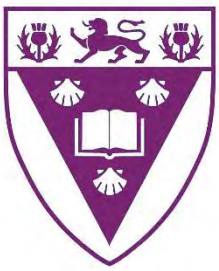

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Where leaders learn

Phylogeography and reproductive isolation of the brown mussel, *Perna perna,* on the South African coastline

By

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A thesis submitted in the fulfilment of the requirements for the degree of
Master of Science in Marine Biology

Department of Zoology and Entomology
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Abstract

Maintenance of a species' abundance and adaptive potential is partially dependent on its genetic diversity. Distinct genetic lineages within a species can differ significantly in their resistance and resilience to environmental pressure. An assessment of such differences is key to grasp the adaptive potential of a species. Physical environmental conditions are significant determinants of the distribution of species and their genetic lineages. Under climate change scenarios, the assessment of the temporal stability of the spatial distribution of genetic structure has important consequences for conservation as it offers key insights into the adaptive potential and evolutionary capacity of a species. This thesis investigated the phylogeography and reproductive isolation of *Perna perna*, the brown mussel. This species is an ecologically and economically important intertidal mussel on South Africa's coastline. It was determined in 2007 that there were two distinct genetic lineages of *P. perna* present on South Africa's coastline, the Eastern and Western lineage. This thesis compared mitochondrial DNA between samples collected in 2007 and samples collected in 2019 to determine differences in genetic structure of *P. perna* over time. This thesis further investigated the reproductive timing of the two lineages to determine if this contributes to the maintenance of the genetic structure of this species. The results show that there is a shift in the distribution of the Eastern lineage since 2007, an increase in the range of the overlap region, and there was a change in genetic diversity in the form of private haplotypes between 2007 and 2019. The lineages have unsynchronised spawning and reproductive patterns are more closely linked to changes in temperature. While the lineages have shown slight distributional changes over time, the influence of environmental conditions and the predicted changes in sea surface temperatures could see a change in future populations' fitness and dispersal. Thus, changes in sea surface temperature could affect reproductive timing and the future genetic stability of the species.

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Declaration

I hereby declare that this thesis has not been submitted, either in the same or different form, to this or any other university for a degree and that it represents my own work. I know the meaning of plagiarism and declare that all the work in this thesis, save for that which is properly acknowledged, is my own.

The ethical clearance number for this project is RU-DZE-2018-03-018.

CBarker

Cassandra Barker

May 2021

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Chapter 1: General introduction

Genetic diversity is critical to the maintenance of populations and a species' adaptive potential (Consuegra et al. 2005, Holderegger et al. 2006, Hoffmann & Willi 2008). Mounting evidence shows that distinct genetic variations within a species are often characterised by different adaptive potentials (Hauser & Carvalho 2008, Valladares et al. 2014). It is now clear that the successful management and conservation of a species and the understanding of its ecological and evolutionary significance requires a determination of the adaptive traits of distinct portions of the species' genetic pool (Goetze et al. 2015, Jueterbock et al. 2018).

1.1 Genetic structure in marine species

With a reshuffling of the distributional patterns of a wide variety of taxa due to climate change, a species' genetic structure, that is genetic diversity within and among populations, may also be affected (Pauls et al. 2013, Schierenbeck 2017). The existence of distinct genetic entities within a species raises the possibility that different entities will respond differently to climate stressors (Pauls et al. 2013). Thus, a species genetic structure can be destabilised and undergo significant spatial shifts over relatively short temporal scales (Anderson et al. 2010). Until recently, there have been few studies of genetic temporal stability in marine species. Those that have taken place have mainly focused on commercially significant taxa; e.g., cod (Ruzzante et al. 1997, Dahle et al. 2018), and salmon (Vähä et al. 2008, Walter et al. 2009). There is increasing modelling and experimental evidence showing climate-induced modifications of biogeographic dynamics as the

cause of changes in species genetic structures globally (Teske et al. 2008, Zardi et al. 2011, Fenberg et al. 2015, Lourenço et al. 2017), with significant long-term consequences for diversification or speciation processes and the species' adaptive potential against stressful environments (Lexer & Fay 2005).

The genetic structure of marine species in biogeographical transition zones is often the result of complex interactions over time. Some examples of the factors involved in such complex interaction are: historical events, like the last glacial maximum (Cunha et al. 2011, Crandall et al. 2012); contemporary dispersal barriers, such as upwelling and currents that limit demographic connectivity among populations (Munro & Burg 2017); environmental gradients in temperature, food availability or salinity that promote local adaptation; reproductive strategies that promote sustaining self-recruitment and mating incompatibility (Brochier et al. 2009, Barshius et al. 2011).

Gene flow and genetic stability in marine species are often attributed to the species' pelagic larval phase and its potential for dispersal. It is important to note that high gene flow due to larval dispersal does not always cause phylogeographic stability, it is also true that low gene flow does not cause genetic instability (Jueterbock et al. 2016). Thus, there is not necessarily a connection between a species' capacity for dispersal, as influenced by its life history, and its genetic stability over time.

Most studies of genetic variation have focused on distribution, rather than time; how genetic diversity can differ within and among populations rather than how phylogeographic stability changes over time. Thus, sampling is generally done across species' habitats, but rarely includes a time scale of variability across generations. Many genetic studies have sampled a given population only once and inferred genetic resilience from a single time point. Such studies have provided little information on stability in the allelic frequency of the species (Gold & Richardson

1991, Thollesson 1999, Teske et al. 2008). An alternative to this genetic caveat would be resampling through time of the populations of the species in question (Lenfant & Planes 2002, Holmes 2015). Measuring the genetic stability of a species over time potentially gives valuable insight into the adaptive potential of a species to changes in environmental conditions (Jueterbock et al. 2016).

A species' adaptive potential is mainly dependent on that species' genetically significant size, or the size of an ideal sample population that undergoes a genetic change at the same rate as the whole population (Wright 1990). When there is low gene flow between populations, genetic drift plays a vital role, neutralising selection, and resulting in eroded genetic diversity, a possible genetic bottleneck with a loss of allelic variation (Charlesworth 2009, Bijlsma & Loeschcke 2012). The study of the adaptive potential of a species is a useful tool to estimate the vulnerability of species to climate change. This requires an assessment of the genetic stability of a species across its latitudinal and thermal range distribution over time (Jueterbock et al. 2016). Changes in thermal regimes and climate profiles will pose a challenge for organisms to adapt and maintain fitness. Physiological adjustments to temperature changes are better achieved when there is high genetic differentiation already present across populations of the same species throughout a latitudinal or altitudinal cline (Dahlhoff & Rank 2000, Read et al. 2016). Species that show high genetic differentiation that allows for better adaptation across the habitats they occupy will be more resilient to the current climate change trend.

There are several factors that affect genetic drift over time and space. Firstly, a mechanism contributing to genetic stability, when the genome of an organism does not undergo rapid and excessive mutations as those seen in bacteria (Bhagavan and Ha 2015), is migration which enables hereditary transfer among populations and thus increases gene flow (Østergaard et al. 2003).

Secondly, factors that affect temporal stability in allelic frequencies include high fertility levels and early mortality rates. Species that reproduce through mass spawning have large populations that can cause high genetic diversity compared to species with fewer offspring, potentially reducing adaptive potential by increasing the number of possible recruits that are not adapted to the environmental conditions they face, and leading to low survivorship offspring despite high reproductive effort (Turner et al. 2002). While mass spawning or an r-strategy life cycle has the benefit of mass reproduction to better prepare for high propagule mortality, the selection of offspring that achieve adulthood is likely to be random and can lead to genetic dilution and small population sizes (Turner et al. 2002, Árnason 2004, Hauser & Carvalho 2008). High genetic variance and randomly selected survivorship give little room for local adaptation and likely lower fitness for a species (Hauser & Carvalho 2008). Environmental factors that affect genetic stability across a species' distribution include extreme weather events like hurricanes, droughts, and anthropogenic disrupting factors like installing artificial structures within a species' habitat (Allison et al. 2003). Overall, the genetic diversity of a species is highly influenced by dispersal potential and environmental factors.

Intertidal habitats provide complex environments (Kostylev et al. 2005, Denny et al. 2011, Meager et al. 2011) that are regularly immersed and emersed during tidal cycles. These unique ecosystems are very diverse and productive, supplying vital ecosystem services such as the production of food (Abrantes et al. 2015, Horn et al. 2017), stabilisation of shorelines (Chowdhury et al. 2019), and nutrient turnover (Menge 2000). As intertidal habitats are effectively buffering regions between land and sea, organisms living within this habitat are routinely exposed to air temperature extremes, desiccation, high hydrodynamic stress, and seawater temperatures during immersion (Tomanek & Helmuth 2002, Helmuth et al. 2006, Petes et al. 2008a). As a result of

these substantial daily temperature fluctuations, intertidal organisms frequently live close to their thermal limits (Zippay & Hofmann 2010, Nguyen et al. 2011, Jueterbock et al. 2016). The current gradual global warming and increasingly intensifying acute temperature stress events, such as heatwaves, are having a dynamic effect on these ecosystems. Intertidal organisms – particularly those with limited mobility such as sessile or sedentary invertebrates – have been the focus of studies aimed at assessing new climate-induced species distributional shifts and forecasting future range contractions and extinctions (Hiddink et al. 2015, Hare et al. 2016, Macfadyen et al. 2018). Due to the importance of intertidal organisms and their relevance in the study of the effects of climate change, intertidal mussels were used in the investigations of this thesis.

1.2 The brown mussel, *Perna perna*

Mussels are ecosystem engineers in intertidal systems and are of great ecological and economic significance (Smaal 1991, Beadman et al. 2002, Hammond and Griffiths 2006, Carranza et al. 2009). Their beds provide favourable environments to many small invertebrates by providing habitat complexity and ameliorating abiotic conditions such as reducing hydrodynamic, temperature, and desiccation stress (Dittmann 1990, Hammond and Griffiths 2004, Jordaan 2010). Studies have shown that when mussel beds are absent; there is a decrease in intertidal and subtidal diversity and abundance (Karatayev et al. 2002, Borthagaray & Carranza 2007, Arribas et al. 2014). Mussels are widespread and have an r-selected lifestyle, but their population genetic structure is often influenced by shifts in environmental conditions (Suchanek 1981, Gilg & Hilbish 2003, Nicastro et al. 2008). Ocean currents will dictate the dispersal of mussel larvae among populations while recruitment into the population will be shaped by environmental factors

(McQuaid and Phillips 2000, Nicastro et al. 2008, Lourenço et al. 2017). However, a coast's topographic features also influence the genetic connectivity among populations and self-replenishment rates of individual populations. For instance, coastal bays act as source populations with higher migration rates out of bays rather than into them (Nicastro et al. 2008). In comparison, the greater hydrodynamic stress of the open coast generates higher mortality, resulting in genetic turnover (Gawarkiewicz et al. 2007). Thus, gene flow in this important ecosystem engineer is dependent on local and larger scale oceanography.

The indigenous brown mussel, *Perna perna*, dominates the south and east coasts of the South African rocky intertidal shore. Within South Africa, this subtidal and intertidal mussel has a range stretching from the Mozambique border on the east coast to Cape Town in the Western Cape (Fig. 1.1, Zardi et al. 2007b). *Perna perna* is found on the west coast of South Africa but the distribution is patchy, and it can be very difficult to find individuals as the seawater temperatures are cold (Tagliarolo et al. 2016). The abundance of *P. perna* becomes more numerous in northern Namibia, and its range continues up the west coast of Africa (Fig 1.2, Zardi et al. 2007b, Lourenço et al. 2012, Cunha et al. 2014). Previous genetic work using mitochondrial DNA (mtDNA) sequences and Internal Transcribed Spacer (ITS) data identified a phylogeographic break for this species on the south-east coast of South Africa (Zardi et al. 2007b, Cunha et al. 2014). The two lineages have a non-sister relationship, possibly reflecting an Indo-Pacific origin followed by dispersal into the Atlantic through the Tethys seaway. Following that, there was an independent southward expansion along the African continent's western and eastern shores by the two lineages and recent secondary contact on the south-east coast of South Africa (Cunha et al. 2014). The genetic break of the lineages is maintained by a combination of local adaptation to thermal conditions and limited dispersal connectivity, and essentially coincides with the oceanography-

linked biogeographical patterns of South Africa (Zardi et al. 2011). The two genetic lineages overlap in distribution along approximately 200 km of coastline, from Haga Haga to Kenton-on-Sea, close to the interface of the Agulhas and Natal biogeographic regions (Fig. 1.1). With *P. perna* being an ecosystem engineer and showing marked intraspecific variation, it is a suitable candidate for investigating genetic stability over time.

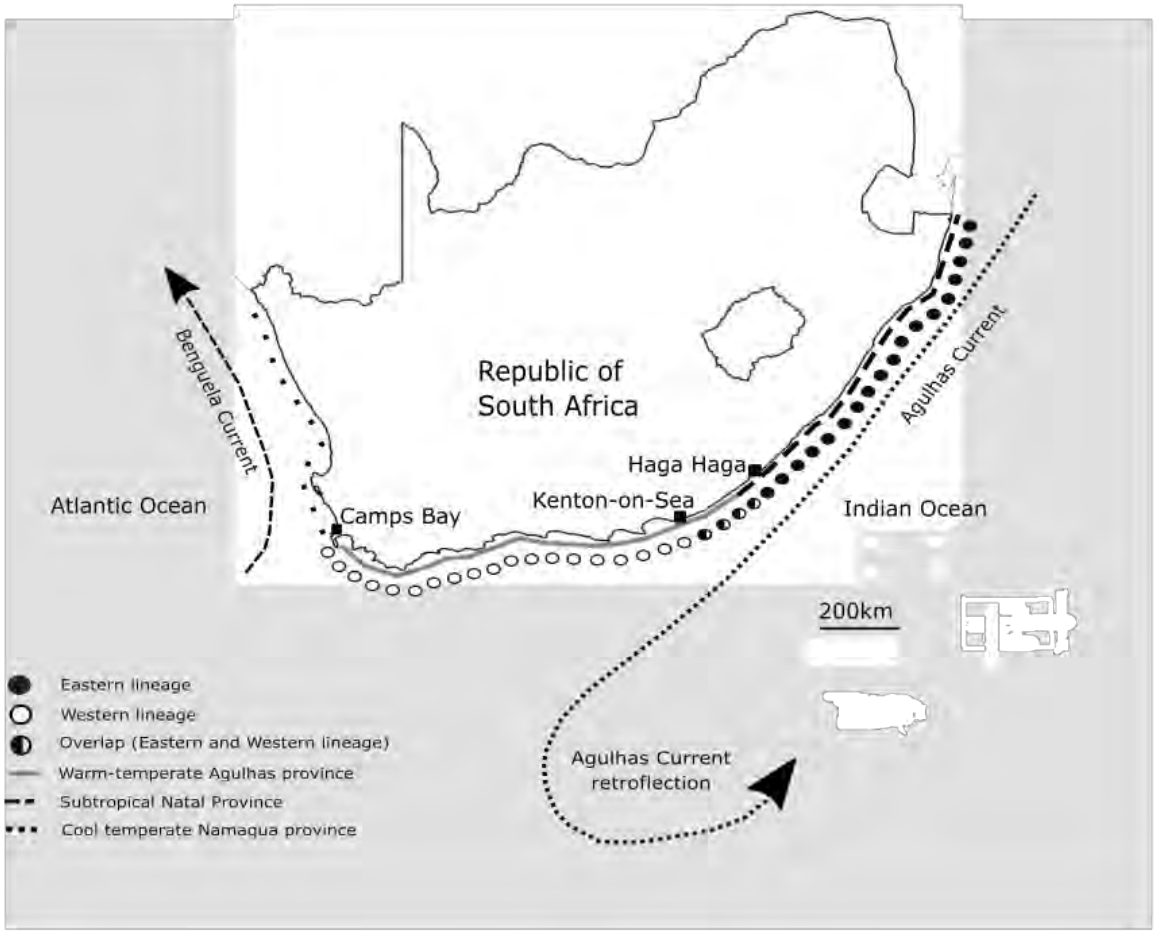


Figure 1.1: Distribution of two genetic lineages of *Perna perna* on the South African coastline. The Eastern lineage is found from the border of South Africa and Mozambique until Kenton-on-Sea in the Eastern Cape. The Western lineage is found from Haga Haga to Camps Bay in the Western Cape. Its distribution does not end at Camps Bay but continues sporadically up the west coast to northern Namibia where the population becomes more abundant. The lineages overlap in distribution between Haga Haga and Kenton-on-Sea. The distribution of the lineages of *P. perna* were taken from Zardi et al. (2007b) and are based on mtDNA COI data. The locations of the Agulhas and Benguela Currents and biogeographic provinces are according to Emanuel et al. (1992).

Figure 1.2: The global distribution of *Perna perna*. Distributions are based on information from Wood et al. (2007) and Gardner et al. (2016). *Perna perna* was introduced to the shores of North America and Mexico supposedly by ballast water from ships (Hicks and Tunnell 1995). The area of interest for this thesis, South Africa, is outline by a grey block.

South Africa has a coastline of 3650 km, and three primary biogeographic provinces characterise this coastline: the cool-temperate Namaqua province (west coast), warm-temperate Agulhas province (south coast), and the subtropical Natal province (east coast) (Fig. 1.1, Anderson et al. 2009, Griffiths et al. 2010). Two ocean currents directly affect their adjacent terrestrial climates: the tropical Agulhas in the east and the temperate Benguela in the west (Smith 1961, Griffiths et al. 2010). These two currents exhibit different temperature conditions and nutrient availability and result in different species occurrences and abundances on the west, south, and east coasts. The oceanographic profile of these coasts has cascading effects as follows:

On the east coast, the warm-water Agulhas Current leads to a low surface nutrient load (Fig. 1.1, Lutjeharms et al. 1996). The low nutrient availability results in high species diversity but low abundance as species must specialise to make use of the available food (Griffiths et al. 2010). By contrast, the more uniform habitat of the west coast has cold, nutrient-rich water from the Benguela upwelling, allowing for high species abundance but low biodiversity (Fig. 1.1, Smith 1961, Griffiths et al. 2010). This pattern is not limited to the subtidal regions of South Africa's coastline and translates to the intertidal zone. The sharp distinction in biogeographical zones makes the South African coastline suitable for the study of genetic adaptive potential and genetic variability since gene flow is often dependent on environmental conditions.

Predicted climate change trends on the South African coastline suggests that the sea surface temperatures on the west coast will decrease by 0.5 °C per decade due to an increase in upwelling, while sea surface temperatures on the east coast will increase by 0.55 °C due to increases in warmer trade winds in the South Indian Ocean (Rouault et al. 2010). The predicted changes in water temperatures might affect the present coastal marine invertebrate populations and result in distributional shifts or disruptions (Whitfield et al. 2016).

1.3 Rationale for the study

Perna perna has an important role as an ecosystem engineer on South Africa's coastline and as an asset in artisanal fisheries (Siegfried et al. 1985). This makes it an important candidate for the investigation of the possible effects of climate change on its genetic lineage distribution and reproductive health. This will provide information for consideration concerning its conservation and possible shifts in distribution.

1.4 Hypotheses, Aims, and Objectives

Mitochondrial DNA (mtDNA) was used to investigate the genetic structure of *P. perna* populations along the South African coastline. Those data were compared to mtDNA data collected 12 years ago (Zardi et al. 2007b) to determine if the phylogeography of the species had been altered over time. The timing of *P. perna* breeding was investigated to determine if it had a restrictive effect on gene flow.

Due to the *P. perna* lineages having differing thermal tolerances, it was hypothesised that the current climate change trend would influence their distributions. The genetic distinction between the lineages was expected to be reenforced by differences in the timing of spawning that and this would contribute to the sharp genetic cline on the south-east coast.

Chapter 2: Revisiting the population genetic structure of the intertidal mussel *Perna perna* along the South African coastline

2.1 Introduction

Physical environmental conditions are key drivers of species' distributions and the genetic structure of their populations (Young et al. 2015, Johansson et al. 2015, Zhang et al. 2016). In the marine realm, barriers to dispersal (e.g., upwelling, currents) and environmental clines (e.g., temperature, salinity) that limit demographic connectivity among populations through local species selection are often responsible for genetic discontinuities among coastal populations (Young et al. 2015). Importantly, over the last two decades, experimental and modelling approaches have shown that marked alterations to oceanographic dynamics due to climatic change are reshuffling species' genetic patterns and distributions globally (Kordas et al. 2011, Poloczanska et al. 2016, Alabia et al. 2018). Under fast-changing climatic conditions, the assessment of the temporal stability of the spatial distribution of genetic structure has fundamental implications for conservation as it provides critical insights into the adaptive potential and evolutionary capacity of a species (Pulido & Berthold 2004).

Contemporary climate change trends show an increase of 0.11 °C per decade in oceanic water temperatures and an increase in marine heatwaves (IPCC 2014, Oliver et al. 2018). Increases in water temperature have a more significant effect on the range limits on intertidal ectotherms

when compared to increases in air temperature (Okey et al. 2014, Assis et al. 2015, Lourenço et al. 2016, Seabra et al. 2016). Thus, coastal areas with distinctive biogeographical characteristics, like tropical or temperate sea temperature profiles, are likely to experience shifts in intertidal biodiversity (Okey et al. 2014).

Changes in sea surface temperatures along the South African coast differ from the global trend significantly. On the west coast, affected by the Benguela Current, there is an average cooling trend in sea surface temperatures of 0.5 °C, with an increase in sea surface temperature along the east coast, affected by the Agulhas Current, of 0.55 °C per decade from 1982 to 2009 (Rouault et al. 2010). While these two bioregions seem to be changing in temperature profiles by the same magnitude, the cooling trend on the west coast and warming trend on the east coast means that the difference in temperature profiles between the two bioregions is growing greater (Rouault et al. 2010). With the distributional range of intertidal ectotherms being significantly governed by sea surface temperature (Lourenço et al. 2016), it is likely that there will be a fracturing in species distributions in the Cape Agulhas region of the South African coastline and not a gradual change in biodiversity as presently seen. This will have a significant effect on the ecological and economically important species found on the South African coast.

The brown mussel, *Perna perna* (Linnaeus, 1785) is a widely distributed warm temperate/tropical and subtropical, habitat-forming species. Mussels are ecologically and economically important species with numerous bioengineering features that are key to the functioning of intertidal habitats and the maintenance of biodiversity (Smaal 1991, Beadman et al. 2002, Arribas et al. 2014). In southern Africa, *P. perna* is a common mussel species on intertidal rocky shores, from central Mozambique to the Cape of Good Hope (Fig. 1.1, Berry and Schleyer 1983, Zardi et al. 2007b, Cunha et al. 2014). The distribution of *P. perna* is patchy on the west

coast of South Africa due to the cold, upwelled waters of the Benguela system (Tagliarolo et al. 2016).

Analyses of mitochondrial DNA (mtDNA, Zardi et al. 2007b), nuclear DNA (Zardi et al. 2015, Ntuli et al. 2020) and other nuclear gene (ITS; Cunha et al. 2014) sequences have revealed a sharp phylogeographic break between two genetic lineages that coincides with the transition between warm-temperate and the subtropical bioregions (Zardi et al. 2007b, 2015). The Western lineage includes mussels from the southeast coast of South Africa (from Haga Haga) and Namibia (despite a patchy distribution of more than 1000 km across the Benguela upwelling system, Fig. 1.1). The second, Eastern lineage comprises of mussels from the southeast and east coasts of South Africa (Fig. 1.1). The distributions of the two lineages overlap over almost 200 km on the southeast coast (Fig. 1.1). A more recent study has revealed a non-sister relationship between the two geographically contiguous lineages (Cunha et al. 2014). This is most likely explained by a biogeographic scenario that involved an Indo-Pacific origin followed by dispersal into the Mediterranean and Atlantic through the Tethys seaway, and recent secondary contact after southward expansion along the western and eastern shores of the African continent (Fig.1.2, Cunha et al. 2014).

The combination of differential selection and oceanographic barriers to larval dispersal are believed to be the main determinants maintaining genetic divergence between the two *P. perna* lineages (Zardi et al. 2011). Nearshore oceanographic drifters have shown very limited overlap between the trajectories of drifters released to the north and south of the genetic cline (Zardi et al. 2011). Such restricted dispersal between shores inhabited by the two *P. perna* lineages has been further confirmed by microsatellite analyses; these have shown very moderate and predominantly east-to-west gene flow (Zardi et al. 2015). In addition, laboratory and field reciprocal transplant

experiments have highlighted significant inter-lineage physiological and behavioural differences, and have clearly indicated that mussels on the east coast (Eastern lineage) are physiologically more tolerant of thermal stress than conspecifics on the south coast (Western lineage; Zardi et al. 2011).

2.1.1 Aims

This chapter aimed to revisit the investigation by Zardi et al. (2007b) using mitochondrial DNA (mtDNA) to establish if there has been a distributional shift of the Eastern and Western lineages of *P. perna*.

2.2 Methods and Materials

2.2.1 Sampling, DNA extraction, amplification, and sequencing

Perna perna were sampled at ten sites along the South African coastline in 2019 (Fig. 2.1). At each site, 10 mussels were collected across the intertidal zone, including individuals from the low, mid, and high mussel zones. Mantle tissue (approximately 1cm^2) was excised from the mussels collected and stored in ~98 % ethanol. The cellular fluid of the mussels dilutes the ethanol during storage and compromises preservation integrity (Williams & Van Syoc 2007). Thus, ethanol was changed, to maintain ethanol concentration at ~98 %, approximately every six hours for the first 72 hours. The samples were stored at -20 ˚C.

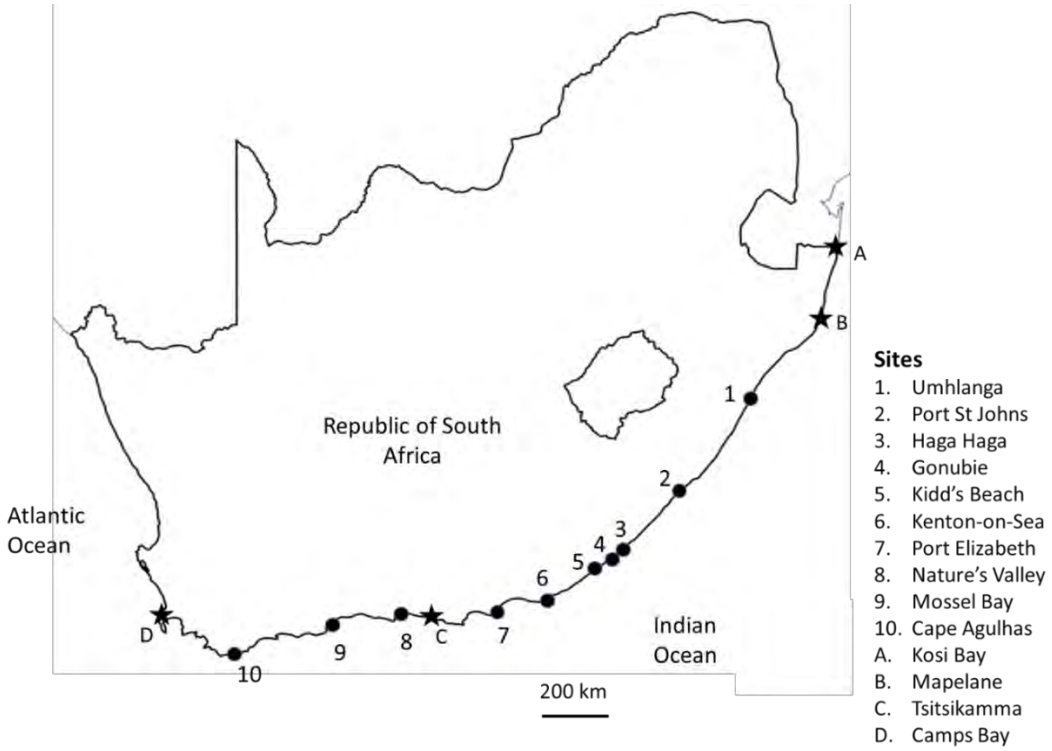


Figure 2.1: Sampling sites of *Perna perna* along the South African coastline. The east coast region comprised of populations from Umhlanga to Kidd's Beach (sites 1 – 5) and the south coast region comprised populations from Kenton-on-Sea to Cape Agulhas (sites 6 – 10). Site A – D were included in Zardi et al. (2007b) but samples were not collected from those sites in 2019.

Genomic DNA was isolated from the tissue samples (n = 100) with a standard salt extraction method (Bruford et al. 1992) using lysis (Buffer ATL; Qiagen) and elution (Buffer AE; Qiagen) buffers. Standard PCR procedures were utilized to amplify a portion of the mitochondrial cytochrome oxidase subunit I gene (mtDNA COI). The PCR was carried out using the primer pairs LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG-3') and HCO2198 (5'-GGT CAA CAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1995). Amplification of the selected gene was done in a 25 µl solution containing 1– 2 µl of DNA (~50 ng/µl), 2 µl of each primer (10 µM), 12.5 µl iTaq Universal SYBR Green Supermix (1725121, BioRad), and 7.5 – 6.5 µl distilled water. The PCR cycling profile consisted of an initial denaturation step at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 45 s and a final extension step at 72 °C for 8 min. The PCR products were then sent to Macrogen Inc. (Amsterdam, Netherlands) or the Central Analytical Facility (CAF) at Stellenbosch University and sequenced in the forward direction with the LCO1490 primer. The sequences generated by Macrogen or CAF will from hereon be referred to as the 2019 sequences. The sequences (n = 100) used in Zardi et al. (2007b) were provided by GI Zardi and will be hereon referred to as the 2007 sequences.

2.2.1 Data analysis

The 2019 DNA sequences were checked using the BLAST Sequence Analysis Tool (Madden 2002) to check the assumption that the sequences generated were from *P. perna*. The 2007 and 2019 DNA sequences were aligned using ClustalW (weight 1.6) in the program MEGA7: Molecular Evolutionary Genetics Analysis (v7.0, Kumar et al. 2016). Population Analysis with Reticulated Trees (PopART v1.7, Bandelt et al. 1999, Leigh and Bryant 2015) was used to generate

a median joining haplotype network for the 2007 and 2019 sequences separately. Haplotype frequencies were calculated in PopART and visualised as pie charts.

Fu's F_S test of genetic neutrality (Fu 1997) was performed on the clusters from the 2007 and 2019 haplotype networks in DnaSp (v6.12, Rozas et al. 2017). A negative Fu's F_S value shows an excess number of alleles and indicates a recent population expansion or genetic hitchhiking. A positive F_S value is evidence of a deficiency of alleles and indicates a recent population bottleneck.

The Bayesian Analysis of Population Structure (BAPS) of the lineages in each data set was statistically analysed in R Studio (v1.3.1073, R v3.6, R Core Team 2013) using the package "fastbaps" (Tonkin-Hill et al. 2019). This package analyses the nucleotide differences in each individual sequence and assigns an individual to a group or "clusters" similar sequences together (Tonkin-Hill et al. 2019). This was done to check assumptions of the lineage assignments in the 2007 and 2019 sequences.

Analysis of Molecular Variance (AMOVA) was performed for each year (the 2007 and the 2019 sequences) in ARLEQUIN (v3.5, Excoffier & Lischer 2010), populations were defined by the sites where the samples were collected. For the 2007 and 2019 sequences data sets, nucleotide (π) and haplotype (Hd) diversity (Nei 1987) were calculated in DnaSP (v6.12, Rozas et al. 2017). When Hd is high, there are a large number of haplotypes, while a high π value indicates that the present haplotypes are very different from each other. A pairwise comparison with an uncorrected p–distance model (Nei & Kumar 2000) was performed for the 2007 and 2019 sequences separately in MEGA X: Molecular Evolutionary Genetic Analysis (Kumar et al. 2018). This was done to compare the genetic differences between the Eastern and Western specific haplotypes and to determine, within the lineages, any indication of reduced gene flow.

2.3 Results

Haplotype networks

The 100 sequences from each data set were collapsed into 30 haplotypes for the 2007 sequences and 28 haplotypes for the 2019 sequences. The haplotype networks showed a similar pattern of clustering between the years of sampling (Figs 2.2 and 2.3). There were three distinct, most common haplotypes, Haplotype 1, 2 and 3, present in both 2007 and 2019 (Figs 2.2 and 2.3). In each of the haplotype networks, there were two main clusters, Cluster 1, and Cluster 2 (Figs 2.2 and 2.3). Both Cluster 1 and Cluster 2 included samples from Gonubie in 2019 (Fig. 2.3). Haplotype 5 in 2007 was shared among the sites between Port St Johns to Port Elizabeth and has split from the middle of the network (Fig. 2.2). In the 2019 haplotype network, Haplotype 5 was only shared between Umhlanga and Mossel Bay but was again split from the middle of the network (Fig. 2.3).

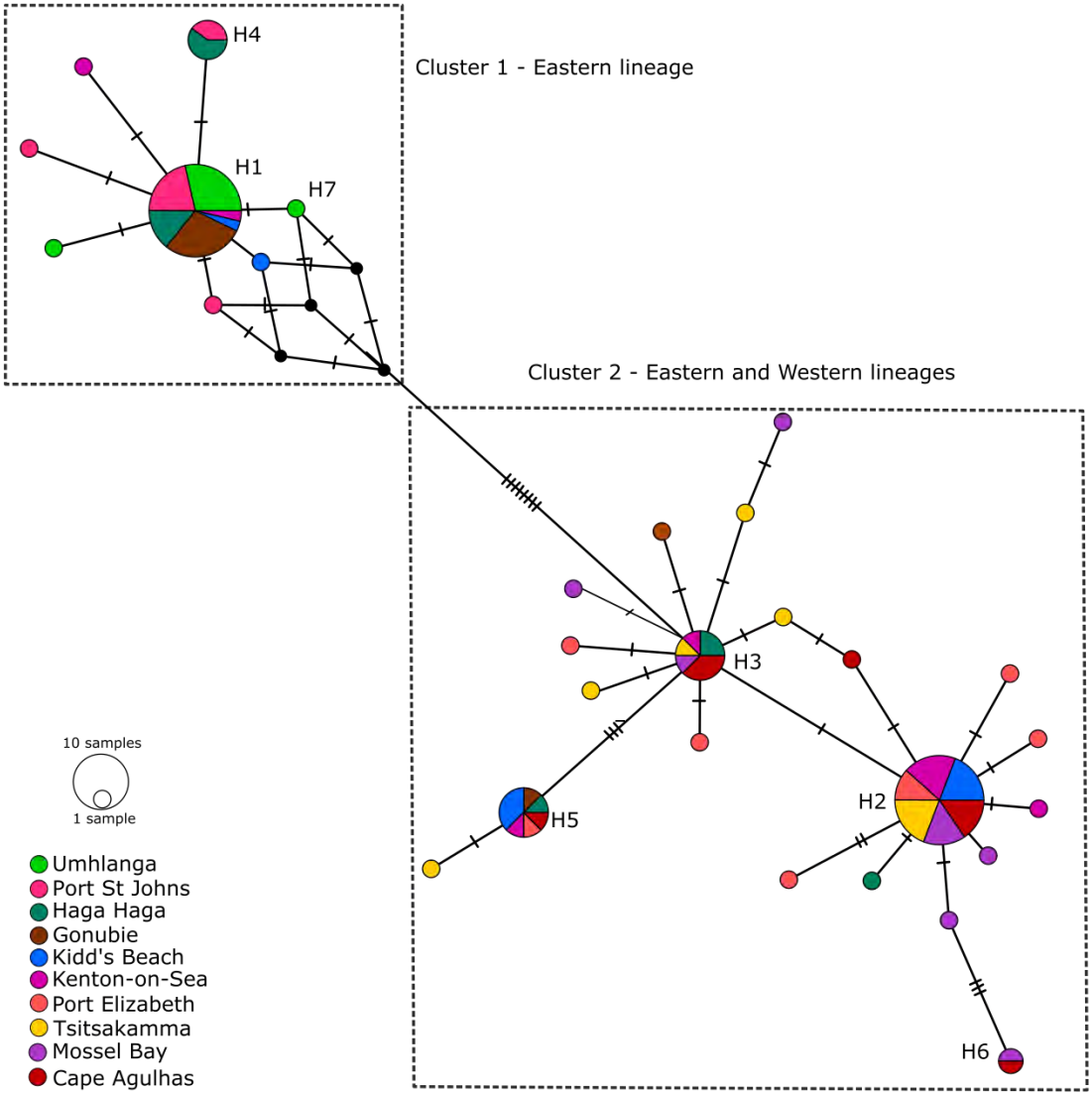


Figure 2.2: A median-joining haplotype network of 2007 mtDNA COI sequences of *Perna perna*. Haplotypes are shown by the circles whose size is proportional to haplotype frequency. The vertical lines indicate the nucleotide differences among haplotypes and the sampling sites were colour coded. In the figure 'H' means haplotype and only the shared haplotypes (present in >1 individual) were numbered.

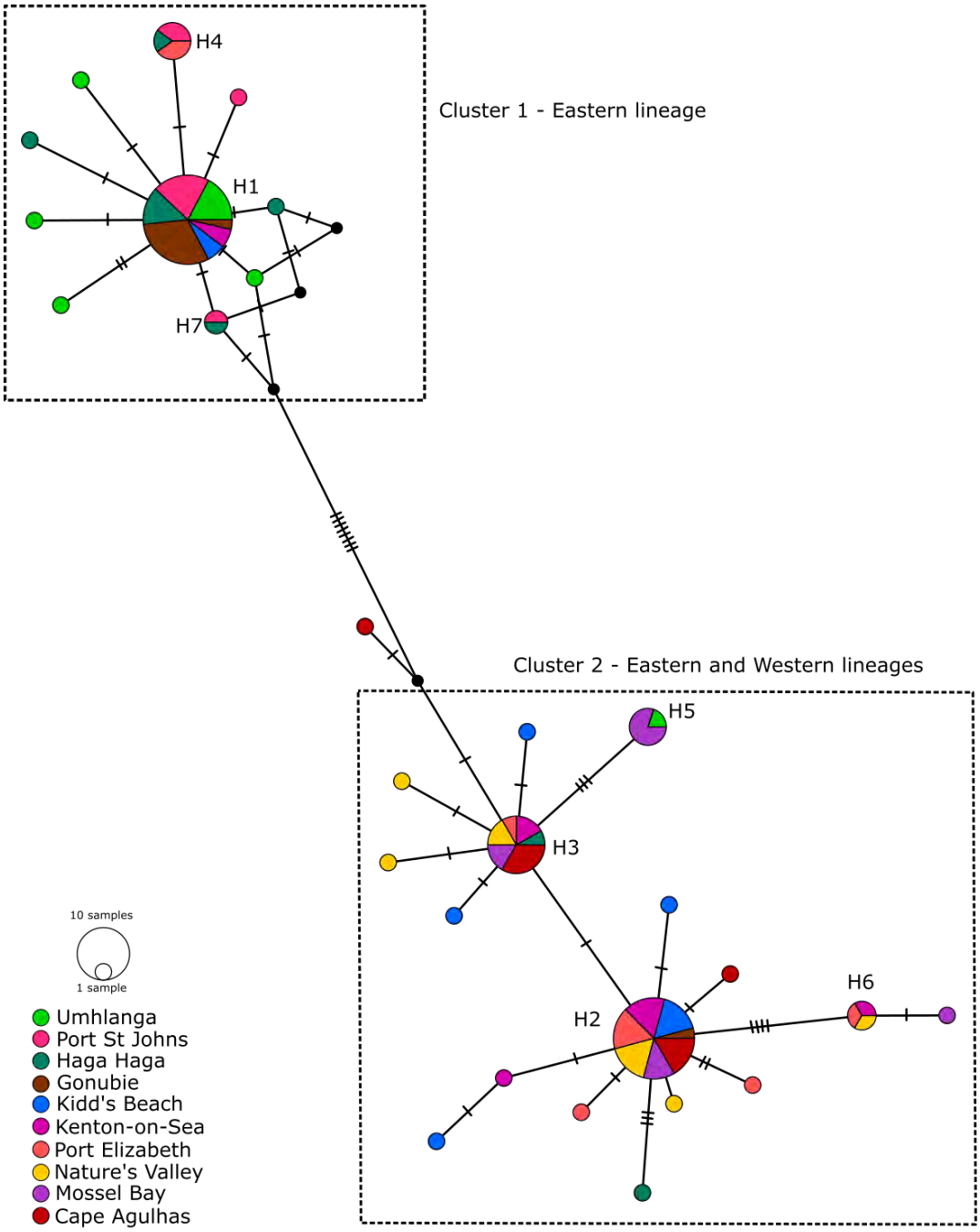


Figure 2.3: Median-joining haplotype network of 2019 COI sequences. Haplotypes are shown by the circles whose size is proportional to haplotype frequency. The vertical lines indicate the nucleotide differences among haplotypes and the sampling sites were colour coded. In the figure 'H' means haplotype and only the shared haplotypes (present in >1 individual) were numbered.

Haplotype frequency

The most abundant haplotypes in the 2007 sequences were Haplotypes 1 and 4, on the east coast, and Haplotype 2, on the south coast (Fig. 2.4). Haplotype 6 was found infrequently and only found on the south coast (Fig 2.4). Both Haplotypes 1 and 2 were present at Kidd's Beach and Kenton-on-Sea (Fig. 2.4). Haplotypes 1 and 2 remained abundant on their respective coastlines in the 2019 sequences and was present in Port Elizabeth (Fig. 2.5). Haplotypes 3 and 5 were present on both coastlines in 2007 and 2019 (Figs 2.4 and 2.5). Haplotype 4 shifted between surveys from the east coast to the south coast and was found in Port Elizabeth while Haplotype 6 remained in the south coast sample sites between the years of sampling (Figs 2.4 and 2.5). In 2019, Haplotype 7 was found at Port St Johns and Haga Haga but was only found in Umhlanga in 2007 (Figs. 2.4 and 2.5). Between 2007 and 2019 there was a change in the incidence of private haplotypes, most notably at Kidd's Beach (an increase from 10% to 40%), Port Elizabeth (a decrease from 60% to 20%), and Mossel Bay (a decrease from 40% to 10%, Figs 2.4 and 2.5).

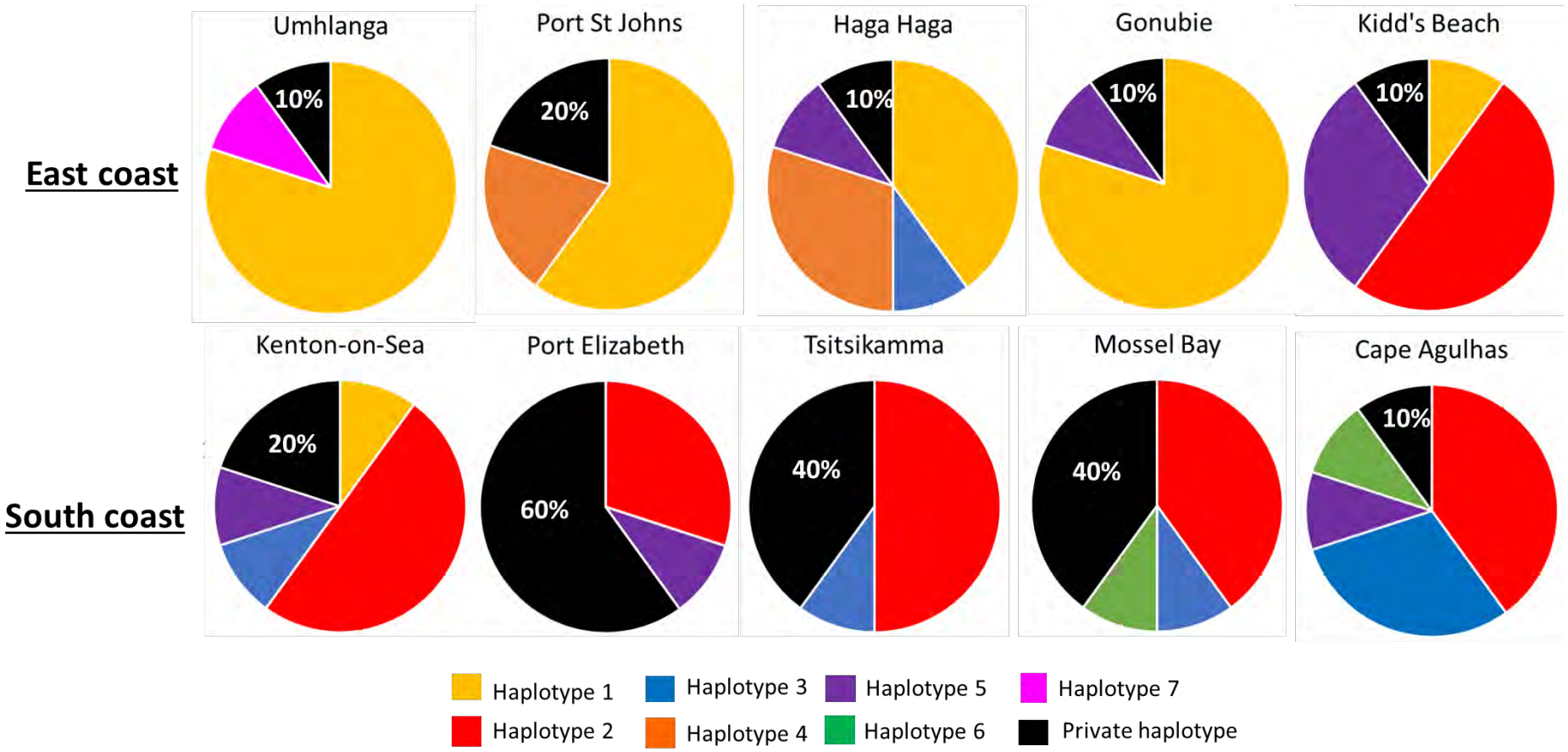


Figure 2.4: Pie chart of haplotype frequency for the 2007 mtDNA COI sequences. The colours denote haplotypes and the proportion of the private haplotypes (haplotypes only found at those sample sites) are given as a percentage.

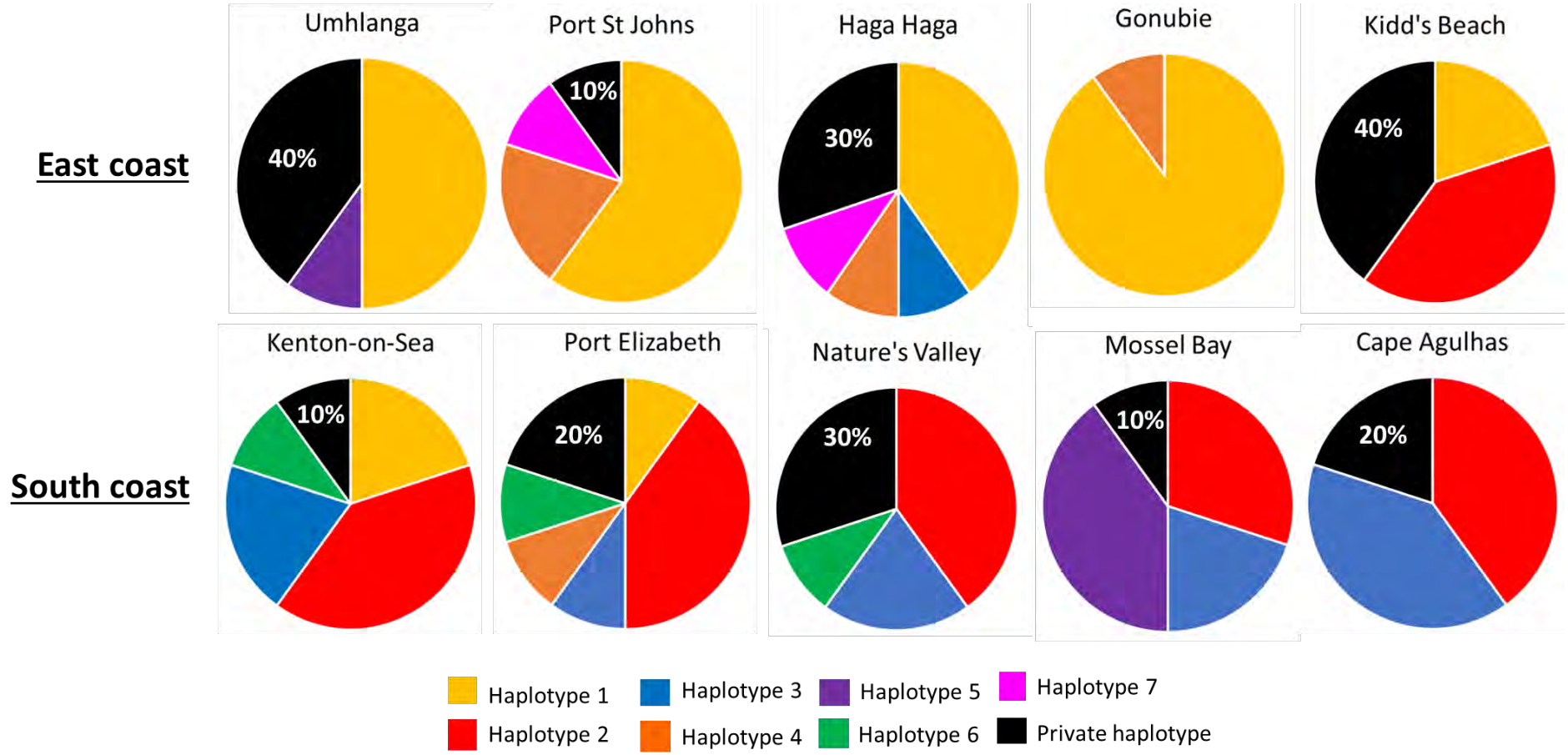


Figure 2.5: Pie chart of haplotype frequency for the 2019 mtDNA COI sequences. The colours denote haplotypes and the proportion of the private haplotypes (haplotypes only found at those sites) is given as a percentage.

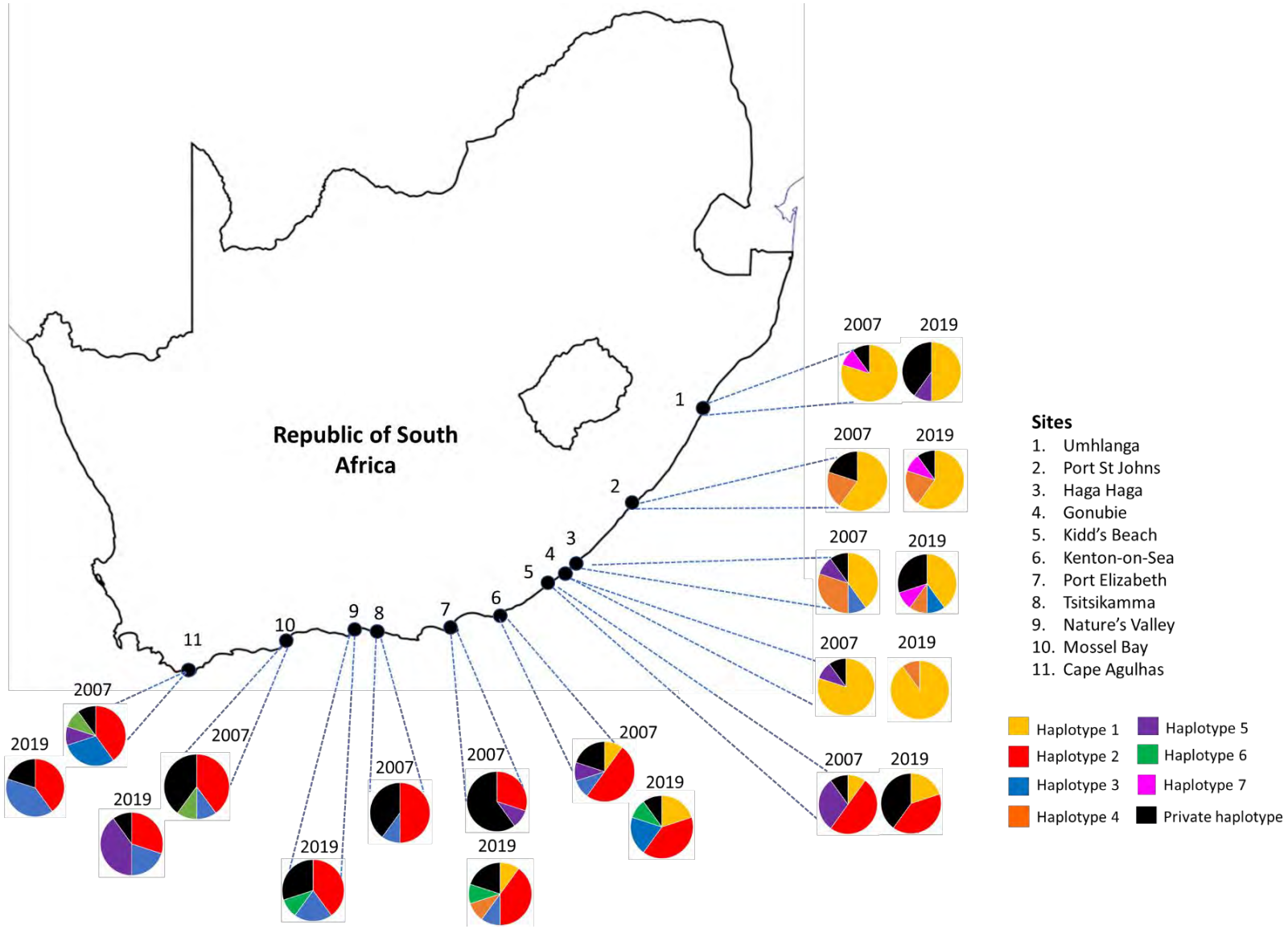


Figure2.6: Haplotype frequency from the 2007 and 2019 mtDNA COI sequences along the South African coastline.

Neutrality test

In Cluster 1 in 2007 and 2019, negative Fu's F_S value were not significant (F_S = -5.149 and -8.633 respectively, $p > 0.02$, Tables 2.1 and 2.2). Cluster 2 had significant negative Fu's F_S values (F_S = -14.960 and -8.722 respectively, $p < 0.02$ Tables 2.1 and 2.2).

Table 2.1: Fu's F_S test of neutrality on 2007 COI mtDNA sequences. n: the number of sequences, S: number of polymorphic sites, k: average number of pairwise nucleotide differences, π: nucleotide diversity, Eta(s): total number of singleton mutations, Hd: haplotype diversity. Significant values are in bold font.

Clusters	n	S	k	π	Eta(s)	Hd	Fu's FS	P-value
1	38	6	0.498	0.001	5	0.448	-5.149	**<0.05**
2	62	35	2.524	0.006	25	0.802	-14.960	**<0.02**

Table 2.2: Fu's F_S test of neutrality on 2019 COI mtDNA sequences. n: the number of sequences, S: number of polymorphic sites, k: average number of pairwise nucleotide differences, π: nucleotide diversity, Eta(s): total number of singleton mutations, Hd: haplotype diversity. Significant values are in bold font.

Clusters	n	S	k	π	Eta(s)	Hd	Fu's FS	P-value
1	42	42	0.650	0.002	8	0.520	-8.633	**<0.05**
2	58	58	2.374	0.006	20	0.785	-8.722	**<0.02**

Bayesian analysis of Population Structure (BAPS)

From the Bayesian analysis, Haplotypes 1, 4, 5, and 7 belong to the Eastern lineage and Haplotypes 2, 3, and 6 belong to the Western lineage (Tables 2.3 and 2.4) The clustering analysis showed that in 2007, the Eastern lineage was distributed from Umhlanga to Kenton-on-Sea (Fig 2.7). The Western lineage was distributed from Haga Haga to Cape Agulhas (Fig 2.7). From the 2019 sequences, the Eastern lineage was distributed from Umhlanga to Port Elizabeth and the Western lineage from Haga Haga to Cape Agulhas (Fig 2.7). Thus, the overlap region between the Eastern and Western lineages increased in range from 2007 to 2019 (Fig 2.7).

Table 2.3: Allocation of haplotypes to lineage from the 2007 COI mtDNA sequences according to the Bayesian Analysis of Population Structure (BAPS). Haplotypes 1 – 6 were found at numerous sites and Haplotypes 7 – 30 were found at a single site. Site numbers correspond to Figure 2.6.

Haplotype	Lineages	Site/s
1	Eastern	1, 2, 3, 4, 5, 6
2	Western	5, 6, 7, 8, 10, 11
3	Western	3, 6, 8, 10, 11
4	Eastern	2, 3
5	Eastern	3, 4, 5, 6, 7, 11
6	Western	10, 11
7	Eastern	1
8	Eastern	1
9	Eastern	2
10	Eastern	2
11	Western	3
12	Western	4
13	Eastern	5
14	Western	6
15	Western	6
16	Western	7
17	Western	7
18	Western	7
19	Western	7
20	Western	7
21	Western	7
22	Western	8
23	Western	8
24	Western	8
25	Western	8
26	Western	10
27	Western	10
28	Western	10
29	Western	10
30	Western	11

Table 2.4: Allocation of haplotypes to lineage from the 2019 COI mtDNA sequences according to the Bayesian analysis of Population Structure. Haplotypes 1 – 7 were found at numerous sites and Haplotypes 8 – 28 were found at a single site. Site numbers correspond to Figure 2.6.

Haplotype	Lineages	Site/s
1	Eastern	1, 2, 3, 4, 5, 6, 7, 9, 10, 11
2	Western	4, 5, 6, 7, 9, 10, 11
3	Western	2, 6, 7, 9, 10, 11
4	Eastern	2, 3, 7
5	Eastern	1, 10
6	Western	6, 7, 9
7	Eastern	2, 3
8	Eastern	1
9	Eastern	1
10	Eastern	1
11	Eastern	1
12	Eastern	2
13	Western	3
14	Eastern	3
15	Eastern	3
16	Western	5
17	Western	5
18	Western	5
19	Western	5
20	Western	5
21	Western	7
22	Western	7
23	Western	9
24	Western	9
25	Western	9
26	Western	10
27	Western	11
28	Western	11

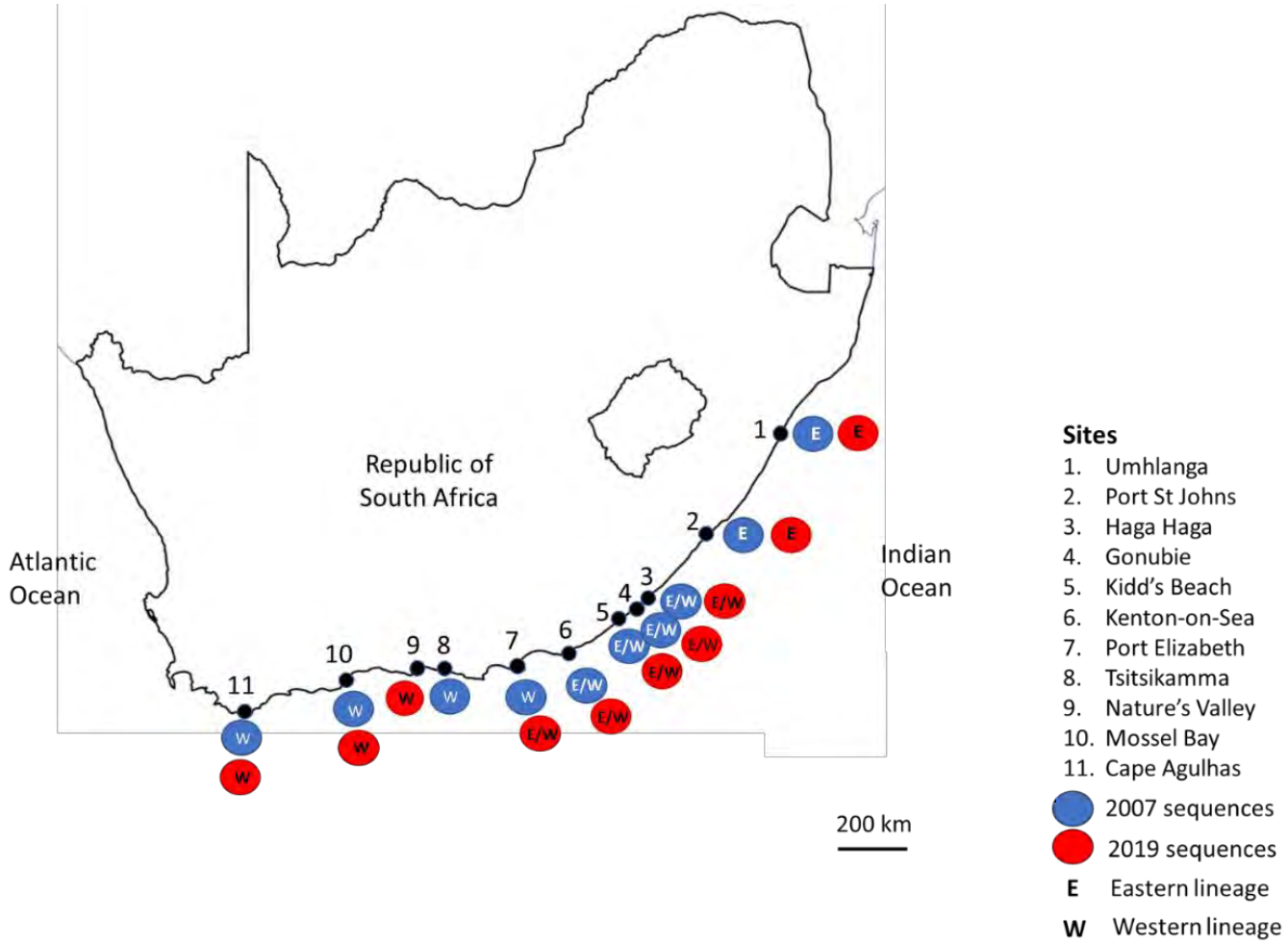


Figure 2.7: Distribution of the Eastern and Western lineages for the 2007 and 2019 sequences based on results from Bayesian Analysis of Population Structure.

Genetic diversity

There was a mean value of 55.54 % variation among the sites, and 44.46 % variation within those sampled sites (Table 2.5). There is a slight change in variation in the 2019 sequences, with 51.88 % variation among populations and 48.12 % variation within the sampled sites (Table 2.6).

Table 2.5: Analysis of Molecular Variance (AMOVA) of the 2007 sequences. This shows the genetic variation for 30 haplotypes within populations and between populations with Φ_{ST} correction. Significant values are in bold font.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	9	196.74	2.02	55.54	**<0.001**
Within populations	90	145.80	1.62	44.46	**<0.001**
Total	99	342.54	3.64		

Table 2.6: Analysis of Molecular Variance (AMOVA) of the 2019 sequences. This shows the genetic variation for 28 haplotypes within populations and between populations with Φ_{ST} correction. Significant values are in bold font.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	9	185.66	1.89	51.88	**<0.001**
Within populations	90	157.60	1.75	48.12	**<0.001**
Total	99	343.26	3.64		

From the 2007 sequences, the highest haplotype diversity was in Port Elizabeth (Hd = 0.933) and Mossel Bay (Hd = 0.867), but these sites did not have very high nucleotide diversity (π = 0.006, π = 0.006 respectively, Table 2.7). Umhlanga and Gonubie had the lowest haplotype diversity (Hd = 0.378 for both), but the nucleotide diversity differed between these sites (π = 0.001, π = 0.012 respectively, Table 2.7).

In the 2019 sequences data set, Port Elizabeth and Nature's Valley had the highest haplotype diversity (Hd = 0.867 and Hd = 0.844 respectively, Table 2.8) and the nucleotide diversity in Port Elizabeth had increased since 2007 (π = 0.006 in 2007 and π = 0.144 in 2019, Tables 2.7 and 2.8). In the 2019 sequences, Gonubie had the lowest haplotype diversity (Hd = 0.006, Table 2.8).

Table 2.7: Nucleotide diversity (π) and haplotype diversity (Hd) for the sites from the 2007 sequences.

Sites	No. of haplotypes	Haplotype diversity (Hd)	Nucleotide diversity (π)
Umhlanga	3	0.378	0.001
Port St Johns	4	0.640	0.002
Haga Haga	5	0.800	0.017
Gonubie	3	0.378	0.012
Kidd's Beach	4	0.711	0.014
Kenton-on-Sea	6	0.778	0.014
Port Elizabeth	8	0.933	0.006
Tsitsakamma	6	0.778	0.005
Mossel Bay	7	0.867	0.006
Cape Agulhas	5	0.800	0.005
Total		0.846	0.017

Table 2.8: Nucleotide diversity (π) and haplotype diversity (Hd) for the sites from the 2019 sequences.

Sites	No. of haplotypes	Haplotype diversity (Hd)	Nucleotide diversity (π)
Umhlanga	6	0.778	0.009
Port St Johns	4	0.644	0.002
Haga Haga	7	0.867	0.001
Gonubie	2	0.200	0.006
Kidd's Beach	6	0.844	0.014
Kenton-on-Sea	5	0.822	0.013
Port Elizabeth	7	0.867	0.014
Nature's Valley	6	0.844	0.048
Mossel Bay	4	0.778	0.008
Cape Agulhas	4	0.733	0.003
Total		0.845	0.017

Pairwise distances

In the 2007 COI mtDNA sequences there was a 0.019 (± 0.014 S.D.) mean p-distance difference between sequences from the Eastern, and a mean difference of 0.014 (± 0.014 S.D.) among the Western sequences (Table 2.9). There was a mean difference of 0.022 (± 0.012 S.D.) between the Eastern and Western lineages in 2007 (Table 2.9). From the 2019 data set, the Eastern lineage sequences had a mean difference of 0.011 (± 0.012 S.D.) and a mean difference of 0.009 (± 0.004 S.D.) among the Western lineage sequences (Table 2.9). The mean difference between the lineages in 2019 was 0.031(± 0.007 S.D., Table 2.9). There was no change in genetic flow between 2007 and 2019 but there was a trend towards greater genetic similarity within the lineages, resulting in the difference between the lineages becoming larger between 2007 and 2019.

Table 2.9: Mean and standard deviation uncorrected p-distance differences between and among the COI mtDNA sequences from 2007 and 2019. The table of pairwise comparison between individual sequences can be found in Tables A2.1 and A2.2.

Lineages	2007		2019	
	Mean	Standard deviation	Mean	Standard deviation
Eastern	0.019	0.014	0.011	0.012
Western	0.014	0.014	0.009	0.004
Eastern - Western	0.022	0.012	0.031	0.007

2.4 Discussion

Investigating temporal shifts in the genetic structure of marine species can have important implications for assessing the effective population size for good marine stock management (Hare et al. 2011). It can also shed light on genetic 'patchiness' for future population fitness (Larson & Julian 1999), and can be used to temper assumptions of population connectivity in high gene flow populations based on 'snapshot' spatial investigations (Sun & Hedgecock 2017). This chapter has further shown the necessity of investigating temporal genetic shifts by adding to the narrative of genetic stability overtime. This chapter aimed to compare the lineage distribution of the Eastern and Western lineages of *P. perna* between 2007 and 2019. The results from this chapter show that the Eastern lineage's distribution has shifted south. The distribution in 2007 ranged from Umhlanga to Kenton-on-Sea and in 2019, the distribution extended to Port Elizabeth.

In both haplotype networks Cluster 1 represents the Eastern lineage and Cluster 2 combined the Western lineage and overlap region (Figs 2.2 and 2.3). In 2007, Cluster 1 had a loop like structure amongst Haplotype 1 and the private haplotypes, this could indicate that there were alternative splits between Haplotype 1 and the private haplotypes in the Eastern lineage (Bandelt and Dress 1992, Fig. 2.2). The starburst pattern, i.e., low frequency haplotypes branch off from high frequency haplotypes, of Haplotypes 1, 2 and 3 shows that there has been a population expansion with these haplotypes being ancestral (Slatkin and Hudson 1991) and having multiple descendent haplotypes, e.g., Haplotype 6 is a descendent of Haplotype 2. The presence of Haplotype 5 in the Eastern and Western lineage distributions could indicate either that this is a comparatively young haplotype, or one that has been dispersed through limited wind-dispersal up the east coast from the Western lineage distribution, or one that was transported through ship's

ballast water. Overall, between 2007 and 2019, the shape of the haplotype network did not change. However, it is important to note that Cluster 1 in 2007 included Haplotype 1 from Umhlanga to Kenton-on-Sea (Fig. 2.2) and Cluster 2 in the 2019 haplotype network included Haplotype 2 from Port Elizabeth (Fig. 2.3). Interestingly, in both years, Cluster 2 included Haplotype 5 (Figs 2.2 and 2.3). Haplotype 5 is more closely related to Haplotype 3 than Haplotype 1, even though it was classified as an Eastern lineage haplotype (Tables 2.1 and 2.2) and it is likely that over time, random mutations have resulted in Haplotype 5 being more closely related to the Western lineage than the Eastern. Ntuli et al. (2020) did find Eastern lineage haplotypes in Gansbaai and Mossel Bay, which are sites within the range of the Western lineage. This shows long distance migration and could indicate that the distribution of the Eastern lineage could be shifting towards Mossel Bay.

The negative Fu's F_S of Cluster 2 in both 2007 and 2019 provide evidence of a population expansion within that Cluster between the years (Tables 2.1 and 2.2). This agrees with the narrative from Cunha et al. (2014), their findings indicated the divergence of the lineages of *P. perna* after the drying up of the Tethys seaway. The lineages diverged in the north of Africa and took time to move down either side of Africa and accumulate new/novel haplotypes on either side (Cunha et al. 2014). Cluster 2 included the Western lineage and haplotypes from the Eastern lineage which would give good reason for seeing a population expansion and an increase in divergence within the cluster.

The haplotype frequencies changed only slightly between the two years sampled. Similarities between the two years include that Haplotype 1 was most common in the east coast for both investigations but has also spread to the south coast sites by 2019 and that Haplotype 2 remained abundant on the south coast (Fig. 2.6). The spread of Haplotype 1 from the east coast to

the south coast populations could be explained by the flow of the Agulhas Current and accords with results from Zardi et al. (2015). These authors found a general east to west migration of *P. perna* microsatellite loci attributed to the direction of the Agulhas Current. Hedgecock (1986) has shown that marine species with pelagic larval phases that last about two weeks will show high levels of gene flow over time. *Perna perna* has a pelagic larval phase which lasts from two weeks to months (Hicks and Tunnell 1995) and the long larval phase *of P. perna* could influenced gene flow similarly to the result in Hedgecock (1986).

The changes in frequencies of Haplotypes 3, 4, and 5 between the 2007 and 2019 frequencies could reflect the small sample sizes, with some of these haplotypes being missed in either 2007 or 2019, rather than having disappeared from those sites (Figs 2.4 and 2.5). The change in frequency of Haplotype 5 could also be attributed to transport of mussel larvae to Umhlanga through ship's ballast water (Cariton & Geller 1993, Barry et al. 2008, Asif & Krug 2012) as Umhlanga is near a major harbour in Durban. It is recommended that a larger sample size be used in future investigations to have a better scope of the genetic profile of such an abundant species.

In the 2019 haplotype frequencies, Haplotype 7 was present at Port St Johns and Haga Haga (Fig. 2.5). There was one instance of this haplotype in the 2007 sequences in Umhlanga and it was classified as Haplotype 7 rather than a private haplotype as it showed that this haplotype has spread down the east coast. The shift in distribution of Haplotype 7 to Port St Johns and Haga Haga indicates long distance dispersal of haplotypes from northern-eastern populations. It would be interesting to investigate the genetic profile of *P. perna* again in the future and see how far Haplotype 7 has dispersed over time.

While there were only slight differences in haplotype frequencies between 2007 and 2019 (Figs 2.3 and 2.4), there does seem to be a shift in the lineage distributions between 2007 and 2019

(Fig. 2.7). In 2007, the overlap region was between Kidd's Beach and Kenton-on-Sea, but the region has shifted to between Kenton-on-Sea and Port Elizabeth, that would mean the distribution of the Eastern lineage has shifted to the south-west (Fig. 2.7), which would accord with the direction of flow of the Agulhas Current. The BAPS analysis has also shown that Haplotype 3 is specific to the Western lineage and this haplotype ranges up the east coast to Haga Haga (Table 2.1, Fig. 2.7). It is likely that Haplotype 3 was introduced to Haga Haga through limited wind-driven dispersal against the flow of the Agulhas Current (McQuaid and Phillips 2000, Zardi et al. 2015). It is important to note that any differences in results for 2007 and 2019 could be due to the small sample sizes used. Small sample sizes raise the possibility that some haplotypes were missed by chance, and it would be beneficial that future research of the haplotype diversity of *P. perna* includes a larger samples size. Ntuli et al. (2020) used a sample size of 50 sequences from each site the authors investigated. The larger sample size and the use of microsatellite data, which gives an indication of higher variation between the lineages, was beneficial as it showed 3 clusters in their STRUCTURE analysis of the lineages whereas this investigation only found two clusters. Overall, the results from the BAPS analysis suggest that there was a shift in the distribution of the Eastern lineage from 2007 to 2019 but that the genetic lineages seem to have retained spatial stability.

There were only slight differences in haplotype diversity between the 2007 and 2019 sequences. The haplotype diversity in Umhlanga increased from 2007 to 2019 (Tables 2.6 and 2.7) and this is thought to be due to migration of haplotypes from northern populations into Umhlanga since 2007. Port Elizabeth showed the greatest genetic diversity in both 2007 and 2019 (Tables 2.6 and 2.7). This could be due to the commercial harbour at Port Elizabeth, as the ballast water from

ships coming in could influence gene flow between lineages and introduce haplotypes from both lineages to this site (Cariton & Geller 1993, Barry et al. 2008, Asif & Krug 2012).

The pairwise comparison of haplotypes within years showed that there was no reduction in gene flow among the sites but the increase in difference between private haplotypes and shared haplotypes at some sites could indicate self-recruitment leading to genetic isolation of specific haplotypes and thus increased genetic difference between shared and private haplotypes. It would have be expected that the highest p-distance differences in the pairwise analysis would have been between private haplotypes from sites situated in bays rather than the open coast. Nicastro et al. (2008) found that there was more haplotype endemism in *P. perna* in bays than on the open coast whereas in this study the private haplotypes (e.g., Haplotype 25) that showed the most difference from others were usually from open coastal sites (Tables A2.1 and A2.2).

The dynamic change in genetic distinction seen in this investigation concurs with other studies focussed on temporal genetic drift in marine ectotherms. Riquet et al. (2016) investigated genetic drift over nine years in the invasive mollusc, *Crepidula fornicata.* They found significant genetic drift over time and changes in allele frequencies, results that were attributed to widespread larval dispersal and recruitment (Riquet et al. 2016). It is speculated that the same result was seen in this investigation due to *P. perna* also having a long pelagic larval phase (Lasiak & Barnard 1995) and the direction of the Agulhas Current assisting dispersal of larvae.

Another economically important mollusc on the South African coastline, *Haliotis midae* (abalone), showed a significant temporal genetic shift (Rhode et al. 2017) similar to this investigation. Rhode et al. (2017) attributed the genetic shifts in *H. midae* to changes in selective pressures over time, such as changes in fisheries management (Rhode et al. 2017). However, *P. perna* faces fishing pressures primarily from subsistence fisheries (Berry and Schleyer 1983) and

it is more likely that the change in the distribution of the Eastern lineage is linked to changes in sea surface temperature and natural long-term dispersal.

This Chapter has shown that there has been only a slight shift in the distribution of the Eastern lineage onto the south coast since 2007 and shows decadal stability in the phylogeography of *P. perna* on the east and south coasts of South Africa. It is likely that this shift in distribution is due to the flow of the Agulhas Current and might also be a result of the warming sea temperatures on the south coast.

Chapter 3: Reproductive isolation between two lineages of *Perna perna*

3.1 Introduction

Synchronised reproductive cycles within species are key to the evolution of marine invertebrates with complex life cycles, such as bivalves, providing the vital selective benefits of maximising larval production and survival (Olive 1995). A clear understanding of variations in the temporal reproductive patterns of distinct populations is essential to the evaluation of the spatial structure and dynamics of the species under environmental fluctuations such as global climate change (Pauls et al. 2013, Emery & La Rosa 2019, Rilov et al. 2019). This knowledge can only be gained from the robust assessment of heterogeneity in a species' reproductive patterns across broad geographical scales covering the environmental gradients and clines across the species' distributional range (Hanski 1982). Data on the seasonal trends of intraspecific reproductive patterns across populations are particularly relevant for habitat-forming species that play a crucial role in maintaining local and regional biodiversity (Lemieux & Cusson 2014).

Within a species, a variety of adaptive and plastic traits coordinate reproductive events with the environment to maximise reproductive success. Although the reproductive patterns of many bivalves have been investigated (Newell et al. 1982, Pouvreau et al. 2000, Kang et al. 2006), we still have a limited understanding of the intricate relationships between exogenous (e.g., food availability, temperature, salinity) and endogenous (e.g., genotype) factors that control the initiation and duration of the various reproductive stages. Further, synchronised reproduction might represent an evolutionary trait of mussels to promote gene flow amongst populations and to prevent bottlenecking in isolated populations.

Looking at endogenous factors that might affect bivalve spawning; if mussels, for example, were to coordinate spawning events with environmental conditions favourable for mussel larvae, this could serve to maximise reproductive success (Newell et al. 1982, Starr et al. 1990). If mussels spawn according to environmental conditions, this could mean that the recruited propagules are particularly fit and can lead to high recruitment rates. This is beneficial as biogeographic population limits are influenced by recruitment rates rather than by spawning productivity (McQuaid & Payne 1998). If an area has low primary production during larval development before spawning, there will be a decrease in recruitment due to larval mortality or poor larval quality regardless of spawning output. There have been numerous attempts, to correlate mussel reproductive behaviour with external environmental conditions, such as temperature (Myrand et al. 2000, Eads et al. 2016), food availability (Newell et al. 1982, Thorarinsdóttir et al. 2013, Sreedevi et al. 2014, Asaduzzaman et al. 2019), and photoperiod (e.g. Domínguez et al. 2010). But temperature and food availability have been deemed the most critical environmental conditions to understand the timing of spawning (Aji 2011). This is a promising avenue to follow as it gives insight into the conditions that may affect wild populations of mussels and thus provide information behind fluctuations in population abundance.

While there is ample knowledge of the effects of environmental conditions on reproductive output, however, there is a school of thought that invertebrates that share the same shore or habitat must maintain biological clocks to have synchronised spawning across populations regardless of the influence of ecological conditions (Mercier & Hamel 2010). By isolating reproduction to an event that does not overlap with reproduction in co-occurring species, hybridisation can be reduced or avoided, and genetic purity maintained. This behaviour has been seen in co-occurring mussel species: *Aulacomya ater* and *Choromytilus meridionalis*. When collected from the same shore and

exposed to the same environmental conditions, they spawned during different months over the same time period (van Erkom Schurink & Griffiths 1991). Thus, it is thought that endogenous factors influences mussel reproductive timing and maintain reproductive isolation between conspecifics (Himmelman et al. 2008).

Beds of the brown mussel *Perna perna* are a conspicuous feature of intertidal systems in Africa. *Perna perna*, is a species widely distributed around the African continent (Fig. 1.2). In the East, it occurs through the Gulf of Aden into the Red Sea, along the west coast of Madagascar, and from Mozambique to False Bay on the southern African coastline (Fig. 1.2). In the west, it extends along the west coast of Africa, except for a patchy distribution in the upwelling influenced Benguela region (Zardi et al. 2007b, 2011), from the Cape of Good Hope through the Strait of Gibraltar to the Gulf of Tunis (Fig 1.2, Wood et al. 2007). This species also appears on the coast of Sri Lanka, southern India and in the Atlantic coast of South America in Venezuela, Uruguay, and Brazil, and the West Indies (Fig 1.2, Berry 1978, Vakily 1989, Wood et al. 2007, Gardner et al. 2016). In areas of the Gulf of Mexico, and east coast of southern Mexico, *P. perna* has been classified as an invasive species, and it owes its introduction to transportation through ballast water (Hicks & Tunnell 1993). The invasive status of *P. perna* has been classified as 'low priority' on the coasts of Western Australia but no information on the actual distribution on this coastline has been published at present (Hayes et al. 2005). *Perna perna* has been reported on the Portuguese coast recently and this is thought to have been due to a range extension from North African shores (Lourenço et al. 2012).

In South Africa, *P. perna* densely populates rocky shores of the east coast and competes for space with *Mytilus galloprovincialis* on the south coast (Berry 1978, van Erkom Schurink & Griffiths 1991). *Perna perna* is usually associated with wave-exposed conditions and occupies the

rocky shore from the lower balanoid zone to a depth of 5 m (Lasiak 1986). Zardi et al. (2007b) determined that the population of *P. perna* on the South African coastline falls into two distinct genetic lineages. According to Zardi et al. (2007b), the two lineages have a distinct overlap region that extends from Haga Haga to Kenton-on-Sea in the Eastern Cape (Fig. 1.1). However, the distribution of the Eastern lineage seems to have shifted since 2007 to Port Elizabeth (Chapter 2) and within the lineage overlap region, there are instances of hybridisation between the lineages (Zardi et al. 2015).

Despite several studies that have investigated the reproductive patterns of *P. perna*, a definitive spawning season remains unclear. The studies that have investigated spawning profiles of populations of *P. perna* across its distribution in South Africa have shown that there are distinct differences in the timing of spawning peaks in distinct regions and in relation to environmental conditions regardless of lineage (van Erkom Schurink & Griffiths 1991, Ndzipa 2002, Zardi et al. 2007a). Zardi et al. (2007a) and van Erkom Schurink & Griffiths (1991) showed a positive correlation between spawning and temperature on the south coast, while a negative correlation between spawning and temperature was seen on the east coast (van Erkom Schurink & Griffiths 1991). Specifically, these studies showed a major reproductive event between September and January or January to March, and a minor event between March to June or between June and July (van Erkom Schurink & Griffiths 1991, Zardi et al. 2007a).

3.1.1 Aims and Hypotheses

Here, the aim was to test for possible synchronisation of spawning over five sites on the South African coast and to determine if differences in reproductive output are related to genetic

identity. This chapter also aimed to determine if there is a correlation between the timing of reproduction and temperature. It was hypothesised that the Eastern and Western lineages will have differences in reproductive timing with peak spawning occurring during different months, which allows for the persistence of the overlap region on the South Africa coastline. It was also hypothesised that reproductive timing is positively correlated with favourable temperature conditions that populations experience during their reproductive cycle.

3.2 Materials and Methods

This study was initially planned to run from September 2019 to August 2020. However, due to the South African government declaring a National State of Disaster due to the SARS-COVID-19 pandemic and the country going into an extended lockdown, the fieldwork was interrupted in March 2020. This investigation was carried out at five sites along South Africa's coastline. In addition, at all five sites, temperature was recorded. The sites were chosen based on the known distribution of the two *P. perna* lineages present (Zardi et al. 2007b) hereafter referred to as Eastern and Western lineages (Fig. 3.1). Two sites were selected within the distribution of the Eastern lineage: Port Edward (31˚03'45.6" S, 30˚13'22.5" E) and Port St Johns (31˚38'55.8" S, 29˚31'17.1" E); and two sites within the distribution of the Western lineage: Nature's Valley (33˚59'08.6" S, 23˚32'54.6") and Mossel Bay (34˚10'52.1" S, 22˚09'28.8" E, Fig. 3.1). An additional fifth site was located in the transition area where the two lineages overlap in distribution. At this site (Old Woman's River (33˚28'56.6" E, 27˚09'05.6" S)), a manipulative transplant experiment was set up.

3.2.1 Field surveys of natural populations

At each of the four lineage abundant population sites (Port Edward, Port St Johns, Nature's Valley, Mossel Bay), quadrats (n = 3; 15 cm x 15 cm) were haphazardly placed on the shore within the mussel zone every month. Mussels (n = 10, 3 – 6 cm in shell length) were collected from each quadrat each month and preserved in ~98% ethanol (n = 30 per site, per month).

In the laboratory, the shell length of each individual was measured to the nearest mm with a pair of callipers (GRIP GV 9370). The soft tissue was excised from the shells with a sterile

surgical scalpel blade and dried at 60 °C in glass vials for 48 h in an oven (SMC, no: 11/07/006). Dried tissue was weighed to the nearest mg using an analytical balance (Mettler Toledo, MS105DU).

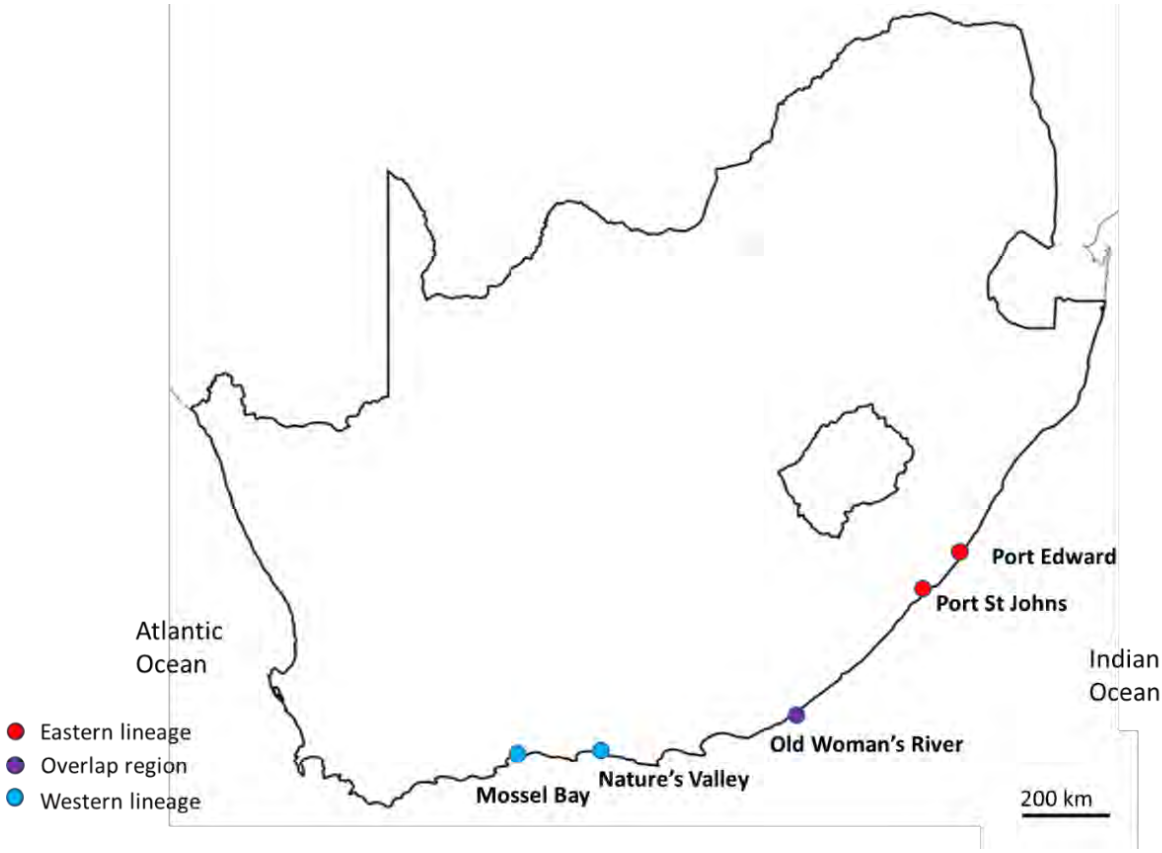


Figure 3.1: Fives sites used for this investigation. The sites where the lineages exist naturally in isolation were Port Edward and Port St Johns (Eastern lineage, red dot), and Nature's Valley and Mossel Bay (Western lineage, blue dot). The lineages co-occur at Old Woman's River (purple dot).

3.2.2 Temperature measurements

At each site, three temperature loggers encapsulated in hard acrylic (EnvLogger variety C, ElectricBlue, CRL, 21 mm x 18 mm x 10 mm) were deployed using Splash Zone 2-Part Epoxy Compound A788. The loggers were placed haphazardly within the low to mid-mussel zone (approximately 1 – 2 m from the low tide mark) to measure water temperature from August 2019

to March 2020. The loggers were set to capture temperature every hour with 0.1 °C resolution. The water temperature data were separated from air temperature data by comparing the logged times with tide charts for each site (obtained from https://tides4fishing.com) and selecting the water temperatures (five values per high tide, twice a day) where the loggers would be underwater during high tide.

3.2.3 Manipulative transplant experiment

The two lineages of *P. perna* cannot be identified morphologically and thus, to determine if the lineages spawn at different times in the overlap region, a manipulative transplant experiment was set up.

In August 2019, before the beginning of the experiment, approximately 1000 individuals of each of the Eastern and Western lineages were collected from Port St Johns and Nature's Valley respectively (Fig. 3.1). Mussels were kept moist and cool in insulated boxes with travel times to the laboratory of between four and six hours. In the laboratory, mussels were kept in aerated aquaria with unfiltered seawater at 21 °C for one to two days before deployment. The collection and translocation of the mussels was done over the same spring tide period.

Stainless steel quadrats (15 cm x 15 cm) were installed on the rocky shore at Old Woman's River within the mid-mussel zone. Mussels (n = 30) were placed inside each quadrat and kept in place with plastic netting (mesh size =1.5 cm x 1.5 cm) and cable ties. Quadrats (n = 21 per lineage) were randomly assigned to each lineage to avoid spatial bias. From September 2019 (one month after deployment) to March 2020, a subset of mussels (n = 10) was collected from three quadrats monthly (n = 30 per lineage).

In the laboratory, mussels were measured, and the dried tissue was weighed as above (n = 30 mussels per month, per lineage).

3.2.4. Statistical analysis

The statistical analyses were performed in R Studio v1.3.1073 (R v3.6, R Core Team 2013). The dry body weight data from all five sites were plotted on a histogram to determine the distribution (Appendix, Fig. A3.1a) and $\log_{10}$-transformed. The $\log_{10}$-transformed data fitted the assumptions of normal distribution (Appendix, Fig. A3.1b) and were treated as a parametric data set. The $\log_{10}$-transformed body weight data were regressed against $\log_{10}$-transformed shell length for each month per site (Appendix, Fig. A3.2, n = 30), and the predicted weight of a standard 60 mm individual read from the regression equation (Berry 1978, Grant & Tyler 1983, van Erkom Schurink & Griffiths 1991). The $\log_{10}$-transformed predicted body weight data were plotted against time (in months) to provide an index of temporal cycles of gamete accumulation and gamete release using "ggplot2" (Wickham 2016).

A three-way nested ANCOVA was used to explore the differences in reproductive output among the four Sites (Port Edward, Port St Johns, Nature's Valley, Mossel Bay) (n = 30 per site, per month). Port Edward and Port St Johns were Eastern lineage abundant Sites and Nature's Valley, and Mossel Bay Sites where the Western lineage was abundant. The $\log_{10}$-transformed body weight (mg) was used as the dependent variable with the $\log_{10}$-transformed shell length (mm) as the covariate with Lineage (fixed, two levels: Eastern and Western), Month (fixed, seven levels: September 2019 – March 2020), and Site (nested in lineage, random, four levels: Port Edward, Port St Johns, Nature's Valley, Mossel Bay) as factors, followed by a Tukey HSD post-hoc test (Tukey 1949). A two-way ANCOVA was performed on the $\log_{10}$-transformed body weight

(dependent variable) and $\log_{10}$-transformed shell length (covariate) data collected from Old Woman's River with Lineage (fixed, two levels: Eastern and Western) and Month (fixed, seven levels: September 2019 - March 2020) as factors.

The average monthly water temperature for each site was plotted over time and analysed using two-way ANOVA with Site (fixed, five levels: Port Edward, Port St Johns, Old Woman's River, Nature's Valley, Mossel Bay) and Month (fixed, seven levels: March 2019 – September 2020) as factors. A cross-correlation analysis was performed between the average monthly water temperature and monthly mean $\log_{10}$-transformed body weight for each site, with a separate analysis done for each lineage at Old Woman's River.

3.3 Results

3.3.1 Reproductive Isolation – single lineage sites

There was very little synchrony among sites in spawning events suggesting very little consistency among sites in minor or major spawning events. For example, the Mossel Bay population had a major spawning event between September and October 2019 whereas Nature's Valley was possibly going to have a major spawning event between March and April 2020 (Fig. 3.2). Port Edward and Port St Johns did not show a clear major spawning event, the data suggesting protracted rather than pulsed spawning.

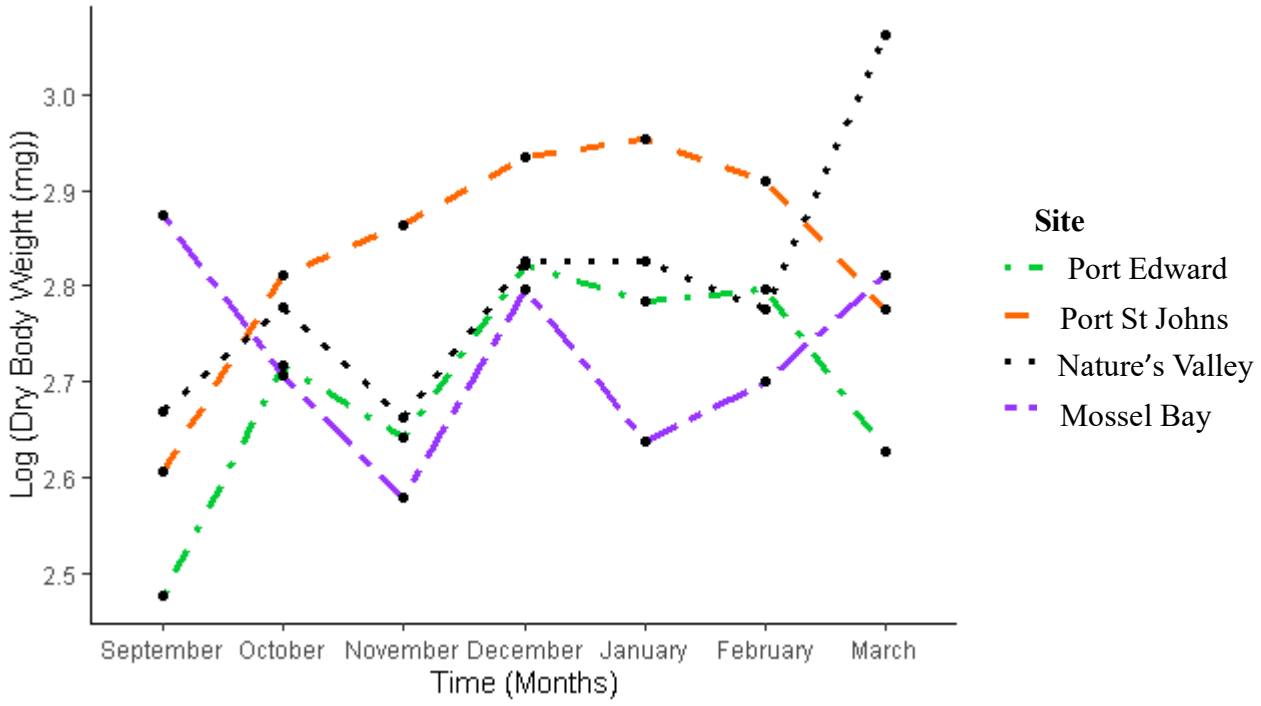


Figure 3.2: Temporal variation in $\log_{10}$-transformed body weight (mg) of a standard 60 mm mussel at each location from September 2019 to March 2020. Potential spawning periods are indicated by an abrupt drop in weight between consecutive months.

Log_{10}-transformed shell length had a significant effect on log_{10}-transformed dried tissue weight in all cases (Shell length: $F_{(1,811)} = 889.76$, $p < 0.0001$; Table 3.1). The reproductive cycle differed between lineages (Lineage x Month: $F_{(6,811)} = 40.21$, $p < 0.0001$; Table 3.1) indicating lack of synchrony between the two genetic entities. The post-hoc Tukey HSD analysis showed that there were significant differences in log_{10}-transformed body weight among all sites rather than between lineages (post-hoc, Table 3.2).

Table 3.1: Three-way nested ANCOVA of log_{10}-transformed body weight (mg) of *Perna perna* across four lineage abundant sites with interaction between Lineages, Sites (nested in lineage), and Months. Significant p-values are in bold font.

	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	P-value
Lineage	1	0.136	0.136	14.5	**0.0002**
Month	6	4.745	0.791	84.39	**<0.0001**
Shell length	1	8.339	8.339	889.76	**<0.0001**
Site (Lineage)	2	4.296	2.148	229.19	**<0.0001**
Lineage x Month	6	2.261	0.377	40.21	**<0.0001**
Residuals	811	7.600	0.009		

Table 3.2: Post-hoc Tukey HSD analysis of log_{10}-transformed body weight (mg) of *Perna perna* between lineage abundant sites with adjusted p-values using Bonferroni correction. Significant adjusted p-values are in bold font.

Sites		Difference in observed means	95% confidence interval		Adjusted p-values
			Lower	Upper	
Port Edward	Nature's Valley	-0.110	-0.14	-0.092	**<0.0001**
	Mossel Bay	-0.034	-0.058	-0.0097	**0.0019**

Port St Johns	Port Edward	0.150	0.13	0.17	**<0.0001**
	Nature's Valley	0.034	0.0097	0.058	**0.0019**
	Mossel Bay	0.110	0.092	0.14	**<0.0001**
Nature's Valley	Mossel Bay	0.082	0.058	0.11	**<0.0001**

3.3.2 Reproductive Isolation - Old Woman's River

The Western lineage decreased in body mass between September and October 2019, indicating a minor spawning event, most likely caused by translocation stress. The two lineages experienced a major spawning event between December 2019 and February 2020.

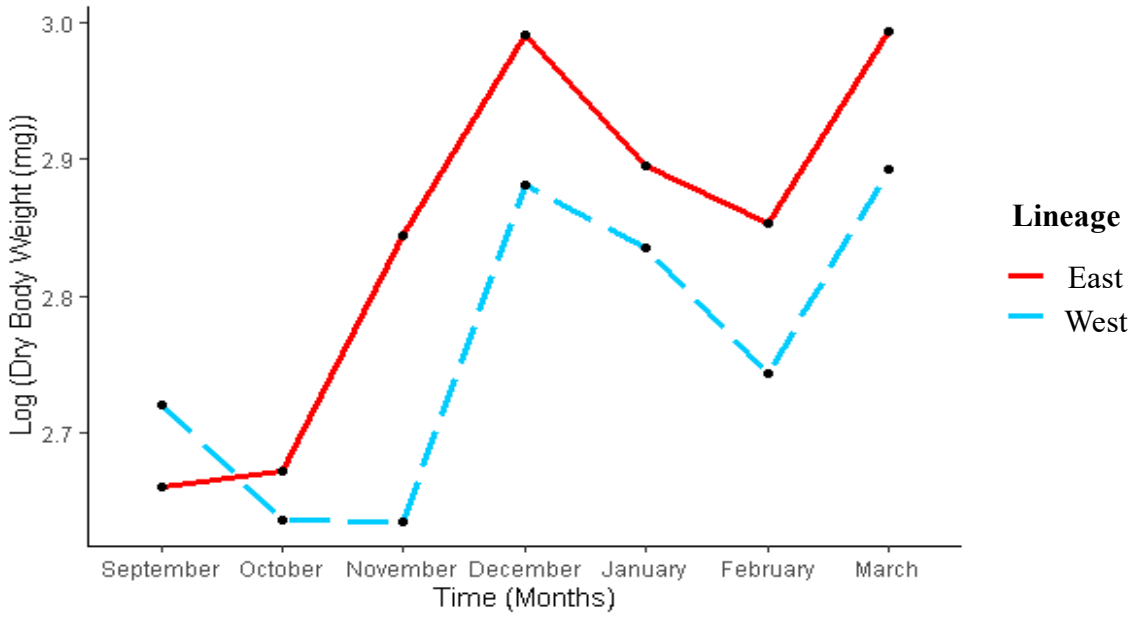


Figure 3.3: Body weight of two lineages of *Perna perna* on Old Woman's River from September 2019 to March 2020. A sudden drop in weight between months can be interpreted as a spawning event.

There was significant effect of $\log_{10}$-transformed shell length on $\log_{10}$-transformed body weight ($F_{(1,405)} = 487.13$, $p < 0.05$, Table 3.3). The interaction between Lineage and Month ($F_{(6,405)} = 7.43$, $p = 0.003$ (Table 3.3)) reflects the fact that the Eastern lineage's body mass increased from September to October 2019, while the Western lineage decreased, the Western lineage then maintained body mass while the Eastern lineage increased between October to November 2019. After November, the two lineages show very similar patterns in body mass fluctuations from month to month (Fig.3.3).

Table 3.3: Two-way ANCOVA of $\log_{10}$-transformed body weight (mg) of Eastern and Western lineages of *Perna perna* at Old Woman's River. Interaction was between Lineages and Months. Significant p-values are in bold font.

	Degrees of Freedom	Sum of Squares	Mean sum of Squares	F-value	P-value
Lineage	1	0.05	0.05	4.65	**0.0316**
Month	6	6.87	1.15	103.08	**<0.0001**
Shell length (mm)	1	5.41	5.41	487.13	**<0.0001**
Lineage x Month	6	0.49	0.08	7.43	**0.0003**
Residuals	405	4.49	0.01		

3.3.3 Temperature across sites

Across all sites, the range of temperatures was minimal in spring (August or September), with greater within-month variability in summer. Although the broad pattern was similar among sites, there were important differences, with some sites showing strong seasonality while others did not, leading to a significant Site x Month interaction (Month x Site: $F_{(24,70)} = 15.61$, $p = <0.0001$, Table 3.4).

Highest temperatures were recorded at Port Edward (26.8 °C) followed by Port St Johns (25.8 °C). Lowest temperatures were recorded Nature's Valley (10.8 °C) followed by Old Woman's River (12.5 °C). The greatest temperature variation within months was recorded at Port St Johns (Fig. 3.5) where temperatures ranged from 13.6 °C to 25. 8 °C between December 2019 to January 2020 (Fig. 3.5).

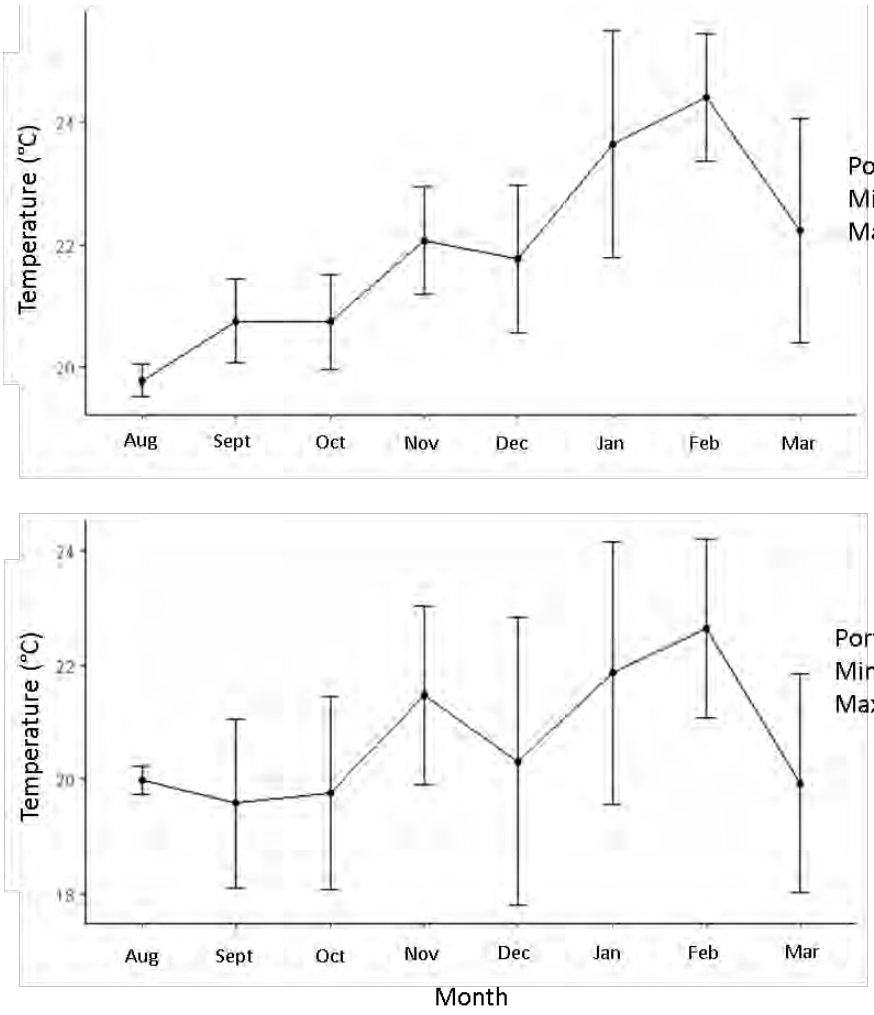
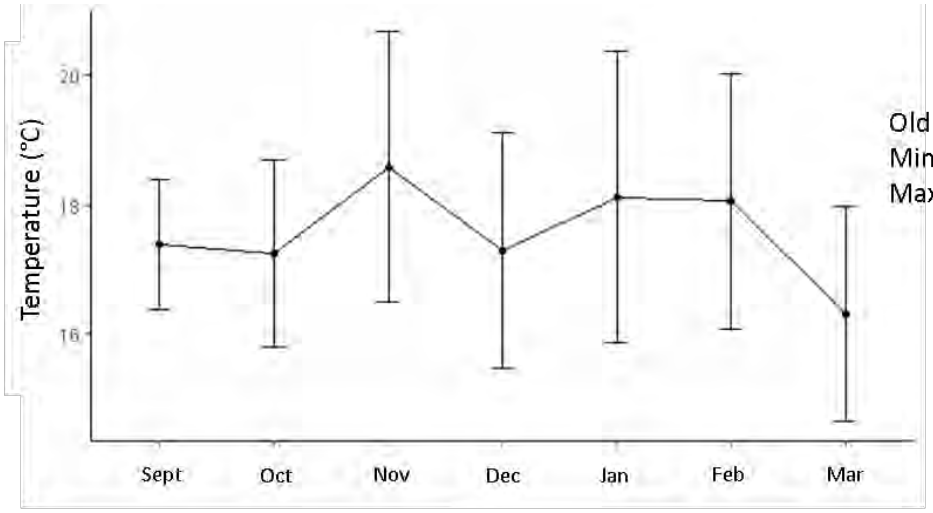
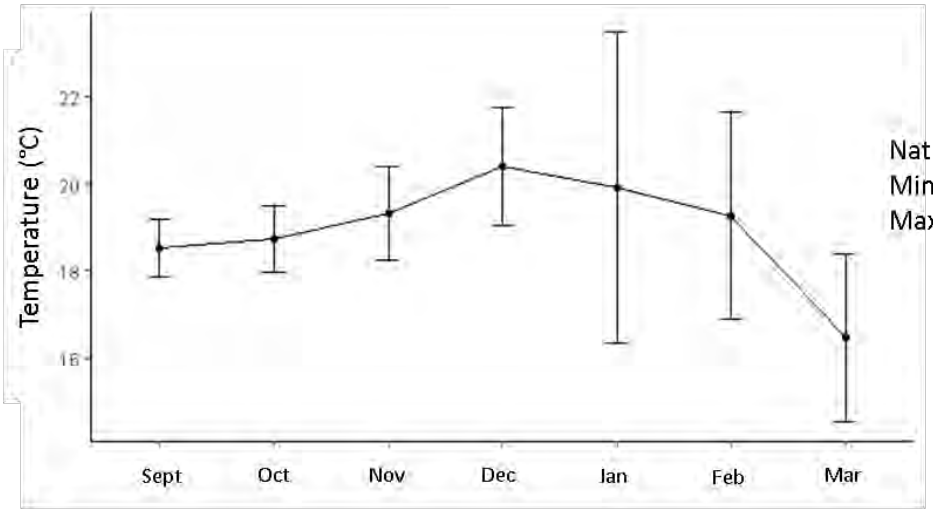


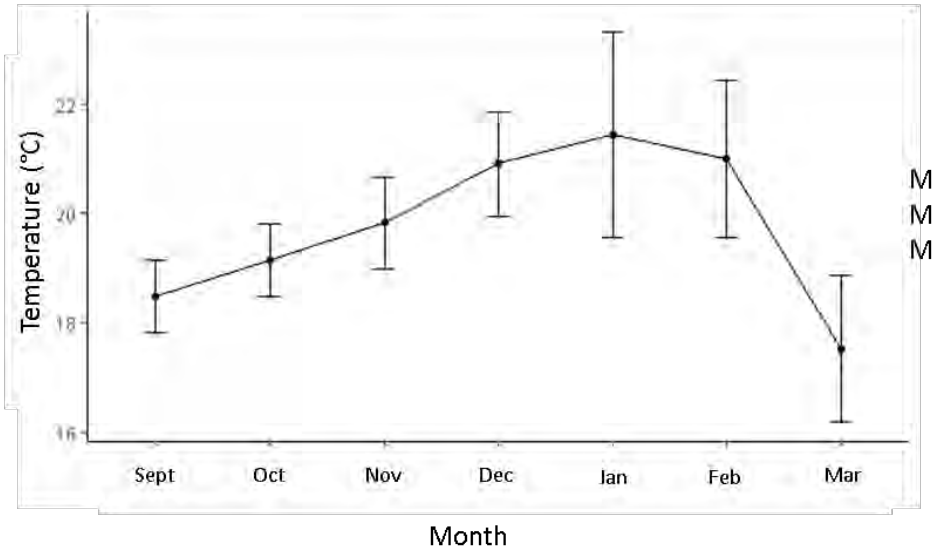
Figure 3.4: Average (±SD error bars) monthly water temperature from four lineage abundant sites (Port Edward, Port St Johns, Nature's Valley, Mossel Bay) and manipulative transplant site (Old Woman's River).



Old Woman's River
Min temperature: 12.5°C (March 2020)
Max temperature: 22.8°C (Jan 2020)



Nature's Valley
Min temperature: 10.8°C (March 2020)
Max temperature: 24.8°C (Jan 2020)



Mossel Bay
Min temperature: 14.1°C (March 2020)
Max temperature: 24.9°C (Jan 2020)

Figure 3.4 continued.

Table 3.4: Two-way ANOVA of monthly water temperature (°C) across all sites with interaction between sites and months. Significant p-values are in bold font.

	Degrees of freedom	Sum of Squares	Mean sum of squares	F-value	p-value
Site	4	235.57	58.89	537.65	**<0.0001**
Month	6	105.81	17.63	161.00	**<0.0001**
Site x Month	24	41.04	1.71	15.61	**<0.0001**
Residuals	70	7.67	0.11		

There was significant positive correlation between water temperature and $\log_{10}$-transformed body weight only at Port Edward with a one-month lag (Lag = 1, h = 0.742, Table 3.5).

Table 3.5: Auto-correlation function between monthly water temperature and $\log_{10}$-transformed body weight across five sites from September 2019 to March 2020. Significant correlation values are in bold font. The Auto-correlation function graphs can be found in Figure A3.3.

Site	Lag	Correlation
Port Edward	1	**0.742**
Port St Johns	1	0.601
Old Woman's River (East Lineage)	2	-0.722
Old Woman's River (West Lineage)	2	-0.342
Nature's Valley	0	-0.485
Mossel Bay	0	-0.531

3.4 Discussion

The data collected show an interesting trend in reproductive isolation between two lineages of *P. perna* on the South African coastline over five sites. This investigation showed that the reproductive cycle at Nature's Valley and Mossel Bay are similar to results from Van Erkom Shurink and Griffiths (1991) with two distinctive reproductive peaks during the annual cycle; one between September and January and one between March and June (Fig. 3.2). The second body mass peak indicated possible spawning after March, and this seems to have been the major reproductive event for *P. perna* in the context of this study. The two more easterly sites did not show this trend of two peaks during the annual cycle. The population at Port Edward seemed to have two peaks between September and January, while the population at Port St Johns exhibited a bell curve like pattern of body mass, with a spawning peak in January and a downward trend in March (Fig. 3.2). The patterns observed in Port Edward and Port St Johns are likely due to "trickling" spawning, where the spawning season is made up of a collection of small spawning events (Kinlan & Gaines 2003, Knights et al. 2006). Such non-synchronous or "trickle" spawning could be due to the higher temperatures experienced at Port Edward and Port St Johns as spikes in temperature can induce spawning (Sreedevi et al. 2014). This investigation does show that locality of mussel populations affects reproductive timing since the manipulative transplant experiment has shown that, when exposed to the same environmental conditions, the two lineages exhibited similar patterns of change in body mass with a peak in December and a possible second peak in March. An obvious *caveat* in this investigation is the limitation of data and a full data set would have allowed for a clearer interpretation.

It is not clear that the difference in spawning profiles was influenced as strongly as expected by the temperature conditions experienced at each site. Water temperature has been

identified as having an influence on mussel reproductive trends (Myrand et al. 2000, Eads et al. 2016) but the different reproductive profiles observed could only be partially explained by the water temperatures experienced at each site. Vélez and Epifanio (1981) showed that gametogenesis is inhibited when *P. perna,* from the Western lineage, were exposed to temperatures above 21 °C, although somatic tissue growth proceeds even when mussels were exposed to temperatures above 28 °C (Vélez & Epifanio 1981). This could explain the spike in body mass at Nature's Valley between February and March 2020 (Fig. 3.2). The Nature's Valley population experienced temperature means closer to 21°C leading up to the major spawning event (18 ± 2°C – 19 ± 4 °C, Fig. 3.4), which are better conditions for reproduction in *P. perna* from the Western lineage (Vélez & Epifanio 1981). In contrast, Sreedevi et al. (2014) have shown that a sudden spike in temperature can induce spawning in *Perna viridis* and it is likely that if this study collected data at a higher resolution, such as biweekly rather than monthly, spawning as result of sudden changes in temperature might be observed in *P. perna*. Nevertheless, only one sites showed a significant correlation between sea temperature and body mass a month later (Table 3.5).

The genetic distinction between the lineages does seem to influence how they respond to the temperatures faced at the different sites. Using the higher body mass as a proxy of body condition, the Eastern lineage seems to prefer cooler and more nutrient abundant conditions on Old Woman's River compared to conditions in Port Edward and Port St Johns (Figs 3.2 and 3.3). While the Western lineage was more productive between September 2019 and January 2020 at Old Woman's River, the reproductive output seems to be most significant at Nature's Valley for the March peak (Figs 3.2 and 3.3). The higher temperatures at Old Woman's River when compared to Nature's Valley (Fig. 3.4) may have caused a stress response that reduced reproductive output. When stressed, mussels will allocate fewer resources to growth and reproduction (Petes et al.

2008b). This investigation has shown that the lineages react to the same environmental conditions through different levels of body mass (Fig. 3.3). However, with fluxes of environmental conditions, reproductive peaks can likely change annually from month to month. It would be recommended that these results be expanded on by repeating this experiment and running the experiment for longer than one annual cycle to illustrate better how the fluctuations in environmental conditions can influence peaks in reproductive output. A more direct evaluation of reproduction, such as separate weighing of soma and gonads is also recommended.

The two lineages of *P. perna* might be genetically distinct based on the results from Zardi et al. (2007b) and Chapter 2, but this investigation has found that the environmental conditions, such as sea temperature, play more of a significant role on reproductive timing than genetic makeup.

Chapter 4: Discussion

Climate change can significantly affect species' genetic structure (Pauls et al. 2013). Genetic variations within and among populations is the result of the interactions of gene flow, and selection, involving larval dispersal ability, reproductive timing, thermal adaptation, climate change related disturbances, e.g., heat waves and increasing temperatures (Lowe & Allendorf 2010, Davies et al. 2016). Resilience to climate change is dependent on how a species' genetic diversity allows it to respond to changes in environmental trends (Sintayehu 2018).

High levels of genetic diversity at the population level typically manifests as high levels of heterozygosity and helps species to resist environmental changes (Jump et al. 2009). A model simulation has shown that a species with low heterozygosity, but a large distribution will show differing patterns in heterozygosity among populations over time depending on the severity and frequency of disturbance (Davies et al. 2016). On the other hand, taxa with limited distributions that experience severe and frequent disturbances will experience a rapid decline in heterozygosity regardless of the initial level of heterozygosity (Davies et al. 2016).

Understanding a species' genetic diversity and how it responds to changes in abiotic conditions is valuable in understanding a species' ecology. Investigations into a species' genetic diversity must go a step further, however, by investigating temporal changes in genetic diversity and determining whether genetic diversity within a species and across its populations changes over time. This can be achieved by resampling the genetic profile of a species and relating any possible changes in genetic profile to a species' life history, e.g., reproductive timing, distribution, and dispersal etc. This also applies to the separate lineages within a species and how the distributions of such lineage are likely to shift with changes in abiotic conditions.

This thesis aimed to revisit the genetic structure of *Perna perna* on the South African coastline using mtDNA COI data after an initial investigation in 2007 (Zardi et al. 2007b). The evidence in Chapter 2 showed that the geographic distribution of *P. perna* intraspecific diversity has changed over the last twelve years and the overlap region has shifted by approximately 100km (Fig. 2.7), though this interpretation must be tempered by a recognition of the small sample size used. The results of Chapter 2 showed that the genetic diversity within some sites had increased with a higher occurrence of private haplotypes than reported in 2007 (e.g., Umhlanga). It is likely that the reported increase in private haplotypes is related to natural long-term dispersal of haplotypes from northern populations in this investigation, self-recruitment of larvae, and human induced transportation of larvae or adults through shipping. The results from Chapter 2 concurred with results from other temporal genetic studies based on marine species which showed an increase in genetic diversity and spatial stability over time (Ruzzante et al. 1997, Vähä et al. 2008, Walter et al. 2009).

Chapter 2 was limited in that the sample sizes of the genetic sequences used were quite small and this limited insight into the haplotype diversity seen at each site. It is likely that due to the small samples sizes that the individuals collected simply did not possess certain haplotypes making it appear that those haplotypes are missing from the sites between the years (e.g., Haplotype 5 was not found in Umhlanga in 2007 but was found in 2019, Fig. 2.6).

This thesis also aimed to investigate how reproduction was influenced by local conditions and whether the two lineages of *P. perna* would exhibit synchronized spawning rates when present on the same shore. Using changes in body mass as a proxy for the reproductive cycle, Chapter 3 showed that populations at different sites exhibit different patterns of spawning (as indicated by abrupt loss of mass) and that the Western and Eastern lineages differed in magnitude of body mass

(Figs 3.2 and 3.3). Nevertheless, their reproductive spawning events were synchronized when the lineages were present on the same shore (Fig. 3.3). When comparing the reproductive timing of the natural populations and the manipulated field experiment, it was clear that the reproductive timing was based on location rather than genetic distinction. Thus, the timing of spawning is determined by differences in temperature across the investigated sites rather than dependent on genetic distinction and it is unlikely that reproductive timing maintains genetic isolation of the lineages.

The fact that the timing of reproductive events differed with geography rather than genetic identity shows that, although intraspecific variation exists in *P. perna* this does not influence reproductive potential, timing, or output This is similar to the findings from Sanford and Kelly (2011). The results from that study showed that in species with high levels of gene flow, phenotypic plasticity is favoured over local adaptation so long as there is no great cost to plasticity (Sanford & Kelly 2010). Phenotypic plasticity might be the reason behind the similar reproductive profiles between the Eastern and Western lineages in the manipulated transplant experiment in Chapter 3. An important limitation to the present investigation was that I was unable to collect a full year's worth of temperature and spawning data due to the national lockdown in 2020.

Rouault et al. (2010) predicted that there will be an average increase in water temperature by 0.5 °C on the east coast and a decrease in water temperature of 0.55 °C on the west coast of South Africa per decade. With changing climates and the south coast sea surface temperature increasing, it is likely that the south coast and the southern section of the east coast are becoming more suitable for *P. perna* than the east coast as seen from the results in Chapter 3. This might indicate a reduced fitness in the northern east coast population of *P. perna* or an increase in trickle

spawning seen along the east coast rather than a pulsed spawning event. If this is the case, it is an interesting development in the distribution of *P. perna*.

The results did give evidence that there had been a genetic shift with the Eastern lineage distribution extending onto the south coast but did not support the hypothesis that the genetic distinction was maintained by unsynchronised reproduction. These results have shown the value of investigating temporal genetic variation and possible causes of changes in genetic profiles between lineages within a species. These results were similar to previously seen results when investigating temporal genetic variation and thus strengthened the narrative. This study could be related to other species that have the similar genetic patterns with populations that are distributed over different bioregions. The influence of intraspecific variability of reproductive timing and output may have an effect on the population health of *P. perna* in the face of predicted climate change. While the lineages stay genetically distinct, increasing temperatures may affect reproductive potential and lead to a reduction in recruitment and the sustainability of north east populations in South Africa.

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Appendix

Tables

Table A2.1: Uncorrected p-distance pairwise comparison of 2007 COI mtDNA sequences. The assignment of haplotypes to sites can be found in Table 2.1.

Haplotypes	1	4	5	7	8	9	10	13	2	3	6	11	12	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
4	0.003																													
5	0.035	0.038																												
7	0.003	0.005	0.033																											
8	0.030	0.033	0.010	0.028																										
9	0.003	0.005	0.033	0.005	0.028																									
10	0.003	0.005	0.038	0.005	0.033	0.005																								
13	0.030	0.033	0.010	0.028	0.005	0.028	0.033																							
2	0.030	0.033	0.010	0.028	0.005	0.028	0.033	0.005																						
3	0.028	0.030	0.008	0.025	0.003	0.025	0.030	0.003	0.003																					
6	0.035	0.038	0.020	0.033	0.015	0.033	0.038	0.015	0.010	0.013																				
11	0.003	0.005	0.038	0.005	0.033	0.005	0.005	0.033	0.033	0.030	0.038																			
12	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.013	0.035																		
14	0.003	0.005	0.033	0.005	0.028	0.005	0.005	0.028	0.028	0.025	0.033	0.005	0.030																	
15	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.013	0.035	0.005	0.030																

Table A2.1 continued.

16	0.003	0.005	0.038	0.005	0.033	0.005	0.005	0.033	0.033	0.030	0.038	0.005	0.035	0.005	0.035														
17	0.030	0.033	0.010	0.028	0.005	0.028	0.033	0.005	0.005	0.003	0.015	0.033	0.008	0.028	0.008	0.033													
18	0.030	0.033	0.010	0.028	0.005	0.028	0.033	0.005	0.005	0.003	0.015	0.033	0.008	0.028	0.008	0.033	0.005												
19	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.013	0.035	0.005	0.030	0.005	0.035	0.008	0.008											
20	0.035	0.038	0.015	0.033	0.010	0.033	0.038	0.010	0.005	0.008	0.010	0.038	0.008	0.033	0.008	0.038	0.010	0.010	0.008										
21	0.033	0.035	0.013	0.030	0.008	0.030	0.030	0.008	0.003	0.005	0.013	0.035	0.005	0.030	0.005	0.035	0.008	0.008	0.005	0.008									
22	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.013	0.035	0.005	0.030	0.005	0.035	0.008	0.008	0.005	0.008	0.005								
23	0.030	0.033	0.010	0.028	0.005	0.028	0.033	0.005	0.005	0.003	0.015	0.033	0.008	0.028	0.008	0.033	0.005	0.005	0.008	0.010	0.008	0.008							
24	0.030	0.033	0.010	0.028	0.005	0.028	0.033	0.005	0.005	0.003	0.015	0.033	0.008	0.028	0.008	0.033	0.005	0.005	0.008	0.010	0.008	0.008	0.005						
25	0.038	0.040	0.003	0.035	0.013	0.035	0.040	0.013	0.013	0.010	0.023	0.040	0.015	0.035	0.015	0.040	0.013	0.013	0.015	0.018	0.015	0.015	0.013	0.013					
26	0.033	0.035	0.013	0.030	0.003	0.030	0.035	0.008	0.008	0.005	0.018	0.035	0.010	0.030	0.010	0.035	0.008	0.008	0.010	0.013	0.010	0.010	0.008	0.008	0.015				
27	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.008	0.035	0.005	0.030	0.005	0.035	0.008	0.008	0.005	0.008	0.005	0.005	0.008	0.008	0.015	0.010			
28	0.030	0.033	0.010	0.028	0.005	0.028	0.033	0.005	0.005	0.003	0.015	0.033	0.008	0.028	0.008	0.033	0.005	0.005	0.008	0.010	0.008	0.008	0.005	0.005	0.013	0.008	0.008		
29	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.013	0.035	0.005	0.030	0.005	0.035	0.008	0.008	0.005	0.008	0.005	0.005	0.008	0.008	0.015	0.010	0.005	0.008	
30	0.033	0.035	0.013	0.030	0.008	0.030	0.035	0.008	0.003	0.005	0.013	0.035	0.005	0.030	0.005	0.035	0.008	0.008	0.005	0.008	0.005	0.005	0.008	0.003	0.015	0.010	0.005	0.008	0.005

Table A2.2: Uncorrected p-distance pairwise comparison of 2019 COI mtDNA sequences. The assignment of haplotypes to sites can be found in Table 2.2.

Haplotypes	1	4	5	7	8	9	10	11	12	14	15	2	3	6	13	16	17	18	19	20	21	22	23	24	25	26	27	28
1																												
4	0.003																											
5	0.035	0.038																										
7	0.003	0.005	0.033																									
8	0.003	0.005	0.033	0.005																								
9	0.005	0.008	0.040	0.008	0.008																							
10	0.003	0.005	0.038	0.005	0.005	0.008																						
11	0.003	0.005	0.038	0.005	0.005	0.008	0.005																					
12	0.003	0.005	0.038	0.005	0.005	0.008	0.005	0.005																				
14	0.003	0.005	0.038	0.005	0.005	0.008	0.005	0.005	0.005																			
15	0.003	0.005	0.033	0.005	0.005	0.008	0.005	0.005	0.005	0.005																		
2	0.030	0.033	0.010	0.028	0.028	0.035	0.033	0.033	0.033	0.033	0.028																	
3	0.028	0.030	0.008	0.025	0.025	0.033	0.030	0.030	0.030	0.030	0.025	0.003																
6	0.035	0.038	0.020	0.033	0.033	0.040	0.038	0.038	0.038	0.038	0.033	0.010	0.013															
13	0.038	0.040	0.018	0.035	0.035	0.043	0.040	0.040	0.040	0.040	0.035	0.008	0.010	0.018														
16	0.033	0.035	0.013	0.030	0.030	0.038	0.035	0.035	0.035	0.035	0.030	0.003	0.005	0.013	0.010													
17	0.035	0.038	0.010	0.033	0.033	0.040	0.038	0.038	0.038	0.038	0.033	0.005	0.008	0.015	0.013	0.008												
18	0.030	0.033	0.010	0.028	0.028	0.035	0.033	0.033	0.033	0.033	0.028	0.005	0.003	0.015	0.013	0.008	0.010											
19	0.030	0.033	0.010	0.028	0.028	0.035	0.033	0.033	0.033	0.033	0.028	0.005	0.003	0.015	0.013	0.008	0.010	0.005										
20	0.033	0.035	0.013	0.030	0.030	0.038	0.035	0.035	0.035	0.035	0.030	0.003	0.005	0.013	0.010	0.005	0.003	0.008	0.008									

Table A2.2 continued.

21	0.030	0.028	0.015	0.028	0.028	0.035	0.033	0.033	0.033	0.033	0.028	0.005	0.008	0.015	0.013	0.008	0.010	0.010	0.010	0.008								
22	0.033	0.035	0.013	0.030	0.030	0.038	0.035	0.035	0.035	0.035	0.030	0.003	0.005	0.013	0.010	0.005	0.008	0.008	0.008	0.005	0.008							
23	0.030	0.033	0.010	0.028	0.028	0.035	0.033	0.033	0.033	0.033	0.028	0.005	0.003	0.015	0.013	0.008	0.010	0.005	0.005	0.008	0.010	0.008						
24	0.033	0.035	0.013	0.030	0.030	0.033	0.035	0.035	0.035	0.035	0.030	0.003	0.005	0.013	0.010	0.005	0.008	0.008	0.008	0.005	0.008	0.005	0.008					
25	0.030	0.033	0.010	0.028	0.028	0.035	0.033	0.033	0.033	0.033	0.028	0.005	0.003	0.015	0.013	0.008	0.010	0.005	0.005	0.008	0.010	0.008	0.005	0.008				
26	0.038	0.040	0.023	0.035	0.035	0.043	0.040	0.040	0.040	0.040	0.035	0.013	0.015	0.003	0.020	0.015	0.018	0.018	0.018	0.015	0.018	0.015	0.018	0.015	0.018			
27	0.033	0.035	0.013	0.030	0.030	0.038	0.035	0.035	0.035	0.035	0.030	0.003	0.005	0.013	0.010	0.005	0.008	0.008	0.008	0.005	0.008	0.005	0.008	0.005	0.008	0.015		
28	0.028	0.030	0.013	0.025	0.025	0.033	0.030	0.030	0.030	0.030	0.030	0.008	0.005	0.018	0.015	0.010	0.013	0.008	0.008	0.010	0.013	0.010	0.008	0.010	0.008	0.020	0.010	

Figures

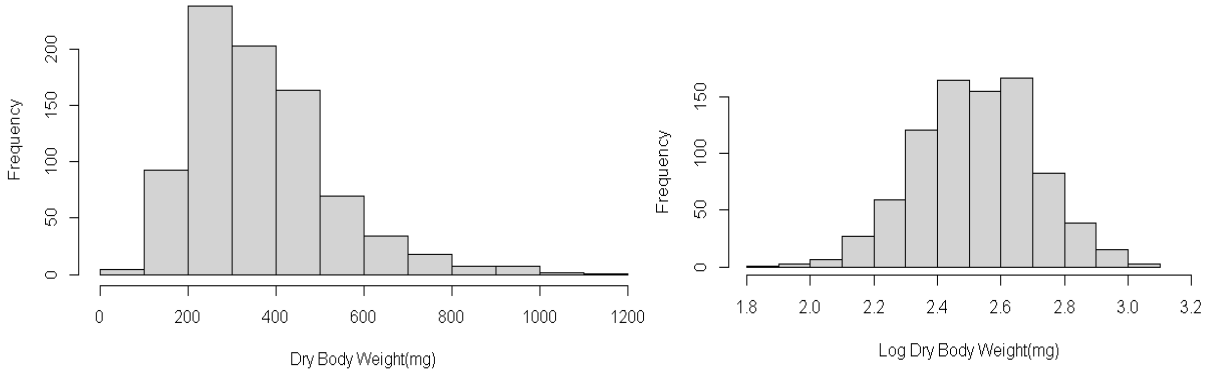

Figure A3.1: a) Histogram of body weight and b) log10-transformed body weight data of *Perna perna* from natural populations sites and the manipulative transplant site.

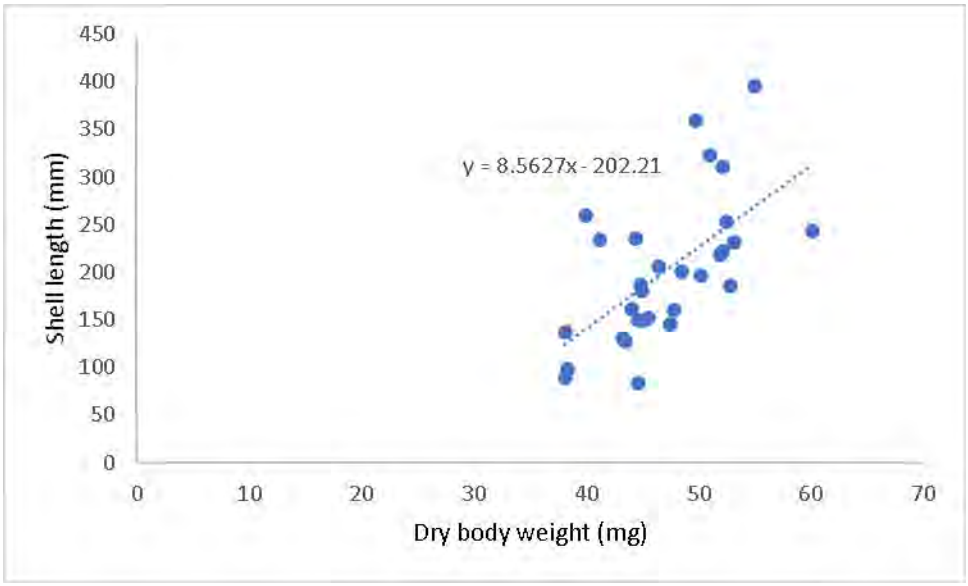

Figure A3.2: Example of regression plot from data collected from Port Edward in September. The regression equation was used to calculate the hypothetical weight of a 6cm mussel.

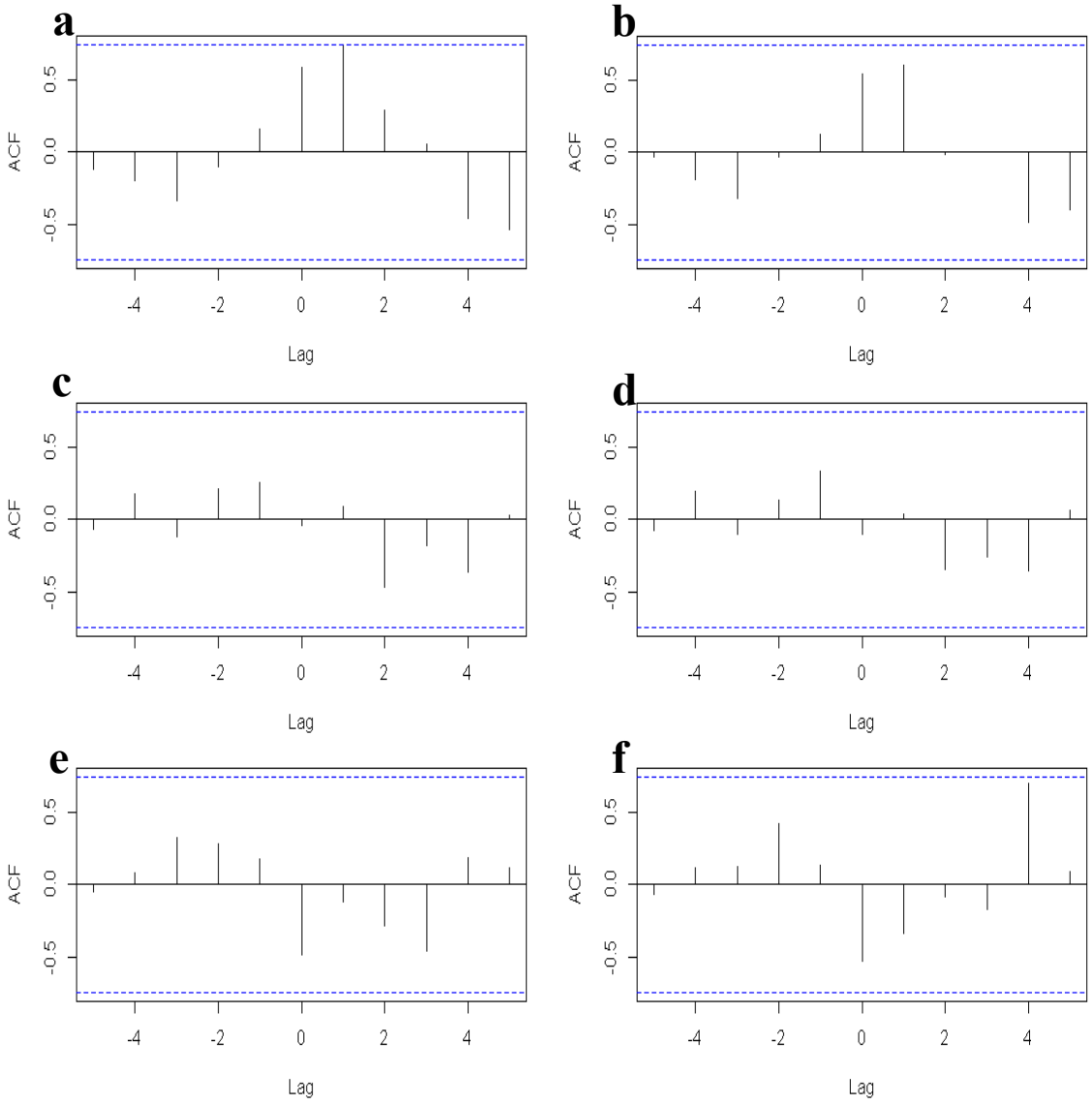

Figure A3.3: Autocorrelation functions (ACF) between average monthly water temperature and $\log_{10}$-transformed body mass at a) Port Edward, b) Port St Johns, c) Old Woman's River (Eastern lineage), d) Old Woman's River (Western lineage), e) Nature's Valley, and f) Mossel Bay.