Associations between biota and substrata on rocky seashores in temperate southern Australia

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Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed

Nathan Janetzki

Date: 21/05/2019

Abstract

Empirical fieldwork enhances our understanding of the associations between intertidal assemblages and the physical attributes of rocky substrata. Research investigating associations between intertidal biota and rock type has largely overlooked established assemblages on rock platforms and later-successional (i.e. >1 year) assemblages on boulders. Moreover, data for the hardness, temperature behaviour and mineralogy of different seashore rocks is often lacking. This makes it difficult to reliably associate these physical attributes with any rock-related differences in the structure, richness and abundances of individual taxa for rocky seashore assemblages. Here I have addressed these knowledge gaps by investigating under-studied biota and rock physical attributes for rocky seashores in temperate southern Australia.

Field-based surveys were used in Chapter 2 to quantify the hardness of seven rock types and to investigate associations between established platform assemblages and rock hardness. No general association between intertidal biota and rock hardness was identified, with most hardness-related biotic differences specific to the bioregion sampled. A boulder transplant experiment was used in Chapter 3 to investigate changes across later-successional (i.e. 5-6 year) assemblages on limestone and siltstone boulders. Limestone supported assemblages with a greater richness and taxonomic abundances than siltstone, with these biotic differences most-strongly correlated with the greater surface complexity of limestone. These results show that rock type is associated with the development of different assemblages on boulders from earliest colonisation and recruitment until the later stages of succession six years later.

The temperature behaviour, in relation to mineralogy, for boulders of six rock types was established in Chapter 4 using a common-garden experiment. This experiment showed that during sunny weather each rock type had patterns of temperature difference which consisted of warmer and cooler areas at the scale of millimetres to centimetres. The maximum temperature of boulder surfaces differed consistently between rocks, with purple and grey siltstone the hottest and white limestone and quartzite the coolest. The temperature maxima were associated with the major mineral and trace element content of rocks, with rock types with the hottest temperatures having the highest metallic content. The biological relevance of this small-scale temperature heterogeneity on boulder lower surfaces was investigated in Chapter 5. Seashore sampling showed that three snail species were strongly associated with lower-surface temperature patterns. Snails generally occupied cooler areas on boulder surfaces, with stronger associations detected on hotter versus cooler rock types. Overall, these studies contribute much to our understanding of rock physical attributes how intertidal biodiversity is associated with different rocks and their physical attributes as substratum.

Acknowledgements

Bilbo Baggins, a fresh-faced young hobbit, once excitedly shouted to the world ‘I’m going on an adventure!’ Way back in March 2014 I too was fresh-faced and excited to be embarking on an adventure of my own: starting a PhD in marine ecology. However, I soon learnt, as did young Bilbo and his kin in the cinematic masterpieces that are The Hobbit & The Lord of the Rings, adventures aren’t always what they are cracked up to be. In fact, my PhD experience at times was more akin to Frodo Baggins and Samwise Gamgee’s desperate journey to throw the ring of power into the fires of Mount Doom. I bogged the university Hilux on the beach and needed a lobster fisherman to tow it out with his tractor for a fee of $100. Two days later I bogged the same vehicle on a slightly sandy track near Robe. For the remainder of my PhD I walked any time I was within 1 km of a coastline. A rogue wave got the better of me at Enchanted Forest, with the Southern Ocean subsequently claiming the majority of my sampling equipment. The sea giveth and the sea taketh away. We got helplessly lost in dense shrubbery near Portland, and my trusty sidekick Nick tumbled down a small waterfall, as Google Earth is not an appropriate substitute for an actual map. There was also the terrifying moment I had to tell my supervisor that 30 tiles in the shower of his house we borrowed had fallen off the wall and shattered. All this was on top of the normal day-to-day challenges I faced while grappling with collecting, analysing, interpreting and communicating my research.

Each of these calamities triggered a small meltdown on my behalf. I am therefore extremely fortunate and grateful to have been supervised throughout my journey by Peter

Fairweather and Kirsten Benkendorff. Without their immense dedication, investment of time, knowledge, knowing when to support me, motivating me to push on and believing in me when I had given up hope, the submission of this thesis would never have been possible. What they saw in me back in 2014 I will never know, but their tireless efforts over these past 5 years have helped mould the scientist, and person, that I am today. I will always be indebted for what you have invested in me.

I am also indebted to my laboratory colleagues and field volunteers who have variously assisted me these past five years. First and foremost to Nick Thyer, my trusty sidekick who spent many weeks by my side in the south-east of the country poking around on rocky seashores. Thanks for putting up with me, entertaining us on the long 6-7 hour drives, getting up at 4am in the dark and freezing cold to go sampling, and for being my fishing buddy on the Beachport Jetty or Portland Breakwater when the weather made sampling seem nothing more than a pipe dream. Secondly to Josh Nitschke and Michelle Clanahan, who got roped into helping flip boulders and count snails on days of extreme summer heat. Without the two of you, that sampling would ever have happened. A big thankyou to my other field volunteers who helped out over the years including Linh Nguyen, Aidan Janetzki, Sam Davidson and Mark Barrett. To my chums in Laboratory 141, including Sasha Whitmarsh, Ryan Baring, Thomas Clarke, David Dodemaide, Hayley Jessup-Case, Clare Dicker & Nick Modra, thanks for the discussions about anything and everything, and for being a soundboard to bounce ideas off and to vent frustrations at.

My final thankyou is reserved for my family. Five years is a long investment of time, and outside of this PhD we have shared in many ups and downs. Firstly, the downs. My grandparents meant the world to me, and they were intrigued, even a little proud, that one day I might become a doctor. Sadly their time in this world passed before they could see this happen. In loving memory of Owen & Esme Janetzki and Cyril Lieschke. To my family, Mum, Dad, Paula & Aidan, thanks for your support, especially over these last five years. And finally to the moments of joy. A fiancé, Naomi Furniss. And a son, Riley James. You both mean the world to me. Naomi, thankyou for always putting Riley's needs and my wants first, you are the most selfless person I know and an amazing mother. Riley, you are the cheekiest little boy on Earth, you make your parents so proud every day. However, please learn to eat so your parents stress and anxiety can diminish. Hopefully with less things to juggle I can be at home a little more often, and we can concentrate on being the best little family we can.

Author contributions, permits & funding

The following outlines the author contributions for each data chapter or appendix:

Chapter 3. Janetzki N, Fairweather PG, Benkendorff K (2018) Assemblages on limestone and siltstone boulders diverge over six years in a primary-succession transplant experiment. Marine Ecology Progress Series 604: 21-32

Nathan Janetzki (NJ), Peter G. Fairweather (PGF) and Kirsten Benkendorff (KB) conceived and designed the study. NJ collected and analysed the data. PGF and KB advised on data analysis. NJ wrote the manuscript. PGF and KB contributed to revising the manuscript.

Appendix 5. Janetzki N, Fairweather PG, Benkendorff K (2015) Inventory of rock types, habitats, and biodiversity on rocky seashores in South Australia's two south-east marine parks. A report to the South Australian Department of Environment and Water. Flinders University, Adelaide, South Australia.

https://data.environment.sa.gov.au/Content/_layouts/15/WopiFrame.aspx?sourcedoc=/Content/Publications/INVENTORY%20OF%20ROCK%20TYPES,%20HABITATS,%20AND%20BIODIVERSITY%20ON%20ROCKY%20SEASHORES%20IN%20SOUTH%20AUSTRALIA%E2%80%99S%20TWO%20SOUTH-EAST%20MARINE%20PARKS%20(1).pdf&action=default&DefaultItemOpen=1

PGF conceived this study, NJ and KB contributed to its design. NJ collected and analysed the data. PGF and KB advised on data analysis. NJ wrote the report. PGF and KB contributed to revising the report.

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Thesis glossary

Hardness class: Rocks may be assigned to one of two hardness classes based on their Moh's scratch hardness value. The soft class encompasses all rocks with a Moh's scratch hardness ≤4, while the hard class encompasses all rocks with a Moh's scratch hardness >4.

Rock: specific term for the identity of the material making up a boulder or platform (see examples below). The rocks investigated in this thesis include: calcarenite; Mount Gambier limestone; fossiliferous limestone; siltstone; felsic volcanic rock; Kanmantoo schist; basalt; flint; grey siltstone; purple siltstone; quartzite; white fossiliferous limestone; orange fossiliferous limestone; and fossiliferous sandstone.

Rock type: Describes any typology (e.g. could be based on colour or hardness or temperature behaviour, etc.) for the different geological rocks sampled in this thesis. This general term has been applied by rocky seashore ecologists to describe the rocks that constitute the seashore since at least McGuiness & Underwood's (1986) research comparing shale versus sandstone. This identity of any rock includes what specific values of all the other physical attributes have been described for it.

Seashore: Describes the specific intertidal rock platform or boulderfield where field surveys or transplant experiments were completed. A total of 18 seashores were investigated in this thesis.

Chapter 1

General introduction

Rocky seashores, located at the interface of the land and sea, constitute the areas of coastline exposed to air during the lowest tides, extending up to a level on the seashore that is periodically submerged underwater during the highest tides or reached by the spray of waves (Raffaelli and Hawkins 1996; Garcia and Smith 2013). Consequently, the biota that use rocky seashores as habitat, which include algae, molluscs, crustaceans, echinoderms, sea anemones, sponges, ascidians, and polychaetes are subjected to alternating aquatic (high tide) and aerial (low tide) climatic regimes (Helmuth et al. 2006a). This makes rocky seashores one of the most stressful and variable habitats on Earth, especially at low tide when any biota of marine origin must contend with the terrestrial environment (Raffaelli and Hawkins 1996; Helmuth and Hofmann 2001; Helmuth et al. 2006a). Besides habitat-related stress, intertidal biota are subjected to a multitude of anthropogenic stressors including habitat modification or loss (Martins et al. 2009; Chapman and Underwood 2011; Green et al. 2012), being trampled, physically disturbed, crushed (Keough et al. 1993; Underwood 1993; Alexander and Gladstone 2013) or exploited as food or bait (Underwood 1993; Cooling and Smith 2015; Coppa et al. 2015). With concerns already raised for the survival and persistence of some intertidal species (Madeira et al. 2012; Coppa et al. 2015), ecologists and managers must seek to better understand vulnerable intertidal taxa and assemblages to ensure their long-term management and conservation.

Such an understanding requires a baseline knowledge of the assemblages on rocky seashores that establishes how their structure, richness and abundance is associated with the complex interactions between abiotic and biotic characteristics of the seashore (Dayton 1971; Menge 1976; Chapman 2002a). Developing this baseline is made all the more difficult by rocky seashores not being equal in terms of their abiotic characteristics such as wave exposure, salinity gradients or biogeographic setting, nor the biological assemblages that they support. The type of rock that comprises the seashore represents one abiotic characteristic that differs between seashores and may be associated with the intertidal biota inhabiting them. However, many larger-scale studies of biotic spatial variation on rocky substrata seem to have overlooked the role of rock type (e.g. O'Riordan et al. 2004; Bertness et al. 2006), despite strong evidence of its importance from many smaller-scale studies (e.g. McGuinness and Underwood 1986; Raimondi 1988). These smaller-scale studies have variously reported specific differences in assemblage structure, species richness, and the abundances of individual taxa from comparisons of different rock types (Table 1.1). However, see Caffey (1982), Burt et al. (2009), and Cox et al. (2013) as points of contrast (Table 1.1).

Table 1.1: Summary of studies discussed in Chapter 1 that investigate associations between marine biota and rock type. For each study, the rocks compared, study location, substrata assessed, biotic variables measured (* denotes a significant rock-type difference), and the key findings are reported. Literature has been reviewed up to April 4, 2019, with the keywords searched for this review including rock, rock type, substrate, intertidal, rugosity, microhabitat, temperature, mineralogy, hardness, species richness, abundance, and structure.

Rocks compared	**Study location & substrata assessed**	**Biotic variable**	**Key findings**	**Reference**
Limestone, chalk	Southern England, intertidal rocky reef	Abundance*	Higher abundances of barnacles *Chthamalus stellatus* & *Balanus balanoides* on limestone versus chalk.	Moore & Kitching 1939
Andesite, basalt, limestone	Hawaii, Guam, Palau & Singapore intertidal rocky reef	Abundance*	High intertidal neritids & limpets showed species specific preferences for volcanic (andesite, basalt) or limestone seashores. Littorinids, planaxids, low intertidal neritids and most lower intertidal limpets displayed no rock preference.	Vermeij 1971a
Shale, sandstone, mudstone, gabbro	NSW Australia, intertidal plates cut from boulders	Abundance	No differences in recruitment for the barnacle *Tessoropora rosea* on shale, sandstone, mudstone,& gabbro.	Caffey 1982
Sandstone, shale, laterite	NSW, intertidal boulders	Abundance*	Green alga *Ulva lactuca* was more abundant on sandstone versus shale or laterite; spirorbid worms were more abundant on shale or laterite versus sandstone.	McGuinness & Underwood 1986
Granite, basalt	North-west Mexico, intertidal rocky reef & stones	Abundance*	Higher recruitment of barnacle *Chthamalus anisopoma* on granite versus basalt.	Raimondi 1988; 1990
Granite, limestone	South Island, New Zealand, subtidal rocky reef	Abundance*, assemblage structure*	Rock-related assemblage differences, with limestone characterised by foliose red algae & brown macroalgae, & granite characterised by crustose coralline algae; abundance of grazing limpets & gastropods was higher on granite versus limestone.	Davidson & Chadderton 1994
Shale, sandstone	NSW, intertidal boulders	Abundance*	Recruitment of spirorbid worms was higher on shale versus sandstone.	James & Underwood 1994

Rocks compared	**Study location & substrata assessed**	**Biotic variable**	**Key findings**	**Reference**
Quartz, marble, basalt, adensite, dolerite, sandstone, limestone, schist, gneiss, larvikite, slate	South-west Scotland, subtidal plates cut from natural rock & polished	Abundance*	Higher recruitment of barnacle *Balanus balanoides* on slate, quartz, marble & basalt versus all other rocks tested; lower recruitment on sandstone & gneiss versus all other tested rocks.	Holmes et al. 1997
Serpentinite, basalt, gabbro, sandstone	Italy, subtidal rocky reef	Abundance*, species richness*	Basalt and sandstone had the greatest algal space occupancy, serpentinite and gabbro had the greatest algal richness. Rock-related abundance differences detected for red algae (highest on sandstone), brown algae (highest on basalt) & sessile invertebrates (highest on gabbro).	Cattaneo-Vietti et al. 2002
Granite, limestone	North-east Sardinia, subtidal rocky reef	Abundance*	The serranid *Serranus scriba* was more abundant on granite than limestone, while *S. cabrilla* was more abundant on limestone than granite.	Guidetti & Cattaneo-Vietti 2002
Amphibolite, diabase, porphyry, gneiss, granite, sandstone	Sweden, subtidal rocky reef	Abundance*	Recruitment of the brown alga *Fucus vesiculous* was significantly higher on alkaline (amphibolite & diabase) than acidic (gneiss, granite, porphyry & sandstone) rocks. Plant density and biomass was always greatest on sandstone.	Malm et al. 2003
Granite, limestone	North-east Sardinia, subtidal rocky reef	Abundance*	Sessile gastropod *Dendropoma petraeum* was more abundant on granite versus limestone, while *Vermetus triquetrus* displayed no rock differences; erect macroalgae had a greater percentage cover on limestone versus granite.	Schiaparelli et al. 2003
Granite, limestone	North-east Sardinia, subtidal rocky reef	Abundance*, assemblage structure*, species richness*	Algal mats had lower species richness but greater percentage cover on granite versus limestone. Fish assemblage structure differed between granite & limestone.	Guidetti et al. 2004
Cementstone, limestones, chalk	Southern England, intertidal tiles cut from boulders	Abundance*	Lower recruitment of barnacle *Chthamalus montagui* on cementstone versus chalk & two types of limestone.	Herbert & Hawkins 2006
Sandstone, gabbro, granite	United Arab Emirates, subtidal plates cut from natural rock.	Assemblage structure	No differences in assemblage structure for coral, algae & ascidians on plates of sandstone, gabbro, granite & several artificial substrata.	Burt et al. 2009
Sandstone, volcanic rock	Argentina, intertidal rocky reef	Abundance*	Barnacle *Balanus glandula* had a greater density on sandstone versus volcanic rock.	Savoya & Schwindt 2010

Rocks compared	Study location & substrata assessed	Biotic variable	Key findings	Reference
Basalt, sandstone	NSW, intertidal boulders	Abundance*, assemblage structure*	Rock-related assemblage differences, with basalt having a greater abundance of oysters & barnacles than sandstone, & sandstone having a greater abundance of turf-forming algae, foliose algae, & tubeworms than basalt.	Green et al. 2012
Basalt, limestone	Hawaii, intertidal reef	Assemblage structure	No differences in the structure of mixed algal and invertebrate assemblages between basalt & granite reefs.	Cox et al. 2013
Granite, basalt, quartzite, limestone	South Australia, intertidal boulders	Abundance*, assemblage structure*, species richness	Rock-related assemblage differences, with granite, basalt, & quartzite boulders characterised by a higher abundance of common chiton species versus limestone; chiton species richness did not differ among rocks.	Liversage & Benkendorff 2013
Siltstone, limestone	South Australia, intertidal boulders	Abundance*, assemblage structure*	Rock-related assemblage differences, with siltstone having higher abundances of gastropods *Nerita atramentosa* & *Notoacmea* spp, & lower abundances of crab *Ozius truncatus* versus limestone.	Liversage et al. 2014
Basalt, conglomerate	Sicily, subtidal rocky reef	Abundance*, species richness*	Algae & mollusc richness & abundance were greater on basalt versus conglomerate substrata.	Cosentino & Giacobbe 2015
Dolostone, limestone, phosphorite	Puerto Rico, intertidal reef	Assemblage structure, abundance*	No differences in the endolithic assemblage between rocks. Species-specific rock preferences for some cyanobacteria identified.	Couradeau et al. 2017
Serpentinite, metagabbros	Italy, intertidal & subtidal rocky reef	Abundance*, species richness*	Serpentinite had a higher algal richness and abundance of the barnacle *Chthamalus stellatus* versus metagabbros. Individual alga spp. had higher space occupancies on one or the other tested rocks.	Bavestrello et al. 2018
Granite, limestone	South-west Western Australia, intertidal reef & boulders	Abundance*, assemblage structure*, species richness*	Granite and limestone reefs supported distinct mixed algal and invertebrate assemblages. Granite reefs had a higher invertebrate richness and abundance than limestone reefs.	Bessey et al. 2018
Pelitic mudstone, limestone, conglomeradic subulitic sandstone, grey sandstone, volcanic rock	Argentina, intertidal tiles cut from rocky reef	Abundance*	Higher recruitment of oyster *Crassostrea gigas* on pelitic mudstone versus the other four rocks investigated.	Carrasco et al. 2019

Rock-related differences in assemblage structure have been identified for mixed algal and invertebrate assemblages (Green et al. 2012; Bessey et al. 2018) and mollusc assemblages (Liversage and Benkendorff 2013; Liversage et al. 2014) from intertidal reefs in Australia. On subtidal reefs, rock-related differences have been identified for algal and grazer assemblages from New Zealand's South Island (Davidson and Chadderton 1994) and for epibenthic and fish assemblages from Italy (Guidetti and Cattaneo-Vietti 2002; Guidetti et al. 2004) (Table 1.1). For species richness, rock-related differences have been identified intertidally for invertebrates from Western Australia (Bessey et al. 2018), from Italian intertidal and subtidal reefs for algae (Cattaneo-Vietti et al. 2002; Guidetti et al. 2004; Bavestrello et al. 2018) and subtidally for algae and molluscs from Sicily (Cosentino and Giacobbe 2015). Rock-related differences in abundance for individual taxa have been identified for a variety of barnacle species from around the world (Moore and Kitching 1939; Raimondi 1988; 1990; Holmes et al. 1997; Herbert and Hawkins 2006; Savoya and Schwindt 2010; Bavestrello et al. 2018). Furthermore, rock-related differences in abundance have been identified from intertidal boulderfields for green algae and spirorbid tubeworms (McGuinness and Underwood 1986) and molluscs (Liversage and Benkendorff 2013; Liversage et al. 2014), and from intertidal and subtidal reefs for a variety of algae and invertebrate species (Vermeij 1971a; Cattaneo-Vietti et al. 2002; Malm et al. 2003; Cosentino and Giacobbe 2015; Bavestrello et al. 2018; Bessey et al. 2018; Carrasco et al. 2019) (Table 1.1).

To date, much of our understanding about the associations between rock type and intertidal biota has been derived from early-successional (i.e. <1 year old) assemblages that developed on either initially bare (e.g. McGuinness and Underwood 1986; Green et al. 2012; Liversage et al. 2014) or seasoned (e.g. Chapman 2007) transplanted boulders, or for established assemblages on subtidal reefs (e.g. Davidson and Chadderton 1994; Guidetti et al. 2004; Cosentino and Giacobbe 2015). Consequently, little is known about any such association for later-successional (i.e. >1 year old) assemblages on transplanted boulders, or for established assemblages from other intertidal habitats, including rock platforms. A baseline understanding of the abiotic factors influencing intertidal biota is paramount for their effective management (Chapman 2005; Bessey et al. 2018), and research that investigates associations between understudied intertidal assemblages and rock type will go some way towards establishing this baseline.

Some studies have also investigated whether any rock-related biotic patterns are associated with some specific physical attributes of the rocks investigated (Table 1.2). However, relatively few studies have quantified, and thus compared, the physical attributes of multiple seashore rock types. Consequently, our understanding of the associations between biota and rock physical attributes is derived largely from studies on a single seashore investigating a single rock type or artificial substratum only (Table 1.2). Some of the rock physical attributes that have been investigated include colour (James and Underwood 1994), the presence of micro-habitats (McGuinness and Underwood 1986; Moreira et al. 2007; Chapman and Underwood 2011; Liversage et al. 2014), surface rugosity (Raimondi 1990; Chapman and Underwood 1994; Herbert and Hawkins 2006; Liversage et al. 2014; Loke and Todd 2016), mineralogy (Amor et al. 1991; Cerrano et al. 1999; Bavestrello et al. 2000; Cerrano et al. 2007; Pozzolini et al. 2010), surface temperature (Raimondi 1988; Marshall et al. 2010), hardness (Evans 1968; Thomsen et al. 2004; Liversage and

Benkendorff 2013; Bagur et al. 2014; Liversage et al. 2014; Gutiérrez et al. 2018; Russell et al. 2018), and porosity (Carrasco et al. 2019) (Table 1.2).

Not all physical attributes of seashore rocks have been investigated equally in the published literature. The surface rugosity of different substrata has been well documented, and the biotic associations with differences in surface rugosity established (see Raimondi 1990; Berntsson et al. 2000; 2004; Herbert and Hawkins 2006; Savoya and Schwindt 2010) (Table 1.2). However, there remains a lack of empirical data quantifying rock-related differences in mineralogy, hardness, or surface temperature (Table 1.2).

Our understanding of rock temperature characteristics is bolstered by studies that describe a single rock from a single location (Table 1.2); however, this information cannot be used to discern how rock differences in surface temperature may affect intertidal biota. One approach increasingly employed by ecologists to measure the surface temperature of rocky substrata, and of intertidal biota inhabiting rocky substrata, is thermal imagery (e.g. Caddy-Retalic et al. 2011; Chapperon and Seuront 2011a; b; Cox and Smith 2011;). Thermal imagery measures mid to long-wave infrared radiation, creating a thermograph (or thermal image) where the radiation emitted, transmitted, and reflected by objects are shown as temperature (Tattersall 2016). How an object emits, transmits and reflects radiation is dependent upon a number of factors including composition, surface colour, surface complexity, and specific emissivity (Tattersall 2016; Seuront et al. 2018). Darker-coloured objects absorb more radiation than lighter-coloured objects, with darker-coloured objects attaining hotter temperatures than lighter-coloured objects (Marshall et al. 2010; Judge et al. 2011). The composition of an object will often determine its colour, such as mineral composition affecting the colour of rocks. Specific emissivity, the ability of an object to emit thermal radiation is also important, with an objects emissivity independent of colour, ranging between 0 (a perfect reflector) and 1 (a blackbody or prefect absorber), with all objects in nature neither a perfect reflector or absorber (Tattersall 2016; Seuront et al. 2018). Water content also affects an objects emissivity as water is an excellent emitter of infrared radiation (Seuront et al. 2018). In intertidal research, where object surfaces are often wet or damp, this may lead to an underestimation of surface temperature, as a wet surface emits more infrared radiation than if the surface was dry, especially in sunlight (Seuront et al. 2018). To overcome any caveats from measuring the surface temperature of damp or wet objects, it is recommended to temporarily shade the area being imaged on the seashore (Lathlean et al. 2012; Lathlean and Seuront 2014; Seuront et al. 2018).

Other than the recent work of Bavestrello et al. (2018), few studies detail the major mineral and trace element composition of seashore rocks, with most studies of substratum mineralogy focusing on the content of silica and carbonate minerals in rocks (Bavestrello et al. 2000; Cerrano et al. 2007; Pozzolini et al. 2010). Furthermore, for hardness, most published investigations have confounded rock hardness with rock type by not replicating rock types within hardness classes (Table 1.2). Until rock-related differences for each of these physical attributes are better known, it remains difficult to reliably associate these attributes with rock-related biotic differences. Therefore, observational studies and experimental manipulations are needed to better understand rocky seashore assemblages and to quantify some of the under-studied physical attributes of seashore rocks. Only then will it be possible to establish how biota in the rocky intertidal zone are associated overall with rock physical attributes.

Table 1.2: Summary of studies discussed in Chapter 1 that investigate associations between marine biota and rock physical attributes. For each physical attribute, the substrata tested & the key findings are reported. Literature has been reviewed up to April 4, 2019, with the keywords searched for this review including rock, rock type, substrata, intertidal, rugosity, microhabitat, temperature, mineralogy, hardness, species richness, abundance, and structure.

Substratum characteristic	**Substrata tested**	**Key findings**	**Reference**
Colour	Shale & sandstone boulders	Higher recruitment of spirorbid worms to naturally-dark shale & dark-painted sandstone versus naturally-light sandstone & light-painted shale.	James & Underwood 1994
Surface temperature	Basalt & granite reefs & stones	Basalt was hotter than granite. This temperature difference was associated with a higher vertical limit & recruitment of the barnacle *Chthamalus anisopoma* on granite versus basalt.	Raimondi 1988
	Granite reefs	Highest mortality of limpet *Lottia scabra* was associated with areas of granite with the hottest surface temperatures.	Harley 2008
	Sandstone reefs	Darker-coloured ferruginous sandstone was hotter than lighter-coloured sandstone. Models of the snail *Echinolittorina malaccana* had higher body temperatures on darker than lighter coloured sandstone.	Marshall et al. 2010
	Exposed rock, lithology not specified	Black rock was hotter than white rock.	Judge et al. 2011
	Siltstone reefs	Higher recruitment & first-week post-settlement growth for barnacle *Tessoropora rosea* was associated with areas of siltstone with the coolest surface temperatures.	Lathlean et al. 2013
Microhabitat presence	Artificial concrete blocks	Addition of pit & groove microhabitats on the underside of concrete increased the richness of sessile fauna assemblages; addition of pit microhabitats on the upper-side of concrete increased the abundance of the snails *Bembicium nanum* & *N.atramentosa,* which used these pits for shelter.	McGuinness & Underwood 1986
	Sandstone reefs & artificial seawalls	Abundance of chiton *Sypharochiton pelliserpentis* was higher in crevice microhabitats between sandstone blocks than on exposed surfaces; chitons were observed to return to crevice microhabitats during translocation experiments.	Moreira et al. 2007
	Sandstone reefs & artificial seawalls	Pit & groove microhabitats on sandstone blocks were not associated with the species richness or total abundance of the invertebrate assemblage sampled.	Chapman & Underwood 2011
	Siltstone & limestone boulders	Fossiliferous limestone had more crack & depression micro-habitats >13 mm than siltstone. Rock-related assemblage differences were not associated with these physical differences.	Liversage et al. 2014
Rugosity	Quartz stones	Barnacle *Chthamalus anisopoma* only recruited to naturally rough quartz. No recruitment whatsoever was recorded on artificially smooth quartz.	Raimondi 1990

Substratum characteristic	**Substrata investigated**	**Key findings**	**Reference**
Rugosity	Sandstone reefs	Snail *Nodilittorina pyramidalis* was observed to return to complex areas of substratum when translocated onto areas of flat substratum.	Chapman & Underwood 1994
	Artificial plexiglas panels	Recruitment of barnacle *Balanus improvisus* was 82 % higher on smooth versus rough panel surfaces; cyprids spent more time swimming & less time exploring smooth versus rough surfaces.	Berntsson et al. 2000
	Artificial Polymethyl-methacrylate panels	Recruitment of barnacle *Balanus improvisus* was higher on smooth versus rough panel surfaces.	Berntsson et al. 2004
	Boulders (rock type unspecified)	Macrofaunal richness, abundance, & biomass were highest in areas with the greatest substratum complexity.	Le Hir and Hily 2005
	Cementstone, limestone & chalk plates cut from boulders	Higher recruitment of barnacle *Chthamalus montagui* was recorded on tiles with the roughest surface texture.	Herbert & Hawkins 2006
	Sandstone, commercial tiles (composition not specified)	Recruitment & survival of barnacle *Balanus glandula* was highest on rough versus smooth surfaces.	Savoya & Schwindt 2010
	Sandstone reefs	Invertebrate species richness was highest on platforms with the greatest substratum complexity.	Meager & Schlacher 2013
	Natural basalt seashores & artificial (basalt or concrete) seawalls	Species-specific associations with surface rugosity; some species had higher abundances on smooth-textured substrata while others had higher abundances on rough-textured surfaces.	Cacabelos et al. 2016
	Artificial concrete tiles	Concrete tiles with a greater surface complexity supported a higher algal & invertebrate species richness & abundance versus tiles with simpler surfaces.	Loke & Todd 2016
Mineralogy	Sedimentary rock (type not specified)	The bivalve *Pholadidea darwini* was generally absent from harder rock samples with the highest calcite content.	Amor et al. 1991
	Carbonatic & quartzite sediments	Abundance of zoobenthos decreased as the proportion of quartz in sediments increased.	Cerrano et al. 1999
	Carbonatic & quartzite sediments	Abundance of hydroid *Eudendrium glomeratum was* five times higher on carbonatic versus quartzite sediments.	Bavestrello et al. 2000
	Carbonatic & quartzite sediments	Growth of the sponge *Cliona nigricans* was inhibited in quartzite sediments, with the greatest size & mass reported from specimens buried in carbonatic sediments.	Cerrano et al. 2007
	Quartz & marble	Primmorphs of the sponge *Petrosia ficiformis* grew thicker on quartz versus marble discs, with quartz having a positive influence on sponge metabolism has shown by the greater production of the silicate in beta gene.	Pozzolini et al. 2010

Substratum characteristic	Substrata investigated	Key findings	Reference
Hardness	Greywacke sandstone, arkosic sandstone & metagreywacke	Rock hardness influenced the shape and angle of burrows that the rock-boring clam *Penitella penita* bored into the substratum.	Evans 1968
	Sandstone, granite & limestone	The kelp *Ecklonia radiata* was significantly smaller and more-strongly attached to the substratum on harder sandstone and granite than softer limestone.	Thomsen et al. 2004
	Sandstone & calcrete	The density of endolithic invertebrates was greater in calcrete (harder) than sandstone (softer).	Bagur et al. 2014
	Basalt, granite, quartzite & limestone boulders	Hardness-related assemblage differences detected; common chiton species had higher abundances on hard (basalt, granite & quartzite) versus soft (limestone) rocks.	Liversage & Benkendorff 2013
	Siltstone & limestone boulders.	Hardness-related assemblage differences detected; hard rocks (siltstone) supported higher *Nerita atramentosa* & *Notoacmea* spp abundances, & lower *Ozius truncatus* abundances, versus soft rocks (limestone).	Liversage et al. 2014
	Pampean loess & Ordovicic orthoquartzite	The mussel *Brachidontes rodriguezii* increased byssus production to compensate for increased potential of substratum failure when attaching to softer Pampean loess.	Gutiérrez et al. 2018
	Mudstone, fine-grained sandstone, coarse-grained sandstone, granite	Hardness-related differences in the rate of pit formation detected for the sea urchin *Stronglyocentrotus purpuratus.* Pit formation, and thus bioerosion, was faster on softer mudstone and sandstone than harder granite.	Russell et al. 2018
Porosity	Plates of pelitic mudstone, limestone, conglomeradic subulitic sandstone, grey sandstone, volcanic rock	Highest recruitment of oyster *Crassostrea gigas*was recorded on the rock type with the highest porosity, pelitic mudstone.	Carrasco et al. 2019

Thesis aim and objectives

Figure 1.1 provides a schematic of the overall aim and specific objectives for this thesis. The main objectives are to:

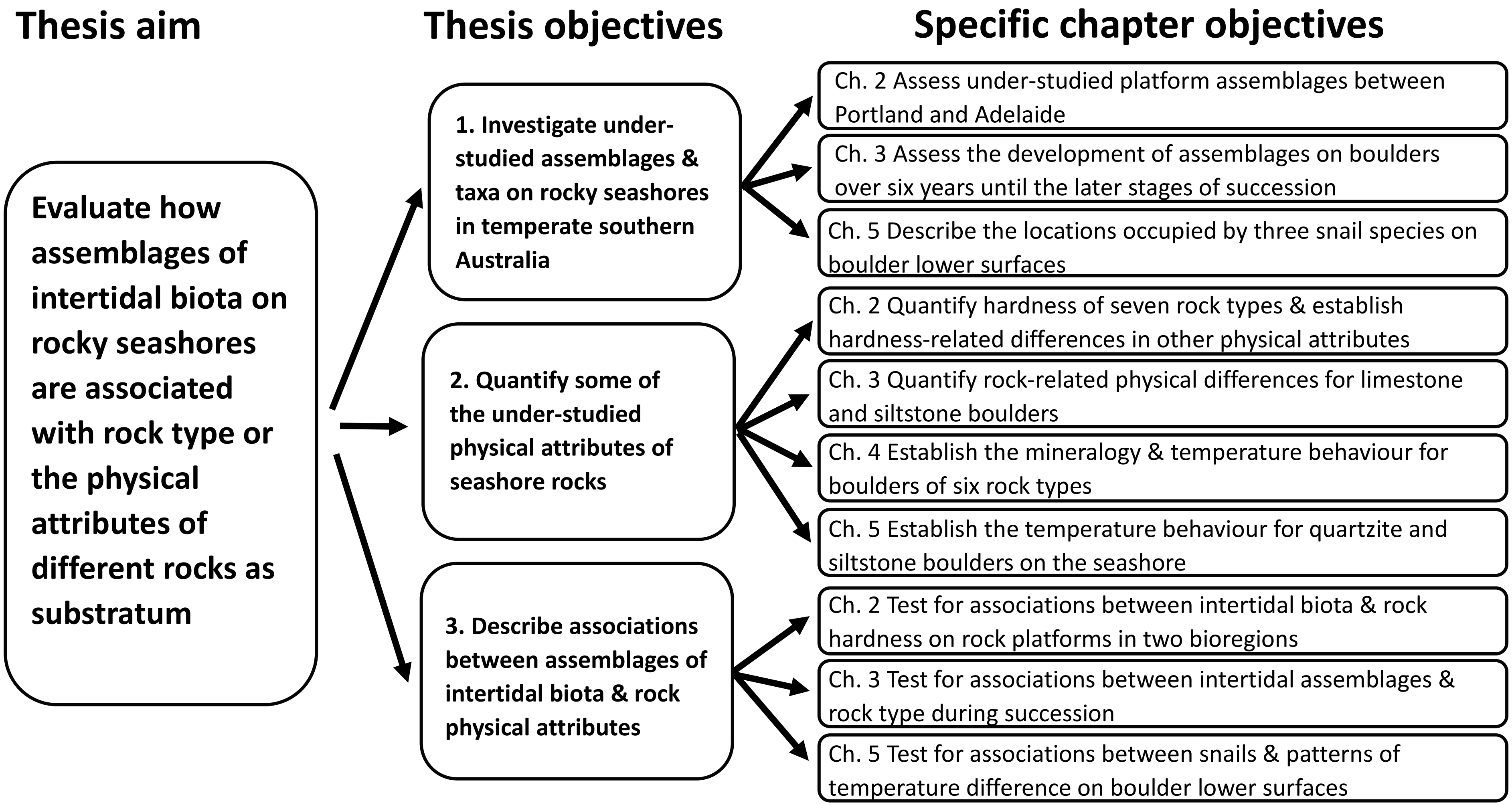
1) enhance our understanding of under-studied assemblages and taxa on rocky seashores in temperate southern Australia;

2) investigate some of the under-studied physical attributes, especially hardness, surface temperature and mineralogy of seashore rocks; and

3) determine the associations between assemblages of intertidal biota & rock physical attributes.

I present here a series of different studies to achieve my thesis aim. Thesis data chapters have been designed to address one or more thesis objectives, with the contribution of each chapter, and its specific objectives, outlined in Figure 1.1. To address the first objective I investigate and describe: a) established platform assemblages between Portland (Victoria) and Adelaide (South Australia, SA), a region of temperate southern Australia that has been seldom studied (Chapter 2 & Appendix 5); and b) later-successional assemblages developing on boulders transplanted over 6 years along SA's Fleurieu Peninsula (Chapter 3). To address the second objective I quantify some of physical attributes of seashore rocks, including several that have been under-studied. These include hardness (Chapter 2), surface rugosity (Chapters 2 &3), microhabitat density (Chapters 2 & 3), surface temperature (Chapters 4 & 5) and mineralogy (Chapters 2 & 4). To address the third objective I test whether established platform assemblages (Chapter 2), developing later-successional assemblages on boulders

(Chapter 3), and three snail species underneath boulders (Chapter 5) are associated with rock physical attributes. Each of these data chapters contribute to the principal aim (Figure 1.1) of this thesis by evaluating whether assemblages of intertidal biota are associated with rock type or the physical attributes of different rocks as substrata.



Thesis aim

Evaluate how assemblages of intertidal biota on rocky seashores are associated with rock type or the physical attributes of different rocks as substratum

Thesis objectives

1. Investigate under-studied assemblages & taxa on rocky seashores in temperate southern Australia

2. Quantify some of the under-studied physical attributes of seashore rocks

3. Describe associations between assemblages of intertidal biota & rock physical attributes

Specific chapter objectives

Ch. 2 Assess under-studied platform assemblages between Portland and Adelaide

Ch. 3 Assess the development of assemblages on boulders over six years until the later stages of succession

Ch. 5 Describe the locations occupied by three snail species on boulder lower surfaces

Ch. 2 Quantify hardness of seven rock types & establish hardness-related differences in other physical attributes

Ch. 3 Quantify rock-related physical differences for limestone and siltstone boulders

Ch. 4 Establish the mineralogy & temperature behaviour for boulders of six rock types

Ch. 5 Establish the temperature behaviour for quartzite and siltstone boulders on the seashore

Ch. 2 Test for associations between intertidal biota & rock hardness on rock platforms in two bioregions

Ch. 3 Test for associations between intertidal assemblages & rock type during succession

Ch. 5 Test for associations between snails & patterns of temperature difference on boulder lower surfaces

Figure 1.1: Schematic depicting the overall aim & three main objectives of this thesis with the contribution of each chapter to those objectives and overall thesis aim.

Thesis structure

This thesis is comprised of six chapters, which include a general introduction (Chapter 1), four data chapters (Chapters 2-5) and a general discussion (Chapter 6). A pilot study that was published as a report to government during my PhD is also available as Appendix 5. Although this pilot study is not formally part of my thesis, it is relevant to my thesis aim because it describes under-studied assemblages on three rock types in south-east SA.

Chapter 1 is a review of the published literature. It is intentionally brief to avoid repetition of information presented in the introduction to each data chapter (Chapters 2-5) and detailed summaries of the published literature can be found in the summary tables (Tables 1.1 & 1.2).

Chapter 2 is an observational study that describes associations between established platform assemblages and rock hardness in two marine bioregions in temperate southern Australia. This chapter also quantifies some other hardness-related differences in rock physical attributes including mineralogy, microhabitat density and surface rugosity.

Chapter 3 is an experimental study that examines associations between later-successional assemblages and rock type for limestone and siltstone boulders transplanted over six years. This chapter also quantifies the surface area, microhabitat density and surface rugosity of each rock type to determine whether rock-related physical differences are associated with rock-related biotic patterns. This chapter was published in Marine Ecology Progress Series in October 2018.

Chapter 4 describes a common-garden experiment that was used to quantify the temperature behaviour and mineralogy of local seashore rock types. Data on the temperature behaviour and major mineral and trace element composition of six rock types are presented.

Chapter 5 is an observational study that describes associations between three snail species and patterns of temperature difference on boulder lower surfaces for two rock types during low tide.

Chapter 6 is a synthesis of the outcomes from Chapters 2-5. This general discussion summarises the implications of my research, describing the knowledge gaps that have been addressed and identifying key areas for future research.

Appendix 5 is a pilot study describing baseline inventories for rocky seashores in two marine parks completed for the SA government Department of Environment and Water. These inventories describe under-studied assemblages for seashores comprised of three rock types.

Chapter 2

Lack of general associations between intertidal assemblages and rock hardness

Abstract

Different assemblages of intertidal biota may be associated with the hardness of the rock that comprises the seashore. However, because most published studies investigating rock hardness are confounded by a limited selection of rocks from different hardness classes, it is difficult to reliably associate assemblages of intertidal biota with rock hardness. To remedy this, I investigated the physical attributes and biotic assemblages on 12 rock platforms comprised of seven rock types from soft or hard classes. Soft versus hard rocks differed physically, with established platform assemblages showing few general differences between hardness classes. Most hardness-related biotic differences were specific to the rocks sampled in each marine bioregion. Hardness-related assemblage differences were only weakly-to-moderately correlated with hardness differences in mineralogy or microhabitat density. The detection of bioregion-specific hardness differences for intertidal assemblages, rather than general hardness class trends, indicate that the type of rock comprising the seashore in each bioregion may be more strongly associated with the biotic patterns identified than hardness *per se*.

Introduction

Intertidal biota are influenced by the complex interactions of a number of biological and physical features, operating across a hierarchy of different spatial and temporal scales (Dayton 1971; Menge 1976; Chapman 2002a). By untangling some of these complex interactions it has been possible to better understand species distribution and abundance (e.g. Moore and Kitching 1939), recruitment success (e.g. Berntsson et al. 2000), growth rate (e.g. Cerrano et al. 2007), survival and persistence (e.g. Raimondi 1988) and overall assemblage composition (e.g. Liversage and Benkendorff 2013) in the rocky intertidal zone. Because of sessile or sedentary nature of biota on the rocks, the type of rock that comprises the seashore is one physical feature that influences intertidal organisms. Field-based observational studies and experimental manipulations have variously reported differences in the abundances of individual taxa (e.g. Moore and Kitching 1939; McGuinness and Underwood 1986; Raimondi 1988), taxon richness (e.g. Cattaneo-Vietti et al. 2002; Guidetti et al. 2004; Bavestrello et al. 2018), and assemblage composition (e.g. Davidson and Chadderton 1994; Guidetti et al. 2004; Liversage and Benkendorff 2013) from comparisons among different rock types.

Many of these rock-related biotic differences have been attributed to specific physical attributes of the rocks investigated. To date, physical attributes that have been associated with rock-type differences in marine assemblages include: rugosity (Raimondi 1990; Chapman and Underwood 1994; Berntsson et al. 2000; Le Hir and Hily 2005; Herbert and Hawkins 2006; Savoya and Schwindt 2010), the presence of micro-habitats (McGuinness and Underwood 1986; Chapman 2000; Moreira et al. 2007; Chapman and Underwood 2011), mineralogy (Bavestrello et al. 2000; Schiaparelli et al. 2003; Cerrano et al. 2007; Pozzolini et al. 2010), substratum colour (James and Underwood 1994), surface temperature (Raimondi 1988), and porosity (Carrasco et al. 2019).

Another physical attribute that can differ among rocks, and may therefore be associated with biotic differences, is hardness. Rocks may be classed as either ‘soft’ or ‘hard’ based on their hardness (Evans 1968). Hardness, the measure of a rock's or mineral's resistance to being scratched, can be measured using Moh's scale of scratch hardness, which arranges 10 minerals in increasing order of scratch hardness, from talc (1 = softest) to diamond (10 = hardest) (Tabor 1954; 1956). Using this scale, the soft class encompasses all rocks with a scratch hardness ≤4 and includes a variety of sedimentary and metamorphic rocks such as chalk, calcarenite, limestone and some sandstones (Herbert and Hawkins 2006; Liversage and Benkendorff 2013). In contrast, the hard class encompasses all rocks with a scratch hardness >4 using Moh's scale, and includes a variety of metamorphic, igneous and some sedimentary rocks such as gneiss, basalt, granite, quartzite, some sandstones and some siltstones (Glindemann et al. 2005; Liversage and Benkendorff 2013).

Rock hardness has been associated with greater abundances of some endolithic invertebrates in hard than soft rock (Bagur et al. 2014), a greater algal biomass and density on soft than hard rock (Malm et al. 2003), increased byssus production by mussels on soft than hard rock (Gutiérrez et al. 2018), stronger holdfast attachment for kelp on hard than soft rock (Thomsen et al. 2004), a faster rate of pit formation by sea urchins on soft than hard rock (Russell et al. 2018) and burrow morphology in clams (Evans 1968). What currently remains poorly understood is whether rock hardness may be associated with the overall composition of the benthic marine plant and invertebrate assemblages inhabiting rocks of differing hardness. Some evidence of assemblage differences has emerged for invertebrates under boulders composed of soft versus hard rock (Liversage and Benkendorff 2013; Liversage et al. 2014). However, as both of these studies confounded rock hardness with rock type *per se* by only investigating a single type of soft rock (i.e. limestone), it remains difficult to reliably associate rock hardness with the biotic patterns identified.

To remedy this knowledge gap, twelve seashores comprised of seven rock types of differing hardness were sampled. Specifically, this study aimed to quantify whether the structure or richness of assemblages, or abundances of individual taxa, differed between hardness classes for mixed marine plant and invertebrate assemblages. Seashores from two marine bioregions were sampled, as distinct biogeographical regions might show different biotic associations with rock hardness. This study also measured some of the other physical attributes of these rocks (i.e. rugosity, microhabitat density, mineralogy) to quantify how they differed between hardness classes, and to determine how these physical attributes may be associated with any hardness-related biotic patterns. This allowed me to address the following three null hypotheses (H1-H3):

H1) the physical attributes of rocky seashores will not differ between hardness classes in each marine bioregion;

H2) intertidal assemblages will not differ between hardness classes in each marine bioregion; and

H3) associations between intertidal assemblages and hardness classes are not correlated with any differences in physical attributes.

Materials & methods

Sampling locations

Sampling was completed at 12 rocky seashores, spanning approximately 650 km of southern Australia's geologically-diverse coastline (Figure 2.1). This section of coastline encompasses three marine bioregions (Day et al. 2008; Kirkman 2013), with rocky seashores occurring in the Otway Bioregion to the east, and the Gulf St Vincent (GSV) Bioregion to the west (Figure 2.1). Three seashores, from each hardness class, were sampled in each bioregion (Figure 2.1, Table 2.1). These seashores were collectively composed of a variety of sedimentary, metamorphic, or igneous rock types (Table 2.1), with each sampled seashore dominated by a rock platform. The sampled seashores in each bioregion were not stratified for shore exposure or tidal elevation as not enough seashores were sampled to completely cross-stratify. However, the survey design that was employed used three representative shore perpendicular transects that covered all levels of the shore at each site. Hence exposure and tidal elevation was representative of each site rather than factored into the design.

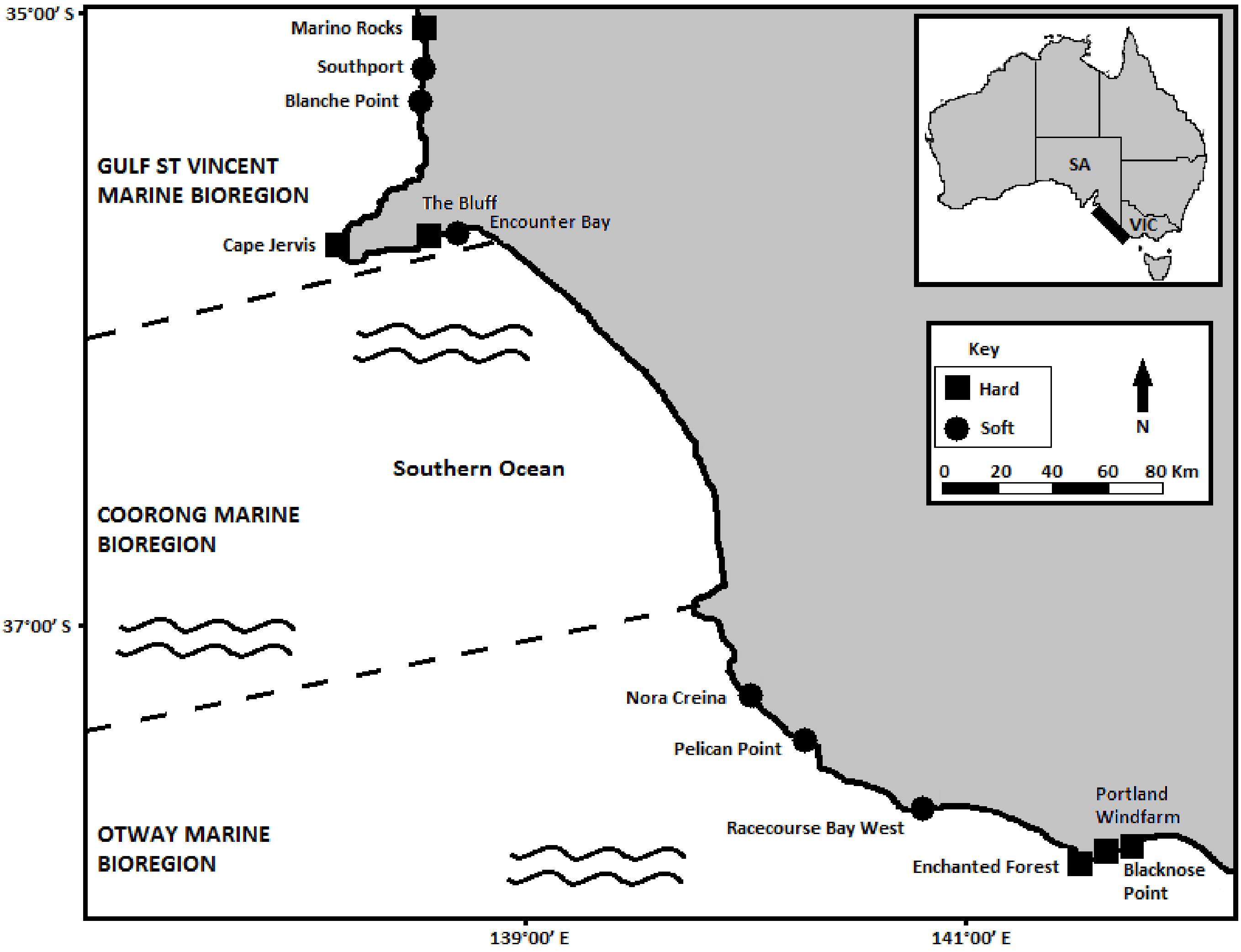


Figure 2.1: Seashores from the soft versus hard classes sampled in the Gulf St Vincent and Otway Marine Bioregions. Inset map shows the location of the study region in Australia.

Table 2.1: The hardness class, rock, and locations for each seashore sampled in two marine bioregions in southern Australia.

Marine Bioregion	**Hardness class**	**Seashore**	**Rock**	**Co-ordinates**
Otway	Soft	Racecourse Bay West	Mount Gambier limestone	38°03’ S, 140°45’ E
		Pelican Point	Mount Gambier limestone	37°55’ S, 140°25’ E
		Nora Creina	Calcarenite	37°19’ S, 139°50’ E
	Hard	Blacknose Point	Basalt	38°23’ S, 141°38’ E
		Portland Windfarm	Basalt	38°23’ S, 141°37’ E
		Enchanted Forest	Felsic volcanic rock	38°24’ S, 141°34’ E
Gulf St Vincent	Soft	Encounter Bay	Fossiliferous limestone	35°34’ S, 138°36’ E
		Blanche Point	Fossiliferous limestone	35°14’ S, 138°27’ E
		Southport	Fossiliferous limestone	35°10’ S, 138°27’ E
	Hard	The Bluff	Kanmantoo Schist	35°35’ S, 138°36’ E
		Cape Jervis	Kanmantoo Schist	35°36’ S, 138°05’ E
		Marino Rocks	Siltstone	35°02’ S, 138°30’ E

Sampling design

Sampling was completed during three seasons: summer 2015 (January); autumn 2015 (March-April); and summer 2016 (January). Due to an unseasonably mild and wet summer persisting over 2014/5, additional sampling was completed during the summer of 2015/6, to ensure more representative summer data were collected. No sampling during winter was possible due to the persistence of dangerous sampling conditions of waves, swells and tides, with seashores in the Otway Bioregion typically exposed to larger waves and swell than seashores in the GSV Bioregion. Sampling was completed during suitable daytime low tides (predicted low tide ≤0.60 m Australian Height Datum).

At each seashore for each season, three transects were sampled. These transects ran perpendicular to the ocean and were spaced approximately 10 metres apart, extending from the low tide level at the time of sampling to the top of the seashore. Consequently, transect lengths were variable within and among seashores, with the longest transect of 188.0 metres sampled at Racecourse Bay West and the shortest transect of 21.2 metres sampled at Portland Windfarm. Each transect was divided into thirds (= heights, lower, middle and upper), with three randomly-placed quadrats (size = 30 cm x 30 cm) sampled for each transect height. Quadrats were only sampled for platform habitats, with any randomly-placed quadrats encompassing rock pools or sediment patches re-randomised. Nine quadrats were thus sampled per transect, with a total of 27 quadrats sampled at each seashore for each season (total N = 972).

Quadrats were used to separately sample the sessile (marine plants and sessile invertebrates) and mobile (invertebrates only) assemblages inhabiting rock platforms. Each quadrat was photographed using an Olympus Tough TG-820 digital camera, with archived images subsequently processed in the laboratory. The identification and abundances of all mobile invertebrates ≥4 mm in size was recorded to species level except for limpets from the genera *Notoacmea* and *Siphonaria*. Instead, species guilds were used for both genera due to difficulties in distinguishing among congeners in archived images. The *Siphonaria* spp. guild included *S. tasmanica*, *S. zelandica* and *S. diemenensis*, while the *Notoacmea* spp. guild encompassed *N. flammea*, *N. mayi* and *N. petterdi*. An organism size of ≥ 4 mm was selected as it was equal to the width of the quadrat frame (= fixed reference point in archived photographs). The only mobile invertebrates regularly recorded at sizes <4 mm were limpets. Due to the difficulty in assigning individuals of this size to a species, another species guild encompassing all small limpets (*Cellana tramoserica*, *Notoacmea* spp., *Patelloida alticostata*, *P. insignis*, *P. latistrigata*, *Montfortula rugosa*, and *Siphonaria* spp.) <4 mm was established.

Sessile space occupancy was quantified using Coral Point Count with Excel extensions (CPCe), a program for the determination of substratum coverage using random point count methodology (Kohler and Gill 2006). Each quadrat photo was magnified to 300 % of its original size, with 50 points distributed inside the quadrat frame using a simple random technique. Marine plant and sessile invertebrate space occupancy was recorded at species level, with each species then assigned to one of 12 functional groups (see Appendix 1, Tables 8.1 - 8.6 for more details). A functional group approach, where species were assigned groups based on functional aspects of their morphology, was employed to better assess sessile assemblages (Steneck and Dethier 1994). Counts were also kept for the number of points distributed over bare rock or sediment (i.e. unoccupied space), with a total of 14 space-occupancy groups recognised. Once all 50 random points had been assigned a category, CPCe calculated the percentage of points overlying each of the 14 benthic categories. Sessile space occupancy was also measured along transects, but as transect-scale data showed the same biotic patterns as quadrat-scale data, transect sampling methods and results are presented in Appendix 1.

Four physical attributes were measured for each seashore *in situ*, which were: colour; scratch hardness; microhabitat density; and rugosity. Colour and scratch hardness were measured at the seashore scale, while rugosity and microhabitat density were measured at the quadrat scale. Colour was measured using comparison with Munsell colour charts, with a descriptive colour determined for bare rock at each seashore and expressed as Hue Value/Chroma. Hardness was measured using Moh's scale of scratch hardness, and was quantified by scratching three dry rock surfaces at each seashore with a sample of the nine softest minerals on this scale. The scratch hardness of the softest mineral capable of scratching the rock surface was assigned to each rock surface.

Microhabitat density was quantified by counting all cracks and/or depressions in the rock surface that were ≥18 mm wide, ≥22 mm long, and ≥13 mm deep for each quadrat. To determine microhabitat size, an empty shell of the snail *Nerita atramentosa* with these dimensions was fitted to likely microhabitats. This size of microhabitat may be deemed biologically relevant, as it would adequately encapsulate the majority of adult *N. atramentosa* (McGuinness and Underwood 1986). Rugosity was measured by using an inelastic piece of cotton string to closely contour vertical profiles of the substratum surface (Liversage et al. 2014). Ratios of substratum surface linear length (30 cm = quadrat dimensions) to substratum surface contour length (≥30 cm) were used to produce rugosity indices between 0 and 1 (indices approaching 1 are almost flat, with lower values being more rugose, Frost et al. 2005; Wilding et al. 2010).

Substratum mineralogy was determined through X-ray fluorescence (XRF), with separate tests completed for major mineral and trace element composition for three rock samples from each seashore except Blanche Point, where no samples were collected. XRF analysis of each rock sample (see Appendix 1 for specific XRF methods) tested for 11 major minerals and 40 trace elements, which were returned as % composition and parts per thousand, respectively.

Statistical analyses

All analyses were completed using either PRIMER v7/PERMANOVA+ or SYSTAT v13 software. All tests of PERMutational ANalysis Of VAriance (PERMANOVA) were completed using permutations of residuals with 9999 permutations as described in Anderson et al. (2008). Differences ($\alpha = 0.05$) among bioregions (Otway vs GSV) and hardness classes (soft vs hard) were considered to be fixed factors, while differences between seashores (12 seashores listed in Table 2.1), transect heights (lower, middle, upper) or seasons (Summer 2015, Autumn 2015, Summer 2016) were considered to be random factors. A hierarchical design was used for all PERMANOVA, where hardness class was nested in bioregion and seashores nested in hardness class. Bray-Curtis resemblances were used for assemblage data and Euclidean distances for univariate biotic data (species richness, total abundance or percent cover) and physical variables. For analyses based on multivariate assemblage data, a dummy variable of 1 was added because of the sparsity of non-zero values among samples (Anderson et al. 2008). PERMANOVA pair-wise tests were used to distinguish significant differences among significant factor levels or interactions. SIMilarity PERcentages (SIMPER) were used to calculate the average dissimilarity between factor levels, and to identify any consistent (i.e. SD/Similarity Ratio > 1) indicators associated with compositional differences. Non-metric Multi-Dimensional Scaling (nMDS) ordination plots or Multi-Dimensional Scaling (MDS) bootstrapped averages plots were used to visualise significant multivariate compositional differences.

Hardness-related rugosity or microhabitat density differences (H1) were tested separately using untransformed data with a five-factor (same five factors described above) PERMANOVA. No analyses were completed for scratch hardness or colour, as measurements were generalised at the seashore scale. For mineralogy, hardness class differences were tested separately for the multivariate measures of major mineral composition and trace element composition. A three-factor PERMANOVA was used to test for differences between bioregions, hardness classes and rock nested in hardness class (a fixed factor specific to mineralogy analyses only) using untransformed major mineral and fourth-root transformed trace element data. This same three-factor PERMANOVA was then used to test for univariate differences in the content of each major mineral.

Biotic differences between hardness classes (H2) were tested separately for sessile and mobile assemblages. Prior to analysis, the categories of bare rock and sediment were removed from all sessile multivariate data sets, and analysed separately, to ensure that every sample did not necessarily add up to 100 % space occupancy. Square-root transformations were applied to all biotic data except species richness, with a five-factor PERMANOVA model (as above) used to test for assemblage differences.

To determine whether hardness related biotic differences were correlated with the other physical variables measured (H3), BIOENV analyses were completed for multivariate biotic data and correlations for univariate biotic data. As mineralogy was measured for just three rock samples per seashore, the average content of each major mineral and trace element per seashore was determined (except Blanche Point), and these averages applied to replicate quadrats. At Blanche Point, averaged major mineral and trace element composition for fossiliferous limestone (based on samples collected from Southport and Encounter Bay) was applied. Data were averaged across heights, for each replicate transect for each seashore and season sampled. This reduced the number of samples by two-thirds, and was applied to all biotic and physical data.

A Principal Components Analysis (PCA) was completed to eliminate any non-independence among the 53 quadrat dependent (response) variables and reduce them into a smaller number of principal components (PC). Eight trace elements (silver, molybdenum, ytterbium, antimony, selenium, tin, tantalum and tellurium) were immediately excluded as their quantities could not be definitively determined through XRF. PCA using Varimax axis rotation was used to identify the PC axes (axis eigenvalues >1) that explained the greatest variation in the 45 remaining physical variables included in the model. Varimax component rotated loadings were used to identify which physical variables contributed strongly

(Varimax component loadings ≥0.90) to each axis identified. The factor scores calculated for each PC axis for each of the 324 averaged quadrat samples was then used in correlations.

BIOENV analyses between multivariate biotic data and PC factor scores were completed. The BEST (BIOENV) procedure searches for a combination of environmental variables whose inter-sample resemblances best match those arising from multivariate biological data (Anderson et al. 2008). A BEST analysis was completed between PC factor scores and Bray-Curtis similarity matrices of square-root transformed mobile invertebrate or sessile assemblage data to test the significance and correlation strength between biological data and the best combination of PC axes. A RELATE analysis between Euclidean distances of PC factor scores and Bray-Curtis resemblances of assemblage structure tested for a significant relationship between biological and physical variables. Correlations were completed to determine whether un-transformed measurements of species richness, total space occupancy and total invertebrate abundance were correlated with each PC axis that had an eigenvalue >1. Correlations were also completed for specific taxa whose abundances were found to contribute significantly to assemblage differences, to determine whether abundance differences were associated with rock physical attributes sampled at the quadrat scale.

Results

H1: The physical attributes of rocky seashores will not differ between hardness classes in each marine bioregion.

Physical attributes differed between hardness classes in each bioregion (Table 2.2) and among rocks (Appendix 1, Table 8.7). Rocks from the soft class (Moh's scratch hardness of 2.0 – 3.0) were moderately to highly friable, to the extent where limestone and calcarenite platforms crumbled underfoot (Table 2.2, Appendix 1, Table 8.7). In contrast, rocks from the hard class (Moh's scratch hardness of 5.0 – 7.5) had little or no friability (Table 2.2, Appendix 1, Table 8.7). The softest rock was Mount Gambier limestone from Racecourse Bay West and Pelican Point which had a Moh's scratch hardness of 2, while the hardest rock was Kanmantoo schist at Cape Jervis which had a Moh's scratch hardness of 7.5.

Colour was highly variable, with colour differences detected between hardness classes, and within and among the seven sampled rocks (Table 2.2, Appendix 1, Table 8.7). Rocks from the soft class were generally lighter in colour than rocks from the hard class. Rock colour was unaffected by encrusting lichens or algae, which generally had a sparse space-occupancy at each of the studied seashores.

Table 2.2: The physical attributes of hardness classes in each marine bioregion.

	Bioregion:	**GSV**		**Otway**	
	Hardness class:	**Soft**	**Hard**	**Soft**	**Hard**
Physical attribute					
Rock type(s)		Fossiliferous limestone	Siltstone, Kanmantoo schist	Mount Gambier limestone, calcarenite	Basalt, felsic volcanic rock
Scratch hardness (Moh's scale)		3.0	5.0 – 7.5	2.0 – 2.5	6.0 - 7.0
Friability		Moderate	Low - none	Moderate - high	None
Descriptive colour (range)		Light grey	Greyish red, brownish grey, grey	Light grey, dull yellow orange	Brownish black, brownish grey, brown
Mineralogy: Major minerals (%, mean ± SE)	SiO_2	51.2 ± 10.3	62.4 ± 3.3	8.4 ± 3.8	43.1 ± 4.4
	CaO	24.2 ± 6.0	5.1 ± 2.1	49.2 ± 2.4	14.4 ± 3.7
	Al_2O_3	1.0 ± 0.2	12.9 ± 1.2	0.6 ± 0.2	13.8 ± 1.5
	Fe_2O_3	0.7 ± 0.1	5.7 ± 0.9	0.4 ± 0.1	10.4 ± 1.0
	MgO	0.7 ± 0.1	3.4 ± 0.5	0.9 ± 0.1	4.8 ± 0.6
	Na_2O	0.5 ± 0.1	2.2 ± 0.3	0.5 ± 0.1	3.6 ± 0.5
	K_2O	0.3 ± 0.1	2.6 ± 0.1	0.2 ± 0	0.9 ± 0.1
	TiO_2	0.1 ± 0	0.7 ± 0	0 ± 0	1.6 ± 0.2
	P_4O_{10}	0 ± 0	0.2 ± 0	0.1 ± 0	0.3 ± 0
	MnO_2	0 ± 0	0.1 ± 0	0 ± 0	0.2 ± 0
	SO_3	0.2 ± 0	0 ± 0	0.2 ± 0	0.1 ± 0
Mineralogy: Dominant trace elements (ppm, mean ± SE)		Cl 4027 ± 736	Cl 573 ± 175	Cl 5846 ± 1222	Cl 2312 ± 419
		Sr 398 ± 113	Mn 524 ± 39	Sr 515 ± 87	Mn 1114 ± 174
		Ba 64 ± 11	Ba 437 ± 61	Mn 145 ± 24	Sr 445 ± 51

Nine major minerals were recorded at higher quantities in the hard versus soft class in each bioregion (Table 2.2). However, these differences were only significant for manganese oxide, magnesium oxide and potassium oxide (PERMANOVA smallest permuted *p*-value = 0.0001 for magnesium oxide). The total content of each major mineral was similar between bioregions, with the higher content of magnesium oxide in the Otway than GSV Bioregion the only significant difference (PERMANOVA permuted *p*-value = 0.0031). Major mineral composition was not significantly different between hardness classes (PERMANOVA permuted *p*-value = 0.1528) or bioregions (PERMANOVA permuted *p*-value = 0.0865), but was significantly different among rocks (PERMANOVA permuted *p*-value = 0.0029). Each rock had its own distinct major mineral composition (Appendix 1, Table 8.8 & Figure 8.1a). Mount Gambier limestone and calcarenite were characterised by a higher calcium oxide (CaO) content, fossiliferous limestone, siltstone, Kanmantoo schist and basalt were characterised by a higher silicon dioxide (SiO_2) content, while felsic volcanic rock contained approximately equal quantities of SiO_2 and CaO (Appendix 1, Table 8.8).

For trace element composition, the soft class was characterised by higher quantities of chlorine and strontium than the hard class in both bioregions (Table 2.2, Appendix 1, Table 8.9). In contrast, the hard class was characterised by higher quantities of barium, zirconium and chromium, plus a variety of other metallic trace elements in smaller quantities, than the soft class. These hardness-class compositional differences were significant (Appendix 1, Figure 8.1b, PERMANOVA permuted *p*-value = 0.0186), and were associated with the higher content of chlorine in the soft class, although chlorine was only a reliable indicator in the GSV bioregion (SIMPER SD/Sim ratio = 1.30). A significant difference in trace element composition was also detected among rocks (PERMANOVA permuted *p*-value = 0.0002), with each rock having a very distinct trace element composition (Appendix 1, Table 8.10 & Figure 8.1b). Trace element composition was not significantly different between bioregions (PERMANOVA permuted *p*-value = 0.1082).

Seashores composed of soft rocks generally had greater microhabitat densities, lower rugosity indices, and thus more-complex surfaces, than hard seashores (Appendix 1, Figure 8.2). When data were pooled across seasons, soft seashores had significantly greater (more than 2x) microhabitat densities than hard seashores in each bioregion (Appendix 1, Table 8.11). Microhabitat density ranged between 0.56 ± 0.06 per quadrat for the hard class in the Otway Bioregion and 3.72 ± 0.16 per quadrat for the soft class in the GSV Bioregion. No differences in surface rugosity were detected between hardness classes (Appendix 1, Table 8.11). Surface rugosity was relatively flat, ranging between 0.85 ± 0.01 per quadrat for the soft class in the GSV Bioregion and 0.92 ± 0.01 per quadrat for the hard class in the Otway Bioregion.

Seashores in the Otway Bioregion generally had fewer microhabitat features, higher rugosity indices, and thus simpler surfaces, than seashores in the GSV Bioregion (Appendix 1, Figure 8.2). Microhabitat density differed between bioregions, with differences interacting with height (Appendix 1, Table 8.11). For both hardness classes and all three heights, the GSV Bioregion had significantly higher microhabitat densities than the Otway Bioregion (Appendix 1, Figure 8.2a, PERMANOVA smallest permuted pair-wise *p*-value = 0.0022 for the lower height). For rugosity, significant bioregional differences interacted with height (Appendix 1, Table 8.11). The GSV Bioregion had smaller rugosity indices than the Otway Bioregion for all heights, although these differences were only significant for the lower height (PERMANOVA permuted pair-wise *p*-value = 0.0038, Appendix 1, Figure 8.2b).

H2: Intertidal assemblages will not differ between hardness classes in each marine bioregion

Sessile assemblages.

Bioregion-specific differences in the structure, richness and space occupancy of sessile assemblages were detected between hardness classes (Tables 2.3 & 2.4, Figure 2.2, Appendix 1, Tables 8.12 & 8.13). In the Otway Bioregion, hardness class interacted with height for structural differences (Table 2.4), although no height-specific hardness-class differences were identified (PERMANOVA smallest pair-wise permuted *p*-value = 0.0845 for the lower height). The hard class was characterised by a higher richness and space occupancy of sessile functional groups than the soft class (Figure 2.2c-f, Table 2.3). However, only invertebrate richness and space occupancy significantly differed, with significant differences interacting with height (Tables 2.3 & 2.4), such that hardness-class differences were specific to lower and middle heights only (Figure 2.2, smallest PERMANOVA pair-wise permuted *p*-value = 0.0002 for invertebrate richness at the lower height). No significant difference for the coverage of bare rock (PERMANOVA pair-wise permuted *p*-value = 0.8199) or sediment (PERMANOVA pair-wise permuted *p*-value = 0.0682) was detected between hardness classes in the Otway Bioregion.

Table 2.3: Univariate assemblage differences (mean ± SE values) for hardness classes in each marine bioregion. * = *p* <0.05, L = lower, M = middle and U = upper heights.

	Bioregion:	**Otway**				**GSV**			
Dependent variable	**Height**	**Soft**		**Hard**		**Soft**		**Hard**	
Marine plant species richness		1.1 ± 0.1	<	1.4 ± 0.1		3.4 ± 0.1	>	0.6 ± 0.1	*
Sessile Invertebrate species richness	L	0.1 ± 0	<	2.0 ± 0.2	*	1.5 ± 0.1	>	1.4 ± 0.1	
	M	0.1 ± 0	<	1.3 ± 0.1	*	1.8 ± 0.1	>	0.4 ± 0.1	
	U	0	<	0.4 ± 0.1		1.9 ± 0.1	>	0.1 ± 0	*
Marine plant space occupancy (%)		10.9 ± 1.5	<	18.7 ± 2.0		28.9 ± 1.3	>	3.9 ± 0.7	*
Sessile Invertebrate space occupancy (%)	L	0.1 ± 0	<	7.5 ± 1.0	*	10.5 ± 1.5	>	4.3 ± 0.9	
	M	0.1 ± 0	<	4.1 ± 0.8	*	12.2 ± 1.5	>	0.6 ± 0.2	*
	U	0.1 ± 0	<	2.2 ± 0.7		7.7 ± 1.0	>	0.1 ± 0	*
Bare rock (%)		79.5 ± 2.0	>	75.1 ± 2.0		49.5 ± 1.7	<	91.3 ± 1.1	*
Sediment (%)		9.5 ± 1.2	>	1.6 ± 0.4		11.4 ± 1.1	>	3.1 ± 0.6	*
Mobile invertebrate species richness	L	1.7 ± 0.1	<	2.8 ± 0.2		0.9 ± 0.1	<	1.9 ± 0.1	
	M	1.7 ± 0.1	<	2.5 ± 0.2		1.3 ± 0.1	>	0.8 ± 0.1	
	U	1.4 ± 0.1	<	1.9 ± 0.2		1.7 ± 0.1	>	0.2 ± 0.1	*
Mobile invertebrate abundance		36.5 ± 2.9	<	46.6 ± 3.4		10.5 ± 0.9	>	7.8 ± 1.4	

Table 2.4: Summary of PERMANOVA results testing for differences in eight dependent variables (all univariate variables except assemblage structure which is multivariate). AS = assemblage structure; PR = marine plant richness; IR = invertebrate richness; P % = marine plant occupancy; I % = invertebrate occupancy; BR = bare rock coverage; SR = species richness; TA = total abundance; NS = not significant; * = *p* <0.05; ** = *p* <0.01; *** = *p* <0.001.

Assemblage type:	**Sessile**						**Mobile**		
Factor Dependent variable:	**AS**	**PR**	**IR**	**P %**	**I %**	**BR**	**AS**	**SR**	**TA**
Bioregion, B	NS	**	NS	NS	*	NS	**	**	***
Height, H	***	***	**	**	**	*	**	*	NS
Season, S	NS	NS	NS	NS	NS	NS	NS	*	*
Hardness class(bioregion), HC(B)	***	***	**	***	***	**	NS	NS	NS
B x H	NS	NS	NS	NS	NS	NS	NS	NS	NS
B x S	NS	NS	*	NS	**	NS	NS	NS	NS
H x S	NS	**	NS	NS	NS	NS	NS	NS	NS
Seashore(HC(B))	***	*	***	**	***	NS	***	***	NS
HC(B) x H	**	NS	***	NS	*	NS	*	**	NS
HC(B) x S	NS	NS	NS	NS	NS	NS	NS	NS	NS
B x H x S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Seashore(HC(B)) x H	***	***	***	***	**	***	***	***	***
Seashore(HC(B)) x S	***	NS	NS	*	NS	***	***	**	NS
HC(B) x H x S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Seashore(HC(B)) x H x S	***	*	*	*	***	*	***	NS	*

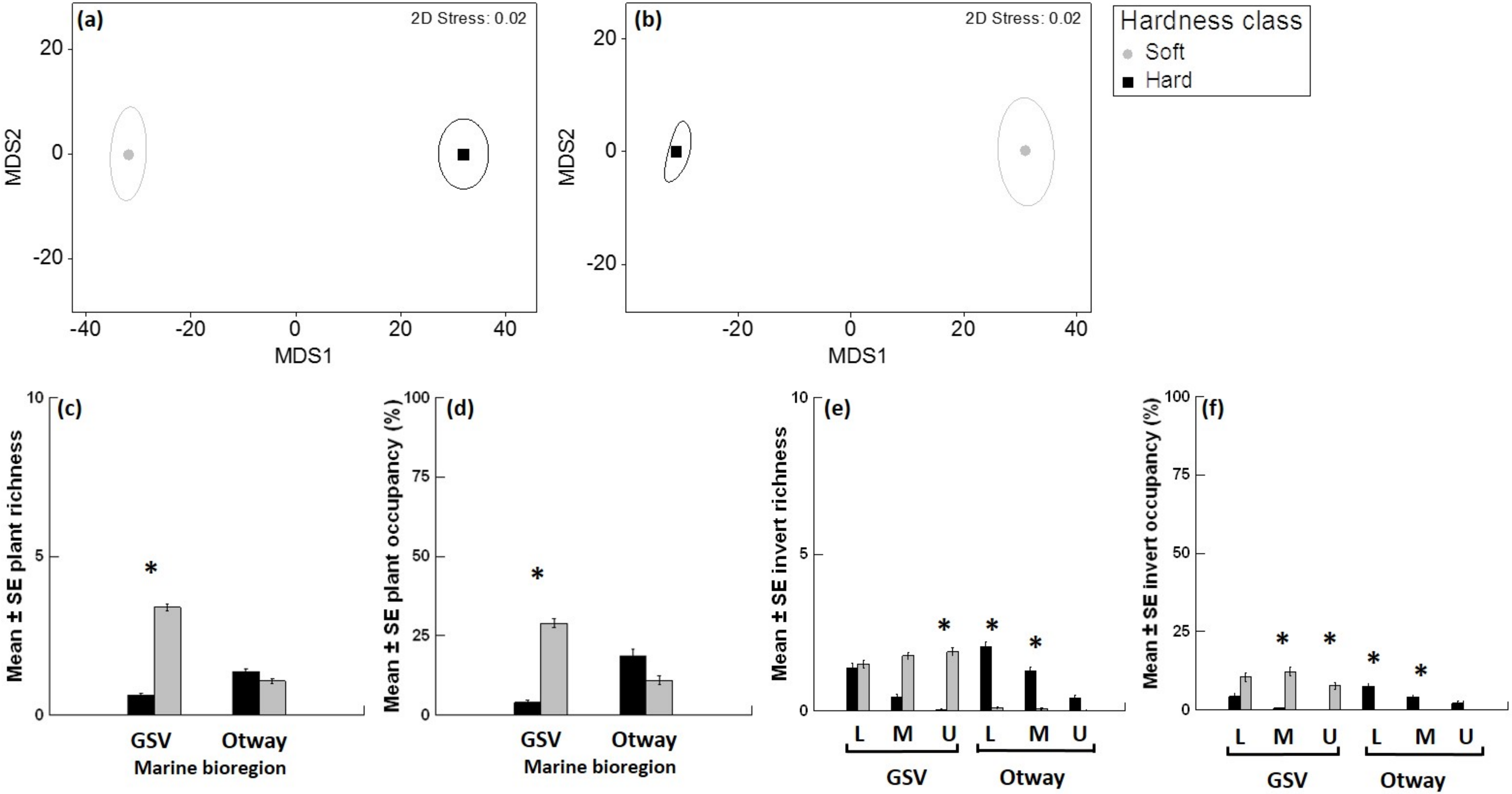


Figure 2.2: Sessile assemblage differences between hardness classes. MDS bootstrapped averages ordination plots, which show the mean and 95 % confidence envelope of the mean, depicting structural differences between hardness classes for (a) middle height in the GSV Bioregion and (b) upper height in the GSV Bioregion. Mean ± SE differences between hardness classes for (c) marine plant richness, (d) marine plant occupancy as % cover, (e) sessile invertebrate richness and (f) sessile invertebrate occupancy as % cover. Each y-axis on bar charts extends to encompass the range of the raw data. L = lower height; M = middle height; U = upper height; GSV = Gulf St Vincent; * = significant difference detected between hardness classes.

In the GSV Bioregion, hardness structural differences interacted with height (Table 2.4), with differences detected for the middle and upper heights (Figure 2.2a-b, PERMANOVA smallest pair-wise permuted *p*-value = 0.0112 for the upper height). However, neither difference could be associated reliably with specific functional groups (all SIMPER SD/Sim ratios <1). The soft class was characterised by a significantly higher richness and space occupancy of marine plants versus the hard class (Figure 2.2c-d, Tables 2.3 & 2.4). A significant interaction between hardness class and height was detected for invertebrate richness and space occupancy (Table 2.4), with the soft class characterised by a higher invertebrate richness and space occupancy (Figure 2.2e-f, Table 2.3). Hardness-class differences in richness were specific to the upper height (Figure 2.2e, PERMANOVA pair-wise permuted *p*-value = 0.0135), while space occupancy differences were specific to the middle and upper heights (Figure 2.2f, PERMANOVA smallest pair-wise permuted *p*-value = 0.0076 for the upper height). The soft class had significantly higher sediment cover, and significantly less bare rock, versus the hard class (Table 2.3, Appendix 1, Table 8.13).

The structure, richness and space occupancy of sessile assemblages was highly variable among seashores and heights, with several assemblage differences between bioregions also identified (Table 2.4). No significant bioregional differences in assemblage structure were detected, with GSV seashores having a higher richness and space occupancy of sessile functional groups than Otway seashores (Table 2.4, Appendix 1, Figure 8.3). Significant bioregional differences were detected for marine plant richness, invertebrate richness and invertebrate space occupancy, although significant invertebrate differences interacted with season, and were therefore specific to autumn 2015 only (Appendix 1, Figure 8.3). No bioregional differences in the coverage of sediment or bare rock were detected (Appendix 1, Table 8.13). Bioregion-specific hardness differences were also detected for sessile assemblages at the transect scale, with richness and space occupancy differences generally similar to quadrat-scale differences (see Appendix 1 for more detail). Only the coverage of turf-forming algae at the transect scale showed a consistent hardness-related trend, with more on soft than hard rock (Appendix 1, Table 8.22).

Mobile assemblages

Fewer assemblage differences due to hardness were identified for mobile invertebrates, with all structure or richness differences interacting with height (Table 2.4, Appendix 1, Table 8.14). Structural differences were specific to the middle (Figure 2.3a, PERMANOVA pair-wise *p*-value = 0.0370) and upper (Figure 2.3b, PERMANOVA pair-wise *p*-value = 0.0124) heights of the GSV Bioregion. These differences were associated with the higher abundances of *Siphonaria* spp. (SIMPER SD/Sim ratio >1) on soft than hard seashores. Richness differences were specific to the upper height of the GSV Bioregion (PERMANOVA pair-wise *p*-value = 0.0214), where the soft class had a higher species richness than the hard class (Figure 2.3c, Table 2.3). Mobile invertebrate abundance did not significantly differ between hardness classes (Table 2.4).

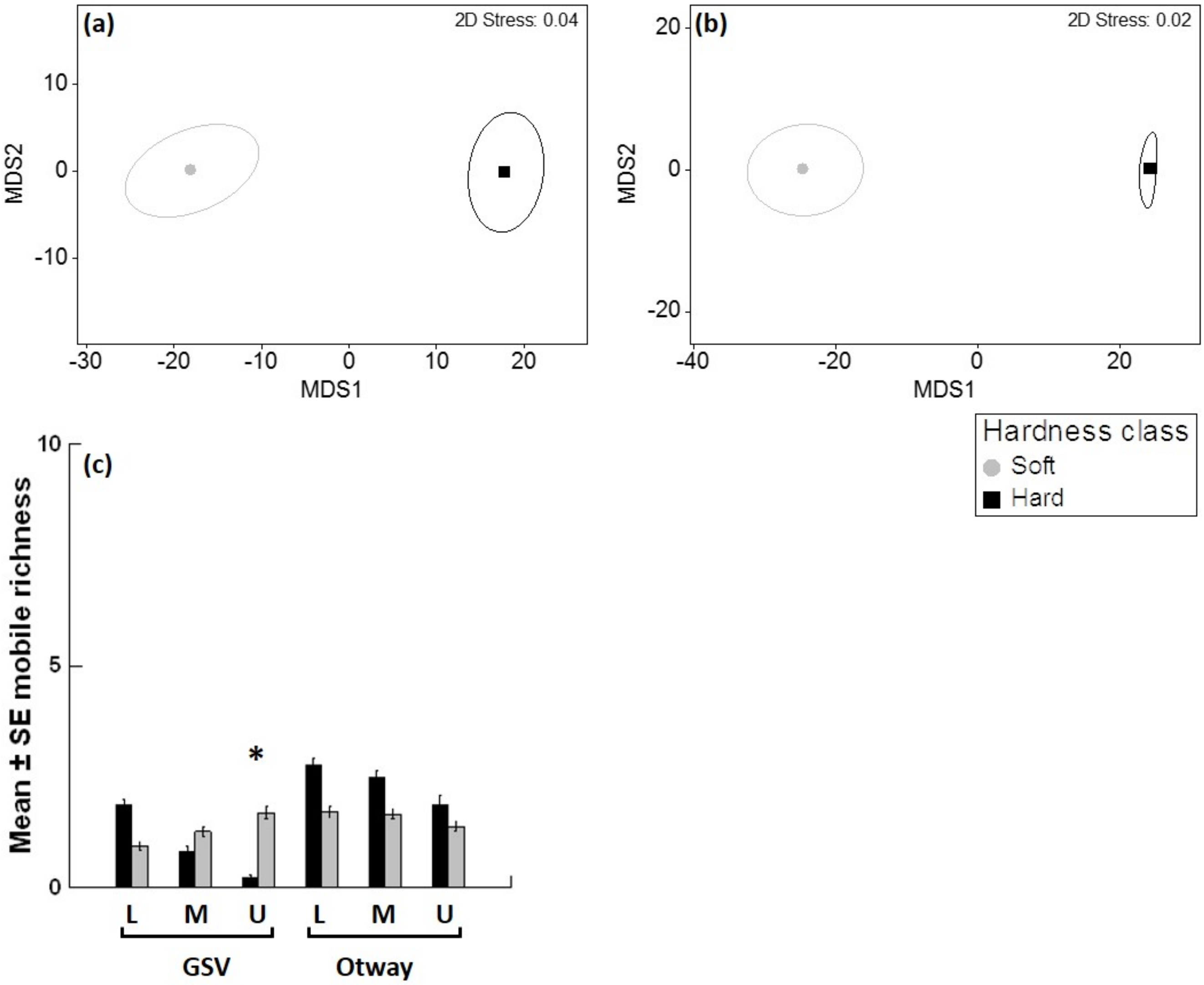


Figure 2.3: Assemblage differences between hardness classes for mobile invertebrates. MDS bootstrapped averages ordination plots depicting structural differences due to the factor Hardness class for (a) the middle height in the GSV Bioregion and (b) the upper height in the GSV Bioregion, with (c) the mean ± SE mobile species richness for each height in each bioregion. L = lower height; M = middle height; U = upper height; GSV = Gulf St Vincent; * = significant difference detected between hardness classes.

Mobile invertebrate assemblages were significantly different between bioregions and seashores (Table 2.4, Appendix 1, Table 8.14). The Otway and GSV Bioregions supported very distinct mobile invertebrate assemblages (Appendix 1, Figure 8.4a), although no consistent indicator species were reliably associated with structural differences (all SIMPER SD/Sim ratios <1). The species richness and total abundance of mobile invertebrate assemblages significantly differed between bioregions (Table 2.4), with the Otway Bioregion recording almost twice the species richness, and more than four times the abundances than the GSV Bioregion (Appendix 1, Figure 8.4b-c).

H3) Associations between intertidal assemblages and hardness classes are not correlated with any differences in physical attributes.

Principal components analysis reduced 45 raw physical variables into six PC axes with eigenvalues >1 (Appendix 1, Table 8.15). The first three PC axes were strongly associated (i.e. Varimax rotated loadings ≥0.90) with several major mineral or trace elements (Appendix 1, Table 8.15), and accounted for 37.7 %, 30.7 % and 8.0 %, respectively, of the total variation in physical attributes measured. PC axes four through six were less-strongly associated (Varimax rotated loadings <0.90) with specific trace elements or microhabitat density (Appendix 1, Table 8.15). See temperature data section and Tables 8.16 & 8.17 in Appendix 1 for other approaches investigated when modelling environmental data.

A significant relationship was detected between ordinations of sessile assemblage structure and an ordination of the six PC axes with eigenvalues >1 (RELATE Spearman Rho p = 0.111, *p*

= 0.0010). However, sessile assemblage structure was only weakly, albeit significantly, correlated with the first, second and fifth PC axes (BEST p = 0.183, p = 0.0100). Each of these PC axes were most-strongly associated with hardness-class differences in the content of major minerals or trace elements and microhabitat density (Appendix 1, Table 8.15). Generally there was a higher content of metallic oxides and trace elements in the hard versus soft class. A significant relationship was also detected between ordinations of mobile assemblage structure and the same six PC axes (RELATE Spearman Rho p = 0.224, p = 0.0010). Mobile assemblage structure was weakly correlated with the fourth PC axis (BEST p = 0.237, p = 0.0010), which was most-strongly associated with the content of samarium (Appendix 1, Table 8.15).

Each univariate biotic variable was weakly to moderately, but significantly, correlated with at least two PC axes (Appendix 1, Table 8.18). Sessile invertebrate richness and percent cover were significantly correlated with all six PC axes, while mobile invertebrate abundance and richness were each correlated with just two PC axes. Each invertebrate (mobile and sessile) variable was most-strongly correlated with the second PC axis, which was associated with hardness-related differences in the content of potassium oxide and several trace metals (Appendix 1, Table 8.18). In contrast, each marine plant variable was most-strongly correlated with the fifth PC axis, which was associated with hardness-related differences in microhabitat density (Appendix 1, Table 8.18). All six univariate biotic variables were each correlated with several other PC axes, with each of these axes associated with hardness-related differences in the content of metallic oxides or trace metals.

The higher abundance of *Siphonaria* spp. at middle and upper heights on soft versus hard platforms in the GSV Bioregion may be associated with the greater surface complexity of soft rocks. Statistically-significant but only weak-to-moderate correlations were detected between *Siphonaria* spp. abundance and microhabitat density (Spearman's Rho = 0.523, *p*-value < 0.01), and between abundance and surface rugosity (Spearman's Rho = - 0.294, *p*-value <0.01). A statistically weak negative correlation was also detected between microhabitat density and surface rugosity for these same quadrats (Spearman's Rho = - 0.424, *p*-value <0.01). Therefore, *Siphonaria* spp. abundance was highest on rocks with a higher microhabitat density and thus smaller rugosity indices.

Discussion

This research identified hardness-related differences for several physical attributes including microhabitat density and mineralogy. Few general associations between intertidal assemblages and rock hardness were detected, with the associations that were identified generally specific to just one of the marine bioregions sampled. These biotic differences were most strongly associated with hardness-related differences in the content of metallic oxides and trace metals or microhabitat densities.

In the Otway Bioregion, hard platforms generally supported assemblages with a greater richness, space occupancy and abundance than soft platforms. This pattern was reversed in the GSV Bioregion. The detection of bioregion-specific associations indicate intertidal biota may be less-strongly associated with rock hardness than some of the other rock physical attributes (e.g. rugosity, microhabitat density, mineralogy) known to influence biota elsewhere (McGuinness and Underwood 1986; Raimondi 1990; Bavestrello et al. 2000). Alternatively, the detection of bioregion-specific differences may be a product of each bioregions lithology, as rock types were specific to each bioregion and thus could not be replicated across bioregions. Consequently, rock type may be more-strongly associated with the biotic trends identified here than hardness *per se*, with intertidal biota strongly associated with rock type elsewhere (e.g. Chapter 3; Moore and Kitching 1939; McGuinness and Underwood 1986; Green et al. 2012). Moreover, as the 12 seashores investigated were interspersed over 650 km, there are a myriad of factors other than rock characteristics that varied among seashores, such as wave action and sea surface or air temperatures. Therefore, environmental differences other than the characteristics of the rock may also influence the biotic patterns observed within and between bioregions.

This study untangles associations between sessile biota and rock hardness by investigating multiple independent soft versus hard rocks. Before this study, our only insight into such associations came from studies comparing multiple hard rocks versus a single soft rock (e.g. Malm et al. 2003; Thomsen et al. 2004) or a single soft versus a single hard rock (e.g. Davidson and Chadderton 1994; Guidetti et al. 2004; Bavestrello et al. 2018; Bessey et al. 2018). Despite such hardness comparisons being confounded by a limited selection of rocks from each hardness class, most studies detected potential hardness-related differences for specific functional or morphological groups. Comparing across studies, no overall hardness-class association is evident, with higher space occupancies detected on both softer (Davidson and Chadderton 1994; Schiaparelli et al. 2003; Bessey et al. 2018) and harder rocks (Davidson and Chadderton 1994; Guidetti et al. 2004). Similar associations were detected here, with few consistent hardness differences identified. Instead, specific functional groups were usually associated with one hardness class, in one bioregion.

Like earlier studies, my comparisons of rock hardness may be influenced by rock type, which may account for the bioregional patterns that emerged. The two softest rocks sampled, Mount Gambier limestone and calcarenite, were found only in the Otway Bioregion and accounted for all soft platforms sampled there. As the softest rocks experience the fastest erosion rates (Bagur et al. 2014), sessile biota that settle on these rocks are more likely to experience substratum failure (Thomsen et al. 2004; Gutiérrez et al. 2018), and thus have a shortened life span (Menge et al. 2010). When coupled with the Otway Bioregion’s rough ocean conditions of waves and swell, the high erodibility of soft rocks may make them unfavourable substrata for settling sessile species. This may account for the sparse sessile space occupancy, especially for invertebrates, on soft rocks in the Otway Bioregion. In contrast, the softest (fossiliferous limestone) platforms of the GSV Bioregion had a greater scratch hardness than Mount Gambier limestone or calcarenite. When combined with the GSV Bioregion’s calmer ocean conditions in comparison to the Otway Bioregion, fossiliferous limestone may erode more slowly. This may make fossiliferous limestone a suitable substratum for sessile species to settle and persist on, accounting for the GSV Bioregions higher sessile space occupancy on soft rocks.

The only exception where an overall hardness-class association was identified was for turf-forming algae sampled at the transect scale. Turf-forming algae always had greater space occupancy on platforms from the soft versus hard class. This association may be related to seashore height. Soft platforms had a low height in relation to sea level, and generally had little or no shore slope compared with hard platforms, resulting in soft platforms staying submerged longer than hard platforms during a tidal cycle. Given thermal and desiccation stresses increase with emersion time (Somero 2002; Harley 2003), and that some algal turfs are unable to persist where thermal stress is highest (Harley 2003), then algae that are submerged longer are subjected to fewer thermal extremes (Nielsen 2007), thus improving their chances of persistence. Consequently, algal turfs may persist better on soft platforms due to their lower relief and reduced emersion times, with flatter shore slopes positively associated with algal turfs elsewhere (Whorff et al. 1995).

Few associations between mobile invertebrates and rock hardness were detected. Specifically, soft platforms had a higher species richness and also abundances of *Siphonaria* spp., at middle or upper heights in the GSV Bioregion, compared to hard platforms. These associations contrast strongly with those previously reported, where higher abundances of invertebrates were recorded on hard versus soft rock (Liversage and Benkendorff 2013; Liversage et al. 2014; Bessey et al. 2018). These contrasting hardness-related associations may be related to specific physical attributes of fossiliferous limestone as a platform habitat. Soft rocks had a greater surface complexity than hard rocks, with the abundance of *Siphonaria* spp. weakly-to-moderately but significantly correlated with these microhabitat and rugosity differences. Complex areas of substratum afford intertidal biota some protection from predation and physical stress (Levings and Garrity 1983; Atkinson and Newbury 1984; Branch and Cherry 1985), with *Siphonaria* spp. potentially occupying more-complex limestone substrata to reduce exposure to these risks. Alternatively, *Siphonaria* species construct home scars, where their shells fit exactly into the depressions they have constructed, thereby reducing their risk of desiccation during emersion (Garrity and Levings 1983; Branch and Cherry 1985). These scars are presumably easier to construct in softer rocks like limestone, so individuals may be better protected there than they may be on hard rocks. Mobile invertebrate species richness differences are also likely associated with the greater surface complexity of limestone, as it has been well documented that substratum with the greatest surface complexity often support the highest species richness (Blanchard and Bourget 1999; Guidetti et al. 2004; Le Hir and Hily 2005).

The two bioregions supported distinct assemblages. The GSV Bioregion had a greater richness and space occupancy of sessile biota versus the Otway Bioregion, while the Otway Bioregion had twice the richness and four times the abundance of mobile invertebrates versus the GSV Bioregion. Each bioregion differs in its environmental characteristics, any number of which may also influence the biotic patterns identified. Some of the environmental characteristics differing between these bioregions that have been associated with biotic differences elsewhere include wave exposure (Underwood 1981; Steneck and Dethier 1994; Martins et al. 2009), ambient air temperatures (Lathlean et al. 2015b), sea-surface temperatures (Blanchette et al. 2008; Wieters et al. 2009), upwelling events (Kämpf et al. 2004) and rock type (Moore and Kitching 1939; McGuinness and Underwood 1986). It is also possible that bioregional differences may result from the sandy beaches of the Coorong Bioregion (Figure 2.1) acting as a biogeographic barrier potentially restricting the distribution of some species across these bioregions (Hidas et al. 2007; Ayre et al. 2009).

Rocks from the hard class contained higher quantities of most metallic oxides and metallic trace elements, and significantly less chlorine, than rocks from the soft class. Hard rocks also had fewer microhabitat features, and thus simpler surfaces, than soft rocks. Hardness-related biotic differences were predominantly correlated with PCs associated with differences in the content of metallic oxides and trace metals. However, for the univariate measures of marine plant richness and space occupancy, biotic differences were also correlated with microhabitat densities. The associations between intertidal biota and mineralogy differ from the few that are described elsewhere. These previous studies investigated substratum differing in their content of quartz (silica) minerals, reporting that quartz influenced the growth and development of some species (Cerrano et al. 2007; Pozzolini et al. 2010) and that the presence of silica was negatively associated with invertebrate abundance in sediments (Cerrano et al. 1999; Bavestrello et al. 2000). My results highlight the potential importance of rarer major minerals and trace elements, which until now have been largely overlooked from a biological perspective, other than the work of Malm et al. (2003) on associations between algae and alkaline rocks.

In conclusion, rocks from the soft versus hard classes differ physically and in mineralogy. Few consistent differences in intertidal assemblages were detected between hardness classes, with the differences that were identified mostly specific to marine bioregions. Hardness-related assemblage differences were weakly-moderately correlated with differences in the content of metallic oxides and trace metals or microhabitat densities. Overall, the detection of bioregion-specific hardness differences, rather than general hardness trends, indicate intertidal biota may be less strongly associated with rock hardness than several other rock physical attributes, especially rock type.

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Chapter 3

Assemblages on limestone and siltstone boulders diverge over six years in a primary-succession transplant experiment

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Abstract

Transplant experiments have been used to test for associations between different rock types and developing assemblages during succession in intertidal boulderfields, with all previous transplants concluding within one year. Therefore, differences in colonisation and recruitment on different rocks have been well established but any late-successional associations remain unknown. To remedy this, a previous transplant experiment contrasting limestone and siltstone was extended so that the assemblages that developed on transplanted bare boulders were investigated over six years. The structure and richness of the later-successional assemblages differed between rocks, with limestone assemblages on both upper and lower surfaces being characterised by more species, and higher abundances of some species, than siltstone. These assemblage differences were most strongly correlated with rock-related differences in surface rugosity and microhabitat density. The later-successional assemblages sampled after 5 and 6 years were generally similar to earlier-successional assemblages sampled after 11 months. However, the subtle successional changes that transpired were such that all rock-related differences after five and six years were unique to later-successional assemblages only. These rock-related differences, that appear to take multi-year time-scales to manifest, would have remained unknown had this experiment concluded within the historical timeframe of <1 year.

Introduction

Successional change occurs in biological assemblages following a perturbation that opens up a bare space (Connell and Slatyer 1977; Connell 1987). In intertidal boulderfields, the intermittent overturning of boulders by wave action often opens up such space, subjecting the algal and invertebrate assemblages on boulder surfaces to successional change (Sousa 1979a; 1980; 1984). Succession may be cut short and started again on frequently-disturbed boulders, while progressing to later stages on less-often disturbed boulders (Connell and Slatyer 1977; Sousa 1979a). As a result, boulder assemblages may be viewed as a highly-variable mosaic of successional stages (Sousa 1979a; Connell 1985).

Successional processes in intertidal boulderfields are not solely influenced by physical disturbances initiating change. Rather, succession may also be associated with a suite of environmental variables, including desiccation stress at low tide (Sousa 1979b), boulder size (Sousa 1979a), and lithology of the rock comprising the boulders (often referred to as 'rock type' in the published literature, McGuinness and Underwood 1986; McGuinness 1988; Liversage et al. 2014). Presently, successional patterns on different types of rocks remain under-examined, with all published experiments targeting colonisation and recruitment onto initially-bare boulders and concluding within 12 months (see McGuinness and Underwood 1986; James and Underwood 1994; Green et al. 2012; Liversage et al. 2014). Therefore, rock-related differences have only been established for boulder assemblages early in succession. These include differences in the space occupancy of algae and tubeworms after four months (McGuinness and Underwood 1986), spirorbid-worm recruitment after three months (James and Underwood 1994), the space occupancy of algae and oysters over 10 months (Green et al. 2012), and limpet abundance over 11 months (Liversage et al. 2014). However, other longer-term successional studies on a single rock type in intertidal reef systems do provide evidence of community changes well beyond 12 months. Some species increased, whereas others continued to decline, for over three years after experimental clearing on boulders (Sousa 1979b) and rock platforms (Turner 1983;

Petraitis and Dudgeon 2005), for over ten years after an oil spill and subsequent cleaning with toxic dispersants (Southward and Southward 1978; Hawkins et al. 2017), or after natural ice scour events (McCook and Chapman 1997). Divergent successional pathways after disturbance on sheltered intertidal reefs can lead to alternative-state communities (Petraitis and Dudgeon 2005; Petraitis et al. 2009). There is also evidence to suggest that different assemblages can form on intertidal reefs of different rock type (McGuinness and Underwood 1986; Davidson and Chadderton 1994; Savoya and Schwindt 2010; Green et al. 2012; Liversage and Benkendorff 2013; Bavestrello et al. 2018). However, longer-term successional studies (i.e. >1 year) are required to establish whether the assemblages diverge as a result of rock type or other factors that may vary at the reef scale.

Each rock type possesses a specific set of physical attributes as substratum, and rock-related differences in physical attributes may be associated with some of the biotic patterns identified. Some of these physical attributes include boulder size and hence surface area (Chapman 2002a; Liversage et al. 2014), substratum colour (James and Underwood 1994), microhabitat density (McGuinness and Underwood 1986; Chapman 2000; Moreira et al. 2007; Chapman and Underwood 2011), rugosity (Underwood and Chapman 1989; Chapman and Underwood 1994; Herbert and Hawkins 2006), mineralogy (Bavestrello et al. 2000; 2018; Cerrano et al. 2007) and hardness (Liversage and Benkendorff 2013; Liversage et al. 2014). Generally, associations between assemblages of intertidal biota and rock physical attributes have been assessed in studies of a single rock only, mostly for platform areas on rocky seashores. Furthermore, most biotic associations with rock-related differences in physical attributes have been identified through laboratory- or field-based experimental manipulations that have used small, modified pieces (i.e. tiles) of either the tested rocks or anthropogenic materials (e.g. asbestos). With the exception of Liversage et al. (2014), very few published studies have evaluated multiple physical attributes of naturally-occurring intertidal boulders of differing rock, and examined how multiple physical attributes may be associated with assemblages during succession.

To extend time frames, an earlier boulder-transplant experiment (Liversage et al. 2014) was re-sampled after several more years to determine whether later-successional invertebrate-dominated assemblages diverged on limestone and siltstone boulders. By re-sampling an existing experiment, comparisons between earlier- and later-successional assemblages were possible, thus enabling me to investigate rock-type associations across all stages of succession. When the experiment by Liversage et al. (2014) concluded after 11 months, the early-successional assemblages on both the upper and lower surfaces of limestone and siltstone had a similar mobile-invertebrate assemblage structure and abundances of common invertebrate species. The only exception was for abundances of limpets belonging to the genus *Notoacmea*, which were consistently denser on siltstone than limestone (Liversage et al. 2014). Due to their consistently sparse space occupancies, sessile invertebrates were not evaluated for these early successional assemblages. My research builds upon these short-term results (<1 year) by investigating assemblage structure, species richness and the abundances of individual taxa for the upper and lower surfaces of boulders transplanted over six years. These biotic dependent variables were investigated for both mobile and sessile assemblages later in succession. As Liversage et al. (2014) reported significant differences in the rugosity and microhabitat density of limestone and siltstone boulders, here I also determined whether these physical attributes, along with boulder surface area to account for possible species-area relationships, were associated with rock-related biotic patterns. This appears to represent the first boulder-transplant experiment with a duration exceeding one year, presenting a unique opportunity to consider the following four null hypotheses (H1-H4) for later-successional stages on boulders of differing rock:

H1) Assemblages will not differ between transplanted limestone and siltstone; I predict that the assemblages on different rocks will continue to diverge as the communities develop over 5-6 years since initial transplantation;

H2) Assemblages will not differ between native and transplanted boulders of the same rock; I predict that the assemblages on transplanted boulders that are of the same rock type as the reef substratum will converge on the native boulders over time;

H3) Assemblages will not differ between the earlier (11 months) and later (5 or 6 years) stages of succession; I predict that the communities that establish on transplanted boulders after 5-6 years will differ to those that had developed after 11 months; and

H4) Patterns of association between later-successional assemblages and different rocks are not correlated with rock physical attributes; I predict differences in the assemblages will be correlated with the greater surface complexity of limestone compared to siltstone boulders (Liversage et al. 2014).

Materials & methods

Experimental locations and rock types

Boulder translocations were completed at four rocky seashores along South Australia's geologically-diverse Fleurieu Peninsula (Figure 3.1, Liversage et al. 2014). All four seashores have a westerly aspect and are subject to similar oceanic and environmental conditions such as wave exposure and air and sea surface temperature. Each seashore was located along an 80 km stretch of coast on the south-eastern side of a large inverse estuary, Gulf St Vincent, which provides protection from the Southern Ocean’s large waves and swell. Two seashores were dominated by siltstone, Myponga Beach (35°22’ S, 138°23’ E) and Marino Rocks (35°02’ S, 138°30’E), while two seashores were dominated by fossiliferous limestone, Blanche Point (35°14’ S, 138°27’ E) and Southport (35°10’ S, 138°27’ E) (Figure 3.1). Transplants were completed using representative bare boulders sourced from above the tide line at Southport for limestone and Marino Rocks for siltstone.

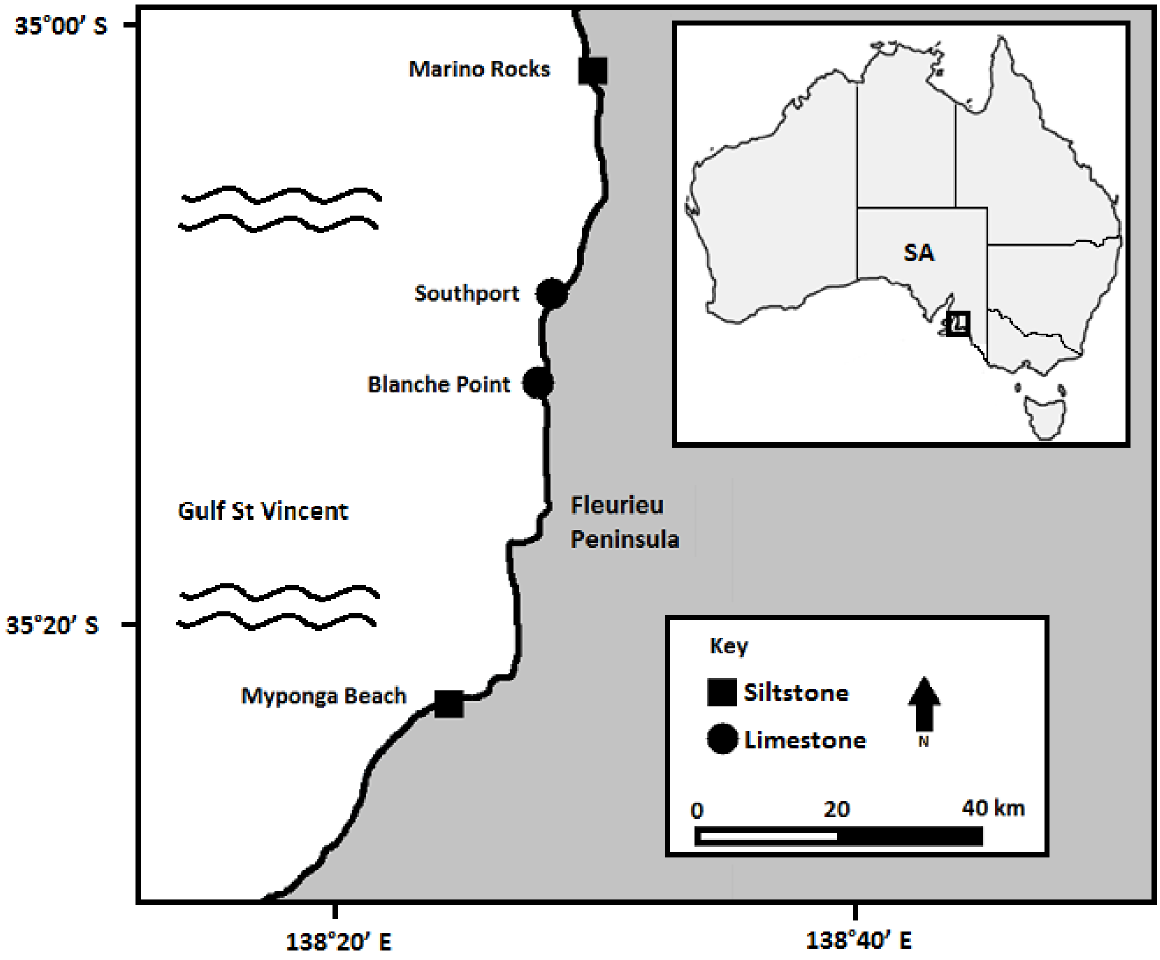

Figure 3.1: Rocky seashores used during experimental boulder translocations along South Australia's Fleurieu Peninsula. Inset map shows the location of the study region in Australia.

Fossiliferous limestone boulders were a light grey in colour (Appendix 2, Figure 8.6) and had a softer scratch hardness of 4 on Moh's scale, while siltstone boulders were a darker greyish yellow-brown in colour and had a harder scratch hardness of 8 (Appendix 2, Table 8.25). Both rocks had a mineralogy dominated by silicon dioxide (SiO_2) although limestone contained a higher percentage (71.6 %) of SiO_2 than siltstone (55.4 %, Appendix 2, Table 8.25). Siltstone had a higher content of several major minerals and trace metals than limestone including aluminium oxide, iron oxide, magnesium oxide, barium, manganese and zirconium (Appendix 2, Table 8.25). Siltstone boulders, which were much heavier in weight than limestone, were generally ovoid in shape, while fossiliferous limestone formed prism-shaped boulders (Appendix 2, Figure 8.6). Consequently, boulder weight, rather than shape, may have influenced disturbance regimes, as lighter-weight limestone boulders were presumably more susceptible to disturbance by waves.

Experimental design

Bare boulder translocations were completed between November 2009 and February 2010. All boulders (length or width up to 30 cm) were collected from above the high tide mark at source seashores, and thus lacked any established marine assemblages on their surfaces including visible biofilms (see Liversage et al. 2014 for full description). Transplanted boulders (60 per rock type) were tagged and randomly allocated on each shore with alternative rock types placed 10 m apart along mid-low shore parallel transects. Independent boulders were sampled by Liversage et al. (2014) after 1, 4 and 11 months, then I resampled as many as could be found at each seashore (Table 3.1) during suitable daytime low tides (predicted low tide ≤0.60 m Australian Height Datum) during the austral summer some 5 years later in February 2015, and again in February-March 2016 after 6 years had elapsed. Controls, consisting of non-transplanted and untagged boulders native to each seashore (hereafter referred to as '*native'* boulders), were also sampled (Table 3.1). At Myponga Beach and Marino Rocks native boulders were grey siltstone, while at Blanche Point and Southport native boulders were fossiliferous limestone. Native boulders were sampled to determine whether the species composition on transplanted boulders of the same rock had converged with the assemblages naturally present, over a timeframe of five or six years.

Table 3.1: Number of transplanted limestone, transplanted siltstone, native limestone, and native siltstone boulders sampled at each seashore, for each sampling time. Initially 60 limestone and 60 siltstone boulders were transplanted onto each seashore between November 2009 and February 2010. The tabulated numbers refer to the number of boulders that could be relocated and sampled 5 or 6 years later. '-' = not applicable as no native rocks of that sort occurred at that seashore.

History:	**Transplanted**				**Native**			
Rock:	**Limestone**		**Siltstone**		**Limestone**		**Siltstone**	
Sampling time (yr):	**5**	**6**	**5**	**6**	**5**	**6**	**5**	**6**
Seashore								
Marino Rocks	10	7	10	7	-	-	10	7
Myponga Beach	10	7	10	7	-	-	10	7
Southport	10	4	10	6	10	6	-	-
Blanche Point	7	7	10	7	10	7	-	-

Sampling procedures

Lower surfaces are defined as the underside of the boulder that was in contact with the substratum, while upper surfaces are defined as all remaining surfaces that were not in contact with the substratum. Consequently, upper surfaces generally encompassed both the top and sides of boulders and so were larger (Appendix 2, Figure 8.7). Upper and lower surfaces were sampled separately as they often support different assemblages (McGuinness and Underwood 1986). I therefore hypothesised that associations between rock type and later-successional assemblages would be surface specific. All invertebrate and algal species >5 mm in size were identified and recorded *in situ* wherever possible (Appendix 2, Table 8.26). Digital photographs were used to subsequently identify any ambiguous specimens that could not be accurately identified in the field. Sampling was restricted to macroscopic species that could be observed *in situ*. Species richness was estimated as the number of species per boulder. The space occupancy of sessile species (algae, tubeworms and mussels) was quantified using a flexible but inelastic quadrat marked in cm^2. Sessile assemblage coverage was recorded as the total percentage of the boulder surface occupied by all sessile species. Barnacles did not form dense aggregations and hence their space occupancies were often zero, so they were counted and analysed separately. The abundance of all mobile-invertebrate species was counted per boulder surface and recorded as the 'mobile assemblage'. Boulder surface area, microhabitat density (i.e. surface features >13 mm in size; *sensu* Liversage et al. 2014) and surface rugosity were all measured using the methods described in Liversage et al. (2014).

Statistical analyses

All analyses were completed using PRIMER v7/PERMANOVA+ software. All tests of PERMutational ANalysis Of VAriance (PERMANOVA) were completed using permutations of residuals with 9999 permutations as described in Anderson et al. (2008). A Monte Carlo (MC) *p*-value replaced a permuted *p*-value when the number of available unique permutations was <100 (Anderson et al. 2008). Differences ($\alpha = 0.05$) among rocks (limestone, siltstone), seashores (Myponga Beach, Blanche Point, Southport, Marino Rocks) and sampling times (specific to each analysis) were assumed to be fixed factors or analysed separately. Separate PERMANOVAs were completed for upper versus lower surfaces, and for the five- and six-year transplant times to ensure data independence, as the upper and lower surfaces of the same boulders were sampled, and I could not be certain whether the same subset of boulders had been sampled after five and six years. Bray-Curtis resemblances were used for species assemblage data and Euclidean distances were used for univariate data, including physical variables. For analyses based on multivariate assemblage data, a dummy variable of 1 was added because of the sparsity of non-zero values among samples (Anderson et al. 2008). Multi-dimensional scaling (MDS) bootstrapped-averages ordination plots were used to visualise significant assemblage differences. SIMilarity PERcentages (SIMPER) were used to calculate the average dissimilarity between rocks, and to identify any consistent (i.e. SD/Similarity Ratio > 1) indicators associated with rock-related differences.

Biotic differences among native and transplanted boulders were tested using a one-factor PERMANOVA with *a priori* planned comparisons among four levels of the factor boulder history (i.e. levels were transplanted limestone, transplanted siltstone, native limestone, and native siltstone; a fixed factor). This approach was employed because I predicted that each contrast between a pair of boulder histories and at each seashore could yield results specific to that pairing. A planned comparison between transplanted limestone versus transplanted siltstone was used to test the first hypothesis (H1), while a comparison between transplanted and native boulders of the same rock was used to test the second hypothesis (H2). For the PERMANOVA, all univariate biodiversity measurements were square-root transformed. To overcome limitations with the hierarchical experimental design (i.e. whilst transplanted boulders were orthogonal with relation to shores, native boulders could only be nested within seashores), separate planned comparisons were completed for data from each seashore, for each surface and sampling time.

To assess assemblage development during succession (H3), comparisons were made between later-successional assemblages and the 11-month early-successional assemblages as reported by Liversage et al. (2014). Square-root transformations were applied to all biodiversity measurements. A three-factor PERMANOVA tested for differences between rocks, sampling times (11 months, 5 years and 6 years) and seashores. The interaction term Seashore x Rock x Sampling time was excluded from analyses as the same subset of boulders may have been sampled after 5 and 6 years. PERMANOVA pair-wise tests were completed for the interaction term Rock x Sampling time to determine significantly-different sampling times for each rock.

Univariate PERMANOVAs confirmed that limestone had significantly more microhabitats and a lower surface rugosity than siltstone boulders (rugosity index closer to 1) (Appendix 2, Figure 8.7) and, as expected, the upper surfaces of boulders had a greater surface area than lower surfaces for both rocks and some surface- and year-specific differences in the surface area was found between rocks. Thus to determine whether rock physical attributes were correlated with assemblage differences (H4), a BIOENV analysis was completed separately for each surface. The BEST (BIOENV) procedure searches for a combination of environmental variables whose inter-sample resemblances best match those arising from multivariate biological data (Anderson et al. 2008). Physical attribute data (rugosity, microhabitat density and surface area) were normalised, with square-root transformations applied to assemblage data. A BEST analysis tested the significance and correlation strength between the best combination of normalised physical attributes and Bray-Curtis resemblances of mobile or sessile assemblage structure. A RELATE analysis between Euclidean distances of physical attributes and Bray-Curtis resemblances of assemblage structure tested for a significant relationship between biological and physical variables.

Results

H1: Assemblages will not differ between transplanted limestone and siltstone.

Later-successional assemblages were often different between transplanted limestone and siltstone (Table 3.2, Appendix 2, Table 8.27). For upper surfaces, 16 from 32 analyses detected significant assemblage differences, while 10 from 32 analyses detected assemblage differences for lower surfaces (Table 3.2). These significant assemblage differences for both surfaces were highly variable among seashores and sampling times (Appendix 2, Table 8.27).

Table 3.2: Tally of significant (S) versus non-significant (NS) PERMANOVA results out of 8 tests per surface [from 4 seashores each sampled twice after 5 & 6 years] for each biotic variable comparing between transplanted limestone versus siltstone, and between transplanted and native boulders of the same rock. See Tables 8.27 & 8.29 in Appendix 2 for PERMANOVA tests.

Comparison:	**Limestone v siltstone**				**Transplant v native**			
Surface:	**Upper**		**Lower**		**Upper**		**Lower**	
PERMANOVA result:	**S**	**NS**	**S**	**NS**	**S**	**NS**	**S**	**NS**
Biotic variable								
Mobile assemblage structure	4	4	1	7	1	7	1	7
Sessile assemblage structure	4	4	5	3	0	8	0	8
Species richness	5	3	3	5	1	7	0	8
Barnacle abundance	3	5	1	7	1	7	1	7
Totals	**16**	**16**	**10**	**22**	**3**	**29**	**2**	**30**

There was strong evidence of rock-related differences for later-successional assemblages on siltstone seashores (Appendix 2, Table 8.27 & Figures 8.8 - 8.10). At Marino Rocks, rock-related differences in species richness were evident on both surfaces after five and six years, and the mobile invertebrate assemblage was different on upper surfaces in both years and lower surfaces after six years (Figure 3.2, Appendix 2, Table 8.27 & Figures 8.8 - 8.10). At Myponga Beach, only upper surfaces appeared to develop significantly divergent assemblages, with most assemblage and barnacle abundance differences specific to boulders sampled after five years. On lower surfaces at Myponga Beach, only sessile assemblage structure was significantly different. At the two limestone seashores there was less evidence of rock-related assemblage differences (Appendix 2, Table 8.27), with the exception of barnacle abundance differing on upper and lower surfaces at Southport and upper surfaces at Blanche Point after five years. Generally, the few differences detected between rocks were unique to a specific surface and sampling time (Appendix 2, Table 8.27 & Figures 8.8 - 8.10).

For mobile assemblage structure, a greater number of rock-related differences were detected on upper than lower surfaces (Table 3.2). These structural differences were associated with a small number of consistent indicator species (Appendix 2, Table 8.28). The snail *Bembicium nanum* was always more abundant on the upper surfaces of transplanted limestone. *Notoacmea* spp. was typically more abundant on siltstone, although after six years at Marino Rocks, these limpets were more abundant on limestone. The rock-related differences in sessile assemblage structure were consistently associated with a higher space occupancy of the tubeworm *Galeolaria caespitosa* and a visible biofilm on limestone (Appendix 2, Table 8.28).

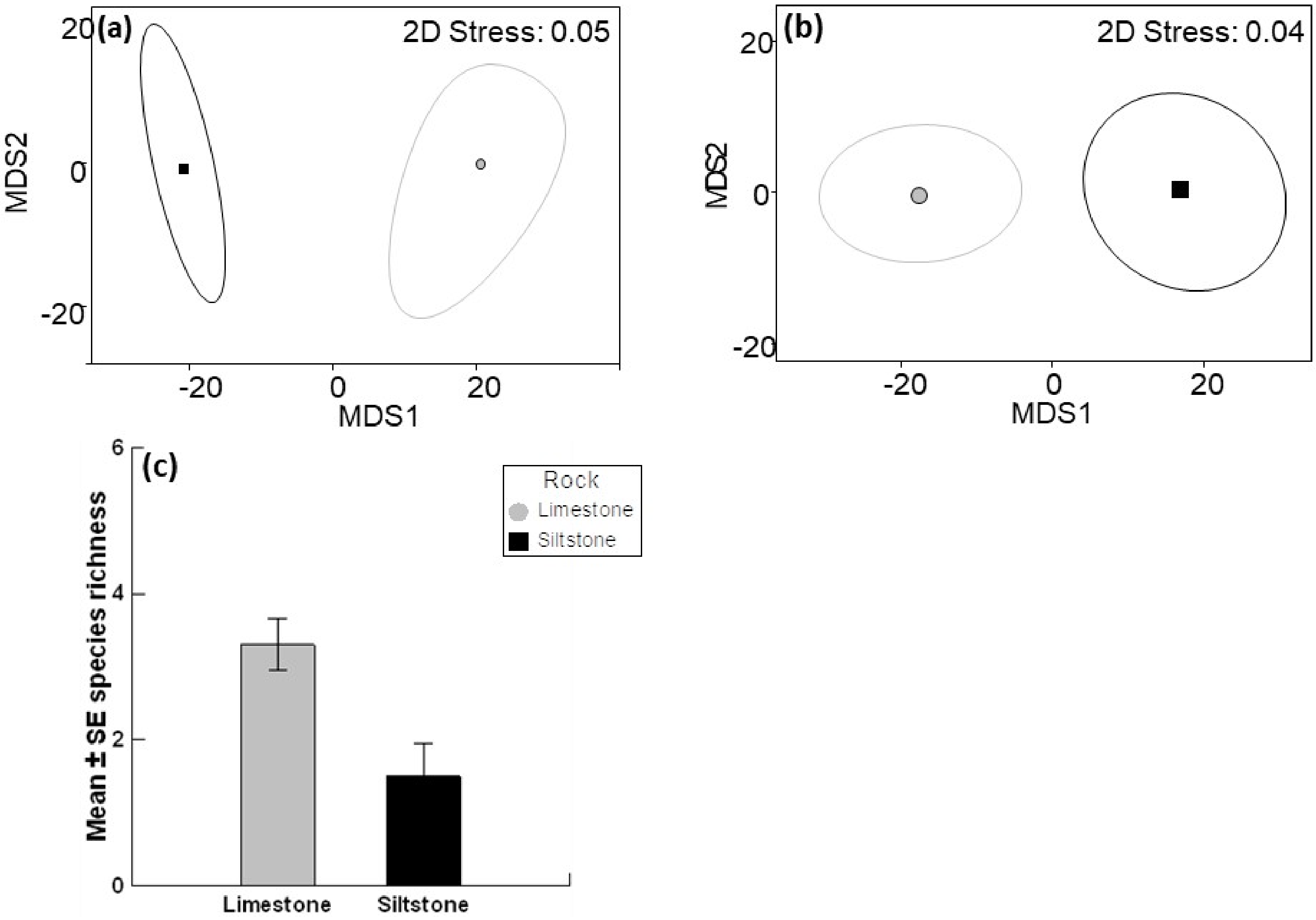

Figure 3.2: Significantly divergent assemblages that developed over 5 years on the upper surfaces of transplanted limestone versus siltstone at Marino Rocks. Multi-dimensional scaling (MDS) bootstrapped-averages ordination plots depicting rock differences for (a) mobile assemblage structure and (b) sessile assemblage structure, and mean (± SE) differences between rocks for (c) species richness (number of species per boulder). Please see Figures 8.3-8.5 in Appendix 2 for complete summary plots of all significant differences detected.

The species richness of later-successional assemblages on transplanted boulders was significantly different between rocks, with a greater number of differences on upper than lower surfaces (Table 3.2, Appendix 2, Figure 8.10). Species richness differences were consistent across surfaces and sampling times, with the assemblages on limestone generally having a higher species richness than siltstone (Appendix 2, Figure 8.10). The abundance of barnacles was generally similar between rocks, with rock-related differences recorded on just four occasions overall and no obvious rock-related trend apparent (Table 3.2, Appendix 2, Figure 8.10).

H2: Assemblages will not differ between native and transplanted boulders of the same rock. Assemblage structure and species richness were generally similar between native and transplanted boulders of the same rock for both sampling times (Table 3.2, Appendix 2, Table 8.29). For upper surfaces, just 3 from 32 analyses detected significant assemblage differences (Table 3.2). No significant differences were detected between transplanted and native boulders, for either surface, for any of the variables measured after five years (Appendix 2, Table 8.29). Twelve months later, mobile-invertebrate assemblage differences were detected for siltstone upper surfaces at Marino Rocks and limestone lower surfaces at Southport. Species richness was different on lower surfaces at both limestone seashores, whilst barnacle abundance was different on upper surfaces at Southport (Appendix 2, Table 8.29).

H3: Assemblages will not differ between the earlier (11 months) and later (5 or 6 years) stages of succession.

For the upper and lower surfaces of both rocks, there was limited evidence for a change in species composition between earlier- and later-successional assemblages (Table 3.3). Later-successional assemblages shared 12-16 species in common with assemblages sampled after 11 months, predominantly abundant limpet and snail species (Table 3.3). However, there was an exchange of rarer species, which were generally recorded as singletons, between earlier- and later-successional assemblages, with the greatest changes recorded on limestone lower surfaces (Table 3.3). Nevertheless, the following later successional species were only recorded after five and six years on both rock types: spirorbid sp., *Actinia tenebrosa*, *Austrocochlea constricta*, *Zuzara venosa*, with all of these except the spirorbid only occurring on the underside of boulders (Table 3.3). Two small gastropods (*Cantharidella balteata* and *Rissoina* sp.) were detected on both rocks after 11 months, but not on any transplanted boulders after five and six years.

For both surfaces, the mean species richness of early-successional assemblages were lower than in native assemblages on the same rock after one and four months (Figures 3.3 & 3.4). At 11 months these parameters had converged between transplanted and native boulders (see also Liversage et al. 2014). This convergence of species richness was maintained between later-successional and native assemblages on the same rock, as evidenced on limestone (Figure 3.3a & c) and siltstone (Figure 3.4a & c) after five and six years for both surfaces.

Table 3.3: Species compositions on the upper and lower surfaces of transplanted limestone and siltstone for assemblages sampled after 11 months, five years, and six years. Changes in algal species composition could not be evaluated because no data were collected for algae on boulders transplanted for 11 months.

Rock:		**Limestone**						**Siltstone**					
Surface:		**Upper**			**Lower**			**Upper**			**Lower**		
Sampling time: **Species**	**Life history$^{\#}$**	**11**	**5**	**6**	**11**	**5**	**6**	**11**	**5**	**6**	**11**	**5**	**6**
Notopsilus sp.	M, FS								x				
Galeolaria caespitosa	S, FS	x	x	x	x	x	x	x	x	x	x	x	x
Spirorbid sp.	S, FS		x	x		x	x		x	x		x	x
Notoplana australis	M, IF, PL		x						x		x	x	x
Actinia tenebrosa	MS, C, FS					x	x					x	x
Isanemonia australis	S, C, FS					x	x	x				x	x
Ischnochiton elongatus	M, FS				x	x	x				x	x	x
Ischnochiton smaragdinus	M, FS										x		
Montfortula rugosa	M, IF, PL		x	x		x							
Notoacmaea spp.	M, IF, PL	x	x	x	x	x	x	x	x	x	x	x	x
Bembicium nanum	M, E, PL	x	x	x	x	x	x		x	x		x	x
Bembicium vittatum	M, E, PL	x	x	x		x				x		x	
Cellana tramoserica	M, IF, PL	x	x	x	x	x	x	x	x	x	x	x	
Nerita atramentosa	M, E, PL	x	x	x	x	x	x	x		x	x	x	x
Siphonaria diemenensis	M, E, PL	x	x	x	x	x	x	x	x	x	x	x	x
Siphonaria zelandica	M, E, PL	x	x		x	x		x			x		
Austrocochlea constricta	M, IF, PL					x	x					x	x
Austrocochlea porcata	M, IF, PL				x						x	x	
Diloma concamerata	M, IF, PL	x	x	x	x	x	x	x		x	x	x	x
Cantharidella balteata	M, IF, PL	x			x			x			x		
Rissoina sp.	M, IF, PL				x						x		
Haustrum vinosum	M, E, PL					x							
Brachidontes rostratus	S, FS		x									x	
Xenostrobus pulex	S, FS	x	x		x	x	x	x	x		x	x	x
Halicarcinus ovatus	M, B, PL	x											
Ozius truncatus	M, B, PL				x	x	x				x	x	x
Cyclograpsus granulosus	M, B, PL						x						
Euidotea bakeri	M, B, PL						x						x
Zuzara venosa	M, B, PL					x	x						x
Chthamalus antennatus	S, IF, PL	x	x	x	x	x	x	x	x	x	x	x	x
Tetraclitella purpurascens	S, IF, PL	x		x	x	x	x			x	x	x	x
Total species		**14**	**15**	**12**	**16**	**21**	**19**	**11**	**10**	**11**	**17**	**20**	**18**

M = Mobile; S = Sessile; MS = mostly sessile, limited pedal locomotion; C = clonal reproduction; B = brooding; E = benthic egg mass, IF = internal fertilisation; FS = free spawning; PL = pelagic larvae.

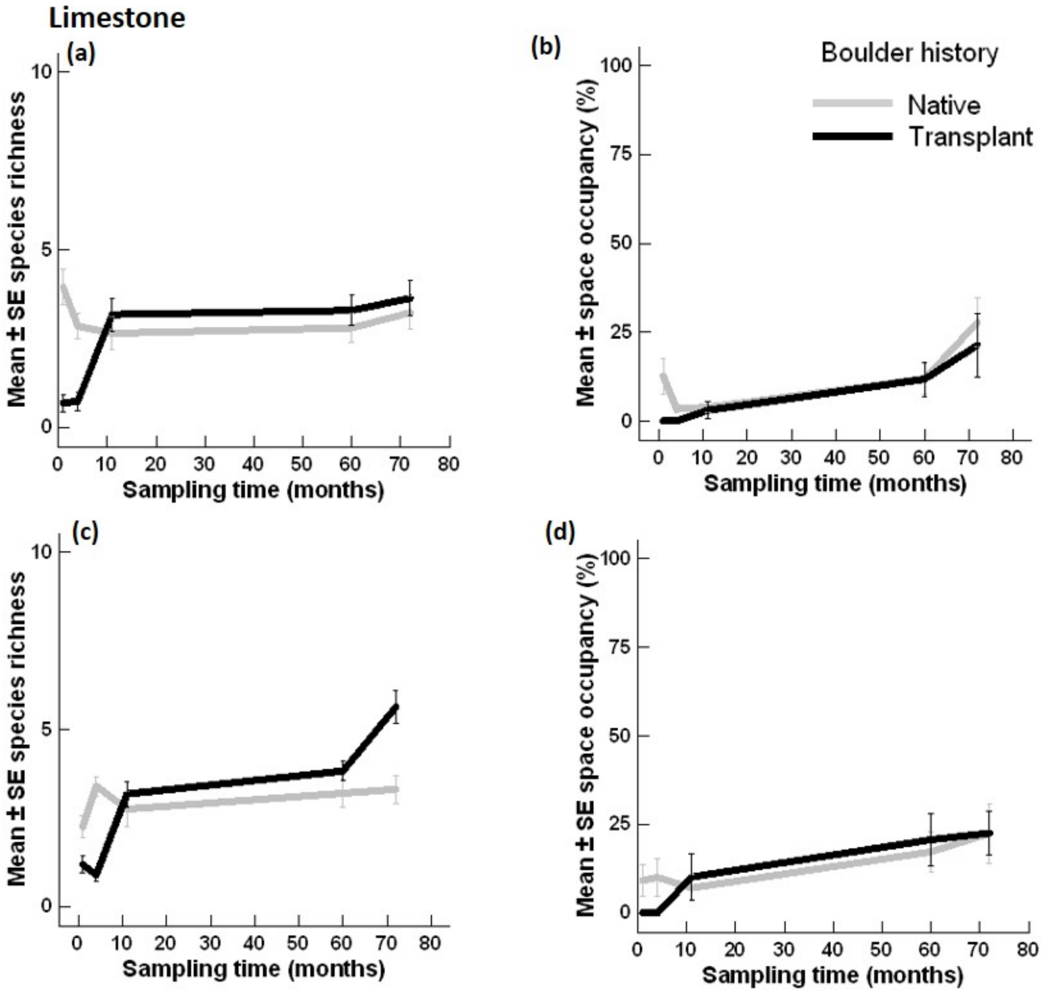

Figure 3.3: Trajectories over time, averaged across limestone seashores, showing convergence of assemblages on transplanted with native boulders for upper (a-b) and lower (c-d) surfaces for species richness (a, c) and space occupancy (b, d). Each y-axis extends to encompass the full range of the raw data.

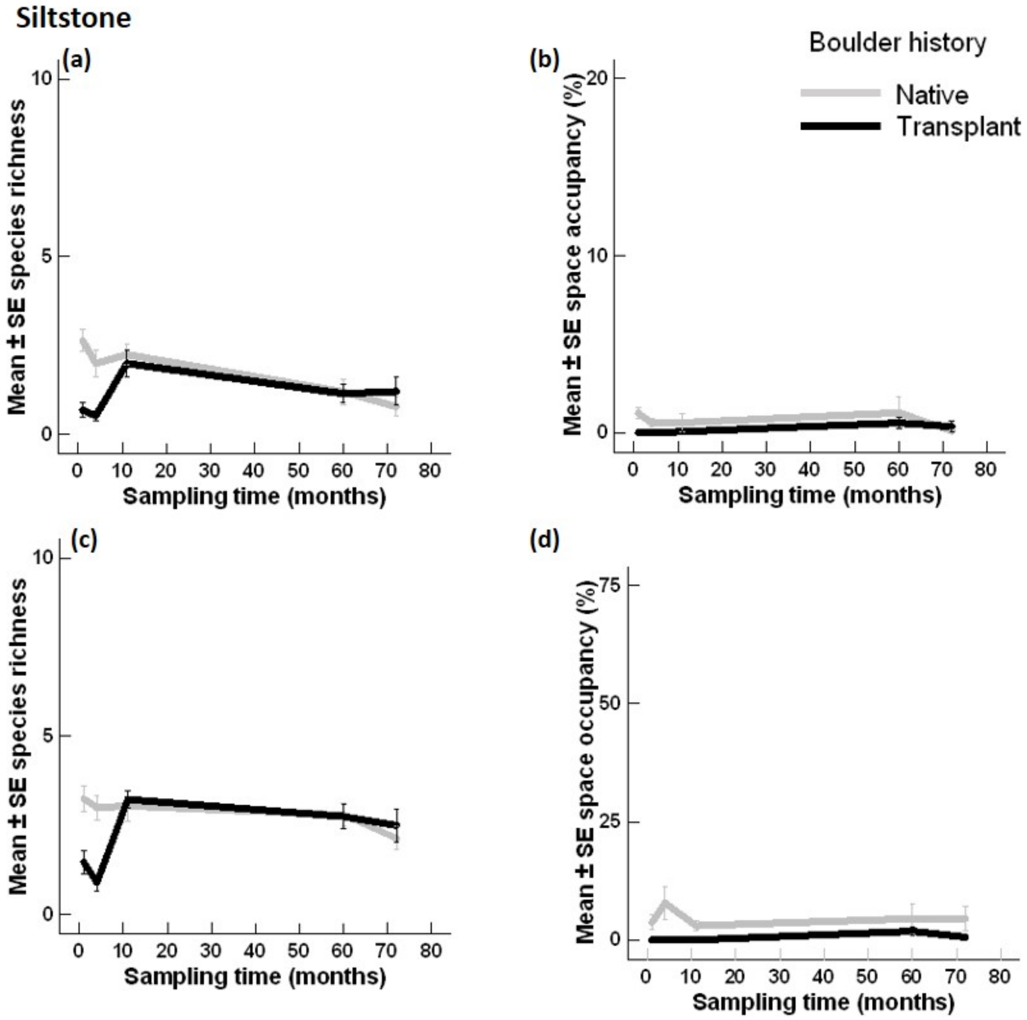

Figure 3.4: Trajectories over time, averaged across siltstone seashores, showing convergence of assemblages on transplanted siltstone with native boulders for upper (a-b) and lower (c-d) surfaces for species richness (a, c) and space occupancy (d, e). Each y-axis extends to encompass the full range of the raw data.

At limestone seashores, a similar convergence over time was observed on upper and lower surfaces for the mean sessile-assemblage space occupancy on transplanted versus native limestone boulders (Figure 3.3b & d). Minimal recruitment was recorded during the first four months (mean space occupancy <0.1 % per boulder surface) but, by 11 months, the sessile assemblage that had recruited to transplanted limestone had a similar space occupancy to native limestone assemblages (Figure 3.3b & d). At siltstone seashores total space occupancy was consistently low, with the sessile space occupancy on transplanted siltstone after 11 months (mean space occupancy <0.1 % per boulder surface) markedly lower than on native siltstone, especially on lower surfaces (Figure 3.4b). By five years, sessile space occupancy on transplanted boulders converged with that on native siltstone (Figure 3.4b & d).

On upper and lower boulder surfaces, the later-successional assemblages sampled after five and six years were generally similar to the earlier-successional assemblages sampled after 11 months described by Liversage et al. (2014) (Appendix 2, Table 8.30). However, I detected significant Sampling time x Rock interactions for sessile assemblage structure on both surfaces and species richness on lower surfaces (Appendix 2, Table 8.30). Post hoc tests detected differences in the sessile assemblages on lower surfaces of limestone after 11 months compared to 6 years, and siltstone after 11 months compared to both later-successional times (PERMANOVA pair-wise permuted *p*-values <0.05). These structural differences were associated with *G. caespitosa* space-occupancy (SIMPER SD/Sim ratio >1), which more than doubled on both rocks between earlier (11-month grand mean = 3.8 ± 1.8 %) and later (5-year grand mean = 10.2 ± 2.0 %, 6-year grand mean = 8.6 ± 2.1 %) times.

H4) Patterns of association between later-successional assemblages and different rocks are not correlated with rock physical attributes.

A significant relationship was detected between ordinations of mobile invertebrate assemblage structure and physical attributes for upper (RELATE Spearman Rho $\rho = 0.102$, $p = 0.0013$), but not lower surfaces (RELATE Spearman Rho $\rho = -0.019$, $p = 0.6846$). For upper surfaces, mobile-assemblage structure was weakly, but significantly, correlated with rock differences in microhabitat density combined with surface area (BEST $\rho = 0.106$, $p = 0.0040$). Likewise, significant relationships were detected between ordinations of sessile assemblage structure and rock physical attributes for both upper (RELATE Spearman Rho $\rho = 0.118$, $p = 0.0082$) and lower surfaces (RELATE Spearman Rho $\rho = 0.059$, $p = 0.0459$). Rock-related patterns of sessile assemblage structure for upper surfaces were weakly, but significantly, correlated with rock differences in microhabitat density combined with surface rugosity (BEST $\rho = 0.127$, $p = 0.0170$) but no physical attributes were correlated to sessile invertebrate structure on lower surfaces, (BEST $\rho = 0.059$, $p = 0.1330$).

The higher abundances of *B. nanum* on upper limestone surfaces may be associated with their greater surface area and complexity than siltstone. Statistically-significant weak-to-moderate relationships were detected between *B. nanum* abundance and surface area (r value = 0.348, p-value = <0.01), rugosity (r value = -0.281, p-value <0.01) and microhabitat density (r value = 0.560, p-value <0.01). However, the r^2 values for each of these relationships explained less than one-third of the total variation between *B. nanum* abundance and each physical attribute. The higher space occupancy of *G. caespitosa* on limestone was only related with rock differences in microhabitat density on both upper (r value = -0.230, p-value =0.02) and lower surfaces (r value = 0.270, p-value <0.01). The r^2

values for these relationships explained <10 % of the total variation between *G. caespitosa* abundance and microhabitat density.

Discussion

By re-sampling an earlier transplant experiment (Liversage et al. 2014) after five and then six years, the present study tracked assemblage development on different bare rocks from earliest recruitment through to later stages of succession. Before this work, transplant experiments addressing hypotheses about the effects of rock on succession were completed within one year, so only early differences in colonisation were known (McGuinness and Underwood 1986; James and Underwood 1994; Green et al. 2012). My study overall identified some early-successional rock-type differences (Liversage et al. 2014), then later confirmed distinct rock differences in the structure and richness of later-successional assemblages.

The assemblages that developed on transplanted boulders were dominated by sparse mobile or sessile invertebrates. This composition contrasts strongly with the algal dominance on boulders manipulated along the California coast that first tested hypotheses about succession in intertidal boulderfields (Sousa 1979a; 1979b; 1980). Those pioneering experiments identified a highly-variable mosaic of successional stages on boulders, with species richness peaking earlier in succession and declining through later stages (Sousa 1979a; 1979b; 1980; Connell and Keough 1985). The assemblages on my transplanted boulders appeared to follow a similar pattern of succession. Transplanted boulders supported a highly-variable mosaic of later-successional assemblages, differing between seashores and sampling times. Moreover, siltstone assemblages peaked in species richness earlier in succession (i.e. by 11 months), before declining thereafter. In contrast, limestone assemblages peaked in species richness later during the overall succession (i.e. by five years), highlighting how rock as an environmental variable can influence succession.

My results showed that later-successional assemblages were in many ways similar to earlier successional assemblages sampled after 11 months. The only consistent rock difference identified by 11 months was higher abundance of the limpets *Notoacmea* spp. on siltstone (Liversage et al. 2014). This difference appeared to persist after five years but not after six, where abundances were often higher on limestone. At 11 months there was also a trend (albeit not significant) for higher species richness and abundances of *Bembicium nanum* and *Galeolaria caespitosa* on limestone versus siltstone. These rock-related trends ultimately manifested into significant differences late in succession. Similarly, in previous long-term successional studies on intertidal rock platforms, depending on the patch size, some changes in community structure took over four years to manifest (McCook and Chapman 1997; Petraitis and Dudgeon 2005; Petraitis et al. 2009). My results indicate that the later-successional assemblages that developed on different rocks were subtly different from early assemblages, such that early trends became later differences. Consequently, had this experiment concluded within the historical timeframe of <1 year, successional differences between limestone and siltstone that took multi-year timescales to manifest would never have been identified.

The species richness and space occupancy of earlier-successional assemblages generally converged with assemblages on native boulders within 11 months (Liversage et al. 2014), although sessile-taxa space occupancy on siltstone took longer. This 11-month convergence was consistent with other transplant experiments, which had reported rapid recruitment onto initially-bare boulders over several weeks to months (McGuinness and Underwood 1986; James and Underwood 1994; Chapman 2002b). Despite no major changes in assemblage structure from 11 months to five years, there was some evidence of species turnover and several species were only found on transplanted boulders after 5 or 6 years. Of these, spirorbids are *r*-selected free spawners that have been found to recruit to panels within 6 months in other Australian studies (Anderson and Underwood 1997). Therefore, the late arrival of this species may simply reflect the timing of a settlement event, followed by sufficient growth for detection with the naked eye. Conversely, sea anemones may be later successional species, as a study of intertidal succession in South Africa found no *Actinia equina* after 8 or 9 years across three locations (Dye 1992). Some mobile species like the brooding isopod *Zuzara venosa* and gastropod *Austrocochlea constricta* were also only detected after 5-6 years on transplants, where as two small gastropods were only detected within the first year (see Table 3.3). However, as all these species were uncommon, I cannot confirm successional patterns over shorter temporal variation. Unlike previous studies on algal communities (Sousa 1980), I did not detect any successional patterns that aligned with specific life-history traits.

When the test results from every planned comparison between transplanted limestone versus siltstone were summed for both surfaces, 26 significant differences were detected (Table 3.2). In contrast, just five significant differences were detected from planned comparisons between transplanted and native boulders of the same rock. This provides strong evidence for an association between rocks and later-successional assemblages. My prediction that such associations may be surface dependent was not supported. Six more rock-related biotic differences were detected on upper than lower surfaces, but the structural and richness differences identified, and the species associated with those differences, were similar for both surfaces. Ten more (18 versus 8) significant rock-type differences were detected on siltstone versus limestone seashores, with one reef-scale (i.e. between seashores) rock-type difference detected (for mobile invertebrates on lower surfaces after 5 years, Appendix 2, Table 8.31 & Figure 8.11). This result emphasises the scale dependency of rock-related differences in assemblages, with differences generally being specific to either the reef or boulder scale (McGuinness 1988, Green et al. 2012, Liversage et al. 2014). The recurrence of this outcome from multiple transplant experiments highlights the importance of studying multiple spatial scales to fully assess the associations between lithology and boulder assemblages during succession.

The consistently higher species richness on limestone than siltstone is a finding that is unique to later-successional assemblages, with no significant rock-related richness differences identified earlier during succession (Liversage et al. 2014) or for earlier-successional assemblages on boulders transplanted elsewhere (McGuinness and Underwood 1986; Green et al. 2012). Instead, these results mirror those reported for established assemblages on Italian subtidal reefs, where limestone supported a higher benthic species richness than granite or quartzite (Bavestrello et al. 2000; Guidetti et al. 2004). It has been well established that substrata with the greatest surface complexity often support the highest species richness (Blanchard and Bourget 1999; Le Hir and Hily 2005; Cosentino and Giacobbe 2015; Loke and Todd 2016). Consequently, it is likely that the surface complexity of limestone provides suitable habitat for later-succession species than the relatively-flat featureless siltstone.

Rock-related differences have been reported from earlier-successional studies, associated with higher abundances of one or more species on one of the tested rocks (McGuinness and Underwood 1986; James and Underwood 1994; Green et al. 2012; Liversage et al. 2014). I identified a range of different taxa contributing to the rock-related differences in assemblage structure but, unlike earlier-successional studies, the identity of taxa associated with abundance differences was inconsistent, often varying among seashores, sampling times, and surfaces. Only *B. nanum* and *G. caespitosa* displayed a consistent rock association, recorded at higher abundances on limestone than siltstone. For *B. nanum*, abundances were most-strongly related with the higher microhabitat density on limestone, with this snail frequently recorded within depression microhabitats (personal observation), and thus a moderate relationship was detected between their abundance and microhabitat density. It is possible that, by sheltering in depression microhabitats on boulder upper surfaces, *B. nanum* is afforded some protection from the extremes of temperature and desiccation during emersion. This is consistent with this species sheltering in pit microhabitats elsewhere (McGuinness and Underwood 1986). For *G. caespitosa*, abundance differences were also associated with the greater microhabitat density of limestone. Microhabitats provide potential refuges from a range of abiotic stressors and biotic interactions (McGuinness and Underwood 1986) and may facilitate successful establishment of *G. caespitosa* recruits. *G. caespitosa* settlement into disturbed patches shows a behavioural response resulting in gregarious metamorphs (Minchinton 1997). This is consistent with low levels of settlement in the early successional stages of our study, then conspecific attraction from the successful early recruits could facilitate further accumulation in microhabitats on limestone. It is also possible that rock differences in *G. caespitosa* abundance are associated with the lighter colour of limestone, with higher settlement of these white tubeworms recorded on lighter versus darker substrata (Marsden and Anderson 1981).

Despite detecting rock-related differences for later-successional assemblages, surface area, rugosity and microhabitat density accounted for little of this biotic variability on either surface. This contrasts strongly with many other studies, which have shown rock differences in microhabitat density (McGuinness and Underwood 1986; Chapman 2000; Moreira et al. 2007; Chapman and Underwood 2011) and rugosity (Raimondi 1990; Chapman and Underwood 1994; Berntsson et al. 2000; Le Hir and Hily 2005; Herbert and Hawkins 2006; Savoya and Schwindt 2010) to be strongly associated with biota. Additional rock-related physical and chemical differences could be included in future models to further examine the factors influencing intertidal succession. For example, limestone has cooler surface temperatures than siltstone (see Chapter 4) and rock-related temperature differences have been associated with biological patterns elsewhere (Raimondi 1988). Furthermore, mineralogy varies between rocks and could influence settlement, recruitment, persistence or competitive abilities of some species. For example, I detected different amounts of quartz (silica) minerals (i.e. the dominant mineral in both rocks) and differences in silica content are strongly associated with marine biotic patterns elsewhere (Bavestrello et al. 2000; Cerrano et al. 2007).

In conclusion, this study documents assemblage structure and the abundances of individual taxa for later-successional boulder assemblages, with limestone developing assemblages characterised by a higher species richness and higher abundances of some species, than siltstone. These assemblage differences were most-strongly correlated with rock-related differences in surface complexity. Assemblages that developed on transplanted boulders were in most ways similar to established communities on native boulders after both 11 months and 5-6 years, although the subtle changes that transpired allowed early-successional rock-related trends to manifest into significant later-successional differences. These later-successional differences that took multi-year time scales to develop would remain unknown had this experiment concluded within the historical timeframe of <1 year. These results show that succession in intertidal boulderfields is not solely influenced by physical disturbance regimes, with rock type influencing both the early and later stages of succession.

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Chapter 4

Rocks of different mineralogy show different thermal behaviour: implications for biodiversity on rocky seashores

Abstract

Exposure to extreme heat during low tide can subject biota living on rocky seashores to desiccation and heat stress, which may challenge the survival and persistence of some species. Most studies that investigate associations between biota and the temperature of substrata in the rocky intertidal zone provide only limited empirical data about the range of temperature characteristics of the substratum investigated. Moreover, associations between the temperature of substrata and rock mineral composition have rarely been tested. Consequently, a common-garden experiment using intertidal boulders of six rock types tested whether temperature characteristics differed among rocks and whether thermal behaviour was associated with rock mineralogy. This experiment found that temperature of the upper and lower surfaces of all six rocks was heterogeneous at the millimetre to centimetre scale. Three patterns of temperature differences were identified on boulder surfaces: gradients; mosaics; and limited heterogeneity. The frequency of occurrence of these temperature patterns was heavily influenced by cloud cover. Temperature range did not differ consistently between rocks or surfaces, although maximum temperature did. Upper surfaces were generally hotter than lower surfaces, plus purple siltstone and grey siltstone consistently had the hottest temperatures and white limestone and quartzite the coolest. Each rock type had unique major mineral and trace elemental compositions (i.e. mineralogy), with maximum temperatures correlated with the highest metallic oxide and trace metal content of rocks. These baseline data enhance our understanding of rock mineralogy and the temperature characteristics of different rocks and surfaces, such that knowledge can now be used to guide the design of studies aiming to further understand any associations between the temperature of substrata and the biota that use these rocks as habitat.

Introduction

Rocky seashores are one of the most thermally-variable and stressful habitats on Earth. Rocky seashore substrata exposed to insolation can warm by as much as 10 – 20 ˚C while emersed during low tide (Bertness 1999; Helmuth and Hofmann 2001; Harley 2008). This extreme heat and temperature variability presents risks of desiccation and heat stress to intertidal ectotherms (i.e. animals) that use rocky seashores as habitat (Connell 1972; Bertness 1999). Ectotherms do not physiologically regulate their body temperature, instead they track that of the surrounding environment (Caddy-Retalic et al. 2011; Chapperon and Seuront 2011a; Madeira et al. 2012). Exposure to extreme heat during emersion may challenge the survival and ultimately the persistence of some intertidal species. Examples of the impacts of heat stress on intertidal species include mass-mortality events during heatwaves (Helmuth et al. 2002; Harley 2008), lower survival rates in thermally less-favourable habitats (Jones and Boulding 1999; Harley 2008; Gedan et al. 2011; Lathlean et al. 2013), and restricted vertical seashore distributions of some species (Raimondi 1988; Somero 2002; Harley 2003).

In response to these deleterious impacts from exposure to extreme heat, intertidal ectotherms can employ behavioural thermoregulation and/or morphological adaptations to minimise the risks posed by desiccation and heat stress (Pörtner and Farrell 2008). One mode of behavioural thermoregulation involves retreating to the underside of boulders at low tide (Chapman 2003; Chapperon and Seuront 2011a). Boulder lower surfaces are sheltered from insolation and may remain damp when sitting on wet substratum (e.g. rock pools or wet sediment) when emersed. Thus they are purported to provide a cooler and more thermally-stable microhabitat, relative to dry, sun-exposed microhabitats such as rock platforms and boulder upper surfaces (Chapman 2003; Chapperon and Seuront 2011a). Various studies have identified temperature differences between sun-exposed and sun-protected microhabitats in the rocky intertidal zone (e.g. Garrity 1984; Denny et al. 2011; Chapperon et al. 2017), although relatively few have included boulder upper and lower surfaces in their comparisons. Chapperon and Seuront (2011b) showed that sun-exposed flat rock surfaces were 4.2 °C hotter than sun-protected under-rock surfaces, although this was not a direct comparison of boulder upper and lower surfaces *per se*. Therefore, a better understanding of the temperature differences between upper and lower surfaces is necessary to assess the potential magnitude of thermal relief that intertidal ectotherms may experience when retreating under boulders.

With predictions of hotter air temperatures and an increased frequency of extreme-heat events associated with global climate change (IPCC 2013), the survival and persistence of some intertidal species is likely to be challenged further. To make predictions regarding the biological ramifications of global climate change, we must first establish a baseline of the temperature characteristics for intertidal habitats (Helmuth et al. 2002; Gedan et al. 2011; Lough and Hobday 2011). However, we currently lack ongoing physical observations in many shallow-water coastal habitats, including rocky seashores, to adequately describe their baseline environmental conditions (Lough and Hobday 2011). On rocky seashores, including intertidal boulderfields, this is especially true for the general temperature characteristics of the substratum during low tide. While several studies have shown that rock temperature is influenced by its colour (Raimondi 1988; Judge et al. 2011), mineral composition (Gómez-Heras et al. 2006) or size and orientation relative to the sun (Bertness 1999; Chapperon et al. 2016; Chapperon et al. 2017), there is dearth of empirical data detailing the temperature characteristics of seashore rocks.

It is possible that the specific mineral constituents of different rocks influence their temperature characteristics. Investigations of small granitic test cubes have shown that their different mineral constituents can have a temperature range of up to 4 °C (Gómez-Heras et al. 2006). The few studies that tested biotic associations with substratum mineralogy (e.g. Bavestrello et al. 2000; 2008; Cattaneo-Vietti et al. 2002) have focused specifically on the silica content of rocks (i.e. for a range of rocks that vary in quartz minerals). As such, differences in the major mineral and trace elemental composition of seashore rocks are not always investigated, and how the specific mineralogy of each rock is associated with their temperature characteristics untested.

The temperature characteristics of rocks are also influenced by their type. For example, Raimondi (1988) showed that basalt was hotter than granite while Marshall et al. (2010) reported lighter-coloured sandstone was cooler than darker-coloured ferruginous sandstone. Furthermore, while Judge et al. (2011) failed to identify their rock lithology, they reported that black rock was hotter than white rock. These studies aside, relatively few intertidal studies have made temperature comparisons between rock types, with most studies instead describing the thermal characteristics of a single rock from just a single location (e.g. Harley 2008; Chapperon and Seuront 2011a; Seuront and Ng 2016). Many of these studies have shown that observed substratum temperature is not homogenous (e.g. Huey et al. 1989; Gómez-Heras et al. 2006; Helmuth et al. 2006b; Lathlean et al. 2012). Instead, the temperature of substrata is heterogeneous, with temperature differences up to

25.5 °C identified between the hottest and coolest locations (e.g. Huey et al. 1989; Denny et al. 2011; Lathlean et al. 2012). The scale of these patterns of temperature difference can vary enormously, from many kilometres down to millimetres (Helmuth et al. 2006b; Denny et al. 2011; Judge et al. 2011; Lathlean et al. 2012; 2013). Most temperature patterns have been described in terms of comparisons made between hotter microhabitats exposed to sunlight (e.g. flat rock platforms) and cooler microhabitats sheltered from sunlight (e.g. crevices, boulder lower surfaces, overhangs) (e.g. Garrity 1984; Helmuth et al. 2006b; Denny et al. 2011). What currently remains unclear is the type of patterns and magnitude of temperature differences that can develop within a single type of microhabitat, such as over boulder surfaces.

To address these knowledge gaps, I tested whether intertidal boulders of six rock types consistently had patterns of temperature difference (i.e. cooler and warmer areas) on upper and lower surfaces. I also quantified the major mineral and trace elemental composition of each rock type and investigated which minerals were correlated with rocks showing different temperature behaviours. To measure the temperature of six seashore rocks simultaneously exposed to the same conditions, a common-garden experiment, using small boulders, was established. Such an experiment standardises the conditions under which all rocks are studied, thus allowing their temperature performances to be contrasted. Consequently, I tested whether temperature behaviour differed among rock types, and whether rock temperature behaviour was associated with rock mineralogy, allowing me to address the following five objectives:

1) establish whether boulders of different rocks have patterns of temperature differences under common-garden conditions;

2) quantify whether maxima differ between rocks or surfaces;

3) quantify whether temperature ranges differ between rocks or surfaces;

4) quantify whether major mineral or trace elemental composition differs between rocks; and

5) determine whether rock-related differences in maxima are correlated with their mineral composition.

Materials & methods

Boulder selection

The geologically-diverse Fleurieu Peninsula of South Australia (SA) is comprised of a variety of rocks. Six of these, in the form of small boulders (*n* = 6 boulders per rock type, maximum length ≤30 cm), were collected from four seashores (Appendix 3, Figure 8.13). From Southport (35°10′ S, 138°27′ E) boulders of either white fossiliferous limestone or orange fossiliferous limestone were collected, while fossiliferous sandstone that was yellowish brown in colour was collected from Seaford (35°11′ S, 138°28′ E) (Appendix 3, Figures 8.13 & 8.14). The two limestones and the fossiliferous sandstone had coarse surface textures and complex surfaces that were interspersed by cracks and depressions. From Marino Rocks (35°02′ S, 138°30′E) boulders of both purple siltstone and grey siltstone were collected, while quartzite that was greyish to yellow-brown in colour, was collected from O’Sullivan Beach (35°07′ S, 138°28′E) (Appendix 3, Figures 8.13 & 8.14). The two siltstones and the quartzite had smooth surface textures and featureless surfaces that generally lacked cracks or depressions. Six boulders of each rock type were collected (total *N* = 36), with selected boulders spanning the range of thicknesses that occurred for each type on each seashore.

Experimental location

Boulders were transplanted into a paddock on a farm at Kangarilla, which was located approximately 20 km inland from the coast (Appendix 3, Figure 8.13). This setting was selected, over using one of the seashores where boulders were sourced, because there were too many variables that could not be controlled or monitored on the seashore (e.g. wave splash, tidal movement, sediment or wrack deposition, shading by cliffs, under-boulder substratum, angle of repose). Setting the boulders into a sandy beach was not attempted due to the boulder losses sustained during an earlier translocation experiment in the same region (Chapter 3). Issues with sand scour or burial of boulders by sand were also likely on a sandy beach. Moreover, given the large population of the Adelaide region (1.3 million people in 2016), interference with boulders left lying on a beach was considered likely.

Design of the common-garden experiment

A square plot measuring 3 x 3 metres was excavated to a depth of approximately 10 centimetres (Appendix 3, Figure 8.14). The ironstone and soil matrix unearthed was replaced by washed yellow beach sand to simulate substratum matrices where experimental boulders were sourced (Appendix 3, Figure 8.14). The location of this plot was selected to ensure it had an east-west orientation (i.e. to follow the movement of the sun) free from any physical obstructions (e.g. buildings, trees) that might shade the plot. Boulders were arranged on the sand matrix in four groups that each contained nine boulders (Appendix 3, Figure 8.14). Boulders were randomly assigned to each group and their location was re-randomised on four occasions. The boulder plot was covered with a tarpaulin when not in use to restrict weed growth and to keep foreign objects out, and to shelter boulders from insolation prior to sampling.

Rock temperatures were measured on 17 days spread over an 18 month period (Table 4.1) to build up a picture of the range of temperature behaviour. Each day was targeted for its forecast cloud cover and air temperature to investigate generally how boulder temperature may be influenced by seasonality and daily weather conditions. Sampling was completed on days where no rainfall was forecast, as wet surfaces were likely to confound my ability to accurately measure rock temperature (Lathlean and Seuront 2014; Seuront et al. 2018). Approximately 20 minutes before the commencement of each day’s sampling, the tarpaulin was removed from the boulder plot and each boulder was submersed individually in a tub filled with seawater. Submersion wetted the boulder surfaces to simulate conditions on the seashore, where boulder surfaces are wet when first emersed by the receding tide. Each boulder was returned to its specific spot in the plot where it was allowed to drain and dry (this took no longer than 5 – 10 minutes as water rarely permeated boulder surfaces). There was no evidence of differential patterns of drying over this 5 - 10 minute timeframe among the six rock types investigated.

Surface temperature of each boulder was measured at one hour intervals, commencing at 0900 hours local time and concluding at 1400 hours daily. Consequently, six measurements of surface temperature, at one hour intervals, were recorded for each replicate boulder on a given day. This five-hour time course was selected to simulate the average length of time boulders on local mid-lower seashores are emersed during a single low tide, which was approximately four hours at several of the seashores where experimental boulders were sourced (unpublished data). The 0900 – 1400 start and finish times were selected to simulate the timing of summer daytime low tides for seashores on the Fleurieu Peninsula. Sunrise occurred between 0555 hours during the height of summer and 0724 hours during the depths of winter.

Table 4.1: The maximum cloudiness and air temperature recorded during sampling on each day, and the weather condition category that each day was subsequently allocated to based on its maximum cloudiness. Days are arranged in each weather condition category according to increasing maximum air temperatures.

Weather condition	**Date**	**Maximum cloudiness (Okta)**	**Maximum air temperature (˚C)**
Cloudy	09/09/2015	8	15
	18/09/2015	7	17
	06/10/2015	7	21
	25/11/2015	8	30
	19/12/2015	8	38
Sunny	16/07/2016	0	12
	10/09/2015	0	15
	17/10/2015	0	22
	17/04/2017	3	23
	02/10/2015	0	24
	07/01/2016	0	26
	07/02/2016	0	29
	06/02/2016	0	31
	09/10/2015	3	33
	19/11/2015	0	34
	08/02/2017	0	39
	18/11/2015	0	40

Surface temperature was measured with a Fluke Ti20 thermal imaging camera (Fluke Corporation, Everett). The thermal resolution of this camera was ≤0.2 °C at 30 °C, with accuracy to 2 % or 2 °C, whichever was greater. Default camera settings were employed, including emissivity (i.e. the ratio of how well a material emits infrared energy between 0 and 1, with objects measuring 1 a perfect emitter), which was set at 0.95. This default emissivity was deemed appropriate, as previous studies have shown that the emissivity of dry rock generally ranges between 0.95 and 1 (Rivard et al. 1995; Cox and Smith 2011; Lathlean et al. 2012). Surface temperature was captured *in situ*, with archived thermal images processed later. Upper and lower surfaces were imaged separately. In this study, lower surfaces are defined as the underside of the boulder that was in contact with the substratum and thus sheltered from insolation. Upper surfaces are defined as all remaining surfaces that were not in contact with the substratum and were potentially exposed to insolation.

Thermal images were recorded for all upper surfaces first without touching boulders. For lower surfaces, each boulder was briefly flipped upside down, and a thermal image recorded, before the boulder was returned to its original position. Care was taken to avoid shading the plot while recording thermal images. Overall, 72 thermal images were recorded each hour (36 upper and 36 lower surfaces), and 432 images were recorded each day (72 surfaces sampled 6 times). Thus, a total 7344 individual thermal images were collected. Air temperature and cloud cover were also recorded at one-hour intervals when taking images. Air temperature was measured in the shade to the nearest degree Celsius with a glass thermometer. Cloud cover (i.e. sky condition) was estimated by how many eighths of sky were covered by cloud, which ranged from zero oktas (sunny, no clouds) to eight oktas (sky completely cloudy, no sunshine) (Li and Lam 2001). Each day sampled was assigned to one of two weather condition categories based on their cloud cover. Days where the cloud cover never exceeded 3 oktas were assigned the ‘sunny’ category, while days where the cloud cover exceeded 3 oktas during sampling were assigned the ‘cloudy’ category (Table 4.1).

Archived thermal images were subsequently processed using the InsideIR version 4.0 software (Fluke Corporation), taking advantage of the one to eight distinct false colours used to portray temperature on the image (see Appendix 3 for more information). The maximum and minimum temperature of substrata were determined using this false-colour scale for each replicate surface, and a temperature range (maximum - minimum) for each surface was calculated. The orientation, relative to the sun, for the maximum temperature, was categorised as either occurring on the boulder side facing the sun, or on any other boulder side. Transects were drawn on images of boulder surfaces from the centre of the boulder side facing the sun to the centre of the side opposite to quantify millimetre-to-centimetre scale patterns of temperature difference. Analysis of the temperature patterns along transects was undertaken on three sunny and three cloudy days, spanning the range of maximum daily air temperatures sampled. Images for the zero-hour and four-hour exposure times were investigated to look at changes across the day, with the four-hour timeframe simulating the average length of time boulders on the seashore were exposed to insolation during low tide. I focused primarily on maxima due to extreme temperatures having a greater impact on organism survival and fitness (e.g. Jones and Boulding 1999; Harley 2008; Gedan et al. 2011; Monaco et al. 2015).

XRF analyses

The mineralogy of each rock type was determined through X-Ray diffraction (XRF), with separate tests completed for major mineral and trace elemental composition for three samples of each rock type. XRF analysis of each rock sample tested for 11 major minerals and 40 trace elements, with concentrations returned as % and parts per thousand, respectively. For major minerals, approximately one gram of each oven-dried sample (at 105 °C) was accurately weighed with four grams of 12-22 lithium borate flux (Norrish and Hutton 1969). The mixtures were heated to 1050 °C in a platinum/gold crucible for 20 minutes to completely dissolve the sample, and then poured into a 32mm platinum/gold mould heated to a similar temperature (Norrish and Hutton 1969). The melt was cooled rapidly over a compressed air stream and the resulting glass disks were analysed on a PANalyticalAxios Advanced wavelength dispersive XRF system using the CSIRO in-house silicates calibration program. For trace elements, approximately four grams of each oven-dried sample (at 105 °C) was accurately weighed with one gram of Licowax binder and mixed well (Norrish and Hutton 1969). The mixtures were pressed in a 32 mm die at 12 tons pressure and the resulting pellets were analysed on a PANalyticalAxios Advanced wavelength dispersive XRF system using the CSIRO in-house powders program (Norrish and Hutton 1969).

Statistical analyses and data presentation

Frequencies of occurrence (%) for three patterns of temperature difference were tallied on the upper and lower surfaces for boulders of all six rock types after four hours exposure to insolation. Only data for three cloudy versus sunny days are presented, because each day is not strictly independent of each other and the 7000+ thermal images collected showed that weather condition (i.e. whether cloudy or sunny) was the largest determinant of the patterns of temperature difference that developed on boulders. A formal analysis (i.e. Chi square contingency tables) of these frequencies was not reliable because there were too few replicates (for *N* = 36 boulders) for each possible combination of interest of temperature pattern and rock type.

Line charts plotting temperature dependent variables (maxima, range) versus exposure time were used to rank rocks in descending order from 6 (largest) to 1 (smallest) for their mean temperature range and maxima, for each surface on each day after four hours. The sum of ranks allocated to each rock was then used to assign an overall rank to each rock from 6 (largest) to 1 (smallest) for their temperature range and maxima, with the highest ranked rocks having the highest sum of ranks. Upper and lower surfaces were ranked separately. To quantify changes over time exposed, dependent variables for the upper and lower surface of each replicate boulder after four hours were subtracted from the same dependent variables for the same replicate surface at zero hours. The mean difference between zero and four hours for each dependent variable was then determined for the upper and lower surfaces of each rock, on each day sampled, to determine whether four-hourly changes in dependent variables differed between rocks or surfaces. To establish whether dependent variables differed between surfaces, upper-surface dependent variables were subtracted from lower-surface dependent variables, for each replicate boulder, for each exposure time on each day sampled. The resulting difference data were plotted as line charts to visually investigate surface differences over the exposure period.

Analyses were completed using PRIMER v7/PERMANOVA+ (PRIMER-e, Plymouth, UK), with significance set at $\alpha = 0.05$. To test for mineralogical differences between rocks, separate multivariate analyses were completed for major mineral composition and trace element composition, with separate univariate analyses completed for the total content of each major mineral. Untransformed major-mineral data (measured as % composition) were used, while fourth-root-transformed trace-element data (measured as parts per million) were used. Euclidean-distance resemblance matrices were prepared, and PERMutational Analyses Of VAriance (PERMANOVAs) were run to test for mineralogical differences between rocks (purple siltstone, grey siltstone, quartzite, white limestone, orange limestone and fossiliferous sandstone; a fixed factor). For multivariate data, constrained ordination Canonical Analysis of Principal coordinate (CAP) plots (Anderson et al. 2008) were used to visualise rock-related mineralogical differences. A leave-one-out procedure was used to test the allocation success of the discriminant function for rock groupings in CAP, with permutation tests used to test the significance of the trace test statistic and first canonical eigenvalue. Vector overlays of Spearman Rank correlations (for Rho values >0.8) were used to identify the major minerals and trace elements that best characterised the mineralogy of each rock. This vector overlay was then used to investigate how the specific mineral constituents correlated with each rock were associated with substratum maximum temperature.

Results

Objective 1: patterns of temperature difference on upper or lower surfaces of different rocks. Boulder upper and lower surfaces, for all six rock types, had generally a heterogeneous surface temperature differing in maxima and minima after four hours exposure to insolation on all days sampled (i.e. a range of temperatures, Figure 4.1). Moving in any direction across boulder surfaces (a representative thermal image showing each rock type’s surface temperature is provided in Figure 4.2), temperature consisted of warmer and cooler areas with patch sizes <10 cm. When transects were drawn on images from the centre of the boulder side facing the sun to the centre of the side opposite, three patterns of temperature difference were identified. The first pattern was a temperature mosaic, which consisted of heterogeneous temperatures across the entire boulder surface (Figure 4.3a). The temperature difference between the warmest and coolest mosaic areas along transects was ≥5 °C. The second pattern was a temperature gradient, where temperature gradually decreased from the side nearest the sun to the side opposite (Figure 4.3b). The temperature difference between the warmest and coolest gradient areas along transects was ≥5 °C. The third pattern was limited temperature heterogeneity, which consisted of only small temperature differences <5 °C between the warmest and coolest areas along transects (Figure 4.3c).

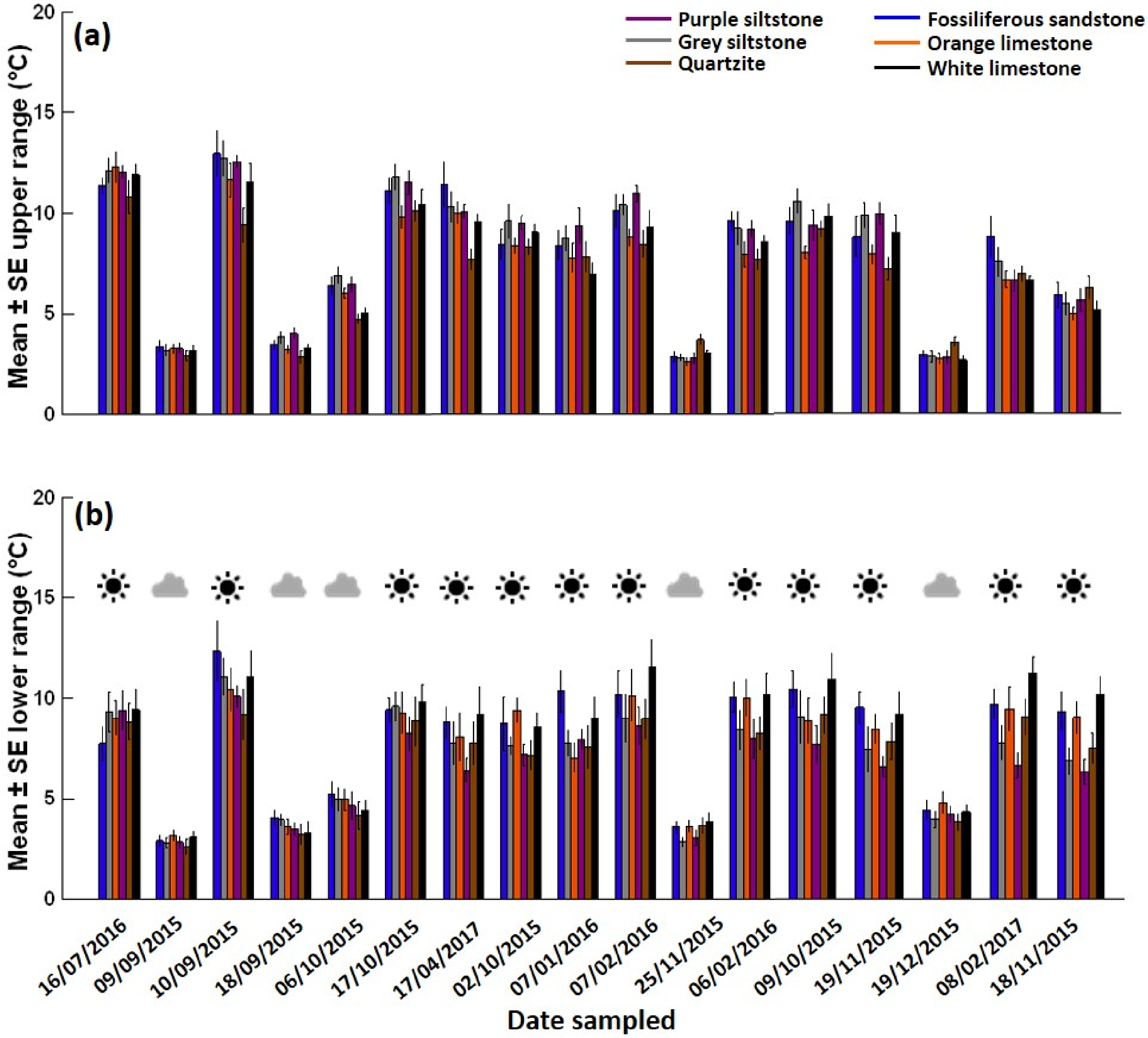

Figure 4.1: Mean ± SE temperature range (*n* = 6 boulders per rock type per day) for (a) upper surfaces and (b) lower surfaces on different days (days ordered by the daily maximum air temperature during sampling) during the common garden experiment. The sun or cloud symbols in panel b denote the day condition for each date sampled.

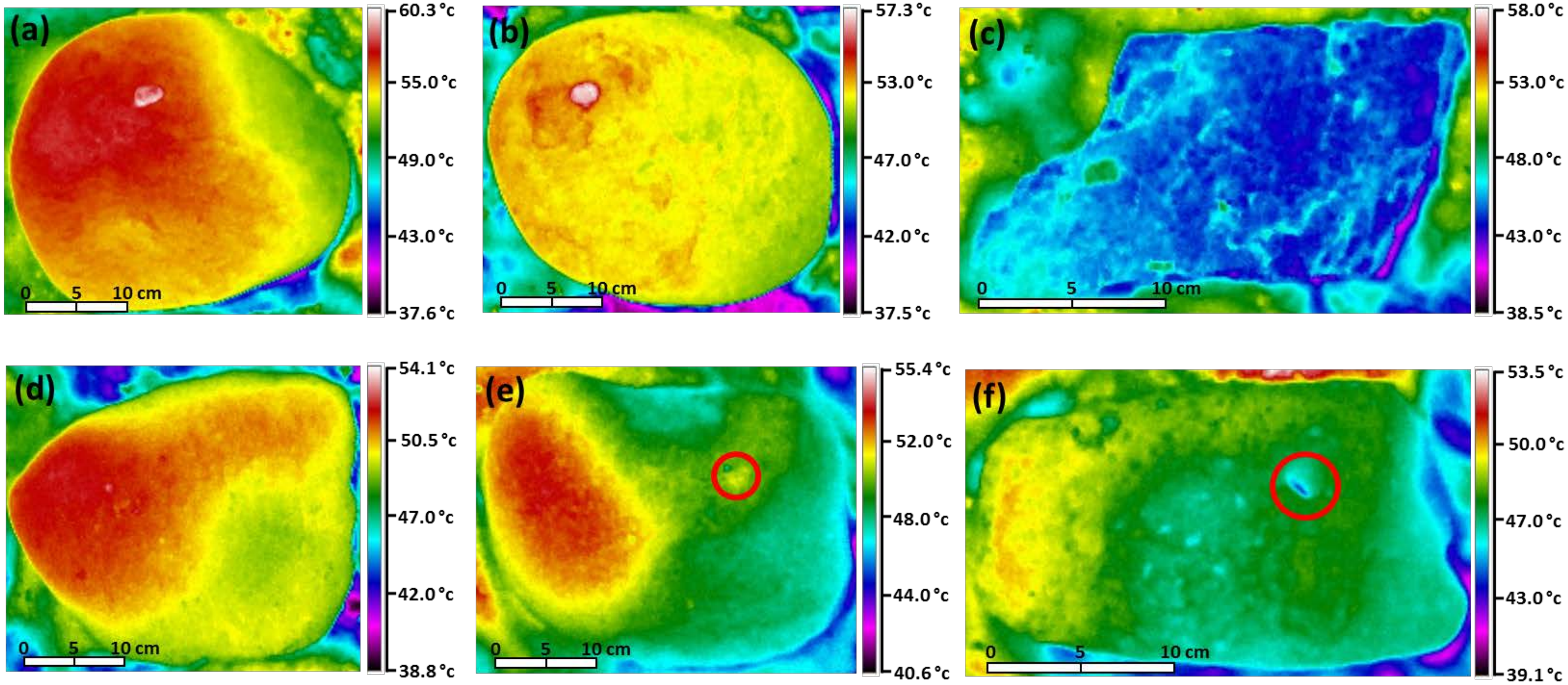

Figure 4.2: Thermal images showing patterns of temperature difference on the upper surfaces of (a) grey siltstone, (b) purple siltstone, (c) quartzite, (d) fossiliferous sandstone, (e) orange limestone and (f) white limestone. Each thermal image was recorded after boulders were exposed to insolation for four hours on the same sunny day (air temperature = 39 °C). Temperature scales (at right of each image) are specific to each rock type. The red circles in panels (e) and (f) depict examples of the interruption of gradient mosaics by microhabitat features.

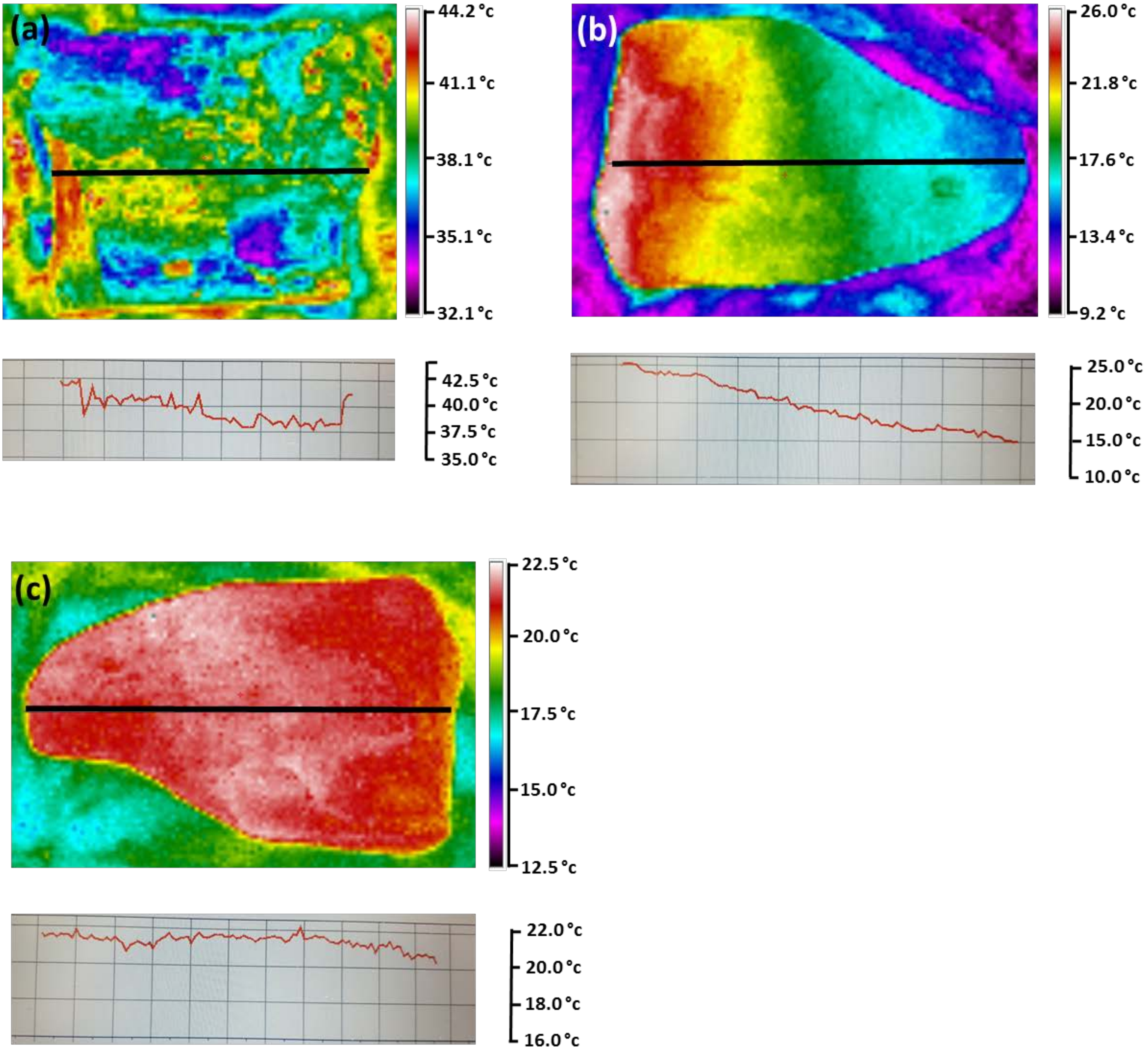

Figure 4.3: Thermal images showing patterns of temperature difference on boulder surfaces: a) mosaic on the upper surface of quartzite; b) gradient on the upper surface of grey siltstone; and c) limited heterogeneity on the upper surface of grey siltstone. Temperature scales (at right of each image) are specific to each image. The black horizontal line denotes the transect drawn on each image to quantify temperature patterns, while the temperature profile (generated using InsideIR v4.0) under each image shows the temperature changes along each transect. The sun is on the left of each image.

The frequency of occurrence for these three patterns of temperature difference was strongly influenced by daily weather conditions (Table 4.2). On cloudy days, almost all (>94 %) boulder surfaces for all six rocks were categorised as having limited temperature heterogeneity (Table 4.2), with temperature differences between the warmest and coolest areas on boulder surfaces almost always <5 °C (Figure 4.1). Temperature mosaics and gradients were seldom, if ever, observed on cloudy days (Table 4.2). On the two cooler sunny days, almost all (>94 %) boulder surfaces for all six rocks were categorised as having either temperature gradients or mosaics (Table 4.2). Temperature differences between the warmest and coolest areas on boulder surfaces were almost always ≥5 °C (Figure 4.1). Temperature gradients were far more common than temperature mosaics, in a ratio of 3:1, on these sunny days (Table 4.2). On the hottest sunny day, all three patterns of temperature difference were observed (Table 4.2), however, boulder surfaces became generally hot, with temperature differences between the warmest and coolest areas mostly <5 °C (Table 4.2, Figure 4.1). As a result, limited temperature heterogeneity was the most common (≥78 %) temperature pattern identified on the hottest sunny day (Table 4.2), with gradients and mosaics observed at much lower frequencies than on the other sunny days.

On sunny days patterns of temperature difference differed among rocks. The generally flat and featureless surfaces of quartzite had a mosaic of fine millimetre to centimetre scale patches of heterogeneous temperature across the surface (Figure 4.2c, Appendix 3, Table 8.33). The five remaining rocks all generally had temperature gradients, although the spatial arrangement of these gradients differed among rocks. The generally flat and featureless surfaces of siltstone had simple gradients of warmer through cooler areas (Figure 4.2a-b , Appendix 3, Table 8.33). In contrast, the two limestones and fossiliferous sandstone had complex surfaces intersected by shallow depressions and pits (<1 cm depth). Consequently, their temperature gradients were interrupted intermittently by these depressions and pits, which could be either warmer or cooler (by up to 2-3 °C) than the flatter surfaces immediately around them (Figure 4.2d-f). All three patterns of temperature difference were related to boulder orientation relative to the sun, with the hottest temperatures generally recorded for the side of boulders facing the sun (>92 %, Table 4.2). Each rock generally had the same pattern of temperature difference on its upper and lower surfaces.

Table 4.2: Frequencies of occurrence (%), pooled across rocks, for the three patterns of temperature difference and the orientation of the maximum in relation to the sun for boulder upper and lower surfaces (*n* = 36) for a subset of the cloudy and sunny days sampled.

				Temperature pattern (%)			Orientation (%)	
Weather	Date	Maximum air temperature (°C)	Surface	Mosaic	Gradient	Limited heterogeneity	Side facing sun	Any other side
Cloudy	09/09/2015	15	Upper	0	0	100	91.6	8.4
			Lower	0	0	100	100	0
	25/11/2015	30	Upper	0	0	100	100	0
			Lower	0	0	100	100	0
	19/12/2015	38	Upper	0	0	100	97.2	2.8
			Lower	0	5.6	94.4	94.4	5.6
Sunny	16/07/2016	12	Upper	13.9	86.1	0	100	0
			Lower	13.9	80.5	5.6	100	0
	07/02/2016	29	Upper	22.2	75.0	2.8	100	0
			Lower	25.0	75.0	0	94.4	5.6
	18/11/2015	40	Upper	2.8	19.4	77.8	100	0
			Lower	5.6	33.3	61.1	94.4	5.6

Objective 2: maximum temperature differs between rocks and surfaces.

After four hours exposure the mean maximum temperature was hotter than the air temperature for all six rocks on both surfaces, especially on sunny days (Figure 4.4a-b). The maximum temperature of upper and lower surfaces generally increased with time exposed on each day, with maxima often peaking around four hours and plateauing thereafter (Figure 4.5a). The largest increases in maxima were generally recorded during the first two hours exposure to insolation, with smaller increases (and sometimes decreases) recorded thereafter (Appendix 3, Figures 8.15 & 8.16). After four hours, the hottest maximum recorded was 57.8 °C (sunny day, air temperature = 39 °C) for the upper surface of grey siltstone while the coolest maximum was 14.4 °C (sunny day, air temperature = 12 °C) for the lower surface of quartzite. Over four hours, increases in mean maximum surface temperature >20 °C were recorded for some rocks on several days, with the greatest increases recorded for upper surfaces on sunny days (Appendix 3, Figure 8.17).

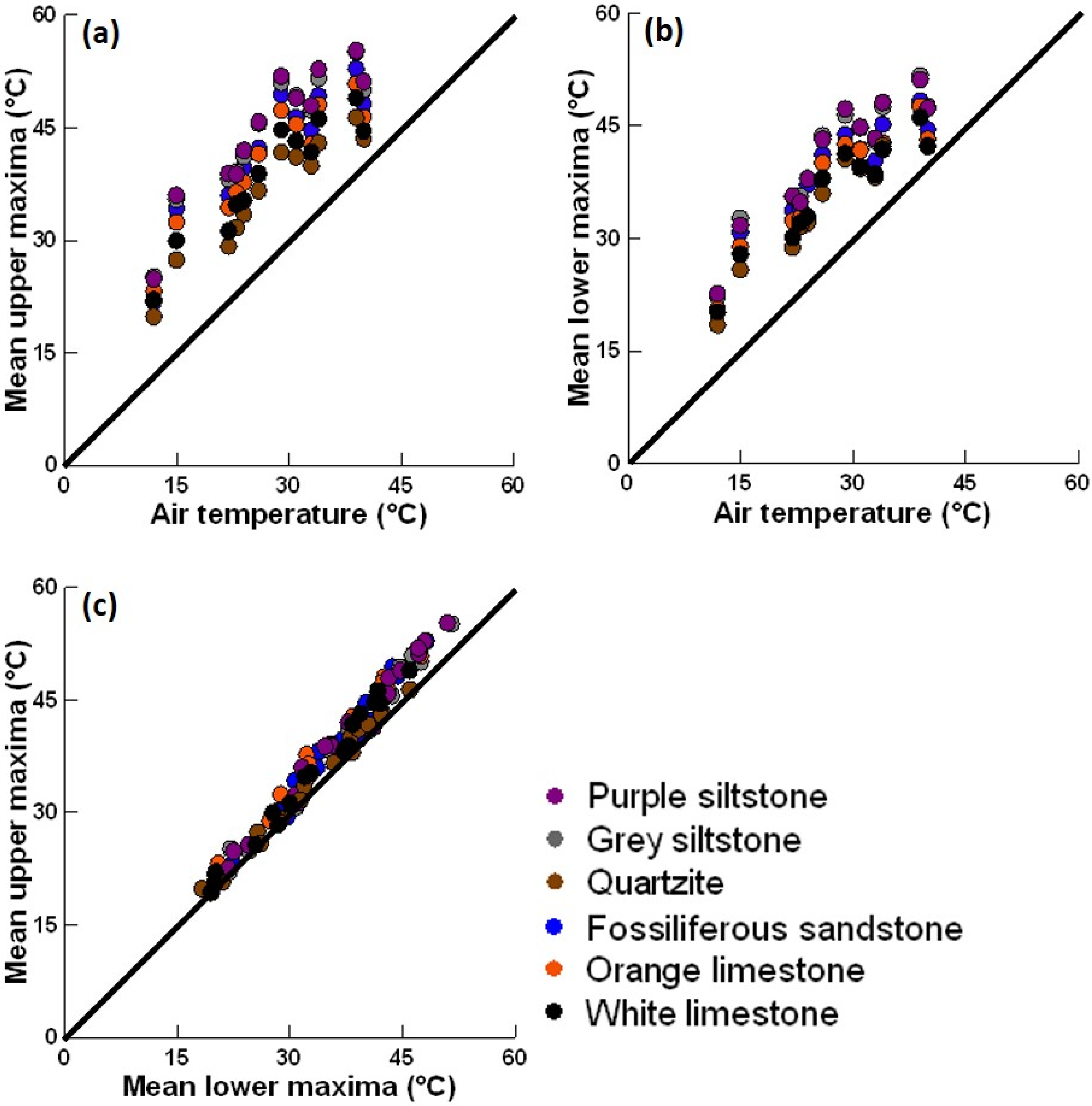

Figure 4.4: Scatter plots (1:1 line shown) showing the association between (a) air temperatures and mean upper maxima; (b) air temperatures and mean lower maxima; and (c) mean upper maxima and mean lower maxima after four hours for each rock on each sunny day.

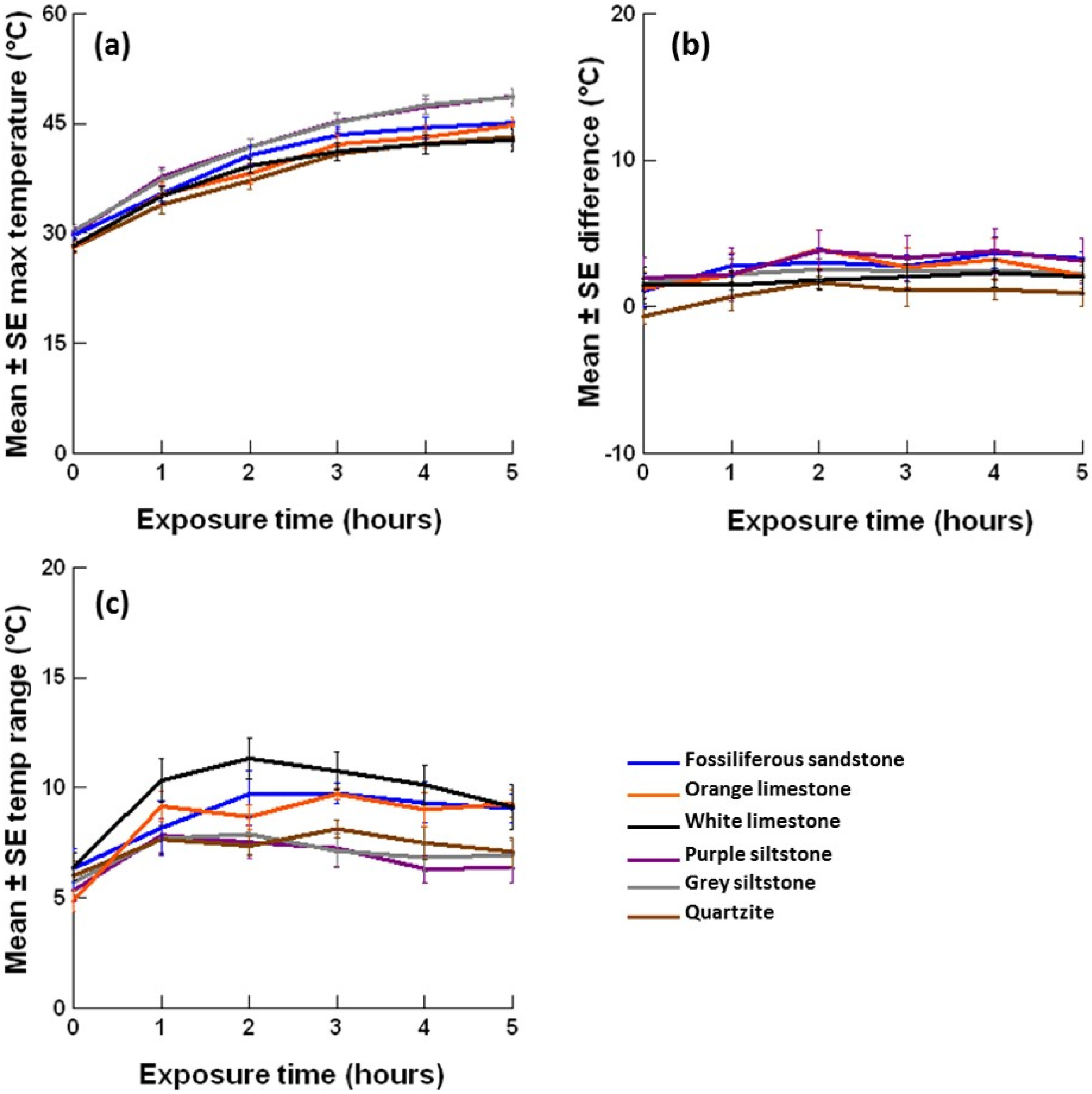

Figure 4.5: Mean ± SE (a) lower-surface maximum temperature; (b) maxima difference between upper and lower surfaces and (c) lower-surface temperature range for 6 rocks (*n* = 6 per rock) over five hours of exposure to insolation on November 18, 2015. Each y-axis extends to encompass the range of raw data. Please see Appendix 3 Figures 8.15-8.22 for the upper surfaces and other days sampled.

When rocks were ranked from hottest to coolest mean maximum temperature after four hours, a consistent rank order across replicate days was identified, irrespective of weather conditions (Table 4.3). For both surfaces, the two siltstones consistently recorded the hottest maxima, followed by fossiliferous sandstone and orange limestone in descending rank order (Table 4.3, Figure 4.5a). On upper surfaces, white limestone consistently recorded the second coolest maxima and quartzite the coolest, while on lower surfaces both rocks were equally ranked in terms of the coolest maxima (Table 4.3). These rock differences in maxima were generally largest on sunny compared to cloudy days and at later versus earlier exposure times (Appendix 3, Figures 8.15 & 8.16). After four hours exposure for the 17 replicate days, the smallest difference for mean maxima across the six rocks was 2.5 °C, while the largest difference was 10.2 °C. A similar rank order was identified for the change in maxima over four hours for both surfaces (Table 4.3, Appendix 3, Figure 8.17). The two siltstones generally had the largest increase in maximum temperature, while white limestone and quartzite had the smallest (Table 4.3, Appendix 3, Figure 8.17). Thus, rock types with the hottest maximum temperature after four hours also had the greatest increase in maximum temperature.

Generally, maxima behaved similarly on upper and lower surfaces (Figure 4.4c). At the commencement of sampling on most days, small negative differences were detected between upper and lower surface maxima for all rocks, with lower surfaces having mean maxima that were slightly hotter (<2 °C) than upper surfaces (Appendix 3, Figure 8.18). Thereafter, small positive differences were detected between upper and lower surfaces for most rocks, with upper surfaces having hotter mean maxima than lower surfaces, although these differences never exceeded 5 °C (Figure 4.5b, Appendix 3, Figure 8.18). The only notable exception to this trend was quartzite, which generally had small negative differences throughout, with lower surfaces sometimes having hotter mean maxima than upper surfaces (Figure 4.5b). The difference in maxima between upper and lower surfaces was always smallest on cloudy days, with larger differences detected on sunny days (Appendix 3, Figure 8.18). Over four hours, a larger increase in maxima was recorded on upper compared to lower surfaces on 16 of the 17 sampling days. Minima behaved similarly to maxima over four hours exposure to insolation with the same trends identified for rock type, surface and time exposed (refer to minima sub-section in Appendix 3).

Table 4.3: The daily rank order of rocks from largest to smallest (6 = largest, 1 = smallest) maximum temperature after four hours and change in maximum temperature over four hours for upper and lower surfaces. Entries are the cumulative number of occurrences of that daily rank for each rock. The rank sum (sum of daily ranks) was used to assign an overall rank to each rock from largest to smallest (6 = largest value, 1 = smallest).

	Surface	Upper								Lower							
	Daily rank	6	5	4	3	2	1	Rank sum	**Overall Rock rank**	6	5	4	3	2	1	Rank sum	**Overall Rock rank**
Measure	Rock																
Maximum temperature after four hours	Purple siltstone	14	3	0	0	0	0	99	**6**	7	10	0	0	0	0	92	**5**
	Grey siltstone	3	14	0	0	0	0	88	**5**	10	7	0	0	0	0	95	**6**
	Fossiliferous sandstone	0	0	16	1	0	0	67	**4**	0	0	16	1	0	0	67	**4**
	Orange limestone	0	0	1	16	0	0	52	**3**	0	0	1	15	1	0	51	**3**
	White limestone	0	0	0	0	13	4	30	**2**	0	0	0	0	9	8	26	**1**
	Quartzite	0	0	0	0	4	13	21	**1**	0	0	0	1	7	9	26	**1**
Change in maximum temperature over four hours	Purple siltstone	8	7	1	0	1	0	89	**6**	9	3	4	0	0	1	86	**5**
	Grey siltstone	7	8	0	1	1	0	87	**5**	5	10	0	2	0	0	86	**5**
	Fossiliferous sandstone	2	0	14	1	0	0	71	**4**	1	4	6	6	0	0	68	**4**
	Orange limestone	0	1	2	13	1	0	54	**3**	1	0	6	6	4	0	56	**3**
	White limestone	0	1	0	1	9	6	32	**2**	1	0	0	2	3	11	29	**2**
	Quartzite	0	0	0	1	5	11	24	**1**	0	0	1	1	10	5	32	**1**

Objective 3: temperature range does not consistently differ between rocks or surfaces. Generally, temperature range behaved similarly on upper and lower surfaces (Appendix 3, Figures 8.19 & 8.20). Temperature range was influenced by weather conditions (Figure 4.1). A larger temperature range (5 – 15 °C) that was more variable between rocks and exposure times was recorded on sunny days for both surfaces (Figure 4.5c, Appendix 3, Figures 8.19 & 8.20). In contrast, a smaller temperature range (generally <5 °C) that was less variable between rocks and exposure times was recorded on each cloudy day for both surfaces (Appendix 3, Figures 8.19 & 8.20). After four hours, the largest temperature range recorded was 16.3 °C (sunny day, air temperature = 15 °C) on the upper surface of grey siltstone while the smallest temperature range was 1.6 °C (cloudy day, air temperature = 38 °C) on the upper surface of purple siltstone.

When rocks were ranked from largest to smallest for the mean temperature range after four hours, there was little evidence of a consistent ranking across replicate days (Table 4.4). For both upper and lower surfaces, the ranking of rocks was highly variable, with each rock having one of the largest temperature ranges on some days and one of the smallest on others (Table 4.4, Appendix 3, Figures 8.19 & 8.20). Rankings were similarly variable after both shorter and longer exposure times, with rank order often changing from one exposure time to the next (Appendix 3, Figures 8.19 & 8.20). No consistent ranking was identified either for the change in mean temperature range over four hours (Table 4.4, Appendix 3, Figure 8.21). For both surfaces, a highly-variable rank order of rocks was detected, with each rock having one of the largest range changes on some days and one of the smallest on others (Table 4.3, Appendix 3, Figure 8.21).

The temperature range difference between upper and lower surfaces (i.e. upper range – lower range) was always smallest on cloudy days, with larger differences detected on sunny days (Appendix 3, Figure 8.22). On cooler days with a maximum daily air temperature <30 °C, regardless of the day condition, small positive differences were generally detected, with the upper surfaces of most rocks having a larger temperature range than lower surfaces (Appendix 3, Figure 8.22). In contrast, on hotter days with a maximum daily air temperature ≥30 °C, small positive differences were detected only for the two siltstones. Small negative differences were often measured for the two limestones and quartzite, with lower surfaces having a larger temperature range than upper surfaces (Appendix 3, Figure 8.22). Overall, the two siltstones generally had the largest range difference between upper and lower surfaces, while white limestone and quartzite often had the smallest (Appendix 3, Figure 8.22). Over four hours, larger changes in temperature range were recorded for lower versus upper surfaces on 11 of 17 days.

Table 4.4: The daily rank order of rocks from largest to smallest (6 = largest value, 1 = smallest) temperature range after four hours and change in temperature range over four hours for upper and lower surfaces. Entries are the cumulative number of occurrences of that daily rank for each rock. The rank sum (sum of daily ranks) was used to assign an overall rank to each rock from largest to smallest (6 = largest value, 1 = smallest).

	Surface	Upper								Lower							
	Daily rank	6	5	4	3	2	1	Rank sum	**Overall rock rank**	6	5	4	3	2	1	Rank sum	**Overall rock rank**
Measure	Rock																
Temperature range after four hours	Purple siltstone	4	4	5	2	1	1	73	**4**	0	1	1	4	3	8	35	**1**
	Grey siltstone	4	9	1	3	0	0	82	**6**	0	3	2	6	5	1	52	**3**
	Fossiliferous sandstone	5	2	7	2	1	0	76	**5**	5	8	2	1	0	1	82	**5**
	Orange limestone	1	0	1	5	6	4	41	**2**	3	0	9	3	1	1	66	**4**
	White limestone	0	2	2	4	7	2	46	**3**	9	4	2	0	2	0	86	**6**
	Quartzite	3	0	1	1	2	10	39	**1**	0	1	1	3	6	6	36	**2**
Change in temperature range over four hours	Purple siltstone	3	4	3	4	2	1	67	**5**	1	0	1	5	5	5	40	**1**
	Grey siltstone	7	3	3	2	1	1	78	**6**	0	4	2	1	8	2	49	**3**
	Fossiliferous sandstone	2	5	3	2	2	3	62	**4**	4	8	3	0	0	1	77	**6**
	Orange limestone	1	1	4	4	4	3	50	**2**	7	2	4	1	0	3	74	**5**
	White limestone	3	2	3	1	6	2	57	**3**	5	3	4	2	2	1	72	**4**
	Quartzite	1	2	1	4	2	7	43	**1**	0	0	2	8	2	5	41	**2**

Objective 4: major mineral and trace elemental composition differs between rocks.

Silicon dioxide (SiO_2) and calcium oxide (CaO) were the dominant major minerals detected, with orange limestone having a CaO-dominated mineralogy and all other rocks a SiO_2-dominated mineralogy (Appendix 3, Table 8.35). Major mineral composition significantly differed among rocks (PERMANOVA permuted *p*-value = 0.0001). For rock differences, a CAP constrained-ordination plot used five axes to discriminate major-mineral differences, with the first two axes accounting for 99.54 % (prop. G) of the total mineralogical variability (Figure 4.6a). All samples were correctly classified using a leave-one-out procedure, and permutation tests for both the trace test statistic ($p = 0.0001$) and first canonical eigenvalue ($p = 0.0001$) were highly significant. The vector overlay of Spearman rank correlations (for rho values>0.8) for major minerals associated with rock differences showed that each rock had a specific major-mineral composition (Figure 4.6a). Grey siltstone was characterised by a higher aluminium oxide and potassium oxide contents, quartzite by the highest SiO_2 content, and orange limestone and fossiliferous sandstone by higher CaO contents (Figure 4.6a). Rock-related differences in the content of specific major minerals were also detected for 10 from 11 major minerals (largest significant permuted PERMANOVA *p*-value = 0.0330 for magnesium oxide), with only sulfur trioxide not differing between rocks (PERMANOVA permuted *p*-value = 0.1244).

Trace-element composition significantly differed between rocks (PERMANOVA permuted *p*-value = 0.0001). For rock differences, a CAP constrained-ordination plot used two axes to discriminate trace-element differences, with these two axes accounting for 79.8 % (prop. G) of the total mineralogical variability (Figure 4.6b). Some 88.9 % of samples were correctly classified using a leave-one-out procedure, and permutation tests for both the trace test statistic (p = 0.0001) and first canonical eigenvalue (p = 0.0001) were highly significant. The vector overlay of Spearman rank correlations (for rho values >0.8) for trace elements associated with rock differences suggested that each rock had a specific trace-element composition (Figure 4.6b). The two siltstones were characterised by a higher trace-metal content (manganese and zirconium especially), quartzite by generally low trace-element quantities (although it had the highest ytterbium content), and the two limestones and fossiliferous sandstone by a higher chlorine content (Figure 4.6b, Appendix 3, Table 8.36).

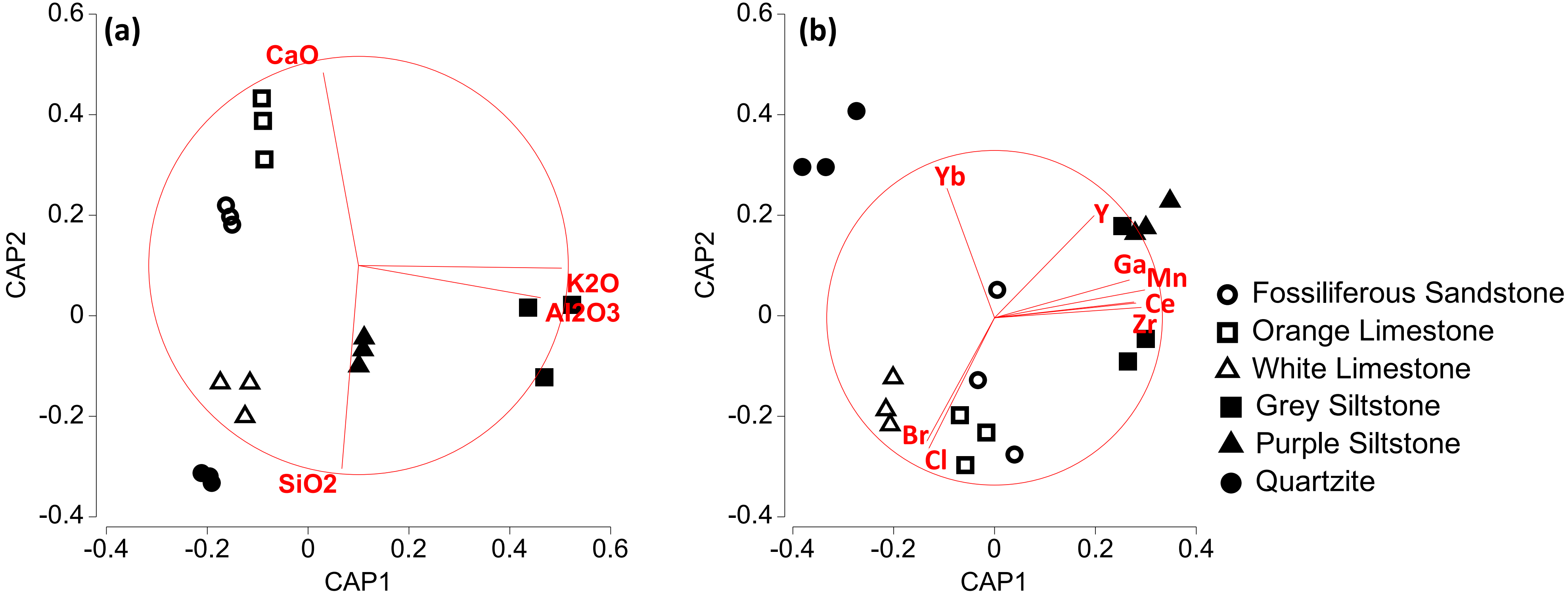

Figure 4.6: Constrained ordination CAP plots with vector overlay of Spearman rank correlations (for rho values>0.8) for (a) major minerals and (b) trace elements contributing to differences in mineralogy among rocks. Each point represents a single rock sample tested. CaO = calcium oxide; SiO_2 = silicon dioxide; K_2O = potassium oxide; Al_2O_3 = aluminium oxide; Yb = ytterbium; Y = Yttrium; Br = bromine; Cl = chlorine; Zr = zirconium; Ce = cerium; Mn = manganese and Ga = gallium.

Objective 5: rock-related differences in maximum temperature are correlated with their mineral composition.

The vector overlay of Spearman rank correlations (for rho values >0.8) in CAP plots (Figure 4.6) showed that the two siltstones, which consistently had the hottest maxima, had mineralogies that were characterised by a higher content of metallic oxides and trace metals versus all other rocks (Figure 4.6, Appendix 3, Tables 8.35 & 8.36). White limestone and quartzite, which consistently had the coolest maxima, were characterised by the highest content of SiO_2 and the lowest content of most metallic oxides and trace metals versus all other rocks (Figure 4.6, Appendix 3, Tables 8.35 & 8.36). Meanwhile, orange limestone and fossiliferous sandstone, which had intermediate maximum temperatures, were characterised by higher contents of CaO and chlorine, and metallic oxide and trace metal quantities that were generally lower than the two siltstones but greater than white limestone and quartzite (Figure 4.6, Appendix 3, Tables 8.35 & 8.36). Thus, it appears that the content of metallic oxides and trace metals may be associated with rock-surface temperature, with the hottest maxima recorded for rocks with the highest metallic oxide and trace metal content.

Discussion

Under common-garden conditions that simulated low tide, I was able to isolate temperature behaviour that was a function of the rocks themselves, not their setting. Weather condition was the single largest determinant of boulder temperature behaviour, with cloud cover moderating all temperature dependent variables. Both boulder upper and lower surfaces had patterns of temperature difference, with three patterns identified: gradients, mosaics, and limited heterogeneity. On cloudy days, limited heterogeneity was identified on most surfaces for all rocks. On the hottest sunny day sampled and rocks much heated, limited heterogeneity was again the dominant pattern identified, although some temperature gradients and mosaics were observed for some combinations of surface and rock type. On the remaining sunny days, most quartzite surfaces developed temperature mosaics, while the surfaces of all other rocks generally developed temperature gradients. The maximum (and minimum) temperature differed consistently between rocks and surfaces. Upper surface maxima were generally somewhat hotter (<5 °C) than lower surface maxima, with the two siltstones consistently being the hottest and quartzite and white limestone the coolest. Each rock had a unique mineralogy. The maximum temperature correlated with the metallic oxide and trace metal content of rocks, with the hottest rocks having the highest metallic oxide and trace metal contents.

These results provide evidence of millimetre-to-centimetre-scale patterns of temperature difference on boulder upper and lower surfaces for all six rocks. While within-microhabitat temperature differences have been described previously in terrestrial habitats (e.g. Huey et al. 1989), these results are novel for intertidal rocky substrata. Most previous research on small-scale temperature heterogeneity for intertidal rocky substrata have investigated differences between sun-exposed and sun-protected microhabitats (e.g. Garrity 1984; Denny et al. 2011; Chapperon et al. 2017). Here I have shown that temperature patterns in the form of gradients and mosaics occur on both the sun-exposed surface and the sun-protected surface of boulders on most sunny days. On the hottest sunny day and all cloudy days sampled, gradients and mosaics generally disappear with patterns of only limited heterogeneity dominating instead.

Patterns of temperature difference were also related to boulder orientation relative to the sun, with the hottest locations on all rocks generally recorded on the side of boulders facing the sun. Substratum orientation, relative to the sun, appears to effect the spatial arrangement of temperatures on rocky seashores generally, with rock faces orientated towards the sun (Harley 2008; Seabra et al. 2011; Chapperon et al. 2016; 2017) having the hottest surface temperatures. The potential biological relevance of within-microhabitat patterns of temperature difference have not yet been quantified (but see Chapter 5). However, given periwinkles will select cooler locations when offered centimetre-scale temperature gradients (Soto and Bozinovic 1998), and intertidal ectotherms can respond to habitat-scale temperature mosaics (e.g. Garrity 1984; Chapperon and Seuront 2011a; Judge et al. 2011; Chapperon et al. 2013), it is likely that intertidal ectotherms will also respond to within-microhabitat temperature patterns on boulder surfaces.

On the hottest sunny day sampled, boulder surfaces became generally uniformly hot with limited temperature heterogeneity. Given the risks that desiccation and heat stress pose to organism survival and fitness (e.g. Jones and Boulding 1999; Harley 2008; Gedan et al. 2011; Monaco et al. 2015), and the exacerbation of these risks at the hottest environmental temperatures (Harley 2008), the disappearance of temperature mosaics and gradients on hot, sunny days may be problematic for intertidal biota. If the cooler areas of mosaics or gradients are found to function as thermal refuges for intertidal biota but these refuges disappear on the hottest days when they are needed most, then the thermal quality of boulder habitats may be diminished on hot, sunny days. Consequently, organism survival and fitness may be challenged on these days. Given predictions of an increased frequency of heatwaves and generally hotter air temperatures associated with global climate change (IPCC 2013), hot boulder surfaces with limited heterogeneity may be observed more often. It is therefore critical that we evaluate the interactions between biota and substratum temperature on these hot sunny days to understand how organism survival and fitness is likely to be impacted by predicted future climate-change scenarios.

Maximum temperatures differed consistently among rocks, with the two siltstones always hottest and white limestone and quartzite the coolest. Rocks with the coolest temperatures were also the most thermally stable, as they had the smallest temperature increases while exposed to insolation over four hours. Thus some rocks possibly minimise thermal stress to biota more than others. These characteristics have not been recognised to date but could allow some predictions of the future fate of populations and assemblages on rocky seashores.

These results under standardised conditions are consistent with field based studies on rocky seashores. Raimondi (1988) found basalt stones could be 5-6 °C hotter than granite stones after three hours emersion while Marshall found that lighter-coloured sandstone was cooler than darker-coloured ferruginous sandstone. Unfortunately Judge et al. (2011) only specified colour *per se* and not the lithology of their two rocks, finding black-rock microhabitats were hotter than white-rock microhabitats. In each of these studies, temperature differences were attributed to the differences in colouration and/or surface texture of the rocks (Raimondi 1988; Marshall et al. 2010; Judge et al. 2011). In this study, the metallic oxide and trace metal content of rocks correlated positively with higher maximum temperatures. I also identified colour and surface texture differences between rocks, with darker rocks of the same type (i.e. purple versus grey siltstone and orange versus white limestone, Appendix 3, Figure 8.14) attaining hotter temperatures. Presumably the major mineral and trace elemental composition of each rock type determines its colour. However, as colour and surface texture differences between rocks were not quantitatively measured, and any colour differences among rocks are confounded by mineralogical differences, it is difficult to reliably associate either variable with any rock-related temperature differences.

Cooler rock temperatures have been positively associated with intertidal biota on seashores globally. In Mexico, the higher vertical distribution of barnacles on granite than basalt shores was attributed to granite’s cooler surface temperatures (Raimondi 1988), while in Brunei Darussalam robotic snail mimics had cooler body temperatures on lighter-coloured sandstone than darker-coloured ferruginous sandstone (Marshall et al. 2010). Moreover, on grey siltstone platforms in Australia, barnacle recruitment and growth rate was higher on cooler than hotter areas of siltstone (Lathlean et al. 2013); while on igneous seashores in Panama gastropod body temperatures and mortality were highest in areas with the hottest temperature (Garrity 1984). Therefore, the cooler and more-thermally stable rocks identified here such as white limestone and quartzite may function as thermal refuges for some intertidal biota. If boulders comprised of cooler rock are available to biota seeking refuge, then the thermal benefits that cooler rocks potentially confer may improve the chances of biota surviving while emersed, and thus persisting, compared to hotter rocks such as siltstone.

Lower surfaces generally had cooler maximum temperatures (<5 °C) than upper surfaces. Over four hours exposure to insolation, a greater increase in maxima was measured on boulder upper than lower surfaces. Therefore, lower surfaces have some temperature benefits over upper surfaces due to their shaded surfaces generally providing cooler maxima and slower temperature increases. The thermal benefits of living underneath boulders are often cited (Evans 1948; Chapperon and Seuront 2011a; Chapperon et al. 2013). However, my results, in conjunction with those published previously for the same siltstone boulders (Chapperon and Seuront 2011a; Chapperon et al. 2013), suggest that the magnitude of temperature relief that biota experience under boulders is actually quite small (<5 °C) when compared to temperature differences of up to 25.5 °C between sun-exposed and sun-protected microhabitats elsewhere on rocky seashores (e.g. Denny et al. 2011; Lathlean et al. 2012). Nevertheless, from a physiological perspective, this 5 °C difference between the top and bottom of boulders may help to ensure organisms remain within their thermal tolerance limits (Helmuth et al. 2002). Moreover, the combined benefits of cooler surface temperatures plus under-boulder dampness and shading from insolation (Evans 1948; Chapman 2003; Chapperon et al. 2013) may interact to make lower surfaces a thermally favourable habitat for intertidal biota at low tide.

Each rock had its own unique major mineral and trace elemental composition. As with previous studies of Mediterranean seashore mineralogy that investigated granite, quartzite, limestone, sandstone, serpentinites and metagabbros (e.g. Bavestrello et al. 2000; 2018; Cattaneo-Vietti et al. 2002), the six rocks investigated here also contained varying quantities of silica. Five of the six rocks investigated had a SiO_2-dominated mineralogy, with only orange limestone having a mineralogy dominated by a different major mineral, CaO. These baseline mineralogy data are likely to be useful for investigating how specific major minerals or trace elements may influence the distribution of biota on rocky seashores. To date, several studies completed in the Mediterranean have investigated biotic responses of a range of taxa to the varying silica content of rocks or sediment (Bavestrello et al. 2000; 2018; Cattaneo-Vietti et al. 2002). How intertidal biota may associate with some of the other mineral constituents of rocks remains largely unknown (although see Chapter 2). Furthermore, with the metallic oxide and trace metal content of rocks positively correlated with maximum temperatures, how substratum mineralogy and temperature behaviour collectively impact intertidal species and assemblages warrants further investigation.

Temperature range was highly variable among the six rocks, with no single rock having a temperature range that was consistently distinct from the others. If these sorts of results extend to the seashore, then biota would have ample opportunity to respond to the range of temperatures on all rocks, as no single rock had a temperature range larger or smaller than the others. In this study, a temperature range as large as 16.3 °C was recorded across boulder surfaces with a maximum length of <30 cm. This temperature range is larger than the 8.2 °C maximum range recorded for replicate 400 cm^2 quadrats on a grey siltstone platform (Lathlean et al. 2012), but somewhat smaller than the 24.0 °C maximum range detected between the edge and centre of rocks (maximum length <2 m) sheltering garter snakes (Huey et al. 1989) or the 25.5 °C maximum range detected between different seashore microhabitats (Chapperon and Seuront 2011a; Chapperon et al. 2017). Consequently, these new results for boulder surfaces indicate that temperature range is likely be specific to the type(s) of substratum, habitat and region investigated.

Cloud cover shaded boulders from direct insolation, and moderated all measured temperature dependent variables. On the seashore, tidal movement generally has the greatest impact on rock temperature, with the incoming tide rapidly cooling rocky substrata as soon as they get wet (Harris 1990). My experimental results are consistent with how rocks warm on the seashore when emersed, with cooler substratum temperatures measured on cloudy versus sunny days (Garrity 1984) and rapid increases in surface temperature reported once sunlight penetrated through cloud cover (Southward 1958). Furthermore, these results are consistent with shading experiments, which showed that shaded patches on rock platforms were 1.6 °C cooler than patches exposed to insolation (Lamb et al. 2014) and that shaded cobbles were 3-8 °C cooler than those exposed to sunlight (Bertness 1989). Thus, for the biota using these boulders as seashore habitat locally, the hottest substratum temperatures will occur when sunny conditions and hot air temperatures coincide with daytime low tides. Daytime low tides and hot sunny days are a common occurrence locally, especially during summer neap tides.

In conclusion, under common-garden conditions weather condition moderated all measured temperature dependent variables. Both upper and lower surfaces of boulders had patterns of temperature difference, with three patterns identified: gradients; mosaics; and limited heterogeneity. Maximum temperature differed consistently between rocks and surfaces. Each rock had a unique major mineral and trace elemental composition, with the content of metallic oxides and trace metals in rocks correlating with their maximum temperature. Consequently, the lower surface of rocks with the lowest metallic oxide and trace metal content (quartzite and white limestone in this study) potentially offer the best thermal refugia for intertidal biota on the seashore during summer. With these baseline data, it is possible to design biotic studies which evaluate the biological relevance of within-microhabitat temperature differences and to investigate how rock and surface-related differences in temperature may impact intertidal biota. Once a better understanding of present-day associations between the temperature of substrata and intertidal biota are developed it may be possible to make better predictions regarding the potential biological ramifications of global climate change in the rocky intertidal zone.

Acknowledgements

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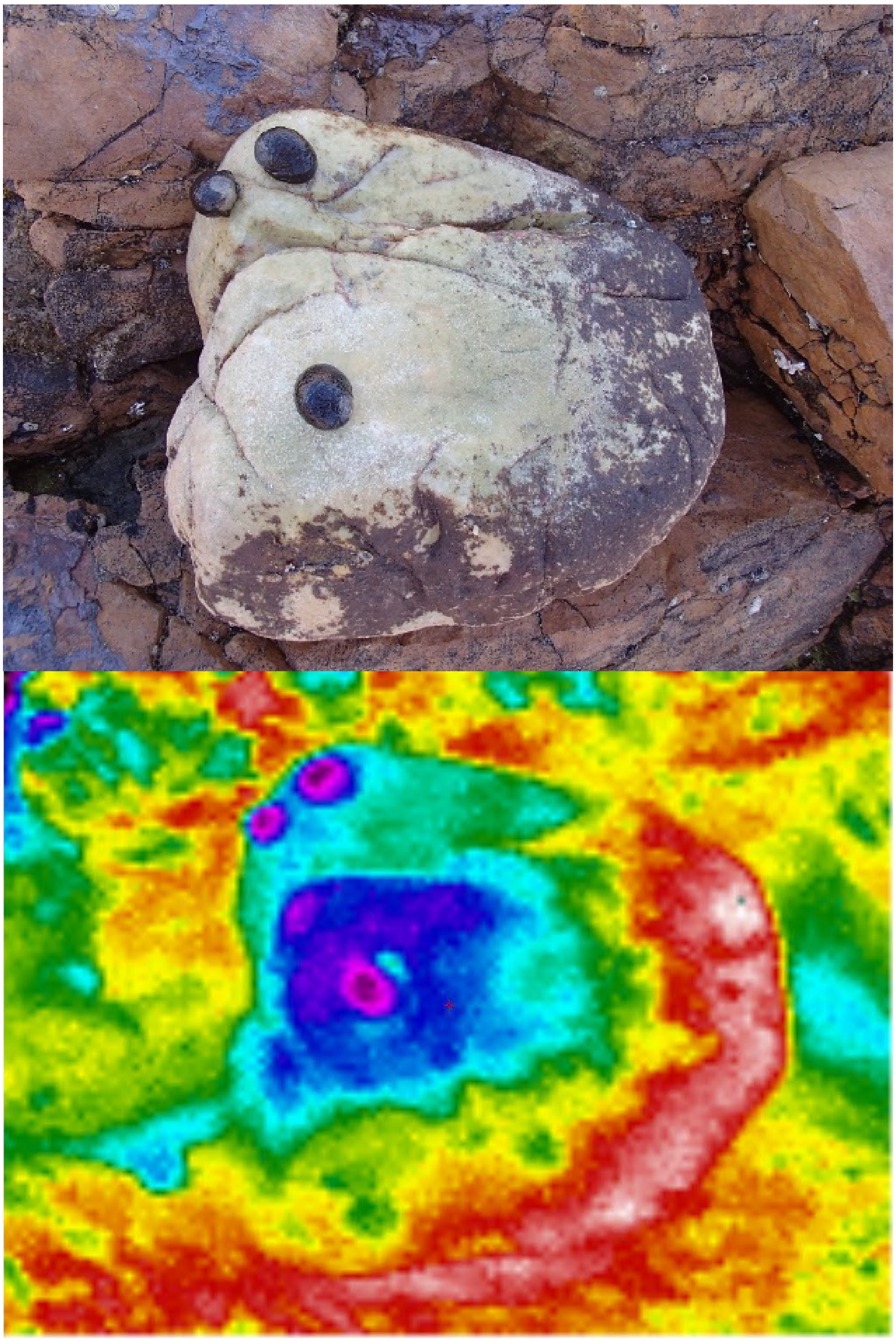
Chapter 5

Where three snail species sit while emersed in relation to the temperature of substrata underneath intertidal boulders



Abstract

With predictions of warming temperatures associated with climate change, it is critical to better understand how biota respond to temperature in intertidal habitats. Many intertidal gastropods employ behavioural thermoregulation to mitigate thermal stress, which often includes retreating to cooler microhabitats such as underneath boulders when emersed. However, little is known about temperatures experienced under boulders, and how gastropod occupancy of under-boulder habitats is associated with any temperature variation. I used thermal imagery to measure the temperature of boulder lower surfaces and investigated how three snail species were associated at low tide with the maximum and mean temperature on grey siltstone and quartzite. Boulders showed several distinct patterns of temperature difference, with grey siltstone having temperature gradients and quartzite temperature showing mosaics. Temperature differences between the hottest and coolest gradient or mosaic locations were >5 °C; thus there was a range of temperatures that snails could potentially interact with. All three snail species occupied cooler parts of temperature mosaics or gradients, avoiding the hottest areas. Stronger associations were detected on the hotter grey siltstone and for the more-thermally sensitive *Nerita atramentosa* and *Diloma concameratum*. Even though snails were associated with cooler areas, some individuals were still exposed to extreme heat (>50 °C). This highlights the importance of evaluating biotic associations with environmental temperature.

Introduction

Intertidal gastropods are periodically exposed to the terrestrial environment during low tide and, as marine ectotherms, do not physiologically regulate their body temperature; instead their body temperature tracks that of the surrounding environment (Wolcott 1973; Madeira et al. 2012). Many intertidal gastropods live in close contact with the substratum, with their large muscular foot keeping them thermally coupled to the substratum underneath (Vermeij 1971b; McMahon 1990; Miller et al. 2015). Consequently, gastropod body temperature is often positively correlated with substratum temperature at low tide (Soto and Bozinovic 1998; Caddy-Retalic et al. 2011; Chapperon and Seuront 2011b). Rock surfaces exposed to direct insolation when emersed can warm by 10 - 20 °C (Southward 1958; Harris 1990; Bertness 1999), with gastropods inhabiting the mid and upper levels of the seashore spending 14 - 98 % of their lives in these thermally-challenging conditions (McMahon 1988; Harley 2003; Helmuth et al. 2006a). Thus, rocky seashores represent a thermally-variable and potentially-stressful habitat for the gastropod populations inhabiting them (Helmuth and Hofmann 2001; Harley 2008).

For intertidal gastropods, exposure to extreme heat and low humidity when emersed can challenge their development, survival and ultimately persistence through heat stress, desiccation, or a combination of both (Bertness 1999; Helmuth and Hofmann 2001; Harley 2003). Biotic responses to extreme heat are highly variable, often differing among species or life stages of the same species (Pörtner 2001; Przeslawski 2004; Byrne and Przeslawski 2013). Depending on the intensity, rate of warming, and duration of extreme-heat events (Evans 1948; Pörtner 2001), both non-lethal and lethal impacts may result from exposure to sub-optimal heat. Non-lethal impacts can include reduced growth rates (Jones and Boulding 1999; Lathlean et al. 2013; Lamb et al. 2014) or the onset of heat coma (Evans 1948; McMahon 1990; Miller et al. 2015); while lethal impacts can include mass mortality events during heatwaves (Harley 2008), overall lower survival rates in less thermally-favourable habitats (Jones and Boulding 1999; Harley 2008; Gedan et al. 2011), or limitations on the vertical seashore distribution of some species (Wolcott 1973; Raimondi 1988; Somero 2002; Harley 2003).

Due to their physiological inability to regulate body temperature, intertidal gastropods can instead employ behavioural thermoregulation to help mitigate some of the deleterious impacts associated with exposure to extreme heat during emersion (Soto and Bozinovic 1998; Ng et al. 2017; Hayford et al. 2018). Each thermoregulatory behaviour employed by intertidal ectotherms includes either a fight-or-flight response *sensu* Ng et al. (2017). A fight response, where organisms can modify their own environment to minimise thermal extremes (Ng et al. 2017), may include: shell-posturing behaviour (Seuront and Ng 2016; Chapperon et al. 2017; Ng et al. 2017); aggregating with conspecifics (Underwood 1976; Chapperon et al. 2013); remaining inactive at low tide by withdrawing into the shell and cementing the aperture to the substratum with mucus (Vermeij 1971b; McMahon 1990); or attaching to the substratum with a mucus holdfast (Marshall and Chua 2012; Seuront et al. 2018). A flight response, where organisms limit exposure to extreme heat by retreating to cooler microhabitats (Ng et al. 2017), may include taking refuge: on the lower surfaces of boulders or mangrove roots (Chapperon and Seuront 2011a,b); in pits or crevices on rock platforms (Garrity 1984; Bates and Hicks 2005); on substratum surfaces orientated away (i.e. shaded) from direct insolation (Williams and Morritt 1995); or in rock pools, algal or barnacle patches (Evans 1948; Jones and Boulding 1999).

These cooler refuge microhabitats, which are often protected from direct insolation for some or all of low tide, can be up to 25.5 °C cooler than adjacent sun-exposed (i.e.

horizontal rock) microhabitats (Garrity 1984; Denny et al. 2011; Seabra et al. 2011; Chapperon et al. 2017). Consequently, these warmer and cooler microhabitats create centimetre-scale temperature differences on the seashore (Garrity 1984; Helmuth et al. 2006b; Denny et al. 2011). Much of the research investigating biotic associations with small-scale intertidal temperature differences has quantified how temperatures of substrata and ectotherm bodies differ between hotter, sun-exposed (usually horizontal rock) microhabitats and cooler, sun-protected refuge microhabitats. Generally, substratum and body temperatures are hotter in sun-exposed than sun-protected microhabitats, as shown for: snails (Garrity 1984; Chapperon and Seuront 2011a; Marshall and Chua 2012; Chapperon et al. 2013; 2017; Marshall et al. 2013), limpets (Wolcott 1973; Garrity 1984; Williams and Morritt 1995; Seabra et al. 2011; Lathlean et al. 2015a), barnacles (Lathlean et al. 2015a), and sea stars (Monaco et al. 2015).

Several studies have identified patterns of temperature difference at even smaller centimetre-to-millimetre scales within 400 cm^2 quadrats on rock platforms (Lathlean et al. 2012; 2013) and within some refuge microhabitats (Huey et al. 1989; Soto and Bozinovic 1998; Chapperon et al. 2017). For intertidal biota, Soto and Bozinovic (1998) found that periwinkles avoided the hottest areas of substratum when offered a centimetre-scale temperature gradient of warmer through cooler locations under laboratory conditions. Meanwhile in the terrestrial realm, Huey et al. (1989) found that garter snakes adjusted their positioning under rocks (<2 metres length or width) in response to temperature differences.

Boulder lower surfaces are one type of intertidal refuge microhabitat that gastropods retreat to when emersed (Chapperon and Seuront 2011a). Given boulder lower surfaces (length or width ≤30 cm) can develop centimetre scale temperature differences of up to 15.5 °C between the hottest and coolest areas (see Chapter 4), it is likely that gastropods may also move in relation to such a temperature range. Consequently, gastropod thermoregulatory behaviour may extend beyond simply retreating to cooler microhabitats, as these organisms may also respond to centimetre-scale temperature differences within the refuge microhabitats they retreat to. However, few intertidal studies have investigated associations between biota and the hotter or cooler areas within microhabitats (i.e. across a single boulder surface).

To address each of these knowledge gaps, I used thermal imagery to quantify patterns of temperature difference under boulders and then investigated where three snail species sit when emersed in relation to these temperature differences. This was achieved by comparing the substratum temperature where snails were attached while emersed, hereafter termed the “target temperature”, to the maximal temperature of the boulder surface. As in previous studies (see Monaco et al. 2015), I focused primarily on maxima due to extreme temperatures having a greater impact on organism survival and fitness (e.g. Jones and Boulding 1999; Harley 2008; Gedan et al. 2011). Boulders of two rock types were sampled because surface temperatures can differ among rocks (Chapter 4), and this in turn can influence responses of ectotherms to the temperature of substrata (Raimondi 1988; Marshall et al. 2010; Judge et al. 2011). Untangling associations between intertidal gastropods and the temperature of substrata is important given predictions of hotter air temperatures and an increased frequency of extreme-heat events associated with global climate change (IPCC 2013). Such changes in climate may exacerbate the temperature extremes and variability currently experienced by intertidal ectotherms while emersed, which may be problematic given some intertidal biota already live near or at their upper tolerable thermal limits (Somero 2002; Helmuth et al. 2002; Madeira et al. 2012). I tested the following four null hypotheses (H1-H4) to improve our understanding of any low-tide associations between snails and small-scale temperature heterogeneity on boulder lower surfaces:

H1) emersed boulder lower surfaces will not display patterns of temperature difference;
H2) temperature characteristics will not differ between grey siltstone and quartzite;
H3) target temperature does not differ from the maximum or average boulder temperature; and
H4) differences between target temperature and either thermal maxima or minima on boulders do not differ between grey siltstone and quartzite.

Materials & methods

Sampling location

South Australia’s (SA) geologically-diverse Fleurieu Peninsula is comprised of a variety of rocks. Two of these, grey siltstone and quartzite, were sampled for temperatures over 18 months in a larger common-garden experiment, and so shown to have very different surface temperatures, with grey siltstone consistently hotter than quartzite (Chapter 4). Therefore, two intertidal boulderfields in Gulf St Vincent, SA, were sampled, grey siltstone at Marino Rocks (35°02’ S, 138°30’E) and quartzite at O’Sullivan Beach (35°07’ S, 138°28’E). Both seashores have a westerly aspect and are located just 20 km apart on the south-eastern side of Gulf St Vincent, a large inverse estuary with a geographic setting that protects the coastline from swell. Both seashores are subjected to similar oceanic and environmental conditions.

Sampling design

Grey siltstone and quartzite boulders were observed to develop noticeable patterns of temperature difference during the common-garden experiment (Chapter 4). On grey siltstone, a gradient of surface temperatures was identified, where temperature gradually decreased from the side nearest the sun to the side opposite. The temperature difference between the warmest and coolest gradient areas was ≥5 °C. On quartzite a mosaic of surface temperatures was generally observed, which consisted of heterogeneous temperatures across the entire boulder surface. The temperature difference between the warmest and coolest mosaic areas was ≥5 °C. Two observations regarding the development of temperature mosaics or gradients were noted: 1) boulders must be exposed to sunlight to develop temperature differences ≥5 °C; and 2) even small-sized boulders (length or width up to 30 cm) still take at least four hours to approach their thermal maxima when exposed to uninterrupted sunshine (Chapter 4, Appendix 4, Figure 8.23). Consequently, only sunny days with little or no cloud cover were sampled. Boulders were sampled approximately one hour either side of predicted daytime low tides (low tide ≤0.40 m Australian Height Datum), as pilot studies at each seashore showed boulders had been emersed for at least four hours by this time. Uninterrupted sunshine (i.e. no cloud cover) and daytime low tides are typical for the Fleurieu Peninsula during summer, so the days sampled were representative of the conditions that intertidal biota are often exposed to.

All sampling was completed between December 2016 and February 2017 during the austral summer to target days of extreme aerial and substratum temperatures. Air temperature measurements collected over the past 63 years (1955 - 2017 inclusive) from the weather station nearest the sampled seashores (Adelaide Airport) showed this region of the coast experiences a mean summer maximum air temperature of approximately 28 °C (Commonwealth of Australia 2017). Therefore, sampling was completed on four days at each seashore that had maximum air temperatures above this summer average (i.e. hotter days), and on four days that had maximum air temperatures at or below this summer average (i.e. cooler days) (Table 5.1). Maximum daily air temperatures were recorded during sampling at low tide, and confirmed by measuring air temperature at half hour intervals in the shade with a mercury thermometer. Air temperature was measured in the shade as the thermometer's dark-coloured mercury absorbed heat when exposed to insolation, producing inflated temperature readings.

Table 5.1: Mean difference (°C) for target - maximum temperature across replicate boulders on each day for *Bembicium nanum, Diloma concameratum* and *Nerita atramentosa* on grey siltstone and quartzite. Entries are ordered by the maximum air temperature for each rock. All mean temperature differences (α = 0.05), as determined by paired *t*-tests, are significant. *n* = the number of snails sampled daily for each species.

			Species:	*Bembicium nanum*		*Diloma concameratum*		*Nerita atramentosa*	
Rock	Date	Predicted time of low tide (24 h clock)	Max air temp (˚C)	*n*	Target - maximum	*n*	Target - maximum	*n*	Target - maximum
Grey siltstone	20/12/16	14:27	23	30	-4.09	30	-5.61	30	-5.58
	16/12/16	13:07	26	30	-4.89	22	-7.34	30	-7.19
	27/1/17	12:12	27	30	-5.10	30	-6.06	30	-6.23
	10/1/17	11:19	28	28	-4.26	30	-4.96	30	-4.88
	28/1/17	12:32	29	30	-5.66	30	-6.46	30	-6.83
	4/1/17	14:42	33	30	-5.06	19	-6.11	30	-6.98
	5/1/17	15:11	35	30	-4.84	28	-7.22	30	-6.87
	17/1/17	13:52	39	30	-4.67	18	-8.01	30	-7.91
			Grand mean		-4.82		-6.36		-6.56
Quartzite	18/12/16	13:47	25	30	-4.65	30	-5.13	30	-5.44
	21/12/16	14:56	25	30	-4.36	30	-5.02	30	-4.96
	12/1/17	12:22	28	30	-3.96	30	-5.09	30	-5.26
	3/1/17	14:17	28	30	-4.64	30	-5.40	30	-4.91
	29/1/17	12:52	30	30	-4.68	30	-5.17	30	-5.12
	16/1/17	13:35	34	30	-4.84	24	-5.71	30	-5.20
	9/2/17	12:03	38	30	-4.41	27	-5.13	30	-5.89
	6/1/17	15:46	40	30	-4.29	23	-5.60	30	-5.52
			Grand mean		-4.48		-5.26		-5.29

Each intertidal boulderfield was inhabited by a variety of mobile gastropods. Pilot studies found the grazing snails *Nerita atramentosa* (Reeve, 1855), *Diloma concameratum* (W. Wood, 1828), and *Bembicium nanum* (Lamarck, 1822) to be most abundant, with sampling subsequently targeting these species. *Nerita atramentosa* and *D. concameratum* generally had a mean shell length ≥15 mm, and were hence identified as adults, with *N. atramentosa* reaching reproductive maturity at a mean shell length of 13.5 mm (Underwood 1975). The highest abundances of *N. atramentosa* and *D. concameratum* were recorded lower on the seashore, approximately 5 - 15 metres shoreward of the low-tide mark. In contrast, *B. nanum* generally had a mean shell breadth ≤5 mm, with individuals identified as juveniles, with this species reaching reproductive maturity at a mean shell breadth of 11.0 mm (Underwood 1975). The highest abundances of *B. nanum* occurred higher up the seashore, approximately 15 - 25 metres shoreward of the low-tide mark. These species-specific seashore distributions were factored into this sampling methodology by completing all sampling activities within two shore-parallel zones. Each shore-parallel zone had an across-shore width of 5 metres and an along-shore length of 80 metres, with this length maximising the amount of boulderfield sampled. One zone was established approximately 5 metres shoreward of the low-tide mark to sample adult *N. atramentosa* and *D. concameratum*, and the other established 15 metres shoreward of the low tide mark to sample juvenile *B. nanum*.

Randomly-selected boulders were only sampled if they met the required sampling criteria. Specifically, their lower surfaces had to be inhabited by an individual from one or more target species, and each individual had to occur as an independent organism. In this study, an independent organism was defined as any individual that had no direct physical contact with conspecifics. Consequently, organisms clustered in groups (predominantly *N. atramentosa* and *D. concameratum*) were only sampled if the individual organisms constituting each cluster were not in physical contact with one another, so as to constrain their choice. Touching conspecifics were not sampled, because it was not possible to guarantee the independence of temperature measurements for an individual touching other snails. Only one individual, from each target species, per lower surface was sampled, with sampled individuals being randomly selected. Multiple target species per surface were sampled if surfaces were cohabited.

To sample lower surfaces, boulders were flipped upside down and shaded from direct sunlight. Boulders were only sampled if snails remained attached during the flipping process. Where possible, 30 under-boulder surfaces inhabited by each target species were sampled at each seashore on each day. On the hottest days sampled, especially for *D. concameratum*, few surfaces were inhabited, so as many inhabited surfaces as could be found were sampled (Table 5.1). Target snail species exhibited a patchy and over-dispersed distribution (Chapman and Underwood 1996; Chapman 2005), with many surfaces unoccupied by any target species. Consequently, many more than the desired maximum of 90 inhabited boulders had to be flipped on each day, with the total number of boulders flipped daily ranging between 121 and 248.

Imagery was used to capture associations between snails and temperatures *in situ*. For every sampled surface (N = 1152), a digital photograph and a thermal image were recorded, and archived images were subsequently processed in the laboratory. Digital photographs were captured using an Olympus Tough TG-820 digital camera, and were used as a reference to identify the species and location of individual snails (Figure 5.1). Thermal images were captured using a Ti20 Fluke thermal imaging camera. The thermal resolution of this camera was ≤0.2 °C at 30 °C, with an accuracy to 2 % or 2 °C, whichever was greater. Default camera settings were employed, including emissivity, which was set at 0.95. This default emissivity was deemed appropriate, as previous studies have shown that the emissivity values of invertebrates and dry rocky substrata, including those investigated here, range somewhere between 0.95 - 1.0 (Chapperon and Seuront 2011a; Lathlean et al. 2012; Chapperon et al. 2013). To avoid measuring inaccurate temperatures from increased amounts of thermal energy being reflected by wet surfaces (Lathlean et al. 2012; Lathlean and Seuront 2014; Seuront et al. 2018), boulders from wet habitats (i.e. rock pools or wet sediment) were not sampled.

Due to their large size and shell temperatures generally somewhat cooler than the substratum, *N. atramentosa* and *D. concameratum* were easy to locate in archived thermal images (Figure 5.1). In contrast, the smaller *B. nanum* generally had shell temperatures more similar to the substratum, and were often difficult to locate on the image (Figure 5.1). To rectify this, empty *N. atramentosa* shells were chilled on ice and were then placed approximately 1 cm to the lower right of *B. nanum* individuals when recording thermal images (Figure 5.1). As the chilled shells were much cooler than the substratum, they acted as an easily-identifiable reference point, making it easier to locate *B. nanum* in archived thermal images (Figure 5.1).

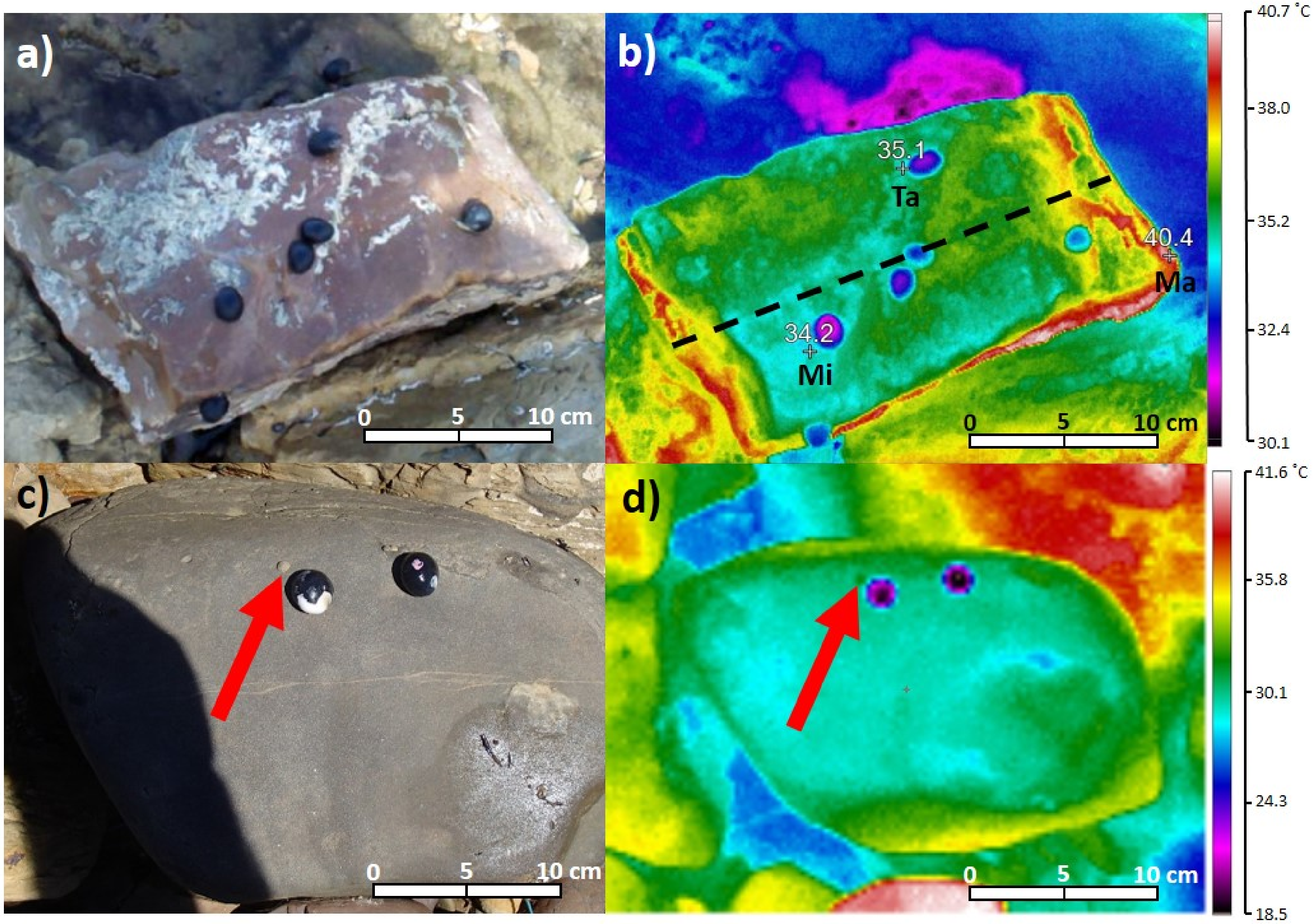


Figure 5.1: Digital photograph (a) and thermal image (b) of *Nerita atramentosa* on the lower surface of a quartzite boulder (range: 40.4 – 34.2 = 6.2 °C) where Ma = maximum surface temperature, Mi = minimum surface temperature and Ta = target temperature for an individual *N. atramentosa*. The solid black line in panel b denotes how transects were drawn on each image to quantify patterns of temperature difference. Digital photograph (c) and thermal image (d) showing the use of two chilled *N. atramentosa* shells to assist with locating juvenile *Bembicium nanum* (one marked with the red arrow) on the lower surface of a siltstone boulder (range: 36.9 – 25.8 = 11.1 °C).

Archived thermal images were processed using the InsideIR version 4.0 software (Fluke Corporation), taking advantage of the one to eight distinct false colours used to portray temperature on the image (see Appendix 4 for more details). For each image of lower surfaces, maximum, minimum and average boulder temperatures were quantified by tracing around each boulder and instructing the software to quantify these temperature parameters using only the temperature pixels within the traced ellipse. A temperature range (i.e. maximum - minimum = range) for each surface was then quantified (daily *n* = 62 – 80, Figure 5.1). A target temperature was recorded for each sampled snail, and, for purposes of standardisation, was measured to the immediate left of each snail (Figure 5.1). The maximum, minimum, and average temperatures of each lower surface were subtracted from each target temperature, to determine how snails were associated with substratum temperatures. Due to the low probability that individual snails will be randomly recorded only in areas with the maximum (or minimum) substratum temperature, comparisons between snail target temperatures and the average temperature of substrata were also completed to better test the hypothesis that snails occupy cooler areas on boulder surfaces.

Within each traced ellipse, a transect was drawn from the centre of the boulder side facing the sun to the centre of the side opposite to quantify millimetre-to-centimetre-scale patterns of temperature difference (Figure 5.1). Transects were drawn for 20 randomly-selected images for three days per seashore, spanning the range of maximum daily air temperatures sampled. This subset of boulders was deemed sufficient to quantify the frequency of occurrence for three different temperature patterns (gradients, mosaics, limited heterogeneity) previously identified for these rocks during the common garden experiment (Chapter 4). Moreover, as I targeted sunny days where temperature mosaics or gradients were likely to form, I was primarily interested in establishing whether the patterns observed in the boulder plot (Chapter 4) were also observed on the seashore.

Statistical analyses

Analyses were completed using either PRIMER v7/PERMANOVA+ (PRIMER-e, Plymouth, UK) or SYSTAT v13 (Systat Software Inc) statistical software, with significance set at α = 0.05, unless otherwise noted. To establish whether boulders on the seashore developed patterns of temperature difference (H1), frequencies of occurrence (%) for each temperature pattern (gradients, mosaics, limited heterogeneity) were tallied separately for 20 boulders per seashore, on each day sampled. Temperature pattern tallies were then pooled across the three days sampled per rock, with a Chi-Square two-way contingency table analysis testing (α = 0.05) for any associations between rock and temperature patterns using these pooled tallies.

To test whether temperature characteristics differed between rocks (H2), means for boulder temperature range and maxima on each sampling day were used in separate one-factor PERMutational ANalyses of COVAriance (PERMANCOVA). Analyses were completed using their untransformed means, with Euclidean distance resemblance matrices prepared separately for each temperature characteristic as dependent variables. To test whether mean temperature characteristics were related to air temperature, maximum daily air temperatures were used as the co-variate in PERMANCOVA models. Permutations of residuals were completed using a reduced model with 9999 permutations.

To test whether snail target temperatures were significantly different from the maximum or average temperature of boulders (H3), paired *t*-tests were completed for each target species on each day sampled for each rock type. As the same target temperatures were used in comparisons against maximum and average temperatures, a Bonferroni correction was applied to reduce the likelihood of making a Type I error. Consequently, a corrected significance level of $\alpha = 0.025$ was applied. Means were then calculated from the replicate boulders on each day for the difference between target temperatures and boulder maxima, and between target temperatures and boulder minima. Mean differences were also used in one-factor PERMANCOVA, as described above (with air temperature as the covariate), to test for differences between grey siltstone and quartzite in how each snail species was associated with the temperature range on lower surfaces (H4). As the same mean target temperature was used to calculate each temperature difference measure for each species, a Bonferroni correction (corrected $\alpha = 0.025$) was applied.

Results

H1) emersed boulder lower surfaces will not display patterns of temperature difference.

The lower surfaces of grey siltstone and quartzite boulders generally had a heterogeneous surface temperature. This heterogeneous temperature differed in maxima and minima by >5 °C on each day sampled at each seashore (Figure 5.2). The type of temperature pattern identified on lower surfaces differed between rocks (Pearson Chi-Square *p*-value < 0.001), with siltstone generally developing gradients (Figure 5.2a) whereas quartzite developed mosaics (Figure 5.2b). Quartzite (60 %) had an overall higher frequency of mosaics versus siltstone (none), while siltstone (76.7 %) had more gradients than quartzite (15 %) when data were pooled across all days analysed (Table 5.2, Appendix 4, Figure 8.24).

Overall occurrences of limited temperature heterogeneity were similar between grey siltstone (23.3 %) and quartzite (25 %) when data were pooled across all days analysed. Occurrences of limited heterogeneity were low (15 %) for both rocks on the two cooler days sampled at each seashore, where gradients dominated on siltstone (85 %) and mosaics on quartzite (≥70 %, Table 5.2, Appendix 4, Figure 8.24). However, on the hottest day sampled for both rocks, occurrences of limited heterogeneity increased to 40-45 % of all boulders sampled (Table 5.2), with a corresponding decrease in the occurrence of gradients and mosaics, especially mosaics on quartzite (Table 5.2, Appendix 4, Figure 8.24).

Table 5.2: Frequencies of occurrence (%) for the three patterns of temperature difference on the lower surfaces of grey siltstone versus quartzite boulders (n = 20 per day sampled) for a subset of the total days sampled.

			Temperature pattern (% of occurrence)		
Rock	Date	Maximum air temperature (°C)	Gradient	Mosaic	Limited heterogeneity
Grey siltstone	20/12/16	23	85	0	15
	28/1/17	29	85	0	15
	17/1/17	39	60	0	40
Quartzite	18/12/16	25	5	80	15
	29/1/17	30	15	70	15
	6/1/17	40	25	30	45

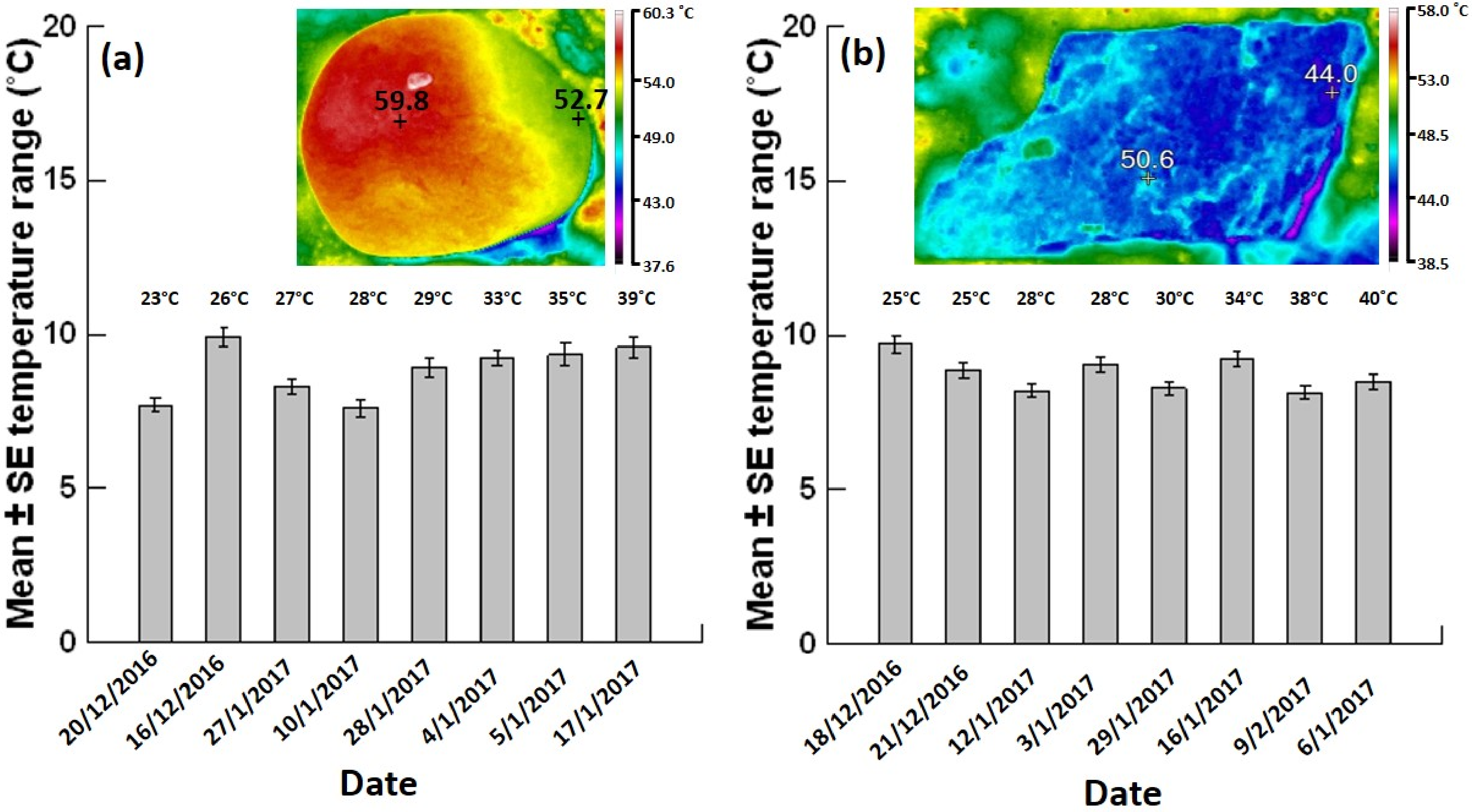


Figure 5.2: Mean ± SE temperature range (*n* = 62 – 80 boulders per day) for (a) grey siltstone and (b) quartzite on different days (ordered by the daily maximum air temperature during sampling at low tide, specified above each individual bar) during the 2016-7 austral summer. Inset thermal images above each bar chart show a representative temperature gradient on a siltstone boulder (range: 59.8 – 52.7 = 7.1 ˚C) and a temperature mosaic on a quartzite boulder (range: 50.6 - 44.0 = 6.6 ˚C) exposed to four hours sunshine at an air temperature of 39 ˚C.

H2) Temperature characteristics will not differ between grey siltstone and quartzite.

The mean temperature range on boulders across sampling days did not significantly differ between grey siltstone and quartzite (univariate PERMANCOVA *p*-value = 0.77, Figures 5.2 & 5.3, Appendix 4, Table 8.37). Temperature range was not related to the daily maximum air temperature (PERMANCOVA covariate *p*-value = 0.64, Figure 5.3a, Appendix 4, Table 8.37). For grey siltstone, the smallest temperature range for an individual surface was 2.3 °C, while the largest temperature range was 18.5 °C. For quartzite, the smallest temperature range was 3.2 °C, while the largest temperature range was 16.9 °C.

Mean maximum temperature differed between rocks (PERMANCOVA *p*-value = 0.0073, Figure 5.3b, Appendix 4, Table 8.37), with grey siltstone (grand mean = 41.1 ± 0.2 °C) having significantly hotter maxima than quartzite (grand mean = 39.3 ± 0.2 °C). The mean maxima were significantly correlated with the maximum air temperatures on the day of sampling (PERMANCOVA covariate *p*-value = 0.0001, Appendix 4, Table 8.37), with hotter maxima recorded on days with hotter air temperatures (Figure 5.3b). The maximum temperature of each replicate boulder was generally hotter than the air temperature at sampling (Figure 5.3c). For grey siltstone, the coolest temperature measured was 20.1 °C, while the hottest temperature was 53.6 ˚C. For quartzite, the coolest temperature was 18.5 °C, while the hottest temperature was 52.2 °C.

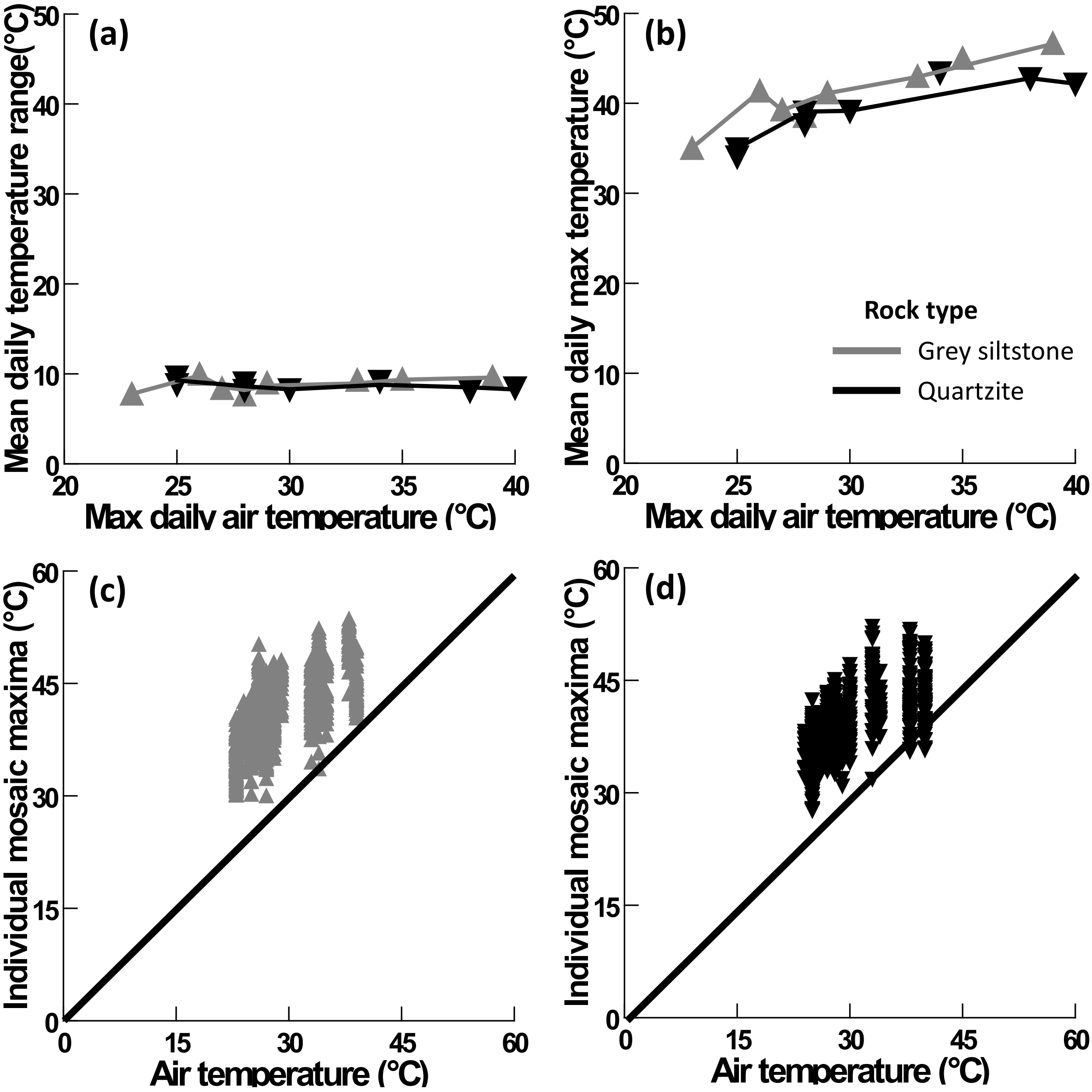


Figure 5.3: The relationship between (a) mean temperature range on each day and maximum air temperature (LOWESS smoother fitted), (b) mean maximum temperature on each day and maximum air temperature (LOWESS smoother fitted), (c) maximum temperature for all replicate grey siltstone boulders and maximum air temperature (1:1 line shown), and (d) maximum temperature for all replicate quartzite boulders and maximum air temperature (1:1 line shown). Each y-axis extends to encompass the range of the raw data, with total *n* = 1152.

H3) Target temperature does not differ from the maximum or average boulder temperature.

Target temperatures, immediately adjacent to snails, were significantly cooler than the maximum temperature of boulders, on each day sampled for both rocks, for all three species (all paired *t*-test *p*-values <0.001, Figures 5.4 & 5.5, Appendix 4, Table 5.3). Somewhat larger differences between target and maximum temperatures were recorded for areas of the rock surface occupied by *D. concameratum* and *N. atramentosa* than for *B. nanum* (Figure 5.4, Table 5.1). For *B. nanum*, mean target temperatures were 4.1 – 5.7 ˚C and 4.0 – 4.8 ˚C cooler than mean maxima on each day sampled for grey siltstone and quartzite, respectively. For *D. concameratum* mean target temperatures were 5.0 – 8.0 ˚C cooler on siltstone and 5.0 – 5.7 ˚C cooler on quartzite than mean maxima, while for *N. atramentosa*, mean target temperatures were 4.9 – 7.9 ˚C cooler on siltstone and 4.9 – 5.9 ˚C cooler on quartzite than mean maxima on each day sampled. The hottest individual target temperatures measured immediately adjacent to snails for each species were 50.7 °C for *B. nanum*, 49.1 °C for *D. concameratum* and 46.3 °C for *N. atramentosa*. The association between target temperature and boulder maxima developed during the first hour that boulders were emersed, and was maintained during the four hour low-tide period. The position of snails on the boulders and associated target temperatures was not related to the amount of surface moisture retained on boulders (see Appendix 4 for additional hypotheses tested and results).

Evidence for snails seeking cooler locations on boulders is provided by paired *t*-tests between means for target temperatures and the average temperature of boulders on each day sampled (Appendix 4, Table 5.3). For both *D. concameratum* and *N. atramentosa*, target temperatures were always cooler than average boulder temperatures on each day sampled for both rocks, with differences of up to 1.81 °C (Appendix 4, Table 5.3). When observations are pooled across rock type, this difference was significant on 13 out of 16 days sampled for *D. concameratum* and 11 out of 16 days for *N. atramentosa*. In contrast, *B. nanum* target temperatures were generally similar (always <1 °C difference) to mean boulder temperatures on each day sampled for both rocks (Appendix 4, Table 5.3). Target temperatures, adjacent to the snails, were significantly warmer than the minimum temperature of boulders, on each day sampled for each rock, for all three species (paired *t*-test *p*-values <0.001, Figure 5.4, Appendix 4, Table 5.3).

Table 5.3: Paired *t*-tests testing for significant differences between snail target temperature and the maximum, minimum and average temperatures of boulders for *Bembicium nanum, Diloma concameratum* and *Nerita atramentosa* on grey siltstone and quartzite for each day sampled. Significant differences (α = 0.025) between temperature measurements are shown in bold. x̄ = mean temperature difference.

	Species:	*Bembicium nanum*						*Diloma concameratum*						*Nerita atramentosa*					
	Measure	Target - maximum		Target - minimum		Target - average		Target - maximum		Target - minimum		Target - average		Target - maximum		Target - minimum		Target - average	
Rock	Max air temp (˚C)	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value	x̄	*p*-value
Grey siltstone	23	-4.09	**<0.001**	3.20	**<0.001**	0.17	0.586	-5.61	**<0.001**	2.77	**<0.001**	-0.89	**0.007**	-5.58	**<0.001**	2.35	**<0.001**	-1.06	**<0.001**
	26	-4.89	**<0.001**	3.76	**<0.001**	-0.22	0.540	-7.34	**<0.001**	2.93	**<0.001**	-1.62	**<0.001**	-7.19	**<0.001**	4.40	**<0.001**	-0.77	0.030
	27	-5.10	**<0.001**	3.49	**<0.001**	-0.13	0.728	-6.06	**<0.001**	2.05	**<0.001**	-1.30	**<0.001**	-6.23	**<0.001**	2.30	**<0.001**	-1.09	**<0.001**
	28	-4.26	**<0.001**	3.56	**<0.001**	0.20	0.489	-4.96	**<0.001**	2.61	**<0.001**	-0.48	0.037	-4.88	**<0.001**	2.46	**<0.001**	-0.53	0.067
	29	-5.66	**<0.001**	3.17	**<0.001**	-0.35	0.267	-6.46	**<0.001**	2.63	**<0.001**	-1.05	**<0.001**	-6.83	**<0.001**	2.14	**<0.001**	-1.29	**<0.001**
	33	-5.06	**<0.001**	3.82	**<0.001**	-0.22	0.543	-6.11	**<0.001**	2.72	**<0.001**	-1.10	**0.006**	-6.98	**<0.001**	2.82	**<0.001**	-1.60	**<0.001**
	35	-4.84	**<0.001**	4.25	**<0.001**	0.20	0.607	-7.22	**<0.001**	2.63	**<0.001**	-1.74	**<0.001**	-6.87	**<0.001**	2.61	**<0.001**	-1.37	**0.007**
	39	-4.67	**<0.001**	4.14	**<0.001**	-0.03	0.925	-8.01	**<0.001**	2.51	**<0.001**	-1.81	**0.001**	-7.91	**<0.001**	2.68	**<0.001**	-1.40	**<0.001**
	Grand means (GM)	-4.82		3.67		-0.05		-6.36		2.59		-1.21		-6.56		2.72		-1.14	
	Standard error of GM	0.14		0.15		0.12		0.19		0.10		0.11		0.17		0.11		0.11	
Quartzite	25	-4.65	**<0.001**	5.07	**<0.001**	0.63	0.045	-5.13	**<0.001**	4.58	**<0.001**	-0.26	0.244	-5.44	**<0.001**	4.72	**<0.001**	-0.34	0.221
	25	-4.36	**<0.001**	5.07	**<0.001**	0.88	**0.017**	-5.02	**<0.001**	3.61	**<0.001**	-0.48	0.064	-4.96	**<0.001**	3.62	**<0.001**	-0.47	0.067
	28	-3.96	**<0.001**	4.19	**<0.001**	0.53	0.114	-5.09	**<0.001**	3.15	**<0.001**	-0.79	**0.007**	-5.26	**<0.001**	3.19	**<0.001**	-0.79	**0.004**
	28	-4.64	**<0.001**	4.40	**<0.001**	0.26	0.299	-5.40	**<0.001**	3.70	**<0.001**	-0.55	**0.005**	-4.91	**<0.001**	3.93	**<0.001**	-0.49	0.072
	30	-4.68	**<0.001**	4.04	**<0.001**	0.16	0.600	-5.17	**<0.001**	2.92	**<0.001**	-0.77	**0.001**	-5.12	**<0.001**	3.13	**<0.001**	-0.87	**<0.001**
	34	-4.84	**<0.001**	4.97	**<0.001**	0.53	0.070	-5.71	**<0.001**	3.01	**<0.001**	-1.14	**<0.001**	-5.20	**<0.001**	3.59	**<0.001**	-0.77	**0.014**
	38	-4.41	**<0.001**	4.37	**<0.001**	0.51	0.113	-5.13	**<0.001**	2.33	**<0.001**	-1.06	**<0.001**	-5.89	**<0.001**	2.25	**<0.001**	-1.45	**<0.001**
	40	-4.29	**<0.001**	3.90	**<0.001**	0.18	0.442	-5.60	**<0.001**	2.68	**<0.001**	-1.15	**<0.001**	-5.52	**<0.001**	3.43	**<0.001**	-0.74	**0.010**
	Grand means (GM)	-4.48		4.50		0.46		-5.26		3.28		-0.75		-5.29		3.48		-0.74	
	Standard error of GM	0.12		0.14		0.10		0.13		0.11		0.08		0.12		0.11		0.09	

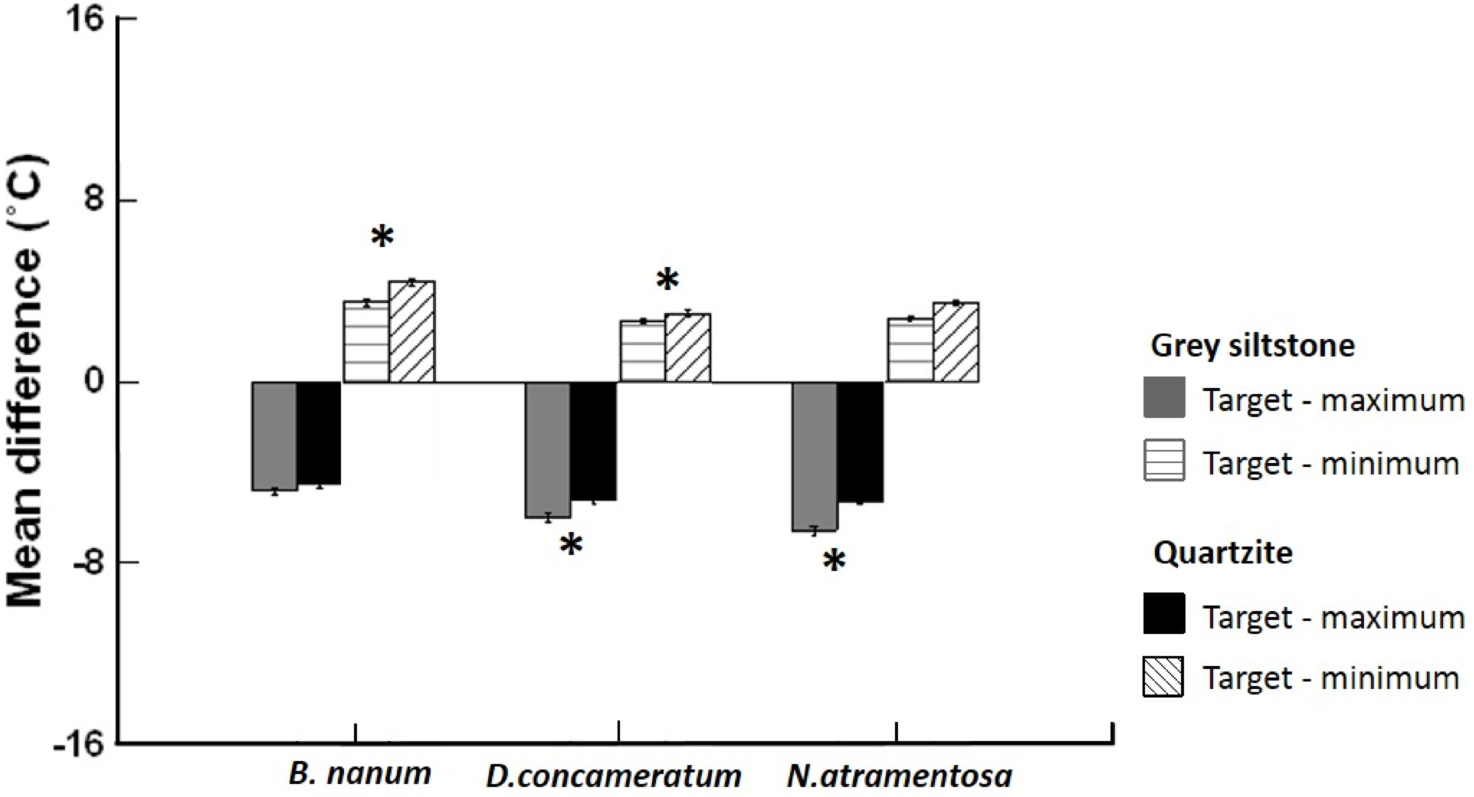


Figure 5.4: Grand mean ± SE (total *n* = 1389) for the differences between target temperature and maximum or minimum boulder temperatures on grey siltstone and quartzite for *Bembicium nanum*, *Diloma concameratum* and *Nerita atramentosa*. * = a significant difference detected between siltstone and quartzite for target – maximum or minimum temperatures for that snail species.

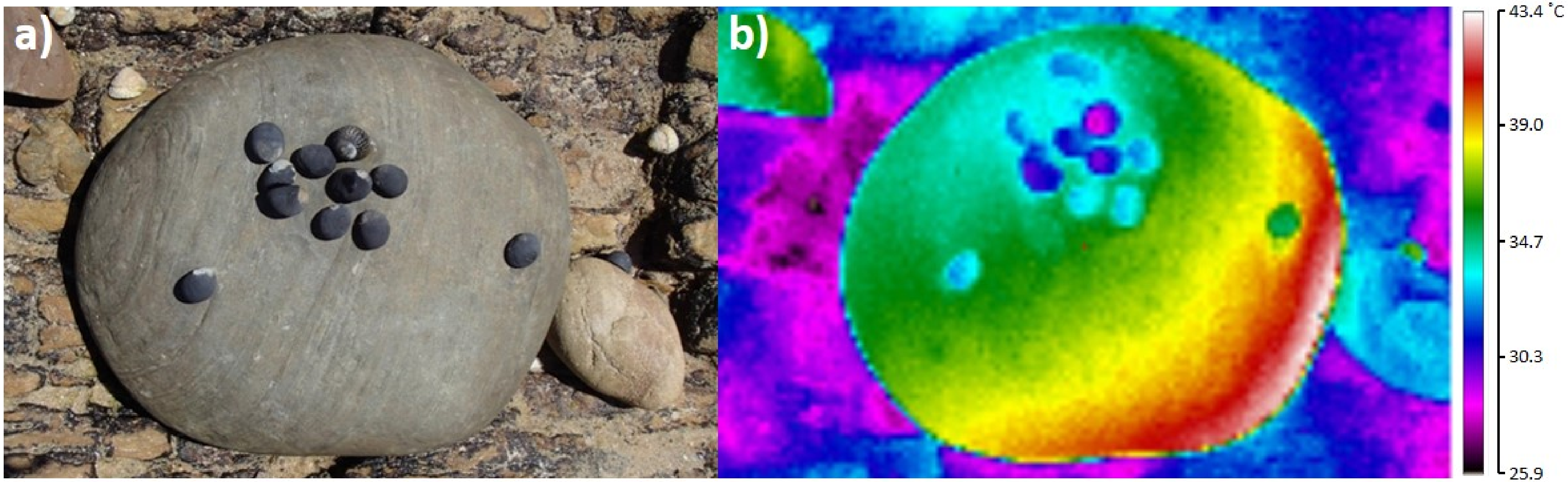


Figure 5.5: Example digital photograph (a) and thermal image (b) showing the spatial separation of snails from boulder-surface maxima for 10 *Nerita atramentosa* and one *Diloma concameratum* on the lower surface of grey siltstone. Ten out of eleven snails have occupied the cooler (shown by the blue and green colours) parts of this temperature gradient and are generally avoiding warmer (shown by the yellow and red colours) gradient locations.

H4) differences between target temperature and either thermal maxima or minima on boulders do not differ between grey siltstone and quartzite.

Target temperatures were further from the maxima, nearer to the minima, and cooler relative to the average, on grey siltstone versus quartzite for all three snail species (Appendix 4, Table 5.3). Together, these results show there was a stronger association between snails and the cooler areas of boulders on hotter siltstone (Figure 5.4). For *D. concameratum*, significantly larger negative mean differences were detected for grey siltstone than quartzite for target - maximum temperature of substrata (PERMANCOVA *p*-value = 0.0014, Appendix 4, Table 8.37), while significantly smaller positive mean differences were detected for grey siltstone than quartzite for target - minimum temperatures (PERMANCOVA *p*-value = 0.0030, Figure 5.4, Appendix 4, Table 8.37). For *N. atramentosa*, significantly larger negative mean differences were identified for grey siltstone than quartzite for target - maximum temperatures (PERMANCOVA *p*-value = 0.0026, Appendix 4, Table 8.37), although the smaller positive mean differences for siltstone than quartzite for target - minimum temperatures were not significantly different after Bonferroni correction (PERMANCOVA *p*-value = 0.0380, Figure 5.4, Appendix 4, Table 8.37). For *B. nanum*, this rock-related difference was not significant for mean target - maximum temperature of substrata (PERMANCOVA *p*-value = 0.12, Appendix 4, Table 8.37), although significantly smaller positive mean differences were recorded on siltstone than quartzite for target - minimum temperature of substrata (PERMANCOVA *p*-value = 0.0019, Figure 5.4, Appendix 4, Table 8.37).

These mean differences between snail target temperature and the maxima and minima of boulders, on each rock, were not strongly influenced by the air temperature at sampling for any species (smallest PERMANCOVA covariate *p*-value = 0.0329 for target – minimum temperatures for *D. concameratum*, which was not significant after Bonferroni correction, Appendix 4, Table 8.37).

Discussion

This research shows that lower boulder surfaces often have patterns of temperature difference, including cooler refuge microhabitats, at a scale of millimetres to centimetres. On grey siltstone, gradients of surface temperature were most frequently observed, while on quartzite temperature mosaics were most common. Siltstone had hotter maximum surface temperatures than quartzite. At low tide, three snail species retreated to these lower-surface microhabitats, with snail occupancy of under-boulder habitats associated with these patterns of temperature difference. All three species had target temperatures significantly cooler than the maxima, and similar to or significantly cooler than the average temperature of boulders. These results provide clear evidence that snails generally avoided the hottest areas on boulders. Stronger associations between target and boulder temperatures were recorded for *N. atramentosa* and *D. concameratum*, and on the hotter of the two rocks tested, grey siltstone.

Patterns of temperature difference during periods of seashore emersion have been extensively documented (e.g. Garrity 1984; Denny et al. 2011; Marshall et al. 2013). The scale of these temperature differences can range from just millimetres to centimetres

(Judge et al. 2011; Lathlean et al. 2012; 2013) to tens or hundreds of kilometres (Helmuth et al. 2006b). My results provide evidence of small-scale temperature differences within a specific type of intertidal microhabitat, boulder lower surfaces. While within-microhabitat temperature differences have been described previously in terrestrial habitats (Huey et al. 1989), these results are novel for intertidal habitats. Most previous research at the small-scale on intertidal reefs have instead investigated temperature heterogeneity between sun-exposed microhabitats and sun-protected refuge microhabitats (e.g. Garrity 1984; Denny et al. 2011; Chapperon et al. 2017). Moreover, while the capacity of boulder lower surfaces to act as cooler refuge habitats has been recognised previously (Huey et al. 1989; Bertness 1999; Chapperon and Seuront 2011a), temperature variability within refuge microhabitats on the seashore has not. Other seashore refuge microhabitats such as crevices generally have only limited temperature heterogeneity (Chapperon et al. 2016; 2017). In the present study, the maximum temperature range of 18.5 °C under an individual siltstone boulder was more than 10 °C larger than the maximum range between replicate 400 cm^2 quadrats on the seashore (Lathlean et al. 2012). However, this 18.5 °C range was smaller than the 24.0 °C range detected on the lower surfaces of terrestrial rocks (<2 metres length or width) that sheltered garter snakes during summer (Huey et al. 1989).

Grey siltstone attained hotter surface temperatures than quartzite, with a gradient of temperatures observed on siltstone and a mosaic of temperatures generally identified on quartzite. While temperature differences between various seashore rocks have been identified elsewhere (Chapter 4; Raimondi 1988; Marshall et al. 2010; Judge et al. 2011), rock-related differences in the patterns of temperature difference (i.e. gradients versus mosaics) have not been observed or reported previously. The temperature of substrata characteristics reported here are largely similar to those identified during the common-garden experiment (Chapter 4). Therefore, observations of boulder thermal behaviour from the common-garden experiment can be extended to boulders on the seashore. On sunny days, limited heterogeneity was most common on days with hotter air temperature. However, frequencies of limited heterogeneity on the seashore (40-45 % occurrence for both rocks) were lower compared to during the common-garden experiment (66-83 % occurrence for both rocks, Chapter 4) on the hottest day sampled in the field and during experimentation, respectively. This difference in occurrence may be attributable to the dynamic properties of seashores. Moisture under boulders, shading by the surrounding substratum, wave splash and wrack deposition may all interact to prevent some boulder surfaces from becoming generally uniformly hot at the hottest air temperatures. In turn, this may enable a higher frequency of temperature gradients and mosaics to persist on the seashore during extreme heatwaves.

Intertidal gastropods under siltstone boulders were subjected to a hotter range of temperatures (20.1 – 53.6 °C) than those under quartzite (18.5 – 52.2 °C). Consequently, siltstone may offer a lower-quality thermal habitat on hot, sunny days. This was reflected by snails on siltstone having stronger associations with boulder temperatures than on quartzite, suggesting that only locations further from the maxima and nearer to the minima offer adequate thermal relief under siltstone. The notion that different rocks provide different quality thermal habitats is supported by other intertidal studies, with Raimondi (1988) attributing the higher vertical seashore distribution of barnacles on granite compared to basalt to granites cooler temperatures. Moreover, the cooler body temperatures recorded by robotic snail mimics on lighter versus darker-coloured rocks was the result of overall cooler surface temperatures on lighter-coloured rocks (Marshall et al. 2010; Judge et al. 2011).

Quartzite may also represent a better thermal habitat for snails due to its temperature mosaics. Snails on siltstone needed to occupy cool positions at the end of a gradient to achieve maximum thermal relief (e.g. Figure 5.2a). In contrast, as quartzite had small millimetre to centimetre scale patches of temperature across the entire surface (e.g. Figure 5.2b), snails could potentially move much shorter distances to achieve thermal relief. Under various climate change scenarios thermal refuges may be beneficial for the persistence of some intertidal biota (Chapman 2005; Gedan et al. 2011). This study indicates that quartzite seashores could potentially function as thermal refuges because of their cooler temperatures and finer-scale temperature mosaics. However, the capacity of quartzite to act as a thermal refuge may be limited during heat waves, as the frequency of boulder surfaces becoming generally uniformly hot, and thus showing limited heterogeneity, increased at the hottest air temperatures. With predications of an increased frequency of heatwaves and hotter air temperatures associated with global climate change (IPCC 2013), environmental conditions where boulder surfaces become generally hot, and hence uniform, may become increasingly common. In turn, this may challenge the survival and persistence of some intertidal biota, as cooler microhabitats under boulders are likely to be most-beneficial on the hottest sunny days.

Nerita atramentosa, *D. concameratum* and *B. nanum* all retreated to the lower surfaces of boulders when emersed, with upper surfaces at low tide rarely occupied by any mobile biota (personal observation). This avoidance of dry, sun-exposed surfaces is consistent with observations made for a variety of gastropod species on seashores in both Panama (Garrity 1984) and Hong Kong (Yeung and Williams 2012). It is thought that the protected lower surface of boulders, relative to more exposed substrata (e.g. upper boulder surfaces, rock platforms), afford intertidal gastropods their best chance of survival when emersed (Chapman 2003; Chapperon and Seuront 2011a). The substrata adjacent to all three snail species had target temperatures that were significantly cooler than the maxima, and that were similar to or cooler than the average temperature of boulders (Figure 5.4). This finding builds upon earlier studies investigating the interactions between intertidal gastropods and habitat temperature (e.g. Garrity 1984; Jones and Boulding 1999; Chapperon and Seuront 2011a) by showing that behavioural thermoregulation in gastropods may be more complex than simply retreating to cooler refuge microhabitats. Instead, as previously reported for garter snakes under rocks in terrestrial habitats (Huey et al. 1989), there is a range of within-microhabitat temperatures under intertidal boulders that mobile gastropods are associated with. My results showed that three snail species were associated with the cooler areas under boulders, clearly avoiding the hottest areas (e.g. Figure 5.5).

This fine-scale association between snails and cooler locations has not previously been reported from under intertidal boulders. However, it is consistent with observations from other intertidal habitats where periwinkles selected the coolest locations when offered centimetre-scale temperature gradients (Soto and Bozinovic 1998; Jones and Boulding 1999). As emersed *N. atramentosa* and *B. nanum* aggregate in clumps in microhabitats that minimise desiccation and heat stress (Underwood 1976; Chapperon et al. 2013), the association I found between snails and cooler temperatures of substrata may be related to the aggregating behaviour of these species. As both species are inactive during low tide

(Underwood 1977), how they identify cooler locations within one hour of emersion (see Appendix 4), before boulders have reached their thermal maxima several hours later, remains unclear. Some congeners of these species will home to refuge microhabitats after feeding by following chemical cues in the mucus trails they create (Vannini and Chelazzi 1978; Chelazzi et al. 1984; 1985). Therefore, after a period of high-tide feeding, the snails investigated here may home to specific locations under boulders using similar cues because of their favourable thermal properties.

Somewhat larger differences between target and maximum temperatures of substrata were recorded for *N. atramentosa* and *D. concameratum* than for *B. nanum*. This is likely related to an interaction between vertical distributions and thermal tolerances, with higher-shore species generally having higher thermal tolerances than lower-shore species (McMahon 1990; Somero 2002). *Bembicium* is a mid-high shore genus and so have a higher thermal tolerance than either of the lower shore species *N. atramentosa* and *D. concameratum* (Underwood 1975; McMahon 1990). Therefore, the less-thermally sensitive *B. nanum* may have been able to withstand, and thus occupy, warmer locations, as indicated by the smaller temperature difference measured for them.

Retreating under boulders may not be sufficient alone to always avoid deleterious heat during daytime summer low tides, with some boulder and target temperatures on the hottest days sampled >50 °C in this study. In conjunction with the behavioural thermoregulation by microhabitat selection observed in this study, each of these species may also employ several other behavioural or physiological mechanisms to mitigate the risk of desiccation and heat stress. These include evaporative cooling or withdrawing deeply into the shell and sealing the aperture with the operculum (Vermeij 1971b; Suryanarayanan and Nair 1979; McMahon 1990). Consequently, these additional mechanisms may allow these snails to withstand exposure to short periods of extreme heat when emersed, such as those recorded under boulders in this study. Nevertheless, exposure to deleterious heat could be problematic, as some intertidal species presently live near their tolerable thermal limits (Helmuth et al. 2002; Somero 2002; Madeira et al. 2012), and exceedance of these limits can result in issues ranging from reduced growth rates (Jones and Boulding 1999; Lathlean et al. 2013; Lamb et al. 2014) to mass mortality events (Harley 2008).

In conclusion, this study shows that three snail species occupied cooler areas within under-boulder habitats. Thus, gastropod behavioural thermoregulation appears more complex than simply retreating to protected locations. Stronger associations between target temperatures adjacent to snails and boulder temperatures were recorded on grey siltstone than quartzite, and for the more-thermally sensitive *N. atramentosa* and *D. concameratum*. Therefore, associations between gastropods and the temperature of substrata also exist within microhabitats, with these associations being both species and rock specific. This new information allows us to better-understand the associations between biota and the temperature of substrata in the thermally-extreme and variable rocky intertidal zone, which is important given predictions of a warming climate associated with global climate change.

Acknowledgements

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Chapter 6

General discussion

This research addresses my overall thesis aim by showing that rock type is an abiotic characteristic of the seashore that can influence intertidal assemblages in temperate southern Australia (Figures 6.1 & 6.2). Different types of rock were shown to vary in hardness (Chapter 2), mineralogy (Chapters 2 & 4), microhabitat density (Chapters 2 & 3), rugosity (Chapters 2 & 3) and temperature behaviour (Chapters 4 & 5). Several of these physical attributes appear to be interrelated (Figure 6.2), with hardness-related differences in microhabitat density and rugosity identified (Chapter 2), and boulder temperature behaviour correlated with mineralogy (Chapter 4).

Rock-related differences in physical attributes accounted for some of the variation observed in intertidal assemblages (Figures 6.1 & 6.2). The greater species richness and abundances of specific taxa on platforms (Chapter 2) or boulders (Chapter 3) comprised of softer rocks, such as limestone or calcarenite, was correlated with the greater rugosity and microhabitat density of these softer rocks. Despite a lack of general associations between intertidal biota and rock hardness (Chapter 2), turf-forming algae were always more abundant on soft versus hard platforms. Assemblage structural differences on rock platforms were most strongly associated with hardness-related differences in mineralogy (Chapter 2). Moreover, rock-related differences in mineralogy and temperature behaviour (Chapters 4 & 5) affected how three species of mobile gastropods associated with patterns of temperature difference on boulder lower surfaces (Chapter 5). Overall, this research shows that the physical attributes that vary among rocks appear to influence the habitat suitability of those rocks for certain taxa, giving rise to the biotic patterns identified.

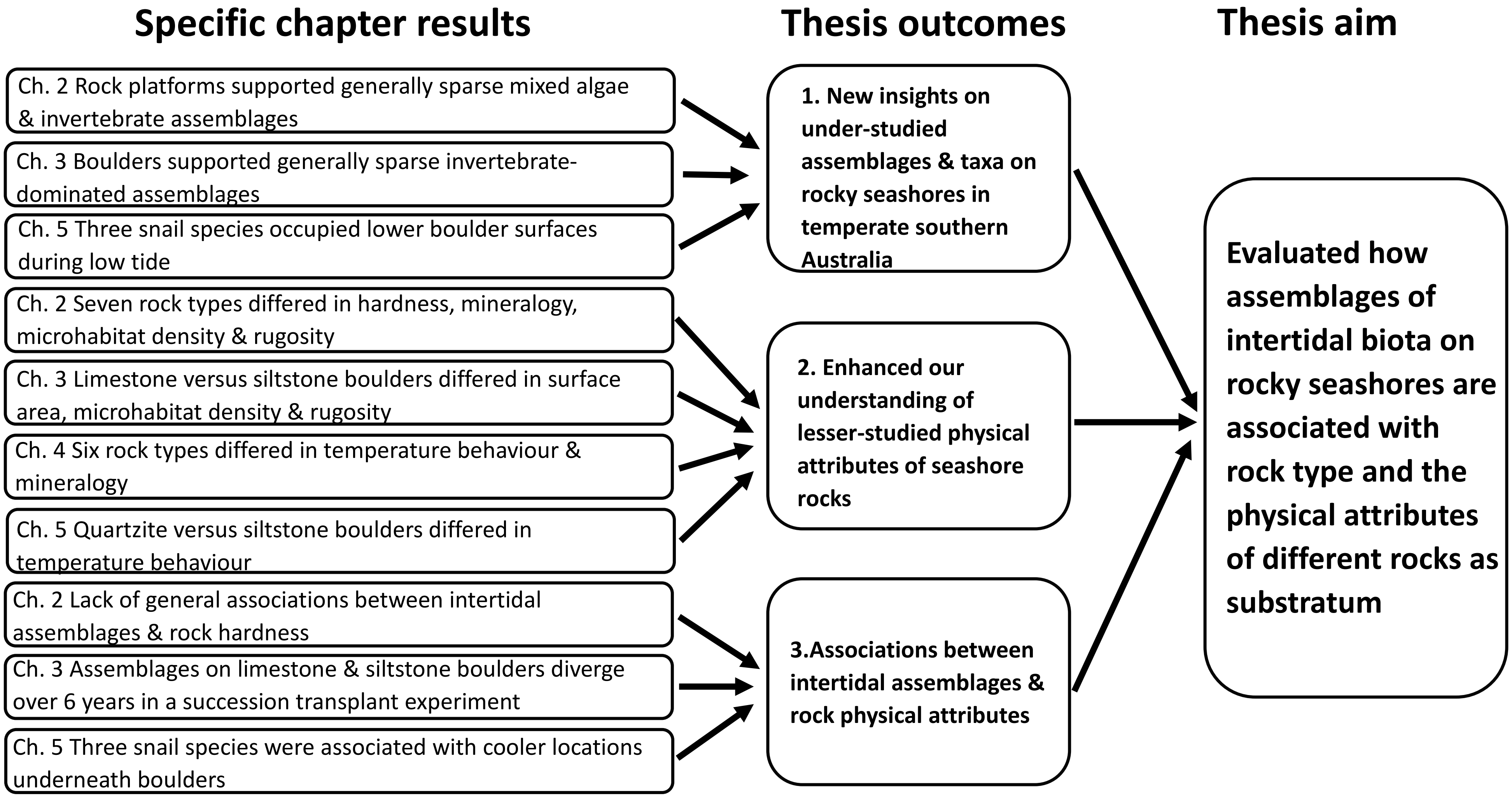

Figure 6.1: Schematic showing how the results from each data chapter contribute to my three thesis outcomes. These three outcomes address my overall thesis aim.

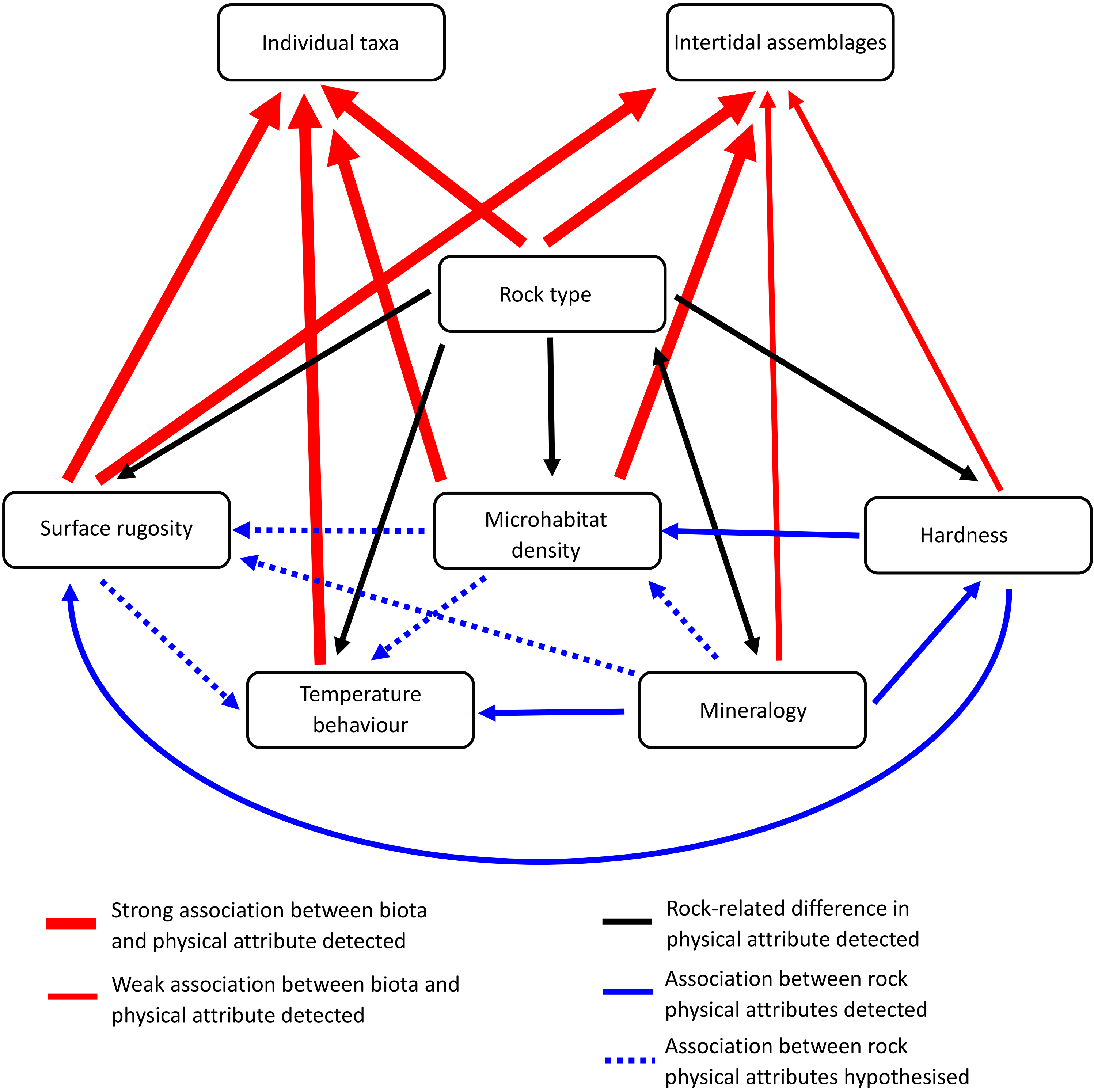

Figure 6.2: Conceptual model showing how assemblages of intertidal biota and individual taxa are associated with rock type and rock-related differences in physical attributes in temperate southern Australia (red arrows). Rock-related differences in physical attributes (black arrows) and the interrelations between various rock physical attributes (blue arrows) are also shown.

Outcome 1: New insights on under-studied assemblages and taxa on rocky seashores in temperate southern Australia.

I established a baseline of the intertidal biodiversity inhabiting seashores between Portland and Adelaide (Figure 6.3), thereby accomplishing my first thesis outcome. This baseline showed that platform habitats support generally sparse mixed algae and invertebrate assemblages (Chapter 2, Appendix 5), while boulder habitats support sparse invertebrate-dominated assemblages (Chapters 3 & 5). The structure, richness and species abundances of these assemblages was highly variable spatially and temporally, differing between bioregions (Chapter 2), individual seashores (Chapters 2-3, Appendix 5), seasons (Chapter 2) and successional stages (Chapter 3).

Of the platform habitats sampled, only Blacknose Point was identified as a biodiversity hotspot (sensu Benkendorff and Davis 2002), with an invertebrate richness (27) more than two standard deviations greater than the overall mean ($\bar{x}$ = 14.9 ± standard deviation = 5.9, Chapter 2). The seashores around Portland (Figure 6.3) may also be a hotspot for invertebrates, with both Portland Windfarm (21) and Enchanted Forest (22) having a high invertebrate richness (Chapter 2). This observation corroborates previous research conducted by Thyer (2015), who reported a similarly-high invertebrate richness across the Portland region. No seashore was a biodiversity hotspot for marine plants (Chapter 2, Appendix 5). The identification of biodiversity hotspots may provide a foundation for the management of seashores in southern Australia, as seashores that support the greatest species richness should be prioritised for conservation (Benkendorff and Davis 2002; Benkendorff 2005; Blanchette et al. 2008).

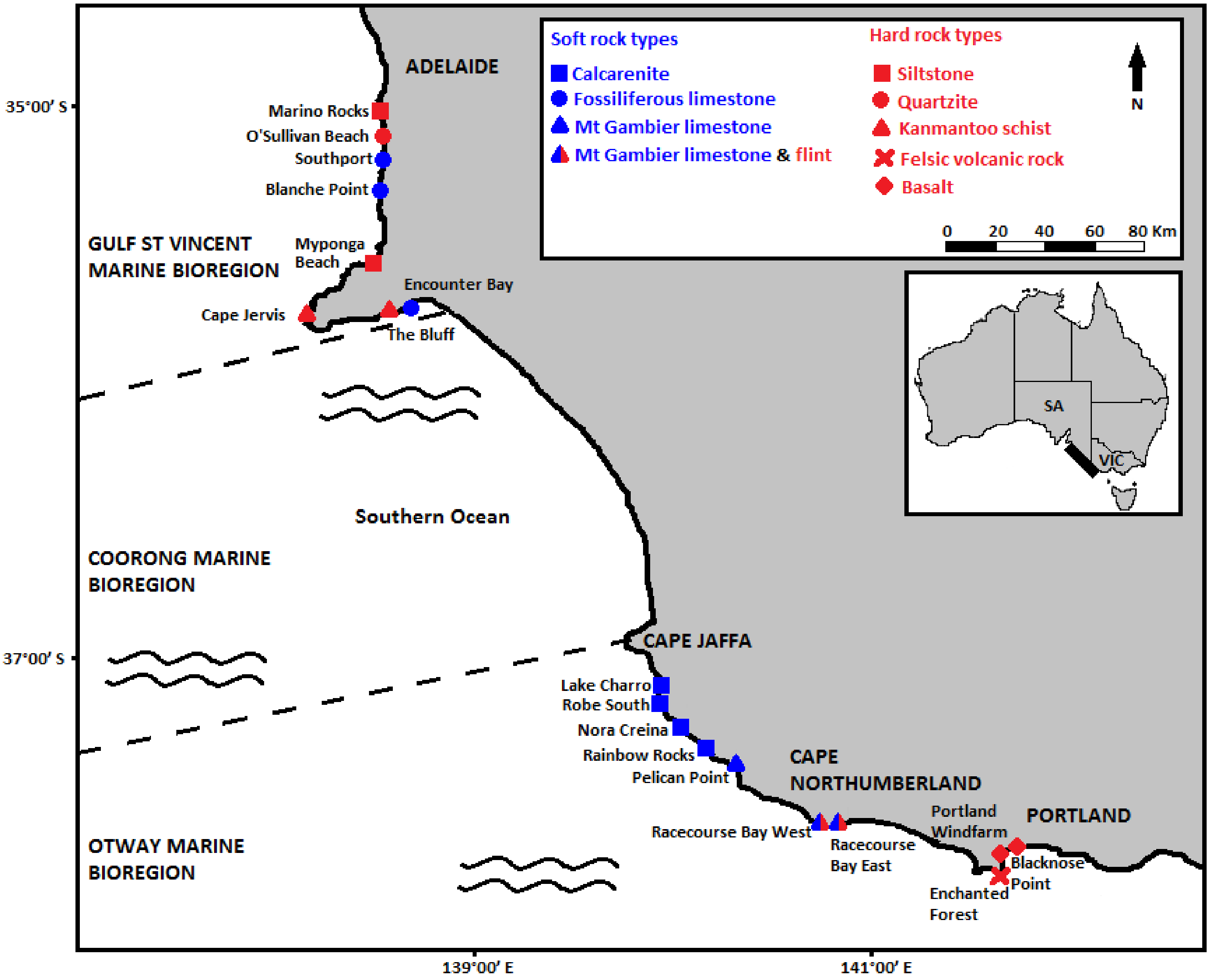

Figure 6.3: Rocks from the soft (blue) versus hard (red) classes sampled in the Gulf St Vincent and Otway Marine Bioregions in this thesis. Inset map shows the location of the study region in temperate southern Australia.

Bioregional differences in the spatial variability of intertidal assemblages have been identified globally, including longitudinally from Portugal (Martins et al. 2018), latitudinally from the west coast of the Americas (Wieters et al. 2009; 2012; Martins et al. 2018), and now longitudinally for temperate southern Australia (Chapter 2; Thyer 2015). The GSV Marine Bioregion was characterised by a greater richness and space occupancy of sessile biota than the Otway Bioregion, while the Otway Bioregion was characterised by a greater overall biomass of invertebrates (Chapter 2). The calcarenite and Mount Gambier limestone seashores between Cape Jaffa and Racecourse Bay East (Figure 6.3) supported a mobile invertebrate richness and abundance considerably lower than that described from inventories for other parts of SA (Chapter 2; Appendix 5; Benkendorff 2005; Benkendorff et al. 2007; Benkendorff and Thomas 2007). The distinct intertidal assemblages identified in each bioregion may be associated with a host of environmental characteristics that differed between bioregions (Chapter 2), including wave action, upwelling events, and air or sea surface temperatures.

Biogeographic barriers can restrict the distribution of species across bioregions (Hidas et al. 2007; Ayre et al. 2009; Thyer 2015), potentially accounting for the bioregional patterns identified (Chapter 2). Thyer (2015) reported that the large sandy beaches of Discovery Bay (situated between Cape Northumberland and Portland) and Encounter Bay (the entire coast of the Coorong Bioregion) act as biogeographic barriers, with Discovery Bay being the more effective barrier (Figure 6.3). SIMilarity PERcentages (SIMPER) analyses comparing seashores on the eastern (Nora Creina, Pelican Point and Racecourse Bay West) versus western (Enchanted Forest, Blacknose Point, Portland Windfarm) sides of Discovery Bay for all seasons sampled found high dissimilarity for mobile invertebrate assemblages (92 %) and sessile assemblages (95 %). However, no reliable indicator taxa were associated with this large dissimilarity (all SIMPER SD/Sim ratios <1). Biogeographic barriers may therefore account for the very distinct assemblages identified either side of Discovery Bay.

For the intertidal assemblages that developed on transplanted boulders, early-successional assemblages after 11 months were subtly different from the later-successional assemblages that developed after five or six years (Chapter 3). These temporal differences for developing intertidal assemblages on transplanted boulders are novel, as previous boulder transplant experiments in the rocky intertidal zone only investigated succession over timeframes of <12 months (e.g. McGuinness and Underwood 1986; James and Underwood 1994; Green et al. 2012; Liversage et al. 2014). Temporal differences were also detected among seasons for the intertidal assemblages inhabiting rock platforms (Chapter 2). While data were only collected from spring through autumn due to the persistence of dangerous sampling conditions during winter, the structure of mixed algal and invertebrate assemblages varied seasonally. Seasonal variation in intertidal assemblages has been documented for algal colonisation in Canada (Scheibling et al. 2009), algal assemblage structure in the Caribbean (Steneck and Dethier 1994), patterns of invertebrate distribution in eastern Australia (O'Gower and Meyer 1965; 1971) and now for the structure of mixed algal and invertebrate assemblages in temperate southern Australia (Chapter 2).

Outcome 2: Enhanced our understanding of lesser-studied physical attributes of seashore rocks.

The research in this thesis contributes to our understanding of rock-related differences for several lesser-studied physical attributes including hardness (Chapter 2 & Appendix 5), mineralogy (Chapters 2 & 4) and surface temperature (Chapters 4 & 5). These physical attributes were quantified for nine types of rock as boulders and platforms (Chapters 2-5, Appendix 5), thus accomplishing my second thesis outcome (Figure 6.1).

The scratch hardness of Mount Gambier limestone, calcarenite and fossiliferous limestone was ≤4, thus these rocks were assigned to the soft class (Chapter 2, Appendix 5). Conversely, basalt, felsic volcanic rock, Kanmantoo schist, siltstone, quartzite and flint all had a hardness >4, thus these rocks were assigned to the hard class (Chapter 2, Appendix 5). The softest rock was Mount Gambier limestone with a hardness of two (Chapter 2, Appendix 5), while the hardest rock was boulders of siltstone and flint, which both had a hardness of eight (Chapter 3, Appendix 5). The range of hardness (2.0 - 8.0) measured for these rocks falls within the range of hardness measured for other seashore rocks in SA (Liversage and Benkendorff 2013; Liversage et al. 2014).

My results indicate that hardness may influence the types of habitat seashore rocks can form (Chapters 2-3, Appendix 5). White limestone and siltstone occurred as both platforms and boulders on the same seashores, with boulders (limestone hardness = 4 & siltstone hardness = 8, Chapter 3) having a greater hardness than platforms comprised of the same rock (limestone hardness = 3 & siltstone hardness = 5, Chapter 2). This result is likely related to the erodibility of rocks, with the softest rocks having the fastest erosion rates, and therefore potentially having a limited capacity to persist as boulders in the rocky intertidal zone (Spencer 1985). Thus, as platforms erode, only the hardest pieces of rock may be able to persist as boulders. This not only accounts for the hardness differences between boulders and platforms of the same rock, but is consistent with the complete absence of boulders on those intertidal reefs comprised of the two softest rocks sampled, Mount Gambier limestone and calcarenite (Chapters 2-3, Appendix 5).

For the mineralogy of seashore rocks as platforms (Chapter 2) and boulders (Chapter4), all rocks investigated were dominated by silicon dioxide or calcium oxide. These dominant major minerals are the same as those reported from studies in the Mediterranean, where a silica or calcite dominated mineralogy was identified (e.g. Cattaneo-Vietti et al. 2002; Guidetti et al. 2004; Bavestrello et al. 2018). I was able to extend our understanding of rock mineralogy further by quantifying the content of 9 additional major minerals and 40 trace elements in each rock (Chapters 2 & 4). Each rock had a unique major mineral and trace elemental composition, and this composition was quite variable among replicate samples of the same rock, even when samples were sourced from a relatively small area on the same seashore (Chapters 2 & 4).

The major mineral and trace elemental composition of each rock was correlated with its temperature behaviour (Figure 6.2, Chapter 4). There was also some evidence that rock colour may be associated with temperature behaviour, with darker-coloured rocks generally hotter than lighter-coloured rocks (Chapter 4). However, as the major mineral and trace elemental composition of rocks presumably determine their colour, the association between rock colour and temperature may simply be a derivative of the mineralogy differences identified among rocks. Rocks with the highest metallic oxide and trace metal content had the hottest maximum temperatures, while rocks with the least metallic content had the coolest maximum temperatures (Chapter 4). This relationship between mineralogy and temperature behaviour has not been previously reported in rocky intertidal studies (e.g. Cattaneo-Vietti et al. 2002; Guidetti et al. 2004; Bavestrello et al. 2018). By showing mineral composition may affect rock temperature behaviour (Chapter 4) I hypothesised that rock-related differences in the associations between snails and temperature difference patterns under boulders may be related to rock type differences in mineralogy and temperature (Chapter 5). Persistent cool, cloudy and wet weather during seasonal sampling of rock platforms prevented the same correlations being tested for other rock and habitat types (Chapter 2).

Both the rock platforms (Chapter 2) and boulders (Chapters 4 & 5) sampled here did not have homogenous surface temperatures. Instead, patterns of temperature difference that consisted of warmer and cooler areas at the scale of millimetres to centimetres were identified (Chapters 2, 4-5). The detection of patterns of temperature difference within a type of microhabitat (boulder upper and lower surfaces) has not been reported previously from intertidal studies. However, within-microhabitat temperature heterogeneity has been observed previously for rocks in terrestrial habitats (Huey et al. 1989). Temperature heterogeneity in the rocky intertidal zone exists at a variety of spatial scales including between seashores (Helmuth et al. 2006b), between replicate quadrats on the same seashore (Chapter 2; Lathlean et al. 2012), between seashore microhabitats (Garrity 1984; Chapperon and Seuront 2011a; Denny et al. 2011; Chapperon et al. 2013), and now for boulder upper and lower surfaces (Chapters 4-5). The maximum (and minimum) temperature of boulder surfaces differed consistently between rocks and surfaces. Upper surfaces were generally hotter than lower surfaces (Chapter 4), with purple siltstone and grey siltstone consistently the hottest, and white limestone and quartzite the coolest (Chapters 4-5). Consequently, maximum temperatures of substrata on the seashore not only differ among different rock types (Raimondi 1988; Chapters 4 & 5; Marshall et al. 2010), rock colours (Judge et al. 2011) and rock orientations (Bertness 1999; Chapperon et al. 2017), but also between different rock mineralogies (Chapter 4) and the upper and lower surfaces of the same boulder (Chapter 4).

For both platforms (Chapter 2) and boulders (Chapters 3-4), the softer limestone, calcarenite and sandstone had a greater microhabitat density and lower rugosity indices (i.e. more complex surfaces) than harder rocks. Thus, it appears that surface complexity is associated with rock hardness (Figure 6.2). With softer rocks being more susceptible to erosion (Spencer 1985), it is likely that softer rocks erode to produce more complex surfaces that are interspersed by microhabitat features. Surface complexity also appears to be associated with the patterns of temperature difference that develop on boulders (Figure 6.2, Chapter 4). The generally featureless surfaces of harder siltstone had gradients of temperature moving from the boulder side facing the sun to the side opposite (Chapters 4-5). In contrast, softer rocks with their complex surfaces had temperature gradients that were interrupted by microhabitat features (Chapter 4). These observations show how rock physical attributes can variously impact one another and co-vary with rock type (Figure 6.2).

Outcome 3: Associations between intertidal assemblages & rock physical attributes.

This research showed assemblages of intertidal biota and individual taxa were associated with rock type and the physical attributes of different rocks as substrata, thus accomplishing the third thesis outcome (Figures 6.1 & 6.2). Rock type affected later-successional boulder assemblages (Chapter 3) and may also affect established assemblages on rock platforms (Chapter 2 & Appendix 5). Later-successional boulder assemblages had a greater richness and abundance of invertebrates on limestone than siltstone (Chapter 3). Thus, rock type affects several stages of succession, from earliest colonisation and recruitment (McGuinness and Underwood 1986; Green et al. 2012; Liversage et al. 2014) through to later successional stages 4 or 5 years later (Chapter 3). Some hardness-related biotic patterns detected for platform assemblages are likely associated with specific rocks, such as Mount Gambier limestone platforms having an invertebrate richness and abundance consistently lower than any other rock (Chapter 2). The detection of biotic associations with rock type (Figure 6.2), for both platform and boulder habitats, highlights the importance of rock type as an abiotic variable affecting intertidal biota.

No general associations between intertidal biota and rock hardness were detected, with hardness-related differences generally being specific to each bioregion (Chapter 2). As the same types of hard and soft rock did not occur in each bioregion (Figure 6.3), the bioregion-specific differences detected may be related to the different rocks that occur in each bioregion. Consequently, these results are similar to other rock hardness investigations, in that biotic differences appear to be related to the types of soft or hard rock investigated (e.g. Savoya and Schwindt 2010; Liversage and Benkendorff 2013; Liversage et al. 2014). These rock-specific results from multiple sources show that the allocation of rocks into simple hardness classes (i.e. soft versus hard) is inappropriate, as biotic differences appear to be attributable to the specific rock types that were compared rather than their hardness *per se*. Therefore, replication at the rock-type scale rather than the hardness-class scale may be desirable to better-understand the biotic patterns that each study identified.

Snails under boulders were associated with the cooler areas of the temperature patterns that developed during emersion (Chapter 5). In the rocky intertidal zone, most previous relationships between ectotherms and cooler temperatures have been identified from comparisons involving hotter versus cooler microhabitat types (Garrity 1984; Chapperon and Seuront 2011a; Marshall and Chua 2012; Chapperon et al. 2013; 2017; Marshall et al. 2013). Intertidal biota were also associated with substratum mineralogy, with the structure, richness and abundance of established platform assemblages weakly correlated with several major minerals and trace elements (Chapter 2). Until now, most previous research investigating biotic interactions with substratum mineralogy has focused on responses to varying quantities of silica (e.g. Bavestrello et al. 2000; 2018; Cattaneo-Vietti et al. 2002). Whilst silica was also a dominant major element in these rocks (Chapters 2 & 4), my results indicate that minerals other than silica may also affect intertidal biota.

When the rock-related physical and biotic differences from each thesis chapter are considered collectively, multiple physical attributes appear to be associated with the rock-related biotic differences identified (Figure 6.2). This conceptual model differs considerably from previous studies, where rock-related biotic differences were generally attributed to just one or two physical attributes (e.g. Chapter 3; Chapter 5; McGuinness and Underwood 1986; Herbert and Hawkins 2006; Liversage et al. 2014). The softer rocks sampled in this thesis (limestone, calcarenite and sandstone) harboured a higher abundance of turf-forming algae on platform habitats (Chapter 2) and a higher richness and taxonomic abundance of invertebrates in boulder habitats (Chapter 3). These biotic differences are likely associated with rock-related differences in microhabitat density, surface rugosity and surface temperature (Figure 6.2, Chapters 2-5). It has been well established that the most complex surfaces support the highest species richness (Blanchard and Bourget 1999; Le Hir and Hily 2005; Cosentino and Giacobbe 2015) and attract greater settlement of algal spores (Fletcher and Callow 1992). These complex surfaces give rise to cooler and damp microhabitat features that may shelter intertidal biota from desiccation and heat stress, especially juveniles and small species (McGuinness and Underwood 1986; Fletcher and Callow 1992). Thus the surface complexity and cooler microhabitat features of softer rocks may combine to provide a more-suitable habitat for some species on hot days than other rocks, accounting for the overall biotic patterns identified.

Areas for future research

Future research should continue investigating the lesser-studied physical attributes of seashore rocks to establish how intertidal biota may be associated with rock-related differences in these physical attributes. Due to budget limitations, X-ray diffraction (XRF) was only completed on three samples for each rock type investigated in Chapters 2 & 4. Consequently, I was only able to establish a baseline of mineralogy for each rock. Many more samples per rock type should be analysed to establish how variable mineralogy is both within and among the rock types sampled. Once the major mineral and trace elemental composition of rocks are established, it may be possible to better-identify which mineral constituents are associated with any rock-related biotic patterns. Experiments that manipulate the concentrations of these mineral constituents in artificial units of substratum (e.g. tiles, pavers) or sediment, like those conducted in Italy (e.g. Cerrano et al. 1999; 2007), could then be used to directly test how specific major minerals and trace elements affect intertidal biota.

Investigations of substratum mineralogy should also quantify whether the major mineral and trace elemental composition of rocks determine their colour, which I have hypothesised in several thesis chapters (Chapters 4 & 6). Untangling the associations between mineralogy and colour are necessary, as rock-related differences in both surface colour (Chapter 4; Raimondi 1988; Marshall et al. 2010; Judge et al. 2011) and mineralogy (Chapter 4) have been associated with temperature differences among rocks. Experiments that manipulate mineral constituents in artificial units of substratum can be used to determine how specific minerals affect substratum colour. Should mineralogy determine a rocks colour, it becomes self-evident that mineralogy would also affect rock surface temperature, as the correlations in Chapter 4 have already shown. This would vindicate the use of the complex mineralogical analyses (Chapter 4) when investigating rock temperatures, rather than a simple ranking of rocks from darkest to lightest colouration.

Sampling that collects temperature data for platform habitats should be completed. Specifically, this sampling should target warm, sunny days over summer, so that a baseline of temperature behaviour for rock platforms can be established. These temperature data can be used in correlations with the mineralogy data already collected (Chapter 2) to establish whether temperature behaviour and mineralogy are also correlated for platform rock types. If additional biotic data are collected to coincide with temperature readings, it may be possible to establish whether surface temperature, and any interactions between surface temperature and mineralogy, are associated with rock-related biotic patterns for platform habitats.

While I was able to establish an association between snails and cooler areas on boulder surfaces (Chapter 5), how and when snails identify the coolest locations when retreating underneath boulders remains unclear. If snails were tagged when active at high tide or at night, it may be possible to track their movements across tidal cycles via radio transmitter (Hayford et al. 2018), videography (Taylor et al. 2017) or time-lapse photography (Liversage and Benkendorff 2017). This may make it possible to determine how and when snails respond to the patterns of temperature difference that develop during daytime low tides.

It also remains unclear how the temperatures measured underneath boulders may directly affect the three snail species investigated (Chapter 5). Temperature of substrata is positively correlated with gastropod body temperature at low tide (Soto and Bozinovic 1998; Caddy-Retalic et al. 2011; Chapperon and Seuront 2011b), although this correlation has not yet been quantified for either *D. concameratum* or *B. nanum*. Moreover, despite gastropod body and substratum temperatures being positively correlated, Seuront and Ng (2016) showed snail shell temperature can be up to 10°C cooler than the substratum nearby. Therefore, to accurately quantify how the temperature of substrata affects snail body temperature, thermocouples that simultaneously measure substratum and snail body temperatures could be used (e.g. Southward 1958; Caddy-Retalic et al. 2011; Lathlean et al. 2012). To determine whether the snail body temperatures measured by thermocouples exceed species thermal tolerance limits, the upper thermal tolerance of each snail species while emersed must be established. Presently, laboratory experiments have only quantified the thermal tolerances of *N. atramentosa* and *D. concameratum* submerged in heated seawater (McMahon 1990). Establishing the maximum tolerable temperature for these species while emersed will enhance our understanding of whether intertidal biota can survive and persist during periods of environmental stress at low tide. This is especially important given predictions of warming temperatures associated with global climate change.

Management implications

This research can be used for evaluating whether current intertidal management frameworks adequately represent intertidal biota and habitats in temperate southern

Australia. In SA, a marine parks network was established in 2014 that affords intertidal areas varying levels of protection through a series of zones (DEW 2014). This supplements existing legislation that prohibits the removal of any biota from the rocky-reef seashore to a water depth of two metres, although compliance is often lacking (personal observation). As intertidal biota were associated with rock type (Chapters 2-3 & 5) and the bioregion sampled (Chapter 2), an effective marine parks network should protect representative seashores (Alexander and Gladstone 2013) of each rock type within each bioregion. In the GSV Bioregion, this representation is generally achieved, with sanctuary zones (the highest level of protection with extractive activities generally prohibited) encompassing seashores comprised of fossiliferous limestone, quartzite, siltstone and Kanmantoo schist. In contrast, adequate representation of all rock types in sanctuary zones may not be achieved in the Otway Bioregion, with just two from five sanctuary zones encompassing rocky seashores, and both those seashores comprised of calcarenite. Thus, Mount Gambier limestone platforms and flint boulderfields, and the assemblages unique to those rocks (Chapter 2 & Appendix 5), may currently be underrepresented and therefore should be considered for future protection.

The associations identified between intertidal biota and rock physical attributes should be integrated into rocky seashore management frameworks. For example, if two seashores support similar habitats and assemblages, but one is comprised of a hotter rock type than the other, protecting only the seashore with the hotter rock type may not achieve desired management outcomes under future predicted conditions. That is because some intertidal species already live near or at their upper tolerable thermal limits (Harley 2008; Madeira et al. 2012). Therefore, warming temperatures associated with global climate change (IPCC 2013) may challenge the survival and persistence of those species, especially on hotter seashores. However, if rock-related differences in thermal behaviour are considered when developing future management frameworks, the seashore comprised of the cooler rock type could be prioritised for inclusion. Cooler seashores may provide a thermal refuge and offer some buffering against the effects of warming temperatures. This could ensure the survival and persistence of vulnerable taxa, thus maintaining an adequate representation of intertidal assemblages in suitably protected areas. This outcome may not be achieved if rock temperature behaviour is overlooked in management frameworks.

Conclusion

This research describes understudied biota and rock physical attributes for rocky seashores in temperate southern Australia. For 18 seashores between Portland and Adelaide (Figure 6.3), platform habitats supported generally sparse mixed algae and invertebrate assemblages, while boulder habitats supported sparse invertebrate-dominated assemblages (Figures 6.1 & 6.2). For the rocks that comprised these seashores, their hardness, mineralogy, rugosity, microhabitat density and temperature behaviour was quantified (Figures 6.1 & 6.2). Developing assemblages on transplanted boulders, established assemblages on rock platforms, and snail interactions with patterns of temperature difference were associated with rock type (Figure 6.1). Intertidal biota were not generally associated with rock hardness, due to more variability between rocks within hardness classes. Assemblages of intertidal biota and individual taxa were variously associated with rock-related differences in mineralogy, microhabitat density, rugosity and temperature behaviour (Figures 6.1 & 6.2). Consequently, the research in this thesis adds considerably to the body of knowledge regarding how intertidal biota is associated with rock type and the physical attributes of different rocks as substratum.

Chapter 7

List of references

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Chapter 8

Appendices

Appendix 1: Chapter 2

Description of functional groups

For invertebrates, three functional groups were recognised: tubeworms; mussels; and barnacles. For marine plants, five of the seven functional groups established by Steneck and Dethier (1994) were applied, which included: visible microalgae; filamentous algae; foliose algae; corticated macrophytes; and leathery macrophytes. Four additional marine plant functional groups were created to cover the local marine plant assemblage. These were: turf-forming algae; crustose algae; seagrasses; and marine lichens. Turf-forming algae were defined as any algal turf formed by larger (>1 cm) macroscopic algae, that grew to form a densely-packed mat (Carpenter 2007). A list of the marine plant and sessile invertebrate species recorded, and the functional group that each species was assigned, can be found in Tables 8.1 – 8.6.

XRF analyses (completed at CSIRO Land and Water Adelaide)

For major minerals, approximately one gram of each oven-dried sample (at 105 °C) was accurately weighed with four grams of 12-22 lithium borate flux (Norrish and Hutton 1969). The mixtures were heated to 1050 °C in a platinum/gold crucible for 20 minutes to completely dissolve the sample, and then poured into a 32 mm platinum/gold mould heated to a similar temperature (Norrish and Hutton 1969). The melt was cooled rapidly over a compressed air stream and the resulting glass disks were analysed on a PANalytical Axios Advanced wavelength dispersive XRF system using the CSIRO in-house silicates calibration program. For trace elements, approximately four grams of each oven-dried sample (at 105 °C) was accurately weighed with one gram of Licowax binder and mixed well (Norrish and

Hutton 1969). The mixtures were pressed in a 32 mm die at 12 tons pressure and the resulting pellets were analysed on a PANalytical Axios Advanced wavelength dispersive XRF system using the CSIRO in-house powders program (Norrish and Hutton 1969).

Surface temperature data

The surface temperature of benthic features in quadrats was measured with a Ti20 Fluke thermal imaging camera. The resolution of the thermal imaging camera was ≤0.2 °C at 30 °C, with an accuracy to 2 % or 2 °C, whichever was greater. Default camera settings were employed, including emissivity, which was set at 0.95. This default emissivity was deemed appropriate, as previous studies have shown that the emissivity of rocky seashore invertebrates and substrata generally ranges between 0.95 and 1 (Rivard et al. 1995; Cox and Smith 2011; Lathlean et al. 2012). To avoid measuring inaccurate temperatures from increased amounts of thermal energy being reflected by damp or wet surfaces, quadrats were temporarily shaded while thermal images were captured (Lathlean et al. 2012; Lathlean and Seuront 2014). Archived thermal images were processed using the InsideIR version 4.0 software (Fluke Corporation), with the maximum, minimum, and average surface temperatures for benthic features in each quadrat determined. Despite attempting to sample favourable thermal conditions (i.e. sunny skies and dry substrata), several factors (i.e. cloud cover, rainfall and/or wave splash) confounded temperature measurements for some seashores and seasons. As such, not all collected temperature data were comparable, so only un-confounded temperature data collected for some seashores during the 2015 and 2016 summers was used when modelling environmental data (Table 8.16).

A PCA that included temperature data for specific seashores during the summers of 2015 and 2016 (Table 8.16) was run. The addition of temperature changed little in the overall PCA model (Table 8.17), with temperature only strongly associated with the seventh PC axis, and most other associations between independent variables and PC axes similar to those reported from the model excluding temperature (Tables 8.15 & 8.17). Therefore, the PCA model that encompassed all seashores and seasons, but lacked any temperature data, was used in all correlations for H3.

Transect-scale sessile space occupancy

Methods

At each seashore for each season, three line-intercept transects (total *N* = 108) were established to quantify the space occupancy of dominant marine plants and sessile invertebrates. Briefly, this method involved measuring the length of a transect intercepted by dominant space occupiers or bare substratum (resolution = patches ≥10 cm in length) as continuous segments (Lucas and Seber 1977). These measurements were then summed for each species or category of bare substratum, and converted into a percentage of the total tape length (Lucas and Seber 1977). Each line-intercept transect was videoed with an Olympus Tough TG-820 digital camera, and archived videos were processed in the laboratory for species identification and space occupancy. Space occupancy was recorded at species level, with each species then assigned to one of the 12 functional groups described for quadrats. Areas of substratum that were devoid of sessile space occupiers were recorded as bare rock. Consequently, a total of 13 space-occupancy categories were recognised.

To test for biotic differences between hardness classes, the statistical methods described for quadrats were applied to transect data, with a four-factor PERMANOVA (same model as quadrats minus the factor height) used in place of the quadrat five-factor model. To determine whether hardness related biotic differences were correlated with substratum mineralogy, BIOENV analyses (multivariate biotic data) and correlations (univariate biotic data) using PCA factor scores were completed.

Results

Bioregion-specific differences in the structure, richness and space occupancy of sessile assemblages were detected between hardness classes (Tables 8.19 – 8.21). In the Otway Bioregion, structural differences were associated with a higher space occupancy of turf-forming algae on seashores from the soft class but more corticated macrophytes and tubeworms on seashores from the hard class (Figure 8.5a, Table 8.22). The hard class was characterised by a higher richness and space occupancy of sessile functional groups than the soft class (Table 8.19), although these differences were only significant for invertebrate richness (PERMANOVA pair-wise permuted *p*-value = 0.0018) and invertebrate space occupancy (PERMANOVA pair-wise permuted *p*-value = 0.0028) (Figure 8.5, Table 8.19). In the GSV Bioregion, structural differences were associated with a higher space occupancy of turf-forming algae, filamentous algae and mussels on the soft versus hard class (Figure 8.5a, Table 8.22). The soft class generally had a higher richness and space occupancy of sessile functional groups than the hard class (Table 8.19), although these differences were only significant for marine plant occupancy (PERMANOVA pair-wise permuted *p*-value = 0.0055).

The hard class had significantly more bare surface than the soft class in the GSV Bioregion (PERMANOVA pair-wise permuted *p*-value = 0.0019) (Figure 8.5e, Table 8.19).

The structure, richness, and space occupancy of sessile assemblages was highly variable among seashores and seasons (Tables 8.20 & 8.21). Several time-specific differences between bioregions were identified, with the GSV Bioregion generally having a higher richness and space occupancy of marine plants and invertebrates versus the Otway Bioregion. The structural and richness differences between bioregions were not significant, although significant Bioregion x Season interactions were identified for the space occupancy of marine plants, sessile invertebrates and bare rock. Pair-wise tests were unable to identify temporal bioregional differences for marine plant or sessile invertebrate space occupancy (PERMANOVA smallest pair-wise permuted *p*-value = 0.0514 in Autumn 2015) but did show that seashores in the Otway Bioregion had more bare rock than seashores in the GSV Bioregion during the 2015 summer and autumn (PERMANOVA smallest pair-wise permuted *p*-value = 0.0038 in summer 2015).

Principal components analysis reduced 43 raw physical variables into six PC axes with eigenvalues >1 (Table 8.23). The first two PC axes were strongly associated (Varimax rotated loadings ≥0.90) with several major mineral or trace elements (Table 8.23), and accounted for 38.9 % and 31.7 %, respectively, of the total variation in physical attributes. PC axes three through six were less-strongly associated (Varimax rotated loadings >0.90) with specific trace elements. A significant relationship was detected between ordinations of assemblage structure and the six PC axes with eigenvalues >1 identified during PCA (RELATE

Spearman Rho $\rho = 0.312$, $p = 0.0100$). Assemblage structure was moderately, but significantly, correlated with the first four PC axes (BEST $\rho = 0.384$, $p = 0.0100$). Each of these PC axes were most-strongly associated with hardness differences in the content of major minerals or trace elements (Table 8.23).

Each univariate biotic variable was weakly-moderately correlated with at least two PC axes (Table 8.24). Sessile invertebrate richness was significantly correlated with five principal component axes, while marine plant richness was correlated with just two axes. Most PC axes were most-strongly associated with hardness differences in the content of metallic oxides and trace metals (Table 8.24). Consequently, univariate biotic differences between hardness classes for transects were associated with differences in the content of major minerals and trace elements.

Table 8.1: Species list of marine algae, lichens, and seagrasses recorded at each seashore using line-intercept transects and photo quadrats for 12 rocky seashores sampled across temperate southern Australia between January 2015 and January 2016. BP = Blacknose Point; PW = Portland Windfarm; EF = Enchanted Forest; RB = Racecourse Bay West; PP = Pelican Point; NC = Nora Creina; EB = Encounter Bay; TB = The Bluff; CJ = Cape Jervis; BLP = Blanche Point; SP = Southport; MR = Marino Rocks; FBA = filamentous brown alga.

				Bioregion:	Otway						GSV						
				Hardness class:	Hard			Soft			Hard			Soft			
Division	Class	Family	Species	Assigned functional group	BP	PW	EF	RB	PP	NC	TB	CJ	MR	BLP	SP	EB	Total shores observed
			Macroscopic biofilm	Microalgae	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	11
Chlorophyta	Ulvophyceae	Codiaceae	*Codium pomoides*	Foliose algae		**X**	**X**										2
		Ulvaceae	*Enteromorpha* sp.	Filamentous algae		**X**	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**	9
			Ulva australis	Foliose algae	**X**	**X**		**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	10
			Ulva compressa	Foliose algae				**X**			**X**			**X**			3
Heterokontophyta	Phaeophyceae		FBA sp 1	Filamentous algae	**X**	**X**	**X**	**X**			**X**		**X**	**X**	**X**		8
			FBA sp 2	Filamentous algae				**X**		**X**	**X**		**X**	**X**	**X**	**X**	7
			FBA sp 3	Filamentous algae									**X**	**X**	**X**	**X**	4
			FBA sp 4	Filamentous algae					**X**							**X**	2
			FBA sp 5	Filamentous algae											**X**	**X**	2
		Alariaceae	*Ecklonia radiata*	Leathery macrophytes	**X**					**X**					**X**		3
			Cystophora monilifera	Corticated macrophytes	**X**	**X**	**X**	**X**		**X**		**X**		**X**	**X**	**X**	9
		Dictyotaceae	*Dictyota dichotoma*	Corticated macrophytes								**X**					1
		Durvillaeaceae	*Durvillaea potatorum*	Leathery macrophytes	**X**												1
		Hormosiraceae	*Hormosira banksii*	Corticated macrophytes	**X**	**X**	**X**	**X**	**X**	**X**	**X**			**X**		**X**	9
		Notheiaceae	*Notheia anomala*	Corticated macrophytes	**X**		**X**										2
		Sargassaceae	*Sargassum* sp.	Leathery macrophytes	**X**								**X**				2
		Scytosiphonaceae	*Colpomenia sinuosa*	Foliose algae			**X**	**X**			**X**	**X**		**X**	**X**	**X**	7
		Seirococcaceae	*Phyllospora comosa*	Leathery macrophytes	**X**												1
		Splachnidiaceae	*Splachnidium rugosum*	Foliose algae		**X**	**X**	**X**			**X**	**X**		**X**	**X**	**X**	8
Rhodophyta	Florideophyceae	Corallinaceae	Amphiroa anceps	Turf-forming algae				**X**				**X**			**X**		3
			Mixed coralline turf	Turf-forming algae	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	12
			Pink & white paint	Crustose algae	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	12
		Gelidiaceae	*Capreolia implexa*	Turf-forming algae	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	12
Magnoliophyta	Liliopsida	Zosteraceae	*Heterozostera nigricaulis*	Seagrasses				**X**								**X**	2
Ascomycota	Lecanoromycetidae	Teloschistaceae	*Caloplaca* sp.	Marine lichens	**X**							**X**					2
	Lichenotheliaceae		*Lichinia* sp.	Marine lichens	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**			9
Marine plant species richness per seashore					15	12	13	16	6	10	13	12	11	15	15	15	
Marine plant richness per hardness class(bioregion)					19			18			19			20			
Marine plant richness per bioregion					24						23						

Table 8.2: Species list of invertebrates recorded at each seashore using line-intercept transects and photo quadrats for 12 rocky seashores sampled across temperate southern Australia between January 2015 and January 2016. See Table A1 for site codes, M = mobile; S = sessile.

				Bioregion:	Otway						GSV						
				Hardness class:	Hard			Soft			Hard			Soft			
Phyla	Class	Family	Species	Type	BP	PW	EF	RB	PP	NC	TB	CJ	MR	BLP	SP	EB	Total shores observed
Cnidaria	Anthozoa	Actiniidae	*Actinia tenebrosa*	M	**X**	**X**	**X**	**X**									4
			Isanemonia australis	M	**X**	**X**	**X**								**X**	**X**	5
Platyhelminthes	Rhabditophora	Notoplanidae	*Notoplana australis*	M											**X**		1
Annelida	Polychaeta	Serpulidae	*Galeolaria caespitosa*	S	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	11
			Pomatoceros taeniata	S												**X**	1
			Spirorbid sp.	S	**X**						**X**	**X**					3
Mollusca	Gastropoda	Nacellidae	*Cellana tramoserica*	M	**X**	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**		9
		Lottidae	*Notoacmea* spp.	M	**X**	**X**	**X**			**X**		**X**	**X**	**X**	**X**		8
			Patelloida alticostata	M	**X**	**X**	**X**			**X**			**X**	**X**	**X**		7
			Patelloida insignis	M	**X**	**X**	**X**			**X**							4
			Patelloida latistrigata	M	**X**		**X**			**X**			**X**				4
		Neritopsidae	*Nerita atramentosa*	M	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**			8
		Fisurellidae	*Montfortula rugosa*	M	**X**	**X**	**X**			**X**		**X**	**X**	**X**	**X**	**X**	9
			Scutus antipodes	M							**X**						1
		Trochidae	*Austrocochlea constricta*	M	**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	11
			Chlorodiloma adelaidae	M						**X**							1
			Diloma concamerata	M	**X**						**X**	**X**					3
		Turbinidae	*Lunella undulata*	M			**X**										1
		Littorinidae	*Afrolittorina praetermissa*	M	**X**	**X**	**X**	**X**		**X**							5
			Austrolittorina unifasciata	M	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			10
			Bembicium nanum	M	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	11
			Bembicium vittatum	M							**X**	**X**	**X**	**X**	**X**		5
		Buccinidae	*Cominella lineolata*	M	**X**			**X**						**X**			3
		Batillariidae	*Eubittium lawleyanum*	M					**X**								1
		Muricidae	*Dicathais orbita*	M	**X**	**X**				**X**							3
			Haustrum vinosum	M		**X**	**X**	**X**	**X**								4
		Siphonariidae	*Siphonaria* spp.	M	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	12
		Onchidiidae	*Onchidella nigricans*	M	**X**												1
	Bivalvia	Mytilidae	*Brachidontes erosus*	S												**X**	1
			Brachidontes rostratus	S	**X**	**X**	**X**										3
			Xenostrobus pulex	S	**X**	**X**	**X**		**X**				**X**	**X**	**X**		7
	Polyplacophora	Mopaliidae	*Plaxiphora albida*	M	**X**												1
Arthropoda	Maxillopoda	Catophragmidae	*Catomerus polymerus*	S	**X**		**X**										2
		Chthamalidae	*Chamaesipho tasmanica*	S	**X**	**X**	**X**			**X**							4
			Chthamalus antennatus	S	**X**	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**		9

Phyla	Class	Family	Species	Type	BP	PW	EF	RB	PP	NC	TB	CJ	MR	BLP	SP	EB	Total shores observed
		Tetraclitidae	*Tetraclitella purpurascens*	S	**X**	**X**							**X**				3
	Malacostraca	Eriphiidae	*Ozius truncatus*	M											**X**		1
Echinodermata	Asteriodea	Asterinidae	*Meridiastra calcar*	M			**X**										1
Invertebrate species richness per seashore					27	21	22	9	8	17	12	12	15	14	14	8	
Invertebrate species richness per hardness class(bioregion)					30			22			19			19			
Invertebrate species richness per bioregion					32						24						

Table 8.3: Seasonal species occurrence of marine algae, lichens, and seagrasses recorded at each seashore using line-intercept transects and photo-quadrats for 6 rocky seashores in the Otway Bioregion. See Table A1 for site codes, FBA = filamentous brown alga. Seasonal species richness is pooled across hardness classes.

Season:	Summer 2015						Autumn 2015						Summer 2016					
Hardness class:	Hard			Soft			Hard			Soft			Hard			Soft		
Species Seashore:	BP	PW	EF	RB	PP	NC	BP	PW	EF	RB	PP	NC	BP	PW	EF	RB	PP	NC
Macroscopic biofilm	**X**	**X**	**X**	**X**		**X**			**X**	**X**		**X**		**X**	**X**	**X**		**X**
Codium pomoides								**X**	**X**						**X**			
Enteromorpha sp.		**X**	**X**					**X**	**X**	**X**				**X**		**X**		
Ulva australis	**X**			**X**	**X**	**X**	**X**			**X**	**X**	**X**		**X**		**X**	**X**	**X**
Ulva compressa																**X**		
FBA sp 1		**X**	**X**	**X**				**X**	**X**	**X**			**X**	**X**	**X**	**X**		
FBA sp 2										**X**								**X**
FBA sp 4																	**X**	
Ecklonia radiata	**X**						**X**					**X**						
Cystophora monilifera		**X**				**X**		**X**	**X**			**X**	**X**	**X**	**X**	**X**		**X**
Durvillaea potatorum							**X**						**X**					
Hormosira banksii	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Notheia anomala	**X**						**X**		**X**				**X**		**X**			
Sargassum sp.	**X**												**X**					
Colpomenia sinuosa									**X**						**X**	**X**		
Phyllospora comosa	**X**						**X**						**X**					
Splachnidium rugosum		**X**	**X**						**X**	**X**					**X**	**X**		
Amphiroa anceps										**X**								
Mixed coralline turf	**X**	**X**	**X**	**X**	**X**		**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Pink & white paint			**X**					**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**
Capreolia implexa	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			**X**	**X**
Heterozostera nigricaulis										**X**						**X**		
Caloplaca sp.	**X**						**X**						**X**					
Lichinia sp.	**X**	**X**		**X**		**X**	**X**	**X**		**X**		**X**	**X**	**X**	**X**	**X**		**X**
Seashore species richness	**11**	**9**	**8**	**7**	**4**	**6**	**10**	**8**	**12**	**13**	**3**	**9**	**12**	**10**	**11**	**13**	**6**	**9**
Hardness class species richness	**16**			**8**			**18**			**15**			**18**			**16**		
Seasonal species richness	**16**						**21**						**22**					

Table 8.4: Seasonal species occurrence of marine algae, lichens, and seagrasses recorded at each seashore using line-intercept transects and photo-quadrats for 6 rocky seashores sampled in the GSV Bioregion. See Table A1 for site codes. Seasonal species richness is pooled across hardness classes.

Season:	Summer 2015						Autumn 2015						Summer 2016					
Hardness class:	Hard			Soft			Hard			Soft			Hard			Soft		
Species Seashore:	TB	CJ	MR	BLP	SP	EB	TB	CJ	MR	BLP	SP	EB	TB	CJ	MR	BLP	SP	EB
Macroscopic biofilm			**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Enteromorpha sp.	**X**		**X**				**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**		
Ulva australis				**X**	**X**		**X**		**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**
Ulva compressa							**X**			**X**								
FBA sp 1				**X**	**X**		**X**			**X**	**X**				**X**	**X**		
FBA sp 2	**X**		**X**	**X**	**X**	**X**				**X**	**X**	**X**			**X**	**X**	**X**	**X**
FBA sp 3				**X**	**X**	**X**			**X**	**X**	**X**				**X**	**X**	**X**	
FBA sp 4						**X**												
FBA sp 5					**X**	**X**												**X**
Ecklonia radiata																	**X**	
Cystophora monilifera				**X**	**X**	**X**		**X**			**X**	**X**		**X**				**X**
Dictyota dichotoma														**X**				
Hormosira banksii	**X**			**X**		**X**						**X**	**X**					**X**
Sargassum sp.									**X**									
Colpomenia sinuosa	**X**	**X**		**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Splachnidium rugosum	**X**			**X**	**X**			**X**		**X**	**X**	**X**		**X**		**X**	**X**	**X**
Amphiroa anceps								**X**			**X**							
Mixed coralline turf	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**
Pink & white paint		**X**						**X**			**X**		**X**	**X**	**X**	**X**	**X**	**X**
Capreolia implexa	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Heterozostera nigricaulis						**X**						**X**						**X**
Caloplaca sp.		**X**						**X**						**X**				
Lichinia sp.	**X**	**X**	**X**				**X**	**X**	**X**	**X**			**X**	**X**	**X**			
Seashore species richness	**8**	**6**	**6**	**11**	**11**	**11**	**7**	**10**	**8**	**12**	**13**	**11**	**8**	**10**	**9**	**11**	**10**	**12**
Hardness class species richness	**11**			**14**			**16**			**17**			**16**			**16**		
Seasonal species richness	**18**						**19**						**19**					

Table 8.5: Seasonal species list for invertebrates recorded at each seashore using line-intercept transects and photo quadrats for 6 rocky seashores sampled in the Otway Bioregion. See Table A1 for site codes. Seasonal species richness is pooled across hardness classes.

Season:	Summer 2015						Autumn 2015						Summer 2016					
Hardness class:	Hard			Soft			Hard			Soft			Hard			Soft		
Species Seashore:	BP	PW	EF	RB	PP	NC	BP	PW	EF	RB	PP	NC	BP	PW	EF	RB	PP	NC
Galeolaria caespitosa	X	X	X				X	X	X			X	X	X	X	X		X
Pomatoceros taeniata									X									
Spirorbid sp.							X											
Actinia tenebrosa	X						X	X	X	X			X					
Isanemonia australis							X							X	X			
Cellana tramoserica	X	X	X			X	X	X	X			X	X	X	X			X
Notoacmea spp.	X	X	X					X	X			X	X		X			
Patelloida alticostata	X	X	X			X	X	X	X			X	X	X	X			X
Patelloida insignis			X			X		X	X				X		X			X
Patelloida latistrigata			X			X			X				X		X			
Nerita atramentosa	X				X	X	X	X				X	X				X	X
Montfortula rugosa			X				X	X	X			X	X		X			
Austrocochlea constricta	X		X	X	X	X		X	X	X	X	X			X	X	X	X
Chlorodiloma adelaidae												X						
Diloma concamerata	X																	
Lunella undulata			X												X			X
Afrolittorina praetermissa				X		X	X	X	X	X		X			X	X		
Austrolittorina unifasciata	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Bembicium nanum	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cominella lineolata	X			X														
Eubittium lawleyanum					X						X							
Dicathais orbita	X							X				X						
Haustrum vinosum			X					X			X					X	X	
Siphonaria spp.	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X
Onchidella nigricans							X											
Brachidontes rostratus	X	X	X					X	X				X	X	X			
Xenostrobus pulex	X	X	X				X	X	X				X	X	X		X	
Plaxiphora albida							X											
Catomerus polymerus	X						X		X				X					
Chamaesipho tasmanica	X	X	X				X	X	X				X	X	X			X
Chthamalus antennatus		X				X	X	X						X	X			
Tetraclitella purpurascens	X							X					X					
Meridiastra calcar									X						X			
Seashore species richness	18	11	16	6	5	11	18	20	19	6	5	13	17	11	19	7	7	10
Hardness class species richness	24			12			28			16			23			14		
Seasonal species richness	26						30						24					

Table 8.6: Seasonal species occurrence of for invertebrates recorded at each seashore using line-intercept transects and photo quadrats for 6 rocky seashores sampled in the GSV Bioregion. See Table A1 for site codes. Seasonal species richness is pooled across hardness classes.

Season:	Summer 2015						Autumn 2015						Summer 2016					
Hardness class:	Hard			Soft			Hard			Soft			Hard			Soft		
Species Seashore:	TB	CJ	MR	BLP	SP	EB	TB	CJ	MR	BLP	SP	EB	TB	CJ	MR	BLP	SP	EB
Galeolaria caespitosa	**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Pomatoceros taeniata						**X**			**X**	**X**		**X**						
Spirorbid sp.							**X**	**X**					**X**					
Isanemonia australis			**X**									**X**					**X**	**X**
Notoplana australis					**X**													
Cellana tramoserica			**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**		**X**		**X**	**X**	**X**	
Notoacmea spp.		**X**	**X**	**X**	**X**			**X**	**X**	**X**						**X**	**X**	
Patelloida alticostata									**X**						**X**	**X**	**X**	
Patelloida latistrigata			**X**						**X**									
Nerita atramentosa	**X**	**X**	**X**				**X**	**X**	**X**	**X**			**X**	**X**	**X**			
Montfortula rugosa			**X**	**X**	**X**							**X**		**X**		**X**	**X**	
Scutus antipodes							**X**											
Austrocochlea constricta	**X**		**X**	**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**			**X**	**X**	
Diloma concamerata	**X**	**X**					**X**	**X**					**X**	**X**				
Austrolittorina unifasciata	**X**	**X**	**X**				**X**	**X**	**X**	**X**			**X**	**X**	**X**			
Bembicium nanum		**X**			**X**	**X**	**X**	**X**		**X**	**X**			**X**	**X**	**X**	**X**	
Bembicium vittatum			**X**	**X**	**X**		**X**	**X**	**X**	**X**								
Cominella lineolata				**X**														
Siphonaria spp.	**X**	**X**	**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Onchidella nigricans						**X**												
Brachidontes erosus						**X**						**X**						
Xenostrobus pulex			**X**	**X**	**X**				**X**	**X**	**X**				**X**	**X**	**X**	
Chthamalus antennatus	**X**	**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**	
Tetraclitella purpurascens			**X**						**X**						**X**			
Ozius truncatus											**X**						**X**	
Seashore species richness	**8**	**7**	**14**	**10**	**11**	**6**	**10**	**10**	**13**	**12**	**8**	**6**	**8**	**8**	**10**	**10**	**12**	**3**
Hardness class species richness	**17**			**15**			**18**			**16**			**14**			**12**		
Seasonal species richness	**22**						**22**						**17**					

Table 8.7: Physical attributes of the rocks in each hardness class. Colour is expressed as the descriptive colour Hue Value/Chroma on Munsell colour charts.

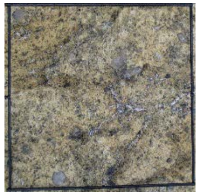
Hardness class:	**Soft**			**Hard**			
Rock: **Physical attribute**	**Mount Gambier limestone**	**Calcarenite**	**Fossiliferous limestone**	**Siltstone**	**Kanmantoo Schist**	**Felsic volcanic rock**	**Basalt**
Appearance (including biota)							
Scratch hardness (Moh's scale)	2.0	2.5	3.0	5.0	5.0 – 7.5	6.0	7.0
Friability	High	High	Moderate	Low	Low - none	None	None
Colour (Munsell colour chart)	Light grey 10YR 8/1, Dull yellow orange 10YR 7/3	Dull yellow orange 10YR 6/3	Light grey 10YR 8/1	Greyish red 2.5YR 5/2, Brownish grey 10YR 5/1	Brownish grey 10YR 4/1, Grey 7.5Y 4/1	Brownish black 7.5YR 3/2, Brown 2.5Y 4/4	Brownish grey 10YR 4/1
Dominant major minerals (% ± SE)	CaO 53.7 ± 0.2 SiO_2 1.2 ± 0.2 MgO 1.0 ± 0	CaO 40.2 ± 3.0 SiO_2 22.7 ± 4.6 Al_2O_3 1.2 ± 0.5	SiO_2 51.2 ± 10.3 CaO 24.2 ± 6.0 Al_2O_3 1.0 ± 0.2	SiO_2 55.4 ± 6.6 CaO 12.0 ± 4.2 Al_2O_3 8.8 ± 0.6	SiO_2 65.9 ± 3.2 Al_2O_3 14.9 ± 1.1 Fe_2O_3 6.5 ± 1.2	CaO 27.6 ± 5.8 SiO_2 27.3 ± 6.3 Al_2O_3 8.2 ± 1.5	SiO_2 51.0 ± 0.1 Al_2O_3 16.5 ± 0.4 Fe_2O_3 13.3 ± 0.3
Dominant trace elements (ppm ± SE)	Cl 7085 ± 1499 Sr 396 ± 33 Mn 152 ± 36	Cl 3369 ± 1438 Sr 753 ± 208 Mn 130 ± 11	Cl 4027 ± 736 Sr 398 ± 113 Ba 64 ± 11	Mn 622 ± 77 Ba 288 ± 32 Zr 198 ± 13	Cl 764 ± 222 Ba 511 ± 74 Mn 475 ± 32	Cl 3582 ± 606 Mn 1329 ± 505 Sr 267 ± 83	Cl 1677 ± 326 Mn 1006 ± 120 Sr 534 ± 11

Table 8.8: Major mineral content (%, mean ± SE) for the rocks in each hardness class.

Hardness class:	**Soft**			**Hard**			
Major mineral / **Rock:**	**Mount Gambier limestone**	**Calcarenite**	**Fossiliferous limestone**	**Siltstone**	**Kanmantoo schist**	**Felsic volcanic rock**	**Basalt**
Silicon dioxide	1.2 ± 0.2	22.7 ± 4.6	51.2 ± 10.3	55.4 ± 6.6	65.9 ± 3.2	27.3 ± 6.3	51.0 ± 0.1
Aluminium oxide	0.3 ± 0.1	1.2 ± 0.5	1.0 ± 0.2	8.8 ± 0.6	14.9 ± 1.1	8.2 ± 1.5	16.5 ± 0.4
Iron oxide	0.2 ± 0	0.8 ± 0.3	0.7 ± 0.1	4.1 ± 0.6	6.5 ± 1.2	6.6 ± 1.0	12.3 ± 0.3
Magnesium oxide	1.0 ± 0	0.9 ± 0.2	0.7 ± 0.1	3.3 ± 0.1	3.4 ± 0.8	4.8 ± 1.8	4.7 ± 0.6
Potassium oxide	0.1 ± 0	0.3 ± 0.1	0.3 ± 0.1	2.1 ± 0.1	2.9 ± 0.1	0.5 ± 0.1	1.1 ± 0
Titanium dioxide	0 ± 0	0.1 ± 0	0.1 ± 0	0.6 ± 0.1	0.7 ± 0.1	1.0 ± 0.2	2.0 ± 0.1
Calcium oxide	53.7 ± 0.2	40.2 ± 3.0	24.2 ± 6.0	12.0 ± 4.2	1.7 ± 0.2	27.6 ± 5.8	7.8 ± 0.4
Sodium oxide	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	1.3 ± 0.4	2.6 ± 0.2	2.0 ± 0.6	4.5 ± 0.1
Manganese oxide	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.1 ± 0
Phosphorus pentoxide	0.1 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0	0.2 ± 0	0.2 ± 0	0.4 ± 0
Sulfur trioxide	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.0 ± 0.0	0 ± 0	0.2 ± 0	0.1 ± 0

Table 8.9: Trace element composition (parts per million, mean ± SE) for each hardness class in each bioregion. Silver, molybdenum, ytterbium, antimony, selenium, tin, tantalum and tellurium were also detected but were too low a concentration to be quantitatively measured. Type of elements NM = non-metallic element, M = metallic element.

	Bioregion:		**GSV**			**Otway**		
Element	**Symbol**	**Type**	**Soft**		**Hard**	**Soft**		**Hard**
Chlorine	Cl	NM	4027 ± 736	>	573 ± 175	5846 ± 1222	>	2312 ± 419
Manganese	Mn	M	52 ± 14	<	524 ± 39	145 ± 24	<	1114 ± 174
Barium	Ba	M	64 ± 11	<	437 ± 61	45 ± 4	<	217 ± 38
Zirconium	Zr	M	65 ± 8	<	194 ± 12	27 ± 6	<	128 ± 15
Vanadium	V	M	14 ± 3	<	92 ± 10	4 ± 2	<	125 ± 16
Strontium	Sr	M	398 ± 113	>	185 ± 19	515 ± 87	>	445 ± 51
Cobalt	Co	M	27 ± 13	<	36 ± 11	3 ± 1	<	51 ± 4
Copper	Cu	M	1 ± 0	<	23 ± 7	1 ± 0	<	31 ± 2
Rubidium	Rb	M	14 ± 2	<	120 ± 11	8 ± 2	<	19 ± 3
Chromium	Cr	M	16 ± 2	<	74 ± 9	11 ± 1	<	222 ± 23
Zinc	Zn	M	4 ± 1	<	67 ± 3	2 ± 0	<	99 ± 9
Arsenic	As	M	10 ± 3	>	5 ± 1	5 ± 2	>	2 ± 0
Bismuth	Bi	M	2 ± 1	=	2 ± 1	1 ± 0	=	1 ± 0
Bromine	Br	NM	25 ± 9	>	1 ± 0	31 ± 2	>	7 ± 1
Cadmium	Cd	M	2 ± 0	=	2 ± 0	2 ± 0	<	3 ± 1
Cerium	Ce	M	8 ± 5	<	69 ± 13	1 ± 0	<	34 ± 5
Caesium	Cs	M	5 ± 3	<	8 ± 3	12 ± 3	>	2 ± 1
Gallium	Ga	M	3 ± 0	<	18 ± 2	3 ± 0	<	19 ± 2
Germanium	Ge	M	1 ± 0	<	2 ± 0	1 ± 0	<	2 ± 0
Iodine	I	NM	11 ± 6	>	1 ± 0	19 ± 2	>	2 ± 1
Lanthanum	La	M	6 ± 5	<	42 ± 10	9 ± 3	<	18 ± 4
Niobium	Nb	M	2 ± 0	<	12 ± 1	1 ± 0	<	22 ± 4
Neodymium	Nd	M	7 ± 3	<	36 ± 6	2 ± 1	<	25 ± 2
Nickel	Ni	M	1 ± 0	<	17 ± 4	1 ± 0	<	112 ± 7
Lead	Pb	M	2 ± 1	<	14 ± 2	1 ± 0	<	2 ± 0
Scandium	Sc	M	1 ± 0	<	9 ± 2	1 ± 0	<	11 ± 3
Samarium	Sm	M	1 ± 0	=	1 ± 0	2 ± 1	>	1 ± 0
Thorium	Th	M	10 ± 0	<	22 ± 2	9 ± 1	<	11 ± 1
Thallium	Tl	M	5 ± 1	<	7 ± 1	6 ± 0	<	6 ± 0
Uranium	U	M	6 ± 1	<	7 ± 1	7 ± 1	>	7 ± 0
Yttrium	Y	M	4 ± 1	<	29 ± 3	4 ± 1	<	22 ± 3
Hafnium	Hf	M	1 ± 0	<	2 ± 1	1 ± 0	<	2 ± 1

Table 8.10: Trace element composition (parts per million, mean ± SE) for the rocks in each hardness class. Silver, molybdenum, ytterbium, antimony, selenium, tin, tantalum and tellurium were also detected but were too small to be quantitatively measured.

Hardness class:	**Soft**			**Hard**			
Rock: **Element**	**Mount Gambier limestone**	**Calcarenite**	**Fossiliferous limestone**	**Siltstone**	**Kanmantoo schist**	**Felsic volcanic rock**	**Basalt**
Chlorine	7085 ± 1499	3369 ± 1438	4027 ± 736	192 ± 105	764 ± 222	3582 ± 606	1677 ± 326
Manganese	152 ± 36	130 ± 11	52 ± 14	622 ± 77	475 ± 32	1329 ± 505	1006 ± 120
Barium	37 ± 2	59 ± 7	64 ± 11	288 ± 32	511 ± 74	66 ± 17	292 ± 6
Zirconium	17 ± 3	47 ± 5	65 ± 8	198 ± 13	192 ± 17	69 ± 12	158 ± 4
Vanadium	1 ± 0	10 ± 5	14 ± 3	84 ± 19	96 ± 12	73 ± 31	151 ± 7
Strontium	396 ± 33	753 ± 208	398 ± 113	145 ± 46	205 ± 14	267 ± 83	534 ± 11
Cobalt	3 ± 1	3 ± 2	27 ± 13	66 ± 30	22 ± 1	37 ± 2	58 ± 4
Copper	1 ± 0	1 ± 0	1 ± 0	35 ± 17	17 ± 6	23 ± 4	35 ± 1
Rubidium	5 ± 1	13 ± 3	14 ± 2	79 ± 5	140 ± 5	9 ± 2	23 ± 1
Chromium	9 ± 1	15 ± 1	16 ± 2	52 ± 6	85 ± 10	147 ± 10	259 ± 19
Zinc	3 ± 1	1 ± 0	4 ± 1	68 ± 9	67 ± 3	65 ± 8	116 ± 3
Arsenic	2 ± 0	11 ± 1	10 ± 3	2 ± 1	6 ± 1	1 ± 0	3 ± 0
Bismuth	1 ± 0	1 ± 0	2 ± 1	2 ± 1	2 ± 1	1 ± 0	2 ± 1
Bromine	30 ± 3	32 ± 1	25 ± 9	1 ± 0	2 ± 1	10 ± 2	6 ± 1
Cadmium	3 ± 1	2 ± 1	2 ± 0	2 ± 1	3 ± 1	2 ± 1	3 ± 1
Cerium	1 ± 0	1 ± 0	8 ± 5	39 ± 5	85 ± 15	18 ± 9	42 ± 3
Caesium	8 ± 4	19 ± 1	5 ± 3	7 ± 6	8 ± 3	1 ± 0	3 ± 2
Gallium	3 ± 0	4 ± 1	3 ± 0	12 ± 1	20 ± 2	12 ± 2	23 ± 0
Germanium	1 ± 0	1 ± 0	1 ± 0	2 ± 0	3 ± 0	1 ± 0	2 ± 0
Iodine	19 ± 3	18 ± 5	11 ± 6	1 ± 0	1 ± 0	3 ± 2	1 ± 0
Lanthanum	7 ± 4	13 ± 6	6 ± 5	17 ± 9	55 ± 11	7 ± 6	24 ± 2
Niobium	1 ± 0	2 ± 1	2 ± 0	9 ± 1	13 ± 1	9 ± 2	29 ± 1
Neodymium	1 ± 0	4 ± 3	7 ± 3	20 ± 3	44 ± 7	17 ± 3	29 ± 1
Nickel	1 ± 0	1 ± 0	1 ± 0	9 ± 3	20 ± 5	96 ± 20	121 ± 3
Lead	1 ± 0	1 ± 0	2 ± 1	7 ± 1	17 ± 3	3 ± 1	2 ± 1
Scandium	1 ± 0	1 ± 0	1 ± 0	3 ± 2	13 ± 2	1 ± 0	17 ± 2
Samarium	3 ± 2	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Thorium	9 ± 1	10 ± 1	10 ± 0	18 ± 1	24 ± 2	9 ± 1	11 ± 1
Thallium	5 ± 1	6 ± 1	5 ± 1	8 ± 1	6 ± 0	6 ± 1	7 ± 0
Uranium	7 ± 1	8 ± 2	6 ± 1	6 ± 2	7 ± 1	6 ± 0	8 ± 0
Yttrium	2 ± 0	7 ± 2	4 ± 1	21 ± 2	32 ± 3	21 ± 9	23 ± 1
Hafnium	1 ± 0	1 ± 0	1 ± 0	1 ± 0	2 ± 1	1 ± 0	2 ± 1

Table 8.11: PERMANOVAs testing for physical differences between heights, hardness classes and seashores in two marine bioregions. Significant results (α = 0.05) are shown in bold.

Dependent variable:		**Rugosity**			**Microhabitats < 13 mm**		
Source	**df**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**
Bioregion, B	1	0.96	6.34	**0.0173**	624	10.44	**0.0050**
Height, H	2	0.09	6.67	**0.0068**	33	7.78	**0.0033**
Hardness class(bioregion), HC(B)	2	0.02	0.27	0.9791	335	8.82	**0.0006**
Bioregion x height, B x H	2	0.06	4.01	**0.0390**	27	6.31	**0.0106**
Seashore(HC(B))	8	0.01	6.78	**0.0011**	33	7.75	**0.0006**
HC(B) x H	4	0.03	2.13	0.1279	5	1.23	0.3347
Seashore(HC(B)) x H	16	0.01	2.07	**0.0085**	4	1.53	0.0819
Residual	936	0.01			3		
Total	971						

Table 8.12: PERMANOVAs testing for sessile structural or richness differences across three heights, two hardness classes, three seasons and several seashores in two marine bioregions. Significant results (α = 0.05) are shown in bold.

Dependent variable:		**Assemblage structure**			**Marine plant richness**			**Invertebrate richness**		
Source	**df**	**MS**	**Pseudo -*F***	***p*-value**	**MS**	**Pseudo -*F***	***p*-value**	**MS**	**Pseudo -*F***	***p*-value**
Bioregion, B	1	60913	1.36	0.1672	151	3.99	**0.0065**	66	2.09	0.0813
Height, H	2	66055	6.39	**0.0001**	133	6.97	**0.0006**	36	6.94	**0.0015**
Season, S	2	4245	1.06	0.3951	2	0.38	0.9533	1	1.08	0.4200
Hardness class(bioregion), HC(B)	2	217880	3.61	**0.0003**	478	11.38	**0.0001**	156	2.95	**0.0054**
B x H	2	6151	0.78	0.8007	10	0.97	0.4860	4	0.74	0.6586
B x S	2	6174	1.53	0.0598	3	1.25	0.3218	5	3.75	**0.0147**
H x S	4	2478	1.43	0.1046	7	4.08	**0.0090**	1	0.30	0.8731
Seashore(HC(B))	8	40660	3.76	**0.0001**	27	1.86	**0.0481**	25	4.41	**0.0001**
HC(B) x H	4	19085	2.02	**0.0041**	14	1.24	0.3078	28	5.11	**0.0004**
HC(B) x S	4	2620	0.81	0.8475	1	0.54	0.9019	1	1.57	0.1647
B x H x S	4	2001	1.16	0.2832	1	0.33	0.8513	1	1.06	0.3987
Seashore(HC(B)) x H	16	8129	4.70	**0.0001**	12	6.49	**0.0001**	5	5.86	**0.0001**
Seashore(HC(B)) x S	16	3160	1.83	**0.0007**	3	1.81	0.0719	1	0.89	0.5952
HC(B) x H x S	8	2190	1.27	0.1425	1	0.64	0.7362	1	0.71	0.6828
Seashore(HC(B)) x H x S	32	1730	1.64	**0.0001**	2	1.64	**0.0158**	1	1.60	**0.0188**
Residual	864	1055			1			1		
Total	971									

Table 8.13: PERMANOVAs testing for coverage differences (%) across three heights, three seasons, two hardness classes and several seashores in two marine bioregions. Significant results (α = 0.05) are shown in bold.

Dependent variable:		**Marine plant space occupancy**			**Invertebrate space occupancy**			**Coverage of bare rock**			**Coverage of sediment**		
Source	**df**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**
Bioregion, B	1	64	0.86	0.5976	168	2.53	**0.0423**	22	1.08	0.4271	60	0.83	0.6298
Height, H	2	285	5.80	**0.0030**	53	5.43	**0.0024**	179	2.99	**0.0348**	21	0.72	0.6771
Season, S	2	11	0.88	0.5432	1	1.17	0.3656	6	0.37	0.9498	1	0.23	0.9960
Hardness class(bioregion), HC(B)	2	960	4.66	**0.0002**	342	4.07	**0.0008**	483	3.68	**0.0013**	291	3.21	**0.0034**
B x H	2	3	0.17	0.9993	1	0.27	0.9903	1	0.07	1	36	1.45	0.2402
B x S	2	2	0.35	0.9625	14	4.76	**0.0056**	1	0.18	0.9989	4	0.47	0.8937
H x S	4	7	1.24	0.3118	2	0.63	0.6520	9	2.15	0.0959	12	2.36	0.0725
Seashore(HC(B))	8	139	2.61	**0.0060**	54	5.52	**0.0001**	88	1.30	0.2394	80	2.13	**0.0242**
HC(B) x H	4	58	1.27	0.2894	25	2.56	**0.0256**	41	0.80	0.6509	10	0.49	0.9337
HC(B) x S	4	17	1.16	0.3612	5	1.99	0.0720	18	0.93	0.5424	10	0.63	0.8298
B x H x S	4	10	1.72	0.1733	2	0.74	0.5839	8	1.85	0.1429	4	0.77	0.5541
Seashore(HC(B)) x H	16	43	7.51	**0.0001**	9	3.40	**0.0023**	52	12.83	**0.0001**	24	4.89	**0.0001**
Seashore(HC(B)) x S	16	12	2.15	**0.0285**	2	0.63	0.8310	19	4.62	**0.0003**	16	3.23	**0.0027**
HC(B) x H x S	8	7	1.29	0.2775	2	0.85	0.5703	5	1.12	0.3692	7	1.45	0.2163
Seashore(HC(B)) x H x S	32	6	1.66	**0.0137**	3	2.60	**0.0001**	4	1.56	**0.0232**	5	2.08	**0.0008**
Residual	864	3			1			3			2		
Total	971												

Table 8.14: PERMANOVAs testing for mobile invertebrate structural, richness, or abundance differences across three heights, three seasons, two hardness classes and several seashores in two marine bioregions. Significant results ($\alpha = 0.05$) are shown in bold.

		Assemblage structure			**Total species richness**			**Total abundance**		
Source	**df**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**
Bioregion, B	1	184000	3.06	**0.0062**	173	4.71	**0.0019**	2530	11.32	**0.0001**
Height, H	2	35780	3.11	**0.0039**	22	3.15	**0.0299**	143	2.23	0.0868
Season, S	2	8655	1.51	0.0677	11	2.98	**0.0389**	87	4.11	**0.0104**
Hardness class(bioregion), HC(B)	2	88929	1.46	0.0660	44	0.86	0.6337	85	0.71	0.8024
B x H	2	9438	0.98	0.5133	3	0.54	0.8236	51	0.82	0.5939
B x S	2	5643	1.17	0.2703	5	1.54	0.2196	49	2.04	0.1109
H x S	4	2539	1.37	0.1351	1	0.86	0.5075	7	0.79	0.5358
Seashore(HC(B))	8	48440	3.59	**0.0001**	30	3.29	**0.0007**	122	1.69	0.0776
HC(B) x H	4	18846	1.74	**0.0319**	31	4.32	**0.0014**	102	1.65	0.1388
HC(B) x S	4	3070	0.73	0.9380	2	0.63	0.8246	6	0.65	0.8112
B x H x S	4	2006	1.08	0.3544	1	0.71	0.5955	12	1.38	0.2543
Seashore(HC(B)) x H	16	9567	5.14	**0.0001**	6	6.08	**0.0001**	61	6.79	**0.0001**
Seashore(HC(B)) x S	16	4430	2.38	**0.0001**	3	3.09	**0.0027**	16	1.81	0.0797
HC(B) x H x S	8	2301	1.24	0.1787	1	1.02	0.4427	7	0.74	0.6553
Seashore(HC(B)) x H x S	32	1860	1.57	**0.0001**	1	0.95	0.5487	9	1.48	**0.0441**
Residual	864	1183			1			6		
Total	971									

Table 8.15: PCA identifying PC axes (eigenvalues >1) that best account for the total variability in 45 physical variables measured across 12 seashores over three seasons. The independent variables associated with each PC axis are specified, with very strongly-associated independent variables (Varimax rotated loadings ≥0.90) shown in bold.

Principal component	**Eigenvalue**	**% of total variance explained**	**Associated independent variables**	**VARIMAX rotated loading**	**Interpretation of PC**
1	16.94	37.7	Chromium	**0.99**	Trace metals & metallic oxide
			Titanium dioxide	**0.98**	
			Nickel	**0.97**	
			Phosphorus pentoxide	**0.96**	
			Iron oxide	**0.94**	
			Niobium	**0.94**	
			Sodium oxide	**0.91**	
2	13.81	30.7	Rubidium	**0.99**	Other trace metals & metallic oxide
			Lead	**0.96**	
			Potassium oxide	**0.96**	
			Thorium	**0.95**	
			Lanthanum	**0.92**	
3	3.59	8.0	Uranium	**0.90**	Trace metal
4	2.61	5.8	Samarium	-0.66	Trace metal
5	2.72	6.1	Microhabitats >13 mm	-0.79	Habitat feature
6	2.29	5.1	Bismuth	0.80	Trace metal

Table 8.16: Summary of temperature data availability. ✓ = data collected for dry substrata on a sunny day and was included in PCA; X (C) = data collected for damp substratum on a cloudy day and was not included in PCA; X (W) = data collected for wet substratum and was not included in PCA.

Bioregion	Seashore	Season Summer 2015	Summer 2016
Otway	Blacknose Point	X (C)	✓
	Portland Windfarm	✓	✓
	Enchanted Forest	X (W)	X (W)
	Racecourse Bay West	✓	X (C)
	Pelican Point	✓	✓
	Nora Creina	X (C)	✓
Gulf St Vincent	Encounter Bay	✓	✓
	The Bluff	✓	✓
	Cape Jervis	✓	✓
	Blanche Point	✓	✓
	Southport	✓	✓
	Marino Rocks	✓	✓

Table 8.17: PCA identifying PC axes (eigenvalues >1) that best account for the total variability in 48 physical variables (i.e. temperature included) across selected seashores over two summers. The independent variables associated with each PC axis are specified, with very strongly-associated independent variables (Varimax rotated loadings ≥0.90) shown in bold.

Principal component	**Eigenvalue**	**% of total variance explained**	**Associated independent variables**	**VARIMAX rotated loading**	**Interpretation of PC**
1	17.84	37.2	Chromium	**1.00**	Trace metals & metallic oxides
			Nickel	**0.99**	
			Titanium dioxide	**0.98**	
			Niobium	**0.97**	
			Phosphorus pentoxide	**0.96**	
			Iron oxide	**0.95**	
			Sodium oxide	**0.92**	
			Manganese oxide	**0.92**	
			Manganese	**0.90**	
2	12.96	27.0	Rubidium	**0.96**	Other trace metals
			Lead	**0.94**	
			Thorium	**0.92**	
3	2.86	6.0	Caesium	**0.91**	Trace metal
4	3.57	7.4	Samarium	-0.84	Trace metal
5	2.01	4.2	Bismuth	**0.90**	Trace metal
6	3.70	7.7	Thallium	0.83	Trace metal
7	3.27	6.8	Average temperature	0.87	Surface temperature

Table 8.18: Correlations between univariate biotic variables and each of the six PC axes (eigenvalue >1) identified from PCA of 45 raw physical variables. Significant correlations (α = 0.05) are shown in bold.

PC axis:	1st		2nd		3rd		4th		5th		6th	
Dependent variable	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value
Mobile invertebrate total abundance	0.086	>0.05	-0.420	**<0.01**	-0.019	>0.05	-0.069	>0.05	0.130	**<0.05**	0.079	>0.05
Mobile invertebrate species richness	0.147	**<0.02**	-0.402	**<0.01**	-0.105	>0.05	0.091	>0.05	0.055	>0.05	0.091	>0.05
Marine plant occupancy	-0.075	>0.05	-0.407	**<0.01**	-0.021	>0.05	0.199	**<0.01**	-0.255	**<0.01**	-0.140	**<0.02**
Sessile invertebrate occupancy	0.282	**<0.01**	-0.239	**<0.01**	-0.376	**<0.01**	0.313	**<0.01**	-0.398	**<0.01**	0.177	**<0.01**
Marine plant richness	-0.153	**<0.01**	-0.482	**<0.01**	-0.084	>0.05	0.181	**<0.01**	-0.332	**<0.01**	-0.055	>0.05
Sessile invertebrate richness	0.306	**<0.01**	-0.185	**<0.01**	-0.361	**<0.01**	0.307	**<0.01**	-0.398	**<0.01**	0.152	**<0.01**

Table 8.19: Univariate assemblage differences (mean ± SE values) at the transect scale for hardness classes in each marine bioregion. * = p <0.05.

Bioregion:	**Otway**				**GSV**			
Dependent variable	**Soft**		**Hard**		**Soft**		**Hard**	
Marine plant species richness	4.0 ± 0.4	<	4.3 ± 0.4		4.6 ± 0.3	>	3.3 ± 0.3	
Invertebrate species richness	0	<	2.1 ± 0.2	*	1.6 ± 0.1	<	1.7 ± 0.3	
Marine plant space occupancy (%)	23.5 ± 3.4	<	28.8 ± 2.9		54.2 ± 4.8	>	11.4 ± 2.0	*
Invertebrate space occupancy (%)	0	<	8.2 ± 1.3	*	19.4 ± 3.6	>	5.4 ± 1.6	
Bare rock (%)	76.5 ± 3.4	>	63.0 ± 3.4		26.4 ± 3.6	<	83.1 ± 2.0	*

Table 8.20: Sessile assemblages sampled at the transect scale: PERMANOVAs testing for structural or richness differences across two hardness classes in three seasons, and several seashores in two marine bioregions. Significant results (α = 0.05) are shown in bold.

Dependent variable:		**Assemblage structure**			**Marine plant richness**			**Invertebrate richness**		
Source	**df**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**
Bioregion, B	1	19758	2.00	0.0755	1	0.13	0.9962	8	0.92	0.5047
Season, S	2	2185	2.11	**0.0330**	36	23.19	**0.0001**	1	1.63	0.2308
Hardness class (bioregion), HC(B)	2	21654	2.17	**0.0215**	13	0.99	0.4735	31	3.32	**0.0384**
B x S	2	1006	0.97	0.4807	2	1.43	0.2650	1	0.22	0.8081
Seashore (HC(B))	8	9376	9.06	**0.0001**	14	8.88	**0.0002**	9	24.85	**0.0001**
HC(B) x S	4	1094	1.06	0.4221	1	0.24	0.9113	1	0.20	0.9336
Seashore (HC(B)) x S	16	1035	2.15	**0.0001**	2	0.98	0.4888	1	1.32	0.2083
Residual	72	480			2			1		
Total	107									

Table 8.21: Sessile assemblages sampled at the transect scale: PERMANOVAs testing for space occupancy differences for biota and univariate PERMANOVAs for bare rock across three seasons, two hardness classes an several seashores in two marine bioregions. Significant results (α = 0.05) are shown in bold.

Dependent variable:		**Marine plant space occupancy**			**Invertebrate space occupancy**			**Coverage of bare rock**		
Source	**df**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**	**MS**	**Pseudo-*F***	***p*-value**
Bioregion, B	1	2	0.13	0.9973	52	2.40	0.1270	45	2.71	0.1072
Season, S	2	1	0.22	0.8066	7	7.02	**0.0058**	6	4.57	**0.0256**
Hardness class(bioregion), HC(B)	2	116	5.35	**0.0066**	83	4.11	**0.0193**	126	12.74	**0.0003**
B x S	2	12	5.97	**0.0129**	4	4.09	**0.0388**	11	8.54	**0.0036**
Seashore (HC(B))	8	21	10.92	**0.0001**	18	17.97	**0.0001**	7	5.33	**0.0022**
HC(B) x S	4	1	0.33	0.8517	3	2.55	0.0827	3	2.73	0.0675
Seashore (HC(B)) x S	16	2	1.69	0.0671	1	1.49	0.1222	1	3.13	**0.0004**
Residual	72	1			1			1		
Total	107									

Table 8.22: SIMPER analyses identifying consistent indicators amongst functional groups (SD/Sim ratio ≥1) associated with sessile assemblage structural differences at the transect scale between hardness classes run separately in each bioregion. Space occupancies are expressed as the mean percentage cover along transects.

Bioregion	Functional group	SD/Sim ratio	% contribution	Space occupancy (%) Soft		Hard	Hardness-class preference
Otway	Turf-forming algae	1.28	29.42	14.12	>	9.43	Soft
	Corticated macrophytes	1.17	20.80	6.40	<	9.97	Hard
	Tube worms	1.40	10.56	0.00	<	4.21	Hard
Gulf St Vincent	Turf-forming algae	1.38	25.29	22.59	>	6.20	Soft
	Filamentous algae	1.13	24.30	17.92	>	0.96	Soft
	Mussels	1.14	20.67	16.36	>	2.35	Soft

Table 8.23: PCA identifying PC axes (eigenvalues >1) that best account for the total variability in 43 physical variables for transects. The independent variables associated with each PC axis are specified, with very strongly-associated independent variables (Varimax rotated loadings ≥0.90) shown in bold.

Principal component	**Eigenvalue**	**% of total variance explained**	**Associated independent variables**	**VARIMAX rotated loading**	**Interpretation of PC**
1	16.71	38.9	Chromium	**0.99**	Trace metals & metallic oxides
			Titanium dioxide	**0.98**	
			Nickel	**0.98**	
			Phosphorus pentoxide	**0.97**	
			Iron oxide	**0.94**	
			Niobium	**0.94**	
			Sodium oxide	**0.91**	
2	13.65	31.7	Rubidium	**0.99**	Other trace metals & metallic oxide
			Lead	**0.96**	
			Potassium oxide	**0.95**	
			Thorium	**0.95**	
			Lanthanum	**0.92**	
3	3.49	8.1	Uranium	0.87	Trace metal
4	2.89	6.7	Samarium	-0.83	Trace metal
5	2.19	5.1	Thallium	0.67	Trace metal
6	1.99	4.7	Bismuth	0.86	Trace metal

Table 8.24: Correlations between univariate biotic variables measured at the transect scale and each of the six PC axes (eigenvalue >1) identified from PCA of 45 raw physical variables. Significant correlations (α = 0.05) are shown in bold.

PC axis:	1st		2nd		3rd		4th		5th		6th	
Dependent variable	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value	*p*	*p*-value
Marine plant occupancy	-0.263	**<0.01**	-0.313	**<0.01**	0.088	>0.05	0.084	>0.05	-0.224	**<0.01**	-0.086	>0.05
Sessile invertebrate occupancy	0.043	>0.05	-0.026	>0.05	-0.451	**<0.01**	0.473	**<0.01**	-0.090	>0.05	0.476	**<0.01**
Marine plant richness	-0.103	>0.05	-0.100	>0.05	0.012	>0.05	0.086	>0.05	-0.205	**<0.01**	-0.388	**<0.01**
Sessile invertebrate richness	0.319	**<0.01**	0.186	**<0.01**	-0.332	**<0.01**	0.465	**<0.01**	0.041	>0.05	0.346	**<0.01**

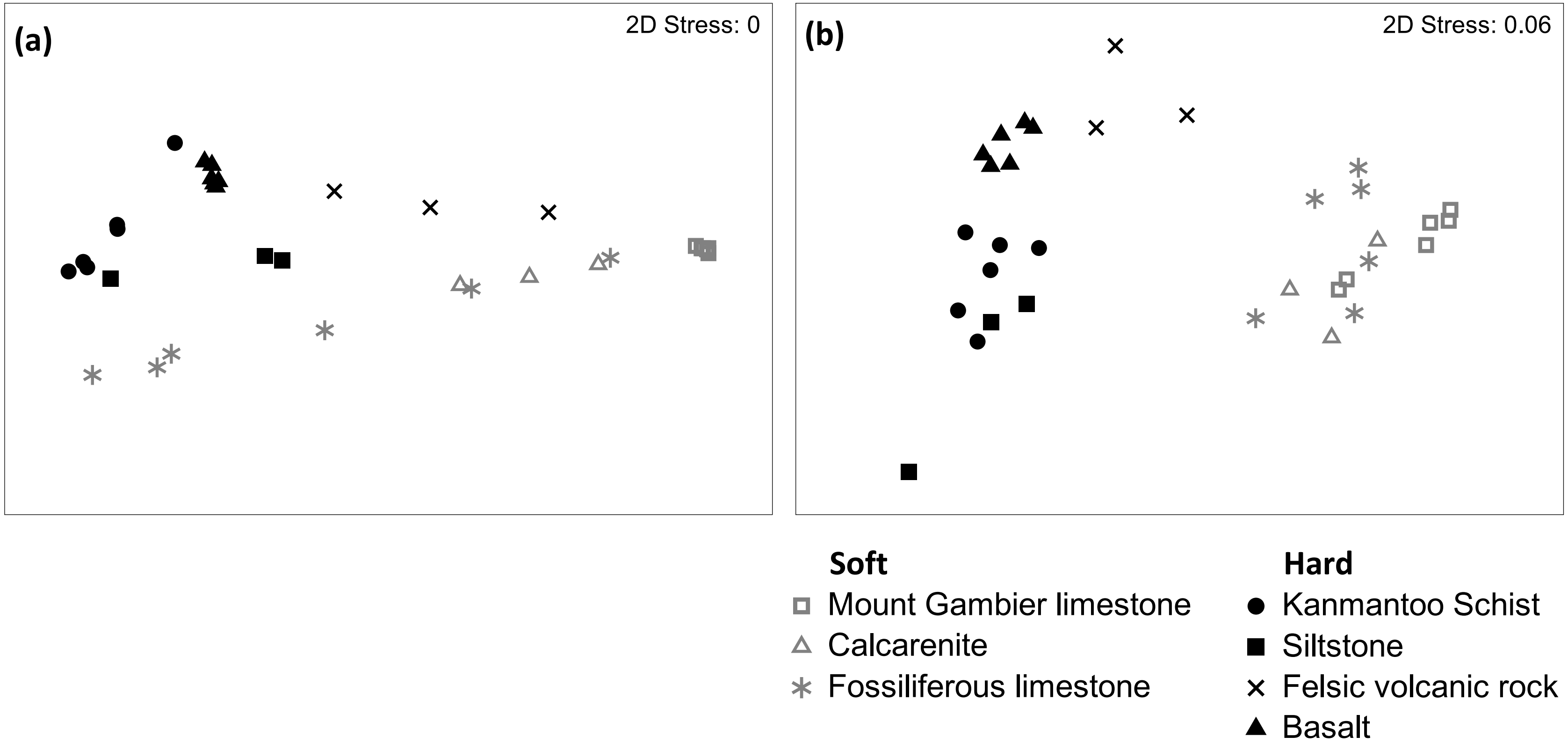


Figure 8.1: Two-dimensional nMDS ordination plots (based on Euclidean distance) depicting the distinct (a) major mineral and (b) trace element composition associated with rocks in the soft (grey unfilled shapes) versus hard (black filled shapes) classes. Each point represents a single rock sample tested.

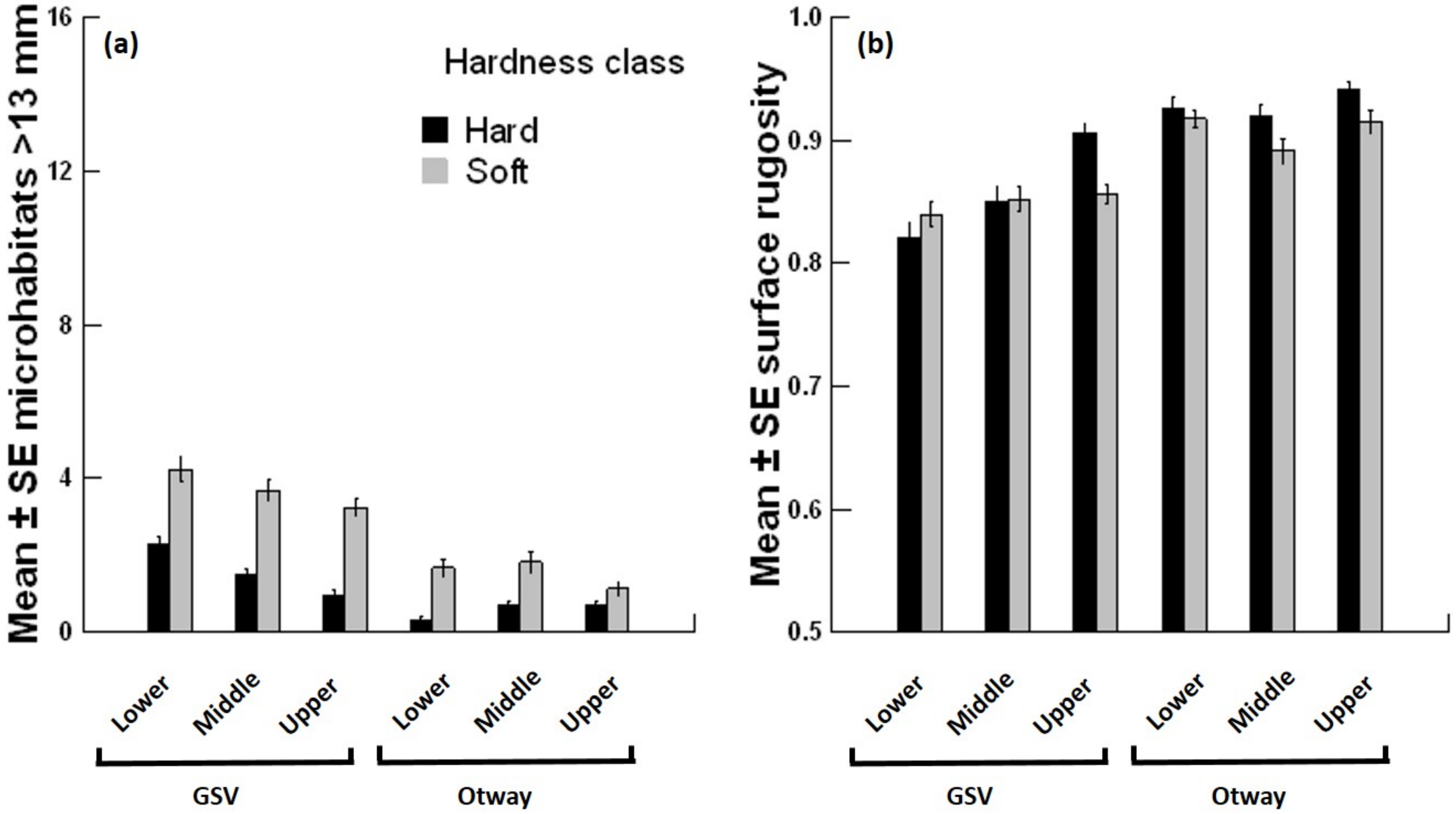


Figure 8.2: Mean ± SE (a) microhabitat density and (b) surface rugosity for quadrats on seashores from the soft versus hard classes for three seashore heights in two marine bioregions. Each y-axis extends to encompass the range of the raw data. GSV = Gulf St Vincent.

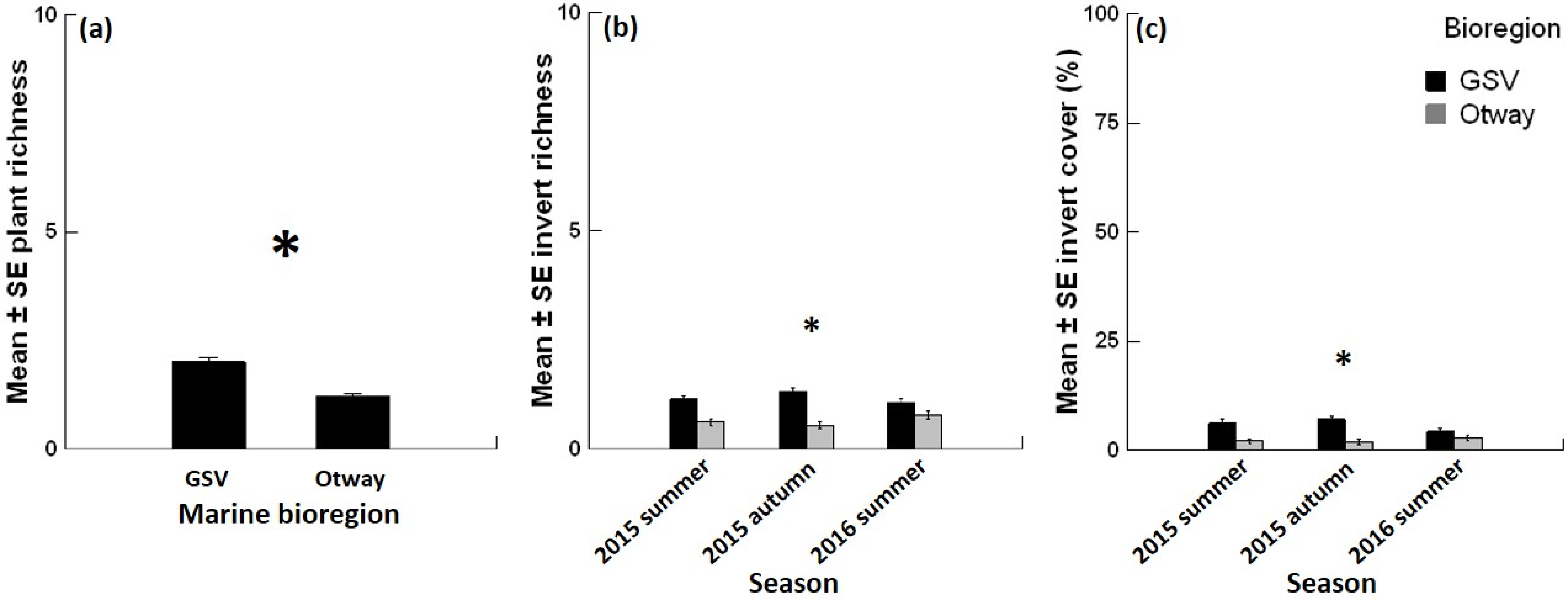


Figure 8.3: Mean ± SE (a) marine plant richness, (b) seasonal sessile invertebrate (“invert”) richness and (c) seasonal sessile invertebrate occupancy in each bioregion. Each y-axis extends to encompass the range of the raw data. * = significant difference detected between bioregions.

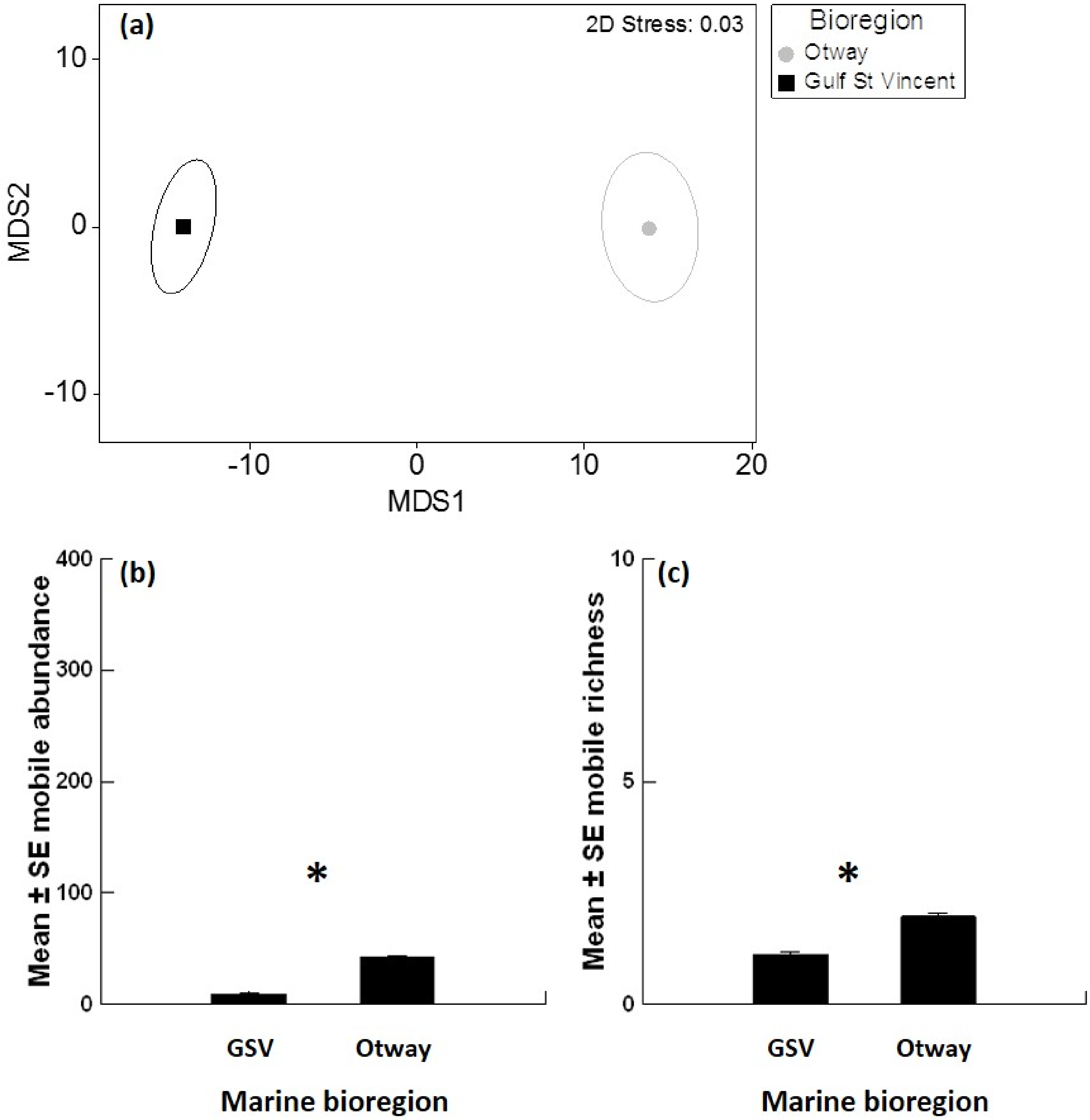


Figure 8.4: Assemblage differences between bioregions for mobile invertebrates sampled at the quadrat scale: (a) MDS bootstrapped averages ordination plot depicting structural differences between marine bioregions (*n* = 300 runs per bioregion); (b) mean ± SE total abundance; and (c) mean ± SE species richness. Each y-axis on bar charts extends to encompass the range of the raw data. GSV = Gulf St Vincent; and * = significant difference detected.

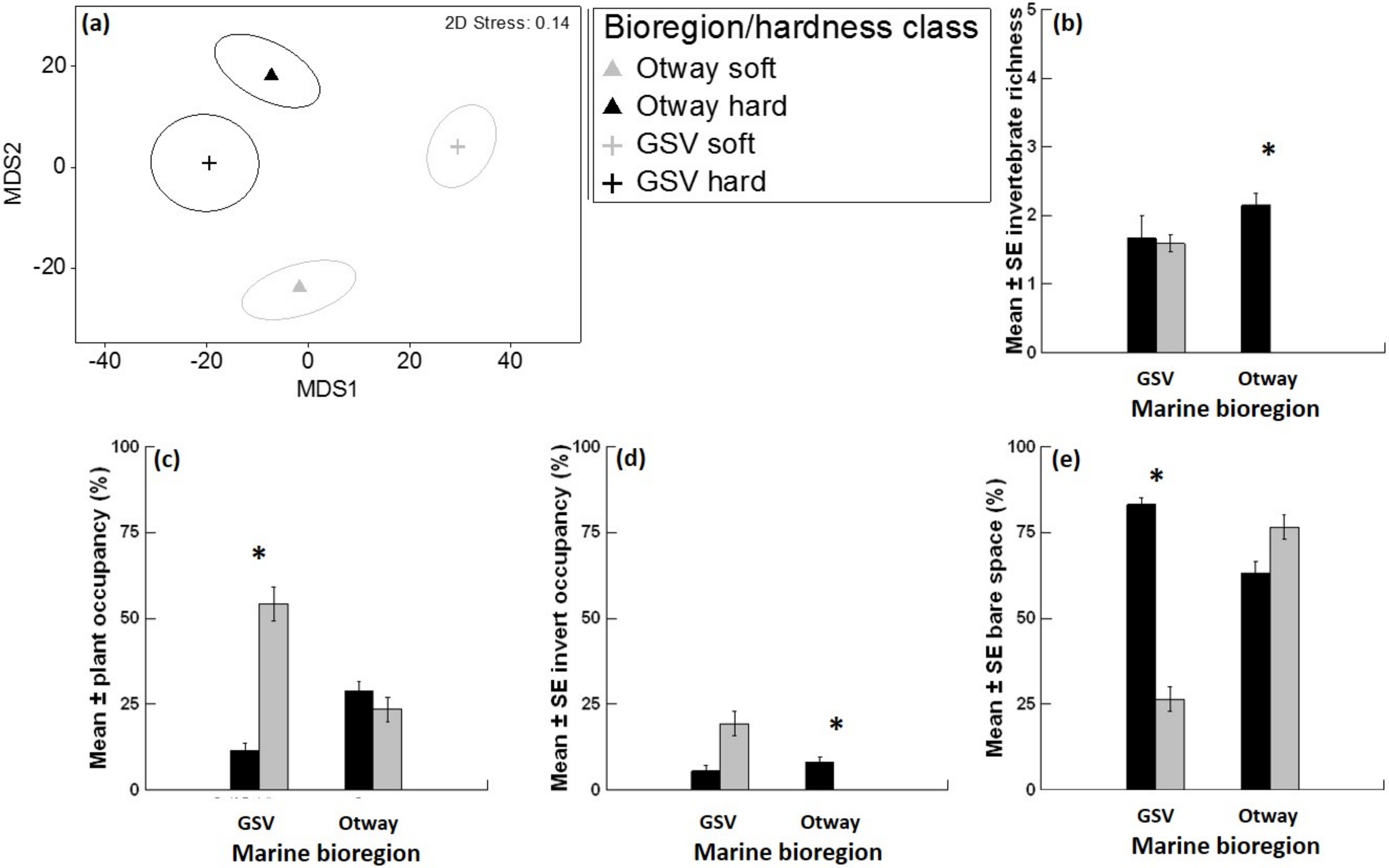


Figure 8.5: Sessile assemblage differences between hardness classes (black fill = hard class, grey fill = soft class) at the transect scale: (a) MDS bootstrapped averages ordination plot depicting structural differences due to the factor Hardness class(Bioregion) and mean ± SE differences between hardness classes for (b) sessile invertebrate richness, (c) marine plant occupancy as % cover, (d) sessile invertebrate occupancy as % cover and (e) bare rock as % cover. Each y-axis on bar charts extends to encompass the range of the raw data. GSV = Gulf St Vincent * = significant difference detected between hardness classes.

Appendix 2: Chapter 3

Physical comparisons of limestone and siltstone boulders

To develop a comprehensive physical description of each boulder, three main physical attributes were measured. Boulder surface area, the number of crack or depression micro-habitats (i.e. surface features >13 mm in size; Liversage et al. 2014), and surface rugosity indices were all measured using the methods described in Liversage et al. (2014). Additional descriptors were assessed using a small subset of representative boulders for each rock type. Hardness was measured using Moh's scale of scratch hardness, quantified by scratching boulder surfaces with a sample of the nine softest minerals on this scale. The scratch hardness of the softest mineral capable of scratching the boulder surface was assigned to each rock.

Surface-area data were square-root-transformed; data for rugosity and microhabitat density were not transformed. Analysis of rock differences for univariate variables was completed using Euclidean distances, with separate one-factor PERMANOVAs completed for upper versus lower surfaces, and for the five- and six-year transplant times. This was done to ensure data independence, as the upper and lower surfaces of the same boulders were sampled, and I could not be certain whether the same subset of boulders had been sampled after five and six years.

The transplanted limestone and siltstone boulders had different physical attributes (Table 8.25 & Figures 8.6 - 8.7). The lighter-coloured limestone had a Moh's scratch hardness of four and was moderately friable, while the darker-coloured siltstone had a Moh's scratch hardness of eight and displayed no friability (Table 8.25 & Figure 8.6). Limestone and siltstone had different-sized upper and lower surfaces, although these differences were not significant at all sampling times (Figure 8.7 & Table 8.25). The upper surfaces of limestone were 5 % and 19 % larger than the upper surfaces of siltstone after five and six years, respectively, while the lower surfaces of siltstone were 14 % and 4 % larger than the lower surfaces of limestone for the same sampling times (Figure 8.7a-b).

Surface rugosity and microhabitat density were significantly different between limestone and siltstone, for both boulder surfaces and both sampling times (Figure 8.7). Limestone had complex, undulating surfaces interspersed by crack and depression microhabitats, thus lowering its rugosity index. In contrast, siltstone generally had flat, featureless surfaces devoid of microhabitat features, giving it a high rugosity index close to 1 (i.e. quite smooth, Figures 8.6 & 8.7).

Rock mineralogy

Mineralogy was determined by using X-ray fluorescence (XRF), with separate tests completed for major mineral and trace element composition for three samples each of limestone and siltstone. XRF analysis tested for 11 major minerals and 40 trace elements, which were returned as % composition and parts per thousand, respectively. For major minerals, approximately one gram of each oven-dried sample (at 105 °C) was accurately weighed with four grams of 12-22 lithium borate flux (Norrish and Hutton 1969). The mixtures were heated to 1050 °C in a platinum/gold crucible for 20 minutes to completely dissolve the sample, and then poured into a 32mm platinum/gold mould heated to a similar temperature (Norrish and Hutton 1969). The melt was cooled rapidly over a compressed air stream and the resulting glass disks were analysed on a PANalytical Axios Advanced wavelength dispersive XRF system using the CSIRO in-house silicates calibration program. For trace elements, approximately four grams of each oven-dried sample (at 105 °C) was accurately weighed with one gram of Licowax binder and mixed well (Norrish and Hutton 1969). The mixtures were pressed in a 32 mm die at 12 tons pressure and the resulting pellets were analysed on a PANalytical Axios Advanced wavelength dispersive XRF system using the CSIRO in-house powders program (Norrish and Hutton 1969).

The total major mineral content in limestone and siltstone was similar, while limestone contained more than double the trace element content (although this accounted for <1 % of the total sample) versus siltstone (Table 8.25). Both rocks also had a similar-sized 'missing' component (Table 8.25), which was comprised of metallic oxide weight gains via oxidation minus water and carbon dioxide loss during the XRF fusion process (M. Raven CSIRO pers. comm). As neither oxidative weight gains or water and carbon dioxide loss were measured directly, they were unable to be attributed to specific minerals or elements, and were subsequently pooled as the 'missing' component for each sample.

Mineralogy differences between rocks were identified, with transplanted limestone and siltstone having a different major mineral composition (Table 8.25). Both rocks had a mineralogy dominated by silicon dioxide (SiO_2), although limestone contained a higher percentage of SiO_2 than siltstone. Siltstone had a higher content of aluminium oxide, iron oxide, and magnesium oxide (Table 8.25). Trace element composition also differed between limestone and siltstone. Limestone contained high levels of chlorine, while siltstone contained small amounts of a variety of metallic elements including barium, manganese and zirconium (Table 8.25).

Biological observations

Twenty-eight marine species were recorded on boulders sampled after five years, while 27 marine species were recorded on boulders sampled after six years (Table 8.26). When pooled across both sampling times, a total of 33 marine species, spanning nine phyla, were recorded. Of these 33 species, 21 mobile species were identified, which included anemones, flatworms, chitons, limpets, snails, isopods, and crabs. Twelve sessile taxa were recorded which included a visible biofilm, algae, tubeworms, mussels, and barnacles. The most commonly-encountered species were the barnacle *Chthamalus antennatus*, the snail *Bembicium nanum*, limpets belonging to the genus *Notoacmea*, and the tubeworm *Galeolaria caespitosa* (Table 8.26).

Of the two rocks transplanted as bare boulders, limestone supported a higher species pool of 30 when observations were combined across both sampling times. Twenty-five species were recorded on limestone after five years, while 26 species were recorded 12 months later (Table 8.26). In contrast, 25 species were recorded on siltstone, with 21 species recorded after five years and 22 species after six years (Table 8.26). Similar patterns of species richness were also recorded for native boulders, with native limestone supporting a higher species richness than native siltstone. When combined across both sampling times, a species richness of 26 was recorded for native limestone, with 21 species recorded after five years and 15 species recorded after six years. In contrast, a combined species richness of only 16 was recorded for native siltstone, with 14 species recorded after five years, and just 7 species recorded after six years (Table 8.26). Lower boulder surfaces appeared to support a greater mean richness of marine species when compared to upper surfaces (Figure 8.10).

Table 8.25: Physical attributes of transplanted limestone versus siltstone. Colour is expressed as descriptive colour Hue Value/Chroma on Munsell colour charts where YR = yellow-red.

Physical attribute	**Rock:**	**Limestone**	**Siltstone**
Scratch hardness (Moh's scale)		4	8
Friability		Moderate	None
Colour (Munsell colour chart)		Light grey 10 YR 8/1	Greyish yellow-brown 10 YR 5/2
Composition (%, mean ± SE)	Major elements	87.9 ± 1.4	87.9 ± 3.8
	Trace elements	0.5 ± 0.1	0.2 ± 0
	'Missing'	11.5 ± 1.4	11.9 ± 3.8
Mineralogy: Major elements (%, mean ± SE)	SiO_2	71.6 ± 3.1	55.4 ± 6.6
	Al_2O_3	1.2 ± 0.3	8.8 ± 0.6
	Fe_2O_3	0.9 ± 0.1	4.1 ± 0.6
	MgO	0.6 ± 0.1	3.3 ± 0.1
	K_2O	0.5 ± 0.1	2.1 ± 0.1
	TiO_2	0.1 ± 0.0	0.6 ± 0.1
	CaO	12.3 ± 1.8	12.0 ± 4.2
	Na_2O	0.6 ± 0.1	1.3 ± 0.4
	MnO_2	0 ± 0	0.1 ± 0.0
	P_4O_{10}	0.1 ± 0	0.1 ± 0
	SO_3	0.2 ± 0.0	0.0 ± 0.0
Mineralogy: Dominant trace elements (ppm, mean ± SE)		Cl 4884 ± 882	Mn 622 ± 77
		Sr 157 ± 16	Ba 288 ± 32
		Zr 76 ± 11	Zr 198 ± 13
		Co 46 ± 22	Cl 192 ± 105

Table 8.26: Species list for algae and invertebrates recorded on boulder surfaces for each sampling time at each seashore. TL = transplanted limestone; TS = transplanted siltstone; NS = native siltstone; and NL = native limestone.

				Seashore:	Marino Rocks						Myponga Beach						Blanche Point						Southport					
				Sampling time (years):	5			6			5			6			5			6			5			6		
Phyla	Class	Family	Species	Boulder History:	TL	TS	NS	TL	TS	NS	TL	TS	NS	TL	TS	NS	TL	TS	NL	TL	TS	NL	TL	TS	NL	TL	TS	NL
Algae			Biofilm					x						x						x	x	x				x		x
Chlorophyta	Ulvophyceae	Ulvaceae	*Enteromorpha* sp.																	x		x	x			x		x
Heterokontophyta	Phaeophyceae		Unknown filamentous brown																		x	x						
Rhodophyta	Florideophyceae	Corallinaceae	*Corallina officinalis*																						x			
			Crustose 'pink paint'											x												x		x
		Gelidiaceae	*Capreolia implexa*								x			x						x			x		x			
Animals																												
Cnidaria	Anthozoa	Actiniidae	*Actinia tenebrosa*												x		x	x	x	x	x		x	x	x		x	
			Isanemonia australis				x																x	x	x	x		
Platyhelminthes	Rhabditophora	Notoplanidae	*Notoplana australis*			x	x		x										x									
Annelida	Polychaeta	Oenonidae	*Notopsilus* sp.									x					x											
		Serpulidae	*Galeolaria caespitosa*		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
			Spirorbid sp.			x					x	x		x		x	x		x				x	x	x	x	x	x
Mollusca	Polyplacophora	Ischnochitoniidae	*Ischnochiton elongatus*			x	x										x	x	x	x	x		x		x	x	x	
	Gastropoda	Fisurellidae	*Montfortula rugosa*										x										x		x	x		
		Lottidae	*Notoacmaea* spp.		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
		Littorinidae	*Bembicium nanum*		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x
			Bembicium vittatum		x	x											x									x		
		Nacellidae	*Cellana tramoserica*				x	x				x	x										x	x	x	x		
		Neritopsidae	*Nerita atramentosa*		x	x	x	x	x	x	x	x	x	x	x	x		x										
		Siphonariidae	*Siphonaria diemenensis*		x	x		x			x		x	x			x	x	x	x	x	x	x	x	x	x	x	x
			Siphonaria zelandica																x				x		x			
		Trochidae	*Austrocochlea constricta*															x	x				x	x	x	x		
			Austrocochlea porcata															x	x									
			Diloma concamerata		x	x	x	x	x	x	x	x	x	x	x	x				x								
		Muricidae	*Haustrum vinosum*								x																	
	Bivalvia	Mytilidae	*Brachidontes rostratus*																				x	x	x			
			Xenostrobus pulex		x	x	x							x			x	x	x	x		x	x	x	x	x	x	x
Arthropoda	Malacostraca	Eriphiidae	*Ozius truncatus*		x	x			x		x		x		x		x	x	x	x	x	x	x	x	x		x	x
		Grapsidae	*Cyclograpsus granulosus*																							x		
		Idoteidae	*Euidotea bakeri*					x																		x		
		Sphaeromatidae	*Zuzara venosa*																				x		x	x		
	Maxillopoda	Chthamalidae	*Chthamalus antennatus*		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
		Tetraclitidae	*Tetraclitella purpurascens*					x			x			x	x		x	x		x		x		x				x
Total species richness per boulder (summed)					**10**	**12**	**11**	**11**	**7**	**6**	**12**	**9**	**10**	**13**	**9**	**7**	**13**	**13**	**14**	**14**	**10**	**11**	**19**	**14**	**19**	**18**	**10**	**12**

Table 8.27: *P* values from PERMANOVA planned comparisons testing for assemblage differences between transplanted limestone and siltstone. Analyses were completed separately for each seashore, sampling time, and surface. Significant results (α = 0.05) are shown in bold, with MC = *p*-value used from Monte Carlo tests. The total degrees of freedom for each comparison ranged from 15 for surfaces sampled at SP after 6 years to 29 for surfaces sampled at MB, MR and SP after 5 years. MB = Myponga Beach, MR = Marino Rocks, SP = Southport; and BP = Blanche Point.

Surface		**Upper**				**Lower**			
	Seashore	**MB**	**MR**	**SP**	**BP**	**MB**	**MR**	**SP**	**BP**
Measure	**Year**								
Mobile invertebrate assemblage structure	5	**0.0384**	**0.0002**	0.2773	0.1811	0.856	0.2207	0.0945	0.1207
	6	**0.0068**	**0.0022**	0.887 (MC)	0.2582	0.2659	**0.028**	0.2376	0.6759
Sessile assemblage structure	5	**0.0006**	**0.0009**	0.1097	0.0913 (MC)	**0.0019**	**0.0001**	0.3075	0.0882
	6	0.3965 (MC)	**0.0332 (MC)**	0.0559	**0.0005**	**0.0197**	**0.0013 (MC)**	0.4319	**0.0037**
Species richness	5	**0.0006 (MC)**	**0.0077**	0.1728	**0.0246 (MC)**	0.1154	**0.0181**	0.2383	0.1900
	6	0.4331 (MC)	**0.0004 (MC)**	0.6593	**0.0154 (MC)**	0.4650	**0.0043**	0.1555 (MC)	**0.0023**
Barnacle abundance	5	**0.0031 (MC)**	0.2295 (MC)	**0.0007**	**0.0282 (MC)**	0.0839	0.0698 (MC)	**0.0025**	0.3346 (MC)
	6	0.4049 (MC)	0.0561 (MC)	0.2198 (MC)	0.4687	0.0719 (MC)	0.2317 (MC)	0.1399 (MC)	0.3379 (MC)

Table 8.28: SIMPER analyses identifying consistent (SD/Sim ratio >1) indicator species associated with assemblage differences on transplanted limestone (TL) versus transplanted siltstone (TS). Abundances are expressed as the mean number of individuals per boulder surface. Some combinations of seashore/boulder surface/ sampling time are missing, when PERMANOVAs detected no significant differences between rocks.

Measure	Year	Boulder surface	Seashore	Average dissimilarity (%)	Indicator species	TL abundance		TS abundance	Rock preference
Mobile assemblage structure	5	upper	Myponga Beach	98.1	*Bembicium nanum*	1.09	>	0.00	Limestone
					Notoacmea spp.	0.27	<	0.70	Siltstone
			Marino Rocks	97.4	*Bembicium nanum*	1.45	>	0.00	Limestone
					Notoacmea spp.	0.10	<	0.81	Siltstone
	6	upper	Myponga Beach	92.0	*Notoacmea* spp.	0.14	<	2.56	Siltstone
					Bembicium nanum	1.16	>	0.14	Limestone
			Marino Rocks	89.3	*Bembicium nanum*	1.50	>	0.14	Limestone
					Notoacmea spp.	0.40	>	0.14	Limestone
		Lower	Marino Rocks	65.7	*Nerita atramentosa*	1.81	>	0.61	Limestone
					Notoacmea spp.	1.56	>	1.49	Limestone
					Bembicium nanum	0.84	>	0.14	Limestone
Sessile assemblage structure	5	Upper	Myponga Beach	85.3	*Galeolaria caespitosa*	1.63	>	0.22	Limestone
			Marino Rocks	81.1	*Galeolaria caespitosa*	1.91	>	0.41	Limestone
		Lower	Myponga Beach	73.4	*Galeolaria caespitosa*	3.82	>	0.79	Limestone
			Marino Rocks	89.7	*Galeolaria caespitosa*	2.92	>	0.21	Limestone
	6	Upper	Marino Rocks	100.0	*Galeolaria caespitosa*	0.73	>	0.00	Limestone
			Blanche Point	88.1	Biofilm	1.87	>	0.59	Limestone
					Galeolaria caespitosa	1.57	>	0.10	Limestone
		Lower	Myponga Beach	57.6	*Galeolaria caespitosa*	2.30	>	0.75	Limestone
			Marino Rocks	90.4	*Galeolaria caespitosa*	2.23	>	0.25	Limestone
			Blanche Point	80.7	Biofilm	2.11	>	0.28	Limestone
					Galeolaria caespitosa	2.88	>	0.67	Limestone

Table 8.29: *P* values for PERMANOVA planned comparisons testing for assemblage differences between native and transplanted boulders of the same rock. Analyses were completed separately for each seashore, sampling time, and surface. Significant results (α = 0.05) are shown in bold, with MC = *p*-value used from Monte Carlo tests. The total degrees of freedom for each comparison ranged from 15 for surfaces sampled at SP after 6 years to 29 for surfaces sampled at MB, MR and SP after 5 years. MB = Myponga Beach, MR = Marino Rocks, SP = Southport; and BP = Blanche Point.

	Surface	**Upper**				**Lower**			
Measure	**Seashore**	**MB**	**MR**	**SP**	**BP**	**MB**	**MR**	**SP**	**BP**
	Year								
Mobile invertebrate assemblage structure	5	0.1413 (MC)	0.1293 (MC)	0.4667	0.1126	0.5631	0.5152	0.2546	0.8126
	6	**0.0284 (MC)**	0.72656 (MC)	0.5086 (MC)	0.8483	0.0602	0.7089	**0.0373**	0.4018
Sessile assemblage structure	5	0.1222	0.0903	0.4993	0.3497	0.1959	0.1071	0.6472	0.8609
	6	0.3207 (MC)	0.1455 (MC)	0.9327	0.3584	0.0660	0.7013 (MC)	0.9422	0.2093
Barnacle abundance	5	0.1635 (MC)	0.3232	0.1673	0.0971 (MC)	0.3201 (MC)	0.3315 (MC)	**0.0428 (MC)**	0.4784 (MC)
	6	0.0603 (MC)	0.1223 (MC)	**0.0138 (MC**)	0.4671	0.3275 (MC)	0.8733 (MC)	0.2398 (MC)	0.2386 (MC)
Total species richness	5	0.0600 (MC)	0.0538 (MC)	0.7865	0.1491 (MC)	0.7524	0.8169	0.2742	0.4161
	6	0.1414 (MC)	0.6452 (MC)	0.9613 (MC)	0.4999 (MC)	0.3088 (MC)	0.4733 (MC)	**0.0194 (MC)**	**0.0202 (MC)**

Table 8.30: *P* values for PERMANOVAs testing for assemblage differences among sampling times (11 months, 5 years and 6 years) for transplanted limestone versus siltstone across all seashores. Analyses were completed separately for upper versus lower surfaces. Significant results (α = 0.05) are shown in bold. The interaction term Seashore x Rock x Sampling time was excluded from analyses.

	Measure	**Mobile assemblage structure**		**Sessile assemblage structure**		**Species richness**		**Barnacle abundance**	
Source	**df**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
Seashore	3	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Rock	1	**0.0001**	**0.0004**	**0.0288**	0.0877	**0.0289**	0.1130	0.0576	0.063
Sampling time	2	**0.0001**	**0.0004**	0.1034	**0.0026**	0.7999	0.0725	0.5317	0.1047
Seashore x Rock	3	0.0915	0.6488	0.1737	**0.0001**	0.5596	**0.0118**	**0.0048**	**0.0001**
Seashore x Sampling time	6	**0.0006**	**0.0001**	**0.0001**	0.0556	0.1106	**0.0373**	0.7831	0.1612
Rock x Sampling time	2	0.2964	0.2971	**0.0116***	**0.0031**	0.5819	**0.0031***	0.6387	0.3951
Residual	177								

* I detected significant interactions between sampling time and rock for sessile assemblage structure on upper surfaces and species richness on lower surfaces, but the changes over time for either rock were too subtle to be determined via post hoc tests (all PERMANOVA pair-wise permuted *p*-values >0.05).

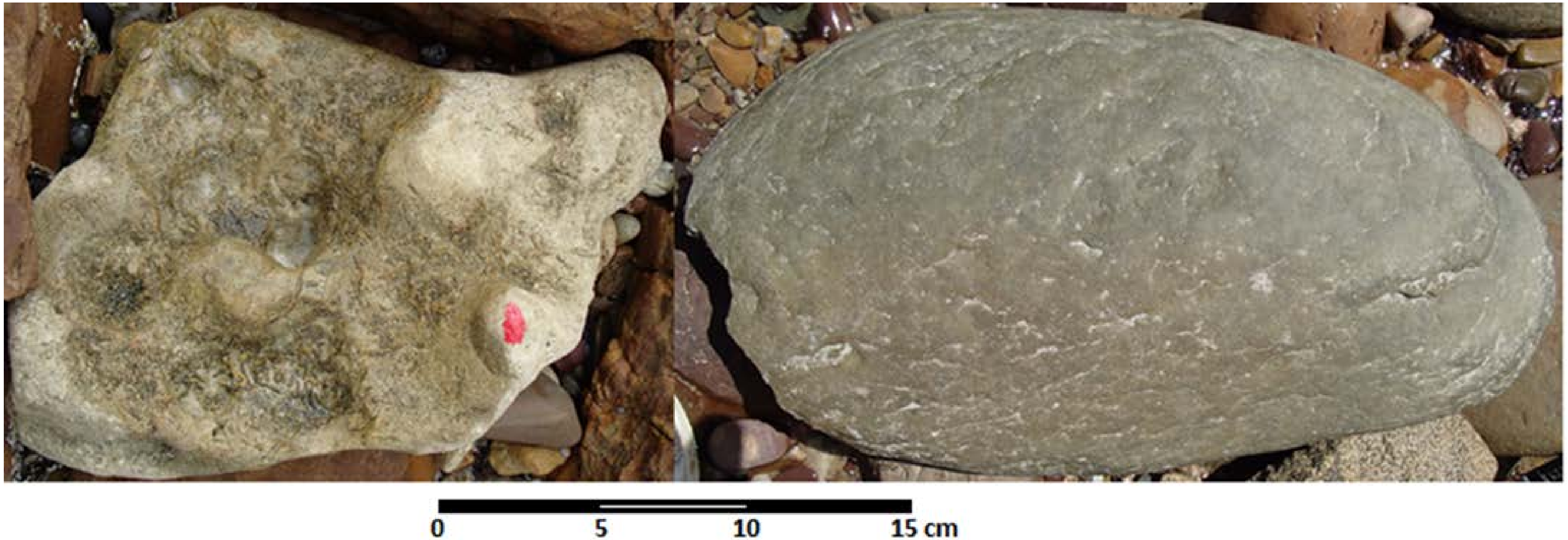


Figure 8.6: Upper surfaces of a transplanted limestone (left image) and transplanted siltstone (right image) boulder sampled at Marino Rocks after six years.

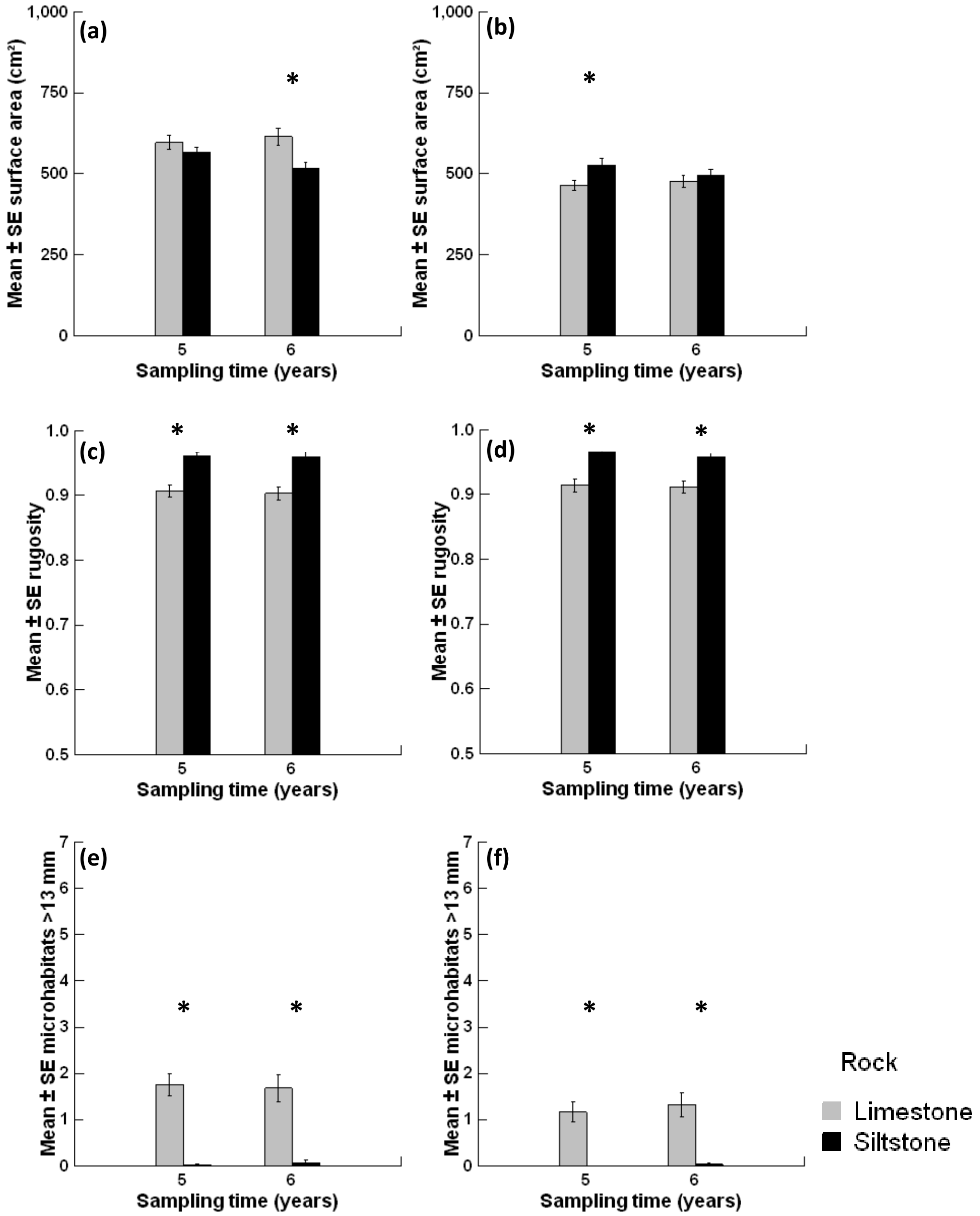


Figure 8.7: Mean ± SE surface area (a-b), rugosity (c-d), and microhabitat >13 mm density (e-f) for the upper (a, c, e) and lower (b, d, f) surfaces of transplanted limestone versus siltstone for each sampling time. Averages calculated from totals of 37 limestone and 40 siltstone boulders sampled after 5 years, and 25 limestone and 27 siltstone boulders sampled after 6 years. Each y-axis extends to encompass the range of the raw data. * = significant (*p* < 0.05) difference detected between rocks

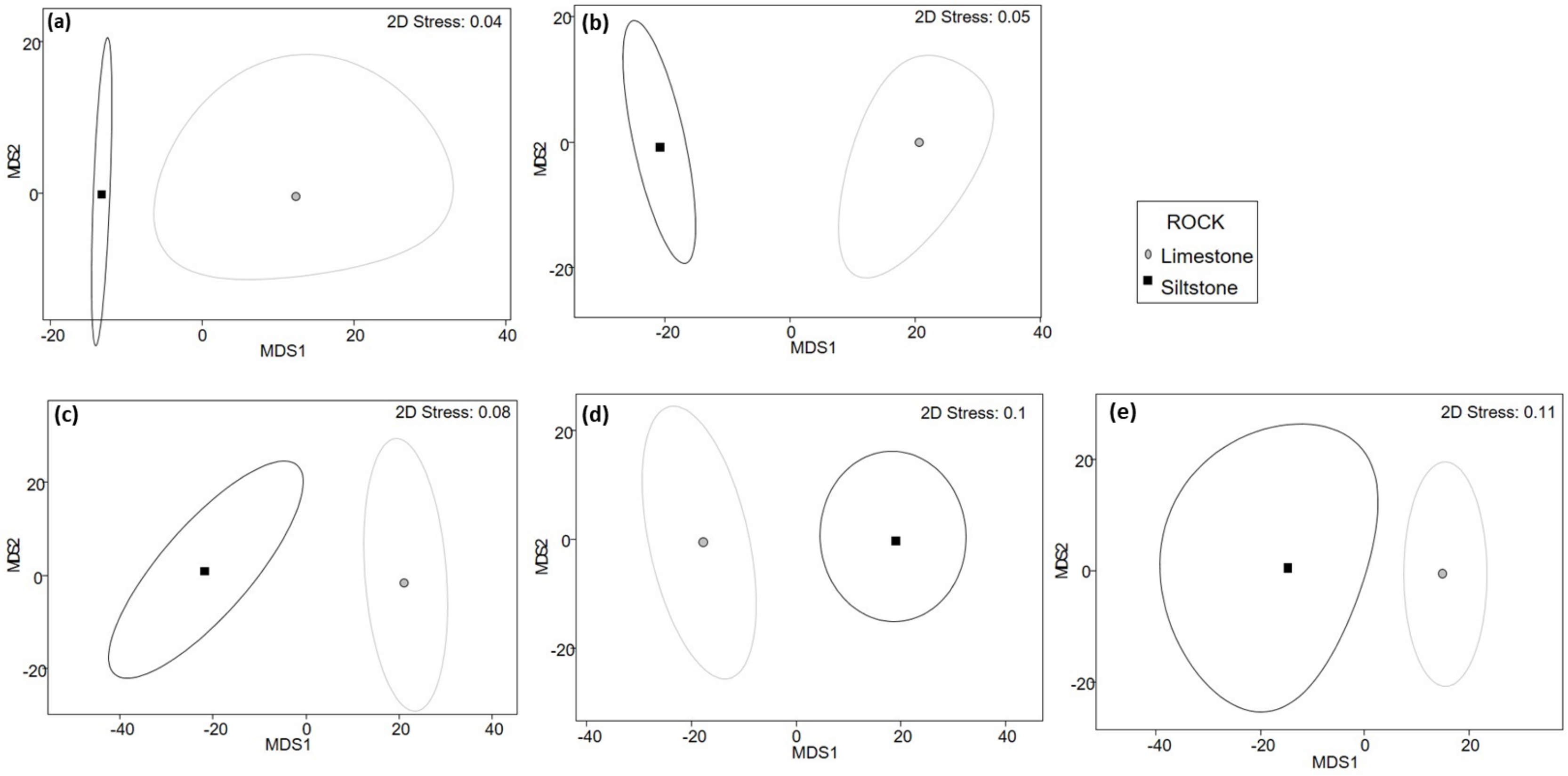


Figure 8.8: Bootstrapped averages ordination plots depicting significantly-divergent mobile invertebrate assemblages detected during PERMANOVA planned comparisons between transplanted limestone versus siltstone: (a) upper surfaces at Myponga Beach after 5 years; (b) upper surfaces at Marino Rocks after 5 years; (c) upper surfaces at Myponga Beach after 6 years; (d) upper surfaces at Marino Rocks after 6 years; (e) lower surfaces at Marino Rocks after 6 years.

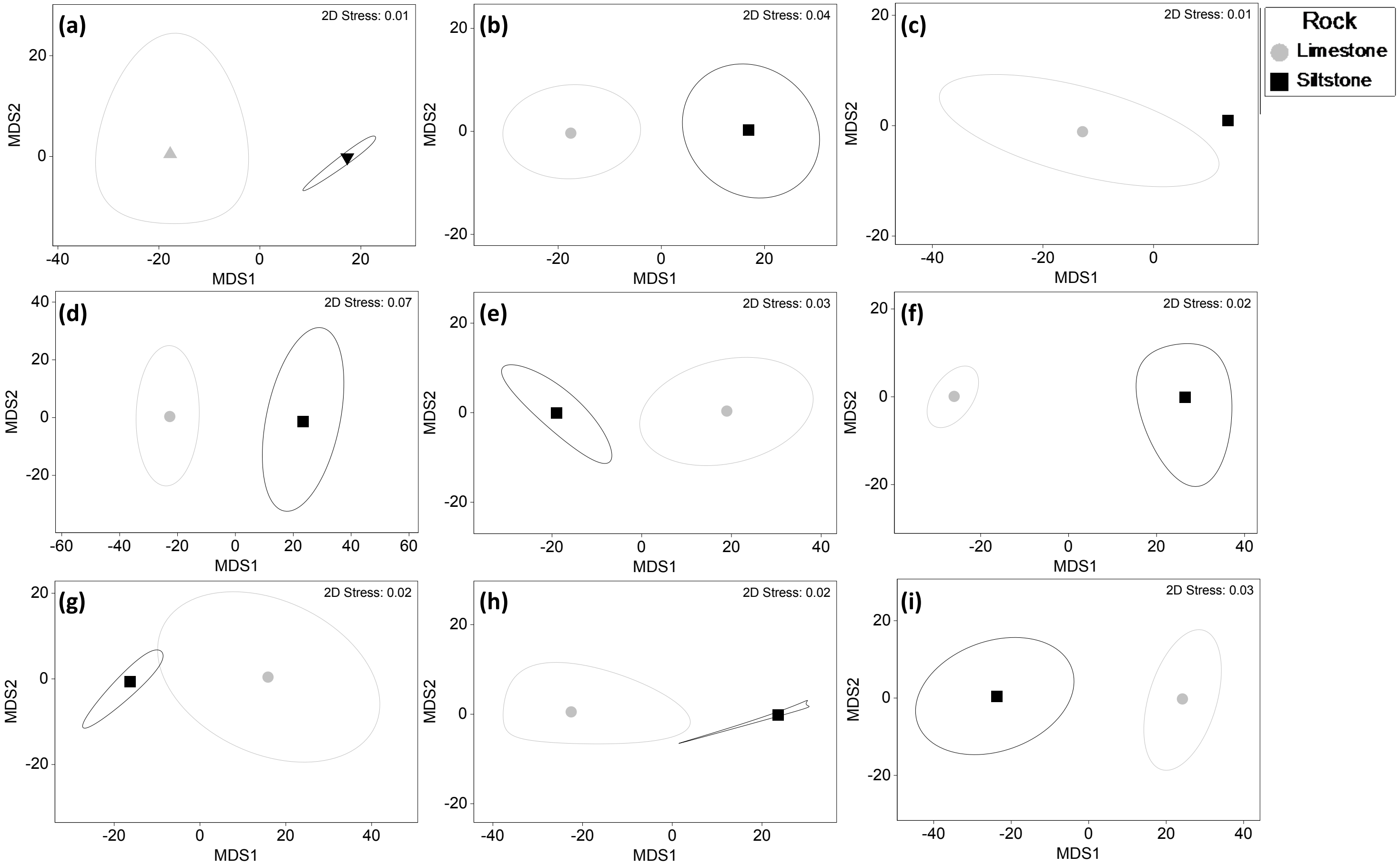


Figure 8.9: Bootstrapped averages ordination plots depicting significantly-divergent sessile assemblages detected during PERMANOVA planned comparisons between transplanted limestone versus siltstone: (a) upper surfaces at Myponga Beach after 5 years; (b) upper surfaces at Marino Rocks after 5 years; (c) upper surfaces at Marino Rocks after 6 years; (d) upper surfaces at Blanche Point after 6 years; (e) lower surfaces at Myponga Beach after 5 years; (f) lower surfaces at Marino Rocks after 5 years; (g) lower surfaces at Myponga Beach after 6 years; (h) lower surfaces at Marino Rocks after 6 years; and (i) lower surfaces at Blanche Point after 6 years.

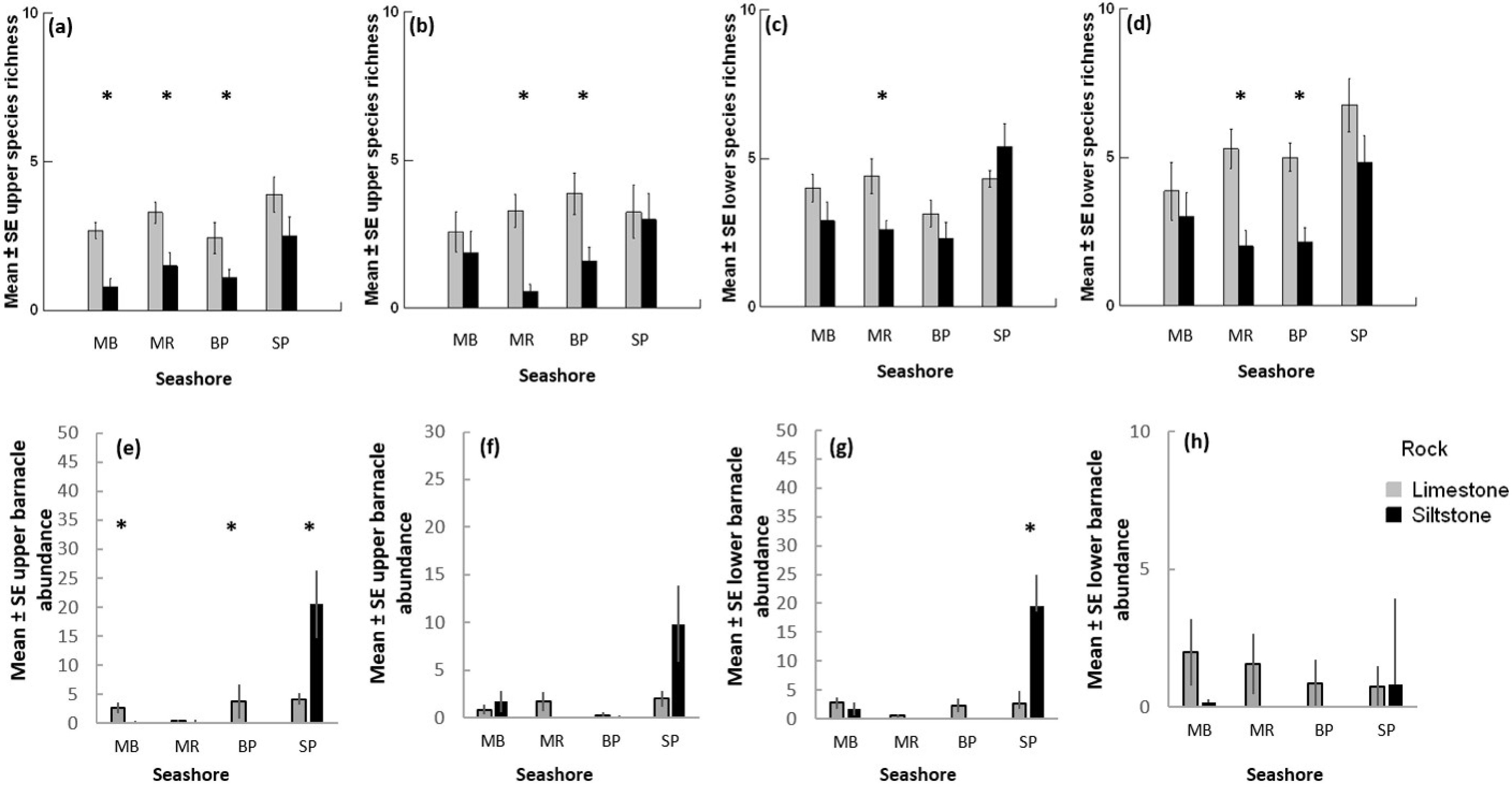


Figure 8.10: Mean ± SE species richness (a-d) and barnacle abundance (e-h) for upper (a,b,e,f) versus lower (c,d,g,h) transplanted boulders for each sampling time: a) 5 years upper species richness; b) 6 years upper species richness; c) 5 years lower species richness; d) 6 years lower species richness; e) 5 years upper barnacle abundance; f) 6 years upper barnacle abundance; g) 5 years lower barnacle abundance; and h) 6 years lower barnacle abundance. Each y-axis extends to encompass the full range of the raw data. MB = Myponga Beach; MR = Marino Rocks; BP = Blanche Point; and SP = Southport. * = significant ($p < 0.05$) difference detected between rocks.

Comparison of boulder assemblages between limestone versus siltstone reefs and representative seashores of these reefs

Several boulder transplant experiments have shown that associations between assemblages and rocks can be scale dependent, with patterns observed at the scale of reefs not applicable at the scale of individual boulders transplanted on those reefs (see McGuinness 1988; Green et al. 2012; Liversage et al. 2014). To assess associations between boulder assemblages and different rocks at the reef-scale, and at representative seashores of those reefs, two-factor PERMANOVA models were used to test ($\alpha = 0.05$) for differences among reef types (limestone versus siltstone; a fixed factor), and seashores nested in reef types (Marino Rocks, Myponga Beach, Southport, or Blanche Point; a random factor). For all PERMANOVA tests, permutations of residuals were completed using a reduced model with 9999 permutations, with a Monte Carlo (MC) p-value replacing a PERMANOVA p-value when the number of available unique permutations was <100 (Anderson et al. 2008). Only transplanted boulders were included in analyses, with PERMANOVAs completed separately for the multivariate measures of mobile or sessile assemblage structure, and for the univariate measures of species richness and barnacle abundance. Separate PERMANOVAs were also completed for upper versus lower surfaces, and for both sampling times. MDS bootstrapped-averages ordination plots were used to visualise significant multivariate differences between seashores.

Assemblage structure was generally similar between siltstone versus limestone reefs, with lower-surface mobile assemblage structure the only significant reef difference (Table 8.31). Limestone reefs usually supported a higher species richness and barnacle abundance than siltstone reefs, for both boulder surfaces and both sampling times, although these differences were never significant (Table 8.31).

The structure and species richness of assemblages was often different among representative seashores of limestone versus siltstone reefs. Mobile invertebrate assemblage structure was significantly different among seashores for all surface and time combinations except upper surfaces after 5 years, while sessile assemblage structure was significantly different among seashores for upper surfaces only (Table 8.31 & Figure 8.11). Generally, the assemblages sampled at limestone seashores were very distinct from each other, while the assemblages sampled at siltstone seashores clustered more closely, and at times overlapped with the assemblages sampled on limestone at Blanche Point (Figure 8.11). Significant differences among seashores within reefs were also detected for the univariate measures of species richness after five years and barnacle abundance in both years (Table 8.31). Generally, boulders sampled at Southport supported assemblages with the highest species richness and barnacle abundance (Figure 8.12). I postulate that the very distinct assemblages recorded at Southport may be attributed to that locations low relief, such that boulders might not be exposed to stressful conditions for as long during hot daytime summer low tides compared to the other seashores sampled. Thus the boulders at Southport may be able to support more species in greater abundances.

Table 8.31: PERMANOVA tests for assemblage differences among the factors of reef type (R) and seashores nested within reef types (S(R)). Analyses completed separately for upper versus lower surfaces, and for the 5 (total *df* = 116) and 6 year (total *df* = 78) sampling times. Significant results (α = 0.05) are shown in bold, with MC = *p*-value used from Monte Carlo tests

		Surface:	**Upper**		**Lower**	
Measure	**Years**	**Factor**	**Pseudo-*F***	***p*-value**	**Pseudo-*F***	***p*-value**
Mobile invertebrate assemblage structure	5	R	1.13	0.4024	4.99	**0.016 (MC)**
		S(R)	1.02	0.4433	3.29	**0.0011**
	6	R	1.25	0.2896	1.46	0.2879 (MC)
		S(R)	2.56	**0.0002**	6.17	**0.0001**
Sessile assemblage structure	5	R	0.35	0.77 (MC)	1.12	0.41 (MC)
		S(R)	7.26	**0.0001**	1.64	0.17
	6	R	3.22	0.09 (MC)	1.62	0.29 (MC)
		S(R)	3.79	**0.0005**	2.38	0.05
Species richness	5	R	0.18	0.72 (MC)	0.06	0.83 (MC)
		S(R)	5.75	**0.0063**	8.13	**0.0009**
	6	R	12.04	0.07 (MC)	1.16	0.39 (MC)
		S(R)	0.24	0.78	2.88	0.07
Barnacle abundance	5	R	0.19	0.8012	0.37	0.7103 (MC)
		S(R)	3.98	**0.0027**	5.97	**0.0007**
	6	R	1.18	0.2849	0.58	0.5852 (MC)
		S(R)	3.08	**0.0057**	3.43	**0.0152**

Table 8.32: *P*-values from PERMANOVA tests for assemblage differences for the composite factor boulder history (transplanted limestone, transplanted siltstone, native limestone, and native siltstone). Analyses were completed separately for each seashore, sampling time, and boulder surface. Significant results (α = 0.05) are shown in bold. The total degrees of freedom for each comparison ranged from 15 for surfaces sampled at SP after 6 years to 29 for surfaces sampled at MB, MR and SP after 5 years. MB = Myponga Beach, MR = Marino Rocks, SP = Southport and BP = Blanche Point.

	Surface	**Upper**				**Lower**			
	Shore	**MB**	**MR**	**SP**	**BP**	**MB**	**MR**	**SP**	**BP**
Measure	**Years**								
Mobile invertebrate assemblage structure	5	0.0799	**0.0001**	2311	0.0817	0.9235	0.275	**0.0485**	0.0525
	6	**0.0007**	**0.0004**	0.8812	0.4916	0.2263	0.1089	0.0686	0.7274
Sessile assemblage structure	5	**0.0021**	**0.0001**	0.07	**0.0195**	**0.0044**	**0.0001**	0.80	0.28
	6	0.26	**0.0193**	0.08	**0.0124**	0.05	**0.0008**	0.71	**0.0063**
Species richness	5	**0.0017**	**0.0004**	0.12	0.06	0.19	**0.0405**	0.12	0.32
	6	0.07	**0.0033**	0.86	**0.0331**	0.22	**0.0016**	0.05	**0.0028**
Barnacle abundance	5	**0.0099**	0.0822 (MC)	**0.0014**	**0.0355**	**0.0361**	0.1627	**0.0001**	0.2264
	6	0.1292 (MC)	0.1772 (MC)	**0.0146**	0.5687	0.0715 (MC)	0.2437 (MC)	**0.0079**	0.2124 (MC)

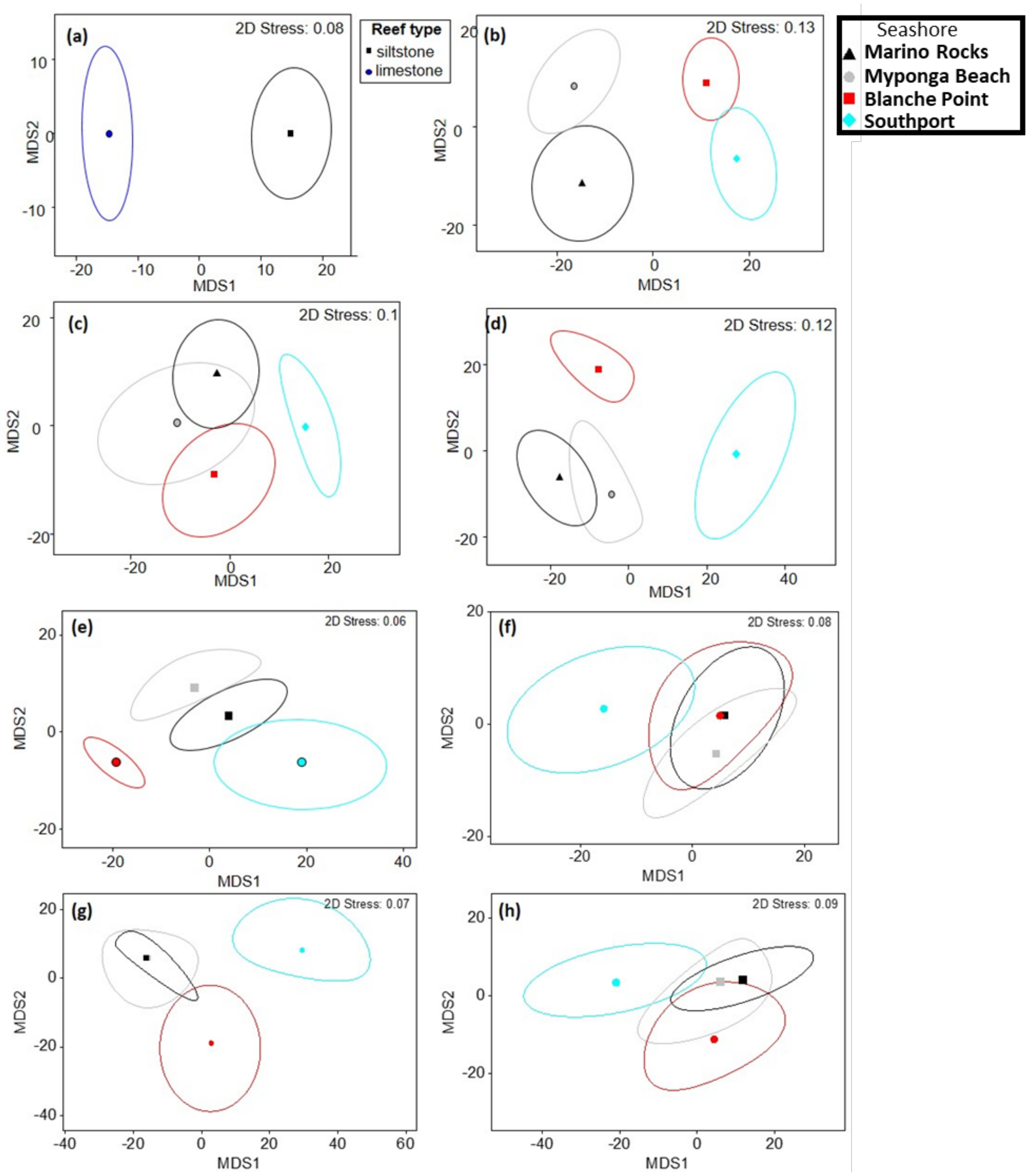


Figure 8.11: Bootstrapped-averages ordination plots depicting significant differences in mobile assemblages (a-d) and sessile assemblages (e-h) between (a) reef types for lower surfaces after 5 years; and sites for (b) lower surfaces after 5 years; (c) upper surfaces after 6 years; (d) lower surfaces after 6 years; (e) upper surfaces after 5 years; (f) lower surfaces after 5 years; (g) upper surfaces after 6 years; and (h) lower surfaces after 6 years.

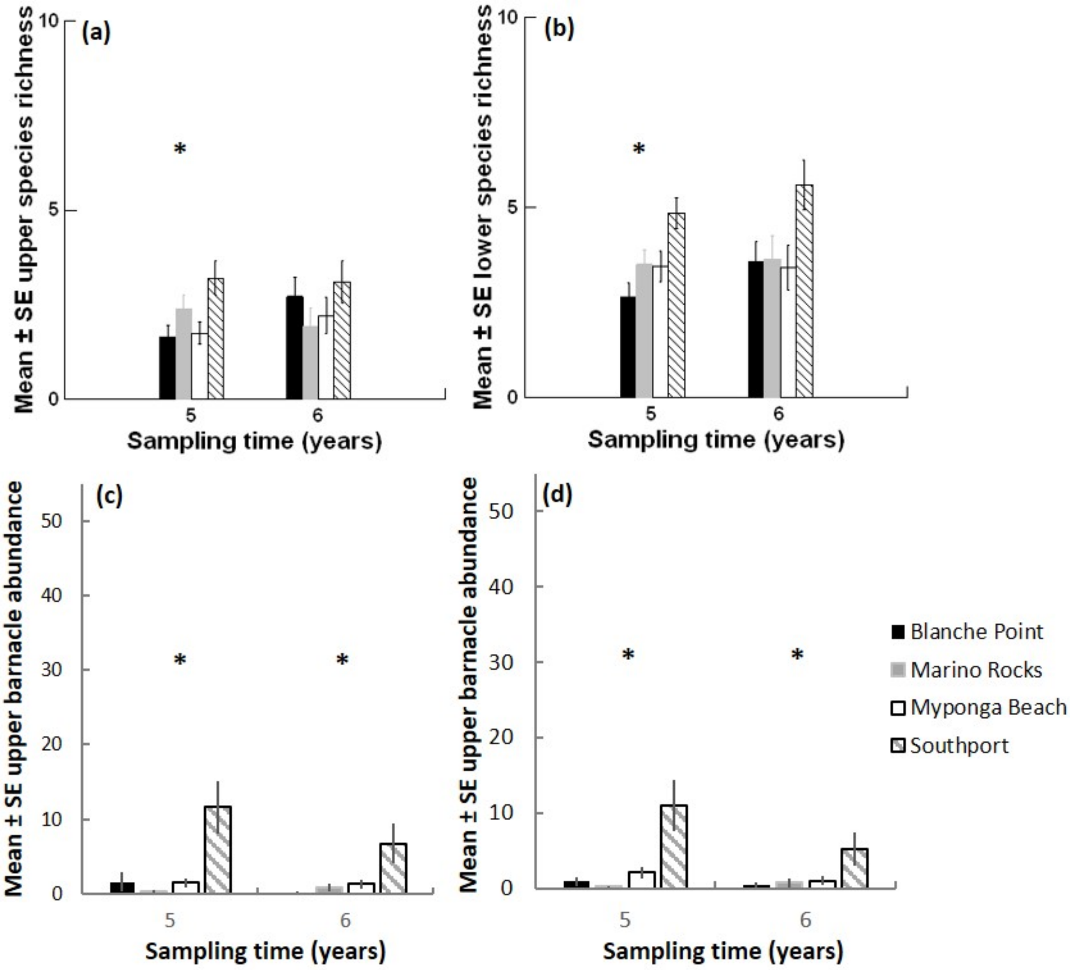


Figure 8.12: Mean ± standard error (SE) species richness (a-b) and barnacle abundance (c-d) for transplanted boulders sampled at each seashore for upper (a, c) and lower (b, d) surfaces, and both sampling times. The range displayed on each y-axis extends to encompass the range of the raw data. * = significant difference detected amongst seashores.

Appendix 3: Chapter 4

Thermal imagery and false-colour scales

Thermal imagery captures the amount of infrared energy (i.e. heat) emitted by objects in an image. When a false-colour scale is applied to each image, differences in the amount of infrared energy emitted by objects are represented by the different colours that appear (Figure 4.3). This false-colour scale, which is scaled separately for a range on each image, consists of eight distinct colours from black (coldest) to white (hottest). Black, purple, blue and green colours represent objects emitting less infrared energy and are thus cooler in temperature, while yellow, orange, red and white represent objects emitting more infrared energy and are thus hotter in temperature (Figure 4.3).

Minimum temperatures

Minima behaved similarly to maxima over four hours exposure with the same trends identified for weather condition (hotter minima on sunny days) and exposure time (increased with time exposed and peaked at four hours). After four hours, the hottest minimum recorded was 50.9 °C (sunny day, air temperature = 39 °C) for the upper surface of grey siltstone while the coolest minimum was 7.0 °C (sunny day, air temperature = 12 °C) for the lower surface of fossiliferous sandstone. Over four hours, increases in minimum surface temperature of >20 °C were recorded for some rocks on several days, with the greatest increases recorded for upper surfaces on sunny days. When rocks were ranked from hottest to coolest for mean minimum temperature after four hours, the same consistent ranking identified for maxima was observed (Table 8.34). The rock rank order for maxima and minima was significantly correlated for both upper (Spearman's Rho = 1.00, *p*-value < 0.01) and lower (Spearman's Rho = 0.94, p-value < 0.01) surfaces. Therefore, the lower surfaces of white limestone had the coolest temperatures of the six rocks investigated. These coolest areas on white limestone potentially offer intertidal biota the greatest refuge from extreme heat on sunny days with the hottest air temperatures. The rank order of rocks according to changes in minima over four hours conformed to the consistent rankings identified elsewhere, with the two siltstones having the largest overall increases and white limestone and quartzite generally having the smallest (Table 8.34).

Table 8.33: Frequencies of occurrence (%) for the three patterns of temperature difference identified on boulder upper and lower surfaces, for each rock, for a subset of the cloudy and sunny days sampled.

				Rock:	Purple siltstone	Grey siltstone	Quartzite	Fossiliferous sandstone	Orange limestone	White limestone	Total
Weather	Date	Maximum air temperature (°C)	Surface	Temperature pattern							
Cloudy	09/09/2015	15	Upper	Mosaic	0	0	0	0	0	0	0
				Gradient	0	0	0	0	0	0	0
				Limited heterogeneity	100	100	100	100	100	100	100
			Lower	Mosaic	0	0	0	0	0	0	0
				Gradient	0	0	0	0	0	0	0
				Limited heterogeneity	100	100	100	100	100	100	100
	25/11/2015	30	Upper	Mosaic	0	0	0	0	0	0	0
				Gradient	0	0	0	0	0	0	0
				Limited heterogeneity	100	100	100	100	100	100	100
			Lower	Mosaic	0	0	0	0	0	0	0
				Gradient	0	0	0	0	0	0	0
				Limited heterogeneity	100	100	100	100	100	100	100
	19/12/2015	38	Upper	Mosaic	0	0	0	0	0	0	0
				Gradient	0	0	0	0	0	0	0
				Limited heterogeneity	100	100	100	100	100	100	100
			Lower	Mosaic	0	0	0	0	0	0	0
				Gradient	0	0	0	16.7	0	16.7	5.6
				Limited heterogeneity	100	100	100	83.3	100	83.3	94.4
Sunny	16/07/2016	12	Upper	Mosaic	0	0	83.3	0	0	0	13.9
				Gradient	100	100	16.7	100	100	100	86.1
				Limited heterogeneity	0	0	0	0	0	0	0
			Lower	Mosaic	0	0	83.3	0	0	0	13.9

				Rock:	Purple siltstone	Grey siltstone	Quartzite	Fossiliferous sandstone	Orange limestone	White limestone	Total
Weather	Date	Maximum air temperature (°C)	Surface	Temperature pattern							
				Gradient	83.3	100	16.7	100	83.3	100	80.5
				Limited heterogeneity	16.7	0	0	0	16.7	0	5.6
	07/02/2016	29	Upper	Mosaic	0	0	83.3	33.3	0	16.7	22.2
				Gradient	100	100	16.7	66.7	100	66.6	75
				Limited heterogeneity	0	0	0	0	0	16.7	2.8
			Lower	Mosaic	0	0	83.3	33.3	33.3	0	25
				Gradient	100	100	16.7	66.7	66.7	100	75
				Limited heterogeneity	0	0	0	0	0	0	0
	18/11/2015	40	Upper	Mosaic	0	0	16.7	0	0	0	2.8
				Gradient	33.3	50	0	16.7	0	16.7	19.4
				Limited heterogeneity	66.7	50	83.3	83.3	100	83.3	77.8
			Lower	Mosaic	0	0	33.3	0	0	0	5.6
				Gradient	33.3	16.7	0	66.7	33.3	50	33.3
				Limited heterogeneity	66.7	83.3	66.6	33.3	66.7	50	61.1

Table 8.34: The daily rank order of rocks from largest to smallest (6 = largest, 1 = smallest) minimum temperature after four hours and change in minimum temperature over four hours for upper and lower surfaces. The rank sum (sum of daily ranks) was used to assign an overall rank to each rock from largest to smallest (6 = largest value, 1 = smallest).

	Surface	Upper								Lower							
	Daily rank	6	5	4	3	2	1	Rank sum	**Overall rock rank**	6	5	4	3	2	1	Rank sum	**Overall rock rank**
Measure	Rock																
Minimum temperature after four hours	Purple siltstone	14	3	0	0	0	0	99	**6**	14	3	0	0	0	0	99	**6**
	Grey siltstone	3	14	0	0	0	0	88	**5**	3	14	0	0	0	0	88	**5**
	Fossiliferous sandstone	0	0	13	4	0	0	64	**4**	0	0	16	1	0	0	67	**4**
	Orange limestone	0	0	4	13	0	0	55	**3**	0	0	1	12	4	0	48	**3**
	White limestone	0	0	0	0	14	3	31	**2**	0	0	0	0	4	13	21	**1**
	Quartzite	0	0	0	0	3	14	20	**1**	0	0	0	4	9	4	34	**2**
Change in minimum temperature over four hours	Purple siltstone	13	3	0	1	0	0	96	**6**	12	5	0	0	0	0	97	**6**
	Grey siltstone	3	9	4	0	0	1	80	**5**	5	12	0	0	0	0	90	**5**
	Fossiliferous sandstone	0	4	10	2	1	0	68	**4**	0	0	8	4	5	0	54	**4**
	Orange limestone	0	0	2	12	2	1	49	**3**	0	0	3	6	5	3	43	**2**
	White limestone	1	0	0	1	9	6	33	**2**	0	0	1	1	4	11	26	**1**
	Quartzite	0	1	1	1	5	9	31	**1**	0	0	5	6	3	3	47	**3**

Table 8.35: Major mineral content (%, mean ± SE) of six seashore rocks. The dominant major mineral in each rock is bolded.

Major mineral	Chemical formula	Grey siltstone	Purple siltstone	Quartzite	Fossiliferous sandstone	White limestone	Orange limestone
Silicon dioxide	SiO_2	**55.4 ± 6.6**	**71.2 ± 3.8**	**96.6 ± 0.1**	**44.0 ± 4.7**	**71.6 ± 3.1**	11.3 ± 2.3
Calcium oxide	CaO	12.0 ± 4.2	1.9 ± 0.2	0 ± 0	24.5 ± 0.6	12.3 ± 1.8	**46.9 ± 1.4**
Aluminium oxide	Al_2O_3	8.8 ± 0.6	8.2 ± 1.6	0.7 ± 0.1	1.1 ± 0.1	1.2 ± 0.3	0.7 ± 0.1
Iron oxide	Fe_2O_3	4.1 ± 0.6	7.3 ± 1.7	0.3 ± 0.1	3.1 ± 0.1	0.9 ± 0.1	2.1 ± 0.2
Sodium oxide	Na_2O	1.3 ± 0.4	2.9 ± 1.1	0.1 ± 0	0.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.1
Titanium dioxide	TiO_2	0.6 ± 0.1	2.0 ± 0.6	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Magnesium oxide	MgO	3.3 ± 0.1	1.6 ± 0.1	0.1 ± 0	3.0 ± 1.9	0.6 ± 0.1	1.0 ± 0.1
Potassium oxide	K_2O	2.1 ± 0.1	0.9 ± 0.2	0 ± 0	0.4 ± 0	0.5 ± 0.1	0.3 ± 0.1
Manganese oxide	MnO_2	0.1 ± 0	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Phosphorus pentoxide	P_4O_{10}	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Sulfur trioxide	SO_3	0 ± 0	0.1 ± 0.1	0 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0

Table 8.36: Trace element content (parts per million, mean ± SE) of six seashore rocks. Silver, molybdenum, hafnium, antimony, selenium, tin, tantalum and tellurium were also detected but were too small to be quantitatively measured. The dominant trace element in each rock is bolded.

Element	Grey siltstone	Purple siltstone	Quartzite	Fossiliferous sandstone	White limestone	Orange limestone
Chlorine	192 ± 105	1 ± 0	116 ± 6	**1526 ± 887**	**4884 ± 882**	**3687 ± 1041**
Barium	288 ± 32	**1798 ± 1358**	185 ± 30	62 ± 8	42 ± 3	54 ± 2
Manganese	**622 ± 77**	852 ± 133	7 ± 3	133 ± 13	30 ± 11	255 ± 80
Zirconium	198 ± 13	235 ± 39	23 ± 1	126 ± 24	76 ± 11	52 ± 6
Strontium	145 ± 46	78 ± 22	25 ± 5	265 ± 125	157 ± 16	320 ± 64
Cobalt	66 ± 30	155 ± 66	**286 ± 131**	24 ± 4	46 ± 22	11 ± 6
Copper	35 ± 17	115 ± 57	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Vanadium	84 ± 19	83 ± 8	3 ± 2	104 ± 6	16 ± 2	20 ± 2
Rubidium	79 ± 5	37 ± 8	2 ± 1	13 ± 1	17 ± 3	12 ± 2
Chromium	52 ± 6	28 ± 5	16 ± 10	45 ± 6	18 ± 3	12 ± 2
Zinc	68 ± 9	37 ± 15	1 ± 0	7 ± 1	6 ± 1	6 ± 1
Arsenic	2 ± 1	7 ± 1	3 ± 1	55 ± 3	6 ± 1	20 ± 1
Bismuth	2 ± 1	3 ± 1	2 ± 1	2 ± 1	2 ± 1	1 ± 0
Bromine	1 ± 0	1 ± 0	1 ± 0	4 ± 2	11 ± 2	7 ± 1
Cadmium	2 ± 1	3 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1
Cerium	39 ± 5	32 ± 7	1 ± 0	16 ± 8	1 ± 0	7 ± 6
Caesium	7 ± 6	1 ± 0	1 ± 0	8 ± 4	1 ± 0	11 ± 6
Gallium	12 ± 1	9 ± 1	2 ± 0	1 ± 0	3 ± 1	3 ± 0
Germanium	2 ± 0	3 ± 0	2 ± 0	1 ± 0	1 ± 0	1 ± 0
Iodine	1 ± 0	1 ± 0	1 ± 0	23 ± 6	1 ± 0	14 ± 4
Lanthanum	17 ± 9	6 ± 5	7 ± 6	1 ± 0	1 ± 0	1 ± 0
Niobium	9 ± 1	18 ± 4	1 ± 0	3 ± 0	2 ± 1	2 ± 0
Neodymium	20 ± 3	15 ± 8	8 ± 4	4 ± 3	4 ± 3	7 ± 6
Nickel	9 ± 3	7 ± 6	1 ± 0	14 ± 13	1 ± 0	1 ± 0
Lead	7 ± 1	9 ± 2	4 ± 2	3 ± 1	4 ± 0	3 ± 0
Scandium	3 ± 2	5 ± 2	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Samarium	1 ± 0	5 ± 4	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Thorium	18 ± 1	16 ± 2	10 ± 0	13 ± 0	10 ± 0	11 ± 1
Thallium	8 ± 1	8 ± 1	8 ± 2	7 ± 1	6 ± 1	7 ± 1
Uranium	6 ± 2	3 ± 0	2 ± 1	5 ± 1	5 ± 0	9 ± 1
Ytterbium	1 ± 0	7 ± 3	12 ± 1	1 ± 0	1 ± 0	1 ± 0
Yttrium	21 ± 2	25 ± 5	14 ± 1	4 ± 0	4 ± 0	3 ± 0

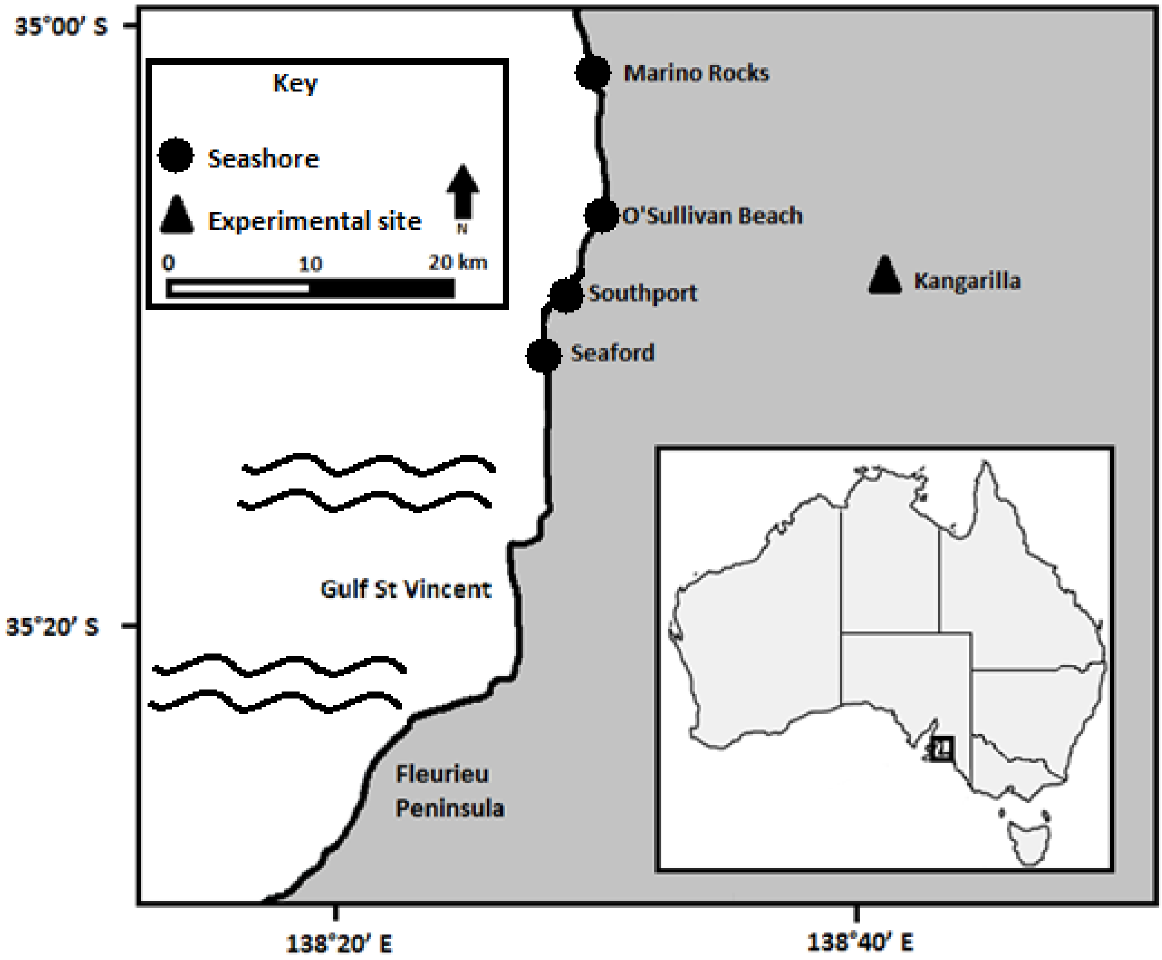


Figure 8.13: Four seashores where rocks were collected from South Australia's Fleurieu Peninsula, and the inland location, Kangarilla, where the common-garden experiment was completed. Purple or grey siltstone was collected from Marino Rocks, quartzite from O'Sullivan Beach, white or orange fossiliferous limestone from Southport and fossiliferous sandstone from Seaford. Inset map shows the location of the study region within Australia.

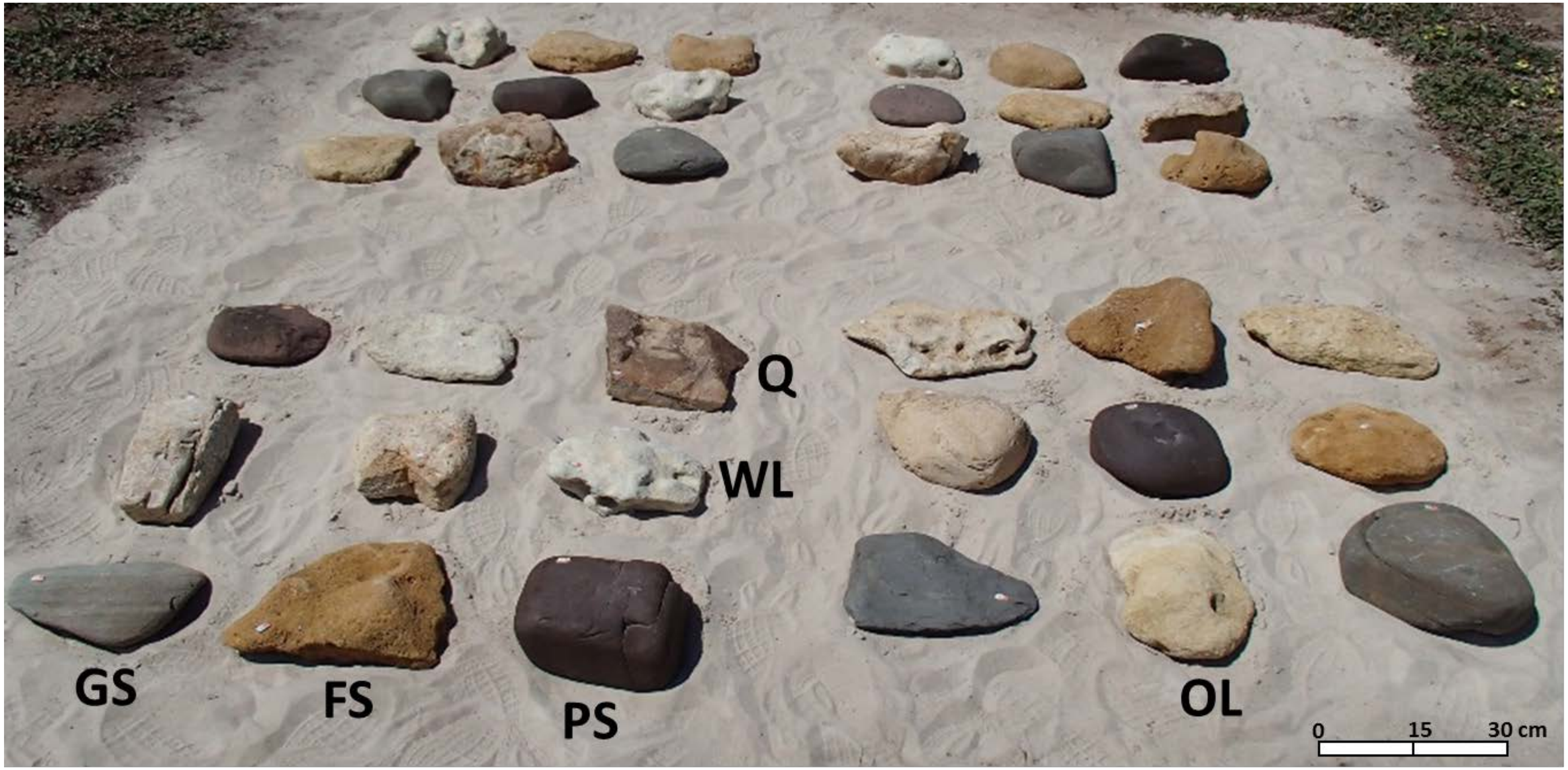


Figure 8.14: The six rocks in the boulder plot of the common-garden experiment constructed in a paddock at Kangarilla. Q = quartzite; WL = white limestone; GS = grey siltstone; FS = fossiliferous sandstone; PS = purple siltstone; and OL = orange limestone.

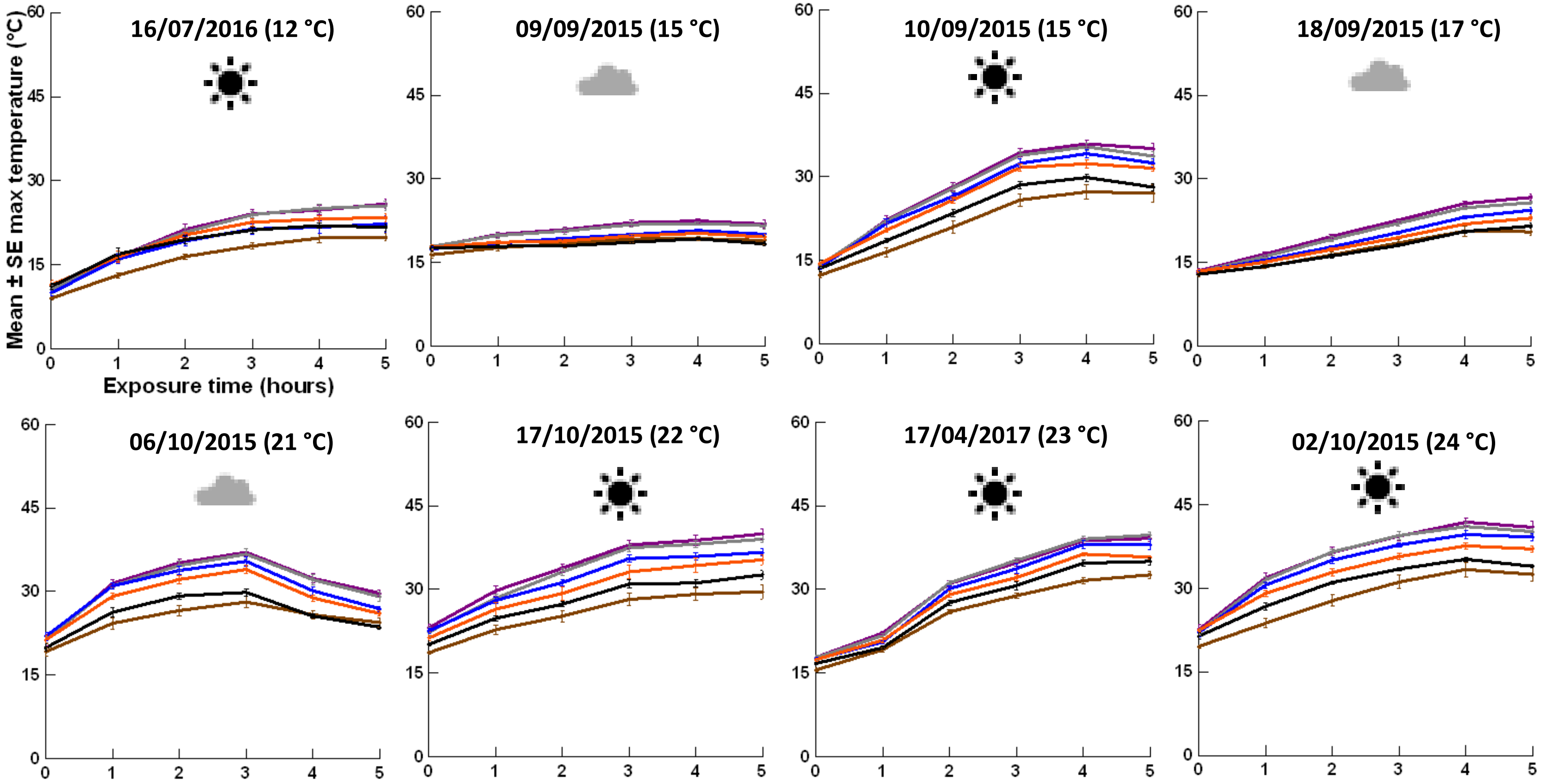


Figure 8.15: Mean ± SE maximum upper surface temperature for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

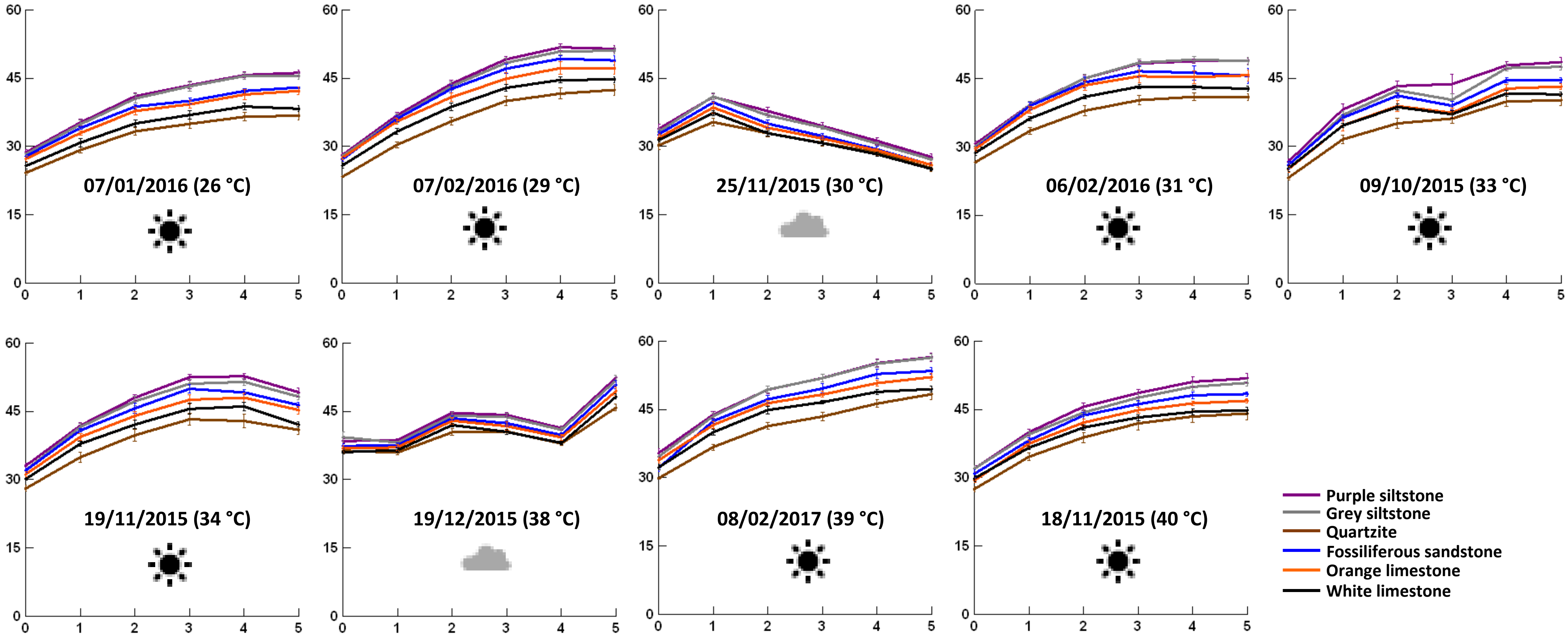


Figure 8.15 (continued): Mean ± SE maximum upper surface temperature for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

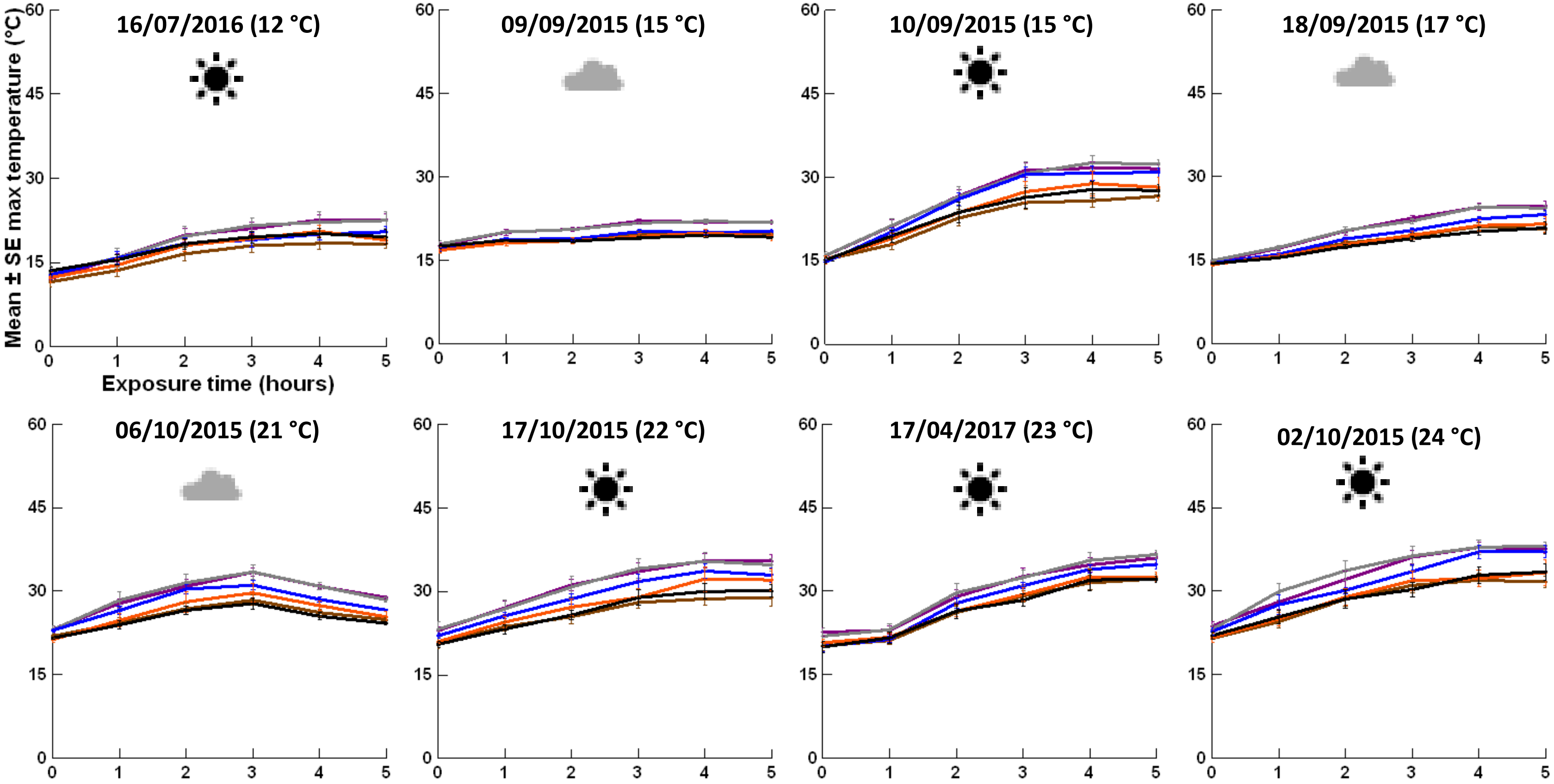


Figure 8.16: Mean ± SE maximum lower surface temperature for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

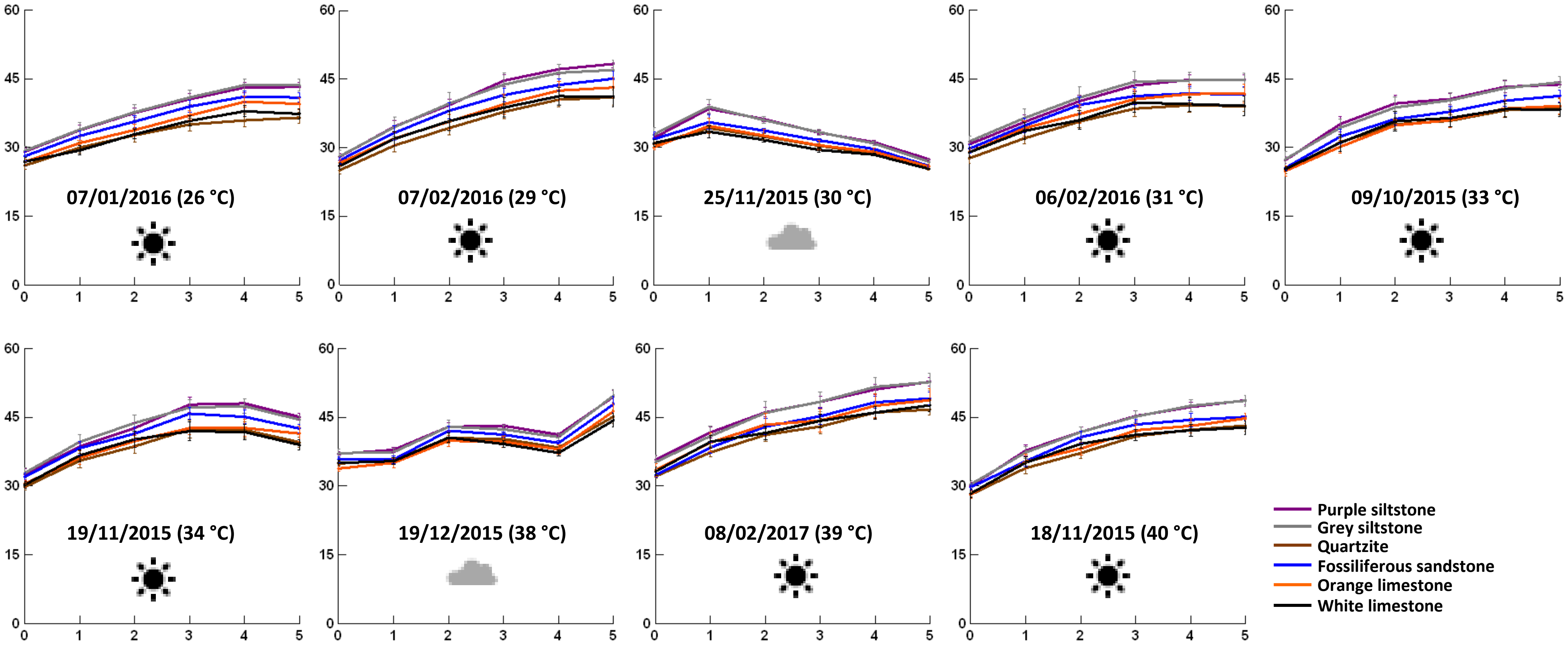


Figure 8.16 (continued): Mean ± SE maximum lower surface temperature for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

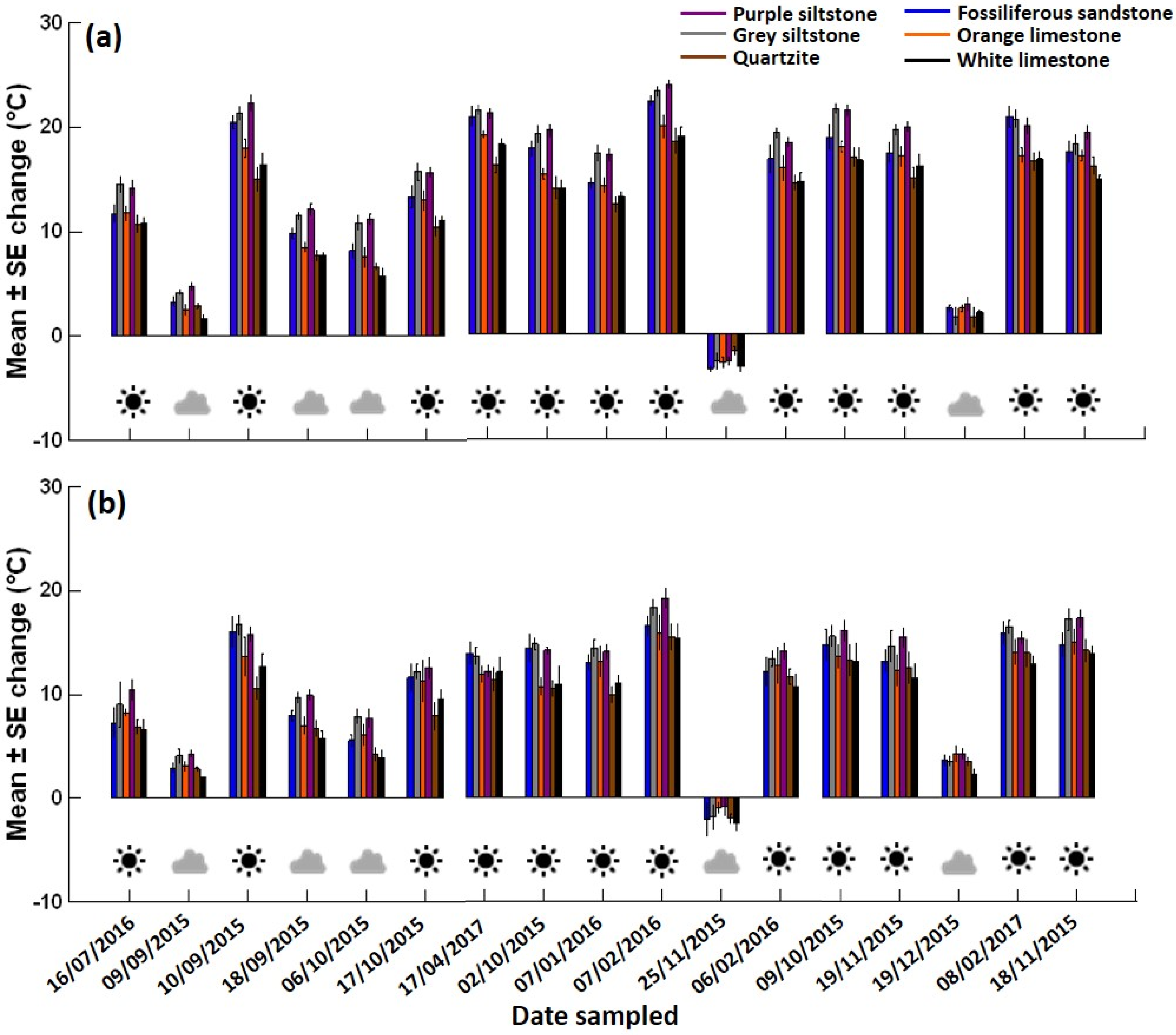


Figure 8.17: Change over four hours in maxima for each rock on each day for (a) upper and (b) lower surfaces. Dates on the x-axis are arranged from coolest to hottest maximum air temperatures moving from left to right.

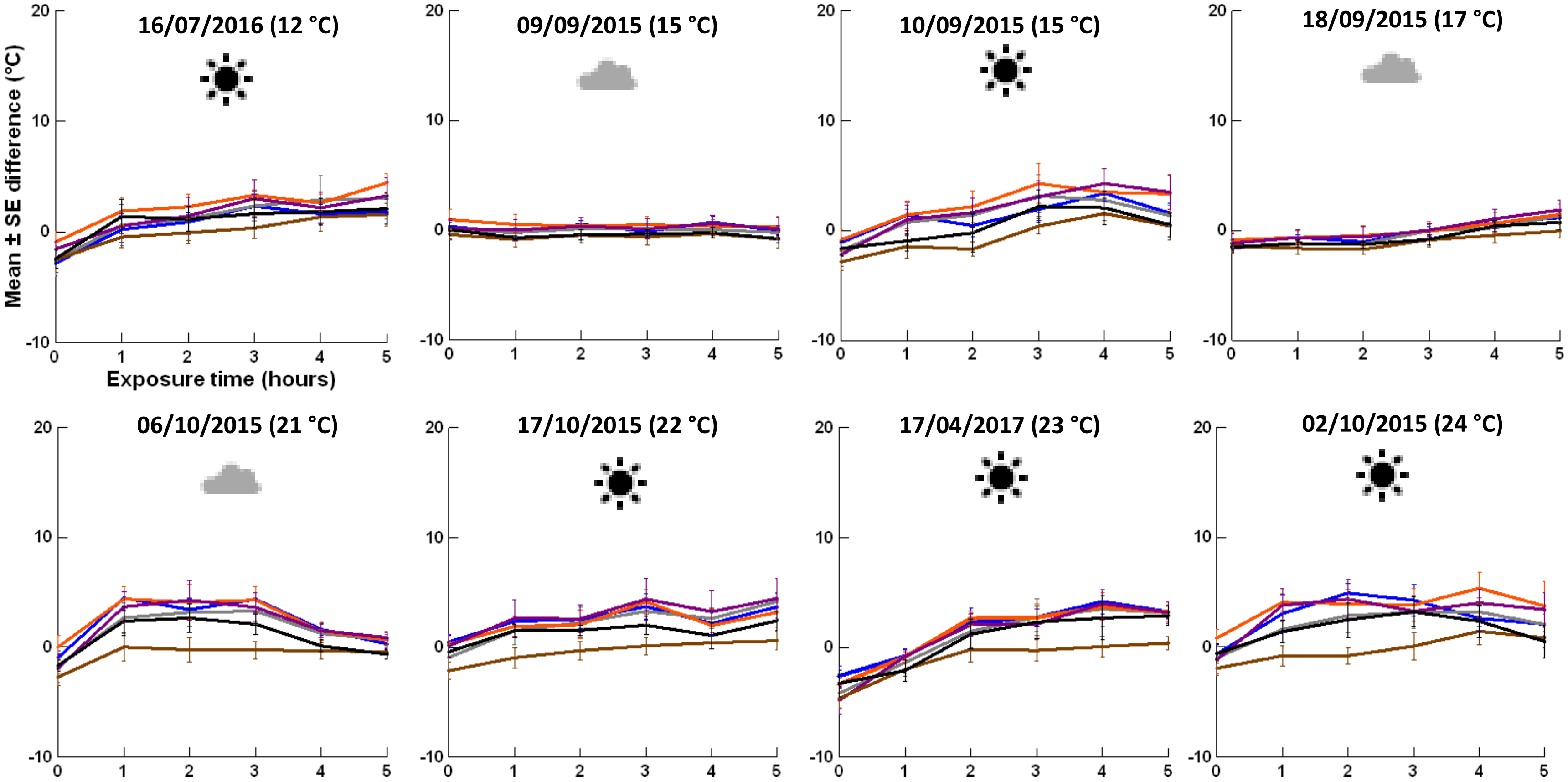


Figure 8.18: Mean ± SE maxima difference between boulder upper and lower surfaces for 6 rocks (n = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

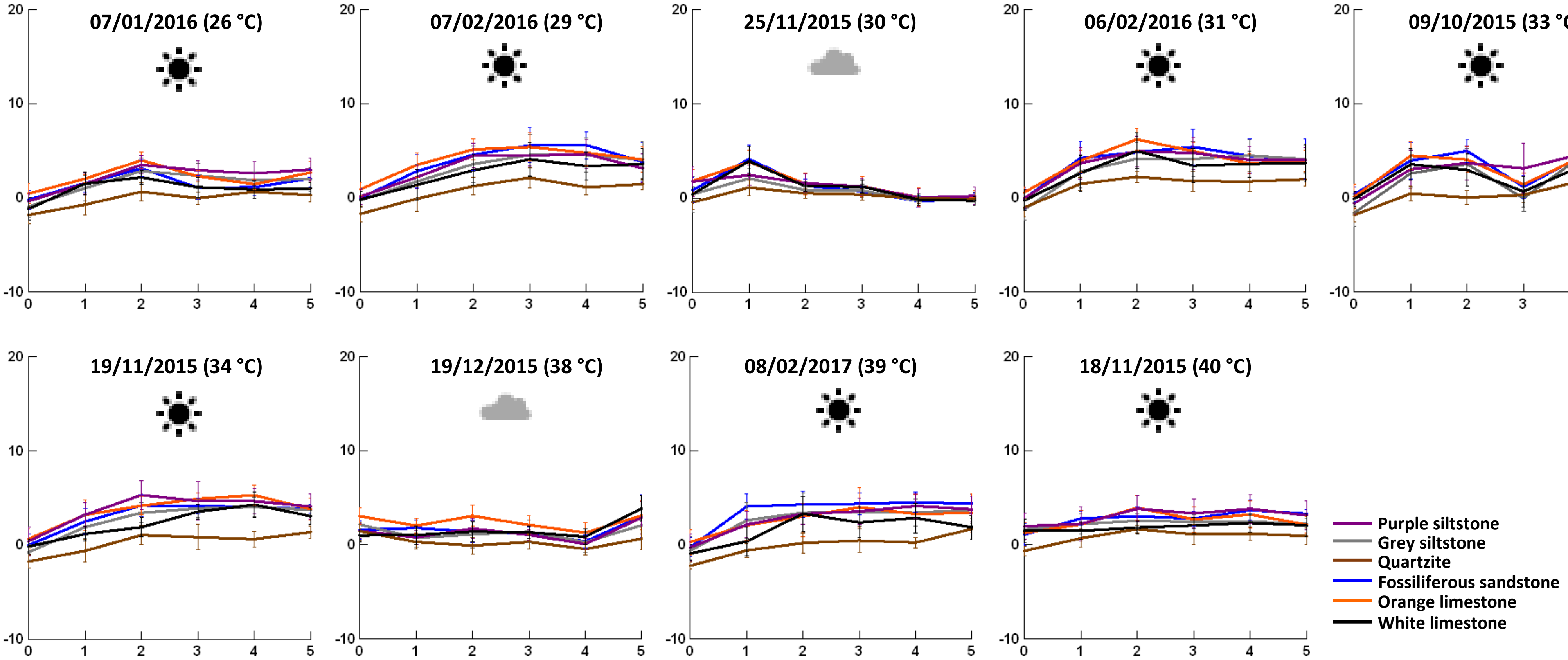


Figure 8.18 (continued): Mean ± SE maxima difference between boulder upper and lower surfaces for 6 rocks (n = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

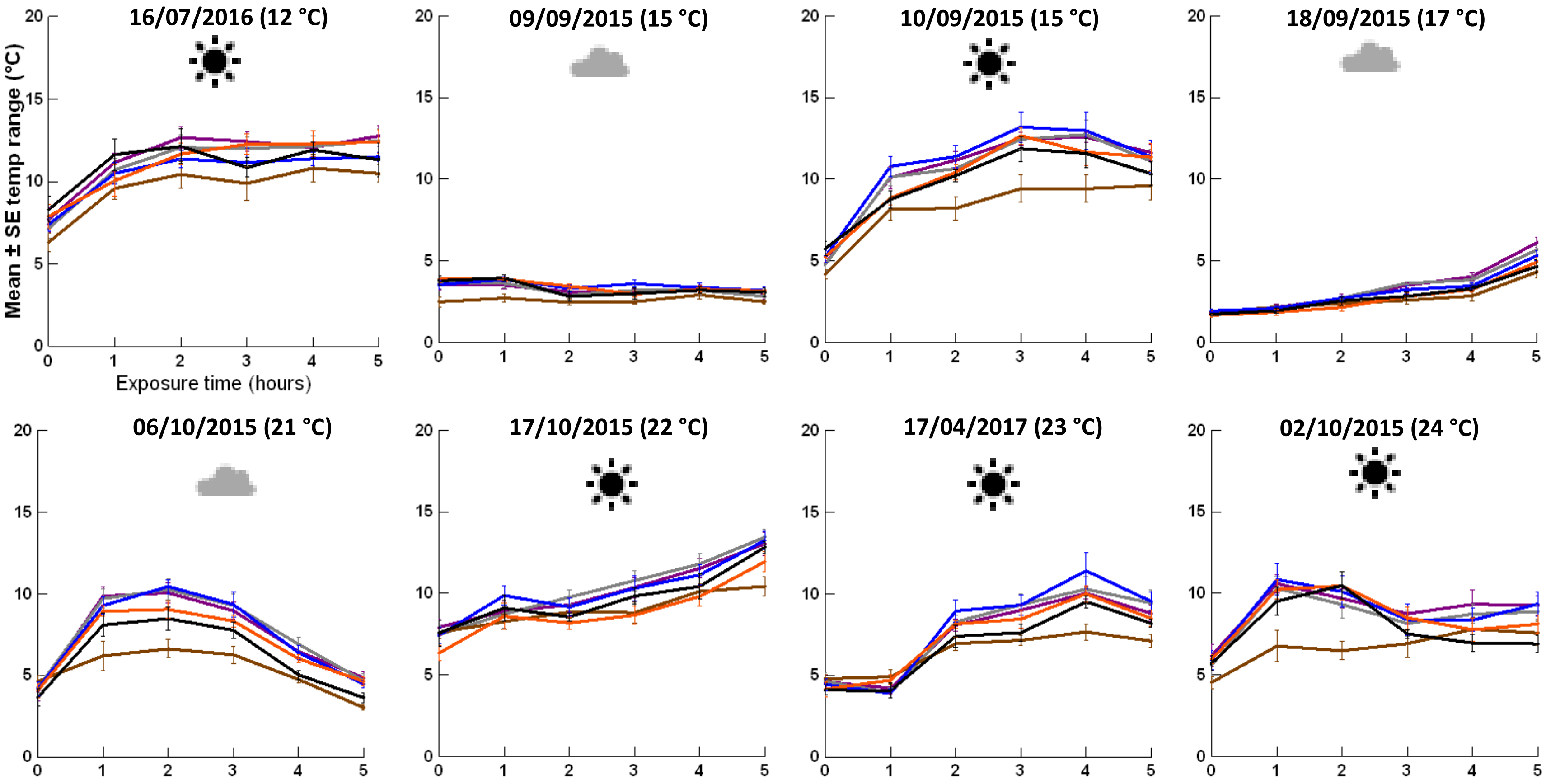


Figure 8.19: Mean ± SE upper temperature range for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

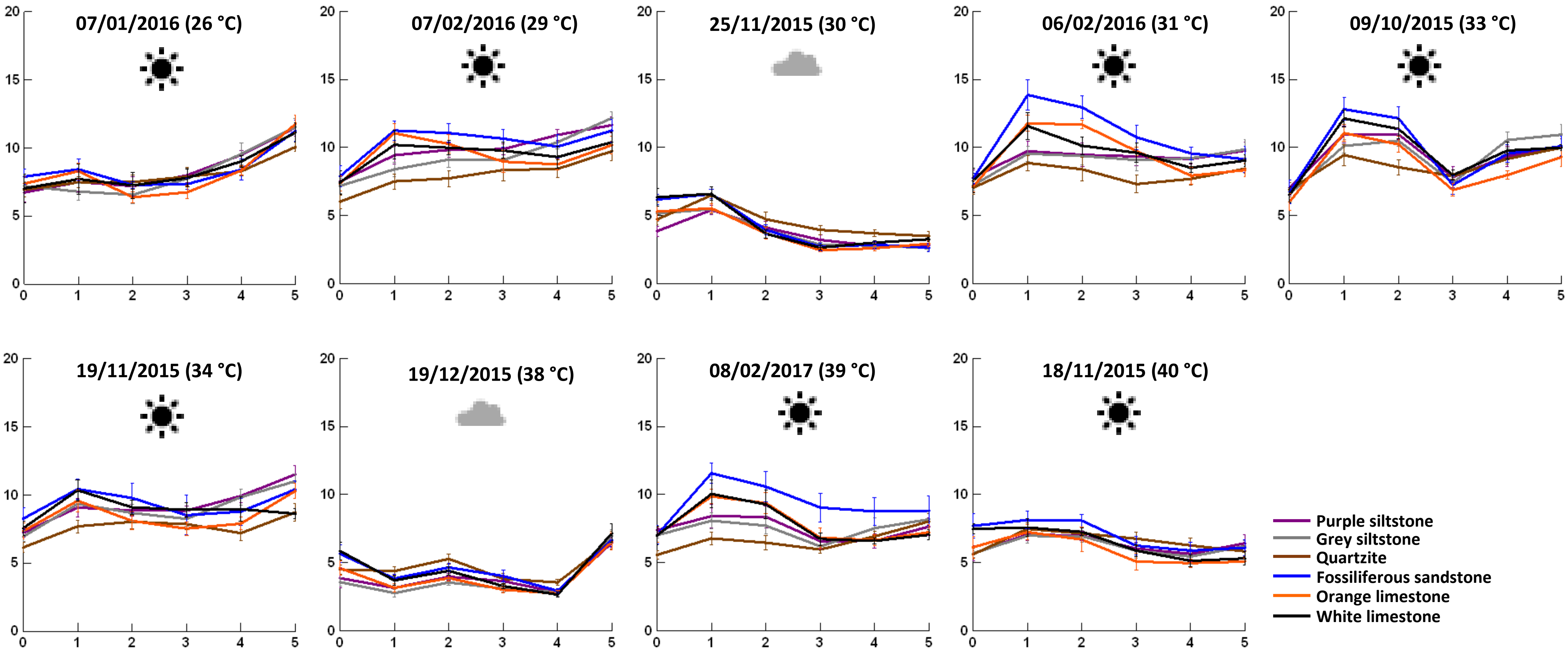


Figure 8.19 (continued): Mean ± SE upper temperature range for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

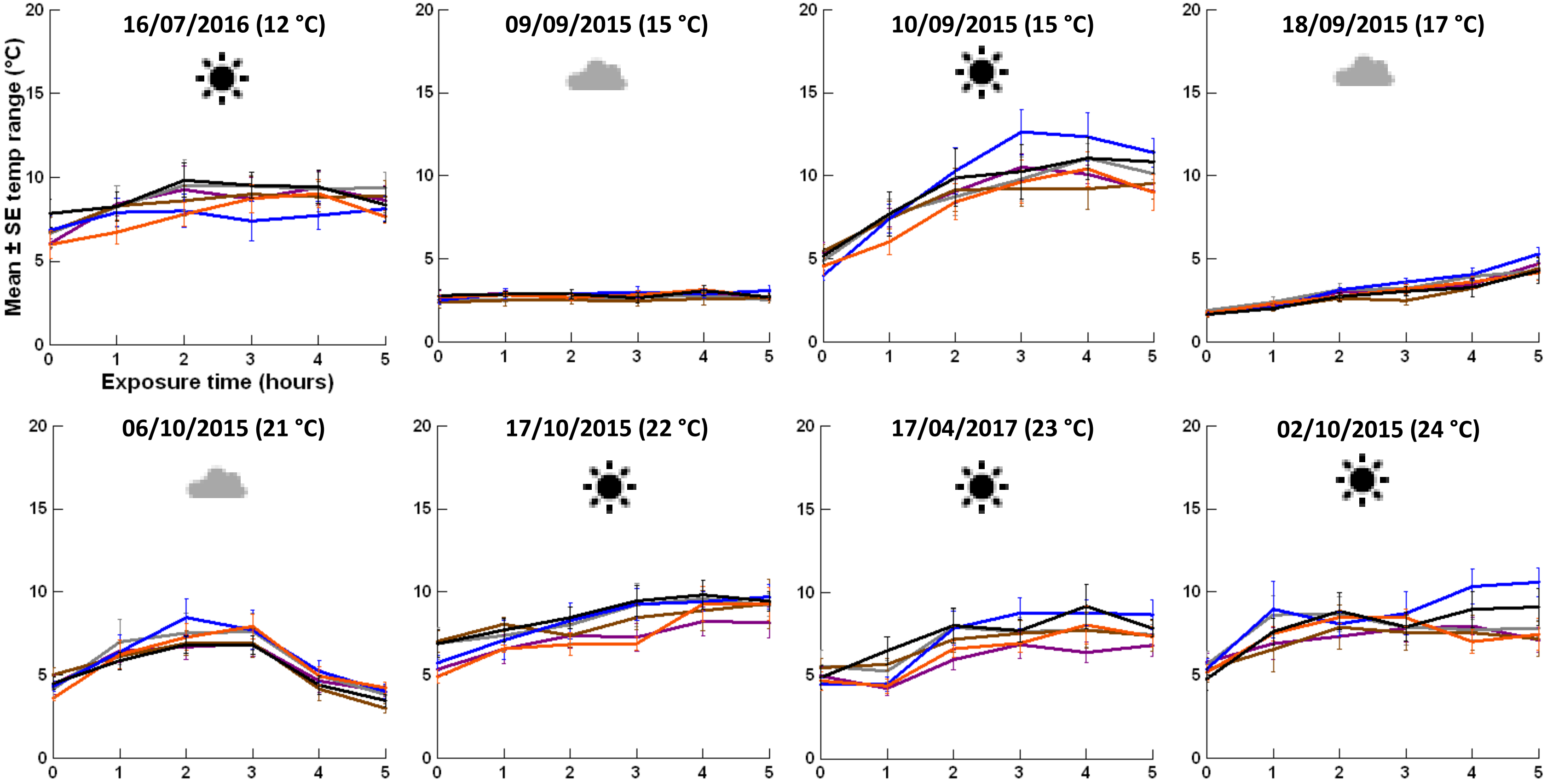


Figure 8.20: Mean ± SE lower temperature range for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

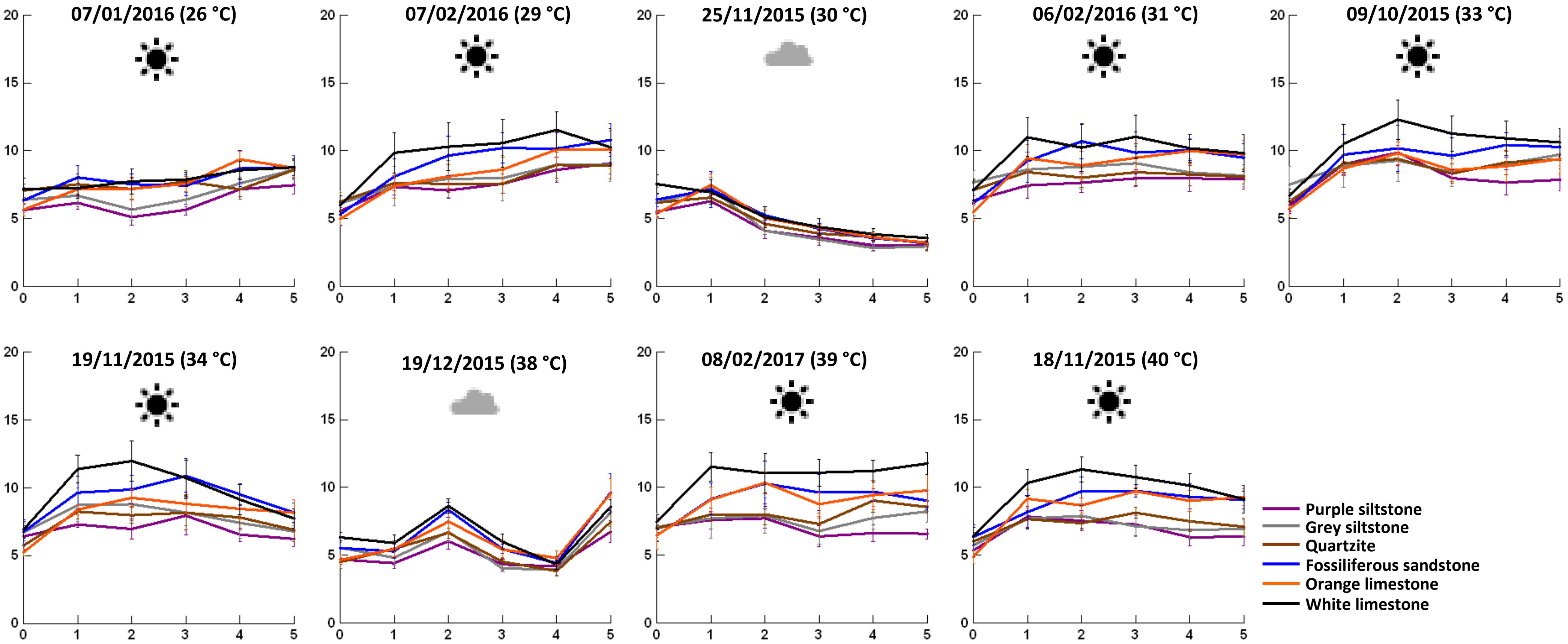


Figure 8.20 (continued): Mean ± SE lower temperature range for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

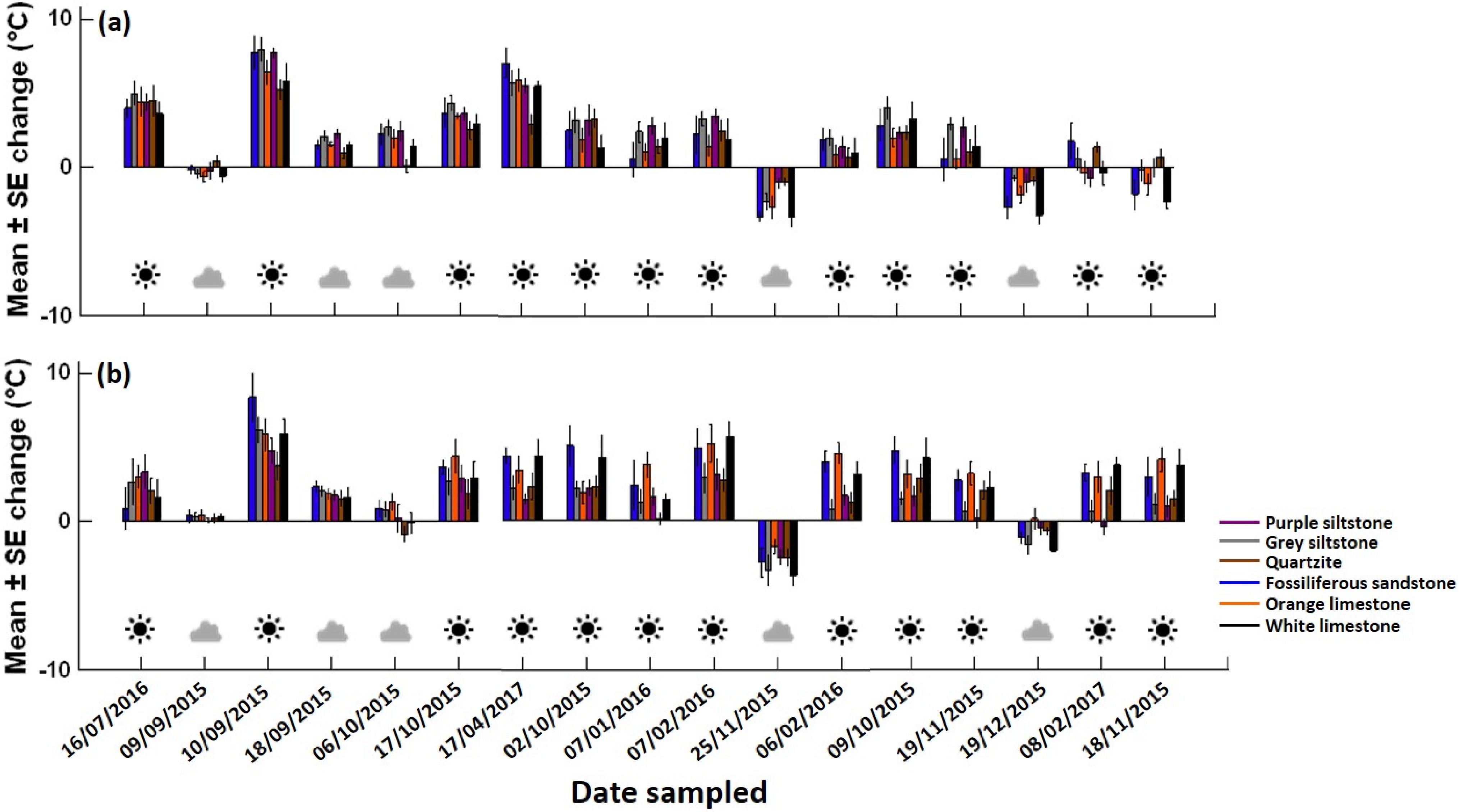


Figure 8.21: Change over four hours in temperature range for each rock on each day for (a) upper and (b) lower surfaces. Dates on the x-axis are arranged from coolest to hottest maximum air temperatures moving from left to right.

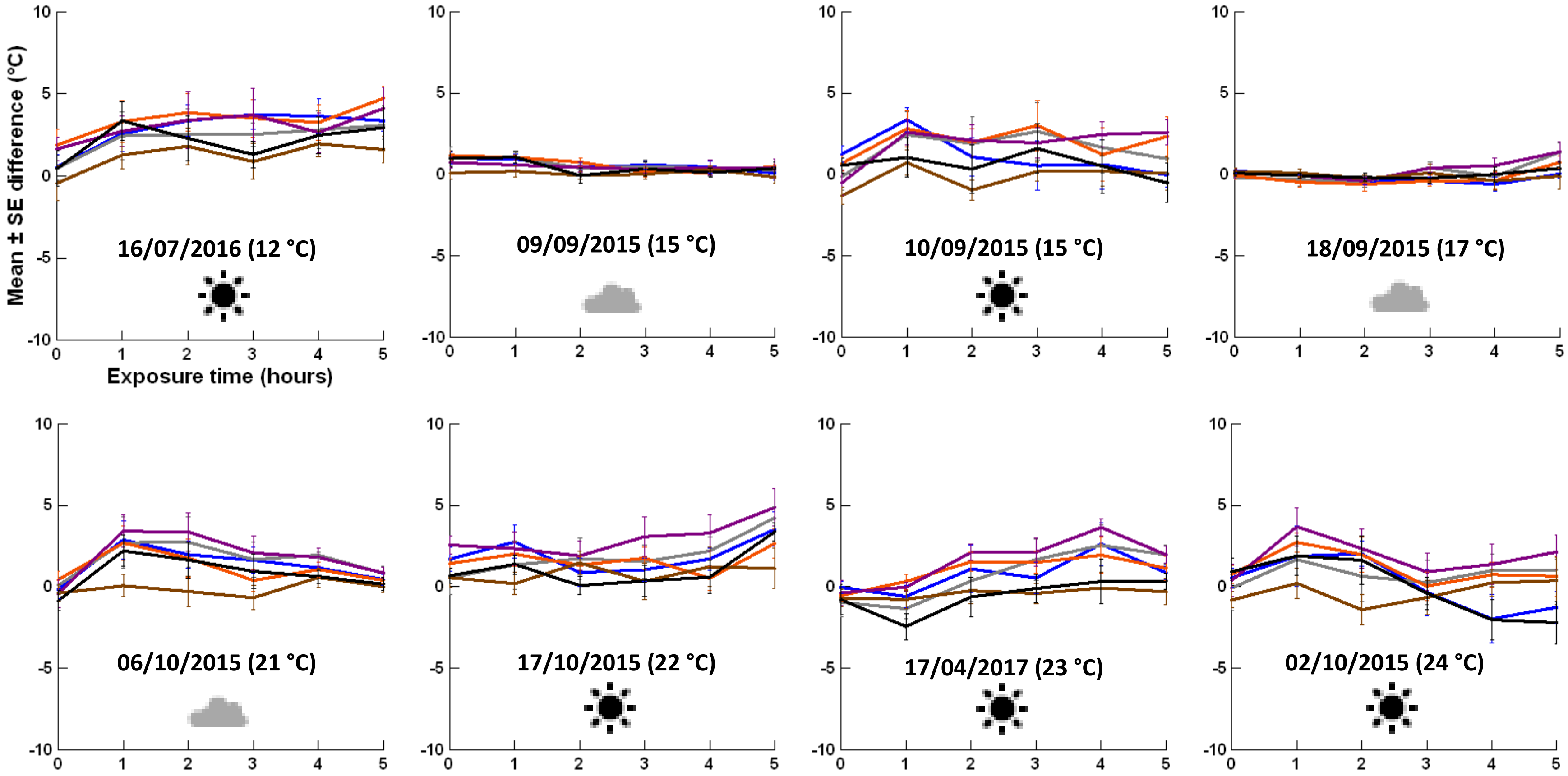


Figure 8.22: Mean ± SE temperature range difference between boulder upper and lower surfaces for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

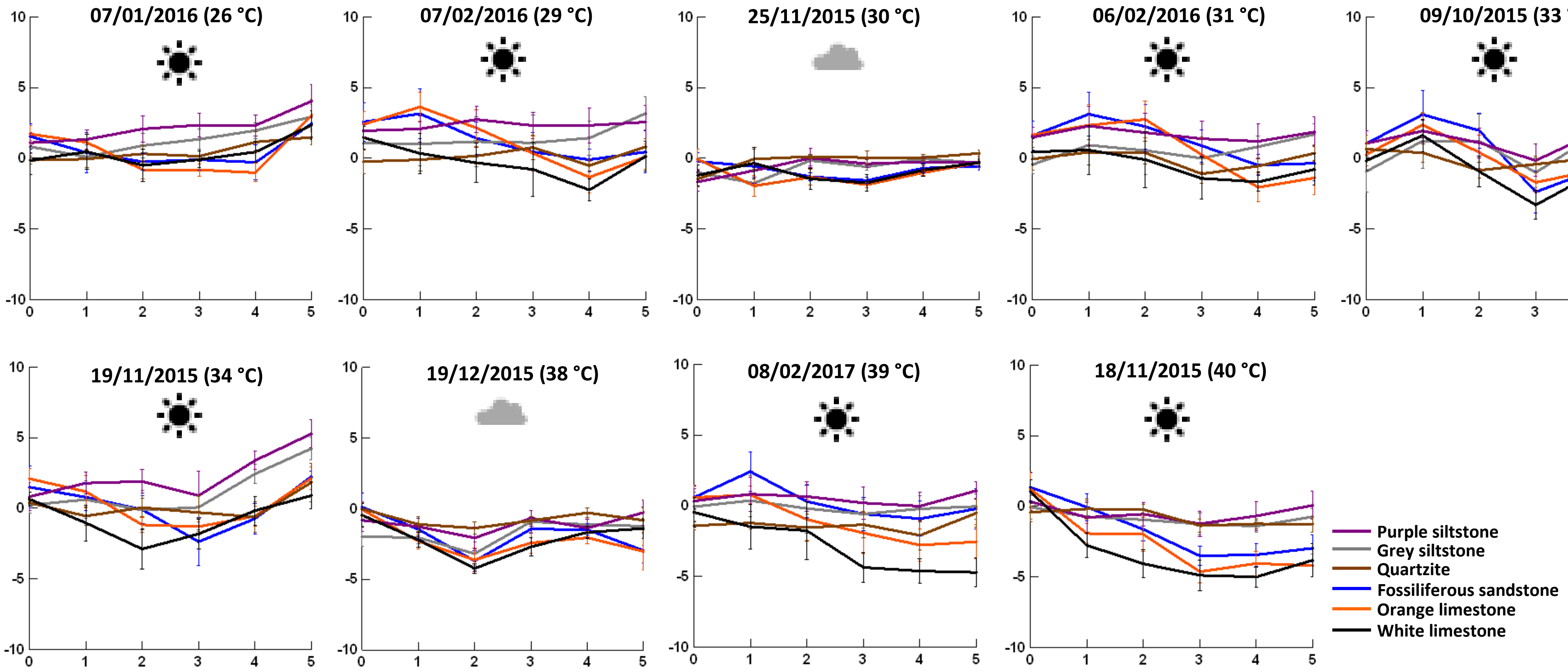


Figure 8.22 (continued): Mean ± SE temperature range difference between boulder upper and lower surfaces for 6 rocks (*n* = 6 per rock) over five hours exposure to insolation. The maximum air temperature and weather condition (sunny or cloudy) are specified for each date sampled, with days arranged from coolest to hottest maximum air temperature. Each y-axis extends to encompass the range of raw data.

Appendix 4: Chapter 5

Thermal imagery and false-colour scales

Thermal imagery captures the amount of infrared energy (i.e. heat) emitted by objects in an image. When a false-colour scale is applied to each image, differences in the amount of infrared energy emitted by objects are represented by the different colours that appear (Figure 5.1). This false-colour scale, which is scaled separately for a range on each image, consists of eight distinct colours from black (coldest) to white (hottest). Black, purple and blue colours represent objects emitting less infrared energy and are thus cooler in temperature, while green, yellow, red and white represent objects emitting more infrared energy and are thus hotter in temperature (Figure 5.1).

First additional hypothesis: any association between the target temperature and the maximum temperature does not change with time emersed.

A consistent association between snails and maximum temperatures was recorded for each target species when sampling boulders emersed for at least four hours. However, this sampling was unable to identify when this association manifested during emersion, and whether it increased in strength with time emersed. Given these target species remain virtually motionless when emersed (Underwood 1977; McMahon 1990), I hypothesised that associations between snails and maximum temperatures would manifest shortly after boulders were emersed by the receding tide. To test this, some additional sampling using boulders emersed for one hour, two hours and four hours during a single low tide was completed. The same shore-parallel zones using the same boulder selection criteria described previously was used, with 30 independent lower surfaces, inhabited by each target species, sampled at each emersion time where possible (Table 8.38). To ensure the independence of measurements across emersion times, each flipped boulder was marked with chalk to ensure it was not re-sampled at a later time. As a larger number of inhabited surfaces per species had to be sampled ($N = 90$), only *N. atramentosa* and *B. nanum* were sampled, as *D. concameratum* was too scarce. Each target species was sampled on separate days, as it was not possible to find 30 surfaces inhabited by each target species within the one-hour sampling window between the one- and two-hour emersion times. Sampling was completed for grey siltstone only, with one day of cooler and hotter air temperatures sampled per species (Table 8.38).

To establish whether snail target or boulder temperatures changed across emersion times, separate PERMutational ANalyses Of VAriance (PERMANOVA) were completed for the untransformed measures of snail target temperature, boulder maxima, boulder temperature range, and for the difference between snail target – boulder maximum temperature for the factor Time emersed (i.e. 1 hour, 2 hours and 4 hours; a fixed factor) on each day sampled. Euclidean distance resemblance matrices were prepared for each temperature variable, and permutations of residuals were completed using an unrestricted permutation of raw data model with 9999 permutations. When a significant difference was detected, pair-wise tests were used to distinguish significantly-different emersion times. Paired *t*-tests were used to test for a significant difference between snail target and maximum lower-surface temperature for each emersion time on each day sampled.

A range of temperatures was recorded on the lower surfaces of grey siltstone sampled after one, two and four hours of emersion (Figure 8.25a). The size of this temperature range increased significantly with time emersed on all days except February 3, where a significant decrease with emersion time was observed (Figure 8.25a & Table 8.39, largest PERMANOVA *p*-value = 0.0025). Maximum temperature generally increased significantly with time emersed (Figure 8.25b & Table 8.39, all significant PERMANOVA *p*-values = 0.0001). As the temperature range increased and maxima warmed, there was a corresponding significant increase in *B. nanum* and *N. atramentosa* target temperatures at later emersion times (Figure 8.25c & Table 8.39, largest PERMANOVA *p*-value = 0.0003). For each emersion time on each day sampled, the association between snail target temperatures and boulder maxima was identical to that identified for H3, with *B. nanum* and *N. atramentosa* target temperatures significantly cooler than maxima (Figure 8.25d & Table 8.40, all paired *t*-test *p*-values <0.001). The large differences between snail target and the maximum temperature of substrata showed that snails avoided the hottest areas, with some evidence of this association strengthening with time emersed, especially for *N. atramentosa* (Figure 8.25d). There was a general trend for the difference between snail target and maximum surface temperatures to increase with emersion time, except on February 3 for *B. nanum*, where temperature differences decreased with emersion time (Figure 8.25d, Tables 8.39 & 8.418.40).

Second additional hypothesis: any association between the target temperature and maxima does not change with surface condition (damp versus dry).

As air temperatures became increasing hot, fewer boulders were inhabited by *N. atramentosa* or *D. concameratum*. Furthermore, on the surfaces that were inhabited, individual snails appeared to retreat to any damp patches that remained (personal observation). Given these damp patches were also generally the coolest, I wondered whether *N. atramentosa* and *D. concameratum* were responding solely to the temperature of the substratum, substratum moisture, or whether there was some sort of synergistic response. To test this, 30 naturally damp and 30 naturally dry surfaces inhabited by *N. atramentosa* were sampled on one day each of cooler and hotter air temperatures for grey siltstone (Table 8.38). Due to their scarcity on lower surfaces, especially on the hottest days sampled, it was not possible to also test this hypothesis for *D. concameratum*. Sampling was completed in the lower shore zone, using the same boulder-selection criteria detailed previously to select 30 damp and 30 dry surfaces, after boulders had been emersed for at least four hours during daytime low tides.

Paired *t*-tests were used to test for significant differences between snail target and boulder maximum temperatures for damp versus dry surfaces on each day sampled. This difference between target and maximum temperatures for replicate boulders was then used in univariate PERMANOVAs that tested whether this difference changed according to surface condition (damp versus dry; a fixed factor). PERMANOVAs were completed separately for each day sampled using Euclidean distance resemblance matrices derived from untransformed data.

Regardless of the surface condition (i.e. damp or dry), the association between *N. atramentosa* target temperature and the maximum temperature of grey siltstone was identical to that identified more-generally when testing H3 (Figure 8.26 & Table 8.40). All *N. atramentosa* target temperatures were significantly cooler than boulder maxima (Table 8.40). There was no evidence that this association between target temperature and boulder maxima differed between damp and dry surfaces, with the difference between snail target temperature and boulder maxima not differing between damp versus dry surfaces on either day sampled (PERMANOVA *p*-values = 0.18 on February 26 and 0.79 on February 27).

Table 8.37: Univariate PERMANCOVAs, where the maximum daily air temperature during low tide was included as a covariate (Air), testing for differences between Rocks for boulder temperature characteristics and the difference between snail target and boulder maximum or minimum temperatures for each snail species. Significant differences (α = 0.05 for boulder temperature characteristics and α = 0.025 for snail temperature differences) are shown in bold.

Variable	Source	df	SS	MS	Pseudo-F	*p*-value
Lower surface temperature range	Air	1	0.12	0.12	0.22	0.6397
	Rock	1	0.05	0.05	0.09	0.7715
	Residual	13	7.22	0.56		
	Total	15	7.40			
Lower surface maximum temperature	Air	1	140	140	57.50	**0.0001**
	Rock	1	27	27	11.18	**0.0073**
	Residual	13	32	2		
	Total	15	199			
B. nanum target – maximum temperature	Air	1	0.01	0.01	0.05	0.8390
	Rock	1	0.49	0.49	2.82	0.1170
	Residual	13	2.24	0.17		
	Total	15	2.74			
B. nanum target – minimum temperature	Air	1	0.06	0.06	0.29	0.5908
	Rock	1	2.69	2.69	13.12	**0.0019**
	Residual	13	2.66	0.20		
	Total	15	5.41			
D. concameratum target – maximum temperature	Air	1	1.40	1.40	3.33	0.0888
	Rock	1	6.28	6.28	14.97	**0.0014**
	Residual	13	5.46	0.42		
	Total	15	13.14			
D. concameratum target – minimum temperature	Air	1	1.11	1.11	5.74	0.0329
	Rock	1	1.93	1.93	10.01	**0.0030**
	Residual	13	2.51	0.19		
	Total	15	5.56			
N. atramentosa target – maximum temperature	Air	1	1.68	1.68	4.55	0.0487
	Rock	1	7.18	7.18	19.50	**0.0026**
	Residual	13	4.79	0.37		
	Total	15	13.65			
N. atramentosa target – minimum temperature	Air	1	0.63	0.63	1.34	0.2791
	Rock	1	2.59	2.59	5.47	0.0380
	Residual	13	6.16	0.47		
	Total	15	9.38			

Table 8.38: The species, dates, air temperatures and number of snails (*n*) sampled on grey siltstone for hypotheses testing whether the associations between snail target temperature and boulder maxima changed with time emersed or surface condition. Surface condition for all the dashes were dry.

Hypothesis tested	Species	Date	Time emersed (hours)	Surface condition	Air temperature at each emersion time (˚C)	*n*
Time emersed	*Bembicium nanum*	3/2/2017	1	-	26	30
			2	-	26	30
			4	-	24	30
		28/2/2017	1	-	29	30
			2	-	30	30
			4	-	33	30
	Nerita atramentosa	10/2/2017	1	-	27	25
			2	-	29	26
			4	-	32	30
		14/2/2017	1	-	22	30
			2	-	23	30
			4	-	25	30
Surface condition	*Nerita atramentosa*	26/2/2017	4	Damp	22	30
			4	Dry	22	30
		27/2/2017	4	Damp	27	30
			4	Dry	27	30

Table 8.39: Univariate PERMANOVAs testing for differences among emersion times (1, 2, and 4 hours) for target temperatures, the temperature range and maximum temperature of lower surfaces, and for the difference between target – boulder maximum temperature for each day sampled for each species on grey siltstone. When a significant difference was detected between emersion times, PERMANOVA pair-wise tests were used to identify which times were significantly different. Significant differences (α = 0.05) are shown in bold. The dashes denote where no pair-wise tests were completed, as no main effect for time emersed was detected.

Species	Date	Hours compared	Target	Maximum	Range	Target - maximum
Bembicium nanum	3/2/2017	1,2,4	**0.0003**	0.0679	**0.0010**	**0.0078**
		1,2	**0.0044**	**-**	0.2608	**0.0379**
		1,4	**0.0001**	**-**	**0.0003**	**0.0033**
		2,4	0.2750	-	**0.0059**	0.3885
	28/2/2017	1,2,4	**0.0001**	**0.0001**	**0.0014**	**0.0380**
		1,2	**0.0001**	**0.0001**	**0.0073**	**0.0072**
		1,4	**0.0001**	**0.0001**	**0.0011**	0.0656
		2,4	**0.0001**	**0.0001**	0.2722	0.5657
Nerita atramentosa	10/2/2017	1,2,4	**0.0001**	**0.0001**	**0.0001**	**0.0001**
		1,2	**0.0001**	**0.0001**	**0.0065**	**0.0429**
		1,4	**0.0001**	**0.0001**	**0.0001**	**0.0001**
		2,4	**0.0001**	**0.0001**	**0.0243**	**0.0136**
	14/2/2017	1,2,4	**0.0001**	**0.0001**	**0.0025**	0.0675
		1,2	**0.0001**	**0.0001**	0.1475	-
		1,4	**0.0001**	**0.0001**	**0.0007**	**-**
		2,4	**0.0001**	**0.0001**	**0.0304**	**-**

Table 8.40: Paired *t*-tests testing for significant differences between target and maximum boulder temperature for *Bembicium nanum* and *Nerita atramentosa* on grey siltstone for time emersed or surface condition. Significant differences (α = 0.05) are shown in bold. x̄ = mean temperature difference between target and maximum boulder temperature.

Species	Date	Time emersed or surface condition	x̄	*p*-value
Bembicium nanum	3/2/2017	1 hour	-6.73	**<0.001**
		2 hours	-5.42	**<0.001**
		4 hours	-4.98	**<0.001**
	28/2/2017	1 hour	-5.17	**<0.001**
		2 hours	-6.51	**<0.001**
		4 hours	-6.19	**<0.001**
Nerita atramentosa	10/2/2017	1 hour	-4.06	**<0.001**
		2 hours	-5.30	**<0.001**
		4 hours	-6.96	**<0.001**
	14/2/2017	1 hour	-4.37	**<0.001**
		2 hours	-4.98	**<0.001**
		4 hours	-5.56	**<0.001**
	26/2/2017	Damp	-6.58	**<0.001**
		Dry	-7.40	**<0.001**
	27/2/2017	Damp	-6.31	**<0.001**
		Dry	-6.16	**<0.001**

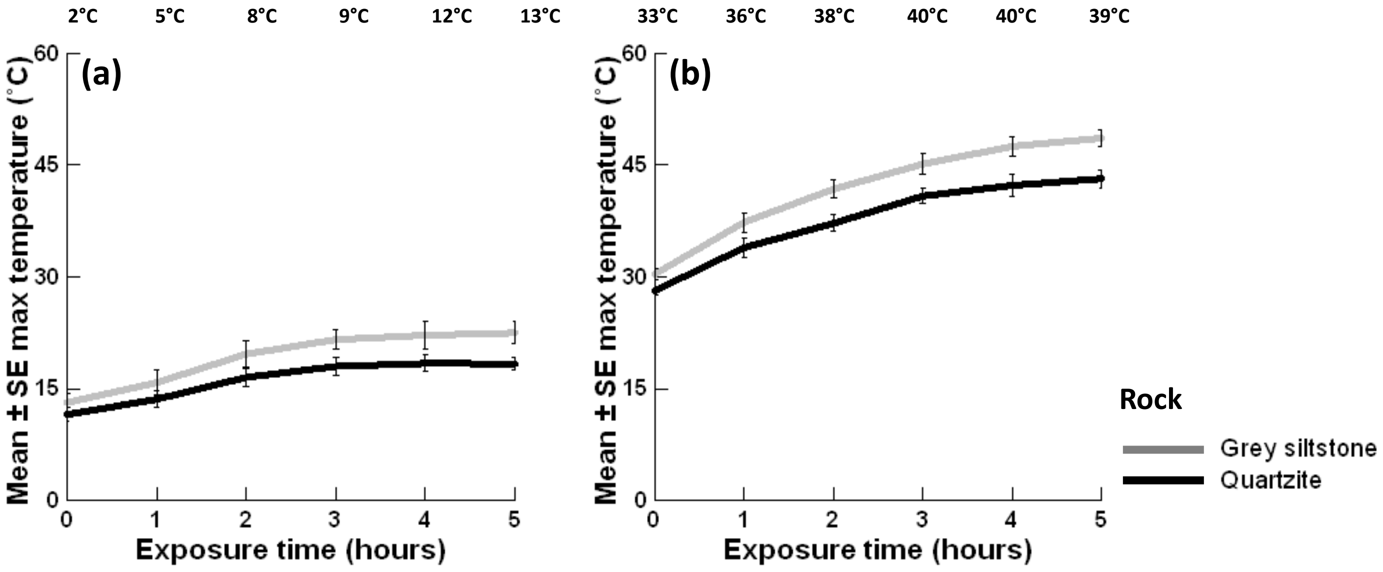


Figure 8.23: Mean ± SE maximum lower-surface temperatures for grey siltstone and quartzite boulders on sunny days with (a) cooler and (b) hotter air temperatures. Rock temperatures were measured at one hour intervals over a time course of five hours using thermal imagery in a common-garden experiment (see Chapter 4). The air temperature at the time of sampling is shown above each exposure time.

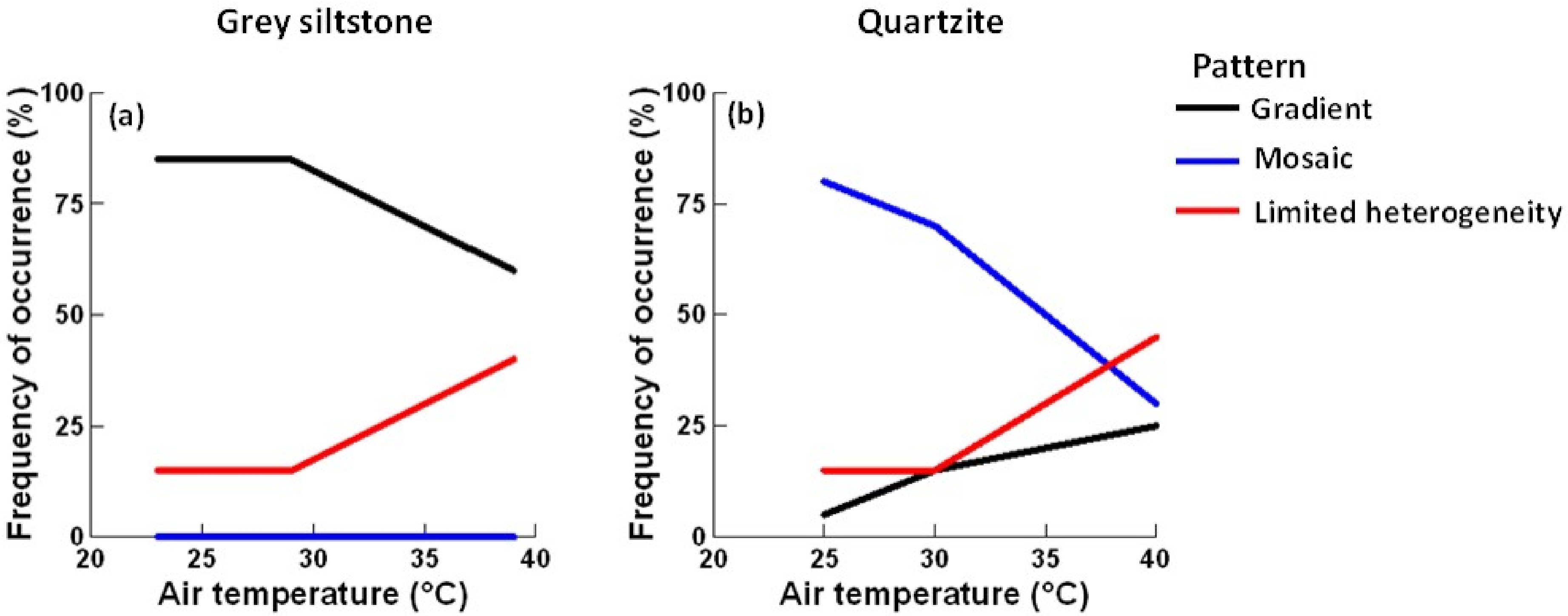


Figure 8.24: Frequencies of occurrence (%) for patterns of temperature difference on the lower surfaces of (a) grey siltstone and (b) quartzite boulders (*n* = 20 per day sampled) for three days of differing air temperature per rock.

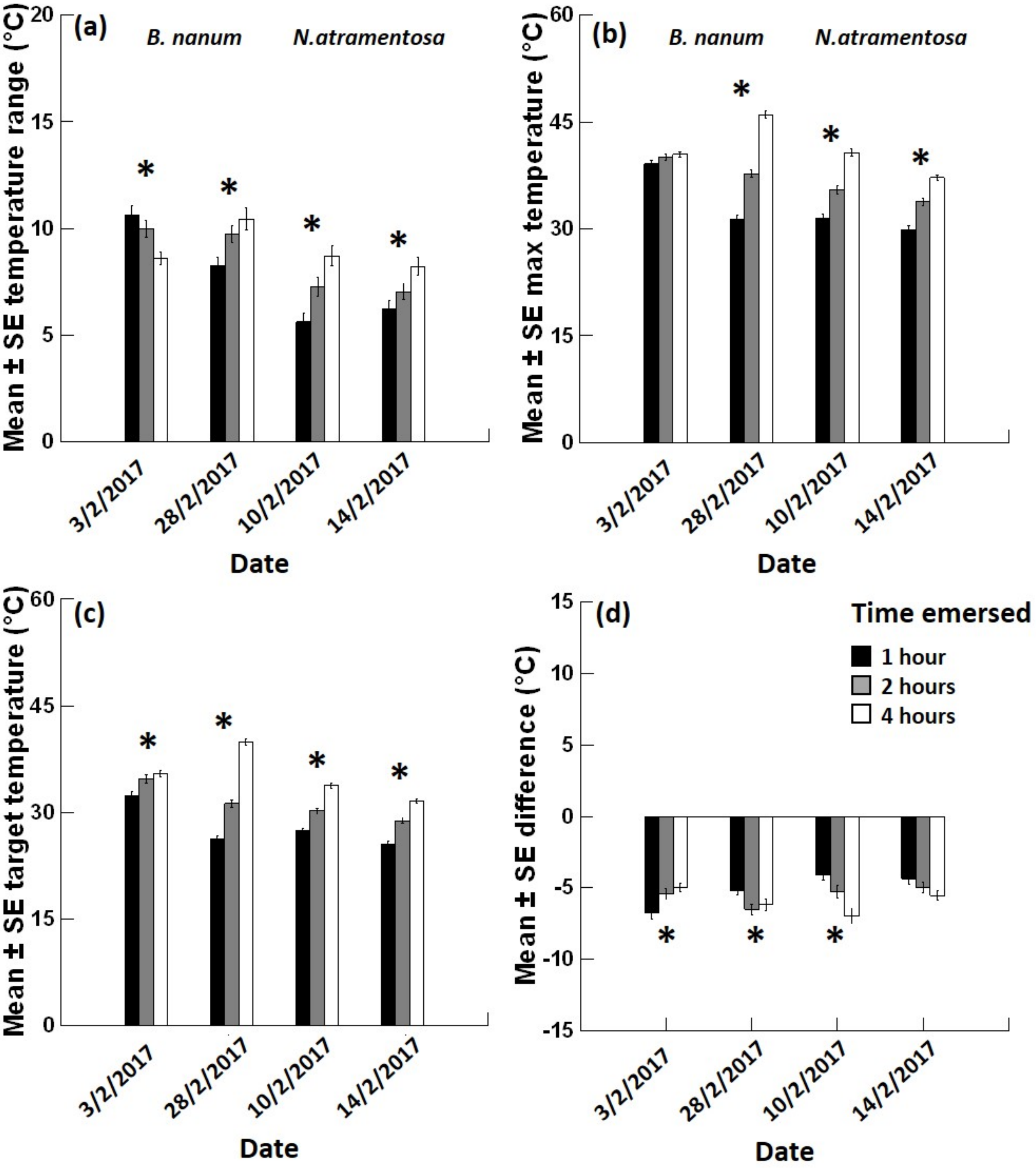


Figure 8.25: The changes with time emersed on grey siltstone for the mean ± SE (a) lower-surface temperature range, (b) maximum lower-surface temperature, (c) target temperature and (d) difference between target – boulder maximum temperature. *Bembicium nanum* was sampled on February 3 & 28 while *Nerita atramentosa* was sampled on February 10 & 14. Each y-axis extends to encompass the range of the raw data. * = significant difference detected between emersion times.

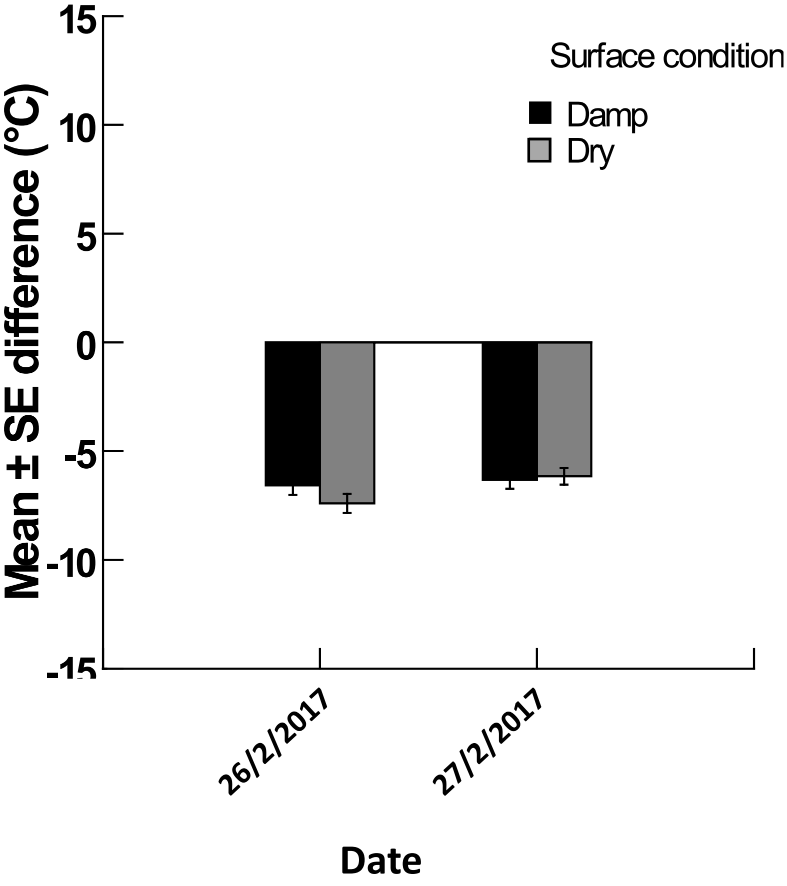


Figure 8.26: Mean ± SE difference for target – maximum temperature for damp versus dry lower surfaces of grey siltstone on each day sampled.   All damp-dry comparisons were NS.

Appendix 5: Pilot study

Inventory of rock types, habitats, and biodiversity on rocky seashores in South Australia's two south-east marine parks

Nathan Janetzki, Peter G. Fairweather & Kirsten Benkendorff

A report to the South Australian Department of Environment and Water. This report was published online in June 2015 https://data.environment.sa.gov.au/Content/_layouts/15/WopiFrame.aspx?sourcedoc=/Content/Publications/INVENTORY%20OF%20ROCK%20TYPES,%20HABITATS,%20AND%20BIODIVERSITY%20ON%20ROCKY%20SEASHORES%20IN%20SOUTH%20AUSTRALIA%E2%80%99S%20TWO%20SOUTH-EAST%20MARINE%20PARKS%20(1).pdf&action=default&DefaultItemOpen=1

Abstract

Geological, habitat, and biodiversity inventories were conducted across six rocky seashores in South Australia's (SA) two south-east marine parks during August 2014, prior to the final implementation of zoning and establishment of management plans for each marine park. These inventories revealed that the sampled rocky seashores in SA's South East Region were comprised of several rocks: a soft calcarenite, Mount Gambier limestone, and/or a harder flint. Furthermore, these inventories identified five major types of habitat across the six sampled rocky seashores, which included: emersed substratum; submerged substratum; boulders; rock pools; and sand deposits. Overall, a total of 12 marine plant species and 46 megainvertebrate species were recorded across the six sampled seashores in the Lower South East and Upper South East Marine Parks. These species richness values are considerably lower than those recorded previously for rocky seashores in other parts of SA. Low species richness may result from the type of rock that constitutes south-east rocky seashores, the interaction between rock type and strong wave action and/or large swells, or may reflect the time of year (winter) during which these inventories were conducted. The species richness and space occupancy of marine plants displayed no significant difference among sampled sites, although a significant difference in the structure of marine plant assemblages was detected among sites. Exploration of patterns within the megainvertebrate assemblage revealed very strong correlations between invertebrate assemblage structure and the type of habitats that were sampled. With a megainvertebrate species richness two standard deviations greater than the regional average, Racecourse Bay West was identified as a potential hotspot for intertidal megainvertebrates. Due to the short timeframe of the current pilot project, the data presented here should be viewed as the first step in creating a baseline of the geology, habitats, and biodiversity for rocky seashores in

SA’s two south-east marine parks. Ideally, this report should be supplemented by a replicated sampling regime, that spans multiple seasons, a greater spread of sites, and is balanced across seashores of hard, soft, and mixed rock types, to capture data on the true variability within marine plant and megainvertebrate assemblages present on rocky seashores in SA’s south-east marine parks.

Introduction

A management tool for protecting marine environments worldwide is the establishment of marine parks, which have protocols in place to govern accessibility and the nature of activities that can be undertaken within their boundaries (Agardy et al. 2003). The South Australian (SA) government established a network of nineteen multiple-use marine parks across the state to protect its iconic marine species and habitats from growing anthropogenic pressures (DEW 2014). Represented within this network of marine parks are rocky seashores, which constitute the areas of rocky coastline exposed to air during the lowest tides, extending up to a level on the shore that is periodically submerged underwater during the highest tides, or reached by the spray of waves (Benkendorff et al. 2008; Garcia and Smith 2013). Rocky seashores support a diverse suite of benthic marine plants and invertebrates which include, but are not limited to algae, seagrasses, lichens, molluscs, crustaceans, echinoderms, sea anemones, sponges, ascidians, and polychaetes (Benkendorff et al. 2008). The potential biological importance of SA rocky seashores is reflected in the fact that over 90 % of the marine invertebrate fauna of southern Australia is endemic (Gowlett-Holmes 2008). Furthermore, the diversity and rate of endemism of southern Australian marine invertebrate fauna is higher than for many other temperate marine regions globally (Gowlett-Holmes 2008).

Effective conservation and management programmes preferably integrate our current understanding of how the structure of marine assemblages are influenced through the interaction of biological and physical variables, drawing upon a detailed understanding of distribution, abundance and life history of each species in response to these interactions (Brooks et al. 2004; Banks and Skilleter 2007). Regrettably, the links between biota and environment remain largely unstudied for any suite of species and their associated habitats (Davidson and Chadderton 1994; Underwood and Chapman 2001; Brooks et al. 2004; Banks and Skilleter 2007). Despite their likely biological importance, SA's rocky seashores are no exception, with the types of rock that constitute them generally unknown, and the benthic assemblages that inhabit them largely unstudied. This is problematic, as a baseline understanding of intertidal geology and biodiversity is necessary to know what is being protected, design effective management and conservation strategies, and for assessing how effective these strategies are in achieving their specified management and conservation objectives.

Consequently, the principal aim of this pilot project was to provide a preliminary assessment of six rocky seashores currently protected in the Lower South East and Upper South East Marine Parks along SA's south-east coast. Specifically, dedicated geological, habitat, and biodiversity inventories were conducted for four shores in General Managed Use Zones and two shores in Habitat Protection Zones during the austral winter of 2014. Rocky seashores in Sanctuary Zones or Restricted Access Zones were not assessed during this study due to their limited availability. Each inventory assessed the abiotic characteristics of each shore, capturing information on the type(s) of rock and habitats that dominate each site. Additionally, biological surveys were conducted for each type of identified habitat, for each sampled shore, to quantify the algal and seagrass (hereafter *marine plant*) and megainvertebrate assemblage specific to each habitat type. While these inventories should not be considered as definitive for the overall biodiversity at each site, they do mark the beginning of establishing a baseline of intertidal biodiversity for these seashores. Additional inventories that assess a greater spread of sites, with seasonal replication, are necessary to build a comprehensive baseline of intertidal biodiversity on rocky seashores throughout SA's south-east marine parks. However, these inventories can be used as the cornerstone for developing and implementing an ongoing south-east marine parks intertidal monitoring program. This would not only facilitate the development of a comprehensive baseline for south-east rocky seashore biodiversity, but could ultimately be used to evaluate and enhance the management objectives of each marine park in relation to rocky seashores.

Materials & methods

Due to unfavourable weather and tidal conditions over winter, just six sites were selected for geological, habitat, and biodiversity inventories (Figure 1). Four of these sites were located in General Managed Use Zones, which were Lake Charro, Robe South, Racecourse Bay West, and Racecourse Bay East (Table 1). The remaining two sites were situated in Habitat Protection Zones, which were Rainbow Rocks and Nora Creina (Table 1). No sites within Sanctuary Zones or Restricted Access Zones were assessed during this study. Restricted Access Zones were not sampled because this level of marine park protection is not represented within SA's two south-east marine parks, while Sanctuary Zones were not sampled due to the difficulty in finding a suitable rocky seashore within this zone to sample during winter. In total, three sites each were located in the Upper South East and Lower South East Marine Parks, respectively (Figure 1). All inventories were undertaken during the final week of August 2014, during suitable daytime low tides (predicted low tide ≤0.70 m AHD).

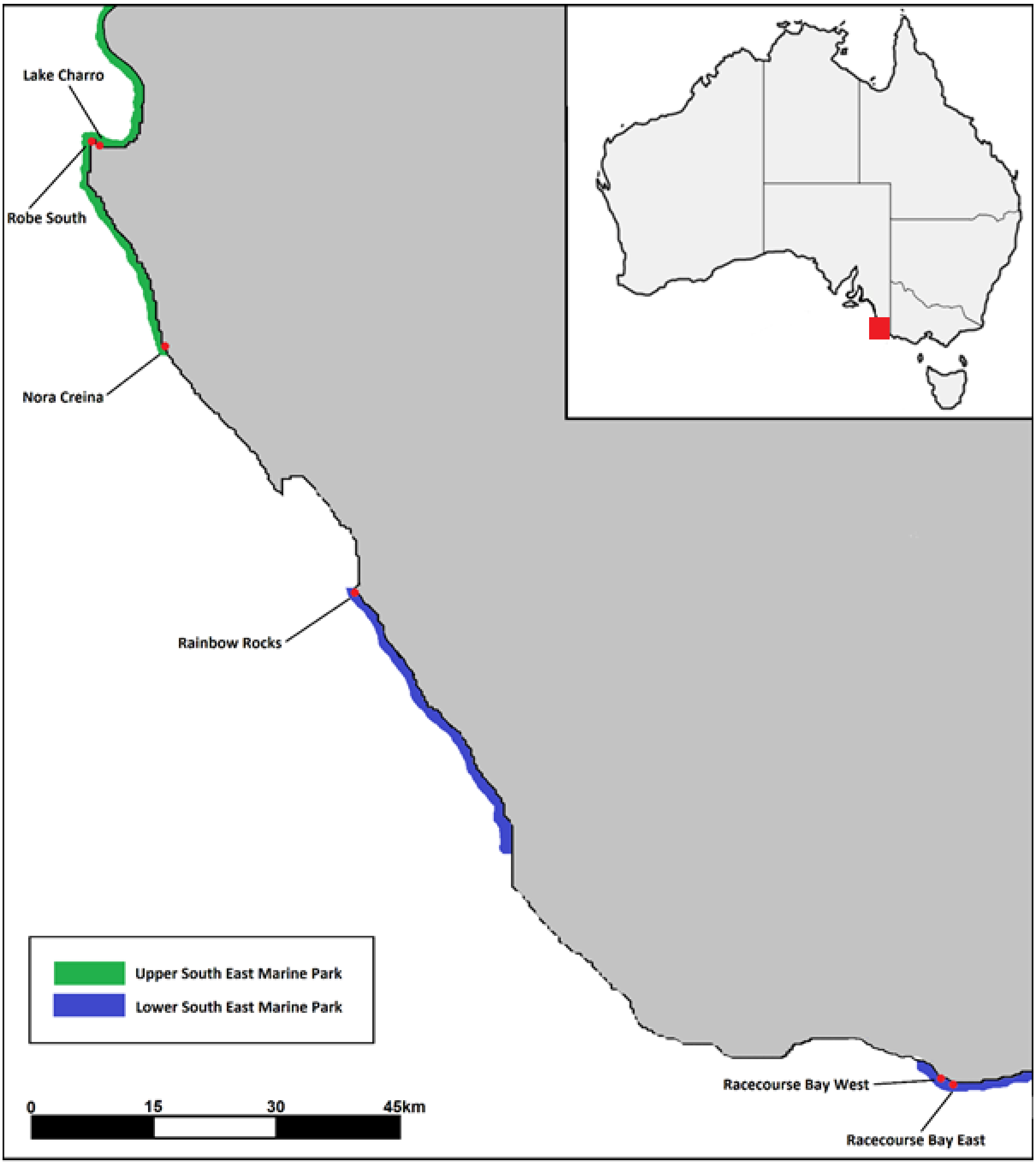


Figure 1: Map depicting sites sampled during geological, habitat, and biodiversity inventories in the Lower South East and Upper South East Marine Parks during winter 2014.

Table 1: The location, date surveyed, predicted tidal and observed weather conditions for geological, habitat, and biodiversity inventories in SA's South East Region. The aspect of each site is provided for the principal direction looking out to sea. The average slope and dominant rock types for each shore are also specified.

Site	**Latitude**	**Longitude**	**Marine park name**	**Marine park zone**	**Survey date**	**Predicted tidal height at sampling (m) AHD**	**Weather at sampling**	**Aspect of the shoreline**	**Wave exposure (DEWNR class)**	**Shore slope (%)**	**Dry substratum colour**	**Rock type(s)**
Racecourse Bay East	38°03'27.62" S	140°46'01.49"E	Lower South East	General Managed Use	28/08/2014	0.4	Sunny	South	Low	0.84	Light grey	Flint
Racecourse Bay West	38°03'31.52" S	140°44'53.94"E	Lower South East	General Managed Use	29/08/2014	0.5	Sunny	South	Low	0.68	Light grey-yellow	Mount Gambier limestone & flint
Rainbow Rocks	37°34'16.51" S	140°06'42.47"E	Lower South East	Habitat Protection	30/08/2014	0.7	Sunny	South-west	Moderate	-1.49*	Dull orange	Calcarenite
Nora Creina	37°19'46.34" S	139°50'54.97"E	Upper South East	Habitat Protection	30/08/2014	0.5	Sunny	South-west	Moderate	3.32	Light yellow	Calcarenite
Robe South	37°09'55.30" S	139°44'34.84"E	Upper South East	General Managed Use	27/08/2014	0.7	Cloudy	South-west	Low	5.61	Dull orange	Calcarenite
Lake Charro	37°09'44.29" S	139°45'51.31"E	Upper South East	General Managed Use	27/08/2014	0.4	Sunny	North-east	Low	0.8	Light brown	Calcarenite

* The negative shore slope % recorded at Rainbow Rocks is driven by this rocky seashore sloping downwards from front to back (i.e. the shore is highest closest to the sea).

Abiotic characteristics of the shore

A summary of the date, observed weather and predicted tidal conditions at the time sampling was conducted are provided in Table 1. The aspect of each shore, relative to the ocean, was measured with a compass (Table 1). Shore slope was quantified for each site along a minimum of three transects perpendicular to the ocean, which extended from the low tide mark at the time of sampling to the top of the rocky seashore. A clinometer and graduated staff were used to measure the slope of the shore along each transect, with an average value calculated per site (Table 1). Substratum colour was approximated by comparing the colour of apparently dry substratum samples against standardised soil colour charts (Table 1). The rock type(s) that dominated each site were determined by collecting three small representative samples of each rock type observed, and presenting these rock samples to Claudia Flaxman, a geologist at the University of Adelaide, for accurate identification. Hardness, the measure of a rock's or mineral's resistance to being scratched or indented, was measured using Moh's scale of scratch hardness, which arranges 10 minerals in increasing order of scratch hardness, ranging from talc (1 = softest) through to diamond (10 = hardest) (Tabor 1954,1956). Using this scale, it was possible to classify all rocks into two general classes based on their hardness, soft versus hard. The soft class encompassed rocks with a scratch hardness ≤4 using Moh's scale, while the hard class encompassed rocks with a scratch hardness >4 using Moh's scale (Liversage and Benkendorff 2013). The scratch hardness of the softest mineral capable of scratching the rock surface (as opposed to the mineral scratching off on the rock's surface) was assigned to each rock. Additionally, the modelled wave exposure for each site was recorded using DEW's online GIS mapping system (Table 1).

Space occupancy of habitats

Line-intercept transects were employed to quantify the dominant types of intertidal habitat present at each site. Briefly, this method involved measuring the length of transect tape intercepted by the dominant forms of habitat (≥10 cm in length) as continuous segments (Lucas and Seber 1977; Dutton 2007). These measurements were then summed for each type of habitat, and converted into a percentage of the total tape length (Lucas and Seber 1977; Dutton 2007). Categories of habitat included: emersed substratum (i.e. dry bedrock); submerged substratum (i.e. bedrock covered by ≤10 cm of water at low tide); boulders (i.e. rock not attached to the substratum that was fist-sized or larger); rock pools (i.e. areas of the shore covered by >10 cm of water at low tide and where no water exchange occurred with the subtidal environment); and sand deposits (Benkendorff and Thomas 2007). Each transect was deployed perpendicular to the ocean, extending from the low tide mark at the time of sampling to the top of the rocky seashore (Underwood 1981). Due to the six sampled rocky seashores having vastly different alongshore lengths, three replicate transects were measured at the smaller shores (Rainbow Rocks, Nora Creina, Robe South, and Lake Charro), and five replicate transects were measured at the larger shores (Racecourse Bay East and Racecourse Bay West).

Biodiversity inventories

Each site was stratified according to the dominant types of habitat identified using line-intercept transects, with biodiversity inventories conducted separately for emersed substratum, submerged substratum, boulders, and rock pools. Previous studies have shown distinct differences in marine assemblages across these four intertidal habitats (Underwood 1981; Underwood and Chapman 2001; Smith 2005; Goodsell et al. 2007). No inventories were conducted during this study for the sand deposit habitat. The space occupancy of sessile species (algae, seagrass, tube worms, and mussels) was quantified using the same line-intercept transects employed when identifying habitat types (described above).

For the remaining intertidal invertebrate assemblage (including barnacles, which do not generally form dense aggregations on any of the six sampled seashores), 30-minute timed-search (TS) surveys were conducted. Because many intertidal species often display a patchy and over-dispersed distribution (Underwood and Chapman 2001; Chapman 2002a; b; 2005; Grayson and Chapman 2004), or have naturally low abundances on rocky seashores (Benkendorff 2003; Goodsell et al. 2007), TS surveys represent the most effective means of surveying large sections of the seashore, to ensure data is captured on the rare or over-dispersed abundances of some intertidal species (Benkendorff 2003). For TS surveys, all faunal species >5 mm (hence 'megainvertebrates') encountered within a 30-minute timeframe were recorded and ranked according to their relative abundance (Dutton 2007). Categories of abundance included: absent (0 individuals); very uncommon (1-2 individuals); moderately common (3-10 individuals); common (11-50 individuals); and abundant (≥51 individuals). Biota was identified in the field to species level wherever possible with the exception of the limpet genera *Notoacmea* and *Siphonaria*, due to the difficulty in distinguishing between each species belonging to those genera *in situ.* For the genus *Notoacmea*, a species complex including *Notoacmea flammea*, *N. mayi* and *N. alta* was acknowledged, while for *Siphonaria*, a species complex including *Siphonaria diemenensis* and *S. funiculata* was recognised. In any ambiguous cases of species identification, specimens were digitally photographed for later identification in the laboratory.

The total number of marine plant and megainvertebrate species per site (i.e. species richness) was determined by pooling the data collected from each line-intercept transect and TS survey. Sites with a species richness at least two standard deviations higher than the mean (the mean being the average species richness across the six sampled sites) were identified as potential biodiversity hotspots for marine plants and megainvertebrates, respectively, on rocky seashores in SA's south-east (Benkendorff and Davis 2002).

Data analysis and presentation

To determine whether the percentage cover or species richness of sessile species measured using line-intercept transects differed among sites, univariate analyses were conducted using SYSTAT v13. Where data did not meet the assumptions for normality of distribution or homogeneity of variances, a square-root transformation was performed on the raw data. Once complete, one-factor ANOVAs were designed and run to test for differences ($\alpha = 0.05$) among sites for the total percentage cover of marine plants, the total percentage cover of marine plant divisions, and the species richness of marine plant divisions. The divisions of marine plants examined included: Rhodophyta (red algae); Heterokontophyta (brown algae); Chlorophyta (green algae); and Magnoliophyta (seagrass). Histograms were prepared to compare the mean (± standard error) percentage cover or species richness of marine plants, and divisions of marine plants, among sites from replicated transects. To determine whether the sampled sessile assemblage differed among sites, univariate analyses were conducted using the PRIMER version 6 and PERMANOVA+ add-on statistical package (Anderson et al. 2008). A similarity matrix was prepared using Bray-Curtis similarity on untransformed sessile species data with the addition of a dummy variable (value = 1). From this matrix, a non-metric multidimensional scaling (nMDS) ordination plot was generated, and a one-factor PERMANOVA designed and run to test for differences (α = 0.05) in the structure of sessile assemblages among sites. When a significant difference was detected among sites, pair-wise tests were run to distinguish which sites were significantly different (α = 0.05) from one another, with a *p*-value from Monte Carlo tests used in place of a permutation *p*-value whenever the number of unique permutations <100 (Anderson et al. 2008). A SIMPER analysis was performed to determine which species characterised the sessile assemblage at each site, and which species contributed most to differences in assemblage structure among sites.

To examine the characteristics of the remaining intertidal invertebrate assemblage sampled using semi-quantitative abundance rankings from TS surveys, patterns within the data were explored using PRIMER & PERMANOVA+. A CLUSTER analysis that encompassed all TS surveys for each site and habitat type was performed, as the habitats searched during TS surveys (emersed substratum, submerged substratum, boulders, and rock pools) have been shown previously (e.g. Underwood 1981; Underwood and Chapman 2001; Smith 2005; Goodsell et al. 2007) to support distinct intertidal assemblages. This group average linkage approach identified four distinct invertebrate assemblages at the 45-50 % Bray-Curtis similarity level. A constrained canonical analysis of principal coordinates (CAP) plot was generated to illustrate differences in two-dimensional ordination space among these four invertebrate assemblages (Anderson et al. 2008). Vectors corresponding to strong Spearman rank correlations (for lengths >0.8) of individual species were superimposed over this CAP plot to illustrate which species best characterised each of the four identified assemblages. This approach was supported by a SIMPER analysis, which was performed to determine which species characterised each assemblage, and which species contributed most to the dissimilarities detected among assemblages. Correlation analyses were conducted in SYSTAT, and scatterplots generated, to examine the relationship between sampled habitats and biodiversity at each site.

Results

Rock type

Three rock types were identified during geological, habitat, and biodiversity inventories in the Lower South East and Upper South East Marine Parks (Table 2). These were a softer calcarenite and Mount Gambier limestone, and a harder flint (Table 2, Figure A2). Calcarenite was recorded at Rainbow Rocks, Nora Creina, Robe South, and Lake Charro, where it occurred as extensive platform areas or heterogeneous reef (Table 2, Figure A1). Calcarenite had a Moh's scratch hardness value of 2.5 and consisted of coarse-grained sand particles cemented together, producing a highly friable form of rock that could be easily fractured by hand (Figure A2). Calcarenite was a light-coloured substratum, ranging from a dull orange to light brown in colour. Mount Gambier limestone was recorded at Racecourse Bay West, where it occurred as extensive platform areas (Table 2, Figure A1). Mount Gambier limestone had a Moh's scratch hardness value of 2.0 and consisted of a matrix of fine-grained calcium carbonate particles that produced a highly friable form of rock that could be easily fractured by hand (Figure A2). It had a lighter pale-yellow colouration. Flint was recorded at both Racecourse Bay East and Racecourse Bay West, where it only occurred as small, complex boulders (Table 2, Figure A1). Flint boulders had a Moh's scratch hardness value of 8.0 and displayed negligible friability (Table 2). Each flint boulder had a coarse-grained, rough surface texture that was a lighter grey to pale yellow in colouration. The surface conditioning of flint boulders was the result of extensive weathering (C. Flaxman pers. comm.), with un-weathered flint inside of boulders smoother in texture, and a darker black in colouration (Figure A2).

Habitats

Five types of habitat were identified using line-intercept transects during geological, habitat, and biodiversity inventories in the Lower South East and Upper South East Marine Parks. These were: emersed substratum; submerged substratum; boulders; rock pools; and sand deposits (Table 3). Emersed substratum was recorded at five of the six sampled sites, and was the dominant type of habitat at each site where it occurred, with a mean percentage cover (± standard error) ranging from 46.6 (± 7.5) % at Racecourse Bay West to 83.2 (± 3.4) % at Robe South (Table 3). Submerged substratum was only recorded at Racecourse Bay West, where it had a mean percentage cover of 28.8 (± 3.6) % (Table 3). Boulders were recorded across three sampled sites, with a mean percentage cover ranging from 5.3 (± 5.3) % at Nora Creina to 100 (± 0) % at Racecourse Bay East, where boulders were observed to completely cover the shore (Table 3). Rock pools were recorded at four of the six sampled sites, with a mean percentage cover ranging from 8.7 (± 5.5) % at Racecourse Bay West to 24.1 (± 11.9) % at Nora Creina (Table 3). Although recorded at five of the six sampled sites, sand deposits generally covered only small areas of the seashore, with a mean percentage cover ranging from 0.9 (± 0.9) % at Racecourse Bay West to 16.8 (± 3.4) % at Robe South (Table 3). A graphical representation of the space occupancy of each habitat type, for each replicate transect at each site, can be found in Figures A3a-f.

Table 2: The types of rock, the form in which they were observed, the Moh's scratch hardness value, the hardness class, the number of sites each rock type was recorded at, and the number of habitats observed for each rock type identified during geological, habitat and biodiversity inventories in SA's South East Region.

Rock type	Form	Moh's scratch hardness value	Hardness class	Number of sites where rock was recorded	Number of habitats observed
Mount Gambier limestone	Platforms	2.0	Soft	1	3
Calcarenite	Platforms & heterogeneous reef	2.5	Soft	4	3
Flint	Boulders	8.0	Hard	2	1

Table 3: Mean percent cover (± standard error) of the habitat types identified at each site using line-intercept transects during geological, habitat, and biodiversity inventories in SA's South East Region. Key: - habitat not present at sampled site, * habitat present at sampled site but not measured on transects.

Habitat	Racecourse Bay East	Racecourse Bay West	Rainbow Rocks	Nora Creina	Robe South	Lake Charro	Total sites with that habitat observed
Emersed substratum	-	46.6 (± 7.5)	69.0 (± 13.5)	67.3 (± 15.0)	83.2 (± 3.4)	66.1 (±14.9)	**5**
Submerged substratum	-	28.8 (± 3.6)	-	-	-	-	**1**
Boulders	100 (± 0)	15.0 (±9.7)	-	5.3 (± 5.3)	-	-	**3**
Rock pools	-	8.7 (± 5.5)	29.3 (±13.2)	24.1 (±11.9)	*	19.2 (± 10.4)	**5**
Sand deposits	-	0.9 (± 0.9)	1.7 (± 1.7)	3.4 (± 3.4)	16.8 (± 3.4)	14.7 (± 8.1)	**5**
Habitats per site	**1**	**5**	**3**	**4**	**3**	**3**	

Site descriptions at time of 2014 winter inventory

Racecourse Bay East (38°03'27.62"S, 140°46'01.49"E) is located in a General Managed Use Zone in the lower segment of the Lower South East Marine Park (Figure 1). This south-facing rocky seashore (relative to the ocean) consists of an extensive boulder/rubble field of light-grey-coloured flint boulders. These boulders are generally of a small size (majority having a major length <40 cm), with weathered and flattened surfaces (Figure A1). No underlying bedrock was observed, with multiple layers of boulders (i.e. boulders on top of boulders) often observed. This site is considered to experience a low wave exposure, has an extremely gentle average shore slope of 0.84 % (Table 1), and is sufficiently elevated to make sampling possible during suitable daytime winter low tides (<0.60 m AHD). Significant sedimentation was observed over the lower two-thirds of the flint boulder field at the time of winter inventories.

Racecourse Bay West (38°03'31.52"S**,** 140°44'53.94"E) is located in a General Managed Use Zone in the lower segment of the Lower South East Marine Park (Figure 1). This south-facing rocky seashore (relative to the ocean) consists of an extensive, pale yellow-coloured Mount Gambier limestone platform, which is interspersed throughout by large rock pools and areas of submerged substratum (Figure A1). Small light-grey-coloured flint boulders (majority having a major length <40 cm) with weathered and flattened surfaces dominated the mid-upper levels of the eastern side of this shore, with these boulders forming a single layer (i.e. no boulders on top of boulders) on the limestone platform. This site is considered to experience a low wave exposure, has an extremely gentle average shore slope of 0.68 % (Table 1), and is sufficiently elevated to make sampling possible during suitable daytime winter low tides (<0.60 m AHD). Significant sedimentation was observed across the entirety of the limestone platform and flint boulder field at the time of winter inventories.

Rainbow Rocks (37°34'16.51"S, 140°06'42.47"E) is located in a Habitat Protection Zone in the upper segment of the Lower South East Marine Park (Figure 1). This south-west-facing rocky seashore (relative to the ocean) consists of a small, elevated segment of dull-orange-coloured calcarenite platform, which is interspersed throughout by a number of small, yet very deep (average water depth >1 m) rock pools (Figure A1). As this platform slopes gently (average 1.49 %) downwards from east at west, and from front to back (i.e. the shore is highest closest to the sea), when waves wash over the seaward extremities of this platform at high tide, water trickles down the platform, flowing from one rock pool to another. This site is considered to experience a moderate wave exposure (Table 1) and is sufficiently elevated to make sampling possible during suitable daytime winter low tides (<0.60 m AHD).

Nora Creina (37°19'46.34"S, 139°50'54.97"E) is located in a Habitat Protection Zone at the southern-most extreme of the Upper South East Marine Park (Figure 1). This south-west-facing rocky seashore (relative to the ocean) consists of a small, highly complex and friable, light-yellow-coloured calcarenite platform, which is interspersed in several places by large, deep rock pools (Figure A1). This site is considered to experience a moderate wave exposure, has a steeper average shore slope (when compared against the other sampled sites) of 3.32 % (Table 1), and is sufficiently elevated to make sampling possible during suitable daytime winter low tides (<0.60 m AHD).

Robe South (37°09'55.30"S, 139°44'34.84"E) is located in a General Managed Use Zone towards the northern end of the Upper South East Marine Park (Figure 1). This south-west-facing rocky seashore (relative to the ocean) is dominated by two dull-orange-coloured calcarenite platforms, each located at different heights on the shore (Figure A1). The lower calcarenite platform appears to only be emersed during the lowest tides, being rapidly washed over by waves shortly after low tide. This lower platform is interspersed by several large, deep rock pools (water depth >2 m) that were unable to be sampled during winter inventories due to the height of the incoming tide. The upper calcarenite platform appears to be almost permanently emersed, with waves only washing over the seaward extremities of this platform at high tide (personal observation). A small area of vertical shore marks the transition between the upper and lower platforms, giving this shore the steepest average slope (5.61 %) of the six sampled sites. This site is considered to experience a low wave exposure (Table 1), with the lower rock platform having an elevation that makes winter sampling challenging, even during suitable low tides (<0.60 m AHD).

Lake Charro (37°09'44.29"S, 139°45'51.31"E) is located in a General Managed Use Zone towards the northern end of the Upper South East Marine Park (Figure 1). This north-east-facing rocky seashore (relative to the ocean) consists of a small segment of very flat, light-brown-coloured calcarenite platform, which is divided into distinct sections by several very deep rock pools (Figure A1). This site is considered to experience a low wave exposure, has an extremely gentle average shore slope of 0.80 % (Table 1), and has a low elevation that makes winter sampling challenging, even during suitable daytime low tides (<0.60 m AHD). Substantial sedimentation was recorded at the time of winter inventories on the mid-lower levels of this calcarenite platform.

Biological observations

The sessile assemblage measured using line-intercept transects consisted of marine algae and seagrass only, with no sessile filter-feeding invertebrates recorded along transects at any of the six sampled sites. A graphical representation of the space occupancy of each sessile species, for each replicate transect at each site, can be found in Figures A3a-f. While sessile filter-feeding invertebrate species such as the tube-worms *Galeolaria caespitosa* and members of the spirorbid subfamily, plus the barnacle *Tetraclitella purpurascens* were observed under boulders during TS surveys, they were never recorded along transects. Likewise, the mussel *Xenostrobus pulex* was only recorded in several locations at Lake Charro during TS surveys, with no ascidians or sponges recorded at any of the six sampled sites.

A total of 12 marine plant species were recorded across the six sampled sites using line-intercept transects (Table 4). Overall, three species of green algae, four species of brown algae, four species of red algae, and one species of seagrass constituted the 12 recorded plant species (Table 4). Of these species, the green alga *Ulva rigida* was recorded at all six sites, while the brown alga *Hormosira banksii* was recorded at five sites (Table 4). Two species of red algae recorded the fewest observations, with *Amphiroa anceps* and *Laurencia* spp. observed at just one site each (Table 4). For the six sites sampled across SA's South East Region, the mean marine plant species richness per site was 5.67 species, with a standard deviation (SD) ±2.49 species (Figure 2a). Racecourse Bay West recorded the highest marine pant species richness with nine species, while the lowest species richness of marine plants was recorded at Robe South with two species (Figure 2a). None of the six sampled sites were identified as potential biodiversity hotspots for marine plants, as no site had a marine plant species richness two standard deviations higher than the regional mean (Figure 2a).

The species richness for each division of marine algae was variable among sites, with Racecourse Bay East recording the highest species richness of four for brown algae (Heterokontophyta). Racecourse Bay West had the highest species richness of three for red algae (Rhodophyta), and Racecourse Bay West and Rainbow Rocks the highest species richness of two for green algae (Chlorophyta) (Figure 2b). Similarity percentages were generated to characterise the marine plant assemblage sampled using line-intercept transects for each site (Table A1). Nora Creina, Lake Charro, and Racecourse Bay East were all dominated by the green alga *U. rigida*, a coralline red-algal turf dominated Robe South, the green alga *Ulva compressa* dominated Racecourse Bay West, while the brown alga *Scytosiphon lomentaria* dominated Rainbow Rocks (Table A1).

For the remaining megainvertebrate assemblage sampled using semi-quantitative abundance rankings during TS surveys, a total of 46 megainvertebrate species were recorded across the six sampled sites (Table 5). Of these species, only the air-breathing limpet *Siphonaria* spp. and the snails *Lunella undulata* and *Cominella lineolata* were recorded at all six sampled sites (Table 5). A further eight invertebrate species were recorded at five of the six sampled sites (Table 5). Eighteen species of invertebrate were only recorded at one site (Table 5). For the six sites sampled across SA’s South East Region, the mean megainvertebrate species richness per site was 21.0 species, with a SD = ±5.51 species (Figure 2c). Racecourse Bay West recorded the highest megainvertebrate species richness with 33 species, while the lowest species richness of megainvertebrates was recorded at Rainbow Rocks with 17 species (Figure 2c). Racecourse Bay West was identified as a potential biodiversity hotspot for megainvertebrates, as it had a megainvertebrate species richness more than two standard deviations higher than the regional mean (Figure 2c). The species richness for specific intertidal invertebrate phyla was generally highest at Racecourse Bay West, with the highest species richness of molluscs (20 species), arthropods (7 species) and cnidarians (2 species) all recorded there (Figure 2d). Two species of cnidarians were recorded at Lake Charro, which also had the highest species richness of Echinodermata, with two species recorded (Figure 2d).

Table 4: Species list outlining the mean percent cover (± standard error) of the dominant marine algae and seagrass identified at each site using line-intercept transects during geological, habitat, and biodiversity inventories in SA's South East Region. Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; LC = Lake Charro; - = not present at sampled site; and * = present at sampled site but not recorded on transects.

Division	**Class**	**Family**	**Species**	**RBE**	**RBW**	**RR**	**NC**	**RS**	**LC**	**Total sites observed**
Chlorophyta	Ulvophyceae	Codiaceae	*Codium pomoides*	-	-	-	*	-	*	**2**
		Ulvaceae	*Ulva compressa*	-	23.3 (±4.3)	1.6 (±1.6)	-	-	-	**2**
			Ulva rigida	38.3 (±16.9)	8.7 (±5.3)	2.6 (±1.8)	26.8 (±14.4)	2.7 (±2.7)	17.7 (±10.2)	**6**
Heterokontophyta	Phaeophyceae	Hormosiraceae	*Hormosira banksii*	1.3 (±0.6)	11.2 (±5.0)	*	2.1 (±0.9)	-	10.5 (±6.3)	**5**
		Splachnidiaceae	*Splachnidium rugosum*	3.2 (±1.5)	0.5 (±0.3)	-	-	-	1.6 (±1.1)	**3**
		Scytosiphonaceae	*Colpomenia sinuosa*	0.3 (±0.2)	-	-	-	-	0.4 (±0.4)	**2**
			Scytosiphon lomentaria	3.2 (±1.5)	13.6 (±3.7)	15.1 (±4.5)	-	-	-	**3**
Rhodophyta	Florideophyceae	Corallinaceae	*Amphiroa anceps*	-	0.5 (±0.3)	-	-	-	-	**1**
			Mixed coralline turf	3.2 (±1.5)	4.1 (2.5)	-	-	56.5 (±8.3)	5.3 (±5.3)	**4**
		Gelidiaceae	*Capreolia implexa*	0.3 (±0.2)	1.7 (1.1)	-	5.2 (±2.3)	-	-	**3**
		Rhodomelaceae	*Laurencia* spp.	-	-	-	-	-	10.9 (9.0)	**1**
Magnoliophyta	Liliopsida	Zosteraceae	*Zostera tasmanica*	-	1.2 (±1.2)	-	-	-	4.9 (±4.9)	**2**
Marine plant species richness per site				**7**	**9**	**4**	**4**	**2**	**8**	

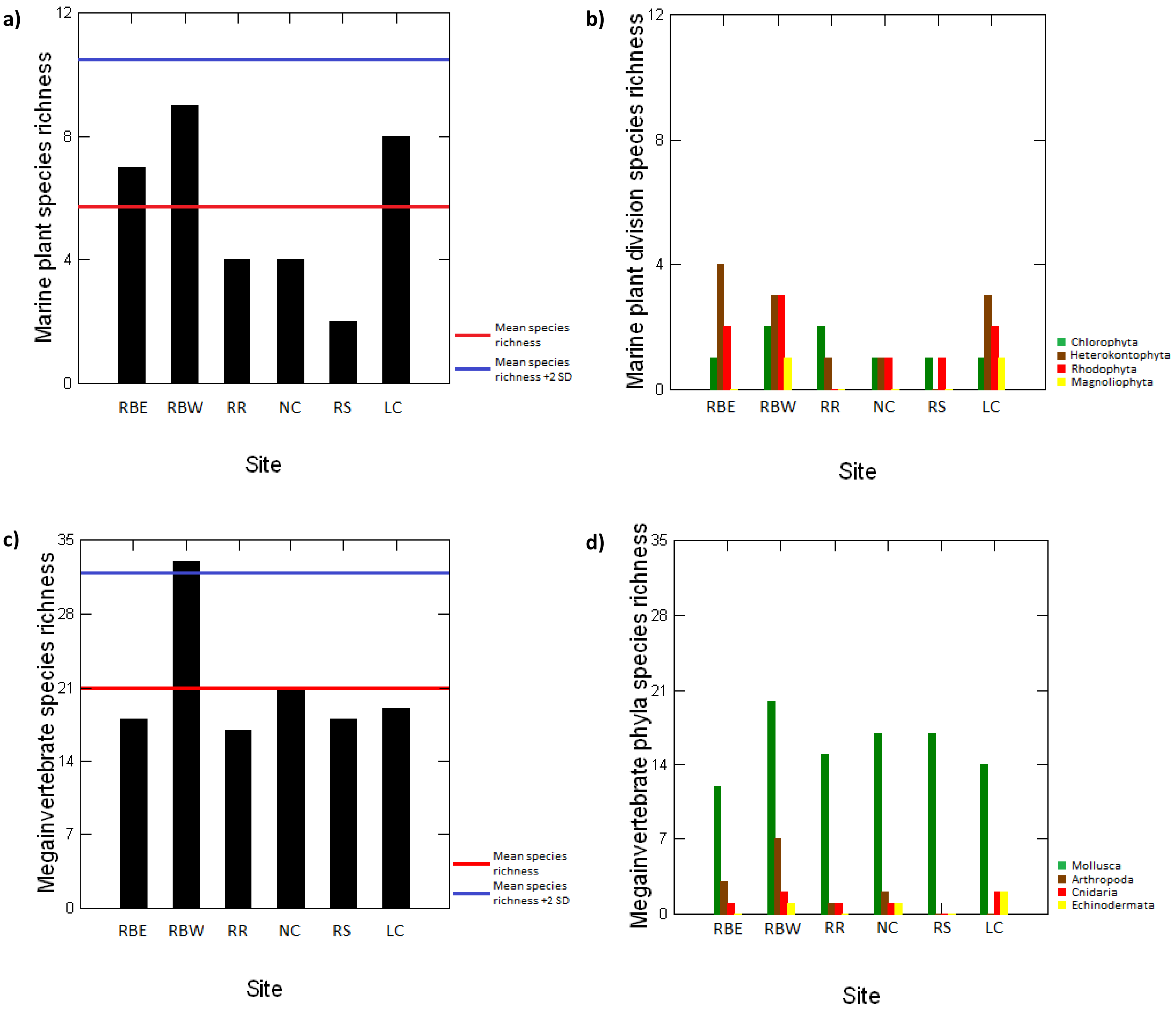


Figure 2: Species richness recorded for each site by pooling marine plant species for replicate transects and megainvertebrate species across TS surveys for: a) marine plants; b) divisions of marine plants; c) megainvertebrates; and d) phyla of megainvertebrates. Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; and LC = Lake Charro.

Table 5: Species list for megainvertebrate identified at each site during geological, habitat, and biodiversity inventories in SA's south-east. The megainvertebrate assemblage column specifies which megainvertebrate assemblage(s) (from cluster analysis) that each species was found in. Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; and LC = Lake Charro.

Phyla	**Class**	**Family**	**Species**	**RBE**	**RBW**	**RR**	**NC**	**RS**	**LC**	**Total sites observed**	**Megainvertebrate assemblage(s)**
Cnidaria	Anthozoa	Actiniidae	*Actinia tenebrosa*		**X**	**X**	**X**		**X**	**4**	**2, 3, 4**
			Isanemonia australis	**X**	**X**				**X**	**3**	**1, 2, 3, 4**
Platyhelminthes	Rhabditophora	Notoplanidae	*Notoplana australis*		**X**					**1**	**2**
Annelida	Polychaeta	Serpulidae	*Galeolaria caespitosa*	**X**	**X**			**X**	**X**	**4**	**1**
			Spirorbid	**X**	**X**					**2**	**1**
Echinodermata	Asteroidea	Goniasteridae	*Tosia australis*						**X**	**1**	**4**
	Echinoidea	Echinometridae	*Heliocidaris erythrogramma*		**X**		**X**			**2**	**2, 4**
		Temnopleuridae	*Amblypneustes ovum*						**X**	**1**	**4**
Mollusca	Gastropoda	Nacellidae	*Cellana tramoserica*		**X**	**X**	**X**	**X**	**X**	**5**	**1, 3, 4**
		Lottidae	*Notoacmea petterdi*					**X**		**1**	**3**
			Notoacmea spp.	**X**	**X**		**X**	**X**	**X**	**5**	**1, 2, 3**
			Patelloida alticostata			**X**	**X**	**X**	**X**	**4**	**3, 4**
		Neritopsidae	*Nerita atramentosa*	**X**	**X**	**X**	**X**	**X**		**5**	**1, 3, 4**
		Haliotidae	*Haliotis laevigata*		**X**					**1**	**2**
			Haliotis rubra						**X**	**1**	**4**
		Fisurellidae	*Montfortula rugosa*	**X**	**X**					**2**	**1, 2**
		Trochidae	*Austrocochlea constricta*	**X**	**X**	**X**	**X**			**4**	**1, 2, 3, 4**
			Cantharidella balteata						**X**	**1**	**4**
			Chlorodiloma adelaidae		**X**	**X**	**X**	**X**	**X**	**5**	**2, 3, 4**
			Diloma concamerata	**X**	**X**	**X**		**X**		**4**	**1, 3**
			Phasianotrochus eximius					**X**		**1**	**3**
		Turbinidae	*Lunella undulata*	**X**	**X**	**X**	**X**	**X**	**X**	**6**	**1, 2, 3, 4**
		Littorinidae	*Afrolittorina praetermissa*		**X**	**X**	**X**	**X**	**X**	**5**	**1, 3**
			Austrolittorina unifasciata		**X**	**X**	**X**	**X**	**X**	**5**	**1, 3**
			Bembicium nanum	**X**	**X**	**X**	**X**	**X**		**5**	**1, 3**
			Bembicium vittatum			**X**		**X**		**2**	**3**

Phyla	Class	Family	Species	RBE	RBW	RR	NC	RS	LC	Total sites observed	Megainvertebrate assemblage(s)
		Buccinidae	*Cominella lineolata*	**X**	**X**	**X**	**X**	**X**	**X**	**6**	**1, 2, 3, 4**
		Fasciolariidae	*Australaria australasia*				**X**			**1**	**4**
		Batillariidae	*Eubittium lawleyanum*		**X**					**1**	**2**
		Muricidae	*Dicathais orbita*	**X**	**X**		**X**		**X**	**4**	**1, 2, 3, 4**
			Haustrum vinosum	**X**	**X**			**X**		**3**	**1, 2, 3**
		Conidae	*Conus anemone*		**X**	**X**	**X**			**3**	**2, 3, 4**
		Siphonariidae	*Siphonaria* spp.	**X**	**X**	**X**	**X**	**X**	**X**	**6**	**1, 2, 3, 4**
		Aplysiidae	*Aplysia parvula*		**X**		**X**			**2**	**1, 2, 3, 4**
	Polyplacophora		Polyplacophora sp.	**X**						**1**	**1**
		Mopalidae	*Plaxiphora albida*		**X**	**X**	**X**	**X**	**X**	**5**	**1, 2, 3, 4**
	Bivalvia	Mytilidae	*Xenostrobus pulex*						**X**	**1**	**3**
Arthropoda	Maxillopoda	Tetraclitidae	*Tetraclitella purpurascens*	**X**	**X**					**2**	**1, 2**
	Malacostraca	Grapsidae	*Leptograpsus variegatus*				**X**			**1**	**4**
		Hymenosomatidae	*Halicarcinus ovatus*		**X**					**1**	**2**
		Leucosiidae	*Bellidilia laevis*	**X**	**X**					**2**	**1, 2**
		Plagusiidae	*Guinusia chabrus*				**X**			**1**	**4**
		Varunidae	*Cyclograpsus granulosus*	**X**	**X**	**X**				**3**	**1, 2, 3**
		Sphaeromatidae	*Zuzara venosa*		**X**					**1**	**1, 2**
		Ligiidae	*Ligia australiensis*		**X**					**1**	**1**
		Palaemonidae	*Palaemon serenus*		**X**					**1**	**2**
Invertebrate species richness per site				**18**	**33**	**17**	**21**	**18**	**19**		

Percent coverage of marine plants

No significant difference in the space occupancy of marine plants, sampled using line-intercept transects, was detected among sites (Figure 3a, ANOVA *p*-value >0.05). The grand mean for marine plant percentage cover across the six sampled sites was 48.3 % cover, with a SE = 5.9 %. Racecourse Bay West was observed to have the highest percentage cover of marine plants, with a mean percentage cover of 64.7 (± 10.8) %, while Rainbow Rocks had the lowest percentage cover of marine plants, with a mean percentage cover of 19.3 (± 7.7) % (Figure 3a).

A significant difference in the percentage cover of red algae was detected among sites (Figure 3b, ANOVA *p*-value = <0.001). Pair-wise tests indicate that Robe South was significantly different from all other sampled sites, with this difference associated with the much higher percentage cover of a red algal coralline turf at Robe South (mean = 56.5 ± 8.3 %, Table 4) compared to the five other sampled sites (grand mean = 6.0 ± 1.9 %) (Figure 3b, Tables A2 & A3a).

A significant difference in the percentage cover of brown algae was also detected among sites (Figure 3c, ANOVA *p*-value = 0.017). Pair-wise tests indicate that the percentage cover of brown algae at Racecourse Bay West (mean = 25.2 ± 5.9 %) was significantly different from the percentage cover of brown algae at Nora Creina (mean = 2.1 ± 0.9 %) and Robe South (zero) (Figure 3c, Tables A2 & A3b). These pair-wise differences were associated with the significantly higher percentage cover of the brown alga *S. lomentaria* at Racecourse Bay West (Table 4).

No significant difference in the percentage cover of green algae was detected among sites (Figure 3d, ANOVA *p*-value >0.05). The grand mean for green algal percentage cover across the six sampled sites was 23.0 % cover, with a SE = 5.2 %. Racecourse Bay East was observed to have the highest percentage cover of green algae, with a mean percentage cover of 38.3 (± 16.9) %, while Robe South had the lowest coverage of green algae, with a mean percentage cover of 2.7 (± 2.7) % (Figure 3d).

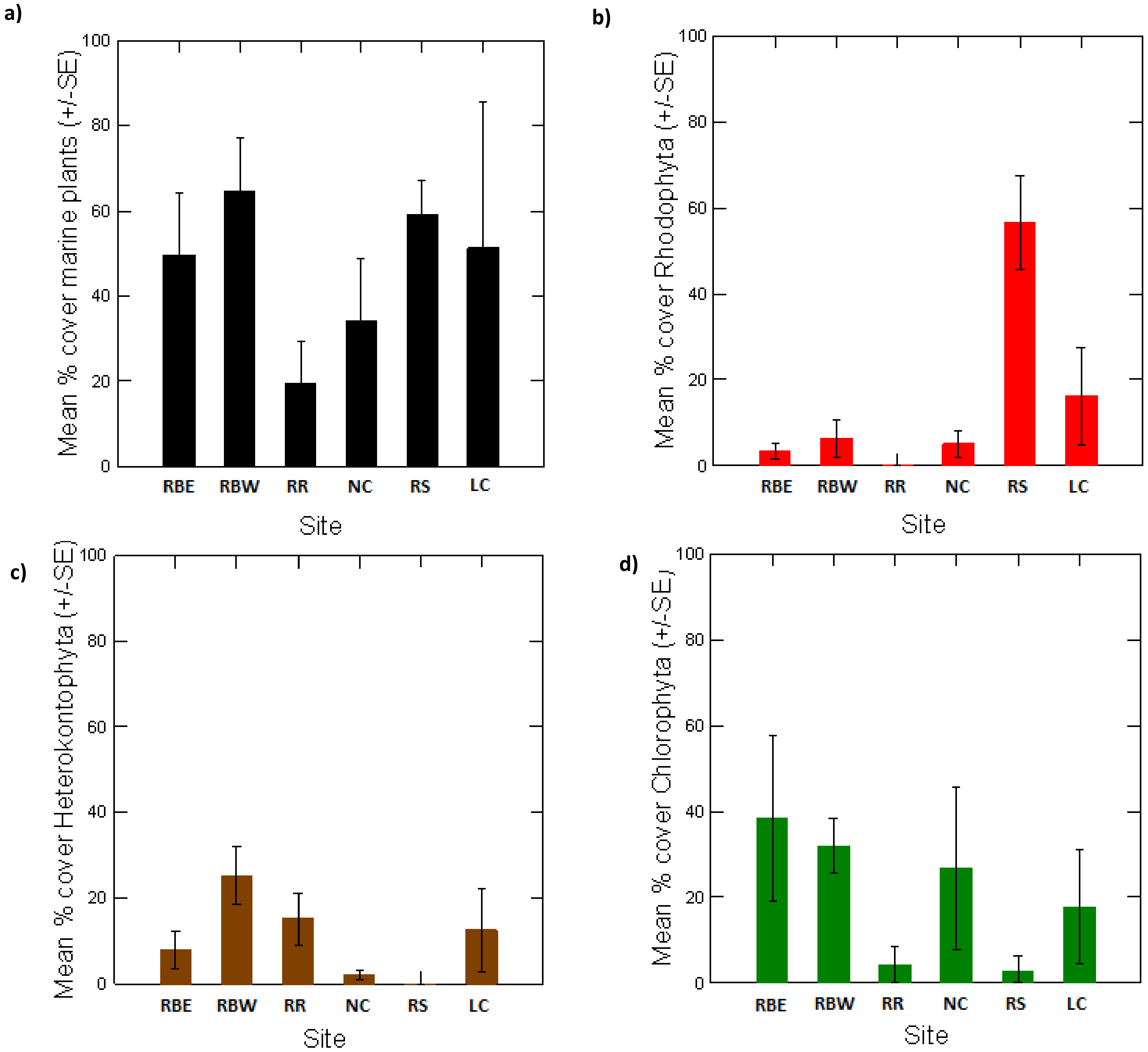


Figure 3: Mean (± standard error) percentage cover of marine plants recorded along line-intercept transects at each site for: a) the whole marine plant assemblage; b) the division Rhodophyta; c) the division Heterokontophyta; and d) the division Chlorophyta. Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; and LC = Lake Charro.

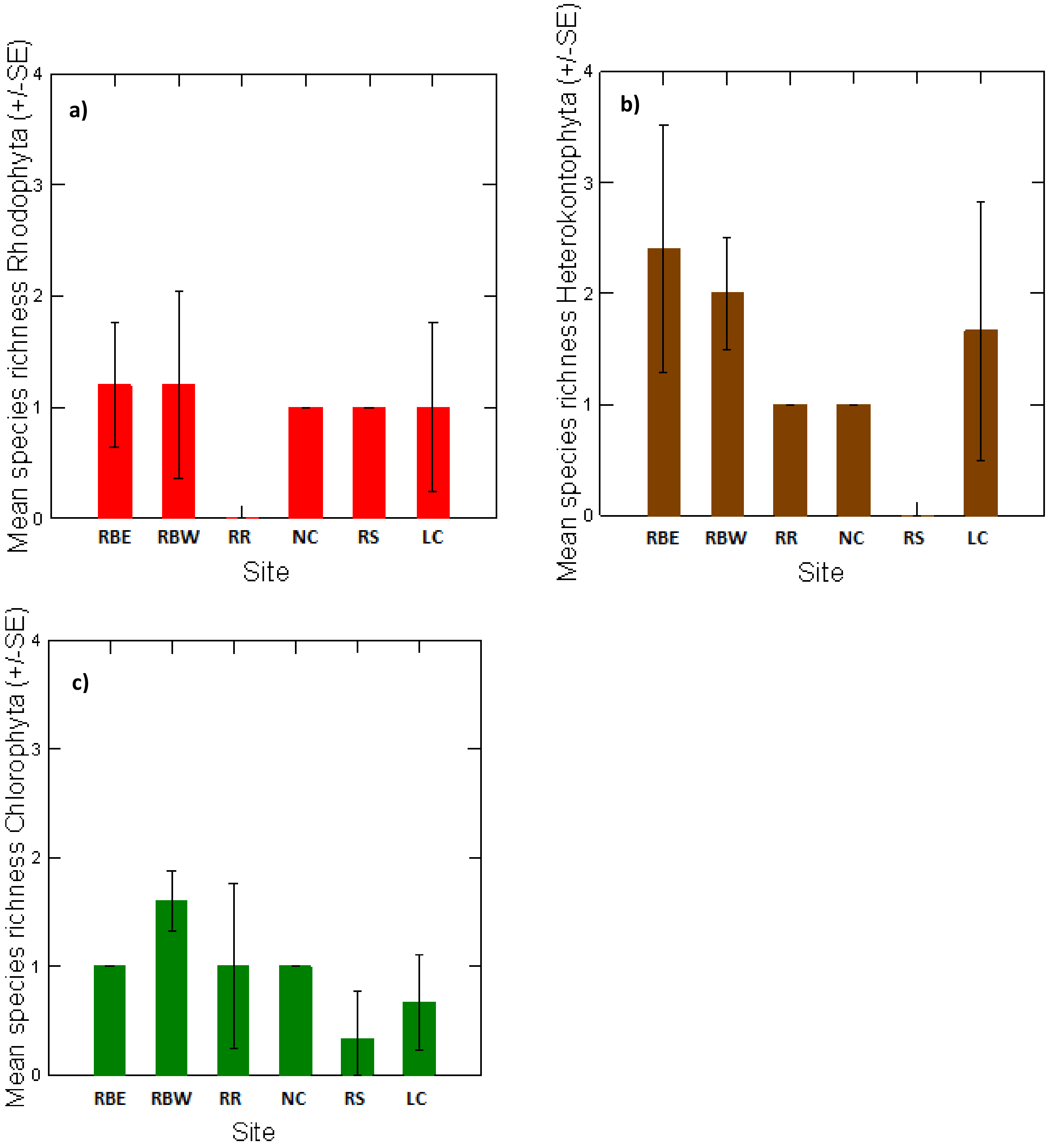


Figure 4: Mean (± standard error) species richness of algae divisions recorded along line-intercept transects at each site for: a) Rhodophyta; b) Heterokontophyta; and c) Chlorophyta Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; and LC = Lake Charro.

Species richness of marine plant divisions

No significant difference in the species richness of red algae, sampled using line-intercept transects, was detected among sites (Figure 4a, ANOVA *p*-value >0.05). The grand mean for red algae species richness across the six sampled sites was 0.95 species, with a SE = 0.21. No significant difference in the species richness of brown algae was detected among sites (Figure 4b, ANOVA *p*-value >0.05). The grand mean for brown algae species richness across the six sampled sites was 1.50 species, with a SE = 0.30. No significant difference in the species richness of green algae was detected among sites (Figure 4c, ANOVA *p*-value >0.05). The grand mean for green algae species richness across the six sampled sites was 1.00 species, with a SE = 0.13.

Structure of the marine plant assemblage

An nMDS ordination plot (2D stress = 0.17) from the space occupancy of marine plant species sampled using line-intercept transects was created to examine assemblage structure differences among sites (Figure 5). In particular, the marine plant assemblage at Robe South was quite distinct when compared against the assemblages sampled elsewhere (Figure 5). A subsequent PERMANOVA that examined the distinctness of marine plant assemblage structure differences among sites produced a significant result (PERMANOVA *p*-value = 0.0001, Table A4). Pair-wise tests indicate that the structure of marine plant assemblages was significantly different between each pair of sites except Lake Charro and Racecourse Bay East, Lake Charro and Nora Creina, and Nora Creina and Racecourse Bay East (Monte Carlo test *p*-value >0.05, Table A5). A SIMPER analysis revealed that where a significant difference was detected, average dissimilarity in the structure of marine plant assemblages among sites ranged between 69.2 % for the comparison between Racecourse Bay West and

Rainbow Rocks, and 97.8 % for the comparison between Robe South and Rainbow Rocks (Table A6). Generally, assemblage structure differences among sites were driven by the higher percentage cover of a single algal species at one of the compared sites (Table A6).

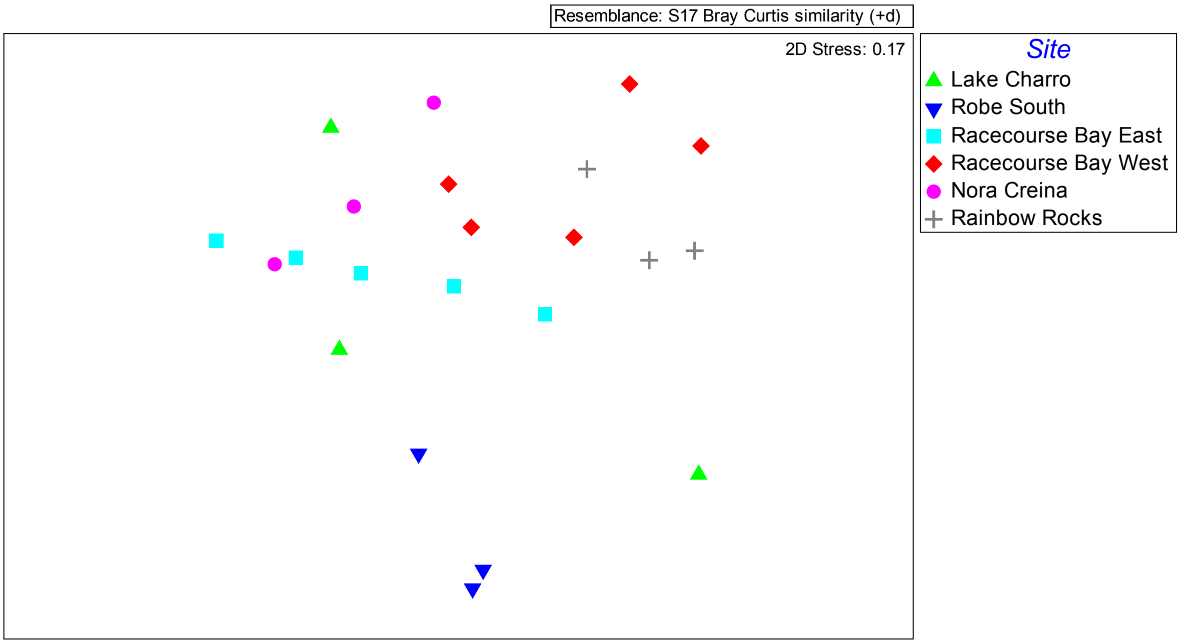


Figure 5: Two-dimensional nMDS ordination plot (based on Bray-Curtis similarity) depicting differences in the structure of marine plant assemblages among sites sampled during geological, habitat, and biodiversity inventories in SA’s South East Region. Each point represents a single transect.

Structure of the intertidal megainvertebrate assemblage

A cluster analysis of the megainvertebrate assemblage sampled using semi-quantitative abundance rankings from TS surveys indicated that at approximately 45-50 % Bray-Curtis similarity, surveys clustered into four distinct groups (Figure 6). It is important to acknowledge that each of these groups does not represent a specific location, but rather a specific type of megainvertebrate assemblage (Figure 6). TS surveys from boulder habitats at Racecourse Bay (East and West) clustered into one group (first assemblage), although boulder habitats at Racecourse Bay West were different from those at Racecourse Bay East at approximately 70% Bray Curtis similarity (as indicated by the solid black line differentiating Racecourse Bay East and Racecourse Bay West in the first assemblage box in Figure 6). Surveys from submerged habitats (bedrock and pools) at Racecourse Bay clustered into another (second assemblage), all surveys from emersed habitats plus rock pools at Rainbow Rocks clustered into a third group (third assemblage), while surveys from rock pools at Lake Charro and Nora Creina formed a fourth group (fourth assemblage) (Figure 6). Patterns in the intertidal megainvertebrate data were explored further by using these four assemblages.

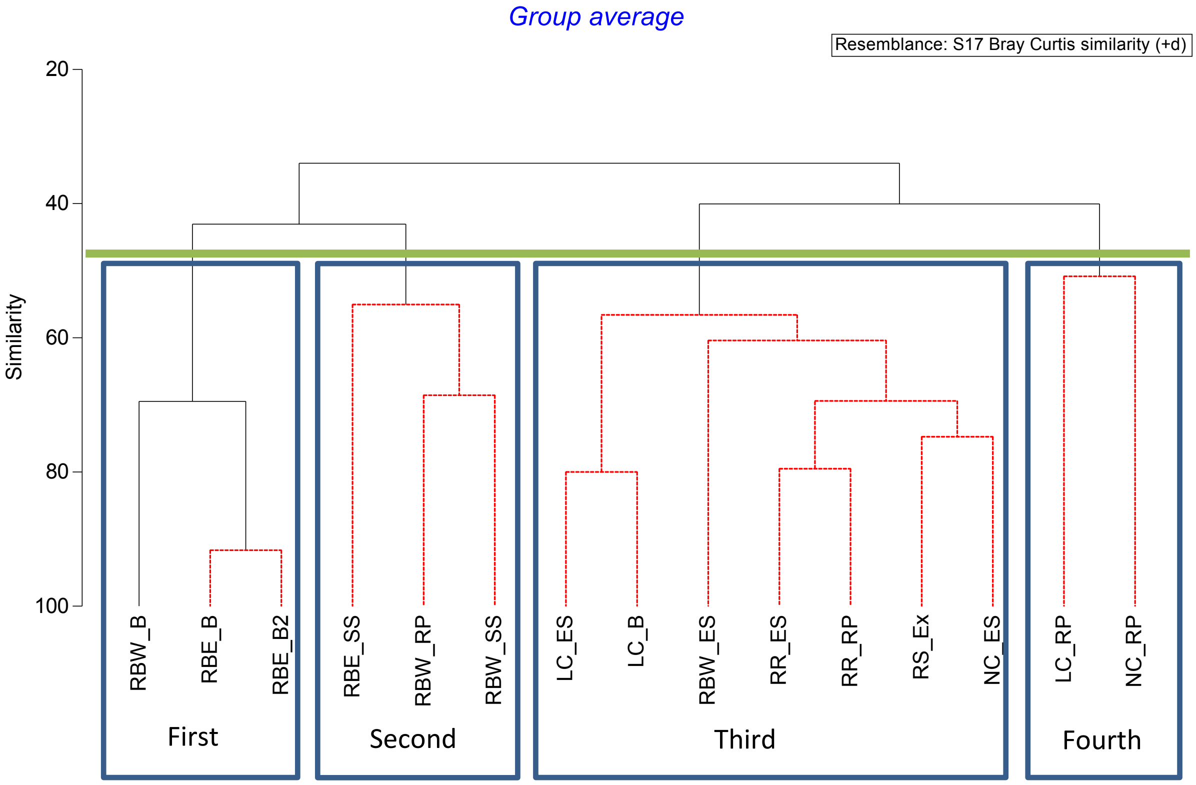


Figure 6: Cluster analysis depicting how individual TS surveys were differentiated into four distinct groups at approximately 45-50 % (green horizontal line) Bray Curtis similarity value (clear groups denoted by solid black lines but less-certain groups by red lines). Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; LC = Lake Charro; _B = Boulders*; _SS = submerged substratum; _ES = emersed substratum; and _RP = rock pools. *Boulder habitats were sampled twice at Racecourse Bay East due to their extensive coverage of the intertidal shore, with the second sampling of boulders denoted by _B2.

A constrained CAP ordination produced very strong canonical correlations ($\delta 1 = 0.98$ and $\delta 2 = 0.85$), strongly supporting the distinction of the four assemblages identified during cluster analysis (Figure 7a). By superimposing vectors (for rho >0.8) over this CAP ordination plot (Figure 7b), and conducting a SIMPER analysis on these four assemblages, it was possible to determine which species characterised each assemblage (Table A7). The first assemblage, identified on boulder habitats at Racecourse Bay, was characterised by the relatively high abundances of the snails *N. atramentosa*, *Diloma concamerata*, and *Austrocochlea constricta*, limpet *Notoacmea* spp., and barnacle *T. purpurascens* sheltering underneath boulders (Figure 7b & Table A7). The second assemblage, associated with submerged substratum habitats at Racecourse Bay, was characterised by the relatively high abundances of the anemone *Isanemonia australis* and the snail *A. constricta* (Figure 7b & Table A7). The third assemblage, identified on emersed substrata at all sites and the small yet very deep rock pools at Rainbow Rocks, was characterised by the periwinkles *Austrolittorina unifasciata* and *Afrolittorina praetermissa*, and the limpets *Siphonaria* spp. and *Cellana tramoserica* (Figure 7b & Table A7). The fourth assemblage, identified in rock pools at Lake Charro and Nora Creina, was characterised by the relatively high abundances of the snails *Chlorodiloma adelaidae*, *Lunella undulata*, and *C. lineolata* (Figure 7b & Table A7).

A SIMPER analysis also revealed which species contributed most to structural differences being detected among the four megainvertebrate assemblages identified during cluster analysis (Table A8). Average dissimilarity in the structure of intertidal megainvertebrate assemblages ranged between 58.5 % for the comparison between the first (Racecourse Bay boulders) and second (Racecourse Bay submerged substrata) assemblages, and 81.9 % for the comparison between the first and fourth (rock pools at Nora Creina and Lake Charro) assemblages (Table A8). Generally, structural differences among assemblages were associated with the higher abundance of several megainvertebrate species for one assemblage when compared against the other (Table A8).

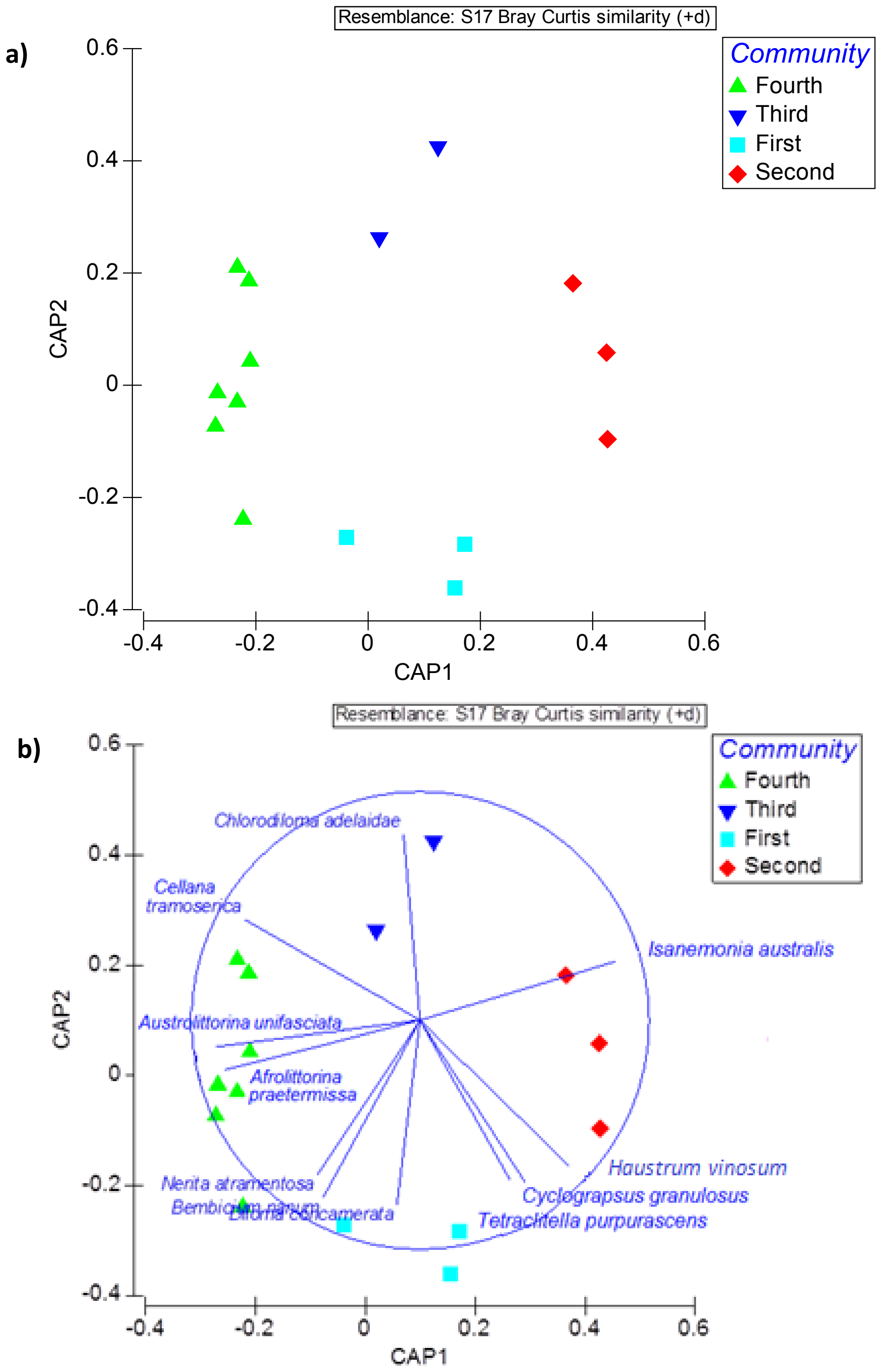


Figure 7: Constrained CAP ordination plot depicting the separation of the four assemblages identified during cluster analysis for: a) the first two canonical correlations (axes); and with b) vector overlay of Spearman rank correlations (for rho >0.8) for individual species contributing to differences in assemblage structure among the four examined groups.

Correlations between sampled habitats and biodiversity

A positive linear correlation (r value = 0.584) was detected between the species richness of marine plants sampled using line-intercept transects and the number of habitats sampled at each site (Figure 8a & Table A9). However, due to the small number of sites sampled (n = 6), this relationship was not statistically significant (p-value >0.05, Table A9). The r^2 value for this relationship was 0.34, indicating only a small proportion (approximately one-third) of the total variability in the species richness of marine plants may be accounted for by variation in the number of habitats that were sampled at each site. This indicates that the number of habitats present at a given site will not necessarily act as a strong indicator for the likely marine plant species richness at that site.

A positive linear correlation (r value = 0.766) was detected between the species richness of megainvertebrates sampled using TS surveys and the number of habitats sampled at each site (Figure 8b & Table A9). However, due to the small number of sites sampled (n = 6), this relationship was again not statistically significant (p-value >0.05, Table A9). Furthermore, this relationship was strongly influenced by the outlier Racecourse Bay West, which positively influenced the strength of the Pearson correlation (r value = 0.11) (Figure 8b). Consequently, the r^2 value of 0.59 for this relationship should be interpreted cautiously. Despite these limitations, this positive linear relationship indicates that as the number of habitats sampled at a site increases, a greater diversity of megainvertebrates may be recorded. Further sampling across a larger number of sites in the region is required to confirm the validity of this relationship. If a significant positive correlation is detected between megainvertebrate species richness and the number of habitats sampled, it may be possible to use the number of habitats present at a site as a viable surrogate to identify which rocky seashores are likely to support a greater diversity of intertidal invertebrates.

A positive linear correlation (*r* value = 0.594) was detected between the species richness of marine plants sampled using line-intercept transects, and the species richness of megainvertebrates sampled using TS surveys at each site (Figure 8c & Table A9). However, again due to the small number of sites sampled (*n* = 6), this correlation was not statistically significant (*p*-value >0.05, Table A9). Furthermore, this relationship was driven almost entirely by the outlier Racecourse Bay West, which positively influenced the strength of the Pearson correlation (*r* value = 0.52) (Figure 8c). Upon its removal, almost no correlation whatsoever was detected between marine plant and megainvertebrate species richness (*r* value = 0.067). This indicates that the species richness of marine plants sampled using line-intercept transects will not indicate the potential species richness of megainvertebrates sampled using TS surveys (and vice-versa) at a given site.

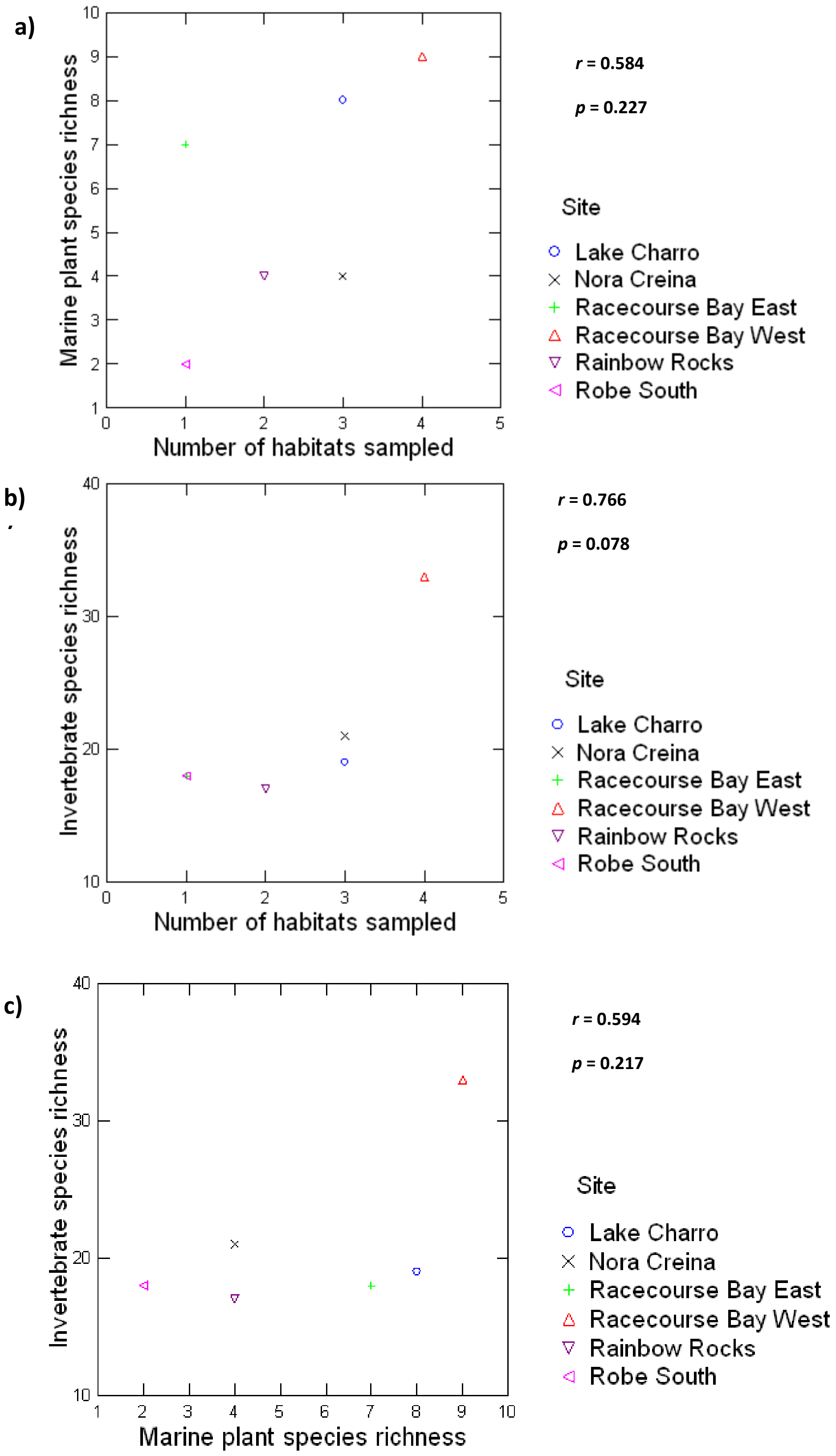


Figure 8: Scatterplots depicting the relationships between: a) marine plant species richness and the number of habitats sampled; b) invertebrate species richness and the number of habitats sampled; and c) invertebrate species richness and marine plant species richness. Racecourse Bay West is an outlier in Figures 8b and 8c, where it positively influences the strength of the Pearson correlation by 0.11 and 0.52 respectively.

Discussion

Overall, a total of 12 marine plant and 46 megainvertebrate species were recorded across six sites in the state's South East Region using line-intercept transects and TS surveys, respectively. Of these 46 megainvertebrate species, 25 mollusc and three echinoderm species were recorded. The species richness values from these preliminary surveys are considerably lower than those recorded previously during biodiversity assessments using similar methods for rocky seashores in other locations around SA. For example, Benkendorff (2005) recorded 82 mollusc and eight echinoderm species pooled across 10 granite or limestone seashores from Althorpe Island and the Yorke Peninsula. Benkendorff et al. (2007) recorded 94 mollusc and echinoderm species and 55 marine plant species when conducting biodiversity inventories across five limestone seashores on Kangaroo Island. Furthermore, Benkendorff and Thomas (2007) recorded 141 mollusc and echinoderm species and 49 marine plant species during biodiversity assessments for 17 seashores of differing rock types along the Fleurieu Peninsula.

The comparatively low species richness of marine plants and megainvertebrates recorded during this study may be accounted for by several factors. The first of these is differences in the amount of sampling effort invested during each biodiversity inventory conducted for SA rocky seashores (Table 6). While each study employed an un-replicated sampling regime (i.e. each rocky seashore was only sampled once using TS surveys), there were differences in the number of sites sampled, the total time spent conducting TS surveys, the number of line-intercept transects sampled, and the number of habitats sampled within each study region (Table 6). For example, Benkendorff (2005) examined four habitat types across 10 rocky seashores, and spent 600 minutes conducting TS surveys for mollusc and echinoderm species on Althorpe Island and the Yorke Peninsula. Moreover, Benkendorff and Thomas (2007) examined 11 habitat types across 17 rocky seashores, and spent 1020 minutes conducting TS surveys for mollusc and echinoderm species along the Fleurieu Peninsula. In contrast, the present study examined four habitat types across only six rocky seashores, and spent just 450 minutes conducting TS surveys that encompassed the entire intertidal invertebrate assemblage (Table 6). Consequently, it is possible that the smaller sampling effort employed during this study, especially for the number of sites sampled and the total time spent conducting TS surveys, may help to account for the comparatively low species richness reported here.

Table 6: A comparison among studies for the sampling effort invested during biodiversity inventories for rocky seashores in SA. Key: N/A = not sampled in described study.

Location assessed	**Number of sites assessed**	**Region of shore assessed**	**Number of habitats assessed**	**Number of transects sampled**	**Number of TS surveys completed**	**Total sampling time (minutes)**	**Number of marine plant species recorded**	**Number of invertebrate species recorded**	**Reference**
Yorke Peninsula & Althorpe Island	10	Lower & middle only	4	0	10	600	N/A	90	Benkendorff (2005)
Kangaroo Island	5	Lower & middle only	6	0	5	195	55	94	Benkendorff et al. (2007)
Fleurieu Peninsula	17	Lower & middle only	11	85	17	1020	49	141	Benkendorff & Thomas (2007)
South-east	6	Entire shore	5	22	15	450	12	46	Present study

Another factor that may help to explain the comparatively low marine plant and megainvertebrate species richness recorded for SA's South East Region is the type of rock that constitutes each seashore. Across the six seashores sampled, two rocks from the soft class were recorded, Mount Gambier limestone and calcarenite. One rock from the hard class was recorded, boulders comprised of flint. The previously described biodiversity assessments for the Yorke and Fleurieu Peninsulas, plus several honours theses produced at Flinders University, have reported differences among intertidal assemblages inhabiting different rocks (Benkendorff 2005; Benkendorff and Thomas 2007; Dutton 2007; Liversage and Benkendorff 2013; Liversage et al. 2014). Specifically, limestone supported a lower species richness of invertebrates, or abundances of individual invertebrate taxa, when compared against rock types from the hard class (Benkendorff and Thomas 2007; Dutton 2007; Liversage and Benkendorff 2013). However, see the species richness results for later-successional assemblages on boulders in Chapter 3 as a point of contrast.

In the present study, the lack of a balanced sampling design for rocks within each hardness class precludes any statistical analyses investigating how rock type or rock hardness may impact intertidal assemblage structure from being completed. However, given that four of the six sites sampled were comprised of the very soft, and highly friable calcarenite (Table 1), it's possible that calcarenite may support megainvertebrate assemblages with a lower species richness. Moreover, while flint boulders at Racecourse Bay East had a similar megainvertebrate species richness to the four calcarenite seashores, the mixed Mt Gambier limestone and flint rock types at Racecourse Bay West supported the highest megainvertebrate species richness recorded (Figure 2c). This observation indicates that rock type may have an additive effect on species richness, supporting previous studies (e.g.

Benkendorff 2005; Benkendorff and Thomas 2007; Dutton 2007; Liversage and Benkendorff 2013; Liversage et al. 2014) that have reported invertebrate assemblage differences across different types of rock.

It has been well documented that rocks from the soft class display faster erosion rates than rocks from the hard class, and that the fastest rates of erosion are recorded in environments with the greatest wave energy (Kirk 1977; Spencer 1985). Given the south-east coast is frequently exposed to strong waves and swell, especially during winter, the soft and highly friable rocks that dominate this region are likely to be highly susceptible to weathering and erosion. Consequently, the dynamic nature of the predominantly soft-rock substratum across SA’s South East Region may provide an unsuitable habitat for some intertidal species. This concept is exemplified by the almost complete absence of several sessile invertebrate species on platform areas that require a stable substratum for semi-permanent or permanent attachment including tube-worms, barnacles, and mussels. Furthermore, both calcarenite and Mount Gambier limestone are both softer than the fossiliferous limestone (Moh’s scratch hardness = 3.0) found in other parts of SA including the Fleurieu Peninsula and Kangaroo Island (see Chapter 2). Consequently, the softer rocks of the South East region may offer less resistance to the forces of weathering and erosion than fossiliferous limestone, potentially providing a less-suitable habitat for some intertidal species. This may help to account for the low invertebrate and plant species richness recorded in the present study when compared to that recorded on Kangaroo Island (Benkendorff et al. 2007), which also examined intertidal assemblages on rocks from the soft class for a similar number of sites (Table 6).

An alternative explanation that may account for the comparatively low marine plant and invertebrate species richness recorded in the South East region is the time of year that sampling was conducted. The biodiversity assessments conducted for rocky seashores along the Fleurieu Peninsula, Yorke Peninsula, and Kangaroo Island all conducted sampling during suitable daytime low tides over mid-spring and summer (Benkendorff 2005; Benkendorff et al. 2007; Benkendorff and Thomas 2007). The present study conducted biodiversity inventories during suitable daytime low tides during the final week of winter. While these inventories were effective in collecting baseline data for this region, they did not effectively assess intertidal biodiversity on the lower extremes of the rocky seashore. Over winter, low-pressure atmospheric cells (i.e. cold fronts), strong winds, large swells, and relatively high low-tides (low tide height never <0.40 m AHD) interact to prevent the lower-most extremes of rocky seashores from becoming emersed at low tide. This was particularly poignant at the four calcarenite-dominated sites with their apparently lower elevation, which effectively prevented the lower intertidal shore from being examined whatsoever during these winter inventories. Consequently, it is possible that any species solely living on the lower intertidal shore were not recorded in the inventories reported here (up to half the algal species and a quarter of the invertebrate species, PGF, pers. obs.).

During the final week of the 2014 winter, no significant difference in the space-occupancy or species richness of marine plants was detected among the six sampled sites. However, a significant difference was detected among sites for the assemblage structure of marine plants, except amongst Lake Charro, Racecourse Bay East and Nora Creina (see Figure 5). Racecourse Bay West, located in a General Managed Use Zone, recorded both the greatest percentage cover (64.7 ± 10.8 %) and highest species richness (nine) of marine plants (Table

3, Figure 3a). No site recorded a marine plant species richness two standard deviations higher than the regional mean (Figure 2a). As such, no sites sampled during this study were identified as potential marine plant hotspots. The higher species richness and space-occupancy of marine plants at Racecourse Bay West may be explained by the occurrence of multiple rock types at the same location, which may offer some marine plant species a greater diversity of exploitable habitats and growing conditions. This is reflected by Racecourse Bay West having the greatest number of habitats (five) across the six sampled sites (Table 3), ranging from water-filled depressions (i.e. both submerged substratum and rock pools) and sand deposits on the soft Mount Gambier limestone platform, to the sheltered and shaded undersides of hard flint boulders. However, the correlation between marine plant species richness and the number of habitats sampled produced a non-significant result, although this may be driven by the small number of sites sampled (*n* = 6). Therefore, it would be prudent to sample a greater number of sites to better understand any relationship that may exist between marine plant species richness and the number of habitats sampled, and to determine whether the presence of multiple rock types at a single location contributes to this relationship in any way (i.e. is the greatest number of habitats generally found at sites comprised by multiple types of rock).

For the intertidal invertebrate assemblage, TS surveys were successful in establishing a baseline of the assemblages associated with each type of habitat. By exploring patterns in the invertebrate data collected during the final week of the 2014 winter, it was possible to identify four distinct megainvertebrate assemblages from the six sites sampled (Figure 6). Each assemblage was generally characterised by several dominant species that were strongly associated with a specific type of habitat. For example, flint boulders at Racecourse

Bay East and West were characterised by high abundances of several megainvertebrate species sheltering under boulders, including the barnacle *T. purpurascens*. This species has been shown previously to require shaded habitats in order to survive the harsh physical stresses of the intertidal environment (Denley and Underwood 1979). The undersides of flint boulders at Racecourse Bay may afford *T. purpurascens* such a shaded intertidal habitat, helping to account for the higher abundances recorded there.

Further supporting an association between intertidal invertebrates and habitats, a positive linear relationship was detected between the species richness of megainvertebrates and the number of habitats sampled (Figure 8b). Generally, megainvertebrate species richness increased as the number of habitats sampled increased (Figure 8b). However, intertidal invertebrate assemblages for a larger number of seashores must be examined to determine the statistical validity of this relationship, as it produced a non-significant result and was strongly influenced by the outlier Racecourse Bay West (Table A9). With a megainvertebrate species richness of 33, Racecourse Bay West had a species richness that was more than two standard deviations greater than the regional mean (Figure 2c). Consequently, Racecourse Bay West may be considered a biodiversity hotspot for intertidal megainvertebrates, an observation not recorded for any of the other five sampled sites.

The higher megainvertebrate species richness recorded at Racecourse Bay West may be a product of multiple rock types offering a greater diversity of exploitable habitats and environmental conditions. For example, the soft Mount Gambier limestone platform was interspersed by numerous water-filled depressions providing habitat for the anemone *I. australis*, while the undersides of hard flint boulders provided a sheltered, shaded habitat for several gastropod species and the barnacle *T. purpurascens* (Figure 7b). Consequently, if only one type of rock was present at Racecourse Bay West, the habitats associated with the alternative rock type may not be available for exploitation by intertidal megainvertebrates, potentially reducing the species richness at this site. Alternatively, the higher species richness and identification of Racecourse Bay West as a potential biodiversity hotspot could simply be an artefact of the sampling design employed, in which sites with the greatest number of habitats were searched for the greatest length of time during TS surveys. Therefore, to untangle such possibilities it would be prudent to sample each site for a standardised length of time to ensure that differences in the sampling effort employed do not potentially confound the data that has been collected. Furthermore, a greater number of sites should also be sampled to better understand any relationship that may exist between megainvertebrate species richness and the number of habitats sampled, and to determine whether the presence of multiple rock types at a single location interacts with this relationship in any way.

The general observation that megainvertebrate species richness appeared to increase with the number of habitats sampled is consistent with the Habitat Diversity Hypothesis, which predicts that the greatest diversity of species will occur where the greatest diversity of habitats exists (Connor and McCoy 1979). Furthermore, this observation is consistent with biodiversity inventories conducted elsewhere for rocky seashores in SA (Benkendorff 2005). Consequently, it may be possible to use habitat counts as a viable surrogate for identifying sites that may make valuable inclusions in any ongoing south-east rocky seashore monitoring program. From the six sites sampled to date, Racecourse Bay West, with its highest space occupancy and species richness of marine plants, and potential classification as a megainvertebrate biodiversity hotspot, would be worthy of inclusion in any ongoing monitoring program. However, for any monitoring program to comprehensively evaluate the marine plant and megainvertebrate assemblages of the South East region's rocky seashores, a sampling regime that spans multiple seasons, and encompasses a greater spread of sites balanced across rock type must be implemented.

Of the 12 marine plant and 46 megainvertebrate species recorded using line-intercept transects and TS surveys respectively, at least 11 megainvertebrate species have been identified as potentially being vulnerable to anthropogenic disturbances. In Australia, intertidal organisms are harvested by recreational fishers for bait or by some cultural groups for food (Keough et al. 1993; Underwood 1993; Alexander and Gladstone 2013). The species targeted by human harvesting can include the whole range of organisms present on the rocky seashore, although highly sought after species locally can include: the limpet *C. tramoserica* and snail *N. atramentosa* for bait and/or food; and the abalone *Haliotis* spp., and snails *L. undulata*, and *A. constricta* for food (Keough et al. 1993; Underwood 1993; Alexander and Gladstone 2013). These harvesting activities not only impact intertidal assemblages through the direct removal of individual organisms but indirectly by: 1) altering the size-structure of some invertebrate populations; 2) changing ecological interactions; 3) damaging intertidal habitats during the foraging process; and/or 4) by trampling vulnerable algae and invertebrate assemblages while accessing and/or foraging on the shore (Keough et al. 1993; Underwood 1993; Alexander and Gladstone 2013).

In SA, the harvesting of benthic organisms on rocky seashores out to a depth of two metres has been prohibited by law since January 1, 1996. Regrettably, this legislation has been largely ineffective in achieving its desired conservation outcomes, with many users of rocky seashores either unaware of or choosing to ignore the current legislative framework (pers. obs.). Accentuating this point, the three authors of this report have observed individuals and groups actively foraging for gastropods across a number of SA rocky seashores, with the heaviest foraging observed on seashores within close proximity to urban centres, despite this activity being illegal. A higher species diversity, abundances of individual invertebrate taxa, and larger size-classes for some mollusc species were reported from comparisons among seashores inside and outside the exclusion zone surrounding the former Port Stanvac oil refinery in metropolitan Adelaide (Dutton and Benkendorff 2008; Baring et al. 2010). It appears that the fences surrounding the refinery site prevent human intrusion onto the rocky seashore, affording the intertidal assemblages that inhabit these seashores protection from illegal harvesting (Dutton and Benkendorff 2008; Baring et al. 2010). Likewise, in Port Phillip Bay, Victoria, the illegal harvesting of gastropods was found to reduce the size structure and abundance of several mollusc species including *C. tramoserica*, *A. constricta*, and *N. atramentosa,* when comparisons were made between rocky seashores with and without exclusion from human harvesting (Keough et al. 1993).

Given the rocky seashores of the South East Region support substantial populations that include very large individuals of the limpets *C. tramoserica* and *Patelloida alticostata*, and the snails *N. atramentosa*, *A. constricta*, *C. adelaidae*, *D. concamerata*, *L. undulata*, *Bembicium nanum*, and *Dicathais orbita*, it is highly likely that these taxa may be subjected to illegal harvesting activities in the future. This same threat is also likely to apply to the highly sought after, but far less abundant, abalone *Haliotis laevigata* and *Haliotis rubra*, which were also observed on some rocky seashores. Therefore, it is recommended that these 11 species form the basis of a targeted and longer-term monitoring program that assesses the threat that illegal harvesting activities, both directly and indirectly, may pose to populations of these taxa, and to the entire marine plant and invertebrate assemblage within these marine parks.

The marine park zones that afford the highest levels of protection, Sanctuary Zones and Restricted Access Zones (which prohibit all extractive activities and access by the general public, respectively), were not sampled during this study. Restricted Access Zones were not sampled because this level of marine park protection is not represented within SA's two south-east marine parks. In contrast, Sanctuary Zones were not sampled due to the difficulty in finding a suitable rocky seashore within this zone to sample during winter. Of the five sanctuary zones located in the two south-east marine parks, rocky seashores are only represented in the Canunda Sanctuary Zone in the Lower South East Marine Park, and the Cape Dombey Sanctuary Zone in the Upper South East Marine Park. The calcarenite rocky seashores in both of these zones are located in areas where accessibility can be problematic (i.e. access via steep cliffs, refer to photo on front cover of this report, or via 4WD through sand dunes), and are frequently subjected to strong winds, swells, and wave action. Furthermore, the rocky seashore in the Sanctuary Zone at Cape Dombey is quite small in area, making sampling with replication difficult. Consequently, finding sections of suitable rocky seashore in Sanctuary Zones that can be regularly sampled, especially during winter, was not achievable. Additionally, given these shores are comprised of flat calcarenite platforms, their generally low elevation subjects them to near permanent submersion during winter. This observation suggests that these shores may be vulnerable to permanent submersion if sea levels rise, which is predicted to be a consequence of global climate change (IPCC 2013). It is therefore possible that rocky seashores may not be adequately protected by Sanctuary Zones in the South East region if such changes were to transpire.

Future research

To adequately evaluate the potential role of rock type and rock hardness on marine plant and invertebrate assemblages along SA's south-east coast, it is recommended that a replicated sampling design that spans multiple rocks from each hardness class be implemented (see Chapter 2). Ideally, this sampling design would encapsulate a greater spread of sites, including several sites along the Fleurieu Peninsula (where softer fossiliferous limestone and harder siltstone, granite, and schist shores occur). Thus, it would be possible to contrast the marine plant and invertebrate assemblages between a number of different locations and rocks, to develop the emerging understanding of how the predominantly calcarenite and Mount Gambier limestone seashores of the South East region compare to rocky seashores east and west of this region (see Chapter 2).

Furthermore, this recommended sampling design would ideally span multiple seasons, ensuring sampling is also conducted during summer when high-pressure atmospheric cells, lighter winds, smaller swells, and lower low-tides persist. This would enable the lower-most extremes of each rocky seashore to be assessed, and new species richness values determined for the marine plant and invertebrate assemblage across the entire shore. In doing so, it would be possible to determine whether the low species richness values reported here are truly indicative of this region, or an artefact of a winter sampling regime that was unable to sample the lower extremes of the rocky seashore.

Conclusions

The inventories conducted in the two south-east marine parks during the final week of the 2014 winter have been successful in establishing a baseline of the geology, habitats, and biodiversity currently protected on rocky seashores in the Lower South East and Upper South East Marine Parks. Across the six sampled sites, three rocks (harder flint and softer calcarenite and Mount Gambier limestone) and five types of habitat (submerged substratum, emersed substratum, rock pools, boulders, and sand deposits) were identified. A total of 12 marine plant species were recorded using line-intercept transects, while 46 species of megainvertebrates were recorded during TS surveys. This sampling approach revealed a significant difference in the structure of the marine plant assemblage among sites, while the structure of the megainvertebrate assemblage was strongly correlated with the number and/or types of habitat that were sampled. A species rich hotspot for megainvertebrates was potentially identified at Racecourse Bay West. Due to the short timeframe of the current pilot survey, the data presented here should be supplemented by a replicated sampling regime which spans multiple seasons, a greater spread of sites, and multiple rocks from each hardness class, to capture data on the true variability present within marine plant and megainvertebrate assemblages on rocky seashores in SA's south-east marine parks.

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Figure A1: Rocky seashores examined during geological, habitat, and biodiversity inventories in SA's South East Region: a) boulder field at Racecourse Bay East; b) boulder field and platform at Racecourse Bay West; c) platform and rock pools at Rainbow Rocks; d) platform and rock pools at Nora Creina; e) platform at Robe South; and f) platform and rock pools at Lake Charro.

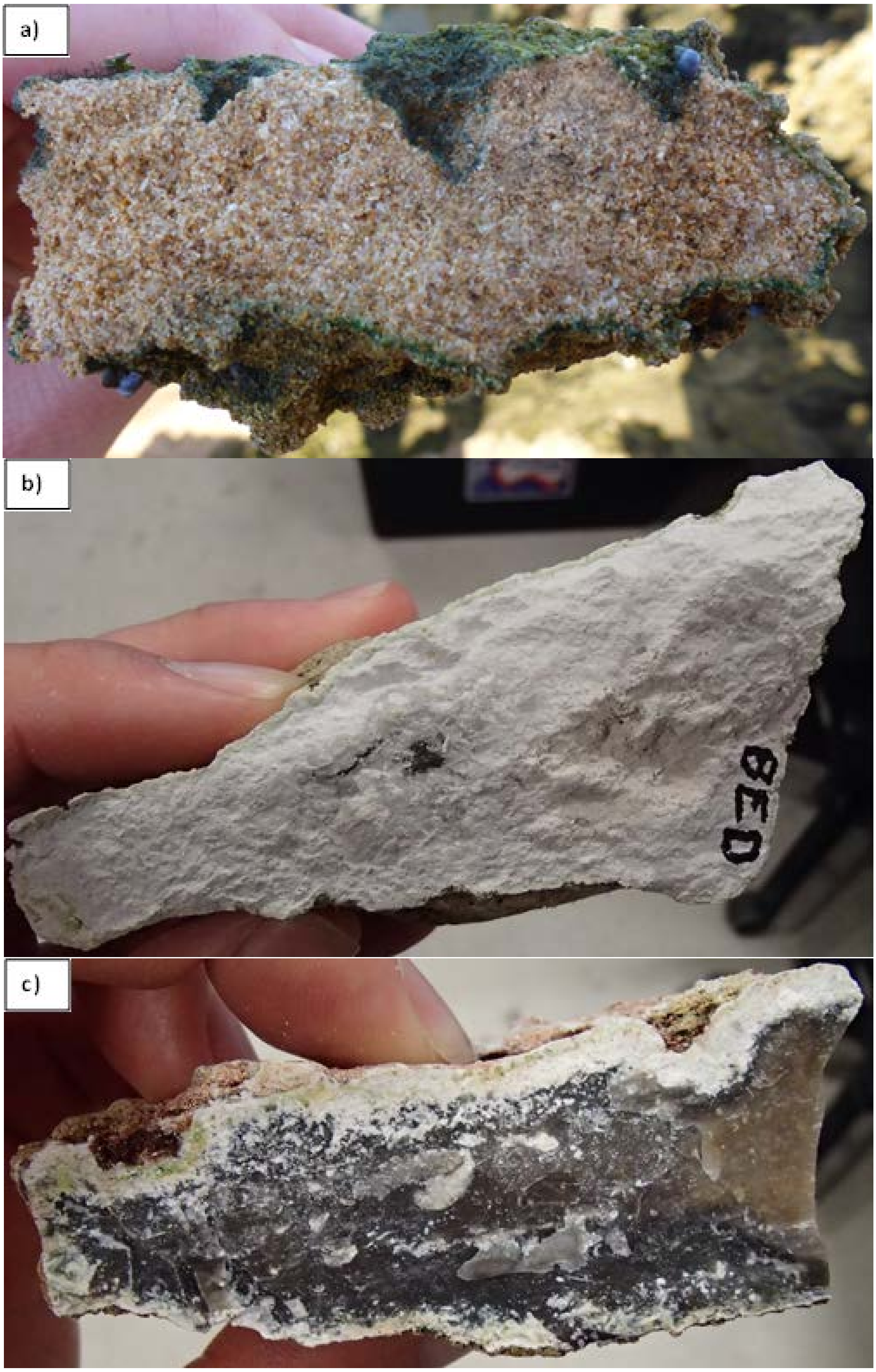

Figure A2: Rock types identified during geological, habitat, and biodiversity inventories in South Australia’s South East Region: a) calcarenite; b) Mount Gambier limestone; and c) flint.

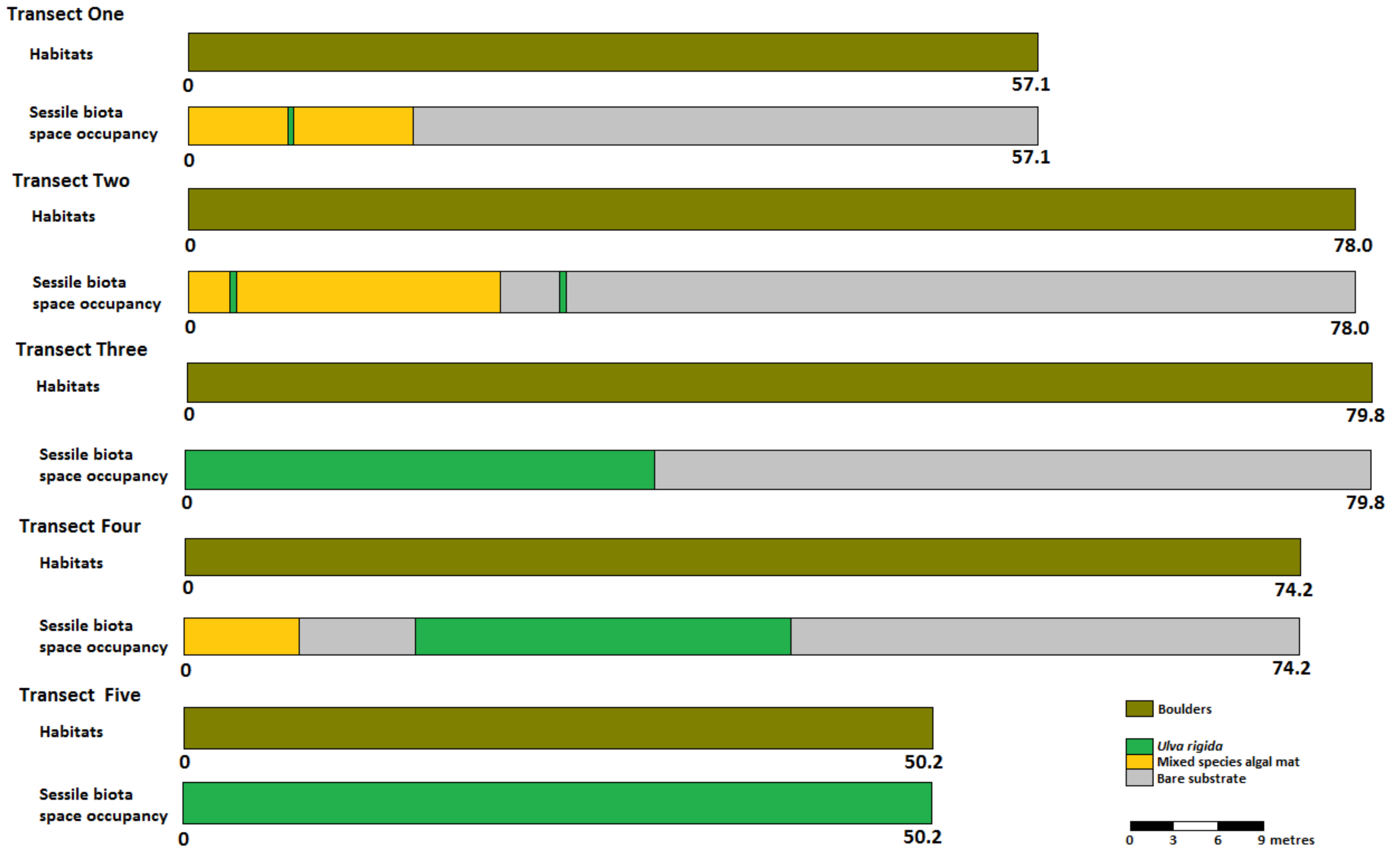

Figure A3a: Space occupancy of habitats and sessile biota recorded along line-intercept transects at Racecourse Bay East. The numbers under each transect denote its overall length (m), with each transect extending from the low tide level at the time of sampling to the top of the rocky shore. At Racecourse Bay East the mixed-species algal mat was comprised of *S. rugosum, H. banksii, S. lamentaria, C. sinuosa, C. officinalis,* and *C. implexa*.

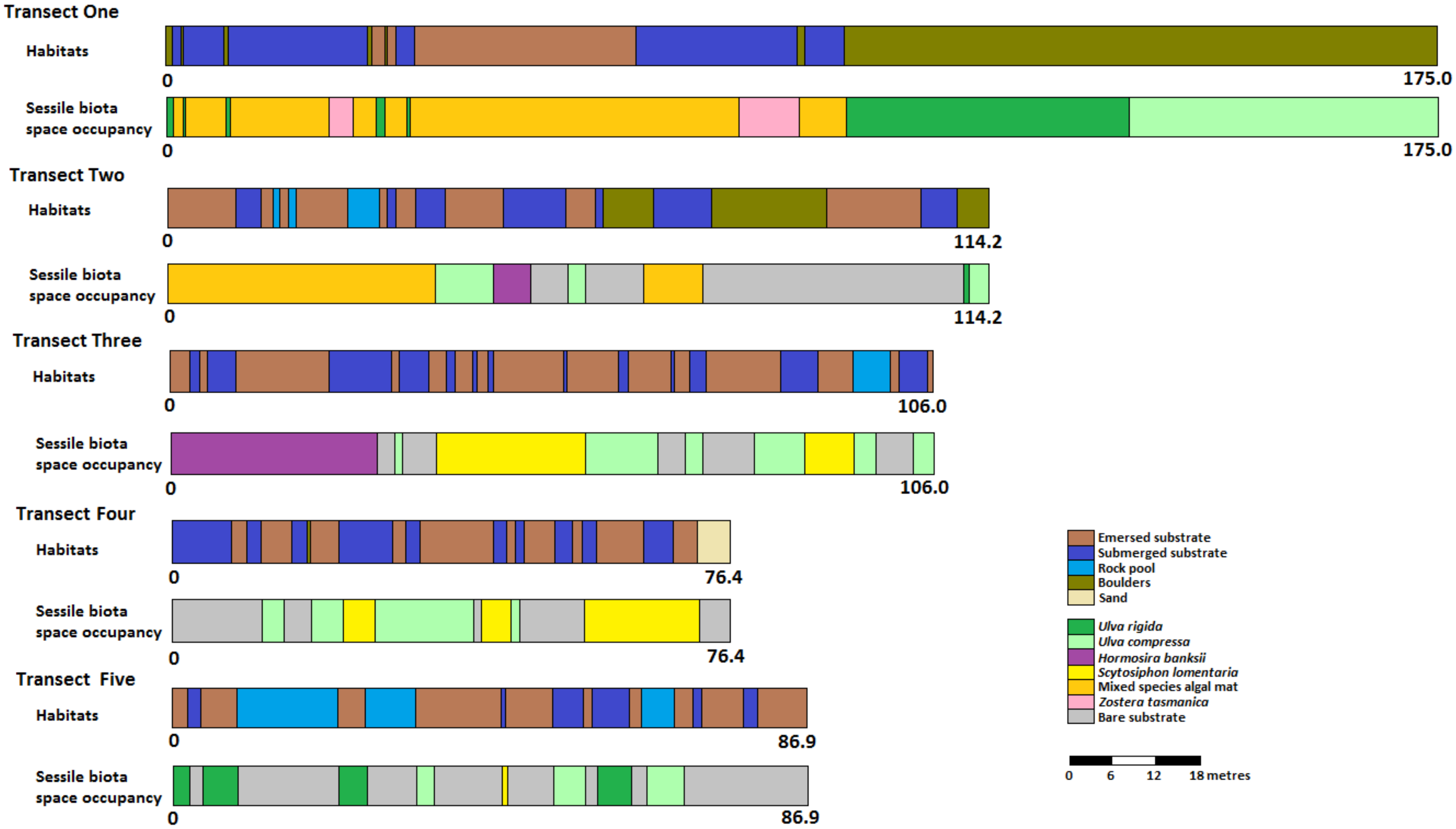

Figure A3b: Space occupancy of habitats and sessile biota recorded along line-intercept transects at Racecourse Bay West. The numbers under each transect denote its overall length (m), with each transect extending from the low tide level at the time of sampling to the top of the rocky shore. At Racecourse Bay West the mixed-species algal mat was comprised of *H. banksii, S. lamentaria, S. rugosum, C. officinalis, C. implexa*, and *A. anceps*.

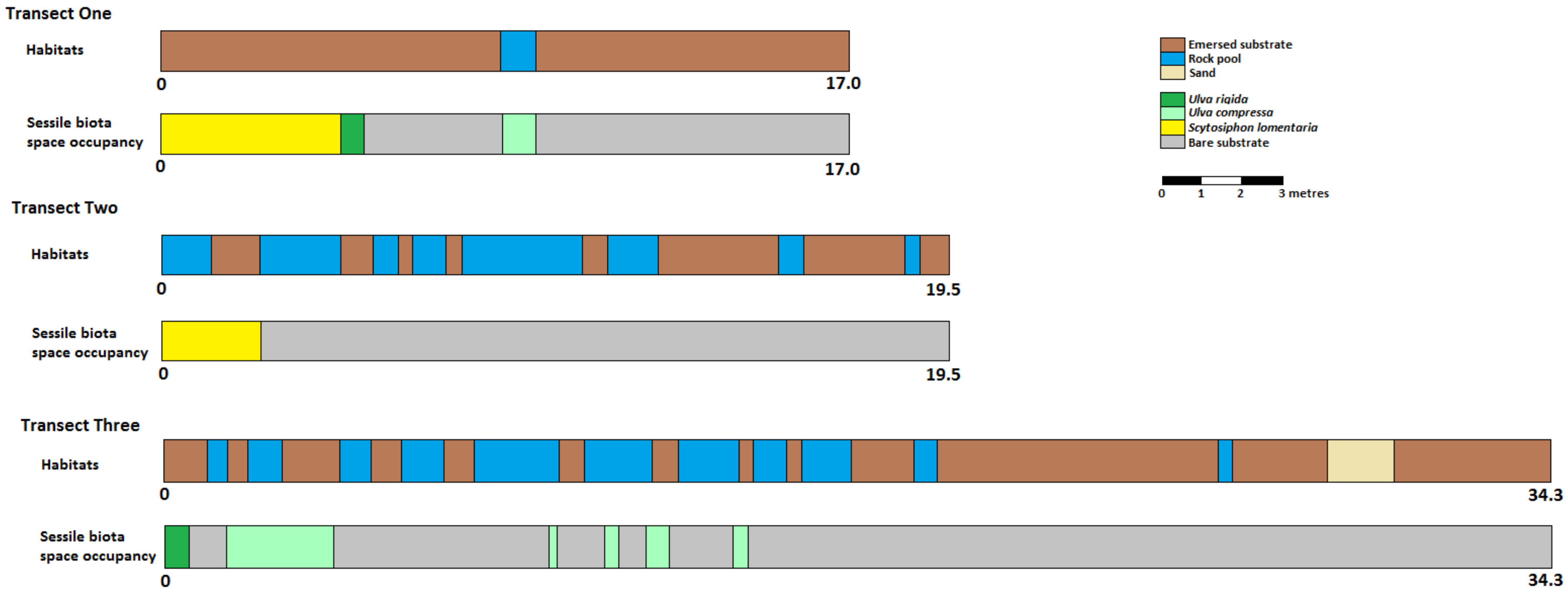

Figure A3c: Space occupancy of habitats and sessile biota recorded along line-intercept transects at Rainbow Rocks. The numbers under each transect denote its overall length (m), with each transect extending from the low tide level at the time of sampling to the top of the rocky shore.

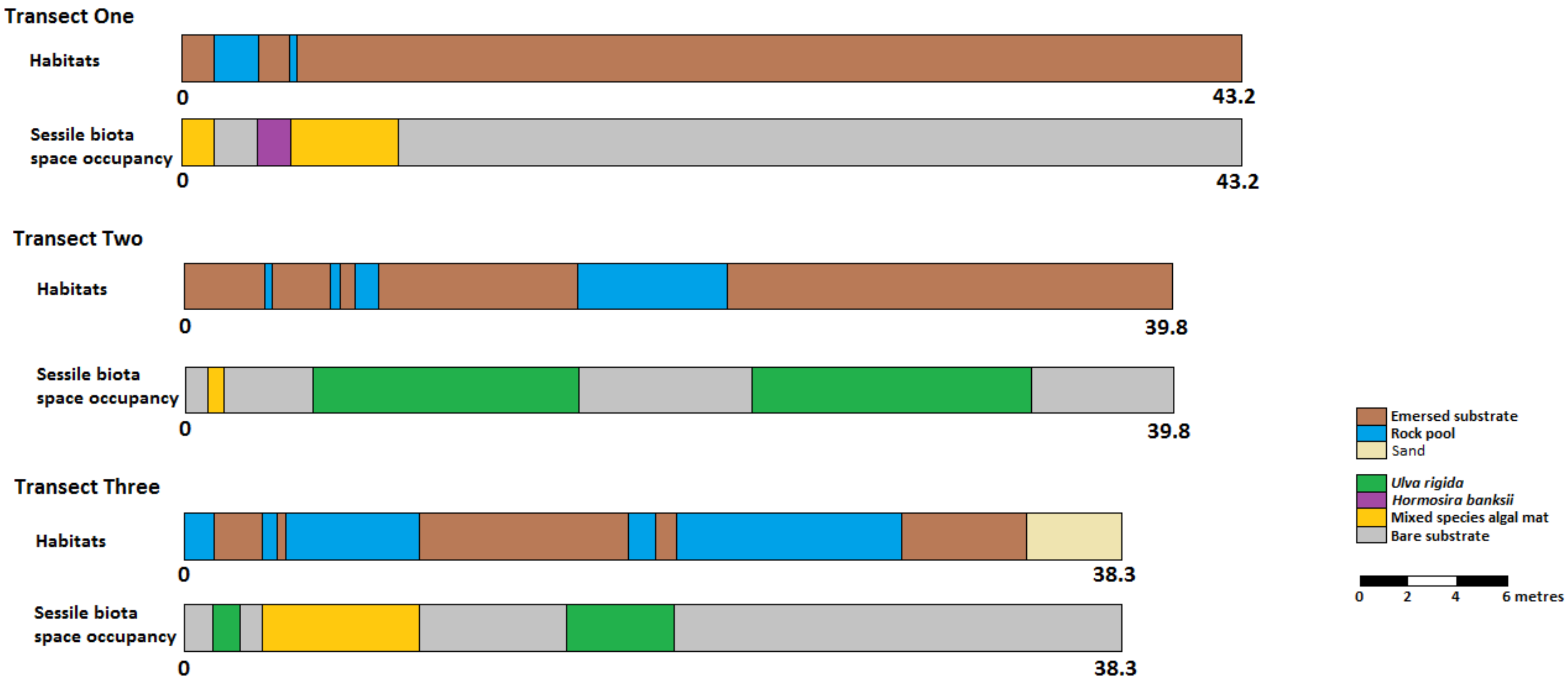

Figure A3d: Space occupancy of habitats and sessile biota recorded along line-intercept transects at Nora Creina. The numbers under each transect denote its overall length (m), with each transect extending from the low tide level at the time of sampling to the top of the rocky shore. At Nora Creina the mixed-species algal mat was comprised of *U. rigida, H. banksii*, and *C. implexa*.

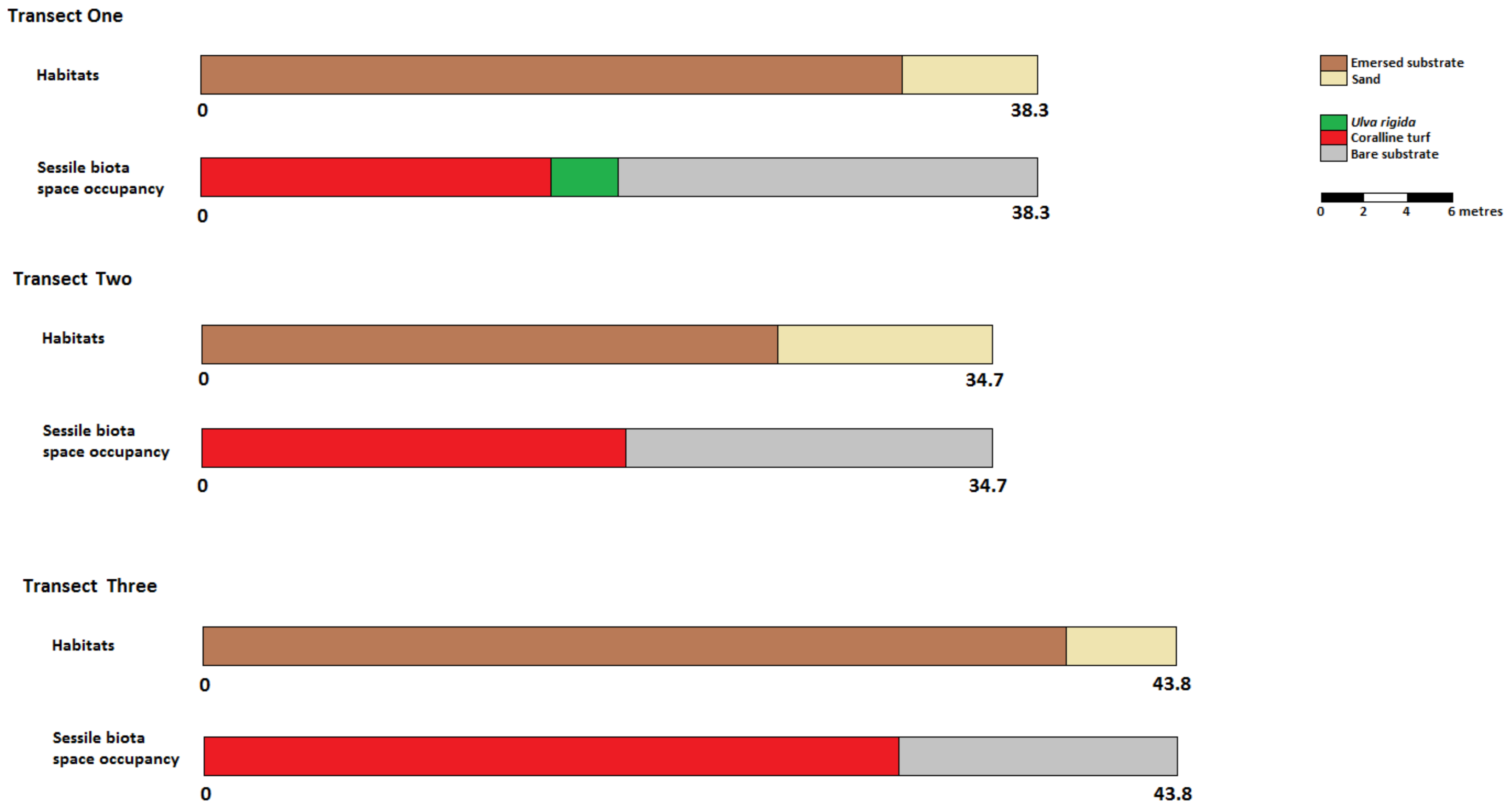

Figure A3e: Space occupancy of habitats and sessile biota recorded along line-intercept transects at Robe South. The numbers under each transect denote its overall length (m), with each transect extending from the low tide level at the time of sampling to the top of the rocky shore.

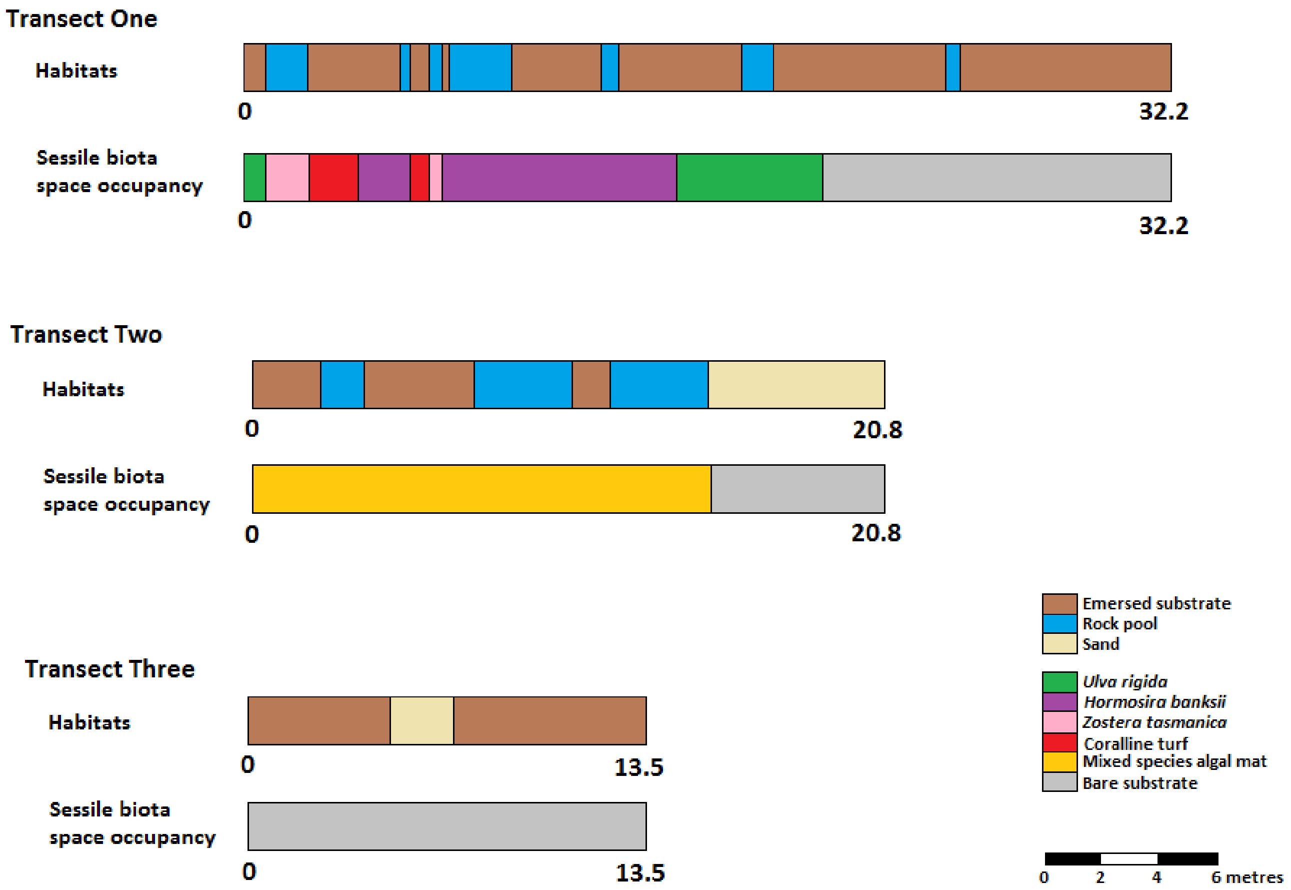

Figure A3f: Space occupancy of habitats and sessile biota recorded along line-intercept transects at Lake Charro. The numbers under each transect denote its overall length (m), with each transect extending from the low tide level at the time of sampling to the top of the rocky shore. At Lake Charro the mixed-species algal mat was comprised of *U. rigida, H. banksii*, and *Laurencia* spp.

Table A1: Output from SIMPER analysis done in PRIMER/PERMANOVA+ examining the average similarity among replicate line-intercept transects for each site, and the dominant species characterising this average similarity at each site.

Site	Average % similarity among transects	Characterising species	That species % contribution to average similarity
Racecourse Bay East	42.82	*Ulva rigida*	71.32
Racecourse Bay West	50.84	*Ulva compressa*	53.75
Rainbow Rocks	59.35	*Scytosiphon lamentaria*	95.68
Nora Creina	43.29	*Ulva rigida*	66.23
Robe South	79.14	Coralline turf	100
Lake Charro	14.31	*Ulva rigida*	54.49

Table A2: Output from ANOVA analyses done in SYSTAT testing for differences in the percentage cover/species richness of marine plants and marine plant divisions among sites. Significant results (α = 0.05) are shown in bold.

Parameter	Source	Type III SS	df	Mean squares	*F*-ratio	*p*-value
Total marine plants percentage cover	Site	33.785	5	6.757	1.274	0.323
	Error	84.856	16	5.304		
Red algal percentage cover	Site	7073.614	5	1414.723	18.282	**<0.001**
	Error	1238.113	16	77.382		
Brown algal percentage cover	Site	54.484	5	10.897	3.904	**0.017**
	Error	44.663	16	2.791		
Green algal percentage cover	Site	72.331	5	14.466	2.643	0.063
	Error	87.569	16	5.473		
Red algal species richness	Site	2.130	5	0.426	0.966	0.467
	Error	7.057	16	0.441		
Brown algal species richness	Site	3.913	5	0.783	1.779	0.174
	Error	7.038	16	0.440		
Green algal species richness	Site	1.840	5	0.368	2.268	0.097
	Error	2.596	16	0.162		

Table A3: Output from pair-wise tests done in SYSTAT examining differences in the total percentage cover among sites for: a) red algae and b) brown algae. Significant results (α = 0.05) are shown in bold. Key: RBE = Racecourse Bay East; RBW = Racecourse Bay West; RR = Rainbow Rocks; NC = Nora Creina; RS = Robe South; and LC = Lake Charro.

a)

Site	NC	RBE	RBW	RR	RS
LC	0.646	0.393	0.647	0.266	**<0.001**
NC		1.000	1.000	0.977	**<0.001**
RBE			0.995	0.993	**<0.001**
RBW				0.916	**<0.001**
RR					**<0.001**

b)

Site	NC	RBE	RBW	RR	RS
LC	0.747	0.982	0.469	0.999	0.590
NC		0.953	**0.038**	0.552	1.000
RBE			0.090	0.894	0.853
RBW				0.687	**0.021**
RR					0.401

Table A4: Output from multivariate PERMANOVA done in PRIMER/PERMANOVA+ testing for differences in the structure of marine plant assemblages among sites. Significant results (α = 0.05) are shown in bold.

Source	SS	df	Mean squares	F-ratio	*p*-value	Unique permutations
Site	42159	5	8431.7	5.055	**0.0001**	9909
Residual	26690	16	1668.1			
Total	68849	21				

Table A5: Output from PERMANOVA pair-wise tests done in PRIMER/PERMANOVA+ testing for differences in the structure of marine plant assemblages among sites. Significant results (α = 0.05) are shown in bold.

Sites compared	*t*	Permutation *p*-value	Unique permutations	Monte Carlo *p*-value
Lake Charro, Robe South	1.993	0.095	10	**0.046**
Lake Charro, Racecourse Bay East	1.172	0.234	56	0.271
Lake Charro, Racecourse Bay West	1.776	0.019	56	**0.046**
Lake Charro, Nora Creina	1.186	0.301	10	0.288
Lake Charro, Rainbow Rocks	1.966	0.104	10	**0.041**
Robe South, Racecourse Bay East	2.860	0.019	56	**0.006**
Robe South, Racecourse Bay West	3.856	0.020	56	**0.001**
Robe South, Nora Creina	3.350	0.099	10	**0.007**
Robe South, Rainbow Rocks	4.765	0.105	10	**0.002**
Racecourse Bay East, Racecourse Bay West	2.414	**0.015**	126	0.007
Racecourse Bay East, Nora Creina	1.166	0.323	56	0.293
Racecourse Bay East, Rainbow Rocks	2.280	0.034	56	**0.019**
Racecourse Bay West, Nora Creina	2.524	0.019	56	**0.008**
Racecourse Bay West, Rainbow Rocks	2.321	0.017	56	**0.011**
Nora Creina, Rainbow Rocks	2.701	0.096	10	**0.013**

Table A6: Output from SIMPER analysis done in PRIMER examining the average dissimilarity between pairs of sites for marine plant assemblage structure. As assemblage differences were generally driven by the higher percentage cover of a single algal species at one of the compared sites, the site which recorded a higher percentage cover of the characterising species is presented in bold. Key: NS = No significant difference detected among sites at α = 0.05.

Sites compared	Average dissimilarity %	Characterising species	That species % contribution to average dissimilarity
Lake Charro, **Robe South**	89.67	Coralline turf	61.60
Lake Charro, Racecourse Bay East NS	75.25	*Ulva rigida*	45.64
Lake Charro, **Racecourse Bay West**	82.79	*Ulva compressa*	28.37
Lake Charro, Nora Creina NS	77.89	*Ulva rigida*	43.00
Lake Charro, **Rainbow Rocks**	96.72	*Scytosiphon lomentaria*	39.55
Robe South, Racecourse Bay East	88.37	Coralline turf	56.93
Robe South, Racecourse Bay West	91.91	Coralline turf	47.32
Robe South, Nora Creina	94.32	Coralline turf	65.49
Robe South, Rainbow Rocks	97.80	Coralline turf	74.22
Racecourse Bay East, Racecourse Bay West	78.11	*Ulva rigida*	34.46
Racecourse Bay East, Nora Creina NS	60.31	*Ulva rigida*	59.04
Racecourse Bay East, Rainbow Rocks	80.85	*Ulva rigida*	56.15
Racecourse Bay West, Nora Creina	82.50	*Ulva compressa*	29.65
Racecourse Bay West, Rainbow Rocks	69.21	*Ulva compressa*	39.15
Nora Creina, Rainbow Rocks	91.21	*Ulva rigida*	44.35

Table A7: Output from SIMPER analysis done in PRIMER examining the average similarity among TS surveys within each of the four intertidal invertebrate assemblages identified from cluster analysis, and the dominant species characterising this average similarity within each assemblage.

Assemblage	Average % similarity among surveys	Characterising species	That species % contribution to average similarity
First (Racecourse Bay boulders)	76.36	*Nerita atramentosa*	12.50
		Diloma concamerata	12.50
		Austrocochlea constricta	12.50
		Notoacmea spp.	12.50
		Tetraclitella purpurascens	12.50
Second (Racecourse Bay submerged substratum)	58.21	*Isanemonia australis*	22.54
		Austrocochlea constricta	18.59
Third (Lake Charro & Nora Creina rock pools)	49.12	*Chlorodiloma adelaidae*	28.57
		Cominella lineolata	21.43
		Lunella undulata	21.43
Fourth (emersed substratum & rock pools at Rainbow Rocks)	61.82	*Austrolittorina unifasciata*	17.20
		Afrolittorina praetermissa	13.49
		Siphonaria spp.	10.22
		Cellana tramoserica	9.84

Table A8: Output from SIMPER analysis done in PRIMER examining the average dissimilarity between pairs of assemblages for intertidal invertebrates sampled using semi-quantitative abundance rankings from TS surveys.

Assemblages compared	Average dissimilarity %	Characterising species	That species % contribution to average dissimilarity
Fourth, third	61.82	*Austrolittorina unifasciata*	9.82
		Afrolittorina praetermissa	8.76
		Siphonaria spp.	5.74
Fourth, first	59.32	*Tetraclitella purpurascens*	8.55
		Diloma concamerata	6.47
		Cyclograpsus granulosus	6.41
Third, first	81.94	*Diloma concamerata*	7.07
		Notoacmea spp.	7.07
		Tetraclitella purpurascens	7.07
Fourth, second	72.86	*Austrolittorina unifasciata*	8.11
		Isanemonia australis	7.79
		Afrolittorina praetermissa	7.16
Third, second	66.73	*Isanemonia australis*	7.46
		Lunella undulata	6.34
		Cellana tramoserica	6.31
First, second	58.51	*Nerita atramentosa*	9.56
		Diloma concamerata	9.56
		Bembicium nanum	8.70

Table A9: Output from correlation analyses done in SYSTAT exploring the linear relationships between sampled habitats and biodiversity.

Correlation	r value	p-value	r^2 value
Marine plant species richness, number of habitats sampled	0.584	0.227	0.34
Intertidal invertebrate species richness, number of habitats sampled	0.766	0.078	0.59
Intertidal invertebrate species richness, marine plant species richness	0.594	0.217	0.35