Interactive effects of pH, temperature and exposure period on native and invasive mussels from the West Coast of South Africa

By

[1]Martin Phillippe Emanuel

Supervisors: [1]Dr. Deena Pillay, [2]Dr. Mathilde van der Merwe

Dissertation presented in partial fulfilment of the requirements for the degree of
Master of Science in Applied Marine Science

in the

Department of Biological Sciences

[1]Department of Biological Sciences, University of Cape Town, Cape Town, South Africa
[2]Centre for Higher Education Development, University of Cape Town, Cape Town, South Africa

09th July 2013

Contents

List of Figures and Tables

Figures:

Tables:

PLAGIARISM DECLARATION

1. I know that plagiarism is wrong. Plagiarism is to use another's work and pretend that it is one's own.

2. I have used the footnote convention for citation and referencing. Each contribution to and quotation in this assignment from the work(s) of other people has been attributed, cited and referenced.

3. I acknowledge that copying someone else's assignment or essay, or part of it, is wrong and that this assignment is my own work.

4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Signature: _____

Date: _____

Abstract

Global warming and ocean acidification due to an increase in anthropogenic carbon dioxide can impact marine calcifying organisms. Shells of marine calcifying organisms protect their internal soft tissue and may be key in determining the susceptibility of marine calcifiers to these environmental stressors. To test this, the effects of pH, temperature, exposure period and their interactions on the performance of native and alien mussels with varying shell thickness was studied. Listed in order of decreasing shell thickness, I compared shell dissolution, shell growth, shell breaking force and condition index of *Aulacomya ater, Choromytilus meridionalis* (both native), *Mytilus galloprovincialis* and *Semimytilus algosus* (both invasive) found on the Western Cape coast of South Africa. Live mussels and bare shells were exposed to seawater temperatures of 14^{o}C and 20^{o}C set at two pH levels (7.5 and 8.0) for roughly 40 days. Live mussels were either exposed to aerial drying for four hours per day or fully submerged for the duration of the study. The results suggest that shell thickness determines the susceptibility of mussels to environmental stressors, in terms of shell dissolution and breaking force, but does not affect internal growth. Invasive mussels showed increased shell dissolution at low pH but their growth rates were unaffected. They also exhibited higher condition indices than native mussels under low pH and high temperatures. On the other hand, the thicker shelled native mussels showed no significant changes in shell dissolution among the treatments and exhibited increases in growth rates in low pH treatments. *C. meridionalis*, being cold water adapted, exhibited a reduction in condition index in high temperature treatments. The study indicates that native and invasive mussels have different compensatory mechanisms to respond to anthropogenic impacts. These mechanisms allow them to maintain their specific life history strategies

under short term exposure to warming and acidification. It was also elucidated that mussels exposed to low temperature aerial conditions exhibit increased shell and tissue growth as periodic exposure minimises the deleterious effects of ocean acidification and warming. The findings suggest that native and invasive mussels respond differently to ocean acidification and warming depending on their specific physiologies and life history strategies.

Keywords: Ocean acidification, temperature, mussels, dissolution, metabolism.

Acknowledgements

I would like to thank the Applied Centre for Climate and Earth System Science (ACCESS) and the department of environmental affairs (DEA) for funding my Master's degree; Dr Deena Pillay and Dr Mathilde van der Merwe for their supervision and assistance during this study. I would also like to thank Dr Lutz Auerswald for his insight and guidance into climate change and ocean acidification research design; Ralph Watson for his assistance and guidance with ADOBE Photoshop; and Stewart James Norman and Suzanne Zeeman for their assistance in mussel collection and preparation.

CHAPTER 1: General Introduction

Anthropogenic increases in greenhouse gases are responsible for drastic changes in global climate and ocean chemistry (Widdicombe and Needham 2007; Wood et al. 2008). Since the beginning of the industrial revolution there has been an increase in the levels of atmospheric carbon dioxide (CO_2) from approximately 280 to 387 parts per million (ppm), with as much as 50% of the increase occurring in the last three decades (Bibby et al. 2008; Feely et al. 2009). This is largely due to the liberal burning of fossil fuels from industrial and agricultural activities (Feely et al. 2009). Atmospheric CO_2 concentration is now greater than it has been for approximately 800,000 years (Lüthi et al. 2008) and is expected to increase at an accelerated rate (Feely et al. 2009). The presence of excessive amounts of atmospheric CO_2 has resulted in an increase in global atmosphere and ocean temperatures as well as an increase in the CO_2 concentration of the world's oceans (Tunnicliffe et al. 2009). Climate change models predict significant alterations to temporal patterns of extreme climatic events and an increase in frequency of their occurrence (Bertocci et al. 2007; Hiebenthal et al. 2012).

The world's oceans act as natural sinks for CO_2 and have so far curtailed the harmful impacts of global warming (Caldeira and Wickett 2005). This absorption of CO_2 however, modifies chemical reactions that ultimately lead to a reduction in seawater pH, carbonate ion concentration and also the saturation states of biologically important calcium carbonate minerals (calcite and aragonite). This process is widely referred to as "Ocean Acidification" (Caldeira and Wickett 2003, 2005; Orr et al. 2005; Wood et al. 2008; Doney et al. 2009; Bechmann et al. 2011). Ocean acidification occurs via the following reaction:

$$CO_{2\,(aqueous)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad (1)$$

Carbon dioxide (CO_2) and seawater (H_2O) bond to form carbonic acid (H_2CO_3), which then dissociates and releases hydrogen (H^+) and bicarbonate (HCO_3^-). This results in most of the free H^+ bonding with carbonates (CO_3^{2-}) to form additional bicarbonate ions. CO_2 absorption into the ocean therefore increases H^+ which decreases the pH of seawater and decreases CO_3^{2-} concentrations.

In addition to ocean acidification, an increase in atmospheric CO_2 levels can significantly accelerate rates of warming of the atmosphere and the ocean surface in the near future (Feely et al. 2009). This is largely due to the ability of atmospheric CO_2, along with other greenhouse gases, to trap infrared radiation which would otherwise be radiated back into space. Seawater, being a good conductor of heat, therefore absorbs heat which in turn leads to warming of ocean waters.

Due to increasing CO_2 emissions, mean seawater pH has already decreased by 0.1 units and mean global surface temperature has increased by 0.76^{o}C (IPCC 2007). The pH of sea water is expected to decrease by 0.3 to 0.5 units by 2100 under current CO_2 emission rates (Caldeira and Wickett 2005; Orr et al. 2005; IPCC 2007; Widdicombe and Needham 2007). Also, the Intergovernmental Panel on Climate Change (IPCC) models forecast a 1.7 to 4.4^{o}C increase in the temperature of the earth's surface due to increasing anthropogenic CO_2 levels by the end of the 21st century (IPCC 2007). The stress of high temperature and low seawater pH, due to increased anthropogenic CO_2 levels, can affect the biology, physiology and performance of marine calcifying organisms either independently or synergistically. This may lead to altered distribution patterns of sensitive species and could directly affect the diversity and abundance of ecologically sensitive species (Hiebenthal et al. 2012).

Temperature is one of the most important factors affecting the biology and physiology of marine organisms. There is a plethora of research demonstrating the strong effect of temperature on the growth, survival, reproduction and distribution of marine organisms (Hochachka and Somero 2002; Jones et al. 2009; Lesser et al. 2010; Kordas et al. 2011). The effects of increasing temperature on marine organisms has been the focus of considerable research in recent years due to the potential threats posed by global warming (Jones et al. 2009; Lesser et al. 2010; Lathlean and Minchinton 2012; Madeira et al. 2012). The distribution and abundance of a variety of organisms varies across spatial and temporal thermal gradients and there are well documented accounts of distributional shifts attributed to climate change (Hochachka and Somero 2002; Helmuth et al. 2006; Jones et al. 2009; Kordas et al. 2011).

The physiology of ectothermic organisms in particular is drastically affected by slight changes in temperature. It is evident that many species occupy specific thermal niches within which they function optimally (Hochachka and Somero 2002; Fields et al. 2006; Rayssac et al. 2010). An increase in seawater temperatures increases metabolic rates in aquatic organisms (Hiebenthal et al. 2012). This is of particular concern for marine invertebrates that produce calcium carbonate shells (Michaelidis et al. 2005; Gazeau et al. 2007; Feely et al. 2009), as increased metabolic activity results in increased CO_2 production during cellular respiration. This further reduces the pH of seawater, thereby reducing the availability of free carbonates which is essential for shell formation (Michaelidis et al. 2005; Gazeau et al. 2007).

Organisms have specific temperature requirements and even closely related species respond to changes in temperature differently (Braby and Somero 2005). Experiments conducted to quantify the effect of extreme heat and cold stress on the invasive marine

mussel *Mytilus galloprovincialis* and a native marine mussel *Mytilus californianus* revealed that *M. galloprovincialis* could tolerate wider temperature ranges, suffer less DNA damage due to temperature stress and was better able to repair DNA relative to *Mytilus californianus* (Yao and Somero 2012). These features give *M. galloprovincialis* a competitive edge over *M. californianus*. The variability in response to temperature could either be due to species specific thermal optima for biological functions or differences in thermal sensitivity (e.g. steep increase in performance with increased temperature as opposed to a gradual increase in response to temperature) (Kordas et al. 2011).

In spite of specific thermal requirements, organisms are able to compensate for changes in external temperature through alterations in their morphology, life history stages, behaviour and physiology. This fluid organismal response is broadly referred to as phenotypic plasticity (Pigliucci 2001; Byrne 2011; Melatunan et al. 2013). For example, when temperature increases are imposed on calcifying organisms, the energy required to counter the stress is diverted from other physiological functions (Hiebenthal et al. 2012; Melatunan et al. 2013). Temperature, which is such a stressor, may speed up metabolic rates, but continually increasing temperatures could work to the organism's detriment, causing oxidation of specific cellular components and greater energy investment in repair and leaving less energy for shell growth, reproduction and maintenance (Abele et al. 2002; Hiebenthal et al. 2012). Laboratory experiments on the marine mussel *Mytilus edulis* indicated a marked increase in shell growth rate up to temperatures of 20^{o}C but a decrease in growth rate between temperatures of 20 to 25^{o}C (Hiebenthal et al. 2012).

Temperature-induced reductions in shell growth due to increased investment in tissue repair can affect shell stability in marine organisms (Irie 2006; Hiebenthal et al. 2012). Shell stability, which is a measure of the ability of the shell to resist breakage, is influenced

by shell thickness, shape, height and size (Hiebenthal et al. 2012). Changes in growth of shells under high temperature may thus affect the susceptibility of shelled organisms to mechanical damage and predation. While some research has shown that increasing temperatures can reduce shell stability, others have reported increases. For example, Nagarajan et al. (2006) found that the shell breaking force in the mussel *Mytilus edulis* increased at high temperatures (24^{o}C), and suggested that this is due to increased shell thickness.

While early climate change research focused on increasing temperatures in marine ecosystems, more effort has focused on ocean acidification recently, which is a consequence of elevated diffusion of atmospheric CO_2 in oceans (Feely et al. 2009). Since the inception of climate modelling based on past and present CO_2 emission scenarios from the IPCC, there has been a drive to understand the direct and underlying effects of ocean acidification on ecologically sensitive marine systems and marine calcifying organisms as they may be most susceptible to the deleterious effects of ocean acidification (Gazeau et al. 2010). There are a number of research publications that support the notion that ocean acidification poses a significant threat to marine calcifying organisms (Gazeau et al. 2007; O'Donell et al. 2009; Yamamoto-Kawai et al. 2009; Hofmann et al. 2010; Byrne 2011; Gaylord et al. 2011; Hiebenthal et al. 2012). There is a greater need to study organisms that inhabit cooler waters, as they may have to deal with higher rates and levels of ocean acidification than warm water species (Moy et al. 2009). This is because of a negative linear relationship between CO_2 absorption and seawater temperatures. Cooler seawater absorbs a greater amount of CO_2 than warmer seawater, thereby reducing the amount of free carbonates to a greater extent (Findlay et al. 2010; Bechmann et al. 2011). Marine calcifying

organisms require free carbonates (CO_3^{2-}) for the deposition of their calcium carbonate ($CaCO_3$) shells and skeletons (equation 2).

$$\mathbf{Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_{3\,(solid)}} \quad \mathbf{(2)}$$

These free carbonates occur in the ocean in the form of calcite and aragonite, which are the products of dissolved shells and skeletons (dissolution) of dead marine calcifying invertebrates (Feely et al. 2004; Fabry et al. 2008; Feely et al. 2009; Bechmann et al. 2011). With less carbonate available, because of their bonding with free H^+ during CO_2 absorption (see equation 1), the saturation state of $CaCO_3$ is reduced. This directly affects the ability of marine calcifying invertebrates to produce calcium carbonate structures, thereby putting them at greater risk from the harmful effects of ocean acidification (Feely et al. 2009).

Low pH seawater can also increase CO_2 diffusion into organismal tissue and body fluids, which in turn can decrease intra and extracellular pH (Michaelidis et al. 2005). However, under conditions of ocean acidification, calcifying organisms are able to regulate their intra and extracellular pH by increasing the level of bicarbonates available to bond with CO_2 from their calcium carbonate exoskeletons (Portner et al. 1998; Michaelidis et al. 2005; Portner 2008; Bechmann et al. 2011). Therefore, under conditions of temporary acid-base fluctuations, marine calcifying organisms are still able to produce shell material, albeit at a reduced rate. Beesley et al. (2008) showed that the growth rate of the mussel, *Mytilus edulis*, was significantly reduced during temporary exposure to low pH, but that tissue structures were not impacted. It was suggested that the decrease in growth was due to the build-up of Ca^+ ions in the mussels' haemolymph by the dissolution of their shells. This revealed that *M. edulis* has strong physiological mechanisms which protect its tissues when temporarily exposed to low pH seawater. Beesley et al. (2008) however, concluded that

these mechanisms are energetically costly and may result in reduced shell growth, health and survival of *M. edulis* under periods of long term exposure to low pH.

Guppy and Withers (1999) showed that anostracan crustaceans (*Artemia* spp.), like various other marine organisms, exhibit metabolic depression as a strategy to survive under low pH. A reduction in metabolic rate would likely reduce growth and reproduction of organisms in response to ocean acidification. This is supported by Wood et al. (2008), who showed that although calcification increased at low pH (6.8) in the ophiuroid brittlestar *Amphiura filiformis*, it came at the expense of arm muscle mass, as muscle was used as an energy source for calcification and repair, resulting in metabolic depression. It is suggested that low pH has a narcotic effect on the metabolism of marine calcifying organisms (Wood et al. 2008; Melzner et al. 2011; Byrne 2011). If the seawater pH is low enough to hamper shell production, it may have fatal consequences for marine calcifying organisms (Michaelidis et al. 2005).

The existing literature shows that different species react differently to acidification, depending on life history, growth rate and population size and dynamics (Kurihara 2008). Kurihara and Shirayama (2004) showed that the fertilisation rate of the urchins *Hemicentrotus pulcherrimus* and *Echinometra mathaei* was reduced when the pH of seawater was decreased from 8.1 to 6.8. In contrast the fertilisation rates of the mussel *Mytilus galloprovincialis* and the oyster *Crassostrea gigas* were unaffected in lower pH (7.4) water (Kurihara et al. 2007; Ries et al. 2009) and fertilisation in the giant scallop *Placopecten megallanicus* increased under pH less than 7.5 (Desrosiers et al. 1996).

In addition to interspecific differences in response to acidification, adult-juvenile responses also differ significantly, with larval or early life stages of marine calcifying invertebrates considered to be most sensitive. For example, studies by Gazeau et al. (2007)

on *Mytilus edulis* showed that juveniles were particularly susceptible to ocean acidification and that the calcification rate in juveniles declined linearly with an increase in CO_2 concentrations of seawater. A seawater pH of less than 7.5 was found to reduce the pH of haemolymph in juvenile mussels (*Mytilus galloprovincialis*) and increase the haemolymph bicarbonate concentration due to shell dissolution (Michaelidis et al. 2005). A pH of 7.5 or lower also negatively impacted juvenile *M. galloprovincialis* growth rate and metabolism (Michaelidis et al. 2005; Berge et al. 2006).

Organismal acid-base balance, metabolic depression and reproduction are not the only physiological processes affected by ocean acidification. It is well accepted that calcium carbonate is the primary building block of support structures such as shells and spines in marine invertebrates and that these structures may be severely impacted by ocean acidification through dissolution (Wood et al. 2008). Nienhuis et al. (2010), working on the rocky intertidal snail, *Nucella lamellosa*, reported a decrease in shell growth with decreasing pH, which was paralleled by a linear increase in shell dissolution. This finding suggested that the observed loss in shell growth at low pH was due to shell dissolution rather than reduced shell production. In line with this finding, Lischka et al. (2011) showed that ocean acidification causes a more pronounced effect on the dissolution of the shell of the marine polar pteropod *Limacina helicina* than on shell deposition. The onset of shell dissolution in the bivalve *Mytilus edulis* due to exposure to low pH seawater was found to occur at pH 7.5 or lower (Michaelidis et al. 2005; Gazeau et al. 2007; Melzner et al. 2011).

Dissolution of shells of calcifying organisms under acidic conditions is of particular concern due to reductions in shell stability. For example, Gaylord et al. (2011) found that juvenile mussels (*Mytilus californianus*) exhibited a linear decrease in shell breaking force (which is an indicator of low stability) with decreasing pH. In contrast, Hiebenthal et al.

(2012) found that the breaking force for shells of young mussels (*M. edulis* and *Arctica islandica*) was unaffected by low pH. However, both Gaylord et al. (2011) and Hiebenthal et al. (2012) agreed that shell calcification and thickness may not be the only factors determining shell strength in mussels, arguing that complex interactions between shell thickness and other shell properties such as height, shape and size are involved.

The available research documenting the effect of anthropogenic climate change on marine calcifying invertebrates, for the most part, considers single stressor effects (Lesser et al. 2010; Nienhuis et al. 2010; Byrne 2011; Gaylord et al. 2011). However, ocean change involves the additive, antagonistic or synergistic effect of multiple stressors (Byrne 2011; Wahl et al. 2011; Hiebenthal et al. 2012). Therefore, in order to predict the fate of marine calcifying invertebrates under changing climate conditions, it is important to conduct multi-factorial studies to identify those organisms with a physiological advantage under predicted climate change scenarios (Byrne 2011). The response of ecologically important species that structure communities and regulate ecosystem functions is of particular interest, as their exclusion due to climate change may drastically alter marine ecosystems (Byrne 2011).

In recent years, the importance of testing the interactive effects of these stressors has come to light, as it aids in drawing real world conclusions about the future of marine calcifying organisms and marine ecosystems (Widdicombe and Spicer 2008; Byrne 2011). A transplantation experiment near an active volcanic CO_2 vent by Rodolfo-Metalpa et al. (2011) revealed that the harmful effect of low seawater pH on shell growth rate and dissolution of the limpet *Patella caerulea*, the mussel *Mytilus galloprovincialis*, and corals *Cladocora caespitosa* and *Balanophyllia europaea* was exacerbated when combined with high temperatures. In support of these findings, Melatunan et al. (2013) showed that the intertidal gastropod, *Littorina littorea,* exhibited a far greater reduction in shell growth rate

and an increase in shell dissolution under conditions of elevated temperature and low pH as compared to any of these factors in isolation. However this study also revealed a complex interaction between temperature and pH on shell thickness. *L. littorea* produced thicker shells in low pH, high temperature treatments as compared to low pH, normal temperature treatments. It was suggested that the latter response was due to an increase in calcite and aragonite saturation states at higher temperatures. Research by Hiebenthal et al. (2012) concluded that exposure to low pH (7.5) and high temperature (25^{o}C) seawater had a greater negative impact on the growth rate of the bivalve molluscs, *Arctica islandica* and *M. edulis*, than the independent effects of these stressors. Lischka et al. (2011), studying the interactive effects of low pH and higher temperatures on the juvenile, cold water marine pteropod, *Limacina helicina*, concluded that the pteropod is negatively impacted by both pH and temperature. However each stressor had a more pronounced individual than combined effect on different aspects of the physiology of *L. helicina*. High temperature was the overriding stressor responsible for increased metabolic rates and hence mortality, while shell growth and shell degradation were significantly affected by decreasing pH. The study suggests that increased metabolic activity at high temperatures, and increased shell dissolution in low pH seawater, results in higher metabolic repair costs. Energy is thus diverted away from important physiological functions in *L. helicina*, ultimately resulting in increased mortality during long term exposure to warm acidic conditions (Lischka et al. 2011).

There is currently no research published on the interactive effects of pH, temperature and exposure period on intertidal organisms. This is of particular importance as the latter two stressors are considered to be amongst the most influential determinants of rocky shore community structure around the globe (Griffiths and Hockey 1987; Bertocci et

al. 2007). Rocky intertidal ecosystems are ideal environments for studying the interactive effects of climate change stressors on marine calcifying organisms as these ecosystems are dynamic and sensitive to change. Rocky shores are located between the high and low water marks along coastlines and may be viewed as ecotones between marine and terrestrial environments (Thompson et al. 2002). Intertidal rocky shores are particularly challenging environments, as resident organisms are exposed to multiple stressors from both aerial and aquatic regimes (Hammond and Griffiths 2004; Bertocci et al. 2007). Aerial exposure causes thermal fluctuations and desiccation, which affect spatio-temporal distribution patterns on rocky shores (Bertocci et al. 2007). Events such as global warming, sea-level rise and ocean acidification may exacerbate inherent variability and environmental stresses and may indirectly affect biological interactions between and within species. Organisms that are resident on rocky shores often live close to the upper limit of their thermal tolerance and may therefore act as indicators or early warning systems to the possible effects of climate change (Somero 2002; Schneider et al. 2010).

Given the challenging conditions faced by intertidal rocky shore species, their persistence at the upper thresholds of tolerances and the threat they potentially face from changing climate, it is important to determine the response of important rocky shore species under projected climate change scenarios in order to track future changes in species diversity, abundance and distribution in coastal systems at both regional and global scales.

CHAPTER 2: Interactive effects of pH, temperature and exposure period on native and invasive mussels from the West Coast of South Africa

2.1. Introduction

The challenges posed by changing temperature and pH in marine systems for calcifying organisms have been outlined in Chapter 1. However, a modern phenomenon that needs to be considered within the context of climate change is the spread of non-indigenous species across the globe. As a result of increases in shipping intensity over the last century, marine ecosystems around the world have been subjected to increases in colonisation by invasive species (Bownes and McQuaid 2006; Simon-Blecher et al. 2008). These species in general have *r*-selected life history characteristics, including high fecundity, growth and recruitment rates (Van Erkom Schurink and Griffiths 1991; Griffiths et al. 1992) and it is suggested that these characteristics increase their invasive potential. There has been growing interest in the effects of alien invasive species on intertidal systems (Kado 2003; Braby and Somero 2005; Schwindt 2007; Laird and Griffiths 2008; Nalepa et al. 2009), along with growing concerns that their persistence may remove indigenous species permanently (Branch and Steffani 2004; Nalepa et al. 2009).

In the South African context, there are three major invasive species found on the intertidal rocky shores of the Western Cape viz. the Mediterranean mussel *Mytilus galloprovincialis* (Griffiths et al. 1992; Bownes and McQuaid 2006), the barnacle *Balanus glandula* (Simon-Blecher et al. 2008) and the Pacific South American mussel *Semimytilus algosus*. *M. galloprovincialis* was probably first introduced to South African shores in the 1970s (Grant and Cherry 1985). It has since replaced the indigenous intertidal mussels *Choromytilus meridionalis* and *A. ater* as the dominant species on the West Coast of South Africa (Griffiths et al. 1992). *Balanus glandula* is a fairly newly discovered invasive barnacle

in South Africa, reported for the first time in South Africa by Simon-Blecher et al. (2008), in Camp's Bay on the Cape Peninsula. *Semimytilus algosus* is the most recently discovered invasive species in the Western Cape and there are currently no publications available on it. It is, however, assumed that these stocks may have extended down the West Coast of South Africa from Namibia.

Mussels are important calcifying organisms found on rocky shores and are highly susceptible to climate change (Michaelidis et al. 2005; Gazeau et al. 2007; Hiebenthal et al. 2012; Range et al. 2012). Recently, there has been growing interest in the effect of climate change variables on mussels (Michaelidis et al. 2005; Beesley et al. 2008; Bibby et al. 2008; Gaylord et al. 2011; Hiebenthal et al. 2012; Range et al. 2012). Studies suggest that mussels have mixed responses when subjected to climate change stressors (Byrne 2011; Range et al. 2012).

The Western Cape of South Africa, which is the focal area in this study (Figure 2.1), is a cool-temperate bioregion that typically has water temperatures ranging from ~14^{o}C in winter to ~18^{o}C in the summer (Dufois and Rouault 2012). In terms of mussels, there are long stretches of this coastline in which both native and invasive mussels co-occur, albeit at different tidal heights on the intertidal zone. There are four major mussel species that occur on these rocky shores; the natives *Choromytilus meridionalis* (black mussel) and *Aulacomya ater* (ribbed mussel) and the invasives *Mytilus galloprovincialis* (Mediterranean mussel) and *Semimytilus algosus* (Pacific South American mussel) (Branch et al. 2010).

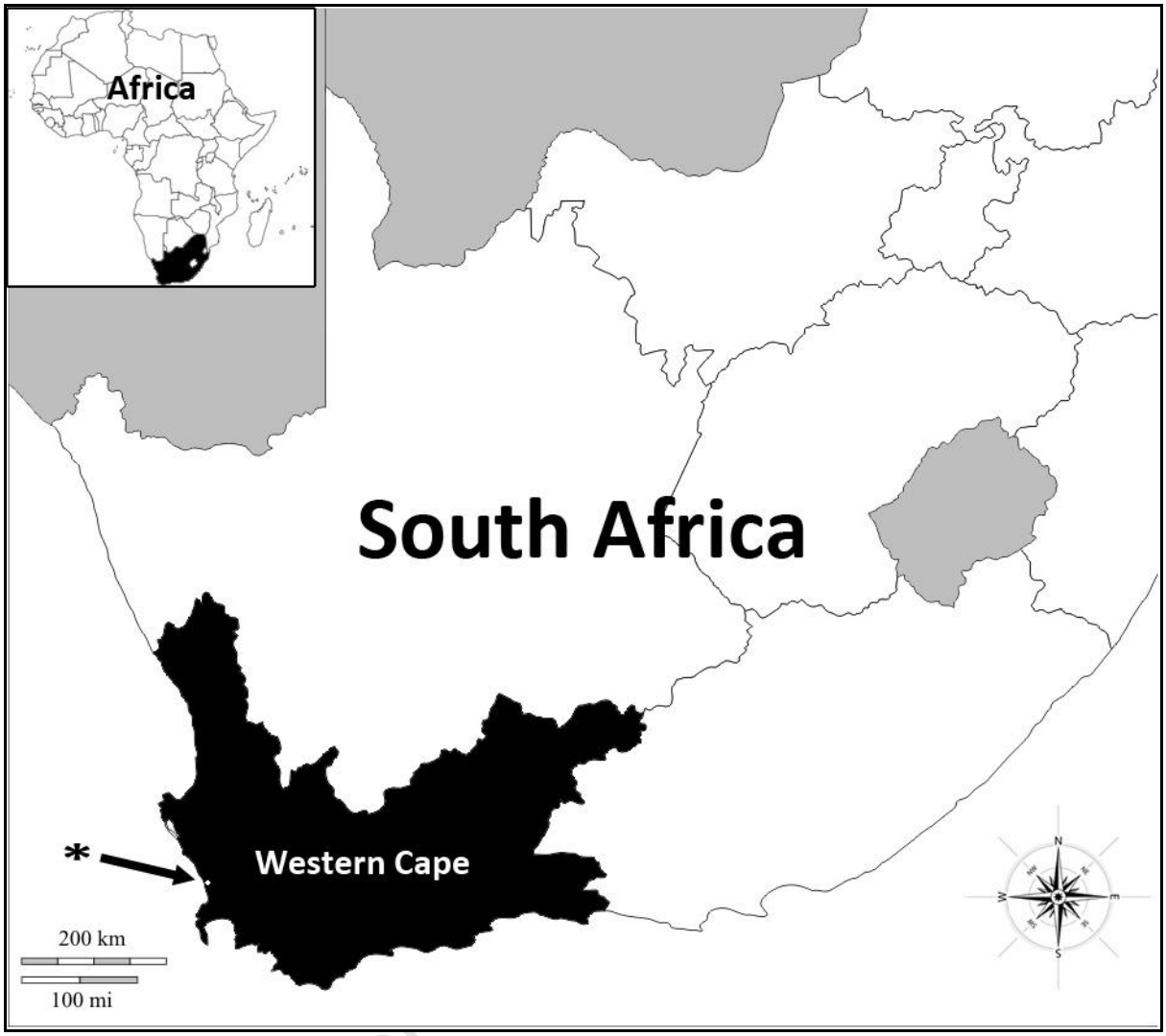

Figure 2. 1: Map of South Africa highlighting the Western Cape Province and Bloubergstrand (*) where samples were collected for the experiment

Aim

The responses of native and invasive marine mussels to the interactive effects of ocean acidification and temperature change is not well documented and needs to be incorporated into current thinking in order to track changes in species distribution and community structure over time (Byrne 2011). The general aim of this study was therefore to assess the interactive effects of pH, temperature and exposure period on the performance

of two native and two invasive species of mussels found on the rocky intertidal zone of the West Coast of South Africa. It was hypothesised that shell thickness would determine the susceptibility of the mussels in terms of growth, performance, shell dissolution and shell breaking force to acidification, and that increasing temperature and aerial exposure will reduce susceptibility to acidification.

2.2 Materials and Methods

A mesocosm experiment was undertaken to experimentally quantify the effect of temperature (14^{o}C and 20^{o}C), pH (7.5 and 8.0) and exposure period (complete submersion vs. four hours exposure per day) on the performance of native and invasive mussels. Two invasive (*Semimytilus algosus* and *Mytilus galloprovincialis*) and two native (*Aulacomya ater* and *Choromytilus meridionalis*) mussels, which occur sympatrically, were chosen for the experiment. Two approaches were used to test the hypotheses posed above. Firstly, living mussels were used to measure the effect of the different treatments on mussel survival, growth rate and tissue condition index. Secondly, empty shell valves were utilised to test the effect of treatments on shell dissolution and shell breaking force.

Animal Collection and Preparation

One hundred and twenty live adults of each mussel species were collected from Bloubergstrand (33^{o} 48’ S; 18^{o} 27’ E, Figure 2.1). Mussels were held in a 1000L aquarium tank containing recirculating natural seawater (temperature = 10^{o}C, pH = 8.0) for 10 days prior to experimentation, after which their lengths and weights were recorded. The lengths were measured using a digital vernier calliper to a precision of 0.1mm and mass was measured on a digital balance recorded to the nearest 0.1g. Adult mussels, which was the dominant cohort encountered at the site were used in the experiment, due to the large

number of mussels (180 per species) needed for the experiment. For *Aulacomya ater*, *Choromytilus meridionalis* and *Mytilus galloprovincialis* the relationship between adult shell length and shell thicknesses had previously been studied by van Erkom Schurink and Griffiths (1993), who produced equations relating these two variables. For *A. ater*, *C. meridionalis* and *M. galloprovincialis* shell thicknesses at particular mussel sizes were calculated using the equations of van Erkom Schurink and Griffiths (1993). The equations used to calculate the shell thickness of each species are summarised in Table 2.1.

Table 2. 1: Equations describing the relationship between shell length and thickness of three mussel species occurring in the West Coast of South Africa. y = shell thickness (mm), x = shell length (mm)

Species	Equation
Aulacomya ater	$y = (0.028774) * (x^{1.025})$
Choromytilus meridionalis	$y = (0.024322) * (x^{0.994})$
Mytilus galloprovincialis	$y = (0.021727) * (x^{1.028})$

However due to a lack of information on the relationships between shell length and thickness for *S. algosus*, ten individuals of *S. algosus* were collected and shell thicknesses were measured directly. Shells were cleaned and the left shell valve of each species was sawed into halves at the highest point of the shell. The shell thickness at the highest point of the shell was measured with the aid of a digital vernier calliper and recorded to the nearest 0.01mm. The mean shell thickness was calculated and then used to generate a linear regression comparing the effect of shell thickness on shell loss, breaking force, and growth rate and condition index among the four species of mussel.

CO_2 and temperature system

The experimental design used in this study is illustrated in Figure 2.2. Seawater was transferred into 12 120L tanks with individual aeration lines and circulation pumps. Six tanks

were maintained at a temperature of 14°C ± 2°C (low temperature) and the remaining six were kept at 20°C ± 2°C (high temperature). The temperature was measured to a precision of 0.1°C. Water temperature in the low temperature treatment was maintained by the aquarium feeder pumps and was heated to 20°C using 300W Eheim Jager heaters for the high temperature treatments. Within each of these temperature treatments, there were two different pH treatments viz. 7.5 (hypercapnic) or 8.0 (normocapnic) (Figure 2.2). The pH of the seawater was measured to the nearest 0.01 pH units.

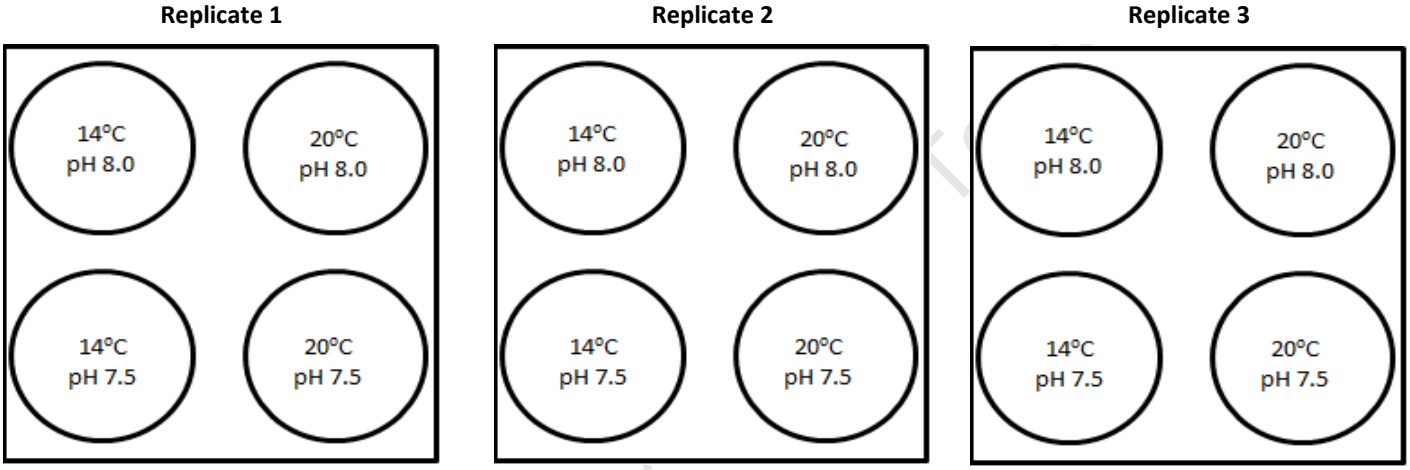

Figure 2. 2: Experimental design used to test the interactive effect of temperature (14°C and 20°C) and pH (7.5 and 8.0) on native and invasive mussels. All treatments were replicated 3 times and randomly distributed

For hypercapnic treatments, pH was lowered by using Aqua Medic systems, which bubbled gaseous CO_2 from a cylinder into the seawater via a M-Ventil electronic solenoid regulator valve connected to a pH 2001C pH monitor and low pressure electrode. Seawater pH is predicted to decline by 0.3 – 0.5 units by 2100 (Caldeira and Wickett 2005; Orr et al. 2005; IPCC 2007; Widdicombe and Needham 2007). pH of sea water from Bloubergstrand, where mussels were collected and Seapoint, where water was collected for experiments was 8.0. Hypercapnic treatments were therefore set at 7.5 pH units to test mussel perforamce at the most extreme projected (0.5) decline in sea water pH by 2100. Unmanipulated seawater was used for the normocapnic treatments. A handheld pH meter

and a thermometer were utilised to measure the pH and temperature of each tank daily to ensure that the pH monitors and heaters were operating at the required level. Water in each tank was changed weekly, to prevent build-up of unwanted nutrients in tanks. This involved preparation of tanks with water at the required temperatures and pH in advance and then transferring mussels into these tanks after a week. Ammonium, nitrate and nitrite test kits were used to monitor water quality daily. Concentrations of ammonium, nitrate and nitrate were stable over the duration of the experiment.

Live Mussels

In order to test the effect of the different treatments on mussel survival, condition index and growth rate, two batches of mussels, each consisting of five individuals of each of the four species was placed into each tank. Each batch of five individuals was set in a flexible mesh tube, separated by a plastic movable cable-tie. Each mussel was given an alphanumeric label in order to track individual mussel performance for the duration of the experiment. One batch of mussels was submerged throughout the experiment, while the second batch was exposed to aerial conditions for four hours per day (average duration of exposure to aerial conditions in the field). The mussels were subjected to experimental treatments for a period of 42 days between November and December 2012. Prior studies have shown that experiments running for roughly 6 weeks are adequate to test the effects of temperature and pH on calcifying organisms (Widdicombe and Needham 2007; Wood et al. 2008; Melzner et al. 2011). During the experiment, mussels were fed on a diet of a live concentrated culture of *Pavlova lutheri*, a species of phytoplankton ranging from 3 - 6µm in size. Mussels were exposed to a 12 hour light and 12 hour dark cycle for the duration of the experiment. Each tank received 100 ml of concentrate every three days. During the study,

mussel survival was recorded daily. Those which perished were replaced by new mussels. The replacements were not utilised in the results, but were inserted to maintain consistent stocking densities. The mussels used in the experiment were not reproductively mature.

Condition index

After the 42 day exposure period all mussels were removed from the tanks. The shell lengths of all the live mussels were recorded and the mussels were then sacrificed. The tissue of each mussel was removed and dried in an oven until constant mass was achieved. This was achieved by drying the tissue of five mussels of each species and periodically weighing them until there was no measurable change in tissue mass. *Aulacomya ater* and *Semimytilus algosus* were dried at 70^{o}C for ~24 hours while *Choromytilus meridionalis* and *Mytilus galloprovincialis* were dried at 90^{o}C for ~24 hours. Because of the different sizes of the mussel species, different drying temperatures had to be used to prevent combustion of the samples. Thereafter the dry tissue mass was recorded with a digital balance to a precision of 0.0001g. The empty shells were air dried for 4 days and also weighed and recorded to the nearest 0.0001g. After drying, the Condition Index was calculated by using the following equation:

Condition index (CI) = [tissue weight/shell length]

Growth rate

Growth rates of mussels in response to different experimental treatments were calculated as a change in shell length over the duration of the experiment (42 days).

Mussel Shells

Shell dissolution and shell breaking force in response to the different temperature and pH treatments were determined by exposing empty shells to the treatments in a separate test, running simultaneously with the live mussel experiment. Sixty individual mussels of each of the two native and two invasive species were collected and were sacrificed. The left shell valve of each mussel was cleaned, dried, weighed and labelled and exposed to treatments for 39 days. In this experiment, the effect of exposure period was not tested.

Shell Dissolution

The weight of the empty shell valves was measured to a precision of 0.0001g every 7 days for the duration of the experiment. After 39 days, all empty shell valves were removed from the treatments and their final weight was measured. Shell dissolution was determined as the change in shell weight between the start and end of the experiment expressed as a proportion of the original shell weight.

Shell Breaking Force

At the termination of the experiment, the left shell valves of all empty shells were used to determine the shell breaking force for each species among the different treatments. A Tensometer Compression Tester was utilised to measure the shell breaking force, which was the force (N) required to cause a shell to break. All valves were orientated in the same direction to maintain consistency of measurements. Forces were always applied to the highest point on each valve in a downward crushing motion. The shell breaking force was measured to a precision of 0.0001N.

Statistical Analysis

Statistical analyses of the data were performed using IBM SPSS 21. Single factor analysis of variance (ANOVA) was used to test the main and interaction effects of treatments on response variables. Data normality and homogeneity of variance were assessed using Kolmogorov-Smirnov tests and Levene tests. All data displayed normal distributions and homogeneous variance. For all cases the significance level was set at $P < 0.05$.

2.3. Results

The mean shell length and mean thickness of each mussel species is summarised in Table 2.2 and the temperature and pH levels recorded per treatment are summarised in Table 2.3. One hundred and twenty adults of each mussel species was exposed to experimental conditions. At the end of the exposure period (42 days) 5 *Aulacomya ater*, 6 *Choromytilus meridionalis*, 3 *Mytilus galloprovincialis* and 15 *Semimytilus algosus* had perished.

Table 2. 2: Mean shell length, standard deviation and shell thickness of four species of mussel used in the experiment

Species	Range of Lengths (mm)	Mean Shell Length (mm)	Shell Thickness (mm)
Aulacomya ater	37 - 42	40 ± 1.65	1.26
Choromytilus meridionalis	48 - 53	50 ± 1.63	1.19
Mytilus galloprovincialis	42 – 47	45 ± 1.83	1.09
Semimytilus algosus	32 – 37	45 ± 1.89	0.42

Table 2. 3: Minimum and maximum seawater pH and temperatures in the four experimental treatments for the duration of the experiment

Tank	pH	Temperature (^{O}C)
Hypercapnic, low temperature	7.46 – 7.52	13 - 15
Hypercapnic, high temperature	7.45 – 7.52	18 - 20
Normocapnic, low temperature	7.98 - 8.0	13 – 15
Normocapnic, high temperature	7.98 - 8.0	18 - 20

On average, all four mussel species lost more shell material in the low pH 7.5 treatments (Figure 2.3, Table 2.4) relative to high pH treatments. Shell loss at low pH (7.5) was significantly greater in the thinner shelled invasive mussels *S. algosus* (Table 2.4) and *M. galloprovincialis* (Table 2.4), but pH had non-significant effects on dissolution of the thicker shelled native mussels' *A. ater* (Table 2.4) and *C. meridionalis* (Table 2.4). Temperature did not affect the percentage shell loss over the duration of the experiment for *Semimytilus algosus* (Table 2.4), *A. ater* (Table 2.4) and *C. meridionalis* (Table 2.4). However *M. galloprovincialis* was significantly affected by temperature, (Table 2.4), showing at high temperature (shell loss = 0.0144) when compared to lower temperatures (shell loss = 0.0104), (Figure 2.3). The results of the ANOVA indicated there was no significant interactive effect of pH and temperature on shell loss in any of the four species (Table 2.4).

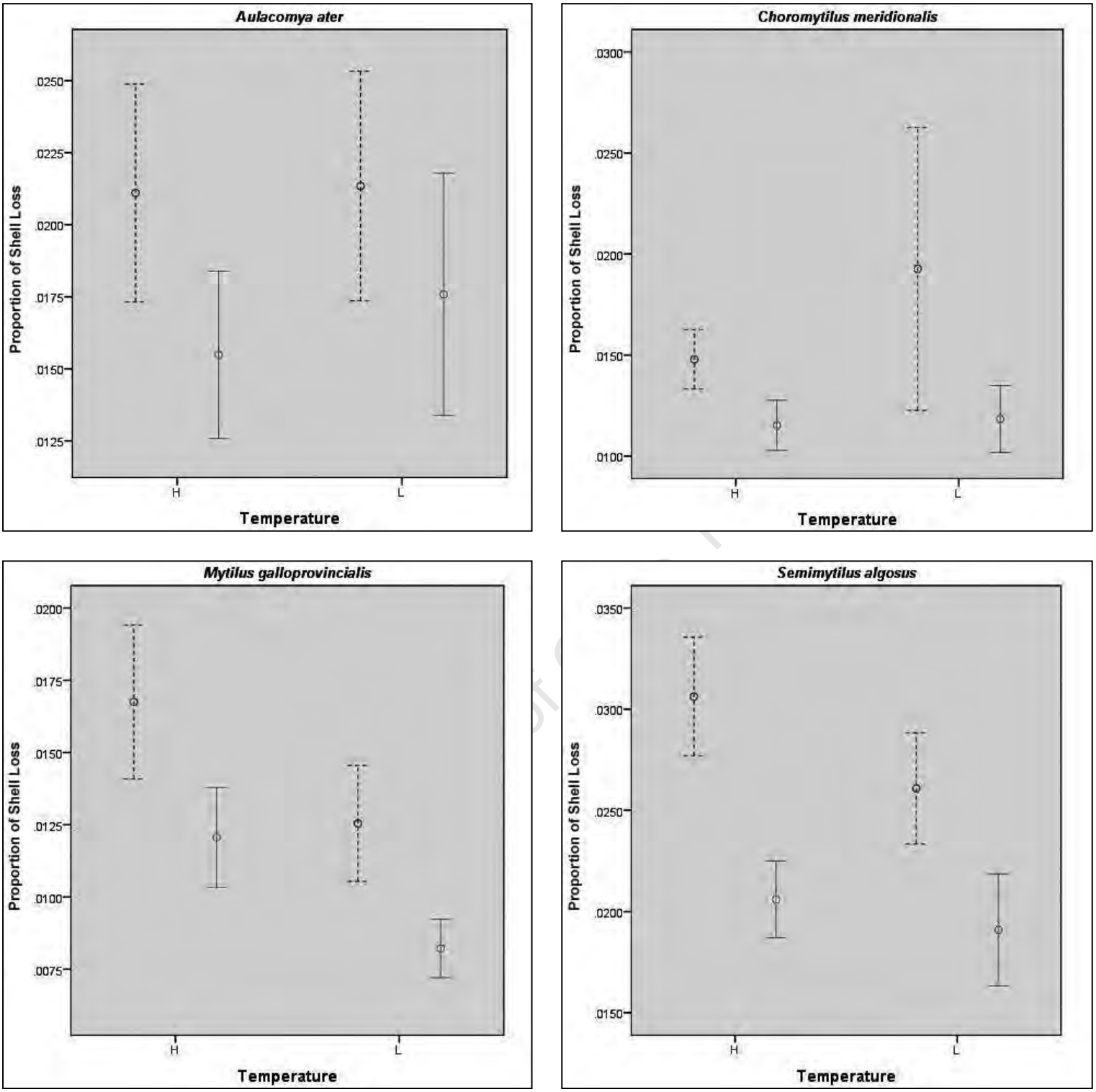

Figure 2. 3: Mean proportion of shell loss under different temperatures (14^{o}C = L and 20^{o}C = H) and pH (solid line = 8.0, dashed line = 7.5) of empty shells of *Aulacomya ater*, *Choromytilus meridionalis, Mytilus galloprovincialis* and *Semimytilus algosus*; each error plot indicates the mean and standard error (n=5)

Table 2. 4: Results of ANOVA testing the effects of pH, temperature and their interaction on mean proportion of shell loss in empty shells of the four mussel species

Species	Treatment									
	Degrees of Freedom	pH			Temperature			pH * Temperature		
		Significance			Significance			Significance		
		(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power
A. ater	1, 56	1.562	0.217	0.233	0.097	0.756	0.061	0.061	0.805	0.057
C. meridionalis	1, 56	2.065	0.156	0.292	0.414	0.523	0.097	0.313	0.578	0.085
M. galloprovincialis	1, 56	5.374	0.024	0.625	4.276	0.043	0.529	0.009	0.927	0.051
S. algosus	1, 56	10.547	0.002	0.891	1.331	0.253	0.205	0.335	0.565	0.088

pH and temperature, when acting independently, had no significant effects on the shell breaking force of any of the four mussel species (Figure 2.4,Table 2.5). Shell breaking force in *S. algosus* was significantly affected by the interaction between pH and temperature (Table 2.5). At high temperature, shell breaking force of *S. algosus* was significantly greater at normal pH (breaking force = 83.50N) than at low pH (breaking force = 62.95N). The interactive effects of pH and temperature had non-significant effects on the breaking force of shells of the other three mussel species.

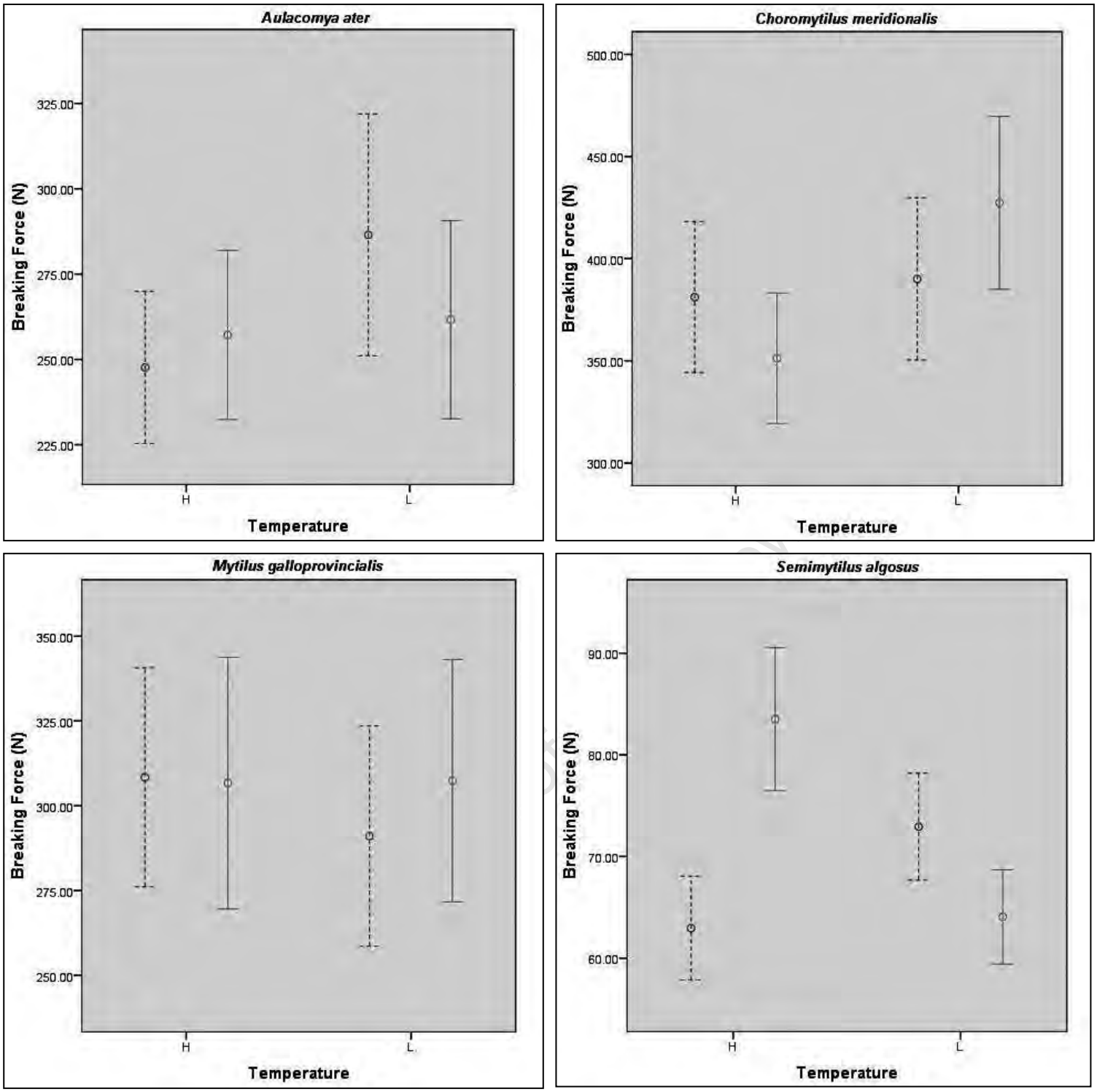

Figure 2. 4: Mean shell breaking force (N) under different temperatures (14°C = L and 20°C = H) and pH (solid line = 8.0, dashed line = 7.5) of empty shells of *Aulacomya ater*, *Choromytilus meridionalis, Mytilus galloprovincialis* and *Semimytilus algosus*; each error plot indicates the mean and standard error (n = 5)

Table 2. 5: Results of ANOVA testing the effects of pH and temperature and their interaction on mean shell breaking force (N) in empty shells of the four mussel species

Species	Treatment									
	Degrees of Freedom	pH			Temperature			pH * Temperature		
		Significance			Significance			Significance		
		(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power
A. ater	1, 56	0.073	0.788	0.058	0.585	0.448	0.117	0.368	0.547	0.092
C. meridionalis	1, 56	0.009	0.924	0.051	1.255	0.267	0.196	0.783	0.38	0.140
M. galloprovincialis	1, 56	0.045	0.832	0.055	0.058	0.81	0.056	0.068	0.795	0.058
S. algosus	1, 56	1.089	0.301	0.177	0.717	0.401	0.132	6.932	0.011	0.735

pH had a significant negative effect on the growth rate of the native mussel, *C. meridionalis*, showing an increase in growth rate at low pH (growth rate = 0.008mm.42d^{-1}) when compared to high pH (growth rate = 0.0055mm.42d^{-1}), regardless of water temperature or exposure to aerial conditions (Figure 2.5, Table 2.6). The growth rate of *C. meridionalis* decreased as pH increased. The thickest shelled mussel, *A. ater*, also exhibited increased growth rates in low pH, high temperature and fully submersed treatments although this was statistically insignificant. Temperature and exposure period had no significant effect on the growth rate of this native mussel (Table 2.6). Invasive mussels exhibited no significant changes in growth rate across all treatments (Figure 2.5, Table 2.6). An exceptionally large variance in growth rate of *M. galloprovincialis* was observed in the normal pH, low temperature, exposed treatment and was due to an unusually high growth rate recorded for a single individual. The ANOVA indicated no significant interactions between temperature, pH and exposure period in terms of their effect on the growth rate of the four species, although there was a marginally non-significant interactive effect of pH and temperature for *M. galloprovincialis* (Figure 2.5, Table 2.6).

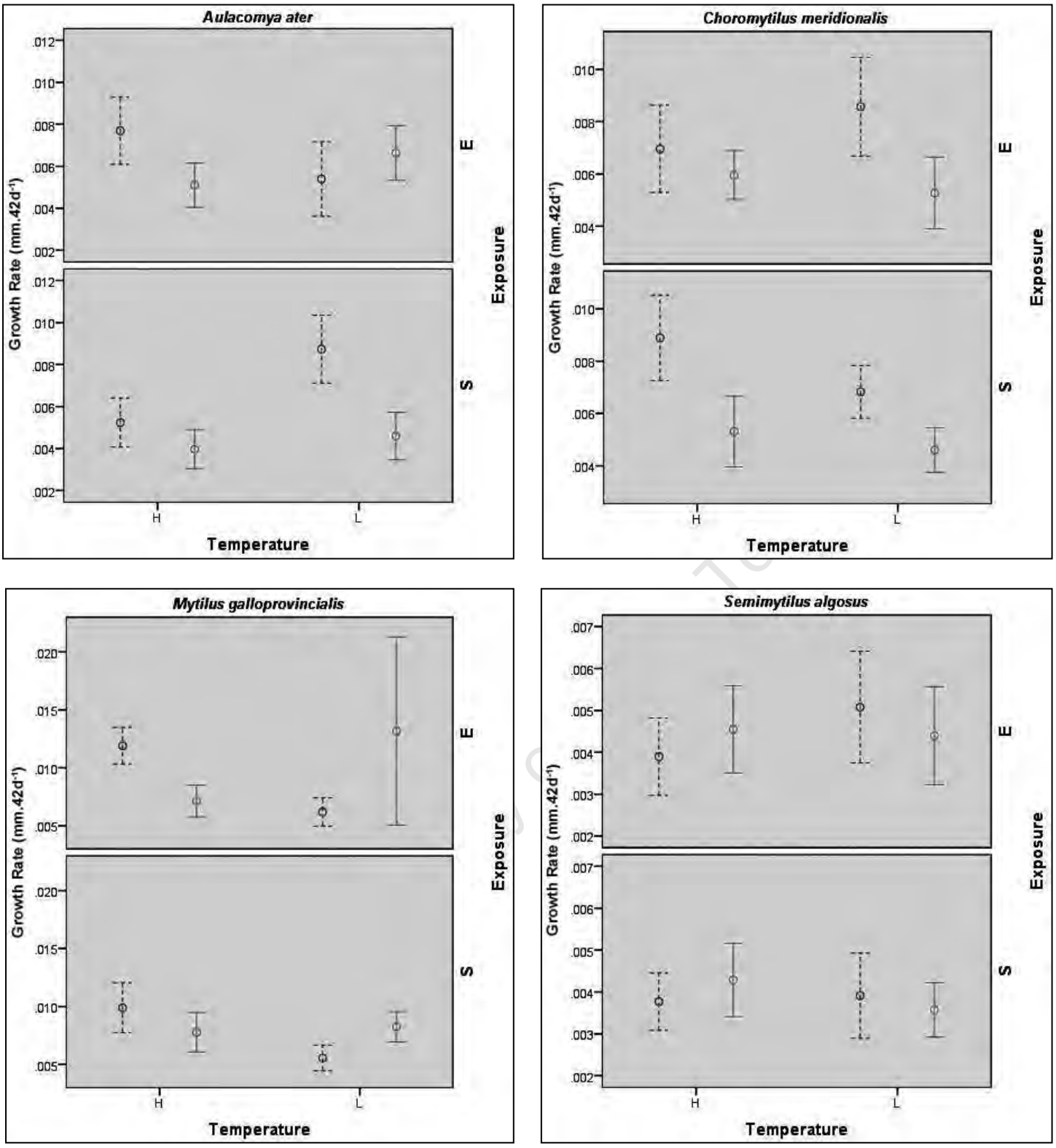

Figure 2. 5: Mean shell growth rate (mm.42d^{-1}) under different temperatures (14^{o}C = L and 20^{o}C = H) and pH (solid line = 8.0, dashed line = 7.5) of live mussels of *Aulacomya ater*, *Choromytilus meridionalis, Mytilus galloprovincialis* and *Semimytilus algosus* at different exposure periods (exposure to aerial conditions = E and submersed permanently = S); each error plot indicates the mean and standard error (n = 5)

Table 2. 6: Results of ANOVA testing the effects of pH, temperature and exposure period and their interaction on mean growth rate (mm.42d^{-1}) in live samples the four mussel species

Species	Treatment											
	Degrees of Freedom	pH			Exposure Period			Temperature			pH * Exposure Period		
		Significance			Significance			Significance			Significance		
		(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power
A. ater	1, 107	3.111	0.081	0.416	0.358	0.551	0.091	0.764	0.384	0.139	1.118	0.293	0.182
C. meridionalis	1, 106	6.643	0.011	0.724	0.083	0.774	0.059	0.22	0.64	0.075	0.145	0.704	0.066
M. galloprovincialis	1, 109	0.092	0.762	0.060	0.563	0.454	0.115	0.147	0.702	0.067	0.031	0.86	0.054
S. algosus	1, 97	0.002	0.961	0.050	0.69	0.408	0.130	0.026	0.872	0.053	0.005	0.942	0.051

Species	Treatment									
	Degrees of Freedom	pH * Temperature			Exposure Period * Temperature			pH * Exposure Period * Temperature		
		Significance			Significance			Significance		
		(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power
A. ater	1, 107	0.062	0.803	0.057	1.621	0.206	0.243	3.049	0.084	0.409
C. meridionalis	1, 106	0.057	0.812	0.056	0.892	0.347	0.155	0.865	0.354	0.152
M. galloprovincialis	1, 109	3.211	0.076	0.427	0.204	0.652	0.073	0.563	0.454	0.115
S. algosus	1, 97	0.584	0.446	0.118	0.315	0.576	0.086	0.028	0.868	0.053

Although the condition index of *A. ater* was higher in the low temperature, low pH and submersed treatment, the ANOVA indicated no significant effect of pH, temperature or exposure period on *A. ater* (Figure 2.6, Table 2.7). pH did not significantly affect the condition indices of *S. algosus* (Table 2.7) and *C. meridionalis* (Table 2.7), but the condition index of *M. galloprovincialis* significantly increased (Table 2.7) as pH decreased, and was most evident in mussels periodically exposed to aerial conditions. The high temperature, low pH and exposed treatment recorded the highest mean condition index in *M. galloprovincialis* (0.0312). An increase in temperature resulted in a decrease in the condition index of *C. meridionalis* (Table 2.7), while the condition indices of the thinner shelled invasive mussels were not significantly affected by temperature (Table 2.7). There was no significant effect of exposure period on the condition index of all mussel species. The condition index of *S. algosus* was significantly affected by the interaction of pH, temperature and exposure period (Table 2.7) with condition index being greatest in the high temperature, normal pH and periodically exposed treatment (0.0280). The results of the ANOVA indicated there were no significant interactive effects on the condition index.

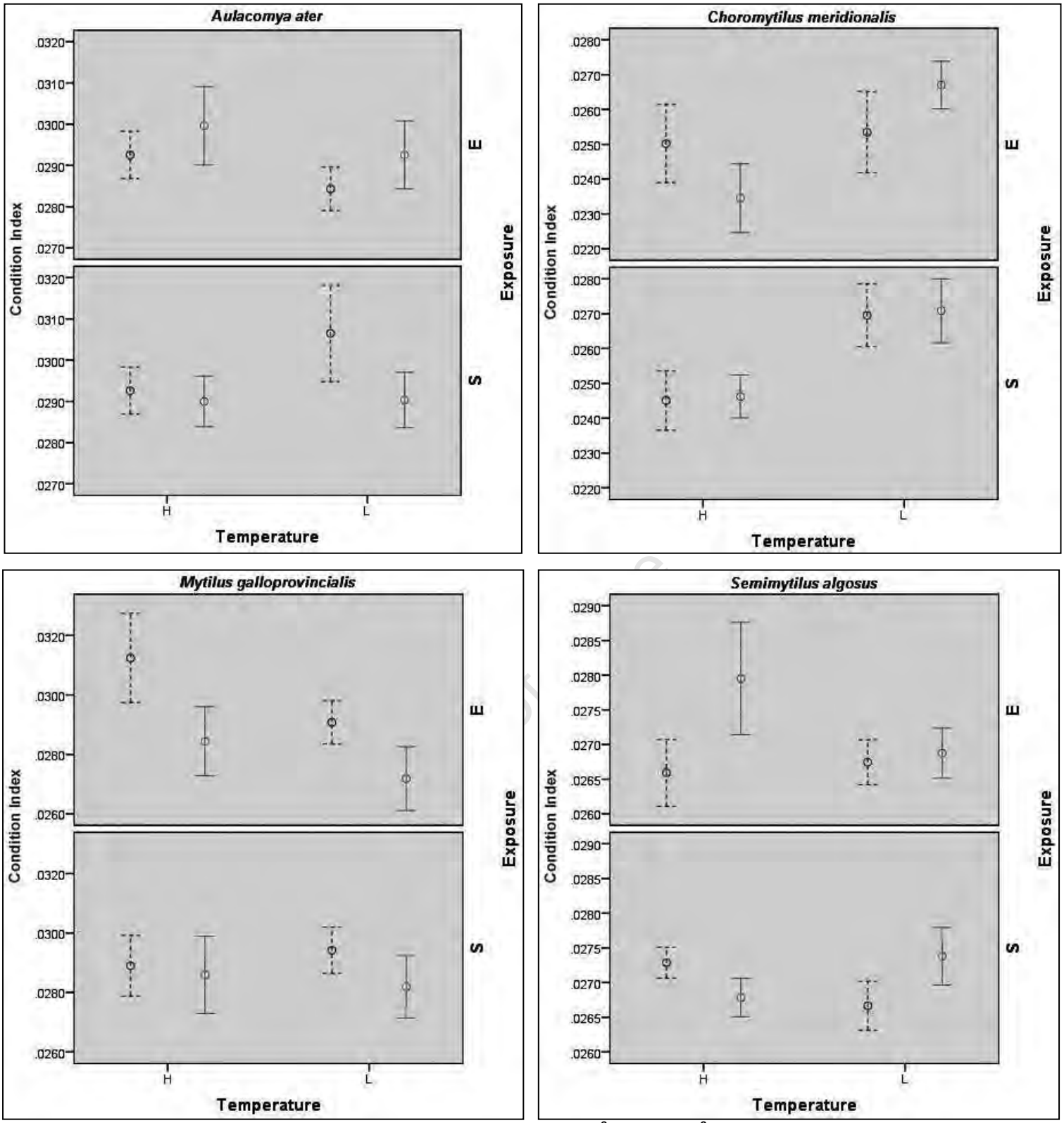

Figure 2. 6: Mean mussel condition index under different temperatures (14°C = L and 20°C = H) and pH (solid line = 8.0, dashed line = 7.5) of live mussels of *Aulacomya ater*, *Choromytilus meridionalis, Mytilus galloprovincialis* and *Semimytilus algosus* at different exposure periods (exposure to aerial conditions = E and submersed permanently = S); each error plot indicates the mean and standard error (n = 5)

Table 2. 7: Results of ANOVA testing the effects of pH, temperature and exposure period and their interaction on mean condition index in live samples of the four mussel species

Species	Treatment											
	Degrees of Freedom	pH			Exposure Period			Temperature			pH * Exposure Period		
		Significance			Significance			Significance			Significance		
		(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power
A. ater	1, 107	0.025	0.875	0.053	0.226	0.635	0.076	0.002	0.962	0.050	2.433	0.122	0.340
C. meridionalis	1, 106	0	0.992	0.050	1.01	0.317	0.169	10.414	0.002	0.892	0.032	0.858	0.054
M. galloprovincialis	1, 109	3.965	0.049	0.505	0.073	0.788	0.058	1.118	0.293	0.182	1.018	0.315	0.170
S. algosus	1, 97	2.096	0.151	0.300	0.001	0.969	0.050	0.653	0.421	0.126	1.188	0.278	0.190

Species	Treatment									
	Degrees of Freedom	pH * Temperature			Exposure Period * Temperature			pH * Exposure Period * Temperature		
		Significance			Significance			Significance		
		(F)	(P)	Power	(F)	(P)	Power	(F)	(P)	Power
A. ater	1, 107	0.319	0.574	0.087	1.829	0.179	0.268	0.454	0.502	0.102
C. meridionalis	1, 106	1.246	0.267	0.198	0.256	0.614	0.079	1.224	0.271	0.195
M. galloprovincialis	1, 109	0	0.996	0.050	1.28	0.26	0.202	0.346	0.558	0.090
S. algosus	1, 97	0	0.992	0.050	0.586	0.446	0.118	4.333	0.04	0.540

The results for shell loss, shell breaking force, growth rate and condition index across all treatments are summarised in Table 2.8. A linear regression analysis of the relationship between shell thickness and four response variables (shell loss, shell breaking force, growth rate and condition index) was also performed and these results are summarised in Table 2.9.

Table 2. 8: Summary of the response of the four mussel species to pH, temperature and exposure period

Species	Response Variable			
	Shell Loss	**Shell Breaking Force**	**Growth Rate**	**Condition Index**
Aulacomya ater	-	-	-	-
Choromytilus meridionalis	-	-	↑ at low pH	↑ at low temperature
Mytilus galloprovincialis	↑ at low pH; ↑ at high Temperature	-	-	↑ at low pH
Semimytilus algosus	↑ at low pH	↑ at low pH-low temperature & at high pH-high temperature	-	↑at high pH, high temperature & exposure

There was a negative linear relationship between shell thickness and shell loss across all four treatments (Table 2.9), indicating that increasing shell thickness resulted in a decrease in shell loss. The shell breaking force increased from thinner to thicker shelled mussels, with a significant positive linear effect of shell thickness on shell breaking force in all four treatments ($P < 0.001$).

Although there was a positive relationship between shell thickness and growth rate (Table 2.9), this finding was only significant in the low pH, high temperature, exposed and the low pH, low temperature, submersed treatments. There was a positive linear relationship between shell thickness and condition index (Table 2.9), but this was only significant in the low pH, low temperature and submersed treatment.

Table 2. 9: Summary of linear regression analysis showing the relationship between shell thickness and four response variables under different temperature (14°C and 20°C), pH (7.5 and 8.0) and exposure periods. Data for all mussel species were pooled for the regression analysis.

Bare Shells												
Response Variable	Treatment											
	pH 7.5; 20°C			pH 7.5; 14°C			pH 8.0; 20°C			pH 8.0; 14°C		
	R	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)
% Shell loss	**0.435**	**-0.016**	**0.001**	0.175	-0.009	0.181	**0.359**	**-0.009**	**0.005**	0.200	-0.007	0.125
Breaking Force	**0.664**	**308.294**	**< 0.001**	**0.649**	**319.122**	**< 0.001**	**0.633**	**274.141**	**< 0.001**	**0.639**	**335.723**	**< 0.001**
Live Mussels												
Response Variable	Treatment											
	pH 7.5; 20°C; Exposed			pH 7.5; 20°C; Submersed			pH 7.5; 14°C; Exposed			pH 7.5; 14°C; Submersed		
	R	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)
Growth Rate	**0.284**	**0.005**	**0.042**	0.231	0.004	0.090	0.110	0.002	0.402	**0.311**	**0.005**	**0.016**
Condition Index	0.137	0.002	0.335	0.026	0.000	0.852	0.097	0.001	0.462	**0.287**	**0.003**	**0.028**
Response Variable												
	pH 8.0; 20°C; Exposed			pH 8.0; 20°C; Submersed			pH 8.0; 14°C; Exposed			pH 8.0; 14°C; Submersed		
	R	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)	*R*	Slope (*B*)	Significance (*P*)
Growth Rate	0.113	0.001	0.420	0.074	0.001	0.581	0.073	0.004	0.595	0.169	0.002	0.206
Condition Index	0.047	-0.001	0.736	0.094	0.001	0.481	0.152	0.001	0.262	0.109	0.001	0.415

* Bold values indicate significant responses

2.4. Discussion

The aim of this study was to assess the interactive effects of pH, temperature and exposure period on native and invasive mussels found on the rocky intertidal zone of the West Coast of South Africa. This was done by subjecting empty shells of the mussels *Aulacomya ater* and *Choromytilus meridionalis* (native), and *Mytilus galloprovincialis* and *Semimytilus algosus* (invasive) to two different pH levels (8.0 and 7.5) at high (20^{o}C) and low (14^{o}C) temperatures. In addition, live mussels were subjected to the above treatments at two levels of exposure period. In general, it was found that these three factors affected shell loss, breaking force, and growth rate and condition index of mussels to varying degrees, with species specific responses.

A few major patterns emerged from the study. Firstly, from the study on bare shells, low pH was found to be the key stressor responsible for increases in average shell dissolution. Regression analysis also showed a linear decrease in shell dissolution with increasing shell thickness (Table 2.8). The thinner shelled alien species (*M. galloprovincialis* and *S. algosus*) exhibited greater losses in shell material than native mussels (Figure 2.3). Lischka et al. (2011) showed that shell dissolution in the marine pteropod, *Limacina helicina*, increased linearly with decreasing pH. The bare shells of the marine gastropod, *Littorina littorea* also exhibited greater shell dissolution when subjected to pH 7.7 relative to pH 8.0 seawater for 30 days (Melatunan et al. 2013). Similarly, McClintock et al. (2009) found a significant increase in shell dissolution of empty shells of the Antarctic bivalves, *Laternula elliptica* and *Yoldia eightsi*, the limpet *Nacella concinna* and the brachiopod *Liothyrella uva* when exposed to low pH (7.4) seawater relative to controls at pH of 8.2. The study by McClintock et al. (2009) also suggested that thin shelled marine calcifying organisms were at

greater risk of shell dissolution compared with thicker shelled marine calcifiers. These lines of evidence are in agreement with the finding that thinner shelled mussels (*M. galloprovincialis* and *S. algosus*) lose more shell material than the thicker shelled ones (*A. ater* and *C. meridionalis*).

In the experiment dealing with bare mussel shells, shell loss can be attributed to dissolution of $CaCo_3$ structures when exposed to low pH seawater and not because of insufficient deposition of shell material (e.g. Michaelidis et al. 2005, Nienhuis et al. 2010, Melatunan et al. 2013). Shells of marine calcifying organisms can either be composed of $CaCO_3$ in the form of aragonite, high magnesium-calcite or low magnesium-calcite (Ries et al. 2009). Calcite is less dense and less soluble than aragonite (Morse et al. 2007). However, shells formed with magnesium rich calcite may be more soluble than aragonite shells (Morse et al. 2007). Ries et al. (2009), testing the effect of ocean acidification on 18 marine calcifying species, found that of the six species that experienced net dissolution, five had shells composed of predominantly aragonite and high magnesium-calcite. Taking this into account, the invasive mussels in the present study may produce shells that are composed primarily of aragonite and high magnesium-calcite, whereas the shells of the native mussels may be composed of primarily low magnesium-calcite. However, Mclintock et al. (2009), studying the effect of ocean acidification on the dissolution rates of the aragonite shells of the bivalves *Laternula elliptica* and *Yoldia eightsi* and the calcite shells of the gastropod limpet *Nacella concinna* and the brachiopod *Liothyrella uva*, found no consistent pattern that calcitic shells have slower dissolution rates than aragonitic shells. Hence the relative solubility of calcite and aragonite shells is still debatable.

Mussel shells are generally composed of an inner nacreous layer, a central prismatic layer and an outer periostracal layer (Mclintock et al. 2009). The outer periostracum is a

protective layer secreted by mussels (Ries et al. 2009; Lischka et al. 2011). Rodolfo-Metalpa et al. (2011), studying the effect of ocean acidification and warming on the mussel *M. galloprovincialis* and the limpet *Patella caerulea*, showed that although both species had an outer calcitic shell layer, *P. caerulea* dissolved faster than *M. galloprovincialis*. *P. caerulea* does not have an outer periostracum, while *M. galloprovincialis* does. It was therefore suggested that the presence and extent of this outer protective layer is the key determinant of the susceptibility of marine calcifying organisms to ocean acidification. Although this was not tested, there is a possibility that the thicker shelled native mussels in the present study have a thicker periostracum, thus making them less susceptible to dissolution during short term exposure to acidification.

At high temperature, the shell breaking force of the thinnest shelled mussel, *S. algosus* was significantly reduced at low pH conditions, suggesting an interactive effect of pH and temperature (Figure 2.4). The lower breaking force may be the result of increased shell dissolution under the aforementioned treatment conditions, which weakened the structural integrity of the shells of *S. algosus* (see Figure 2.3). No other mussel species in the study showed a significant difference in breaking force across the four treatments. The robustness of shells of *A. ater*, *C. meridionalis* and *M. galloprovincialis,* in terms of shell breaking force even under conditions favouring dissolution, suggests that the shells of these species may have not been sufficiently dissolved during the experiment. This is in line with findings by Hiebenthal et al. (2012), who reported no significant change in shell breaking force of *A. islandica* and *M. edulis* when exposed to low pH seawater (7.5) and temperatures as high as 25^{o}C. In the current study, however, the regression analysis showed a positive linear relationship between shell thickness and breaking force (Table 2.9), suggesting that shell integrity increases with shell thickness. Therefore thinner shelled mussels may be more

susceptible to predation, provided that their predators are not adversely affected by climate change as well (Hiebenthal et al. 2012).

Bare shells of invasive mussels, S*. algosus* and *M. galloprovincialis,* dissolved more in low pH seawater than thicker shelled native species (Table 2.8). Increased shell dissolution would be predicted to decrease the growth rate of S*. algosus* and *M. galloprovincialis* but this was not upheld as growth rates of both invasives were not affected by low pH. This may indicate that there are species specific compensatory mechanisms to counter dissolution or that dissolution rates on shells of the invasive species may insignificantly affect their growth rates.

The condition index of *M. galloprovincialis* increased at low pH, while the condition index of *S. algosus* only significantly increased in the high temperature, normal pH and exposed treatment. These findings, however, differ from that of Michealidis et al. (2005), who found a uniform decrease in tissue growth rate when the invasive mussel, *Mytilus galloprovincialis*, was exposed to low pH (7.3) seawater. A study by Range et al. (2012), however, found a linear increase in somatic tissue mass and no change in growth rate of *M. galloprovincialis* when subjected to decreasing pH levels (8.0 – 7.4 units). This finding was further supported by Fernandez-Reiriz et al. (2012), who found that the dry tissue weight increased linearly with decreasing pH. The findings on somatic tissue and shell growth in *Mytilus galloprovincialis* concurs with Range et al. (2012) and Fernandez-Reiriz et al. (2012), while that of *S. algosus* is not significantly affected by the applied levels of acidification, even under conditions favouring shell dissolution.

Although the condition index of *S. algosus* increased in the high temperature, normal pH and exposed treatment, it must be noted that this result may have been confounded by the constraints of the experimental design. Organisms exposed to aerial conditions on rocky

shores experience relatively higher temperatures as compared to permanently submerged organisms (Bertocci et al. 2007; Lathlean and Minchinton 2012). In this study, the ambient temperature of the experimental area had to be lowered to 14^{o}C in order to keep the temperature of the low temperature treatment tanks constant. This in effect resulted in mussels in the exposed treatments to be subjected to low temperatures instead of significantly higher temperatures, therefore resulting in an unnatural exposure condition for mussels.

The present study indicates that some invasive mussels may respond to the stress of ocean acidification, by allocating a larger portion of their energy budget to tissue growth, at the expense of their shell stability. Melzner et al. (2011) suggests that this response is an adaptation to survive in extreme environmental conditions, such as those observed in rocky intertidal zones. However, in the present study, native species increased growth rates in low pH seawater, but exhibited no significant change in condition index due to ocean acidification. Studies by van Erkom Schurink and Griffiths (1990 and 1991) show that the invasive mussel, *M. galloprovincialis,* has a rapid growth rate and high fecundity, which are key factors that make them good invaders. Therefore, it is likely that invasive mussels in this study may in fact employ *r*-type strategies, and redirect energy from shell deposition to somatic growth in order to reach reproductive success under unfavourable conditions. This also suggests that the response of different species to ocean acidification may involve physiological trade-offs between shell deposition, growth and metabolism, in order to fulfil their life histories (Findlay et al. 2011). Beesley et al. (2008) showed that the mussel *Mytilus edulis* has strong physiological mechanisms which protect its body tissue when temporarily exposed to low pH seawater. They, however, concluded that these mechanisms are energetically costly and may result in reduced shell growth, health and survival of *M. edulis*

under periods of long term exposure to low pH. That being said, if this is in fact a life history strategy of invasive species, there is a possibility that, in invasive mussels, shell dissolution may be countered by allocating more energy to somatic growth.

The experiment revealed that the condition index of the native *C. meridionalis* was significantly reduced in the high temperature treatments, while its growth rate increased in low pH treatments. The thicker shelled native mussel, *A. ater* was however not affected by the applied pH and temperature levels. Although *C. meridionalis* and *A. ater* are primarily cold-water adapted mussels, *A. ater* had in previous studies demonstrated a higher tolerance for increases in temperatures up to 20^{o}C (van Erkom Schurink and Griffiths 1991). *C. meridionalis* exhibited a greater increase in respiration and excretory losses at high temperatures and exhibited greater population densities in cool temperate waters (± 15^{o}C) as opposed to warmer waters (20^{o}C). Under the current experimental conditions, the effect of increased metabolic rates due to high temperature, coupled with the stress of dissolution due to low pH seawater, may have incurred greater metabolic repair costs to *C. meridionalis*, therefore leaving less energy for internal tissue growth.

The results of this experiment on live mussels are in agreement with research by Melzner et al. (2011), who show that mussels exhibit high levels of phenotypic plasticity in response to environmental change due to increased anthropogenic CO_2. Melzner et al. (2011) reported phenotypic plasticity in the blue mussel, *M. edulis*, where resources were allocated to somatic growth to the detriment of shell stability in response to low pH and high temperature. Conversely, Melatunan et al. (2013), working on the marine gastropod, *Littorina littorea*, demonstrated that the gastropod devoted a substantially larger amount of energy to changes in shell morphology, in order to maintain its integrity, at the cost of reduced growth and metabolism.

Studies by Hiebenthal et al. (2012) and Melatunan et al. (2013) showed that shell production is energetically costly and may result in reduced tissue growth, as more energy was transferred to shell production and repair under acidic conditions, therefore reducing metabolic activity. Taking this into consideration, it is possible that *C. meridionalis* and *A. ater* increase shell production rather than reproductive output. On the other hand the invasive mussels, *Mytilus galloprovincialis* and *Semimytilus algosus* may not require thicker shells but instead increase growth and fecundity in a shorter period.

Chapter 3: Conclusion and future research

This is the first study comparing the interactive effect of pH, temperature and exposure period on native and invasive mussels in South Africa. The findings suggest that shell thickness determines the susceptibility of the external shell properties of mussels to ocean acidification and high temperatures, but does not mirror effects on internal physiology in terms of somatic tissue and shell growth. Invasive mussels had thinner shells than native mussels and although they incurred most shell loss across all treatments, they produced more somatic tissue than the native species, suggesting phenotypic plasticity among these mussels. This allows them to compensate for unfavourable environmental conditions, by reallocating energy to shell repair and deposition or somatic tissue growth and reproduction. However, the reallocation of energy under prolonged exposure to unfavourable conditions may incur increases in metabolic repair costs, resulting in decreased metabolic rates which will affect mussel growth, performance and ultimately survival.

Mixed compensatory responses of native and invasive mussels to climate change variables were elucidated from the study, suggesting that mussel responses to low pH and high temperature are largely dependent on their specific life history strategies. A review by Sorte et al. (2013), comparing the effects of CO_2 induced changes in temperature and pH on 29 native and 22 invasive marine species, concluded that invasive species outcompeted native species and under acid and warmer conditions, they could possibly replace native species. Our short-term experiment, however, suggests that native and invasive species have regulatory mechanisms to cope with short term changes in the intensity of

environmental stressors, and that both are equally susceptible to exposure to CO_2 induced climate change and ocean acidification.

Here, bare mussels were used to measure shell dissolution. The degree of dissolution is not a true reflection of dissolution in live mussels as mussels are able to regulate their internal acid-base balance (Michaelidis et al. 2005; Lischka et al. 2011; Lischka and Riebesell 2012; Range et al. 2012). Therefore the inner shell layer of live mussels is not directly exposed to low pH seawater and may not be subjected to the same degree of dissolution as its corresponding outer surface. It is therefore important also to study shell dissolution in live marine calcifying organisms, in order to determine the true impact of ocean acidification on them.

Many results in this study suggest that there may be an effect of the different treatments on the mussel species, but the sample size may have been too small to detect these effects statistically. This is indicated by the low power values obtained from the ANOVAs (see Table 2.4 to 2.7). It may therefore be useful to determine the optimal sample size for this type of experimentation in future studies. From this experiment, we have a basis for measuring samples sizes for future research. This study suggests that different bivalve species have significantly different sensitivities to ocean acidification and warming, which makes it difficult to predict the future impacts of climate change variables on marine bivalves. The extent of the impact of ocean acidification and warming depends on a combination of biotic and abiotic factors between and among species in rocky shore communities. In order to track changes of rocky shore communities both on a local and global scale, future research needs to encompass an array of physical and biological factors (inter and intraspecific species interactions) in order to make robust predictions on the

future of coastal habitats in a high CO_2 world. Furthermore, long term studies are important in order to determine the effect of climate change and ocean acidification at a species level.

It is important to understand the underlying mechanisms by which shell production, reproduction and physiological activity are governed and to test their responses to acidification and thermal stress, including invertebrate development in seawater undersaturated in terms of biologically important calcium carbonate minerals. Of particular importance to marine calcifying organisms, is the effect of climate change on carbonic anhydrase, which is an enzyme that catalyses the reversible formation of carbonates from carbon dioxide. Carbonic anhydrase plays an important role in calcification, respiration and acid-base regulation in marine calcifying organisms (Medakovic 2000; Zhenyan et al. 2006).

The magnitude of the effect of ocean warming and acidification on species distribution, community structure and ecosystem functioning depends largely on biological interactions among species and shifts in biotic interactions and abiotic factors (Hiebenthal et al. 2012). Therefore studies need to be focused on community interactions encompassing the stressors of climate change as individual populations act upon each other and may influence the species specific ranges and distributions to a greater extent than climate change alone.

References

Abele D., Heise K., Portner H.O. and Puntarulo S. (2002). Temperature dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. Journal of Experimental Biology. 205 (13),1831–1841.

Bechmann R.K., Taban I.C., Westerlund S., Goadl B.F., Arnberg M., Vingen S., Ingavarsdottir A. and Baussant T. (2011). Effects of ocean acidification on early life stages of shrimp (*Pandeus borealis*) and mussel (*Mytilus edulis*). Journal of Toxicology and Environmental Health, Part A. 74, 424-438.

Beesley A., Lowe D.M., Pascoe C.K. and Widdicombe S. (2008). Effects of CO_2-induced seawater acidification on the health of *Mytilus edulis*. Climate Research. 37, 215-225.

Berge J.A., Bjerkeng B., Pettersen O., Schaanning M.T. and Oxnevad S. (2006). Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. Chemosphere. 62 (4), 681–687.

Bertocci I., Vaselli S., Maggi E. and Benedetti-Cecchi L. (2007). Changes in temporal variance of rocky shore organism abundances in response to manipulation of mean intensity and temporal variability of aerial exposure. Marine Ecology Progress Series. 338, 11-20.

Bibby R., Widdicombe S., Parry H., Spicer J. and Pipe R. (2008). Effects of ocean acidification on the immune response of the blue mussel *Mytilus edulis*. Aquatic Biology. 2, 67-74.

Bownes S.J. and McQuaid C.D. (2006). Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? Journal of Experimental Marine Biology and Ecology. 338, 140-151.

Braby C.E. and Somero G.N. (2005). Ecological gradients and relative abundances of native (*Mytilus trossulus*) and invasive (*Mytilus galloprovincialis*) blue mussels in the California hybrid zone. Marine Biology. 148 (6), 1249-1262.

Branch G.M. and Steffani C.N. (2004). Can we predict the effects of alien species? A case history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). Journal of Experimental Biology and Ecology. 300, 189-215.

Branch G.M., Branch M.L., Beckley, L.E. and Griffiths C.L. (2010). Two oceans: a guide to the marine life of southern Africa. Random House Struik Publishers.

Byrne M. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: Vulnerabilities and potential for persistence in a changing ocean. Oceanography and Marine Biology: An Annual Review. 49, 1-42.

Caldeira K. and Wickett M.E. (2003). Anthropogenic carbon and ocean pH. Nature. 425, 365.

Caldeira K. and Wickett M.E. (2005). Ocean model prediction of chemistry changes from carbon dioxide emission to the atmosphere and ocean. Journal of Geophysical Research. 110, 1-12.

Desrosiers R.R., Desilets J. and Dube F. (1996). Early developmental events following fertilization in the giant scallop *Placopecten magellanicus*. Canadian Journal of Fisheries and Aquatic Sciences. 53, 1382-1392.

Doney S.C., Fabry V.J., Feely R.A. and Kleypas J.A. (2009). Ocean acidification: The other CO_2 problem. Annual Review of Marine Science. 1, 169-192.

Dufois F. and Rouault M. (2012). Sea surface temperature in False Bay (South Africa): Towards a better understanding of its seasonal and inter-annual variability. Continental Shelf Research. 43, 24-35.

Fabry V.J., Seibel B.A., Feely R.A. and Orr J.C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science. 65, 414-432.

Feely R.A., Doney S.C. and Cooley S.R. (2009). Ocean acidification: Present conditions and future changes in a high CO_2 world. Oceanography. 22 (4), 36-47.

Feely R.A., Sabine C.L., Lee K., Berelson W., Kleypas J., Fabry V.J. and Millero J. (2004). Impact of anthropogenic CO_2 on the $CaCo_3$ system in the oceans. Science. 305 (5682), 362-366.

Fernández-Reiriz M.J., Range P., Alvarez-Salgado X.A., Espinosa J. and Labarta U. (2012). Tolerance of juvenile *Mytilus galloprovincialis* to experimental seawater acidification. Marine Ecology Progress Series. 454, 65–74.

Fields P.A., Rudomin E.L. and Somero G.N. (2006). Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. Journal of Experimental Biology. 209, 656-667.

Findlay H.S., Kendall M.A., Spicer J.I. and Widdicombe S. (2010). Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. Estuarine, Coastal and Shelf Science. 86, 675–682.

Findlay H.S., Wood H.L., Kendall M.A., Spicer J.I., Twitchett R.J. and Widdicombe S. (2011). Comparing the impact of high CO2 on calcium carbonate structures in different marine organisms. Marine Biology Research. 7, 565–575.

Gaylord B., Hill T.M., Sanford E., Lenz E.A., Jacobs L.A., Sato K.N., Russell A.D. and Hettinger A. (2011). Functional impacts of ocean acidification in an ecologically critical foundation species. Journal of Experimental Biology. 214, 2586-2594.

Gazeau F., Gattuso J-P., Dawber C., Pronker A.E., Peene F., Heip C.H.R. and Middelburg J.J. (2010). Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. Biogeosciences. 7, 2051-2060.

Gazeau F., Quiblier C., Jansen J.M., Gattuso J-P., Middelburg J.J. and Heip C.H.R. (2007). Impact of elevated CO_2 on shellfish dissolution. Geophysical Research Letters. 34, 1-5.

Grant W.S. and Cherry M.I. (1985). *Mytilus galloprovincialis* Lmk. in Southern Africa. Journal of Experimental Marine Biology and Ecology. 90, 179–191.

Griffiths C.L. and Hockey, P.A.R. (1987). A model describing the interactive roles of predation, competition and tidal elevation in structuring mussel populations. South African Journal of Marine Science. 5, 547-556.

Griffiths C.L., Hockey P.A.R.,Van Erkom Schurink C. and Le Roux P.J. (1992). Marine invasive aliens on South African rocky shores: implications for community structure and trophic functioning. South African Journal of Marine Science. 12, 713–722.

Guppy M. and Withers P. (1999). Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biological Reviews of the Cambridge Philosophical Society. 74, 1-40.

Hammond W. and Griffiths C.L. (2004). Influence of wave exposure on South African mussel beds and their associated infaunal communities. Marine Biology. 144, 547-552.

Helmuth B., Mieszkowska N., Moore P. and Hawkins S.J. (2006). Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. Annual Review of Ecology, Evolution and Systematics. 37, 373-404.

Hiebenthal C., Philipp E.E.R., Eisenhauer A. and Wahl M. (2012). Effects of seawater $_pCO_2$ and temperature on shell growth, shell stability, condition index and cellular stress of

Western Baltic Sea *Mytilus edulis* (L.) and *Arctica islandica* (L.). Marine Biology. 160 (8), 2073-2087.

Hochachka P.W. and Somero G.N.. (2002). Biochemical adaptation: Mechanism and process in physiological evolution. Oxford University Press, New York.

Hofmann G.E., Barry J.P., Edmunds P.J., Gates R.D., Hutchins D.A., Klinger T. and Sewell M.A. (2010). The effect of ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective. Annual Review of Ecology, Evolution and Systematics. 41, 127-147.

IPCC (2007). Climate Change 2007, the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press.

Irie T. (2006). Geographical variation of shell morphology in *Cypraea annulus* (Gastropoda: Cypraeidae). Journal of Molluscan Studies. 72, 31-38.

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. Biology Bulletin. 217, 73-85.

Kado R. (2003). Invasion of Japanese shores by the NE Pacific barnacle *Balanus glandula* and its ecological and biogeographical impact. Marine Ecology Progress Series. 246, 199-206.

Kordas R.L., Harley C.D.G. and O'Connor M.I. (2011). Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. Journal of Experimental Marine Biology and Ecology. 400, 218-226.

Kurihara H. (2008). Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Marine Ecology Progress Series. 373, 275-284.

Kurihara H. and Shirayama Y. (2004). Effects of increased atmospheric CO_2 on sea urchin early development. Marine Ecology Progress Series. 274, 161–169.

Kurihara H., Kato S. and Ishimatsu A. (2007). Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*, Aquatic Biology. 1, 91–98.

Laird M.C. and Griffiths C.L. (2008). Present distribution and abundance of the introduced barnacle *Balanus glandula* Darwin in South Africa. African Journal of Marine Science. 30 (1), 93-100.

Lathlean J.A. and Minchinton T.E. (2012). Manipulating thermal stress on rocky shores to predict patterns of recruitment of marine invertebrates under a changing climate. Marine Ecology Progress Series. 467, 121-136.

Lesser M.P., Bailey M.A., Merselis D.G. and Morrison J.R. (2010). Physiological response of the blue mussel *Mytilus edulis* to differences in food and temperature in the Gulf of Maine. Comparative Biochemistry and Physiology, Part A. 156, 541-551.

Lischka S. and Riebesell U. (2012). Synergistic effects of ocean acidification and warming on overwintering pteropods in the Arctic. Global Change Biology. 18, 3517-3528.

Lischka S., Budendender J., Boxhammer T. and Riebesell U. (2011). Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limancina helicina*: mortality, shell degradation, and shell growth. Biogeosciences. 8, 919-932.

Luthi D., Le Floch M., Bereiter B., Blunier T., Barnola J.M., Siegenthaler U., Raynaud D., Jouzel J., Fischer H., Kawamura K. and Stocker T.F. (2008). High-resolution carbon dioxide concentration record 650,000‐800,000 years before present. Nature. 453,379‐382.

Madeira D., Narciso L., Cabral H.N. and Vanagre C. (2012). Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. Journal of Sea Research. 70, 32-41.

McClintock J.B., Angus R.A., Mcdonald M.R., Amsler C.D., Catledge S.A. and Vohra Y.K. (2009). Rapid dissolution of shells of weakly calcified Antarctic benthic macroorganisms indicates high vulnerability to ocean acidification. Antarctic Science. 21 (5), 449-456.

Medakovic D. (2000). Carbonic anhydrase activity and biomineralization process in embryos, larvae and adult blue mussels *Mytilus edulis*. Helgoland Marine Research. 54, 1-6.

Melatunan S., Carlosi P., Rundle S.D., Widdicombe S. and Moody A.J. (2013). Effects of ocean acidification and elevated temperature on shell plasticity and its energetic basis in an intertidal gastropod. Marine Ecology Progress Series. 472, 155-168.

Melzner F., Stange P., Trubenbach K., Thomsem J., Casties I., Panknin U., Gorb S.N. and Gutowska M.A. (2011). Food supply and seawater $_pCO_2$ impact calcification and internal dissolution in the blue mussel *Mytilus edulis*. PLos ONE. 6 (9), 1-9.

Michaelidis B., Ouzounis C., Paleras A. and Portner H.O. (2005). Effects of long-term moderate hypercapnia on acid-base balance and growth in mussels *Mytilus galloprovincialis*. Marine Ecology Progress Series. 293, 109-118.

Morse J.W., Arvidson R.S. and Luttge A. (2007). Calcium carbonate formation and dissolution. Chemical Reviews. 107, 342-381.

Moy A.D., Howard W.R., Bray S.G. and Trull T.W. (2009). Reduced calcification in modern Southern Ocean planktonic foraminifera. Nature Geoscience. 2, 276-280.

Nagarajan R., Lea S.E.G. and Goss-Custard J.D. (2006). Seasonal variations in mussel, *Mytilus edulis* L. shell thickness and strength and their ecological implications. Journal of Experimental Marine Biology and Ecology. 339, 241-250.

Nalepa T.F., Fanslow D.L. and Lang G.A. (2009). Transformation of the offshore benthic community in Lake Michigan: recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. Freshwater Biology. 54, 466-479.

Nienhuis S., Palmer A.R. and Harley C.D.G. (2010). Elevated CO2 affects shell dissolution rate but not calcification rate in a marine snail. Proceedings of the Royal Society B. 277, 2553–2558.

O'Donell M.J., Hammond L.M. and Hofmann G.E. (2009). Predicted impact of ocean acidification on a marine invertebrate: elevated CO_2 alters response to thermal stress in sea urchin larvae. Mar. Biol. 156, 439-446.

Orr J.C., Fabry V.J., Aumunt O., Bopp L., Doney, S.C., Feely R.A., Gnanadesikan A., Gruber N., Ishida A., Joos F., Key R.M., Lindsay K., Maier-Reimer E., Matear R., Monfray P., Mouchet A., Najjar R.G., Plattner G.K., Rodgers K.B., Sabine C.L., Sarmiento J.L., Schlitzer R., Slater R.D., Totterdell I.J., Weirig M.F., Yamanaka Y. and Yool A. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature. 437, 681-686.

Pigliucci M. (2001). Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, MD.

Portner H.O. (2008). Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology Progress Series. 373, 203-217.

Portner H.O., Reipschlager 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, 43-55.

Range P., Pilo D., Ben-Hamadou R., Chicharo M.A., Matias D., Joaquuim S., Oliviera A.P. and Chicharo L. (2012). Seawater acidification by CO_2 in a coastal lagoon environment:

Effects on life history traits of juvenile mussels *Mytilus galloprovincialis*. Journal of Experimental Marine Biology and Ecology. 424-425, 89-98.

Rayssac N., Pernet F., Lacasse O. and Tremblay R. (2010). Temperature effect on survival, growth and triacylglycerol content during the early ontogeny of *Mytilus edulis* and *M. trossulus*. Marine Ecology Progress Series. 417, 183-191.

Ries J.B., Cohen A.L. and McCorkle D.C. (2009). Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology. 37 (12), 1131-1134.

Rodolfo-Metalpa R., Houlbreque F., Tambutte E., Boisson F., Baggini C., Patti F.P., Jeffree R., Fine M., Foggo A., Gattuso J-P. and Hall-spencer J.M. (2011). Coral and mollusc resistance to ocean acidification adversely affected by warming. Nature Climate Change. 1, 308-312.

Schneider K.R., Van Thiel L.E. and Helmuth B. (2010). Interactive effects of food availability and aerial body temperature on the survival of two intertidal *Mytilus* species. Journal of Thermal Biology. 35, 161-166.

Schwindt E. (2007). The invasion of the acorn barnacle *Balanus glandula* in the south-western Atlantic 40 years later. Journal of the Marine Biological Association of the United Kingdom. 87, 1219-1225.

Simon-Blecher N., Granevitz Z. and Achituv Y. (2008). *Balanus glandula*: from North-West America to the west coast of South Africa. African Journal of Marine Science. 30 (1), 85-92.

Somero G.N. (2002). Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. Integrative and Comparative Biology. 42, 780‑789.

Sorte C.J.B., Ibanez I., Blumenthal D.M., Molinari N.A., Miller L.P., Grosholz E.D., Diez J.M., D'Antonio C.M., Olden J.D., Jones S.J. and Dukes J.S. (2013). Poised to prosper? A cross-

system comparison of climate change effects on native and non-native species performance. Ecology Letters. 16, 261-270.

Thompson R.C., Crowe T.P. and Hawkins S.J. (2002). Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. Environmental Conservation. 29 (2), 168-191.

Tunnicliffe V., Davies K.T.A., Butterfield D.A., Embley R.W., Rose J.M. and Chadwick Jr W.W. (2009). Survival of mussels in extremely acidic waters on a submarine volcano. Nature Geoscience. 2, 344-348.

Van Erkom Schurink C. and Griffiths C.L. (1990). Marine mussels of southern Africa—their distribution patterns, standing stocks, exploitation and culture. Journal of Shellfish. Research. 9, 75–85.

Van Erkom Schurink C. and Griffiths C.L. (1991). A comparison of reproductive cycles and reproductive output in four Southern African mussel species. Marine Ecology Progress Series. 76, 123–134.

van Erkom Schurink C. and Griffiths C.L. (1993). Factors affecting relative rates of growth in four South African mussel species. Aquaculture. 109, 257-273.

Wahl M., Jormalainen V., Eriksson B.K., Coyer J.A., Molis M., Schubert H., Dethier M., Ehlers A., Karez R., Kruse I., Lenz M., Pearson G., Rohde S., Wikstrom S.A. and Olsen J.L. (2011). Stress ecology in FUCUS: abiotic, biotic and genetic interactions. Advances in Marine Biology. 59, 37–105.

Widdicombe S. and Needham H.R. (2007). Impact of CO2-induced seawater acidification on the burrowing activity of *Nereis virens* and sediment nutrient flux. Marine Ecology Progress Series. 341, 111–122.

Widdicombe S. and Spicer J.I. (2008). Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? Journal of Experimental Marine Biology and Ecology. 366, 187-197.

Wood H.L., Spicer J.I. and Widdicombe S. (2008). Ocean acidification may increase calcification rates, but at a cost. Proceedings of the Royal Society B. 275, 1767–1773.

Yamamoto-Kawai M., McLaughlin F. A., Carmack E. C., Nishino S. and Shimada K. (2009). Aragonite undersaturation in the Arctic ocean: Effects of ocean acidification and sea ice melt. Science. 326, 1098-1100.

Yao C.L. and Somero G.N. (2012). The impact of acute temperature stress on hemocytes of invasive and native mussels (*Mytilus galloprovincialis* and *Mytilus californianus*): DNA damage, membrane integrity, apoptosis and signaling pathways. Journal of Experimental Biology. 215, 4267-4277.

Zhenyan Y., Liping X., Seunghwan L. and Rongqing Z. (2006). A novel carbonic anhydrase from the mantle of the pearl oyster (*Pinctada fucata*). Comparative Biochemistry and Physiology, Part B. 143, 190-194.