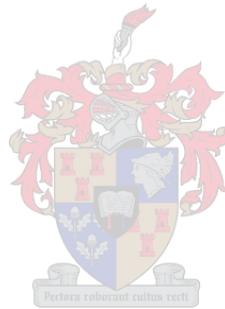


*Using multi-species seascape genomics to conserve areas of
evolutionary importance*

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Dissertation presented for the degree of Doctor of Philosophy (PhD) in the
Faculty of Science at Stellenbosch University



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Declaration

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This dissertation includes one original paper published in a peer-reviewed journal, and two unpublished publications, which are in review. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and a declaration is included in the dissertation (at the end of each chapter) indicating the nature and extent of the contributions of co-authors.

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Abstract

Understanding the environmental footprints on species and genetic biodiversity is a key concern in molecular ecology and conservation genetics. As species are increasingly under pressure from anthropogenic climate change, understanding how rapid environmental changes will influence intra- and interspecific diversity is essential if we are to conserve functioning ecosystems. This PhD thesis used the unique environmental backdrop of the South African coastline to infer how environmental variables over space and time shape multiple facets of biological variation. Specifically, this thesis utilised seascape genomic analyses to test the strong environmental gradients within South Africa against the molecular variation of three rocky intertidal species: Cape urchin (*Parechinus angulosus*), Common shore crab (*Cyclograpsus punctatus*), and Granular limpet (*Scutellastra granularis*). The first chapter evaluated which contemporary seascape features most strongly correlate with neutral and adaptive intraspecific diversity across species. Here, the results show that gene-environment relationships are species-specific, with the crab showing less population differentiation, strongly influenced by sea-surface salinity, and the urchin and limpet showing a west-east population differentiation predominantly influenced by sea-surface and air temperature. Chapter Two tested the relative influence of historical climatic stability versus contemporary species distributions in shaping patterns of neutral diversity of the three species. The results from this chapter indicate that historical climatic refugia since the Last Glacial Maximum are potentially stronger predictors of contemporary molecular diversity hotspots than the species' current distribution. The third research chapter evaluated the vulnerability of the three study species with regards to future climatic change, both at two time-points and under two emission scenarios. Here, the results highlight how future responses to global change will likely differ among species, as well as among populations within each species. In the final chapter, the patterns uncovered in the three data chapters, pertaining to genomic diversity and vulnerability, climatic stability, and adaptive potential, are combined in a conservation planning framework to identify areas of evolutionary importance, which can be thought of as priority areas for forward-thinking conservation action. As a whole, this thesis used novel ecological and evolutionary models to understand the spatio-temporal interplay between species, genes, and environment, and used this information to guide conservation action within South Africa.

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List of Abbreviations

2X outliers: outliers selected by two or more outlier-detection methods

ABC: Approximate Bayesian Computation

ABF: Additive Benefit Function

AICc: Akaike Information Criteria corrected for small sample sizes

BF: Bayes Factor

BH: Benjamini-Hochberg

BPA: BayPass Auxiliary model

BPC: BayPass Core model

CAF-SU: Central Analytical Facility at Stellenbosch University

CBD: Convention on Biological Diversity

CCSM: Community Climate System Model

CI: Confidence Interval

COI: Cytochrome Oxidase-1

dbRDA: distance-based Redundancy Analysis

EBSA: Ecologically or Biologically Significant Area

EEZ: Exclusive Economic Zone

ESU: Evolutionary Significant Unit

GCC: Global Climate Change

GCM: General Circulation Model

GEA: Gene-Environment Association

GF: Gradient Forest

GO: Gene Ontology

h: Haplotype Diversity

HADCM3: Hadley Centre Ocean-Atmosphere Climate Model

H_E : Expected Heterozygosity

HPC: High Performance Cluster

IBD: Isolation-by-Distance

IBE: Isolation-by-Environment

kya: Thousand Years Ago

LD: Linkage Disequilibrium

LFMM: Latent Factor Mixed Models
LGM: Last Glacial Maximum
MAF: Minor Allele Frequency
MEM: Moran's Eigenvector Map
MH: Mid-Holocene
MIROC: Model for Interdisciplinary Research on Climate
MIROC-ESM: Model for Interdisciplinary Research on Climate Earth System Model
MPA: Marine Protected Area
MSOD: Moran Spectral Outlier Detection
MSP: Marine Spatial Planning
mtDNA: mitochondrial DNA
NBA: National Biodiversity Assessment
 N_e : Effective Population Size
NGS: Next Generation Sequencing
PCA: Principal Components Analysis
PCoA: Principal Coordinates Analysis
PCNM: Principal Coordinates of Neighbour Matrice
PLD: Pelagic Larval Duration
POD: Pseudo-Observed Dataset
Pool-seq: Pooled DNA sequencing
RAD-seq: Restriction Site Associated DNA sequencing
RCP: Representative Concentration Pathway
RDA: Redundancy Analysis
ROC: Receiver Operating Characteristic
SDM: Species Distribution Model
SNP: Single Nucleotide Polymorphism
SSS: Sea Surface Salinity
SSSmean: mean Sea Surface Salinity
SSSrange: range in Sea Surface Salinity
SST: Sea Surface Temperature
SSTmean: mean Sea Surface Temperature
SSTrange: range in Sea Surface Temperature

Tmax: maximum Air Temperature

Tmin: minimum Air Temperature

Trange: range in Air Temperature

TSS: True Skill Statistic

VIF: Variance Inflation Factor

~

General Introduction

*Extending seascape genomics to characterise evolutionary processes,
both in a global context, and within South Africa*

~

Introduction

Unsustainable resource use and increasing carbon dioxide emissions by human populations are major threats to ecological systems globally, and in South Africa in particular (Mead et al., 2013; Miraldo et al., 2016; Pecl et al., 2017; Pörtner et al., 2019). Broadly, conservation measures aim to counteract the negative impacts of human interactions with the environment through protecting and managing biodiversity and ecosystem functioning (Brooks et al., 2006). To protect the planet's biodiversity, we must know how it is arranged over space and time, as well as how it responds to the both historical and contemporary processes shaping its trajectory. Moreover, there are multiple levels of biodiversity, ranging from ecosystems- and species-level, to genetic diversity, although their uptake in conservation actions varies significantly (Bax et al., 2016; Carvalho et al., 2011a; Hoban et al., 2020).

Conservation planning is built upon prioritising areas that capture both representation and persistence of biodiversity (Margules & Pressey, 2000; Moritz, 2002; Pressey, 2004). Yet there are recent calls for conservation practice to promote resilience to climate change, which means that reserve designs should aim to incorporate species and ecosystem vulnerability (Groves et al., 2012; Tittensor et al., 2019). Vulnerability is thought to be composed of three facets: exposure (i.e. the rate and magnitude of climatic change), sensitivity (i.e. a species' tolerance thresholds against climatic change), and adaptive capacity (i.e. a species' ability to adapt to climatic change via demographic, evolutionary, or behavioural mechanisms; Dawson et al., 2011). Arguably, one of the best tools to quantify species sensitivity and adaptive capacity to global change for conservation planning is with molecular datasets (Beger et al., 2014; Moritz, 2002; von der Heyden, 2009).

Over the last 20 years, the field of molecular ecology has broadly advanced from using genetic markers, here defined as markers from a single or few loci, to using genomic markers, defined here as pertaining to hundreds to thousands of loci spread across the entire genome (Allendorf, 2017; Ellegren, 2014). This technological advance allows for more robust inferences regarding population structure and connectivity, as well as understanding the impacts of local selective pressures on natural populations. Further, with the advancement of landscape genomics statistical models, we can now identify putatively important drivers of adaptation within natural populations, by taking into consideration

local environmental differences (Balkenhol et al., 2019; Epps & Keyghobadi, 2015; Manel & Holderegger, 2013). However, these models are still relatively simplistic, compared to the complex evolutionary trajectories of species composed of interactions with environmental, biotic and ecological changes over space and time (Feinberg & Irizarry, 2010; Hand et al., 2015; Liggins et al., 2019). It is beneficial if these models can incorporate biologically relevant information, and that they compare the genomic variation between species, as this will help us understand how ecosystems will respond to global climate change (Grummer et al., 2019; Selmoni et al., 2020; Sork et al., 2010). As the field of conservation genetics evolves into conservation genomics, and applications of next-generation approaches for non-model species become more accessible (da Fonseca et al., 2016; Ellegren, 2014), estimates of putative adaptive variation and drivers of selection can be used to inform conservation action (Funk et al., 2012, 2019; Ouborg et al., 2010; Stapley et al., 2010).

Land- and seascape genomics

The field of landscape genetics, and the subsequent fields of riverscape and seascape genetics, integrate methodologies from geography, ecology, and population genetics to explain the spatial and temporal distribution of genetic variation (Manel et al., 2003; Manel & Holderegger, 2013; Riginos et al., 2016; Selkoe et al., 2016; Storfer et al., 2018).

Landscape genetic analyses use spatial statistics to address landscape-scale research questions on evolutionary processes such as gene flow, natural selection, and genetic drift (Manel et al., 2003; Sork et al., 2013; Storfer et al., 2018). With recent advances in high-throughput sequencing and bioinformatic programs, the field of landscape genetics is evolving into landscape genomics, by assessing how environmental variation interacts with genome-wide variation (Balkenhol et al., 2019; Schwartz et al., 2010). The advent of landscape genomics greatly increases our ability to disentangle the environmental drivers of neutral and adaptive variation, as neutral markers are generally not as equipped to capture quantitative and adaptive traits (Holderegger et al., 2006; Mittell et al., 2015).

Specifically, gene-environment association analyses (GEAs) are one of the most widely used tools to identify putatively adaptive loci, referred to here as outlier loci, which should reflect patterns of local adaptation (Balkenhol et al., 2019; Liggins et al., 2019;

Riginos & Liggins, 2013), although they can also relate to neutral patterns via false positives (Hoban et al., 2016; Lotterhos & Whitlock, 2014, 2015). Broadly, GEAs test for correlations between allelic and environmental variation, and a wide variety of GEA methods exist (Storfer et al., 2018), often making different assumptions about past demography (Forester et al., 2017), and potentially leading to differences in outlier loci detected (Dalongeville et al., 2018). GEAs are helpful tools to explore selection forces acting on natural populations and identify candidate loci for adaptation, but often report false positives when geographic/demographic processes mimic selection (Rellstab et al., 2015). However, GEAs are quite robust when historical demographic expansions and ecological variation are aligned in space (Frichot et al., 2015). Despite this, many studies conducting GEAs do not consider past demographic histories, nor explore how genetic isolation-by-distance may influence outlier loci detection (Nadeau et al., 2016). Moreover, most studies to date have conducted GEAs on single species, and within strong environmental gradients at coarse spatial resolution, especially within the marine environment (Selkoe et al., 2016).

Of the influx of landscape genetics studies over the past decade, there are several marine examples, yet they still make up a smaller proportion than terrestrial examples (Selkoe et al., 2016; Storfer et al., 2010, 2018). Seascape genetics aims to answer specific questions based on the biology of marine taxa and the fluid medium they occur in (Carr et al., 2003; Galindo et al., 2010; Riginos & Liggins, 2013; Selkoe et al., 2010). These questions are often challenging, since in marine, compared to terrestrial realms, environmental heterogeneity is less distinct, dispersal barriers are not as obvious, and most marine invertebrate species have multiple life stages, as well as a wide variety of life history traits that are often not well understood (Galindo et al., 2006, 2010; Liggins et al., 2015, 2019). Disentangling neutral genetic structure and adaptive divergence is particularly challenging within the marine realm (Riginos et al., 2016; Sandoval-Castillo et al., 2018; Selmoni et al., 2020), as many marine species, especially marine invertebrates, often have large effective population sizes (N_e) and high dispersal abilities, increasing their potential for selective sweeps despite high levels of gene flow (Leiva et al., 2019; Selkoe et al., 2010, 2014).

Specifically, seascape-genomic studies on marine invertebrates are difficult to interpret as the functional roles of putatively adaptive loci are still poorly understood for these non-model species, due to a general lack of well-annotated genomes (Ekblom & Galindo, 2011; Ellegren, 2014; Hornett & Wheat, 2012). Seascape genomic studies of marine

invertebrates have largely identified population differentiation within species, despite high dispersal capabilities, predominantly driven by ocean temperatures (Selkoe et al., 2016). For example, Wei et al. (2013) found that average Sea Surface Temperature (SST) best explained the genetic structure in the greenshell mussel (*Perna canaliculus*) in New Zealand, and Sandoval-Castillo et al. (2018) identified minimum SST and oxygen concentration as drivers of the genomic structure of the greenlip abalone (*Haliotis laevis*) in Australia. Identifying putatively differentially adapted populations, and the environmental force(s) likely creating these differences, is of conservation importance as it can inform which populations within a species may be most at risk to future climatic change (Jahnke et al., 2018; Selmoni et al., 2020).

Landscape/seascape genetics and conservation

The field of landscape genetics is inherently connected to conservation planning, as the preservation of spatially explicit evolutionary and ecological processes is one of the key pillars of conservation (Coates et al., 2018; Lankau et al., 2011; Moilanen et al., 2009). By identifying the landscape features linked with genetic and genomic patterns, we are able to better understand the processes driving the persistence and adaptation of populations, which gives us more clarity in the use of genetic information for conservation management (Lean et al., 2017; Wagner & Fortin, 2013). There have been several landscape/seascape genetic studies with a focus on conservation planning strategies, such as Melià et al. (2016) who used community level seascape approaches to assess community connectivity for MPA design. There are also calls for landscape genetic studies to evolve from descriptive to predictive analyses, so that the field can better inform conservation planning decisions in a changing world (Manel & Holderegger, 2013). However, for this to happen, landscape genetics studies should aim to capture the patterns of multiple species and ecosystem dynamics, and over multiple timeframes (Epps & Keyghobadi, 2015; Manel & Holderegger, 2013). Including data from multiple time points allows for investigations into 'genetic time lags', or rate of response of molecular data to climatic changes over time (Hall & Beissinger, 2014).

Influence of past climatic change on seascape genomic analyses

Considerations of scale are pertinent to most genetic inferences and thus, issues of temporal scale are pertinent to landscape genetic analyses (Dungan et al., 2002). One of the major challenges in seascape genomics is to identify key processes affecting the genomic variation at one point in time, in an environment that varies both spatially and temporally (Selkoe et al., 2015, 2016). Within this context, it has been shown that multiple sampling seasons can better unravel stochastic patterns of genomic variation (Henriques et al., 2016; Rhode et al., 2017), yet historical factors may also play important roles in shaping the contemporary patterns of marine taxa (Dawson et al., 2014; Hart & Marko, 2010; Toms et al., 2014). However, until recently, there has been little exploration into the effects of temporal scale on landscape genetic inferences (Balkenhol et al., 2019).

The interpretation of genetic variation can be challenging, as it is difficult to disentangle the effects of historical and contemporary evolutionary processes. Moreover, current species distributions and patterns of genetic variation may have resulted from historical environmental conditions that no longer exist and may be difficult to reconstruct (James et al., 2007; Roe et al., 2009; Wiens & Graham, 2005). It is suggested that contemporary species distributions and population connectivity (Eckert et al., 2008), as well as past climatic stability (Hewitt 2000), are important drivers of extant genetic diversity, which is often used as a proxy for evolutionary potential (Catullo et al., 2015; Waldvogel et al., 2020). Multiple studies have tested the roles of contemporary or historical influences on genetic diversity (Assis et al., 2014; Chefaoui & Serrão, 2017; Phair et al., 2019; Sinclair et al., 2016), but the relative strengths of past and present climatic variation on extant molecular diversity remains poorly tested (Guo, 2012). Understanding how historical species distributions have shaped current genomic variation may give us an indication of how future climate change might affect species' ranges and adaptive variation (Beatty & Provan, 2011), assuming that climate is the key driver of species range shifts and adaptation.

Genes and species into the future

How future climatic change will affect molecular variation, both on a species and population level, is still largely unknown. There are a number of studies already demonstrating that species are altering their physiological tolerance and/or shifting their distributions in response to rapid environmental changes (Aitken et al., 2008; Jump & Peñuelas, 2005; Reusch, 2014). Globally, the trend is for marine species to move polewards (or deeper) in their distributions in response to warming temperatures (Chen et al., 2011; Walther et al., 2002). Within coastal communities, there are multiple reports of synchronous shifts in abundance and distributions of microalgae and invertebrates, which is likely a result of increased annual temperatures associated with Global Climate Change (GCC; Harley et al. 2006; Mieszkowska et al. 2006; Hawkins et al. 2008). However, there are also known differences in coastal species' responses to recent temperature changes (Harley, 2011; Hawkins et al., 2009; Rubal et al., 2013). For example, Rubal et al. (2013) found that an increase in SST lead to the range retraction of the molluscs *Littorina saxatilis* and *Nucella lapillus*, but also to the range expansion of *Siphonaria pectinata* along the Iberian Peninsula from 1949 to 2010. Thus, further work is needed to understand species-specific responses to global change, and which life history traits lead to increased or decreased climatic resilience.

Most studies assessing the effects of GCC on biodiversity and distributional shifts have focused on the species level of biodiversity, specifically with species distribution models (SDMs). SDMs use correlations between known species occurrences and environmental variables to predict species distributions in space and time (Elith & Leathwick, 2009). SDMs are still limited in the marine environment, especially in marine invertebrate species (Bosch et al., 2018; Robinson et al., 2011). Although widely used to predict species responses to GCC, SDMs are relatively simplistic, and fail to incorporate evolutionary processes of phenotypic plasticity and adaptation (Rilov et al., 2019). SDMs can benefit from including the intraspecific level of biodiversity, such as distributional shifts of genetic clusters, as this facet of biodiversity will also be impacted by global change (Jahnke et al., 2018; Prates et al., 2016; Taubmann et al., 2011; Wielstra et al., 2013; Yannic et al., 2014). There are a growing number of studies adopting interdisciplinary approaches to incorporate aspects of phylogenetic or intraspecific genetic variation into SDMs (Bálint et al., 2011; Benito Garzón et al., 2011; Bystriakova et al., 2014; D'Amen et al., 2013; Jay et al., 2012; Sork et al., 2010). In fact, there is mounting evidence that including intraspecific

variation into SDMs leads to an increase in overall model performance (Pearman et al., 2010).

Recent studies suggest that species' distributions, their genetic composition, and their predicted loss of genetic diversity may vary between sympatric species (Alsos et al., 2012; Beatty & Provan, 2011; Prates et al., 2016). For example, Alsos et al. (2012) assessed the genetic consequences of a range reduction in 27 plant species using eight SDM approaches under two climate change scenarios, and found that the loss of genetic diversity varied widely across species, but could be explained by species dispersal adaptation (57%) and genetic structure (61%). However, Alsos et al. (2012) used life history traits to estimate dispersal adaptation, and therefore did not fully assess the potential adaptive capacity of the study species, as the molecular evidence of adaptation was not tested.

There are also recent developments in projecting molecular composition in space, and using this to infer genomic vulnerability to GCC (Bay et al., 2018; Fitzpatrick & Keller, 2015). More specifically, Gradient Forest (GF) models have been extended from mapping species, to genomic composition and turnover patterns (Ellis et al., 2012; Fitzpatrick & Keller, 2015). These analyses offer unique insights into patterns of intraspecific vulnerability, highlighting areas where populations may have to adapt the most to track future environmental changes (Bay et al., 2018; Ruegg et al., 2018). Studies where intraspecific genetic variation is projected into the future are steadily increasing, but remain predominantly focused on modelling the intraspecific genetic distribution of single species and in terrestrial settings (Bay et al., 2018; Martins et al., 2018; Morgan et al., 2020; Ruegg et al., 2018). Thus, understanding how genetic composition of multiple marine species may respond to GCC is still largely unexplored.

Current knowledge gaps in the field of seascape genomics

The field of seascape genomics is rapidly advancing, but knowledge gaps still remain. Specifically, these include: 1) a lack of multi-species comparisons, 2) a lack of multi-marker comparisons, 3) testing the effect of different gene-environment association models on outlier loci identification, 4) testing the influence of historical environmental features on contemporary molecular variation, and 5) understanding how gene-environmental

relationships will shift into the future under projected climate change scenarios. This thesis used seascape genomic analyses in a novel comparative context, by assessing the imprints of past, present, and future climatic variables on the genetic and genomic patterns of three ecologically important southern African marine invertebrates. Multispecies comparative seascape genomic analyses, such as the ones used here, are an important step towards understanding how communities and ecosystems may respond to GCC (Barley et al., 2015; Borneman et al., 2014; Gamboa & Watanabe, 2019; Heupel & Simpfendorfer, 2014; Sork et al., 2010). This is especially true in regions where high biodiversity and endemism align with above-average climatic warming velocities, such as in South Africa.

South African seascape

South Africa is an especially interesting region to study how species respond to climatic changes, because the strong environmental gradients along its coastline act as a natural laboratory to test gene-environment interactions (Fig. i; Bustamante et al., 1995; Griffiths et al., 2010; Sink et al., 2012). South Africa is unique in that it is bounded by two contrasting ocean currents co-existing mainly along a longitudinal gradient (Fig. i). Within the Indian Ocean on the east coast of South Africa is the Agulhas Current, consisting of warm, nutrient poor water flowing predominantly southward from Mozambique along the continental shelf, deflecting southwards from the Transkei region to the Agulhas Bank (Lutjeharms et al., 1989; Schumann & Li van Heerden, 1988). On the western coast, the Atlantic Ocean is dominated by the northward flowing cold Benguela Current, which is characterized by nutrient rich waters and seasonal upwelling events (Andrews & Hutchings, 1980; Hutchings et al., 2009). Thus, climatic shifts within South Africa will likely lead to many coastal species shifting their distributions predominantly east or west, rather than the general global polewards trend (Chen et al., 2011), and even shifts over relatively small spatial scales may lead to extreme changes in environment and habitat (Branch & Branch, 2018; Whitfield et al., 2016). This environmental heterogeneity drives in part the high and complex biological diversity of this region, with over 12,000 identified marine species identified (Griffiths et al., 2010), approximately 30% of which are endemic (Costello et al., 2010). Broadly, endemism

and species richness within South Africa are greatest along the south coast, and lowest on the west coast (Fig. i; Awad et al., 2002; Griffiths et al., 2010).

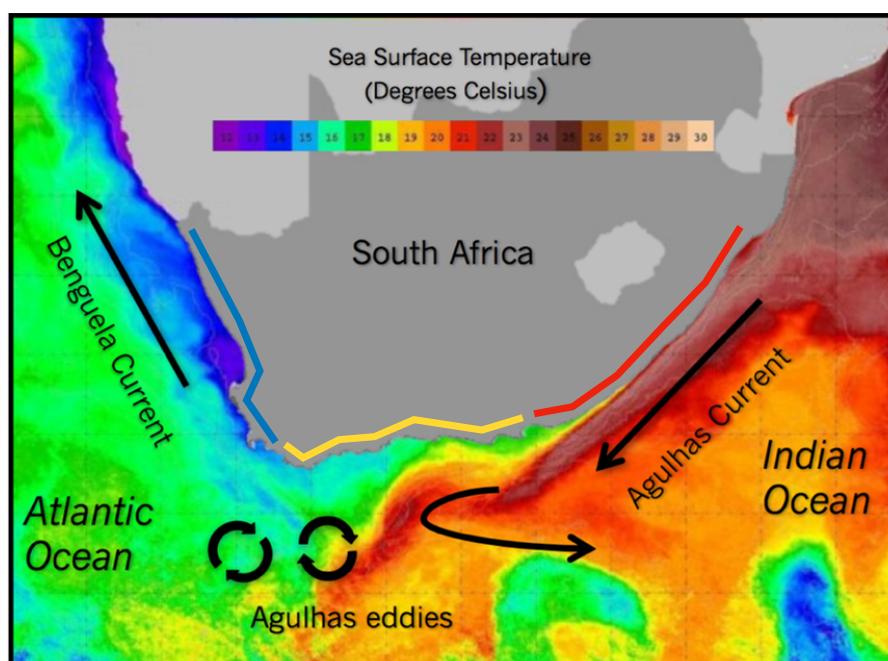


Figure i – The South African seascape, with mean sea surface temperatures (from World Ocean Database 2009) and main oceanographic current systems indicated. The South African coastline is here broadly defined into the west coast shown in blue, the south coast shown in yellow, and the east coast shown in red.

Many South African coastal communities exhibit biogeographic and phylogeographic breaks following four inshore bioregions: Southern Benguela, Agulhas, Natal, and Delagoa (Fig. ii; Teske et al., 2011; Turpie et al., 2000; van der Bank et al., 2019). While these bioregions are broadly distinct in their abiotic and biotic characteristics, there are transition zones between regions where flora and fauna overlap (Awad et al., 2002; Bolton et al., 2004; Bustamante & Branch, 1996; Harrison & Whitfield, 2006). The classification of bioregions is important, as it allows us to understand the spatial arrangement of biodiversity, which is valuable for systematic conservation planning (Turpie et al., 2000; Whittaker et al., 2005), and understanding how biodiversity patterns may change in response to future environmental change (Opdam & Wascher, 2004; Thuiller, 2004). Within this thesis, the study species show population differentiation, which can broadly be categorized into western and eastern clusters, with the western cluster consisting of sites

predominantly within the Southern Benguela bioregion, and the eastern cluster consisting mostly of sites in the Agulhas and Natal bioregions (Fig. ii).

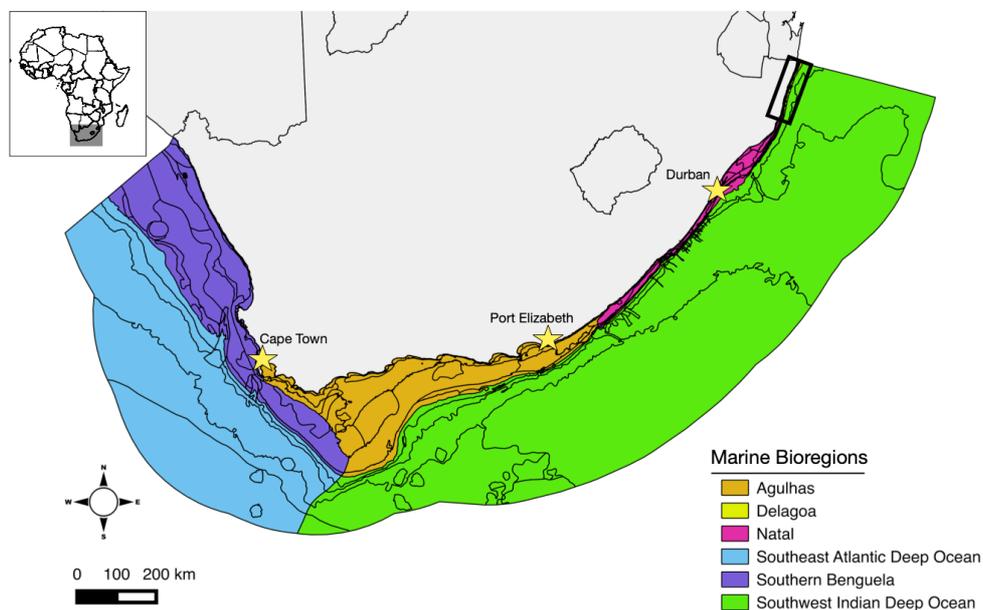


Figure ii – Local bathymetry and bioregions (from the 2018 National Biodiversity Assessment) within South Africa’s Exclusive Economic Zone, with the black box indicating the Delagoa bioregion for clarity.

South African paleoceanography

The oceanographic patterns of South Africa are not only spatially variable, but also dynamic through time. For instance, the southern Africa coastline experienced pronounced environmental changes during the Quaternary period, such as shifts in ocean currents and changes in sea level (Ramsay & Cooper, 2002). It has been suggested that at the beginning of the last interglacial period, i.e. the Mid-Holocene (~6 thousand years ago [kya]), SSTs were higher, the Agulhas Current was stronger, and the upwelling along the west coast of South Africa was weaker (Cohen & Tyson, 1995; Crowley & North, 1991). During the Last Glacial Maximum (~21 kya), South Africa is thought to have experienced cooler SSTs (Stone, 2014), increased sea surface salinities (SSS; Holloway et al., 2016), stronger upwelling along the west coast (Romero et al., 2002), and a weakened Agulhas Current on the southern and

eastern coasts (Franzese et al., 2006). Additionally, the coastal topography is thought to have changed dramatically since the late Quaternary period, with sea levels being at the -120m and +4m bathymetries during the LGM and Mid-Holocene, respectively (Cooper et al., 2018).

As areas of high species endemism align with areas of low paleoclimate variability globally (Svenning et al., 2015), these local glacial-interglacial climatic cycles are also expected to have pronounced effects on the composition of South Africa's marine biodiversity. Previous phylogeographic studies suggest that historical demographic persistence of coastal species varies by taxa and region, with populations of barnacle and abalone persisting mostly on the western coast (Evans et al., 2004; Reynolds et al., 2014), and urchin and limpet populations persisting mostly on the southern and eastern coasts (Mmonwa et al., 2015; Muller et al., 2012), during past glacial and interglacial events. The availability of rocky shore during lowered sea levels at the LGM has also been shown to be a possible driver of phylogenetic structure (Toms et al., 2014), with specifically the area around Cape Agulhas having seen drastic changes in the extent of exposed continental shelf (Dingle & Rogers, 1972). However, the southwestern cape region surrounding the area from Cape Point to Cape Agulhas (at the transition between the Southern Benguela and Agulhas bioregions) has also been shown to be climatically stable with regards to temperature since the LGM (Phair et al., 2019).

Predicted changes to South Africa's seascape

South Africa is currently experiencing rapid environmental changes (Hobday & Pecl, 2014), which will likely affect the biogeography and biodiversity patterns along the coastline (Bolton et al., 2012; Cockcroft et al., 2008; Crawford et al., 2008; Griffiths et al., 2010; Rouault et al., 2010). For example, South Africa had an increase in mean annual air temperature of 0.13°C from 1960 to 2003 (Kruger & Shongwe, 2004), and an increase in SST of 1.5°C in the Agulhas Current from 1980 to 2008 (Rouault et al., 2010). South African rainfall patterns also changed over the past few decades, with an increase of both seasonal drought and wet periods (Kruger, 2006; Rouault & Richard, 2003). These changes were not uniform throughout the country, as a general cooling and drying trend was seen on the

western coast and a general wet, warming trend was observed on the eastern coast (Rouault et al., 2010). As the South African coastline runs mostly from west to east, there is little room for coastal, inshore species to move polewards to track their thermal preferences and environmental niches. This is compounded by the fact that the distributions of most marine invertebrates are also heavily influenced by larval transport by ocean currents, which could further impede their ability to track their thermal niches (Galindo et al., 2010; Lal et al., 2017; Riginos et al., 2016). This could thus result in local extinctions throughout the region.

Variability in recent sea level changes have also been described for South Africa, with observed sea level increases being the highest on the eastern coast, and lowest on the southern coast (Mather et al., 2009). In addition to sea level rise, Guastella & Rossouw (2009) found an increase in wave height of 0.5m from 1994 to 2008 in offshore waters during winter storms. Beside the ecological consequences of GCC, the predicted changes in the South African marine environment will also likely have knock-on effects on the economic (Allison, 2005; Bryan et al., 2009; Clark, 2006) and social (Russell et al., 2013; Sandifer et al., 2015) well-being of those who interact with this environment.

South African rocky shore ecosystems

Coastal marine environments not only have aesthetic and cultural value, but also provide a range of goods and services to human populations. However, due to their close proximity to human populations, coastal ecosystems are exposed to multiple anthropogenic pressures (Crain et al., 2009; Harris et al., 2015; Lu et al., 2018), and are predicted to experience increased pollution, alien species introductions, coastal modification, and intensified climatic events within the next decade (Bakun et al., 2015; Cloern et al., 2016; Mead et al., 2013). Furthermore, GCC has already been shown to alter coastal habitats and species distributions (Pecl et al., 2017; Perry et al., 2005).

This PhD focused specifically on rocky shores, which are some of the most diverse and dynamic coastal systems in the world (Thompson et al., 2002). Rocky and mixed shores make up ~60% of South Africa's coastline, the majority of which are found along the south and west coasts (Griffiths et al., 2010). Here, rocky shore ecosystems harbor diverse

communities (Emanuel et al., 1992; Sink et al., 2005), many of which have a high socio-economic relevance (Liebau, 2013). However, South African rocky shores are under increasing pressures, ranging from the introduction and spread of alien species (Branch & Steffani, 2004; Griffiths et al., 1992), to coastal development, as well as oil extraction, and diamond mining (Majiedt et al., 2013; Sink et al., 2012).

Current status of conservation planning in South Africa

South Africa is home to one of the most biodiverse marine realms on earth, and thus a considerable amount of marine protection is likely required to conserve its richness (van der Bank et al., 2019). Compared to Africa as a whole, South Africa’s coastal waters are relatively well protected, and the marine protection status of the country recently increased to 5% of the Exclusive Economic Zone (EEZ), with the establishment of 20 new Marine Protected Areas (MPAs; Fig. iii; van der Bank et al., 2019). However, the newly proposed MPA network focuses primarily on offshore, rather than coastal regions (Fig. iii).

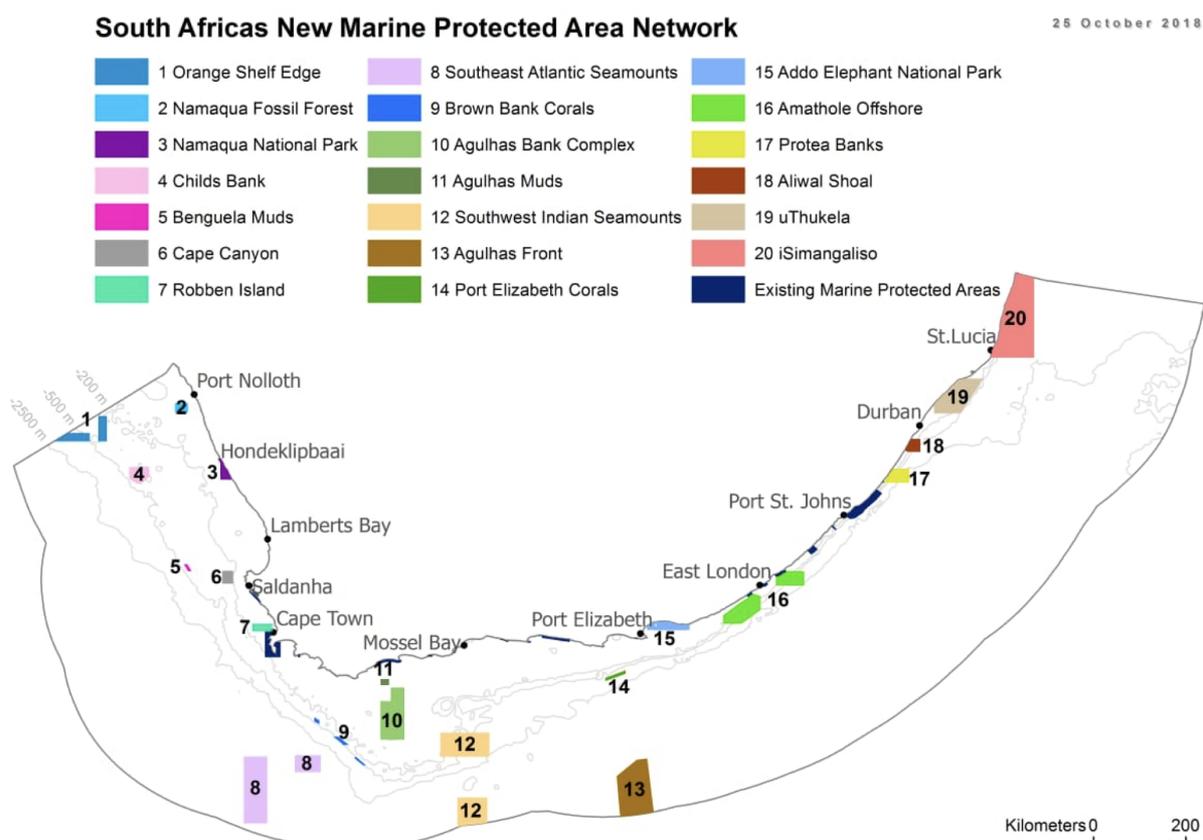


Figure iii – The 20 recently proposed Marine Protected Areas (MPAs) are shown within South Africa’s Exclusive Economic Zone, each a colored polygon representing a new MPA within the network, which are overlaid on the previously existing MPAs shown in dark blue (image from www.environment.gov.za).

Conservation research and management efforts are ongoing in South Africa to better protect its unique biodiversity with anthropogenic climate change. This is mirrored by global calls for MPAs to incorporate the spatio-temporal responses of species to global change (Tittensor et al., 2019; Wilson et al., 2020), as several studies indicate that marine species are already responding to recent climatic changes (Calosi et al., 2016; Grant et al., 2017). Within South Africa, recent genomic work showed anthropogenic pressures such as fishing, habitat loss, and mining are likely already leading to genetic erosion in the eelgrass, *Zostera capensis*, which can have detrimental effects on the resilience of its populations (Phair et al., 2020).

As genetic and genomic datasets have the ability to capture snapshots of evolutionary adaptive capacity of many South African species, this information could be included into the country’s adaptive management strategies (Nielsen et al., 2020; von der Heyden, 2009, 2017; Xuereb et al., 2020). One of the dominant methods to identify adaptive capacity, and the drivers of adaptive variation, is with seascape genomic analyses (Balkenhol et al., 2019; Selkoe et al., 2015). However, seascape genetic and genomic analyses are currently sparse within South Africa, leaving the potential seascape features driving genomic variation in southern African coastal species largely unknown (but see Singh et al., 2018 and Phair et al., 2019).

Molecular patterns of South African coastal taxa

Evolutionary histories are well documented for a multitude of South African marine taxa, especially for coastal species (Mertens et al., 2018; Nielsen et al., 2017; Phair et al., 2019, 2020; Teske et al., 2011; von der Heyden, 2009; Wright et al., 2015). Largely, the genetic diversity of rocky shore species follows the trend of species richness, with the south coast harboring the highest levels of genetic diversity for these species (Dalongeville in prep; Wright et al., 2015). There have also been multiple studies describing the genetic structuring

of South African coastal and inshore species (Evans et al., 2004; Mbongwa et al., 2019; Reynolds et al., 2014; Rhode et al., 2017; Teske et al., 2007, 2008, 2009; Zardi et al., 2015), many of which point to the presence of at least one phylogeographic break along the coastline. Overall, it is acknowledged that South African marine taxa display phylogeographic breaks that broadly correspond with known bioregions (Fig. ii), as well as an additional break around Cape Agulhas (Teske et al., 2011; von der Heyden, 2009). Many phylogeographic analyses of marine species in South Africa suggest that contemporary oceanographic and habitat conditions are prominent drivers of genetic structure along the coastline (Emami-Khoyi et al., 2020; Teske et al., 2011, 2019). In contrast, several other studies have found paleoclimatic oceanic oscillations better correspond with contemporary patterns of genetic variation of intertidal species (Marko et al., 2010; Reynolds et al., 2014; Toms et al., 2014). Therefore, the environmental and biotic factors shaping the genetic structure of coastal species are likely to be not only composed of complex relationships, but also dynamic through time (Henriques et al., 2016).

Most phylogeographic studies within South Africa are based on traditional genetic markers, leaving genome-wide inferences largely unexplored. Compared to traditional markers such as mitochondrial (mtDNA) and nuclear DNA genes and introns, genome-wide single nucleotide polymorphism (SNP) datasets are unique in that they allow for robust identifications of genes putatively associated with environmental variability (Davey et al., 2011; Stapley et al., 2010). Genomic phylogeographic studies are also capable of identifying more recent evolutionary processes, owing to the increased number of alleles to calculate molecular variation from (Wang, 2011).

Genomic studies are limited within the South African marine environment, but existing genomic datasets reveal structuring at a finer resolution than traditional genetic markers. For example, Teske et al. (2019) found that while neutral genomic markers of the goby, *Psammogobius knysnaensis*, showed homogeneity across the coastline, putative adaptive outlier loci showed structuring mirroring temperature-defined bioregions. A comparative genomic study on two rocky shore invertebrates, the Cape urchin (*Parechinus angulosus*) and the Granular limpet (*Scutellastra granularis*), found that while the two species showed highly distinct population structuring with mtDNA markers, they had highly similar genomic structure with SNP data (Nielsen et al., 2018). However, Nielsen et al. (2018) only investigated the genomic variation along the west coast of South Africa, and

therefore it is uncertain how these patterns may change when considering these species' entire distributional ranges throughout the South African coastline.

This PhD thesis aimed to build on conservation genomic analyses of *P. angulosus*, and *S. granularis* within South Africa (Nielsen et al., 2018, 2020), by including a wider geographic extent, an additional study species, and more in-depth genomic inferences. Broadly, this thesis used a seascape genomics approach to identify putative environmental drivers of neutral and adaptive genomic variation in three southern African rocky shore species. This project was not only the first to describe the environmental features that are shaping the evolutionary trajectories of South African coastal species across different taxa, but also compared the environmental features that are correlated with different molecular markers. Furthermore, it investigated the relative importance of historical versus contemporary evolutionary/ecological processes in shaping extant genetic and genomic patterns. Finally, this study was also the first to project both species and genomic variation into the future, under multiple emission scenarios, to characterise both inter- and intraspecific vulnerability to climate change. These analyses were conducted on three ecologically relevant rocky shore species: Cape urchin (*Parechinus angulosus*), Common shore crab (*Cyclograpsus punctatus*), and Granular limpet (*Scutellastra granularis*). These species were chosen as their distributions span the environmental gradients in the region, they are generally abundant on rocky shores, and each play key ecological roles within different rocky shore zonation.

Study species

Parechinus angulosus (Leske, 1778)

The Cape sea urchin, *P. angulosus*, is found from Lüderitz, in Namibia, to northern KwaZulu-Natal, South Africa, making it the most widespread echinoid in southern Africa (Branch, 2017; Velimirov et al., 1977). *Parechinus angulosus* plays a key role in the intertidal ecosystem, as it helps control kelp abundance in the lower rocky shore, while also providing shelter for juvenile abalone (Day & Branch, 2002). Blamey (2010) found that *P. angulosus* predominantly feeds on drift kelp, but shifts to grazing when drift kelp is not available. The

observed temperature range of *P. angulosus* is quite broad (9-31⁰C, Mertens pers. comm.), but the species is known to prefer cool-temperate waters, which is why it is most abundant on the west and south-west coasts (Greenwood & Bennett, 1981). Usually, individuals are found in dense aggregations in the lower to mid-rocky shore zonation, and tend to settle on rocky overhangs that are sheltered from wave shocks (Day & Branch, 2002; Farquhar, 1994).

Parechinus angulosus individuals reach sexual maturity after one to two years and then produce offspring via seasonal broadcast spawning (Greenwood, 1975, 1980), with a major spawning event in August-September and a minor in April-May (Greenwood & Bennett, 1981; Hodgson, 2010). The species has a Pelagic Larval Duration (PLD) that lasts around 50 days, at the end of which it actively selects its substrate (Cram, 1971). If the preferred substrate is not available, the larval phase is predicted to extend up to 11 days (Cram, 1971).

Parechinus angulosus has several different color morphs, varying between pink, red and purple, yet there is no significant genetic distinction between the different color morphs based on the mitochondrial *COI* and nuclear *SpREJ9* genes (Fig. iv; Muller et al., 2012). Previous mitochondrial DNA (mtDNA) analyses of *P. angulosus* along the west coast show strong population structuring, strong signatures of isolation-by-distance (IBD) and bidirectional gene flow (Mertens et al., 2018; Muller et al., 2012). Recent genomic analyses along the South African west coast display very low levels of genetic structuring and no significant IBD (Nielsen et al., 2018). Further, analyses based on outlier SNPs suggest that populations within the northern range of the species' distribution show distinct signals of local selection, yet a lack of an annotated genome prevents the identification of the functional traits corresponding to putatively adaptive loci (Nielsen et al., 2018).



Figure iv – The purple color morph of the Cape urchin, *Parechinus angulosus*.

Scutellastra granularis (Linnaeus, 1758)

The Granular limpet, *S. granularis*, is endemic to southern Africa, occurring from Swakopmund, Namibia, to Durban, South Africa (Branch, 2017). Individuals are normally brown with white speckles, but shift to a broken, striped pattern when amongst barnacles, or to a dark brown pattern when living amidst mussels (Fig. v; Branch & Branch, 2018). *Scutellastra granularis* individuals can endure high air temperatures, with an observed thermal range between -1°C and 36°C (Mertens pers. comm.). The preferred niche of *S. granularis* is the mid-upper intertidal, where it uses its radula to scrape algae off of the rocky surface (Branch, 2017). *Scutellastra granularis* is also a key intertidal species, as it acts as a secondary habitat for other invertebrates that settle on top or beneath its shell, and by controlling algal growth through grazing (Hawkins & Hartnoll, 1983). Its individuals mostly feed during daytime low tides, when they consume various available micro-algae and generally forage in a random pattern, but return to a small home range of about 5 cm^2 (Gray & Hodgson, 1997).



Figure v – Granular limpet, *Scutellastra granularis*, individuals on an exposed rock surface.

Scutellastra granularis is a broadcast spawner, with spawning events observed in June along the west coast, and from June to September in the south coast (Hodgson 2010). The annual quantity of gonadal material produced depends on body size, with the zygotes developing into planktonic larvae (Bosman & Hockey 1988; Kay 2002). The PLD of *S. granularis* larvae is currently unknown, however we can assume that it is similar to that of its congeners, *Patella vulgata* and *P. caerulea*, which is roughly 4-10 days (Sá-Pinto et al., 2008). The larvae of *S. granularis* settle in the low tidal zone, where they are safeguarded from stresses such as desiccation, and then migrate into the high intertidal zone as they develop into adults (Branch & Branch, 2018; Nakin, 2008).

Along the South African west coast, *S. granularis* displayed low levels of genetic differentiation and no significant IBD and bidirectional gene flow when measured with mtDNA (Mertens et al., 2018). A recent analysis by Nielsen et al. (2018) found that *S. granularis* continued to show no significant genetic structure or IBD along the west coast with genome-wide SNPs. West coast individuals of *S. granularis* also displayed signatures of local adaptation, with the northern populations harboring a larger number of total and private outlier SNPs (Nielsen et al., 2018). Along the entire South African coastline, *S.*

granularis displayed genetic structuring (using mtDNA *COI* region), with a break between Mossel Bay and Tsitsikamma (Mmonwa et al., 2015).

Cyclograpsus punctatus H. Milne-Edwards, 1837

The common shore crab, *C. punctatus*, is characterized by red-brown spotted body coloring, and sizes ranging from ~2-50mm (Fig. vi; Branch, 2017; Le Roux, 1991). The species is also found in various environmental niches, inhabiting the high- to mid-rocky intertidal as well as the muddy banks of estuarine environments (Alexander & Ewer, 1969; Barnard, 1950; Broekhuysen, 1940). As a high shore species, *C. punctatus* has the highest thermal tolerance of the three study species, with an observed thermal range of -1°C to 46°C (Mertens pers. comm.). Within rocky shore environments, *C. punctatus* primarily feeds on kelp wrack, along with green and brown algae and molluscs (Griffiths et al., 1992), and within estuarine environments is known to feed on cordgrasses and salt marsh plants (Richoux & Froneman 2008). Further, Alexander and Ewer (1969) describe *C. punctatus* as a general scavenger, feeding on vegetable matter and various isopods, zoea and polychaete worms. Within the rocky shore, *C. punctatus* is found mainly in between boulders and within shallow burrows underneath flat rocks (Le Roux, 1991).



Figure vi – A common shore crab specimen, *Cyclograpsus punctatus*.

Cyclograpsus punctatus has two breeding seasons, a major one between May and September and a minor one between January and February, which was observed in crabs from two southwest and southeastern South African sites (Broekhuysen, 1940). This is a brooding species, which releases multiple batches of eggs in the principal breeding season in winter (Broekhuysen, 1940). The minimum PLD is 14 days (Wright et al., 2015), with larval development taking up to 18 days at 20°C (Fagetti & Campodonico, 1971).

Cyclograpsus punctatus has been shown to be physiologically robust, with high tolerance to fluctuating water temperatures (Le Roux, 1991), as well as salinity (Heeg & Cannone, 1966). Bolt and Heeg (1975) found that *C. punctatus* uses both behavioral mechanisms, along with osmoregulatory techniques, to withstand changes in salinity, while Winch and Hodgson (2007) found that the species changes its oxygen consumption to withstand changes in temperature. A genetic analysis, using the *COI* mitochondrial gene, of *C. punctatus* by Wright et al. (2015) found high levels of genetic structure and significant IBD among populations along the South African coastline.

Thesis rationale

Understanding species- and gene-environment relationships is critical if we are to predict biodiversity changes, and proactively manage biodiversity and ecosystem functioning under global change. While many studies have assessed either the historical or future environmental footprints on species and genetic biodiversity patterns, studies projecting biodiversity patterns into both the past and future are limited (Espíndola et al., 2012; Yannic et al., 2014). Further, at present, existing seascape genomic studies are restricted to single species, leaving comparative analyses unexplored (but see Gamboa & Watanabe, 2019 for a terrestrial example). Moreover, there is also a lack of comparative analyses investigating how genomic diversity patterns differ between genetic and genomic markers, as well as putatively neutral and adaptive markers (Camacho-Sanchez et al., 2020; Epps & Keyghobadi, 2015; Hall & Beissinger, 2014). Lastly, while genomic data is increasingly being generated, it is still generally unknown how it compares to other data types, such as climatic velocity or species richness, in selecting areas of evolutionary importance in conservation planning frameworks (Hanson et al., 2020a). This thesis aimed to address all of these knowledge gaps

by assessing the influence of past, present, and predicted future climatic variation on the genetic and genomic composition of three southern African marine invertebrates. Inferences from these ecological and evolutionary processes were used to identify possible conservation priority areas to promote inter- and intra-specific persistence under climate change.

Thesis layout

This thesis is composed of an introduction, four data-driven chapters (Chapters 1-4; Fig. vii), and one conclusion chapter. Chapter One generated fine-scale genomic data for three rocky shore species, *C. punctatus*, *P. angulosus*, and *S. granularis*, and compared patterns of putative neutral and adaptive variation among species. The first chapter also compared the influence of environmental features on the genomic diversity of the study species. Chapter Two focused on the influence of historical versus contemporary environmental features on both genetic (mtDNA) and genomic (SNP) variation of the three study species. Within Chapter Two I also conducted species distribution modelling to reconstruct past distributions for each species, to understand how historical distributions at different time-points in the past correlate with extant molecular diversity. Chapter Three then forecasted species distributions and genomic variation into the future under predicted climate change scenarios, to compare how species and population level vulnerability can differ in space and time. Lastly, the climatic, ecological, and evolutionary patterns observed in the three data chapters were combined into a spatial conservation plan, and their use in guiding future conservation efforts were discussed in Chapter Four. The main findings and future research avenues are discussed in the conclusion.

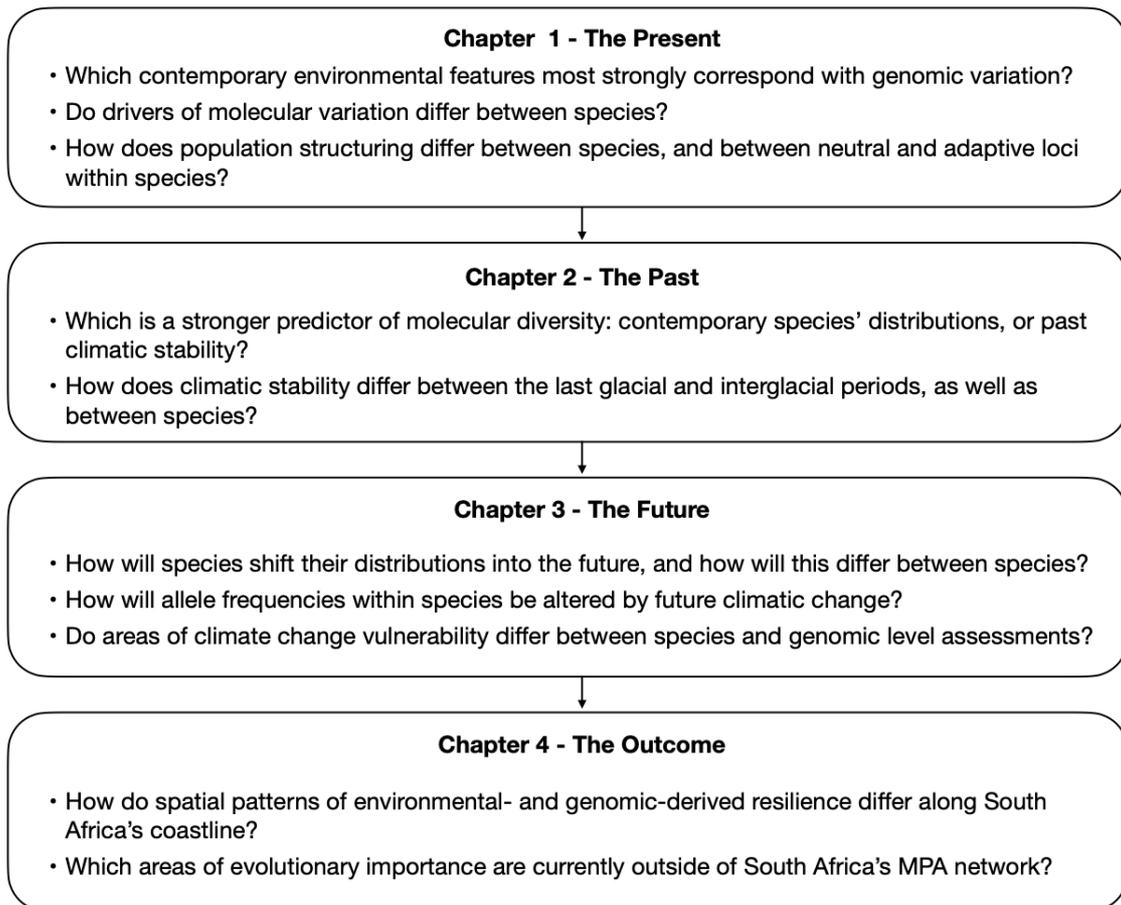


Figure vii – A diagram of the four data chapters, highlighting the questions that each section aims to answer.

Chapter One

With regards to Chapter One (pages 27-54), the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Sampled and extracted DNA, conducted all analyses, and led writing	60%

The following co-authors have contributed to Chapter 1 (pages 27-53):

Name	Email address	Nature of contribution	Extent of contribution (%)
Sophie von der Heyden	svdh@sun.ac.za	drafting of MS, editing	15%
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Robert Toonen	rjtoonen@gmail.com	financial assistance for sequencing, editing of MS	5%

Signature of candidate:



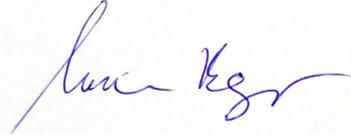
Date: Oct 26 2020

Declaration by co-authors

The undersigned hereby confirm that:

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter One (pages 27-53),
2. No other authors contributed to Chapter One (pages 27-53), besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter One (pages 27-53), of this dissertation.

Signature	Institutional affiliation	Date

	Stellenbosch University	19 October 2020
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Chapter One

Multi-model seascape genomics identifies distinct environmental drivers of selection among sympatric marine species

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Introduction

Anthropogenic climate change is altering the physical and chemical properties of coastal ecosystems at an unprecedented rate, ultimately threatening the persistence of biological communities (Harvey et al., 2013; Pecl et al., 2014). Nearshore environments are especially at risk from anthropogenic change as they are exposed to threats from both the terrestrial and marine realms (Glavovic et al., 2015). Coastal systems experience strong environmental gradients, caused by complex interactions among features such as wind and wave action, ocean currents and upwelling cells, and exposure to sunlight and precipitation (Helmuth et al., 2006). Environmental heterogeneity in coastal systems should therefore impose differential selection pressures, facilitating local adaptation and genetic differentiation (Riginos et al., 2016). While many marine species are thought to exhibit low genetic differentiation due to large effective population sizes and high dispersal potential, there is growing evidence suggesting that many coastal organisms display surprisingly fine-scale population structuring and local adaptation (De Wit et al., 2020; Selkoe et al., 2014; Stanley et al., 2018; Tisthammer et al., 2020). Along with oceanographic patterns and coastal topography, the support for climatic environmental gradients acting as barriers to gene flow is steadily increasing (Benestan et al., 2016; Bernatchez et al., 2019; Saenz-Agudelo et al., 2015; Sandoval-Castillo et al., 2018). Uncovering patterns of genetic differentiation and possible local adaptation, and distinguishing which environmental conditions shape such patterns, is critical for effective conservation management in the face of global change (Barshis et al., 2018; Narum et al., 2013; Razgour et al., 2018; von der Heyden, 2017).

Quantifying genomic differentiation and putative adaptive variation of marine species, and the resultant field of seascape genomics, relies on recent advances in Next Generation Sequencing (NGS; Aldrich & Meyer, 2015; Stapley et al., 2010). One of the main goals of seascape genomics is to use NGS to identify loci that differ significantly over environmental gradients, using gene-environment association analyses (GEAs; Balkenhol et al., 2019; Joost et al., 2007). GEAs are powerful tools to detect putative adaptive loci (commonly termed 'outlier loci') by directly associating allele frequencies with environmental variables (Forester et al., 2017; Rellstab et al., 2015; Riginos et al., 2016). Sea surface temperature (SST) is the most common environmental structuring force identified

among seascape studies to date (Selkoe et al., 2016), and has been shown to strongly correlate with genomic variation in abalone (Sandoval-Castillo et al., 2018), mussels (Wei et al., 2013), oysters (Bernatchez et al., 2019; Takeuchi et al., 2020), sea cucumbers (Xuereb et al., 2018), and lobsters (Benestan et al., 2016). As SST is consistently identified as one of the prominent drivers of genomic variation in marine invertebrates, it shows promise as a proxy for evolutionary processes, such as local selection, in conservation (Hanson et al., 2017). However, previous studies have solely investigated single-species GEAs, which means that the effects of SST and other environmental variables on coastal species with similar distributions, but different micro-environmental niches, are still largely unexplored (Harvey et al., 2013). Furthermore, there are a multitude of GEA methods available, which differ in their statistical analyses and assumptions of demographic histories, often leading to diverse outputs (Dalongeville et al., 2018; Lotterhos & Whitlock, 2015). Even though many studies use two or three outlier detection methods to account for false positives (Forester et al., 2017), there has yet to be a comprehensive comparison of various methods in their ability to identify the dominant selection forces acting on wild marine populations.

This study focuses on the environmental drivers of genomic differentiation in three rocky shore invertebrates: Cape urchin (*Parechinus angulosus*), Granular limpet (*Scutellastra granularis*), and Common shore crab (*Cyclograpsus punctatus*), that are widely distributed along the southern African coastline, which is known for its strong biogeographic gradients of temperature, productivity and other environmental variables (Fig. 1.1; Smit et al., 2013). Previous studies, consisting of mitochondrial DNA (mtDNA) data, have suggested multiple lineages for each species, broadly differentiated into western and eastern clades (with population distinctions mainly falling between the Benguela and Agulhas bioregions; Fig. ii; Fig. 2.S1.2, Appendix 2.S1; Mmonwa et al., 2015; Muller et al., 2012; Wright et al., 2015), with evidence of isolation-by-distance (IBD; Wright et al., 2015). However, a recent study using NGS data from the estuarine-restricted seagrass *Z. capensis* suggested that isolation-by-environment (IBE) plays a significant role in shaping the genomic differentiation (Phair et al., 2019), although the extent that IBD and IBE characterise the genomic variation of other marine species in the region currently remains unknown.

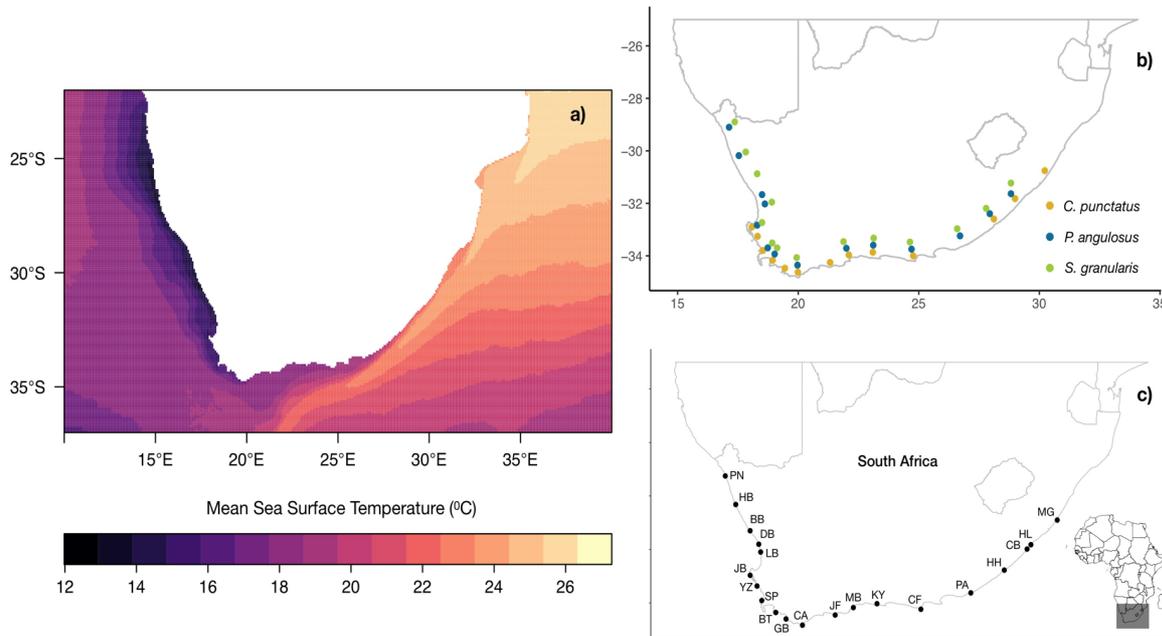


Figure 1.1 – The distributions of the study species (*Cyclograpsus punctatus*, *Parechinus angulosus*, *Scutellastra granularis*), imposed over average sea surface temperatures within the study region (a), and sample sites per species (b) plus abbreviations of the 20 total sample sites included in the study (c).

Broadly, the objectives of this chapter are to characterise phylogeographic patterns of three ecologically important rocky shore species, and to identify the dominant environmental drivers of putative adaptive variation within southern African rocky shore communities. A pooled (Pool-seq), restriction-site associated DNA sequencing (RAD-seq) approach was used to characterise genomic variation across at least 13 sites per species, and describe population diversity within and differentiation amongst these species. Seven outlier detection methods were used to distinguish the principal environmental drivers of selection in each species. I hypothesized that: 1) each species will exhibit eastern and western population differentiation in accordance with mtDNA population structure, 2) each species will show significant IBD and IBE, 3) SST will be the dominant driver of putative selection for all three species.

Materials and Methods

Study region and species

The study domain lies along the South African coastline, which is one of the most biodiverse marine systems in the world (Griffiths et al., 2010). This region has also been identified as a hotspot for ocean warming as it is experiencing environmental change at a faster rate than predicted (Hobday & Pecl, 2014). In South Africa, the coastline is characterised by SST increasing with longitude, from the cool-temperate Benguela bioregion on the west coast to the sup-tropical Natal bioregion on the east coast (Fig. 1.1).

The study species were selected as their distributions span several bioregions and the natural environmental gradients of southern Africa (Fig. 1.S1.1, Appendix 1.S1), and can represent the high- (*C. punctatus*), mid- (*S. granularis*), and low- (*P. angulosus*) rocky shore ecotypes (Branch & Branch, 2018). They also differ in life history traits with *C. punctatus* being a brooder, and *S. granularis* and *P. angulosus* being broadcast spawners, with PLDs varying from ~ 5-15 days (*S. granularis* and *C. punctatus*) to potentially up to 50 days (*P. angulosus*; Mertens et al., 2018; Muller et al., 2012; Wright et al., 2015). These species are each ecologically important; either as dominant grazers or scavengers, as substrates for other species to either live on, or as shelter for juvenile abalone (Branch & Branch, 2018).

A total of 14 sites, spanning ~2,200 km of the South African coastline, were sampled for *S. granularis* and *P. angulosus*, and 13 sites spanning ~1,800 km were sampled for *C. punctatus* (Fig. 1.1). These sites incorporate the natural environmental (e.g. SST, salinity, air temperature) gradients in the region, as well as the distributional range per study species (Branch, 2017).

Laboratory protocols and bioinformatics

DNA samples were collected from three species, including 13 or 14 sites each, per species (Fig. 1.1; Tables 1.S1.1-3, Appendix 1.S1). Genomic data consisted of pooled ezRAD-seq samples, as it is a cost-effective approach to obtain precise allele frequency data (Schlötterer et al., 2014; Toonen et al., 2013). Dorant et al. (2019) found that Pool-seq inflated F_{ST} values relative to individual-based sequencing approaches, but still gave highly similar allele frequency outputs and patterns of population structure. Thus, while the absolute magnitude of F_{ST} values may be upwardly biased relative to sequencing individuals,

for a fraction of the cost Pool-seq data still allow us to infer relative patterns of population structure with confidence (Kurland et al., 2019).

A pooled ezRAD sequencing and *de novo* assembly approach was used, as this allowed for larger contig lengths (e.g. > 1,000 base pairs; bp) compared to other RAD-seq approaches (Toonen et al., 2013). Further, the ezRAD approach does not rely on a PCR step to amplify sequences during library preparation, which removes potential biases from PCR duplicates, and is a unique RAD-seq method which allows for high coverage at specific RAD loci in combination with low coverage of across the entire genome (Forsman et al., 2017). Additionally, the effectiveness of ezRAD has been verified with Pool-seq, which is a cost-effective method of sequencing multiple individuals, and is increasingly being used to characterize population level differentiation (Kofler et al., 2016a, 2016b). To avoid any potential sequencing biases, best practices were followed, by including a large number of individuals per pool (~40), as well as using a stringent coverage cut-off of > 20X per pool (Schlötterer et al., 2014), which are discussed further below.

Genomic RAD-seq data was previously obtained for the study species from 11 of the 20 sample sites (Nielsen et al., 2018; Mertens pers. comm.). Additional sampling was conducted at the remaining sites during July 2018, with 30-40 individuals collected from each site (Tables 1.S1.1-3, Appendix 1.S1). Individuals were preserved in 100% ethanol, from which < 25mg tissue (gonad from *P. angulosus*, foot from *S. granularis*, and muscle from *C. punctatus*) was taken for DNA extractions. Extractions were performed with the Qiagen DNeasy Blood & Tissue kit following the manufacturer's protocols. The quality of the DNA extractions was assessed on 1% agarose gels and quantity was determined using the Qubit Quant iT dsDNA HS Assay system at the Central Analytical Facility at Stellenbosch University (CAF-SU). All extractions passing quality and quantity checks were stored at -20°C. For each species, equimolar amounts of DNA from each individual were pooled per sample site, flash frozen and sent to the Hawaii Institute of Marine Biology (HIMB) for library preparation following Knapp et al. (2016; further outlined in Nielsen et al., 2018). Equimolar pooled ezRAD libraries (Toonen et al., 2013) were sequenced (V3, 2x300PE) on the Illumina Mi-Seq platform at University of California, Riverside.

The quality of raw FASTA reads was assessed with FastQC (Andrews, 2010), and then the reads were uploaded onto the CAF-SU high performance cluster (HPC) for further analyses (see Table 1.S1.4, Appendix 1.S1 for outline of analyses). Bases with low quality

scores ($Q < 20$), overrepresented sequences and adapter sequences were removed using TrimGalore! (Krueger, 2015).

As mitochondrial DNA (mtDNA) markers have different evolutionary characteristics than nuclear markers (Epps & Keyghobadi, 2015; Teske et al., 2018), mtDNA-mapped reads were filtered from the complete datasets (Pujolar et al., 2014). In order to separate mtDNA from nuclear sequences, the quality-trimmed reads were first mapped onto mitogenome references of closely related species obtained from GenBank (Purple mottled shore crab, *Cyclograpsus granulatus*, Accession number: NC_025571.1; Rea sea urchin, *Loxechinus albus*, Accession number: JX888466.1; Fingered limpet, *Lottia digitalis*, Accession number: DQ238599.1) using BWA-MEM (Li, 2013; Table 1.S1.5, Appendix 1.S1). The mapped reads were converted to BAM files, sorted and filtered using SAMtools v.1.3 (Li et al., 2009), and then merged using BAMtools (Barnett et al., 2011). The merged BAM files were converted back to SAM and used to filter the quality-trimmed reads, removing putative mtDNA markers before mapping, using the 'filterbyname' command in BBMap (Bushnell, 2015).

Given that there are no reference genomes for these or closely-related species, *de novo* assemblies were created, using quality-trimmed reads that were normalized to a coverage of 100X with BBMap 'bbnorm', and using k-mer value ranges identified with K-mer Genie (Chikhi & Medvedev, 2014). The reads were assembled with three different programs: ABySS (Simpson et al., 2009), MEGAHIT (Li et al., 2015) and SPAdes (Bankevich et al., 2012). Because SPAdes can only handle nine input samples at a time, half of each species' samples were assembled at a time, and then merged the two SPAdes assemblies using GARM (Mayela Soto-Jimenez et al., 2014). The outputs of the three assemblers were compared using QUAST v.4.1.1 (Gurevich et al., 2013) and the NCBI BLASTN v.2.4.0+ algorithm (Camacho et al., 2009). Metrics such as N50 and L50 values, and number of BLAST hits, were used to select a *de novo* assembly for further analysis.

The mtDNA-filtered reads were also mapped to available reference genomes of the Purple urchin (GCA_000002235.4; 990.915 Mb), Owl limpet (GCA_000327385.1; 359.506 Mb), and Chinese mitten crab (GCA_003336515.1; 258.8 Gb) for comparison. Because these species are distantly related to the study species, SNP calling parameters were relaxed (mapping quality > 10 , minimum pool coverage = 10), but it was found that overall patterns of population structure were consistent between both approaches, mirroring the findings of Rellstab et al., (2013). As *de novo* assemblies have been shown to lead to more robust

inferences than mapping onto loosely related genomes (Tripp et al., 2017), only the more stringent *de novo* assembly approach is presented here.

The mtDNA-free, but not normalized, reads were mapped onto the *de novo* assemblies with BWA-MEM. The subsequent SAM files were converted into BAM files, sorted, indexed and filtered with SAMtools. To control for sequencing biases, the sampled SAM files were downsampled to the median number of reads across all pools with SAMtools. A synchronized multiple pileup file was created for each species with SAMtools 'mpileup', followed by the Popoolation2 'mpileup2sync.jar' commands (Kofler et al., 2011). Final SNP calling was performed with the 'popsync2pooldata' function of the *poolfstat* v.0.0.1 R package (Hivert et al., 2018). To avoid potential biases associated with unequal sequencing of individuals within the pool, and since fewer SNPs at higher coverage have been shown to be more effective than a greater number of SNPs at lower coverage (Graham et al., 2020), stringent SNP calling parameters were chosen: minimum coverage > 20X, minimum read count > 4, maximum read count < 400X, and a minor allele frequency (MAF) > 0.01 in each pool (Dorant et al., 2019; Leblois et al., 2018). To account for the possibility of loci being physically linked (linkage disequilibrium: LD) custom R scripts were used to randomly select one SNP per 1,000bp per contig.

Assessing gene flow and potential drivers of population structuring

Characterising genomic differentiation

To assess genomic population structuring, pairwise Weir and Cockerham's F_{ST} values (Weir & Cockerham, 1984) from the LD-pruned SNP dataset were calculated using the 'computeFST' function of *poolfstat*, the confidence interval (CI) values of which were computed with a custom bash script from (Dorant et al., 2019) using 1,000 bootstrap iterations. Nei's genetic distances (Nei, 1973) matrices were generated with the 'stampNeisD' function of the R package *StAMPP*, and visualized in Principal Coordinates Analyses (PCoAs) generated with the 'pco' function in the *ecodist* R package (Goslee & Urban, 2007).

Additionally, the allele frequencies of all SNPs per species were input into the core model of BayPass v.2.1 (Gautier, 2015) to estimate scaled covariance (Ω) matrices. BayPass is specifically designed to handle Pool-seq data, and uses allele-frequencies to create a Ω matrix, which can be interpreted as pairwise estimates of differentiation and population structure. BayPass was run under default conditions to create the Ω matrices, which were then converted into correlation matrices using the 'cov2cor' function in R *stats* package, and visualized as similarity matrix heatmaps.

Additional population structuring analyses were run to test if altering coverage cut-off parameters influences genomic differentiation patterns. To do so, subsets of the LD-filtered SNP dataset described above underwent additional coverage filters of either: 1) maximum coverage < 200, or 2) minimum coverage > 40. It was subsequently assessed how the different coverage scenarios influenced population structure, performing a singular value decomposition of the Ω matrices (from the core BayPass model) per scenario per species (Dorant et al., 2019; Leblois et al., 2018).

Seascape features

The various seascape genomic analyses included a standard set of environmental features as predictor variables. A total of 20 environmental features were considered (Appendix 1.S3), including air temperature and precipitation of the coldest month, warmest month, the range between coldest and warmest months, as well as annual mean between 1970 and 2000, which were downloaded from the WorldClim database at a resolution of ~ 1 km (Fick & Hijmans, 2017). Annual mean, monthly minimum and maximum, and the range in SST between 2002 and 2010 and annual mean, monthly minimum and maximum, and range in sea surface salinity between 1955 and 2006 were downloaded from the MARSPEC database at a resolution of ~ 1 km (Sbrocco & Barber, 2013). Mean surface dissolved oxygen, diffuse attenuation coefficient, pH, and chlorophyll concentration between 2000 and 2014 were downloaded from the BIO-ORACLE database at a resolution of ~ 9.2 km (Tyberghein et al., 2012). Environmental features were downloaded for each sample site with the 'load_layers' function of the *sdmpredictors* R package (Bosch et al., 2017). Collinearity between predictor variables was tested using pairwise Spearman's correlation coefficients (Spearman, 1961)

and Benjamini-Hochberg (BH) corrected p-values ($p < 0.05$; Benjamini & Hochberg, 1995). All variables that were significantly correlated ($r > 0.65$), and those with a variance inflation factor (VIF) >10 were removed.

Isolation-by-distance (IBD) versus isolation-by-environment (IBE)

Isolation-by-distance (IBD) and isolation-by-environment (IBE) were tested using Mantel tests (Mantel, 1976). Mantel tests are widely used in landscape genetics to test which spatial features are significant drivers of genetic differentiation (Diniz-Filho et al., 2013). IBD was assessed with a standard Mantel test, which evaluates the relationship between two matrices (i.e. geographic versus genetic distances) and IBE was tested with Partial Mantel tests, which compare the relationship between two matrices while taking into account the effect of a third (i.e. temperature versus genetic distance, accounting for geographic distance; Diniz-Filho et al., 2013).

IBD analyses consisted of Slatkin's linearized pairwise F_{ST} ($F_{ST} = [F_{ST} / (1 - F_{ST})]$; Slatkin, 1995), and log-transformed geographic distances along the coastline calculated with the roadmap tool in QGIS (Team, 2009), starting from the western-most site for each species. IBE analyses additionally included pairwise Euclidean climatic distances. Partial Mantel tests were performed for each climatic variable separately, with geographic distance as a conditioning variable. Individual Mantel test significance was assessed in *ecodist*, using 1,000 permutations. To account for multiple tests, p - were converted to q -values and significance was assessed using a False Discovery Rate of 0.05 (FDR) based on BH criteria with the *qvalue* R package (Dabney et al., 2010).

A multi-model approach to identifying environmental associations with SNPs

To investigate possible associations between SNPs and environmental variables, seven different outlier detection methods, using the same seascape features as stated above as predictor variables, were used. As GEA methods have been shown to vary in the type and number of outliers detected (Dalongeville et al., 2018; Forester et al., 2017), the multi-

model approach used here allows for more robust inferences. The protocol pertaining to each outlier detection method are outlined below.

BayPass Bayesian hierarchical models

For an F_{ST} -like outlier detection approach, the core model of BayPass was run, which uses a hierarchical Bayesian model to create per-locus XTX values, which can be interpreted as an F_{ST} values corrected for the scaled Ω of population allele frequencies (Gautier, 2015). BayPass v.2.1 was run under default conditions to create XTX values. As described in (Gautier, 2015), a pseudo-observed dataset (POD) was created to estimate the posterior predictive distribution of XTX values, and candidate SNPs were selected if they fell within the 99.9% quantile of the POD XTX distribution.

For a GEA-like approach, the auxiliary model in BayPass was run to identify candidate SNPs due to associations with environmental variables. The auxiliary covariate model includes a binary auxiliary variable to classify the association and compute a Bayes Factor (BF) for each locus while accounting for multiple testing (Gautier, 2015). After running the model under default conditions, the general rule derived from Jefferys (1961), which identifies outliers as those having a \log_{10} Bayes factor (db) >20 (Gautier, 2015), was followed.

Latent factor mixed models (LFMM)

Latent factor mixed models (LFMM) use mixed linear models to test for correlation between allele frequencies and an environmental predictor variable while correcting for population structure with latent factors (Frichot et al., 2013). As such, these models require *a priori* knowledge of the number of genetic clusters (K). K was inferred from previous mtDNA clustering analyses ($K = 2$ for each species; Mmonwa et al., 2015; Muller et al., 2012; Wright et al., 2015), as it is recommended to estimate K from independent genetic datasets (Frichot et al., 2013). LFMMs were run separately for each environmental variable using the R package *LEA* (Frichot & François, 2015) with 10,000 cycles of the Gibbs sampling algorithm, 5,000 burn-in cycles, and 10 replicate runs. For all runs per predictor variable, z-scores were

combined, genomic inflation factor was calculated, and candidate loci were selected following using R scripts available from: <http://membres-timc.imag.fr/Olivier.Francois/LEA>.

Moran spectral outlier detection (MSOD) & Moran spectral randomization (MSR)

Moran spectral outlier detection (MSOD) uses Moran's eigenvector maps (MEMs) to create power spectrums for each individual SNP, by taking the squared correlation coefficient of allele frequencies with MEM eigenvectors (Wagner et al., 2017). Candidate SNPs are then identified as having power spectra outside of the average spectrum across all SNPs. Moran spectral randomization (MSR), is then used to identify candidate SNPs that show a strong correlation to environmental variables by building the observed spatial structure into the null model, while accounting for spatial autocorrelation (Wagner et al., 2017).

MEM axes were first created from geographic coordinates using the *spdep* R package (Bivand et al., 2011), then power spectra corresponding MAFs and MEMs at each site were calculated. Z-scores were calculated for each locus based on the deviation from the average power spectrum following R code from: https://popgen.nescent.org/2016-12-13_MEM_outlier.html. The outlier loci identified by MSOD were then subjected to the MSR randomization approach, which tests the correlation between outlier MAFs and environmental variables, given the power spectra of each SNP. Using the *adespatial* R package, the MSR was run individually for each environmental variable, with 1,000 permutations. The suggested cut-offs of (Wagner et al., 2017) of 0.01 and 0.05 for MSOD and MSR candidates, respectively, were followed in these analyses.

Redundancy analysis (RDA)

Redundancy analyses (RDAs) are an extension of linear regressions that compare a matrix of dependent variables with multiple independent predictor variables. Linear regressions are calculated between allele frequencies and the climate variables at each site, while the fitted values are simultaneously constrained using a PCA. Environmental variables were centred and scaled, and allele frequencies were Hellinger transformed (Legendre & Gallagher, 2001). All RDAs were performed with the 'rda' function of the *vegan* R package (Oksanen et al.,

2013). Significance was assessed from the adjusted R^2 value and with an ANOVA following 1,000 permutations. Candidate loci were those that had loading scores ± 3 Standard Deviations (SD) of the mean loading for each of the first two constrained axes (Dalongeville et al., 2018; Xuereb et al., 2018).

Distance-based RDAs (dbRDAs) were also run to account for autocorrelation between environmental and geographic distance. Distance-based Moran's eigenvector maps (dbMEMS), which decompose Euclidean distances into a set of spatial variables (Dray et al., 2006), were created with the R package *adespatial* (Dray et al., 2017). Significant dbMEMS were selected by first running an RDA solely using the dbMEMS as predictor variables, then using the adjusted R^2 value from that RDA as the threshold for the forward selection procedure with the 'forward.sel' function in the *packfor* R package (Blanchet et al., 2008).

Outlier variation and functional annotation

Loci that were selected by two or more detection methods (2X outliers) were used to create a statistical 'outlier dataset', and these loci were removed from the total SNP dataset to create a 'putatively neutral dataset'. Intraspecific outlier and putatively neutral variation were compared by running PCA ordinations on the MAFs of each dataset with the *vegan* package, and plotting the ordinations with the *ggplot2* package in Rstudio (Wickham, 2016).

Furthermore, the potential functional roles of outlier SNPs selected by two or more detection methods (2X outliers) was investigated. The contigs containing the outliers selected by at least two methods were BLASTed against NCBI non-redundant protein sequence database for crustaceans (for *C. punctatus*), molluscs (for *S. granularis*), and sea urchins (for *P. angulosus*) using Blast2GO (Conesa et al., 2005). Search results were filtered to only include those that had an E-value less than 10^{-4} , and a minimal alignment length of 20 bp. Gene Ontology (GO) mapping and annotation was conducted on BLAST searches passing quality filters, using default parameters in Blast2GO.

Results

Sequencing and bioinformatic processing

To assure retained SNPs best reflect nuclear genome-wide variation, possible mtDNA reads were removed, as well as compared the performance of three *de novo* assemblers. The average number of reads per pool that mapped onto the reference mitogenomes was 12,363 for *C. punctatus*, 20,342 for *P. angulosus*, and 234 for *S. granularis* (Table 1.S1.5, Appendix 1.S1). These mitochondrial reads were subsequently removed from the raw reads during the mapping stage, as they reflect distinct evolutionary processes compared to nuclear loci (Morin et al., 2004). As there are no reference genomes for these or closely related species, *de novo* assemblies were compared between three programs, SPAdes, AbySS, and MEGAHIT, for each species. There are multiple measures to assess *de novo* assemblies, and here common practice of choosing the assembly with higher N50 and L50 values, and with longer contig lengths, was followed (Narzisi & Mishra, 2011). *De novo* assemblies were also blasted to the NCBI database, but less weight was put on this analysis as it can be biased toward model genomes (Moreton et al., 2016). SPAdes resulted in the more robust assembly, with the longest contig length, N50, and L50, as well as a higher number of NCBI hits on average for all three species (Table 1.S1.6, Appendix 1.S1), and thus was used for all downstream analyses.

The number of raw reads per species ranged from ~29 million for *C. punctatus* to ~47 million for *P. angulosus* (Table 1.S1.7, Appendix 1.S1). The average number of raw reads per pool was ~2.2 million for *C. punctatus*, ~2.5 million for *S. granularis*, and ~3.5 million for *P. angulosus* (Tables 1.S1.8-10, Appendix 1.S1). A total of 17,309, 3,946, and 10,416 SNPs were identified for each species, respectively (Table 1.S1.7, Appendix 1.S1). After filtering for biallelic SNPs and pruning the SNP datasets to one SNP per 1,000bp (to account for linkage disequilibrium; LD), *C. punctatus*, *P. angulosus*, and *S. granularis* had 1,190, 822, and 1,658 SNPs, respectively (Table 1.S1.7, Appendix 1.S1).

Genomic structuring

To assess population structuring, all filtered and LD-pruned SNPs were used to calculate pairwise Weir and Cockerham's F_{ST} values and Nei's genetic distances. Population structure

was investigated with scaled Ω matrices produced by the BayPass v.2.1 core model, which explicitly accounts for Pool-seq data (Gautier, 2015). The scaled Ω matrix characterises the covariation of allele frequencies both within and between pools, and can be interpreted as pairwise relatedness estimates of population structure. Isolation-by-distance patterns were assessed by comparing genomic and geographic distance per species.

Pairwise F_{ST} values varied between species, with ranges of: *C. punctatus* $F_{ST} = 0 - 0.021$, *P. angulosus* $F_{ST} = 0 - 0.127$, and *S. granularis* $F_{ST} = 0 - 0.059$ (Appendix 1.S2). The PCoAs from Nei's genetic distance and the Ω heatmap matrices show no clear spatial clustering for *C. punctatus* and *P. angulosus*, but slight differentiation between western and eastern sites for *S. granularis* (Fig. 1.2). Mantel tests suggest that of the three species, only *S. granularis* populations are characterised by IBD ($r = 0.48$, $p < 0.01$; Table 1.1).

The results also show that altering coverage cut-off parameters has little influence on patterns of population structure, as the two-dimensional visualizations of genomic differentiation (derived from Ω matrices), show similar genomic clustering across three coverage scenarios per species (Fig. 1.S1.2-4, Appendix 1.S1), confirming other studies which found that Pool-seq population differentiation patterns are robust to coverage variances (Hivert et al., 2018; Rellstab et al., 2013).

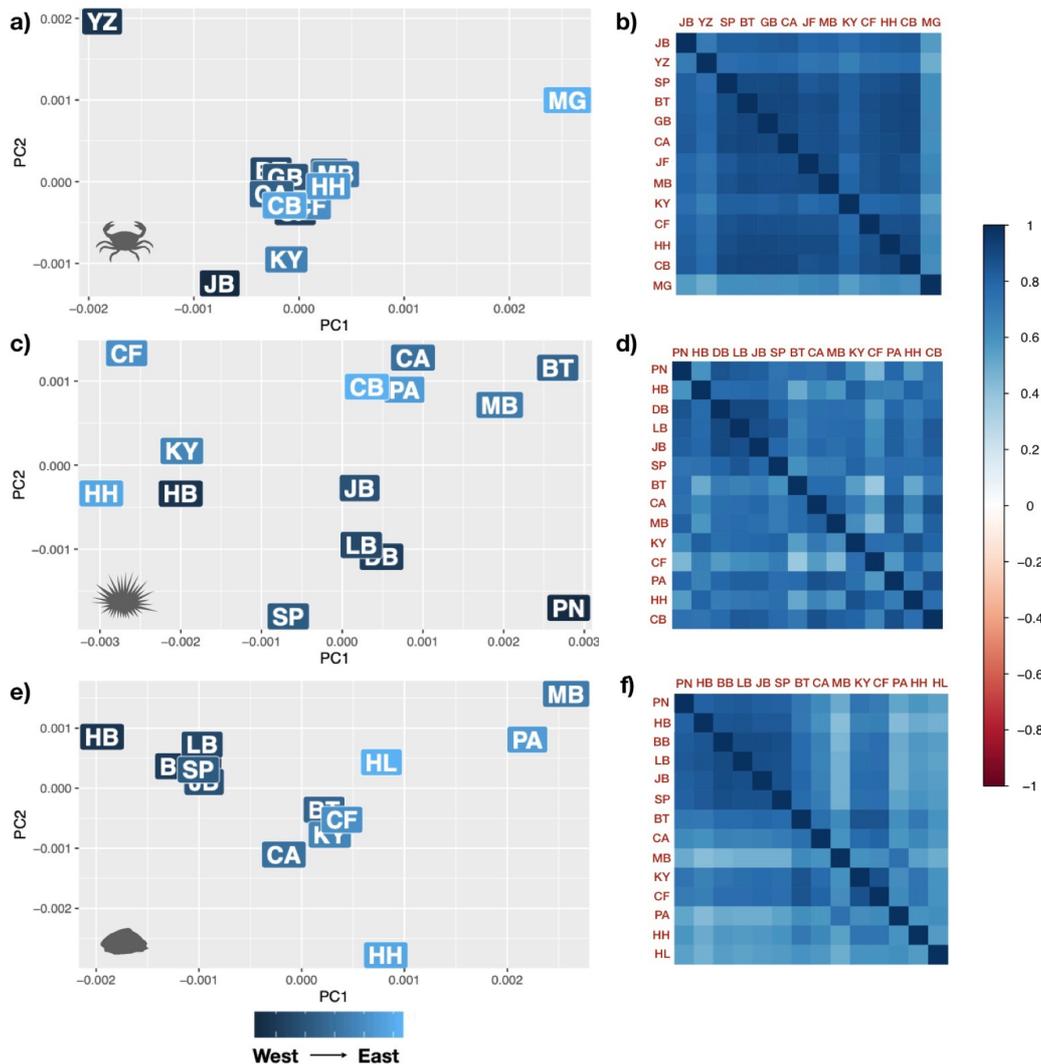


Figure 1.2 – Population differentiation is shown by PCoAs of Nei's genetic distance from all quality-filtered SNPs (a, c, e) and covariance (Ω) matrices represented as heatmaps (b, d, f), shown for *C. punctatus* (a, b), *P. angulosus* (c, d), and *S. granularis* (e, f). Letters in the PCoAs (a, c, e) correspond to the sample sites shown in Figure 1.1, with darker shaded letters corresponding to western sites, and lighter shaded letters corresponding to eastern sites.

Potential environmental drivers of genomic structuring

To assess possible environmental drivers of genomic structuring, IBE tests were run, which compare genomic and environmental distance, accounting for geographic distance. To identify environmental variables for the IBE and GEA analyses, a total of 20 environmental variables were originally included, and subsequently filtered based on Spearman's correlation coefficients < 0.65 and variance inflation factors < 10 . There were multiple correlations between the 20 environmental predictor variables (Appendix 1.S3). After

filtering for collinearity, five final environmental predictor variables remained: mean sea surface salinity (SSSmean), sea surface salinity range (SSSrange), mean sea surface temperature (SSTmean), sea surface temperature range (SSTrange) and air temperature range (Trange; Appendix 1.S3).

Partial Mantel tests showed significant IBE by SSTmean for *C. punctatus* ($r = 0.43$, $p < 0.05$), but this did not remain significant after correcting for multiple testing ($q = 0.19$; Table 1.1). SSTmean was also found to significantly correlate with genomic differentiation in *S. granularis* ($r = 0.40$, $p < 0.01$), which remained significant after multiple testing correction ($q = 0.001$; Table 1.1). The partial Mantel tests did not find a significant correlation between any of the three environmental predictor variables and genomic differentiation in *P. angulosus* (Table 1.1).

Table 1.1 – Mantel and partial Mantel test results for *C. punctatus*, *P. angulosus*, and *S. granularis*.

Test	<i>C. punctatus</i>			<i>P. angulosus</i>			<i>S. granularis</i>		
	r	p	q	r	p	q	r	p	q
$F_{ST} \sim D$	0.18	0.20	0.39	0.13	0.18	0.54	0.48	0.00	0.01
$F_{ST} \sim SSSmean D$	0.18	0.45	0.67	-0.02	0.87	0.87	-0.23	0.27	0.34
$F_{ST} \sim SSSrange D$	-0.50	0.06	0.19	-0.02	0.77	0.87	-0.23	0.03	0.06
$F_{ST} \sim SSTmean D$	0.43	0.03	0.19	-0.20	0.14	0.54	0.40	0.00	0.01
$F_{ST} \sim SSTrange D$	0.04	0.79	0.79	-0.08	0.46	0.87	-0.14	0.39	0.39
$F_{ST} \sim Trange D$	0.08	0.61	0.73	-0.06	0.71	0.87	-0.19	0.28	0.34

Correlation coefficients (r), p -values (p), and q -values (q) are given for tests between genetic distance (F_{ST}) and geographic distance (D), and distance matrices between each of the five environmental variables: mean sea surface salinity (SSSmean), sea surface salinity range (SSSrange), mean sea surface temperature (SSTmean), sea surface temperature range (SSTrange), and surface air temperature range (Trange). Significant values are denoted in bold.

Characterising possible selection signals via outlier loci identification

As GEA methods have been shown to vary in the type and number of outliers detected (Dalongeville et al., 2018; Forester et al., 2017), seven different outlier-detection methods were compared, including six GEAs to investigate possible associations between SNPs and

environmental variables. The analyses included BayPass Bayesian hierarchical models (both core and auxiliary models), Latent factor mixed models (LFMM), Moran spectral outlier detection (MSOD) and Moran spectral randomization outlier detection (MSR), and Redundancy analyses (RDA) and distance-based redundancy analyses (dbRDA).

Overall, there was a large range in the number of outliers detected, with little overlap between models (Table 1.2). LFMM detected the most outliers and had the highest number of unique outliers, followed by MSOD (Table 1.2). Generally, *S. granularis* had the highest number of outliers detected for each model, with the exception of LFMM (Table 1.2). The model type with the lowest number of outliers selected was dbRDA (Table 1.2). For the dbRDA analyses, a forward selection process retained zero dbMEMs for *C. punctatus* and *P. angulosus*, and one dbMEM for *S. granularis*. The dbRDA for *S. granularis* had an adjusted R^2 value of 0.020 ($p = 0.33$), with one outlier locus selected. The standard RDAs had adjusted R^2 values of 0.021 ($p = 0.31$), 0.021 ($p = 0.65$), and 0.084 ($p = 0.01$) for *C. punctatus*, *P. angulosus*, and *S. granularis*, respectively. The single population-differentiation based outlier detection method, BayPass core model (BPC), identified nine outliers for *C. punctatus*, five outliers in *P. angulosus*, and 19 in *S. granularis*, with two, two, and eight outliers unique to that method, respectively (Table 1.2).

Table 1.2 – Comparisons in number of outlier SNPs detected between seven outlier detection methods.

Method (Abbreviation)	Model type	Correction for spatial or population structure	<i>C. punctatus</i> # outliers (# unique)	<i>P. angulosus</i> # outliers (# unique)	<i>S. granularis</i> # outliers (# unique)
BayPass core model (BPC)	Bayesian	Yes, population	9 (2)	5 (0)	19 (5)
BayPass auxiliary model (BPA)	Bayesian	Yes, population	0	0	4 (0)
Latent factor mixed model (LFMM)	Mixed model	Yes, population	134 (121)	72 (60)	125 (101)
Moran spectral outlier detection (MSOD)	Multivariate model	Yes, spatial	15 (14)	9 (7)	20 (18)
Moran spectral randomization	Multivariate model	Yes, spatial	3 (NA)	3 (NA)	8 (NA)

outlier detection (MSR)					
Redundancy analysis (RDA)	Multivariate model	No	9 (3)	9 (1)	16 (2)
Distance-based redundancy analysis (dbRDA)	Multivariate model	Yes, spatial	0	0	1 (1)

Descriptions of outlier detection methods, and the number of total and unique outliers (restricted to that method) detected by each method for each species. Note that MSR could not have unique outliers as it uses those identified by MSOD.

The environmental variable that most strongly correlates with genomic variation differed between outlier detection methods and across species. The majority of methods for *C. punctatus* identified the most outlier loci in association with SSSmean, with the exception of LFMM that identified the most outliers with Trange (Fig. 1.3). Trange and SSTmean were the two variables that identified outliers in at least three models for *P. angulosus* (Fig. 1.3). SSTmean identified the most outlier loci in all methods except LFMM for *S. granularis* (Fig. 1.3).

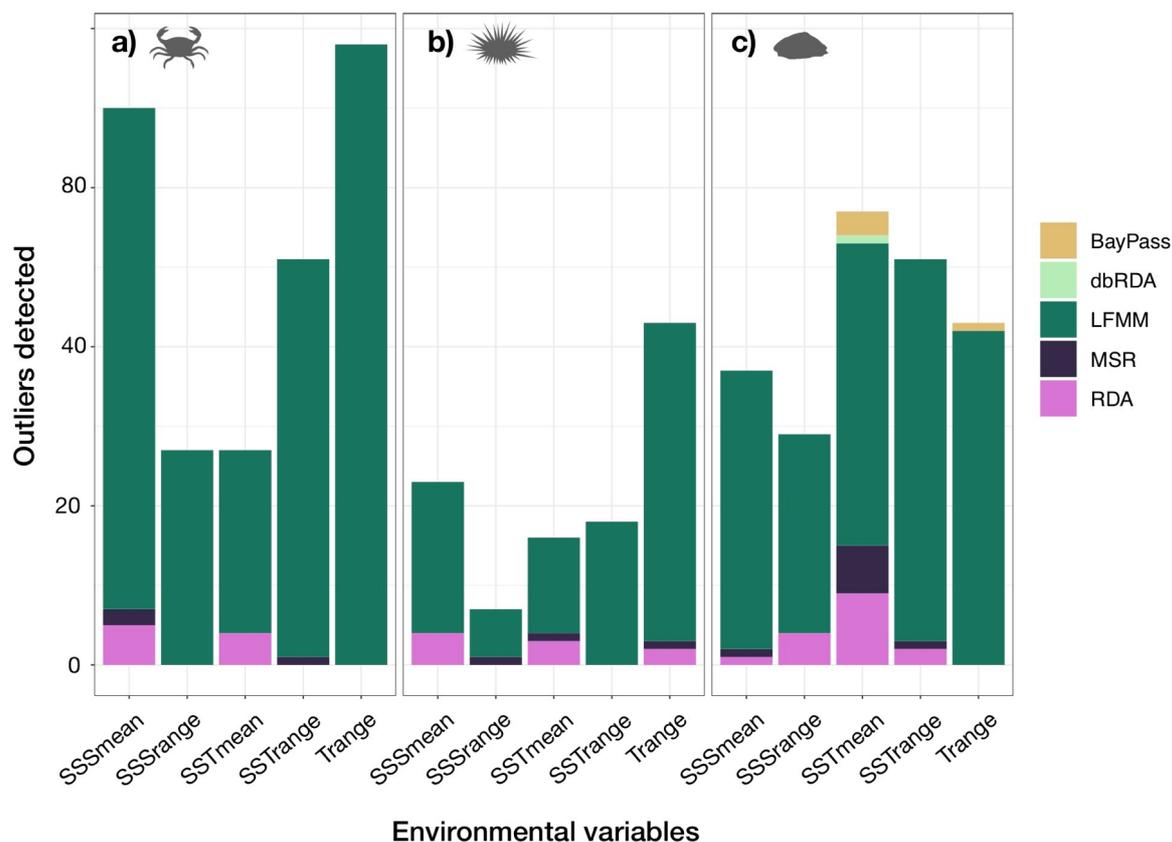


Figure 1.3 – The number of outlier SNPs detected per method for *C. punctatus* (a), *P. angulosus* (b), and *S. granularis* (c). See Table 1.2 for method abbreviations.

Genomic structure of putatively neutral vs. outlier SNPs

Loci that were selected by two or more outlier detection methods (2X outliers) were used to create an ‘outlier dataset’ (Dalongeville et al., 2018; Forester et al., 2017; Nadeau et al., 2016; Phair et al., 2019), and these loci were removed from the total SNP dataset to create a ‘putatively neutral dataset’. The genomic structuring between putative neutral and outlier SNPs were compared via Principal Components Analysis (PCA) ordinations of allele frequencies from each dataset.

The number of SNPs used to create ‘outlier’ datasets was 13, 12, and 26 for *C. punctatus*, *P. angulosus*, and *S. granularis*, respectively. The PCAs of allele frequencies differed between the putatively neutral and outlier SNP datasets for all three species (Fig. 1.4). For *C. punctatus*, the putatively neutral SNPs show most of the sites within one main cluster, with the YZ and MG sample sites each forming individual clusters. In contrast, the outliers show more differentiation between sites, with MG and YZ as most divergent. The putatively neutral SNPs of *P. angulosus* do not separate sites following any geographical pattern, however the outlier SNPs clearly distinguish between the eastern and western sites (Fig. 1.4). In *S. granularis*, the putatively neutral dataset separates eastern and western sites, a pattern even more pronounced when examining the outlier dataset, where sampling sites are clearly differentiated according to geography (Fig. 1.4).

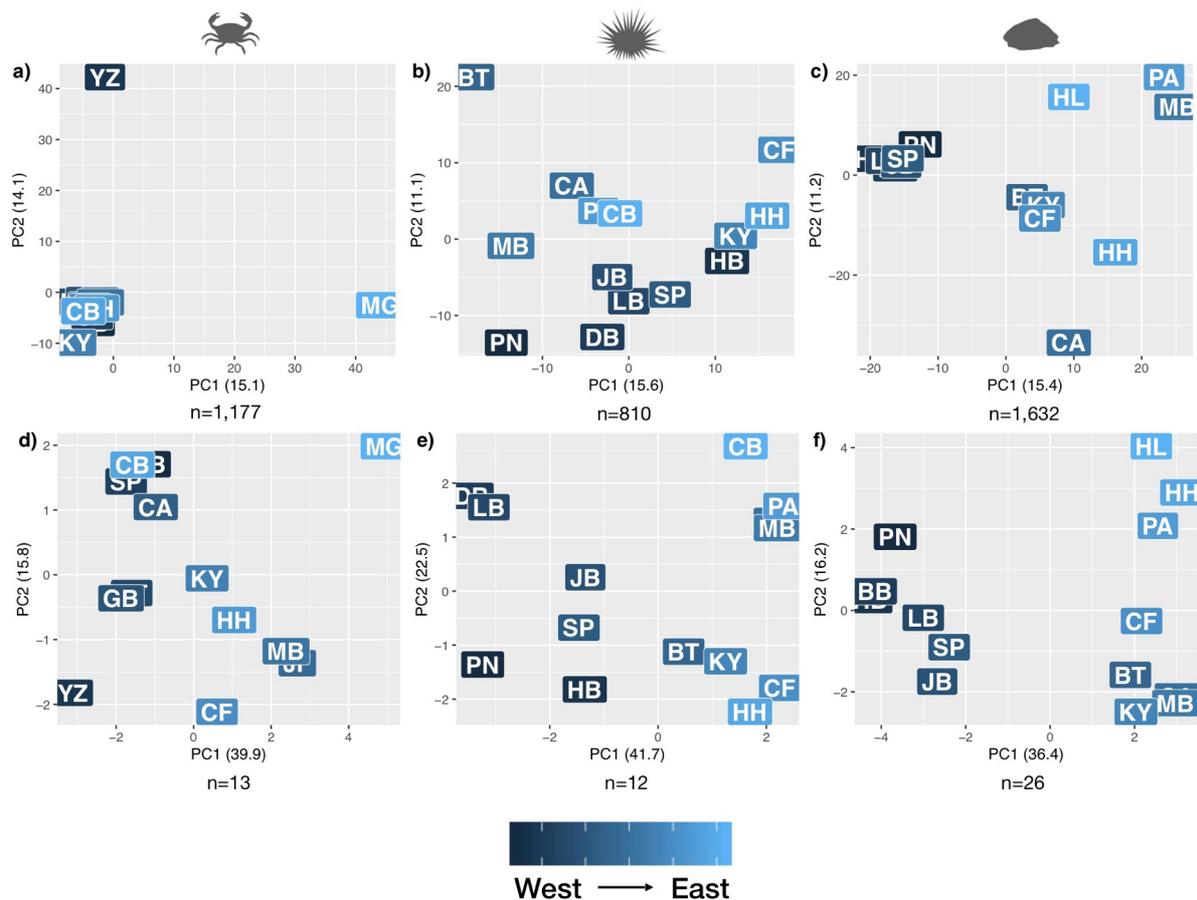


Figure 1.4 – Genomic differentiation as shown by PCAs of allele frequencies in either the putatively neutral (a-c) or outlier (d-f) datasets for *C. punctatus* (a, d), *P. angulosus* (b, e), and *S. granularis* (c, f). Letters correspond to the sample sites shown in Figure 1.1, with darker shaded letters corresponding to western sites, and lighter shaded letters corresponding to eastern sites.

Potential functionality of outlier SNPs

The potential functional roles of the outlier SNPs selected by two or more detection methods (2X outliers) were investigated, by BLASTing them to the NCBI non-redundant protein sequence database, and assessing gene ontology (GO) with Blast2GO.

Of the 2X outliers (which are selected by two or more outlier detection methods), seven contigs from *C. punctatus*, six contigs from *P. angulosus*, and 11 contigs from *S. granularis* led in BLAST results passing quality filters (Appendix 1.S4). Several contigs from *P. angulosus*, and *S. granularis* matched to histone complexes, with GO terms relating to DNA-binding, protein heterodimerization activity, and regulation of DNA recombination and chromatin silencing (Appendix 1.S4). The remaining contigs with BLAST hits for *S. granularis* had GO terms relating to regulation of transcription, GTPase activity, and cell adhesion

(Appendix 1.S4). The GO terms relating to the BLAST hits for *C. punctatus* include protein and ion transport, carbohydrate metabolism, DNA binding and synthesis, and the anaphase-promoting complex (Appendix 1.S4).

Discussion

This study builds on single-marker genetic analyses, by utilising high-throughput genomic data to elucidate phylogeographic patterns of three southern African coastal marine invertebrates. It was hypothesized that the study species would each demonstrate western versus eastern population differentiation as well as IBD and IBE. However, these hypotheses were only confirmed in the limpet, *S. granularis*. A multispecies approach was used to explore putative environmental drivers of genomic variation within this unique marine biogeographical gradient. Here it was hypothesized that SST would be the dominant driver of genomic variation, and yet again this hypothesis was rejected in all species except for *S. granularis*. These findings display how high-throughput sequencing can elucidate distinctive population structuring and gene-environment associations, and offer exciting avenues for future research which investigate these evolutionary processes at even finer scales. Overall, the results reveal species-specific evolutionary patterns, highlighting the complexity of interacting factors shaping natural genomic variation, which is discussed in detail in the following sections.

Genomic markers elucidate distinct patterns of population structuring

The first hypothesis was that each species would reflect previously described mtDNA patterns, with two clusters separated into western and eastern clusters, reflecting the biogeographic breakpoint around the southwestern Cape. However, only the limpet *S. granularis* follows this pattern, with *C. punctatus* showing high connectivity between populations, and *P. angulosus* showing no clear population structuring (Fig. 1.2; Appendix 1.S2). The discordance in genomic differentiation found between mtDNA datasets in previous studies and the SNPs datasets here could be owing to the differences between the two marker types, as mtDNA markers are comprised of a single maternally inherited locus,

while SNP markers represent a broad range of loci across the nuclear genome (Morin et al., 2004). Additionally, mtDNA markers are expected to reflect relatively historical evolutionary events compared to the more contemporary processes captured by genome-wide SNP markers which have larger sample sizes to capture genomic legacies from (Epps & Keyghobadi, 2015).

Scutellastra granularis was also the only species which supported the hypothesis of IBD and IBE influencing genomic structure (Table 1.1). This pattern of IBD and IBE in *S. granularis* could also result from repeated founder effects and allele surfing, caused by colonization generating an allele frequency gradient which co-varies with the environmental gradient (de Lafontaine et al., 2013; Nadeau et al., 2016). However, *S. granularis* and *P. angulosus* were shown to have similar evolutionary histories (Mmonwa et al., 2015; Muller et al., 2012), and thus it seems more likely that contemporary environmental, rather than historical demographic, processes are leading to the distinct patterns found in *S. granularis*. The distinct IBD and IBE patterns found in *S. granularis* could be owing to this species having the shortest pelagic larval duration of the three study species, or due to it being a habitat specialist, preferring sheltered boulder shores (Blamey & Branch, 2009). Additionally, *S. granularis* was found to have the lowest thermal tolerance of four co-occurring mid-shore limpets (Kankondi et al., 2018), indicating that it may be more sensitive to the temperature gradient within the region compared to the other the crab and urchin.

Even though *S. granularis* is the only species to show distinct west versus east differentiation in all SNPs, both *P. angulosus* and *S. granularis* show strong west and east population differentiation when using only outlier SNPs (Fig. 1.4). The western versus southern and eastern bioregions exhibit profound differences in not only temperature, but other environmental variables such as primary productivity (van der Bank et al., 2019), which can potentially lead to local selection despite high levels of connectivity (Teske et al., 2019). This finding builds on multiple other studies which have found outlier SNPs showing fine-scale genomic structuring in populations characterised by high genetic connectivity, yet situated within strong environmental gradients (Diopere et al., 2018; Pujolar et al., 2014; Sandoval-Castillo et al., 2018), and suggests that environmental variation along coastal South Africa plays an important role in the evolutionary dynamics of species in the region.

In contrast to the other two species, the crab *C. punctatus*, did not show a strong separation between the western and eastern sites. Instead, two range-edge sampling

locations (YZ and MG) are highly differentiated in both the putatively neutral and outlier SNP datasets (Fig. 1.4). It could be that this species is less affected by large-scale environmental gradients of the coastline, but rather that an edge effect driven by demographic processes explains this pattern (Eckert et al., 2008). Populations on the edge of a species' distribution are generally more likely to experience historical distributional range shifts, which in combination with contemporary gene flow patterns, can explain the differentiation of these two populations in the putatively neutral SNPs (Eckert et al., 2008; Pujolar et al., 2014). In addition, these sites are also on the edges of the species' ecological niche, and may experience unique environmental variation, potentially leading to the increased differentiation seen in the outlier SNPs (Johannesson & André, 2006; Peluso et al., 2018). There may also be selection forces specific to these two populations which were not included in the GEAs, but which explain evolutionary dynamics of this species (Selkoe et al., 2016). Ultimately, more comprehensive genomic data, such as whole-genome sequencing, and increased fine-scale ecological and environmental assessments are needed to confidently assess the unique population variation seen in *C. punctatus*.

The distinct distribution of genomic variation of *C. punctatus* could also result from it being the most generalist of the three species, inhabiting both estuarine and marine environments (Branch & Branch, 2018; Branch, 2017). A previous study by Ayre et al. (2009) found that among 10 rocky intertidal invertebrates, the ability to utilize sheltered habitat was the strongest predictor of genetic structure. Of the three species here, *S. granularis* is the most restricted in its habitat, compared to *P. angulosus* and *C. punctatus*, which are both able to shelter under rocks and macroalgae rather than remain exposed on rocky surfaces (Branch & Branch, 2018). While dispersal abilities could also potentially be driving intraspecific differences in population structure, it is unlikely that PLD is driving this pattern, as *S. granularis* and *C. punctatus* have similar PLDs (~5-15 days) compared to *P. angulosus* (~50 days; Wright et al., 2015), yet the patterns of genomic differentiation do not reflect the PLD similarities between species. However, additional ecological characteristics pertaining to the larval stages of each species, such as the effect of temperature on larval development (O'Connor et al., 2007; Reitzel et al., 2004), in combination with the spatial and temporal variation in near-shore currents affecting larval dispersal (Galindo et al., 2010), could be influencing population structure. Broadly, while the results show that each species exhibits weak levels of genomic variation, possibly owing to high levels of gene flow, there are fine-

scale interspecific differences in genomic variation, which appear to vary based on the ecology of the species.

Identifying drivers of selection using a multi-model approach

The number of outlier loci, as well as the environmental variable most strongly associated with outlier loci, varied across GEA methodologies (Table 1.2; Fig. 1.3), which mirrors previous studies describing differences in outlier detection methods (e.g., Dalongeville et al., 2018; Joost et al., 2007; Lotterhos & Whitlock, 2015). Each GEA accounts for demographic histories differently, leading to differences in outliers detected (Lotterhos & Whitlock, 2015; Storfer et al., 2018), and thus it has been suggested to use multiple models in combination when the principal environmental variables are unknown (Forester et al., 2017). At present, most studies use one or two outlier detection methods, and identify the most important environmental drivers of selection based on which variable identifies the most outlier SNPs (Forester et al., 2017). However, the results here, as well as those from (Dalongeville et al., 2018), indicate that the number of SNPs identified as outliers varies greatly among detection methods. Therefore, the importance of environmental variables should not be measured by the total number of outliers it detects, but rather by the number of GEA approaches in which the variable identifies outliers. For example, even though the temperature range identified the most outliers for *C. punctatus*, it can be argued that the most important environmental driver of genetic differentiation for *C. punctatus* is mean SSS, because it identified outliers by three detection methods rather than only one for Trange (Fig. 1.3). Given that outlier detection methods are highly variable and subject to false positives (Lotterhos & Whitlock, 2015), using multi-model approaches should increase the robustness of GEAs, especially in studies identifying potential drivers of selection across species with varying evolutionary histories. Hence, in the following section, I discuss the dominant environmental drivers identified for each species based on the number of GEA models in which outliers were identified.

Different environmental drivers of selection across species

Of the three species, only *S. granularis* supported the hypothesis of SST being the most important environmental predictor of genomic variation (Fig. 1.3). Previous seascape genomic studies in temperate regions have frequently identified some measure of SST as the best predictor of genomic variation of marine invertebrates (Benestan et al., 2016; Bernatchez et al., 2019; Coscia et al., 2019; Selkoe et al., 2016; Xuereb et al., 2018), which is most likely due to SST affecting both cellular processes, and life-history events such as spawning and larval development (O'Connor et al., 2007). However, for *P. angulosus*, Trange and SST_{mean} best explained genomic variation, whereas SSS_{mean} best explained the structure of *C. punctatus*. Salinity emerging as a major selective force on *C. punctatus* is understandable, as this species is an osmoconformer that inhabits estuarine environments (Winch & Hodgson, 2007), and because the larval development of decapods is influenced by changes in salinity (Anger, 2003). The lack of clear correlations with any environmental variables is unexpected for the urchin *P. angulosus*, given that previous studies have shown genomic variation corresponding to SST gradients in other echinoderms (Pespeni & Palumbi, 2013; Xuereb et al., 2018). Additionally, the paucity of annotated genomes for marine invertebrates makes it difficult to identify the functionality of outlier loci, which likely led to the limited number of BLAST hits for the 2X outliers (i.e. selected by two or more methods) in each species (Appendix 1.S4). Despite this limitation, outliers from all species indicated a relation to DNA processing, which has previously been highlighted as a response to environmental pressures in marine invertebrates (Suarez-Ulloa et al., 2015). However, other than DNA synthesis and binding, the outliers BLASTed to a variety of proteins, involved in biological processes such as metabolism, cell adhesion, and protein transport (Appendix 1.S4). Overall, the Gene Ontology results suggest that the biological pathways influenced in gene-environment interactions are species-specific, yet further work including more complete genomic information is needed to uncover the environmental footprints on the biology of these species.

Previous terrestrial comparative GEA studies have found distinct results in co-occurring species, which the authors attribute to either differences in ecological niche ranges (Prates et al., 2018) or phenotypic plasticity (Nadeau et al., 2016). A multitude of factors could be driving the interspecific differences observed here, as the study species not

only inhabit different ecological niches, but also exhibit differential behaviours to remain within their physiological niches (Branch & Branch, 2018). It is also likely that the study species exhibit phenotypic plasticity in response to environmental pressures, as plasticity and epigenetic effects have been noted in response to temperature and salinity at multiple life stages in marine invertebrates (Foo & Byrne, 2016). Additionally, the rocky shore is a highly variable environment, and it is likely that species within different rocky shore zones are under differential selection pressures at fine spatial scales (Andrade & Solferini, 2007; Osorio et al., 2018), which might interact with large-scale environmental gradients to create complex patterns of genomic variation.

Conclusions

The results of this comparative genomic study suggest that environmental drivers, and the impacts from their future change, may be highly species-specific, even among co-occurring species living within regions of strong environmental gradients. Further, the results contrast many single-species marine GEA studies by showing that SST does not consistently emerge as an important environmental force structuring the distribution of genomic variation in marine organisms. This finding brings into question the use of SST clines as simple surrogates for selection in marine conservation spatial planning with regards to global change. Yet these results provide exciting opportunities to investigate the relationships between ecological or behavioural traits and environmental drivers of selection across species, which can be further assessed with common garden or physiological experiments.

This is one of the first comparative seascape genomic studies to date, and it is imperative that future seascape genomic studies aim to understand how climatic change will impact not just individual species, but communities (Gamboa & Watanabe, 2019). Multispecies GEA studies remain a challenge due to costs associated with high-throughput sequencing and the lack of annotated genomes in non-model species, particularly marine invertebrates (GIGA Community of Scientists, 2014; Goodwin et al., 2016). Here a pooled RAD-seq approach was used, which allowed us to conduct a multispecies comparative GEA study with relatively low costs, albeit with some limitations such as low coverage alleles being lost due to filtering sequencing errors, and the identification of individuals and polygenic scores being unavailable (Lowry et al., 2017; Tiffin & Ross-Ibarra, 2014).

Additionally, using a reduced representation sequencing approach such as RAD-seq means that portions of the genome remain unknown, and many adaptive loci may not be captured (Lowry et al., 2017). However, for the research questions at hand, a pooled RAD-seq approach is beneficial as it allowed us to maximize the number of individuals per location to obtain accurate population allele frequency estimates (Dorant et al., 2019; Schlötterer et al., 2014), as well as maximize the number of sample sites, both of which are essential for GEAs (Fischer et al., 2013; Storfer et al., 2018), without the full cost of sequencing every individual. One of the greatest disadvantages of using Pool-seq is that admixture analyses to infer population structure are not possible, and as such, this project was forced to follow Pool-seq practices to discern population structure, such as PCAs and SVDs (Dorant et al., 2018; Kahnt et al., 2018; Schulze et al., 2020). As this study is a first step in elucidating the putative adaptive potential of coastal invertebrate species in this unique marine realm, further studies using more complete genomic sequencing strategies are needed to characterize the full breadth of selection processes. Finally, I also provide a novel approach to identify drivers of selection across a diverse array of species, by using multiple GEA methods and inferring the importance of each variable across methods. Ultimately, the future seascape genomics studies can benefit from widening their scope with species and model comparisons, to more robustly identify environmental drivers of selection.

Chapter Two

With regards to Chapter Two (pages 57-75), the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Obtained data, conducted all analyses, and led writing	65%

The following co-authors have contributed to Chapter 2 (pages 56-69):

Name	Email address	Nature of contribution	Extent of contribution (%)
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Maria Beger	m.beger@leeds.ac.uk	drafting of MS, editing	10%

Signature of candidate:

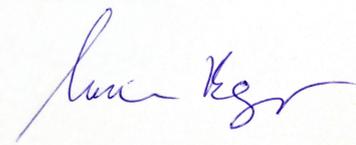


Date: Oct 26 2020

Declaration by co-authors

The undersigned hereby confirm that:

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter Two (pages 57-69),
2. No other authors contributed to Chapter Two (pages 57-69) besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter Two (pages 57-69) of this dissertation.

Signature	Institutional affiliation	Date
	Stellenbosch University	19 October 2020
	University of Leeds	19 Oct 2020

	Technical University of Denmark	20 October 2020
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Chapter Two

Comparing drivers of molecular diversity: testing central-margin and refugial-persistence hypotheses in the marine environment

This paper is in review as: Nielsen E.S., Beger M., Henriques R., von der Heyden S. (2020)

Comparing drivers of molecular diversity: testing central-margin and refugial-persistence hypotheses in the marine environment. *Ecography*

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Introduction

Characterising the impacts of historical and contemporary processes shaping extant genetic variation has gained recent interest to identify populations with heightened evolutionary potential (Hoban et al., 2019). Intraspecific molecular diversity is derived from a complex interplay of spatial and temporal variation, including demographic processes, lineage interactions, local adaptation, and genetic drift (Rödin-Mörch et al., 2019). Currently, two main hypotheses focus on historical versus current processes driving contemporary patterns of intra-and-interspecific genetic diversity. The first is the ‘abundant-centre’, or here termed ‘central-margin’ hypothesis, which proposes that the core of a species’ distribution should harbour greater genetic diversity than range edges (Eckert et al., 2008). This hypothesis assumes that range edges experience more variable or potentially unfavourable ecological conditions, as well as more pronounced range shifts over time (Brown, 1984), resulting in lower genetic diversity (Lawton, 1993). Many empirical studies support this pattern (reviewed in Eckert et al., 2008), but there are several exceptions, possibly caused by processes such as high gene flow between core and edge populations, or environmental pressures historically selecting on range edges inducing greater genetic variation (Maggs et al., 2008). In contrast, several studies provide evidence for an alternative hypothesis, stating that areas that remained climatically stable and habitable over time should correlate with areas of higher extant genetic diversity (Hewitt, 2004). This ‘refugial-persistence’ hypothesis assumes that areas of stability during paleoclimatic oscillations, i.e. glacial refuges, should harbour larger population sizes over time, and thus lead to higher genetic (and species) diversity, compared to areas that experienced historical range shifts and bottlenecks (Beatty & Provan, 2011; Bennett et al., 1991). Multiple studies have confirmed refugial-persistence within cool-temperate marine species in the Northern Hemisphere, showing higher diversity in southern refugial areas, compared to the northern regions, which were formerly glaciated (Francisco et al., 2014; Neiva et al., 2014; Waltari & Hickerson, 2013).

Although a multitude of studies have assessed central-margin or refugial-persistence patterns in natural populations, these hypotheses have almost always been investigated separately. While these two hypotheses are not mutually exclusive, and are also likely not the only aspects shaping molecular diversity (e.g. secondary contact, ocean currents, and migrational behaviour), they are highly tested and reported in the literature, warranting

their investigation in a comparative manner. To evaluate the relative strengths of these hypotheses, it is necessary to assess them in a comparative framework, as many studies reporting central-margin patterns have not accounted for latitudinal gradients, and thus cannot discount the effect of refugial-persistence (Guo, 2012). Additionally, processes shaping extant molecular diversity of cool-temperate coastal species have predominantly been investigated in the Northern Hemisphere, where glaciation events and variation in ice sheets differ profoundly from southern coastal systems (Velichko et al., 1997). Many inferences of molecular diversity are also often obtained from a single marker, which may also influence results, as markers like mitochondrial DNA (mtDNA) are expected to reflect more historical processes compared to markers such as nuclear genomic single nucleotide polymorphisms (SNPs; Wang, 2011).

Here the central-margin and refugial-persistence hypotheses are compared across three rocky shore species endemic to southern Africa. South Africa is an ideal system to compare the relative contribution of historical and contemporary processes shaping molecular variation, due to many marine species harbouring high levels of intraspecific genetic variation within the region (Teske et al., 2011). The coastline is unique with only ocean to the south, and it bordering two contrasting boundary current systems (Fig. 2.1), leading to strong gradients in temperature, salinity, and productivity (Branch & Branch, 2018). Unlike in many coastal systems, South African coastal species are mostly restricted to shifting along a west-east gradient, where even small shifts can result in a dramatically different environment. During the Last Glacial Maximum (LGM), South Africa was not glaciated, but paleoclimatic and sea level changes are thought to have led to shifts in species distributions along the longitudinal gradient within the region (Toms et al., 2014). Many phylogeographic studies within South Africa have attributed extant patterns of genetic variation to ecological divergence via contemporary selection (Teske et al., 2019; Phair et al., 2020), or due to historical changes such as sea level variability since the LGM (von der Heyden et al., 2011, Toms et al., 2014, Phair et al., 2019). Here, the processes driving molecular diversity along South Africa's coastline are elucidated with a combination of paleoclimatic reconstructions, Species Distribution Models (SDMs), and phylogeographic analyses for three southern African rocky shore species. Specifically, this chapter aims to: 1) characterise habitat stability since the LGM from SDMs and paleoclimatic reconstructions, 2) characterise molecular diversity for both genetic mtDNA and genomic SNP markers, 3)

explore the relationships between climatic stability, distance from range-edges, and molecular diversity for each species. I hypothesize that patterns of past range shifts and contemporary genetic diversity will differ between species, given their different life-history features, and that the central-margin pattern will best describe genetic diversity, as South Africa did not experience as much climatic instability with regards to glaciation as compared to Northern cool-temperate coastal regions (Velichko et al., 1997).

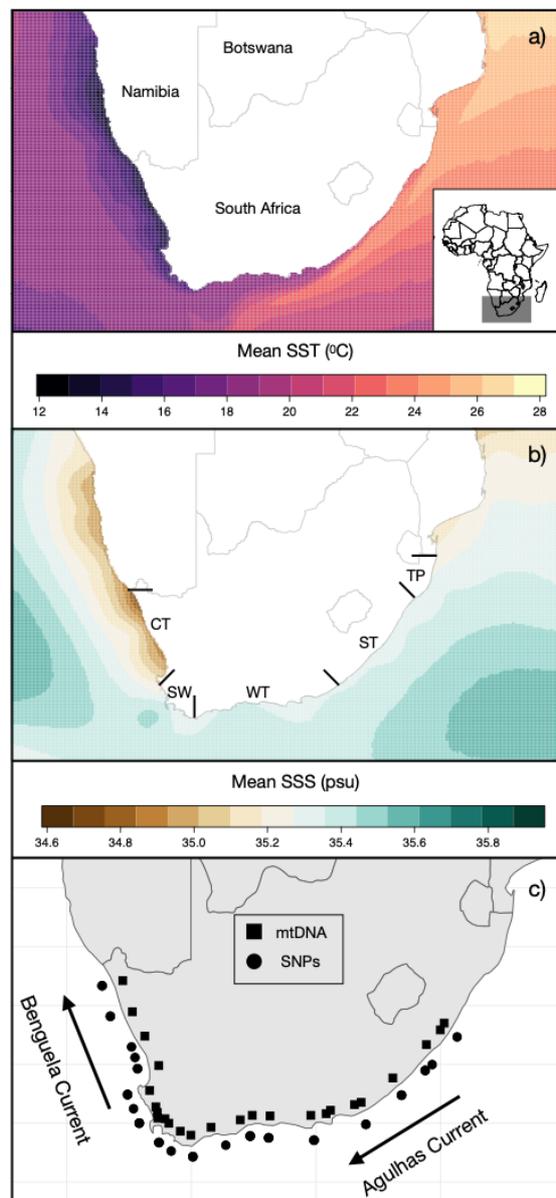


Figure 2.1 – Mean sea surface temperatures (Mean SST; a), mean sea surface salinity (Mean SSS; b) based on monthly averages from MARSPEC data (Sbrocco & Barber 2013) and the Cool-temperate (CT), Southwest (SW), Warm-temperate (WT), Subtropical (ST) and Tropical (TP) bioregions of South Africa (b), as well as the dominant current systems and the genetic (mtDNA) and genomic (SNPs) sample sites (c) are shown.

Materials and Methods

Study species

Study species include the Cape urchin, *Parechinus angulosus*, the Granular limpet, *Scutellastra granularis*, and the Common shore crab, *Cyclograpsus punctatus*. These species are abundant members of South Africa rocky shore communities, have sympatric distributions, and are found in the low, medium and high intertidal zonations, respectively (Branch & Branch, 2018). The study species have differing dispersal abilities that may affect their distributions and genetic diversity. *Scutellastra granularis* and *P. angulosus* are broadcast spawners, while *C. punctatus* is a brooder, and pelagic larval durations estimates vary from ~ 5-15 days for *S. granularis* and *C. punctatus*, and ~50 days for *P. angulosus* (Wright et al., 2015).

Distributional range shifts since the Last Glacial Maximum

SDMs were used to hindcast species distributions to the LGM, predicting the extent of historical ranges based on statistical relationships between known contemporary species distributions and environmental variables (Guisan & Thuiller, 2005). Models included environmental variables which are known to influence the genomic variation of the study species (Chapter One); namely mean sea surface temperature (SST) and salinity (SSS), and mean air temperature of warmest and coolest ice-free month (Tmax and Tmin, respectively). Environmental variables were obtained from the MARSPEC (Sbrocco, 2014) and WorldClim (Fick & Hijmans, 2017) databases. As different General Circulation Models have been shown to lead to distinct SDM projections (Vega et al., 2010), models were based on both the Community Climate System Model (CCSM4) and the Model for Interdisciplinary Research on Climate (MIROC5; Assis et al., 2014), for three available time snapshots: present day, Mid-Holocene (MH; 6 thousand years ago - kya) and Last Glacial Maximum (LGM; 21 kya). Collinearity was assessed from the variance inflation factor (VIF), and layers

with $VIF > 10$ were removed.

Extant distributional ranges were obtained from Branch (2017), Emmerson (2017), coastal habitat maps from van der Bank et al. (2018), and observational knowledge based on extensive fieldwork, to create occurrence points at every rocky shore habitat patch within the distributional range of each species, spaced at 10km intervals (Fig. 2.S1.1, Appendix 2.S1). Pseudo-absences and ensemble models, including six model types, were generated within the R package *biomod2* (Thuiller et al., 2013; see Supplementary Material, Appendix 2.S2 for SDM methodology). Ensemble models were run separately for CCSM4 and MIROC5, the outputs of which were merged into a single layer per time point, portraying habitat suitability ranging from 0-1,000.

Characterising patterns of genetic and genomic diversity

Diversity was characterised by genetic haplotype diversity (h) and genomic expected heterozygosity (H_E), which were chosen as they represent frequency-weighted variation (Nei, 1973, 1987), and are widely used to infer coancestry, bottlenecks, and effective population sizes.

Genetic data, consisting of the mtDNA cytochrome oxidase-1 (*COI*) gene region, was previously generated for 150, 529 and 257 individuals from 11, 17 and 18 sample sites, for *C. punctatus*, *P. angulosus*, and *S. granularis*, respectively (Mertens et al., 2018; Mmonwa et al., 2015; Muller et al., 2012; Wright et al., 2015) and were filtered to include only sites with at least five individuals. As the genetic data for *S. granularis* were derived from two studies with large differences in sampled individuals (Mertens et al., 2018; Mmonwa et al., 2015), those from Mertens et al. (2018) were downsampled by randomly choosing 10 individuals per site. Population-level haplotype diversity was calculated using the program DNAsp v.5 (Librado & Rozas, 2009).

Available genomic data, derived from pooled restriction-site associated DNA sequencing protocol, generated 1,175, 804, and 1,628 putatively neutral single nucleotide polymorphisms (SNPs) for *C. punctatus*, *P. angulosus*, and *S. granularis*, respectively (see Chapter One for details on genomic data generation). H_E was calculated for each site following Fischer et al. (2017; see Equation 2.S1.1, Appendix 2.S1).

A lineage level assessment of climatic stability and molecular variation

Previous assessments of genetic differentiation, using *COI* mtDNA data, of the study species suggest that each species is composed of eastern and western lineages/clusters with separate evolutionary histories (Muller et al., 2012; Mmonwa et al., 2015; Wright et al., 2015; Fig. 2.S1.2, Appendix 2.S1). Population differentiation and niche divergence was further assessed here by conducting redundancy analyses (RDAs) on the allele frequencies of the SNP sets used within the chapter, using the same four environmental predictor variables used in the SDMs. The RDAs were run using the *vegan* R package.

To assess whether past climatic stability varied per lineage, and per species, and to investigate whether regional level climatic changes led to differences in genomic diversity, additional SDMs were also run at the lineage level. Here the occurrence points were partitioned into to eastern or western lineages (see Fig. 2.S1.2, Appendix 2.S1 for site distinctions), and the models were run for each lineage independently, using the same methodology stated above for the same three time points. From the lineage SDMs, shifts in distributional ranges between the present, Mid-Holocene, and LGM were calculated from the binary species presences outputs, using the 'BIOMOD_RangeSize' function of *biomod2*. As the SDMs at the LGM were mapped onto different map cells, due to the lowered sea level and exposed continental shelf, the habitat suitability values at this time point were interpolated onto the map cells of the present and Mid-Holocene models using thine-spline interpolation with the *fields* R package. The change in available/suitable cells per time point, per lineage, were visually compared to the H_E values per lineage, using the R package *ggplot2*.

Influence of paleoclimatic changes and current distribution on molecular diversity

Linear models were built including habitat suitability from the SDMs, as well as three other explanatory covariates: 1) climatic variability, 2) sea level variability (as the region experienced significant changes with sea level lows of -120m; Toms et al., 2014) and 3) distance from the centre of the current distribution. Climatic variability was characterised

using paleoclimatic layers projecting mean SST and SSS, precipitation, and air temperature to the LGM, based on the Hadley Centre ocean-atmosphere climate model (HADCM3; Singarayer et al., 2017). These layers have coarse spatial resolutions of > 1.25 degrees, and thus were not used for the SDMs. Each variable was projected back 21 kya at 1,000-year intervals, and climatic variability was determined by calculating the standard deviation of each variable across the 21 time-steps. Sea level variability was calculated by measuring the Euclidean distance between the zero to -120m bathymetry lines. The distance along the coastline between each sample site and the centre point of the current distribution was calculated per species as the per-site 'marginal distance'.

Linear models were run with the 'lm' R function, comparing nine models including the following predictor variables: 1) Current habitat suitability, 2) MH habitat suitability, 3) LGM habitat suitability, 4) Climatic variability, 5) Sea level variability, 6) Marginal distance, 7) Marginal distance + Current habitat suitability, 8) Sea level + Climatic variability, and 9) MH + LGM habitat suitability. The response variables in the linear models were either genetic or genomic metrics (h and H_E , respectively). Individual models were ranked using the Akaike Information Criteria corrected for small sample sizes (AICc), specifically Δ AICc and AICc weights (ω_i).

Results

Hindcasting distributions to Last Glacial Maximum

The environmental variables had $VIFs < 10$ at each time-step (Table 2.S2.1, Appendix 2.S2), and were kept for further analyses. Individual models used in the ensembles each had high ROC and TSS values (Table 2.S2.2, Appendix 2.S2). Of the four predictor variables, SSS best described the distribution of *C. punctatus*, and SST was most important in inferring the distribution of *P. angulosus* and *S. granularis* (Fig. 2.S1.3, Appendix 2.S1).

The current and projected distributional ranges differed among species and time points (Fig. 2.2). *Cyclograpsus punctatus* likely experienced a slight range reduction during the Mid-Holocene, and a drastic range reduction with a few small refugia eastern South Africa during the LGM (Fig. 2.2). *Parechinus angulosus* showed a range reduction in the Mid-

Holocene with a distributional shift towards the west, and an expanded range during the LGM, with most of the South African and Namibian coastlines being habitable (Fig. 2.2). Compared to *P. angulosus*, *S. granularis* showed a less severe range reduction during the Mid-Holocene, and a slightly larger range expansion during the LGM (Fig. 2.2).

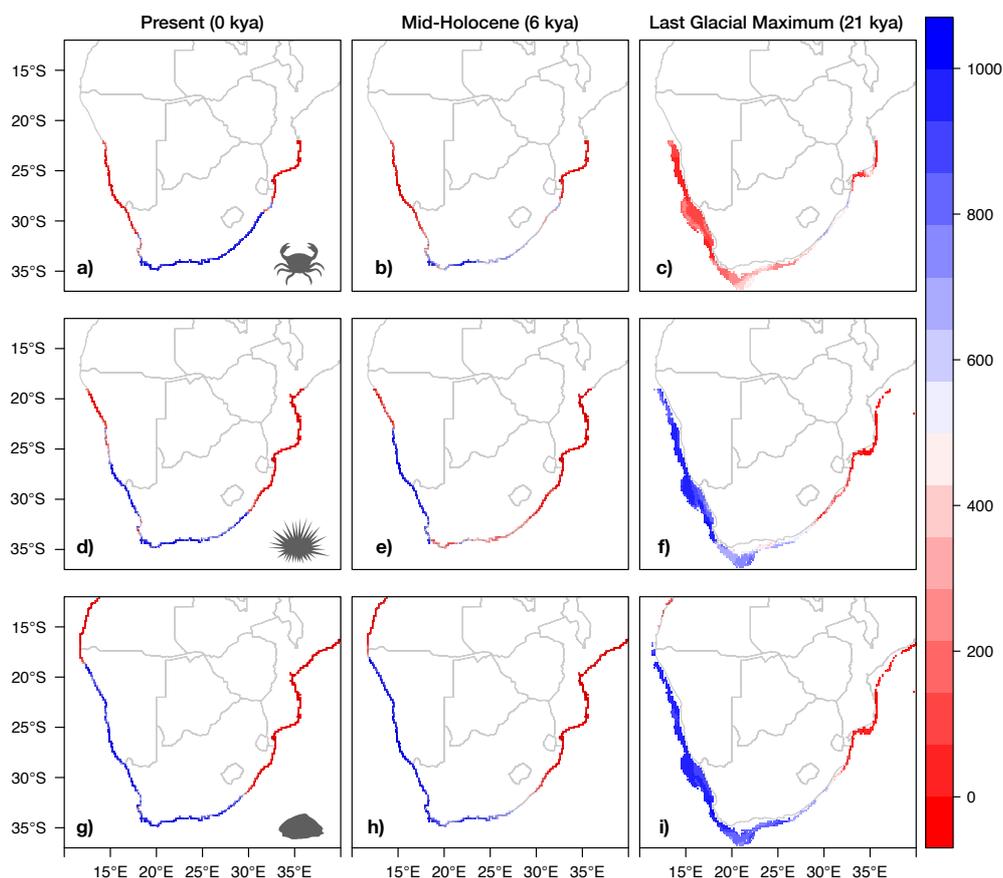


Figure 2.2 – Species distribution model results projecting distributions within the exposed coastal habitat at the present day (Current; a, d, g), Mid-Holocene (b, e, h), and Last Glacial Maximum (c, f, i), for *C. punctatus* (a-c), *P. angulosus* (d-f), and *S. granularis* (g-i). Habitat suitability ranges from zero to 1,000, with areas in blue representing high habitat suitability, whereas areas in red represent those of low suitability. The map cells of the coastline at the LGM differ from the present day and Mid-Holocene due to the drop in sea level and exposure of the continental shelf.

Spatial distributions of molecular diversity

Spatial patterns of molecular diversity within each species were similar between mtDNA and SNP datasets (Fig. 2.3). Broadly, the southwest coast had highest diversity levels for *C. punctatus*, with a few sites along the south coast also harbouring high diversity, and the

edge sites having generally lower diversity levels in both molecular datasets (Fig. 2.3; Tables 2.S1.1-2, Appendix 2.S1). Both *P. angulosus* and *S. granularis* generally showed higher diversity along the west coast, with *P. angulosus* also having high diversity along the south-eastern coast at Cape St. Francis (Fig. 2.3; Tables 2.S1.1-2, Appendix 2.S1).

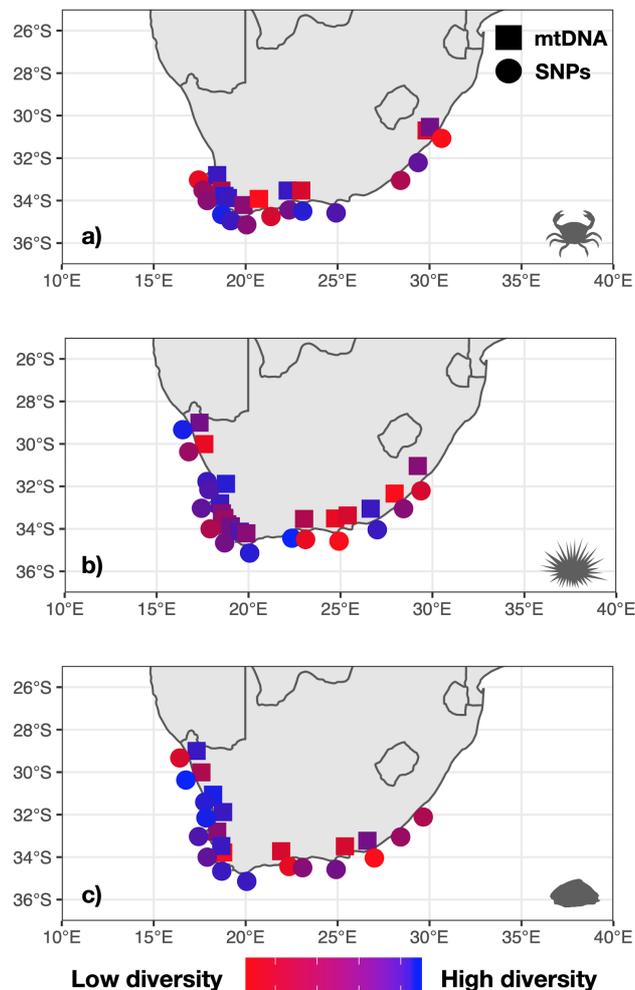


Figure 2.3 – Present day patterns of molecular diversity for *C. punctatus* (a), *P. angulosus* (b), and *S. granularis* (c) with the landward squares representing mtDNA values and seaward circles representing SNP values. Sites in red represent lower diversity and sites in blue represent higher diversity values per species.

Comparing region-wide habitat stability and genomic diversity

The RDAs support niche divergence of eastern and western coast sites in each species as described in previous studies (Fig. 2.S1.4, Appendix 2.S1; Mmonwa et al., 2015; Muller et al.,

2012; Wright et al., 2015). Broadly, the SDMs run at the lineage level revealed similar hindcasted distributions of the species as a whole, with *C. punctatus* showing a range reduction through the past, and *P. angulosus* and *S. granularis* expanding up western southern Africa into the LGM (Fig. 2.S1.5-7, Appendix 2.S1). The SDMs run per each lineage per species show intraspecific differences in range shifts since the LGM (Fig. 2.4). Both eastern and western lineages in *C. punctatus* show range reductions in the past compared to present day, but with the eastern population showing larger range losses (Fig. 2.4). For *P. angulosus* and *S. granularis*, the western lineages had larger range sizes during the Mid-Holocene and LGM compared to present day, while the eastern lineages had reduced range sizes (Fig. 2.4). Eastern and western lineage sites do not significantly differ in H_E for *C. punctatus* or *P. angulosus*, but in *S. granularis*, the western sites have significantly higher H_E values (Fig. 2.4).

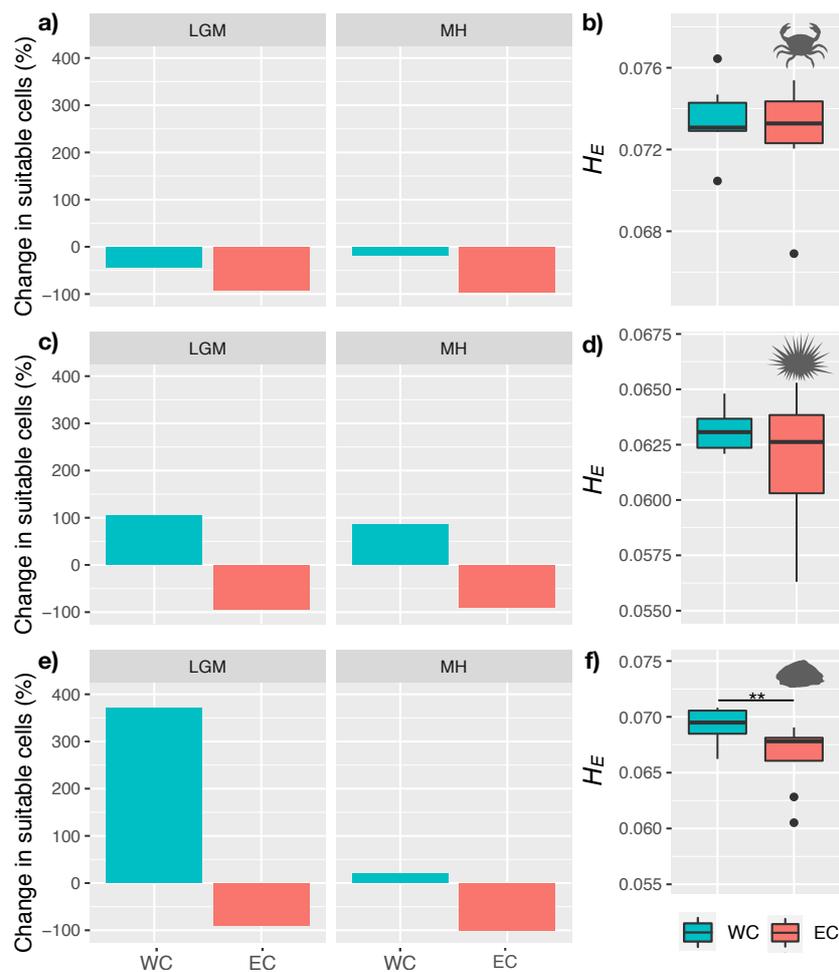


Figure 2.4 – Change in suitable map cells from the lineage SDMs (compared to the present day) is shown for either the west coast (WC) or east coast (EC) sites (see Figure 2.S1.2, Appendix 2.S1 for

eastern and western site distinctions), at the Last Glacial Maximum (LGM; 21 kya), Mid-Holocene (MH; 6 kya) for *C. punctatus* (a), *P. angulosus* (c), and *S. granularis* (e). H_E values per lineage are also shown for *C. punctatus* (b), *P. angulosus* (d), and *S. granularis* (f). * = significant at $p < 0.05$, ** = significant at $p < 0.01$.

Relationships between paleoclimatic stability and diversity

Linear models assessing the relationship between molecular diversity and habitat suitability at the three time points, as well as climatic variability, were not significant for either h or H_E across all species (Tables 2.1-2). The only significant relationship between sea level variability and molecular diversity was for *C. punctatus* ($p = 0.038$; $R^2 = 0.37$). The relationship between marginal distance and molecular diversity was only significant with h for *C. punctatus* ($p = 0.02$; $R^2 = 0.47$), and with H_E for *S. granularis* ($p = 0.04$; $R^2 = 0.25$).

Marginal distance best explained *C. punctatus* genetic diversity ($\omega_i = 0.53$), yet the null model ranked highest in explaining genomic diversity of *C. punctatus* (Tables 2.1-2). The null model, followed by climatic variability, was the best ranking model for both genetic and genomic diversity of *P. angulosus*, and the null model best explained genetic diversity, and marginal distance best explained genomic diversity of *S. granularis* ($\omega_i = 0.43$; Tables 2.1-2).

Table 2.1 – Linear models explaining mtDNA haplotype diversity (h) for *C. punctatus*, *P. angulosus*, and *S. granularis*. Number of parameters (K), Akaike Information Criteria corrected for small sample sizes (AICc), differences in (Δ AICc), and AICc weights (ω_i) are shown per model. Models are listed by rank, with Δ AICc < 2 models highlighted in bold.

Genetic diversity (h) model outputs				
Model	K	AICc	Δ AICc	ω_i
<i>C. punctatus</i>				
Marginal distance	3	-7.29	0	0.53
Sea-level variability	3	-5.48	1.81	0.21
Null	2	-4.04	3.25	0.1
Current suitability	3	-2.39	4.9	0.05
MH suitability	3	-2.06	5.23	0.04
Marginal distance + Current suitability	4	-1.49	5.8	0.03

Sea-level + Climatic variability	4	0.18	7.47	0.01
LGM suitability	3	0.19	7.47	0.01
Climatic variability	3	0.22	7.5	0.01
MH + LGM suitability	4	3.77	11.06	0
<i>P. angulosus</i>				
Null	2	-45.36	0	0.24
Climatic variability	3	-45.34	0.02	0.24
Marginal distance	3	-44.15	1.21	0.13
MH suitability	3	-43.82	1.54	0.11
LGM suitability	3	-42.88	2.48	0.07
Current suitability	3	-42.45	2.91	0.06
Sea-level variability	3	-42.38	2.98	0.05
Sea-level + Climatic variability	4	-41.85	3.51	0.04
Marginal distance + Current suitability	4	-41.02	4.34	0.03
MH + LGM suitability	4	-40.33	5.02	0.02
<i>S. granularis</i>				
Null	2	-21.12	0	0.51
Current suitability	3	-18.17	2.95	0.12
LGM suitability	3	-17.86	3.26	0.1
Climatic variability	3	-17.14	3.98	0.07
Sea-level variability	3	-17	4.12	0.07
Marginal distance	3	-16.95	4.17	0.06
MH suitability	3	-16.84	4.28	0.06
Marginal distance + Current suitability	4	-12.32	8.8	0.01
MH + LGM suitability	4	-12.24	8.88	0.01
Sea-level + Climatic variability	4	-11.18	9.93	0

Table 2.2 – Linear models explaining SNP expected heterozygosity (H_E) for *C. punctatus*, *P.*

angulosus, and *S. granularis*. Number of parameters (K), Akaike Information Criteria corrected for small sample sizes (AICc), differences in (Δ AICc), and AICc weights (ω_i) are shown per model. Models are listed by rank, with Δ AICc < 2 models highlighted in bold.

Genomic diversity (H_E) model outputs				
Model	K	AICc	ΔAICc	ω_i
<i>C. punctatus</i>				
Null	2	-115.84	0	0.27
Marginal distance	3	-115.78	0.05	0.26
MH suitability	3	-114.45	1.38	0.14
LGM suitability	3	-113.51	2.33	0.08
Current suitability	3	-113.14	2.7	0.07
Climatic variability	3	-112.62	3.22	0.05
Sea-level variability	3	-112.6	3.23	0.05
Marginal distance + Current suitability	4	-111.78	4.06	0.04
MH + LGM suitability	4	-111.08	4.76	0.03
Sea-level + Climatic variability	4	-108.47	7.36	0.01
<i>P. angulosus</i>				
Null	2	-126.17	0	0.3
Climatic variability	3	-125.47	0.69	0.21
MH suitability	3	-124.72	1.45	0.14
LGM suitability	3	-123.59	2.58	0.08
Sea-level variability	3	-123.27	2.89	0.07
Current suitability	3	-123.03	3.14	0.06
Marginal distance	3	-122.98	3.19	0.06
Sea-level + Climatic variability	4	-122.21	3.96	0.04
MH + LGM suitability	4	-120.92	5.25	0.02
Marginal distance + Current suitability	4	-119.12	7.05	0.01
<i>S. granularis</i>				
Marginal distance	3	-121.97	0	0.34

Sea-level variability	3	-120.67	1.3	0.18
Null	2	-120.18	1.79	0.14
Marginal distance + Current suitability	4	-119.54	2.43	0.1
LGM suitability	3	-119.08	2.9	0.08
MH suitability	3	-118.44	3.53	0.06
Climatic variability	3	-117.42	4.56	0.03
Sea-level + Climatic variability	4	-117	4.97	0.03
Current suitability	3	-117	4.98	0.03
MH + LGM suitability	4	-115.03	6.94	0.01

Discussion

Overall, the multi-species multi-locus used here results offer greater support for refugial-persistence driving diversity patterns than the central margin hypothesis, although some variation remains unexplained. Intraspecific molecular diversity showed distinct spatial patterns between species (Fig. 2.3), in line with the first hypothesis. Climatic refuges varied per species, as well as between the Mid-Holocene and LGM oscillations (Fig. 2.2; Fig. 2.4). Whereas regions of high habitat suitability in the past roughly correspond to those of high molecular diversity today (Fig. 2.2-4), habitat, climatic, and sea level variability did not significantly correlate with patterns of molecular diversity (Tables 2.1-2). Linear regression models showed significant relationships between marginal distance and molecular diversity, yet this was not consistent across molecular markers or species (Tables 2.1-2).

Looking to the past: multi-species range shifts through time

During the late Quaternary, southern Africa's coastline experienced pronounced environmental changes. The Mid-Holocene was characterised by warmer temperatures, a stronger Agulhas Current in the east, with weakened upwelling in the Benguela Current

along the west coast (Cohen & Tyson, 1995). Going back further to the LGM, South Africa experienced cooler SSTs (Stone, 2014), a weakened Agulhas Current (Franzese et al., 2006), and an increase in SSS (Holloway et al., 2016). These climatic changes likely influenced the distributions of species in distinct ways. As such, the results suggest that historical habitat suitability differed among species, with the crab *C. punctatus* having the most severe range-shifts between the present and LGM (Fig. 2.2). The SDMs for *C. punctatus* suggest a range-reduction during this time (Fig. 2.2), possibly linked to an influx of low salinity waters from the Mozambique Current due to an increase in freshwater run-off from the Zambezi River during the last interglacial (Holloway et al. 2016), and lowered sea surface temperatures during the last glacial period. Both salinity and temperature have been shown to play an important role in the physiology of adult *C. punctatus* individuals (Winch & Hodgson, 2007), as well as in the larval development of other decapod species (Anger, 2003). The importance of salinity in predicting the distribution of *C. punctatus* highlights how marine SDMs based solely on temperature can lead to potentially over- or under-estimating distributions.

In contrast, the historical distributions of *P. angulosus* and *S. granularis* are predominantly influenced by SSTs, consistent with findings on other coastal species (Assis et al., 2014; Chefaoui & Serrão, 2017; Neiva et al., 2014). The importance of SST in predicting *P. angulosus* and *S. granularis* distributions likely relates to the physiology of these species, as they occupy the intertidal niche, and are subjected to several hours of warmer SSTs during low tide. It is also well known that temperature influences all biological structures and processes (Somero, 2002), and can indirectly affect the distributions of *P. angulosus* and *S. granularis* by influencing the distributions of predator, prey and competing species (Kordas et al., 2011).

Both *P. angulosus* and *S. granularis* display a slight shift toward the west coast of southern Africa in the Mid-Holocene, which is most likely a response to the increase in SST during the last interglacial period (Fig. 2.2; Crowley & North, 1991). Both species also show a range expansion going from the Mid-Holocene to the LGM as SSTs decreased (Fig. 2.2). The findings here differ from the majority of other hindcasting SDMs on temperate marine species, which show range reductions during the LGM (Assis et al., 2014; Neiva et al., 2014; Waltari & Hickerson, 2013). However, these studies were conducted in the Northern Hemisphere, where ice-sheets inhibited northern range edges (Provan, 2013), compared to

the study system used here, which lacked permanent ice cover, and where sea level change and associated changes in the topology and near-shore currents along the coastline were the primary barriers to dispersal of coastal species (Otto-Bliesner et al., 2006; Toms et al., 2014).

Comparing historical and contemporary drivers of molecular variation

This chapter tested whether areas near the centre of the species' distribution or areas of past refugial-persistence better correspond to areas of high extant molecular diversity. The SDM results offer support for refugial-persistence explaining diversity in all species, as areas of higher suitability at the MH and LGM generally correspond with areas of contemporary elevated molecular diversity (Fig. 2.2, Fig. 2.3). The results also show that lineages that experienced higher habitat stability since the LGM generally have slightly higher diversity at present (Fig. 2.4), although this was only significant in the limpet, and was not strongly supported by linear models for either marker type (Tables 2.1-2). Similarly, linear models comparing climatic and sea level variation to molecular diversity also offer little support for the refugial-persistence hypothesis (Tables 2.1-2).

Numerous studies relate hindcasted distributions to genetic patterns, providing evidence for refugial-persistence, although contrasting patterns do exist. For example, Bermejo et al. (2018) identified incongruence between LGM suitability and genetic variation in the algae *Cystoseira tamariscifolia*, which they expect is due to range expansions during the Mid-Holocene. Sunday et al. (2012) suggest that compared to terrestrial counterparts, marine species are expected to show more immediate responses to climatic variation. Therefore, the molecular diversity of marine invertebrates with high fecundity and large dispersal ranges may reflect climatic variation from the Mid-Holocene rather than LGM. The results show substantial differences between habitat suitability in the LGM and Mid-Holocene, and thus both time points should be included in future studies to best estimate the rate at which climatic fluctuations imprint on the molecular diversity across the distributional ranges of marine species.

When testing central-margin patterns, linear models offered inconsistent support across species and marker types (Tables 2.1-22). The inconsistencies found here mirror

several of the existing studies testing the central-margin hypothesis. For example, previous studies, assessing species abundances of southern African sandhoppers *Talorchestia capensis* and *Africorchestia quadrispinosa* (Baldanzi et al., 2013) and North Pacific coastal invertebrates (Sagarin & Gaines, 2002) both offer little support for the central-margin hypothesis, ultimately suggesting that this pattern cannot be generalized across intertidal species. In contrast, in their review the central-margin hypothesis, Eckert et al. (2008) found support for the hypothesis, with ~65% of 134 studies showed a decline in molecular diversity towards the range edges, although this finding was likely influenced by including studies that considered exclusively the northern range boundary with increased climatic variability during past glaciations (Eckert et al., 2008; Guo, 2012). In more recent studies, there are inconsistencies in core-edge patterns between marker types such as in the results shown here, which could be attributed to some markers taking into account different sample sizes, and thus are more sensitive to changes in diversity (Cahill & Levinton, 2015; Ntuli et al., 2020). Furthermore, Ntuli et al. (2020) propose that natural populations may only reflect central-margin patterns when population connectivity levels are below a certain threshold, allowing evolutionary, rather than ecological, processes to be the more dominant pattern within the genetic mosaic.

Even though this chapter aimed to independently test central-margin and refugial-persistence, the two processes could not be completely disentangled, as for example in *C. punctatus*, the centre of the distribution, the highest sea level variability, and the highest probability of occurrence at the Mid-Holocene all overlap within the southwest South African coast. These findings highlight how these two hypotheses are not mutually exclusive, and how a multitude of abiotic and biotic features co-vary and interact to shape demographic and evolutionary histories in complex ways (Rödin-Mörch et al., 2019).

Conclusions

While multiple studies have tested the central-margin hypothesis in marine species, many have not explicitly considered the effect of glacial persistence within their analyses (Liggins et al., 2015). Although the multi-species, multi-marker results here offer greater support for refugial-persistence, conclusive evidence for either hypothesis was not shown. It is likely

that both the central-margin and refugial-persistence hypotheses oversimplify the processes that shape extant patterns of molecular diversity of species (Lal et al., 2017; Maggs et al., 2008). Further investigation of species demographic histories, using methods such as Approximate Bayesian Computation, is needed to more accurately describe how historical dynamics influence diversity patterns (Hoban et al., 2019). Overall, the results suggest that coastal marine species within temperate bioregions in the Southern Hemisphere may have more complex biogeographic and evolutionary histories than those in the North, and further work is needed to assess the processes that have shaped, and will continue to shape, the molecular diversity of these organisms.

Chapter Three

With regards to Chapter Three (pages 78-101), the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Obtained data, conducted all analyses, and led writing	70%

The following co-authors have contributed to Chapter 3 (pages 73-94):

Name	Email address	Nature of contribution	Extent of contribution (%)
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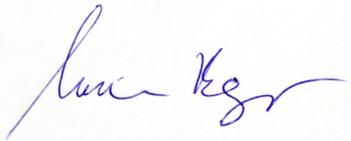
Date: Oct 26 2020

Declaration by co-authors

The undersigned hereby confirm that:

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter Three (pages 73-94),
2. No other authors contributed to Chapter Three (pages 73-94) besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter Three (pages 73-94) of this dissertation.

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Chapter Three

Distinct inter- and intra-specific vulnerability of coastal species to global change

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Distinct inter- and intra-specific vulnerability of coastal species to global change. *Global*

Change Biology

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Introduction

Anthropogenic climate change is causing worldwide species redistributions and local extinctions, altering community compositions and ecosystem functioning (Babcock et al., 2019; Román-Palacios & Wiens, 2020). As global environmental change forces species to adapt or shift their distributional ranges, conservation actions need to anticipate individual species' responses and how these will in turn affect ecosystem functioning and human wellbeing (Bonebrake et al., 2018; Pecl et al., 2017; Tittensor et al., 2019). Thus, one of the emerging objectives within conservation science is to protect areas of heightened resilience and/or evolutionary potential (Funk et al., 2019; Razgour et al., 2019), which requires vulnerability assessments from methods such as physiological experiments, land- or seascape genomics, or forecasting species distribution models (Grummer et al., 2019; Wilson et al., 2020).

A taxon's vulnerability to climate change is often assessed with species distribution models (SDMs; Guisan & Thuiller, 2005) that predict the probability of occurrence from correlations between known occurrences and environmental variables (Elith & Leathwick, 2009). However, most assessments of species range shifts from SDMs disregard the occurrence of intraspecific climatic tolerances, local adaptation, and gene flow (Rilov et al., 2019). This is problematic, as genetic variation is a crucial component of a species' resilience, with areas of high neutral diversity inferring more raw material for adaptation to occur, and high adaptive diversity inferring pre-adapted populations (Nielsen et al., 2020). There have been efforts to assess lineage (D'Amen et al., 2013; Espíndola et al., 2012) and population (Banta et al., 2012; Jay et al., 2012) level responses to climate change with 'genetic SDMs', often showing a disproportionate loss of genetic variation over the species' range. However, to date, most studies including genetic variation in SDMs applied neutral loci to delineate population level variation, which may not capture differentially adapted populations (Mittell et al., 2015). Studies including candidate or 'outlier' loci, i.e. presumed to be under selection, are thus necessary to identify differentially adapted populations (Xuereb et al., 2020). Yet, comparisons of neutral and outlier loci to assess intraspecific vulnerability to global change remain limited, especially in the marine environment. In a recent terrestrial study, Razgour et al. (2019) used outlier loci to identify differentially adapted populations in two bat species, finding that projected distributions into the future

differed between populations.

Developing separate SDMs on differentially adapted populations is an important step in understanding the intraspecific responses to future environmental change. However, this methodology does not account for nonlinear gene-environment relationships (Fitzpatrick & Keller, 2015) that are better captured with Gradient Forest (GF) models. Originally used as a community-level approach to predict species assemblages and species turnover (Ellis et al., 2012; Pitcher et al., 2012), GF models can also serve to predict within-species 'allele communities', and map areas of 'genomic-turnover' (Fitzpatrick & Keller, 2015). Gradient Forest models can also be used to determine intraspecific 'genomic vulnerability', which is derived from the mismatch between current and future spatial genomic composition (Bay et al., 2018). Such approaches to predict intraspecific vulnerability present a hitherto unexplored opportunity to quantify climate change responses in marine species, which are generally more sensitive to warming than terrestrial organisms (Pinsky et al., 2019). Further, comparing the predicted responses to global change across levels of taxonomic organisation with SDMs and GF models has yet to be conducted. Characterising spatio-temporal vulnerability from models accounting for different biological levels is essential to support the identification of climatic refugia for future conservation efforts (Carroll et al., 2017; Morelli et al., 2016).

For the first time in a marine context, this study applies a comparative approach to characterise both intra- and interspecific vulnerability based on species- and gene-environment interactions. This was performed within South Africa, one of the world's most biodiverse marine regions (Griffiths et al., 2010), an ocean warming hotspot (Hobday & Pecl, 2014), and currently underrepresented in marine range shift studies (Sorte et al., 2010). Coastal South Africa is unique, as it is bordered by two contrasting boundary currents, with striking transitions in habitat conditions taking place over relatively short distances (Fig. 3.1; van der Bank et al., 2019). The coast is composed of five bioregions (Fig. 3.2), defined by changes in environment (i.e. temperature, salinity, precipitation), habitat type, and species composition (van der Bank et al., 2019). Intraspecific phylogeographic breaks of South African marine species often correlate with these biogeographic breaks, possibly owing to similar environmental features shaping species and genetic level biodiversity patterns (Teske et al., 2011). The region is currently experiencing strong environmental changes, such as an increase in mean annual air temperature (Kruger & Shongwe, 2004), and sea

surface temperatures (Rouault et al., 2010). The combination of unique oceanographic features and the marked environmental gradients in this region could easily drive species into vastly different physical conditions and substantially alter community compositions. There are however, few predictions of future species range shifts within this highly unique and threatened marine region (but see Bolton et al., 2012 for an empirical range shift example). Moreover, in other regions of the world, studies suggest species-specific responses to future change (Bates et al., 2014; Sunday et al., 2015), highlighting the need to identify future climatic refugia across multiple species and regions. Thus, this study compares vulnerability patterns across three ecologically important southern African marine invertebrates, to identify areas of multispecies evolutionary potential in a known marine biodiversity hotspot.

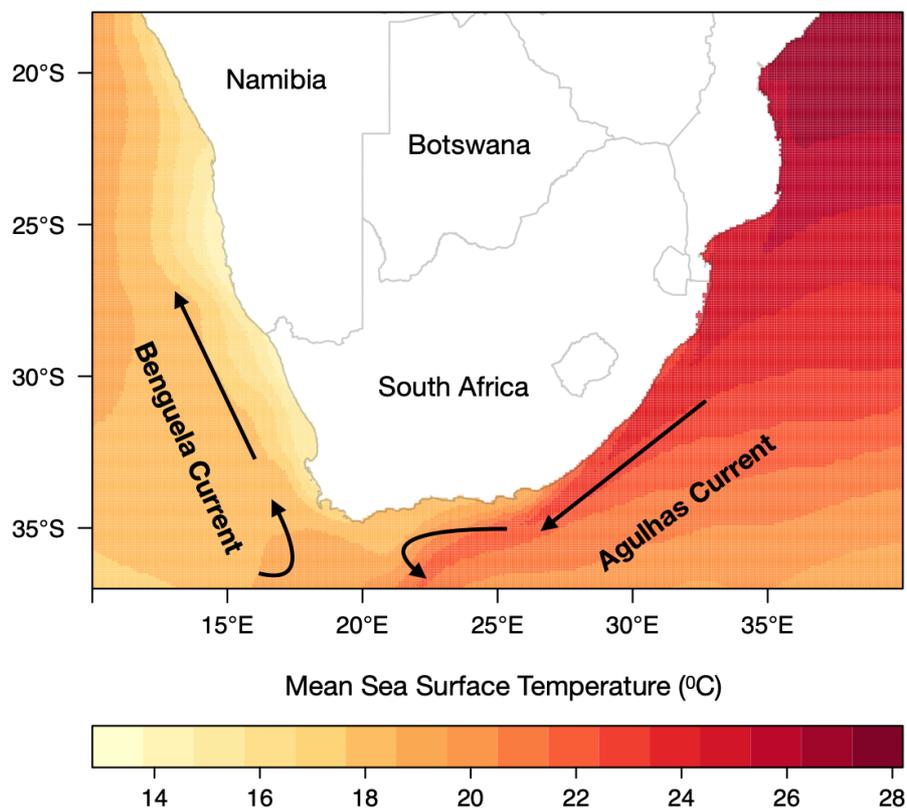


Figure 3.1 – Sea surface temperatures (averaged over 2000–2014 and from Bio-Oracle2.0; Assis et al., 2018), increasing from yellow to red, and major ocean currents within the study region.

Specifically, this study aims to: 1) characterise spatial genomic composition and predict genomic vulnerability based on neutral and outlier loci per species; 2) predict species distributional shifts into the future, and the environmental drivers of these range

shifts, and 3) compare species- and gene-environment relationships and vulnerability footprints between species and populations. I hypothesize a mismatch between species and genomic vulnerability, as this has been previously shown within SDMs run at both the species and population level (Jay et al., 2012; Razgour et al., 2019). However, this is the first time that species forecasts from SDMs will be compared to genomic forecasts from GF models, the latter of which are capable of capturing complex gene-environment relationships. Here, I expect that areas in which SDMs predict range losses (i.e. high vulnerability) might correlate with areas of low genomic vulnerability, as these populations may be pre-adapted to climatic changes such as warming. Verifying this hypothesis will increase our understanding of how climatic resilience differs between species and populations of co-distributed taxa occurring in a climate change hotspot. More broadly, this work is an essential step in predicting the trajectories of coastal ecosystems under global change.

Materials and Methods

Gradient Forest modelling

To infer how climatic variables shape, and will continue to shape, the genomic variation of southern African rocky shore species, allele frequency data from genome-wide single nucleotide polymorphism (SNP) was input into Gradient Forest (GF) models. These models assess the relationships between environmental variables and biological abundances by creating an ensemble of individual decision trees (Ellis et al., 2012). More specifically, a machine-learning algorithm is used to partition splits between values of each environmental variable, then calculate the change in allele frequency at each environmental split value, for each allele (Ellis et al., 2012). The amount of variation explained at each split value, termed the 'split importance', is summed along the environmental gradient for each allele and aggregated across alleles to create a genome-wide turnover function, per environmental variable.

Gradient Forest models were fitted to the allele frequencies of putatively neutral and outlier SNP datasets for three rocky shore species, namely the Cape urchin (*Parechinus*

angulosus), Common shore crab (*Cyclograpsus punctatus*), and Granular limpet (*Scutellastra granularis*). These species are ecologically important and broadly distributed along the environmental gradients of the southern African coastline, but exhibit different ecological niches and life histories (Branch, 2017; Branch & Branch, 2018). The datasets were previously obtained in Chapter One, including ~40 individuals each from 13 to 14 sites for *C. punctatus*, *P. angulosus* and *S. granularis* (Fig. 3.2). A pooled restriction site-associated sequencing (RAD-seq) approach was used to generate allele frequencies, and seven outlier detection methods were used to identify a set of candidate SNPs, which are presumed to be under selection (see Chapter One for details). Alleles which were selected as candidate loci by at least two outlier-detection methods went to create the 'outlier dataset' per species, and were removed from the full SNP list to create a 'neutral dataset'. The neutral datasets consist of 1,177, 810, and 1,632 SNPs, and the outlier datasets consist of 13, 12, and 26 SNPs, for *C. punctatus*, *P. angulosus*, and *S. granularis*, respectively. These allele frequencies served as the response variables in the GF models.

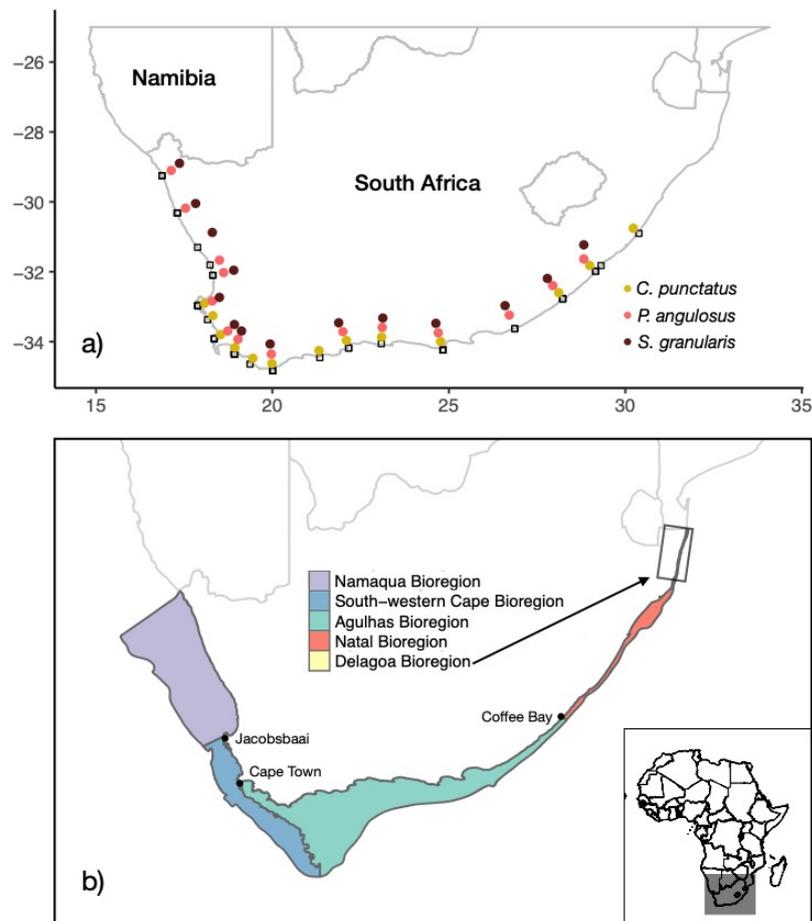


Figure 3.2 – Samples sites from which genomic data was obtained for *C. punctatus*, *P. angulosus*, and *S. granularis* (a), as well as the bioregions obtained from van der Bank et al. (2019), with the Delagoa Bioregion indicated in the box for clarity (b).

Eight environmental variables were selected for the GF models based on their importance for rocky shore ecology (Branch & Branch, 2018), marine species distributions (Bosch et al., 2018), and significance in shaping genomic variation within the study species (Chapter One). These variables were the means and ranges of sea surface temperature (SST), sea surface salinity (SSS), air temperature (T) and precipitation (P). Atmospheric variables were obtained from WorldClim (Fick & Hijmans, 2017) at a ~1km resolution, for three time periods: present day (averaged over 1950-2000), 2050 (averaged over 2041-2060), and 2070 (averaged over 2061-2080). Both the Community Climate System Model (CCSM4) and Model for Interdisciplinary Research on Climate Earth System Model (MIROC-ESM) General Circulation Models (GCMs) were downloaded for atmospheric variables, and

cell values were averaged over the two GCMs using the ‘overlay’ function of the *raster* R package (Hijmans et al., 2015). Additionally, two separate Representative Concentration Pathways (RCPs) were considered for the future variables, namely the intermediate emissions scenario RCP 4.5 and ‘worst case’ scenario RCP 8.5. Oceanic variables were obtained from Bio-Oracle2.0 (Assis et al., 2018), at a resolution of ~9km, for present day (averaged over 2000–2014), 2050 (averaged over 2040–2050), and 2100 (averaged over 2090–2100). The downloaded oceanic variables were already an ensemble of different GCMs (CCSM4, HadGEM2-ES and MIROC5; Assis et al., 2018). Marine variables also corresponded to RCPs 4.5 and 8.5. As both the atmospheric and oceanic variables showed little variation between the two RCP scenarios, averaging the cell values between RCP 4.5 and 8.5 (as above with GCMs) to obtain a single map per time-point in the future was conducted.

Since GF models cannot directly accommodate spatial variables, principal coordinates of neighbour matrices (PCNMs) were also included as predictors to account for spatial autocorrelation and unmeasured environmental variation. PCNMs were calculated with the *vegan* R package and the first half of the positive PCNMs were retained as inputs into the GF models (Manel et al., 2010; Sork et al., 2013). To account for collinearity between environmental predictor variables, conditional permutations within the GF models was implemented, following the protocol outlined by Strobl et al. (2008), using a correlation threshold (r) of 0.5. Default values were used for the number of predictor variables randomly sampled as candidates at each split, number of regression trees fit per allele, and the proportion training and testing samples per tree. Five hundred trees were run per dataset. Gradient Forest models were assessed by the overall goodness of fit (R^2) per allele, and the significance of each environmental variable was assessed by the relative importance weighted by R^2 . Only alleles with $R^2 > 0$ are included in the aggregate turnover function per environmental variable.

Turnover functions from the GF models were used to transform the environmental variables into a common biological importance scale, termed here as ‘genomic importance values’ (*sensu* Fitzpatrick & Keller, 2015). The genomic importance values were mapped in biological and geographic space in a manner similar to ordination, but accounting for non-linear and/or threshold changes that occur within the environmental gradients. Specifically, a Principal Components Analyses (PCA) was used to transform the environmental variation into three principal components (PCs). I followed Fitzpatrick and Keller (2015), by centering

but not scaling the transformed environmental variables, to retain the magnitude of the genomic importance among variables. The difference in allele frequencies between map cells was assigned to the first three PCs and partitioned into red/green/blue (RGB) colour palette, which were then mapped using the 'plotRGB' function of the *raster* R package. The resultant 'genomic turnover' maps indicate areas of genomic similarity by similar coloured map cells, and a change in allele frequencies by a change in colour. Individuals on either side of these turnover areas are presumed to have different demographic histories (if using neutral loci), or be under different selection pressures (if using outlier loci).

The GF turnover functions were also used to create 'genomic vulnerability' maps, which indicate areas where gene-environment relationships will be most disrupted under future climatic conditions (Fitzpatrick & Keller, 2015; Bay et al., 2018). These maps were created by first transforming the future environmental variables in a similar manner as described above for the current day variables, and then calculating the Euclidean distance between the current and future genomic importance values for each map cell. Areas in which the Euclidean distances are high indicate populations that will experience the greatest impact from future environmental shifts, due to their alleles being less likely to match the predicted climatic changes. These genomic vulnerability maps are limited by the simplified gene-environment interactions used to identify the adaptive optima of alleles, but are still a novel and valuable tool to characterise relative vulnerability, without accounting for migration or multi-gene interactions allowing alleles to track climatic changes.

As only the SNPs with an $R^2 > 0$ are included in the turnover functions and mapping analyses, the allele frequencies of the subset of SNPs retained in the GF models were visualised in PCAs. This was done to assess how well the SNPs in the GF models reflect patterns seen in the entire SNP datasets, which were previously used to assess neutral and outlier population structure (i.e. in Chapter One).

Species Distribution Modelling

Species distributions were projected into the future using correlative SDMs. These assume that environmental variables are the main determinant of species' distributions, and use correlations between these variables and contemporary species occurrence patterns to

predict species distributions into new environmental space (Guisan & Thuiller, 2005; Guisan & Zimmermann, 2000). Unlike GF models, SDMs cannot inherently account for collinearity between environmental predictor variables. Therefore, four environmental variables were used, which are not correlated (Spearman's $r < 0.6$; Variance Inflation Factor < 10), but expected to be important drivers of rocky shore ecological niches, and have been used previously to hindcast distributions of the study species (Chapter 2). Therefore, mean SST, mean SSS, maximum air temperature, and minimum air temperature were used as predictors in the SDMs (all based on monthly averages). These variables were downloaded from the same databases, for the same GCMs, RCPs, and time points as those used in the GF models. RCPs 4.5 and 8.5 were averaged into a single map per time point as in the GF models.

As the study species are highly abundant, and their ranges well documented (Branch, 2017), coastal habitat maps (from van der Bank et al., 2019) were used to create occurrence points at rocky shore habitats within the known distributions, spaced 10km apart (Fig. 3.S1.1, Appendix 3.S1). An equal number of pseudo-absences (to presences) were randomly selected over five replicate runs using the *biomod2* R package (Bermejo et al., 2018; Thuiller et al., 2013). As an ensemble of multiple model types has been shown to increase overall accuracy (Araújo & New, 2007; Forester et al., 2013), an ensemble of six models (Flexible Discriminant Analysis [FDA]; Generalized Additive Model [GAM]; Generalized Boosting Model [GBM], Generalized Linear Model [GLM]; Multivariate Adaptive Regression Splines [MARS]; Random Forest [RF]) was created in *biomod2*. All six models were used in the ensembles for *C. punctatus* and *S. granularis*, and all but FDA and GLM were used for *P. angulosus*, as these two models overestimated the distribution of this species in preliminary runs. Models were run with default parameters, with the exception of using 1,000 trees for GBM the 'mgcv' function for GAM. Data was randomly subset into 70% for calibration and 30% for validation, over ten evaluation runs and three permutations. Following best practice for marine SDMs (Bermejo et al., 2018; Bosch et al., 2018; Chefaoui et al., 2017) only models having Receiver Operating Characteristic (ROC; Fielding & Bell, 1997) greater than 0.8, and True Skill Statistic (TSS; Allouche et al., 2006) greater than 0.55 were retained in the ensemble per species.

To assess the extent to which the SDMs extend the relationships between current environmental conditions and species distributions into novel environmental conditions,

two-dimensional convex hulls of the environmental space were created at five time points into the past and future. Specifically, the environmental variation of the four predictor variables (mean SSS and SST, and minimum and maximum air temperature) were compared across the present day, the Mid-Holocene (6 thousand years ago; kya), Last Glacial Maximum (21 kya), 2050, and 2070/2100. Future environmental variables consisted of cell values averaged over the RCP 4.5 and 8.5 scenarios. The environmental values were extracted from 28 points, spaced 200km apart along the coastline, over the full extent of the SDMs (i.e. the extent used for *S. granularis*). Values were extracted for each time point, with the 28 points extrapolated to the -120m bathymetry line of the Last Glacial Maximum (Fig. 3.S1.2, Appendix 3.S1). Euclidean distances were calculated between time points, and two principal components from a PCA were used to maximize the amount of variation explained by the environmental variables. The global convex hull was mapped, including all five time points, onto which each time step's hull was mapped to compare the extent of environmental change across time periods (McWilliam et al., 2018).

Results

Gradient Forest modelling

The R² weighted importance of environmental variables was higher in the outlier, compared to neutral SNPs, for all species (Fig. 3.3). Turnover in outlier SNP frequencies of the crab, *C. punctatus*, was strongly influenced by mean SSS, while those of the urchin, *P. angulosus*, and the limpet, *S. granularis*, were most strongly influenced by mean SST (Fig. 3.3). The cumulative importance plots (representing the GF turnover functions) also suggest that mean SSS and SST are important in driving the turnover of outlier frequencies of all species (Fig. 3.S21-3, Appendix 3.S2). *Parechinus angulosus* and *S. granularis* show congruent cumulative importance curves, with similar values of precipitation, and mean SSS and SST, leading to changes in outlier frequencies (Fig. 3.S2.1-3, Appendix 3.S2).

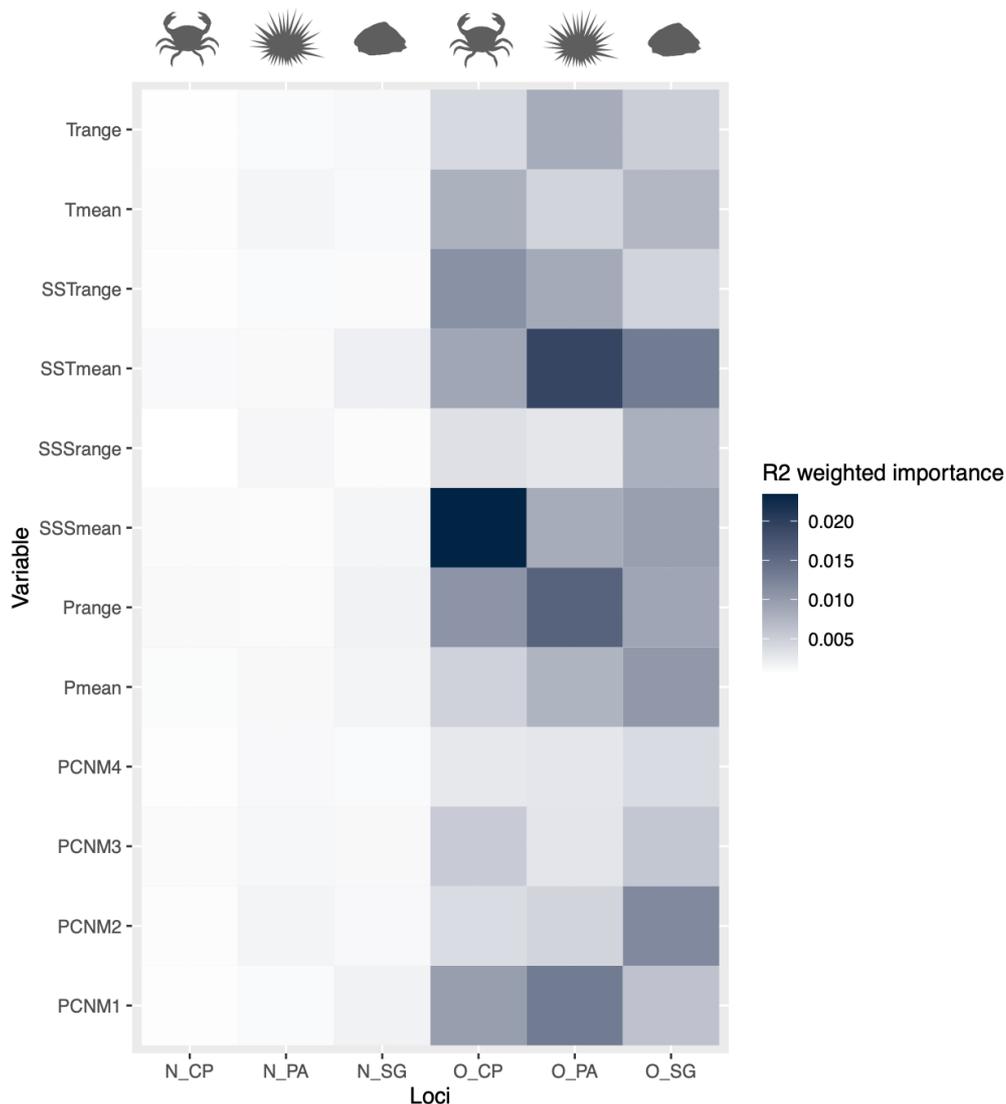


Figure 3.3 – The relative importance of each environmental predictor variable in describing the turnover in allele frequencies from the Gradient Forest models based on either neutral (N_*) or outlier (O_*) loci for *C. punctatus* (*_CP), *P. angulosus* (*_PA) and *S. granularis* (*_SG). Predictor variables are the means and ranges of air temperature (T), precipitation (P), sea surface salinity (SSS) and sea surface temperature (SST), and four principal coordinates of neighbour matrices (PCNMs). Darker shaded squares represent higher importance of predictor variables per genomic dataset.

The number of SNPs selected to create turnover functions differed between neutral and outlier SNPs per species, with 149, 112, and 340 neutral SNPs, and three, six, and 14 outlier SNPs having $R^2 > 0$ for *C. punctatus*, *P. angulosus*, and *S. granularis*, respectively (Table 3.S2.1, Appendix 3.S2). These subsets of loci generally reflect the genomic structuring seen in the entire SNP datasets, with the putative outlier loci showing more distinct eastern vs. western clustering than the neutral loci (Chapter One; Fig. 3.S2.4, Appendix 3.S2). The

patterns of genomic turnover, where map cells are coloured similarly if they portray similar allele frequencies, differed between the neutral and outlier loci, as well as across the three species. The neutral loci display higher genomic turnover along the eastern coast, while putative outlier loci show higher turnover along the southwest coast (Fig. 3.4). The differences between genomic turnover between the outlier and neutral loci, calculated as Procrustes residuals, show distinct areas of high differentiation in marker types between species (Fig. 3.S2.5, Appendix 3.S2). The highest discrepancies between neutral and outlier turnover are seen along the north-eastern coast for *C. punctatus* and the south coast for *P. angulosus* and *S. granularis* (Fig. 3.S2.5, Appendix 3.S2). The genomic turnover patterns within the putative outlier loci generally follow the biogeographic breaks of the marine environment in the region (Fig. 3.2, Fig. 3.4).

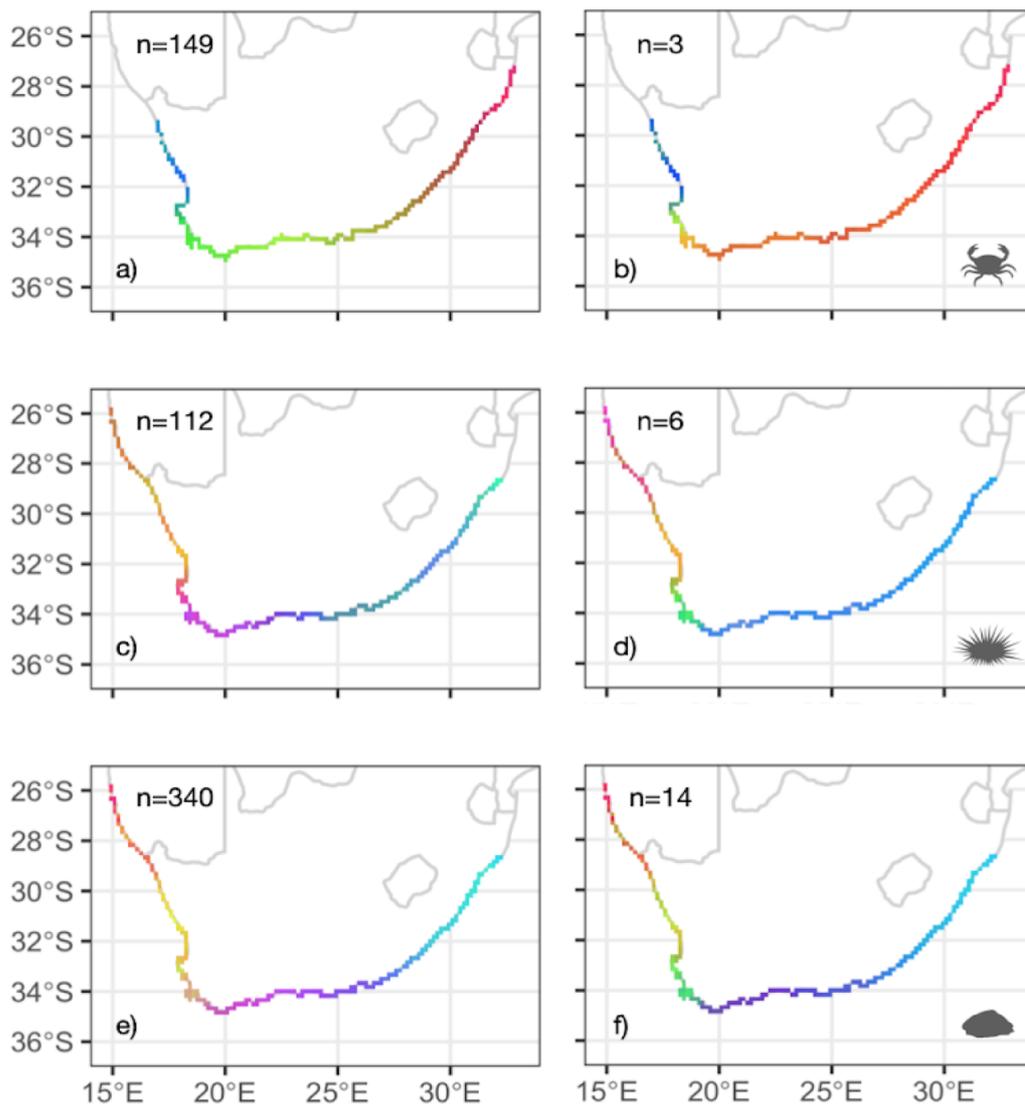


Figure 3.4 – The composition of genomic turnover shown in geographic space, in which the first three PCs of the Gradient Forest transformation of allele frequencies are partitioned into the red, green, and blue colour palette and each palette is overlaid, creating the colours seen on the maps. The colours are arbitrary, but similar coloured map cells indicate similar allele frequencies in either putatively neutral (a, c, e) or outlier (b, d, f) loci for *C. punctatus* (a, b), *P. angulosus* (c, d), and *S. granularis* (e, f). Only alleles with a $R^2 > 0$ were included in the Gradient Forest transformations (number in the top left corner).

When putative adaptive genomic composition is projected into the predicted environmental space of 2050, and 2070/2100, the results highlight areas of increased genomic vulnerability where outlier allele frequencies will have to respond more rapidly to track future environmental changes (Fig. 3.5). Higher genomic vulnerability is shown predominantly along the eastern South African coastline for *C. punctatus* (Fig. 3.5). In contrast, individuals within the western sites display higher genomic vulnerability for the

other two species (Fig. 3.5). These patterns are generally mirrored by the putatively neutral loci, with slight differences such as *P. angulosus* and *C. punctatus* along the western coast predicted to be less vulnerable to future climatic conditions (Fig. 3.S2.6, Appendix 3.S2).

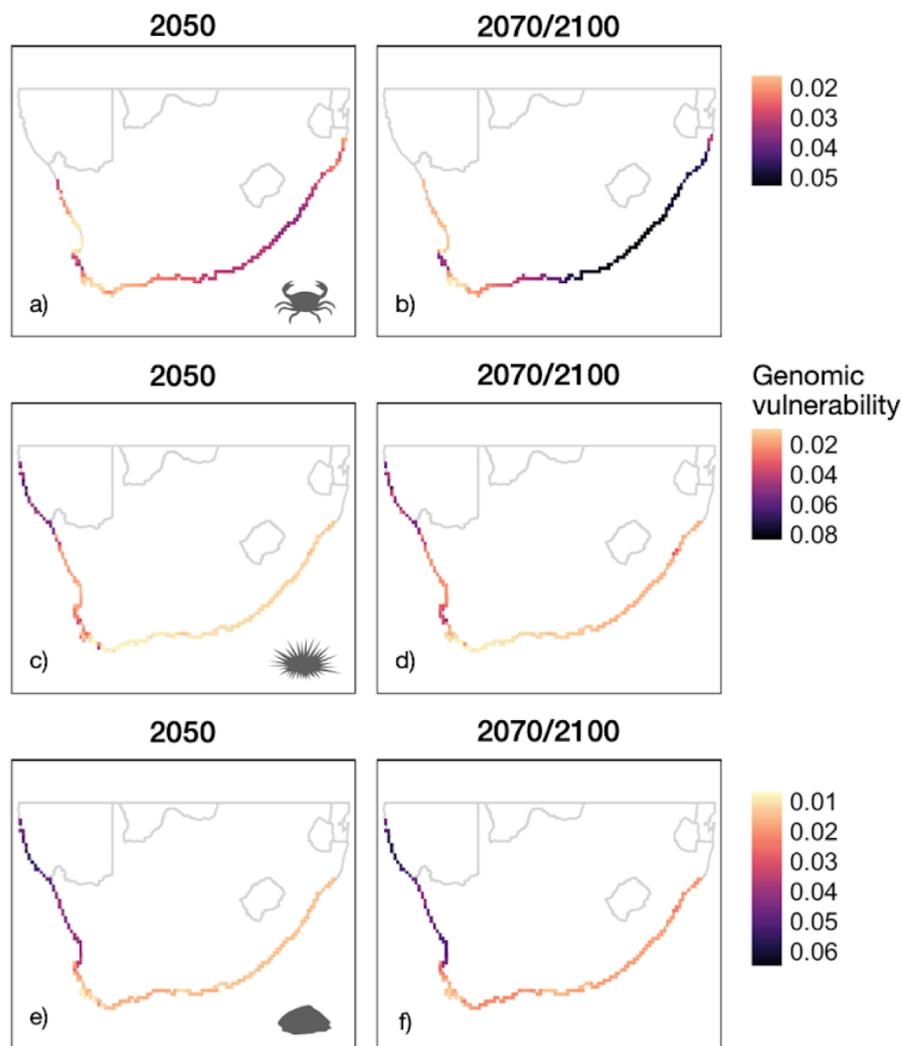


Figure 3.5 – Spatial patterns of outlier genomic vulnerability, calculated as Euclidean distance between current and future genetic spaces, averaged across RCPs 4.5 and 8.5, for 2050 (a, c, e), and 2070/2100 (b, d, f; 2070 for landscape features, 2100 for seascape features), for *C. punctatus* (a, b), *P. angulosus* (c, d), and *S. granularis* (e, f). Areas with darker coloration indicate areas of high vulnerability, where genomic composition will have to change the most to track environmental change.

Species Distribution Modelling

Individual models used in the ensemble SDMs per species had high ROC and TSS scores (Table 3.S1.1, Appendix 3.S1), and were used to assess future distributional shifts per

species. Similar to the GF models, mean SSS was the most important predictor of *C. punctatus*, and mean SST was the most important predictor of *P. angulosus* and *S. granularis* distributions in the SDMs (Fig. 3.S1.3, Appendix 3.S1). The species distribution models show distinct distributional changes both between and within species at each time step (Fig. 3.6). At the 2050 projection, *C. punctatus* shows an increase in habitat suitability along the western coast, *P. angulosus* shows a decrease in suitability along the east and western portions of the coastline, and *S. granularis* shows a slight reduction in suitability along its range edges (Fig. 3.6). These changes are even more pronounced in the 2070/2100 projections, with *C. punctatus* further increasing, and *S. granularis* and *P. angulosus* further decreasing their ranges (Fig. 3.6).

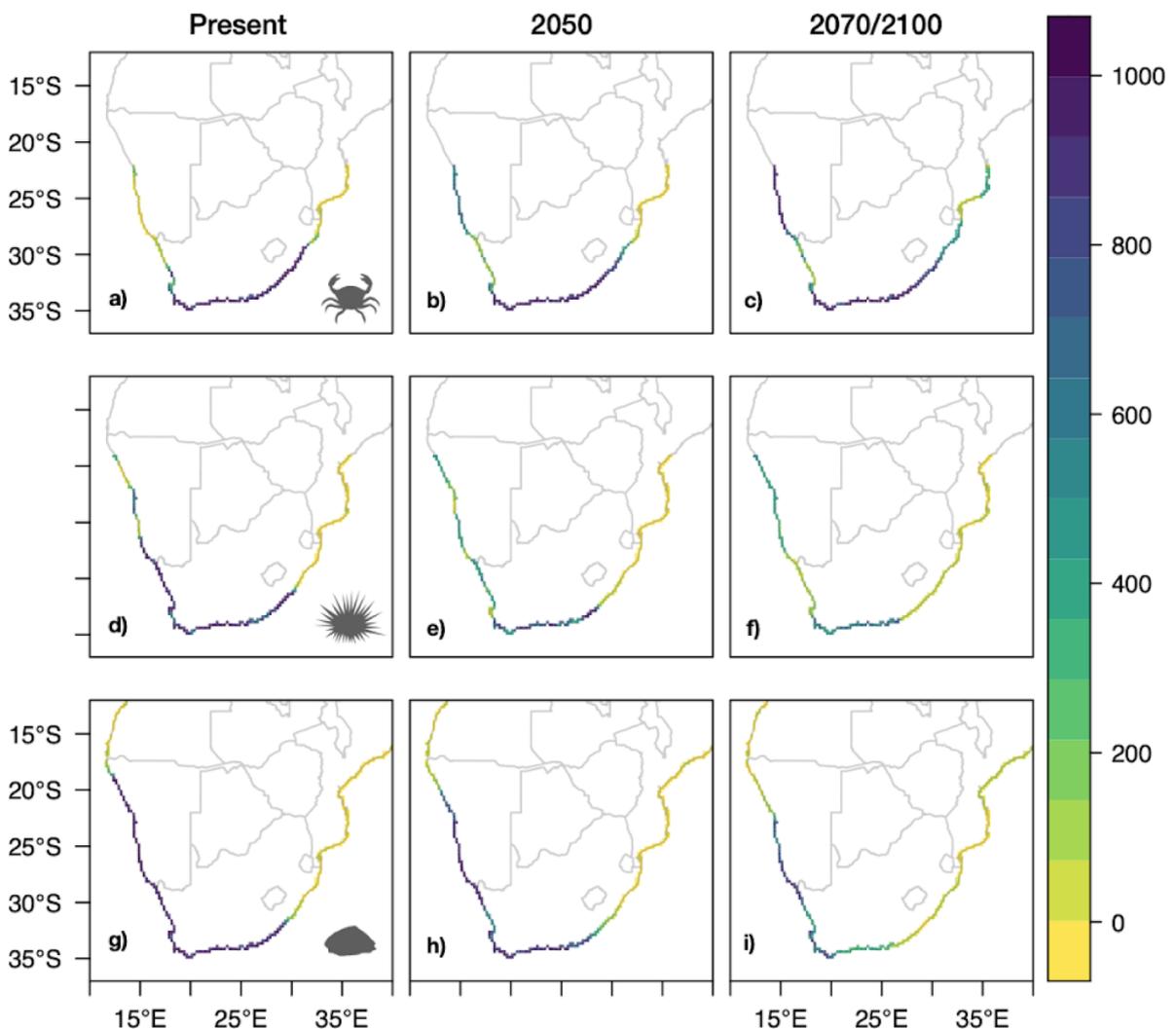


Figure 3.6 – Habitat suitability (ranging from zero, where species will likely be absent, to 1,000, where species are most likely to be present) is shown for present day (a, d, g), 2050 (b, e, h), and a

combination of 2070 for the two terrestrial variables and 2100 for the two seascape variables (c, f, i), for *C. punctatus* (a-c), *P. angulosus* (d-f), and *S. granularis* (g-i). Darker shaded regions represent higher habitat suitability.

The convex hulls of the environmental space show that going further into the past and/or future leads to greater deviations from the reference environmental space used in the SDMs (Fig. 3.7). Therefore, as expected, the models in 2070/2100 are less certain than those for 2050. However, overall the model environmental space does not substantially change from the current environmental space, validating their use.

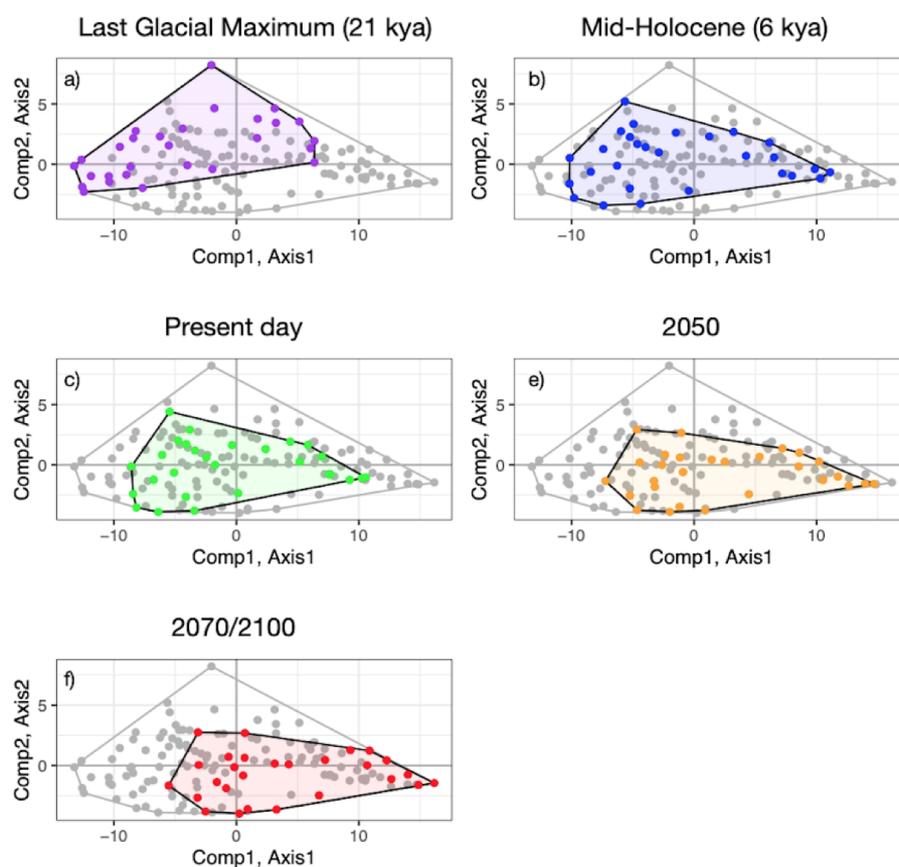


Figure 3.7 – Environmental variation within the species distribution models, shown in two-dimensional space, with the global environmental space across all five time points shown in grey, and the environmental space pertaining to each time period overlaid in colour. The Present day (c), as well as two past time points: Last Glacial Maximum (a), Mid-Holocene (b); and two future time points: 2050 (e), 2070/2100 (f; 2070 for terrestrial layers, and 2100 for oceanic layers) are shown.

Discussion

This study provides novel insights into assessing the vulnerability of species to global

change, in which vulnerability was shown to vary both within and between species. Species-specific range shifts were shown, yet the modelled predictions did not capture the high genomic vulnerability that some populations will experience within these species level changes. Thus, SDMs alone may misrepresent the vulnerability of a species to climate change by assuming all populations have similar adaptive capacity to novel environments. This chapter shows that inferences of intraspecific vulnerability by SDMs can be further supported by genomic approaches, which can offer snapshots of selection patterns across populations. However, the SDM and GF models identified the same principal environmental drivers of spatial variation for the study species, specifically salinity for the crab, and sea-surface temperature for the urchin and limpet. Additionally, while the genomic turnover patterns varied among species, and between putative neutral and adaptive markers, they broadly correlated with known breaks between bioregions (Fig. 3.2). Such breaks may be useful proxies for intraspecific evolutionary distinctness (Teske et al., 2011), but further work is needed to understand how shifts in biogeographic breaks due to global change will influence species and population dynamics.

As promoting adaptation to, and mitigating the effects of, climate change is increasingly being included into marine conservation objectives (Rilov et al., 2020; Wilson et al., 2020), it is important to understand how vulnerability differs between metrics pertaining to species and populations. This study demonstrates how assessments of vulnerability differ between ecological processes shaping species distributions and evolutionary processes shaping population dynamics. The results indicate that resilience hotspots may vary depending on the vulnerability metric and/or species assessed, and provide a basis for future investigations into the complex ecological-genomic interactions within marine environments.

Patterns of genomic composition between markers and species

Broadly, differences in vulnerability were found both among three co-distributed coastal species, as well as between putatively neutral and outlier markers within each species (Fig. 3.5, 3.6). This is expected, as vulnerability to climate change has been shown to be highly species-specific (Román-Palacios & Wiens, 2020; Sunday et al., 2015). In addition, statistical

outlier loci often show distinct patterns of genomic variation compared to putative neutral loci datasets (Grummer et al., 2019; Phair et al., 2019). Yet, despite the species differing in ecology and life history, all species display genomic turnover (shown by the changes of colour in Fig. 3.4) that relates to the biogeographic breaks in the region (Fig. 3.2). These findings suggest that the environmental features shaping biogeography may extend to the molecular level of biodiversity patterns. For example, Stanley et al. (2018) found that genomic patterns corresponded to bioregion delineations for five marine species with varying distributions and life histories in the northwest Atlantic. However, the findings here provide a deeper level of insight by delineating turnover patterns between putatively neutral and outlier loci, in which it was found that both data types roughly follow biogeographic breaks. Broadly, the findings demonstrate how environmental gradients, and the evolutionary processes they enact on species, can lead to intraspecific genomic clusters which have distinct evolutionary trajectories compared to the species as a whole (Prates et al., 2018; Razgour et al., 2018). Identifying these genomic clusters can be especially powerful when combined across taxa, which can indicate biogeographically significant units for conservation (Quiroga et al., 2019).

Environmental drivers of species and genomic level composition

Although the results show similar genomic turnover patterns across species, the environmental variables driving these patterns are species-specific. This work suggests that the principal environmental variables in genomic turnover functions differ between putatively neutral and adaptive loci. Similar to Chapters One and Two, both the GF and SDM analyses highlight the importance of SSS for the crab, and SST for the limpet and urchin. Sea-surface temperature is a prevailing determinant of marine invertebrate distributions globally (Bosch et al., 2018), which is reflected here in the limpet and urchin distributions. In contrast, the distribution of the crab is best explained by salinity, which is unexpected as this species also inhabits estuarine environments, and has a wide salinity tolerance (Boltt & Heeg, 1975). However, the importance of salinity in the crab's distribution could be driven by larval and juvenile life history stages, as salinity is a key parameter in larval development of decapod crustaceans (Anger, 2003). This notion is further supported by salinity proving to

have a higher effect on the osmoregulatory abilities of juvenile *C. punctatus* individuals (Winch & Hodgson, 2007). Furthermore, the physiology of adult decapods, such as ingestion and respiration rates being higher at low salinities (Falconer et al., 2020; Urbina et al., 2010).

Overall, the two marine variables, SST and SSS, were stronger predictors than mean and maximum air temperature. Although all three of the study species live at the land-sea interface, many important biological processes (such as feeding, fertilization, and larval development) occur when the species are submerged (Branch, 1979; Byrne, 2011; Newell et al., 1971), thus leading to the importance of marine over terrestrial predictor variables as a whole. Consequently, mean or maximum sea temperature have been shown to be better predictors of mortality events in the intertidal organisms than maximum air temperature (Jones et al., 2012; Rivadeneira & Fernández, 2005).

While SST appears to be an important predictor for the genomic turnover functions of the urchin and limpet (Fig. 3.3.; Fig. 3.S2.2-3, Appendix 3.S2), the resultant genomic vulnerability patterns differ between the two species, with the limpet having higher genomic vulnerability across southern Africa, in both neutral and outlier SNPs (Fig. 3.5; Fig. 3.S2.6, Appendix 3.S2). Similarly, although the SDMs highlighted SST as the most important predictor of the limpet and urchin distributions (Fig. 3.S1.3, Appendix 3.S1), the predicted habitat suitability of the two species differs into the future (Fig. 3.6). Similar discrepancies in forecasted distributions were found between two co-distributed *Anolis* lizard species, which the authors attribute to species-specific ecological and demographic constraints (Prates et al., 2016). Therefore, even though temperature has been shown to be a principal driver of biological patterns in marine systems (Bosch et al., 2018), individual species responses to fluctuations in temperature are likely to differ based on their ecology and demographic histories (Chapter One; Sunday et al., 2015).

While species- or gene-environment association analyses are vital tools to predict species' responses to forecasted climatic change, they solely encapsulate correlations with the environment, disregarding integral biological processes and interactions. The inclusion of the latter may thus lead to further interspecific differences in vulnerability, particularly with the emergence of novel communities in response to climatic change (Catullo et al., 2015). Further work, such as mechanistic and joint species distribution models, physiological measurements, and adaptive and demographic models are essential to fully understand

each species' responses to global change (Bush et al., 2016; Rilov et al., 2019). As a whole, the results stress the importance of comparative species distribution and seascape genomic analyses, which are an essential step towards elucidating ecosystem level resilience to global change.

Species and genomic level forecasts identify distinct vulnerability hotspots

The SDM and GF models offer distinct insights into areas where the study species will be at increased risk to future climatic change. The limpet, and especially the urchin, show range reductions, with only the western and southern coastline projected being habitable into the future. This region is characterised by the Benguela upwelling bioregion, which is experiencing a cooling trend due to increased upwelling, compared to the Agulhas current on the eastern coast, which is predicted to follow global warming trends (Rouault et al., 2010). The Benguela upwelling system may thus act as an important climatic refuge for other marine species which are predominantly within cool-temperate regions of southern Africa (Greenstein & Pandolfi, 2008; Riegl & Piller, 2003). In contrast, for the crab, the SDMs predict that exposure to unfavourable environmental conditions will be far less pronounced than for the two study species, which have distributions extending further into cool-temperate waters. Specifically, it is forecasted that *C. punctatus* will expand its range both up the western and eastern southern African coast, as these marine environments become warmer and more saline (Fig. 3.6). However, the GCMs used here predict ocean warming along the entire South African coastline, due to the warm bias of coupled GCM simulations along the west coast of Africa (Weber et al., 2018), and thus do not account for the local cooling trend and increased upwelling within the Benguela current on the west coast (Rouault et al., 2010). Unfortunately, even less is known about predicted salinity changes, and its potential biases in GCMs within southern Africa, meaning that our understanding of how the crab and its distribution, which is suggested to be strongly influenced by salinity, may shift in the future is limited. While this caveat limits the inferences from this chapter, GCMs such as those used here are currently the best possible available climatic forecasting data, and their use to explain biodiversity patterns within the region is a valid starting point to build future models on (Yemane et al., 2020).

Additionally, the SDMs assume that there is available habitat within the entire region, that species cannot respond by adaptation and plasticity, and that environmental changes act on adult and larval stages equally, all leading to uncertainty in their predictions (Reusch, 2014). Furthermore, even though the SDMs identify the crab as a climate change ‘winner’, due to its predominantly warm-temperate, rather than cool-temperate range, it is argued that warm-adapted coastal species are actually the most at risk to climate change due to their upper thermal limits being closer to their thermal optima (Somero, 2002). It should also be noted that predicted species distributional changes do not account for behavioural mechanisms, such as actively selecting microhabitats, to remain within their thermal optima (Chappon et al., 2017; Seabra et al., 2011). While the results show clear species differences in responses to climatic change, further analyses are ideally needed to assess the uncertainties in the SDMs, such as incorporating physiological tolerance (Franco et al., 2018), habitat condition (Hattab et al., 2014), and species interactions (Fulton, 2011) into the models.

In addition to the SDMs, areas of high genomic vulnerability were also identified, in which populations will likely have to drastically adapt to track future environmental changes (Fitzpatrick & Keller, 2015). As expected, the results show a mismatch between species and population level sensitivity to future environmental change. For the crab, populations inhabiting the eastern South African coastline are suggested to be highly sensitive to climatic changes based on their genomic vulnerability, yet this area is predicted to be highly stable based on the SDM (Fig. 3.5, 3.6). Further, both the urchin and limpet individuals existing within the Benguela region within western southern Africa are expected to be highly vulnerable to future environmental change, yet this region is also predicted to remain more habitable than the eastern coastline within the species’ SDMs (Fig. 3.5, 3.6). Even though there are only a few outlier loci from which the genomic vulnerability measures are derived, similar patterns are shown by the larger subsets of putatively neutral loci (Fig. 3.S2.6, Appendix 3.S2), indicating that putatively neutral and adaptive loci may have similar gene-environment relationships, but to different extents. For example, Martins et al. (2018) also found similar spatial patterns of genomic vulnerability between all loci or solely environmental-associated loci (in the oak species *Quercus rugosa*), yet the latter of the two datasets displayed larger offset values. The findings support the concept that local adaptation along environmental clines is not only shaped by selection, but also by neutral

processes such as demographic history, contemporary gene flow, and standing genomic variation (Cayuela et al., 2020; Nadeau et al., 2016).

Overall, the results suggest that climatic exposure (i.e. the extent of environmental change experienced by a species) may not directly relate to climatic sensitivity and adaptive potential (i.e. how well equipped species are to respond to environmental change; Dawson et al. 2011). For example, even though limpet and urchin individuals within western portion of the southern African coastline may remain within each species' known environmental space, many of the cold adapted individuals in these populations may not be genetically equipped to adapt to warming temperatures (if warming does indeed happen here). Alternatively, the crabs inhabiting eastern South Africa may not be able withstand temperature or salinity changes due to genomic, rather than ecological, constraints. Changes to these gene-environment associations may lead to changes in species abundances, consequently altering community composition, further impacting species distributions (Harley et al., 2006). However, further testing is needed, such as with whole genome sequencing and/or common garden experiments, to validate the assumption that the correlations between allele frequencies and environmental variables reflect patterns of local adaptation and fitness (Fitzpatrick et al., 2018). In addition, measures of genomic vulnerability cannot account for shifts in allele frequencies due to selection, genetic drift, or gene flow, and therefore may overestimate (Exposito-Alonso et al., 2018) or underestimate (Crisci et al., 2017) vulnerability. It should also be noted that the study species are expected to have relatively high adaptive capacity, as they have large effective population sizes, overlapping generations, and dispersive reproductive modes, and thus spatial vulnerability patterns should also be assessed in species with traits making them sensitive to change (Bennett et al., 2019; Catullo et al., 2015).

Potential implications for conservation

Climatic refuges identified from SDMs often inform conservation plans to promote species resilience (Morelli et al., 2016; Wilson et al., 2020). It is show here that genomic and other population level measures of vulnerability are also crucial to predict species' responses to climate change. Due to the differences in environmental space restricting species and

genomic composition found here, and presumed differences in how species and genes will respond to environmental change, prioritising areas over an array of climatic velocities may be the best way to ensure species persistence. This ‘portfolio approach’ of prioritising a range of current and future suitable habitats and environmental conditions is key to ensure that adapted populations can actively or passively enhance the gene pools of those populations less likely to track future environmental change (Beyer et al., 2018; Matz et al., 2020). While further work is needed to fully describe the adaptive capacity of coastal marine systems in this study region and elsewhere (Munday et al., 2013), this study is an essential step in understanding marine species’ sensitivity to global change across biological scales, and offers a unique framework to further understand species resilience to changes in biological-environmental interactions.

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Chapter Four

Conserving evolutionary resilience within coastal South Africa

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Introduction

The increase of human activities in the marine space is leading to considerable degradation of oceanic and coastal ecosystems (Halpern et al., 2015; McCauley et al., 2015; Poloczanska et al., 2013). To counteract the negative impacts of anthropogenic extraction and alteration within marine environments, establishing and effectively managing Marine Protected Areas (MPAs) is a global priority (CBD, 2010; UN, 2016). Broadly, the aim of marine conservation planning is to protect the most important areas for the representation and persistence of biodiversity (Margules & Pressey, 2000), while maximizing returns on conservation investments via ecosystem services and human well-being (Brooks et al., 2006). It is recognized that for MPAs to function optimally, they need to capture not only biological, but also social and economic features (Ban et al., 2013; Cinner et al., 2012). However, as anthropogenic climate change is altering biological and socio-economic factors at an unprecedented rate, conservation planning must also adapt and identify areas of importance based on future projections of social-ecological systems (Griggs et al., 2013; Harley et al., 2006; Peterson et al., 2019). Anticipating, and proactively managing for, climate change resilience in marine conservation is difficult, as it must incorporate multiple facets of vulnerability across biological levels of organization such as genes, species, and ecosystems (Coates et al., 2018; Moritz & Agudo, 2013; Reside et al., 2018).

Recent reviews show that, globally, MPAs with practical climate change considerations are sparse (Tittensor et al., 2019), and are predominantly restricted to tropical coral reef systems (Wilson et al., 2020). One of the main barriers to including adaptation to climate change into MPA design/management is the uncertainty associated with the vulnerability of biodiversity to global change (Wilson et al., 2020). Intraspecific adaptive capacity is an essential component to consider when designing MPAs to withstand global change, and is arguably best captured by molecular data (Flanagan et al., 2018; Hoffmann et al., 2015; Rilov et al., 2019). However, there is a missing link between genomic research, and genomic data in conservation plans (Bernos et al., 2020; Sandström et al., 2019; Shafer et al., 2015). In addition, recent calls have been made for molecular data to be better incorporated to conservation frameworks such as the IUCN red list and the

Convention on Biological Diversity (CBD; Garner et al., 2020; Hoban et al., 2020; Laikre et al., 2020).

Conservation prioritisation methods based on genetic information, such as selecting evolutionary significant units (ESUs) across several species, or areas with high phylogenetic diversity as conservation priorities, have become an integral sector of conservation planning research (Fraser & Bernatchez, 2001; Funk et al., 2012; Moritz, 1994; von der Heyden, 2017). A multitude of population genetic studies have described conservation implications based on various levels of genetic differentiation, as well as from various marker types (Cadotte & Davies, 2010; Hanson et al., 2020a; Laity et al., 2015; Nielsen et al., 2017; Paz-Viñas et al., 2018). Most studies to date have derived conservation relevance from a few neutral loci. However, neutral loci for the most part do not capture adaptive evolutionary processes, which are important in the context of environmental change (Fraser & Bernatchez, 2001; Hanson et al., 2020a; Mittell et al., 2015; Moritz, 1994; Schlötterer, 2000, 2004). As next generation sequencing technologies are becoming more available with decreases in costs, researchers are now better able to identify genome-wide patterns of variation, as well as putatively adaptive ‘outlier’ loci in non-model species (Allendorf et al., 2010; Faure & Joly, 2015; Seeb et al., 2011). Genomic data has increased power in identifying population structure, gene-environment associations, and demographic histories (Garner et al., 2020; Hoban et al., 2020; Laikre et al., 2020).

Although limited in scope, previous empirical comparisons display little overlap in conservation solutions from different genetic and genomic metrics. For example, measures of diversity or differentiation based on genetic nuclear microsatellite or mtDNA markers highlight different conservation priority areas, shown both within a single clam species (Beger et al., 2014), or across five marine invertebrates (Nielsen et al., 2017). Moreover, Xuereb et al. (2020) found that putatively neutral and outlier loci from RAD-seq data from a sea cucumber resulted in highly differentiated conservation solutions. In contrast, Nielsen et al. (2020) found that neutral genetic data (mtDNA *COI* gene regions) from five rocky shore species inadvertently captured putative genomic adaptive patterns (from RAD-seq SNPs) from two species, indicating that neutral data may be an alternative to adaptive markers when combined over multiple species.

Other work suggests the utility of ‘molecular surrogates’, where other types of data can provide the same information as molecular markers, such as environmental gradients

adequately capturing genetic variation in alpine tree species (Hanson et al., 2017), or species distribution patterns capturing genetic clusters in Australian freshwater fish species (Hermoso et al., 2016). Even though environmental heterogeneity has been theoretically proposed (Carvalho et al., 2011a), and empirically shown (Hanson et al., 2017), to potentially be a viable or simple surrogate to capture differentially adapted populations, environmental stability has been shown to be an inadequate substitute for genetic diversity (Hanson et al., 2020b). However, these studies have tested the correlation between environmental gradients or stability with molecular patterns derived from genetic markers, which are known to lead to less robust measures of variation and diversity than genome-wide SNP markers (Camacho-Sanchez et al., 2020). Therefore, there is currently a knowledge gap relating to how environmental and genomic features influence conservation solutions aimed at ensuring species persistence under global change. Understanding how genomic data relates to past and future climatic changes will hopefully illuminate some of the many unknowns regarding how different molecular data, as well as non-molecular data, alters spatial conservation plans (Hanson et al., 2017, 2020b; Nielsen et al., 2017, 2020; Paz-Viñas et al., 2018).

In order to explore how different molecular and environmental data types can be utilized to capture evolutionary resilience in conservation plans, a series of spatial conservation scenarios were run within the South African coastal environment in this chapter. South Africa has embraced and supported systematic conservation planning and marine spatial planning (MSP) to design 20 new MPAs in recent years (Balmford, 2003; van der Bank et al., 2019). Additionally, South Africa has recently identified Ecologically or Biologically Significant Areas (EBSAs) in its marine space as part of reaching the Aichi Target 11 of the CBD, which aims to have 10% of the world's oceans under protection within an ecologically representative and well connected marine reserve network (CBD, 2010; Kirkman et al., 2019). However, important challenges remain. Lombard et al. (2019) highlighted six key challenges with regards to ocean planning in South Africa, one of which being the limited understanding of how climate change will alter local ecosystems. In their work, the authors argue that models to predict the sensitivity of species to climate change are required along with adaptive management strategies.

The main goal of this chapter is to assess how areas identified as of evolutionary importance differ between genomic and climatic evolutionary metrics, for the first time in

the marine environment. This is explored within the unique environmental, biological, and socio-economic backdrop of South Africa's coastline. In this context, this chapter aims to address this knowledge gap in South Africa, by developing a novel framework on how to integrate resilience into marine conservation planning, using ecological, environmental, and genomic models from three South African marine species: Cape urchin (*Parechinus angulosus*), Granular limpet (*Scutellastra granularis*), and Common shore crab (*Cyclograpsus punctatus*). These species are ecologically important, both as dominant grazers and/or providing habitat for other species, making their persistence within South Africa vital to sustain local ecosystem stability (Branch & Branch, 2018). Specifically, conservation scenarios including spatial data from Chapters One to Three will be compared (i.e. climatic stability, genomic diversity, outlier abundance, genomic vulnerability), and combined for all three species to identify regions signalling evolutionary importance and potential conservation priority for rocky shore invertebrates. The goal of this chapter is thus to identify potential gaps in South Africa's current MPA network that can be prioritised as 'evolutionary hotspots', in order to better protect multispecies persistence through climatic changes. Based on the findings of Chapters One to Three, I expect that conservation solutions will differ between putatively neutral and adaptive genomic scenarios, and that of the three species, solutions from the limpet and the urchin will be the most similar. Understanding and mapping these links between genomic variation, species distributions, and climatic change is critical if we are to actively conserve ecological systems and protect local communities from both the short and long-term impacts of global climate change (Bourne et al., 2016).

Materials and Methods

Resilience features

Four resilience features were included as spatial priorities in conservation planning scenarios, namely: climatic stability, genome-wide neutral diversity, outlier abundance, and genomic vulnerability (see Table 4.1 for a background). These features were obtained per species (i.e. crab, limpet, and urchin) from Chapters One to Three. To create the climatic

stability layer, SDM outputs from Chapters Two and Three were turned into binary species presence/absence maps. This analysis was performed using a minimum training presence cut-off script adapted from: <https://babichmorrowc.github.io/post/2019-04-12-sdm-threshold/>. If the species was present at each time point, from the Last Glacial Maximum, 21 thousand years ago (kya), to what is predicted for the year 2100 in the future, then it was included in the final binary map portraying climatic stability per species. As the LGM map cells reflect the -120m bathymetry coastline, values were interpolated onto the present day 0m bathymetry line, using thin plate spline interpolation with the ‘tps’ function of the *fields* R package (Nychka et al., 2017).

Genome-wide diversity was estimated as expected heterozygosity (H_E) per site, as obtained in Chapter Two. To obtain outlier abundance per species, per site, allele frequencies of the outlier loci identified in Chapter One were summed per sample site within each species. Following standard protocol for the interpolation of molecular data (Hanson et al., 2020a; Nielsen et al., 2020; Xuereb et al., 2020), genomic diversity and outlier abundance point data was interpolated across the planning region using inverse distance weighting. The measure of genomic vulnerability used here reflects the outlier loci-derived genomic vulnerability obtained from the Gradient Forest models in Chapter Three. Each of the three molecular resilience features were categorised into low, medium, and high ranking groups, pertaining to natural breaks within each variable. Areas falling within the high-ranking groups of outlier abundance and genomic diversity, and falling within the low-ranking group of genomic vulnerability, were included as binary priority maps per species. Thus, conservation planning scenarios had the overall objective of conserving areas of high climatic stability, genomic diversity, and outlier abundance, and low genomic vulnerability (Table 4.1). All resilience features were converted into raster files using the *raster* R package (Hijmans et al., 2015).

Table 4.1 – The four resilience features included in conservation planning scenarios, with data origin and conservation importance shown per feature.

Resilience Feature	Data Origin	Conservation Importance
Climatic stability	Hindcasted & forecasted species distributions from Chapters Two and Three	Areas of past climatic stability (i.e. historical refugia) are likely to have allowed species to persist, diversify, and expand after environmental conditions improved. Historical refugia are associated with higher

		<p>levels of genetic diversity and thus may contribute to increased resilience to future change</p> <p>Areas of future climatic stability can act as refuges where species can remain in their thermal niches</p> <p>Areas of past and future climatic stability are potential hotspots of low species sensitivity and climatic velocity</p>
Genomic diversity	Complete set of neutral SNPs from Chapter One	Populations with high neutral diversity can be assumed to have large effective population sizes, more raw genomic material for selection to act on, and be connected to other populations via gene flow, all of which should increase adaptive capacity
Outlier abundance	Complete set of outlier SNPs from Chapter One	High abundance of outlier SNPs infers local selection pressures from salinity and temperature, and it can be assumed that since these areas have higher frequencies of SNPs associated with these environmental variables, that these areas are better equipped to respond to future selection by these environmental pressures
Genomic vulnerability	Subset of outlier SNPs from Gradient Forest models in Chapter Three	Low genomic vulnerability suggests that the population is better equipped to respond to predicted environmental changes, based on contemporary gene-environment relationships being less altered by climate change

Conservation planning scenarios

Conservation scenarios were run using the decision support tool Zonation v4 (Moilanen et al., 2005, 2014). Zonation iteratively discards the least prioritised map cells, based on the provided input features, in a way that minimises the overall loss of conservation value across the planning domain. This ‘loss of conservation’ value, aggregated across features occurring within a map cell, depends on the ‘cell-removal rule’ (Moilanen et al., 2014). Here the Additive Benefit Function (ABF) removal rule was used, which removes cells so that the summed conservation loss across all biodiversity features declines the least per removal (i.e. tries to maximize species/feature richness in the reserve design; Moilanen

et al., 2005, 2011). All four resilience features per species were given equal weights within all Zonation scenarios, and all other Zonation parameters were set on default. In total, eight scenarios were run: all resilience features per each species individually, all species per each resilience feature individually, and all features for all species (Table 4.2). Scenarios were compared by their output rank maps, in which each map cell is valued from zero (lowest conservation priority) to one (highest conservation priority). The similarities between output maps were quantified by running Pearson's r correlations between raster cell values (Rogers & Nicewander, 1988). Additionally, the output map from the scenario including all features for all species (*ALL* scenario), was compared to habitat protection and threat status from the 2018 National Biodiversity Assessment (NBA; van der Bank et al., 2019). Here, rocky and mixed shore habitats classified in the NBA as being poorly protected, or being endangered (i.e. 'EN' Red List Ecosystem category), were overlaid on top of the *ALL* scenario priority rank map.

Table 4.2 – The eight scenarios run within this chapter, listing relevant features and species within each.

Scenario	Features included	Species included
<i>Climatic stability</i>	Climatic stability from hindcasting & forecasting SDMs	all
<i>Genomic diversity</i>	Genomic diversity (H_E) from neutral SNPs	all
<i>Outlier abundance</i>	Outlier abundance from all outliers identified by at least two outlier detection models	all
<i>Genomic vulnerability</i>	Genomic vulnerability identified by subset of outlier SNPs in Gradient Forest models	al
<i>C. punctatus</i>	all	<i>C. punctatus</i> (crab)
<i>P. angulosus</i>	all	<i>P. angulosus</i> (urchin)
<i>S. granularis</i>	all	<i>S. granularis</i> (limpet)
<i>ALL</i>	all	all

Results

In this chapter, I created conservation scenarios capturing different aspects of evolutionary importance (Tables 4.1, 4.2), which when combined into the *ALL* scenario, highlight regions of multispecies evolutionary resilience over multiple timeframes. The output rank map of the *ALL* scenario identified high priority conservation areas along most of the coastline, with the exception of the northeast Delagoa bioregion (Fig. 4.1a). Specifically, high priority areas include Coffee Bay on the southeast coast, Knysna to Port Elizabeth and Cape Point to Cape Agulhas on the south coast, Jacobs Bay to the Lamberts Bay on the west coast (Fig. 4.1a). Rocky shore areas of evolutionary significance that are also poorly protected are predominantly on the west coast, but also include Cape St. Francis and Port Elizabeth on the southeast coast (Fig. 4.1b). In contrast, most of the resilience hotspots that fall within endangered rocky shore habitat are along the southwest coast (Fig. 4.1c). Areas that are highly endangered, poorly protected, and have high evolutionary resilience, include the areas around Lamberts Bay, Cape Point, and Port Elizabeth (Fig. 4.1)

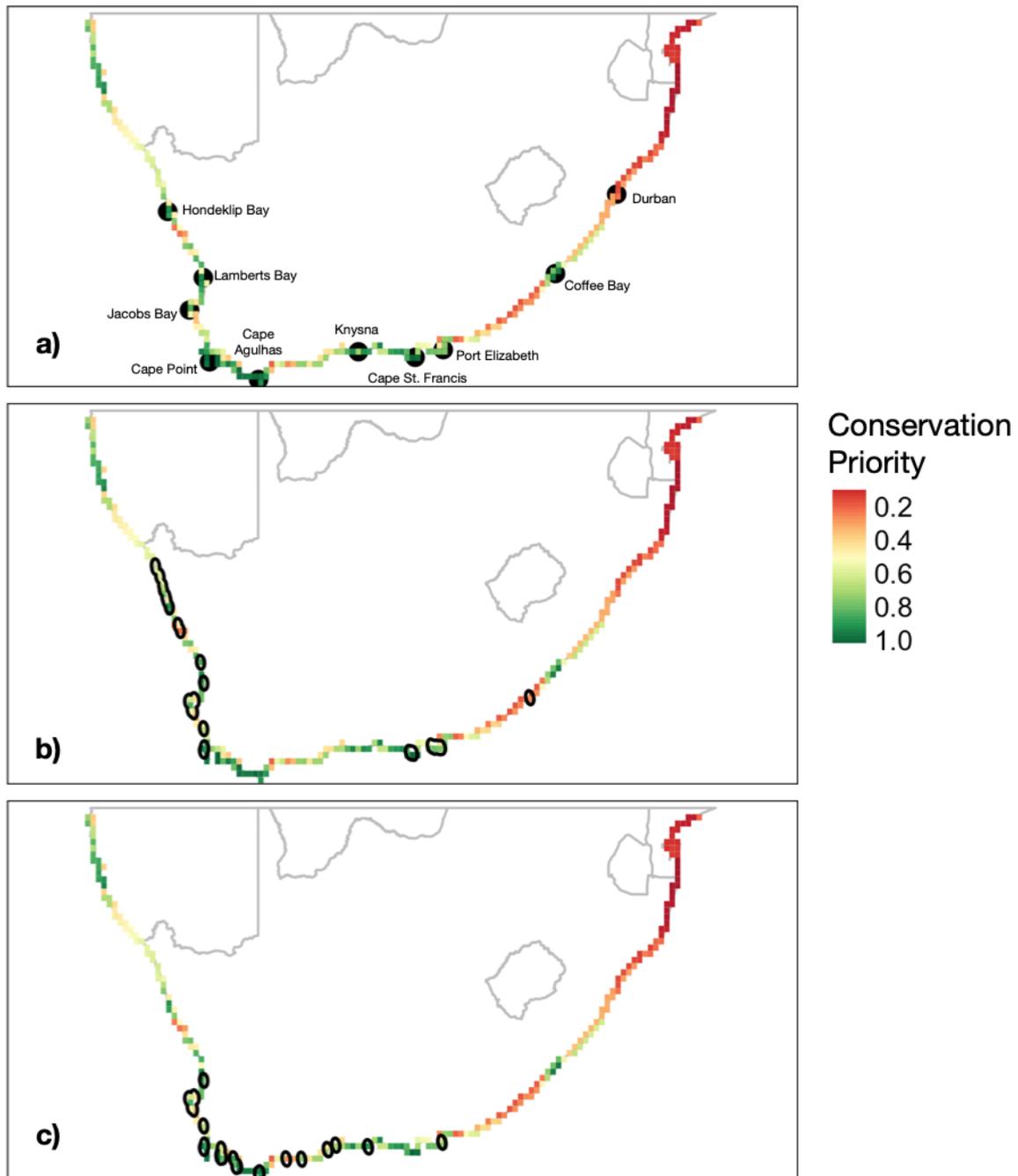


Figure 4.1 – The output rank map from the Zonation scenario including all four resilience features for all species (i.e. *ALL* scenario), with green shading representing high conservation priority areas, and coastal towns indicated by black place markers for reference (a). Rocky shore habitats classified as poorly protected (b) or as being endangered (c) within the latest National Biodiversity Assessment (van der Bank et al., 2019) are enlarged for clarity and outlined in black.

Pearson's r correlations between scenarios that included a change in species were more similar within themselves (r ranging from 0.23 - 0.71) than those including a change in resilience feature (r ranging from -0.08 - 0.79). The most similar spatial plans were C.

punctatus and *S. granularis* between species (Table 4.3), but even still the similarity was moderate ($r = 0.47$). Of the scenarios with change in resilience feature, *Genomic Diversity* and *Climatic Stability* were the most similar ($r = 0.62$). The two 'adaptive scenarios' including outlier loci, *Genomic Vulnerability* and *Outlier Abundance*, were the most distinct of all scenario comparisons ($r = -0.08$). Specifically, *Genomic Vulnerability* had spatial solutions with the least overlap with the scenarios derived from other resilience features for all species (Table 4.4).

Table 4.3 – Pearson's r correlations between the output rank maps including all resilience features per individual species, or including all features from all species (*ALL*).

	<i>C. punctatus</i>	<i>P. angulosus</i>	<i>S. granularis</i>
<i>C. punctatus</i>			
<i>P. angulosus</i>	0.31		
<i>S. granularis</i>	0.47	0.23	
<i>ALL</i>	0.71	0.68	0.70

Table 4.4 – Pearson's r correlations between the output rank maps including all species per resilience feature or including all features from all species (*ALL*).

	<i>Climatic Stability</i>	<i>Genomic Diversity</i>	<i>Outlier Abundance</i>	<i>Genomic Vulnerability</i>
<i>Climatic Stability</i>				
<i>Genomic Diversity</i>	0.62			
<i>Outlier Abundance</i>	0.16	0.47		
<i>Genomic Vulnerability</i>	0.14	0.20	-0.08	
<i>ALL</i>	0.69	0.79	0.51	0.42

Discussion

This chapter uses an innovative framework to identify conservation priority areas that meet the objective of ensuring multispecies adaptive capacity and persistence under climate change. Here the results show that conservation priorities change more depending on which

resilience feature is included, rather than species. Specifically, the two ‘adaptive’ scenarios, *Outlier Abundance* and *Genomic Vulnerability*, varied substantially in their conservation outputs, suggesting that the genomic data used to identify intraspecific adaptive capacity should be carefully selected and weighted appropriately within conservation plans. Such differential weighting has been previously suggested with regards to uncertainty in other biodiversity features, such as with future species distributions (Carvalho et al., 2011b). However, *Climatic Stability* and *Genomic Diversity* were the most similar of all scenarios, which suggests that climatic stability may be a viable proxy for genomic diversity. This work is an important step towards understanding how to conserve coastal areas to ensure multi-species resilience under global change, and offers exciting research questions pertaining to ‘evolutionary surrogates’ going forward. On a local scale, this is the first spatio-temporal assessment of species and habitat evolutionary resilience within coastal South Africa, and should be utilized to inform conservation plans in the region.

Comparing evolutionary hotspots between species and features

This is the first time that multispecies genomic and climatic evolutionary hotspots have been identified together, both globally, and within South Africa. The results show that including resilience features per species individually leads to distinct outcomes (Table 4.3), highlighting how co-distributed species within a strong environmental gradient can have varied evolutionary trajectories, and stressing the importance for multi-species studies. Previous studies assessing the spatial patterns of resilience within co-distributed terrestrial species support the species-specific patterns found here (Hanson et al., 2020a; Prates et al., 2016; Razgour et al., 2019). Interestingly, the limpet and the crab were most similar in their spatial priorities (Table 4.3). This is unexpected as throughout Chapters One to Three, the limpet and the urchin had the most similar results, as both were more influenced by SST, whereas the crab's distribution and genomic variation were better explained by SSS. While it could be argued that the crab and limpet have similar scenarios due to them being more similar in number of SNPs compared to the urchin, this is unlikely to be the case as the limpet and urchin showed similar results in previous chapters despite the differences in their number of SNPs. Further, Xuereb et al. (2020) tested whether conservation scenarios

differed between putatively neutral and adaptive SNP datasets in the sea cucumber, *Paranthropus californicus*, owing to the large discrepancies in number of neutral and adaptive SNPs, finding that this was not the case, as similar patterns were found using subsets of neutral SNPs equal to the number of outlier SNPs. The unexpected similarities in conservation outcomes among the three study species within this chapter is likely due to the high discrepancies between the four resilience features (Table 4.4). The results indicate that changing the resilience feature included, rather than species, leads to greater differences in spatial priorities (Tables 4.3, 4.4). This finding differs from Nielsen et al. (2017), who found that including different species led to a greater change in conservation outcomes than different genetic metrics. However, Nielsen et al. (2017) only included evolutionary metrics pertaining to mtDNA markers, which have less variation than NGS genomic markers, as well as five instead of three study species, which included a vertebrate fish species. Here, the resilience features include both neutral and adaptive genomic metrics, as well as a climatic metric, all of which capture different evolutionary processes, likely leading to the broad range of conservation solutions.

Previous studies assessing conservation scenarios based on neutral and outlier loci from genomic markers highlight large discrepancies, arguing that these data types cannot be used interchangeably for conservation (Hanson et al., 2020a; Xuereb et al., 2020). Here, the neutral loci used to calculate genomic diversity and outlier loci used to calculate outlier abundance had relatively similar spatial outputs (Table 4.4). This indicates that populations with higher diversity in genomic neutral loci may be more likely to have higher outlier abundance due to greater standing diversity for selection to act on (Catullo et al., 2015; Kelly, 2019). It has also been shown that areas where multiple species harbour highly diverse and unique neutral genetic variation correspond to areas of high adaptive genomic variation in single species (Nielsen et al., 2020). Therefore, the multispecies comparisons in the South African coastal system suggest that high neutral diversity may be an adequate surrogate of adaptive capacity. However, examples from other marine and terrestrial systems offer diverse conclusions on the effectiveness of surrogates of adaptive potential (Hanson et al., 2017, 2020b; Hermoso et al., 2016; Ponce-Reyes et al., 2014; Xuereb et al., 2020). Thus, a more comprehensive analysis of the applicability of environmental and molecular data types to capture adaptive patterns across taxa and ecosystems is needed to

create an overarching framework for practitioners to use to incorporate evolutionary processes in conservation plans.

In comparison, the two 'adaptive' scenarios, including either genomic vulnerability or outlier abundance, were highly distinct (Table 4.4). Xuereb et al. (2020) also found discrepancies between adaptive scenarios, with areas of high heterozygosity in all outlier loci or high allele frequencies in warm temperature-associated loci, resulting in contrasting conservation solutions. The authors argue that the two metrics should be used to meet different conservation objectives, where prioritising high abundances of temperature-adapted loci is essentially 'picking climate change winners' (from past divergent selection), and prioritising high total outlier diversity lets 'nature choose the winners' (from standing variation). However, one of the critiques of prioritising areas based on candidate loci is that having strong associations with contemporary environmental variation does not necessarily mean that these loci will be of benefit to future environmental change (Funk et al., 2012; Harrison et al., 2014; Luikart et al., 2003). Here, the results show that high outlier abundance does not align with low genomic vulnerability, meaning that areas where selection has created uniquely adapted individuals do not necessarily align with areas where selection will impact individuals the least in the future. This has novel implications for conservation planning, as prioritizing high frequencies of adaptive loci is generally considered the best way to enhance adaptive capacity in conservation (Manel et al., 2018; Xuereb et al., 2020), but this metric fails to consider future environmental changes. This study challenges the assumption of increased outlier abundance inferring heightened genomic resilience to future climatic change, and suggests that projections of genomic composition under climate change scenarios is necessary to predict future adaptive capacity for conservation.

Few studies have compared conservation solutions from either molecular diversity or climatic stability, but terrestrial examples suggest a mismatch between the two. For example, areas of future climatic stability do not correlate with areas of high diversity from neutral genomic loci in two amphibian species within the Iberian Peninsula (Hanson et al., 2020b), or from microsatellite markers in a tree species in Brazil (Diniz-Filho et al., 2020). Yet, it is important to also consider areas of past climatic stability (Hoffmann et al., 2015), as these have been shown to generally harbour higher molecular and species diversity (Manel et al., 2020; Nicotra et al., 2015). This is the first spatial comparison of molecular diversity

with climatic stability relating to both past and future. Here, the results show that areas of climatic stability through time are highly correlated with areas of high genomic diversity, highlighting how environmental change over time may be a viable surrogate for genomic diversity patterns. This result contrasts the findings of Hanson et al. (2020b), who found that historically climatically stable areas are not a good substitute for molecular diversity within conservation plans. However, the authors used microsatellite markers, which better reflect contemporary environmental changes (such as contemporary human disturbances), rather than historical climatic legacies (such as stability through glacial-interglacial periods; Hall & Beissinger, 2014). Ultimately, the links between past and future climatic stability and molecular diversity need further exploration, within both marine and terrestrial contexts, to understand how they can be used to meet conservation objectives pertaining to climate change.

Considering evolutionary potential in South Africa's coastal MPA network

This chapter uses a novel framework to conserve multispecies evolutionary potential, highlighting unique resilience hotspots along South Africa's coastline. Broadly, conservation priority areas identified from all resilience features and species fall within the south and southwest coasts of South Africa (Fig. 4.1). When poorly protected and endangered rocky shore habitats are overlaid onto evolutionary importance, areas around Lambert's Bay, Cape Point, and Port Elizabeth stand out as areas where high evolutionary importance is threatened, yet under-protected (Fig. 4.1). Each of these areas fall into the periphery of EBSAs within the country, supporting their locations and importance (Fig. 4.2). It is highly unlikely that the entire identified EBSA areas can be conserved, due to their number and size (Fig. 4.2), and thus the approach here can refine important areas for rocky shore protection within EBSAs. This approach also aligns with recent calls to incorporate climate change refugia into EBSAs (Johnson & Kenchington, 2019). Here, I show how including different resilience features can identify multispecies climate change refugia spanning evolutionary processes, which can further pinpoint areas to mitigate biodiversity threats within the South African coastal environment.

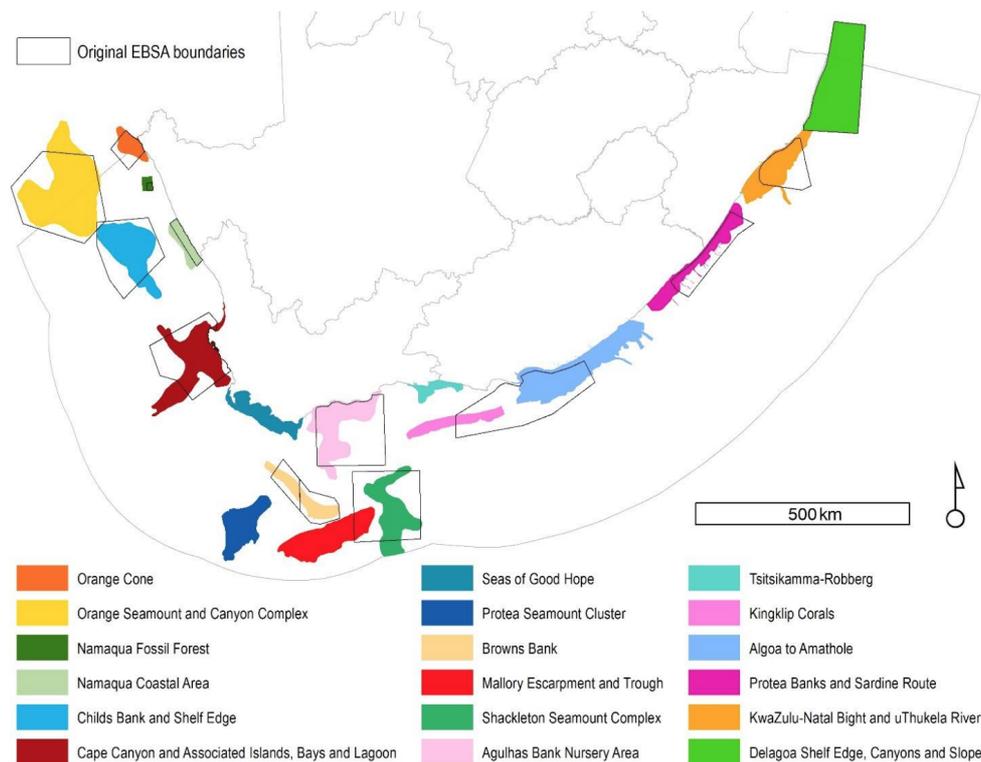


Figure 4.2 – Original and newly proposed Ecologically or Biologically Significant Marine Areas (EBSAs) in South Africa, obtained from the 2018 NBA (van der Bank et al. 2019).

Broadly, results from the three study species indicate that the marine species and populations within the eastern coast of South Africa may be highly vulnerable to novel climatic changes (Fig. 4.1). The east coast is also expected to be the most impacted by local sea level changes (Mather et al., 2009). Furthermore, the southeast coast has been reported as having the highest levels of subsistence harvesting of marine intertidal organisms (Liebau, 2013), and overall higher levels of natural resource dependence (Hamann et al., 2015). Thus, ecosystem shifts within this region may have detrimental effects on the human communities that rely on local coastal resources. This stretch of coastline has the most protection overall, but effective and adaptive management must be enforced to conserve coastal ecosystem functioning under the threat of global change (Reside et al., 2018; Tittensor et al., 2019). In contrast, populations along the southern coast are highlighted as the most resilient (Fig. 4.1). This region is also of interest as it generally has the highest species and genetic richness along the coast (Griffiths et al., 2010; Wright et al., 2015), as well as high endemism (Henriques et al., 2020). Ideally, further protection would be implemented within the south and especially southwest regions of the coast, as to ensure

that these areas of increased resilience to climate change can persist, and potentially be used to replenish/support more vulnerable regions of the coastline (Fredston-Hermann et al., 2018). However, it is also recognized that the conservation planning scenarios used here are highly specific, and do not include other important biological and socio-economic features such as larval dispersal (Crochelet et al., 2016), invasive species (Giakoumi et al., 2016), waste water discharge (Wickramasinghe et al., 2009), coastal development (Palmer et al., 2010), coastal harvesting (Branch et al., 2002; Crowe et al., 2000), and rare or endangered species (Dias et al., 2017), all of which could expand further application of the framework used here.

Conclusions

Recent advances have been made to include species' resilience to climate change, from measures such as climatic velocity and species sensitivity, into marine conservation planning (Coleman et al., 2017; Magris et al., 2014; Munday et al., 2013; Wilson et al., 2020). However, the use of genomic data to inform resilience for spatial conservation planning is still lacking within scientific literature (but see Hanson et al., 2020a). This chapter creates a new framework to identify evolutionary resilience hotspots based on seascape genomics and ecological modelling outputs. The results offer unique insights into how neutral and adaptive genomic metrics can be used to infer multispecies adaptive potential. Within South Africa, areas of evolutionary resilience mainly lie within the south coast, which has previously been suggested as a refugia based on local environmental trends 'squeezing' species towards the south (Blamey et al., 2015; Whitfield et al., 2016). However, this study included species predominantly inhabiting cool- and warm-temperate bioregions, and thus resilience patterns of species which predominantly inhabit the warm and subtropical bioregions in South Africa still need to be characterized. Additionally, further work is needed to combine vulnerability assessments from an interdisciplinary perspective, including not just genomic and climatic data, but also socio-economic and ecological data, to fully capture and protect coastal social-ecological systems under global change (Rilov et al., 2019; Thiault et al., 2018).

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Chapter Five

Concluding statements and future directions

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Thesis summary and conclusions

Understanding the spatio-temporal environmental processes shaping biodiversity patterns is crucial if we are to preserve ecosystem functioning in the age of the Anthropocene. The fields of landscape and seascape genomics are increasingly characterising the links between the environment, ecology, and genetics (Balkenhol et al., 2019; Liggins et al., 2019). However, our understanding of how species may respond to novel environmental and biological changes associated with global change is still poor (Logan & Cox, 2020; Miller et al., 2018) and comparative approaches, such as investigating multiple species, molecular markers, and timeframes within seascape genomic studies remain limited. These types of comprehensive analyses are an essential step if we are to assess the vulnerability of marine ecosystems to global change, and use this information to proactively conserve biodiversity into the future (Waldvogel et al., 2020). Here, I showed how that incorporating multiple species and timescales into seascape genomic analyses can elucidate important environmental drivers of biodiversity, and the legacies of environmental change on intraspecific genomic composition. Furthermore, by conducting multiple seascape genetic models, I assessed the uncertainty surrounding the influence of various seascape features in shaping the evolutionary trajectories of three marine species. Specifically, I consistently found that sea surface salinity (SSS) and sea surface temperature (SST) were distinctive features driving patterns in the crab, or limpet and urchin, which provided relatively decisive evidence for the importance of these seascape variables, compared to if a single gene-environment association analysis was performed, which would have led to less conclusive results. The results also indicate that the biodiversity level at which seascape genomics analyses are performed, from between species to populations within a species, can greatly alter inferences of environmental footprints on biodiversity patterns, and in result lead to different conservation strategies. As the future of marine ecosystems is highly uncertain (Deser et al., 2012), approaching seascape genomic analyses from a holistic approach (i.e. including multiple molecular markers, species, and timeframes), is preferable to understand and conserve evolutionary processes going forward.

In this thesis, I employed a comparative seascape genomics approach, with the aims of identifying how environmental change through space and time influence populations of three ecologically important marine invertebrates. These species vary in their reproductive

mode, distributions, pelagic larval durations, and rocky shore zonation (Branch & Branch, 2018). Broadly, throughout the chapters I found that results were similar between the urchin and limpet, yet more distinct for the crab. This is not entirely surprising, as the crab is a brooding species with a more warm-temperate distribution, compared to the urchin and limpet, which are both broadcast spawners with the centre of their abundance in a cool-temperate region. It is also interesting to note that while the limpet was originally thought to be most influenced by air temperature, due to it being the most exposed of the three species during low tides (Branch & Branch 2018), it is the species showing the strongest association to SST.

Future work should ideally include additional warm-temperate species, so that we can further understand whether the discrepancies between species found here are due to their distributional ranges. For example, SST may be an important feature shaping other cool-temperate species, and SSS could be the dominant variable shaping most warm-temperate species. In comparison, it might alternatively be that decapods are especially influenced by SSS due to their developmental and adult physiology being highly sensitive to this variable (Anger, 2003; Falconer et al., 2020), and other warm-temperate species are rather predominantly influenced by SST.

This thesis is also novel in that it empirically showed that high neutral genomic diversity aligns with past climatic stability, and with high outlier abundance, suggesting that all three of these evolutionary metrics are linked, possibly because similar environmental and demographic processes have shaped them accordingly. The use of neutral genetic diversity to infer resilience to climate change has been previously demonstrated with microsatellite markers in kelp (Wernberg et al., 2018), and seagrass (Ehlers et al., 2008), showing how increased diversity leads to changes in phenotype increasing fitness. A recent example with genome-wide SNP data has shown that increased diversity leads to less drastic distributional ranges losses between four lineages in a cold-adapted plant (Theodoridis et al., 2018), further highlighting the importance of genetic diversity to persistence under global change. However, these studies did not specifically investigate which underlying processes brought about higher genetic diversity levels to begin with. This thesis provides a novel view on the importance of historical climatic events, as these may be important drivers of extant genetic diversity, which in turn may predict resilience into the future. Future explorations into how past climatic changes shape neutral and adaptive

patterns, and how these patterns then alter physiology in response to climatic change, offer exciting prospects in the field of molecular ecology. Overall, this project shows how incorporating past, present, and future in seascape genomic analyses allow us to identify overarching spatio-temporal processes driving intraspecific resilience in coastal species.

Main findings and future prospects per chapter

Within Chapter One, I conducted additional sampling of the study species to build on previous genomic work (Nielsen et al. 2018) to create the most extensive genomic datasets for South African coastal species to date. This RAD-seq dataset was then used to assess population structure, isolation-by-distance (IBD) and isolation-by-environment (IBE), and putative selection patterns. The results in Chapter One suggested that the crab and urchin have shallow population structure, with no evidence of IBD or IBE, and experience selection predominantly driven by sea surface salinity and air temperature, respectively. In contrast, the limpet showed shallow population structure, IBD and IBE in response to SSTs, and selection patterns most closely associated with SSTs. Chapter One also highlighted the discrepancies between six commonly used gene-environment association (GEA) analyses, and offered a new multi-model methodology to identify prominent selection forces within natural populations.

From Chapter One, I concluded that different contemporary seascape features are acting on the three study species, and that local adaptation is an important evolutionary force despite high levels of gene flow. However, this chapter has several limitations, which must be considered when evaluating the results. For example, even though multiple GEA models were used to identify outlier loci, false positives and negatives may still exist in the data. Furthermore, gene ontologies were unavailable for many of the outlier loci, which is often the case for non-model marine invertebrates (Lopez et al., 2019). Therefore, even though the outliers detected here are statistically significant, they may have no functional role in physiological adaptation, which is a hindrance to many land- and seascape genomic studies on non-model species. The full spectrum of selection, including how environmental pressures lead to genomic differentiation, phenotypic plasticity, epigenetic modifications, and gene interactions is still largely unknown within molecular ecology (Hoban et al., 2016),

particularly for non-model species without well annotated reference genomes. Further work is needed to conclude the selective importance of seascape features on populations of these species, which could include common garden experiments and transcriptomic sequencing (da Fonseca et al., 2016; Waldvogel et al., 2020).

That being said, the results from Chapter Two offered further support for the importance of the environmental drivers of salinity for the crab, and temperature for limpet and urchin population dynamics, as these two variables were found to also be the most correlated with past and current distribution patterns of these species. More broadly, Chapter Two aimed to assess the historical and contemporary drivers of extant molecular diversity in a multispecies framework. Here, two common biogeographic hypotheses were compared: the central-margin hypothesis, which states that the centre of a species contemporary distribution should harbour highest diversity levels, and the refugial-persistence hypothesis, which states that areas of past climatic stability should correlate with extant high diversity (Guo, 2012). The results showed how areas of past climatic stability spatially align with areas of high diversity, in both mtDNA and nuclear SNP markers. This finding supports the refugial-persistence hypothesis within all three study species. In contrast, linear models offered little statistical support for either the refugial-persistence or central-margin hypothesis across species.

Another possible explanation for the observed patterns of contemporary molecular diversity in the study species could be secondary contact, which has also been proposed to increase intraspecific genetic diversity (Maggs et al., 2008). Future work could further explore the influence of past climatic events on extant molecular patterns by modelling past demographic histories of the study species (Hoban et al., 2019). For example, Prates et al. (2016) used Approximate Bayesian Computation (ABC) to model historical demographic shifts in three co-distributed lizard species in a South American forest to infer genetic diversity changes over time. However, in order to conduct demographic modelling on the study species used here, species mutation rates are required, and individual sequencing (instead of the Pool-seq) should be performed on the species, so that population assignment tests can be conducted and input into past demographic simulations (Leblois et al., 2018; Riginos et al., 2016; Titus et al., 2019).

Individual sequencing could also help inform predictions of intraspecific changes due to future climate change. For example, Razgour et al. (2019) modelled the future

distributions of either cool- or warm-adapted bat individuals, finding that the ‘warm genotype’ individuals are predicted to expand their ranges at the expense of the ‘cool genotype’ individuals. Yet, despite restrictions associated with Pool-seq data, this thesis was still able to assess both inter- and intra-specific vulnerability of the study species to global change. Chapter Three used allele frequencies from Chapter One to assess patterns of genomic vulnerability, finding that eastern sites within the crab, and western sites of the limpet and urchin, are potentially more vulnerable to future climatic changes. These patterns of genomic vulnerability (i.e. where populations will have to adapt more to track climatic change) contrasted with patterns of future habitat suitability within each species. Therefore, some areas, like the eastern sites for the crab, may experience climatic changes which are within the species’ ecological niche, but outside of the populations’ selective niche. The implications around this process are uncertain, but may possibly lead to outcomes such as species persistence but with decreased abundance in these regions. However, for this to be tested, biodiversity monitoring is required to compare climate change responses to baseline data (Pereira & Cooper, 2006). To fully understand the vulnerability to climate change of the study species, the outputs of the Gradient Forest models should be validated showing that increased genomic vulnerability does lead to decreased fitness into the future (Fitzpatrick et al., 2018). Moreover, even if high genomic vulnerability reflects changes in fitness, species may still persist in high vulnerability areas due to adaptation or phenotypic plasticity (Reusch, 2014). The models used in Chapter Three also did not specifically account for environmental influences on multiple life history stages, and thus vulnerability assessments may differ as the larval dynamics of these species are better understood (Álvarez-Romero et al., 2018).

Lastly, in Chapter Four, data from the first three chapters were combined into a marine conservation plan with the objective of prioritising areas to enhance evolutionary resilience. The results show how including different resilience features, specifically climatic stability, neutral genomic diversity, outlier abundance, or outlier genomic vulnerability, lead to greater changes in spatial priorities than including different species. The two scenarios based on putative adaptive variation were the most distinct, which highlights how contemporary selective pressures may not reflect high adaptive capacity under novel climatic changes associated with global change (Rilov et al., 2019). The results also showed similar spatial outcomes from climatic stability and genomic diversity, reflecting the findings

from Chapter Two, and offering promise for climatic stability as a potential surrogate for neutral genomic diversity when no genetic data is available. However, simple surrogates of molecular variation need to be investigated further, as previous studies highlight the uncertainty in the use of environmental gradients or past climatic histories to capture genomic patterns (Carvalho et al., 2011a; Hanson et al., 2017, 2020b; Ponce-Reyes et al., 2014).

When all features and species were combined into a single conservation plan, the south and southwest coasts of South Africa are identified as priority areas, relating to high evolutionary resilience. In contrast, the eastern coast had lower priority, potentially meaning that coastal species and populations in this region are more at risk to global change. Yet, this study was conducted mainly on cool-temperate species, and thus the east coast might be less vulnerable to warm-temperate and subtropical marine species. Additionally, conservation planning analyses are rarely this specific, and rather include broader multispecies and habitat features to meet objectives (Kukkala & Moilanen, 2013; Schmiing et al., 2015). As ecosystem-based conservation planning and adaptive management are becoming more prominent, a more holistic approach to identify conservation priority areas for coastal resilience is needed (Toonen et al., 2011; Wondolleck & Yaffee, 2017). It is also increasingly recognized that including in-depth socio-economic information into conservation planning can lead to more effective reserve design and management (Ban et al., 2013; Folke et al., 2005; Naidoo et al., 2006). Thus, human coastal use, and how this may shift under global change, should also be included into a conservation plan to best design additional MPAs within the country's network. The spatial analyses within this thesis were also at a relatively coarse resolution, and accounting for microhabitats acting as refuges, near-shore currents impacting gene flow, and fine-scale cost features may change conservation outcomes (Cheek et al., 2016). Lastly, it is also acknowledged that the study species used here do not solely occur within South Africa, and that future work should consider conservation planning analyses in neighbouring countries.

Final comments

This thesis offered in-depth insights into the evolutionary patterns of coastal species within the uniquely biodiverse, yet highly threatened, South African marine environment. Across chapters, the results exposed species-specific responses to the seascape, specifically the crab in response to SSS, and the urchin and limpet in response to SST. Although biodiversity patterns of the limpet and urchin were both predicted to be driven by SST, the spatial patterns were still species-specific, highlighting how strong climatic gradients can impose different selection pressures on species, possibly depending on life histories and ecology. Another novel finding from this thesis was the importance of historical climatic changes on the biodiversity patterns of today, which may have lasting legacies on intraspecific resilience into the future. It is important that seascape genomic studies shift from focusing solely on contemporary timeframes to integrating gene-environment interactions across timescales. Moreover, seascape genomics can benefit from comparisons to species level inferences, as the findings here provide evidence of distinct species- and gene-environment associations across taxa. Finally, this thesis demonstrated how seascape genomics can inform conservation planning, and highlights how there is no easy surrogate to capture putative adaptive patterns across species.

As conserving biodiversity and ecosystem functioning under global change is arguably one of the greatest challenges in the Anthropocene, it is important to understand the effectiveness of genomic data to meet global conservation goals. This thesis is an important step in our understanding of how genomics can be used to assess species vulnerability and spatially prioritise evolutionary resilience. The findings here offer the essential groundwork for future explorations linking evolution, ecology and the environment to better ensure species persistence during the world's 6th mass extinction.

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Appendix 1.S1: Sampling information, bioinformatic pipeline parameters, results of mitogenome mapping and *de novo* assembly comparisons, and single nucleotide polymorphism (SNP) results are shown per species. Population structure based on three coverage scenarios are also shown per species. Species distributions are shown in comparison to the regional sea surface temperature gradient.

Table 1.S1.1 – Sample sites with the corresponding abbreviation, date of collection, and number of collected individuals for *Cyclograpsus punctatus*.

Sample Site	Abbreviation	Date collected	N
Jacobsbaai	JB	Aug 2015	38
Yzerfontein	YZ	July 2018	40
Sea Point	SP	July 2015	40
Bettys Bay	BT	July 2018	40
Gansbaai	GB	July 2018	40
Cape Agulhas	CA	Aug 2015	40
Jongensfontein	JF	July 2018	40
Mossel Bay	MB	July 2018	40
Knysna	KY	Aug 2015	40
Cape St. Francis	CF	Aug 2015	37
Haga Haga	HH	Oct 2015	40
Coffee Bay	CB	July 2018	39
Margate	MG	Nov 2018	40

Table 1.S1.2 – Sample sites with the corresponding abbreviation, date of collection and number of collected individuals for *Parechinus angulosus*.

Sample Site	Abbreviation	Date collected	N
Port Nolloth	PN	July 2015	40
Hondeklipbaai	HB	July 2015	40

Doringbaai	DB	Aug 2015	40
Lambertsbaai	LB	July 2015	40
Jacobsbaai	JB	July 2015	40
Sea Point	SP	July 2015	40
Bettys Bay	BT	July 2018	40
Cape Agulhas	CA	Aug 2015	39
Mossel Bay	MB	July 2018	39
Knysna	KY	Aug 2015	40
Cape St. Francis	CF	Oct 2015	40
Port Alfred	PA	July 2018	40
Haga Haga	HH	Oct 2015	40
Coffee Bay	CB	July 2018	40

Table 1.S1.3 – Sample sites with the corresponding abbreviation, date of collection and number of collected individuals for *Scutellastra granularis*.

Sample Site	Abbreviation	Date collected	N
Port Nolloth	PN	July 2015	40
Hondeklipbaai	HB	July 2015	40
Brandsebaai	BB	July 2015	40
Lambertsbaai	LB	July 2015	40
Jacobsbaai	JB	July 2015	40
Sea Point	SP	July 2015	40
Bettys Bay	BT	July 2018	40
Cape Agulhas	CA	Aug 2015	40
Mossel Bay	MB	July 2018	39
Knysna	KY	Aug 2015	40

Cape St. Francis	CF	Oct 2015	40
Port Alfred	PA	July 2018	40
Haga Haga	HH	Oct 2015	40
Hluleka	HL	July 2018	30

Table 1.S1.4 – The bioinformatic steps used to generate SNP datasets per species, with the associated program and parameters.

Step	Program	Parameters
1. Filter raw reads	TrimGalore!	<ul style="list-style-type: none"> • Q = 20 • length = 50 • a/a2 = adapter sequences • stringency = 10 • e = 0.01
2. Map reads onto mitogenomes	BWA-MEM	<ul style="list-style-type: none"> • a • T = 20
3. Convert SAM to BAM format	SAMtools	<ul style="list-style-type: none"> • default
4. Filter mtDNA-mapped BAMs	SAMtools	<ul style="list-style-type: none"> • F 0X0004 • Q = 10
5. Merge mtDNA-mapped BAMs	BAMtools	<ul style="list-style-type: none"> • default
6. Convert merged BAMs to SAM files	SAMtools	<ul style="list-style-type: none"> • default
7. Filter mtDNA-mapped reads from quality-trimmed reads	BBMap	<ul style="list-style-type: none"> • include = f • minlen = 50
8. Normalize reads for de novo assemblies	BBMap	<ul style="list-style-type: none"> • tossbadreads = t • target = 100
9. Identify k-mers for de novo assemblies	KmerGenie	<ul style="list-style-type: none"> • default
10. AbySS assembly	Abyss	<ul style="list-style-type: none"> • c = 3
11. Megahit assembly	Megahit	<ul style="list-style-type: none"> • default
12. Spades assembly	Spades	<ul style="list-style-type: none"> • careful
13. GARM merge Spades assemblies	GARM	<ul style="list-style-type: none"> • default
14. Compare assemblies	QUAST BUCSO NCBI BLAST +	<ul style="list-style-type: none"> • default • default using metazoan database

		<ul style="list-style-type: none"> • default using nucleotide database
15. Map reads onto de novo assembly	BWA-MEM	<ul style="list-style-type: none"> • a • T = 20
16. Convert SAM to BAM format	SAMtools	<ul style="list-style-type: none"> • default
17. Filter mapped BAMs	SAMtools	<ul style="list-style-type: none"> • F 0X0004 • F 0X0008 • f 0X0002 • Q = 20
18. Subsample BAMs to median # of quality-filtered mapped reads	SAMtools	<ul style="list-style-type: none"> • default
19. Create a multiple-pileup file	SAMtools	<ul style="list-style-type: none"> • B • Q = 20
20. Create a sync file	PopPoolation2	<ul style="list-style-type: none"> • min-qual = 20
21. Call SNPs	Poolfstat	<ul style="list-style-type: none"> • min.cov.per.pool = 20 • max.cov.per.pool = 400 • min.maf = 0.01 • min.rc = 4

Table 1.S1.5 – Reference mitogenomes, their size, and average number of raw reads per sample that mapped to the mitogenomes, shown for *C. punctatus*, *P. angulosus*, and *S. granularis*.

	<i>C. punctatus</i>	<i>P. angulosus</i>	<i>S. granularis</i>
Reference mitogenome / Accession number	<i>Cyclograpsus granulosus</i> / NC_025571.1	<i>Loxechinus albus</i> / JX888466.1	<i>Lottia digitalis</i> / DQ238599.1
Reference mitogenome size (in base pairs; bp)	16300 bp	15260 bp	26835 bp
Average number of reads per sample mapped onto mitogenome	12363	20342	234

Table 1.S1.6 – Quast outputs from three different de novo assembly methods (AbySS, MEGAHIT, and SPAdes) shown for *C. punctatus*, *P. angulosus*, and *S. granularis*.

Quast output	AbySS	MEGAHIT	SPAdes
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<i>C. punctatus</i>			
N50	713	576	1609
L50	475	537	755
Number contigs > 1000 bp	164	21	1592
Largest contig	4174	1691	8615
Total length	955536	747360	3813367
BLASTN % nodes matched	0.01	0.06	0.20
<i>P. angulosus</i>			
N50	659	614	1434
L50	8555	7399	1309
Number contigs > 1000 bp	1567	552	2432
Largest contig	5354	2116	10665
Total length	14836022	11240686	5791195
BLASTN % nodes matched	0.04	0.10	0.19
<i>S. granularis</i>			
N50	553	586	886
L50	67	7933	6307
Number contigs > 1000 bp	0	156	4505
Largest contig	806	1965	7721
Total length	83626	10866182	16058421
BLASTN % nodes matched	0.01	0.03	0.07

Table 1.S1.7 – The number (#) of total raw and mapped reads, number of reads passing filtering, number of total SNPs and SNPs passing filtering, for *C. punctatus*, *P. angulosus* and *S. granularis*.

Species	# Raw reads	# Mapped reads	# Reads after filtering	# Total SNPs	# SNPs after filtering
<i>C. punctatus</i>	29246761	23431343	20641431	17309	1190

<i>P. angulosus</i>	47663829	33686426	20570929	3946	822
<i>S. granularis</i>	39463412	29734860	27443789	10416	1658

Table 1.S1.8 – Site-specific number (#) of raw reads, quality-mapped reads, total filtered SNPs, and mean depth coverage per pool are shown for *C. punctatus*.

Site	# Raw reads	# Mapped reads	# of SNPs	Mean depth coverage
JB	1255227	1094399	682	60.7
YZ	655848	566740	552	39.7
SP	2691816	2395053	908	138.4
BT	2656600	2132438	995	158.1
GB	2443284	1883225	976	149.7
CA	2690586	2109919	955	152.5
JF	2431546	1964114	928	144.9
MB	2813579	2169307	946	141.2
KY	1132946	971259	712	65.8
CF	1268052	1003981	760	76.1
HH	4703307	3674480	962	148.1
CB	3718276	2963114	982	159.1
MG	785694	503314	448	37.2

Table 1.S1.9 – Site-specific number (#) of raw reads, quality-mapped reads, total filtered SNPs, and mean depth coverage per pool are shown for *P. angulosus*.

Site	# Raw reads	# Mapped reads	# of SNPs	Mean depth coverage
PN	2278589	1614913	520	110.9
HB	1751423	1261453	484	76.9
DB	2316543	1779152	590	116.5

LB	2226368	1604554	631	125.7
JB	3237292	2461858	612	129.2
SP	1809007	1033637	441	57.3
BT	862961	655017	375	52.0
CA	1737662	1296096	491	90.3
MB	2588031	1880341	551	116.3
KY	17984447	13096132	577	115.8
CF	2369908	1478938	364	55.9
PA	2526174	1860987	572	107.0
HH	3242187	1706892	513	99.2
CB	2733237	1956456	821	110.9

Table 1.S1.10 – Site-specific number (#) of raw reads, quality-mapped reads, total filtered SNPs, and mean depth coverage per pool are shown for *S. granularis*.

Site	# Raw reads	# Mapped reads	# of SNPs	Mean depth coverage
PN	2458390	2098877	1073	85.3
HB	3068493	2320497	1157	105.3
BB	3347812	2449271	1217	111.7
LB	2574014	1894418	1178	99.9
JB	3227994	2345470	1196	113.4
SP	3051925	2420161	1151	108.4
BT	4745447	3685996	1223	114.3
CA	886627	629450	756	38.3
MB	1146977	813173	793	51.6
KY	3142000	2327798	1187	106.0
CF	2708281	2065118	1189	109.2

PA	1253857	904309	794	53.5
HH	1179705	908600	798	45.9
HL	6671890	4871722	1121	120.6

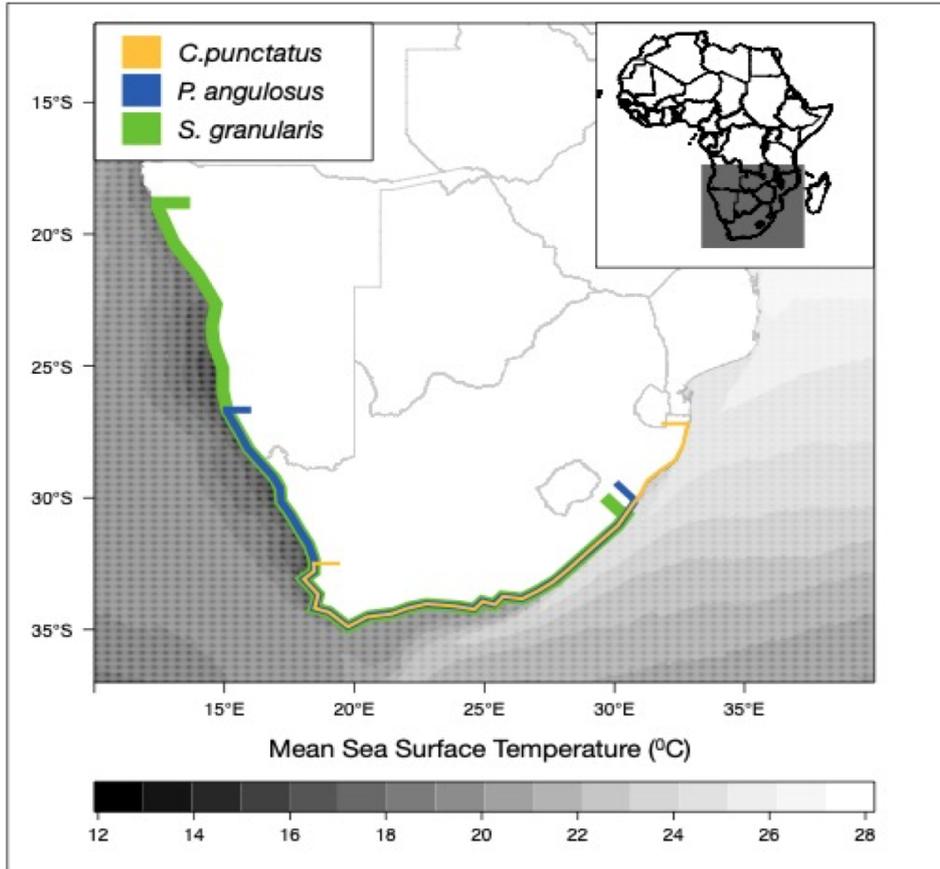


Figure 1.S1.1 – The species distributions with *C. punctatus* in yellow, *P. angulosus* in blue and *S. granularis* is green, shown over the average sea surface temperatures from the MARSPEC database (Sbrocco & Barber, 2013).

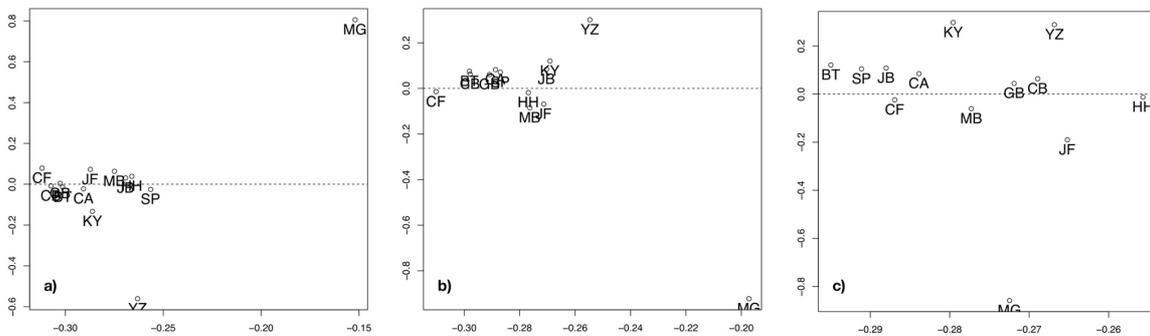


Figure 1.S1.2– Clustering of *C. punctatus* populations derived from a singular value decomposition of the scaled covariance matrices of population allele frequencies (Ω) from the core Bayesian

hierarchical model implemented in BayPass (Gautier, 2015), shown for three coverage parameter scenarios: minimum coverage = 20, maximum coverage = 200 (a); minimum coverage = 20, maximum coverage = 400 (b); minimum coverage = 40, maximum coverage = 400 (c). Refer to Table S1 for sample site abbreviations.

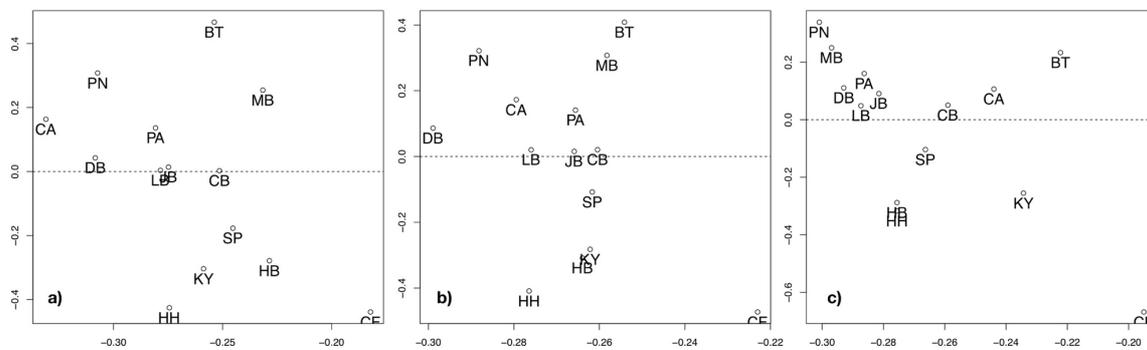


Figure 1.S1.3 – Clustering of *P. angulosus* populations derived from a singular value decomposition of the scaled covariance matrices of population allele frequencies (Ω) from the core Bayesian hierarchical model implemented in BayPass (Gautier, 2015), shown for three coverage parameter scenarios: minimum coverage = 20, maximum coverage = 200 (a); minimum coverage = 20, maximum coverage = 400 (b); minimum coverage = 40, maximum coverage = 400 (c). Refer to Table S2 for sample site abbreviations.

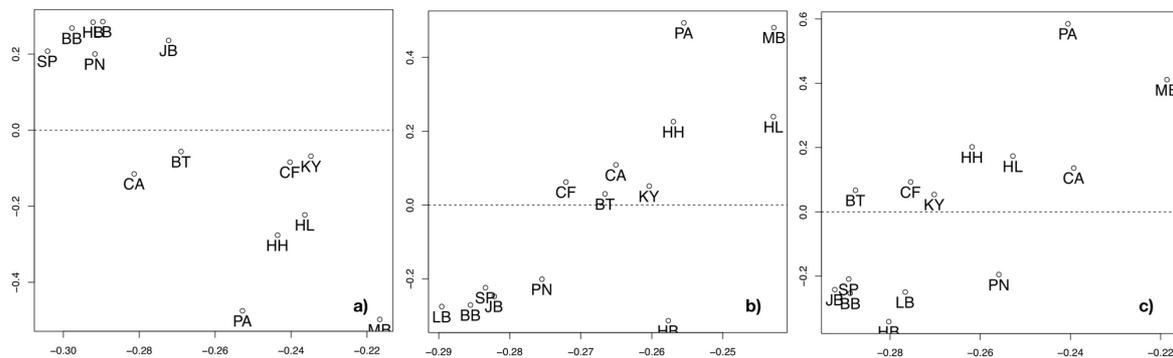


Figure 1.S1.4 – Clustering of *S. granularis* populations derived from a singular value decomposition of the scaled covariance matrices of population allele frequencies (Ω) from the core Bayesian hierarchical model implemented in BayPass (Gautier, 2015), shown for three coverage parameter scenarios: minimum coverage = 20, maximum coverage = 200 (a); minimum coverage = 20, maximum coverage = 400 (b); minimum coverage = 40, maximum coverage = 400 (c). Refer to Table S3 for sample site abbreviations.

Appendix 1.S1 References

Gautier, M. (2015). *BAYPASS version 2.1 User Manual*.

Sbrocco, E. J., & Barber, P. H. (2013). MARSPEC: Ocean climate layers for marine spatial ecology. *Ecology*, 94(4), 979–979. <https://doi.org/10.1890/12-1358.1>

For *Appendices 1.S2-1.S4*, please refer to the Additional Files 2-4, respectively, in the publication ‘Multi-model seascape genomics identifies distinct environmental drivers of selection among sympatric marine species’, available online at:

<https://bmcevolbiol.biomedcentral.com/articles/10.1186/s12862-020-01679-4>

Appendix 2.S1- Additional information regarding hindcasting species distribution models and genetic and genomic diversity for *S. granularis*, *P. angulosus*, and *C. punctatus*.

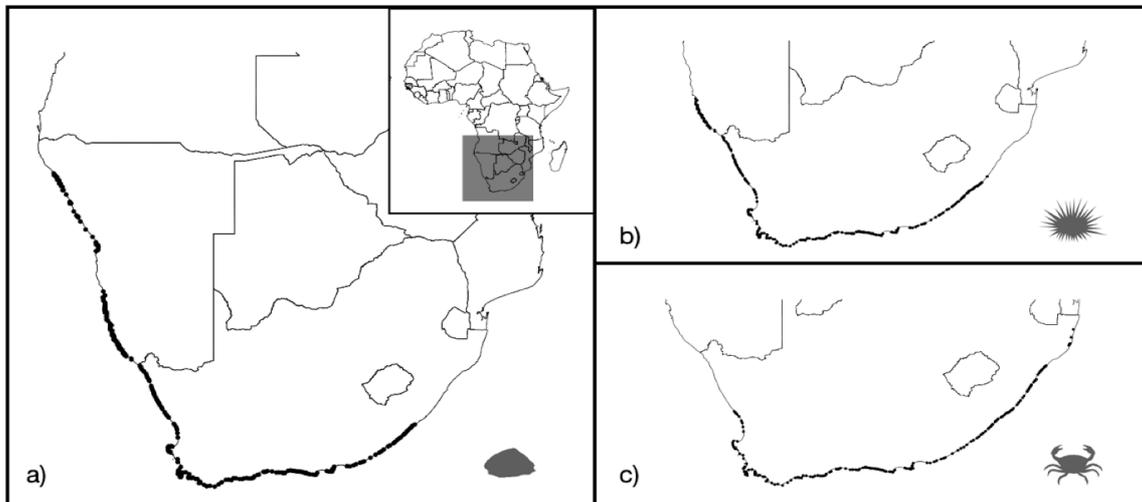


Figure 2.S1.1 – The presence points included in the species’ distribution models, shown for *S. granularis* (a), *P. angulosus* (b), and *C. punctatus* (c).

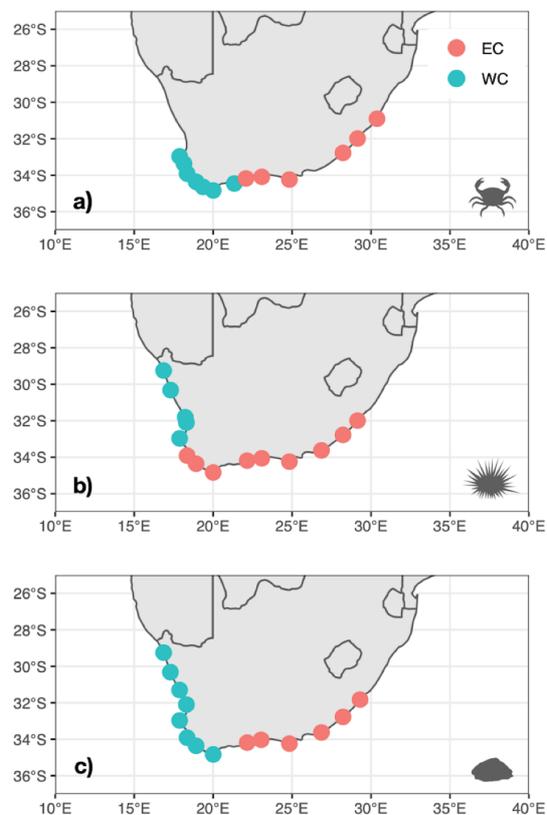


Figure 2.S1.2 – The genomic sample sites, and whether they fall into the west coast (WC) or east (EC) coast lineages for *C. punctatus* (a), *P. angulosus* (b), and *S. granularis* (c). Lineages are from mtDNA data from Muller et al. (2012), Mmonwa et al. (2015), and Wright et al. (2015).

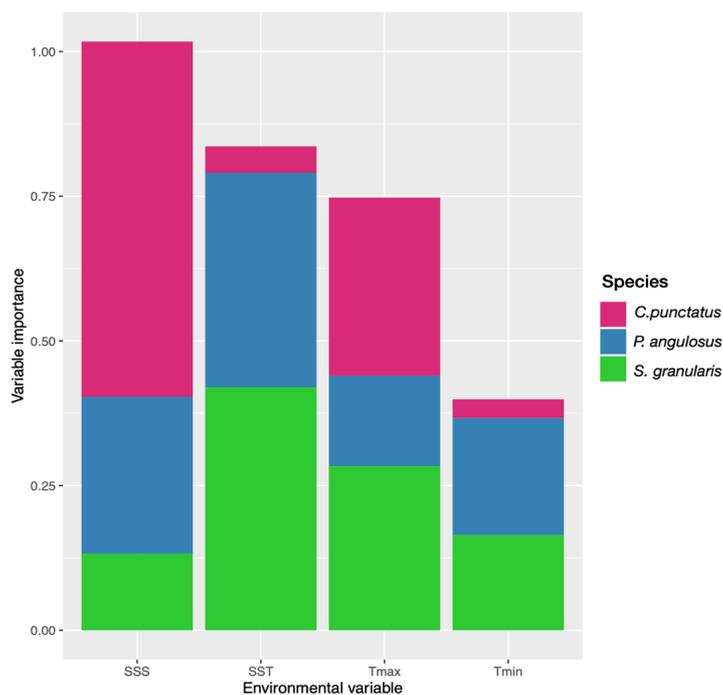


Figure 2.S1.3 – The relative importance of the four environmental predictor variables (mean sea surface salinity – SSS; mean sea surface temperature – SST; maximum air temperature – Tmax; minimum air temperature – Tmin), averaged across all hindcasting species distribution models, shown for *C. punctatus*, *P. angulosus* and *S. granularis*.

Equation 2.S1.1 – Calculation of expected heterozygosity (following Fischer et al., 2017):

$$H_E = (\sum(2 \times \text{MAF}) \times (1 - \text{MAF})) / n\text{MAF}$$

Where MAF is the minor allele frequency per locus, and nMAF is the number of loci per population.

Table 2.S1.1 – The latitude and longitude, and haplotype diversity value (h) for each sample site per species.

Site	Latitude	Longitude	<i>C. punctatus</i>	<i>P. angulosus</i>	<i>S. granularis</i>
PN	16.86	-29.25	-	0.94	0.95
HB	17.26	-30.32	-	0.84	0.86
BB	17.88	-31.30	-	-	0.98
LB	18.31	-32.09	-	0.98	0.95
JB	17.88	-32.97	0.54	0.97	0.87
SP	18.38	-33.92	NA	-	0.76

MP	18.40	-33.90	-	0.92	-
KM	18.32	-34.14	-	0.95	0.95
WP	18.45	-34.13	0.68	0.90	-
RO	18.813	-34.30	0.74	0.90	-
BT	18.93	-34.36	0.79	0.94	-
GB	19.34	-34.58	-	0.96	-
CA	20.01	-34.83	0.79	0.92	-
CI	20.87	-34.44	0.96	-	-
MB	22.09	-34.21	-	-	0.86
HB	22.40	-34.07	0.87	-	-
SB	25.53	-34.04	-	-	0.86
KOS	26.67	-33.70	-	-	0.93
KY	23.09	-34.06	0.86	0.89	-
JFB	24.92	-34.07	-	0.84	-
PE	25.67	-33.99	-	0.85	-
PA	26.86	-33.63	-	0.97	-
HH	28.23	-32.77	-	0.77	-
PSJ	29.54	-31.65	-	0.92	-
MU	30.24	-31.06	0.87	-	-
MG	30.38	-30.90	0.46	-	-

Table 2.S1.2 – The latitude and longitude, and expected heterozygosity value (H_E) for each sample site per species.

Site	Latitude	Longitude	<i>C. punctatus</i>	<i>P. angulosus</i>	<i>S. granularis</i>
PN	16.86	-29.25	-	0.068	0.067
HB	17.26	-30.32	-	0.063	0.072
BB	17.88	-31.30	-	-	0.071
DB	18.23	-31.80	-	0.066	-
LB	18.31	-32.09	-	0.066	0.071

JB	17.88	-32.97	0.071	0.065	0.070
YZ	18.16	-33.37	0.074	-	-
SP	18.38	-33.92	0.074	0.064	0.069
BT	18.93	-34.36	0.077	0.065	0.069
GB	19.34	-34.58	0.076	-	-
CA	20.01	-34.83	0.074	0.065	0.069
JF	21.34	-34.45	0.073	-	-
MB	22.09	-34.21	0.074	0.066	0.063
KY	23.09	-34.06	0.076	0.060	0.069
CF	24.83	-34.24	0.075	0.056	0.069
PA	26.86	-33.63	-	0.065	0.061
HH	28.23	-32.77	0.073	0.063	0.068
CB	29.15	-31.98	0.075	0.062	-
HL	29.31	-31.82	-	-	0.069
MG	30.38	-30.90	0.068	-	-

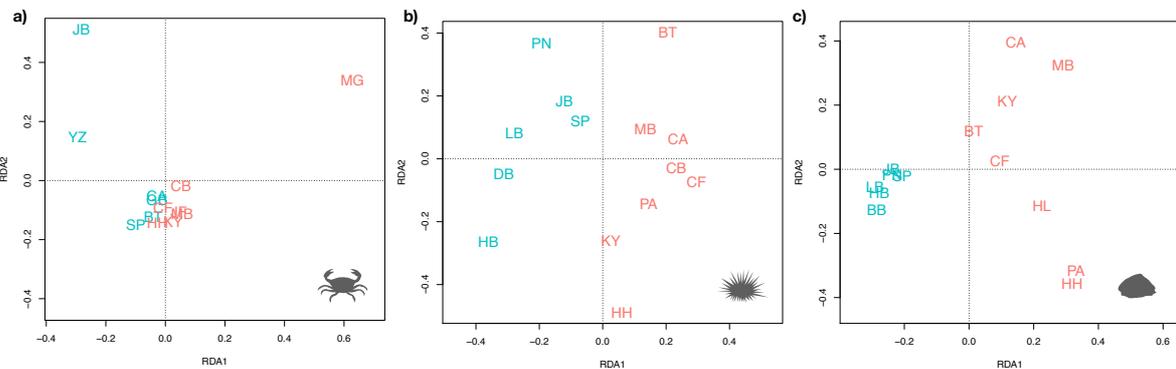


Figure 2.S1.4 – Redundancy analyses based on the allele frequencies used to generate genomic diversity, using the same four environmental predictor variables used in the SDMs of Chapter Two, with western lineage sites shown in blue and eastern sites shown in red for *C. punctatus* (a), *P. angulosus* (b), and *S. granularis* (c).

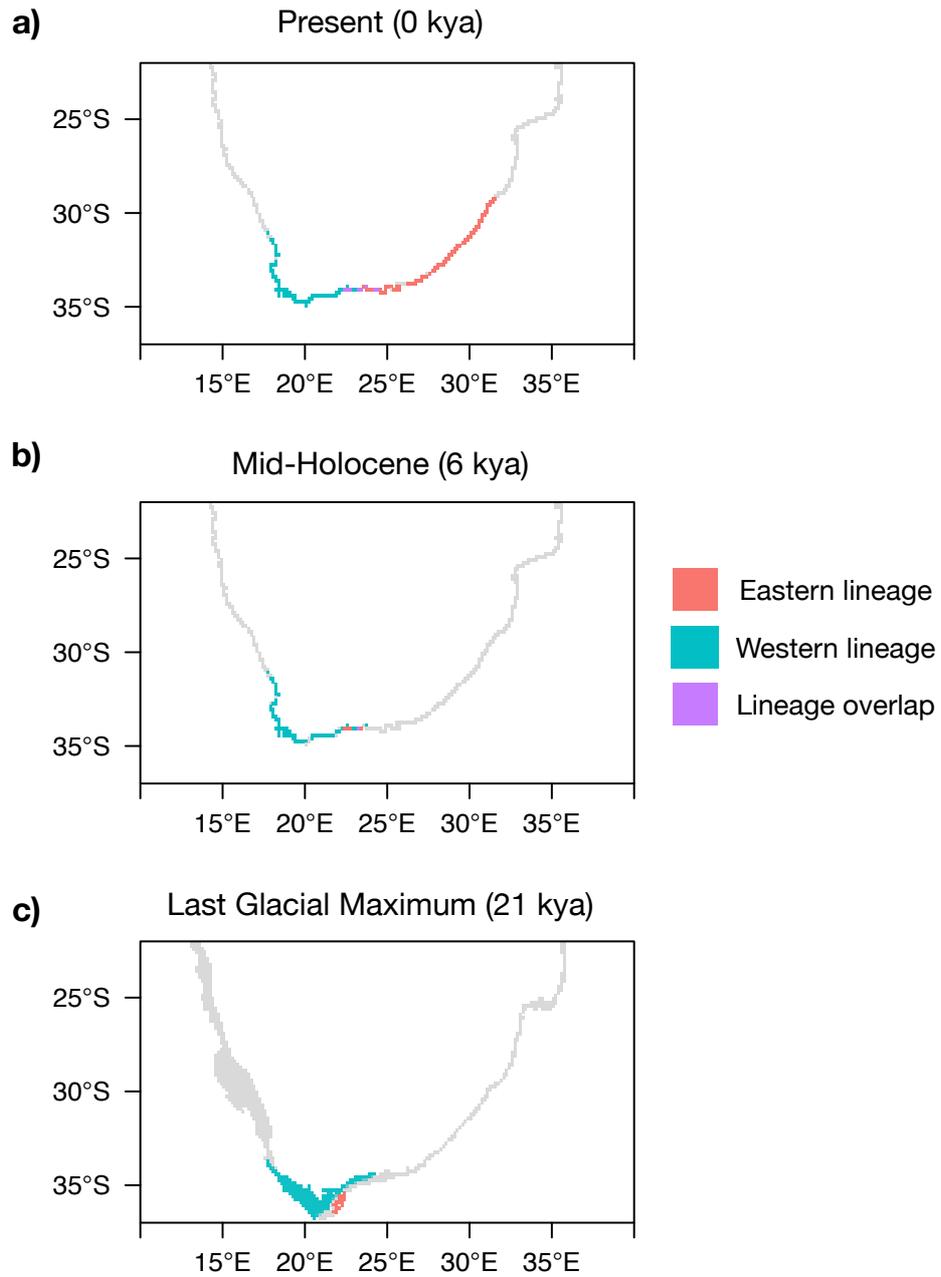


Figure 2.S1.5 – Binary presence outputs from SDMs run on either western or eastern lineage sites, shown for *C. punctatus*, for the present day (a), Mid-Holocene (b), and Last Glacial Maximum (c), at zero, six, and 21 thousand years ago (kya), respectively.

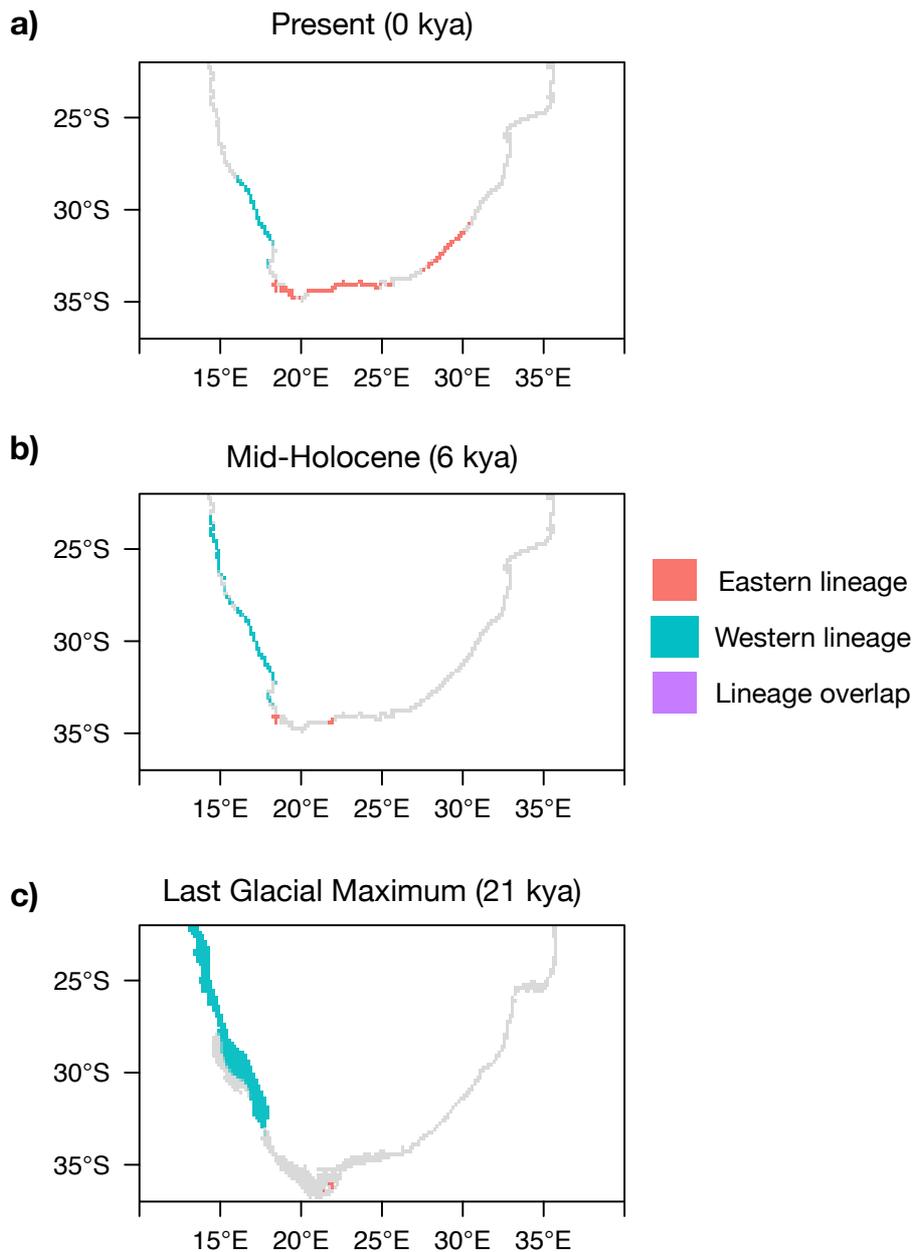


Figure 2.S1.6 – Binary presence outputs from SDMs run on either western or eastern lineage sites, shown for *P. angulosus*, for the present day (a), Mid-Holocene (b), and Last Glacial Maximum (c), at zero, six, and 21 thousand years ago (kya), respectively.

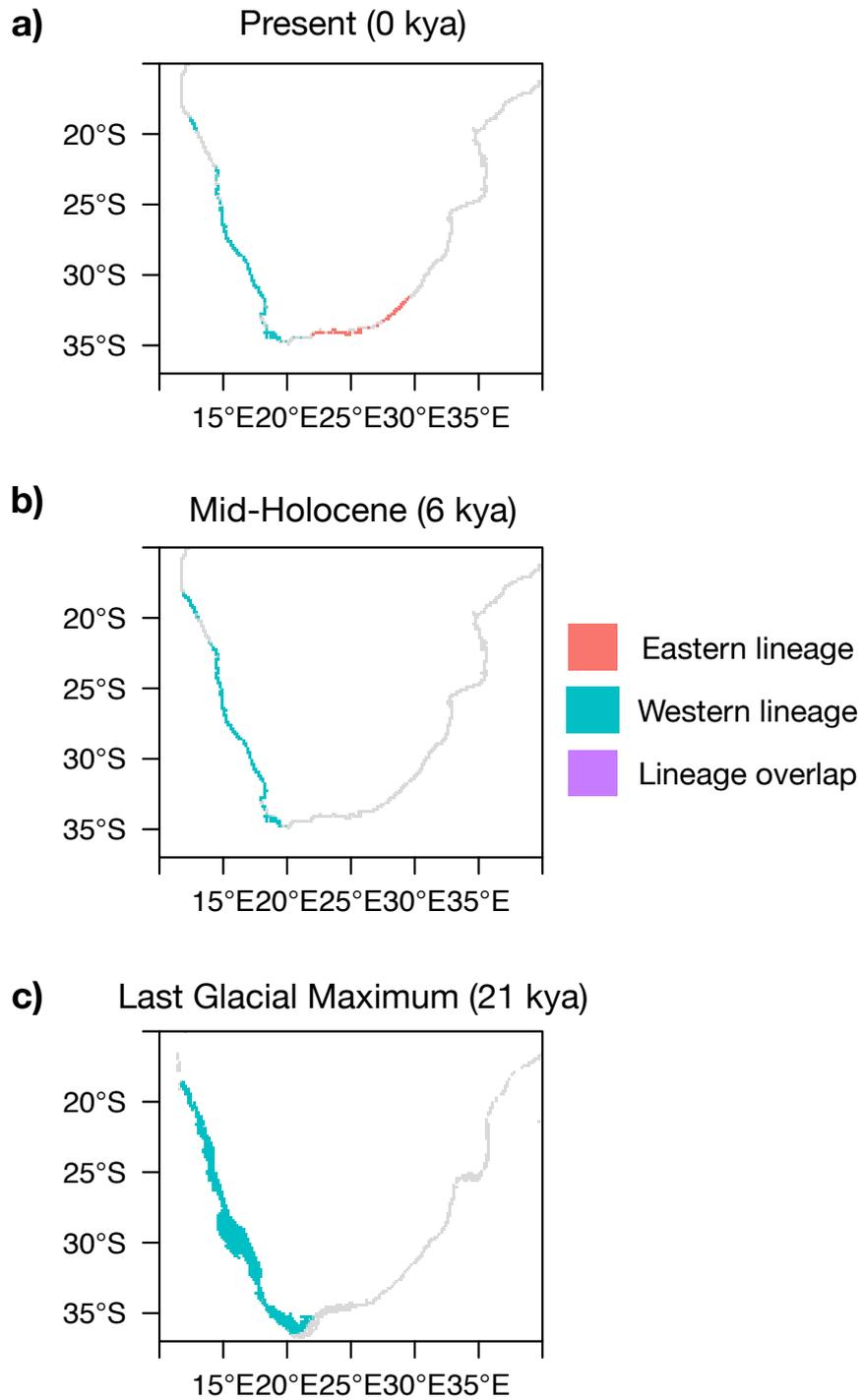


Figure 2.S1.7 – Binary presence outputs from SDMs run on either western or eastern lineage sites, shown for *S. granularis*, for the present day (a), Mid-Holocene (b), and Last Glacial Maximum (c), at zero, six, and 21 thousand years ago (kya), respectively.

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Appendix 2.S2 – Information regarding hindcasting SDM methodology, and model outputs for each species.

Supplementary Methods: Hindcasting distributions to Last Glacial Maximum

Species distribution models (SDMs) were run independently for three rocky shore species, from which only presence data were available. Pseudo-absences were generated within the R package *biomod2* (Thuiller et al., 2013), randomly generating an equal number of absences to presences per species, replicated over five separate runs (Bermejo et al., 2018). SDMs were run within *biomod2*, following an ensemble approach that can significantly improve model accuracy over single models (Araújo & New, 2007; Forester et al., 2013). Six standard model types were used within ensemble models; (1) Flexible Discriminant Analysis (FDA), (2) Generalized Additive Model (GAM), (3) Generalized Boosting Model (GBM), (4) Generalized Linear Model (GLM), (5) Multivariate Adaptive Regression Splines (MARS) and (6) Random Forest (RF; Brown & Yoder 2015; Chefaoui et al., 2016; Engler et al., 2011; Yannic et al., 2014,). Default parameters were used for all algorithms, except for GAM models, which were executed with the GAM ‘mgcv’ function, GBM models which were run with 1,000 trees, and GLM models which were fitted with a quadratic term (Phair et al., 2019). All six statistical model types were used to create the ensemble models for *C. punctatus* and *S. granularis*, and all but the GLM were used in the *P. angulosus* ensemble model, due to the GLM over-estimating the current distribution.

The relative importance of each environmental predictor variable was assessed by averaging the values from the ‘get_variables_importance’ function of *biomod2* across all models. To assess model performance, a random subset of the data was used for calibration

(70%) and validation (30%), with ten evaluation runs and three permutations. Models were scored with an area under the Receiver Operating Characteristic (ROC) curve (AUC; Fielding & Bell, 1997), and True Skill Statistic (TSS; Allouche et al., 2006), with only models having TSS > 0.55 and ROC > 0.8 producing the ensemble model. The ensemble models were combined into a weighted mean SDM used to project the present-day habitat suitability. The ensemble models were conducted using both the CCSM4 and MIROC5 models for the Mid-Holocene and Last Glacial Maximum, the outputs of which were merged using the *raster* R package (Hijmans et al., 2015).

Table 2.S2.1 – The variance inflation factors (VIFs) for mean sea-surface temperature (SST), mean sea-surface salinity (SSS), as well as mean air temperature of warmest and coolest ice-free month (Tmax and Tmin, respectively), calculated for the present day, Mid-Holocene (MH) and Last Glacial Maximum (LGM). The values for MH and LGM are averaged between the MIROC and CCSM General Circulation Models.

Environmental variable	Present	MH	LGM
SST	7.3	7.6	8.4
SSS	1.1	1.4	1.7
Tmax	4.7	5.3	3.4
Tmin	3.8	4.2	3.8

Table 2.S2.2 – The average receiver operating characteristic (ROC) and true skill statistic (TSS) values for each individual model included in the ensemble, as well as for the ensemble model per species. Individual models included the following: (1) Flexible Discriminant Analysis (FDA), (2) Generalized Additive Model (GAM), (3) Generalized Boosting Model (GBM), (4) Generalized Linear Model (GLM), (5) Multivariate Adaptive Regression Splines (MARS) and (6) Random Forest (RF).

Model	<i>C. punctatus</i>		<i>P. angulosus</i>		<i>S. granularis</i>	
	ROC	TSS	ROC	TSS	ROC	TSS
FDA	0.82	0.60	0.83	0.57	0.87	0.63
GLM	0.80	0.59	NA	NA	0.86	0.62
GAM	0.83	0.62	0.84	0.59	0.88	0.66

GBM	0.85	0.65	0.87	0.62	0.88	0.65
RF	0.85	0.64	0.88	0.65	0.90	0.67
MARS	0.83	0.62	0.82	0.57	0.87	0.65
Ensemble	0.92	0.69	0.96	0.75	0.96	0.74

Appendix 2.S2 References:

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Appendix 3.S1 – Individual and ensemble model scores, and input presence points for the forecasting species distribution models (SDMs), and the variable importance in SDMs per species are shown. The points used to generate change in overall environmental space over the SDMs for the convex hulls are also shown.

Table 3.S1.1 – Individual and ensemble model scores, shown as the average Receiver Operating Characteristic (ROC) and True Skill Statistic (TSS) per individual model, namely: Flexible Discriminant Analysis (FDA), Generalized Additive Model (GAM), Generalized Boosting Model (GBM), Generalized Linear Model (GLM), Multivariate Adaptive Regression Splines (MARS) and Random Forest (RF). Models which were not included in the ensemble are represented by ‘NA’.

	<i>C. punctatus</i>		<i>P. angulosus</i>		<i>S. granularis</i>	
Model	ROC	TSS	ROC	TSS	ROC	TSS
FDA	0.81	0.60	NA	NA	0.88	0.73
GLM	0.81	0.59	NA	NA	0.88	0.69
GAM	0.83	0.63	0.88	0.68	0.89	0.75
GBM	0.83	0.61	0.89	0.69	0.89	0.74
RF	0.83	0.61	0.89	0.69	0.90	0.74
MARS	0.81	0.61	0.86	0.66	0.88	0.74
Ensemble	0.92	0.66	0.96	0.84	0.96	0.77

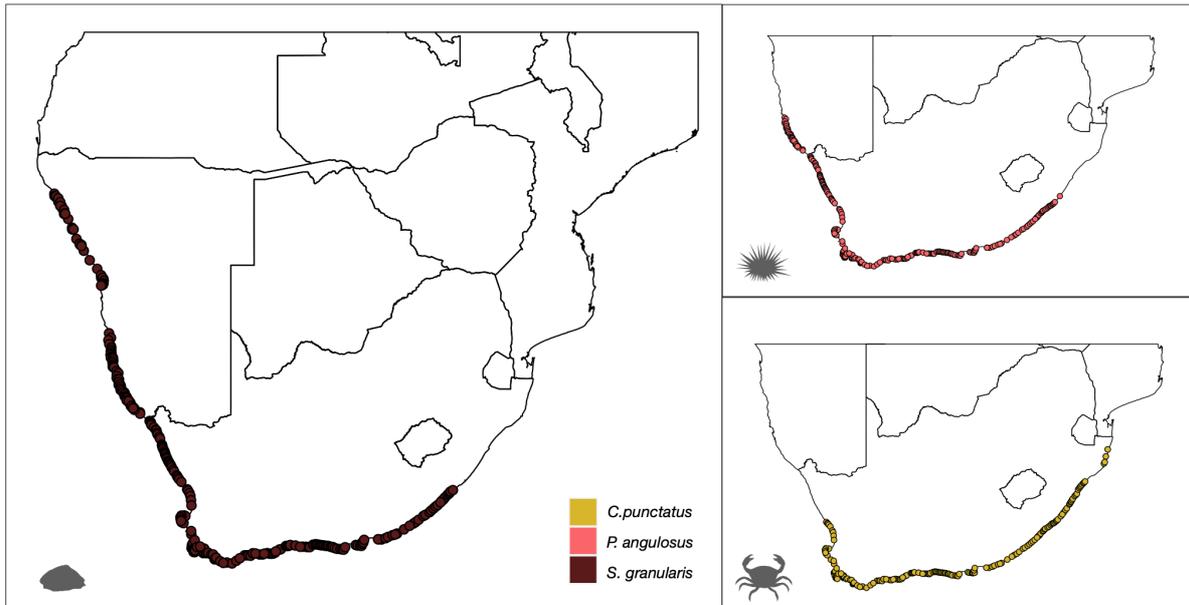


Figure 3.S1.1 – The input presence coordinates for the species distribution models, from rocky shore habitat (spaced at 10km intervals) within the known distributions of each species.

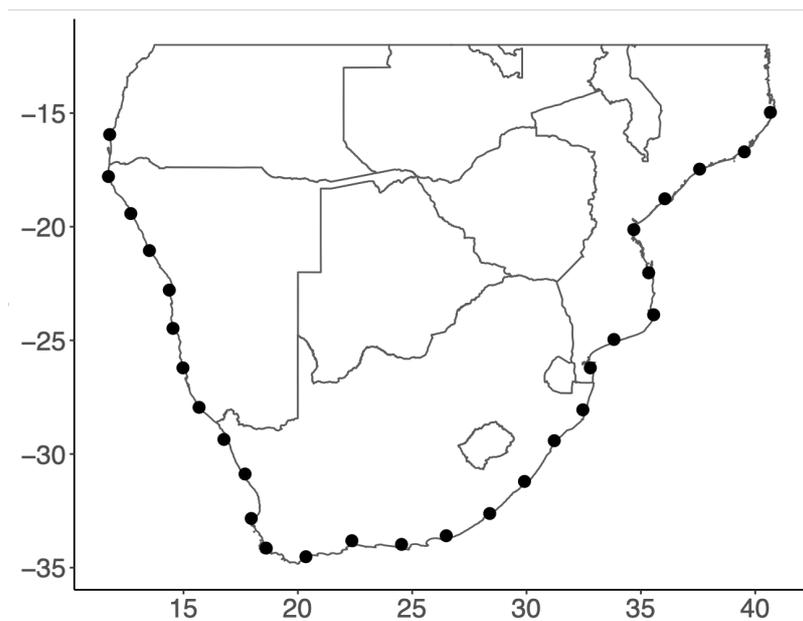


Figure 3.S1.2 – The localities from which environmental data was extracted to create two-dimensional environmental hulls across time points.

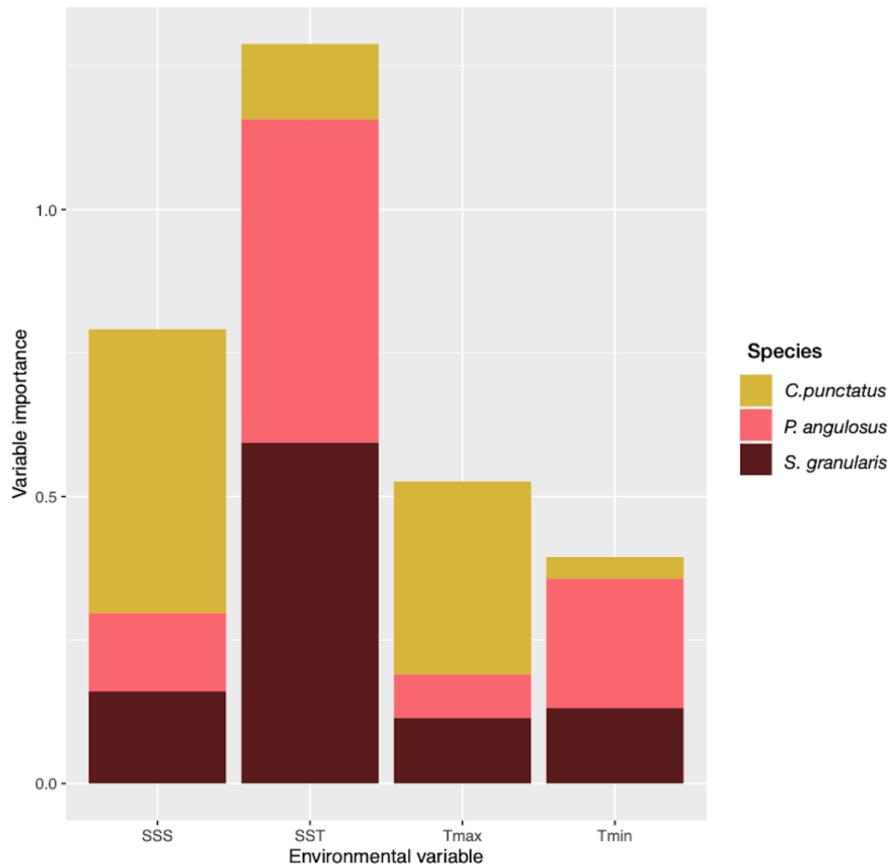


Figure 3.S1.3 – The importance of each environmental variable in describing the distributions of *C. punctatus*, *P. angulosus*, and *S. granularis* within the species distribution model ensembles. The four environmental variables are as follows: mean sea-surface salinity (SSS), mean sea-surface temperature (SST), maximum air temperature (Tmax), and minimum air temperature (Tmin).

Appendix 3.S2 – The number of loci and mean R² of those loci in the Gradient Forest (GF) models, cumulative importance curve outputs from GF models, Principal Components Analyses of allele frequencies from the subset of SNPs included in the GF models, difference in spatial genomic turnover patterns between putatively neutral and outlier SNPs, and genomic vulnerability of neutral SNPs, are shown per species.

Table 3.S2.1 – The number (#) of loci which had a R² value greater than (>) zero, and the mean R² values from the Gradient Forest models run on putatively neutral, or statistically outlier allele datasets, for *C. punctatus*, *P. angulosus*, and *S. granularis*.

	<i>C. punctatus</i>	<i>P. angulosus</i>	<i>S. granularis</i>
# of neutral loci with R² > 0	149	111	340
# of outlier loci with R² > 0	3	6	14
Mean R² of neutral loci	0.154	0.162	0.206
Mean R² of outlier loci	0.283	0.442	0.390

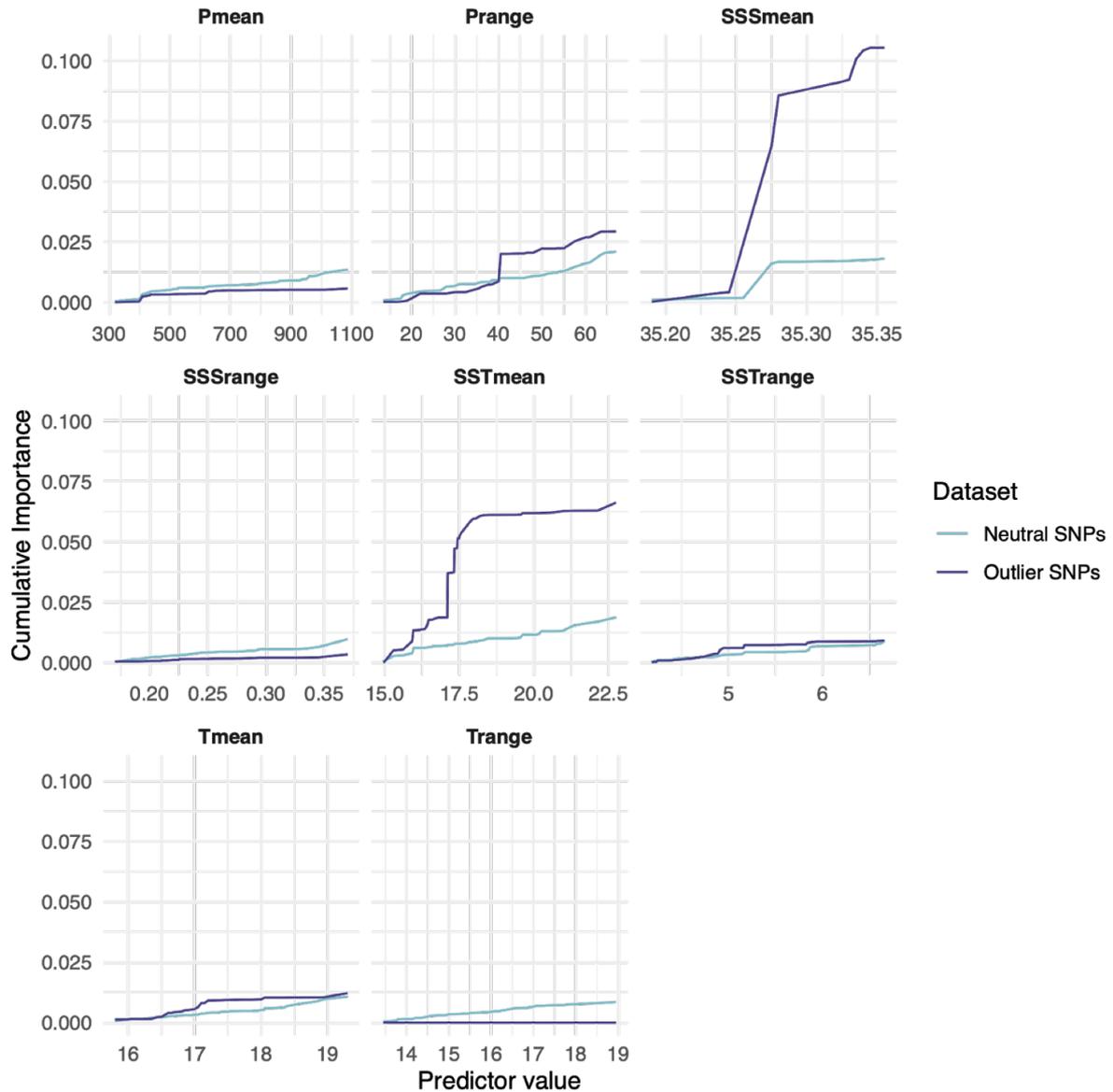


Figure 3.S2.1 – Cumulative importance curves based on the turnover functions from each environmental variable in the Gradient Forest models for the crab, *C. punctatus*. The curve shape indicates the rate of change in allele frequencies along the environmental gradient, and the maximum height indicates the total turnover in allele frequencies. Curves are shown for both neutral and outlier datasets, and the number of loci within these datasets is shown in Table S1.

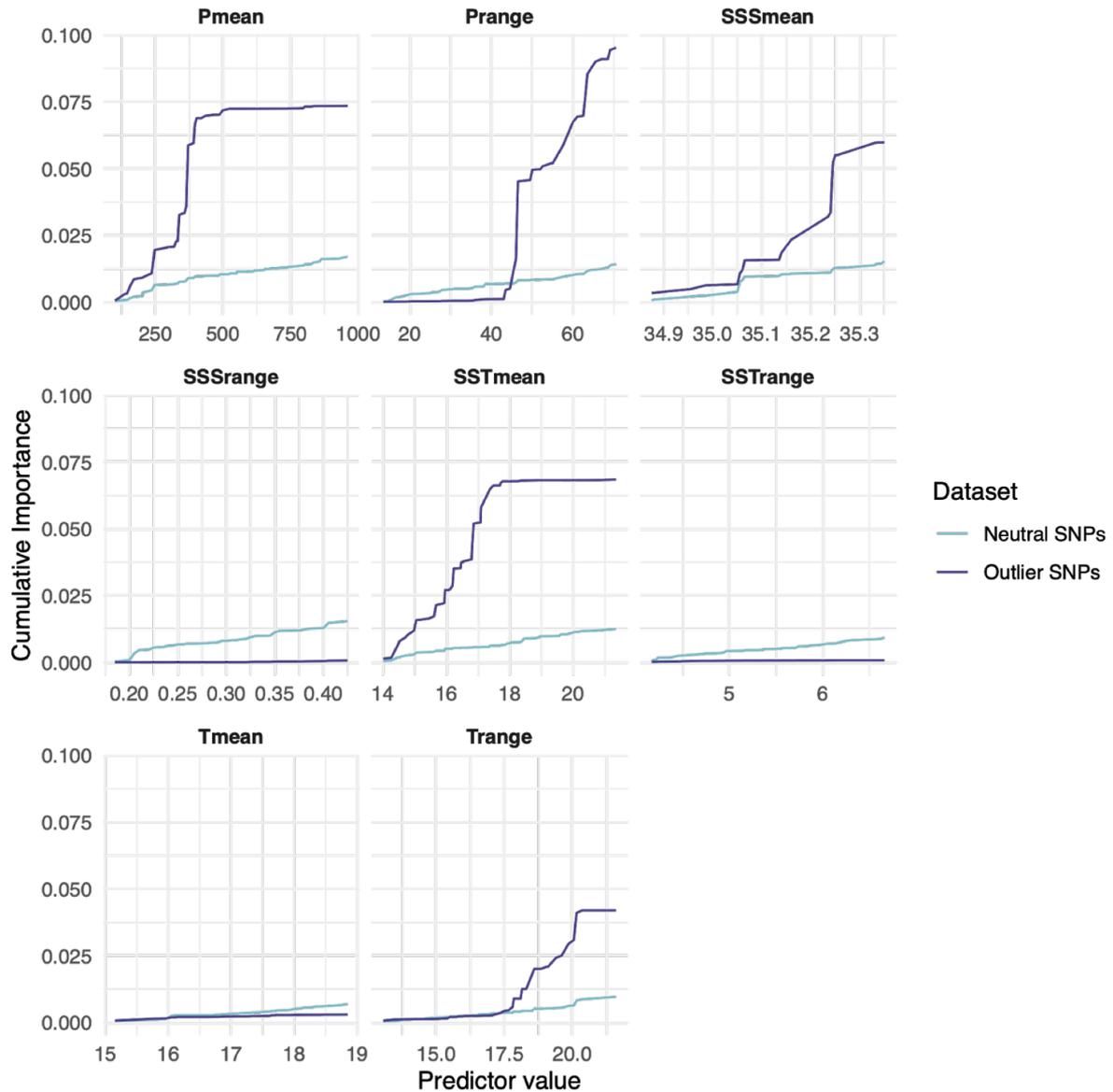


Figure 3.S2.2 – Cumulative importance curves based on the turnover functions from each environmental variable in the Gradient Forest models for the urchin, *P. angulosus*. The curve shape indicates the rate of change in allele frequencies along the environmental gradient, and the maximum height indicates the total turnover in allele frequencies. Curves are shown for both neutral and outlier datasets, and the number of loci within these datasets is shown in Table S1.

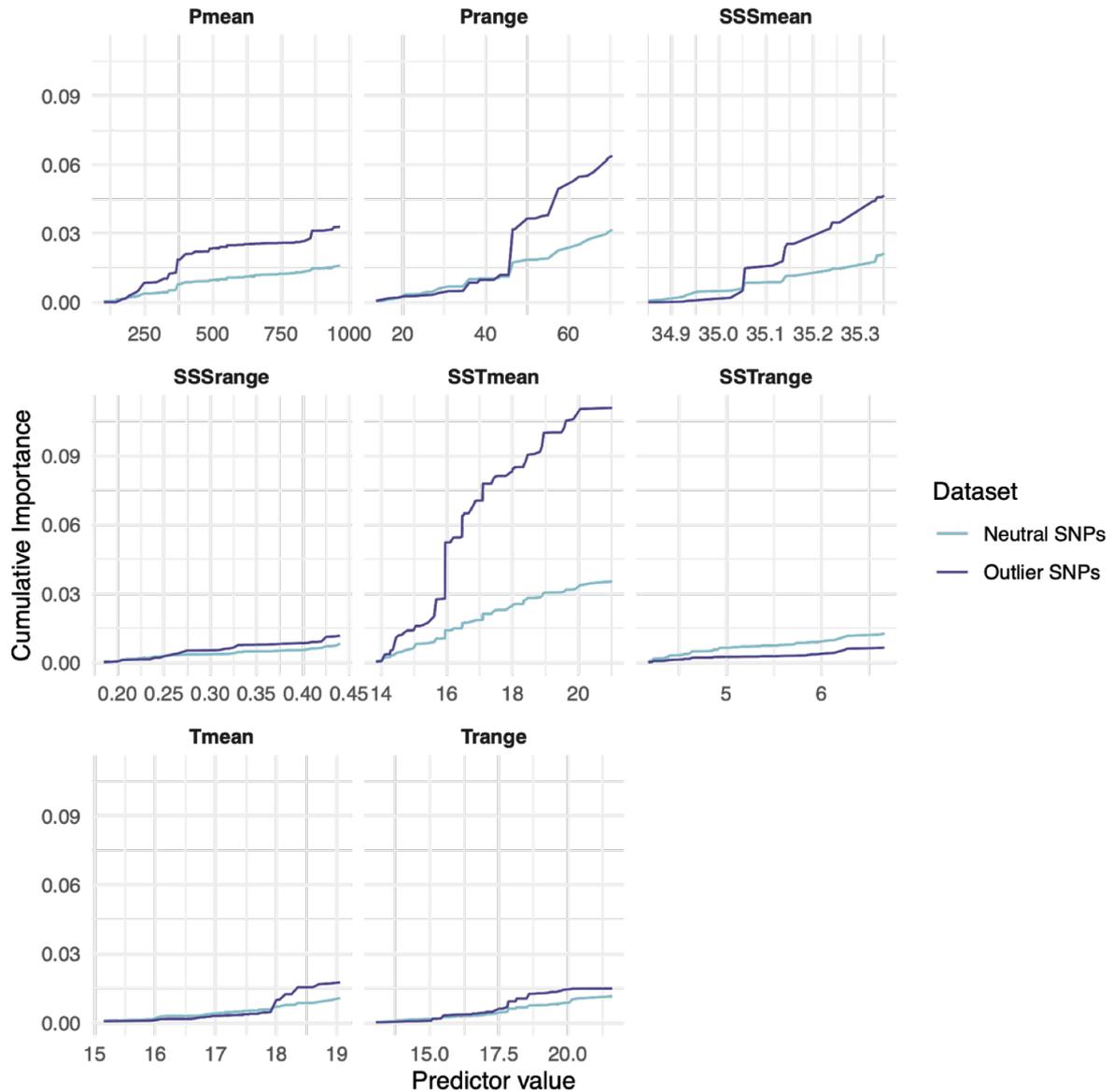


Figure 3.S2.3 – Cumulative importance curves based on the turnover functions from each environmental variable in the Gradient Forest models for the limpet, *S. granularis*. The curve shape indicates the rate of change in allele frequencies along the environmental gradient, and the maximum height indicates the total turnover in allele frequencies. Curves are shown for both neutral and outlier datasets, and the number of loci within these datasets is shown in Table S1.

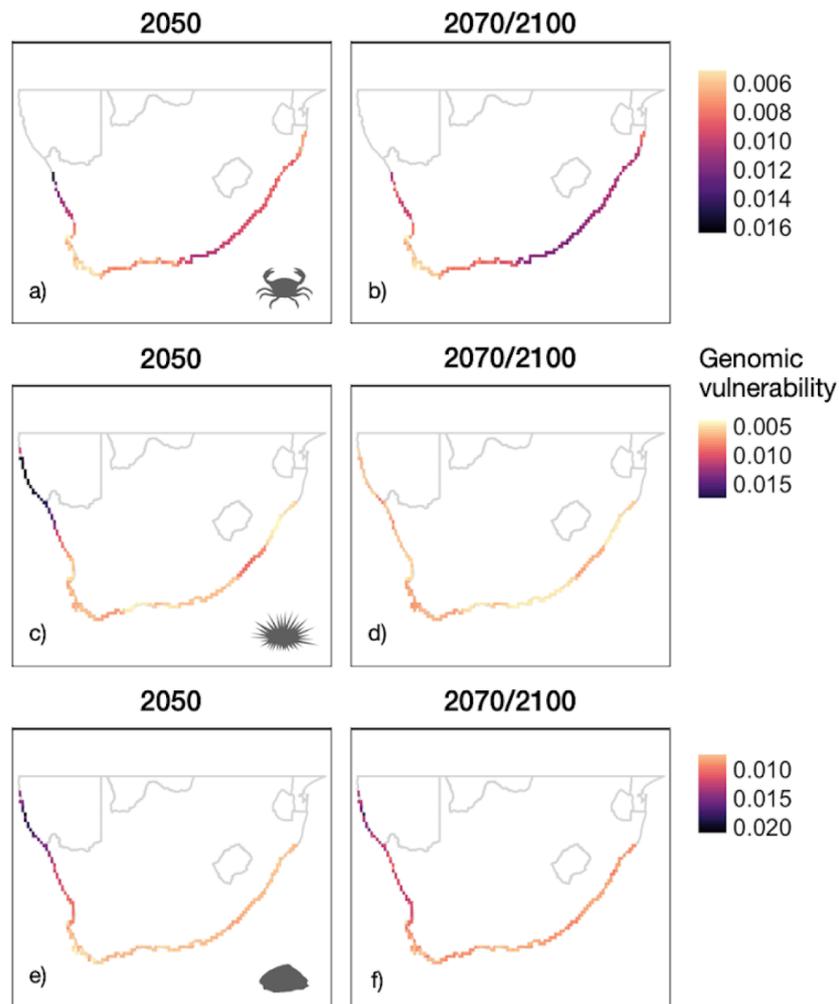


Figure 3.S2.6 – Genomic vulnerability patterns of neutral SNPs, calculated as Euclidean distance between current and future genetic spaces, averaged over RCP 4.5 and 8.5 scenarios for 2050 (a, c, e), and 2070/2100 (b, d, f; 2070 for landscape features, 2100 for seascape features), for *C. punctatus* (a, b), *P. angulosus* (c, d), and *S. granularis* (e, f). Darker shaded regions represent areas of higher genomic vulnerability, where genomic composition will be altered the most to track environmental changes.

The End

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“We do not inherit the earth from our ancestors. We borrow it from our children.” – Native American Proverb

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