Does The Transkei Population Of *Haliotis Midae* At The Eastern Edge Of Its Distributional Range Have A Higher Thermal Tolerance Than Those In More Temperate Waters?

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ABSTRACT

The South African perlemoen abalone (*Haliotis midae*) may be vulnerable to rising temperatures on the East coast of South Africa due to climate change. *H. midae* is South Africa's most valuable aquaculture species, and it is thus important to understand its physiological ability to adapt to the expected changes and to identify possible management measures to mitigate the impacts of rising temperatures. This project aimed to understand thermal tolerance and growth rates of offspring from two *H. midae* populations originating from the warm-temperate Central region (Port Elizabeth) and warmer Eastern edge of the species' distribution in the Transkei region.

To determine the effects of temperature on the physiological performance of the two abalone populations, growth rates, oxygen consumption rates and critical thermal maxima (CTM) were compared. A laboratory growth trial was conducted at three controlled temperatures, visibly, the 'optimal' temperature for *H. midae* growth (18 - 20°C), 'pejus' (stressful) temperature (21 - 22°C) and 'critical' temperature (23 - 24°C). Oxygen consumption rates were recorded at optimum (20°C), pejus (22°C) and critical (24°C) temperature using a respirometer. In addition, a farm growth trial with simultaneously spawned cohorts of abalone from the two populations was conducted at ambient temperature (19.5 and 20.0°C).

The laboratory growth trial revealed no significant differences in growth rate between the two populations at the three temperature treatments (ANOVA, $P > 0.05$; df = 2). However, mortalities were high and growth rates low, indicating that the rearing conditions were sub-optimal, possibly masking genetic differences. No significant differences were observed in oxygen consumption rates (ANOVA, $P = 0.18$; df = 2), and CTM (t-Test, $P = 0.31$; df = 3) between the two populations. The CTM for both populations was between 29 - 30°C. The farm trial yielded no significant differences in growth rate during the Nursery phase (t-Test, P = 0.25; df = 2), however significant differences in growth rate were observed during the grow out phase with the Central region abalone offspring growing faster than the Eastern edge population (t-Test, P = 0.04; df = 4) indicating the possibility of a genetic difference between the two

populations. Further experiments will be required to determine whether the differences observed in the growth trial were genetically or environmentally induced.

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Lastly Thank you Lord

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Adenosine triphosphate	ATP
Analysis of variance	ANOVA
Average growth rate	AGR
Average growth rate	AGR
Change in oxygen consumption	ΔO2
Critical temperature	Tc
Critical thermal maxima	CTM
Department of Ichthyology and fisheries science	DIFS
Heat shock factor 1	HSF1
Heat shock protein	Hsp
Heat shock protein 70	Hsp70
Heat shock proteins	Hsps
Heat shock response	HSR
Length	L
Marine heatwaves	MHWs
Pejus temperature	Tp
Sea surface temperature	SST
Standard length	SL
Time	t
Weight	W

DEDICATION

To my mother Johanna Nyanisi Nxumalo, you taught me how to fly and motivated me when I felt like giving up.
I no longer fly just for myself, but I do it for the both of us.
I appreciate the time spent with you in this world.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Problem statement

Increasing sea surface temperature (SST) due to climate change is having a profound effect on the ocean ecosystem, but the physiological responses of different marine organisms are still poorly understood (Pörtner 2001; Fusi *et al.* 2015; Claësson *et al.* 2016). Climate change is understood to be the change in long term weather patterns, both on a regional and global scale due to anthropogenic effects such as carbon emissions (Rahman 2013). Sea surface temperature (SST) effects can be observed through changes in water chemistry, organisms' ecology and biology (Vosloo *et al.* 2013). With an increase in climate change induced temperature anomalies, there are accompanying changes in the dissolved oxygen concentration available to the organisms which may potentially disrupt physiological and biochemical processes (Vosloo *et al.* 2013). An increase in temperature influences the immune response of an organism, thus increasing the susceptibility to disease outbreaks (Chen *et al.* 2019).

The physiological responses of different populations of the same species to increasing SST may vary due to their adaption to the specific local temperature regime (Chen *et al.* 2019). On the South African coast, fish and invertebrates from the east coast have a vulnerability to climate change (Ortega-Cisneros *et al.* 2018). *Haliotis midae* is one of the species that is predicted to be affected by climate change, with changes expected in its distributional range (Ortega-Cisneros *et al.* 2018). *Haliotis midae* is an important aquaculture and fishery species, and it is thus important to understand the species' physiological response to increasing temperature to find ways to mitigate and manage the effects of climate change (Britz *et al.* 1997; Sales and Britz 2001; Gordon and Cook 2004; Vosloo *et al.* 2013).

The environmental temperature directly determines the rates of physiological and biochemical processes of ectotherms such as abalone, including rates of growth, gonadal recrudescence, thermal tolerance, foraging activity and development of the immune response (Britz *et al.* 1997; Claireaux *et al.* 2000; Gillooly *et al.* 2001: Pörtner 2001; Vosloo *et al.* 2013; Fusi *et al.* 2015; Chen *et al.* 2016). An increase in temperature to stressful levels may negatively affect larval development, growth rate, feed consumption, survival rates and oxygen consumption rate (Britz *et al.* 1997; Pörtner 2001; Vosloo *et al.* 2013, Vosloo and Vosloo 2010; Venter *et al.* 2018). There are various ways to measure the effects that temperature may have on an ectothermic organism including growth rate, oxygen consumption, critical thermal maximum, heart rate and genetic expression (Hecht 1994; Tarr 1995; Diaz et al. 2000; Vosloo and Vosloo 2010; Chen *et al*. 2016; Chen *et al*. 2019). There is however an information gap on the South African abalone physiology and its response to climate change.

The current thesis assesses the effect of temperature on oxygen consumption, CTM and growth of two abalone populations from the central and eastern edge of the Eastern Cape. It also tested the hypothesis that the Central region population would perform better within the 'optimum' temperature range, and the Eastern edge population would perform better at stressful 'pejus' and near lethal 'critical' temperatures due to possible adaptation to higher environmental temperatures.

1.2 Literature Review

1.2.1 Effects of climate change induced increasing SST on oceans and aquatic organisms

Climate change influences marine organism physiology through changes in water temperature, cyclic and seasonal natural phenomena, the length and frequency of upwelling events, increasing ocean acidification and low oxygen zones, changes in ocean current patterns, and the rising sea level (Figure 1.1) (Pörtner and Peck 2010). For the scope of this study the effects of increasing sea surface temperature (SST) on marine organisms is discussed.

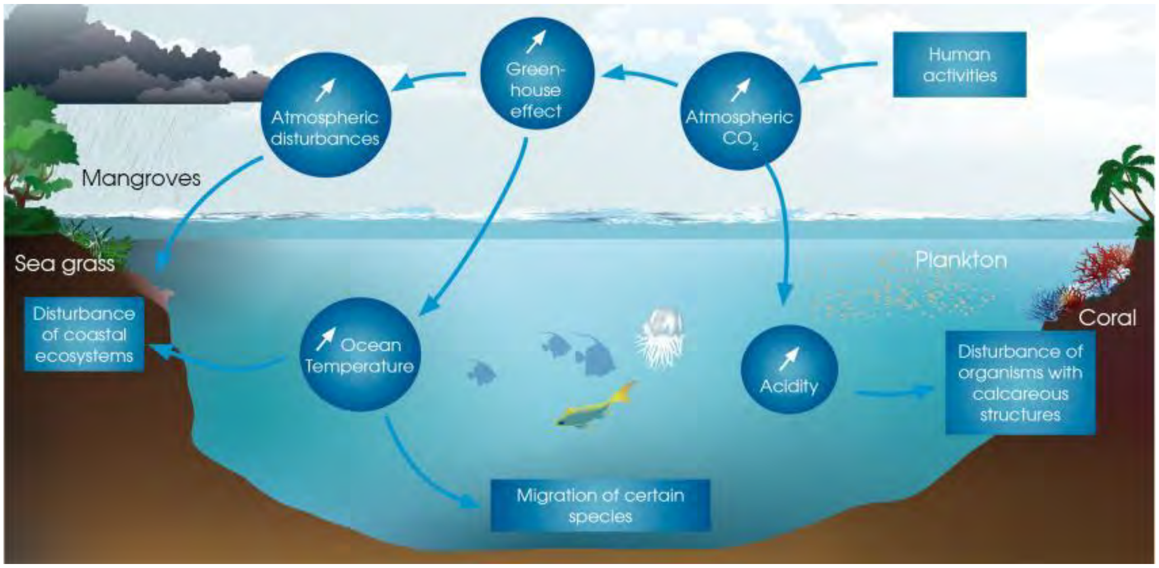


Figure 1.1: Schematic showing the main causes and effects of climate change on the oceans. Source: https://www.iucn.org/resources/issues-briefs/ocean-and-climate-change.

Increasing sea surface temperature (SST) due to climate change is one of the main stressors affecting the future economic and ecological sustainability of the aquaculture and the fisheries sectors (Chen *et al.* 2016). SST is one of the effects that will influence aquatic organism physiology, with increases greater than 0.1°C per decade having been observed; and with a predicted 1 – 3°C increase in temperature by the end of the 21st century (Pörtner *et al.* 2014; Wessel *et al.* 2018). If the predicted temperature increase is correct, there will be further changes that can prove detrimental to the biosphere (Thomas *et al.* 2004; Somero 2005; Pörtner *et al.* 2014; Wessel *et al.* 2018).

With increasing climate induced SST, there is an increase in extreme warming events known as marine heat waves (MHWs) (Hobday *et al.* 2016; Schlegel *et al*. 2017; Schlegel *et al.* 2017b). Marine heatwaves are long distinct abnormal prolonged warm water events, with temperatures of 3 – 5°C above normal temperature (Hobday *et al.* 2016). Marine heatwaves are mainly due to anomalies in wind and current patterns, and regions with the most changes in both wind and current patterns will have an increase in MHWs occurrence (Schlegel *et al*. 2017). MHWs are spontaneous and have been well documented (Hobday *et al.* 2016). For example, in 2003, the Mediterranean Sea experienced MHW, with temperatures 1 – 3°C above average wiping out over 80% of the

Gorgonian fan colonies (Garrabou *et al.* 2009). On the west coast of Australia in 2011, the SST was above normal by 2 - 4°C for over 2 months (Wernberg *et al.* 2012). This event occurred over 2000 km of the west coast and influenced biodiversity and endemism by wiping out Kelp forests (Wernberg *et al.* 2012). This also shifted species composition favouring warm water adapted fish species and further supressed the recovery of the Kelp forests (Wenberg *et al.* 2012). MHWs will also prove detrimental to commercial fisheries and other aquatic animals through diseases as well as thermal stress (Hobday *et al.* 2016; Oliver *et al.* 2017; Schlegel *et al.* 2017). Furthermore, with an increase in temperature there may be an increase in the occurrence of algal blooms which may influence coastal organisms and aquaculture farms that are located on the coast (Whitfield *et al.* 2016). In 2011 between early February and early March Western Australia experienced marine heatwaves that affected the Roe's abalone (*Haliotis roei*) population (Pearce *et al.* 2011). SST were recorded between 26 and 30 °C in late February after 2 months of abnormal high temperatures (Pearce *et al.* 2011). Due to the inability to move away from unfavourable conditions, abalone were more susceptible to high mortalities during the marine heatwave (Pearce *et al.* 2011). Commercial fisher surveys were conducted during March and June 2011 and no abalone populations were recorded north of the Murchison River due to marine heatwaves (Pearce *et al.* 2011). The high mortalities were also associated with algal blooms (Pearce *et al.* 2011). Thermal stress threatens an organisms physiological and biological responses to increasing temperature and thus leaving the organisms susceptible to diseases (Schlegel *et al.* 2017). During summer 2015/2016 in Australia in Tasmania, another MHW occurred with a recorded 3 – 4°C above natural observed temperatures during this time (Oliver *et al.* 2017). The MHWs had a devastating impact on farmed (abalone, Pacific oyster and Atlantic salmon industries) and coastal fisheries (Oliver *et al.* 2017). Marine heatwaves increase water temperature which causes thermal stress and with increased thermal stress organisms are susceptible to disease outbreak for commercially valuable species (Schlegel *et al.* 2017).

With temperature observed as one of the main physical elements that have a biological effect on marine organisms (Chen and Chen 1999; Romo *et al.* 2010). Determining the effects that sublethal stress may have on marine organisms, can aid in understanding the effects that the stress may have on an ecological scale (Chen and Chen 1999).

Temperature has been determined as a controlling factor in the survival and distribution of aquatic organisms (Romo *et al.* 2010). The survival and growth in the early life stages of marine invertebrates has an influence in population dynamics through recruitment (Takami *et al.* 2008). Due to their smaller size, earlier life stages may be more susceptible to changes in temperature compared to their older counterparts (Takami *et al.* 2008). Using young of the year(YOY) for the species *Haliotis discus hannai*, an experiment was carried out to determine if there was a correlation between the winter sea temperature and the recruitment fluctuations, and to also determine if winter sea temperature regulates the distribution limit for the northern most abalone in Kadonohama Bay, northern Japan (Takami *et al.* 2008). Winter mortalities were observed for 2000, 2001 and 2003 - 2006-year classes (Takami *et al.* 2008). There was an observed variation in the winter modalities between the different years, with the variation ranging from 0 - 97.4% (Takami *et al.* 2008). There was an inverse relationship between the winter temperature and rapid mortality rates of the YOY populations (Takami *et al.* 2008). High mortality rates in the experiment were observed when temperatures were below 5°C due to the Oyashio current extending into the study area causing water temperatures to drop between February 2001 and March 2006 (Takami *et al.* 2008). In the other years of study, the Oyashio current did not extended to the study area and temperatures were higher than 6°C (Takami *et al.* 2008). With observed shell length variations between early and post winter, it was suggested that low temperature tolerance may be size-dependent. It was also determined that temperature is one of the factors affecting YOY survival (Takami *et al.* 2008).

With an increase in temperature there is a decrease in oxygen solubility, therefore decreasing the amount of oxygen available for consumption by an organism, which may also lead to stress (Chen *et al*. 2019). Respiration rates can thus be a good indicator of the level of stress exerted on the organism due to an increase in temperature (Harris *et al.* 1999). The oxygen consumption rate of an organism is usually linked with the metabolic rate, which gives a picture of the organism's energy expenditure or activity level and general health with regards to a stressor (Harris *et al.* 1999; Kang *et al*. 2019). Oxygen consumption rates may also show the organism's propensity for growth and or metabolic adaptions to a changing environment (Harris *et al.* 1999; Kang *et al*. 2019). Evolutionary and recent thermal history also has an influence on thermal tolerance (Pörtner 2002; Tomanek and Somero 2002).

Ectotherms' overall fitness including growth, reproductive success, and performance are optimal over a specific temperature range, termed the 'optimum thermal range' (Huey and Kingsolver 1993). Outside the organism's optimum thermal range stress is induced, and this negatively influences growth and reproduction (Vosloo and Vosloo 2010). When temperatures are too high, the organism focuses its metabolic resources on survival and not growth (Vosloo *et al.* 2013). The ability of an animal to adapt to fluctuating temperatures has been defined using the Shelford's law of tolerance (Jost *et al.* 2012), where temperature tolerance is described using three temperature ranges namely the 'optimum', 'pejus' and 'critical' thermal ranges. The optimal thermal range is where the overall physiological performance and fitness of the organism is optimal (Huey and Kingsolver 1993; Jost *et al.* 2012). In the pejus thermal range, overall fitness, activity, and growth of an organism start to decrease as metabolic processes become increasingly compromised (Huey and Kingsolver 1993; Jost *et al.* 2012). In the critical thermal range growth and activity are very low and mortalities may occur (Huey and Kingsolver 1993; Jost *et al.* 2012). Within critical thermal range there is also a shift from aerobic metabolism to anaerobic metabolism (Anestis *et al.* 2007; Pörtner and Farrell 2008; Pörtner 2012).

A functional and precise measurement for evaluating the upper limit of thermal tolerance is vital for both wild and farmed abalone (Chen *et al*. 2016). Conventionally thermal tolerance has been determined through the critical thermal maxima (CTM), where temperature is increased by 1°C every 30 minutes to an hour, until an animal detaches from the substrate (Hecht 1994; Diaz *et al*. 2000; Chen *et al*. 2016). When 50% of the animals have detached from the substrate, this is said to be the critical thermal maxima (Hecht 1994; Diaz *et al*. 2000; Chen *et al*. 2016). The critical thermal maximum provides a relevant ecological index for a species to determine temperatures where thermal limits are reached (Diaz *et al*. 2000). With continued rising temperatures, understanding thermal limits will aid in finding ways to mitigate the effects of rising temperature (Diaz *et al.* 2000). In abalone, changes in temperature may activate long or short-term acclimation

(Vosloo and Vosloo 2010). Acclimation to changing temperatures, occurs at both the physiological and biochemical level, for example, as observed changes in membrane fluidity, enzyme kinetics and function (Vosloo and Vosloo 2010). Abalone possess an inherent ability to sustain physiological function even during body temperature fluctuations because they are poikilotherms (Vosloo *et al.* 2013). It is now accepted that the thermal optima of abalone have a degree of plasticity, the plasticity depends on evolutionary and thermal history of the animal (Vosloo *et al.* 2013). There has been a lot of research done looking at thermal tolerance for different abalone species. A study looking at temperature tolerance for different abalone size classes was conducted by Searle *et al.* (2006), using three size classes (10, 30 and 60mm) from the *Halitios iris* species at 14, 18, 22 and 26℃. Optimal growth was determined to be at 22℃ for the 10 and 30mm size classes and 18℃ for the 60mm size class (Searle *et al.* 2006). The CTM was also determined for the different size classes, at 10mm CTM was 28.8℃, at 30mm it was observed as 27.7 and at 60mm CTM was 27.8℃ (Searle *et al.* 2006). It was determined that temperature tolerance is dependent on size, with the smaller abalone having an increased temperature tolerance compared to the larger abalone (Searle *et al.* 2006). Temperature tolerance was also determined for *Haliotis diversicolor supertexter* at 20, 25 and 30°C for three salinities (25, 30 and 35 psu), the temperature tolerance was determined by increasing and decreasing temperature by 1°C per hour (Chen and Chen 1999). The upper thermal tolerance and lower thermal tolerance were also determined using a higher temperature of 30 - 36°C and a low temperature of 5 - 15°C water baths respectively (Chen and Chen 1999). The CTM (critical thermal maximum and critical thermal minimum) for the species was also determined (Chen and Chen 1999). It was observed that with increasing temperature there was also an increase for both the upper and lower thermal tolerance, however, the upper and lower thermal tolerance for the species was found to decrease with an increase in salinity (Chen and Chen 1999). In some other research that has been done it has been determined that maintaining *H. diversicolor supertexta* at a salinity of 25 - 35 psu and a temperature of 23 - 28°C is optimal for growth of the species (Yang and Ting 1986), while another suggests that increased growth rate for the species can be obtained at 30 - 35 psu and 24 - 30°C (Chen 1986).

For South African abalone, a few previous studies have investigated the effect of temperature on *Haliotis midae* physiology (Hecht 1994; Britz *et al.* 1997; Vosloo and Vosloo 2010). For *H. midae,* it has been determined that the optimum thermal range for growth and feed conversion efficiency is between 12 – 20 °C (Britz *et al.* 1997). It can be inferred from the results of Britz *et al.* (1997) that the pejus range for *H. midae* occurs between 20 – 24°C as growth rate as growth rate and feed conversion efficiency declined sharply with increasing temperature above 20°C, while the mortality rate increased. The critical thermal range for *H. midae* appears to 24°C and above as Britz *et al.* (1997) observed very low growth and feed conversion efficiency and significant mortality over a three-month growth trial. A CTM study was done by Hecht (1994), where CTM was recorded as 27.9°C for *Haliotis midae*. However, a long-term study by Britz *et al.* (1997) determined that 24°C was the critical temperature for *H. midae*, which is lower than the CTM observed by Hecht (1994). Vosloo and Vosloo (2010) investigated the effects of chronic (one month) and acute (24 hours) temperature exposure for cold acclimated *H. midae.* During the investigation three temperatures were used 16, 19 and 22°C. It was determined that cold acclimated *H. midae* can acclimate to increasing temperature, if the temperature is within the optimum thermal range for their growth (Vosloo and Vosloo 2010). It was also determined that *H. midae* acclimates better to temperatures that are stable, with no short and or high temperature spikes (Vosloo and Vosloo 2010).

1.2.2 The biology and distribution of *Haliotis midae* (Perlemoen) on the South African coast

Haliotis midae commonly known as the 'perlemoen' is endemic to South Africa (Britz *et al.* 1997). It is one of five species that are distributed along the rocky coastal South African coast, reaching sexual maturity between 20 – 25mm in size (Wood and Buxton 1996). It is dioecious and release eggs and sperm into the water column (Wood and Buxton 1996). Temperature serves as a reproduction cue for abalone and influences larval success after spawning (Visser-Roux 2011). With an increase in temperature, there is a decrease in the survival of larvae (Visser-Roux 2011). Lower temperatures mean slower larval development (Visser-Roux 2011)

Haliotis midae is distributed along a wide temperature range, from the warm Agulhas current in the east coast to the cold Benguela current in the west coast (Figure 1.2) (Hecht 1994; Britz *et al.* 1997; Sales and Britz 2001; van de Merwe 2009). However, the distributional range of *H. midae* has been divided into three biogeographic regions (Bester- van der Merwe *et al.* 2011; Whitfield *et al.* 2016). These three regions are the west coast, which is a cool temperate region, the south coast, which is a warm temperate region and the east coast, which is a subtropical region (Bester- van der Merwe *et al.* 2011). Oceanographic conditions and ecological factors acting on the different environments were important in determining these regions (Bester- van der Merwe *et al.* 2011). Research looking at the genetic differences between the abalone regions has been undertaken to determine management practices for farmed abalone, as well as abalone ranching practices (van der Merwe 2009). Subtle genetic differences have been observed between the east coast and west coast abalone populations, suggesting two genetically separate populations (Evans *et al.* 2004; van de Merwe 2009). A hydrogeographic barrier was identified between the east coast and west coast populations, inhibiting genetic flow between the two regions (van de Merwe 2009). The genetic evidence has suggested that the abalone population East of Cape Agulhas was more recent ‘founder’ population originating from the Western region that is genetically homogenous (Evans *et al.* 2004; Bester- van der Merwe *et al.* 2011).

The optimal and pejus temperature range for *H. midae* corresponds to its natural distribution, with the monthly average SST ranging from a minimum of 12 – 13°C on the west coast and 21 - 22°C in the East coast (Figure 1.2) (Britz *et al.* 1997). On the east coast, absolute temperatures can reach up to a maximum of over 25ºC, which is outside the optimal temperature preference of abalone (Lyon 1995).

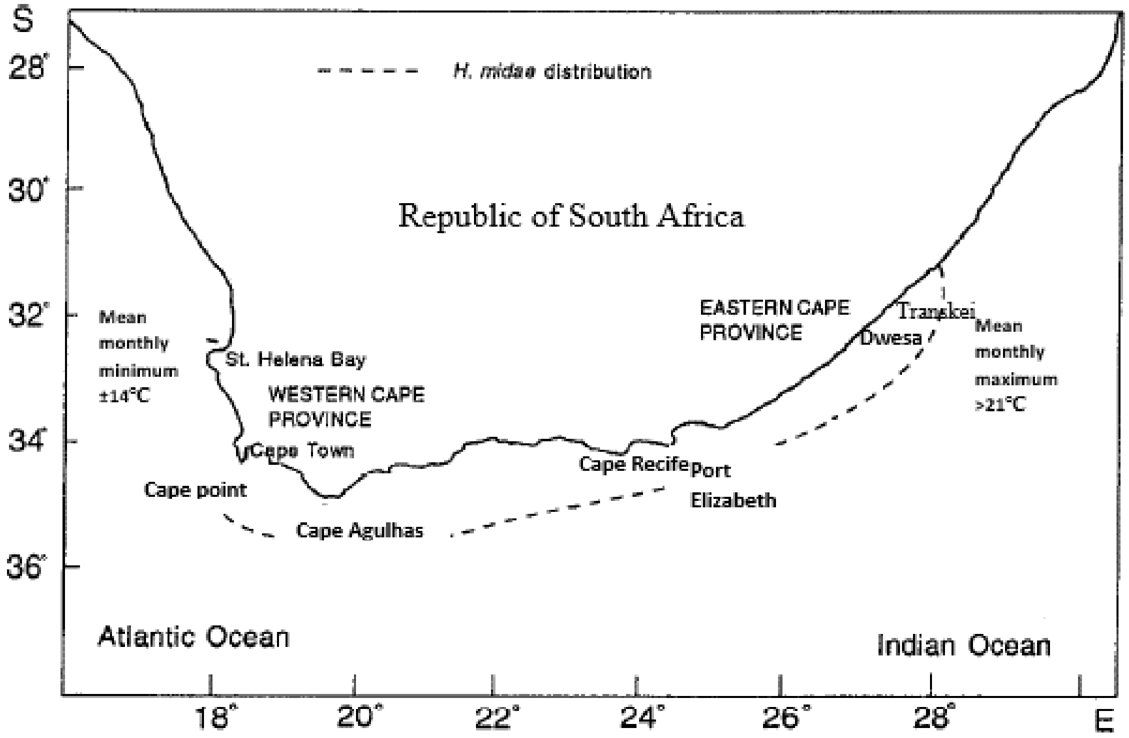


Figure 1.2: The distribution of *Haliotis midae* and the monthly thermal averages. Source: Britz *et al.* (1997).

On the east coast there are further differences in temperature, Port Elizabeth which is central, abalone experience average monthly temperatures between 15 – 22°C, while in Transkei in the eastern edge, which is the transition to the sub-tropical zone, average monthly temperatures range between 16 – 25°C (Mann *et al.* 2003; Goschen *et al.* 2012; Wait and Schoeman 2012). In the light of these observed differences in temperature, it was hypothesised that there may be temperature related differences in growth and survival between the Central (Cape Recife, Port Elizabeth, Eastern Cape) and the Eastern edge population (Transkei, Eastern Cape), with the Eastern edge population possibly performing better at higher temperatures compared to the Central population. Determining performance differences, if any, in growth and survival can aid in mitigating the influence of climate change associated increasing temperature on abalone. The performance of hatchery spawned offspring originating from Central and Eastern edge origin broodstock was thus compared under different temperature conditions.

1.2.3 The influence of climate change on the South African coast and *H. Midae*

Research about the effects of climate change, especially on distributional range are vital in understanding the impact of climate change on populations (Blamey *et al.* 2015; Whitfield *et al.* 2016; Ortega-Cisneros *et al.* 2018). There is an observed trend of a decrease in temperature on the west and south coast, with an increase on east coast (Moloney *et al.* 2013; Whitfield *et al.* 2016). The South and West coast have experienced a substantial cooling in SST approximately by 0.2 – 0.6°C from January to the month of August, while the East coast, including the Transkei region, has warmed up by 0.1 – 0.5°C from January to December (Rouault *et al.* 2010).An increase in temperature on the east coast is causing a distributional shift south-westward by the warmer water species (Whitfield *et al.* 2016). If the observed trend continues, the distribution of warm-temperate species such as abalone will potentially shrink to a confined range in the southern Cape (Whitfield *et al.* 2016; Ortega-Cisneros *et al.* 2018).

Ortega-Cisneros *et al.* (2018) adapted and applied a trait-based sensitivity assessment for the key marine species on the Benguela system to assess the effects and sensitivity to climate-based changes in the marine ecosystem, by looking at abundance distribution and phenology. The study looked at a range of commercially valuable species, ranging from invertebrates (abalone and mussels) and different types of fish, sharks and rays (Ortega-Cisneros *et al.* 2018). Characteristics such as depleted stocks, age at maturity, endemism and or dependence to an environmental cue increased the overall sensitivity to climate change (Ortega-Cisneros *et al.* 2018). For *H. midae*, it was determined they were moderately sensitive to the effects of climate change (Ortega-Cisneros *et al.* 2018). Anthropogenic factors such as high fishing pressure, habitat loss, poaching and pollution were identified that increase their vulnerability to climate change (Ortega-Cisneros *et al.* 2018). Abalone were also determined to have a high sensitivity ranking to climate change in terms of distribution, with spawning highly dependent on the environment (Ortega-Cisneros *et al.* 2018). The overall sensitivity to climate change was ranked as medium-high for *H. midae*.

Cooling and warming events due to climate change have been recorded in parts of the South African coast (Rouault *et al.* 2010; Schlegel *et al.* 2017). The east coast has experienced an increase in sea surface temperature (SST) of 0.5˚C per decade (Rouault *et al.* 2010). The Agulhas current has intensified due to a poleward shift in westerly winds and the increase in trade winds in the south Indian ocean at specific latitudes, hence the increase in SST in the east coast (Rouault *et al.* 2010). The west coast has had a 0.55°C decrease in SST for the past few decades, because of the occurrence in upwelling due to favourable south easterly and easterly winds (Rouault *et al.* 2010).

On the South African coast, future MHW occurrences are predicted (Schlegel *et al*. 2017). It has been determined that during abnormal ocean circulation, warm water is forced onto the South African coast which was the strongest oceanic characteristic during MHWs, while the increase in atmospheric temperature over the subcontinent during onshore and alongshore winds was the strongest atmospheric characteristic during MHWs (Schlegel *et al.* 2017). The South African coast is divided into three regions the west, south and east coast (Schlegel *et al.* 2017; Schlegel *et al.* 2017b). The west coast is distinctively different from the other two regions because it is characterized by the eastern boundary upwelling system, which is the Benguela current characterized by cold water (Schlegel *et al.* 2017; Schlegel *et al.* 2017b). The south and east coast regions are characterized by the western boundary Agulhas current which is characterized by warm water (Schlegel *et al.* 2017; Schlegel *et al.* 2017b). However, the South coast differs from the east coast because it experiences both shear-forced and wind-driven upwelling, it also has greater thermal variability compared to the other two regions due to the influence from both the Agulhas and Benguela current (Schlegel *et al.* 2017; Schlegel *et al.* 2017b). MHWs are said to occur for shorter periods on the east coast compared to the south and west coast, with MHWs intensity also weaker on the east coast compared to the south and west coast (Schlegel *et al.* 2017b). MHWs have been on the rise over 3 decades, with a MHW event occurring every decade in each region but with varying duration and intensity (Schlegel *et al.* 2017b). Oceanic and atmospheric thermal increases are not the only variables influencing MHWs, therefore the continued increase in temperature does not necessarily mean there will be an increase in MHWs (Schlegel *et al.* 2017). However, the changes in climate systems will influence the occurrence in wind and current patterns associated with MHWs which may influence the frequency in MHWs (Schlegel *et al.* 2017). Predictions on the South African coast showed MHWs would occur more in autumn, and spring months compared to the Summer months which has increased SST (Schlegel *et al.* 2017). With marine heatwaves predicted for autumn and spring months, abalone might experience more frequent periods of high temperature due to the increased temperatures during a MHW event and during normal summer months.

1.2.4 Molecular response to thermal stress (Heat shock proteins)

It has also been determined that with climate change influenced SST increase, an organism's survival ability will depend on the protein repair response (Tomanek 2008). Organisms found in different thermal regions have been observed to display variability in the heat shock response (HSR) (Tomanek 2010). The HSR is responsible in determining thermal limits for an organism's physiology (Tomanek 2008). When a protein is heavily damaged Heat shock proteins (Hsps) facilitate the removal of the damaged protein through proteolysis (The breaking down of proteins) (Tomanek and Somero 2002). However, under normal circumstances, Hsps function as chaperone molecules that inhibit protein denaturation and aid in the refolding of the protein into its original conformation (Snyder *et al.* 2001; Tomanek and Somero 2002). Heat shock proteins have been used in determining temperature sensitivity of organisms (Chen *et al.* 2016). When animals are under thermal stress the expression of the heat shock gene is induced increasing the production of Hsps (Tomanek and Somero 2002). When thermal stress is introduced, the multi-chaperone complex dissociates, exposing Heat shock factor 1 (HSF1) monomers and the hsp40, 70 and 90 binds to an unfolded protein (Tomanek and Somero 2002). During normal functioning, the HSF1 is encased in a multi-chaperone complex by hsp40, 70 and 90 (Tomanek and Somero 2002). The free HSF1 enables the transcription of hsp-encoding genes (Tomanek and Somero 2002). Heat shock protein 70 (Hsp70) has been identified as one of the proteins vital in mitigating thermal related stress in invertebrates (Tomanek and Somero 2002; Li *et al.* 2012). It was determined that an increase in thermal stress, has an increase in hsp70 expression for the Pacific abalone *Haliotis discus hannai* (Cheng *et al* 2007; Li *et al.* 2012). In South Africa, there has been research done looking at the hsp expression of abalone with increasing temperature during acute (24hours) and chronic (1 month) exposure to increasing temperature (16, 19 and 22°C) (Vosloo and Vosloo 2010). It was determined that during acute exposure, hsp70 expression was high in all three temperatures, while during chronic exposure hsp70 expression was only high at 22°C (Vosloo and Vosloo 2010).

1.2.5.1 Aim

The project aimed to compare the physiological responses of the Central and Eastern edge population in respect of growth at different temperatures and thermal tolerance.

1.2.5.2 Research Objectives

i. Assess growth and survival rates of the Central and Eastern edge populations at optimal, pejus and critical temperature.

ii. Assess growth and survival rates of the central and Eastern edge population at different temperatures during nursery and grow-out stages

iii. Assess thermal tolerance for both the Central and Eastern edge populations.

iv. Evaluate oxygen consumption rates of the Eastern edge and Central populations at optimal, pejus and critical temperature.

1.3 Research Approach

The study evaluated the effects of temperature on the physiology of two east coast populations, originating from the central and eastern edge distributional ranges. The study tested the hypothesis that the population distributed at the eastern edge in warmer water would have a higher thermal tolerance and grow better at high temperature compared to the Central region population. A laboratory growth trial and oxygen consumption rates were recorded and compared under optimal, stressful (pejus) and critical temperature conditions for both populations. The critical thermal maxima (CTM) was also determined for both populations. A farm experiment was also done to compare growth and survival rates at two different developmental stages (Nursery and grow-out phase)

CHAPTER 2

OXYGEN CONSUMPTION RATES FOR THE EASTERN EDGE AND CENTRAL POPULATION FROM THE SOUTH AFRICAN EAST COAST

2.1 Introduction

The metabolic rate and hence oxygen consumption rate of poikilotherms increases with temperature (Pörtner 2002; Vosloo *et al.* 2013). With every 10°C increase in temperature there is also an increase in the rate of biological processes by a factor of two, which causes an elevation in ATP use, and in-turn elevates oxygen demand to keep the biological processes going (Frederich and Pörtner 2000; Zielinski and Pörtner 2000). When oxygen demand exceeds oxygen delivery to the cells at high temperatures, thermal tolerance becomes affected (Pörtner and Knust 2007; Vosloo *et al.* 2013).

In determining the extent to which temperature affects an organism, one must look at metabolic rate, because it is an essential biological process in energy uptake and transformation, and it is governed by temperature and size of the animal (Pörtner 2002; Gillooly *et al.* 2001; Brown *et al.* 2004). Metabolic rate is the rate at which an organism takes up and transforms energy through the process of respiration (Brown *et al.* 2004). The metabolic rate controls the organism's resource demand from the environment, and it acts as a limiting factor to resource allocation for all elements of fitness (Brown *et al.* 2004). It is determined by finding the difference between the maximum and minimum oxygen consumption rates (Clark *et al.* 2013). The metabolic rate of an organism is equivalent to its respiration rate, because heterotrophs obtain energy from carbon compound oxidation, which can be shown by the following reaction:

$$CH_2O + O_2 \text{ -> Energy} + CO_2 + H_2O$$

The oxygen consumption rate of an organism is thus a direct indication of its respiration rate and is measured by determining the difference between the maximum and minimum oxygen consumption (Brown *et al.* 2004, Clark *et al.* 2013).

When temperature increases, demand for oxygen may surpass oxygen availability, and individuals may experience functional hypoxia irrespective of environmental oxygen levels (Vosloo *et al.* 2013). Ventilation and circulation processes are thus limited by temperature extremes (Frederich and Pörtner 2000; Zielinski and Pörtner 2000).

An abalone farm on the east coast, has reported an increase in mortalities during summer months when temperatures are high (Khuzwayo 2014). This may be due to the decrease in oxygen solubility in water with an increase in oxygen consumption by the abalone, while oxygen delivery to the cells is decreased (Chen *et al.* 2019). With increasing temperatures, the maintenance of cellular processes will determine the tolerance of an organism to temperature changes (Vosloo *et al.* 2013).

With the continued increase in temperature, an organism's sensitivity to temperature can be tested using oxygen consumption rates at three temperature ranges namely: optimal, pejus and critical temperature range. In the optimal temperature range, oxygen consumption rates are normal, with no stress observed on the organism, where normal body and molecular functioning are fully functional, there is also an increase in growth rate (Britz *et al.* 1997; Gilroy and Edwards 1998; Diaz *et al.* 2000). Outside the 'optimal' temperature range is the 'pejus' and 'critical' temperature ranges (Anestis *et al.* 2007; Pörtner 2012). At pejus temperature (T_p), the first signs of a decrease in performance are observed due to limitation in oxygen supply to maintain biological processes (Anestis *et al.* 2007; Pörtner 2012). At T_p, animals reduce their metabolic scope to balance the change in temperature with metabolic needs (Anestis *et al.* 2007; Pörtner and Knust, 2007; Pörtner 2012; Vosloo *et al.* 2013). At critical temperature (T_c), animals can no longer depend on aerobic metabolism and a transition occurs where anaerobic metabolism together with protection mechanisms are initiated to deal with the change in temperature (Anestis *et al.* 2007; Pörtner and Farrell 2008; Pörtner 2012).

Metabolic processes are fuelled by ATP energy release, which supports growth, reproduction, and stress alleviation (Venter *et al.* 2018). During hypoxic conditions, processes that use-up oxygen are stopped and pathways that are not oxygen based are initiated in the form of anaerobic metabolism (Venter *et al.* 2018). Abalone can shift from aerobic to anaerobic metabolism for a short period during decreased oxygen levels due to its ability to use phospho-arginine as an energy source (Morash and Alter 2015; Venter *et al.* 2018). The changes in respiration rates in abalone can vary according to the length of exposure to the varying temperatures (Vosloo and Vosloo 2010). When abalone are exposed to 24 hours of the varying temperatures, there will be an increase in respiration rates, while exposure for a month, would cause a decrease in the respiration rates with an increase in temperature (Vosloo and Vosloo 2010). Understanding the changes in oxygen consumption rates with increasing temperature, may aid in determining temperatures leading to aerobic stress in abalone.

With increasing temperature, there is a significant change in respiratory rate which may provide an indication of possible differences in the physiological adaptive ability between abalone populations (Vosloo and Vosloo 2010). To test whether the central region and Eastern edge abalone populations differed in their physiological response at different temperatures, their respiratory rates were measured, with parameters adapted from a study done by Britz *et al.* (1997), where optimum temperature for abalone growth was recorded at 20°C, and at temperatures above 20°C abalone started showing signs of stress, while 24°C was determined as the critical temperature. The respiratory rates parameters used for the current study were optimum 20°C (optimum), 22°C (pejus) and 24°C (critical).

2.1.1 Research Objectives

Compare oxygen consumption rates of the *H. midae* Eastern edge and Central populations at optimum, pejus and critical temperature.

2.1.2 Hypothesis

H_0 – There would be no significant differences in oxygen consumption rates between the Eastern edge and Central population.

H_a – There would be a significant difference in oxygen consumption rates between the Eastern edge and Central population

2.2 Methods and Materials

2.2.1 Husbandry

The wild original broodstock spawned in this study represented two *Haliotis midae* populations in the Eastern Cape Province; the first broodstock population was collected from Cape Recife, Port Elizabeth which is ‘centrally’ located (33.9608° S, 25.6022° E), and the second population was collected from Dwesa, Transkei (32.2541° S, 28.8772° E) – which has an ‘eastern edge’ distribution. The animals are kept at the Wild Coast Abalone (Pty) Ltd farm in environmentally controlled conditions as part of the farm’s commercial breeding stock. Broodstock from the Eastern edge distribution has been on the farm for over 6 years, while the centrally distributed broodstock has been on the farm for over 10 years. Broodstock for the two populations are kept in separate broodstock rooms. The males and females are held in separate tanks. The tanks are supplied with oxygen and the seawater is filtered at ~1µm. The water flow is at a rate of ~90 L h^{-1}. The central broodstock is maintained at 18.5°C, while the Eastern edge broodstock is maintained at 20°C, as these are found to produce optimal spawning by the farm management. The Eastern Edge population had originally been maintained at 18.5°C but did not come into spawning condition until the temperature was increased to 20°C. This suggested a possible adaptation to higher ambient water temperatures. The broodstock are fed twice a week. The tanks are also cleaned twice a week and the animals are kept on a 12-hour day and night photoperiod. The two broodstock populations were spawned on the same day at the same time in October 2018 to provide animals for this study. The Eastern edge population is spawned at 22°C and the Central population is spawned at 20°C according to the standard hatchery operating procedures.

Spawned eggs from each population are collected into a 20L bucket, and sperm collected from the male tanks is then mixed in. The fertilized eggs are then transferred to a hatching tank. Within hours of fertilization, the trochophores, which are free swimming larvae with yolk reserves, hatch from the eggs. The two larval batches were kept in separate tanks. During the yolk-stage the larvae are monitored daily. After a few days the larvae develop into veliger, at this stage the yolk reserves can no longer sustain the larvae. The veliger larvae are then moved to a settlement section. The larvae are settled in diatom bags with micro-algae where they metamorphose from larvae into spat and attach on to the surface of the plates and feed on the micro-algae. After about three months, the spat are anaesthetized and moved to the weaning section. During weaning, the abalone are weaned off the natural diet and introduced to a formulated diet (Abfeed). The abalone are fed three times a week, and the tanks are also cleaned three times a week. The animals are kept in the weaning section for about 3 months. After three months some of the abalone are moved to the nursery section, while others were sent to the Department of Ichthyology and Fisheries Science (Hereafter referred to as DIFS) in order to carry out the laboratory experiments.

2.2.2 Laboratory Holding System

The abalone used for this experiment were early juveniles (8 – 13mm shell length) and they were kept in the DIFS General lab during the oxygen consumption rates experiment. 180 abalone from each population were collected from the Farm. The abalone were kept in 1.5L holding chambers, in 3 separate water-baths at 18 – 20°C, with salinity 35 ± 1.01ppt and oxygen 100 ± 0.98% saturation. The abalone were kept in three separate water bathes as a form of replication, and to make sure that if there is any influencing factor in the tanks it can be observed and accounted for. The abalone were fed Abfeed with 34% protein content every second day. The abalone were acclimated to lab conditions for two weeks before the commencement of the experiment. The abalone were starved for about 36hours before experiments were conducted. The animals were starved to make sure that the respiratory rates recorded do not

2.2.3 Respirometry

Respirometry to determine oxygen consumption rates for both the Central and Eastern edge population, were conducted using three temperatures, the optimum (20°C), pejus (22°C) and critical (24°C) temperature. The oxygen consumption rates were recorded for the early juvenile stage using static respirometry (PreSense, SDR SensorDish® Reader, Germany) (Figure 2.1). The respirometry sensors were pre-calibrated by the manufacturer. Before the oxygen consumption experiments were conducted, the water for the respirometer was filtered using a 0.45-micron gridded filter paper (MCE membrane filters) (PRIMA) and treated using 12,5% sodium hypochlorite which was neutralised using sodium thiosulphate. The water was treated to decrease the amount of background respiration during oxygen consumption readings.

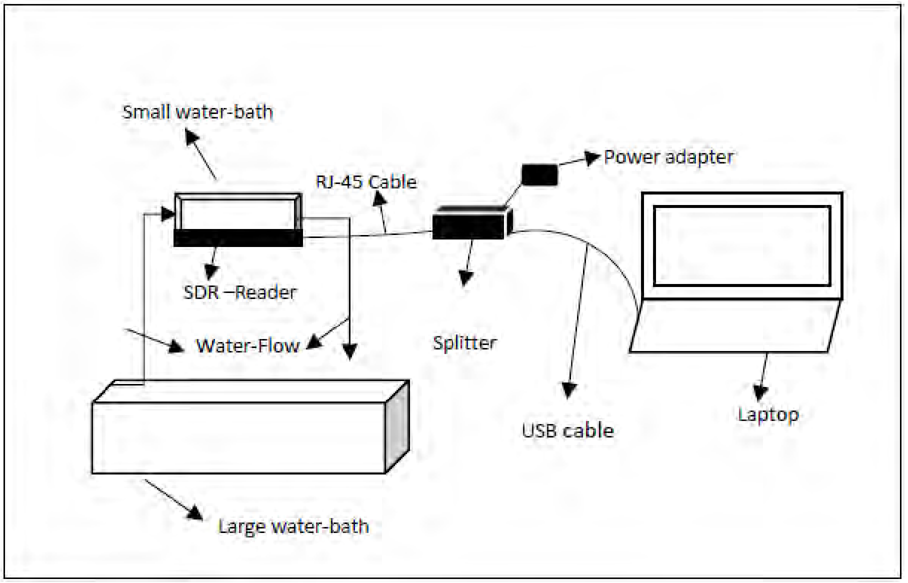


Figure 2.1: The PreSens set-up for measuring oxygen consumption rate. Water from the large water-bath was fed into the small water-bath; the water then flowed out of the small water-bath back into the large water bath. Oxygen consumption was measured by means of An SDR – Reader with micro-plate sensors which was in the small water-bath. The was recorded by a laptop computer connected to the PreSens system.

The experimental abalone were removed from the holding chambers and placed into 1000ml beakers containing sterilised water, which were then placed into the PreSense large water-bath. The abalone were left in the water-bath for an hour to acclimate to the experimental conditions. The abalone were then transferred into a 1700µL plate (Loligo® System), with 24 chambers. For each run an average of 18 animals were placed randomly in a microplate with 6 chambers used to account for any background respiration. The animals were placed in each chamber using fine forceps and sterilized seawater was loaded into the chambers using a pipette. The microplate was loaded into the small water-bath with the SDR Reader and the experiment ran for 30 minutes for each population at each temperature treatment and this was replicated three times.

When the oxygen consumption rates were recorded, the length of each animal was measured, to account for any differences in oxygen consumption rates in relation to size. The length was converted to weight using the equation;

$$W = a \times L^b$$

where W = weight, a = 0.00014, L = length and b = 3.03042, where a and b are constants (Rogers-Bennett *et al.* 2007).

Oxygen consumption rate per unit mass (MO_2) was calculated using the formula;

$$MO_2 = \frac{[\Delta O_2] \times volume/\ M}{t}$$

where ΔO_2 is the change in oxygen consumption in the respirometry chamber (O_{2max} – O_{2min}), volume is the volume of the respirometry chamber in litres, M is the converted weight of an individual animal in a chamber and t is the total time that the respirometry experiment was run.

2.3 Data analysis

Analysis of variance (ANOVA) was used to analyse oxygen consumption rates for both populations and determine whether there were significant differences. Simple regression was used to determine statistical significance for the observed oxygen consumption trends with an increase in temperature. Statistical analysis was done using STATISTICA 13 (Dell Inc. 2015).

2.4 Results

Mean oxygen consumption rates decreased with increasing temperature (optimum, pejus and critical temperature treatments), however this trend was not statistically significant due to the variability of the data (Regression, $P > 0.05$) (Figure 2.2). Oxygen consumption rates did not differ significantly between the two populations for each temperature treatment (ANOVA, $P = 0.18$; df = 2).

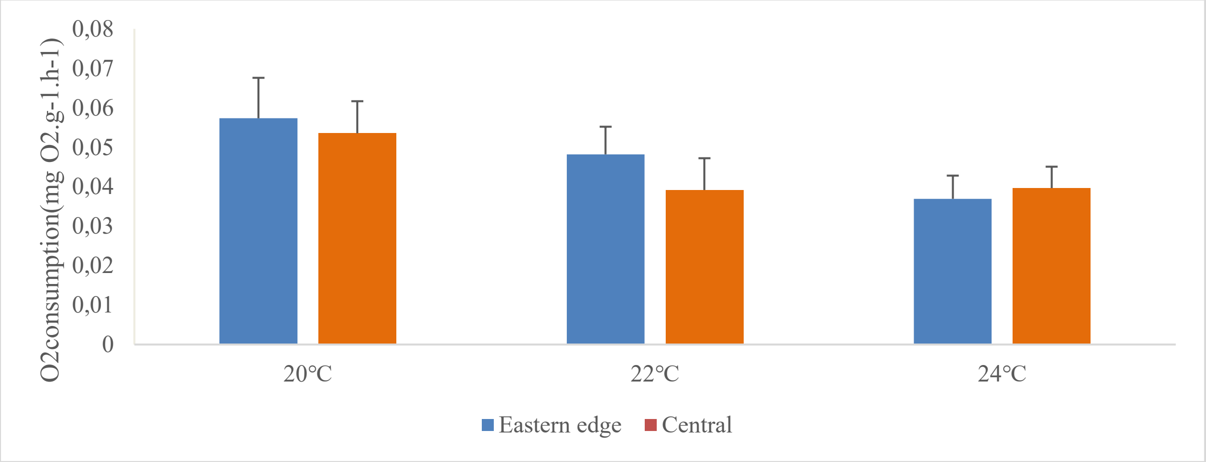


Figure 2.2: The mean oxygen consumption rates and confidence interval of the Eastern edge and Central *H. midae* population. Oxygen consumption rates recorded in three temperature treatments for both populations.

2.5 Discussion

The current study indicated that there were no significant differences in oxygen consumption rates between the Eastern edge and Central abalone populations under the experimental conditions that were tested. Previous studies done by Vosloo and Vosloo (2010) and Khuzwayo (2014) have investigated the effects temperature has on oxygen consumption rates of *H. midae*. Vosloo and Vosloo (2010) examined the effects of an acute temperature change on abalone exposed for 24 hours to three temperatures (16, 19 and 22°C), and observed a trend of increasing oxygen consumption rates with increasing temperature. In the same study, a second longer term exposure experiment was performed, where the abalone were exposed to the three temperature treatments for a month. This yielded a trend of decreasing oxygen consumption rates with increasing temperature (Vosloo and Vosloo 2010). However, during the current study no significant differences in oxygen consumption rates with an increase in temperature were observed. The acclimation period may have influenced the outcome of the results, a longer acclimation to the temperature treatments may yield a difference in results. For the scope of this project a longer acclimation period could not be conducted.

Abalone from the current study did not acclimate well to the living conditions in the lab, before the experiment started. Available evidence from the following chapter will indicate that the animals were chronically stressed as shown by low growth and high mortality. Stress could have reduced oxygen consumption and possibly masked genetic differences in respiration rate for both populations.

The current study failed to reject the null hypothesis, which states that there would be no differences in oxygen consumption rates between the Eastern edge and Central population.

2.6 Recommendations

For future research purposes, it is recommended to use smaller abalone between 2 – 4mm and or larvae to see if there will be differences in oxygen consumption at different life stages. Another recommendation would be to test acute (24hrs) and chronic (1 month) exposure for the two populations to see if any differences in oxygen consumption rates would be observed between the two population over a longer acclimation period.

CHAPTER 3

THE EFFECTS OF TEMPERATURE ON LABORATORY AND FARM GROWTH OF *HALIOTIS MIDAE*

3.1 Introduction

Water temperatures both in the sea and the land-based aquaculture system can fluctuate widely between the summer and winter months (Stone *et al.* 2014; Kang *et al.* 2019). However, ectotherms can regulate their physiological processes to decrease thermal sensitivity and energetic demand during fluctuating temperatures for a short period of time (Kang *et al.* 2019). Energy optimisation during changes in temperature may determine rates of an organism's development, growth and survival (Kang *et al.* 2019).

With temperatures increasing due to climate change, summer mortalities in the aquaculture industry are becoming a serious threat (Chen *et al.* 2016). During summer months abalone may experience water temperatures which are outside their optimum temperature, which has an influence on their growth and survival rate (Stone *et al.* 2014). For examples, in Australia abalone farms have recorded an increase in mortality during summer months for *H. laevigata* (greenlip abalone), and they have also observed an increase in larger abalone mortalities during summer (Lange *et al.* 2014; Stone *et al.* 2014). Similarly, increased summer mortalities have been observed in South Africa, with abalone from the Wild Coast Abalone farm (Wild Coast Abalone Farm production records). It has been suggested that critical temperatures promote tissue breakdown, and bacteria proliferates and becomes pathogenic and increased temperature may increase the chances of bacterial and viral infections (Lange *et al.* 2014; Stone *et al.* 2014). Nutritional supplementation during the tissue breakdown can help ease the breakdown (Lange *et al*. 2014). During increased temperatures abalone are more susceptible to diseases and infection such as *Vibrio* spp (Stone *et al.* 2014; Hooper *et al.* 2014). It has been determined there is a difference in the tolerance of large and small abalone to increased temperature, with the small abalone having greater temperature tolerance compared to larger abalone (Britz *et al.* 1997; Vosloo and Vosloo 2010; Lange *et al.* 2014). The decrease in survival rates during summer months in abalone populations can be attributed to multifaceted interactions between physiology and metabolism, and the abiotic and biotic environmental effects produced in high water temperatures (Stone *et al.* 2014). Farming practises such as spawning procedures, maturation, handling and nutritional factors have been implicated as some of the factors that may influence the increase in mortality for abalone during increased water temperature (Stone *et al.* 2014).

Although abalone can to an extent acclimate to changes in temperature, it is still yet to be determined if they will be able to survive the expected rise in temperature due to climate change. Hence this study aimed to determine the effects of increasing temperature on the growth performance of two geographic populations *H. midae* under aquaculture conditions.

Two farmed abalone populations originating from broodstock collected at Cape Recife (Central region) and the Transkei (Eastern edge) where used. The Central region population is distributed in a region with a monthly average temperature range of 15 – 22°C (Wait and Schoeman 2012). While the Eastern edge population experiences a monthly average temperature of 16 - 25°C (Mann *et al.* 2003). With the observed differences in temperature experienced by the two populations, it was hypothesised that the Eastern edge population could have a higher thermal tolerance and better growth performance at higher temperature compared to the Central population. By performing research to understand the species physiological response to increasing temperature, ways to mitigate and manage the effects of climate change can be identified.

3.1.1 Research Objectives

To assess possible differences in growth and survival rates for the Central and Eastern edge abalone populations:

1) under ambient temperature conditions on an abalone farm and
2) under controlled optimal, pejus and critical temperatures in a laboratory recirculating system.

3.1.2 Hypotheses

H_0 – There are no significant differences in growth and survival rates between the Eastern edge and Central populations under ambient temperature conditions on the farm.

H_a – There are significant differences in growth and survival rates between the two populations under ambient temperature conditions on the farm.

H_0 – There are no significant differences in growth and survival rates between the Eastern edge and Central populations under different temperatures in controlled laboratory conditions.

H_a – There are significant differences in growth and survival rates between the two populations under different temperatures in controlled laboratory conditions.

3.2 Methods and Materials

3.2.1 Experimental Animals

The animals used in this study were from the same batch spawned at the Wild Coast Abalone (Pty) Ltd as described in Chapter 2. Refer to section 2.2.1 for a detailed description of the husbandry.

3.2.1a Laboratory Growth Trial

The juvenile abalone spawned from the two broodstock populations (Central and Eastern edge population) were collected from Wild Coast Abalone farm (hereafter referred to as the farm) for laboratory growth trial experiments under controlled temperature conditions. 315 animals from each population with an average standard length (SL) of 10 ± 0.68mm were used.

Before transport from the abalone farm to Rhodes University, the abalone was starved for two days. The abalone were sedated and placed on a wet cloth in a labelled crate. The crate was placed in a tightly closed oxygenated plastic bag, then placed in a Styrofoam box. Two ice blocks were placed in the box with sponges to restrict movement of the crate. The box was taped closed and then transported by car to the DIFS General lab. The animals were removed from the crates but left in the cloth. They were then acclimated overnight by placing them into separate ~100L water baths (Figure 3.1) with temperatures ranging from 18.5 - 19°C, 100% oxygen saturation, with a salinity of 34 – 35ppt and a pH between 7.9 and 8.5. The abalone were then anaesthetised using magnesium sulphate, and the SL measurements and weighing was carried out over a two-day period.

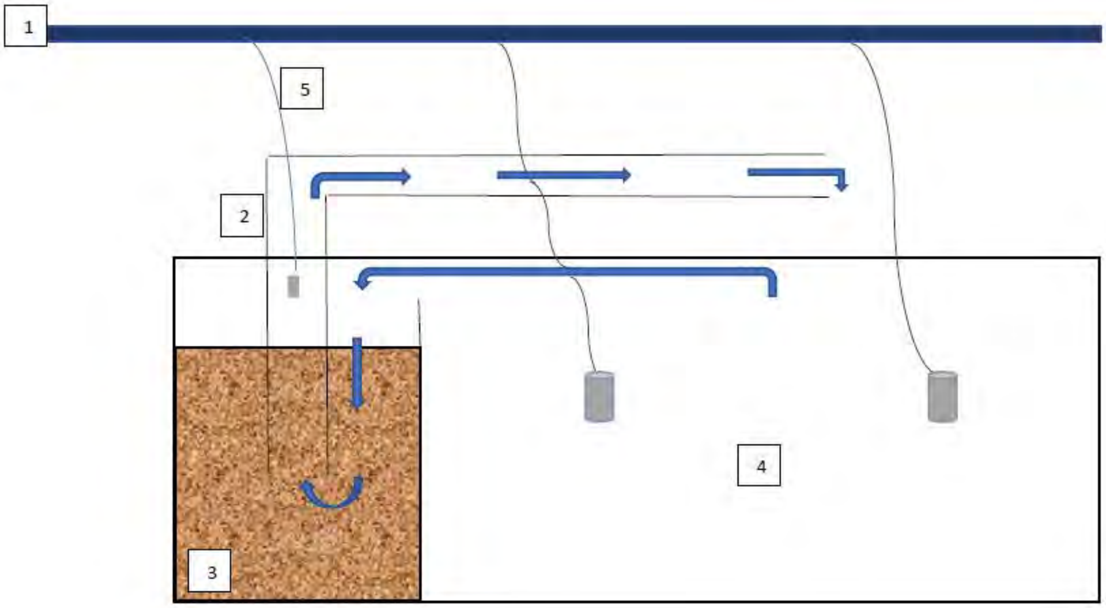


Figure 3.1: Schematic of the recirculation tank units (~100L) used for the experiment. 1 – the main airline, with 5mm air pipes attached to airstones to supply oxygen into the tank. 2 – The water pipe connected to the sand biofilter, pumping filtered water back into the tank. 3 – The biofilter, 4 – The tank with seawater and 5 – the uplift pipe attached to an airstone.

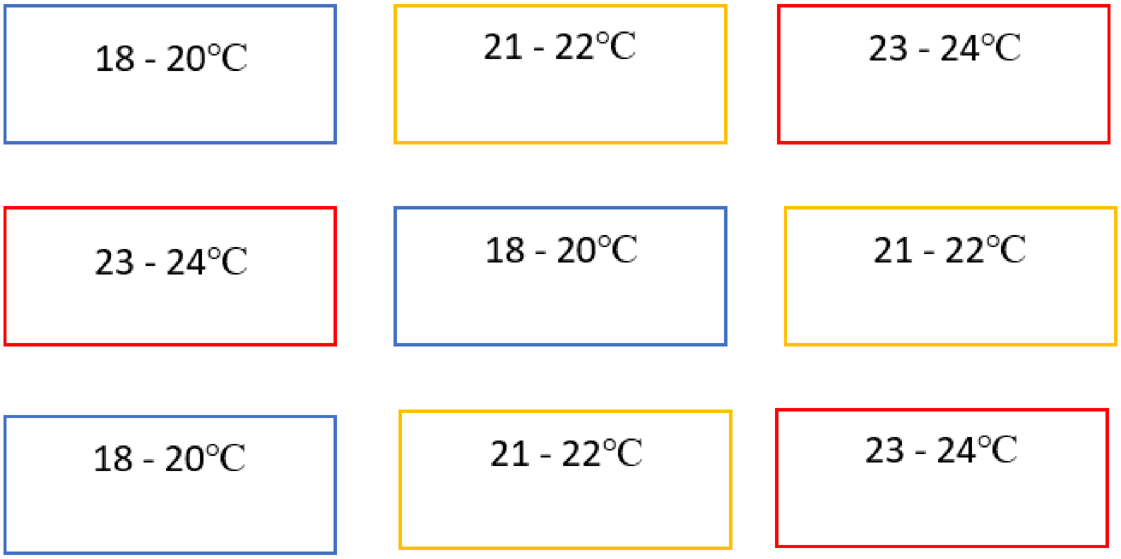
Standard length measurements were made using callipers and the weight was measured using a scale accurate to 0.01g. Measurements were taken every month for 3 months during the growth trial. The average growth rate (AGR) was calculated using SL measurements.

$AGR = \frac{Final(SL) - Initial(SL)}{t}$ where final (SL) was the average final standard-length measurement for the whole experiment, initial (SL) was the average initial standard length for the whole experiment and *t* was the time in months.

The growth trial was initiated in April 2019 and ended early August 2019. The first measurements taken after the initiation of the experiment were in June, due to the high mortalities observed in April and May during the study.

I. Experimental Treatments and Systems

Three temperature treatments were used during the experiment, treatment 1 was the optimal thermal range (18 - 20°C), treatment 2 was pejus thermal range (21 – 22°C) and treatment 3 was the critical thermal range (23 - 24°C) (Figure 3.2).



18 - 20°C	21 - 22°C	23 - 24°C
23 - 24°C	18 - 20°C	21 - 22°C
18 - 20°C	21 - 22°C	23 - 24°C

Figure 3.2: Schematic drawing of the tank set- up for the lab growth trial. The blue indicates the optimal thermal, while the yellow indicates the pejus thermal range and the red indicates the critical temperature range. The replicates from each treatment were randomly placed in each row.

After measurements and weighing, the experimental animals were counted and placed into separate experimental replicate containers (Figure 3.4). 35 animals were placed into each of the 1.5 L transparent plastic containers which were covered in black tape, with a mesh placed at the bottom of the chamber to decrease the amount of light penetrating the chamber. This was done because abalone are sensitive to light and prefer the dark. The lids on the chambers were perforated, to permit the exchange of water between the water bath and the chamber. A pipe was inserted through the 25mm hole on lids of the chambers and an air-stone was inserted into it to circulate water through the jar and to prevent stratification in the chamber. The chambers were then placed into a recirculating aquarium with a biofilter (water-bath). Each water-bath had a representative of both populations in separate chambers, and this was replicated three times for the three temperature treatments. 200W heaters were used to maintain the temperatures in the tanks and an air conditioner was used to maintain ambient room temperature at 18°C. The water in each water-bath was changed every second day, each water change was about 20 - 80% depending on the total mortality in a water-bath. The water was warmed up to the different temperature treatments before it could be put into the water-bath to avoid temperature shock on the animals. The animals were fed a formulated feed (Abfeed) with 34% protein to satiation every second day. Uneaten pellets were removed using a siphon pipe.

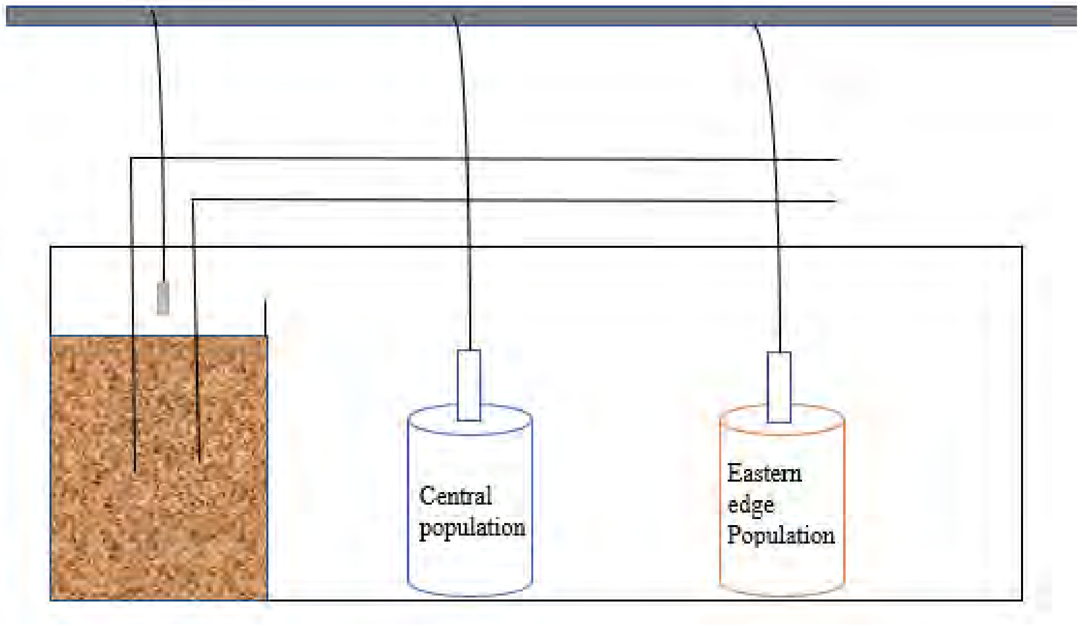


Figure 3.3: Schematic showing the experimental set-up of the populations in each water bath.

Water quality measurements, visibly; temperature, pH and oxygen saturation were taken every second day using the HACH HQ 30d(flexi) multi-meter (Danaher, United States). Salinity was also recorded every second day using a HANNA seawater refractometer HI 96822 (Hanna Instruments (Pty) Ltd). Ammonia levels were measured once a week using the HACH D900 meter (Danaher, United States).

3.2.1b Farm Growth Trial

An experiment was conducted at the farm, to determine the differences in growth rates between the Eastern edge and Central populations under uncontrolled ambient environmental temperature conditions. Abalone (9 – 11mm) from the Eastern edge and Central populations were used for a growth trial which spanned the ‘nursery’ and ‘grow-out’ phases as part of the normal farm grow-out protocol which included size grading.

The abalone were moved from the weaning section of the hatchery into the nursery section at the beginning of the trial. The abalone were anaesthetized using magnesium sulphate, to help detach the animals from their cones in the weaning tank and then weighed and counted according to the standard farm procedure. Three baskets for each population with 2000 animals in each basket were placed in one of the nursery section raceways (Figure 3.4) and subsequently placed in the grow out section raceways (Figure 3.5). The farm growth trial commenced a week after the laboratory growth trial was initiated in April 2019.

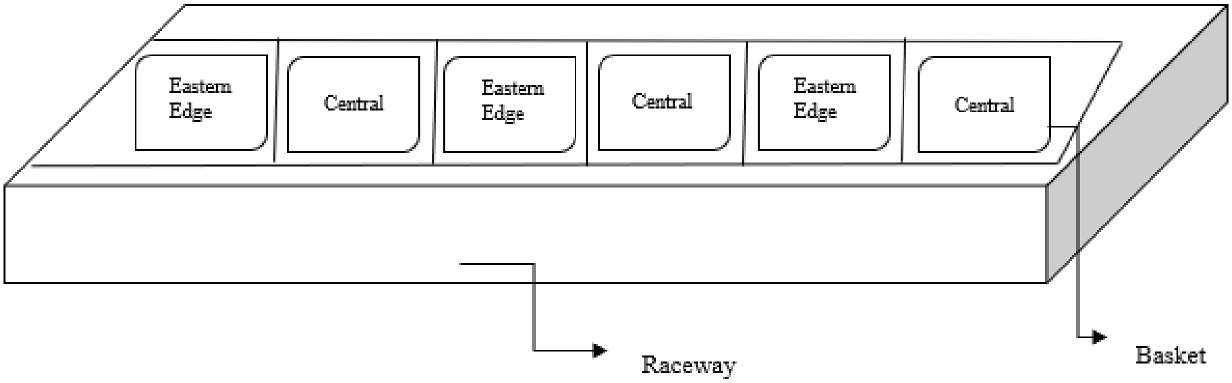


Figure 3.4: A schematic showing the raceway set-up with the baskets containing each population from the Eastern edge and Central distribution for the farm growth trial, in the nursery section.

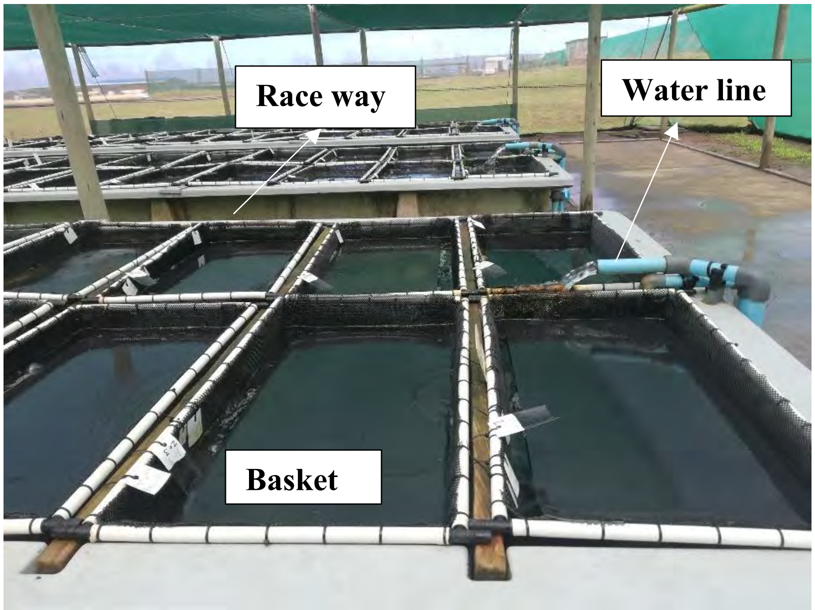


Figure 3.5: Photographic representation of the grow out section raceway, the abalone populations were randomly placed in a basket.

During the experiment the animals were fed Ulva, Gracilaria and formulated feed. Temperature and Oxygen concentrations (99 – 100% saturation) were recorded monthly. The average temperature at the farm was 19.5 – 20.0°C.

Growth was determined by measuring the SL monthly for both populations during the nursery and grow out phase (Figure 3.6). 35 animals were randomly selected from each basket and the SL was measured using a digital Vernier calliper. The animals were left in the farm nursery section and they were grown as per farm conditions. Standard length measurements of the animals were taken every second month. In accordance with the farm procedure, five months into the growth trial in September, the animals were size graded and moved to the grow out section (Figure 3.6). Average growth rates (AGR) were calculated using the SL measurements for both populations, in both the nursery and grow out phase using the formula $AGR = \frac{Final(SL) - Initial(SL)}{t}$ as described above.

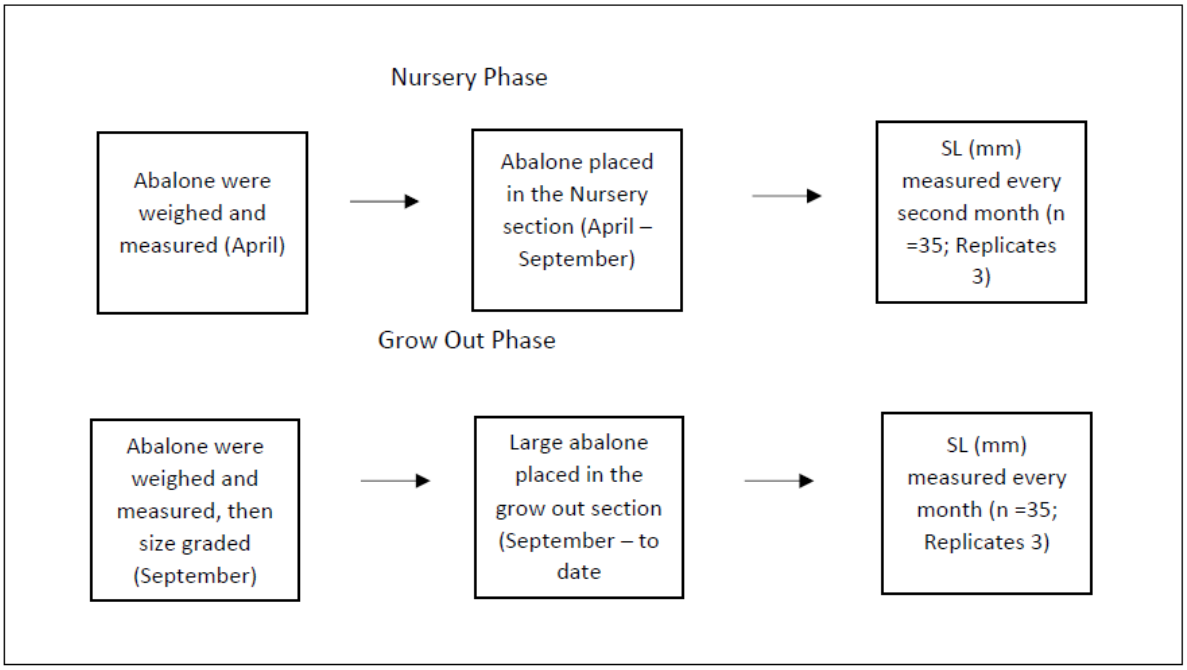


Figure 3.6: A schematic showing the data collection protocol for the farm growth trial for both the nursery and grow out section. The data collection protocol was followed for both the Eastern edge and Central population.

After routine farm size grading and splitting in the nursery, the larger animals were moved into the grow out section (Figure 3.6). Approximately 2000 animals were placed per basket and this was replicated three times for each population in the grow out section. 35 individuals from each replicate were randomly collected, and SL was measured for each population.

3.3 Data Analysis

Analysis of variance (ANOVA) was carried out for the laboratory growth trial and a t-Test for the farm growth trial, to determine if there are any statistically significant differences between the means that were recorded for growth in both populations. Statistical analysis was done using STATISTICA 13 (Dell Inc. 2015).

3.4 Results

3.4.1 Laboratory Growth Trial

I. Water quality parameters

The recorded temperatures were within the target thermal ranges (Table 3.1); but there was a slight decrease in pH and dissolved oxygen with increasing temperature (Table 3.1). Salinity was constant at an average of 35ppt for all three temperature treatments (Table 3.1). Ammonia readings were very low (< 0.02mg/L).

Table 3.1. Mean water quality parameters measured during the laboratory growth trials, with the standard deviations for optimum, pejus, and critical temperature range.

	OPTIMUM (18 -20°C)	**PEJUS (21 – 22°C)**	**CRITICAL (23 – 24°C)**
TEMPERATURE (°C)	19.9 ± 0.40	21.7 ± 0.32	23.4 ± 0.52
PH	7.71 ± 0.09	7.62 ± 0.11	7.58 ± 0.14
DO_2 (MG/L)	8.62 ± 0.17	8.35 ± 0.08	8.24 ± 0.16
SALINITY (PPT)	35 ± 1.01	35 ± 1.05	35 ± 0.96

II. Growth Rates

The observed average SL growth rates between the two populations, did not differ significantly for the optimal, pejus and critical thermal treatments (ANOVA, $P > 0.05$; df = 2) (Table 3.2).

Table 3.2: The initial and final Standard length (mm) and weight (g), for both the Eastern edge and Central population during the laboratory growth trial, with the average growth rate(mm/month) for each population of *H. midae* with the standard deviation (P > 0.05).

	EASTERN EDGE POPULATION					**CENTRAL POPULATION**				
	WEIGHT(g)		**STANDARD LENGTH (mm)**		**AGR**	**WEIGHT(g)**		**STANDARD LENGTH (mm)**		**AGR**
	INITIAL	**FINAL**	**INITIAL**	**FINAL**		**INITIAL**	**FINAL**	**INITIAL**	**FINAL**	
OPTIMUM THERMAL RANGE (18 – 20°C)	0.21 ± 0.02	0.29 ± 0.04	10.2 ± 0.12	12.5 ± 0.28	0.78 ± 0.04	0.26 ± 0.01	0.33 ± 0.02	11.0 ± 0.43	13.5 ± 0.70	0.74 ± 0.26
PEJUS THERMAL RANGE (21 – 22°C)	0.21 ± 0.01	0.33 ± 0.01	10.3 ± 0.37	12.9 ± 0.23	0.86 ± 0.14	0.27 ± 0.02	0.36 ± 0.05	11.0 ± 0.22	13.1 ± 0.37	0.71 ± 0.26
CRITICAL THERMAL RANGE (23 – 24°C)	0.21 ± 0.02	0.34 ± 0.01	10.1 ± 0.23	13.0 ± 0.42	0.96 ± 0.14	0.28 ± 0.02	0.36 ± 0.08	11.1 ± 0.12	13.3 ± 1.05	0.83 ± 0.31

III. Mortality Rates

During the laboratory growth trials there was a high rate of mortality ranging from 25 to 45 individuals per treatment. There were however no significant differences in mortality rates between the two populations (ANOVA, P = 0.38; df = 2). and between the three temperature treatments (Regression, P > 0.05) (Figure 3.7).

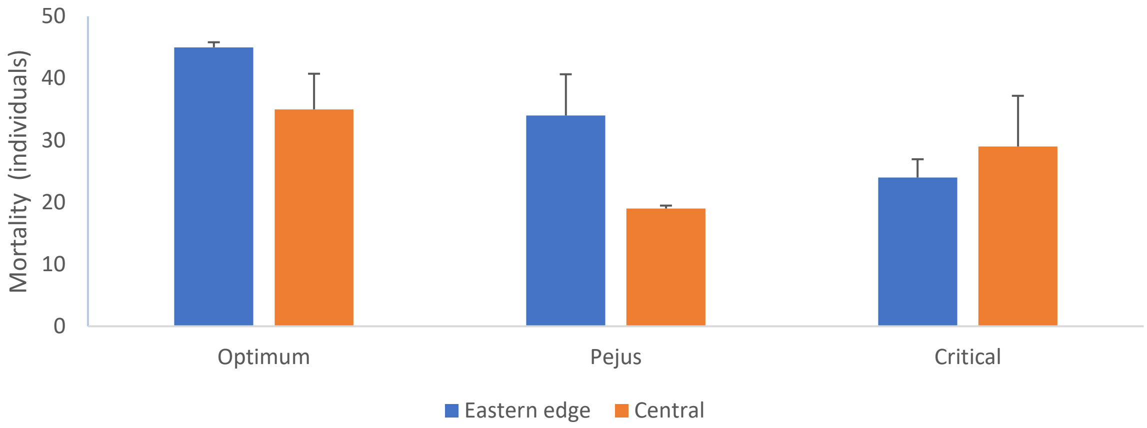


Figure 3.7: The mean mortality rates with the standard deviation of the Eastern edge and Central *H. midae* population grown under laboratory conditions. Mortality rates were recorded every second day of the week at three temperature treatments. The mean mortality rates expressed are from the second and third month of the laboratory growth trial.

3.4.2 Farm Growth Trial

The farm temperature averaged at 19.5 ± 0.76℃ during the nursery phase and 20.0 ± 0.35℃ during grow out phase of the growth trial.

I. Nursery Section Growth

During the nursery phase which began in April, growth rates were calculated from May 2019 to August 2019 (Table 3.3). The observed growth rates differences between the two populations were not statistically significant (Table 3.3) (t-Test, P = 0.25; df = 2).

Table 3.3: The initial and final average standard length(mm) and the average growth rates (mm/month) with the standard deviation for both the Eastern edge and Central population, over a four-month period in the nursery section.

	INITIAL SL (mm)	**FINAL SL (mm)**	**GROWTH RATE (mm/month)**
EASTERN EDGE POPULATION	10.6 ± 0.21	12.6 ± 0.20	0.51 ± 0.10
CENTRAL POPULATION	11.5 ± 0.11	13.3 ± 0.09	0.45 ± 0.02

II. Grow Out Section Growth

Significant growth rate differences were however observed between the two population's in the grow out section from September 2019 to January 2019 (t-Test, P = 0.04; df = 4) (Table 3.4), with the Central population growing significantly faster (Table 3.4).

Table 3.4: The initial and final standard length(mm) measurements and growth rate (mm/month) with the standard deviation, for the farm growth trial in the grow-out section ($P < 0.05$).

	INITIAL SL (mm)	**FINAL (mm)**	**GROWTH RATE (mm/month)**
EASTERN EDGE POPULATION	14.4 ± 0.60	29.4 ± 0.23	3.76 ± 0.17
CENTRAL POPULATION	15.3 ± 0.38	31.7 ± 0.28	4.10 ± 0.13

The growth trial at the farm is ongoing, to see if any long-term growth differences will arise between the two populations. This is because abalone are long lived and slow growing animals. Therefore, long-term growth trials are recommended to determine any long-term variances that may arise between the two populations.

3.5 Discussion

3.5.1 Laboratory Growth Trial

In the laboratory growth trial at optimum, pejus and critical temperatures, average growth rate and mortality did not differ significantly between the Eastern Edge and Central population offspring. The was also no significant differences in growth and mortality observed between the three temperature treatments. The findings are contradictory to those of Britz *et al.* (1997), where with increasing temperature growth rates increased between 12 - 20°C. However, there was a decrease in growth rates for *H. midae* at temperatures greater than 20°C (Britz *et al.* 1997). Stone *et al.* (2014) investigated the effects that increasing temperature at 18, 22 and 26°C may have on 2 and 3year old green lip abalone and found that there were no effects on both populations at 18 and 22°C. However, at a critical temperature of 26°C the survival of 3year old abalone had decreased, while there was no effect on the two-year-old abalone (Stone *et al.* 2014). The abalone used in the current study were less than a year old (9 – 11mm), which may have influenced increased temperature tolerance. The abalone used in this study were also smaller than those from the Britz *et al.* 1997 study (~17mm), which may have also influenced the differences in the findings.

The abalone from the current study did not perform well, displaying low growth and high mortality rates. Growth rates were less than 1mm per month, which were well below the commercial benchmark of 2mm/month for acceptable growth. Furthermore, the high mortality rates in all temperature treatments indicated that the culture conditions were not optimum, and the animals were stressed. Any genetic difference in growth performance may thus have been masked by the sub-optimal rearing conditions. There was insufficient time to rerun the laboratory growth trial within the scope of the present masters study. The trial can however be regarded a useful pilot study to set up the methodology for a more rigorous controlled laboratory trial.

Thus, in conclusion, the current study failed to reject the null hypothesis stating that there would be no significant differences in growth and survival rates between the two populations at optimum, pejus and critical temperatures.

3.5.2 Farm Growth Trial

I. Nursery Section Growth

The growth trial under farm ambient conditions was undertaken to supplement the laboratory growth trials and compare performance under normal commercial aquaculture conditions of fluctuating environmental temperature. During the nursery phase of experiment at an average temperature of 19.5 ± 0.76°C, growth rates did not differ significantly between the two treatments. According to farm standards, the growth rate recorded during the nursery phase growth was slow (< 1mm/month). It was determined that the liners of the baskets had restricted the circulation of water, thus probably affecting oxygen distribution in the baskets and reducing growth rates.

II. Grow Out section growth

After size grading in September, when animals were moved to the grow out section very good growth was observed for the two populations, at an average temperature of 20.0 ± 0.35°C. The findings of an increase in growth rate at 20°C, coincide with those observed by Britz *et al.* (1997), where growth rates and feed consumption were increased between 12 – 20°C. The overall increase in growth may have also been due to the re-introduction of artificial feed into the abalone diet and combined with sufficient water and oxygen circulation (Naidoo *et al.* 2006; Naylor *et al.* 2011; Naylor *et al.* 2014). Under these conditions, a significant difference in growth rate was evident with the 'central' broodstock offspring performing slightly better. The difference in growth may indicate a possibility of a genetic basis influencing the growth performance of the two populations in the farm. There may be a difference in the hsp70 gene expression between the two populations due to the differences in thermal distribution (Tomanek 2008; Tomanek 2010). Further experiments are thus planned to determine what genetic differences, if any, influence the growth rates of the two populations at the farm.

3.5 Recommendations

The laboratory growth rates should be carried out for long than 3 months, especially if there are low mortalities observed during the experiment, to see if there will be any differences in growth and survival rates between the two populations over time. The farm growth trial can be also observed over a longer period.

CHAPTER 4

CRITICAL THERMAL MAXIMA FOR THE EASTERN EDGE AND CENTRAL ABALONE POPULATIONS ON THE EAST COAST OF SOUTH AFRICA

4.1 Introduction

Climate change influences aquatic organisms through the continued increase in water temperature, with aquatic organisms exposed to high temperatures outside their normal thermal range (Diaz *et al.* 2000). Organisms naturally experience temperature fluctuations in the marine environment, the ability to alter their behaviour and internal function daily or seasonally becomes vital in order to survive the changes in temperature (Dahlhoff and Somero 1993). Although temperature fluctuations are common, high temperatures have a negative impact on organism physiology, thus understanding the effects that temperature may have on physiology will aid in determining ways to mitigate these effects on aquatic organisms (Diaz *et al.* 2000; Cheng *et al.* 2006). The continued increase in temperature due to climate change may pose great risk to the abalone aquaculture industry (Chen *et al.* 2016).

The continued increase in temperature impacts the metabolism of an organism, the metabolic rate is the main physiological process that determines the energy required by an organism (Chen *et al.* 2019; Kang *et al.* 2019). When temperatures are high there is an increase in the amount of oxygen required to maintain biological processes, while oxygen availability to the cells is decreased (Pörtner and Knust 2007; Vosloo *et al.* 2013; Lange *et al.* 2014; Duong *et al.* 2016). With increasing temperature abalone feeding, growth and oxygen consumption are also increased, until thermal limits are reached where abalone health and growth decrease (Duong *et al.* 2016; Lange *et al.* 2014).

Increasing temperature has an impact on the thermal tolerance of abalone, it has been determined that abalone have a low thermal adaptation capacity for fluctuating temperatures (Gilroy and Edwards 1998). It has also been determined that abalone cannot withstand severe temperature shock, in the wild abalone may experience quite wide fluctuating temperatures for long- or short-term periods (Gilroy and Edwards 1998; Diaz *et al.* 2000; Stone *et al.* 2014). During summer months farms have recorded an increase in mortalities due to the increase in temperature (Stone *et al.* 2014). Stone *et al.* (2014) observed that an increase in temperature above 22°C during summer showed an increase in mortality for *H. laevigata*. South African farms also experience an increase mortalities summer, when temperatures are often over 20°C and can reach up to 25°C (Britz *et al.* 1997; Khuzwayo 2014; Wild Coast Abalone farm production records).

There are different methods that can be employed to determine the effects that temperature may have on aquatic organisms, among these methods is the use of the critical thermal maximum (CTM) (Diaz *et al.* 2000). The CTM is used to determine the practical ecological index, because the temperature experienced by the organism during the experiment may occur in the wild for either a long or short period of time in the ocean (Diaz *et al.* 2000). CTM can also be used to determine the upper thermal tolerance limits of invertebrates and vertebrates (Becker and Genoway 1979). Generally, the CTM is determined by an increase in water temperature by 1°C every 30 minutes to an hour, until an animal detaches from the substrate. When 50% of the animals have detached from the substrate, this is said to be the CTM of that organism (Hecht 1994; Diaz *et al.* 2000; Chen *et al.* 2016).

There have been multiple studies conducted to determine the CTM of abalone. In a study done by Gilroy and Edwards (1998) on two abalone species *Haliotis rubra* an *Haliotis laevigata*, it was determined that the cold adapted *H. rubra* had a lower CTM which was 26.9°C and a preferred temperature which was 16.9°C, compared to the warm adapted *H. laevigata* which had a CTM of 27.5°C and a preferred temperature of 18.9°C (Gilroy and Edwards 1998). For the abalone on the South African coast, two abalone populations from the west coast (cold adapted) and east coast (warm adapted) were collected and their preferred temperature and CTM were determined (Hecht 1994). It was observed that there were no significant differences in temperature preference and CTM between the two populations. The preferred temperature was between 24.1 and 24.5°C, while CTM was 27.9°C.

Two populations of *Haliotis midae* from the east coast were used to determine and compare CTM. The populations are found in two regions with differences in SST. With the Central region (Cape Recife) experiencing minimum temperatures of about 15°C and the Eastern edge region (Transkei) experiencing minimum temperatures of about 16°C (Mann *et al.* 2003; Wait and Schoeman 2012). Due to the differences in temperature with the Eastern edge population adapted to slightly higher temperatures, it was inferred that there would be a difference in thermal tolerance between the two populations. With the eastern edge population having a considerable higher CTM compared to the Central region population.

4.1.1 Research Objective

i. Determine CTM of the Eastern edge and Central distribution *H. midae* populations.

4.1.2 Hypothesis

H_0 – There is no significant differences in CTM between the Eastern edge and Central populations.

H_a – There is a significant difference in CTM between the Eastern edge and Central population, with the Eastern edge having greater thermal tolerance compared to the Central population.

4.2 Methods and Materials

For the purposes of this study, the critical thermal maximum of a population was defined to be the temperature at which 50% of the organisms had detached from a substrate.

4.2.1 Experimental Animals

The animals used in the study were collected from the Wild Coast Abalone (Pty) Ltd, detailed methods of husbandry are in Chapter 2; section 2.2.1. The CTM of the Central and Eastern edge populations was determined using early juveniles ranging from about 8 – 14mm in standard length.

4.2.2 Experimental system

The abalone used in the experiment, were the same animals that were used for the oxygen consumption rates experiment (Chapter 2). After this experiment, the abalone were put back into the holding chambers and left to recover for a month. They were maintained at an average temperature range of 18 – 20°C, salinity was 35 ± 1.01ppt and oxygen was at 100 ± 0.98% saturation. The abalone were fed a pelleted diet (Abfeed) with 34% protein content.

For the CTM experiments three tanks (~100L) with a recirculating sand filter were used to determine CTM. One tank was used for the experimental treatment, the second for the control and the third for recovery. Two chambers were placed into each of the water-bath, with 1 chamber representing each population (This was repeated three times). Before the experiment commenced, the tape used to darken the chambers was removed, and the animals starved for 24 hours. Twenty animals were placed in the experimental chamber and the control respectively. The abalone from the control, were not subjected to any temperature changes and no animals detached from the control substrate.

During the CTM experiment, the temperature in the experimental tank was increased every hour by 1 ± 0.5°C, from the 18 ± 1°C acclimation temperature. The movement and the number of animals that detached from the substrate were recorded. The experiment was repeated three times. The detached animals were placed in a recovery tank overnight which was 23 ± 1°C so that they could recover from the experiment. When 50% of the animals in a chamber had detached from the substrate, the final temperature was recorded as the CTM.

4.3 Data analysis

For this experiment, a Student's t-Test was used to determine if there are any statistically significant differences between the means that were recorded for the CTM for both populations. The statistical analysis was done using STATISTICA 13 (Dell Inc. 2015)

4.4 Results

The critical thermal maxima for the *H. midae* populations was 30.0 ± 1.41℃ for the Central Population and 29.0 ± 0.94℃ for the Transkei population. The means did not differ significantly (t-Test, P = 0.31; df = 3).

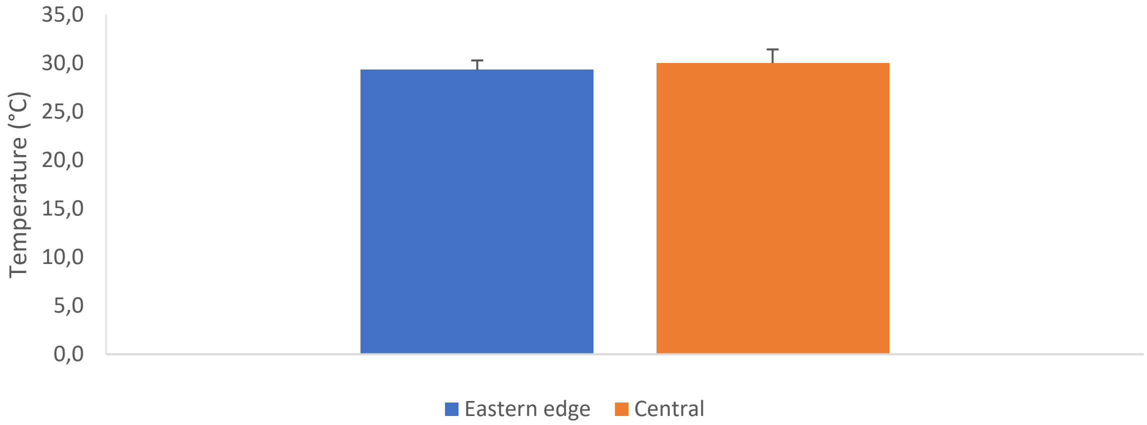


Figure 4.1: The critical thermal maxima and standard deviation for the Eastern edge and Central *H. midae* population.

The behaviour of the experimental animals the increasing temperature was observed and is summarised in Table 4.1. The table below shows the average of the total observations for the three repetitions of the CTM experiment. Low levels of stress were observed in both populations at temperatures below 20℃. From 20-22℃, the abalone began to display behaviour indicating stress, but none detached from the substrate (Table 4.1). From 23-25℃ the abalone were clearly in distress, hanging from the substrate with two detaching (Table 4.1). From 26-28℃, the animals were highly stressed with their shells hanging away from their bodies, and several detaching. The 50% CTM was attained for both populations at 29-30℃ (Table 4.1).

Table 4.1: The summary of observations for the Eastern edge and Central population during the CTM experiments. The number of individuals detaching from a substrate were also recorded over time.

TIME (HOURS)	**TEMPERATURE (°C)**	**CENTRAL POPULATION (n = 20)**	**EASTERN EDGE POPULATION (n = 20)**	**OBSERVATIONS**
1 - 2	18 - 19	1	0	Normal activity, some movement in chamber
3 -5	20 - 22	0	0	Most of the animals started showing signs of stress through a decrease in activity
6-8	23 - 25	2	0	20 – 30% of the animals were hanging from the substrate.
9 - 11	26 - 28	3	4	Some of the animals were very stressed, the shell was extended away from the body
12	29	0	7	50% of the Eastern edge population has detached from the substrate
13	30	6	0	50% of the Central population has detached from the substrate

4.5 Discussion

The experiment aids in understanding thermal tolerance in abalone from two varying thermal regions. The experiment was used to determine the thermal tolerance of the Eastern edge population which is found in warmer waters and compared it to the Central population which is found in cooler waters on the east coast of South Africa. The results showed that there were no significant differences in thermal tolerance between the two populations. This was similar to the findings of Hecht (1994), who found there were no significant differences in CTM between the warm-temperate and cold adapted populations from the east coast and west coast respectively. The CTM for abalone in the study by Hecht (1994), was 27.9°C for abalone ranging from 30 – 40mm in length, was slightly lower than the CTM of 29 – 30°C observed in the current study. Hecht (1994) noted that abalone with different thermal histories are likely to have different critical thermal maxima.

The current study failed to reject the null hypothesis, which stated that there would be no significant differences in CTM between the two populations. In conclusion the current study showed no evidence for higher thermal tolerance in the Eastern edge population compared to the Central population.

CHAPTER 5

GENERAL DISCUSSION

The study aimed to assess the effects of increasing temperature on abalone physiology by comparing the Eastern edge (warmer water) and the Central region (Cooler water) populations of *H. midae* on the east coast. The current laboratory-based study found no significant differences in the thermal tolerance and physiological responses to increasing temperature between the Eastern edge and Central population. The findings from the current study supports finding of Hecht (1994), who found no significant differences in thermal tolerance between the warm-temperate adapted east coast population and the cold adapted west coast *H. midae* population. While no significant differences in the response of the two populations to increasing temperature were observed, the stressful captive conditions which resulted in low growth rates and a relatively high mortalities may have masked possible differences. The current study also aimed to develop a methodology, upon which future studies of this kind could be based.

Thermal tolerance and the effects of increasing temperature on abalone physiology were determined by comparing growth, oxygen consumption and the CTM. A farm growth under ambient temperature conditions was also conducted at near ‘optimal’ temperature. It was hypothesised that the Eastern edge population would have a higher thermal tolerance compared to that of abalone from the warm-temperate central region, but that the central population would perform better under ‘optimal’ temperature conditions (16-20°C). The laboratory growth trial found no significant differences in growth rate or oxygen consumption between the two populations for the three temperature treatments (optimum, pejus and critical thermal ranges). Other studies investigating the effects of increasing temperature on abalone did however show a significant difference in growth and oxygen consumption rates with increasing temperature (Britz *et al.* 1997; Vilchis *et al.* 2005; Vosloo and Vosloo 2010). The lack of statistical differences in the present study was probably due to the high degree of variance in the data, and the stressed state of the experimental abalone.

There were also no significant differences observed in CTM between the two populations with the CTM for both populations between 29 – 30°C.

In summary, the findings from the current study showed no differences in performance and overall fitness between the Eastern edge and Central population with increasing temperature. A significant difference was however observed in growth rate under optimal temperature conditions on an abalone farm, suggesting a possible genetic difference between the two populations.

The high mortality and slow growth rates observed during the current laboratory studies indicated sub-optimal culture conditions, which may have masked possible genetic effects on performance. It has been observed that handling and nutrition may influence high mortality rates for abalone during an increase in sea surface temperature (Stone *et al.* 2014). The trial should be repeated under better conditions to conclusively determine whether the two populations perform differently at the optimum, pejus and critical thermal range. There have not been many of studies of this nature, and this study thus serves as a pilot for future studies. A future genetic study will be carried out, to determine if the slight differences in growth on the farm are adaptive or genetic. The farm growth trial is ongoing, to determine if these differences in growth between the two populations during the grow out phase will continue adulthood.

For future studies to get a better understanding on the effects of temperature on abalone physiology and thermal tolerance, temperature experiments should be carried out for all different life stages for both populations.

REFERENCES

Anestis A, Lazou A, Pörtner HO, Michaelidis, B. 2007. Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient temperature. *American Journal of Physiology -Regulatory, Integrative and Comparative Physiology* 293(2): R911 – R921.

Becker CD, Genoway RG. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes* 4(3): 245 – 256.

Bester-van der Merwe AE, Roodt-Wilding R, Volckaert FAM, D'Amato ME. 2011. Historical isolation and hydrodynamically constrained gene flow in declining populations of the South-African abalone, *Haliotis midae*. *Conservation Genetics* 12: 543 – 555.

Blamey LK, Shannon LJ, Bolton JJ, Crawford RJM, Dufois F, Evers-King H, Griffiths CL, Hutchings L, Jarre A, Rouault M, Watermeyer KE, Winker H. 2015. Ecosystem change in the southern Benguela and the underlying processes. *Journal of Marine System* 144: 9 – 29.

Britz PJ, Hecht T, Mangold S. 1997. Effects of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed formulated diet. *Aquaculture* 152: 191-203

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004. Toward a metabolic theory of ecology. *Ecology* 85(7): 1771 – 1789.

Chen JC, Chen WC (1999) Temperature tolerance of Haliotis *diversicolor supertexta* at different salinity and temperature levels. *Comparative Biochemistry and Physiology. A, Molecular and Integrative Physiology* 124: 73 – 80.

Chan N, Huang Z, Lu C, Shen Y, Luo X, Ke C, You W. 2019. Different transcriptomic responses tp thermal stress in heat-tolerant and heat-sensitive Pacific abalones indicated by cardiac performance. *Frontier in Physiology* 9: 1895.

Chen N, Luo X, Gu Y, Han G, Dong Y, You W, Ke C. 2016. Assessment of the thermal tolerance of abalone based on cardiac performance in *Haliotis discus hannai*, *H. gigantea* and their interspecific hybrid. *Aquaculture* 465: 258 – 264.

Cheng P, Liu X, Zhang G, Deng Y. 2006. Heat-shock protein70 gene expression in four hatchery Pacific Abalone *Haliotis discus hannai* Ino populations using for marker-assisted selection. *Aquaculture Research* 37: 1290 – 1296.

Cheng PZ, Liu X, Zhang GF, He JG. 2007. Cloning and expression analysis of a HSP70 gene from Pacific abalone (Haliotis discus hannai). *Fish & Shellfish Immunology* 22(1-2): 77 – 87.

Claësson D, Wang T, Malte H. 2016. Maximal oxygen consumption increases with temperature in the European eel (*Anguilla anguilla*) through increased heart rate and arteriovenous extraction. *Conserv Physiology* 4(1): 1 – 8.

Claireaux G, Webber DM, Lagardère, JP, Kerr SR. 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). *Journal of Sea Research* 44: 257 – 265.

Clark TD, Sandblom E, Jutfelt F. 2013. Review: Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendation. *The Journal of Experimental biology* 216: 2771 – 2782.

Dahlhoff E, Somero GN. 1993. Effects of temperature on mitochondria from abalone (genus Haliotis): adaptive plasticity and its limits. *Journal of Experimental Biology* 185: 151 – 168.

Diaz F, del Rio-Portilla MA, Sierra E, Aguilar M, Re-Araujo AD. 2000. Preferred temperature and critical thermal maxima of red abalone *Haliotis rufescens*. *Journal of Thermal Biology* 25(3): 257 – 261.

Evans BS, Sweijd NA, Bowie RCK, Cook PA, Elliott NG. 2004. Population genetic structure of the perlemoen *Haliotis midae* in South Africa: evidence of range expansion and founder events. *Marine Ecology Progress Series* 270: 163 – 172.

Frederich M, Pörtner HO. 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilator performance in the spider crab, *Maja squinado. American Journal of Physiology* 279: R1531 – R1538

Frederich M, Pörtner HO. 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado. American Journal of Physiology Regulatory Integrative and Comparative Physiology* 279: R1531 – R1538.

Fusi M, Giomi F, Babbini S, Daffonchio D, McQuaid CD, Porri F, Cannicci S. 2015. Thermal specialization across large geographical scales predicts the resilience of mangrove crab population to global warming. *Oikos* 124: 784 – 795.

Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonne P, Ciglianos M, Diaz D, Harmelin JG, Gambi MC, Kersting DK, Ledoux JB, Lejeusne C, Linares C, Marschal C, Perez T, Ribes M, Romano JC, Serrano E, Teixido N, Torrents O, Zabala M, Zuberer F, Cerrano C. 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effect of the 2003 heat wave. *Global Change Biology* 15: 1090 – 1103.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL. 2001. Effects of size and temperature on metabolic rate. *Science* 293: 2248 – 2251.

Gilroy A, Edwards SJ. 1998. Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenlip abalone, *Haliotis laevigata* (Leach). *Aquaculture Research* 29: 481 – 485.

Gordon HR, Cook PA. 2004. World abalone fisheries and aquaculture update: supply and market dynamics. *Journal of Shellfish Research*. 23(4): 935 – 939.

Goschen WS, Schumann EH, Bernard KS, Bailey SE, Deyzel SHP. 2012. Upwelling and ocean structures off Algoa Bay and the south-east coast of South Africa. *African Journal of Marine Science* 34(4): 525 – 536.

Harris JO, Maguire GB, Edwards SJ, Johns DR. 1999. Low dissolved oxygen reduces growth rate and oxygen consumption rate of juvenile greenlip abalone, *Haliotis laevigata* Donovan. *Aquaculture* 174: 265 – 278.

Hecht T. 1994. Behavioural thermoregulation of the abalone *Haliotis midae*, and the implication for intensive culture. *Aquaculture* 126: 171 – 181

Hobday AJ, Alexander LV, Perkins SE, Smale DA, Straub SC, Oliver ECJ, Benthuysen JA, Burrows MT, Donat MG, Feng M, Holbrook NJ, Moore PJ, Scannell HA, Gupta AS, Wernberg T. 2016. A hierarchal approach to defining marine heatwaves. *Progress in Oceanography* 141: 227 – 238.

Hooper C, Day R, Slocombe R, Benkendorff K, Handlinger J, Goulias J. 2014. Effects of severe heat stress on immune function, biochemistry and histopathology in farmed Australian abalone (hybrid *Haliotis laevigata* × *Haliotis rubra*). *Aquaculture* 432: 26 – 37.

Huey RB, Kingsolver JG. 1993. Evolution of resistance to high temperatures in ectotherms. *The American Naturalist* 142: S21 – S46.

Jost JA, Podolski SM, Frederich M. 2012. Enhancing thermal tolerance by eliminating the pejus range: a comparative study with three decapod crustaceans. *Marine Ecology Progress Series* 444: 263 – 274.

Kang HY, Lee Y, Song W, Kim T, Lee W, Kim TY, Kang C. 2019. Physiological responses of the abalone *Haliotis discus hannai* to daily and seasonal temperature variations. *Scientific Reports* 9: 8019

Khuzwayo S. 2014. Evaluating the thermal stress response of South African abalone, *Haliotis midae,* to biogeographical temperature variability. MSc thesis. University of KwaZulu-Natal. Durban.

Lange B, Currie K, Howarth GS, Stone DAJ. 2014. Grape seed extract and dried macroalgae, *Ulva lactuca Linnaeus*, improve survival of greenlip abalone, *Haliotis laevigata* Donovan, at high water temperature. *Aquaculture* 433: 348 – 360.

Li J, He Q, Sun H, Liu X. 2012. Acclimation-dependent expression of heat shock protein 70 in Pacific abalone (*Haliotis discus hannai* Ino) and its acute response to thermal exposure. *Chines Journal of Oceanology and Limnology* 30(1): 146 – 151.

Lyon RG. 1995. Aspects of the physiology of the South African abalone *Haliotis midae* L., and implications for intensive abalone culture. MSc Thesis, Rhodes University. Grahamstown.

Mann BQ, McDonald AM, Sauer WHH. Hecht T. 2003. Evaluation of Participation in and Management of the Transkei Shore Linefishery. *African Journal of Marine Science* 25(1): 79 – 97.

Moloney CL, Fennessy ST, Gibbons MJ, Roychoudhury A, Shillington FA, von der Heyden BP, Watermeyer K. 2013. Reviewing evidence of marine ecosystem change off South Africa. *African Journal of Marine Science* 35(3): 427 – 448.

Morash AJ, Alter K. 2015. Effects of environmental and farm stress on abalone physiology: perspectives for abalone aquaculture in the face of global climate change. *Reviews in Aquaculture* 7: 1 – 27.

Naidoo K, Maneveldt G, Ruck K, Bolton JJ. 2006. A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *Journal of Applied Phycology* 18: 437 – 443.

Naylor MA, Kaiser H, Jones CLW. 2011. Water quality in a serial-use raceway and its effect on the growth of South African abalone, *Haliotis midae* Linnaeus, 1758. *Aquaculture Research* 42: 918 – 930.

Naylor MA, Kaiser H, Jones CLW. 2014. The effect of free ammonia nitrogen, pH and supplementation with oxygen on growth of South African abalone, *Haliotis midae* L. in an abalone serial-use raceway with three passes. *Aquaculture Research* 45: 213 – 224.

Oliver ECJ, Benthuysen JA, Bindoff NL, Hobday AJ, Holbrook NJ, Mundy CN, Perkins-Kirkpatrick SE. 2017. The unprecedented 2015/16 Tasman Sea marine heatwave. *Nature Communications* 8: 16101.

Ortega-Cisneros K, Yokwana S, Sauer W, Cochrane K, Cockcroft A, James NC, Potts WM, Singh L, Smale M, Wood A, Pecl G. 2018. Assessment of the likely sensitivity to climate change for the key marine species in the southern Benguela system. *African Journal of Marine Science* 40(3): 279 – 292.

Pearce A, Lenanton R, Jackson G, Moore J, Feng M, Gaughan D, 2011. The ‘‘marine heat wave’’ off Western Australia during the summer of 2010/11. Fisheries Research Report No. 222. Department of Fisheries, Government of Western Australia, Perth.

Pearce A, Lenanton R, Jackson G, Moore J, Feng M, Gaughan D. 2011. The “marine heat wave” off Western Australia during the summer of 2010/11. Fisheries Research Report No. 222. Department of Fisheries, Western Australia. 40pp.

Pörtner HO, Farrell AP. 2008. Physiology and climate change. *Science* 322(5902): 690 – 692.

Pörtner HO, Karl DM, Boyd PW, Cheung WWL, Lluch-Cota SE, Nojiri Y, Schmidt DN, Zavialov PO. 2014. Ocean systems. in: climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 411 – 484.

Pörtner HO, Knust R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315(5808): 95 – 97.

Pörtner HO, Peck MA. 2010. Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *Journal of Fish Biology* 77: 1745 – 1779.

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

Pörtner HO. 2002. Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *The Journal of Experimental Biology* 205: 2217 – 2230.

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

Proudfoot LA, Kaehler S, McQuaid CD. 2008. Using growth band autofluorescence to investigate large-scale variation in growth of the abalone *Haliotis midae*. *Marine Biology* 153: 789 – 796.

Rahman MI. 2013. Climate change: A theoretical review. *Interdisciplinary Description of Complex Systems* 11(1): 1 – 13.

Romo ZM, Re AD, Diaz F, Mena A (2010) Physiological responses of pink abalone *Haliotis corrugate* (Gray, 1828) exposed to different combinations of temperature and salinity. *Aquaculture Research* 41: 953 – 960.

Rouault M, Pohl B, Penven P. 2010. Coastal oceanic climate change and variability from 1982 to 2009 around South Africa. *African Journal of Marine Science* 32(2): 237 – 246.

Sales J, Britz PJ. 2001. Research on abalone (*Haliotis midae* L.) cultivation in South Africa. *Aquaculture Research* 32(11): 863 – 874.

Schlegel RW, Oliver ECJ, Perkins-Kirkpatrick S, Kruger A, Smit AJ. 2017. Predominant atmospheric and oceanic patterns during coastal marine heatwaves. *Frontiers in Marine Science* 4: 1 – 15

Schlegel RW, Oliver ECJ, Wernberg T, Smit AJ. 2017. Nearshore and offshore co-occurrence of marine heatwaves and cold-spells. *Progress in Oceanography* 151: 189 – 205.

Searle T, Roberts RD, Lokman PM (2006) Effects of temperature on growth of juvenile blackfoot abalone, *Haliotis iris* Gmelin. *Aquaculture Research* 37: 1441 – 1449.

Snyder MJ, Girvetz E, Mulder EP. 2001. Induction of marine mollusc stress proteins by chemical or physical stress. *Archives of Environmental Contamination and Toxicology* 41: 22 – 29.

Somero G. 2005. Linking biogeography to physiology: evolutionary and acclamatory adjustments of thermal limits. *Frontiers in Zoology* 2(1): 1 – 9.

Stone DAJ, Bansemer MS, Lange B, Schaefer EN, Howarth GS, Harris JO. 2014. Dietary intervention improves the survival of cultured greenlip (*Haliotis laevigata* Donovan) at high water temperature. *Aquaculture* 430: 230 – 240.

Takami H, Saldo T, Endo T, Noro T, Musashi T, Kawamura T (2008) Overwinter mortality of young-of-the year Ezo abalone in relation to seawater temperature on the North Pacific coast of Japan. *Marine Ecology Progress Series* 367: 203 – 212.

Tarr RJQ. 1995. Growth and movement of the South African abalone *Haliotis midae*: a reassessment. *Marine Freshwater Resource* 46: 583 – 590.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, Ferreira de Siqueira M, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Townsend Peterson A, Phillips OL, Williams SE. 2004. Extinction risk from climate change. *Nature* 427 (6970): 145 – 148.

Tomanek L, Somero GN. 2002. Interspecific and acclimation induced variation in levels of heat-shock proteins 70 (hsp70) and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus *Tegula*): implication for regulation of hsp gene expression. *Journal of Experimental Biology* 205: 677 – 685.

Tomanek L. 2008. The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat-shock response. *Physiological and Biochemical Zoology* 81(6): 709 – 717.

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

van der Merwe AE. 2009. Population genetic structure and demographical history of South African abalone, *Haliotis midae*, in a conservation context. PhD thesis. Stellenbosch University. Stellenbosch.

Venter L, Loots D, Mienie LJ, van Rensburg PJJ, Mason S, Vosloo A, Lindeque JZ. 2018. Uncovering the metabolic response of abalone (*Haliotis midae*) to environmental hypoxia through metabolomics. *Metabolomics* 14: 49.

Vilchis L.I, Tegner MJ, Moore JD, Friedman CS, Riser KL, Robbins TT, Dayton PK. 2005. Ocean warming effects on growth, reproduction, and survivorship of the southern California abalone. *Ecological Applications* 15(2): 469 – 480.

Visser-Roux A. 2011. Reproduction of the South Africa abalone, *Haliotis midae*. PhD Thesis, University of Stellenbosch. Stellenbosch.

Vosloo D, Vosloo A, Morillion EJ, Samuels JN, Sommer P. 2013. Metabolic readjustment in juvenile South African abalone (*Haliotis midae*) acclimated to combinations of temperature and dissolved oxygen levels. *Journal of Thermal Biology* 38(7): 458 – 466.

Vosloo D, Vosloo A. 2010. Response of cold-acclimated, farmed South African abalone (*Haliotis midae*) to short-term and long-term changes in temperature. *Journal of Thermal Biology* 35(7): 317 – 323.

Wait M, Schoeman DS. 2012. Shell use, population structure, and reproduction of the hermit crab, *Clibanarius Virescens* (Kraus, 1843) at Cape Recife, South Africa. *Journal of Crustacean Biology* 32(2): 203 – 214.

Wernberg T, Smale DA, Tuya F, Thomsen MS, Langlois TJ, de Bettignies T, Bennet S, Rousseaux CS. 2012. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nature Climate Change* 3: 78 – 82.

Wessel N, Martin S, Badou A, Dubois P, Huchette S, Julia V, Nunes F, Harney E, Paillard C, Auzoux-Bordenave S. 2018. Effect of CO_2-induced ocean acidification on the early development and shell mineralization of the European abalone (*Haliotis tuberculate*). *Journal of Experimental Marine Biology and Ecology* 508: 52 – 63.

Whitfield AK, James NC, Lamberth SJ, Adams JB, Perissinotto R, Rajkaran A, Bornman TG. 2016. The role of pioneers as indicators of biogeographic range expansion caused by global change in southern African coastal waters. *Estuarine, Coastal and Shelf Science* 172: 138 – 153.

Wood AD, Buxton CD. 1996. Aspects of the biology of the abalone, *Haliotis midae* Linneaus, 1758, on the east coast of South Africa. *South African Journal of Marine Science* 17(1): 69 – 78.

Zielinski S, Pörtner HO. 2000. Oxidative stress and antioxidative defense on cephalopods: a function of metabolic rate or age? *Comparative Biochemistry and Physiology Part B* 125: 147 – 160.