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Integrating genetics into marine conservation planning in South Africa

MSc Thesis
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March 2017

Declaration

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Chapter One is accepted in *Conservation Biology* - please see declaration of authors contribution on the last page of this chapter.

Erica Spotswood Nielsen

March 2017

Abstract

The mounting threats to biodiversity and global alteration of habitat and species distributions make it increasingly necessary to consider evolutionary patterns in conservation decision-making. Yet there is no clear-cut guidance on how genetic features can be incorporated into conservation planning processes, with several genetic metrics with different ecological and evolutionary relevance to choose from. Genetic patterns also differ between species, but the potential trade-offs amongst different genetic objectives for multiple species in conservation planning are currently understudied. Therefore, the first chapter of this thesis compares spatial conservation prioritizations derived from two metrics of both genetic diversity (nucleotide and haplotype diversity) and genetic isolation (private haplotypes and local genetic differentiation) for five marine species. The results from Chapter One show that conservation plans based solely on habitat representation noticeably differ from those additionally including genetic data, and that all four genetic metrics select similar conservation priority areas. The second chapter builds on the findings of Chapter One by comparing conservation solutions from three marker types (mitochondrial DNA, neutral nuclear DNA, and adaptive nuclear DNA) for the two most genetically distinct species from the multi-species data set. Next generation sequencing was used to identify single nucleotide polymorphism (SNP) variation in both the Cape urchin (*P. angulosus*) and the Granular limpet (*S. granularis*), both of which showed high levels of genomic diversity and signals of adaptation to local ecotypes. When comparing the genetic variation between the mitochondrial DNA (mtDNA) and SNP markers within a spatial conservation framework, the solutions show a wide range of spatial priorities, yet the spatial similarities between the different marker types are not consistent across the different species approaches. Largely, the findings from this project suggest that selected species and genetic marker(s) chosen will alter all conservation solutions. Importantly, increasing the amount of genetic information leads to more distinct conservation priorities, resulting in a clearer picture of community-level evolutionary hotspots within the planning region.

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Introduction

Nearshore marine environments not only have aesthetic, cultural and natural value, but also provide a range of goods and services to humans. Consequently, owing to their close proximity to human populations, coastal and nearshore ecosystems are exposed to increased anthropogenic pressures (Mead et al. 2013, McCauly et al. 2015). Such pressures are expanding within South Africa with the advent of Operation Phakisa in 2014, which states multiple objectives focused on maximizing the blue economy by increasing aquaculture farming, marine transportation, and oil and gas exploitation in the region (http://www.operationphakisa.gov.za/). To counteract the increase in anthropogenic activities within the South African marine realm, further marine protection is required. Establishing Marine Protected Areas (MPAs) is arguably one of the most successful strategy to sustain marine resources and protect marine biodiversity (Lubchenco et al. 2003), along with other strategies such as effective fisheries management (Hilborn et al. 2004). Yet the effectiveness of an MPA in reaching its objectives depends on several factors such as a clear definition of its objectives, along with effective management, enforcement and design. In order to design an effective MPA, it is essential to include not only current patterns of biodiversity, but also predict how these patterns may evolve in a changing world (Myers & Knoll 2001, Pressey et al. 2007).

In the past, conservation prioritization was mostly based on patterns of species abundance or on protection of economically important species (Hockey & Branch 1997), but within the past few decades it was recognized that these measures alone fail to represent the evolutionary history of species (Cowling & Pressey 2003, Lombard et al. 2003, Rouget et al. 2003, 2005, Pio et al. 2011). It is now accepted that the evolutionary processes that have shaped extant patterns of biodiversity may also shape future biodiversity patterns, and therefore require protection (Cowling et al. 1999, Desmet et al. 2002, Rouget et al. 2003, Klein et al. 2009). Therefore, to ensure that marine biodiversity and ecosystem functions continue to benefit both natural systems and society, it is crucial to understand the evolutionary processes that have created the diverse and unique marine life of South Africa.

The South African seascape

Biodiversity & oceanographic characteristics of South Africa

South Africa is home to one of the most environmentally and biologically diverse coastlines in the world, with over 12,000 species identified (Griffiths et al. 2010). Of these, approximately 30% are endemic, making the region one of the most unique on earth (Costello et al. 2010). Further, much of the biodiversity is yet to be described, with estimates of up to 25% of endemic fish species remaining unknown to science (von der Heyden 2011). Broadly, species endemism increases from west to east, with a peak along the south coast (Awad et al. 2002, Scott et al. 2010). Species richness portrays a similar pattern, with the south and east coasts supporting more diverse communities compared to the west coast (Griffiths et al. 2010).

High levels of species richness and endemism are in part driven by the atmospheric and oceanographic regimes of the South African coastline. South Africa is situated at the contact zone between the warm Indian Ocean and the cool Atlantic Ocean (Fig. 1). The Indian Ocean feeds the Agulhas Current, consisting of warm, nutrient poor water flowing southward along the East Coast from Mozambique to Cape Agulhas (Schumann & van Heerden 1988, Lutjeharms 1989). The Agulhas Current then retroflects offshore near Cape Agulhas and creates an eastward flowing countercurrent (Lutjeharms 1981, Hutchings et al. 2009). The west coast is dominated by the Benguela Current, which flows from Cape Point northward towards Namibia and is characterized by its cool-temperate, nutrient rich water (Andrews & Hutchings 1980, Hutchings et al. 2009).

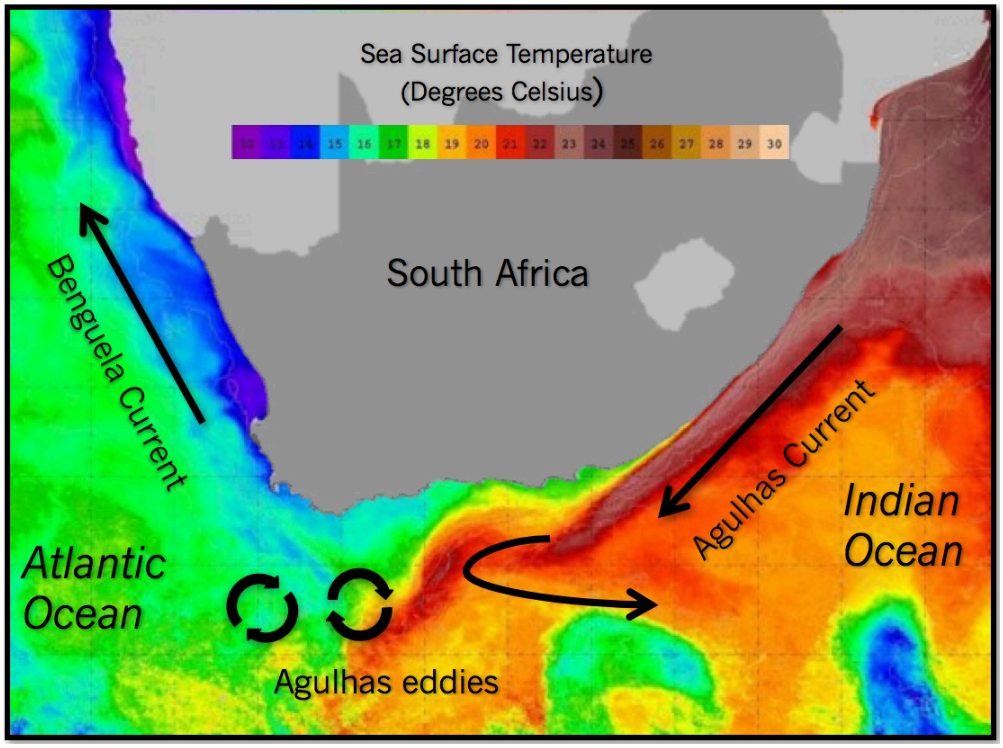


Figure 1- The sea surface temperatures (World Ocean Database 2009) and major currents of South Africa.

Within the Benguela system there is wind-driven upwelling, which occurs between September and March in the southern sub-system, and throughout the year in the northern sub-system (Peterson & Stramma 1991, Rae et al. 2005). Upwelling systems transport surface water offshore and replace the surface with cool, nutrient rich water from greater depths. The supply of nutrients to the euphotic zone increases overall productivity and sustains high fishery intakes (Pauly & Christensen 1995). Nutrient transport between the two currents is facilitated by the Agulhas eddies, which originate off of Cape Agulhas and connect the Indian Ocean with the Atlantic Ocean (Andrews & Hutchings 1980, Richardson et al. 2003). Eddie systems are also capable of entrapping and translocating marine organisms, such as zooplankton (Mackas & Galbraith 2002) and fish larvae (Lobel 2011).

Biogeographic & phylogeographic patterns along a dynamic coastline

The South African coastline can be divided into five distinct biogeographic zones: Namaqualand, South western Cape, Agulhas, Natal and Delagoa (Lombard et al. 2004; Fig. 2), with each region reflecting differences in temperature, nutrients,

productivity and species composition (Sink et al. 2004). The five inshore biogeographic regions can be further classified into 21 biozones, consisting of habitats such as kelp forest, estuaries, coral reefs, as well as rocky and sandy shores (Sink et al. 2012). The breaks between these biogeographic boundaries are not abrupt, but instead consist of transition zones (with the exception of Cape Point), where flora and fauna overlap along the coastline. For some species, these transition zones coincide with genetic breaks, which indicate an interruption in gene flow between coastal and estuarine populations (Teske et al. 2006, Teske et al. 2011). However, some marine species show either no genetic variation across these bioregions, or genetic breaks that do not correlate with the known transition zones (Teske et al. 2011). The spatial distribution of genetic variation within or among closely related species is referred to as phylogeography, and thus a genetic break across a biogeographic region is known as a phylogeographic break (Avise 2009).

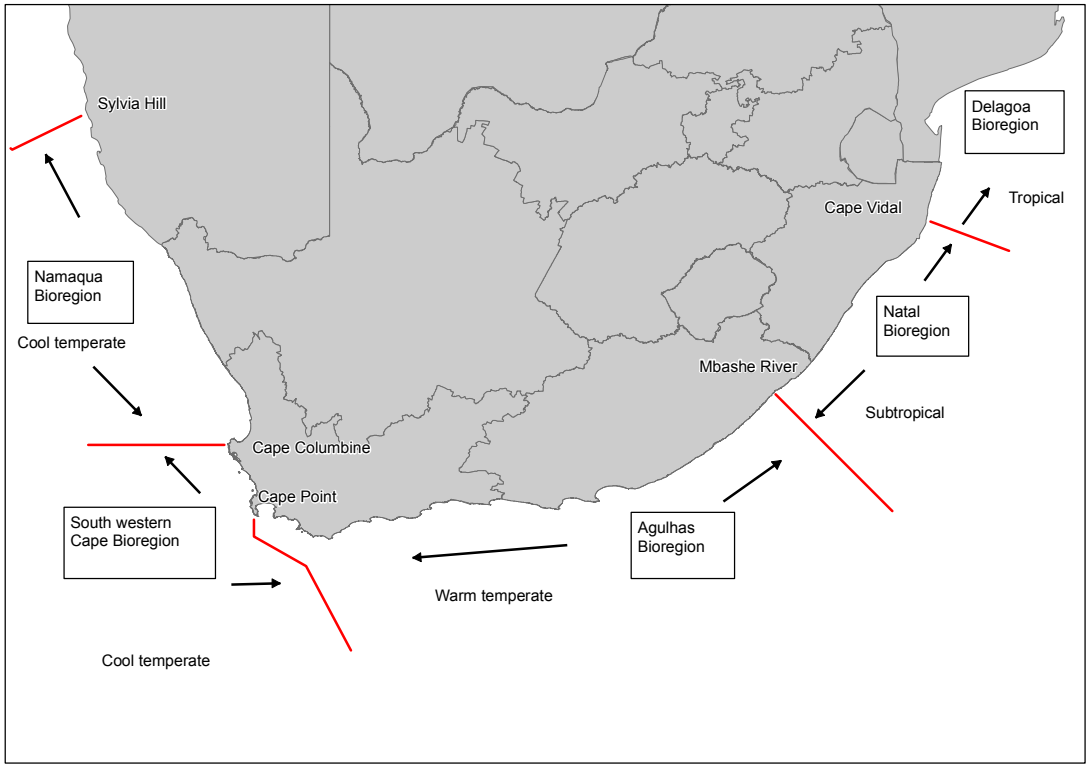


Figure 2 - The five inshore South African bioregions(separated by the red lines) and associated temperature regimes (base map downloaded from www.esri-southafrica.com/basemaps).

Phylogeographic breaks across multiple species can be compared to assess the overall gene flow patterns within a targeted area (Avise 2009). Within the South African

marine environment, studies show significant phylogeographic breaks at Cape Point, Cape Agulhas, Algoa Bay, central Wild Coast, and St. Lucia (von der Heyden 2009, Teske et al. 2011). While the processes that produce phylogeographic breaks within South Africa are not well understood, some studies provide evidence of oceanographic and climate oscillations shaping population divergence (Teske et al. 2007, Reynolds et al. 2010, Muller et al. 2012, Toms et al. 2014). For instance, Toms et al. (2014) found that lowered sea levels during glacial periods correspond with the molecular dating of divergence within two divergent lineages of the klipfish *Clinus cottoides,* which may be a result of reduced rocky intertidal habitats during sea level changes.

Historical events may create phylogeographic breaks, but contemporary processes such as oceanographic systems, behavioral mechanisms, changes in habitat availability, life history traits and local adaptation are necessary to maintain them (Patarnello et al. 2007, Pelc et al. 2009, Teske et al. 2011). Ocean currents, previously believed to enhance larval transport, are now suggested to also hinder dispersal with systems such as upwelling and downwelling eddies (Beckley et al. 1995, Gaylord & Gaines 2000, Gaines et al. 2003, Henriques et al. 2012, 2014a, b). Behavioral mechanisms such as lunar or tidal associated spawning (Lobel 1989, Skov et al. 2005, Gladstone 2007) and larval swimming (Leis 2005, Fiksen 2007, Paris et al. 2007) may also increase larval retention within spawning sites. A key life history trait associated with dispersal and recruitment is pelagic larval duration (PLD). PLD was originally considered to positively correlate positively with dispersal range and gene flow, yet it is currently disputed as a substandard predictor of genetic connectivity between populations (Weersing & Toonen 2009, Selkoe et al. 2014). However, while PLD may provide poor genetic estimates for brooders and spawners, life history traits are shown to be stronger predictors of genetic structure in live-bearing species (Kelly & Palumbi 2010, Selkoe & Toonen 2011, Wright et al. 2015). Lastly, if there is local adaptation across phylogeographic breaks, selection forces may be driving genetic differentiation, with temperature and sand inundation as possible drivers in South Africa (Teske et al. 2008, Teske et al. 2011). These processes are involved in the shaping the evolutionary trajectories of South African marine fauna and may contribute towards maintaining high levels of biodiversity over the relatively short coastline of ~3700km.

Designing marine reserves: A global and local perspective

A review of marine protected area design

Human activities such as overfishing, introduction of alien species, habitat alteration and human mediated climate change are causing irreparable damage to marine environments (McCauley et al. 2015). Owing to difficulties in moderating human activities, physical solutions to resource management issues are often adopted. Thus, MPAs are commonly implemented to counteract threats against ecosystem and species biodiversity, and to sustain resource extraction (Pauly et al. 2002, Botsford et al. 2003). The International Union for Conservation of Nature (IUCN) defines an MPA as "any area of intertidal or subtidal terrain, together with its overlying water and associated flora, fauna, historical, and cultural features, which has been reserved by law or other effective means to protect part or all of the enclosed environment". MPAs vary in protection level, terminology and management type, and are often a combination of coastal sanctuaries, refuges, ecological reserves, natural monuments and marine parks (Hyrenbach et al. 2000).

Multiple studies have evaluated the efficacy of MPAs, finding that they can be effective at maintaining biological diversity, whilst also sustaining fishery yields (Lubchenco et al. 2003, Kleczkowski et al. 2008, Barrett et al. 2009, Harrison et al. 2012, Kerwath et al. 2013). Further, MPAs were also found to be successful in preserving habitat condition (Selig & Bruno 2010), supplying spill-over of individuals into other MPAs and unprotected waters (Christie et al. 2010), and mitigating marine diseases (Lamb et al .2016). A study conducted by Lester et al. (2009) reviewed 124 MPAs in 29 countries to find that, on average, MPAs increased biomass, numerical density, species richness and organism size within their boundaries, regardless of MPA size. Within South Africa, MPAs were found to enhance the sustainability of commercially exploited fish species (Roberts et al. 2005) as well as facilitate the recovery of surf-zone fish assemblages after exploitation (Bennett & Attwood 1991).

Even though the formerly mentioned studies suggest that MPAs are effective at protecting marine biodiversity, they often fail to meet conservation objectives (Jameson et al. 2002, Mora et al. 2006). In contrast to the study by Lester et al. (2009), an empirical meta-analysis by Halpern (2003) found that out of 70 MPAs, 37% produced

no increase in population densities, 4–10% produced no increase in the average organism biomass, 11–20% produced no increase in mean organism size, and 24–41% produced no increase in species diversity. There are multiple reasons why an MPA may fail to reach its biodiversity objectives, such as lack of enforcement, degradation of the surrounding unprotected ecosystems, natural or human induced catastrophes, or a poor design to begin with (Agardy et al. 2011). Arguably one of the most important aspects to ensure that MPAs meet their defined objectives is an adequate and representative design, but unfortunately, there is no 'blueprint' for designing MPAs (Burgess et al. 2014, Rossiter & Levine 2014). As MPAs serve a multitude of purposes, the size, shape, and implementation will be a function of the primary objectives that it sets out to achieve (Agardy 2000).

While the number, size and shape of MPAs are dependent on their specific conservation objectives, there are certain characteristics that lead to more effective designs. For instance, it is widely accepted that a network of small, well connected MPAs is an optimal design to increase species diversity and fishery benefits (Neubert 2003, Costello & Polasky 2008, Planes et al. 2009, Gaines et al. 2010). By definition, a MPA network implies that the reserves are connected via movement of individuals between them, whether it is by larval, juvenile or adult migration, ontogenetic movement or adjacency (Jones 2008). Connectivity between reserves can be further classified as either demographic or genetic, both of which are recognized as important processes in maintaining contemporary biodiversity and population viability, as well as providing resilience to habitat alteration and climate change (Sgrò et al. 2011).

Demographically-connected populations are those where population dynamics (e.g. growth, survival, and birth rates) are affected by immigration or emigration (Mills 2007). Demographic connectivity helps MPAs buffer ecological or environmental uncertainty by enhancing meta-population resilience to recruitment stochasticity (Crowder et al. 2000, Allison et al. 2003, Planes et al. 2009). For example, if an environmental disaster occurs in one reserve, populations can be replenished from another unaffected reserve. The benefits of ontogenetic connectivity were evaluated in an empirical study by Olds et al. (2011), which revealed that connectivity increased fish (piscivore and herbivore) abundance within the reserves compared to non-reserve waters, in contrast to isolated reserve habitats, which showed fish abundances similar to those in non-reserve areas.

In contrast, genetically connected populations are those linked by gene flow

(Lowe et al. 2010, Baco et al. 2016), which can be achieved by a lower number of successful dispersers than that required for demographic connectivity (Slatkin 1987). A network of genetically connected MPAs may help maintain the genetic diversity of protected populations because the MPAs can act as 'stepping stones' for dispersal and gene flow (Hellberg et al. 2002). To illustrate, the allelic richness of the commercially exploited fish *Diplodus sargus* (White seabream) was found to be greater within populations from two small closely-spaced MPAs compared to those in surrounding unprotected waters (Pérez-Ruzafa et al. 2006). Further, a study by Bell & Okamura (2005) found evidence of inbreeding and decreased genetic diversity in the gastropod *Nucella lapillus* within a single isolated marine reserve. Currently, there remains a lack of studies assessing how a network of MPAs compares to a single reserve in effectively maintaining genetic diversity.

Despite the presumed benefits of a connected reserve network, most MPA designs are based on local geography, biodiversity patterns and ecological knowledge, rather than marine connectivity (Salm et al. 2000, Sale et al. 2005, Botsford et al. 2009, Tulloch et al. 2016). This is largely owing to difficulties in quantifying dispersal in the marine realm, as well as the overall variability in the dispersal ranges of marine organisms (Levin 2006, Baco et al. 2016). Within marine environments, dispersal often occurs during larval stages, which makes observational and mark-and-recapture methods unfeasible for many marine organisms (Shanks et al. 2003). In this context, studies have utilized a variety of methods such as tagging, elemental fingerprinting, and genetic monitoring to find estimates of dispersal distances varying from a few kilometers to hundreds of kilometers (Palumbi 2003, Levin 2006).

For example, Kinlan and Gaines (2003) used an isolation-by-distance (IBD) model (which relates genetic differentiation to geographic distance) incorporating over 60 marine species to reveal dispersal distances ranging from ~10- 500km, with marine invertebrates displaying the highest variability. In contrast, a review by Jones et al. (2009) found that corals and reef fishes show significantly shorter dispersal ranges, advocating reserves spaced 1-50km apart. In South Africa, an analysis of genetic dispersal estimates showed that dispersal is probably at the scale of tens, rather than 100s of kilometers (Wright et al. 2015). These studies, along with others (e.g. Cowen & Sponaugle 2009, Pelc et al. 2010, Pinsky et al. 2010, Berumen et al. 2012), illustrate the diversity in dispersal ranges of marine fauna, therefore making it difficult to ensure connectivity across a variety of species. Designing MPAs with regards to genetic

connectivity is also complicated by the fact that dispersal is usually only estimated from a small portion of the entire population and over a limited time scale (Pinsky et al. 2010), however recent advances have been made with regards to estimating genetic connectivity and dispersal kernels (D'Aloia et al. 2013, Pinsky et al. 2016).

Another complication in designing MPAs with regards to connectivity is that dispersal distances are not static over time. MPA designs should also consider possible changes in connectivity due to climate change, such as distributional shifts toward higher latitudes and deeper depths (Harley et al. 2006, Dulvy et al. 2008), and increased water temperatures expediting developmental rates, thus shortening PLD and dispersal ranges (O'Connor et al. 2007, Munday et al. 2009, Andrello et al. 2015). There are suggestions for MPA designs regarding possible changes in marine connectivity, such as larger reserves to compensate for reduced habitats and closely spaced reserves to accommodate for reduced larval input (Gerber et al. 2014). However, due to conflicting interests, it is highly unlikely that multiple large and closely spaced reserves can be implemented feasibly, hence the need for explicit methods on how to facilitate conservation priorities to account for dynamic biodiversity processes and threats (Pressey et al. 2007). Because climate change and how biota will respond to climate change remains unknown, a mixture of small and large MPAs should be implemented whenever possible to best account for any changes in ecological and demographic processes.

As shown with expected shifts in connectivity, MPA designs must not only incorporate current biological patterns, but also account for future changes. While it is impossible to predict future biodiversity patterns or evolutionary outcomes, we can make meaningful estimates of past and current evolutionary patterns (Myers & Knoll 2001). Analyzing genetic data from molecular markers is widely applied to document the evolutionary processes that have and are influencing biodiversity patterns (Moritz 2002), and much progress has been made on the use of genetic markers to understand historical and contemporary demography and population dynamics (reviewed in Sunnucks & Taylor 2008). Subsequently, much is known on how to spatially delineate populations and conservation units using genetic data (Moritz 2002, Waples et al. 2008, Funk et al. 2012), yet the actual implementation of genetic data into MPA designs by resource managers remains an exception and not the rule (Laikre 2010, von der Heyden 2014).

Currently, there is a gap in the marine spatial planning literature on how to

include genetic data into conservation decision-making processes (Shafer et al. 2014), and thus levels of genetic diversity and divergence are rarely included in suites of conservation objectives, particularly for marine systems. Several studies advocate the importance of ecological data into marine spatial planning (see for example Crowder & Norse 2008, Foley et al. 2010), but there is limited support for the inclusion of genetic data into marine spatial plans (Waples et al. 2008, Beger et al. 2014). Although genetic data may potentially increase the long-term success of MPA networks, it is often excluded from marine conservation planning processes due to difficulties in interpretation and implementation (Beger et al. 2014, Shafer et al. 2014). Therefore, this study focuses on the incorporation of genetic data into marine conservation planning, and the conservation priority outcomes associated with different types of genetic information. This theoretical work will be applied to create a systematic marine biodiversity plan for the South African west coast, which is recognized as an area requiring further conservation management (von der Heyden 2009, Sink et al. 2012).

Marine protection status of South Africa

Coastal marine systems are at great risk to anthropogenic threats owing to their location on the land-ocean interface (Harley et al. 2006, Mead et al. 2013). For example, some of the threats experienced by the rocky and sandy shore biological communities of South Africa include pollution, introduced species, trampling, increased wave action, exploitation, coastal erosion and coastal development (Mead et al. 2013). Sink et al. (2012) calculated that 17% of South Africa's coastline has some form of development within 100m of the shore, with a likely increase in the near future, which leaves a pressing need for marine conservation planning to counteract the threats associated with human activities.

Compared to Africa as a whole, South Africa's coastal waters are relatively well protected, and the marine protection status of the country is expected to increase with 21 new MPAs gazetted for comment in 2016 (Fig. 3 and 4). However, no genetic information was used to inform either the existing or the newly proposed MPAs. As previously mentioned, information regarding population structuring and connectivity is a vital part of designing a connected, resilient and effective MPA network. While South Africa's relatively linear coastline makes it is possible to think of connectivity in terms

of geographic distance, there are local environmental factors such as eddies, upwelling cells and headlands that make geographic distance a poor measurement of connectivity (Waters et al. 2014). Hence, there is a growing awareness that the marine species within South Africa may not be as panmictic (genetically connected via gene flow) as previously believed (Evans et al. 2004, Teske et al. 2006, 2011, von der Heyden et al. 2007, von der Heyden 2009, Muller et al. 2012, Reynolds et al. 2014, Wright et al. 2015). Further, in a multi-species study using genetic isolation-by-distance analyses, Wright et al. (2015) propose that even the two closest reserves within South Africa (approx. 20km) might not be close enough to encompass the short dispersal potentials of some intertidal marine species. Additionally, Mann et al. (2016) suggest a distance of 15-20km between marine reserves, determined from tag and recapture records of surf-zone angling fish species. While the inclusion of new MPAs will most likely help act as 'stepping stones' for connectivity, there are still wide gaps between the newly proposed MPAs, especially along the west coast.

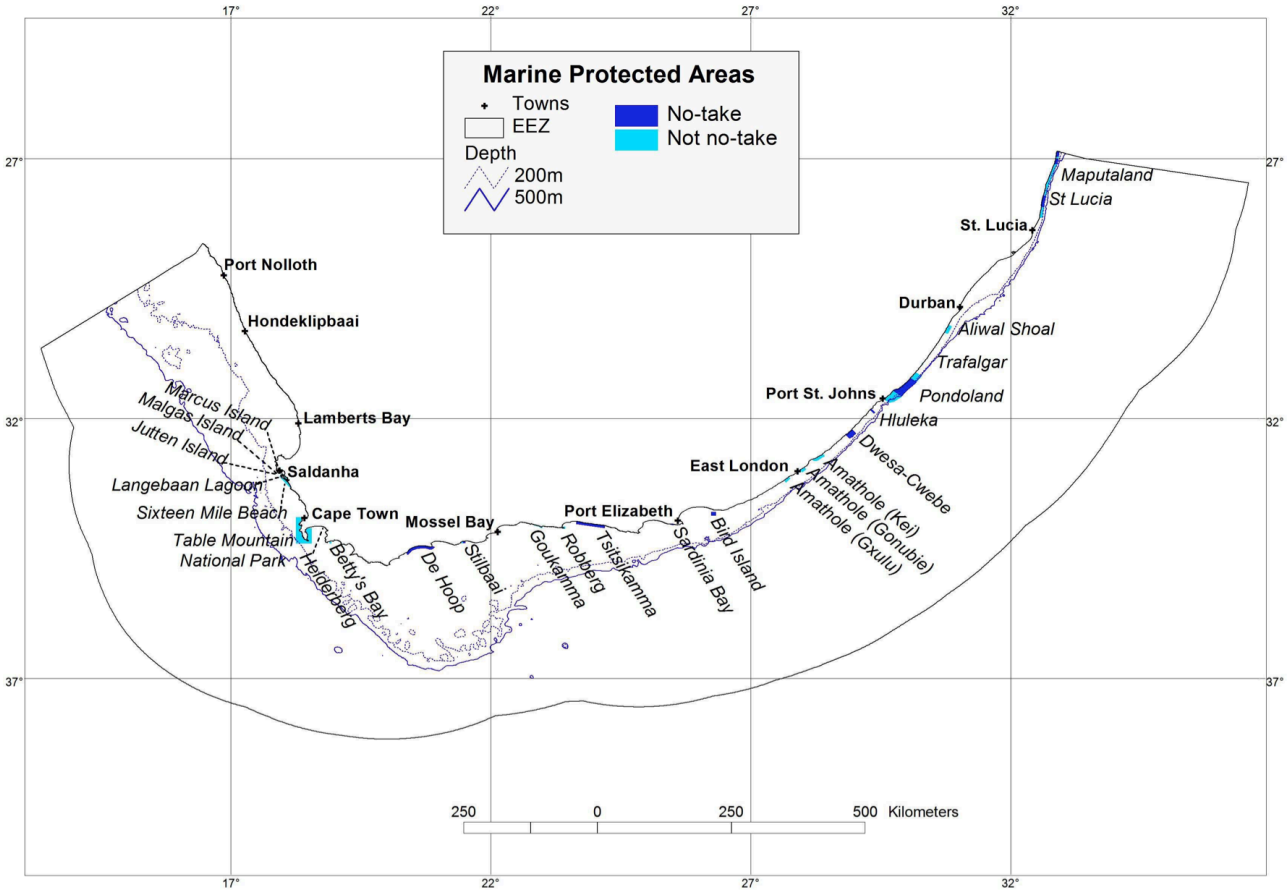


Figure 3 - The current marine protected area network of South Africa. Dark blue polygons represent no-take reserves and light blue polygons represent non no-take reserves (Sink et al. 2012).

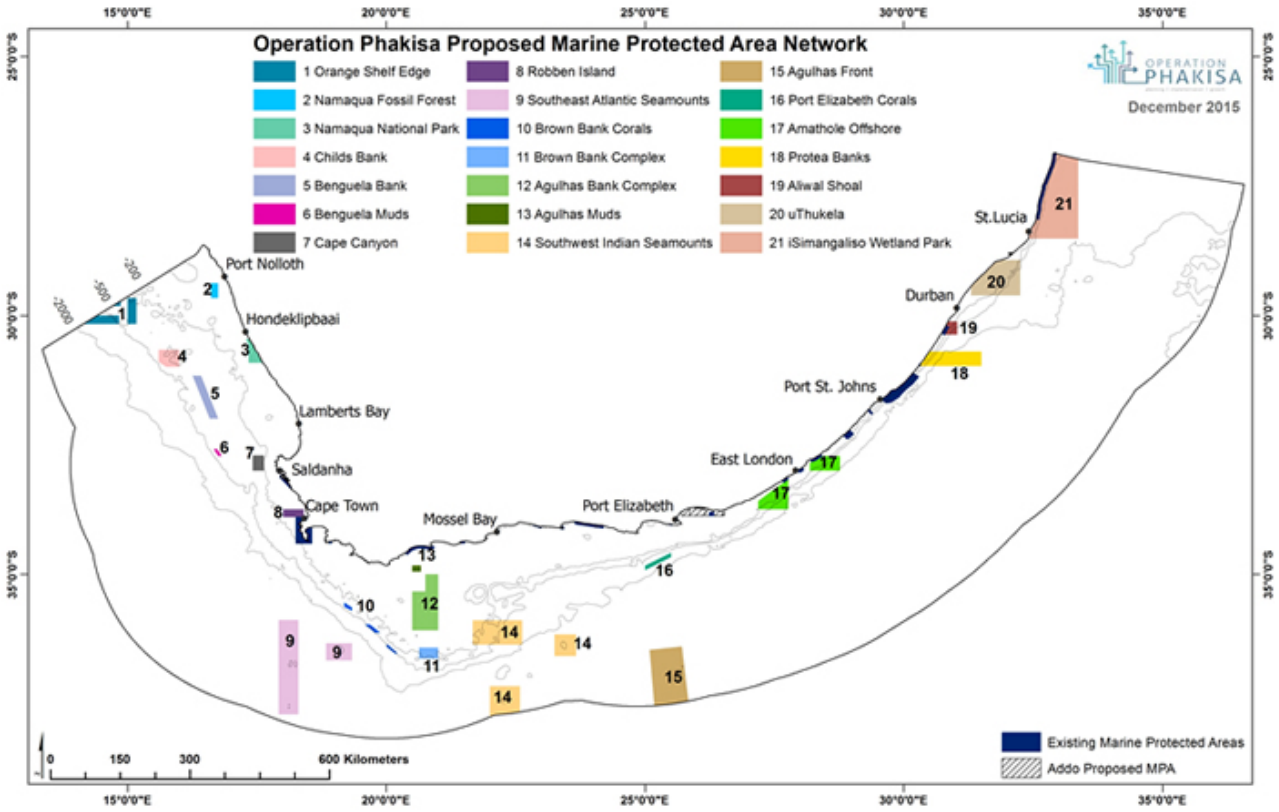


Figure 4 - South Africa's 21 recently proposed marine protected areas (MPAs), with each colored polygon representing a different MPA within the network (image from www.environment.gov.za).

Both the existing and proposed marine reserve networks show a spatial bias against the west coast, with the majority of MPAs located within the south and east coasts (Fig. 3 and 4). This coastal bias is most likely owing to the west coast having lower levels of species richness and endemism compared to the rest of the coastline (Awad et al. 2002, Scott et al. 2010). Although the west coast displays lower levels of biodiversity and endemism, Emanuel et al. (1992) argue that the region is unique on a global scale, due to the dense assemblages of limpets, which have a large impact on the rocky shore communities. Furthermore, the west coast is known to be the most threatened region of the South African coastline, with exposure to diamond, oil and gas mining as well as fishing pressures (Lombard et al. 2004, Sink et al. 2012).

In addition to unique species assemblages, genetic diversity patterns also distinguish the west coast as a conservation priority area. Based on mitochondrial data (mtDNA) for 11 rocky shore and reef dwelling marine species, Wright et al. (2015) found that the genetic diversity along the west coast is significantly lower compared to the south and east coasts. However, these genetic estimates were strongly influenced by

the low genetic diversity of *Oxystele variegata* (Variegated topshell) and *Clinus cottoides* (Bluntnose klipfish), while other species such as *Clinus superciliosus* (Super klipfish) and *Tetraclita serrata* (Grey volcano barnacle) displayed high levels of haplotype diversity (Wright et al. 2015). Population structuring within the west coast has also been detected in sea stars (*Parvulastra exigua*, Mertens et al. in review), urchins (*Parechinus angulosus,* Muller et al. 2012), and hake (*Merluccius capenis,* Henriques et al. 2016). The unique evolutionary histories of west coast species are further illustrated in a phylogenetic diversity study focusing on chitons (Volkmann 2015), which showed that while the west coast had low levels of endemism and species richness, it had relatively high levels of genetic diversity (in this case evolutionary diversity measured as branch lengths on a phylogenetic tree). The variable genetic patterns, and little congruence in phylogeographic breaks across species with similar life history characteristics and/or distributions, suggests that several MPAs may be required to protect the unique evolutionary processes within this region.

Genetic aspects of conservation planning

The 1992 Earth Summit in Rio de Janeiro established the Convention on Biological Diversity (CBD), which aims to monitor biological diversity as well as the "processes or activities that are likely to have significant impacts on the conservation and sustainable use of biological diversity" (www.cbd.int). With 195 nations as signatories, the CBD is the largest international political apparatus for halting biodiversity loss. The CBD recognizes three levels of biodiversity (ecosystems, species, and genes) that are to be conserved and used sustainably. While ecosystems, habitats and species are often prioritized in conservation planning, the genetic level lags behind in conservation action, likely owing to genetic data requiring interpretation and extrapolation within the conservation planning process (Laikre et al. 2009, Tulloch et al. 2016). For instance, out of 24 National Biodiversity Strategy and Action Plans (NBSAP) globally one third did not include genetic variation and only 20% recognized the need for genetic monitoring (Laikre et al. 2010). This is alarming as genetic processes are recognized in shaping patterns of species distributions, as well as influencing population viability (Reed & Frankham 2003, Frankham 2005). Within South Africa, the recent National Biodiversity Assessments (terrestrial, estuarine, freshwater and marine) of

2011 only included genetic knowledge and perspectives in the marine component, highlighting the further need for inclusion of this vital level of biodiversity in conservation planning. Therefore, to identify areas with long-term conservation outcomes for the preservation of global biodiversity, genetic data should no longer be neglected in conservation planning.

Examples of genetic data in marine conservation planning are limited, with only approximately five percent of conservation planning prioritizations including some sort of genetic information in their planning scenarios (Tolluch et al. 2016), most likely owing to implementation difficulties in the field. Nonetheless, there are several cases of genetic studies conducted within a conservation framework, with varying degrees of applied management (see von der Heyden et al. 2009, 2014). Because species and ecosystem representation does not account for phylogenetic diversity (which considers the phylogenetic relationships between species) or evolutionary processes, these studies are essential to represent all aspects of biodiversity (Rodrigues & Gatson 2002, Pio et al. 2011). Therefore, the inclusion of genetic data should not only adjust conservation planning priorities, but also strengthen management plans (Beger et al. 2014). For example, Vasconcelos et al. (2012) found reserve networks including evolutionary significant units (identified from genetic data) to be more effective at protecting rare endemics than reserve designs based only on species distributions.

Some applications of genetic data in biodiversity planning include identifying individuals and species, as well as the demographic parameters (e.g. effective population size, migration rates), population structure and adaptive potential within a species (Schwartz et al. 2006, Selkoe et al. 2008, Sink et al. 2012, von der Heyden et al. 2014). Genetic data can also highlight patterns of larval dispersal, identifying where larvae disperse from (sources), and where they settle (sinks; Levin 2006). Many studies suggest that source populations warrant protection because they are likely self-replenishing and will increase the resilience of other populations (Crowder et al. 2000, Neubert 2003). Others indicate that sink populations also require protection because they are likely depositories of genetic diversity (Almany et al. 2009) and may provide demographic benefits to meta-populations (Howe et al. 1990).

While identifying source and sink dynamics is important in marine conservation planning, it is not the only way in which genetic monitoring can target areas of conservation interest. Genetic surveys can also provide insight into future biodiversity patterns by detecting intraspecific adaptive variation (Gebremedhin et al. 2009). For

instance, Funk et al. (2012) suggest that adaptive variation can help determine evolutionary significant units (ESUs), with the most divergent populations warranting protection to maintain high adaptive diversity. Understanding adaptive variation is also important when deciding which populations to use as sources for translocation, supplementation and assisted migration efforts (Sgrò et al. 2010, Shafer et al. 2014), and it has been shown that supplementing a declining population with maladapted individuals can result in outbreeding depression (Frankham et al. 2011). Adaptive genetic monitoring is also a powerful tool for conservation strategies with regards to climate change (Harrison et al. 2014), as genome scans can highlight genes linked to traits associated with climate change in non-model organisms, augmenting current knowledge of gene-environment interactions (see Hoffman & Daborn 2007 and Porcelli et al. 2015 for reviews). The quantitative assessment of adaptive genetic variation therefore has the potential to strengthen marine conservation plans and help conserve future species distributions (Bonin et al. 2007).

Generally, estimates of genetic diversity and divergence are produced from various molecular markers that correlate to regions of the genome with different characteristics (e.g. mutation rate, selection; Mariette et al. 2002). This leads to different molecular markers often displaying various rates of genetic diversity. For instance, DiBattista et al. (2012) compiled 14 case studies that identified population structure from both mtDNA and nuclear microsatellite data, and found that only eight of those cases showed concordance between the two markers. Several studies advocate the use of multiple markers in detecting population structure for conservation purposes (see for example Bowen et al. 2005, Keeney et al. 2005, Stapley et al. 2010, Funk et al. 2012, Jackson et al. 2014). However, there remains a lack of multiple markers actually being included into conservation planning analyses. Therefore, further theoretical work is required to better understand how different molecular tools compare in their conservation solutions within marine environments.

Beger et al. (2014) showed that adding different types of genetic data (structure, allelic richness, local genetic differentiation, and recent migration rates) from a single marker type (microsatellites) resulted in different conservation priorities, insisting on the need for careful evaluation when applying genetic data to conservation planning scenarios. Others have debated the applicability of different measures of genetic differentiation, such as F_{ST}, D_{est}, and Φ_{ST} within conservation planning processes (Pearse

& Crandall 2004, Bird et al. 2011). Bird et al. (2011) suggested that population genetic studies should include both a fixation index (F_{ST} or Φ_{ST}) and an index of genetic differentiation (F'_{ST} or D_{est}) in their data sets because they represent different properties of population partitioning. Yet it is still unclear how F_{ST}, Φ_{ST}, F'_{ST} and D_{est} compare in conservation planning scenarios. An additional problem with integrating genetic information into conservation planning is deciding how to standardize genetic divergence cut-offs across multiple species, as scientific evidence on how to correct for both species and marker variation is limited. For example, Kelly and Palumbi (2010) use a Φ_{ST} value of 0.11-0.6 to indicate strong structure, but this value for one species may be on a completely different scale to another species with different levels of heterozygosity (Jost et al. 2008, Bird et al. 2011). In order to circumnavigate this problem, Toonen et al. (2011) used significance, rather than the actual value of divergence in order to delineate genetically distinct units in Hawaii.

Overall, much progress remains to be made with regards to integrating evolutionary patterns into conservation objectives, prioritization methodologies and decision-making (Carvalho et al. 2010, Benestan et al 2016). Therefore, this project aims to answer the following questions on the integration of genetic data into conservation planning processes: 1) Do conservation plans including genetic data result in different conservation priorities compared to conservation plans based solely on habitat data?; 2) How do multi-species genetic spatial priorities compare to single-species genetic priorities?; 3) Does including different genetic metrics from multiple species result in different conservation priorities?; 4) How does including different genetic and genomic marker types affect conservation prioritizations?; and 5) What is the effect of including different amounts of genetic and genomic information on conservation prioritizations?

These questions will be explored in the following chapters. Chapter One will first examine how conservation solutions solely based on habitat features compare to those also based on genetic metrics. This chapter also investigates multi-species versus single-species conservation solutions, as well as conservation solutions derived from several genetic metrics from a single marker. Chapter Two will then identify the genomic variation from the two most genetically distinct species within Chapter One, namely the Cape urchin (*Parechinus angulosus*) and the Granular limpet (*Scutellastra granularis*). Subsequently, Chapter Two then compares the genetic variation from Chapter One with the genomic variation of Chapter Two for *S. granularis* and *P. angulosus* (both

individually and combined) within a conservation planning framework.

Chapter One: Multi-species genetic objectives in spatial conservation planning

(Accepted in *Conservation Biology* - please see declaration of authors contribution on the last page of this chapter)

Abstract

The increasing threats to biodiversity and global alteration of habitat and species distributions make it increasingly necessary to consider evolutionary patterns in conservation decision-making. Yet there is no clear-cut guidance on how genetic features can be incorporated into conservation planning processes, with multiple molecular markers and several genetic variation measures for each marker type to choose from. Genetic patterns also differ between species, but the potential trade-offs amongst genetic objectives for multiple species in conservation planning are currently understudied. This study compares spatial conservation prioritizations derived from two metrics of both genetic diversity (nucleotide and haplotype diversity) and genetic isolation (private haplotypes and local genetic differentiation) for five marine species. The findings show that conservation plans based solely on habitat representation noticeably differ from those additionally including genetic data, with habitat-based conservation plans selecting fewer conservation priority areas. Furthermore, all four genetic metrics selected approximately similar conservation priority areas, which is likely a result of prioritizing genetic patterns across a genetically diverse array of species. Largely, the results suggest that multi-species genetic conservation objectives are vital to create protected area networks that appropriately preserve community-level evolutionary patterns.

Introduction

Nearshore marine environments not only harbor an array of diverse species assemblages, but also have significant cultural and economic value. For example, Martínez et al. (2007) recorded that coastal ecosystems amount to 77% of the global ecosystem-service value calculated by Costanza et al. (1997). But with 41% of the human population living within 100km of the coastline (Martínez et al. 2007), coastal ecosystems are highly exposed to anthropogenic threats on a global scale. At a local scale, the alteration of nearshore ecosystems may in turn affect local communities as many South Africans utilize coastal species for either food (Kyle et al. 1997, Troell et al. 2006, Harris et al. 2007) or traditional medicine (Herbert et al. 2003).

In order to ensure that both the economic and cultural value of rocky shore ecosystems are preserved, conservation planning objectives must be aimed at the community level, as rocky shore ecosystems are highly connected through ecological interactions (Menge et al. 1986, Bustamante & Branch 1996). Ecosystem-based management has become widely accepted within marine conservation planning (Levin & Lubchenco 2008) and aims to understand biological communities and the processes that drive their heterogeneity, and identify management units based on potential resistance and resilience to stressors (Crowder & Norse 2008). While marine reserves are often aimed at protecting entire ecosystems, many conservation planning guidelines typically only consider ecological processes of single species (White et al. 2010, Andrello et al. 2015), or use habitat or species surrogates to represent community level assemblages, which are regarded as weak substitutes for multi-species data sets (Margules & Pressey 2000, Rodrigues & Brooks 2007).

Nevertheless, developing and meeting multi-species conservation objectives is not trivial, as compromises often need to be made between species (Beaudry et al. 2016, Melià et al. 2016). This is especially true for multi-species genetic objectives, as there are many indications of multiple species with similar distributions and/or life history characteristics (such as PLD) portraying incongruent genetic histories (Teske et al. 2006, Selkoe & Tonnen 2011, von der Heyden et al. 2013, Wright et al. 2015, Mertens et al. in review). In contrast, there is also evidence of prominent environmental or biotic features, such as ocean currents or chlorophyll a concentrations, shaping genetic trajectories of entire communities (Melià et al. 2016, Selkoe et al. 2016).

Although the environmental and ecological variables shaping population divergences between South African marine species are poorly understood, genetic information from multiple coastal marine species is available to be included into conservation plans. For example, Wright et al. (2015) used mtDNA genetic variation from 11 nearshore species to identify areas for preserving genetic diversity along the South African coastline. Yet from a management point of view, the selection of evolutionary significant areas may not be as straightforward as prioritizing areas with high genetic diversity. For example, Petit et al. (1998) argue that population genetic distinctiveness is just as important as population genetic diversity in delineating conservation priority areas. Furthermore, certain species might be weighted differently, or different measures of genetic diversity may be considered for different conservation objectives. The method of implementation of multi-species genetic data sets into conservation planning processes may also affect conservation priorities. Overall, it remains unclear how to best incorporate measures of genetic variation from multiple species into conservation planning software, as well as the conservation outcomes that occur when including different genetic diversity metrics. Moreover, there is no current framework on how to create a single integrated management plan using various genetic metrics across multiple species as conservation features.

The aims of this chapter are to compare conservation scenarios using various genetic metrics for five phylogenetically and functionally diverse species, and to provide a baseline for the incorporation of multi-species genetic data sets into conservation planning. Furthermore, this study aims to disentangle the conservation outcomes that may occur when including multiple genetic metrics from species with dissimilar genetic patterns. This chapter combines habitat and socio-economic data with genetic data for five obligate rocky shore species (from Mertens et al. in review) into a conservation optimization software to compare conservation priorities along roughly 800km of the west coast of South Africa. Largely this chapter asks the following questions: 1) Do priorities differ between habitat- and genetic-based conservation plans?; 2) Do priorities differ between conservation plans based on different genetic diversity and isolation metrics?; 3) What is the effect of averaging genetic dimensions from multiple species rather than incorporating them as individual layers?; and 4) Do multiple species and genetic metrics contribute equally to the combined conservation outcome? Answers to these questions are a prerequisite to formulating a generalizable framework for conserving multi-species genetic patterns.

Materials & Methods

This study focuses on the west coast of South Africa (bounded by 18.3'E, -34.1'S and 16.8'E, -29.3'S), and includes genetic data from five obligate rocky shore species that share similar distributions along the South African coastline. All species were collected from the same seven sites along the South African west coast, one of South Africa's most threatened marine environments (Fig. 5, Sink et al. 2012).

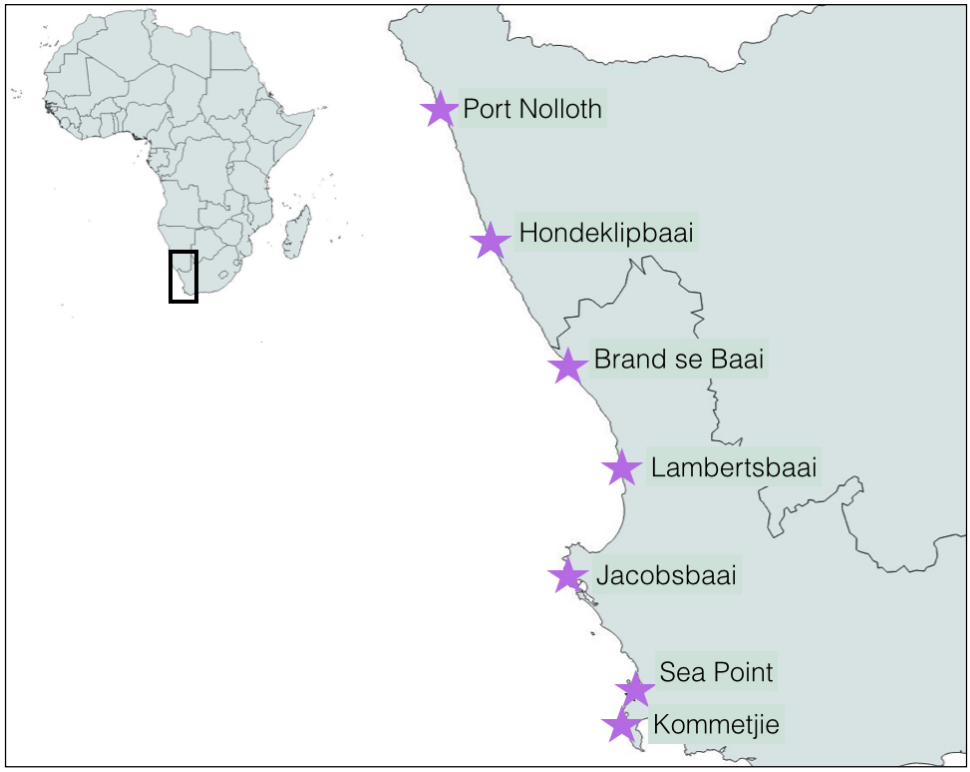


Figure 5 - Sampling localities from which roughly 30 individuals of each species were collected, indicated by the purple stars (base map downloaded from www.esri-southafrica.com/basemaps).

Study species

The five species for which I included genetic data are the Granular limpet (*Scutellastra granularis*), Super klipfish (*Clinus superciliosus*), Cape urchin (*Parechinus angulosus*), Tiger topshell winkel (*Oxystele tigrina*) and Cushion star (*Parvulastra exigua*; Fig. 6). These species were chosen because of their different life history traits, reproductive strategies and functional roles within the rocky shore

community (Mertens et al. in review, Table 1 of Supplementary Materials 1). Several studies suggest that these five species exhibit complex evolutionary histories along the west coast of South Africa (von der Heyden et al. 2011; Muller et al. 2012; Wright et al. 2015). Based on mtDNA data sets, the five focal species display variable genetic structure, different migration rates and a wide range of genetic diversity values (Table 1 Supplementary Materials; Mertens et al. in review). Therefore, they are expected to represent the genetic spectrum of species within the regional rocky shore community.

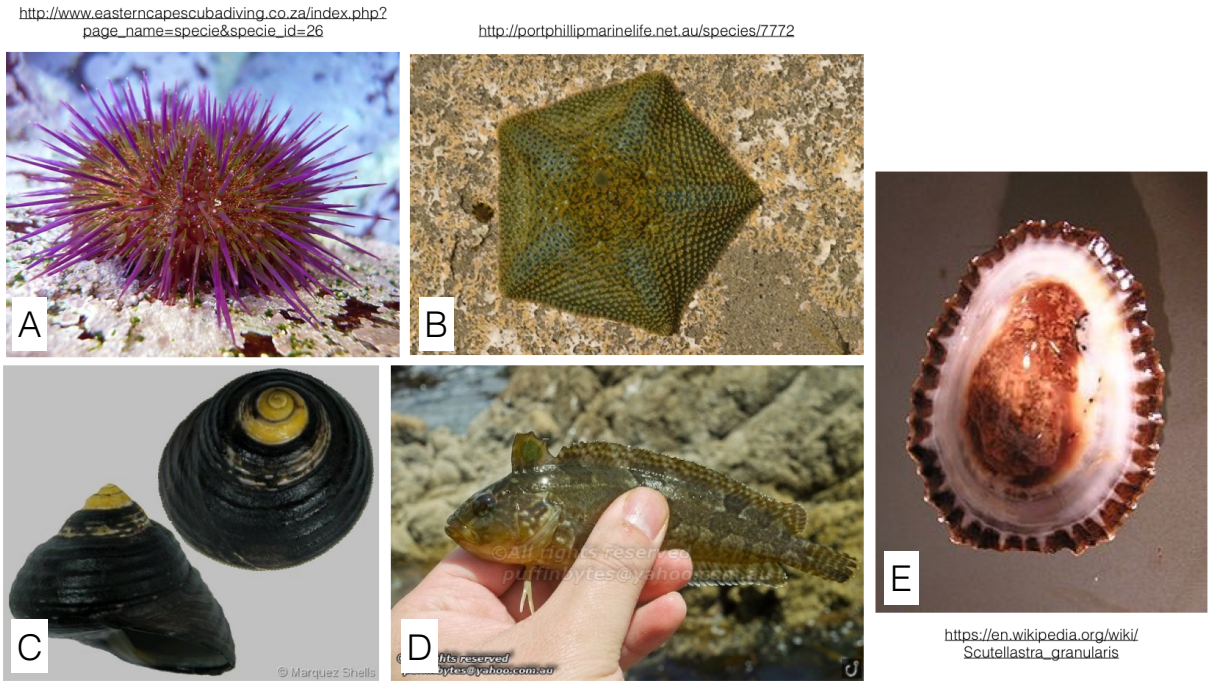


Figure 6 - The five study species included in Chapter One, A) *Parechinus angulosus* (Cape urchin), B) *Parvulastra exigua* (Cushion star), C) *Oxystele tigrina* (Tiger topshell winkle), D) *Clinus superciliosus* (Super klipfish), E) *Scutellastra granularis* (Granular limpet). Sources are indicated next to each photograph.

Clinus superciliosus (Linnaeus, 1758)

Clinidae (klipfish) have a global distribution, inhabiting rocky intertidal environments within the Americas, Australia, New Zealand, the Mediterranean, the Indo-Pacific and Southern Africa. Locally, South Africa hosts 44 species of Clinidae, most of which are endemic (von der Heyden et al. 2008, Holleman et al. 2012). South African clinids are viviparous, giving birth to fully developed juveniles after internal fertilization (Veith 1980). To counteract the energetic costs and reduced fecundity

associated with viviparity, clinids perform superembryonation, developing several broods of different ages simultaneously (Veith 1978, 1979).

This study focuses specifically on *C. superciliosus*, which is distributed from the Orange River to Durban, South Africa. *Clinus superciliosus* individuals reach a maximum size of 30 cm, and are found in rocky shore tidal pools and subtidal gullies (von der Heyden et al. 2008), where they feed on invertebrates (Branch et al. 2007). Klipfish are highly abundant within local rocky shore habitats, with collection efforts showing that clinids make up 88-98% of all recorded fish within South African rocky shore tide pool communities (Prochazka & Griffiths 1992).

Previous molecular studies have shown that *C. superciliosus* is composed of three distinct lineages (von der Heyden et al. 2011), which were later recognized and described as species (Holleman et al. 2012). *Clinus superciliosus* also shows population structuring along the west coast of South Africa, with a genetic break near Jacobsbaai (Mertens et al. in review). Because *C. superciliosus* adults have adequate swimming abilities, this genetic break is likely a result of the nearshore upwelling cell off of Cape Columbine (Shannon 1985, Mertens et al. in review) acting as a barrier to larval dispersal from north to south. However, all previous studies on *C. superciliosus* have utilized mtDNA gene regions to infer genetic patterns, leaving contemporary genetic processes still unknown.

Oxystele tigrina (Anton, 1839)

Oxystele tigrina, or the Tiger topshell winkel, has a round black shell with a white mouth, which is closed by a flexible operculum (Branch et al. 2007). Individuals can grow up to 43 mm in diameter, and inhabit the low shore where they graze on encrusting algae (Branch et al. 2007). *Oxystele tigrina* is endemic to South Africa and can be found from the Orange River to approximately Durban (Branch et al. 2007).

Much is still unknown about the life history and ecological traits of *O. tigrina*. Based on information from the family Trochidea, we can assume that *O. tigrina* is a broadcast spawner with lecithotrophic larvae (Moran 1997). We can also predict based on life histories of sister species, that the pelagic larval duration of *O. trigina* is approximately five days (Muteveri 2013, Wright et al 2015). While the temporal patterns of reproduction are unknown for *O. tigrina*, two sister species *O. variegata* and

O. tabularis are known to have continuous spawning, with seasonal increases during February and September (Joska & Branch 1983, Lasiak, 1987).

Molecular analyses based on the mtDNA Cytochrome oxidase 1 (CO1) gene region show that *O. tigrina* is composed of a single panmitic population along the west coast of South Africa (Mertens et al. in review) with shallow yet significant structuring along the entire coast (Wright et al. 2015). Further, *O. tigrina* does not show signals of isolation-by-distance along the South African coastline (Wright et al. 2015), with high and bi-directional gene flow between subpopulations (Muteveri 2013).

Parechinus angulosus (Leske, 1778)

Parechinus angulosus, or the Cape sea urchin, is the most widespread echinoid in southern Africa, found from Luderitz, Namibia to northern KwaZulu-Natal, South Africa (Velimirov et al. 1997, Branch et al. 2007). *Parechinus angulosus* plays a key role in the intertidal ecosystem not only as a grazer, but also by providing shelter for juvenile abalone (Day & Branch 2002). The species has a relatively wide temperature range (10-23^{o}C), but prefers cool-temperate waters, which is why it is most abundant on the west coast (Greenwood & Bennet 1981). *Parechinus angulosus* is found in dense aggregations in the lower- to mid-zone of rocky shore habitats, and tends to settle on rocky overhangs that are sheltered from wave shocks (Farquhar 1994, Day & Branch 2002). A study by Blamey (2010) showed that *P. angulosus* is predominantly a drift feeder, but shifts to grazing when drift kelp is not available.

Parechinus angulosus is a broadcast spawner with individuals reaching sexual maturity after 1-2 years (Greenwood 1975, Greenwood 1980). There is a seasonal inverse relationship between gonad and body growth, with a decrease in body growth between April and August (Greenwood 1980). The second increase in body mass corresponds to the post-spawning period of rapid growth which occurs from August to November (Greenwood 1980). The species displays seasonal spawning, with a major spawning event in August-September and a minor in April-May (Greenwood & Bennet 1981, Hodgson 2010). After a larval stage that lasts around 50 days, *P. angulosus* actively selects its substrate, and if the preferred substrate is not available, the larval phase can be extended by about 11 days (Cram 1971).

Parechinus angulosus has several different color morphologies (pink, red and

purple), yet there is no genetic distinction between the different color morphs based on the mitochondrial COI and nuclear SpREJ9 genes (Muller et al. 2012). Previous mtDNA analyses of *P. angulosus* along the west coast revealed strong population structure (Mertens et al. in review, Muller et al. 2012), following a possible IBD scenario, with migration analyses suggesting bidirectional gene flow (Mertens et al. in review, Muller et al. 2012).

Parvulastra exigua (Lamarck, 1816)

Parvulastra exigua, or the Dwarf cushion-star, is found in temperate intertidal habitats widespread across the southern hemisphere, from Namibia to Mozambique, southeastern Australia and several oceanic islands (Clark & Downey 1992). It is a prominent member of intertidal communities where it serves as important grazer on microalgae (Arrontes & Underwood 1991, Jackson et al. 2009). *Parvulastra exigua* grows to a maximum size of 20mm and exhibits external colorations of khaki green, orange, blue, brown, often in variegated and geometrical patterns (Payne et al 2015).

In South Africa, *P. exigua* occurs in sympatry with endemic *Parvulastra dyscrita*, and the two species mainly differ in gonopore position, with *P. dyscrita* releasing eggs into the water column via aboral gonopores, and *P. exigua* laying egg masses onto rocks via oral gonopores (Payne et al. 2015). The sticky egg masses deposited by *P. exigua* give rise to benthic lecitotrophic larvae, which therefore lack a pelagic dispersal phase (Bryne & Anderson 1994). *Parvulastra exigua* is an ovipositor, spawning predominantly from August to October (Lawson–Kerr & Anderson 1978, Byrne 1992).

Using the mtDNA CO1 gene, Payne et al. (2015) found little intraspecific genetic variation across seven sample locations from the mid-west to the mid-south coast of South Africa. Furthermore, the authors found that two main haplotypes dominate the haplotype network, and the average distance between haplotypes is relatively short (with a maximum of five mutational steps). Further, they also found three haplotypes that were unique to the South African west coast. Also using the mtDNA CO1 gene region, Mertens et al. (in review) found moderate, but significant population structure along the west coast of South Africa (Port Nolloth to Kommetjie), with a haplotype network dominated by a single highly abundant haplotype, bi-directional gene flow between populations, and no signal of IBD.

Scutellastra granularis (Linnaeus, 1758)

Scutellastra granularis is a small cap-shaped limpet, with a maximum size of ~60mm. The species is endemic to South Africa and occurs from Orange River to Durban (Branch et al. 2007). Individuals are normally brown with white speckles, but shift to a dark brown form when living amidst mussels, or to a broken, striped form when amongst barnacles (Branch & Branch 1981). Their preferred niche is in the upper intertidal, where *S. granularis* uses its radula to scrape algae off of the rocky surface (Branch et al. 2007). *Scutellastra granularis* is also a key intertidal species, controlling algal growth by grazing and acting as a secondary habitat for other invertebrates that settle on top or beneath its shell (Hawkins & Hartnoll 1983). Individuals mostly feed during daytime low tides, during which they consume all available micro-algae (Gray & Hodgson 1997). *Scutellastra granularis* is observed to forage in a random pattern, yet return to a small home range of about 5cm (Gray & Hodgson 1997).

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 quantity of gonadal material produced annually is dependent on body size, and the gametes develop into planktonic larvae (Bosman & Hockey 1988, Kay 2002). *Scutellastra granularis* settle in the low tidal zone, where smaller juveniles are safeguarded from stresses such as desiccation, and then they migrate into the high intertidal zone as they develop into adults (Branch &Branch 1981, Nakin 2008). Individuals grow at an intermediate rate, yet faster in the cooler waters along the west coast (Branch 1974). There is also evidence of differences in life history traits along the west coast, possibly owing to discrepancies in food availability (Bosman & Hockey 1988).

While *S. granularis* shows population structuring along the South African coastline (using mtDNA CO1 region), with a break between Mossel Bay and Tsitsikamma (Mmonwa et al. 2015), the west coast shows no population differentiation (Mertens et al. in review). Further, an IBD analysis (conducted from mtDNA CO1 region) along the west coast showed that there is no relationship between geographic and genetic differentiation, suggesting unrestricted gene flow throughout the region (Mertens et al. in review). Gene flow was also found to be bidirectional at Lamberts Bay

(Mertens et al. in review). While this asymmetrical movement at Lamberts Bay did not indicate a decrease in gene flow, it could possibly lead to population divergence over a longer time scale.

Genetic metrics

Genetic metrics were derived from mtDNA regions, specifically a fragment of the COI gene for the invertebrates and a section of the mtDNA control region for *C. superciliosus* (Table 1 of Supplementary Materials 2). The evolutionary mechanisms of mtDNA are well understood from a comparative phylogeographic and evolutionary perspective (Bowen et al. 2014), making mtDNA regions useful markers for integrative genetic conservation planning efforts. The analyses in Chapter One include four genetic metrics, two of diversity: haplotype diversity (*h*), nucleotide diversity (π; *sensu* Nei 1987); and two of uniqueness: the number of private haplotypes, and local genetic differentiation (Fig. 7). These metrics are highly relevant to conservation as they capture historical and contemporary processes shaping extant patterns of biodiversity (Table 1 and 2).

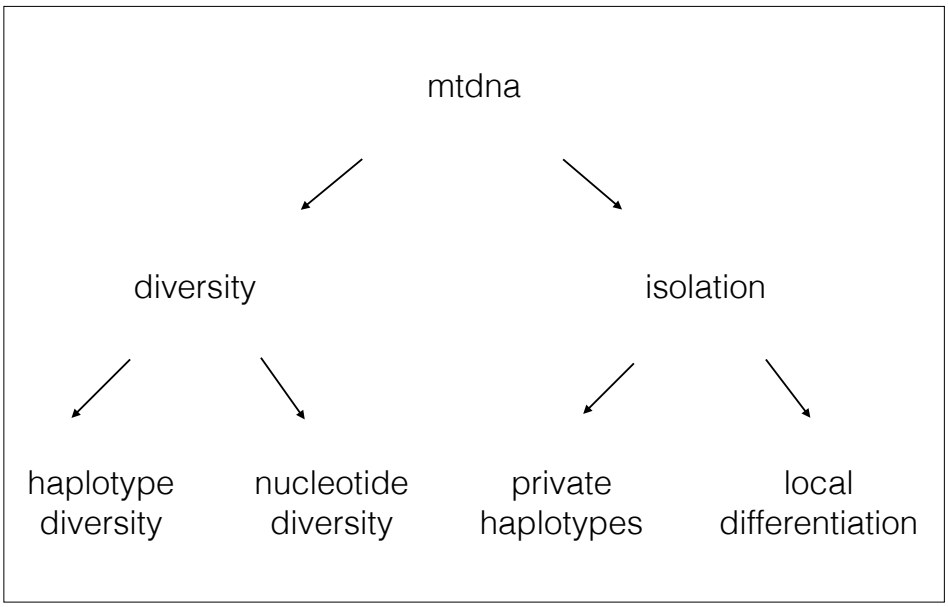


Figure 7 - The different genetic data types being compared within a conservation planning framework for Chapter One. The genetic scenarios include information from a single marker (mitochondrial DNA), two measurement types (diversity and isolation), with two metrics of each measure (haplotype and nucleotide are diversity metrics and private haplotypes and local differentiation are isolation metrics).

Table 1- The four genetic features compared in this study, what they measure, and their relevance to conservation planning.

Genetic feature	Definition	Conservation relevance
Haplotype diversity (*h*)	-The probability that two randomly sampled individuals differ in their haplotypes (a.k.a. mitochondrial DNA allele types)	-As haplotype diversity represents frequency-weighted variation (Nei 1987), it incorporates gene flow, which may make it a more suitable metric to identify management units (Funk et al. 2012)
Nucleotide diversity (π)	- The average number of nucleotide differences per site between any two DNA sequences chosen randomly from the sample population	- Nucleotide diversity represents the absolute standing genetic variation, which may make it a more suitable metric to identify evolutionary significant units (Funk et al. 2012)
Number of private alleles	- Private haplotypes (or alleles) are unique to a single population -A measure of how unique a site is compared to other sites	- A site with a high number of private haplotypes might be genetically isolated, rendering it less resilient to stochastic, catastrophic features such as oil spills (Lande & Shannon 1996) - Genetically unique populations may be interpreted as evolutionary hotspots (Beger et al. 2014)
Local genetic differentiation	- A measure of how much a population's genetic diversity differs from the mean of all of the populations combined	- If a population is genetically isolated from the other populations then it may be less resilient - A population may also be genetically distinct due to local evolutionary processes, in this case the site can play an important role in the meta-population (Beger et al. 2014)

Table 2- Examples of how different levels of genetic diversity and differentiation would be used to meet different conservation objectives.

Genetic feature	Conservation objective
High genetic diversity (high π and h)	To protect populations with high 'adaptive potential' in which there is more genetic material for selection to act on.
Low genetic diversity (low π and h)	To identify and safeguard populations that are at risk of inbreeding depression.
High levels of differentiation (high F_{ST} and number of private alleles)	To protect populations which are adapted to local environmental characteristics. To protect populations that are genetically disconnected from the meta-population, and are therefore at greater risk of catastrophes.
Low levels of genetic differentiation (low F_{ST} and number of private alleles)	To protect 'genetic source populations', which supply other populations with genetic variants and therefore enhance meta-population stability.

Conservation relevance of chosen genetic metrics

Genetic diversity is recognized as being an important conservation feature as high levels of genetic diversity and variation in genotypes/haplotypes can increase individual fitness and population resilience (Hughes et al. 2008) and is the raw material for natural selection to act on (Lande & Shannon 1996; Table 2). Further, there is evidence that genetic diversity may correlate with species richness (Messmer et al. 2012; Wright et al. 2015; Selkoe et al. 2016), and potentially enhance ecosystem function and resilience (Reusch et al. 2005; Bernhardt & Leslie 2012). Conversely, low genetic diversity makes a population more susceptible to inbreeding depression and possible extinction (Charlesworth & Charlesworth 1987; Table 2).

Additionally, meta-population persistence and individual population resilience can be inferred by comparing the genetic distinctiveness of populations (Mortiz 2002; Beger et al. 2014). If a population is genetically isolated, it may be less likely to persist (Van Oppen & Gates 2006; Vollmer & Palumbi 2007) and should be delineated as an individual management unit (Palumbi 2003). Therefore, such populations have conservation importance simply because they are different, making them analogous to a rare species. Further, unique genotypes/haplotypes or rare allele frequencies may be a

result of natural selection, which in the absence of markers that measure adaptive variation could indicate local adaptation if ecological or environmental factors are driving genetic patterns (Baltazar-Soares & Eizaguirre 2016; Table 2). On the contrary, low distinctiveness and uniqueness is also of conservation value because populations that are not in isolation are genetically and demographically connected, making them potentially more resistant and resilient to change (Table 2). The middle classes of each genetic metric also have significance, as these areas may turn into low or high ranking sites in the future.

Data generation and implementation

While mtDNA data sets for the five species were previously generated (Table 1 of Supplementary Materials 2), there was no existing mtDNA data for *P. angulosus* from Brand se Baai. Therefore, mtDNA data was generated within this project. Universal primers jgLCO and jgHCO (Geller et al. 2013) were used to amplify the mtDNA Cytochrome oxidase 1 gene region. Optimal polymerase chain reaction (PCR) parameters were 35 cycles of 30 seconds at 95^0C, 30 seconds at 48^0C, 45 seconds at 72^0C and lastly five minutes at 72^0C. To confirm amplification, 5µl of PCR product was visualized in 1% agarose gel. Successful amplification products were sequenced at the Central Analytical Facility located at Stellenbosch University. The sequences were confirmed by BLAST searches (Altschul et al. 1990) and then edited and aligned manually within Geneious v.1.7.5. The final length of the aligned sequences was 790 base pairs.

I used TCS (Clement 2000) to collapse all genetic data sets into haplotypes and Arlequin v3.5 (Excoffier et al. 2010) to calculate π and *h*. Local genetic differentiation was calculated in Arlequin, with a sequential AMOVA including two populations; one being the site of interest, and the other being all sites combined. Unique haplotypes were counted and labeled as private haplotypes for each population.

After being described for each species, genetic values were then interpolated from the seven point localities using an inverse distance weighting technique in ArcGIS v10.2 (Fig. 8, ESRI 2014). This procedure does result in a simplified version of natural genetic patterns, and ideally genetic data should rather be predicted using environmental parameters, however there is currently no framework on how to model genetic patterns

in marine environments (Beger et al. 2014), particularly in spatially and temporally heterogeneous environments such as the South African west coast rocky shores.

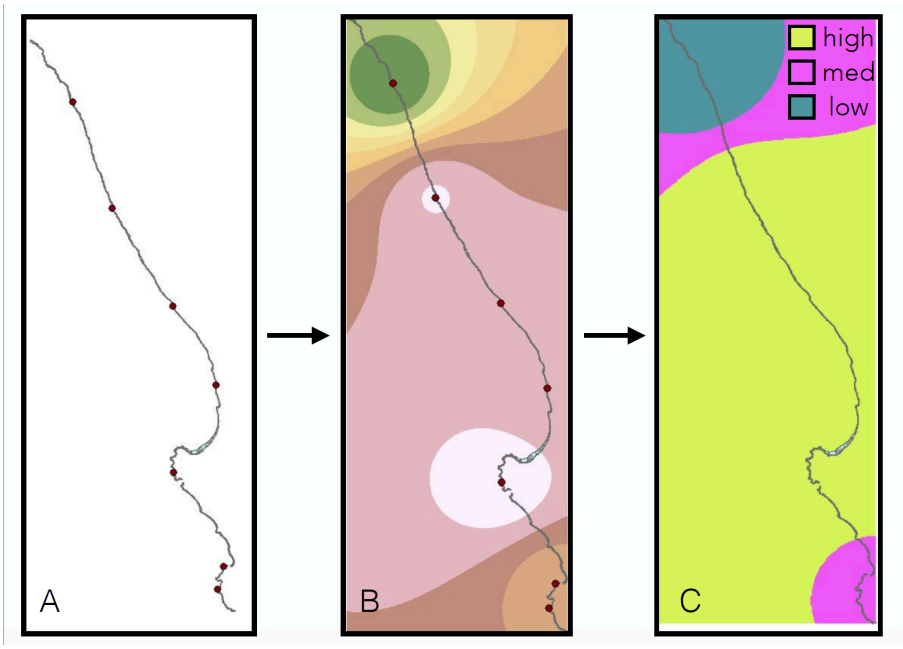


Figure 8 - A visual example of the interpretation of genetic data for conservation planning processes. The genetic point values (A) are interpolated using inverse distance weighting (B), followed by reclassification using equal intervals (C).

For each genetic feature (haplotype diversity (h), nucleotide diversity (π), number of private haplotypes, and local genetic differentiation), I created three classes (low, medium, high) using equal intervals across their measured range of values and set conservation targets for each class (Fig. 8). However, to set appropriate targets for each genetic feature, it is important to first identify conservation objectives (Carwardine et al. 2009). Here, the chosen conservation objective was to represent regional genetic variability to include evolutionary significant areas into a marine reserve network. I then followed a similar protocol to Beger et al. (2014) and set targets to represent 50% of the high and low classes, and 30% of the medium class, as each class may have different evolutionary value (Table 1).

Spatial prioritizations incorporating genetic features were carried out for each of the five species individually, as well as a sixth scenario including values averaged across all five species for each of the seven sampling locations. Averaging the values for each genetic feature summarizes the interspecific genetic composition within the planning region, and may identify important areas for conserving ecosystem function (Whitham et al. 2006; Hersch-Green et al. 2011). This ‘community genetics’ approach may be

more effective with large data sets (such as in Wares et al. 2002; Selkoe et al. 2016), but its applicability to spatial management has yet to be explored.

Conservation prioritization analyses

The conservation of biodiversity is constrained by limited space and resources, and therefore investments in conservation must be prioritized (Margules & Pressey 2000). While the ocean is thought to have more untapped space and resources compared to land, it is often more difficult to conduct spatial prioritization within the ocean, owing to the fact that most marine realms are governed by a patchwork of uncoordinated and often disjointed rules and regulations (Foley et al. 2010). The spatial prioritization of conservation action is invaluable to preserve marine ecosystems, and is achieved through analyzing quantitative data to identify locations for conservation investment (Wilson et al. 2009). One of the most effective methods to analyze data for spatial prioritization is with the use of interactive planning software (Pressey et al. 2007).

An example of such planning software is Marxan, a decision support tool that uses a simulated annealing algorithm to balance the representation of biological features, such as species distributions, with socioeconomic features, such as reserve cost and size (http://www.uq.edu.au/marxan/, Ball et al. 2009). For example, the algorithm can be used to minimize the reserve network cost (by selecting areas with lower economic value) as well as the boundary length of the entire network (to reduce edge effects), whilst maximizing the protection of biodiversity features (Ball et al. 2009). Globally, it is the most widely used conservation planning software, with 2600 individuals and 1,500 organizational users in 110 countries (Watts et al. 2009). Marxan creates a starting point for stakeholders to build on, with areas more frequently selected by the algorithm indicating the level of priority of a pool of sites available for further negotiation (Klein et al. 2008). Within this study I used Marxan to create several MPA network scenarios for South Africa's west coast.

The planning domain included nearshore intertidal areas along the ~800km length of the west coast of South Africa (Fig 1A), extending 500m seaward to 500m inland. The planning units were created with the QMarxan plug-in in QGIS, resulting in a total of 214 units. Hexagons were chosen because they have a larger area to edge ratio,

and a side length of 3km was chosen to correspond with the scale of the habitat and cost data layers.

The baseline conservation features are five rocky shore habitat types identified in the 2011 National Biodiversity Assessment (Sink et al. 2012), namely exposed, sheltered, mixed, boulder and hard ground rocky shores. In practice, conservation targets have great importance, and require sensitivity analyses and clear justification for why they are chosen (Levin et al. 2015). However, as this is a theoretical study, the conservation targets were chosen by testing several target proportions (20%-60% of each feature), and then selecting the lowest possible target to where changes were seen in the conservation solutions. Therefore for the purpose of this study, the conservation target was set to include 40% of each habitat.

To represent lost exploitation opportunities, I included cost data from Majiedt et al. (2013), which quantifies a diverse array of socio-economic pressures, such as commercial fisheries, mining activities and coastal development, currently identified along the South African west coast. The cost values were ranked from one to four, with one having the lowest economic cost, to four having the highest. The Marxan algorithm then gives priority to the low cost areas over the high cost areas. The habitat and cost features remained constant across all planning scenarios and are termed ‘baseline’ for the remainder of this study.

In order to explore the effects of each genetic metric, as well as each of the five species on conservation priorities, trade-offs were compared between variables using the following scenarios: 1) A genetic metric approach where each metric was included separately for all species (change in genetic metric); 2) A species approach where all genetic metrics were included for each species separately (change in species); 3) A combined approach where each species combined with each genetic metric was treated separately (termed ALL); and 4) An averaged approach where genetic metrics were averaged across the five species resulting in one spatial data set per genetic metric (termed AVG; Table 3). The conservation targets of 50% and 30% remained the same for each genetic feature across the scenarios.

Additionally, to examine the effects of targeting a combination high and low ranking areas, I chose a single metric, local genetic differentiation, and solely protected either high or low ranking areas. For the objective of conserving genetically distinct areas, the conservation target was set to protect 60% of high-ranking areas, and zero percent of the medium and low ranking areas. For the counter objective of conserving

genetically connected sites I set the target to conserve 60% of low ranking areas and zero percent of the medium and high ranking areas.

For each of the scenarios, Marxan was run 100 times to account for variability across solutions, and calibration parameters maintained constant. To view similarities between scenarios, selection frequency maps were created using QMarxan, which display how often each planning unit was selected to be within the MPA network out of the 100 solutions. I then followed the protocols in Harris et al. (2014) to analyze similarities between scenarios, performing non-metric multi-dimensional scaling (nMDS) ordination based on Jaccard resemblance matrices in R 3.2.2 (R Development Core Team 2012).

Finally, to quantify the similarity between scenarios, Pearson correlation coefficients were calculated from selection frequency values for each planning unit between each pair of scenarios. To obtain the average amount of congruence between scenarios with either a change in species or genetic feature, I then took the average of the Pearson correlation coefficients for each of the two scenario groupings. To further quantify the trade-offs associated with either a change in species or genetic metric, I calculated the range in number of selected planning units, as well as Marxan cost and score from both scenarios with a change in species or genetic metric. The Marxan score is calculated for every solution, with a lower score representing more biodiversity features within a smaller area and at a lower cost (ie. the more efficient the reserve network, the lower the score).

Table 3 - Describes the various scenarios compared in Chapter One using Marxan.

Scenario No.	Conservation features included	Abbreviation
1	Habitat type (baseline)	B
2	Haplotype diversity	H
3	Nucleotide diversity	N
4	Local genetic differentiation	L
5	Private alleles	P
6	All genetic metrics for *C. superciliosus*	CS
7	All genetic metrics for *O. tigrina*	OT
8	All genetic metrics for *P. angulosus*	PA
9	All genetic metrics for *P. exigua*	PE
10	All genetic metrics for *S. granularis*	SG
11	All genetic metrics as five individual layers corresponding to each species	ALL
12	Each genetic metric as single layer averaged over the five species	AVG

Results

Spatial conservation priorities

High-priority sites for conservation differ between the baseline scenario and each genetic scenario (Fig. 9 A-H), yet all scenarios highlight areas along the entire coastline as priority sites. There are minor differences between the genetic scenarios, with each one identifying multiple clusters of conservation priority areas, roughly extending from those chosen in the baseline scenario (Fig. 9 E-H). The haplotype diversity scenario has the most definitive high priority clusters (Fig. 9 E), followed by the local genetic differentiation scenario (Fig. 9 G). Both the private haplotypes and nucleotide diversity scenarios show smaller conservation priority clusters that are more spread out along the coastline (Fig. 9 F and H). Lastly, the planning units chosen throughout all genetic scenarios (Scenarios 2-5) indicate that the northern region, as well as select areas throughout the mid-and southern west coast are conservation genetic ‘focal areas’ (Fig. 9 D).

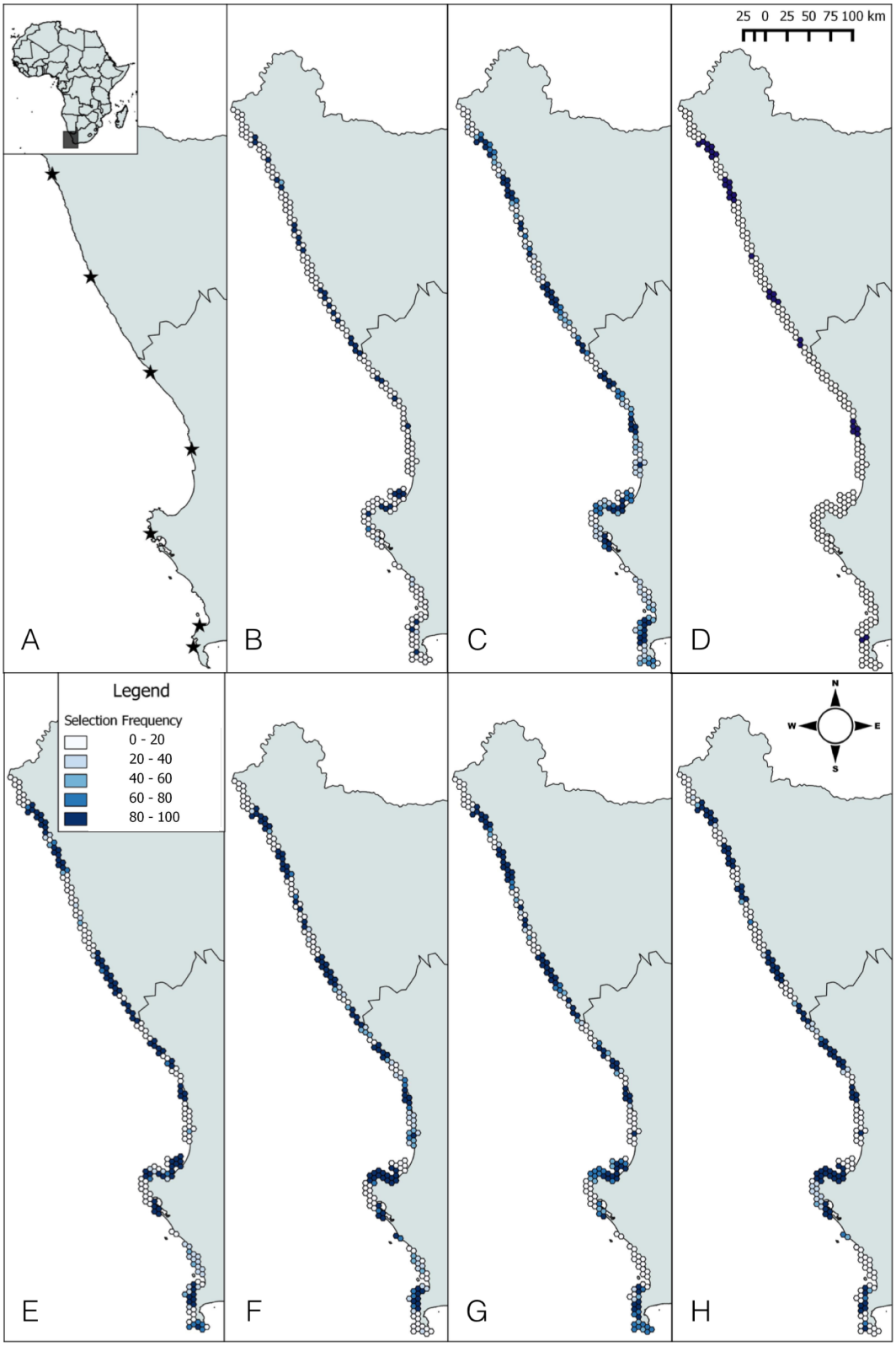


Figure 9 - The seven sampling locations (A) and conservation priorities from the Baseline (B) and ALL (C) scenarios, as well as planning units chosen in each genetic metric scenario (D). Haplotype diversity (E), Nucleotide diversity (F), Local genetic differentiation (G), and Private haplotype (H) scenarios are also shown. Conservation priority maps are based on selection frequencies; darker planning units have a higher selection frequency.

The selection frequencies of the two scenarios with a change in objective (Fig. 10) show greater dissimilarities in spatial priorities compared to the scenarios with a change in genetic metric. A larger number of priority areas are selected in the scenario aimed to conserve areas with low levels of genetic uniqueness compared to the alternative scenario of conserving areas with genetic uniqueness. However, as indicated by the red circles, several areas along the coastline are selected as high priority clusters for both objectives.

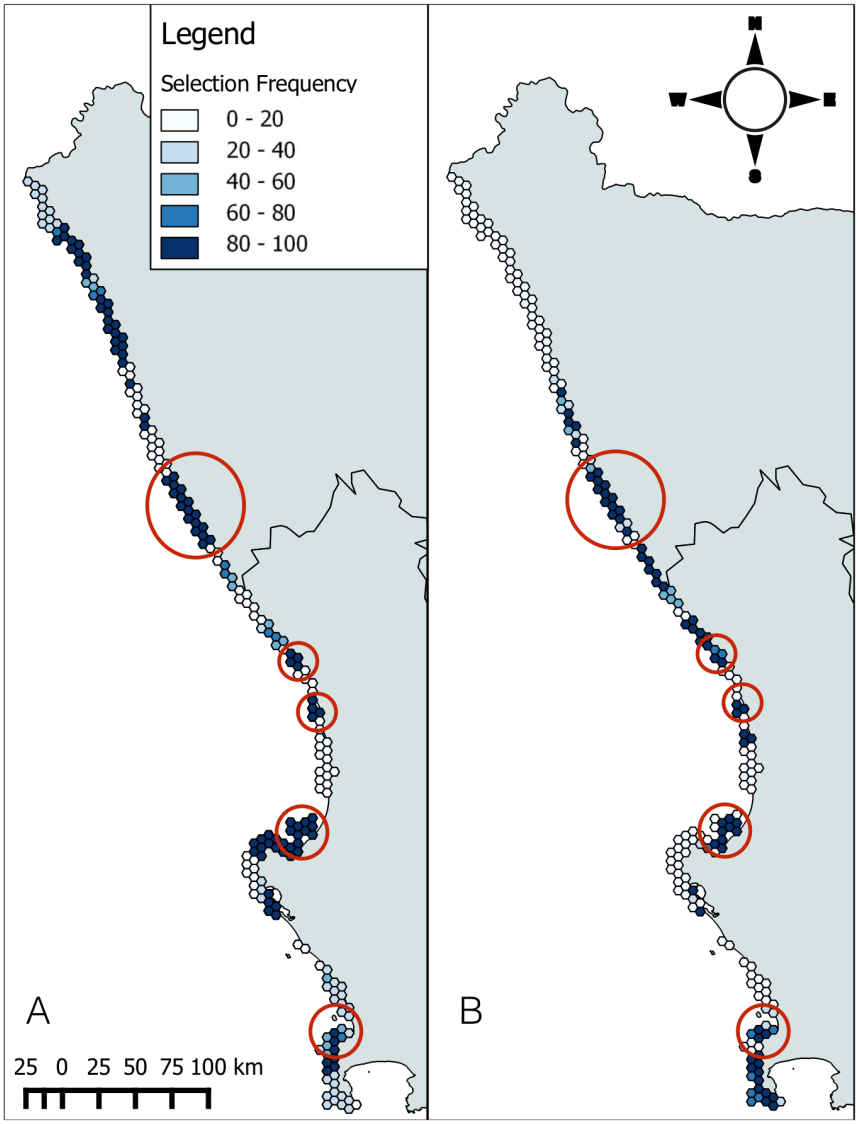


Figure 10 - The selection frequency patterns derived from conserving 60% of either low genetic differentiation (A) or high differentiation (B). Areas highlighted in red are selected with both objectives.

Scenario dissimilarities

The baseline scenario forms a distinct cluster and is highly dissimilar from the genetic scenarios (Fig. 11 A). Solutions from each genetic scenario form a distinct cluster, with little overlap between scenarios (Fig. 11 B). The scenarios including nucleotide diversity and number of private haplotypes for all species are the most similar, followed by those including haplotype diversity and local genetic differentiation. The ALL scenario shows a broad range of solutions, of relatively equal similarity to each of the scenarios including one genetic metric. Lastly, the scenario with the averaged genetic metrics is most dissimilar to all of the other genetic scenarios and there is no congruence between the two scenarios that include all genetic metrics (ALL and AVG).

The nMDS plot based on the dissimilarities between single species and multi-species genetic scenarios (Fig. 11 C) shows little concordance between the solutions, with each species highlighting different conservation priority areas. Most single-species scenarios form tight clusters with highly similar solutions, with the exception of the *S. granularis*, which shows a broader range of spatial solutions. The two scenarios including all species (ALL and AVG) show no congruence, with the AVG scenario displaying the most divergent set of solutions.

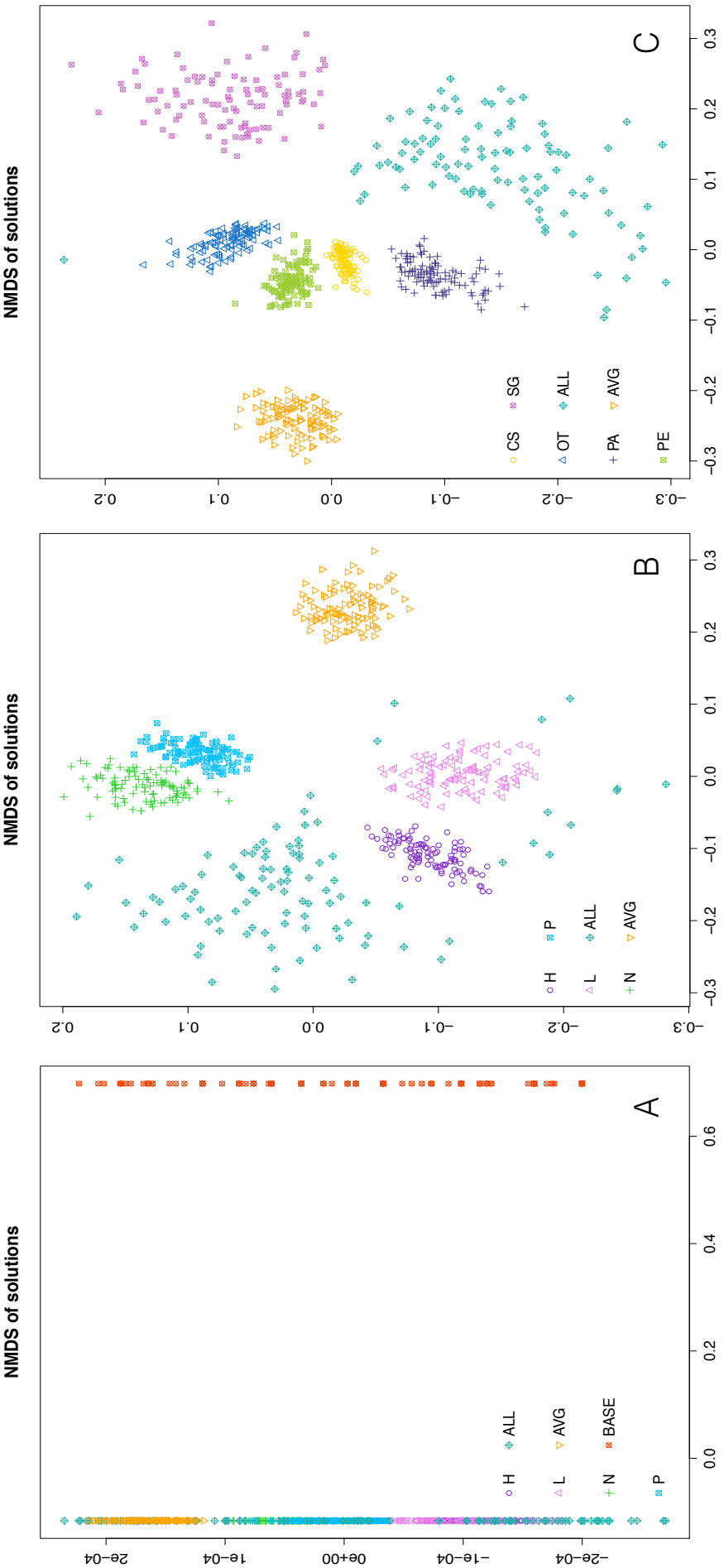


Figure 11 - Non-metric multi-dimensional scaling ordination plots illustrating the dissimilarities among the 100 solutions of the baseline and genetic scenarios (A), solely the genetic scenarios (B), as well as the single-species scenarios (C).

Quantified conservation trade-offs

The Pearson correlation coefficients mirror the nMDS plots (Tables 1 and 2 of Supplementary Materials 3) and show that no one solution is highly dissimilar to the others with the exception of the baseline scenario. The average similarity between scenarios with a change in genetic metric is just slightly lower than the scenarios with a change in species (Table 4). However, the ranges in number of selected planning units, Marxan cost and score are larger across the scenarios with a change in species versus a change in genetic metric (Table 4).

Table 4 - Measures of dissimilarity across scenarios altering either the species or genetic feature included in the scenarios for Chapter One.

Measure of dissimilarity	Change in species	Change in genetic feature
Average Pearson correlation	0.61	0.56
Range in cost	95	50
Range in score	91	44
Range in planning units	7	5

Discussion

Intraspecific genetic variation is the foundation of biological diversity, and thus conserving the adaptive potential of organisms is pivotal to their long-term persistence. Despite calls to inform conservation decisions with genetic and genomic information (von der Heyden 2009; Funk et al. 2012; Shafer et al. 2014), few examples exist where evolutionary patterns have been translated into actionable conservation objectives (Laikre 2010) with existing studies focusing solely on single species (Sork et al. 2009; Beger et al. 2014; von der Heyden et al. 2014), that probably do not capture community level patterns (Selkoe et al. 2016). The results demonstrate that no single species can adequately represent multi-species genetic patterns because spatial conservation priority sites vary between different species. These results mirror the findings of Lombard (1995), who found little congruence in conservation priorities areas across six vertebrate

taxa across South Africa. Further, within the context of understanding habitat-only versus genetic scenarios, each scenario including a genetic metric highlights noticeably more priority areas compared to the baseline scenario. This indicates that not accounting for community genetic metrics in conservation plans will underrepresent genetic patterns in MPA networks, thereby jeopardizing the protection of the processes driving spatial patterns of biodiversity (Klein et al. 2009).

Conservation planning with and without genetic data

The results show a clear separation between conservation priority areas derived from the baseline scenario and the genetic scenarios, confirming similar results for data from a single species (Beger et al. 2014). While conservation priority areas from each genetic metric seem to roughly correlate with those in the baseline scenario, the priority sites chosen throughout all genetic scenarios (Fig. 9 D) are not representative of the baseline, meaning that genetic focal areas are not spatially associated with the different habitat types in the west coast. Using multi-species conservation objectives, the conservation comparisons between habitat-based and genetics-based conservation plans result in highly different spatial solutions, further supporting the need to include genetic information into conservation planning (von der Heyden 2009). In the context of a rapidly changing climate, this finding has important implications for the persistence of species and communities, as failing to protect standing genetic variation increases the likelihood of losing genetic variants which may be more resilient to change (Barrett & Schulter 2008).

Conservation trade-offs between genetic measures

All genetic scenarios choose approximately similar areas as conservation priorities, with slight discrepancies in conservation selection patterns (Fig. 9 E-H). This suggests that protecting a percentage of high, medium and low ranking areas for a single genetic metric from multiple species will most likely also capture priority sites arising from other genetic metrics. The broadly similar conservation priorities between the different genetic metrics are unexpected, as different evolutionary and demographic processes and statistical approaches relate to the different metrics (Table 1). The

similarities between the conservation priority areas from the separate genetic metrics could be a result of the broad spectrum of genetic patterns within the five study species. For instance, when different conservation objectives (conserving only high or low ranking areas) are compared from just a single metric (local genetic differentiation), some sites are chosen as conservation priority areas for both objectives (Fig. 10). This illustrates that while the genetic metrics may have different spatial patterns, these differences can be captured in the conservation solutions in some instances without spatial rearrangement of priorities. The finding that different genetic metrics select similar areas to represent multiple levels of genetic variability suggests that solely targeting different genetic rankings of a single metric may also incidentally capture areas representative of other genetic metrics.

Whilst the different genetic metrics broadly select similar conservation priority areas along the coastline, there are discrepancies between the different genetic scenarios. For instance, the scenarios including nucleotide diversity and private haplotypes leads to smaller, but more widely spread, areas of conservation priority when compared to those based on haplotype diversity and local genetic differentiation (Fig. 9 E-H). The similar conservation priorities between nucleotide diversity and private haplotypes, and haplotype diversity and local genetic differentiation are unexpected, as it would be likely that the two scenarios including either a diversity (h / π) or isolation (private haplotypes / local genetic differentiation) metric would be more similar to each other. However, the similar conservation spatial patterns between nucleotide diversity and private haplotypes in this study are most likely because there is little agreement in the genetic values between species, which leads to the more widely spread selection of planning units.

Conservation trade-offs across different species

Each of the five study species shows highly variable conservation solutions (which is expected since each species is characterized by unique genetic characteristics), with little congruence between scenarios representing different species (Fig. 11 B). Larval dispersal is recognized as an important driver of these differences (White et al. 2010), but the interaction between pelagic larval duration and population structure varies greatly between species (Selkoe & Toonen 2011). Furthermore, interspecific genetic differences can be owed to forces unrelated to dispersal, such as habitat availability and

time since re-colonization (Selkoe et al. 2014; Selkoe et al. 2016). Therefore, the inclusion of genetic information from multiple species, even if they have similar biological characteristics (e.g. distribution ranges, life history) is critical, as even functionally similar species can be characterized by very different evolutionary histories and contemporary genetic patterns (von der Heyden et al. 2013, Wright et al. 2015). Moreover, the results show little congruence between phylogeographic patterns and conservation spatial patterns, as the two most highly structured species (*P. angulosus* and *P. exigua*) and the two panmitic species (*S. granularis* and *O. tigrina*) do not have spatial solutions that are more similar to each other than those species with different phylogeographic patterns (Fig. 11 C; Table 1 of Supplementary Materials 2). In addition, the number of selected planning units also does not correspond with phylogeographic patterns, as the two species with the most planning units chosen are *P. angulosus* and *S. granularis*, which have the highest and lowest genetic structure respectively (Table 4; Table 1 of Supplementary Materials 2). This suggests that if the objective is to identify genetically diverse or unique areas, then solely including phylogeographic patterns may not capture the full extent of genetic relationships between sites.

The findings also show that distinct conservation priorities occur with the inclusion of either single-species or multi-species genetic metrics (Fig. 11, C). While the inclusion of multi-species objectives is recommended in conservation planning (von der Heyden 2009; Toonen et al 2011; Magris et al. 2015), no previous studies have explored how conservation objectives aimed at protecting community-level genetic composition compare with those aimed at single species as indicators for overall genetic variability. This study shows that including genetic information for multiple species independently (ALL scenario) gives conservation priorities that are equally similar to the priorities derived from genetic data from each individual species (Fig. 11, C; Table 2 of Supplementary Materials 3). Thus, my findings support the notion that including multiple species as features individually will lead to more representative conservation priorities than using the multi-species average as a single conservation feature in conservation planning (Fig. 11, B and C). However, averaging genetic metrics may be a viable approach with larger or more homogeneous data sets. For example, Selkoe et al. (2016) found that within a 47 species genetic data set, many species showed compatible genetic patterns, which lends some support for averaging genetic measures. Further, the effects of averaging genetic data sets with missing data has yet to be explored, as well as

the potential trade-offs of having multiple species with averaged values versus having fewer species with non-averaged values.

Conservation trade-offs across genetic metrics and species

The average similarity between spatial priorities is only slightly larger with a change in species versus change in genetic metric (Table 4), implying that the inclusion of either an additional genetic metric or species will alter the conservation priorities to a similar degree. However, the results also show that the scenarios with a change in species lead to a greater range in number of planning units chosen (Table 4), as well as Marxan cost and score, which means a change in species is more likely to result in conservation solutions with a broader range in priority areas chosen in the 'optimum' spatial plan. Overall, the results suggest that a change in species leads to an overall greater change in number of planning units selected (which in turn leads to greater trade-offs in cost and score), yet the areas where the planning units are selected will spatially be more similar to each other with a change in species than genetic metric.

Concluding remarks

This chapter shows that, using mtDNA as a marker, conservation plans can be developed to preserve not only habitat features, but also the evolutionary aspects of species distributions. Given that a majority of studies dealing with population genetic structure to date have used mtDNA as one of the markers (Bowen et al. 2014; Keyse et al. 2014), there is ample opportunity for exploring the approaches laid out here with different species and geographical areas. For example, there are a large number of single and multi-species genetic data sets available for the Indo-Pacific (see Horne et al. 2008; Gaither et al. 2010; Keyse et al. 2014) and the Mediterranean (see Carlsson et al. 2004; Duran et al. 2004; Carreras et al. 2007), which could be utilized and included into management plans. A key hurdle is the mismatch in scales between genetic variability and planning areas; but genetic data is well suited to inform regional-scale and multi-lateral conservation efforts. The findings from this chapter provide a first step on how to account for differences in genetic objectives, specifically different genetic metrics and

species, within conservation planning frameworks. The next step is to compare conservation priority areas derived from neutral and adaptive markers, and including genetic measures from multiple markers, which will be investigated in the following chapter.

Chapter Two: Characterizing and comparing genome-wide versus mitochondrial genetic patterns within a conservation planning framework for two obligatory rocky shore species

Introduction

The first chapter compared several genetic measures from a single marker, yet it remains unclear how genetic information from multiple markers affect conservation priorities. Because there is a wide array of molecular markers to choose from in the molecular ecologist toolkit, it is important to identify whether multiple markers perform differently in meeting specific conservation objectives. Locally, within South Africa, there are a multitude of studies that have utilized different markers to understand evolutionary processes driving the distribution of genetic variation in marine taxa (Muteveri et al. 2015, Mmonwa et al. 2015, Ridgeway et al. 2008, Chow et al. 2000, Bojangles et al. 2001, Zardi et al. 2007, Evans et al. 2004, Teske et al. 2007, von der Heyden et al. 2015, Henriques et al. 2014 a,b, Drost et al. 2016), with mtDNA and microsatellites being the predominant molecular markers. Globally, as well as locally, this picture is changing, with the increasing popularity of next generation sequencing techniques on non-model organisms (i.e. organisms without annotated genomes; Mardis 2008), which allows for a substantial increase in genome coverage and resolution of genomic variation.

Due to their unique genetic characteristics, different molecular markers have varying levels of conservation relevance (Beger et al. 2014; Nielsen et al. in press). Mitochondrial DNA (mtDNA) can be used to determine historical population genetic diversity and structure, while microsatellites and next-generation markers are better suited to identify recent demographic events (Dudgeon et al. 2012). Further, next generation markers can also include outlier or adaptive loci, which can be used to identify signals of selection within populations (Allendorf et al. 2010), and thus populations with more adaptive loci are presumed to have increased resilience to environmental change.

While the multi-marker genetic information is available for a range of South

African marine organisms, none of this information has been used to prioritize areas for marine protection, likely owing to difficulties in the interpretation and weighting of different genetic data types. This research and management gap (von der Heyden 2009, Shafer et al. 2014) is also likely owing to the lack of development and execution of objectives requiring multi-marker genetic information being currently unexplored in conservation planning processes. In order for genetic and genomic findings to be relevant for conservation, methods must be developed to include the ever-increasing number of molecular tools into conservation planning.

Molecular markers

With the advancement of genetic applications and the growing number of molecular markers (Liu & Cordes 2004), it is important to understand the genetic advantages, as well as disadvantages, unique to each marker type. This study aims to compare different molecular markers within a spatial conservation framework, yet in order to compare the conservation outcomes from these genetic markers, it is imperative to understand how each marker reflects different evolutionary processes. Broadly, a molecular marker can be classified as a 'traditional' marker, which reflects the evolutionary characteristics of a specific gene region, or as a 'next generation' marker, which reflects the evolutionary characteristics of the entire genome.

'Traditional' markers

Presently, 'traditional' markers, such as mtDNA, microsatellites, and nuclear genes, are the most widely applied tools in assessing gene flow and population structure to determine conservation units (Hoffmann & Willi 2008). Although genes within protein coding regions of mtDNA (such as Cytochrome C oxidase and Cytochrome B genes) are known to show signals of selection (Meiklejohn et al. 2007), and length variants in microsatellites are known to influence expression patterns of linked genes (reviewed in Kashi & King 2006), many population genetic studies assume that mtDNA, microsatellite and nuclear loci mostly reflect neutral patterns. Neutral genetic variation is predominantly governed by the interactions between genetic drift, mutation, recombination and migration (Kohn et al. 2006), which allow these markers to provide

insight into processes governing population demography and patterns of gene flow.

Mitochondrial DNA consists of a single linked array of genes (a single locus) and has neither introns nor long noncoding spacers, hence producing a clearer sequence composition than nuclear DNA (Avise 1994). Mitochondrial DNA is also commonly used because of its 'universal' primers that can be applied for a wide variety of taxa, and is only inherited through the maternal line (Morin et al. 2004, Wan et al. 2004), resulting in a four-fold decrease in effective population size. Mitochondrial DNA is well equipped to identify genetic management units and areas of conservation priority from an evolutionary perspective, yet due to the relatively slow mutation rate compared to other markers, it is less effective at characterizing demographic parameters such as effective population size and migration rates, especially when these parameters fluctuate (Moritz 2008).

In contrast, microsatellites are bi-parentally inherited hypervariable repeat units scattered throughout the genome, with different lengths representing alleles (Chambers & MacAvoy 2000). Because microsatellites have an even faster mutation rate than mtDNA, they are better suited to estimate effective population size and migration rates (Avise 2010). However, one of their main caveats is that with their high mutation rate, they are more likely to display size homoplasy, which can lead to underestimates in genetic diversity (Wan et al. 2004, Henriques et al. 2016). Furthermore, the mutation rate of a single microsatellite locus can vary within even closely related species, making microsatellites ill-suited for interspecific comparisons of genomic variability (Brumfield et al. 2003). Nonetheless, microsatellites are currently one of the most widely applied molecular markers in population genetic studies due to their high sensitivity and utility (Christiakov et al. 2006, Larsson et al. 2007).

Multiple genetic studies have used mtDNA and microsatellites in tandem to reveal population differentiation within marine species (see for example Bowen et al. 2005, Dickerson et al. 2010, Henriques et al. 2014 a,b, Jackson et al. 2014). Because the two markers assess genetic variation through different inheritance patterns (uni- and bi-parental) and on different time scales (historical and contemporary), mtDNA and microsatellite data provide separate estimates of population structure, and discordance between the two markers can leading to discoveries of hybridization, sex-biased dispersal, or deviations from mutation-drift equilibrium (Dickerson et al. 2010, DiBattista et al. 2012). If the markers are congruent, it provides even stronger estimates of population structure than either marker on its own. Overall, an increase in the number

of markers leads to more powerful results because more of the genome is represented (Avise 2010, Ezaguirre 2014).

However, even using a combined approach, traditional neutral markers are generally poor predictors of quantitative genetic variation and adaptive potential (Karhu et al. 1996, Reed & Frankman 2001, Kohn et al. 2006, Bos et al. 2008, Gebremedhin et al. 2009). This discordance can be a result of adaptive genetic divergence and genetic drift occurring at different rates, or if gene flow is high enough to prevent neutral marker differentiation but is not high enough to prevent local adaptation (Conover et al. 2006). The latter scenario may be more apparent in marine species because populations are expected to have large effective population sizes (N_e), which may decrease genetic drift, allowing selection to be more efficient (Li 1978, Allendorf et al. 2010). Previous studies conducted on Atlantic cod (Pampoulie et al. 2011) and European flounder (Hemmer-Hansen et al. 2007) discovered no diversity in microsatellite markers, yet high variation in adaptive genes. Further, Bonin et al. (2007) found that adding adaptive variation into biodiversity planning led to more areas of conservation significance than neutral diversity alone. These studies give evidence to the benefits of incorporating adaptive variation in conservation planning methods.

Molecular markers: The next generation

To represent an even larger portion of the genome, Next Generation Sequencing (NGS) can be used to detect patterns of neutral and adaptive evolution. NGS performs highly parallel DNA sequencing where hundreds of thousands of reads (i.e. DNA sequences) are combined in a single sequencing run (Stapley et al. 2010). Compared to mtDNA and microsatellite studies, which generally include no more than 20 loci for non-model organisms, NGS studies analyze 1,000s of loci and, more importantly, can be utilized for studying genomes of non-model organisms. Next generation sequencing has multiple applications such as quantitative trait locus (QTL) mapping (Narum 2013), expression profiling (Hoffman & Willi 2008), taxonomic identification (Shafer et al. 2014), along with detecting hybridization (Allendorf et al. 2010) and inbreeding depression (Kohn et al. 2006). Within this chapter, NGS will be used to detect neutral genetic diversity by scanning the genome for single nucleotide polymorphisms (SNPs). Because the majority of loci are not under selection, a genome-wide SNP survey should

reflect overall neutral processes (Angeloni 2012).

As the previously mentioned studies compared mtDNA to microsatellite data, this chapter will compare mtDNA to 'neutral' SNP data. Genome-wide SNPs are similar to microsatellites in that they are bi-parentally inherited and can capture contemporary genetic variation, and therefore may produce similar estimates in population genetic studies (Morin et al. 2004). Even though NGS captures genetic patterns across the entire genome (including both slow and fast mutating gene regions), it has been shown that next-generation sequencing methodologies detect even shallower genetic structure than microsatellites (Puritz et al. 2012, da Fonseca et al. 2016, Maroso et al. 2016). Further, because genome-wide SNPs capture genetic variation on a much larger scale, SNPs will most likely identify even more recent population genetic patterns and higher diversity measures than microsatellites (Seeb et al. 2011).

Thus, it is predicted that SNPs will gradually come to replace microsatellites in population genetic studies because they reflect similar genetic processes, but with greater power (Väli et al. 2008, Seeb et al. 2011). For example, Puckett and Eggert (2016) found that SNPs have increased accuracy compared to microsatellites in estimating natal assignments of black bear individuals across North America and Rengmark et al. (2006) suggest using SNPs as a substitute for microsatellites for parentage testing. Glover et al. (2010) also demonstrated that a panel of SNPs gave significantly more accurate individual genetic self-assignment compared to any combination of microsatellite loci. In addition, SNP calling with NGS may make stronger predictions of neutral variation because it can facilitate the exclusion of loci under selection (Allendorf et al. 2010).

Detecting adaptive loci with next generation sequencing

Local adaptation is a principal component of evolutionary diversification, and should be an integral factor in conservation objectives (Angeloni et al. 2012, Narum et al. 2013). Until recently, studies focused on identifying local adaptation have focused primarily on model organisms, with non-model species (i.e. species without reference genomes) poorly represented in research. It has been shown that adaptive processes can occur on contemporary time scales, especially in response to invasions, habitat alterations, or range expansions (Conover et al. 2006, Hoffmann & Daborn 2007,

Gebremedhin et al. 2009). Therefore, detecting intraspecific adaptive variation could potentially highlight populations of conservation priority in order to counter anthropogenic threats. An increasing number of studies are utilizing genetic markers to assess the adaptive potential of species and evaluate how genetic changes are linked to the environment (see for example Baird et al. 2008, Schmidt et al. 2008, Rietzel 2013, Lexer et al. 2014). This is in part a result of the technological advancements only recently allowing for the sequencing of thousands of loci and genome assembly in non-model species.

Identifying adaptive variation in non-model species is simplified by the availability of NGS methodology. One of the techniques used to detect adaptive differentiation is to screen for outlier SNPs. A genome-wide average is used as a baseline for variation in neutral processes such as mutation rate and genetic drift, and any outliers from this baseline indicate the action of selection (Avise 2010, Willette et al. 2014). When populations are compared, any locus that varies from the average divergence between populations (F_{ST}) has a high chance of being acted on by selection or being linked to a gene under selection (Angeloni et al. 2012). If selected loci show greater divergence among populations and decreased variation within a population compared to the null expectation, it suggests directional selection at those loci (Hoffmann & Willi 2008, Angeloni et al. 2012). In contrast, when loci have a lower divergence than the null expectation between populations, stabilizing selection is presumed (Schmidt et al. 2008). Several studies have applied this method to capture adaptive diversity and find evidence of both divergent selection (Hohenlohe et al. 2014, Lexer et al. 2014) and stabilizing selection (Reitzel et al. 2013, Hohenlohe et al. 2014).

However, applying NGS to detect outlier SNPs has its potential biases and disadvantages. For example, it is now expected that the majority of selective sweeps in natural populations are soft sweeps (Messer & Petrov 2013), which means that instead of few genes of large effect, there are many genes of small magnitude under selection. Next generation sequencing may not be as effective in detecting soft selective sweeps because the combined contribution of multiple loci may be overlooked (Harrison et al. 2014). Genome-wide selection scans are also limited in detecting pleiotropic genes, which are single genes that control multiple (usually unrelated) phenotypic traits (Liu et al. 2009). Furthermore, it is argued that linkage disequilibrium decay occurs at a faster rate in many marine species owing to a large N_e (Hemmer-Hansen 2014). Linkage disequilibrium refers to the nonrandom association of alleles at two or more loci, or in

other words, the likelihood that two loci are 'linked' on a chromosome (Slatkin 2008). Thus, in populations with a large N_e, more recombination is likely to occur and lead to the decay of linkage disequilibrium (Nordborg & Tavaré 2002), which will in turn decrease the likelihood of SNP detection because fewer genes are linked to the outlier loci. However, SNP detection can be increased with restriction-site-based genotyping, using frequently cutting enzymes in combination with larger volumes of sequencing (Nielsen et al. 2011, Hemmer-Hansen 2014).

Molecular markers in conservation

Multiple studies have theoretically explored the use of different marker types and genomic tools for different conservation objectives (Wan et al. 2004, Beger et al. 2014, Funk et al. 2012, Corlett 2016). For example, if the objective is to identify management units (such as fisheries stocks), microsatellites are an ideal marker because their fast mutation rate can be used to estimate population demographic patterns, and the non-coding repeat motifs are thought to reflect neutral processes (Schlötterer et al. 1999). But if the objective is to identify evolutionary significant units (such as areas with high biodiversity), outlier 'adaptive' SNPs may be more informative because they should reflect selective processes and adaptive potential (Funk et al. 2012). In order to extend the theoretical basis of multi-marker conservation objectives, this chapter aims to empirically compare three marker types, namely mtDNA, 'neutral' SNPs and outlier SNPs, within a spatial conservation planning framework.

While empirical conservation planning comparisons between molecular markers are lacking in the marine realm, there are examples of conservation trade-offs associated with different marker types within terrestrial and freshwater ecosystems. For example, Sork et al. (2009) found that to conserve genetic diversity of the California valley oak across 37 sites, six sites were required to capture chloroplast allelic diversity, eight sites to capture nuclear allelic diversity and 11 sites to capture the allelic diversity of both markers. Using neutral and adaptive amplified fragment length polymorphism (AFLP) markers to identify conservation priority areas for the Austrian dragonhead (*Dracocephalum austriacum*) and the common frog (*Rana temporaria*), Bonin et al. (2006) found no significant correlation between the marker types for the two species, and that relatively few populations were required to conserve neutral diversity compared

to adaptive diversity. Lastly, Asmyhr et al. (2014), used both the mtDNA CO1 and the nuclear 18S regions to calculate phylogenetic diversity for freshwater stygofauna, and then used phylogenetic diversity to identify high biodiversity areas for conservation. They found that fewer sites were needed to conserve 18S phylogenetic diversity than CO1 diversity, but that CO1 marker might include significant diversity not captured by the 18S marker. Broadly, the results from these studies suggest that including genetic information from separate molecular markers will lead to different conservation solutions.

If there are inconsistencies in conservation priorities between different marker types, then it suggests that marker choice will depend on the conservation objective at hand. However, if there are negligible trade-offs between marker types, then it means that the inclusion of additional markers, such as next generation markers, may not be necessary. As conservation planning and implementation of reserve areas is a long and delicate process even without the generation and inclusion of genetic information, it is important to understand the most cost and time effective manner to protect genetic patterns and the evolutionary process that drive them.

I chose to use a multi-species approach to compare the conservation priorities between markers, as Chapter One showed that genetic patterns from a single species will most likely not reflect genetic patterns of entire communities. However, because next generation sequencing is much more cost and time consuming compared to traditional sequencing methods, only two species were included in this chapter. To best represent the genetic range of regional rocky shore organisms, I included the most genetically distinct species (based on mtDNA) from Chapter One, *P. angulosus* and *S. granularis*, into the analyses for Chapter Two. However, in order to compare the different marker types for *P. angulosus* and *S. granularis* within a conservation framework, I first had to generate next generation SNP data for the two species. Therefore, the first part of this chapter characterizes genomic patterns for *P. angulosus* and *S. granularis*, whereas the second part of the chapter compares conservation scenarios based on several genomic metrics for the two species.

Chapter Two, Part One: Characterizing genomic variation of the Granular limpet (*S. granularis*) and Cape urchin (*P. angulosus*) along South Africa's west coast

Introduction

This sub-chapter aims to use next generation sequencing (NGS) to characterize neutral and adaptive patterns for two non-model obligatory rocky shore species along South Africa's west coast. A pooled, reduced representation sequencing approach was implemented as it is a cost effective method to describe population-wide differences. However, the costs are still much greater for NGS than traditional methods, so only the two most genetically distinct species from Chapter One, *S. granularis* and *P. angulosus,* were included in Chapter Two. Thus, the first section of this chapter will describe genome-wide single nucleotide polymorphism (SNP) variation and suggest potential environmental and biological drivers of both neutral and adaptive patterns for the two study species.

Materials & Methods

Study species and sample collection

The focal species for this chapter are the Cape urchin (*P. angulosus*) and the Granular limpet (*S. granularis*). Firstly, these species were chosen as they are highly abundant along the South African west coast, making it feasible to collect 40 specimens at each of the six sampling localities (Fig. 12). Secondly, these species were selected because of their highly differentiated genetic patterns based on the CO1 region (Fig. 11 C; Table 1 of Supplementary Materials 2). The variation in the CO1 gene region displayed the highest population structuring for *P. angulosus* and no genetic structure for *S. granularis* (Table 1 of Supplementary Materials 2), yet this pattern may not hold with the increase in genomic data available with next generation sequencing.

Samples were collected from six rocky intertidal localities along the west coast of

South Africa (Fig. 12). Forty samples of each species (*S. granularis* and *P. angulosus*) were collected from each site. Sampling was conducted from June to August 2015. Samples were stored in 100% ethanol and transported to the Evolutionary Genomics Group (EGG) lab at Stellenbosch University. Genomic sequences are included for all six sites for the limpet *S. granularis*, however, only four sites are included for the urchin *P. angulosus* owing to library preparation and sequencing difficulties. The individuals from each of the six sample locations are labeled as separate 'populations' for the remainder of the study.

Figure 12- The sampling localities from which 40 individuals of each species were collected, indicated by the purple stars. The stars enclosed in blue circles represent the sampling localities from which genomic sequences are unavailable for the urchin *P. angulosus* (base map downloaded from www.esri-southafrica.com/basemaps).

DNA extraction protocols

Twenty-five micrograms of tissue was taken from each sample (gonad tissue from *P. angulosus*, foot tissue from *S. granularis*). Genomic DNA was extracted using Qiagen DNeasy Blood & Tissue kit following the manufacturer's protocols and

extractions were then stored at -20^{0}C. At least 3 µg of high molecular weight DNA was measured into a concentration of >40ng/µl using molecular grade H_20. The DNA concentration was quantified using the Qubit Quant iT dsDNA HS Assay system available at the Central Analytical Facilities at Stellenbosch University. To check the quality of the DNA samples, I ran a 1% agarose gel to visualize any degradation. For each species, all 40 individuals from each location were pooled to create 12 final samples. The pooled samples were flash frozen and sent to the Hawaii Institute of Marine Biology for library construction and Mi-Seq Illumina sequencing.

Restriction-site associated sequencing

Next generation sequencing offers unprecedented access to genomic information at comparatively low costs and high speeds. Yet the cost of sequencing entire genomes remains a hindrance to the majority of studies on non-model organisms (especially organisms with large genomes; Etter et al. 2011). However, most research questions within phylogenetics, phylogeography, and population genetics do not require whole genome sequencing, but rather a spread of loci across the genome. As a result, cost-effective approaches such as restriction-site associated DNA sequencing (RAD-seq) have been adopted to target a reduced representation of the genome. With RAD-seq, only the short DNA fragments that lie adjacent to the restriction endonuclease sites are sequenced, thus providing high coverage of homologous portions of the genome from multiple individuals (Baird et al. 2008). A multitude of RAD-seq strategies have been developed, such as mbRAD (Miller et al. 2007 & Baird et al. 2008), ddRAD (Peterson et al. 2012), 2bRAD (Wang et al. 2012) and ezRAD (Toonen et al. 2013), all of which have trade-offs to consider (reviewed in Andrews et al. 2014, Puritz et al. 2014). ezRAD is the only method that utilizes the Illumina Tru-Seq kits, which means the sequencing can be performed by any commercial laboratory with the Illumina library preparation services. Because the EGG lab at Stellenbosch University does not have the resources or equipment to develop in-house RAD capability, the library preparation and sequencing were performed at the Hawaii Institute of Marine Biology using ezRAD technology.

The ezRAD protocol (Toonen et al. 2013), which was performed at the Hawaii Institute of Marine Biology, uses high-frequency cutter isochimozer enzymes MboI and Sau3AI (which cut the same site) to perform a DNA double digest. The digested DNA is

inserted into an Illumina TruSeq library preparation kit, where the digesting libraries are end-repaired and TruSeq adapters are ligated to the genomic fragments. Libraries are then size-selected on a 2100 Agilent Bioanalzer, quantified using qPCR (following Kapabiosystems Illumina quantification kit protocol), and paired end sequencing using 600 cycles on the Illumina Mi-Seq platform.

Pooled sequencing

While the cost of NGS has decreased significantly over the last decade, the expenses are still much greater than traditional sequencing techniques. One method to reduce the cost of high throughput sequencing is by sequencing pools of individuals (termed Pool-Seq). Sequencing pooled DNA samples allows for more individuals to be analyzed, which increases the power to estimate allele frequencies (Futschik & Schlötterer 2010, Gautier et al. 2013), but at the cost of less accurate base calling (Schlötterer et al. 2014). While Pool-Seq has been shown to be a viable approach to identify population genomic variation and detect adaptive loci (Guo et al. 2015, 2016), there are several limitations associated with pooled sequencing (Benestan et al. 2016). For example, distinguishing low-frequency alleles from sequencing errors is more difficult with Pool-Seq because reads at the same position represent a random sample from the population, meaning variants present in only a few reads may be either a sequencing error or a sampling artifact. However, the differential representation of individuals in pooled samples only has a substantial effect when the pool sizes are very small (Schlötterer et al. 2014). Furthermore, pooled sequencing also has the drawback of sequencing biases being specific to populations instead of individuals. For example, one population may seem less variable, but this might be an artifact of lower coverage or quality. One way to circumvent this problem is by subsampling the raw reads to obtain uniform coverage before calling variants, which is possible with various SNP-calling platforms. Lastly, because Pool-Seq is not as prevalent as the individual sequencing methodologies, many analyses cannot yet accommodate Pool-Seq data sets, such as individual assignment methods (e.g. STRUCTURE) genome scans and parental assignment analyses (Cutler & Jensen 2010). Nonetheless, as this study aims to compare population-level genomic diversity and adaptive potential from pools of 40 individuals with stringent quality filtering, any Pool-Seq related biases are expected to have a

negligible effect on the results (Schlötterer et al. 2014, Fu et al. 2016).

Bioinformatics protocol

Assembly, mapping and variant calling

All pooled samples successfully sequenced with Illumina Mi-Seq were received as raw reads, and thus multiple bioinformatics steps were taken to perform quality control, *de novo* reference assembly, and variant calling (Fig. 13). Illumina reads were first analyzed on the Basespace Illumina platform, using FASTQC and FASTQ toolkits (Andrews 2010) to trim adapter sequences, overrepresented sequences, and reads with a Phred quality score less than 25. Reads were then converted from Illumina +64 quality scoring to Sanger +33 scoring using FASTQ Groomer (Blankenberg et al. 2010) tool on the Galaxy platform (Afgan et al. 2016).

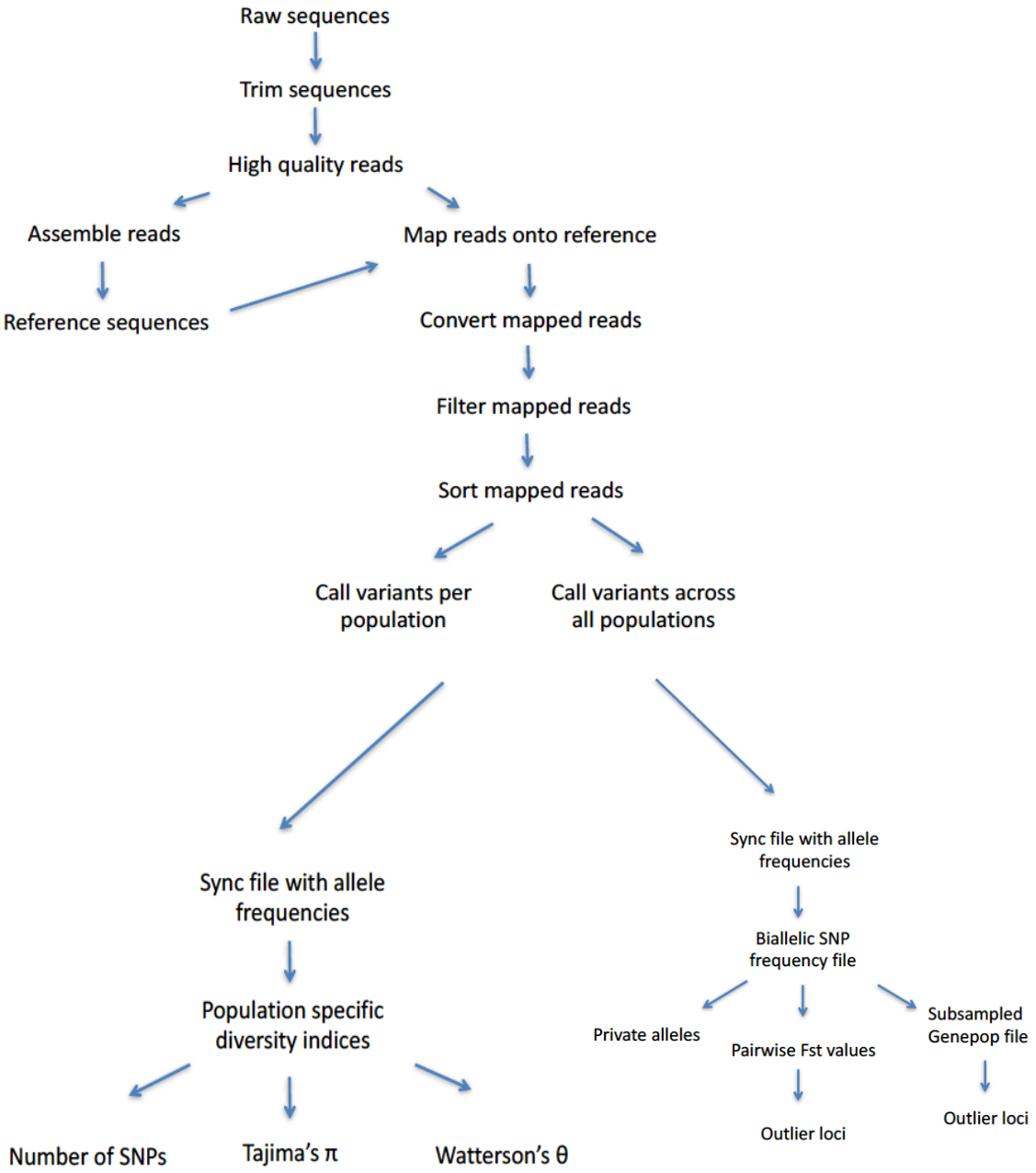


Figure 13 - The various steps of the bioinformatics analyses, which include trimming reads, assembling reads, mapping, converting and sorting reads to then call variants. The variant files were further analyzed to calculate population specific diversity measures, as well as identify potential outlier single nucleotide polymorphisms (SNPs).

All further analyses were conducted using Perl, Shell and Bash scripts modified from those written by Guo et al. (2016; Supplementary Materials 4). Quality reads were assembled *de novo* using the 'velvetoptimiser' command in Velvet v.1.2.10, as optimizing k-mer lengths for RAD sequences produces the highest quality assemblies (Davey et al. 2012). The contig sequences corresponding to each sample were combined

and assembled with Cap3 (Huang & Madan 1999) using default parameters, creating the reference sequences. Filtered reads were first mapped onto the reference sequences with ALN and SAMPE commands within BWA v.0.7.13 (Li & Durbin 2009), which are specifically tailored for long (>100bp) paired-end reads and the semi-global alignment and realignment of unmapped reads is preferred for pooled samples (Schlötterer et al. 2014). Mapping and read group attachment was performed for each population using default parameters. Mapping results (number of mapped versus unmapped reads) were calculated using the 'stats.idx' command in SAMtools v.1.3 (Li 2011). The resulting BAM files were filtered in SAMtools v.1.3, discarding all reads with a mapping quality score (MAPQ) < 15. SAM files were then converted to BAM format and sorted by alignment position using SAMtools v.1.3. The sorted BAM files were used to call variants with SAMtools 'mpileup' command, with a minimum quality score of 15 and maximum depth of 1,000 reads per locus. The mpileup variant file was converted with PoPoolation2 (Kofler et al. 2011b), producing a sync file indicating allele counts across all populations.

Population genomic variation

To characterize population-specific genetic variation, the number of SNPs, nucleotide diversity (Tajima's π) and population mutation rate (Watterson's θ_W), were calculated for each population using the 'variance-sliding' command in PoPoolation 1.2.2 (Kofler et al. 2011a). The number of SNPs, π, and θ_W were all calculated using a sliding window of 1,000 base pairs (bp), which was chosen after testing windows of 100, 1,000 and 10,000 bp. In order to standardize for sequencing biases, I first subsampled for uniform coverage (minimum coverage of 10 and maximum coverage of 500) and set a minimum allele count of 2 and a quality score of 10. Private alleles were identified from the population-specific allele counts given by the 'snp-frequency-diff' command in Popoolation2. All population genetic estimates were calculated from biallelic SNPs, as the majority of SNPs in nature are thought to be biallelic (Kumar et al. 2012) and thus non-biallelic SNPs are likely to be sequencing errors.

Detection of selection footprints

Outlier loci were identified using three methodologies, all calculated from the sync file produced by Popoolation2, using a minimum allele count of 2 with a target coverage of 10. The first method follows an empirical outlier detection approach (Akey et al. 2010), using Popoolation2 to identify pairwise F_{ST} values for each SNP, and then selecting the SNPs falling into the 99.5 percentile of the empirical distribution of pairwise F_{ST} values. The second method follows a Bayesian approach (Foll & Gaggiotti 2008), where sync files were first converted and exported as Genepop files with Popoolation2. Genepop files were further edited using Perl script, to merge all contigs and identify locus positions. The edited Genepop files were converted into Bayescan files using PGDSpider2 v.2.1.03 (Lischer & Excoffier 2012), using custom spid parameters (Supplementary Materials 4). To identify candidate outlier loci, 20 pilot runs of 5,000 iterations and a burn-in of 50,000 were performed with Bayescan v.2.1(Foll & Gaggiotti 2008), using prior odds of 100, 55,000 reversible-jump MCMC chains, a thinning interval of 10 and a false discovery rate (FDR) of 0.05. The third approach, LOSITAN (Antao et al. 2008), uses the relationship between the expected heterozygosity (H_E) and F_{ST} in an island model of migration with neutral markers to describe the expected distribution of Wright's inbreeding coefficient. The loci with excessively high or low F_{ST} values compared to the neutral expectations of the distribution are then identified as outlier loci. LOSITAN was run with 1,000,000 simulations, using the "neutral mean F_{ST}" and "force mean F_{ST}" options, a confidence interval of 0.95, FDR of 0.05, and subsample size of 40.

In order to obtain the functional roles of the outlier loci that were chosen by at least two of the three methods, the contigs associated with each outlier locus were subject to BLASTX searches, using the non-redundant protein sequences database and all other default parameters (Altschul et al. 1997).

Results

Sequencing and assembly

A total of 35.4 million paired reads were obtained from the *S. granularis* samples, with the average number of paired reads per population being 5.9 million. The *de novo*

assembly produced a total of 415,329 contig sequences, ranging from 100 to 2,350 bp in length, which were combined to create the reference sequence for all downstream analyses. A total of 4.59 million reads were mapped onto the reference sequences, with number of mapped reads ranging from 0.97 to 2.05 million for each population (Table 5).

Table 5 - Sequencing, assembly and mapping quality of *S. granularis* (Granular limpet) reads per sample site (SP= Sea Point, JB= Jacobsbaai, LB= Lambertsbaai, BB= Brand se Baai, HB= Hondeklipbaai, PN= Port Nolloth).

Sample Site	No. reads sequenced	No. reads after quality trimming	Avg. insert size (base pairs)[a]	% High quality	% Assembled	No. Mapped reads (after quality filtering)
SP	6,103,850	5,595,480	518	0.92	0.91	1,584,064
JB	6,455,988	5,967,348	533	0.91	0.89	1,905,470
LB	5,148,028	4,652,058	527	0.87	0.91	1,606,380
BB	6,695,624	6,124,314	539	0.91	0.91	2,054,810
HB	6,136,986	5,390,052	532	0.86	0.86	1,843,492
PN	4,916,780	3,595,364	495	0.64	0.73	977,910

a. Insert size is the length of the DNA sequenced between the adapters (including both read 1 and 2).

Due to complications in library preparation, two urchin populations are still awaiting Illumina Mi-Seq sequencing at the Hawaii Institute of Marine Biology (Fig. 11). Of the four populations that were sequenced, 17.3 million paired reads were acquired. A total of 165,237 contig sequences were obtained from the *de novo* assembly, with each individual contig ranging from 100 to 2,733 bp in length. After mapping, 8.7 million reads were aligned to the reference sequence, with total mapped reads ranging from 0.72 to 1.65 million for each population (Table 6).

Table 6 - Sequencing, assembly and mapping qualities of *P. angulosus* (Cape urchin) reads (SP= Sea Point, LB= Lambertsbaai, BB= Brand se Baai, PN= Port Nolloth).

Sample Site	No. reads sequenced	No. reads after quality trimming	Avg. insert size (base pairs)[a]	% High quality	% Assembled	No. Mapped reads (after quality filtering)
SP	3,618,014	3,315,950	499	0.84	0.47	722,318
LB	4,452,736	3,857,728	450	0.86	0.83	1,651,136
BB	4,633,086	4,235,634	506	0.87	0.80	1,072,332
PN	4,557,178	3,400,394	616	0.75	0.86	1,150,778

a. Insert size is the length of the DNA sequenced between the adapters (including both read 1 and 2).

Genome-wide variation

Scutellastra granularis

The total number of SNPs identified with PoPoolation v.2 across all *S. granularis* populations was 18,178, with the number of SNPs within each population ranging from 11,638 to 15,807 (Table 7). The within-population genome-wide average nucleotide diversity ranged from 0.0127 to 0.0135 for Tajima's π and 0.0157 to 0.0196 for Watterson's θ_W. The number of private SNPs varied greatly across populations, ranging from 135 to 511, with the percentage of population-specific private SNPs ranging between 0.012% to 0.036% (Table 7).

Table 7 - Number of single nucleotide polymorphisms (SNPs) and private SNPs, nucleotide diversity, and population mutation rate for each *S. granularis* (Granular limpet) population (SP= Sea Point, JB= Jacobsbaai, LB= Lambertsbaai, BB= Brand se Baai, HB= Hondeklipbaai, PN= Port Nolloth).

Sample Site	No. of SNPs	Tajima's π	Watterson's θ_W	No. of private SNPs	% of SNPs that are private	No. of outlier SNPs*	% of SNPs that are outlier*	No. of private outlier SNPs*
SP	15,807	0.0129	0.0188	391	2.47%	16	0.101%	0
JB	15,044	0.0131	0.0182	223	1.48%	8	0.053%	0
LB	13,199	0.0132	0.0178	194	1.47%	4	0.030%	0
BB	14,832	0.0135	0.0196	195	1.31%	13	0.087%	0
HB	14,053	0.0135	0.0187	511	3.64%	33	0.234%	19
PN	11,638	0.0127	0.0157	135	1.16%	7	0.060%	0

*These outlier SNPs refer to the SNPs that were identified by Popoolation as well as LOSITAN.

Parechinus angulosus

For *P. angulosus* populations, Popoolation v.2 identified a total of 5,285 SNPs, with the within population number of SNPs ranging from 1,273 to 2,925 (Table 8). The population specific nucleotide diversity values, Tajima's π and Watterson's θ_W, ranged from 0.0120 to 0.0139 and 0.0190 to 0.0263, respectively (Table 8). The number of private SNPs ranged from 40 to 416, and the percentage of private SNPs ranged from 0.02% to 0.14% (Table 8).

Table 8 - Number of single nucleotide polymorphisms (SNPs) and private SNPs, nucleotide diversity, and population mutation rate for each *P. angulosus* (Cape urchin) population (SP= Sea Point, LB= Lambertsbaai, BB= Brand se Baai, PN= Port Nolloth).

Sample Site	No. of SNPs	Tajima's π	Watterson's θ_W	No. of private SNPs	% of SNPs that are private	No. of outlier SNPs*	No. of private outlier SNPs*
SP	1,273	0.0139	0.0190	51	4.01 %	4	3
LB	2,008	0.0120	0.0177	40	1.99 %	1	0
BB	2,097	0.0128	0.0194	53	2.52 %	1	0
PN	2,925	0.0138	0.0263	416	14.2 %	1	0

*These outlier SNPs refer to the SNPs that were identified by Popoolation as well as LOSITAN.

Detection of outlier loci

A total of 8,722 loci from the *S. granularis* populations were included into all three outlier detection analyses. Bayescan analyses identified zero outlier loci within the *S. granularis* populations. LOSITAN identified 461 outlier loci, 49 of which were under divergent selection and 412 were under balancing selection. The empirical approach identified a total of 306 outlier loci, 39 of which were also selected by LOSITAN. The total number of outlier SNPs (detected by more than one method) varied per population, with Lambertsbaai having the lowest number of outliers (4) and Hondeklipbaai having the largest amount (33; Table 7). Further, Hondeklipbaai was the only location to have private outlier SNPs, with a total of 19 unique outliers (Table 7). The 39 outliers chosen by both Popoolation and LOSITAN are on a total of 12 contigs, most of which were identified as hypothetical proteins in BLASTX searches, however three contigs were highly similar to histone proteins and one contig was fairly similar to the mtDNA CO1 (Table 9).

Table 9 - The top results from the *S. granularis* (Granular limpet) BLASTX searches, including the percent of identical nucleotide bases.

Contig (containing one or more outlier loci)	BLASTX search result	% identical
Contig291466	hypothetical protein	83%
Contig296167	histone H3c	100%
Contig297270	cytochrome c oxidase subunit 1	75%
Contig303805	predicted histone H2B type 2-F-like isoform X1	90%
Contig308129	no significant similarity found	
Contig308387	hypothetical protein	79%
Contig310087	hypothetical protein	36%
Contig31551	predicted histone H2A	99%
Contig321053	hypothetical protein	36%
Contig33640	hypothetical protein	54%
Contig377699	hypothetical protein	78%
Contig380076	uncharacterized protein	59%

Of the 1,025 *P. angulosus* loci analyzed, zero SNPs were selected as outlier loci by Bayescan. LOSITAN identified 14 outlier loci, with 3 divergent loci and 11 balancing loci. The empirical approach with Popoolation2 identified 24 outlier loci, 4 of which were also selected by LOSITAN. Of those four loci selected by both methods, three were under divergent selection, which were all unique to the Sea Point location, and one under balancing selection, which was shared across all populations (Table 8). These four outlier loci are located on three contigs, with each contig aligning to different proteins in the BLASTX searches (Table 10). One contig is highly similar to mtDNA CO1, another is relatively similar to mtDNA NADH dehydrogenase subunit 5, and the third is faintly similar to a protein predicted to be RNA-directed DNA polymerase (Table 10).

Table 10 - The top results from the *P. angulosus* (Cape urchin) BLASTX searches, including the percent of identical nucleotide bases.

Contig (containing one or more outlier loci)	BLASTX search result	% identical
Contig292	NADH dehydrogenase subunit 5	65%
Contig5773	predicted: RNA-directed DNA polymerase	36%
Contig27802	cytochrome c oxidase subunit 1	96%

Discussion

The South African west coast is highly threatened (Sink et al. 2012) and considered a climate change hotspot (Popova et al. 2016). Therefore, it is important to understand the evolutionary patterns and the adaptive potential of marine organisms inhabiting this region. This chapter characterizes genome-wide neutral and adaptive variation for two marine invertebrate species, *S. granularis* and *P. angulosus,* along South Africa's west coast using a pooled RAD sequencing approach. The SNP nucleotide diversity levels are considerably greater than the mtDNA nucleotide diversity levels for *S. granularis*, but only slightly larger for *P. angulosus*, suggesting that the *S. granularis* populations have expanded along the west coast more recently than *P. angulosus*, possibly owing to differences in low and high shore habitat availability during the Last Glacial Maximum (LGM) 22,000 years ago (Flemming et al. 1998, Petit et al. 2003, Seymour unpublished data).

From a genetic divergence perspective, the most genetically unique sites are Hondeklipbaai and Sea Point for *S. granularis*, and Port Nolloth and Sea Point for *P. angulosus*, making it difficult to generalize patterns for entire communities, which has been shown previously for the South African coastline (Teske et al. 2011). While the South African west coast is a relatively small and homogeneous study region compared to other seascapes in which adaptive variation has been identified (Bradbury et al. 2010, Renault et al. 2011, Bourret et al. 2013, Milano et al. 2014), there are still multiple features that could be shaping the genomic patterns of these two species, such as regional oceanographic conditions including upwelling and wave action, or fine scale

changes in temperature, primary productivity and dissolved minerals, or responses to anthropogenic stressors such as pollutants (Trussell et al. 2001, Suarez-Ulloa et al. 2013, Selkoe et al. 2016). The intraspecific differences in number of private and outlier SNPs found within the relatively homogeneous environment of South Africa's west coast suggests that studies aimed at identifying adaptive loci should not only focus on large areas over environmental gradients, but also over regions with fine scale differences in ecotypes.

Genomic variation and selection footprints

Genomic-level population diversity

Overall, noticeably more SNPs were identified for *S. granularis* populations compared to *P. angulosus*, with the average number of SNPs per population being 14,095 and 2,075 respectively (Tables 7 and 8). However, the number of identified SNPs is likely to be biased by the differential sequencing success between the two species. Firstly, six *S. granularis* populations were sequenced compared to four *P. angulosus* populations. Furthermore, the number of raw sequences and high quality sequences are substantially lower in the *P. angulosus* samples compared to the *S. granularis* samples (Tables 5 and 6). As the number of trimmed reads were used to create the reference sequences, this would have led to the larger number of contigs in the *S. granularis* references sequences compared to *P. angulosus.* The number of reference contigs and mapped reads will therefore affect the number of SNPs identified, as well as the number of private SNPs, thereby making it difficult to compare SNP diversity between species.

While the total number of SNPs differ between species, the nucleotide diversity and mutation indices are relatively similar (Tables 7 and 8). The average nucleotide diversity and population mutation rate for *S. granularis* are 0.0132 and 0.0181 compared to the average values of 0.0131 and 0.0206 for *P. angulosus*. Moreover, these diversity indices are quite high compared to other marine organisms, with average genome-wide SNP nucleotide diversity being 0.003 in Three-spine sticklebacks, *Gasterosteus aculeatus* (Guo et al. 2015), 0.0075 in Atlantic herring, *Clupea harengus* (Guo et al. 2016), 0.0006 in the Black turban snail, *Chlorostoma funebralis* (Gleason et al. 2016),

0.004 in the sea anemone *Aiptasia* species (Bellis et al. 2016) and 0.0024 in three hamlet *Hypoplectrus* species (Picq et al. 2016).

The high nucleotide diversity values in this study could possibly be a result of the sequencing or bioinformatics methodologies (Shafer et al. 2016). For instance, none of the above mentioned studies used ezRAD sequencing, which means that the high nucleotide diversity indices in this study could be related to the areas where the specific restriction enzymes splice the DNA. For instance, if the enzymes cut in fast mutating non-coding regions of the genome, this could lead to a larger number of single nucleotide mutations, leading to higher nucleotide diversity and mutation rates (Davey et al. 2013). Another possibility is that the parameters used throughout the bioinformatic analysis contribute to the high nucleotide diversity measures. For example, the number of SNPs will be affected by the *de novo* assembly and mapping parameters, with downstream analyses such as estimating π and θ dependent on initial assembly. Also, it could be that the size of the sliding window, the quality and minimum allele frequency and allele count cut-offs have an effect on the genome-wide nucleotide diversity indices. However, due to time constraints, the comparisons of genome-wide diversity indices derived from various bioinformatics programs and parameters could not be performed within this study and in general are still poorly understood (Shafer et al. 2016). Finally, the observed results are unlikely owing to pooling biases, because several of the above mentioned studies were based on pooled samples (Guo et al. 2015, 2016).

Alternatively, it is possible that the observed genetic diversity levels are a result of biological processes, rather than methodological artifacts. For example, the two species could have exceedingly high levels of genomic diversity due to large effective population sizes (Frankham 1996) and abundances along the South African west coast (Emanuel et al. 1992). The low nucleotide diversity values for *S. granularis* mtDNA CO1 region suggests that the species does not have historically high effective population levels, which could mean that the west coast populations have gone through recent expansions in effective population size. This pattern is supported by the findings of Wright et al. (2015), who found that 11 rocky shore species on average had lower genetic diversity values (from mtDNA regions) along the west coast compared to the south and east coasts of South Africa.

These signals are interesting as paleo-climatic models of air and sea-temperatures suggest that coastal rocky shore species experienced range contractions along the west coast during the LGM 22,000 years ago, with northern populations

probably becoming extinct (Seymour, unpublished data). Post LGM, populations would have expanded northwards again, hence explaining some of the genetic diversity signals for the two species. Interestingly, both air and sea temperature were identified as drivers of historical distribution patterns for six *Patella* limpet species (Seymour, unpublished data), which suggests that *S. granularis* may have had a more recent expansion into the west coast because it is more exposed on the high shore compared to *P. angulosus* which remains submerged at the low shore or in rock pools.

Other possible explanations are the occurrence of sex-biased asymmetries, adaptive introgression of mtDNA, or the inherent differences in mutation rates and sample sizes between the two marker types (Toews & Brelsford 2012). The mtDNA nucleotide diversity values for *P. angulosus* are only slightly lower than those for the SNP markers, indicating the *P. angulosus* populations have historically and contemporarily large effective population sizes. This genetic pattern is particularly interesting, as the BLASTX results show that the outlier loci within the *P. angulosus* populations are similar to the mtDNA CO1 and NADH gene regions, meaning that the historically high levels of genetic diversity and population structuring in *P. angulosus* could be to due selection within mtDNA regions.

Other studies have found both concordance and incongruence in genetic diversity levels between next generation and traditional markers. Fernandez et al. (2015) found that SNPs showed similar diversity patterns to microsatellites but not mtDNA in white-sided dolphins (*Lagenorhynchus obliquidens*), and Ford et al. (2015) also found that SNPs showed more similar patterns to microsatellites in cichlids (genus *Alcolapia*). Overall, there are many cases of discordance between molecular markers, and generally the trend seems to be that SNP markers will result in higher levels of population structuring and genetic diversity, compared to mtDNA markers (Blance-Bercial & Bucklin 2016, Benestan et al. 2015, Reitzel et al. 2013, Ogden et al. 2013, Jackson et al. 2014). The increased similarities between SNP and microsatellite compared to SNP and mtDNA patterns is most likely because SNPs and microsatellites share similar marker characteristics, such as bi-parental inheritance, as well as increased detection of recent demographic events owing to increased marker variability (Seeb et al. 2011, da Fonseca et al. 2016, Maroso et al. 2016).

Genomic-level population differentiation

The NGS results show different genomic patterns between populations of *S. granularis* and *P. angulosus*. For instance, the average percentage of private SNPs is higher in *P. angulosus* populations compared to *S. granularis* (Tables 7 and 8), which is expected as *P. angulosus* had higher structuring in the mtDNA gene region. It is interesting that *P. angulosus* has higher levels of genetic structure and genomic uniqueness despite having a pelagic larval duration (PLD) of 49-56 days, compared to the expected PLD of 4-10 days for *S. granularis* (Dodd 1957, Muller et al. 2012). This scenario of higher genetic variability in both the mtDNA and SNP analyses for the species with a higher PLD supports the notion that PLD is not an effective predictor of evolutionary patterns (Weersing & Toonen 2009, Selkoe et al. 2014). However, the higher percent of private SNPs may also be a result of *P. angulosus* having fewer populations for SNPs to be shared between, which remains to be tested.

Further, the population differences in private SNPs are inconsistent between species, with the population with the highest percentage of private SNPs being Hondeklipbaai for *S. granularis* and Port Nolloth for *P. angulosus*, although both are representative of the northern range of both species (Tables 7 and 8). However, Sea Point is the second most unique site for both species. Interestingly, Port Nolloth is the most unique site for *P. angulosus* and the least unique site for *S. granularis*. This illustrates how two obligate rocky shore species with similar distributions and life history traits are capable of having highly distinct genomic patterns.

In addition, the number of outlier SNPs seems to be linked with the percentage of private SNPs within the *S. granularis* populations, but not within *P. angulosus* populations (Tables 7 and 8). The association between private SNPs and outlier SNPs is expected, as the potential causes of genetic uniqueness are either a break in gene flow or localized selection forces, and these selective forces should similarly influence patterns of outlier SNPs (Morin et al. 2004, Seeb et al. 2011). Highly distinct populations, such as the Hondeklipbaai *S. granularis* population, could also have an effect on outlier detection, as it will likely affect the 'neutral' F_{ST} values from which the outlier F_{ST} values are derived from. However, when the Hondeklipbaai *S. granularis* population is excluded from the LOISTAN analyses, 35% of the outlier SNPs are still identified, meaning that there are still outliers that are under both balancing and divergent selection

associated with the other *S. granularis* populations.

In contrast, there is little congruence between the number of private SNPs and outlier SNPs in the *P. angulosus* populations, probably as a result of fewer outlier SNPs identified. As with *S. granularis*, the number of private outlier SNPs appears to be linked with the number of outlier SNPs (Table 8). However, it is unexpected that private outlier SNPS were only found in single populations of *S. granularis* and *P. angulosus*. With the detection of highly localized unique outliers, and all private outliers labeled as under divergent selection, these loci are likely to be linked to environmental or biological pressures unique to the respective locations (which are discussed further in the following section).

Potential drivers of genomic differentiation

Scutellastra granularis

Each *S. granularis* population was characterized by different levels of private and outlier SNPs (Table 7). For example, the most distinct population for *S. granularis* is Hondeklipbaai, with the highest number of private SNPs, outlier SNPs and private outlier SNPs. The genomic distinctiveness of Hondeklipbaai is unlikely to be sampling artifact, as all *S. granularis* individuals were collected within the winter months and sampled evenly across each site.

Therefore, numerous environmental features could be behind the genomic uniqueness of this population (Selkoe et al. 2010, Riginos & Liggins 2013), namely wave action, exposure to temperature, shore slope, and biological features such as primary productivity and food availability, or competition within the high shore community (Trussell et al. 2001). Furthermore, the northern region of South Africa's west coast experiences seasonal and localized upwelling events (Jury & Taunton-Clark 1986, Hagen et al. 2001), which can be driving the observed pattern. Upwelling cells can have multiple effects on the local seascape, including lowering pH, as well as increasing CO_2 and primary productivity levels (Feely et al. 2008, 2010, Erga et al. 2012), all of which can act as selective forces on marine invertebrates. De Wit and Palumbi (2013) identified outlier loci associated with biomineralization proteins in red abalone individuals located within a frequent upwelling ecotype, which suggests that

upwelling events may lead to adaptation in the development of mineralized tissues, such as the shells of marine mollusks. Further, Evans et al. (2013) found that purple urchin larvae adjust skeletogenic pathways to sustain calcification in the low pH environment associated with chronic upwelling systems.

Additionally, Hondeklipbaai harbors localized mining activity that result in approximately 14km of the coastline being characterized by dark red/black sand year-round due to shallow copper deposits (Fig. 14). Although the effect of metal exposure on the adaptive potential of marine species is poorly understood, it is likely that the presence of copper deposits in this region could lead to increased levels of local adaptation, and to adaptive variants of biomineralization and metallothionein proteins (English et al. 2012, Wit & Palumbi 2012). This was shown for the mussel *Mytilus galloprovincialis,* with increased iron and cadmium exposure leading to increased resilience in anoxic environments (Viarengo et al. 1999).

Figure 14 - Evidence of the dark red/black coloration of the sand along the 14km stretch of mining areas near Hondeklipbaai.

The BLASTX results (excluding those which were not hypothetical proteins) suggest that the private outliers in this location are related to histones (Table 9). Broadly, the function of histones is to assist with the formation of DNA within a chromosome, yet histone variants have several other functional roles, such as regulating transcription and signaling cell-cycle regulation (Suganuma & Workman 2011). Santoro

and Dulac (2012) also found that a variant of histone H2B controls the expression of olfactory genes in mice, and suggest that isoforms of histones may have specialized functions in specific tissues.

Within marine invertebrates, histone variants have been found to alter chromatin structure and regulate chromatin-related genes in response to environmental signals such as harmful algal blooms (Suarez-Ulloa et al. 2013). However, Hondeklipbaai is not known to experience algal blooms, compared to other areas of the coastline where they occur periodically (Pitcher & Calder 2000). The epigenetic function of histones (meaning they control gene expression) can actually lead to heritable changes and long-term adaptation (Gapp et al. 2014, Suarez-Ulloa et al. 2015). Suarez-Ulloa et al. (2015) suggest using epigenetic markers, such as the replacement of canonical histones with variant histones, as pollutant biomonitoring tools in marine organisms. They argue that pollution, especially with genotoxic characteristics, prompt genetic reprogramming (such as remodeling chromatin fibers, DNA methylation, modifying histones) in order to preserve genome integrity. Therefore, copper deposits and/or other pollutants could be driving selection for histone variants within *S. granularis* individuals in Hondeklipbaai. A further explanation for the identification of histones as outlier loci in *S. granularis* could be a result of genetic hitchhiking, which is when genes are spaced within close proximity along a chromosome and therefore selection on one gene leads to a change in allele frequencies of other genes (Barton 2000), however this could not be verified due to the inability of linkage disequilibrium tests to operate on pooled RAD samples (Schlötterer et al. 2014, Benestan et al. 2016).

After Hondeklipbaai, the Sea Point population of *S. granularis* has the second largest amount of both private and outlier SNPs. Because Sea Point is located within the urban Cape Town city center, it experiences high levels of human disturbance and pollution, both of which could have a selective effect on *S. granularis* individuals in the area. The Sea Point population includes an outlier locus that is 100% similar to histone H3C (Table 9), which could be adapted to water pollutants specific to Sea Point, supporting the findings of Suarez-Ulloa et al. (2015).

Several environmental features could be shaping the unique genomic make-up of the Hondeklipbaai and Sea Point *S. granularis* populations, such as fine scale differences in temperature, rocky shore zonation or wave action (Trussell et al. 2001). Other genomic studies on marine snails have identified outlier loci associated with proteins related to temperature, such as molecular chaperone heat shock proteins

(Tomanek & Somero 1999, 2002, Kelly et al. 2012, Chu et al. 2014). Further, a study by Dong and Somero (2009) compared the enzymatic activity of NADH dehydrogenase across six marine snail species within the *Lottia* genus, and found that high shore species performed better at higher temperatures compared to low-shore species. Expanding on this, Galindo et al. (2010) identified outliers associated with shell matrix, muscle and metabolic proteins (including NADH dehydrogenase) and reverse transcriptases from the periwinkle, *Littorina saxatilis* individuals within either the high- or mid-shore ecotypes. There is also evidence of wave action driving adaptive differentiation in *L. saxatilis*, with Martinez-Fernandez et al. (2010) suggesting that variants of the CO1 gene are involved in increasing the metabolic activity of wave-adapted individuals.

It is difficult to ascertain which exact environmental or biological features are mostly responsible for the genomic patterns within west coast *S. granularis* populations, although it is likely that there are additive, rather than single, processes shaping the evolutionary dynamics of marine species in the ocean (Andrello et al. 2015). To further understand the evolutionary processes within this dynamic system, seascape genomic and isolation by distance/environment analyses should be performed, including environmental and or biological features in comparison with the SNP data generated within this study.

Parechinus angulosus

In comparison to the population-level genetic patterns of *S. granularis,* the two most genomically distinct *P. angulosus* populations are located in Port Nolloth, with noticeably more private SNPs, and Sea Point with markedly more outlier and private outlier SNPs (Table 8). As before, it is difficult to discern if genomic patterns in private and outlier SNPs are a result of sampling biases, but all sampling efforts were conducted in a way as to minimize any potential biases. Furthermore, with the Hondeklipbaai sample removed from analyses, there is a large spatial gap in the sampling regime, which might lead to the higher amount of private SNPs found in Port Nolloth.

The increased number of private SNPs in Port Nolloth could also be owing to the population being closest to the Orange River upwelling cone (Lett et al. 2007). The highly localized and seasonal upwelling along the west coast leads to different oceanographic microclimates along the coastline (Andrews & Hutchings 1980, Monterio

& Largier 1999, Hagen et al. 2001). For instance, upwelling occurrences can lead to localized differences in CO_2 and pH levels (Feely et al. 2008, Hales et al. 2005). Previous studies on the purple urchin, *S. purpuratus*, found that increased CO_2 and decreased pH levels lead to a decrease in larval size (Pauline et al. 2011, Kelly et al. 2013). Evans et al. (2013) also found increased pH tolerance in purple urchin populations that were exposed to re-occurring upwelling events, with adaptations including altered bioavailability of calcium and adjusted skeletogenic pathways, both of which could help individuals sustain calcification levels in a low pH environment. In addition, Foo et al. (2012) found that increased CO_2 levels decreased the percentage of cleavage and that increased temperature levels decreased the percentage of gastrulation in embryos for the urchin *C. rodersii.* Together these studies suggest that, through altering localized oceanographic regimes, upwelling can drive genomic variation within Cape urchin individuals along the west coast.

The distinctly high number of private SNPs in Port Nolloth could also be a result of other environmental features such as wave action, sea and surface temperatures, substrate type, or biological features such food availability, larval behavior or competition with other species (Trussell et al. 2001). Although South Africa's west coast can be largely thought of as homogeneous in environmental features such as wave action and sea temperature, there are signals of fine scale differences in temperature and primary productivity along the coastline (Bustamante et al. 1995, Gremillet et al. 2008). Several other genomic studies have attributed genome-wide genetic patterns to regional oceanographic features, including ocean currents (Jackson et al. 2014), sea level changes (Zarraonaindia et al. 2014), sea surface temperature (De Wit & Palumbi 2013), as well as anthropogenic related stressors (Rietzel et al. 2013).

Similar reasoning can be applied to the high number of outlier and private outlier SNPs found in Sea Point, as local oceanographic features can also be driving the genomic uniqueness of this population. Yet in addition to the above mentioned features, Sea Point is also the most metropolitan of the six sample sites, which could also explain the increased number of outlier loci. The BLASTX searches showed that the three contigs including outlier loci are 96% similar to mtDNA CO1 gene region, 65% similar to NADH dehydrogenase subunit 5, and 36% similar to the predicted RNA-directed DNA polymerase (Table 10). The mtDNA CO1 gene has a functional role within energy metabolism (Tsukihara et al. 1996) and has been found to be under selection in several species (Goldberg et al. 2003, Ward et al. 2007, Castoe et al. 2008). For instance,

Consuegra et al. (2015) suggest that selection on the mtDNA genome may be linked to low temperatures and increased metabolic efficiency in Atlantic salmon, and Silva et al. (2014) found evidence for temperature driving the distribution of mtDNA frequencies in the European anchovy.

Furthermore, NADH dehydrogenase subunit 5 was also selected by the BLASTX searches to be under selection in this population. Interestingly, NADH dehydrogenase is also functionally associated with metabolic pathways (Porcelli et al. 2015). Moreover, several studies have shown that NADH dehydrogenase and other genes related to energy metabolism may be under selection forces associated with changes in temperature in marine mollusks (Pante et al. 2012, De Wit & Palumbi 2013, Gleason & Burton 2016). Additional studies found evidence of differential metabolic adaptations within purple urchin and red abalone populations sampled across temperature gradients (De Wit and Palumbi 2013, Pespeni et al. 2013).

Temperature was not anticipated to be a prominent selection force on west coast *P. angulosus* populations, because the South African west coast is not generally considered to have a strong temperature gradient, and the low resolution temperature information that is available shows minor differences between Sea Point and the other sample locations (Gremillet et al. 2008). However, the association between thermal regimes and genetic patterns found in the above mentioned studies may be owing to the availability of temperature data compared to other environmental data sets. Further, there may be other environmental features that are linked with temperature which are selecting on metabolic genes such as CO1 and NADH dehydrogenase. With the increase in seascape genomics studies as well as the number of environmental features included in seascape analyses (Selkoe et al. 2008, Riginos & Liggins 2013), the driving forces of adaptive genomic patterns of *P. angulosus* might become more apparent.

Concluding remarks

Within conservation planning it is important to not only protect biodiversity patterns, but also processes that shape biodiversity, such as gene flow and natural selection (Bowen 1999). However, it is difficult to confidently pinpoint the evolutionary processes that are shaping the genetic patterns within and between species. Yet with the increase in genetic information associated with NGS, we are able to distinguish genetic

patterns with greater resolution, as well as identify specific loci that are expected to be under selection. This chapter was able to capture genome-wide diversity patterns for *S. granularis* and *P. angulosus*, within a relatively small and fairly homogeneous coastal region. The results show that for high-dispersing marine species along a linear coastline with no obvious environmental gradients (at least compared with other adaptive genomics studies; Wilding et al. 2001, Gremillet et al. 2008, Wit & Palumbi 2012, Pepensi et al. 2012), there are still signals of selection. However, the west coast is a highly dynamic marine system, and environmental information at a finer resolution will be invaluable to better understand how ecosystem characteristics interact with genetic patterns of the local marine species. Lastly, the SNP data set in this chapter identified distinct genetic patterns compared to the mtDNA data in Chapter One, and thus differences in their conservation spatial solutions can be expected in the following sub-chapter.

Chapter Two, Part Two: Comparing molecular markers within a conservation planning framework for two rocky shore species

Introduction

Chapter One showed that different genetic metrics from a single marker (mitochondrial DNA or 'mtDNA'), lead to similar spatial conservation priorities within multi-species scenarios. However, it may be that the conservation objective is not only to conserve neutral genetic diversity (to prioritize populations with a large effective population sizes (N_e) to ensure persistence), but also to conserve adaptive genetic diversity (to prioritize populations that are most resistant to environmental change). In this setting, several metrics from a single marker may not be sufficient to meet the conservation objectives at hand (Funk et al. 2012). Therefore, it is necessary to understand how genetic metrics from multiple markers compare in their conservation spatial priorities. To do this, I compared conservation spatial solutions from mtDNA, 'neutral' single nucleotide polymorphisms (SNPs) and 'adaptive' SNPs for two species individually, as well as combined.

This sub-chapter will compare the spatial priorities from the genetic data from Chapter One with the genomic data from Chapter Two, for the limpet *S. granularis* and the urchin *P. angulosus*. I chose to explore differences between marker types within a spatial conservation framework because there is currently no baseline for interpreting and implementing data from different molecular markers in conservation planning processes. Therefore, in this chapter I not only describe the conservation relevance of three marker types (mtDNA, SNPs and outlier SNPs), but also describe a methodological framework for informing conservation planning processes with different marker types.

An additional aim of this sub-chapter is to compare the conservation solutions between different sampling regimes, inspired by the missing genomic sequences for *P. angulosus* (Fig. 12). While the absence of genomic sequences for these populations does not allow inferences of genetic patterns in as high resolution for *P. angulosus* as for *S. granularis*, making comparisons between the two species difficult, it does present an

opportunity to examine the effects of incomplete genetic data sets in conservation planning. The discordance in sample designs between species is one example of a practical issue with integrating genetic information into conservation planning, as sampling protocols worldwide are far from uniform, and are likely both spatially and taxonomically biased (Rodrigues et al. 2011, Selkoe et al. 2016). As there is no existing framework to account for sampling inequalities, this study provides the first step towards understanding how disparate genetic information can affect spatial conservation prioritization.

Lastly, this section aims to compare conservation priorities based on different amounts of genetic and genomic information. A key question within conservation planning is how much information is required to effectively reach defined objectives (Grantham et al. 2009, Mazor et al. 2016). As very few studies have empirically compared different types of genetic data in conservation planning, it is still unknown how much genetic information is needed to fully capture the evolutionary patterns that are of conservation interest. Here, I assessed the effects of including different amounts of genetic information by comparing the spatial solutions from including all genetic information for Chapter One (four metrics from one marker for five species) to those including all genomic information for Chapter Two (three metrics from three markers from two species; Fig. 15).

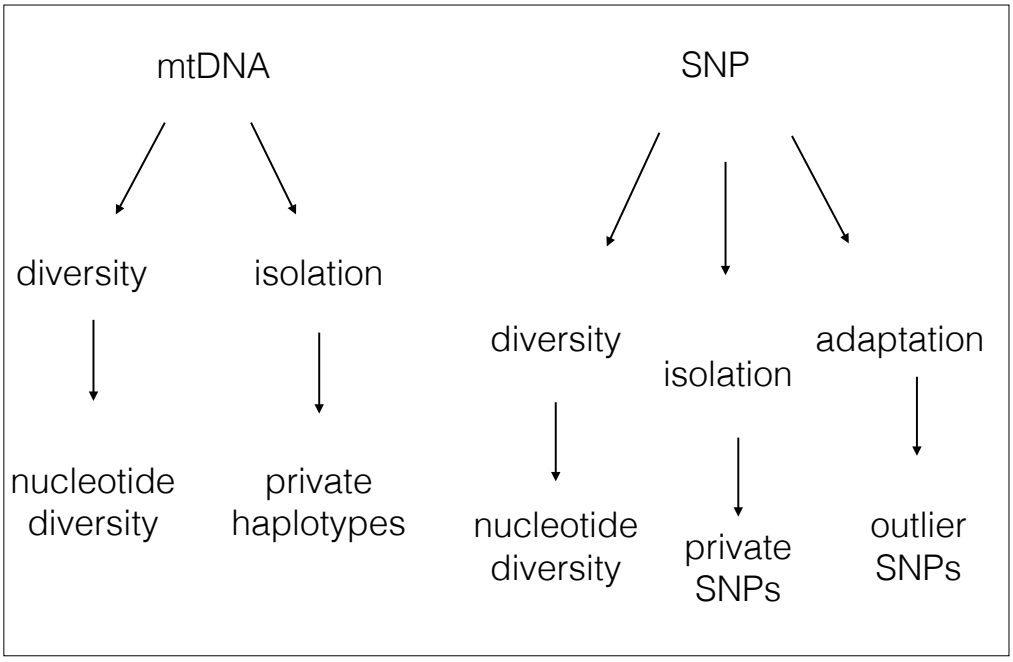


Figure 15 - The genomic features that were included into Marxan for Chapter Two, which are comprised of three genetic marker types (mtDNA, SNPs, and Outlier SNPs),

three different measures (diversity, isolation, and adaption), and five metrics (mtDNA nucleotide diversity, number of private haplotypes, SNP nucleotide diversity, percentage of private SNPs, and percentage of outlier SNPs).

Molecular markers and conservation objectives

The conservation scenarios in this chapter include three different molecular markers (mtDNA, SNPs, and outlier SNPs) for two species, *S. granularis* and *P. angulosus*. These three marker types differ in what evolutionary patterns they represent, and thus will have different implications for conservation (Table 11). Broadly, mtDNA markers will reflect historical neutral and potentially adaptive variation, 'neutral' SNPs will reflect contemporary neutral variation, and outlier SNPs will reflect adaptive variation.

Table 11 - The three markers compared in Chapter Two, what they measure, and their relevance to conservation planning.

Genetic feature	Genetic representation	Conservation Relevance
mtDNA	- Historic genetic patterns - Control region is neutral, other regions such as CO1 under selection - Genetic patterns from a single locus - Maternally inherited	- Identifies genetic management units that are historically genetically disconnected, and acts as a baseline diversity measure to compare more recent genetic patterns to (Begg et al. 1999, Crandall et al. 2000) - mtDNA CO1, the 'barcode' gene is utilized to support morphological or cryptic species identification - mtDNA can also be used to calculate phylogenetic diversity, another conservation feature (Mortiz & Faith 1998) - Detects bottlenecks and hybridization zones (Mortiz et al. 1987)
Genome-wide SNPs	- Contemporary genetic patterns - As most SNPs throughout the genome are neutral, all SNPs detected by NGS methodologies should broadly identify neutral processes - Genetic patterns captured from over 10,000's loci (on average) - Bi-parental inheritance	- Identifies recent demographic changes and contemporary genetic management units (Benestan et al. 2015, Silva-Brandão et al. 2015) - Because the sample size is much larger, SNPs should provide a more powerful estimate of N_e than mtDNA or microsatellites (Pujolar et al. 2013) - SNPs should have increased power to identify fisheries stocks & stock restoration (Gruenthal et al. 2014) - Reconstructing phylogenetic trees with greater resolution (Wagner et al. 2013)
Outlier SNPs	- Putatively adaptive genetic patterns - Usually range from 10's to 100's of loci - Can either be loci with highly distinct F_{ST} values or loci where the F_{ST} value is highly correlated with an environmental parameter	- May indicate the 'adaptive potential' of a population with candidate genes, and identify adaptive units (Funk et al. 2012) - Conserves the genetic 'resources' of fisheries stocks, which Limborg et al. (2012) define as the diversity at the DNA level that leads to phenotypic expression at ecologically important traits - Identifies locally adapted populations for conservation translocations/reintroductions (Shafer et al. 2014)

In order to compare the spatial priorities from different marker types and their relevant metrics (Fig. 15), it is important to first understand how the different metrics and markers compare with the context of conservation objectives. Chapter One discussed the reasoning behind objectives aimed at protecting either low or high ranking areas of both genetic diversity and isolation from mtDNA data sets. The same reasoning can be applied to high and low ranking areas from 'neutral' SNP data sets because, in theory, neutral evolutionary processes should affect mtDNA and SNP data sets in similar ways (Allendorf et al. 2010). However, the resulting genetic patterns may differ between the marker types owing to differences in inheritance, number of loci, and recombination and mutation rates (Morin et al. 2004, Blanco-Bercial & Bucklin 2016). Mitochondrial DNA and SNPs can be used to meet similar conservation objectives, which could include protecting populations with large effective population sizes or populations that are genetically connected, to better ensure persistence into the future (Morin et al. 2004, 2009). However, current SNP data sets are highly biased towards areas with strong environmental gradients, as well as towards model organisms with reference genomes. Therefore, it is important to understand if mtDNA data sets identify similar priority areas as SNP data sets, as mtDNA data exists for a far wider range of species and environments.

Outlier SNPs have similar conservation relevance to private haplotypes and private SNPs, as they are also potentially influenced by natural selection and/or gene flow. Because outlier loci indicate that a population is adapted to its local environment, it can be assumed that populations with greater amounts of outlier SNPs have increased adaptive potential and resilience. However, one could argue that populations with low amounts of outlier SNPs also require protection, as they are more susceptible to environmental change. Therefore, similar to genetic diversity and isolation, areas with both high and low levels of 'adaptive potential' can be of conservation relevance, depending on the overall objective.

Materials & Methods

Conservation prioritization analyses

Integration of genomic data

For both species, I chose to include two metrics for both the mtDNA and SNP markers, and a single metric for outlier SNPs (Fig. 15). As Chapter One showed that including two diversity or isolation metrics from a single marker resulted in fairly similar conservation solutions, I chose to include one metric for diversity and isolation for both mtDNA and SNP markers within the conservation prioritizations. To represent genomic diversity, I chose to use nucleotide diversity, because there are currently no bioinformatics programs that will calculate haplotype diversity for pooled restriction-site associated DNA (RAD) sequences. Further, nucleotide diversity is readily available as a 'standard' result reported in many molecular population studies. To represent genomic isolation, I chose to use the number of private haplotypes/SNPs instead of genetic differentiation (F_{ST}), as the allele frequency differences in the SNP results may potentially be biased by the pooled sequencing (i.e. a high frequency allele might be one individual sequenced many times, or many individuals sequenced a few times). I used the percentage instead of number of private SNPs to account for potential sequencing biases leading to differences in the total number of SNPs per population.

As outlier SNPs inherently characterize adaptive patterns (instead of diversity or isolation), I chose to use the percentage of outlier SNPs as a proxy for population uniqueness or 'adaptive potential' (Luikart et al. 2003). Again, the percentage of outlier SNPs were included rather than the absolute number of outliers, to account for the differences in total number of SNPs per population. Furthermore, because only four outlier loci were identified for *P. angulosus* populations, outlier SNPs were only included into *S. granularis* conservation scenarios. While the percentage of outlier SNPs within a population may not be linked to local adaptation, possibly due to false positives (Bierne et al. 2013), linkage disequilibrium (Pan et al. 2009) or polygenetic traits, outlier SNPs (even those with unknown adaptive functional roles) can be considered as important to conserve simply because they are different. Being outliers, they have unique allele frequencies, making them analogous to rare species, and in that sense they

have conservation relevance, even if they are not actively being selected on (Cadotte et al. 2010). In addition, genomic seascape analyses frequently show outlier SNPs correspond to adaptation to local marine environmental conditions (Galindo et al. 2010, De Wit & Palumbi 2013, Pespeni et al. 2013, Reitzel et al. 2013, Hohenlohe et al. 2014, Guo et al. 2015). As the genomics era is advancing at an exceptional rate, and as we are becoming more aware of the processes that are driving evolutionary, and especially adaptive patterns, it is vital to start developing theoretical and empirical evidence for how outlier SNPs may inform conservation prioritizations.

Marxan scenarios

To compare the different metrics for each marker type, I followed similar protocols to the first chapter. The planning domain and baseline scenario are identical to those used in Chapter One, and genetic metrics were interpolated across the entire region using the inverse distance weighting (IDW) tool in ArcMap. Each genetic feature was subdivided into three equal-interval classes (low, medium and high) for each species. The habitat and genetic feature conservation targets were identical to those in Chapter One, with 40% of each habitat, 50% for the 'low', 30% for the 'medium' and 50% for the 'high' genetic classes. For each scenario, Marxan was run 100 times with 1,000,000 iterations, and with a boundary length and species penalty factor of one.

Because there is genomic data for six sample sites for *S. granularis* and only for four sample sites for *P. angulosus*, I wanted to determine how excluding two sites would affect conservation priorities. This comparison (Table 12) included all genomic metrics for *S. granularis*, and all mtDNA metrics for *P. angulosus*, interpolated from either four or six sample locations.

Table 12 - The Marxan scenarios with a change in sample design.

Scenario I.D.	Species	Genetic Features	Sample Design
1	*S. granularis*	All genetic features	4 locations
2	*S. granularis*	All genetic features	6 locations
3	*P. angulosus*	All mtDNA features	4 locations
4	*P. angulosus*	All mtDNA features	6 locations

I also employed both a single and multi-species approach to compare conservation priorities from the different genetic metrics and marker types. The single species approach included all genetic features individually for either *S. granularis* (Table 13) or *P. angulosus* (Table 14), and the multi-species approach included all genetic features in a similar manner for both species (Table 15). For the multi-species approach, four locations were included for *P. angulosus* and six locations were included for *S. granularis*.

Table 13 - The Marxan scenarios with a change in genomic metric for the limpet *S. granularis*.

Species I.D.	Species	Genetic Features(s)	Sample Design
5	*S. granularis*	mtDNA - π	6 locations
6	*S. granularis*	mtDNA - private alleles	6 locations
7	*S. granularis*	SNPs - π	6 locations
8	*S. granularis*	SNPs - private alleles	6 locations
9	*S. granularis*	SNPs - outlier richness	6 locations

Table 14 - The Marxan scenarios with a change in genomic metric for the urchin *P. angulosus*.

Scenario I.D.	Species	Genetic Feature(s)	Sample Design
10	*P. angulosus*	mtDNA - π	4 locations
11	*P. angulosus*	mtDNA - private alleles	4 locations
12	*P. angulosus*	SNPs - π	4 locations
13	*P. angulosus*	SNPs - private alleles	4 locations

Table 15 - The Marxan scenarios with a change in genomic metric for the combined species approach.

Scenario I.D.	Species	Genetic Feature(s)	Sample Design
14	Both	mtDNA - π	4 & 6 locations
15	Both	mtDNA - private alleles	4 & 6 locations
16	Both	SNPs - π	4 & 6 locations
17	Both	SNPs - private alleles	4 & 6 locations

Lastly, to observe the effects of including fewer molecular markers for more species versus more markers for fewer species, I compared the ALL scenarios (those including all genetic information for all species), as well as the 'genetic focal areas', which are the planning units selected across all scenarios, for Chapters One and Two. I created selection frequency maps using QMarxan to view similarities between scenarios, which display how often each planning unit was selected to be within the MPA network out of the 100 solutions. I then quantified the similarities between scenarios by calculating Pearson correlation coefficients between the selection frequency values per planning unit for each scenario pairing.

Results

The effects of incomplete genetic data sets on conservation priorities

Scutellastra granularis

The selection frequency maps illustrate that a change in sample design (Fig. 16 A and B) does not greatly alter spatial priorities for *S. granularis* populations (Fig. 16 C and D). Broadly, the scenario including genetic information from fewer sample sites selects more planning units at lower frequencies, especially within the southern coastline (Fig. 16 C). However, the planning units with the highest selection frequency are largely similar between the scenarios with differential sampling designs (Fig. 16 C and D).

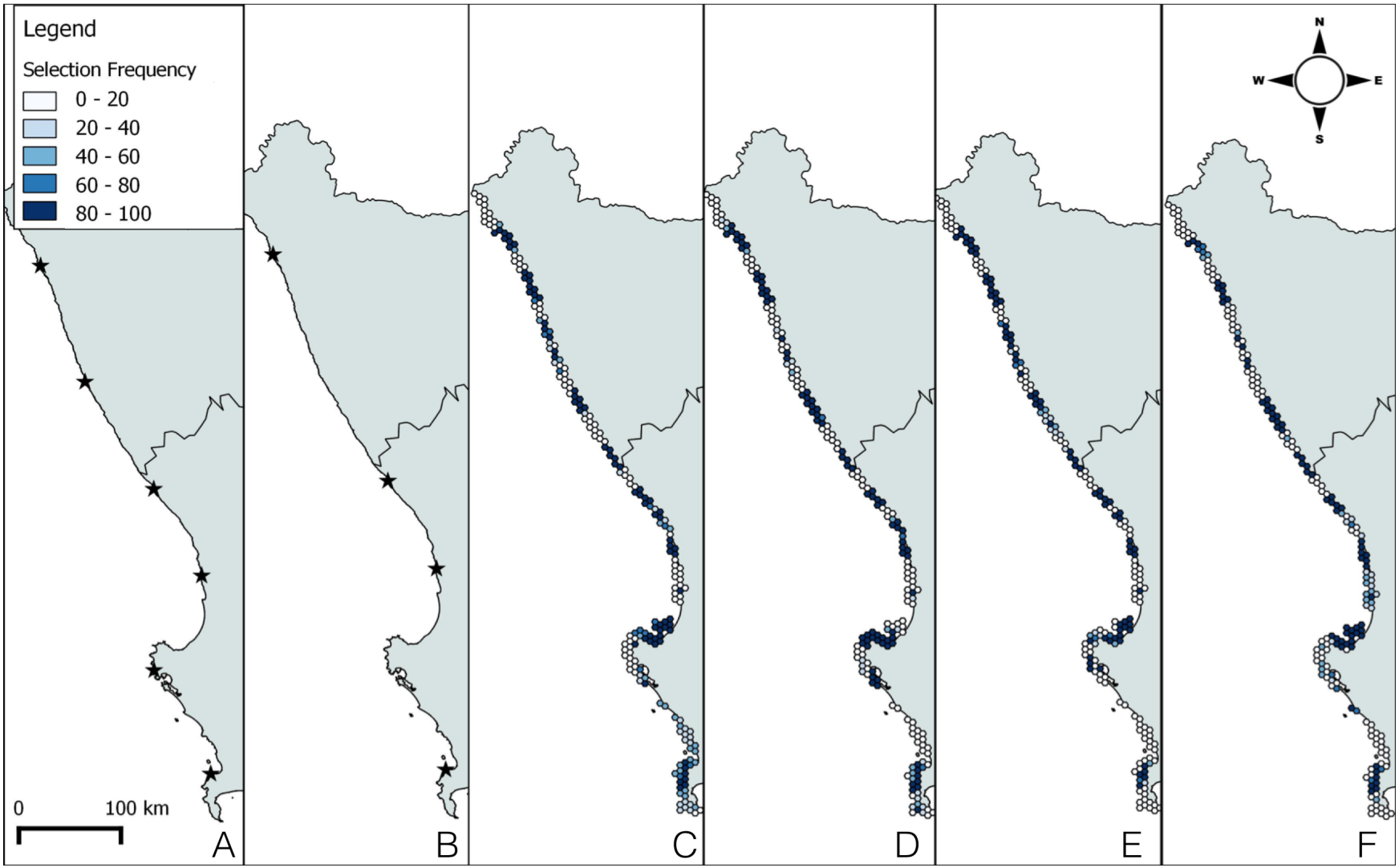
When the differences in sample regimes are compared by individual metrics for *S. granularis*, it is apparent that the SNP metrics result in highly similar conservation

solutions from either four or six sample sites (Table 16). In contrast, the inclusion of mtDNA metrics from either four or six locations leads to dissimilar conservation solutions (Table 16).

Table 16 - Pearson correlation coefficients between scenarios with genetic information from either four or six sample sites (MN= mtDNA nucleotide diversity, MP= mtDNA private haplotypes, SN= SNP nucleotide diversity, SP= private SNPs, SO= outlier SNPS).

Scenario	*S. granularis*	*P. angulosus*
MN	0.64	0.65
MP	0.57	0.92
SN	0.89	N/A
SP	0.83	N/A
SO	0.87	N/A

Figure 16 - The sample sites for which genomic sequences were obtained for the two study species, A) *S. granularis* (Granular limpet) , B) *P. angulosus* (Cape urchin), as well as the selection frequencies corresponding to the following scenarios: C) All *S. granularis* genomic information from four sample sites, D) All *S. granularis* genomic information from six sample sites, E) All *P. angulosus* genetic information from four sample sites, F) All *S. granularis* genetic information from six sample sites.



Parechinus angulosus

Due to the incomplete SNP data set for *P. angulosus*, the effects of differential sampling on conservation priorities could only be assessed for mtDNA metrics. The selection frequency maps show slight discrepancies in the spatial solutions for the scenarios including either four or six sample sites for *P. angulosus* (Fig. 16 E and F). Broadly, the scenario based on the incomplete data set gives higher priority to the northern coastline, compared to the scenario with the full genetic data set, in which the priorities are shifted southwards (Fig. 16 E and F). The scenarios with a change in sampling design for each individual metric show large discrepancies in the nucleotide diversity solutions and only minor differences in private haplotype solutions (Table 16).

A spatial comparison of different marker types in single species conservation plans

Scutellastra granularis

The conservation priorities for the *S. granularis* scenarios vary depending on both the metric and marker type. The two most spatially similar scenarios are those derived from private and outlier SNPs (Fig. 17, Table 17). There are noticeable differences between the private/outlier SNP spatial priorities and those of the other metrics, with the majority of the planning units selected by private and outlier SNPs being within a large cluster in the middle of the coastline, compared to the small, spread out clusters selected by the other scenarios (Fig. 17). On average, a greater number of planning units were selected in the private and outlier SNPs scenarios compared to the other genetic metrics (Fig. 17, Table 17).

Table 17 - Pearson correlation coefficients between the scenarios with a change in genomic metric for *S. granularis* (Granular limpet).

	MN	MP	SN	SP	SO
MP	0.75				
SN	0.75	0.68			
SP	0.45	0.63	0.70		
SO	0.46	0.63	0.71	0.98	
ALL	0.74	0.58	0.56	0.39	0.38

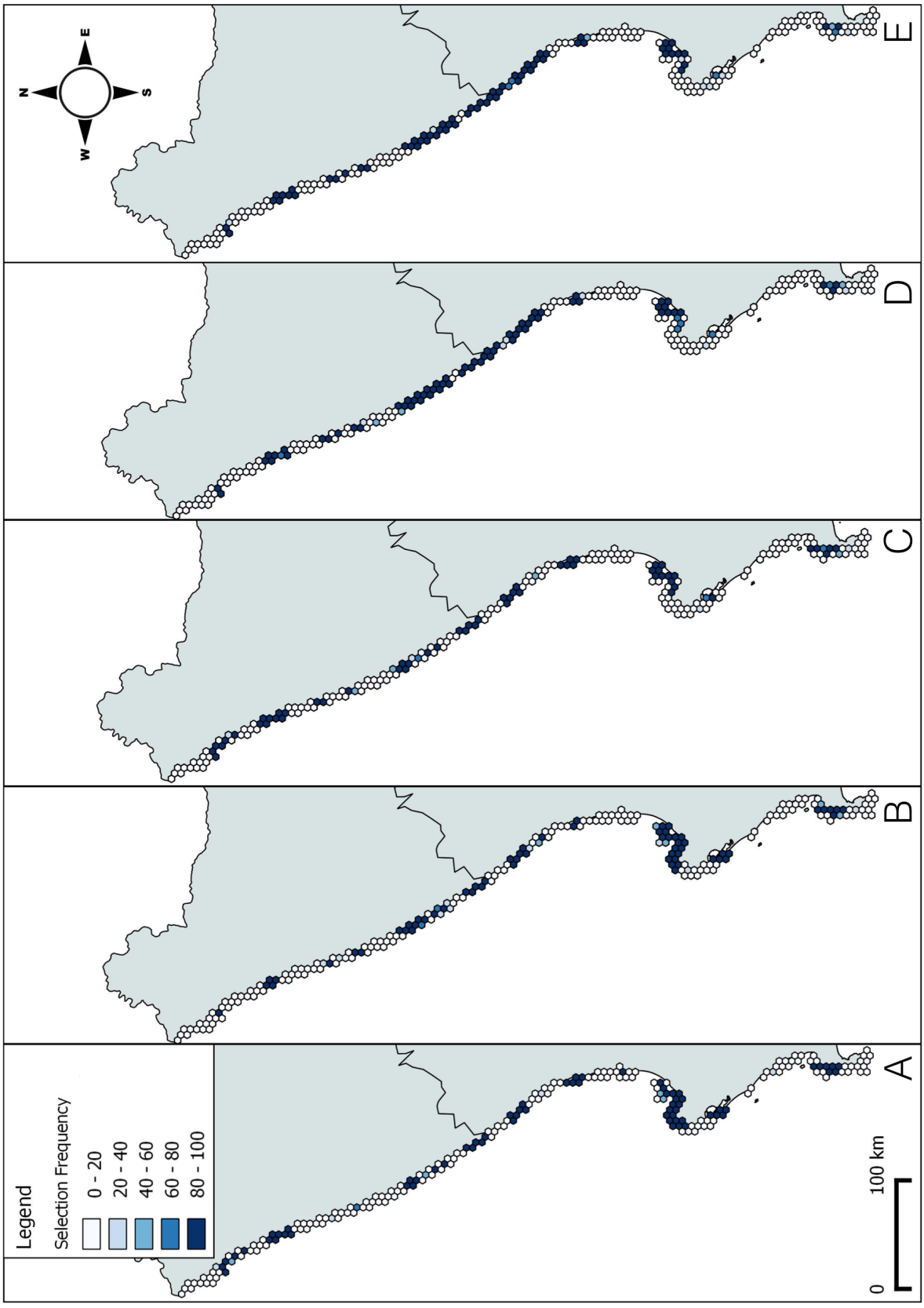


Figure 17 - Selection frequencies of the scenarios with a change in genetic metric for *S. granularis* (Granular limpet), including A) mtDNA nucleotide diversity, B) mtDNA private haplotypes, C) SNP nucleotide diversity, D) Private SNPs, E) Outlier SNPS.

Parechinus angulosus

When comparing the different genetic metrics for *P. angulosus* populations, the two most similar metrics are mtDNA nucleotide diversity and private SNPs (Fig. 18, Table 18). These two metrics mainly highlight the central coastline as a conservation priority area, similar to the conservation solutions of the private and outlier SNP scenarios for *S. granularis* (Fig 17, Table 17). The second most similar metrics are private mtDNA haplotypes and SNP nucleotide diversity (Table 18), which both select smaller and more spread out high priority clusters (Fig. 18).

Table 18 - Pearson correlation coefficients between the scenarios with a change in genomic metric for *P. angulosus*.

	MN	MP	SN	SP
MP	0.78			
SN	0.63	0.80		
SP	0.87	0.75	0.62	
ALL	0.66	0.74	0.81	0.68

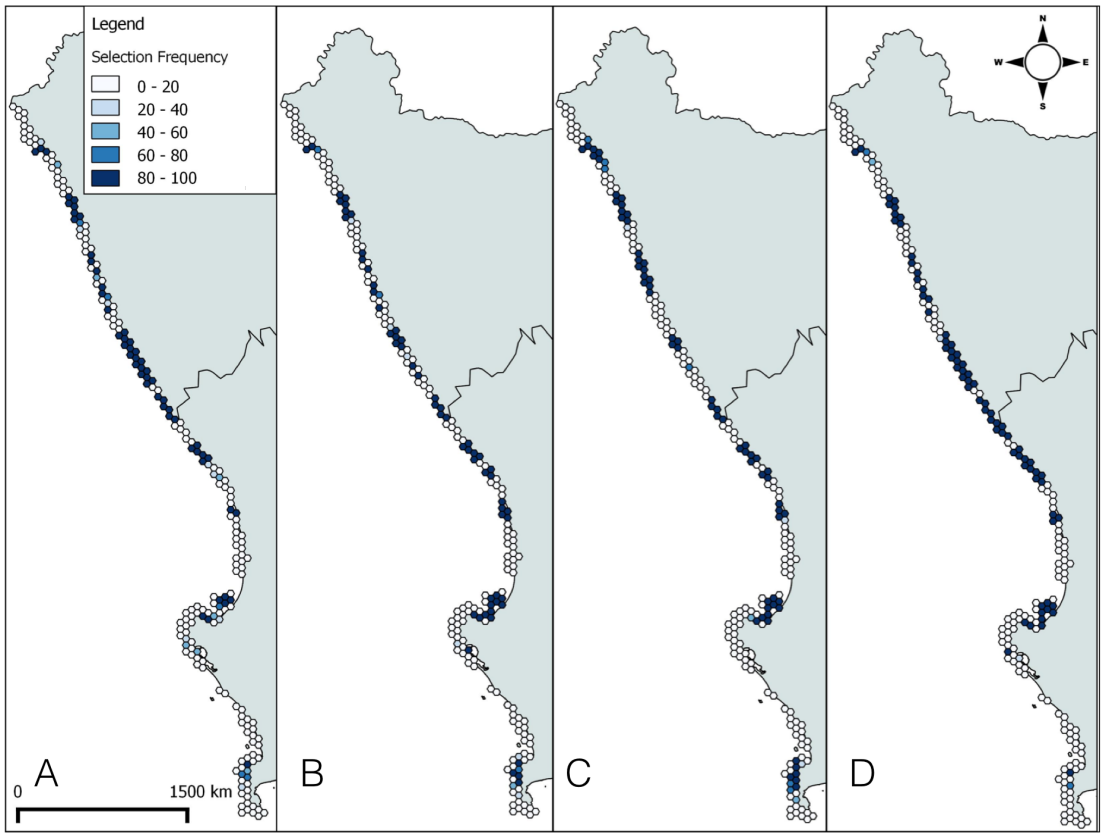


Figure 18 - Selection frequencies of the scenarios with a change in genetic metric for *P. angulosus* (Cape urchin), including A) mtDNA nucleotide diversity, B) mtDNA private haplotypes, C) SNP nucleotide diversity, D) Private SNPs.

Further investigation of spatial similarities between metrics

The two most similar scenarios for both species are from different makers, which is unanticipated as the genetic patterns should be distinct between marker types owing to differences in mutation rate, number of loci, and inheritance (Blanco-Bercial & Bucklin 2016). In order to better understand why these different markers lead to similar conservation priorities, I compared the raw spatial patterns of the genetic metrics included in the scenarios. When the actual genetic patterns are scrutinized, it is apparent that the two most similar metrics for each species actually have very different spatial patterns (Fig. 19 & 20).

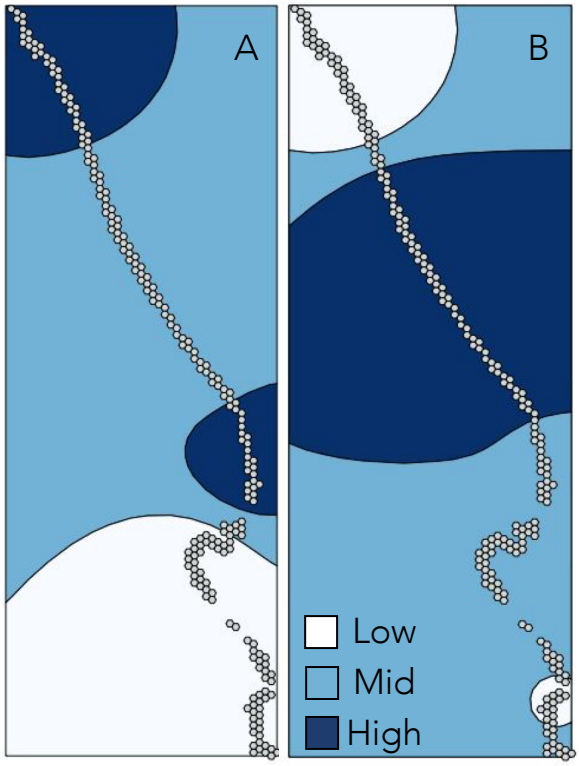


Figure 19 - The spatial genetic patterns, after interpolation and reclassification of genetic values, for the two most similar metrics within the *S. granularis* (Granular limpet) scenarios, A) mtDNA nucleotide diversity and B) SNP nucleotide diversity.

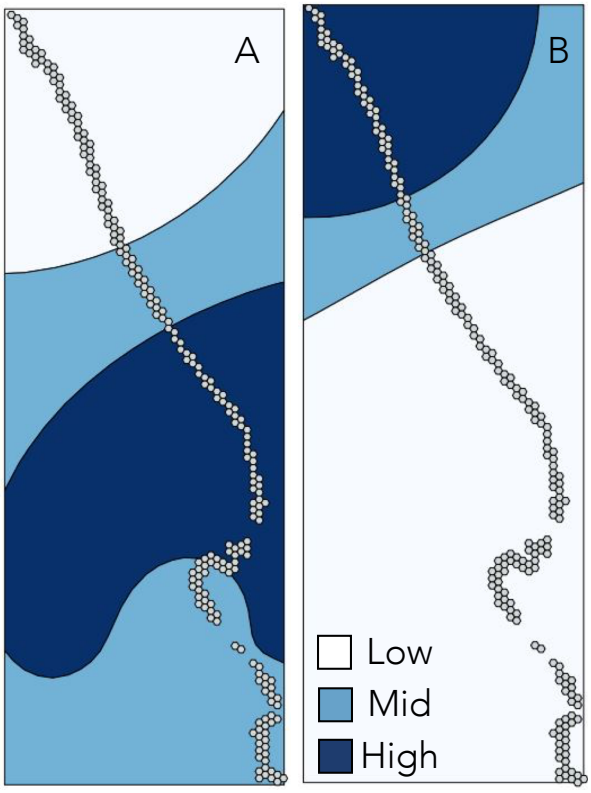


Figure 20 - The spatial genetic patterns, after interpolation and reclassification of genetic values, for the two most similar metrics within the *P. angulosus* (Cape urchin) scenarios, A) mtDNA nucleotide diversity and B) private SNPs.

A spatial comparison of different marker types in multi-species conservation plans

The scenarios including genomic metrics for both *S. granularis* and *P. angulosus* show noticeable differences in spatial priorities between the different metrics (Fig. 19). When genomic metrics are included for both species, the two most similar metrics are private haplotypes and SNP nucleotide diversity (Table 19), with both scenarios selecting high priority areas in small clusters along the entire coastline (Fig. 21). The most spatially distinct scenario is that including private SNPs, which highlights a large portion along the central coastline as a conservation priority area (Fig. 21).

Table 19 - Pearson correlation coefficients between the scenarios with a change in genomic metric for both species.

	MN	MP	SN	SP
MP	0.72			
SN	0.65	0.74		
SP	0.47	0.62	0.69	
ALL	0.83	0.73	0.75	0.61

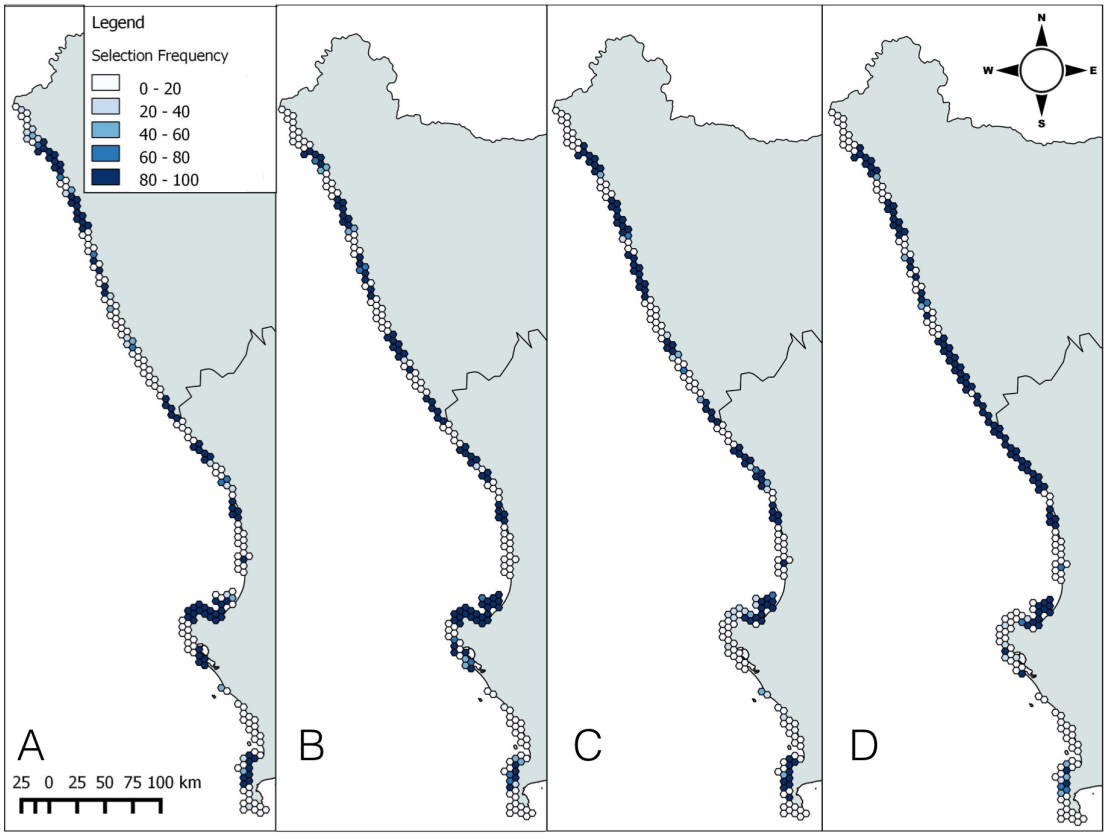


Figure 21 - Selection frequencies of the scenarios with a change in genomic metric within the combined species approach, including A) mtDNA nucleotide diversity, B) mtDNA private haplotypes, C) SNP nucleotide diversity, D) private SNPs.

Quantified marker trade-offs between conservation scenarios

Overall, there is little consistency of the spatial similarities for the scenarios between the different species approaches (Tables 17-19). For instance, there are discrepancies between the two most distinct scenarios, with the most dissimilar scenarios being SNP nuclear diversity and private SNPs for *P. angulosus*, and mtDNA nuclear diversity and private SNPs for *S. granularis* and both species combined. On average, the correlation between spatial priorities of the different metrics is higher for *P. angulosus* (0.73) than for *S. granularis* (0.63). Furthermore, the genetic metric with the highest cost is also not consistent across the different species approaches, with *S. granularis* having a higher average cost (341) than *P. angulosus* (329). The scenario selecting the least planning units is SNP nucleotide diversity for *S. granularis*, and mtDNA nucleotide diversity for *P. angulosus*; yet both of these scenarios choose the same number of planning units when the two species are combined (Table 20). In

contrast, private SNPs select the largest area for each species individually as well as combined (Table 20).

Lastly, the spatial dissimilarities are greater between rather than within the different marker types for *S. granularis*, yet for *P. angulosus,* they are greater between the two SNP metrics than between the SNP and mtDNA markers (Table 21). When both species are combined, the most similar conservation priorities are between mtDNA metrics and the most dissimilar priorities are between the different marker types (Table 21).

Table 20 - The average cost and number of selected planning units of scenarios with a change in genomic metric, for both the individual and combined species approaches.

Scenario	Average Marxan cost	Average # of planning units chosen
	S. granularis - 6 sites	
MN	382	70
MP	348	68
SN	307	62
SP	335	72
SO	335	72
	P. angulosus - 4 sites	
MN	307	64
MP	316	65
SN	353	68
SP	343	73
	Both species - 4 & 6 sites	
MN	537	88
MP	498	90
SN	491	88
SP	497	96

Table 21 - Pearson correlation coefficients within and between marker types, for each individual species as well as combined.

	MN vs. MP	SN vs. SP	mtDNA vs. SNP
S. granularis	0.74	0.77	0.71
P. angulosus	0.70	0.62	0.69
Both species	0.71	0.69	0.62

Comparing different amounts of genetic information in conservation scenarios

Comparing the ALL scenarios from Chapters One and Two, the selection frequency maps are quite similar between the two scenarios (Fig. 22). The scenarios including all genetic information from Chapters One and Two select multiple high priority clusters along the coastline, with almost the entire coastline being selected as some level of conservation priority in both scenarios. The planning units selected by each genetic metric for both species included in Chapter Two display several conservation genetic 'focal areas' spanning the entire coastline (Fig. 23). When compared to the genetic focal areas of the mtDNA metrics from Chapter One, the consistently chosen planning units of Chapter Two select an overall larger area, with noticeably more units chosen along the mid and southern west coast (Fig. 23).

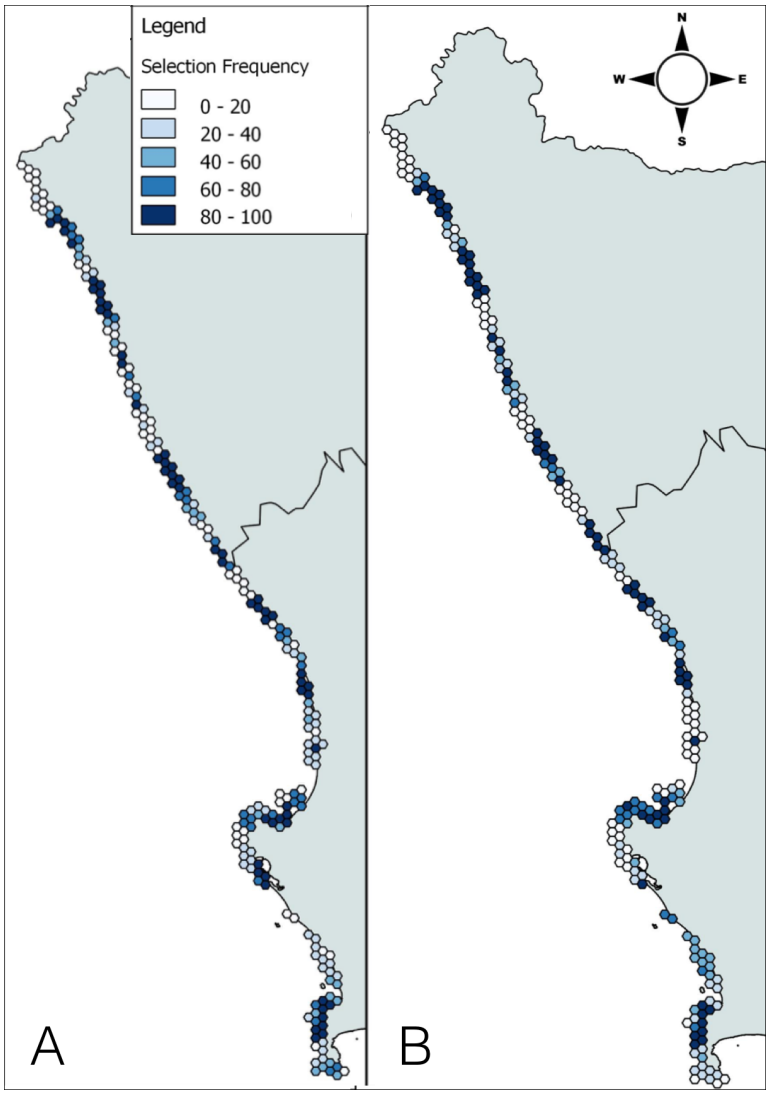


Figure 22- The selection frequencies of the scenarios including all genetic or genomic information (ALL) for A) Chapter One, B) Chapter Two.

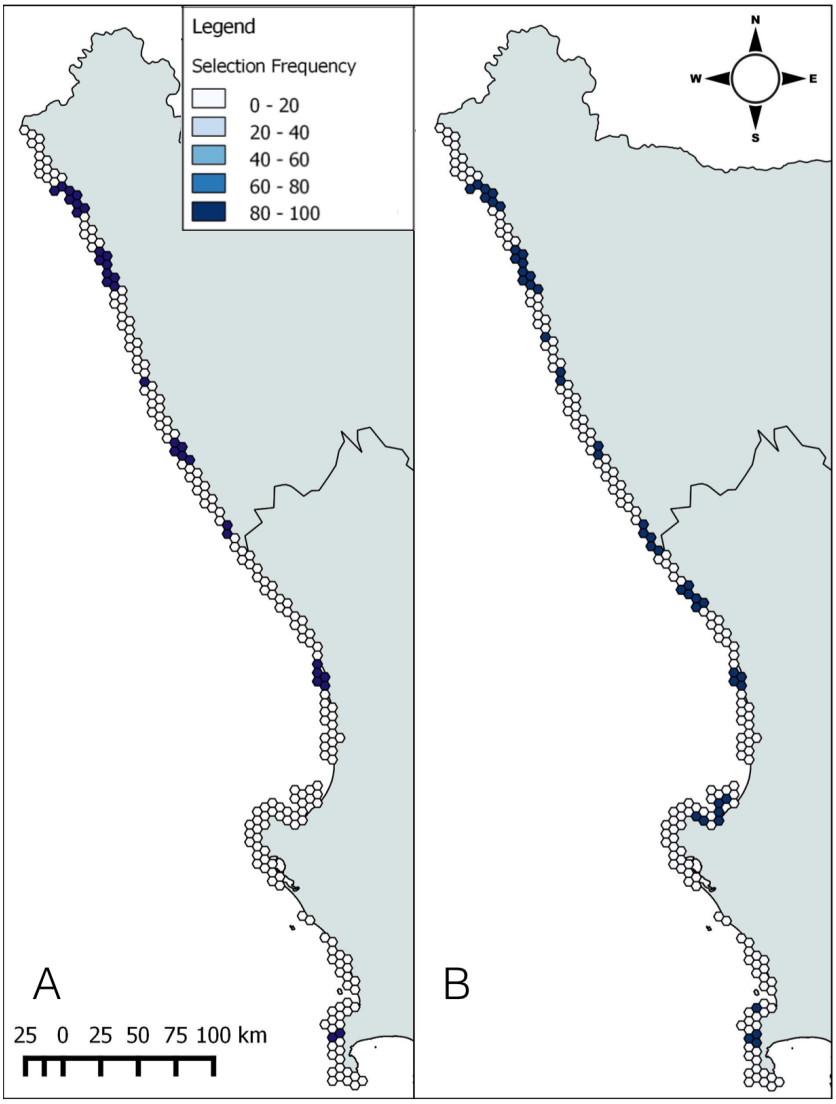


Figure 23 - The genetic 'focal areas' which were chosen as high priority sites within all scenarios with a change in genetic metric for either A) Chapter One, or B) Chapter Two.

Discussion

In this sub-chapter I found noticeable differences in conservation priorities for conservation objectives aiming to conserve either neutral genetic diversity (to prioritize populations with a large effective population sizes to ensure persistence) or adaptive genetic diversity (to prioritize populations that are most resistant to environmental change). These findings suggest that next generation SNP data will be most beneficial to conservation planning in its ability to identify populations with heightened 'adaptive potential'. Furthermore, the metrics presumably associated with adaptive processes (private and outlier SNPs) consistently selected the largest reserve areas, mirroring the

findings of Bonin et al. (2006), who also found that larger reserve networks were required to conserve adaptive compared to neutral amplified fragment length polymorphism (AFLP) genetic patterns. Furthermore, there are moderate dissimilarities in spatial priorities between the two species, with mtDNA metrics showing larger trade-offs between species than the SNP metrics. Moderate discrepancies are also found in the spatial priorities between scenarios including either four or six sample sites, with the differences varying between metrics. Interestingly, trade-offs are not consistently greater between than within the marker types, meaning that a change in marker type does not necessarily lead to greater trade-offs than a change in metric. Broadly, the results suggest that different markers lead to distinct conservation solutions when included individually, yet the spatial differences between markers decreases as the number of included metrics and species per marker type increases.

The effects of incomplete genetic data sets on conservation spatial solutions

Effective conservation of biodiversity patterns and processes requires fine-scale and detailed spatial data sets for each biodiversity feature (Margules & Pressey 2000). However, species occurrence data sets are often incomplete, and spatially and/or taxonomically biased (Rodrigues et al. 2011). This also holds true for genetic datasets, as few studies collect and generate data for multiple species from the same area. Therefore, to test the effects of incomplete genetic data sets on conservation solutions, this chapter included genetic information from either four or six sample sites for two species. The results show that differences in sampling regimes lead to slight or moderate trade-offs in conservation priorities, depending on the genetic metric (Fig. 16, Table 16).

For *S. granularis*, different sampling designs have little effect on the conservation priorities for the SNP metrics, but do cause sufficient changes in the priorities derived from the mtDNA metrics. These results are unexpected as Hondeklipbaai is the most genomically unique site for the *S. granularis* SNP metrics, yet the conservation priorities do not change when it is excluded. The minute differences in the conservation scenarios either including or excluding the most genomically unique site could be a result of the genetic ranking and interpolation techniques. The drawback of classifying genetic values into equal interval ranks is that if a site is highly distinct (either

significantly lower or higher than the other sites), this significant difference will be lost, which in turn affects the ranking of the other values. One possibility is to exclude the 'outlier sites' that have significantly different genetic values from genetic ranking methodologies, and to give outlier sites their own specific conservation target. However, comparing the effects of different genetic implementation methods on conservation priorities is outside the scope of this study.

With the mtDNA metrics for *P. angulosus*, differential sampling results in negligible differences between the private haplotypes scenarios, but substantial differences in the nucleotide diversity scenarios, which highlights how separate metrics respond differently to missing data. When the species are combined, the scenarios including all metrics from either four or six sample sites have moderate differences in spatial priorities (Fig. 16 C-F). This suggests that including incomplete genetic data sets may not lead to highly distinct conservation spatial results if included for multiple genetic metrics, because the influence of missing data is balanced between the different metrics. Furthermore, there is likely a trade-off in the number of samples sites and the number of biodiversity features, so that including more genetic metrics for fewer sample sites leads to similar conservation results as fewer metrics from more sample sites (Börger et al. 2006). In addition, including genetic information for more sites will likely lead to more concise spatial priorities, as described by Grand et al. (2007), who found that spatial prioritization based on reduced species occurrence data sets resulted in larger reserve areas compared to full data sets.

There are several possible avenues to account for missing genetic data or biased genetic sampling within a conservation planning framework. For instance, Beger et al. (2014) suggested sampling at high densities and over environmental gradients to account for any biases in conservation priorities derived from genetic point data. In order to account for sites with missing phylogenetic diversity (PD) data, Moritz & Faith (1998) suggested either assigning an average PD value to missing taxa, or estimating the combined PD by jack-knifing across species. Another method to circumvent missing genetic data is with the use of environmental surrogates, which were tested for neutral and genetic diversity of Mediterranean herpetofauna (Carvalho et al. 2010), and two bird species (Ponce-Reyes et al. 2014), yet the effectiveness of environmental surrogates of genetic diversity is still in question (Carvalho et al. 2010, Ponce-Reyes et al. 2014). In the marine realm, Abesamis et al. (2016) found that ocean currents and fish assemblages were effective surrogates for larval connectivity in coral reef fish. However, the

accuracy of genetic diversity and differentiation surrogates within the marine realm are still unexplored.

Exploring conservation trade-offs between marker types for single species

When analyzed independently for each species, the genetic metrics display an array of conservation spatial priorities. For example, conservation scenarios based on genomic metrics from *S. granularis* show noticeable variations in spatial priorities with the inclusion of different markers as well as metrics. Largely, the mtDNA metrics result in similar conservation solutions, with multiple small priority clusters spanning the coastline (Fig. 17 A and B). Similar conservation solutions from the mtDNA metrics are expected, as Chapter One showed that the inclusion of various mtDNA metrics resulted in minor differences in spatial priorities. Within the SNP scenarios, private and outlier SNPs display highly similar conservation priorities, both selecting a large amount of planning units along the central coast (Fig. 17 D and E), owing to the strong correlation between the metrics (R=0.92). This suggests that private SNPs may be an effective substitute for outlier SNPs and vice versa, however further evidence is needed to solidify the relationship between these two genomic metrics.

There are however large discrepancies in spatial priorities between the private/outlier SNPs and SNP nucleotide diversity *S. granularis* scenarios, with SNP nucleotide diversity selecting priority areas more similar to the mtDNA metrics than to other SNP metrics (Fig. 17 C). This is likely owing to distinct evolutionary processes shaping SNP nucleotide diversity (mutation rate and N_e), compared to those acting on private and outlier SNPs (gene flow and natural selection). The SNP nucleotide diversity scenario is most similar to the mtDNA nucleotide diversity scenario (R=0.75), which is unanticipated as the two marker types should capture different evolutionary patterns (Blanco-Bercial & Bucklin 2016). Interestingly, the two most similar scenarios for *P. angulosus* are those based on private haplotypes and SNP nucleotide diversity (R=0.87) which is also unexpected as they are not only from two different marker types, but also describe either diversity or isolation processes.

One explanation for the similar conservation priorities between mtDNA and SNPs scenarios can also be due to the genetic patterns having similar ‘transition zones’, where

it shifts from one ranking to another (Fig. 19 & 20). The transition areas are likely important factors in the resulting conservation priorities, as conserving the transition areas results in a reserve network that incorporates different genetic features within a small area (Ball & Possingham 2009). However, the potential effects of transition zones on conservation solutions may vary with conservation planning techniques, such as between the 'minimum set' approach of the Marxan algorithm (which selects planning units that reach targets at lowest cost; Ball et al. 2009), and the maximum cover approach of Zonation (which selects the planning units that reach the maximum amount of targets at a fixed cost; Moilanen et al. 2009). It has been shown that the different prioritization approaches of the two programs lead to different conservation results (Delavenne et al. 2012), therefore it can be expected that the spatial patterns of the biodiversity features (including the transition zones of genetic classes) will have different effects on the conservation solutions derived from the separate algorithms.

The similar conservation solutions between metrics with dissimilar genetic patterns raises the concern that conservation prioritizations might be biased by implementation techniques within the conservation planning process. The transition zones are created when interpolating the genetic data from the selected sampling sites, therefore the sample sites, genetic classifications and interpolation methods likely all have an effect on the placement of the transition zones, and thus also the conservation solutions (Levy et al. 2013, Lechner et al. 2014). For example, Wilson et al. (2005) compared conservation solutions from various types of predicted species distribution data and found that the various prediction models differed in their conservation priorities and expected species representation. This suggests that the method in which genetic point data is interpolated or modeled will have an effect on spatial prioritizations. Therefore, it is important to quantify the trade-offs associated with different interpolation and ranking techniques, which is outside the scope of this study.

Importantly, this is the first time these 'transition zones' between different biodiversity features have been reported as potential drivers of conservation solutions. The relationship between transition zones and conservation priorities is easily derived from the results in this study because there are only three genetic classes, and the planning domain is relatively small and linear. It is more difficult to compare the transition zones and resulting priorities within planning domains that expand over large and non-linear regions, as well as with biodiversity features that have many classes and complex spatial patterns. For example, Beger et al. (2014) interpolated five genetic

rankings over a large area around a complex arrangement of islands within the Indo-Pacific, and thus the genetic patterns and conservation solutions are more difficult to interpret. Another example is that from Carvalho et al. (2010), who compared conservation scenarios including different biodiversity features from Portugal, Spain and Morocco. Their study included highly complex patterns for five species richness classes, resulting in scenarios in which the relationship between the transition zones and conservation priorities are also challenging to interpret. Therefore, it is difficult to determine if the relationship between transition zones and conservation priorities is a re-occurring pattern within spatial prioritizations, or whether it is unique to this study.

Explaining conservation spatial priorities between species

Comparing the spatial prioritizations from each genetic metric between the two species reveals some highly similar scenarios, such as the ones obtained with private SNPs, as well as highly dissimilar scenarios, such the ones obtained with mtDNA nucleotide diversity (Fig. 17, 18). The spatial similarities in the SNP metrics and dissimilarities in the mtDNA metrics between the two species could be a result of different historical evolutionary patterns, but similar contemporary patterns. This situation could be explained by *S. granularis* having a more recent expansion into the west coast of South Africa compared to *P. angulosus* (Excoffier 2003), possibly owing to differences in historical glacial refugia and ecosystem stability patterns within the intertidal habitat (Petit et al. 2003, Carnaval et al. 2009). As very little is known both about the historical and contemporary processes shaping the evolutionary trajectories of intertidal species on the west coast of South Africa, it is not possible at this stage to provide firm evidence for the patterns observed.

In addition to biological differences, laboratory and analytical processes could also have resulted in species differences, respectively within each step of the process from extraction, library preparation, sequencing, *de novo* assembly, mapping or SNP calling analyses (Davey et al. 2013, Fountain et al. 2016, Rodríguez-Ezpeleta et al. 2016, Shafer et al. 2016). For instance, a study by Fountain et al. (2016) found that decreased coverage in RAD-seq bioinformatics analyses led to an increase in number of total SNPs detected, as well as an increase in the proportion of incorrect parentage assignments. As *P. angulosus* samples on average had lower coverage, as well as lower

sequencing quality and yield than *S. granularis,* the sequencing and bioinformatics analyses could have an impact on the resulting genetic patterns of the two species.

Exploring conservation trade-offs between marker types for multi-species data sets

On average, the spatial priorities of each genetic metric do not differ between the individual and combined species approaches (Fig. 17, 18 and 21). Private haplotypes, private SNPs and SNP nucleotide diversity all display similar conservation priorities in the combined species scenarios as they do in each individual species scenarios, albeit with slight discrepancies in the number and position of selected planning units. However, the mtDNA scenario for the combined species approach is noticeably more similar to the mtDNA scenario of *S. granularis*, selecting small spread out priority clusters instead of the larger and more northern clusters of *P. angulosus*.

The two most similar metrics within the combined species comparisons are mtDNA nucleotide diversity and SNP nucleotide diversity, which is not consistent with either of the individual species comparisons. This again illustrates that multi-species conservation solutions are distinct from single-species solutions (Lombard 1995, Roberge & Angelstam 2004) and that no one species is likely to capture genetic or genomic patterns of entire communities (Melià et al 2016). Multi-species approaches differ from single species conservation scenarios in their objectives, trade-offs (Moilanen et al. 2005, Nicholson & Possingham 2006), and reliability, as umbrella species and/or surrogates show inconsistent efficiency in adequately representing species distributions and assemblages (Roberge & Angelstam 2004, Rodgrigues & Brooks 2007, Wiens et al. 2008), as well as genetic patterns (Garnier-Géré et al. 2001, Carvalho et al. 2010, Ponce-Reyes 2014).

Comparing spatial priorities, reserve size and conservation costs between marker types

The Pearson correlation coefficients show little consistency in the spatial similarities between metrics for the individual and combined species approaches (Tables

16-18). These inconsistencies lead to the inability to draw explicit conclusions regarding the use of one genomic metric as a surrogate for another. Furthermore, there is also little consistency in the differences between metric and marker types, as *P. angulosus* has greater dissimilarities between the two SNP metrics than between marker types, but in *S. granularis* and the combined species approach, the conservation priorities are greater with a change in marker than change in metric (Table 21). Thus it cannot be concluded that a change in marker will lead to larger conservation trade-offs than a change in metric. Furthermore, there is no uniformity regarding which genomic metric has the highest cost between the different species approaches (Table 20). The metric resulting in the costliest reserve network is SNP nucleotide diversity for *P. angulosus*, and mtDNA nucleotide diversity for *S. granularis* and both species combined. It is also interesting how in Chapter One the number of planning units chosen was correlated with the reserve cost, which is not found in the results in Chapter Two. Therefore, it cannot be assumed that the species or genetic metric selecting the most priority areas will be the costliest to conserve.

There is consistency in the metric with the highest number of planning units chosen, with private SNPs on average selecting a larger area than the other metrics (with outlier SNPs also selecting the most planning units for *S. granularis*). Similarly, Bonin et al. (2006) found that larger reserve networks were required to conserve adaptive diversity than neutral diversity with AFLP markers for the Austrian dragonhead (*Dracocephalum austriacum*) and the Common frog (*Rana temporaria*). While the results from this chapter show that a larger number of planning units were selected from the 'adaptive' outlier SNPs scenario compared to the 'neutral' SNP nucleotide diversity scenario, this could only be assessed for *S. granularis*. However, private SNPs consistently selected the largest number of planning units compared to the other metrics, and the percentage of private SNPs is more likely to be affected by adaptive processes than the nucleotide diversity of SNPs (Hale et al. 2013, Hartmann et al. 2014), and in that regard, the results from this chapter are comparable to those in Bonin et al. (2006). Conversely, Carvalho et al. (2010), found that surrogates of adaptive genetic diversity (environmental gradients) required less area to conserve than surrogates of neutral diversity (biotic elements), however this is probably because the environmental gradients were defined within the biotic elements, making it so that adaptive variation was on a smaller scale than neutral variation. Further work is required to create effective genetic surrogates in conservation planning, and to understand how neutral and adaptive

genetic patterns compare within conservation planning results.

Genetic data in conservation planning: how much information is needed?

This study compared the different conservation spatial results from altering the included species and genetic metrics, but how do different amounts of genetic information from a different number of species affect conservation scenarios? In order to provide some insights, I compared the ALL scenarios, as well as the 'genetic focal areas' (i.e. planning units chosen across all scenarios) from Chapters One and Two. Interestingly, four genetic metrics from a single marker from five species (Chapter One) results in highly similar conservation priorities as two metrics from two markers from two species (Chapter Two; Fig. 22). Furthermore, the genetic focal areas from Chapter One are only slightly different from those in Chapter Two, with a few additional areas along the central and southern west coast (Fig. 23). Within this study, the similar conservation priorities between mtDNA data for five species and SNP data for two species could be a result of the genomic patterns being strongly influenced by the outlier SNPs detected within the mitochondrial genome that would prioritize the same regions. The similar conservation solutions could also suggest that there is a cut-off point at which including additional genetic information leads to similar conservation solutions, or that selecting the species with the two most distinct mtDNA patterns leads to these species acting as genetic surrogates, possibly capturing the genetic patterns of multiple rocky shore organisms.

Due to the socio-economic nature of conservation planning, it is important to determine how much information is required to meet conservation targets, as well as the monetary trade-offs associated with including broader versus finer scale biodiversity features (Andelman & Fagan 2000, Wilson et al. 2007). For instance, Grantham et al. (2008) found that increasing the conservation investment (specifically surveying costs) from US $100,000 to US $2.5 million led to almost no difference in the amount of *Protea* species effectively protected within the Cape Floristic Region within South Africa. The authors attribute these diminishing conservation returns to the strong correlation in species richness values between partial and complete databases, therefore protea-rich areas are likely to be captured with small initial investments. In addition,

Rodrigues et al. (2011) found that incomplete PD data sets led to similar conservation outcomes as complete PD data sets, again suggesting that there is a cut-off point to where additional information does not change conservation priorities. Along with these previous studies, the results from this project suggest that there is a point at which including additional biodiversity information is no longer cost effective. However, this is the first study to compare conservation solutions from different amounts of genetic and genomic material from multiple species, and thus further evidence is required to better understand how genetic and genomic data types compare in their conservation efficiency.

Concluding Remarks

In contrast to Chapter One, where there were only negligible differences between metrics, the results from this chapter display large discrepancies in spatial priorities between metrics from different markers. Interestingly, there were also scenarios more similar to each other than those in Chapter One, such as the private and outlier SNP scenarios for *S. granularis*. This sub-chapter showed that similarities in conservation solutions do not only arise because the metrics have highly correlated genetic patterns, such as in the private and outlier SNP scenarios, but also because the metrics have similar transition zones. This novel finding has real implications for the inclusion of genetic data into conservation planning, as the sampling and interpolation of genetic data will likely bias outcomes. Currently, the best way to avoid these potential biases is by fine scale sampling regimes over a broad environmental range. Including more species, as well as more metrics, may also help avoid sampling and interpolation biases, as the spatial differences between the different biodiversity features seem to balance out when combined. This balance was seen not only in scenarios with different sample regimes being more similar when they included more genetic metrics, but also in the mtDNA data for five species in Chapter One having similar conservation priorities to the SNP data from Chapter Two. This suggests that for regions where funding for NGS studies is unavailable, the more accessible marker of mtDNA will most likely be sufficient in identifying community evolutionary patterns, if analyzed over multiple sample sites and several species. Ultimately, the comparison of spatial conservation prioritizations from different genetic markers is required for additional species as well as

in other environmental regimes to better understand how they compare in a conservation planning framework.

Conclusion

The field of conservation planning has recently expanded from focusing on protected areas that effectively conserve standing biodiversity features, such as habitats and species, to those that additionally incorporate the persistence of biodiversity features through protecting ecological and evolutionary processes (Klein et al. 2009, Ferrier & Drielsma 2010, Pressey et al. 2007). Inferences of biodiversity processes can be made with genetic patterns, which are expected to vary based on the molecular marker and genetic metric used (Allendorf et al. 2010). Therefore, this study compared conservation objectives and resulting priorities from multi-species genetic patterns derived from various genetic metrics and markers.

The first chapter compared four mtDNA genetic metrics for five rocky shore species, finding that each metric resulted in similar conservation solutions. However, when mtDNA and SNP metrics from two rocky shore species were compared in Chapter Two, there were noticeable differences in the spatial priorities between the genetic metrics. This suggests that including different marker types, corresponding to either neutral or adaptive evolutionary processes, will lead to larger trade-offs in conservation prioritizations than including a single marker. This finding is not surprising, as other studies have found similar trade-offs when comparing different molecular markers (Bonin et al. 2006, Stork et al. 2009, Asmyhr et al. 2014). Overall, there were greater spatial distinctions in scenarios with a change in species in Chapter One and in scenarios with a change in genetic metric in Chapter Two, which suggests that there may be a trade-off between the number of molecular markers and the number of species included. While the most efficient amount of genetic information and marker types will differ with the conservation objective (Beger et al. 2014, Shafer et al. 2015), it is important to quantify the economic trade-offs between conservation scenarios including more species versus more genetic metrics.

It is also important to understand how the different implementation techniques affect conservation priorities, as sampling, classification and interpolation methods likely bias the conservation solutions (Levy et al. 2013, Lechner et al. 2014). Ideally, genetic data should be modeled over the entire planning region, rather by simply interpolating the values from sample sites. Further, as the environmental drivers of community genetic patterns become more apparent within the marine realm (Selkoe et

al. 2008, Liggins et al. 2013, Riginos & Liggins 2013, Selkoe et al. 2016), it would be beneficial to develop 'genetic distribution models' that can be used to map and predict genetic patterns for conservation purposes.

Few studies have examined spatially modeling genetic information, and only include genetic structure for terrestrial mammals (Cushman et al. 2006, Corander et al. 2008), leaving spatial genetic diversity modeling and marine genetic modeling still unexplored. While interpolation techniques have yet to be empirically tested within a marine conservation planning framework, interpolation of species distribution data from point localities is theoretically thought to be less informative than predicted distribution data, because with predictive modeling the probabilities of either presence or absence are derived from statistical models, which better account for uncertainties (Rondinini et al. 2006).

In theory, uncertainty values should be associated with genetic patterns within conservation planning processes, potentially even with levels of uncertainty varying with the molecular marker, yet quantifying the uncertainty of genetic patterns is not trivial (Dudaniec et al. 2016). For instance, it can be argued that an increase in the number of loci (or sample size) may lead to greater certainty with SNP markers compared to mtDNA; however, mtDNA sequencing has greater repeatability than RAD sequencing, as the restriction enzymes with RAD-seq will not always splice in the exact same regions of the genome with consistent coverage (Davey et al. 2013). This in itself may lead to inconsistencies within the raw SNP data, as well as downstream analyses (Narum et al. 2013). As the integration of genetic data types is still emerging within the conservation planning field, the interpolation and levels of uncertainty within genetic metrics are just a few of the topics that will require further careful research and analysis.

Another future research topic includes quantifying the incidental genetic representation within MPAs, as Chapter One showed that each metric roughly had similar conservation solutions, which is likely a result of the priorities of one metric incidentally capturing priorities of the other genetic metrics. Bridge et al. (2015) examined the factors leading to increased amounts of incidental species representation within MPAs in the Great Barrier Reef, finding that incidental representation increased with higher conservation targets and the partitioning of priority areas over biophysical gradients. However, this study did not quantify the incidental representation of different genetic metrics, therefore it would be interesting to perform a gap analysis within South Africa to examine how much of the available genetic patterns are incidentally captured

within the current MPA network.

Lastly, it is also suggested that comprehensive conservation plans (such as those including predicted species distributions, connectivity patterns or genetic diversity) are only effective when they can be implemented immediately. For example, Mier et al. (2004) found that conservation plans with relatively simple conservation targets (such as protecting high species richness or irreplaceability) outperformed both ad-hoc and comprehensive conservation plans. Therefore, it is vital that genetic data sets can be simplified and tailored for conservation objectives (to expedite the conservation planning process), as genetic biodiversity features will likely just play a small part in the development of protected areas, compared to other ecological and socioeconomic constraints.

Ultimately, it is highly unlikely that genetic and genomic information from multiple non-model organisms will be generated simply to be included into a single conservation plan (Ferrier 2002, Grantham et al. 2009). However, genetic and genomic patterns, as well as the drivers of these patterns, are becoming more and more accessible to conservation managers due to advancements in fields of molecular ecology, population genomics and seascape genetics (Kohn et al. 2006, Stapley et al. 2010, Liggins et al. 2013, Willette et al. 2014, Selkoe et al. 2016). Therefore, it is important for conservation planners to understand what genetic information is relevant to their objectives, and how to interpret the genetic patterns as well as the conservation results associated with different genetic metrics. In this context, this study acts a theoretical and empirical prerequisite to building a framework for including genetic data into conservation planning, and provides a baseline for further research focused on genetic objectives in conservation planning.

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Supplementary Materials

Supplementary Materials 1 - Life history characteristics of the five study species

Table 1- Summary of life history characteristics

The five study species represent a phylogenetically diverse assemblage of rocky shore taxa and are generally abundant on South African rocky shores. The table below summarizes some of their life history characteristics (after Table 1 in Wright et al. 2015). Additional information was taken from Payne et al. (2015).

Species	Common Name	Phylum	Fertilization type	Min pelagic larval duration (days)	Diet	Position on rocky shore
Clinus superciliosus	Super klipfish	Chordata	Internal, live young	0	Carnivore	Subtidal-high
Oxystele tigrina	Tiger topshell	Mollusca	Spawns	4-6 (sister species)	Grazer	Mid shore
Scutellastra granularis	Granular limpet	Mollusca	Spawns	4-10 (sister species)	Grazer	High shore
Parechinus angulosus	Cape urchin	Echinodermata	Spawns	49-56	Grazer	Mid to low shore
Parvulastra exigua	Cushion star	Echinodermata	Brooder, crawl-away young	0	Grazer	Mid to low shore

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Supplementary Materials 2 - Genetic data acquisition and genetic values included into Marxan analyses

Genetic data generation

Data sets were generated by and taken from Mertens et al. (in review; *S. granularis*, *O. tigrina*, *P. exigua*, *C. superciliosus*), with the exception of *P. angulosus*, which correspond to sequences from Muller et al. (2012) and some sequences of *C. superciliosus* (von der Heyden et al. 2011). Briefly, DNA was extracted using the Nucleo-Spin DNA extraction kit (Machery-Nagel), followed by PCR amplification following the protocols of Folmer et al. (1994) for mtDNA Cytochrome Oxidase I (CO1), Palumbi et al. (1991) for mtDNA COI and Lee et al. (1995) for the mtDNA control region. After visualizing on a 1% agarose gel stained with ethidium bromide, PCR products were sent for sequencing on an ABI-3100 automated sequencer (Applied Biosystems) at the Central Analytical Facility at the University of Stellenbosch. Alignments were created in BioEdit v7.0.9.0 (Hall 1999) and all new sequences were deposited in GenBank under the following Accession Numbers: KU64040 - KU640590, KU640591 - KU640755 and KU640756 - KU640952.

Table 1- Genetic metrics that were normalized, interpolated and included into Marxan as conservation features

Location	Kommetjie	Sea Point	Jacobs Bay	Lamberts Bay	Brand Se Bay	Hondeklip Bay	Port Nolloth
				Haplotype Diversity (*h*)			
C. superciliosus	0.90	0.94	0.93	0.92	N/A	0.91	0.96
P. exigua	0.80	0.61	0.46	0.56	0.80	0.68	0.34
P. angulosus	0.95	0.92	0.97	0.97	0.83	0.84	0.93
S. granularis	0.88	0.83	0.90	0.95	0.96	0.89	0.94
O. tigrina	0.88	0.91	0.92	0.90	0.87	0.89	0.91

				Nucleotide Diversity (π)			
C. superciliosus	0.0080	0.0089	0.0059	0.0128	N/A	0.0121	0.0123
P. exigua	0.0001	0.0015	0.0008	0.0015	0.0028	0.0019	0.0009
P. angulosus	0.0114	0.0134	0.0114	0.0163	0.0027	0.0033	0.0031
S. granularis	0.0040	0.0036	0.0037	0.0053	0.0044	0.0043	0.0050
O. tigrina	0.0051	0.0052	0.0061	0.0046	0.0055	0.0049	0.0052
				Private Haplotypes (#)			
C. superciliosus	8	8	9	1	N/A	8	13
P. exigua	1	3	3	0	1	4	2
P. angulosus	11	8	13	12	5	8	11
S. granularis	5	6	4	10	9	8	8
O. tigrina	8	4	6	5	2	1	3
				Local genetic differentiation			
C. superciliosus	0.0663	0.1094	0.0617	0.0650	N/A	0.1343	0.0333
P. exigua	0.0885	0.0553	0.0414	0.2205	0.0833	0.3755	0.0231
P. angulosus	0.3519	0.2392	0.0116	0.0231	0.1625	0.0166	0.1712
S. granularis	0.0008	0.0023	0.0201	0.0137	0.0074	0.0128	0.0009
O. tigrina	0.0103	0.0087	0.0019	0.0133	0.0137	0.0013	0.0028

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Supplementary Materials 3 - Quantitative trade-offs between scenarios with a change in species versus change in genetic metric.

Table 1- Pearson correlation coefficients corresponding to each pair of scenarios with a change genetic metric.

	B	H	N	L	P	ALL
B						
H	0.37					
N	0.27	0.62				
L	0.33	0.72	0.61			
P	0.26	0.59	0.74	0.63		
ALL	0.38	0.81	0.80	0.78	0.70	
AVG	0.37	0.50	0.56	0.57	0.52	0.61

Table 2 - Pearson correlation coefficients corresponding to each pair of scenarios with a change in species.

	B	CS	OT	PA	PE	SG	ALL
B							
CS	0.35						
OT	0.39	0.74					
PA	0.41	0.83	0.72				
PE	0.41	0.83	0.71	0.70			
SG	0.29	0.67	0.68	0.63	0.69		
ALL	0.38	0.78	0.81	0.83	0.76	0.81	
AVG	0.37	0.60	0.61	0.62	0.64	0.47	0.61

Supplementary Materials 4 – All steps and the corresponding scripts used for the bioinformatics analyses conducted within Chapter Two, Part One.

1. Find optimum assembly kmer value with velvetoptimiser

```
VelvetOptimiser.pl -s 19 -e 31 -t 8 -f '-shortPaired -separate -fastq p1.read1.fq
p1.read2.fq'
```

2. Running Velvet assembler

```
velveth /home/esnielsen/work/limpet_velvt_New/ 71 -shortPaired -separate -fastq
p1.read1.fq p1.read2.fq

velvetg /home/esnielsen/work/limpet_velvt_New/  -ins_length 518 -exp_cov 3 -
min_contig_lgth 200 -scaffolding no -unused_reads yes
```

3. Combine contigs and perform assembly with caps

```
cat *contigs.fa >All_contig.fa
nohup cap3 All_contig.seq
```

4.Create the 'reference sequences'

```
cat All_contig.fa.cap.contigs  All_contig.fa.cap.singlets > ref.seq
```

5. Map onto the reference

a) BWA ALN

```
bwa index ref.fa
bwa aln -t 20 ref.fa p1.read2.fq > p1.r2.sai
```

b) BWA SAMPE

```
bwa index ref.fa
bwa sampe -n 1 -a 550 -r '@RG\tID:pop6\tSM:SP\tLB:library6' ref.fa p6.r1.sai p6.r2.sai
p6.read1.fq p6.read2.fq > A_SP.sam
```

6. Convert SAM to BAM in Samtools

```
samtools view -q 20 -bS A_SP.sam > A_SP.bam
```

7. Sort BAM file

```
samtools sort -T A.SP.sorted -o A.SP.sorted.bam A_SP.bam
```

8. Index BAM files

```
samtools index A_SPsorted.bam
```

9. Create SAMtools mpileup variant file

samtools mpileup -d 10000 -Q 20 -B -f L.ref.fa L.SS.sorted.bam L.SJ.sorted.bam L.SL.sorted.bam L.SB.sorted.bam L.SH.sorted.bam L.SP.sorted.bam > L.6pop.mpileup

10. Create a sync file from mpileup using Popoolation2

mpileup2sync.pl --fastq-type sanger --min-qual 1 --input hc.q10.mpileup --output hc.q10.sync

11. Calculate pi, theta, and number of SNPs with Popoolation1

perl /apps/PoPoolation/1.2.2/Variance-sliding.pl --fastq-type sanger --measure theta --input EN7_velvt.pileup --min-count 2 --min-coverage 10 --max-coverage 500 --min-qual 0 --pool-size 80 --window-size 100 --step-size 100 --output Gal_EN7_cov10.velvt.pis --snp-output pop3.gal.l.v.snps

12. Calculate pi and theta only from contig regions that have SNPs in each population

more pop1.pi |awk '{if($3!=”0”)print$1"\t"$2} > pop1.snp.list
cat *snp.list |sort | uniq -c | awk '{if($1==4)print$2"\t"$3}' > SNP_sharedBYall.list
/usr/bin/perl split.pl SNP_sharedBYall.list.snp pop1.pi > pop1.shared.pi

13. Calculate SNP frequency differences per population (this gives you the number of SNPs and their allele counts)

snp-frequency-diff.pl --input q10.q5.uv.sync --output-prefix q10.q5.cov10_diff --min-count 2 --min-coverage 10 --max-coverage 500

14. Get a list of only biallelic SNPs

more *_rc|awk '{if($4==2)print $1'\t'$2}' >biallelic_SNP.list

15. Calculate FST values for each individual SNP

fst-sliding.pl --input q10.q5.uv.sync --output q10.q5.uv.cov10.w100.fst --suppress-noninformative --min-count 2 --min-coverage 10 --max-coverage 500 --pool-size 80 --window-size 100 --step-size 100

16. Get the number of private SNPs per population

more q10.q5.cov10_diff_rc|awk '{if($4==2)print $10 "\t" $11 "\t" $12 "\t" $13 "\t" $14 "\t" $15 "\t" $16 "\t" $17}' > q10.cov10.bial.list
more *maa.txt|awk '{if($1==$2&&$3==$4&&$5==$6&&$7!=$8)print}' > q10.q5.cov10.prv.list.POP6

18. Run BayeScan

a) Build up a file containing all reference sequences with SNPs you are interested, called region.txt

```
more biallelic_SNP.list|awk '{print $1"pos"$2"\t"$1":"$2"-"$2}' >region.txt
```

b) Run the following commands to get GenePop format file for SNPs in each reference sequence.

```
more region.txt|awk '{print "perl
/apps/PoPoolation/2.svn204/export/subsample_sync2GenePop.pl --input q10.q5.uv.sync
--output "$1".GenePop --method fraction --min-count 2 --target-coverage 10 --max-
coverage 500 --region "$2" --diploid"}' > sync2GenePop.sh
```

c) Merge all GenePop files into a single file

```
/usr/bin/perl merge.gpop.pl
```

d) Add 'gt' to each line

```
/usr/bin/perl gt.pl pool_GenePop.txt > pool_GenePop.txt.md
```

e) Use PGDSpider to convert GenePop file to BayeScan file

```
nohup java -Xmx1024m -Xms512m -jar PGDSpider_2.0.2.0/PGDSpider2-cli.jar -
inputfile Pool_GenePop.txt -inputformat GENEPOP -outputfile BayeScan.txt -
outputformat GESTE_BAYE_SCAN -spid
PGDSpider_2.0.2.0/GENEPOP_to_GESTE_BAYE_SCAN.spid &
```

f) Running BayeScan

```
nohup BayeScan2.1/source/bayescan_2.1 BayeScan.txt -snp -od . -threads 8 -pr_odds
100 -out_pilot -out_freq&
```