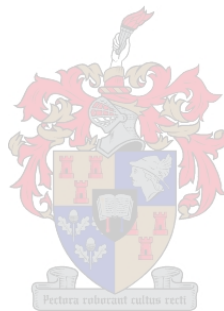


Assessing the evolutionary and physiological resilience of southern African marine species

by Lisa Mertens

*Dissertation presented for the degree of Doctor of Philosophy
in the Faculty of Science
at Stellenbosch University*



Supervisor:
Prof. John Measey

March 2021

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2021

Abstract

Assessing the genomic basis of local adaptation and critical thermal limits is essential for anticipating species persistence and distribution under climate change. Environmental gradients are associated with genomic and physiological differences between populations. South Africa's two ocean regime creates a thermal gradient, which delimits distinct bioregions between its cold-temperate west coast, warm-temperate south coast and warm-subtropical east coast. Three co-distributed key rocky shore invertebrate species representing different phyla were selected for a multi-species approach. The objectives of this dissertation were to 1) identify selectively neutral genomic loci and neutral population structure, 2) determine putatively adaptive loci and adaptive population structure, 3) explore functional annotations and 4) measure critical thermal limits (CT_{min} , CT_{max}).

Pooled RAD-Seq (ezRAD) was utilised to identify selectively neutral shared and population-specific single nucleotide polymorphisms (SNPs) in six populations of shore crab *Cyclograpsus punctatus* (CP), granular limpet *Scutellastra granularis* (SG), and Cape sea urchin *Parechinus angulosus* (PA). Population-specific SNPs were detected in all populations. Nucleotide diversity indices (Tajima's π , Watterson's θ) appear heightened in PA's northern west coast population compared to the remaining sites. Estimated pairwise F_{ST} values range from 0.043-0.055 (CP), 0.044-0.066 (SG) to 0.039-0.089 (PA). Selectively neutral genomic population structure indicates instances of intraspecific subdivisions present in all populations. All species populations harbour unique SNPs, yet increased nucleotide diversity is only detected in PA.

The empirical F_{ST} -method, BayeScan and BayeScEnv identified overall 1102 outliers under positive selection, of which 69 (CP), 11 (SG) and 27 (PA) could be functionally annotated. Candidate loci are involved in various cellular functions including membrane transport, vesicle signalling, protein folding/modification and cytoskeleton function. Identified loci related to energy cycling might point to selection on metabolic capacity to counter environmental stressors. Environmental differentiation of sea surface temperature (SST), salinity and air temperature could be associated with several putative outliers. There is no isolation-by-distance (IBD), but isolation-by-environment (IBE) suggests salinity variation to account for 48% of genomic variation in *P. angulosus* and SST and air temperature for 45% in *S. granularis*. Outlier-based population structure indicates possible intraspecific subdivision in some species.

Critical thermal limits (CT_{min} , CT_{max}) were investigated with thermal tolerance trials and compared to local min/max environmental temperature for warming and cooling tolerance. Across populations, mean CT_{min} ranges from -1.5-0.6°C (CP), -0.4-3.2°C (SG) to 5-10.9°C

(PA). Mean CT_{max} ranges from 43.8-46.1°C (CP), 34.4-35.7°C (SG) to 28.9-32.4°C (PA). West coast crabs have significantly higher CT_{max} than east coast crabs. CT_{min} is negatively and thermal breadth positively correlated with body mass (CP, PA). Significant regional differences in mass were detected (SG, PA). Warming and cooling tolerance appears sufficient, requiring further investigation with *in situ* microhabitat data.

East coast rocky shore populations likely face future warm-edge range contractions, whereas the south coast might experience distributional shifts depending on local thermal conditions. The west coast is an anchor for rocky shore species in South Africa and represents a possible climate change refugium. Strategic recognition in regional marine conservation management is warranted.

keywords

rocky shore, marine invertebrates, local adaptation, RADseq, SNPs, thermal tolerance, thermal gradient, seascape genomics

Acknowledgements

Financial support

Department of Botany and Zoology, Departmental Bursary (2015)
Deutsche Kreditbank AG Berlin Germany (DKB), Education loan program (2015-2017)
National Research Foundation South Africa (NRF), Innovation Doctoral Grant (2016-2017)
National Research Foundation South Africa (NRF), Extension Support (2017-2018)

Academic guidance

Prof. John Measey

Fieldwork, aquaria, experimental and analytical support

Fieldwork (Genomic): Erica Nielsen, Amir Rezai, Nozibusiso Mbongwa, Akhona Stofile

Fieldwork (Thermal): Molly Czachur, Dirk Warnich, Melissa Schulze,

Benedikt Hammerschmid, Craig Campbell

Aquaria: Casey Broom, Jen McShane, Anthony Madden, Jessica Toms, Henry Witbooi

Experiments: Erika Nortje, Casey Broom

Analytics: Dr. Nikki Phair, Erica Nielsen, Dr. Romina Henriques, Dr. Ingrid Minnaar,
Charl Moller and Gerhard van Wageningen, Dr. Julia L. Riley, Dr. James Baxter-Gilbert

Administrational support

Stellenbosch University International Office

Saudah Jacobs, Janine Basson, Marí Sauerman, Fawzia Gordon, Janette Law-Brown

Rozelle Petersen, Marié Theron

Visa Facilitation Services Global, South Africa

2018 Departmental Postgraduate Representative

Additional support

Stellenbosch University Campus Health Service

Centre for Student Counselling and Development

Winelands Radiology Coetzenburg

Special Thanks

Dr. Sören Häfker, DB, Jasmin Döhling-Wölm

Families

Mertens, Warnich, Van der Merwe



SFH150702122874

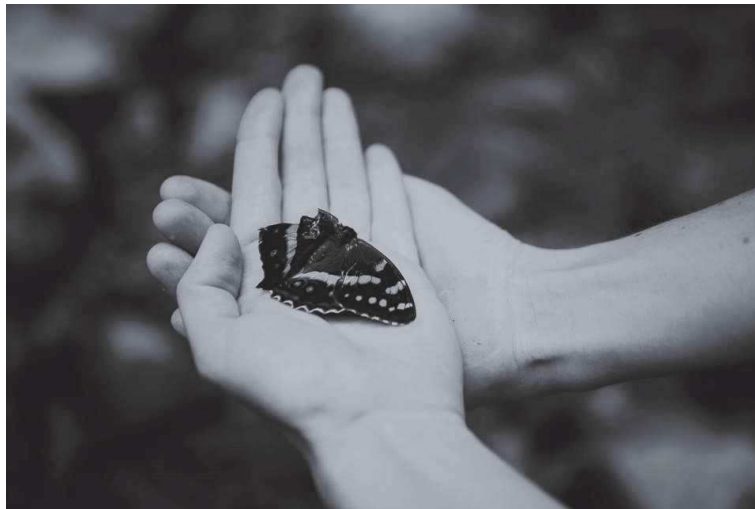
AEMD170601236034

IN MEMORIAM

LIDA-MARI GROENEWALD

Requiescat in pace.

{2019}



IN MEMORIAM

ANNA HENNIG

*“There is only one happiness in this life,
to love and be loved.” (A. Dupin)*

{2018}



Contents

Declaration.....	i
Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	v
List of Figures	x
List of Tables	xiv

Chapter I

1. Introduction	1
1.1 Climate change and its impact on species	1
1.2 Evolutionary potential and molecular diversity	4
1.3 From the genetic to the genomic perspective	7
1.4 Phenotypic plasticity.....	10
1.5 Marine biodiversity and oceanographic setting of South Africa.....	12
1.5.1 Marine biodiversity and conservation	12
1.5.2 Genetic breaks and the spatio-temporal variation of molecular variation.....	15
1.6 Paleoclimate and future climate change impacts in South Africa.....	18
1.6.1 Influence of paleoclimate on marine species	18
1.6.2 Climate change in South Africa's marine environment.....	19
1.7 Study species.....	22
1.7.1 Granular limpet - <i>Scutellastra granularis</i> (Patellidae, Patelloidea).....	22
1.7.2 Cape urchin - <i>Parechinus angulosus</i> (Echinidea, Echinoida)	25
1.7.3 Shore crab - <i>Cyclograpsus punctatus</i> (Brachyura, Varunidae).....	26
1.8 Aims and outline of study	28
1.9 References.....	30

Chapter II

Chapter II: Characterising the neutral genomic variation of selected marine invertebrates in South Africa

2.1 Introduction	58
2.1.1 RAD-Seq and Pool-Seq for non-model organisms	60
2.1.2 RAD-Seq literature review	60

2.1.3 Potential drivers of South African intertidal species population structure.....	66
2.1.4 Comparisons between traditional markers and SNPs	69
2.1.5 Expectations based on previous findings	70
2.2 Materials and Methods	71
2.2.1 Sample collection	71
2.2.2 DNA extraction and NGS sequencing	72
2.2.3 Statistical analysis	72
2.3 Results.....	77
2.3.1 Assembly metrics	77
2.3.2 Total and private SNPs	79
2.3.3 Nucleotide diversity (Tajima's π) and Watterson's theta (θ_w)	81
2.3.4 Pairwise F_{ST} values.....	82
2.3.5 Cluster analyses	83
2.4 Discussion.....	85
2.4.1 Genomic diversity	85
2.4.2 Genomic differentiation	88
2.4.3 Comparison of SNP-based and traditional marker findings	90
2.4.4 Evolutionary resilience	92
2.4.5 Comparisons across different SNP parameters	94
2.4.6 <i>De novo</i> assemblies	95
2.4.7 Conclusion.....	96
2.5 Appendix.....	98
2.5.1 Population metrics (12 scenarios).....	98
2.5.2 Clustering analyses	110
2.5.2.1 Mixture analysis based on mixture of groups of individuals	110
2.5.2.2 Population structure inference with fastSTRUCTURE	111
2.6 References.....	113

Chapter III

Chapter III: Characterising the putatively adaptive potential of selected marine invertebrates in South Africa

3.1 Introduction	137
3.1.1 Adaptive variation and evolutionary potential.....	140
3.1.2 Outlier detection approaches	141
3.1.2.1 Outlier differentiation method (F_{ST}).....	141

3.1.2.2 Environmental association method.....	143
3.1.2.2.1 Background.....	143
3.1.2.2.2 Environmental variables.....	144
3.1.2.2.3 BayeScEnv, isolation-by-distance (IBD) and isolation-by-environment (IBE).....	147
3.1.2.3 Outlier detection approaches summary.....	148
3.1.3 Goals and expectations.....	149
3.2 Methods and Materials.....	152
3.2.1 Data collection.....	152
3.2.2 Statistical analysis.....	152
3.2.2.1 Empirical F_{ST} method.....	152
3.2.2.2 BayeScan.....	152
3.2.2.3 Environmental and geographic variables.....	153
3.2.2.4 BayeScEnv.....	153
3.2.2.5 Annotation with BlastX.....	154
3.2.2.6 IBD and IBE.....	154
3.2.2.7 BAPS and fastSTRUCTURE.....	155
3.3 Results.....	156
3.3.1 Empirical F_{ST} method and BayeScan.....	156
3.3.2 BayeScEnv.....	156
3.3.3 Summary of detected outliers.....	157
3.3.4 Outlier F_{ST} estimates.....	159
3.3.5 Outlier annotation (BlastX).....	160
3.3.6 IBD and IBE testing.....	165
3.3.7 BAPS and fastSTRUCTURE.....	166
3.4 Discussion.....	167
3.4.1 Detection of outlier loci.....	167
3.4.2 Outliers with potential environmental association.....	169
3.4.3 Spatial differentiation of outlier loci and outlier F_{ST} estimates.....	172
3.4.4 Functional association of outliers.....	174
3.4.4.1 Established genomic elements of initially viral or bacterial origin.....	178
3.4.4.2 Outlier loci and (metabolic) adaptive divergence.....	180
3.4.5 Population structure estimates.....	185
3.4.6 Implications for resilience.....	190
3.4.7 Conclusion.....	192
3.5 Appendix.....	194
3.5.1 Geographic and environmental variables (RDA analysis).....	194

3.5.2 Outlier SNPs (empirical F_{ST} , BayeScan, PoPoolation)	196
3.5.3 Comparison F_{ST} estimates	196
3.5.4 List of BlastX protein domains	198
3.6 References.....	207

Chapter IV

Chapter IV: Assessing the thermal tolerance in three intertidal marine invertebrates

4.1 Introduction and background.....	242
4.1.1 Temperature and species distribution	242
4.1.2 The South African ocean temperature gradient.....	243
4.1.3 Intertidal zonation of the rocky shore	243
4.1.4 Impacts of climate change on intertidal communities	245
4.1.5 Thermal tolerance limits.....	247
4.1.6 Preferred intertidal niches of study species.....	252
4.1.7 Research aims and expectations	253
4.2 Materials and Methods.....	254
4.2.1 Field collection.....	254
4.2.2 Acclimation and maintenance	254
4.2.3 Thermal tolerance experiments	257
4.2.3.1 <i>Cyclograpsus punctatus</i>	258
4.2.3.2 <i>Scutellastra granularis</i>	259
4.2.3.3 <i>Parechinus angulosus</i>	260
4.2.4 Habitat temperature	261
4.2.5 Thermal breadth and warming and cooling tolerance (WT, CT)	263
4.2.6 Statistical analysis	263
4.3 Results.....	264
4.3.1 Preliminary analyses: Body mass and sex differences.....	264
4.3.2 Critical lower limits (CT_{min})	264
4.3.3 Critical upper limits (CT_{max})	264
4.3.4 Thermal breadth (T_{br})	264
4.3.5 Warming (WT) and cooling tolerance (CT).....	269
4.4 Discussion.....	271
4.4.1 Body mass differences associated with primary productivity and SST	271
4.4.2 Mass influences thermal tolerance.....	272
4.4.3 Intraspecific regional differences in thermal limits	274

4.4.4 Highest thermal breadth in <i>C. punctatus</i>	276
4.4.5 Warming and cooling tolerance	277
4.4.6 Interspecific comparison of 'winners' and 'losers'	278
4.4.7 Regional variation of climate change impacts	281
4.4.8 Summary	286
4.5 Appendix	289
4.6 References.....	295
Conclusion	
5. Conclusion	320
References	322

List of Figures

Chapter I:

Figure 1.1. Changing oceanic, atmospheric and terrestrial conditions, anthropogenic influences and the resulting abiotic and biotic stress factors from the perspective of an intertidal organism. Symbols mark increase (+), decrease (-) or change (~). Source: L. Mertens..... 4

Figure 1.2. South Africa's thermal environmental gradient, marine bioregions and recognised coastal genetic breaks. The cold Benguela Current on the west coast and the warm Agulhas Current on the east coast are associated with four temperature-delimited marine bioregions: cool-temperate, warm-temperate, subtropical and tropical. Four locations have been suggested based on genetic data as genetic breaks: Cape Point, Cape Agulhas, Algoa Bay and St. Lucia (taken from Teske et al. 2011)..... 15

Figure 1.3. Study species and their distribution in southern Africa. *Scutellastra granularis* (A), *Parechinus angulosus* (B), *Cyclograpsus punctatus* (C) (Adapted from: Branch et al. 2017). It is debated whether *C. punctatus* occurs on the entire west coast as shown in the map or only up to Jacob's Bay, where sampling could be successfully conducted. (Images: bit.ly/2V9w73w (PA), bit.ly/3dm0HgF (CP), L. Mertens (SG)). 24

Chapter II:

Figure 2.1. Map of the South African coastline showing the sea surface temperature gradient (December 2013) and sampling locations: Port Nolloth (PN), Jacob's Bay (JC) (*C. punctatus* only), Sea Point (SP), Cape Agulhas (CA), Knysna (KY), Cape St. Francis (CF) and Haga Haga (HH). Source: NASA, ID: 30487, <http://svs.gsfc.nasa.gov/30487>. 72

Figure 2.2. Software tools used to create the assemblies (left) and to process the reads (right). 73

Figure 2.3. Number of total SNPs in *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.2. 80

Figure 2.4. Number of private SNPs in *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.2. 81

Chapter II – Appendix:

Figure S2.1. Coloured partition of clusters suggested for *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.2. 110

Figure S2.2a. Suggested admixture proportions for *C. punctatus* over the suggested range (overall K = 1-4) with (1) K = 1, (2) K = 2, (3) K = 3, (4) K = 4..... 111

Figure S2.2b. Suggested admixture proportions for *S. granularis* over the suggested range (overall K = 3-5) with (1) K = 3, (2) K = 4, (3) K = 5. 112

Figure S2.2c. Suggested admixture proportions for *P. angulosus* over the suggested range (overall K = 1-3) with (1) K = 1, (2) K = 2, (3) K = 3. 112

Chapter III:

Figure 3.1. Outliers detected for *C. punctatus* in BayeScan, BayeScEnv and the empirical F_{ST} method..... 158

Figure 3.2. Outliers detected for *S. granularis* in BayeScan, BayeScEnv and the empirical F_{ST} method..... 158

Figure 3.3. Outliers detected for *P. angulosus* in BayeScan, BayeScEnv (salinity) and the empirical F_{ST} method..... 159

Figure 3.4. Coloured partition of clusters suggested by BAPS (left) and fastSTRUCTURE (right) for *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.2..... 166

Chapter IV:

Figure 4.1. The thermal performance curve indicates the relationship between environmental temperature and the physiological rate of an ectotherm. The optimum temperature (T_{OPT}) indicates the temperature at optimum performance. The thermal performance breadth is determined by the thermal minimum (CT_{min}) and maximum (CT_{max}) (Figure adapted from Tuff, Tuff and Davies, 2016)..... 248

Figure 4.2. Thermal performance curves under different warming scenarios. (a) Climate warming can shift the distributions of T_b (body temperature). If warming raises T_b closer to T_{OPT} of a species (e.g. shift from A to B), warming can enhance fitness. If warming increases T_b higher than T_{OPT} (e.g. shift from B to C), the fitness will decline. (b,c) Increases in T_b from warming can have much bigger effects on (b) thermal specialists than on (c) thermal generalists (Figures adapted from Huey et al. 2012)..... 249

Figure 4.3. *Parechinus angulosus* individuals engaging in “covering” behaviour, photographed on the west coast (Port Nolloth) during low tide. White circles are added for better identification. (Source: L. Mertens). 253

Figure 4.4. (A) Interpolated summertime sea surface temperature (SST) measurements across 87 sites (Port Nolloth to Sodwana Bay; broadly grouped by red boxes into west, south and east coast) from varying periods ranging between 1972 and 2012. The data was collected *in situ* either manually with hand-held thermometers or electronically with underwater temperature recorders (UTRs) within 400 m from the coast at depths ranging from 20 cm to 9 m by different institutions (compare Figure 1 in Smit et al. 2013). The compiled data records were used to calculate a monthly temperature climatology, which served to produce an interpolated dataset representing temperature records at evenly spaced sites along the coast. These same data are also plotted in the lower panel (B) to further highlight the alongshore gradients. The middle and upper panels in (B) show the seasonal mean monthly *in situ* temperature for August and February respectively representing winter and summer (Smit et al. 2013)..... 255

Figure 4.5. Schematic setup of the artificial Perspex island for the acclimation of *S. granularis*, which are depicted as oval circles. The arrow indicates the direction of the water flow generated by the aquaria pump. 257

Figure 4.6. Schematic setup of the CT _{min} and CT _{max} trials for <i>C. punctatus</i>	259
Figure 4.7. Schematic setup of the CT _{min} and CT _{max} trials for <i>S. granularis</i>	260
Figure 4.8. Schematic setup of the CT _{min} and CT _{max} trials for <i>P. angulosus</i>	261
Figure 4.9. Body mass and CT _{min} shown by region. Significant differences are marked with asterisks (* = p<0.05). Box and whisker plot showing quartiles and median. Small circles represent outliers.	266
Figure 4.10. CT _{max} and thermal breadth shown by region. Significant differences are marked with asterisks (* = p<0.05). Box and whisker plot showing quartiles and median. Small circles represent outliers.	268

List of Tables

Chapter I:

Table 1.1. Previous genetic and genomic findings research on study species (South Africa)	28
---	----

Table 1.2. Components of study, utilised data and details.	29
--	----

Chapter II:

Table 2.1. RAD-Seq studies conducted on marine invertebrates since 2013.	62
--	----

Table 2.2. List of study species, including life history traits and shore height preference.	68
--	----

Table 2.3. Previous findings on population differentiation, nucleotide diversity and suggested lineages.	68
--	----

Table 2.4. Amount of q20 reads, non-ambiguously mapped reads and subsampled non-ambiguously mapped reads, shown by species and location.	75
--	----

Table 2.5a. Quast metrics from assemblies created for <i>C. punctatus</i> in SPAdes, ABySS and Rainbow.	78
---	----

Table 2.5b. Quast metrics from assemblies created for <i>S. granularis</i> in SPAdes, ABySS and Rainbow.	78
--	----

Table 2.5c. Quast metrics from assemblies created for <i>P. angulosus</i> in SPAdes, ABySS and Rainbow.	79
---	----

Table 2.6. Total assembly length shown per species, indicating the total number of bases in the SPAdes assemblies together with the nearest available reference genome assembly.	79
--	----

Table 2.7. Number of private SNPs shown as percentage by species and location.	81
--	----

Table 2.8. Number of SNPs, Tajima's π and Watterson's θ_w values as estimated in PoPoolation. Location abbreviations are listed in Figure 2.	82
---	----

Table 2.9. F_{ST} as estimated in PoPoolation2 (scenario 6) incorporating 14,392 (A), 5,440 (B) and 4,235 (C) SNPs. Location abbreviations are listed in Figure 2.	83
--	----

Table 2.10. Number of estimated population clusters according to program and species.	83
---	----

Table 2.11. Findings from mtDNA and SNPs: study species pairwise F_{ST} values, nucleotide diversity, suggested lineages and potential clusters.	84
--	----

Chapter II – Appendix:

Table S2.1. Settings used for PoPoolation (individual pileups) and PoPoolation 2 (meta-pileup).	98
---	----

Table S2.2. <i>Cyclograpsus punctatus</i> (Shore crab). Number of SNPs and values for π and θ shown per location in scenario 1-9 (PoPoolation).	99
--	----

Table S2.3. <i>Scutellastra granularis</i> (Granular limpet). Number of SNPs and values for pi, theta and D shown per location in scenario 1-9 (PoPoolation).	100
Table S2.4. <i>Parechinus angulosus</i> (Cape urchin). Number of SNPs and values for pi and theta shown per location in scenario 1-9 (PoPoolation).	101
Table S2.5. <i>Cyclograpsus punctatus</i> (Shore crab). Number of SNPs and private SNPs shown per location in scenario 4-12 (PoPoolation2).	102
Table S2.6. <i>Scutellastra granularis</i> (Granular limpet). Number of SNPs and private SNPs shown per location in scenario 4-12 (PoPoolation2).	103
Table S2.7. <i>Parechinus angulosus</i> (Cape urchin). Number of SNPs and private SNPs shown per location in scenario 4-12 (PoPoolation2).	104
Table S2.8. <i>Cyclograpsus punctatus</i> (Shore crab). Pairwise F_{ST} values shown between locations in scenario 4-12 (PoPoolation2).	105
Table S2.9. <i>Scutellastra granularis</i> (Granular limpet). Pairwise F_{ST} values shown between locations in scenario 4-12 (PoPoolation2).	106
Table S2.10. <i>Parechinus angulosus</i> (Cape urchin). Pairwise F_{ST} values shown between locations in scenario 4-12 (PoPoolation2).	107
Table S2.11. Range of selected SNP-based nucleotide diversity estimates (pi and theta) of (marine) Arthropoda, Mollusca and Echinodermata.	108
Table S2.12. Summary of the range of findings for varying SNP parameters (see Table A1) for study species (see Table A2-A10) shown for Sea Point population.	109

Chapter III:

Table 3.1. Detected outliers with empirical F_{ST} method and BayeScan. The total number of loci differs due to the different methodological approaches to identify outlier loci.	156
Table 3.2. Number of outliers detected by BayeScEnv, which are putatively influenced by differentiation in sea surface temperature, air temperature and salinity across the study populations.	157
Table 3.3. Summary of outliers detected by the selected methods in comparison.	157
Table 3.4. F_{ST} estimated in PoPoolation2 (scenario 6) incorporating 494 (A), 165 (B) and 121 (C) outliers. Asterisk(*) indicates significance according to Fisher's exact test. Location abbreviations are listed in Figure 1, Chapter I.	160
Table 3.5. Number of outlier contigs, BlastX database results per E-value cut off, hypothetical and putatively identified BlastX findings listed per species. Percentage in brackets relates to the total number of outlier contigs.	161
Table 3.6. Suggested protein domains of empirical outliers listed per species with contig query length, query cover, respective E-value and the percentage of the contig identical with the putative protein.	161
Table 3.7. Suggested protein domains of BayeScan and BayeScEnv outliers listed per species with contig query length, query cover, respective E-value and the percentage of the contig	

identical with the putative protein. Protein domains suggested across BayeScan and BayeScEnv or between multiple environmental parameters appear in grey. 164

Table 3.8. Number of estimated population clusters based on putatively adaptive loci listed by application and species compared to population clusters estimated in the previous Chapter with selectively neutral loci. 166

Chapter III – Appendix:

Table S3.1. GPS coordinates utilised to extract environmental variables used in BayeScEnv and RDA analysis (Abbreviations listed in Figure 1, Chapter I). 194

Table S3.2. Geographic along-shore distances (km) between sampling locations utilised in the RDA analysis. Source: SANBI (South African National Biodiversity Institute). 194

Table S3.3. Environmental variables (SST and air temperature in °C, salinity in ppt) utilised in the RDA analysis and BayeScEnv. Source: World Ocean Atlas, 2013. 195

Table S3.4. Number of unique potential outliers detected with the empirical F_{ST} method pairwise population estimation. 196

Table S3.5. F_{ST} estimated in PoPoolation2 (scenario 6) for selectively neutral loci (left) and outlier loci (right). Location abbreviations as listed in Figure 1, Chapter I. 197

Table S3.6. Putatively identified and hypothetical or uncharacterised protein domains from empirical outliers listed per species with contig query length, query cover, respective E-value and the percentage of the contig identical with the suggested protein domain. 198

Table S3.7. Putatively identified and hypothetical or uncharacterised protein domains from BayeScan and BayeScEnv outliers listed with contig query length, query cover, respective E-value and the percentage of the contig identical with the suggested protein domain. Protein domains suggested across BayeScan and BayeScEnv or between multiple environmental parameters appear grey. 204

Chapter IV:

Table 4.1. Air temperature minima and maxima for the seven study locations (averaged monthly mean (2009-2019; Coffee Bay: 2010-2019); Source: South African Weather Service (SAWS)) or the closest available locations (*Cape Columbine (<20 km), **Cape Town Yacht Harbour (<5km), *** Coffee Bay (~200 km)). 262

Table 4.2. Monthly average sea surface temperature (SST) for the seven study locations (2017-2019; Source: South African Weather Service) or the closest available locations (*Saldanha Bay (distance: 5 km), **Mosselbay (~100 km), ***Port Elizabeth (~100 km), ****East London, Orient Beach (~50 km)). 262

Table 4.3. Model parameter estimates of linear mixed models fitted for mass. t-tests use Satterthwaite's method. 265

Table 4.4. Model parameter estimates of linear mixed models fitted for CT_{min} . t-tests use Satterthwaite's method. 265

Table 4.5. Model parameter estimates of linear mixed models fitted for CT _{max} . t-tests use Satterthwaite's method.....	267
--	-----

Table 4.6. Model parameter estimates of linear mixed models fitted for thermal breadth. t-tests use Satterthwaite's method.	267
--	-----

Table 4.7. Warming and cooling tolerance (in °C) calculated in relation to upper/lower critical limits and mean maximum/minimum air temperature per population and month in <i>C. punctatus</i> and <i>S. granularis</i>	269
--	-----

Table 4.8. Warming and cooling tolerance (in °C) calculated in relation to upper/lower critical limits and mean SST temperature per population and month in <i>P. angulosus</i>	270
---	-----

Chapter IV – Appendix:

Table S4.1. Shapiro Wilk's Test, distribution skewness coefficient, Levene's Test and Bartlett's Test values per coastal region across species for body mass, CT _{max} , CT _{min} and T _{br} (thermal breadth). Significant values are indicated in bold.	289
---	-----

Table S4.2. Summary of mass data for all three species, including sample size (N), mean and median (in g), standard deviation and standard error per region and population.	290
--	-----

Table S4.3. Summary of CT _{min} data set for all three species, including sample size (N), mean and median (in °C), standard deviation and standard error per region and population.	292
--	-----

Table S4.4. Summary of CT _{max} data set for all three species, including sample size (N), mean and median (in °C), standard deviation and standard error per region and population.	293
--	-----

Table S4.5. Summary of thermal breadth per species population including the mean (in °C), standard deviation and standard error per region and population.....	294
--	-----

1. Introduction

1.1 Climate change and its impact on species

Global climate change is recognised as a threat, with widespread impacts on biodiversity (Parmesan, 2006; Pereira et al. 2010; Bellard et al. 2012; Poloczanska et al. 2013; Archer et al. 2018). Climate change effects are multi-faceted and affect marine environments through ocean acidification, changes in ocean current patterns, melting of glaciers, the retraction of sea ice, sea level rise, changes in sea temperature and expanding hypoxia zones, with varying impact between regions (Trenberth et al. 2007; IPCC, 2013; Mora et al. 2013; Wolff et al. 2017). During the 21st century, the global mean sea level is predicted to rise between 0.26 to 0.55 m (RCP2.6 scenario, representative of <2°C warming above pre-industrial temperatures; Representative Concentration Pathway (RCP)) and between 0.45 to 0.82 m (RCP8.5 scenario, representative of the highest emissions) compared to present day levels (2081-2100 relative to 1986-2005) (IPCC, 2013). Some researchers suggest a potential sea level rise of up to 2.0 m to account for uncertainties regarding the rate and magnitude of ice sheet loss in a warming ocean (Parris et al. 2012). Moreover, the global average surface temperature has been predicted to rise 0.3 to 1.7°C above the pre-industrial level in the lowest emissions scenario (RCP2.6) and 2.6 to 4.8°C in the highest emission scenario (RCP8.5) (2081-2100 relative to 1986-2005) (IPCC, 2013). Global sea surface temperatures (SST) increased at an average rate of 0.05°C per decade from 1880-2012 (IPCC, 2013). The surface warming rates vary depending on the region and while most parts of the world's oceans experience warming trends, instances of cooling sea surface temperatures have been identified for example in parts of the North Atlantic and southern Africa (Rouault et al. 2010; NOAA, 2016). With the current rate of emissions, it is becoming less plausible to contain a temperature increase below 2°C by 2100 (Rafferty et al. 2017). Rates of warming might be even higher in tropical and subtropical regions, which could experience a departure from historical levels in temperature significantly earlier than temperate regions (Mora et al. 2013; Khaliq et al. 2014).

There have been numerous studies focusing on how species respond to changing environments (Mawdsley, O'Malley and Ojima, 2009; Pacifici et al. 2015; Miller et al. 2018; Foden et al. 2019). While some species may be able to acclimate to altered environmental conditions through phenotypic plasticity, other species may experience shifts in their distribution and changes in life history and phenology (Foden et al. 2019); yet other studies focus on evolutionary adaptation, although evidence for the latter remains limited at present (Miller et al. 2018). Climate change related shifts, mostly in poleward direction, have been reported for several species in the marine environment (Parmesan and Yohe, 2003; Perry et

al. 2005; Lima et al. 2007; Barton et al. 2016; Beaugrand and Kirby, 2018; Wilson, Skinner and Lotze, 2019). Temperature is thought to predict between 53-99% of the current biogeographic structure of shallow-marine benthic fauna along coastlines (Belanger et al. 2012), which possibly makes it the most important factor defining biogeographic boundaries and can be expected to project large-scale biotic responses to climate change in the future (Roy et al. 1998; Tittensor et al. 2010; Belanger et al. 2012). Overall, changing environmental conditions can lead to range extensions or contractions (Walther et al. 2002), which is particularly relevant for species living in areas characterised by specific climatic conditions with no possibility to track the preferred conditions in adjacent areas (Bellard et al. 2012; Ralston et al. 2017). For example, the prevalent poleward shifts observed for species in the northern hemisphere with often North-South oriented coastlines (see for example Perry et al. 2005) would not apply to geographical settings such as southern Africa, where the coastline has a predominant West-East orientation. Where a large scale poleward shift is geographically not possible, species are limited by available habitat offering environmental conditions within their (importantly thermal) tolerance limits, possibly resulting in a climate-induced range reduction. In general, species extinctions and a loss in overall biodiversity are projected as a consequence of shifting temperature regimes and other factors (Bellard et al. 2012), but the determinants linked to extinctions include diverse abiotic and biotic factors, which might act synergistically (Brook, Sodhi and Bradshaw, 2008). While there is evidence for species extinctions due to climate change, the exact mechanisms allowing populations to persist are still poorly understood (Cahill et al. 2013).

The potential of species resilience towards climate change is also closely linked to intraspecific diversity, which is seen as the most fundamental level of biodiversity (May, 1994), yet the impact of changing climatic conditions on the spatio-temporal distribution of genomic variation is also understudied. As such, it is unclear how species and their geographical distribution and spatial patterns of molecular variation may be affected by the wide range of shifting environmental factors potentially affecting them (Figure 1.1). This is because populations or lineages potentially already adjusted to different climatic conditions across their distribution, may not interact with changing environments in the same way (Mergeay and Santamaria, 2012; Exposito-Alonso et al. 2018). In addition, studying species distributions and the genomic variation of their populations under forecasted conditions is seen as critical for adaptive management frameworks (Rilov et al. 2019). Some studies have tried to predict the distribution of intraspecific molecular variation under changing climatic conditions (see for example Balint et al. 2011; Rubidge et al. 2012; Alsos et al. 2012; Pauls et al. 2013; Yannic et al. 2014; Jueterbock et al. 2018). A terrestrial study widely noticed for its conservation implications reports that the caribou, *Rangifer tarandus*, comprises two

genetically unique clades, shaped by relative climate stability in the past 21 kyr. However, strong shifts to the caribou distribution driven by warming climatic conditions are forecasted in the next 70 years. Models predict that climate change will impact the two identified clades of *R. tarandus* differently, with one potentially vanishing from its current range due to suitable habitat reduction of up to 89% (Yannic et al. 2014). The second example focuses on the poleward shifting intertidal seaweed *Fucus serratus*, a habitat-forming ecosystem engineer for rocky shores, with a contemporary distribution from northern Portugal to northern Norway (Jueterbock et al. 2018). Decadal sampling (2000 and 2010) showed that while genetic diversity values of the species mid-range, located at one of the species large former glacial refugia in north-western France, remained stable overall, there was a strong decline of genetic diversity (multi-locus heterozygosity) along the southern edge of the species distribution, which has been linked to a local temperature increase. The *F. serratus* lineage associated with the most southern occurrence is regarded as genetically unique and expected to largely disappear within the next 80 years without intervention (Jueterbock et al. 2018).

The southern African coastline is a prime study system for species potential response to forecasted climatic changes, just as this system has been used to illustrate past selection and its role in shaping ecology (e.g. Toms et al. 2014; Wright et al. 2015; Mmonwa et al. 2015; Teske et al. 2019). South Africa's coast is a natural gradient of contrasting environmental conditions, where populations of the same species inhabit cool-temperate to almost subtropical conditions (Branch et al. 2007; Griffiths et al. 2010; Sink et al. 2012). Geographic and associated environmental variation may provide the basis for some populations to be more resilient to climate change (see for example Teske et al. 2019). However, our understanding of possible local geographic variations in marine animals and plants with potential relevance towards climate change adaptation remains not widely understood at present (but see Baldanzi et al. 2017). This can be attributed to comparatively recent empirical interest in the matter, but also methodological constraints related to linking genomic and environmental variation (von der Heyden, personal communication). While the importance of studying species responses to climate change is well established, there is also a need for studies incorporating multiple taxonomic groups from the same habitat, exploring understudied species (non-model organisms) and applying interdisciplinary approaches to further the knowledge of species adaptation capacities. This project explores genomic and physiological variation within three abundant and common intertidal species, representing different taxonomic groups (Crustacea, Echinodermata, Mollusca; selection criteria are provided in section 1.7), within the context of climate change in South African marine

systems. The concepts of evolutionary potential, species resilience and phenotypic plasticity, which are central to studying genomic and physiological traits of species, are discussed in the following sections to provide a theoretical background for the research questions.

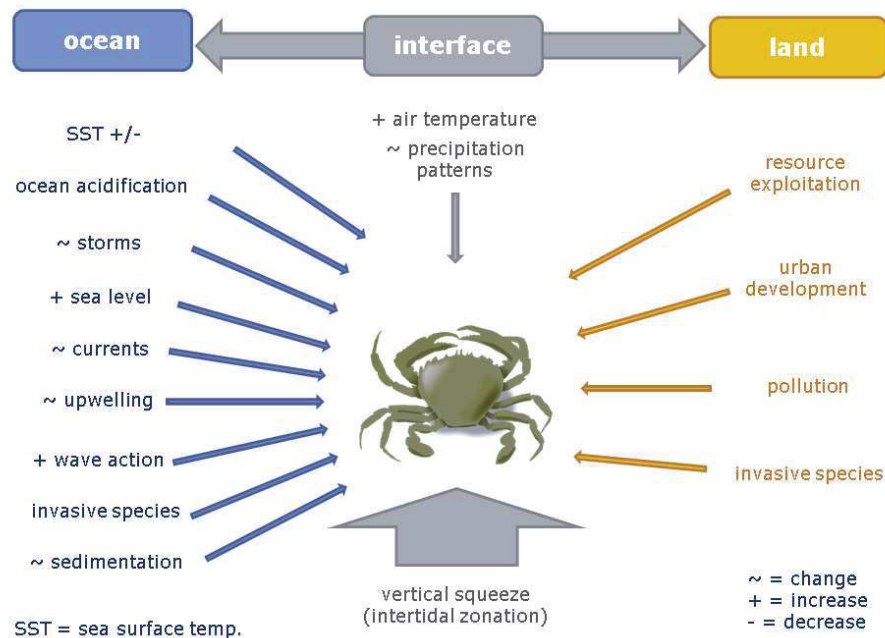


Figure 1.1. Changing oceanic, atmospheric and terrestrial conditions, anthropogenic influences and the resulting abiotic and biotic stress factors from the perspective of an intertidal organism. Symbols mark increase (+), decrease (-) or change (~). Source: L. Mertens.

1.2 Evolutionary potential and molecular diversity

Anthropogenic environmental change widely affects global biodiversity levels (Rands et al. 2010), with climate change (IPCC, 2013), habitat fragmentation and environmental degradation increasingly impacting the distribution, population size and genetic diversity of both terrestrial and marine species (Pereira et al. 2010; Bellard et al. 2012; Archer et al. 2018). It is of critical importance to estimate how species might react to rapidly changing environmental conditions and how their potential to adapt, disperse or perish can underpin their conservation management (Hoffmann and Sgrò, 2011; MacLean and Beissinger, 2017; Rilov et al. 2019). The ability to withstand adversity or to cope with challenges is assessed in terms of the resilience of an organism or a system, derived from the Latin term *resiliens* ("act of rebounding") (Simpson and Weiner, 1989, p. 714). Building on the ecological definition of resilience (Gunderson, 2000; Thrush et al. 2009), Sgrò formulated an evolutionary definition of resilience as "the ability of populations to persist in their current state [...] and to undergo evolutionary adaptation in response to changing environmental conditions" (Sgrò et al. 2011; p. 327; also see Gunderson, 2000; Thrush et al. 2009). Over time, the term "evolutionary

resilience” morphed into the more widely and often synonymously utilised “evolutionary potential”, which has been suggested as *“the property of a biological entity to be able to experience heritable change in some of its components between times t and $t + \Delta t$. This entity can be for example a genome, a trait, a population, a species, an ecosystem, or something else.”* (Milot, Béchet and Maris, 2020; p. 1365). The basis of evolutionary resilience (henceforth used synonymously with evolutionary potential) is thought to be the standing, or intraspecific genetic diversity of species populations across their range. Directional and fluctuating selective forces acting on the existing genetic composition of species shapes their ability to adapt genetically to novel circumstances (Barrett and Schluter, 2008; Forsman et al. 2011; Alsos et al. 2012; Pauls et al. 2013; Sunde et al. 2018). Therefore, genetic diversity has been identified as a key factor to the evolutionary past and future of species and their potential response to climate change (Garner et al. 2005; Alsos et al. 2012). Low levels of genetic diversity are thought to decrease species adaptive potential (Allendorf and Luikart, 2007), which underlines the importance of spatial genetic diversity patterns to assist the conservation of threatened populations (Provan, 2013). Further, widespread species are estimated to possess high genetic variation for numerous traits potentially involved in climatic adaptation (Hoffmann and Sgrò, 2011), which might put small populations of restricted range more at risk of population decline and possible extinction. Interestingly, a comparison of genetic diversity patterns between terrestrial and marine species revealed that endemic (and sometimes isolated) populations in the marine environment do not necessarily have low genetic diversity by default (Gaston, 1994; Gaston et al. 1997) and instances of high haplotype and nucleotide diversity were detected (Hobbs et al. 2011; Hobbs et al. 2013). Moreover, marine endemics were observed to have a higher abundance and therefore, the detrimental effects of genetic drift or inbreeding in small populations might be reduced compared to terrestrial endemic species (Frankham, 1996; Hobbs et al. 2011; Hobbs et al. 2013). While it is possible that high genetic diversity may reduce the extinction risk of species via the potentially large genetic spectrum from which to adapt to changing climatic conditions (Hobbs et al. 2011; Hobbs et al. 2013), it has been debated that demographic and environmental stochasticity, particularly for small population sizes or isolated populations, might have an overall stronger impact on extinction risk than genetic variation alone (Willi and Hoffmann, 2009). A review of 136 case studies investigating potential climate-change related extinctions found only seven instances in which approximate causes of reported local extinctions could be associated with anthropogenic climate change, demonstrating that the exact mechanisms are under ongoing investigation and currently not understood in detail (see Table 1 in Cahill et al. 2013).

Genetic diversity is thought to be impacted by climate change through a range of effects including spatial and temporal changes in the distribution and abundance of different genetic variants associated with shifting species ranges (Riddle et al. 2008; Pauls et al. 2013). Shifting ranges following contemporary climatic change have been observed across numerous terrestrial and marine species (Parmesan and Yohe, 2003; Perry et al. 2005; Hickling et al. 2006; Lima et al. 2007; Kelly and Goulde, 2008; Barton et al. 2016; Beaugrand and Kirby, 2018; Wilson, Skinner and Lotze, 2019). However, genetic variation is rarely distributed equally across species ranges, which has been attributed to synergistic effects of historical and contemporary influences (Provan, 2013). It is debated and likely species-specific if the highest genetic diversity is found in the core of the species range, at the leading edge or at the trailing edge of the distribution (Gibson, van der Marel and Starzomski, 2009; Parisod and Joost, 2010; Pfenniger et al. 2012). For instance, geographical areas which might have served as refugia in the past may comprise a large portion of the species overall genetic diversity and/or unique adaptive variation not present in the later established populations (Hampe and Petit, 2005). Moreover, the impact of species distributional shifts can include changes in the dynamics of metapopulations, as for instance a geographical shift of the core of a species range (Pfenniger et al. 2012). Populations located at the leading edge of the shifting species distribution may experience changes of their genetic composition due to expansion effects or colonisation bottlenecks (Garcia-Ramos and Kirkpatrick, 1997; Vucetich and Waite, 2003), while populations in the trailing edge might be unable to shift and their intraspecific diversity threatened by extirpation (Arenas et al. 2012; Pfenniger et al. 2012).

Phylogeographic lineages and other geographically-distinct features such as local adaptive divergence (discussed in more detail in section 1.3) as part of intraspecific genetic diversity play an important role for ecological plasticity, evolutionary potential and the future persistence of species in changing climatic conditions on a global scale (Hughes et al. 2008; Jump, Marchant and Peñuelas, 2009). Identifying regions or populations of high evolutionary potential as resources of high molecular diversity may serve conservation of species and help to mitigate climate change impacts (Pauls et al. 2013). An example of a predicted climate change-induced loss of genetic diversity has been reported for brown alga *Bifurcaria bifurcata*, whose likely poleward shift might result in the loss of a spatially restricted distinct southern lineage or perhaps the overall extirpation of a diversity hotspot in Morocco, representing the species southern range limit (Neiva et al. 2015). Scenarios such as forecasted for *B. bifurcata*, indicating the effect of spatial redistribution and the contrasting future outlooks of divergent lineages, may decrease the levels of genetic diversity globally and have the potential to remove unique evolutionary lineages from species genetic

spectrum in the long term (Neiva et al. 2015). Further, there is a concern for instances where species may respond with a temporal lag to shifted environmental conditions (Tilman et al. 1994), meaning that some species might already follow an extinction trajectory at larger spatial scales, which is not recognised or underestimated in the present (Dullinger et al. 2013). With the probability and extent of delayed extinction risks still under debate, it has been hypothesised that the impact of climate change on contemporary biodiversity and genetic diversity specifically might only become fully evident within the coming decades (Dullinger et al. 2013).

1.3 From the genetic to the genomic perspective

In the past, molecular studies assessed genetic patterns based on mitochondrial and nuclear markers as well as microsatellites, thus covering only a small portion of the overall genomic variation, although it has been advocated that single marker approaches may retain their value in signalling first insights into a species genetic composition and their evolutionary history (Bowen et al. 2014; von der Heyden, 2017). Particularly mitochondrial DNA (mtDNA) *“still likely delivers a very strong and heuristically valuable first approximation of geographic genetic architecture”* (Riddle, 2016; p. 7973). Traditionally utilised markers are thought to be mostly neutral markers and hence limited for the detection of adaptive variation in species, which has been associated with non-neutral (adaptive) markers (i.e. genes under selection) in the genome (Kirk and Freeland, 2011). Advances in sequencing technology have opened the door to whole-genome studies, which allow access to genetic signatures at a genome-wide scale, leading to the differentiation between genetics (studies on certain genes or parts of genes) and genomics (studies on the entirety or a comparatively large proportion of an organism's genes) (Luikart et al. 2003; Schlötterer, 2003; Miller et al. 2007; Baird et al. 2008; Hohenlohe et al. 2010; Li, 2011; Behjati and Tarpey, 2013; Buermans and den Dunnen, 2014). Different techniques have been developed to harness the possibilities of whole-genome sequencing (next generation sequencing (NGS)), with RAD-Seq (restriction site-associated DNA sequencing), one of the reduced-representation sequencing approaches, widely utilised in its various forms (Andrews et al. 2016). RAD-Seq focuses on a subset of the overall genome, which is associated with greater coverage per locus (gene) and can be conducted without prior information of the study species (Andrews et al. 2016). Which percentage of the genome is covered by the RAD-Seq subset depends on the restriction enzyme and the species genome composition, but estimates range from 0.1%-10% (Floragenex, 2015) to 2%-25% (Lowry et al. 2017). Hence, RAD-Seq can be advantageous for studies on non-model organisms (Ekblom and Galindo, 2010). The controlled combination of individuals from one population or geographic location to form a 'meta-

sample' (pool) for sequencing while still using the RAD-Seq approach (pool-Seq), reduces the cost of sequencing individuals (Futschik and Schlötterer, 2010; Toonen et al. 2013; Schlötterer et al. 2014), which is important for comparative phylogeographic studies of non-model species. Moreover, it has been estimated that the cost of next-generation sequencing per base pair might be 1/1000th the investment of traditional sequencing or lower (Bowen et al. 2014). Importantly, genomic data derived from RAD-Seq studies allows for the differentiation between selectively neutral and non-neutral (potentially adaptive) genomic diversity (Luikart et al. 2003; Storz, 2005; Helyar et al. 2011; Grummer et al. 2019) with more methodological ease than in the past (Holderegger et al. 2006; Storfer et al. 2010). This is achieved by separating putatively neutral single nucleotide polymorphisms (SNPs) from putatively adaptive SNPs. Neutral genomic variation is shaped by a variety of determinants encompassing random drift, mutation, population size and connectivity between populations (Frankham, Briscoe, and Ballou, 2002; Gaggiotti et al. 2009; Bragg et al. 2015; Gómez-Fernández, Alcocer, and Matesanz, 2016). In contrast, outlier SNPs signal loci (or genome regions) which are highly differentiated compared to neutral SNPs, as a result of potentially experiencing divergent selection or as a result of genetic hitchhiking (Barton, 2000; Luikart et al. 2003; Storz, 2005; Akey et al. 2010). Conversely, signals of outlier loci may also be artefacts of outlier detection programs (Lotterhos and Whitlock, 2015; Meirmans, 2015).

Population differentiation and signals for possible local adaptation are commonly expected across large-scale environmental gradients as a response to varying environmental pressures (Bradbury et al. 2010; Renaut et al. 2011; Bourret et al. 2013; Guo et al. 2015; Guo, Li and Merilä, 2016; Milano et al. 2014; Stanley et al. 2018; Phair et al. 2019). Spatial molecular variation can be tested for association of genomic patterns with differentiation of environmental variables, the latter might explain differences between populations through driving possible local adaptive divergence (Storz, 2005; Coop et al. 2010; Villemereuil and Gaggiotti, 2015). Past studies on various marine taxa point to genomic population patterns influenced by naturally occurring geographic differences in abiotic factors like temperature, salinity and primary productivity (Jump et al. 2006; Bourret et al. 2013; Milano et al. 2014; Berg et al. 2015; Benestan et al. 2016; Dennenmoser et al. 2017; Dalongeville et al. 2018). However, there is also evidence that populations in broadly homogenous marine environments can also show differentiation of outlier loci, suggesting potential adaptive divergence in close geographical distance or environmental parameters that have not been measured (Nielsen et al. 2009; Freamo et al. 2011; Zarraonaindia et al. 2012; Milano et al. 2014; Ravinet et al. 2016; Wagner et al. 2017; Nielsen et al. 2018). Thus, local selective pressures of the environment can play an important role for structure between populations and potentially restrain gene exchange due to fine-scale "*semi-independent adaptive*

evolutionary trajectories" (Nielsen et al. 2009; p.7), which should be accounted for in the context of delimiting conservation units (Funk et al. 2012; Bernatchez, 2016; von der Heyden, 2017). Moreover, differentiation in outlier loci, associated in most instances with potential local adaptation and historical population factors, has been identified in numerous studies between populations of high gene flow species (Nielsen et al. 2009; André et al. 2011; Milano et al. 2014; Dierickx et al. 2015; Fernández et al. 2016; Cure et al. 2017; Nielsen et al. 2018). This sheds new light on the paradigm that, particularly in marine systems, high gene flow might limit local adaptation effects by facilitating uniform allelic frequency among populations (Hauser and Carvalho, 2008). Weak population structure is possibly not the effect of high gene flow, but owed to large effective population sizes of species constraining the effects of genetic drift (Hauser and Carvalho, 2008). Evidence for highly structured outlier loci between populations indicate that local adaptation to environmental conditions might occur despite shallow neutral population structure, which can be utilised to expand marine conservation and management (Hemmer-Hansen et al. 2007).

Assessing the distribution and scale of adaptive divergence in the marine environment on a local, regional and global scale of marine species natural ranges has been initiated for marine fishes (Conover et al. 2006; Nielsen et al. 2009; Limborg et al. 2012), but the genomic composition and patterns of potential adaptation are currently unknown for the majority of marine taxa, including invertebrates. Comparative phylogeographic approaches with genomic data for multiple, co-distributed species remain underrepresented (but see Gaither et al. 2015; Barrow et al. 2018; Bunnefeld et al. 2018; Nielsen et al. 2018; Crane et al. 2018). Multi-species comparisons allow the exploration of whether genomic responses are shared across different species, or whether species respond differently (Conte et al. 2012; Westram et al. 2014; Nielsen et al. 2018; Stanley et al. 2018). Moreover, testing multiple species across the same geographical range can possibly reveal the scale of selective forces (see for example Ravinet et al. 2016; Nielsen et al. 2018; Stanley et al. 2018). Species distribution and their corresponding evolutionary trajectory are also influenced by historical processes (which is discussed in more detail in section 1.6.1), which can make it challenging to resolve the effects of environmental selective forces and evolutionary history (Hart and Marko, 2010; Marko and Hart, 2011; von der Heyden, 2017). Leveraging advanced molecular technologies to characterise both neutral or potentially local adaptation, is a crucial step towards estimating the evolutionary consequences of climate change on species (Parmesan and Yohe, 2003; Reusch and Wood, 2007; Brown et al. 2016). Importantly, due to the challenge of matching genotype to phenotype, exploring the actual adaptive benefit of a particular locus under natural conditions is a field of ongoing

research (Reusch and Wood, 2007; Hughes, 2007; Holderegger et al. 2010; Hoban et al. 2016; Jones, Arnold and Bürger, 2019).

1.4 Phenotypic plasticity

When faced with changing or novel conditions, organisms can respond within the range of their phenotypic plasticity, which describes the ability of an individual genotype to express varying phenotypes in response to environmental conditions, or with microevolutionary change (Dufty et al. 2002; West-Eberhard, 2003; Hoffmann and Sgrò, 2011; Fox et al. 2019). Moreover, phenotypic plasticity is also regarded as an individual's ability to regulate its physiological processes to withstand current or shifting environmental conditions (Canale and Henry, 2010). Evidence shows that climate change conditions can lead to genetic (evolutionary) changes and phenotypic (plastic) adaptation in some populations, but the empirical differentiation between the two remains challenging to resolve (Hoffmann and Sgrò, 2011; Merilä and Hendry, 2014). While the importance of plasticity in phenotypic adaptation to rapid environmental change is well recognised and frequently regarded as a rapid response mechanism, plasticity can act on different timescales and not all plastic responses are equally important to adapt for fast paced environmental change (Fox et al. 2019). A notable constraint has been identified in the case of plasticity possibly decelerating adaptation through transiently blocking genes from natural selection by shifting the population's phenotype distribution closer to the optimum (Huey, Hertz and Sinervo, 2003). To roundup the perspective on plasticity facilitating adaptation to environmental conditions, plastic responses can also possibly be maladaptive or neutral for an individual's fitness (Ghalambor et al. 2007; Merilä and Hendry, 2014).

Stronger climate variations apply increasing selective pressure on characteristics that are linked to a wider phenotypic plasticity as species are forced to adjust to a decreasingly predictable environment (Pauls et al. 2013). It has been demonstrated that phenotypes with low plasticity in relevant (heritable) traits are selected against in an environment which favours high phenotypic plasticity (Nussey et al. 2005). Despite the importance of phenotypic plasticity as a mechanism to cope with climate change, details on genotypic and phenotypic plasticity are not well-known for many species' groups, particularly for marine species (Hallegraeff, 2010). Furthermore, although laboratory experiments have demonstrated that genetic adaptation can occur within a relatively rapid timescale in certain instances (Schlüter et al. 2014; Listmann et al. 2016; Padfield et al. 2016; Schaum et al. 2017), it remains speculative if species at large can adapt as quickly in the natural environment. Therefore, an individual's phenotypic plasticity is an important potential buffer against the onset of climate

change effects (Somero, 2010). With regards to characterising phenotypic plasticity, studies have shown that species responses explored across environmental gradients or to changing climatic conditions help to estimate the organisms potential adaptive capacity by demonstrating the degree of phenotypic plasticity (of the studied traits) in the overall gene pool of the species populations (Jensen et al. 2008; Fischer and Karl, 2010).

From a physiological perspective, species thermal tolerance to temperature changes and extremes is a key factor in the context of climate change impacts. Phenotypic plasticity (in terms of increased tolerance towards the impacting stress factor) of certain traits might support the survival of individuals before genetic (microevolutionary) changes possibly contribute to local adaptation in the population (phenotypic buffering) (Waddington, 1942; Bradshaw, 1965; Chevin et al. 2013; Reusch, 2014). Changes in temperature, including a possible frequency increase of extreme climatic events, are among the major consequences associated with climate change (IPCC, 2013), making the study of temperature tolerance urgent. However, critical thermal limits and thermal tolerance breadths remain unknown for the majority of organisms (Vinagre et al. 2013). Moreover, temperature is recognised as the main factor determining marine species distributions across the globe (Perry et al. 2005; Sorte et al. 2010; Kleisner et al. 2017; Stuart-Smith et al. 2017) and it has been shown that altered temperature conditions can impose substantial physiological pressure on populations (Pörtner and Knust, 2007; Deutsch et al. 2008; Pörtner and Peck, 2010; Hoffmann and Sgrò, 2011). This makes it crucial to estimate intra- and inter populations thermal tolerance and plasticity, as it can help to plan conservation management frameworks under future change scenarios (Levy and Ban, 2013; Rilov et al. 2019). While most of the world's oceans experience warming sea surface temperature trends, the predicted trends in southern Africa vary from warming to cooling depending on the geographic region, owed to South Africa's dynamic oceanographic setting (discussed in more detail below, but also see Rouault et al. 2009; Rouault et al. 2010). Hence, not only upper, but also lower thermal tolerance limits are relevant for South African marine species (Teske et al. 2019). Upper and lower critical thermal limits (CT_{max} , CT_{min}) are regarded as the "*arithmetic mean of the collective thermal points at which the endpoint is reached*" (Lowe and Vance, 1955; p. 74). The endpoint, considered as a state from which the organism can still recover, is broadly indicated by the organism's loss of equilibrium (Bonin, Lee and Rinne, 1981); for instance the animal stops swimming, is unable to righten itself when inverted or does not exhibit a response to mechanical stimulus. Even though some endpoints may be utilised across similar species, it is required to confirm existing or devise novel endpoints in the experimental setup with the specific study species. The upper thermal maximum (CT_{max}) is regarded as the most reliable parameter for macro-physiological studies on ectotherms (Cowles et al. 1944; Lutterschmidt

and Hutchison, 1997), and as highly useful to investigate particularly upper thermal limits across different taxonomic groups (Deutsch et al. 2008; Huey et al. 2009; Somero, 2010; Vinagre et al. 2013).

1.5 Marine biodiversity and oceanographic setting of South Africa

1.5.1 Marine biodiversity and conservation

South Africa's environment is home to a wide array of terrestrial and marine species and has a high overall level of biodiversity, measured in terms of species richness and levels of endemism (terrestrial: Wynberg, 2002; Driver et al. 2005; Midgley and Thuiller, 2011; marine: Awad et al. 2002; Griffiths et al. 2010; Sink et al. 2012). At least one third of marine species are recognised as endemic (Griffiths et al. 2010). The contemporary southern African coastline formed in the transition from the late Pleistocene (500–20 kya) to the early Holocene (commencing ~11,700 years ago), which determined the contemporary distribution of rocky shores (27%), sandy beaches (42%) and mixed shores (31%) (Davies, 1973; Compton, 2001; Compton, 2011; Fisher et al. 2010; Sink et al. 2012). Across the 136 recognised marine habitat types (58 coastal, 62 offshore benthic, 16 offshore pelagic), at least 47% are threatened (Lombard et al. 2004; Sink et al. 2012). Despite strong research effort over decades, the full extent of marine biodiversity remains unknown, especially for many of the less-well studied and small-bodied taxonomic groups (von der Heyden, 2009; Zemlak et al. 2009; Costello et al. 2010; von der Heyden, 2011; von der Heyden et al. 2011). South African marine species richness is overall high across different taxonomic groups, but was shown to form a gradient, with substantially lower species numbers found on the cold-temperate west coast compared to high numbers of species located on the tropical and subtropical east coast (Harrison, 2002; Awad et al. 2002; Griffiths et al. 2010). This is also mirrored in genetic diversity, which has been hypothesised to follow a trend of increasing haplotype diversity from the west coast eastwards (Wright et al. 2015), providing some indication that historical and contemporary processes shape species and genetic diversity together.

It is established that molecular characteristics of species populations greatly inform conservation planning processes (Moritz, 2002; Funk et al. 2012; Bowen et al. 2014; Selkoe et al. 2016; Xuereb et al. 2019; Lopez et al. 2019), but the integration is currently still not commonly applied (von der Heyden, 2009; Laikre, 2010; Begger et al. 2014; von der Heyden et al. 2014; Xuereb et al. 2019). Marine spatial planning can be enhanced by utilising even basal population genetic characteristics (Nielsen et al. 2017b; Begger et al. 2014; von der Heyden, 2017). The higher resolution power of genomic data has sparked a growing number

of studies aiming to detect adaptive potential and to determine how genomic variation could be shaped by varying environmental conditions (Baird et al. 1998; Schmidt et al. 2008; Lexer et al. 2014; Stanley et al. 2018; Teske et al. 2019; Xuereb et al. 2019), which allows for more complex population genomic patterns to be considered in delineating conservation areas (Loeschcke, Tomiuk and Jain, 2013; Funk et al. 2012; Narum et al. 2013; Bowen et al. 2014; Shafer et al. 2015; Selkoe et al. 2016; Xuereb et al. 2019). In South Africa, the importance of integrating findings from molecular population analyses into marine conservation planning has been advocated for more than a decade (von der Heyden, 2009). Established marine protected areas (MPAs) were historically skewed towards the east coast with its higher number of species than the south and west coast (von der Heyden, 2009; Griffiths et al. 2010; Sink et al. 2012; Majiedt et al. 2013). South African MPAs are further limited by lacking habitat type representation between coastal regions, lack of consistent regulation enforcement and inadequate acknowledgement of social impacts (von der Heyden, 2009; Griffiths et al. 2010; Sink et al. 2011; Wright et al. 2015; Sowman, 2015; Sowman and Sunde, 2018). Only 0.4% of the South African mainland ocean territory had some degree of protection (Sink, 2016), when twenty new MPAs were announced in October 2018, which increases the percentage of protected ocean space within the borders of the South African Exclusive Economic Zone to 5% (DEA, 2018). Importantly, the new Namaqua National Park MPA (500 km²) constitutes the first coastal marine protection in the west coast region, which hosts numerous rocky shore populations (Sink et al. 2012; Majiedt et al. 2013). The importance of marine conservation efforts on the west coast, and elsewhere in South Africa, was emphasised in multiple studies (Sink et al. 2012; Majiedt et al. 2013; Wright et al. 2015; Nielsen et al. 2017b; Mertens, Treml, and von der Heyden, 2018) and genomic insights can contribute to ongoing conservation efforts by analysing potential regional differences in species molecular diversity and population structure.

South African oceanography and marine bioregions

Patterns of biodiversity in the South African region are shaped to a large extent by the different environmental conditions created by two dominant current systems driving differences in sea surface temperature and primary productivity (Bustamante et al. 1995) (Figure 1.2). The west coast is characterised by the influence of the cold water of the northwards flowing Benguela Current system, which makes this part of the southern Atlantic Ocean one of the most dynamic and productive upwelling regions in the world (Nelson and Hutchings, 1983; Shannon, 1985; Shannon and Nelson, 1996). The Benguela system is characterised by seasonal upwelling and relatively cool temperatures, but high primary productivity. In contrast, the warm-water Agulhas Current of the east coast forms part of the

southernmost reaches of the western Indian Ocean and flows southwards along South Africa's coastline (Lutjeharms and Ballegooyen, 1988; Lutjeharms and Ruijter, 1996). It then retroflects along the Agulhas bank on the southern Cape coast. The Agulhas and the Benguela currents form an oceanographic convergence zone mainly at the southern tip of the African continent, which has characteristics of both oceanographic systems (Nelson and Hutchings, 1983; Lutjeharms and Ballegooyen, 1988; Lutjeharms and Ansorge, 2001). In addition, large anti-cyclonic Agulhas rings and filaments cause warm Agulhas water to flow into the South Atlantic at certain times throughout the year and have been implicated in the transport of larvae along the coastline (Ballegooyen et al. 1994; Lutjeharms and Cooper, 1996). Another characteristic of the oceanography in the region is the Agulhas counter-current, which flows west- and northwards and in close proximity along the south-eastern shore (Duncan, 1970; Wyrski, 1973). To a smaller degree, nearshore counter currents and regional eddies have also been identified for the South African west coast (Boyd et al. 1992; Mertens, Trembl and von der Heyden, 2018).

The Benguela Current and the Agulhas Current form a temperature gradient from west (cold-temperate) to east (tropical) (Figure 1.2), with mean temperatures on the west coast ranging seasonally from 11-15°C with short-term fluctuations, whereas for the warmer east coast mean temperatures range from 18-27°C (Smit et al. 2013). Moreover, the south coast broadly forms a continuum of temperature between the South African west and east coast (Smit et al. 2013). There is evidence that the thermal gradient along the coast might have led to temperature-defined marine bioregions (cool-temperate, warm-temperate, subtropical and tropical; Figure 1.2) (Teske et al. 2011a). Comparing the genomic structure between populations of the sand goby *Psammogobius knysnaensis* inhabiting different coastal regions showed that temperature-driven selection likely resulted in early signs of ecological speciation (Teske et al. 2019). Differences in the species gene regions linked to temperature suggest that the divergent thermal environment may delimit temperature-defined marine bioregions. Importantly, the thermal gradient across the southern African coast could drive ecological divergence between presumed locally adapted populations of *P. knysnaensis* (Teske et al. 2019). Based on environmental and species composition, the South African coastline (ca. 3,650 km) has been broadly categorised into four coastal ecoregions (Benguela, Agulhas, Natal and Delagoa) and two offshore ecoregions (South-East and South-West Atlantic; Sink et al. 2012). The largest habitat type proportions are rocky shore (27%), sandy beaches (42%) and mixed shore (31%) (Sink et al. 2012), but there are other habitats including seagrass meadows, mangrove systems and coral reefs, thus supporting a broad array of species.

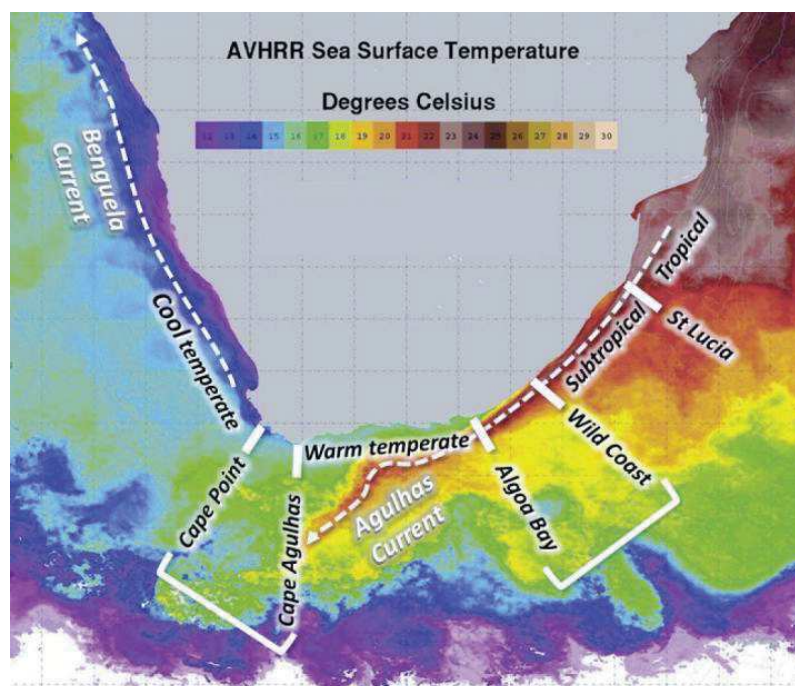


Figure 1.2. South Africa's thermal environmental gradient, marine bioregions and recognised costal genetic breaks. The cold Benguela Current on the west coast and the warm Agulhas Current on the east coast are associated with four temperature-delimited marine bioregions: cool-temperate, warm-temperate, subtropical and tropical. Four locations have been suggested based on genetic data as genetic breaks: Cape Point, Cape Agulhas, Algoa Bay and St. Lucia (Source: Teske et al. 2011).

1.5.2 Genetic breaks and the spatio-temporal variation of molecular variation

In South Africa, numerous marine species inhabit multiple biogeographic regions and there are on the one hand examples of the absence of genetic structure across species distribution (see for example Neethling et al. 2008; Teske et al. 2010; Mmonwa et al. 2015) and on the other hand examples of multiple species indicating population structure between marine bioregions (Teske et al. 2006; Teske et al. 2011a; Zardi et al. 2015; Baldanzi et al. 2016). Interestingly, various phylogeographic and phylogenetic studies suggest regionally distinct genetic lineages, whose phylogeographic breaks often align with the temperature-delimited marine bioregions (von der Heyden, 2009; Teske et al. 2011a). For instance, Cape Point (which is also a major biogeographic break; Teske et al. 2007a; von der Heyden, Prochazka and Bowie, 2008) and Cape Agulhas (Evans et al. 2004; Teske et al. 2007b), broadly forming the disjunction between the cool-temperate west coast and the warm-temperate south coast, have been identified as major genetic breaks (von der Heyden, 2009; Teske et al. 2011a). Moreover, the dynamic oceanographic transition zone between Cape Point and Cape Agulhas has revealed instances of genetically distinct signals in populations (Figure 1.2) (Teske et al. 2006; Teske et al. 2007a). Apart from the brown mussel *Perna perna* (Zardi et al. 2007), genetic breaks are less pronounced between the warm-temperate south coast and subtropical east coast. However, genetic disjunctions have been located in

the area of Algoa Bay (Teske et al. 2007c; von der Heyden, Prochazka and Bowie, 2008) and on the central Wild Coast (Teske et al. 2006; Teske et al. 2008). Furthermore, phylogeographic breaks have been detected in the region of St. Lucia (Teske et al. 2007d; Teske et al. 2009), broadly representing the transition from subtropical to tropical east coast, or even further north near Mozambique (Gopal et al. 2005; Ridgway et al. 2008). Comparative mtDNA data of eleven South African rocky shore and reef-dwelling marine species indicates overall steep isolation-by-distance (IBD) slopes for all species (but see Teske et al. 2018) and found that several broadcast-spawning species demonstrate similar levels of population structure as live-bearing clinid fishes (Wright et al. 2015). This unexpected finding might be influenced by factors such as egg size, spawning time and frequency, larval type, larval behaviour and ecological transitions across biogeographic regions (Wright et al. 2015). It has also been shown that the two closely related intertidal topshells *Oxystele variegata* and *O. tigrina* yielded strongly contrasting signals of genetic differentiation in the context of similar habitat requirements (Wright et al. 2015). In addition, the sympatric beach lice *Excirolana natalensis* and *E. latipes* show distinct differences in phylogeographic structure, with one species defined by a strong genetic break across Cape Point and one showing connectivity between the west and south coast populations (Mbongwa, 2018). The aforementioned studies primarily pertain to rocky shore species and near shore coastal species and less is currently known about the phylogeographic structure of sandy shore species (Grant and da Silva-Tatley, 1997; Laudien et al. 2003; Muteveri et al. 2015; Baldanzi et al. 2016; Mbongwa et al. 2019), despite international acclaim for South African beaches ranking among the best-studied globally (Nel et al. 2014). Populations of the sandy beach plough shell *Bullia digitalis* displayed no population structure over their range of 2,400 km based on allozymes (Grant and da Silva-Tatley, 1997). A second allozyme study conducted with the surf clam *Donax serra* in Namibia and South Africa noted strong geographic separation between two Namibian populations, but unexpected genetic similarity between the remaining populations despite marked morphological differences (Laudien et al. 2003). Findings of mtDNA from the co-distributed plough shell *Bullia rhodostoma* revealed shallow genetic differentiation between False Bay (east of Cape Point) and Port St. John's on the east coast (Muteveri et al. 2015). In contrast, the sandhopper *Talorchestia capensis* consists of three separate evolutionary significant units (Baldanzi et al. 2016) and populations of the sandy beach isopod *Tylos granulatus* appear to be genetically isolated from each other across the species distribution spanning South Africa and Namibia (Mbongwa et al. 2019). The phylogeography of marine flora remains understudied in the region, but a recent genomic study on the seagrass *Zostera capensis* distributed in southern and eastern Africa identified low genomic variation (but high clonality)

between populations spanning nearly 10,000 km and suggested two geographically distinct population clusters (Phair et al. 2019).

In summary, genetic and genomic studies find congruent phylogeographic breaks for some South African coastal marine species, but more complex patterns for others, with genetic boundaries often appear to associate with biogeographic differences between the coastal regions (von der Heyden, 2009; Teske et al. 2011a). Recent evidence suggests that thermal regimes may drive incipient ecological speciation along the coastline (Teske et al. 2019).

Drivers of molecular connectivity

Species reproductive strategies (for example brooding, live-bearing and broadcast spawning), larval behaviour, recruitment and retention rates, dispersal scale, pelagic larval duration (PLD), settlement processes and other life history traits and ecological variables are known to impact spatial genetic population patterns of marine species (von der Heyden, 2009; Teske et al. 2011a; Baguette et al. 2012; Pfaff et al. 2015; Selkoe et al. 2016; Bowen et al. 2016; Mertens, Trembl and von der Heyden, 2018). During their pelagic phase, larvae can disperse or be hindered to disperse by land masses or dynamic ocean currents (Wares, Gaines and Cunningham, 2001; White et al. 2010). South Africa's coastline is broadly "V-shaped" with two different ocean current regimes on either side, which impacts connectivity between coastal populations (Teske et al. 2011a). Apart from the "V-shaped" coastline, South Africa's coastal topography does not feature prominent geographical barriers, but dispersal of species can be influenced by local-scale oceanographic features like near-shore counter currents and eddies (see for example Teske, Bader and Golla, 2015; Mertens, Trembl and von der Heyden, 2018). It has been suggested for the majority of benthic marine species with complex life cycles, particularly for species with sessile adult stages or with limited mobility, that the exchange of individuals between populations primarily occurs during the (pelagic) larval stage (Cowen and Sponaugle, 2009). If the larva (or adult individual) settles in a population different from its origin and succeeds to reproduce, it is regarded as a migrant and contributes to the genetic/genomic connectivity of the two populations (Pineda et al. 2007). It has been hypothesised that, if the local environmental conditions of the new population exceed the migrant's physiological capacities, such as an individual from a subtropical area attempting to settle in a population in a temperate area (see for example the mudprawn *Upogebia africana* and the brown mussel *Perna perna* (Teske et al. 2008; 2011; Zardi et al. 2011)), it might not be able to survive and hence populations become geographically disjunct and province-specific adaptations might become more pronounced (Cowen and Sponaugle, 2009; Teske et al. 2011a; Baguette et al. 2012; Nosil, 2012). Thus,

it is not unexpected to find biogeographic and phylogenetic patterns overlap, as local environmental conditions in different geographic regions, representing contrasting selective forces, can maintain molecular structure between populations by reducing gene exchange (Teske et al. 2011a; Zardi et al. 2011; Nosil, 2012; Teske et al. 2019). Temperature differences between coastal regions and possible regionally specific physiological adaptations have been suspected as drivers of species divergence and possible speciation in *P. knysnaensis* (Teske et al. 2019). In summary, marine species genetic structure is known to be influenced by ecological, environmental, oceanographic and physiological factors (von der Heyden, 2009; Teske et al. 2011; Bowen et al. 2016; Selkoe et al. 2016), with an important additional potential driver, paleo-climatic fluctuations, explored in the following section.

1.6 Paleoclimate and future climate change impacts in South Africa

1.6.1 Influence of paleoclimate on marine species

Global climate oscillations associated with glacial periods comprised sea level fluctuations of 115-130 m below current level, which caused changes in ocean currents, altered the thermal dynamics of the water column and shifted habitable area for marine intertidal communities (Pillans, Chappell and Naish, 1998; Bintanja, van de Wal and Oerlemans, 2008; Ludt and Rocha, 2015). These changes have influenced the genetic composition of intertidal species (Hewitt, 2000; Ludt and Rocha, 2015), although it is not always easy to disentangle historical and contemporary drivers of population genetic parameters. The magnitude of habitat availability changes has been studied in tropical coral reefs, where sea-level lows decreased up to 92% of habitable shelf area (<60 m depth), from which it has been estimated that habitat-related separation between populations could be associated with population genetic bottlenecks in marine species globally (Ludt and Rocha, 2015). In the southern African region, paleoenvironmental models show that the Pleistocene (2.6 Ma–11.7 ka) glacial cycles brought periods of drastic changes in not only sea levels and ocean currents, but also temperature and salinity (Davies, 1973; Hewitt, 2000; Compton, 2001; Fisher et al. 2010; Compton, 2011), which impacted the distribution of intertidal communities and the connectivity of populations (von der Heyden et al. 2010; Teske et al. 2011; Toms et al. 2014; Seymour, 2016). The periodic reduction of habitable coastal area separating populations has been linked to numerous signals for long-standing historical isolation and in some instances might have given rise to divergent lineages in marine species (von der Heyden et al. 2010; Teske et al. 2011b; Muller et al. 2012; Henriques et al. 2012; Phair et al. 2015; Reynolds et al. 2014; Toms et al. 2014; Mmonwa et al. 2015; von der Heyden et al. 2015; Swart et al. 2016; Potts et al. 2016). Furthermore, fluctuating habitat availability has been suspected to

play an important role for genetic breaks or instances of speciation in marine species in South Africa (Toms et al. 2014; Potts et al. 2016). Periodically decreasing and increasing sea levels have been documented to lead to several episodes of complex patterns of population colonisation, expansion and contraction (Wares, 2002; Hewitt, 2004). Signs for such demographic expansions have been reported for a variety of marine taxa in South Africa, including fishes and invertebrates, both for rocky and sandy shore species (der Heyden et al. 2008; der Heyden et al. 2011; Muller et al. 2012; Reynolds et al. 2014), although these must be interpreted with caution (Grant and Bowen, 1998).

Briefly, the evolutionary history and population structure of numerous South African marine species may have been profoundly influenced by paleo-climatic shifts during glacial periods (Teske et al. 2011b; Toms et al. 2014). The current South African marine environment incorporates multiple drivers which can act to maintain historical patterns or influence contemporary molecular patterns of population differentiation including the temperature differences and other environmental variation between the coastal regions (von der Heyden, 2009; Teske et al. 2011a; Teske et al. 2019), as well as local-scale oceanographic features like near-shore counter currents and eddies (Teske, Bader and Golla, 2015; Mertens, Trembl and von der Heyden, 2018). Moreover, regionally specific physiological adaptations might lead to population divergence or even speciation (see for example Teske et al. 2008; Teske et al. 2011a; Zardi et al. 2011; Teske et al. 2019). Further, ecological factors such as life history traits, particularly related to reproduction or dispersal and other ecological variables are known to impact genetic population patterns of marine species (von der Heyden, 2009; Teske et al. 2011a; Baguette et al. 2012; Pfaff et al. 2015; Selkoe et al. 2016; Mertens, Trembl and von der Heyden, 2018).

1.6.2 Climate change in South Africa's marine environment

In South Africa, as elsewhere, there have been several signals of changing marine climates. Signs of the onset of changes in South African climatic conditions include an increase in mean annual air temperature by 0.13°C per decade (measured between 1960 and 2003) (Kruger and Shongwe, 2004), with further increases expected depending on the emission levels relative to pre-industrial values (Engelbrecht and Engelbrecht, 2016; Maúre et al. 2018). Moreover, rainfall patterns have changed in recent decades, with an increase in drought and wet periods on a seasonal scale (most pronounced on the south and lower east coast) (Rouault and Richard, 2003; Kruger, 2006), which has knock-on effects on run-off into marine estuarine and marine systems. A noticeable change in sea surface temperature (SST) has been recorded for the Agulhas Current, which has warmed by 1.5°C since the

1980s due to changing wind patterns in the southern Indian Ocean (Rouault et al. 2009). As a result, the east coast has a positive trend in annual SST (Rouault et al. 2010). In contrast, a significant cooling SST trend of up to 0.5°C per decade in the southern Benguela has been reported in the months January to August and to a lesser magnitude also for the south coast in the region of Port Elizabeth/Port Alfred in the months May to August (Rouault, 2011). Cooling on the west coast is thought to be caused by an increase in upwelling intensity and frequency, which is driven by increased southerly winds, particularly in summer (Rouault et al. 2010). Moreover, the west coast is periodically influenced by the ENSO (El Niño-Southern Oscillation), which affects air temperature, wind, rainfall and SST (Rouault et al. 2010; Dufois and Rouault, 2012). ENSO events and their strength, duration and impact are challenging to anticipate, which makes ecological forecasts based on climate predictions more complex in the South African context (James and Hermes, 2011).

Sea level increase varies on the South African coast (Mather et al. 2009). The west coast showed an average increase of 1.87 mm y⁻¹ (from 1959-2006), the south coast of 1.48 mm y⁻¹ (1957-2006) and the east coast of 2.74 mm y⁻¹ (1967-2006) (Mather et al. 2009). After correction of the values for changes in the barometric pressure in the regions and vertical crust movements, sea level appears to increase from west to east, which is influenced by the Agulhas and Benguela currents and their interactions (Mather et al. 2009), but also impacted by the recent warming of the Agulhas Current (Rouault et al. 2009). Increasing sea levels are currently regarded as a minor threat to South Africa's coast, as intertidal species are supposed to *"simply move higher up on the shore"* (Griffiths et al. 2010; p. 10). This suggestion is challenged by the observation that appropriate coastal habitat does not extend infinitely inland. If sea levels rise as much as predicted in the higher IPCC emission scenarios (IPCC, 2013), the limitation of coastal habitat availability poses a long-term concern, particularly in those areas where the coastal margin is narrow. Sea level change would impact different habitat types in different ways; for example from a rocky shore perspective, the most affected shores would be situated along the east coast, where rock platforms are located on the lower shore and bounded by sandy habitat further above (Griffiths et al. 2010). As a consequence of changing horizontal species distribution in the intertidal, a vertical squeezing was observed for the upper limit of species due to rising air temperature on the northern west coast of North America (Harley, 2003), which may also develop into a significant challenge for South African intertidal species in the future. Due to steep topography and anthropogenic constructions preventing species migrating inland, it has been estimated that certain ecologically important bays in North America might face a reduction of 20-70% of intertidal habitat area during the next 100 years (Galbraith et al.

2002), illustrating how drastic the effect of vertical squeezing might impact on intertidal communities in other parts of the world.

In addition to rising sea levels, a significant increase in wave height around South Africa of 5 cm decade⁻¹ has been recorded (Trenberth et al. 2007). Moreover, peak wave height during winter storms has increased in offshore waters (1994-2008; ~0.5 m over 14 years) (Guastella and Rossouw, 2009). Wave action and the impact of storm events play an important role for intertidal ecosystems (Blamey and Branch, 2009), as both can influence species presence/absence and the associated trophic structure (McQuaid and Branch, 1984; McQuaid and Branch, 1985). Rising sea levels and increased extreme weather events could in turn also affect disturbance to coastal ecosystems, by increasing sediment transport in (Drinkwater et al. 2010) and changes in ocean current patterns can impact on pelagic larval dispersal and later recruitment success (Menge et al. 1999).

In a sensitivity assessment of the southern Benguela region, 40 marine species' sensitivity and capacity to face climate change were estimated, which revealed several species including the abalone *Haliotis midae*, the soupfin shark *Galeorhinus galeus* and the white steenbras *Lithognathus lithognathus* as highly vulnerable to impacts from changing climatic conditions (Ortega-Cisneros et al. 2018). Moreover, continued changes in air and sea temperatures may shift currently recognised biogeographic zones in South Africa, which in turn drive alterations in species distributions (Mead 2011; Mead et al. 2013; Blamey et al. 2015). Considering the observed trends for regional warming and cooling in South Africa, it might be for example expected that the south coast climatic zone with its warm-temperate conditions might be reduced, which means that intertidal species might face physiological pressure from which a successful shift into either direction along the shoreline is not possible. Examples of current and imminent climate-driven coastal distribution changes in South Africa have been identified in species including fishes, zoobenthos and mangroves (Potts, Götz and James, 2015; Whitfield et al. 2016), but shifting communities and ecosystems are not well understood at present. Strong evidence for species on the move comes from a multi-year study on a South African estuarine fish community showing indications for a climate-induced change in community composition (James et al. 2008). James et al. (2008) showed that with increasing sea surface temperatures, subtropical and tropical fish species started occurring in the East Kleinemonde Estuary, indicating a southward extension of the fishes from the tropical into the warm-temperate region (James et al. 2008). Interestingly, potential southward shifts by (sub)tropical species are limited by signals of decreasing sea temperatures along the west and parts of the south coast (Rouault et al. 2009), thus potentially restricting adequate habitat in the future. Possibly as a result of

the gradually cooling temperature in these areas, the distributional limits of *Ecklonia maxima* kelp beds have been observed to shift eastwards along the south coast since 2006, where they were not historically found (Bolton et al. 2012). This study hypothesised that a continued cooling trend might cause *E. maxima* kelp beds and the species depending on the habitat-forming ecosystem engineer to gradually expand eastwards, until warming temperatures on the southern east coast have a limiting effect to further eastward expansion (Bolton et al. 2012). Moreover, a shift in community composition in False Bay (900 km²) (a strong transition zone between the cold-temperate west coast and warm-temperate south coast) has been partially associated with climate change, where a decline in native warm-water mussel *Perna perna* and an increase in alien cold-water mussel *Mytilus galloprovincialis* (and in kelp) have been recorded (Mead, 2011). False Bay's species composition changed significantly based on a dataset spanning 76 years, although the shift is thought to be associated with factors beyond changing water temperatures alone (Mead, 2011). Regarding rocky shore communities throughout South Africa, Mead highlights that the number of cold-water species increased and warm-water species decreased on the west and south-east coast, which might be linked to cooling of near-shore water as part of complex biological interactive mechanisms (Rouault et al. 2010; Mead, 2011). These instances of large-scale shifts illustrate that it is essential to understand and possibly anticipate coastal species response to climate change conditions.

1.7 Study species

An important factor for the selection of the study species was the availability of genetic findings from past studies (Muller et al. 2012; Wright et al. 2015; Mmonwa et al. 2015; Mertens, Treml and von der Heyden, 2018; Nielsen et al. 2018). Moreover, all three species are broadly co-distributed across multiple marine bioregions. The invertebrates represent non-model species, which are not commercially harvested and abundant in South African rocky shore communities. Overall, the species have a diverse range of life history traits, inhabit different microhabitats and ecological niches in the intertidal and belong to three phyla (Mollusca, Echinodermata, Arthropoda).

1.7.1 Granular limpet - *Scutellastra granularis* (Patellidae, Patelloidea)

Scutellastra granularis (Linnaeus 1758) (max. 60 mm; Figure 1.3) is morphologically characterised by a cap-shaped shell with a distinct reddish spot on top and a ring of gills around the foot. The geographical distribution in southern Africa spans the coastline of Namibia to approximately Port St. John's on the southern east coast (Branch et al. 2017). As

a grazer, *S. granularis* has an important function for the intertidal community by maintaining bare rock surface (Hawkins and Hartnoll, 1983), but it is also recorded as a facultative generalist feeder. Adult individuals are assumed to maintain a limited home range in the high shore (Gray and Hodgson, 1997; Branch et al. 2007), but the duration, distance and frequency of foraging excursions are unknown. *Scutellastra granularis* is a broadcast spawner and its veliger larvae have been observed to stay in the water column for up to 7-14 days, before they settle in the lower shore (Kilburn and Rippey, 1982; Kay, 2002). There appears to be variability in spawning, which has been observed from June to August (Branch, 1974), but possibly also throughout the year with peaks in winter and summer (Vat, 2000; Hodgson, 2010). Juvenile *S. granularis* move upwards into the upper balanoid zone to settle (Nakin and McQuaid, 2014).

The population structure of *S. granularis* has been the focus of several studies (Mmonwa et al. 2015; Nielsen et al. 2018; Mertens, Trembl and von der Heyden, 2018) (Table 1.1). While *S. granularis* tested panmictic along the west coast based on the COI gene (cytochrome c oxidase subunit I) (mtDNA; pairwise Φ_{ST} : 0-0.05) (Mertens, Trembl and von der Heyden, 2018), a broader scale including also the south and east South African coast reports that *S. granularis* is possibly separated into two distinct lineages between the west coast populations (1) and the southern/eastern coast populations (2) with limited genetic exchange between the two groups (mtDNA (COI); global Φ_{ST} : 0.023) (Mmonwa et al. 2015). The first genomic study on the species, conducted across six populations on the west coast, confirms no strong signal of structure in selectively neutral and outlier data (SNPs; pairwise Φ_{ST} : 0.008-0.013) in the region (Nielsen et al. 2018). Yet, the study documents that Hondeklip Bay, one of the northernmost populations, might be evolutionary unique based on the number of detected private SNPs (226 private SNPs; remaining populations: 9-42) and potential outlier SNPs (30 outlier SNPs; remaining populations: 8-19). Values for nucleotide diversity range from 0.009-0.012 (Tajima's π) to 0.010-0.013 (Watterson's θ), with no geographical pattern (Nielsen et al. 2018).

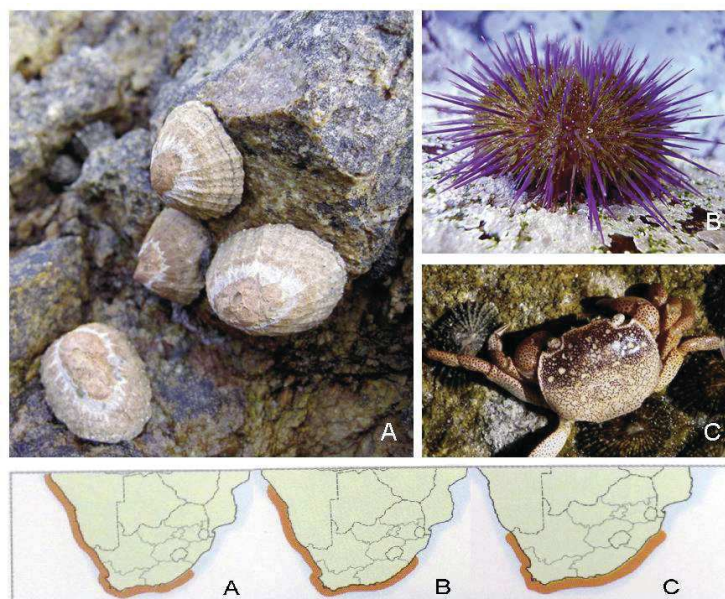


Figure 1.3. Study species and their distribution in southern Africa. *Scutellastra granularis* (A), *Parechinus angulosus* (B), *Cyclograpsus punctatus* (C) (Adapted from: Branch et al. 2017). It is debated whether *C. punctatus* occurs on the entire west coast as shown in the map or only up to Jacob's Bay, where sampling could be successfully conducted.

Granular limpets are functionally sessile (sedentary) during low tide and are occasionally observed to form aggregates, but do not create home scars as some other limpet species (Branch and Branch, 1981; Branch et al. 2017; personal observation). Juvenile *S. granularis* improve their recruitment by settling onto shells of the invasive mussel *Mytilus galloprovincialis* as secondary substratum (Branch et al. 2010), but adult limpet densities decline in the presence of *M. galloprovincialis* (Sadchatheeswaran et al. 2015). *Mytilus galloprovincialis* may reduce the abundance of adult limpets and decrease their median size by half over time (Griffiths et al. 1992; Hockey and van Erkom Schurink, 1992).

In terms of physiology, *S. granularis* has been tested in a South African study alongside another patellogastropod limpet in comparison with two pulmonate intertidal limpets regarding their upper thermal tolerance measured as median lethal temperature (LT50) and cardiac Arrhenius breakpoint temperature (ABT) (Kankondi, McQuaid and Tagliarolo, 2018). The study reports no differences in the overall upper thermal tolerance limits between patellogastropod and pulmonate limpets in both air and water despite their differences in respiratory morphology. In both media, *S. granularis* had lower LT50 and ABT values than the other three species, based on overall small *S. granularis* sample sizes (LT50: $n = 3$; ABT: $n = 10$). The final cardiac breakpoint temperature in *S. granularis* appears to be, similar as shown in (mid to high shore) patellogastropod limpet *Lottia digitalis* (Bjelde and Todgham, 2013), higher in air than in water, which might be associated with the efficient oxygen

absorption via the strongly vascularised mantel cavity of high-shore patellid limpets (Kankondi, McQuaid and Tagliarolo, 2018).

1.7.2 Cape urchin - *Parechinus angulosus* (Echinidea, Echinoida)

Sea urchin *Parechinus angulosus* (Leske 1778) (max. 60 mm; Figure 1.3) is characterised by short pointed spines of various colour variations. It inhabits rocky shores and kelp beds from Torra Bay region in Namibia to northern Kwazulu-Natal in the area of Margate (Branch et al. 2017), although its peak distribution appears to be on the west and south-west coasts, with lower densities of urchins found along the south-east and eastern coastlines (von der Heyden, personal communication). It counts as the most widespread southern African echinoid (Day and Branch, 2002; Branch et al. 2007). *Parechinus angulosus* has a key functional position in the ecosystem as grazer, controlling the recruitment of young kelp plants (Fricke, 1979a; Stuart and Field, 1981; Branch et al. 2007). Specimens can be found to a depth of 30 m, although they are most abundant in the intertidal and shallow sub-tidal. The Cape urchin does generally not adjust its selected position during low tide, which can be in low shore to mid shore rock pools or in low shore open water. For the purpose of this study, all urchins were collected from open water. Reproduction is thought to peak twice a year, with a major spawning event in August/September (spring) and a minor spawning event in April/May (autumn), but this observation has not been empirically evaluated since the 1980s (Fricke, 1979b; Hodgson, 2010). Experiments showed that the echinopluteus larvae of *P. angulosus* can stay in the water column for a few weeks (49 to 56 days) and their active choice of settlement substrate can be delayed by 11 days if no suitable substrate is available (Cram, 1971; Greenwood, 1975). Adult individuals reach maturity after 1-2 years (Greenwood, 1975).

Despite factors including the planktonic larval stage, the ability to spend an extended period in the water column and substrate-selective larval behaviour, genetic connectivity between populations on the southern African coast appears to be limited based on the mitochondrial COI marker and the nuclear egg jelly protein (SpREJ9) marker (Muller et al. 2012) (Table 1.1). Two distinct geographic groupings (west coast populations (1), south and east coast populations (2)), separated by the documented biogeographic barrier Cape Peninsula (Cape Point), were reported (Muller et al. 2012). Interestingly, there is also a high degree of COI (mtDNA) differentiation between west coast populations (Table 1.1), including a significant signal for isolation-by-distance (IBD) for the species (Mertens, Treml and von der Heyden, 2018). Conversely, a SNP-based study sampling six locations on the South African west coast did not recover strong signals of genomic structure in selectively neutral and outlier

data (Nielsen et al. 2018). For *P. angulosus*, the most northern population Port Nolloth appears to be evolutionary unique based on numbers of identified private SNPs (14 private SNPs; remaining populations: 2-6). The potential outlier SNPs ranged from 4-8 across all populations. The study also suggested that values of nucleotide diversity are highest in Port Nolloth (Tajima's π : 0.011; Watterson's θ : 0.012) (Nielsen et al. 2018). Patterns between genetic and genomic findings of *P. angulosus*' west coast populations differ, which is illustrated by the contrasting population structure (pairwise Φ_{ST}) values of COI (mtDNA) (0.15-0.62) and SNPs (0.006-0.019) (Table 1.1); and a significant signal of IBD (mtDNA) versus no signal of IBD (SNPs). Mitochondrial DNA (mtDNA) is uni-parentally inherited and is thought to indicate species' evolutionary history (Avice, 2000; Dudgeon et al. 2012; von der Heyden, 2017), whereas SNPs are bi-parentally inherited and show, utilising a much larger scale than mtDNA markers, contemporary genomic variation, more recent population structure and potential adaptive variation (loci under selection) (Morin, Luikart and Wayne, 2004; Allendorf, Hohenlohe and Luikart, 2010; Funk et al. 2012; Bowen et al. 2014; von der Heyden, 2017). For marine species, it has been suggested that mtDNA can in many cases, but not in every circumstance, constitute the first locus to reveal molecular structure based on its smaller effective population size (Timm et al. 2012, DeBoer et al. 2014). While mtDNA findings can provide a strong initial assessment of species historical population structure, genome-wide analyses are necessary to differentiate between neutral and selective patterns (Bowen et al. 2014).

Critical thermal limits of adult *P. angulosus* have not been previously investigated. The Cape urchin uses debris and shells as a form of sun-shading (Branch, personal communication; personal observation), which might alleviate thermal stress and conceal from predators. Gametes of *P. angulosus* have a tolerance limit towards ambient temperature and salinity levels (Greenwood and Bennett, 1981). Globally, data on critical thermal limits in adult sea urchins is comparatively scarce (discussed in more detail in section 4.4.6). Across several temperate urchin species, upper thermal limits were shown to range from 19.5 to 26.8°C (Morley et al. 2016).

1.7.3 Shore crab - *Cyclograpsus punctatus* (Brachyura, Varunidae)

Cyclograpsus punctatus H. Milne Edwards 1853 (max. 30 mm; Figure 1.3) is distributed from the west coast in the area of Jacob's Bay to the east coast in the area of Margate (northern KwaZulu-Natal; Branch et al. 2017). It possesses a carapace with convex sides and generally smooth body and nippers (Branch et al. 2007). The body is typically dark brown to black at the front of the carapace and blends into a grey-green pattern at the back. The legs

range from brown to orange and carry red spots (Peer et al. 2014). *Cyclograpsus punctatus* scavenges, preferably during the night and at low tide, on seaweeds and available animal matter (Branch et al. 2007). The shore crab is mobile and seeks shelter from sun exposure and predators in crevices during low tide. Juvenile shore crabs hatch from eggs which are held by female crabs and develop into planktonic larvae with an estimated pelagic larval duration (PLD) of 14 days (Branch et al. 2007; Wright et al. 2015). Two breeding seasons have been observed in *C. punctatus*, with a major peak in July (mid-winter) and a minor peak during January/February (summer) (Broekhuysen, 1940; Hodgson, 2010; Bliss and Mantel, 2012). Winter is also the main reproduction period on the warmer eastern coast and evidence has been recorded that more than one brood is produced during breeding season (Broekhuysen, 1940; Hodgson, 2010).

Molecular data revealed a high degree of genetic structure in the species (COI (mtDNA); Table 1.1) in comparison to ten other rocky shore organisms and a significant signal of isolation by distance (IBD) when tested across the South African west, east and south coast (see Wright et al. 2015; Table 1-2, Figure 1.3a). This study constitutes the first SNP-based analysis of *C. punctatus*. From an ecological perspective, *C. punctatus* benefits from assemblages of invasive mussel *M. galloprovincialis*, resulting in higher overall levels of crab biomass, although this was only recorded for the predominantly sandy Langebaan Lagoon (Robinson and Griffiths, 2002). It is not entirely resolved if *C. punctatus* lives predominantly high on the shore (intertidal; Fagetti and Campodonico, 1971; Branch et al. 2007) or mainly in potentially overlapping lower regions of estuaries (estuaries; Alexander and Ewer, 1969; Hill, 1981), where the shore crab has been portrayed as “a common member of the estuarine brachyuran assemblage” (Winch and Hodgson, 2007; p. 118). For the purposes of this study all individuals were collected from high shore rocky intertidal areas. Past studies have been conducted on the physiology of *C. punctatus* collected from estuaries (Bolt and Heeg, 1975; Kowie Estuary; Dye and Veen, 1980; Swartkops Estuary; Winch and Hodgson, 2007; Bushman’s River estuary), where it was shown that adult *C. punctatus* are less tolerant to low salinity levels and show a progressive decrease in osmoregulatory capacity during hyposaline exposure (Bolt and Heeg, 1975). Oxygen consumption increases significantly at the combination of high temperature/low salinity (Winch and Hodgson, 2007). Temperature and salinity extremes influence oxygen consumption most in small individuals of *C. punctatus*, indicating that perhaps their greater surface area to volume ratio leads to higher physiological stress (Winch and Hodgson, 2007). Lastly, newly hatched larvae of *C. punctatus* raised at 25°C water temperature led to 100% larval mortality (Fagetti and Campodonico, 1971); (however, the authors express uncertainty if temperature alone was the reason for the high mortality).

Table 1.1. Previous genetic and genomic findings on the study species (South Africa = SA).

Species	Markers	Population structure (Φ_{ST})	Sample region	Reference
<i>S. granularis</i>	mtDNA	0.023	west, south, east SA	Mmonwa et al. 2015
<i>S. granularis</i>	mtDNA	0-0.05	west coast SA	Mertens et al. 2018
<i>S. granularis</i>	SNPs	0.008-0.013	west coast SA	Nielsen et al. 2018
<i>P. angulosus</i>	mtDNA,	0-0.87	west, south,	Muller et al. 2012
	nDNA	0-0.71	south-east SA	
<i>P. angulosus</i>	mtDNA	0.15-0.62	west coast SA	Mertens et al. 2018
<i>P. angulosus</i>	SNPs	0.006-0.019	west coast SA	Nielsen et al. 2018
<i>C. punctatus</i>	mtDNA	0.49	west, south, east SA	Wright et al. 2015

1.8 Aims and outline of the study

Utilising a multi-species approach, the purpose of this dissertation is to use genomic data obtained from pooled RAD-sequencing to expand the current knowledge on population genomic structure in selected rocky shore species (*S. granularis*, *P. angulosus*, *C. punctatus*) across the South African marine environmental gradient. Previous studies based on single markers suggested signals for isolation-by-distance in two species (*P. angulosus*, *C. punctatus*) (Wright et al. 2015; Mertens, Treml and von der Heyden, 2018) and distinct geographic clusters (*S. granularis*, *P. angulosus*) (Muller et al. 2012; Mmonwa et al. 2015), but it is unknown whether these findings are reflected at genome-wide scale (except for the west coast: *S. granularis*, *P. angulosus*; Nielsen et al. 2018). Further, this study seeks to explore species ability to withstand temperature extremes and aims to establish their tolerance thresholds regarding high and low temperatures through thermal tolerance experiments. A previous study conducted on *S. granularis* suggests that its tolerance limits are lower compared to three other limpet species tested in the same experimental setup (Kankondi, McQuaid and Tagliarolo, 2018), but critical thermal limits are unknown for *P. angulosus* and *C. punctatus* and it is also not understood if geographical differences occur in the thermal limits between populations from different biogeographic regions.

In summary, the study conducts a multi-species comparison across three intertidal phyla (Crustacea, Echinodermata, Mollusca) co-distributed in rocky shore populations throughout the South African west, south and east coast (Table 1.2) in order to (1) assess selectively neutral population structure based on genome-wide SNPs and compare with previously detected genetic patterns, (2) explore signals for adaptive processes based on outlier loci

and test their association with geographic distance between populations and environmental factors including sea surface temperature, air temperature and salinity; and (3) to establish the species upper and lower critical thermal limits (CT_{max} , CT_{min}), testing for possible differences along the natural thermal gradient. The overarching aim of the genomic and physiological testing is to characterise the species potential evolutionary potential and thermal resilience to withstand the range of changing climatic conditions predicted for the different coastal regions in the future.

Table 1.2. Components of the study with utilised data and further details.

Parts	Data	Details
Chapter I	Literature review	Introduction and literature review
Chapter II	Neutral SNPs	Assessing selectively neutral SNPs: <i>De novo</i> assemblies, total and private SNPs, pairwise F_{ST} , nucleotide diversity (Tajima's π , Watterson's θ), cluster analyses
Chapter III	Outlier SNPs	Assessing outlier SNPs: Empirical F_{ST} method, BayeScan, BayeScEnv, blasting outliers with BlastX, Testing for IBD and IBE (isolation by distance, isolation by environment)
Chapter IV	Critical thermal limits	Testing critical thermal limits (CT_{max} , CT_{min}) in heating and cooling experimental setups
Conclusion	All	Summary of findings

- Baird, Nathan A., Paul D. Etter, Tressa S. Atwood, Mark C. Currey, Anthony L. Shiver, Zachary A. Lewis, Eric U. Selker, William A. Cresko, and Eric A. Johnson. 2008. "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers." *PloS One* 3 (10): e3376.
- Baldanzi, Simone, Gavin Gouws, Nigel P. Barker, and Sara Fratini. 2016. "Molecular evidence of distinct evolutionary units in the sandhopper *Talorchestia capensis* (Amphipoda, Talitridae) along South African coasts." *Hydrobiologia* 779, no. 1:35-46.
- Baldanzi, Simone, Ralph Watson, Christopher D. McQuaid, Gavin Gouws, and Francesca Porri. 2017. "Epigenetic variation among natural populations of the South African sandhopper *Talorchestia capensis*." *Evolutionary Ecology* 31, no. 1: 77-91.
- Bálint, Miklós, Sami Domisch, Christine H. M. Engelhardt, Peter Haase, Stephanie Lehrian, Jan Sauer, Kathrin Theissinger, Steffen U. Pauls, and Carsten Nowak. 2011. "Cryptic biodiversity loss linked to global climate change." *Nature Climate Change* 1, no. 6: 313.
- Ballegooyen, Roy C., Marten L. Gründlingh, and Johann R. E. Lutjeharms. 1994. "Eddy Fluxes of Heat and Salt from the Southwest Indian Ocean into the Southeast Atlantic Ocean: A Case Study." *Journal of Geophysical Research, C: Oceans* 99 (C7): 14053–70.
- Barrett, Rowan D. H., and Dolph Schluter. 2008. "Adaptation from Standing Genetic Variation." *Trends in Ecology & Evolution* 23 (1): 38–44.
- Barrow, Lisa N., Alan R. Lemmon, and Emily M. Lemmon. 2018. "Targeted Sampling and Target Capture: Assessing Phylogeographic Concordance with Genome-Wide Data." *Systematic Biology*, syy021.
- Barton, Andrew D., Andrew J. Irwin, Zoe V. Finkel, and Charles A. Stock. 2016. "Anthropogenic climate change drives shift and shuffle in North Atlantic phytoplankton communities." *Proceedings of the National Academy of Sciences* 113, no. 11: 2964-2969.
- Barton, Nicholas H. 2000. "Genetic hitchhiking." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 355, no. 1403: 1553-1562.
- Beaugrand, Grégory, and Richard R. Kirby. 2018. "How do marine pelagic species respond to climate change? Theories and observations." *Annual Review of Marine Science* 10 (2018): 169-197.
- Behjati, Sam, and Patrick S. Tarpey. 2013. "What Is next Generation Sequencing?" *Archives of Disease in Childhood. Education and Practice Edition* 98 (6): 236–38.
- Belanger, Christina L., David Jablonski, Kaustuv Roy, Sarah K. Berke, Andrew Z. Krug, and James W. Valentine. 2012. "Global Environmental Predictors of Benthic Marine Biogeographic Structure." *Proceedings of the National Academy of Sciences of the United States of America* 109 (35): 14046–51.
- Bellard, Céline, Cleo Bertelsmeier, Paul Leadley, Wilfried Thuiller, and Franck Courchamp. 2012. "Impacts of Climate Change on the Future of Biodiversity." *Ecology Letters* 15 (4): 365–77.

- Benestan, Laura, Brady K. Quinn, Halim Maaroufi, Martin Laporte, Fraser K. Clark, Spencer J. Greenwood, Rémy Rochette, and Louis Bernatchez. 2016. "Seascape Genomics Provides Evidence for Thermal Adaptation and Current-Mediated Population Structure in American Lobster (*Homarus americanus*)."
Molecular Ecology 25 (20): 5073–92.
- Berg, Paul R., Sissel Jentoft, Bastiaan Star, Kristoffer H. Ring, Halvor Knutsen, Sigbjørn Lien, Kjetill S. Jakobsen, and Carl André. 2015. "Adaptation to Low Salinity Promotes Genomic Divergence in Atlantic Cod (*Gadus morhua* L.)."
Genome Biology and Evolution 7 (6): 1644–63.
- Bernatchez, Louis. 2016. "On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes." *Journal of Fish Biology* 89, no. 6: 2519-2556.
- Bjelde, Brittany E., and Anne E. Todgham. 2013. "Thermal physiology of the fingered limpet *Lottia digitalis* under emersion and immersion." *Journal of Experimental Biology* 216, no. 15: 2858-2869.
- Bintanja, Richard, van de Wal, Roderik S.W. and Oerlemans, Johannes. 2005. "Modelled atmospheric temperatures and global sea level over the past million years." *Nature*, 437, 125–128.
- Blamey, Laura K., and George M. Branch. 2009. "Habitat Diversity Relative to Wave Action on Rocky Shores: Implications for the Selection of Marine Protected Areas." *Aquatic Conservation: Marine and Freshwater Ecosystems* 19 (6): 645–57.
- Blamey, Laura K., Lynne J. Shannon, John J. Bolton, Robert J. M. Crawford, Francois Dufois, Hayley Evers-King, Charles L. Griffiths, L. Hutchings, A. Jarre, M. Rouault and K. E. Watermeyer. 2015. "Ecosystem Change in the Southern Benguela and the Underlying Processes." *Journal of Marine Systems* 144 (April): 9–29.
- Bliss, Dorothy, and Linda Mantel. 2012. "Integument, Pigments, and Hormonal Processes: Volume 9: Integument, Pigments and Hormonal Processes." *Academic Press, Biology of Crustacea* 9.
- Boltt, Gillian, and J. Hebg. 1975. "The osmoregulatory ability of three grapsoid crab species in relation to their penetration of an estuarine system." *African Zoology* 10, no. 2 (1975): 167-182.
- Bolton, John J., Robert J. Anderson, Albertus J. Smit, and Mark D. Rothman. 2012. "South African Kelp Moving Eastwards: The Discovery of *Ecklonia maxima* (Osbeck) Papenfuss at De Hoop Nature Reserve on the South Coast of South Africa." *African Journal of Marine Science* 34 (1): 147–51.
- Bonin, John D., Raymond M. Lee, and John N. Rinne. 1981. "Measuring thermal limits of fish." *Transactions of the American Fisheries Society* 110, no. 5: 662-664.
- Bourret, Vincent, Matthew P. Kent, Craig R. Primmer, Anti Vasemägi, Sten Karlsson, Kjetil Hindar, Philip McGinnity, Eric Verspoor, Louis Bernatchez, and Sigbjørn Lien. 2013. "SNP-Array Reveals Genome-Wide Patterns of Geographical and Potential Adaptive Divergence across the Natural Range of Atlantic Salmon (*Salmo salar*)."
Molecular Ecology 22 (3): 532–51.
- Bowen, Brian W., Michelle R. Gaither, Joseph D. DiBattista, Matthew Iacchei, Kimberly R. Andrews, W. Stewart Grant, Robert J. Toonen, and John C. Briggs. 2016. "Comparative

- Phylogeography of the Ocean Planet." *Proceedings of the National Academy of Sciences of the United States of America* 113 (29): 7962–69.
- Bowen, Brian W., Kartik Shanker, Nina Yasuda, Maria Celia, Maria Celia Machel D. Malay, Sophie von der Heyden, Gustav Paulay, Luiz A. Rocha, Kimberly A. Selkoe, and Paul H. Barber. 2014. "Phylogeography Unplugged: Comparative Surveys in the Genomic Era." *Bulletin of Marine Science* 90 (1): 13–46.
- Boyd, Alan J., J. Taunton-Clark, and G. P. J. Oberholster. 1992. "Spatial Features of the near-Surface and Midwater Circulation Patterns off Western and Southern South Africa and Their Role in the Life Histories of Various Commercially Fished Species." *South African Journal of Marine Science* 12 (1): 189–206.
- Bradbury, Ian R., Sophie Hubert, Brent Higgins, Tudor Borza, Sharen Bowman, Ian G. Paterson, Paul VR Snelgrove et al. 2010. "Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature." *Proceedings of the Royal Society B: Biological Sciences* 277, no. 1701: 3725–3734.
- Bradshaw, Anthony D. 1965. "Evolutionary Significance of Phenotypic Plasticity in Plants." In *Advances in Genetics*, 13:115–55. Academic Press.
- Branch, George, and Margo Branch. 1981. *The Living Shores of Southern Africa*. Struik publishers.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2017. *Two Oceans: A Guide to the Marine Life of Southern Africa*. Penguin Random House South Africa.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2007. *Two Oceans: A Guide to the Marine Life of Southern Africa*. New Holland Publishers, Cape Town, South Africa.
- Branch, George M., Francois Odendaal, and Tamara B. Robinson. 2010. "Competition and Facilitation between the Alien Mussel *Mytilus galloprovincialis* and Indigenous Species: Moderation by Wave Action." *Journal of Experimental Marine Biology and Ecology* 383 (1): 65–78.
- Branch, George M. 1974. "The Ecology of *Patella linnaeus* from the Cape Peninsula, South Africa. 2. Reproductive Cycles." *Transactions of the Royal Society of South Africa* 41 (2): 111–60.
- Broekhuysen, Gerry J. 1940. "The Life-History of *Cyclograpsus punctatus*, M. Edw.: Breeding and Growth." *Transactions of the Royal Society of South Africa* 28 (4): 331–66.
- Brown, Jason L., Jennifer J. Weber, Diego F. Alvarado-Serrano, Michael J. Hickerson, Steven J. Franks, and Ana C. Carnaval. 2016. "Predicting the Genetic Consequences of Future Climate Change: The Power of Coupling Spatial Demography, the Coalescent, and Historical Landscape Changes." *American Journal of Botany* 103 (1): 153–63.
- Buermans, Henk P. J., and Johan T. den Dunnen. 2014. "Next Generation Sequencing Technology: Advances and Applications." *Biochimica et Biophysica Acta* 1842 (10): 1932–41.
- Bunnefeld, Lynsey, Jack Hearn, Graham N. Stone, and Konrad Lohse. 2018. "Whole-

- Genome Data Reveal the Complex History of a Diverse Ecological Community." *Proceedings of the National Academy of Sciences of the United States of America* 115 (28): E6507–15.
- Bustamante, Rodrigo H., George M. Branch, Sean Eekhout, Bruce Robertson, Peter Zoutendyk, Michael Schleyer, Arthur Dye, N. Hanekom, D. Keats, M. Jurd and Christopher McQuaid. 1995. "Gradients of Intertidal Primary Productivity around the Coast of South Africa and Their Relationships with Consumer Biomass." *Oecologia* 102 (2): 189–201.
- Cahill, Abigail E., Matthew E. Aiello-Lammens, M. Caitlin Fisher-Reid, Xia Hua, Caitlin J. Karanewsky, Hae Yeong Ryu, Gena C. Sbeglia, F. Spagnolo, J. B. Waldron, O. Warsi and J. J. Wiens. 2013. "How does climate change cause extinction?" *Proceedings of the Royal Society B: Biological Sciences* 280, no. 1750: 20121890.
- Canale, Cindy I., and Pierre-Yves Henry. 2010. "Adaptive Phenotypic Plasticity and Resilience of Vertebrates to Increasing Climatic Unpredictability." *Climate Research* 43 (1): 135–47.
- Chevin, Luis-Miguel, Sinéad Collins, and François Lefèvre. 2013. "Phenotypic Plasticity and Evolutionary Demographic Responses to Climate Change: Taking Theory out to the Field." *Functional Ecology* 27 (4): 967–79.
- Compton, John S. 2001. "Holocene Sea-Level Fluctuations Inferred from the Evolution of Depositional Environments of the Southern Langebaan Lagoon Salt Marsh, South Africa." *Holocene* 11 (4): 395–405.
- Compton, John S. 2011. "Pleistocene Sea-Level Fluctuations and Human Evolution on the Southern Coastal Plain of South Africa." *Quaternary Science Reviews* 30 (5): 506–27.
- Connell, Joseph H. 1972. "Community Interactions on Marine Rocky Intertidal Shores." *Annual Review of Ecology and Systematics* 3 (1): 169–92.
- Conover, David O., Lorraine M. Clarke, Stephan B. Munch, and Glenn N. Wagner. 2006. "Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation." *Journal of Fish Biology* 69: 21–47.
- Conte, Gina L., Matthew E. Arnegard, Catherine L. Peichel, and Dolph Schluter. 2012. "The probability of genetic parallelism and convergence in natural populations." *Proceedings of the Royal Society B: Biological Sciences* 279, no. 1749: 5039–5047.
- Coop, Graham, David Witonsky, Anna Di Rienzo, and Jonathan K. Pritchard. 2010. "Using Environmental Correlations to Identify Loci Underlying Local Adaptation." *Genetics* 185 (4): 1411–23.
- Costello, Mark John, Marta Coll, Roberto Danovaro, Pat Halpin, Henn Ojaveer, and Patricia Miloslavich. 2010. "A Census of Marine Biodiversity Knowledge, Resources, and Future Challenges." *PloS One* 5 (8): e12110.
- Cowen, Robert K., and Su Sponaugle. 2009. "Larval Dispersal and Marine Population Connectivity." *Annual Review of Marine Science* 1: 443–66.
- Cowles, Raymond Bridgman, Charles Mitchill Bogert, and Others. 1944. "A Preliminary Study of the Thermal Requirements of Desert Reptiles. Bulletin of the AMNH; v. 83, Article 5." <http://digitallibrary.amnh.org/handle/2246/1237>.

- Cram, David L. 1971. "Life History Studies on South African Echinoids (Echinodermata) 1. *Parechinus angulosus* (Leske) (Echinidae, Parechininae)." *Transactions of the Royal Society of South Africa* 39 (3): 321–37.
- Crane, Nicole L., Juliette Tariel, Jennifer E. Caselle, Alan M. Friedlander, D. Ross Robertson, and Giacomo Bernardi. 2018. "Clipperton Atoll as a Model to Study Small Marine Populations: Endemism and the Genomic Consequences of Small Population Size." *PloS One* 13 (6): e0198901.
- Dalongeville, Alicia, Laura Benestan, David Mouillot, Stephane Lobreaux, and Stéphanie Manel. 2018. "Combining Six Genome Scan Methods to Detect Candidate Genes to Salinity in the Mediterranean Striped Red Mullet (*Mullus surmuletus*)." *BMC Genomics* 19 (1): 217.
- Davies, Oliver. 1973. "Pleistocene Shorelines in the Western Cape and South-West Africa." *Annals of the Natal Museum* 21 (3): 719–65.
- Day, Elizabeth, and George M. Branch. 2002. "Effects of Sea Urchins (*Parechinus angulosus*) on Recruits and Juveniles of Abalone (*Haliotis midae*)." *Ecological Monographs* 72 (1): 133–49.
- Dayton, Paul K., Mia J. Tegner, Peter B. Edwards, and Kristin L. Riser. 1998. "Sliding Baselines, Ghosts, and Reduced Expectations in Kelp Forest Communities." *Ecological Applications: A Publication of the Ecological Society of America* 8 (2): 309–22.
- Department of Environmental Affairs (DEA). 2018. "Cabinet approves a representative network of Marine Protected Areas in the South African exclusive zone" 25 October 2018. Web source: https://www.environment.gov.za/mediarelease/cabinetapproves_representativenetworkofMPAs
- DeBoer, Timery S., Ma Rio Abdon Naguit, Mark V. Erdmann, Maria Carmen A. Ablan-Lagman, Kent E. Carpenter, Abdul Hamid A. Toha, and Paul H. Barber. 2014. "Concordant phylogenetic patterns inferred from mitochondrial and microsatellite DNA in the giant clam *Tridacna crocea*." *Bulletin of Marine Science* 90, no. 1: 301-329.
- Dennenmoser, Stefan, Steven M. Vamosi, Arne W. Nolte, and Sean M. Rogers. 2017. "Adaptive Genomic Divergence under High Gene Flow between Freshwater and Brackish-water Ecotypes of Prickly Sculpin (*Cottus asper*) Revealed by Pool-Seq." *Molecular Ecology* 26, no. 1: 25-42.
- Deutsch, Curtis A., Joshua J. Tewksbury, Raymond B. Huey, Kimberly S. Sheldon, Cameron K. Ghalambor, David C. Haak, and Paul R. Martin. 2008. "Impacts of Climate Warming on Terrestrial Ectotherms across Latitude." *Proceedings of the National Academy of Sciences of the United States of America* 105 (18): 6668–72.
- Drinkwater, Kenneth F., Gregory Beaugrand, Masahide Kaeriyama, Suam Kim, Geir Ottersen, R. Ian Perry, Hans-Otto Pörtner, Jeffrey J. Polovina, and Akinori Takasuka. 2010. "On the Processes Linking Climate to Ecosystem Changes." *Journal of Marine Systems* 79 (3): 374–88.
- Driver, Amanda, Kristal Maze, Mathieu Rouget, Amanda T. Lombard, Jeannee Nel, Jane K. Turpie, Richard M. Cowling et al. 2005. "National spatial biodiversity assessment 2004: priorities for biodiversity conservation in South Africa." *Strelitzia* 17.

- Dudgeon, Christine L., Blower, Dean C., Broderick, D., Giles, J.L., Holmes, B.J., Kashiwagi, T., Krück, N.C., Morgan, J.A.T., Tillett, B.J. and Ovenden, J.R. 2012. "A review of the application of molecular genetics for fisheries management and conservation of sharks and rays." *Journal of Fish Biology*, 80: 1789-1843.
- Dufois, François, and Mathieu Rouault. 2012. "Sea Surface Temperature in False Bay (South Africa): Towards a Better Understanding of Its Seasonal and Inter-Annual Variability." *Continental Shelf Research* 43: 24–35.
- Duffy, Alfred M., Jean Clobert, and Anders P. Møller. 2002. "Hormones, Developmental Plasticity and Adaptation." *Trends in Ecology & Evolution* 17 (4): 190–96.
- Dullinger, Stefan, Franz Essl, Wolfgang Rabitsch, Karl-Heinz Erb, Simone Gingrich, Helmut Haberl, Karl Hülber et al. 2013. "Europe's other debt crisis caused by the long legacy of future extinctions." *Proceedings of the National Academy of Sciences* 110, no. 18: 7342-7347.
- Duncan, Charles Peter. 1970. "The Agulhas Current." *The Agulhas Current* Scholarspace, Manoa.Hawaii.edu.
- Dye, Arthur H., and L. Van Der Veen. 1980. "Respiratory Responses of Winter Acclimated Grapsoid Crabs to a Number of Environmental Parameters." *Comparative Biochemistry and Physiology. Part A, Physiology* 67 (4): 643–47.
- Edwards, Matthew S. 2004. "Estimating Scale-Dependency in Disturbance Impacts: El Niños and Giant Kelp Forests in the Northeast Pacific." *Oecologia* 138 (3): 436–47.
- Eklom, Robert, and Juan Galindo. 2011. "Applications of next generation sequencing in molecular ecology of non-model organisms." *Heredity* 107, no. 1:1.
- Engelbrecht, Christien J., and Francois A. Engelbrecht. 2016. "Shifts in Köppen-Geiger Climate Zones over Southern Africa in Relation to Key Global Temperature Goals." *Theoretical and Applied Climatology* 123 (1): 247–61.
- Evans, Brad S., Neville A. Sweijid, Rauri C. K. Bowie, Peter A. Cook, and N. G. Elliott. 2004. "Population genetic structure of the perlemoen *Haliotis midae* in South Africa: evidence of range expansion and founder events." *Marine Ecology Progress Series* 270: 163-172.
- Exposito-Alonso, Moises, François Vasseur, Wei Ding, George Wang, Hernán A. Burbano, and Detlef Weigel. 2018. "Genomic Basis and Evolutionary Potential for Extreme Drought Adaptation in *Arabidopsis thaliana*." *Nature Ecology & Evolution* 2 (2): 352–58.
- Fagetti, Elda, and Italo Campodonico. 1971. "The Larval Development of the Crab *Cyclograpsus punctatus* H. Milne Edwards, Under Laboratory Conditions (Decapoda Brachyura, Grapsidae, Sesarminae*)." *Crustaceana* 21 (2): 183–95.
- Fischer, Klaus, and I. Karl. 2010. "Exploring Plastic and Genetic Responses to Temperature Variation Using Copper Butterflies." *Climate Research* 43 (1): 17–30.
- Fisher, Erich C., Miryam Bar-Matthews, Antonieta Jerardino, and Curtis W. Marean. 2010. "Middle and Late Pleistocene Paleoscape Modeling along the Southern Coast of South Africa." *Quaternary Science Reviews* 29 (11): 1382–98.
- Florgenex Inc., United States. 2015. "RAD Sequencing: Discovery and Genotyping using

- Next Gen DNA Sequencing." <https://www.floragenex.com/s/2015-RAD-Seq-Technical-Whitepaper-v10.pdf> (accessed: 25.04.2019)
- Foden, Wendy B., Bruce E. Young, H. Resit Akçakaya, Raquel A. Garcia, Ary A. Hoffmann, Bruce A. Stein, Chris D. Thomas et al. 2019. "Climate change vulnerability assessment of species." *Wiley Interdisciplinary Reviews: Climate Change* 10, no. 1: e551.
- Forsman, Anders, Magnus Karlsson, Lena Wennersten, Jenny Johansson, and Einat Karpestam. 2011. "Rapid evolution of fire melanism in replicated populations of pygmy grasshoppers." *Evolution: International Journal of Organic Evolution* 65, no. 9: 2530-2540.
- Fox, Rebecca J., Jennifer M. Donelson, Celia Schunter, Timothy Ravasi, and Juan D. Gaitán-Espitia. 2019. "Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change." *Philosophical Transactions Royal Society B* 374: 20180174.
- Frankham, Richard, David A. Briscoe, and Jonathan Ballou. 2002. *Introduction to Conservation Genetics*. Cambridge, UK; New York: Cambridge University Press.
- Frankham, Richard. 1996. "Relationship of Genetic Variation to Population Size in Wildlife." *Conservation Biology: The Journal of the Society for Conservation Biology* 10 (6): 1500–1508.
- Freamo, Heather, Patrick O'Reilly, Paul R. Berg, Sigbjörn Lien and Elizabeth G. Boulding. 2011. "Outlier SNPs show more genetic structure between two Bay of Fundy metapopulations of Atlantic salmon than do neutral SNPs." *Molecular Ecology Resources* 11: 254-267.
- Fricke, Alexander Hermann. 1979a. "Aspects of the Community Structure and Reproductive Ecology of the Common Sea Urchin *Parechinus angulosus* around the Cape Peninsula, South Africa." *Annual Report of the Director. Fishing Industry Research Institute, University of Cape Town/Jaarverslag van Die Direkteur. Visnywerheid-Navorsingsinstituut Universiteit van Kaapstad* MSc Thesis.
- Fricke, Alexander Hermann. 1979b. "Kelp Grazing by the Common Sea Urchin *Parechinus angulosus* (Leske) in False Bay, Cape Town, South Africa." *South African Journal of Zoology* 14 (3): 143–48.
- Funk, W. Chris, John K. McKay, Paul A. Hohenlohe, and Fred W. Allendorf. 2012. "Harnessing genomics for delineating conservation units." *Trends in Ecology & Evolution*, 27: 489-96.
- Futschik, Andreas, and Christian Schlötterer. 2010. "The next Generation of Molecular Markers from Massively Parallel Sequencing of Pooled DNA Samples." *Genetics* 186 (1): 207–18.
- Gaggiotti, Oscar E., Dorte Bekkevold, Hanne B. H. Jørgensen, Matthieu Foll, Gary R. Carvalho, Carl Andre, and Daniel E. Ruzzante. 2009. "Disentangling the Effects of Evolutionary, Demographic, and Environmental Factors Influencing Genetic Structure of Natural Populations: Atlantic Herring as a Case Study." *Evolution; International Journal of Organic Evolution* 63 (11): 2939–51.
- Gaither, Michelle R., Moisés A. Bernal, Richard R. Coleman, Brian W. Bowen, Shelley A. Jones, W. Brian Simison, and Luiz A. Rocha. 2015. "Genomic Signatures of

- Geographic Isolation and Natural Selection in Coral Reef Fishes." *Molecular Ecology* 24 (7): 1543–57.
- Galbraith, Hector, Richard Jones, R. Park, J. Clough, Susan Herrod-Julius, Brian Harrington, and Gary Page. 2002. "Global climate change and sea level rise: Potential losses of intertidal habitat for shorebirds." *Waterbirds*, 25 , 173 – 183.
- García-Ramos, Gisela, and Mark Kirkpatrick. 1997. "Genetic models of adaptation and gene flow in peripheral populations." *Evolution* 51, no. 1: 21-28.
- Garner, Alisse, Janet L. Rachlow, and Jason F. Hicks. 2005. "Patterns of Genetic Diversity and Its Loss in Mammalian Populations." *Conservation Biology: The Journal of the Society for Conservation Biology* 19 (4): 1215–21.
- Gaston, Kevin J. 1994. *What Is Rarity?* Springer Netherlands, Population and Community Biology Series.
- Gaston, Kevin J., Tim M. Blackburn, and John H. Lawton. 1997. "Interspecific Abundance-Range Size Relationships: An Appraisal of Mechanisms." *The Journal of Animal Ecology*, 579–601.
- Ghalambor, Cameron K., John K. McKay, Scott P. Carroll, and David N. Reznick. 2007. "Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments." *Functional Ecology* 21, no. 3: 394-407.
- Gibson, Sachiko Y., Ryan C. Van der Marel, and Brian M. Starzomski. 2009. "Climate change and conservation of leading-edge peripheral populations." *Conservation Biology* 23, no. 6: 1369-1373.
- Gopal, Keshni, Krystal A. Tolley, Johan C. Groeneveld, and Conrad A. Matthee. 2006. "Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the southwestern Indian Ocean." *Marine Ecology Progress Series* 319: 191-198.
- Grant, William Stewart, and Brian W. Bowen. 1998. "Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation." *Journal of Heredity* 89, no. 5: 415-426.
- Grant, William Stewart, and Fernanda M. Da Silva-Tatley. 1997. "Lack of genetically-subdivided population structure in *Bullia digitalis*, a southern African marine gastropod with lecithotrophic development." *Marine Biology* 129, no. 1: 123-137.
- Gray, David R., and Alan N. Hodgson. 1997. "Temporal Variation in Foraging Behaviour of *Patella granularis* (Patellogastropoda) and *Siphonaria concinna* (Basommatophora) on a South African Shore." *The Journal of Molluscan Studies* 63 (2): 121–30.
- Greenwood, Peter John. 1975. "Population Dynamics and Ecological Energetics of *Parechinus angulosus* at Robben Island and False Bay, South Africa." *M. Sc. Thesis, University of Cape Town, Cape Town*.
- Greenwood, Peter John, and T. Bennett. 1981. "Some Effects of Temperature-Salinity Combinations on the Early Development of the Sea Urchin *Parechinus angulosus* (Leske). Fertilization." *Journal of Experimental Marine Biology and Ecology* 51 (2): 119–31.

- Griffiths, Charles L., Tamara B. Robinson, Louise Lange, and Angela Mead. 2010. "Marine Biodiversity in South Africa: An Evaluation of Current States of Knowledge." *PloS One* 5 (8): e12008.
- Griffiths, Charles L., Philip A. R. Hockey, Corine Van Erkom Schurink, and Petrus J. Le Roux. 1992. "Marine Invasive Aliens on South African Shores: Implications for Community Structure and Trophic Functioning." *South African Journal of Marine Science/Suid-Afrikaanse Tydskrif Vir Seewetenskap* 12 (1): 713–22.
- Grummer, Jared A., Luciano B. Beheregaray, Louis Bernatchez, Brian K. Hand, Gordon Luikart, Shawn R. Narum, and Eric B. Taylor. 2019. "Aquatic Landscape Genomics and Environmental Effects on Genetic Variation." *Trends in Ecology & Evolution*. 0169-5347
- Guastella, L. A., and J. Rossouw. 2009. "Coastal Vulnerability: Are Coastal Storms Increasing in Frequency and Intensity along the South African Coast." In *Abstracts, International Multipurpose Reefs (IMPR) Conference, Jeffery's Bay*, 10.
- Gunderson, Lance H. 2000. "Ecological Resilience--in Theory and Application." *Annual Review of Ecology and Systematics*, 425–39.
- Guo, Baocheng, Jacquelin DeFaveri, Graciela Sotelo, Abhilash Nair, and Juha Merilä. 2015. "Population genomic evidence for adaptive differentiation in Baltic Sea three-spined sticklebacks." *BMC Biology* 13, no. 1: 19.
- Guo, Baocheng, Zitong Li, and Juha Merilä. 2016. "Population genomic evidence for adaptive differentiation in the Baltic Sea herring." *Molecular Ecology* 25, no. 12: 2833–2852.
- Hallegraeff, Gustaaf M. 2010. "Ocean Climate Change, Phytoplankton Community Responses, and Harmful Algal Blooms: A Formidable Predictive Challenge 1." *Journal of Phycology* 46, no. 2: 220-235.
- Hampe, Arndt, and Rémy J. Petit. 2005. "Conserving biodiversity under climate change: the rear edge matters." *Ecology Letters* 8, no. 5: 461-467.
- Harley, Christopher D. G. 2003. "Abiotic Stress and Herbivory Interact to Set Range Limits across a Two-Dimensional Stress Gradient." *Ecology* 84 (6): 1477–88.
- Harley, Christopher D. G., A. Randall Hughes, Kristin M. Hultgren, Benjamin G. Miner, Cascade J. B. Sorte, Carol S. Thornber, Laura F. Rodriguez, Lars Tomanek, and Susan L. Williams. 2006. "The Impacts of Climate Change in Coastal Marine Systems." *Ecology Letters* 9 (2): 228–41.
- Harrison, Trevor D. 2002. "Preliminary Assessment of the Biogeography of Fishes in South African Estuaries." *Marine and Freshwater Research* 53 (2): 479–90.
- Hart, Michael W., and Peter B. Marko. 2010. "It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates." *Integrative and Comparative Biology* 50, no. 4: 643-661.
- Hawkins, Stephen J., and Richard G. Hartnoll. 1983. "Changes in a Rocky Shore Community: An Evaluation of Monitoring." *Marine Environmental Research* 9 (3): 131–81.
- Helmuth, Brian. 2002. "How Do We Measure the Environment? Linking Intertidal Thermal

- Physiology and Ecology Through Biophysics." *Integrative and Comparative Biology* 42 (4): 837–45.
- Helmuth, Brian. 2009. "From Cells to Coastlines: How Can We Use Physiology to Forecast the Impacts of Climate Change?" *The Journal of Experimental Biology* 212 (6): 753–60.
- Helmuth, Brian, Nova Mieszkowska, Pippa Moore, and Stephen J. Hawkins. 2006. "Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change." *Annual Review of Ecology, Evolution, and Systematics*, 373–404.
- Helmuth, Brian S., and Gretchen E. Hofmann. 2001. "Microhabitats, Thermal Heterogeneity, and Patterns of Physiological Stress in the Rocky Intertidal Zone." *The Biological Bulletin* 201 (3): 374–84.
- Helyar, Sarah J., J. Hemmer-Hansen, Dorte Bekkevold, M. I. Taylor, R. Ogden, M. T. Limborg, Alessia Cariani et al. 2011. "Application of SNPs for Population Genetics of Nonmodel Organisms: New Opportunities and Challenges." *Molecular Ecology Resources* 11 Suppl 1: 123–36.
- Henriques, Romina, Warren M. Potts, Warwick H. H. Sauer, and Paul W. Shaw. 2012. "Evidence of Deep Genetic Divergence between Populations of an Important Recreational Fishery Species, *Lichia amia* L. 1758, around Southern Africa." *African Journal of Marine Science* 34 (4): 585–91.
- Hewitt, Godfrey M. 2004. "Genetic Consequences of Climatic Oscillations in the Quaternary." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359 (1442): 183–95.
- Hewitt, Godfrey. 2000. "The Genetic Legacy of the Quaternary Ice Ages." *Nature* 405 (6789): 907–13.
- Hickling, Rachael, David B. Roy, Jane K. Hill, Richard Fox, and Chris D. Thomas. 2006. "The distributions of a wide range of taxonomic groups are expanding polewards." *Global Change Biology* 12, no. 3: 450–455.
- Hill, B. J. 1981. "Adaptations to Temperature and Salinity Stress in Southern African Estuaries." *Estuarine Ecology with Particular Reference to Southern Africa*, 187–96.
- Hoban, Sean, Joanna L. Kelley, Katie E. Lotterhos, Michael F. Antolin, Gideon Bradburd, David B. Lowry, Mary L. Poss, Laura K. Reed, Andrew Storfer, and Michael C. Whitlock. 2016. "Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions." *The American Naturalist* 188, no. 4: 379–397.
- Hobbs, Jean-Paul A., Lynne van Herwerden, Dean R. Jerry, Geoffrey P. Jones, and Philip L. Munday. 2013. "High Genetic Diversity in Geographically Remote Populations of Endemic and Widespread Coral Reef Angelfishes (genus: *Centropyge*)." *Diversity* 5 (1): 39–50.
- Hobbs, Jean-Paul, Geoffrey P. Jones, and Philip L. Munday. 2011. "Extinction Risk in Endemic Marine Fishes." *Conservation Biology: The Journal of the Society for Conservation Biology* 25 (5): 1053–55.
- Hockey, Philip A. R., and Corine van Erkom Schurink. 1992. "The Invasive Biology of the Mussel *Mytilus galloprovincialis* on the Southern African Coast." *Transactions of the*

- Royal Society of South Africa* 48 (1): 123–39.
- Hodgson, Alan N. 2010. "Reproductive Seasonality of Southern African Inshore and Estuarine Invertebrates—a Biogeographic Review." *African Zoology* 45 (1): 1–17.
- Hoffmann, Ary A., and Carla M. Sgrò. 2011. "Climate Change and Evolutionary Adaptation." *Nature* 470 (7335): 479–85.
- Hohenlohe, Paul A., Susan Bassham, Paul D. Etter, Nicholas Stiffler, Eric A. Johnson, and William A. Cresko. 2010. "Population Genomics of Parallel Adaptation in Threespine Stickleback Using Sequenced RAD Tags." *PLoS Genetics* 6 (2): e1000862.
- Holderegger, Rolf, Urs Kamm, and Felix Gugerli. 2006. "Adaptive vs. Neutral Genetic Diversity: Implications for Landscape Genetics." *Landscape Ecology* 21 (6): 797–807.
- Huey, Raymond B., Curtis A. Deutsch, Joshua J. Tewksbury, Laurie J. Vitt, Paul E. Hertz, Héctor J. Álvarez Pérez, and Theodore Garland Jr. 2009. "Why Tropical Forest Lizards Are Vulnerable to Climate Warming." *Proceedings. Biological Sciences / The Royal Society* 276 (1664): 1939–48.
- Huey, Raymond B., Paul E. Hertz, and B. Sinervo. 2003. "Behavioral drive versus behavioral inertia in evolution: a null model approach." *The American Naturalist* 161, no. 3: 357–366.
- Hughes, A. Randall, Brian D. Inouye, Marc TJ Johnson, Nora Underwood, and Mark Vellend. 2008. "Ecological consequences of genetic diversity." *Ecology Letters* 11, no. 6: 609–623.
- IPCC. 2013. "IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change." Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- James, Nicola C., Alan K. Whitfield, and Paul D. Cowley. 2008. "Preliminary Indications of Climate-induced Change in a Warm-temperate South African Estuarine Fish Community." *Journal of Fish Biology* 72 (7): 1855–63.
- James, Nicola C., and Juliet Hermes. 2011. "Insights into Impacts of Climate Change on the South African Marine and Coastal Environment. Pretoria." *South African Environmental Observation Network (SAEON)*.
- Jensen, Lasse Fast, Michael M. Hansen, Cino Pertoldi, Gert Holdensgaard, Karen-Lise Dons Mensberg, and Volker Loeschcke. 2008. "Local Adaptation in Brown Trout Early Life-History Traits: Implications for Climate Change Adaptability." *Proceedings. Biological Sciences / The Royal Society* 275 (1653): 2859–68.
- Jones, Adam G., Stevan J. Arnold, and Reinhard Bürger. 2019. "The Effects of Epistasis and Pleiotropy on Genome-Wide Scans for Adaptive Outlier Loci." *Journal of Heredity*, esz007
- Jueterbock, Alexander, James A. Coyer, Jeanine L. Olsen, and Galice Hoarau. 2018. "Decadal stability in genetic variation and structure in the intertidal seaweed *Fucus serratus* (Heterokontophyta: Fucaceae)." *BMC Evolutionary Biology* 18, no. 1: 94.
- Jump, Alistair S., Jenny M. Hunt, José A. Martínez-Izquierdo, and Josep Penuelas. 2006.

- "Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*." *Molecular Ecology* 15, no. 11: 3469-3480.
- Jump, Alistair S., Rob Marchant, and Josep Peñuelas. 2009. "Environmental change and the option value of genetic diversity." *Trends in Plant Science* 14, no. 1: 51-58.
- Kankondi, Sebbi L., Christopher D. McQuaid, and Morgana Tagliarolo. 2018. "Influence of respiratory mode on the thermal tolerance of intertidal limpets." *PloS One* 13, no. 9: e0203555.
- Kay, Catherine M. 2002. "Recruitment in the Intertidal Limpet *Lottia digitalis* (Patellagastropoda: Lottiidae) May Be Driven By Settlement Cues Associated with Adult Habitat." *Marine Biology* 141: 467-77.
- Kelly, Anne E., and Michael L. Goulden. 2008. "Rapid shifts in plant distribution with recent climate change." *Proceedings of the National Academy of Sciences* 105, no. 33: 11823-11826.
- Khaliq, Imran, Christian Hof, Roland Prinzinger, Katrin Böhning-Gaese, and Markus Pfenninger. 2014. "Global Variation in Thermal Tolerances and Vulnerability of Endotherms to Climate Change." *Proceedings. Biological Sciences / The Royal Society* 281 (1789): 20141097.
- Kilburn, Richard, and Elizabeth Rippey. 1982. *Sea Shells of Southern Africa*. Intl Specialized Book Service Inc.
- Kirk, Heather, and Joanna R. Freeland. 2011. "Applications and implications of neutral versus non-neutral markers in molecular ecology." *International Journal of Molecular Sciences* 12, no. 6: 3966-3988.
- Kitzes, Justin A., and Mark W. Denny. 2005. "Red Algae Respond to Waves: Morphological and Mechanical Variation in *Mastocarpus papillatus* along a Gradient of Force." *The Biological Bulletin* 208 (2): 114-19.
- Kleisner, Kristin M., Michael J. Fogarty, Sally McGee, Jonathan A. Hare, Skye Moret, Charles T. Perretti, and Vincent S. Saba. 2017. "Marine Species Distribution Shifts on the U.S. Northeast Continental Shelf under Continued Ocean Warming." *Progress in Oceanography* 153 (April): 24-36.
- Kruger, Andries C. 2006. "Observed Trends in Daily Precipitation Indices in South Africa: 1910-2004." *International Journal of Climatology* 26 (15): 2275-85.
- Kruger, Andries C., and Stephen Shongwe. 2004. "Temperature Trends in South Africa: 1960-2003." *International Journal of Climatology* 24 (15): 1929-45.
- Laudien, Jürgen, N. S. Flint, F. H. Van der Bank, and Thomas Brey. 2003. "Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern African sandy beaches." *Biochemical Systematics and Ecology* 31, no. 7: 751-772.
- Leslie, Heather M., Erin N. Breck, Francis Chan, Jane Lubchenco, and Bruce A. Menge. 2005. "Barnacle Reproductive Hotspots Linked to Nearshore Ocean Conditions." *Proceedings of the National Academy of Sciences of the United States of America* 102 (30): 10534-39.

- Levy, Jessica S., and Natalie C. Ban. 2013. "A method for incorporating climate change modelling into marine conservation planning: An Indo-west Pacific example." *Marine Policy* 38: 16-24.
- Li, Heng. 2011. "A Statistical Framework for SNP Calling, Mutation Discovery, Association Mapping and Population Genetical Parameter Estimation from Sequencing Data." *Bioinformatics* 27 (21): 2987–93.
- Lima, Fernando P., Pedro A. Ribeiro, Nuno Queiroz, Stephen J. Hawkins, and António M. Santos. 2007. "Do distributional shifts of northern and southern species of algae match the warming pattern?" *Global Change Biology* 13, no. 12: 2592-2604.
- Limborg, Morten T., Sarah J. Helyar, Mark De Bruyn, Martin I. Taylor, Einar E. Nielsen, R. O. B. Ogden, Gary R. Carvalho, FPT Consortium, and Dorte Bekkevold. 2012. "Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*)." *Molecular Ecology* 21, no. 15: 3686-3703.
- Listmann, Luisa, Maxime LeRoch, Lothar Schlüter, Mridul K. Thomas, and Thorsten B. H. Reusch. 2016. "Swift Thermal Reaction Norm Evolution in a Key Marine Phytoplankton Species." *Evolutionary Applications* 9 (9): 1156–64.
- Lombard, Amanda T., T. Strauss, Jean Harris, Kerry Sink, Colin Attwood, and Larry Hutchings. 2004. "South African National Spatial Biodiversity Assessment 2004: Technical Report. Volume 4: Marine Component." *South African National Spatial Biodiversity Assessment 2004*, 4.
- Lotterhos, Katie E., and Michael C. Whitlock. 2015. "The relative power of genome scans to detect local adaptation depends on sampling design and statistical method." *Molecular Ecology* 24, no. 5: 1031-1046.
- Lowe, Charles H., and Velma J. Vance. 1955. "Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*." *Science* 122, no. 3158: 73-74.
- Lowry, David B., Sean Hoban, Joanna L. Kelley, Katie E. Lotterhos, Laura K. Reed, Michael F. Antolin, and Andrew Storfer. 2017. "Breaking RAD: An evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation." *Molecular Ecology Resources* 17, no. 2: 142-152.
- Ludt, William B., and Luiz A. Rocha. 2015. "Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa." *Journal of Biogeography* 42, no. 1: 25-38.
- Luikart, Gordon, Phillip R. England, David Tallmon, Steve Jordan, and Pierre Taberlet. 2003. "The Power and Promise of Population Genomics: From Genotyping to Genome Typing." *Nature Reviews. Genetics* 4 (12): 981–94.
- Lutjeharms, Johann R. E., and Isabelle J. Ansorge. 2001. "The Agulhas Return Current." *Journal of Marine Systems* 30 (1): 115–38.
- Lutjeharms, Johann R. E., and Roy C. Van Ballegooyen. 1988. "The Retroflexion of the Agulhas Current." *Journal of Physical Oceanography* 18 (11): 1570–83.
- Lutjeharms, Johann R. E., and John Cooper. 1996. "Interbasin Leakage through Agulhas Current Filaments." *Deep Sea Research Part I: Oceanographic Research Papers* 43 (2): 213–38.

- Lutjeharms, Johann R. E., and Wilhelmus P. M. De Ruijter. 1996. "The Influence of the Agulhas Current on the Adjacent Coastal Ocean: Possible Impacts of Climate Change." *Journal of Marine Systems* 7 (2): 321–36.
- Lutterschmidt, William I., and Victor H. Hutchison. 1997. "The Critical Thermal Maximum: History and Critique." *Canadian Journal of Zoology* 75 (10): 1561–74.
- Majiedt, Prideel, Stephen Holness, Kerry Sink, Ané Oosthuizen, and Peter Chadwick. 2013. "Systematic Marine Biodiversity Plan for the West Coast of South Africa." *South African National Biodiversity Institute, Cape Town*, 46.
- Marko, Peter B., and Michael W. Hart. 2011. "The complex analytical landscape of gene flow inference." *Trends in Ecology & Evolution* 26, no. 9: 448–456.
- Massa, Sónia Isabel, Sophie Arnaud-Haond, Gareth A. Pearson, and Ester A. Serrão. 2009. "Temperature Tolerance and Survival of Intertidal Populations of the Seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal)." *Hydrobiologia* 619 (1): 195–201.
- Mather, Andrew A., Gerald G. Garland, and Derek D. Stretch. 2009. "Southern African Sea Levels: Corrections, Influences and Trends." *African Journal of Marine Science* 31 (2): 145–56.
- Maúre, Genito, Izidine Pinto, Mzime Ndebele-Murisa, Mavhungu Muthige, Chris Lennard, Grigory Nikulin, Alessandro Dosio, and Arlindo Meque. 2018. "The Southern African Climate under 1.5° C and 2° C of Global Warming as Simulated by CORDEX Regional Climate Models." *Environmental Research Letters: ERL* 13 (6): 065002.
- Mawdsley, Jonathan R., Robin O'Malley, and Dennis S. Ojima. 2009. "A review of climate-change adaptation strategies for wildlife management and biodiversity conservation." *Conservation Biology* 23, no. 5: 1080–1089.
- May, Robert Mccredie. "Biological diversity: differences between land and sea." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 343, no. 1303 (1994): 105–111.
- Mbongwa, Nozibusiso, Hui, Cang, Pulfrich Andrea, and Sophie von der Heyden. 2019. Every beach an island - deep population divergence and possible loss of genetic diversity in *Tylos granulatus*, a sandy shore isopod. *Marine Ecology Progress Series*, 614: 111–123
- Mbongwa, Nozibusiso. 2018. "Testing phylogeographic and biogeographic patterns of southern African sandy beach species." M. Sc. Thesis, University of Stellenbosch, South Africa
- MacLean, Sarah A., and Steven R. Beissinger. 2017. "Species' traits as predictors of range shifts under contemporary climate change: A review and meta-analysis." *Global Change Biology* 23, no. 10: 4094–4105.
- McQuaid, Christopher D., and George M. Branch. 1984. "Influence of Sea Temperature, Substratum and Wave Exposure on Rocky Intertidal Communities: An Analysis of Faunal and Floral Biomass." *Marine Ecology Progress Series. Oldendorf* 19 (1): 145–51.
- McQuaid, Christopher D., and George M. Branch. 1985. "Trophic Structure of Rocky

- Intertidal Communities: Response to Wave Action and Implications for Energy Flow." *Marine Ecology Progress Series. Oldendorf* 22 (2): 153–61.
- Mead, Angela, Charles L. Griffiths, George M. Branch, Christopher D. McQuaid, Laura K. Blamey, John J. Bolton, Robert J. Anderson, Francois Dufois, Mathieu Rouault, P. William Froneman, Alan K. Whitfield, Linda R. Harris, Ronel Nel, Deena Pillay, Janine B. Adams. 2013. "Human-Mediated Drivers of Change - Impacts on Coastal Ecosystems and Marine Biota of South Africa." *African Journal of Marine Science* 35 (3): 403–25.
- Mead, Angela. 2011. "Climate and Bioinvasives Drivers of Change on South African Rocky Shores?" University of Cape Town. Phd Thesis, February 2011.
- Meirmans, Patrick G. 2015. "Seven common mistakes in population genetics and how to avoid them." *Molecular Ecology* 24, no. 13: 3223–3231.
- Menge, Bruce A., Bryon A. Daley, Jane Lubchenco, Eric Sanford, Elizabeth Dahlhoff, Patricia M. Halpin, Gregory Hudson, and Jennifer L. Burnaford. 1999. "Top-down and Bottom-up Regulation of New Zealand Rocky Intertidal Communities." *Ecological Monographs* 69 (3): 297–330.
- Mergeay, Joachim, and Luis Santamaria. 2012. "Evolution and Biodiversity: The Evolutionary Basis of Biodiversity and Its Potential for Adaptation to Global Change." *Evolutionary Applications* 5 (2): 103–6.
- Merilä, Juha, and Andrew P. Hendry. 2014. "Climate Change, Adaptation, and Phenotypic Plasticity: The Problem and the Evidence." *Evolutionary Applications* 7 (1): 1–14.
- Mertens, Lisa, Eric Treml, and Sophie Von Der Heyden. 2018. "Genetic and Biophysical Models Help Define Marine Conservation Focus Areas." *Frontiers in Marine Science* 5: 268.
- Midgley, Guy F., and Wilfried Thuiller. 2011. "Potential responses of terrestrial biodiversity in Southern Africa to anthropogenic climate change." *Regional Environmental Change* 11, no. 1: 127–135.
- Milano, Ilaria, Massimiliano Babbucci, Alessia Cariani, Miroslava Atanassova, Dorte Bekkevold, Gary R. Carvalho, Montserrat Espiñeira et al. 2014. "Outlier SNP Markers Reveal Fine-scale Genetic Structuring across European Hake Populations (*Merluccius merluccius*)." *Molecular Ecology* 23 (1): 118–35.
- Miller, Dana D., Yoshitaka Ota, Ussif Rashid Sumaila, Andrés M. Cisneros-Montemayor, and William W. L. Cheung. 2018. "Adaptation Strategies to Climate Change in Marine Systems." *Global Change Biology* 24 (1): e1–14.
- Miller, Michael R., Joseph P. Dunham, Angel Amores, William A. Cresko, and Eric A. Johnson. 2007. "Rapid and Cost-Effective Polymorphism Identification and Genotyping Using Restriction Site Associated DNA (RAD) Markers." *Genome Research* 17 (2): 240–48.
- Milot, Emmanuel, Arnaud Béchet, and Virginie Maris. 2020. "The dimensions of evolutionary potential in biological conservation." *Evolutionary Applications* 13:1363–1379
- Mmonwa, Kolobe Lucas, Peter R. Teske, Christopher D. McQuaid, and Nigel P. Barker. 2015. "Historical Demography of Southern African Patellid Limpets: Congruence of

- Population Expansions, but Not Phylogeography." *African Journal of Marine Science* 37, no. 1: 11-20.
- Mora, Camilo, Abby G. Frazier, Ryan J. Longman, Rachel S. Dacks, Maya M. Walton, Eric J. Tong, Joseph J. Sanchez, Lauren R. Kaiser, Yuko O. Stender, and James M. Anderson. 2013. "The Projected Timing of Climate Departure from Recent Variability." *Nature* 502 (7470): 183–87.
- Mora, Camilo, Chih-Lin Wei, Audrey Rollo, Teresa Amaro, Amy R. Baco, David Billett, Laurent Bopp, Qi Chen, Mark Collier, and Roberto Danovaro. 2013. "Biotic and Human Vulnerability to Projected Changes in Ocean Biogeochemistry over the 21st Century." *PLoS Biology* 11 (10): e1001682.
- Morin, Phillip A., Luikart, Gordon and Wayne, Robert K. 2004. "SNPs in ecology, evolution and conservation." *Trends in Ecology & Evolution*, 19: 208-216.
- Morley, Simon Anthony, Amanda E. Bates, Miles Lamare, Joelle Richard, Khanh Dung Nguyen, J. Brown, and Lloyd S. Peck. 2016. "Rates of Warming and the Global Sensitivity of Shallow Water Marine Invertebrates to Elevated Temperature." *Journal of the Marine Biological Association of the United Kingdom* 96 (1): 159–65.
- Muller, Cornelius M., Sophie von der Heyden, Rauri C. K. Bowie, and Conrad A. Matthee. 2012. "Oceanic Circulation, Local Upwelling and Palaeoclimatic Changes Linked to the Phylogeography of the Cape Sea Urchin *Parechinus angulosus*." *Marine Ecology Progress Series* 468: 203.
- Muteveri, Tinashe, Conrad A. Matthee, Rauri C. K. Bowie, and Sophie von der Heyden. 2015. "High population connectivity and Pleistocene range expansion in the direct-developing plough shell *Bullia rhodostoma* along the South African coast." *African Journal of Marine Science* 37, no. 1: 21-31.
- Nakin, Motebang Dominic Vincent, and Christopher D. McQuaid. 2014. "Marine Reserve Effects on Population Density and Size Structure of Commonly and Rarely Exploited Limpets in South Africa." *African Journal of Marine Science* 36 (3): 303–11.
- Neethling, Marlene, Conrad A. Matthee, Rauri C. K. Bowie, and Sophie Von der Heyden. 2008. "Evidence for panmixia despite barriers to gene flow in the southern African endemic, *Caffrogobius caffer* (Teleostei: Gobiidae)." *BMC Evolutionary Biology* 8, no. 1: 325.
- Neiva, João, Jorge Assis, Nelson C. Coelho, Francisco Fernandes, Gareth A. Pearson, and Ester A. Serrão. 2015. "Genes left behind: climate change threatens cryptic genetic diversity in the canopy-forming seaweed *Bifurcaria bifurcata*." *PloS One* 10, no. 7: e0131530.
- Nel, Ronel, Eileen E. Campbell, Linda Harris, Lorenz Hauser, David S. Schoeman, Anton McLachlan, Derek R. du Preez, Karien Bezuidenhout, and Thomas A. Schlacher. 2014. "The status of sandy beach science: Past trends, progress, and possible futures." *Estuarine, Coastal and Shelf Science* 150: 1-10.
- Nelson, Greville, and Larry Hutchings. 1983. "The Benguela Upwelling Area." *Progress in Oceanography* 12 (3): 333–56.
- Nielsen, Erica S., Romina Henriques, Robert J. Toonen, Ingrid S. S. Knapp, Baocheng Guo, and Sophie von der Heyden. 2018. "Complex Signatures of Genomic Variation of Two

- Non-Model Marine Species in a Homogeneous Environment." *BMC Genomics* 19 (1): 347.
- Nielsen, Einar E., Jakob Hemmer-Hansen, Nina A. Poulsen, Volker Loeschcke, Thomas Moen, Torild Johansen, Christian Mittelholzer, Geir-Lasse Taranger, Rob Ogden, and Gary R. Carvalho. 2009. "Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*)." *BMC Evolutionary Biology* 9, no. 1: 276.
- NOAA (National Oceanic and Atmospheric Administration). 2016. Extended reconstructed sea surface temperature (ERSST.v4). National Centers for Environmental Information. www.ncdc.noaa.gov/data-access/marineocean-data/extended-reconstructed-sea-surface-temperature-ersst.
- Nosil, Patrik. 2012. Ecological speciation. Oxford University Press.
- Nussey, Daniel H., Erik Postma, Phillip Gienapp, and Marcel E. Visser. 2005. "Selection on Heritable Phenotypic Plasticity in a Wild Bird Population." *Science* 310 (5746): 304–6.
- Ortega-Cisneros, Kelly, Sibusiso Yokwana, Warwick Sauer, K. Cochrane, A. Cockcroft, N. C. James, W. M. Potts et al. 2018. "Assessment of the likely sensitivity to climate change for the key marine species in the southern Benguela system." *African Journal of Marine Science* 40, no. 3: 279-292.
- Pacifici, Michela, Wendy B. Foden, Piero Visconti, James E. M. Watson, Stuart H. M. Butchart, Kit M. Kovacs, Brett R. Scheffers et al. 2015. "Assessing species vulnerability to climate change." *Nature Climate Change* 5, no. 3: 215.
- Padfield, Daniel, Genevieve Yvon-Durocher, Angus Buckling, Simon Jennings, and Gabriel Yvon-Durocher. 2016. "Rapid Evolution of Metabolic Traits Explains Thermal Adaptation in Phytoplankton." *Ecology Letters* 19 (2): 133–42.
- Parisod, Christian, and Stéphane Joost. 2010. "Divergent selection in trailing-versus leading-edge populations of *Biscutella laevigata*." *Annals of Botany* 105, no. 4: 655-660.
- Parmesan, Camille. 2006. "Ecological and Evolutionary Responses to Recent Climate Change." *Annual Review of Ecology, Evolution, and Systematics*, 637–69.
- Parmesan, Camille, and Gary Yohe. 2003. "A Globally Coherent Fingerprint of Climate Change Impacts across Natural Systems." *Nature* 421 (6918): 37–42.
- Parris, Adam, Peter Bromirski, Virginia Burkett, Daniel R. Cayan, Mary Culver, John Hall, Radley Horton, Kevin Knuuti, Richard Moss, and Jayantha Obeysekera. 2012. *Global Sea Level Rise Scenarios for the United States National Climate Assessment*. US Department of Commerce, National Oceanic and Atmospheric Administration, Oceanic and Atmospheric Research, Climate Program Office.
- Pauls, Steffen U., Carsten Nowak, Miklós Bálint, and Markus Pfenninger. 2013. "The Impact of Global Climate Change on Genetic Diversity within Populations and Species." *Molecular Ecology* 22 (4): 925–46.
- Pearson, Richard G., and Terence P. Dawson. 2003. "Predicting the Impacts of Climate Change on the Distribution of Species: Are Bioclimate Envelope Models Useful?" *Global Ecology and Biogeography: A Journal of Macroecology* 12 (5): 361–71.

- Peer, Nasreen, Renzo Perissinotto, Ricky H. Taylor, and Nelson A. F. Miranda. 2014. "Temporal Variations in the Diversity of True Crabs (Crustacea: Brachyura) in the St Lucia Estuary, South Africa." *African Invertebrates* 55 (1): 39–65.
- Pereira, Henrique M., Paul W. Leadley, Vânia Proença, Rob Alkemade, Jörn P. W. Scharlemann, Juan F. Fernandez-Manjarrés, Miguel B. Araújo, P. Balvanera, R. Biggs, W. W. Cheung and L. Chini. 2010. "Scenarios for Global Biodiversity in the 21st Century." *Science* 330 (6010): 1496–1501.
- Perry, Allison L., Paula J. Low, Jim R. Ellis, and John D. Reynolds. 2005. "Climate Change and Distribution Shifts in Marine Fishes." *Science* 308 (5730): 1912–15.
- Pfaff, Maya C., George M. Branch, Jennifer L. Fisher, Vera Hoffmann, Allan G. Ellis, and John L. 2015. "Delivery of marine larvae to shore requires multiple sequential transport mechanisms." *Ecology* 96, 1399–1410.
- Pfenninger, Markus, Miklós Bálint, and Steffen U. Pauls. 2012. "Methodological framework for projecting the potential loss of intraspecific genetic diversity due to global climate change." *BMC Evolutionary Biology* 12, no. 1: 224.
- Phair, Nikki Leanne, Robert John Toonen, Ingrid Knapp, and Sophie von der Heyden. 2019. "Shared genomic outliers across two divergent population clusters of a highly threatened seagrass." *PeerJ* 7: e6806.
- Phair, Nikki Leanne, Jaco Barendse, M. Kyle S. Smith, and Sophie von der Heyden. 2015. "Molecular analyses confirm genetically distinct populations of two indigenous estuarine fish species in an isolated coastal lake: implications for the management of introduced ichthyofauna." *Conservation Genetics* 16, no. 4: 801-809.
- Pineda, Jesús, Jonathan A. Hare, and S. U. Sponaugle. 2007. "Larval Transport and Dispersal in the Coastal Ocean and Consequences for Population Connectivity." *Oceanography* 20 (3): 22–39.
- Pillans, Brad, John Chappell, and Tim R. Naish. 1998. "A review of the Milankovitch climatic beat: template for Plio–Pleistocene sea-level changes and sequence stratigraphy." *Sedimentary Geology* 122, no. 1-4: 5-21.
- Poloczanska, Elvira S., Christopher J. Brown, William J. Sydeman, Wolfgang Kiessling, David S. Schoeman, Pippa J. Moore, Keith Brander, J. F. Bruno, L. B. Buckley, M. T. Burrows and C. M. Duarte. 2013. "Global Imprint of Climate Change on Marine Life." *Nature Climate Change* 3 (August): 919.
- Pörtner, Hans Otto, and Rainer Knust. 2007. "Climate Change Affects Marine Fishes through the Oxygen Limitation of Thermal Tolerance." *Science* 315 (5808): 95–97.
- Pörtner, Hans Otto, and Myron A. Peck. 2010. "Climate Change Effects on Fishes and Fisheries: Towards a Cause-and-effect Understanding." *Journal of Fish Biology* 77, no. 8: 1745-1779.
- Potts, Alastair J., Dimitri A. Veldkornet, and Janine B. Adams. 2016. "A Phylogeographic Break in a South African Coastal Saltmarsh Macrophyte, *Juncus kraussii*." *South African Journal of Botany* 107: 80–83.
- Potts, Warren Mason, Albrecht Götz, and N. James. 2015. "Review of the projected impacts of climate change on coastal fishes in southern Africa." *Reviews in Fish Biology and*

Fisheries 25, no. 4: 603-630.

- Provan, Jim. 2013. "The effects of past, present and future climate change on range-wide genetic diversity in northern." Issue: *Frontiers of Biogeography*, 5 (1).
- Przeslawski, Rachel, A. R. Davis, and Kirsten Benkendorff. 2005. "Synergistic Effects Associated with Climate Change and the Development of Rocky Shore Molluscs." *Global Change Biology* 11 (3): 515–22.
- Rafferty, Adrian E., Alec Zimmer, Dargan M. W. Frierson, Richard Startz, and Peiran Liu. 2017. "Less than 2°C Warming by 2100 Unlikely." *Nature Climate Change* 7 (July): 637.
- Ralston, Joel, William V. DeLuca, Richard E. Feldman, and David I. King. 2017. "Population Trends Influence Species Ability to Track Climate Change." *Global Change Biology* 23 (4): 1390–99.
- Rands, Michael R. W., William M. Adams, Leon Bennun, Stuart H. M. Butchart, Andrew Clements, David Coomes, Abigail Entwistle, I. Hodge, V. Kapos, J. P. Scharlemann and W. J. Sutherland. 2010. "Biodiversity Conservation: Challenges beyond 2010." *Science* 329 (5997): 1298–1303.
- Ravinet, Mark, Anja Westram, Kerstin Johannesson, Roger Butlin, Carl André, and Marina Panova. 2016. "Shared and nonshared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale." *Molecular Ecology* 25: 287–305.
- Renaut, Sebastien, Arne W. Nolte, Sean M. Rogers, Nicolas Derome, and Louis Bernatchez. 2011. "SNP signatures of selection on standing genetic variation and their association with adaptive phenotypes along gradients of ecological speciation in lake whitefish species pairs (*Coregonus* spp.)." *Molecular Ecology* 20, no. 3: 545-559.
- Reusch, Thorsten B. H. 2014. "Climate Change in the Oceans: Evolutionary versus Phenotypically Plastic Responses of Marine Animals and Plants." *Evolutionary Applications* 7 (1): 104–22.
- Reusch, Thorsten B. H., and Troy E. Wood. 2007. "Molecular Ecology of Global Change." *Molecular Ecology* 16 (19): 3973–92.
- Reynolds, Terry V., Conrad A. Matthee, and Sophie von der Heyden. 2014. "The Influence of Pleistocene Climatic Changes and Ocean Currents on the Phylogeography of the Southern African Barnacle, *Tetraclita serrata* (Thoracica; Cirripedia)." *PloS One* 9 (7): e102115.
- Riddle, Brett R. 2016. "Comparative phylogeography clarifies the complexity and problems of continental distribution that drove AR Wallace to favor islands." *Proceedings of the National Academy of Sciences* 113, no. 29 (2016): 7970-7977.
- Riddle, Brett R., Michael N. Dawson, Elizabeth A. Hadly, David J. Hafner, Michael J. Hickerson, Stacy J. Mantooth, and Anne D. Yoder. 2008. "The role of molecular genetics in sculpting the future of integrative biogeography." *Progress in Physical Geography* 32, no. 2: 173-202.
- Ridgway, Tyrone, Cynthia Riginos, Jasmine Davis, and Ove Hoegh-Guldberg. 2008. "Genetic connectivity patterns of *Pocillopora verrucosa* in southern African Marine Protected Areas." *Marine Ecology Progress Series* 354 (2008): 161-168.

- Rilov, Gil, Antonios D. Mazaris, Vanessa Stelzenmüller, Brian Helmuth, Martin Wahl, Tamar Guy-Haim, Nova Mieszkowska, Jean-Baptiste Ledoux, and Stelios Katsanevakis. 2019. "Adaptive marine conservation planning in the face of climate change: What can we learn from physiological, genetic and ecological studies?." *Global Ecology and Conservation* : e00566.
- Robinson, Tamara B., and Charles L. Griffiths. 2002. "Invasion of Langebaan Lagoon, South Africa, by *Mytilus galloprovincialis*—effects on Natural Communities." *African Zoology* 37 (2): 151–58.
- Root, Terry L., Jeff T. Price, Kimberly R. Hall, Stephen H. Schneider, Cynthia Rosenzweig, and J. Alan Pounds. 2003. "Fingerprints of Global Warming on Wild Animals and Plants." *Nature* 421 (6918): 57–60.
- Rouault, Marjolaine. 2011. "Agulhas Current Variability Determined from Space: A Multi-Sensor Approach." University of Cape Town.
- Rouault, Mathieu, Pierrick Penven, and Benjamin Pohl. 2009. "Warming in the Agulhas Current System since the 1980's." *Geophysical Research Letters* 36 (12).
- Rouault, Mathieu, Benjamin Pohl, and Pierrick Penven. 2010. "Coastal Oceanic Climate Change and Variability from 1982 to 2009 around South Africa." *African Journal of Marine Science* 32 (2): 237–46.
- Rouault, Mathieu, and Yves Richard. 2003. "Spatial Extension and Intensity of Droughts since 1922 in South Africa." *Water SA* 29: 489–500.
- Roy, Kaustuv, David Jablonski, James W. Valentine, and Gary Rosenberg. 1998. "Marine Latitudinal Diversity Gradients: Tests of Causal Hypotheses." *Proceedings of the National Academy of Sciences of the United States of America* 95 (7): 3699–3702.
- Rubidge, Emily M., James L. Patton, Marisa Lim, A. Cole Burton, Justin S. Brashares, and Craig Moritz. 2012. "Climate-induced range contraction drives genetic erosion in an alpine mammal." *Nature Climate Change* 2, no. 4: 285.
- Sadchatheeswaran, Saachi, George M. Branch, and Tamara B. Robinson. 2015. "Changes in Habitat Complexity Resulting from Sequential Invasions of a Rocky Shore: Implications for Community Structure." *Biological Invasions*, 1–18.
- Sagarin, Raphael D., James P. Barry, Sarah E. Gilman, and Charles H. Baxter. 1999. "Climate-Related Change in an Intertidal Community over Short and Long Time Scales." *Ecological Monographs* 69 (4): 465–90.
- Scavia, Donald, John C. Field, Donald F. Boesch, Robert W. Buddemeier, Virginia Burkett, Daniel R. Cayan, Michael Fogarty, Mark A. Harwell, Robert W. Howarth, and Curt Mason. 2002. "Climate Change Impacts on US Coastal and Marine Ecosystems." *Estuaries* 25 (2): 149–64.
- Schaum, C. Elisa, Samuel Barton, Elvire Bestion, Angus Buckling, Bernardo Garcia-Carreras, Paula Lopez, Chris Lowe, S. Pawar, N. Smirnov, M. Trimmer and G. Yvon-Durocher. 2017. "Adaptation of Phytoplankton to a Decade of Experimental Warming Linked to Increased Photosynthesis." *Nature Ecology & Evolution* 1 (4): 94.
- Schlötterer, Christian. 2003. "Hitchhiking Mapping—functional Genomics from the Population Genetics Perspective." *Trends in Genetics: TIG* 19 (1): 32–38.

- Schlötterer, Christian, Raymond Tobler, Robert Kofler, and Viola Nolte. 2014. "Sequencing Pools of Individuals - Mining Genome-Wide Polymorphism Data without Big Funding." *Nature Reviews. Genetics* 15 (11): 749–63.
- Schlüter, Lothar, Kai T. Lohbeck, Magdalena A. Gutowska, Joachim P. Gröger, Ulf Riebesell, and Thorsten B. H. Reusch. 2014. "Adaptation of a Globally Important Coccolithophore to Ocean Warming and Acidification." *Nature Climate Change* 4 (September): 1024.
- Selkoe, Kimberly A., Cassidy C. D. Aloia, Eric D. Crandall, Matthew Iacchei, Libby Liggins, Jonathan B. Puritz, Sophie von der Heyden, and Robert J. Toonen. 2016. "A decade of seascape genetics: contributions to basic and applied marine connectivity." *Marine Ecology Progress Series*. 554: 1–19.
- Seymour, Sian. 2016. "How paleoclimatic changes in sea surface temperature and sea level have shaped contemporary species richness along the coastline of South Africa." Honour's thesis. University of Stellenbosch.
- Sgro, Carla M., Andrew J. Lowe, and Ary A. Hoffmann. 2011. "Building Evolutionary Resilience for Conserving Biodiversity under Climate Change." *Evolutionary Applications* 4 (2): 326–37.
- Shannon, L. Vere. 1985. "The Benguela Ecosystem. I: Evolution of the Benguela Physical Features and Processes." *Oceanography and Marine Biology: An Annual Review* 23: 105–82.
- Shannon, L. Vere, and Greville Nelson. 1996. "The Benguela: Large Scale Features and Processes and System Variability." In , 163–210. The South Atlantic. Springer.
- Simkanin, Christina, Anne Marie Power, Alan Myers, David McGrath, Alan Southward, Nova Mieszkowska, Rebecca Leaper, and Ruth O'Riordan. 2005. "Using Historical Data to Detect Temporal Changes in the Abundances of Intertidal Species on Irish Shores." *Journal of the Marine Biological Association of the United Kingdom. Marine Biological Association of the United Kingdom* 85 (06): 1329–40.
- Simpson, John A., and Edmund S. C. Weiner. 1989. *The Oxford English dictionary (2nd ed. Vol. XIII)*. New York: Oxford University Press.
- Sink, Kerry. 2016. "The Marine Protected Areas Debate: Implications for the Proposed Phakisa Marine Protected Areas Network." *South African Journal of Science* 112 (9/10).
- Sink, Kerry, Stephen Holness, Linda Harris, Prideel Majiedt, Lara Atkinson, Tamara Robinson, Steve Kirkman, Larry Hutchings, Robin Leslie, Lamberth Stephen et al. 2012. "National Biodiversity Assessment 2011: Technical Report. Volume 4: Marine and Coastal Component. Pretoria: South African National Biodiversity Institute." *Volume 4*: 325.
- Sink, Kerry, Colin G. Attwood, Amanda T. Lombard, Hedley Grantham, Robin Leslie, Toufiek Samaai, Sven Kerwath et al. 2011. "Spatial Planning to Identify Focus Areas for Offshore Biodiversity Protection in South Africa 2011. Final Report for the Offshore Marine Protected Area Project. Cape Town: South African National Biodiversity Institute." *Unpublished Report. Cape Town: South African National Biodiversity Institute*.
- Smit, Albertus J., Michael Roberts, Robert J. Anderson, Francois Dufois, Sheldon F. J.

- Dudley, Thomas G. Bornman, Jennifer Olbers, and John J. Bolton. 2013. "A Coastal Seawater Temperature Dataset for Biogeographical Studies: Large Biases between in Situ and Remotely-Sensed Data Sets around the Coast of South Africa." *PloS One* 8 (12): e81944.
- Somero, George N. 2010. "The Physiology of Climate Change: How Potentials for Acclimatization and Genetic Adaptation Will Determine 'Winners' and 'Losers.'" *The Journal of Experimental Biology* 213 (6): 912–20.
- Sorte, Cascade J. B., Susan L. Williams, and James T. Carlton. 2010. "Marine Range Shifts and Species Introductions: Comparative Spread Rates and Community Impacts." *Global Ecology and Biogeography: A Journal of Macroecology* 19 (3): 303–16.
- Sowman, Merle. 2015. "Governability Challenges Facing Small-Scale Fishers Living Adjacent to Marine Protected Areas in South Africa." In *Interactive Governance for Small-Scale Fisheries: Global Reflections*, 359–77. Cham: Springer International Publishing.
- Sowman, Merle, and Jackie Sunde. 2018. "Social Impacts of Marine Protected Areas in South Africa on Coastal Fishing Communities." *Ocean & Coastal Management* 157 (May): 168–79.
- Stanley, Ryan R. E., Claudio DiBacco, Ben Lowen, Robert G. Beiko, Nick W. Jeffery, Mallory Van Wyngaarden, Paul Bentzen et al. 2018. "A climate-associated multispecies cryptic cline in the northwest Atlantic." *Science Advances* 4, no. 3: eaaq0929.
- Stocker, T. F., D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, B. Bex, and B. M. Midgley. 2013. "Climate Change 2013 (IPCC): The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change."
- Storfer, Andrew, Melanie A. Murphy, Stephen F. Spear, Rolf Holderegger, and Lisette P. Waits. 2010. "Landscape Genetics: Where Are We Now?" *Molecular Ecology* 19 (17): 3496–3514.
- Storz, Jay F. 2005. "Using Genome Scans of DNA Polymorphism to Infer Adaptive Population Divergence." *Molecular Ecology* 14 (3): 671–88.
- Stuart-Smith, Rick D., Graham J. Edgar, and Amanda E. Bates. 2017. "Thermal Limits to the Geographic Distributions of Shallow-Water Marine Species." *Nature Ecology & Evolution* 1 (12): 1846–52.
- Stuart, Venetia, and J. G. Field. 1981. "Respiration and Ecological Energetics of the Sea Urchin *Parechinus angulosus*." *South African Journal of Zoology* 16 (2): 90–95.
- Sunde, Johanna, Carl Tamario, Petter Tibblin, Per Larsson, and Anders Forsman. 2018. "Variation in salinity tolerance between and within anadromous subpopulations of pike (*Esox lucius*)." *Scientific Reports* 8, no. 1: 1–11.
- Swart, Belinda L., Aletta E. Bester-van der Merwe, Sven E. Kerwath, and Rouvay Roodt-Wilding. 2016. "Phylogeography of the Pelagic Fish *Seriola lalandi* at Different Scales: Confirmation of Inter-Ocean Population Structure and Evaluation of Southern African Genetic Diversity." *African Journal of Marine Science* 38 (4): 513–24.
- Tenggardjaja, Kimberly A., Brian W. Bowen, and Giacomo Bernardi. 2018. "Comparative

- Phylogeography of Widespread and Endemic Damselfishes in the Hawaiian Archipelago." *Marine Biology* 165 (8): 139.
- Teske, Peter R., Jonathan Sandoval-Castillo, Tirupathi Rao Golla, Arsalan Emami-Khoyi, Mbaye Tine, Sophie von der Heyden, and Luciano B. Beheregaray. 2019. "Thermal selection as a driver of marine ecological speciation." *Proceedings of the Royal Society B* 286, no. 1896: 20182023.
- Teske, Peter R., Tirupathi Rao Golla, Jonathan Sandoval-Castillo, Arsalan Emami-Khoyi, Carl D. van der Lingen, Sophie von der Heyden, Brent Chiazzari, Bettine Jansen van Vuuren, and Luciano B. Beheregaray. 2018. "Mitochondrial DNA Is Unsuitable to Test for Isolation by Distance." *Scientific Reports* 8 (1): 8448.
- Teske, Peter R., Sophie Bader, and Golla, Tirupathi R. 2015. "Passive dispersal against an ocean current." *Marine Ecology Progress Series* 539, 153–163.
- Teske, Peter R., Sophie von der Heyden, Christopher D. McQuaid, and Nigel P. Barker. 2011a. "A Review of Marine Phylogeography in Southern Africa." *South African Journal of Science* 107 (5-6): 43–53.
- Teske, Peter R., Isabelle Papadopoulos, K. Lucas Mmonwa, Tgiven Matumba, Christopher D. McQuaid, Nigel P. Barker, and Luciano B. Beheregaray. 2011b. "Climate-driven Genetic Divergence of Limpets with Different Life Histories across a Southeast African Marine Biogeographic Disjunction: Different Processes, Same Outcome." *Molecular Ecology* 20 (23): 5025–41.
- Teske, Peter R., Fabien R. G. Forget, Paul D. Cowley, Sophie von der Heyden, and Luciano B. Beheregaray. 2010. "Connectivity between marine reserves and exploited areas in the philopatric reef fish *Chrysoblephus laticeps* (Teleostei: Sparidae)." *Marine Biology* 157, no. 9: 2029-2042.
- Teske, Peter R., Henning Winker, Christopher D. McQuaid, and Nigel P. Barker. 2009. "A Tropical/subtropical Biogeographic Disjunction in Southeastern Africa Separates Two Evolutionarily Significant Units of an Estuarine Prawn." *Marine Biology* 156 (6): 1265–75.
- Teske, Peter R., Isabelle Papadopoulos, Brent K. Newman, Peter C. Dworschak, Christopher D. McQuaid, and Nigel P. Barker. 2008. "Oceanic dispersal barriers, adaptation and larval retention: an interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn." *BMC Evolutionary Biology* 8, no. 1: 341.
- Teske, Peter R., Isabelle Papadopoulos, Gerardo I. Zardi, Christopher D. McQuaid, Max Thabiso Edkins, Charles L. Griffiths, and Nigel P. Barker. 2007a. "Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development." *Marine Biology* 152, no. 3: 697-711.
- Teske, Peter R., P. William Froneman, Nigel P. Barker, and Christopher D. McQuaid. 2007b. "Phylogeographic structure of the caridean shrimp *Palaemon peringueyi* in South Africa: further evidence for intraspecific genetic units associated with marine biogeographic provinces." *African Journal of Marine Science* 29, no. 2: 253-258.
- Teske, Peter R., Isabelle Papadopoulos, Christopher D. McQuaid, Brent K. Newman, and Nigel P. Barker. 2007c. "Climate change, genetics or human choice: why were the

- shells of mankind's earliest ornament larger in the Pleistocene than in the Holocene?." *PloS One* 2, no. 7: e614.
- Teske, Peter R., Nigel P. Barker, and Christopher D. McQuaid. 2007d. "Lack of genetic differentiation among four sympatric southeast African intertidal limpets (Siphonariidae): phenotypic plasticity in a single species?." *Journal of Molluscan Studies* 73, no. 3: 223–228.
- Teske, Peter R., Christopher D. McQuaid, P. William Froneman, and Nigel P. Barker. 2006. "Impacts of Marine Biogeographic Boundaries on Phylogeographic Patterns of Three South African Estuarine Crustaceans." *Marine Ecology Progress Series* 314: 283–93.
- Thrush, Simon F., Judi E. Hewitt, Paul K. Dayton, Giovanni Coco, Andrew M. Lohrer, Alf Norkko, Joanna Norkko, and Mariachiara Chiantore. 2009. "Forecasting the Limits of Resilience: Integrating Empirical Research with Theory." *Proceedings. Biological Sciences / The Royal Society* 276 (1671): 3209–17.
- Tilman, David, Robert M. May, Clarence L. Lehman, and Martin A. Nowak. 1994. "Habitat destruction and the extinction debt." *Nature* 371, no. 6492: 65.
- Timm Janne, Planes Serge, Kochzius Mark. 2012. "High similarity of genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*) found in microsatellite and mitochondrial control region analysis." *Conservation Genetics* 13:693–706.
- Tittensor, Derek P., Camilo Mora, Walter Jetz, Heike K. Lotze, Daniel Ricard, Edward Vanden Berghe, and Boris Worm. 2010. "Global Patterns and Predictors of Marine Biodiversity across Taxa." *Nature* 466 (7310): 1098–1101.
- Toms, Jessica A., John S. Compton, Malcolm Smale, and Sophie von der Heyden. 2014. "Variation in Palaeo-Shorelines Explains Contemporary Population Genetic Patterns of Rocky Shore Species." *Biology Letters* 10 (6): 20140330
- Toonen, Robert J., Jonathan B. Puritz, Zac H. Forsman, Jonathan L. Whitney, Iria Fernandez-Silva, Kimberly R. Andrews, and Christopher E. Bird. 2013. "ezRAD: A Simplified Method for Genomic Genotyping in Non-Model Organisms." *PeerJ* 1: e203.
- Trenberth, Kevin E., Philip D. Jones, P. Ambenje, Roxana Bojariu, D. Easterling, T. Klein, D. Parker, J. Renwick, M. Rusticucci, and B. Soden. 2007. "Observations: Surface and Atmospheric Climate Change." *IPCC, Climate Change*, 235–336.
- Vat, Laura Suzanne. 2000. "The growth and reproduction of *Patella granularis* (Mollusca: Patellogastropoda) on the south-east coast of South Africa." PhD Thesis, Rhodes University.
- Villemereuil, Pierre de, and Oscar E. Gaggiotti. 2015. "A New *FST*-based Method to Uncover Local Adaptation Using Environmental Variables." *Methods in Ecology and Evolution / British Ecological Society* 6, no. 11: 1248–1258.
- Vinagre, Catarina, Marta Dias, Joana Roma, Ana Silva, Diana Madeira, and Mário S. Diniz. 2013. "Critical Thermal Maxima of Common Rocky Intertidal Fish and Shrimps — A Preliminary Assessment." *Journal of Sea Research* 81 (August): 10–12.
- von der Heyden, Sophie. 2009. "Why Do We Need to Integrate Population Genetics into South African Marine Protected Area Planning?" *African Journal of Marine Science* 31 (2): 263–69.

- von der Heyden, Sophie, Kim Prochazka, and Rauri C. K. Bowie. 2008. "Significant population structure and asymmetric gene flow patterns amidst expanding populations of *Clinus cottoides* (Perciformes, Clinidae): application of molecular data to marine conservation planning in South Africa." *Molecular Ecology* 17, no. 22: 4812-4826.
- von der Heyden, Sophie, Enelge Gildenhuys, Giacomo Bernardi, and Rauri C. K. Bowie. 2013. "Fine-Scale Biogeography: Tidal Elevation Strongly Affects Population Genetic Structure and Demographic History in Intertidal Fishes." *Frontiers of Biogeography* 5 (1).
- von der Heyden, Sophie, Rauri C. K. Bowie, Kim Prochazka, Paulette Bloomer, Nicole L. Crane, and Giacomo Bernardi. 2011. "Phylogeographic patterns and cryptic speciation across oceanographic barriers in South African intertidal fishes." *Journal of Evolutionary Biology* 24, no. 11: 2505-2519.
- von der Heyden, Sophie. 2011. "'Carry on sampling!'—assessing marine fish biodiversity and discovery rates in southern Africa." *Diversity and Distributions* 17, no. 1: 81-92.
- von der Heyden, Sophie, Marek R. Lipinski, and Conrad A. Matthee. 2010. "Remarkably Low mtDNA Control Region Diversity in an Abundant Demersal Fish." *Molecular Phylogenetics and Evolution* 55 (3): 1183–88.
- von der Heyden, Sophie, Kim Prochazka, and Rauri C. K. Bowie. 2008. "Significant Population Structure and Asymmetric Gene Flow Patterns amidst Expanding Populations of *Clinus cottoides* (Perciformes, Clinidae): Application of Molecular Data to Marine Conservation Planning in South Africa." *Molecular Ecology* 17 (22): 4812–26.
- von der Heyden, Sophie, Jessica A. Toms, Peter R. Teske, Stephen J. Lamberth, and Wouter Holleman. 2015. "Contrasting Signals of Genetic Diversity and Historical Demography between Two Recently Diverged Marine and Estuarine Fish Species." *Marine Ecology Progress Series* 526 (April): 157–67.
- von der Heyden, Sophie. 2017. "Making evolutionary history count: biodiversity planning for coral reef fishes and the conservation of evolutionary processes." *Coral Reefs* 36, no. 1: 183-194.
- Vucetich, John A., and Thomas A. Waite. 2003. "Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics." *Conservation Genetics* 4, no. 5: 639-645.
- Waddington, Conrad H. 1942. "Canalization of development and the inheritance of acquired characters." *Nature* 150 (November): 563.
- Wagner, Dominique Neitzel, Tara Z. Baris, David I. Dayan, Xin Du, Marjorie F. Oleksiak, and Douglas L. Crawford. 2017. "Fine-scale genetic structure due to adaptive divergence among microhabitats." *Heredity* 118, no. 6: 594.
- Walther, Gian-Reto, Eric Post, Peter Convey, Annette Menzel, Camille Parmesan, Trevor J. C. Beebee, Jean-Marc Fromentin, Ove Hoegh-Guldberg, and Franz Bairlein. 2002. "Ecological Responses to Recent Climate Change." *Nature* 416 (6879): 389–95.
- Wares, John P., Steven D. Gaines, and Clifford W. Cunningham. 2001. "A comparative study of asymmetric migration events across a marine biogeographic boundary." *Evolution* 55, no. 2: 295-306.

- Wares, John P. 2002. "Community Genetics in the Northwestern Atlantic Intertidal." *Molecular Ecology* 11 (7): 1131–44.
- Wernberg, Thomas, and Nisse Goldberg. 2008. "Short-Term Temporal Dynamics of Algal Species in a Subtidal Kelp Bed in Relation to Changes in Environmental Conditions and Canopy Biomass." *Estuarine, Coastal and Shelf Science* 76 (2): 265–72.
- West-Eberhard, Mary Jane. 2003. *Developmental Plasticity and Evolution*. Oxford University Press.
- Westram, Anja M., Juan Galindo, Magnus Alm Rosenblad, John W. Grahame, Marina Panova, and Roger K. Butlin. 2014. "Do the same genes underlie parallel phenotypic divergence in different *Littorina saxatilis* populations?." *Molecular Ecology* 23, no. 18: 4603–4616.
- White, Crow, Kimberly A. Selkoe, James Watson, David A. Siegel, Danielle C. Zacherl, and Robert J. Toonen. 2010. "Ocean currents help explain population genetic structure." *Proceedings of the Royal Society B: Biological Sciences* 277, no. 1688: 1685–1694.
- Whitfield, Alan K., Nicola C. James, Stephen J. Lamberth, Janine B. Adams, Renzo Perissinotto, Anusha Rajkaran, and Thomas G. Bornman. 2016. "The role of pioneers as indicators of biogeographic range expansion caused by global change in southern African coastal waters." *Estuarine, Coastal and Shelf Science* 172: 138–153.
- Willi, Yvonne, and Ary A. Hoffmann. 2009. "Demographic factors and genetic variation influence population persistence under environmental change." *Journal of Evolutionary Biology* 22, no. 1: 124–133.
- Wilson, Kristen L., Marc A. Skinner, and Heike K. Lotze. 2019. „Projected 21st-century distribution of canopy-forming seaweeds in the Northwest Atlantic with climate change.“ *Diversity and Distributions*; 00: 1– 21.
- Winch, James J. W. W., and Alan N. Hodgson. 2007. "The Effect of Temperature and Salinity on Oxygen Consumption in the Brachyuran Crab *Cyclograpsus punctatus* (Crustacea: Decapoda: Grapsidae)." *African Zoology* 42 (1): 118–23.
- Witman, Jon D., and Franz Smith. 2003. "Rapid Community Change at a Tropical Upwelling Site in the Galápagos Marine Reserve." *Biodiversity & Conservation* 12 (1): 25–45.
- Wolff, E., N. Arnell, P. Friedlingstein, J. M. Gregory, J. Haigh, A. Haines, E. Hawkins, et al. 2017. "Climate Updates: Progress since the Fifth Assessment Report (AR5) of the IPCC." In *Climate Updates: What Have We Learnt since the IPCC 5th Assessment Report?* The Royal Society.
- Wright, Daniel, Jacqueline M. Bishop, Conrad A. Matthee, and Sophie von der Heyden. 2015. "Genetic isolation by distance reveals restricted dispersal across a range of life histories: implications for biodiversity conservation planning across highly variable marine environments." *Diversity and Distributions* 21, no. 6: 698–710.
- Wynberg, Rachel. 2002. "A decade of biodiversity conservation and use in South Africa: tracking progress from the Rio Earth Summit to the Johannesburg World Summit on Sustainable Development." *South African Journal of Science* 98, no. 5: 233–243.
- Wyrtki, Klaus. 1973. "Physical Oceanography of the Indian Ocean." In , 18–36. The Biology

of the Indian Ocean. Springer.

Yannic, Glenn, Loïc Pellissier, Joaquín Ortego, Nicolas Lecomte, Serge Couturier, Christine Cuyler, Christian Dussault et al. 2014. "Genetic diversity in caribou linked to past and future climate change." *Nature Climate Change* 4, no. 2: 132.

Zardi, Gerardo I., Christopher D. McQuaid, Peter R. Teske, and Nigel P. Barker. 2007. "Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis*." *Marine Ecology Progress Series* 337: 135-144.

Zardi, Gerardo I., Katy R. Nicastro, Christopher D. McQuaid, Rita Castilho, Joana Costa, Ester A. Serrão, and Gareth A. Pearson. 2015. "Intraspecific genetic lineages of a marine mussel show behavioural divergence and spatial segregation over a tropical/subtropical biogeographic transition." *BMC Evolutionary Biology* 15, no. 1:100.

Zardi, Gerardo I., Katy R. Nicastro, Christopher D. McQuaid, Lisa Hancke, and Brian Helmuth. 2011. "The combination of selection and dispersal helps explain genetic structure in intertidal mussels." *Oecologia* 165, no. 4: 947-958.

Zarraonaindia, Iratxe, Mikel Iriondo, Aitor Albaina, Miguel Angel Pardo, Carmen Manzano, W. Stewart Grant, Xabier Irigoien, and Andone Estonba. 2012. "Multiple SNP markers reveal fine-scale population and deep phylogeographic structure in European anchovy (*Engraulis encrasicolus* L.)." *PloS One* 7, no. 7: e42201.

Zemlak, Tyler S., Robert D. Ward, Allan D. Connell, Bronwyn H. Holmes, and Paul D. N. Hebert. 2009. "DNA barcoding reveals overlooked marine fishes." *Molecular Ecology Resources* 9: 237-242.

Chapter II: Characterising the neutral genomic variation of selected marine invertebrates in South Africa

2.1 Introduction

Changing climatic and environmental conditions challenge species on a global scale, which makes it essential to estimate their evolutionary potential to withstand or perhaps adapt (Mergeay and Santamaria, 2012; IPCC, 2013; Graudal et al. 2014; Exposito-Alonso et al. 2018; Archer et al. 2018). Evolutionary potential is commonly approximated from levels of species intraspecific genomic variation across their geographic distribution, because the genome forms the available foundation for natural selection to act on (see section 1.2; Lande and Shannon, 1996; Pressey et al. 2007; Barrett and Schluter, 2008; Ehlers et al. 2008; Sgro et al. 2011; Alsos et al. 2012; Pauls et al. 2013). Spatio-temporal genomic variation allows the identification of populations with high diversity, which could indicate overall population resilience (Hughes et al. 2008) and benefit the functioning and resilience of ecosystems (Reusch et al. 2005; Bernhardt and Leslie, 2013; Pauls et al. 2013). Low levels of molecular diversity are expected to increase the risk of inbreeding depression and leave populations more vulnerable to extinction (Charlesworth and Charlesworth, 1987). To differentiate between selectively neutral and putatively adaptive parts of the genome, it is required to distinguish selectively neutral loci indicating processes such as drift and gene flow, from outlier loci potentially signalling adaptive processes (Barrett and Schluter, 2008; Sgro, Lowe, and Hoffmann, 2011; Alsos et al. 2012; Pauls et al. 2013).

Multi-species phylogeographic analyses with either closely related taxonomic groups or different phyla are conducted to gain insights into community dynamics (Ellegren, 2014; Delord et al. 2018), but despite the recognised importance in conservation planning processes, these type of studies are still not widely represented in the literature on the marine environment (von der Heyden, 2009; Toonen et al. 2011; Funk et al. 2012; Bowen et al. 2014; Magris et al. 2015; Xuereb et al. 2019). Of course, species molecular composition differs across taxonomic groups, which constrains comparisons, but the potential limitations of analysing multiple species in parallel are not well understood at present (Nielsen et al. 2017b; Nielsen et al. 2018). However, the empirical value of multi-species comparisons is established for single markers (mitochondrial DNA, nuclear DNA) and microsatellite data (Waples, 1987; McMillan and Palumbi, 1995; Avise, 1998; von der Heyden, 2009; Teske et al. 2011; Wright et al. 2015; Bowen et al. 2016; Selkoe et al. 2016; Riddle, 2016). Single markers and microsatellites can cover only a limited range of genome regions, but genome-

wide called single nucleotide polymorphisms (SNPs) allow resolution of spatial patterns with greater power (Morin, Luikart and Wayne, 2004; Funk et al. 2012; Bowen et al. 2014; Selkoe et al. 2016; Xuereb et al. 2019). Identifying distinct population genomic clusters or potential regional adaptations limited to certain populations is highly valuable for conservation and management (Allendorf, Hohenlohe and Luikart, 2010; Funk et al. 2012; Bowen et al. 2014; Shafer et al. 2015; Selkoe et al. 2016; Xuereb et al. 2019; Lopez et al. 2019). For instance, investigating multiple species can reveal whether they are similarly impacted by environmental drivers such as temperature, salinity and primary productivity and if these impacts differ across study species and their distribution (Conte et al. 2012; Westram et al. 2014; Stanley et al. 2018), which can reveal fundamental processes influencing molecular variation across land and seascapes. A genomic comparison across five marine species from different phyla indicates the presence of a climate-associated multi-species cryptic cline in the northwest Atlantic, where a biogeographic break coincides with a steep climatic gradient driven by seasonal temperature differences (Stanley et al. 2018). Dynamic ocean currents or land masses are thought to play a decisive role if gene flow is reduced across multiple species (Wares, Gaines and Cunningham, 2001). An example for this is found on the South African coastline, where the Cape peninsula (Cape Point; located south of Cape Town), appears to impede gene flow in multiple species and constitutes a biogeographic barrier (von der Heyden, 2008; Griffiths et al. 2010; Teske et al. 2011; Muller et al. 2012, Wright et al. 2015). There is further evidence from multi-species studies for varying degrees of genetic structure and diversity in the South African marine environment (von der Heyden, 2009; Teske et al. 2011; Wright et al. 2015; Mertens, Trembl, and von der Heyden, 2018). Overall, the number of studies demonstrating congruent molecular patterns across multiple species is growing (Bernardi, Findley and Rocha-Olivares, 2003; Gaither et al. 2015; Bunnefeld et al. 2018; Barrow et al. 2018; Stanley et al. 2018; Nielsen et al. 2018; Crane et al. 2018), but the marine environment and the forces shaping and maintaining population structure are complex, making it a field of ongoing investigation. Concordant spatial patterns can indicate important macroecological drivers of population dynamics and the composition of marine communities (Kuo and Avise, 2005). Several determinants or a combination of them have been invoked to influence multi-species patterns in the marine realm, including environmental gradients, ocean currents and historic divergence due to vicariance during glacial cycles (Stanley et al. 2018). In South Africa, such drivers have been associated with the contemporary genetic structure of numerous marine species, indicating that the southern African marine environment is a prime study region to further explore potentially congruent multi-species patterns suggested by genetic data with genomic tools (von der Heyden, 2009; Teske et al. 2011; Toms et al. 2014; Wright et al. 2015; Mmonwa et al. 2015; Mertens, Trembl, and von der Heyden, 2018; Nielsen et al. 2018).

2.1.1 RAD-Seq and Pool-Seq for non-model organisms

Genomic studies were previously limited to selected model organisms and associated with high technical and budgetary investments, before high-throughput sequencing (HTS) became the standard practise for studying the genomic architecture of species (Allendorf, Hohenlohe, and Luikart, 2010; Davey et al. 2011; Ellegren, 2014). As opposed to whole-genome sequencing, reduced representation sequencing approaches are a more widely accessible method (restriction site-associated DNA sequencing (RAD-Seq); Miller et al. 2007; Baird et al. 2008). The original RAD-Seq protocol (Miller, 2007; Baird, 2008) diversified into different technical approaches, including ddRAD (Peterson et al. 2012), 2b-RAD (Wang et al. 2012) and ezRAD (Toonen et al. 2013; utilised in this study). An emerging approach, which is utilised for this study in conjunction with the ezRAD method, is Pool-Seq (pooled sequencing approach) (Schlötterer et al. 2014), which entails pooling equimolar concentrations of DNA from individuals of one species or one population into a joint sample (pool) before sequencing. The pools form the basis for libraries, which are used for genotyping and quantifying thousands of single nucleotide polymorphisms (SNPs) throughout the genome after sequencing (Baird et al. 2008). As this study documents, SNPs can be called based on specifying their minimum (allele) count, minimum (sequencing) coverage and maximum (sequencing) coverage; influencing the number of SNPs incorporated in subsequent analyses. One of the ground-breaking benefits of RAD-Seq-based sequencing approaches is the ability to genotype species for which no or very few prior genomic resources are available as for example in the form of an annotated reference genome (Seeb et al. 2011; Ekblom and Galindo, 2011; Rellstab et al. 2013). With the shift in time and budgetary investments of genomic sequencing approaches, studying the genomic composition of non-model species has become more accessible (Davey et al. 2011; Seeb et al. 2011; Ekblom and Galindo, 2011).

2.1.2 RAD-Seq literature review

RAD-Seq studies performed on marine invertebrates since 2013 (reviewed until 15th September 2018) were retrieved in Web of Science (WoS) and later Google Scholar (GS) with search terms “RADseq”, “RAD-seq”, “RAD”, “ezRAD”, “marine” and “invertebrate”. GS is utilised to detect sources which might not be included in traditional databases (Shariff et al. 2013; Orduña-Malea et al. 2014). While it is possible that individual studies might have been undetected by the search terms, Table 2.1 captures the available studies (N=56) as exhaustively as possible. An important observation is the large geographical bias of the

studies, which leaves a gap for the southern hemisphere, but especially the African continent (Table 2.1). Although equal splits across taxa cannot be expected, the review reveals a research bias towards bivalve and gastropod molluscs (31%; N=16), Arthropoda (21%; N=11) and corals (21%; N=11) as study subjects (Table 2.1). Echinoderms are among the understudied taxonomic groups (Table 2.1). There are several studies conducted on two species or studies assessing within phylum species complexes (Combosch and Vollmer, 2015; Forsman et al. 2017; Gutierrez et al. 2017; Bongaerts et al. 2017; Combosch et al. 2017; McFadden et al. 2017; Johnston et al. 2017; Gagnaire et al. 2018; Simmonds et al. 2018; Spano et al. 2018), but multi-species studies across different phyla are still rare (but see Benestan et al. 2017; Nielsen et al. 2018; Stanley et al. 2018). The literature review suggests that until 15th September 2018, no prior study with RAD-Seq data sets of more than two phyla for the purpose of exploring population structure and molecular diversity could be identified. The present study, through assessing species from three different phyla, including an echinoderm, a gastropod mollusc and a crustacean across a large environmental gradient, contributes towards extending the genomic knowledge on co-distributed rocky shore marine invertebrates.

Table 2.1. RAD-Seq studies conducted on marine invertebrates since 2013.

Authors	Year	Species	Common name	Geographic coverage
Arthropoda				
Deagle et al.	2015	<i>Euphausia superba</i>	Antarctic krill	Antarctica
Benestan et al.	2016	<i>Homarus americanus</i>	American lobster	North America, Atlantic coast
Blanco-Bercial and Bucklin	2016	<i>Centropages typicus</i>	NN [copepod]	North Atlantic Ocean
Jeffery et al.	2017a	<i>Carcinus maenas</i>	European green crab	eastern North America
Jeffery et al.	2017b	<i>Carcinus maenas</i>	European green crab	eastern North America
Forsström, Ahmad, and Vasemägi	2017	<i>Rhithropanopeus harrisii</i>	Mud crab	Baltic Sea
Villacorta-Rath et al.	2017	<i>Jasus edwardsii</i>	Spiny lobster	southeast Australia
Jeffery et al.	2018	<i>Carcinus maenas</i>	European green crab	eastern North America
Wu et al.	2018	<i>Portunus pelagicus</i>	Blue swimming crab	Indo-West Pacific Ocean
Dexter et al.	2018	<i>Pseudodiaptomus inopinus</i>	Calanoid copepod	North America, Pacific coast
Lima and Willett	2018	<i>Tigriopus californicus</i>	Tiger copepod	North America, Pacific coast
Echinodermata				
Tay et al.	2016	<i>Protoreaster nodosus</i>	Chocolate chip sea star	Singapore
Paterno et al.	2017	<i>Paracentrotus lividus</i>	Purple sea urchin	central Mediterranean Sea
Galaska et al.	2017	<i>Astrofoma agassizii</i>	White brooding brittle star	Antarctic Polar Front (APF)
Addison and Kim	2018	<i>Strongylocentrotus droebachiensis</i> , <i>S. pallidus</i>	Green sea urchin	North Atlantic Ocean
Xuereb et al.	2018	<i>Parastichopus californicus</i>	Giant California sea cucumber	North America, Pacific coast
Bivalvia & Gastropoda				

Chu et al.	2014	<i>Nucella lapillus</i>	Dog whelk	north-western Atlantic Ocean
Ravinet et al.	2016	<i>Littorina saxatilis</i>	Rough periwinkle	Sweden, Skagerrak coast
Kess, Gross, and Harper	2016	<i>Littorina saxatilis</i>	Rough periwinkle	north-western Atlantic Ocean
Gleason and Burton	2016	<i>Chlorostoma funebris</i>	Black turban snail	North America, California
Van Wyngaarden et al.	2016	<i>Placopecten magellanicus</i>	Atlantic deep-sea scallop	north-western Atlantic Ocean
Miller et al.	2016	<i>Haliotis rubra</i>	Blacklip abalone	south-eastern Australia
Lal et al.	2017	<i>Pinctada margaritifera</i>	Black-lip pearl oyster	Indo-Pacific Ocean
Gutierrez et al.	2017	<i>Crassostrea gigas</i> , <i>Ostrea edulis</i>	Pacific oyster, European flat oyster	North Atlantic Ocean
Vendrami et al.	2017	<i>Pecten maximus</i>	Great scallop	North Atlantic Ocean
Kess, Galindo, and Boulding	2018	<i>Littorina saxatilis</i>	Rough periwinkle	northeast Atlantic Ocean
Maas et al.	2018	<i>Brachidontes</i> spp.	mussels (family Mytilidae)	Indonesia
Sandoval-Castillo et al.	2018	<i>Haliotis laevigata</i>	Greenlip abalone	south-western Australia
Gagnaire et al.	2018	<i>Crassostrea gigas</i> , <i>Crassostrea angulata</i>	Pacific oyster, Portuguese oyster	north-western Pacific Ocean
Song et al.	2018	<i>Crassostrea gigas</i>	Pacific oyster	Chinese coast
Van Wyngaarden et al.	2018	<i>Placopecten magellanicus</i>	Atlantic deep-sea scallop	north-western Atlantic Ocean
Lehnert et al.	2018	<i>Placopecten magellanicus</i>	Atlantic deep-sea scallop	north-western Atlantic Ocean
Corals (Anthozoa)				
Combosch and Vollmer	2015	<i>Pocillopora</i> (3 species)	stony corals (family Pocilloporidae)	Tropical Eastern Pacific, Central West Pacific
Everett et al.	2016	<i>Swiftia simplex</i>	NN [deep sea coral]	North America, Eastern Pacific
McFadden et al.	2017	<i>Ovabunda</i> spp.	soft corals (family Xenidiidae)	Red Sea
Johnston et al.	2017	<i>Pocillopora</i> (7 species)	stony corals (family Pocilloporidae)	Tropical East Pacific, Hawai'i, Australia

Bongaerts et al.	2017	<i>Agaricia fragilis</i> , <i>Stephanocoenia intersepta</i>	Fragile Saucer coral, Blushing Star coral	Bermuda, Western Atlantic
Devlin-Durante and Baums	2017	<i>Acropora palmata</i>	Caribbean elkhorn coral	Caribbean Sea
Forsman et al.	2017	<i>Porites lobata</i> , <i>Porites compressa</i>	Lobe coral, Finger coral	Hawai'i
Leydet et al.	2018	<i>Oculina patagonica</i>	NN [stony coral]	Spanish Mediterranean coast
Hammerman et al.	2018	<i>Agaricia lamarcki</i>	Lamarck's sheet coral	Caribbean Sea
Pratlong et al.	2018	<i>Corallium rubrum</i>	Red coral	Mediterranean Sea
Simmonds et al.	2018	<i>Porites</i> (12 species)	stony corals (family Poritidae)	Indo-Pacific Ocean
Cnidaria: Anthozoa (excluding corals) and Hydrozoa				
Reitzel et al.	2013	<i>Nematostella vectensis</i>	Starlet sea anemone	North America, Pacific and Atlantic coast
Bellis et al.	2016	<i>Aiptasia</i> spp.	Sea anemone(s)	North America and Hawai'i
Chang, Orive and Cartwright	2018	<i>Ectopleura larynx</i>	Ringed tubularia (Hydrozoa)	North Atlantic Ocean
Spano et al.	2018	<i>Anthothoe</i> spp.	sea anemones (Sagartiidae)	South Atlantic coast, South Pacific coast
Cephalopoda				
Gillanders et al.	2016	<i>Sepia apama</i>	Giant Australian cuttlefish	southern Australia
Combosch et al.	2017	<i>Nautilidae</i> (6 species)	Nautilus	South Pacific Ocean
Morse et al.	2018	<i>Hapalochlaena maculosa</i>	Blue-ringed octopus	southern Australia
Porifera				
Schuster et al.	2018	<i>Porifera</i> (6 species)	sponges	Puerto Rico, Galapagos, Panama, Florida, Jamaica
Tunicata (included despite belonging to Urochordata)				
Gao, Li, and Zhan	2018	<i>Botryllus schlosseri</i>	Golden star tunicate	Mediterranean Sea

Studies across different taxonomic groups				
Toonen et al.	2013	<i>Cellana talcosa</i> ; <i>Cryptasterina hystera</i> , <i>Cryptasterina pentagona</i> , <i>Patiria miniata</i> ; <i>Porites lobata</i> , <i>P. compressa</i> , <i>Pocillopora damicornis</i> ; [<i>Paracirrhites arcatus</i>]; [<i>Stenella longirostris</i>]	Turtle limpet; sea star (family Asterinidae), sea star (family Asterinidae), Bat star; Lobe coral, Finger coral; Cauliflower coral; [Arc-eye hawkfish]; [Spinner dolphin]	Hawai'i
Benestan et al.	2017	<i>Homarus americanus</i> , [<i>Salvelinus alpinus</i>]	American lobster, [Arctic Char]	North America, Atlantic coast
Nielsen et al.	2018	<i>Scutellastra granularis</i> , <i>Parechinus angulosus</i>	Granular limpet, Cape urchin	South Africa, west coast
Stanley et al.	2018	<i>Homarus americanus</i> , <i>Placopecten magellanicus</i> , <i>Pandalus borealis</i> , <i>Carcinus maenas</i> , [<i>Gadus morhua</i>]	American lobster, Atlantic deep-sea scallop, Northern shrimp, European green crab, [Atlantic cod]	north-western Atlantic Ocean

2.1.3 Potential drivers of South African intertidal species population structure

In South Africa, there are a range of possible drivers of molecular patterns of population structure, in which the region's geography and oceanography play an important role (von der Heyden, 2009; Teske et al. 2011; Teske et al. 2019). South Africa's coastline is characterised by the cold Benguela Current on the west coast and the warm Agulhas Current on the east coast, shaping a natural environmental gradient with high variation in sea surface temperature (SST) and levels of primary productivity (Figure 1.2) (Bustamante et al. 1995; Griffiths et al. 2010; Smit et al. 2013). Province-specific adaptations have been speculated for species including the mudprawn *Upogebia africana*, the brown mussel *Perna perna* and the sand goby *Psammogobius knysnaensis* (Teske et al. 2008; 2011; 2019; Zardi et al. 2011). Moreover, there is evidence that the thermal gradient characterising the coastline strongly contributes towards shaping temperature-defined marine bioregions (Figure 1.2), for which an indication is found in the genome of the sand goby *P. knysnaensis* (Teske et al. 2019). The southern African coast and its thermal gradient could potentially harbour regionally specific adaptations in marine species. Populations might differ in their standing intraspecific genomic diversity and hence in their evolutionary potential to withstand changing conditions. Indeed, evidence from partial COI and D-loop sequences (mtDNA) in multiple intertidal species suggests that genetic diversity might be lower on the west coast relative to the remaining coastline, which raised the hypothesis that the South African coastline might have a spatial gradient with molecular diversity increasing from low on the west coast and increasing on the south and east coast (Wright et al. 2015). However, although more studies on a wider range of species need to be conducted, there are two recent studies across the South African coastline, which did not observe neutral genomic diversity differentiation in the abalone *Haliotis midae* (Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017) and the sand goby *P. knysnaensis* (Teske et al. 2019; see section 2.1.4).

Aside from the role of temperature differences, the influence of paleo-climatic shifts associated with drastic sea-level changes in the southern African region during glacial cycles (see section 1.6.1) and their influence on population structure have been recognised (Toms et al. 2014). The historic contraction and expansion of rocky shore populations in South Africa is thought to have impacted their molecular structure (Teske et al. 2011; Toms et al. 2014), but the details of genetic signatures left by expansions and contractions are not well understood (Grant and Bowen, 1998). Aside from the thermal gradient and paleo-oceanographic influences, contemporary local oceanographic features such as eddies and near-shore counter currents have been suggested to hinder larval dispersal between

adjacent populations, thus contributing to differentiation in southern Africa (Teske, Bader and Golla, 2015; Mertens, Trembl and von der Heyden, 2018). Across many molecular studies of population structure, life history traits, specifically reproductive strategies and larval characteristics driving potential dispersal or retention, have been discussed to influence biogeographic population dynamics in the last two decades (Teske et al. 2007; Vähä et al. 2007; Sherman, Hunt and Ayre, 2008; Ross et al. 2009; von der Heyden, 2009; Weersing and Toonen, 2009; Kelly and Palumbi, 2010; Sivasundar and Palumbi, 2010; Selkoe and Toonen, 2011; Teske et al. 2011; Freamo et al. 2011; Bowen et al. 2016; Selkoe et al. 2016). However, it is far from well-established how larval dynamics shape or contribute to genomic population structure (Weersing and Toonen, 2009; Selkoe and Toonen, 2011; Faubry and Barber, 2012; Trembl et al. 2012; Giangrande, Gambi and Gravina, 2017). But pioneering work on factors such as uncoupling between larval phase and settlement and post-settlement biotic and abiotic mechanisms is paving the way to hopefully disentangle the impact better in the future (Porri et al. 2006; Pineda et al. 2010).

Reviews on the marine biogeography of South Africa reveal a breadth of genetic variation, including the detection of cryptic species, and areas of reduced gene flow, termed genetic breaks or splits (see section 1.5.3), in certain species between the coastal regions (von der Heyden, 2009; Teske et al. 2011). Improving the understanding of the driving forces of marine population structure in the region is a field of ongoing development and the possibilities of RAD-Seq and differentiation between selectively neutral (this chapter) and outlier loci (Chapter III) provide new tools to assess molecular patterns in this context. Overall, neutral genomic variation, the focus of this chapter, is known to be shaped by a range of factors encompassing natural selection, random drift, population size and genetic connectivity between populations (Frankham, Briscoe, and Ballou, 2002; Gaggiotti et al. 2009; Bragg et al. 2015; Gómez-Fernández, Alcocer, and Matesanz, 2016).

Table 2.2. List of study species, including life history traits and shore height preference.

Life history	Common name	Scientific name	Phylum	Descriptor	Fertilisation	PLD*	Shore height	Mobility during low tide	Reference
Brooder	Shore crab	<i>Cyclograpsus punctatus</i>	Arthropoda	H. Milne-Edwards, 1853	Brooding, then pelagic larvae	14 days	High shore	mobile	Wright et al. 2015; Branch et al. 2017
Broadcast spawner	Granular limpet	<i>Scutellastra granularis</i>	Mollusca	Linnaeus, 1758	Spawning	7-14 days	High shore	functionally sessile	Kilburn and Rippey, 1982; Kay, 2002; Branch et al. 2017
Broadcast spawner	Cape urchin	<i>Parechinus angulosus</i>	Echinodermata	Leske, 1778	Spawning	~ 50 days	Rock pools from mid-shore to low shore	functionally sessile	Cram, 1971; Greenwood, 1975; Branch et al. 2017

Table 2.3. Previous findings on population differentiation, nucleotide diversity and suggested lineages (COI; cytochrome c oxidase subunit I).

Species	Markers	Population structure (pairwise F_{ST})	Global F_{ST} (ϕ_{ST})	Nucleotide diversity (π)	Average $\bar{\pi}$	Sample region South Africa	Suggested lineages	Signal for IBD (isolation-by-distance)	Reference
<i>C. punctatus</i>	COI	0.15-0.91	0.49	0.002-0.018	0.009	west, south, east	-	✓	Wright et al. 2015
<i>S. granularis</i>	COI	0-0.473	0.023	NA	NA	west, south, east	west & east lineage**	-	Mmonwa et al. 2015
<i>S. granularis</i>	COI	0-0.05	0.00	0.0036-0.0052	0.0043	west coast	-	-	Mertens et al. 2018
<i>S. granularis</i>	SNPs	0.008-0.013	NA	0.009-0.012	0.011	west coast	-	-	Nielsen et al. 2018
<i>P. angulosus</i>	COI, SpREJ9 (nDNA)	0-0.87 0-0.71	NA	0.007-0.069	0.022	west, south, south-east	west coast; south/east coast***	-	Muller et al. 2012
<i>P. angulosus</i>	COI	0.15-0.62	0.32	0.0031-0.0163	0.0098	west coast	-	✓	Mertens et al. 2018
<i>P. angulosus</i>	SNPs	0.006-0.019	NA	0.006-0.011	0.009	west coast	-	-	Nielsen et al. 2018

NA = not available; *pelagic larval duration, **phylogeographic break along the central South Coast, ***phylogeographic break in the area of Cape Point

2.1.4 Comparisons between traditional markers and SNPs

Molecular patterns obtained from mitochondrial DNA (mtDNA) are thought to indicate species past demographic history on a larger evolutionary scale (Avice, 2000; Dudgeon et al. 2012; von der Heyden, 2017). There are significant differences between traditional markers such as mtDNA compared to single nucleotide polymorphisms markers, which can indicate, on a much larger scale than traditional markers, contemporary population structure and genomic variation as well as potential adaptive loci (Morin, Luikart and Wayne, 2004; Allendorf, Hohenlohe and Luikart, 2010; Funk et al. 2012; Bowen et al. 2014; von der Heyden, 2017). Expectedly, findings derived from traditional markers might differ compared to SNP marker findings (for the study species see Table 2.3 and section 1.7). Mito-nuclear discordance identified between mtDNA and SNP marker findings can be influenced by factors including differences in mutation rates and sample sizes, as well as adaptive mtDNA introgression and possible sex-biased demographic history resulting in asymmetries between male and female effective population size (Toews and Brelsford, 2012). Studies on other marine invertebrates have documented both discordance and congruence across different marker types for patterns of population differentiation and molecular diversity. For instance, studies undertaken with traditional markers (mtDNA, microsatellites and allozymes) on the Atlantic dogwinkle *Nucella lapillus* in the North Atlantic reported weak or no genetic population structure (Chu et al. 2014). In contrast, SNP-based findings (and the identification of putatively adaptive loci) show strong evidence for a split in *N. lapillus* populations into southern and northern clades in the north western Atlantic (Chu et al. 2014). An example for congruence across molecular markers has been reported for brittle star *Astrofoma agassizii* populations across the Antarctic Polar Front (APF), where findings from both mitochondrial and nuclear DNA confirm that the APF constitutes a strong, but not insurmountable barrier between *A. agassizii* populations (Galaska et al. 2017). However, evidence from traditional and genomic markers might also both confirm the absence of population structure, as found in the abalone *Haliotis rubra* (Miller et al. 2016). Overall, congruence across molecular patterns obtained with traditional and SNP markers could also be shown in the sea star *Protoreaster nodosus* (Tay et al. 2016), the sea cucumber *Parastichopus californicus* (Xuereb et al. 2018), the mud crab *Rhithropanopeus harrisi* (Forsström, Ahmad and Vasemägi, 2017) and in *Planes* spp. crabs (Pfaller et al. 2019). In summary, these examples demonstrate that a wide spectrum of studies exists for marine invertebrates where past and contemporary marker patterns may either be congruent or discordant. Thus, it is difficult to predict in advance whether single marker findings are possibly reflected in SNP-based patterns.

2.1.5 Expectations based on previous findings

The goal of this chapter is to test the hypothesis of spatial selectively neutral genomic structure and potential differences in molecular diversity as possible result of environmental differences across the South African coastal regions with a RAD-Seq approach incorporating pooled population samples of three rocky shore invertebrates (Table 2.2): the Cape urchin (*Parechinus angulosus*, low to mid-intertidal zone), the granular limpet (*Scutellastra granularis*, high shore) and the shore crab (*Cyclograpsus punctatus*, mid to high shore) (see section 1.7). Selecting species which represent three important taxonomic groups in the intertidal community (multi-species approach) potentially allows insights into community dynamics. The aim is to analyse selectively neutral SNPs for levels of neutral genomic diversity (Tajima's π and Watterson's θ) and potential spatial variation thereof. Further, ratios of total versus private (unique to a certain population) SNPs are investigated for possible differentiation between sampling locations. Moreover, the obtained SNPs are analysed for putatively neutral population structure based on pairwise F_{ST} values and cluster analyses (BAPS, fastSTRUCTURE).

Lower genetic diversity was measured with the COI and D-loop markers in several rocky shore species on the South African west coast compared to the remaining coastline (Wright et al. 2015), which gave rise to the preliminary hypothesis that molecular variation might follow a geographic gradient with increasing genetic diversity from the South African west to the east coast (Wright et al. 2015). However, recent genomic marker studies do not report strong differences in selectively neutral genomic diversity across sampled populations (Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017; Nielsen et al. 2018; Teske et al. 2019). If evidence for spatial molecular variation is detected in this study, it might be expected to be comparatively low (Hypothesis I). In this instance, "low" could be defined as observing shallow (no significant F_{ST} values) levels of genomic variation. Although only a limited number of genetic studies suggests the occurrence of a geographic molecular diversity gradient on the South African coastline, this study contributes to testing whether such a gradient exists.

Despite partial COI and D-loop (mtDNA) and nuclear markers indicating strong population differentiation in several marine South African species (particularly for the study species; see Table 2.3; section 1.7), recent genomic studies with SNP markers did not observe pronounced evidence of neutral population structure (Table 2.3; see section 2.1.4) (Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017; Nielsen et al. 2018; Teske et al. 2019). This suggests that patterns detected with mtDNA or nuclear markers do not necessarily

translate to SNP marker population structure due to marker system differences. It can be hypothesised that despite the evidence for population structure in the study species with the COI marker, population structure in neutral SNP markers across the field sites might be low (no geographically separated clusters) (Hypothesis II). Finally, the study species differ in biological factors such as inter-phyletic differences, reproductive modes and dispersal traits (Table 2.2), which may lead to possibly varying interspecific patterns of genomic diversity and population structure (Hypothesis III). Overall, this study contributes to a growing number of genomic findings of eukaryotic South African marine species (Picone, Rhode, and Roodt-Wilding, 2015; Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017; Spano et al. 2018; Nielsen et al. 2018; Mullins et al. 2018; Teske et al. 2019; Phair et al. 2019).

2.2 Materials and Methods

2.2.1 Sample collection

Field sites were selected to span three South African coastal regions with a wide array of environmental conditions, particularly temperature (Figure 2.1). Covering ~1,800 km of the coastline, two sites from the west coast (Port Nolloth/Jacob's Bay, Sea Point), south coast (Cape Agulhas, Knysna) and east coast (Cape St. Francis, Haga Haga) were chosen. Field collections took place from April to October 2015 and included six field sites per species (*Scutellastra granularis*, *Parechinus angulosus*, *Cyclograpsus punctatus*). The distribution of *C. punctatus* does not extend to Port Nolloth, with Jacob's Bay the most northern site on the west coast for which animals could be obtained. With 120 samples overall per site, a total of 720 samples were collected for DNA extraction. Forty individuals per species and location were selected to counter limitations associated with small numbers of individuals forming the foundation of pooled population samples (Pool-Seq) (Futschik and Schlötterer, 2010) and to ensure comparability with a closely related study (Nielsen et al. 2018). In this study, "pooled" always refers to joint DNA samples ("pools") of multiple individuals (Pool-Seq; see section 2.2.2). Animals occupying rock pools at the time of collection were not sampled for this study. Limpets (*S. granularis*) were carefully detached from rock surfaces with a spatula, urchins (*P. angulosus*) were retrieved from approximately the same water depth at all sites (~30 – 50 cm) and crabs (*C. punctatus*) were caught manually from under rocks and boulders. Sex has only been recorded for the shore crabs collected at a later stage for Chapter IV, as equimolar amounts of DNA were combined into a pooled sample (Pool-Seq; see section 2.2.2). Sampling has been conducted at the lowest point of low tide and independent of body size and covered around 200 meters of each coastal site. Samples were preserved in ethanol (100%).

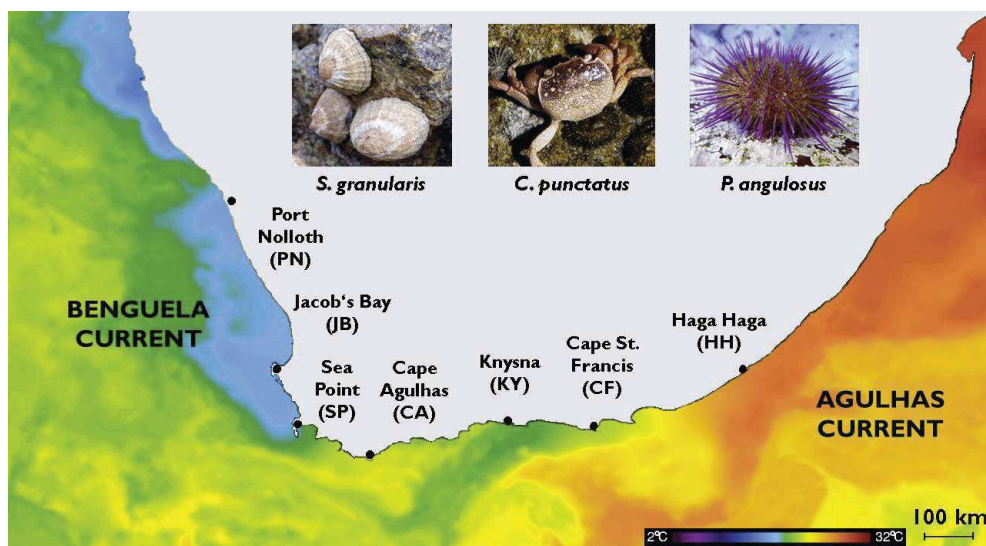


Figure 2.1. Map of the South African coastline showing the sea surface temperature gradient (December 2013) and sampling locations: Port Nolloth (PN), Jacob's Bay (JC) (*C. punctatus* only), Sea Point (SP), Cape Agulhas (CA), Knysna (KY), Cape St. Francis (CF) and Haga Haga (HH). Source: NASA, ID: 30487, <http://svs.gsfc.nasa.gov/30487>.

2.2.2 DNA extraction and NGS sequencing

Genomic DNA was extracted from equally sized pieces of sample tissue with DNeasy tissue extraction kits (Qiagen), according to the manufacturer's protocol. Extracted DNA stems from tissue from muscle (*C. punctatus*), foot (*S. granularis*) and gonads (*P. angulosus*). The choice of tissue minimises the risk of contamination from symbionts or parasites. DNA concentration of the extracted samples was determined via Qubit fluorometry (Qubit Quant iT dsDNA HS Assay system at Central Analytical Facilities (CAF) of Stellenbosch University). The 40 individual DNA extractions were pooled per species and per location to a final concentration per pool of 2000 ng/μl (*C. punctatus*), 3000 ng/μl (*S. granularis*) and 2500 ng/μl (*P. angulosus*), mirroring preceding pool concentrations for the same species (Nielsen, 2017a). One pooled sample per species per site (18 pools overall) were obtained after the pooling protocol. Individuals were not labelled, as this technology was not available yet at the time of DNA sample processing. The pools were freeze-dried and shipped to the Genetics Core Facility (GCF) at the Hawai'i Institute of Marine Biology. The pools served as basis for library preparation (Knapp et al. 2016) and subsequent v3 2 × 300 PE Mi-Seq Illumina sequencing, following the ezRAD method protocol (Toonen et al. 2013). The pooled DNA sample for *P. angulosus* from Cape Agulhas could not be included in the study.

2.2.3 Statistical analysis

As pipelines that could analyse pooled samples of non-labelled individuals were not available at the time this study was carried out, analyses were conducted, in a stepwise

manner, carefully testing a variety of available software tools at each stage. This approach also allows for greater control of settings and parameters. Sequenced pooled reads from the Illumina Mi-Seq are from here on referred to as “reads”. In the first step, quality metrics of the obtained paired-end reads, forward and reverse, were calculated with FASTQC (Andrews, 2010). Trimming and removing adapter sequences was executed with TrimGalore! (Krueger, 2015). Reads were trimmed twice, with the phred quality score threshold of 25 (q25) for creating the assemblies and with the phred score quality threshold of 20 (q20) for downstream analyses (Figure 2.2). In the absence of an assembled genome, a draft genome may be assembled *de novo* from the sequenced reads or the reads can be mapped to a closely related species with an available genome (Rellstab et al. 2013; da Fonseca et al. 2016). The intricacies of assembling a genome or partial genome without a reference have led to a wide range of assembling algorithms, strategies and quality parameters. Importantly, the quality of the assembled genome strongly influences downstream analyses of the entire dataset (da Fonseca et al. 2016), requiring cautious choice of programmes and their parameters.

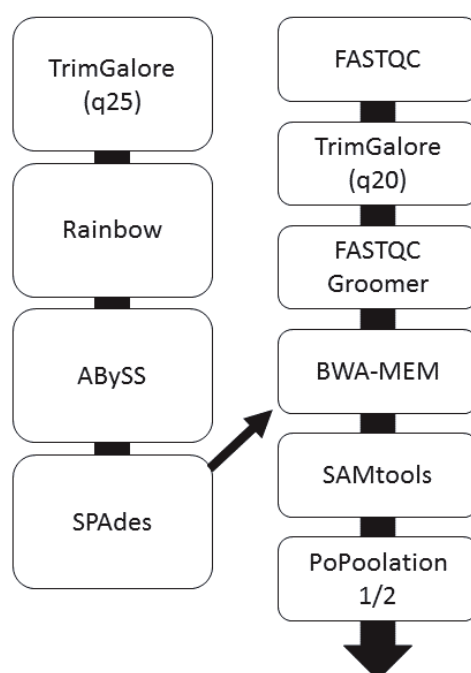


Figure 2.2. Software tools used to create the assemblies (left) and to process the reads (right).

This study tests three assembly programs that allow for pooled data, SPAdes v.3.11.1 (Bankevich et al. 2012), ABySS v.1.9.0 (Simpson et al. 2009) and Rainbow v.2.0.4 (Chong et al. 2012) to obtain the highest quality draft genome for each species. Mapping to closely related species with annotated genomes was not performed, as the available genomic resources differ between the study species and using a consistent *de novo* assembly

approach across all species renders the results more comparable (Toonen et al. 2011; Bowen et al. 2014; Selkoe et al. 2016). Comparison of the obtained assemblies via Quast reports (Quast Genome Assembly Quality, Galaxy v.4.1.1, www.usegalaxy.org) led to utilising SPAdes for the *de novo* assemblies of the three species (Table 2.4). The assemblies' k-mer length, which describes all possible sub-sequences with length k from a read, were tested with the assistance of KmerGenie v.1.7016 (Chikhi and Medvedev, 2014) to optimise the draft assemblies (Davey et al. 2013). According to the program, the estimated k-mer lengths range from 19 (*C. punctatus*) to 21 (*S. granularis*) and 111 (*P. angulosus*). Basing an assembly on a computationally suggested k-mer value alone can lead to suboptimal assemblies and in two instances (*S. granularis*, *P. angulosus*) SPAdes failed to create assemblies with low range k-mer values. Thereby multiple SPAdes assemblies with different k-mer values were created per species and rated according to their Quast-reported total assembly length, contig N50 (definition follows) and the size of the largest contig. This process led to assemblies, which were created by SPAdes with the k-mer range 41-61-81. The contig N50 is an assembly quality metric describing a weighted median value of the distribution of contig lengths in the assembly, as described by the International Human Genome Sequencing Consortium in 2001.

The second set of reads trimmed in TrimGalore (q20) were uploaded to the Galaxy platform (<http://usegalaxy.org>) for grooming in FastQC Groomer (v.1.1.1) to ensure correct formatting of the sequences (Blankenberg et al. 2010). Afterwards the Burrows-Wheeler Aligner (BWA-MEM algorithm) was applied to map the groomed and trimmed reads against the assembled *de novo* reference (Table 2.4) (Li, 2013). Post-processing read alignments from BWA was achieved with SAMtools v.1.3 (Li et al. 2009; Li, 2011). Essential steps performed in SAMtools included removing potentially ambiguously mapped reads and the subsampling of reads to the minimum available pool size per species subset (Table 2.4). The purpose of the latter step is to avoid over-representing populations in the analyses due to a higher number of available reads (Cárcer et al. 2011). Concluding the SAMtools stage, individual pileups (maximum depth 10,000) and meta-pileups (maximum depth 1,000) of reads with quality score 20 were created per species for further analyses.

Table 2.4. Amount of q20 reads, non-ambiguously mapped reads and subsampled non-ambiguously mapped reads, shown by species and location.

<i>C. punctatus</i>			
	#q20reads	#mapped reads	#subsampled
JC	2,479,596	1,691,878	1,522,491
SP	5,338,886	3,735,625	1,529,437
CA	5,330,090	3,618,472	1,522,505
KY	2,244,068	1,538,767	1,538,767
CF	2,507,078	1,660,041	1,527,144
HH	9,256,962	6,335,549	1,518,232
<i>S. granularis</i>			
	#q20reads	#mapped reads	#subsampled
PN	4,864,068	3,154,567	1,198,597
SP	6,050,940	4,034,381	1,211,710
CA	1,761,766	1,223,489	1,223,489
KY	6,238,706	4,250,166	1,187,725
CF	5,386,378	3,795,305	1,214,248
HH	2,344,056	1,624,923	1,217,982
<i>P. angulosus</i>			
	#q20reads	#mapped reads	#subsampled
PN	4,526,062	3,663,773	2,599,470
SP	3,561,922	2,640,735	2,614,544
KY	35,575,174	28,996,029	2,609,508
CF	4,576,674	2,614,279	2,614,279
HH	6,331,076	3,469,651	2,604,195

To allow for downstream compatibility, the meta-pileup had to be created using SAMtools v.0.1.18 instead of v.1.3, which was used for every other step requiring SAMtools. The difference concerns merely the data formatting and does not affect the reads themselves. The calculations described in the following were conducted with PoPoolation v.1.2.2 and PoPoolation2 v.1201 (Kofler et al. 2011a; Kofler et al. 2011b) initially using 12 different scenarios overall (see Table S2.1, Appendix) to document which parameters in terms of minimum (allele) count, minimum (sequencing) coverage and maximum (sequencing) coverage yield which results. Large SNP window sizes, detecting larger numbers of SNPs, are theoretically expected to lead to more false positives, whereas smaller window sizes, detecting smaller numbers of SNPs, may increase the risk for false negative findings (Beissinger et al. 2015; O'Leary et al. 2018). However, additional testing to document the

impact of SNP parameter variety is at present rarely performed or published in studies, which would provide a reference frame for novel genomic findings. Testing the SNP data with 12 different SNP calling parameters indicates that total numbers of SNPs are higher with wide (less stringent) parameters for minimum allele count and maximum sequencing coverage and conversely lower with stringent parameters (see Table S2.1, Appendix). For this study, the selected parameters were set in the middle, which is neither on the broad nor the narrow end of the spectrum (scenario 6). For both PoPoolation and PoPoolation2, scenario 6 utilises minimum (allele) count 4, minimum (sequencing) coverage 10 and maximum (sequencing) coverage 200.

To assess selectively neutral population patterns over patterns potentially affected by outlier loci, detected outliers (see Chapter III) were removed manually from the overall dataset and calculations conducted with the selectively neutral part of the overall genomic dataset. Two measures of nucleotide diversity, Tajima's π (π) (Nei and Li, 1979) and Watterson's θ_w (θ_w) (Watterson, 1975), were computed from the individual pileups with PoPoolation (Kofler et al. 2011a). The total number of SNPs and the number of private SNPs, which are geographically limited to a specific population and are hence considered "private", were obtained with PoPoolation2 from the meta-pileup. Private SNPs were also calculated as a percentage of the total number of SNPs per population for comparative purposes. The presence of private SNPs in populations signals genomic markers which are geographically distinct, which may be associated with localised evolutionary and environmental processes. Among the possible evolutionary processes, gene flow and genetic drift are assumed to have a higher impact on the occurrence of private SNPs over mutation and selection (Hutchison and Templeton, 1999). Moreover, the spatial variation of the proportion of private SNPs in relation to the overall number of SNPs can indicate populations with a higher degree of molecular geographic distinctness possibly associated with a certain degree of demographic isolation (Hutchison and Templeton, 1999). Known geographic uniqueness of species populations relative to others is a valuable foundation for future research and can aid decisions regarding spatial prioritisation in conservation planning processes (von der Heyden, 2017). Finally, PoPoolation2 and the meta-pileup were utilised to estimate the fixation index (F_{ST}) values for each pairwise comparison with a sliding window approach, exploring potential divergence between populations (Kofler et al. 2011b). As part of PoPoolation2, Fisher's exact test was used to estimate the significance of allele frequency differences between populations (Fisher, 1922; Kofler et al. 2011b).

Potential clustering among the populations was tested with BAPS v.5.4 (Bayesian Analysis of Population Structure) group mixture (clustering of groups of individuals) analysis (P .

angulosus K=1-5; *S. granularis* K=1-6; *C. punctatus* K=1-6) (Corander and Marttinen, 2006; Corander et al. 2006). For the instance that the suggested optimal partition equals the number of sampled species populations, a potentially higher number of clusters was tested with a vector approach (5 10 15). fastSTRUCTURE v1.0 was utilised for the same purpose, testing K=1-5 for *P. angulosus* and K=1-6 for *S. granularis* and *C. punctatus* (Raj, 2014) on the selectively neutral dataset with the prior logistic model and seed parameter 100.

2.3 Results

2.3.1 Assembly metrics

Three assembly programs were used to create *de novo* assemblies (SPAdes v.3.11.1, ABySS v.1.9.0 and Rainbow v.2.0.4), which were compared with Quast reports (Gurevich, 2013) to assess genome assembly quality (Table 2.5a-c). The SPAdes assemblies surpass the results of the other two applications in values including total length, N50 and the size of the largest contig. The total length of the SPAdes assembly of the Cape urchin *P. angulosus* (416 mega base pairs, Mbp) reaches around half of the documented genome assembly length of the purple urchin *Strongylocentrotus purpuratus* (814 Mbp) (Sodergren et al. 2006). Further, total sequence length in the granular limpet *S. granularis* assembly (159 Mbp) is smaller compared to the genome assembly of the owl limpet *Lottia gigantea* (360 Mbp) (JGI, 2012). The largest difference in total sequence length between assemblies is found in the shore crab *C. punctatus* (301 Mbp) in comparison to the (more distantly related) Chinese mitten crab *Eriocheir sinensis* (1.12 giga base pairs, Gbp; Song et al. 2016; 1.55 Gbp, IOCAS, 2018). The total obtained assembly lengths notably differ from previous *de novo* assemblies of two of the species (Nielsen et al. 2018; Table 2.6). Constructing *de novo* assemblies is a field of ongoing developments, thereby differences across assembly program outputs demonstrate the value of testing multiple algorithms.

Table 2.5a. Quast metrics from assemblies created for *C. punctatus* in SPAdes, ABySS and Rainbow.

Assembly	SPAdes	ABySS	Rainbow
# contigs (≥ 0 bp)	713,932	4,424,788	192,205
# contigs (≥ 1000 bp)	81,505	13,064	45
Total length (≥ 0 bp)	423,811,236	780,855,861	37,692,017
Total length (≥ 1000 bp)	116,609,349	16,016,864	53,358
# contigs	354,486	162,024	867
Largest contig	23,947	4,537	1,773
Total length	301,060,503	112,560,268	550,936
GC (%)	42.85	43	44
N50	863	675	597
N75	639	572	536
L50	118,049	63,784	356
L75	220,292	109,255	601
# N's per 100 kbp	0	0	0

Table 2.5b. Quast metrics from assemblies created for *S. granularis* in SPAdes, ABySS and Rainbow.

Assembly	SPAdes	ABySS	Rainbow
# contigs (≥ 0 bp)	559,934	2,882,823	684,970
# contigs (≥ 1000 bp)	37,975	686	34
Total length (≥ 0 bp)	254,301,125	641,592,484	98,651,348
Total length (≥ 1000 bp)	51,345,516	782,835	42,935
# contigs	200,797	50,253	539
Largest contig	9,127	2,904	2,141
Total length	159,310,926	30,540,454	357,771
GC (%)	36.53	37	42
N50	790	586	631
N75	606	534	549
L50	69,908	21,765	216
L75	128,009	35,454	368
# N's per 100 kbp	0	0	0

Table 2.5c. Quast metrics from assemblies created for *P. angulosus* in SPAdes, ABySS and Rainbow.

Assembly	SPAdes	ABySS	Rainbow
# contigs (≥ 0 bp)	368,464	19,703,262	439,765
# contigs (≥ 1000 bp)	145,763	6,322	384
Total length (≥ 0 bp)	466,183,889	1,728,124,915	100,965,369
Total length (≥ 1000 bp)	359,424,766	8,141,286	450,683
# contigs	227,992	79,185	7,493
Largest contig	28,426	4,937	1,996
Total length	416,409,222	54,519,890	5,005,584
GC (%)	35.6	36	36
N50	2,471	659	644
N75	1,385	563	566
L50	49,211	30,925	3,069
L75	105,757	53,415	5,140
# N's per 100 kbp	0	0	0

Table 2.6. Total assembly length shown per species, indicating the total number of bases in the SPAdes assemblies together with the nearest available reference genome assembly.

Species	Nielsen et al. 2018	This study	nearest reference	Source
<i>C. punctatus</i>	-	301 Mbp	1.12 Gbp 1.55 Gbp	<i>E. sinensis</i> (Song et al. 2016; IOCAS, 2018)
<i>S. granularis</i>	180 Mbp	159 Mbp	360 Mbp	<i>L. gigantea</i> (JGI, 2012)
<i>P. angulosus</i>	200 Mbp	416 Mbp	814 Mbp	<i>S. purpuratus</i> (Sodergren et al. 2006)

2.3.2 Total and private SNPs

The number of total SNPs and private SNPs as calculated by PoPoolation2, are presented in Figure 2.3-4 (see also Appendix S2.5-7). The total number of SNPs ranges between 50,000 to 60,000 in *P. angulosus* and *S. granularis*, with an exception of the most western population (Port Nolloth) in *P. angulosus*, which returned over 100,000 SNPs. Substantially more SNPs are found in *C. punctatus*, ranging from 120,000 to almost 150,000. The number

of private SNPs amounts to 1-2 % in *S. granularis* and *C. punctatus*, but is substantially higher in *P. angulosus*, ranging from 5-9% (Table 2.7). There is overall no geographical variation across SNP numbers, the exception being, as mentioned above, a higher number of total and private SNPs found in the urchin *P. angulosus* in Port Nolloth.

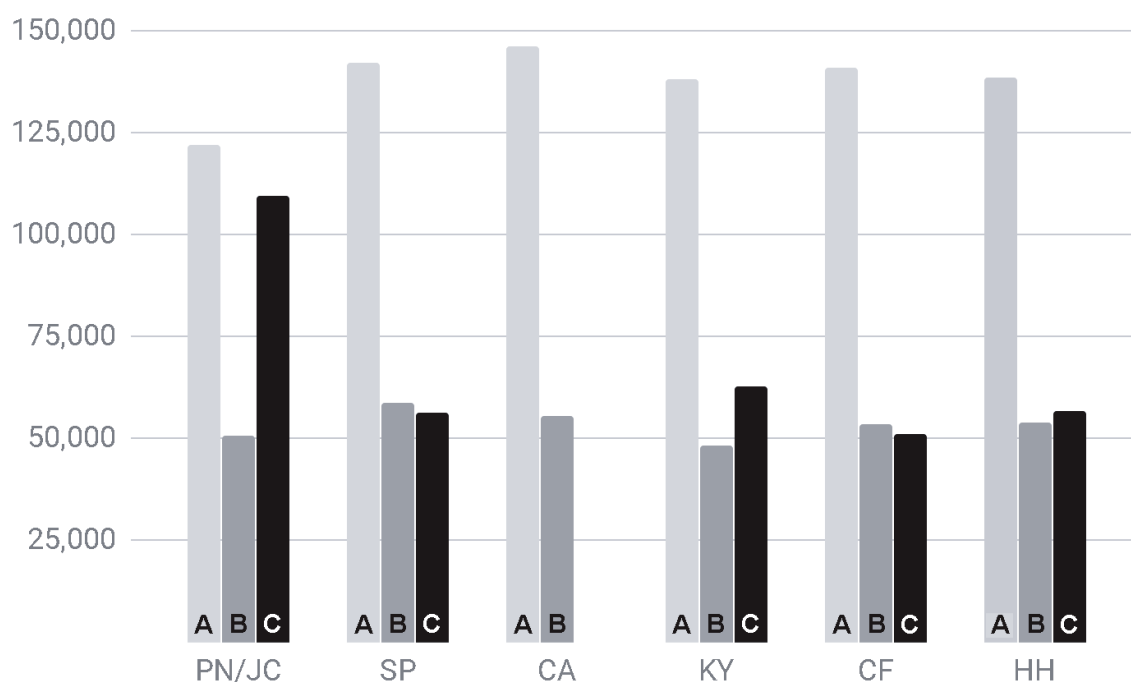


Figure 2.3. Number of total SNPs in *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.1.

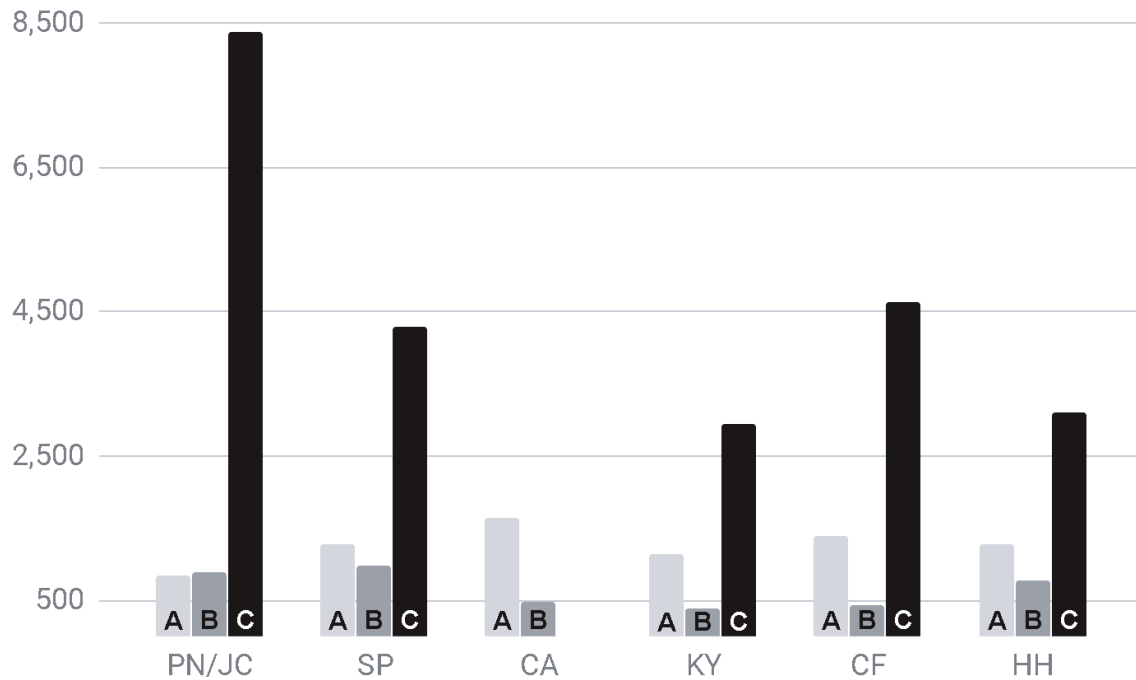


Figure 2.4. Number of private SNPs in *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.1.

Table 2.7. Number of private SNPs shown as percentage by species and location.

Location	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>
Port Nolloth / Jacob's Bay	0.7%	1.8%	7.6%
Sea Point	0.9%	1.7%	7.6%
Cape Agulhas	1.1%	0.8%	-
Knysna	0.8%	0.8%	4.7%
Cape St. Francis	1.0%	0.8%	9.1%
Haga Haga	0.9%	1.4%	5.5%

2.3.3 Nucleotide diversity (Tajima's π) and Watterson's theta (θ_w)

Nucleotide diversity (Tajima's π) and Watterson's theta (θ_w) was estimated by PoPoolation based on the individual pileups (Table 2.8; see Table S2.2-4, Appendix). Values of Tajima's π range from 0.010-0.012 (*C. punctatus*) to 0.009-0.010 (*S. granularis*) and 0.009-0.015 (*P. angulosus*). Watterson's theta (θ_w) ranges from 0.011-0.013 (*C. punctatus*) to 0.009-0.011 (*S. granularis*) and 0.010-0.017 (*P. angulosus*). There was no significant difference in diversity values between sampling sites for the granular limpet and the shore crab. For the Cape urchin, a paired-samples t-test was conducted to compare the values of π and θ_w across populations. There is a significant difference in the scores for π ($M = 0.011$, $SD = 0.0025$) and θ_w ($M = 0.012$, $SD = 0.0029$); $t(4) = 2.776$, $p = 0.005$.

Table 2.8. Number of SNPs, Tajima's π and Watterson's θ_w values as estimated in PoPoolation. The last column indicates the average values across populations. Location abbreviations are listed in Figure 2.1.

<i>C. punctatus</i>	JC	SP	CA	KY	CF	HH	Ø average
# SNPs	21,257,499	31,319,803	36,545,844	27,545,887	32,910,660	29,735,374	29,885,845
pi	0.010	0.010	0.012	0.010	0.011	0.010	0.011
theta	0.011	0.011	0.013	0.011	0.011	0.011	0.011
<i>S. granularis</i>	PN	SP	CA	KY	CF	HH	
# SNPs	5,951,362	9,101,835	7,169,491	4,238,802	5,790,902	5,096,943	6,224,889
pi	0.010	0.010	0.010	0.009	0.009	0.009	0.010
theta	0.010	0.011	0.010	0.009	0.010	0.010	0.010
<i>P. angulosus</i>	PN	SP	-	KY	CF	HH	
# SNPs	122,319,835	62,779,686		71,632,295	63,025,279	62,558,141	76,463,047
pi	0.015	0.011		0.009	0.010	0.009	0.011
theta	0.017	0.012		0.010	0.012	0.010	0.012

2.3.4 Pairwise F_{ST} values

The estimated pairwise F_{ST} values range from 0.039-0.089 (*P. angulosus*), 0.044-0.066 (*S. granularis*) and 0.043-0.055 (*C. punctatus*) (Table 2.9; see Appendix S2.8-10). According to Fisher's exact test, there are no significant differences between the sampled populations in the three species.

Table 2.9. F_{ST} as estimated in PoPoolation2 (scenario 6) incorporating 14,392 (A), 5,440 (B) and 4,235 (C) SNPs. Location abbreviations listed in Figure 2.1.

<i>C. punctatus</i> (A)	JC	SP	CA	KY	CF	HH
JC	-					
SP	0.049	-				
CA	0.050	0.045	-			
KY	0.050	0.044	0.044	-		
CF	0.052	0.049	0.043	0.046	-	
HH	0.055	0.052	0.048	0.049	0.046	-
<i>S. granularis</i> (B)	PN	SP	CA	KY	CF	HH
PN	-					
SP	0.051	-				
CA	0.061	0.055	-			
KY	0.061	0.059	0.051	-		
CF	0.061	0.055	0.044	0.050	-	
HH	0.066	0.063	0.053	0.053	0.051	-
<i>P. angulosus</i> (C)	PN	SP	-	KY	CF	HH
PN	-					
SP	0.066	-				
KY	0.074	0.049	-	-		
CF	0.089	0.061	-	0.053	-	
HH	0.082	0.055	-	0.039	0.059	-

2.3.5 Cluster analyses

The number of estimated potential clusters differs between BAPS and fastSTRUCTURE (Table 2.10). The optimal partitions estimated in BAPS range from 4 (*S. granularis*, *P. angulosus*) to 5 (*C. punctatus*) and in fastSTRUCTURE from 1 (*C. punctatus*, *P. angulosus*) to 3 (*S. granularis*). Importantly, all partitions are present at all populations and the suggested clusters do not follow any geographical pattern (see Appendix, Figure S2.1-2).

Table 2.10. Number of estimated population clusters per species and application.

	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>
BAPS	5	4	4
BAPS Log (ml)*	-2076450.8497	-475298.4289	-187512.439
fastSTRUCTURE	1	3	1

*log (marginal likelihood) of optimal partition

Table 2.11. Findings from mtDNA and SNPs: study species pairwise F_{ST} values, nucleotide diversity, suggested lineages and potential clusters.

Species	Markers	Population structure (pairwise F_{ST})	Global F_{ST} (ϕ_{ST})	Nucleotide diversity (π)	Average $\bar{\pi}$	Sample region South Africa*	Suggested lineages based on mt/nDNA	Signal for IBD	Clusters (BAPS, fast-structure)	Reference
<i>C. punctatus</i>	COI**	0.15-0.91	0.49	0.002-0.018	0.009	west, south, east (11)	-	✓	-	Wright et al. 2015
<i>C. punctatus</i>	SNPs	0.043-0.055	NA	0.010-0.012	0.011	west, south, east (6)		NA	5/1	This study
<i>S. granularis</i>	COI	0-0.473	0.023	NA	0.09078	west, south, east (16)	west & east lineage***	-	-	Mmonwa et al. 2015, Mmonwa, 2013
<i>S. granularis</i>	COI	0-0.05	0.00	0.0036-0.0052	0.0043	west coast (7)	-	-	-	Mertens et al. 2018
<i>S. granularis</i>	SNPs	0.008-0.013	NA	0.009-0.012	0.011	west coast (6)	-	-	-	Nielsen et al. 2018
<i>S. granularis</i>	SNPs	0.044-0.066	NA	0.009-0.010	0.010	west, south, east (6)			4/3	This study
<i>P. angulosus</i>	COI, SpREJ9 (nDNA)	0-0.87 0-0.71	NA	0.007-0.069	0.022	west, south, south-east (18)	west coast; south/east coast****	-	-	Muller et al. 2012
<i>P. angulosus</i>	COI	0.15-0.62	0.32	0.0031-0.0163	0.0098	west coast (6)	-	✓	-	Mertens et al. 2018
<i>P. angulosus</i>	SNPs	0.006-0.019	NA	0.006-0.011	0.009	west coast (6)	-	-	-	Nielsen et al. 2018
<i>P. angulosus</i>	SNPs	0.039-0.089	NA	0.009-0.015	0.011	west, south, east (5)		NA	4/1	This study

IBD = isolation-by-distance; NA = not available; *number of sampled populations shown in brackets, ** cytochrome c oxidase subunit I, ***phylogeographic break along the central South Coast, ****phylogeographic break in the area of Cape Point.

2.4 Discussion

The focus of this chapter is to investigate the following hypotheses (see section 2.1.6): 1) Based on past genomic studies, neutral genomic diversity might not show an east-west gradient and appear rather uniform across populations, 2) neutral genomic population structure (if detected) might not indicate geographically separated clusters and 3) findings are expected to display possible interspecific differences in levels of genomic diversity and population structure based on inter-phyletic differences.

2.4.1 Genomic diversity

The intraspecific number of total SNPs does not vary greatly across the sampled populations (~50,000), but there are two notable exceptions. While less SNPs relative to the remaining populations are detected in the shore crab *C. punctatus* in Jacob's Bay, the opposite is true for the urchin *P. angulosus*, which shows a roughly doubled total SNP count (>100,000) in Port Nolloth (both west coast sites) (Figure 2.3). The number of private SNPs, referring to single nucleotide polymorphisms which are unique to the sampled population, ranges without an apparent spatial pattern between 500-1,000 per population (1-2% of the total SNPs) in *C. punctatus* and *S. granularis*. As expected from the high number of *P. angulosus*' total SNPs in Port Nolloth, this population also shows with ~8,500 by far the highest occurrence of unique SNPs. This finding is congruent with Nielsen also reporting the highest amount of private SNPs in Port Nolloth, which has been attributed to complex "patterns of genetic drift and gene flow" rather than mutation and selection (Nielsen et al. 2018; p. 8). A comparatively large number of private SNPs may be a sign of subtle demographic isolation of a population (Nielsen et al. 2018). However, the species is not constrained by rocky shore habitat availability in the Port Nolloth area (Majiedt et al. 2013; Figure 4) and hydrodynamic larval dispersal models suggest theoretical larval connectivity with other west coast populations (Mertens, Treml and von der Heyden, 2018; Figure 3). Interestingly, the Cape urchin further shows increased values (~ 4,500) of private SNPs in the second west coast population (Sea Point) and in Cape St. Francis on the southern east coast (Figure 2.4, Table 2.7), which might point to possible local unique evolutionary and environmental processes at these localities. In general, the amount of private SNPs appears to be significantly higher in the urchin *P. angulosus* compared to the other two species. The samples were prepared and analysed with the same procedures to reduce confounding methodological influences, which increases the possibility that the observation may be linked to taxonomic differences such as higher levels of polymorphisms in the genome of urchins or even in individual urchins (Balhoff and Wray, 2005; Cameron et al. 2005; Cameron et al. 2009).

Nucleotide diversity estimates do not demonstrate variation across populations (with the exception of Port Nolloth in *P. angulosus*). From a genetic perspective, it is unexpected to recover such similar nucleotide diversity estimates, because there is for instance evidence for highly polymorphic individual sea urchins and a high degree of polymorphism in urchin genomes (Balhoff and Wray, 2005; Cameron et al. 2005; Cameron et al. 2009; Sodergren et al. 2006). Differences in life history traits (see Table 2.2) could perhaps have contributed to spatial variations in nucleotide diversity, but nucleotide diversity estimates are remarkably congruent across study species and similar to other South African coastal marine species (Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017; Nielsen et al. 2018; Teske et al. 2019). Interestingly, a neutral genetic diversity survey across 167 species from 14 phyla suggests that despite patchy availability of species estimates, broader patterns indicate that significant differences in genetic diversity across phyla appear to exist (Leffler et al. 2012). Further, there is evidence for phyla with a wide range of nucleotide diversity levels, pointing to pronounced genetic variation among species of the same phylum and also across phyla (Leffler et al. 2012). Although some limited whole genomic data was incorporated by Leffler et al., it will be valuable to investigate the estimates and ensuing patterns of nucleotide diversity derived from SNP markers in future research to revisit the observations across multiple phyla. Returning to the study results, Tajima's π and Watterson's θ_w values in the urchin *P. angulosus* however indicate significant geographic differentiation (Table 2.8). Both indices suggest Port Nolloth on the northern South African west coast as an area of increased nucleotide diversity relative to the remaining coastline, which is congruent with similar estimates for this species in the Port Nolloth region (π : 0.011, θ_w : 0.012; Nielsen et al. 2018). The present study confirms that this population of *P. angulosus* is possibly "evolutionary unique" (Nielsen et al. 2018; p. 8), which should be considered for regional conservation plans as an indication of evolutionary potential.

A study across eleven South African rocky shore and reef-dwelling marine species with (partial) COI and D-loop markers suggested the preliminary hypothesis of a nucleotide diversity gradient at the South African coastline with lower molecular diversity on the west coast compared to higher estimates on the south and east coast (Wright et al. 2015). However, this observation is strongly influenced by the low diversity measured in topshell *Oxystele variegata* and fish *Clinus cottoides* in the west coast region (Wright et al. 2015). The present study contributes evidence against this hypothesis, because genomic SNP markers across three species suggest no spatial variation between the populations. The urchin's Port Nolloth population constitutes the notable exception, as it appears to turn the hypothetical gradient upside down with comparatively higher molecular diversity on the

northern west coast. Importantly, more genomic studies are required to assess molecular diversity in South African marine species beyond the study species. Overall, the evidence contributes to the observation that molecular patterns from traditional markers such as COI might not be aligned with patterns by genomic markers.

Overall, neutral genomic diversity can be impacted by a range of determinants, which include natural selection, random drift, effective population size (N_e) and genetic connectivity between populations (Frankham, Briscoe, and Ballou, 2002; Gaggiotti et al. 2009; Bragg et al. 2015; Gómez-Fernández, Alcocer, and Matesanz, 2016). Regarding the lack of spatial genomic population structure across study species, it could be hypothesised that the species large effective population size in the intertidal ecosystem might contribute to the observation. However, the species effective population size across the South African coastline has at present not been empirically studied. Moreover, life history traits differ strongly between study species (Table 2.2). But it is not established with certainty which effect reproductive strategies (e.g. broadcasting vs. brooding followed by pelagic larvae), PLD (pelagic larval duration) and other larval traits such as feeding mode (planktotrophic, lecithotrophic) and larval behaviour have on the genomic structure of intertidal populations at the South African coastline. For example, genomic connectivity and oceanographic current patterns between populations might be one of the factors facilitating the observed similar nucleotide diversity levels. A further possible influence stems from the paleo-climatic history in southern Africa, which is discussed in more detail in the next section. The lack of neutral nucleotide diversity differentiation (exception: urchins/Port Nolloth) is likely not attributable to a single factor, but the result of multiple influences and further research is necessary to unravel which drivers are at play.

RAD-Seq studies on marine species from the same phyla as the study species (Arthropoda, Echinodermata, Mollusca) have been reviewed for nucleotide diversity estimates (see Table S2.11, Appendix) to gain a preliminary reference frame for nucleotide diversity variation in other marine ecosystems across the world. Directly comparing nucleotide diversity estimates from SNPs across multiple studies is impeded by a multitude of differences including number of sampled populations, sampled geographical area, selected RAD-Seq approach, applied filtering protocols, utilisation of subsampling, varying software tools and analytical parameters. Investigating estimates from the available marine species RAD-Seq literature revealed that reporting nucleotide diversity metrics appears to some extent less common compared to past phylogeographic studies based on traditional markers (Benestan et al. 2016; Kess et al. 2016; 2018; Vendrami et al. 2017; Jeffery et al. 2017b; Wu et al. 2018) or alternative diversity indices are reported (Ravinet et al. 2016; Jeffery et al. 2017a; Villacorta-

Rath et al. 2017; Paterno et al. 2017; Forsström, Ahmad, and Vasemägi, 2017; Galaska et al. 2017; Xuereb et al. 2018; Sandoval-Castillo et al. 2018). From other marine species (see Table S2.11, Appendix), it is evident that there is a spectrum of nucleotide diversity values across arthropods, molluscs and echinoderms. For instance, nucleotide diversity may vary between 0.0002 to 0.0086 in marine Arthropoda, between 0.0003 to 0.94 in Mollusca and between 0.00146 to 0.307 in Echinodermata (Table S2.11, Appendix). Such a range is expected, as these taxa inhabit different geographical locations, have different life history traits and were sampled at different spatial scales. These methodological differences complicate analyses across broader geographical scales, which may well influence the inferences that can be made across studies. This includes a discussion on the definition of what constitutes low or high genomic diversity. In marine arthropods, SNP-based nucleotide diversity found in the shore crab *C. punctatus* is noticeably higher than in red king crab (Grant and Cheng, 2012; Table S2.11), but not far removed from shrimps, copepods and barnacles (Daizhen et al. 2016; Nunez et al. 2018; Dexter et al. 2018; Zhang et al. 2019; Choquet et al. 2019). Nucleotide diversity estimates for the granular limpet *S. granularis* are congruent with earlier estimates of the same species (Nielsen et al. 2018) and comparable with genomic diversity in the Pacific and the Portuguese oyster (Gagnaire et al. 2018; Song et al. 2018). Although nucleotide diversity as high as 0.94 ($\theta\pi$) has been found in *Brachidontes* spp. mussels (Maas et al. 2018), values estimated for *S. granularis* (0.010; Table S2.11) are notably higher than for two other intertidal molluscs (Chu et al. 2014; Gleason and Burton, 2016). In the Cape urchin *P. angulosus*, nucleotide diversity is comparable to values estimated for the same species (Nielsen et al. 2018; Table S2.11), but noticeably lower than observed in two other urchin species by Addison and Kim (2018). Nucleotide diversity values appear broadly similar when obtained from multiple species in the same study (for instance, Gagnaire et al. 2018; Nielsen et al. 2018; Addison and Kim, 2018; Choquet et al. 2019; this study), which may signal the influence of the RAD-Seq approach, data filtering protocols and software tools among other reasons. SNP-based estimates for nucleotide diversity (π ; θ_w) might be available for a larger number of species in the near future, as RAD-Seq studies on marine invertebrates will likely increase.

2.4.2 Genomic differentiation

To detect potential population structure, pairwise F_{ST} values were estimated and two tests for genomic clustering conducted. While some degree of F_{ST} -based differentiation is detected across study species populations, it is not significant according to Fisher's exact test. The lowest range of pairwise F_{ST} values is found in *C. punctatus* (0.043-0.055; Table 2.9). The Cape urchin *P. angulosus* has the highest range of F_{ST} values (0.039-0.089), which

is likely associated with the species relatively high numbers of private SNPs. The level of divergence, when regarded as the widest range of F_{ST} values, is much higher in *P. angulosus* compared to the other two species, but does not appear to occur in a specific spatial pattern (e.g. in relation to suggested phylogeographic or biogeographic breaks, see section 1.5.3). This may be regarded as evidence that population structure found with mtDNA does not necessarily reflect findings from SNP markers (see section 2.4.3). To test for the presence of population clusters, BAPS v.5.4 and fastSTRUCTURE v1.0 were utilised (Table 2.10; Figure S2.1-2, Appendix). BAPS estimates a higher number of possible clusters across the species, ranging from 4 (*S. granularis*, *P. angulosus*) to 5 (*C. punctatus*). However, fastSTRUCTURE suggests three clusters in *S. granularis* and no subdivision of a single population in the other two species. Importantly, and this is congruent across both programs, there is no spatial separation between possible clusters, meaning all potential subdivisions (where estimated) occur in all sampled populations (Figure S2.1-2, Appendix). Differences in the optimal number of estimated clusters across BAPS, fastSTRUCTURE and Structure (Pritchard et al. 2000; precursor of the improved inference model used in fastSTRUCTURE) have also been observed in other studies with some estimates yielding vastly contrasting numbers of clusters (Steiner et al. 2018; Mura-Jornet et al. 2018; Paz et al. 2019; Pinho, Cardoso and Hey, 2019), which may demonstrate the different methodological approaches of the underlying models (Wilkinson et al. 2011). For instance, BAPS is thought to identify finer genetic differentiation patterns compared to other Bayesian genotypic clustering methods and thus might estimate a higher number of underlying population clusters, which appears congruent with the findings of this study (Wilkinson et al. 2011). Lastly, low population differentiation is thought to decrease the overall power of clustering algorithms to detect population clusters (Latch et al. 2006; Wollstein and Lao, 2015).

The absence of significant differentiation in selectively neutral loci across the study species populations is concordant with findings of other South African marine genomic studies (Nielsen et al. 2018; Mullins et al. 2018; Teske et al. 2019; but see Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017). Internationally, multiple studies found spatial genomic differentiation across different geographic scales with F_{ST} -based approaches in marine invertebrates with a range of life history traits (e.g. *Arthropoda*: Tepolt and Palumbi, 2015; Benestan et al. 2016; Jefferey et al. 2017a; Forsström, Ahmad, and Vasemägi, 2017; Pfaller et al. 2019; Choquet et al. 2019; *Mollusca*: Chu et al. 2014; Ravinet et al. 2016; Vendrami et al. 2017; Lal et al. 2017; Kess, Galindo, and Boulding, 2018; *Echinodermata*: Galaska et al. 2017; Paterno et al. 2017; Xuereb et al. 2018). On the contrary, there is also evidence for the absence of or very low genomic differentiation across marine invertebrate populations

(e.g. *Arthropoda*: Villacorta-Rath et al. 2017; Nunez et al. 2018; Hay, Jorge, and Poulin, 2018; Dexter et al. 2018; *Mollusca*: Gleason and Burton, 2016; Miller et al. 2016; Sandoval-Castillo et al. 2018; *Echinodermata*: Tay et al. 2016; Addison and Kim, 2018). For the South African context, it may become clearer with future research whether the absence of significant genomic differentiation in selectively nuclear markers applies to other marine invertebrates.

2.4.3 Comparison of SNP-based and traditional single marker findings

Traditional single markers (for instance from species mitochondrial DNA) are commonly used to elucidate past demographic history (Avise, 2000; Dudgeon et al. 2012; von der Heyden, 2017), whereas single nucleotide polymorphisms markers reveal contemporary population structure and loci with adaptive potential (Morin, Luikart and Wayne, 2004; Allendorf, Hohenlohe and Luikart, 2010; Funk et al. 2012; Bowen et al. 2014; von der Heyden, 2017). Patterns derived from single marker studies and SNPs studies can therefore be expected to differ, even though this is not automatically the case. Several contrasting signals between the traditionally used COI locus (mtDNA) and SNP markers are observed in this study. While nucleotide diversity estimates are broadly similar across marker types in the study species, pairwise F_{ST} values between populations contrast strongly with generally higher F_{ST} -based differentiation in the partial COI locus (mtDNA) compared to SNP markers (Table 2.11). Further, the presence of pronounced spatial population structure is indicated in COI data (mtDNA) (Table 2.3), but not confirmed with SNP markers across study species (see section 2.4.2). While a certain degree of molecular subdivision appears to perhaps exist depending on the Bayesian cluster model (i.e. BAPS and fastSTRUCTURE), these are not occurring in spatial separation from each other (Table 2.10).

Analyses of the study species population structure with the COI marker (mtDNA) detected significant levels of spatial differentiation across the South African coastline (Muller et al. 2012; Wright et al. 2015; Mmonwa et al. 2015; see section 1.7; Table 2.3). The absence of genomic structure across populations in this study in selectively neutral SNP markers is however congruent with previous regionally limited findings (Nielsen et al. 2018). Low levels of genomic population structure can be influenced by a range of environmental and biological factors such as for instance oceanographic current patterns (Belanger et al. 2012; Bowen et al. 2014; Selkoe et al. 2016). The South African oceanographic seascape is particularly dynamic due to the influence of the warm Agulhas Current associated with the Indian Ocean in the east and the cold Benguela Current associated with the southern Atlantic Ocean in the west (Bustamante et al. 1995). However, how prevalent current

systems influence the genomic population structure of species in coastal ecosystems is not established in detail at the South African coast (Siegel et al. 2008; Pfaff et al. 2015; Mertens, Trembl and von der Heyden, 2018), but there are pioneering studies on oceanographic larval dynamics across the Agulhas bank (Porri et al. 2006; Porri et al. 2008; Porri et al. 2014; Weidberg et al. 2015; Weidberg et al. 2019). Furthermore, species life history traits are regarded as important influencing factors for marine genomic population structure (Teske et al. 2007; Vähä et al. 2007; Freamo et al. 2011; Zarraonaindia et al. 2012; Young et al. 2015; McKeown et al. 2017). The three study species possess a wide range of life history traits, including differences in pelagic larval duration (PLD) from estimated 7-14 days (*S. granularis* (Kilburn and Rippey, 1982; Kay, 2002); *C. punctatus* (Branch et al. 2007; Wright et al. 2015)) to around 50 days (*P. angulosus*; Cram, 1971; Greenwood, 1975). Hydrodynamic dispersal networks constructed for larvae of the urchin *P. angulosus* demonstrate a hypothetically high dispersal potential for the species larvae at the west coast (Mertens, Trembl and von der Heyden, 2018), but this is not evidenced in the high population structure indicated by the COI locus (Muller et al. 2012) and the high (neutral) nucleotide diversity in Port Nolloth (Nielsen et al. 2018; this study). Other life history traits include preferred intertidal microhabitat niche and attributes like mobility during low tide and reproductive traits such as spawning frequency throughout the year and variable recruitment. However, it is fiercely disputed if and then to which degree the often not well established life history traits of marine invertebrates serve as reliable indicators for genomic population structure in the ocean (Weersing and Toonen, 2009; Selkoe and Toonen, 2011; Faubry and Barber, 2012; Trembl et al. 2012; Giangrande, Gambi and Gravina, 2017).

Apart from oceanography and life history traits, population differentiation may also arise from changes of the species demographic history. For instance, contemporary population structure based on the mtDNA control region (D-loop) across South African intertidal fish populations has been attributed to intermittent spatial isolation caused by changes in paleo-oceanography during the Pleistocene (Toms et al. 2014). It has been suggested that since the historic population divergence, factors such as oceanographic patterns and near shore coastal dynamics may have decreased the effect of potential past isolation (Toms et al. 2014). The evidence for the three study species specific historic demography is limited (e.g. Mmonwa et al. 2015; Seymour, unpublished data), but as they widely share the same rocky shore habitat across the coastline, it might be possible if not likely that past spatial separation and subsequently re-established connectivity between historic intertidal populations may have played a role in shaping the detected differences between mtDNA and SNP marker findings. Evidence for the evolutionary effects of Pleistocene climate oscillations (and ocean current patterns) on species phylogeography has been observed in several

South African coastal marine species (Reynolds et al. 2014; Toms et al. 2014; Muteveri et al. 2015; von der Heyden et al. 2015). Discordance between mitochondrial DNA and SNPs has been associated with adaptive mtDNA introgression, varying sample sizes, differing mutation rates and sex-bias in demographic history causing asymmetries of effective population size between males and females (Toews and Brelsford, 2012). Due to the complex nature of biological and environmental factors potentially shaping population structure or the absence thereof in the marine environment, it is important to conduct further investigations across the range of co-distributed intertidal species occupying the heterogeneous South African coastline.

Both congruence and discordance between traditional single markers and SNP markers has been observed in marine invertebrates. An example for discordance is a study on calanoid copepod *Centropages typicus*, where significant population structure became evident with RAD-Seq derived SNPs after traditional markers did not indicate differentiation (Blanco-Bercial and Bucklin, 2016). Similarly, discordant observations across marker types were made in scallop *Pecten maximus* (Vendrami et al. 2017), intertidal snail *Chlorostoma funebris* (Gleason and Burton, 2016) and in the Atlantic dogwinkle *Nucella lapillus* (Chu et al. 2014; see section 2.1.4). Examples for congruent findings across marker types include evidence for population differentiation in the urchin *Paracentrotus lividus* across the Mediterranean Sea (Paterno et al. 2017) and in the crab *Carcinus maenas* in eastern North America (Jefferey et al. 2017a). Congruent patterns are indicated in a range of marine invertebrates (Tay et al. 2016; Forsström, Ahmad and Vasemägi, 2017; Xuereb et al. 2018; Pfaller et al. 2019), but both marker types might also equally confirm no presence of population structure, such as in the abalone *Haliotis rubra* (Miller et al. 2016). It is evident that findings obtained with traditional single markers do not allow a prediction of SNP-based patterns. The possible ongoing relevance of traditional single markers has been advocated for insights into the genetic composition and corresponding evolutionary history of species (see section 1.3; Bowen et al. 2014; von der Heyden, 2017; Riddle, 2016), because the coverage achieved with whole genome sequencing remains costly and might not be feasible for comparative multi-species studies.

2.4.4 Evolutionary resilience

The concept of evolutionary potential or resilience seeks to infer species capacity to respond to changing conditions based on their genomic diversity or signals for adaptive variation (the latter is subject of Chapter III) (Bonin et al. 2007; Sgro et al. 2011). High molecular diversity in populations and species may lower their risk of extinction and vice versa (Reed and

Frankham, 2003). Populations with high genomic diversity and thus a broader molecular foundation to potentially adapt from, might indicate regional species groups with higher evolutionary resilience compared to other populations in their geographic range. Signs of regional variation in genomic diversity may warrant recognition in spatial prioritisation processes along coastlines and thus harbour value for conservation management of species (von der Heyden, 2017; Flanagan et al. 2018; Xuereb et al. 2019). For instance, one may actively focus on low diversity areas and coordinate protection for high diversity regions (Drury et al. 2017). There is evidence from two mtDNA markers (COI, D-loop) for species with lower molecular diversity on the west coast than on the south and east coast (Wright et al. 2015). However, the SNP markers findings (see section 2.4.1), indicate against the occurrence of a molecular diversity gradient across the South African coastline. Moreover, roughly equal levels of selectively neutral nucleotide diversity may suggest that evolutionary resilience related to neutral SNPs appears to be evenly distributed across the species populations. Observing comparable molecular diversity across three co-distributed species is interesting in the context of the vast geographical distance and the heterogeneous environmental conditions across the South African coastline. A striking exception is Port Nolloth on the northern west coast, which shows significantly higher nucleotide diversity estimates in the urchin *P. angulosus* (Table 2.8). This regional signal is congruent with a previous study (Nielsen et al. 2018; incorporating outlier loci) and may thus perhaps constitute a real biological signal for the evolutionary uniqueness and distinct molecular signatures of this keystone species in the specific geographical area.

Molecular diversity estimates have been utilised in other studies in the marine environment as indicators for varying degrees of inferred evolutionary resilience. For instance, molecular diversity has been investigated across the taxonomic subclass of sharks and rays against the background of the species estimated level of threat (Domingues, Hilsdorf and Gadig, 2018). On the intraspecific level, distinct marine populations with comparatively high molecular diversity have been identified (Drury et al. 2017; Cahill et al. 2017). In one example, data across multiple species allowed to compute entire bioregions with outstanding overall genetic diversity (Pope et al. 2015). Further, there is evidence that low molecular diversity, among other potential factors, can be associated with the impact of human activities and that in turn, low human population densities may correlate with higher molecular diversity (Cahill et al. 2017). Human population densities vary across the seven combined South African sites in this study. For instance, Sea Point is located close to the commercial port of Cape Town, whereas the other six sampled locations serve as recreational destinations and thus experience fluctuating impact from human activities. It remains speculative at present how the significantly higher genomic diversity of the urchin *P.*

angulosus might be associated with human presence around Port Nolloth (>6,092 inhabitants, statssa.gov.za, Census 2011), because the town is historically associated with mining of diamonds from the sea (Majiedt et al. 2013). The influence of sea diamond mining and other possible industry-related effects on local genomic population structure at the west coast remain to be determined.

2.4.5 Comparisons across different SNP parameters

Single nucleotide polymorphisms derived from RAD-Sequencing and the application of pooled sampling strategies facilitate the exploration of large numbers of loci in non-model species (Delord et al. 2018; Xuereb et al. 2019). RAD-Seq and Pool-Seq studies are increasing overall (Therkildsen and Palumbi, 2017), but the impact of different bioinformatic procedures and the lack of standardised analysing (and reporting) is not well understood yet (Shafer et al. 2017). As genomic findings obtained in these studies may be drawn on to inform conservation and management processes, it is important to better understand the reliability (or potential variability) of results (Shafer et al. 2017). Findings from RAD-Seq and Pool-Seq studies may be influenced through several factors (Kofler et al. 2016; Shafer et al. 2017), but this study explores how the results can vary depending on different parameters of what may constitute a SNP in terms of minimum allele count, minimum coverage and maximum coverage (Table S2.1, Appendix). In this study, stringent and less stringent SNP parameters are represented with minimum allele count ranging from 2-16, minimum coverage from 10-32 and maximum coverage 50-200 (Table S2.1), with the understanding that the selected values can vary in studies and for instance reach up to 500 for maximum coverage (Pandey et al. 2011). The range of PoPoolation's estimates under stringent and less stringent SNP definitions for number of SNPs, number of private SNPs, nucleotide diversity (π , θ_w) and F_{ST} values, is summarised in Table S2.12 and the full estimates for all locations are shown in Table S2.2-10 (Appendix). To illustrate the range of results, the Sea Point site is selected in this instance, because it has been sampled across all three study species. Some of the most drastic differences between varying minimum allele count, minimum coverage and maximum coverage are observed in *P. angulosus* (Table S2.12, Appendix). For instance, the number of total SNPs and private SNPs calculated by PoPoolation2 can range from 1,243-56,185 (total SNPs) and 41-4,287 (private SNPs). Moreover, nucleotide diversity indices vary from 0.008-0.016 (π) and 0.008-0.020 (θ_w), depending on which parameter stringency is selected. Lastly, pairwise F_{ST} estimates (between Sea Point and Knysna, which is calculated for all three species) range from 0.019-0.063 (Table S2.12). As expected, the estimates derived from stringent parameters are overall lower than results obtained under less stringent parameters. Aside from *P.*

angulosus, the other two study species also show notable variation across SNP parameters (Table S2.12). This demonstrates, while the investigation towards understanding the impact and reliability of estimates obtained from different bioinformatic processes and pipelines is ongoing, there is a need to report prominently which SNP parameters were utilised and if possible to also report potential variation under different parameters. This would help some way towards making RAD-Seq (and Pool-Seq) studies more comparable and better the scientific understanding of genomic estimates in different geographical contexts. SNP parameters have been specifically reported in studies utilising PoPoolation (e.g. Guo et al. 2015; 2016; Nielsen et al. 2018; Phair et al. 2019), but with an increasing number of bioinformatic pipelines for RAD-Seq data (for instance Stacks (Catchen et al. 2013); assessPool (Barba et al. 2018)), there is a need to strive for better understanding of how findings might vary in different scenarios and how potential variation might be accounted for in decision making processes. Several authors are advocating for more standardisation in Pool-Seq data analyses paths (Anderson, Skaug, and Barshis, 2014; Kofler et al. 2016). In this study, estimates derived from minimum allele count 4, minimum coverage 10 and maximum coverage 200 (scenario 6; Table S2.1) in PoPoolation and PoPoolation2 were selected to broadly represent a middle ground across stringent and less stringent SNP parameters. Reporting across multiple SNP parameters might, despite the substantial time investment, facilitate enhanced comparison between study organisms in different geographical settings and serve to demonstrate the reliability of estimates based on their documented variation.

2.4.6 *De novo* assemblies

Traditional genome assemblies require substantiated research endeavours and resources, which has concentrated past and continued efforts on a selected range of model organisms and their reference genomes (Seeb et al. 2011). The reduced investment of SNPs derived from RAD-Seq approaches and the utilisation of pooled samples has made genotyping available for non-model species and paved the way for comparative multi-species studies (Delord et al. 2018). The development gave rise to the necessity of assemblies created from scratch (*de novo*) in the absence or very small amount of genomic information or resources available for the majority of species. Multiple statistical approaches and algorithms have been developed to create *de novo* assemblies (see section 2.3) and three of them allowing for pooled data were tested to create draft genomes for the study species (SPAdes v.3.11.1 (Bankevich et al. 2012), ABySS v.1.9.0 (Simpson et al. 2009) and Rainbow v.2.0.4 (Chong et al. 2012)). The highest values for total assembly length, contig N50 and the size of the largest contig were obtained with the assemblies created by SPAdes v.3.11.1 (Table 2.5a-c)

and refined with k-mer length optimisation (Davey et al. 2013; Chikhi and Medvedev, 2014). The final draft genome assemblies of the three study species have notably less Mbp than their nearest identified reference genomes (Table 2.6), which is expected based on for instance the different sequencing approaches (e.g. whole-genome shotgun and bacterial artificial chromosome (BAC) sequences as opposed to pooled RAD-Seq) and multi-year research efforts conducted to assemble, for example, the genome of the purple urchin *Strongylocentrotus purpuratus* (a model organism for developmental and systems biology; Sodergren et al. 2006). Differences are also evident between draft genomes of two species (*S. granularis*, *P. angulosus*) created for this study (159 Mbp, 416 Mbp) and a previous RAD-Seq study (180 Mbp, 200 Mbp) (Table 2.6; Nielsen et al. 2018). These differences could have arisen from a number of factors, for example from utilising different versions of SPAdes (v.3.11.1 vs. v.3.5.0), sampling geographically different populations (exception: Port Nolloth, Sea Point), determining different optimised k-mer values and the subsampling approach applied in this study (see Table 2.4). The differences between the draft genomes created for the same species signal the cumulative impact varying determinants might have in the assembling process. As is to be expected, draft genomes showed different assembly lengths across phyla (159 Mbp, 301 Mbp, 416 Mbp), which is indicated by the taxonomic distance connected to different genomic compositions between the study species and can also be observed in the vast size contrasts between the nearest identified reference genomes (see Table 2.6). Notably, two previous RAD-Seq draft genomes appear similar in size across species (180 Mbp, 200 Mbp), which was attributed to the enzymatic activity of RAD-Seq leading to similar sized fragments influencing the draft genome size (Nielsen et al. 2018). This was not observed for the same two species and their *de novo* assemblies in this study. Finally, the Quast genome assembly quality reports across multiple assemblers (see Table 2.5a-c) emphasise the benefit of testing multiple assembly algorithms to identify the highest draft genome quality. Advances in both RAD-Seq methodology and *de novo* assembly programs will facilitate continually improved draft genomes of non-model organisms, thus expanding the genomic knowledge and the foundation for genomic analyses for a wide range of species in the future.

2.4.7 Conclusion

Congruent with initial expectation, nucleotide diversity indices (π , θ_w) in *C. punctatus*, *S. granularis* and *P. angulosus* do not show geographical variation across the selected populations (with the exception of *P. angulosus*' Port Nolloth population) and F_{ST} -estimated population differentiation is not significant. These findings contraindicate the hypothesis of a west-east molecular diversity gradient (Wright et al. 2015), but are broadly in congruence

with other genomic studies on marine animals in the region (Rhode, Bester-van der Merwe, and Roodt-Wilding, 2017; Nielsen et al. 2018; Mullins et al. 2018; Teske et al. 2019). Contrary to theoretical expectations, diversity indices show similar levels of nucleotide diversity across species. This is unexpected, because there are inter-phyletic differences such as very polymorphic individual sea urchins and a high number of polymorphisms in the genome of urchin (Balhoff and Wray, 2005; Cameron et al. 2005; Cameron et al. 2009; Sodergren et al. 2006). But as expected, distinct geographical lineages in *S. granularis* and *P. angulosus* identified with two single markers (see Table 2.3) could not be confirmed with SNP-based F_{ST} estimates and cluster analyses. Potential genomic subdivision into multiple clusters across populations is indicated by both clustering algorithms in *S. granularis*, but only in BAPS for *C. punctatus* and *P. angulosus*. However, there is, as initially expected, no indication of a spatial division into separate geographic clusters. The findings are congruent with several previous genomic studies in the South African marine environment, which did not detect population structure in their species selectively neutral markers (Nielsen et al. 2018; Mullins et al. 2018; Teske et al. 2019). Overall, this study contributes evidence towards discordant patterns between traditional markers such as COI and SNP markers in marine invertebrates (Chu et al. 2014; Blanco-Bercial and Bucklin, 2016; Gleason and Burton, 2016; Vendrami et al. 2017), which cautions against predicting species (neutral) genomic population differentiation with past findings derived from mtDNA, microsatellites and allozymes. Finally, methodological comparisons between calculations based on different SNP parameters demonstrate how genomic estimates can vary, which advocates for careful selection and clear reporting of SNP parameters.

2.5 Appendix

2.5.1 Population metrics (12 scenarios)

As described in section 2.3 (Statistical analysis), the results of overall 12 different scenarios (Table S2.1) constituting different definitions of putative SNPs are shown in their variation between locations in terms of total number of SNPs, nucleotide diversity measured as Tajima's π (π) and Watterson's θ_w (θ_w) in PoPoolation v.1.2.2 (scenario 1-9) (Kofler et al. 2011a) and pairwise F_{ST} values and total SNPs in comparison to private SNPs in PoPoolation 2 (Kofler et al. 2011b) (scenario 4-12) (Table S2.2-10).

Table S2.1. Settings used for PoPoolation (individual pileups) and PoPoolation 2 (meta-pileup).

Scenario	Minimum count	Minimum coverage	Maximum coverage
1	2	10	50
2	2	10	100
3	2	10	200
4	4	10	50
5	4	10	100
6	4	10	200
7	8	20	50
8	8	20	100
9	8	20	200
Scenario	Minimum count	Minimum coverage	Maximum coverage
4	4	10	50
5	4	10	100
6	4	10	200
7	8	20	50
8	8	20	100
9	8	20	200
10	16	32	50
11	16	32	100
12	16	32	200

Table S2.2. *Cyclograpsus punctatus* (Shore crab). Number of SNPs and values for pi and theta shown per location in scenario 1-9 (PoPoolation).

Location	Parameter	1	2	3	4	5	6	7	8	9
Jacob's Bay	# SNPs	52,336,240	62,568,057	67,760,222	15,846,250	19,333,188	21,267,399	3,249,104	4,808,579	5,593,130
	pi	0.015	0.015	0.015	0.010	0.010	0.010	0.008	0.008	0.008
	theta	0.019	0.020	0.020	0.010	0.010	0.011	0.009	0.009	0.009
Sea Point	# SNPs	74,435,146	89,625,104	100,440,717	21,923,350	27,160,823	31,330,966	4,110,050	6,346,464	8,012,299
	pi	0.016	0.016	0.016	0.010	0.010	0.010	0.008	0.008	0.008
	theta	0.020	0.021	0.022	0.011	0.011	0.011	0.008	0.008	0.009
Cape Agulhas	# SNPs	90,946,783	107,476,785	117,424,971	26,552,414	32,772,546	36,564,139	4,779,875	7,696,646	9,201,562
	pi	0.019	0.019	0.019	0.012	0.012	0.012	0.009	0.009	0.009
	theta	0.023	0.024	0.024	0.012	0.012	0.013	0.009	0.009	0.009
Knysna	# SNPs	65,572,161	78,970,430	86,893,531	20,011,976	24,790,595	27,559,328	3,760,334	5,904,986	7,095,303
	pi	0.016	0.016	0.016	0.010	0.010	0.010	0.008	0.008	0.008
	theta	0.019	0.020	0.021	0.011	0.011	0.011	0.008	0.008	0.008
Cape St. Francis	# SNPs	75,277,595	91,862,536	100,978,777	23,486,804	29,532,469	32,934,261	4,713,753	7,563,394	8,973,891
	pi	0.016	0.016	0.016	0.011	0.011	0.011	0.009	0.009	0.009
	theta	0.019	0.020	0.021	0.011	0.011	0.011	0.009	0.009	0.009
Haga Haga	# SNPs	70,590,834	85,361,965	94,358,052	21,541,146	26,720,904	29,752,579	4,431,257	6,760,123	8,047,062
	pi	0.015	0.015	0.015	0.010	0.010	0.010	0.009	0.009	0.009
	theta	0.019	0.020	0.020	0.010	0.011	0.011	0.009	0.009	0.009

Table S2.3. *Scutellastra granularis* (Granular limpet). Number of SNPs and values for pi, theta and D shown per location in scenario 1-9 (PoPoolation).

Location	Parameter	1	2	3	4	5	6	7	8	9
Port Nolloth	# SNPs	13,978,004	16,914,029	19,065,384	4,100,410	5,120,032	5,962,855	704,370	1,192,122	1,656,937
	pi	0.015	0.015	0.015	0.010	0.010	0.010	0.006	0.006	0.006
	theta	0.019	0.019	0.019	0.010	0.010	0.010	0.006	0.007	0.007
Sea Point	# SNPs	21,467,587	25,303,480	27,864,122	6,621,616	8,054,955	9,111,996	1,077,945	1,712,855	2,182,612
	pi	0.016	0.015	0.015	0.011	0.010	0.010	0.007	0.007	0.007
	theta	0.018	0.019	0.019	0.011	0.011	0.011	0.007	0.007	0.007
Cape Agulhas	# SNPs	18,565,615	21,715,855	23,777,691	5,335,379	6,405,137	7,176,152	956,999	1,426,261	1,835,911
	pi	0.016	0.016	0.015	0.010	0.010	0.010	0.007	0.007	0.007
	theta	0.019	0.019	0.020	0.010	0.010	0.010	0.007	0.007	0.007
Knysna	# SNPs	11,883,049	14,115,000	14,655,079	3,301,664	4,069,197	4,244,029	556,544	893,421	972,014
	pi	0.014	0.014	0.014	0.009	0.009	0.009	0.006	0.006	0.006
	theta	0.018	0.018	0.018	0.009	0.009	0.009	0.006	0.006	0.006
Cape St. Francis	# SNPs	15,162,801	17,993,012	19,419,406	4,201,181	5,231,317	5,794,681	715,425	1,179,481	1,438,644
	pi	0.016	0.015	0.015	0.010	0.010	0.009	0.007	0.007	0.007
	theta	0.019	0.019	0.019	0.010	0.010	0.010	0.007	0.007	0.007
Haga Haga	# SNPs	14,002,665	16,463,919	17,355,814	3,906,406	4,793,732	5,101,154	729,650	1,140,679	1,293,210
	pi	0.015	0.015	0.015	0.010	0.010	0.009	0.006	0.006	0.006
	theta	0.019	0.019	0.019	0.010	0.010	0.010	0.006	0.006	0.006

Table S2.4. *Parechinus angulosus* (Cape urchin). Number of SNPs and values for pi and theta shown per location in scenario 1-9 (PoPoolation).

Location	Parameter	1	2	3	4	5	6	7	8	9
Port Nolloth	# SNPs	241,577,002	290,850,149	319,197,953	90,302,002	110,788,178	122,365,447	15,069,715	23,146,642	27,663,338
	pi	0.021	0.020	0.020	0.016	0.016	0.015	0.011	0.010	0.010
	theta	0.023	0.024	0.025	0.016	0.016	0.017	0.011	0.011	0.011
Sea Point	# SNPs	144,208,724	172,895,279	194,199,718	44,974,685	54,744,483	62,821,927	7,589,471	11,704,229	14,971,803
	pi	0.016	0.016	0.016	0.011	0.011	0.011	0.008	0.008	0.008
	theta	0.019	0.020	0.020	0.011	0.011	0.012	0.008	0.008	0.008
Knysna	# SNPs	151,731,269	200,324,980	236,672,805	43,023,692	58,649,726	71,686,624	7,851,036	13,691,447	18,452,300
	pi	0.014	0.014	0.014	0.009	0.009	0.009	0.006	0.006	0.006
	theta	0.018	0.019	0.020	0.009	0.010	0.010	0.006	0.006	0.007
Cape St. Francis	# SNPs	123,490,435	171,956,186	203,064,103	34,764,131	50,988,422	63,036,008	6,315,824	12,511,086	17,166,864
	pi	0.016	0.016	0.017	0.010	0.010	0.010	0.008	0.008	0.008
	theta	0.020	0.022	0.023	0.011	0.011	0.012	0.008	0.008	0.009
Haga Haga	# SNPs	150,957,240	199,168,924	225,956,567	38,162,507	52,949,962	62,600,326	6,546,170	11,638,162	15,153,067
	pi	0.016	0.016	0.016	0.009	0.009	0.009	0.006	0.006	0.006
	theta	0.021	0.022	0.023	0.010	0.010	0.010	0.006	0.007	0.007

Table S2.5. *Cyclograpsus punctatus* (Shore crab). Number of SNPs and private SNPs shown per location in scenario 4-12 (PoPoolation2).

Location	Parameter	4	5	6	7	8	9	10	11	12
Jacob's Bay	# SNPs	61,950	98,145	122,116	10,141	29,488	46,699	1,555	7,462	16,064
	# private SNPs	538	746	849	2	3	7	0	0	0
Sea Point	# SNPs	72,186	114,611	142,443	11,788	33,811	52,950	2,048	8,748	17,943
	# private SNPs	628	1,061	1,289	2	13	21	0	1	1
Cape Agulhas	# SNPs	76,967	119,326	146,323	13,147	35,178	53,943	2,343	9,088	18,240
	# private SNPs	1,225	1,522	1,650	32	32	32	3	3	3
Knysna	# SNPs	70,422	111,285	138,140	11,683	33,030	51,669	1,944	8,487	17,563
	# private SNPs	659	971	1,131	7	10	11	0	0	0
Cape St. Francis	# SNPs	73,145	114,977	141,243	12,276	34,143	52,438	2,181	8,974	17,994
	# private SNPs	902	1,261	1,396	59	65	67	10	11	11
Haga Haga	# SNPs	70,207	111,604	138,584	12,034	33,240	51,348	2,141	8,790	17,600
	# private SNPs	643	1,057	1,268	9	18	21	2	3	3

Table S2.6. *Scutellastra granularis* (Granular limpet). Number of SNPs and private SNPs shown per location in scenario 4-12 (PoPoolation2).

Location	Parameter	4	5	6	7	8	9	10	11	12
Port Nolloth	# SNPs	28,487	41,734	50,681	4,511	10,536	16,646	932	2,662	5,738
	# private SNPs	424	705	888	8	20	32	1	4	9
Sea Point	# SNPs	35,433	50,352	59,877	5,149	11,855	18,328	1,237	3,248	6,457
	# private SNPs	397	769	993	4	19	32	0	0	0
Cape Agulhas	# SNPs	33,029	47,208	55,586	4,647	11,275	17,389	979	2,866	5,918
	# private SNPs	238	389	471	0	1	2	0	0	4
Knysna	# SNPs	28,380	40,707	48,294	3,961	9,836	15,424	781	2,355	5,151
	# private SNPs	207	318	380	5	5	7	0	0	0
Cape St. Francis	# SNPs	31,759	45,320	53,568	4,554	10,959	16,960	955	2,720	5,687
	# private SNPs	261	382	437	2	3	4	0	0	1
Haga Haga	# SNPs	32,751	45,912	53,988	4,443	10,700	16,597	974	2,682	5,615
	# private SNPs	361	620	771	2	12	30	0	1	6

Table S2.7. *Parechinus angulosus* (Cape urchin). Number of SNPs and private SNPs shown per location in scenario 4-12 (PoPoolation2).

Location	Parameter	4	5	6	7	8	9	10	11	12
Port Nolloth	# SNPs	82,221	94,658	109,515	11,810	15,792	21,671	3,240	5,476	7,662
	# private SNPs	6,853	7,428	8,372	226	245	291	23	32	34
Sea Point	# SNPs	31,863	42,550	56,185	5,233	8,290	13,722	1,243	2,473	4,287
	# private SNPs	3,964	4,108	4,287	206	225	242	41	59	65
Knysna	# SNPs	31,118	45,454	62,817	6,311	9,897	16,167	1,818	3,331	5,402
	# private SNPs	2,014	2,471	2,938	53	64	66	3	5	5
Cape St. Francis	# SNPs	26,544	37,291	51,036	5,192	8,416	14,054	1,546	3,048	5,116
	# private SNPs	4,145	4,336	4,623	307	326	332	46	56	56
Haga Haga	# SNPs	29,469	41,839	56,851	5,348	8,524	14,135	1,450	2,769	4,615
	# private SNPs	2,088	2,630	3,106	49	52	58	4	4	5

Table S2.8. *Cyclograpsus punctatus* (Shore crab). Pairwise F_{ST} values shown between locations in scenario 4-12 (PoPoolation2).

	4	5	6	7	8	9	10	11	12
#SNPS used	10,245	13,199	14,392	1,984	4,766	5,960	84	1,604	2,721
JCvsSP	0.060	0.052	0.049	0.037	0.031	0.028	0.023	0.023	0.019
JCvsCA	0.059	0.053	0.05	0.036	0.031	0.028	0.027	0.023	0.019
JCvsKY	0.060	0.053	0.049	0.037	0.031	0.028	0.027	0.022	0.019
JCvsCF	0.061	0.055	0.052	0.037	0.033	0.030	0.030	0.024	0.021
JCvsHH	0.062	0.057	0.055	0.039	0.034	0.033	0.029	0.024	0.023
SPvsCA	0.054	0.048	0.045	0.034	0.028	0.025	0.027	0.020	0.016
SPvsKY	0.054	0.047	0.044	0.034	0.027	0.024	0.024	0.019	0.016
SPvsCF	0.057	0.052	0.049	0.036	0.030	0.028	0.026	0.022	0.019
SPvsHH	0.060	0.054	0.052	0.039	0.034	0.032	0.029	0.024	0.022
CAvsKY	0.053	0.047	0.044	0.031	0.027	0.024	0.022	0.019	0.016
CAvsCF	0.050	0.045	0.043	0.031	0.025	0.023	0.022	0.017	0.015
CAvsHH	0.054	0.050	0.048	0.033	0.029	0.028	0.024	0.021	0.019
KYvsCF	0.055	0.049	0.046	0.034	0.029	0.026	0.024	0.020	0.017
KYvsHH	0.057	0.051	0.049	0.035	0.031	0.029	0.024	0.022	0.020
CFvsHH	0.053	0.048	0.046	0.033	0.029	0.027	0.023	0.020	0.018

Table S2.9. *Scutellastra granularis* (Granular limpet). Pairwise F_{ST} values shown between locations in scenario 4-12 (PoPoolation2).

	4	5	6	7	8	9	10	11	12
#SNPS used	3,175	4,713	5,440	400	1,361	2,023	16	336	866
PNvsSP	0.063	0.055	0.051	0.037	0.034	0.030	0.026	0.026	0.023
PNvsCA	0.070	0.064	0.061	0.043	0.039	0.038	0.024	0.030	0.030
PNvsKY	0.071	0.065	0.062	0.042	0.040	0.039	0.027	0.031	0.031
PNvsCF	0.071	0.065	0.062	0.046	0.040	0.039	0.035	0.031	0.031
PNvsHH	0.075	0.069	0.066	0.045	0.043	0.042	0.030	0.031	0.032
SPvsCA	0.064	0.058	0.055	0.043	0.036	0.034	0.038	0.028	0.025
SPvsKY	0.069	0.063	0.059	0.046	0.040	0.037	0.032	0.032	0.029
SPvsCF	0.065	0.059	0.055	0.042	0.036	0.034	0.039	0.029	0.026
SPvsHH	0.072	0.066	0.063	0.048	0.043	0.040	0.037	0.031	0.028
CAvsKY	0.061	0.055	0.051	0.036	0.031	0.028	0.025	0.023	0.020
CAvsCF	0.055	0.048	0.044	0.031	0.026	0.023	0.015	0.018	0.016
CAvsHH	0.063	0.057	0.053	0.035	0.033	0.030	0.022	0.022	0.022
KYvsCF	0.061	0.054	0.050	0.036	0.030	0.026	0.026	0.022	0.018
KYvsHH	0.064	0.057	0.053	0.037	0.033	0.030	0.029	0.023	0.021
CFvsHH	0.060	0.055	0.051	0.036	0.032	0.029	0.020	0.022	0.021

Table S2.10. *Parechinus angulosus* (Cape urchin). Pairwise F_{ST} values shown between locations in scenario 4-12 (PoPoolation2).

	4	5	6	7	8	9	10	11	12
#SNPS used	1,484	3,021	4,235	101	582	1,240	2	101	425
PNvsSP	0.083	0.074	0.066	0.055	0.052	0.044	0.018	0.037	0.033
PNvsKY	0.088	0.081	0.074	0.064	0.062	0.053	0.029	0.051	0.045
PNvsCF	0.101	0.095	0.089	0.075	0.069	0.061	0.039	0.058	0.050
PNvsHH	0.099	0.089	0.082	0.068	0.065	0.056	0.084	0.051	0.047
SPvsKY	0.063	0.054	0.049	0.037	0.036	0.031	0.019	0.032	0.026
SPvsCF	0.072	0.066	0.061	0.041	0.040	0.037	0.018	0.034	0.029
SPvsHH	0.072	0.062	0.055	0.042	0.039	0.034	0.054	0.030	0.028
KYvsCF	0.064	0.058	0.053	0.038	0.033	0.030	0.038	0.026	0.021
KYvsHH	0.057	0.045	0.039	0.034	0.025	0.021	0.077	0.018	0.016
CFvsHH	0.073	0.065	0.059	0.040	0.035	0.032	0.023	0.023	0.022

Table S2.11. Range of selected SNP-based nucleotide diversity estimates (π and θ) of marine species from the phyla Arthropoda, Mollusca and Echinodermata.

Phylum/Species	Common name	Nucleotide diversity (π , θ_w)	Reference
Arthropoda			
<i>Semibalanus balanoides</i>	Acorn barnacle	0.0048-0.0058 (π)	Nunez et al. 2018
<i>Pseudodiaptomus inopinus</i>	copepod	0.0011-0.0043 (π)	Dexter et al. 2018
<i>Calanus finmarchicus</i>	copepod	0.0063-0.0073 (π)	Choquet et al. 2019
<i>Calanus glacialis</i>	copepod	0.0074-0.0082 (π)	Choquet et al. 2019
<i>Oratosquilla oratoria</i>	Mantis shrimp	0.00409-0.00669 (π)	Daizhen et al. 2016
<i>Litopenaeus vannamei</i>	White leg shrimp (king prawn)	0.00369 (π)	Zhang et al. 2019*
<i>Paralithodes camtschaticus</i>	Red king crab	0.0002-0.0086 (π)	Grant and Cheng, 2012
<i>Cyclograpsus punctatus</i>	Shore crab	0.011 (π), 0.011 (θ_w)	This study
Mollusca			
<i>Crassostrea gigas</i>	Pacific oyster	0.0101 (π)	Gagnaire et al. 2018
<i>Crassostrea gigas</i>	Pacific oyster	0.0077 (π), 0.0119 (θ_w)	Song et al. 2018
<i>Crassostrea angulata</i>	Portuguese oyster	0.0096 (π)	Gagnaire et al. 2018
<i>Brachidontes</i> spp.	bivalve mussels	0.81-0.94 ($\theta\pi$)*, 0.0091-0.0124 (θ_w)	Maas et al. 2018
<i>Nucella lapillus</i>	Dog whelk	0.0003-0.0005 (π)	Chu et al. 2014
<i>Tegula funebris</i> **	Black turban snail	0.0006 (π)	Gleason and Burton, 2016
<i>Scutellastra granularis</i>	Granular limpet	0.011 (π), 0.012 (θ_w)	Nielsen et al. 2018
<i>Scutellastra granularis</i>	Granular limpet	0.010 (π), 0.010 (θ_w)	This study
Echinodermata			
<i>Apostichopus japonicus</i>	Japanese sea cucumber	0.00146 (π)	Jo et al. 2017
<i>Protoreaster nodosus</i>	Chocolate chip sea star	0.305-0.307 (π)	Tay et al. 2016
<i>Strongylocentrotus droebachiensis</i>	Green urchin	0.155-0.161 (π)	Addison and Kim, 2018
<i>Strongylocentrotus pallidus</i>	Pale urchin	0.238-0.263 (π)	Addison and Kim, 2018
<i>Parechinus angulosus</i>	Cape urchin	0.009 (π), 0.010 (θ_w)	Nielsen et al. 2018
<i>Parechinus angulosus</i>	Cape Urchin	0.011 (π), 0.012 (θ_w)	This study

*wild population, ** (until recently known as *Chlorostoma funebris*), ***theta pi ($\theta\pi$), calculated according to Nei, 1987.

Table S2.12. Summary of the range of findings for varying SNP parameters (see Table S2.1) for study species (see Table S2.2-10) shown for Sea Point population.

	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>
SNPs (PoPoolation)	4,110,050 - 100,440,717	1,077,945 - 27,864,122	7,589,471 - 194,199,718
pi	0.008 - 0.016	0.007 - 0.016	0.008 - 0.016
theta	0.008 - 0.022	0.007-0.019	0.008 - 0.020
SNPs (PoPoolation2)	2,048 -142,443	1,237-59,877	1,243 - 56,185
private SNPs	0 - 1,289	0 - 993	41 - 4,287
F_{ST} [SP vs. KY]	0.016 - 0.054	0.029 - 0.069	0.019 - 0.063

2.5.2 Clustering analyses

2.5.2.1 Mixture analysis based on mixture of groups of individuals

Each grouping is automatically assigned a colour, with each clustered individual represented by a vertical bar with the colour pointing to the associated cluster. Optimal partitions estimated in BAPS range from 5 (*C. punctatus*) to 4 (*S. granularis*, *P. angulosus*). All estimated partitions are present at all populations and the suggested clusters do not follow a geographical order (Figure S2.1).

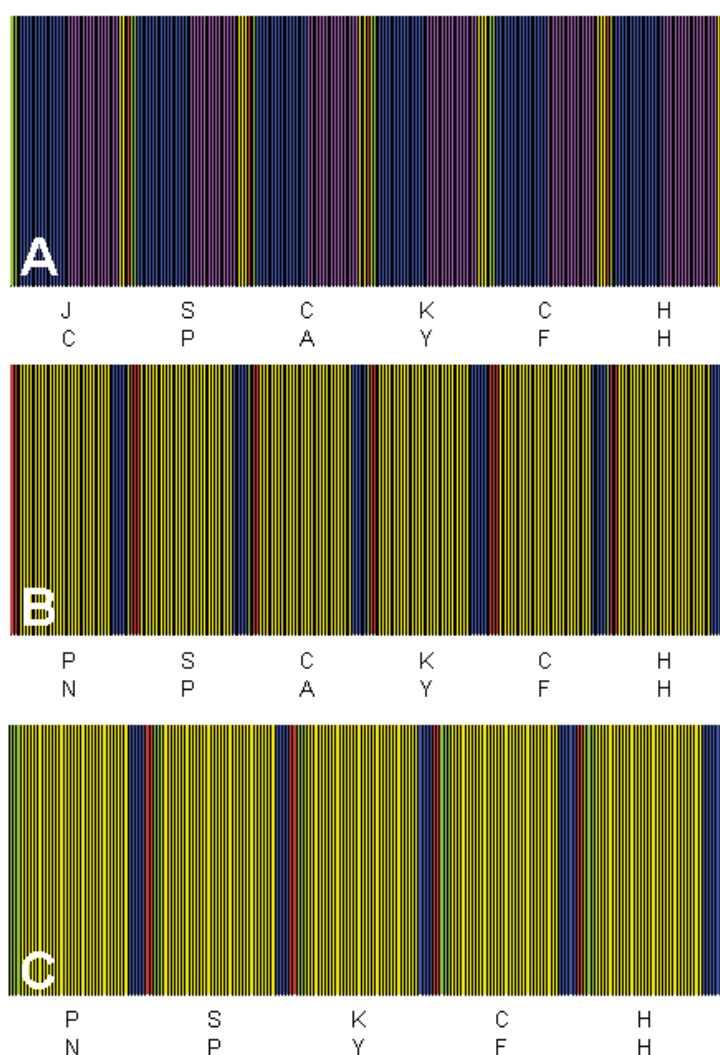


Figure S2.1. Coloured partition of clusters suggested for *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.1.

2.5.2.2 Population structure inference with fastSTRUCTURE

Suggested admixture proportions are visualised by plotting the mean of the variational posterior distribution over the admixture proportions, grouped according to geographic location. Each population is automatically assigned a colour and “K” denominates the number of estimated clusters (Figure S2.2a-c). The K means clustering analysis estimates a range of potential K values (*C. punctatus*: K= 1-4, *S. granularis*: K = 3-5, *P. angulosus*: K = 1-3). The admixture graph analysis suggests one overall population for *C. punctatus* and *P. angulosus* and three for *S. granularis*, which are present at all sampled geographic locations.

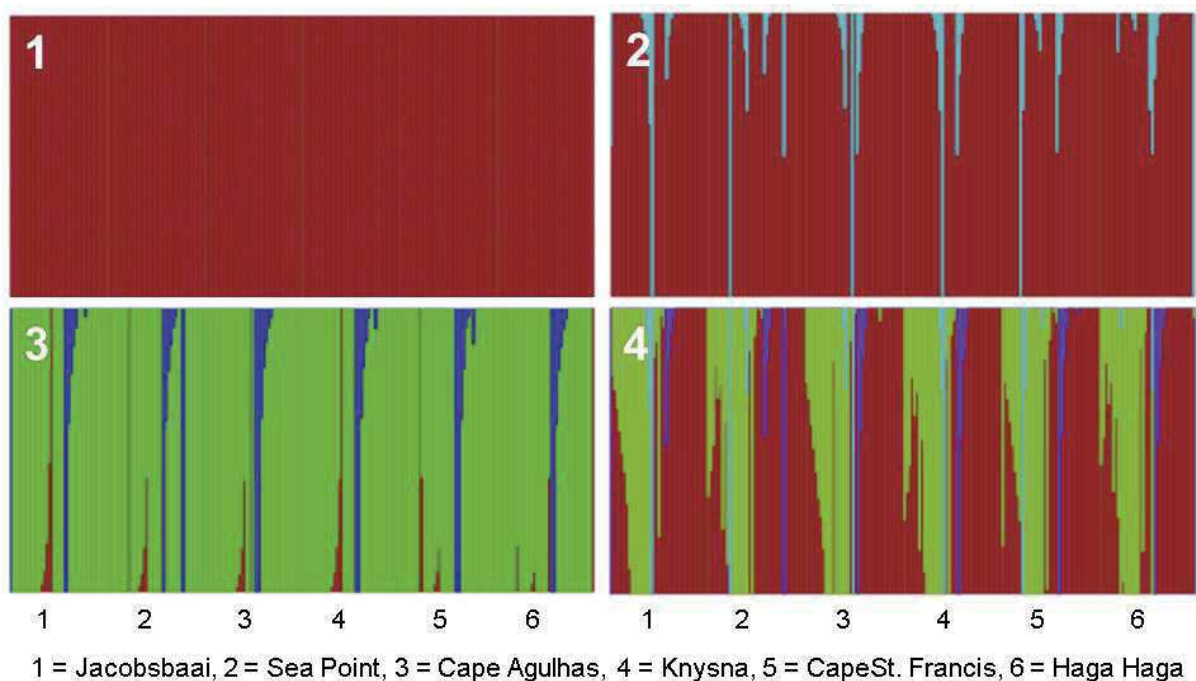


Figure S2.2a. Suggested admixture proportions for *C. punctatus* over the suggested range (overall K = 1-4) with (1) K = 1, (2) K = 2, (3) K = 3, (4) K = 4.

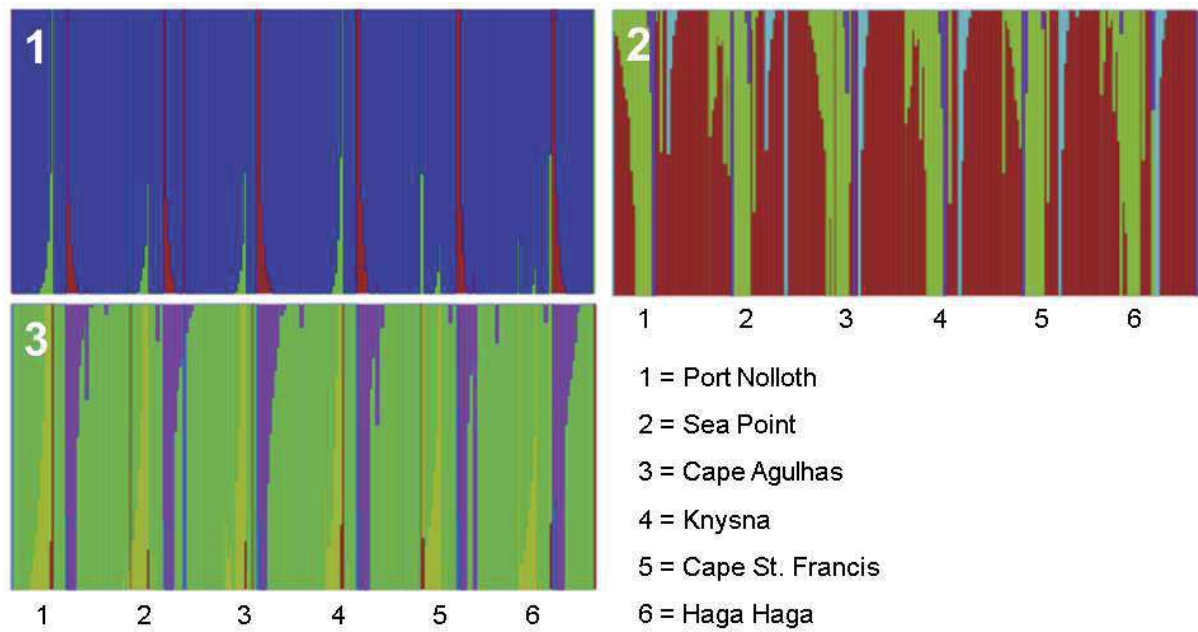


Figure S2.2b. Suggested admixture proportions for *S. granularis* over the suggested range (overall K = 3-5) with (1) K = 3, (2) K = 4, (3) K = 5.

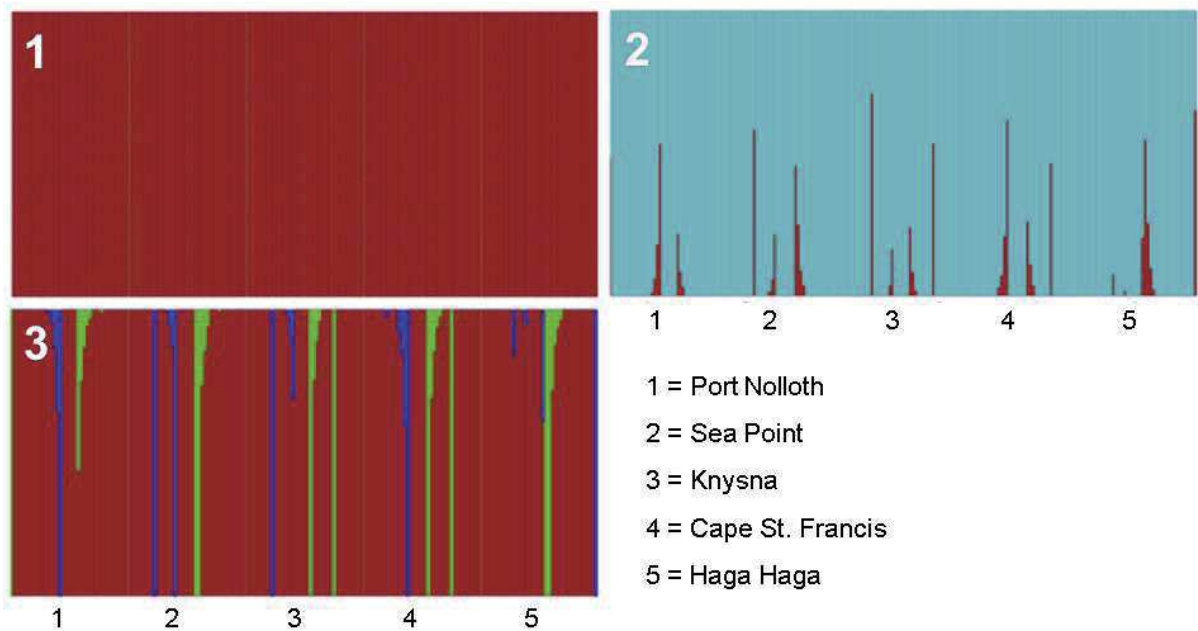


Figure S2.2c. Suggested admixture proportions for *P. angulosus* over the suggested range (overall K = 1-3) with (1) K = 1, (2) K = 2, (3) K = 3.

2.6 References

- Addison, Jason A., and Jin-Hong Kim. 2018. "Cryptic species diversity and reproductive Isolation among sympatric lineages of *Strongylocentrotus* sea urchins in the northwest Atlantic." *FACETS* 3, no. 1: 61-78.
- Allendorf, Fred W., Paul A. Hohenlohe, and Gordon Luikart. 2010. "Genomics and the Future of Conservation Genetics." *Nature Reviews. Genetics* 11 (10): 697–709.
- Alsos, Inger Greve, Dorothee Ehrich, Wilfried Thuiller, Pernille Bronken Eidesen, Andreas Tribsch, Peter Schönswetter, Claire Lagaye, Pierre Taberlet, and Christian Brochmann. 2012. "Genetic Consequences of Climate Change for Northern Plants." *Proceedings. Biological Sciences / The Royal Society* 279 (1735): 2042–51.
- Anderson, Eric C., Hans J. Skaug, and Daniel J. Barshis. 2014. "Next-generation Sequencing for Molecular Ecology: A Caveat Regarding Pooled Samples." *Molecular Ecology* 23, no. 3: 502-512.
- Andrews, Simon. 2010. "FastQC: A Quality Control Tool for High Throughput Sequence Data." <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
- Archer, Emma R. M., L. E. Dziba, K. J. Mulongoy, Malebajoa A. Maoela, and Michele Walters. 2018. *The IPBES regional assessment report on biodiversity and ecosystem services for Africa*. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), Bonn, Germany.
- Avice, John C. 2000. *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.
- Avice, John C. 1998. "The history and purview of phylogeography: a personal reflection." *Molecular Ecology* 7, no. 4: 371-379.
- Baird, Nathan A., Paul D. Etter, Tressa S. Atwood, Mark C. Currey, Anthony L. Shiver, Zachary A. Lewis, Eric U. Selker, William A. Cresko, and Eric A. Johnson. 2008. "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers." *PloS One* 3 (10): e33376.
- Balhoff, James P., and Gregory A. Wray. 2005. "Evolutionary Analysis of the Well Characterized endo16 Promoter Reveals Substantial Variation within Functional Sites." *Proceedings of the National Academy of Sciences of the United States of America* 102 (24): 8591–96.
- Bankevich, Anton, Sergey Nurk, Dmitry Antipov, Alexey A. Gurevich, Mikhail Dvorkin, Alexander S. Kulikov, Valery M. Lesin, et al. 2012. "SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing." *Journal of Computational Biology: A Journal of Computational Molecular Cell Biology* 19 (5): 455–77.
- Barba, Evan, Emily Conklin, D. Kraft, Jonathan Whitney [Manual by Emily Conklin]. 2018. *assessPool*. <https://github.com/ToBoDev/assessPool/wiki>
- Barrett, Rowan D. H., and Dolph Schluter. 2008. "Adaptation from Standing Genetic Variation." *Trends in Ecology & Evolution* 23 (1): 38–44.

- Barrow, Lisa N., Alan R. Lemmon, and Emily Moriarty Lemmon. 2018. "Targeted Sampling and Target Capture: Assessing Phylogeographic Concordance with Genome-Wide Data." *Systematic Biology*, syy021.
- Bay, Line Kolind, Ross H. Crozier, and M. Julian Caley. 2006. "The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef." *Marine Biology* 149, no. 5: 1247-1256.
- Beissinger, Timothy M., Guilherme J. M. Rosa, Shawn M. Kaeppler, Daniel Gianola, and Natalia de Leon. 2015. "Defining Window-Boundaries for Genomic Analyses Using Smoothing Spline Techniques." *Genetics Selection Evolution* 47 (1): 30.
- Belanger, Christina L., David Jablonski, Kaustuv Roy, Sarah K. Berke, Andrew Z. Krug, and James W. Valentine. 2012. "Global Environmental Predictors of Benthic Marine Biogeographic Structure." *Proceedings of the National Academy of Sciences of the United States of America* 109 (35): 14046–51.
- Bellis, Emily S., Dana K. Howe, and Dee R. Denver. 2016. "Genome-wide polymorphism and signatures of selection in the symbiotic sea anemone *Aiptasia*." *BMC Genomics* 17, no. 1: 160.
- Benestan, Laura, Jean-Sébastien Moore, Ben J. G. Sutherland, Jérémy Le Luyer, Halim Maaroufi, Clément Rougeux, Eric Normandeau, et al. 2017. "Sex Matters in Massive Parallel Sequencing: Evidence for Biases in Genetic Parameter Estimation and Investigation of Sex Determination Systems." *Molecular Ecology* 26 (24): 6767–83.
- Benestan, Laura, Brady K. Quinn, Halim Maaroufi, Martin Laporte, Fraser K. Clark, Spencer J. Greenwood, Rémy Rochette, and Louis Bernatchez. 2016. "Seascape Genomics Provides Evidence for Thermal Adaptation and Current-Mediated Population Structure in American Lobster (*Homarus americanus*)." *Molecular Ecology* 25 (20): 5073–92.
- Bensch, Staffan, and Mikael Akesson. 2005. "Ten Years of AFLP in Ecology and Evolution: Why so Few Animals?" *Molecular Ecology* 14 (10): 2899–2914.
- Beger, Maria, Kimberly A. Selkoe, Eric Treml, Paul H. Barber, Sophie von Der Heyden, Eric D. Crandall, Robert J. Toonen, and Cynthia Riginos. 2014. "Evolving coral reef conservation with genetic information." *Bulletin of Marine Science* 90, no. 1: 159-185.
- Bernardi, Giacomo, Lloyd Findley, and Axayacatl Rocha-Olivares. 2003. "Vicariance and dispersal across Baja California in disjunct marine fish populations." *Evolution* 57, no. 7: 1599-1609.
- Bernhardt, Joanna R., and Heather M. Leslie. 2013. "Resilience to climate change in coastal marine ecosystems." *Annual Review of Marine Science* 5:1, 371-392
- Blanco-Bercial, Leocadio, and Ann Bucklin. 2016. "New View of Population Genetics of Zooplankton: RAD-Seq Analysis Reveals Population Structure of the North Atlantic Planktonic Copepod *Centropages typicus*." *Molecular Ecology* 25 (7): 1566–80.
- Blankenberg, Daniel, Assaf Gordon, Gregory Von Kuster, Nathan Coraor, James Taylor, Anton Nekrutenko, and Galaxy Team. 2010. "Manipulation of FASTQ Data with Galaxy." *Bioinformatics* 26 (14): 1783–85.

- Bongaerts, Pim, Cynthia Riginos, Ramona Brunner, Norbert Englebert, Struan R. Smith, and Ove Hoegh-Guldberg. 2017. "Deep Reefs Are Not Universal Refuges: Reseeding Potential Varies among Coral Species." *Science Advances* 3 (2): e1602373.
- Bonin, Aurelie, Florence Nicole, Francois Pompanon, Claude Miaud, and Pierre Taberlet. 2007. "Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation." *Conservation Biology* 21, no. 3: 697-708.
- Bowen, Brian W., Michelle R. Gaither, Joseph D. DiBattista, Matthew Iacchei, Kimberly R. Andrews, W. Stewart Grant, Robert J. Toonen, and John C. Briggs. 2016. "Comparative Phylogeography of the Ocean Planet." *Proceedings of the National Academy of Sciences of the United States of America* 113 (29): 7962-69.
- Bowen, Brian W., Kartik Shanker, Nina Yasuda, Maria Celia, Maria Celia Machel D. Malay, Sophie von der Heyden, Gustav Paulay et al. 2014. "Phylogeography unplugged: comparative surveys in the genomic era." *Bulletin of Marine Science* 90, no. 1: 13-46.
- Bradbury, Ian R., Benjamin Laurel, Paul V. R. Snelgrove, Paul Bentzen, and Steven E. Campana. 2008. "Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history." *Proceedings of the Royal Society B: Biological Sciences* 275, no. 1644: 1803-1809.
- Bragg, Jason G., Megan A. Supple, Rose L. Andrew, and Justin O. Borevitz. 2015. "Genomic Variation across Landscapes: Insights and Applications." *The New Phytologist* 207 (4): 953-67.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2017. *Two Oceans: A Guide to the Marine Life of Southern Africa*. Penguin Random House South Africa.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2007. *Two Oceans: A Guide to the Marine Life of Southern Africa*. New Holland Publishers, Cape Town, South Africa.
- Branch, George and Margo Branch. 1981. *The Living Shores of Southern Africa*. Struik Publishers.
- Britten, Roy J., Lee Rowen, John Williams, and R. Andrew Cameron. 2003. "Majority of Divergence between Closely Related DNA Samples Is due to Indels." *Proceedings of the National Academy of Sciences of the United States of America* 100 (8): 4661-65.
- Bunnefeld, Lynsey, Jack Hearn, Graham N. Stone, and Konrad Lohse. 2018. "Whole-Genome Data Reveal the Complex History of a Diverse Ecological Community." *Proceedings of the National Academy of Sciences of the United States of America* 115 (28): E6507-15.
- Bustamante, Rodrigo H., George M. Branch, Sean Eekhout, Bruce Robertson, Peter Zoutendyk, Michael Schleyer, Arthur Dye, et al. 1995. "Gradients of Intertidal Primary Productivity around the Coast of South Africa and Their Relationships with Consumer Biomass." *Oecologia* 102 (2): 189-201.
- Cahill, Abigail E., Aurelien De Jode, Sophie Dubois, Zoheir Bouzaza, Didier Aurelle, Emilie Boissin, Olivier Chabrol et al. 2017. "A multispecies approach reveals hot spots and cold spots of diversity and connectivity in invertebrate species with contrasting dispersal modes." *Molecular Ecology* 26, no. 23: 6563-6577.

- Cameron, R. Andrew, Manoj Samanta, Autumn Yuan, Dong He, and Eric Davidson. 2009. "SpBase: The Sea Urchin Genome Database and Web Site." *Nucleic Acids Research* 37 (Database): D750–54.
- Cameron, R. Andrew, Suk Hen Chow, Kevin Berney, Tsz-Yeung Chiu, Qiu-Autumn Yuan, Alexander Krämer, Argelia Helguero, Andrew Ransick, Miron Yun, and Eric H. Davidson. 2005. "An Evolutionary Constraint: Strongly Disfavored Class of Change in DNA Sequence during Divergence of Cis-Regulatory Modules." *Proceedings of the National Academy of Sciences of the United States of America* 102 (33): 11769–74.
- Cárcer, Daniel Aguirre de, Stuart E. Denman, Chris McSweeney, and Mark Morrison. 2011. "Evaluation of Subsampling-Based Normalization Strategies for Tagged High-Throughput Sequencing Data Sets from Gut Microbiomes." *Applied and Environmental Microbiology* 77 (24): 8795–98.
- Catchen, Julian, Paul A. Hohenlohe, Susan Bassham, Angel Amores, and William A. Cresko. 2013. "Stacks: an analysis tool set for population genomics." *Molecular Ecology* 22, no. 11: 3124–3140.
- Chang, E. Sally, Maria E. Orive, and Paulyn Cartwright. 2018. "Nonclonal Coloniality: Genetically Chimeric Colonies through Fusion of Sexually Produced Polyps in the Hydrozoan *Ectopleura larynx*." *Evolution Letters* 2 (4): 442–55.
- Charlesworth, Deborah, and Brian Charlesworth. 1987. "Inbreeding depression and its evolutionary consequences." *Annual Review of Ecology and Systematics* 18, no. 1: 237–268.
- Chikhi, Rayan, and Paul Medvedev. 2013. "Informed and automated k-mer size selection for genome assembly." *Bioinformatics* 30, no. 1: 31–37.
- Chong, Zechen, Jue Ruan, and Chung-I Wu. 2012. "Rainbow: An Integrated Tool for Efficient Clustering and Assembling RAD-Seq Reads." *Bioinformatics* 28 (21): 2732–37.
- Choquet, Marvin, Irina Smolina, Anusha K. S. Dhanasiri, Leocadio Blanco-Bercial, Martina Kopp, Alexander Jueterbock, Arvind Y. M. Sundaram, and Galice Hoarau. 2019. "Towards population genomics in non-model species with large genomes: a case study of the marine zooplankton *Calanus finmarchicus*." *Royal Society Open Science* 6, no. 2: 180608.
- Chu, Nathaniel D., Stefan T. Kaluziak, Geoffrey C. Trussell, and Steven V. Vollmer. 2014. "Phylogenomic Analyses Reveal Latitudinal Population Structure and Polymorphisms in Heat Stress Genes in the North Atlantic Snail *Nucella lapillus*." *Molecular Ecology* 23 (7): 1863–73.
- Combosch, David J., Sarah Lemer, Peter D. Ward, Neil H. Landman, and Gonzalo Giribet. 2017. "Genomic Signatures of Evolution in Nautilus-An Endangered Living Fossil." *Molecular Ecology* 26 (21): 5923–38.
- Combosch, David J., and Steven V. Vollmer. 2015. "Trans-Pacific RAD-Seq Population Genomics Confirms Introgressive Hybridization in Eastern Pacific *Pocillopora* Corals." *Molecular Phylogenetics and Evolution* 88 (July): 154–62.
- Compton, John S. 2011. "Pleistocene Sea-Level Fluctuations and Human Evolution on the Southern Coastal Plain of South Africa." *Quaternary Science Reviews* 30 (5): 506–27.

- Conte, Gina L., Matthew E. Arnegard, Catherine L. Peichel, and Dolph Schluter. 2012. "The probability of genetic parallelism and convergence in natural populations." *Proceedings of the Royal Society B: Biological Sciences* 279, no. 1749: 5039-5047.
- Corander, Jukka, Pekka, Marttinen, Jukka Sirén, and Jing Tang. 2006. "BAPS: Bayesian Analysis of Population Structure, Manual v. 4.1." Department of Mathematics and Statistics, University of Helsinki.
- Corander, Jukka, and Pekka Marttinen. 2006. "Bayesian Identification of Admixture Events Using Multilocus Molecular Markers." *Molecular Ecology* 15 (10): 2833–43.
- Cowen, Robert K., and Su Sponaugle. 2009. "Larval dispersal and marine population connectivity." *Annual Review of Marine Science* 1: 443-466.
- Cowen, Robert K., Kamazima M. M. Lwiza, Su Sponaugle, Claire B. Paris, and Donald B. Olson. 2000. "Connectivity of marine populations: open or closed?." *Science* 287, no. 5454: 857-859.
- Cram, David L. 1971. "Life History Studies on South African Echinoids (Echinodermata) 1. *Parechinus angulosus* (Leske)(Echinidae, Parechininae)." *Transactions of the Royal Society of South Africa* 39 (3): 321–37.
- Crane, Nicole L., Juliette Tariel, Jennifer E. Caselle, Alan M. Friedlander, D. Ross Robertson, and Giacomo Bernardi. 2018. "Clipperton Atoll as a Model to Study Small Marine Populations: Endemism and the Genomic Consequences of Small Population Size." *PloS One* 13 (6): e0198901.
- Crawford, Douglas L., and Marjorie F. Oleksiak. 2016. "Ecological Population Genomics in the Marine Environment." *Briefings in Functional Genomics* 15 (5): 342–51.
- Daizhen, Zhang, Ding Ge, Ge Baoming, Zhang Huabin, and Tang Boping. 2016. "Germplasm Authentication of Mantis Shrimps (*Oratosquilla oratoria*) in China Sea by SNP and AS-PCR Method." *Indian Journal of Geo-Marine Sciences* 45(11):1471-1473
- Davey, John W., Timothée Cezard, Pablo Fuentes-Utrilla, Cathlene Eland, Karim Gharbi, and Mark L. Blaxter. 2013. "Special features of RAD Sequencing data: implications for genotyping." *Molecular Ecology* 22, no. 11: 3151-3164.
- Davey, John W., Paul A. Hohenlohe, Paul D. Etter, Jason Q. Boone, Julian M. Catchen, and Mark L. Blaxter. 2011. "Genome-Wide Genetic Marker Discovery and Genotyping Using Next-Generation Sequencing." *Nature Reviews. Genetics* 12 (7): 499–510.
- Department of Environmental Affairs (DEA). 2018. "Cabinet approves a representative network of Marine Protected Areas in the South African exclusive zone" 25 October 2018. Web source: https://www.environment.gov.za/mediarelease/cabinetapproves_representativenetworkofMPAs
- Deagle, Bruce E., Cassandra Faux, So Kawaguchi, Bettina Meyer, and Simon N. Jarman. 2015. "Antarctic Krill Population Genomics: Apparent Panmixia, but Genome Complexity and Large Population Size Muddy the Water." *Molecular Ecology* 24 (19): 4943–59.
- Delord, Chrystelle, Gilles Lassalle, Adrien Oger, Dominique Barloy, Marie-Agnes Coutellec, Adline Delcamp, Guillaume Evanno, et al. 2018. "A Cost-and-Time Effective Procedure

- to Develop SNP Markers for Multiple Species: A Support for Community Genetics." *Methods in Ecology and Evolution* / *British Ecological Society* 9 (9): 1959–74.
- Devlin-Durante, Meghann K., and Iliana B. Baums. 2017. "Genome-Wide Survey of Single-Nucleotide Polymorphisms Reveals Fine-Scale Population Structure and Signs of Selection in the Threatened Caribbean Elkhorn Coral, *Acropora palmata*." *PeerJ* 5 (November): e4077.
- Dexter, Eric, Stephen M. Bollens, Jeffery Cordell, Ho Young Soh, Gretchen Rollwagen-Bollens, Susanne P. Pfeifer, Jérôme Goudet, and Séverine Vuilleumier. 2018. "A genetic reconstruction of the invasion of the calanoid copepod *Pseudodiaptomus inopinus* across the North American Pacific Coast." *Biological Invasions* 1-19.
- Domingues, Rodrigo Rodrigues, Alexandre Wagner Silva Hilsdorf, and Otto Bismarck Fazzano Gadig. 2018. "The importance of considering genetic diversity in shark and ray conservation policies." *Conservation Genetics* 19, no. 3: 501-525.
- Drury, Crawford, Stephanie Schopmeyer, Elizabeth Goergen, Erich Bartels, Ken Nedimyer, Meaghan Johnson, Kerry Maxwell, Victor Galvan, Carrie Manfrino, and Diego Lirman. 2017. "Genomic patterns in *Acropora cervicornis* show extensive population structure and variable genetic diversity." *Ecology and Evolution* 7, no. 16: 6188-6200.
- Dudgeon, Christine L., Blower, Dean C., Broderick, D., Giles, J. L., Holmes, B. J., Kashiwagi, T., Krück, N. C., Morgan, J. A. T., Tillett, B. J. and Ovenden, J. R. 2012. "A review of the application of molecular genetics for fisheries management and conservation of sharks and rays." *Journal of Fish Biology*, 80: 1789-1843.
- Ehlers, Anneli, Boris Worm, and Thorsten B. H. Reusch. 2008. "Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming." *Marine Ecology Progress Series* 355: 1-7.
- Ekblom, Robert, and Juan Galindo. 2011. "Applications of next Generation Sequencing in Molecular Ecology of Non-Model Organisms." *Heredity* 107 (1): 1–15.
- Ellegren, Hans. 2014. "Genome Sequencing and Population Genomics in Non-Model Organisms." *Trends in Ecology & Evolution* 29 (1): 51–63.
- Everett, Meredith V., Linda K. Park, Ewann A. Berntson, Anna E. Elz, Curt E. Whitmire, Aimee A. Keller, and M. Elizabeth Clarke. 2016. "Large-Scale Genotyping-by-Sequencing Indicates High Levels of Gene Flow in the Deep-Sea Octocoral *Swiftia simplex* (Nutting 1909) on the West Coast of the United States." *PloS One* 11 (10): e0165279.
- Exposito-Alonso, Moises, François Vasseur, Wei Ding, George Wang, Hernán A. Burbano, and Detlef Weigel. 2018. "Genomic Basis and Evolutionary Potential for Extreme Drought Adaptation in *Arabidopsis thaliana*." *Nature Ecology & Evolution* 2 (2): 352–58.
- Faubry, Sören, and Paul H. Barber. 2012. "Theoretical limits to the correlation between pelagic larval duration and population genetic structure." *Molecular Ecology* 21, no. 14: 3419-3432.
- Fisher, Ronald A. 1922. "On the interpretation of χ^2 from contingency tables, and the calculation of P." *Journal of the Royal Statistical Society* 85, no. 1: 87-94.
- Flanagan, Sarah P., Brenna R. Forester, Emily K. Latch, Sally N. Aitken, and Sean Hoban.

2018. "Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation." *Evolutionary Applications* 11, no. 7: 1035-1052.
- Fonseca, Rute R. da, Anders Albrechtsen, Gonçalo Espregueira Themudo, Jazmín Ramos-Madrigal, Jonas Andreas Sibbesen, Lasse Maretty, M. Lisandra Zepeda-Mendoza, Paula F. Campos, Rasmus Heller, and Ricardo J. Pereira. 2016. "Next-Generation Biology: Sequencing and Data Analysis Approaches for Non-Model Organisms." *Marine Genomics* 30 (December): 3–13.
- Forsman, Zac H., Ingrid S. S. Knapp, Kaho Tisthammer, Deren A. R. Eaton, Mahdi Belcaid, and Robert J. Toonen. 2017. "Coral Hybridization or Phenotypic Variation? Genomic Data Reveal Gene Flow between *Porites lobata* and *P. compressa*." *Molecular Phylogenetics and Evolution* 111: 132–48.
- Forsström, Tiia, Freed Ahmad, and Anti Vasemägi. 2017. "Invasion Genomics: Genotyping-by-Sequencing Approach Reveals Regional Genetic Structure and Signatures of Temporal Selection in an Introduced Mud Crab." *Marine Biology* 164 (9): 186.
- Frankham, Richard, David Briscoe, and Jonathan Ballou. 2002. *Introduction to Conservation Genetics*. Cambridge, UK; New York: Cambridge University Press.
- Freemo, Heather, Patrick O'Reilly, Paul R. Berg, Sigbjørn Lien, and Elizabeth G. Boulding. 2011. "Outlier SNPs show more genetic structure between two Bay of Fundy metapopulations of Atlantic salmon than do neutral SNPs." *Molecular Ecology Resources* 11: 254-267.
- Fricke, Alexander Hermann. 1979. "Kelp Grazing by the Common Sea Urchin *Parechinus angulosus* (Leske) in False Bay, Cape Town, South Africa." *South African Journal of Zoology* 14 (3): 143–48.
- Funk, W. Chris, Robert E. Lovich, Paul A. Hohenlohe, Courtney A. Hofman, Scott A. Morrison, T. Scott Sillett, Cameron K. Ghalambor, et al. 2016. "Adaptive Divergence despite Strong Genetic Drift: Genomic Analysis of the Evolutionary Mechanisms Causing Genetic Differentiation in the Island Fox (*Urocyon littoralis*)." *Molecular Ecology* 25 (10): 2176–94.
- Funk, W. Chris, John K. McKay, Paul A. Hohenlohe, and Fred W. Allendorf. 2012. "Harnessing genomics for delineating conservation units." *Trends in Ecology & Evolution* 27, no. 9: 489-496
- Futschik, Andreas, and Christian Schlötterer. 2010. "The next Generation of Molecular Markers from Massively Parallel Sequencing of Pooled DNA Samples." *Genetics* 186 (1): 207–18.
- Gaggiotti, Oscar E., Dorte Bekkevold, Hanne B. H. Jørgensen, Matthieu Foll, Gary R. Carvalho, Carl Andre, and Daniel E. Ruzzante. 2009. "Disentangling the Effects of Evolutionary, Demographic, and Environmental Factors Influencing Genetic Structure of Natural Populations: Atlantic Herring as a Case Study." *Evolution; International Journal of Organic Evolution* 63 (11): 2939–51.
- Gagnaire, Pierre-Alexandre, Jean-Baptiste Lamy, Florence Cornette, Serge Heurtebise, Lionel Dégremont, Emilie Flahauw, Pierre Boudry, Nicolas Bierne, and Sylvie Lapègue. 2018. "Analysis of Genome-Wide Differentiation between Native and Introduced Populations of the Cupped Oysters *Crassostrea gigas* and *Crassostrea angulata*."

Genome Biology and Evolution 10, no. 9: 2518-2534.

- Gaither, Michelle R., Georgios A. Gkafas, Menno de Jong, Fatih Sarigol, Francis Neat, Thomas Regnier, Daniel Moore, et al. 2018. "Genomics of Habitat Choice and Adaptive Evolution in a Deep-Sea Fish." *Nature Ecology & Evolution* 2 (4): 680–87.
- Gaither, Michelle R., Moisés A. Bernal, Richard R. Coleman, Brian W. Bowen, Shelley A. Jones, W. Brian Simison, and Luiz A. Rocha. 2015. "Genomic Signatures of Geographic Isolation and Natural Selection in Coral Reef Fishes." *Molecular Ecology* 24 (7): 1543–57.
- Galaska, Matthew P., Chester J. Sands, Scott R. Santos, Andrew R. Mahon, and Kenneth M. Halanych. 2017. "Crossing the divide: admixture across the Antarctic polar front revealed by the brittle star *Astrothoma agassizii*." *The Biological Bulletin* 232, no. 3: 198-211.
- Gao, Yangchun, Shiguo Li, and Aibin Zhan. 2018. "Genome-Wide Single Nucleotide Polymorphisms (SNPs) for a Model Invasive Ascidian *Botryllus schlosseri*." *Genetica* 146 (2): 227–34.
- Gautier, Mathieu, Julien Foucaud, Karim Gharbi, Timothée Cézard, Maxime Galan, Anne Loiseau, Marian Thomson, Pierre Pudlo, Carole Kerdelhué, and Arnaud Estoup. 2013. "Estimation of Population Allele Frequencies from next-Generation Sequencing Data: Pool-versus Individual-Based Genotyping." *Molecular Ecology* 22 (14): 3766–79.
- Giangrande, Adriana, M. Cristina Gambi, and M. Flavia Gravina. 2017. "Paradigm shifts in community ecology: Open versus closed units, challenges and limits of connectivity studies." *Marine Ecology* 38, no. 6: e12480.
- Gillanders, Bronwyn, Stephen Charles Donnellan, Thomas A. A. Prowse, Damien A. Fordham, Christopher Izzo, Steven Anthony Myers, Keith P. Rowling, Michael A. Steer, and Skye H. Woodcock. 2016. "Giant Australian Cuttlefish in South Australian Waters". Fisheries Research and Development Corporation.
- Gleason, Lani U., and Ronald S. Burton. 2016. "Genomic Evidence for Ecological Divergence against a Background of Population Homogeneity in the Marine Snail *Chlorostoma funebris*." *Molecular Ecology* 25 (15): 3557–73.
- Gómez-Fernández, Alicia, Irene Alcocer, and Silvia Matesanz. 2016. "Does Higher Connectivity Lead to Higher Genetic Diversity? Effects of Habitat Fragmentation on Genetic Variation and Population Structure in a Gypsophile." *Conservation Genetics* 17 (3): 631–41.
- Grant, William Stewart, and Brian W. Bowen. 1998. "Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation." *Journal of Heredity* 89, no. 5: 415-426.
- Grant, William Stewart, and Wei Cheng. 2012. "Incorporating deep and shallow components of genetic structure into the management of Alaskan red king crab." *Evolutionary Applications* 5, no. 8: 820-837.
- Graudal, Lars, Filippou Aravanopoulos, Zohra Bennadji, Suchitra Changtragoon, Bruno Fady, Erik D. Kjær, Judy Loo, Lolona Ramamonjisoa, and Giovanni G. Vendramin. 2014. "Global to Local Genetic Diversity Indicators of Evolutionary Potential in Tree Species within and Outside Forests." *Forest Ecology and Management* 333

(December): 35–51.

- Gray, David R., and Alan N. Hodgson. 1997. "Temporal Variation in Foraging Behaviour of *Patella granularis* (Patellogastropoda) and *Siphonaria concinna* (Basommatophora) on a South African Shore." *The Journal of Molluscan Studies* 63 (2): 121–30.
- Greenwood, Peter John. 1975. "Population Dynamics and Ecological Energetics of *Parechinus angulosus* at Robben Island and False Bay, South Africa." M. Sc. Thesis, University of Cape Town, Cape Town.
- Griffiths, Charles L., Tamara B. Robinson, Louise Lange, and Angela Mead. 2010. "Marine Biodiversity in South Africa: An Evaluation of Current States of Knowledge." *PloS One* 5 (8): e12008.
- Gunderson, Lance H. 2000. "Ecological Resilience--in Theory and Application." *Annual Review of Ecology and Systematics*, 425–39.
- Guo, Baocheng, Zitong Li, and Juha Merilä. 2016. "Population genomic evidence for adaptive differentiation in the Baltic Sea herring." *Molecular Ecology* 25, no. 12: 2833–2852.
- Guo, Baocheng, Jacquelin DeFaveri, Graciela Sotelo, Abhilash Nair, and Juha Merilä. 2015. "Population Genomic Evidence for Adaptive Differentiation in Baltic Sea Three-Spined Sticklebacks." *BMC Biology* 13 (March): 19.
- Gurevich, Alexey, Vladislav Saveliev, Nikolay Vyahhi, and Glenn Tesler. 2013. "QUAST: Quality Assessment Tool for Genome Assemblies." *Bioinformatics* 29 (8): 1072–75.
- Gutierrez, Alejandro P., Frances Turner, Karim Gharbi, Richard Talbot, Natalie R. Lowe, Carolina Peñaloza, Mark McCullough, Paulo A. Prodöhl, Tim P. Bean, and Ross D. Houston. 2017. "Development of a Medium Density Combined-Species SNP Array for Pacific and European Oysters (*Crassostrea gigas* and *Ostrea edulis*)." *G3* 7 (7): 2209–18.
- Hammerman, Nicholas M., Ramon E. Rivera-Vicens, Matthew P. Galaska, Ernesto Weil, Richard S. Appeldoorn, Monica Alfaro, and Nikolaos V. Schizas. 2018. "Population Connectivity of the Plating Coral *Agaricia lamarcki* from Southwest Puerto Rico." *Coral Reefs* 37 (1): 183–91.
- Harrison, Susan, and Alan Hastings. 1996. "Genetic and Evolutionary Consequences of Metapopulation Structure." *Trends in Ecology & Evolution* 11 (4): 180–83.
- Hawkins, Stephen J., and Richard G. Hartnoll. 1983. "Changes in a Rocky Shore Community: An Evaluation of Monitoring." *Marine Environmental Research* 9 (3): 131–81.
- Helmuth, Brian, Nova Mieszkowska, Pippa Moore, and Stephen J. Hawkins. 2006. "Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change." *Annual Review of Ecology, Evolution, and Systematics*, 373–404.
- Herrera, Santiago, Paula H. Reyes-Herrera, and Timothy M. Shank. 2015. "Predicting RAD-Seq Marker Numbers across the Eukaryotic Tree of Life." *Genome Biology and Evolution* 7 (12): 3207–25.

- Hess, Jon E., Nathan R. Campbell, David A. Close, Margaret F. Docker, and Shawn R. Narum. 2013. "Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species." *Molecular Ecology* 22, no. 11: 2898-2916.
- Hughes, Jennifer B., Gretchen C. Daily, and Paul R. Ehrlich. 1997. "Population diversity: its extent and extinction." *Science* 278, no. 5338: 689-692.
- Hutchison, Delbert W., and Alan R. Templeton. 1999. "Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability." *Evolution* 53, no. 6: 1898-1914.
- International Human Genome Sequencing Consortium. 2001 "Initial sequencing and analysis of the human genome." *Nature* 409.6822: 860-921.
- IOCAS. 2018. "*Eriocheir sinensis* (Chinese Mitten Crab) Genome Assembly (ASM333651v1). Institute of Oceanology Chinese Academy of Sciences (IOCAS)." https://www.ncbi.nlm.nih.gov/assembly/GCA_003336515.1/.
- IPCC. 2013. "IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change." Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jeffery, Nicholas W., Ian R. Bradbury, Ryan R. E. Stanley, Brendan F. Wringe, Mallory Van Wyngaarden, J. Ben Lowen, Cynthia H. McKenzie, Kyle Matheson, Philip S. Sargent, and Claudio DiBacco. 2018. "Genomewide Evidence of Environmentally Mediated Secondary Contact of European Green Crab (*Carcinus maenas*) Lineages in Eastern North America." *Evolutionary Applications* 11 (6): 869–82.
- Jeffery, Nicholas W., Claudio DiBacco, Mallory Van Wyngaarden, Lorraine C. Hamilton, Ryan R. E. Stanley, Renée Bernier, Jennifer FitzGerald, et al. 2017a. "RAD Sequencing Reveals Genomewide Divergence between Independent Invasions of the European Green Crab (*Carcinus maenas*) in the Northwest Atlantic." *Ecology and Evolution* 7 (8): 2513–24.
- Jeffery, Nicholas W., Claudio DiBacco, Brendan F. Wringe, Ryan RE Stanley, Lorraine C. Hamilton, Praveen N. Ravindran, and Ian R. Bradbury. 2017b. "Genomic Evidence of Hybridization between Two Independent Invasions of European Green Crab (*Carcinus maenas*) in the Northwest Atlantic." *Heredity* 119 (3): 154–65.
- JGI. 2012. "*Lottia gigantea* (Owl Limpet) Genome Assembly (Helro1). Joint Genome Institute (JGI)." https://www.ncbi.nlm.nih.gov/assembly/GCF_000327385.1/.
- Jo, Jihoon, Jooseong Oh, Hyun-Gwan Lee, Hyun-Hee Hong, Sung-Gwon Lee, Seongmin Cheon, Elizabeth Kern, Soyeong Jin, Sung-Jin Cho, Joong-Ki Park, Chungoo Park. 2017. "Draft genome of the sea cucumber *Apostichopus japonicus* and genetic polymorphism among color variants." *Gigascience* 6, no. 1: 1-6, giw006
- Johnston, Erika C., Zac H. Forsman, Jean-François Flot, Sebastian Schmidt-Roach, Jorge H. Pinzón, Ingrid S. S. Knapp, and Robert J. Toonen. 2017. "A Genomic Glance through the Fog of Plasticity and Diversification in *Pocillopora*." *Scientific Reports* 7 (1): 5991.
- Kay, Catherine M. 2002. "Recruitment in the Intertidal Limpet *Lottia digitalis* (Patellagastropoda: Lottiidae) May Be Driven By Settlement Cues Associated with Adult

- Habitat." *Marine Biology* 141: 467–77.
- Kelly, Ryan P., and Stephen R. Palumbi. 2010. "Genetic structure among 50 species of the northeastern Pacific rocky intertidal community." *PLoS One* 5, no. 1: e8594.
- Kess, Tony, Jeffrey Gross, Fiona Harper, and Elizabeth G. Boulding. 2016. "Low-cost ddRAD method of SNP discovery and genotyping applied to the periwinkle *Littorina saxatilis*." *Journal of Molluscan Studies* 82, no. 1: 104–109.
- Kess, Tony, Juan Galindo, and Elizabeth G. Boulding. 2018. "Genomic Divergence between Spanish *Littorina saxatilis* Ecotypes Unravels Limited Admixture and Extensive Parallelism Associated with Population History." *Ecology and Evolution* 8 (16): 8311–27.
- Kilburn, Richard, and Elizabeth Rippey. 1982. *Sea Shells of Southern Africa*. Intl. Specialized Book Service Inc.
- Knapp, Ingrid, Jonathan Puritz, Christopher Bird, Jonathan Whitney, Mareike Sudek, Zac Forsman, and Robert J. Toonen. 2016. "ezRAD—an accessible next-generation RAD sequencing protocol suitable for non-model organisms v3. 1." *Protocols. io Life Sciences Protocol Repository*.
- Kofler, Robert, Anna Maria Langmüller, Pierre Nouhaud, Kathrin Anna Otte, and Christian Schlötterer. 2016. "Suitability of different mapping algorithms for genome-wide polymorphism scans with pool-seq data." *G3: Genes, Genomes, Genetics* 6, no. 11: 3507–3515.
- Kofler, Robert, Pablo Orozco Wengel, Nicola De Maio, Ram Vinay Pandey, Viola Nolte, Andreas Futschik, Carolin Kosiol, and Christian Schlötterer. 2011a. "PoPoolation: A Toolbox for Population Genetic Analysis of next Generation Sequencing Data from Pooled Individuals." *PloS One* 6 (1): e15925.
- Kofler, Robert, Ram Vinay Pandey, and Christian Schlötterer. 2011b. "PoPoolation2: Identifying Differentiation between Populations Using Sequencing of Pooled DNA Samples (Pool-Seq)." *Bioinformatics* 27 (24): 3435–36.
- Krueger, Felix. 2015. "Trim Galore!: A Wrapper Tool around Cutadapt and FastQC to Consistently Apply Quality and Adapter Trimming to FastQ Files." https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
- Kuo, Chih-Horng, and John C. Avise. 2005. "Phylogeographic breaks in low-dispersal species: the emergence of concordance across gene trees." *Genetica* 124, no. 2-3: 179–186.
- Lachance, Joseph, and Sarah A. Tishkoff. 2013. "SNP Ascertainment Bias in Population Genetic Analyses: Why It Is Important, and How to Correct It." *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 35 (9): 780–86.
- Laikre, Linda. 2010. "Genetic diversity is overlooked in international conservation policy implementation." *Conservation Genetics* 11, no. 2: 349–354.
- Lal, Monal M., Paul C. Southgate, Dean R. Jerry, Cyprien Bosserelle, and Kyall R. Zenger. 2017. "Swept Away: Ocean Currents and Seascape Features Influence Genetic Structure across the 18,000 km Indo-Pacific Distribution of a Marine Invertebrate, the Black-Lip Pearl Oyster *Pinctada margaritifera*." *BMC Genomics* 18 (1): 66.

- Lande, Russell, and Susan Shannon. 1996. "The role of genetic variation in adaptation and population persistence in a changing environment." *Evolution* 50, no. 1: 434-437.
- Latch, Emily K., Guha Dharmarajan, Jeffrey C. Glaubitz, and Olin E. Rhodes. 2006. "Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation." *Conservation Genetics* 7, no. 2: 295-302.
- Le Corre, Valérie, and Antoine Kremer. 2012. "The Genetic Differentiation at Quantitative Trait Loci under Local Adaptation." *Molecular Ecology* 21 (7): 1548–66.
- Leffler, Ellen M., Kevin Bullaughey, Daniel R. Matute, Wynn K. Meyer, Laure Segurel, Aarti Venkat, Peter Andolfatto, and Molly Przeworski. 2012. "Revisiting an old riddle: what determines genetic diversity levels within species?." *PLoS Biology* 10, no. 9: e1001388.
- Lehnert, Sarah J., Claudio DiBacco, Mallory Van Wyngaarden, Nicholas W. Jeffery, J. Ben Lowen, Emma VA Sylvester, Brendan F. Wringe, Ryan R. E. Stanley, Lorraine C. Hamilton, and Ian R. Bradbury. 2018. "Fine-scale temperature-associated genetic structure between inshore and offshore populations of sea scallop (*Placopecten magellanicus*)." *Heredity* 122, no. 1: 69.
- Lexer, Christian, Rafael O. Wüest, Sophia Mangili, Myriam Heuertz, Kai N. Stölting, Peter B. Pearman, Félix Forest, Nicolas Salamin, Niklaus E. Zimmermann, and Eligio Bossolini. 2014. "Genomics of the divergence continuum in an African plant biodiversity hotspot, I: drivers of population divergence in *Restio capensis* (Restionaceae)." *Molecular Ecology* 23, no. 17: 4373-4386.
- Leydet, Karine Posbic, Carsten G. B. Grupstra, Rafel Coma, Marta Ribes, and Michael E. Hellberg. 2018. "Host-Targeted RAD-Seq Reveals Genetic Changes in the Coral *Oculina patagonica* Associated with Range Expansion along the Spanish Mediterranean Coast." *Molecular Ecology* 27 (11): 2529–43.
- Li, Heng. 2011. "A Statistical Framework for SNP Calling, Mutation Discovery, Association Mapping and Population Genetical Parameter Estimation from Sequencing Data." *Bioinformatics* 27 (21): 2987–93.
- Li, Heng. 2013. "Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM." *arXiv [q-bio.GN]*. arXiv. <http://arxiv.org/abs/1303.3997>.
- Li, Heng, Bob Handsaker, Alec Wysoker, Tim Fennell, Jue Ruan, Nils Homer, Gabor Marth, Goncalo Abecasis, and Richard Durbin, and 1000 Genome Project Data Processing Subgroup. 2009. "The Sequence Alignment/Map Format and SAMtools." *Bioinformatics* 25 (16): 2078–79.
- Lima, Thiago G., and Christopher S. Willett. 2018. "Using Pool-Seq to Search for Genomic Regions Affected by Hybrid Inviability in the Copepod *T. californicus*." *The Journal of Heredity* 109 (4): 469–76.
- Loeschcke, Volker, Jürgen Tomiuk, and Subodh K. Jain, eds. 2013. Conservation genetics. Vol. 68. Birkhäuser.
- Lopez, Jose V., Bishoy Kamel, Mónica Medina, Timothy Collins, and Iliana B. Baums. 2019. "Multiple Facets of Marine Invertebrate Conservation Genomics." *Annual Review of Animal Biosciences* 7:1, 473-497

- Lowry, David B., Sean Hoban, Joanna L. Kelley, Katie E. Lotterhos, Laura K. Reed, Michael F. Antolin, and Andrew Storfer. 2017. "Breaking RAD: An Evaluation of the Utility of Restriction Site-Associated DNA Sequencing for Genome Scans of Adaptation." *Molecular Ecology Resources* 17 (2): 142–52.
- Maas, Diede L., Stefan Prost, Ke Bi, Lydia L. Smith, Ellie E. Armstrong, Ludi P. Aji, Abdul Hamid A. Toha, Rosemary G. Gillespie, and Leontine E. Becking. 2018. "Rapid Divergence of Mussel Populations despite Incomplete Barriers to Dispersal." *Molecular Ecology* 27 (7): 1556–71.
- Magris, Rafael A., Eric A. Treml, Robert L. Pressey, and Rebecca Weeks. 2016. "Integrating multiple species connectivity and habitat quality into conservation planning for coral reefs." *Ecography* 39, no. 7: 649–664.
- Majiedt, Prideel, Stephen Holness, Kerry Sink, Ané Oosthuizen, and Peter Chadwick. 2013. "Systematic Marine Biodiversity Plan for the West Coast of South Africa." South African National Biodiversity Institute, Cape Town, 46.
- McFadden, Catherine S., Roxanne Haverkort-Yeh, Alexandra M. Reynolds, Anna Halász, Andrea M. Quattrini, Zac H. Forsman, Yehuda Benayahu, and Robert J. Toonen. 2017. "Species Boundaries in the Absence of Morphological, Ecological or Geographical Differentiation in the Red Sea Octocoral Genus *Ovabunda* (Alcyonacea: Xeniidae)." *Molecular Phylogenetics and Evolution* 112 (July): 174–84.
- McKeown, Niall J., Lorenz Hauser, and Paul W. Shaw. 2017. "Microsatellite genotyping of brown crab *Cancer pagurus* reveals fine scale selection and 'non-chaotic' genetic patchiness within a high gene flow system." *Marine Ecology Progress Series* 566: 91–103.
- McMillan, W. Owen, and Stephen R. Palumbi. 1995. "Concordant evolutionary patterns among Indo-West Pacific butterflyfishes." *Proceedings of the Royal Society of London. Series B: Biological Sciences* 260, no. 1358: 229–236.
- Mergeay, Joachim, and Luis Santamaria. 2012. "Evolution and Biodiversity: The Evolutionary Basis of Biodiversity and Its Potential for Adaptation to Global Change." *Evolutionary Applications* 5 (2): 103–6.
- Mertens, Lisa, Eric Treml, and Sophie von der Heyden. 2018. "Genetic and Biophysical Models Help Define Marine Conservation Focus Areas." *Frontiers in Marine Science* 5: 268.
- Miller, Adam D., Anthony van Rooyen, Gordana Rašić, Daniel A. Ierodionou, Harry K. Gorfine, Rob Day, C. Wong, Ary A. Hoffmann, and Andrew R. Weeks. 2016. "Contrasting Patterns of Population Connectivity between Regions in a Commercially Important Mollusc *Halotis rubra*: Integrating Population Genetics, Genomics and Marine LiDAR Data." *Molecular Ecology* 25 (16): 3845–64.
- Miller, Michael R., Joseph P. Dunham, Angel Amores, William A. Cresko, and Eric A. Johnson. 2007. "Rapid and Cost-Effective Polymorphism Identification and Genotyping Using Restriction Site Associated DNA (RAD) Markers." *Genome Research* 17 (2): 240–48.
- Mimura, Makiko, Tetsukazu Yahara, Daniel P. Faith, Ella Vázquez-Domínguez, Robert I. Colautti, Hitoshi Araki, Firouzeh Javadi, et al. 2017. "Understanding and Monitoring the

- Consequences of Human Impacts on Intraspecific Variation." *Evolutionary Applications* 10 (2): 121–39.
- Mmonwa, Kolobe Lucas, Peter R. Teske, Christopher D. McQuaid, and Nigel P. Barker. 2015. "Historical Demography of Southern African Patellid Limpets: Congruence of Population Expansions, but Not Phylogeography." *African Journal of Marine Science* 37, no. 1: 11-20.
- Mmonwa, Kolobe Lucas. 2013. "Molecular Phylogeny, Phylogeography and Evolutionary Adaptation of Foraging Behaviour Amongst Sympatric Patellid Limpets Along the Southern African Shoreline." PhD dissertation, Rhodes University.
- Morjan, Carrie L., and Loren H. Rieseberg. 2004. "How Species Evolve Collectively: Implications of Gene Flow and Selection for the Spread of Advantageous Alleles." *Molecular Ecology* 13 (6): 1341–56.
- Morin, Phillip A., Gordon Luikart, and Robert K. Wayne. 2004. "SNPs in ecology, evolution and conservation." *Trends in Ecology & Evolution* 19, no. 4: 208-216.
- Morse, Peter, Shannon R. Kjeldsen, Mark G. Meekan, Mark I. McCormick, Julian K. Finn, Christine L. Huffard, and Kyall R. Zenger. 2018. "Genome-Wide Comparisons Reveal a Clinal Species Pattern within a Holobenthic Octopod-the Australian Southern Blue-Ringed Octopus, *Hapalochlaena maculosa* (Cephalopoda: Octopodidae)." *Ecology and Evolution* 8 (4): 2253–67.
- Moritz, Craig. 2002. "Strategies to protect biological diversity and the evolutionary processes that sustain it." *Systematic Biology* 51, no. 2: 238-254.
- Muller, Cornelius M., Sophie von der Heyden, Rauri C. K. Bowie, and Conrad A. Matthee. 2012. "Oceanic Circulation, Local Upwelling and Palaeoclimatic Changes Linked to the Phylogeography of the Cape Sea Urchin *Parechinus angulosus*." *Marine Ecology Progress Series* 468: 203.
- Mullins, Rachel B., Niall J. McKeown, Warwick H. H. Sauer, Paul W. Shaw, and Handling editor: W. Stewart Grant. 2018. "Genomic analysis reveals multiple mismatches between biological and management units in yellowfin tuna (*Thunnus albacares*)." *ICES Journal of Marine Science* 75, no. 6: 2145-2152.
- Mura-Jornet, Isidora, Carolina Pimentel, Gisele P. M. Dantas, Maria Virginia Petry, Daniel González-Acuña, Andrés Barbosa, Andrew D. Lowther, Kit M. Kovacs, Elie Poulin, and Juliana A. Vianna. 2018. "Chinstrap penguin population genetic structure: one or more populations along the Southern Ocean?." *BMC Evolutionary Biology* 18, no. 1: 90.
- Muteveri, Tinashe, Conrad A. Matthee, Rauri C. K. Bowie, and Sophie von der Heyden. 2015. "High population connectivity and Pleistocene range expansion in the direct-developing plough shell *Bullia rhodostoma* along the South African coast." *African Journal of Marine Science* 37, no. 1: 21-31.
- Narum, Shawn R., C. Alex Buerkle, John W. Davey, Michael R. Miller, and Paul A. Hohenlohe. 2013. "Genotyping-by-Sequencing in Ecological and Conservation Genomics." *Molecular Ecology* 22 (11): 2841–47.
- Nei, Masatoshi. 1987. *Molecular Evolutionary Genetics*. New York, NY: Columbia University Press

- Nei, Masatoshi, and Wen-Hsiung Li. 1979. "Mathematical model for studying genetic variation in terms of restriction endonucleases." *Proceedings of the National Academy of Sciences* 76, no. 10: 5269-5273.
- Nielsen, Erica S., Romina Henriques, Robert J. Toonen, Ingrid S. S. Knapp, Baocheng Guo, and Sophie von der Heyden. 2018. "Complex Signatures of Genomic Variation of Two Non-Model Marine Species in a Homogeneous Environment." *BMC Genomics* 19 (1): 347.
- Nielsen, Erica S., Maria Beger, Romina Henriques, Kimberly A. Selkoe, and Sophie von der Heyden. 2017b. "Multispecies genetic objectives in spatial conservation planning." *Conservation Biology* 31, no. 4: 872-882.
- Nielsen, Erica S. "Integrating genetics into marine conservation planning in South Africa". 2017a. MSc Thesis, Department of Botany and Zoology, Stellenbosch University, South Africa
- Nunez, Joaquin C. B., Rebecca G. Elyanow, David A. Ferranti, and David M. Rand. 2018. "Population Genomics and Biogeography of the Northern Acorn Barnacle (*Semibalanus balanoides*) Using Pooled Sequencing Approaches." pp. 1-30, In: . Population Genomics. Springer, Cham
- O'Leary, Shannon J., Jonathan B. Puritz, Stuart C. Willis, Christopher M. Hollenbeck, and David S. Portnoy. 2018. "These Aren't the Loci You're Looking for: Principles of Effective SNP Filtering for Molecular Ecologists." *Molecular Ecology* 27, no. 16: 3193-3206.
- Orduña-Malea, Enrique, Juan Manuel Ayllón, Alberto Martín-Martín, and Emilio Delgado López-Cózar. 2014. "About the size of Google Scholar: playing the numbers." *EC3 Working Papers* 18
- Palumbi, Stephen R. 2003. "Population genetics, demographic connectivity, and the design of marine reserves." *Ecological Applications* 13, no. sp1: 146-158.
- Pandey, Ram Vinay, Robert Kofler, Pablo Orozco-terWengel, Viola Nolte, and Christian Schlötterer. 2011. "PoPoolation DB: a user-friendly web-based database for the retrieval of natural polymorphisms in *Drosophila*." *BMC Genetics* 12, no. 1: 27.
- Paterno, Marta, Marcello Schiavina, Giorgio Aglieri, Jamila Ben Souissi, Elisa Boscari, Renato Casagrandi, Aureo Chassanite, et al. 2017. "Population Genomics Meet Lagrangian Simulations: Oceanographic Patterns and Long Larval Duration Ensure Connectivity among *Paracentrotus lividus* Populations in the Adriatic and Ionian Seas." *Ecology and Evolution* 7 (8): 2463–79.
- Pauls, Steffen U., Carsten Nowak, Miklós Bálint, and Markus Pfenninger. 2013. "The Impact of Global Climate Change on Genetic Diversity within Populations and Species." *Molecular Ecology* 22 (4): 925–46.
- Paz, Andrea, Zoe Spanos, Jason L. Brown, Mariana Lyra, Célio Haddad, Miguel Rodrigues, and Ana Carnaval. 2019. "Phylogeography of Atlantic Forest glassfrogs (*Vitreorana*): when geography, climate dynamics and rivers matter." *Heredity* 122, no. 5: 545.
- Pérez-Figueroa, Andrés, María Jesús García-Pereira, María Saura, Emilio Rolán-Alvarez, and Armando Caballero. 2010. "Comparing three different methods to detect selective loci using dominant markers." *Journal of Evolutionary Biology* 23, no. 10: 2267-2276.

- Peterson, Brant K., Jesse N. Weber, Emily H. Kay, Heidi S. Fisher, and Hopi E. Hoekstra. 2012. "Double Digest RADseq: An Inexpensive Method for de Novo SNP Discovery and Genotyping in Model and Non-Model Species." *PloS One* 7 (5): e37135.
- Pfaff, Maya C., George M. Branch, Jennifer L. Fisher, Vera Hoffmann, Allan G. Ellis, and John L. Largier. 2015. "Delivery of marine larvae to shore requires multiple sequential transport mechanisms." *Ecology* 96, no. 5: 1399-1410.
- Picone, Barbara, Clint Rhode, and Rouvay Roodt-Wilding. 2015. "Transcriptome Profiles of Wild and Cultured South African Abalone, *Haliotis midae*." *Marine Genomics* 20 (April): 3–6.
- Pineda, Jesús, Francesca Porri, Victoria Starczak, and Jonathan Blythe. 2010. "Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity." *Journal of Experimental Marine Biology and Ecology* 392, no. 1-2: 9-21.
- Pinho, Catarina, Vera Cardoso, and Jody Hey. 2019. "A population genetic assessment of taxonomic species: the case of Lake Malawi cichlid fishes." *Molecular Ecology Resources* pp: 1755-0998.13027.
- Pope, Lisa C., Cynthia Riginos, Jennifer Ovenden, Jude Keyse, and Simon P. Blomberg. 2015. "Population genetic diversity in the Australian 'seascape': a bioregion approach." *PLoS One* 10, no. 9: e0136275.
- Porri, Francesca, Jennifer M. Jackson, Charles E. O. Von der Meden, Nicolas Weidberg, and Christopher D. McQuaid. 2014. "The effect of mesoscale oceanographic features on the distribution of mussel larvae along the south coast of South Africa." *Journal of Marine Systems* 132: 162-173.
- Porri, Francesca, Christopher D. McQuaid, S. M. Lawrie, and S. J. Antrobus. 2008. "Fine-scale spatial and temporal variation in settlement of the intertidal mussel *Perna perna* indicates differential hydrodynamic delivery of larvae to the shore." *Journal of Experimental Marine Biology and Ecology* 367, no. 2: 213-218.
- Porri, Francesca, Christopher D. McQuaid, and Sarah Radloff. 2006. "Spatio-temporal variability of larval abundance and settlement of *Perna perna*: differential delivery of mussels." *Marine Ecology Progress Series* 315: 141-150.
- Pratlong, Marine, Anne Haguénauer, Kelly Brener, Guillaume Mitta, Eve Toulza, Joaquim Garrabou, Nathaniel Bensoussan, Pierre Pontarotti, and Didier Aurelle. 2018. "Separate the Wheat from the Chaff: Genomic Analysis of Local Adaptation in the Red Coral *Corallium rubrum*." *bioRxiv*, 306456.
- Pressey, Robert L., Mar Cabeza, Matthew E. Watts, Richard M. Cowling, and Kerrie A. Wilson. 2007. "Conservation planning in a changing world." *Trends in Ecology & Evolution* 22, no. 11: 583-592.
- Pritchard, Jonathan K., Matthew Stephens, and Peter Donnelly. 2000. "Inference of population structure using multilocus genotype data." *Genetics* 155, no. 2: 945-959.
- Raj, Anil, Matthew Stephens, and Jonathan K. Pritchard. 2014. "fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets." *Genetics* 197 (2): 573–89.

- Ravinet, Mark, Anja Westram, Kerstin Johannesson, Roger Butlin, Carl André, and Marina Panova. 2016. "Shared and Nonshared Genomic Divergence in Parallel Ecotypes of *Littorina saxatilis* at a Local Scale." *Molecular Ecology* 25 (1): 287–305.
- Reed, David H., and Richard Frankham. 2003. "Correlation between fitness and genetic diversity." *Conservation Biology* 17, no. 1: 230-237.
- Reitzel, Adam M., Santiago Herrera, Michael J. Layden, Mark Q. Martindale, and Timothy M. Shank. 2013. "Going Where Traditional Markers Have Not Gone before: Utility of and Promise for RAD Sequencing in Marine Invertebrate Phylogeography and Population Genomics." *Molecular Ecology* 22 (11): 2953–70.
- Rellstab, Christian, Stefan Zoller, Andrew Tedder, Felix Gugerli, and Martin C. Fischer. 2013. "Validation of SNP Allele Frequencies Determined by Pooled Next-Generation Sequencing in Natural Populations of a Non-Model Plant Species." *PloS One* 8 (11): e80422.
- Reusch, Thorsten B. H., Anneli Ehlers, August Hämmerli, and Boris Worm. 2005. "Ecosystem recovery after climatic extremes enhanced by genotypic diversity." *Proceedings of the National Academy of Sciences* 102, no. 8: 2826-2831.
- Reynolds, Terry V., Conrad A. Matthee, and Sophie von der Heyden. 2014. "The Influence of Pleistocene Climatic Changes and Ocean Currents on the Phylogeography of the Southern African Barnacle, *Tetraclita serrata* (Thoracica; Cirripedia)." *PloS One* 9 (7): e102115.
- Rhode, Clint, Aletta E. Bester-van der Merwe, and Rouvay Roodt-Wilding. 2017. "An assessment of spatio-temporal genetic variation in the South African abalone (*Haliotis midae*), using SNPs: implications for conservation management." *Conservation Genetics* 18, no. 1: 17-31.
- Riddle, Brett R. 2016. "Comparative phylogeography clarifies the complexity and problems of continental distribution that drove AR Wallace to favor islands." *Proceedings of the National Academy of Sciences* 113, no. 29: 7970-7977.
- Ross, Philip M., Ian D. Hogg, Conrad A. Pilditch, and Carolyn J. Lundquist. 2009. "Phylogeography of New Zealand's coastal benthos." *New Zealand Journal of Marine and Freshwater Research* 43, no. 5: 1009-1027.
- Sandoval-Castillo, Jonathan, Nick A. Robinson, Anthony M. Hart, Lachlan W. S. Strain, and Luciano B. Beheregaray. 2018. "Seascape Genomics Reveals Adaptive Divergence in a Connected and Commercially Important Mollusc, the Greenlip Abalone (*Haliotis laevigata*), along a Longitudinal Environmental Gradient." *Molecular Ecology* 27 (7): 1603–20.
- Schlegel, Robert W., Eric C. J. Oliver, Thomas Wernberg, and Albertus J. Smit. 2017. "Coastal and Offshore Co-Occurrences of Marine Heatwaves and Cold-Spells." *Progress in Oceanography* 151: 189-205.
- Schlötterer, Christian, Raymond Tobler, Robert Kofler, and Viola Nolte. 2014. "Sequencing Pools of Individuals - Mining Genome-Wide Polymorphism Data without Big Funding." *Nature Reviews. Genetics* 15 (11): 749–63.

- Schmidt, Paul S., Ester A. Serrao, Gareth A. Pearson, Cynthia Riginos, Paul D. Rawson, Thomas J. Hilbish, Susan H. Brawley, Geoffrey C. Trussell, Emily Carrington, David S. Wetthey, John W. Grahame, François Bonhomme and David M. Rand. 2008. "Ecological genetics in the North Atlantic: environmental gradients and adaptation at specific loci." *Ecology* 89, no. sp11: S91-S107.
- Schuster, Astrid, Sergio Vargas, Ingrid S. Knapp, Shirley A. Pomponi, Robert J. Toonen, Dirk Erpenbeck, and Gert Wörheide. 2018. "Divergence Times in Demosponges (Porifera): First Insights from New Mitogenomes and the Inclusion of Fossils in a Birth-Death Clock Model." *BMC Evolutionary Biology* 18 (1): 114.
- Seeb, James E., Gary Carvalho, Lorenz Hauser, Kerry Naish, Steven Roberts, and Lisa W. Seeb. 2011. "Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms." *Molecular Ecology Resources* 11: 1-8.
- Selkoe, Kimberly A., Cassidy C. D. Aloia, Eric D. Crandall, Matthew Iacchei, Libby Liggins, Jonathan B. Puritz, Sophie von der Heyden, and Robert J. Toonen. 2016. "A decade of seascape genetics: contributions to basic and applied marine connectivity." *Marine Ecology Progress Series*. 554: 1–19.
- Selkoe, Kimberly A., Oscar E. Gaggiotti, Eric A. Trembl, Johanna L. K. Wren, Mary K. Donovan, Hawai'i Reef Connectivity Consortium, and Robert J. Toonen. 2016. "The DNA of coral reef biodiversity: predicting and protecting genetic diversity of reef assemblages." *Proceedings of the Royal Society B: Biological Sciences* 283, no. 1829: 20160354.
- Selkoe, Kimberly A., Oscar E. Gaggiotti, ToBo Laboratory, Brian W. Bowen, and Robert J. Toonen. 2014. "Emergent patterns of population genetic structure for a coral reef community." *Molecular Ecology* 23, no. 12: 3064-3079.
- Selkoe, Kimberly A., and Robert J. Toonen. 2011. "Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal." *Marine Ecology Progress Series* 436: 291-305.
- Seymour, Sian. 2016. "How paleoclimatic changes in sea surface temperature and sea level have shaped contemporary species richness along the coastline of South Africa." Honour's thesis. University of Stellenbosch.
- Sgro, Carla M., Andrew J. Lowe, and Ary A. Hoffmann. 2011. "Building Evolutionary Resilience for Conserving Biodiversity under Climate Change." *Evolutionary Applications* 4 (2): 326–37.
- Shafer, Aaron, Claire R. Peart, Sergio Tusso, Inbar Maayan, Alan Brelsford, Christopher W. Wheat, and Jochen B. W. Wolf. 2017. "Bioinformatic Processing of RAD-Seq Data Dramatically Impacts Downstream Population Genetic Inference." *Methods in Ecology and Evolution / British Ecological Society* 8 (8): 907–17.
- Shafer, Aaron B. A., Jochen B. W. Wolf, Paulo C. Alves, Linnea Bergström, Michael W. Bruford, Ioana Brännström, Guy Colling et al. 2015. "Genomics and the challenging translation into conservation practice." *Trends in Ecology & Evolution* 30, no. 2: 78-87.
- Shafer, Aaron, and Jochen B. W. Wolf. 2013. "Widespread Evidence for Incipient Ecological Speciation: A Meta-analysis of Isolation-by-ecology." *Ecology Letters* 16 (7): 940–50.
- Shariff, Salimah Z., Shayna A. D. Bejaimal, Jessica M. Sontrop, Arthur V. Iansavichus, R.

- Brian Haynes, Matthew A. Weir, and Amit X. Garg. 2013. "Retrieving clinical evidence: a comparison of PubMed and Google Scholar for quick clinical searches." *Journal of Medical Internet Research* 15, no. 8: e164.
- Sherman, Craig D. H., Alison Hunt, and David J. Ayre. 2008. "Is life history a barrier to dispersal? Contrasting patterns of genetic differentiation along an oceanographically complex coast." *Biological Journal of the Linnean Society* 95, no. 1: 106-116.
- Siegel, David A., Satoshi Mitarai, Christopher J. Costello, Steven D. Gaines, Bruce E. Kendall, Robert R. Warner, and K. B. Winters. 2008. "The stochastic nature of larval connectivity among nearshore marine populations." *Proceedings of the National Academy of Sciences* 105, no. 26: 8974-8979.
- Simmonds, Sara E., Vincent Chou, Samantha H. Cheng, Rita Rachmawati, Hilconida P. Calumpong, G. Ngurah Mahardika, and Paul H. Barber. 2018. "Evidence of Host-Associated Divergence from Coral-Eating Snails (genus *Coralliophila*) in the Coral Triangle." *Coral Reefs* 37 (2): 355–71.
- Simpson, Jared T., Kim Wong, Shaun D. Jackman, Jacqueline E. Schein, Steven J. M. Jones, and Inanç Birol. 2009. "ABYSS: A Parallel Assembler for Short Read Sequence Data." *Genome Research* 19 (6): 1117–23.
- Sink, Kerry. 2016. "The Marine Protected Areas Debate: Implications for the Proposed Phakisa Marine Protected Areas Network." *South African Journal of Science* 112 (9/10).
- Sink, Kerry, Stephen Holness, Linda Harris, Prideel Majiedt, Lara Atkinson, Tamara Robinson, Steve Kirkman, Larry Hutchings, Robin Leslie, and Stephen Lamberth. 2012. "National Biodiversity Assessment 2011: Technical Report. Volume 4: Marine and Coastal Component. Pretoria: South African National Biodiversity Institute." Volume 4: 325.
- Sivasundar, Arjun, and Stephen R. Palumbi. 2010. "Life history, ecology and the biogeography of strong genetic breaks among 15 species of Pacific rockfish, *Sebastes*." *Marine Biology* 157, no. 7: 1433-1452.
- Smit, Albertus J., Michael Roberts, Robert J. Anderson, Francois Dufois, Sheldon F. J. Dudley, Thomas G. Bornman, Jennifer Olbers, and John J. Bolton. 2013. "A Coastal Seawater Temperature Dataset for Biogeographical Studies: Large Biases between in Situ and Remotely-Sensed Data Sets around the Coast of South Africa." *PloS One* 8 (12): e81944.
- Sodergren, Erica, George M. Weinstock, Eric H. Davidson, R. Andrew Cameron, Richard A. Gibbs, Robert C. Angerer, Lynne M. Angerer et al. Sea Urchin Genome Sequencing Consortium. 2006. "The genome of the sea urchin *Strongylocentrotus purpuratus*." *Science* 314, no. 5801: 941-952.
- Song, Linsheng, Chao Bian, Yongju Luo, Lingling Wang, Xinxin You, Jia Li, Ying Qiu, et al. 2016. "Draft Genome of the Chinese Mitten Crab, *Eriocheir sinensis*." *GigaScience* 5 (January): 5.
- Song, Kai, Li Li, and Guofan Zhang. 2018. "Relationship Among Intron Length, Gene Expression, and Nucleotide Diversity in the Pacific Oyster *Crassostrea gigas*." *Marine Biotechnology* 20, no. 5: 676-684.
- Spano, Carlos A., Vreni Häussermann, Fabián H. Acuña, Charles Griffiths, Lisa W. Seeb,

- and Daniel Gomez-Uchida. 2018. "Hierarchical Biogeographical Processes Largely Explain the Genomic Divergence Pattern in a Species Complex of Sea Anemones (Metridioidea: Sagartiidae: Anthothoe)." *Molecular Phylogenetics and Evolution* 127 (October): 217–28.
- Stanley, Ryan R. E., Claudio DiBacco, Ben Lowen, Robert G. Beiko, Nick W. Jeffery, Mallory Van Wyngaarden, Paul Bentzen, et al. 2018. "A Climate-Associated Multispecies Cryptic Cline in the Northwest Atlantic." *Science Advances* 4 (3): eaaq0929.
- Steiner, Florian M., Sándor Csősz, Bálint Markó, Alexander Gamisch, Lukas Rinnhofer, Clemens Folterbauer, Sarina Hammerle, Christian Staufer, Wolfgang Arthofer, and Birgit C. Schlick-Steiner. 2018. "Turning one into five: Integrative taxonomy uncovers complex evolution of cryptic species in the harvester ant *Messor 'structor'*." *Molecular Phylogenetics and Evolution* 127: 387–404.
- Stuart, Venetia, and J. G. Field. 1981. "Respiration and Ecological Energetics of the Sea Urchin *Parechinus angulosus*." *South African Journal of Zoology* 16 (2): 90–95.
- Sunnucks, Paul. 2000. "Efficient Genetic Markers for Population Biology." *Trends in Ecology & Evolution* 15 (5): 199–203.
- Tay, Ywee Chieh, M. W. P. Chng, W. W. Genevieve Sew, Frank Erwin Rheindt, Karenne P. P. Tun, and Rudolf Meier. 2016. "Beyond the Coral Triangle: High Genetic Diversity and near Panmixia in Singapore's Populations of the Broadcast Spawning Sea Star *Protoreaster nodosus*." *Royal Society Open Science* 3 (8): 160253.
- Tepolt, Carolyn K., and Stephen R. Palumbi. 2015. "Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader." *Molecular Ecology* 24, no. 16: 4145–4158.
- Teske, Peter R., Jonathan Sandoval-Castillo, Tirupathi R. Golla, Arsalan Emami-Khoyi, Mbaye Tine, Sophie von der Heyden, and Luciano B. Beheregaray. 2019. "Thermal selection as a driver of marine ecological speciation." *Proceedings of the Royal Society B* 286, no. 1896: 20182023.
- Teske, Peter R., Sophie Bader, and Tirupathi Rao Golla. 2015. "Passive dispersal against an ocean current." *Marine Ecology Progress Series* 539: 153–163.
- Teske, Peter R., Gerardo I. Zardi, Christopher D. McQuaid, and Katy R. Nicastro. 2013. "Two sides of the same coin: extinctions and originations across the Atlantic/Indian Ocean boundary as consequences of the same climate oscillation." *Frontiers of Biogeography* 5, no. 1.
- Teske, Peter R., Sophie von der Heyden, Christopher D. McQuaid, and Nigel P. Barker. 2011. "A Review of Marine Phylogeography in Southern Africa." *South African Journal of Science* 107 (5–6): 43–53.
- Teske, Peter R., Isabelle Papadopoulos, Brent K. Newman, Peter C. Dworschak, Christopher D. McQuaid, and Nigel P. Barker. 2008. "Oceanic dispersal barriers, adaptation and larval retention: An interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn." *BMC Evolutionary Biology* 8:834.
- Teske, Peter R., Isabelle Papadopoulos, Gerardo I. Zardi, Christopher D. McQuaid, Max Thabiso Edkins, Charles L. Griffiths, and Nigel P. Barker. 2007. "Implications of life

history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development." *Marine Biology* 152, no. 3: 697-711.

- Therkildsen, Nina Overgaard, and Stephen R. Palumbi. 2017. "Practical Low-Coverage Genomewide Sequencing of Hundreds of Individually Barcoded Samples for Population and Evolutionary Genomics in Nonmodel Species." *Molecular Ecology Resources* 17 (2): 194–208.
- Thrush, Simon F., Judi E. Hewitt, Paul K. Dayton, Giovanni Coco, Andrew M. Lohrer, Alf Norkko, Joanna Norkko, and Mariachiara Chiantore. 2009. "Forecasting the Limits of Resilience: Integrating Empirical Research with Theory." *Proceedings. Biological Sciences / The Royal Society* 276 (1671): 3209–17.
- Toews, David P. L., and Alan Brelsford. 2012. "The biogeography of mitochondrial and nuclear discordance in animals." *Molecular Ecology* 21, no. 16: 3907-3930.
- Toms, Jessica A., John S. Compton, Malcolm Smale, and Sophie von der Heyden. 2014. "Variation in Palaeo-Shorelines Explains Contemporary Population Genetic Patterns of Rocky Shore Species." *Biology Letters* 10 (6): 20140330
- Toonen, Robert J., Jonathan B. Puritz, Zac H. Forsman, Jonathan L. Whitney, Iria Fernandez-Silva, Kimberly R. Andrews, and Christopher E. Bird. 2013. "ezRAD: A Simplified Method for Genomic Genotyping in Non-Model Organisms." *PeerJ* 1: e203.
- Toonen, Robert J., Kimberly R. Andrews, Iliana B. Baums, Christopher E. Bird, Gregory T. Concepcion, Toby S. Daly-Engel, Jeff A. Eble, Anuschka Faucci, Gaither, Michelle R., Matthew Iacchei, and Jonathan B. Puritz. 2011. "Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian Archipelago". *Journal of Marine Biology*, vol. 2011, Article ID 460173.
- Trembl, Eric A., Jason J. Roberts, Yi Chao, Patrick N. Halpin, Hugh P. Possingham, and Cynthia Riginos. 2012. "Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations." *Integrative and Comparative Biology* 52, No. 4: 525-537.
- Van Wyngaarden, Mallory, Paul V. R. Snelgrove, Claudio DiBacco, Lorraine C. Hamilton, Naiara Rodríguez-Ezpeleta, Nicholas W. Jeffery, Ryan R. E. Stanley, and Ian R. Bradbury. 2017. "Identifying Patterns of Dispersal, Connectivity and Selection in the Sea Scallop, *Placopecten magellanicus*, Using RAD Seq-Derived SNPs." *Evolutionary Applications* 10 (1): 102–17.
- Van Wyngaarden, Mallory, Paul V. R. Snelgrove, Claudio DiBacco, Lorraine C. Hamilton, Naiara Rodríguez-Ezpeleta, Luyao Zhan, Robert G. Beiko, and Ian R. Bradbury. 2018. "Oceanographic Variation Influences Spatial Genomic Structure in the Sea Scallop, *Placopecten magellanicus*." *Ecology and Evolution* 8 (5): 2824–41.
- Vähä, Juha-Pekka, Jaakko Erkinaro, Eero Niemelä, and Craig R. Primmer. 2007. "Life-history and habitat features influence the within-river genetic structure of Atlantic salmon." *Molecular Ecology* 16, no. 13: 2638-2654.
- Vendrami, David L. J., Luca Telesca, Hannah Weigand, Martina Weiss, Katie Fawcett, Katrin Lehman, Melody Susan Clark, et al. 2017. "RAD Sequencing Resolves Fine-Scale Population Structure in a Benthic Invertebrate: Implications for Understanding Phenotypic Plasticity." *Royal Society Open Science* 4 (2): 160548.

- von der Heyden, Sophie. 2017. "Making evolutionary history count: biodiversity planning for coral reef fishes and the conservation of evolutionary processes." *Coral Reefs* 36, no. 1: 183-194.
- von der Heyden, Sophie, Jessica A. Toms, Peter R. Teske, Stephen J. Lamberth, and Wouter Holleman. 2015. "Contrasting Signals of Genetic Diversity and Historical Demography between Two Recently Diverged Marine and Estuarine Fish Species." *Marine Ecology Progress Series* 526 (April): 157–67.
- von der Heyden, Sophie, Maria Beger, Robert J. Toonen, Lynne van Herwerden, Marie Antonette Juinio-Meñez, Rachel Ravago-Gotanco, Cecile Fauvelot, and Giacomo Bernardi. 2014. "The application of genetics to marine management and conservation: examples from the Indo-Pacific." *Bulletin of Marine Science* 90, no. 1: 123-158
- von der Heyden, Sophie, Rauri C. K. Bowie, Kim Prochazka, Paulette Bloomer, Nicole L. Crane, and Giacomo Bernardi. 2011. "Phylogeographic patterns and cryptic speciation across oceanographic barriers in South African intertidal fishes." *Journal of Evolutionary Biology* 24, no. 11: 2505-2519.
- von der Heyden, Sophie. 2009. "Why Do We Need to Integrate Population Genetics into South African Marine Protected Area Planning?" *African Journal of Marine Science* 31 (2): 263–69.
- von der Heyden, Sophie, Kim Prochazka, and Rauri C. K. Bowie. 2008. "Significant population structure and asymmetric gene flow patterns amidst expanding populations of *Clinus cottoides* (Perciformes, Clinidae): application of molecular data to marine conservation planning in South Africa." *Molecular Ecology* 17, no. 22: 4812-4826.
- Villacorta-Rath, Cecilia, Carla A. Souza, Nicholas P. Murphy, Bridget S. Green, Caleb Gardner, and Jan M. Strugnell. 2018. "Temporal Genetic Patterns of Diversity and Structure Evidence Chaotic Genetic Patchiness in a Spiny Lobster." *Molecular Ecology* 27 (1): 54–65.
- Wang, Shi, Eli Meyer, John K. McKay, and Mikhail V. Matz. 2012. "2b-RAD: A Simple and Flexible Method for Genome-Wide Genotyping." *Nature Methods* 9 (8): 808–10.
- Waples, Robin S. 1987. "A multispecies approach to the analysis of gene flow in marine shore fishes." *Evolution* 41, no. 2: 385-400.
- Wares, John P., Steven D. Gaines, and Clifford W. Cunningham. 2001. "A comparative study of asymmetric migration events across a marine biogeographic boundary." *Evolution* 55, no. 2: 295-306.
- Watterson, G. A. 1975. "On the number of segregating sites in genetical models without recombination." *Theoretical Population Biology* 7, no. 2: 256-276.
- Weersing, Kimberley, and Robert J. Toonen. 2009. "Population genetics, larval dispersal, and connectivity in marine systems." *Marine Ecology Progress Series* 393: 1-12.
- Weidberg, Nicolás, Wayne Goschen, Jennifer M. Jackson, Paula Pattrick, Christopher D. McQuaid, and Francesca Porri. 2019. "Fine scale depth regulation of invertebrate larvae around coastal fronts." *Limnology and Oceanography* 64, no. 2: 785-802.
- Weidberg, Nicolás, Francesca Porri, Charles E. O. Von der Meden, Jennifer M. Jackson,

- Wayne Goschen, and Christopher D. McQuaid. 2015. "Mechanisms of nearshore retention and offshore export of mussel larvae over the Agulhas Bank." *Journal of Marine Systems* 144: 70-80.
- Westram, Anja M., Juan Galindo, Magnus Alm Rosenblad, John W. Grahame, Marina Panova, and Roger K. Butlin. 2014. "Do the same genes underlie parallel phenotypic divergence in different *Littorina saxatilis* populations?." *Molecular Ecology* 23, no. 18: 4603-4616.
- Wilkinson, Samantha, Chris Haley, Lawrence Alderson, and Pamela Wiener. 2011. "An empirical assessment of individual-based population genetic statistical techniques: application to British pig breeds." *Heredity* 106, no. 2: 261.
- Wollstein, Andreas, and Oscar Lao. 2015. "Detecting individual ancestry in the human genome." *Investigative Genetics* 6, no. 1: 7.
- Wright, Daniel, Jacqueline M. Bishop, Conrad A. Matthee, and Sophie von der Heyden. 2015. "Genetic Isolation by Distance Reveals Restricted Dispersal across a Range of Life Histories: Implications for Biodiversity Conservation Planning across Highly Variable Marine Environments." *Diversity and Distributions* 21, no. 6: 698-710.
- Wright, Sewall. 1943. "Isolation by distance." *Genetics* 28, no. 2: 114.
- Wu, Qingyang, Guidong Miao, Xincang Li, Wenhua Liu, Mhd Ikhwanuddin, and Hongyu Ma. 2018. "De Novo Assembly of Genome and Development of Polymorphic Microsatellite Loci in the Blue Swimming Crab (*Portunus pelagicus*) Using RAD Approach." *Molecular Biology Reports* 45, no. 6: 1913-1918.
- Xuereb, Amanda, Laura Benestan, Éric Normandeau, Rémi M. Daigle, Janelle M. R. Curtis, Louis Bernatchez, and Marie-Josée Fortin. 2018. "Asymmetric Oceanographic Processes Mediate Connectivity and Population Genetic Structure, as Revealed by RADseq, in a Highly Dispersive Marine Invertebrate (*Parastichopus californicus*)." *Molecular Ecology* 27 (10): 2347-64.
- Xuereb, Amanda, Cassidy C. D'Aloia, Rémi M. Daigle, Marco Andrello, Alicia Dalongeville, Stéphanie Manel, David Mouillot, Frédéric Guichard, Isabelle M. Côté, Janelle M. Curtis, Louis Bernatchez and Marie-Josée Fortin. 2019. "Marine Conservation and Marine Protected Areas." *Population Genomics* 1-24. Springer, Cham
- Young, Emma F., Mark Belchier, Lorenz Hauser, Gavin J. Horsburgh, Michael P. Meredith, Eugene J. Murphy, Sonia Pascoal, Jennifer Rock, Niklas Tysklind, and Gary R. Carvalho. 2015. "Oceanography and life history predict contrasting genetic population structure in two Antarctic fish species." *Evolutionary Applications* 8, no. 5: 486-509.
- Zardi, Gerardo I., Katy R. Nicastro, Christopher D. McQuaid, Lisa Hancke, and Brian Helmuth. 2011. "The combination of selection and dispersal helps explain genetic structure in intertidal mussels." *Oecologia*. 2011;165:947-958.
- Zarraonaindia, Iratxe, Mikel Iriondo, Aitor Albaina, Miguel Angel Pardo, Carmen Manzano, W. Stewart Grant, Xabier Irigoien, and Andone Estonba. 2012. "Multiple SNP markers reveal fine-scale population and deep phylogeographic structure in European anchovy (*Engraulis encrasicolus* L.)." *PloS one* 7, no. 7: e42201.

Zhang, Xiaojun, Jianbo Yuan, Yamin Sun, Shihao Li, Yi Gao, Yang Yu, Chengzhang Liu et al. 2019. "Penaeid shrimp genome provides insights into benthic adaptation and frequent molting." *Nature Communications* 10, no. 1: 356.

Chapter III: Characterising the putatively adaptive potential of selected marine invertebrates in South Africa

3.1 Introduction

Exploring the molecular basis of adaptive divergence in species is a fundamental pursuit in evolutionary biology (Ford, 1975; Tenaillon and Tiffin, 2008; Balkenhol et al. 2017). Local adaptation arises when individuals possess a higher average fitness in their local habitat conditions relative to individuals from different populations (Williams, 1966; Kawecki and Ebert, 2004; Savolainen, Lascoux and Merilä, 2013), which has traditionally been studied with resource-intensive reciprocal transplant or common garden experiments (Flanagan et al. 2018). The occurrence of local adaptation is thought to rise with larger molecular variation across species populations and with larger environmental and phenotypic disparity among populations (Lande, 1976; Endler, 1977; García-Ramos and Kirkpatrick, 1997). Geographic variation of environmental variables can lead to natural selection shaping the spatial distribution of species traits (Linhart and Grant, 1996). Patterns of local adaptation are often associated with spatially diverging environmental conditions between populations, which can be observed through phenotypic and/or molecular differentiation along environmental gradients (such as latitude, altitude, water depth) or across different types of habitats (Becker et al. 2006; Hereford and Winn, 2008; Conover, Duffy and Hice, 2009). For instance, geographic phenotypic variation is central to Bergmann's rule, which observes that body size increases with latitude in several species (Bergmann, 1847; Atkinson and Sibly, 1997). Also, geographic phenotypic variation in the shape of a latitudinal cline has for example been observed for flowering time in plants (Stinchcombe et al. 2004). Further, evidence for geographical patterns of genetic variation influenced by an ecological gradient have been for example recovered in sticklebacks (*Gasterosteus aculeatus*) in the Baltic Sea, indicating local adaptation to spatial variation in salinity levels (DeFaveri and Merilä, 2014). Evidence of spatially varying selective pressures in numerous studies involve pronounced environmental gradients (Bradbury et al. 2010; Milano et al. 2014; Guo et al. 2015; Guo, Li and Merilä, 2016; Dalongeville et al. 2018; Stanley et al. 2018), often revealing clinal gene frequency patterns for putatively adaptive loci (Eckert et al. 2010). Interestingly, spatially varying molecular patterns in areas characterised by relatively homogeneous environmental conditions have also been discovered (Nielsen et al. 2018). Further evidence suggests that selective pressures can impact relatively closely related species inhabiting the same geographical area differently, pointing to dissimilar processes shaping their evolutionary trajectory (Cullingham, Cooke and Coltman, 2014). Importantly, divergent selection driving

local adaptation over species populations exposed to heterogeneous environmental conditions across their range may initiate early phases of speciation (Nosil, Funk and Ortiz-Barrientos, 2009; Nosil 2012; Ravinet et al. 2017; see for example Teske et al. 2019; Tisthammer et al. 2020).

While local adaptation and associated higher average fitness can benefit organisms in locally experienced environmental conditions, there is a potential trade-off in terms of consequently lower average fitness in other environments (Hereford, 2009). Possible trade-offs related to local adaptation to the environmental conditions of a certain area might stimulate molecular variation between species populations across their range, which can result in physiological and/or morphological differentiation (Futuyma and Moreno, 1988; Jasmin and Kassen, 2007). A range of factors, as for example low molecular variation in populations, can influence or limit local adaptation potential (Hereford, 2009). High levels of gene flow may potentially homogenise allele frequencies between populations and hence reduce local adaptation, but it has been demonstrated that adaptive divergence can arise in the presence of gene flow (Schluter, 2009; Leinonen et al. 2013; see for example Guo et al. 2015). Further, the occurrence of local adaptation in species might be impacted by genetic drift, large (effective) population size and by selection processes related to short term environmental variability (Kawecki and Ebert, 2004; Cano et al. 2008; Nosil, Funk and Ortiz-Barrientos, 2009; Lotterhos and Whitlock, 2015). Next to limited standing genetic variation, the molecular composition of relevant genes (Le Corre and Kremer, 2012; Gagnaire and Gaggiotti, 2016) and wide phenotypic plasticity (Fraser et al. 2011) are also thought to hinder adaptive divergence in populations. Hence, detected local adaptation may be shaped by a range of factors and might not be solely the result of divergent selection due to spatial ecological variation (Kawecki and Ebert, 2004; Leinonen et al. 2013; Blanquart et al. 2013).

Putatively adaptive regions in the genome, which may signal local adaptation patterns, have been discovered in a wide range of species (Lewontin and Krakauer, 1973; Leimu and Fischer, 2008; Hereford, 2009; Feng, Jiang and Fan, 2015; Dayan, 2018). There is evidence from studies linking genomic variation (genotypes), the evolutionary basis of adaptation, to phenotypic variation (phenotypes) in some species (Hohenlohe et al. 2010; Renaut et al. 2011; Deagle et al. 2012; Jones et al. 2012; McGaughan et al. 2016; Marques et al. 2018). However, the molecular basis of adaptive divergence is still not well understood (Savolainen, Lascoux and Merilä, 2013; Harrisson et al. 2014). As established in section 1.3, high-throughput sequencing and RAD-Seq (restriction-site-associated sequencing) technology have made detecting potentially adaptive genomic regions more widely accessible and cost effective (Manel et al. 2016; Andrews et al. 2016; but see Lowry et al. 2017), particularly for

species with no or few existing molecular resources (non-model organisms) (Luikart et al. 2003; Manel et al. 2010, 2016; Ekblom and Galindo, 2011; Ellegren, 2014; Delord et al. 2018; Weigand and Leese, 2018). Moreover, the scale of genomic data (as opposed to traditional markers) helps to facilitate comparative studies exploring biogeographic processes of co-distributed species (Papadopoulou and Knowles, 2016). However, studies based on genomic data obtained by scanning for SNPs can be subject to genotyping errors (Xue et al. 2009; O'Leary et al. 2018) and the discovery of false positive loci (Excoffier, Hofer and Foll, 2009). Utilising limited genome-wide data, such as the reduced representation approach (RAD-Seq), as opposed to whole genome data might also risk missing signals of adaptive selection (Toonen et al. 2013; Puritz et al. 2014; Hoban et al. 2016; Lowry et al. 2017). Further, low coverage of genomic data and strict bioanalytical filtering protocols can additionally hinder the detection of signs of adaptive evolutionary change (Hoban et al. 2016; O'Leary et al. 2018). Conversely, it is recommended to use stringent filter parameters to increase the confidence of detected candidate loci and to lower the rate of false positives (Cantarel et al. 2014; Nevado and Pérez-Enciso, 2015; O'Leary et al. 2018). For instance, large SNP window sizes are thought to lead to more false positives, whereas smaller window sizes may raise false negative findings (Beissinger et al. 2015; O'Leary et al. 2018). Lastly, sampling design is thought to impact analyses of genomic data and sampling many populations with a few individuals has since been suggested above selecting a small number of populations with numerous individuals (De Mita et al. 2013; Lotterhos and Whitlock, 2015).

At the same time as the above-mentioned quickly expanding biotechnological possibilities, natural populations are being adversely impacted by changing climatic conditions, anthropogenic pressures and invasive species (Parmesan, 2006; Hoffmann and Sgrò, 2011; Mead et al. 2013; Hillebrand et al. 2018). Species may respond to changing environmental conditions in their habitat by phenotypic plasticity (Chevin, Lande and Mace, 2010), geographically shifting their range to an appropriate area if available (Loarie et al. 2009) and/or genetically adapting to the new conditions (Franks and Hoffmann, 2012). Populations which are unable to adapt or shift geographically might face extinction (Aitken et al. 2008; Cahill et al. 2013). Importantly, climate change-induced shifts of environmental conditions have the capacity to influence prevalent selection processes in the local environment of species populations (Franks and Hoffmann, 2012). Evidence of spatially varying adaptive signals across populations can contribute to estimate how species occupying environmental gradients or latitudinal clines might be able to respond to changing conditions, if for instance some populations already face challenging or extreme conditions (Alberto et al. 2013; Guo et al. 2015; Balkenhol et al. 2017; Stanley et al. 2018; Exposito-Alonso et al. 2018). Species

estimated capacity to respond to changes of climatic conditions on the genomic level might be inferred from the detection and geographic distribution of putatively adaptive loci to help conserve and maximise their molecular diversity and evolutionary potential (Fraser and Bernatchez, 2001).

3.1.1 Adaptive variation and evolutionary potential

Species capacity to respond to changing climatic conditions may be estimated from the molecular diversity and ‘adaptive uniqueness’ of their populations, as the genome builds the basis for natural selection to act on (Lande and Shannon, 1996; Fraser and Bernatchez, 2001; Barrett and Schluter, 2008; Hereford, 2009; Alsos et al. 2012; Pauls et al. 2013; Graudal et al. 2014). Genomic uniqueness of populations can be incorporated into conservation strategies (Sgrò, Lowe and Hoffmann, 2011; Flanagan et al. 2018), although the best avenues to implement species spatial adaptive characteristics are not well established yet (Harrisson et al. 2014; Hendricks, Schweizer and Wayne, 2019; Hoelzel, Bruford and Fleischer, 2019). The rationale is that adaptive evolution potential arising from standing genomic variation and the distribution of putatively adaptive loci across the species native range might indicate the persistence of populations under changing climatic conditions (Exposito-Alonso et al. 2018; Lai et al. 2019). For instance, flowering plant *Arabidopsis thaliana* is widely found across Europe, but populations in the Mediterranean and Scandinavian regions demonstrate larger variety of drought resistance alleles (linked to higher drought resistance) compared to Central European populations, which influences the respective resilience of their populations to changing climatic conditions (Exposito-Alonso et al. 2018). Adaptations to environmental heterogeneity or extreme conditions at the edges of the species range, which are based on intraspecific genomic heterogeneity, might be the key to enable an evolutionary response under climate change. It is possible that the distinct environmental differences between the most western and most eastern South African coastal study sites (Port Nolloth, Haga Haga) could facilitate the basis for within-species adaptive variation in the populations. Nevertheless, the long-term persistence of species depends on both neutral and adaptive elements of genomic diversity and it is still in question to which extent and with which spatio-temporal patterns both elements determine species evolutionary potential (Romiguier et al. 2014; Mittell, Nakagawa and Hadfield, 2015). Thus, it has been advocated that genomic markers may likely not directly translate into future adaptive potential of populations (Christmas, Breed and Lowe, 2016) and that evolutionary potential possesses an additional epigenetic component and possible variation thereof (Harrisson et al. 2014). Functional annotations are not widely available for non-model organisms at present, RAD-Seq data covers less markers relative to whole-genome scan

data (see Lowry et al. 2017) and outlier detection approaches have technical limitations (Weigand and Leese, 2018), which means putatively adaptive loci in this study serve as an early indication of possible evolutionary potential. The identification of interesting candidate loci has been described as a starting point (Lotterhos and Whitlock, 2015; Gagnaire and Gaggiotti, 2016; Catchen et al. 2017), which can be expanded in the future with ongoing innovation of genomic tools and evidence of the specific functional role of outliers (Pardo-Diaz, Salazar and Jiggins, 2015).

3.1.2 Outlier detection approaches

3.1.2.1 Outlier differentiation method (F_{ST})

Identifying loci potentially showing patterns of local adaptation is practised with two broadly adapted methods: the frequency-based outlier differentiation approach, which focuses on loci which display distinctively high differentiation across the remaining genomic data and the correlation-based environmental association approach, which investigates loci which may be impacted by the influence of environmental variables. The former approach follows the rationale that alleles associated with local adaptation are likely to possess a higher frequency in populations where they improve fitness and reversely a lower frequency if they decrease fitness (Hoban et al. 2016). Hence, determining which alleles display above average differentiation (i. e. higher variance compared to putatively neutral loci) in the genome could point to loci under selection among species populations (Lewontin and Krakauer, 1973; Storz, 2005; Beaumont, 2005; Schilling et al. 2014; but see Nei and Maruyama, 1975). Genomic differentiation is widely estimated with fixation index (F_{ST} ; Wright, 1949; Ahrens et al. 2018) approaches, coining the term ' F_{ST} outliers' (Lotterhos and Whitlock, 2014). Theoretically, loci affected by adaptive selection processes exhibit high F_{ST} values and reversely low F_{ST} values if they are under balancing selection (Excoffier, Hofer and Foll, 2009; Leinonen et al. 2013). A drawback of the outlier differentiation method is that loci can deviate from neutrality (neutral processes) due to other processes than adaptive divergence (Lotterhos and Whitlock, 2014; Gagnaire and Gaggiotti, 2016), such as background selection, pre-/post-zygotic isolation, stochastic effects, selection attributed to deleterious alleles and differential introgression (Bierne et al. 2011; Bierne, Roze and Welch, 2013; Gosset and Bierne, 2013; Lotterhos and Whitlock, 2015; Fraïsse et al. 2016; Matthey-Doret and Whitlock, 2019). In spite of the various factors possibly influencing F_{ST} estimates throughout the genome, this outlier detection method is widely applied and serves as valuable initial step to establish candidate loci which might be targets of selection among a large range of species and their populations (Beaumont, 2005; Jensen, Foll and

Bernatchez, 2016; Ahrens et al. 2018; but see Bierne et al. 2003; Bierne, Roze and Welch, 2013).

This study conducts two F_{ST} -based outlier differentiation approaches, of which the first one is the empirical F_{ST} method. The empirical F_{ST} method designates SNPs as outliers whose pairwise F_{ST} estimates between populations are so strongly differentiated that they are found in the upper 0.5% (alternatively 0.1%) proportion of the empirical distribution of all estimated values (99.5 percentile; Akey et al. 2010; Feulner et al. 2013). For instance, the empirical F_{ST} method has been used to identify the molecular basis of phenotypic variation across 275 modern domesticated dogs from phenotypically diverse breeds (Akey et al. 2010). The outlier detection through the empirical F_{ST} distribution is thought to reveal a list of candidate genes of interest, but it may be influenced by how susceptible the footprints of selection are to the demographic background of the species (Teshima, Coop and Przeworski, 2006; Lotterhos and Whitlock, 2015). Also, the method might lower representation of adaptive divergence from standing molecular variation or from recessive variation (Teshima, Coop and Przeworski, 2006; Lai et al. 2019). Early studies which utilise this empirical outlier detection path include investigations on fruit fly *Drosophila melanogaster* (Kolaczowski et al. 2011) and mosquito complex *Anopheles gambiae* (Cheng et al. 2012). In the marine environment, this approach has been used for instance in three-spined sticklebacks *Gasterosteus aculeatus* (Feulner et al. 2013; Guo et al. 2015), rainwater killifish *Lucania parva* (Kozak et al. 2014) and Atlantic herring *Clupea harengus* (Guo, Li and Merilä, 2016).

The second conducted outlier detection approach infers from a Bayesian perspective that gene frequencies in a neutrally structured population model might be estimated with a multinomial Dirichlet distribution (Beaumont, 2005). BayeScan version 2.1 (Foll and Gaggiotti, 2008) utilises a Bayesian-based framework where F_{ST} serves as a model parameter to estimate the posterior probability that a locus is under directional selection (Foll and Gaggiotti, 2008). The application scans for a “*departure from neutrality*” in the genome based on differences in allele frequencies between populations (Fischer et al. 2011; p. 1453; also see Foll and Gaggiotti, 2008; Foll et al. 2010). Moreover, BayeScan estimates q-values for each locus which indicate the minimum false discovery rate (FDR) at which the individual locus might be considered significant. BayeScan has been suggested as more efficient at detecting outlier loci in comparison to other approaches (Pérez-Figueroa et al. 2010; Narum and Hess, 2011; De Mita et al. 2013), but this may strongly depend on the criteria utilised to judge significance (Lotterhos and Whitlock, 2014). Also, it is thought that BayeScan might be potentially impacted by the presence of population structure such as isolation-by-distance (IBD) and range expansions (Lotterhos and Whitlock, 2014). BayeScan has been used

across a broad range of species populations and examples for the application in marine invertebrates include the California sea cucumber *Parastichopus californicus* (Xuereb et al. 2018), the spiny lobster *Panulirus homarus* (Al-Breiki et al. 2018) and the Eastern oyster *Crassostrea virginica* (Bernatchez et al. 2019).

3.1.2.2 Environmental association method

3.1.2.2.1 Background

The second approach to detect outlier loci seeks correlations of population allele frequencies with spatial differentiation of environmental variables occurring across a species range or a section thereof (Endler, 1986). If an allele imparts a benefit under specific environmental conditions, it is expected to appear at an increased frequency in species populations with the conditions or is likely identified by a strong geographic allele frequency difference between populations (Coop et al. 2010; Bragg et al. 2015). Methods following this approach aim to investigate if alleles are stronger correlated with tested environmental variables than presumed from neutral processes alone (Coop et al. 2010; Günther and Coop, 2013; Bradburd, Ralph and Coop, 2013; Villemereuil and Gaggiotti, 2015). For instance, a comparative genomic approach revealed candidate loci presumably associated with high-altitude adaptation in Tibetans, who are exposed to strongly different environmental conditions than humans inhabiting lowland regions (Simonson et al. 2010; Beall et al. 2010). Aside from factors such as altitude, climatic conditions are recognised as influential selective pressures acting on species populations (Parmesan, 2006; Hancock et al. 2008; Urban, 2015; Foden et al. 2019). Exploring the molecular basis of local adaptation is therefore key in the context of climate change, which is associated with comparatively rapid changes in climatic conditions (IPCC, 2013). Moreover, environmental association approaches have the potential to reveal signals of local adaptation which might not be detectable by population genomic differentiation methods or be able to support and add to them (Rellstab et al. 2015).

Nevertheless, the environmental association approach has drawbacks: It is restricted to detecting loci based on the tested environmental variables and therefore may possibly overlook other environmental variables or indicate a potential multicollinearity between them (Cullingham, Cooke and Coltman, 2014; Bragg et al. 2015; Rellstab et al. 2015). Moreover, this approach depends not only on the estimated environmental variables which might be most biologically relevant for the species populations (Lotterhos and Whitlock, 2014), but at the same time on the resolution of the available environmental data (Hoban et al. 2016). Further, without direct natural selection being involved, several instances, including hidden demographic effects (De Mita et al. 2013; Lotterhos and Whitlock, 2015), variation in the

mutation and recombination rate (Roesti et al. 2012; Tine et al. 2014), isolation by distance (Vasemägi, 2006), hitchhiking genes (Bierne et al. 2011; Lotterhos and Whitlock, 2015), secondary contact following allopatric divergence (Bierne et al. 2003) and allele surfing related to range expansions (Klopfstein, Currat and Excoffier, 2006; Travis et al. 2007) might lead to correlations between genomic patterns and environmental variables. Parameters such as for instance recombination rate estimates are not known yet for many non-model organisms, which means genome scans for natural selection need to be interpreted cautiously with the potential influence of genomic heterogeneity in mind (Haas and Payseur, 2016). Nonetheless, environmental association studies allow an exploration of the genome (or sections thereof in case of RAD-Seq) which was not possible with the technical limitations of the pre-genomic investigation era (Rellstab et al. 2015).

3.1.2.2.2 Environmental variables

The strength of environmental association approaches depends on predicting which variables might be most relevant to the populations across their spatial distribution, which might be challenging to forecast (Lotterhos and Whitlock, 2014). In the marine environment, sea surface temperature (SST) is a dominant factor influencing the geographical distribution, physiology and reproduction of numerous marine species (Perry et al. 2005; Pörtner and Farrell, 2008; Marbà et al. 2015; Hiddink, Burrows and García-Molinos, 2015; Riginos et al. 2016; Free et al. 2019). Depending on the niche marine species are inhabiting, alternatives to SST might be SBT (sea bottom temperature) (Gaudin et al. 2018) or seasonal measures such as average winter or summer sea temperature (Stanley et al. 2018; Van Wyngaarden et al. 2018). For example, spatial molecular differentiation in five marine species appears to be best explained by spring bottom temperature and winter sea surface temperature in the northwest Atlantic (Stanley et al. 2018). Other examples include mean bottom temperature putatively driving local adaptation in populations of California sea cucumber *Parastichopus californicus* (Xuereb et al. 2018) and seasonal phases of thermal minima in sea scallop *Placopecten magellanicus* (Lehnert et al. 2019). Apart from sea water temperature, another potential determinant are salinity levels. For instance, due to the very distinct salinity gradient in the Baltic Sea (from ~25 psu to <1 psu (practical salinity unit); Bonsdorff, 2006), it is not unexpected to recover patterns of likely local adaptation to salinity variation in numerous marine species inhabiting the region (e.g. Atlantic cod (*Gadus morhua*), Berg et al. 2015; Malachowicz and Wenne, 2019; (marine) three-spined stickleback (*Gasterosteus aculeatus*), Guo et al. 2015; blue mussel (*Mytilus* spp.), Larsson et al. 2017). Nevertheless, examples for putatively divergent selection driven by salinity differences are not limited to the Baltic Sea and were discovered across other geographic areas as well (e.g. European hake

(*Merluccius merluccius*) (Milano et al. 2014); striped red mullet (*Mullus surmuletus*) (Dalongeville et al. 2018); turbot (*Scophthalmus maximus*) (Do Prado et al. 2018); small yellow croaker (*Larimichthys polyactis*) (Zhang et al. 2019a); oyster (*Crassostrea ariakensis*) (Liu et al. 2019)).

Detailed environmental data availability permitting, studies on marine species have furthermore tested genome-environment association with a wide range of factors including for instance seawater density, chlorophyll A (as proxy for primary productivity), inorganic nutrient concentrations (Van Wyngaarden et al. 2018), current velocity, dissolved oxygen concentration (Xuereb et al. 2018) and turbidity (Zhang et al. 2019a). While the factor air temperature might not appear as decisive for the majority of marine species, it can pose a challenge for those living at the marine-terrestrial interface such as intertidal rocky shore ecosystems. Species inhabiting the mid- to high shore experience different degrees of air temperature variation during low tide phases (Helmuth et al. 2006a; Somero, 2012). Therefore, testing genomic data for environmental association with air temperature might yield insights into possible signs of local adaptation in those species. Overall, air temperature has been previously considered as a factor influencing molecular patterns in sea birds (Tigano et al. 2017) and a range of freshwater fish populations (Matala, Hess and Narum, 2011; Perrier et al. 2017; Chen et al. 2018). In intertidal species, air temperature has been investigated regarding adaptive divergence in the intertidal oyster *Crassostrea gigas* (Li et al. 2017) and air temperature is speculated to contribute to a genetic cline in the barnacle *Balanus glandula* (Wares and Skoczen, 2019). The latter two studies do not utilise RAD-Seq approaches, but testing intertidal species for impacts of air temperature is for example also well established in the field of experimental physiology (Miller, Harley and Denny, 2009; see Chapter IV).

Potential associations between environmental driving factors and genomic patterns have been previously detected in the South African coastal marine environment. Putatively adaptive loci of the sea grass *Zostera capensis* demonstrated signs of influence by spatial variation in sea temperature and precipitation across the species distribution in southern Africa (Phair et al. 2019). Moreover, environmental association with the thermal gradient across the South African coastline has also been recovered in the genome of the sand goby *Psammogobius knysnaensis* (Teske et al. 2019). And although more of circumstantial nature, the authors by a study on the South African abalone *Haliotis midae* suggest that their findings might point to the influence of varying local environmental conditions across coastal regions in South Africa (Rhode, Bester-van der Merwe and Roodt-Wilding, 2017). Further, studies on DNA methylation in populations of the brown mussel *Perna perna* (Watson et al.

2018) and the South African sandhopper *Talorchestia capensis* suggest that local environmental conditions might drive differences between their epigenetic profiles (Baldanzi et al. 2017).

Contributing to the research on genome-environment associations in South Africa's marine environment and with time constraints of a multi-species approach in mind, this study tests the RAD-Seq data of three intertidal species for potential association with mean annual sea surface temperature, salinity and air temperature. Sea surface temperature (SST) and salinity influence egg buoyancy, hatching, larval retention/survival and the density of adults in marine species and have hence been suggested as "*functional and operative proxies of the variation of complex environmental and oceanographic conditions*" of a greater geographical area (Milano et al. 2014, p. 119, and references therein; also see Porri et al. 2014). Beyond SST, salinity and air temperature, future empirical endeavours will be able to expand on a wider range of environmental variables insofar as available for the geographical locations of the species populations. Lastly, this study explores the potential impact of environmental variables on multiple co-distributed species, which is at present still comparatively rare in the field of population genomics (see Chapter II). Comparative phylogeographic studies have the potential to shine light on drivers of spatial genomic differentiation (Toonen et al. 2011; von der Heyden et al. 2014; Stanley et al. 2018). Selection footprints may vary among populations and species (Conte et al. 2012; Westram et al. 2014; Ravinet et al. 2016) and it is not well understood whether environmental factors commonly have similar or contrasting impacts on co-distributed species. For example, a past study describes two Canadian pine sister species likely subjected to different evolutionary selection pressures despite inhabiting the same geographic area (Cullingham, Cooke and Coltman, 2014). However, there is evidence for concordant patterns across multiple species with clines or similar genetic discontinuities (Barber et al. 2000; Bernardi, Findley and Rocha-Olivares, 2003). The advances of genotyping technology and the utilisation of genome-environment association methods might reveal more occurrences of congruent multi-species patterns. For instance, a congruent climate-associated multi-species cline has been found for five marine species with contrasting life history traits across a strong environmental gradient in the north-western part of the Atlantic, where sea water temperature appears to play a decisive role for population structure (Stanley et al. 2018). While the impact of climate change on species and populations is complex (Parmesan, 2006; Hoffmann and Sgrò, 2011; Cahill et al. 2013; Hillebrand et al. 2018), comparative phylogeographic studies of intraspecific genomic patterns across environmental gradients can serve to explore population structure and win insights on the potential influence of climate change on species (Avisé, 2000; Kelley et al. 2016).

3.1.2.2.3 BayeScEnv, isolation-by-distance (IBD) and isolation-by-environment (IBE)

BayeScEnv (Villemereuil and Gaggiotti, 2015), which was selected to conduct the environmental association approach in this study, compares the differentiation of the environmental variables with the genomic variation. The application estimates the empirical patterns of covariance between allele frequencies from different populations and utilises this as a null model for testing individual SNPs (Villemereuil and Gaggiotti, 2015). The correlation is not estimated between the allele frequencies and the environmental variables, but the patterns of differentiation between both (Villemereuil and Gaggiotti, 2015). Using a Bayesian approach, the application runs a Reversible Jump MCMC to estimate the posterior probability of a specific loci being affected by local adaptation. Importantly, possible divergent selection and other influences apart from local adaptation such as range expansions, background selection and varying mutation rates, are accounted for (Villemereuil and Gaggiotti, 2015). The authors claim that BayeScEnv has a lower rate of false discovery compared to methods based on F_{ST} estimates alone. Lastly, the tool may be best suited for species with a medium to high dispersal capacity, which specifically includes marine species with those attributes (Villemereuil and Gaggiotti, 2015). In the South African marine environment, the application has been utilised to investigate sea grass populations *Zostera capensis*, revealing signs of local adaptation to temperature and precipitation clines (Phair et al. 2019). Internationally, BayeScEnv contributed to studies on marine invertebrates including the red coral *Corallium rubrum* (association with sea water temperature) (Pratlong et al. 2018), the American lobster *Homarus americanus* (association with sea surface temperature) (Benestan et al. 2016), and the green crab *Carcinus maenas* (association with winter sea surface temperature and other variables) (Jeffery et al. 2018). Another important aspect of genome scans for local adaptation pertains to accounting for the role of geographic factors across the spatial distribution of populations.

Apart from the potential impact of environmental variables, geographic distance or barriers related to prominent landscape features can influence patterns of species spatial genomic variation (Wright, 1943; 1946; Bragg et al. 2015). Theoretically, geographical factors may limit dispersal, whereby the levels of migration can be higher in adjacent populations and decreasing among remote populations, which means that genomic differentiation across species populations can increase with geographic distance, ecological distance (if present) or both (Bradburd, Ralph and Coop, 2013). An occurrence of isolation by environment can arise if local adaptation is so prevalent that it may decrease the dispersal and settlement ability beyond the environmental conditions experienced at the origin (Bragg et al. 2015).

The relative strength of environmental variables and geographic distance in population structure can be assessed by testing genomic data for the impact of isolation-by-distance (IBD) and isolation-by-environment (IBE) (Orsini et al. 2013; Shafer and Wolf, 2013; Tiffin and Ross-Ibarra, 2014; Sexton, Hangartner and Hoffmann, 2014). Nevertheless, assessing the influence of IBD and IBE can be challenging in case geographic distance and environmental variables may be covarying (Sexton, Hangartner and Hoffmann, 2014). Constrained ordinations such as performed with redundancy analysis (RDA) (Legendre and Legendre, 2012), are suggested to outperform univariate approaches in detecting signals of selection (Forester et al. 2018). Importantly, the South African coastline might be regarded as a principal study system for potential IBD/IBE patterns in marine species owed to the roughly linear coastline, the total distance of over 2,500 km and the distinct thermal gradient (Branch and Branch, 1981; Branch et al. 2017).

3.1.2.3 Outlier detection approaches summary

Overall, it has been acknowledged that outlier detection methods each have methodological limitations to detect statistical outliers in genomic data (Excoffier, Hofer and Foll, 2009; Narum and Hess, 2011; Hoban et al. 2016; Haasl and Payseur, 2016). Therefore, as suggested by a number of past studies, it has become established to utilise multiple methods in conjunction to decrease the potential of false-positive outliers (Narum and Hess, 2011; Nunes et al. 2011; De Mita et al. 2013; Rellstab et al. 2015). Due to the different strengths of outlier detection strategies (Lotterhos and Whitlock, 2015), combining outlier differentiation and environmental association methods can assist to capture a wider range of outlier loci than a single approach (Rellstab et al. 2015). It is expected that contrasting methods might detect different outliers, which can overall contribute to the effort to understand signs of local adaptation in species (Vasemägi and Primmer, 2005; Nunes et al. 2011; Rellstab et al. 2015). It has been suggested that outlier differentiation approaches appear to yield higher numbers of false positives as opposed to environmental association methods (De Mita et al. 2013), but both methods are subject to inherent limitations and dismissing either technical approach might result in missing interesting candidate loci. Generally, loci identified by more than one detection method are considered as more robust (Pérez-Figueroa et al. 2010; Narum and Hess, 2011; Nunes et al. 2011; De Mita et al. 2013; Rellstab et al. 2015; Lotterhos and Whitlock, 2015), but exploring only loci identified by multiple methods typically reduces the overall amount of outliers (Guo, Li and Merilä, 2016). It is also possible that there might be few to no overlap between the identified outliers across detection methods (Villemereuil et al. 2014; Lotterhos and Whitlock, 2015). Conservative exclusion of outlier loci to reduce false positives could result in losing loci of interest for

future research (Gosset and Bierne, 2013), therefore this study aims to combine outlier lists yielded by different methods. Outlier loci are subsequently tested for a potential functional association with a general blast search in NCBI's protein sequence reference database BlastX (see section 3.2.2.5). Further, outlier loci are evaluated for population structure patterns in BAPS (Bayesian Analysis of Population Structure; Corander and Marttinen, 2006; Corander et al. 2006) and fastSTRUCTURE (see section 3.2.2.7; Raj, Stephens and Pritchard, 2014) as in Chapter II tested with the selectively neutral loci. It is important to acknowledge the difficulties to ascertain which outliers are potentially false positives. Essentially, every method yields a number of loci per species which can be regarded as hypothetical adaptive loci (Jensen, Foll and Bernatchez, 2016; Gagnaire and Gaggiotti, 2016), which require to be assessed and confirmed for instance by functional analyses of candidate genes, common garden or reciprocal transplant experiments and gene expression studies in future research efforts (also see section 3.4.7) (Hereford, 2009; Lotterhos and Whitlock, 2015; Pardo-Diaz, Salazar and Jiggins, 2015). Candidate loci provide a foundation to uncover the species molecular basis of adaptive changes (Pardo-Diaz, Salazar and Jiggins, 2015), which is particularly useful for species with few or no prior molecular resources (Tiffin and Ross-Ibarra, 2014; Bragg et al. 2015; Catchen et al. 2017).

3.1.3 Goals and expectations

The main objective of this chapter is to combine outlier differentiation (F_{ST}) and genome-environment association approaches to explore the presence and distribution of putative signals of adaptation and assess potential drivers of adaptive variation in three co-distributed intertidal non-model species across the natural environmental gradient of the South African coastline (as previously described: Granular limpet *Scutellastra granularis*, Cape urchin *Parechinus angulosus*; shore crab *Cyclograpsus punctatus*). Based on findings in Chapter II, selectively neutral loci show low genomic variation and overall limited genomic evidence for underlying population structure in the three species. However, other studies on marine species have reported low neutral genomic variation with simultaneously distinct differences between populations putatively adaptive loci (Guo et al. 2015; Guo, Li and Merilä, 2016; Gleason and Burton, 2016; Sandoval-Castillo et al. 2018; Nielsen et al. 2018; Phair et al. 2019; Teske et al. 2019). On that account, footprints of selection possibly related to localised processes such as the influence of environmental factors were expected, despite the study species relatively low neutral genomic background structure (see Chapter II). This study builds on a previous genomic investigation, which demonstrated putatively adaptive signals for two species on the comparatively environmentally homogeneous South African west coast (*S. granularis*, *P. angulosus*; Nielsen et al. 2018). Hence, additional candidate loci

possibly related to adaptive processes can be expected with an extension of the study area to the south and southern east coast of South Africa (spanning the thermal environmental gradient; Smit et al. 2013; Teske et al. 2019). Sea water temperature has been recognised as a dominant factor shaping marine population structure (Perry et al. 2005; Hiddink, Burrows and García-Molinos, 2015; Riginos et al. 2016; Free et al. 2019) and evidence for molecular signs of thermal adaptation to different coastal areas in the South African marine environment have been reported (Teske et al. 2008; 2019). Therefore, genome-environmental association can be expected to possibly recover SST (sea surface temperature) to be associated with the largest number of putatively adaptive loci. Alternatively, high-shore specialists *C. punctatus* and *S. granularis*, which are exposed to air for extended periods during low tide phases, might perhaps indicate more air- than sea temperature-associated outlier loci. As the salinity differences across the sampled populations are less pronounced, they might play a lesser role in proportion to potential temperature-associated outliers. Moreover, it is possible that two or all three environmental variables influence the same putatively adaptive loci in the species genomes, which may provide some evidence towards how the environment might driving selection.

A South African west coast study on *S. granularis* and *P. angulosus* functionally associated some of the detected genomic outliers via blasting against a reference database (Nielsen et al. 2018). Observing identical outliers might be expected, which however could be influenced by different sampling scales (two vs. six west coast populations sampled per study) and utilised analytical parameters. Moreover, more outlier loci from *S. granularis* and *P. angulosus* can be expected to be attributed due to the existing annotations in the genomes of the owl limpet *Lottia gigantea* and the purple sea urchin *Strongylocentrotus purpuratus*. Less closely related genomic resources are available for *C. punctatus*, which could translate into functionally associating only a small number of outlier loci compared to the other two study species.

Apart from environmental differences and the possible presence of IBE (isolation-by-environment), the geographical distance between the species populations of around 1,800 km might contribute to the occurrence of IBD (isolation-by-distance). The species differ in their biological characteristics such as pelagic larval duration (PLD) and the ability of adult individuals for dispersal (migration between populations). The estimated PLD ranges from >50 days in *P. angulosus* to ~14 days in *S. granularis* and *C. punctatus*, which perhaps makes a genomic sign of IBD in the latter two more likely (but see Weersing and Toonen, 2009; Selkoe and Toonen, 2011). Further, there is evidence from comparative multi-species phylogeographic studies for clines or shared genetic breaks (Barber et al. 2000; Bernardi,

Findley and Rocha-Olivares, 2003; Toonen et al. 2011; Stanley et al. 2018), but given the complex environmental heterogeneity across the South African coastline and the different life history traits of the study species (such as taxonomic differences and reproductive parameters), it cannot be predicted with confidence whether a shared environmentally-associated cline might exist across species. Nevertheless, there is the possibility that the species cold west coast populations (Port Nolloth/Jacob's Bay, Sea Point) and the warm east coast populations (Cape St. Francis, Haga Haga) demonstrate spatially distinct adaptive signals. Nielsen et al. found Port Nolloth to show evolutionary distinct localised adaptive signals in *P. angulosus* (and nearby Hondeklip Bay appears to harbour distinct signals in *S. granularis*), which might be similarly the case in this study (Nielsen et al. 2018). Further, it is possible that the two east coast populations (CSF, HH) with the comparatively higher sea water and air temperatures might reflect these selection pressures with a potentially increased number of localised adaptive signals. Overall, a combination of outliers shared across and outliers unique to specific populations were expected. Potential intraspecific geographic variation of adaptive signals and the identification of interesting candidate loci may provide a starting point to explore future resilience towards changing climatic conditions.

In summary, the overarching goal is to explore the three study species intraspecific putatively adaptive signals across six coastal populations in southern Africa with a range of outlier detection methods to investigate possible footprints of selection in RAD-Seq-derived genomic markers. The potential role of environmental heterogeneity across South Africa's coastal thermal gradient is explored by testing SST, salinity and air temperature as selection factors in the species genomes and investigate the potential occurrence of isolation-by-distance (IBD) and isolation-by-environment (IBE). The following questions were addressed: 1) whether a functional role of outlier loci can be assigned and to which extent this is possible in each species depending on molecular resources, 2) whether loci found in Nielsen et al. 2018 from two study species are detected again, 3) whether spatially distinct outliers occur in populations, 4) whether SST appears as the dominant environmental factor over salinity and air temperature, 5) whether the two high shore species are impacted more by air temperature than by sea water temperature and 6) whether there are specific outliers showing signs of selection by multiple environmental factors.

3.2 Methods and Materials

3.2.1 Data collection

The outlier detection has been conducted with RAD-Seq pools from Chapter II sampled from six different rocky shore populations of three species *S. granularis*, *C. punctatus* and *P. angulosus* at the South African coastline (see Figure 2.1). The shore crab *C. punctatus* is not found at the most western site Port Nolloth, thereby it was collected from Jacob's Bay instead. The pooled sample from Cape Agulhas is not available for *P. angulosus*.

3.2.2 Statistical analysis

Three methods were utilised to detect potential outlier loci: the empirical F_{ST} method (1), BayeScan (2) and BayeScEnv (3).

3.2.2.1 Empirical F_{ST} method

The empirical F_{ST} approach has been performed with the species RAD-Seq SNP datasets as described by Akey (Akey et al. 2010). Scripts used in this chapter were adapted from Baocheng Guo (Guo et al. 2015; Guo, Li and Merilä, 2016), with the SNP parameters remaining consistent as reported in Chapter II (minimum allele count 4, minimum coverage 10 and maximum coverage 200). To detect signals of selection, F_{ST} values were first estimated for each SNP and pairwise population comparison (6 populations: *C. punctatus*, *S. granularis*; 5 populations: *P. angulosus*) in Popoolation2 (Kofler, Pandey and Schlötterer, 2011). The SNPs contained in the 99.5 percentile of the empirical distribution of the pairwise F_{ST} estimates were treated as possible outlier loci (Akey et al. 2010; Guo et al. 2015; Guo, Li and Merilä, 2016).

3.2.2.2 BayeScan

BayeScan version 2.1 (Foll and Gaggiotti, 2008; Foll et al. 2010; Fischer et al. 2011) utilises a Bayesian framework to assess SNPs on the basis of GenePop-formatted files, which store high-throughput sequencing reads and associated details (<http://genepop.curtin.edu.au>). While GenePop formatting software is available as standalone application (Rousset, 2008), it has been adapted into Popoolation2 (Kofler, Pandey and Schlötterer, 2011) (sync2GenePop.pl). GenePop files were processed via Popoolation2 on the University of Stellenbosch Central Analytical Facilities HPC2 (<http://www.sun.ac.za/hpc>). The obtained GenePop files, which contain individual level genotype data with loci which have an allele

variant count of >2, were converted with PGDSpider version 2.1.1.5 (Lischer and Excoffier, 2012) into GESTE/BayScan format. The BayScan-compatible GenePop files were constructed with minimum allele count 4, minimum coverage 10 and maximum coverage 200, which yields different numbers of total loci (*S. granularis*: 10 462, *P. angulosus*: 4638, *C. punctatus*: 46 527). The chain parameters in BayScan were set to performing 5000 reversible jumps with a thinning interval of 10, after 20 pilot runs with 5000 runs each and a burn-in length of 50 000. The prior odds for the neutral model were set to 10. The q-value threshold was set to 5%, meaning that 5% of the outliers which have a q-value lower than 5% may be expected to report false positives.

3.2.2.3 Environmental and geographic variables

To test the potential influence of environmental variables, the annual mean sea surface temperature (SST in °C) and salinity (in ppt) measured in 1° grid cells, were obtained from the National Oceanic and Atmospheric Administration (NOAA) World Ocean Atlas 2013 version V2 (Locarnini et al. 2013; Zweng et al. 2013; Garcia et al. 2010; <https://www.nodc.noaa.gov/OC5/woa13/woa13data.html>; The latest World Ocean Atlas (2019) was published after the analyses had been finalised.) Annual mean air temperature (in °C) at the sites was obtained from WorldClim at the smallest grid cell size reporting data for all sites (res10) (Fick and Hijmans, 2017). Environmental variables were extracted with QGIS (v2.8.9; QGIS Development Team (2016); WGS84; EPSG: 32734) according to the GPS coordinates of the sampling locations (Table S3.1-3, Appendix). The variables were standardised by subtracting the mean and dividing by the standard deviation across all populations, as is required by BayScEnv (Villemereuil and Gaggiotti, 2015; see section 3.2.2.4), and the values were used in their original form for the RDA analysis, as required by the application (Legendre and Legendre, 2012; see section 3.2.2.6). Geographic along-shore distances (in km) between sampling locations were calculated in QGIS (v2.8.9) utilising the shapefile “Africa.shp” provided by SANBI (South African National Biodiversity Institute).

3.2.2.4 BayScEnv

In BayScEnv (Villemereuil and Gaggiotti, 2015), 5000 reversible-jump MCMC chains with a thinning interval of 10 were utilised, after 20 pilot runs with each 2000 iterations and a burn-in length of 50 000. The MCMC chains were evaluated for convergence in the R package CODA version 0.19.1 (Plummer et al. 2006) with the Heidelberger and Welch's Convergence Diagnostic test (Heidelberger and Welch, 1981; 1983; Schruben, 1982). All chains

converged except for the chain testing the variable 'sea surface temperature' in *S. granularis*. In the latter case, the MCMC was calibrated to perform 5000 reversible-jump chains with a thinning interval of 20, after performing 25 pilot runs with each 3000 iterations and a burn-in length of 50 000. Loci with a *qvalue_g* below 0.05 were identified as potential outlier loci.

3.2.2.5 Annotation with BlastX

To determine if outlier loci are shared between populations or private to certain populations, customised Unix bash commands were utilised. This step seeks to confirm whether potential adaptive signals are shared or unique among the species populations. Further, a general blast search was conducted to assess the potential functional role (or general possible genomic association) of the outlier loci in NCBI's BlastX, the protein sequence reference database by the National Center for Biotechnology Information (<https://bit.ly/2KgiuMz>; Altschul et al. 1997), with the nucleotide sequence of the contigs associated with the identified locus and 1,000 bp on either side of the contig, as the signal of selection might possibly point to a region adjacent to the suggested outlier.

3.2.2.6 IBD and IBE

The subject of the IBD/IBE testing are the minor allele frequencies of the SNPs, which describe the rate at which the minor vs. the major allele occurs, which indicates genomic variability and serves as response variable in the RDA models. The models evaluate geographic distances between populations and selected environmental variables (SST, air temperature, salinity) as possible predictors for the species minor allele frequencies. Three environmental variables (SST, air temperature, salinity) and geographic distances between the respective populations were tested across the study species. *Parechinus angulosus* was not tested for air temperature variation, since the individuals are not exposed to air during low tide if they can avoid it. The redundancy analysis (RDA) (Legendre and Legendre, 2012) was conducted in R (version 3.5.1) with the *rda* function in the *vegan* package (Oksanen et al. 2018). The *pcnm* function was used to transform geographic distances to a PCNM matrix (Principal Coordinates of Neighbourhood Matrix). Environmental variables were supplied to the model as shown in Table S3.2 (Appendix). The best fitting model across the environmental variables was selected with the *ordistep* function. The first RDA involved testing the species minor allele frequency against the geographic distance matrix of their respective sampling sites. The second RDA tested which environmental variables or combinations of environmental variables could predict the minor allele frequencies. The third

and fourth (partial) RDAs assessed whether the retained environmental variation or the geographic distance matrix could be partitioned from the total variation of the different models. The significance of the RDA outputs was tested with the ANOVA function (999 permutations).

3.2.2.7 BAPS and fastSTRUCTURE

Outlier loci were tested with BAPS v.5.4 (Bayesian Analysis of Population Structure) for potential population clustering with the following settings: minimum size of population 40, number of iterations 50, number of reference individuals from each population 40, number of reference individuals iterations 10 (*P. angulosus* K=1-5; *S. granularis* K=1-6; *C. punctatus* K=1-6) (Corander and Marttinen, 2006; Corander et al. 2006). Moreover, fastSTRUCTURE v1.0 was used with K=1-5 for *P. angulosus* and K=1-6 for *S. granularis* and *C. punctatus* (Raj, Stephens and Pritchard, 2014) seed parameter 100 and the prior logistic model.

3.3 Results

3.3.1 Empirical F_{ST} method and BayeScan

The empirical F_{ST} method detects loci, which fall into the 99.5 percentile of the empirical distribution of the pairwise F_{ST} estimates (Table 3.1). The percentage of detected outliers per species compared to the overall loci analysed ranges from 2.86%-3.43% (empirical F_{ST} method) to 0.03%-0.34% (BayeScan). The spatial distribution of the empirical outliers is shown in table S3.4 (Appendix). Outliers detected with BayeScan did not show spatial variation, i.e. are found in all populations.

Table 3.1. Detected outliers with empirical F_{ST} method and BayeScan. The total number of loci differs due to the different methodological approaches to identify outlier loci.

	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>
empirical F_{ST} method			
Outlier loci	493	165	121
Total loci	14392	5440	4235
% outliers/total	3.43%	3.03%	2.86%
BayeScan			
Outlier loci	15	36	7
Total loci	46527	10462	4638
% outliers/total	0.03%	0.34%	0.15%

3.3.2 BayeScEnv

BayeScEnv tested differentiation of environmental variables including sea surface temperature (SST), air temperature and salinity against the allele frequencies of the study species populations to suggest outlier loci which are putatively influenced by the respective environmental variable (Table 3.2). The total number of outliers estimated across species ranges from 225 (*C. punctatus*) to 45 (*S. granularis*) and 4 (*P. angulosus*) (after deducting outliers suggested by multiple factors). The BayeScEnv outliers are found in all populations and therefore do not show spatial variation across sampling sites.

Table 3.2. Number of outliers detected by BayeScEnv, which are putatively influenced by differentiation in sea surface temperature, air temperature and salinity across the study populations.

Environmental variable	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>
Sea surface temperature (SST)	56	13	0
Air temperature	80	4	-
Salinity	91	32	4
Shared between variables			
SST : salinity	2	4	-
SST : air temperature	-	-	-
Salinity : air temperature	-	-	-

3.3.3 Summary of detected outliers

To summarise the findings from the outlier detection methods, a numerical summary is presented in Table 3.3, which is followed by species-specific Venn diagrams (Figure 3.1 to 3.3) illustrating outlier loci detected across two or all outlier detection methods.

Table 3.3. Summary of outliers detected by the selected methods in comparison.

Summary	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>
F_{ST} Outliers	493	165	121
BayeScan	15	36	7
BayeScEnv	56 (sea surface temperature) 80 (air temperature) 91 (salinity)	13 (sea surface temperature) 4 (air temperature) 32 (salinity)	0 (sea surface temperature) - 4 (salinity)

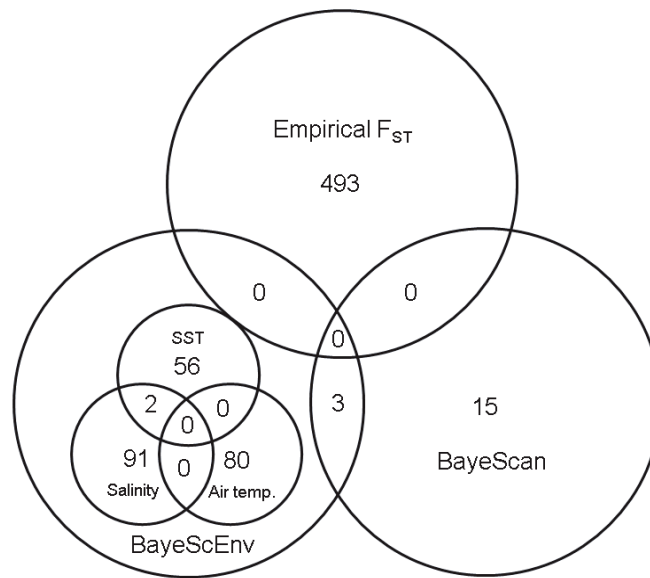


Figure 3.1. Outlier loci detected for *C. punctatus* in BayeScan, BayeScEnv and the empirical F_{ST} method.

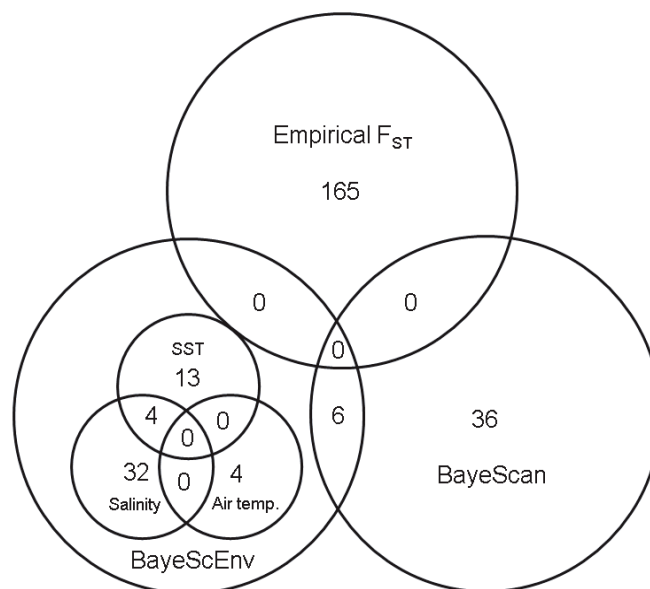


Figure 3.2. Outlier loci detected for *S. granularis* in BayeScan, BayeScEnv and the empirical F_{ST} method.

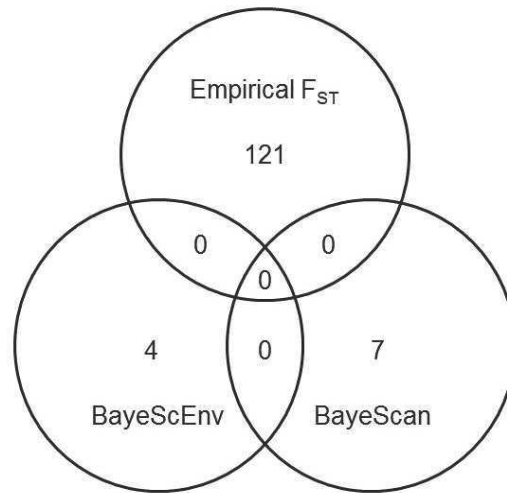


Figure 3.3. Outlier loci detected for *P. angulosus* in BayeScan, BayeScEnv (salinity) and the empirical F_{ST} method.

3.3.4 Outlier F_{ST} estimates

Outliers detected by BayeScan, BayeScEnv and the empirical F_{ST} method were combined to obtain outlier F_{ST} estimates (Table 3.4) for the study species in PoPoolation (Kofler et al. 2011a,b). Selected SNP parameters (minimum allele count 4, minimum coverage 10 and maximum coverage 200) were applied to all calculations. The estimated F_{ST} values range from 0.108-0.185 in *C. punctatus* and 0.108-0.287 in *S. granularis* to 0.109-0.310 in *P. angulosus*. Overall, estimates indicate increasing differentiation between the most peripheral populations. Fisher's exact test suggests that the pairwise comparisons between populations are significant across all species populations. A comparison between estimates obtained from outlier loci alongside selectively neutral loci (previously discussed in Chapter II) is included in the Appendix (Table S3.5).

Table 3.4. F_{ST} estimated in PoPoolation2 (scenario 6) incorporating 494 (A), 165 (B) and 121 (C) outliers. Asterisk(*) indicates significance according to Fisher's exact test. Location abbreviations are listed in Figure 2.1.

<i>C. punctatus</i> (A)	JC	SP	CA	KY	CF	HH
JC	-					
SP	0.116*	-				
CA	0.113*	0.108*	-			
KY	0.135*	0.112*	0.110*	-		
CF	0.153*	0.128*	0.119*	0.114*	-	
HH	0.185*	0.160*	0.153*	0.128*	0.111*	-
<i>S. granularis</i> (B)	PN	SP	CA	KY	CF	HH
PN	-					
SP	0.131*	-				
CA	0.242*	0.195*	-			
KY	0.242*	0.190*	0.113*	-		
CF	0.258*	0.195*	0.109*	0.111*	-	
HH	0.289*	0.239*	0.138*	0.151*	0.133*	-
<i>P. angulosus</i> (C)	PN	SP	KY	CF	HH	
PN	-					
SP	0.213*	-				
KY	0.261*	0.125*	-			
CF	0.310*	0.162*	0.121*	-		
HH	0.286*	0.141*	0.109*	0.143*	-	

3.3.5 Outlier annotation (BlastX)

The total number of outlier contigs obtained in this chapter varies between species and approach from 4 to 375 (Table 3.5). Proteins or protein domains suggested by the BlastX database are listed per species in Table 3.6 (empirical outliers) and Table 3.7 (BayeScan and BayeScEnv outliers). Further, numerous outlier contigs point to uncharacterised or hypothetical proteins (see Table 3.5), highlighting the still unknown dimensions of non-model invertebrate genomes (for the full list see Appendix, Table S3.6-7).

Table 3.5. Number of outlier contigs, BlastX database results per E-value cut off, hypothetical and putatively identified BlastX findings listed per species. Percentage in brackets relates to the total number of outlier contigs.

[Empirical]	Outlier contigs	E-value >1.0E-5	Hypothetical	Identified	E-value <1.0E-5
<i>C. punctatus</i>	375	111 (30%)	68 (18%)	43 (11%)	264 (70%)
<i>S. granularis</i>	116	29 (25%)	21 (18%)	8 (7%)	87 (75%)
<i>P. angulosus</i>	100	35 (35%)	13 (13%)	22 (22%)	65 (65%)
[BayeScan]					
<i>C. punctatus</i>	12	4 (33%)	2 (17%)	2 (17%)	8 (67%)
<i>S. granularis</i>	19	5 (26%)	3 (16%)	2 (11%)	14 (74%)
<i>P. angulosus</i>	7	5 (71%)	1 (14%)	4 (57%)	2 (29%)
[BayeScEnv]					
	<i>C. punctatus</i>				
SST	51	15 (29%)	8 (16%)	7 (14%)	36 (71%)
Salinity	91	22 (24%)	13 (14%)	9 (10%)	69 (76%)
Air	68	18 (26%)	10 (15%)	8 (12%)	50 (74%)
[BayeScEnv]					
	<i>S. granularis</i>				
SST	11	3 (27%)	3 (27%)	-	8 (73%)
Salinity	25	4 (16%)	3 (12%)	1 (4%)	21 (84%)
Air	4	3 (75%)	3 (75%)	-	1 (25%)
[BayeScEnv]					
	<i>P. angulosus</i>				
Salinity	4	1 (25%)	-	1 (25%)	-

Table 3.6. Suggested protein domains of empirical outliers listed per species with contig query length, query cover, respective E-value and the percentage of the contig identical with the putative protein.

Query length	Protein domain name	Query cover	E-values	%identical
<i>C. punctatus</i>				
1306	Retrovirus-related Pol polyprotein from type-1 retrotransposable element R2	26%	5E-23	50.43%
1291	ATP-binding cassette sub-family F member 2	38%	2E-30	66.67%
1160	wsv191-like protein	34%	2E-28	47.41%
1009	ATP-binding cassette sub-family F member 2	49%	4E-64	66.47%
1077	wsv220-like protein	99%	9E-36	27.93%
1140	RTBS	23%	1E-16	61.90%
1069	Craniofacial development protein 2-like	31%	2E-28	46.96%
1138	Putative nuclease HARBI1	69%	3E-97	57.09%
1289	PiggyBac transposable element-derived protein 4-like	21%	2E-11	43.96%
1148	Transposon Ty3-G Gag-Pol polyprotein	99%	2E-127	47.52%

1105	LIM domain kinase 1-like isoform X2	27%	1E-17	74.58%
1048	Tectonin beta-propeller repeat-containing protein	12%	4E-09	69.05%
1742	E3 ubiquitin-protein ligase MYCBP2-like	25%	9E-17	63.24%
1226	Prefoldin subunit 3	9%	3E-12	97.30%
1309	Proline/betaine transporter	14%	5E-06	45.45%
1265	Myc box-dependent-interacting protein 1-like	35%	2E-20	34.53%
1302	RNA-directed DNA polymerase	39%	2E-34	50.49%
1147	Protein flightless-1	17%	3E-34	96.92%
1465	Methyltransferase-like protein 7A	21%	2E-18	67.24%
1414	dtw domain-containing protein 2	36%	7E-16	32.60%
1270	Glutenin, high molecular weight subunit DY10-like	94%	2E-22	29.21%
1189	Nck-associated protein 5	99%	4E-164	75.89%
1303	Growth hormone secretagogue receptor type 1	18%	1E-25	68.35%
1323	Reticulophagy regulator 3-like	12%	3E-06	53.03%
1382	Repressor of the inhibitor of the protein kinase-like (52 kDa)	38%	3E-15	38.98%
1435	THAP domain-containing protein 6-like	12%	3E-12	56.67%
1253	N-acetylglucosamine-6-phosphate deacetylase	11%	1E-17	95.74%
1159	RNA-directed DNA polymerase	36%	8E-15	50.98%
1094	Dynein intermediate chain 1, axonemal-like	12%	3E-08	62.22%
1104	RTXE	75%	6E-30	37.50%
1084	Putative protein in type-1 retrotransposable element R1DM	67%	1E-111	70.49%
1075	Putative disco-interacting protein 2 isoform X2	20%	6E-27	100.00%
1005	RNA-directed DNA polymerase from mobile element jockey-like	97%	2E-60	38.21%
932	RNA-directed DNA polymerase from mobile element jockey-like	96%	4E-68	41.59%
866	Adaptin ear-binding coat-associated protein 1	83%	1E-31	36.56%
1112	Formyl-CoA transferase	57%	7E-118	86.38%
933	NADH dehydrogenase subunit 6	46%	6E-30	50.34%
859	Putative sidestep protein, partial	17%	5E-06	53.06%
826	KRAB-A domain-containing protein 2, partial	70%	3E-59	68.87%
358	Protein SpAN-like	56%	8E-24	65.67%
449	NADH dehydrogenase subunit 4	99%	7E-51	83.89%
215	Putative RNA-directed DNA polymerase from transposon BS	99%	1E-29	73.24%
181	Ribosome-binding protein 1-like	100%	8E-06	52.73%
<i>S. granularis</i>				
9127	Cytochrome b	11%	2E-158	76.76%

1263	Putative nuclease HARBI1	30%	6E-32	49.61%
1241	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit	22%	1E-10	70.45%
843	Beta-1,4-N-acetylgalactosaminyltransferase bre-4-like	24%	3E-30	81.16%
676	Sodium-independent anion transporter	99%	5E-70	48.89%
833	Phospholipase A2 AP-PLA2-I	17%	7E-07	79.59%
572	Cytochrome c oxidase subunit I	99%	4E-94	82.63%
587	Putative RNA-directed DNA polymerase from transposon BS	97%	7E-61	48.19%
<i>P. angulosus</i>				
1052	Bromodomain adjacent to zinc finger domain protein 2B isoform X7	25%	3E-19	87.93%
1283	Myophilin-like	12%	1E-23	89.09%
1410	Protein LCHN	14%	4E-14	71.01%
1283	Gastrula zinc finger protein XICGF8.2DB-like	53%	8E-60	47.52%
1401	Roundabout homolog 1 isoform X2	47%	5E-57	86.10%
1255	Band 4.1-like protein 4A isoform X2	7%	4E-05	90.00%
1340	GPI ethanolamine phosphate transferase 2(*)	36%	1E-31	72.63%
1488	Putative growth factor receptor-bound protein 14	21%	4E-20	62.50%
1312	Nuclear pore complex protein Nup214	19%	2E-40	87.21%
1501	Vesicle-associated membrane protein 7	23%	3E-31	86.96%
1406	Fibrillin-1	14%	6E-09	78.57%
1442	Serine/arginine repetitive matrix protein 2 isoform X4	13%	1E-21	85.94%
1183	Protein disulfide-isomerase TMX3	11%	2E-18	91.30%
1183	Beta-galactosidase-1-like protein 2	7%	6E-09	93.55%
1275	Toll-like receptor 3	84%	4E-70	41.16%
1124	Hydroxylysine kinase isoform X2	18%	1E-21	72.86%
1292	Tubulin polyglutamylase TTLL5	14%	1E-07	100.00%
1077	Serine/threonine-protein kinase H1	28%	2E-31	94.23%
1013	Echinoderm microtubule-associated protein (77 kDa)	15%	3E-24	96.23%
1125	Krev interaction trapped protein 1 isoform X2	13%	7E-05	92.31%
617	Endonuclease-reverse transcriptase	98%	2E-98	69.31%
426	ABC transporter ATP-binding protein	94%	9E-42	54.48%

(*) potentially similar protein domain identified in Nielsen et al. 2018 for *P. angulosus*.

Table 3.7. Suggested protein domains of BayeScan and BayeScEnv outliers listed per species with contig query length, query cover, respective E-value and the percentage of the contig identical with the putative protein. Protein domains suggested across BayeScan and BayeScEnv or between multiple environmental parameters appear in grey.

Query length	Protein domain name	Query cover	E-values	%identical
<i>C. punctatus</i>				
(BayeScan)				
1113	Lysosomal-trafficking regulator	13%	7E-22	92.16%
1021	putative RNA-directed DNA polymerase from transposon X-element	99%	4E-127	56.93%
(BayeScEnv, sea surface temperature (SST))				
975	R2DM Retrovirus-related Pol polyprotein from type II retrotransposable element	76%	6E-37	34.13%
977	E3 ubiquitin-protein ligase SHPRH	27%	5E-40	82.95%
1035	putative RNA-directed DNA polymerase from transposon BS	56%	2E-83	65.13%
1091	Guanine nucleotide-releasing factor 2-like isoform X6	32%	1E-09	44.53%
1109	RTXE	43%	3E-19	40.91%
964	Kinesin-like protein KIF20B isoform X1	35%	7E-25	86.57%
512	Solute carrier family 22 member 3	28%	3E-18	77.55%
(BayeScEnv, salinity)				
1035	R2DM Retrovirus-related Pol polyprotein from type II retrotransposable element	71%	1E-36	34.13%
1201	RTJK, partial	74%	9E-57	54.63%
1096	RNA-directed DNA polymerase from mobile element jockey-like	56%	3E-21	31.78%
1105	WD repeat-containing protein 35	11%	2E-05	81.40%
1104	MAM and LDL-receptor class A domain-containing protein 1-like, partial	15%	4E-05	42.11%
1152	RNA-directed DNA polymerase from mobile element jockey-like	79%	5E-63	39.34%
1119	Protein pangolin, isoforms A/H/I/S-like	13%	2E-18	98.04%
1043	Craniofacial development protein 2-like	59%	2E-67	57.89%
834	ATP synthase F0 subunit 6	80%	3E-106	87.05%
(BayeScEnv, air temperature)				
975	R2DM Retrovirus-related Pol polyprotein from type II retrotransposable element	76%	6E-37	34.13%
1075	Retrovirus-related Pol polyprotein from transposon 17.6	23%	2E-14	48.98%
998	Zinc finger protein 2	19%	9E-08	54.55%
1095	putative RNA-directed DNA polymerase from transposon	53%	6E-83	65.13%

BS				
904	Kinesin-like protein KIF20B isoform X1	35%	5E-21	86.67%
878	TPA: Endonuclease-Reverse Transcriptase	82%	7E-51	43.33%
272	Solute carrier family 22 member 3	60%	2E-19	66.13%
417	SDR family oxidoreductase	99%	1E-92	96.38%
<i>S. granularis</i>				
(BayeScan)				
903	Beta-1,4-N-acetylgalactosaminyltransferase bre-4-like	22%	4E-30	81.16%
454	UDP-N-acetylmuramate dehydrogenase	97%	2E-47	55.26%
(BayeScEnv, salinity)				
471	Protein ZBED8-like	99%	2E-79	86.57%
<i>P. angulosus</i>				
(BayeScan)				
5400	Baculoviral IAP repeat-containing protein 6 isoform X6	13%	5E-18	92.50%
1105	Monocarboxylate transporter 9	21%	1E-07	56.96%
1328	Iron-sulfur protein NUBPL isoform X2	10%	6E-15	91.11%
1252	Inositol 1,4,5-trisphosphate receptor isoform X8	27%	7E-26	98.72%
(BayScEnv, salinity)				
10126	Baculoviral IAP repeat-containing protein 6 isoform X6	10%	1E-42	96.59%

3.3.6 IBD and IBE testing

The first RDA (redundancy analysis), testing genomic variation against the geographic distance matrix, did not indicate significance in any study species. The second RDA, relating environmental variables and combinations thereof, tested significant for the following two species: The optimal model for *P. angulosus* suggests that salinity variation may account for 48% of genomic variation ($\text{Pr}(> F) = 0.025$). The optimal model for *S. granularis* suggests that the joint effect of SST and air temperature variation may account for 45% of genomic variation ($\text{Pr}(> F) = 0.0236$). Models of *S. granularis* containing only SST or only air temperature were not significant. The partial RDAs, partitioning the effect of geographic distance against the retained most informative environmental variables and vice versa, were not significant (*S. granularis*, *P. angulosus*). In *S. granularis*, the reduction of the model for the partial RDAs to one factor (SST vs. air temperature partitioned against geographical distance) indicated no significance. While differentiation in the tested environmental variables might contribute to genomic variation in two of the study species, this could not be confirmed for the possible influence of geographic distance across species populations.

3.3.7 BAPS and fastSTRUCTURE

FastSTRUCTURE does not suggest structure across the study species putatively adaptive loci, whereas BAPS estimates four potential divergent clusters in two of the species (*C. punctatus*, *P. angulosus*) (Table 3.8). Each possible grouping is automatically assigned a colour, with each clustered individual represented by a vertical bar with the colour pointing to the associated cluster (Figure 3.4).

Table 3.8. Number of estimated population clusters based on putatively adaptive loci listed by application and species compared to population clusters estimated in Chapter II with selectively neutral loci.

	<i>C. punctatus</i>	<i>S. granularis</i>	<i>P. angulosus</i>	
BAPS	4	1	4	Outlier loci
fastSTRUCTURE	1	1	1	Outlier loci
BAPS	2	2	1	Neutral loci
fastSTRUCTURE	1	3	1	Neutral loci

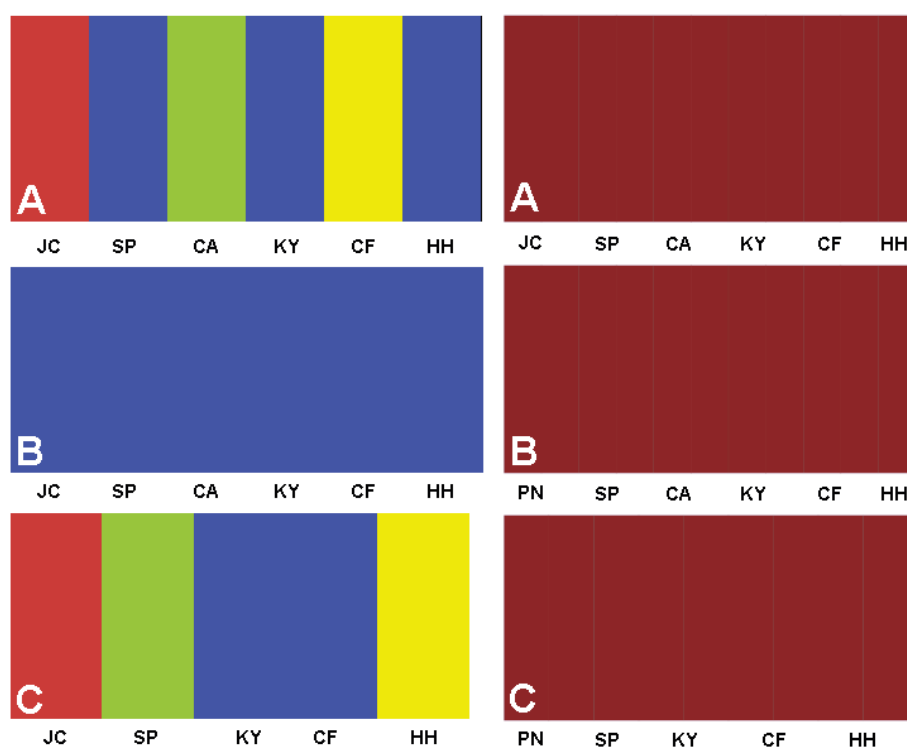


Figure 3.4. Coloured partition of clusters suggested by BAPS (left) and fastSTRUCTURE (right) for *C. punctatus* (A), *S. granularis* (B) and *P. angulosus* (C). Location abbreviations are listed in Figure 2.1.

3.4 Discussion

Across three South African rocky shore species, a total of 1102 candidate outlier loci potentially implicated with adaptive processes were identified. The findings are utilised to address the questions, 1) whether adaptive signals are present despite the low neutral genomic background structure, 2) whether SST or air temperature (the latter for the high shore species) play a dominant role for environmentally-associated loci over salinity, 3) if outliers impacted by multiple environmental factors can be identified, 4) whether previously detected outlier loci in two study species are recovered, 5) if spatially distinct loci occur, 6) if functional associations can be obtained to similar extent across the species, 7) if signs for isolation-by-distance or -environment can be detected and 8) if there is evidence for distinct evolutionary uniqueness in populations.

3.4.1 Detection of outlier loci

Recovering different numbers of outliers per species is expected based on differentiation between the methodological detection frameworks and interspecific genomic differences. For instance, the empirical F_{ST} approach yielded a higher number of potential outliers than utilising BayeScan (Table 3.1). Remarkably, the latter method was conducted based on a comparatively larger quantity of SNPs, highlighting the inherent methodological differences between the two approaches (see section 3.1.2.1). Detecting only small numbers of BayeScan outliers or none at all compared to other outlier methods has been reported across a wide range of species, which has been attributed to the application conservatively dismissing smaller footprints of selection (Buckley, Butlin and Bridle, 2012; Huang et al. 2012; Dillon et al. 2014; Zhan et al. 2015; Miller et al. 2016; Al-Breiki et al. 2018). Comparing detection levels between species reveals that both the empirical F_{ST} approach and BayeScan find the lowest number of potential outliers in the Cape urchin *P. angulosus*, whereas the highest number of outliers are found in the shore crab *C. punctatus* (empirical F_{ST}) and the granular limpet *S. granularis* (BayeScan) (Table 3.1). Further differences in outlier detection relate to the spatial occurrence. Identified BayeScan-outliers are detected across all populations of the respective species, whereas the pairwise comparison of populations with the empirical F_{ST} approach indicates differences between some sampling locations. The estimated number of potentially spatially distinct outliers appears slightly higher between the most western population (Port Nolloth in *S. granularis* and Jacob's Bay in *C. punctatus*) and the nearest sampled adjacent population Sea Point (Table S3.4, Appendix). The remaining pairwise population estimates indicate varying degrees of putatively unique outliers in no apparent geographical pattern. As the shore crab *C.*

punctatus demonstrates the highest total number of empirical F_{ST} -outliers, it is expected to also find the highest number of potential outliers shared across all pairwise population estimates in this species (Table S3.4, Appendix).

The outliers potentially associated with environmental parameters are discussed further in section 3.4.2. and 3.4.4, but it is important to note that the total number of outliers detected by BayeScEnv differs strongly between species (Table 3.2-3). Congruent to the observed BayeScan and empirical F_{ST} -outliers, the lowest number of BayeScEnv outliers is found in the urchin *P. angulosus* (Table 3.2). This is notable as there is evidence for highly polymorphic individuals in sea urchins and a high degree of polymorphism in urchin genomes (Balhoff and Wray, 2005; Cameron et al. 2005; Cameron et al. 2009; Sodergren et al. 2006). The interpretation of what constitutes few or many outliers is biased by the other two study species relatively higher outlier numbers and it is important to acknowledge that it is presently not understood how many outliers can be regarded low or high. The number of identified outliers reported across urchin RAD-Seq studies varies from 12 (Nielsen et al. 2018), 17 (Paterno et al. 2017) to 304 (Addison and Kim, 2018), which is also influenced by the size of the geographical area and other study design parameters. In general, the disparity between species outlier detection numbers highlights a challenge specific to multi-species studies, where for example a wider SNP window might have yielded a higher number of outliers in *P. angulosus*, but in turn perhaps diluted findings in the other two species. This quandary warrants further consideration and possible avenues of resolution may emerge with growing numbers of multi-species studies.

Contrary to the outlier detection numbers in the urchin *P. angulosus*, the crab *C. punctatus* demonstrates the highest number of outliers across BayeScEnv and the empirical F_{ST} -approach. Overall, only nine outliers are jointly identified by two detection approaches and none by all three methods (Figure 3.1-3). In fact, only BayeScan and BayeScEnv jointly identified 3 outlier loci in *C. punctatus* and 6 in *S. granularis*. Hence, the advocated cautionary approach to minimise false positive outlier identification by only treating loci suggested by two or more methods as robust “true” outliers (Pérez-Figueroa et al. 2010; Narum and Hess, 2011; Nunes et al. 2011; Zhao et al. 2013; De Mita et al. 2013; Rellstab et al. 2015) may prove too limiting. These findings expand the evidence towards recovering few or no outliers across multiple approaches (de Villemereuil et al. 2014; Lotterhos and Whitlock, 2015). Some past studies might have focused only on outliers suggested by multiple applications, but all outliers across the three methods and species in this study are considered as putative adaptive signals and assessed for functional association (see section 3.4.4).

In general, the findings in this chapter suggest the possibility that despite a largely similar neutral genomic background, outlier loci may signal the presence of adaptive processes in the species genomes. Present day technology has facilitated the advance into non-model species genomes, but there are caveats which need to be briefly acknowledged before the results are discussed in more detail. RAD-Seq is a pioneering technique for genomic exploration at a large scale (in terms of numbers of samples), with the drawback that RAD-Seq loci represent a fraction of all loci possibly under divergent selection (Lowry et al. 2017). Further, detected loci and their functional association are strictly candidate loci and remain promising but hypothetical until conclusively evidenced by future directed research. The expertise regarding adaptive marker identification is growing rapidly, but of course, as described in earlier sections, there may be other processes which might promote patterns of genomic divergence. These include demographic effects, background selection, selective sweeps, pre-/post-zygotic isolation, stochastic effects, variation in the mutation and recombination rate, differential introgression and hitchhiking genes (Bierne et al. 2011; Roesti et al. 2012; De Mita et al. 2013; Bierne, Roze and Welch, 2013; Gosset and Bierne, 2013; Lotterhos and Whitlock, 2014; Tine et al. 2014; Lotterhos and Whitlock, 2015; Fraïsse et al. 2016; Gagnaire and Gaggiotti, 2016; Matthey-Doret and Whitlock, 2019). In this study, measures such as stringent quality filtering and conservative SNP parameters (see section 3.2.2.1) contribute to the quality of detected putatively adaptive candidate outliers.

3.4.2 Outliers with potential environmental association

The number of outliers possibly impacted by environmental factors differs notably between species (as discussed in 3.4.1; for functional associations see section 3.4.4). Across all environmental factors, water temperature such as SST is known to strongly shape populations in the marine environment (Perry et al. 2005; Pörtner and Farrell, 2008; Riginos et al. 2016; Free et al. 2019). Hence, the majority of environmentally-associated outliers were expected to be linked to SST variation. Furthermore, salinity differentiation is in comparison less pronounced than SST and air temperature throughout the sampling region, which might result in small numbers or the absence of salinity-associated outliers. Contrary to these speculations, most outliers were linked to salinity over air temperature (where applicable) and SST variation across the study species (Table 3.2). It is important to interpret this preliminary observation cautiously and treat the outliers as candidate loci under the consideration of methodological caveats which have been discussed for environmental association methods (see section 3.1.2.2.1). Nevertheless, there is evidence from marine species populations for which salinity parameters ranked as the most influential

environmental factor for adaptive molecular signals over SST and other variables (Van Wyngaarden et al. 2018; Carreras et al. 2019; Yang et al. 2020; also see Montes et al. 2016; Xuereb et al. 2018). Strictly excluding Baltic Sea species affected by the steep regional salinity gradient, this study contributes to these instances where salinity differentiation appears to play a dominant role. For instance, the overall only four BayeScEnv outliers detected in the Cape urchin *P. angulosus* appear to be environmentally associated with salinity variation. Sea urchins have poor abilities to regulate osmosis and ion concentrations, leaving them sensitive to salinity conditions (Binyon, 1972; Stickle and Diehl, 1987; Russell, 2013). Although different populations may have varying capabilities of acclimation and tolerance, past studies have reported that salinity levels can constitute a notable selective pressure for Echinodermata (Kaack and Pomory, 2011; Delorme and Sewell, 2014; Agüera et al. 2015; Xuereb et al. 2018; Carreras et al. 2019). Importantly, salinity conditions in rocky shore micro niches may fluctuate depending on shore height, tidal phase and proximity to freshwater input from rivers, which can create a mosaic of salinity levels over small spatial scales (Morris and Taylor, 1983; Drouin, Himmelman and Béland, 1985; Bible and Sanford, 2016). As has been shown in Hawaiian limpets (Bird, 2011; Bird et al. 2011), localised fine scale salinity measurements with spatial, tidal and seasonal considerations are paramount to further elucidate the role of salinity as potential selective force on intertidal species molecular composition.

Concerning air temperature, the two high-shore specialists (*C. punctatus*, *S. granularis*) could hypothetically demonstrate air temperature differentiation as a factor driving genomic divergence due to their long exposure during low tide. The putative environmental association with air temperature differentiation appears more potent in *C. punctatus* (80 loci) over *S. granularis* (4), which is contrary to their ability to avoid temperature stress (e.g. through hiding under rocks). Nevertheless, interspecific genomic differences and different numbers of tested loci per species may not support a direct side-by-side comparison and the impact of putatively adaptive loci is not defined by their absolute detection numbers. Although genome-environment association investigations with air temperature are still a rarity for the marine environment, studies testing air temperature variables for adaptive significance in the mussel *Mytilus galloprovincialis* (Han and Dong, 2020) and the Atlantic salmon *Salmo salar* (Bourret et al. 2013; Sylvester et al. 2018) found that this factor appears to notably impact spatial genomic structure in the species populations. The intertidal environment is thermally heterogeneous at diel, tidal, seasonal and microhabitat level (Helmuth et al. 2006a,b; Sinclair, Thompson and Seebacher, 2006) and therefore the use of one air temperature or environmental variable per site lacks consideration of the fine-scale patterns of thermal (or other) conditions experienced by local organisms (Lathlean, Seuront

and Ng, 2017). Further investigation which could employ detailed temperature measurements at various scales will greatly expand the knowledge of species local adaptation capacity in the region. With climatic conditions changing globally, air temperature might overall grow in importance as a driver for ecological divergence in relevant marine populations next to the widely established influence of sea water temperature variables such as SST.

Sea surface temperature variation appears to account for 51 outliers in *C. punctatus* and 11 outliers in *S. granularis* with putative environmental -association (Table 3.2; none in the third species). Water temperature differences are regarded as a strong driver for molecular divergence and even early stages of speciation (Van Wyngaarden et al. 2018; Jeffery et al. 2018; Zhao et al. 2018; Stanley et al. 2018; Teske et al. 2019), although in this study with less dominance than expected relative to other tested factors. The shore crab depends on moist crevices during low tide and lives submerged in sea water during high tide phases, therefore it is not unexpected that a number of loci with suspected influence from sea water temperature are observed. The candidate loci for selection through SST variation can help to forge a path to better understanding of how environmental conditions shape species genomic composition. As with fluctuating patterns of air temperature and salinity levels in the intertidal, establishing the latitudinal spectrum of SST variation experienced by the species microhabitats represents a challenge towards obtaining more detailed observations.

Environmental conditions, their possible interactions and impacts on species are far from simplistic in any marine habitat, but the intertidal with its drastic daily extremes constitutes a particularly complex habitat. One of the initial expectations was that potential outliers might be influenced by multiple tested environmental factors. In fact, both SST and salinity differentiation are suggested to influence four loci in *S. granularis* and two in *C. punctatus*, which could not be observed for the third study species or any other combination of the tested variables (Table 3.2). There is evidence for putatively adaptive markers jointly influenced by water temperature and salinity in other marine species (Jeffery et al. 2018; Zhang et al. 2019a; Carreras et al. 2019; Bernatchez et al. 2019). In this study only SST, salinity and air temperature were considered, while it is plausible that other combinations than water temperature/salinity such as for instance water/air temperature (Micheletti et al. 2018; Han and Dong, 2020) or alternative factors including wave action, pollution and desiccation (Sanford and Kelly, 2011; Puritz and Toonen, 2011; Tisthammer et al. 2020) might drive genomic divergence at the species outlier loci. Other variables were not investigated as the focus of this study is on the environmental factors likely to shape broad-scale population patterns of the three species.

Testing multiple species with the same environmental variables might reveal which drivers are most dominant or which joint factors may account for molecular divergence. Nevertheless, the findings demonstrate that species showed contrasting numbers of putative environmentally associated outliers. Apart from inherent molecular differences between phyla, it is possible that the various intertidal microhabitats with their dynamic conditions impact the genome in a way which conceals broad-scale patterns of environmental influence identified in multiple species (see e.g. Stanley et al. 2018). It is important to acknowledge that the environmental data in this study has a coarse resolution, which might impact detected putatively adaptive loci. Further comparative multi-species testing of more comprehensive (higher resolution) environmental data sets will benefit the understanding of selective processes affecting co-distributed species.

3.4.3 Spatial differentiation of outlier loci and outlier F_{ST} estimates

The spatial distribution of outlier loci might indicate the presence of spatially distinct loci in populations, which could be a sign of the species potential local adaptation (Coop et al. 2010; Bragg et al. 2015; Flanagan et al. 2018). It was hypothesised to recover a combination of shared outliers and unique population-specific outliers. Due to the varying environmental conditions across South African coastal regions, there might be an increased number of unique outlier loci as putative signals of local adaptation in the most western (cold-temperate) populations (Port Nolloth/Jacob's Bay, Sea Point) and the most eastern (subtropical) populations (Cape St. Francis, Haga Haga) relative to the populations located on the south coast between them. The two most eastern populations (CSF, HH) experience the highest sea water and air temperature, possibly acting as strong selection pressures. Hence, these two populations might reveal the overall highest numbers of outlier loci relative to the remaining populations. Contrary to these expectations, the outliers detected with BayeScan and BayeScEnv are detected in all populations, not demonstrating geographic patterns (see section 3.4.3). These shared BayeScan and BayeScEnv outliers might represent large scale genomic dynamics and environmental processes affecting all populations. For instance, a small number of loci is detected by both methods in *C. punctatus* and *S. granularis* (Figure 3.1-2), which could indicate that there might be an overarching process or combination of factors leading to high differentiation of these loci across all populations (not observed in *P. angulosus*, Figure 3.3). The BayeScan/BayeScEnv loci constitute candidates for further investigation, as a range of other factors such as background selection, differential introgression, stochastic effects and variation in mutation and recombination rate may contribute to detected molecular signals

(see section 3.1.2.1, 3.1.2.2.1). In general, BayeScan has been found to conservatively dismiss low selection signals (Narum and Hess, 2011; and similarly BayeScEnv, Villemereuil and Gaggiotti, 2015), thereby identifying fewer outliers and there are cases where the detection number was so low that population structure in terms of spatial occurrence of these outliers could not be assessed (for instance Gonçalves da Silva, Appleyard and Upston, 2015; Stockwell et al. 2016; Maroso et al. 2016; Galaska et al. 2017; Al-Breiki et al. 2018). As opposed to BayeScan and BayeScEnv, the empirical F_{ST} method (discussed further below) is not designed to identify spatially unique outliers in populations due to the nature of pairwise estimations. There is also the fact that a large number of studies are not reporting or specifically investigating location-specific outliers and instead use for instance STRUCTURE and BAPS (see section 3.4.5) to determine potential structure of neutral and adaptive SNPs. Finally, it is important to acknowledge that RAD-Seq-derived SNPs and outlier loci represent a small portion of the overall species genome and it is possible for location-specific outliers to be identified in future research.

The empirical F_{ST} outlier detection method estimates a range of 22 to 73 outliers as shared across the populations of the respective species (Table S3.4, Appendix). The remaining species outliers are suggested as uniquely occurring among specific population pairs (as per the F_{ST} method, assessing pairs of populations, see e.g. Kess, Galindo and Boulding, 2018; therein Table 3), ranging in numbers from up to 10 (*P. angulosus*) to 18 (*S. granularis*) and 30 (*C. punctatus*) (Table S3.4, Appendix). Nevertheless, distinct spatial patterns such as regionally higher or lower F_{ST} outliers are not evident. By definition, outlier loci constitute significant deviations from neutral expectations in genomic data, hence high differentiation between them as evidenced in the outlier F_{ST} estimates between the loci is expected (Table 3.4). The significant outlier F_{ST} estimates are highest in *P. angulosus* (0.310) and *S. granularis* (0.287), which is not too distant from the peak value in *C. punctatus* (0.185). The decisive factor appears to be the distance between populations, as the outlier F_{ST} estimate is highest, across all species, in the respective most geographically distant populations. Other studies on marine invertebrates have interpreted outlier F_{ST} estimates of 0.40-0.48 (Plough, 2017) and 0.44 (Lal et al. 2017) as high, which brings *P. angulosus* and *S. granularis* into the vicinity of what may be considered as the upper end of the outlier F_{ST} range. Nevertheless, outlier F_{ST} estimates for marine invertebrates can reach 0.64-0.74 (Kess, Galindo and Boulding, 2018), which would rank the obtained values in this study in the “medium” outlier F_{ST} range (Sandoval-Castillo et al. 2018). Which estimates constitute high and low and in which context and phyla might only be elucidated in more detail with a growing body of published marine invertebrate F_{ST} outlier estimates.

Findings in Chapter II showed that every population possesses a certain quantity of selectively neutral location-specific SNPs (see section 2.3.2; Figure 3.5), indicating that all sampled locations harbour unique genomic signatures. Interestingly, the Cape urchin *P. angulosus* demonstrated the highest number of neutral location-specific SNPs across its populations, peaking at almost 8% of total SNPs in Port Nolloth at the west coast. Despite higher rates of neutral private SNPs relative to the other two species, *P. angulosus* revealed the lowest numbers of putatively adaptive outliers (Table 3.3). As evidenced by the findings in this chapter, location-specific neutral components are not necessarily an indication for the presence or quantity of unique outlier loci or unique environmentally-associated outliers. Instead, BayeScan and BayeScEnv uncovered loci occurring across all populations, which may be caused by broad-scale selection processes or other presently unknown factors. Although speculative, another hypothesis might be that a set of SST-associated environmental outliers is, while present in all populations, subjected to different directions of selection pressure (Siepielski et al. 2013). To stay with the factor SST, warming sea water conditions on the southern east coast may drive divergence at physiologically relevant loci which are conversely under selection by cooling sea water conditions on the west coast. It remains unknown whether the Bayes-outliers represent selection processes where the driving factor (e.g. SST, air temperature) might differ in its directional impact in populations.

3.4.4 Functional association of outliers

Cumulatively, 1102 outlier loci were detected and blasted in the BlastX database across the three methods and study species, providing pointers to their potential functional association. The outlier loci primarily matched to protein-coding sequences in the BlastX database identified in the horse crab *Portunus trituberculatus*, the owl limpet *Lottia gigantea* and the purple sea urchin *Strongylocentrotus purpuratus*. Typically, few or no genomic data or reference resources exist for non-model species (Ellegren, 2014; Delord et al. 2018; Weigand and Leese, 2018). This is evidenced by the number of outliers which bear no resemblance to documented protein sequences or whose E-value did not meet the $>10e-5$ threshold. Further, matched sequences with relevant E-values are in some instances still hypothetical or uncharacterised proteins (Table 3.5). It is necessary to acknowledge these outlier loci with presently unknown functional association, as they harbour presently inaccessible insights. Overall, 69 (*C. punctatus*), 11 (*S. granularis*) and 27 outlier contigs (*P. angulosus*) could be matched to non-hypothetical protein sequences, leaving an association gap of 90% relative to the overall 1102 loci (Table 3.6-7). Genomes of non-model species are still widely uncharted territory, which demands to document and retain unidentified candidate loci for future investigative use and to focus on the functionally associated outliers.

The degree of successful functional association was expected to be relatively high in echinoderm *P. angulosus* and mollusc *S. granularis* due to genomic resources on functional roles derived from the model organisms *Strongylocentrotus purpuratus* and *Lottia gigantea* (Greenspan, 2005; Hamdoun et al. 2018). Interestingly, slightly higher numbers of functional associations were obtained for the shore crab *C. punctatus*, which is to some extent facilitated by profound research interest in decapod genomic resources and particularly genes and protein sequences related to immune system dynamics by the global aquaculture sector (Lai and Aboobaker, 2017; discussed in section 3.4.4.1). Across the study species, it is demonstrated that genomic resources from a relatively closely related organism are only one of several factors determining the functional association rate of outliers.

Shore crab (C. punctatus)

Across the three species, the associated functional roles of the detected outliers fall into a variety of categories and functions such as developmental proteins, membrane transport, vesicle signalling, protein folding/modification, enzymes and cytoskeleton functional components (Table 3.6-7). This spectrum is particularly evident in the shore crab *C. punctatus*, whose 69 putatively associated functions include established elements of historically viral or bacterial origin commonly found in numerous species genomes (retrovirus-related Pol polyprotein, RTXE, wsv191-like protein, further discussed in 3.4.4.1), essential components of the electron transport chain (NADH dehydrogenase subunits, ATP synthase, see section 3.4.4.2) and diverse other protein domains and families such as solute carriers and ubiquitin-protein ligases. In this study, the solute carrier family 22 member 3 is suggested to be putatively under selection by both sea surface temperature and air temperature differentiation in *C. punctatus*. In functional terms, this solute carrier facilitates transmembrane transport of organic cations (Verri et al. 2012). Although speculative for *C. punctatus*, the solute carrier family 22 member 3 emerged in another marine invertebrate as likely molecular actor during heat shock response (Li and Xu, 2018). The potential adaptive significance of this type of solute carrier is presently not understood, but may become clearer with expanding knowledge of molecular adaptive mechanisms in invertebrates. Another type of protein appearing under selection are two E3 ubiquitin-protein ligases (BayeScEnv/SST: E3 ubiquitin-protein ligase SHPRH; emp. F_{ST} : E3 ubiquitin-protein ligase MYCBP2-like). E3 ligases are tasked with attaching ubiquitin to proteins, which regulates signal transduction, protein degradation and serves in DNA repair and cell cycle control (Hershko and Ciechanover, 1998; Ardley and Robinson, 2005; Teixeira and Reed, 2013). Further, the ubiquitin system has been associated with organism development, immune response, apoptosis and environmental stress response (Hershko and Ciechanover, 1998; Ciechanover, Orian and Schwartz, 2000; Pespeni et al. 2012). The involvement in these

essential processes may make identified E3 ligases plausible candidates for selection. The E3 ubiquitin-protein ligase SHPRH assists with DNA repair to counter genotoxic stress (Motegi et al. 2006). In marine crustacean *Penaeus monodon*, multiple E3 ligases including SHPRH were detected as part of the immune response to disease (WSSV, also see section 3.4.4.1; Robinson et al. 2014). The E3 ubiquitin-protein ligase MYCBP2 ortholog has been linked to key neural development such as axon guidance and synaptogenesis in organisms (James, Key and Beverdam, 2014). This study appears as the first instance where MYCBP2 was detected in relation to a marine invertebrate, but it has been associated with physiological stress in a marine fish (Mu et al. 2015). In general, several E3 ligases were found to be among adaptive signals in other marine invertebrates (Pespeni et al. 2012; Gleason, 2015; Wang et al. 2016; Silliman, 2019a,b; Vera et al. 2019; Leiva et al. 2019), which underpins their potential as putative signs of selection in *C. punctatus*.

Granular limpet (S. granularis)

From the eleven functionally associated outliers in the limpet *S. granularis*, the empirical F_{ST} -outliers represent a range of protein functions, which interestingly includes two outliers pointing to the mitochondrial electron transport chain (Cytochrome b; Esposti et al. 1993; Cytochrome c oxidase subunit I; Tsukihara et al. 1996; Table 3.6; further discussed in section 3.4.4.2). Two outliers identified with BayeScan and BayeScEnv (salinity) respectively (Table 3.7) include a protein from the oxidoreductase family (UDP-N-acetylmuramate dehydrogenase; Schomburg and Stephan, 1995) and a putative ancient transposon (ZBED8-like protein; Hayward et al. 2013). Further, both BayeScan and the empirical F_{ST} -approach detected an ortholog of Beta-1,4-N-acetylgalactosaminyltransferase as outlier loci (loci to contig reference; Table 3.6-7). This protein transfers galactose onto proteins and lipids as part of protein glycosylation (Kawar, Van Die and Cummings, 2002; Griffiths et al. 2003). Invertebrate glycobiology is not understood in sufficient detail to speculate about a functional adaptive role (Zhu, Li and Chen, 2019). However, Beta-1,4-N-acetylgalactosaminyl-transferase appears to play a role in the immune response in disease-resistant abalone *Haliotis iris* (Neave et al. 2019) and has been detected in molluscs under ocean acidification stress (Koh et al. 2015; Timmins-Schiffman et al. 2019). Identified across two outlier detection methods, the Beta-1,4-N-acetylgalactosaminyltransferase might represent a possible candidate loci to further elucidate molecular actors of adaptive signals in marine molluscs.

Cape urchin (P. angulosus)

The empirical F_{ST} -outliers in the urchin *P. angulosus* span a range of molecular functions from vesicle transport to cytoskeletal structural elements (Table 3.6; ABC transporter ATP-

binding protein is discussed further in 3.4.4.2). It was expected to observe previously identified outliers in *P. angulosus*. Among the functionally associated outliers, there appears to be only one instance where a potential outlier (GPI ethanolamine phosphate transferase; Table 3.6) may perhaps belong to a similar protein family (here: structural domain associated with protein family) which was previously detected (Endonuclease/Exonuclease/Phosphatase family; Nielsen et al. 2018; therein Supplementary Table S2). There were no similarities for previously detected *S. granularis* outliers (Nielsen et al. 2018) and *C. punctatus* (no previous outlier data). Importantly, it is possible that unidentified or uncharacterised proteins in this study (Table S3.6-7, Appendix) may demonstrate similarities to previously detected outliers, which can only be confirmed with expanding genomic resources of the species.

Functional associations detected for the Bayesian-based outliers of *P. angulosus* are related to membrane transport (Monocarboxylate transporter 9; Nakayama et al. 2013), assembly of the mitochondrial respiratory complex (Iron-sulfur protein NUBPL isoform X2; Sheftel et al. 2009) and a receptor mediating cytosolic calcium (Inositol 1,4,5-trisphosphate receptor isoform X8; Picard, Coquil and Mauger, 1998; Table 3.7). The loci to contig reference process revealed an identical outlier contig jointly identified across BayeScan and BayeScEnv (salinity), the Baculoviral IAP (inhibitor of apoptosis) repeat-containing protein 6 isoform X6 (BIRC6) (Table 3.7). The BIR protein domain is involved in regulating apoptosis and cell division by controlling caspases (Bartke et al. 2004; Pohl and Jentsch, 2008). BIR serves as one of three possible subdomains of NLR proteins (nucleotide-binding domain and leucine-rich repeat containing gene family; Ting et al. 2008; Wilmanski, Petnicki-Ocwieja and Kobayashi, 2008), which are tasked with detecting cellular stress patterns. Beyond evidence in vertebrates, NLR proteins were first confirmed in sea urchins as part of the invertebrate immune system in terms of pathogen recognition (Rast et al. 2006; Davis, Wen and Ting, 2011). It is presently not understood in detail how BIRC6 may influence both immune response and apoptosis in echinoderms, but there are apparently profound evolutionary and functional links across regulatory networks overseeing apoptosis and immune response (pathogen defence) (Robertson et al. 2006). This study constitutes the first instance in which a Baculoviral IAP repeat protein is detected as a putatively adaptive loci in sea urchins (but see Todgham and Hofmann, 2009). Nevertheless, there are studies on other marine invertebrates who found Baculoviral IAPs to be implicated with immune response and physiological stressors, which may carry significance for adaptive processes (Lesser and MacManes, 2016; Détrée et al. 2017; Feis et al. 2018). The fact that both BayeScan and BayeScEnv point to BIRC6 as putatively under selection possibly makes it an interesting candidate loci for further evaluation.

3.4.4.1 Established genomic elements of initially viral or bacterial origin

Overall, suggested proteins and protein domains appear to span a wide array of molecular functions and dynamics in the study species (Table 3.6-7). Nevertheless, a number of them are particularly interesting for various reasons. For instance, one particular marker in *C. punctatus* appears as putatively under selection by all BayeScEnv-tested environmental parameters. This retrovirus-related Pol polyprotein (Table 3.7) is thought to have aspartic-type endopeptidase activity (Fujita et al. 2017). Generally, large numbers of endogenous viral elements (EVE; Holmes, 2011) and specifically retroviral sequences (endogenous retroviruses (ERV); Cotton, 2001) are found in all species genomes, where their functional roles are varied and extend beyond potential host-virus interactions (Katzourakis and Gifford, 2010; Blomberg, Ushameckis and Jern, 2013). Frequently, ERVs can be dominating parts of the genome and as such increase genomic diversity (Blomberg, Ushameckis and Jern, 2013). From the different types of polyproteins (Pol, Env, Gag; Coffin, Hughes and Varmus, 1997), specifically the env protein is known for rapid gene sequence divergence possibly due to high selective pressure related to regulating the host immune response (Malik, Henikoff and Eickbush, 2000; Cotton, 2001). The polyprotein genes are located in close proximity, suggesting that high divergence in one of them may likely contribute to the Pol polyprotein being identified as outlier. Importantly, genomic signals for endogenous viral elements do not equate to contamination, because genome sequences resembling or originating historically from viruses are widespread in marine arthropods (such as *C. punctatus*) and may serve functional roles, which remain to be further explored (Flegel, 2009; Hauton, 2017). Integrated viral genome elements are hypothesised to perhaps aid with the recognition and suppression of virus replication and thus increase host immunity (Flegel, 2009; Hauton, 2017). For instance, there is genomic evidence that a variety of reverse transcriptase (RT) sequences overlap between decapod crustaceans and the purple sea urchin *S. purpuratus*, which might indicate that these RT sequences occur in other marine invertebrates and could be specific to the marine realm (Harms et al. 2013). Further, a study across 7407 malacostracan genes related to immune response derived from 55 species demonstrated the dynamic divergent evolutionary adaptations of these immunity components (Lai and Aboobaker, 2017), which might generally contribute to sequences associated with pathogen recognition being identified as outliers.

Interestingly, the empirical F_{ST} outlier method identified two sequences regarded as signs of possible pathogenic presence in *C. punctatus* (wsv191-like protein, wsv220-like protein; Table 3.6). The wsv220-like protein is found in all six populations, whereas the wsv191-like

protein is limited to the south coast sites (Cape Agulhas, Knysna). Wsv proteins are associated with white spot syndrome virus (WSSV), which is a feared crustacean pathogen in the aquaculture industry (Cavalli et al. 2013). The virus has been detected in wild crustacean populations (Cavalli et al. 2013; Macías-Rodríguez et al. 2014) and in non-crustacean host invertebrates (Desrina et al. 2013). White spot syndrome has been described as “*most dangerous and devastating marine pathogen affecting crustaceans*” (Gong, Ju and Zhang, 2018; p. 2). Two sequences showing similarity to wsv proteins in the present study are not conclusive evidence for the occurrence of WSSV in local populations of *C. punctatus*, but future investigation is warranted. There is currently no recorded outbreak of white spot syndrome in South Africa, but the disease occurred in Mozambique in the last decade (Tang, Le Groumellec and Lightner, 2013; Oakey et al. 2019). Until proven otherwise, proteins with high similarity to wsv proteins may not be treated as indication for white spot syndrome. However, WSSV has been demonstrated to show regional genotypic differences between strains (Oakey et al. 2019) and the ecological dynamics of the pathogen are still not well understood (Sánchez-Paz et al. 2015). A possible alternative hypothesis might be that the sequences resembling wsv-like proteins in *C. punctatus* might be involved with recognising pathogens such as WSSV as part of a protective immune function (see Flegel, 2009; Hauton, 2017), which would make sense for the wsv220-like protein found present in all six populations.

Aside from endogenous viral elements, there is evidence for the presence of endogenous bacteria-associated proteins in *C. punctatus* (not in the other two species) in the form of RTX, a cytotoxic ATP-binding protein (Table 3.6, Linhartová et al. 2010). Virulence factors such as RTX (repeats in toxin) proteins originate from a wide variety of bacterial pathogen genera (Linhartová et al. 2010) and create pores to permeate the membrane of eukaryotic (preferably immune) target cells (Chenal, Sotomayor-Perez and Ladant, 2015). For instance, evidence from *Pseudovibrio* spp. suggests high bacterial metabolic flexibility and varied adaptations to a range of marine invertebrate hosts (Alex and Antunes, 2018; Versluis et al. 2018). To summarise, roughly a third or more of all putatively functionally associated outlier sequences in *C. punctatus* (Table 3.6-7) point to the occurrence or similarity of endogenous viral elements in the genome (and to a smaller extent endogenous elements of bacterial origin) across the three detection methods. In comparison, this applies to only three functionally associated markers in *S. granularis* and *P. angulosus*, which may be influenced by an overall larger quantity of *C. punctatus* outliers. In general, instances of outlier sequences identified as polyproteins, transposable elements (retrotransposons, transposons) or other elements of endogenous viral nature have similarly been reported from population studies of marine invertebrates in the phyla Mollusca and Arthropoda

(Crustacea), but to our knowledge not yet in Echinodermata (Galindo, Grahame and Butlin, 2010; Plough, 2017; Metivier, Kim and Addison, 2017; Rhode, Bester-van der Merwe and Roodt-Wilding, 2017; Silliman, 2019a,b; Cheng et al. 2020). It is plausible that transposable elements are experiencing selection in sea urchins, as for instance the genome of the purple sea urchin *S. purpuratus* contains 8.36% transposons (Zhang et al. 2017a). Evidence for *P. angulosus* might only open up with expanding the level of functional associations of its detected outlier loci. Overall, it is important to emphasise that these genomic elements may not be directly targeted by selection, but linked to markers under selection (Metivier, Kim and Addison, 2017). To conclude this section, there is evidence that transposable elements, due to their capacity to drive gene and genome evolution (Joly-Lopez et al. 2012; Hoen and Bureau, 2015) and to regulate gene expression levels (González, Macpherson and Petrov, 2009), have been suggested to play a significant role in local adaptation to environmental conditions (González et al. 2008; van't Hof et al. 2016; Rey et al. 2016). It is possible that the identified outlier loci discussed in this section are impacted by positive selection and might therefore play a role in the adaptive divergence of *C. punctatus* and *S. granularis*, who both demonstrated transposable elements among their functionally associated loci.

3.4.4.2 Outlier loci and (metabolic) adaptive divergence

Outlier loci in the shore crab *C. punctatus* point to NADH dehydrogenase subunits, ATP synthase and ATP-binding proteins (Table 3.6-7). Further outlier loci detected in the other study species include the ABC transporter ATP-binding protein (*P. angulosus*), cytochrome b and the cytochrome c oxidase subunit I (both *S. granularis*) (Table 3.6-7). Facing changing climatic conditions or other environmental stressors is energetically costly for species (Sørensen and Loeschcke, 2007; Sokolova et al. 2012) and prompts high intracellular demand for energy production (Somero, 2002; Harvey et al. 2014). The required additional energy to withstand environmental stress decreases the energy balance to the disadvantage of growth, reproduction and development (Sokolova et al. 2012). Adaptive shifts of metabolic functions are thought to be a key contributor to coping with stressful conditions (Somero, Lockwood and Tomanek, 2017). Evolutionary processes detected in metabolic gene networks have been suggested as an attempt to adjust tolerance limits to stress factors (De Wit, Dupont and Thor, 2016). In the marine environment, genes related to energy metabolism appear to be under selection in several species (Galindo, Grahame and Butlin, 2010; De Wit and Palumbi, 2013; Xu et al. 2017; Zhang et al. 2019a,b; Tisthammer et al. 2019; Lou, Gao and Han, 2019). Around 95% of energy capacity of eukaryotic cells stems from mitochondria (da Fonseca et al. 2008). Mitochondrial protein-coding genes are at the centre of energy metabolism and several mechanisms of biosynthesis (Green and Reed,

1998; Newmeyer and Ferguson-Miller, 2003; Mitchell, 2011). There is mounting evidence that the often regarded as neutral mitochondrial genome can be a target of positive selection (da Fonseca et al. 2008; Galtier et al. 2009; Czarnomska et al. 2019). Non-synonymous changes in SNPs contained in genes encoding components of the electron transport chain may influence essential subunit binding sites and overall electron flow (Beckstead et al. 2009). Such mutations can potentially impact the electron transport chain functionality and ultimately metabolic capacity, associated biosynthetic processes and species fitness traits (Ballard and Whitlock, 2004; Gershoni, Templeton and Mishmar, 2009). Importantly, a more flexible respiratory metabolism can serve as an adaptive advantage (Ballard and Rand, 2005; Dowling, Friberg and Lindell, 2008).

To provide a brief overview before going into detail, the oxidative phosphorylation via the electron transport chain involves five complexes: nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase, cytochrome bc1 complex, cytochrome c oxidase, and ATP synthase (Carroll et al. 2009; McKenzie, Lazarou and Ryan, 2009). The five complexes consist of numerous subunits, which are encoded in mitochondrial genes, but in part also in nuclear genes (Rand, Haney and Fry, 2004; Burton and Barreto, 2012). The highest energy capacity is achieved when these sets of genes interact smoothly, meaning that a dissonance in the mito-nuclear evolution can interfere with molecular population structure to create instances in which species mtDNA and nuclear DNA deviate from fully complementary roles (Burton and Barreto, 2012). The complexity of the respiratory subunit origins and the mito-nuclear interactions highlight the impact even small changes may have on traits associated with species physiological fitness (Brandt, 2006; Coyle et al. 2019). Evidence shows that positive selection on the mitochondrial genome can drive adaptive divergence (Ballard and Whitlock, 2004; Galtier et al. 2009; Jacobsen et al. 2016). Signs for adaptive evolution have been detected in genes linked to essential electron transport chain complexes such as NADH dehydrogenase (Xu et al. 2007; Yu et al. 2011; Zhang et al. 2017b; Sun et al. 2018), cytochrome b (da Fonseca et al. 2008), cytochrome c oxidase (Luo et al. 2008) and ATP synthase (Hassanin et al. 2009; Zhou et al. 2014; Zhang et al. 2017b; Sun et al. 2018).

NADH dehydrogenase

Three outliers in *C. punctatus* are associated with NAD⁺/NADH dynamics: NADH dehydrogenase subunit 4 and 6 and possibly the outlier identified belonging to the family of short-chain dehydrogenases/reductases (SDR), of which many of the latter are NAD- or NADP-dependent oxidoreductases (Table 3.6-7; Jörnvall et al. 1995). As the detailed functional association of the SDR outlier is not determined at present, the following section

focuses on the NADH outliers. NADH dehydrogenase serves as the first and largest enzyme complex in the respiratory electron transport chain and facilitates the electrochemical proton gradient required to generate ATP (Kaniuga, 1963; Brandt, 2006). Molecular changes in subunits or associated enzymes might impact the process of protons pumped to drive the gradient (da Fonseca et al. 2008). NADH subunit 4 (ND4) has been suggested to constitute one of the designated proton pumping components of the complex (Brandt, 2006), hence potential mutations may interfere with its efficiency. The detailed role of NADH subunit 6 (ND6) is less well understood, even though mutations in the corresponding gene (MT-ND6) have been related to certain disease patterns (Jun, Brown and Wallace, 1994; Ronchi et al. 2011). Generally, ND2 and ND4 have widely been used as markers for phylogeographic research and a number of studies on mitogenomes emphasise the importance of the NADH dehydrogenase genes for adaptive evolutionary processes (Xu et al. 2007; da Fonseca et al. 2008; Ning et al. 2010; Yu et al. 2011). There are a number of instances where NADH dehydrogenase-components have been identified as putative signals for adaptive divergence in marine invertebrates. These include molluscs (Galindo, Grahame and Butlin, 2010; Saunier et al. 2014; Zhong et al. 2016; Gleason and Burton, 2016; Rhode, Bester-van der Merwe and Roodt-Wilding, 2017; Silliman, 2019a,b), crustaceans (Nunez et al. 2018; Wang et al. 2017; Zhang et al. 2019b) and echinoderms (Hart and Foster, 2013; Mu, Liu and Zhang, 2018). While the detected NADH-related outliers in *C. punctatus* must still be regarded as candidate loci for selection, there is evidence from aforementioned studies that they might indeed to some extent constitute a signal for adaptive evolution associated with NADH dehydrogenase capacity.

Cytochrome b and c

Cytochrome b and c oxidase are positioned downstream of the NADH dehydrogenase complex in the electron transport chain (Carroll et al. 2009). Cytochrome b facilitates the electron transfer between ubiquinol and cytochrome c, which is linked to the translocation of protons against the gradient ultimately producing ATP (Saraste, 1999; Mitchell, 2011). Similar to NADH, cytochrome b and c markers have widely been used to assess phylogenetic population structure (Avice, 2000; Ballard and Whitlock, 2004; Karlsen et al. 2014). In the limpet *S. granularis*, cytochrome b and the cytochrome c oxidase subunit I are detected as putatively adaptive loci (Table 3.6-7). To draw from other marine invertebrates, several cytochrome c oxidase genes are upregulated when polychaete *Protolaeospira stalagmia* is exposed to thermal stress (Nieva, 2019). The higher expression of these genes is interpreted as a possible metabolic response to high energy demand such as heat shock protein (Hsps) production (Nieva, 2019). There are other lines of evidence from several marine species that divergent selection in the cytochrome b (Cytb) gene may be correlated

with the impact of sea temperature (Foote et al. 2011; Silva et al. 2014; Xu et al. 2017; Deng et al. 2019). Overall, temperature has been recognised as a major driver of selection on the mitogenome (Ballard and Whitlock, 2004; Coyle et al. 2019). Nevertheless, cytochrome b has also been identified as putatively adaptive in relation to other selection pressures in the aquatic environment apart from temperature (Bélanger-Deschênes et al. 2013) and the full range of possible influencing factors is not well understood at present. Hence, there is a certain possibility that the two cytochrome outliers detected in *S. granularis* with the empirical F_{ST} -method (Table 3.6-7) might be impacted by sea or air temperature, but this requires further exploration in the future, as testing the genomic data for association of these two factors with BayeScEnv did not point to similar markers (which could be functionally associated) in the species.

ATP synthase and ATP-binding

As the last enzyme complex, the ATP synthase utilises the transmembrane proton gradient generated by upstream respiratory chain components to generate adenosine triphosphate (ATP) from adenosine diphosphate (Saraste, 1999; Mitchell, 2011). Ubiquitous in all life forms, ATP is regarded as a universal molecular currency to transfer energy in cells and to drive processes such as chemical synthesis and muscle contraction (Knowles, 1980). Molecular variation in the ATP synthase (ATPase) are expected to influence the production of ATP and ultimately energy metabolism (Mishmar et al. 2003; Wallace, 2007). Past studies have associated changes in the ATP synthase dynamics with species capacity to adapt to different environmental conditions (Hassanin et al. 2009; Zhou et al. 2014; Slimen et al. 2017; Zhang et al. 2017b). In ectotherms, it has even been advocated that persisting environmental variability is determined by the capability to regulate ATP production (Seebacher et al. 2010). For instance, diversifying selection pressure might impact ATP-related pathways or networks of metabolic genes to redirect ATP and possibly enhance tolerance to environmental stressors (Evans et al. 2017). In the shore crab *C. punctatus*, the ATP synthase was identified to have possible adaptive association (Table 3.6). Studies on marine crustaceans have detected the ATP synthase in the context of environmental adaptation in the blue crab *Callinectes sapidus* and the alvinocaridid shrimp *Shinkaicaris leurokolos* (Yednock and Neigel, 2014; Sun et al. 2018).

In *C. punctatus* and *P. angulosus*, further outlier sequences point to ATP-binding proteins possibly under selection (Table 3.6). Although ATP-binding proteins are not directly part of the electron transport chain, they are included here in the context of ATP-related cellular energy dynamics. Generally, ATP-binding cassette proteins (ABC transporters) facilitate transmembrane transport of diverse substances in cells, where ATP enables the transport

process (Jones and George, 2004; Linton, 2007). Interestingly, ATP-binding proteins have been detected among the most frequent genomic signals driving metazoan's evolutionary response to thermal changes in their environment (Porcelli et al. 2015). Signs for selection related to ATP-binding have been found in intertidal crustaceans (Rock et al. 2009) and sea urchins (Kober and Pogson, 2017; Uthicke et al. 2019). For the sake of completeness regarding the limpet *S. granularis*, none of the functionally annotated loci point to ATP dynamics, but there is mounting evidence from other marine molluscs (ATP synthase: Rhode, 2013; De Wit and Palumbi, 2013; Saunier et al. 2014; Sandoval-Castillo et al. 2018; Pante et al. 2019; ATP-binding: Ravinet et al. 2016). It is biologically plausible that such loci might be contained in the detected putatively adaptive loci whose functional association could not be determined with the presently available genomic database resources.

In summary, adaptive divergence in a wide variety of species has been linked to genes coding for energy metabolism functions. Further, the electron transport chain and its enzyme complexes are at the centre of cellular energy production. The three study species show putatively adaptive genomic outliers related to key energy production components (NADH dehydrogenase, cytochrome b and c oxidase, ATP synthase), which have been associated in other marine invertebrates with divergent selection driven by evolutionary pressures such as environmental stressors. Testing SST, salinity and air temperature with BayeScEnv yielded a small number of putatively associated loci with functional association (see section 3.4.1-2). To reiterate, this study only considered these particular environmental variables and other factors such as ocean currents, wave exposure, upwelling, pH and small scale biotic interactions can also drive selection (Pespeni et al. 2012; Galindo and Grahame, 2014; Jeffery et al. 2018). Nevertheless, temperature acts as a major selective force on the genome and particularly mitochondrial DNA (Ballard and Whitlock, 2004). Therefore, it can be suggested that further relevant candidate loci related to sea and air temperature (and likely other factors) will become evident once a higher degree of functional genomic association is achieved, alternative environmental-association methods might have been consulted and higher resolution environmental data is established. Overall, scouring largely uncharacterised genomes of non-model species for loci associated with adaptive evolution poses a distinct challenge. It needs to be emphasised that the putatively adaptive loci detected with the described outlier methods in this study can be characterised as candidate loci and require empirical validation. The adaptive significance of identified target loci appearing under selection needs to be further empirically explored. Based on multiple previous studies discussed in this section, it might be promising to examine loci linked to genes associated with energy metabolism dynamics as target candidates for adaptive divergence. Because SNPs reflect contemporary genomic patterns (see section 2.1.5), it is

an unresolved question in this context how potential past historic adaptive processes driven by paleo-climatic changes in sea surface temperature, oceanic currents, air temperature and other environmental factors (see section 1.6.1) might have laid the ground for current adaptive signals.

3.4.5 Population structure estimates

Analyses based on the COI locus (and the nuclear egg jelly protein (SpEJ9) marker in *P. angulosus*) pointed to various signs of population structure across the study species South African range (see Table 2.3; Muller et al. 2012; Wright et al. 2015; Mmonwa et al. 2015), but findings based on selectively neutral loci in Chapter II (see section 2.3.4-5) suggest no significant division across sampled populations. There are instances in other species where outlier loci have indicated population divergence despite the lack of population structure in selectively neutral genomic composition (Guo et al. 2015; Funk et al. 2016; Gaither et al. 2018) and even in smaller regions with relatively homogeneous environmental conditions (Nielsen et al. 2018; and references therein). This phenomenon and differences in environmental conditions across the South African sampling range, the study species varying biological characteristics and microhabitat preferences could be expected to contribute to population divergence among detected outliers. In the South African oceanographic context, it might be plausible that the west coast populations (Port Nolloth/Jacob's Bay, Sea Point) and the east coast populations (Cape St. Francis, Haga Haga) constitute spatially distinct outlier groups. Nevertheless, in summary, the findings suggest either the absence of structure or possible divergent groups, but no distinct separation of populations into regional clusters supported by multiple approaches.

Absence of IBD, but some signals of IBE effects

Across study species, no signals for isolation-by-distance (IBD) were detected (see section 3.3.6). Testing for isolation-by-environment (IBE) indicates that a combination between geographic distance, SST and air temperature variation may possibly account for up to 45% of genomic divergence in the limpet *S. granularis*. This hints the potential role of synergistic effects between geographical and environmental distance on the species genomic differentiation in this system. The influence of multi-factorial IBE or joint IBE/IBD on species molecular patterns has been confirmed in other marine species (e. g. Nanninga et al. 2014; Whittaker and Rynearson, 2017; Phair et al. 2019). In the Cape urchin *P. angulosus*, RDA models suggest that salinity variation might influence up to 48% of molecular variation. Echinoderms are known to experience strong selection pressure by salinity conditions (e.g.

Agüera et al. 2015; Xuereb et al. 2018; Carreras et al. 2019), but it is important to remember that these are preliminary observations and future research with widely assessed *in situ* measurements is necessary to ascertain the role of environmental factors for the molecular composition of South African marine invertebrates. From a comparative multi-species perspective in this study, it could be noted that the possible presence of IBE effects on species does not seem to be influenced by the number of detected putatively environmentally-associated outliers. For instance, *C. punctatus* revealed the highest total number of this type of outlier (see Table 3.2), but as tested here, there is no IBE signal in the shore crab. In accordance with what has previously been discussed on environmental association approaches, it is of course possible that factors not tested here might influence molecular processes in the species.

Population structure: Different levels of resolution

FastSTRUCTURE determines the absence of structure across all species outliers, which is congruent tested with BAPS in the limpet *S. granularis*. Hence, the outlier markers detected in the granular limpet populations demonstrate a lack of population structure according to fastSTRUCTURE, BAPS and IBD, except for the possible combined influence of IBE/IBD described above. BAPS groups outliers of the shore crab and the Cape urchin into four units (Table 3.8), which appears at first glance as a contrasting finding (to fastSTRUCTURE). In this estimation, *P. angulosus* populations in Knysna and Cape St. Francis might form a group and BAPS further assumes one cluster in *C. punctatus* including Sea Point, Knysna and Haga Haga populations, which does have different degrees of geographical plausibility (see Figure 2.1). In general, accurate estimations of population structure are influenced by factors such as uneven sample sizes per population (Fenderson, Kovach and Llamas, 2020), which was controlled for by utilising pooled samples constructed from equal numbers of individuals per location (see section 2.2.1-2). Further influence on assessing population divergence is exerted by the number of molecular markers and their variability (Puechmaille, 2016), which notably varied among study species (see section 3.3.3). Adapted from its precursor STRUCTURE (Pritchard, Stephens and Donnelly, 2000), every component of a sample in fastSTRUCTURE models has equal prior probability to belong to any of K populations (Raj, Stephens and Pritchard, 2014), whereas BAPS (Corander and Marttinen, 2006; Corander et al. 2006) employs a clustering approach with an extent of spatial autocorrelation, where the likelihood of samples to cluster together decreases with growing geographical distance. It is possible that the difference between lack of population division (fastSTRUCTURE, IBD testing) and four units (BAPS) in *C. punctatus* and *P. angulosus* might to some extent be influenced by different modelling assumptions. Additionally, BAPS

appears to have a tendency to overestimate the number of clusters, because it may detect finer molecular variation and indicate low threshold groupings dismissed by other applications (Wilkinson et al. 2011; Pinho, Cardoso and Hey, 2019). Another important aspect is that the BayeScan and BayeScEnv outliers are present in all populations and merely a limited number of empirical F_{ST} outliers are estimated as exclusive to certain population pairs (see section 3.4.3, Table S3.4, Appendix). With numerous outliers shared across the species populations, fastSTRUCTURE may speculatively place higher importance on shared outlier sequences across populations (no structure) and BAPS might place more emphasis on the small number of outlier sequences which are not shared by all populations. Isolation-by-distance, which was tested for, and other demographic processes further discussed later are known to impact patterns of apparent population divergence (Lawson, van Dorp and Falush, 2018). Finally, low levels of molecular differentiation, as attested by fastSTRUCTURE and IBD testing across species, might decrease the capacity of clustering applications to detect relevant clusters (Latch et al. 2006; Wollstein and Lao, 2015).

In summary, there is strong evidence from IBD testing and fastSTRUCTURE pointing to the absence of population structure in all study species. On the level of individual species, there is no support for outlier-based population structure in the limpet *S. granularis*, except for the signal of a joint IBE/IBD effect possibly influencing genomic variation. In the shore crab *C. punctatus*, only BAPS suggests a division into four divergent groups, which might be influenced by the methodological factors discussed above, particularly by empirical F_{ST} outliers occurring in some, but not all populations. Lastly, the Cape urchin *P. angulosus* might experience genome-environment influence from salinity conditions (IBE) and was assessed into four possible groups by BAPS. It can be acknowledged that the latter findings may be influenced by the overall low numbers of outliers in the Cape urchin compared to the other two species. Overall, no explicit population division into regional clusters is supported by multiple approaches.

Neutral and outlier-based population structure estimates

Reviewing the species population structure estimates for selectively neutral markers (Chapter II) and putatively adaptive markers, it becomes evident that notably less structuring was identified than anticipated based on the large scale environmental gradient. Across neutral and outlier markers, as discussed in the previous section, BAPS appears to estimate generally more signals of population structure compared to fastSTRUCTURE (Table 3.8), which might stem from varying sensitivities and differences in underlying model assumptions

leading to different levels of resolution or overestimation of clusters due to low levels of molecular differentiation. Across species and types of SNP markers, fastSTRUCTURE estimates a single population, with the exception of three divergent groups of selectively neutral markers in the limpet *S. granularis*. As discussed in Chapter II, these divergent groups are present in all sampled locations and do not indicate spatially distinct clustering. Perhaps most notable is that both programs indicate the absence of structure in *S. granularis* outliers, but pick up on two (BAPS) or three (fastSTRUCTURE) possible divergent groups among the neutral markers (see Chapter II).

For the urchin *P. angulosus*, the only signal for population divergence is identified by BAPS among the outlier markers, which is contrary to fastSTRUCTURE estimating a single population. In comparison with *S. granularis*, the Cape urchin represents the opposite case, where selectively neutral markers are found to lack structure and signs of divergence are limited to the outlier loci. This is a valuable take-away from a multi-species study, cautioning that patterns of population structure, neutral or outlier-oriented, cannot be extrapolated easily across phyla boundaries or perhaps even species boundaries.

The shore crab *C. punctatus* constitutes the only species where possible signals for population divergence are not limited to either selectively neutral markers (as in *P. angulosus*) or potential outliers (as in *S. granularis*). The evidence is however not conclusive, as the two divergent groups of neutral loci and four groups of outlier loci (both estimated by BAPS) are opposed by fastSTRUCTURE detecting a single group each. With the tendency of BAPS to possibly overestimate clusters (Pinho, Cardoso and Hey, 2019), the number of suggested *C. punctatus* groups may be lower than estimated, as advocated by fastSTRUCTURE testing.

Lack of population structure: Possible causes of low population differentiation

In this study, the granular limpet *S. granularis* demonstrates no population structure in its outlier loci and the Cape urchin *P. angulosus* in its selectively neutral markers (less clear-cut in the shore crab *C. punctatus*, where fastSTRUCTURE assesses no structure across all marker types and BAPS suggests multiple groups). Several processes, which are not mutually-exclusive, can lead to the absence of significant population structure. For instance, lack of geographic structure may arise from migration of individuals between populations. Assuming migration as the underlying cause appears tempting, as the study species are broadcast spawners with their larvae dispersing during a pelagic phase (see section 1.7). Nevertheless, this may be too simplistic, as it has been established that reproductive

strategy and pelagic larval duration (PLD) are not reliable proxies for genetic and genomic population structure (Weersing and Toonen, 2009; Selkoe and Toonen, 2011; Faurby and Barber, 2012; Riginos et al. 2016). Instead of a steady exchange of migrants, it might be plausible that genetic exchange between populations is facilitated by a series of stochastic or rare dispersal episodes (Knowles, 2009; Hock and Mumby, 2015). In a neutral framework, as few as one to ten migrants per generation can decrease genomic heterogeneity and create a setting perceived as panmictic (Mills and Allendorf, 1996; Waples and Gaggiotti, 2006), but inferences about dispersal can not be made with non-neutral loci (Kimura, 1991; Ohta, 1992; Gillespie, 1994; Whitlock and McCauley, 1999). The impact of mesoscale oceanographic features and near shore current patterns on marine population connectivity in the highly dynamic southern African region (see section 1.5.2) are the focus of continued research (Porri, McQuaid and Radloff, 2006; Pineda et al. 2010; Porri et al. 2014; McQuaid et al. 2015; Weidberg et al. 2015). *In situ* measurements of near shore current dynamics are necessary to better understand which role they might play towards the lack of genomic structure in populations (White et al. 2010). Moreover, high abundance of species can contribute to apparent lack of molecular heterogeneity, because large population sizes may promote a lack of drift leading to genomic similarity (Whitlock and McCauley, 1999; Kelly and Palumbi, 2010; Riginos et al. 2016). All three species are fairly abundant in South African rocky shores, with local caveats (e.g. settling space competition for *S. granularis* from barnacles at Buffel's Bay (Knysna), personal observation). Further, changing environmental conditions driven by glacial cycles in South Africa were associated with population shifts (see section 1.6.1). These past population shifts might conceal genomic differentiation through high numbers of ancestral polymorphisms and shared standing genomic variation (Knowles, 2009; Riginos et al. 2016), which may be treated as a sign for non-equilibrium patterns of drift-migration (Slatkin, 1993). Thereby, genomic similarity might be a molecular signature of the past, where shared population history might mimic what may be interpreted as high rates of contemporary larval exchange (Hart and Marko, 2010; Marko and Hart, 2011). To some extent, species evolutionary history might be inferred from traditional marker studies, where the three study species demonstrated significant differentiation between their partial COI sequences across their geographic range (Muller et al. 2012; Wright et al. 2015; Mmonwa et al. 2015). Beyond these studies, the species suspected demographic history in southern Africa is not well known, which limits interpretations towards the impact of past events. The causes of apparent lack of population structure suggested across certain marker types of the study species are likely multi-faceted and further investigation is necessary to determine their definite causes and drivers.

Outlier loci and signals for divergence

Lack of population structure might be associated with the absence of diversifying loci (e.g. Tay et al. 2016). Importantly, this is not the case in this study, where the three species indicate outlier markers, environmentally-associated outliers (Table 3.3) and potential divergent groups among their populations (BAPS: *P. angulosus*; *C. punctatus*). The limpet *S. granularis* may be impacted by a joint IBE/IBD effect and the urchin *P. angulosus* showed an IBE signal for salinity conditions (see section 3.3.6). The wealth of putatively environmentally-associated outliers and the lack of isolation-by-distance contribute to the hypothesis that genomic divergence might be driven by environmentally mediated selection processes rather than geographical distance. It can be acknowledged that molecular patterns of diversifying processes may under circumstances resemble signals created by allelic surfing, which however becomes less likely with large population sizes and some degree of genomic connectivity (Klopfstein, Currat and Excoffier, 2006; Excoffier and Ray, 2008). Other possible similarities can arise from hitchhiking genes (Bierne et al. 2011; Lotterhos and Whitlock, 2015), hidden demographic effects (De Mita et al. 2013; Lotterhos and Whitlock, 2015) and varying recombination and mutation rates (Roesti et al. 2012; Tine et al. 2014). It can be hypothesised that the main source of adaptive divergence (outlier loci) in the study species possibly stems from processes related to heterogeneous environmental conditions. RAD-Seq derived SNPs represent only a small portion of the overall genome, therefore it could be suggested that the impact of environmental variables on the species genome might be larger than detected here and that further research is necessary to elucidate the extent of environmentally-mediated selection processes in the study species populations across the South African coastal gradient.

3.4.6 Implications for resilience

Changing climatic conditions and other anthropogenic disturbances challenge species resilience and capacity to cope on a global scale (Pereira et al. 2010; Bellard et al. 2012; Archer et al. 2018). Adaptive divergence has been associated with evolutionary potential, where species with signals of adaptive processes might be at an advantage in the face of adversity (Barrett and Schluter, 2008; Alsos et al. 2012; Pauls et al. 2013) and spatial differentiation of outliers can possibly indicate areas of unique evolutionary distinctness. High genomic diversity, lack of population structure and absent diversifying loci have been interpreted as “*high resilience*” against “*still minimal*” impact from anthropogenic stressors in the sea star *Protoreaster nodosus* (Tay et al. 2016; p. 1; p. 12). It is important to question whether this can be conclusively deducted from a single study without incorporating possible

environmental influences. On the contrary, analyses of adaptive divergence indicated several spatially distinct clusters in abalone *Haliotis laevis*, which is treated as strong evidence that they should be considered as separate management units in a marine conservation framework (Sandoval-Castillo et al. 2018). Both instances demonstrate that adaptive divergence or the apparent lack thereof create a spectrum of molecular potential for resilience, which makes management considerations challenging for less straightforward patterns. In this study, distinct spatial patterns of putative adaptive divergence are not detected (*S. granularis*) or are not supported by multiple approaches (*C. punctatus*, *P. angulosus*). Nevertheless, all three species revealed potential selective processes in the shape of putatively adaptive markers and environmentally-associated outliers. The candidate loci cover a wide range of possible cellular functions, including a number of markers involved in energy metabolism dynamics. It can be hypothesised that the candidate outliers probably influence the resilience of *S. granularis*, *P. angulosus* and *C. punctatus* populations at present and in future environments. Further evidence for selective processes comes from the likely joint influence of SST, air temperature and geographic distance on the genomic variation in *S. granularis* and the impact of salinity conditions on genomic variation in *P. angulosus*. The significantly higher neutral nucleotide diversity found in the Cape urchins northern west coast population (Port Nolloth) is an important observation (Chapter II), which may indicate unique evolutionary distinctness in this area (congruent with Nielsen et al. 2018). Following a precautionary approach, it might be prudent to consider genomic monitoring of the species populations for temporal allelic fluctuations and to investigate additional environmental variables (Toonen and Grosberg, 2011). Further, the avenue of a candidate gene approach (Yednock and Neigel, 2014) could be promising to explore adaptive processes. In the case of the Cape urchin, the Port Nolloth population appears to hold a special position with regards to neutral nucleotide diversity, which warrants recognition in possible conservation measures. In summary, this study confirms the presence of hundreds of putatively adaptive loci across the intertidal species, which demonstrates the likely presence of adaptive selective processes across the examined South African populations. It can be hypothesised that the detected candidate markers might, together with other possibly not yet discovered divergent markers, serve as the foundation for spatially distinct adaptive patterns to emerge in the future. From the multi-species perspective of the study, it became evident that adaptive divergence differs notably across phyla, which advocates considering multiple rather than single species for genomic monitoring to facilitate effective conservation of marine communities for generations to come.

3.4.7 Conclusion

The findings in this chapter contribute to the growing empirical knowledge of adaptive divergence in marine invertebrates inhabiting coastal environmental gradients and introduce candidate loci putatively associated with selective environmental processes. Possible signals for adaptive divergence in populations of the shore crab *C. punctatus*, the granular limpet *S. granularis* and the Cape urchin *P. angulosus* were explored across the majority of their distribution in South Africa. In total, 1102 putatively adaptive loci were detected across species and assessed for possible functional association and population structure. Moreover, sea surface temperature, air temperature and salinity differentiation were tested for putative genome-environment association, which revealed against initial expectations that the largest number of candidate markers appears to be associated with salinity conditions over sea surface temperatures. Further, it became evident that levels of likely genome-environment associations differ notably between species and that markers exist which may be impacted by two environmental factors in *C. punctatus* and *S. granularis*. Contrary to the hypothesis of detecting site-specific (unique) outlier loci, the outliers detected with BayeScan and BayeScEnv were found present in all sampled populations. However, the empirical F_{ST} -method suggests a number of outliers which occur only in two sites (pairwise population estimates), which hints at a certain degree of spatial variation. Functional associations pointing to a large variety of cellular functions could be determined for around 10% of the outlier loci. Congruent with past studies, a number of these markers appear to incorporate endogenous viral elements such as retrotransposons or might form part of key metabolic components such as the electron transport chain. These types of loci have been implicated with local adaptation processes in studies on other marine invertebrates. In contrast to COI marker findings, there is no indication for isolation-by-distance between populations, but two species suggest the possible influence of isolation-by-environment (*P. angulosus*) or a joint IBE/IBD effect (*S. granularis*) on their genomic divergence. Contrary to the expectation of spatial differentiation in outlier loci across the populations, testing population structure for possible clustering suggests that there are no patterns supported by multiple approaches and that there are no geographical clusters of outliers. Likely attributable to varying model sensitivities, the species might represent a single population each or consist of multiple slightly divergent groups (*C. punctatus*, *P. angulosus*). Importantly, all study species demonstrate multiple loci which might be ecologically relevant and carry adaptive significance, regardless of the largely similar genomic neutral background levels established in Chapter II. In terms of resilience and evolutionary potential, it can be recommended to conduct genomic monitoring of temporal allele fluctuations across the populations, as the detected outlier markers possibly represent

the molecular foundation on which spatially distinct clusters may emerge. Moreover, the identified candidate loci likely play a role for the species resilience in future environments, which warrants their further validation and investigation. The study system spans a vast geographical area, where factors such as near shore current patterns and detailed *in situ* measurements of environmental variables in the intertidal are not well understood or available at present. The candidate markers obtained with RAD-Seq genomic data may constitute only a small percentage of loci affected by adaptive processes in the study species and further assessment is necessary to confirm detailed functional roles of candidate loci. Whole-genome sequencing, reciprocal transplant studies and transcriptome-based physiological experiments are promising avenues to better elucidate and validate markers putatively under selection, as well as to gain more insights into the impact of environmental factors (Larsen et al. 2008; Hereford, 2009; Limborg et al. 2012; Lotterhos and Whitlock, 2015; Pardo-Diaz, Salazar and Jiggins, 2015). For instance, candidate SNPs with putative environmental association can be verified with physiological experiments combined with transcriptomic approaches, where groups of the study species are exposed to variations of the environmental variable of interest such as SST, air temperature or salinity under laboratory controlled conditions. Exposure to physiological pressure such as increased sea water temperature upregulates the expression of genes involved in the species metabolic response. For example, a study on the Japanese mantis shrimp (*Oratosquilla oratoria*) exposed to different sea water temperatures identified adaptive genes underpinning the species molecular response to regulate the environmental stress (Lou, Gao and Han, 2019). Similar approaches have been utilised to explore genes linked to salinity stress in marine species (Liu et al. 2019; Malachowicz et al. 2019). Identified candidate genes can be subjected to qRT-PCR analysis (Real-Time Quantitative Reverse Transcription PCR; see for example Larsen et al. 2008) to validate genes with presumed adaptive potential and their possible involvement in physiological pathways. Comparing marine invertebrate populations from the South African east coast and west coast with a transcriptomic approach can reveal whether gene expression displays regional differences and possibly verify candidate loci detected in this study. Different levels of sea water and air temperature conditions have been shown to incite different genome expression trends (Gleason and Burton, 2015; Lou, Gao and Han, 2019), hence it might be hypothesised that regional transcriptomic differences exist in South African marine species inhabiting the coastal gradient.

3.5 Appendix

3.5.1 Geographic and environmental variables (RDA analysis)

Table S3.1. GPS coordinates utilised to extract environmental variables used in BayeScEnv and RDA analysis (Abbreviations listed in Figure 2.1).

Site	<i>C. punctatus</i> *	<i>S. granularis</i> **	<i>P. angulosus</i> **
JC*/PN**	-32.977944, 17.885978	-29.266415, 16.870284	-29.266415, 16.870284
SP	-33.915117, 18.386147	-33.915117, 18.386147	-33.915117, 18.386147
CA	-34.828587, 20.011403	-34.828587, 20.011403	-
KY	-34.089115, 22.973161	-34.089115, 22.973161	-34.089115, 22.973161
CF	-34.210609, 24.837540	-34.210609, 24.837540	-34.210609, 24.837540
HH	-32.766773, 28.241982	-32.766773, 28.241982	-32.766773, 28.241982

Table S3.2. Geographic along-shore distances (km) between sampling locations utilised in the RDA analysis. Source: SANBI (South African National Biodiversity Institute).

<i>C. punctatus</i>	JC	SP	CA	KY	CF	HH
JC		240.459	598.727	958.626	1165.07	1592.21
SP	240.459		359.717	719.254	925.274	1352.44
CA	598.727	359.717		359.896	565.915	992.744
KY	958.626	719.254	359.896		202.982	633.238
CF	1165.07	925.274	565.915	202.982		430.416
HH	1592.21	1352.44	992.744	633.238	430.416	
<i>S. granularis</i>	PN	SP	CA	KY	CF	HH
PN		753.391	1110.39	1470.78	1673.76	2102.6
SP	753.391		359.717	719.254	925.274	1352.44
CA	1110.39	359.717		359.896	565.915	992.744
KY	1470.78	719.254	359.896		202.982	633.238
CF	1673.76	925.274	565.915	202.982		430.416
HH	2102.6	1352.44	992.744	633.238	430.416	
<i>P. angulosus</i>	PN	SP	KY	CF	HH	
PN		753.391	1470.78	1673.76	2102.6	
SP	753.391		719.254	925.274	1352.44	
KY	1470.78	719.254		202.982	633.238	
CF	1673.76	925.274	202.982		430.416	
HH	2102.6	1352.44	633.238	430.416		

Table S3.3. Environmental variables (SST and air temperature in °C, salinity in ppt) utilised in the RDA analysis and BayeScEnv. Source: World Ocean Atlas, 2013.

<i>C. punctatus</i>					
sites	y	x	SST	salinity	air
JC	-32.9779	17.88598	14.945	35.022	17.8
SP	-33.9151	18.38615	16.175	35.200	16.8
CA	-34.8286	20.0114	18.172	35.389	16.9
KY	-34.0891	22.97316	18.752	35.314	16.6
CF	-34.2106	24.83754	18.861	35.298	17.1
HH	-32.7668	28.24198	23.103	35.484	18.5
<i>S. granularis</i>					
sites	y	x	SST	salinity	air
PN	-29.2664	16.87028	13.807	34.879	14.7
SP	-33.9151	18.38615	16.175	35.200	16.8
CA	-34.8286	20.0114	18.172	35.389	16.9
KY	-34.0891	22.97316	18.752	35.314	16.6
CF	-34.2106	24.83754	18.861	35.298	17.1
HH	-32.7668	28.24198	23.103	35.484	18.5
<i>P. angulosus</i>					
sites	y	x	SST	salinity	air
PN	-29.2664	16.87028	13.807	34.879	-
SP	-33.9151	18.38615	16.175	35.200	-
KY	-34.0891	22.97316	18.752	35.314	-
CF	-34.2106	24.83754	18.861	35.298	-
HH	-32.7668	28.24198	23.103	35.484	-

3.5.2 Outlier SNPs (empirical F_{ST} , BayeScan, PoPoolation)

The total number of estimated empirical F_{ST} outliers across species is shown in Table 3.1. Table S3.4 below shows how many unique potential outliers were detected in the pairwise comparison across locations and how many are shared by all.

Table S3.4. Number of unique potential outliers detected with the empirical F_{ST} method pairwise population estimation.

<i>C. punctatus</i>	JC	SP	CA	KY	CF	HH
JC	-					
SP	30	-				
CA	19	28	-			
KY	15	24	15	-		
CF	9	13	16	23	-	
HH	4	4	5	11	24	-
Shared by all	73					
<i>S. granularis</i>	PN	SP	CA	KY	CF	HH
PN	-					
SP	18	-				
CA	1	-	-			
KY	2	2	10	-		
CF	2	-	10	13	-	
HH	-	2	4	6	10	-
Shared by all	28					
<i>P. angulosus</i>	PN	SP	KY	CF	HH	
PN	-					
SP	6	-				
KY	-	10	-			
CF	3	7	9	-		
HH	2	7	10	10	-	
Shared by all	22					

3.5.3 Comparison F_{ST} estimates

The following table shows a comparison between estimates obtained from outlier loci alongside selectively neutral loci (previously discussed in Chapter II) (Table S3.5).

Table S3.5. F_{ST} estimated in PoPoolation2 (scenario 6) for selectively neutral loci (left) and outlier loci (right).
Location abbreviations as listed in Figure 2.1.

<i>C. punctatus</i> (A)	Neutral Loci						Outlier Loci					
	JC	SP	CA	KY	CF	HH	JC	SP	CA	KY	CF	HH
JC	-						-					
SP	0.049	-					0.116*	-				
CA	0.050	0.045	-				0.113*	0.108*	-			
KY	0.050	0.044	0.044	-			0.135*	0.112*	0.110*	-		
CF	0.052	0.049	0.043	0.046	-		0.153*	0.128*	0.119*	0.114*	-	
HH	0.055	0.052	0.048	0.049	0.046	-	0.185*	0.160*	0.153*	0.128*	0.111*	-
<i>S. granularis</i> (B)	PN	SP	CA	KY	CF	HH	PN	SP	CA	KY	CF	HH
PN	-						-					
SP	0.051	-					0.131*	-				
CA	0.061	0.055	-				0.242*	0.195*	-			
KY	0.061	0.059	0.051	-			0.242*	0.190*	0.113*	-		
CF	0.061	0.055	0.044	0.050	-		0.258*	0.195*	0.109*	0.111*	-	
HH	0.066	0.063	0.053	0.053	0.051	-	0.289*	0.239*	0.138*	0.151*	0.133*	-
<i>P. angulosus</i> (C)	PN	SP	KY	CF	HH		PN	SP	KY	CF	HH	
PN	-						-					
SP	0.066	-					0.213*	-				
KY	0.074	0.049	-				0.261*	0.125*	-			
CF	0.089	0.061	0.053	-			0.310*	0.162*	0.121*	-		
HH	0.082	0.055	0.039	0.059	-		0.286*	0.141*	0.109*	0.143*	-	

3.5.4 List of BlastX protein domains

The full list of protein sequences with an E-value above $>1.0E-5$ per study species from the empirical outlier detection method (Table S3.5) and the BayeScan and BayeScEnv outlier identification method (Table S3.6).

Table S3.6. Putatively identified and hypothetical or uncharacterised protein domains from empirical outliers listed per species with contig query length, query cover, respective E-value and the percentage of the contig identical with the suggested protein domain.

Query length	Protein domain name	Query cover	E-values	%identical
<i>C. punctatus</i>				
1266	HAZT_HAZT007701 (hypothetical protein)	17%	1E-23	93.06%
1271	LOC107337606 (uncharacterized protein)	48%	5E-06	30.32%
1184	hypothetical protein	28%	4E-62	89.19%
1113	DSY42_03570 (hypothetical protein)	25%	6E-07	37.00%
1306	Retrovirus-related Pol polyprotein from type-1 retrotransposable element R2	26%	5E-23	50.43%
1328	DSY42_04590 (hypothetical protein)	75%	7E-113	50.89%
1258	DSY42_03545 (hypothetical protein)	44%	3E-78	61.62%
1192	T4E_7988, partial (hypothetical protein)	37%	6E-30	60.27%
1291	ATP-binding cassette sub-family F member 2	38%	2E-30	66.67%
1215	LOC114966011 (uncharacterized protein)	99%	6E-135	50.58%
1130	ECANGB1_2401, partial (hypothetical protein)	15%	1E-17	77.19%
1160	wsv191-like protein	34%	2E-28	47.41%
1160	LOC105323971 (uncharacterized protein)	84%	3E-122	56.40%
1009	ATP-binding cassette sub-family F member 2	49%	4E-64	66.47%
1047	hypothetical protein	45%	2E-12	31.65%
1093	LOC108672789 (uncharacterized protein)	66%	2E-26	27.87%
1124	F54H12.2-like (uncharacterized protein)	43%	4E-10	28.40%
1164	E1301_Tti011794 (hypothetical protein)	38%	1E-39	52.35%
1032	hypothetical protein	27%	1E-33	64.89%
1153	hypothetical protein	21%	3E-05	53.57%
1131	C7M84_002030 (hypothetical protein)	61%	1E-52	48.97%
1068	BRAFLDRAFT_97280 (hypothetical protein)	29%	3E-09	34.91%
1077	wsv220-like protein	99%	9E-36	27.93%
1072	E2C01_015619 (hypothetical protein)	95%	1E-107	50.15%
1167	LOC108182213, partial (uncharacterized protein)	58%	4E-26	31.15%

1067	E2C01_088635 (hypothetical protein)	82%	2E-104	58.98%
1181	hypothetical protein	20%	4E-17	54.43%
1140	RTBS	23%	1E-16	61.90%
1069	Craniofacial development protein 2-like	31%	2E-28	46.96%
1138	Putative nuclease HARBI1	69%	3E-97	57.09%
1121	hypothetical protein	16%	5E-10	51.61%
1289	PiggyBac transposable element-derived protein 4-like	21%	2E-11	43.96%
1099	E2C01_024578 (hypothetical protein)	10%	6E-12	92.31%
4107	hypothetical protein	72%	7E-175	34.99%
1148	Transposon Ty3-G Gag-Pol polyprotein	99%	2E-127	47.52%
1105	LIM domain kinase 1-like isoform X2	27%	1E-17	74.58%
1129	C7M84_011008 (hypothetical protein)	13%	5E-20	96.00%
1049	AVEN_124460_1 (hypothetical protein)	20%	5E-18	53.42%
1186	E2C01_079680 (hypothetical protein)	51%	2E-39	51.90%
1130	CAPTEDRAFT_211086 (hypothetical protein)	70%	2E-62	42.11%
1117	hypothetical protein	52%	3E-43	54.00%
1077	K02A2.6-like (uncharacterized protein)	99%	7E-51	33.79%
1317	CAPTEDRAFT_215442 (hypothetical protein)	12%	1E-14	59.65%
1055	KP79_PYT00846 (hypothetical protein)	29%	6E-09	35.92%
1104	LOC111106872 (uncharacterized protein)	59%	1E-42	42.04%
1048	Tectonin beta-propeller repeat-containing protein	12%	4E-09	69.05%
1206	ECANGB1_2036 (hypothetical protein)	34%	4E-44	62.86%
1076	hypothetical protein	12%	7E-09	73.33%
1201	ECANGB1_2401, partial (hypothetical protein)	41%	4E-18	41.07%
1203	AC249_AIPGENE14849, partial (hypothetical protein)	62%	3E-42	41.54%
1742	E3 ubiquitin-protein ligase MYCBP2-like	25%	9E-17	63.24%
2636	E2C01_093774 (hypothetical protein)	5%	1E-08	61.22%
1226	Prefoldin subunit 3	9%	3E-12	97.30%
1309	Proline/betaine transporter	14%	5E-06	45.45%
2898	LOC107341966 (uncharacterized protein)	45%	4E-121	46.52%
1265	Myc box-dependent-interacting protein 1-like	35%	2E-20	34.53%
1302	RNA-directed DNA polymerase	39%	2E-34	50.49%
1147	Protein flightless-1	17%	3E-34	96.92%
1086	E2C01_023748 (hypothetical protein)	26%	4E-21	72.60%
1465	Methyltransferase-like protein 7A	21%	2E-18	67.24%
1652	hypothetical protein	16%	2E-06	35.56%
1414	dtw domain-containing protein 2	36%	7E-16	32.60%

1331	ECANGB1_2401, partial (hypothetical protein)	66%	6E-32	46.86%
1500	LOC113393431 (uncharacterized protein)	10%	1E-06	51.28%
1270	Glutenin, high molecular weight subunit DY10-like	94%	2E-22	29.21%
1189	Nck-associated protein 5	99%	4E-164	75.89%
1266	C7M84_008981 (hypothetical protein)	51%	5E-98	67.89%
1303	Growth hormone secretagogue receptor type 1	18%	1E-25	68.35%
1323	Reticulophagy regulator 3-like	12%	3E-06	53.03%
1382	52 kDa repressor of the inhibitor of the protein kinase-like	38%	3E-15	38.98%
1435	THAP domain-containing protein 6-like	12%	3E-12	56.67%
1253	N-acetylglucosamine-6-phosphate deacetylase	11%	1E-17	95.74%
1210	hypothetical protein	90%	4E-82	44.29%
1159	RNA-directed DNA polymerase	36%	8E-15	50.98%
1133	XENTR_v90017050mg (hypothetical protein)	18%	8E-08	36.62%
1094	Dynein intermediate chain 1, axonemal-like	12%	3E-08	62.22%
1104	RTXE	75%	6E-30	37.50%
1159	hypothetical protein	14%	3E-19	73.68%
1084	Putative protein in type-1 retrotransposable element R1DM	67%	1E-111	70.49%
1075	Putative disco-interacting protein 2 isoform X2	20%	6E-27	100.00%
1689	hypothetical protein, partial	65%	1E-82	37.97%
1012	hypothetical protein	66%	3E-23	30.90%
1034	hypothetical protein	76%	3E-79	64.68%
1005	RNA-directed DNA polymerase from mobile element jockey-like	97%	2E-60	38.21%
1015	LOC109468467 (uncharacterized protein)	39%	5E-60	75.56%
932	RNA-directed DNA polymerase from mobile element jockey-like	96%	4E-68	41.59%
866	Adaptin ear-binding coat-associated protein 1	83%	1E-31	36.56%
1195	D6690_01995 (hypothetical protein)	99%	2E-51	35.73%
925	LOC101239284, partial (uncharacterized protein)	71%	6E-51	42.27%
1112	Formyl-CoA transferase	57%	7E-118	86.38%
917	unnamed protein product	42%	2E-48	55.81%
1061	BSL78_07387 (hypothetical protein)	93%	2E-108	50.00%
1039	C7M84_018246, partial (hypothetical protein)	23%	5E-28	66.27%
1010	Evm_004501 (hypothetical protein)	67%	3E-17	30.67%
985	ECANGB1_2309, partial (hypothetical protein)	15%	4E-15	81.13%
949	LOC114544175, partial (uncharacterized protein)	21%	7E-06	47.14%
933	NADH dehydrogenase subunit 6	46%	6E-30	50.34%

859	Putative sidestep protein, partial	17%	5E-06	53.06%
826	KRAB-A domain-containing protein 2, partial	70%	3E-59	68.87%
754	LOC113814571 isoform X1 (uncharacterized protein)	21%	8E-06	72.00%
727	hypothetical protein	80%	4E-51	65.96%
358	Protein SpAN-like	56%	8E-24	65.67%
345	ECANGB1_2036 (hypothetical protein)	89%	7E-26	73.33%
449	NADH dehydrogenase subunit 4	99%	7E-51	83.89%
433	DSY59_01135, partial (hypothetical protein)	81%	6E-40	58.47%
411	ECANGB1_252 (hypothetical protein)	100%	5E-74	75.91%
406	B7P43_G02324, partial (hypothetical protein)	98%	9E-29	46.38%
360	AVEN_15752_1 (hypothetical protein)	95%	3E-20	40.87%
215	Putative RNA-directed DNA polymerase from transposon BS	99%	1E-29	73.24%
181	Ribosome-binding protein 1-like	100%	8E-06	52.73%
153	predicted protein, partial	100%	4E-12	62.75%
Query length	Protein domain name	Query cover	E-values	%identical
<i>S. granularis</i>				
9127	Cytochrome b	11%	2E-158	76.76%
1198	CRN43_01760 (hypothetical protein)	70%	6E-61	38.24%
1134	KP79_PYT00092 (hypothetical protein)	67%	1E-24	32.03%
1146	LOTGIDRAFT_161039 (hypothetical protein)	65%	5E-50	40.32%
1208	LOTGIDRAFT_155952 (hypothetical protein)	92%	4E-83	40.48%
1263	Putative nuclease HARBI1	30%	6E-32	49.61%
1241	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit	22%	1E-10	70.45%
1159	EGW08_018866, partial (hypothetical protein)	22%	2E-07	57.14%
1198	LOC111344944 (hypothetical protein)	99%	3E-111	46.15%
998	LOTGIDRAFT_234217 (hypothetical protein)	12%	3E-10	78.05%
1027	LOC114525576 (uncharacterized protein)	75%	6E-68	45.08%
843	Beta-1,4-N-acetylgalactosaminyltransferase bre-4-like	24%	3E-30	81.16%
946	LOTGIDRAFT_237366 (hypothetical protein)	19%	3E-19	72.58%
1076	LOTGIDRAFT_160045 (hypothetical protein)	64%	9E-97	64.53%
808	LOTGIDRAFT_176514, partial (hypothetical protein)	45%	8E-13	29.92%
676	Sodium-independent anion transporter	99%	5E-70	48.89%
833	Phospholipase A2 AP-PLA2-I	17%	7E-07	79.59%
642	hypothetical protein, partial	98%	5E-77	58.96%
650	LOTGIDRAFT_228644 (hypothetical protein)	33%	1E-26	68.06%

572	Cytochrome c oxidase subunit I	99%	4E-94	82.63%
727	C0Q70_17168 (hypothetical protein)	21%	5E-24	90.20%
709	CRYPA_1252 (hypothetical protein)	52%	6E-18	37.80%
669	LOTGIDRAFT_168316 (hypothetical protein)	72%	2E-56	57.14%
587	Putative RNA-directed DNA polymerase from transposon BS	97%	7E-61	48.19%
559	AC249_AIPGENE8055 (hypothetical protein)	75%	1E-60	70.21%
554	LOC114974970 (uncharacterized protein)	86%	7E-62	61.01%
341	CRYPA_469 (hypothetical protein)	99%	4E-51	73.45%
279	LOC111948367 (uncharacterized protein)	97%	1E-30	65.93%
277	LOC115316855, partial (uncharacterized protein)	88%	7E-23	56.10%
Query length	Protein domain name	Query cover	E-values	%identical
<i>P. angulosus</i>				
1052	Bromodomain adjacent to zinc finger domain protein 2B isoform X7	25%	3E-19	87.93%
1283	Myophilin-like	12%	1E-23	89.09%
1410	Protein LCHN	14%	4E-14	71.01%
1372	LOC589927 (uncharacterized protein)	21%	6E-25	81.69%
1451	LOC576652 (uncharacterized protein)	19%	4E-15	85.11%
1283	Gastrula zinc finger protein XICGF8.2DB-like	53%	8E-60	47.52%
1401	Roundabout homolog 1 isoform X2	47%	5E-57	86.10%
1255	Band 4.1-like protein 4A isoform X2	7%	4E-05	90.00%
1340	GPI ethanolamine phosphate transferase 2	36%	1E-31	72.63%
1464	"deleted in malignant brain tumors 1 protein, partial"	16%	2E-16	67.14%
1443	LOC105440060 (uncharacterized protein)	11%	4E-06	52.73%
1488	Putative growth factor receptor-bound protein 14	21%	4E-20	62.50%
1167	Putative DDB_G0271606 isoform X1 (uncharacterized protein)	23%	2E-16	69.31%
1312	Nuclear pore complex protein Nup214	19%	2E-40	87.21%
1476	LOC579728 (uncharacterized protein)	15%	8E-25	68.83%
1501	Vesicle-associated membrane protein 7	23%	3E-31	86.96%
1406	Fibrillin-1	14%	6E-09	78.57%
1442	Serine/arginine repetitive matrix protein 2 isoform X4	13%	1E-21	85.94%
1229	BSL78_26173 (hypothetical protein)	32%	4E-18	48.11%
1183	Protein disulfide-isomerase TMX3	11%	2E-18	91.30%
1286	LOC100893564 (uncharacterized protein)	39%	2E-12	51.92%
1183	Beta-galactosidase-1-like protein 2	7%	6E-09	93.55%
1275	Toll-like receptor 3	84%	4E-70	41.16%

1124	Hydroxylysine kinase isoform X2	18%	1E-21	72.86%
1292	Tubulin polyglutamylase TTLL5	14%	1E-07	100.00%
1077	Serine/threonine-protein kinase H1	28%	2E-31	94.23%
1013	Echinoderm microtubule-associated protein (77 kDa)	15%	3E-24	96.23%
1125	Krev interaction trapped protein 1 isoform X2	13%	7E-05	92.31%
912	LOC594540 (uncharacterized protein)	24%	4E-12	88.64%
1096	"deleted in malignant brain tumors 1 protein"	34%	2E-57	81.15%
1149	LOC105438137 (uncharacterized protein)	20%	8E-22	66.23%
663	LOC100893246 (uncharacterized protein)	23%	2E-11	64.71%
617	Endonuclease-reverse transcriptase	98%	2E-98	69.31%
426	ABC transporter ATP-binding protein	94%	9E-42	54.48%
323	BSL78_04809, partial (hypothetical protein)	83%	1E-47	80.00%

Table S3.7. Putatively identified and hypothetical or uncharacterised protein domains from BayeScan and BayeScEnv outliers listed with contig query length, query cover, respective E-value and the percentage of the contig identical with the suggested protein domain. Protein domains suggested across BayeScan and BayeScEnv or between multiple environmental parameters appear grey.

Query length	Protein domain name	Query cover	E-values	%identical
<i>C. punctatus</i>				
(BayeScan)				
1113	Lysosomal-trafficking regulator	13%	7E-22	92.16%
1077	XENTR_v90000373mg, partial (hypothetical protein)	32%	2E-12	33.00%
895	D6690_01995 (hypothetical protein)	95%	3E-38	40.00%
1021	putative RNA-directed DNA polymerase from transposon X-element	99%	4E-127	56.93%
(BayeScEnv, sea surface temperature (SST))				
980	C7M84_008981 (hypothetical protein)	54%	2E-66	58.99%
966	LOC113808154 (uncharacterized protein)	65%	4E-23	33.33%
1046	hypothetical protein	34%	3E-15	46.25%
975	R2DM Retrovirus-related Pol polyprotein from type II retrotransposable element	76%	6E-37	34.13%
984	predicted protein	27%	9E-21	60.67%
977	E3 ubiquitin-protein ligase SHPRH	27%	5E-40	82.95%
1035	putative RNA-directed DNA polymerase from transposon BS	56%	2E-83	65.13%
1091	Guanine nucleotide-releasing factor 2-like isoform X6	32%	1E-09	44.53%
1109	RTXE	43%	3E-19	40.91%
1041	hypothetical protein	21%	3E-05	54.10%
964	Kinesin-like protein KIF20B isoform X1	35%	7E-25	86.57%
1010	hypothetical protein	23%	3E-06	45.00%
895	D6690_01995 (hypothetical protein)	95%	3E-38	40.00%
795	ECANGB1_2036 (hypothetical protein)	19%	2E-18	75.00%
512	Solute carrier family 22 member 3	28%	3E-18	77.55%
(BayeScEnv, salinity)				
1089	DSY42_09150 (hypothetical protein)	35%	1E-59	80.00%
1031	DEA37_0001408, partial (uncharacterized protein)	91%	8E-58	39.34%
1046	hypothetical protein	34%	3E-15	46.25%
1011	C7M84_008216 (hypothetical protein)	56%	6E-33	45.27%
1019	hypothetical protein	50%	8E-62	59.41%
1035	R2DM Retrovirus-related Pol polyprotein from type II retrotransposable element	71%	1E-36	34.13%

1201	RTJK, partial	74%	9E-57	54.63%
1096	RNA-directed DNA polymerase from mobile element jockey-like	56%	3E-21	31.78%
1086	hypothetical protein	23%	6E-21	53.49%
1105	WD repeat-containing protein 35	11%	2E-05	81.40%
1104	MAM and LDL-receptor class A domain-containing protein 1-like, partial	15%	4E-05	42.11%
1152	rna-directed dna polymerase from mobile element jockey-like	79%	5E-63	39.34%
1068	ECANGB1_2401, partial (hypothetical protein)	26%	4E-32	80.85%
1119	protein pangolin, isoforms A/H/I/S-like	13%	2E-18	98.04%
1043	Craniofacial development protein 2-like	59%	2E-67	57.89%
1042	LOC108673117 isoform X2 (uncharacterized protein)	22%	5E-05	45.10%
935	hypothetical protein	26%	1E-09	50.91%
1050	ECANGB1_208 (hypothetical protein)	65%	6E-71	53.22%
1070	hypothetical protein	21%	3E-06	45.00%
895	D6690_01995 (hypothetical protein)	95%	3E-38	40.00%
834	ATP synthase F0 subunit 6	80%	3E-106	87.05%
716	hypothetical protein	64%	2E-72	72.73%
(BayeScEnv, air temperature)				
1040	C7M84_008981 (hypothetical protein)	57%	1E-77	60.10%
975	R2DM Retrovirus-related Pol polyprotein from type II retrotransposable element	76%	6E-37	34.13%
1020	hypothetical protein	50%	2E-57	57.89%
1075	Retrovirus-related Pol polyprotein from transposon 17.6	23%	2E-14	48.98%
966	hypothetical protein	14%	3E-07	56.52%
998	Zinc finger protein 2	19%	9E-08	54.55%
1095	putative RNA-directed DNA polymerase from transposon BS	53%	6E-83	65.13%
923	LOC113825078 isoform X2 (uncharacterized protein)	20%	1E-27	95.24%
904	Kinesin-like protein KIF20B isoform X1	35%	5E-21	86.67%
950	hypothetical protein	24%	2E-06	45.00%
835	D6690_01995 (hypothetical protein)	95%	3E-38	40.96%
878	TPA: endonuclease-reverse transcriptase	82%	7E-51	43.33%
795	ECANGB1_2036 (hypothetical protein)	19%	2E-18	75.00%
820	putative uncharacterized protein K02A2.6-like	99%	9E-95	57.72%
246	unnamed protein product, partial	95%	2E-06	39.74%
272	Solute carrier family 22 member 3	60%	2E-19	66.13%
417	SDR family oxidoreductase	99%	1E-92	96.38%

241	hypothetical protein	99%	1E-21	76.62%
Query length	Protein domain name	Query cover	E-values	%identical
<i>S. granularis</i>				
(BayeScan)				
1045	AC249_AIPGENE27713 (hypothetical protein)	99%	1E-152	60.63%
903	Beta-1,4-N-acetylgalactosaminyltransferase bre-4-like	22%	4E-30	81.16%
464	EAZ74_07105 (hypothetical protein)	56%	5E-36	69.32%
454	UDP-N-acetylmuramate dehydrogenase	97%	2E-47	55.26%
426	PPERSA_06560 (hypothetical protein)	93%	3E-30	61.04%
(BayeScEnv, sea surface temperature (SST))				
997	LOTGIDRAFT_174945 (hypothetical protein)	25%	4E-13	86.05%
1204	LOTGIDRAFT_157217 (hypothetical protein)	61%	1E-35	35.48%
727	C0Q70_17168 (hypothetical protein)	21%	5E-24	90.20%
(BayeScEnv, salinity)				
1129	LOC105347521 isoform X3 (uncharacterized protein)	15%	5E-05	55.56%
1105	AC249_AIPGENE27713 (hypothetical protein)	99%	6E-166	61.68%
727	C0Q70_17168 (hypothetical protein)	21%	5E-24	90.20%
471	protein ZBED8-like	99%	2E-79	86.57%
(BayeScEnv, air temperature)				
1117	LOTGIDRAFT_174945 (hypothetical protein)	23%	7E-13	86.05%
1204	LOTGIDRAFT_157217 (hypothetical protein)	61%	1E-35	35.48%
727	C0Q70_17168 (hypothetical protein)	21%	5E-24	90.20%
Query length	Protein domain name	Query cover	E-values	%identical
<i>P. angulosus</i>				
(BayeScan)				
5400	Baculoviral IAP repeat-containing protein 6 isoform X6	13%	5E-18	92.50%
1152	LOC100889761 (uncharacterized protein)	9%	6E-12	92.11%
1105	Monocarboxylate transporter 9	21%	1E-07	56.96%
1328	Iron-sulfur protein NUBPL isoform X2	10%	6E-15	91.11%
1252	Inositol 1,4,5-trisphosphate receptor isoform X8	27%	7E-26	98.72%
(BayeScEnv, salinity)				
10126	Baculoviral IAP repeat-containing protein 6 isoform X6	10%	1E-42	96.59%

3.6 References

- Addison, Jason A., and Jin-Hong Kim. 2018. "Cryptic Species Diversity and Reproductive Isolation among Sympatric Lineages of *Strongylocentrotus* Sea Urchins in the Northwest Atlantic." *FACETS* 3 (1): 61–78.
- Agüera, Antonio, Tim Schellekens, Jeroen M. Jansen, and Aad C. Smaal. 2015. "Effects of Osmotic Stress on Predation Behaviour of *Asterias rubens* L." *Journal of Sea Research* 99 (May): 9–16.
- Ahrens, Collin W., Paul D. Rymer, Adam Stow, Jason Bragg, Shannon Dillon, Kate D. L. Umbers, and Rachael Y. Dudaniec. 2018. "The Search for Loci under Selection: Trends, Biases and Progress." *Molecular Ecology* 27 (6): 1342–56.
- Aitken, Sally N., Sam Yeaman, Jason A. Holliday, Tongli Wang, and Sierra Curtis-McLane. 2008. "Adaptation, Migration or Extirpation: Climate Change Outcomes for Tree Populations." *Evolutionary Applications* 1 (1): 95–111.
- Akey, Joshua M., Alison L. Ruhe, Dayna T. Akey, Aaron K. Wong, Caitlin F. Connelly, Jennifer Madeoy, Thomas J. Nicholas, and Mark W. Neff. 2010. "Tracking Footprints of Artificial Selection in the Dog Genome." *Proceedings of the National Academy of Sciences of the United States of America* 107 (3): 1160–65.
- Alberto, Florian J., Sally N. Aitken, Ricardo Alía, Santiago C. González-Martínez, Heikki Hänninen, Antoine Kremer, François Lefèvre, et al. 2013. "Potential for Evolutionary Responses to Climate Change - Evidence from Tree Populations." *Global Change Biology* 19 (6): 1645–61.
- Al-Breiki, Rufaida Dhuhai, Shannon R. Kjeldsen, Hasifa Afzal, Manal Saif Al Hinai, Kyall R. Zenger, Dean R. Jerry, Mohammed Ali Al-Abri, and Madjid Delghandi. 2018. "Genome-Wide SNP Analyses Reveal High Gene Flow and Signatures of Local Adaptation among the Scalloped Spiny Lobster (*Panulirus homarus*) along the Omani Coastline." *BMC Genomics* 19 (1): 690.
- Alex, Anoop, and Agostinho Antunes. 2018. "Genus-Wide Comparison of *Pseudovibrio* Bacterial Genomes Reveal Diverse Adaptations to Different Marine Invertebrate Hosts." *PLoS ONE* 13 (5).
- Alsos, Inger Greve, Dorothee Ehrich, Wilfried Thuiller, Pernille Bronken Eidesen, Andreas Tribsch, Peter Schönswetter, Claire Lagaye, Pierre Taberlet, and Christian Brochmann. 2012. "Genetic Consequences of Climate Change for Northern Plants." *Proceedings. Biological Sciences / The Royal Society* 279 (1735): 2042–51.
- Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman. 1997. "Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs." *Nucleic Acids Research* 25 (17): 3389–3402.
- Andrews, Kimberly R., Jeffrey M. Good, Michael R. Miller, Gordon Luikart, and Paul A. Hohenlohe. 2016. "Harnessing the Power of RADseq for Ecological and Evolutionary Genomics." *Nature Reviews. Genetics* 17 (2): 81–92.
- Archer, Emma R. M., L. E. Dziba, K. J. Mulongoy, Malebajoa A. Maoela, and Michele Walters. 2018. *The IPBES regional assessment report on biodiversity and ecosystem*

- services for Africa*. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), Bonn, Germany.
- Ardley, Helen C., and Philip A. Robinson. 2005. "E3 Ubiquitin Ligases." *Essays in Biochemistry* 41 (1): 15.
- Atkinson, David, and Richard M. Sibly. 1997. "Why Are Organisms Usually Bigger in Colder Environments? Making Sense of a Life History Puzzle." *Trends in Ecology & Evolution* 12 (6): 235–39.
- Avise, John C. 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press.
- Baldanzi, Simone, Ralph Watson, Christopher D. McQuaid, Gavin Gouws, and Francesca Porri. 2017. "Epigenetic variation among natural populations of the South African sandhopper *Talorchestia capensis*." *Evolutionary Ecology* 31, no. 1: 77-91.
- Balhoff, James P., and Gregory A. Wray. 2005. "Evolutionary Analysis of the Well Characterized Endo16 Promoter Reveals Substantial Variation within Functional Sites." *Proceedings of the National Academy of Sciences of the United States of America* 102 (24): 8591–96.
- Balkenhol, Niko, Rachael Y. Dudaniec, Konstantin V. Krutovsky, Jeremy S. Johnson, David M. Cairns, Gernot Segelbacher, Kimberly A. Selkoe, et al. 2017. "Landscape Genomics: Understanding Relationships Between Environmental Heterogeneity and Genomic Characteristics of Populations." In , 261–322. Springer, Cham.
- Ballard, J. William O., and David M. Rand. 2005. "The Population Biology of Mitochondrial DNA and Its Phylogenetic Implications." *Annual Review of Ecology, Evolution, and Systematics* 36 (1): 621–42.
- Ballard, J. William O., and Michael C. Whitlock. 2004. "The Incomplete Natural History of Mitochondria." *Molecular Ecology* 13 (4): 729–44.
- Barber, Paul H., Stephen R. Palumbi, Mark V. Erdmann, and M. Kasim Moosa. 2000. "A Marine Wallace's Line?" *Nature* 406 (6797): 692–93.
- Barrett, Rowan D. H., and Dolph Schluter. 2008. "Adaptation from Standing Genetic Variation." *Trends in Ecology & Evolution* 23 (1): 38–44.
- Bartke, Till, Christian Pohl, George Pyrowolakis, and Stefan Jentsch. 2004. "Dual Role of BRUCE as an Antiapoptotic IAP and a Chimeric E2/E3 Ubiquitin Ligase." *Molecular Cell* 14 (6): 801–11.
- Beall, Cynthia M., Gianpiero L. Cavalleri, Libin Deng, Robert C. Elston, Yang Gao, Jo Knight, Chaohua Li, et al. 2010. "Natural Selection on EPAS1 (HIF2alpha) Associated with Low Hemoglobin Concentration in Tibetan Highlanders." *Proceedings of the National Academy of Sciences of the United States of America* 107 (25): 11459–64.
- Beaumont, Mark A. 2005. "Adaptation and Speciation: What Can *Fst* Tell Us?" *Trends in Ecology & Evolution* 20 (8): 435–40.
- Becker, Ute, Guy Colling, Petr Dostal, Anna Jakobsson, and Diethart Matthies. 2006. "Local Adaptation in the Monocarpic Perennial *Carlina vulgaris* at Different Spatial Scales

- across Europe.” *Oecologia* 150 (3): 506–18.
- Beckstead, Wesley A., Mark T. W. Ebbert, Mark J. Rowe, and David A. McClellan. 2009. “Evolutionary Pressure on Mitochondrial Cytochrome b Is Consistent with a Role of Cytb17T Affecting Longevity during Caloric Restriction.” *PLoS ONE* 4 (6): e5836.
- Beissinger, Timothy M., Guilherme J. M. Rosa, Shawn M. Kaeppler, Daniel Gianola, and Natalia de Leon. 2015. “Defining Window-Boundaries for Genomic Analyses Using Smoothing Spline Techniques.” *Genetics Selection Evolution* 47 (1): 30.
- Bélanger-Deschênes, Sébastien, Patrice Couture, Peter G. C. Campbell, and Louis Bernatchez. 2013. “Evolutionary Change Driven by Metal Exposure as Revealed by Coding SNP Genome Scan in Wild Yellow Perch (*Perca flavescens*).” *Ecotoxicology* 22 (5): 938–57.
- Bellard, Céline, Cleo Bertelsmeier, Paul Leadley, Wilfried Thuiller, and Franck Courchamp. 2012. “Impacts of Climate Change on the Future of Biodiversity.” *Ecology Letters* 15 (4): 365–77.
- Benestan, Laura, Brady K. Quinn, Halim Maaroufi, Martin Laporte, Fraser K. Clark, Spencer J. Greenwood, Rémy Rochette, and Louis Bernatchez. 2016. “Seascape Genomics Provides Evidence for Thermal Adaptation and Current-Mediated Population Structure in American Lobster (*Homarus americanus*).” *Molecular Ecology* 25 (20): 5073–92.
- Berg, Paul R., Sissel Jentoft, Bastiaan Star, Kristoffer H. Ring, Halvor Knutsen, Sigbjørn Lien, Kjetill S. Jakobsen, and Carl André. 2015. “Adaptation to Low Salinity Promotes Genomic Divergence in Atlantic Cod (*Gadus morhua* L.).” *Genome Biology and Evolution* 7 (6): 1644–63.
- Bergmann, Carl. 1847. *Über Die Verhältnisse Der Wärmeökonomie Der Thiere Zu Ihrer Grösse*. Stud. 1. Göttingen.
- Bernardi, Giacomo, Lloyd Findley, and Axayacatl Rocha-Olivares. 2003. “Vicariance and Dispersal across Baja California in Disjunct Marine Fish Populations.” *Evolution* 57 (7): 1599–1609.
- Bernatchez, Simon, Amanda Xuereb, Martin Laporte, Laura Benestan, Royce Steeves, Mark Laflamme, Louis Bernatchez, and Martin A. Mallet. 2019. “Seascape Genomics of Eastern Oyster (*Crassostrea virginica*) along the Atlantic Coast of Canada.” *Evolutionary Applications* 12 (3): 587–609.
- Bible, Jillian M., and Eric Sanford. 2016. “Local Adaptation in an Estuarine Foundation Species: Implications for Restoration.” *Biological Conservation* 193 (January): 95–102.
- Bierne, Nicolas, Denis Roze, and John J. Welch. 2013. “Pervasive Selection or Is It...? Why Are F_{ST} Outliers Sometimes so Frequent?” *Molecular Ecology* 22 (8): 2061–64.
- Bierne, Nicolas, John Welch, Etienne Loire, François Bonhomme, and Patrice David. 2011. “The Coupling Hypothesis: Why Genome Scans May Fail to Map Local Adaptation Genes.” *Molecular Ecology* 20 (10): 2044–72.
- Bierne, Nicolas, Claire Daguin, François Bonhomme, Patrice David, and Philippe Borsa. 2003. “Direct selection on allozymes is not required to explain heterogeneity among marker loci across a *Mytilus* hybrid zone”. *Molecular Ecology* 12(9), pp.2505-2510.

- Binyon, John. 1972. *Physiology of Echinoderms*. Oxford: New York: Pergamon Press.
- Bird, Christopher E., Brenden S. Holland, Brian W. Bowen, and Robert J. Toonen. 2011. "Diversification of sympatric broadcast-spawning limpets (*Cellana* spp.) within the Hawaiian archipelago." *Molecular Ecology* 20, no. 10 (2011): 2128-2141.
- Bird, Christopher E. 2011. "Morphological and behavioral evidence for adaptive diversification of sympatric Hawaiian limpets (*Cellana* spp.)". *Integrative and Comparative Biology* 51, no. 3: 466-473.
- Blanquart, François, Oliver Kaltz, Scott L. Nuismer, and Sylvain Gandon. 2013. "A practical guide to measuring local adaptation." *Ecology Letters* 16, no. 9: 1195-1205.
- Blomberg, Jonas, Dmitrijs Ushameckis, and Patric Jern. 2013. "Evolutionary Aspects of Human Endogenous Retroviral Sequences (HERVs) and Disease." *Madame Curie Bioscience Database. Landes Bioscience*.
- Bonsdorff, Erik. 2006. "Zoobenthic Diversity-Gradients in the Baltic Sea: Continuous Post-Glacial Succession in a Stressed Ecosystem." *Journal of Experimental Marine Biology and Ecology* 330 (1): 383–91.
- Bourret, Vincent, Mélanie Dionne, Matthew P. Kent, Sigbjørn Lien, and Louis Bernatchez. 2013. "Landscape Genomics in Atlantic Salmon (*Salmo salar*): Searching for Gene-Environment Interactions Driving Local Adaptation." *Evolution* 67 (12): 3469–87.
- Bradburd, Gideon S., Peter L. Ralph, and Graham M. Coop. 2013. "Disentangling the Effects of Geographic and Ecological Isolation on Genetic Differentiation." *Evolution* 67 (11): 3258–73.
- Bradbury, Ian R., Sophie Hubert, Brent Higgins, Tudor Borza, Sharen Bowman, Ian G. Paterson, Paul V. R. Snelgrove, et al. 2010. "Parallel Adaptive Evolution of Atlantic Cod on Both Sides of the Atlantic Ocean in Response to Temperature." *Proceedings. Biological Sciences / The Royal Society* 277 (1701): 3725–34.
- Bragg, Jason G., Megan A. Supple, Rose L. Andrew, and Justin O. Borevitz. 2015. "Genomic Variation across Landscapes: Insights and Applications." *The New Phytologist* 207 (4): 953–67.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2017. *Two Oceans: A Guide to the Marine Life of Southern Africa*. Penguin Random House South Africa.
- Branch, George and Margo Branch. 1981. *The Living Shores of Southern Africa*. Struik Publishers.
- Brandt, Ulrich. 2006. "Energy Converting NADH: Quinone Oxidoreductase (Complex I)." *Annual Review of Biochemistry* 75 (1): 69–92.
- Buckley, James, Roger K. Butlin, and Jon R. Bridle. 2012. "Evidence for Evolutionary Change Associated with the Recent Range Expansion of the British Butterfly, *Aricia agestis*, in Response to Climate Change." *Molecular Ecology* 21 (2): 267–80.
- Burton, Ronald S., and Felipe S. Barreto. 2012. "A Disproportionate Role for MtDNA in Dobzhansky-Muller Incompatibilities?" *Molecular Ecology* 21 (20): 4942–57.
- Cahill, Abigail E., Matthew E. Aiello-Lammens, M. Caitlin Fisher-Reid, Xia Hua, Caitlin J.

- Karanewsky, Ryu Hae Yeong, Gena C. Sbeglia, et al. 2013. "How Does Climate Change Cause Extinction?" *Proceedings of the Royal Society B: Biological Sciences* 280 (1750): 20121890.
- Cameron, R. Andrew, Manoj Samanta, Autumn Yuan, Dong He, and Eric Davidson. 2009. "SpBase: The Sea Urchin Genome Database and Web Site." *Nucleic Acids Research* 37 (Database): D750–54.
- Cameron, R. Andrew, Suk Hen Chow, Kevin Berney, Tsz-Yeung Chiu, Qiu-Autumn Yuan, Alexander Krämer, Argelia Helguero, Andrew Ransick, Mirong Yun, and Eric H. Davidson. 2005. "An Evolutionary Constraint: Strongly Disfavored Class of Change in DNA Sequence during Divergence of Cis-Regulatory Modules." *Proceedings of the National Academy of Sciences of the United States of America* 102 (33): 11769–74.
- Cano, José M., Takahito Shikano, Anna Kuparinen, and Juha Merilä. 2008. "Genetic Differentiation, Effective Population Size and Gene Flow in Marine Fishes: Implications for Stock Management." *Journal of Integrative Field Biology* 5: 1–10.
- Cantarel, Brandi L., Daniel Weaver, Nathan McNeill, Jianhua Zhang, Aaron J. Mackey, and Justin Reese. 2014. "BAYSIC: A Bayesian Method for Combining Sets of Genome Variants with Improved Specificity and Sensitivity." *BMC Bioinformatics* 15 (1): 104.
- Carreras, Carlos, Alex García-Cisneros, Owen S. Wangensteen, Víctor Ordóñez, Creu Palacín, Marta Pascual, and Xavier Turon. 2019. "East Is East and West Is West: Population Genomics and Hierarchical Analyses Reveal Genetic Structure and Adaptation Footprints in the Keystone Species *Paracentrotus lividus* (Echinoidea)." *Diversity and Distributions*, December, ddi.13016.
- Carroll, Joe, Ian M. Fearnley, Qing Wang, and John E. Walker. 2009. "Measurement of the Molecular Masses of Hydrophilic and Hydrophobic Subunits of ATP Synthase and Complex I in a Single Experiment." *Analytical Biochemistry* 395 (2): 249–55.
- Catchen, Julian M., Paul A. Hohenlohe, Louis Bernatchez, W. Chris Funk, Kimberly R. Andrews, and Fred W. Allendorf. 2017. "Unbroken: RADseq Remains a Powerful Tool for Understanding the Genetics of Adaptation in Natural Populations." *Molecular Ecology Resources* 17 (3): 362–65.
- Cavalli, Lissandra Souto, Carolina Reyes Batista, Bruna F. S. Nornberg, Fabiana Quoos Mayer, Fabiana K. Seixas, Luis Alberto Romano, Luis Fernando Marins, and Paulo César Abreu. 2013. "Natural Occurrence of White Spot Syndrome Virus and Infectious Hypodermal and Hematopoietic Necrosis Virus in *Neohelice granulata* Crab." *Journal of Invertebrate Pathology* 114 (1): 86–88.
- Chen, Zhongqi, Anthony P. Farrell, Amanda Matala, and Shawn R. Narum. 2018. "Mechanisms of Thermal Adaptation and Evolutionary Potential of Conspecific Populations to Changing Environments." *Molecular Ecology* 27 (3): 659–74.
- Chenal, Alexandre, Ana Cristina Sotomayor-Perez, and Daniel Ladant. 2015. "Structure and Function of RTX Toxins." In *The Comprehensive Sourcebook of Bacterial Protein Toxins*, 677–718. Elsevier Inc.
- Cheng, Jiao, Min Hui, Yulong Li, and Zhongli Sha. 2020. "Genomic Evidence of Population Genetic Differentiation in Deep-Sea Squat Lobster *Shinkaia crosnieri* (Crustacea: Decapoda: Anomura) from Northwestern Pacific Hydrothermal Vent and Cold Seep." *Deep-Sea Research Part I: Oceanographic Research Papers* 156: 103188.

- Cheng, Changde, Bradley J. White, Colince Kamdem, Keithanne Mockaitis, Carlo Costantini, Matthew W. Hahn, and Nora J. Besansky. 2012. "Ecological Genomics of *Anopheles gambiae* along a Latitudinal Cline: A Population-Resequencing Approach." *Genetics* 190 (4): 1417–32.
- Chevin, Luis-Miguel, Russell Lande, and Georgina M. Mace. 2010. "Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory." *PLoS Biology* 8 (4): e1000357.
- Christmas, Matthew J., Martin F. Breed, and Andrew J. Lowe. 2016. "Constraints to and Conservation Implications for Climate Change Adaptation in Plants." *Conservation Genetics* 17 (2): 305–20.
- Ciechanover, Aaron, Amir Orian, and Alan L. Schwartz. 2000. "Ubiquitin-Mediated Proteolysis: Biological Regulation via Destruction." *BioEssays* 22 (5): 442–51.
- Coffin, John M., Stephen Hughes, and Harold E. Varmus. 1997. *Retroviruses*. New York: Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
- Conover, David O., Tara A. Duffy, and Lyndie A. Hice. 2009. "The Covariance between Genetic and Environmental Influences across Ecological Gradients." *Annals of the New York Academy of Sciences* 1168 (1): 100–129.
- Conte, Gina L., Matthew E. Arnegard, Catherine L. Peichel, and Dolph Schluter. 2012. "The Probability of Genetic Parallelism and Convergence in Natural Populations." *Proceedings. Biological Sciences* 279 (1749): 5039–47.
- Coop, Graham, David Witonsky, Anna Di Rienzo, and Jonathan K Pritchard. 2010. "Using Environmental Correlations to Identify Loci Underlying Local Adaptation." *Genetics* 185 (4): 1411–23.
- Corander, Jukka, Pekka Marttinen, Jukka Sirén, and Jing Tang. 2006. "BAPS: Bayesian Analysis of Population Structure, Manual v. 4.1." *Department of Mathematics and Statistics, University of Helsinki*.
- Corander, Jukka, and Pekka Marttinen. 2006. "Bayesian Identification of Admixture Events Using Multilocus Molecular Markers." *Molecular Ecology* 15 (10): 2833–43.
- Cotton, James. 2001. "Retroviruses from Retrotransposons." *Genome Biology* 2 (2): reports0006.
- Corre, Valérie Le, and Antoine Kremer. 2012. "The Genetic Differentiation at Quantitative Trait Loci under Local Adaptation." *Molecular Ecology* 21 (7): 1548–66.
- Coyle, Aidan F., Erin R. Voss, Carolyn K. Tepolt, and David B. Carlon. 2019. "Mitochondrial Genotype Influences the Response to Cold Stress in the European Green Crab, *Carcinus maenas*." *Journal of Experimental Biology* 222 (17).
- Cunningham, Catherine I., Janice E. K. Cooke, and David W. Coltman. 2014. "Cross-Species Outlier Detection Reveals Different Evolutionary Pressures between Sister Species." *New Phytologist* 204: 215–29.
- Czarnomska, Sylwia D., Magdalena Niedziałkowska, Tomasz Borowik, and Bogumiła Jędrzejewska. 2019. "Winter Temperature Correlates with MtDNA Genetic Structure of

- Yellow-Necked Mouse Population in NE Poland.” *PLOS ONE* 14 (5): e0216361.
- Dalongeville, Alicia, Laura Benestan, David Mouillot, Stephane Lobreaux, and Stéphanie Manel. 2018. “Combining Six Genome Scan Methods to Detect Candidate Genes to Salinity in the Mediterranean Striped Red Mullet (*Mullus surmuletus*).” *BMC Genomics* 19 (1): 217.
- Davis, Beckley K., Haitao Wen, and Jenny P. Y. Ting. 2011. “The Inflammasome NLRs in Immunity, Inflammation, and Associated Diseases.” *Annual Review of Immunology* 29 (1): 707–35.
- Dayan, David I. 2018. “Clinal Adaptation in the Marine Environment.” In , 1–27. Springer, Cham.
- Deagle, Bruce E., Felicity C. Jones, Yingguang F. Chan, Devin M. Absher, David M. Kingsley, and Thomas E. Reimchen. 2012. “Population Genomics of Parallel Phenotypic Evolution in Stickleback across Stream–Lake Ecological Transitions.” *Proceedings of the Royal Society B: Biological Sciences* 279 (1732): 1277–86.
- DeFaveri, Jacquelin, and Juha Merilä. 2014. “Local Adaptation to Salinity in the Three-Spined Stickleback?” *Journal of Evolutionary Biology* 27 (2): 290–302.
- Delord, Chrystelle, Gilles Lassalle, Adrien Oger, Dominique Barloy, Marie-Agnes Agnes Coutellec, Adline Delcamp, Guillaume Evanno, et al. 2018. “A Cost-and-Time Effective Procedure to Develop SNP Markers for Multiple Species: A Support for Community Genetics.” *Methods in Ecology and Evolution* 9 (9): 1959–74.
- Delorme, Natalí J., and Mary A. Sewell. 2014. “Temperature and Salinity: Two Climate Change Stressors Affecting Early Development of the New Zealand Sea Urchin *Evechinus chloroticus*.” *Marine Biology* 161 (9): 1999–2009.
- Deng, Zhaochao, Shengyong Xu, Tianxiang Gao, and Zhiqiang Han. 2019. “Thermal Adaptation of Japanese Sand Lance (*Ammodytes personatus*) in Different Ocean Currents Revealed by the Cytochrome-b Gene.” *Marine and Freshwater Research* 70 (10): 1484.
- Desrina, Haryadi, Johan A. J. Verreth, Slamet Budi Prayitno, Jan H. W. M. Rombout, Just M. Vlak, and Marc C. J. Verdegem. 2013. “Replication of White Spot Syndrome Virus (WSSV) in the Polychaete *Dendronereis* Spp.” *Journal of Invertebrate Pathology* 114 (1): 7–10.
- Détrée, Camille, Edgar López-Landavery, Cristian Gallardo-Escárate, and Fabiola Lafarga-De la Cruz. 2017. “Transcriptome Mining of Immune-Related Genes in the Muricid Snail *Concholepas concholepas*.” *Fish and Shellfish Immunology* 71 (December): 69–75.
- Dillon, Shannon, Rachel McEvoy, Darren S. Baldwin, Gavin N. Rees, Yvonne Parsons, and Simon Southerton. 2014. “Characterisation of Adaptive Genetic Diversity in Environmentally Contrasted Populations of *Eucalyptus camaldulensis* Dehnh. (River Red Gum).” *PloS One* 9 (8): e103515.
- Dowling, Damian K., Urban Friberg, and Johan Lindell. 2008. “Evolutionary Implications of Non-Neutral Mitochondrial Genetic Variation.” *Trends in Ecology and Evolution* 23.10: 546–554.
- Drouin, Guy, John H. Himmelman, and Pierre Béland. 1985. “Impact of Tidal Salinity

- Fluctuations on Echinoderm and Mollusc Populations." *Canadian Journal of Zoology* 63 (6): 1377–87.
- Eckert, Andrew J., Andrew D. Bower, Santiago C. González-Martínez, Jill L. Wegrzyn, Graham Coop, and David B. Neale. 2010. "Back to Nature: Ecological Genomics of Loblolly Pine (*Pinus taeda*, Pinaceae)." *Molecular Ecology* 19 (17): 3789–3805.
- Ekblom, Robert, and Juan Galindo. 2011. "Applications of next Generation Sequencing in Molecular Ecology of Non-Model Organisms." *Heredity* 107 (1): 1–15.
- Ellegren, Hans. 2014. "Genome Sequencing and Population Genomics in Non-Model Organisms." *Trends in Ecology & Evolution* 29 (1): 51–63.
- Endler, John A. 1977. *Geographic Variation, Speciation, and Clines. Monographs in Population Biology*. 10th ed. Princeton University Press, Princeton, NJ.
- Endler, John A. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.
- Esposti, Mauro Degli, Simon De Vries, Massimo Crimi, Anna Ghelli, Tomaso Patarnello, and Axel Meyer. 1993. "Mitochondrial Cytochrome b: Evolution and Structure of the Protein." *BBA - Bioenergetics*. vol: 1143 (3): 243–271
- Evans, Tyler G., Melissa H. Pespeni, Gretchen E. Hofmann, Stephen R. Palumbi, and Eric Sanford. 2017. "Transcriptomic Responses to Seawater Acidification among Sea Urchin Populations Inhabiting a Natural pH Mosaic." *Molecular Ecology* 26 (8): 2257–75.
- Excoffier, Laurent, Tamara Hofer, and Matthieu Foll. 2009. "Detecting Loci under Selection in a Hierarchically Structured Population." *Heredity* 103 (4): 285–98.
- Excoffier, Laurent, and Nicolas Ray. 2008. "Surfing during Population Expansions Promotes Genetic Revolutions and Structuration." *Trends in Ecology and Evolution*. Elsevier Current Trends 23, no. 7: 347–351.
- Exposito-Alonso, Moises, François Vasseur, Wei Ding, George Wang, Hernán A. Burbano, and Detlef Weigel. 2018. "Genomic Basis and Evolutionary Potential for Extreme Drought Adaptation in *Arabidopsis thaliana*." *Nature Ecology & Evolution* 2 (2): 352–58.
- Faurby, Søren, and Paul H. Barber. 2012. "Theoretical Limits to the Correlation between Pelagic Larval Duration and Population Genetic Structure." *Molecular Ecology* 21 (14): 3419–32.
- Feis, Marieke E., Uwe John, Ana Lokmer, Pieternella C. Luttikhuisen, and K. Mathias Wegner. 2018. "Dual Transcriptomics Reveals Co-Evolutionary Mechanisms of Intestinal Parasite Infections in Blue Mussels *Mytilus edulis*." *Molecular Ecology* 27 (6): 1505–19.
- Fenderson, Lindsey E., Adrienne I. Kovach, and Bastien Llamas. 2020. "Spatiotemporal Landscape Genetics: Investigating Ecology and Evolution through Space and Time." *Molecular Ecology* 29 (2): 218–46.
- Feng, Xiao-Jing, Guo-Fang Jiang, and Zhou Fan. 2015. "Identification of Outliers in a Genomic Scan for Selection along Environmental Gradients in the Bamboo Locust, *Ceracris kiangsu*." *Scientific Reports* 5 (1): 13758.

- Feulner, Philine G. D., Frédéric J. J. Chain, Mahesh Panchal, Christophe Eizaguirre, Martin Kalbe, Tobias L. Lenz, Marvin Mundry, et al. 2013. "Genome-Wide Patterns of Standing Genetic Variation in a Marine Population of Three-Spined Sticklebacks." *Molecular Ecology* 22 (3): 635–49.
- Fick, Stephen E., and Robert J. Hijmans. 2017. "WorldClim 2: New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas." *International Journal of Climatology* 37 (12): 4302–15.
- Fischer, Martin C., Matthieu Foll, Laurent Excoffier, and Gerald Heckel. 2011. "Enhanced AFLP Genome Scans Detect Local Adaptation in High-Altitude Populations of a Small Rodent (*Microtus arvalis*)." *Molecular Ecology* 20 (7): 1450–62.
- Flanagan, Sarah P., Brenna R. Forester, Emily K. Latch, Sally N. Aitken, and Sean Hoban. 2018. "Guidelines for Planning Genomic Assessment and Monitoring of Locally Adaptive Variation to Inform Species Conservation." *Evolutionary Applications* 11 (7): 1035–52.
- Flegel, Timothy W. 2009. "Hypothesis for Heritable, Anti-Viral Immunity in Crustaceans and Insects." *Biology Direct* 4 (1): 32.
- Foden, Wendy B., Bruce E. Young, H. Resit Akçakaya, Raquel A. Garcia, Ary A. Hoffmann, Bruce A. Stein, Chris D. Thomas, et al. 2019. "Climate Change Vulnerability Assessment of Species." *Wiley Interdisciplinary Reviews: Climate Change* 10 (1): e551.
- Foll, Matthieu, Martin C. Fischer, Gerald Heckel, and Laurent Excoffier. 2010. "Estimating Population Structure from AFLP Amplification Intensity." *Molecular Ecology* 19 (21): 4638–47.
- Foll, Matthieu, and Oscar Gaggiotti. 2008. "A Genome-Scan Method to Identify Selected Loci Appropriate for Both Dominant and Codominant Markers: A Bayesian Perspective." *Genetics* 180 (2): 977–93.
- Fonseca, Rute R. da, Warren E. Johnson, Stephen J. O'Brien, Maria Ramos, and Agostinho Antunes. 2008. "The Adaptive Evolution of the Mammalian Mitochondrial Genome." *BMC Genomics* 9 (1): 119.
- Foot, Andrew D., Phillip A. Morin, John W. Durban, Robert L. Pitman, Paul Wade, Eske Willerslev, M. Thomas P. Gilbert, and Rute R. Da Fonseca. 2011. "Positive Selection on the Killer Whale Mitogenome." *Biology Letters* 7 (1): 116–18.
- Ford, Edmund Brisco. 1975. *Ecological Genetics*. Chapman and Hall.
- Forester, Brenna R., Jesse R. Lasky, Helene H. Wagner, and Dean L. Urban. 2018. "Comparing Methods for Detecting Multilocus Adaptation with Multivariate Genotype-Environment Associations." *Molecular Ecology* 27 (9): 2215–33.
- Fraïsse, Christelle, Khalid Belkhir, John J. Welch, and Nicolas Bierne. 2016. "Local Interspecies Introgression Is the Main Cause of Extreme Levels of Intraspecific Differentiation in Mussels." *Molecular Ecology* 25 (1): 269–86.
- Franks, Steven J., and Ary A. Hoffmann. 2012. "Genetics of Climate Change Adaptation." *Annual Review of Genetics* 46 (1): 185–208.
- Fraser, Dylan J., Laura K. Weir, Louis Bernatchez, Michael Møller Hansen, and Eric B.

- Taylor. "Extent and Scale of Local Adaptation in Salmonid Fishes: Review and Meta-Analysis." *Heredity* 106 (3): 404.
- Fraser, Dylan J., and Louis Bernatchez. 2001. "Adaptive Evolutionary Conservation: Towards a Unified Concept for Defining Conservation Units." *Molecular Ecology* 10 (12): 2741–52.
- Free, Christopher M., James T. Thorson, Malin L. Pinsky, Kiva L. Oken, John Wiedenmann, and Olaf P. Jensen. 2019. "Impacts of Historical Warming on Marine Fisheries Production." *Science* 363 (6430): 979–83.
- Fujita, Yuko, Panpaki Seekaki, Norichika Ogata, and Kazuhiro Chiba. 2017. "Physiological Effects of a Novel Artificially Synthesized Antimalarial Cyclic Peptide: Mahafacyclin B." *PLOS ONE* 12 (11): e0188415.
- Funk, W. Chris, Robert E. Lovich, Paul A. Hohenlohe, Courtney A. Hofman, Scott A. Morrison, T. Scott Sillett, Cameron K. Ghalambor, et al. 2016. "Adaptive Divergence despite Strong Genetic Drift: Genomic Analysis of the Evolutionary Mechanisms Causing Genetic Differentiation in the Island Fox (*Urocyon littoralis*)." *Molecular Ecology* 25 (10): 2176–94.
- Futuyma, Douglas J., and Gabriel Moreno. 1988. "The Evolution of Ecological Specialization." *Annual Review of Ecology and Systematics* 19 (1): 207–33.
- Gagnaire, Pierre-Alexandre Alexandre, and Oscar E. Gaggiotti. 2016. "Detecting Polygenic Selection in Marine Populations by Combining Population Genomics and Quantitative Genetics Approaches." *Current Zoology* 62 (6): 603–16.
- Gaither, Michelle R., Georgios A. Gkafas, Menno de Jong, Fatih Sarigol, Francis Neat, Thomas Regnier, Daniel Moore, et al. 2018. "Genomics of Habitat Choice and Adaptive Evolution in a Deep-Sea Fish." *Nature Ecology & Evolution* 2 (4): 680–87.
- Galaska, Matthew P., Chester J. Sands, Scott R. Santos, Andrew R. Mahon, and Kenneth M. Halanych. 2017. "Geographic Structure in the Southern Ocean Circumpolar Brittle Star *Ophionotus victoriae* (Ophiuridae) Revealed from MtDNA and Single-Nucleotide Polymorphism Data." *Ecology and Evolution* 7 (2): 475–85.
- Galindo, Juan, John W. Grahame, and Roger K. Butlin. 2010. "An EST-Based Genome Scan Using 454 Sequencing in the Marine Snail *Littorina saxatilis*." *Journal of Evolutionary Biology* 23 (9): 2004–16.
- Galindo, Juan, and John W. Grahame. 2014. "Ecological Speciation and the Intertidal Snail *Littorina saxatilis* ." *Advances in Ecology* 2014: 1–9.
- Galtier, Nicolas, Benoit Nabholz, Sylvain Glémin, and Gregory D. D. Hurst. 2009. "Mitochondrial DNA as a Marker of Molecular Diversity: A Reappraisal." *Molecular Ecology* 18 (22): 4541–50.
- Garcia, Hernan E., Timothy P. Boyer, Ricardo A. Locarnini, John I. Antonov, Alexey V. Mishonov, Olga K. Baranova, Melissa M. Zweng, James R. Reagan, Daphne R. Johnson, and Sydney Levitus. 2010. "World Ocean Atlas 2013. Volume 3, Dissolved Oxygen, Apparent Oxygen Utilization, and Oxygen Saturation." repository.library.noaa.gov.
- García-Ramos, Gisela, and Mark Kirkpatrick. 1997. "Genetic Models of Adaptation and Gene

- Flow in Peripheral Populations.” *Evolution* 51 (1): 21–28.
- Gaudin, François, Nicolas Desroy, Stanislas F. Dubois, Caroline Broudin, Louis Cabioch, Jérôme Fournier, Franck Gentil, et al. 2018. “Marine Sublittoral Benthos Fails to Track Temperature in Response to Climate Change in a Biogeographical Transition Zone.” *ICES Journal of Marine Science* 75 (6): 1894–1907.
- Gershoni, Moran, Alan R. Templeton, and Dan Mishmar. 2009. “Mitochondrial Bioenergetics as a Major Motive Force of Speciation.” *BioEssays* 31 (6): 642–50.
- Gillespie, John H. 1994. Alternatives to the neutral theory. In *Non-Neutral Evolution* (pp. 1–17). Springer, Boston, MA.
- Gleason, Lani U., and Ronald S. Burton. 2016. “Genomic Evidence for Ecological Divergence against a Background of Population Homogeneity in the Marine Snail *Chlorostoma funebris*.” *Molecular Ecology* 25 (15): 3557–73.
- Gleason, Lani Uriarte. 2015. “Ecological Genomics of an Intertidal Marine Snail: Population Structure and Local Adaptation to Heat Stress in *Chlorostoma* (Formerly *Tegula*) *funebris*. PhD Thesis.” University of California San Diego.
- Gonçalves da Silva, Anders, Sharon A. Appleyard, and Judy Upston. 2015. “Establishing the Evolutionary Compatibility of Potential Sources of Colonizers for Overfished Stocks: A Population Genomics Approach.” *Molecular Ecology* 24 (3): 564–79.
- Gong, Yi, Chenyu Ju, and Xiaobo Zhang. 2018. “Shrimp MiR-1000 Functions in Antiviral Immunity by Simultaneously Triggering the Degradation of Two Viral MRNAs.” *Frontiers in Immunology* 9: 2999.
- González, Josefa, J. Michael Macpherson, and Dmitri A. Petrov. 2009. “A Recent Adaptive Transposable Element Insertion Near Highly Conserved Developmental Loci in *Drosophila melanogaster*.” *Molecular Biology and Evolution* 26 (9): 1949–61.
- González, Josefa, Kapa Lenkov, Mikhail Lipatov, J. Michael Macpherson, and Dmitri A. Petrov. 2008. “High Rate of Recent Transposable Element–Induced Adaptation in *Drosophila melanogaster*.” *PLoS Biology* 6 (10): e251.
- Gosset, Céline C., and Nicolas Bierne. 2013. “Differential Introgression from a Sister Species Explains High F_{ST} Outlier Loci within a Mussel Species.” *Journal of Evolutionary Biology* 26 (1): 14–26.
- Graudal, Lars, Filippou Aravanopoulos, Zohra Bennadji, Suchitra Changtragoon, Bruno Fady, Erik D. Kjær, Judy Loo, Lolona Ramamonjisoa, and Giovanni G. Vendramin. 2014. “Global to Local Genetic Diversity Indicators of Evolutionary Potential in Tree Species within and Outside Forests.” *Forest Ecology and Management* 333: 35–51.
- Green Douglas R., and Reed John C. 1998. “Mitochondria and Apoptosis.” *Science* 281 (5381): 1309–12.
- Greenspan, Ralph J. 2005. “No Critter Left behind: An Invertebrate Renaissance.” *Current Biology* 15, no. 17: R671–R672.
- Griffitts, Joel S., Danielle L. Huffman, Johanna L. Whitacre, Brad D. Barrows, Lisa D. Marroquin, Reto Müller, Jillian R. Brown, Thierry Hennet, Jeffrey D. Esko, and Raffi V. Aroian. 2003. “Resistance to a Bacterial Toxin Is Mediated by Removal of a Conserved

- Glycosylation Pathway Required for Toxin-Host Interactions." *Journal of Biological Chemistry* 278 (46): 45594–602.
- Günther, Torsten, and Graham Coop. 2013. "Robust Identification of Local Adaptation from Allele Frequencies." *Genetics* 195 (1): 205–20.
- Guo, Baocheng, Jacquelin DeFaveri, Graciela Sotelo, Abhilash Nair, and Juha Merilä. 2015. "Population Genomic Evidence for Adaptive Differentiation in Baltic Sea Three-Spined Sticklebacks." *BMC Biology* 13: 19.
- Guo, Baocheng, Zitong Li, and Juha Merilä. 2016. "Population Genomic Evidence for Adaptive Differentiation in the Baltic Sea Herring." *Molecular Ecology* 25 (12): 2833–52.
- Haas, Ryan J., and Bret A. Payseur. 2016. "Fifteen Years of Genomewide Scans for Selection: Trends, Lessons and Unaddressed Genetic Sources of Complication." *Molecular Ecology* 25 (1): 5–23.
- Hamdoun, Amro, Catherine S. Schrankel, Katherine T. Nesbit, and Jose A. Espinoza. 2018. "Sea Urchins as Lab Animals for Reproductive and Developmental Biology." In *Encyclopedia of Reproduction*, 696–703. Elsevier.
- Han, Guo-Dong, and Yun-Wei Dong. 2020. "Rapid Climate-Driven Evolution of the Invasive Species *Mytilus galloprovincialis* over the Past Century." *Anthropocene Coasts* 3 (1): 14–29.
- Hancock, Angela M., David B. Witonsky, Adam S. Gordon, Gidon Eshel, Jonathan K. Pritchard, Graham Coop, and Anna Di Rienzo. 2008. "Adaptations to Climate in Candidate Genes for Common Metabolic Disorders." *PLoS Genetics* 4 (2): e32.
- Harms, Lars, Stephan Frickenhaus, Melanie Schiffer, Felix C. Mark, Daniela Storch, Hans Otto Pörtner, Christoph Held, and Magnus Lucassen. 2013. "Characterization and Analysis of a Transcriptome from the Boreal Spider Crab *Hyas araneus*." *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* 8 (4): 344–51.
- Harrisson, Katherine A., Alexandra Pavlova, Marina Telonis-Scott, and Paul Sunnucks. 2014. "Using Genomics to Characterize Evolutionary Potential for Conservation of Wild Populations." *Evolutionary Applications* 7 (9): 1008–25.
- Hart, Michael W., and Adam Foster. 2013. "Highly Expressed Genes in Gonads of the Bat Star *Patiria miniata*: Gene Ontology, Expression Differences, and Gamete Recognition Loci." *Invertebrate Biology* 132 (3): 241–50.
- Hart, Michael W. and Marko, Peter B. 2010. "It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates". *Integrative and Comparative Biology* 50(4), pp.643-661.
- Harvey, Ben P., Balsam Al-Janabi, Stefanie Broszeit, Rebekah Cioffi, Amit Kumar, Maria Aranguren-Gassis, Allison Bailey, et al. 2014. "Evolution of Marine Organisms under Climate Change at Different Levels of Biological Organisation." *Water* 6, no. 11: 3545-3574.
- Hassanin, Alexandre, Anne Ropiquet, Arnaud Couloux, and Corinne Cruaud. 2009. "Evolution of the Mitochondrial Genome in Mammals Living at High Altitude: New Insights from a Study of the Tribe Caprini (Bovidae, Antilopinae)." *Journal of Molecular Evolution* 68 (4): 293–310.

- Hauton, Chris. 2017. "Recent Progress toward the Identification of Anti-Viral Immune Mechanisms in Decapod Crustaceans." *Journal of Invertebrate Pathology* 147 (July): 111–17.
- Hayward, Alexander, Awaisa Ghazal, Göran Andersson, Leif Andersson, and Patric Jern. 2013. "ZBED Evolution: Repeated Utilization of DNA Transposons as Regulators of Diverse Host Functions." *PloS One* 8 (3): e59940.
- Heidelberger, Philip, and Peter D. Welch. 1981. "A Spectral Method for Confidence Interval Generation and Run Length Control in Simulations." *Communications of the ACM* 24 (4): 233–45.
- Heidelberger, Philip. 1983. "Simulation Run Length Control in the Presence of an Initial Transient." *Operations Research* 31 (6): 1109–44.
- Helmuth, Brian, Bernardo R. Broitman, Carol A. Blanchette, Sarah Gilman, Patricia Halpin, Christopher D. G. Harley, Michael J. O'Donnell, Gretchen E. Hofmann, Bruce Menge, and Denise Strickland. 2006a. "Mosaic Patterns of Thermal Stress in the Rocky Intertidal Zone: Implications for Climate Change." *Ecological Monographs* 76 (4): 461–79.
- Helmuth, Brian, Nova Mieszkowska, Pippa Moore, and Stephen J. Hawkins. 2006b. "Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change." *Annual Review of Ecology, Evolution, and Systematics*, 373–404.
- Hendricks, Sarah A., Rena M. Schweizer, and Robert K. Wayne. 2019. "Conservation Genomics Illuminates the Adaptive Uniqueness of North American Gray Wolves." *Conservation Genetics* 20 (1): 29–43.
- Hereford, Joe. 2009. "A Quantitative Survey of Local Adaptation and Fitness Trade-Offs." *The American Naturalist* 173 (5): 579–88.
- Hereford, Joe, and Alice A. Winn. 2008. "Limits to Local Adaptation in Six Populations of the Annual Plant *Diodia teres*." *New Phytologist* 178 (4): 888–96.
- Hershko, Avram, and Aaron Ciechanover. 1998. "The Ubiquitin System." *Annual Review of Biochemistry* 67 (1): 425–79.
- Hiddink, Jan G., Michael T. Burrows, and Jorge García Molinos. 2015. "Temperature Tracking by North Sea Benthic Invertebrates in Response to Climate Change." *Global Change Biology* 21 (1): 117–29.
- Hillebrand, Helmut, Thomas Brey, Julian Gutt, Wilhelm Hagen, Katja Metfies, Bettina Meyer, and Aleksandra Lewandowska. 2018. "Climate Change: Warming Impacts on Marine Biodiversity." In *Handbook on Marine Environment Protection : Science, Impacts and Sustainable Management*, 353–73. Cham: Springer International Publishing.
- Hoban, Sean, Joanna L. Kelley, Katie E. Lotterhos, Michael F. Antolin, Gideon Bradburd, David B. Lowry, Mary L. Poss, Laura K. Reed, Andrew Storfer, and Michael C. Whitlock. 2016. "Finding the Genomic Basis of Local Adaptation: Pitfalls, Practical Solutions, and Future Directions." *The American Naturalist* 188 (4): 379.
- Hock, Karlo, and Peter J. Mumby. 2015. "Quantifying the Reliability of Dispersal Paths in

- Connectivity Networks." *Journal of the Royal Society Interface* 12 (105).
- Hoelzel, A. Rus, Michael W. Bruford, and Robert C. Fleischer. 2019. "Conservation of Adaptive Potential and Functional Diversity." *Conservation Genetics* 20 (1): 1–5.
- Hoen, Douglas R., and Thomas E. Bureau. 2015. "Discovery of Novel Genes Derived from Transposable Elements Using Integrative Genomic Analysis." *Molecular Biology and Evolution* 32 (6): 1487–1506.
- Hoffmann, Ary A., and Carla M. Sgrò. 2011. "Climate Change and Evolutionary Adaptation." *Nature* 470 (7335): 479–85.
- Hohenlohe, Paul A., Susan Bassham, Paul D. Etter, Nicholas Stiffler, Eric A. Johnson, and William A. Cresko. 2010. "Population Genomics of Parallel Adaptation in Threespine Stickleback Using Sequenced RAD Tags." *PLoS Genetics* 6 (2): e1000862.
- Holmes, Edward C. 2011. "The Evolution of Endogenous Viral Elements." *Cell Host and Microbe* 10, no. 4: 368–377.
- Huang, K., Raj Whitlock, Malcolm C. Press, and Julie D. Scholes. 2012. "Variation for Host Range within and among Populations of the Parasitic Plant *Striga hermonthica*." *Heredity* 108 (2): 96–104.
- IPCC. 2014. "Climate Change 2014 Synthesis Report Summary for Policymakers."
- IPCC. 2013. "IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change."
- Jacobsen, Magnus W., Rute R. da Fonseca, Louis Bernatchez, and Michael M. Hansen. 2016. "Comparative Analysis of Complete Mitochondrial Genomes Suggests That Relaxed Purifying Selection Is Driving High Nonsynonymous Evolutionary Rate of the NADH2 Gene in Whitefish (*Coregonus Ssp.*)." *Molecular Phylogenetics and Evolution* 95 (February): 161–70.
- James, Gregory, Brian Key, and Annemiek Beverdam. 2014. "The E3 Ubiquitin Ligase Mycbp2 Genetically Interacts with Robo2 to Modulate Axon Guidance in the Mouse Olfactory System." *Brain Structure and Function* 219 (3): 861–74.
- Jasmin, Jean-Nicolas, and Rees Kassen. 2007. "On the Experimental Evolution of Specialization and Diversity in Heterogeneous Environments." *Ecology Letters* 10 (4): 272–81.
- Jeffery, Nicholas W., Ian R. Bradbury, Ryan R. E. Stanley, Brendan F. Wringe, Mallory Van Wyngaarden, J. Ben Lowen, Cynthia H. McKenzie, Kyle Matheson, Philip S. Sargent, and Claudio DiBacco. 2018. "Genomewide Evidence of Environmentally Mediated Secondary Contact of European Green Crab (*Carcinus maenas*) Lineages in Eastern North America." *Evolutionary Applications* 11 (6): 869–82.
- Jensen, Jeffrey D., Matthieu Foll, and Louis Bernatchez. 2016. "The Past, Present and Future of Genomic Scans for Selection." *Molecular Ecology* 25 (1): 1–4.
- Joly-Lopez, Zoé, Ewa Forczek, Douglas R. Hoen, Nikoleta Juretic, and Thomas E. Bureau. 2012. "A Gene Family Derived from Transposable Elements during Early Angiosperm Evolution Has Reproductive Fitness Benefits in *Arabidopsis thaliana*." *PLoS Genetics* 8

(9): e1002931.

- Jones, Peter M., and Anthony M. George. 2004. "The ABC Transporter Structure and Mechanism: Perspectives on Recent Research." *Cellular and Molecular Life Sciences* 61, no. 6: 682-699.
- Jones, Felicity C., Manfred G. Grabherr, Yingguang Frank Chan, Pamela Russell, Evan Mauceli, Jeremy Johnson, Ross Swofford, et al. 2012. "The Genomic Basis of Adaptive Evolution in Threespine Sticklebacks." *Nature* 484 (7392): 55–61.
- Jörnvall, Hans, Bengt Persson, Maria Krook, Silvia Atrian, Roser Gonzalez-Duarte, Jonathan Jeffery, and Debashis Ghosh. 1995. "Short-Chain Dehydrogenases/Reductases (SDR)." *Biochemistry* 34 (18): 6003–13.
- Jun, Albert S., Michael D. Brown, and Douglas C. Wallace. 1994. "A Mitochondrial DNA Mutation at Nucleotide Pair 14459 of the NADH Dehydrogenase Subunit 6 Gene Associated with Maternally Inherited Leber Hereditary Optic Neuropathy and Dystonia." *Proceedings of the National Academy of Sciences of the United States of America* 91 (13): 6206–10.
- Kaack, Katrina E., and Christopher M. Pomory. 2011. "Salinity Effects on Arm Regeneration in *Luidia clathrata* (Echinodermata: Asteroidea)." *Marine and Freshwater Behaviour and Physiology* 44 (6): 359–74.
- Kaniuga, Zbigniew. 1963. "The Transformation of Mitochondrial NADH Dehydrogenase into NADH: Cytochrome c Oxidoreductase." *Biochimica et Biophysica Acta (BBA) - Specialized Section on Enzymological Subjects* 73 (4): 550–64.
- Karlsen, Bård O., Åse Emblem, Tor E. Jørgensen, Kevin A. Klingan, Jarle T. Nordeide, Truls Moum, and Steinar D. Johansen. 2014. "Mitogenome Sequence Variation in Migratory and Stationary Ecotypes of North-East Atlantic Cod." *Marine Genomics* 15: 103–8.
- Katzourakis, Aris, and Robert J. Gifford. 2010. "Endogenous Viral Elements in Animal Genomes." *PLoS Genetics* 6 (11): e1001191.
- Kawar, Ziad S., Irma Van Die, and Richard D. Cummings. 2002. "Molecular Cloning and Enzymatic Characterization of a UDP-GalNAc:GlcNAc β -R B1,4-N-Acetylgalactosaminyltransferase from *Caenorhabditis elegans*." *Journal of Biological Chemistry* 277 (38): 34924–32.
- Kawecki, Tadeusz J., and Dieter Ebert. 2004. "Conceptual Issues in Local Adaptation." *Ecology Letters* 7 (12): 1225–41.
- Kelley, Joanna L., Anthony P. Brown, Nina Overgaard Therkildsen, and Andrew D. Foote. 2016. "The Life Aquatic: Advances in Marine Vertebrate Genomics." *Nature Reviews Genetics* 17 (9): 523–34.
- Kelly, Ryan P., and Stephen R. Palumbi. 2010. "Genetic Structure Among 50 Species of the Northeastern Pacific Rocky Intertidal Community." *PLoS ONE* 5 (1): e8594.
- Kess, Tony, Juan Galindo, and Elizabeth G. Boulding. 2018. "Genomic Divergence between Spanish *Littorina saxatilis* Ecotypes Unravels Limited Admixture and Extensive Parallelism Associated with Population History." *Ecology and Evolution* 8 (16): 8311–27.
- Kimura, Motoo 1991. "Recent development of the neutral theory viewed from the Wrightian

- tradition of theoretical population genetics". *Proceedings of the National Academy of Sciences* 88(14), pp.5969-5973.
- Klopfstein, Seraina, Mathias Currat, and Laurent Excoffier. 2006. "The Fate of Mutations Surfing on the Wave of a Range Expansion." *Molecular Biology and Evolution* 23 (3): 482–90.
- Knowles, Jeremy R. 1980. "Enzyme-Catalyzed Phosphoryl Transfer Reactions." *Annual Review of Biochemistry* 49 (1): 877–919.
- Knowles, L. Lacey. 2009. "Statistical Phylogeography." *Annual Review of Ecology, Evolution, and Systematics* 40 (1): 593–612.
- Kober, Kord M., and Grant H. Pogson. 2017. "Genome-Wide Signals of Positive Selection in Strongylocentrotid Sea Urchins." *BMC Genomics* 18 (1): 555.
- Kofler, Robert, Ram Vinay Pandey, and Christian Schlötterer. 2011. "PoPoolation2: Identifying Differentiation between Populations Using Sequencing of Pooled DNA Samples (Pool-Seq)." *Bioinformatics* 27 (24): 3435–36.
- Koh, Hye Yeon, Jun Hyuck Lee, Se Jong Han, Hyun Park, Seung Chul Shin, and Sung Gu Lee. 2015. "A Transcriptomic Analysis of the Response of the Arctic Pteropod *Limacina helicina* to Carbon Dioxide-Driven Seawater Acidification." *Polar Biology* 38 (10): 1727–40.
- Kolaczowski, Bryan, Andrew D. Kern, Alisha K. Holloway, and David J. Begun. 2011. "Genomic Differentiation between Temperate and Tropical Australian Populations of *Drosophila melanogaster*." *Genetics* 187 (1): 245–60.
- Kozak, Genevieve M., Reid S. Brennan, Emma L. Berdan, Rebecca C. Fuller, and Andrew Whitehead. 2014. "Functional and Population Genomic Divergence within and between Two Species of Killifish Adapted to Different Osmotic Niches." *Evolution* 68 (1): 63–80.
- Lai, Alvina G., and A. Aziz Aboobaker. 2017. "Comparative Genomic Analysis of Innate Immunity Reveals Novel and Conserved Components in Crustacean Food Crop Species." *BMC Genomics* 18 (1): 389.
- Lai, Yu-Ting, Carol K. L. Yeung, Kevin E. Omland, Er-Li Pang, Yu Hao, Ben-Yang Liao, Hui-Fen Cao, et al. 2019. "Standing Genetic Variation as the Predominant Source for Adaptation of a Songbird." *Proceedings of the National Academy of Sciences* 116 (6): 2152–57.
- Lal, Monal M., Paul C. Southgate, Dean R. Jerry, Cyprien Bosserelle, and Kyall R. Zenger. 2017. "Swept Away: Ocean Currents and Seascape Features Influence Genetic Structure across the 18,000 Km Indo-Pacific Distribution of a Marine Invertebrate, the Black-Lip Pearl Oyster *Pinctada margaritifera*." *BMC Genomics* 18 (1): 66.
- Lande, Russell. 1976. "Natural Selection and Random Genetic Drift in Phenotypic Evolution." *Evolution* 30 (2): 314–34.
- Lande, Russell, and Susan Shannon. 1996. "The Role of Genetic Variation in Adaptation and Population Persistence in a Changing Environment." *Evolution; International Journal of Organic Evolution* 50 (1): 434–37.
- Larsen, Peter Foged, Einar Eg Nielsen, Tim D. Williams, and Volker Loeschcke. 2008.

- "Intraspecific variation in expression of candidate genes for osmoregulation, heme biosynthesis and stress resistance suggests local adaptation in European flounder (*Platichthys flesus*)."
Heredity 101, no. 3: 247-259.
- Larsson, Josefine, Emma E. Lind, Hanna Corell, Mats Grahn, Katarzyna Smolarz, and Mikael Lönn. 2017. "Regional Genetic Differentiation in the Blue Mussel from the Baltic Sea Area." *Estuarine, Coastal and Shelf Science* 195 (August): 98–109.
- Latch, Emily K., Guha Dharmarajan, Jeffrey C. Glaubitz, and Olin E. Rhodes. 2006. "Relative Performance of Bayesian Clustering Software for Inferring Population Substructure and Individual Assignment at Low Levels of Population Differentiation." *Conservation Genetics* 7 (2): 295–302.
- Lathlean, Justin A., Laurent Seuront, and Terence P. T. Ng. 2017. "On the Edge: The Use of Infrared Thermography in Monitoring Responses of Intertidal Organisms to Heat Stress." *Ecological Indicators*. Elsevier B.V. 81: 567-577.
- Lawson, Daniel J., Lucy van Dorp, and Daniel Falush. 2018. "A Tutorial on How Not to Over-Interpret STRUCTURE and ADMIXTURE Bar Plots." *Nature Communications* 9 (1): 1–11.
- Legendre, Pierre, and Louis. Legendre. 2012. *Numerical Ecology*. Elsevier.
- Lehnert, Sarah J., Claudio DiBacco, Mallory Van Wyngaarden, Nicholas W. Jeffery, J. Ben Lowen, Emma V. A. Sylvester, Brendan F. Wringe, Ryan R. E. Stanley, Lorraine C. Hamilton, and Ian R. Bradbury. 2019. "Fine-Scale Temperature-Associated Genetic Structure between Inshore and Offshore Populations of Sea Scallop (*Placopecten magellanicus*)."
Heredity 122 (1): 69–80.
- Leimu, Roosa, and Markus Fischer. 2008. "A Meta-Analysis of Local Adaptation in Plants." *PLoS ONE* 3 (12): e4010.
- Leinonen, Tuomas, R. J. Scott McCairns, Robert B. O'Hara, and Juha Merilä. 2013. "QST–FST Comparisons: Evolutionary and Ecological Insights from Genomic Heterogeneity." *Nature Reviews Genetics* 14 (3): 179–90.
- Leiva, Carlos, Sergi Taboada, Nathan J. Kenny, David Combosch, Gonzalo Giribet, Thibaut Jombart, and Ana Riesgo. 2019. "Population Substructure and Signals of Divergent Adaptive Selection despite Admixture in the Sponge *Dendrilla antarctica* from Shallow Waters Surrounding the Antarctic Peninsula." *Molecular Ecology* 28 (13): mec.15135.
- Lesser, Michael P., and Matthew MacManes. 2016. "Transcriptomic Resources for the Rocky Intertidal Blue Mussel *Mytilus edulis* from the Gulf of Maine." *Journal of Shellfish Research* 35 (2): 435–65.
- Lewontin, Richard C., and Jesse Krakauer. 1973. "Distribution of Gene Frequency as a Test of the Theory of the Selective Neutrality of Polymorphisms." *Genetics* 74 (1).
- Li, Chao, and Dongxue Xu. 2018. "Understanding MicroRNAs Regulation in Heat Shock Response in the Sea Cucumber *Apostichopus japonicus*." *Fish and Shellfish Immunology* 81 (October): 214–20.
- Li, Ao, Li Li, Kai Song, Wei Wang, and Guofan Zhang. 2017. "Temperature, Energy Metabolism, and Adaptive Divergence in Two Oyster Subspecies." *Ecology and Evolution* 7 (16): 6151–62.

- Limborg, Morten T., Sarah J. Helyar, Mark De Bruyn, Martin I. Taylor, Einar E. Nielsen, R. O. B. Ogden, Gary R. Carvalho, FPT Consortium, and Dorte Bekkevold. 2012. "Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*)." *Molecular Ecology* 21, no. 15: 3686-3703.
- Linhart, Yan B., and Michael C. Grant. 1996. "Evolutionary Significance of Local Genetic Differentiation in Plants." *Annual Review of Ecology and Systematics* 27 (1): 237–77.
- Linhartová, Irena, Ladislav Bumba, Jiř Mařn, Marek Basler, Radim Osička, Jana Kamanová, Kateřina Procházková, et al. 2010. "RTX Proteins: A Highly Diverse Family Secreted By a Common Mechanism." *FEMS Microbiology Reviews* 34(6): 1076-1112.
- Linton, Kenneth J. 2007. "Structure and Function of ABC Transporters." *Physiology* 22, no. 2: 122-130.
- Lischer, Heidi E. L., and Laurent Excoffier. 2012. "PGDSpider: An Automated Data Conversion Tool for Connecting Population Genetics and Genomics Programs." *Bioinformatics* 28 (2): 298–99.
- Liu Xingyu, Li Li, Ao Li, Yingxiang Li, Wei Wang, and Guofan Zhang. 2019. "Transcriptome and Gene Coexpression Network Analyses of Two Wild Populations Provides Insight into the High-Salinity Adaptation Mechanisms of *Crassostrea ariakensis*." *Marine Biotechnology*, June, 1–17.
- Loarie, Scott R., Philip B. Duffy, Healy Hamilton, Gregory P. Asner, Christopher B. Field, and David D. Ackerly. 2009. "The Velocity of Climate Change." *Nature* 462 (7276): 1052–55.
- Locarnini, Ricardo A., Alexey V. Mishonov, John I. Antonov, Timothy P. Boyer, Hernan E. Garcia, Olga K. Baranova, Melissa M. Zweng, et al. 2013. "World Ocean Atlas 2013, Volume 1: Temperature" A. *Mishonov Technical Ed. , NOAA Atlas NESDIS* 73: 40.
- Lotterhos, Katie E., and Michael C. Whitlock. 2014. "Evaluation of Demographic History and Neutral Parameterization on the Performance of F_{ST} Outlier Tests." *Molecular Ecology* 23 (9): 2178–92.
- Lotterhos, Katie E., and Michael C. Whitlock. 2015. "The Relative Power of Genome Scans to Detect Local Adaptation Depends on Sampling Design and Statistical Method." *Molecular Ecology* 24 (5): 1031–46.
- Lou, Fangrui, Tianxiang Gao, and Zhiqiang Han. 2019. "Transcriptome Analyses Reveal Alterations in Muscle Metabolism, Immune Responses and Reproductive Behavior of Japanese Mantis Shrimp (*Oratosquilla oratoria*) at Different Cold Temperature." *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* 32: 100615.
- Lowry, David B., Sean Hoban, Joanna L. Kelley, Katie E. Lotterhos, Laura K. Reed, Michael F. Antolin, and Andrew Storfer. 2017. "Breaking RAD: An Evaluation of the Utility of Restriction Site-Associated DNA Sequencing for Genome Scans of Adaptation." *Molecular Ecology Resources* 17 (2): 142–52.
- Luikart, Gordon, Phillip R. England, David Tallmon, Steve Jordan, and Pierre Taberlet. 2003. "The Power and Promise of Population Genomics: From Genotyping to Genome Typing." *Nature Reviews. Genetics* 4 (12): 981–94.

- Luo, Yongjun, Wenxiang Gao, Yuqi Gao, Sha Tang, Qingyuan Huang, Xiaoling Tan, Jian Chen, and Taosheng Huang. 2008. "Mitochondrial Genome Analysis of *Ochotona curzoniae* and Implication of Cytochrome c Oxidase in Hypoxic Adaptation." *Mitochondrion* 8 (5–6): 352–57.
- Macías-Rodríguez, Norma A., Nathalie Mañón-Ríos, Jesús L. Romero-Romero, Erika Camacho-Beltrán, Marco A. Magallanes-Tapia, Norma E. Leyva-López, Jorge Hernández-López, et al. 2014. "Prevalence of Viral Pathogens WSSV and IHNV in Wild Organisms at the Pacific Coast of Mexico." *Journal of Invertebrate Pathology* 116 (1): 8–12.
- Malachowicz, Magdalena, and Roman Wenne. 2019. "Microarray Analysis of Gene Expression of Atlantic Cod from Different Baltic Sea Regions: Adaptation to Salinity." *Marine Genomics* 48: 100681.
- Malik, Harmit S., Steve Henikoff, and Thomas H. Eickbush. 2000. "Poised for Contagion: Evolutionary Origins of the Infectious Abilities of Invertebrate Retroviruses." *Genome Research* 10 (9): 1307–18.
- Manel, Stéphanie, Charles Perrier, Marine Pratlong, Laurent Abi-Rached, Julien Paganini, Pierre Pontarotti, and Didier Aurelle. 2016. "Genomic Resources and Their Influence on the Detection of the Signal of Positive Selection in Genome Scans." *Molecular Ecology* 25 (1): 170–84.
- Manel, Stéphanie, Stéphane Joost, Bryan K. Epperson, Rolf Holderegger, Andrew Storfer, Michael S. Rosenberg, Kim T. Scribner, Aurélie Bonin, and Marie-Josée Fortin. 2010. "Perspectives on the Use of Landscape Genetics to Detect Genetic Adaptive Variation in the Field." *Molecular Ecology* 19 (17): 3760–72.
- Marbà, Núria, Gabriel Jordà, Susana Agustí, Coraline Girard, and Carlos M. Duarte. 2015. "Footprints of Climate Change on Mediterranean Sea Biota." *Frontiers in Marine Science* 2 (August): 56.
- Marko, Peter B. and Hart, Michael W. 2011. "The complex analytical landscape of gene flow inference". *Trends in Ecology & Evolution* 26(9), pp.448-456.
- Maroso, Francesco, Raffaella Franch, Giulia Dalla Rovere, Marco Arculeo, and Luca Bargelloni. 2016. "RAD SNP Markers as a Tool for Conservation of Dolphinfinh *Coryphaena hippurus* in the Mediterranean Sea: Identification of Subtle Genetic Structure and Assessment of Populations Sex-Ratios." *Marine Genomics* 28: 57–62.
- Marques, David A., Felicity C. Jones, Federica Di Palma, David M. Kingsley, and Thomas E. Reimchen. 2018. "Experimental Evidence for Rapid Genomic Adaptation to a New Niche in an Adaptive Radiation." *Nature Ecology & Evolution* 2 (7): 1128–38.
- Matala, Andrew P., Jon E. Hess, and Shawn R. Narum. 2011. "Resolving Adaptive and Demographic Divergence among Chinook Salmon Populations in the Columbia River Basin." *Transactions of the American Fisheries Society* 140 (3): 783–807.
- Matthey-Doret, Remi, and Michael C. Whitlock. 2019. "Background Selection and F_{ST} : Consequences for Detecting Local Adaptation." *Molecular Ecology*, mec.15197.
- McGaughan, Angela, Christian Rödelberger, Dominik G. Grimm, Jan M. Meyer, Eduardo Moreno, Katy Morgan, Mark Leaver, et al. 2016. "Genomic Profiles of Diversification and Genotype–Phenotype Association in Island Nematode Lineages." *Molecular*

Biology and Evolution 33 (9): 2257–72.

- McKenzie, Matthew, Michael Lazarou, and Michael T. Ryan. 2009. "Chapter 18: Analysis of Respiratory Chain Complex Assembly with Radiolabeled Nuclear- and Mitochondrial-Encoded Subunits." *Methods in Enzymology* 456: 321-339.
- McQuaid, Christopher D., Francesca Porri, Katy R. Nicastro, and Gerardo I. Zardi. 2015. "Simple, scale-dependent patterns emerge from very complex effects: an example from the intertidal mussels *Mytilus galloprovincialis* and *Perna perna*." *Oceanography Marine Biology* 53: 127-156.
- Mead, Angela, Charles L. Griffiths, George M. Branch, Christopher D. McQuaid, Laura K. Blamey, John J. Bolton, Robert J. Anderson, Francois Dufois, Mathieu Rouault, P. William Froneman, Alan K. Whitfield, Linda R. Harris, Ronel Nel, Deena Pillay, Janine B. Adams. 2013. "Human-Mediated Drivers of Change - Impacts on Coastal Ecosystems and Marine Biota of South Africa." *African Journal of Marine Science* 35 (3): 403–25.
- Metivier, Stacy L., Jin-Hong Kim, and Jason A. Addison. 2017. "Genotype by Sequencing Identifies Natural Selection as a Driver of Intraspecific Divergence in Atlantic Populations of the High Dispersal Marine Invertebrate, *Macoma petalum*." *Ecology and Evolution* 7 (19): 8058–72.
- Micheletti, Steven J., Amanda R. Matala, Andrew P. Matala, and Shawn R. Narum. 2018. "Landscape Features along Migratory Routes Influence Adaptive Genomic Variation in Anadromous Steelhead (*Oncorhynchus mykiss*)." *Molecular Ecology* 27 (1): 128–45.
- Milano, Ilaria, Massimiliano Babbucci, Alessia Cariani, Miroslava Atanassova, Dorte Bekkevold, Gary R. Carvalho, Montserrat Espiñeira et al. 2014. "Outlier SNP Markers Reveal Fine-scale Genetic Structuring across European Hake Populations (*Merluccius merluccius*)." *Molecular Ecology* 23 (1): 118–35.
- Miller, Luke P., Christopher D. G. Harley, and Mark W. Denny. 2009. "The Role of Temperature and Desiccation Stress in Limiting the Local-scale Distribution of the Owl Limpet, *Lottia gigantea*." *Functional Ecology* 23 (4): 756–67.
- Miller, Adam D., Anthony van Rooyen, Gordana Rašić, Daniel A. Ierodiaconou, Harry K. Gorfine, Rob Day, C. Wong, Ary A. Hoffmann, and Andrew R. Weeks. 2016. "Contrasting Patterns of Population Connectivity between Regions in a Commercially Important Mollusc *Halotis rubra*: Integrating Population Genetics, Genomics and Marine LiDAR Data." *Molecular Ecology* 25 (16): 3845–64.
- Mills, L. Scott, and Fred W. Allendorf. 1996. "The One-Migrant-per-Generation Rule in Conservation and Management." *Conservation Biology* 10 (6): 1509–18.
- Mishmar, Dan, Eduardo Ruiz-Pesini, Pawel Golik, Vincent Macaulay, Andrew G. Clark, Seyed Hosseini, Martin Brandon, et al. 2003. "Natural Selection Shaped Regional MtDNA Variation in Humans." *Proceedings of the National Academy of Sciences of the United States of America* 100 (1): 171–76.
- Mita, Stéphane De, Anne-Céline Céline Thuillet, Laurène Gay, Nourollah Ahmadi, Stéphanie Manel, Joëlle Ronfort, and Yves Vigouroux. 2013. "Detecting Selection along Environmental Gradients: Analysis of Eight Methods and Their Effectiveness for Outbreeding and Selfing Populations." *Molecular Ecology* 22 (5): 1383–99.

- Mitchell, Peter. 2011. "Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation." *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1807, no. 12: 1507-1538.
- Mittel, Elizabeth A., Shinichi Nakagawa, and Jarrod D. Hadfield. 2015. "Are Molecular Markers Useful Predictors of Adaptive Potential?" *Ecology Letters* 18 (8): 772–78.
- Mmonwa, Kolobe Lucas, Peter R. Teske, Christopher D. McQuaid, and Nigel P. Barker. 2015. "Historical Demography of Southern African Patellid Limpets: Congruence of Population Expansions, but Not Phylogeography." *African Journal of Marine Science* 37, no. 1: 11-20.
- Montes, Iratxe, Iratxe Zarraonaindia, Mikel Iriondo, W. Stewart Grant, Carmen Manzano, Unai Cotano, Darrell Conklin, Xabier Irigoien, and Andone Estonba. 2016. "Transcriptome Analysis Deciphers Evolutionary Mechanisms Underlying Genetic Differentiation between Coastal and Offshore Anchovy Populations in the Bay of Biscay." *Marine Biology* 163 (10): 205.
- Morley, Simon Anthony, Khanh Dung Nguyen, Lloyd S. Peck, Chien-Houng Lai, and Koh Siang Tan. 2017. "Can Acclimation of Thermal Tolerance, in Adults and across Generations, Act as a Buffer against Climate Change in Tropical Marine Ectotherms?" *Journal of Thermal Biology* 68: 195–99.
- Morris, S., and Alan C. Taylor. 1983. "Diurnal and Seasonal Variation in Physico-Chemical Conditions within Intertidal Rock Pools." *Estuarine, Coastal and Shelf Science* 17 (3): 339–55.
- Motegi, Akira, Raman Sood, Helen Moinova, Sanford D. Markowitz, Pu Paul Liu, and Kyungjae Myung. 2006. "Human SHPRH Suppresses Genomic Instability through Proliferating Cell Nuclear Antigen Polyubiquitination." *Journal of Cell Biology* 175 (5): 703–8.
- Mu, Wendan, Jun Liu, and Haibin Zhang. 2018. "Complete Mitochondrial Genome of *Benthodytes marianensis* (Holothuroidea: Elasipodida: Psychropotidae): Insight into Deep Sea Adaptation in the Sea Cucumber." *PLOS ONE* 13 (11): e0208051.
- Mu, Xingjiang, Maoliang Su, Lang Gui, Xuemei Liang, Peipei Zhang, Pan Hu, Zhenhao Liu, and Junbin Zhang. 2015. "Comparative Renal Gene Expression in Response to Abrupt Hypoosmotic Shock in Spotted Scat (*Scatophagus argus*)." *General and Comparative Endocrinology* 215 (May): 25–35.
- Muller, Cornelius M., Sophie von der Heyden, Rauri C. K. Bowie, and Conrad A. Matthee. 2012. "Oceanic Circulation, Local Upwelling and Palaeoclimatic Changes Linked to the Phylogeography of the Cape Sea Urchin *Parechinus angulosus*." *Marine Ecology Progress Series* 468: 203.
- Nakayama, Akiyoshi, Hirotaka Matsuo, Takuya Shimizu, Hiraku Ogata, Yuzo Takada, Hiroshi Nakashima, Takahiro Nakamura, et al. 2013. "A Common Missense Variant of Monocarboxylate Transporter 9 (MCT9/SLC16A9) Gene Is Associated with Renal Overload Gout, but Not with All Gout Susceptibility." *Human Cell* 26 (4): 133–36.
- Nanninga, Gerrit B., Pablo Saenz-Agudelo, Andrea Manica, and Michael L. Berumen. 2014. "Environmental Gradients Predict the Genetic Population Structure of a Coral Reef Fish in the Red Sea." *Molecular Ecology* 23 (3): 591–602.

- Narum, Shawn R., and Jon E. Hess. 2011. "Comparison of F_{ST} Outlier Tests for SNP Loci under Selection." *Molecular Ecology Resources* 11: 184–94.
- Neave, Matthew J., Serge Corbeil, Kenneth A. McColl, and Mark S. J. Crane. 2019. "Investigating the Natural Resistance of Blackfoot Pāua *Haliotis iris* to Abalone Viral Ganglioneuritis Using Whole Transcriptome Analysis." *Diseases of Aquatic Organisms* 135 (2): 107–19.
- Nei, Masatoshi, and Wen-Hsiung Li. 1979. "Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases." *Proceedings of the National Academy of Sciences of the United States of America* 76 (10): 5269–73.
- Nei, Masatoshi, and Takeo Maruyama. 1975. "Lewontin-Krakauertest for neutral genes." *Genetics* 80, no. 2: 395.
- Nevado, Bruno, and Miguel Perez-Enciso. 2015. "Pipeliner: Software to Evaluate the Performance of Bioinformatics Pipelines for next-Generation Resequencing." *Molecular Ecology Resources* 15 (1): 99–106.
- Newmeyer, Donald D., and Shelagh Ferguson-Miller. 2003. "Mitochondria: Releasing Power for Life and Unleashing the Machineries of Death." *Cell* 112, no. 4: 481–490.
- Nielsen, Erica S., Romina Henriques, Robert J. Toonen, Ingrid S. S. Knapp, Baocheng Guo, and Sophie von der Heyden. 2018. "Complex Signatures of Genomic Variation of Two Non-Model Marine Species in a Homogeneous Environment." *BMC Genomics* 19 (1): 347.
- Nieva, Leyre Villota. 2019. "Effects of in Situ Oceanic Warming on Marine Benthic Recruitment and Community Development in Antarctica. (PhD Thesis)." Bangor University.
- Ning, T., H. Xiao, J. Li, S. Hua, and Y. P. Zhang. 2010. "Adaptive Evolution of the Mitochondrial ND6 Gene in the Domestic Horse." *Genetics and Molecular Research*. Vol. 9, no. 1: 144–150.
- Nosil, Patrik. 2012. *Ecological Speciation*. Oxford University Press.
- Nosil, Patrik, Daniel J. Funk, and Daniel Ortiz-Barrientos. 2009. "Divergent Selection and Heterogeneous Genomic Divergence." *Molecular Ecology* 18 (3): 375–402.
- Nunes, Vera L., Mark A. Beaumont, Roger K. Butlin, and Octávio S. Paulo. 2011. "Multiple Approaches to Detect Outliers in a Genome Scan for Selection in Ocellated Lizards (*Lacerta lepida*) along an Environmental Gradient." *Molecular Ecology* 20 (2): 193–205.
- Nunez, Joaquin C. B., Rebecca G. Elyanow, David A. Ferranti, and David M. Rand. 2018. "Population Genomics and Biogeography of the Northern Acorn Barnacle (*Semibalanus balanoides*) Using Pooled Sequencing Approaches." In , 1–30. Springer, Cham.
- Oakey, J., C. Smith, D. Underwood, M. Afsharnasab, V. Alday-Sanz, A. Dhar, S. Sivakumar, A. S. Sahul Hameed, K. Beattie, and A. Crook. 2019. "Global Distribution of White Spot Syndrome Virus Genotypes Determined Using a Novel Genotyping Assay." *Archives of Virology* 164, no. 8: 2061–2082.
- Ohta, Tomoko. 1992. "The nearly neutral theory of molecular evolution". *Annual Review of Ecology and Systematics* 23(1), pp.263–286.

- O'Leary, Shannon J., Jonathan B. Puritz, Stuart C. Willis, Christopher M. Hollenbeck, and David S. Portnoy. 2018. "These Aren't the Loci You're Looking for: Principles of Effective SNP Filtering for Molecular Ecologists." *Molecular Ecology* 27 (16): 3193–3206.
- Oksanen, Jari, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, et al. 2018. "Vegan: Community Ecology Package. R Package Version 2.5-2."
- Orsini, Luisa, Joost Vanoverbeke, Ine Swillen, Joachim Mergeay, and Luc De Meester. 2013. "Drivers of Population Genetic Differentiation in the Wild: Isolation by Dispersal Limitation, Isolation by Adaptation and Isolation by Colonization." *Molecular Ecology* 22 (24): 5983–99.
- Pante, Eric, Vanessa Becquet, Amélia Viricel, and Pascale Garcia. 2019. "Investigation of the Molecular Signatures of Selection on ATP Synthase Genes in the Marine Bivalve *Limecola balthica*." *Aquatic Living Resources* 32: 3.
- Papadopoulou, Anna, and L. Lacey Knowles. 2016. "Toward a Paradigm Shift in Comparative Phylogeography Driven by Trait-Based Hypotheses." *Proceedings of the National Academy of Sciences* 113 (29): 8018–24.
- Pardo-Diaz, Carolina, Camilo Salazar, and Chris D. Jiggins. 2015. "Towards the Identification of the Loci of Adaptive Evolution." *Methods in Ecology and Evolution* 6 (4): 445–64.
- Parmesan, Camille. 2006. "Ecological and Evolutionary Responses to Recent Climate Change." *Annual Review of Ecology, Evolution, and Systematics* 37 (1): 637–69.
- Paterno, Marta, Marcello Schiavina, Giorgio Aglieri, Jamila Ben Souissi, Elisa Boscari, Renato Casagrandi, Aurore Chassanite, et al. 2017. "Population Genomics Meet Lagrangian Simulations: Oceanographic Patterns and Long Larval Duration Ensure Connectivity among *Paracentrotus lividus* Populations in the Adriatic and Ionian Seas." *Ecology and Evolution* 7 (8): 2463–79.
- Pauls, Steffen U., Carsten Nowak, Miklós Bálint, and Markus Pfenninger. 2013. "The Impact of Global Climate Change on Genetic Diversity within Populations and Species." *Molecular Ecology* 22 (4): 925–46.
- Peck, Lloyd S. 2011. "Organisms and Responses to Environmental Change." *Marine Genomics* 4(4): 237–243.
- Pereira, Henrique M., Paul W. Leadley, Vânia Proença, Rob Alkemade, Jörn P. W. Scharlemann, Juan F. Fernandez-Manjarrés, Miguel B. Araújo, et al. 2010. "Scenarios for Global Biodiversity in the 21st Century." *Science* 330 (6010): 1496–1501.
- Pérez-Figueroa, Andrés, María Jesús García-Pereira, María Saura, Emilio Rolán-Alvarez, and Armando Caballero. 2010. "Comparing Three Different Methods to Detect Selective Loci Using Dominant Markers." *Journal of Evolutionary Biology* 23 (10): 2267–76.
- Perrier, Charles, Anne-Laure Ferchaud, Pascal Sirois, Isabel Thibault, and Louis Bernatchez. 2017. "Do Genetic Drift and Accumulation of Deleterious Mutations Preclude Adaptation? Empirical Investigation Using RADseq in a Northern Lacustrine Fish." *Molecular Ecology* 26 (22): 6317–35.

- Perry, Allison L., Paula J. Low, Jim R. Ellis, and John D. Reynolds. 2005. "Climate Change and Distribution Shifts in Marine Fishes." *Science* 308 (5730): 1912–15.
- Pespeni, Melissa H., David A. Garfield, Mollie K. Manier, and Stephen R. Palumbi. 2012. "Genome-Wide Polymorphisms Show Unexpected Targets of Natural Selection." *Proceedings of the Royal Society B: Biological Sciences* 279 (1732): 1412–20.
- Phair, Nikki Leanne, Robert John Toonen, Ingrid Knapp, and Sophie von der Heyden. 2019. "Shared Genomic Outliers across Two Divergent Population Clusters of a Highly Threatened Seagrass." *PeerJ* 7 (e27517v1): e6806.
- Picard, Laurent, Jean François Coquil, and Jean Pierre Mauger. 1998. "Multiple Mechanisms of Regulation of the Inositol 1,4,5-Trisphosphate Receptor by Calcium." *Cell Calcium* 23 (5): 339–48.
- Pineda, Jesús, Francesca Porri, Victoria Starczak, and Jonathan Blythe. 2010. "Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity." *Journal of Experimental Marine Biology and Ecology* 392, no. 1-2: 9-21.
- Pinho, Catarina, Vera Cardoso, and Jody Hey. 2019. "A Population Genetic Assessment of Taxonomic Species: The Case of Lake Malawi Cichlid Fishes." *Molecular Ecology Resources* 19 (5): 1755-0998.13027.
- Plough, Louis V. 2017. "Population Genomic Analysis of the Blue Crab *Callinectes sapidus* Using Genotyping-By-Sequencing." *Journal of Shellfish Research* 36 (1): 249–61.
- Plummer, Martyn, Nicky Best, Kate Cowles, and Karen Vines. 2006. "CODA: Convergence Diagnosis and Output Analysis for MCMC." *R News* 6 (1): 7–11.
- Pohl, Christian, and Stefan Jentsch. 2008. "Final Stages of Cytokinesis and Midbody Ring Formation Are Controlled by BRUCE." *Cell* 132 (5): 832–45.
- Porcelli, Don, Roger K. Butlin, Kevin J. Gaston, D. Joly, and Rhonda R. Snook. 2015. "The Environmental Genomics of Metazoan Thermal Adaptation." *Heredity* 114, no. 5: 502-514.
- Porri, Francesca, Jennifer M. Jackson, Charles E. O. Von der Meden, Nicolas Weidberg, and Christopher D. McQuaid. 2014. "The effect of mesoscale oceanographic features on the distribution of mussel larvae along the south coast of South Africa." *Journal of Marine Systems* 132: 162-173.
- Porri, Francesca, Christopher D. McQuaid, and Sarah Radloff. 2006. "Spatio-temporal variability of larval abundance and settlement of *Perna perna*: differential delivery of mussels." *Marine Ecology Progress Series* 315: 141-150.
- Pörtner, Hans Otto, and Anthony P. A. P. Farrell. 2008. "Ecology: Physiology and Climate Change." *Science* 322 (5902): 690–92.
- Pritchard, Jonathan K., Matthew Stephens, and Peter Donnelly. 2000. "Inference of Population Structure Using Multilocus Genotype Data." *Genetics* 155 (2).
- Prado, Fernanda Dotti do, Manuel Vera, Miguel Hermida, Carmen Bouza, Belén G. Pardo, Román Vilas, Andrés Blanco, et al. 2018. "Parallel Evolution and Adaptation to

- Environmental Factors in a Marine Flatfish: Implications for Fisheries and Aquaculture Management of the Turbot (*Scophthalmus maximus*)." *Evolutionary Applications* 11 (8): 1322–41.
- Pratlong, Marine, Anne Haguenauer, Kelly Brener, Guillaume Mitta, Eve Toulza, Joaquim Garrabou, Nathaniel Bensoussan, Pierre Pontarotti, and Didier Aurelle. 2018. "Separate the Wheat from the Chaff: Genomic Scan for Local Adaptation in the Red Coral *Corallium rubrum*." *BioRxiv*, December, 306456.
- Puechmaille, Sebastien J. 2016. "The Program Structure Does Not Reliably Recover the Correct Population Structure When Sampling Is Uneven: Subsampling and New Estimators Alleviate the Problem." *Molecular Ecology Resources* 16 (3): 608–27.
- Puritz, Jonathan B., Mikhail V. Matz, Robert J. Toonen, Jesse N. Weber, Daniel I. Bolnick, and Christopher E. Bird. 2014. "Demystifying the RAD fad". *Molecular Ecology* 23(24), pp.5937-5942.
- Puritz, Jonathan B., and Robert J. Toonen. 2011. "Coastal pollution limits pelagic larval dispersal." *Nature Communications* 2, no.1: 1-8.
- QGIS Development Team. 2016. "QGIS."
- Raj, Anil, Matthew Stephens, and Jonathan K. Pritchard. 2014. "FastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets." *Genetics* 197 (2): 573–89.
- Rand, David M., Robert A. Haney, and Adam J. Fry. 2004. "Cytonuclear Coevolution: The Genomics of Cooperation." *Trends in Ecology and Evolution* 19, no. 12: 645-653
- Rast, Jonathan P., L. Courtney Smith, Mariano Loza-Coll, Taku Hibino, and Gary W. Litman. 2006. "Genomic Insights into the Immune System of the Sea Urchin." *Science* 314, no. 5801: 952-956.
- Ravinet, Mark, Rui Faria, Roger K. Butlin, Juan Galindo, Nicolas Bierne, Marina Rafajlović, Mohamed A. F. Noor, Bernhard Mehlig, and Anja M. Westram. 2017. "Interpreting the Genomic Landscape of Speciation: A Road Map for Finding Barriers to Gene Flow." *Journal of Evolutionary Biology* 30 (8): 1450–77.
- Ravinet, Mark, Anja Westram, Kerstin Johannesson, Roger Butlin, Carl André, and Marina Panova. 2016. "Shared and Nonshared Genomic Divergence in Parallel Ecotypes of *Littorina saxatilis* at a Local Scale." *Molecular Ecology* 25 (1): 287–305.
- Rellstab, Christian, Felix Gugerli, Andrew J. Eckert, Angela M. Hancock, and Rolf Holderegger. 2015. "A Practical Guide to Environmental Association Analysis in Landscape Genomics." *Molecular Ecology* 24 (17): 4348–70.
- Renaut, Sébastien, Arne W. Nolte, Sean M. Rogers, Nicolas Derome, and Louis Bernatchez. 2011. "SNP Signatures of Selection on Standing Genetic Variation and Their Association with Adaptive Phenotypes along Gradients of Ecological Speciation in Lake Whitefish Species Pairs (*Coregonus* Spp.)." *Molecular Ecology* 20 (3): 545–59.
- Rey, Olivier, Etienne Danchin, Marie Mirouze, Céline Loot, and Simon Blanchet. 2016. "Adaptation to Global Change: A Transposable Element-Epigenetics Perspective." *Trends in Ecology and Evolution* 31, no. 7: 514-526.

- Rhode, Clint. 2013. "Signatures of Selection in Natural and Cultured Abalone (*Haliotis midae*): A Population Genomics Study. Phd Thesis." Stellenbosch University.
- Rhode, Clint, Aletta E. Bester-van der Merwe, and Rouvay Roodt-Wilding. 2017. "An Assessment of Spatio-Temporal Genetic Variation in the South African Abalone (*Haliotis midae*), Using SNPs: Implications for Conservation Management." *Conservation Genetics* 18 (1): 17–31.
- Riginos, Cynthia, Eric D. Crandall, Libby Liggins, Pim Bongaerts, and Eric A. Trembl. 2016. "Navigating the Currents of Seascape Genomics: How Spatial Analyses Can Augment Population Genomic Studies." *Current Zoology* 62 (6): 581–601.
- Robertson, Anthony J., Jenifer Croce, Seth Carbonneau, Ekaterina Voronina, Esther Miranda, David R. McClay, and James A. Coffman. 2006. "The Genomic Underpinnings of Apoptosis in *Strongylocentrotus purpuratus*." *Developmental Biology* 300 (1): 321–34.
- Robinson, Nicholas A., Gopalapillay Gopikrishna, Matthew Baranski, Vinaya Kumar Katneni, Mudagandur S. Shekhar, Jayakani Shanmugakarathik, Sarangapani Jothivel, et al. 2014. "QTL for White Spot Syndrome Virus Resistance and the Sex-Determining Locus in the Indian Black Tiger Shrimp (*Penaeus monodon*)." *BMC Genomics* 15 (1): 731.
- Rock, Jenny, Julia L. Magnay, S. Beech, Alicia J. El Haj, Geoff Goldspink, David H. Lunt, and Nia M. Whiteley. 2009. "Linking Functional Molecular Variation with Environmental Gradients: Myosin Gene Diversity in a Crustacean Broadly Distributed across Variable Thermal Environments." *Gene* 437 (1–2): 60–70.
- Roesti, Marius, Andrew P. Hendry, Walter Salzburger, Daniel Berner, Walter Salzburger, and Daniel Berner. 2012. "Genome Divergence during Evolutionary Diversification as Revealed in Replicate Lake-Stream Stickleback Population Pairs." *Molecular Ecology* 21 (12): 2852–62.
- Romiguier, Jonathan, Philippe Gayral, Marion Ballenghien, Arnaud Bernard, Vincent Cahais, A. Chenuil, Ylenia Chiari et al. 2014. "Comparative Population Genomics in Animals Uncovers the Determinants of Genetic Diversity." *Nature* 515 (7526): 261–63.
- Ronchi, Dario, Alessandra Cosi, Davide Tonduti, Simona Orcesi, Andreina Bordoni, Francesco Fortunato, Mafalda Rizzuti, et al. 2011. "Clinical and Molecular Features of an Infant Patient Affected by Leigh Disease Associated to m.14459G > A Mitochondrial DNA Mutation: A Case Report." *BMC Neurology* 11 (1): 85.
- Rousset, Francois. 2008. "Genepop'007: A Complete Re-implementation of the Genepop Software for Windows and Linux." *Molecular Ecology Resources* vol: 8 (1): 103-106
- Russell, Michael P. 2013. "Echinoderm Responses to Variation in Salinity." In *Advances in Marine Biology*, 66:171–212.
- Sánchez-Paz, Arturo, Berenice Terán-Díaz, Tania Enríquez-Espinoza, Trinidad Encinas-García, Isela Vázquez-Sánchez, and Fernando Mendoza-Cano. 2015. "The Tidepool Shrimp, *Palaemon ritteri* Holmes, Constitutes a Novel Host to the White Spot Syndrome Virus." *Journal of Fish Diseases* 38 (7): 613–20.
- Sandoval-Castillo, Jonathan, Nick A. Robinson, Anthony M. Hart, Lachlan W. S. Strain, and Luciano B. Beheregaray. 2018. "Seascape Genomics Reveals Adaptive Divergence in a Connected and Commercially Important Mollusc, the Greenlip Abalone (*Haliotis*

- laevigata*), along a Longitudinal Environmental Gradient.” *Molecular Ecology* 27 (7): 1603–20.
- Sanford, Eric, and Morgan W. Kelly. 2011. “Local Adaptation in Marine Invertebrates.” *Annual Review of Marine Science* 3 (1): 509–35.
- Saraste, Matti. 1999. “Oxidative Phosphorylation at the Fin de Siecle.” *Science* 283, no. 5407: 1488-1493.
- Saunier, Alice, Pascale Garcia, Vanessa Becquet, Nathalie Marsaud, Frédéric Escudié, and Eric Pante. 2014. “Mitochondrial Genomes of the Baltic Clam *Macoma balthica* (Bivalvia: Tellinidae): Setting the Stage for Studying Mito-Nuclear Incompatibilities.” *BMC Evolutionary Biology* 14 (1): 259.
- Savolainen, Outi, Martin Lascoux, and Juha Merilä. 2013. “Ecological Genomics of Local Adaptation.” *Nature Reviews Genetics* 14 (11): 807–20.
- Schilling, Martin P., Paul G. Wolf, Aaron M. Duffy, Hardeep S. Rai, Carol A. Rowe, Bryce A. Richardson, and Karen E. Mock. 2014. “Genotyping-by-Sequencing for *Populus* Population Genomics: An Assessment of Genome Sampling Patterns and Filtering Approaches.” *PloS One* 9 (4): e95292.
- Schluter, Dolph. 2009. “Evidence for Ecological Speciation and Its Alternative.” *Science (New York, N.Y.)* 323 (5915): 737–41.
- Schomburg, Dietmar, and Dörte Stephan. 1995. “UDP-N-Acetylmuramate Dehydrogenase.” In *Enzyme Handbook* 10, 29–32. Springer Berlin Heidelberg.
- Schruben, Lee W. 1982. “Detecting Initialization Bias in Simulation Output.” *Operations Research* 30 (3): 569–90.
- Seebacher, Frank, Martin D. Brand, Paul L. Else, Helga Guderley, Anthony J. Hulbert, and Christopher D. Moyes. 2010. “Plasticity of Oxidative Metabolism in Variable Climates: Molecular Mechanisms.” *Physiological and Biochemical Zoology: PBZ* 83 (5): 721–32.
- Selkoe, Kimberly A. and Toonen, Robert J. 2011. “Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal”. *Marine Ecology Progress Series* 436, pp.291-305.
- Sexton, Jason P., Sandra B. Hangartner, and Ary A. Hoffmann. 2014. “Genetic Isolation by Environment or Distance: Which Pattern of Gene Flow Is Most Common?” *Evolution* 68 (1): 1–15.
- Sgrò, Carla M., Andrew J. Lowe, and Ary A. Hoffmann. 2011. “Building Evolutionary Resilience for Conserving Biodiversity under Climate Change.” *Evolutionary Applications* 4 (2): 326–37.
- Shafer, Aaron, and Jochen B. W. Wolf. 2013. “Widespread Evidence for Incipient Ecological Speciation: A Meta-analysis of Isolation-by-ecology.” *Ecology Letters* 16 (7): 940–50.
- Sheftel, Alex D., Oliver Stehling, Antonio J. Pierik, Daili J. A. Netz, Stefan Kerscher, Hans-Peter Elsässer, Ilka Wittig, Janneke Balk, Ulrich Brandt, and Roland Lill. 2009. “Human Ind1, an Iron-Sulfur Cluster Assembly Factor for Respiratory Complex I.” *Molecular and Cellular Biology* 29 (22): 6059–73.

- Siepielski, Adam M., Kiyoko M. Gotanda, Michael B. Morrissey, Sarah E. Diamond, Joseph D. Dibattista, and Stephanie M. Carlson. 2013. "The Spatial Patterns of Directional Phenotypic Selection." *Ecology Letters* 16, no. 11: 1382-1392.
- Silliman, Katherine. 2019a. "Population Structure and Local Adaptation in the Olympia Oyster (*Ostrea lurida*)". PhD Thesis. The University of Chicago.
- Silliman, Katherine. 2019b. "Population Structure, Genetic Connectivity, and Adaptation in the Olympia Oyster (*Ostrea lurida*) along the West Coast of North America." *Evolutionary Applications* 12 (5): 923–39.
- Silva, Gonçalo, Fernando P. Lima, Paulo Martel, and Rita Castilho. 2014. "Thermal Adaptation and Clinal Mitochondrial DNA Variation of European Anchovy." *Proceedings of the Royal Society B: Biological Sciences* 281 (1792).
- Simonson, Tatum S., Yingzhong Yang, Chad D. Huff, Haixia Yun, Ga Qin, David J. Witherspoon, Zhenzhong Bai, et al. 2010. "Genetic Evidence for High-Altitude Adaptation in Tibet." *Science* 329 (5987): 72–75.
- Sinclair, Elektra L. E., Michael B. Thompson, and Frank Seebacher. 2006. "Phenotypic Flexibility in the Metabolic Response of the Limpet *Cellana tramoserica* to Thermally Different Microhabitats." *Journal of Experimental Marine Biology and Ecology* 335 (1): 131–41.
- Slatkin, Montgomery. 1993. "Isolation by Distance in Equilibrium and Non-Equilibrium Populations." *Evolution* 47 (1): 264–79.
- Slimen, Hichem Ben, Helmut Schaschl, Felix Knauer, and Franz Suchentrunk. 2017. "Selection on the Mitochondrial ATP Synthase 6 and the NADH Dehydrogenase 2 Genes in Hares (*Lepus capensis* L., 1758) from a Steep Ecological Gradient in North Africa." *BMC Evolutionary Biology* 17 (1): 46.
- Smit, Albertus J., Michael Roberts, Robert J. Anderson, Francois Dufois, Sheldon F. J. Dudley, Thomas G. Bornman, Jennifer Olbers, and John J. Bolton. 2013. "A Coastal Seawater Temperature Dataset for Biogeographical Studies: Large Biases between in Situ and Remotely-Sensed Data Sets around the Coast of South Africa." *PLOS One* 8 (12): e81944.
- Sodergren, Erica, Yufeng Shen, Xingzhi Song, Lan Zhang, Richard A. Gibbs, and George M. Weinstock. 2006. "Shedding Genomic Light on Aristotle's Lantern." *Developmental Biology* 300, no. 1: 2-8.
- Sokolova, Inna M., Markus Frederich, Rita Bagwe, Gisela Lannig, and Alexey A. Sukhotin. 2012. "Energy Homeostasis as an Integrative Tool for Assessing Limits of Environmental Stress Tolerance in Aquatic Invertebrates." *Marine Environmental Research* 79: 1–15.
- Somero, George N. 2002. "Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living." *Integrative and Comparative Biology* 42 (4): 780–89.
- Somero, George N. 2012. "The Physiology of Global Change: Linking Patterns to Mechanisms." *Annual Review of Marine Science* 4 (1): 39–61.
- Somero, George N., Brent L. Lockwood, and Lars Tomanek. 2017. *Biochemical Adaptation:*

Response to Environmental Challenges from Life's Origins to the Anthropocene. Oxford University Press.

- Sørensen, Jesper G., and Volker Loeschcke. 2007. "Studying Stress Responses in the Post-Genomic Era: Its Ecological and Evolutionary Role." *Journal of Biosciences* 32, no. 3: 447-456.
- Stanley, Ryan R. E., Claudio DiBacco, Ben Lowen, Robert G. Beiko, Nick W. Jeffery, Mallory Van Wyngaarden, Paul Bentzen, et al. 2018. "A Climate-Associated Multispecies Cryptic Cline in the Northwest Atlantic." *Science Advances* 4 (3): eaaq0929.
- Stickle, William B., and Walter J. Diehl. 1987. "Effects of Salinity on Echinoderms." *Echinoderm Studies* 2: 235-285.
- Stinchcombe, John R., Cynthia Weinig, Mark Ungerer, Kenneth M. Olsen, Charlotte Mays, Solveig S. Halldorsdottir, Michael D. Purugganan, and Johanna Schmitt. 2004. "A Latitudinal Cline in Flowering Time in *Arabidopsis thaliana* Modulated by the Flowering Time Gene FRIGIDA." *Proceedings of the National Academy of Sciences of the United States of America* 101 (13): 4712-17.
- Stockwell, Brian L., Wesley A. Larson, Ryan K. Waples, Rene A. Abesamis, Lisa W. Seeb, and Kent E. Carpenter. 2016. "The Application of Genomics to Inform Conservation of a Functionally Important Reef Fish (*Scarus niger*) in the Philippines." *Conservation Genetics* 17 (1): 239-49.
- Storz, Jay F. 2005. "Using Genome Scans of DNA Polymorphism to Infer Adaptive Population Divergence." *Molecular Ecology* 14 (3): 671-88.
- Sun, Shao'e, Ming Hui, Minxiao Wang, and Zhongli Sha. 2018. "The Complete Mitochondrial Genome of the Alvinocaridid Shrimp *Shinkaicaris leurokolos* (Decapoda, Caridea): Insight into the Mitochondrial Genetic Basis of Deep-Sea Hydrothermal Vent Adaptation in the Shrimp." *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics* 25: 42-52.
- Sylvester, Emma V. A., Robert G. Beiko, Paul Bentzen, Ian Paterson, John B. Horne, Beth Watson, Sarah Lehnert, et al. 2018. "Environmental Extremes Drive Population Structure at the Northern Range Limit of Atlantic Salmon in North America." *Molecular Ecology* 27 (20): 4026-40.
- Tang, Kathy F. J., Marc Le Groumellec, and Donald V. Lightner. 2013. "Novel, Closely Related, White Spot Syndrome Virus (WSSV) Genotypes from Madagascar, Mozambique and the Kingdom of Saudi Arabia." *Diseases of Aquatic Organisms* 106 (1): 1-6.
- Tay, Ywee Chieh, M. W. P. Chng, Genevieve Sew, Frank Erwin Rheindt, Karenne P. P. Tun, and Rudolf Meier. 2016. "Beyond the Coral Triangle: High Genetic Diversity and near Panmixia in Singapore's Populations of the Broadcast Spawning Sea Star *Protoreaster nodosus*." *Royal Society Open Science* 3 (8): 160253.
- Teixeira, Leonardo K., and Steven I. Reed. 2013. "Ubiquitin Ligases and Cell Cycle Control." *Annual Review of Biochemistry* 82 (1): 387-414.
- Tenaillon, Maud I., and Peter L. Tiffin. 2008. "The Quest for Adaptive Evolution: A Theoretical Challenge in a Maze of Data." *Current Opinion in Plant Biology* 11(2):110-15.

- Teshima, Kosuke M., Graham Coop, and Molly Przeworski. 2006. "How Reliable Are Empirical Genomic Scans for Selective Sweeps?" *Genome Research* 16 (6): 702–12.
- Teske, Peter R., Isabelle Papadopoulos, Brent K. Newman, Peter C. Dworschak, Christopher D. McQuaid, and Nigel P. Barker. 2008. "Oceanic Dispersal Barriers, Adaptation and Larval Retention: An Interdisciplinary Assessment of Potential Factors Maintaining a Phylogeographic Break between Sister Lineages of an African Prawn." *BMC Evolutionary Biology* 8 (1): 341.
- Teske, Peter R., Jonathan Sandoval-Castillo, Tirupathi Rao Golla, Arsalan Emami-Khoyi, Mbaye Tine, Sophie von der Heyden, and Luciano B. Beheregaray. 2019. "Thermal Selection as a Driver of Marine Ecological Speciation." *Proceedings of the Royal Society B* 286 (1896): 20182023.
- Tiffin, Peter, and Jeffrey Ross-Ibarra. 2014. "Advances and Limits of Using Population Genetics to Understand Local Adaptation." *Trends in Ecology & Evolution* 29 (12): 673–80.
- Tigano, Anna, Allison J. Shultz, Scott V. Edwards, Gregory J. Robertson, and Vicki L. Friesen. 2017. "Outlier Analyses to Test for Local Adaptation to Breeding Grounds in a Migratory Arctic Seabird." *Ecology and Evolution* 7 (7): 2370–81.
- Timmins-Schiffman, Emma, José M. Guzmán, Rhonda Elliott, Brent Vadopalas, and Steven B. Roberts. 2019. "Dynamic Response in the Larval Geoduck Clam Proteome to Elevated PCO₂." *BioRxiv*, 1–27.
- Tine, Mbaye, Heiner Kuhl, Pierre-Alexandre Gagnaire, Bruno Louro, Erick Desmarais, Rute S. T. Martins, Jochen Hecht, et al. 2014. "European Sea Bass Genome and Its Variation Provide Insights into Adaptation to Euryhalinity and Speciation." *Nature Communications* 5 (1): 5770.
- Ting, Jenny P. Y., Ruth C. Lovering, Emad S. Alnemri, John Bertin, Jeremy M. Boss, Beckley K. Davis, Richard A. Flavell, et al. 2008. "The NLR Gene Family: A Standard Nomenclature." *Immunity* 28, no. 3: 285–287.
- Tisthammer, Kaho H., Zac H. Forsman, Robert J. Toonen, and Robert H. Richmond. 2020. "Genetic structure is stronger across human-impacted habitats than among islands in the coral *Porites lobata*." *PeerJ* 8: e8550.
- Tisthammer, Kaho H., Emma Timmins-Schiffman, Francois O. Seneca, Brook L. Nunn, and Robert H. Richmond. 2019. "Physiological and Molecular Responses Suggest Local Adaptation of the Lobe Coral *Porites lobata* to the Nearshore Environment." *BioRxiv*, September, 786673.
- Todgham, Anne E., and Gretchen E. Hofmann. 2009. "Transcriptomic Response of Sea Urchin Larvae *Strongylocentrotus purpuratus* to CO₂-Driven Seawater Acidification." *Journal of Experimental Biology* 212 (16): 2579–94.
- Toonen, Robert J., Puritz, Jonathan B., Forsman, Zac H., Whitney, Jonathan L., Fernandez-Silva, Iria, Andrews, Kimberly R. and Bird, Christopher E. 2013. "ezRAD: a simplified method for genomic genotyping in non-model organisms". *PeerJ* 1, p.e203.
- Toonen, Robert J., Kimberly R. Andrews, Iliana B. Baums, Christopher E. Bird, Gregory T. Concepcion, Toby S. Daly-Engel, Jeff A. Eble, et al. 2011. "Defining Boundaries for

Ecosystem-Based Management: A Multispecies Case Study of Marine Connectivity across the Hawaiian Archipelago.” *Journal of Marine Biology* Article ID 460173

- Toonen, Robert J. and Grosberg, Richard K. 2011. Causes of chaos: spatial and temporal genetic heterogeneity in the intertidal anomuran crab *Petrolisthes cinctipes*. In: Held, Koenemann & Schubart (Eds.) *Phylogeography and Population Genetics in Crustacea*. CRC Press. pp.75-107.
- Tsukihara, Tomitake, Hiroshi Aoyama, Eiki Yamashita, Takashi Tomizaki, Hiroshi Yamaguchi, Kyoko Shinzawa-Itoh, Ryosuke Nakashima, Rieko Yaono, and Shinya Yoshikawa. 1996. “The Whole Structure of the 13-Subunit Oxidized Cytochrome c Oxidase at 2.8 Å.” *Science* 272 (5265): 1136–44.
- Travis, Justin M. J., Tamara Münkemüller, Olivia J. Burton, Alex Best, Calvin Dytham, and Karin Johst. 2007. “Deleterious Mutations Can Surf to High Densities on the Wave Front of an Expanding Population.” *Molecular Biology and Evolution* 24 (10): 2334–43.
- Urban, Mark C. 2015. “Climate Change. Accelerating Extinction Risk from Climate Change.” *Science (New York, N.Y.)* 348 (6234): 571–73.
- Uthicke, Sven, Nandan P. Deshpande, Michelle Liddy, Frances Patel, Miles Lamare, and Marc R. Wilkins. 2019. “Little Evidence of Adaptation Potential to Ocean Acidification in Sea Urchins Living in ‘Future Ocean’ Conditions at a CO₂ Vent.” *Ecology and Evolution* 9 (17): 10004–16.
- van’t Hof, Arjen E., Pascal Campagne, Daniel J. Rigden, Carl J. Yung, Jessica Lingley, Michael A. Quail, Neil Hall, Alistair C. Darby, and Ilik J. Saccheri. 2016. “The Industrial Melanism Mutation in British Peppered Moths Is a Transposable Element.” *Nature* 534 (7605): 102–5.
- Vasemägi, Anti, and Craig R. Primmer. 2005. “Challenges for Identifying Functionally Important Genetic Variation: The Promise of Combining Complementary Research Strategies.” *Molecular Ecology* 14 (12): 3623–42.
- Vasemägi, Anti. 2006. “The Adaptive Hypothesis of Clinal Variation Revisited: Single-Locus Clines as a Result of Spatially Restricted Gene Flow.” *Genetics* 173 (4): 2411–14.
- Vera, Manuel, Belén G. Pardo, Asunción Cao, Román Vilas, Carlos Fernández, Andrés Blanco, Alejandro P. Gutierrez, et al. 2019. “Signatures of Selection for Bonamiosis Resistance in European Flat Oyster (*Ostrea edulis*): New Genomic Tools for Breeding Programs and Management of Natural Resources.” *Evolutionary Applications* 12 (9): 1781–96.
- Verri, Tiziano, Genciana Terova, Alessandro Romano, Amilcare Barca, Paola Pisani, Carlo Storelli, and Marco Saroglia. 2012. “The SoLute Carrier (SLC) Family Series in Teleost Fish.” In *Functional Genomics in Aquaculture*, 219–320. Oxford, UK: Wiley-Blackwell.
- Versluis, Dennis, Bart Nijse, Mohd Azrul Naim, Jasper J. Koehorst, Jutta Wiese, Johannes F. Imhoff, Peter J. Schaap, Mark W. J. Van Passel, Hauke Smidt, and Detmer Sipkema. 2018. “Comparative Genomics Highlights Symbiotic Capacities and High Metabolic Flexibility of the Marine Genus *Pseudovibrio*.” *Genome Biology and Evolution* 10 (1): 125–42.
- Villemereuil, Pierre, and Oscar E. Gaggiotti. 2015. “A New *FST*-based Method to Uncover Local Adaptation Using Environmental Variables.” *Methods in Ecology and Evolution* 6,

no. 11: 1248-1258.

- Villemereuil, Pierre de, Éric Frichot, Éric Bazin, Olivier François, and Oscar E. Gaggiotti. 2014. "Genome Scan Methods against More Complex Models: When and How Much Should We Trust Them?" *Molecular Ecology* 23 (8): 2006–19.
- von der Heyden, Sophie, Maria Beger, Robert J. Toonen, Lynne van Herwerden, Marie Antonette Juinio-Meñez, Rachel Ravago-Gotanco, Cecile Fauvelot, and Giacomo Bernardi. 2014. "The Application of Genetics to Marine Management and Conservation: Examples from the Indo-Pacific." *Bulletin of Marine Science* 90 (1): 123–58.
- Wallace, Douglas C. 2007. "Why Do We Still Have a Maternally Inherited Mitochondrial DNA? Insights from Evolutionary Medicine." *Annual Review of Biochemistry* 76 (1): 781–821.
- Wang, Juan, Dong-Xiu Xue, Bai-Dong Zhang, Yu-Long Li, Bing-Jian Liu, and Jin-Xian Liu. 2016. "Genome-Wide SNP Discovery, Genotyping and Their Preliminary Applications for Population Genetic Inference in Spotted Sea Bass (*Lateolabrax maculatus*)." *PLOS ONE* 11 (6): e0157809.
- Wang, Zhengfei, Xuejia Shi, Linxia Sun, Yuze Bai, Daizhen Zhang, and Boping Tang. 2017. "Evolution of Mitochondrial Energy Metabolism Genes Associated with Hydrothermal Vent Adaption of Alvinocaridid Shrimps." *Genes and Genomics* 39 (12): 1367–76.
- Waples, Robin S., and Oscar E. Gaggiotti. 2006. "What Is a Population? An Empirical Evaluation of Some Genetic Methods for Identifying the Number of Gene Pools and Their Degree of Connectivity." *Molecular Ecology* 15, no. 6: 1419-1439.
- Wares, John P., and Katelyn M. Skoczen. 2019. "Maintenance of a Genetic Cline in the Barnacle *Balanus glandula*." *The Biological Bulletin* 236 (3): 199–206.
- Watson, R. G. A., Simone Baldanzi, Andrés Pérez-Figueroa, Gavin Gouws, and Francesca Porri. 2018. "Morphological and Epigenetic Variation in Mussels from Contrasting Environments." *Marine Biology* 165 (3): 50.
- Watterson, G. A. 1975. "On the Number of Segregating Sites in Genetical Models without Recombination." *Theoretical Population Biology* 7 (2): 256–76.
- Weersing, Kimberley and Toonen, Robert J. 2009. "Population genetics, larval dispersal, and connectivity in marine systems". *Marine Ecology Progress Series* 393, pp.1-12.
- Weidberg, Nicolás, Francesca Porri, Charles E. O. Von der Meden, Jennifer M. Jackson, Wayne Goschen, and Christopher D. McQuaid. 2015. "Mechanisms of nearshore retention and offshore export of mussel larvae over the Agulhas Bank." *Journal of Marine Systems* 144: 70-80.
- Weigand, Hannah, and Florian Leese. 2018. "Detecting Signatures of Positive Selection in Non-Model Species Using Genomic Data." *Zoological Journal of the Linnean Society* 184 (2): 528–83.
- Westram, Anja M., Juan Galindo, Magnus Alm Rosenblad, John W. Grahame, Marina Panova, and Roger K. Butlin. 2014. "Do the Same Genes Underlie Parallel Phenotypic Divergence in Different *Littorina saxatilis* Populations?" *Molecular Ecology* 23 (18): 4603–16.

- White, Crow, Kimberly A. Selkoe, James Watson, David A. Siegel, Danielle C. Zacherl, and Robert J. Toonen. 2010. "Ocean currents help explain population genetic structure". *Proceedings of the Royal Society B: Biological Sciences* 277(1688), pp.1685-1694.
- Whitlock, Michael C. and McCauley, David E. 1999. "Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$ ". *Heredity* 82(2), pp.117-125.
- Whittaker, Kerry A., and Tatiana A. Ryneerson. 2017. "Evidence for Environmental and Ecological Selection in a Microbe with No Geographic Limits to Gene Flow." *Proceedings of the National Academy of Sciences of the United States of America* 114 (10): 2651–56.
- Wilkinson, Samantha, Chris Haley, Lawrence Alderson, and Pamela Wiener. 2011. "An Empirical Assessment of Individual-Based Population Genetic Statistical Techniques: Application to British Pig Breeds." *Heredity* 106 (2): 261–69.
- Williams, George Christopher. 1966. *Adaptation and Natural Selection*. Princeton University Press, Princeton, NJ.
- Wilmanski, Jeanette M., Tanja Petnicki-Ocwieja, and Koichi S. Kobayashi. 2008. "NLR Proteins: Integral Members of Innate Immunity and Mediators of Inflammatory Diseases." *Journal of Leukocyte Biology* 83 (1): 13–30.
- Wit, Pierre De, Sam Dupont, and Peter Thor. 2016. "Selection on Oxidative Phosphorylation and Ribosomal Structure as a Multigenerational Response to Ocean Acidification in the Common Copepod *Pseudocalanus acuspes*." *Evolutionary Applications* 9 (9): 1112–23.
- Wit, Pierre De, and Stephen R. Palumbi. 2013. "Transcriptome-Wide Polymorphisms of Red Abalone (*Haliotis rufescens*) Reveal Patterns of Gene Flow and Local Adaptation." *Molecular Ecology* 22 (11): 2884–97.
- Wollstein, Andreas, and Oscar Lao. 2015. "Detecting Individual Ancestry in the Human Genome." *Investigative Genetics* 6 (1): 7.
- Wright, Sewall. 1943. "Isolation by Distance." *Genetics* 28 (2): 114–38.
- Wright, Sewall. 1946. "Isolation by Distance under Diverse Systems of Mating." *Genetics* 31 (1): 39–59.
- Wright, Sewall. 1949. "The Genetical Structure of Populations." *Annals of Eugenics* 15 (1): 323–54.
- Wright, Daniel, Jacqueline M. Bishop, Conrad A. Matthee, and Sophie von der Heyden. 2015. "Genetic Isolation by Distance Reveals Restricted Dispersal across a Range of Life Histories: Implications for Biodiversity Conservation Planning across Highly Variable Marine Environments." *Diversity and Distributions* 21 (6): 698–710.
- Wyngaarden, Mallory Van, Paul V. R. R. Snelgrove, Claudio DiBacco, Lorraine C. Hamilton, Naiara Rodríguez-Ezpeleta, Luyao Zhan, Robert G. Beiko, and Ian R. Bradbury. 2018. "Oceanographic Variation Influences Spatial Genomic Structure in the Sea Scallop, *Placopecten magellanicus*." *Ecology and Evolution* 8 (5): 2824–41.
- Xu, Sheng Yong, Dian Rong Sun, Na Song, Tian Xiang Gao, Zhi Qiang Han, and Bo Nian Shui. 2017. "Local Adaptation Shapes Pattern of Mitochondrial Population Structure in *Sebastes marmoratus*." *Environmental Biology of Fishes* 100 (7): 763–74.

- Xu, Shuqing, Jiangbai Luosang, Sang Hua, Jian He, Asan Ciren, Wei Wang, Xiaomei Tong, Yu Liang, Jian Wang, and Xiaoguang Zheng. 2007. "High Altitude Adaptation and Phylogenetic Analysis of Tibetan Horse Based on the Mitochondrial Genome." *Journal of Genetics and Genomics* 34 (8): 720–29.
- Xue, Yali, Xuelong Zhang, Ni Huang, Allan Daly, Christopher J. Gillson, Daniel G. Macarthur, Bryndis Yngvadottir, et al. 2009. "Population Differentiation as an Indicator of Recent Positive Selection in Humans: An Empirical Evaluation." *Genetics* 183 (3): 1065–77.
- Xuereb, Amanda, Christopher M. Kimber, Janelle M. R. Curtis, Louis Bernatchez, and Marie-Josée Fortin. 2018. "Putatively Adaptive Genetic Variation in the Giant California Sea Cucumber (*Parastichopus californicus*) as Revealed by Environmental Association Analysis of Restriction-site Associated DNA Sequencing Data." *Molecular Ecology* 27 (24): mec.14942.
- Yang, Tian Yan, Tian Xiang Gao, Wei Meng, and Yan Lin Jiang. 2020. "Genome-Wide Population Structure and Genetic Diversity of Japanese Whiting (*Sillago japonica*) Inferred from Genotyping-by-Sequencing (GBS): Implications for Fisheries Management." *Fisheries Research* 225: 105501.
- Yednock, Bree K., and Joseph E. Neigel. 2014. "Detecting Selection in the Blue Crab, *Callinectes sapidus*, Using DNA Sequence Data from Multiple Nuclear Protein-Coding Genes." *PLoS ONE* 9 (6).
- Yu, Li, Xiaoping Wang, Nelson Ting, and Yaping Zhang. 2011. "Mitogenomic Analysis of Chinese Snub-Nosed Monkeys: Evidence of Positive Selection in NADH Dehydrogenase Genes in High-Altitude Adaptation." *Mitochondrion* 11 (3): 497–503.
- Zhan, Xiangjiang, Andrew Dixon, Nyambayar Batbayar, Eugene Bragin, Z. Ayas, Lucia Deutschova, Jozef Chavko, et al. 2015. "Exonic versus Intronic SNPs: Contrasting Roles in Revealing the Population Genetic Differentiation of a Widespread Bird Species." *Heredity* 114 (1): 1–9.
- Zhang, Bai Dong, Dong Xiu Xue, Yu Long Li, and Jin Xian Liu. 2019a. "RAD Genotyping Reveals Fine-Scale Population Structure and Provides Evidence for Adaptive Divergence in a Commercially Important Fish from the Northwestern Pacific Ocean." *PeerJ* 7: e7242.
- Zhang, Bo, Yingying Wu, Xin Wang, Wei Jiang, Jianping Yin, and Qiang Lin. 2019b. "Comparative Analysis of Mitochondrial Genome of a Deep-Sea Crab *Chaceon granulatus* Reveals Positive Selection and Novel Genetic Features." *Journal of Oceanology and Limnology* 1-11.
- Zhang, Xiaojun, Lina Sun, Jianbo Yuan, Yamin Sun, Yi Gao, Libin Zhang, Shihao Li, et al. 2017a. "The Sea Cucumber Genome Provides Insights into Morphological Evolution and Visceral Regeneration." *PLoS Biology* 15 (10).
- Zhang, Bo, Yan-Hong Zhang, Xin Wang, Hui-Xian Zhang, and Qiang Lin. 2017b. "The Mitochondrial Genome of a Sea Anemone *Bolocera* Sp. Exhibits Novel Genetic Structures Potentially Involved in Adaptation to the Deep-Sea Environment." *Ecology and Evolution* 7 (13): 4951–62.
- Zhao, Shancen, Pingping Zheng, Shanshan Dong, Xiangjiang Zhan, Qi Wu, Xiaosen Guo, Yibo Hu, et al. 2013. "Whole-Genome Sequencing of Giant Pandas Provides Insights

- into Demographic History and Local Adaptation.” *Nature Genetics* 45 (1): 67–71.
- Zhao, Yunfeng, Wenzhu Peng, Huayang Guo, Baohua Chen, Zhixiong Zhou, Jian Xu, Dianchang Zhang, and Peng Xu. 2018. “Population Genomics Reveals Genetic Divergence and Adaptive Differentiation of Chinese Sea Bass (*Lateolabrax maculatus*).” *Marine Biotechnology* 20 (1): 45–59.
- Zhong, Xiaoxiao, Dandan Feng, Hong Yu, Lingfeng Kong, and Qi Li. 2016. “Genetic Variation and Breeding Signature in Mass Selection Lines of the Pacific Oyster (*Crassostrea gigas*) Assessed by SNP Markers.” *PLOS ONE* 11 (3): e0150868.
- Zhou, Taicheng, Xuejuan Shen, David M. Irwin, Yongyi Shen, and Yaping Zhang. 2014. “Mitogenomic Analyses Propose Positive Selection in Mitochondrial Genes for High-Altitude Adaptation in Galliform Birds.” *Mitochondrion* 18: 70–75.
- Zhu, Feifei, Dong Li, and Keping Chen. 2019. “Structures and Functions of Invertebrate Glycosylation.” *Open Biology* 9, no. 1: 180232.
- Zweng, Melissa M., James R. Reagan, John I. Antonov, Ricardo A. Locarnini, Alexey V. Mishonov, Timothy P. Boyer, Hernan E. Garcia, et al. 2013. “World Ocean Atlas 2013. Volume 2, Salinity.”

Chapter IV: Assessing the thermal tolerance in three intertidal marine invertebrates

4.1 Introduction and background

4.1.1 Temperature and species distribution

Temperature impacts the physiology, ecology and evolution of species (Angilletta, 2009). Hence, globally changing climatic conditions are likely to significantly influence species fitness and interactions and thus ecosystem functioning (Castañeda, Lardies and Bozinovic, 2004; Mora and Maya, 2006; Nguyen et al. 2011; Stillman, 2019). Further, temperature is a main factor in determining species abundance and distributions (Helmuth, 2002; Parmesan and Yohe, 2003; Loarie et al. 2009; Thomas, 2010; Chen et al. 2011). With the onset of climate change, a wide range of taxonomic groups have shifted their range poleward to evade rising temperature (Parmesan and Yohe, 2003; Hickling et al. 2006; Parmesan, 2006; Smith, Dowling and Brown, 2019). Range-shifts attributed to temperature changes could also be observed in multiple marine species (Perry et al. 2005; Loarie et al. 2009; Ling et al. 2009; Hawkins et al. 2009; Chen et al. 2011; Flagor and Bourdeau, 2018). Sea surface temperature (SST) appears as a stronger determinant for the distribution of coastal shallow water species compared to pelagic organisms (Belanger et al. 2012), which might also face additional constraints by lack of available habitat to shift into (Fenberg, Posbic and Hellberg, 2014). There are also physiological differences between species inhabiting temperate and tropical regions (Stillman, 2002; Bonebrake and Deutsch, 2012). Thermal conditions in the tropics are generally more stable relative to the seasonal variability in temperate areas (Richard et al. 2012). Tropical ectotherms are thought to already exist closer to their upper thermal limits (Deutsch et al. 2008; Huey et al. 2009; Duarte et al. 2012; Sunday, Bates and Dulvy, 2012), which might make them more vulnerable to warming conditions (Sinervo et al. 2010; Bonebrake and Deutsch, 2012; Pinsky et al. 2019). To predict effects of changing temperatures on ecosystems, it is essential to assess species' capacity to cope with these changes through plastic and/or evolutionary responses or by shifting their distribution (Stillman, 2003; Deutsch et al. 2008; Hughes et al. 2018). Knowledge about temperature responses and adaptations is therefore of key importance for managing biodiversity or conservation planning and to explore the possible regional impact of climate change across populations.

4.1.2 The South African ocean temperature gradient

Ocean current regimes can create natural gradients of thermal conditions across coastlines and are able to affect the distribution of intertidal organisms and the evolution of temperature-associated traits (Stillman, 2002; Somero, 2005; Pulgar, Bozinovic and Ojeda, 2005). The South African coastline has one of the most defined marine thermal gradients (Bustamante et al. 1995; Teske et al. 2019; see section 1.5.2; Figure 1.2), where mean temperatures on the west coast can seasonally range from 11-15°C with short-term fluctuations and temperatures on the east coast range from 18-27°C (Smit et al. 2013). Climate change predictions for South Africa differ depending on the coastal region (see section 1.6.2), with increasing temperatures on the east coast (Rouault, Penven and Pohl, 2009) and likely decreasing temperatures on the west coast (Rouault, Pohl and Penven, 2010; Dufois and Rouault, 2012). At the south coast, some areas might experience warming and others cooling temperature conditions (Rouault, 2011). Several endemic rocky shore species are distributed across the cold-temperate western, warm-temperate southern and subtropical eastern coastal regions of South Africa (Branch, 2017). Populations of the same species inhabiting these different temperature-defined bioregions might possess physiological adaptations to local temperature conditions (Somero, 2005; Gaitán-Espitia et al. 2014). For instance, larvae of the subtropical lineage of South African mud prawn *Upogebia africana* apparently fail to successfully settle in populations of the warm-temperate mud prawn lineage, suggesting that population-specific temperature adaptations limit dispersal and exchange between habitats (Teske et al. 2008). Further, a study on the sand goby *Psammogobius knysnaensis* uncovered evidence for geographically-distinct differences in temperature-associated gene regions across populations (Teske et al. 2019). Selection pressure from regional temperature differences might possibly drive ecological speciation in *P. knysnaensis* (Teske et al. 2019).

In species populations spanning multiple coastal regions, the temperature differences could be associated with geographic variation in critical thermal limits. Thermal limits of coastal species should hypothetically be highest on the subtropical east coast and lowest on the cold-temperate west coast, with the warm-temperate south coast forming an intermediate region.

4.1.3 Intertidal zonation of the rocky shore

The rocky shore is a highly dynamic environment under constant influence of tides and wave action and with drastic fluctuations of abiotic conditions such as water temperature, air

temperature, salinity, dissolved oxygen and pH (Stillman, 2002; Gaines and Denny, 2007). Further, environmental conditions for intertidal species have diel, tidal, lunar and seasonal variations in the additional context of latitudinal clines, vertical zonation and microhabitats (Harley and Helmuth, 2003; Helmuth et al. 2006; Sinclair, Thompson and Seebacher, 2006). Rocky shore species require physiological, morphological and behavioural adaptations to withstand the stress imposed by their dynamic environment (Somero, 2002; Muñoz et al. 2005; Williams et al. 2005; Harley et al. 2009). Although there can be exceptions, most intertidal organisms are distributed in horizontal bands across a vertical zonation of upper, middle and low shore, which creates a high variety of microhabitats (Gaines and Denny, 2007). The upper limits of intertidal species distribution on the high shore are set by thermal stress from extended exposure to high or low air temperature and increased desiccation risk (Helmuth and Hofmann, 2001). Further, lower limits of rocky shore species distribution are structured by competition and predation (Harley, 2011; Sorte et al. 2019). The body temperature of intertidal species is determined by seawater temperature during high tide, but can sharply increase or decrease during low tide due to air temperature, solar radiation, wind speed, cloud cover, wave height, relative humidity levels and the timing of low tide (Helmuth and Hofmann, 2001; Helmuth et al. 2011). For instance, biomimetic loggers placed inside mussel shells demonstrated that water temperature can range from 10°C to more than 40°C over the course of a single low tide period in temperate coastal regions (Harley and Helmuth, 2003). In tropical coastal regions, temperature might even surpass 50°C (Williams and Morritt, 1995). Overall, intertidal invertebrates are thought to already exist at or near their upper thermal tolerance limit (Somero, 2002) and possess limited capacity for acclimation (Hopkin et al. 2006; Tomanek, 2010; Somero, 2010). Changing climatic conditions may put these species further at risk, because the highly dynamic fluctuations in factors such as maximum or minimum habitat temperature in the intertidal might respectively exceed their upper or lower thermal limits (Somero, 2010). Species occupying the high shore are usually most impacted by extreme temperature events during emersion, which can result in “*high shore kills*” during summer heat waves (Williams et al. 2005; p. 213; Williams, Chan and Dong, 2019; p. 384) and substantial population decline during winter cold waves (Crisp, 1964; Firth, Knights and Bell, 2011; Firth et al. 2015). Further, preferred shore height in species is associated with physiological differences, where high shore species likely have a higher thermal tolerance compared to low shore or shallow subtidal species (Stillman and Somero, 2000; Nguyen et al. 2011; Sorte et al. 2019). Distinct differences between high-shore and low shore thermal limits could for instance be shown in *Petrolisthes* crabs (Stillman, 2002). For this study, the high-shore species (*S. granularis*, *C. punctatus*) are expected to show higher thermal tolerance than the low shore species (*P. angulosus*). Across the three species, the granular limpet *S. granularis* might possibly have the highest

tolerance to high temperatures due to its extended direct sun exposure compared to the shore crab *C. punctatus*.

4.1.4 Impacts of climate change on intertidal communities

Environmental and body temperatures are closely associated in ectotherms, thus changing climatic conditions will impact their distribution, abundance and survival (van Heerwaarden, Malmberg and Sgrò, 2016). Impacts of climate change might occur more rapidly in shallow coastal waters (Helmuth et al. 2006; Menge, Chan and Lubchenco, 2008; Hawkins et al. 2009; Rastrick et al. 2014; Peck et al. 2014; Vinagre et al. 2016), which thus have been suggested to serve to some extent as early warning systems for climate change effects (Helmuth et al. 2006; Eslami-Andergoli et al. 2015; Chemello, Vizzini and Mazzola, 2018). Shallow coastal ecosystems such as rocky shores possess lower thermal inertia in comparison with open ocean areas and thermal impacts are more directly influenced by oceanographic and atmospheric conditions (Vinagre et al. 2018). Populations in temperate climate regions experience a wider temperature range with pronounced seasonal variation compared to populations with more stable temperatures in the tropics (Richard et al. 2012). Thus, populations in temperate regions might be more tolerant to larger shifts in temperature conditions than tropical populations (Tewksbury, Huey and Deutsch, 2008; Hofmann and Todgham, 2010; Nguyen et al. 2011; Vinagre et al. 2019). Further, tropical and polar marine ectotherm species are likely to have decreased physiological capacity for acclimation compared to similar species living in temperate regions due to the more pronounced seasonal temperature variability in the latter (Peck et al. 2014; but see Brahim, Mustapha and Marshall, 2019). Due to global warming, the frequency, duration and severity of extreme heat waves is predicted to increase and future heat waves are expected to take place in a wider time frame from late spring to early fall (IPCC, 2014; Angélil et al. 2017; Frölicher, Fischer and Gruber, 2018). Summer heat wave mortality has increasingly occurred during the last decade (Buckley and Huey, 2016; Stillman, 2019) and annual marine heat wave days rose by 54% from 1925 to 2016 globally (Oliver et al. 2018). For intertidal species, summertime temperature extremes can have severe impacts (Garrahou et al. 2009; Leung, Connell and Russell, 2017), because they face strong temperature fluctuations with often pronounced peaks during low tide. Further, rocky shore organisms may suffer greater mortality from future heat waves due to the adverse effects of accumulated heat exposure (Vinagre et al. 2018; Siegle, Taylor and O'Connor, 2018). Apart from heat waves, cold waves are predicted to decrease in many parts of the world due to global warming (Chust et al. 2011; IPCC, 2013; van Oldenborgh et al. 2019), but certain regions such as the South

African west coast, are forecasted to undergo further cooling temperatures, while warming is expected in other regions (Rouault, Pohl and Penven, 2010; Dufois and Rouault, 2012).

Populations inhabiting the edge of the species range commonly face the extreme ends of environmental stress gradients, which the species is able to tolerate (Gaston, 2009; Sexton et al. 2009; Sorte et al. 2019). Range edge populations might therefore already experience strain by the contemporary environmental conditions and hence may be more vulnerable to further changes in climatic conditions (Sorte and Hofmann, 2004; Sagarin and Somero, 2006; Lima et al. 2016; Han et al. 2019). Assessing responses to thermal stress across populations spanning a gradient of different conditions of the species distribution is essential to explore the species phenotypic and genotypic variability and its potential resilience to climate change. In this study, the populations located on the southern east coast represent the sampled range edge most exposed to high temperatures in all three species (Figure 2.1) and might thus exist under most current and potentially future temperature stress. The range of the Cape urchin *P. angulosus* and the granular limpet *S. granularis* extends to the southern Namibian coast (Branch, 2017; see section 1.7), therefore their most western South African populations in this study are by definition not range edge populations. However, the crab *C. punctatus* has not been found further North of Jacob's Bay (Figure 2.1), which might constitute its most western range edge population. Generally, changes in temperature conditions have led to poleward shifts in multiple species in order to track their accustomed habitat and preferred thermal range (Bellard et al. 2012; Ralston et al. 2017; see section 4.1.1). Importantly, poleward shifts are geographically limited in South Africa due to the West-East orientation of the coastline (Figure 2.1). For instance, while the predicted temperature changes on the south coast are complex, an attempted poleward shift for west coast populations could be associated with an increase in water temperature. In the absence of adequate habitat to shift into, climate change might cause a range compression for local species. Lastly, interspecific physiological differences in intertidal species can occur due to vertical zonation patterns, where high-shore species typically have higher thermal tolerance limits compared to low shore species (Stillman and Somero, 2000; Sorte et al. 2019; see section 4.1.3). However, the higher thermal upper limits of high-shore species place them more at risk from climate change impacts compared to species lower on the shore (Somero, 2010; see section 4.1.6). Assessing thermal tolerance limits is an important step towards predicting species fitness, abundance and ecosystem composition in the future.

4.1.5 Thermal tolerance limits

Physiological tolerance limits demonstrate high intra- and interspecific variability and can differ according to the developmental stage (Stillman and Somero, 2000; Stillman, 2003; Folguera et al. 2010; Sorte, Jones and Miller, 2011). In some aquatic invertebrates, thermal stress response may vary with sex (Sornom et al. 2010; Madeira et al. 2012b; Vaughn, Turnross and Carrington, 2014; Bedulina et al. 2017; Grilo et al. 2018). For example, female *Pachygrapsus marmoratus* crabs were shown to have higher critical upper limits than their male counterparts (Madeira et al. 2012b). Sex-dependent differences in thermal traits have been associated with physiological differences and varying energy allocation between males and females (Grilo et al. 2018). The body size of animals can also influence thermal tolerance (Peck et al. 2007; Peck et al. 2009; Madeira et al. 2012b; Muñoz, Kelley and De Rivera, 2017; Gunderson et al. 2019). For instance, smaller crabs can prove more heat tolerant than larger crabs (Jensen and Armstrong, 1991; Madeira et al. 2012b; Gunderson et al. 2019). In this context, body mass is often used as proxy for body size. Importantly, there is also the possibility that size confounds the measurement of physiological parameters (see for example Woiwode and Adelman, 1992).

Small-scale spatial and temporal variation of thermal conditions (thermal mosaic) commonly found for example in rocky shores can give rise to varying thermal performance (Helmuth et al. 2011; Richter-Boix et al. 2015). This constrains the deduction of community-level responses to thermal changes (Clark et al. 2017). Most organisms have an optimal thermal range for their physiological performance, which is broadly congruent with the range of thermal conditions experienced in their habitat (Pörtner et al. 2000; Pörtner, 2010; Somero, 2010). This means that within this optimal range, species can show the highest activity level, the fastest growth and produce the most offspring. Thermal performance is usually characterised by a bell-shaped curve (Figure 4.1), where the optimum temperature (T_{OPT}) describes the temperature at optimal performance for the respective organism, whereas the critical upper (CT_{max}) and lower thermal tolerance limit (CT_{min}) set the upper and lower boundary of thermal tolerance beyond which long term survival is not ensured. A critical limit is reached when the organism is able to recover after displaying physiological failure such as loss of righting capacity (ability to invert when placed on dorsal surface), knockdown or lack of response to a stimulus (Schmidt-Nielsen, 1997; Lutterschmidt and Hutchison, 1997a; Blackburn et al. 2014). Returning to the performance curve, the breadth characterises the thermal breadth (T_{br}), which is regarded as the range of body temperatures for species to perform well (Angilletta, 2006; Rohr et al. 2018). If the ambient temperature is below a species' optimum, climate change conditions can increase body temperature in ectotherms

towards their optimal temperature, which would have a positive effect on their fitness (Figure 4.2; Huey et al. 2012). However, if body temperature rises beyond optimum temperature, fitness and associated thermal performance will decrease (Figure 4.2; Huey et al. 2012).

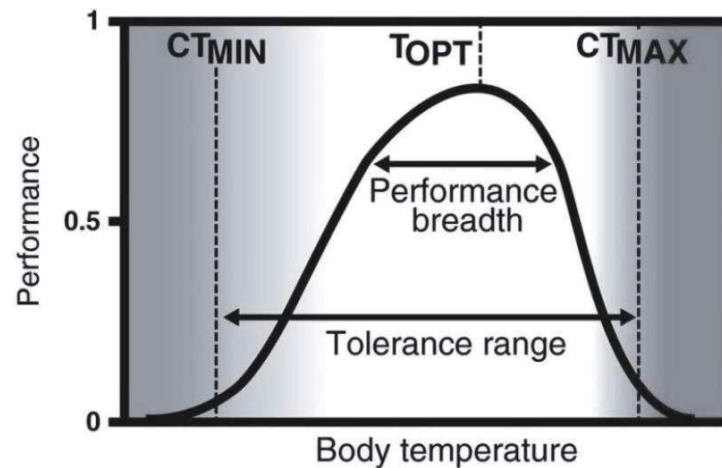


Figure 4.1. The thermal performance curve indicates the relationship between environmental temperature and the physiological rate of an ectotherm. The optimum temperature (T_{OPT}) indicates the temperature at optimum performance. The thermal performance breadth is determined by the thermal minimum (CT_{min}) and maximum (CT_{max}) (Figure adapted from Tuff, Tuff and Davies, 2016).

Warming and cooling tolerance

Warming tolerance (WT) is considered as the gap between the upper critical thermal limit (CT_{max}) and the maximum habitat temperature (T_{max}) and thus approximates the capacity to tolerate further warming of the habitat due to for instance climate change (Deutsch et al. 2008). Theory suggests that the larger the warming tolerance is, the better species populations might be able to withstand rising temperatures (Deutsch et al. 2008). On the other end of the spectrum, the suggested cold equivalent is the difference between lower critical thermal limits (CT_{min}) and minimum habitat temperature (T_{min}) as cooling tolerance (CT) (Gutiérrez-Pesquera et al. 2016). In general, cold tolerance in temperate rocky shore organisms has received comparatively less empirical investigation than heat tolerance (Qari and Aljarari, 2014; Chiba et al. 2016). In some parts of the world, marine ectotherms can experience temperatures below their freezing point in winter low tides, which requires tolerance to extracellular ice formation to persist (Ronges et al. 2012). Nevertheless, most marine intertidal ectotherms do not survive at low temperatures above their freezing point and the processes underpinning non-freezing cold injury are not well understood (Ronges et al. 2012). On the South African coast, extended periods of below-zero temperatures are uncommon, but species may still suffer from cold damage induced by marine cold-spells (Schlegel et al. 2017). Cooling tolerance has previously been studied in ectothermic

vertebrates (Oyamaguchi et al. 2018; Pintanel et al. 2019), but further investigations are needed to ascertain the predictive power of cooling tolerance for the complex circumstances of low temperature tolerance potential in rocky shore invertebrates. Moreover, there is evidence that lower critical thermal limits might exhibit substantial intra- and interspecific variation, while upper thermal limits are considered as more conserved across lineages (Araújo et al. 2013). This could be associated with different physiological mechanisms governing thermal limits when species face high and low temperatures (Araújo et al. 2013). Asymmetric variation in upper and lower thermal limits might make warming tolerance estimates overall firmer than cooling tolerance estimates. Additionally, the upper critical thermal limit (CT_{max}) appears to be highly repeatable (Morgan, Finnøen and Jutfelt, 2018) and might be directly compared across diverse taxonomic groups (Bates and Morley, 2020). Patterns of geographic variation of warming and cooling tolerance can serve as valuable tool to assess the impact of extreme temperatures on populations across species ranges (Sorte, Jones and Miller, 2011; Morley et al. 2016). In general, warming tolerance is expected to be lower in tropical species compared to species in higher latitudes (Comte and Olden, 2017). Furthermore, it is likely that cooling tolerance is higher in temperate regions relative to tropical regions, based on the greater seasonal temperature variability (Stuart-Smith, Edgar and Bates, 2017).

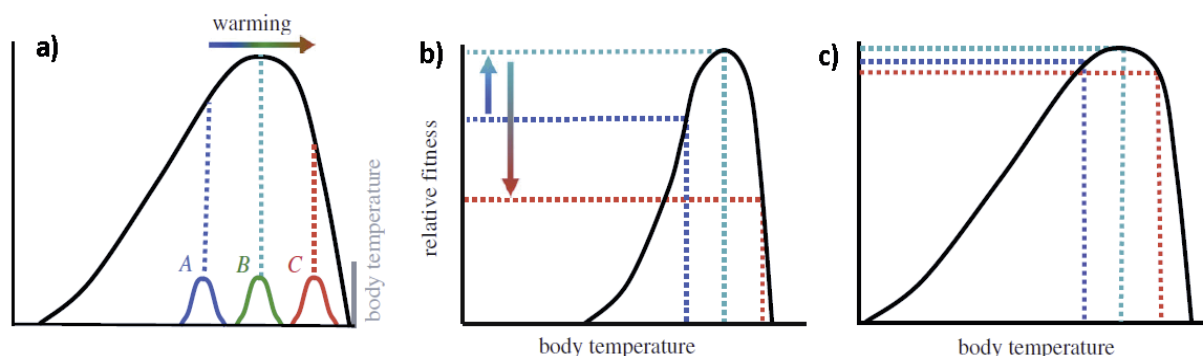


Figure 4.2. Thermal performance curves under different warming scenarios. (a) Climate warming can shift the distributions of T_b (body temperature). If warming raises T_b closer to T_{OPT} of a species (e.g. shift from A to B), warming can enhance fitness. If warming increases T_b higher than T_{OPT} (e.g. shift from B to C), the fitness will decline. (b,c) Increases in T_b from warming can have much bigger effects on (b) thermal specialists than on (c) thermal generalists (Figures adapted from Huey et al. 2012).

Influence of experimental parameters

When thermal limits are assessed, the experimental design can influence the measured temperature tolerance for instance by the selected starting temperature and the rate of temperature increase (Terblanche et al. 2007; Faulkner et al. 2014; Morley et al. 2016; Kingsolver and Umbanhowar, 2018; Bates and Morley, 2020). The starting temperature is typically the same constant temperature at which animals are held prior to heating or cooling

(Terblanche et al. 2007). Ectotherms typically have higher thermal limits at faster rates of temperature change and lower limits at slower rates (Morley et al. 2016; Kingsolver and Umbanhowar, 2018; Bates and Morley, 2020). For instance, intertidal crustaceans from different geographic regions showed lower maximum thermal tolerance with lower rates of change, whereas fast rates of temperature change led to a higher measured maximum thermal tolerance (Faulkner et al. 2014; Noisette et al. 2014; Gunderson and Stillman, 2015). Finally, acclimatisation might lead to different thermal tolerance limits during winter than during summer due to changes in animal physiology related to seasonal changes in temperature (Schmidt-Nielsen, 1997). Thermal limits are considered ecologically relevant, because they provide insight into the activity range of individuals under varying temperature conditions (Vannier, 1994; Somero, 2005) and can be utilised in climate envelope models to project future patterns of biodiversity or local extinctions (Helmuth, 2009; Peck et al. 2009; Rilov et al. 2019).

Oxygen limitation and the intertidal

Oxygen supply can also play a role in thermal performance, as for instance hypoxia limits the oxygen carrying capacity in species (Wang et al. 2014; Devor et al. 2016; Sibraus et al. 2017). In aquatic animals, the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis postulates that species response to temperature increase above their optimum temperature is determined by the transition from aerobic to anaerobic metabolic processes and that thermal parameters such as critical upper limits can be considered oxygen-limited (Pörtner, 2010; Pörtner, Bock and Mark, 2017; Box 1; p. 2687). Though there is certain evidence to support the OCLTT, the generality and the boundaries of the concept remain debated and expanded upon (Jutfelt et al. 2018; Pörtner, Bock and Mark, 2018; Ern, 2019; MacMillan, 2019). Intertidal tide pools can, depending on shore height and heating during low tide, experience drastic fluctuations of oxygen (Truchot and Duhamel-Jouve, 1980; Richards, 2011) and the effects of oxygen limitation are well documented in for instance intertidal fish inhabiting them (Mandic, Todgham and Richards, 2009; Richards, 2011; Craig et al. 2014; McArley, Hickey and Herbert, 2020). The subtidal zone, located below the shore, can also have fluctuations in oxygen and temperature. However, these variations are typically much less pronounced than in any other part of the intertidal zone (Richards, 2011). Upper high shore species are usually adapted to longer periods of full aerial exposure during low tide and are most threatened by extreme temperatures, desiccation and/or predation. In this study, the shore crab *C. punctatus* and the limpet *S. granularis* are both high shore specialists and the urchin *P. angulosus* was only collected from below the shore's waterline at low tide, not from tide pools.

Limits of physiological plasticity and acclimatisation

Species responses to thermal stress might include the upregulation of physiological processes such as ventilation (Sokolova et al. 2012) and cardiac activity (Braby and Somero, 2006) with associated energy costs increasing disproportionately. Moreover, defensive cellular responses to stress may involve changes of metabolic rate, the cell cycle and apoptosis (Kültz, 2005; Sokolova et al. 2012). Thermal limits are determined by the duration and magnitude of temperature exposure in ectotherms (Morley et al. 2016). The impact of changing climatic conditions is subject to species thermal limits and their acclimatisation capacity, both of which are understudied in the majority of species (Vinagre et al. 2016). Existing phenotypic plasticity in populations is the foundation for acclimatisation, which is a crucial process to withstand changes in environmental temperature conditions (Lucassen et al. 2006; Brahim, Mustapha and Marshall, 2019; Neel et al. 2020). Nevertheless, species are constrained by the plasticity limits of their thermal tolerance to keep up with more extreme temperature events (Stillman, 2019). Thus, it is possible that physiological plasticity might not be sufficient for long-term survival of populations in future climate change (Gunderson and Stillman, 2015; van Heerwaarden, Kellermann and Sgrò, 2016; Gunderson, Dillon and Stillman, 2017). The increased occurrence of extreme temperatures could exceed species capacity for acclimatisation (Stillman, 2019). Further, increased tolerance towards higher or lower temperatures in species might be hindered by physiological and genetic constraints (Addo-Bediako, Chown and Gaston, 2000; Hoffmann, Chown and Clusella-Trullas, 2013; Araújo et al. 2013; Meester, Stoks and Brans, 2018). For instance, it appears as if the phenotypic plasticity of upper thermal limits is substantially reduced compared to variation in lower limits (Araújo et al. 2013). If limits of physiological plasticity are exceeded, species can only shift their geographic range, adapt on an evolutionary scale or face extinction (Chevin, Lande and Mace, 2010; Urban, 2015; Stillman, 2019). Thermal adaptation has a multivariate genetic basis and the capacity of populations to respond to warming or cooling temperatures is underpinned by available genomic variation and covariation in traits under selection (Williams et al. 2012; Healy et al. 2018). There is evidence that the evolutionary scope to extend upper thermal limits in species could be limited and determined by the type of thermal stress experienced (van Heerwaarden and Sgrò, 2013; Blackburn et al. 2014). Furthermore, it is questionable whether molecular adaptation can keep up with the contemporary rate of climate change (Hoffmann and Sgrò, 2011; Reusch, 2014; Fox et al. 2019).

4.1.6 Preferred intertidal niches of study species

The three study species, the shore crab *Cyclograpsus punctatus*, the granular limpet *Scutellastra granularis* and the Cape urchin *Parechinus angulosus*, are prominent members of South African rocky shore communities and belong to the three phyla Arthropoda, Mollusca and Echinodermata, respectively (Branch, 2017; see section 1.7). The shore crab and the granular limpet are high-shore specialists, whereas the Cape urchin prefers the low shore and tide pools, which allow the species to preferentially remain submerged throughout the tidal phases (Branch, 2017; see section 1.7). The shore crab retains its mobility during emersion and typically shelters in crevices to behaviourally regulate body temperature and reduce the risk of predation (personal observation). Behavioural thermoregulation by moving and/or hiding is an important mechanism against changing temperature conditions, but it might not be sufficient to persist climate change for ectotherms (Blackburn et al. 2014). High shore crabs in particular are thought to already live close to their temperature maxima (Stillman and Somero, 1996; Somero, 2005; Madeira et al. 2012a). The granular limpet is functionally sessile in low tide phases and remains attached to vertical or horizontal high-shore rock surfaces, which limits its ability to evade extreme temperatures (personal observation). Certain limpet species including *S. granularis* have been found to utilise “mushrooming behaviour”, whereby the animal raises its shell from the rock surface for assumed temporary relief from heat stress (Williams et al. 2005; p.1; also see Williams and Morritt, 1995). Nevertheless, shell raising for evaporative cooling might be of little effect in these small-bodied animals with restricted internal water reservoirs (Denny, Miller and Harley, 2006; Diederich, 2015). Tide pools or low shore areas can undergo drastic temperature fluctuations and remaining submerged is not guaranteed for the Cape urchin, whose settling position is generally stationary during low tide (personal observation). The species displays covering behaviour (Figure 4.3), which could be interpreted as an attempt to shield itself with small debris from radiation and/or predators during low tide (Brothers and McClintock, 2015).

Preferred shore height is likely decisive for impacts from climate change on intertidal species (see section 4.1.4). The highest temperature and desiccation stress occurs on high-shore and horizontal surfaces (Williams and Morritt, 1995; Denny, Miller and Harley, 2006; Miller, Harley and Denny, 2009). Both the shore crab *C. punctatus* and the granular limpet *S. granularis* are high-shore species, but the latter is severely limited in its ability to evade temperature stress during emersion and might thus be most at risk from changing climatic conditions across the three species. The Cape urchin *P. angulosus* has the thermal advantage of inhabiting the low shore, but as a functionally sessile organism, is unable to

substantially change its position during low tide. If the physical geography allows, intertidal sea urchin species can generally hide under large platform-type rocks during low tide and can create moist “micro-pools” in the presence of malleable dune rock (Francesca Porri, personal communication). It is possible that the shore crab *C. punctatus*, despite being a high-shore organism, might be best prepared to cope with changing climatic conditions due to its mobility and thermoregulatory behaviour (sheltering).



Figure 4.3. *Parechinus angulosus* individuals engaging in “covering” behaviour, photographed on the west coast (Port Nolloth) during low tide. White circles are added for better identification.
(Source: L. Mertens).

4.1.7 Research aims and expectations

The main objective of this chapter is to investigate upper and lower critical thermal limits (CT_{max} , CT_{min}) and thermal breadth (T_{br}) in three intertidal invertebrate species from three coastal regions to establish whether populations across the South African temperature gradient exhibit different thermal limits (and T_{br}) between biogeographic regions. (1) Thermal tolerance theory suggests that the highest intraspecific critical thermal limits would be expected on the east coast and the lowest thermal limits on the west coast. The south coast might hypothetically indicate ‘intermediate’ thermal limits between the west and east coast. (2) Thermal breadth is predicted to be narrower at the east coast compared to the west coast (3). Interspecifically, high-shore limpet species *S. granularis* would be expected to demonstrate the highest thermal tolerance due to its direct exposure to solar radiation and air temperature on horizontal and vertical rock surfaces compared to the shade-seeking crab *C. punctatus* during low tide. In contrast, the constantly submerged low shore urchin species *P. angulosus* might have the lowest thermal limits. A further objective of this chapter is to calculate warming and cooling tolerance (WT, CT) to investigate which population(s) would be most at risk from predicted regional temperature trends in South Africa. Based on local

temperature data, (4) eastern populations (Cape St. Francis, Haga Haga) are expected to live closest to their upper thermal limits (lowest warming tolerance) and western populations (Port Nolloth, Jacob's Bay, Sea Point) are expected to exist closest to their lower thermal limits (lowest cooling tolerance).

4.2 Materials and Methods

4.2.1 Field collection

The study area on the South African coast stretched from 29°S to 32°S and from 16°E to 28°E. Field sites (Port Nolloth, Sea Point, Cape Agulhas, Knysna, Cape St. Francis and Haga Haga; Figure 2.1) were visited between July and mid-September 2017 to collect individuals for the thermal tolerance trials. Port Nolloth and Haga Haga broadly represent the western and eastern range edge of the study species distribution in South Africa, respectively (Branch, 2017). Jacob's Bay was substituted for Port Nolloth given the more limited distribution of *C. punctatus*. Around 25 individuals per species were collected over an area of roughly 200 m at all sites during low tide during daytime. All field collections commenced at the lowest tidal height according to public tidal charts. Shore crabs (*C. punctatus*) were caught on the high shore from underneath large rocks and boulders. As sex differences might play a role for *C. punctatus* (Madeira et al. 2012b), equal numbers of male and female individuals were collected. The granular limpets (*S. granularis*) were haphazardly removed from sun-exposed vertical and horizontal rock surfaces on the upper high shore. After removal from the substratum, all animals were inspected for foot damage. The Cape urchins (*P. angulosus*) were collected from ~50 cm water depth within 30 minutes of the lowest tidal height to ensure similar microhabitat conditions. No sea urchins were collected from tide pools (Vinagre et al. 2018). With around 75 individuals overall per site, a total of 450 individuals were collected for the thermal experiments and transported separated by species and field site back to Stellenbosch University's Department of Botany and Zoology in battery-pump aerated dark transport containers filled with fresh sea water. Transport times ranged from one hour to twelve hours, depending on the distance to the collection site.

4.2.2 Acclimation and maintenance

Collected *S. granularis* and *P. angulosus* individuals were acclimated at 18±1°C (mean±SE) for 7-10 days in 60-litre perspex aquaria (600 x 300 x 300 mm) with a salinity of 35±2 ‰ (mean±SE). The acclimation temperature of 18°C was selected, because it lies within the temperature range experienced by all populations (Figure 4.4). This approach was utilised by Broom *et al.* for similar experiments with South African rocky shore klipfish *Clinus*

superciliosus (Broom, 2016). Shore crab *C. punctatus* specimens were kept in plastic boxes (10x10x8 cm) covered with shading net with small rocks providing shelter and a small amount of sea water, mimicking the conditions under which they are found in the field. Tests in advance of the field collection proved the importance of keeping this species separate, as intraspecific aggression including cannibalism may occur (personal observation). Keeping the crustaceans separate prevents the potential stress induced by conspecifics. All animals were fed small pieces of kelp ad libitum. Animals were not fed 24 hours before trials. An acclimation period of 1-2 weeks has been used in similar experiments on rocky shore invertebrates (Madeira et al. 2012a,b; 2014; Vinagre et al. 2012; 2013). Salinity levels were monitored daily with a salinity meter (Model 30, YSI Inc., Yellow Springs, OH). Sea water was prepared from sea salt kits (Seachem Reef Salt). Every 2 days, ~40-50% of the aquaria's water volume was changed and accumulated organic matter waste removed. The filters of the pumps were cleaned every 2-3 days. Test kits were used twice a week to monitor the levels of pH, nitrate and ammonium (Sera GmbH, Heinsberg, Germany). *Cyclograpsus punctatus* was sexed according to the shape of its abdominal flap, which is established for decapod crabs (Lee, Yamauchi and Yamazaki, 1994; Branch et al. 2007).

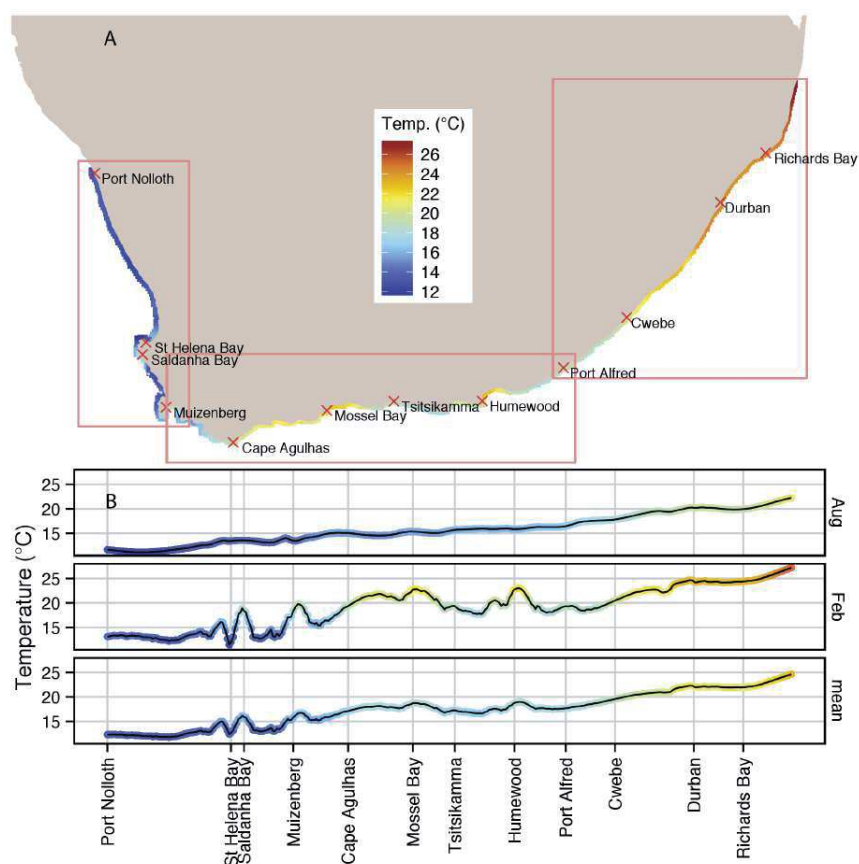


Figure 4.4. (A) Interpolated summertime sea surface temperature (SST) measurements across 87 sites (Port Nolloth to Sodwana Bay; broadly grouped by red boxes into west, south and east coast) from varying periods ranging between 1972 and 2012. The data was collected *in situ* either manually

with hand-held thermometers or electronically with underwater temperature recorders (UTRs) within 400 m from the coast at depths ranging from 20 cm to 9 m by different institutions (compare Figure 1 in Smit et al. 2013). The compiled data records were used to calculate a monthly temperature climatology, which served to produce an interpolated dataset representing temperature records at evenly spaced sites along the coast. These same data are also plotted in the lower panel (B) to further highlight the alongshore gradients. The middle and upper panels in (B) show the seasonal mean monthly *in situ* temperature for August and February respectively representing winter and summer (Smit et al. 2013).

Acclimation setup for S. granularis

For the limpet *S. granularis*, the following method has proven successful for maintaining intertidal limpets in circumstances when flow through tanks or tidal variation systems are not available. As a high shore animal, *S. granularis* is used to being fully exposed to air during low tide for multiple hours per day. These circumstances perhaps reflect in their settling preference in the aquaria, such when given free choice on where to position themselves, individuals choose to be semi-emerged or completely emerged over the waterline. In rocky shore habitats, tidal phases naturally regulate the range of *S. granularis*, as the animals merely wait for the upcoming high tide to submerge them or splash them again with fresh sea water. When housed in aquaria, *S. granularis* was expected to self-regulate its internal water levels for instance by occasional nightly foraging. However, without intervention, animals would desiccate beyond recovery (personal observation). The circumstances of self-regulation behaviour for internal water levels or the absence thereof in *S. granularis* would be an interesting issue for future research. To prevent desiccation and to standardise the acclimation temperature, the animals were placed on a Perspex plate as an artificial island. The water level and the air pump were adjusted to constantly cover the island in a thin, moving water layer, which also helped to flush organic waste away (Figure 4.5). The bottom of the tank was filled with a layer of sand to discourage the molluscs to cross over to the walls for uncontrolled emergence. This simple setup allows to maintain limpets without access to tanks with regulated water levels or climbing confinements like nets or grids. Due to limitations of available aquaria and crab containers, two populations could be maintained in the laboratory facility at the same time. New populations were acclimated as soon as the trials for examined ones were completed.

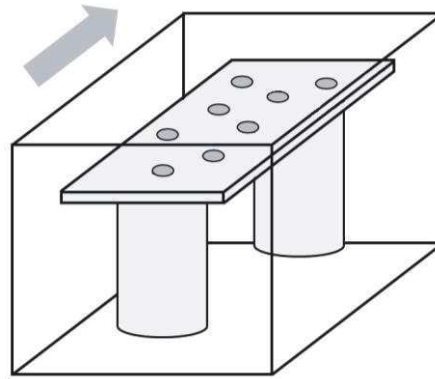


Figure 4.5. Schematic setup of the artificial Perspex island for the acclimation of *S. granularis*, which are depicted as oval circles. The arrow indicates the direction of the water flow generated by the aquaria pump.

4.2.3 Thermal tolerance experiments

In the limpet *S. granularis*, individuals were distinguished by combinations of coloured dots on the shell and overall shell length. For the urchin *P. angulosus*, length, weight and naturally occurring colour variations as well as multi-coloured cotton threads were utilised for identification. Shore crabs were kept separately in labelled plastic containers. Following the acclimation period of 7 days, including the pre-trial 24 hour period of starvation, experiments were conducted in species-specific setups (Figure 4.6-8). Limpets were carefully transferred to a small high-walled container and allowed to firmly attach to its walls for 15-20 minutes. Both the crab *C. punctatus* and the limpet *S. granularis* specimen were assessed in air, which corresponds to low tide field conditions, when both species are fully emerged from sea water and exposed to air. The Cape urchin *P. angulosus* was tested in sea water (volume ~2L), congruent with its habitat position during low tide. All trials were conducted in thermal baths (Grant GP200 R4, Grant Instruments, Cambridge, UK) containing water (CT_{max}) or a glycol:water (50:50) mix (CT_{min}). A wide range of different heating rates has been utilised for various intertidal organisms in thermal tolerance studies (Lutterschmidt and Hutchison, 1997b). Similar studies on intertidal invertebrates have applied heating rates between 0.1-0.5°C per minute (*Limpets and mussels*: Braby and Somero, 2006; Dong and Williams, 2011; Logan, Kost and Somero, 2012; Tagliarolo and McQuaid, 2015; Kankondi, McQuaid and Tagliarolo, 2018; *Sea urchins*: Sherman, 2015; Collin et al. 2018; *Crabs*: Metzger et al. 2007; Walther et al. 2009; Vinagre et al. 2013; Madeira et al. 2014). In this study, a heating and cooling rate of 0.5°C per minute is utilised to enable comparisons of populations and possibly species while maintaining a rate within the range reported in the literature. Consistency of heating rate was confirmed in preliminary tests. Temperature loggers used to capture the endpoint during the trials (Thermochron iButtons, DS1922L, Maxim Integrated

Products Inc., Sunnydale, CA; resolution $\sim 0.5^{\circ}\text{C}$; time interval 60 sec) were positioned with reusable putty adhesive Prestik® (*C. punctatus*, *S. granularis*) or submerged in a thin, sealed silicone capsule (*P. angulosus*) as close as possible to the animal. The time at which the pre-determined endpoints (see section 4.2.3.1-3) occurred was noted immediately in every trial and the temperature measurement retrieved from the iButton recordings afterwards, ensuring that times were synchronized to the second for both. Past studies have used hand-held thermometers or thermocouples in this context, but the iButton position was consistent across species trials and minimised interference with the setup. CT_{\min} was first assessed by cooling and afterwards animals were given 12 hours to recover in their maintenance tanks. CT_{\max} was then tested by heating in the same individuals, which were then returned to the maintenance tanks. Every individual was measured once for CT_{\min} and once for CT_{\max} . Obtained lower and upper thermal limits were only included in analyses, if the animal was in good condition after the recovery period following both tests. The number of animals tested for both lower and upper critical thermal limits is listed in Table S4.2 (Appendix).

4.2.3.1 *Cyclograpsus punctatus*

Congruent with the maintenance containers, the experimental chamber for the *C. punctatus* crabs required a high rim. A high-walled plastic container was placed in the thermal bath with a weight at the bottom preventing floating (Figure 4.6). For the trial, the individual animal and the iButton were positioned in the container, separated from the weight at the bottom by a layer of 3 mm cardboard (Figure 4.6). The thermal bath was covered with a 1 mm plastic sheet and a custom piece of foam board to maintain the temperature inside the chamber. To begin, the thermal bath was set to the acclimation temperature (18°C) and allowed to equilibrate. The trial started after the animal was placed in the experimental chamber. The animal was turned on its back with tweezers, following a method described by Vinagre and Madeira for assessing $\text{CT}_{\min}/\text{CT}_{\max}$ on intertidal crustaceans (Madeira et al. 2012c; Vinagre et al. 2015). For both CT_{\max} and CT_{\min} trials, the animal was inverted every 10 minutes in the initial phase of the experiment (first 50 minutes), then the interval shortened to 5 minutes until the individual was unable to right itself (invert when placed on dorsal surface) within 60 seconds. The loss of righting response was noted as the endpoint of the trial as critical thermal minimum and critical thermal maximum, respectively. Once CT_{\max} was determined, individuals were removed from the chamber, weighed, measured and splashed with a few drops of sea water before returning to their respective maintenance containers for recovery.

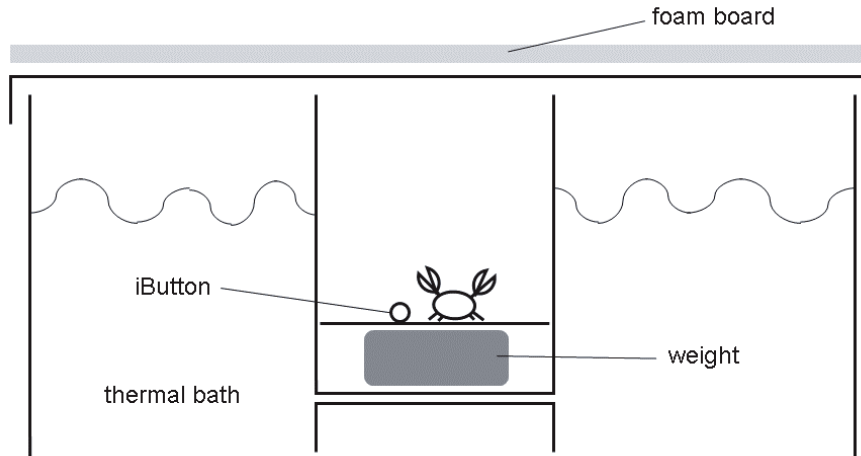


Figure 4.6. Schematic setup of the CT_{min} and CT_{max} trials for *C. punctatus*.

4.2.3.2 *Scutellastra granularis*

Among the approaches to assess thermal limits in limpets is the duration for which the animal is able to remain attached to a substratum with muscle capacity (Morley et al. 2011). Other possibilities include the assessment of cardiac performance during heat stress (Dong and Williams, 2011; Han et al. 2013) or the evaluation of heat shock protein 70 (HSP70) as indication of near lethal stress (Miller, Harley and Denny, 2009; Han et al. 2013). During heat stress, some limpet species including *S. granularis* display mushrooming behaviour, where the shell is lifted off the substrate by several millimetres to likely provide temporary relief (Williams et al. 2005; Harley et al. 2009). In this study, the heating endpoint (CT_{max}) was recorded when the shell was lifted off the surface on all sides by 1-2 millimetres. For the trial, a limited number of animals (6-10) were distributed over the walls of a plastic chamber and allowed to settle. Limpets were spaced as far apart as possible and did not move or interact with each other during a trial. Low temperatures do not induce mushrooming behaviour and there are at present no established, widely applicable approaches to measure CT_{min} in limpets. To avoid drastically different setups between CT_{max} and CT_{min} , the latter was determined with a mechanical stimulus by prodding the shell with the backend of a scalpel until the animal was unable to stay attached to the substrate (wall). The CT_{min} endpoint was noted as soon as the animal was unable to hold its attached position on the vertical surface (Figure 4.7). During the trial, a thin plastic sheet and foam board were used to maintain the temperature. Prior tests confirmed that briefly lifting the cover to assess or prod the animals did not affect the temperature conditions inside. For the CT_{max} trials, the animals were observed for the occurrence of mushrooming behaviour throughout the experiment. During the CT_{min} trials, the mechanical stimulus was applied every 10 minutes in the initial phase of

the experiment (first 70 minutes) and then every 5 minutes till the endpoint occurred (detachment).

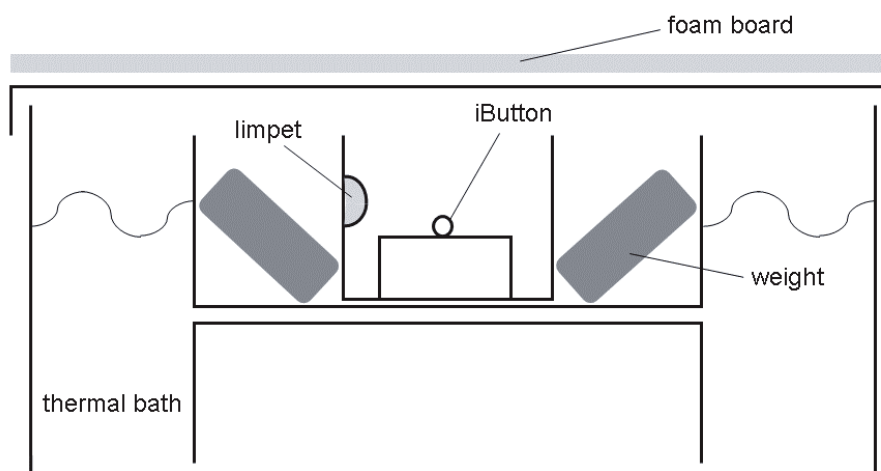


Figure 4.7. Schematic setup of the CT_{min} and CT_{max} trials for *S. granularis*.

4.2.3.3 *Parechinus angulosus*

Different approaches to assess thermal limits in sea urchins have been suggested, including whether the urchin is able to right itself quickly when inverted (Sherman, 2015) or remains attached to a vertical surface (Hernández et al. 2004). Both strategies are not applicable to *P. angulosus*, which prefers not to remain attached to vertical surfaces during trials and its capacity to right itself after inversion is highly dependent on the individual and the number of times the animal is inverted (preliminary tests at 18°C and 25°C sea water). Testing revealed that *P. angulosus* responds with strong tube feet activity to a mechanical stimulus, such as briefly prodding with large tweezers. The sub-lethal points (CT_{min} and CT_{max}) were noted when the tube feet no longer display a response to prodding. Further, tests showed that *P. angulosus* does not habituate to the prodding during the trial and shows the same tube feet response intensity throughout. To commence the trial, an individual urchin was submerged together with the silicone capsuled iButton in a container filled with sea water (18°C) inside the thermal bath. The setup was covered with a thin Perspex sheet and foam board. In an unfamiliar environment, *P. angulosus* displays a brief period of increased tube feet activity to explore the immediate surroundings. The trial only started when the animal returned to not using the tube feet (resting state), which usually took under 2 minutes. For the CT_{max} trials, the animals were prodded every 10 minutes in the initial phase of the experiment (first 30 minutes) and then every 3 minutes until the endpoint was observed. To assess CT_{min} , animals were prodded every 10 minutes in the initial phase of the experiment (first 50 minutes) and then every 5 minutes until the endpoint was observed (with an \pm accuracy of 2.5 °C). The animal was removed from the experimental chamber as soon as no response

occurred to the stimulus, measured, weighed and placed in the maintenance tank to begin the recovery period. As with the other species, every animal was tested once for CT_{min} and once for CT_{max} .

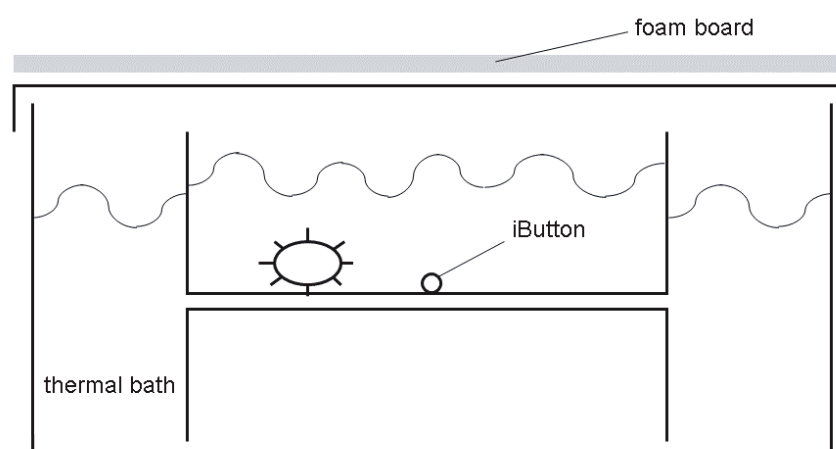


Figure 4.8. Schematic setup of the CT_{min} and CT_{max} trials for *P. angulosus*.

4.2.4 Habitat temperature

Measurements of habitat temperature allow the approximation of species possible warming (Deutsch et al. 2008) and cooling tolerance (Gutiérrez-Pesquera et al. 2016). For upper high shore species such as the limpet *S. granularis* and the crab *C. punctatus*, air temperature is likely more decisive than SST during vulnerable low tide phases. In contrast, SST is most relevant to the low shore urchin *P. angulosus*, which remains submerged throughout tidal phases. Maximum and minimum air temperature (averaged monthly mean, 2009-2019) of the field sites or the closest available locations were provided by the South African Weather Service (SAWS) (Table 4.1). Further, SAWS oversees data collection of coastal sea surface temperature, which is recorded with alcohol thermometers at the same place and time each day from the shore (Table 4.2). Coastal SST data was made available for the years 2017-2019. Maximum and minimum measurements are not captured by the data collection protocol. Collected data from Sea Point was excluded due to insufficient data quality.

Table 4.1. Air temperature minima and maxima for the seven study locations (averaged monthly mean (2009-2019; Coffee Bay: 2010-2019); Source: South African Weather Service (SAWS)) or the closest available locations (*Cape Columbine (<20 km), **Cape Town Yacht Harbour (<5km), ***Coffee Bay (~200 km)).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Port Nolloth												
Max °C	21.5	21.6	21.0	21.2	19.8	19.1	19.6	18.5	19.1	19.7	20.3	21.3
Min °C	13.9	13.6	13.2	11.8	10.4	9.1	8.8	8.4	9.4	11.0	12.2	13.8
Jacob's Bay*												
Max °C	21.3	22.0	21.0	20.4	18.3	16.8	16.8	16.5	17.6	19.2	19.9	21.1
Min °C	13.9	14.1	13.6	12.8	11.9	10.7	10.3	10.1	10.9	12.0	12.6	13.7
Sea Point**												
Max °C	27.8	28.0	26.4	24.7	21.4	18.8	19.0	19.3	20.4	22.9	24.8	26.6
Min °C	17.6	17.5	16.3	15.0	13.1	11.2	11.0	11.4	12.4	13.9	15.2	16.8
Cape Agulhas												
Max °C	24.2	24.1	23.1	21.1	19.5	17.8	17.6	17.6	18.6	19.8	21.1	23.1
Min °C	18.8	18.6	17.4	15.4	13.8	11.7	11.3	11.4	12.6	14.3	15.5	17.6
Knysna												
Max °C	27.0	27.1	26.3	24.4	23.1	20.8	20.4	20.5	20.9	22.6	23.7	25.8
Min °C	17.3	17.2	16.1	13.3	11.3	8.8	8.5	9.0	10.6	12.6	13.7	16.0
Cape St. Francis												
Max °C	23.3	22.5	22.0	20.7	20.1	19.1	18.7	18.4	18.6	19.2	20.3	22.1
Min °C	17.7	16.8	16.4	14.7	13.2	10.9	10.9	11.2	12.5	13.8	14.7	16.5
Haga Haga***												
Max °C	27.2	27.6	27.2	25.6	25.0	23.8	23.1	23.1	23.3	23.1	24.4	25.4
Min °C	18.7	18.9	18.5	15.7	13.9	11.2	11.3	11.9	13.3	14.5	16.3	17.6

Table 4.2. Monthly average sea surface temperature (SST) for the seven study locations (2017-2019; Source: South African Weather Service) or the closest available locations (*Saldanha Bay (distance: 5 km), **Mosselbay (~100 km), ***Port Elizabeth (~100 km), ****East London, Orient Beach (~50 km)).

(°C)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Port Nolloth	16.1	16.3	16.5	16.5	16.2	15.9	15.9	15.3	15.3	15.9	16.4	17.0
Jacob's Bay*	18.8	18.5	17.6	16.4	14.6	14.3	14.0	14.1	15.0	16.1	17.8	17.5
Cape Agulhas	18.9	18.3	17.7	14.3	15.0	14.2	14.3	14.0	15.1	16.2	17.3	18.0
Knysna**	22.1	21.0	20.5	18.9	16.3	15.2	15.3	16.0	17.1	17.7	19.0	20.4
St. Francis Bay***	21.3	20.1	20.0	18.4	17.1	16.1	16.1	15.9	17.2	17.6	18.5	19.4
Haga Haga****	19.2	18.6	18.4	18.3	17.2	16.9	17.0	17.2	18.1	18.6	18.4	18.7

It is important to acknowledge that habitat temperature is not an ideal proxy for species body temperature, because the influencing factors of body temperature are vastly complex (Broitman et al. 2009; Potter, Woods and Pincebourde, 2013). Sea surface temperature and air temperature represent a coarse indication of temperature possibly experienced at intertidal microsites. Deploying *in situ* temperature loggers (for instance Lima and Wetthey,

2009) at the seven study sites microhabitats was part of the project planning framework, but could not be technically realised in the time frame of the study. Sea surface data collected by institutions of the Western Indian Ocean Coastal Observing System (WIOCOS) and by Smit et al. (Figure 4.4) were also considered but excluded due to data deficiency at several study sites (Smit et al. 2013).

4.2.5 Thermal breadth and warming and cooling tolerance (WT, CT)

The thermal tolerance range (thermal breadth) was calculated by subtracting CT_{min} from CT_{max} ($T_{br} = CT_{max} - CT_{min}$). Warming and cooling tolerance were calculated from CT_{max}/CT_{min} and the maximum/minimum habitat temperature (T_{max} , T_{min}) respectively ($WT = CT_{max} - T_{max}$; $CT = CT_{min} - T_{min}$) (Deutsch et al. 2008; Gutiérrez-Pesquera et al. 2016). Air temperature was utilised for the exposed upper shore species (*S. granularis*, *C. punctatus*) and water temperature for the low shore species (*P. angulosus*).

4.2.6 Statistical analysis

The goal of this chapter is to test if the three species reveal regional differences in CT_{max} , CT_{min} and T_{br} and how these thermal parameters might be influenced by mass and (in *C. punctatus*) sex. Statistical analyses were performed in R (version 4.0.2; <http://cran.r-project.org>). Sampling sites were regionally grouped into west coast (WC; Port Nolloth (PN)/Jacob's Bay (JC); Sea Point (SP)), south coast (SC; Cape Agulhas (CA), Knysna (KY)) and east coast (EC; Cape St. Francis (CF); Haga Haga (HH)). Data was assessed for normal distribution and homogeneity of variance with Shapiro–Wilk's test (Shapiro and Wilk, 1965), the function skewness (package moments; Komsta and Novomestky, 2015), Levene's Test (Levene, 1960; car package, Fox and Weisberg, 2011) and Bartlett's Test (Bartlett, 1937) (Appendix, Table S4.1). Using the lmer function in the package lme4 (Bates et al. 2015), linear mixed-effect models were fitted with body mass as response variable, region as fixed effect (three levels: west, south and east coast) and site as random effect (six levels: Port Nolloth/Jacob's Bay, Sea Point, Cape Agulhas, Knysna, Cape St. Francis, Haga Haga). In *C. punctatus*, sex was fitted as an additional fixed effect to test for differences between male and female crabs. Identical models were fitted with CT_{min} , CT_{max} and T_{br} as respective response variable. To test the potential effect of mass on critical thermal limits, additional models were fitted with CT_{min} (CT_{max} , T_{br}) as response variable and region and mass as fixed effect and site as random effect. For the latter models, CT_{min} , CT_{max} , T_{br} and mass were log10-transformed for appropriate allometric scaling. For CT_{min} data containing negative values, a constant was added before log10-transformation. Pairwise post hoc

comparisons between regions were conducted by estimating marginal means with the package *emmeans* (formerly *lsmeans*) (Lenth et al. 2018). Values represent mean \pm standard error unless stated otherwise.

4.3 Results

4.3.1 Preliminary analyses: Body mass

The mean body mass of *Scutellastra granularis* was found to be significantly higher in west coast populations compared to populations at the south ($p = 0.021$) and east coast ($p = 0.031$) (Table 4.3, Table S4.2; Figure 4.9). Further, west coast populations of *P. angulosus* indicate higher body mass than south coast populations ($p = 0.041$) (Table 4.3; Figure 4.9). No regional mass differences were detected in *C. punctatus*, but male crabs possess significantly higher body mass than females ($p = <0.001$) (Table 4.3).

4.3.2 Critical lower temperature limits (CT_{min})

Linear mixed effect models suggest no significant CT_{min} differences in *C. punctatus*, *S. granularis* and *P. angulosus* (Table 4.4, Table S4.3; Figure 4.9). CT_{min} is suggested to be negatively correlated with mass in both *C. punctatus* ($p = <0.001$) and *P. angulosus* ($p = 0.007$) (i.e. heavier individuals have lower CT_{min}) (Table 4.4).

4.3.3 Critical upper temperature limits (CT_{max})

Populations of *C. punctatus* demonstrate significantly higher CT_{max} in west coast populations compared to east coast populations ($p = 0.033$) (Table 4.5, Table S4.4; Figure 4.10). The models suggest no significant CT_{max} differences in *S. granularis* and *P. angulosus*. In the urchin *P. angulosus*, CT_{max} is suggested to be positively correlated with mass ($p = 0.005$) (i.e. heavier individuals have higher CT_{max}) (Table 4.5).

4.3.4 Thermal breadth (T_{br})

Linear mixed effect models suggest no significant T_{br} differences in *C. punctatus*, *S. granularis* and *P. angulosus* (Table 4.6, Table S4.5; Figure 4.10). Thermal breadth is suggested to be positively correlated with mass (i.e. heavier individuals have larger thermal breadth) in both *C. punctatus* ($p = <0.001$) and *P. angulosus* ($p = <0.001$) (Table 4.6).

<i>C. punctatus</i>					
(mass) ~ sex + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	1.4616	0.9615	3.4071	1.52	0.215
sexm	2.6300	0.4351	98.3828	6.044	<0.001***
regionSC	4.1012	1.3251	3.0744	3.095	0.052
regionWC	3.9704	1.3199	3.0262	3.008	0.057
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	-4.101	1.33	3.05	-3.095	0.103
EC - WC	-3.97	1.32	3.00	-3.008	0.112
SC - WC	0.131	1.32	2.96	0.099	0.995
<i>S. granularis</i>					
(mass) ~ region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	2.5974	0.6495	2.735	3.999	0.033*
regionSC	-0.5706	0.9289	2.861	-0.614	0.584
regionWC	4.7163	0.9311	2.8876	5.065	0.016*
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	0.571	0.929	2.94	0.614	0.823
EC - WC	-4.716	0.931	2.97	-5.065	0.031*
SC - WC	-5.287	0.941	3.1	-5.616	0.021*
<i>P. angulosus</i>					
(mass) ~ region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	20.791	2.428	2.802	8.561	0.004**
regionSC	-3.792	3.441	2.824	-1.102	0.355
regionWC	11.719	3.463	2.892	3.385	0.045*
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	3.79	3.44	2.94	1.101	0.577
EC - WC	-11.72	3.46	3.01	-3.382	0.085
SC - WC	-15.51	3.47	3.03	-4.467	0.041*

Table 4.4. Model parameter estimates of linear mixed models fitted for CTmin. t-tests use Satterthwaite's method.

<i>C. punctatus</i>					
(log10(CTmin)) ~ (log10(mass)) + sex + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	0.8530	0.06212	4.33816	13.732	<0.001***
log10(mass)	-0.2764	0.0799	99.04419	-3.459	<0.001***
sexm	-0.0137	0.03343	97.75248	-0.410	0.683
regionSC	-0.0186	0.08736	4.22584	-0.213	0.841
regionWC	0.0302	0.08695	4.15853	0.348	0.745
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	0.0186	0.0878	4.20	0.212	0.976
EC - WC	-0.0302	0.0874	4.14	-0.346	0.937
SC - WC	-0.0488	0.0789	2.86	-0.619	0.821
<i>P. angulosus</i>					
(log10(CTmin)) ~ (log10(mass)) + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	1.1882	0.13354	9.74776	8.898	<0.001***
log10(mass)	-0.1916	0.06989	105.4016	-2.742	0.007*
regionSC	-0.1693	0.13989	3.00121	-1.21	0.313
regionWC	-0.0831	0.14064	3.06548	-0.591	0.595
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	0.1693	0.140	3.00	1.210	0.525
EC - WC	0.0831	0.141	3.06	0.591	0.834
SC - WC	-0.0861	0.141	3.13	-0.609	0.825

Table 4.3. (left) Model parameter estimates of linear mixed models fitted for mass. t-tests use Satterthwaite's method.

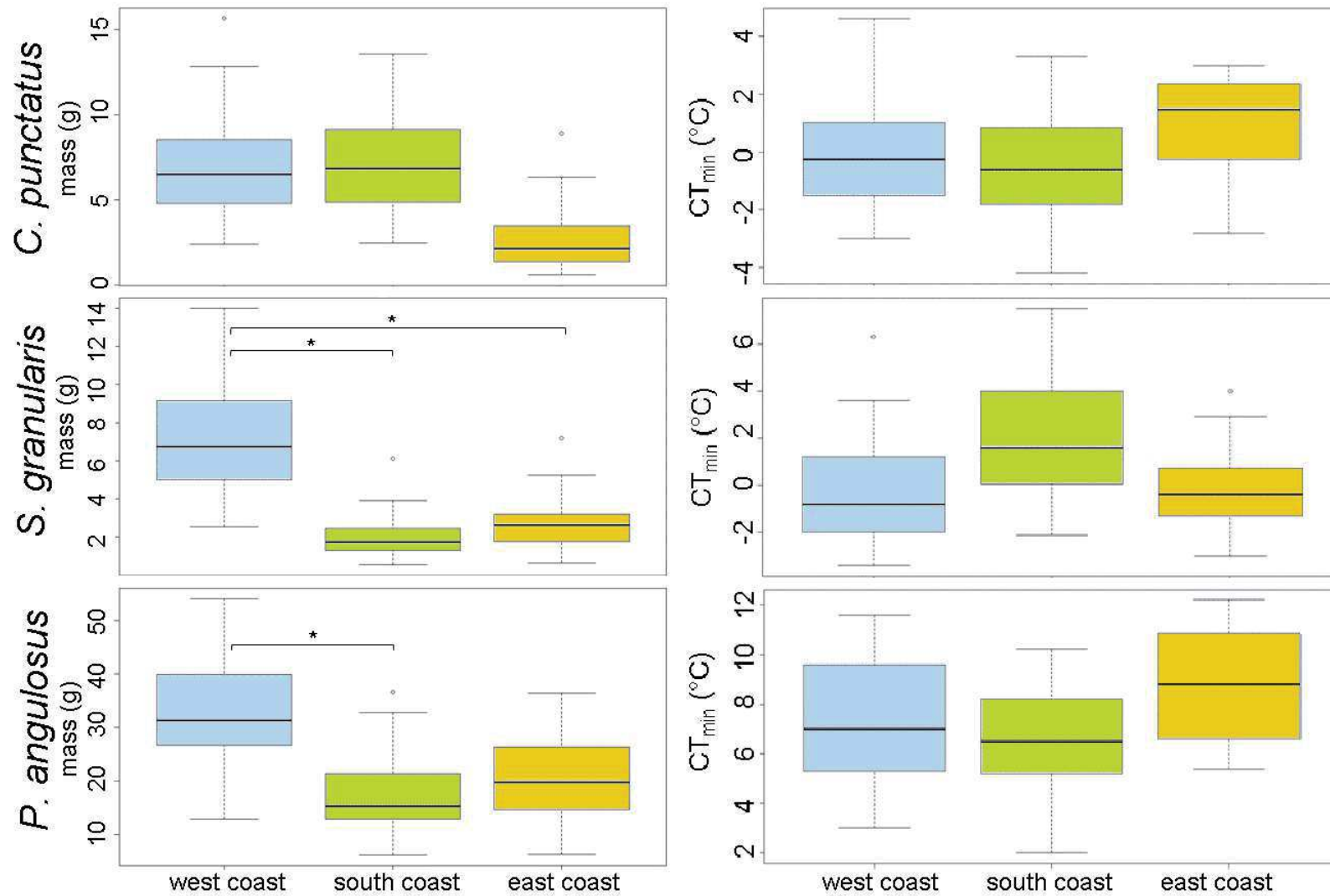


Figure 4.9. Body mass and CT_{min} shown by region. Significant differences are marked with asterisks (* = p < 0.05). Box and whisker plot showing quartiles and median. Small circles represent outliers.

Table 4.5. Model parameter estimates of linear mixed models fitted for CTmax. t-tests use Satterthwaite's method.

<i>C. punctatus</i>					
(log10(CTmax)) ~ (log10(mass)) + sex + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	1.645062	0.002919	5.466814	563.6	<0.001***
log10(mass)	0.004455	0.005102	81.02667	0.873	0.385
sexm	-0.00111	0.002198	98.43329	-0.506	0.614
regionSC	0.007083	0.004073	5.077881	1.739	0.142
regionWC	0.014513	0.004041	4.99	3.592	0.016*
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	-0.00708	0.00417	5.64	-1.700	0.284
EC - WC	-0.01451	0.00412	5.54	-3.522	0.033*
SC - WC	-0.00743	0.0033	2.69	-2.254	0.221
<i>P. angulosus</i>					
(log10(CTmax)) ~ (log10(mass)) + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	1.443528	0.022374	9.732509	64.517	<0.001***
log10(mass)	0.03322	0.011694	105.4078	2.841	0.005**
regionSC	-0.00384	0.023467	3.010837	-0.164	0.880
regionWC	-0.0294	0.023592	3.074974	-1.246	0.299
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	0.00384	0.0235	3.00	0.164	0.985
EC - WC	0.0294	0.0236	3.06	1.246	0.508
SC - WC	0.02555	0.0237	3.13	1.077	0.586

Table 4.6. Model parameter estimates of linear mixed models fitted for thermal breadth. t-tests use Satterthwaite's method.

<i>C. punctatus</i>					
(log10(Tbr)) ~ (log10(mass)) + sex + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	1.625291	0.00557	4.90697	291.78	<0.001***
log10(mass)	0.033484	0.009052	88.89694	3.699	<0.001***
sexm	-0.00073	0.00386	98.11506	-0.188	0.851
regionSC	0.007938	0.007794	4.640686	1.018	0.359
regionWC	0.012399	0.007739	4.553217	1.602	0.176
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	-0.00794	0.00792	5.18	-1.002	0.606
EC - WC	-0.01240	0.00785	5.08	-1.580	0.334
SC - WC	-0.00446	0.00654	2.75	-0.682	0.791
<i>P. angulosus</i>					
(log10(Tbr)) ~ (log10(mass)) + region + (1 site)					
Fixed effects	Estimate	SE	df	t-value	Pr(> t)
(Intercept)	1.201275	0.060781	5.870168	19.764	<0.001***
log10(mass)	0.098574	0.025344	105.2042	3.889	<0.001***
regionSC	0.050144	0.072612	3.005358	0.691	0.539
regionWC	-0.00952	0.072802	3.036836	-0.131	0.904
Pairwise regional comparisons					
Contrasts	Estimate	SE	df	t-value	p
EC - SC	-0.05014	0.0726	3.00	-0.691	0.785
EC - WC	0.00952	0.0728	3.03	0.131	0.991
SC - WC	0.05966	0.073	3.06	0.817	0.719

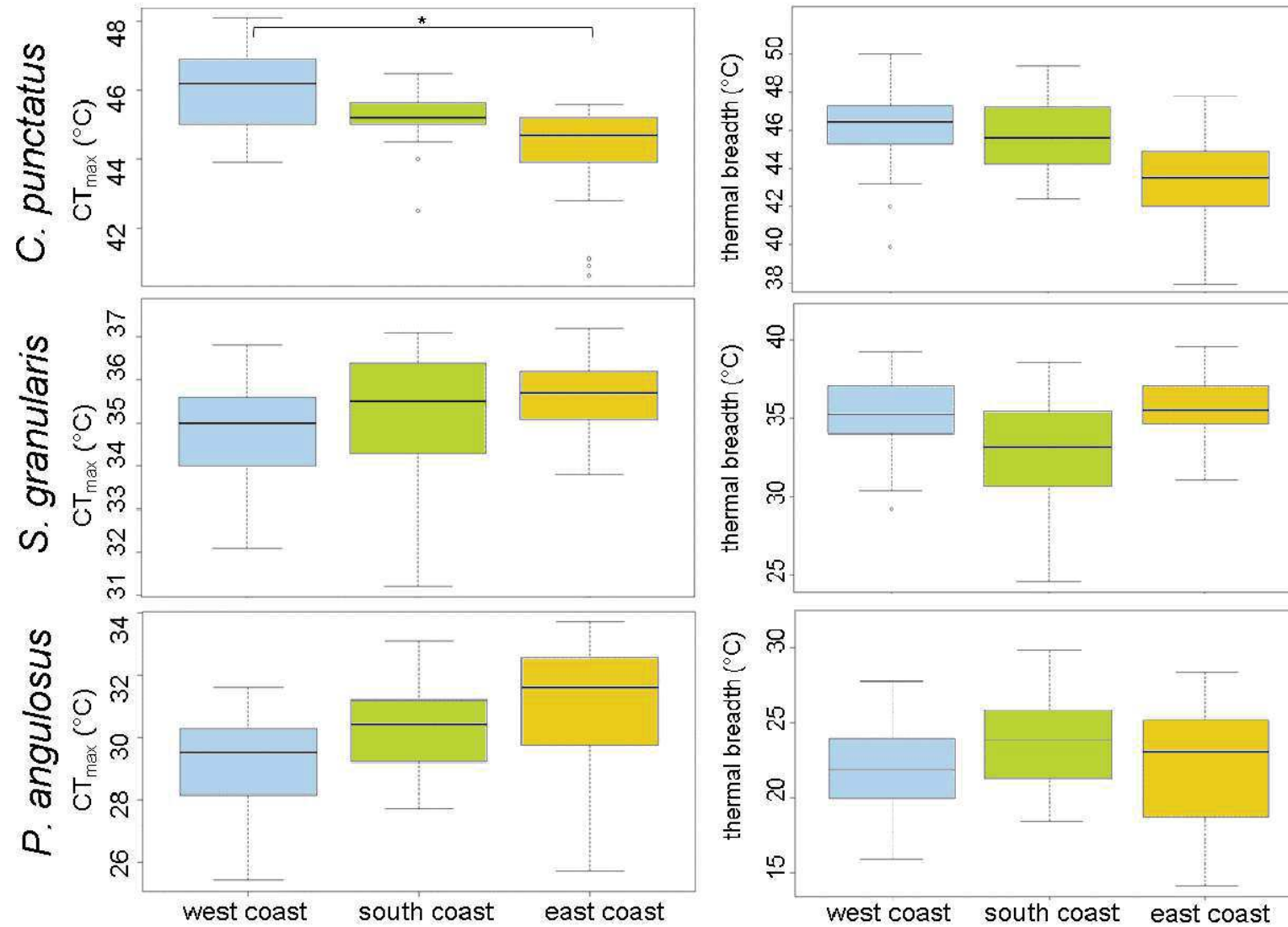


Figure 4.10. CT_{max} and thermal breadth shown by region. Significant differences are marked with asterisks (* = $p < 0.05$). Box and whisker plot showing quartiles and median. Small circles represent outliers.

4.3.5 Warming (WT) and cooling tolerance (CT)

The range between upper critical limits and maximum habitat temperature (warming tolerance, WT), as well as the range between lower critical limits and minimum air temperature (cooling tolerance, CT) are listed in Table 4.7 for the high-shore species *C. punctatus* and *S. granularis*. For instance, the average maximum air temperature in the Jacob's Bay area in January is reported as 21.3°C (Table 4.1) and the mean CT_{max} measured for the population is 45.9°C (Table S4.4, Appendix), signalling a potential warming tolerance of 24.5°C (Table 4.7). For *P. angulosus*, the range between upper and lower critical limits was calculated in reference to the available mean SST data (Table 4.8).

Table 4.7. Warming and cooling tolerance (in °C) calculated in relation to upper/lower critical limits and mean maximum/minimum air temperature per population and month in *C. punctatus* and *S. granularis*.

<i>C. punctatus</i>	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jacob's Bay												
WT (C°)	24.5	23.8	24.9	25.4	27.6	29.1	29.1	29.4	28.3	26.6	26.0	24.8
CT (C°)	13.6	13.8	13.3	12.5	11.6	10.4	10.0	9.8	10.6	11.6	12.3	13.4
Sea Point												
WT (C°)	18.3	18.1	19.8	21.4	24.8	27.3	27.1	26.8	25.7	23.2	21.3	19.6
CT (C°)	18.2	18.1	17.0	15.6	13.7	11.9	11.6	12.0	13.0	14.6	15.8	17.4
Cape Agulhas												
WT (C°)	21.1	21.2	22.2	24.2	25.8	27.5	27.7	27.7	26.7	25.5	24.2	22.2
CT (C°)	18.3	18.1	17.0	15.0	13.3	11.2	10.9	10.9	12.1	13.8	15.1	17.2
Knysna												
WT (C°)	18.1	18.0	18.8	20.7	22.0	24.3	24.7	24.6	24.2	22.6	21.4	19.3
CT (C°)	18.9	18.7	17.6	14.8	12.8	10.3	10.0	10.5	12.1	14.2	15.2	17.5
Cape St. Francis												
WT (C°)	20.5	21.4	21.9	23.2	23.7	24.7	25.1	25.5	25.2	24.7	23.6	21.7
CT (C°)	16.4	15.5	15.1	13.4	11.9	9.6	9.6	9.9	11.2	12.5	13.4	15.2
Haga Haga												
WT (C°)	17.5	17.1	17.5	19.2	19.7	21.0	21.6	21.6	21.4	21.6	20.3	19.3
CT (C°)	18.2	18.3	17.9	15.1	13.4	10.6	10.7	11.3	12.7	13.9	15.8	17.0
<i>S. granularis</i>	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Port Nolloth												
WT (C°)	13.1	13.0	13.7	13.5	14.8	15.5	15.0	16.1	15.5	15.0	14.3	13.3
CT (C°)	14.0	13.7	13.3	11.9	10.6	9.2	8.9	8.5	9.5	11.1	12.3	13.9
Sea Point												
WT (C°)	7.3	7.2	8.8	10.5	13.8	16.4	16.2	15.9	14.7	12.3	10.4	8.6
CT (C°)	18.0	17.9	16.8	15.4	13.5	11.6	11.4	11.8	12.8	14.4	15.6	17.2
Cape Agulhas												
WT (C°)	10.3	10.4	11.3	13.3	15.0	16.6	16.8	16.8	15.8	14.7	13.4	11.4
CT (C°)	15.6	15.4	14.3	12.2	10.6	8.5	8.2	8.2	9.4	11.1	12.3	14.5
Knysna												
WT (C°)	8.7	8.6	9.4	11.3	12.6	14.9	15.3	15.2	14.8	13.2	12.0	10.0
CT (C°)	16.2	16.1	14.9	12.1	10.2	7.7	7.4	7.9	9.5	11.5	12.6	14.8
Cape St. Francis												
WT (C°)	12.3	13.2	13.7	15.0	15.5	16.5	17.0	17.3	17.0	16.5	15.4	13.5
CT (C°)	18.0	17.2	16.7	15.1	13.6	11.2	11.2	11.5	12.8	14.2	15.0	16.8
Haga Haga												
WT (C°)	8.2	7.8	8.3	9.9	10.4	11.7	12.4	12.4	12.2	12.4	11.1	10.0
CT (C°)	18.8	19.0	18.6	15.8	14.0	11.2	11.4	12.0	13.4	14.6	16.4	17.7

Table 4.8. Warming and cooling tolerance (in °C) calculated in relation to upper/lower critical limits and mean SST temperature per population and month in *P. angulosus*.

<i>P. angulosus</i>	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Port Nolloth												
WT (°C)	12.8	12.5	12.4	12.4	12.7	13.0	12.9	13.6	13.5	12.9	12.4	11.9
CT (°C)	10.5	10.7	10.9	10.9	10.6	10.3	10.4	9.7	9.7	10.4	10.8	11.4
Cape Agulhas												
WT (°C)	10.3	11.0	11.6	14.9	14.3	15.1	15.0	15.2	14.1	13.0	12.0	11.2
CT (°C)	10.8	10.2	9.6	6.2	6.8	6.1	6.2	5.9	7.0	8.1	9.2	9.9
Knysna												
WT (°C)	9.0	10.1	10.6	12.2	14.9	15.9	15.8	15.1	14.1	13.5	12.2	10.8
CT (°C)	17.1	16.0	15.5	13.9	11.2	10.2	10.3	11.0	12.0	12.6	14.0	15.3
Cape St. Francis												
WT (°C)	7.8	8.9	9.1	10.6	11.9	12.9	12.9	13.2	11.8	11.4	10.6	9.6
CT (°C)	10.4	9.2	9.1	7.6	6.3	5.2	5.3	5.0	6.3	6.7	7.6	8.6
Haga Haga												
WT (°C)	13.2	13.8	14.0	14.2	15.2	15.5	15.4	15.2	14.4	13.8	14.1	13.7
CT (°C)	12.0	11.4	11.2	11.1	10.0	9.7	9.9	10.0	10.9	11.4	11.2	11.5

4.4 Discussion

Analysing intraspecific variation of physiological traits across environmental gradients in multiple species is key to establish macrophysiological patterns (Osovitz and Hofmann, 2007; Helmuth 2009; Gaston et al. 2009; Richard et al. 2012). Thermal tolerance limits are understudied in the majority of species, yet their investigation is crucial to anticipate potential physiological resilience to climate change impacts (Vinagre et al. 2016). This study contributes upper and lower critical thermal limits of three co-distributed rocky shore invertebrates inhabiting a pronounced marine thermal gradient and indicates that west coast crabs have higher upper thermal limits than east coast crabs. Impacts from climate change can be expected to differ depending on the local thermal conditions which populations experience on the South African coastline.

4.4.1 Body mass differences associated with primary productivity and SST

This chapter contributes evidence for regional body mass differences in South African marine invertebrates in two of three study species. Within sites, animals were collected randomly to minimise potential sampling bias, yet the Cape urchin *P. angulosus* demonstrates significantly higher body mass on the west coast compared to the south coast (Table 4.3; Figure 4.9). Further, west coast limpets (*S. granularis*) are significantly larger than limpets at the south and east coast (Table 4.3; Figure 4.9). Repeated visits to the field sites over multiple years confirm that the findings represent patterns reflected in the greater local populations (personal observation). Detecting significant mass (or size) differences across the South African coast corroborates earlier work on a probable relationship between intertidal primary productivity and consumer biomass in different coastal regions (Kilburn and Rippey, 1982; Bustamante et al. 1995). Assessments of nutrient concentrations including nitrates, nitrites, phosphates and silicates in addition to chlorophyll a levels produced by micro algae confirm the presence of a large scale intertidal primary productivity gradient around the coast (Bustamante et al. 1995). Intertidal primary production and nutrient concentrations are highest on the west coast, intermediate on the south and finally lowest on the east coast (Bustamante et al. 1995). Strong upwelling on the South African west coast is associated with nutrient rich waters and high biological productivity, whereas productivity is comparatively low on the south and east coast due to the nutrient poor waters brought by the Agulhas Current from the tropical Indian Ocean (Hill and McQuaid, 2008; Griffiths et al. 2010). The grazer *S. granularis* and filter feeder *P. angulosus* directly or mostly consume algae biomass, which links them closer to primary productivity than omnivorous scavenger *C. punctatus* (Branch, 2017). It appears probable that intertidal primary productivity plays a

major role in ecological patterns of varying body mass of rocky shore invertebrates on the South African coast (Bustamante et al. 1995; Hill and McQuaid, 2008). Other possible influencing factors could be the impact of wave exposure, other oceanographic variables such as sea surface temperature, predator-prey interactions and demand for intertidal animals as food source (Bustamante et al. 1995; Branch and Odendaal, 2003; Griffiths et al. 2010; Kosloski, Dietl and Handley, 2017). Size clines across species geographic distribution have also been regarded as possible result of phenotypic plastic response to local thermal conditions or the product of optimising natural selection towards local adaptation (Lee and Boulding, 2010; Sanford and Kelly, 2011). Evidence for the former could be observed in several crab species, where larger crabs are found in areas with cooler temperatures, known as the temperature-size rule (Atkinson, 1994; Kelley et al. 2015; Jaramillo et al. 2017; Johnson et al. 2019). However, regional mass differences in *C. punctatus* are not significant in linear mixed effect models (Figure 4.9). Where phenotypic plasticity is exceeded, warmer water temperature might act as selection pressure against larger body size in shore crabs (Muñoz, Kelley and De Rivera, 2017). Regarding Bergmann's rule (Bergmann, 1847), the South African coast represents an interesting case, because the common association of cold water at higher latitude and warm water at lower latitudes does not apply. Upwelling on the west coast drives lower water temperatures than on the south and east coast, which could influence observed body size differences in *P. angulosus* and *S. granularis* (Griffiths et al. 2010). Importantly, geographic variations of size or body mass in marine invertebrates should not be generalised, because these gradients are driven by complex interactions of environmental and biological factors (Angilletta and Dunham, 2003; Malvé, Gordillo and Rivadeneira, 2018). Nevertheless, patterns of intertidal primary productivity and sea surface temperatures are likely key factors for geographic size variation in South African rocky shore species.

4.4.2 Mass influences thermal tolerance

A number of studies have shown that thermal stress response can differ with body size (or its proxy mass) (Peck et al. 2007; Peck et al. 2009; Madeira et al. 2012b; Muñoz, Kelley and De Rivera, 2017; Gunderson et al. 2019). The Cape urchin *P. angulosus* demonstrates negative size correlation in CT_{min} values and positive mass correlation in CT_{max} and thermal breadth (Table 4.4-4.6), which gives larger urchins a thermal advantage over smaller individuals. On the west coast, mean water temperatures can seasonally vary between 11-15°C with short-term fluctuations, whereas water temperatures at the east coast vary between 18-27°C (Smit et al. 2013; Figure 4.4). Mean mass in west coast urchins was measured at 32 g, compared to generally lighter urchins at the east coast (21 g) and south coast (17 g) (Table S4.2). If

larger Cape urchins have a thermal advantage due to their body size, west coast urchins might consequently be better equipped at coping with thermal stress than urchin populations at the south and east coast. Interestingly, the smaller east coast urchins display slightly higher mean critical thermal limits (CT_{max} : 30.9°C; CT_{min} : 8.9°C) than west coast urchins (CT_{max} : 29.2°C; CT_{min} : 7.3°C) (Table S4.3-4; see section 4.4.3). Considering that large Cape urchins have a thermal advantage over smaller ones, it is possible that larger body size at the west coast signals a physiological adaptation to colder temperatures. This expands considerations for regional size differences in South African marine invertebrates (see section 4.4.1). For populations of *P. angulosus* in warmer regions (south and east coast), an increased body size would improve thermal resistance. Essentially, factors such as nutrient concentrations, which might limit body size in *P. angulosus* at the east coast and south coast (see section 4.4.1), could also negatively influence their thermal tolerance capacity. Cape urchins occur in high abundance at the west coast (see section 1.7.2), which indicates that young *P. angulosus* withstand the regionally lower water temperatures sufficiently. One avenue to navigate low water temperatures during earlier life stages is the timing of spawning, which for *P. angulosus* reportedly takes place mostly after low winter temperatures subside (see section 1.7.2). Larvae of the Cape urchin have displayed active substrate settlement behaviour (Cram, 1971; Greenwood, 1975), which might allow them to choose advantageous microsites before reaching maturity.

In marine crustaceans, smaller crabs can be more heat tolerant than larger crabs (Jensen and Armstrong, 1991; Madeira et al. 2012b; Gunderson et al. 2019). Although upper critical thermal limits do not show mass correlation in *C. punctatus*, larger shore crabs tolerate lower temperatures (CT_{min}) and demonstrate wider thermal breadth (Table 4.4, 4.6). The mean mass of *C. punctatus* at the west and south coast lies around ~7 g, compared to below 3 g in east coast crabs (Table S4.2). The mass correlation of CT_{min} and thermal breadth in *C. punctatus* suggests that west and south coast crabs are thermally advantaged to withstand extreme temperatures compared to east coast crabs. Mean CT_{min} is lower in west and south coast crabs (-0.2°C; -0.5°C) compared to east coast crabs (0.9°C) (Table S4.3) and mean thermal breadth in east coast crabs is smaller (43.3°C) than at the west and south coast (46.2°C; 45.7°C) (Table S4.5). This could be a possible signal that *C. punctatus* physiologically adapted through a regional increase in body size at the west and south coast to better tolerate local temperatures. Higher temperatures at the east coast mitigate the physiological pressure to tolerate low temperatures. Mass is not correlated with CT_{max} , which means that for CT_{max} there is no apparent advantage to adaptively increase or decrease body size. In general, body size can confound physiological measurements (see for example Woiwode and Adelman, 1992) and smaller body size in east coast crabs might possibly

reflect environmental limitations to attaining a higher body mass (see section 4.4.1). An unsolved question in this context is the potential influence of maturity and its relationship with thermal tolerance, as it is currently not known how mass (body size) relates to age across regions in *C. punctatus*.

This study also contributes evidence that mass (or body size) does not necessarily influence critical thermal limits or thermal breadth in marine invertebrates. Mass does not impact thermal tolerance in *S. granularis*, even with significant regional mass differences (Figure 4.9). It demonstrates that the role of mass in thermal characteristics of intertidal invertebrates is complex and cannot merely be deduced from co-occurring species. Other studies have shown that body size does not necessarily influence species thermal stress response (Prentice and Schneider, 1979; Taylor, 1982; Lee and Boulding, 2010; Monaco, Brokordt and Gaymer, 2010; Darnell, Nicholson and Munguia, 2015). Aside from body size, thermal traits in aquatic invertebrates can be influenced by sex (Sornom et al. 2010; Madeira et al. 2012b; Vaughn, Turnross and Carrington, 2014; Bedulina et al. 2017; Grilo et al. 2018), where female crabs might for instance demonstrate higher CT_{max} than males (Madeira et al. 2012b). However, other studies report no impact of sex on thermal traits (Todd and Dehnel, 1960; Tagatz, 1969; Simčič et al. 2014; Darnell, Nicholson and Munguia, 2015). This study contributes evidence that marine invertebrates do not necessarily display sex differences in thermal characteristics, as observed across male and female *C. punctatus*. Overall, thermal limits can be influenced by ontogeny, gravidness, nutritional status and desiccation stress (Terblanche et al. 2011; Madeira et al. 2012b; Klockmann, Günter and Fischer, 2017).

4.4.3 Regional difference in CT_{max}

Spanning regions from cool-temperate to warm-temperate to subtropical/tropical (Smit et al. 2013; Teske et al. 2019), the South African coastline is a likely setting for populations to display geographic variation in critical thermal limits in response to local temperature conditions. Species in subtropical/tropical regions are generally associated with higher CT_{min} and CT_{max} limits (Stillman, 2002; Bonebrake and Deutsch, 2012), but variation in species physiological traits across thermal gradients can produce various patterns (Levins, 1968; Conover and Schultz, 1995; Angilletta, 2009; Conover, Duffy and Hice, 2009). The highest thermal limits are expected for populations in the warmest region of the species distribution (Sorte, Jones and Miller, 2011), yet the highest mean CT_{max} values in the shore crab *C. punctatus* are detected on the cool-temperate west coast. The high temperature variability in colder regions possibly shapes animals to withstand more extreme temperatures (Richard et al. 2012; Peck et al. 2014). Moreover, physiological variation in populations is influenced by

both environmental and genomic factors, which can create ‘counter-gradient’ patterns (Angilletta, 2009; Gardiner, Munday and Nilsson, 2010; Marshall and Chua, 2012). The counter-gradient variation model suggests that cool-region populations can exhibit higher thermal limits than warm-region populations if environmental and genomic influences are negatively associated (Angilletta, Niewiarowski and Navas, 2002; Angilletta, 2009; Gardiner, Munday and Nilsson, 2010), which could be the case for *C. punctatus*. In this study, significant difference in mean CT_{max} between west coast and east coast crabs might reflect variation in phenotypic plasticity, epigenetic plasticity and/or local adaptation to the thermal gradient (Gardiner, Munday and Nilsson, 2010; Schulte, Healy and Fanguie, 2011; Yampolsky, Schaer and Ebert, 2014; Collins et al. 2020).

Regional variation in thermal tolerance or in indicators of thermal stress such as heat shock proteins are generally expected with geographic environmental variation and could be shown in intertidal crabs (Tepolt and Somero, 2014; Darnell and Darnell, 2018), gastropods (Kuo and Sanford, 2009; Gleason and Burton, 2015; Wang et al. 2019) and sea urchins (Fujisawa, 1989; Osovitz and Hofmann, 2005; Byrne et al. 2011; Pecorino et al. 2013). For example, Darnell and Darnell (2018) showed that CT_{max} is highest in two fiddler crab species inhabiting the warmest region on the American east coast between southern Texas and New York. In a further example, Osovitz and Hofmann (2005) found that levels of heat stress response in the purple sea urchin *Strongylocentrotus purpuratus* populations are higher in southern California compared to cold-temperate Oregon. Nevertheless, macrophysiological patterns of geographic variation in thermal traits do not occur by default, as demonstrated by studies confirming only weak or no evidence for clinal differences in intertidal crustaceans (Gaitán-Espitia et al. 2014), gastropods (Sagarin and Somero, 2006; Lee and Boulding, 2010) and sea urchins (Hammond, 2010). Here, no significant regional differences were detected in *S. granularis* and *P. angulosus*, which demonstrates the temperature variations these species can tolerate. Other examples include populations of the porcelain crab *Petrolisthes violaceus*, which did not show thermal tolerance differences across a latitudinal gradient of 3000 km in Chile in spite of a latitudinal gradient in environmental temperature (Gaitán-Espitia et al. 2014). Furthermore, two rocky shore gastropods did not demonstrate clinal variation in heat stress response across populations spanning 22 degrees of latitude with marked differences in ambient temperature conditions (Sagarin and Somero, 2006).

Overall, organismal stress response can be influenced by local effects, species-specific differences and complex patterns of environmental conditions along coasts such as oceanography, coastal geomorphology and geographic differences in tidal regimes (Sagarin and Somero, 2006; Kuo and Sanford, 2009). To reiterate, thermal response can also vary

due to phenotypic and/or epigenetic plasticity and genomic local adaptation (Gardiner, Munday and Nilsson, 2010; Schulte, Healy and Fangue, 2011; Yampolsky, Schaer and Ebert, 2014; Baldanzi et al. 2017; Collins et al. 2020). As mentioned before, critical thermal trials are influenced by experimental factors such as acclimation period, length of exposure, heating rate and single compared to repeated thermal stress exposure (Terblanche et al. 2007; Nguyen et al. 2011; Faulkner et al. 2014; Vinagre et al. 2015; Díaz et al. 2017).

4.4.4 Highest thermal breadth in *C. punctatus*

Thermal breadth (T_{br}) or thermal tolerance range in populations inhabiting warmer regions is generally expected to be narrower compared to cold region counterparts (climatic variability hypothesis), due to the higher temperature variability associated with colder areas (Richard et al. 2012; Peck et al. 2014; Gutiérrez-Pesquera et al. 2016). There is an indication for this theoretical expectation in the shore crab *C. punctatus*, which displays lower mean T_{br} in east coast crabs (43.3°C; Table S4.5) compared to west coast crabs (46.2°C; Table S4.5). However, linear mixed effect models determine no significant regional T_{br} differences across the three study species (Figure 4.10; see section 4.4.3). Thermal breadth averaged across all regions shows largest T_{br} in the crab *C. punctatus* (45.1°C) and narrowest T_{br} in the urchin *P. angulosus* (22.6°C) (*S. granularis* (T_{br}): 34.7°C; Table S4.5, Appendix). In the intertidal, high shore species are suggested to have the widest thermal breadth (Davenport and Davenport, 2005), which is interspecifically evidenced in low shore *P. angulosus* demonstrating the narrowest T_{br} . For comparison with literature values, T_{br} was calculated from recorded lower and upper thermal limits as part of the same study on a specific species. The Cape urchin displays a similar T_{br} to the purple urchin *Strongylocentrotus purpuratus* (18.5°C; Farmanfarman and Giese, 1963) and the white urchin *Tripneustes ventricosus* (20.3°C; Collin et al. 2018), whereas seven other urchin species demonstrated broader T_{br} values ranging from 28.8-31.8°C (Collin et al. 2018). In marine gastropods, T_{br} around ~30-31°C for *Cellana radiata* is comparable with *S. granularis*, whereas T_{br} in *Siphonaria atra* is narrower at around 22-23°C (Morley et al. 2014). In marine crabs, T_{br} can vary between 25.0-29.7°C in tropical and subtropical species (Qari and Aljarari, 2014; Azra et al. 2018) and 32.9-33.8°C in temperate species (Tepolt and Somero, 2014; Cumillaf et al. 2016). A study on benthic populations of *Cancer pagurus* in Norway suggests that the species thermal breadth might differ by as much as ~10°C between winter and summer (Bakke, Siikavuopio and Christiansen, 2019). Hence, it appears reasonable to assume a certain degree of seasonal variation in T_{br} across the study species, which remains to be confirmed.

4.4.5 Warming and cooling tolerance

Comparing critical thermal limits against maximum and minimum habitat temperatures provides an important indication for how much species are at risk for their thermal limits to be exceeded. Theoretical expectations (Deutsch et al. 2008; Huey et al. 2009; Sunday, Bates and Dulvy, 2012; Comte and Olden, 2017) would suggest east coast populations to live closest to their upper limits (lowest warming tolerance) and west coast populations closest to their lower thermal limits (lowest cooling tolerance). Moreover, high shore animals are expected to possess lower warming tolerance than low shore species (Vinagre et al. 2016). In the high-shore specialists *C. punctatus* and *S. granularis*, the warming and cooling tolerance is well above 5°C and often much higher (Table 4.7). Nevertheless, it would be misleading to deduct that these species have thermal limits which might easily buffer changing climatic conditions. Mean monthly maximum and minimum air temperatures are a coarse approximation of the temperature mosaic typical for intertidal habitats and their associated thermal niches. The temperature experienced by intertidal species during low tide can drastically rise or drop under the influence of factors such as solar radiation, wind speed, cloud cover, wave height and relative humidity levels (Helmuth and Hofmann, 2001; Helmuth et al. 2011) and further depends on microtopography of rocky substrates such as substrate surface orientation (Harley, 2008; Miller, Harley and Denny, 2009; Seabra et al. 2011).

In the urchin *P. angulosus*, the lowest warming tolerance is suggested for the Cape St. Francis population during summer months (Dec-Feb; Table 4.8), which corroborates expectations to identify low WT in warm regions compared to cooler regions (Comte and Olden, 2017). The highest warming tolerance might be expected for west coast populations during austral winter months. However, local temperature data indicates the highest WT in Knysna instead (Table 4.8), which demonstrates the potential for local thermal variability on the south coast. Interestingly, the Knysna population is also suggested to potentially possess the highest cooling tolerance (Dec-Feb) and Cape St. Francis the lowest cooling tolerance (Jun-Aug; Table 4.8). The latter supports expectations of lower cooling tolerance in warmer regions (Stuart-Smith, Edgar and Bates, 2017), whereas the high variability of local thermal conditions on the south coast (Rouault, 2011; Blamey et al. 2015) likely influences the former finding. These are preliminary considerations, which require further assessment with minimum and maximum temperatures for the field sites.

Only the strategic fine-scale collection of air and water temperature data across the intertidal zone and large-scale data collection across the thermal coastal gradient itself over longer time periods will allow more accurate risk estimations for local rocky shore species. Further,

species thermal limits might be higher in summer compared to (in this study: austral) winter, which would lead to varying warming tolerance (Schmidt-Nielsen, 1997). To reach a better understanding of the study species thermal resilience, future investigation towards summer CT_{max} and CT_{min} would be valuable. This effort could be supported by a recent framework of the South African Department of Environmental Affairs to collect *in situ* intertidal temperature data in numerous rocky shore locations as part of “Operation LIMPET” (Long-term Intertidal Monitoring through Participation, Evaluation and Training).

4.4.6 Interspecific comparison of ‘winners’ and ‘losers’

As famously stated by Somero (2010), climate change has ‘winners’ and ‘losers’ depending on their ability to adjust their thermal limits on a phenotypic and/or evolutionary scale. Ranging across populations from 28.9°C to 32.4°C, average CT_{max} values of low shore species *P. angulosus*, are notably below the thermal limits of the high shore species *C. punctatus* (43.8°C to 45.9°C) and *S. granularis* (34.4°C to 35.7°C; Table S4.3, Appendix), which confirms the expectation of low shore species having lower thermal limits compared to high shore species (Stillman and Somero, 2000; Stillman, 2002; Nguyen et al. 2011; Sorte et al. 2019). Counter to the hypothesis that the limpet *S. granularis* might have higher upper thermal limits due to prolonged direct sun exposure during low tide, the highest CT_{max} values are detected in the shade-seeking crab *C. punctatus*. The order remains the same for lower critical limits, which are lowest in the shore crab (-1.5°C to 1.3°C) and highest in the Cape urchin (5.6°C to 10.9°C), with the granular limpet falling in between (-0.4°C to 3.2°C) (Table S4.2, Appendix). The shore crab *C. punctatus* demonstrates a substantial thermal range, displaying the highest upper critical thermal limits and the largest thermal range (from 43 to almost 47°C), which provides this species a potential advantage in future climate change. Thermal limits may show seasonal differences (Schmidt-Nielsen, 1997; Cuculescu, Hyde and Bowler, 1998; Qari and Aljarari, 2014), but it remains to be confirmed whether the shore crab displays higher CT_{max} in summer, as the values assessed in late winter/spring in this study (43.8–46.1°C) surpass upper thermal limits measured in tropical crabs (Qari and Aljarari, 2014; Cumillaf et al. 2016; Azra et al. 2018; Roni et al. 2019). It needs to be investigated how long *C. punctatus* can survive such peaks in temperature and how repeated exposure would affect the species thermal resilience. Cumillaf et al. (2016) argue that temperate intertidal crabs have a CT_{max} limit around 35°C, seemingly corresponding with low CT_{max} limits of tropical crabs (34–36°C). With *C. punctatus* demonstrating a regional average CT_{max} of 46°C on the cold temperate South African west coast, this study provides evidence against very defined thermal limits between temperate and tropical crustaceans.

In the literature, upper thermal tolerance in intertidal crabs ranges from 20-36.4°C in temperate species (Cuculescu, Hyde and Bowler, 1998; McGaw, 2003; Stillman, 2004; Hopkin et al. 2006; Kelley, de Rivera and Buckley, 2011; Madeira et al. 2012a,b; Tepolt and Somero, 2014; Cumillaf et al. 2016; Darnell and Darnell, 2018; Bakke, Siikavuopio and Christiansen, 2019) to 41.5-44.38°C in subtropical and tropical species (Stillman, 2004; Qari and Aljarari, 2014; Azra et al. 2018; Darnell and Darnell, 2018; Roni et al. 2019). There is a dominant emphasis on upper thermal limits in comparison to lower thermal limits in marine crustaceans, which has been recognised (Qari and Aljarari, 2014; Bakke, Siikavuopio and Christiansen, 2019). Lower critical thermal limits in temperate crab species are reported from -1.3°C to 2.7°C (Stillman, 2004; Tepolt and Somero, 2014; Cumillaf et al. 2016; Bakke, Siikavuopio and Christiansen, 2019) and from 11.3°C to 19.30°C in subtropical and tropical regions (Stillman, 2004; Qari and Aljarari, 2014; Azra et al. 2018).

For limpets, lower thermal limits range between 2.6°C and 15.6°C (Morley et al. 2014), but data for temperate and tropical species is very limited. In temperate limpet species, upper thermal limits are generally observed between 30°C and 42°C (Miller, Harley and Denny, 2009; Bjelde et al. 2015; Miller et al. 2015; Chapperon et al. 2016; Drake, Miller and Todgham, 2017; Vinagre et al. 2019; Wang et al. 2019). The highest upper thermal tolerance limit in a temperate limpet was observed at 42.5°C in *Patella vulgata* (Chapperon et al. 2016). Tropical limpet species show upper limits between 28.3°C and 42°C (Morley et al. 2014; Díaz et al. 2015; Vinagre et al. 2018). A previous study on South African *S. granularis* measured the upper mean LT₅₀ (median lethal temperature) at ~36°C in austral winter across three east coast populations, which are located between the present study's Cape St. Francis and Haga Haga populations (Kankondi, McQuaid and Tagliarolo, 2018). This finding appears congruent with the mean CT_{max} (35.5°C, Table S4.4, Appendix) obtained for *S. granularis* from the east coast region.

Lower critical thermal limits in urchins are reported between 5°C to 19.1°C (Farmanfarmaian and Giese, 1963; Collin et al. 2018), but cold tolerance in urchins is otherwise poorly understood. Upper thermal limits range in temperate species from 19.5 to 26.8°C (Morley et al. 2016). In tropical regions, upper thermal limits have been observed between 23.5°C and 37.1°C (Farmanfarmaian and Giese, 1963; Hernández et al. 2004; Nguyen et al. 2011; Sherman, 2015; Collin and Chan, 2016; Morley et al. 2016; Collin et al. 2018), with the highest thermal limit (38.42°C) reported for the tropical urchin *Arbacia stellata* (Díaz et al. 2017). This study contributes lower and upper critical thermal limits for temperate populations of adult urchins, which are generally underrepresented in the literature as opposed to urchin embryos or larvae (Greenwood and Bennett, 1981; Sconzo et al. 1986; Sewell and Young,

1999; Byrne et al. 2009; Ling et al. 2009; Hammond and Hofmann, 2010; Byrne et al. 2011; Hardy et al. 2014; Collin and Chan, 2016). In general, limited available data for thermal tolerance in adult intertidal marine invertebrates highlights the need for empirical investigation of critical thermal limits across temperate and tropical regions.

Thermal regulation through behaviour

Behavioural thermal regulation influences species temperature resilience, for which the study species display different strategies to persist in their intertidal microhabitat. Shade seeking, as practised by *C. punctatus*, is regarded as the most important thermoregulatory behaviour (Jackson, 2010; Gunderson et al. 2019; Brahim, Mustapha and Marshall, 2019), but it remains questionable whether it will be sufficient for the shore crab to evade climate change impacts (Blackburn et al. 2014). The low shore urchin *P. angulosus* usually remains submerged throughout the tidal cycle, which creates a certain buffer against temperature change. Additionally, the Cape urchin demonstrates behaviour interpreted as protection from solar radiation and potential predators by self-covering with small rocks and shells (Figure 4.3; Millott, 1956; Crook and Barnes, 2001; Brothers and McClintock, 2015; Ziegenhorn, 2017). Further, the low shore position in the intertidal zone makes it more feasible for *P. angulosus* to evade rising temperatures by permanently seeking out deeper waters (Perkins et al. 2015). Thermoregulatory behaviour in intertidal gastropods such as *S. granularis* is influenced by a variety of factors.

Behaviour trade-offs affect thermal resilience in S. granularis

High shore gastropods are already constrained by physiological stress in the intertidal (Garrity, 1984). Apart from microhabitat selection, thermoregulatory behaviour can include shell orientation, mucous holdfast and shell-raising (mushrooming) (Garrity, 1984; Williams and Morritt, 1995; Muñoz et al. 2005; Williams et al. 2005; Chapperon et al. 2016; Seuront and Ng, 2016). Gastropod aggregations might also be considered as thermal regulation behaviour (Garrity, 1984), but there is evidence suggesting otherwise (Coleman, 2010). South African *S. granularis* faces limited possibilities for mitigation of thermal stress during low tide. Aerial exposure likely acts as a strong cue to prime intertidal animals for thermal stress (Drake, Miller and Todgham, 2017). However, the phenomenon termed desiccation-risk-avoidance describes that high-shore gastropods stop crawling when the substrate becomes hot and dry with the receding tide to prevent desiccation, which undermines the selection of a thermally suitable settling position (Monaco, McQuaid and Marshall, 2017). Failure to select settling positions on thermal cues means individuals tend to assume their

low tide position in direct sunlight (Marshall and Chua, 2012; Marshall, Baharuddin and McQuaid, 2013; Monaco, McQuaid and Marshall, 2017). Not surprisingly, high-shore animals may be most threatened by mortality from acute overheating over gradual long term temperature increases (Williams and Morritt, 1995; Marshall and McQuaid, 2011; Marshall et al. 2011). *Scutellastra granularis* has been observed to utilise shell-raising as a temporary heat relief, which does however greatly increase its risk of desiccation (Williams and Morritt, 1995; Williams et al. 2005). Some limpet species maintain home scars, which are specific crevices they return to if not even defend (Branch and Branch, 1981; Gray, 1997), but *S. granularis* does not form home scars. The possible effects of shell-orientation or microhabitat selection have not been studied in the granular limpet and there is evidence that wind-driven convective cooling can marginally reduce temperature impacts (Helmuth et al. 2011), but the persistence of high-shore gastropods is uncertain under ongoing climate change. Around the world, limpets are regarded as key herbivorous grazers, which shape the rocky shore community with control of algal growth (Branch, 1981; Hawkins and Hartnoll, 1983; Geller, 1991; Bustamante, Branch and Eekhout, 1995; Coleman et al. 2006). Hence, the decline of limpet species from thermal stress will impact ecological processes in the intertidal.

Climate change poses a drastic physiological challenge with presumed ‘winners’ and ‘losers’ (Somero, 2010). Risking simplification of this complex matter, critical thermal limits combined with behavioural thermoregulation strategy suggest the crab *C. punctatus* as possible ‘winner’ and the limpet *S. granularis* as ‘loser’, with the sea urchin *P. angulosus* falling between them on the climate change risk estimation scale. However, it could count as opposite argument to regard high shore species in general as more at risk than low shore species such as *P. angulosus* (Somero, 2010).

4.4.7 Regional variation of climate change impacts

Assessing species thermal limits is an important step towards anticipating their ability to cope with climate change. In South Africa, geographically varying climatic predictions lead to different risks from climate change for local rocky shore species. To briefly recapitulate, the South African coastal SST gradient ranges seasonally from 11-15°C on the west coast to 18-27°C on the east coast (see section 1.5.2; Smit et al. 2013). The east coast will warm further (see section 1.6.2; Rouault, Penven and Pohl, 2009), the west coast shows signs of cooling (Rouault, Pohl and Penven, 2010; Dufois and Rouault, 2012) and the south coast is expected to both warm and cool depending on the area (Rouault, 2011; Blamey et al. 2015).

Shore crab C. punctatus

West coast shore crabs possess the overall highest average CT_{max} values, which likely makes them most thermo-resilient. Moreover, west coast crabs benefit from the advantageous regional conditions, such as the highest intertidal primary productivity, which is in turn lowest on the east coast (Bustamante et al. 1995). Importantly, temperatures on the west coast are predicted to cool rather than warm (Rouault, Pohl and Penven, 2010; Dufois and Rouault, 2012), which creates a temperature refugium supported by nutrition-rich waters connected to strong upwelling (Whitfield et al. 2016). In general, areas associated with coastal upwelling are characterised by lower warming rates than adjacent areas, making them a shelter for species from climate change (Lourenço et al. 2020). Additionally, upwelling-driven cool water temperatures create unfavourable conditions for certain pathogens impacting intertidal invertebrates in warmer areas (Lester, Tobin and Behrens, 2007). Changing climate conditions will likely pose the strongest burden on shore crab populations on the east coast, which – in spite of facing the highest environmental temperatures – have the lowest mean CT_{max} limits across all regions and possibly already face limitations from environmental factors such as low primary productivity in the area (Bustamante et al. 1995). Climatic predictions for the south coast are less clear (Rouault, 2011; Blamey et al. 2015), suggesting that the thermal resilience of southern populations will depend on local temperature trends in specific areas.

Granular limpet S. granularis

Granular limpets usually settle in full sunlight high on the shore for low tide (desiccation-risk-avoidance), which greatly increases their risk of overheating. Thus, rising temperatures on the east coast (Rouault, Penven and Pohl, 2009) could drive a population decline and/or local extinctions at the range edge of *S. granularis* and other high shore gastropods. South coast populations might fare better than east coast populations, but predictions at the population level will only become possible with more fine-scale climatic predictions for this coastal stretch. Across South African marine species, the cold-water west coast has been suggested as a climate change shelter (Whitfield et al. 2016). High levels of nutrients linked to upwelling likely facilitate the high *S. granularis* body mass compared to the south and east coasts (see section 4.4.1). Western populations appear to be in a better position to cope with climate change, but it is important to remember that the limpet's low tide settling behaviour greatly increases mortality induced by extreme temperatures (see section 4.4.6), overall lowering the species thermal resilience.

Cape urchin P. angulosus

Further warming of the east coast (Rouault, Penven and Pohl, 2009) is bound to increase the physiological strain on populations of *P. angulosus*. Other urchin species around the world have started to seek out deeper waters or shifted poleward in response to rapidly changing environmental conditions (Ling et al. 2009; Wernberg et al. 2011; Cheung et al. 2012; Perkins et al. 2015; Poloczanska et al. 2016). The Cape urchin occurs up to a depth of 30 m (see section 1.7.2) and is the only species which is able to permanently retreat to deeper waters as a means of tracking its preferred conditions. If shifts towards deeper waters in South African rocky shore urchin populations occur, it is plausible for eastern populations to display them first due to the higher environmental temperatures on the east coast. While it is difficult to predict exact consequences, it has to be considered that vertical shift in the urchin distribution will also shift grazing pressure on intertidal algae communities and may result in altered vertical zonation patterns and ecological interactions. Responses of south coast urchins will depend on whether the local temperature conditions warm or cool (Rouault, 2011; Blamey et al. 2015) and on other relevant factors including the occurrence of extreme temperature events (Schlegel et al. 2017). West coast urchins indicate the highest body mass levels and apparently benefit from upwelling-driven nutrient-rich waters, which appear to provide the most favourable conditions for *P. angulosus* populations across the South African coast. Ultimately, west coast Cape urchins may be buffered to some degree against impending climate change impacts.

Interspecific regional patterns

Of all coastal regions, east coast rocky shore organisms are most at risk from future climate change impacts, which is congruent with expectations for ectotherms in subtropical/tropical regions (Sinervo et al. 2010; Bonebrake and Deutsch, 2012; Pinsky et al. 2019). Additionally, populations on the southern east coast constitute the approximate range edge of the study species distribution (notwithstanding that rocky shore species can occur further east of Haga Haga), where populations already experience strain from higher temperatures at present (Gaston, 2009; Sexton et al. 2009; Sorte et al. 2019). Consequently, these populations are more vulnerable than in other coastal regions to future climate change (Sorte and Hofmann, 2004; Sagarin and Somero, 2006; Han et al. 2019). Species are likely further disadvantaged by environmental limitations including nutrient-poor waters and low intertidal primary productivity (Bustamante et al. 1995).

The south coast is characterised by high variability of local temperature trends (Rouault, 2011; Blamey et al. 2015), which hinders predictions for species future trajectories in

response to climatic changes. Depending on geographic position, species might either benefit from cooling conditions or battle with the effects of warming conditions. An example for east-west coastline orientation and climate-driven effects can be found at the northern Gulf of Mexico and the Gulf of Maine in North America, where depth shifts of invertebrate assemblages and demersal fish to cooler waters have been observed (Nye et al. 2009; Pinsky et al. 2013). In Australia, the south coast also has an east-west orientation and harbours most of the continent's temperate communities (Wernberg et al. 2011). This narrow latitudinal band is expected to undergo 'tropicalisation' with the incipient distribution shift of species from warmer waters (Cheung et al. 2012). The development leads to species gains and losses, with far reaching implications for temperate marine communities. On the South African south coast, a similar development is plausible, yet the oceanographic processes and associated local water temperature variability are not directly comparable with southern Australia. Cooling of the Benguela Current and warming of the Agulhas Current are expected to drive a contraction of warm-temperate conditions on the south coast (Blamey et al. 2015; Whitfield et al. 2016). Upwelling filaments (cold-water tongues) occurring on the south coast (Lutjeharms and Stockton, 1991; Roberts and Van Den Berg, 2005; Jackson et al. 2012) could concentrate temperate species in certain areas, whereas warming areas will attract warmer-water species.

West coast populations continue to benefit from nutrient-rich cold water upwelling, which creates a temperature refugium for local rocky shore species (Whitfield et al. 2016; Lourenço et al. 2020). Predicted cooling for the west coast might affect local rocky shore communities less than warming temperature conditions expected for other regions, because populations in temperate regions are expected to tolerate temperature shifts better than populations in subtropical/tropical regions (Tewksbury, Huey and Deutsch, 2008; Hofmann and Todgham, 2010; Nguyen et al. 2011; Vinagre et al. 2019). Overall, this provides important evidence towards managing and protecting parts of the west coast as possible climate change refugium for rocky shore species, which are likely to suffer from the impact of warming temperatures in other coastal regions (Whitfield et al. 2016). Unlike species in other parts of the world (Perry et al. 2005; Ling et al. 2009; Flagor and Bourdeau, 2018), coastal South African species are unable to shift their range poleward to evade rising temperatures due to the east-west orientation of the coastline. The absence of this escape avenue emphasises the importance of supporting and protecting the South African west coast as thermal refugium (Whitfield et al. 2016). The west coast has received some conservation attention (Majiedt et al. 2013; Sink, 2016), but it is crucial that further recognition is considered to support and protect rocky shore communities.

Climate change impacts on species distribution

Rising temperatures have the potential to drastically reduce or extirpate range edge populations in the warmest regions of species distribution (Stuart-Smith, Edgar and Bates, 2017). The rocky shore species in this study might experience warm range edge contractions on the east coast and may possibly cease to occur in this coastal region in the long term. Distributional shifts could also appear towards deeper waters in the urchin *P. angulosus*, with widespread ecological implications. On the south coast, warming areas might generally become less attractive for rocky shore species (Wernberg et al. 2011; Cheung et al. 2012), which could lead to a species concentration in cooling areas on the south coast. The west coast will increase in its importance as anchor for the distribution of rocky shore species in South Africa. If west coast populations stand to benefit from cooling temperatures in their abundance remains to be established in accordance with factors such as industrial use impacts (Majiedt et al. 2013). Moreover, the occurrence of extreme temperature events can be expected to influence regional distribution patterns.

Impact of extreme temperature events on South African coasts

Aside from gradually changing temperature conditions, climate change is also associated with more frequent and extended extreme temperature events (IPCC, 2014; Angéilil et al. 2017; Frölicher, Fischer and Gruber, 2018). In the intertidal, high shore species are disproportionately at risk from heat waves (Williams et al. 2005; Williams, Chan and Dong, 2019) and cold waves (Firth, Knights and Bell, 2011; Firth et al. 2015) compared to low shore species. Consequently, low shore *P. angulosus* may be least at risk compared to *S. granularis* and *C. punctatus*, which are exposed during low tide. The latter two species are more exposed to temperature extremes and may for instance suffer more extensively from the adverse impacts of accumulated heat exposure (Pasparakis, Davis and Todgham, 2016; Vinagre et al. 2018; Siegle, Taylor and O'Connor, 2018). In South Africa, marine heat waves have increased over the last 20 years and marine cold-spells have decreased, with regional differences in frequency and intensity between coasts (Schlegel et al. 2017). Regionally, cold waves are decreasing more on the east than on the west coast, which is advantageous for population inhabiting the former (Schlegel et al. 2017). However, the occurrence of marine heat waves increases from the west coast to the east coast, rendering intertidal east coast populations most vulnerable to heat-induced mortality (Schlegel et al. 2017). Despite the limited duration of heat waves or cold-spells, extreme temperature events can have profound and lasting adverse impacts on intertidal communities such as collapsing or extirpating marginal populations when physiological thresholds are surpassed (Garrahou et al. 2009;

Firth, Knights and Bell, 2011; Wetthey et al. 2011; Smale and Wernberg, 2013; Rilov, 2016; Thomsen et al. 2019).

Factors limiting acclimation to climate change

Overall, acclimation facilitated by variation in phenotypic plasticity is essential to cope with environmental changes (Brahim, Mustapha and Marshall, 2019; Neel et al. 2020), but physiological and genetic constraints (Hoffmann, Chown and Clusella-Trullas, 2013; Araújo et al. 2013; Meester, Stoks and Brans, 2018) as well as the impact of increasing extreme temperature events are expected to limit its scope (Stillman, 2019). Further, subtropical and tropical populations are likely to have lower acclimation potential compared to temperate populations (Peck et al. 2014). Climate change may exceed species physiological plasticity and ability to acclimate to new conditions in the long term (van Heerwaarden, Kellermann and Sgrò, 2016; Gunderson, Dillon and Stillman, 2017). The concern for limited acclimation ability is very relevant for South African rocky shore species, which are geographically unable to shift polewards in pursuit of lower temperatures. Shifting within the intertidal zonation as a thermal risk mitigation strategy for high shore species is, on a longer time scale, hampered by rising sea levels (IPCC, 2013). Coastal habitat is reduced as tidal boundaries move upslope (Harley, 2003; Schleupner, 2008; Leo et al. 2019). Vertical squeezing constrains species including *S. granularis* and *C. punctatus* specifically when fixed landward boundaries are present. Finally, the resilience of rocky shore communities is, beyond climate change, threatened by non-climate stressors such as environmental pollution and coastal development, which act synergistically to lower species persistence to impacts from storms, invasive species and diseases (Lester, Tobin and Behrens, 2007; Wernberg et al. 2011; Whitfield et al. 2016).

4.4.8 Summary

To briefly recapitulate, the findings in this chapter provide further evidence for regional body mass differences in certain intertidal species across South Africa's coastal regions. It is possible that physiological adaptation to thermal conditions contributes to these regional mass differences. Upper and lower critical thermal limits vary substantially across species and confirm theoretical expectations of higher thermal tolerance in high shore species. Findings indicate no sex-bias in thermal tolerance across male and female *C. punctatus*. Highest upper critical thermal limits were expected to be measured in the high shore limpet *S. granularis* and lowest critical thermal limits in the urchin *P. angulosus*. Surprisingly, both the highest and the lowest thermal limits were measured in the shore crab *C. punctatus*. In

general, thermal limits were expected to be higher in eastern populations over western populations. In contrast, the highest CT_{max} was measured in *C. punctatus*' west coast populations and both *P. angulosus* and *S. granularis* did not reveal regional differences in critical thermal limits. Theoretical expectations suggest that the thermal breadth is usually smaller in populations in warmer compared to colder regions, yet no significant differences were measured in this study. In general, warming and cooling tolerance is suggested to be narrowest in warmer regions, but this study observes that populations at the south coast, which experience high local thermal variation, might have the narrowest warming and cooling tolerance. In light of increasing marine heat waves in South Africa (Schlegel et al. 2017), populations at the east coast are at high risk from future heat waves and further increasing temperatures. Cold water temperatures from upwelling give the west coast region high importance to shelter species from climate change impacts, but general predictions for the south coast are constrained by the high variation of local thermal conditions. While phenotypic plasticity enables intertidal organisms to adjust their physiology in response to their environment and thereby allows them to extend their thermal limits to some degree, temperature changes exceeding their phenotypic limits could prove fatal. To cope with such changes, evolutionary adaptations are required, which will however depend on species genomic potential as well as the rate of adaptation.

Remarkably, the shore crab *C. punctatus* demonstrates a broad thermal range and high upper critical thermal limits compared to other crustaceans (see section 4.4.6). Because the thermal tolerance experiments were conducted after the study species were collected in late winter/early spring, it would be interesting to assess how critical thermal limits measured in midsummer compare at the field sites. Further, thermal tolerance can also be measured with failure of cardiac function (Arrhenius break temperature) and irreversible protein damage (carbonylation), which could offer further insights into the species' thermal resilience (see for example Stillman and Tagmount, 2009; Dong and Williams, 2011; Han et al. 2013; Tepolt and Somero, 2014; Wang et al. 2019). A future question worth investigating is the possible impact of accumulated heat stress from repeated exposure to thermal stress (Terblanche et al. 2007; Faulkner et al. 2014; Vinagre et al. 2015; Siegle, Taylor and O'Connor, 2018). Moreover, *in situ* intertidal water and air temperature data is required to investigate microhabitat conditions experienced by the study species during low tides. For instance, calculating appropriate warming and cooling tolerance in the Cape urchin *P. angulosus* requires near-shore minimum and maximum sea water temperatures at rocky shore sites. Reflecting on the study findings overall, it is remarkable that the shore crab *C. punctatus* indicates a significant regional difference in thermal tolerance between west and east coast crabs (see section 4.4.3), which is not indicated by geographic differences in the species

(selectively neutral) population structure (Chapter II) and detected putatively adaptive loci (Chapter III). A transcriptome-based physiological experimental approach could further explore this observation (see section 3.4.7). With a regional thermal tolerance difference in *C. punctatus*, it could be hypothesised that gene expression geographically varies in this and possibly other marine invertebrates in response to environmental stress. Following up transcriptomic data with qRT-PCR analysis would allow the comparison and functional verification of putatively adaptive loci identified in this study.

4.5 Appendix

Table S4.1. Shapiro Wilk's Test, distribution skewness coefficient, Levene's Test and Bartlett's Test values per coastal region across species for body mass, CT_{max}, CT_{min} and T_{br} (thermal breadth). Significant values are indicated in bold.

		Univariate Shapiro Wilk's Test	Distribution skewness	Levene's Test	Bartlett Test
Body mass	Crab	W = 0.956, p = 0.001	0.67	F(2) = 4.01, p = 0.021	k ² (2) = 8.80, p = 0.012
	Limpet	W = 0.824, p = <0.001	1.55	F(2) = 23.83, p = <0.001	k ² (2) = 57.21, p = <0.001
	Urchin	W = 0.961, p = 0.002	0.60	F(2) = 2.52, p = 0.085	k ² (2) = 5.07, p = 0.079
Crab	CTmax	W = 0.923, p = <0.001	-0.89	F(2) = 4.87, p = 0.010	k ² (2) = 13.84, p = 0.001
	CTmin	W = 0.983, p = 0.214	0.01	F(2) = 0.47, p = 0.626	k ² (2) = 0.514, p = 0.774
	Tbr	W = 0.981, p = 0.133	-0.51	F(2) = 0.35, p = 0.705	k ² (2) = 1.12, p = 0.571
Limpet	CTmax	W = 0.955, p = <0.001	-0.77	F(2) = 6.80, p = 0.002	k ² (2) = 16.68, p = <0.001
	CTmin	W = 0.942, p = <0.001	0.86	F(2) = 4.85, p = 0.009	k ² (2) = 10.16, p = 0.006
	Tbr	W = 0.954, p = <0.001	-0.85	F(2) = 6.51, p = 0.002	k ² (2) = 13.61, p = 0.001
Urchin	CTmax	W = 0.982, p = 0.134	-0.34	F(2) = 2.91, p = 0.059	k ² (2) = 11.68, p = 0.003
	CTmin	W = 0.977, p = 0.048	-0.11	F(2) = 1.89, p = 0.157	k ² (2) = 2.65, p = 0.266
	Tbr	W = 0.988, p = 0.444	-0.23	F(2) = 3.04, p = 0.052	k ² (2) = 5.57, p = 0.062

Table S4.2. Summary of mass data for all three species, including sample size (N), mean and median (in g), standard deviation and standard error per region and population.

<i>C. punctatus</i>	Sample size [N]	Mean [g]	Median [g]	SD	SE
west coast	38	6.79	6.52	2.82	0.46
south coast	35	7.11	6.84	3.20	0.54
east coast	32	2.69	2.13	1.87	0.33
Total/Average	105	5.5	5.2	2.6	0.4
<i>S. granularis</i>	Sample size [N]	Mean [g]	Median [g]	SD	SE
west coast	38	7.31	6.75	2.99	0.48
south coast	40	2.02	1.75	1.07	0.17
east coast	53	2.58	2.63	1.16	0.16
Total/Average	131	4.0	3.7	1.7	0.3
<i>P. angulosus</i>	Sample size [N]	Mean [g]	Median [g]	SD	SE
west coast	35	32.08	31.29	9.59	1.62
south coast	38	17.05	15.35	6.57	1.07
east coast	39	20.77	19.77	7.75	1.24
Total/Average	112	23.3	22.1	8.0	1.3
<i>C. punctatus</i>	Sample size [N]	Mean [g]	Median [g]	SD	SE
Jacob's Bay	18	5.80	5.57	2.42	0.57
Sea Point	20	7.68	7.11	2.91	0.65
Cape Agulhas	18	5.96	5.60	2.60	0.61
Knysna	17	8.34	6.93	3.38	0.82
Cape St. Francis	16	1.99	1.47	1.36	0.34
Haga Haga	16	3.40	2.81	2.08	0.52
Total/Average	105	5.53	4.91	2.46	0.59
<i>S. granularis</i>	Sample size [N]	Mean [g]	Median [g]	SD	SE
Port Nolloth	19	8.37	7.90	2.31	0.53
Sea Point	19	6.26	5.15	3.26	0.75
Cape Agulhas	19	2.28	1.95	1.35	0.31
Knysna	21	1.77	1.69	0.68	0.15
Cape St. Francis	25	3.00	2.79	0.78	0.16
Haga Haga	28	2.20	1.90	1.31	0.25
Total/Average	131	3.98	3.56	1.62	0.36
<i>P. angulosus</i>	Sample size [N]	Mean [g]	Median [g]	SD	SE
Port Nolloth	15	36.94	36.39	8.90	2.30
Sea Point	20	28.43	28.15	8.57	1.92
Cape Agulhas	17	16.38	14.42	5.96	1.45
Knysna	21	17.59	18.57	7.12	1.55
Cape St. Francis	18	21.20	23.67	7.11	1.67
Haga Haga	21	20.40	19.25	8.42	1.84
Total/Average	112	23.49	23.41	7.68	1.79

Table S4.3. Summary of CT_{min} data set for all three species, including sample size (N), mean and median (in °C), standard deviation and standard error per region and population.

<i>C. punctatus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	38	-0.2	-0.3	1.7	0.3
south coast	35	-0.5	-0.6	1.9	0.3
east coast	32	0.9	1.5	1.7	0.3
Total/Average	105	0.1	0.2	1.7	0.3
<i>S. granularis</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	38	-0.3	-0.8	2.2	0.4
south coast	40	2.1	1.6	2.6	0.4
east coast	53	-0.2	-0.4	1.6	0.2
Total/Average	131	0.5	0.1	2.1	0.3
<i>P. angulosus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	35	7.3	7.0	2.7	0.5
south coast	38	6.4	6.5	2.1	0.3
east coast	39	8.9	8.8	2.2	0.3
Total/Average	112	7.5	7.4	2.3	0.4
<i>C. punctatus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Jacob's Bay	18	0.3	0.3	1.8	0.4
Sea Point	20	-0.6	-0.8	1.4	0.3
Cape Agulhas	18	0.5	0.8	1.6	0.4
Knysna	17	-1.5	-1.7	1.6	0.4
Cape St. Francis	16	1.3	1.6	1.7	0.4
Haga Haga	16	0.6	0.7	1.7	0.4
Total/Average	105	0	0.1	1.8	0.2
<i>S. granularis</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Port Nolloth	19	-0.2	-0.8	1.7	0.4
Sea Point	19	-0.4	-1.4	2.6	0.6
Cape Agulhas	19	3.2	2.3	2.2	0.5
Knysna	21	1.1	0.2	2.6	0.6
Cape St. Francis	25	-0.3	-0.6	1.9	0.4
Haga Haga	28	-0.1	-0.4	1.4	0.3
Total/Average	131	0.5	-0.1	2.4	0.2
<i>P. angulosus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Port Nolloth	15	5.6	6.2	1.9	0.5
Sea Point	20	8.6	9.3	2.5	0.5
Cape Agulhas	17	8.1	8.3	1	0.3
Knysna	21	5	5.2	1.7	0.4
Cape St. Francis	18	10.9	11	1	0.2
Haga Haga	21	7.2	6.7	1.3	0.3
Total/Average	112	7.6	7.8	2.5	0.2

Table S4.4. Summary of CT_{max} data set for all three species, including sample size (N), mean and median (in °C), standard deviation and standard error per region and population.

<i>C. punctatus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	38	46.0	46.2	1.1	0.2
south coast	35	45.2	45.2	0.7	0.1
east coast	32	44.3	44.7	1.3	0.2
Total/Average	105	45.2	45.4	1.0	0.2
<i>S. granularis</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	38	34.9	35.0	1.0	0.2
south coast	40	35.1	35.5	1.6	0.3
east coast	53	35.5	35.7	0.9	0.1
Total/Average	131	35.2	35.4	1.2	0.2
<i>P. angulosus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	35	29.2	29.5	1.5	0.3
south coast	38	30.3	30.4	1.4	0.2
east coast	39	30.9	31.6	2.3	0.4
Total/Average	112	30.1	30.5	1.7	0.3
<i>C. punctatus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Jacob's Bay	18	45.9	46	1.1	0.3
Sea Point	20	46.1	46.2	1.1	0.3
Cape Agulhas	18	45.3	45.3	0.5	0.1
Knysna	17	45.1	45.2	0.8	0.2
Cape St. Francis	16	43.8	44.5	1.7	0.4
Haga Haga	16	44.7	44.9	0.6	0.1
Total/Average	105	45.2	45.3	1.3	0.1
<i>S. granularis</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Port Nolloth	19	34.6	34.9	1.1	0.2
Sea Point	19	35.2	35.5	0.8	0.2
Cape Agulhas	19	34.4	34.7	1.4	0.3
Knysna	21	35.7	36.4	1.6	0.3
Cape St. Francis	25	35.7	35.8	0.7	0.1
Haga Haga	28	35.4	35.7	1.1	0.2
Total/Average	131	35.2	35.5	1.2	0.1
<i>P. angulosus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Port Nolloth	15	28.9	28.9	1.4	0.4
Sea Point	20	29.4	29.6	1.6	0.3
Cape Agulhas	17	29.3	29.2	1	0.2
Knysna	21	31.1	31.2	1	0.2
Cape St. Francis	18	29	29.4	2.1	0.5
Haga Haga	21	32.4	32.5	0.9	0.2
Total/Average	112	30.1	30.1	1.9	0.2

Table S4.5. Summary of thermal breadth per species population including the mean (in °C), standard deviation and standard error per region and population.

<i>C. punctatus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	38	46.2	46.5	2.0	0.3
south coast	35	45.7	45.6	1.9	0.3
east coast	32	43.3	43.5	2.2	0.4
Total/Average	105	45.1	45.2	2.1	0.3
<i>S. granularis</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	38	35.2	35.4	2.3	0.4
south coast	40	33.0	33.2	3.3	0.5
east coast	53	35.7	35.6	1.9	0.3
Total/Average	131	34.7	34.7	2.5	0.4
<i>P. angulosus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
west coast	35	21.8	21.8	2.9	0.5
south coast	38	23.9	23.8	3.1	0.5
east coast	39	22.0	23.0	4.2	0.7
Total/Average	112	22.6	22.9	3.4	0.6
<i>C. punctatus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Jacob's Bay	18	45.6	45.9	2.3	0.5
Sea Point	20	46.8	46.8	1.7	0.4
Cape Agulhas	18	44.8	44.5	1.4	0.3
Knysna	17	46.7	47.2	1.9	0.5
Cape St. Francis	16	42.6	42.6	2.6	0.6
Haga Haga	16	44.2	44.2	1.6	0.4
Total/Average	105	45.1	45.2	1.9	0.5
<i>S. granularis</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Port Nolloth	19	34.8	34.8	1.9	0.4
Sea Point	19	35.6	36.6	2.7	0.6
Cape Agulhas	19	31.3	31.7	2.6	0.6
Knysna	21	34.6	35.1	3.1	0.7
Cape St. Francis	25	36	36.3	2.1	0.4
Haga Haga	28	35.5	35.5	1.8	0.3
Total/Average	131	34.6	35	2.4	0.5
<i>P. angulosus</i>	Sample size [N]	Mean [°C]	Median [°C]	SD	SE
Port Nolloth	15	23.3	23.5	2.2	0.6
Sea Point	20	20.8	20.3	2.9	0.6
Cape Agulhas	17	21.1	21.0	1.5	0.4
Knysna	21	26.1	25.7	2.2	0.5
Cape St. Francis	18	18.2	18.3	2.7	0.6
Haga Haga	21	25.2	25.1	1.6	0.4
Total/Average	112	22.5	22.3	2.2	0.5

4.6 References

- Addo-Bediako, Abraham, Steven L. Chown, and Kevin J. Gaston. 2000. "Thermal Tolerance, Climatic Variability and Latitude." *Proceedings. Biological Sciences / The Royal Society* 267 (1445): 739–45.
- Angélil, Oliver, Dáithí Stone, Michael Wehner, Christopher J. Paciorek, Harinarayan Krishnan, and William Collins. 2017. "An Independent Assessment of Anthropogenic Attribution Statements for Recent Extreme Temperature and Rainfall Events." *Journal of Climate* 30 (1): 5–16.
- Angilletta, Michael James. 2006. "Estimating and Comparing Thermal Performance Curves." *Journal of Thermal Biology* 31 (7): 541–45.
- Angilletta, Michael James. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford, UK.
- Angilletta, Michael James, and Arthur E. Dunham. 2003. "The Temperature-Size Rule in Ectotherms: Simple Evolutionary Explanations May Not Be General." *American Naturalist* 162 (3): 332–42.
- Angilletta, Michael James, Peter H. Niewiarowski, and Carlos A. Navas. 2002. "The Evolution of Thermal Physiology in Ectotherms." *Journal of Thermal Biology* 27 (4): 249–68.
- Araújo, Miguel B., Francisco Ferri-Yáñez, Francisco Bozinovic, Pablo A. Marquet, Fernando Valladares, and Steven L. Chown. 2013. "Heat Freezes Niche Evolution." *Ecology Letters* 16 (9): 1206–19.
- Atkinson, David. 1994. "Temperature and Organism Size: A Biological Law for Ectotherms?" In *Advances in Ecological Research*, 25:1–58. Academic Press, London.
- Azra, Mohamad N., Jiann-Chu Chen, Mhd Ikhwanuddin, and Ambok Bolong Abol-Munafi. 2018. "Thermal Tolerance and Locomotor Activity of Blue Swimmer Crab *Portunus Pelagicus* Instar Reared at Different Temperatures." *Journal of Thermal Biology* 74: 234–40.
- Bakke, Snorre, Sten I. Siikavuopio, and Jørgen S. Christiansen. 2019. "Thermal Behaviour of Edible Crab *Cancer Pagurus* Linnaeus, 1758 in Coastal Norway." *Fauna Norvegica* 39: 1–11.
- Baldanzi, Simone, Ralph Watson, Christopher D. McQuaid, Gavin Gouws, and Francesca Porri. 2017. "Epigenetic variation among natural populations of the South African sandhopper *Talorchestia capensis*." *Evolutionary Ecology* 31, no. 1: 77-91.
- Bartlett, Maurice Stevenson. 1937. "Properties of Sufficiency and Statistical Tests." *Proceedings of the Royal Society of London. Series A - Mathematical and Physical Sciences* 160 (901): 268–82.
- Bates, Douglas, Martin Mächler, Ben Bolker, and Steve Walker. 2014. "Fitting linear mixed-effects models using lme4." *arXiv preprint arXiv:1406.5823*
- Bates, Amanda E., and Simon A. Morley. 2020. "Interpreting Empirical Estimates of Experimentally Derived Physiological and Biological Thermal Limits in Ectotherms."

Canadian Journal of Zoology 98 (4): 237–44.

- Bedulina, Daria, Michael F. Meyer, Anton Gurkov, Ekaterina Kondratjeva, Boris Baduev, Roman Gusdorf, and Maxim A. Timofeyev. 2017. "Intersexual Differences of Heat Shock Response between Two Amphipods (*Eulimnogammarus Verrucosus* and *Eulimnogammarus Cyaneus*) in Lake Baikal." *PeerJ* 2017 (2): e2864.
- Belanger, Christina L., David Jablonski, Kaustuv Roy, Sarah K. Berke, Andrew Z. Krug, and James W. Valentine. 2012. "Global Environmental Predictors of Benthic Marine Biogeographic Structure." *Proceedings of the National Academy of Sciences of the United States of America* 109 (35): 14046–51.
- Bellard, Céline, Cleo Bertelsmeier, Paul Leadley, Wilfried Thuiller, and Franck Courchamp. 2012. "Impacts of Climate Change on the Future of Biodiversity." *Ecology Letters* 15 (4): 365–77.
- Bergmann, Carl. 1847. *Über Die Verhältnisse Der Wärmeökonomie Der Thiere Zu Ihrer Grösse*. Stud. 1. Göttingen.
- Bjelde, Brittany E., Nathan A. Miller, Jonathon H. Stillman, and Anne E. Todgham. 2015. "The Role of Oxygen in Determining Upper Thermal Limits in *Lottia Digitalis* under Air Exposure and Submersion." *Physiological and Biochemical Zoology* 88 (5): 483–93.
- Blackburn, Shaun, Belinda Van Heerwaarden, Vanessa Kellermann, and Carla M. Sgrò. 2014. "Evolutionary Capacity of Upper Thermal Limits: Beyond Single Trait Assessments." *Journal of Experimental Biology* 217 (11): 1918–24.
- Blamey, Laura K., Lynne J. Shannon, John J. Bolton, Robert J. M. Crawford, Francois Dufois, Hayley Evers-King, Charles L. Griffiths, et al. 2015. "Ecosystem Change in the Southern Benguela and the Underlying Processes." *Journal of Marine Systems* 144 (April): 9–29.
- Bonebrake, Timothy C., and Curtis A. Deutsch. 2012. "Climate Heterogeneity Modulates Impact of Warming on Tropical Insects." *Ecology* 93 (3): 449–55.
- Braby, Caren E., and George N. Somero. 2006. "Following the Heart: Temperature and Salinity Effects on Heart Rate in Native and Invasive Species of Blue Mussels (Genus *Mytilus*)." *The Journal of Experimental Biology* 209 (Pt 13): 2554–66.
- Brahim, Amalina, Nurshahida Mustapha, and David J. Marshall. 2019. "Non-Reversible and Reversible Heat Tolerance Plasticity in Tropical Intertidal Animals: Responding to Habitat Temperature Heterogeneity." *Frontiers in Physiology* 9 (JAN): 1909.
- Branch, George M., and Francois Odendaal. 2003. "The Effects of Marine Protected Areas on the Population Dynamics of a South African Limpet, *Cymbula Oculus*, Relative to the Influence of Wave Action." *Biological Conservation* 114 (2): 255–69.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2017. *Two Oceans: A Guide to the Marine Life of Southern Africa*. Penguin Random House South Africa.
- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2007. *Two Oceans: A Guide to the Marine Life of Southern Africa*. New Holland Publishers, Cape Town, South Africa.
- Branch, George. 1981. "The Biology of Limpets: Physical Factors, Energy Flow, and

- Ecological Interactions." *Oceanography and Marine Biology: An Annual Review* 19: 235–380.
- Branch, Margo, and George Branch. 1981. *The Living Shores of Southern Africa*. Struik publishers.
- Broitman, Bernardo R., P. Lauren Szathmary, K. A. S. Mislan, Carol A. Blanchette, and Brian Helmuth. 2009. "Predator-Prey Interactions under Climate Change: The Importance of Habitat vs Body Temperature." *Oikos* 118 (2): 219–24.
- Broom, Casey J. 2016. "Thermal Adaptation and Behaviour: The Interplay between Performance and Personality in the Intertidal Teleost *Clinus Superciliosus*." University of Stellenbosch.
- Brothers, Cecilia J., and James B. McClintock. 2015. "The Effects of Climate-Induced Elevated Seawater Temperature on the Covering Behavior, Righting Response, and Aristotle's Lantern Reflex of the Sea Urchin *Lytechinus Variegatus*." *Journal of Experimental Marine Biology and Ecology* 467 (June): 33–38.
- Buckley, Lauren B., and Raymond B. Huey. 2016. "Temperature Extremes: Geographic Patterns, Recent Changes, and Implications for Organismal Vulnerabilities." *Global Change Biology* 22 (12): 3829–42.
- Bustamante, Rodrigo H., George M. Branch, and Sean Eekhout. 1995. "Maintenance of an Exceptional Intertidal Grazer Biomass in South Africa: Subsidy by Subtidal Kelps." *Ecology* 76 (7): 2314–29.
- Bustamante, Rodrigo H., George M. Branch, Sean Eekhout, Bruce Robertson, Peter Zoutendyk, Michael Schleyer, Arthur Dye, et al. 1995. "Gradients of Intertidal Primary Productivity around the Coast of South Africa and Their Relationships with Consumer Biomass." *Oecologia* 102 (2): 189–201.
- Byrne, Maria, Melanie Ho, Paulina Selvakumaraswamy, Hong D. Nguyen, Symon A. Dworjanyan, and Andy R. Davis. 2009. "Temperature, but Not pH, Compromises Sea Urchin Fertilization and Early Development under near-Future Climate Change Scenarios." *Proceedings. Biological Sciences / The Royal Society* 276 (1663): 1883–88.
- Byrne, Maria, Paulina Selvakumaraswamy, Melanie A. Ho, Erika Woolsey, and Hong D. Nguyen. 2011. "Sea Urchin Development in a Global Change Hotspot, Potential for Southerly Migration of Thermotolerant Propagules." *Deep-Sea Research. Part II, Topical Studies in Oceanography* 58 (5): 712–19.
- Castañeda, Luis E., Marco A. Lardies, and Francisco Bozinovic. 2004. "Adaptive Latitudinal Shifts in the Thermal Physiology of a Terrestrial Isopod." *Evolutionary Ecology Research* 6 (4): 579–93.
- Chappon, Coraline, Nils Volkenborn, Jacques Clavier, Sarah Séité, Rui Seabra, and Fernando P. Lima. 2016. "Exposure to Solar Radiation Drives Organismal Vulnerability to Climate: Evidence from an Intertidal Limpet." *Journal of Thermal Biology* 57 (April): 92–100.
- Chemello, Silvia, Salvatrice Vizzini, and Antonio Mazzola. 2018. "Regime Shifts and Alternative Stable States in Intertidal Rocky Habitats: State of the Art and New Trends of Research." *Estuarine, Coastal and Shelf Science* 214: 57-63.

- Chen, I-Ching, Jane K. Hill, Ralf Ohlemüller, David B. Roy, and Chris D. Thomas. 2011. "Rapid Range Shifts of Species Associated with High Levels of Climate Warming." *Science* 333 (6045): 1024–26.
- Cheung, William W. L., Jessica J. Meeuwig, Ming Feng, Euan Harvey, Vicky W. Y. Lam, Tim Langlois, Dirk Slawinski, Chaojiao Sun, and Daniel Pauly. 2012. "Climate-Change Induced Tropicalisation of Marine Communities in Western Australia." *Marine and Freshwater Research* 63 (5): 415.
- Chevin, Luis-Miguel, Russell Lande, and Georgina M. Mace. 2010. "Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory." *PLoS Biology* 8 (4): e1000357.
- Chiba, Susumu, Takeshi Iida, Aya Tomioka, Noriko Azuma, Takeo Kurihara, and Kazuhiro Tanaka. 2016. "Population Divergence in Cold Tolerance of the Intertidal Gastropod *Littorina Brevicula* Explained by Habitat-Specific Lowest Air Temperature." *Journal of Experimental Marine Biology and Ecology* 481 (August): 49–56.
- Chust, Guillem, Ángel Borja, Ainhoa Caballero, Xabier Irigoien, Jon Sáenz, Roberto Moncho, Marta Marcos, et al. 2011. "Climate Change Impacts on Coastal and Pelagic Environments in the Southeastern Bay of Biscay." *Climate Research* 48 (2): 307–32.
- Clark, Melody S., Ulf Sommer, Jaspreet K. Sihra, Michael A. S. Thorne, Simon A. Morley, Michelle King, Mark R. Viant, and Lloyd S. Peck. 2017. "Biodiversity in Marine Invertebrate Responses to Acute Warming Revealed by a Comparative Multi-Omics Approach." *Global Change Biology* 23 (1): 318–30.
- Coleman, Ross A. 2010. "Limpet Aggregation Does Not Alter Desiccation in the Limpet *Cellana Tramoserica*." *Journal of Experimental Marine Biology and Ecology* 386 (1–2): 113–18.
- Coleman, Ross A., Antony J. Underwood, Lisandro Benedetti-Cecchi, Per Aberg, Francisco Arenas, Julio Arrontes, João Castro, et al. 2006. "A Continental Scale Evaluation of the Role of Limpet Grazing on Rocky Shores." *Oecologia* 147 (3): 556–64.
- Collin, Rachel, and Kit Yu Karen Chan. 2016. "The Sea Urchin *Lytechinus Variegatus* Lives Close to the Upper Thermal Limit for Early Development in a Tropical Lagoon." *Ecology and Evolution* 6 (16): 5623–34.
- Collin, Rachel, Francesco Rendina, Valerie Goodwin, and Samantha McCabe. 2018. "Do Tropical Specialist Sea Urchins Have Higher Thermal Tolerances and Optimal Temperatures than Their More Widely Distributed Relatives?" *Marine Ecology Progress Series* 589: 153–66.
- Collins, Christina L., Nicholas P. Burnett, Matthew J. Ramsey, Kaitlyn Wagner, and Mackenzie L. Zippay. 2020. "Physiological Responses to Heat Stress in an Invasive Mussel *Mytilus Galloprovincialis* Depend on Tidal Habitat: Habitat-Specific Stress Response in Mussels." *Marine Environmental Research* 154 (February): 104849.
- Comte, Lise, and Julian D. Olden. 2017. "Climatic Vulnerability of the World's Freshwater and Marine Fishes." *Nature Climate Change* 7 (10): 718–22.
- Conover, David O., Tara A. Duffy, and Lyndie A. Hice. 2009. "The Covariance between Genetic and Environmental Influences across Ecological Gradients: Reassessing the Evolutionary Significance of Countergradient and Cogradients Variation." *Annals of the*

New York Academy of Sciences 1168: 100–129.

- Conover, David O., and Eric T. Schultz. 1995. "Phenotypic Similarity and the Evolutionary Significance of Countergradient Variation." *Trends in Ecology & Evolution* 10 (6): 248–52.
- Craig, Paul M., John L. Fitzpatrick, Patrick J. Walsh, Chris M. Wood, and Grant B. McClelland. 2014. "Coping with Aquatic Hypoxia: How the Plainfin Midshipman (*Porichthys Notatus*) Tolerates the Intertidal Zone." *Environmental Biology of Fishes* 97 (2): 163–72.
- Cram, D. L. 1971. "Life History Studies on South African Echinoids (Echinodermata) 1. *Parechinus angulosus* (Leske) (Echinidae, Parechininae)." *Transactions of the Royal Society of South Africa* 39 (3): 321–37.
- Crisp, Dennis John. 1964. "The Effects of the Severe Winter of 1962-63 on Marine Life in Britain." *Journal of Animal Ecology* 33 (1): 165–210.
- Crook, Anne C., and David K. A. Barnes. 2001. "Seasonal Variation in the Covering-Behaviour of the Echinoid *Paracentrotus Lividus* (Lamarck)." *Marine Ecology* 22 (3): 231–39.
- Cuculescu, Mirela, David Hyde, and Kenneth Bowler. 1998. "Thermal Tolerance of Two Species of Marine Crab, *Cancer Pagurus* and *Carcinus Maenas*." *Journal of Thermal Biology* 23 (2): 107–10.
- Cumillaf, Juan P., Johnny Blanc, Kurt Paschke, Paulina Gebauer, Fernando Díaz, Denisse Re, María E. Chimal, Jorge Vázquez, and Carlos Rosas. 2016. "Thermal Biology of the Sub-Polar-Temperate Estuarine Crab *Hemigrapsus Crenulatus* (Crustacea: Decapoda: Varunidae)." *Biology Open* 5 (3): 220–28.
- Darnell, M. Zachary, and Kelly M. Darnell. 2018. "Geographic Variation in Thermal Tolerance and Morphology in a Fiddler Crab Sister-Species Pair." *Marine Biology* 165 (2).
- Darnell, M. Zachary, Haley S. Nicholson, and Pablo Munguia. 2015. "Thermal Ecology of the Fiddler Crab *Uca Panacea*: Thermal Constraints and Organismal Responses." *Journal of Thermal Biology* 52 (August): 157–65.
- Davenport, John, and Julia L. Davenport. 2005. "Effects of Shore Height, Wave Exposure and Geographical Distance on Thermal Niche Width of Intertidal Fauna." *Marine Ecology Progress Series* 292 (May): 41–50.
- Denny, Mark W., Luke P. Miller, and Christopher D. G. Harley. 2006. "Thermal Stress on Intertidal Limpets: Long-Term Hindcasts and Lethal Limits." *The Journal of Experimental Biology* 209 (Pt 13): 2420–31.
- Deutsch, Curtis A., Joshua J. Tewksbury, Raymond B. Huey, Kimberly S. Sheldon, Cameron K. Ghalambor, David C. Haak, and Paul. R. Martin. 2008. "Impacts of Climate Warming on Terrestrial Ectotherms across Latitude." *Proceedings of the National Academy of Sciences of the United States of America* 105 (18): 6668–72.
- Devor, Devin P., Donald E. Kuhn, Kristin M. O'Brien, and Elizabeth L. Crockett. 2016. "Hyperoxia Does Not Extend Critical Thermal Maxima (CT_{max}) in White- or Red-Blooded Antarctic Notothenioid Fishes." *Physiological and Biochemical Zoology* 89 (1): 1–9.

- Díaz, Fernando, Ana Denisse Re, Alfredo Salas, Clara E. Galindo-Sanchez, Marco A. Gonzalez, Adolfo Sanchez, and Carlos Rosas. 2015. "Behavioral Thermoregulation and Critical Thermal Limits of Giant Keyhole Limpet *Megathura Crenulata* (Sowerby 1825) (Mollusca; Vetigastropoda)." *Journal of Thermal Biology* 54 (December): 133–38.
- Díaz, Fernando, Ana Denisse Re, Clara E. Galindo-Sanchez, Eugenio Carpizo-Ituarte, Leonel Perez-Carrasco, Marco González, Alexei Licea, Adolfo Sanchez, and Carlos Rosas. 2017. "Preferred Temperature, Critical Thermal Maximum, and Metabolic Response of the Black Sea Urchin *Arbacia Stellata* (Blainville, 1825; Gmelin, 1791)." *Journal of Shellfish Research* 36 (1): 219–25.
- Dong, Yun-Wei, and Gray A. Williams. 2011. "Variations in Cardiac Performance and Heat Shock Protein Expression to Thermal Stress in Two Differently Zoned Limpets on a Tropical Rocky Shore." *Marine Biology* 158 (6): 1223–31.
- Drake, Madeline J., Nathan A. Miller, and Anne E. Todgham. 2017. "The Role of Stochastic Thermal Environments in Modulating the Thermal Physiology of an Intertidal Limpet, *Lottia Digitalis*." *The Journal of Experimental Biology* 220 (Pt 17): 3072–83.
- Duarte, Helder, Miguel Tejedo, Marco Katzenberger, Federico Marangoni, Diego Baldo, Juan Francisco Beltrán, Dardo Andrea Martí, Alex Richter-Boix, and Alejandro Gonzalez-Voyer. 2012. "Can Amphibians Take the Heat? Vulnerability to Climate Warming in Subtropical and Temperate Larval Amphibian Communities." *Global Change Biology* 18 (2): 412–21.
- Dufois, Francois, and Mathieu Rouault. 2012. "Sea Surface Temperature in False Bay (South Africa): Towards a Better Understanding of Its Seasonal and Inter-Annual Variability." *Continental Shelf Research* 43: 24–35.
- Ern, Rasmus. 2019. "A Mechanistic Oxygen- and Temperature-Limited Metabolic Niche Framework." *Philosophical Transactions of the Royal Society B: Biological Sciences* 374 (1778): 20180540.
- Eslami-Andergoli, Leila, Pat E. R. Dale, Jon M. Knight, and Hamish McCallum. 2015. "Approaching Tipping Points: A Focussed Review of Indicators and Relevance to Managing Intertidal Ecosystems." *Wetlands Ecology and Management* 23 (5): 791–802.
- Farmanfarmaian, A., and Arthur C. Giese. 1963. "Thermal Tolerance and Acclimation in the Western Purple Sea Urchin, *Strongylocentrotus Purpuratus*." *Physiological Zoology*, 237–43.
- Faulkner, Katelyn T., Susana Clusella-Trullas, Lloyd S. Peck, and Steven L. Chown. 2014. "Lack of Coherence in the Warming Responses of Marine Crustaceans." *Functional Ecology* 28 (4): 895–903.
- Fenberg, Phillip B., Karine Posbic, and Michael E. Hellberg. 2014. "Historical and Recent Processes Shaping the Geographic Range of a Rocky Intertidal Gastropod: Phylogeography, Ecology, and Habitat Availability." *Ecology and Evolution* 4 (16): 3244–55.
- Firth, Louise B., Antony M. Knights, and Susan S. Bell. 2011. "Air Temperature and Winter Mortality: Implications for the Persistence of the Invasive Mussel, *Perna Viridis* in the Intertidal Zone of the South-Eastern United States." *Journal of Experimental Marine Biology and Ecology* 400 (1–2): 250–56.

- Firth, Louise B., Nova Mieszkowska, Lisa M. Grant, Laura E. Bush, Andrew J. Davies, Matthew T. Frost, Paula S. Moschella, et al. 2015. "Historical Comparisons Reveal Multiple Drivers of Decadal Change of an Ecosystem Engineer at the Range Edge." *Ecology and Evolution* 5 (15): 3210–22.
- Flagor, Torre E., and Paul E. Bourdeau. 2018. "First Record of the Predatory Snail *Acanthinucella Spirata* (Blainville, 1832) North of Its Known Range." *Marine Biodiversity Records* 11 (1): 1–6.
- Folguera, Guillermo, Julián Mensch, José L. Muñoz, Santiago G. Ceballos, Esteban Hasson, and Francisco Bozinovic. 2010. "Ontogenetic Stage-Dependent Effect of Temperature on Developmental and Metabolic Rates in a Holometabolous Insect." *Journal of Insect Physiology* 56 (11): 1679–84.
- Fox, John, and Sanford Weisberg. 2011. *An R Companion to Applied Regression, Second Edition*. Thousand Oaks California (CA).
- Fox, Rebecca J., Jennifer M. Donelson, Celia Schunter, Timothy Ravasi, Juan D. Gaitán-Espitia, Fox Rebecca J., Donelson Jennifer M., Schunter Celia, Ravasi Timothy, and Gaitán-Espitia Juan D. 2019. "Beyond Buying Time: The Role of Plasticity in Phenotypic Adaptation to Rapid Environmental Change." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 374 (1768): 20180174.
- Fricke, Alexander Hermann. 1979. "Kelp Grazing by the Common Sea Urchin *Parechinus angulosus* (Leske) in False Bay, Cape Town, South Africa." *South African Journal of Zoology* 14 (3): 143–48.
- Frölicher, Thomas L., Erich M. Fischer, and Nicolas Gruber. 2018. "Marine Heatwaves under Global Warming." *Nature* 560 (7718): 360–64.
- Fujisawa, Hirosuke. 1989. "Differences in Temperature Dependence of Early Development of Sea Urchins with Different Growing Seasons." *The Biological Bulletin* 176 (2): 96–102.
- Gaines, Steven Dean, and Mark W. Denny. 2007. *Encyclopedia of Tidepools and Rocky Shores*. First Edition. University of California Press.
- Gaitán-Espitia, Juan Diego, Leonardo D. Bacigalupe, Tania Opitz, Nelson A. Lagos, Tania Timmermann, and Marco A. Lardies. 2014. "Geographic Variation in Thermal Physiological Performance of the Intertidal Crab *Petrolisthes Violaceus* along a Latitudinal Gradient." *The Journal of Experimental Biology* 217 (24): 4379–86.
- Gardiner, Naomi M., Philip L. Munday, and Göran E. Nilsson. 2010. "Counter-Gradient Variation in Respiratory Performance of Coral Reef Fishes at Elevated Temperatures." *PLoS ONE* 5 (10): e13299.
- Garrabou, Joaquim, Rafael Coma, Nathaniel Bensoussan, Marc Bally, Pierre Chevaldonné, M. Cigliano, David Díaz, and et al. 2009. "Mass Mortality in Northwestern Mediterranean Rocky Benthic Communities: Effects of the 2003 Heat Wave." *Global Change Biology* 15 (5): 1090–1103.
- Garrity, Stephen D. 1984. "Some Adaptations of Gastropods to Physical Stress on a Tropical Rocky Shore." *Ecology* 65 (2): 559–74.

- Gaston, Kevin J. 2009. "Geographic Range Limits of Species." *Proceedings of the Royal Society B: Biological Sciences* 276 (1661): 1391–93.
- Gaston, Kevin J., Steven L. Chown, Piero Calosi, Joseph Bernardo, David T. Bilton, Andrew Clarke, Susana Clusella-Trullas, et al. 2009. "Macrophysiology: A Conceptual Reunification." *American Naturalist* 174 (5): 595–612.
- Geller, Jonathan B. 1991. "Gastropod Grazers and Algal Colonization on a Rocky Shore in Northern California: The Importance of the Body Size of Grazers." *Journal of Experimental Marine Biology and Ecology* 150 (1): 1–17.
- Gleason, Lani U., and Ronald S. Burton. 2015. "RNA-Seq Reveals Regional Differences in Transcriptome Response to Heat Stress in the Marine Snail *Chlorostoma Funnebralis*." *Molecular Ecology* 24 (3): 610–27.
- Gray, David Richard. 1997. "Studies of the Biology and Ecology of the High Shore South African Limpet, *Helcion Pectunculus* (Mollusca: Patellogastropoda)." PhD Thesis. Rhodes University.
- Greenwood, P. J. 1975. "Population Dynamics and Ecological Energetics of *Parechinus angulosus* at Robben Island and False Bay, South Africa." *M. Sc. Thesis, University of Cape Town, Cape Town*.
- Greenwood, Paul J., and T. Bennett. 1981. "Some Effects of Temperature-Salinity Combinations on the Early Development of the Sea Urchin *Parechinus Angulosus* (Leske). Fertilization." *Journal of Experimental Marine Biology and Ecology* 51 (2): 119–31.
- Griffiths, Charles L., Tamara B. Robinson, Louise Lange, and Angela Mead. 2010. "Marine Biodiversity in South Africa: An Evaluation of Current States of Knowledge." *PloS One* 5 (8): e12008.
- Grilo, Tiago F., Ana R. Lopes, Eduardo Sampaio, Rui Rosa, and Patrícia G. Cardoso. 2018. "Sex Differences in Oxidative Stress Responses of Tropical Topshells (*Trochus Histrio*) to Increased Temperature and High PCO₂." *Marine Pollution Bulletin* 131 (June): 252–59.
- Gunderson, Alex R., Metadel Abegaz, Alma Y. Ceja, E. K. Lam, Benjamin F. Souther, Kirsten Boyer, E. E. King, Kayley T. You Mak, Brian Tsukimura, and Jonathon H. Stillman. 2019. "Hot Rocks and Not-So-Hot Rocks on the Seashore: Patterns and Body-Size Dependent Consequences of Microclimatic Variation in Intertidal Zone Boulder Habitat." *Integrative Organismal Biology* 1 (1).
- Gunderson, Alex R., Michael E. Dillon, and Jonathon H. Stillman. 2017. "Estimating the Benefits of Plasticity in Ectotherm Heat Tolerance under Natural Thermal Variability." *Functional Ecology* 31 (8): 1529–39.
- Gunderson, Alex R., and Jonathon H. Stillman. 2015. "Plasticity in Thermal Tolerance Has Limited Potential to Buffer Ectotherms from Global Warming." *Proceedings of the Royal Society of London B: Biological Sciences* 282 (1808): 20150401.
- Gutiérrez-Pesquera, Luis Miguel, Miguel Tejedo, Miguel Á. Olalla-Tárraga, Helder Duarte, A. Nicieza, and M. Solé. 2016. "Testing the Climate Variability Hypothesis in Thermal Tolerance Limits of Tropical and Temperate Tadpoles." *Journal of Biogeography* 43 (6): 1166–78.

- Hammond, Latisha Marie, and Gretchen E. Hofmann. 2010. "Thermal Tolerance of *Strongylocentrotus Purpuratus* Early Life History Stages: Mortality, Stress-Induced Gene Expression and Biogeographic Patterns." *Marine Biology* 157 (12): 2677–87.
- Hammond, LaTisha Marie. 2010. "Physiological Response to Environmental Variation in *Strongylocentrotus Purpuratus* Early Developmental Stages. PhD Thesis." University of California, Santa Barbara.
- Han, Guo dong, Stephen R. Cartwright, Monthon Ganmanee, Benny K. K. Chan, Kee A. A. Adzis, Neil Hutchinson, Jie Wang, Tommy Y. Hui, Gray A. Williams, and Yun Wei Dong. 2019. "High Thermal Stress Responses of *Echinolittorina* Snails at Their Range Edge Predict Population Vulnerability to Future Warming." *Science of the Total Environment* 647 (January): 763–71.
- Han, Guo Dong, Shu Zhang, David J. Marshall, Cai Huan Ke, and Yun Wei Dong. 2013. "Metabolic Energy Sensors (AMPK and SIRT1), Protein Carbonylation and Cardiac Failure as Biomarkers of Thermal Stress in an Intertidal Limpet: Linking Energetic Allocation with Environmental Temperature during Aerial Emersion." *Journal of Experimental Biology* 216 (17): 3273–82.
- Hardy, Natasha A., Miles Lamare, Sven Uthicke, Kennedy Wolfe, Steve Doo, Symon Dworjanyn, and Maria Byrne. 2014. "Thermal Tolerance of Early Development in Tropical and Temperate Sea Urchins: Inferences for the Tropicalization of Eastern Australia." *Marine Biology* 161 (2): 395–409.
- Harley, Christopher D. G. 2008. "Tidal Dynamics, Topographic Orientation, and Temperature-Mediated Mass Mortalities on Rocky Shores." *Marine Ecology Progress Series* 371 (November): 37–46.
- Harley, Christopher D. G., and Brian S. T. Helmuth. 2003. "Local- and Regional-Scale Effects of Wave Exposure, Thermal Stress, and Absolute versus Effective Shore Level on Patterns of Intertidal Zonation." *Limnology and Oceanography* 48 (4): 1498–1508.
- Harley, Christopher D. G. 2011. "Climate Change, Keystone Predation, and Biodiversity Loss." *Science* 334 (6059): 1124–27.
- Harley, Christopher D. G. 2003. "Abiotic Stress and Herbivory Interact to Set Range Limits across a Two-Dimensional Stress Gradient." *Ecology* 84 (6): 1477–88.
- Harley, Christopher D. G., Mark W. Denny, Katharine J. Mach, and Luke P. Miller. 2009. "Thermal Stress and Morphological Adaptations in Limpets." *Functional Ecology* 23 (2): 292–301.
- Hawkins, Stephen J., and Richard G. Hartnoll. 1983. "Grazing of Intertidal Algae by Marine Invertebrates." *Oceanography and Marine Biology* 21.
- Hawkins, Stephen J., Heather E. Sugden, Nova Mieszkowska, Pippa J. Moore, Elvira Poloczanska, Rebecca Leaper, Roger J. H. Herbert et al. 2009. "Consequences of Climate-Driven Biodiversity Changes for Ecosystem Functioning of North European Rocky Shores." *Marine Ecology Progress Series* 396 (December): 245–59.
- Healy, Timothy M., Reid S. Brennan, Andrew Whitehead, and Patricia M. Schulte. 2018. "Tolerance Traits Related to Climate Change Resilience Are Independent and Polygenic." *Global Change Biology* 24 (11): 5348–60.

- Heerwaarden, Belinda van, Vanessa Kellermann, and Carla M. Sgrò. 2016. "Limited Scope for Plasticity to Increase Upper Thermal Limits." *Functional Ecology* 30 (12): 1947–56.
- Heerwaarden, Belinda van, Michelle Malmberg, and Carla M. Sgrò. 2016. "Increases in the Evolutionary Potential of Upper Thermal Limits under Warmer Temperatures in Two Rainforest *Drosophila* Species." *Evolution* 70 (2): 456–64.
- Heerwaarden, Belinda van, and Carla M. Sgrò. 2013. "Multivariate Analysis of Adaptive Capacity for Upper Thermal Limits in *Drosophila Simulans*." *Journal of Evolutionary Biology* 26 (4): 800–809.
- Helmuth, Brian S., and Gretchen E. Hofmann. 2001. "Microhabitats, Thermal Heterogeneity, and Patterns of Physiological Stress in the Rocky Intertidal Zone." *The Biological Bulletin* 201 (3): 374–84.
- Helmuth, Brian. 2002. "How Do We Measure the Environment? Linking Intertidal Thermal Physiology and Ecology Through Biophysics." *Integrative and Comparative Biology* 42 (4): 837–45.
- Helmuth, Brian. 2009. "From Cells to Coastlines: How Can We Use Physiology to Forecast the Impacts of Climate Change?" *The Journal of Experimental Biology* 212 (Pt 6): 753–60.
- Helmuth, Brian, Nova Mieszkowska, Pippa Moore, and Stephen J. Hawkins. 2006. "Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change." *Annual Review of Ecology, Evolution and Systematics*, 373–404.
- Helmuth, Brian, Lauren Yamane, Saurabh Lalwani, Allison Matzelle, Alyson Tockstein, and Nan Gao. 2011. "Hidden Signals of Climate Change in Intertidal Ecosystems: What (Not) to Expect When You Are Expecting." *Journal of Experimental Marine Biology and Ecology* 400 (1–2): 191–99.
- Hernández, Mónica, Fernando Bückle, Chita Guisado, Benjamín Barón, and Naielli Estavillo. 2004. "Critical Thermal Maximum and Osmotic Pressure of the Red Sea Urchin *Strongylocentrotus Franciscanus* Acclimated at Different Temperatures." *Journal of Thermal Biology* 29 (4): 231–36.
- Hickling, Rachael, David B. Roy, Jane K. Hill, Richard Fox, and Chris D. Thomas. 2006. "The Distributions of a Wide Range of Taxonomic Groups Are Expanding Polewards." *Global Change Biology* 12 (3): 450–55.
- Hill, Jaclyn M., and Christopher D. McQuaid. 2008. "δ¹³C and δ¹⁵N Biogeographic Trends in Rocky Intertidal Communities along the Coast of South Africa: Evidence of Strong Environmental Signatures." *Estuarine, Coastal and Shelf Science* 80 (2): 261–68.
- Hoffmann, Ary A., Steven L. Chown, and Susana Clusella-Trullas. 2013. "Upper Thermal Limits in Terrestrial Ectotherms: How Constrained Are They?" *Functional Ecology* 27 (4): 934–49.
- Hoffmann, Ary A., and Carla M. Sgrò. 2011. "Climate Change and Evolutionary Adaptation." *Nature* 470 (7335): 479–85.
- Hofmann, Gretchen E., and Anne E. Todgham. 2010. "Living in the Now: Physiological

- Mechanisms to Tolerate a Rapidly Changing Environment.” *Annual Review of Physiology* 72 (1): 127–45.
- Hopkin, Richard S., Suhaila Qari, Kenneth Bowler, David Hyde, and Mirela Cuculescu. 2006. “Seasonal Thermal Tolerance in Marine Crustacea.” *Journal of Experimental Marine Biology and Ecology* 331 (1): 74–81.
- Huey, Raymond B., Curtis A. Deutsch, Joshua J. Tewksbury, Laurie J. Vitt, Paul E. Hertz, Héctor J. Álvarez Pérez, and Theodore Garland Jr. 2009. “Why Tropical Forest Lizards Are Vulnerable to Climate Warming.” *Proceedings. Biological Sciences / The Royal Society* 276 (1664): 1939–48.
- Huey, Raymond B., Michael R. Kearney, Andrew Krockenberger, Joseph A. M. Holtum, Melissa Jess, and Stephen E. Williams. 2012. “Predicting Organismal Vulnerability to Climate Warming: Roles of Behaviour, Physiology and Adaptation.” *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 367 (1596): 1665–79.
- Hughes, A. Randall, Torrance C. Hanley, Althea F. P. Moore, Christine Ramsay-Newton, Robyn A. Zerebecki, and Erik E. Sotka. 2018. “Predicting the Sensitivity of Marine Populations to Rising Temperatures.” *Frontiers in Ecology and the Environment* 17 (1): fee.1986.
- IPCC. 2013. “IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.”
- IPCC. 2014. “Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Edited by Core Writing Team, Pachauri RK, Meyer LA. Geneva, Switzerland: IPCC, 2014.”
- Jackson, Angus C. 2010. “Effects of Topography on the Environment.” *Journal of the Marine Biological Association of the United Kingdom* 90 (1): 169–92.
- Jackson, Jennifer M., Luc Rainville, Michael J. Roberts, Christopher D. McQuaid, and Johann R. E. Lutjeharms. 2012. “Mesoscale Bio-Physical Interactions between the Agulhas Current and the Agulhas Bank, South Africa.” *Continental Shelf Research* 49 (October): 10–24.
- Jaramillo, Eduardo, Jenifer E. Dugan, David M. Hubbard, Heraldo Contreras, Cristian Duarte, Emilio Acuña, and David S. Schoeman. 2017. “Macroscale Patterns in Body Size of Intertidal Crustaceans Provide Insights on Climate Change Effects.” *PLoS One* 12 (5).
- Jensen, Gregory C., and David A. Armstrong. 1991. “Intertidal Zonation among Congeners: Factors Regulating Distribution of Porcelain Crabs *Petrolisthes* Spp. (Anomura: Porcellanidae).” *Marine Ecology Progress Series* 73: 47–60.
- Johnson, David Samuel, Cynthia Crowley, Katherine Longmire, James Nelson, Bethany Williams, and Serina Wittingham. 2019. “The Fiddler Crab, *Minuca Pugnax*, Follows Bergmann’s Rule.” *Ecology and Evolution* 9 (24): 14489–97.
- Jutfelt, Fredrik, Tommy Norin, Rasmus Ern, Johannes Overgaard, Tobias Wang, David J. McKenzie, Sjannie Lefevre, et al. 2018. “Oxygen- and Capacity-Limited Thermal

- Tolerance: Blurring Ecology and Physiology.” *Journal of Experimental Biology* 221 (1).
- Kankondi, Sebbi L., Christopher D. McQuaid, and Morgana Tagliarolo. 2018. “Influence of Respiratory Mode on the Thermal Tolerance of Intertidal Limpets.” *PloS One* 13 (9): e0203555.
- Kelley, Amanda L., Catherine E. de Rivera, Edwin D. Grosholz, Gregory M. Ruiz, Sylvia Behrens Yamada, and Graham Gillespie. 2015. “Thermogeographic Variation in Body Size of *Carcinus Maenas*, the European Green Crab.” *Marine Biology* 162 (8): 1625–35.
- Kelley, Amanda L., Catherine E. de Rivera, and Bradley A. Buckley. 2011. “Intraspecific Variation in Thermotolerance and Morphology of the Invasive European Green Crab, *Carcinus Maenas*, on the West Coast of North America.” *Journal of Experimental Marine Biology and Ecology* 409 (1): 70–78.
- Kilburn, Richard, and Elizabeth Rippey. 1982. *Sea Shells of Southern Africa*. Intl Specialized Book Service Inc.
- Kingsolver, Joel G., and James Umbanhowar. 2018. “The Analysis and Interpretation of Critical Temperatures.” *The Journal of Experimental Biology* 221 (12).
- Klockmann, Michael, Franziska Günter, and Klaus Fischer. 2017. “Heat Resistance throughout Ontogeny: Body Size Constrains Thermal Tolerance.” *Global Change Biology* 23 (2): 686–96.
- Komsta, Lukasz, and Frederick Novomestky. 2015. “Moments: Moments, Cumulants, Skewness, Kurtosis and Related Tests. R Package Version 0.14.” <https://cran.r-project.org/package=moments>.
- Kosloski, Mary E., Gregory P. Dietl, and John C. Handley. 2017. “Anatomy of a Cline: Dissecting Anti-Predatory Adaptations in a Marine Gastropod along the U.S. Atlantic Coast.” *Ecography* 40 (11): 1285–99.
- Kültz, Dietmar. 2005. “Molecular and Evolutionary Basis of the Cellular Stress Response.” *Annual Review of Physiology* 67 (1): 225–57.
- Kuo, Evelyne S. L., and Eric Sanford. 2009. “Geographic Variation in the Upper Thermal Limits of an Intertidal Snail: Implications for Climate Envelope Models.” *Marine Ecology Progress Series* 388 (August): 137–46.
- Lee, Hyuk J. E., and Elizabeth G. Boulding. 2010. “Latitudinal Clines in Body Size, but Not in Thermal Tolerance or Heat-Shock Cognate 70 (HSC70), in the Highly-Dispersing Intertidal Gastropod *Littorina Keenae* (Gastropoda: Littorinidae).” *Biological Journal of the Linnean Society* 100 (3): 494–505.
- Lee, Tai-Hung, Masato Yamauchi, and Fumio Yamazaki. 1994. “Sex Differentiation in the Crab *Eriocheir Japonicus* (Decapoda, Grapsidae).” *Invertebrate Reproduction and Development* 25 (2): 123–37.
- Lenth, Russell, Henrik Singmann, Jonathon Love, Paul Buerkner, and Maxime Herve. 2018. “emmeans: Estimated marginal means, aka least-squares means. R package.” <https://CRAN.R-project.org/package=emmeans>
- Leo, Kelly L., Chris L. Gillies, James A. Fitzsimons, Lynne Z. Hale, and Michael W. Beck. 2019. “Coastal Habitat Squeeze: A Review of Adaptation Solutions for Saltmarsh,

- Mangrove and Beach Habitats." *Ocean and Coastal Management* 175: 180-190.
- Lester, Sarah E., Elizabeth D. Tobin, and Michael D. Behrens. 2007. "Disease Dynamics and the Potential Role of Thermal Stress in the Sea Urchin, *Strongylocentrotus Purpuratus*." *Canadian Journal of Fisheries and Aquatic Sciences* 64 (2): 314–23.
- Leung, Jonathan Y. S., Sean D. Connell, and Bayden D. Russell. 2017. "Heatwaves Diminish the Survival of a Subtidal Gastropod through Reduction in Energy Budget and Depletion of Energy Reserves." *Scientific Reports* 7 (1): 1–8.
- Levene, Howard. 1960. "Contributions to Probability and Statistics." *Essays in Honor of Harold Hotelling*, 278–92.
- Levins, Richard. 1968. *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton University Press.
- Lima, Fernando P., Filipa Gomes, Rui Seabra, David S. Wetthey, Maria I. Seabra, Teresa Cruz, António M. Santos, and Thomas J. Hilbish. 2016. "Loss of Thermal Refugia near Equatorial Range Limits." *Global Change Biology* 22 (1): 254–63.
- Lima, Fernando P., and David S. Wetthey. 2009. "Robolimpets: Measuring Intertidal Body Temperatures Using Biomimetic Loggers." *Limnology and Oceanography: Methods* 7 (5): 347–53.
- Ling, Scott D., Craig R. Johnson, Ken Ridgway, Alistair J. Hobday, and Malcolm Haddon. 2009. "Climate-driven Range Extension of a Sea Urchin: Inferring Future Trends by Analysis of Recent Population Dynamics." *Global Change Biology* 15 (3): 719–31.
- Loarie, Scott R., Philip B. Duffy, Healy Hamilton, Gregory P. Asner, Christopher B. Field, and David D. Ackerly. 2009. "The Velocity of Climate Change." *Nature* 462 (7276): 1052–55.
- Logan, Cheryl A., Laurie E. Kost, and George N. Somero. 2012. "Latitudinal Differences in *Mytilus Californianus* Thermal Physiology." *Marine Ecology Progress Series* 450: 93–105.
- Lourenço, Carla R., Katy R. Nicastro, Christopher D. McQuaid, Lilian A. Krug, and Gerardo I. Zardi. 2020. "Strong Upwelling Conditions Drive Differences in Species Abundance and Community Composition along the Atlantic Coasts of Morocco and Western Sahara." *Marine Biodiversity* 50 (2): 1–18.
- Lucassen, Magnus, Nils Koschnick, Lars G. Eckerle, and Hans Otto Pörtner. 2006. "Mitochondrial Mechanisms of Cold Adaptation in Cod (*Gadus Morhua* L.) Populations from Different Climatic Zones." *Journal of Experimental Biology* 209 (13): 2462–71.
- Lutjeharms, Johann R., and P. L. Stockton. 1991. "Aspects of the Upwelling Regime between Cape Point and Cape Agulhas, South Africa." *South African Journal of Marine Science* 10 (1): 91–102.
- Lutterschmidt, William I., and Victor H. Hutchison. 1997a. "The Critical Thermal Maximum: Data to Support the Onset of Spasms as the Definitive End Point." *Canadian Journal of Zoology* 75 (10): 1553–60.
- Lutterschmidt, William I., and Victor H. Hutchison. 1997b. "The Critical Thermal Maximum: History and Critique." *Canadian Journal of Zoology* 75 (10): 1561–74.

- MacMillan, Heath A. 2019. "Dissecting Cause from Consequence: A Systematic Approach to Thermal Limits." *Journal of Experimental Biology* 222 (4).
- Madeira, Diana, Luís Narciso, Henrique N. Cabral, and Catarina Vinagre. 2012a. "Thermal Tolerance and Potential Impacts of Climate Change on Coastal and Estuarine Organisms." *Journal of Sea Research* 70: 32–41.
- Madeira, Diana, Luís Narciso, Henrique N. Cabral, Mario S. Diniz, and Catarina Vinagre. 2012b. "Thermal Tolerance of the Crab *Pachygrapsus Marmoratus*: Intraspecific Differences at a Physiological (CTMax) and Molecular Level (Hsp70)." *Cell Stress and Chaperones* 17 (6): 707–16.
- Madeira, Diana, Luís Narciso, Henrique N. Cabral, Catarina Vinagre, and Mario S. Diniz. 2012c. "HSP70 production patterns in coastal and estuarine organisms facing increasing temperatures." *Journal of Sea Research* 73: 137–147.
- Madeira, Diana, Luís Narciso, Mário Sousa Diniz, and Catarina Vinagre. 2014. "Synergy of Environmental Variables Alters the Thermal Window and Heat Shock Response: An Experimental Test with the Crab *Pachygrapsus Marmoratus*." *Marine Environmental Research* 98: 21–28.
- Majiedt, Prideel, Stephen Holness, Kerry Sink, Ané Oosthuizen, and Peter Chadwick. 2013. "Systematic Marine Biodiversity Plan for the West Coast of South Africa." *South African National Biodiversity Institute, Cape Town*, 46.
- Malvé, Mariano E., Sandra Gordillo, and Marcelo M. Rivadeneira. 2018. "Connecting pH with Body Size in the Marine Gastropod *Trophon Geversianus* in a Latitudinal Gradient along the South-Western Atlantic Coast." *Journal of the Marine Biological Association of the United Kingdom* 98 (3): 449–56.
- Mandic, Milica, Anne E. Todgham, and Jeffrey G. Richards. 2009. "Mechanisms and Evolution of Hypoxia Tolerance in Fish." *Proceedings of the Royal Society B: Biological Sciences* 276 (1657): 735–44.
- Marshall, David J., Nursalwa Baharuddin, and Christopher D. McQuaid. 2013. "Behaviour Moderates Climate Warming Vulnerability in High-Rocky-Shore Snails: Interactions of Habitat Use, Energy Consumption and Environmental Temperature." *Marine Biology* 160 (9): 2525–30.
- Marshall, David J., and Teddy Chua. 2012. "Boundary Layer Convective Heating and Thermoregulatory Behaviour during Aerial Exposure in the Rocky Eulittoral Fringe Snail *Echinolittorina Malaccana*." *Journal of Experimental Marine Biology and Ecology* 430–431 (November): 25–31.
- Marshall, David J., Yun Wei Dong, Christopher D. McQuaid, and Gray A. Williams. 2011. "Thermal Adaptation in the Intertidal Snail *Echinolittorina Malaccana* Contradicts Current Theory by Revealing the Crucial Roles of Resting Metabolism." *Journal of Experimental Biology* 214 (21): 3649–57.
- Marshall, David J., and Christopher D. McQuaid. 2011. "Warming Reduces Metabolic Rate in Marine Snails: Adaptation to Fluctuating High Temperatures Challenges the Metabolic Theory of Ecology." *Proceedings of the Royal Society B: Biological Sciences* 278 (1703): 281–88.
- McArley, Tristan J., Anthony J. R. Hickey, and Neill A. Herbert. 2020. "Acute High

- Temperature Exposure Impairs Hypoxia Tolerance in an Intertidal Fish." *PLOS ONE* 15 (4): e0231091.
- McGaw, Iain J. 2003. "Behavioral Thermoregulation in *Hemigrapsus Nudus*, the Amphibious Purple Shore Crab." *Biological Bulletin* 204 (1): 38–49.
- Meester, Luc De, Robby Stoks, and Kristien I. Brans. 2018. "Genetic Adaptation as a Biological Buffer against Climate Change: Potential and Limitations." *Integrative Zoology* 13 (4): 372–91.
- Menge, Bruce A., Francis Chan, and Jane Lubchenco. 2008. "Response of a Rocky Intertidal Ecosystem Engineer and Community Dominant to Climate Change." *Ecology Letters* 0 (0): 151–62.
- Metzger, Rebekka, Franz J. Sartoris, Martina Langenbuch, and Hans Otto Pörtner. 2007. "Influence of Elevated CO₂ Concentrations on Thermal Tolerance of the Edible Crab *Cancer Pagurus*." *Journal of Thermal Biology* 32 (3): 144–51.
- Miller, Luke P., Bengt J. Allen, Felicia A. King, Daisy R. Chilin, Vanessa M. Reynoso, and Mark W. Denny. 2015. "Warm Microhabitats Drive Both Increased Respiration and Growth Rates of Intertidal Consumers." *Marine Ecology Progress Series* 522 (March): 127–43.
- Miller, Luke P., Christopher D. G. Harley, and Mark W. Denny. 2009. "The Role of Temperature and Desiccation Stress in Limiting the Local-scale Distribution of the Owl Limpet, *Lottia Gigantea*." *Functional Ecology* 23 (4): 756–67.
- Millott, Norman. 1956. "The Covering Reaction of Sea-Urchins: I. A Preliminary Account Of Covering in the Tropical Echinoid *Lytechinus Variegatus* (Lamarck), and Its Relation to Light." *The Journal of Experimental Biology* 33 (3): 508–23.
- Monaco, Cristián J., Katherina B. Brokordt, and Carlos F. Gaymer. 2010. "Latitudinal Thermal Gradient Effect on the Cost of Living of the Intertidal Porcelain Crab *Petrolisthes Granulosus*." *Aquatic Biology* 9 (1): 23–33.
- Monaco, Cristián J., Christopher D. McQuaid, and David J. Marshall. 2017. "Decoupling of Behavioural and Physiological Thermal Performance Curves in Ectothermic Animals: A Critical Adaptive Trait." *Oecologia* 185 (4): 583–93.
- Mora, Camilo, and Maria F. Maya. 2006. "Effect of the Rate of Temperature Increase of the Dynamic Method on the Heat Tolerance of Fishes." *Journal of Thermal Biology* 31 (4): 337–41.
- Morgan, Rachael, Mette H. Finnøen, and Fredrik Jutfelt. 2018. "CTmax Is Repeatable and Doesn't Reduce Growth in Zebrafish." *Scientific Reports* 8 (1): 1–8.
- Morley, Simon Anthony, Amanda E. Bates, Miles Lamare, Joelle Richard, Khanh Dung Nguyen, J. Brown, and Lloyd S. Peck. 2016. "Rates of Warming and the Global Sensitivity of Shallow Water Marine Invertebrates to Elevated Temperature." *Journal of the Marine Biological Association of the United Kingdom* 96 (1): 159–65.
- Morley, Simon Anthony, Chien Hsiang Lai, Andrew Clarke, Koh Siang Tan, Michael A. S. Thorne, and Lloyd S. Peck. 2014. "Limpet Feeding Rate and the Consistency of Physiological Response to Temperature." *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 184 (5): 563–70.

- Morley, Simon Anthony, V. Lemmon, Birgit E. Obermüller, John I. Spicer, Melody S. Clark, and Lloyd S. Peck. 2011. "Duration Tenacity: A Method for Assessing Acclimatory Capacity of the Antarctic Limpet, *Nacella Concinna*." *Journal of Experimental Marine Biology and Ecology* 399 (1): 39–42.
- Muñoz, José L. P., G. Randall Finke, Patricio A. Camus, and Francisco Bozinovic. 2005. "Thermoregulatory Behavior, Heat Gain and Thermal Tolerance in the Periwinkle *Echinolittorina Peruviana* in Central Chile." *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 142 (1): 92–98.
- Muñoz, L. S. T., Amanda L. Kelley, and Catherine De Rivera. 2017. "The Effect of Salinity Acclimation on the Upper Thermal Tolerance Threshold of the European Green Crab." *Fisheries and Oceanography* 4 (1).
- Neel, Lauren K., John D. Curlis, Chase T. Kinsey, Christian L. Cox, and Lance D. McBrayer. 2020. "Acclimatization in the Physiological Performance of an Introduced Ectotherm." *The Journal of Experimental Biology* 223 (6).
- Nguyen, Khanh Dung T., Simon A. Morley, Chien-Houng Hough Lai, Melody S. Clark, Koh Siang Tan, Amanda E. Bates, and Lloyd S. Peck. 2011. "Upper Temperature Limits of Tropical Marine Ectotherms: Global Warming Implications." *PloS One* 6 (12): e29340.
- Noisette, Fanny, Joëlle Richard, Ines Le Fur, Lloyd S. Peck, Dominique Davoult, and Sophie Martin. 2014. "Metabolic Responses to Temperature Stress under Elevated $p\text{CO}_2$ in *Crepidula Fornicata*." *The Journal of Molluscan Studies* 81 (2): 238–246.
- Nye, Janet A., Jason S. Link, Jonathan A. Hare, and William J. Overholtz. 2009. "Changing Spatial Distribution of Fish Stocks in Relation to Climate and Population Size on the Northeast United States Continental Shelf." *Marine Ecology Progress Series* 393: 111–29.
- Oldenborgh, Geert Jan Van, Eli Mitchell-Larson, Gabriel A. Vecchi, Hylke de Vries, Robert Vautard, and Friederike Otto. 2019. "Cold Waves Are Getting Milder in the Northern Midlatitudes." *Environmental Research Letters* 14: 114004.
- Oliver, Eric C. J., Markus G. Donat, Michael T. Burrows, Pippa J. Moore, Dan A. Smale, Lisa V. Alexander, Jessica A. Benthuisen, et al. 2018. "Longer and More Frequent Marine Heatwaves over the Past Century." *Nature Communications* 9 (1): 1–12.
- Osovitz, Christopher J., and Gretchen E. Hofmann. 2005. "Thermal History-Dependent Expression of the Hsp70 Gene in Purple Sea Urchins: Biogeographic Patterns and the Effect of Temperature Acclimation." *Journal of Experimental Marine Biology and Ecology* 327 (2): 134–43.
- Osovitz, Christopher J., and Gretchen E. Hofmann. 2007. "Marine Macrophysiology: Studying Physiological Variation across Large Spatial Scales in Marine Systems." *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 147, no. 4: 821–827.
- Oyamaguchi, Hilton M., Pacific Vo, Kiran Grewal, Robert Do, Eugene Erwin, Narin Jeong, Katherine Tse, et al. 2018. "Thermal Sensitivity of a Neotropical Amphibian (*Engystomops Pustulosus*) and Its Vulnerability to Climate Change." *Biotropica* 50 (2): 326–37.

- Parmesan, Camille. 2006. "Ecological and Evolutionary Responses to Recent Climate Change." *Annual Review of Ecology, Evolution and Systematics* 37 (1): 637–69.
- Parmesan, Camille, and Gary Yohe. 2003. "A Globally Coherent Fingerprint of Climate Change Impacts across Natural Systems." *Nature* 421 (6918): 37–42.
- Pasparakis, Christina, Brittany E. Davis, and Anne E. Todgham. 2016. "Role of Sequential Low-Tide-Period Conditions on the Thermal Physiology of Summer and Winter Laboratory-Acclimated Fingered Limpets, *Lottia Digitalis*." *Marine Biology* 163 (2): 23.
- Peck, Lloyd Samuel, Simon Anthony Morley, Joëlle Richard, and Melody S. Clark. 2014. "Acclimation and Thermal Tolerance in Antarctic Marine Ectotherms." *Journal of Experimental Biology* 217 (1): 16–22.
- Peck, Lloyd Samuel, Melody S. Clark, Simon Anthony Morley, Alison Massey, and Helen Rossetti. 2009. "Animal Temperature Limits and Ecological Relevance: Effects of Size, Activity and Rates of Change." *Functional Ecology* 23 (2): 248–56.
- Peck, Lloyd Samuel, Simon Anthony Morley, Hans Otto Pörtner, and Melody Susan Clark. 2007. "Thermal Limits of Burrowing Capacity Are Linked to Oxygen Availability and Size in the Antarctic Clam *Laternula Elliptica*." *Oecologia* 154 (3): 479–84.
- Pecorino, Danilo, Miles D. Lamare, Mike F. Barker, and Maria Byrne. 2013. "How Does Embryonic and Larval Thermal Tolerance Contribute to the Distribution of the Sea Urchin *Centrostephanus Rodgersii* (Diadematidae) in New Zealand?" *Journal of Experimental Marine Biology and Ecology* 445 (July): 120–28.
- Perkins, Nicholas R., Nicole A. Hill, Scott D. Foster, and Neville S. Barrett. 2015. "Altered Niche of an Ecologically Significant Urchin Species, *Centrostephanus Rodgersii*, in Its Extended Range Revealed Using an Autonomous Underwater Vehicle." *Estuarine, Coastal and Shelf Science* 155 (March): 56–65.
- Perry, Allison L., Paula J. Low, Jim R. Ellis, and John D. Reynolds. 2005. "Climate Change and Distribution Shifts in Marine Fishes." *Science* 308 (5730): 1912–15.
- Pinsky, Malin L., Anne Maria Eikeset, Douglas J. McCauley, Jonathan L. Payne, and Jennifer M. Sunday. 2019. "Greater Vulnerability to Warming of Marine versus Terrestrial Ectotherms." *Nature* 569 (7754): 108–11.
- Pinsky, Malin L., Boris Worm, Michael J. Fogarty, Jorge L. Sarmiento, and Simon A. Levin. 2013. "Marine Taxa Track Local Climate Velocities." *Science* 341 (6151): 1239–42.
- Pintanel, Pol, Miguel Tejedo, Santiago R. Ron, Gustavo A. Llorente, and Andrés Merino-Viteri. 2019. "Elevational and Microclimatic Drivers of Thermal Tolerance in Andean *Pristimantis* Frogs." *Journal of Biogeography* 46 (8): 1664–75.
- Poloczanska, Elvira S., Michael T. Burrows, Christopher J. Brown, Jorge García Molinos, Benjamin S. Halpern, Ove Hoegh-Guldberg, Carrie V. Kappel, et al. 2016. "Responses of Marine Organisms to Climate Change across Oceans." *Frontiers in Marine Science* 3 (May): 62.
- Pörtner, Hans Otto. 2010. "Oxygen- And Capacity-Limitation of Thermal Tolerance: A Matrix for Integrating Climate-Related Stressor Effects in Marine Ecosystems." *Journal of Experimental Biology* 213 (6): 881–93.

- Pörtner, Hans-Otto, Peter L. M. Van Dijk, Iris Hardewig, and Angela Sommer. 2000. "Levels of Metabolic Cold Adaptation: Tradeoffs in Eurythermal and Stenothermal Ectotherms." In *Antarctic Ecosystems: Models for Wider Ecological Understanding*, edited by C. Howard Williams W. Davison. Caxton Press, Christchurch New Zealand.
- Pörtner, Hans Otto, Christian Bock, and Felix C. Mark. 2017. "Oxygen-and Capacity-Limited Thermal Tolerance: Bridging Ecology and Physiology." *Journal of Experimental Biology* 220 (15): 2685–96.
- Pörtner, Hans Otto, Christian Bock, and Felix C. Mark. 2018. "Connecting to Ecology: A Challenge for Comparative Physiologists? Response to 'Oxygen- and Capacity-Limited Thermal Tolerance: Blurring Ecology and Physiology.'" *Journal of Experimental Biology* 221 (1).
- Potter, Kristen A., H. Arthur Woods, and Sylvain Pincebourde. 2013. "Microclimatic Challenges in Global Change Biology." *Global Change Biology* 19 (10): 2932–39.
- Prentice, Earl F., and David E. Schneider. 1979. "Respiration and Thermal Tolerance of the Dungeness Crab, *Cancer Magister* Dana." *Comparative Biochemistry And Physiology. Part A, Physiology* 63 (4): 591–97.
- Pulgar, Jose M., Francisco Bozinovic, and F. Patricio Ojeda. 2005. "Local Distribution and Thermal Ecology of Two Intertidal Fishes." *Oecologia* 142 (4): 511–20.
- Qari, Suhaila, and Rabab Aljarari. 2014. "The Effect of Season and Acclimation on the Heat and Cold Tolerance of the Red Sea Crab, *Portunus Pelagicus*." *Life Science Journal* 11: 145–48.
- Ralston, Joel, William V. DeLuca, Richard E. Feldman, and David I. King. 2017. "Population Trends Influence Species Ability to Track Climate Change." *Global Change Biology* 23 (4): 1390–99.
- Rastrick, Samuel P. S., Piero Calosi, Ruth Calder-Potts, Andy Foggo, Gregory Nightingale, Stephen Widdicombe, and John I. Spicer. 2014. "Living in Warmer, More Acidic Oceans Retards Physiological Recovery from Tidal Emersion in the Velvet Swimming Crab, *Necora Puber*." *Journal of Experimental Biology* 217 (14): 2499–2508.
- Reusch, Thorsten B. H. 2014. "Climate Change in the Oceans: Evolutionary versus Phenotypically Plastic Responses of Marine Animals and Plants." *Evolutionary Applications* 7 (1): 104–22.
- Richard, Joelle, Simon Anthony Morley, Michael A. S. Thorne, and Lloyd Samuel Peck. 2012. "Estimating Long-Term Survival Temperatures at the Assemblage Level in the Marine Environment: Towards Macrophysiology." *PLoS One* 4 (7).
- Richards, Jeffrey G. 2011. "Physiological, Behavioral and Biochemical Adaptations of Intertidal Fishes to Hypoxia." *Journal of Experimental Biology* 214 (2): 191–99.
- Richter-Boix, Alex, Marco Katzenberger, Helder Duarte, María Quintela, Miguel Tejedo, and Anssi Laurila. 2015. "Local Divergence of Thermal Reaction Norms among Amphibian Populations Is Affected by Pond Temperature Variation." *Evolution* 69 (8): 2210–26.
- Rilov, Gil. 2016. "Multi-Species Collapses at the Warm Edge of a Warming Sea." *Scientific Reports* 6 (1): 1–14.

- Rilov, Gil, Antonios D. Mazaris, Vanessa Stelzenmüller, Brian Helmuth, Martin Wahl, Tamar Guy-Haim, Nova Mieszkowska, Jean-Baptiste Ledoux, and Stelios Katsanevakis. 2019. "Adaptive Marine Conservation Planning in the Face of Climate Change: What Can We Learn from Physiological, Ecological and Genetic Studies?" *Global Ecology and Conservation* 17: e00566.
- Rohr, Jason R., David J. Civitello, Jeremy M. Cohen, Elizabeth A. Roznik, Barry Sinervo, and Anthony I. Dell. 2018. "The Complex Drivers of Thermal Acclimation and Breadth in Ectotherms." *Ecology Letters* 21 (9): 1425–39.
- Ronges, Daria, Jillian P. Walsh, Brent J. Sinclair, and Jonathon H. Stillman. 2012. "Changes in Extreme Cold Tolerance, Membrane Composition and Cardiac Transcriptome during the First Day of Thermal Acclimation in the Porcelain Crab *Petrolisthes Cinctipes*." *Journal of Experimental Biology* 215 (11): 1824–36.
- Roni, Nasdwiana, Nadiarti Nurdin Kadir, Shinta Werorilangi, and Wayne A. Bennett. 2019. "Impact of Changing Temperature on Critical Thermal Maximum and Metabolic Rate of *Uca Perplexa* and *Uca Crassipes*." *Ecological Questions* 30 (3): 17–24.
- Rouault, Marjolaine. 2011. "Agulhas Current Variability Determined from Space: A Multi-Sensor Approach." PhD Thesis. University of Cape Town.
- Rouault, Mathieu, Pierrick Penven, and Benjamin Pohl. 2009. "Warming in the Agulhas Current System since the 1980's." *Geophysical Research Letters* 36 (12).
- Rouault, Mathieu, Benjamin Pohl, and Pierrick Penven. 2010. "Coastal Oceanic Climate Change and Variability from 1982 to 2009 around South Africa." *African Journal of Marine Science* 32 (2): 237–46.
- Sagarin, Raphael D., and George N. Somero. 2006. "Complex Patterns of Expression of Heat-Shock Protein 70 across the Southern Biogeographical Ranges of the Intertidal Mussel *Mytilus Californianus* and Snail *Nucella Ostrina*." *Journal of Biogeography* 33 (4): 622–30.
- Samraus, Florian, Rolf Erik Olsen, Mette Remen, Tom Johnny Hansen, Thomas Torgersen, and Per Gunnar Fjellidal. 2017. "Water Temperature and Oxygen: The Effect of Triploidy on Performance and Metabolism in Farmed Atlantic Salmon (*Salmo Salar* L.) Post-Smolts." *Aquaculture* 473 (April): 1–12.
- Sanford, Eric, and Morgan W. Kelly. 2011. "Local Adaptation in Marine Invertebrates." *Annual Review of Marine Science* 3 (1): 509–35.
- Schlegel, Robert W., Eric C. J. Oliver, Thomas Wernberg, and Albertus J. Smit. 2017. "Coastal and Offshore Co-Occurrences of Marine Heatwaves and Cold-Spells." *Progress in Oceanography* 151: 189-205
- Schleupner, Christine. 2008. "Evaluation of Coastal Squeeze and Its Consequences for the Caribbean Island Martinique." *Ocean and Coastal Management* 51 (5): 383–90.
- Schmidt-Nielsen, Knut. 1997. *Animal Physiology: Adaptation and Environment*. Cambridge University Press.
- Schulte, Patricia M., Timothy M. Healy, and Nann A. Fangue. 2011. "Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure." *Integrative and Comparative Biology* 51 (5): 691–702.

- Sconzo, Gabriella, Maria Carmela Roccheri, Maria La Rosa, Daniele Oliva, A. Abrignani, and Giovanni Giudice. 1986. "Acquisition of Thermotolerance in Sea Urchin Embryos Correlates with the Synthesis and Age of the Heat Shock Proteins." *Cell Differentiation* 19 (3): 173–77.
- Seabra, Rui, David S. Wetthey, António M. Santos, and Fernando P. Lima. 2011. "Side Matters: Microhabitat Influence on Intertidal Heat Stress over a Large Geographical Scale." *Journal of Experimental Marine Biology and Ecology* 400 (1–2): 200–208.
- Seuront, Laurent, and Terence P. T. Ng. 2016. "Standing in the Sun: Infrared Thermography Reveals Distinct Thermal Regulatory Behaviours in Two Tropical High-Shore Littorinid Snails." *Journal of Molluscan Studies* 82 (2): 336–40.
- Sewell, Mary A., and Craig M. Young. 1999. "Temperature Limits to Fertilization and Early Development in the Tropical Sea Urchin *Echinometra Lucunter*." *Journal of Experimental Marine Biology and Ecology* 236 (2): 291–305.
- Sexton, Jason P., Patrick J. McIntyre, Amy L. Angert, and Kevin J. Rice. 2009. "Evolution and Ecology of Species Range Limits." *Annual Review of Ecology, Evolution, and Systematics* 40 (1): 415–36.
- Shapiro, Samuel Sanford, and Martin B. Wilk. 1965. "An Analysis of Variance Test for Normality (Complete Samples)." *Biometrika* 52 ((3–4)): 591–611.
- Sherman, Elizabeth. 2015. "Can Sea Urchins Beat the Heat? Sea Urchins, Thermal Tolerance and Climate Change." *PeerJ* 3: e1006.
- Siegle, Matthew R., Eric B. Taylor, and Mary I. O'Connor. 2018. "Prior Heat Accumulation Reduces Survival during Subsequent Experimental Heat Waves." *Journal of Experimental Marine Biology and Ecology* 501 (April): 109–17.
- Simčič, Tatjana, Franja Pajk, Martina Jaklič, Anton Brancelj, and Al Vrezec. 2014. "The Thermal Tolerance of Crayfish Could Be Estimated from Respiratory Electron Transport System Activity." *Journal of Thermal Biology* 41 (1): 21–30.
- Sinclair, Elektra L. E., Michael B. Thompson, and Frank Seebacher. 2006. "Phenotypic Flexibility in the Metabolic Response of the Limpet *Cellana Tramoserica* to Thermally Different Microhabitats." *Journal of Experimental Marine Biology and Ecology* 335 (1): 131–41.
- Sinervo, Barry, Fausto Mendez-De-La-Cruz, Donald B. Miles, Benoit Heulin, Elizabeth Bastiaans, Maricela Villagrán-Santa Cruz, Rafael Lara-Resendiz et al. 2010. "Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches." *Science* 328 (5980): 894–99.
- Sink, Kerry. 2016. "The Marine Protected Areas Debate: Implications for the Proposed Phakisa Marine Protected Areas Network." *South African Journal of Science* 112 (9/10).
- Smale, Dan A., and Thomas Wernberg. 2013. "Extreme Climatic Event Drives Range Contraction of a Habitat-Forming Species." *Proceedings of the Royal Society B: Biological Sciences* 280 (1754): 20122829.
- Smit, Albertus J., Michael Roberts, Robert J. Anderson, Francois Dufois, Sheldon F. J. Dudley, Thomas G. Bornman, Jennifer Olbers, and John J. Bolton. 2013. "A Coastal

- Seawater Temperature Dataset for Biogeographical Studies: Large Biases between *in Situ* and Remotely-Sensed Data Sets around the Coast of South Africa." *PLOS One* 8 (12): e81944.
- Smith, Kimberley A., Christopher E. Dowling, and Joshua Brown. 2019. "Simmered Then Boiled: Multi-Decadal Poleward Shift in Distribution by a Temperate Fish Accelerates During Marine Heatwave." *Frontiers in Marine Science* 6 (407).
- Sokolova, Inna M., Markus Frederich, Rita Bagwe, Gisela Lannig, and Alexey A. Sukhotin. 2012. "Energy Homeostasis as an Integrative Tool for Assessing Limits of Environmental Stress Tolerance in Aquatic Invertebrates." *Marine Environmental Research* 79 (August): 1–15.
- Somero, George N. 2002. "Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living." *Integrative and Comparative Biology* 42 (4): 780–89.
- Somero, George N. 2005. "Linking Biogeography to Physiology: Evolutionary and Acclimatory Adjustments of Thermal Limits." *Frontiers in Zoology* 2 (1): 1.
- Somero, George N. 2010. "The Physiology of Climate Change: How Potentials for Acclimatization and Genetic Adaptation Will Determine 'Winners' and 'Losers.'" *The Journal of Experimental Biology* 213 (6): 912–20.
- Sornom, Pascal, Vincent Felten, Vincent Médoc, Sophie Sroda, Philippe Rousselle, and Jean Nicolas Beisel. 2010. "Effect of Gender on Physiological and Behavioural Responses of *Gammarus Roeseli* (Crustacea Amphipoda) to Salinity and Temperature." *Environmental Pollution* 158 (5): 1288–95.
- Sorte, Cascade J. B., Geneviève Bernatchez, K. A. S. Mislán, Lauren L. M. Pandori, Nyssa J. Silbiger, and Piper D. Wallingford. 2019. "Thermal Tolerance Limits as Indicators of Current and Future Intertidal Zonation Patterns in a Diverse Mussel Guild." *Marine Biology* 166 (1): 1–13.
- Sorte, Cascade J. B., Sierra J. Jones, and Luke P. Miller. 2011. "Geographic Variation in Temperature Tolerance as an Indicator of Potential Population Responses to Climate Change." *Journal of Experimental Marine Biology and Ecology* 400 (1–2): 209–17.
- Sorte, Cascade J. B., and Gretchen E. Hofmann. 2004. "Changes in Latitudes, Changes in Aptitudes: *Nucella Canaliculata* (Mollusca: Gastropoda) Is More Stressed at Its Range Edge." *Marine Ecology Progress Series* 274 (June): 263–68.
- Stillman, Jonathon. 2002. "Causes and Consequences of Thermal Tolerance Limits in Rocky Intertidal Porcelain Crabs, Genus *Petrolisthes*." *Integrative and Comparative Biology* 42 (4): 790–96.
- Stillman, Jonathon. 2003. "Acclimation Capacity Underlies Susceptibility to Climate Change." *Science* 301 (5629): 65.
- Stillman, Jonathon. 2004. "A Comparative Analysis of Plasticity of Thermal Limits in Porcelain Crabs across Latitudinal and Intertidal Zone Clines." *International Congress Series / Excerpta Medica* 1275: 267–74.
- Stillman, Jonathon. 2019. "Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities." *Physiology* 34

(2): 86–100.

- Stillman, Jonathon, and George N. Somero. 2000. "A Comparative Analysis of the Upper Thermal Tolerance Limits of Eastern Pacific Porcelain Crabs, Genus *Petrolisthes*: Influences of Latitude, Vertical Zonation, Acclimation, and Phylogeny." *Physiological and Biochemical Zoology* 73 (2): 200–208.
- Stillman, Jonathon H., and Abderrahmane Tagmout. 2009. "Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, *Petrolisthes cinctipes*." *Molecular Ecology* 18, no. 20: 4206–4226.
- Stuart-Smith, Rick D., Graham J. Edgar, and Amanda E. Bates. 2017. "Thermal Limits to the Geographic Distributions of Shallow-Water Marine Species." *Nature Ecology and Evolution* 1 (12): 1846–52.
- Sunday, Jennifer M., Amanda E. Bates, and Nicholas K. Dulvy. 2012. "Thermal Tolerance and the Global Redistribution of Animals." *Nature Climate Change* 2 (9): 686–90.
- Tagatz, Marlin E. 1969. "Some Relations of Temperature Acclimation and Salinity to Thermal Tolerance of the Blue Crab, *Callinectes Sapidus*." *Transactions of the American Fisheries Society* 98 (4): 713–16.
- Tagliarolo, Morgana, and Christopher D. McQuaid. 2015. "Sub-Lethal and Sub-Specific Temperature Effects Are Better Predictors of Mussel Distribution than Thermal Tolerance." *Marine Ecology Progress Series* 535 (September): 145–59.
- Taylor, Phillip R. 1982. "Environmental Resistance and the Ecology of Coexisting Hermit Crabs: Thermal Tolerance." *Journal of Experimental Marine Biology and Ecology* 57 (2): 229–36.
- Tepolt, Carolyn K., and George N. Somero. 2014. "Master of All Trades: Thermal Acclimation and Adaptation of Cardiac Function in a Broadly Distributed Marine Invasive Species, the European Green Crab, *Carcinus Maenas*." *Journal of Experimental Biology* 217 (7): 1129–38.
- Terblanche, John S., Jacques A. Deere, Susana Clusella-Trullas, Charlene Janion, and Steven L. Chown. 2007. "Critical Thermal Limits Depend on Methodological Context." *Proceedings. Biological Sciences / The Royal Society* 274 (1628): 2935–42.
- Terblanche, John S., Ary A. Hoffmann, Katherine A. Mitchell, Lea Rako, Peter C. Le Roux, and Steven L. Chown. 2011. "Ecologically Relevant Measures of Tolerance to Potentially Lethal Temperatures." *Journal of Experimental Biology* 214 (22): 3713–25.
- Teske, Peter R., Isabelle Papadopoulos, Brent K. Newman, Peter C. Dworschak, Christopher D. McQuaid, and Nigel P. Barker. 2008. "Oceanic Dispersal Barriers, Adaptation and Larval Retention: An Interdisciplinary Assessment of Potential Factors Maintaining a Phylogeographic Break between Sister Lineages of an African Prawn." *BMC Evolutionary Biology* 8 (1): 341.
- Teske, Peter R., Jonathan Sandoval-Castillo, Tirupathi Rao Golla, Arsalan Emami-Khoyi, Mbaye Tine, Sophie von der Heyden, and Luciano B. Beheregaray. 2019. "Thermal Selection as a Driver of Marine Ecological Speciation." *Proceedings of the Royal Society B* 286 (1896): 20182023.
- Tewksbury, Joshua J., Raymond B. Huey, and Curtis A. Deutsch. 2008. "Putting the Heat on

- Tropical Animals." *Science* 320 (5881): 1296–97.
- Thomas, Chris D. 2010. "Climate, Climate Change and Range Boundaries." *Diversity and Distributions* 16 (3): 488–95.
- Thomsen, Mads S., Luca Mondardini, Tommaso Alestra, Shawn Gerrity, Leigh Tait, Paul M. South, Stacie A. Lilley, and David R. Schiel. 2019. "Local Extinction of Bull Kelp (*Durvillaea* Spp.) Due to a Marine Heatwave." *Frontiers in Marine Science* 6 (MAR): 84.
- Todd, Mary-Elizabeth, and Paul A. Dehnel. 1960. "Effect of Temperature and Salinity on Heat Tolerance in Two Grapsoid Crabs, *Hemigrapsus Nudus* and *Hemigrapsus Oregonensis*." *The Biological Bulletin* 118 (1): 150–72.
- Tomanek, Lars. 2010. "Variation in the Heat Shock Response and Its Implication for Predicting the Effect of Global Climate Change on Species' Biogeographical Distribution Ranges and Metabolic Costs." *Journal of Experimental Biology* 213 (6): 971–79.
- Truchot, Jean Paul, and A. Duhamel-Jouve. 1980. "Oxygen and Carbon Dioxide in the Marine Intertidal Environment: Diurnal and Tidal Changes in Rockpools." *Respiration Physiology* 39 (3): 241–54.
- Tuff, Kika T., Ty Tuff, and Kendi F. Davies. 2016. "A Framework for Integrating Thermal Biology into Fragmentation Research." *Ecology Letters* 19 (4): 361–74.
- Urban, Mark C. 2015. "Accelerating Extinction Risk from Climate Change." *Science (New York, N.Y.)* 348 (6234): 571–73.
- Vannier, Guy. 1994. "The Thermobiological Limits of Some Freezing Tolerant Insects: The Supercooling and Thermostupor Points." *Acta Oecologica* 15: 31–42.
- Vaughn, Dawn, Olivia R. Turnross, and Emily Carrington. 2014. "Sex-Specific Temperature Dependence of Foraging and Growth of Intertidal Snails." *Marine Biology* 161 (1): 75–87.
- Vinagre, Catarina, Marta Dias, Rui Cereja, Francisca Abreu-Afonso, Augusto A. V. Flores, and Vanessa Mendonça. 2019. "Upper Thermal Limits and Warming Safety Margins of Coastal Marine Species – Indicator Baseline for Future Reference." *Ecological Indicators* 102 (July): 644–49.
- Vinagre, Catarina, Marta Dias, Joana Roma, Ana Silva, Diana Madeira, and Mário S. Diniz. 2013. "Critical Thermal Maxima of Common Rocky Intertidal Fish and Shrimps - A Preliminary Assessment." *Journal of Sea Research* 81 (August): 10–12.
- Vinagre, Catarina, Inês Leal, Vanessa Mendonça, and Augusto A. V. Flores. 2015. "Effect of Warming Rate on the Critical Thermal Maxima of Crabs, Shrimp and Fish." *Journal of Thermal Biology* 47: 19–25.
- Vinagre, Catarina, Inês Leal, Vanessa Mendonça, Diana Madeira, Luís Narciso, Mário S. Diniz, and Augusto A. V. Flores. 2016. "Vulnerability to Climate Warming and Acclimation Capacity of Tropical and Temperate Coastal Organisms." *Ecological Indicators* 62 (March): 317–27.
- Vinagre, Catarina, Diana Madeira, Luís Narciso, Henrique N. Cabral, and Mário S. Diniz. 2012. "Effect of Handling, Confinement and Crowding in HSP70 Production in *Pachygrapsus Marmoratus*, a Model Species for Climate Change Experiments." *Journal*

of Sea Research 72 (August): 64–68.

- Vinagre, Catarina, Vanessa Mendonça, Rui Cereja, Francisca Abreu-Afonso, Marta Dias, Damián Mizrahi, and Augusto A. V. Flores. 2018. “Ecological Traps in Shallow Coastal Waters-Potential Effect of Heat-Waves in Tropical and Temperate Organisms.” *PLoS ONE* 13 (2).
- Walther, Kathleen, Franz J. Sartoris, Christian Bock, and Hans Otto Pörtner. 2009. “Impact of Anthropogenic Ocean Acidification on Thermal Tolerance of the Spider Crab *Hyas Araneus*.” *Biogeosciences* 6 (10): 2207–15.
- Wang, Terrance, Richelle L. Tanner, Eric J. Armstrong, David R. Lindberg, and Jonathon H. Stillman. 2019. “Plasticity of Foot Muscle and Cardiac Thermal Limits in the Limpet *Lottia Limatula* from Locations with Differing Temperatures.” *Aquatic Biology* 28: 113–25.
- Wang, Tobias, Sjannie Lefevre, Nina K. Iversen, Inge Findorf, Rasmus Buchanan, and David J. McKenzie. 2014. “Anaemia Only Causes a Small Reduction in the Upper Critical Temperature of Sea Bass: Is Oxygen Delivery the Limiting Factor for Tolerance of Acute Warming in Fishes?” *Journal of Experimental Biology* 217 (24): 4275–78.
- Wernberg, Thomas, Bayden D. Russell, Pippa J. Moore, Scott D. Ling, Daniel A. Smale, Alex Campbell, Melinda A. Coleman, Peter D. Steinberg, Gary A. Kendrick, and Sean D. Connell. 2011. “Impacts of Climate Change in a Global Hotspot for Temperate Marine Biodiversity and Ocean Warming.” *Journal of Experimental Marine Biology and Ecology* 400 (1–2): 7–16.
- Wethey, David S., Sarah A. Woodin, Thomas J. Hilbish, Sierra J. Jones, Fernando P. Lima, and Pamela M. Brannock. 2011. “Response of Intertidal Populations to Climate: Effects of Extreme Events versus Long Term Change.” *Journal of Experimental Marine Biology and Ecology* 400 (1–2): 132–44.
- Whitfield, Alan K., Nicola C. James, Stephen J. Lamberth, Janine B. Adams, Renzo Perissinotto, Anusha Rajkaran, and Thomas G. Bornman. 2016. “The Role of Pioneers as Indicators of Biogeographic Range Expansion Caused by Global Change in Southern African Coastal Waters.” *Estuarine, Coastal and Shelf Science* 172 (April): 138–53.
- Williams, Belinda Ruth, Belinda Van Heerwaarden, Damian Kimon Dowling, and Carla Maria Sgrò. 2012. “A Multivariate Test of Evolutionary Constraints for Thermal Tolerance in *Drosophila Melanogaster*.” *Journal of Evolutionary Biology* 25 (7): 1415–26.
- Williams, Gray A., and David Morritt. 1995. “Habitat Partitioning and Thermal Tolerance in a Tropical Limpet, *Cellana Grata*.” *Marine Ecology Progress Series* 124 (1): 89–103.
- Williams, Gray A., Benny K. K. Chan, and Yun-Wei Dong. 2019. “Rocky Shores of Mainland China, Taiwan and Hong Kong: Past, Present and Future.” In *Interactions in the Marine Benthos: Global Patterns and Processes (Systematics Association Special Volume Series)*, 360–90. Cambridge University Press.
- Williams, Gray A., Maurizio De Pirro, Kenneth M. Y. Leung, and David Morritt. 2005. “Physiological Responses to Heat Stress on a Tropical Shore: The Benefits of Mushrooming Behaviour in the Limpet *Cellana Grata*.” *Marine Ecology Progress Series* 292: 213–24.

- Woiwode, John G., and Ira R. Adelman. 1992. "Effects of starvation, oscillating temperatures, and photoperiod on the critical thermal maximum of hybrid striped × white bass." *Journal of Thermal Biology* 17, no. 4-5: 271-275.
- Yampolsky, Lev Y., Tobias M. M. Schaer, and Dieter Ebert. 2014. "Adaptive Phenotypic Plasticity and Local Adaptation for Temperature Tolerance in Freshwater Zooplankton." *Proceedings of the Royal Society B: Biological Sciences* 281 (1776): 20132744.
- Ziegenhorn, Morgan A. 2017. "Sea Urchin Covering Behavior: A Comparative Review." In *Sea Urchin - From Environment to Aquaculture and Biomedicine*, edited by Maria Agnello. InTech, Croatia.

5. Conclusion

South Africa is home to numerous marine and terrestrial endemic species and counts among the most biodiverse countries in the world (Sink et al. 2012; Whitfield et al. 2016; Warner, Sobrevila, and Ledec, 2016). The contrasting influences of the cold Benguela Current and the warm Agulhas Current drive an environmental gradient with temperature-delimited bioregions along the South African coast (Bustamante et al. 1995; Smit et al. 2013; Teske et al. 2019). Rocky shores cover 27% of South Africa's coastal area and also contribute to mixed shore areas (Sink et al. 2012). Among rocky shore species, the Cape urchin *Parechinus angulosus* exerts decisive grazing pressure on ecosystem-engineering kelp and the granular limpet *Scutellastra granularis* contributes to grazing dynamics on the high shore (Branch, 2017). The omnivorous predator *Cyclograpsus punctatus* (shore crab) holds a vital position in the rocky shore ecosystem (Branch, 2017). Due to drastic abiotic variations on multiple scales, the intertidal is a high-stress environment for species (Stillman, 2002), which places its inhabitants at higher risk from adverse climate change impacts (Helmuth et al. 2006). Species persistence and distribution under climate change depend on physiological and genomic characteristics, yet these are unknown for the majority of species (Sanford and Kelly, 2011; Vinagre et al. 2013). The present study investigates thermal tolerance limits and the genomic potential for local adaptation across three species from different phyla and six locations across the coastline to lay the foundation for consideration in integrated cross provincial environmental management frameworks.

Selectively neutral SNPs demonstrated no distinct geographic pattern across the coastline with low to intermediate pairwise F_{ST} values across species (see section 2.3). This finding expands past studies conducted with mitochondrial markers which detected marked genetic population structure across all study species (Muller et al. 2012; Wright et al. 2015; Mmonwa et al. 2015). In this study, substantial amounts of population-specific neutral SNPs point to genomic diversity at the sampled field sites. Moreover, two nucleotide diversity indices (Tajima's π and Watterson's θ) display a significant increase on the northern west coast (Port Nolloth) in the urchin *P. angulosus*, which is an important signal for the molecular significance of this area. Further, 1102 putatively adaptive RAD-Seq loci were identified across species with three different methods (see section 3.3). The findings show that environmental differentiation in sea surface temperature (SST), salinity and air temperature appears, to varying degrees, associated with positive selection in the species genomes. Putatively adaptive loci indicated a lack of geographic population structure, which could for instance be observed in the South African sand goby (Teske et al. 2019). Overall, 69 (*C. punctatus*), 11 (*S. granularis*) and 27 (*P. angulosus*) outliers could be functionally associated with a wide range of cellular processes from membrane transport to protein folding/modification and

cytoskeleton function. Across the study species, there is evidence for possible selection on loci linked to energy cycling, which has been interpreted as response to environmental stress in past studies (see section 3.4.4.2). Compared to patterns of single marker population structure (Muller et al. 2012; Wright et al. 2015; Mmonwa et al. 2015), the study species do not indicate geographic separation into spatially separate lineages or subgroups, which could count as a positive sign for genomic and/or evolutionary resilience. It is sensible to acknowledge that geographic genomic differences between regional populations might still exist, which might only become evident with future investigations. Although the molecular findings are multi-faceted, increased nucleotide diversity in Port Nolloth could signal that the local population of *P. angulosus* possesses higher evolutionary resilience compared to its other South African populations.

Coping with changing temperature conditions is one of the key aspects determining species distribution and resilience under climate change (Helmuth, 2002; Stillman, 2019). The present study establishes upper and lower critical thermal limits (CT_{min} , CT_{max}) in the three study species across the South African coastal thermal gradient (see section 4.3). The shore crab *C. punctatus* indicates significantly higher CT_{max} at the west coast compared to the east coast, but no regional thermal tolerance differences were detected in the other two species. Calculated thermal breadth values are comparable with thermal breadth in related species, with the shore crab *C. punctatus* tolerating a notably vast temperature range. Warming and cooling tolerance might appear adequate, but this requires further investigation with peak and minimum environmental temperatures during low tide. Regional body mass differences found in *P. angulosus* and *S. granularis* corroborate previous findings of regional biomass differences in South Africa (Bustamante et al. 1995). The Cape urchin demonstrates a mass correlation for CT_{min} , CT_{max} and thermal breadth, whereas the shore crab only indicates mass correlation for CT_{min} and thermal breadth. The findings introduce the possibility that regional body mass differences might, among other factors, be associated with physiological adaptation. Combining critical thermal limits with considerations of behaviour and intertidal niche to approximate the study species physiological resilience indicates very different capacities to withstand climate change impacts (see section 4.4.7).

Climate change will likely lead to distribution shifts in South Africa. Rising temperatures on the east coast might surpass the abilities of local rocky shore species and cause decreasing population sizes or even collapse of east coast populations. It is plausible that the thermal variability found across the south coast region will attract warmer-water species to warming areas ('tropicalisation') and concentrate cool-preferring species in colder areas. Attributed to strong upwelling, the cool-temperate conditions on the west coast represent a climate change refugium for South African rocky shore species, which warrants acknowledgement in marine

management frameworks to maintain South Africa's status as one of the most biodiverse nations in the world.

References

- Branch, George, Charles Griffiths, Margo Branch, and Lynnath Beckley. 2017. *Two Oceans: A Guide to the Marine Life of Southern Africa*. Penguin Random House South Africa.
- Bustamante, Rodrigo H., George M. Branch, Sean Eekhout, Bruce Robertson, Peter Zoutendyk, Michael Schleyer, Arthur Dye, et al. 1995. "Gradients of Intertidal Primary Productivity around the Coast of South Africa and Their Relationships with Consumer Biomass." *Oecologia* 102 (2): 189–201.
- Helmuth, Brian. 2002. "How Do We Measure the Environment? Linking Intertidal Thermal Physiology and Ecology Through Biophysics." *Integrative and Comparative Biology* 42 (4): 837–45.
- Helmuth, Brian, Nova Mieszkowska, Pippa Moore, and Stephen J. Hawkins. 2006. "Living on the Edge of Two Changing Worlds: Forecasting the Responses of Rocky Intertidal Ecosystems to Climate Change." *Annual Review of Ecology, Evolution and Systematics*, 373–404.
- Mmonwa, Kolobe Lucas, Peter R. Teske, Christopher D. McQuaid, and Nigel P. Barker. 2015. "Historical Demography of Southern African Patellid Limpets: Congruence of Population Expansions, but Not Phylogeography." *African Journal of Marine Science* 37 (1): 11–20.
- Muller, Cornelius M., Sophie von der Heyden, Rauri C. K. Bowie, and Conrad A. Matthee. 2012. "Oceanic Circulation, Local Upwelling and Palaeoclimatic Changes Linked to the Phylogeography of the Cape Sea Urchin *Parechinus Angulosus*." *Marine Ecology Progress Series* 468: 203.
- Sanford, Eric, and Morgan W. Kelly. 2011. "Local Adaptation in Marine Invertebrates." *Annual Review of Marine Science* 3 (1): 509–35.
- Sink, Kerry, Stephen Holness, Linda Harris, Prideel Majiedt, Lara Atkinson, Tamara Robinson, Steve Kirkman, Larry Hutchings, Robin Leslie, and Stephen Lamberth. 2012. "National Biodiversity Assessment 2011: Technical Report. Volume 4: Marine and Coastal Component. Pretoria: South African National Biodiversity Institute." *Volume 4*: 325.
- Smit, Albertus J., Michael Roberts, Robert J. Anderson, Francois Dufois, Sheldon F. J. Dudley, Thomas G. Bornman, Jennifer Olbers, and John J. Bolton. 2013. "A Coastal Seawater Temperature Dataset for Biogeographical Studies: Large Biases between *in Situ* and Remotely-Sensed Data Sets around the Coast of South Africa." *PLOS One* 8 (12): e81944.
- Stillman, Jonathon. 2002. "Causes and Consequences of Thermal Tolerance Limits in Rocky Intertidal Porcelain Crabs, Genus *Petrolisthes*." *Integrative and Comparative Biology* 42 (4): 790–96.
- Stillman, Jonathon. 2019. "Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities." *Physiology* 34 (2): 86–100.
- Teske, Peter R., Jonathan Sandoval-Castillo, Tirupathi Rao Golla, Arsalan Emami-Khoyi,

- Mbaye Tine, Sophie von der Heyden, and Luciano B. Beheregaray. 2019. "Thermal Selection as a Driver of Marine Ecological Speciation." *Proceedings of the Royal Society B* 286 (1896): 20182023.
- Vinagre, Catarina, Marta Dias, Joana Roma, Ana Silva, Diana Madeira, and Mário S. Diniz. 2013. "Critical Thermal Maxima of Common Rocky Intertidal Fish and Shrimps - A Preliminary Assessment." *Journal of Sea Research* 81 (August): 10–12.
- Warner, Christopher J., Claudia Sobrevila, and George C. Ledec. 2016. "Protecting Biodiversity, Rehabilitating Ecosystems, and Promoting Conservation for Development." In "Making It Happen: Selected Case Studies of Institutional Reforms in South Africa". *World Bank eLibrary* 151-164
- Whitfield, Alan K., Nicola C. James, Stephen J. Lamberth, Janine B. Adams, Renzo Perissinotto, Anusha Rajkaran, and Thomas G. Bornman. 2016. "The Role of Pioneers as Indicators of Biogeographic Range Expansion Caused by Global Change in Southern African Coastal Waters." *Estuarine, Coastal and Shelf Science* 172: 138–53.
- Wright, Daniel, Jacqueline M. Bishop, Conrad A. Matthee, and Sophie von der Heyden. 2015. "Genetic Isolation by Distance Reveals Restricted Dispersal across a Range of Life Histories: Implications for Biodiversity Conservation Planning across Highly Variable Marine Environments." *Diversity and Distributions* 21 (6): 698–710.