# Oxygen limitation and thermal tolerance: a comparison of pulmonate and patellogastropod limpets.

By

**Sebbi Kankondi**

A thesis submitted in fulfilment of the requirements for the MSc (Marine Biology) degree in the Department of Zoology and Entomology of Rhodes University

Supervisor

**Professor C.D. McQuaid (Rhodes University)**

Co-supervisor

**Dr Morgana Tagliarolo (University of KwaZulu-Natal)**

February 2017

# ABSTRACT

Since the scientific community anticipates a general change in the global climate, it has become increasingly important to develop predictive models which encompass mechanisms to generate reliable forecasts of the effects this change on ecological communities and processes. To this end, the oxygen- and capacity- limited thermal tolerance (OCLTT) theory has been developed as a link between various physiological processes, the thermal aspect of climate change and the associated shifts at different levels of biological organization. This study set out to assess the general applicability of the OCLTT theory in eurythermal pulmonate and patellogastropod limpets, whose distributions overlap on the high shore rocks of the warm temperate, south-east coast of South Africa.

This was done by determining their microhabitat use, median lethal temperatures and cardiac, Arrhenius breakpoint temperatures as measures of their upper thermal tolerance limits, in both air and water. The main hypotheses of the study were that the pulmonate limpets would be more common than the patellogastropods in warmer microhabitats during low tide and would have higher thermal limits than the patellogastropods in air and *vice versa* in water. This was based on the assumption that the two limpet groups have different capabilities of oxygen consumption in air and water, due to differences in their respiratory organs and that this would be reflected in their thermal tolerances based on predictions made by the OCLTT. This assumption was important because oxygen consumption was not measured in this study.

Previous research (e.g. Garrity, 1984), showed that a thermal stress gradient exists among rocky intertidal microhabitats. From most to least thermally stressful the gradient is horizontal surfaces > slopes > vertical surfaces > tide pools > crevices. The current study found that, while the pulmonate limpets, *Siphonaria capensis* and *S. serrata*, preferred rock pools, sloped, vertical and horizontal rock surfaces, the patellogastropod limpets, *Cellana capensis* and *Scutellastra granularis*, preferred rock pools and vertical rock surfaces. Furthermore, the pulmonate limpets were only common on horizontal rock surfaces where specific ameliorating conditions would have mitigated thermal stress there. In addition, *C. capensis* had similar thermal tolerance limits to the pulmonate limpets in air and the pulmonate limpets had similar and/or higher thermal tolerance limits compared to *S. granularis* in water. This indicates that the pulmonate limpets did not necessarily prefer warmer microhabitats than the patellogastropod limpets and that there were no differences in the collective upper thermal tolerance limits between the two limpet groups in either medium.

Consequently, there was no indication from this study that an assumed superior capacity for oxygen supply translates into greater thermal tolerance and that the hypotheses based on the OCLTT were not supported. Although this was an indirect test of the OCLTT theory, I conclude that this study does not support the notion of its general applicability and that mechanisms other than those outlined by the OCLTT theory may help explain the patterns of thermal limitation observed in the current study.

# DECLARATION AND AUTHOURITY OF ACCESSS

I, Sebbi Lenga Kankondi, hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or academic institution, and to the best of my knowledge contains no paraphrase or copy of material previously published or written by other persons, except where due reference is made in the text of the thesis. The views and opinions expressed herein are solely those of the author and do not reflect the views of the university.

SIGNATURE: .........................................................DATE……...............................

I empower the University to reproduce, for the purpose of research, either the whole or any portion of the contents of my dissertation in any manner whatsoever.

NAME: Mr. Sebbi Lenga Kankondi STUDENT NO: 10k4325

SIGNATURE: .........................................................DATE…………..........................................

# ACKNOWLEDGEMENTS

Thank you to my supervisor, Prof. Christopher McQuaid for giving me the opportunity to work on this project under his guidance. Thank you for the expert advice, even when I did not think I needed it. Most of all, thank you for giving me the space to make mistakes and to learn from them.

A huge thank you to my co-supervisor Dr Morgana Tagliarolo, for the major effort you put into helping me with my work. Thank you for the patience you showed when I failed to understand some of the core concepts even after explaining it to me for a third and fourth time. Thank you to both my supervisor and co-supervisor for all the assistance with editing and improving the quality of my work.

Thank you to the members of the coastal research team (past and present) for helping me with various aspects of my work. Thank you to Dr Justin Lathlean for making my robolimpets. Thank you to Gwendolyn Johnson and Jaqui Trasseira for making sure I had the material I needed to carry out my experimental work.

A big, big thank you to my parents, Dr Lantine Harases-Kankondi and Petri Pekkarinen for the constant support and for being ultra-patient with me, especially considering you were my main source of financial support.

Thank you to the National Research Fund (NRF) for the financial support.

Finally, thank you to my very good friend Mananya Senona for all the encouragement when things were tough and for re-motivating me to complete my work.

TABLE OF CONTENTS

# Chapter 1 - General Introduction

## Climate Change and its' ecological implications

Climate change is a statistically significant change in the long-term state of the global climate, which is probably caused by a combination of natural processes and external anthropogenic activity (IPCC, 2012). While this change occurs on a global scale, there are variations in its effects at regional and local scales, on land and in the oceans. One of the most important consequences of climate change is the perceived change of temperature at these different scales and in these different environments (IPCC, 2014). Variations in environmental temperature influence the physiological and biochemical processes of most animals (Cossins and Bowler, 1987; Schmidt-Nielsen, 1997). Therefore, through the influence of environmental temperature, climate change is responsible for numerous shifts in ecosystem level processes (Perry *et al.*, 2005; Parmesan, 2006; Rosenzweig *et al.*, 2008). These changes include disruptions in complex species interactions (Parmesan, 2006), latitudinal and altitudinal shifts in species geographic distributions (Brander *et al.*, 2003; Perry *et al.*, 2005; Grebmeier *et al.*, 2006), temporal shifts in biological phenomena (Wiltshire and Manly, 2004) and, alterations or failure of animal migrations (Farrell *et al.*, 2008). Ultimately these can all be driven by differences in organismal responses to climate change. As a result, the development of a conceptual framework to understand the mechanisms that underlie the potential impacts of climate change on individual organisms and various ecosystems has become pivotal to contemporary science (Pörtner and Farrell, 2008). To this end, the oxygen- and capacity- limited thermal tolerance (OCLTT) theory has been developed in an attempt to gain an understanding of the physiological, ecological and evolutionary implications of climate change (Frederich and Pörtner, 2000; Pörtner, 2001; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010; Pörtner, 2012; Bozinovic and Pörtner, 2015).

# Oxygen- and capacity- limited thermal tolerance (OCLTT)

The OCLTT theory hypothesizes that the aerobic scope, which is the difference between minimum and maximum aerobic metabolic rate (metabolic rate ≈ oxygen consumption rate, $M_{O2}$), determines the thermal limitations of ectothermic animals (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). As described by the OCLTT theory, the aerobic scope increases with temperature as heightened cardiorespiratory rates result in an increase of the maximum oxygen consumption ($MO_{2max}$) rate (Fig 1.1; Pörtner, 2001; Pörtner and Knust, 2007). This increase is driven by an increase in mitochondrial $O_2$ demand, as the animal cell attempts to maintain its ATP production in order to meet its energy requirements (Pörtner *et al.,* 1998; Frederich and Pörtner, 2000). As temperatures continue to rise, cardiorespiratory rates remain constant while mitochondrial $O_2$ demand continues to increase until the upper Pejus (Pejus – "getting worse") temperature ($Tp_{\text{II}}$) is reached (Fig 1.1). At $Tp_{\text{II}}$, haemolymph $PO_2$ starts falling, which represents a decrease in the amount of $O_2$ available to the mitochondria and an overall reduction in aerobic scope (Frederich and Pörtner, 2000). This continues until the aerobic scope is completely diminished at the upper critical temperature ($Tc_{\text{II}}$), at which point tissue hypoxia and anaerobiosis set in due to the mismatch between $O_2$ demand and supply (Fig 1.1; Pörtner *et al.*, 1998; Frederich and Pörtner, 2000). When $Tc_{\text{II}}$ is reached, heart and ventilation rates decline rapidly, signalling the collapse of the cardiorespiratory system as anaerobic by-products accumulate in the tissues, eventually resulting in organismal death (Zielinski and Pörtner, 1996; Sommer *et al.*, 1997). The processes that determine these physiological breakpoints at low temperatures are very similar to those at high temperatures. However, at the lower Pejus temperature ($T_{P\,\text{I}}$), the ventilation and heart rates start slowing down while cellular $O_2$ demand keeps rising, which causes the drop in haemolymph $PO_2$ (Frederich and Pörtner, 2000). While animals can still recover fully from temperatures beyond the Pejus temperatures, ecological recovery after critical temperatures have been reached is less likely.

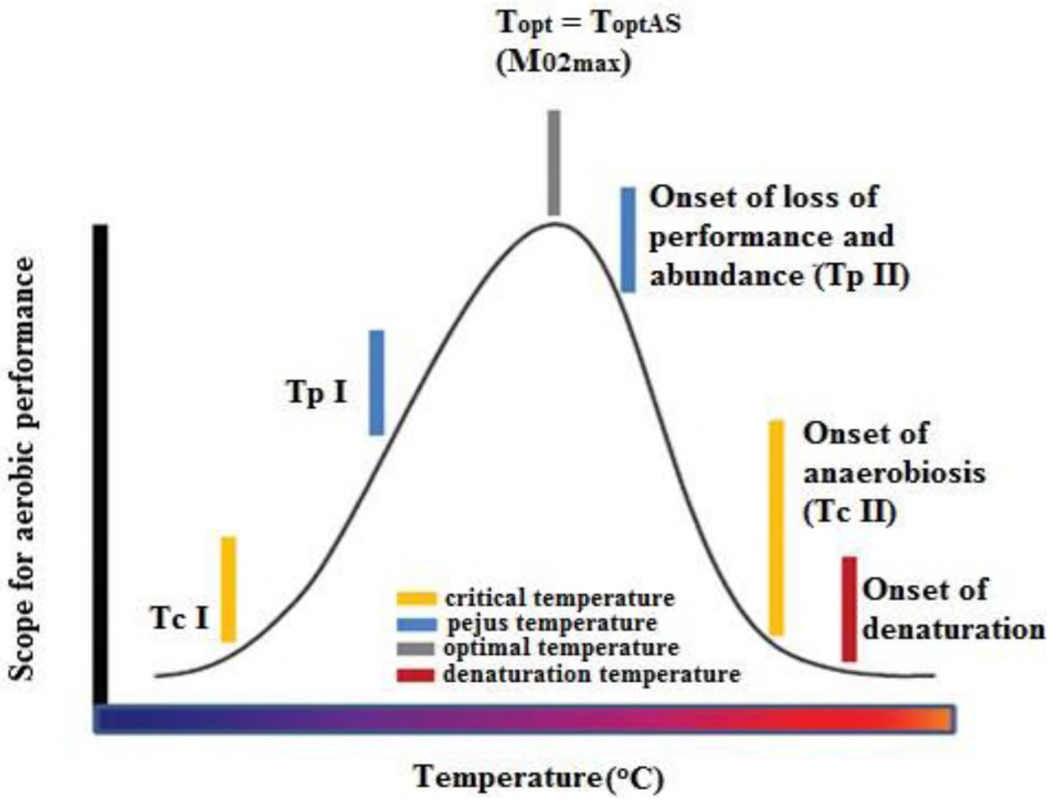

**Fig 1.1: Theoretic performance curve for fishes of the aerobic scopes response to temperature adapted from Pörtner and Farrell (2008) and redrawn by Clark *et al.* (2013).**

According to the OCLTT theory, the aerobic scope is said to be greatest in the range between the two Pejus temperatures, which contains the optimum temperature ($T_{opt}$) for aquatic ectotherms (Fig 1.1). Consequently, various important, fitness related performance parameters such as reproduction, feeding and locomotion are expected to be optimal within this temperature range (Gräns *et al.*, 2014). This means that animals exposed to temperatures outside of this range for extended periods of time will experience limitations in their fitness and therefore, probable reductions in their abundance (Pörtner and Knust, 2007; Farrell *et al.*, 2008; Munday *et al.*, 2009; Pörtner, 2010; Clark *et al.*, 2011; Neuheimer *et al.*, 2011; Donelson *et al.*, 2012; Pörtner, 2012; Comte and Grenouillet, 2013). The OCLTT theory has been used to relate animal thermal tolerances to their general performance and their responses to climate change in several papers (Pörtner and Knust, 2007; Farrell *et al.*, 2008; Pörtner and Farrell, 2008; Farrell, 2013; Pörtner and Giomi, 2013; Pörtner, 2014; Bozinovic and Pörtner, 2015). However, there has been a growing discontent in recent literature regarding the mechanisms governing animal thermal tolerance, as outlined in the OCLTT theory (Overgaard *et al.*, 2012; Clark *et al.*, 2013; Gräns *et al.*, 2014; Norin *et al.*, 2014; Ern *et al.*, 2015). This has resulted in a fervent debate surrounding the merits and generality of aerobic scope as a unifying concept linking animal thermal tolerance to ecology and possibly evolution (Overgaard *et al.*, 2012; Clark *et al.*, 2013; Farrell, 2013; Pörtner and Giomi, 2013; Ern *et al.*, 2014; Gräns *et al.*, 2014; Jutfelt *et al.*, 2014; Norin *et al.*, 2014; Pörtner, 2014; Ern *et al.*, 2015).

These studies point out that most of the literature in support of the OCLTT theory is based on aquatic temporal or polar stenothermal invertebrates and fish (Zielinski and Pörtner, 1996; Pörtner and Zielinski, 1998; Pörtner *et al.*, 1999; Frederich and Pörtner, 2000; Peck *et al.*, 2002; Pörtner and Knust; 2007). In contrast, many studies conducted on eurythermal and air breathing species from temperate and tropical regions do not support the OCLTT theory (Martin and Huey, 2008; Clark *et al*, 2011; Jost *et al.*, 2012; Clark *et al.*, 2013; Gräns *et al.*, 2014; Norin *et al.*, 2014; Ern *et al.*, 2014). Thus, the primary argument levelled against the OCLTT theory is that the aerobic scope of water or air breathing animals in environments that experience high and highly variable temperatures, does not decrease until immediately before the lethal limit is reached (Fig 1.2). Furthermore, Clark *et al.* (2013) indicates that a contrast exists between the $T_{opt}$ and the optimum temperature for the maximum aerobic scope ($T_{optAS}$) (Fig 1.2), which suggests that maximum $O_2$ supply does not determine an organism's optimal fitness.

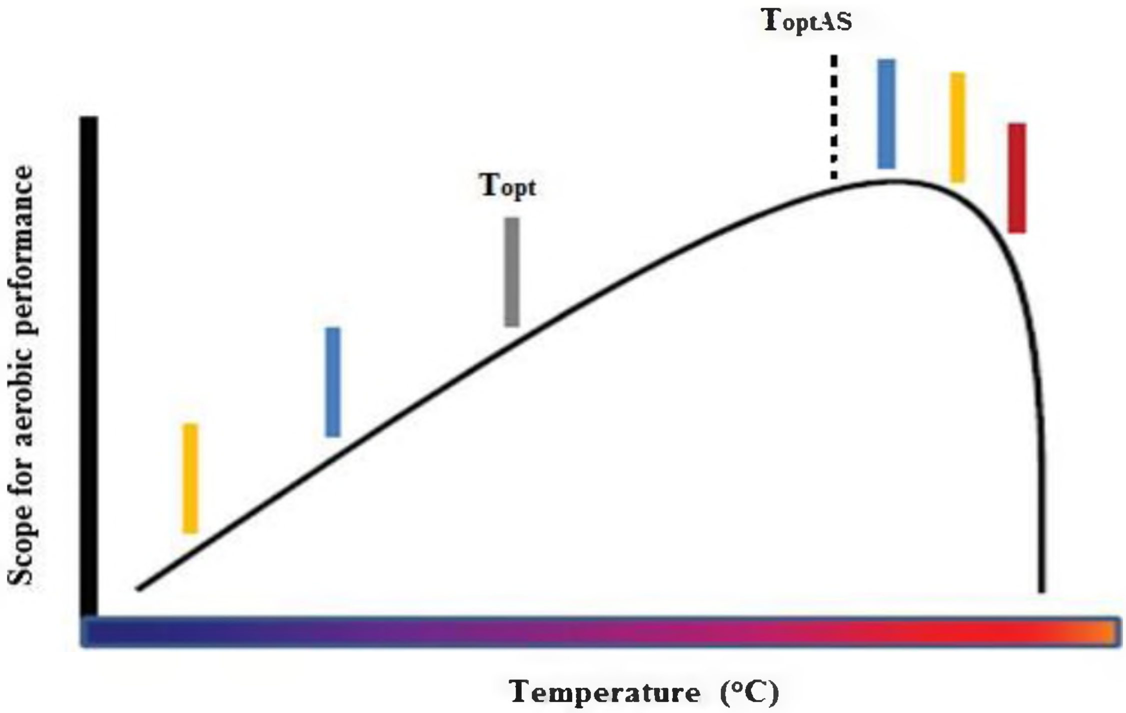

**Fig 1.2: Theoretic, aerobic scope thermal response (aerobic performance) curve generated by Clark *et al.* (2013) as an alternative to the hypothetical curve (Fig 1.1) drawn up by Pörtner and Farrell (2008). This curve illustrates the contrast between $T_{opt}$ and $T_{optAS}$. Colour codes as in Fig. 1.1.**

# Aims

This study therefore aims to contribute to this debate by determining if the OCLTT theory applies to air or water breathing animals in environments with acute temperature variations. This was done by examining the upper thermal tolerance limits and microhabitat use of air and water breathing, rocky intertidal animals. The rocky intertidal environment serves as a suitable environment for this study, because animals from this environment are subjected to rapid and regular temperature fluctuations (see Chapters 2 and 3 for a description of rocky intertidal environments). These extreme temperature fluctuations are driven by the daily or twice daily rise and fall in sea level, particularly during sunny days (Helmuth *et al.*, 2002; Harley and Helmuth 2003; Little *et al.*, 2009). Measures of thermal limitation, such as the Arrhenius Breakpoint Temperature (Chapter 4) and the median lethal temperature (Chapter 3), are often used to examine the responses of intertidal animals to these temperature fluctuations (Dong and Williams, 2011; Huang *et al.*, 2015; Tagliarolo and McQuaid, 2015; Xing *et al.*, 2016). As the tide rises, cool sea water immerses and quickly reduces the body temperatures of intertidal animals, while falling tides expose them and their surroundings to often intense, unabated solar radiation, resulting in a rapid increase in their body temperatures. Furthermore, because of the oscillating tide, rocky intertidal environments are often home to different groups of air and/or water breathing intertidal animals, such as the patellogastropod (water breathing) and pulmonate (air breathing) intertidal limpets (see "study animals and sample sites" below). Based on the OCLTT theory described above, it can be predicted that an increased ability to extract $O_2$ from the surrounding environment should result in an increased ability to survive thermal stress within that environment. Therefore, this study also aims to further assess the general relevance of the OCLTT theory, by determining if differences in the aerial and aquatic respiratory capabilities of pulmonate and patellogastropod limpet species influence their upper thermal limits in air or in water.

As was described, several studies (Clark *et al.*, 2013; Ern *et al.*, 2014; Gräns *et al.*, 2014; Norin *et al.*, 2014) suggest that there is no correlation between $T_{optAS}$ and the temperatures ($T_{opt}$) that optimize fitness-related performance of these animals (Fig 1.2). This, in turn indicates that factors other than those outlined by the OCLTT theory determine thermal limits of these organisms (Overgaard *et al.*, 2012; Clark *et al.*, 2013; Ern *et al.*, 2014; Gräns *et al.*, 2014; Jutfelt *et al.*, 2014; Wang *et al.*, 2014). Such factors include chronic exposure to acidified ocean water (Gräns *et al.*, 2014) and impaired nervous system function (Ern *et al.*, 2015). In addition, Norin *et al.* (2014) suggested that aerobic scope may be modified constantly to suit different circumstances related to temporal shifts in an organisms' requirements. This indicates that the aerobic scope can become temperature independent via processes such as the acclimation of animals to temperatures relevant in their environment (Gräns *et al.*, 2014). Furthermore, as stated by Norin *et al.* (2014; pg. 248), this means that "any perceived benefit of $T_{optAS}$ would be lost and that the aerobic scope would lose its predictive power." One of the ways in which intertidal animals can become acclimated to different environmental temperatures within the same region, is by inhabiting different microhabitats (see Chapter 2) with different thermal regimes (Williams and Morritt, 1995; Sinclair *et al.*, 2006). As shown in several past studies (Tomanek and Somero 1999; Anestis *et al.*, 2008; Dong *et al.*, 2008; Marshall *et al.*, 2011), intertidal animals that occupy microhabitats that attain higher temperatures in the intertidal region often have greater thermal tolerance limits than conspecifics from cooler microhabitats. See Chapter 2 for a description of the different rocky intertidal microhabitats and how animals inhabiting them may be influenced by their thermal regimes. Therefore, an additional aim of this study, is to determine if the OCLTT theory can be used indirectly to predict microhabitat use of the two limpet groups (see Chapter 2).

## Study animals and sampling sites

Limpets are marine gastropod molluscs that normally have a single flattened or conically shaped shell, hence the term 'univalves' sometimes used to describe them (Ponder and Lindberg, 2008). These animals are common in rocky intertidal environments on shores around the entire globe (Powell, 1973; Branch, 1981). They are important grazers of intertidal flora and serve as a key dietary resource to many intertidal predators and humans (Eekhout *et al.*, 1992; Lasiak, 1993). As such, the ecological significance of limpets stems mostly from their influence on the community structure of rocky shores, which has made them important test subjects in various studies (Branch, 1975; Branch, 1981; Hawkins and Hartnoll, 1983). These animals form two broad groups, namely the patellogastropod and pulmonate limpet groups, both of which are well represented along the South African coast (Branch, 1971; Branch, 1975; Kilburn and Rippey, 1982). Along the South African coast, the patellogastropods include limpets from the patellid family, which consists of limpets from the genera *Cymbula, Scutellastra, Cellana and Helcion* (Ridgeway *et al.*, 1988; Weber *et al.*, 1997), while the pulmonates comprise several limpets from the families Siphonaridae and Trimusculidae (Hubendick, 1978; Kilburn and Rippey, 1982). This study focuses on the patellids, *Scutellastra granularis* (Linnaeus, 1758) and *Cellana capensis* (Gmelin, 1791), and the siphonariids, *Siphonaria capensis* (Quoy and Gaimard, 1833) and *S. serrata* (Fischer, 1807). The biology of these two limpet groups differs in several ways. For example, while many of the patellid limpets are gonochoristic external fertilizers (Marshall, 1981), all siphonariid limpets are hermaphroditic (Marshall, 1981; Chambers and McQuaid, 1994; Ansell *et al.*, 1999). In the context of this study, however, the key difference between these two limpet groups lies in their different abilities to breathe in either air or water. This difference has given rise to the terms "true-limpets" and "false-limpets" often used to describe patellids and siphonariids respectively.

This difference arises from anatomical differences in their respiratory structures (Yonge, 1947;

Yonge, 1952; Marcus and Marcus, 1960; Branch, 1981). The siphonariids have a highly vascularised mantle cavity on top of the visceral mass, which functions as a lung and has an inlet for external air known as the pneumatophore (Yonge, 1952). In addition to the mantle cavity lung, siphonariids possess gills formed secondarily from the roof of the mantle, which are called the secondary gills (Yonge, 1952; Marcus and Marcus, 1960). The secondary gills facilitate aquatic respiration but, they are not as adept as the patellid gills at aiding in aquatic respiration (see below). As a result, siphonariids are probably primarily adapted to aerial respiration (Branch, 1981). The patellid patellogastropod limpets have a full ring of pallial gills but lack the "traditional gills" (ctenidia) found in many intertidal molluscs (Yonge, 1947; McMahon, 1988). "Traditional gills" or "primitive" aspidobranch ctenidia are thought to be the molluscan respiratory structure most suited for aquatic respiration (Branch, 1981). Despite this Nuwayhid *et al.* (1978) demonstrated striking structural similarities between pallial gills and the ctenidium in their work on the patellid, *Patella vulgata*. Therefore, in terms of suitability for aquatic respiration, at least amongst intertidal limpets, the pallial gills rank right after the aspidobranch ctenidia (McMahon, 1988). Furthermore, unlike the siphonariids that possess a lung for aerial gas exchange, patellids only have the pallial gills to aid their aerial respiration (Branch, 1981). As a result, the patellids are primarily adapted to aquatic respiration (Innes *et al.*, 1984; McMahon, 1988).

Siphonariid limpets are perhaps the most common limpets on South African rocky shores. One of the limpets from this group, *Siphonaria capensis* is widely distributed along the entire South African coastline (Fig 1.3; Allanson, 1959; Branch *et al.*, 1994). This species occurs beyond the northern borders of South Africa on both the west and east coasts (Allanson, 1959; Branch *et al.*, 1994). *S. serrata* also occurs along an extensive part of the country's coastline (Fig 1.3). Individuals from this species extend from Cape Town on the south coast to Durban on the east coast, while isolated populations occur in Langebaan lagoon and extend north past the Orange river on the west coast (Allanson, 1959; Branch *et al.*, 1994). Both *S. capensis* and *S. serrata* occur on the high shore, however, *S. serrata* is more common in the upper Balanoid zone just below the high shore mark while *S. capensis* extends further up the shore and is rare in the mid shore region (Chambers, 1994; Chambers and McQuaid, 1994). *Scutellastra granularis* is one of the most common patellid limpets on the shores of South Africa (Branch *et al.*, 1994). Like most patellid limpets in South Africa, its distribution extends along the entire west coast and farther north into Namibia, whereas on the south-east coast its distribution ends just north of East London (Fig 1.3; Branch, 1971; Branch and Branch, 1988; Branch *et al.*, 1994). *C. capensis* only occurs on the south-east and east coasts, where its distribution extends from Port Alfred, past the country's northern border, through to the north coast of Mozambique (Fig 1.3;

Kalk, 1959; Branch, 1975). Both patellid species are ubiquitous across the intertidal, extending from the low shore Cochlear zone to the upper Balanoid zone (Branch, 1971, Branch, 1975).

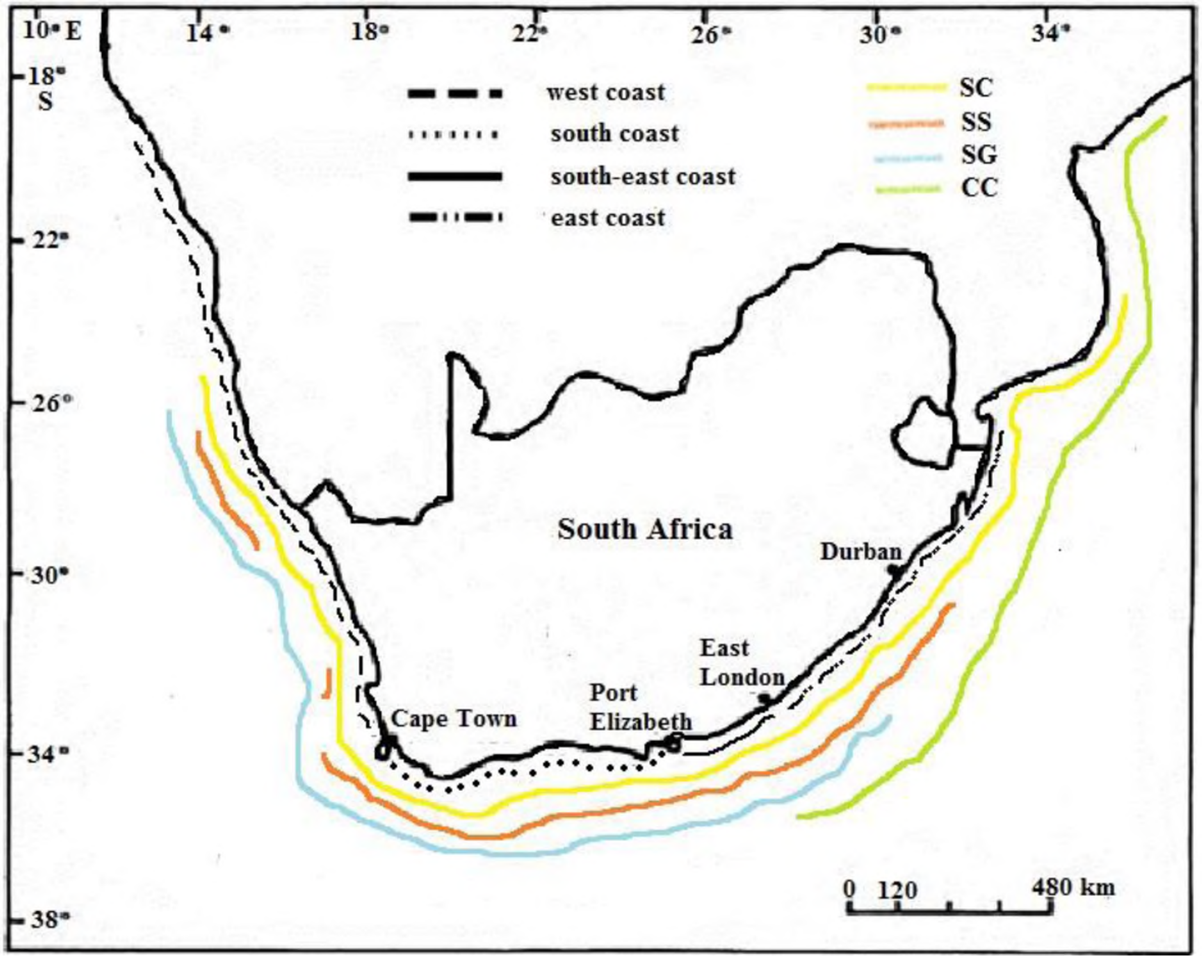

**Fig 1.3: Modified map adapted from Vat (2000) displaying the study animals' distributions along the South African coast line and the biogeographic sub-divisions that constitute this shore line. (SC – *Siphonaria capensis*; SS – *Siphonaria serrata*; SG – *Scutellastra granularis*; CC – *Cellana capensis*). Colours represent the different species biogeographic distributions.**

This study was conducted on limpets found along the south-east coast of South Africa (Fig 1.3). These four limpets were sampled from the rocky shores at Kenton on Sea, Port Alfred and Chintsa West (Fig 1.4). Kenton on sea (referred to as Kenton in this study) and Port Alfred are both found on the south-east coast, while Chintsa West is located on the east coast, close to the "border" between the south-east and east coast (Fig 1.3). See chapter 2 for a more comprehensive description of the South African coastal bioregions and the subdivisions of this coast line.

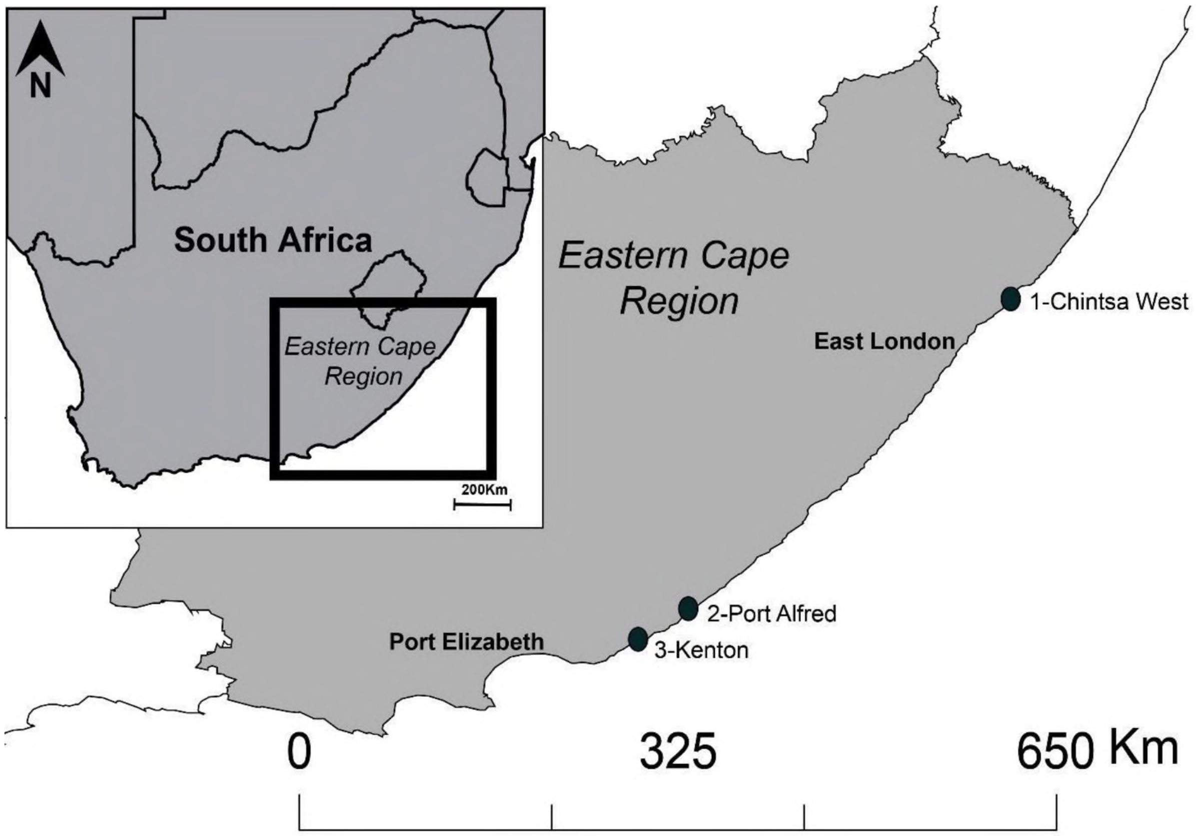

**Fig 1.4: Map of the three sample sites (shown on the right) in the Eastern Cape region (black square) of South Africa.**

Due to the overlap in their geographic distributions and vertical zonation, and the differences in their presumed respiratory capabilities in different media, these two limpet groups serve as excellent model specimens to address these aims. By comparing the thermal limits and microhabitat use of these limpet groups, this study represents an indirect approach to determining if $MO_2$ and/or $O_2$ delivery can be used as a predictor of their response to the forecasted changes in environmental temperature. It is important to note that most other studies that set out to test the relevance of the OCLTT theory have done so by directly measuring the aerobic scope of their study animals under various circumstances (Overgaard *et al.*, 2012; Clark *et al.*, 2013; Gräns *et al.*, 2014; Norin *et al.*, 2014; Ern *et al.*, 2015). In this study, no direct measurements of $MO_2$ were made. Instead, it was assumed that medium related differences in $MO_2$ between the pulmonate and patellogastropod limpets, based on differences in their respiratory structures, would be reflected in their thermal tolerance limits.

# Hypotheses

The study hypothesises that the OCLTT theory can be used to predict the thermal tolerances of intertidal limpets because it is expected that: 1. Species with a respiratory advantage in either medium will have a greater, upper thermal tolerance limits in that medium than limpets lacking that advantage, and 2. The breathing capabilities of the limpets, as determined by their respective respiratory anatomies, should result in upper thermal tolerance limits that correlate with microhabitat use.

If the limpets with respiratory advantages in either medium do not have higher thermal tolerance limits than limpets without the same advantage, this will indicate that respiratory capacity and therefore a greater capacity for aerobic scope, does not play a pivotal role in determining thermal tolerance. Furthermore, if the relationship between microhabitat use and the study animal's thermal tolerance limit is not corroborated by their breathing capabilities, this will suggest that thermal acclimation has a great impact on aerobic scope and that aerobic scope is limited in its use to predict these animal's response to future increases in temperature. Finally, this would indicate that the OCLTT theory does not have a general applicability to all organisms and as a result, the main hypothesis would not hold true.

# Organisation

This thesis consists of 5 chapters. The first chapter is a general introduction, where the study species, study sites and essential concepts of the study are first described. Chapter Two is an investigation of the study animals' microhabitat use across the study sites. Chapters Three and Four examine the lethal thermal tolerance limits and various aspects of cardiac function in response to increasing temperature of the study species, respectively. Finally, Chapter Five provides a synthesis of the study and a general conclusion.

# Chapter 2: Does the oxygen limitation theory predict microhabitat use by pulmonate and patellogastropod limpets?

## Introduction

Intertidal organisms on the South African coastline are exposed to a wide range of environmental conditions due to the country's geographical position at the juncture between the Indian and the Atlantic Oceans. This is because the Indian Ocean on the east is generally warmer than the Atlantic Ocean on the west. In addition to this, the oceanographic regime on South Africa's coast is controlled by the Agulhas current in the warm water Indian Ocean and the cold water Benguela current in the Atlantic Ocean (Fig 2.1; Griffiths *et al.*, 2010). On the east coast, the Agulhas current flows close to the northern part of the coast line, eventually flowing further offshore the farther south it goes (Schumann, 1987). East London is the approximate point at which the current deflects and starts flowing away from the coastline (Gründlingh, 1983). Farther south along this coast line, between Cape Agulhas and Port Elizabeth, the current retroflects (Lutjeharms, 1998), essentially turning away from the coastline and terminating further east in the Indian ocean (Schumann and Beckman, 1984). In contrast, the Benguela current carries cold water from the Southern Ocean northward along the country's western coastline (Fig 2.1; Griffiths *et al.*, 2010). Along the west coast, strong wind driven upwelling is characteristic of the Benguela current closer to the shore. This upwelling brings nutrient rich water to the west coast that supports a high abundance and lower diversity of organisms along that coast (Shannon, 1985; Shannon and Nelson, 1996; Griffiths *et al.*, 2010). In contrast to this, the Agulhas current carries nutrient poor water from the equatorial region of the Indian Ocean (Lutjeharms, 1998).

These differences in the oceanographic regime along South Africa's coastline generate three recognised and broadly defined coastal regions (Branch and Branch, 1988). These coastal regions are the cool temperate Namaqua bioregion along the west coast, the warm temperate Agulhas bioregion on the south coast and the subtropical Natal bioregion on the east coast

(Stephenson and Stephenson, 1972; Brown and Jarman, 1978; Emanuel *et al.*, 1992; Stegenga and Bolton, 1992; Bustamante and Branch, 1996; Turpie *et al.*, 2000; Bolton *et al.*, 2004). More recently, the South-western Cape bioregion between the Namaqua and Agulhas bioregions, and the Delagoa bioregion north of the Natal bioregion have also been identified as separate sub regions of the South African coastal regions (Griffiths *et al.*, 2010). South Africa has a long (3650 km) roughly linear coastline, stretching from Namibia on the west to Mozambique on the east (Griffiths *et al.*, 2010). As described by Branch and Branch (1988), the west coast stretches from the Orange River to Cape Town and the south coast from Cape Town to East London, while the east coast represents the remaining coastline from East London to the Limpopo River. Vat (2000) described another division to the coastline, the south-east coast, which represents the coastline that extends from Cape Town to Port Elizabeth. This means that the south coast covers the area from Cape Town to Port Elizabeth, instead of the area from Cape Town to East London. Even though most studies only recognise the three coastal divisions described by Branch and Branch (1988), this study will include all four divisions (Fig 2.1).

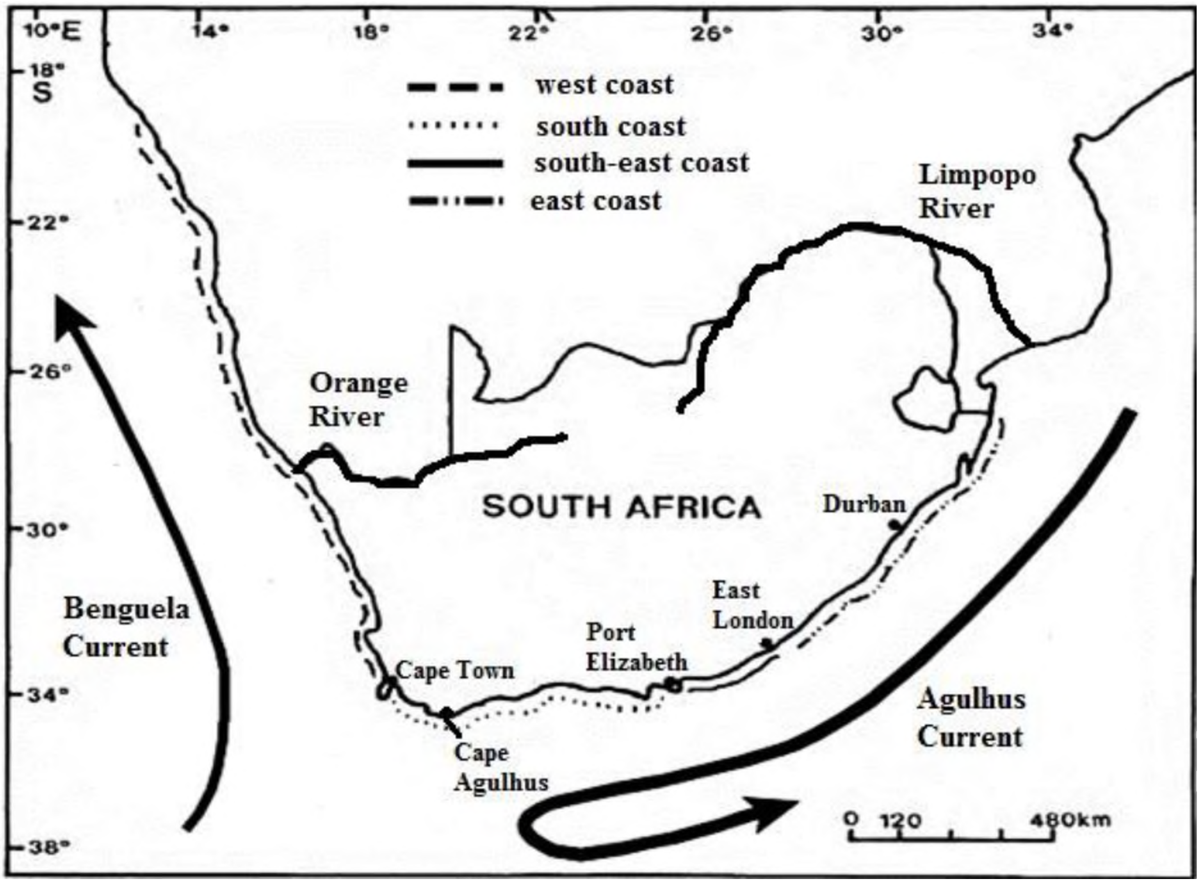

**Fig 2.1 Map adapted from Vat (2000) displaying the major currents either side of South Africa, as well as the four major coastal divisions.**

The rocky intertidal is a very unpredictable habitat in which biotic factors generally set the lower limits of organisms and abiotic factors set the upper limits (Paine, 1969; Connell, 1972). In conjunction with biotic factors such as food availability and predation stress (Thomson *et al.*, 2000; Lord *et al.*, 2011), abiotic factors such as desiccation, osmotic and thermal stress also determine which microhabitats high shore intertidal gastropods occur in (Branch, 1981; Garrity, 1984; Denny *et al.*, 2006). These stressors can become lethal if they are experienced for long periods of time. To avoid potentially lethal conditions, intertidal gastropods may inhabit 'protected' microhabitats as a behavioural response to emersion. These include crevices, rock pools and vertical rock surfaces (Wolcott, 1973; Garrity, 1984; Hawkins and Hartnoll, 1985; Branch *et al.*, 1988; Raimondi, 1990; Liu and Morton, 1994; Williams and Morritt, 1995). Microhabitats are areas at a spatial scale smaller than habitats which differ in several ways, including the abiotic conditions experienced by their inhabitants (Morris, 1987; Olabarria *et al.*, 2002). Rock pools form a unique microhabitat for gastropods in the intertidal zones (Underwood, 1984; Huggett and Griffiths, 1986) because organisms found in rock pools are less likely to experience desiccation compared to those found on emerged surfaces (Newell, 1979; Femino and Mathieson, 1980). Like crevices and vertical rock surfaces, biogenic habitats also offer shelter from harmful conditions to intertidal gastropods (Thompson *et al.*, 1996; Bertness *et al.*, 1999; Castilla *et al.*, 2004; Cartwright and Williams, 2012). Biogenic microhabitats are microhabitats formed by larger organisms, clusters or large beds of other organisms such as bivalves, ascidians or macroalgae (Williams and Morritt, 1995; Mak, 1996; Harper and Williams, 2001; Cartwright and Williams, 2012). Many intertidal limpets also exhibit homing behaviour, using a home scar into which the shell fits closely to help alleviate the effects of some of these abiotic stresses when they are not feeding or foraging (Santina, 1994; Gray and Hodgson, 1998). This is achieved by protecting their soft tissues from the outside environment and its associated stressors (Verderber *et al.*, 1983; Branch and Cherry; 1985). Limpets that do not exhibit homing return to a general area that is normally referred to as the "resting site" during high tide (Hirano, 1979; Kitting, 1980; Garrity, 1984; Little, 1989; Williams and Morritt, 1995). All four of the study species exhibit homing behaviour, where both siphonariids and *Cellana capensis* use home scars, whereas *Scutellastra granularis* does not (Branch, 1975; Branch, 1981; Branch and Cherry, 1985; Branch 1988; Davenport, 1997).

In the current study, microhabitat availability was determined at the rocky shores of Kenton on Sea, Port Alfred and Chintsa West (See Fig 1.4 for site locations). Microhabitat use by the pulmonate limpets *Siphonaria capensis* and *S. serrata*, and the patellogastropod limpets

*Scutellastra granularis* and *Cellana capensis*, was then determined at these three study sites (see Chapter 1 for a description of the study species vertical zonation patterns across the rocky intertidal environment). The South African rocky coastlines are made up of approximately 31% mixed shores, which consist of low lying wave cut rocky platforms that are strongly affected by sand inundation, sandy beaches and 41% pure rocky shores (Bally *et al*., 1984). The rocky shores found along the south-east coast consist of either aeolian calcarenite or quartzitic sandstone rock types. Wave cut rocky platforms are made up of aeolian calcarenite which is a soft rock type with a granular surface (Marker, 1988; Dower, 1989; Vat, 2000). Boulder or reef rocky shores in this region consist of hard quartzitic sandstone, which generally has a smoother surface than the irregular aeolian calcarenite surfaces (Marker, 1988; Dower, 1989; Vat, 2000). The rocky shore at Kenton is composed of an aeolian calcarenite wave cut platform and a cliff which forms a headland. The rocky shores at Port Alfred and Chintsa West consist of reef like quartzitic sandstone ridges that run perpendicular to the ocean (Marker, 1988; Dower, 1989; Vat, 2000). Like much of the South African coastline, the rocky shores at all three study sites offer very little shelter from wave exposure (Shillington and Harris, 1978). However, the rocky headland at Kenton offers the intertidal organisms found there an area of relative calm.

*Siphonaria capensis* is normally associated with encrusting coralline algae in very shallow rock pools, while *S. serrata* normally occurs on bare rock surfaces or crevices (Chambers, 1994). However, previous studies (Branch and Cherry, 1985; Chambers, 1994) have shown that *S. capensis* individuals also make use of open rock surfaces. *S. capensis* use of different microhabitats in the high shore zone may be due to differences in rock substratum type (Chambers, 1994). As mentioned earlier, *S. capensis* is rare or absent from the mid-shore down and its juveniles occur in the same microhabitats as the adults (Chambers, 1994). Unlike *S. capensis*, *S. serrata* adults and juveniles do not always occupy the same microhabitats. There are instances where the juveniles occur in crevices or crustose rock pools while the adults only occur on bare rock surfaces, due to the availability of different food sources (Chambers, 1994). *Scutellastra granularis* juveniles are common on flat bare rocks or amongst barnacles in the lower intertidal zones while the adults normally occur on vertical rock surfaces (Vat, 2000). *C. capensis* juveniles are also common on bare horizontal rock surfaces low on the shore, while they occur in rock pools in the high shore zones (Branch, 1975; 1976). However, *C. capensis* occasionally occupies bare rock surfaces much higher up the shore than *S. granularis* and at similar intertidal heights compared to the siphonariids (Branch, 1975; Chambers, 1994).

Many studies have documented differences in the microhabitat use of various intertidal organisms. However, many of these focused only on how microhabitat use may influence thermal limits (Wolcott, 1973; Garrity, 1984; Williams and Morritt, 1995; Helmuth and Hofmann, 2001; Lord *et al.*, 2011). Unlike the current study, no previous studies intentionally compare microhabitat use between patellogastropod and pulmonate limpets. Two limpet groups with striking differences in their respiratory structure morphology and therefore suspected differences in their $O_2$ consumption abilities (see Chapter 1). The current study aims to determine the microhabitat use of these two limpet groups, while striving to determine if their upper thermal limits, as predicted by their respiratory structure morphology, correlates with their microhabitat use. As described in Chapter 1, and based on predictions made in the OCLTT theory, I predict that the pulmonate limpets will have higher aerial thermal limits compared to the patellogastropod limpets and that the patellogastropod limpets will have higher aquatic thermal limits. This is because the pulmonate limpets are assumed to have a greater aerial respiratory capacity and lower aquatic respiratory capacity compared to the patellogastropod limpets. However, because all high shore intertidal microhabitats except rock pools are emerged during low tide, any findings from this study can only be related to the OCLTT in terms of the limpets overall thermal limits. Therefore, I hypothesise that the OCLTT theory will be supported only if the upper thermal limits, as projected by the respiratory capacity, of the four study species correlates with their preferred microhabitat use. Thus, the pulmonate limpets are expected to prefer warmer microhabitats than the patellogastropod limpets.

## Materials and Methods

### *Study sites*

Low tide microhabitat use of two pulmonate limpets, *Siphonaria capensis* and *S. serrata*, and two patellogastropod limpets, *Scutellastra granularis* and *C. capensis* was examined at 3 sites along the south-east and east coasts of South Africa (Fig 1.4).

### *Robolimpets*

Robolimpets (n = 3) made by Dr Justin Lathlean (Queen's University, Northern Ireland) according to the specifics provided by Lima and Wethey (2009), with empty *S. granularis* shells, were used to estimate general limpet body temperature on the high shore rocks at Kenton. All three robolimpets were secured to horizontal rock surfaces using a Z-spar and epoxy resin mix. This equates to the warmest microhabitat used by these limpets (see "Discussion"). The rock surfaces were cleared of any rough debris using a hammer and chisel before the robolimpets were attached to them. Three random locations, several meters apart, were selected as "homes" for the robolimpets. Before deployment using the OneWireViewer software (Maxim Integrated, USA), the robolimpets were set to record temperature (to 0.001°C) for 5½ months at 30min intervals starting at the end of April until mid-October 2014. The data were collected once every two months and analysed using Statistica 13.

### *Sample area selection*

To develop an understanding of the species' microhabitat use within the selected sites, the study species' abundances within the available microhabitats were determined at each study site. A sampling procedure modified slightly from the SWAT coastal biodiversity survey protocol (2011), developed at the University of California, Santa Cruz for mobile invertebrates, was used. Sample sites that had coastlines with a 20m or longer continuous stretch of rocky intertidal environment were selected, based on knowledge of the study specie's location obtained from previous studies (Branch, 1976; Chambers, 1994; Vat, 2000). Appropriate 20m wide (along shore) by 20m long (across the shore from high to low intertidal zones) sample areas at each site were selected, each with a good representative sample of the study species. If a sample area with a sufficiently wide stretch of rocky intertidal coastline could not be found within a study site, the sample area was split into two 10m wide by 20m long sample area sections. This was only done if selected sample areas were dominated by large sections inundated with sand or large areas with deep pools, as was the case at Kenton.

### *Sampling method*

Sampling at all three sites took place throughout the November 2015 spring low tides (25/11 – Kenton; 26/11 – Port Alfred; 27/11 – Chintsa West), during clear, sunny weather conditions. The relevant tide information was obtained online (South African Navy Hydrographic Office, 2017). The sampling effort was started during the outgoing tide (2 hours before low tide) to ensure enough time was available to complete sampling during a single low tide event. Point intercept quadrats along transect lines were used to sample microhabitat availability and limpet species' abundances within these microhabitats at each site (Aronson, 2001; Hill and Wilkinson, 2004). Before sampling commenced, the sample area was demarcated by setting up two reference lines (one high shore and one low shore line), each 20m long, parallel to the coast using 50m measuring tapes (Fig 2.3). The high shore line was placed 1m above where the highest individual limpet of *Siphonaria capensis* was found and the low shore line 20m below the high shore line. *S. capensis* was chosen because it generally occurs higher up the shore than most intertidal limpets and because it is widespread along South African rocky shores (Chambers, 1994).

Once the reference lines were established, 10 transect lines (20m measuring tape) were extended in a parallel series from the high shore to low shore reference line (Fig 2.3). The parallel line series was created by shifting the transect line every 2m along the reference lines from the 1m to the 19m point (Fig 2.3). Pre-classified microhabitat types (Table 2.1) were then identified and recorded at 2m intervals from the 1m to 19m point along the transect lines. This was done by placing the centre point of a 50 X 50cm quadrat over the relevant 2m demarcation points of the transect lines before visually estimating the microhabitat cover at these points. The quadrat was divided into four smaller sections by two intersecting fishing cords tied to the middle on each side of the square boundary. The slope of the relevant microhabitats (Table 2.1) was measured to within 10° using a clinometer. When there was a mixture of horizontal and non-horizontal surfaces (crevices, vertical and sloped surfaces) in a quadrat, underestimating the cover of the non-horizontal surfaces was sometimes unavoidable using this method. This is because the quadrat was always kept parallel to the horizontal and microhabitat cover was estimated from a "top view" perspective (Fig 2.2). As shown in Fig 2.2, even though the horizontal and non-horizontal surfaces, technically, cover the same length, because of the way surface cover was estimated it seems as though the non-horizontal surface covers a smaller section of the quadrat. Even though the study sought to avoid deep rock pools, this was not always possible. As a result, rock pools were considered to be deep when they had a depth of more than 20cm (determined with a meter stick).

**Table 2.1: The physical descriptions adapted from Garrity (1984) and numerical codes assigned to each microhabitat type.**

| **Microhabitat** | | |
|---|---|---|
| **Type** | **Nr Code** | **Description** |
| Horizontal | 1 | Rock surfaces with an incline between $0^\circ$ – $30^\circ$ |
| Horizontal boulder | 2 | Flat rock surfaces on top of boulders |
| Sloped | 3 | Rock surfaces with an incline between $31^\circ$ – $70^\circ$ |
| Sloped Boulder | 4 | Sloped rock surfaces on the sides of boulders |
| Vertical | 5 | Rock surfaces with an incline between $71^\circ$ – $90^\circ$ |
| Crevice | 6 | “A slit or pock-like recess in the rock” |
| Pool | 7 | A rock pool < 20cm deep |
| Deep Pool | 8 | A rock pool > 20cm deep |

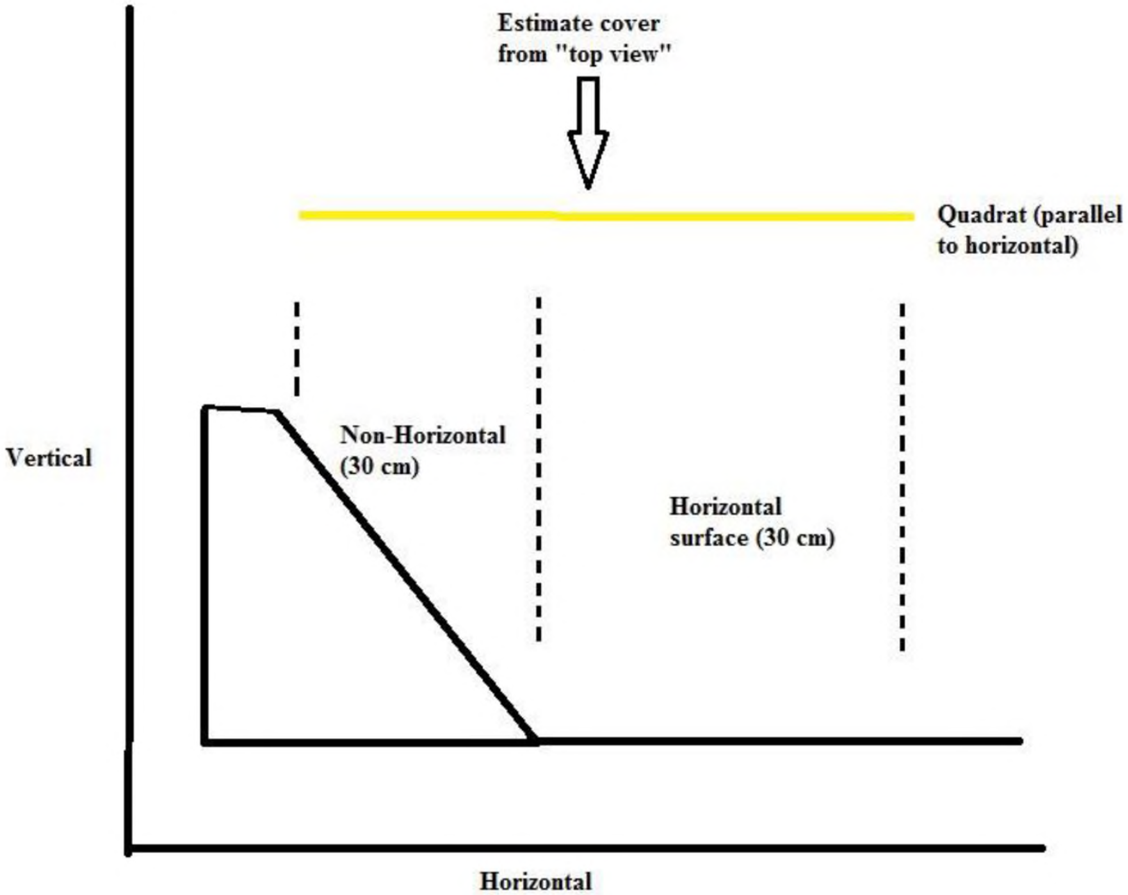

**Fig 2.2: Two - Dimensional scheme displaying how non-horizontal microhabitat cover can be underestimated in a quadrat with mixed (horizontal and non-horizontal) surfaces. Arbitrary length (cm) values for the two microhabitat types displayed in parentheses.**

Once microhabitat cover was established, limpet abundances (abundance/microhabitat type) of each species was determined using the same sample area and procedures described in the previous paragraph. However, limpet abundance was determined by counting all the individuals of each species found within the quadrats. Thus, both microhabitat and limpet abundance within the available microhabitats were recorded at the same points/quadrats within the sample area. The reference and transect lines were held in place during sampling using large rocks found at the study sites (Fig 2.3). The start and end points of the sample areas were photographed and clearly marked in photographs to keep record of the exact sample areas (Fig 2.4).

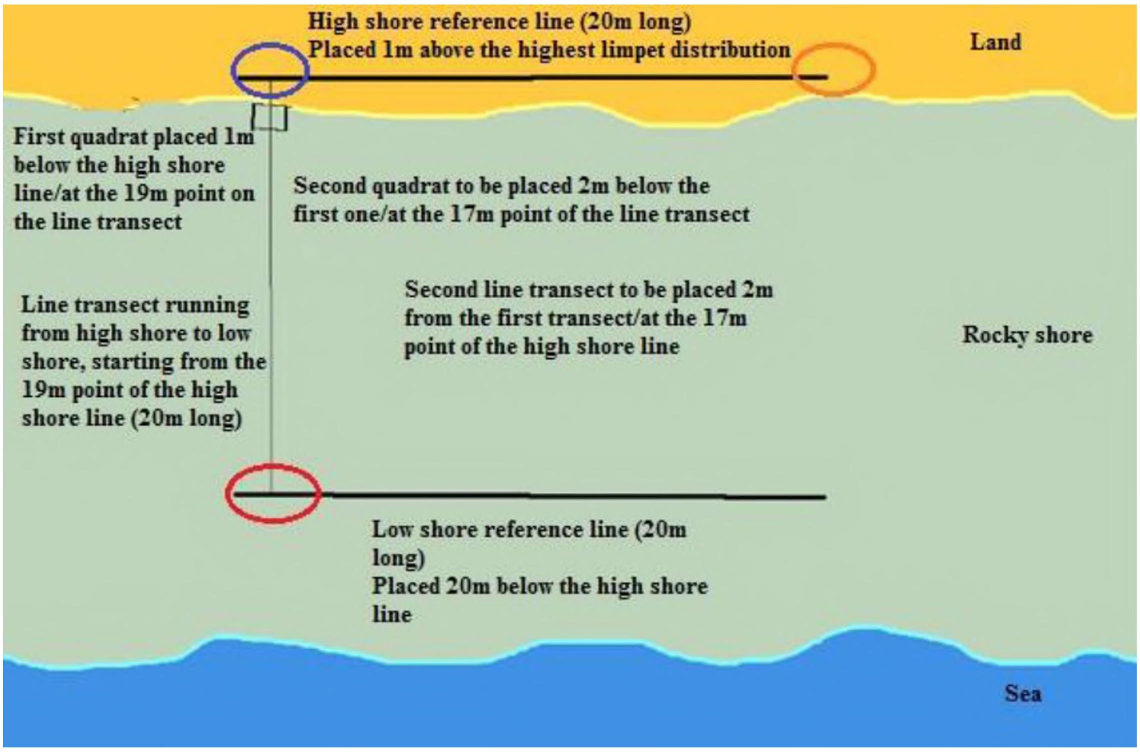

**Fig 2.3: Scheme of how microhabitat availability and microhabitat use were sampled at the 3 study sites, with the marked reference points (see Fig 2.4 below) circled in colour.**

**Fig 2.4 Picture displaying the marked start (blue circle) and end (orange circle) points of the high shore reference line, and the start (red circle) point of the low shore reference line at the Kenton sample area.**

Once microhabitat cover and limpet abundances were recorded, other relevant features, using the same sampling procedure for microhabitat cover at the study sites, were noted (see Table 2.2 in results section). These features included algal and sand cover on rock surfaces, as well as the aspect of vertical or sloped rock surfaces.

### *Data visualisation*

Base maps (Fig 2.8 – 2.10 below) for each study site were prepared in order to display limpet microhabitat use and microhabitat availability across shore within the sample areas, using Excel 2015 and Adobe Illustrator CS6.

### *Statistical analysis*

Chi-squared analyses were used to determine if the study animals were distributed evenly among the available microhabitats at each study site, using Excel 2015. This was done by first identifying all the quadrats in which the respective limpet species were found, in the different sample areas. Thereafter, the microhabitat cover (%) of each microhabitat type was determined, as a proportion of the total area ($cm^2$) covered by the relevant quadrats for each limpet species. Limpet abundance for each species was then determined for each microhabitat type, as a percentage of the total number of individuals found within the total area sampled. The Chi squared analyses were then conducted using microhabitat and percentage limpet abundance as the expected and observed values respectively.

# Results

## *Robolimpets*

Temperature was successfully measured by each of the robolimpets during the 5½ month period. However, the temperature measurements were not correlated to the right daily times. This meant that I could not accurately determine the correct times for important temperature measures such as, the time of day the maximum and minimum temperatures were likely to occur. There is a clear trend in the temperature data, with all three robolimpets displaying increases in mean monthly, maximum and minimum (Fig 2.5) temperatures from May to October. With the highest mean monthly temperatures occurring in August (21°C) and September (21°C), and the lowest mean monthly temperature (17.1°C) occurring in May (Fig 2.5). In addition, the highest maximum temperatures were recorded in September and October, while the lowest maximum and minimum temperatures were both recorded during May (Fig 2.5)

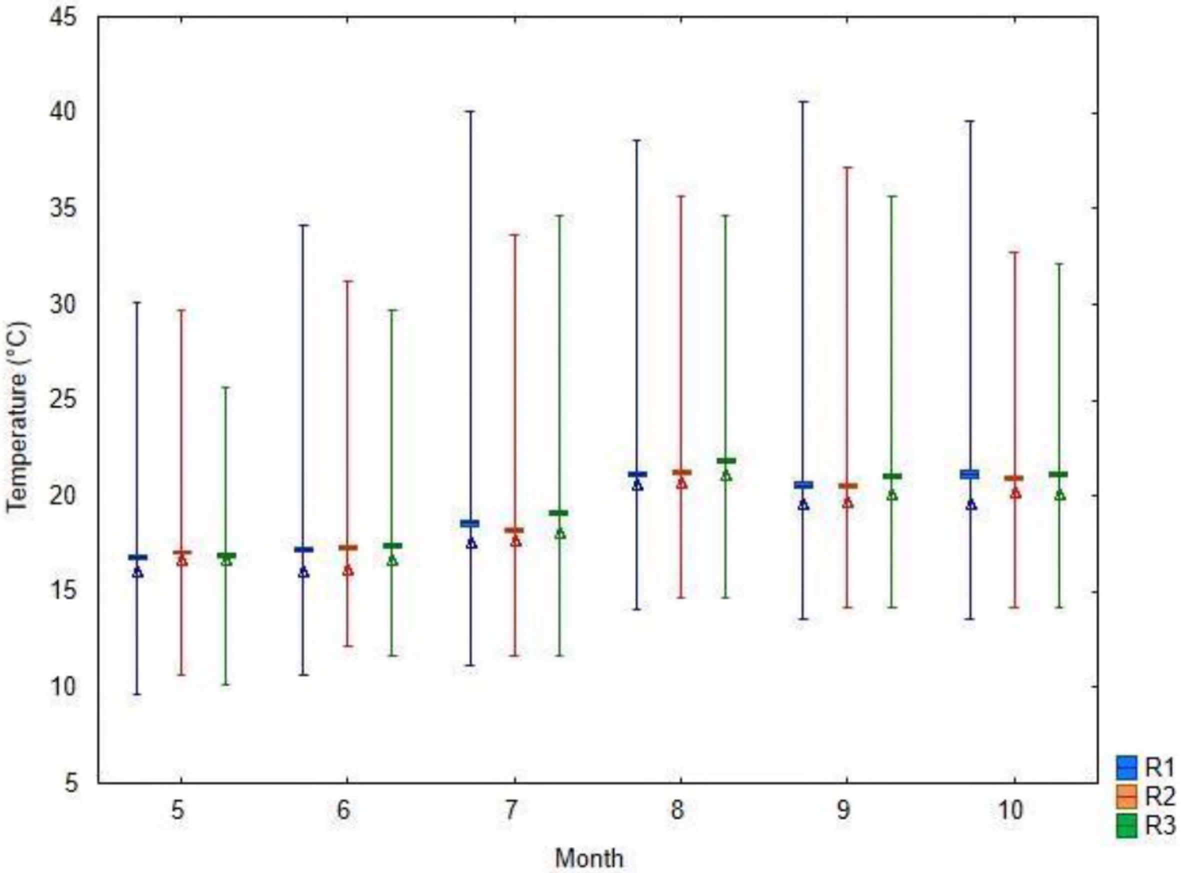

**Fig 2.5: Box and whisker plot displaying the mean (squares), median (triangles) and, the maximum and minimum (whiskers) monthly temperatures (°C) of the robolimpets (n = 3) deployed on the high shore rocks at Kenton. The colours represent individual robolimpet replicates. The x-axis values represent the months in which the robolimpets were active (5 – May; 6 – June; 7 – July; 8 – August; 9 – September; 10 – October).**

### *Microhabitat availability and characteristics*

Flat/horizontal rock surfaces were the dominant microhabitat type at Kenton and Chintsa West (67.8% and 79.7% respectively, of the 25m$^2$ measured at each site), but formed only 27.9% at Port Alfred, where sloped surfaces were more common, making up approximately 45.9% of the total area sampled (Fig 2.6). After flat rock surfaces, rock pools and vertical rock surfaces covered the next largest area at all three sites. Rock pools comprised 16.2%, 9.1% and 10.2%, while vertical rock surfaces made up 11.3%, 13.9% and 7% of the sampled areas in Kenton, Port Alfred and Chintsa West respectively (Fig 2.6). Sloped rock surfaces comprised only 1.2% and 0.8% of the total area sampled at Kenton or Chintsa West (Fig 2.6). The remaining microhabitats covered very small areas and in most cases, were not sampled at all three of the study sites. Crevices were only found in Port Alfred and Chintsa West (1% and 2.3% respectively; Fig 2.6). Boulders with horizontal surfaces were only found in Kenton (1.9%) and Port Alfred (1.1%), while Port Alfred was the only study site where sloped boulder surfaces (1.2%) were found. Finally, because the study sought *a priori* to avoid deep pools, this microhabitat was rare and was found only at Kenton where it was unavoidable, covering approximately 1.8% of the sample area.

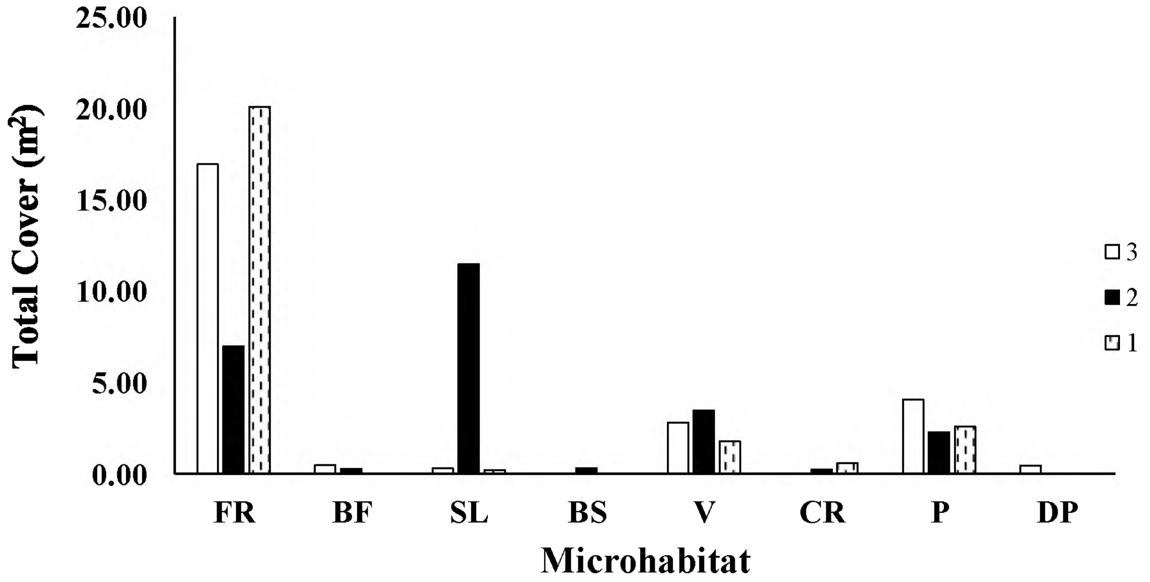

**Figure 2.6: Bar graph displaying the microhabitat cover ($m^2$) in the sampled areas of the study sites at Kenton (3), Port Alfred (2) and Chintsa West (1). The sampled microhabitats are represented as FR (flat rock), BF (boulder with flat surface), SL (sloped surface), BS (boulder with sloped surface), V (vertical surface), CR (crevice), P (pool) and DP (deep pool).**

The predominantly flat rock site at Kenton had a relatively even spread of sand, algae and bare rock cover (Table 2.2). Kenton also had one long west facing rock feature running from high to low shore that formed part of a larger cliff face (Table 2.2). In addition to this, a smaller east facing rock section was sampled as part of a small (approx. 1 m high) rocky outcrop. This east facing rock sample was made up of approximately, 50:50 vertical and sloped rock surfaces (Table 2.2). There were no south or north facing features at Kenton (Table 2.2). In contrast to the Kenton study site, the sample area at Port Alfred was mostly a mixture of sloped and vertical rock surfaces which were east or west facing (Table 2.2). While most the west facing rock features at Port Alfred were vertical rock surfaces, the rest were sloped rock surfaces. In comparison to this, the east facing features consisted almost entirely of sloped rock surfaces and a few vertical surfaces. North and south facing rock features were less common at Port

Alfred and comprised only vertical rock surfaces (Table 2.2). Most of the rock surfaces sampled at Port Alfred were bare, with only a small proportion of this area covered by sand (Table 2.2). In addition to this, no algae were sampled at Port Alfred (Table 2.2). Like Port Alfred, most of the rocky shore sampled at Chintsa West was made up of bare rocks with very little algal or sand cover (Table 2.2). Just like Kenton, the sample site at Chintsa West was predominantly made up of flat rock surfaces (Fig 2.6). Most of the few vertical rock surfaces here were west or south facing but, there were some north facing vertical rock features (Table 2.2). The east facing rock features were comprised mostly of vertical rock surfaces and less so of sloped rock surfaces (Table 2.2).

**Table 2.2: Percentage cover of the major characteristics of the sampled areas at Kenton (3), Port Alfred (2) and Chintsa West (1). The sampled characteristics include sand cover (S), algal cover (AL), bare rocks (B), south (ST), west (W), north (N) and east (E) facing rock features.**

| **Study Site** | **Percentage of total area (%)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Rock Surface Features** | | | **Aspect** | | | |
| | **S** | **AL** | **B** | **ST** | **W** | **N** | **E** |
| **3** | 39.5 | 29.5 | 31 | 0 | 86.67 | 0 | 13.33 |
| **2** | 14 | 0 | 86 | 1.83 | 44.51 | 1.22 | 42.44 |
| **1** | 6 | 0 | 94 | 28.57 | 38.1 | 9.52 | 23.81 |

*Microhabitat use*

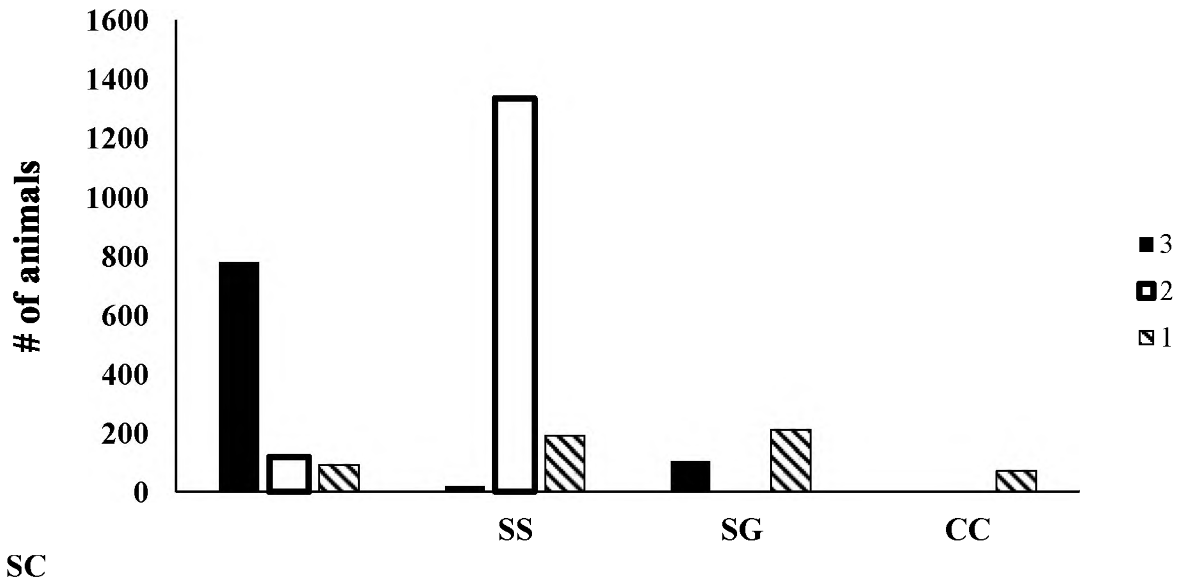

**Figure 2.7: Bar graph displaying the number of limpets counted at the sample areas in Kenton (3), Port Alfred (2) and Chintsa West (1). (SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis*).**

*Kenton*

Three of the four study species were found at Kenton in varying proportions in the different microhabitats (Fig 2.7). *Siphonaria capensis* (n =779) was by far the most common limpet in the area, followed by *Scutellastra granularis* (n = 102) and finally *Siphonaria serrata* (n = 19), which was present in very low numbers compared to the other two species (Fig 2.7). *C. capensis* was the only limpet not sampled in Kenton (Fig 2.7). In addition, *S. capensis* and *S. serrata* were mostly found higher up the shore, while *Scutellastra granularis* could be found from high to low shore (Fig 2.8). Despite being found in the area, *S. granularis* was only present on vertical rock surfaces (Fig 2.8). In fact, it was only found in quadrats consisting entirely of this microhabitat.

Out of the three limpet species found at Kenton, only *Siphonaria capensis* was not distributed equitably ($p < 0.05$; df = 4) among the sampled microhabitats (Table 2.3). The greatest proportion of *S. capensis* occurred on flat rock surfaces (Fig 2.8) and the flat top of large boulders while it avoided rock pools, vertical and sloped rock surfaces (Table 2.3). *S. serrata*

($p > 0.05$; df = 2) did not display a significant preference for any of the available microhabitats within the sampled quadrats (Table 2.3). It was most common on vertical rock surfaces and, to a lesser extent it was also found on flat rock surfaces and boulder flats (Table 2.3). The fact that *S. serrata* was found in these microhabitats in their expected proportions explains the insignificant result obtained from the chi-squared test conducted on *S. serrata* (Table 2.3). Furthermore, *S. serrata* was not found in either the sloped rock and shallow rock pools (Table 2.3). Finally, none of the limpet species at Kenton were found in deep rock pools (Table 2.3).

**Table 2.3: Habitat availability and chi-squared results for each species calculated using data only for quadrats in which that species was found at Kenton. The species are represented as SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis* and their microhabitats as FR = flat rock; BF = boulder flat; SL = sloped surface; V = vertical surface and P = pool. Proportion of animals expected in each microhabitat presented in parentheses. Note CC was absent for this site.**

| **Species** | **Proportion (%) of animals in each microhabitat** | | | | | $\chi^2$ |
|---|---|---|---|---|---|---|
| | **FR** | **BF** | **SL** | **V** | **P** | |
| **SC** | 65.73<br>(60.23) | 22.21<br>(4.3) | 0<br>(0.35) | 8.34<br>(20.93) | 3.72<br>(14.19) | 90.66 |
| **SS** | 21.05<br>(22.22) | 10.53<br>(11.11) | 0 | 68.42<br>(66.67) | 0 | 0.14 |
| **SG** | 0 | 0 | 0 | 100<br>(100) | 0 | N/A |
| **CC** | 0 | 0 | 0 | 0 | 0 | N/A |

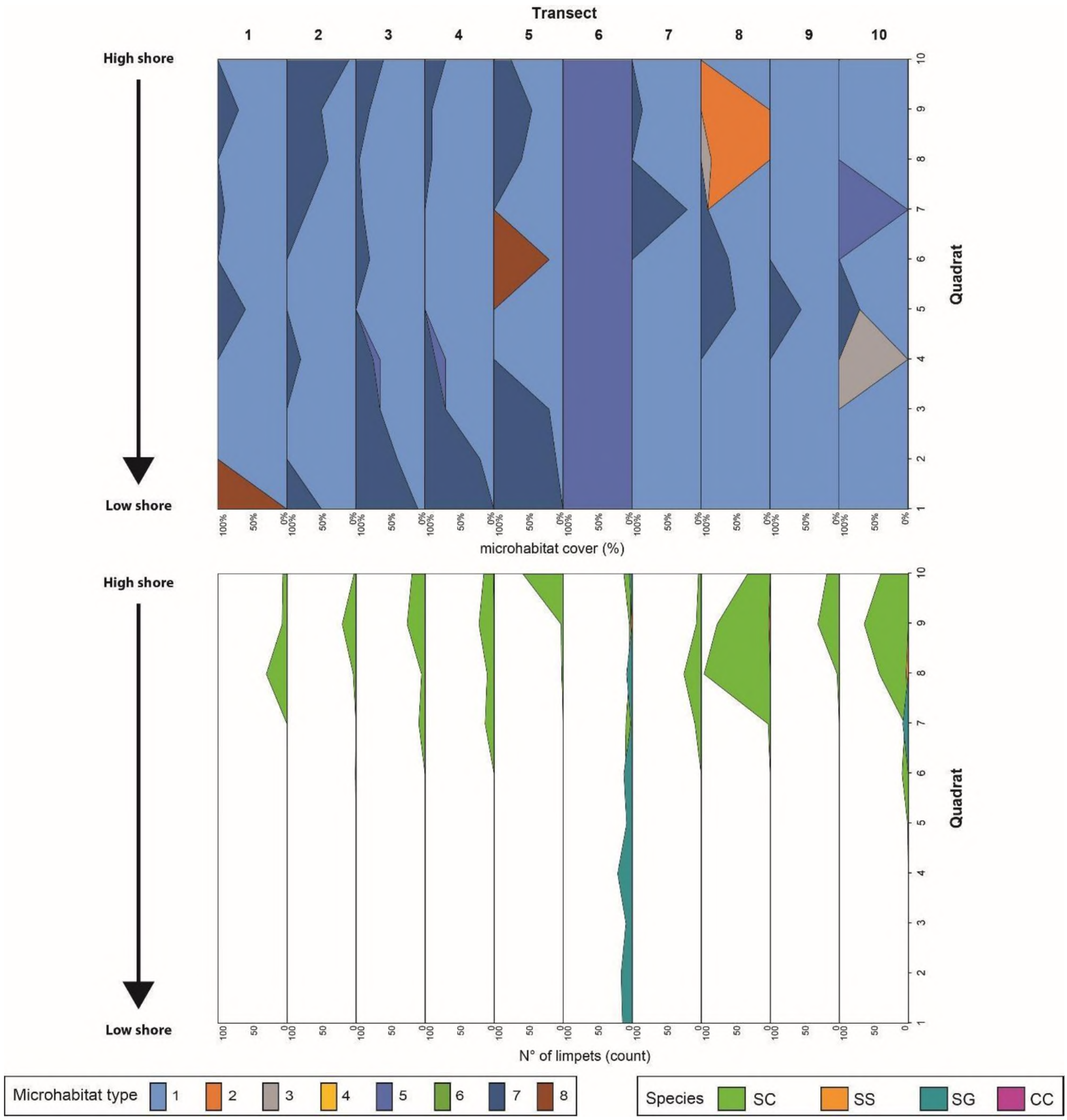

**Fig 2.8: Base map displaying: % microhabitat cover (top panel) and abundances of limpets (bottom panel) at Kenton. The species are represented as SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis* and the numbers represent their microhabitat type (see Table 2.1). Note CC was absent for this site.**

### *Port Alfred*

Compared to Kenton, there was an even poorer representation of the study species in Port Alfred where only two, *Siphonaria serrata* (n = 1334) and *S. capensis* (n = 118), were found (Fig 2.7). Furthermore, while *S. serrata* was common from high to low shore, *S. capensis* was mostly found in the mid-low shore region (Fig 2.9). Both *S. capensis* ($p < 0.05$; df = 3) and *S. serrata* ($p < 0.05$; df = 6) were not distributed among the microhabitats in proportion to the microhabitat availability (Table 2.4). A large proportion of *S. capensis* sampled in Port Alfred made use of rock pools (Fig 2.9). While *S. capensis* was also found on flat and sloped rock surfaces, based on the abundance of these microhabitats, they were mostly avoided. In addition, despite the availability of vertical rock surfaces, *S. capensis* individuals did not occur on them (Table 2.4). *S. serrata* was found in a broader range of the available microhabitats than *S. capensis* (Table 2.4). Sloped rock surfaces were its preferred microhabitat, followed by vertical rock surfaces (Table 2.4). Like *S. capensis*, despite being common on flat rock surfaces, *S. serrata* individuals appeared generally to avoid this microhabitat (Table 2.4). Specimens of *S. serrata* were not as common on boulder flat, boulder slope, crevice and rock pool microhabitats as they were in the aforementioned microhabitats (Table 2.4).

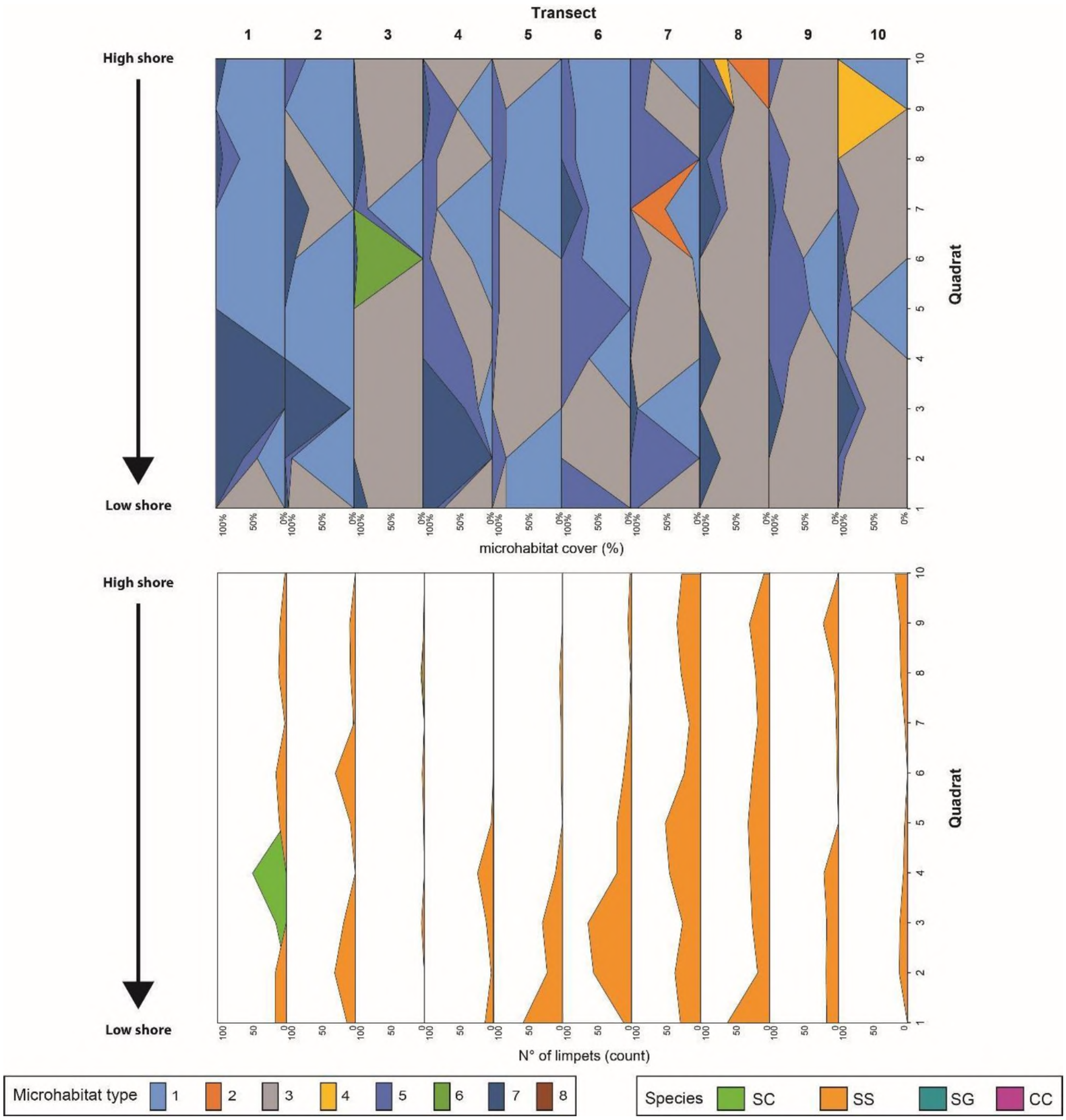

**Fig 2.9: Base map displaying: % microhabitat cover (top panel) and abundances of limpets (bottom panel) at Port Alfred. The species are represented as SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis* and the numbers represent their microhabitat type (see Table 2.1). Note SG and CC were absent from this site.**

**Table 2.4: Habitat availability and chi-squared results for each species calculated using data only for quadrats in which that species was found at Port Alfred. The species are represented as SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis* and their microhabitats as FR = flat rock; BF = boulder flat; SL = sloped surface; BS = boulder sloped; V = vertical surface; CR = crevice and P = pool. Proportion of animals expected in each microhabitat presented in parentheses. Note SG and CC were absent from this site.**

| Species | Proportion (%) of animals in each microhabitat | | | | | | | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|
| | FR | BF | SL | BS | V | CR | P | |
| **SC** | 14.41<br>(30) | 0 | 16.10<br>(43.68) | 0 | 0<br>(5.53) | 0 | 69.49<br>(20.79) | 145.14 |
| **SS** | 16.42<br>(28.61) | 1.65<br>(1.39) | 50.45<br>(44.94) | 0.97<br>(1.52) | 24.74<br>(14.43) | 0.22<br>(1.2) | 5.55<br>(7.91) | 14.98 |
| **SG** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |
| **CC** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A |

### *Chintsa West*

In contrast to Kenton and Port Alfred, all four study species were found in Chintsa West (Fig 2.7). *Scutellastra granularis* (n = 210) was the most abundant species, followed by *Siphonaria serrata* (n = 190), *S. capensis* (n = 91) and the least common species was *C. capensis* (n = 71), which was found only at this site. *S. serrata* was the only species found at all shore heights but it was most common in the mid shore area (Fig 2.10). While *S. capensis* was common in the high to mid shore areas, *Scutellastra granularis* and *C. capensis* were common in the mid-low shore and low shore areas respectively (Fig 2.10). *Siphonaria capensis* ($p < 0.05$; df = 2), *S. serrata* ($p < 0.05$; df = 3), *Scutellastra granularis* ($p < 0.05$; df = 3) and *C. capensis* ($p < 0.05$; df = 3) all had highly significant preferences for particular microhabitats within the Chintsa West study site (Table 2.5). While *Siphonaria capensis* and *C. capensis* preferred rock pools to the other microhabitats, both *Scutellastra granularis* and *Siphonaria serrata* were frequently found in other microhabitats even though they showed a preference for rock pools (Table 2.5). *Scutellastra granularis* was most common on vertical rock surfaces and had a distinct preference for this microhabitat. Neither of the remaining three limpet species were nearly as common on vertical rocks, particularly *S. capensis*, which clearly avoided this microhabitat (Table 2.5).

While horizontal rock surfaces were a dominant feature of Chintsa West, *C. capensis*, *Siphonaria serrata* and *Scutellastra granularis* were not common there and appeared to avoid this habitat (Table 2.5). In contrast, *Siphonaria capensis*, was most common on horizontal rock surfaces, although it displayed no preference for it (Table 2.5). Of the four study species, only *S. capensis* was not recorded in crevices. The remaining three species were all found in crevices but, they were all least common there (Table 2.5). None of the limpets were found on sloped rock surfaces at Chintsa West. This was expected, however, since it formed a very small proportion of the total area at this study site and did not occur in any of the sampled quadrats (Fig 2.6).

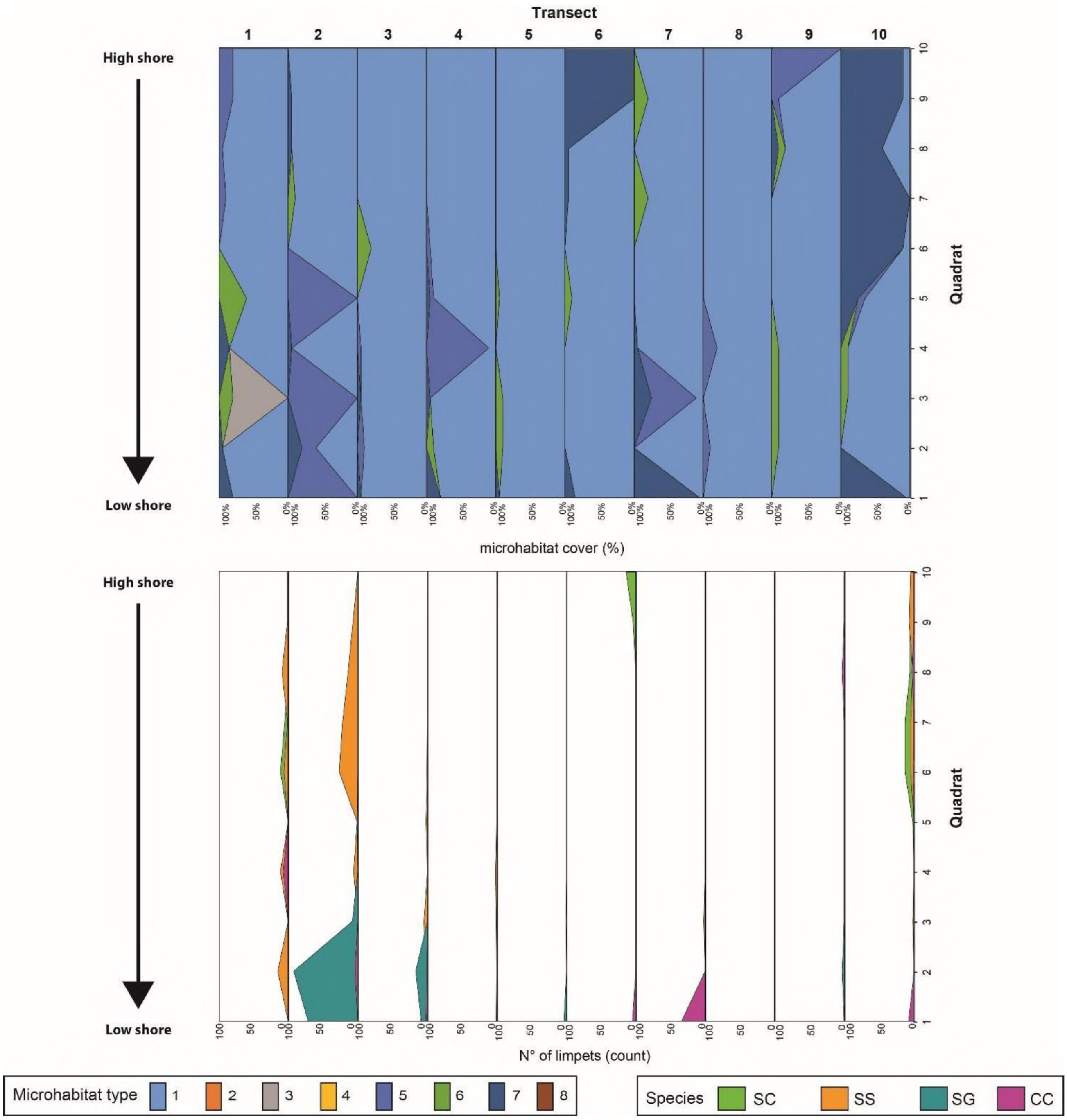

**Fig 2.10: Base map displaying: % microhabitat cover (top panel) and abundances of limpets (bottom panel) at Chintsa West. The species are represented as SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis* and the numbers represent their microhabitat type (see Table 2.1).**

**Table 2.5: Habitat availability and chi-squared results for each species calculated using data only for quadrats in which that species was found at Chintsa West. The species are represented as SC = *Siphonaria capensis*; SS = *Siphonaria serrata*; SG = *Scutellastra granularis*; CC = *Cellana capensis* and their microhabitats as FR = flat rock; V = vertical surface and CR = crevice; P = pool. Proportion of animals expected in each microhabitat presented in parentheses.**

| Species | Proportion of animals/microhabitat (%) | | | | $\chi^2$ |
|---|---|---|---|---|---|
| | FR | V | CR | P | |
| **SC** | 17.58<br>(30.58) | 5.49<br>(15.71) | 0 | 76.92<br>(53.93) | 21.83 |
| **SS** | 55.79<br>(65.38) | 16.84<br>(15) | 6.32<br>(1.43) | 21.05<br>(18.21) | 18.79 |
| **SG** | 2.38<br>(66.11) | 73.81<br>(25.56) | 1.43<br>(3.33) | 22.38<br>(5) | 214.06 |
| **CC** | 0<br>(55.77) | 8.45<br>(10) | 1.41<br>(1.54) | 90.14<br>(32.69) | 156.97 |

# Discussion

As described in the introduction, rocky intertidal habitats include multiple microhabitats, which have different thermal regimes during day time emersion (see below). The robolimpets were used only to gain a preliminary measurement of high shore intertidal temperatures, to gauge the general temperatures these limpets may be subject to in the region (Fig 2.5). It is important to note that most of these measurements occurred during winter, which means that the absolute monthly maxima and mean monthly temperatures, expressed in the results, are likely to be much lower than they would have been in summer. As a result, I relied heavily on past papers to estimate the level of thermal stress the study animals may have experienced in the various microhabitats (Garrity, 1984; Williams and Morrit, 1995). In his study on the adaptations of gastropods to physical stress, Garrity (1984) found that a gradient of most to least (horizontal surfaces > slopes > vertical surfaces > tide pools > crevices) thermally stressful conditions exists among high shore intertidal microhabitats.

Horizontal surfaces are thermally stressful because they are normally exposed to higher levels of solar heating and potentially greater temperatures and desiccation stress than other intertidal microhabitats (Williams and Morritt, 1995; Helmuth and Hofmann, 2001; Denny *et al.*, 2006; Sinclair *et al.*, 2006). High shore horizontal surfaces are therefore normally bare or barren, because most intertidal organisms cannot survive the extreme temperatures on these surfaces. When high shore horizontal surfaces are not barren, it is normally due to some mitigating factor that makes the microhabitat cooler than it would have been otherwise. Branch (1976) suggested that horizontal surfaces covered in sand retain more moisture than bare horizontal surfaces, potentially making them more habitable. The geomorphology of rocky shores has also been shown to positively influence the habitability of horizontal microhabitats. For example, a few studies (Marker, 1988; Dower, 1989; Vat, 2000) have shown that aeolianite rocks retain moisture for longer than quartzitic sandstone rocky shores, due to the high surface rugosity and slightly more porous nature of aeolianite rocks. The retention of water creates damp conditions allowing horizontal surfaces influenced by these factors to remain cooler.

Non-horizontal, intertidal microhabitats such as sloped and vertical rock surfaces are more likely to be shaded and therefore, cooler than horizontal rock surfaces during the day (Wolcott, 1973; Williams and Morritt, 1995; Helmuth, 1998; 2002; Wethey, 2002; Harley, 2008; Lima and Wethey, 2009; Seabra *et al.*, 2011; Lima *et al.*, 2016). However, when considering sloped and vertical rock surfaces, it is important to note that their orientation to the sun ultimately influences levels of solar heating and thus, how hot these microhabitats get during the day (Williams and Morritt, 1995; Helmuth and Hofmann, 2001; Seabra *et al.*, 2011). Vertical or sloped rock surfaces oriented away from full solar insolation are usually cooler than rock surfaces exposed to direct sunlight (Williams and Morritt, 1995; Seabra *et al.*, 2011). This is true even for sunny microhabitats found lower down the shore compared to microhabitats that are in shade most of the time and higher up the shore (Seabra *et al.*, 2011). The angle of these non-horizontal microhabitats to the horizontal also plays an important part in determining the temperature range experienced on them (Wolcott, 1973; Helmuth, 1998; 2002; Wethey, 2002; Harley, 2008; Lima and Wethey, 2009). Generally, the closer the angle is to the horizontal, the higher the maximum temperature is on that substratum (Wethey, 1983; 1984; Williams and Morritt, 1985; Menconi *et al.*, 1999). This means that, sloped rock surfaces generally have higher maximum and average temperatures than vertical rock surfaces (Wethey, 1983; 1984; Williams and Morritt, 1985; Denny *et al.*, 2006).

Rock pools are normally cooler and have more stable thermal conditions than the aforementioned emerged microhabitats, which is mainly due to the buffering effect water has on temperature (Newell, 1979). This buffering effect is intensified in deep rock pools with steep or sloped boundary surfaces that provide the rock pool organisms with a refuge from direct solar insolation (Branch and Cherry, 1985; Williams and Morritt, 1995; Lord *et al.*, 2011). However, when rock pools are relatively shallow and spread over large areas, the water inside them does not have as great a buffering effect on temperature (Branch and Cherry, 1985; Williams and Morritt, 1995). Therefore, like horizontal surfaces these shallow rock pools are subject to high levels of solar radiation during the hottest parts of the day, which could lead to high maximum aquatic temperatures (Ganning, 1971; Morris and Taylor, 1983; Huggett and Griffiths, 1986). As seen in the study by Williams and Morritt (1995) shallow rock pools are a less predictable thermal refuge than deeper rock pools. Crevices are generally considered to be the least thermally stressful microhabitat (Garrity, 1984) however, because the study limpets generally had very low numbers in them they will not be discussed further. While limpet numbers were low in crevices, limpet use of crevices, particularly that of *S. serrata* (Chambers, 1994), may have been higher had there been a greater representation of crevices at the study sites (Fig 2.6).

Sampling of limpet microhabitat use at the three study sites indicated distinct preferences for particular microhabitats. However, not all the study animals were present at each of the study sites. *Scutellastra granularis* did not occur at Port Alfred and *C. capensis* was only present at Chintsa West, while both siphonariid limpets occurred at all three study sites (Fig 2.7). Flat rock surfaces made up much of the area at Kenton and Chintsa West, while sloped rock surfaces were the main microhabitat at Port Alfred (Fig 2.6). *Siphonaria capensis* preferred flat rock surfaces in Kenton and rock pools at the other two study sites (Tables 2.3-2.5). *S. serrata* showed no preference for any of the microhabitats at Kenton though it was most common on vertical and horizontal rock surfaces (Table 2.3). In Port Alfred, *S. serrata* preferred sloped rock surfaces and to a lesser extent, vertical surfaces, while in Chintsa West, it preferred rock pools but, was also very common on flat rock surfaces. *Scutellastra granularis* may have preferred vertical rock surfaces in Kenton (Table 2.3). However, there was no way of testing this statistically using the chi-squared test, because vertical rock surfaces were the only microhabitats sampled in the quadrats where *S. granularis* was present. At Chintsa West, most *S. granularis* individuals preferred vertical rock surfaces while the rest of the individuals from this species preferred rock pools (Table 2.5). *C. capensis* on the other hand, preferred rock pools at Chintsa West, like *Siphonaria capensis* (Table 2.5).

Overall, the results indicate that *S. capensis* and *C. capensis* preferred rock pools to all other microhabitats, while *Scutellastra granularis* preferred vertical surfaces and *Siphonaria serrata* preferred sloped and vertical rock surfaces (Table 2.3-2.5). This is in accordance with previous findings, which show that *S. capensis* (Chambers, 1994) and *C. capensis* (Branch, 1975; 1976) prefer rock pools and that *Scutellastra granularis* (Vat, 2000) individuals prefer vertical rock surfaces. However, *Siphonaria capensis* also preferred horizontal rock surfaces, particularly at Kenton, despite the availability of rock pools at this study site (Table 2.3). During his research, Chambers (1994) found that *S. capensis* normally occurs on "bare rock" surfaces. Even though Chambers (1994) did not clearly state it in his study, we can assume that the "bare rock" surfaces mentioned during his work are equivalent to the horizontal rock surfaces described in the current study. Concerning *S. serrata*, there are no previous studies that record finding this species on sloped rock surfaces and in general, very few studies on intertidal microhabitat use have explicitly identified sloped surfaces as a microhabitat type. During their research, Branch and Cherry (1985) also established that *S. capensis* individuals occasionally occur on horizontal surfaces despite preferring shallow rock pools.

The horizontal rock surfaces at Kenton, which formed the most common microhabitat there, were seldom bare (Table 2.3-2.5). In fact, algae and damp sand were present on large portions of the horizontal rock surfaces at Kenton, regardless of shore height (Table 2.2). This was probably because the headland at this sample area (Marker, 1988; Dower, 1989; Vat, 2000), protects parts of the horizontal surfaces from direct insolation during the day and strong wave action. It is important to note however, that the high shore algal cover at Kenton could also be a result of the nutrient rich water brought up by the upwelling cell in an otherwise nutrient poor coastal region (Lutjeharms, 1998; Hanekom *et al.*, 2009). Unlike the horizontal surfaces at Kenton, large sections at Chintsa West were barren. In addition, Kenton consists of an aeolianite rocky shore unlike Port Alfred and Chintsa West, where the rocky shore is formed from quartzitic sandstone (Marker, 1988; Dower, 1989; Vat, 2000). These differences in rock sand/algal cover and rock geomorphology between the sample sites are important because they potentially make the horizontal microhabitat at Kenton more habitable than the horizontal microhabitat at Chintsa West (Table 2.2). This in turn could explain why *Siphonaria capensis* individuals were found in horizontal microhabitats at Kenton, while they avoided the same microhabitat at Chintsa West (Table 2.3 and 2.5).

Even though the vast majority of the horizontal rock surfaces at Chintsa West were free of sand or algae and exposed to direct solar insolation, a large number of *Siphonaria serrata* individuals occurred there. Small sections of these surfaces were shaded during the day because they were immediately adjacent to vertical rock surfaces (pers. obs.) and were probably cooler than the surrounding horizontal surfaces during the hottest times of the day (Lima et al., 2016). Because these cooler surfaces were sporadic and formed a very small part of the larger horizontal microhabitat area at Chintsa West, many *S. serrata* individuals were found to aggregate there (pers. obs.). Therefore, even though the results may indicate that *S. serrata* individuals were common on the barren horizontal surfaces at Chintsa West, they occurred almost exclusively in these cooler "sub-microhabitats". At Port Alfred, both siphonariid limpets also occurred on horizontal surfaces that were adjacent to sloped rock or vertical rock surfaces (Table 2.4). Additionally, many of the *S. serrata* individuals found on horizontal rock surfaces occurred lower down the shore (Fig 2.9). Several past papers (Branch, 1975; Liu, 1994; Williams and Morritt, 1995; Harper and Williams, 2001) show that intertidal gastropods often migrate down shore, diurnally or seasonally, to avoid stressful conditions higher up the shore, although this migration is often associated with the decrease in food farther up the shore during the warmer months (Underwood, 1984; Williams, 1993; Williams and Morritt, 1995). As described previously, many intertidal gastropods also migrate down shore to take advantage of the shelter offered by biogenic microhabitats that occur in this region of the intertidal (Williams and Morritt, 1995; Mak, 1996; Harper and Williams, 2001; Cartwright and Williams, 2012).

At Chintsa West, the barren nature of the horizontal rock surfaces meant that the limpets favoured other microhabitats (Table 2.5). The most popular of these were rock pools and all four species occurred in these (Table 2.5). However, only *C. capensis* and *Siphonaria capensis* preferred rock pools to the other microhabitats (Table 2.5). The sample area at Port Alfred was not as barren as the area at Chintsa West and, presumably because of this, only *S. capensis* preferred the rock pools here (Table 2.4). Gastropods found in rock pools have body temperatures that are similar to the surrounding water temperature, which allows them to maintain cooler body temperatures than they would have experienced, had they been exposed to air (Newell, 1979; Helmuth and Hofmann, 2001). However, most of the rock pools sampled in this study, particularly the ones at Chintsa West, were quite shallow (approximately < 3cm; pers. obs.). As described above, this meant that the limpet species most likely to make use of this microhabitat were probably subjected to more extreme thermal conditions than they would have been in deeper rock pools (Ganning, 1971; Morris and Taylor, 1983; Huggett and Griffiths, 1986; Williams and Morritt, 1995).

Depending on shore height, all the microhabitats except rock pools were emerged, for a period ranging between 2 - 6 hours, during spring low tide (South African Navy Hydrographic Office, 2017). Previous studies have shown that patellogastropod limpets possess breathing structures that are primarily adapted to function in water (Yonge, 1947; Branch, 1981; Innes *et al.*, 1984; McMahon, 1988). These limpets therefore have to utilise behavioural adaptations to help protect their breathing apparatus. The patellogastropod limpet, *C. capensis* mostly occurred in rock pools where it would not have to adopt any other behavioural measures to protect itself from desiccation (Branch, 1975; 1976). However, *Scutellastra granularis* preferred vertical surfaces and was not as common in rock pools. Linked to this, *S. granularis* individuals display strong homing behaviour (Santina, 1994; Jakob *et al.*, 2001; Shanks, 2002). By clamping down tightly to their substratum these limpets create a microclimate within their shells, which allows them to reduce the desiccation and thermal stress often associated with high shore environments (Verderber *et al.*, 1983; Branch and Cherry, 1985; Santina, 1994; Shanks, 2002). However, these limpets have to deal with the temperature regime of their chosen microhabitats for extended periods because of this homing behaviour (Sinclair *et al.*, 2006). It is probably for this reason that *S. granularis* is common in the thermally more moderate vertical surface microhabitat (Vat, 2000). In their study on *C. grata*, Williams and Morritt (1995) showed that these limpets also preferred settling on vertical surfaces during the day, probably to avoid high thermal stress on horizontal surfaces.

Several studies indicate that the thermal limits of limpets (Wolcott, 1973; Garrity, 1984; Williams and Morritt, 1995; Helmuth and Hofmann, 2001; Denny *et al.*, 2006; Sinclair *et al.*, 2006; Lima *et al.*, 2016) are influenced by the thermal regime of the microhabitats in which they normally occur. As a result, in this study it was expected that *Siphonaria capensis*, which was common on horizontal rock surfaces and in rock pools, will have the highest upper thermal limits. Since there was some overlap in the microhabitat use of *S. capensis* and *C. capensis*, which like *S. capensis* preferred rock pools, *C. capensis* is expected to have thermal limits slightly lower than *S. capensis*. *Scutellastra granularis*, in contrast, was primarily found on vertical rock surfaces so it was expected to have the lowest upper thermal limits. *Siphonaria serrata* preferred sloped rock surfaces but, like *Scutellastra granularis* was also common on vertical rock surfaces. Since sloped and vertical rock surfaces are on opposite ends of the assumed microhabitat thermal stress gradient, with rock pools somewhere in the middle, *Siphonaria serrata* is expected to have upper thermal limits similar to and perhaps lower than *C. capensis*. The biogeography of intertidal organisms has also been shown to influence their upper thermal limits (Stillman, 2003; Somero, 2005; Jones *et al.*, 2009). During their study on porcelain crabs, Stillman and Somero (1996) indicated that *Petrolisthes* spp. from warm sub-tropical regions often had higher lethal thermal limits than congeners from cooler temperate regions. Moreover, Tomanek and Somero (1999) obtained similar results in an earlier study on turban snails (*Tegula* spp.). This could possibly have major implications for the upper thermal limits obtained for *Scutellastra granularis*, which was the only predominantly temperate limpet species among the study animals (Kalk, 1959; Branch, 1971; Branch and Branch, 1988; Branch *et al.*, 1994).

In this study, it can be seen that temperature, along with desiccation stress, played an important role in determining limpet microhabitat use among the limpets, with warm horizontal rock surfaces generally being avoided in favour of cooler non-horizontal rock surfaces. However, several mitigating factors such as substratum rugosity, shading, sand and algal cover seemed to make certain emerged microhabitats less thermally stressful, judging from the use of horizontal microhabitats by *Siphonaria capensis* under these conditions (described above). Furthermore, these mitigating factors all appear to have a similar effect on the emerged substrata, which is to increase substratum moisture retention. This could be linked to a potential decrease in temperature, desiccation stress and greater limpet abundances. For example, while limpets avoid bare horizontal rock surfaces with low moisture retention at Chintsa West, they may prefer rough horizontal rock surfaces with a high moisture retention at Kenton. Furthermore, it was assumed that shading plays an important role in determining rock pool temperatures. Where deeper rock pools with more shade than shallow rock pools (described above), could possibly have lower average temperatures as a result. However, the potential influence of shading on limpet rock pool use is not clear at the moment. Because deep rock pools were purposefully avoided in this study, no observational comparisons could be made between deep and shallow rock pools. It is important to note that the influence of these ameliorating conditions were not experimentally determined. However, a good set of observations involving them shows that there could be a strong argument for the influence of the above-mentioned ameliorating conditions on microhabitat suitability.

This study therefore, highlights the need to obtain a more robust representation of the thermal stress experienced by intertidal organisms in each microhabitat available to them. Future studies of this nature can achieve this by deploying robolimpets in each of the available microhabitats. In addition, these studies could use the empty shells of all the study animals to construct the robolimpets. As described by Lima and Wethey (2009), this would allow for a more accurate assessment of the body temperatures experienced by each limpet, since it would account for differences in the species shell morphologies, size and colour. I did not keep track of the microhabitats used by the individuals in the thermal tolerance experiments, and this could have influenced the results unintentionally (see Chapter 3 and 4). Although most individuals will have been collected from the microhabitats each species preferred, it would be worthwhile to determine and compare the thermal limits of limpets collected from their entire range of microhabitats. The combination of these data sets would allow for a more comprehensive analysis of these limpet's thermal histories, based on their microhabitat preferences, and how, or whether, this relates to individual thermal limits. This would make it possible to determine whether the effects of acclimation within these microhabitats plays a more important role in setting individual limpet upper thermal limits than their ability/inability to breathe air.

It is important to note that factors other than thermal stress may also influence intertidal gastropod microhabitat preferences. During their work on the tortoiseshell limpet, *Testudinalia testudinalis,* Lord *et al.* (2011) found that this limpet preferred vertical rock surfaces. However, it was concluded that this was probably to avoid predation and not a response to thermal stress. In fact, various studies have shown that predation pressure is greatly reduced for limpets that inhabit vertical surfaces, compared to limpets that occur on horizontal surfaces (Kitting, 1980; Little, 1989; Thompson *et al.*, 2000). However, predation pressure may not be an important factor determining microhabitat selection in many siphonariid limpets due to their use of chemical defence against predators (Branch and Cherry, 1985; Branch, 1988; Davenport, 1997; McQuaid *et al.*, 1999). As shown by Branch (1985), many patellogastropod limpets are consumed by predators while siphonariid limpets are rarely consumed, which could also explain why *Scutellastra granularis* was shown to avoid horizontal microhabitats in this study. Tortoiseshell limpets often move to horizontal rock surfaces at night because this is where they feed (Lord *et al.*, 2011). This suggests that the availability of food also plays an important part in determining which microhabitats intertidal gastropods prefer. Wallace (1972) and later, Lord *et al.* (2011) found that tortoiseshell limpets are more likely to be found in rock pools than on horizontal surfaces outside rock pools. According to Lord *et al.* (2011) this may be because the preferred food of tortoiseshell limpets, the encrusting coralline alga *Clathromorphum circumscriptum*, is more abundant in rock pools.

The main purpose of this chapter was to determine whether the microhabitat preferences of the study species were correlated with their upper thermal limits as these might be predicted based on the OCLTT theory. In other words, are respiratory mode, thermal limits and microhabitat use correlated? As described previously, various other studies have documented the microhabitat use of intertidal organisms and even related this to their thermal limits (Wolcott, 1973; Garrity, 1984; Williams and Morritt; 1995; Helmuth and Hofmann; 2001; Lord *et al.*, 2011). However, this study is unique in its attempt to link the OCLTT theory to the microhabitat use of the study limpets, based on the differences in their respiratory structures. Retrospectively, the current study should have sought to concurrently record the temperatures within the various microhabitats and to document the microhabitat use of the study animals. While most previous studies on intertidal microhabitat use did not utilise this data collection approach either, many of these researchers did not have the necessary equipment to efficiently, record the body temperatures of intertidal organisms (Evans, 1948; Southward, 1958; Wethey, 2002). However, recent advances in technology, particularly the advent of robolimpets (Lima and Wethey, 2009), means that there are now efficient ways to accurately and automatically record the body temperature limpets are likely to experience in their respective microhabitats (Helmuth and Hofmann, 2001; Fitzhenry *et al.*, 2004; Lima and Wethey, 2009).

Based on the findings here, I reject the hypothesis that pulmonate limpets are more common in microhabitats that generate higher limpet body temperatures than the microhabitats that patellogastropod limpets prefer, due to the pulmonate limpet's superior ability to breathe air. Moreover, the findings indicate that the thermal limits of intertidal gastropods are more likely to be linked to the thermal regimes associated with their microhabitat preferences than their respiratory capacities. In conclusion, the data from this chapter provide evidence that, despite my prediction of higher upper thermal limits, pulmonates do not inhabit more thermally stressful microhabitats.

# Chapter 3: Does the oxygen limitation theory predict how upper lethal thermal limits ($LT_{50}$) differ between pulmonate and patellogastropod limpets?

## Introduction

The rocky intertidal is a steep gradient of varying environmental stressors, the influence of which on organisms is largely affected by the amount of time those organisms spend submerged versus emerged (McMahon, 1988; Helmuth *et al.*, 2002; Harley and Helmuth, 2003; Little *et al.*, 2009). Because these animals are either sessile or sedentary, with limited mobility, the time they spend emerged, as the tide rises and falls, is largely dependent on their position on the shore (Stephenson and Stephenson, 1949; Southward, 1958; Newell, 1964; Helmuth, 1999; Denny *et al.*, 2006; Finke *et al.*, 2007). Intertidal organisms located high up on the shore are exposed to greater levels of thermal and desiccation stress, than organisms found lower down the shore (Stephenson and Stephenson, 1949; Southward, 1958; McMahon, 1988). Within their intertidal zone, the orientation of the substratum and neighbouring structures (Campbell and Norman, 1998) influences the thermal conditions intertidal animals experience and the degree to which these animals are protected from high temperatures (Harley and Helmuth, 2003). The physiological adaptations of these animals are therefore geared towards helping them cope with frequent exposure to high temperatures, long periods of emersion and, rapid changes in salinity and temperature with each tidal cycle (Wolcott, 1973; Garrity, 1984; McMahon, 1988; Helmuth and Hofmann, 2001; Somero, 2002; Davenport and Davenport, 2005; Bertness *et al.*, 2006).

Abiotic factors like these play an important part in setting the high shore distributional limits of intertidal organisms (Wethey, 1984; Yamada and Boulding, 1996; Harley and Helmuth, 2003; Miller *et al.*, 2009; Lima *et al.*, 2011). While low shore limits are largely set by species interactions (Connell, 1972; Underwood and Jernakoff, 1981; Robles *et al.*, 2009), the high shore limits of intertidal animals are generally set by their physiological adaptations which contribute to determining the upper limits to their intertidal zonation (Wolcott, 1973; Branch, 1981; Garrity, 1984; McMahon, 1990; Somero, 2002; Pörtner and Farrell, 2008; Little *et al.*, 2009). Physiological adaptations to heat stress include acclimation via an upregulation of heat shock proteins (HSPs) and other cellular responses (Pörtner, 2002; Tomanek and Sanford, 2003; Schmidt *et al.*, 2007; Dong and Somero, 2009; Finke *et al.*, 2009; Han *et al.*, 2016) as well as metabolic and heart rate adjustments (See Chapter 4; McMahon, 1990; Horowitz, 2001; 2002; Sokolova and Pörtner, 2001; Camacho *et al.*, 2006; Nguyen *et al.*, 2011). In addition, morphological adaptations such as shell size, shape, colour and texture can also limit heat gain and minimize physiological stress (Britton, 1995; Lang *et al.*, 1998; Sokolova and Berger, 2000; Bates and Hicks, 2005; Harley *et al.*, 2009). Limpets also have behavioural responses to thermal stress that include the formation of aggregations (Feare, 1971; Soto and Bozinovic, 1998, Stafford and Davies, 2004; Chapperon and Seuront, 2012), restricting activity to cool and/or wet periods, evaporative cooling through mushrooming (Lewis, 1963; Marshall and Chua, 2012), and seeking microhabitat refuges (Garrity, 1984; Williams and Morritt, 1995; Chapman and Underwood, 1996; Munoz *et al.*, 2005). Intertidal animals, particularly the mobile species, typically use tidal pools, crevices, high rising structures and biological structures such as mussel beds, as microhabitat refuges (see Chapter 2; Bertness *et al.*, 1999; Castilla *et al.*, 2004).

Although there are exceptions (Marshall and McQuaid, 2011), in general, animal physiological performance increases with temperature until it reaches an optimum level. At a point just above this it begins to decrease with further increases in temperature, a process which has major implications for animal survival under thermal stress (Peck *et al.*, 2007; Ivanina *et al.*, 2009; Tattersall *et al.*, 2012). This process is described by the “Oxygen and capacity limited thermal tolerance” or OCLTT theory (Pörtner and Frederich, 2000; Pörtner, 2001; see Chapter 1) and forms the basis for this study. Currently, there is no research comparing aerial and aquatic upper lethal thermal limits between pulmonate and patellogastropod intertidal gastropods. Most of the previous research on limpet thermal tolerance investigates the thermal tolerance of either limpet group in either medium (Evans, 1948; Southward, 1958; Dong *et al.*, 2015). In the case of a comparative analysis, research has focused on thermal limits between representatives of both groups, but only in one medium (Marshall and McQuaid, 1992a; Davenport, 1997; Harley *et al.*, 2009). Consequently, to test for greater generality, the present study focuses on the differences between the pulmonate limpets *Siphonaria capensis* and *S. serrata*, and the patellogastropod limpets *Scutellastra granularis* and *Cellana capensis,* comparing their upper lethal thermal limits in both water and air. This was done by comparing and relating their median lethal temperatures ($LT_{50}$) to their respiratory capabilities in both media. These animals were chosen as models for this study mainly because one group has a lung and the other does not (see Chapter 1; Yonge, 1947; Yonge, 1952; Marcus and Marcus, 1960; Branch, 1981; Innes and Houlihan, 1985; McMahon, 1988). Additionally, these animals can be found occupying similar geographic locations in southern Africa and they are all also found in the same high shore area (Chapter 1). As a result, it is acceptable to study various aspects of their thermal physiology comparatively.

Based on the OCLTT theory (see Chapter 1), I suggest that intertidal organisms that can maintain aerobic respiration, obviating the need for anaerobiosis at lower temperatures, should have higher, upper lethal thermal limits. It is important to note that it was assumed that pulmonate limpets have a higher $MO_2$ in air than patellogastropod limpets and that the reverse is true in water (see Chapter 1). This means that, in either medium, the animal that can sustain higher levels of $O_2$ consumption under thermal stress should have higher, upper lethal thermal limits (referred to as "lethal thermal limits" throughout the rest of this chapter). From this, I hypothesise that: 1. The lethal thermal limits of pulmonates will be greater than those of patellogastropods in air, whereas patellogastropods will have greater lethal thermal limits than pulmonates in water. 2. The lethal thermal limits for pulmonates will be greater in air than in water, while the opposite will be true for patellogastropods.

If these hypotheses are not rejected, this will mean the lethal thermal limit patterns of the study animals follow the projections I have inferred from the OCLTT theory.

# Materials and Methods

### *Study species*

Two pulmonate limpet species, *Siphonaria capensis* and *S. serrata*, and two patellogastropod limpet species, *Scutellastra granularis* and *C. capensis* were used. See Chapter 1 for a description of their geographical ranges, biology and vertical zonation and see Chapter 2 for a description of their microhabitat use.

### *Collection and Transportation*

A total of 360 individuals of each of the four study species (360 individuals/species) were collected from rocky shores at 3 different sites (see Fig 1.4) along the south-east coast of South Africa during the winter months (June – July) of 2014 and 2015. Similar sized (20-30mm) individuals of pulmonate and patellogastropod limpets were collected from high shore/eulittoral zone rocks during spring low tide. The limpets were detached from the rocks using a scalpel, slid quickly underneath the limpet's muscular foot in order to release its grip on the substratum. Any limpets that were not removed from the substratum on the first attempt were left behind to avoid selecting limpets that were likely to be damaged from repeated detachment attempts. The shell and foot were inspected for damage immediately after collection and any limpets showing signs of damage were replaced with fresh limpets. The specimens were then transported to the laboratory (Dept. of Zoology and Entomology, Rhodes University, Grahamstown) within 3 hours in small containers, moistened with sea water and kept inside an insulated box.

### *Handling and Treatment conditions*

In the laboratory, the specimens were housed in a 20L glass tank filled with 5L of sea water kept at room temperature (22 – 24°C). Air stones were used to keep the water aerated. To allow recovery from the handling stress experienced during collection and transportation, specimens were kept for a minimum of 24 hours and a maximum of 48 hours in the holding tank before use. Although the tank contained small, partially submerged rocks on which the limpets could "home", they settled either on the sides of the tank (emerged) or on the bottom (submerged). Three of the limpet species (*Scutellastra granularis*, *C. capensis* and *Siphonaria capensis*) migrated to the sides of the container, while *S. serrata* either remained on the rocks or migrated to the bottom of the container. Because of this migration, all limpets were submerged in 500 mL containers filled with water (constantly aerated) at room temperature for 1 hour to allow for rehydration before treatment in air on the day of experimentation.

### *Determining the median lethal temperature ($LT_{50}$)*

The lethal thermal limits were determined by finding the median lethal temperature ($LT_{50}$) for each species using a protocol similar to that of Clarke *et al.* (2000b). A total of 3 experimental trials were used to determine the mean $LT_{50}$ value for each species in each medium. Measurements were carried out on 50 limpets per species in each trial. Limpet condition was ascertained before they were used to ensure only limpets that were not damaged were used (see below). To simulate aquatic conditions, small (500mL) containers were filled with sea water and fitted with air stones which kept the water aerated during the tests. In order to determine mortality in air, the containers were dampened with sea water to maintain relatively high humidity levels during the heat dose (Denny *et al.*, 2006), thus ensuring the animals did not experience significant levels of dehydration during this treatment. Subsamples of 10 animals were placed in each container (4 containers at a time) held within a Grant programmable water bath (GP 200, Grant, Germany) set to 20°C at the start of each treatment.

The water bath was programmed to increase temperature, starting from 20°C, at different rates (Table 3.1) to reach the target temperatures within 1 hour (Fig 3.1) and to maintain that temperature for 4 hours (see chapter 4 for a description of how temperature was monitored). In order to avoid influencing the results via repeated thermal shock of the specimens (Clarke *et al.*, 2000a; Jones *et al.*, 2009), fresh specimens were used for each temperature interval. The temperature intervals used to find the limpets' LT50 values were based on preliminary experiments and were within a range found to contain each species' thermal breakpoint (Table 3.1). After each test run, limpet mortality was determined (Fig 3.1) and their shell lengths were measured using Vernier callipers (to nearest 0.02mm). Dead limpets were discarded and limpets still living after the test runs were kept in the holding tank until they could be returned to the sampling site.

Mortality was assessed by probing the limpets for tactile responsiveness using a dissection kit probe. Limpets were classified as dead if they showed no response (i.e. no movement) to having their foot muscle or the edge of their mantle cavity probed. Limpet mortality was assessed immediately after heat shock and after a recovery period of 24 hours in the holding tank to allow for a comparison of $LT_{50}$ values obtained at the two time periods (Fig 3.2).

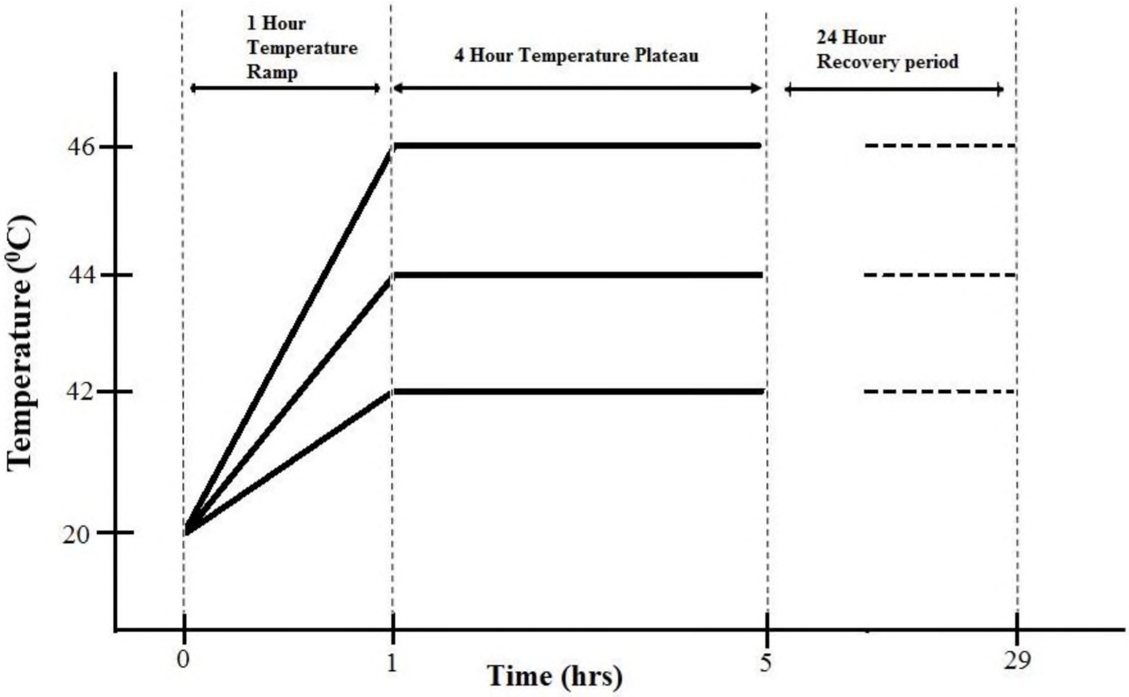

**Fig 3.1: Graphic (not to scale) of the $LT_{50}$ ramping protocol for the three highest temperature intervals used in this study. Mortality (nr of individuals) was determined at the 5 and 29 hour marks.**

**Table 3.1: The temperature intervals and in parentheses the heating rates (°C.min$^{-1}$) used to determine the limpets LT$_{50}$ values in both media.**

| LT$_{50}$ Temperature Intervals (°C) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Medium** | | | | | | | | | |
| | **Air** | | | | | **Water** | | | | |
| ***Siphonaria capensis*** | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 44<br>(0.4) | 46<br>(0.43) | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 44<br>(0.4) | 46<br>(0.43) |
| ***Scutellastra granularis*** | 34<br>(0.23) | 36<br>(0.26) | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 34<br>(0.23) | 36<br>(0.26) | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) |
| ***Siphonaria serrata*** | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 44<br>(0.4) | 46<br>(0.43) | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 44<br>(0.4) | 46<br>(0.43) |
| ***Cellana capensis*** | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 44<br>(0.4) | 46<br>(0.43) | 38<br>(0.3) | 40<br>(0.33) | 42<br>(0.36) | 44<br>(0.4) | 46<br>(0.44) |

### *Data and statistical analysis*

The LT$_{50}$ values for each trial was generated from limpet mortality at each temperature interval using probit analysis (Finney, 1948; 1952). LT$_{50}$ values obtained immediately after heat exposure and after 24 hours of recovery, were compared using a t-test for independent variables. Thereafter, the LT$_{50}$ values were compared between species and between media for each species using a 2-way Analysis of Variance (ANOVA) and, between media and respiration modes using a nested ANOVA with species nested in respiration mode. The limpet species sizes were also compared to each other using a 1-way ANOVA. A Tukey HSD analysis was used to determine where significant differences lay for all the ANOVA models. All statistical analyses were performed with Statistica 13 and the visual representation of analysed data was carried out using Excel 2015.

## Results

### *$LT_{50}$ values determined immediately after experimentation vs 24-hrs later*

Although the differences were not statistically significant, the $LT_{50}$ values measured after limpet recovery were consistently lower, by an average (± S.D) of 1.24±0.2$^0$C in water ($t_{22}$ = 1.21, p = 0.24) and 1.32± 0.01$^0$C in air ($t_{22}$ = 1.31, p = 0.21) than the values obtained immediately after heat shock (Fig 3.2). Therefore, this justified determining limpet mortality only after a 24-hour recovery period to get a more accurate estimate of their $LT_{50}$ values (Logan *et al.*, 2012; Dowd and Somero, 2013; Dong *et al.*, 2015).

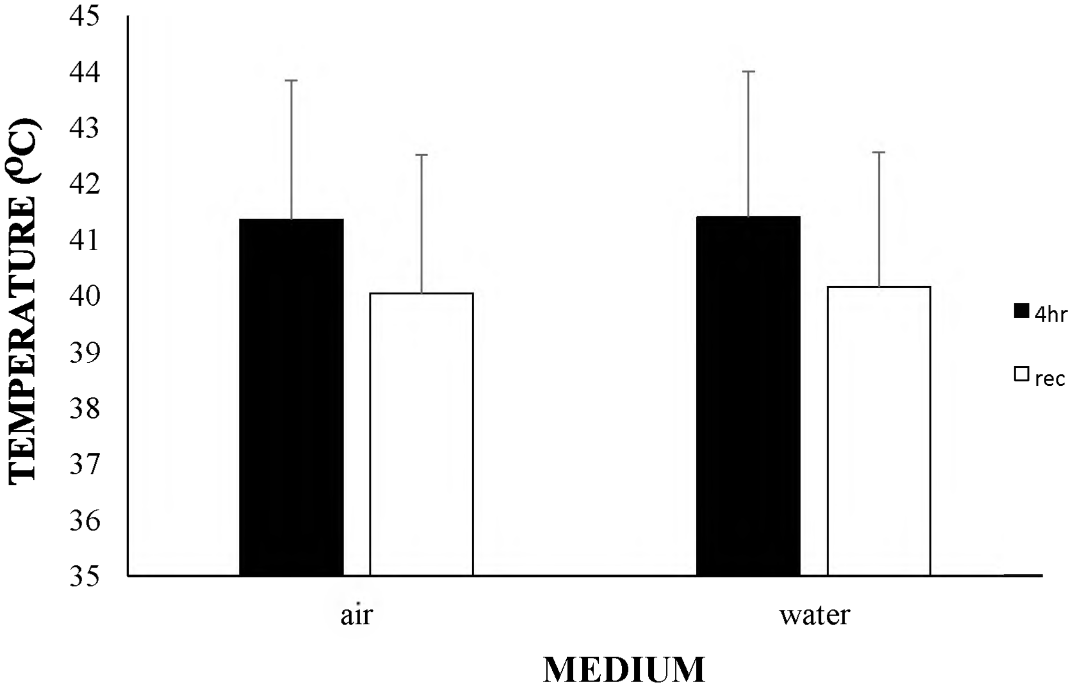

**Fig 3.2: Bar graph displaying the difference in mean (+ S.D) overall $LT_{50}$ (°C) values determined for all four study species immediately after (4hr) and 24 hours after recovery (rec) in both media.**

### *Animal sizes*

Samples of all four species collected were within a 20mm – 30mm size range except for *Siphonaria serrata* (20mm – 27mm) and there were significant size differences between the limpet species (1-way ANOVA, F = 191.6, $p < 0.001$). Mean size for *C. capensis* (Tukey HSD, $p < 0.0001$) was significantly greater than the other three species, followed by *S. capensis* (p <

0.0001) with a mean size significantly greater than the remaining two limpet species (Fig 3.3). The difference in mean size between *S. serrata* and *Scutellastra granularis* was not significant (p = 0.879).

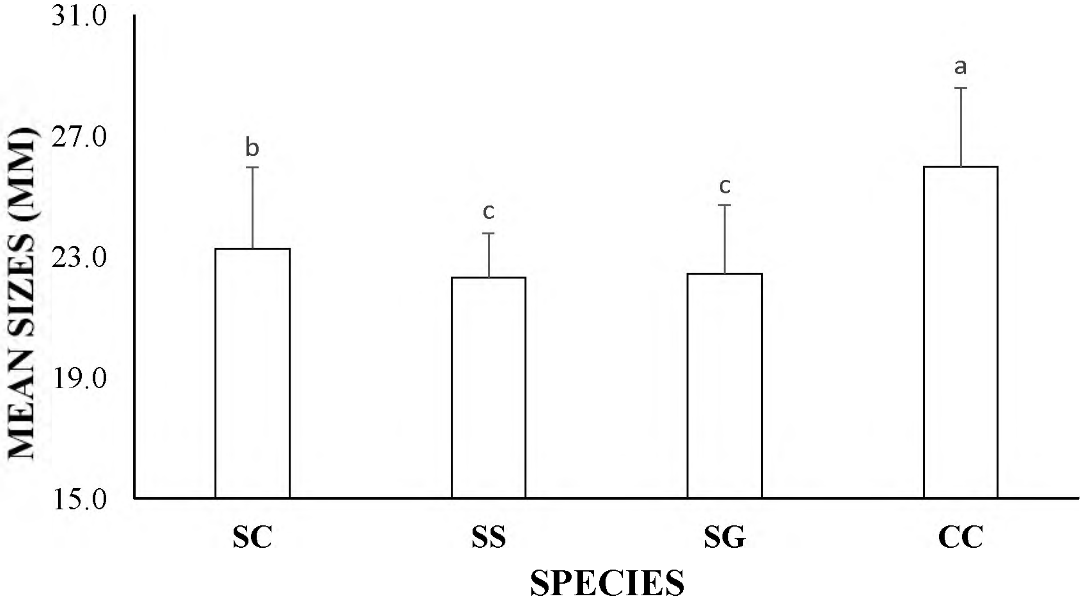

**Fig 3.3: Bar graph of the differences in mean (+ S.D) sizes (mm) of the limpets sampled for the $LT_{50}$ tests with letters indicating homogenous groups (SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; CC – *Cellana capensis*; SS – *Siphonaria serrata*).**

The general linear model (GLM), which considered size effects on the $LT_{50}$ values (Table 3.2) indicated that both size (p = 0.54) and, the interaction between size and species (p = 0.7) had no significant effect on $LT_{50}$ values among species.

**Table 3.2: Results from a GLM analysis conducted on the $LT_{50}$ values obtained via probit analysis of limpet mortality (%) vs temperature (°C) data, pooled from both media. Species, size and species*size were considered as fixed factors.**

| **GLM Factors** | **$LT_{50}$ (°C)** | | | |
|---|---|---|---|---|
| | **F** | **MS** | **df** | **p** |
| **Species** | 0.72 | 0.17 | 3 | p = 0.55 |
| **Size** | 0.39 | 0.09 | 1 | p = 0.54 |
| **Species * Size** | 0.49 | 0.12 | 3 | p = 0.7 |
| **Error** | | 0.24 | 16 | |

### *Did species $LT_{50}$ values differ between media?*

Despite not being statistically significant (2-way ANOVA, $p = 0.56$), there were very small, intraspecific differences in the $LT_{50}$ values between air and water for each limpet species (Fig 3.4). The $LT_{50}$ value for the pulmonate species *Siphonaria capensis* in air was slightly higher than its own $LT_{50}$ in water, whereas $LT_{50}$ values for the other three species were all slightly lower in air than in water.

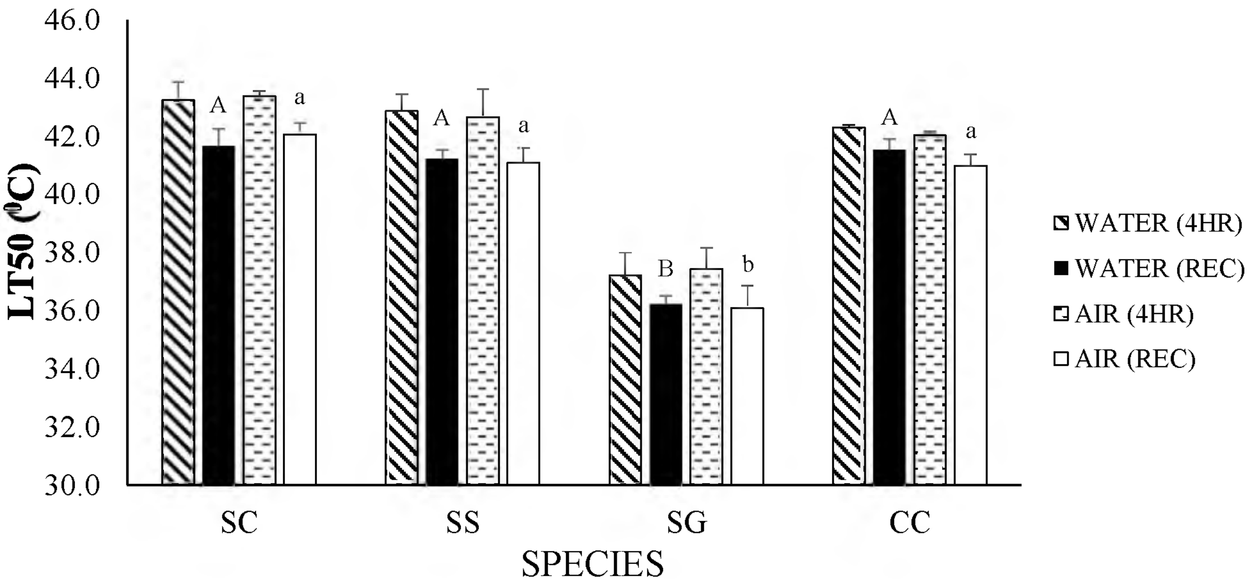

**Fig 3.4: Mean (+ S.D) LT$_{50}$ (°C) values determined by probit analysis for the 4 limpet species (SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; CC – *Cellana capensis*; SS – *Siphonaria serrata*), measured immediately after heat shock (4hr) and 24 hours (rec) later in both media. *Post hoc* tests comparing species were applied only to 24 hr data. Homogenous groups are shown in upper case for water and lower case for air.**

### *Were there differences in LT$_{50}$ values between limpet groups?*

The 2-way ANOVA comparing LT$_{50}$ values between species and media (Table 3.3), showed that there were significant differences among species ($p < 0.0001$) and, that there were no significant differences between media ($p = 0.56$) or the interaction between species and medium ($p > 0.05$). The patellogastropod *Scutellastra granularis* had the lowest LT$_{50}$ (Tukey HSD, $p < 0.001$) of the four species in both media (Fig 3.4). While not significant ($p > 0.05$ for all three species), there were small differences in the LT$_{50}$ values among the other three species. The pulmonate *Siphonaria capensis* had the highest LT$_{50}$ (air = 42°C; water = 41.7°C) in both media, while *C. capensis* had a greater aquatic LT$_{50}$ (41.5°C) than *S. serrata* (41.2°C) and *vice versa* (*S. serrata* = 41.1°C; *C. capensis* = 41°C) in air. It is important to note that the difference in the aquatic LT$_{50}$ values between *S. capensis* and *C. capensis* and, between *S. serrata* and *C. capensis* were very small (~ 0.2 and 0.3°C respectively). This was also the case for the difference in the aerial LT$_{50}$ values between *C. capensis* and *S. serrata* (~ 0.1°C; Fig 3.4). Even though one of the patellogastropod limpets, *Scutellastra granularis*, clearly had the lowest LT$_{50}$ and one of the pulmonate limpets, *Siphonaria capensis*, had the highest LT$_{50}$ values. The pulmonate limpets did not show a clear, collective difference in $LT_{50}$ values from the patellogastropod limpets.

**Table 3.3: Results from a 2-way ANOVA of $LT_{50}$ (°C) values obtained by probit analysis of the limpet mortality (%) vs temperature (°C) data in both media. Medium, species and medium*species were considered as fixed factors.**

| ANOVA Factors | $LT_{50}$ (°C) | | | |
|---|---|---|---|---|
| | F | MS | df | p |
| **Medium** | 0.4 | 0.08 | 1 | $p = 0.56$ |
| **Species** | 190.5 | 42.18 | 3 | $p < 0.0001$ |
| **Medium* Species** | 1 | 0.22 | 3 | $p = 0.41$ |
| **Error** | | 0.22 | 16 | |

This was confirmed by the nested ANOVA comparing $LT_{50}$ values between mode of respiration and media (Table 3.4), which showed that there were no significant differences between media ($p > 0.05$) and the interaction between media and respiration mode ($p > 0.05$) but, that there were significant differences between respiratory modes ($p < 0.0001$) and the effect of species nested in mode of respiration ($p < 0.0001$). *Post hoc* analysis of the data showed that, collectively, the lung bearing limpets had significantly (Tukey HSD, $p < 0.001$) greater $LT_{50}$ values than the gill bearing limpets in both media and, that the $LT_{50}$ values for gill and lung bearing limpets were not significantly ($p > 0.05$) different between media. Furthermore, of the gill bearing limpets, only *Scutellastra granularis* had significantly (Tukey HSD, $p < 0.001$) lower $LT_{50}$ values than the siphonariid limpets (Fig 3.4).

**Table 3.4: Results from the nested design ANOVA run on the $LT_{50}$ (°C) values obtained via probit analysis of the limpet's mortality (%) vs temperature (°C) data in both media. Medium, mode of respiration (R. Mode) and species nested in respiration mode (R. Mode) were considered as fixed factors**

| **ANOVA Factors** | **$LT_{50}$ (°C)** | | | |
|---|---|---|---|---|
| | **F** | **MS** | **df** | **p** |
| **Medium** | 0.4 | 0.8 | 1 | p=0.55 |
| **R. Mode** | 218.5 | 47.06 | 1 | p<0.0001 |
| **Spp (R. Mode)** | 184.5 | 39.74 | 2 | p<0.0001 |
| **Medium*R. Mode** | 1.6 | 0.34 | 1 | p=0.23 |
| **Error** | | 18 | 0.22 | |

# Discussion

Based on the OCLTT theory, I predicted a correlation between mode of respiration and lethal thermal limits. Furthermore, I also predicted that each limpet group would have greater lethal thermal limits in its preferred breathing medium. In the event, the results were species dependent, with no clear differences between the pulmonates and patellogastropods, or between media, then the predictions would not hold true. As indicated by the results, there was no evidence for an effect of either respiratory mode or unexpectedly, medium on lethal thermal limits. However, there were small differences with respect to the predictions.

The siphonariids had higher lethal thermal limits in both media than the patellogastropods, but in the case of *C. capensis*, the differences were not significant. As described in Chapter 1, the siphonariid limpet's respiratory morphology compared to that of patellids, is assumed to give them an advantage over the patellids with respect to their breathing capabilities in air.

Therefore, at high aerial temperatures the respiratory system of the pulmonate limpets should be better suited to maintaining high blood $O_2$ levels and to meeting mitochondrial O2 demand. This should make it easier for the pulmonate limpets to meet the energy demand of respiration during emergence under thermal stress, which allows for the efficient use of energy stores (McMahon, 1988). By doing this, these limpets are assumed to be able to delay the onset of anaerobic metabolism (Zielinski and Pörtner, 1996; Pörtner and Zielinski, 1998; Pörtner *et al.*, 1999; Frederich and Pörtner, 2000; Peck *et al.*, 2002). Thus, by delaying aerial anaerobic respiration and the accumulation of its by-products, the pulmonate limpets should be able to extend their aerial thermal tolerance limits above those of their patellogastropod counterparts (McMahon, 1988). In their work, Marshall and McQuaid (1992a) compared the lethal thermal limits (lethal exposure time) of the pulmonate *Siphonaria oculus* and the patellogastropod *Scutellastra granularis* in air. They discovered that the pulmonate limpet ($LT_{50}$ = 158h) had a higher lethal thermal limit than the patellogastropod limpet ($LT_{50}$ = 89h). This result compares favourably with some of the results presented in this chapter. Both siphonariids in the present study had higher lethal thermal limits in air than *S. granularis*. This also explains to an extent, why *Siphonaria capensis* had the highest lethal thermal limit in air. However, this does not adequately explain why the aerial thermal limits of *C. capensis* and *S. serrata* were so similar, or why neither of the pulmonate limpets had a significantly higher lethal thermal limit than *C. capensis* in air.

Some high shore "gill-bearing" limpets can maintain levels of aerial aerobic metabolism similar to those of their pulmonate counterparts, despite not having a lung (Micallef, 1967; Micallef and Bannister, 1967; Hughes, 1971; McMahon and Russel-Hunter, 1977; Branch and Newell, 1978; Branch, 1979; Houlihan, 1979; Innes, 1982). These patellogastropod limpets improve their aerial gas exchange by a simple increase in the vascularisation of the mantle (Deshpande, 1957; Fretter and Graham, 1962). As described by McMahon (1988). By improving their capacity for aerial respiration during emergence under thermal stress these limpets utilize their energy stores more efficiently, increasing their thermal tolerances. Huang *et al.* (2015) suggested in their study on *Cellana toreuma* that mid- to high shore limpets of this genus could extract aerial $O_2$ efficiently under thermal stress without the accumulation of anaerobic by products. As described above, patellogastropod limpets with this adaptation, possibly including *C. capensis*, can respire effectively in air affording them a higher lethal thermal limit (Deshpande, 1957; Fretter and Graham, 1962; Huang *et al.*, 2015). Dong *et al.* (2015) and, Rao and Ganapati (1972) reported similarly high lethal thermal limits in air for the high shore limpets *C. toreuma* ($LT_{50} = 41.29 – 43.36^0C$) and *C. radiata* ($42^0C$). It is important to note that *C. capensis* is a patellid limpet and it is very likely that there is no vascularisation of its mantle despite the findings of Huang *et al.* (2015). For instance, there is no evidence of a similar respiratory structure in the high shore patellid patellogastropods from the genera *Patella* or *Scutellastra*, e.g. *S. granularis, P. lusitanica and P. vulgata*. Consequently, these limpets almost certainly rely solely on their pallial gills for aerial respiration (McMahon, 1988; Marshall and McQuaid, 1992b). According to McMahon (1988), pallial gills are not as suited to aerial respiration as a lung or a generally vascularised mantle. Therefore, in addition to the respiratory adaptations of *C. capensis*, other factors may contribute towards its high lethal thermal limit in air (Chapter 2).

Surprisingly, the pulmonate limpets had a relatively high lethal thermal limit in water compared to the patellogastropod limpets. Research by McMahon and Russel-Hunter (1981) on the high shore salt marsh snail *Melampus bidentatus* indicates that the aquatic respiration of pulmonates that only have lungs is severely impacted. Unlike these pulmonates, the mud flat snail *Amphibola crenata* (Shumway, 1981; Shumway and Marsden, 1982) and the rocky shore siphonariid limpets *Benhamina obliquata* and *Siphonaria zelandica* (Innes *et al.*, 1984) all have secondary gills. As reported by these researchers, the presence of secondary gills in these pulmonates means they can maintain aquatic $MO_2$ and thus aerobic metabolism at levels similar to their aerial metabolism (Fretter and Peake, 1975; Shumway, 1981; Shumway and Marsden, 1982; Innes *et al.*, 1984). This, as per the predictions based on the OCLTT, should in turn correlate to a higher aquatic thermal tolerance limit for pulmonates with secondary gills (Chapter 1). Like all other siphonariids, *S. capensis* and *S. serrata* also have secondary gills in the dorsal mantle of their pulmonary cavities (Cotrell, 1910; Yonge, 1952; Marcus and Marcus, 1960; Hubendick, 1978). This to some extent explains why their lethal thermal limits in water were higher than or similar to those of the patellogastropods. Despite this, the secondary gills evolved secondarily after loss of the ctenidium, hence the name, which again means these animals are not primarily adapted to aquatic respiration (Yonge, 1947). As shown by Innes *et al.* (1984), *S. zelandica* had significantly lower rates of $O_2$ consumption in water compared to its aerial $MO_2$ despite having secondary gills. Additionally, in the present study the patellogastropod *Scutellastra granularis* had the lowest lethal thermal limit in water despite having pallial gills, which are more appropriate for aquatic $O_2$ consumption than secondary gills (Branch, 1981; McMahon, 1988). Therefore, in theory *S. granularis* along with *C. capensis* should have higher lethal thermal limits in water than the pulmonates. While the aquatic lethal thermal limit of *C. capensis* in water was at least as high as or higher than that of the pulmonates, this was not the case for *Scutellastra granularis*. This indicates that, in conjunction with their efficient aquatic respiration, other factors could be responsible for the high lethal thermal limits of the pulmonates in water.

Of the two pulmonate limpets, only *Siphonaria capensis* had a higher lethal thermal limit in air than water, however insignificant this difference between the media may have been. Air generally has a higher $O_2$ concentration, approximately 30 times higher, than the same volume of $O_2$ saturated water (Randall *et al.*, 1981). Moreover, the $O_2$ concentration of freshwater decreases with temperature and is twice as much at $0^{\circ}C$ as at $30^{\circ}C$ (Wetzel and Limnology, 2001). As this study deals with marine organisms, it is important to note that the solubility of $O_2$ in seawater is even lower than in freshwater (Wetzel and Limnology, 2001). This means that, not only does water have a lower $O_2$ supply for energy production via the mitochondria but also, it is energetically costlier for organisms to respire in water due to its viscosity (Randall *et al.*, 1981). As discussed previously, I suggested, based on the OCLTT that the ability to save energy or produce more of it at high temperatures should stave off anaerobic metabolism, translating into a higher lethal thermal limit (Frederich and Pörtner, 2000; Pörtner, 2001; Pörtner, 2010). The pulmonate limpet lung gives these animals access to high atmospheric $O_2$ concentration levels, levels that are not obtainable in water via their secondary gills (Randall *et al.*, 1981). Also, as discussed previously these siphonariids are primarily adapted to aerial respiration because their mantle cavity lung may be a more efficient respiratory organ than their secondary gills (Cotrell, 1910; Yonge, 1947; 1952; Marcus and Marcus, 1960; Hubendick, 1978; Innes *et al.,* 1984). For example, Dye (1987) demonstrated a lower aquatic $MO_2$ in the high shore pulmonate limpets *Siphonaria capensis* and *S. concinna*. Therefore, not only do these high shore siphonariids have access to more $O_2$ but also, it is energetically cheaper for animals with lungs to respire in air at high temperatures (Randall *et al.*, 1981). While this may explain why *S. capensis* has a slightly higher lethal thermal limit in air, it does not explain why its congener, *S. serrata*, had similar lethal thermal limits in both media.

Although the difference was non-significant, the fact that the lethal thermal limits of both patellogastropod limpets was greater in water than air reflects their superior propensity for aquatic respiration. As I have suggested, based on the OCLLT, this reflects their greater capacity for energy production and aerobic metabolism in water than air (Frederich and Pörtner, 2000; Pörtner, 2001; Pörtner, 2010). It is however, surprising that *Scutellastra granularis* had such a low lethal thermal limit in water ($LT_{50}$ = 36.22°C) compared to *C. capensis* ($LT_{50}$ = 41.53°C). Evans (1948) measured aquatic lethal thermal limits for the high to medium shore patellogastropod limpets *Patella vulgata* ($LT_{50}$ = 42.8°C), *P. depressa* ($LT_{50}$ = 43.3°C) and *P. athletica* ($LT_{50}$ = 41.7°C), and these were similar to those of *C. capensis*. This could suggest that *S. granularis* has an unusually low lethal thermal limit in water, however, the high tolerance limits Evans (1948) found may have been influenced by his methodology, particularly his means of determining limpet mortality. As seen in previous studies (Ospina and Mora, 2004; Mora and Maya, 2006; Angilletta., 2009; Nguyen *et al*., 2011; Terblanche *et al*., 2011) different methods used to determine the thermal tolerances of intertidal organisms can easily influence the results. Evans (1948) placed the animals in water that had to cool down from the test temperatures for a one-hour recovery period before determining mortality, whereas in the present study after cooling down for one hour, the limpets underwent a 24-hour recovery period in cool (20°C) water. Results from this study show that in both media, the lethal thermal tolerance limits would be 2-3°C higher if mortality was determined immediately after heat shock. This shows that some of the limpets that seemed to be alive immediately after heat shock were in fact dead or that they were alive immediately after heat shock but died shortly afterwards. Furthermore, most recent studies have effectively used a 24-hour recovery period to determine lethal thermal limits (Logan *et al*., 2012; Dowd and Somero, 2013; Dong *et al*., 2015). Consequently, the aquatic $LT_{50}$ values for patellids presented by Evans (1948) may be slightly inflated and should be closer to the value presented here for *Scutellastra granularis*.

Research comparing lethal thermal limits of patellogastropod and pulmonate limpets is very rare, particularly when we consider comparative work in both air and water. This makes the current research unique, especially regarding the attempt to take this comparison a step further by relating it to the OCLTT. This was done by attempting to relate the lethal thermal limits of the two limpet groups to differences in the structure of their respiratory organ anatomies and their respiratory capabilities. Having said this, the link between respiratory organ anatomy and respiratory ability, in terms of $MO_2$, in either medium was not tested but rather, it was assumed in this study (Chapter 1). Furthermore, it is important to note that other factors, which I did not test, may be important in setting these lethal thermal limits. These include the limpet's sex, possible parasitism, and feeding and health status of the organisms, which may have influenced how susceptible the limpets were to high temperatures during testing (Tully *et al*., 2000; Huxham *et al*., 2001; Baeza and Fernández, 2002; Cook, 2004; Bates *et al*., 2011). This is especially true for factors that I could not control in this study, including the organism's recent thermal history. This can be influenced by microhabitat use (Britton, 1995; McMahon, 1990; Harley *et al.*, 2009; Chapperon and Seuront, 2011a; b; Cartwright and Williams, 2012), and the formation of aggregations (Feare, 1971; Soto and Bozinovic, 1998; Stafford *et al.*, 2007; 2008).

The recent thermal history of these animals is also subject to the influence of their heat conduction rate controlled by differences in individual morphology, including shell size, shape and colour (Britton, 1995; Lang *et al.*, 1998; Harley *et al.*, 2009). In the current study, there were differences in size between the four limpet species. However, there does not appear to be any real relationship between size and the lethal thermal limits of the study species. As can be seen from the results, neither the largest nor the smallest species had the greatest or lowest lethal thermal limits. In their study, Lee and Boulding (2010) also show that there was no relationship between size and thermal tolerance limits, though they only used one species, the intertidal snail *Littorina keenae*, in their study. It is important to note, however, that many different studies on the effects of body size on thermal tolerance have produced contrasting results (Clark *et al.*, 2000a; Hicks and McMahon, 2002; Nguyen *et al.*, 2011; Madeira *et al.*, 2012) and that size could in fact play a role in determining these limpets' body temperatures. Previous studies show that limpets with high domed (Branch, 1981, 1985, 1986; Marshall and McQuaid, 1992) and lighter coloured shells (Branch, 1981), are generally better at regulating heat gain than limpets with flatter and darker coloured shells. This is a result of the reflective qualities of lighter coloured shells and, because limpets with high domed shells generally have smaller shell surface area to volume ratios and aperture/foot areas than their flatter shelled counterparts (Branch, 1981, 1985, 1986). A smaller foot area means that the limpets would absorb lower amounts of heat via conduction from the substratum whereas, a smaller shell surface area to volume ratio results in a lower rate of heat absorption via solar radiation (Branch, 1981, 1985, 1986). Though no quantitative analyses of shell shape and colour were conducted in the current study, differences in these shell characteristics were observed among the study animals. *Scutellastra granularis* and *Cellana capensis* have high domed shells compared to the siphonariids and, of the four study species *Siphonaria serrata* clearly had the darkest coloured shell. Though it is not clear from this study how these three shell characteristics may have influenced the results, by influencing the maximum body temperatures, the animals experience in the field, shell size, shape and colour may have set the temperatures these animals could survive during testing.

The results reject the null hypotheses that: 1. The lethal thermal tolerance limits of the pulmonates in air will be greater than those of the patellogastropods whereas the patellogastropods will have greater lethal thermal limits in water than the pulmonates; 2. The pulmonates' lethal thermal limits in air will be greater than their lethal thermal limits in water, while the opposite will be true for the patellogastropods.

I therefore conclude that there is not enough evidence provided in the current chapter on lethal thermal limits to support the OCLTT theory. Rather, other factors, including species-identity and the temperatures to which they were acclimated in the field, may have set these limpets' lethal thermal tolerance limits.

# Chapter 4: Does the oxygen limitation theory predict how upper thermal limits (Arrhenius Breakpoint Temperatures) differ between pulmonate and patellogastropod limpets?

## Introduction

The rocky intertidal consists of extreme physical conditions that vary across the shore, from the subtidal and low intertidal, to the high intertidal zones (Tomanek and Somero, 1999; Somero, 2002; Stillman, 2002; Harley and Helmuth, 2003; Fitzhenry *et al.*, 2004; Somero, 2010; Judge *et al.*, 2011; Cartwright and Williams, 2012). Marine organisms that inhabit this environment often have to contend with rapidly changing abiotic conditions as the tide oscillates (Helmuth *et al.*, 2002; Harley and Helmuth, 2003; Little *et al.*, 2009). These extreme conditions include rapidly changing temperature as the tide ebbs and floods, desiccation stress, osmotic stress and very high or low temperatures during low tide (Connell, 1972). Moreover, intertidal organisms are often subject to entirely different thermal regimes during emergence and submergence (Helmuth *et al.*, 2006; Denny *et al.*, 2011). Intertidal organisms therefore have a wide variety of adaptations to these stressors, which influences their biogeographic distribution and vertical zonation on rocky shores (Wolcott, 1973; Garrity, 1984; McMahon, 1988; Helmuth and Hofmann, 2001; Somero, 2002; Davenport and Davenport, 2005; Bertness *et al.,* 2006; Denny and Harley, 2006; Miller *et al*., 2009). One of the most important adaptations is an intertidal organism's physiological adaptation to thermal stress because it is central to setting their distributional limits, both across the shore and biogeographically (Somero, 2002; 2005; Braby and Somero, 2006).

With climate change and its consequences now imminent (Pörtner *et al*., 2001; Stillman, 2003; Pörtner and Knust, 2007; Somero, 2010), generating an understanding of an organism's thermal adaptation to gain some predictive insight into the overall influence of climate change is quickly becoming very important. Several measures of thermal adaptation have been used to help gain an understanding of how these organisms respond to thermal variation in their immediate environment. These include whole organism lethal limit measures, which involve determining an organisms' critical temperatures and their median lethal temperatures (Broekhuysen, 1940; Evans, 1948; McMahon, 1990; Laing and Child, 1996; Davenport and Davenport, 2005; Compton *et al*., 2007), and sub-lethal limit measures such as the detection of heat shock proteins and anaerobic by-products and, the use of Arrhenius breakpoint temperatures (ABTs) (Stillman and Somero, 1996; Chapple *et al*., 1997; Hamdoun *et al*., 2003; Dong and Williams, 2011; Huang *et al*., 2015; Tagliarolo and McQuaid, 2015; Xing *et al*., 2016).

Several studies (Bamber and Depledge, 1997; Hamdoun *et al*., 2003; Stenseng *et al*., 2005; Park *et al*., 2007; Dong and Williams, 2011; Xing *et al*., 2016) have successfully used various parameters of cardiac performance as measures of thermal adaptation in intertidal organisms. In marine intertidal organisms, heart rate is generally related to the oxygen consumption rate ($MO_2$) in marine intertidal organisms and as a result, these parameters of cardiac performance during the increase of temperature can be used to gauge metabolic activity indirectly (Marshall and McQuaid, 1992b; 1994; Santini *et al*., 1999). Moreover, due to the refinements made in heart rate monitoring for gastropod molluscs (Depledge and Anderson, 1990; Aagaard *et al*., 1991; Chelazzi *et al*., 1999; De Pirro *et al*., 1999; McMahon, 1999; Chelazzi *et al*., 2001), cardiac performance during an increase of temperature can be determined non-invasively and without subjecting the animals to the additional stress associated with invasive techniques (Butler *et al*., 2004; Braby and Somero, 2006). Therefore, in the current study, the ABTs and the mean heart rates of intertidal limpets in response to increasing temperature were investigated as parameters of cardiac performance.

The ABT is one of two breakpoint temperatures (it occurs after the Thermoneutral breakpoint temperature (TBPs)), which occurs as heart rate varies with a constant increase in temperature (Stenseng, 2005; Matumba, 2013). While some studies have shown that there is a relationship between an organism's heart rate and $MO_2$/respiration rate (Marshall and McQuaid, 1992b, Bot and Hollander, 2000; Butler *et al.*, 2002; 2004), other studies show that this is only a weak relationship, particularly approaching high temperatures (Frederich and Pörtner, 2000). However, Giomi and Pörtner (2013) show in their study that the ABT of the heart and the upper pejus temperature ($T_{P\ II}$) should normally occur at roughly the same point. As discussed previously, the ABT transpires as the heart rate peaks and falls rapidly with increasing temperature, and the $T_{P\ II}$ is the temperature at which haemolymph oxygenation starts deteriorating and the organism's aerobic scope drops, as the cardiorespiratory system fails to meet the $O_2$ demand (Stillman, 2003; Pörtner *et al.*, 2006; Pörtner and Knust, 2007). It is important to note that the ABT of gastropods does not necessarily coincide with maximum heart rate but rather, with the $T_{opt}$ for the aerobic rate or $T_{P\ II}$ (Marshall *et al.*, 2011). The heart and respiration rate can therefore be related to each other through the co-occurrence of their respective upper thermal limits. This means that an organism's heart rate response to increasing temperature and ABT can be used to make important inferences about the OCLTT theory.

Limpets are important intertidal organisms due to their activity as grazers and their interactions with other intertidal organisms (Branch, 1981). Their ability to adapt to the many physiological challenges in the intertidal zone has made them interesting study subjects over the years (Hawkins and Hartnoll, 1983; Branch, 1985; Little, 1989; Hodgson, 1999). The patellogastropod limpets, *Scutellastra granularis* and *Cellana capensis* and the pulmonate limpets, *Siphonaria capensis* and *S. serrata* were used as model organisms in this study mainly due to differences in their primary mode of respiration (see Chapter 1). While the patellogastropod limpets are primarily adapted to breathing in water, the pulmonate limpets are amphibious and known to breathe just as well in air (Yonge, 1947; Yonge, 1952; Marcus and Marcus, 1960; Branch, 1981). Furthermore, these limpets inhabit similar geographic and intertidal zones along the rocky shores of South Africa (see Chapter 1; Allanson, 1959; Kalk, 1959; Branch, 1971; Vermeij, 1972; Branch, 1975b; Branch and Cherry, 1985; Branch and Branch, 1988; Branch *et al.*, 1994; Chambers, 1994; Chambers and McQuaid, 1994; Vat, 2000). As a result, these two groups of limpets are the ideal test subjects on which to base this study.

The aims of this study are therefore to determine and compare the ABTs of the two limpet groups as measures of their upper thermal tolerance and to relate this to the OCLTT theory in order to ascertain its validity. The hypotheses of the study are that: 1. The pulmonate limpet's aerial ABTs will be greater than those of the patellogastropod limpets, while the patellogastropod limpets' aquatic ABTs will be greater, and 2. The pulmonate limpets will have a greater aerial ABT compared to their aquatic ABT, while the patellogastropod limpets will show the reverse. Furthermore, these hypotheses must be supported for the findings in this study to support the thermal limitation mechanisms outlined in the OCLTT theory. However, it is important to note that $MO_2$ was not measured in this study, and that I made important assumptions regarding differences in $MO_2$ between the two limpet groups, based on differences in their respiratory structures (Chapter 1).

# Materials and Methods

## *Study species*

Two pulmonate limpets, *Siphonaria capensis* and *S. serrata*, and two patellogastropod limpets, *Scutellastra granularis* and *C. capensis* were used. See Chapter 1 for a description of their distribution ranges, biology and vertical zonation and see Chapter 2 for a description of their microhabitat use.

See Chapter 3 for a description of the collection and transport of the specimens, as well as the treatment conditions.

### *Heart rate measurements*

The limpets' upper thermal tolerance limits were determined as cardiac thermal breakpoint temperatures, more specifically as Arrhenius breakpoint temperatures (ABTs). Heart rate measurements were carried out on a sample of 40 individuals of each species exposed to aerial and aquatic experimental conditions (20 individuals/medium/species). Each limpet was probed for tactile responsiveness using a dissection kit probe prior to heart rate measurements to ensure that only healthy individuals were used (see Chapter 3). Both aerial and aquatic conditions were simulated in 500mL containers as described in Chapter 3. Three individuals of the same species were subjected to the same temperature treatment at a time by placing them in a single container. Each container was then held, one at a time, inside a Grant programmable water bath (GP 200, Grant, Germany) set to 20°C at the start of each treatment. These treatments were then repeated until the full complement of sample individuals for each species was subjected to the same experimental conditions.

Heart rate was recorded using non-invasive plethysmography (Depledge and Anderson, 1990) by attaching optoelectronic (infrared) sensors (Vishay semiconductors, V69 CNY70 732/735, Germany) to the shells of each limpet near the heart using Pattex super glue (Henkel (Pty) Ltd, South Africa). These sensors produced signals which were amplified by a custom-built preamplifier, after which Triangular-Bartlett smoothing was used to produce an additional smooth trace on a separate channel. The signal was then filtered before being recorded as beats per minute on a computerised recording system (PowerLab/4SP and 430, Chart version 5 and 7, ADInstruments, Australia). The amplitude ranged between 40 and 1000mV at a sampling rate of 40Hz.

The specimens were exposed to a temperature increase of 30°C from 20-50°C at a rate of 0.25°C.min$^{-1}$, using the Grant programmable water bath heating system whilst simultaneously recording heart rate. Prior to heat dosing the animals, they were allowed to settle inside the water bath container for 30 minutes, as shown in a previous study this is enough for limpets to recover from "handling related stress" (Dong and Williams, 2011). The temperature inside the containers was measured using a Fluke 54II thermometer (Fluke cooperation, USA) fitted with a T-type thermocouple (Fluke cooperation and Cromega) and recorded with a PowerLab recording system. The above-mentioned temperature range ensured the animals were exposed to temperatures higher than their field temperatures (see Chapter 2), thus ensuring that their ABTs would be surpassed. Field temperature data were obtained using RoboLimpets (see Chapter 2) and used to determine the rate of temperature increase *in situ*. After each treatment run, shell length (mm) was measured using Vernier callipers (nearest 0.02mm) and limpet mortality was ascertained after the limpets spent a 24-hour recovery period submerged in the holding tank. All dead limpets were discarded immediately while live limpets were returned to the holding tank to be returned to the field later.

### *Data analysis*

Cardiac thermal response curves (heart rate (bpm) vs temperature (°C)) were plotted using Microsoft Excel 2015 (Fig 4.1). In order to ensure meaningful ABT values were obtained, the cardiac thermal response curves were examined so that only appropriate thermal response curves were used in the ABT analyses. Cardiac thermal response curves that did not show a clear trend between heart rate and temperature were discarded, on the assumption that unclear response curves were probably generated due to limpet movement or problems with the infrared sensors. This meant that the final ABT and heart rate analyses were carried out on fewer than 40 individuals from each species (Table 4.1).

**Table 4.1: Table displaying the final number of replicates per species for each experimental condition. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*.**

| **Species** | **Medium** | |
|---|---|---|
| | **Air** | **Water** |
| **SC** | 9 | 12 |
| **SG** | 11 | 14 |
| **SS** | 11 | 8 |
| **CC** | 13 | 9 |

The temperature ranges used to find the ABTs were 25-45°C for all species in air (except *Scutellastra granularis*; 25-40°C) and 25-40°C for all species in water (except *C. capensis*; 2545°C). These temperature ranges were chosen using the cardiac thermal response curves for each limpet species, generated from the originally chosen temperature range (20-50°C). These showed that the data points were distributed haphazardly from 20 – 25°C and that a heartbeat signal could not be detected above the upper limit of the designated temperature ranges shown above. This is mostly speculative but, the haphazard distribution of these data points was probably a result of the limpets not being completely settled to the experimental environment at the beginning of each heat dose.

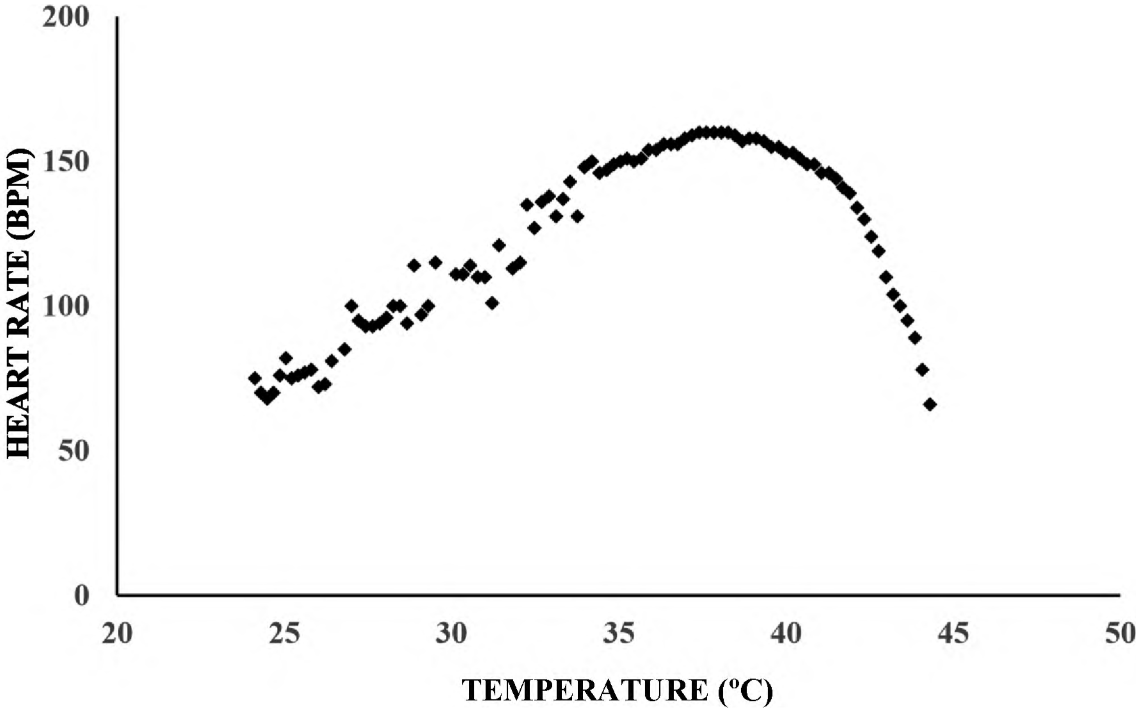

**Fig 4.1: Example of a cardiac thermal response curve for a limpet (*Cellana capensis*) in water.**

Arrhenius plots (Fig 4.2) were then generated from these thermal response curves using equation 4.1 to analyse the effect of temperature on heart rate:

(equation 4.1): $$L_n (HR) = L_n a - \frac{Ea}{R} \text{ x } \frac{1}{T}$$

In equation 4.1, HR represents the heart rate (bpm), a is the normalization constant, $E_a$ is the activation energy ($J.mol^{-1}$), R is the ideal gas constant ($J.K^{-1}.mol^{-1}$) and T is the absolute temperature (K). In addition, since equation 4.1 is clearly a straight-line equation, $E_a/R$ therefore represents the slope of this equation. Put simply, the heart rates (y-axis) were log (natural log) transformed and the reciprocal of temperature (x-axis) was converted into degrees Kelvin prior to determining the limpets' ABTs and the heart rate in response to increasing temperature. Piecewise linear regression (Statistica 13) was used to calculate the breakpoints using transformed heart rate ($L_n$ (HR)) and temperature (1/T) data. These breakpoints were then converted back into degrees' Celsius values, in order to present them as ABTs ($^{\circ}$C) before conducting further analyses on them.

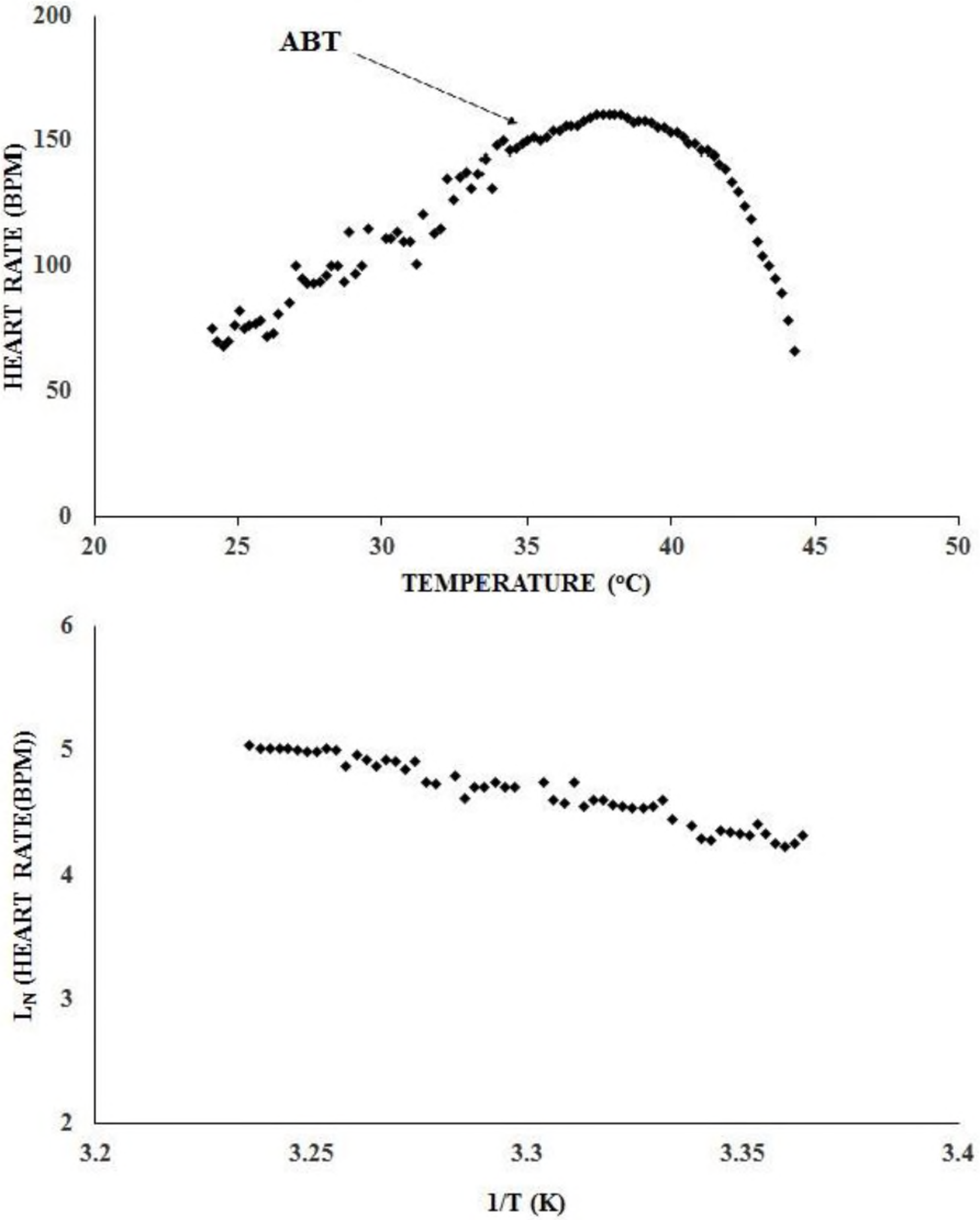

**Fig 4.2: Graphic depiction of the "linearization" (after determining the ABT) of the thermal response curve for a *Cellana capensis* individual treated in water, by using equation 4.1 to generate its Arrhenius plot (Top – Cardiac thermal response curve; Bottom – Resultant linear Arrhenius plot).**

### *Statistical Analysis*

Once the ABT values were determined, the exponential increase in heart rate as a function of temperature was compared among treatments, by considering only the data points below the ABTs when the Arrhenius plots were generated (Fig 4.2). The resultant, linear Arrhenius plots were compared using a Generalized Linear Mixing Model (GLMM) that is considered to be an effective method of analysing non-normal data with random effects (Bolker *et al.*, 2009). Because the experiment was performed using a subset of the natural population, individual replicates were treated as a random categorical variable, while species and medium were set as fixed categorical variables and temperature was set as a continuous fixed variable. In addition, mean untransformed heart rate values, generated over the respective temperature ranges (see above), were used as a proxy for the change in heart rates in response to increasing temperature, to visualise differences in the heart rate responses (Fig 4.7).

To determine if significant interspecific differences in ABTs exist for each medium and if there were significant intraspecific differences in the ABTs between mediums, a factorial ANOVA was used. For the factorial ANOVA, medium and species were set as fixed categorical variables and ABT as a fixed dependent variable. Tukey HSD analyses were then used *post hoc* for both the GLMM and ANOVA to determine where significant differences lay. All the analyses were performed using Statistica 13.

## Results

The cardiac thermal response curves displayed important differences in the cardiac response to increasing temperature between the two limpet groups over the designated temperature ranges in both air (Fig 4.3) and water (Fig 4.4). There were also clear inter-individual differences (Table 4.2) in the cardiac response to increasing temperature, which was especially marked for *C. capensis* in both media (Fig 4.3 and Fig 4.4).

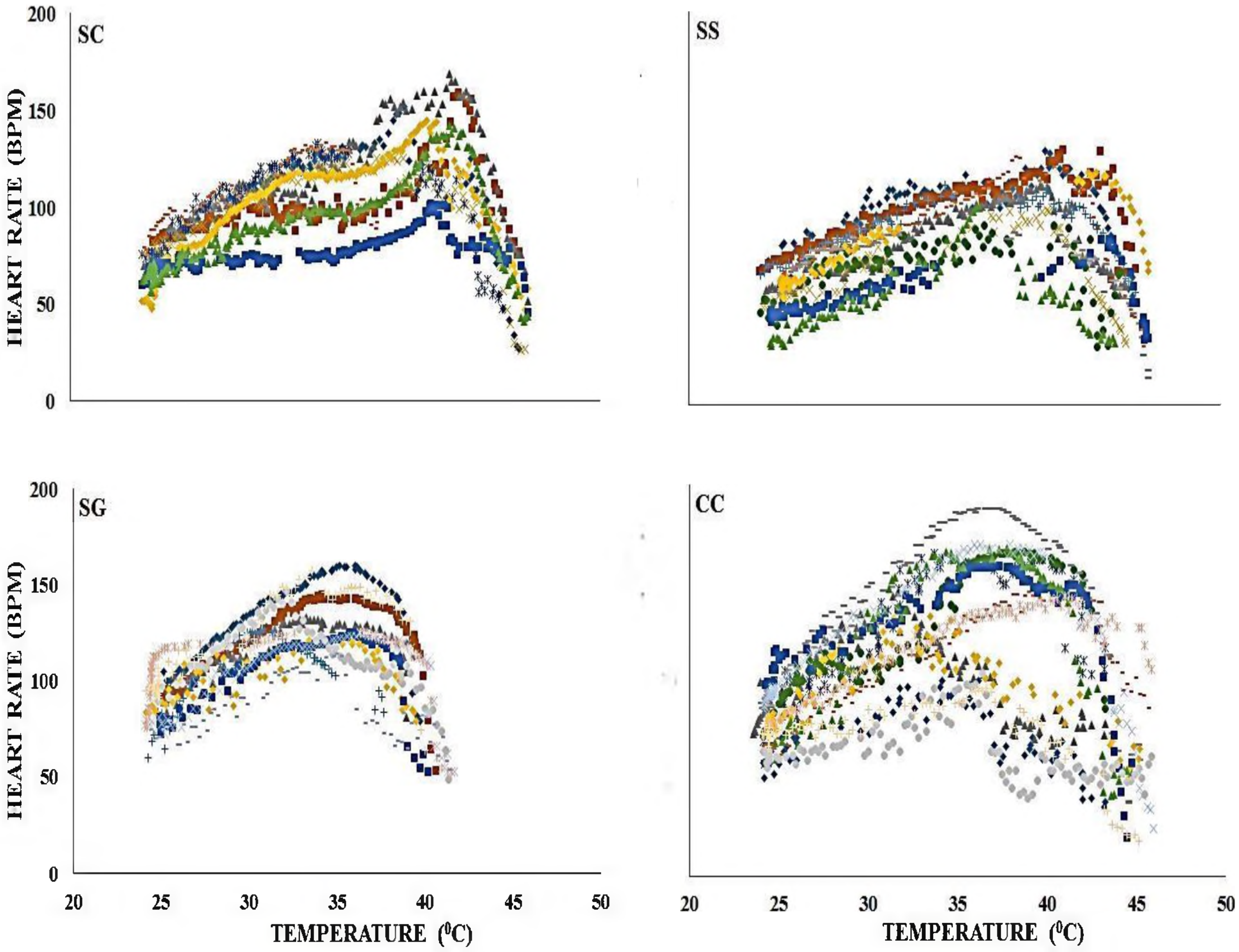

**Fig 4.3: The cardiac thermal response curves of the four study species (SC, n = 9; SG, n = 11; SS, n = 11; CC, n = 13) in air. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*. Each colour represents a different individual.**

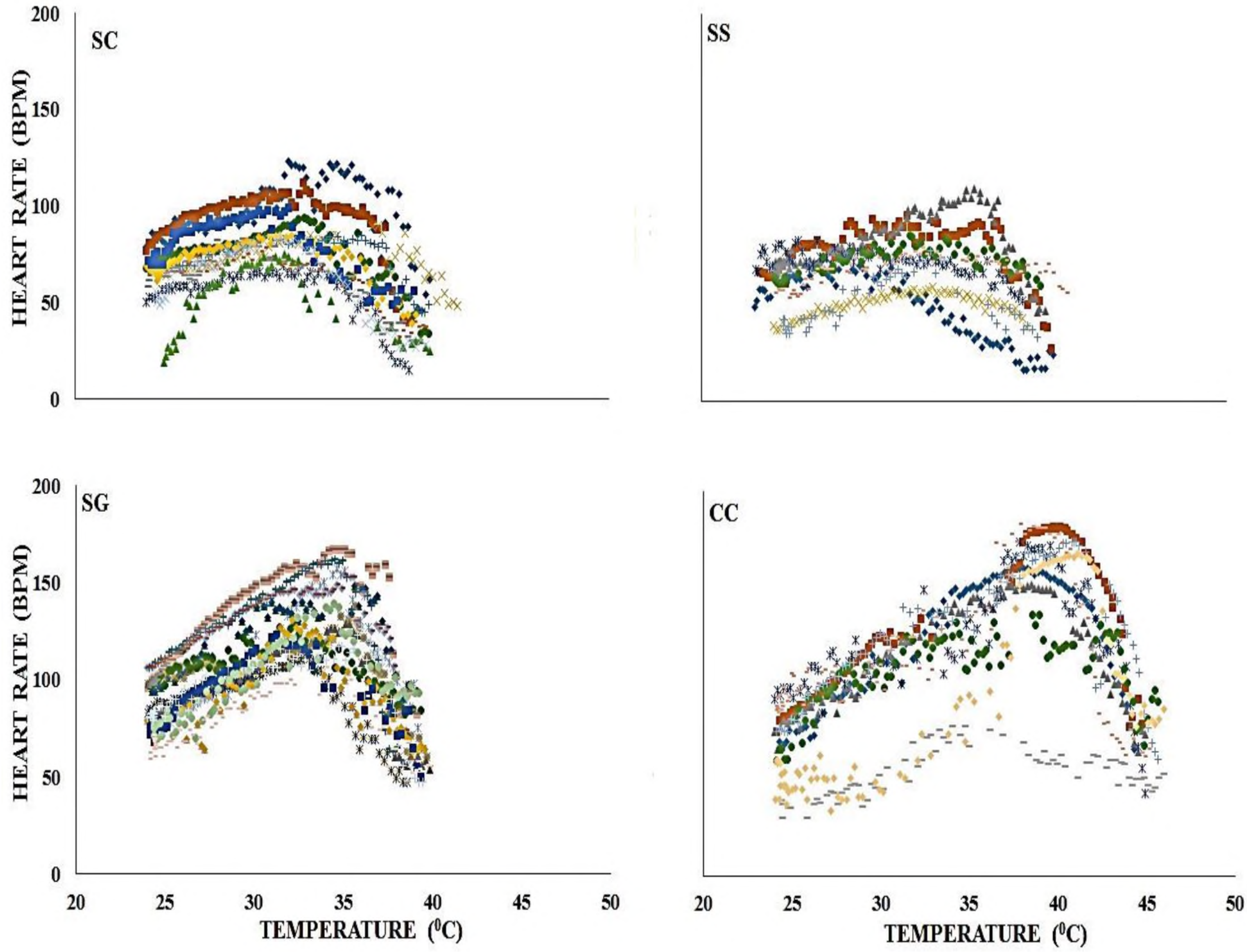

**Fig 4.4: The cardiac thermal response curves of the four study species (SC, n = 12; SG, n = 14; SS, n = 8; CC, n = 9) in water. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*. Each colour represents a different individual.**

### *Relationship between heart rate and temperature*

It can be seen from the Arrhenius plots (Fig 4.5 and 4.6), that there was a strong positive correlation between heart rate and temperature for all sampled species in water (mean $R^2 > 0.65$; Table 4.5). This was particularly true for *Siphonaria capensis* (mean $R^2 = 0.80 \pm 0.14$) and *Scutellastra granularis* (mean $R^2 = 0.85 \pm 0.17$), while the correlation between these parameters were positive, but not as strong for *Siphonaria serrata* (mean $R^2 = 0.69 \pm 0.25$) and *C. capensis* (mean $R^2 = 0.72 \pm 0.30$). Overall, in air there was an even stronger positive correlation (mean $R^2 > 0.75$; Table 4.5) between heart rate and temperature. In air, the correlation between heart rate and temperature for *Scutellastra granularis* (mean $R^2 = 0.82\pm0.21$), *Siphonaria serrata* (mean $R^2 = 0.87\pm0.09$) and *C. capensis* (mean $R^2 = 0.81\pm0.15$) were all higher than $R^2 = 0.8$. Only the correlation for *Siphonaria capensis* (mean $R^2 = 0.78\pm0.22$) was below this value. It is important to note however, that the aforementioned figures only apply to the animal's cardiac response to increasing temperature from 25°C up to their ABT values (Table 4.5).

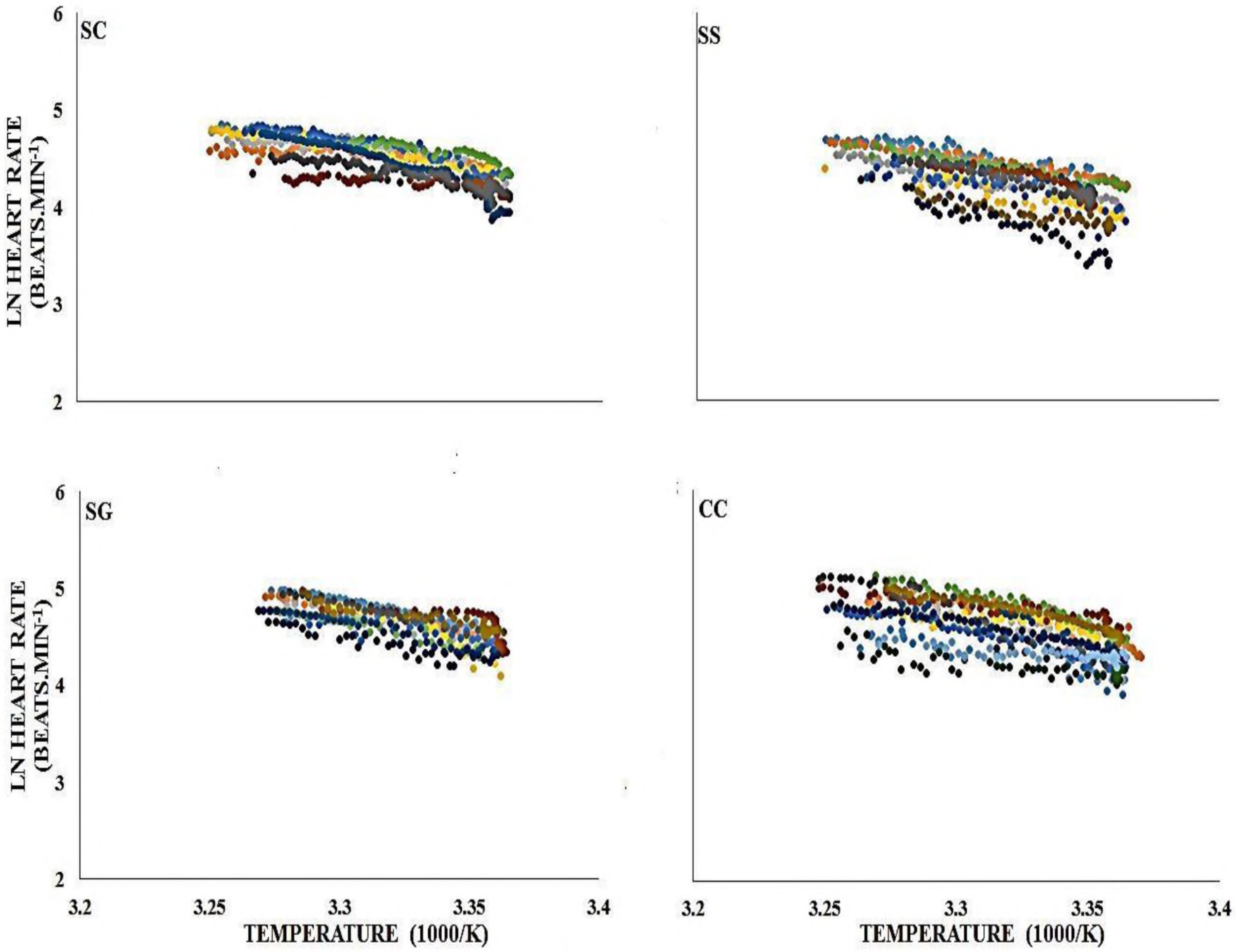

**Fig 4.5: Arrhenius plots ($L_n$ Heart Rate Vs Temperature (1000/K)) of the four study species (SC, n = 12; SG, n = 14; SS, n = 8; CC, n = 9) heat dosed (20°C – 50°C) while submerged in water. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*. Each colour represents a different individual. Average regression parameters presented in Table 4.5 below.**

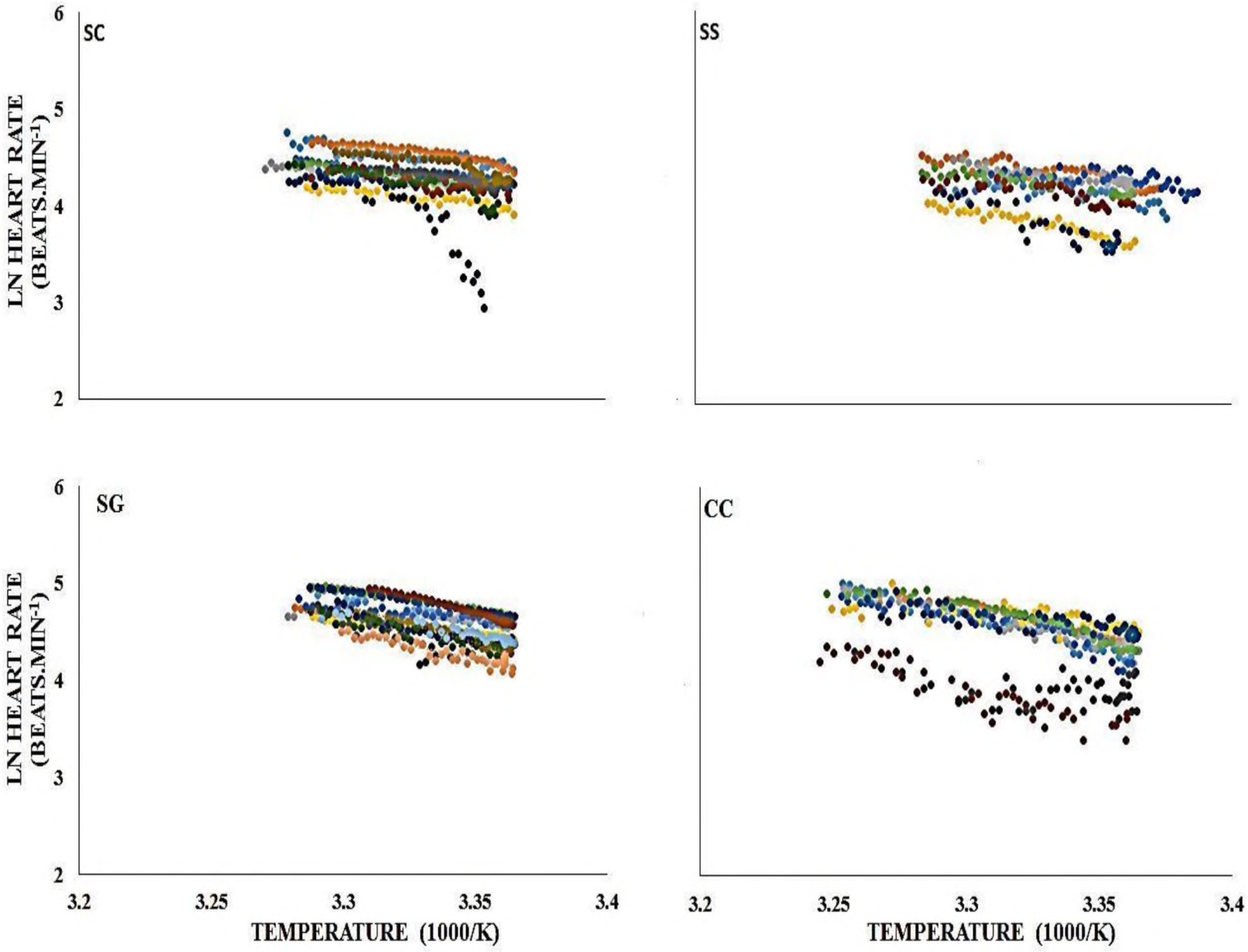

**Fig 4.6: Arrhenius plots ($L_n$ Heart Rate Vs Temperature (1000/K)) of the four study species (SC, n = 12; SG, n = 14; SS, n = 8; CC, n = 9) heat dosed (20°C – 50°C) while emerged. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*. Each colour represents a different individual. Average regression parameters presented in Table 4.5 below.**

**Table 4.2: Results from the ANCOVA analyses run on the Arrhenius plots ($L_n$ Heart Rate vs 1/T) obtained from the linear section of the thermal response curves of each species in air and water. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis.***

| Species | Medium | | | | | |
|---|---|---|---|---|---|---|
| | Water | | | Air | | |
| | F | df | p | F | df | p |
| **SC** | 118 | 11 | p<0.001 | 104 | 8 | p<0.001 |
| **SG** | 309 | 13 | p<0.001 | 110 | 10 | p<0.001 |
| **SS** | 322 | 7 | p<0.001 | 426 | 10 | p<0.001 |
| **CC** | 346 | 8 | p<0.001 | 203 | 12 | p<0.001 |

### *Heart rate response to temperature*

The GLMMs comparing the response of the Arrhenius transformed heart rates to temperature increase (Table 4.3), showed that there were significant differences between species ($p < 0.0001$) and between media ($p < 0.005$), and also that the interaction between species and medium was significant ($p < 0.0001$).

In air, *Scutellastra granularis* and *C. capensis* had a greater mean heart rate response to increasing temperature than *Siphonaria capensis* and *S. serrata* (Tukey HSD, $p < 0.0001$; Fig 4.7). The results for the mean heart rate responses to increasing temperature in water followed an identical trend (*Scutellastra granularis* > *C. capensis* > *Siphonaria capensis* > *S. serrata*) to the results for the same parameter in air ($p < 0.0001$). In addition, all the limpet species had a significantly greater mean heart rate response to increasing temperature in air than they did in water ($p < 0.0001$ for all species except *S. serrata*; $p < 0.001$).

**Table 4.3: Results from the GLMM analysis run on the Arrhenius plots ($L_n$ Heart Rate vs 1/T) obtained from the linear section of the thermal response curves in both media.). Medium, species and medium*species were considered as fixed factors, and replicates as a random factor.**

| **GLMM Factors** | **$L_n$ Heart Rate** | | | |
|---|---|---|---|---|
| | **F** | **Df** | **p** | **MS** |
| **Medium** | 9.31 | 1 | p=0.0023 | 0.27 |
| **Species** | 325.23 | 3 | p<0.0001 | 9.42 |
| **Medium*Species** | 45.96 | 3 | p<0.0001 | 1.33 |
| **Replicate** | 71.09 | 55 | p<0.0001 | 2.06 |
| **Error** | | 3911 | | 0.029 |

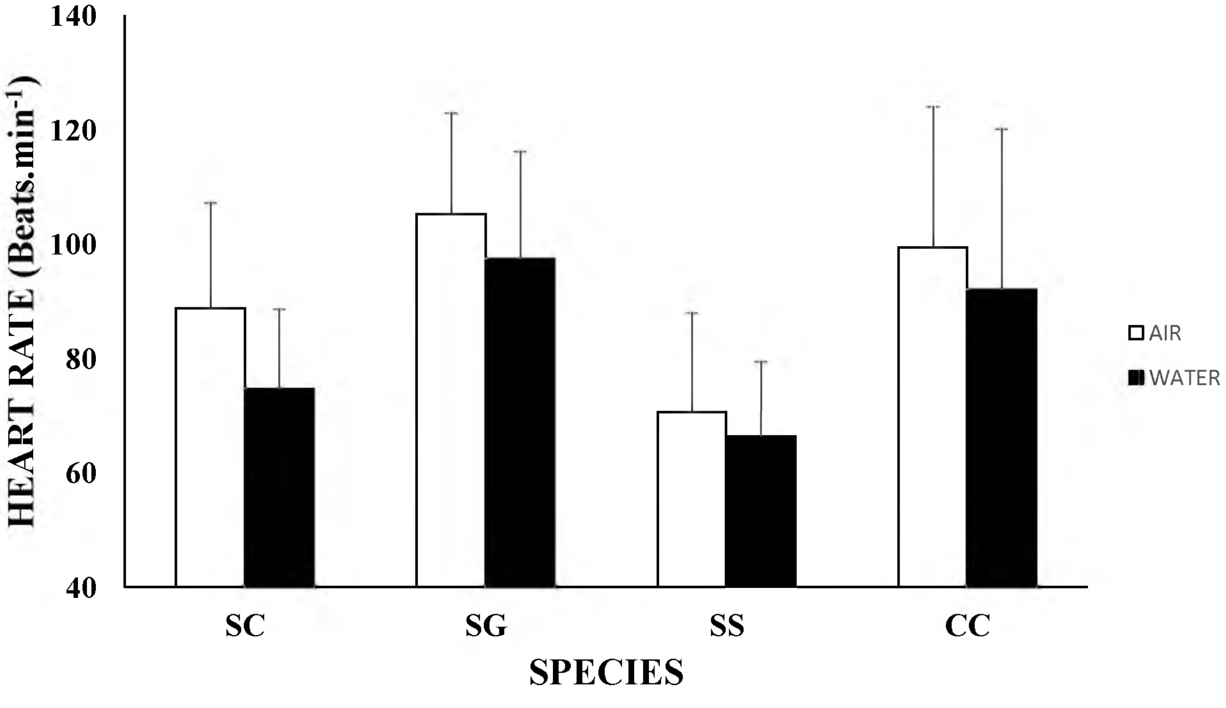

**Fig 4.7: Graph displaying differences in mean (±SD) untransformed heart rates among the study animals in each medium. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*.**

*Were there differences in the ABT values between species and/or media?*

The 2-way ANOVA comparing the ABTs between species in each medium (Table 4.4), showed that there were significant differences between media ($p < 0.0001$) and species ($p < 0.0001$) and that the interaction between medium and species was significant ($p < 0.0001$). In water, *C. capensis* had a significantly greater ABT (Tukey HSD, $p < 0.001$) than the other three species, which did not differ significantly ($p > 0.05$). In air, *Siphonaria capensis*, *S. serrata* and *C. capensis* all had similar ABTs (*C. capensis* 0.1°C lower than both pulmonates) values ($p > 0.05$). *Scutellastra granularis* on the other hand, had a significantly lower aerial ABT ($p < 0.05$) than the other three species. It is worth noting that *Siphonaria capensis* and *S. serrata* had the next two highest cardiac ABTs (*S. serrata* only 0.1°C > *Scutellastra granularis*) in water (Fig 4.8). Other than *C. capensis*, all the limpet species had significantly higher aerial ABTs ($p < 0.05$) than their aquatic ABTs. In contrast, there was no significant difference between *C. capensis* ($p = 0.18$) aerial and aquatic ABT values, however, its aquatic ABT was slightly greater than its aerial ABT value (Fig 4.8).

**Table 4.4: Results from the 2-way ANOVA run on the ABTs obtained from the thermal response curves in both media. Medium, species and medium*species were considered as a factors.**

| **2-Way ANOVA Factors** | **ABT** | | | |
|---|---|---|---|---|
| | **F** | **df** | **p** | **MS** |
| **Medium** | 33.4 | 1 | p<0.0001 | 31.62 |
| **Species** | 24.6 | 3 | p<0.0001 | 23.27 |
| **Medium*Species** | 14.5 | 3 | p<0.0001 | 13.69 |
| **Error** | | 79 | | 0.95 |

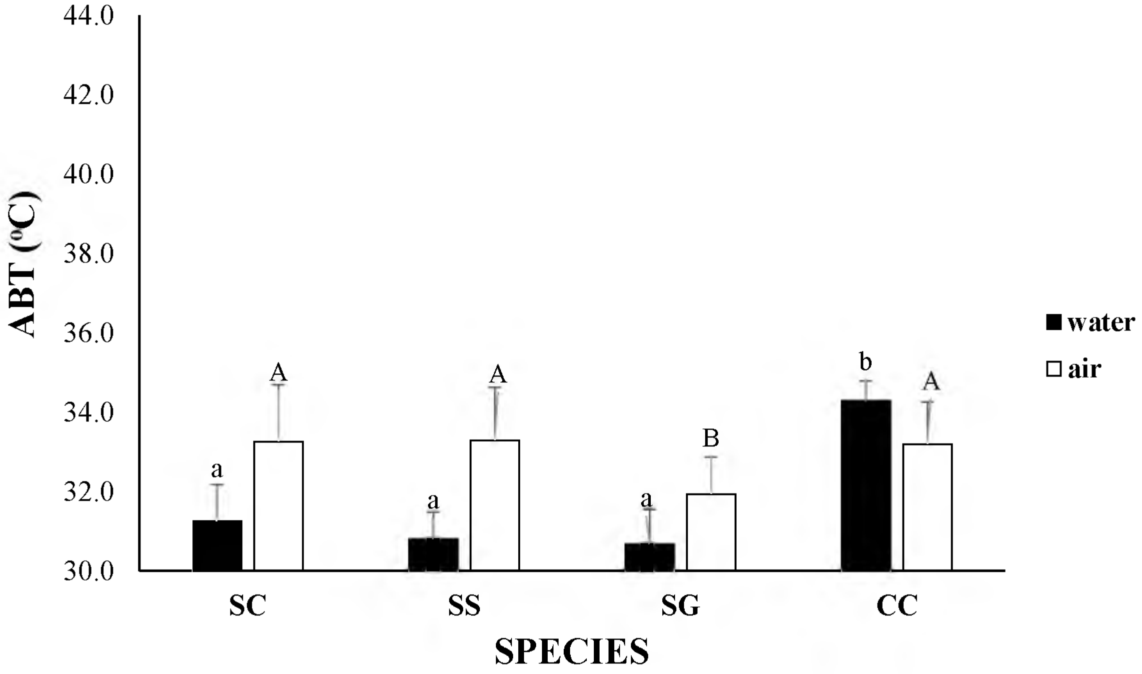

**Fig 4.8: A comparison of the mean (+ SD) aerial and aquatic ABT values of the four study species. SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*. Data labels display homogenous groups (uppercase for air and lowercase for water).**

**Table 4.5: Mean (± SD) values of the Arrhenius plot regression parameters derived from the heart rate measurements in air and water. The $R^2$ values were obtained from the linear section of the thermal response curves ($L_n$ Heart rate vs 1/T). SC – *Siphonaria capensis*; SG – *Scutellastra granularis*; SS – *Siphonaria serrata*; CC - *Cellana capensis*.**

| Species | Water | | | Air | | |
|---|---|---|---|---|---|---|
| | **Mean $L_n$ (hr)** | **Mean Ea/R** | **Mean $R^2$** | **Mean $L_n$ (hr)** | **Mean Ea/R** | **Mean $R^2$** |
| **SC** | 4.28±0.18 | 4.66±4.29 | 0.80±0.14 | 4.50±0.15 | 3.68±2.47 | 0.78±0.22 |
| **SG** | 4.57±0.16 | 4.51±1.86 | 0.85±0.17 | 4.64±0.11 | 5.17±1.83 | 0.82±0.21 |
| **SS** | 4.15±0.21 | 3.93±2.03 | 0.69±0.25 | 4.22±0.22 | 5.19±1.39 | 0.87±0.09 |
| **CC** | 4.47±0.32 | 4.84±2.08 | 0.72±0.30 | 4.55±0.19 | 5.02±2.11 | 0.81±0.15 |

# Discussion

Based on the OCLTT theory, I predicted that differences in the upper thermal limits between and within the two limpet groups would be generated due to differences in their respiratory structure morphology. Therefore, I anticipated that the limpet group with respiratory structures primarily adapted to facilitating respiration in a particular medium, would have a greater upper thermal limit than the limpet group with a respiratory structure that is not similarly/better suited to respiration in that medium. Analysis of the study animals' ABTs revealed that, apart from the marginal species-dependent differences, there were no consistent differences in the upper thermal limits between and within the two limpet groups in either medium.

As discussed in Chapter 1, the pulmonate limpets possess a mantle cavity lung, which is better suited to aerial respiration than the ring of pallial gills used by the patellogastropod limpets (Yonge, 1947; Purchon, 1968; Fretter and Peake, 1975; Branch, 1981; Marshall and McQuaid, 1992b). This mantle cavity lung presumably allows the siphonariids to sustain higher levels of $O_2$ supply to the blood and by extension their mitochondria, compared to the patellids during emersion. Furthermore, based on the OCLTT theory, I assumed that animals with a superior ability to supply the relevant organismal systems and structures with sufficient $O_2$ would have greater upper thermal limits (Frederich and Pörtner, 2000; Peck *et al.*, 2002; Pörtner, 2002; Stillman, 2003; Pörtner *et al.*, 2006; Pörtner and Knust 2007; Pörtner, 2010; Pörtner, 2012; Sokolova *et al.*, 2012). By supplying their mitochondria with more $O_2$, the pulmonate limpets should be able to produce larger amounts of energy aerobically at higher temperatures. As a result, the pulmonate limpets were expected to be able to stave off anaerobic metabolism for longer and have higher ABTs in air than the patellogastropods (McMahon, 1988; Zielinski and Pörtner, 1996; Pörtner and Zielinski, 1998; Pörtner *et al.*, 1999; Frederich and Pörtner, 2000; Peck *et al.*, 2002). This explains why both the siphonariid limpets had slightly higher ABTs in air than *C. capensis* and, why *Scutellastra granularis* clearly had a lower aerial ABT than both pulmonate limpets. This is in accordance with the results from Chapter 3 where the collective upper lethal limits of the pulmonate limpets were greater than that of the patellogastropod limpets in air. In their study on *Siphonaria oculus* and *Scutellastra granularis*, Marshall and McQuaid (1992a) also found that the pulmonate limpet had a higher lethal thermal limit ($LT_{50}$ = 158hrs) than the patellogastropod limpet ($LT_{50}$ = 89hrs) in air. However, as was the case in Chapter 3, the difference among the limpet groups primary respiratory structures may not be enough to explain the comparatively high, aerial ABT of *C. capensis.*

As seen in previous research and described in Chapter 3, several high shore patellid limpets can support aerobic aerial respiration at levels similar to those of high shore pulmonate limpets (Micallef, 1967; Micallef and Bannister, 1967; Hughes, 1971; McMahon and Russel-Hunter, 1977; Branch and Newell, 1978; Branch, 1979; Houlihan, 1979; Innes and Houlihan, 1985) due to an increase in the vascularisation of their mantles (Deshpande, 1957; Fretter and Graham, 1962; Dong and Williams, 2011; Huang *et al.*, 2015). Patellid limpets with this physiological adaptation, particularly those from the genus *Cellana*, can therefore exhibit relatively high aerial ABTs. This was shown in the study by Huang *et al.* (2015) on the mid shore limpet, *C. toreuma* (ABT = 34.2°C) and by Dong and Williams (2011) for *C. toreuma* (ABT = 41.81°C) and the high shore limpet, *C. grata* (ABT = 47°C). In addition, Dong *et al.* (unpubl. data) showed that there was no significant difference between the aerial thermal limit of the pulmonate *Siphonaria japonica* (ABT = 38.9°C) and the patellogastropod *C. toreuma* (ABT = 38.5°C). However, the simple vascularisation of these limpets' mantles only serves to maintain some level of aerobic $O_2$ consumption in air, which keeps down the accumulation of anaerobic by-products at high temperatures (Huang *et al.*, 2015). It is therefore not a respiratory structure primarily adapted to aerial respiration in the way the mantle cavity lung of the siphonariid limpets is (Branch, 1981). It is for this reason that factors other than those stipulated under the OCLTT may be more important to setting the ABT of *C. capensis* and *Scutellastra granularis* in air.

Perhaps an even greater indication that failure of an organism's cardiorespiratory capacity is not always key in setting its thermal limit, is the fact that the patellogastropod limpet *S. granularis* had the lowest aquatic ABT. Previous research has shown that pulmonate limpets with secondary gills can respire aerobically in water at levels similar to patellogastropod limpets (Purchon, 1968; Fretter and Peake, 1975; McMahon and Russel-Hunter 1975; 1981; Shumway, 1981; Shumway and Marsden, 1982; Innes and Houlihan, 1985). As described in Chapter 3, this means that these pulmonate limpets should have aquatic ABTs similar to those of patellogastropod limpets primarily adapted to aquatic metabolism. Therefore, the fact that siphonariid limpets have secondary gills in their pulmonary cavities (Cotrell, 1910; Yonge, 1952; Marcus and Marcus, 1960; Hubendick, 1978) explains to an extent, why both siphonariid limpets in the current study had aquatic ABTs as high as, or higher than the ABT of *S. granularis*. The results from this chapter are similar to the results in Chapter 3, which showed that both siphonariid limpets had aquatic lethal thermal limits that were similar to or slightly higher than that of *S. granularis*. Despite this, it is important to note that the secondary gills are not primarily adapted to aquatic $O_2$ consumption (Yonge, 1947; Innes *et al.*, 1984), which means other factors probably drive the relatively high aquatic ABTs of the siphonariid limpets. Currently, no other research investigating the influence of temperature increase on the cardiac ABTs of submerged limpets from the genus *Scutellastra* have been conducted. It is therefore hard to gain any further insights into how the aquatic ABT of *S. granularis* compares to the other study animals.

Even though there was no clear difference in the aquatic ABTs between the siphonariid limpets and *S. granularis*, the other patellogastropod limpet, *C. capensis*, clearly had the highest aquatic ABT. As seen above, the fact that *C. capensis* is primarily suited to aquatic respiration, cannot be seen as an adequate explanation for its comparatively high aquatic ABT, particularly since *S. granularis* uses the same respiratory structures to facilitate aquatic respiration. Furthermore, Dong *et al.* (unpubl. data) also found that there was no significant difference between the aquatic ABT of another pulmonate, *Siphonaria japonica* (ABT = 35.6°C), and the patellogastropod limpet *C. toreuma* (ABT = 36.5°C). The high aquatic ABT of *C. capensis* is, therefore, probably an effect of its recent thermal history via its acclimation to the thermal conditions in shallow, high shore rock pools along the south east and east coasts of South Africa (Kalk, 1959; Branch, 1975; 1976). Whereas, the other three limpet species are generally emerged during low tide along these coasts (Branch and Cherry, 1985; Chambers, 1994; Chambers and McQuaid, 1994, Vat, 2000). As described in Chapter 2, acclimation to the thermal regimes of specific microhabitats could be an important factor to consider during the examination of these limpets' upper thermal tolerance limits.

The siphonariid limpets and *Scutellastra granularis* all had significantly higher ABTs in air than water. While the reverse was true for *C. capensis*, the difference in ABTs between media was not significant. In their study on *Siphonaria japonica*, Dong *et al.* (unpubl. data) also found that the pulmonate limpet had a higher ABT in air than water. In contrast, as was the case for *C. capensis* in the current chapter, Dong *et al.* (unpubl. data) found that *C. toreuma* had similar ABTs in both media. However, Huang *et al.* (2015) found that *C. toreuma* had a greater ABT in air than in water. Since *C. toreuma* usually occupies tide pools in summer (Williams and Morrit, 2005; Firth and Williams, 2009), and based on the assumptions made in this study (see Chapter 2), it was expected that it would be acclimated to the potentially high temperatures in tide pools, therefore, resulting in it having a higher ABT in water like *C. capensis* in this chapter.

In their study, Huang *et al.* (2015) did not specify which microhabitat they collected their study animals from. It is therefore hard to determine whether or not their results may have been affected by the influence of acclimation on the short-term tolerance of their study animal in its preferred microhabitat (see Chapter 2). These results, regarding the siphonariid limpets are favourable in terms of the OCLTT theory, since they show that the animals primarily adapted to aerial respiration withstand high temperatures in air better than they do in water. However, the same cannot be said about the two patellogastropod limpets, which have higher or similar ABTs in air compared to water.

The aerial, upper thermal limit obtained for *C. capensis* (ABT = 33.2°C) in the current study compares favourably to the upper thermal limit obtained for *C. toreuma* (ABT = 34.2°C) as determined by Huang *et al.* (2015). In their studies, however, Dong and Williams (2011) and Dong *et al.* (unpubl.data) presented aerial ABTs (41.81°C and 38.5°C respectively) that were a lot higher for *C. toreuma*. In terms of the upper thermal limits in water, Dong *et al.* (unpubl. data) recorded a higher ABT (36.5°C) for *C. toreuma* than the value for *C. capensis* (34.3°C) in the current study and Huang *et al.* (2015) reported a lower ABT (30.2°C) for *C. toreuma* in their study. Various factors could be responsible for the contrasting results between the studies on *C. toreuma* and the current study's result for *C. capensis*. The differences in the results from the aforementioned studies are probably due to differences in the geographical zones of the sample sites and their associated temperature ranges (see below). Another likely source of variation between these results could be the differences in the experimental protocols between the studies. In the current study, *C. capensis* limpets were collected from a sample site in a warm temperate geographical zone along the South African coast (Fig 1.4). In contrast, Huang *et al.* (2015) and Dong *et al.* (2011; unpubl. data) collected samples of *C. toreuma* in China from sub-tropical and tropical geographical zones respectively. Several studies (Zakhartsev *et al.*, 2003; Dong *et al.*, 2008; Morley *et al.* 2009; Byrne *et al.*, 2010; Zardi *et al.*, 2011; Zerebecki and Sorte, 2011; Matumba, 2013; Norin *et al.*, 2013; Xing *et al.*, 2016) have shown that an intertidal animal's geographic distribution influences its thermal tolerance, with those found in warmer geographic zones normally having higher thermal tolerances. Regarding differences in study methods, Huang *et al.* (2015) kept their animals at 20°C for more than 24hrs before determining the limpets ABTs, as was done in this study, while Dong *et al.* (2011)

kept their study animals at 28°C. This logic can also be applied to the fact that the siphonariid limpets used in this study, had a lower aerial (*Siphonaria serrata*; ABT = 33.3°C, *S. capensis*; ABT = 33.3°C) and aquatic (*S. serrata*; ABT = 30.8°C, *S. capensis*; ABT = 31.3°C) upper thermal limit than the siphonariid limpet, *S. japonica*, examined by Dong *et al.* (unpubl. data).

To further assess the influence of breathing capabilities and/or medium on the upper thermal tolerance limits of these animals, other measures of cardiac thermal limitation were investigated. The examination of the limpets' heart rates revealed that there was a strong correlation between heart rate and temperature for all the limpet species, even at high aerial temperatures (Table 4.5). This shows that the physiological responses of these limpets were not affected more by desiccation than they were by temperature (Tagliarolo and McQuaid, 2015). Moreover, the relationship between heart rate and temperature varied significantly between the individuals of each species (Table 4.2). Various biotic factors such as intraspecific variation in the limpet species reproductive, nutritional and health status can explain the interindividual variability in the relationship between heart rate and temperature (Lewis, 1971; Bayne *et al.*, 1976; Crear and Forteath, 2000; Huxham *et al.*, 2001; Dahlhoff *et al.*, 2002; Bates *et al.*, 2011). These factors all, either elevate or depress metabolic rates and thermal sensitivities of metabolism among individuals of the same species (Shumway *et al.*, 1993; Huxham *et al.*, 2001; Peak and Veal, 2001; Baeza and Fernández, 2002).

As seen previously (Widows, 1973, Dong and Williams, 2011; Xing *et al.*, 2016), the slope of an organism's heart rate response to temperature is also referred to as its cardiac thermal sensitivity or cardiac sensitivity. In intertidal organisms, the cardiac sensitivity is a measure of responsiveness to temperature changes, with organisms exhibiting high cardiac sensitivities normally having a greater heart rate response to temperature changes than animals with low cardiac sensitivities (Dong and Williams, 2011; Xing *et al.*, 2016). Previous research (Marshall and McQuaid, 1992b; Santini *et al.*, 1999) has also shown that intertidal mollusc heart rates are related to the organism's $MO_2$ and, ultimately to its metabolic rate. This means that an intertidal organism's cardiac sensitivity is related to its thermal metabolic sensitivity (Marshall and McQuaid, 1992b; Matumba, 2013). Animals with high metabolic sensitivities generally require more energy and therefore attain greater levels of anaerobic metabolism during thermal stress, faster than animals with lower metabolic sensitivities (Santini and Chelazzi, 1995; Sokolova and Pörtner, 2001; Marshall *et al.*, 2011; Tagliarolo and McQuaid, 2015). Therefore, in the current study, all the limpet species, except *Siphonaria capensis*, were less likely to endure anaerobic metabolism at high temperatures while submerged.

As organisms reach the limits of their aerobic metabolism during exposure to high temperatures, anaerobic metabolism sets in, which coincides with the production of HSPs and other heat resistant molecular chaperones (Lee and Boulding, 2010; Tomanek, 2010; Judge *et al.*, 2011; Marshall *et al.*, 2011). However, the production of these proteins is energetically expensive and can therefore only be maintained via anaerobic metabolism for a short period of time (Feder and Hofmann, 1999; Pörtner, 2002). Once these organisms can no longer support the synthesis of HSPs they eventually succumb to the heat and toxic environment created by the accumulation of anaerobic by-products (Pörtner, 2002). Several studies (Newell, 1979; Branch, 1981; Dong and Williams, 2011) indicate that intertidal animals with high cardiac sensitivities and therefore, metabolic responses to high temperatures are likely to also have lower, upper thermal tolerance limits. This was displayed in the study by Dong and Williams (2011), in which the mid-shore *C. toreuma* had a higher cardiac sensitivity and a lower ABT than the high shore *C. grata* when exposed to high temperatures. Therefore, since *C. capensis*, *Scutellastra granularis* and *Siphonaria serrata* had lower cardiac sensitivities in water than they did in air, it was expected that they would be more adept at surviving increased thermal exposure in water. Furthermore, the lower cardiac sensitivity in air compared to water shown by *S. capensis* should translate into a higher upper thermal limit in air for this species. While this was reflected in the ABT results for *C. capensis* and *S. capensis* in this chapter (Fig 4.8), both *S. serrata* and *Scutellastra granularis* had higher ABTs in air than water even though they had lower cardiac sensitivities in water (Fig 4.8).

The higher cardiac sensitivities of *Siphonaria serrata* and *Scutellastra granularis* in air may be a general response to emersion for these high shore limpets, as they seek to rapidly repay the energetic debt generated while exposed to a constant temperature increase (Helm and Trueman, 1967; Boyden, 1972; Coleman, 1973; Bayne *et al.*, 1976). In the past, researchers have often found that organisms require high aerobic respiration rates to quickly deal with anaerobic by-products accumulated during physical stress (McMahon, 1988). This could explain why *Siphonaria serrata* and *Scutellastra granularis* had lower aquatic ABTs despite having lower metabolic sensitivities in water than air. However, this logic alone does not clearly explain why most of the study animals had higher metabolic sensitivities in air than water. In marine intertidal zones, temperature change in water is normally more benign than it is in air, where it is often rapid and extreme as the tide rises and falls (Denny *et al.*, 2011). High shore intertidal molluscs are therefore likely to have higher metabolic sensitivities in air than water, in order to cope with the energetic demands associated with the rapid changes in aerial temperatures. This is probably why most of the study animals all had greater cardiac sensitivities in air compared to water.

The low aerial metabolic sensitivity of *Siphonaria capensis* compared to the other three species may be due to a metabolic rate depression response. Previous studies (Storey and Storey, 1990; Marshall and McQuaid, 1991; Storey, 1998; Guderley and St-Pierre, 2002; Pörtner, 2010; Williams *et al.*, 2011) show that various intertidal, marine animals suppress their metabolic rates to save energy during exposure to extreme abiotic conditions, which allows them to survive acute exposure to environmental stressors such as rapidly increasing temperatures. While metabolic depression is common in marine, freshwater and terrestrial pulmonates (Machin, 1975; McMahon, 1983; Riddle, 1983; Marshall and McQuaid, 1991) there is not much evidence of its occurrence in patellogastropod limpets and in particular, Marshall and McQuaid (1991) showed that *Scutellastra granularis* does not undergo a metabolic rate depression during aerial exposure. It is not currently clear why *Siphonaria serrata*, unlike *S. capensis*, had a higher metabolic sensitivity in air than water. However, the fact that, like *S. capensis*, *S. serrata* had lower mean heart rates than the patellogastropods in air (Table 4.5), suggests that it may in fact undergo facultative depression of the heart rate, which could translate into its comparatively high ABT in air. Moreover, this indicates that a high cardiac thermal sensitivity does not always translate into a lower ABT.

Past studies have generally focused on the influence, geographic (Morley *et al.* 2009; Zardi *et al.*, 2011; Matumba, 2013; Xing *et al.*, 2016) or vertical distribution (Broekhuysen, 1940; Evans, 1948; McMahon, 1990; Stillman and Somero, 1996; Davenport and Davenport, 2005; Stenseng *et al.*, 2005; Dong and Williams, 2011; Tagliarolo and McQuaid, 2015) have on the thermal tolerances of intertidal organisms. In addition, most of these studies examined the thermal tolerance of submersed animals and only a few looked at aerial thermal tolerances. Moreover, even fewer studies have compared the thermal tolerance of water breathing organisms between media (Huang *et al.*, 2015; Tagliarolo and McQuaid, 2015), or the aerial thermal limits of an air and water breathing gastropod mollusc (Marshall and McQuaid, 1992a; Davenport, 1997; Harley *et al.*, 2009). Essentially, the current study was the first to consider the influence of medium on the ABTs of two limpet groups, differentially suited to breathing in either medium. The current study also highlights the importance of finding ways to investigate the merits of the OCLTT theory (Pörtner, 2002; Stillman, 2003; Pörtner *et al.*, 2006; Pörtner and Knust 2007; Pörtner, 2012; Sokolova *et al.*, 2012), by reviewing the effect that differences in respiratory organ morphology among organisms have on thermal tolerance. Even though the OCLTT theory highlights mechanisms important to understanding thermal tolerance in intertidal organisms, some of the OCLTT hypothesis core concepts may have to be revisited and revised (Clark *et al.*, 2013; Norin *et al.*, 2013; Ern *et al.*, 2015).

The results show that there is no clear indication of the role different breathing capabilities play in determining either aerial or aquatic ABTs. Therefore, factors outside of an intertidal organisms' breathing capabilities probably had a compound effect on their ABTs. Previous studies indicate that most intertidal organisms exhibit morphological (Vermeij, 1973; Etter, 1988; Harley *et al.*, 2009), physiological (Seebacher and Franklin, 2005) and behavioural (Heath, 1970; Garrity, 1984) thermoregulatory strategies, which could potentially influence their thermal tolerance during testing. Some physiological adaptations include metabolic adjustments and HSP synthesis (Somero, 2010; Tomanek, 2010; Madeira *et al.*, 2012), while shell colour and ornamentation (Bates and Hicks, 2005; Harley *et al.*, 2009), body orientation and microhabitat selection (Marshall and Chua, 2012) serve as examples of morphological and behavioural thermoregulation strategies respectively. As mentioned before (see Chapter 3), these thermoregulatory strategies can influence the thermal tolerance of animals through processes of acclimation (Zakhartsev *et al.*, 2003; Dong *et al.*, 2008; Byrne *et al.*, 2010; Zerebecki and Sorte, 2011; Norin *et al.*, 2013).

Therefore, I reject the null hypotheses that: 1. The ABTs of the pulmonates will be greater than those of the patellogastropods in air whereas the patellogastropods will have a greater ABT than the pulmonates in water; and 2. The ABT of pulmonates will be greater in air than in water, while the opposite will be true for the patellogastropods.

In conclusion, based on evidence garnered in the current study, the OCLTT theory though very useful, is not complete in its coverage of the mechanisms that drive intertidal organisms' thermal tolerances and its predictive powers should be scrutinized further.

# Chapter 5 – General Discussion

It has been established that climate change, through its associated changes in environmental temperature, has the potential to cause significant shifts in the phenology, abundance and distribution of both aquatic and terrestrial animals (Brander *et al.*, 2003; Wiltshire and Manly, 2004; Perry *et al.*, 2005; Grebmeier *et al.*, 2006; Parmesan, 2006; Farrell *et al.*, 2008; Walther, 2010). Additionally, many previous studies indicate that these ecological shifts may be driven by thermally induced changes in physiology (Cossins and Bowler, 1987; Schmidt-Nielsen, 1997; Somero, 2011; Tomanek, 2011; Somero, 2012; Tomanek, 2012; Matumba, 2013). As such, the OCLTT theory was developed to help explain the link between physiology and these ecological systems, and to generate a predictive tool that can help foresee the impact that climate change might have on global biodiversity and evolutionary processes (Frederich and Pörtner, 2000; Pörtner, 2001; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010; Pörtner, 2012; Bozinovic and Pörtner, 2015). This theory is largely based on the idea that an organism's thermal performance is governed by its' aerobic scope such that, as the aerobic scope diminishes during thermal stress the animal is expected to be increasingly limited in its ability to survive, grow and reproduce (Frederich and Pörtner, 2000; Pörtner, 2001; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Farrell *et al.*, 2008).

Therefore, this study investigated the importance of breathing ability in setting the thermal tolerance of patellogastropod and pulmonate limpets, by determining and relating their rocky intertidal distribution patterns to their lethal thermal limits and measures of cardiac thermal limitation, in both air and water. In simpler terms this study sought to determine if the respiratory mode, thermal limits and microhabitat use of the limpets were correlated. The importance of the differences in breathing ability between the two limpet groups, was based on the assumption that it translates into a dissimilarity in $MO_2$ between the two groups (Marshall and McQuaid, 1992b; Bot and Hollander, 2000; Butler *et al.*, 2002). This assumption was important, because $MO_2$ were not measured in this study. As such, it was assumed that the pulmonate limpets had a superior ability for aerial respiration and that the patellogastropod limpets were better suited to aquatic respiration, due to the differences in their respiratory structures (Yonge, 1947; Yonge, 1952; Marcus and Marcus, 1960; Branch, 1981; McMahon, 1988). Thus, by determining the upper thermal limits of these limpet groups in air and water, I could examine how having better, albeit assumed, $O_2$ consumption capabilities in either medium influences their thermal limitation in comparison to each other. Therefore, it was hypothesised that the OCLTT theory would only apply to this study if the prediction that the trend in the thermal tolerances of these limpet groups follows the trend in their different breathing capabilities, is met. This means that the pulmonate limpets were expected to have greater thermal tolerance limits in air than the patellogastropod limpets, while the patellogastropod limpets were expected to have greater aquatic thermal limits. In addition, the pulmonate limpets were expected to have greater aerial thermal tolerance limits and the patellogastropods, greater aquatic thermal tolerance limits.

However, the results indicate that this was not the case and that there was no real difference in the collective thermal limits of the patellogastropod and pulmonate limpets in either medium. The results also indicate that the limpet species recent thermal history, may have been more important in setting their thermal limits than their respective respiratory modes. For example, the fact that *Scutellastra granularis* consistently had the lowest thermal limits in both media probably has more to do with the acclimation, of individuals from this species, to the thermal regime of their preferred microhabitats (Tomanek and Somero 1999; Anestis *et al.*, 2008; Dong *et al.*, 2008) than with their respiratory capabilities (Marshall and McQuaid, 1992b; Dong *et al.*, unpubl data). Since field fresh animals were used in this study, this short-term acclimation probably influenced the thermal tolerance of these limpets during the determination of their thermal limits. In Chapter 2 of this study, it was shown that *S. granularis* preferred the least thermally challenging intertidal microhabitat, vertical rock surfaces, which is assumed to attain the lowest maximum temperatures of the available microhabitats. Similar circumstances also explain why *Siphonaria capensis* along with *Cellana capensis* had the highest thermal tolerance limits in water.

Consequently, there was no evidence to support the expectation that a superior ability to breathe in a specific medium translates into a greater upper thermal tolerance in that medium. As a result, I reject the hypothesis that the OCLTT hypothesis can be used to predict the thermal tolerance of the limpets used in this study. The findings of this study compare favourably with a few other studies (Overgaard *et al.*, 2012; Clark *et al.*, 2013; Gräns *et al.*, 2014; Norin *et al.*, 2014; Ern *et al.*, 2015), conducted to test the general applicability and ecological relevance of the OCLTT theory.

In their studies, Overgaard *et al.* (2012), Wang *et al.* (2014) and Ern *et al.* (2014) all showed that thermal limitations of, the capacity of the cardiorespiratory system to supply $O_2$, does not determine the upper thermal limits of the air breathing toad *Rhinella marina,* the European sea bass and the eurythermal Giant tiger shrimp respectively. Furthermore, in their study on the tropical fish *Lates calcarifer,* Norin *et al.* (2014) suggested that aerobic scope can be regulated independently of temperature. Similarly, Gräns *et al.* (2014) indicated that an increased aerobic scope does not support increased growth in the North Atlantic halibut *Hippoglossus hippoglossus*. Therefore, as in the present thesis, the results from these studies imply that mechanisms other than the ones proposed by the OCLTT theory govern thermal tolerance in various marine and terrestrial organisms. In their review on the subject, Clark *et al.* (2013) outlined protein denaturation, thermal inactivation of enzymes, oxidative stress, cell membrane damage and loss of neural function as factors that could be more important to regulating thermal tolerance in fishes. As suggested above, various factors associated with the study animal's recent thermal history, which was presumably influenced by the acclimation to their preferred microhabitats, could be responsible for setting their thermal limits during testing. One of these includes the expression of heat shock proteins (HSPs) in response to the denaturation of proteins under thermal stress (Feder and Hofmann, 1999; Tomanek and Somero, 1999; 2000; Anestis *et al.*, 2008; Dong *et al.*, 2008; Dong and Williams; 2011; Williams *et al.*, 2011).

Since HSP production is an energetically expensive, time sensitive process that peaks as an organism approaches $T_{C\ II}$, a significant reduction in HSP synthesis during heat stress is associated with mortality (Pörtner, 2010; Dong and Williams, 2011). Furthermore, in their study on the crustaceans *Penaeus monodon* and *Astacus astacus,* Ern *et al.* (2015) indicated that these crustaceans' thermal limits are more closely correlated to their nervous function than to their $O_2$ supply capacities. Ern *et al.* (2015) also point out that a loss of nervous function could coincide with HSP expression, which suggests that thermal tolerance may be more closely related to heat-induced protein denaturation than inadequate $O_2$ supply. Moreover, several past studies (Tomanek and Somero, 1999; Anestis *et al.*, 2008; Dong *et al.*, 2008) have demonstrated that intertidal animals in different microhabitats undergo varying levels of HSP synthesis, associated with differences in the thermal regimes they experience in these microhabitats. Therefore, if the limpets in the current study had different intertidal microhabitat distributions, their recent thermal histories may have been different and as a result, a different upper thermal limit pattern could have been determined amongst them.

It is important to note that individual limpet thermal tolerances within populations could have been influenced by microscale habitat variation, and its associated thermal variability (Helmuth and Denny, 1999; Helmuth and Hofmann, 2001; Jimenez *et al.*, 2015). A factor which could have been controlled for in the current study using a more robust set of data collection measures. This includes establishing limpet body temperatures within the microhabitats across the study sites, recording individual limpet microhabitat preferences and then, determining limpet thermal limits according to their microhabitat preferences. This would have allowed me to test if microhabitat use generates inter-individual variability in limpet thermal tolerance limits and how this may have influenced the results obtained in the current study. Furthermore, in addition to elucidating the limpet's respiratory anatomy, $O_2$ consumption could also have been determined and related to the thermal tolerance of each limpet species to test empirically whether differences in limpet breathing capabilities could be used to predict their thermal tolerances. A combination of these data sets would have provided me with an opportunity to test directly, if the respiratory modes, upper thermal tolerance limits and microhabitat use patterns of the study animals were correlated.

While the influence of intertidal microhabitat preference on the limpets' thermal tolerances was not explicitly examined in the current study, the idea is supported by the findings of Norin *et al.* (2014), which suggest that the tropical fish *Lates calcarifer* shifts its aerobic scope in response to different acclimation temperatures. In their study, Gräns *et al.* (2014) also demonstrate thermal independence of the aerobic scope as a result of acclimation in juvenile Atlantic halibut. Moreover, it is important to note that in both studies, growth was not reduced due to limitations in the fishes' $O_2$ supply capacities. Hence, a higher $T_{optAS}$ due to acclimation need not translate into a more optimal performance of various life history traits (Healy and Schulte, 2012; Clark *et al.*, 2013; Norin *et al.*, 2014; Gräns *et al.*, 2014). This indicates that the OCLTT theory is not a reliable predictor of thermal tolerance and performance for the barramundi and Atlantic halibut fish, or for the limpets in the current study. Therefore, as for other eurythermal temperate and sub-tropical animals, the OCLTT cannot be solely relied on to predict future ecological processes of the study animals considering global climate change.

Clark *et al.* (2013) proposed the multiple performances – multiple optima (MPMO) concept as an alternative and better means of describing thermal tolerance and preference than the OCLTT theory. The MPMO hypothesises that different physiological performance parameters have different $T_{opt}$. It also states that an organism's overall thermal performance and fitness can be limited by any one of these different physiological parameters, and that the limiting physiological parameter can change interspecifically, due to ontogenetic effects or under different thermal challenges. While the MPMO is yet to be developed further or tested extensively (Clark *et al.*, 2013; Farrell, 2013; Pörtner and Giomi, 2013), there are a few studies that have begun to show, albeit indirectly, that this may be a valid concept linking animal physiology and ecology (Munday *et al.*, 2009; Gräns *et al.*, 2014; Norin *et al.*, 2014). Whether this concept applies to the current study is hard to say at the moment and would depend on a more robust experimental analysis (suggested above). However, it seems likely that this concept could prove useful in future, once its ecological relevance has been vigorously investigated. The current study, like several others before it, shows that ecophysiological theory such as the OCLTT should always face strict examination before they are widely applied and accepted. Furthermore, hopefully the current study sets a precedent in terms of finding novel ways to test such conceptual models.

# REFERENCES

Aagaard, A., Andersen, B.B. and Depledge, M.H. 1991. Simultaneous monitoring of physiology and behavioural activity in marine organisms using non-invasive, computer-aided techniques. *Marine Ecology Progress Series* 73: 277-282.

Allanson, B.R. 1959. On the systematics and distribution of the molluscan genus *Siphonaria* in South Africa. *Hydrobiologia* 12: 149-180.

Anestis, A., Pörtner, H.O., Lazou, A. and Michaelidis, B. 2008. Metabolic and molecular responses of sublittoral bearded horse mussel *Modiolus barbatus* to warming sea water: implications for vertical zonation. *The Journal of Experimental Biology* 211: 2889-2898.

Angilletta, Jr. M.A. 2009. Looking for answers to questions about heat stress: researchers are getting warmer. *Functional Ecology* 23: 231-232.

Ansell, A.D., Gibson, R.N. and Barnes, M. 1999. The biology of siphonariid limpets (Gastropoda: Pulmonata). *Oceanography and Marine Biology, An Annual Review 37*: 245-314.

Aronson, R.B. 2001. The limits of detectability: short-term events and short-distance variation in the community structure of coral reefs. *Bulletin of Marine Science* 69: 331-332.

Baeza, J.A. and Fernández, M. 2002. Active brooding *Cancer setosus* (Crustacea: Decapoda): the relationship between female behaviour, embryo oxygen consumption and the cost of brooding. *Functional Ecology* 16: 241-251.

Balaparameswara, R.M. and Ganapati, P.N. 1972. Resistance of the limpet *Cellana radiata* to variations in temperature, salinity and desiccation. *Proceedings of the Indian National Science Academy B* 38: 349-355.

Bally, R., McQuaid, C.D. and Brown, A.C. 1984. Shores of mixed sand and rock: An unexplored marine ecosystem. *South African Journal of Science* 80(11): 500-503.

Bamber, S.D. and Depledge, M.H. 1997. Responses of shore crabs to physiological following exposure to selected environmental. *Aquatic Toxicology* 40: 79-92.

Bates, T.W. and Hicks, D.W. 2005. Locomotory behaviour and habitat selection in littoral gastropods on Caribbean limestone shores. *Journal of Shellfish Research* 24: 75-84.

Bates, A.E., Leiterer, F., Wiedeback, M.L. and Poulin, R. 2011. Parasitized snails take the heat: a case of host manipulation? *Oecologia* 167: 613-621.

Bayne, B.L., Bayne, C.J., Carefoot, T.C. and Thompson, R.J. 1976. The physiological ecology of *Mytilus californianus* Conrad. *Oecologia* 22: 229-250.

Bertness, M.D., Leonard, G.H., Levine, J.M. and Bruno, J.F. 1999. Climate-driven interactions among rocky intertidal organisms caught between rock and hot place. *Oecologia* 120: 446-450.

Bertness, M.D., Crain, C.M., Silliman, B.R., Bazterrica, M.C., Reyna, M., Hildago, F. and Farina, J. 2006. The community structure of western Atlantic Patagonian rocky shores. *Ecological Monographs* 76(3): 439-460.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. and White, J.S.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in ecology and evolution* 24(3): 127-135.

Bolton, J.J., Leliaert, F., De Clerck, O., Anderson, R.J., Stegenga, H., Engledow, H.E. and Coppejans, E. 2004. Where is the western limit of the tropical Indian Ocean seaweed flora? An analysis of intertidal seaweed biogeography on the east coast of South Africa. *Marine Biology* 144(1): 51-59.

Bot, S.D.M. and Hollander, A.P. 2000. The relationship between heart rate and oxygen uptake during non-steady state exercise. *Ergonomics* 43: 1578-1592.

Boyden, C.R. 1972. The behaviour, survival and respiration of the cockles *Cerastoderma edule* and *C. glaucum* in air. *Journal of the Marine Biological Association of the United Kingdom 52*(03): 661-680.

Bozinovic, F. and Pörtner, H.O. 2015. Physiological ecology meets climate change. *Ecology and evolution* 5(5): 1025-1030.

Braby, C.E. and Somero, G.N. 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology* 209: 2554-2566.

Branch, G.M. 1971. The ecology of *Patella* Linnaeus from the Cape Peninsula, South Africa. 1. Zonation, movements and feeding. *African Zoology*. 6: 1-38.

Branch, G.M. 1975. Mechanisms of reducing competition in limpets: migration, differentiation and territorial behaviour. *Journal of Animal Ecology* 44: 575-600.

Branch, G.M. 1976. Interspecific competition experienced by South African *Patella* species. *Journal of Animal Ecology* 45: 507-529.

Branch, G.M. and Newell. R.C. 1978. A comparative study of metabolic energy expenditure in the limpets *Patella cochlear*, *P. oculus* and *P. granularis. Marine Biology* 49: 351-361.

Branch, G.M. 1979. Respiratory adaptations in the limpet *Patella granatina*: A comparison with other limpets. *Comparative Biochemistry and Physiology A* 62: 641-647.

Branch, G.M. 1981. The biology of limpets: Physical factors, energy flow, and ecological interactions. *Oceanography and Marine Biology, An Annual Review* 19: 235-380.

Branch, G.M. 1985. Limpets: their role in littoral and sublittoral community dynamics. Pages 97-116 In: *The Ecology of Rocky Coasts.* (Eds.) Moore, P.G. and Seed, R. Hodder and Stoughton, London.

Branch, G.M. and Cherry, M.I. 1985. Activity rhythms of the pulmonate limpet *Siphonaria capensis* Q. and G. as an adaptation to osmotic stress, predation and wave action. *Journal of Experimental Marine Biology and Ecology* 87: 153-168.

Branch, G.M. and Branch, M. 1988. The living shores of Southern Africa. Struik House, Cape Town, Pages 26-50.

Branch, G.M., Griffiths, C.L., Branch, M. and Beckley, L.E. 1994. Two Oceans, a guide to the marine life of southern Africa. David Philip, Cape Town and Johannesburg.

Brander, K., Blom, G., Borges, M.F., Erzini, K. and Henderson, G. 2003. Changes in fish distribution in the eastern North Atlantic: Are we seeing a coherent response to changing temperature? *ICES Marine Science Symposia* 219: 261-270.

Britton, J.C. 1995. The relationship between position on shore and shell ornamentation in two size-dependent morphotypes of *Littorina striata*, with an estimate of evaporative water loss in these morphotypes and in *Melarhaphe neritoides*. *Hydrobiologia* 309: 129-142.

Broekhuysen, G.J. 1940. A preliminary investigation of the importance of desiccation, temperature, and salinity as factors controlling the vertical distribution of certain intertidal marine gastropods in False Bay, South Africa. *Transactions of the Royal Society of South Africa* 28: 255-292.

Brown, A.C. and Jarman, N. 1978. Coastal marine habitats. Pages 1239–1277 In: *Biogeography and ecology of southern Africa.* (Eds.) Werger, M.J.A. and Van Bruggen, A.C. Springer, Netherlands.

Buckley, B.A., Owen, M.E. and Hofmann, G.E. 2001. Adjusting thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *The Journal of Experimental Biology* 204: 3571-3579.

Bustamante, R. and Branch, G. 1996. Large scale patterns and trophic structure of southern African rocky shores: the roles of geographic variation and wave exposure. *Journal of Biogeography* 23(3): 339-351.

Butler, P.J., Frappell, P.B., Wang, T. and Wikelski. M. 2002. The relationship between heart rate and oxygen consumption in Galapagos marine iguanas (*Amblyrhynchus cristatus*) at two different temperatures. *The Journal of Experimental Biology* 205: 1917-1924.

Butler, P.J., Green, J.A., Boyd, I.L. and Speakman, J.R. 2004. Measuring metabolic rate in the field: The pros and cons of the doubly labelled water and heart rate methods. *Functional Ecology* 18: 168-183.

Byrne, M., Selvakumaraswamy, P., Ho, M.A., Woolsey, E. and Nguyen, H.D. 2010. Sea urchin development in a global change hotspot, potential for southerly migration of thermotolerant propagules. *Deep Sea Research II* 58: 712-719.

Camacho, J., Qadri, S.A., Wang, H. and Worden, M.K. 2006. Temperature acclimation alters cardiac performance in lobster *Homarus americanus*. *Journal of Comparative Physiology Part A* 192: 1327-1334.

Campbell, G.S. and Norman, J.M. 1998. Radiation basics. *An introduction to environmental biophysics.* Springer, New York. Pages 147-165.

Cartwright, S.R. and Williams, G.A. 2012. Seasonal variation in utilization of biogenic microhabitats by littorinid snails on the tropical rocky shores. *Marine Biology* 159: 2323-2332.

Castilla, J.C., Lagos, N.A. and Cerda, M. 2004. Marine ecosystem engineering by the alien ascidian *Pyura praeputialis* on a mid-intertidal rocky shore. *Marine Ecology Progress Series 268*: 119-130.

Chambers, R.J. 1994. The conflict between adaptation and constraint: the case of the siphonariid limpets. PhD Thesis. Rhodes University, South Africa.

Chambers, R.J. and McQuaid, C.D. 1994. A review of larval development in the intertidal limpet genus *Siphonaria* (Gastropoda: Pulmonata). *Journal of Molluscan Studies* 60: 415-423.

Chapman, M.G. and Underwood, A.J. 1996. Influences of tidal conditions, temperature and desiccation on patterns of aggregation of the high-shore periwinkle, *Littorina unifasciata* in New South Wales, Australia. *Journal of Experimental Marine Biology and Ecology* 196: 213237.

Chapperon, C. and Seuront, L. 2011A. Behavioural thermoregulation in tropical gastropod: links to climate change scenarios. *Global Change Biology* 17: 1740-1749.

Chapperon, C. and Seuront, L. 2011B. Space-time variability in environmental thermal properties and snail thermoregulatory behaviour. *Functional Ecology* 25: 1040-1050.

Chapperon, C. and Seuront, L. 2012. Keeping warm in the cold: on the thermal benefits of aggregation behaviour in an intertidal ectotherm. *Journal of Thermal Biology* 37: 640-647.

Chapple, J.P., Smerdon, G.R. and Hawkins, A.J.S. 1997. Stress-70 protein induction in *Mytilus edulis*: responses to elevated temperature reflect relative vulnerability and physiological function. *Journal of Experimental Marine Biology and Ecology* 217: 225-235.

Chelazzi, G., Williams, G.A. and Gray, D.R. 1999. Field and laboratory measurements of heart rate in tropical limpet, *Cellana grata*. *Journal of Marine Biological Association of United Kingdom* 79: 749-751.

Chelazzi, G., De Pirro, M. and Williams, G.A. 2001. Cardiac responses to abiotic factors in two tropical limpets, occurring at different levels of the shore. *Marine Biology* 139: 1079-1085.

Clark, T.D., Jeffries, K.M., Hinch, S. G. and Farrell, A.P. 2011. Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *Journal of Experimental Biology* 214(18): 3074-3081.

Clark, T. D., Sandblom, E. and Jutfelt, F. 2013. Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *Journal of Experimental Biology* 216: 2771-2782.

Clarke, A.P., Mill, P.J. and Grahame, J. 2000A. The nature of heat coma in *Littorina littorea* (Mollusca: Gastropoda). *Marine Biology* 137: 447-451.

Clarke, A.P., Mill, P.J. and Grahame, J. 2000B. Biodiversity in *Littorina* species (Mollusca: Gastropoda): a physiological approach using heat-coma. *Marine Biology* 137: 559-565.

Coleman, N. 1973. The oxygen consumption of *Mytilus edulis* in air. *Comparative Biochemistry and Physiology A* 45: 393-402.

Comte, L. and Grenouillet, G. 2013. Do stream fish track climate change? Assessing distribution shifts in recent decades. *Ecography* 36: 001-011.

Compton, T.J., Rijkenberg, M.J.A., Drent, J. and Piersma, T. 2007. Thermal tolerances ranges and climate variability: a comparison between bivalves from different climates. *Journal of Experimental Marine Biology and Ecology* 352: 200-211.

Connell, J.H. 1972. Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics* 3: 169-192.

Cook, S.J. 2004. Sex-specific differences in cardiovascular performance of a centrarchid fish are only evident during the reproductive period. *Functional Ecology* 18: 398-403.

Cossins, A. R. and Bowler, K. 1987. Temperature Biology of Animals. Chapman and Hall, London.

Cottrell, A.J. 1911. Anatomy of *Siphonaria obliquata* (Sowerby). *Transactions and Proceedings of the New Zealand Institute* 43: 582-594.

Crear, B.J. and Forteath, G.N.R. 2000. The effect of extrinsic and intrinsic factors on oxygen consumption by the southern rock lobster, *Jasus edwardsii*. *Journal of Experimental Marine Biology and Ecology* 252: 129-147.

Dahlhoff, E.P., Stillman, J.H. and Menge, B.A. 2002. Physiological community ecology: variation in metabolic activity of ecologically important rocky intertidal invertebrates along environmental gradients. *Integrative and Comparative Biology* 42: 862-871.

Davenport, J. 1997. Comparisons of the biology of the intertidal sub Antarctic limpets *Nacella concinna* and *Kerguelenella lateralis*. *Journal of Molluscan Studies* 63(1): 39-48.

Davenport, J. and Davenport, J.L. 2005. Effect of shore height, wave exposure and geographical distance on thermal niche width of intertidal fauna. *Marine Ecology Progress Series* 292: 41-50.

Denny, M.W. and Harley, C.D.G. 2006. Hot limpets: predicting body temperature in a conductance-mediated thermal system. *Journal of Experimental Biology* 209: 2420-2431

Denny, M.W., Miller, L.P. and Harley, C.D. 2006. Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *Journal of Experimental Biology* 209(13): 2420-2431.

Denny, M.W., Dowd, W.W., Bilir, L. and Mach, K.J. 2011. Spreading the risk: Small-scale body temperature variations among intertidal organisms and its implications for species persistence. *Journal of Experimental Marine Biology and Ecology* 400: 175-190.

Depledge, M.H. and Andersen, B.B. 1990. A computer-aided physiological monitoring system for continuous, long-term recording of cardiac activity in selected invertebrates. *Comparative Biochemistry and Physiology A* 96: 473-477.

Deshpande, R.D. 1957. Observations on the anatomy and ecology of British trochids. PhD Thesis. University of Reading, United Kingdom.

Donelson, J.M., Munday, P.L., McCormick, M.I. and Pitcher, C.R. 2012. Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change* 2(1): 30-32.

Dong, Y., Dong, S. and Ji, T. 2008. Effect of different thermal regimes on growth and physiological performance of the sea cucumber *Apostichopus japonicas* Selenka. *Aquaculture* 275: 239-334.

Dong, Y. and Somero, G.N. 2009. Temperature adaptation of cytosolic Malate Dehydrogenases of limpets (genus *Lottia*): differences in stability and function due to minor changes in sequence correlates with biogeographic and vertical distributions. *The Journal of Experimental Biology* 212: 169-177.

Dong, Y.W. and Williams, G.A. 2011. Variation in cardiac performance and heat shock protein expression to thermal stress in two differently zoned limpets on a tropical shore. *Marine Biology* 158: 1223-1232.

Dong, Y.W., Han, G.D., Ganmanee, M. and Wang, J. 2015. Latitudinal variability of physiological responses to heat stress of the intertidal limpet *Cellana toreuma* along the Asian coast. *Marine Ecology Progress Series* 529: 107-119.

Dowd, W.W. and Somero, G.N. 2013. Behaviour and survival of *Mytilus* congeners following episodes of elevated body temperature in air and seawater. *Journal of Experimental Biology* 216: 502-514.

Dower, K.M. 1989. Sand inundation on rocky shores: Its effects on species richness and the structure of species assemblages. MSc Thesis. Rhodes University, South Africa.

Dye, A.H. 1987. Aerial and aquatic oxygen consumption in two siphonariid limpets (Pulmonata: Siphonariidae). *Comparative Biochemistry and Physiology* 87(3): 695-698.

Eekhout, S., Raubenheimer, C.M., Branch, G.M., Bosman, A.L. and Bergh, M.O. 1992. A holistic approach to the exploitation of intertidal stocks: limpets as a case study. *South African Journal of Marine Science* 12(1): 1017-1029.

Emanuel, B.P., Bustamante, R.H., Branch, G.M., Eekhout, S. and Odendaal, F.J. 1992. A zoogeographic and functional approach to the selection of marine reserves on the west coast of South Africa. *South African Journal of Marine Science* 12: 341-354.

Ern, R., Huong, D.T.T., Phuong, N.T., Wang, T. and Bayley, M. 2014. Oxygen delivery does not limit thermal tolerance in a tropical eurythermal crustacean. *Journal Experimental Biology* 217**:** 809–814.

Ern, R., Huong, D.T.T., Phuong, N.T., Madsen, P.T., Wang, T. and Bayley, M. 2015. Some like it hot: Thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. *Scientific Reports* 5: 10743.

Etter, R.L. 1988. Physiological stress and colour polymorphism in the intertidal snail *Nucella lapillus*. *Evolution* 42: 660-680.

Evans, R.G. 1948. The lethal temperatures of some common British littoral molluscs. *Journal of Animal Ecology* 17: 165-173.

Farrell, A.P., Hinch, S.G., Cooke, S.J., Patterson, D.A., Crossin, G.T., Lapointe, M. and Mathes, M.T. 2008. Pacific salmon in hot water: applying aerobic scope models and biotelemetry to predict the success of spawning migrations. *Physiological Biochemical Zoology* 81: 697-708.

Farrell, A.P. 2013. Aerobic scope and its optimum temperature: Clarifying their usefulness and limitations. *Journal of Experimental Biology* 216: 4493-4494.

Feare, C.J. 1971. The adaptive significance of aggregation behaviour in the dogwhelk *Nucella lapillus* (L.). *Oecologia* 7: 117-126.

Feder, M.E. and Hofmann, G.E. 1999. Heat-shock proteins, molecular chaperons, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243-282.

Femino, R. J. and Mathieson, A.C. 1980. Investigations of New England marine algae. 4. The ecology and seasonal succession of tide pool algae at bald head cliff, York, Maine, USA. *Botanica Marina* 23(5): 319-332.

Frederich, M. and Pörtner, H.O. 2000. Oxygen of thermal tolerance defined by cardiac and ventilator performance in spider crab, *Maja squinado*. *American Journal of Physiological Regulatory Integrative Comparative Physiology* 279: R1531-R1538.

Fretter, V. and Graham. A. 1962. British prosobranch molluscs. Ray Society, London.

Fretter, V. and Peake, J. 1975. Pulmonates. Volume 1. Functional Anatomy and Physiology. Academic Press, London.

Finke, G.R., Navarrete, S.A. and Bozinovic, F. 2007. Tidal regimes of temperate coasts and their influences on aerial exposure for intertidal organisms. *Marine Ecology Progress Series* 343: 57-62.

Finke, G.R., Bozinovic, F. and Navarrete, A.S. 2009. A mechanistic model to study the thermal ecology of a south eastern Pacific dominant intertidal mussel and implications for climate change. *Physiological and Biological Zoology* 82: 303-313.

Finney, D. J. and Stevens, W.L. 1948. A table for the calculation of working probits and weights in probit analysis. *Biometrika* 35(1-2): 191-201.

Finney, D.J. 1952. Probit Analysis. Cambridge University Press, Cambridge.

Firth, L.B. and Williams, G.A. 2009. The influence of multiple environmental stressors on the limpet *Cellana toreuma* during the summer monsoon season in Hong Kong. *Journal of Experimental Marine Biology and Ecology* 375(1): 70-75.

Fitzhenry, T., Halpin, P.M. and Helmuth, B. 2004. Testing the effects of wave exposure, site and behaviour on intertidal mussel body temperatures: Applications and limits of temperature loggers. *Marine Biology* 145: 339-349.

Ganning, B. 1971. Studies on chemical, physical and biological conditions in Swedish rock pool ecosystems. *Ophelia* 9(1): 51-105.

Garrity, S.D. 1984. Some adaptations of gastropods to physical stress on a tropical rocky shore. *Ecology* 65: 559-574.

Giomi, F. and Pörtner, H.O. 2013. A role for haemolymph oxygen capacity in heat tolerance of eurythermal crabs. *Frontiers in physiology* 4: 110.

Gräns, A., Jutfelt1, F., Sandblom, E., Jönsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Einarsdottir, I., Björnsson, B.T., Sundell, K. and Axelsson, M. 2014. Aerobic scope fails to explain the detrimental effects on growth resulting from warming and elevated $CO_2$ in Atlantic halibut. *Journal of Experimental Biology* 217**:** 711-717.

Gray, D.R. and Hodgson, A.N. 1998. Foraging and homing behaviour in the high-shore, crevice-dwelling limpet *Helcion pectunculus* (Prosobranchia: Patellidae). *Marine Biology* 132(2): 283-294.

Grebmeier, J.M., Overland, J.E., Moore, S.E., Farley, E.V., Carmack, E.C., Cooper, L.W., Frey, K.E., Helle, J.H., McLaughlin, F.A. and McNutt, S.L. 2006. A major ecosystem shift in the northern Bering Sea. *Science* 311(5766): 1461-1464.

Griffiths, H.J. 2010. Antarctic marine biodiversity–what do we know about the distribution of life in the Southern Ocean? *Plos one 5*(8): e11683.

Gründlingh, M.L. 1983. On the course of the Agulhas Current. *South African Journal of Geology* 65: 49-57.

Guderley, H. and St-Pierre, J. 2002. Going with the flow or life in the fast lane: contrasting mitochondrial responses to thermal change. *The Journal of Experimental Biology* 205: 22372249.

Hamdoun, A.M., Cheney, D.P. and Cherr, G.N. 2003. Phenotypic plasticity of HSP and HSP70 gene expression in the Pacific oyster (*Crassostrea gigas*): Implications for resting thermal limits and induction of thermal tolerance. *Biological Bulletin* 205: 160-169.

Han, G., Zhang, S. and Dong, Y., 2016. Anaerobic metabolism and thermal tolerance: the importance of opine pathways on survival of a gastropod after cardiac dysfunction. *Integrative Zoology* DOI: 10.1111/1749-4877.12229.

Hanekom, N., Randall, R.M., Bower, D., Riley, A. and Kruger, N. 2009. Garden Route National Park: The Tsitsikamma Area – State of Knowledge. South African National Parks.

Harley, C.D.G. and Helmuth, B.S.T. 2003. Local- and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. *Limnology and Oceanography* 48: 1498-1508.

Harley, C.D. 2008. Tidal dynamics, topographic orientation, and temperature-mediated mass mortalities on rocky shores. *Marine Ecology Progress Series* 371: 37-46.

Harley, C.D.G., Denny, M.W., Mach, K.J. and Miller, L.P. 2009. Thermal stress and morphological adaptations in limpets. *Functional Ecology* 23: 292-301.

Harper, K.D. and Williams, G.A. 2001. Variation in abundance and distribution of the chiton *Acanthopleura japonica* and associated molluscs on a seasonal, tropical, rocky shore. *Journal of Zoology* 253(3): 293-300.

Hawkins, S.J. and Hartnoll, R.G. 1983. Grazing of intertidal algae by marine invertebrates. *Oceanography and Marine Biology, An Annual Review* 21: 195-282.

Healy, T.M. and Schulte, P.M. 2012. Thermal acclimation is not necessary to maintain a wide thermal breadth of aerobic scope in the common killifish *Fundulus heteroclitus*. *Physiological and Biochemical Zoology* 85: 107-119.

Heath, J.E. 1970. Behavioural regulation of body temperature in poikilotherms. *The Physiologist 13*(4): 399-410.

Helm, M.M. and Trueman, E.R. 1967. The effect of exposure on the heart rate of the mussel, *Mytilus edulis* L. *Comparative Biochemistry and Physiology* 21: 171-177.

Helmuth, B. 1998. Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. *Ecological Monographs* 68: 29-52.

Helmuth, B. 1999. Thermal biology of rocky intertidal mussels: quantifying body temperatures using climatological data. *Ecology* 80(1): 15-34.

Helmuth, B. and Denny, M.W. 1999. Measuring scales of physical stress in the rocky intertidal. *American Zoologist.* 39: 114A (abstract).

Helmuth, B. and Hofmann, G.E. 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in rocky intertidal zone. *Biological Bulletin* 201: 374-384.

Helmuth, B. 2002. How do we measure the environment? Linking intertidal thermal physiology and ecology through biophysics. *Integrative and Comparative Biology* 42(4): 837-845.

Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E. and Blanchette, C.A. 2002. Climate change and latitudinal patterns of intertidal thermal stress. *Science* 298: 1015-1017.

Helmuth, B., Broitman, B.R., Blanchette, C.A., Gilman, S., Halpin, P., Harley, C.D.G., O'Donnell, M.J., Hofmann, G.E., Menge, B. and Strickland, D. 2006. Mosaic patterns of thermal stress in the rocky intertidal zone: implications for climate change. *Ecological Monographs* 76: 461-476.

Hicks, D.W. and McMahon, R.F. 2002. Respiratory responses to temperature and hypoxia in the nonindigenous brown mussel, *Perna perna* (Bivalvia: Mytilidae), from the Gulf of Mexico. *Journal of Experimental Marine Biology* 277: 61-78.

Hill, J. and Wilkinson, C. 2004. Methods for ecological monitoring of coral reefs. Australian Institute of Marine Science, Townsville.

Hirano, Y. 1979. Studies on activity pattern of the patellid limpet *Cellana toreuma* (Reeve). *Journal of Experimental Marine Biology and Ecology* 40(2): 137-148

Hodgson, A.N. 1999. The biology of Siphonariid limpets (Gastropoda Pulmonata). *Oceanography and Marine Biology, An Annual Review* 37: 245-314.

Horowitz, M. 2001. Heat acclimation: phenotypic plasticity and cues to the underlying molecular mechanisms. *Journal of Thermal Biology* 26: 357-363.

Horowitz, M. 2002. From molecular and cellular to integrative heat defence during exposure to chronic heat. *Comparative Biochemistry and Physiology A* 131: 475-483.

Houlihan, D.F. 1979. Respiration in air and water of three mangrove snails. *Journal of Experimental Marine Biology and Ecology* 41: 143-161.

Huang, X., Wang, T., Ye, Z., Han, G. and Dong, Y. 2015. Temperature relations of aerial and aquatic physiological performance in a mid-intertidal limpet *Cellana toreuma*: Adaptation to rapid changes in thermal stress during emersion. *Integrative zoology* 10(1): 159-170.

Hubendick, B. 1978. Systematics and comparative morphology of the Basommatophora. Pages 1-48. In: *Pulmonates.* (Eds.) Fretter, V. and Peake, J. Academic Press, London.

Huggett, J. and Griffiths, C.L. 1986. Some relationships between elevation, physico-chemical variables and biota of intertidal rock pools. *Marine Ecology Progress Series* 29: 189-197.

Hughes, R. N. 1971. Ecological energetics of the keyhole limpet *Fissurella barbadensis* Gmelin. J. *Journal of Experimental Marine Biology and Ecology* 6: 167-178.

Huxham, M., Maitland, D. and Mocogni, M. 2001. Respiration rates in *Littorina littorea* infected with three species of digenean parasite. *Journal of the Marine Biological Association of the United Kingdom* 81: 351-352.

Innes, A.J. 1982. Respiration in air and water of intertidal gastropods. PhD Thesis. University of Aberdeen, United Kingdom.

Innes, A.J., Marsden, I.D. and Wong, P.P.S. 1984. Bimodal respiration of intertidal pulmonates. *Comparative Biochemistry and Physiology Part A: Physiology*: 77(3): 441-445.

Innes, A.J. and Houlihan. D.F. 1985. Aquatic and aerial oxygen consumption of cool temperate gastropods: A comparison with some Mediterranean species. *Comparative Biochemistry and Physiology A* 82: 105-109.

IPCC, 2012: Changes in climate extremes and their impacts on the natural physical environment. *Managing the risks of extreme events and disasters to advance climate change adaptation.*

IPCC, 2014: Climate Change 2014: Synthesis Report. *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.*

Ivanina, A.V., Taylor, C. and Sokolova, I.M. 2009. Effects of elevated temperature and cadmium exposure on stress protein response in eastern oyster *Crassostrea virginica* (Gmelin). *Aquatic Toxicology* 91: 245-254.

Jakob, E.M., Porter, A.H. and Uetz, G.W. 2001. Site fidelity and the costs of movement among territories: an example from colonial web-building spiders. *Canadian Journal of Zoology* 79(11): 2094-2100.

Jimenez, A.G., Jayawardene, S., Alves, S., Dallmer, J. and Dowd, W.W. 2015. Micro-scale environmental variation amplifies physiological variation among individual mussels. *Proceedings of the Royal Society of London. B* 282: 20152273.

Jones, S.J., Mieszkowska, N. and Wethey, D.S. 2009. Linking thermal tolerances and biogeography: *Mytilus edulis* (L.) at its southern limit on the east coast of the United States. *Biological Bulletin* 217: 73-85.

Jost, J.A., Podolski, S.M. and Frederich, M. 2012. Enhancing thermal tolerance by eliminating the pejus range: a comparative study with three decapod crustaceans. *Marine Ecology Progress Series* 444: 263-274.

Judge, M.L., Botton, M.L. and Hamilton, M.G. 2011. Physiological consequences of the supralittoral fringe: microhabitat temperature profiles and stress protein levels in the tropical periwinkle *Cenchritis muricatus* (Linneaus, 1758). *Hydrobiologia* 675: 143-156.

Jutfelt, F., Grӓns, A., Jӧnsson, E., Wiklander, K., Seth, H., Olsson, C., Dupont, S., Ortega-Martinez, O., Sundell, K., Axelsson, M. and Sandblom, E. 2014. Response to ‘How and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope-remarks on the article by Grӓns *et al.*’ *Journal of Experimental Biology* 217(24): 44334435.

Kalk, M. 1959. A general ecological survey of some shores in northern Mozambique. *Revista Brasileira de Biologia* 2: 1-24.

Kilburn, R, and Rippey, E. 1982. Sea shells of southern Africa. Macmillan South Africa, Johannesburg.

Kitting, C.L. 1980. Herbivore_ plant interactions of individual limpets maintaining a mixed diet of intertidal marine algae. *Ecological Monographs* 50(4): 527-550.

Lang, R.C., Britton, J.C. and Metz, T. 1998. What to do when there is nothing to do: the ecology of Jamaican intertidal Littorinidae (Gastropoda: Prosobranchia) in repose. *Hydrobiologia* 378: 161-185.

Laing and Child, 1996. Comparative tolerance of small juvenile palourdes (*Tapes decussatus L.*) and Manila clams (*Tapes philippinarum* Adams and Reeve) to low temperature. *Journal of Experimental Marine Biology and Ecology* 195: 267-285.

Lasiak, T. 1993. Temporal and spatial variations in exploited and non-exploited populations of the intertidal limpet *Cellana capensis*. *Journal of Molluscan Studies* 59(3): 295-307.

Lee, H.J. and Boulding, E.G. 2010. Latitudinal cline in body size, but not in thermal tolerance or heat-shock cognate (Hsc70), in the highly-dispersing intertidal gastropod *Littorina keenae* (Gastropoda: *Littorinidae*). *Biological Journal of the Linnean Society* 100: 494-505.

Lewis, J.B. 1963. Environmental and tissue temperature of some tropical intertidal marine animals. *Biological Bulletin* 124: 277-284.

Lewis, J.B. 1971. Comparative respiration of some tropical intertidal gastropods. *Journal of Experimental Marine Biology and Ecology* 6: 101-108.

Lima, F.P. and Wethey, D.S. 2009. Robolimpets: measuring intertidal body temperatures using biomimetic loggers. *Limnology and Oceanography: Methods* 7: 347-353.

Lima, F.P., Burnett, N.P., Helmuth, B., Kish, N., Aveni-Deforge, K. and Wethey, D.S. 2011. Monitoring the intertidal environment with Biomimetic Devices. Pages 499-522 In: *Biomimetic Based Applications.* (Ed.) Cavrak, M. InTech Publishing, Vienna.

Lima, F.P., Gomes, F., Seabra, R., Wethey, D.S., Seabra, M.I., Cruz, T., Santos, A.M. and Hilbish, T.J., 2016. Loss of thermal refugia near equatorial range limits. *Global Change Biology*, *22*(1): 254-263.

Little, C. 1989. Factors governing patterns of foraging activity in littoral marine herbivorous molluscs. *Journal of Molluscan Studies* 55: 273-284.

Little, C., Williams, G.A. and Trowbridge, C.D. 2009. The biology of rocky shores, Oxford University Press, New York.

Liu, J.H. and Morton, B. 1994. The temperature tolerances of *Tetraclita squamosa* (Crustacea: Cirripedia) and *Septifer virgatus* (Bivalvia: Mytilidae) on a sub-tropical rocky shore in Hong Kong. *Journal of Zoology* 234(2): 325-339.

Logan, C.A., Kost, L.E. and Somero, G.N. 2012. Latitudinal differences in *Mytilus californianus* thermal physiology. *Marine Ecology Progress Series* 450: 93–105.

Lord, J.P., Lyczkowski, E.R. and Wilson, W.H. 2011. Behaviour and microhabitat selection of the tortoiseshell limpet Testudinalia testudinalis in the northwest Atlantic intertidal zone. *Journal of Experimental Marine Biology and Ecology* 407(2): 234-240.

Lutjeharms, J.R.E. 1998. Coastal hydrography. Pages 50–61. In: *Field Guide to the Eastern and Southern Cape Coasts.* (Eds.) Lubke, R. and De Moor, I. University of Cape Town Press, Cape Town.

Machin, J. 1975. Water relationships. Pages 105-164 In: *Pulmonates 1 Functional Anatomy and Physiology.* (Eds.) Fretter, V. and Peake, J. Academic Press, London.

Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C. and Diniz, M.S. 2012B. Thermal tolerance of the crab *Pachygrapsus marmoratus*: intraspecific differences at the physiological (CTMax) and molecular level (Hsp70). *Cell Stress and Chaperons* 17: 707-716.

Mak, Y.M. 1996. The ecology of high-zoned littorinids, *Nodilittorina trochoides, N. radiata* and *N. vidua* on rocky shores in Hong Kong. PhD Thesis. The University of Hong Kong, Hong Kong.

Marcus, E. and Marcus, E. 1960. On *Siphonaria hispida. Bulletin of the Faculty of Philosophy, Sciences and Letters, University of São Paulo. Botany* 23: 107-140.

Marker, M.E. 1988. Geology and geomorphology. Pages 9-18 In: *A field guide to the eastern Cape coast.* (Eds.) Lubke, R.A. Gess, F.W. and Bruton, M.N. Grahamstown centre of the Wildlife Society of Southern Africa, Grahamstown.

Marshall, B.A. 1981. The genus *Williamia* in the western Pacific (Mollusca: Siphonariidae). *New Zealand Journal of Zoology* 8(4): 487-492.

Marshall, D.J. and McQuaid, C.D. 1991. Metabolic rate depression in marine pulmonate snail: pre-adaptation for terrestrial existence? *Oecologia* 88: 274-276.

Marshall, D.J. and McQuaid, C.D. 1992A. Comparative aerial metabolism and water relations of the intertidal limpets *Patella granularis* L. (Mollusca: Prosobranchia) and *Siphonaria oculus* Kr. (Mollusca: Pulmonate). *Physiological Zoology* 65: 1040-1056.

Marshall, D.J. and McQuaid, C.D. 1992B. Relationship between heart rate and oxygen consumption in the intertidal limpets *Patella granularis* and *Siphonaria oculus. Comparative Biochemistry and Physiology A* 103: 297-300.

Marshall, D.J. and McQuaid, C.D. 1994. Seasonal and diel variation of in situ heart rate of the intertidal limpet *Siphonaria oculus* Kr. (Pulmonata). *Journal of Experimental Marine Biology and Ecology* 179: 1-9.

Marshall, D.J., Dong, Y.W., McQuaid, C.D. and Williams, G.A. 2011. Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. *The Journal of Experimental Biology* 214: 3649-3657.

Marshall, D.J. and Chua, T. 2012. Boundary layer convective heating and thermoregulatory behaviour during aerial exposure in the rock eulittoral fringe snail *Echinolittorina malaccana*. *Journal of Experimental Marine Biology and Ecology* 430-431: 25-31.

Martin, T.L. and Huey, R.B. 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *The American Naturalist* 171(3): E102-E118.

Matumba, T.G. 2013. Genetics and thermal biology of littorinid snails of the genera *Afrolittorina*, *Echinolittorina* and *Littorina* (Gastropoda: Littorinidae) from temperate, subtropical and tropical Regions. PhD Thesis. Rhodes University, South Africa.

McMahon, R.F. and Russell-Hunter, W.D. 1977. Temperature relations of aerial and aquatic respiration in six littoral snails in relation to their vertical zonation. *The Biological Bulletin* (Laboratory, Woods Hole, Mass) 152: 182-198.

McMahon, R.F. and Russell-Hunter, W.D. 1981. The effects of physical variables and acclimation on survival and oxygen consumption in the high littoral salt-marsh snail, *Melampus bidentatus*. *The Biological Bulletin* (Laboratory, Woods Hole, Mass) 161: 246-269.

McMahon, R.F. 1983. Physiological ecology of freshwater pulmonates. Pages 359-430 In: *The Mollusca 6 Ecology*. (Ed.) Russell-Hunter, W.D. Academic Press, London.

McMahon, R.F. 1988. Respiratory response to periodic emergence in intertidal molluscs. *American Zoology* 28: 99-114.

McMahon, R.F. 1990. Thermal tolerance, evaporative water loss, air-oxygen consumption and zonation of intertidal prosobranchs: a new synthesis. *Hydrobiologia* 193: 241-260.

McMahon, B.R. 1999. Intrinsic and extrinsic influences on cardiac rhythms in crustaceans. *Comparative Biochemistry and Physiology A* 124: 539-547.

McQuaid, C.D., Cretchley, R. and Rayner, J.L. 1999. Chemical defence of the intertidal pulmonate limpet *Siphonaria capensis* (Quoy and Gaimard) against natural predators. *Journal of Experimental Marine Biology and Ecology* 237(1): 141-154

Menconi, M., Benedetti-Cecchi, L. and Cinelli, F. 1999. Spatial and temporal variability in the distribution of algae and invertebrates on rocky shores in the northwest Mediterranean. *Journal of Experimental Marine Biology and Ecology* 233(1): 1-23.

Micallef, H. 1967. Aerial and aquatic respiration of certain trochids. *Experimentia* 23: 52.

Micallef, H. and Bannister, W.H. 1967. Aerial and aquatic oxygen consumption of *Monodonta turbinata* (Mollusca: Gastropoda). *Journal of Zoology (London)* 151: 479-482.

Miller, L.P., Harley, C.D.G. and Denny, M.W. 2009. The role of temperature and desiccation stress in limiting the local-scale distribution of the owl limpet, *Lottia gigantea*. *Functional Ecology* 23: 756-767.

Mora, C. and Maya, M.F. 2006. Effect of the rate of temperatures increases of the dynamic method on the heat tolerance of fishes. *Journal of Thermal Biology* 31: 337-341.

Morley, S.A., Hirse, T., Pörtner, H.O. and Peck, L.S. 2009. Geographical variation in thermal tolerance within Southern Ocean marine ectotherms. *Comparative Biochemistry and Physiology A* 153: 154-161.

Morris, S. and Taylor, A.C. 1983. Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science* 17(3): 339-355.

Morris, D.W. 1987. Ecological scale and habitat use. *Ecology* 68(2): 362-369.

Munday, P.L., Crawley, N.E. and Nilsson, G.E. 2009. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series* 388: 235-242.

Muñoz, J.L.P., Finke, G.R., Camus, P.A. and Bozinovic, F. 2005. Thermoregulatory behaviour, heat gain and thermal tolerance in the periwinkle *Echinolittorina peruviana* in central Chile. *Comparative Biochemistry and Physiology Part A* 142: 92-98.

Newell, G.E. 1964. Physiological aspects of the ecology of intertidal molluscs. *Physiology of mollusca* 1: 59-81.

Newell, R.C. 1979. Biology of intertidal animals. Marine Ecological Survey, Faversham, United Kingdom.

Neuheimer, A.B., Thresher, R.E., Lyle, J.M. and Semmens, J.M. 2011. Tolerance limit for fish growth exceeded by warming waters. *Nature Climate Change* 1(2): 110-113.

Nguyen, K.D.T., Morley, S.A., Lai, C.H., Clark, M.S., Tan, K.S., Bates, E.A. and Peck, L.S. 2011. Upper temperature limits of tropical marine ectotherms: global warming implications. *Plos One* 6: 1-8.

Norin, T., Malte, H. and Clark, T.D. 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *Journal of Experimental Biology* 217: 244-251.

Nuwayhid, M.A., Davies, P.S. and Elder, H.Y. 1978. Gill structure in the common limpet *Patella vulgata*. *Journal of the Marine Biological Association of the United Kingdom* 58(04): 817-823.

Olabarria, C., Underwood, A. and Chapman, M. 2002. Appropriate experimental design to evaluate preferences for microhabitat: an example of preferences by species of microgastropods. *Oecologia* 132(2): 159-166.

Ospina, A.F. and Mora, C. 2004. Effect of body size on reef fish tolerance to extreme low and high temperatures. *Environmental Biology of Fishes* 70: 339-343.

Overgaard, J., Andersen, J.L., Findsen, A., Pedersen, P.B.M., Hansen, K., Ozolina, K. and Wang, T. 2012. Aerobic scope and cardiovascular oxygen transport is not compromised at high temperatures in the toad *Rhinella marina*. *Journal of Experimental Biology* 215: 3519-3526.

Paine, R.T. 1969. The *Piaster-Tegula* interaction: prey patches, predator food preference, and intertidal community structure. *Ecology* 50: 950-961.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637-669.

Peck, L.S. and Veal, R. 2001. Feeding, metabolism and growth in the Antarctic limpet, *Nacella concinna* (Strebel, 1908). *Marine Biology* 138: 553-560.

Peck, L.S., Pörtner, H.O. and Hardewig, I. 2002. Metabolic demand, oxygen supply, and critical temperatures in the Antarctic bivalve *Laternula elliptica*. *Physiological and Biochemical Zoology* 75: 123-133.

Peck, L.S., Morley, S.A., Pörtner, H.O. and Clark, M.S. 2007. Thermal limits of burrowing capacity are linked to oxygen availability and size in the Antarctic clam *Laternula elliptica*. *Oecologia* 154: 479-484.

Perry, A.L., Low, P.J., Ellis, J.R. and Reynolds, J.D. 2005. Climate change and distribution shifts in marine fishes. *Science* 308: 1912-1915.

Ponder, W. and Lindberg, D.R. 2008. Phylogeny and Evolution of the Mollusca. University of California Press, California.

Pörtner, H.O. and Zielinski, S. 1998. Environmental constraints and the physiology of performance in squids. *African Journal of Marine Science* 20: 207-221.

Pörtner, H.O. Reipschläger, A. and Heisler, N. 1998. Acid-base regulation, metabolism and energetics in *Sipunculus nudus* as a function of ambient carbon dioxide level. *Journal of Experimental Biology* 201.1: 43-55.

Pörtner, H.O., Peck, L., Zielinski, S. and Conway, L.Z. 1999. Intracellular pH and energy metabolism in the highly stenothermal Antarctic bivalve *Limopsis marionensis* as a function of ambient temperature. *Polar Biology* 22: 17-30.

Pörtner, H.O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* 88: 137-146.

Pörtner, H. O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., and Knust, R. 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). *Continental Shelf Research* 21: 1975-1997.

Pörtner, H.O. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology A* 132: 739-761.

Pörtner, H.O., Bennett, A.F., Bozinovic, F., Clarke, A., Lardies, M.A., Lucassen, M., Pelster, B. and Stillman, J.H. 2006. Trade-offs in thermal adaptations: the need for a molecular to ecological integration. *Physiological and Biochemical Zoology* 79: 295-313.

Pörtner, H.O. and Knust, R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315: 95-97.

Pörtner, H.O. and Farrell, A.P. 2008. Ecology. Physiology and climate change. *Science* 322: 690-692.

Pörtner, H.O. 2010. Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate related stressor effects in marine ecosystems. *Journal of Experimental Biology* 213: 881-893.

Pörtner, H.O. 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Marine Ecology Progress Series* 470: 273-290.

Pörtner, H.O. and Giomi, F. 2013. Nothing in experimental biology makes sense except in the light of ecology and evolution. *Journal of Experimental Biology* 216: 4494-4495.

Pörtner, H.O. 2014. How to and how not to investigate the oxygen and capacity limitation of thermal tolerance (OCLTT) and aerobic scope. *Journal of Experimental Biology* 217: 44324433.

Powell, A.W.B., 1973. The patellid limpets of the world (Patellidae). Indo-Pacific Mollusca, Vol, 3: Pages 75-206.

Purchon, R.D. 1977. The biology of the Mollusca. Pergamon Press, Weinheim.

Randall, D.J., Burggren, W.W., Farrell, A.P. and Haswell, M.S. 1981. The Evolution of Air Breathing in Vertebrates. Cambridge University Press, Cambridge.

Raimondi, P.T. 1990. Patterns, mechanisms, consequences of variability in settlement and recruitment of an intertidal barnacle. *Ecological Monographs* 60(3): 283-309.

Riddle, W.A. 1983. Physiological ecology of land snails and slugs. Pages 431-461 In: *The Mollusca 6 Ecology*. (Ed.) Russell-Hunter, W.D. Academic Press, London.

Ridgway, S.A., Reid, D.G., Taylor, J.D., Branch, G.M. and Hodgson, A.N. 1998. A cladistic phylogeny of the family *Patellidae* (Mollusca: Gastropoda). *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 353(1375): 1645-1671.

Roberts, D.A., Hofmann, G.E. and Somero, G.N. 1997. Heat-shock protein expression in *Mytilus californianus*: Acclimatization (seasonal and tidal height comparisons) and acclimation effects. *Biological Bulletin* 192: 309-320.

Robles, C.D., Desharnais, R.A., Garza, C., Donahue, M.J., and Martinez, C.A. 2009. Complex equilibria in the maintenance of boundaries: experiments with mussel beds. *Ecology* 90(4): 985-995.

Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., Menzel, A., Root, T.L., Estrella, N., Seguin, B. and Tryjanowski, P. 2008. Attributing physical and biological impacts to anthropogenic climate change. *Nature* 453(7193): 353-357.

Santina, P.D. 1994. Homing pattern, activity area and trail following of the high shore Mediterranean limpet *Patella rustica* L. (Mollusca Gastropoda). *Ethology ecology and evolution* 6(1): 65-73.

Santini, G. and Chelazzi, G. 1995. Glycogen content and rates of depletion in two limpets with different foraging regimes. *Comparative Biochemistry and Physiology* 111: 271–277.

Santini, G., De Pirro, M. and Chelazzi, G. 1999. In situ and laboratory assessment of heart rate in Mediterranean limpet using a non-invasive technique. *Physiological and Biochemical Zoology* 72: 198-204.

Schmidt, P.S., Phifer-Rixey, M., Taylor, G.M. and Christner, J. 2007. Genetic heterogeneity among intertidal habitats in the flat periwinkle, *Littorina obtusata*. *Molecular Ecology* 16: 2393-2404.

Schmidt-Nielsen, K. 1997. Animal physiology: adaptation and environment. Cambridge University Press, Cambridge.

Schumann, E.H. and Beckman, L.J. 1984. Ocean temperature structures on the Agulhas Bank. *Transactions of the Royal Society of South Africa* 45: 191-203.

Schumann, E.H. 1987. The coastal ocean off the east coast of South Africa. *Transactions of the Royal Society of South Africa* 46: 215-229.

Seabra, R., Wethey, D.S., Santos, A.M. and Lima, F.P. 2011. Side matters: microhabitat influence on intertidal heat stress over a large geographical scale. *Journal of Experimental Marine Biology and Ecology* 400(1): 200-208.

Seebacher, F. and Franklin, C.E. 2005. Physiological mechanisms of thermoregulation in reptiles: a review. *Journal of Comparative Physiology B 175*(8): 533-541.

Shanks, A.L. 2002. Previous agonistic experience determines both foraging behaviour and territoriality in the limpet *Lottia gigantea* (Sowerby). *Behavioural Ecology* 13(4) 467-471.

Shannon, L.V. 1985. The Benguela ecosystem Part I: Evolution of the Benguela, physical features and processes. *Oceanography and Marine Biology - An Annual Review* 23: 105-182.

Shannon, L.V. and Nelson, G. 1996. The Benguela: Large-scale features and process and system variability. 644 p. In: *The South Atlantic: Present and past circulation*. (Eds.) Wefer, G., Berger, W.H., Siedler, G. and Webb, D.J. Springer-Verlag, Berlin.

Shillington, F.A. and Harris, T.F.W. 1978. Surface waves near Cape Town and their associated atmospheric pressure distributions over the South Atlantic. *Ocean Dynamics* 31: 67-82.

Shumway, S.E. 1981. Factors effecting oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *Biological Bulletin* (Laboratory, Woods Hole, Mass) 159: 332-347.

Shumway, S.E. and Marsden, I.D. 1982. The combined effects of temperature, salinity, and declining oxygen tension on oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *Journal of Experimental Marine Biology and Ecology* 61: 133-146.

Shumway, S.E., Lesser, M.P. and Crisp, D.J. 1993. Specific dynamic action demonstrated in the herbivorous marine periwinkle, *Littorina littorea* and *Littorina obtusata* (Mollusca: Gastropoda). *Comparative Biochemistry and Physiology A* 106: 391-395.

Sinclair, E.L.A., Thompson, M.B. and Seebacher, F. 2006. Phenotypic flexibility in the metabolic response of the limpet *Cellana tramoserica* to thermally different microhabitats. *Journal of Experimental Marine Biology and Ecology* 335: 131-141.

Sokolova, I.M. and Berger, V.J. 2000. Physiological variation related to shell colour polymorphism in White Sea *Littorina saxatilis*. *Journal of Experimental Marine Biology and Ecology* 245: 1-23.

Sokolova, I.M. and Pörtner, H.O. 2001. Physiological adaptations to high intertidal life involve improved water conservation abilities and metabolic rate depression in *Littorina saxatilis*. *Marine Ecology Progress Series* 224: 171-186.

Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G. and Sukhotin, A.A. 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research* 79: 1-15.

Somero, G.N. 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integrative Composition Biology* 42: 780-789.

Somero, G.N. 2005. Linking biogeography to physiology: evolutionary and acclamatory adjustments of thermal limits. *Frontiers in Zoology* 21: 1-14.

Somero, G.N. 2010. The physiology of climate change: how potential for acclimatization and genetic adaptation will determine "winners" and "losers." *The Journal of Experimental Biology* 213: 912-920.

Somero, G.N. 2011. Comparative physiology: a "crystal ball" for predicting consequence of global change. *American Journal of Physiology – Regulatory, Integrative and Composition Physiology* 301: R1-R14.

Somero, G.N. 2012. The physiology of global change: linking patterns and mechanisms. *Annual Review of Marine Science* 4: 39-61.

Soto, R.E. and Bozinovic, F. 1998. Behavioural thermoregulation of the periwinkle *Nodilittorina peruviana* inhibiting the rocky intertidal of central Chile: laboratory and field study. *Revista Chilena de Historia Natural* 7: 375-382.

South African Navy Hydrographic Office. 2017. South African Navy, accessed 12/11/2015. <http://www.sanho.co.za/tides/tide_index.htm>

Stafford, R. and Davies, M.S. 2004. Temperature and desiccation do not affect aggregation behaviour in high shore littorinids in north-east England. *Journal of Negative Results: Ecology and Evolutionary Biology* 1: 16-20.

Stafford, R., Davies, M.S. and Williams, G.A. 2007. Computer simulations of high shore littorinids predict small-scale spatial and temporal distribution patterns on rocky shores. *Marine Ecology Progress Series* 342: 151-161.

Stafford, R., Davies, M.S. and Williams, G.A. 2008. Self-organization of intertidal snails facilitates evolution of aggregation behaviour. *Artificial Life* 14: 409-423.

Stegenga, H. and Bolton, J.J. 1992. *Ceramiaceae* (Rhodophyta) of the Cape Province, South Africa: Distribution in relation to concepts of marine provinces. *Botanica marina* 35(2): 99108.

Stenseng, E. 2005. Thermal tolerance limits of heart function in *Tugela* snail congeners of the marine intertidal. *Marine Biology*, <http://web.stanford.edu/group/journal/cgibin/wordpress/wpcontent/uploads/2012/09/Stenseng_NatSci_2005.pdf>

Stenseng, E. Braby, C.E. and Somero, G.N. 2005. Evolutionary and acclimation induced variation in the thermal limits of heart function in congeneric marine snails (Genus *Tugela*): implications for vertical zonation. *Biological Bulletin* 208: 138-144.

Stephenson, T.A. and Stephenson, A. 1949. The universal features of zonation between tidemarks on rocky coasts. *The Journal of Ecology* 289-305.

Stephenson, T. A. and Stephenson, A. 1972. Life between tidemarks on rocky shores. Freeman Press, San Francisco.

Stillman, J.H. and Somero, G.N. 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. *The Journal of Experimental Biology* 199: 1845-1855.

Stillman, J.H. 2002. Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus *Petrolisthes. Integrative and Comparative Biology* 42: 790-796.

Stillman, J.H. 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301: 65.

Sommer, A., Klein, B. and Pörtner, H.O. 1997. Temperature induced anaerobiosis in two populations of the polychaete worm *Arenicola marina* (L.). *Journal of Comparative Physiology B* 167(1): 25-35.

Southward, A.J. 1958. Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution. *Journal of the Marine Biological Association of the United Kingdom 37*(01): 49-66.

Storey, K.B. and Storey, M.S. 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *The Quarterly Review of Biology* 65: 145-174.

Storey, K.B. 1998. Survival under stress: molecular mechanisms of metabolic rate depression in animals. *South African Journal of Zoology* 33: 55-64.

SWAT coastal biodiversity survey protocol. 2011. University of California, Santa Cruz, accessed 21/11/2016.
<http://www.eeb.ucsc.edu/pacificrockyintertidal/biodiversityprotocol.pdf>

Tagliarolo, M. and McQuaid, C.D. 2015. Sub-lethal and sub-specific temperature effects are better predictors of mussel distribution than thermal tolerance. *Marine Ecology Progress Series* 535: 145-159.

Tattersall, G.J., Sinclair, B.J., Withers, P.C., Fields, P.A., Seebacher, F., Cooper, C.E. and Maloney, S.K. 2012. Coping with thermal challenges: physiological adaptations to environmental temperatures. *Comprehensive Physiology* 2: 2151-2202.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., Le Roux, P.C. and Chown, S.L. 2011. Ecological relevant measures of tolerance to potentially lethal temperatures. *Journal of Experimental Biology* 241: 3713-3725.

Thompson, R.C., Wilson, B.J., Tobin, M.L., Hill, A.S. and Hawkins, S.J. 1996. Biologically generated habitat provision and diversity of rocky shore organisms at a hierarchy of spatial scales. *Journal of Experimental Marine Biology and Ecology* 202(1): 73-84.

Thompson, R.C., Jenkins, S.R. and Bussell, J.A. 2000. A method for recording predator–prey encounters between crabs and limpets using wax replicas. *Journal of the Marine Biological Association of the UK* 80(04): 633-638.

Tomanek, L. and Somero, G.N. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snail (Genus *Tugela*) from different thermal habitats: Implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology* 202: 2925-2936.

Tomanek, L. and Somero, G.N. 2000. Time course and magnitude of synthesis of heat-shock proteins in congeneric marine snail (Genus *Tugela*) from different tidal heights. *Physiological and Biochemical Zoology* 73: 249-256.

Tomanek, L. and Sanford, E. 2003. Heat-shock protein 70 (Hsp70) as a biochemical stress indicator: an experimental field test in two congeneric intertidal gastropods (genus: *Tugela*). *Biological Bulletin* 205: 276-284.

Tomanek, L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species biogeographic distribution ranges and metabolic costs. *The Journal of experimental Biology* 213: 971-979.

Tomanek, L. 2011. Environmental proteomics: changes in the proteome of marine organisms in response to environmental stress, pollutants, infection, symbiosis, and development. *Annual Review of Marine Science* 3: 14.1-14.27.

Tomanek, L. 2012. Environmental proteomics of the mussel *Mytilus*: implications for tolerance to stress and change in limits of biogeographic ranges in response to climate change. *Integrative and Comparative Biology* 52: 648-664.

Tully, O., O'Donovan, V. and Fletcher, D. 2000. Metabolic rate and lipofuscin accumulation in juvenile European lobster (*Homarus gammarus*) in relation to stimulated seasonal changes in temperature. *Marine Biology* 137: 1031-1040.

Turpie, J.K., Beckley, L.E. and Katua, S. M. 2000. Biogeography and the selection of priority areas for conservation of South African coastal fishes. *Biological Conservation* 92(1): 59-72.

Underwood, A.J. and Jernakoff, P 1984. The effects of tidal height, wave-exposure, seasonality and rock pools on grazing and the distribution of intertidal macroalgae in New South Wales. *Journal of Experimental Marine Biology and Ecology* 75: 71-96.

Vat, L.S. 2000. The growth and reproduction of Patella granularis (Mollusca: Patellogastropoda) on the south-east coast of South Africa. PhD Thesis. Rhodes University, South Africa.

Verderber, G.W., Cook, S.B. and Cook, C.B. 1983. The role of the home scar in reducing water loss during aerial exposure of the pulmonate limpet, *Siphonaria alternata* (Say). *Veliger* 25(3): 235-243.

Vermeij, G.J. 1972. Intraspecific shore-level size gradients in intertidal molluscs. *Ecology* 54(4): 693-699.

Vermeij, G.J. 1973. Morphological patterns in high-intertidal gastropods: adaptive strategies and their limitations. *Marine Biology 20*(4): 319-346.

Wallace, L.R. 1972. Some factors affecting vertical distribution and resistance to desiccation in the limpet, *Acmaea testudinalis* (Muller). *The Biological Bulletin*: 142(1) 186-193.

Walther, G.R. 2010. Community and ecosystems responses to recent climate change. *Philosophical Transactions of the Royal Society B* 365: 2019-2024.

Wang, T., Lefevre, S., Iversen, N.K., Findorf, I., Buchanan, R. and McKenzie1, D.J. 2014. Anaemia only causes a small reduction in the upper critical temperature of sea bass: is oxygen delivery the limiting factor for tolerance of acute warming in fishes? *Journal of Experimental Biology* 217: 4275-4278.

Weber, L.I., Gray, D.R., Hodgson, A.N. and Hawkins, S.J. 1997. Genetic divergence between South African *Helcion* species and north-east Atlantic *H. pellucidum* (Mollusca: Patellogastropoda). *Journal of the Marine Biological Association of the United Kingdom* 77(04): 1139-1150.

Wethey, D.S. 1983. Geographic limits and local zonation: the barnacles *Semibalanus* (*Balanus*) and *Chthamalus* in New England. *Biological Bulletin*, 165: 330-341.

Wethey, D.S. 1984. Sun and shade mediate competition in the barnacles' geographic limits and local zonation: the barnacles *Chthamalus* and *Semibalanus*. *Biological Bulletin* 167: 176-185.

Wethey, D.S. 2002. Biogeography, competition, and microclimate: the barnacle *Chthamalus fragilis* in New England. *Integrative and Comparative Biology* 42: 872-880.

Wetzel, R. and Limnology, A. 2001. Lake and River Ecosystems. *Limnology* 37: 490-525.

Widdows, J. 1973. Effect of temperature and food on the heartbeat, ventilation rate and oxygen uptake of *Mytilus edulis*. *Marine Biology* 20: 269-276.

Williams, G.A. 1993. Seasonal variation in algal species richness and abundance in the presence of molluscan herbivores on a tropical rocky shore. *Journal of Experimental Marine Biology and Ecology* 167(2): 261-275.

Williams, G.A. and Morritt, D. 1995. Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. *Marine Ecology Progress Series* 124: 89-103.

Williams, G.A., De Pirro, M., Cartwright, S., Khangura, K., Ng, W.C., Leung, P.T.Y. and Morritt, D. 2011. Come rain or shine: the combined effects of physical stresses on physiological and protein-level responses of an intertidal limpet in the monsoonal tropics. *Functional Ecology* 25: 101-110.

Wiltshire, K.H. and Manly, B.F. 2004. The warming trend at Helgoland Roads, North Sea: phytoplankton response. *Helgoland Marine Research* 58(4): 269.

Wolcott, T.G. 1973. Physiological Ecology and intertidal zonation in limpets (*Acmaea*): a critical look at "limiting factors." *Biological Bulletin* 145: 389-422.

Xing, Q., Li, Y., Guo, H., Yu, Q., Huang, X., Wang, S., Hu, X., Zhang, L. and Bao, Z. 2016. Cardiac performance: a thermal tolerance indicator in scallops. *Marine Biology* 163(12): 244.

Yamada, S.B. and Boulding, E.G. 1996. The role of mobile crab predators in the intertidal zonation of their gastropod prey. *Journal of Experimental Biology and Ecology* 204: 59-83.

Yonge, C.M. 1947. The pallial organs in the Aspidobranch Gastropoda and their evolution throughout the Mollusca. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 232: 443-518

Yonge, C.M. 1952. The mantle cavity in *Siphonaria alternata. Proceedings of the Malacological Society of London* 29: 190-199.

Zakhartsev, M.V., De Watcher, B., Sartoris, F.J., Pörtner, H.O. and Blust, R. 2003. Thermal physiology of the common eelpout (*Zoarces viviparus*). *Journal of Comparative Physiology B* 173: 365-378.

Zardi, G.I., Nicastro, K.R., McQuaid, C.D., Hancke, L. and Helmuth, B. 2011. The combination of selection and dispersal helps explain genetic structure in intertidal mussels. *Oecologia* 165: 947-958.

Zerebecki, R.A. and Sorte, C.J.B. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. *Plos One* 6: 1-7.

Zielinski, S. and Pörtner, H.O. 1996. Energy metabolism and ATP free-energy change of the intertidal worm *Sipunculus nudus* below a critical temperature. *Journal of Comparative Physiology B* 166: 492-500.