in spatial patterns of selected priority conservation areas with the addition of genomic measures of diversity (AR), distinctness (PS), adaptive variation (AV), and a combination thereof (ALL) to solely targeting habitat, with units gained in red, lost in grey and remaining selected in blue (number of planning units indicated on the right). 103

Figure 4.7 Threat status of South African estuaries along the cold temperate west coast, warm temperature south coast and subtropical east coast (from the NBA; van Niekerk 2012) compared with the ALL scenario planning unit selection frequency. Circles indicate Operation Phakisa proposed coastal MPAs. 104

Figure S1 FastStructure plots for the analyses of the simulated neutral (left) and complete (right) dataset, with k=2-5 and k=3-6 (top to bottom), respectively. K=1 for the neutral dataset is not shown. 161

Figure S2 The frequency of 10 random neutral loci across sampling sites. 162

Figure S3 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons among the sampling sites, excluding Kenya, for the subset of SNPs contained in the simulated neutral dataset. FST calculated from A) all loci in the full dataset, B) outlier loci and C) neutral loci. 163

Figure S4 R script describing SDM methods 173

List of tables

Table caption	Page
Table 1.1 Sampling locations, biogeographic zone and number of samples per site.	34
Table 1.2. Summary statistics of RAD data and estimates of genomic diversity metrics per sampling site (refer to Table 1.1 for full names of abbreviations) for neutral dataset.	41
Table 1.3. Pairwise F_{ST} values estimated among the 12 sampling sites (refer to Table 1.1 for full names of abbreviations) for the neutral dataset.	42
Table 2.1. Environmental variables included in BayeScEnv and IBE analyses.	54
Table 2.2 Estimates of genomic diversity metrics per sampling site (refer to Table 1.1 for full names of abbreviations) for complete dataset.	56
Table 2.3 Pairwise F_{ST} values estimated among the 12 sampling sites (refer to Table 1.1 for full names of abbreviations) for the complete dataset.	58
Table 2.4 Measures of genomic diversity and distance values used to assess edge effect, with the genomic metric significantly associated with distance from the range core indicated with an asterisk (*).	64
Table 2.5 Allele frequency and counts of candidate outliers in each site identified by PCAdapt, BayeScan, Lositan, BayeScEnv, or combinations thereof. GO terms and BLAST hit sequences are given where matches could be made.	66
Table 3.1 The impact of various measures of genomic variability on the persistence of seagrass species from multiple studies	77
Table 3.2 Effect of environmental stressors (habitat loss, sand mining, fishing effort and change in flow) on genomic diversity indices (nucleotide diversity, heterozygosity and allelic richness) in a GLM.	83
Table 4.1 A description of genomic measures included in this analysis and their relevance to conservation prioritisation (after Beger et al. 2014; Nielsen et al. 2017)	94
Table 4.2 Conservation prioritisation scenarios and planning objectives.	98
Table S1 Outlier allele frequencies across sampling sites (see table 1.1 for full names of abbreviations) and outlier identification methods.	109
Table S2 Five environmental stressors and ecological category (A-F described above) of each site as rated by the NBA (Van Niekerk et al. 2012) as low (L), medium (M), high (H) and very high (VH), as well as the presence (Y) and absence (N) of sand mining.	118

General Introduction

With anthropogenically driven climate and global change being an inevitable feature in the future of our planet, it is important to study its potential impacts on vital resources, such as exploited species, biological and genetic diversity, and essential ecosystems services. This is especially important due to the link between the increasing human population and an increased diversity and intensity of environmental stressors (Goudie 2013). Coastal ecosystems are increasingly subjected to 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 uses of the oceans' resources lies in fisheries industry with catches increasing drastically in the past two decades (Hilborn et al. 2003; Houde & Rutherford 2013). Notably, commercially exploited fisheries have begun to stabilise and even decrease in recent years, not as a result of decreased demand, but due to the suspected depletion of stocks (Hilborn et al. 2003; Houde & Rutherford 2013).

The declines in global biodiversity have been linked to an increase in the rate of resource collapse and a marked decrease in recovery potential, stability and water quality, which forms a negative feedback loop further impairing the ocean's capacity to provide food and ecosystem services (Worm et al. 2006; Hooper et al. 2012; Meyer et al 2016). Historically, habitat loss and over-exploitation were focused on as the main human impacts to the environment (Jackson 2001), but in the last century pollution, invasive species and climate change have become increasingly important as human-associated impacts (Wilcove et al. 1998; Barbier et al. 2011; Meyer et al. 2016).

Estuarine systems are recognised as highly important spawning areas and nurseries for numerous marine and freshwater species, including important fishery species (Blaber & Cyrus 2000; Beck et al. 2001; Vasconcelos & Reis-Santos 2007; Whitfield & Cowley 2010; Unsworth et al. 2018). This capacity is in large part due to seagrasses which, as keystone species, form the habitat in which many other species live at some stage in their life-history (Beckley 1983; Green & Short 2003). As such, the persistence of seagrass through global change is 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 (ie. 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). However, many marine environments, both coastal and offshore are poorly understood in these contexts.

Seagrass communities

Seagrasses are a distinctive feature of many subantarctic, temperate and tropical, as well as estuarine and sub-tidal coastal areas (Den Hartog 1970; Green & Short 2003). Seagrasses are angiosperms, capable of producing flowers and seed, though their aquatic nature results in distinct differences in seed dispersal in comparison to their terrestrial relatives. While all seagrasses are capable of both asexual and sexual reproduction, vegetative reproduction via fragmentation often dominates the maintenance and expansion of beds (Greve & Binzer 2004; Hall et al. 2006). 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 range edge (Phillips et al. 1983; Arriesgado et al. 2015). The seed output, size, buoyancy, dormancy, survival and dispersal all vary greatly among seagrass species (Orth et al. 2006b). Seagrasses generally grow submerged in calm shallow waters with good light availability and low turbidity, and in many places 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, as well as being an important carbon sink (Green and Short 2003; Marba et al 2015; Gullström et al 2016; Arias-Ortiz et al. 2016). 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 et al. 2006a).

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 beds have been found as dense as 4561 shoots per m^2 (Green & Short 2003). The complex web of interactions associated with seagrass beds has both direct and indirect affects within seagrass communities (Siebert & Branch 2006; Stavely et al. 2017). 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; Pusceddu et al. 2016). Seagrass dependent species range from epiphytic algae to large aquatic herbivores, such as the critically endangered green sea turtle which feed directly upon the seagrass (Kitting et al. 1984; Green & Short 2003; York et al. 2018). 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). Resident species, one South African example being the Cape stumpnose, *Rhabdosargus holubi* (Sheppard et al. 2011), may utilise seagrass beds for habitat, shelter, dietary or reproductive requirements (Green & Short 2003). Globally, many seagrass dependent species are endangered or threatened, such as the dugong, manatee, horseshoe crab, green turtle and various grouper fishes and seahorses (Walter & Gillett 1998).

However, communities are not only structured around, but also on seagrasses. 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 these can reduce water movement, reduce desiccation and when algae die, decomposing matter can become a source of nutrients (Fong et al. 2000). This is a finely balanced ecological interaction in undisturbed systems, but fouling becomes hugely problematic with increased eutrophication, a problem in estuaries worldwide (Hughes et al. 2004; Cote et al. 2016; Human et al. 2016).

Seagrass ecosystem services extend beyond the community interactions mentioned above to modifications to the environment. As an ecosystem engineer, seagrass rhizome networks bind sediments thus enhancing nutrient retention, water quality and reducing erosion of the benthos (Orth 1976; Green & Short 2003; Lucas et al. 2012). 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). Additionally, seagrasses are known for their coastal protection (Green & Short 2003; Barbier et al. 2011). The capacity to attenuate waves and diminish the effects of storm surges is strongest in long lived, stable seagrass beds with high biomass (Ondiviela et al. 2014). 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). Despite both the ecological and economical value of seagrasses, many species and bioregions remain under-studied, in particular their population dynamics and resilience to change (Nordlund et al. 2016). Further, understanding the vulnerability of seagrass to future change scenarios requires additional approaches that may help in protecting seagrass populations into the future.

Seagrass declines

While seagrasses occur in all of the world's oceans, except the Arctic and Antarctic Ocean, a marked decline has recently been noted in their cover (Orth et al. 2006a; Waycott et al. 2009a), with 31% of species having declining populations (Short et al. 2011). Estimates indicate that between the late 1880s and 2006 about 30% of the world's seagrass area has been lost. Further, rates of decline have accelerated from 0.9% per year before 1940 to 7% per year since 1990, placing seagrass beds among the most threatened ecosystems in the world (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 et al. 2006a). These declines are largely due 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 et al. 2006a; 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 (Orth et al. 2006a) 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 et al. 2006a). 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 et al. 2006a; Waycott et al. 2009a; Coyer et al. 2013). Major gaps in information exist in Africa, South America and the Indo-Pacific (Waycott et al. 2009; Nordlund et al. 2016). Regardless, large declines have been reported in Zanzibar, Tanzania, with associated impacts on local harvesters' economy and livelihood (Nordlund et al. 2010). Phair et al. 2016 (in prep) illustrated the vulnerability of seagrass along the South African coast with projections estimating a 30% loss of suitable seagrass habitat by the year 2070, with a shift towards the south-east coast. Due to the decline of seagrass systems, the current distribution is uncertain for many species and this uncertainty is exacerbated by a lack of studies in developing regions.

South African Oceanography and Biogeography

The unique oceanographic patterns in southern Africa drive the complexity of the biogeographic patterns of marine and estuarine species in the region. South Africa is the only country globally that experiences two starkly contrasting temperature regimes along its coastline (cold Atlantic and the Benguela Upwelling System on the west; warm Indian Ocean and Agulhas Current on the east), with mixing on the south coast (Nelson & Hutchings 1983; Lutjeharms & Van Ballegooyen 1988). Patterns of biodiversity are determined largely by oceanographic elements such as currents, sea temperatures and continental shelf features. The South African marine coastal environment is distinguished by very high species richness due to its long coastline and variable conditions, with around 30% endemicity (Awad et al. 2002). Many studies have examined the biogeography of species along the South African coast and recognise between two and five broad biogeographic regions, with some slight variation in the naming and region of 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 (Stephenson & Stephenson 1972; Ridgway et al. 1998; Evans et al. 2004, 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 to tropical 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 predominantly 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 inshore regions (Driver et al. 2012; Sink et al. 2012). The cool-temperate Namaqua Bioregion is found on the west coast up to Cape Columbine, where the South-western Cape Bioregion begins and extends to 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. I). 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). The general trend in the distribution of species is that the west coast has the lowest species diversity, with an increase on the south and east coasts (Awad et al. 2002), which also holds for estuarine and marine fish species that generally display a gradient with higher species richness in estuaries on the warmer eastern coast and lower on the cooler western coast (Turpie et al. 2000; Harrison 2002; Harrison & Whitfield 2006).

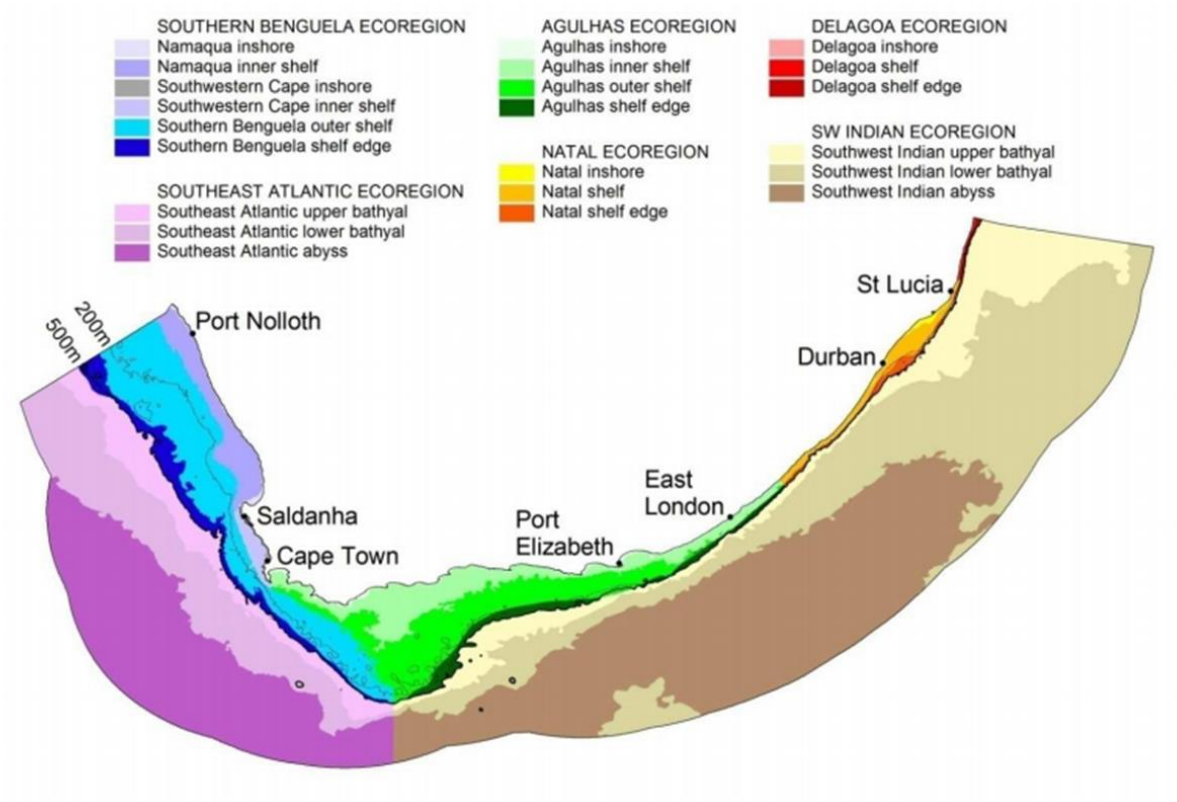

Figure I South Africa's coastal and marine inshore and offshore ecoregions (National Biodiversity Assessment – marine component; Sink et al 2012).

The processes shaping the biogeographic regions probably also act as 'soft' boundaries on connectivity, restricting the dispersal of organisms along the coastline and influencing patterns of genetic structure (Teske et al. 2011). Numerous species, with both active and passive dispersal, have been found to exhibit phylogeographic patterns of genetic structure and differentiation along the three broad bioregions (Teske et al. 2011). For example, estuarine invertebrates such as mudprawns, isopods (Teske et al. 2006), shrimp (Teske et al. 2007) and sandprawns (Teske et al. 2009) all exhibit phylogeographic patterns coinciding with the three broad bioregions. It is hypothesised that these phylogeographic breaks are maintained in two major ways. Firstly, separate genetic lineages are maintained by barriers which restrict dispersal, secondly, genetic lineages are adapted to the environmental conditions distinctive of their biogeographic region, often preventing them from successfully establishing themselves in neighbouring regions (Teske et al. 2011). In other parts of the world, as well as in South Africa, such barriers can take the form of cold-water upwelling (Rocha et al. 2005; Zardi et al. 2007), freshwater discharge (Ridgway et al. 1998), dunefields which represent long stretches of unsuitable habitat for many species (Ayre et al. 2009; Teske et al. 2011), and most predictably near- and off-shore currents (Hare et al. 2005; von der

Heyden et al. 2008; Mertens et al. 2018). The northward flowing Benguela Current on the west coast provides a good example of a driving force of unidirectional gene flow, with east coast klipfish, *Clinus cottoides*, effectively isolated from those on the south and west coasts (von Der Heyden et al. 2008). Conversely, some evidence suggests that passive dispersal can also occur against major ocean currents such as the Agulhas Current (Teske et al. 2015). The Cape sea urchin, *Parechinus angulosus*, for example, was found to exhibit bidirectional gene flow along the east coast (Muller et al. 2012), although this pattern may also be due to other factors such as incomplete lineage sorting.

There are, however, numerous exceptions to the overlap of biogeographic and phylogeographic patterns. This is probably due to the incredibly diverse conditions and palaeo-oceanographic history of the South Africa coastline. The phylogeography of the goby *Caffrogobius caffer* does not conform to described bioregions, but rather exhibits panmixia between populations (Neethling et al. 2008). The genetic structure of mangroves is another example of an exception to these bioregions, displaying strong population structure with high levels of genetic differentiation among populations instead (Maguire & Saenger 2000). Palaeo-oceanography can provide additional explanations for genetically diverged lineages despite the lack of present-day 'hard' barriers (Toms et al. 2014). Models of sea-level change over the last 110 000 years have revealed that although there are no contemporary barriers to dispersal for two genetically diverged lineages of the rocky shore clinid *Clinus cottoides*, its habitat was once separated by large areas of sandy shores for at least 40 000 years (Toms et al. 2014). Further, Teske *et al*. (2006) identified phylogeographic patterns in the cumacean, *Iphinoe truncata*, that reflect palaeo-oceanographic conditions rather than contemporary bioregions. Therefore, in order to explain phylogeographic patterns of a species, one must carefully consider both life history as well as historical events and conditions in the study area.

Notably, population genetic studies on aquatic plants, and on estuarine species in general, are lacking globally in comparison to, for example, commercial fishes and rocky shores (Selkoe et al. 2016). To date, only one molecular study has been published on estuarine plants in South Africa (Potts et al. 2016). Here the authors found that the salt marsh plant, *Juncus kraussii*, exhibited a phylogeographic break along the south coast, despite its high dispersal capacity. This break falls within the warm-temperate coastal

bioregion and does not coincide with other present-day barriers, but could be attributed to the rapid shifting of the shoreline during the Pleistocene, when whole stretches of salt marsh habitat would have been uninhabitable.

East African Biogeography

The Western Indian Ocean (WIO) is commonly divided into five biogeographic regions (Fig. II) based on reef-building organisms, according to the principal currents influencing the region (Obura 2012). The first region (A- Fig. II) is defined by the South Equatorial Current which facilitates gene flow from the Central Indo-Pacific and increases species diversity in the WIO and a decrease in the islands (Veron 2000; Obura 2012). The next region (B-Fig. II) defined by eddies and the Comoros Gyre in the Mozambique Channel, associated with increased connectivity and production (Obura 2012). The East Africa Coastal Current defines another region (C- Fig. II) where it provides linear transport from the northern Mozambique Current to the northern coast of Kenya, and it is associated with a change in coral fauna (Obura 2012). The next region is more complex and is defined by the Somali Current, monsoon reversals and upwelling, associated with colder and higher nutrient conditions leading to a further transition of coral fauna (DeVantier et al. 2004). The most southern region is defined by the Agulhas Current which merges waters from the Mozambique Channel and East Madagascar, resulting in cooler conditions and a decline in coral diversity (Schleyer et al. 2008).

Despite the WIO being widely acknowledged as a biodiversity hotspot, the evolutionary and phylogeographic patterns of this region are still poorly explored compared to the South African coastline (Teske et al. 2011). A review of genetic studies in the WIO found that reef organisms tend to exhibit widespread genetic structuring off the East African coast and greater connectivity amongst the southeast African reefs (Huyghe & Kochzius 2017). Other studies tend to observe no structure (Muths et al. 2012; Muths et al. 2013) and no genetic breaks between Kenya and Tanzania (Minegishi et al. 2008, 2012; Ragionieri et al. 2010; Silva et al. 2010b, 2013; Farhadi et al. 2013). 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 rocky and sandy forms did not differ genetically when examining random amplified polymorphic DNA (RAPD). This seagrass was shown to be highly genetically diverse, indicating the possibility of frequent sexual reproduction or

genetic exchange in southern Mozambican populations. Most genetic variation was detected within rather than between populations, indicating a lack of population structure, with no significant correlation between geographic and genetic distance.

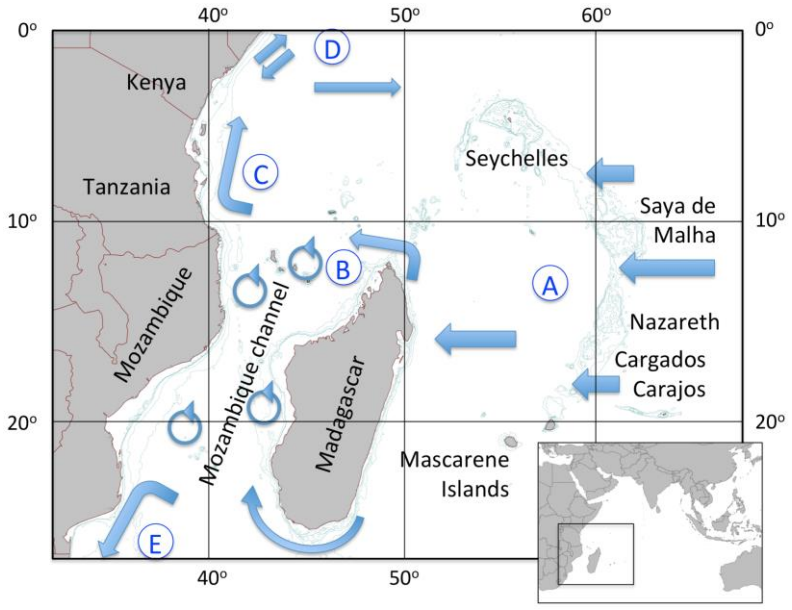

Figure II The Western Indian Ocean, defined by the east African coast and the Saya de Malha, Nazareth and Cargados Carajos banks of the Mascarene Plateau. The principal currents that define the region are coded by the circled letters A–E. A) South Equatorial Current, B) eddies and the Comoros Gyre, C) East Africa Coastal Current, D) Somali Current, E) Agulhas Current. Bathymetric contours were selected to illustrate the main plateau and bank features, at 60, 200 and 1000 m depth (Obura 2012).

Conversely, population structure has been observed within mangrove-associated crabs along the East African coast. A phylogeographic study on the mangrove crab, *Perisesarma guttatum*, revealed two clades with populations in southern Mozambique differentiated from those in northern Mozambique, Tanzania and Kenya (Silva et al. 2010a). This indicates a potential 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. Similar research on the fiddler crab, *Uca annulipes*, found no genetic structure in this region, possibly due to the high dispersal capacity of their planktonic larval phase (Silva et al. 2010b). Further, another possible break has been found Along the East African coast between southern Mozambique and South Africa when investigating the mitochondrial control region of

the spiny lobster, *Palinurus delagoae* (Gopal et al. 2006). However, these few studies do not provide generalised patterns and point to complex and dynamic processes shaping the regions biogeography and phylogeography.

South African estuaries

South Africa's estuarine environment encompasses ~ 90 800 ha in total and estuaries vary in size, turbidity, salinity, mouth condition and density along the coast and generally exhibit a temperature gradient with lower temperatures along the west and higher along the east coast (van Niekerk et al. 2012). The west coast exhibits fewer, well-spaced medium to large estuaries while the east coast, which experiences higher rainfall and has more rivers, and contain the majority of the country's estuaries (see Fig. 6.5 in van Niekerk et al. 2012). The east coast estuaries are more densely distributed and vary in size, as defined by the National Biodiversity Assessment (NBA) of estuaries in 2011 (van Niekerk et al. 2012). During the recent 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, industry (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.

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 by removing top level predators which remove fouling epiphytes on seagrass beds (Mead et al. 2013). An example of seagrass decline in South Africa is from Langebaan Lagoon, where about 38% of seagrass cover 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 specialised to live on *Z. capensis* blades in the mid to upper intertidal of estuarine lagoons (Herbert 1999), is South Africa's most endangered marine invertebrate and is now only restricted to two localities, namely Langebaan Lagoon on the west coast and Knysna estuary on the south

coast (Herbert 1999; Mead et al. 2013). Further, in Knysna Lagoon some seagrass populations have gone extinct linked to increased eutrophication and macroalgal fouling (Human et al. 2016).

Zostera capensis in southern and eastern Africa

Although there are 72 described species of seagrasses, this remains an understudied taxonomic group in Africa (Short et al. 2011). 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; Nordlund et al. 2016. Seagrasses consist of three independent lineages (Hydrocharitaceae, Cymodoceaceae complex, and Zosteraceae) which evolved from one monocotyledonous flowering plant (Les et al. 1997). The Zosteraceae is a largely temperate and subtropical 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).

Zostera capensis is a species of seagrass belonging to the family Zosteraceae. In South Africa it is the most widespread and dominant seagrass species, inhabiting estuaries from the southern west coast to the northern east coast (Fig. III). This species is also listed as present further along the tropical east African coast, with its reported distribution (Fig. III) reaching as far as the southern coast of Kenya (Green and Short 2003). 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.). This range is rare amongst seagrasses as it encompasses cool-temperate, sub-tropical and tropical environments. Throughout its distribution this species is highly fragmented as it is confined to areas with low water movement, such as lagoons, estuaries and intertidal flats (Green & Short 2003). This, together with threats to seagrasses in general, has led to *Z. capensis* being classified as 'vulnerable' on the IUCN Red list of threatened species (www.iucnredlist.org). However, only roughly 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 yet been quantified. As a result, the actual distribution of *Z. capensis*, particularly along the East African coast, is currently uncertain. However, using a molecular approach it was confirmed that *Z. capensis* is indeed present in southern Kenya (Phair 2015, MSc thesis).

Although *Z. capensis* 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, reproduction and dispersal (Adams 2016; 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 globally (Green & Short 2003), yet 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 (Lamberth & Turpie 2003). In 2002 it was estimated that estuarine and estuarine-dependent fisheries in South Africa were worth R1,251 billion (Lamberth & Turpie 2003).

Figure III The range of Zostera capensis, extending from the west coast of South Africa to the southern coast of Kenya.

As observed in seagrasses around the world, *Zostera capensis* has experienced population declines (Pillay et al. 2010, Human et al. 2016), with reports suggesting that some populations have been reduced by around 80% in Mozambique due to bivalve harvesting (Green & Short 2003). Further, seagrasses in southern Africa are usually

poorly monitored and as such, declines and local extinctions may go unnoticed. In addition, many *Z. capensis* populations are highly fouled by epiphytic algae (Källén et al. 2012). Interestingly, in Langebaan Lagoon in South Africa, *Z. capensis* appears to exhibit two morphotypes, one short and stunted on the muddy tidal flats (Geelbek) which experience prolonged exposure to conditions outside the water and the other is longer with a higher biomass on the sandy permanently submerged area (Oostewaal) (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 factors contribute to driving the presence of two morphotypes in some areas of South Africa (D. Pillay, pers. comm.).

Molecular studies on seagrasses vary between regions and species with a bias towards widespread species in more developed regions. For example, several molecular studies have been conducted at both local and regional scales on the widespread marine seagrass, *Zostera marina*, which is distributed throughout the Northern Hemisphere. For this species high clonal diversity (Becheler et al. 2010; Talbot et al. 2016) as well as significant differentiation has been observed at a broad spatial scale between continents (Olsen et al. 2004; Jueterbock et al. 2016), a regional scale between bays (Muñiz-Salazar et al. 2005; Becheler et al. 2010; Kamel et al. 2012; Kim et al. 2017), at a fine scale between meadows within a bay (Becheler et al. 2010; Kamel et al. 2012; Ort et al. 2012; Talbot et al. 2016; Kim et al. 2017), and even between meadows occurring at different depths (Kamel et al. 2012; Ort et al. 2012; Kim et al. 2017). Yet other widespread species such as *Thalassia testudinum*, which is found sheltered bays and lagoons, exhibit a lack of structuring where so-called 'mega clones' consisting of a single genetic individual are dispersed over up to 47 km (Bricker et al. 2018). In more restricted estuarine species of seagrass, such as *Posidonia australis*, low levels of clonal diversity have been observed with shared multilocus genotypes in northern meadows and unique multilocus genotypes in each southern meadow (Evans et al. 2014). Estuarine dependent seagrasses, particularly those in developing regions, such as *Z. capensis*, remain poorly studied from a molecular perspective.

Molecular tools are becoming increasingly recognised as an invaluable resource for conservation, restoration, resource management and marine spatial planning (Hutchinson et al. 2001; Reusch & Hughes 2006; von der Heyden 2009; Beger et al. 2014; von der Heyden et al. 2014; Evans et al. 2017b). In the face of global change molecular tools can be employed to inform management by characterising resistance to stressors (Roger et al. 2012) and resilience to future environmental change (Theodoridis et al. 2017). The ecology or morphology of a species on its own is seldom enough to explain the processes shaping a species distribution or meta-population (von der Heyden 2009). In contrast, population genetic approaches can form an integral part of conservation planning and fisheries management by allowing for the inference of effective population size, diversity and connectivity, which focuses the scale and intensity of management actions (Hutchinson et al. 2001; Beger et al. 2014; von der Heyden et al. 2014; Evans et al. 2017b). Molecular tools are useful for understanding both native species and invasive species, through identifying dispersal corridors (Angeloni et al. 2012), barriers to dispersal (Kelly et al. 2006; Sanford & Kelly 2011), cryptic species (von der Heyden et al. 2011; Glazier & Etter 2014), hybrid zones (Hohenlohe et al. 2011; von der Heyden et al. 2014), demographic history (von der Heyden 2009; Angeloni et al. 2012; Reitzel et al. 2013), evolutionary history and potential (Beger et al. 2014; Nielsen et al. 2017).

More recently, Next-Generation Sequencing (NGS) has come to the fore as a tool for studying various aspects of the molecular ecology of non-model organisms (Ekblom & Galindo 2011; Puritz et al. 2012). NGS has multiple uses in the realms of genomics, transcriptomic and epigenomics, including gene regulation, expression, transcriptome characterization, development of molecular markers, nucleotide profiling and genome assembly (Ekblom & Galindo 2011). Although several different technologies fall under the umbrella of NGS, they are united in generating large quantities of data that can be used to address ecological and evolutionary questions (Metzker 2010). Outputs of these methods consist of relatively short DNA sequence reads (50-100bp), which can be aligned to a reference genome or when a reference genome is not available, as is usually the case for non-model organisms, they are assembled into scaffolds in *de novo* assembly.

A valuable function of NGS is the ability to detect genome-wide diversity by scanning the genome for single nucleotide polymorphisms (SNPs). Neutral SNPs are similar to

microsatellites in terms of reflecting contemporary evolutionary processes including mutations and genetic drift (Morin et al. 2004; Bradbury et al. 2013; Moore et al. 2014; Van Wyngaarden et al. 2017). However, an NGS approach produces a much higher quantity of SNP markers than the amount of microsatellite markers normally used in population genetic studies. Many studies therefore have made use of SNP data to delineate finer-scale units and resolve demographic changes (Oetjen et al. 2009; Willing et al. 2010; Reitzel et al. 2013; Rasic et al. 2014; Benestan et al. 2015; Hernawan et al. 2017; Thomas et al. 2017). A genome-wide search for SNPs, after removing outlier SNPs, will reflect neutral variation, regulated by drift and gene flow, as the majority of loci are not under selection (Angeloni et al. 2012). This in turn can improve our understanding on connectivity and gene flow in an environment with such high dispersal potential. Opposing this, are loci under presumed selection, that can provide insights into potential local adaptation (Angeloni et al. 2012; Tiffin & Ross-Ibarra 2014; Picq et al. 2016).

Restriction site Associated DNA Sequencing (RADseq) is particularly useful in the study of non-model organisms as any restriction enzymes can be used and no reference genome is required (Baird et al. 2008). Instead of whole-genome sequencing, RADseq enables one to take a reduced representation approach, reducing the cost and output complexity of NGS (Lexer et al. 2003; Futschik et al. 2010; Reitzel et al. 2013). Due to the sheer number of loci obtained, this approach reflects variation across the genome arguably better than the small number of microsatellite markers. In fact, RADseq data has shown that the levels genomic diversity of two species of American bumble bees are much more similar than previous microsatellite analysis suggested (Lozier 2014). As such, RADseq is a good compromise between whole genome sequencing, which has a high cost and low number of individuals, and traditional low-cover sequencing across a high number of individuals. Additionally, reduced representation sequencing of pools of individuals further decreases associated costs whilst still allowing the examination of population genetics questions (Schlötterer et al. 2014). RADseq has been utilised to study a diverse range of taxa including marine (Willette et al. 2014; Gaither et al. 2015; Guo et al. 2015; Picq et al. 2016) and freshwater fishes (Kakioka et al. 2013), marine invertebrates (Gruenthal et al. 2014), marine mammals (Fernández et al. 2015), small terrestrial mammals (Sovic et al. 2016), birds (Dierickx et al. 2015) and plants (Eaton & Ree 2013) (also see Narum et al. 2013 and papers therein).

The use of NGS techniques in seagrass studies, and in the Zosteraceae family more specifically, has rapidly expanded, covering transcriptomic profiling under various climate and environmental scenarios (Reusch et al. 2008; Wissler et al. 2009; Wissler & Codoñer 2011; Franssen et al. 2014; Kong et al. 2014; Jueterbock et al. 2016; Ribeiro et al. 2016), as well as whole genome sequencing and annotation (Olsen et al. 2016a, 2016b; Lee et al. 2016; Sablok et al. 2018). However, NGS techniques also have real potential application for restoration ecology (Williams et al. 2014). For instance, one can use NGS techniques to identify populations which are preadapted to specific conditions, for example to increasing temperatures, with which to supplement depleted populations. Alternatively, a more pre-emptive management strategy may use NGS techniques to detect populations with high levels of diversity or connectivity, assisting in the prioritisation of areas for conservation and restoration (Sinclair et al. 2013; Miller et al. 2017; Evans et al. 2018).

Population genetic trends in *P. australis* and *T. testudinum* may give some indication of the patterns to be expected in *Z. capensis*, as they are all restricted to estuaries and sheltered bays. Given that these species have displayed low levels of diversity and variable levels of population differentiation, *Z. capensis* is unlikely to display high levels of connectivity between the disjunct populations found in South Africa. Further, one can expect phylogeographic patterns of *Z. capensis* to be affected by paleo-oceanographic events, such as rapidly shifting shorelines, in a similar manner as the salt marsh plant, *J. kraussii* (Potts et al. 2016). The relative isolation of meadows may promote and retain genetic diversity, however as *Z. capensis* has been experiencing declines (Short et al. 2010; Adams 2016), 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 ecosystem service provider, it still lacks sufficient molecular investigation. Phair (2015, MSc thesis) found no genetic structure among the population of *Z. capensis* in South Africa when examined using the chloroplast maturase K marker. However, this finding is more likely the result of insufficient marker resolution than a biological signal. Here I will be using a fine-scale analysis of single nucleotide polymorphisms (SNPs), rather than a traditional sequencing approach, in order to reveal more about the vulnerability, resilience and adaptations of *Z. capensis*, with implications for its future.

This thesis is structured into four chapters (Fig. IV). The first examines the genomic diversity, differentiation and connectivity of *Z. capensis* throughout its range, by making use of putatively neutral SNP analysis through next-generation sequencing technology. The second chapter assesses adaptive variation by means of outlier SNPs. This chapter also assesses whether putatively adaptive loci are shared or unique amongst populations, and functionally annotates the regions in which they are found. Isolation by distance was also investigated and compared to isolation by environment. Finally, this chapter uses seascape factors to explain the patterns genomic variation. The third chapter examines the association between genomic diversity metrics and estuary condition/environmental stressors, with the aim of assessing the use of *Z. capensis* as an indicator species for environmental status. The fourth and final chapter integrates different measures of genomic variation into spatial conservation planning to determine how to improve the representativeness of Marine Protected Areas (MPAs) and the persistence of the associated species.

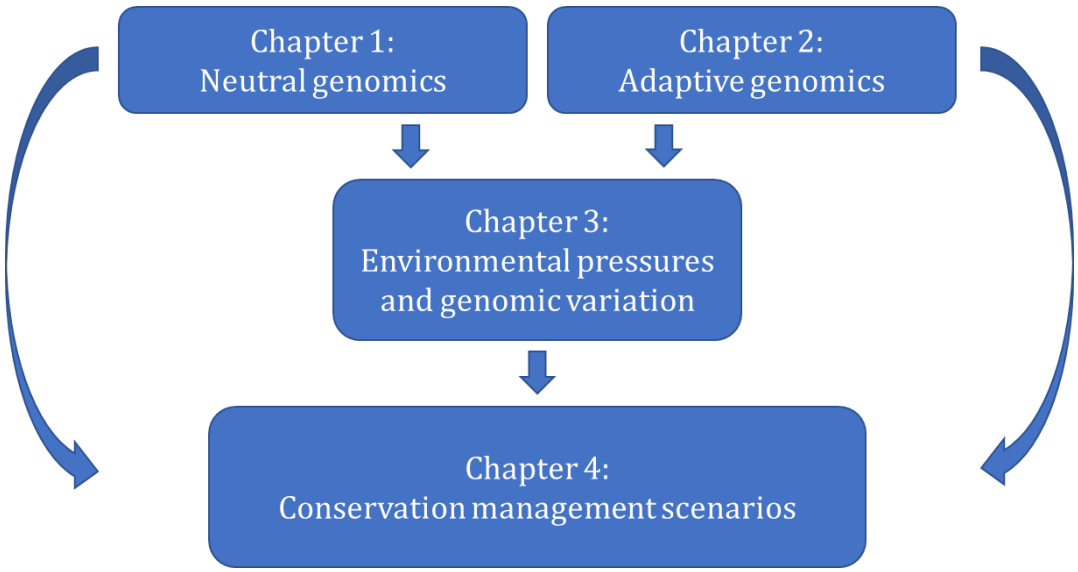

Figure IV. Schematic depicting the flow of information and interconnectivity of the thesis chapters.

Chapter 1: Assessment of the population genomic structure and diversity of *Zostera capensis* using neutral markers

Introduction

One of the key drivers of ecosystem change, and therefore a potential change or loss of function, is a loss of species diversity. The knock-on effects of reduced species diversity are even comparable to those of anthropogenic and climate linked pressures (Hooper et al. 2012), as declining biodiversity in ecosystems has been related to exponential decreases in productivity, stability and resilience (Worm et al. 2006; Duffy et al. 2017; Vallina et al. 2017). Across terrestrial and aquatic environments, consistently, biodiversity positively effects ecosystem function, with species-rich communities maintaining higher multifunctionality than depauperate ones (Lefcheck et al. 2015).

Just as species diversity can be linked to ecosystem function and resilience, so can genetic diversity be linked to evolutionary potential (Moritz 2002; von der Heyden 2009; Pinsky & Palumbi 2014), with higher intraspecific diversity associated with increased resistance and resilience (Hughes & Stachowicz 2004; Ehlers et al. 2008; Hughes et al. 2008; Massa et al. 2013). Globally, seagrasses are facing persistent declines and habitat fragmentation (Orth et al. 2006a; Waycott et al. 2009b), both of which can be linked to loss of genetic diversity (Orth et al. 2006a; Williams 2017). Determining the spatio-temporal patterns of genetic genomic variability and connectivity of populations can therefore be important for understanding persistence and resilience of species, particularly those under threat from environmental pressures. This also provides a baseline against which potential genomic changes can be monitored in the future.

Seagrasses are well documented as keystone species, providing various ecosystem services including habitat provision (Beck et al. 2001; Orth et al. 2006a; Bertelli & Unsworth 2014), increasing primary productivity (Adams 1976; Green & Short 2003), enhancing biodiversity (Hemminga & Duarte 2000) and supporting adjacent ecosystems (Unsworth et al. 2015). Genetic diversity in seagrasses has been linked, not only to the maintenance of ecosystem services, but also to an increased resistance and resilience to environmental pressures (Massa et al. 2013; Unsworth et al. 2015). In this context, high-resolution markers, as employed in NGS techniques, can contribute greatly

to resolving recent changes in demography, such as population declines, as well as signals of local adaptation (Angeloni et al. 2012; da Fonseca et al. 2016).

The southern and eastern African seagrass, *Zostera capensis*, is under threat from various pressures including habitat modification, pollution, urbanization and climate change (Mead et al. 2013). This species ranges from the temperate waters of the South African west coast, to the tropical waters of Mozambique, Tanzania and Kenya (Green and Short 2003, Fig. III). Notably, in South Africa it is limited to the lower reaches of estuaries, and along the East African coast it can also be found in sheltered bays, adjacent to estuaries (Green & Short 2003). While seagrasses are capable of both sexual and vegetative reproduction, the latter often dominates the maintenance and expansion of beds (Greve & Binzer 2004; Hall et al. 2006). As flowering in *Z. capensis* has only been recorded once under specific laboratory conditions (McMillan 1980), it is likely that this species largely relies on vegetative reproduction (Adams 2016). However, Weatherall et al. (2016) demonstrated that vegetative fragment viability and longevity, and hence dispersal potential, differs between species. As such, it is unclear whether vegetative fragments could successfully navigate the currents, harsh coastal conditions and long distances between suitable estuarine habitat, all of which provide somewhat of a barrier to dispersal for *Z. capensis* in southern Africa. Interestingly, infrequent long-distance dispersal of vegetative fragments by water fowl has been suggested (Martinez-Garrido et al. 2017), although if this does occur, it is doubtful that this contributes meaningfully to connectivity.

In South Africa alone, *Z. capensis* supports a fishing industry worth an estimated R1,251 billion in 2002, and includes economically important species of kob, mullet and kingfish (Lamberth & Turpie 2003). Due to its fragmented distribution and declining cover (Pillay et al. 2010), it is rated as 'vulnerable' by the IUCN (Short et al. 2010). Yet only ~13% of seagrass habitat in South Africa is found in currently protected areas (Van Niekerk et al. 2012) and anthropogenic pressures outside of South Africa have not yet been quantified. This provides further urgency for mapping the distribution of genomic diversity and connectivity in *Z. capensis*, as a lack of diversity and connectivity may further increase the risk of decline and impair their capacity to recover.

The distribution of *Z. capensis* along the southern and eastern African coastlines, provides a fascinating 'natural laboratory' in which to study population divergence and adaptive potential due to its gradient of environmental variables. Previous research has

shown that many species exhibit genetic structuring along the South African coastline, often coinciding with biogeographic patterns (Fig. I). The latter were delimited for marine biota (Ridgway et al. 1998; Evans et al. 2004; Teske et al. 2006; Edkins et al. 2007; Teske et al. 2007; Sink et al. 2012; summarised in Teske et al. 2011), based on temperature and habitat distribution, as well as for estuarine biota (Stephenson & Stephenson 1972; Day 1981; Harrison 2002), based on temperature, rainfall and river flow. However, the focus has entirely been on fauna, with only one study published on the population genetics of an estuarine plant in the region (Potts et al. 2016). The authors revealed a phylogeographic break within the warm-temperate bioregion for the salt marsh species, *Juncus kraussii*, and suggested paleo-oceanographic conditions as the main cause (Potts et al. 2016). While biogeography and phylogeography along the East African coast is comparatively understudied, evidence suggests low levels of genetic diversity and structure (Silva et al. 2010b; Muths et al. 2012, 2013; Huyghe & Kochzius 2017), with some species displaying limited south-north structuring (Silva et al. 2010a; Vogler et al. 2012; van der Ven et al. 2016; Huyghe & Kochzius 2017). It is suggested that the splitting of the South Equatorial Current northward into the East Africa Coastal Current, and southward into the Agulhas Current plays an important role in limiting connectivity across this region (Vogler et al. 2012; van der Ven et al. 2016; Huyghe and Kochzius 2017; Fig. II).

The genetic diversity, clonality and connectivity of seagrasses globally is highly context dependent (Jover et al. 2003; Olsen et al. 2004; Procaccini et al. 2007; Sinclair et al. 2014; Arriesgado et al. 2016; Kendrick et al. 2016; Hernawan et al. 2017), with some studies reporting high genetic diversity and population structuring at regional and local scales (Diekmann et al. 2005; van Dijk & van Tussenbroek 2010; Becheler et al. 2010; Sherman et al. 2016), emphasizing the role of near and off-shore currents (Muñiz-Salazar et al. 2005; Nakajima et al. 2014). Conversely, in a few cases, low levels of genetic diversity and shared genotypes, even across exceptionally large spatial scales, have been recorded (van Dijk & van Tussenbroek 2010; Evans et al. 2014; Nakajima et al. 2014; Phan et al. 2017). So-called 'mega clones' of *Thalassia testudinum* can even have genets dispersed over 47km (Bricker et al. 2018) and 'millenary clones' of *Posidonia oceanica* are estimated to be hundreds to thousands of years old (Arnaud-Haond et al. 2012).

NGS techniques are becoming increasingly popular as they enable the study of evolutionary patterns of non-model organisms at a higher resolution, allowing for the identification of distinct evolutionary lineages, genetic breaks and potential management units (Helyar et al. 2011; Williams et al. 2014; da Fonseca et al. 2016). This is also true for seagrasses in particular (Oetjen et al. 2010; Davey et al. 2016; Hernawan et al. 2016). Given the multiple pressures faced by local populations, as well as over-arching anthropogenic and climatic changes in the region (Mead et al. 2013), there is a need to better understand the genomic diversity and structure of *Z. capensis* in a changing African seascape. Therefore, this chapter utilised a Next-Generation Sequencing (NGS) approach to conduct a population genomic study across the distribution of *Z. capensis*, including previously recognised phylogeographic breaks for other estuarine species.

Aims

In the absence of molecular studies on *Z. capensis* in southern and eastern Africa, this chapter aims to investigate the spatial patterns of genomic diversity, population divergence and connectivity in *Z. capensis* meadows across its known range. This was carried out using a genome-wide approach to obtain a greater resolution than traditional markers might allow, enabling the identification of distinct evolutionary lineages, potential management units and genetic breaks in *Z. capensis*. The importance of further investigation of this understudied species is compounded by the potential consequences of its decline for seagrass communities and fishery industries. The metrics calculated in the chapter (genomic diversity, population divergence and connectivity) forms the baseline for the subsequent chapters.

Hypotheses

Due to the disjunct distribution of this species, the lack of sexual reproduction recorded in wild populations and the harsh conditions for vegetative propagules along the coastline, which in combination should reduce potential connectivity, I hypothesised that *Z. capensis* will exhibit strong population structuring and differentiation among sites, coinciding within the general bioregions in South Africa (Fig. I). Further, due to the splitting of the South Equatorial Current (SEC) northward into the East Africa Coastal Current, and southward into the Agulhas Current (Fig. II), I expect meadows in southern African sites to be more closely related to each other than meadows north of the SEC.

Methods

Sample collection

Samples were collected from twelve sites, including nine estuaries/estuarine bays along the South African coast: Olifants, Berg, Langebaan (Oostewaal and Geelbek), Breede, Knysna, Swartkops, Nahoon, Mngazana and Richard's Bay (Mthlatuze estuary). One locality from Inhaca Island, Mozambique, and one location Shimoni, Kenya, was sampled to represent the most northern recorded distribution of *Z. capensis* (Fig. 1.1; Table 1.1). At each location, except Richard's Bay, Mozambique and Kenya, a total of 30 cuttings were collected over five beds at two separate sites in order to minimise the sampling of clones, as it is very difficult to visually disentangle adjacent individuals. Each strand was blotted dry and cleaned of debris before being dried with silica gel crystals to avoid contamination of the plant tissue by mould. Only 23 samples were included from Richard's Bay, due to poor DNA quality, and only 10 separate samples from Mozambique and three from Kenya were obtained. Despite intensive questioning of collaborators and other contacts throughout this study, no samples of *Z. capensis* were obtained from Tanzania, where it has not been recorded in recent times (Nordlund L. pers. comm.).

Laboratory protocols

Accurately estimating genome-wide variation and detecting signals of local adaptation in non-model organisms, such as seagrasses, requires many individuals from many sites to be sequenced, which can be prohibitively expensive despite the advances made by high-throughput sequencing methods such as RADseq (Ellegren 2014; Andrews et al. 2016). As such, this thesis followed the trend in the literature of utilizing a pooled sequencing (pool-seq) approach, where DNA from multiple individuals is combined before sequencing, to decrease the cost while increasing the number of individuals analysed thereby increasing accuracy and providing a more population focussed analysis (Futschik & Schlötterer 2010; Schlötterer et al. 2014).

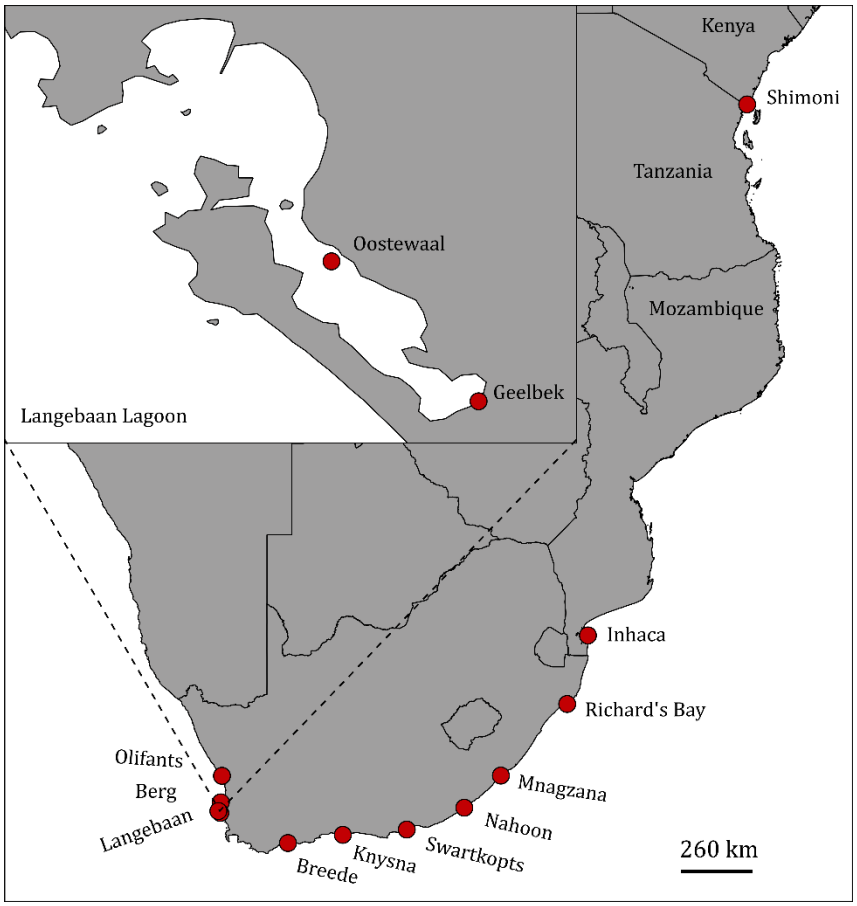

Figure 1.1 Sampling locations at estuaries along the South African and east African coasts with an inset indicating the two sites in Langebaan Lagoon.

Genomic DNA was extracted from dried leaf tissue using the Qiagen DNeasy plant kit (Qiagen, Valencia, USA) following standard protocols, with the exception of eluting the DNA in nuclease-free water instead of elution buffer. High grade nuclease-free water was used instead of the supplied elution buffer so that pooled samples could be freeze-dried without concentrating salts, which may interfere with downstream steps. Genomic DNA quality was then assessed using gel electrophoresis and the concentration of DNA in each sample was determined by Qubit analysis at the Central Analytical Facility of Stellenbosch University (CAF). For each sampling site, at least 60 ng of high molecular weight DNA per individual (Table 1.1) was pooled with equimolar representation to create a total of 12 pooled libraries of 2000 ng/ul concentration for Illumina sequencing.

The two sites at Langebaan, Oostewaal and Geelbek, were kept separate to allow for comparison between the observed morphotypes; one short and stunted on the muddy tidal flats (Geelbek) which experience prolonged exposure to conditions outside the

water and the other is longer with a higher biomass on the sandy permanently submerged area (Oostewaal) (Pillay, pers. Comm.).

Pooled genomic DNA was freeze dried and sent to the Hawaii Institute of Marine Biology (HIMB) for library construction (Knapp et al. 2016) and Mi-Seq Illumina sequencing through the Genetics Core Facility (GCF). Library preparation and sequencing followed the ezRAD method which obtains a reduced representation sequencing library using high frequency restriction enzymes (Toonen et al. 2013; Knapp et al. 2016). The KAPA Hyper Prep kit was used to prepare the size-selected DNA for sequencing. Before sequencing using the v3 2x300 PE kit on the Illumina MiSeq platform, library fragment size was established with a bioanalyzer and quantified with qPCR as quality control measures.

Table 1.1 Sampling locations, biogeographic zone and number of samples per site.

Location	Abbreviation	Coordinates		Biogeographic zone	Samples
Olifants	O	31.7021° S	18.1876° E	Cool-temperate Namaqua	30
Berg	B	32.7697° S	18.1438° E	Cool-temperate Namaqua	30
Geelbek, Langebaan	L1	33.1941° S	18.1211° E	Cool-temperate Namaqua	30
Oostewaal, Langebaan	L2	33.1214° S	18.0447° E	Cool-temperate Namaqua	30
Breede	BR	34.4074° S	20.8453° E	Warm-temperate Agulhas	30
Knysna	K	34.0791° S	23.0562° E	Warm-temperate Agulhas	30
Swartkops	SK	33.8650° S	25.6333° E	Warm-temperate Agulhas	30
Nahoon	N	32.9864° S	27.9517° E	Warm-temperate Agulhas	30
Mngazana	M	31.6921° S	29.4228° E	Suptropical Natal	30
Richards Bay	RB	28.8105° S	32.0947° E	Suptropical Natal	23
Inhaca, Mozambique	MOZ	26.0500° S	32.9297° E	Tropical Delagoa	10
Shimoni, Kenya	KEN	4.6741° S	39.3440° E	Tropical	3

Data quality and formatting

The quality of all raw reads was analysed using FastQC (Andrews 2010). Quality filtering and trimming was then carried out using FastQC Toolkit (Andrews 2010), removing low quality bases (<20 phred score), N's and contaminants. TrimGalore! v 0.4.4 (available at: http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was then used to remove any remaining adapter sequences or poor quality reads (<30bp long). Following filtering and trimming, reads were passed through FastQ groomer (available at: http://usegalaxy.org) to ensure correct formatting (Illumina 1.8+) for downstream analyses.

As an assembled and annotated genome exists for sister species, *Zostera marina* (available from NCBI, BioProject number PRJNA41721, GenBank accession number LFYR00000000, Olsen et al. 2016), BWA-MEM (Li 2013) could be used to map paired-end reads from each pooled site to this reference sequence. Default BWA-MEM parameters were used, except for confining the output to only map scores of greater than 20. SAM files were filtered using SAMtools (Li et al. 2009), removing ambiguously mapped reads, PCR duplicate reads, reads with less than 20 mapping quality and less than 20 base quality, before converting the SAM files to BAM files. Number of mapped and unmapped reads was then calculated using the *idxstats* command in SAMtools. To reduce sequencing bias in the data, mapped reads were subsampled to a median coverage in SAMTools using the *view* command with the '-s' flag. Although subsampling results in a loss of data, it is nonetheless important for correctly interpreting true differences between sites, as opposed to differences in data quality or quantity (Schlötterer et al. 2014). The effectiveness of this approach was confirmed by testing for a correlation between the number of filtered subsampled mapped reads and the number of SNPs and outlier loci identified below, using the *rcorr* function of the 'Hmisc' (Harrell Jr & Dupont 2006) package in R (R Core Development Team 2008). After sorting and indexing BAM files, they were using to call variants by creating pileup files for each individual sampling site with the *mpileup* command in SAMTools(Li et al. 2009), using a minimum quality score of 20 and maximum read depth of 10,000. Finally, a pileup file combining all sites was created using the same parameters in SAMtools and converted to a sync file using PoPoolation2 (Kofler et al. 2011b) for downstream use.

Calling SNPs and simulating data

The total number of SNPs and private SNPs, occurring in only one population, were identified using *snp-frequency-diff.pl* in PoPoolation2 (Kofler et al. 2011b) with genomic sites required to have a minimum minor allele count of four, and coverage between 10 and 500 across all 12 sites (Henriques et al. in review). SNPs were then filtered to retain only those present among sampling sites, and not those present due to differences between the reference sequence (*Z. marina*) and *Z. capensis*. As many software cannot handle pooled data, but require individuals to be specified with in sampling sites, *subsample_sync2GenePop.pl* in PoPoolation2 was used to simulate a multi-locus dataset of a subset of SNPs identified by PoPoolation2 (Kofler et al. 2011b; Guo et al. 2016). As this programme cannot simulate different amounts of individuals across sites, the median of 30 individuals was selected for every site. The resulting GenePop format file was then converted to various formats in PGDspider (Lischer & Excoffier 2012) for downstream analyses.

Outlier loci identification

Due to the uncertainty surrounding RADseq, Pool-seq and outlier detection methods (Narum & Hess 2011; da Fonseca et al. 2016; Mckinney et al. 2016; Lowry et al. 2017), multiple outlier detection methods were employed to increase confidence and decrease false positives. Loci potentially under selection were identified by four approaches, minimising the potential short-comings of each individual method.

Firstly, using the complete simulated dataset, an F_{ST} based approach to detect putative outlier SNPs was implemented in BayeScan v2.1 (Foll & Gaggiotti 2008), which implements a Bayesian approach to directly estimate the probability that each locus is under selection using a reversible-jump Monte Carlo Markov chain (MCMC). This was carried out with a prior odds ratio of 10, 20 pilot runs, burn-in of 50,000 iterations, thinning interval of 50 and a sample size of 5,000. Chain convergence was confirmed using the *coda* package (Plummer et al. 2006) in R.

Secondly, also using the complete simulated dataset, the Beaumont & Nichols Fdist approach (Beaumont & Balding 2004) was implemented in Lositan (Antao et al. 2008). This was carried out using 1 000 000 iterations and a False Discovery Rate (FDR) of 0.05. Loci with an unusually high or low F_{ST} value, conditional on heterozygosity, are considered as potentially under selection.

Thirdly, a genotype-environment correlation approach was implemented in BayeScEnv (de Villemereuil & Gaggiotti 2015), which tests for association between allele frequencies and environmental variables (Table 2.1). Although this method is based on the F model, it is able to consider two locus-specific effects; one due to divergent selection and another due to several processes other than local adaptation (e.g. range expansions, differences in mutation rates across loci or background selection) (de Villemereuil & Gaggiotti 2015). This method was implemented with the complete simulated dataset using 1 000 000 iterations, sample size of 20 000, thinning interval of 50, 20 plot runs with a length of 5 000, burn-in of 50 000 and an FDR of 0.05. Chain convergence was confirmed using the *coda* package (Plummer et al. 2006) in R.

Additionally, allele frequencies from the non-simulated dataset were analysed by means of a principal component analysis in the *pcadapt* package (Luu et al. 2016) in R. As this method is designed to analyse NGS data using the robust Mahalanobis distance, it is less computationally intensive and therefore faster than approaches using Bayesian statistics. Further, Luu et al. (2016) found that their method produces fewer false positive results than, for example, BayeScan. This software presented an interesting opportunity to compare the analyses implemented with the simulated and non-simulated datasets (see chapter 2).

Neutral and outlier loci

All loci putatively identified as being under selection were removed from the dataset to distinguish the effect of adaptive and neutral drivers on the patterns of population structure. The neutral-only multi-locus dataset set was then re-simulated for further analyses. Outlier loci identified by two or more methods were considered candidate outliers and were further analysed in chapter 2, thus the remainder of this chapter only examines the neutral dataset.

Genome-wide variation and differentiation

To characterise genomic diversity, Tajima's nucleotide diversity (π), Watterson's theta (Θw) and Tajima's D were estimated for the neutral-only dataset using a sliding window approach with *Variance-sliding.pl* in PoPoolation v1.2.2 (Kofler et al. 2011a), and averaged over all loci per site. All genomic sites subjected to analysis were required to have a minimum minor allele count of two and coverage between 10 and 500 per sampling site. As the estimation of allele frequencies in pooled individuals is highly

reliant on sequence coverage, a high sequence coverage and large sliding windows were used in order to increase accuracy (Kofler et al. 2011a). Average observed and expected heterozygosity and the inbreeding coefficient (F_{IS}) was estimated from the simulated neutral dataset with the *divBasic* function of the 'DiveRsity' package (Keenan et al. 2013) in R.

To investigate genome-wide levels of differentiation, the fixation index (F_{ST}) for pairwise comparisons of populations was estimated using a sliding window approach with *fst-sliding.pl* in PoPoolation2 (Kofler et al. 2011b), using a minimum minor allele count of four and a coverage between 10 and 500. Pairwise F_{ST} values were averaged over all loci per site. Fisher's exact test was carried out with *fisher-test.pl* in PoPoolation2 to estimate the significance of allele frequency differences between sites. Patterns of differentiation were visualised on a principal coordinate analysis (PCoA) plot generated in R (R Core Development Team 2008) using the *pco* function of the 'labdsv' package (Roberts 2007). The PCoA plot was generated both with and without Kenya in order to account for sampling bias. The simulated neutral dataset was used to investigate population clustering by means of Bayesian Analysis of Population Structure (BAPS) software (Corander & Marttinen 2006; Corander et al. 2006) testing K=1-10 with 100 iterations and a minimum population size of 1, as well as FastStructure (Raj et al. 2014) testing K=1-10 with the logistic prior model and default parameters.

Results

Sequencing and mapping

A total of 54 982 056 paired reads were obtained, with paired reads from each sampling site ranging from 1 368 372 to 7 429 328 (Table 1.2). After filtering reads for quality and adapters, and subsampling to a median value, a total of 7 432 397 reads, ranging from 222 741 to 750 736 per sampling site, were aligned to the *Z. marina* reference sequence (Table 1.2). The number of filtered subsampled mapped reads had no correlation with the number of SNPs ($r = 0.17$; $p>0.05$) or outlier loci ($r = -0.05$; $p>0.05$) identified.

Neutral and outlier loci

The complete simulated dataset consisted of 308 loci. From this dataset, 101 potential outlier loci were detected by Lositan, while BayeScan and BayeScEnv were much more conservative and only detected 25 and five potential outlier loci, respectively. By

analysing allele frequencies of the non-simulated dataset, PCAdapt identified 38 potential outlier loci. All putative outlier loci were removed from the dataset in order to examine only patterns of neutral variation. The complete dataset, including all outlier loci, was retained and utilized in downstream analyses (chapter 2).

Genome-wide variation

The number of SNPs identified by PoPoolation2 ranged from 845 to 1683 per sampling site, with between zero and six private SNPs identified within each sampling site (Table 1.2). The genome-wide average nucleotide diversity (Tajima's π) and the genome-wide average Θ_W ranged from 0.023 to 0.035 and 0.029 to 0.043 respectively, within each sampling site (Table 1.2). The west and south coast sites, with the exception of Oostewaal (L2), exhibited marginally higher nucleotide diversity and Θ_W than the east coast sites. Tests for deviations from neutrality produced genome-wide average Tajima's D that were negative for all sampling sites and ranged from -0.723 to -0.275. Genomic diversity metrics calculated from the simulated dataset included expected heterozygosity (0.04 to 0.06) and no observed heterozygosity within each sampling site when averaged over loci (Table 1.2), and the inbreeding coefficient, F_{IS}, which was uniform across sampling sites and equal to 1.

Genome-wide differentiation

F_{ST} values were estimated for pairwise comparisons of sites (Table 1.3), and Fisher's exact tests did not indicate any significant (p=0.05) differentiation between pairs of sites. However, a trend existed with larger pairwise distances between sites on the east and west/south coasts than sites within the east coast or west/south coasts. Similarly, clustering analysis conducted in BAPS on neutral loci revealed no significant structure between sites ($p < 0.05$), with all sites falling into one cluster (K=1; Fig. 1.2). Although all population group into one cluster, the PCoA (Fig. 1.3) of pairwise F_{ST} values suggests that the west and south coast sites, again with the exception of Oostewaal (L2), are more closely related than the east coast sites. The PCoA generated without Kenya produced the same pattern (S3). Further, the optimal model identified by FastStructure inferred 3-5 clusters (K=1-5; Fig. S1). Nevertheless, the lack of definitive assignment of individuals to particular clusters still indicates weak to non-existent population structuring.

Figure 1.2 Clustering analysis of the twelve sites estimated in BAPS for only neutral loci, with all twelve sites falling into one cluster.

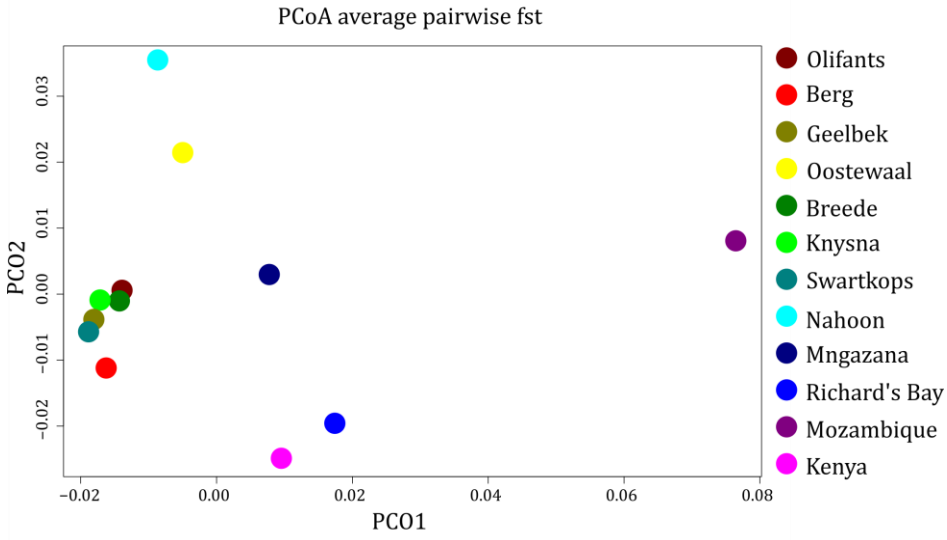

Figure 1.3 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons among the 12 sampling sites, for the subset of SNPs contained in the simulated neutral dataset.

Table 1.2. Summary statistics of RAD data and estimates of genomic diversity metrics per sampling site (refer to Table 1.1 for full names of abbreviations) for neutral dataset.

Sampling site	Number of raw reads	number of mapped reads	number of subsampled mapped reads	Number of SNPs	Number private SNPs	Tajima's π	Watterson's θ	Tajima's D	Average expected heterozygosity	Average observed heterozygosity	F_{IS}
O	5 862 886	1 457 363	743 255	1 278	2	0.034	0.041	-0.714	0.04	0	1
B	4 314 436	1 114 902	746 984	1 683	3	0.035	0.042	-0.722	0.04	0	1
L1	4 997 550	1 153 894	750 031	1 473	2	0.034	0.041	-0.698	0.04	0	1
L2	1 368 372	222 741	222 741	1 027	0	0.025	0.031	-0.616	0.06	0	1
BR	3 105 804	508 608	508 608	1 624	1	0.034	0.041	-0.705	0.05	0	1
K	5 943 674	1 251 227	750 736	1 342	1	0.035	0.041	-0.673	0.04	0	1
SK	5 882 100	1 360 205	748 113	1 387	0	0.035	0.042	-0.675	0.04	0	1
N	4 296 798	568 703	568 703	845	0	0.028	0.034	-0.654	0.05	0	1
M	3 991 420	475 470	475 470	914	1	0.025	0.032	-0.637	0.05	0	1
RB	7 429 328	781 740	750 470	1 105	0	0.022	0.028	-0.646	0.04	0	1
MOZ	4 136 268	719 319	719 319	598	0	0.026	0.028	-0.276	0.05	0	1
KEN	3 653 420	447 966	447 966	1 480	6	0.029	0.043	-0.324	0.04	0	1
Total	54 982 056	10 062 138	7 432 397	-	16	-	-	-	-	-	-
range	1 368 372-7 429 328	222 741-1 457 363	222 741 – 750 736	845 - 1683	0 - 6	0.023-0.035	0.029 - 0.043	(-0.723) - (-0.275)	0.04 - 0.06	0	1

Table 1.3. Pairwise F_{ST} values estimated among the 12 sampling sites (refer to Table 1.1 for full names of abbreviations) for the neutral dataset. No significance detected at P=0.05.

	O	B	L1	L2	BR	K	SK	N	M	RB	MOZ	KEN
O	-											
B	0.017	-										
L1	0.018	0.015	-									
L2	0.040	0.043	0.041	-								
BR	0.024	0.019	0.020	0.035	-							
K	0.019	0.016	0.016	0.033	0.018	-						
SK	0.021	0.017	0.015	0.042	0.022	0.016	-					
N	0.047	0.054	0.050	0.043	0.050	0.048	0.053	-				
M	0.037	0.037	0.038	0.040	0.037	0.035	0.041	0.046	-			
RB	0.050	0.043	0.048	0.058	0.050	0.048	0.049	0.067	0.037	-		
MOZ	0.092	0.095	0.096	0.087	0.092	0.095	0.097	0.095	0.075	0.073	-	
KEN	0.051	0.044	0.050	0.060	0.049	0.049	0.053	0.068	0.043	0.042	0.084	-

Discussion

This chapter sampled *Z. capensis* from 12 meadows across its ~5000 km distribution along the southern and eastern coasts of Africa, resulting in genomic data important for the management of this species and its ecosystem services. Overall, the results of this study revealed that there was no significant differentiation between sites (Fig. 1.2 and Table 1.3) and that genomic diversity varied little between sites (Table 1.2), when considering only neutral SNPs.

Genomic diversity of Z. capensis *across its range*

Although genomic variability did not differ greatly between the sampled sites, west coast sites did exhibit slightly higher levels of variability than east coast sites, with the exception of one site in Langebaan, Oostewaal (L2) (Table 1.2). This site had slightly lower genomic variability than adjacent sites, which is more in line with the level of variability observed in the east coast sites. The genomic variability of populations is influenced by several evolutionary factors, including natural selection, population size, connectivity, and reproductive strategy (Gaggiotti et al. 2009; Bragg et al. 2015; Martin et al. 2016; Gómez-Fernández et al. 2016). With all sites displaying very low heterozygosity and a high level of inbreeding (F_{IS}=1), it is likely that this species does indeed rely heavily, if not solely, on clonal growth and vegetative reproduction, rather than sexual reproduction (Table 1.2). In terms of reproductive strategy, clonality in seagrasses can vary between species with a continuum from monoclonality to meadows

with high clonal diversity (van Dijk & van Tussenbroek 2010), and the predominance of certain clonal lineages may indicate long-term selection on certain phenotypes. This selection may be in response to environmental variables, where conditions are more favourable for the predominant clonal lineages. However, the predominance of certain clonal lineages may also simply be the result of a shared ancestral source meadow prior to historic sea-level fluctuations reshaping the topography of the South African coastline (Ramsay & Cooper 2002; Compton 2011). For example, it is suggested that, following a glacial period, the seagrass *Posidonia oceanica* in the North-Adriatic region recolonised the area from one refugial clone, as all contemporary individuals share a single genotype (Ruggiero et al. 2002). Further, flowering in *P. oceanica* at this site has also not been observed and it is assumed that populations are maintained with low levels of variation through vegetative reproduction (Ruggiero et al. 2002). Similarly, *Z. capensis* is unlikely to be influenced by contemporary gene flow between its fragmented habitat, considering the lack of recorded sexual reproduction in this species (McMillan 1980) and the sheltered nature of seagrass habitats in South African estuaries (Van Niekerk et al. 2012).

Although a high degree of clonality, and therefore low genomic diversity, is considered to negatively impact productivity and resilience (Ehlers et al. 2008; Massa et al. 2013), vegetative reproduction plays a role in short distance dispersal and population maintenance (Alberto et al. 2005; Arnaud-Haond et al. 2012). Further, in long-lived — thousands to tens of thousands of years (Arnaud-Haond et al. 2012)— highly clonal species, vegetative reproduction and somatic mutations can play more of a role in generating and maintaining diversity than sexual reproduction (Wolf et al. 2000; Neigel 2002). This is especially true for disturbed habitats where it has been found that vegetative reproduction is favoured over sexual reproduction (Rasheed 2004). Notably, all sites had few - if any - private SNPs, suggesting that the majority of SNPs are present at all sites, albeit at varying frequencies depending on the level of clonality and selection (Table 1.2). Therefore, it is possible that the overall resilience of this species might be higher than anticipated, as each site carries the same genomic baseline, potentially safeguarding this variation against local site-level extinctions, for example. However, the low level of intra-site diversity would still leave individual sites vulnerable to the effects of environmental and anthropogenic pressures on the environment.

Differentiation and clustering analyses

Counter to my hypothesis of genetically structured populations neutral genomic variation did not reveal any significant differentiation between sites. Nevertheless, west and south coast sites were more closely related, suggesting a more recent origin, than east coast sites. It is therefore possible that the east coast served as a refugium during the Last Glacial Maximum (LGM), with subsequent westward dispersal. The west and south coasts may be less likely to provide suitable refugia for *Z. capensis* during the LGM due to the shifting coastlines and environmental conditions experienced during and after this period, because of the topography of the coastal plains (Compton 2011).

Although the patterns of genetic and genomic diversity and population structure vary widely across different seagrass species and regions, similar patterns to those detected in this study have been identified in other studies. For example, the threatened tropical seagrass, *Halophila beccarii*, displays low levels of diversity and differentiation across its range, with some sites consisting of only one genet (genetic individual; Phan et al. 2017). These authors suggest that low levels of differentiation are likely due to recent bottlenecks, and the dependence on clonal growth may be due to the poor pollen and seed dispersal from the relatively isolated lagoon habitat which the seagrass occupies (Phan et al. 2017). Similarly, another threatened seagrass, *Posidonia australis*, also exhibits a high degree of clonality and little population structure (Evans et al. 2014). As contemporary gene flow is unlikely between the isolated estuarine habitats in which *P. australis* occupies, the low level of differentiation between sites is suggested to be due to a common ancestral source meadow prior to historical sea-level changes (Evans et al. 2014).

These explanations seem a likely fit for *Z. capensis*, as it inhabits similarly isolated estuaries, and if they were to produce seeds, the dormant seed typically produced by the genus are not as buoyant as non-dormant seeds (Kendrick et al. 2016), thus further decreasing their realised dispersal. If this is the case for *Z. capensis*, and connectivity between sites is poor due the isolated nature of the estuarine habitats and harsh coastal conditions for vegetative fragments, careful management of remaining meadows needs to be considered as these may not naturally be replenished by propagules from adjacent estuaries. Restoration projects for this species should be considered, as from a genomic perspective based in neutral markers, they are likely to be successful, with the lack of

observed differentiation between sites enabling meadows to be supplemented with seagrass of similar genotypes from most other sites.

Chapter 2: Adaptive potential and resilience of *Zostera capensis*

Introduction

While standing genetic variation is the material on which selection can act, adaptive variation has been suggested to increase evolutionary resilience by improving the ability to persist through and adapt to changing environmental conditions (Bible & Sanford 2016). One of the major advantages of using an NGS approach is the ability to study potential adaptive variation. This is important as it informs the way in which species are reacting to local environmental conditions and possible future environmental changes. This can be carried out using SNPs by identifying loci that are potentially under selection. Genomic patterns at neutral markers reflect the outcome of gene flow and genetic drift, which in turn affect genome-wide variation within and among populations. This population structure is then acted upon by natural selection resulting in adaptive divergence or local adaptation. Despite high levels of gene flow, local adaptation to salinity and temperature gradients has been identified numerous times in marine species, such as in the three-spined stickleback, *Gasterosteus aculeatus* (Guo et al. 2015). Another example can be found in the riverine prickly sculpin, *Cottus asper*, of North America, where local adaptation to osmotic niches was discovered (Dennenmoser et al. 2016). These studies, amongst others, suggest that signals of local adaptation can override those of gene flow (Savolainen et al. 2013; Yeaman 2013; Huang et al. 2014; Tigano & Friesen 2016; Barth et al. 2017; Cure et al. 2017; Marques 2017). A recent review by Tigano and Friesen (2016) suggested that the mechanisms responsible for this can be divided into four main categories: (i) divergence hitchiking, (ii) increased resistance of linked loci to gene flow following secondary contact, (iii) competition among genetic architectures and (iv) competition among genomic architectures, including mechanisms that reduce or suppress recombination (Yeaman 2013).

Importantly, detecting adaptive variation can assist in pinpointing conservation units, as local adaptation is an important part of evolutionary diversification, even on a contemporary timescale (Funk et al. 2012; Stapley et al. 2010). This may be particularly important in the face of climate change and habitat alteration facing coastal systems in

the present age, because putative outlier loci may confer some adaptive advantages to change into the future. It is especially informative to identify whether adaptive variations lie with gene regions of know function, although this is hampered by a lack of annotated genomes for many species. However, when planning for biodiversity conservation, it is important to include adaptive variation in addition to neutral variation, as it should result in the protection of areas of greater conservation significance (Bonin et al. 2007; Carvalho et al. 2011; Funk et al. 2012; Hanson et al. 2017; Nielsen et al. in review).

However, it is important to note that a range of factors can display correlations between genomic diversity and environmental variables without direct selection (De Mita et al. 2013). For instance, genetic hitchhiking, the process by which the fixation of adaptive mutations by selection leads to the joint fixation of adjacent neutral loci (Kaplan et al. 1989) can result in increased signatures of selection. This effect was observed in a study on the divergence of sticklebacks into lake and stream habitats (Roesti et al. 2012), where the authors found a large number of genes under selection, with genomic divergence becoming increasing biased towards the centre of the chromosome, where recombination is at its lowest and genetic hitchhiking is most prevalent.

Adaptive variation in a population may be determined by identifying putative outlier loci. One approach to accomplishing this, is by averaging the genomic variation across all SNP loci in the population to create a baseline and then scanning the genome for regions deviating from neutral expectations (for example, elevated Fst values), indicating the potential action of selection (Ekblom & Galindo 2011; Willette et al. 2014). Another approach to identify putative outlier loci is by using genotype-environment correlations, using environmental variables such as temperature or salinity (Joost et al. 2007). Work by De Mita et al. (2013) showed that while genotype-environment correlation methods have substantially more power to detect selection than differentiation-based methods, they also generally suffer from higher rates of false positives. There is a chance that departure from Hardy-Weinberg Equilibrium (HWE) may increase the rate of false positives (pers. Comm. Oscar Gaggiotti), and although several studies have found this not to be the case (Chan et al. 2009; Fardo et al. 2009), caution should still be taken when interpreting results from populations outside of HWE (Lotterhos & Whitlock 2015; Hoban et al. 2016; O'Leary et al. 2018).

If outlier SNPs are found within an annotated gene region, this may signal some adaptive importance (Angeloni et al. 2012). Further, one can assume directional selection if selected loci exhibit greater variation between populations than expected (Hoffmann & Willi 2008). In contrast, selected loci exhibiting lower divergence than expected between populations suggest stabilising selection (Schmidt et al. 2008). These methods have been used to investigate adaptive variation and have identified both directional (Hohenlohe et al. 2010; Lexer et al. 2014; Gaither et al. 2015) and stabilising selection patterns in several studies (Hohenlohe et al. 2010; Gaither et al. 2015), providing unique insights into the evolutionary mechanisms of non-model species. However, few studies have addressed the question of whether outlier loci are unique or common among sites and their spatial distribution in natural populations remains fairly undescribed. Limited evidence suggests that complex polygenic traits may arise from multiple evolutionary pathways in response to habitat selection, producing patterns of non-shared divergence across populations under different pressures (Williams & Oleksiak 2008; Perrier et al. 2013; Ravinet et al. 2016). For example, Ravinet et al. (2016) identified putative outlier loci in two ecotypes of the rough periwinkle (*Littorina saxatilis*) across three islands and found only ~2–9% of outlier loci shared between all three islands, despite a high probability of gene flow and neutral genetic variation.

The study of seascape genetics has encouraged a shift from simply describing genetic patterns to investigating the various forces contributing to these patterns (Selkoe et al. 2016). Unlike landscapes, the heterogeneity of the seascape is generally hidden from view, with bathymetry, currents and water chemistry all playing a role in shaping patterns of genetic structure in species. Notably, the vast majority (48%) of seascape studies have focused on fishes, while marine angiosperms have been largely ignored (comprising only about 5% of studies to date; Selkoe et al. 2016). Further, studies are biased towards temperate waters (68%) with fewer studies covering intertidal (15%) or estuarine habitats (14%) (Selkoe et al. 2016). Seascape features such as temperature, salinity, irradiance, turbidity, depth and sediment type have all been shown to act as boundaries and significant drivers of genetic structure (González-Wangüemert et al. 2009; Roy et al. 2012; Viricel & Rosel 2014; Johansson et al. 2015). For example, depth was found to be the only significant environmental factor driving the genetic structure of the highly exploited white hake, *Urophycis tenuis* (Roy et al. 2012). Similarly, distinct genotypes of roundnose grenadier, *Coryphaenoides rupestris*, segregate by depth as they mature (Gaither et al. 2018), and such factors should therefore be incorporated into the

management of this species. However, in addition to present-day environmental conditions, historical processes should also be considered, as they often play an important role in shaping contemporary patterns of genomic diversity and differentiation (Hewitt 2000; Gaither et al. 2011; Toms et al. 2014; Leprieur et al. 2016; Chefaoui et al. 2017; Hernawan et al. 2017).

The highly variable South African coastline is a particularly interesting area in which to investigate the link between patterns of population structure and environmental features. As the seagrass *Z. capensis* occurs in estuaries along the range of southern Africa's estuarine and coastal conditions, it provides an excellent opportunity to study local adaptation and genomic variation along environmental gradients. As adaptive variation occurs along environmental gradients, it is important that studies consider not just geographical distances but also environmental 'distances' which may act in differentiating populations. Distance-limited migration together with local genetic drift produces local differences in allele frequencies which increases with geographic distance, resulting in patterns of Isolation By Distance (IBD). Patterns of IBD have been observed in organisms across a range of life histories, for instance marine invertebrates and fishes (Harris & Taylor 2010; Wright et al. 2015), marine mammals (Moura et al. 2014), estuarine invertebrates (Kelly et al. 2006; Teske et al. 2006) and fishes (Durand et al. 2005), and seagrasses (Olsen et al. 2004; Jones et al. 2008; van Dijk et al. 2009). A recent review of IBD studies found major discrepancies between marker types used to assess IBD and suggested that SNP data from high-throughput sequencing may provide the most power to detect IBD. Further, authors also found that IBD was usually only confirmed for coastal species when distinct regional population were pooled, and not when populations were analysed separately (Teske et al. 2018).

Further, environmental gradients over various spatial scales are able to act as soft barriers, producing similar patterns of isolation, termed Isolation By Environment (IBE) (Wang & Summers 2010; Wang & Bradburd 2014). For example, IBE across a hydro-chemical gradient was found to be a major driver of differentiation of the Amazonian fish, *Triportheus albus* (Cooke et al. 2012). Similarly, the euryhaline prickly sculpin (*Cottus asper*), have diverged along osmotic gradients with freshwater and estuarine populations showing significant differentiation despite high gene flow, suggesting strong selection by environmental conditions (Dennenmoser et al. 2014). A meta-analysis of population genetics studies revealed that although IBD and IBE are both

important in structuring populations, across all studies IBE played a larger role (Sexton et al. 2014). Limited attempts have been made to disentangle the effects and relative contributions of IBD and IBE, including a Bayesian model (Bradbury et al. 2013), redundancy analyses (Lasky et al. 2012), and structural equation modelling (Wang et al. 2013). Nonetheless, it is important to study these effects alongside each other in order to make meaningful contributions to management strategies.

Seascape features can affect not only differentiation between populations but also the distribution of genetic diversity. Theory predicts an edge effect in terms of genetic diversity, where 'rear' edge populations, closest to refugial areas, should harbour the highest diversity and 'leading' edge populations the lowest (Widmer & Lexer 2001; Diekmann & Serrão 2012). For example, this pattern has been observed in *Z. marina*, where populations in northernmost 'leading' edge of the distribution have a lower genetic diversity than central and 'rear' edge populations in the North Atlantic (Diekmann & Serrão 2012). Similarly, the mangrove, *Avicennia marina*, has shown an edge effect, where populations at the extremes of the distribution exhibit lower genetic diversity than those centrally located, likely due to inbreeding (Arnaud-Haond et al. 2006). Despite the genetic load usually associated with inbreeding, this strategy may offer the advantage of reproductive assurance or local adaptation (Arnaud-Haond et al. 2006). Conversely, for the large brown seaweed, *Saccorhiza polyschides*, genetic diversity was found to increase towards the range edge, perhaps reflecting a process of shifting genetic baselines or that genetic diversity at the range core was even higher in the past (Assis et al. 2013).

Aim

Given the wide gradient of conditions along the southern and eastern African coastline, this chapter aims to investigate the patterns of potential adaptive variation in *Z. capensis*, and the drivers thereof, using a seascape genomic approach. Local adaptation was assessed through identifying putative outlier loci and mapping adaptive variation across the range of *Z. capensis*. More specifically, this chapter aims to determine whether putatively adaptive loci are shared or unique amongst sites, and to determine if outlier loci fall within or near functional gene regions, which may inform on their adaptive importance. In addition, this chapter aims to examine the role of IBD and IBE, and to determine if *Z. capensis* exhibits an edge effect in the form of lower genomic diversity at the edge of the species distribution. Finally, using hindcasting of

temperatures and ecological niche modelling, I reconstructed the habitat availability for *Z. capensis* since the Last Glacial Maximum in order to help support the results from this chapter.

Hypotheses

As multiple selective forces can drive non-shared divergence across sites, I hypothesised that sites will exhibit more unique than shared outlier loci, representing alternate alleles from the same contigs, as each site experiences a unique combination of conditions along the diverse coastline. Further, I expected that sites in similar environments will exhibit a higher level of shared outlier loci than sites in dissimilar environments. Although sampling sites are geographically distant and are assumed to have limited dispersal capacity, little neutral variation was observed between sites in chapter 1. This, together with the highly variable conditions along the coastline, lead to the hypothesis that IBE will play a larger role in structuring populations than IBD. Regarding contemporary environmental conditions, I expect temperature and precipitation to be important drivers of adaptive variation in this seagrass, as these factors vary widely along its distribution. Further, I hypothesise that historical conditions will also play an important role in shaping contemporary patterns of genomic variation. In terms of an edge effect, *Z. capensis* meadows at the range edge are expected to display lower levels of genomic diversity than those at the core.

Methods

The complete dataset (putative adaptive and neutral loci) as well as the complete simulated dataset, generated in chapter 1, was utilised for this chapter.

Genomic variation and differentiation

Genomic diversity was characterised by estimating average nucleotide diversity (π), Watterson's theta (θ) and Tajima's D in PoPoolation v1.2.2 (Kofler et al. 2011a) following the same procedure outlined in chapter 1. Patterns of population differentiation, based on average pairwise F_{ST} values calculated as described in chapter 1, was visualised on a Principal Coordinates Analysis (PCoA) plot generated in the R package 'labdsv' (Roberts 2007). This was carried out with and without Kenya, in order to account for sampling bias, for both the complete dataset and for all outlier loci. As in chapter 1, population clustering was investigated in the simulated dataset by means of Bayesian Analysis of Population Structure (BAPS) software (Corander et al. 2006;

Corander and Marttinen 2006) testing K=1-10, and also using FastStructure (Raj et al. 2014), testing K=1-10 with the logistic prior model.

Once putative outliers were identified using BayeScan, Lositan, BayeScEnv and PCAdapt (chapter 1), the frequency at which each outlier appeared in each site was plotted using the 'ggplot2' package (Wickham 2009) in R and listed in Table S1. The frequencies of 10 randomly chosen neutral SNPs were also plotted using the same method in order to compare frequencies of neutral and adaptive loci. The overlap of outliers identified between the different approaches was visualised using a Venn diagram using the 'VennDiagram' package (Chen & Boutros 2011) in R, and provided in Table S1. Outlier loci identified by two or more approaches were considered candidate outliers and used in downstream analyses. The number and proportion of candidate outliers unique to each site and shared between pairwise sites was calculated using a custom script.

Functional annotation of candidate outlier loci

To evaluate the functional roles of candidate outlier loci, 1000 base pairs upstream and downstream of each of the 10 candidate outlier loci were subjected to BLASTx searches, with the non-redundant protein sequence database and an E-value cut off of 10^{-5} (Altschul et al. 1997) using the Blast2Go tool (Conesa et al. 2005). In addition to BLASTing against the general NCBI database, these searches were also carried out against the Zosteraceae family in general, and more specifically, the Northern Hemisphere seagrass, *Zostera marina* (Olsen et al. 2016a) and the Southern Hemisphere distributed *Zostera muelleri* (Lee et al. 2016), for which annotated genomes exist. Gene Ontology (GO) mapping, Interproscan and annotation were performed with Blast2Go default parameters.

Habitat suitability for Z. capensis *in the LGM*

In order to understand the influence of historical environmental conditions on the contemporary patterns of genomic variability, the suitable habitat for *Z. capensis* was hindcast to the LGM (21kya). This was carried out using the methods described in Chefaoui et al. (2017) for guidance. *Zostera capensis* occurrence data was obtained from Adams et al. (2016) and environmental data was downloaded from MARSPEC at 5 arcminute resolution for both the present-day (Sbrocco et al. 2018) and the LGM (CNRM-CM33 model; Braconnot et al. 2007; Sbrocco 2014). As it is important to avoid using strongly correlated variables when carrying out Species Distribution Modelling

(SDM), only Sea Surface Temperature (SST) of the coldest month (Biogeo14) and warmest month (Biogeo15) were utilised. These variables represent relevant present-day and LGM conditions, as they are recognised as important determinants of intertidal seagrass habitat suitability (Short & Neckles 1999; Short et al. 2001; Valle et al. 2014) and they are projected along the present-day (Sbrocco et al. 2018) and LGM coastlines (Braconnot et al. 2007; Sbrocco 2014), respectively. QGIS (QGIS Development Team 2012) was used to crop raster extents, by means of the buffer and crop tools, to focus on the coastal areas including and surrounding the present-day range of *Z. capensis*.

Ecological niche modelling was implemented through an ensemble approach with the 'biomod2' package (Thuiller et al. 2016) in R. As in Chefaoui et al. (2017), the following six presence-absence algorithms were included in the ensemble models: generalized additive model (GAM), flexible discriminant analysis (FDA), generalized boosting model (GBM), multiple adaptive regression splines (MARS), generalized linear model (GLM), and random forest (RF). Default parameters were used for all algorithms, except for the GLM which was fitted with a quadratic term, the GBM which was run with 1000 trees, and the GAM which was executed with the GAM_mgcv function. As the occurrence data (Adams et al. 2016) included reliable presence and absence records for estuaries along the entire South African coastline, no pseudo-absence selection was required. The data was split into a calibration (80%) and a validation (20%) set and three iterations were performed for each algorithm with three permutations to estimate and weight variable importance, for a total of 18 models. Models were assessed with the true skill statistic (TSS; Allouche et al. 2006) and the area under the receiver operating characteristic (ROC) curve (AUC; Fielding and Bell 1997), considering both specificity (true negatives) and sensitivity (true positives). Only models scoring TSS > 0.55 and AUC >0.8 were used to produce ensembles. Retained models were ensembled to produce a weighted mean SDM and first used to project the present-day habitat suitability, in terms of SST, along the South African coastline, and then used to hindcast the habitat suitability to the LGM. The present-day and LGM habitat suitability projections, as well as the changes in habitat suitability between the present-day and LGM were plotted in R. The full R script with SDM and plotting methods are detailed in Figure S4.

IBD vs IBE

A redundancy analysis (RDA) (Legendre & Legendre 2012) was conducted to evaluate the relative contribution of spatial and environmental variation to genomic variability.

As a multivariate regression technique, RDA can be useful when running regression analyses with multivariate predictors (space and environment) and multivariate responses (here, minor allele frequencies of SNPs - which is the frequency at which the alternate allele occurs and is a measure of variability). As spatial distances are not suitable for constrained ordination or regression, such is implemented in RDA, geographic distances were transformed to Principal Coordinates of Neighbourhood Matrix (PCNM) distances with the *pcnm* function in the 'vegan' package (Oksanen et al. 2018) in R. Environmental distances were calculated within the RDA function from the variables in Table 2.1 (excluding the macrophyte species measure, which were only available for South Africa, and therefore excluded from this analysis).

Table 2.1. Environmental variables included in BayeScEnv and IBE analyses.

Environmental variable	**Source**
Macrophyte species measures	
Submerged macrophyte area (ha)	(Adams et al. 2016)
Number of habitat types	
Submerged macrophyte species richness	
the CLiMond dataset	
Annual mean temperature (°C) (Bio1)	(Kriticos et al. 2012)
Max temperature of warmest week (°C) (Bio5)	
Min temperature of coldest week (°C) (Bio6)	
Annual precipitation (mm) (Bio12)	
Precipitation of wettest quarter (mm) (Bio16)	
Precipitation of driest quarter (mm) (Bio17)	
Annual mean radiation (W m-2) (Bio20)	
Annual mean moisture index (Bio28)	
World Ocean Atlas	
Salinity (PSS)	Zweng et al. (2013)
Dissolved Oxygen (ml/l)	Garcia et al. (2013)
Sea Surface Temperature (°C)	Locarnini et al. (2013)

The *ordistep* function from the package 'vegan' was used to select the most informative variables and build the 'optimal' model. Four separate RDAs were conducted with minor allele frequency as the response. Predictor variables in the first RDA were transformed geographic distances. In the second RDA predictor variables were environmental distances. Lastly, two partial RDAs were performed, partitioning out the effect of transformed geographic distance and environmental variation from the total variation respectively. The *anova* function of the package 'vegan' was performed with 999 permutations to test the significance of RDAs.

Edge effect

In order to determine if genomic diversity decreases towards the range edge (Assis et al. 2013), Pearson's correlation tests were carried out on genomic diversity against distance from the range core, using the *rcorr* function in the package 'Hmisc' (Harrell Jr & Dupont 2006) in R. Distance from the range core, defined as the central point of the distribution (the range being from the Olifants estuary on the west coast of South Africa to Shimoni, Kenya), was calculated along the coastline for each site using the 'road graph' plug-in for QGIS (QGIS Development Team 2012). Genomic diversity was measured as nucleotide diversity, number of SNPs and number of private SNPS (chapter 1), as well as allelic richness in the form of average number of alleles per locus, measured with the *divBasic* function in the 'DiveRsity' package (Keenan et al. 2013) in R from the complete simulated dataset.

Results

The complete simulated dataset consisted of 308 loci. From this dataset, 101 potential outlier loci were detected by Lositan, while BayeScan and BayeScEnv were much more conservative and only detected 25 and five potential outlier loci, respectively. The five outlier loci identified by the ecological association approach in BayeScEnv were correlated with precipitation of the driest quarter and annual mean moisture levels. By analysing allele frequencies of the non-simulated dataset, PCAdapt identified 38 potential outlier loci. All outlier loci were retained in the dataset in order to investigate patterns of adaptive variation.

Genome-wide variation

The number of SNPs identified by PoPoolation2 in the full dataset ranged from 913 to 1784 per sampling site, with between zero and six private SNPs identified within each

sampling site (Table 2.2). As stated in chapter one, there was no correlation between the number of filtered subsampled mapped reads and the number of SNPs or outlier loci. The highest number of private SNPs was observed in Kenya. The genome-wide average nucleotide diversity (Tajima's π) and the genome-wide average Θ_W ranged from 0.023 to 0.041 and 0.029 to 0.043 respectively, within each sampling site (Table 2.2). Nucleotide diversity mirrors the clustering described below (Fig. 2.1) and sites in the west/south coast cluster have slighter higher nucleotide diversity than those in the east coast cluster. Tests for deviations from neutrality produced genome-wide average Tajima's D that were negative for all sampling sites and ranged from -0.706 to -0.273. Genomic diversity metrics, heterozygosity and F_{IS}, calculated from the simulated dataset did not differ from chapter 1, with expected heterozygosity ranging from 0.04 to 0.06 within each sampling site and the inbreeding coefficient, F_{IS}, uniform across sampling sites and equal to 1 (Table 2.2).

Table 2.2 Estimates of genomic diversity metrics per sampling site (refer to Table 1.1 for full names of abbreviations) for complete dataset.

Sampling site	**Number of SNPs**	**Number private SNPs**	**Tajima's π**	**Watterson's θ**	**Tajima's D**	**Average expected heterozygosity**	**Average observed heterozygosity**	**F_{IS}**
O	1 362	2	0.034	0.041	-0.716	0.04	0	1
B	1 784	3	0.035	0.043	-0.723	0.04	0	1
L1	1 577	2	0.034	0.041	-0.700	0.04	0	1
L2	1 091	0	0.025	0.031	-0.616	0.06	0	1
BR	1 726	1	0.034	0.041	-0.706	0.05	0	1
K	1 436	1	0.035	0.042	-0.674	0.04	0	1
SK	1 483	0	0.035	0.042	-0.676	0.04	0	1
N	913	0	0.028	0.034	-0.651	0.05	0	1
M	997	1	0.026	0.033	-0.636	0.05	0	1
RB	1 192	0	0.023	0.028	-0.646	0.04	0	1
MOZ	668	0	0.027	0.029	-0.273	0.05	0	1
KEN	1 580	6	0.029	0.043	-0.323	0.04	0	1
Total	-	16	-	-	-	-	-	-
range	913 - 1784	0 - 6	0.023-0.041	0.029 - 0.043	(-0.706) -(-0.273)	0.04 - 0.06	0	1

Genome-wide differentiation

As in chapter 1, F_{ST} values were estimated for pairwise comparisons of sites (Table 2.3), and Fisher's exact tests did not indicate any significant (at p=0.05) differentiation between pairs of sites. However, in contrast to chapter 1, when the clustering analysis in

BAPS included outlier loci, two clusters were detected ($p < 0.05$), with the first cluster comprised of samples from the west and south coasts, and the second cluster comprised of samples from the east coast of South Africa together with the Mozambican and Kenyan samples (Fig. 2.1). Notably, one west coast site in Langebaan, Oostewaal (L2), groups with cluster two rather than cluster one. The optimal model identified by FastStructure inferred 2-6 clusters (K=3-6; Fig. S1). However, as for the putatively 'neutral' data set, there was no definitive assignment of individuals to particular clusters.

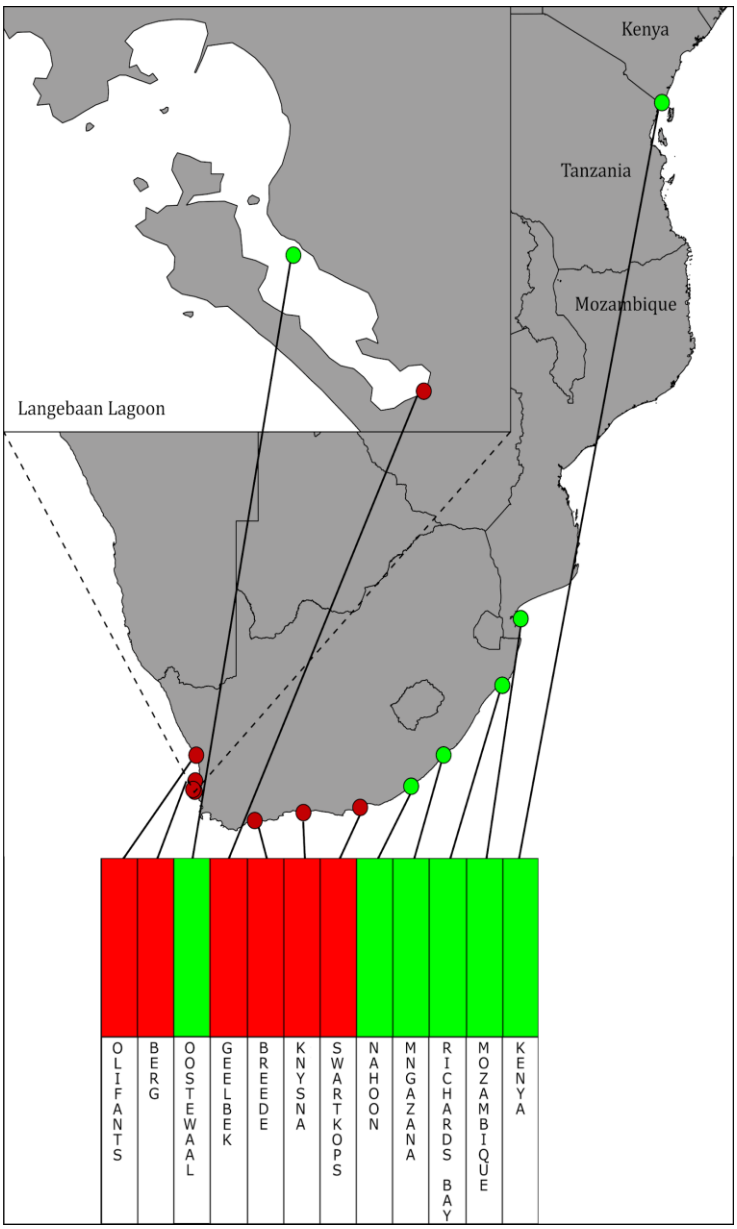

Figure 2.1 Clustering analysis of the twelve sites estimated in BAPS for the complete dataset, with the twelve sites grouped into two clusters.

Table 2.3 Pairwise F_{ST} values estimated among the 12 sampling sites (refer to Table 1.1 for full names of abbreviations) for the complete dataset. No significance detected at P=0.05.

	O	B	L1	L2	BR	K	SK	N	M	RB	MOZ	KEN
O	-											
B	0.023	-										
L1	0.022	0.019	-									
L2	0.048	0.053	0.049	-								
BR	0.027	0.021	0.021	0.042	-							
K	0.022	0.021	0.017	0.041	0.020	-						
SK	0.023	0.022	0.017	0.049	0.024	0.019	-					
N	0.061	0.068	0.059	0.047	0.061	0.058	0.061	-				
M	0.045	0.043	0.040	0.043	0.039	0.038	0.044	0.053	-			
RB	0.059	0.046	0.050	0.067	0.048	0.050	0.054	0.079	0.042	-		
MOZ	0.104	0.102	0.103	0.093	0.098	0.103	0.106	0.104	0.082	0.081	-	
KEN	0.067	0.054	0.060	0.071	0.054	0.060	0.064	0.084	0.050	0.046	0.086	-

While some outlier loci were identified by more than one method, there was little overlap between outlier loci identified using the four different approaches (Fig. 2.2), with only three outliers shared between them. Importantly, all putative outlier loci are shared across all populations (Table 2.4), however, the frequency at which outlier loci occur at each site reflects the two clusters identified using BAPS, with higher frequencies observed in the sites comprising cluster two than those comprising cluster one (Fig. 2.3). This pattern was observed regardless of the outlier identification method or the number of outliers included (Fig. 2.3); even with only three outliers, frequency differences could still define the two clusters. This pattern was not observed in neutral SNPs (Fig. S2). No private outliers were identified as all outlier loci occurred at two or more sites (Table S1).

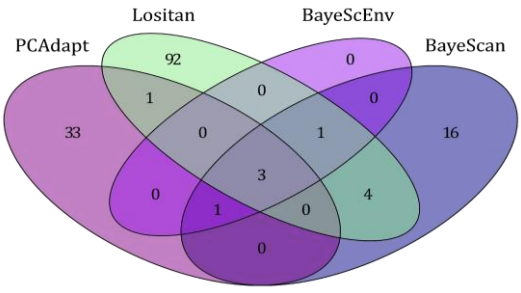

Figure 2.2 A Venn diagram illustrating the overlap between outlier loci identified using the four different approaches. Only three loci were identified by all four programs.

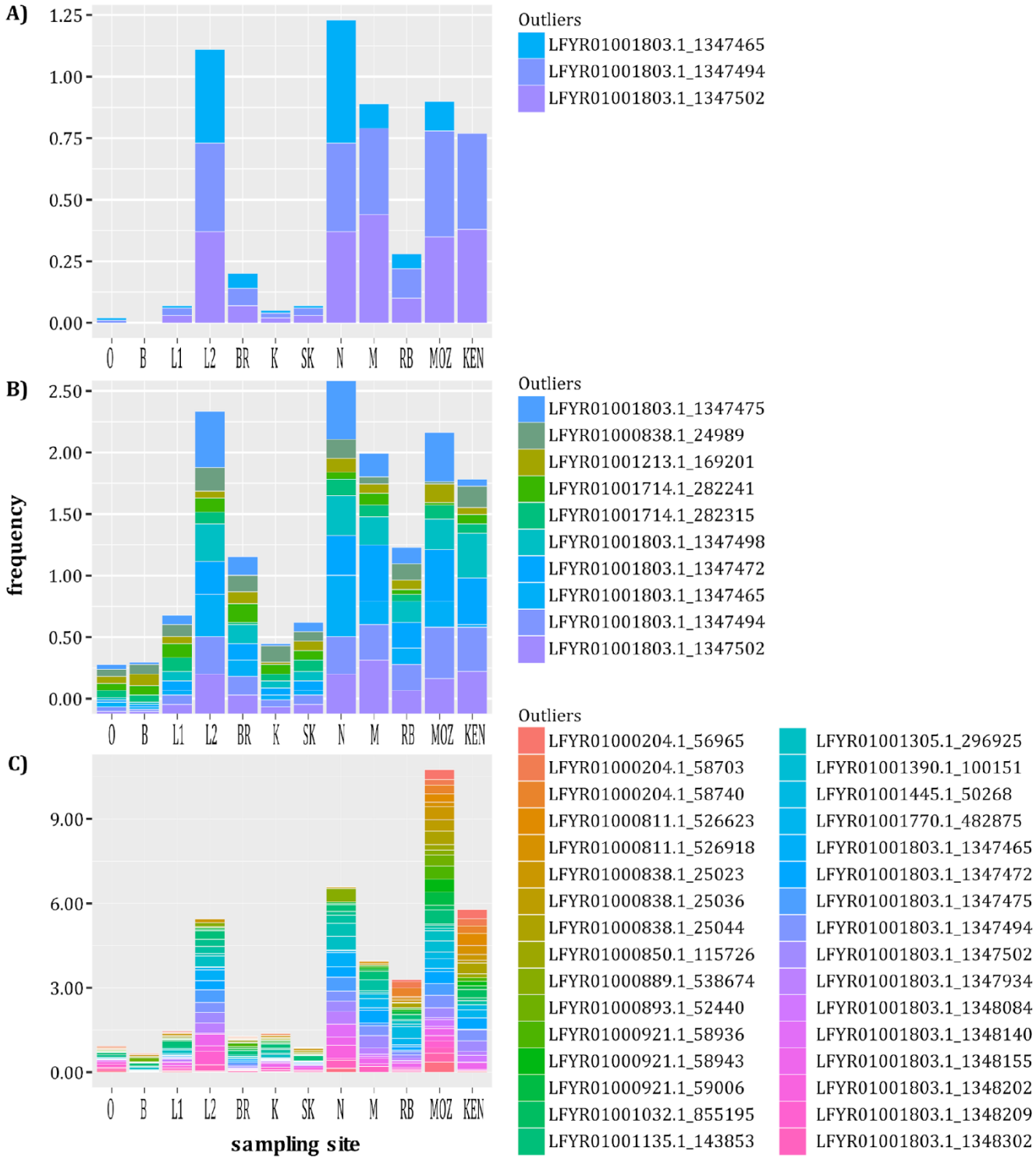

Figure 2.3 The frequency of outlier loci across sampling sites, as identified in chapter 1 by A) all four approaches (only three loci; identified by Lositan, BayeScan, BayeScEnv and PCAdapt), B) at least two approaches (10 loci), C) PCAdapt utilising the non-simulated dataset (32 loci).

PCoAs of pairwise F_{ST} comparisons from the complete dataset (Fig. 2.4) and all outlier loci (Fig. 2.5) resulted in a similar pattern to the outlier allele frequencies, with sites

from cluster one forming a tight group, relatively separate from the remaining sites. As in chapter one, the same pattern was observed when plotting the PCoA with and without Kenya (S3). Sites from cluster two did not group as closely as those from cluster one, with Mozambique most differentiated. Moreover, Mozambique, followed by Kenya, exhibited much higher outlier allele frequencies than other sites (Table S1). Notably, a similar but slightly looser pattern was observed in the neutral data (Chapter one).

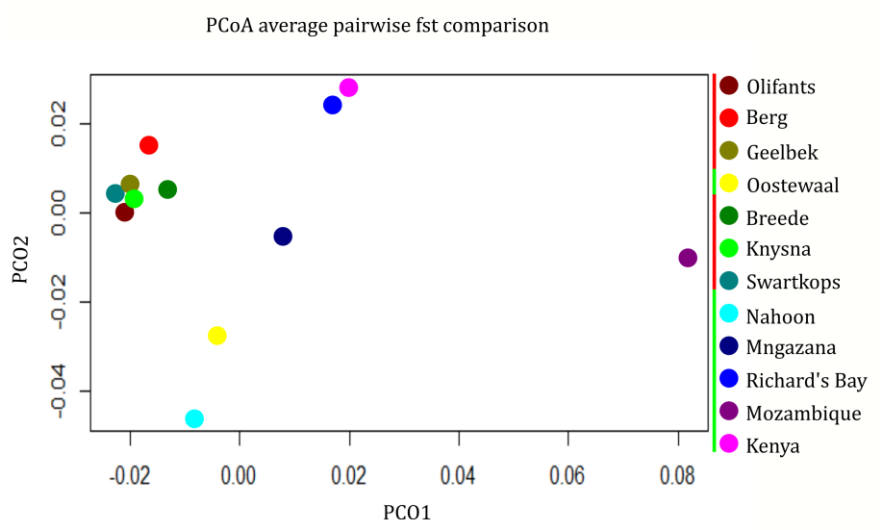

Figure 2.4 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons among the 12 sampling sites for the complete dataset. Sites grouping with cluster one and two are indicated by the red and green bar, respectively, in the legend.

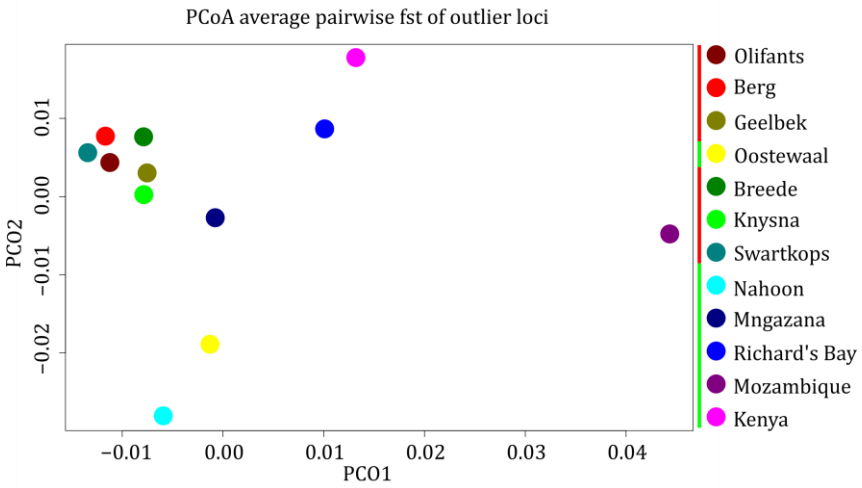

Figure 2.5 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons of outlier loci among the 12 sampling sites. Sites grouping with cluster one and two are indicated by the red and green bar, respectively, in the legend.

Habitat suitability for Z. capensis *in the LGM*

Multiple models from each algorithm met the TSS >0.55 and AUC >0.8 criteria and were retained to produce ensembles. Ensemble models obtained the following average validations scores: TSS=0.654, AUC=0.904, sensitivity=92.11, specificity=73.29. Predicted distributions of suitable habitat, in terms of SST, differed between present-day and LGM conditions, in terms of geographic location, extent and probability of occurrence (Fig. 2.6). The highest probability of occurrence can be seen on the south coast (up to ~25° longitude) and west coast (up to ~18° latitude) for the present-day projection, and on the western-south coast (up to ~21° longitude) and west coast (up to ~18° latitude) for the LGM projection.

Ensemble models project an 11.05% loss and a 10.79% gain of suitable habitat from the LGM to present-day, with a 26.1% range shift. These shifts are most evident in the loss of suitable habitat on the south and south-east coasts (~21-27° longitude), southern-west coast (~30-35° latitude), and west coast (~12-18° latitude), as well as the gain of suitable habitat on the northern-east coast of South Africa (~30-35° latitude), the south coast of Madagascar and the northern-west coast of Africa (~3-8° latitude). Further, within a South African context, the western-south coast represents an area of stable temperature regime, where suitable habitat has occurred from at least as far back as the LGM until the present day (Fig. 2.7). This can also be seen in patches on the west coast and on the east coast of Africa (~-5-25° latitude). By contrast, ensemble models project areas of unsuitable temperature regimes from the LGM to the present-day on the southern-east coast, despite this area overlapping with present-day *Z. capensis* occurrence (Fig. 2.7). This may simply be an artefact of the high number of estuaries in this region with relatelively few harbouring *Z. capensis*, in comparison to regions with more sparsely packed estuaries.

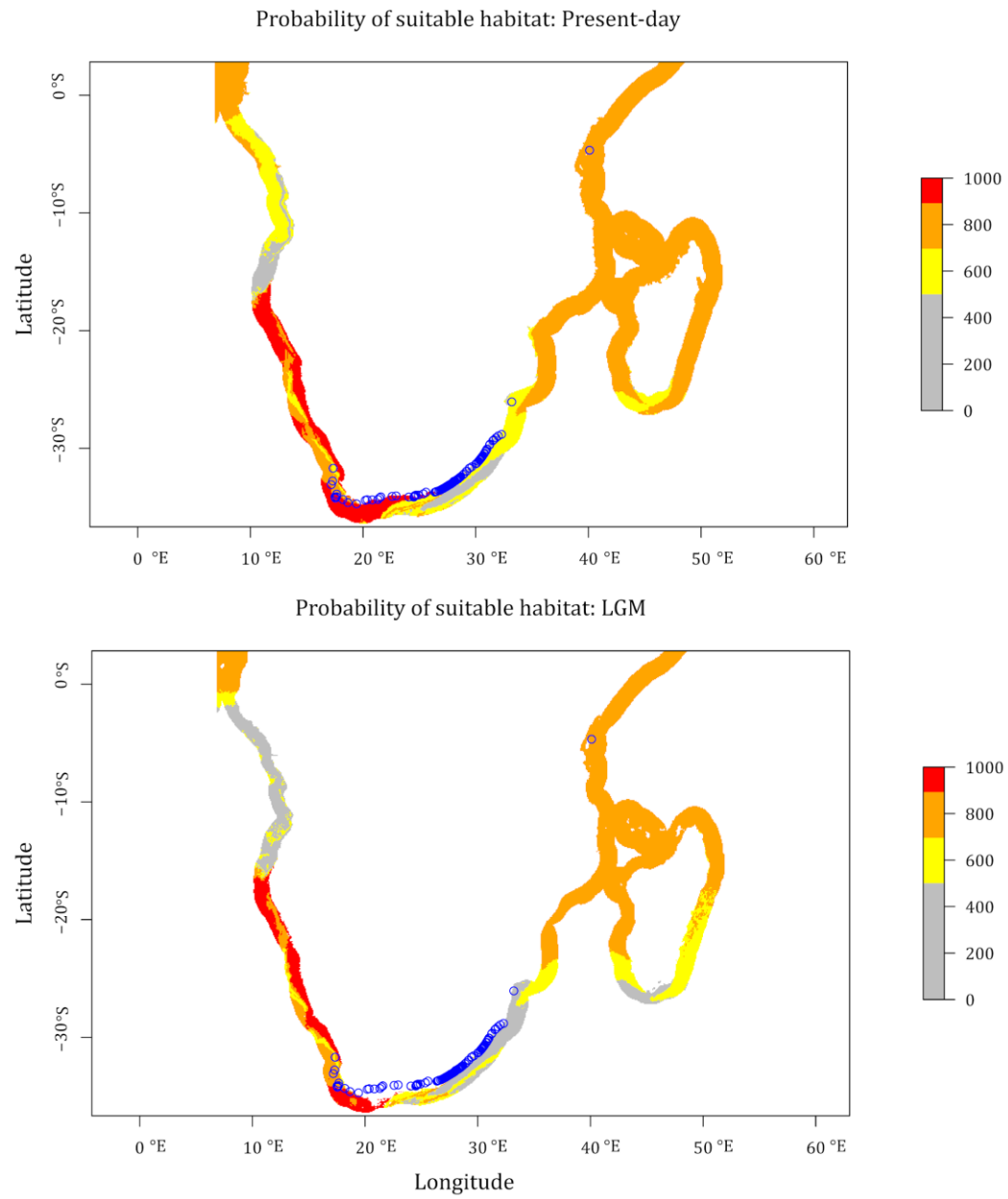

Figure 2.6 Ensemble model projections of probability of habitat suitability for the present-day (top) and the LGM (bottom), with surveyed estuaries represented by blue circles.

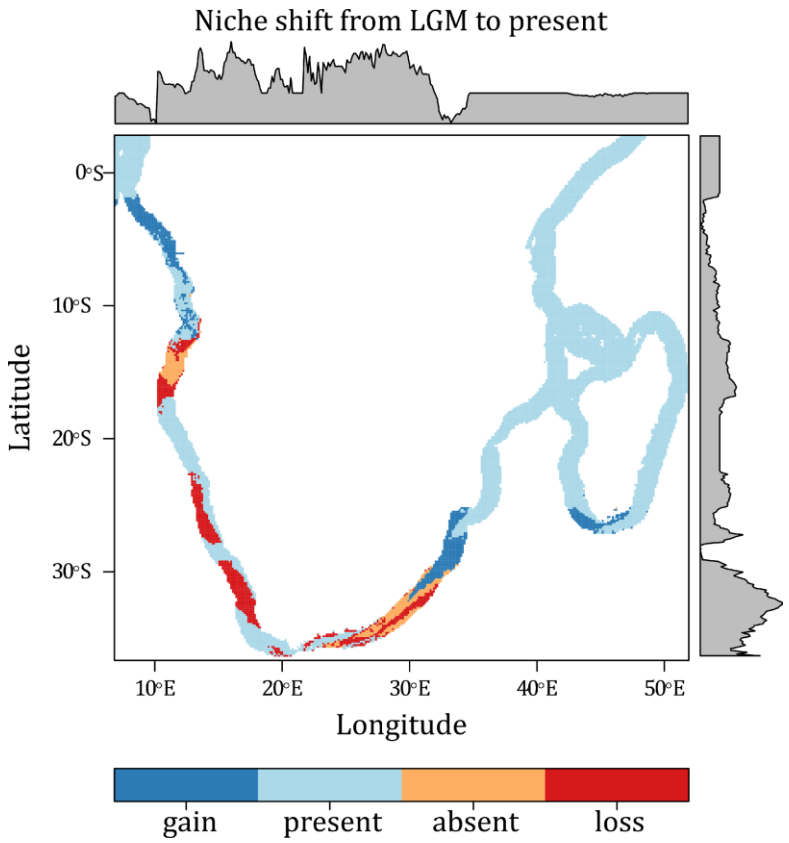

Figure 2.7 Projected changes in suitable habitat, in terms of SST, from the LGM to present with the probability of occurrence graphically represented along the x and y axes.

Functional annotation of candidate outlier loci

2000 base pairs surrounding each of the 10 candidate outlier loci were subject to the Blast2Go pipeline. Although all of the 10 candidate outlier loci yielded significant hits when BLAST searches were conducted against the general NCBI database, less hits were obtained when confining search results to Zosteraceae and further to *Zostera marina*, with no hits when confining search results to *Zostera muelleri*. However, the majority of these hits did not fall within a gene region of know function. GO terms (GO:0016020-IEA 'membrane' and GO:0016021-IEA 'integral component of membrane') were assigned to five of the 10 candidate outlier loci with BLAST matches to hypothetical and predicted proteins (Table 2.4).

IBD vs IBE

Of the 11 environmental variable listed in Table 2.1, seven were selected as the most informative; annual mean moisture index, annual precipitation, annual mean temperature, precipitation of the wettest quarter, maximum temperature of the warmest week, dissolved oxygen and sea surface temperature. The pure RDA of

genomic variation against transformed geographic distance was not significant, but was significant when carried out against environmental variation, with 70.4% of the variation in the data explained by the retained environmental variables. Unexpectedly, neither partial RDA analyses, one conditioned on transformed geographic distance and the other on environmental variation, were significant. Although environmental variation explained such a high percentage of the variation observed in the data, partitioning out the effect of geographic distance on environmental variation rendered the association with genomic variation non-significant.

Edge effect

Edge effect was assessed using distance from the range core and measures of genomic variation including allelic richness, nucleotide diversity, number of SNPs and number of private SNPs (Table 2.4). A positive trend was observed for increased nucleotide diversity, number of SNPs and number of private SNPs towards the range edge (Fig. 2.8). No trend was observed for allelic richness as this metric did not vary much between sites, ranging from 1.23 to 1.36. Further, the number of SNPs was significantly associated ($p<0.05$) with distance from the range core, with sites further from the range core exhibiting a greater number of SNPs.

Table 2.4 Measures of genomic diversity and distance values used to assess edge effect, with the genomic metric significantly associated with distance from the range core indicated with an asterisk (*).

Site	Distance from the range core (km)	Allelic richness	Nucleotide diversity	*Number of SNPs	Number of private SNPs
Olifants	4830	1.24	0.034	1278	2
Berg	4699	1.23	0.035	1683	3
Geelbek(L1)	4580	1.24	0.034	1473	2
Oostewaal(L2)	4563	1.36	0.025	1027	0
Breede	3920	1.3	0.034	1624	1
Knysna	3662	1.29	0.035	1342	1
Swartkops	3338	1.24	0.035	1387	0
Nahoon	3081	1.3	0.028	845	0
Mngazana	2857	1.32	0.026	914	1
Richard's Bay	2411	1.27	0.023	1105	0
Mozambique	2070	1.25	0.027	598	0
Kenya	3980	1.24	0.029	1480	6

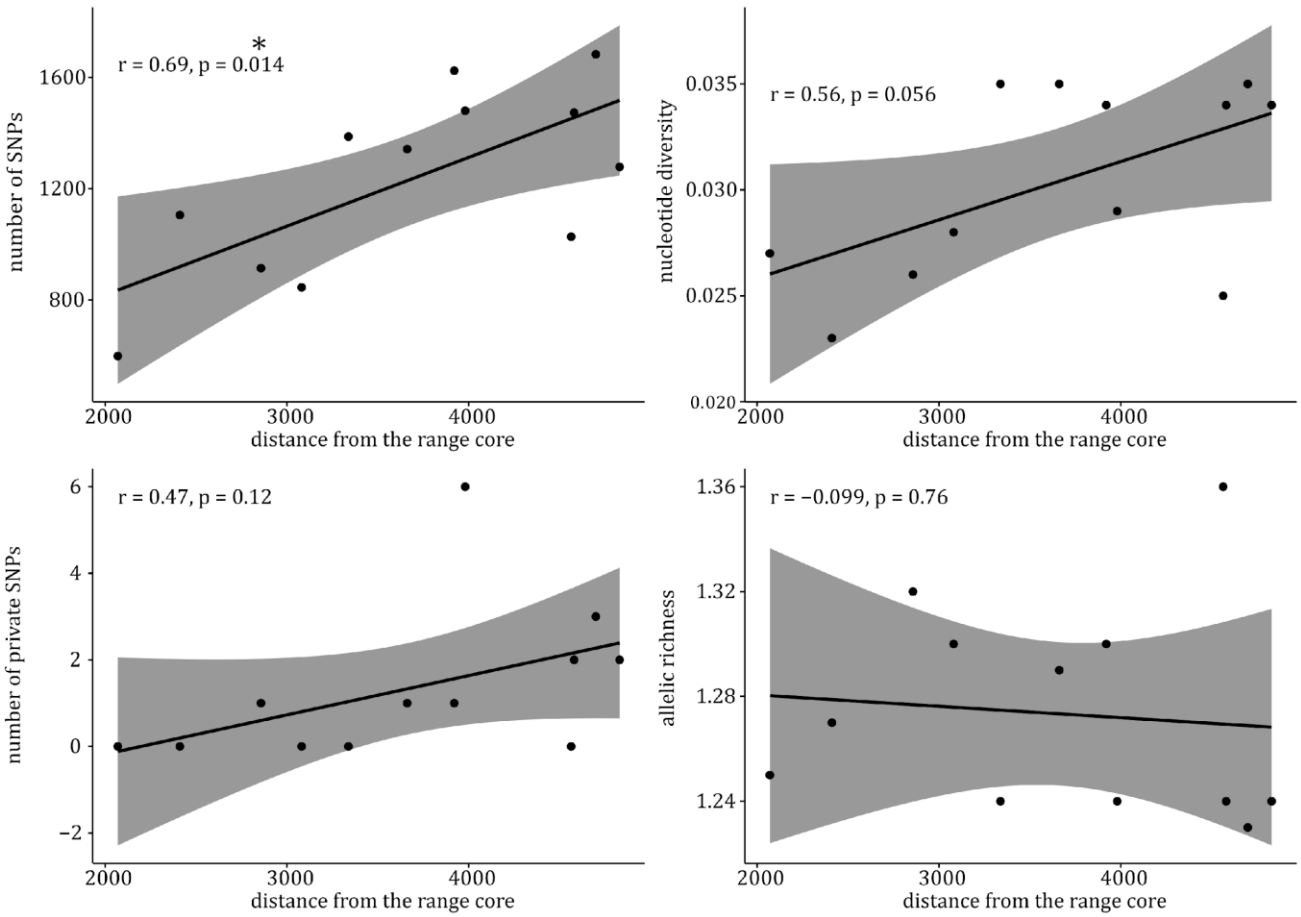

Figure 2.8 Pearson's correlation scatter plot of the association between distance from the range core and measures genomic diversity; number of SNPs, nucleotide diversity, number of private SNPs and allelic richness (from left to right).

Table 2.5 Allele frequency and counts of candidate outliers in each site identified by PCAdapt, BayeScan, Lositan, BayeScEnv, or combinations thereof. GO terms and BLAST hit sequences are given where matches could be made.

Method	**PCAdapt, BayeScan, Lositan, BayeScEnv**			**PCAdapt, BayeScan, BayeScEnv**	**BayeScan, Lositan, BayeScEnv**	**BayeScan, Lositan**				**PCAdapt, Lositan**	
Outlier locus	LFYR01001803.1_1347465	LFYR01001803.1_1347494	LFYR01001803.1_1347502	LFYR01001803.1_1347472	LFYR01001803.1_1347498	LFYR01000838.1_24989	LFYR01001213.1_169201	LFYR01001714.1_282241	LFYR01001714.1_282315	LFYR01001803.1_1347475	Count
O	0.005	0.005	0	0	0	0.02	0.02	0.017	0.02	0.005	7
B	0	0	0	0.004	0.004	0.033	0.041	0.03	0.023	0	6
L1	0.014	0.032	0.032	0.041	0.032	0.04	0.024	0.052	0.045	0.027	10
L2	0.375	0.359	0.368	0.35	0.359	0.104	0.024	0.054	0.041	0.45	10
BR	0.06	0.072	0.073	0.06	0.073	0.064	0.054	0.074	0	0.084	9
K	0.007	0.015	0.015	0.015	0.015	0.063	0	0.038	0.048	0	8
SK	0.013	0.025	0.025	0.025	0.025	0.04	0.028	0.034	0.06	0.025	10
N	0.5	0.361	0.366	0.37	0.493	0.066	0.047	0.022	0.072	0.493	10
M	0.1	0.35	0.438	0.449	0.359	0.019	0.029	0.039	0.039	0.102	10
RB	0.057	0.118	0.109	0.138	0.123	0.061	0.034	0.006	0.021	0.064	10
MOZ	0.118	0.429	0.444	0.443	0.429	0	0.067	0	0.048	0.42	8
KEN	0	0.394	0.375	0.438	0.394	0.079	0.026	0.033	0.03	0.02	9
Count	10	11	10	11	11	11	11	11	11	10	
GO terms	Integral component of membrane; membrane					Integral component of membrane; membrane		Integral component of membrane; membrane	Integral component of membrane; membrane		
BLAST hit	hypothetical					hypothetical protein	hypothetical protein	Predicted: uncharacte	hypothetical protein		

sequence	protein CQW23_33451 [Capsicum baccatum] gi|1270974041|gb|PHT26942.1|		ZOSMA_212G00120 [Zostera marina] gi|901815149|gb|KMZ69491.1| hypothetical protein ZOSMA_71G00010 [Zostera marina] gi|901796154|gb|KMZ58935.1| hypothetical protein ZOSMA_27G01320 [Zostera marina] gi|901811680|gb|KMZ67078.1|	ZOSMA_71G00010 [Zostera marina] gi|901796154|gb|KMZ58935.1|	rized protein LOC108221298 [Daucus carota subsp. sativus] gi|1040882023|ref|XP_017250676.1| hypothetical protein ZOSMA_212G00120 [Zostera marina] gi|901815149|gb|KMZ69491.1|	ZOSMA_212G00120 [Zostera marina] gi|901815149|gb|KMZ69491.1|

Discussion

Despite the generally low levels of genomic variation detected across the distribution of *Z. capensis* (Table 2.2), clustering analyses revealed differentiation of the sites into two major clusters when adaptive variation was considered in addition to neutral variation (Fig. 2.1 & 2.4). The first cluster was comprised of sites from the west and south coast, and the second cluster is comprised of sites from the eastern coast of Africa, except for one west coast site at Langebaan, Oostewaal, also grouping with this cluster (Fig. 2.1). Notably, this split between the clusters roughly coincides with the projected loss of suitable habitat on the south coast (Fig. 2.7) as well as the split between the described temperate and sub-tropical bioregions (Sink et al. 2012) along which phylogeographic breaks have been recorded for marine coastal species (von der Heyden 2009; Teske et al. 2011). Given that putative outlier loci occur at different frequencies within each cluster, this suggests some level of functional variation between the two observed clusters.

Shared adaptive divergence

Interestingly, all outlier loci were shared between all sites, suggesting the same genomic basis for each *Z. capensis* meadow. However, these clusters do not appear to be driven by a variation in alleles in the two clusters, as no private outlier loci were identified (Table 2.5). Rather, it appears that the frequencies of the shared outlier loci across the sites provides the foundation of the two clusters, with sites from cluster one exhibiting outlier loci at significantly lower frequencies compared to cluster two (Fig. 2.3). As most of the outlier loci were shared across all of sites, this suggests that the same suite of genes are possibly under selection across sites in response to the various gradients of environmental variables. Further sampling would be required in order to determine the extent of overlap between these two clusters. Notably, this pattern of differential outlier allele frequencies could be observed even when only considering the three outlier loci that were identified by all four outlier identification methods (Fig. 2.2). This also holds for the analyses on simulated and non-simulated datasets, confirming that regardless of the over-simplifications these simulated datasets could potentially introduce, or the number of SNPs one chooses to employ, this is a biologically significant evolutionary pattern. Further, the fact that there are no frequency differences in neutral loci, suggests that the observed pattern is highly likely a signal of selection, rather than time-dependent processes such as mutation and drift. Importantly, from a conservation

perspective where resource limited situations are often encountered, especially in developing countries, molecular techniques can be more affordably employed with a reduced number of appropriate markers to inform management decisions, once these markers have been identified.

There have been numerous other studies that also report high levels of shared adaptive variation across sites, such as that in Atlantic salmon of eastern Canada, where the allele frequencies of shared outlier loci were used in to assign individuals to their region of origin, assisting with stock management (Freamo et al. 2011). Similarly, in Pacific and Atlantic sticklebacks different allele frequencies of shared outlier loci have been used to distinguish marine and freshwater populations (Jones et al. 2012). Further, on an even smaller scale in western Canada, most outlier loci in sticklebacks were specific to single watershed regions (Deagle et al. 2012), suggesting that the shared or private nature of outlier loci might be highly context specific. In contrast, low levels of shared outlier loci were also observed in the periwinkle, *Littorina saxatilis*, in Sweden (Ravinet et al. 2016). Despite the possibility of high levels of gene flow and similar selective pressures, *L. saxatilis* populations displayed a considerable amount of non-shared genomic divergence, possibly due to complex polygenic traits involved in habitat adaptation.

Neutral vs adaptive variation

Although neutral variation can reveal much about a species demographic history, in many cases, adaptive variation is required to provide unique insight into evolutionary patterns, particularly from an environmental association perspective (Stapley et al. 2010; Guo et al. 2015; Funk et al. 2016; Gaither et al. 2018). In marine systems where gene flow is generally presumed to be high, adaptive variation can uncover population structure otherwise hidden in neutral markers (André et al. 2011; Freamo et al. 2011; Hess et al. 2013; Candy et al. 2015; Araneda et al. 2016; Tigano & Friesen 2016; Attard et al. 2018). For example, golden perch in Australia (Attard et al. 2018), Atlantic herring in the Baltic and North Sea (André et al. 2011), Atlantic salmon in eastern Canada (Freamo et al. 2011), and Chilean blue mussels (Araneda et al. 2016), all exhibit little to no structure in terms of neutral variation. However, increased population structure was observed when taking adaptive variation into account. There are also a few cases, where including adaptive variation did not alter the patterns detected by neutral variation, as observed in certain salmonids (Moore et al. 2014; Hand et al. 2016). Although this has not been investigated in seagrasses, it is plausible that in cases of low gene flow or high

levels of inbreeding, as suspected for *Z. capensis*, the structure detected due to adaptive variation reflects ancestral adaptation in conditions more conducive to gene flow or incomplete lineage sorting in more recent rapid local adaptation. However, it would be difficult to disentangle the signals of these two scenarios. Yet both the neutral and adaptive variation have the same genomic basis across populations sampled over 1000's of kilometers, revealing similar patterns of diversity across sites. As signals of adaptation can appear quickly (Lescak et al. 2015), perhaps too little time has passed for neutral processes, such as drift, to reflect the structure of the more tightly clustered west coast (Fig. 2.4 and 2.5).

How do IBD and IBE contribute towards the spatial arrangement of genomic variability in Z. capensis*?*

Despite the low probability of connectivity between sites, due to both the isolated nature of estuaries and the lack of sexual reproduction recorded for this species, geographic distance (IBD) was not a major driver of the observed genomic variation. Because of the spatial autocorrelation of environmental variables, there appears to be a large spatial component that plays a role in genomic variation, which cannot be separated from the effect of the environmental variables. IBE is likely an important driver of genomic variation in this seagrass, with the analysis of IBE revealing dissolved oxygen, annual mean moisture, precipitation and temperature related environmental variables as potentially important drivers of adaptive variation. Likewise, ecological association analyses correlated precipitation of the driest quarter and annual mean moisture levels with adaptive variation. However, it is important to be cognisant of the possibility of multi-collinearity of environmental variables, which may lead to incorrectly identifying variables as the selective pressure, when the true cause of selection lies in an untested, but correlated variable (Hoban et al. 2016).

Estuaries generally vary greatly in their physico-chemical characteristics over short time-scales as they are influenced by rhythmic tide cycles and often unpredictable inflow (Potter et al. 2015). Nevertheless, the southern and eastern African coastline has a wide gradient of environmental conditions affecting the production and survival of seagrasses. Seagrasses often have a broad temperature tolerance, suggesting some level of plasticity or local adaptation (Georgiou et al. 2016), although populations can respond to environmental change in different ways. For example, transcriptomic studies found that in *Z. marina*, individuals from higher temperature locations often perform

better post simulated warming events than those originating from cooler temperature locations, suggesting adaptation to local conditions (Franssen et al. 2011; Jueterbock et al. 2016). Further, intertidal species which are naturally subjected to greater temperature extremes, as experienced in the shallow waters which *Z. capensis* inhabits, tend to have a higher thermal tolerance than those in subtidal habitats (Franssen et al. 2014). This may provide a competitive advantage to species such as *Z. capensis* as coastal water temperatures rise in response to global warming. Precipitation and freshwater inflow have also been documented as important factors affecting growth and survival of estuarine seagrasses (Borum et al. 2004; Rasheed & Unsworth 2011; Furman & Peterson 2015), such as the tropical *Halodule uninervis* and *Halophila ovalis* which experienced declines in response to decreased precipitation and inflow, and increased exposure (Rasheed & Unsworth 2011). Local extinctions of *Z. capensis* have been recorded, in the Mtata estuary in South Africa for instance, as a result of reduced inflow and therefore a shift in the estuary dynamics (Adams et al. 2002).

Functional annotation of outliers

Although precipitation and temperature related environmental variables were linked with the observed adaptive variation, outlier loci identified here could not be associated gene regions of known function. BLAST hits to predicted and hypothetical proteins which could be assigned assign GO terms GO:0016020-IEA and GO:0016021-IEA suggest that genes linked to the regulation or production of cell 'membranes' and 'integral components of membranes' may be under selection. Similarly, in heat-stressed *Z. marina* genes related to cell wall fortification were upregulated, which authors suggest may increase cell wall thickness and thermotolerance (Franssen et al. 2014). It is plausible that *Z. capensis* reacts in a similar manner to the temperature clines across its distribution. A comparable study on the reef-building coral, *Pocillopora damicornis*, also found signals of selection for heat tolerance and associated processes (Thomas et al. 2017). The outlier loci which BLASTed to hypothetical proteins, but could not be assigned GO terms, could either indicate selection of gene regions of uncharacterised functions or possibly genetic hitchhiking (Barton 2000) involving regions of the genome which were not captured during RAD sequencing.

Clustering suggests divergent evolutionary histories for the south-west and east coast populations of Z. capensis

The patterns of contemporary marine species distributions are profoundly shaped by historical sea-level and climate changes (Hewitt 2000; Toms et al. 2014; Ludt & Rocha 2015). Theory predicts that genetic diversity will be higher at 'rear' edge populations, closest to refugial areas, and lowest at the 'leading' edge due to founder effects (Widmer & Lexer 2001; Eckert et al. 2008; Diekmann & Serrão 2012) and as such, the spatial distribution of diversity can be used to infer aspects of a species demographic history. Contrary to the hypothesis, the edge effect analysis for *Z. capensis* revealed that levels of genomic variation are highest furthest from the range core, with the Kenyan site harbouring the highest number of private SNPs (Table 2.2). This could indicate that genomic variation was even higher at the core in the past but has since decreased or that the edge populations could signal historic refugia. Evidence for the latter is provided by ensemble projections which indicate area of stable temperature along the east African coast (~-5-25° latitude), where suitable habitat could have occurred from at least as far back as the LGM until the present day.

Genomic variation can be affected by a variety of factors, including changes in population size, drift and connectivity (Gaggiotti et al. 2009; Bragg et al. 2015; Martin et al. 2016; Gómez-Fernández et al. 2016). It is likely that when *Z. capensis* population declines occurred, local recovery was facilitated by clonal growth, thereby decreasing genomic diversity. Further, historic sea-level fluctuations would have altered population connectivity substantially as they reshaped the topography of the coastline (Ramsay and Cooper 2002; Compton 2011; Toms et al. 2014). This has been demonstrated for the South African coastline, where sea-level change models revealed that lowered sea-levels during glacial periods reduced rocky intertidal habitat, resulting in the present-day patterns of two genetically diverged lineages of obligate rocky shore fish, *Clinus cottoides* (Toms et al. 2014). Reduced, and further fragmented, seagrass habitat, as suggested by ensemble projections along the south coast, may have also divided *Z. capensis* into two clusters, and higher genomic variation at the present-day range edges may represent refugial areas during this time, with subsequent dispersal into the present-day 'core' area.

Conversely, the topography of the coastal plains on the south and west coasts would have resulted in intensely fluctuating coastlines and environmental conditions during

the LGM (Compton 2011), which in addition to a lack of detailed bathymetric maps that could be used to reconstruct river and estuarine flows, make it impossible to accurately reconstruct potential estuarine sites during the last 21,000 years. PCoAs of both the neutral (Fig. 1.3) and adaptive variation (Fig. 2.4 and 2.5) indicate that east coasts sites are more distantly related from each other than are the west and south coast sites. Perhaps this pattern does not yet reflect strongly enough in neutral population structure to group sites into clusters (Fig. 1.2) due to a lack of time for drift to act in this potentially highly inbred species. As stated in chapter one, the more distantly related east coast sites suggest an earlier origin and support the likelihood of one refugial area on the east coast (Fig. 2.1). Further, the Kenyan site harboured the highest number of private SNPs, which may have been lost in other sites during subsequent south-westward dispersal. Further, the ensemble models indicate a second refugial area on the south-west coast and this area to be climatically stable and suitable habitat from the LGM to present (Fig. 2.7).

Genetic and genomic diversity is not only affected by neutral drivers but also by evolutionary processes such as natural selection (Gaggiotti et al. 2009). Higher genomic variation at the range edge could also be due to adaptive variation, which is expected under high selective pressures (Hampe & Petit 2005; Pearson et al. 2009), experienced at the extremes of the environmental gradient along the southern African coastline. While increased outlier loci, or the frequencies thereof, were not observed at the range edges of *Z. capensis*, increased private SNPs were observed (table 2.4 and 2.5). Nevertheless, these results illustrate the importance of including both neutral and adaptive patterns when considering the evolutionary histories of natural populations.

Chapter 3: Impact of environmental condition on genomic diversity

Introduction

In 2002 the Convention on Biological Diversity (CBD) was set up by world leaders with the aim "to achieve by 2010 a significant reduction of the current rate of biodiversity loss" (Secretariat of the Convention on Biological Diversity 2001). Other recent biodiversity conservation initiatives have had similar goals and include the European Marine Strategy Framework Directive (MSFD, Parliament 2008), Water Framework Directive (WFD, Kallis and Butler 2001), and the South African National Environmental Management: Biodiversity Act (Act 10 of 2004) (NEMBA). Another South African initiative, Operation Phakisa, emphasises sustainable development and preservation of biodiversity through an extensive network of MPAs (Operation Phakisa 2014). To assess the progress of these initiatives, a framework of indicators is often implemented. Indicators can include species' population trends, habitat condition, resource consumption, the presence of alien species and possible impacts of climate change (Butchart et al. 2010). Most of these factors are either directly or indirectly anthropogenic in nature. One thing that conservation initiatives have in common is a major concern over the loss of biodiversity, generally associated with species level and local population level extinctions.

Despite the fact that terrestrial defaunation has been occurring for tens of thousands of years, high impact marine defaunation, which emerged only hundreds of years ago, is rapidly increasing in pace and severity (McCauley et al. 2015). This profoundly impacts functioning and provisioning of services in every ocean (Duffy et al. 2005; Pillay et al. 2010; Mead et al. 2013; De la Torre-Castro et al. 2014; McCauley et al. 2015; Sullivan et al. 2017). In marine environments, where connectivity is generally 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 an increased concern for species level extinctions. This is particularly likely in poorly buffered estuarine systems which host important parts of many marine faunal lifecycles. Estuaries face many potential threats, including habitat modification, exploitation of coastal resources, industry (pollution), urbanisation and climate change

(Bjork et al. 2008; Mead et al. 2013). Further, the state, pressures and threats to South African estuaries are well understood and reported on through the National Biodiversity Assessments (Van Niekerk et al. 2012; NBA), which provides additional opportunities for understanding the evolutionary dynamics of key estuarine species. During the latest published 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). Further, even with the proposed MPA's under Operation Phakisa (Operation Phakisa 2014) estuaries will only receive limited protection.

Intraspecific genetic diversity is the foundation for biodiversity, and as such its conservation has been recognised by the IUCN and emphasised in the CBD (Allendorf 1986a; Laikre et al. 2009; Allendorf et al. 2014). Research supporting the importance of preserving genetic diversity to sustain species and ecosystems continues to build (Whitham and Bailey 2006; Beger et al. 2014; von der Heyden et al. 2014; Nielsen et al. 2017). Nevertheless, genetic and genomic diversity remains largely unmonitored, while ecosystem and species level diversity have received the bulk of the attention. By investigating demographic history under different anthropogenic disturbances, through the study of genomic diversity, population structure and connectivity, one can gain a retrospective view as well as insight into the future evolutionary potential of a population or species (Procaccini et al. 2007). As anthropogenic and climate pressures change into the future, species which are unable to adapt rapidly enough are likely to experience population declines due to loss of suitable habitat, with associated declines in genomic diversity. This has been illustrated for two species of *Anolis* lizards in the Amazonian forests, where models project severe declines in the genetic diversity of both species by the year 2080 in response to climate pressures and decreased suitable habitat (Prates et al. 2016). Further, some evidence exists that adaptive and neutral variation are lost at different rates, with faster declines in adaptive variation (Kirk & Freeland 2011; Hartmann et al. 2014). It is important to monitor both measures neutral and adaptive genomic variation to increase evolutionary resilience thereby safeguarding populations against future change (Bible & Sanford 2016). In addition to this, as adaptive variation is often lost more rapidly in response to population declines, it can be detected before neutral variation, and should thus be included in genetic monitoring efforts (Hartmann et al. 2014).

Genetic variability plays a vital role in increasing resistance and resilience against disturbances (Schaberg et al. 2008; Sgrò et al. 2011; Putnam et al. 2017). However, frequent and high intensity disturbances erode genomic variability through increased mortality and thereby the loss of genotypes. This can result in a negative feedback loop, producing populations that are less resilient to future disturbances and increasing the chance of local extinctions, which can enter species into an inescapable extinction vortex (Blomqvist et al. 2010; Davies et al. 2016; Lloyd et al. 2016; Miraldo et al. 2016). As keystone species in South African estuarine environments (Beckley 1983), the resilience of seagrasses is vital to safeguarding estuarine function in the Anthropocene (Folke et al. 2004; Burgos et al. 2017; O'Leary et al. 2017).

The impact of various measures of genetic variability on seagrass persistence has been summarised in Table 3.1. Allele and genotype diversity in seagrasses have both been found to have a positive influence on the ability to withstand environmental perturbations (resistance) as well as increased post-disturbance recovery (resilience) and productivity (Ehlers et al. 2008; Hughes et al. 2008 and references therein; Massa et al. 2013). Similarly, allele diversity and heterozygosity are also associated with positive population dynamics and the maintenance of ecosystem services in various seagrasses (Massa et al. 2013). For example, a restoration study found that seagrass beds with higher genetic diversity, in terms of allele richness, recovered faster and provided more ecosystem services (invertebrate habitat and nutrient retention) post disturbance than beds with lower genetic diversity (Hughes & Stachowicz 2004). Further, authors found that plants from 'high genetic diversity' beds demonstrated better ecosystem resistance, as fewer died from transplantation stress than in the 'low genetic diversity' beds (Hughes & Stachowicz 2004).

Table 3.1 The impact of various measures of genomic variability on the persistence of seagrass species from multiple studies.

Species	Genomic variable	Impact/buffer	Study
Zostera marina	Genotypic/clonal diversity	Resistance (shoot density)	(Ehlers et al. 2008)
Zostera noltii	Allelic richness, genotypic/clonal diversity	Resistance (first shoot count)	(Massa et al. 2013)
Zostera marina	Genotypic/clonal diversity	Resistance (shoot density)	(Hughes & Stachowicz 2004)
Zostera marina	Genotypic/clonal diversity	Resilience (shoot density, biomass) and function (associated invertebrate abundance)	(Reusch et al. 2005)
Zostera muelleri	Genotypic/clonal diversity	Function (associated invertebrate abundance)	(Macreadie et al. 2014)
Posidonia oceanica	Allelic richness, genotypic/clonal diversity and heterozygosity	Resistance and resilience (presence in disturbed sites)	(Jahnke et al. 2015) Meta-analysis

These trends have also been observed on a broader scale as evidenced by a meta-analysis of 34 datasets across various taxa, including plants, invertebrates, amphibians, marsupials and rodents, which illustrated the negative impact of a loss of heterozygosity on fitness (Reed & Frankham 2003). A study on the forest tree *Prunus africana* in Kenya that looked at adult plants and seedlings to represent before and after intensive human disturbance respectively, provided evidence that both allele richness and heterozygosity were significantly lower in seedlings than in adults. After 80-100 years of human impact these populations showed marked increases in inbreeding and reduction of gene flow (Farwig et al. 2008). Similar trends of genetic diversity increasing resistance have been observed in both corals (Hume et al. 2016) and lichen-forming fungi (Singh et al. 2015) and show that anthropogenic drivers of environmental change play important roles in shaping future patterns of genomic variation and in turn resilience and persistence.

The potential consequences of seagrass declines on genetic diversity have been summarised into four main scenarios: (1) population level extinction; (2) decreased allele or nucleotide diversity, that is the genetic variation on which selection for adaptation operates; (3) decreased genotype diversity through the survival of large clonal beds due to selection, (4) increased allele and genotype diversity resulting from increased sexual reproduction and post disturbance recruitment (Jahnke et al. 2015). However, the influence of disturbances on genetic diversity is likely to be highly complex, as reciprocal causality exists between disturbances that influence genetic diversity and the response of populations to disturbances in turn being influenced by genetic diversity (Hughes et al. 2007). Whilst genomic diversity metrics are known to give insight into historical demographics of species and populations, in an environment dominated by change, genetic monitoring may also prove useful as both status and early warning signs of seagrass declines, as has been shown for other species (Baker et al. 2000; Schwartz et al. 2007a; De Barba et al. 2010). To date, the relationship between anthropogenic impact and molecular variation is poorly understood for species with wide geographic distributions, usually few such data are available for the same geographic locations along which focal taxa might have been sampled. Therefore, given that pressure and threat data is well documented by the NBA, in addition to the genomic resources generated by this study provide an ideal scenario basis from which to test for such correlations.

Aim

This chapter aims to use a combination of neutral and adaptive variation to determine if a relationship exists between the condition of the environment and genomic variation of *Z. capensis*, as well as to assess the extent and direction of any associations. Further, this chapter also discusses the use of *Z. capensis* as an indicator species for assessing ecosystem condition.

Hypotheses

As disturbances and stressors have been found to result in a decrease in genetic diversity across various taxa, I hypothesise that poor environmental conditions and a high level of stressors will be associated with a lower level of genomic diversity (nucleotide diversity, heterozygosity and allelic richness) in *Z. capensis*. Further I

hypothesise that these measures will be higher in less impacted populations of *Z. capensis*.

Methods

Genomic diversity indices

Measures of genome-wide diversity per locus for each site in the form of nucleotide diversity and expected heterozygosity (chapter one), as well as allelic richness (AR) (chapter 2), calculated from the complete dataset containing both neutral and adaptive loci, were selected to be tested for association with environmental status and stressors.

Each of these measures captures a different aspect of genomic variability and therefore may respond differently to environmental pressures and may have different applications as indicators (Table 3.1). As the standing variation on which selection for adaptation may act, allelic richness and nucleotide diversity are important and often used measure of genetic diversity (Hughes et al. 2008; Jahnke et al. 2015). Further, allelic richness is often considered the most useful neutral measure for monitoring changes in population genetic variation as it is most sensitive to population declines (Schwartz et al. 2007b; Hoban et al. 2014; Jangjoo et al. 2016; Gedeon et al. 2017). Whilst nucleotide diversity and AR and are similar, nucleotide diversity captures the degree of variation among individuals and allelic richness captures the total diversity of the population, independent of the combinations of alleles (Reynolds et al. 2012), and is somewhat analogous to alpha diversity in ecology (Hoban et al. 2014). Another often-used measure of genetic diversity is heterozygosity (which is the average proportion of loci carrying two different alleles at a single locus within an individual), which can be considered a measure of evenness (Hoban et al. 2014). Although allelic richness and heterozygosity are correlated, as the maximum number of alleles per locus in a population is dependent on the proportion of heterozygous loci (Jahnke et al. 2015), the two measures have been found to respond to evolutionary processes in slightly different ways (Greenbaum et al. 2014). For example, it has been shown that allelic richness is more sensitive than heterozygosity to founder events followed by expansions, as allelic richness considers only the presence of alleles rather than abundances (Reed & Frankham 2003). Conversely, heterozygosity provides a better indication of the capacity of a population to respond to selection immediately after a bottleneck, with clear implications for resilience (Allendorf, 1986). Allelic richness also better reflects the abundance of rare alleles than heterozygosity (Reed & Frankham

2003). Further, heterozygosity has been linked to both average individual fitness (Reed & Frankham 2003) and ecosystem service provision (Jahnke et al. 2015).

Environmental status and stressors

As part of the NBA (Van Niekerk et al. 2012), estuary condition as well as various stressors were defined for nearly 300 South African estuaries (Fig. 3.1). Estuary condition was characterised by examining the extent to which current abiotic (eg. hydrology, water chemistry and sediment processes) and biotic (faunal and floral groups) components differed from the reference or 'natural' condition. This was described using six categories ranging from A) "unmodified/natural" to F) "Critically/Extremely modified (Table S2). Modifications have reached a critical level and the system has been modified completely, with an almost complete loss of natural habitat and biota. In the worst instances the basic ecosystem functions and processes have been destroyed and the changes are irreversible (Van Niekerk et al. 2012). Each of these categories are associated with a loss of functionality. Three of the study estuaries fell into category B, four into C and one into D, with no estuaries in category A, E or F. Human induced stressors on these systems were also quantified and included change in flow of the estuarine system, pollution, direct habitat loss due to infrastructure development for example, sand mining and fishing effort (Fig. 3.1). These were graded as very high (VH), high (H), medium (M), low (L), absent (N) or present (Y) as applicable (Table S2). As the sites at Langebaan, Mozambique and Kenya did not form part of the NBA, they were excluded from this analysis.

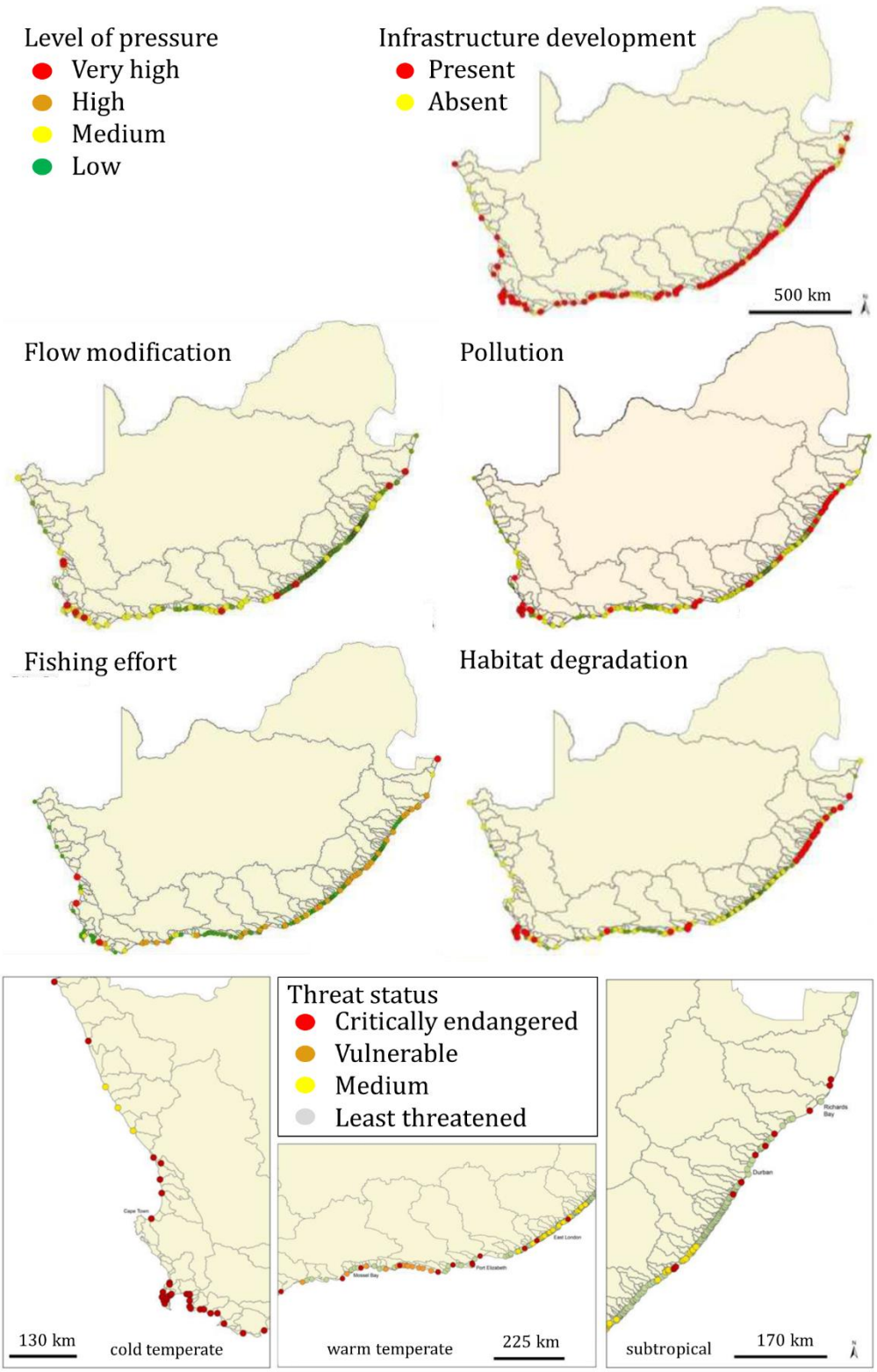

Figure 3.1 Locality maps from the NBA 2011 indicating the level of infrastructure development, flow modification, pollution, fishing effort and habitat degradation in each estuary as well as the overall threat status by biogeographic region.

Statistical analysis

Generalised linear models (GLMs), with a normal distribution (Gaussian) and identity link function, were fitted and used to test for associations between each of the three genomic diversity indices and environmental stressors. This was conducted with the base GLM function in the statistical environment R (R Core Development Team 2008), using Rstudio V 0.98.1102 ("RStudio" 2012). Nucleotide diversity, heterozygosity and allelic richness per locus across sites were each separately fitted as response variables with estuary condition and the 5 environmental stressors (Table S2) as predictor variables. Cross-correlated and Non-informative predictor variables were removed from GLMs using backward selection via the *step* function. Lastly, Spearman's correlation tests were carried out to assess the association between genomic diversity indices and the area of submerged macrophytes per estuary, as estimated by the NBA in 2012 (Van Niekerk et al. 2012), using the base *cor.test* function of R.

Results

Estuary condition was highly collinear with various measures of environmental stress and was therefore removed from the analysis. This is unsurprising as estuary condition was developed as an overall measure, based on environmental stressors. After removing collinear and non-informative variables, fitted GLM's included fishing effort, habitat loss and sand mining as predictors of nucleotide diversity; fishing effort, sand mining and change in flow as predictors of heterozygosity; and fishing effort and habitat loss as predictors of allelic richness. Nucleotide diversity was significantly negatively associated with the presence of sand mining and habitat loss, and positively associated with fishing effort. Heterozygosity was significantly negatively associated with fishing effort and sand mining. Although non-significant, change in flow was positively associated with heterozygosity. Allelic richness was significantly negatively associated with fishing effort. The results of all GLM's are summarised in Table 3.2. None of the diversity indices were significantly associated with the area of submerged macrophytes.

Table 3.2 Effect of environmental stressors (habitat loss, sand mining, fishing effort and change in flow) on genomic diversity indices (nucleotide diversity, heterozygosity and allelic richness) in a GLM.

	Estimate	Standard error	t-value	Pr(>|t|)
Nucleotide diversity ~ habitat loss + sand mining + fishing effort				
Intercept	0.035	0.001	63.960	< 2e-16 ***
Habitat loss (M)	-0.008	0.001	-6.864	7.02e-12 ***
Habitat loss (H)	0.001	0.001	0.872	0.383
Sand mining (Y)	-0.013	0.002	-6.174	6.87e-10 ***
Fishing effort (VH)	0.008	0.001	7.174	7.75e-13 ***
Heterozygosity ~ sand mining + fishing effort + change in flow				
Intercept	0.059	0.001	49.070	< 2e-16 ***
Sand mining (Y)	-0.006	0.002	-2.615	0.00892 **
Fishing effort (VH)	-0.005	0.002	-2.625	0.00866 **
Change in flow (M)	0.003	0.002	1.559	0.11902
Allelic richness ~ habitat loss + fishing effort				
Intercept	1.292	0.017	74.815	< 2e-16 ***
Habitat loss (M)	0.017	0.024	0.686	0.49271
Habitat loss (H)	-0.036	0.024	-1.475	0.14045
Fishing effort (VH)	-0.073	0.024	-2.982	0.00289 **

**P<0.05 and ***P<0.001

Discussion

Patterns of genomic diversity of *Z. capensis* can be linked to environmental condition as nucleotide diversity, heterozygosity and allelic richness were all significantly negatively associated with various environmental stressors. However, it should be noted that it is difficult to disentangle the effects of such contemporary drivers from those of historic processes that may be responsible for shaping patterns of diversity present today. Destructive practices such as fishing, sand mining and habitat loss appear to play the greatest role in decreasing genomic diversity. Fishing effort was the only stressor that could be associated with all three measures of genomic diversity. As fishing effort increased between sites, heterozygosity and allelic richness significantly decreased and, interestingly, nucleotide diversity significantly increased. Likewise, the presence of sand mining activities, habitat loss and a change in flow all had an important impact on the genomic diversity of *Z. capensis*. It is not surprising that each measure of genomic diversity reacted slightly differently to environmental disturbances as they each capture different aspects of variability. For example, nucleotide diversity responded strongly to

pressures that results in the immediate loss of genets (Fig. 3.2). As nucleotide diversity captures the degree of variation among individuals, the removal of groups of individuals will clearly exert a strong impact on this measure of variability. The different reactions of heterozygosity and allelic richness to environmental pressures could possibly be explained by the first principal of conservation genetics, where the immediate impact of population declines is first observed in the number of alleles rather than heterozygosity (Allendorf 1986c; Ryman et al. 1995).

Seagrasses are incredibly sensitive to physical disturbance, and major contributors to global declines include destructive fishing practices, boat damage, dredging and sedimentation (Erftemeijer & Robin Lewis 2006; Orth et al. 2006a; Waycott et al. 2009a). Unsustainable and destructive fishing practises and overfishing can have numerous effects on seagrass meadows (Waycott et al. 2009b). These practices can include poison, blast fishing, trawling and, most commonly, seine-net fishing, which all pose major threats to seagrass meadows globally, and specifically seine-net fishing in South Africa (Van Niekerk et al. 2012), as they cause direct and immediate seagrass loss (Cullen-Unsworth et al. 2013). For example, bivalve harvesting in Maputo, Mozambique, is suspected to be responsible for a staggering ~80% decrease in *Z. capensis* cover (Green & Short 2003). Similarly, *Z. marina* beds in Toralla Island, Spain, impacted by clam harvesting had significantly lower shoot density and biomass than non-impacted beds. Notably, impacted and non-impacted beds displayed different seagrass-associated community structures and bivalve abundances (Barañano et al. 2017). Further, damage caused by destructive gear can often be long lasting as observed in *Z. marina* beds in the coastal bays of the Delmarva Peninsula, USA (Orth et al. 2002). After suffering severe decreases in cover due to hydraulic dredging conducted by clam and oyster fisheries, these beds only displayed a partial recovery after three years (Orth et al. 2002). However, beds in the same area have more recently experienced significant recovery due to restoration efforts (Orth et al. 2006c). Adams (2016) states that 'although fast-growing, *Z. capensis* does not colonise new areas quickly', so it is highly likely that any loss of cover could also result in the loss of some genetic and genomic diversity, although the extent and rate of loss are not possible to determine at this stage.

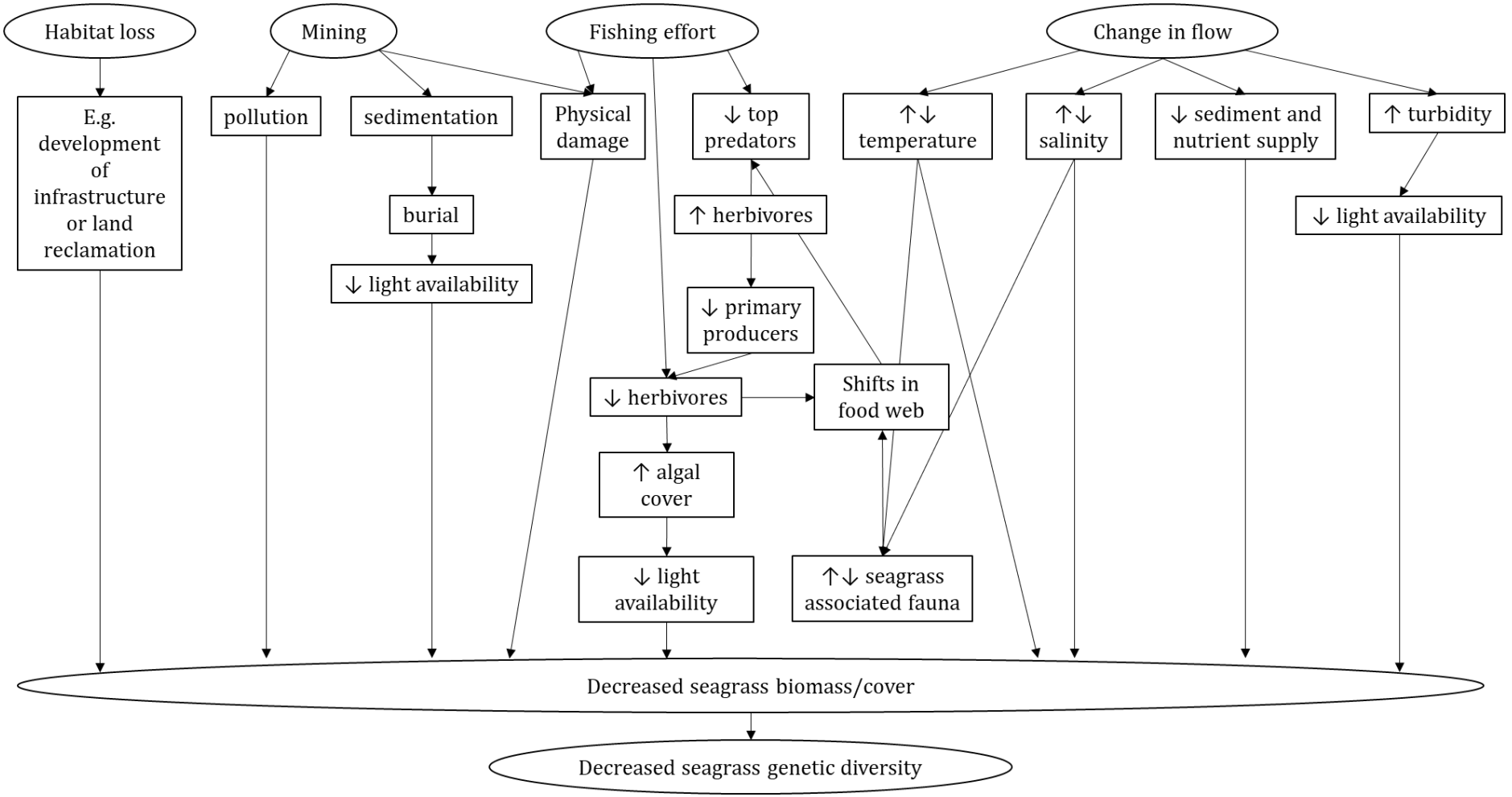

Figure 3.2 Diagram illustrating the impacts of each of the environmental stressors retained by the GLM on seagrass biomass or cover and seagrass genomic diversity.

Fishing effort can have subtle indirect impacts on seagrass. Overfishing of herbivores (Duffy et al. 2005) themselves, or of top predators causing a cascade down the food web, drastically alters the trophic structure of seagrass ecosystems (Jackson et al. 2001; Myers et al. 2007; Moksnes et al. 2008; Huxham et al. 2018 and references therein). Grazing invertebrates play a critical role in seagrass ecosystems as they maintain a low algal biomass on seagrasses, preventing smothering and increasing light availability (Heck & Valentine 2006). There is also a link between grazer diversity and seagrass biomass, with a diverse grazer assemblage increasing seagrass ecosystem functioning (Duffy et al. 2003). While the entire South African coastline suffers from over-fishing, large estuaries in cool temperate estuaries are particularly overexploited (in terms of tonnes per ha removed) (Van Niekerk et al. 2012). The majority of catches are illicit and all fishing efforts are dominated by the use of gillnets, which are highly damaging to both seagrasses, as the weights cause physical damage, and seagrass associated communities with limited selectivity on species or age (Van Niekerk et al. 2012). For example, indiscriminate gillnet fishing over the last 100 years has resulted in populations of the white Steenbras, *Lithognathus lithognathus*, becoming extinct or indiscernible (Van Niekerk et al. 2012). Similarly, Zambezi (bull) sharks, *Carcharhinus leucas*, populations have been depleted due to trophy fishing, the shark-fin industry and being taken as bycatch (Van Niekerk et al. 2012). As a result, they were rated near-threatened in 2005 by the IUCN (Simpfendorfer & Burgess 2009) and further declines in the last 13 years are highly likely. Effects of the removal of this top predator from estuarine ecosystems are likely to cascading through the food web, creating an imbalance of herbivores, grazers, algae and seagrass (Heithaus et al. 2008; Moksnes et al. 2008; Gutiérrez et al. 2012; Donadi et al. 2017). Although difficult to implement, more efficient and dedicated compliance initiatives could achieve a significant reduction in these threats.

The alteration of freshwater inflow to estuaries has varied and cumulative impacts of seagrass ecosystems, but is recognised as a major threat to these ecosystems. As with increased fishing effort, a change in the flow to estuarine systems can impact *Z. capensis* both directly through changes in abiotic conditions and indirectly through disruptions to the food web and trophic cascades. These include reduction of sediment and nutrient supply as well as a change in salinity, turbidity and temperature, all of which can affect the biodiversity, food-web and community assemblages of estuarine systems (Van Ballegooyen et al. 2007). With 8-33% of filter feeder diets consisting on riverine derived

materials (Porter 2009), a reduction of inflow could have various knock-on effects for biodiversity. Further, the effect of reduced freshwater flow extend offshore through significant correlations with commercial line fish catch patterns (Lamberth et al. 2009). It was suggested that reduced catches on the Thukela Banks of KwaZulu-Natal Province on the east coast of South Africa in 14 line fish species, forming over 90% of the total catch, could be correlated with reduced inflow after a short lag phase (Lamberth et al. 2009). This likely results from a combination of cumulative pressures, including the decreased nutrient supply from riverine to estuarine and marine systems, and impairing the estuaries' function as nurseries for many of these commercially important species. Although the West coast is expected to suffer the greatest reduction in freshwater inflow (Van Niekerk et al. 2012), due to decreased regional rainfall, impacts are expected to be most severe on the nutrient-poor east coast (Van Ballegooyen et al. 2007). Reduced inflow also increased mouth closure and lead to closure of estuaries that are normally permanently open to the sea, such as the Kobonqaba Estuary in the Eastern Cape and Uilkraals in the Western Cape (Van Niekerk et al. 2012). Increased frequency and duration of mouth closure can interrupt connectivity and lifecycles by changing spawning, migration and recruitment cues, depriving the fish and invertebrate species of vital nursery services (Whitfield 1998; Lamberth et al. 2009).

Apart from the physical destruction caused by sand mining in estuaries, smothering by sedimentation and decreased light availability due to increased turbidity are among the major impacts to seagrass beds (Van Niekerk et al. 2012). Light availability is a critical environmental resource for all seagrasses (Hemminga & Duarte 2000). While the minimum requirement can vary greatly both within and between seagrass species by as much as 2.5–37% of SI (Erftemeijer & Robin Lewis 2006), smaller seagrasses, such as *Z. capensis*, can survive for shorter periods below critical minimum levels when compared to larger species, likely as a result of differing carbon storage capacity (Cheshire et al. 2002; Peralta et al. 2002). Although the lack of sediment information for South African estuaries (Van Niekerk et al. 2012) makes assessing environmental changes in relation to sand mining problematic, it is clear that these activities have already impacted riverine and estuarine habitats along the east coast and are not sustainable at their current scale (Demetriades 2007; Van Niekerk et al. 2012).

While the impacts of increased fishing effort and reduced inflow on seagrasses may be somewhat obscure, the consequences of habitat loss are clear. Seagrass habitat

degradation and loss in South Africa is largely attributed to development of infrastructure such as marinas, harbours, bridges and land reclamation (Van Niekerk et al. 2012), with meadows in affected areas facing declines in cover and even local extinctions as suitable habitat shrink. Seagrass beds with low genetic diversity are the first to be lost during disturbance (Jahnke et al. 2015), causing a bottleneck which decreases the standing variation even further, reducing survival potential during subsequent disturbances. Restoration efforts by Evans et al. (2017a) found that seagrass beds with higher genetic diversity are more likely to survive the early establishment phase. Similarly, *Posidonia australis* meadows along the east Australian coastline showed differential ability to survive decreased light availability, as experienced under increased turbidity conditions, with low diversity beds exhibiting significantly lower growth rates than high diversity beds (Evans et al. 2017b).

As seagrass beds are sensitive to environmental stressors, and they play such a pivotal role in estuarine and adjacent marine ecosystems (Orth et al. 2006a), *Z. capensis* could be a useful indicator species. The loss of these "coastal canaries" signals the losses of important ecosystem services, with serious implications for biodiversity and fishery industries. As genomic diversity influences resilience and resistance to disturbances (Massa et al. 2013; Evans et al. 2017b), it is imperative that the remaining diversity in South African seagrass beds be conserved through restoration efforts and careful management of pressures, particularly high fishing efforts and sand mining. Although nucleotide diversity represents the standing variation from which populations may adapt and heterozygosity informs on the capacity of populations to respond to selection immediately following declines, conservation managers should rather focus on allelic richness when monitoring genomic variability as this is considered both more sensitive measure, in terms of how fast it responds to population declines, and more important for the long-term response to selection and survival of populations and species (Allendorf 1986c). Therefore, incorporating estuarine areas with high genomic diversity of *Z. capensis* should be prioritised when planning new MPAs for the South African coastline.

Chapter 4: Applying genomic data to conservation planning in South Africa

Introduction

Although estuaries are geologically transient in nature and exhibit stochastic 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 as they retain propagules (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 such as nursery and spawning grounds for many fishery species (Beck et al. 2001; Vasconcelos & Reis-Santos 2007; Whitfield & Cowley 2010; Bertelli & Unsworth 2014; Blandon et al. 2014; Jackson et al. 2015).

Coastal ecosystems, such as estuaries, are under intense anthropogenic pressures due to their proximity to human populations (Mead et al. 2013; Little et al. 2017). Further, these systems are expected to be exposed to increased risk of habitat degradation in future decades due to climate change, which will only be further exacerbated by human induced pressures (Nicholls et al. 2007). South Africa is no exception, even accounting for the progress made in establishing 23 MPAs (Sink et al. 2012) since the founding the initial no-take Tsitsikamma National Park in 1964 (Hockey & Buxton 1989). Yet, many of South Africa's existing coastal MPAs are ineffective, with high fishing pressure often reported even in estuaries with so-called 'no-take' MPA status (Van Niekerk et al. 2012; Fig. 4.1). With the implementation of Operation Phakisa (Harris et al. 2014a), aimed at maximising the use and increasing revenues gained from South Africa's marine environment, it is anticipated that these pressures on coastal systems will intensify. This is despite the proposed expansion of South Africa's MPA coverage from 0.5% to 5% (Harris et al. 2014a), as this largely neglects estuarine systems. For example, increased aquaculture farming, marine transportation, as well as oil and gas exploitation (Harris et al. 2014a) will put South Africa's estuaries, and specifically the seagrass *Z. capensis*, at further risk.

Increased pressures on coastal systems are especially concerning as models project around 30% of seagrass-suitable habitat will be lost along the South African coastline by the year 2070, and remaining suitable habitat will be clustered along the south-east coast due to climate change (Phair et al. in prep). In reality, *Z. capensis* declines may be even more extreme as the effects of climate change are compounded by human pressures, and although *Z. capensis* grows quickly it does not effectively colonise new areas (Adams 2016). As such, once *Z. capensis* is lost in a particular estuary, it's recolonisation is unlikely. Further, evidence indicates that environmental pressures, such as fishing effort, sand mining, habitat loss and flow modification, will have a negative impact on the genomic variability of *Z. capensis* populations through continued population declines and local extinctions, as illustrated in chapter 3. In order to help preserve marine biodiversity, maintain ecosystem services and provide ecological resilience, an increase in quantity and quality of marine and coastal protected areas are needed that safeguards not only the biodiversity, but also the evolutionary diversity of species.

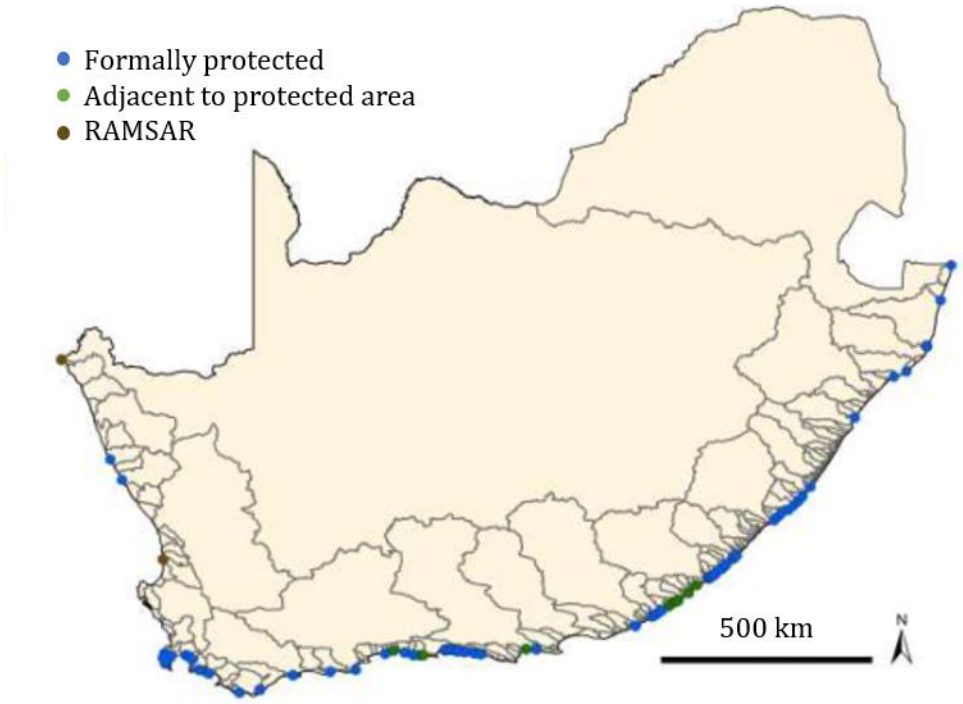

Figure 4.1 Location of formally protected and partially protected estuaries in South Africa (from Van Niekerk et al. 2012).

According to the IUCN, an MPA can be defined as "any area of intertidal or subtidal terrain, together with its overlying water and associated flora, fauna, historical, and

cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment" (Kelleher 1999). MPAs vary in size and protection level, ranging from so-called 'no-take' zones to areas that allow various levels of use (Kelleher 1999). The establishment of MPA's has been found to effectively maintain biodiversity (Barrett et al. 2009; Appolloni et al. 2017; Friedlander et al. 2017; Portugal et al. 2017), sustain fishery yields (Kerwath et al. 2013; Dell et al. 2016; Bucaram et al. 2018) and preserve habitat condition (Selig & Bruno 2010). Further, a review of 124 MPA's in 29 countries found that MPA's can increase biomass, density and species richness regardless of the size of the protected area (Lester & Halpern 2009). MPAs are primarily designed with one or a combination of the following goals: protect ecosystem services/functioning, serve as a source of replenishment of fishery stocks, conserve biodiversity and/or protect a single charismatic species (Kelleher 1999; Agardy et al. 2011 and references therein).

The extent to which protected areas succeed in protecting biodiversity relies primarily on meeting two key objectives, representativeness and persistence (Margules & Pressey 2000). Protected areas should capture a representative sample of the full range of biodiversity across levels of organisation in order to be effective (Austin & Margules 1986; Magris et al. 2018; Mingarro & Lobo 2018). Representativeness has traditionally been interpreted in terms of habitat and species diversity habitats (Hockey & Branch 1997; Carvalho et al. 2011; Hanson et al. 2017), however, as genetic diversity is increasingly recognised as a key component of biological diversity, progress has been made in incorporating genetic and genomic data into conservation planning. Genomic techniques have led to significant advances in marine conservation as they inform fishery stock management, setting conservation priorities for resilience and persistence, and improving our understanding of the mechanisms behind adaptation and speciation (Nielsen et al. 2009; Allendorf et al. 2010; Reitzel et al. 2013; Ribeiro et al. 2016; Selkoe et al. 2016; Oleksiak 2016; Gaither et al. 2018). This is particularly important as traditional measures do not always sufficiently capture evolutionary patterns such as phylogenetic diversity (Pio et al. 2011; Lean & Maclaurin 2016; Mouillot et al. 2016; Carvalho et al. 2017), connectivity (Palumbi 2003; von der Heyden 2009; Luque et al. 2012; Nielsen et al. 2017), and adaptive variation (McMahon et al. 2014; Nielsen et al. 2018).

Evolutionary potential is an important facet in conservation planning as it underpins the capacity of species and populations to adapt to and persist through changing conditions (Mittell et al. 2015; Rey et al. 2016; Paz-Vinas et al. 2018). Protected areas should aim to enhance long-term persistence of species by facilitating natural processes of viable populations, such as connectivity and source-sink dynamics, as well as by reducing threats (Margules & Pressey 2000; Carvalho et al. 2011, 2017). For example, Carvalho et al. (2017) demonstrated the increase in biological representativeness and persistence when including measures of evolutionary potential in conservation planning of amphibian and reptile species of the Iberian Peninsula (Carvalho et al. 2017). Yet often limited resources and a high socio-economic dependence on coastal ecosystem services necessitate a balance between conservation objectives, such as biological representativeness, and the costs associated with management actions (Bottrill et al. 2008; Sowman et al. 2014; Brander et al. 2015).

MPAs often fail in reaching their conservation objectives due to a combination of factors, including poor management and enforcement, degradation of the surrounding environment, natural or human induced disasters, and poor design (Agardy et al. 2011; Roberts et al. 2018). Consequently, clear and appropriate objectives should be set when designing MPA's in order to avoid misallocating resources. Moreover, MPA design should carefully consider climate change to future-proof ecosystem and population management by targeting and enhancing resilience and persistence. More specifically this includes ensuring sufficient connectivity, risk-spreading, and targeting critical areas such as spawning grounds and locally adapted populations in protected areas (McLeod et al. 2009; Carvalho et al. 2017), whilst remaining cognisant of the potential future impacts of climate change and anthropogenic pressures to these systems (Pressey et al. 2007). Both standing genomic diversity and local adaptation can increase evolutionary resilience, and should therefore both be considered when planning MPAs with the aim of enhancing species persistence through climate change (Sgrò et al. 2011; Bible & Sanford 2016). For example, both genomic diversity (Palumbi et al. 2014) and local adaptation via heat stress (Coles & Riegl 2013) have been found to assist corals in surviving further temperature increases, with clear implications for resilience to climate change. However, in this regard, there is very little information on estuaries, which are highly dynamic systems (James & Van Niekerk 2011) and therefore may already harbour populations that can withstand environmental fluctuations.

A molecular approach in the management of marine systems is inherently relevant as it provides estimates of metrics that account for evolutionary history and also act as the basis for functional traits such as behaviour, physiological tolerances, evolutionary potential, and dispersal capacity (Beger et al. 2014). The identification of evolutionary distinct lineages and genetic breaks along the coastline are indispensable as genetically distinct populations warrant protection in order to preserve genetic potential and "to conserve the populations and species of tomorrow" (Rocha et al. 2007). Evolutionary distinct lineages may be on different evolutionary trajectories characterised by differences in adaptive potential, as described for a west and an east coast cluster in Chapter 2.

Due to the ability to identify adaptive variation in addition to neutral variation, genomic approaches have had an invaluable contribution to the delineation of Evolutionary Significant Units (ESUs) and Conservation Units (CUs) (Funk et al. 2012). This proved useful in the case of the near-threatened black footed albatross, *Phoebastria nigripes*. Where previous findings of gene flow and differentiation were controversial, this genomic study found clear evidence of separate management units, influencing the way in which this species should be effectively conserved (Dierickx et al. 2015). Similarly, a study on Chinook salmon, *Oncorhynchus tshawytscha*, used a genomic approach to investigate population structure and adaptive potential, enabling increased accuracy in stock composition/origin assessments (Larson et al. 2014). Measures of genomic variability such as genomic diversity, distinctness and adaptive variation (Table 4.1) are particularly pertinent to effective marine conservation as they inform on the direction and scale of connectivity and resilience among populations (Allendorf et al. 2010; Benestan et al. 2016; R. Taylor et al. 2017; Nielsen et al. 2018).

Table 4.1 A description of genomic measures included in this analysis and their relevance to conservation prioritisation (after Beger et al. 2014; Nielsen et al. 2017).

Genomic measure	Definition	Conservation relevance
	Measure: Diversity	
Nucleotide diversity (N)	Average number of nucleotide differences per site between any two SNPs chosen randomly from a population	Low N can indicate small effective population size and therefore low standing variation from which to adapt, with a increased risk of inbreeding depression and potentially higher extinction risk High N indicates large effective population size and therefore higher standing variation, with potentially increased resilience to environmental change
Heterozygosity (H)	The average proportion of loci carrying two different alleles at a single locus within an individual	Low H is associated with low fitness and decreased capacity to respond immediately following a bottleneck, with the opposite for high H
Allelic Richness (AR)	Average number of alleles per locus	Low AR is associated with low fitness, resilience and long-term persistence, while the opposite is found for high AR (Table 3.1)
Shared SNPs (S)	The number of single nucleotide polymorphisms detected per location detected in neutral loci. These SNPs occur in more than one location	Low S may indicate low genomic variability and potentially decreased resilience to environmental change, with high S indicating the opposite
	Measure: Distinctness	
Proportion Private SNPs (PS)	Neutral loci that are exclusive to specific locations	Low PS per population may indicate high connectivity which could increase metapopulation resilience High PS indicate highly distinct populations with potentially low connectivity and therefore low resilience to stochastic events. However this could also be driven by local adaptation and therefore increase resilience and evolutionary potential
	Measure: Adaptive potential	
Adaptive variation: outlier SNPs – (AV)	Loci that are potentially under selection as they are statistically significantly different from other regions of genome or are strongly correlated with environmental gradients	High AV may indicate local selection, possibly in response to environmental variables, increasing adaptive potential Low AV could indicate a lack of adaptive potential and therefore low resilience to future environmental changes

Despite the importance of genetic data, and the increasing availability of genomic techniques, genomic data is seldom utilised in spatial biodiversity planning (von der Heyden et al. 2014; Shafer et al. 2015b), because of a lack of frameworks to guide their use (Beger et al. 2014; Taylor et al. 2017; von der Heyden 2017; Nielsen et al. in prep). Yet with the incorporation of genomic data into spatial conservation prioritisation tools, the representativeness of MPAs and the persistence of species may be improved (Allendorf et al. 2010; R. Taylor et al. 2017; Nielsen et al. in prep). The effectiveness of conservation efforts is especially important for developing regions that need to balance conservation outcome with development and resource use, such as South Africa, where resources for marine conservation may be limited (Bottrill et al. 2008; Sowman et al. 2014; Brander et al. 2015). For this purpose, it may be useful to apply a genomic approach to the conservation of a keystone estuarine species such as *Z. capensis*, which functions as an umbrella species whose conservation ensures the protection of many other species.

Aim

This chapter explores the conservation implications of integrating different measures of genomic variation that capture genomic diversity, distinctness and adaptive potential (in addition to baseline habitat + cost data) when designing an MPA network for *Z. capensis*. This was carried out by incorporating different measures of genomic diversity generated in the previous chapters into Marxan, a spatial prioritisation tool for biodiversity management. This chapter contributes to building a framework which will allow us to understand the impact different types of genomic data on spatial planning for vulnerable coastal ecosystems.

Hypotheses

Firstly, I hypothesise that conservation priority areas identified by targeting only habitat will differ considerably from those identified by targeting the different genomic measures. Further, I hypothesise that there will be a large degree of overlap between conservation scenarios based on diversity, distinctness and adaptive potential while each identifying different hotspots for conservation along the coastline.

Methods

Genomic measures

In order to identify conservation priority areas under various scenarios, the following measures of genomic diversity were included in the analysis: nucleotide diversity (chapter one), expected heterozygosity (chapter one), allelic richness (chapter two), the number of shared Single Nucleotide Polymorphisms (SNPs) and the private SNPs (chapter one) as a proportion of the number of total SNPs per population, as well as adaptive variation in the form of outlier frequency (chapter two). These measures cover both distinctness and diversity and can inform conservation objectives in different ways; as such their relevance to conservation is summarised in Table 4.1.

Spatial conservation prioritisation

The decision support tool, Marxan v 2.43 (Ball et al. 2009), was used to design networks of MPA's as possible scenarios for the preservation of *Z. capensis* along the South African coastline. Marxan uses an algorithm which minimises reserve cost and size whilst meeting a set of predefined biodiversity targets (Ball et al. 2009). The cost layer was derived from the fishing effort per estuary quantified in the NBA (Van Niekerk et al. 2012) and will represent the cost as lost opportunity for industry if MPAs are established as no-take reserves. A baseline scenario will be established by targeting 20% of each estuarine habitat type as suggested by the National Biodiversity Assessment (NBA; Van Niekerk et al. 2012) whilst applying the cost layer. Habitat data was obtained from Adams et al. (2016). Each genomic scenario was developed using the baseline scenario as a foundation (Table 4.2). The procedures outlined in "Marxan good practices handbook" (Ardron et al. 2010), as well as the methods for integrating genetic data into spatial conservation prioritisation described in Beger et al. (2014), were used to guide the analyses in this chapter. This approach is detailed below and summarised in Figure 4.2.

Conservation decision tools such as Marxan require genetic or genomic point data to be interpolated throughout the entire planning region to form a spatially continuous surface layer. In the past, this has been considered a major barrier to incorporating genetic data into these types of analyses. Accordingly and following Beger et al. (2014) and Nielsen et al. (2017), a resampling procedure in ArcMap v10.1 (ESRI, Redlands CA) was used to interpolate genomic data across South African estuaries to represent a simplified version of the genomic patterns of *Z. capensis*. The reclassification (reclass)

tool in ArcMap v10.1 (ESRI, Redlands CA) was used to reclassify the data from each genomic metric into high, medium and low classes using natural breaks in the data. As both high and low values of genomic diversity are significant in terms of evolutionary processes, targets were set to represent 50% of high and low classes, and 30% of the medium class of each genomic metric following a similar protocol to Beger et al. (2014) and Nielsen et al. (2017).

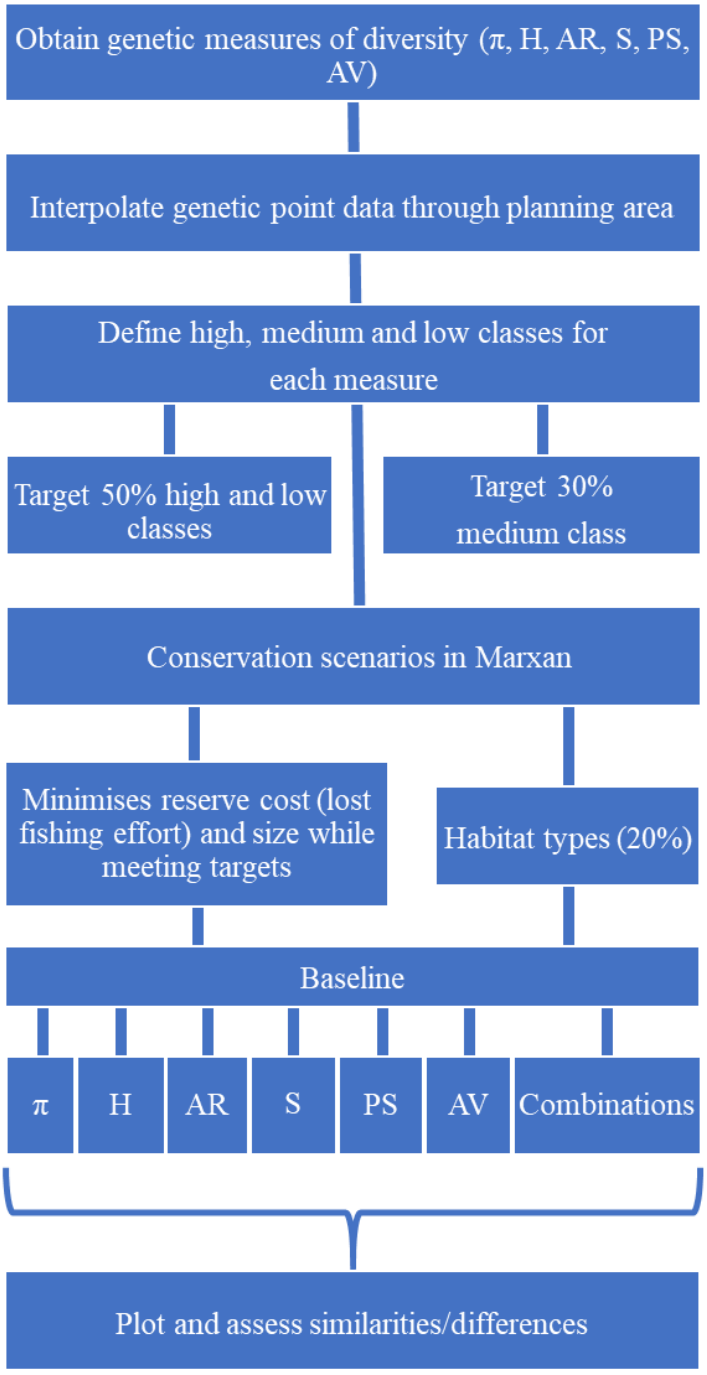

Figure 4.2 A flow of the implementation of genomic data in Marxan for spatial conservation prioritisation.

Conservation prioritisation scenarios calculated in Marxan are outlined in Table 4.2. In addition to the baseline scenario, these scenarios cover different aspects genomic variability, thus allowing for the comparison of the use of different genomic measures in identifying conservation priority areas. Combinations of genomic measures were also included in order to observe how the priority areas identified change with the addition of data. As it is possible for many different configurations of planning units to meet the conservation objectives, each scenario run was repeated 100 times to account for any system variability, allowing Marxan to calculate planning unit selection frequencies and identify the best solution as the one with the lowest cost to target ratio.

Table 4.2 Conservation prioritisation scenarios and planning objectives.

Conservation features	Abbreviation	Planning objective
Baseline (Habitat type + cost)	B	Habitat
Baseline + Nucleotide diversity	N	Diversity
Baseline + Heterozygosity	H	
Baseline + Allelic richness	AR	
Baseline + SNPs	S	
Baseline + Private SNPs	PS	Distinctness
Baseline + Adaptive variation (outliers)	AV	Adaptive potential
Baseline + Allelic richness + Private SNPs + Outliers	ALL	Habitat, diversity, distinctness and adaptive potential

QGIS v2.18.4 (2012) was used to visualise scenario outcomes by means of the QMarxan plugin v 1.3 .1 (Game & Grantham 2008). Planning unit selection frequencies were obtained from the 'ssol' (summed solution) outputs and plotted along the South African coastline for each scenario. In order to understand whether different measures of genomic diversity prioritise different regions, unique and shared priority planning units among diversity scenarios (N, H, AR, S) were identified from the 'best' solution outputs and plotted. As allelic richness is often considered the most useful measure for monitoring even short-term changes in populations, because of its sensitivity to population declines (Schwartz et al. 2007b; Hoban et al. 2014; Jangjoo et al. 2016; Gedeon et al. 2017), allelic richness was chosen as a measure of genomic diversity and formed part of a subset of scenarios (AR, PS, AV, ALL). For these scenarios the differences from and similarities to the baseline scenario, in terms of planning units selected, was obtained from 'best' solution outputs and plotted to visualise the impact of including measures of genomic diversity, distinctness and adaptive variation in conservation planning in addition to habitat data. Planning unit selection frequencies

from the ALL scenario were also plotted separately along the cool temperate west coast, warm temperate south coast and subtropical east coast in order to compare with the ecosystem threat status of estuaries as defined by the NBA (Niekerk & Turpie 2012).

In order to visualise dissimilarities among scenario solutions, I followed the approach in Harris et al. (2014) and applied nonmetric multidimensional scaling (nMDS) ordination based on Jaccard resemblance matrices in the statistical environment R (R Core Development Team 2008) using Rstudio V 0.98.1102 ("RStudio" 2012). Pearson's correlation tests were carried out on the selection frequency values for each planning unit to quantify spatial similarities between each pair of scenarios.

Results

Spatial conservation prioritisation

All scenarios prioritised estuaries for conservation along the entire coastline, however the baseline scenario, selected estuaries at a lower frequency than scenarios targeting genomic measures (Fig. 4.3). Although Pearson correlation tests revealed significant ($p < 0.05$) correlations between all scenarios, there were differences in the spatial distribution of prioritised estuaries and the frequency with which they were selected between scenarios targeting genomic measures (Fig. 4.3). More specifically, H and PS selected fewer estuaries along the west coast, scenarios H, N and S fewer along the south coast, and scenarios AR, H, AV and ALL fewer along the east coast, than other scenarios. Only scenario H selected the prominent St. Lucia estuary on the east coast. Scenarios N, S and SP selected estuaries at a slightly higher frequency than other scenarios (Fig. 4.3). Hotspots for the conservation of genomic diversity, distinctness and adaptive variation exist along the west, south-west and east coasts, as planning units in these regions were selected at high frequencies across genomic scenarios (Fig. 4.3).

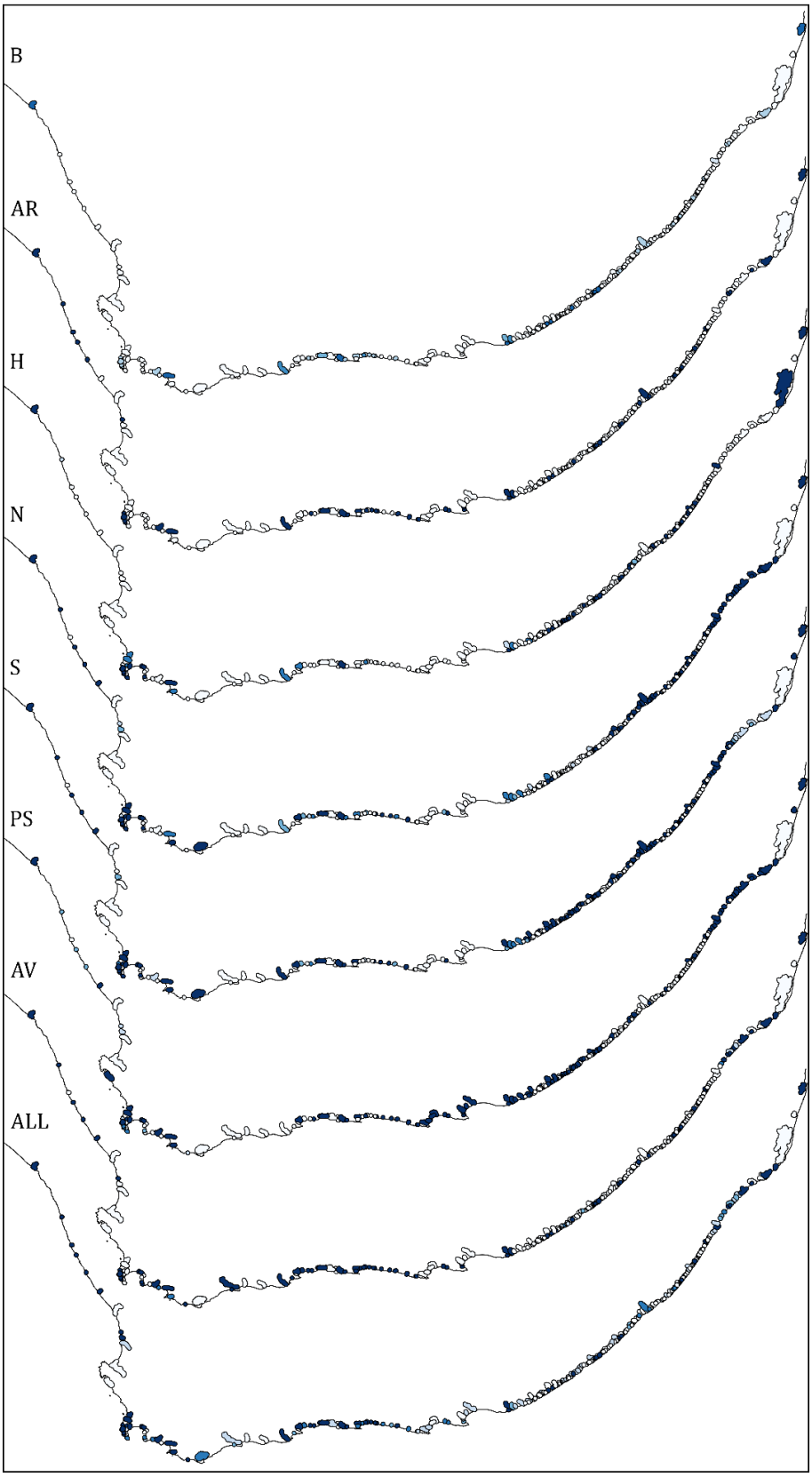

Figure 4.3 The spatial patterns of selected conservation priority areas across all scenarios with high to low planning unit selection frequency represented by dark to light blue. B = baseline, AR = allelic richness, H = heterozygosity, N = nucleotide diversity, S = SNPs, PS = private SNPs, AV = adaptive variation, ALL=combined (also see Table 4.2).

Scenario dissimilarities

When scenario dissimilarities were visualised by means of an nMDS plot, the baseline scenario formed a discrete cluster, which was distant from all other scenarios, with the exception of one outlier solution (Fig. 4.4). Solutions from each genomic scenario formed distinct clusters, with solutions from AR and H scenarios most removed from the other genomic scenario clusters, and the ALL scenario displaying the broadest range of solutions. Further, solutions from N, S and PS group closely together, and those from AV fall almost within the ALL cluster of solutions. For many scenarios, such as B and AR, only a few solutions are visible due to highly overlapping nature of these solutions. In other words, Marxan identified the same configuration of priority estuaries for these scenarios in multiple runs.

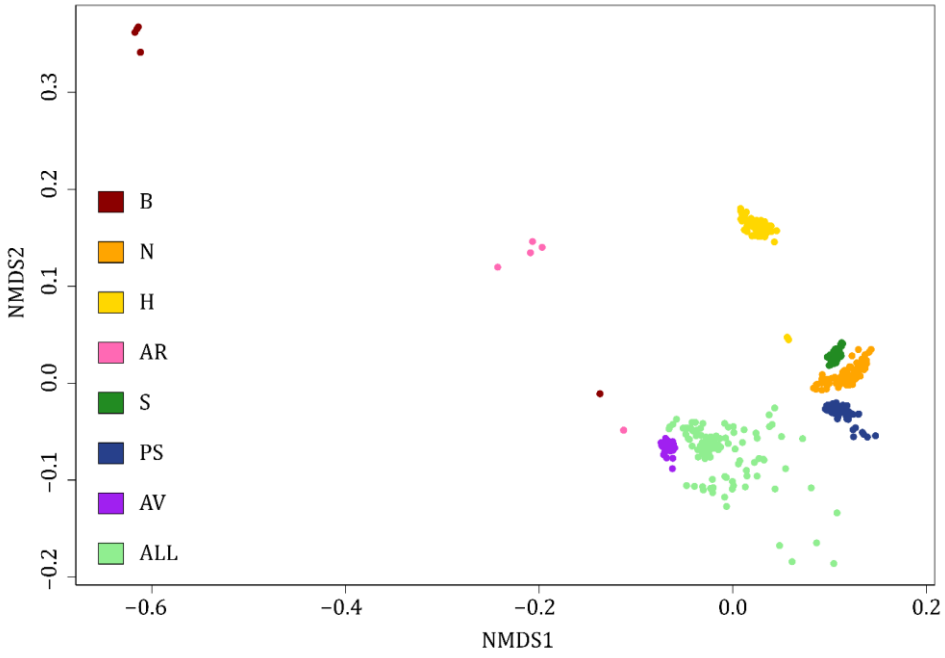

Figure 4.4 nMDS plot displaying dissimilarities among scenarios.

Genomic diversity scenarios

Although most prioritised estuaries overlapped across genomic diversity scenarios (AR, H, N and S), each diversity scenario also highlighted unique estuaries for conservation (Fig. 4.5). Scenario S identified the highest number of unique estuaries for conservation, which were all situated on the south-east coast (Fig. 4.5). Scenario H only identified one unique estuary for conservation, namely St. Lucia estuary (Fig. 4.5). Scenario AR selected unique estuaries for conservation along the south coast and scenario N along the south and east coasts (Fig. 4.5).

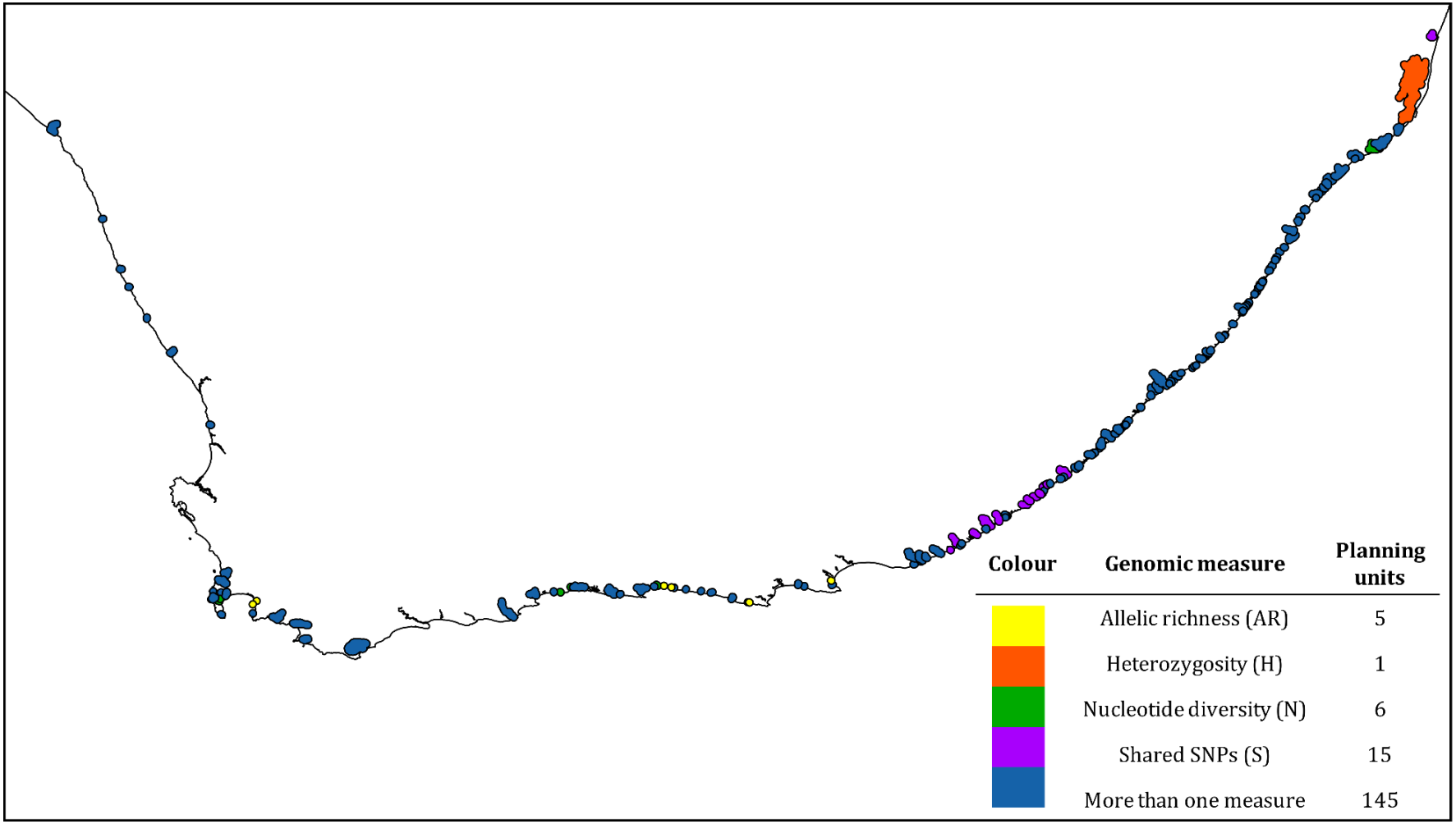

Figure 4.5 Spatial patterns of selected priority conservation areas derived from conserving habitat as well as diversity measures, with units selected by more than one scenario in blue and those selected only by the scenario based on AR in yellow, on H in orange, on N in green and on S in purple.

Genomic diversity vs distinctness vs adaptive variation

Similarly, the majority of prioritised estuaries were identified by both the baseline (habitat) scenario and scenarios targeting diversity (AR), distinctness (PS), adaptive variation (AV) and ALL. However, each of these scenarios also identified unique priority estuaries with respect to the baseline (habitat) scenario, summarised in Figure 4.6. Scenario PS was the most dissimilar from the baseline scenario, as it showed the greatest number of estuaries gained and lost with respect to those selected by the baseline scenario, which is also evident from the nMDS plot (Fig. 4.4).

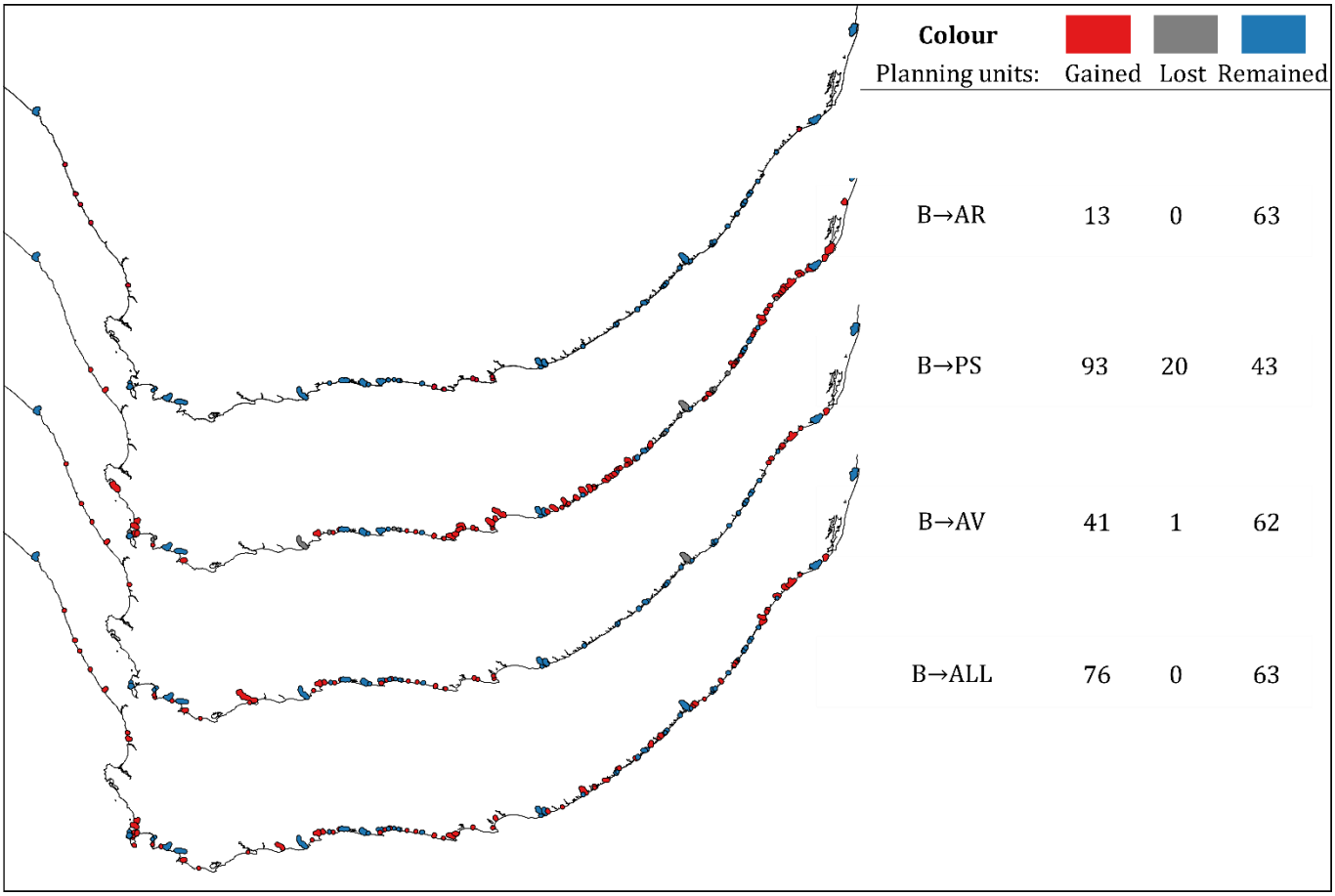

Figure 4.6 Change in spatial patterns of selected priority conservation areas with the addition of genomic measures of diversity (AR), distinctness (PS), adaptive variation (AV), and a combination thereof (ALL) to solely targeting habitat, with units gained in red, lost in grey and remaining selected in blue (number of planning units indicated on the right).

Overlapping threat status and genomic conservation planning

When comparing the planning unit selection frequency of the ALL scenario with the threat status of estuarine systems, the majority of the critically endangered and

vulnerable estuaries are captured (Fig. 4.7). However, these most threatened estuaries do not coincide well with the coastal MPAs proposed by Operation Phakisa, particularly along the west and south coasts (Fig. 4.7).

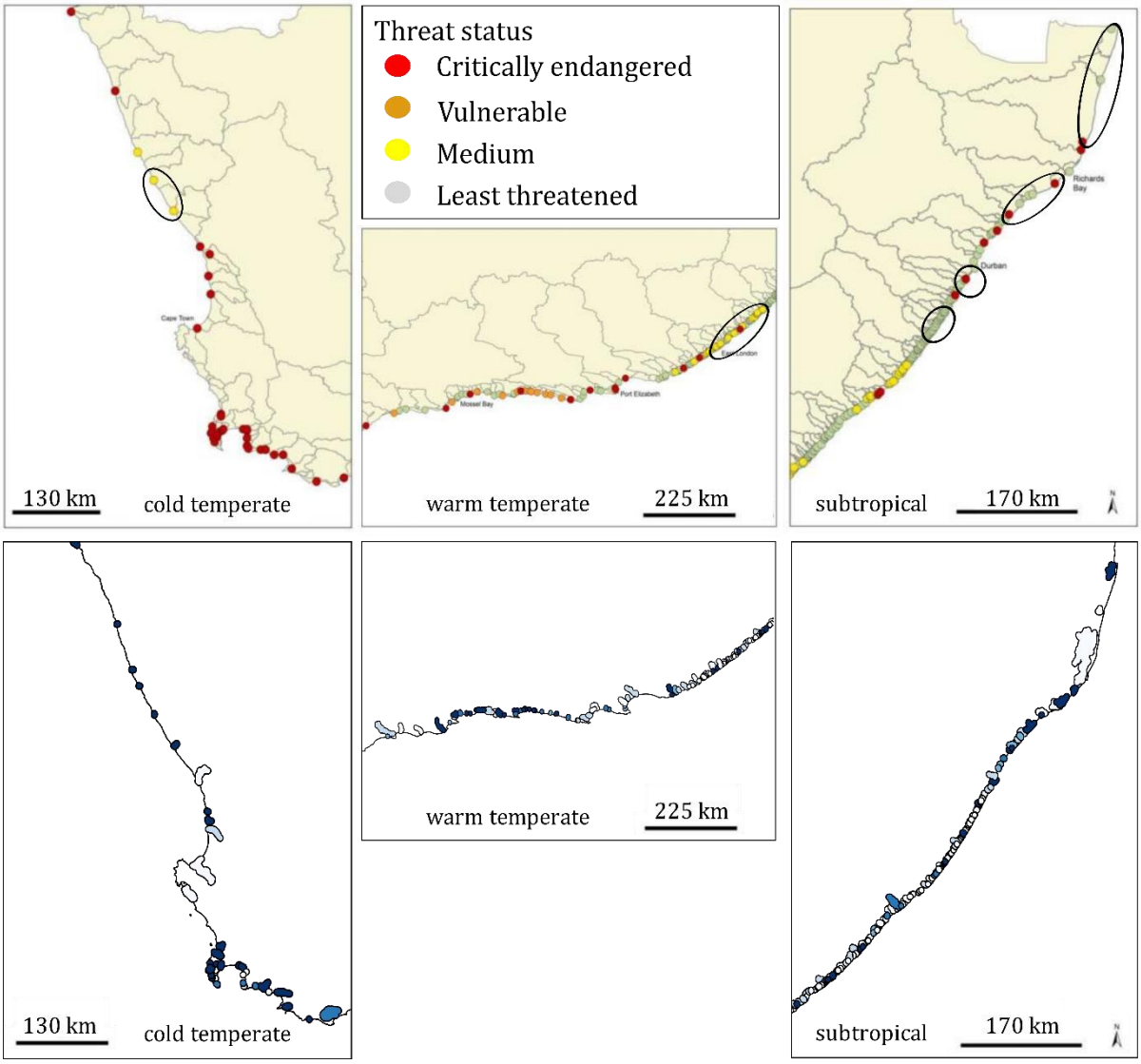

Figure 4.7 Threat status of South African estuaries along the cold temperate west coast, warm temperature south coast and subtropical east coast (from the NBA; van Niekerk 2012) compared with the ALL scenario planning unit selection frequency. Circles indicate Operation Phakisa proposed coastal MPAs.

Discussion

With the threats to coastal systems escalating due to climate change and increasing anthropogenic pressures, resilient MPA networks are vital for the persistence of coastal species and their ecosystem services. Although it has been recognised that genomic diversity is important for biodiversity and resilience of species and ecosystems (Allendorf 2016; Evans et al. 2017a, 2017b; Timpane-Padgham et al. 2017), there are

limited examples of evolutionary patterns, particularly adaptive variation (Pearse 2016), being integrated into actionable conservation and management plans (Sork et al. 2009; Laikre et al. 2009; Laikre 2010; Beger et al. 2014; von der Heyden et al. 2014; von der Heyden 2017; Nielsen et al. 2017). One reason for this is because there is no clear evidence for how different genetic and genomic measures vary within a conservation planning framework, hindering its uptake into a more formalised process identifying priority areas. In this chapter, I generate several spatial plans that not only compare between different metrics capturing genomic diversity, distinctness, and potential signals of local adaptations, but also provide some insights into the feasibility of including these measures into a conservation plan for *Z. capensis* in South Africa. Notably, this chapter illustrates the importance of including genetic and genomic information in MPA network planning, and the risk to the evolutionary processes which drive genomic patterns if management plans are based solely on data that does not include evolutionary history.

Importance of genetic and genomic data for spatial planning

Although priority areas overlapped across all scenarios, as they were all founded on baseline habitat data, baseline and genomic scenarios identified noticeably different estuaries for conservation. This is consistent with findings from other single species (Beger et al. 2014) and even multi-species approaches (Nielsen et al. 2017), and highlights potentially significant omissions in traditional habitat-based MPA design as genetic diversity is the foundation for adaptation and resilience to environmental change (von der Heyden 2009; Beger et al. 2014; von der Heyden et al. 2014). This has important implications for the persistence of *Z. capensis* along the South African coast, where estuaries are under intense pressure (Van Niekerk et al. 2012) and MPA networks only target habitat types and are therefore far from sufficient (Harris et al. 2014a). Further, failing to conserve current genetic variation of *Z. capensis* increases the probability of losing genotypes which may be more resilient to environmental change, indeed I provide some evidence for anthropogenic effects already affecting genomic variation in Chapter 3. This is often the case in seagrasses as genetic diversity has been linked to increased resistance and resilience in various forms (Table 3.1). Although single species approaches in conservation management are often criticised as single species may not be representative of the broader ecosystem (Block et al. 1995; Richardson et al. 2016; Nielsen et al. 2017; Anthonysamy et al. 2018), its use is

recognisably justified when dealing with keystone species (Simberloff 1998; Johnson et al. 2017) such as seagrasses. Conserving such 'umbrella' species can ensure the protection of a large range of other species (Simberloff 1998; Bode et al. 2016).

Measures of genomic diversity, distinctness and adaptation resolve different conservation priorities

Genomic diversity scenarios (AR, H, N, S) all identified similar areas for conservation, with only one to 15 unique planning units selected across measures of genomic diversity (Fig. 4.5). This suggests that conserving a proportion of estuaries with low, medium and high variation for any single genomic diversity measure may sufficiently capture priority estuaries identified by other measures. This has also been observed for measures of genetic diversity (from mtDNA), where Nielsen et al. (2017) employed cytochrome oxidase I and control region as markers to investigate the population genetic structure and diversity of five marine species. The authors consistently identified congruent patterns of spatial prioritisation when targeting haplotype diversity, nucleotide diversity, local genetic differentiation and private alleles (Nielsen et al. 2017), providing support that any one measure of genetic variation can adequately represent the evolutionary patterns observed in other genetic metrics. In this regard the process of integrating genomic information into spatial planning may be somewhat simplified for conservation managers by employing the most easily obtainable genomic measures. This is important as the plethora of genetic and genomic approaches, the measures derived from them and the interpretation thereof can be challenging for non-specialists to grasp (von der Heyden et al. 2014; Shafer et al. 2015a; Hoban 2018). Several recent studies have significantly contributed to a practical framework for implementing genetic and genomic data into actionable conservation objectives (Beger et al. 2014; Shafer et al. 2015b; Nielsen et al. 2017; Nielsen et al. in prep; Hoban 2018).

In addition to measures of diversity it is also important to consider a measure of distinctness, as highly structured populations may harbour unique genetic variants and less structured populations are likely to be highly connected and thus more resilient to short and long-term perturbations (Chust et al. 2013; Grech et al. 2018). Genomic distinctness scenarios (here measured as private SNPs unique to populations) identified estuaries along the entire coastline, representing population with both high and low levels of connectivity (Fig. 4.6). This is beneficial as it safeguards evolutionary potential in a two-pronged approach, firstly, by preserving more homogeneous meadows, which

may be more resilient through rescue of declining populations by adjacent well-connected populations (Mcmahon et al. 2017; Grech et al. 2018) and secondly, by preserving locally adapted populations that may be pre-adapted to specific environmental stressors, e.g. warming. The latter has been observed through reciprocal transplant experiments of the threatened *Posidonia australis* which is mostly restricted to isolated meadows in Australian estuaries and bays (Evans et al. 2017a), comparable to *Z. capensis* habitats.

In order to preserve evolutionary potential, it is important to consider adaptive variation in addition to distinctness and diversity, as locally adapted genetic variants may exhibit higher resilience to environmental pressures (Sgrò et al. 2011; Carvalho et al. 2017; Hoban 2018; Razgour et al. 2018). Notably, the scenario targeting adaptive variation (AV) identified priority areas, distinct from the baseline habitat scenario, on the west, south and north-east coasts (Fig. 4.6). These regions could represent areas of high adaptive potential and therefore resilience to environmental change, under the assumption that the putative outliers, are indeed of adaptive importance and do have conservation relevance. While other genomic scenarios also capture some of these regions, many are unique to AV and are even discounted in other genomic scenarios. Similarly, there is a large degree of overlap between ALL and other genomic scenarios. As such, targeting any one of the measures of genomic variation — diversity, distinctness or adaptive variation — may sufficiently represent the evolutionary processes behind the patterns of variation, while simplifying the conservation prioritisation procedure.

Threats to the evolutionary diversity of Z. capensis

In order to ensure the resilience of MPAs against future environmental change, it is essential to preserve adaptive potential both in the form of standing genomic variation as well as local adaptation (Beger et al. 2014; Shafer et al. 2015b). However, there appears to be a disjunction between coastal MPAs proposed for South Africa under Operation Phakisa (Harris et al. 2014a; Fig. 4.7) and the distribution and intensity of environmental pressures on estuaries along the coastline (Van Niekerk et al. 2012; Fig. 4.7). The majority of the proposed MPAs are located offshore and were designated in order to facilitate the recovery of fishery stocks and sustainable fisheries management (Harris et al. 2014a). While this is an important step in protecting biodiversity and increasing sustainability, it neglects foundational coastal ecosystems, such as estuaries,

which provide many important ecosystem services (Barbier et al. 2011; Costanza et al. 2014; Nordlund et al. 2016). Estuarine ecosystems are estimated to contribute as much as 6.8 trillion USD to the global economy, which in terms of marine ecosystems is only topped in value by coral reefs at 9.9 trillion USD (Costanza et al. 2014), and as such merit increased conservation focus.

The mismatch between proposed MPA placement and estuary threat level is particularly evident on the south and south-west coasts, where there are a high number of estuaries rated vulnerable and critically endangered in terms of a loss of function and structure due to anthropogenic and climate pressures. Further, these estuaries correspond to areas identified as priorities for conservation by genomic scenarios (Fig. 4.7). As such, omitting these estuaries from MPA networks risks the loss of evolutionarily important populations of *Z. capensis* and could threaten the resilience and persistence of not only this keystone species, but also estuarine associated communities, in the long term. Going forward, South African MPAs should be reassessed in order to ensure persistence and representativeness of evolutionary potential of this umbrella species, and thus estuarine associated communities and ecosystem services, in a cost-effective manner.

Chapter 5: General conclusion

Zostera capensis forms a vital part of southern African estuarine systems as it provides critical ecosystem services which support biodiversity, estuary functioning and economically important fishery industry (Hemminga & Duarte 2000; Green & Short 2003; Bertelli & Unsworth 2014; Unsworth et al. 2015). This intertidal seagrass is restricted to estuaries and sheltered bays (Green & Short 2003), and appears to rely chiefly on vegetative reproduction (McMillan 1980; D. Pillay pers. comm.), limiting its dispersal capacity along the often-harsh coastlines of southern Africa. As such, these isolated and highly clonal populations are more vulnerable to the impacts of global change, the effects of which are likely to cascade through the ecosystem. South African estuaries are both highly threatened and poorly protected (Van Niekerk et al. 2012), and threats to estuarine and coastal systems along most of the southern-east African coastline have not been well-defined, increasing the urgency of assessing the status of this keystone estuarine species. As such, a genomic approach was applied to evaluate the vulnerability, resilience and adaptive potential of *Z. capensis*.

Together with the threatened nature of seagrass habitat along the southern African coastline (Van Niekerk et al. 2012), several other factors also contribute to the vulnerability of *Z. capensis*. Results from Chapter one indicate that this seagrass likely exhibits a high degree of clonality, similar to 'mega clones' observed in *T. testudinum* with one genet covering many kilometres of coastline (Bricker et al. 2018). Such low levels of genomic diversity mean that populations are vulnerable to local extinctions in response to changing conditions or extreme events, as it will be less statistically likely that genets will have a suitable genotype that will thrive under new conditions (Evans et al. 2017b). Further, evidence from Chapter three suggests that this forms part of a negative feedback loop with pressures on seagrass habitats decreasing genomic diversity through the loss of meadows and subsequently, the decreased genomic diversity reduces the capacity to respond to further pressure (Hughes et al. 2007). This is compounded by the mismatch between the spatial distribution of threats to estuaries (Van Niekerk et al. 2012) and the currently proposed expansion to the MPA network in South Africa (Driver et al. 2012; Harris et al. 2014a), observed in Chapter four.

Although many factors add to the vulnerability of *Z. capensis*, results from Chapter two also gave insight into its resilience and adaptive potential. The highly heterogeneous South African coastline displays many environmental clines in response to which local adaptation can be expected to occur (Bradbury et al. 2010; Renaut et al. 2011; Guo et al. 2015, 2016), as has been shown for the South African west coast. This is the most environmentally homogeneous of all the South African biogeographic regions, yet for two intertidal species Nielsen et al. (2018) show population-specific private SNPs and outlier loci, suggesting that local adaptation may be acting on small spatial scales. Results from my work in Chapter two suggest that precipitation and temperature gradients along the coastline may be responsible for some of the adaptive variation observed in *Z. capensis*, despite the low levels of genomic diversity. The former was identified by genome-environment associations and the latter by BLAST results of outlier SNPs, corresponding to hypothetical proteins involved in membrane function, suggesting the potential of locally adapted populations of *Z. capensis*. Such populations may be more resilient to climate change, as seen in other species of seagrass. For example, warm-adapted populations of both *Zostera marina* (Franssen et al. 2011; Jueterbock et al. 2016) and *Zostera noltii* (Franssen et al. 2014), display increased stress-resilience and reduced sensitivity to heat waves.

Interestingly, I found evidence of both genomic clusters in Langebaan Lagoon, where *Z. capensis* displays two distinct ecotypes; a shorter, low biomass form on the muddy tidal flats in the cool temperate cluster and a longer, high biomass form on the less exposed sandy banks in the warm temperate/ subtropical cluster. The presence of both clusters points to a complex and dynamic evolutionary history of *Z. capensis* in South Africa, but may point to both lineages surviving in one refugial area, secondary contact after divergence or a rare long-distance dispersal event. The ensemble models indicate the presence of a stable suitable temperature regime from at least the LGM till present and two refugia that were maintained despite historical topographical fluctuations (Compton 2011). These refugia match the break between the two genomic clusters, although it is not clear how *Z. capensis* might have recolonised novel habitat post sea-level rise. Due to ongoing, anthropogenically driven climate change, coastal temperatures are expected to increase and patterns of precipitation are expected to shift, with the decreased rainfall over the west coast and increased rainfall as well as flood frequency and intensity over the east coast (Lumsden et al. 2009). This could have serious implications for the continued persistence of *Z. capensis* and its ecosystem

services into the future if evolutionary potential is neglected when designing MPAs. Adaptive variation in *Z. capensis* differentiates populations into two clusters which correspond to split between cool temperate and warm temperate/ subtropical biogeographic regions along the coastline, despite the lack of differentiation at neutral loci. However, this variation is not due to the presence of different outliers, but rather selection of the same loci at variable frequencies. This could indicate some level of functional variation taking place in the same suite of genes in response to temperature and precipitation gradients, or potentially other environmental variables which I could not account for in my work (Reitzel et al. 2013; Ravinet et al. 2016; Ribeiro et al. 2016). Shared outlier loci across sites may infer some measure of resilience, so at least from a genomic similarity perspective any site could potentially serve as a source to replace individuals from a declining site, although it would make sense to use donor and recipient populations that are geographically close as they are expected to share more similar environmental variation than sites further away.

Due to the apparent poor dispersal capacity of this seagrass, maintenance and restoration projects may be vital in maintaining meadows and their ecosystem services across the distribution. Although *Z. capensis* grows quickly, they do not effectively colonise new areas (Adams 2016) and therefore may be poor candidates for restoration projects, particularly as their low diversity may hinder transplantation success as observed in *P. australis* (Evans et al 2017). Further, successful restoration of seagrasses relies on a complex interaction of factors, including the removal of threats, spatial and temporal scales of effort, as well as associated community feedbacks (Suding 2011; van Katwijk et al. 2015). While several successful restoration efforts have been recorded in the tropical and north Atlantic, as well as southern Australia, none have been recorded in southern Africa (van Katwijk et al. 2015) and Adams (2016) states that restoration experiments in KwaZulu-Natal were not successful due to unfavourable abiotic conditions and turbidity. As such, concerted efforts conserving *Z. capensis* and its habitat going forward are critical. However, current and proposed MPAs are based primarily on habitat data, which does not adequately represent evolutionary potential and risks the loss of the processes which drive the patterns of genomic variation. Through this genomic study of *Z. capensis* I show that incorporating any one measure of genomic variation into conservation planning may be sufficient to represent priority estuaries identified by other measures, as illustrated in Chapter 4. Although there were slight differences in both the selection frequency and spatial distribution of planning

units between genomic conservation prioritisation scenarios, all scenarios identified hotspots for conservation along the entire coastline.

Widespread management is not always feasible, therefore in order capture evolutionary potential and possibly safe guard against future environmental pressures and changes, management could be focused on selected estuaries in each cluster, or Langebaan lagoon where both genetic clusters are present. Another more fisheries-minded approach could be to focus management on estuaries in both clusters under high fishing pressure such as the Olifants estuary on the west coast of South Africa or the Richard's Bay/Mhlathuze Estuary on the east coast of South Africa (Van Niekerk et al. 2012). Future studies are recommended to include transcriptomic analyses of the two clusters of *Z. capensis* in order to better our understanding of the genes involved in local adaptation.

As adaptive variation is often challenging to quantify and interpret in a meaningful manner, environmental patterns have been suggested as effective surrogates for genetic patterns, especially across heterogeneous systems (Carvalho et al. 2011; Funk et al. 2012; Hanson et al. 2017). For example, Hanson et al. (2017) demonstrated that in the absence of genetic data, conservation planners can capture a representative sample of intraspecific adaptive variation using environmental and geographic distance variables. Although this has yet to be tested against NGS data or investigated outside of the plant taxa utilized in Hanson et al. (2017), the emphasis should be on implementing conservation interventions before critical amounts of intraspecific diversity and biodiversity are lost. In this regard, if genetic or genomic data are unavailable, surrogates should be employed wherever necessary to ensure the persistence of biodiversity and ecosystem services. In the context of South African coastal conservation, this may involve conserving a diverse range of habitats in each biogeographic region, with specific emphasis on reducing environmental pressures and protecting umbrella species such as seagrass. In this way it is likely that the representativeness of reserves will be improved in terms of intraspecific diversity, phylogenetic diversity and adaptive potential, thus supporting the safeguarding of unique southern African estuarine systems into the future.

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Supplementary Materials

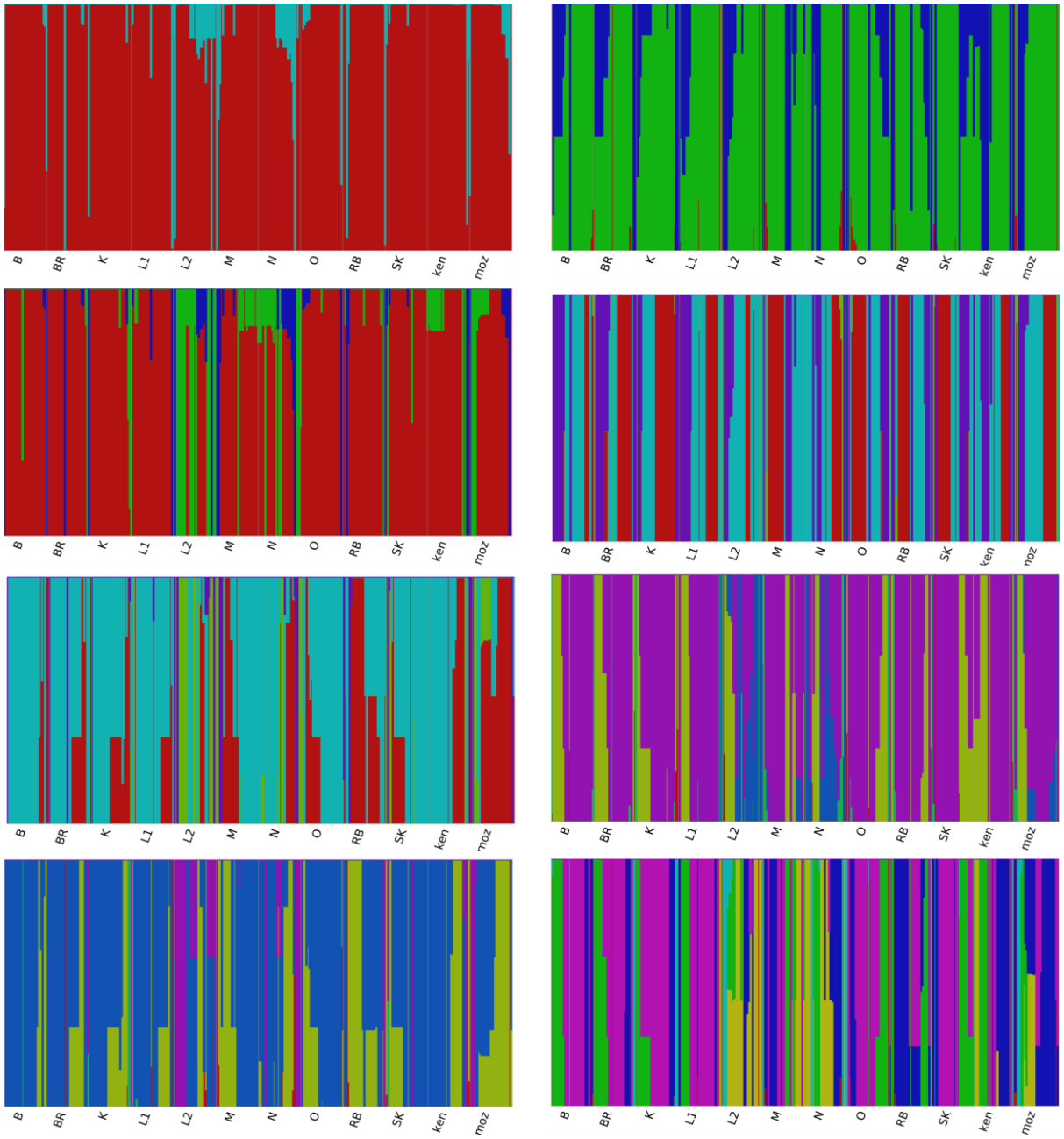

Figure S1 FastStructure plots for the analyses of the simulated neutral (left) and complete (right) dataset, with k=2-5 and k=3-6 (top to bottom), respectively. K=1 for the neutral dataset is not shown.

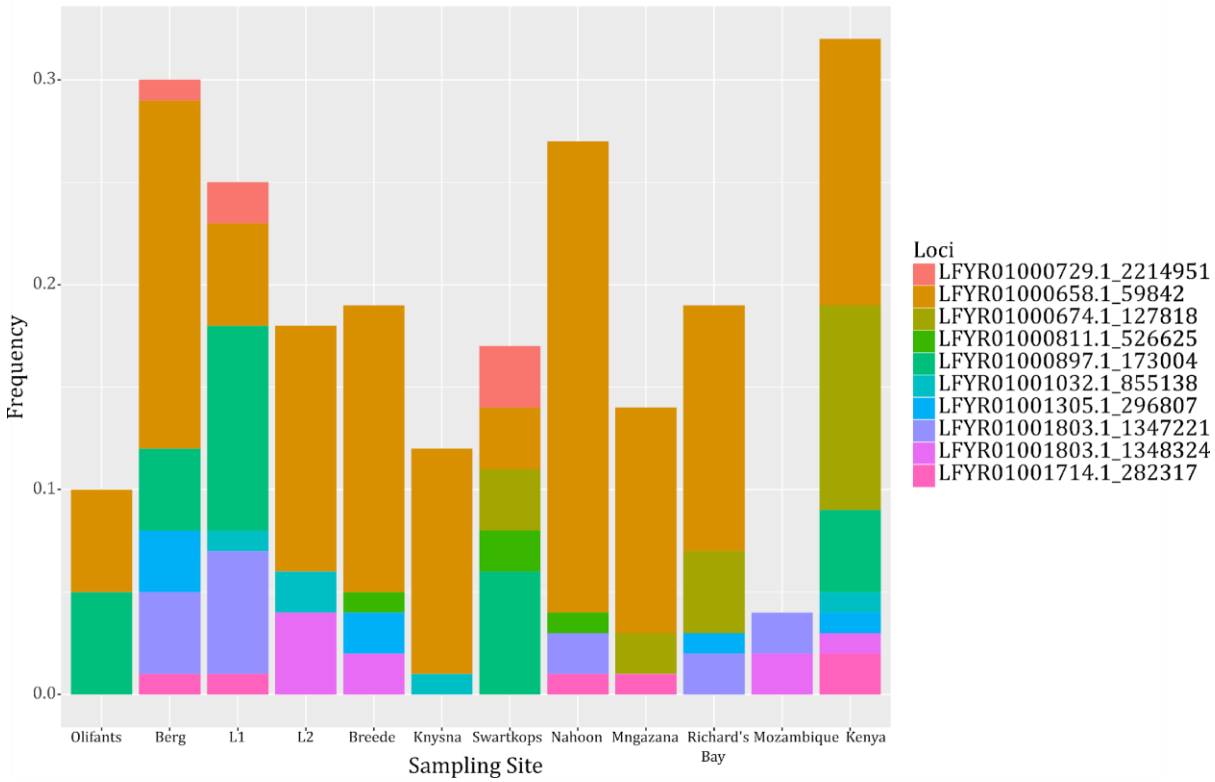

Figure S2 The frequency of 10 random neutral loci across sampling sites.

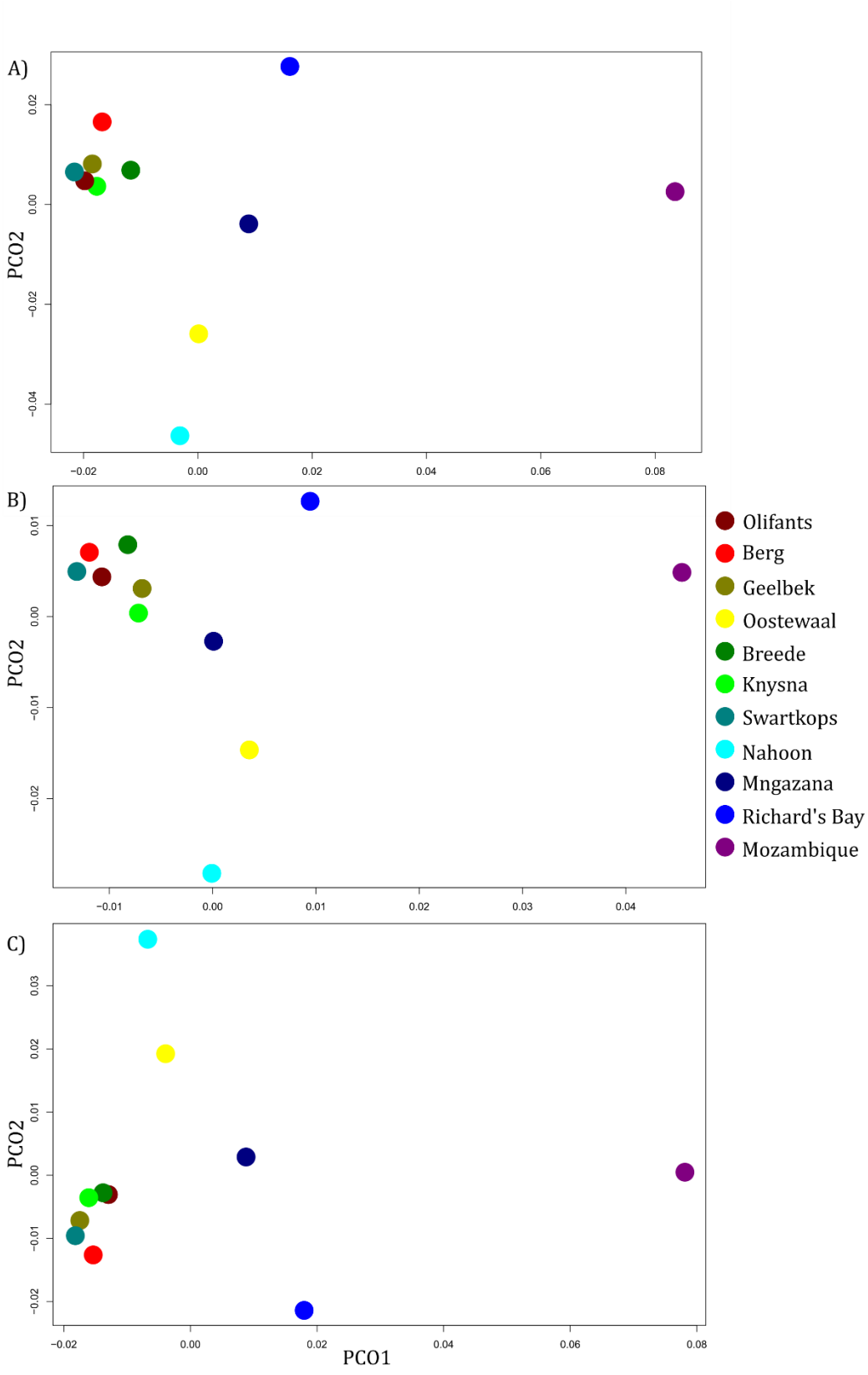

Figure S3 Principal coordinate analysis (PCoA) plot of the average pairwise F_{ST} comparisons among the sampling sites, excluding Kenya, for the subset of SNPs contained in the simulated neutral dataset. FST calculated from A) all loci in the full dataset, B) outlier loci and C) neutral loci.

Table S1 Outlier allele frequencies across sampling sites (see table 1.1 for full names of abbreviations) and outlier identification methods.

Method	Outlier loci	O	B	L1	L2	BR	K	SK	N	M	RB	MOZ	KEN
PCAdapt, BayeScan, Lositan, BayeScEnv	LFYR01001803.1_1347465	0,005	0,000	0,014	0,375	0,060	0,007	0,013	0,500	0,100	0,057	0,118	0,000
	LFYR01001803.1_1347494	0,005	0,000	0,032	0,359	0,072	0,015	0,025	0,361	0,350	0,118	0,429	0,394
	LFYR01001803.1_1347502	0,000	0,000	0,032	0,368	0,073	0,015	0,025	0,366	0,438	0,109	0,444	0,375
PCAdapt, BayeScan, BayeScEnv	LFYR01001803.1_1347472	0,000	0,004	0,041	0,350	0,060	0,015	0,025	0,370	0,449	0,138	0,443	0,438
BayeScan, Lositan, BayeScEnv	LFYR01001803.1_1347498	0,000	0,004	0,032	0,359	0,073	0,015	0,025	0,493	0,359	0,123	0,429	0,394
BayeScan, Lositan	LFYR01000838.1_24989	0,020	0,033	0,040	0,104	0,064	0,063	0,040	0,066	0,019	0,061	0,000	0,079
	LFYR01001213.1_169201	0,020	0,041	0,024	0,024	0,054	0,000	0,028	0,047	0,029	0,034	0,067	0,026
PCAdapt, Lositan	LFYR01001803.1_1347475	0,005	0,000	0,027	0,450	0,084	0,000	0,025	0,493	0,102	0,064	0,420	0,020
PCAdapt	LFYR01000204.1_56965	0,000	0,000	0,041	0,000	0,007	0,000	0,000	0,000	0,000	0,074	0,353	0,320
	LFYR01000204.1_58703	0,059	0,046	0,014	0,000	0,029	0,038	0,012	0,000	0,000	0,226	0,211	0,262
	LFYR01000204.1_58740	0,087	0,034	0,027	0,000	0,042	0,025	0,012	0,000	0,000	0,304	0,313	0,278
	LFYR01000811.1_526623	0,000	0,000	0,000	0,000	0,016	0,000	0,016	0,006	0,034	0,053	0,294	0,429
	LFYR01000811.1_526918	0,011	0,000	0,000	0,000	0,018	0,010	0,000	0,000	0,069	0,068	0,167	0,306
	LFYR01000838.1_25023	0,034	0,053	0,084	0,135	0,033	0,063	0,066	0,060	0,050	0,109	0,455	0,213
	LFYR01000838.1_25036	0,009	0,004	0,007	0,000	0,004	0,022	0,000	0,000	0,000	0,018	0,400	0,116

	LFYR0100 0838.1_25 044	0,000	0,004	0,048	0,000	0,054	0,022	0,084	0,016	0,020	0,180	0,476	0,387
	LFYR0100 0850.1_11 5726	0,000	0,065	0,049	0,000	0,049	0,047	0,036	0,000	0,000	0,000	0,200	0,130
	LFYR0100 0889.1_53 8674	0,000	0,000	0,000	0,135	0,000	0,000	0,000	0,471	0,000	0,000	0,189	0,000
	LFYR0100 0893.1_52 440	0,057	0,196	0,000	0,026	0,036	0,019	0,026	0,000	0,000	0,000	0,600	0,000
	LFYR0100 0921.1_58 936	0,018	0,013	0,026	0,000	0,133	0,036	0,010	0,000	0,057	0,074	0,455	0,125
	LFYR0100 0921.1_58 943	0,018	0,025	0,013	0,000	0,065	0,036	0,010	0,000	0,056	0,089	0,455	0,167
	LFYR0100 0921.1_59 006	0,018	0,019	0,013	0,067	0,063	0,036	0,010	0,059	0,080	0,097	0,455	0,133
	LFYR0100 1032.1_85 5195	0,000	0,010	0,046	0,035	0,041	0,018	0,024	0,051	0,000	0,000	0,167	0,289
	LFYR0100 1135.1_14 3853	0,078	0,115	0,254	0,325	0,121	0,155	0,178	0,244	0,305	0,186	0,500	0,119
	LFYR0100 1305.1_29 6803	0,088	0,000	0,000	0,250	0,000	0,042	0,000	0,130	0,000	0,000	0,049	0,000
	LFYR0100 1305.1_29 6806	0,000	0,075	0,036	0,250	0,083	0,174	0,000	0,304	0,387	0,075	0,098	0,157
	LFYR0100 1305.1_29 6814	0,000	0,028	0,038	0,091	0,043	0,043	0,000	0,455	0,065	0,013	0,093	0,012
	LFYR0100 1305.1_29 6925	0,000	0,101	0,118	0,379	0,059	0,118	0,000	0,487	0,052	0,110	0,364	0,007
	LFYR0100 1390.1_10 0151	0,000	0,000	0,049	0,000	0,000	0,000	0,056	0,000	0,000	0,100	0,400	0,000
	LFYR0100 1445.1_50 268	0,028	0,020	0,016	0,015	0,028	0,035	0,007	0,017	0,174	0,419	0,235	0,202
	LFYR0100 1803.1_13 47434	0,000	0,000	0,000	0,015	0,000	0,000	0,000	0,000	0,000	0,025	0,000	0,009
	LFYR0100 1803.1_13 48084	0,000	0,004	0,000	0,000	0,036	0,000	0,000	0,000	0,077	0,030	0,239	0,239
	LFYR0100 1803.1_13 48140	0,100	0,017	0,064	0,037	0,016	0,087	0,071	0,471	0,071	0,071	0,108	0,013

	LFYR0100 1803.1_13 48155	0,000	0,005	0,133	0,414	0,008	0,130	0,000	0,286	0,185	0,147	0,438	0,258
	LFYR0100 1803.1_13 48202	0,130	0,012	0,059	0,200	0,019	0,000	0,087	0,464	0,220	0,070	0,193	0,069
	LFYR0100 1803.1_13 48209	0,087	0,019	0,059	0,480	0,000	0,100	0,000	0,071	0,000	0,044	0,240	0,009
	LFYR0100 1803.1_13 48302	0,056	0,014	0,057	0,200	0,025	0,069	0,053	0,289	0,209	0,052	0,193	0,003
	LFYR0100 1803.1_13 48408	0,080	0,000	0,041	0,038	0,036	0,000	0,032	0,000	0,000	0,034	0,324	0,017
	LFYR0100 1770.1_48 2875	0,034	0,017	0,000	0,091	0,000	0,030	0,000	0,068	0,310	0,232	0,333	0,290
	LFYR0100 1977.1_46 6221	0,000	0,000	0,011	0,019	0,000	0,024	0,000	0,000	0,000	0,000	0,000	0,000
	LFYR0100 2109.1_40 3289	0,081	0,000	0,033	0,000	0,000	0,000	0,000	0,143	0,000	0,000	0,364	0,029
BayeScan	LFYR0100 1213.1_16 9190	0,105	0,146	0,125	0,205	0,087	0,123	0,057	0,146	0,091	0,129	0,000	0,070
	LFYR0100 1213.1_16 9192	0,398	0,163	0,313	0,333	0,326	0,455	0,414	0,244	0,424	0,388	0,471	0,322
	LFYR0100 1213.1_16 9246	0,077	0,054	0,093	0,056	0,056	0,033	0,085	0,000	0,068	0,086	0,091	0,082
	LFYR0100 1213.1_16 9254	0,023	0,031	0,027	0,000	0,032	0,098	0,056	0,050	0,078	0,017	0,087	0,025
	LFYR0100 1213.1_16 9267	0,047	0,064	0,027	0,053	0,043	0,069	0,029	0,000	0,081	0,053	0,087	0,047
	LFYR0100 1213.1_16 9292	0,106	0,061	0,055	0,105	0,123	0,064	0,066	0,091	0,029	0,020	0,087	0,042
	LFYR0100 1714.1_28 2133	0,077	0,030	0,069	0,057	0,096	0,018	0,049	0,083	0,019	0,056	0,063	0,107
	LFYR0100 1714.1_28 2135	0,211	0,269	0,230	0,108	0,354	0,145	0,217	0,192	0,155	0,256	0,125	0,251
	LFYR0100 1714.1_28 2138	0,212	0,276	0,230	0,108	0,354	0,171	0,217	0,192	0,191	0,270	0,121	0,285
	LFYR0100 1714.1_28 2180	0,262	0,304	0,262	0,096	0,393	0,167	0,298	0,236	0,216	0,277	0,245	0,306

	LFYR0100 1714.1_28 2215	0,109	0,078	0,054	0,071	0,074	0,091	0,069	0,140	0,102	0,093	0,138	0,162
	LFYR0100 1714.1_28 2242	0,379	0,339	0,275	0,308	0,272	0,387	0,394	0,283	0,312	0,329	0,133	0,322
	LFYR0100 1714.1_28 2243	0,527	0,539	0,569	0,500	0,583	0,515	0,500	0,500	0,450	0,500	0,433	0,428
	LFYR0100 1714.1_28 2287	0,189	0,128	0,068	0,157	0,237	0,140	0,121	0,159	0,148	0,119	0,120	0,032
	LFYR0100 1714.1_28 2295	0,044	0,020	0,027	0,000	0,022	0,033	0,048	0,012	0,037	0,052	0,043	0,059
	LFYR0100 1714.1_28 2329	0,160	0,121	0,120	0,043	0,100	0,104	0,191	0,089	0,184	0,155	0,050	0,162
Lositan	LFYR0100 0838.1_24 971	0,020	0,004	0,000	0,000	0,007	0,018	0,000	0,000	0,000	0,000	0,000	0,005
	LFYR0100 0838.1_24 976	0,013	0,007	0,006	0,029	0,014	0,012	0,000	0,000	0,000	0,000	0,000	0,033
	LFYR0100 0838.1_24 978	0,000	0,000	0,000	0,000	0,004	0,012	0,000	0,000	0,000	0,015	0,000	0,000
	LFYR0100 0838.1_24 999	0,000	0,015	0,012	0,000	0,008	0,013	0,000	0,000	0,000	0,000	0,000	0,011
	LFYR0100 0838.1_25 006	0,000	0,004	0,000	0,000	0,000	0,006	0,000	0,000	0,000	0,000	0,000	0,017
	LFYR0100 0838.1_25 010	0,007	0,004	0,000	0,000	0,004	0,000	0,009	0,000	0,000	0,029	0,000	0,006
	LFYR0100 0838.1_25 017	0,000	0,008	0,007	0,000	0,013	0,000	0,009	0,000	0,000	0,000	0,000	0,006
	LFYR0100 1213.1_16 9178	0,040	0,010	0,025	0,026	0,022	0,000	0,014	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1213.1_16 9180	0,000	0,041	0,025	0,000	0,000	0,000	0,043	0,000	0,000	0,000	0,000	0,009
	LFYR0100 1213.1_16 9227	0,047	0,000	0,017	0,057	0,000	0,038	0,017	0,000	0,033	0,020	0,000	0,010
	LFYR0100 1213.1_16 9229	0,000	0,012	0,041	0,000	0,011	0,000	0,014	0,000	0,014	0,010	0,000	0,000
	LFYR0100 1213.1_16 9235	0,011	0,044	0,077	0,100	0,092	0,050	0,062	0,000	0,056	0,019	0,000	0,031

	LFYR0100 1213.1_16 9250	0,023	0,000	0,027	0,000	0,000	0,033	0,014	0,050	0,133	0,000	0,087	0,021
	LFYR0100 1213.1_16 9266	0,047	0,064	0,014	0,000	0,011	0,017	0,015	0,000	0,013	0,009	0,000	0,004
	LFYR0100 1213.1_16 9275	0,026	0,022	0,000	0,025	0,011	0,040	0,016	0,025	0,000	0,009	0,000	0,009
	LFYR0100 1445.1_50 243	0,010	0,008	0,008	0,000	0,012	0,003	0,010	0,009	0,029	0,000	0,000	0,000
	LFYR0100 1445.1_50 245	0,032	0,012	0,019	0,000	0,000	0,022	0,000	0,018	0,029	0,043	0,000	0,000
	LFYR0100 1445.1_50 248	0,000	0,008	0,000	0,000	0,004	0,003	0,000	0,000	0,000	0,000	0,000	0,029
	LFYR0100 1445.1_50 249	0,054	0,075	0,042	0,015	0,028	0,012	0,054	0,036	0,029	0,043	0,000	0,000
	LFYR0100 1445.1_50 254	0,003	0,004	0,000	0,015	0,000	0,000	0,003	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1445.1_50 257	0,006	0,016	0,026	0,000	0,000	0,006	0,005	0,018	0,000	0,000	0,000	0,000
	LFYR0100 1445.1_50 258	0,000	0,008	0,008	0,000	0,008	0,000	0,010	0,000	0,059	0,000	0,000	0,019
	LFYR0100 1445.1_50 259	0,006	0,004	0,003	0,000	0,000	0,003	0,003	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1445.1_50 265	0,000	0,000	0,005	0,000	0,008	0,000	0,010	0,000	0,029	0,000	0,000	0,000
	LFYR0100 1445.1_50 267	0,012	0,008	0,000	0,000	0,008	0,000	0,002	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1640.1_25 1907	0,000	0,000	0,018	0,000	0,000	0,000	0,011	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1640.1_25 1910	0,003	0,017	0,013	0,000	0,028	0,018	0,035	0,011	0,000	0,000	0,000	0,021
	LFYR0100 1640.1_25 1915	0,020	0,014	0,013	0,000	0,020	0,004	0,014	0,000	0,000	0,005	0,000	0,014
	LFYR0100 1640.1_25 1926	0,003	0,007	0,004	0,023	0,007	0,000	0,007	0,011	0,000	0,011	0,000	0,014
	LFYR0100 1640.1_25 1928	0,000	0,005	0,004	0,000	0,007	0,000	0,011	0,000	0,000	0,000	0,000	0,007

	LFYR0100 1640.1_25 1933	0,000	0,007	0,013	0,024	0,028	0,005	0,007	0,000	0,008	0,006	0,000	0,014
	LFYR0100 1640.1_25 1935	0,003	0,014	0,023	0,000	0,030	0,028	0,045	0,000	0,008	0,000	0,000	0,000
	LFYR0100 1640.1_25 1945	0,000	0,003	0,005	0,000	0,000	0,011	0,004	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1714.1_28 2137	0,000	0,000	0,000	0,054	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,010
	LFYR0100 1714.1_28 2141	0,015	0,023	0,041	0,000	0,021	0,009	0,010	0,039	0,075	0,000	0,065	0,034
	LFYR0100 1714.1_28 2146	0,119	0,069	0,078	0,000	0,040	0,045	0,016	0,000	0,033	0,031	0,029	0,014
	LFYR0100 1714.1_28 2157	0,000	0,000	0,000	0,042	0,019	0,014	0,008	0,000	0,015	0,000	0,050	0,000
	LFYR0100 1714.1_28 2165	0,016	0,033	0,026	0,000	0,012	0,025	0,019	0,033	0,025	0,018	0,029	0,024
	LFYR0100 1714.1_28 2172	0,007	0,013	0,007	0,040	0,019	0,007	0,015	0,015	0,000	0,000	0,000	0,000
	LFYR0100 1714.1_28 2173	0,000	0,006	0,000	0,000	0,009	0,007	0,007	0,014	0,000	0,000	0,041	0,008
	LFYR0100 1714.1_28 2174	0,000	0,000	0,021	0,077	0,037	0,000	0,021	0,028	0,013	0,007	0,000	0,039
	LFYR0100 1714.1_28 2177	0,013	0,000	0,000	0,000	0,000	0,000	0,007	0,000	0,024	0,015	0,041	0,000
	LFYR0100 1714.1_28 2178	0,007	0,081	0,028	0,038	0,000	0,013	0,007	0,000	0,024	0,029	0,082	0,039
	LFYR0100 1714.1_28 2182	0,026	0,025	0,042	0,000	0,000	0,020	0,014	0,054	0,006	0,000	0,000	0,019
	LFYR0100 1714.1_28 2184	0,013	0,012	0,014	0,000	0,000	0,013	0,007	0,000	0,012	0,000	0,000	0,000
	LFYR0100 1714.1_28 2194	0,000	0,000	0,000	0,037	0,009	0,000	0,007	0,013	0,018	0,007	0,070	0,008
	LFYR0100 1714.1_28 2213	0,000	0,000	0,007	0,018	0,000	0,000	0,000	0,000	0,028	0,007	0,000	0,004
	LFYR0100 1714.1_28 2214	0,048	0,065	0,027	0,036	0,147	0,096	0,055	0,070	0,034	0,007	0,034	0,069

	LFYR0100 1714.1_28 2222	0,000	0,000	0,007	0,000	0,000	0,000	0,000	0,011	0,000	0,006	0,000	0,054
	LFYR0100 1714.1_28 2223	0,000	0,012	0,007	0,000	0,045	0,000	0,013	0,000	0,006	0,025	0,000	0,023
	LFYR0100 1714.1_28 2224	0,000	0,012	0,019	0,000	0,027	0,006	0,007	0,000	0,022	0,018	0,017	0,031
	LFYR0100 1714.1_28 2232	0,000	0,006	0,019	0,000	0,009	0,000	0,013	0,011	0,011	0,012	0,033	0,000
	LFYR0100 1714.1_28 2233	0,006	0,012	0,013	0,000	0,027	0,012	0,020	0,032	0,017	0,019	0,000	0,048
	LFYR0100 1714.1_28 2237	0,006	0,012	0,026	0,000	0,018	0,049	0,007	0,021	0,022	0,025	0,000	0,036
	LFYR0100 1714.1_28 2246	0,000	0,006	0,000	0,018	0,000	0,000	0,000	0,000	0,022	0,000	0,000	0,000
	LFYR0100 1714.1_28 2247	0,012	0,006	0,006	0,000	0,000	0,006	0,007	0,033	0,000	0,000	0,000	0,008
	LFYR0100 1714.1_28 2248	0,036	0,006	0,026	0,089	0,018	0,070	0,082	0,185	0,073	0,024	0,000	0,040
	LFYR0100 1714.1_28 2255	0,000	0,006	0,006	0,000	0,000	0,000	0,000	0,000	0,000	0,036	0,000	0,037
	LFYR0100 1714.1_28 2259	0,000	0,000	0,000	0,000	0,037	0,000	0,000	0,000	0,000	0,012	0,000	0,000
	LFYR0100 1714.1_28 2260	0,024	0,000	0,019	0,000	0,028	0,000	0,007	0,000	0,011	0,000	0,000	0,012
	LFYR0100 1714.1_28 2265	0,024	0,043	0,013	0,000	0,019	0,055	0,028	0,000	0,011	0,012	0,000	0,004
	LFYR0100 1714.1_28 2284	0,000	0,013	0,007	0,000	0,011	0,013	0,016	0,023	0,019	0,026	0,000	0,009
	LFYR0100 1714.1_28 2298	0,038	0,000	0,000	0,000	0,000	0,007	0,000	0,024	0,013	0,000	0,000	0,014
	LFYR0100 1714.1_28 2299	0,006	0,000	0,014	0,000	0,000	0,013	0,000	0,000	0,000	0,013	0,000	0,018
	LFYR0100 1714.1_28 2309	0,060	0,000	0,000	0,000	0,023	0,060	0,034	0,072	0,026	0,000	0,000	0,000
	LFYR0100 1714.1_28 2316	0,034	0,031	0,000	0,020	0,000	0,007	0,009	0,024	0,013	0,000	0,000	0,020

	LFYR0100 1714.1_28 2323	0,021	0,000	0,008	0,000	0,012	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1714.1_28 2336	0,000	0,000	0,025	0,000	0,015	0,000	0,000	0,000	0,036	0,000	0,054	0,006
	LFYR0100 1714.1_28 2338	0,015	0,027	0,034	0,024	0,059	0,066	0,009	0,013	0,036	0,059	0,000	0,056
	LFYR0100 1714.1_28 2341	0,015	0,000	0,000	0,000	0,015	0,008	0,000	0,000	0,051	0,017	0,000	0,006
	LFYR0100 1770.1_48 2879	0,063	0,015	0,025	0,000	0,062	0,000	0,012	0,028	0,044	0,014	0,000	0,000
	LFYR0100 1770.1_48 2884	0,024	0,007	0,000	0,000	0,000	0,000	0,013	0,000	0,000	0,000	0,048	0,010
	LFYR0100 1770.1_48 2885	0,056	0,037	0,050	0,063	0,018	0,074	0,025	0,121	0,047	0,028	0,000	0,000
	LFYR0100 1770.1_48 2889	0,000	0,036	0,000	0,063	0,053	0,000	0,068	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1770.1_48 2896	0,056	0,066	0,006	0,000	0,035	0,026	0,006	0,000	0,022	0,000	0,000	0,005
	LFYR0100 1770.1_48 2905	0,071	0,000	0,012	0,063	0,018	0,031	0,000	0,000	0,022	0,000	0,000	0,014
	LFYR0100 1770.1_48 2907	0,000	0,015	0,024	0,000	0,000	0,021	0,019	0,028	0,022	0,000	0,000	0,000
	LFYR0100 1803.1_13 47426	0,000	0,007	0,000	0,031	0,000	0,000	0,012	0,000	0,000	0,000	0,018	0,000
	LFYR0100 1803.1_13 47430	0,000	0,000	0,000	0,000	0,000	0,007	0,000	0,000	0,000	0,017	0,008	0,017
	LFYR0100 1803.1_13 47442	0,000	0,008	0,000	0,045	0,010	0,000	0,000	0,023	0,059	0,058	0,033	0,009
	LFYR0100 1803.1_13 47444	0,000	0,000	0,009	0,000	0,019	0,000	0,000	0,000	0,000	0,008	0,000	0,000
	LFYR0100 1803.1_13 47445	0,000	0,000	0,000	0,000	0,020	0,000	0,000	0,102	0,159	0,058	0,000	0,422
	LFYR0100 1803.1_13 47447	0,000	0,004	0,000	0,017	0,000	0,007	0,000	0,000	0,000	0,025	0,025	0,000
	LFYR0100 1803.1_13 47453	0,000	0,000	0,018	0,138	0,010	0,022	0,013	0,046	0,000	0,008	0,061	0,000

	LFYR0100 1803.1_13 47468	0,005	0,000	0,009	0,000	0,024	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	LFYR0100 1803.1_13 47470	0,000	0,015	0,000	0,000	0,012	0,000	0,000	0,000	0,000	0,018	0,013	0,000
	LFYR0100 1803.1_13 47477	0,000	0,004	0,000	0,050	0,000	0,000	0,006	0,000	0,000	0,009	0,000	0,000
	LFYR0100 1803.1_13 47478	0,009	0,000	0,009	0,000	0,000	0,007	0,000	0,000	0,000	0,000	0,000	0,010
	LFYR0100 1803.1_13 47486	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,014	0,082	0,000	0,029	0,071
	LFYR0100 1803.1_13 47495	0,000	0,008	0,018	0,000	0,048	0,029	0,025	0,000	0,000	0,000	0,079	0,000
	LFYR0100 1803.1_13 47504	0,000	0,000	0,009	0,000	0,024	0,000	0,000	0,000	0,000	0,000	0,016	0,000
	LFYR0100 1803.1_13 47934	0,000	0,000	0,077	0,357	0,019	0,042	0,000	0,444	0,211	0,088	0,078	0,138

Table S2 Five environmental stressors and ecological category (A-F described above) of each site as rated by the NBA (Van Niekerk et al. 2012) as low (L), medium (M), high (H) and very high (VH), as well as the presence (Y) and absence (N) of sand mining.

Site	Change in flow	Pollution	Habitat loss	Sand mining	Fishing effort	Ecological category	Submerged macrophyte area (ha)
Olifants	M	M	M	N	VH	C	47.74
Berg	M	H	M	N	VH	D	206
Breede	M	L	L	N	H	B	6
Knysna	L	M	L	N	H	B	238
Swartkops	L	H	H	N	H	C	44.7
Nahoon	M	H	M	N	H	C	2.3
Mngazna	L	M	M	N	H	B	2
Richard's Bay	L	M	H	Y	H	C	28.5

Figure S4 R script describing SDM methods

```
#required libraries
LIB <- c("rgbif", "biomod2", "ggplot2", "gridExtra", "knitr", "raster",
      "ade4", "rworldmap", "cleangeo", "maptools", "rasterVis", "rgdal","rgeos")
for(i in LIB) { library(i, character.only=T) }
# load and stack environmental data
warmest <- raster("data/biogeo15_5m_clipped3.tif", level = 1)
coolest <- raster("data/biogeo14_5m_clipped3.tif", level = 1)
bioclim_world <- stack(warmest,coolest)
plot(bioclim_world)
#load species occurrence data
xy<-read.csv(file="innitial_distribution1.csv", header=TRUE, sep=";", dec=".")
#check location of points
plot(bioclim_world$biogeo15_5m_clipped3)
points(xy[,2:3], pch=19, col="red")
#convert the column named "PRESENCE" to a character class
myResp<-as.numeric(xy$ZosteraCapensis)
myRespName <- 'ZosteraCapensis'
#format for model
SPC_PresAbs <- BIOMOD_FormatingData(resp.var = myResp,
                    expl.var = bioclim_world,
                    resp.xy = xy[,c('x', 'y')],
                    resp.name = myRespName)
SPC_PresAbs
plot(SPC_PresAbs)
#set model options
MySpc_options <- BIOMOD_ModelingOptions(
 GLM = list( type = 'quadratic', interaction.level = 1 ),
 GBM = list( n.trees = 1000 ),
 GAM = list( algo = 'GAM_mgcv' ) )
MySpc_models <- BIOMOD_Modeling( data = SPC_PresAbs,
                    models = c("GLM","GAM", "GBM", "RF","MARS","FDA"),
                    models.options = MySpc_options,
                    NbRunEval = 3,
                    DataSplit = 80,
                    VarImport = 3,
                    models.eval.meth=c('TSS','ROC'),
                    do.full.models = F )
#get models evaluation scores
MyModels_scores <- get_evaluations(MySpc_models)
dim(MyModels_scores)
dimnames(MyModels_scores)
models_scores_graph(MySpc_models, by = "models" , metrics = c("ROC","TSS"), xlim = c(0.5,1), ylim = c(0.5,1))
models_scores_graph(MySpc_models, by = "cv_run" , metrics = c("ROC","TSS"), xlim = c(0.5,1), ylim = c(0.5,1))
models_scores_graph(MySpc_models, by = "data_set" , metrics = c("ROC","TSS"), xlim = c(0.5,1), ylim = c(0.5,1))
#name and load the produced models.
MySpc_glm <- BIOMOD_LoadModels(MySpc_models, models='GLM')
MySpc_gam <- BIOMOD_LoadModels(MySpc_models, models='GAM')
MySpc_gbm <- BIOMOD_LoadModels(MySpc_models, models='GBM')
MySpc_rf <- BIOMOD_LoadModels(MySpc_models, models='RF')
#projection
MySpc_models_proj_current <- BIOMOD_Projection( modeling.output = MySpc_models,
                    new.env = bioclim_world,
                    proj.name = "current",
                    binary.meth = "ROC",
                    output.format = ".img",
                    do.stack = FALSE )
plot(MySpc_models_proj_current)
#ensemble modeling
MySpc_ensemble_models <- BIOMOD_EnsembleModeling( modeling.output = MySpc_models,
                    chosen.models = "all",
                    em.by = 'all', #combine all models
                    eval.metric = 'all',
                    eval.metric.quality.threshold = c(0.55,0.8),
                    models.eval.meth = c('TSS','ROC'),
                    prob.mean = FALSE,
                    prob.cv = TRUE, #coefficient of variation across predictions
                    committee.averaging = TRUE,
                    prob.mean.weight = TRUE,
                    VarImport = 0 )
MySpc_ensemble_models_scores <- get_evaluations(MySpc_ensemble_models)
MySpc_ensemble_models_scores
#ensemble forecast
MySpc_ensemble_models_proj_current <- BIOMOD_EnsembleForecasting(
 EM.output = MySpc_ensemble_models,
 projection.output = MySpc_models_proj_current,
 selected.models = "all",
 binary.meth = c("TSS","ROC"),
 output.format = ".img",
 do.stack = FALSE )
MySpc_ensemble_models_proj_current
#load and stack LGM climate data
lgm_warmest <- raster("data/21kya_CNRM/biogeo15_5m_clipped3.tif", level = 1)
lgm_coolest <- raster("data/21kya_CNRM/biogeo14_5m_clipped3.tif", level = 1)
lgm_bioclim_world <- stack(lgm_warmest,lgm_coolest)
lgm_bioclim_world
#lgm projection
MySpc_models_proj_lgm <- BIOMOD_Projection( modeling.output = MySpc_models,
                    new.env = lgm_bioclim_world,
                    proj.name = "lgm",
                    binary.meth = c("ROC","TSS"),
                    output.format = ".img",
                    do.stack = FALSE )
#LGM ensemble modeling
MySpc_ensemble_models_proj_lgm <- BIOMOD_EnsembleForecasting(
 EM.output = MySpc_ensemble_models,
 projection.output = MySpc_models_proj_lgm,
 binary.meth = "ROC",
 output.format = ".img",
 do.stack = FALSE ,
 build.clamping.mask=F)
pdf(file="Ensemble_predictions_current_to_lgm1.pdf")
plot(MySpc_ensemble_models_proj_current, str.grep = "EMca|EMwmean")
plot(MySpc_ensemble_models_proj_lgm, str.grep = "EMca|EMwmean")
dev.off()
#binary predictions (presence/absence).
MyBinCA_Current <- raster::stack("ZosteraCapensis/proj_current/individual_projections/ZosteraCapensis_EMcaByTSS_mergedAlgo_mergedRun_mergedData.img")
```

```
plot(MyBinCA_Current, main="My Binary ComAverage : Current", col=c("grey","grey","grey","yellow", "orange", "red"))
points(xy[,c("x", "y")], col="blue", pch=1)
dev.off()
#probability of suitable habitat
MyBinCA_lgm <- raster::stack("ZosteraCapensis/proj_lgm/individual_projections/ZosteraCapensis_EMcaByTSS_mergedAlgo_mergedRun_mergedData.img")
plot(MyBinCA_lgm, main="My Binary ComAverage : LGM", col=c("grey","grey","grey","yellow", "orange", "red"))
points(xy[,c("x", "y")], col="blue", pch=1)
## load binary projections
MySpc_bin_proj_current <- stack(
 c(wm = paste("ZosteraCapensis/proj_current/individual_projections/ZosteraCapensis_EMwmeanByTSS_mergedAlgo_mergedRun_mergedData_ROCbin.img")))
MySpc_bin_proj_lgm <- stack(
 c(wm = paste("ZosteraCapensis/proj_lgm/individual_projections/ZosteraCapensis_EMwmeanByTSS_mergedAlgo_mergedRun_mergedData_ROCbin.img")))
plot(MySpc_bin_proj_current + MySpc_bin_proj_lgm)
SRC_current_lgm <- BIOMOD_RangeSize( MySpc_bin_proj_current,
                    MySpc_bin_proj_lgm )
SRC_current_lgm$Compt.By.Models
# Plot the distributions changes in more detail:
#+++++++++++++++++++++++++++++++++++++++++++++
++++++++++++++++++++++++++
MySpc_src_map <- stack(SRC_current_lgm$Diff.By.Pixel)
# set up the color key, open pdf creation,plot and end pdf creation-see file in working directory
my.at <- seq(-2.5,1.5,1)
myColorkey <- list(at=my.at, ## where the colors change
          labels=list(
           labels=c("gain", "pres", "abs","loss"), ## labels
           at=my.at[-1]-0.5 ## where to print labels
          ))
pdf(file=paste("distribution_changes_lgm_to_present.pdf", sep=""))
rasterVis::levelplot( MySpc_src_map,
           main = "Range change from LGM to present",
           colorkey = myColorkey,
           #      col.regions=c("#d01c8b",      "#b8e186", "#f1b6da", "#4dac26"),
           col.regions=c("#2c7bb6",  "#abd9e9",  "#fdae61", "#d7191c"),
           layout = c(1,1) )
plot1<-rasterVis::levelplot( MyBinCA_Current,
           main = "Range change",
           colorkey = FALSE,
           layout = c(1,1) )
plot2<-rasterVis::levelplot( MyBinCA_lgm,
           main = "Range change",
           colorkey = FALSE,
dev.off()
```