THERMAL TOLERANCE AND THE POTENTIAL EFFECTS OF CLIMATE CHANGE ON COASTAL INTERTIDAL AND ESTUARINE ORGANISMS IN THE KARIEGA ESTUARY AND ADJACENT INTERTIDAL COASTLINE, EASTERN CAPE, SOUTH AFRICA

A thesis submitted in fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

By

KERRY-ANN VAN DER WALT

December 2019

ABSTRACT

Temperature changes due to the effects of climate change are evident on all continents and oceans. As a result, there is a growing concern over how marine ectotherms will respond to extreme or fluctuating environmental temperatures. Temperature changes have strong direct and indirect effects on individual, population, and ecosystem functioning traits. A multi-scale approach determining the thermal tolerance and performance of several marine ectotherms belonging to different coastal habitats is rarely considered in thermal physiology studies but is effective for an integrated ecosystem assessment. As such, for this thesis, I aimed to quantify and compare the thermal tolerance and performance of a range of coastal marine ectotherms (fish and macro-invertebrates) with different biogeographical distributions from estuarine, subtidal and rocky intertidal habitats to available and projected *in situ* temperature data. This was also undertaken to gauge the local vulnerability of each species across summer and winter in a warm-temperate region of South Africa. This was done using a multi-method physiological approach, which included the dynamic method (CT_{max} and CT_{min}), static respirometry and maximum heart rate (f_{Hmax}).

Results of the dynamic method on several fish and macro-invertebrate species indicated that there are differences in thermal tolerance according to taxonomy, biogeography and habitat for both summer and winter. Macro-invertebrate species generally had higher CT_{max} endpoints, lower CT_{min} endpoints, higher upper and lower breadths in tolerance, higher upper and lower thermal safety margins and higher thermal scopes than the fish species. This could be a result of the macro-invertebrate species studied being less mobile compared with fish species (which are able to move to more favourable conditions) as well as having broader geographical distributions. In addition, macro-invertebrates from the intertidal rock pool habitat (*Palaemon peringueyi*; *Perna perna*) were more tolerant of high and low temperatures compared with the macro-invertebrates from the estuarine habitat (*Clibanarius virescens*; *Parasesarma catenatum*; *Upogebia africana*). Overall, macro-invertebrates, with the exception of *Parechinus angulosus*, investigated in this study indicated that current temperatures and projected climate change scenarios across seasons would not have a significant impact on them and that they are highly adaptable to changing temperature regimes. This sign of high tolerance was further supported by the heart rates of *P. perna* and *P. catenatum* under an acute increase

in temperature (1.0 °C.h^{-1}) which showed individuals of each species physiologically depressing their metabolism until a final Arrhenius breakpoint temperature was reached (T$_{AB}$).

Among the fish species investigated in this study, tropical species (*Chaetodon marleyi*; *Kuhlia mugil*) had the highest CT$_{max}$ and CT$_{min}$ endpoints when compared with the temperate (*Diplodus capensis*; *Sarpa salpa*), warm-water endemic (*Chelon dumerili*; *Rhabdosargus holubi*) and cool-water endemic (*Chelon richardsonii*) fishes. This suggests that due to their lower breadths in tolerance and thermal safety margins being small, tropical species may be less tolerant of cold temperatures and thermal variability, especially in the form of summer upwelling events which are expected to increase in intensity and frequency in this region as a result of anthropogenic climate change effects. On the other hand, however, if a temperature increase of 2.0 - 4.0 °C takes place at the end of the century as predicted by the Intergovernmental Panel on Climate Change (IPCC), it is likely that tropical species such as *C. marleyi* will become more common. Temperate species such as *D. capensis* and *S. salpa* were able to tolerate a wide range of temperatures (wide thermal scope) compared with the other fish species. These findings may suggest that *D. capensis* and *S. salpa* are thermally resilient and may be the least vulnerable to climate change effects and temperature variability. When evaluating the different life stages of *D. capensis*, however, using the dynamic method (juveniles and adults), static respirometry (juveniles) and maximum heart rate (adults), results suggested that juveniles of this temperate species will be more resilient to increases in ocean temperatures compared with the adults because they have a higher thermal tolerance (CT$_{max}$/T$_{CRIT}$) and a greater metabolic scope (T$_{OPT}$) at higher temperatures. For both juveniles and adults, temperatures beyond 28.0 °C (upper T$_{PEJ}$; T$_{ARR}$) will have a significant impact on their physiology. Using a multi-scale and multi-method approach thus helped to identify which species or community may be vulnerable to the effects of climate change within shallow coastal environments in this warm-temperate climate change hotspot. Adopting this type of approach will assist policy makers in developing comprehensive climate change management frameworks for coastal ecosystems globally and around South Africa.

DECLARATION

I, Kerry-Ann van der Walt, hereby declare that the work described in this thesis was carried out in the Department of Ichthyology and Fisheries Science, Rhodes University and the South African Institute for Aquatic Biodiversity, under the supervision of Dr NC James, Professor WM Potts and Dr F Porri. The components of this thesis comprise original work by the author and have not been submitted to any other university.

_____

Kerry-Ann van der Walt

_12/12/2019_____

Date

DEDICATION

This thesis and work is dedicated to my mother, Penny, and my sister, Amy-Leigh. To the two strongest women I know, thank you for all your unconditional love and support!

ACKNOWLEDGEMENTS

needed at times. To my underwater hockey peeps, thanks for also being there for me too and letting me take my PhD stress out on you in the pool.

To Matthys, Cherise and Barbara. Thank you for your continuous support throughout this chapter in my life. Your kindness and generosity towards me will never be forgotten. You all hold such a special place in my heart, especially Barbara, and knowing that I had an escape from my thesis for a few hours each day to look after such a precious little girl kept me motivated. Barbara (Queen B) you won't realise it now, but your love towards me and your laughter always got me through a really bad day, and I always looked forward to spending that precious time with you, being in the garden looking for insects or building dough Minnie Mouse's. Every minute with you was cherished deeply. Cherise, thank you for your friendship, love and support. Those conversations we shared always helped, especially through everything I have been through. Knowing that I could vent for a few minutes to you, was always such a relief. I also just want to say that you are the kindest woman I have ever met, and you have inspired me in so many ways, so I say thank you.

To my medical doctors, Dr Meihuizen, Dr Zietsman and Dr Walsh, and my therapist, Dr Brown, thank you for taking care of me physically and emotionally. I had quite a big scare health wise during this PhD, and I would just like to thank you all for what you did to make sure I am still here today and that I was able to finish what I started.

To save the best for last, my family, thank you mom, dad and sissy. Mom and dad, without your unconditional love and support and you always pushing me to follow my dreams, I would not have gotten this far. You always supported my goals and dreams and I am extremely grateful. Mom, thank you for being such a great example of a strong woman and for always encouraging me and keeping me calm, a cup of tea does really solve all your problems. Sissy, Amy-Leigh, you are such a supportive sister and your love and endless phone calls on your way home when you were stuck in traffic really got me through some lonely and stressful times, so thank you. Love you all very much.

CONTENTS

GLOSSARY

CT_{max} – critical thermal maximum; upper thermal limit of performance.

CT_{min} – critical thermal minimum; lower thermal limit of performance.

T_{CRIT} – critical temperature; temperature at which an organism's performance is critical.

T_{OPT} – optimum temperature; temperature at which an organism's performance is optimal.

T_{PEJ} – pejus temperature; "getting worse" temperatures that border the thermal optimum temperature.

RMR – routine metabolic rate; mean metabolic rate of a fish in a resting state but exhibiting minor activity.

MMR – maximum metabolic rate; maximum amount of energy that can be metabolized aerobically.

RAS – relative aerobic scope; the difference between the MMR and RMR of aerobic metabolism.

f_{Hmax}/HR_{max} – maximum heart rate; maximum number of beat made by a heart in one minute of effort.

T_{BP1} – first breakpoint temperature; the first temperature that initiates a temperature-insensitive metabolism.

T_{BP2} – second breakpoint temperature 2; the upper limit for a temperature-insensitive metabolism.

T_{AB} – final Arrhenius breakpoint temperature; maximum heart rate stops increasing at a temperature just beyond the optimum temperature.

T_{QB1} – first breakpoint temperature for Q_{10} of f_{Hmax}; temperature that initiates temperature sensitivity of a physiological process due to an increase by 10.0 °C.

T_{QB2} – second breakpoint temperature for Q_{10} of f_{Hmax}; the upper limit for temperature sensitivity of a physiological process due to an increase by 10.0 °C.

T_{QB} – final breakpoint temperature for Q_{10} of f_{Hmax}; temperature at which the incremental Q_{10} of maximum heart rate decreases below a certain value.

T_{ARR} – arrhythmic temperature; cardiac collapse that occurs just before the critical temperatures.

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: GENERAL INTRODUCTION

1.1 OVERVIEW

Covering 71% of the Earth's surface, oceans are complex ecosystems responsible for regulating the world's climate and providing essential services for the maintenance of life (Bounama *et al.*, 2007). Recently, however, there has been overwhelming evidence to suggest that global anthropogenic climate change is threatening our oceans (Hoegh-Guldberg and Bruno, 2010; IPCC, 2014) and life in it. Rising carbon dioxide (CO_2) emissions are globally pervasive and irreversible on ecological timescales (Doney *et al.*, 2012; Munday *et al.*, 2012). The CO_2 in the atmosphere has increased from approximately 280 ppm at the start of the industrial revolution (1750) to over 390 ppm (2012) (IPCC, 2014), and has caused the Earth's mean surface temperature to rise by 0.9 °C between 1880 and 2012. Depending on the magnitude of future CO_2 emissions, these averages are expected to rise by an additional 2.0 – 4.0 °C by the year 2100 (IPCC, 2014). Besides changes in temperature, other climate change impacts include a decrease in the extent of sea-ice, sea-level rise, increased ocean stratification, ocean acidification, altered patterns of ocean circulation and currents, changes in wind strength and direction, increased frequency of extreme weather events, and changes in precipitation (Roessig *et al.*, 2004; Rijnsdorp *et al.*, 2009; Hoegh-Guldberg and Bruno, 2010; Doney *et al.*, 2012).

Temperature is one of the primary factors controlling the physiology and life history functions of marine organisms (Crashaw and O'Connor, 1997). It affects individual traits such as growth, behaviour, longevity, recruitment, reproduction, feeding, swimming ability, development, mortality and distribution (Beaugrand *et al.*, 2003; Perry *et al.*, 2005; Madeira *et al.*, 2012a). Many marine organisms are obligate poikilotherms (ectotherms) and, since they are unable to regulate their body temperature physiologically (Madeira *et al.*, 2012a), they are required to remain in areas within their thermal preference (Pörtner and Peck, 2010). As such, temperature is seen as the most important variable that influences the distribution of marine organisms (Francour *et al.*, 1994). It is predicted that, as temperature rises, the distribution and abundance of species will shift according to their thermal tolerance and ability to adapt (Harley *et al.*, 2006).

1

If environmental changes such as increasing sea temperature are physiologically intolerable, some marine ectotherms may be capable of acclimatization through the adjustment of physiology within individuals or adaptation through the increased abundance and reproduction of tolerant genotypes over generations (Gunderson and Stillman, 2015). While many species will not acclimate or adapt, others that are physiologically tolerant may be exposed to reduced competition or predation and be the "winners" in a changing world (Somero, 2010; Fulton, 2011; Doney *et al.*, 2012). If increasing sea temperatures are, however, physiologically unfavourable, shifts in distributional ranges (by individuals or populations) (Perry *et al.*, 2005; Cheung *et al.*, 2009; Sunday *et al.*, 2011, 2012), changes in phenology (timing of annual events) (Edwards and Richardson, 2004), death, local extinctions and ultimately species extinctions may take place (Roessig *et al.*, 2004; Parmesan, 2006; Hoegh-Guldberg and Bruno, 2010; Pörtner and Peck, 2010). This would result in these marine ectotherms being the "losers" in a changing world (Somero, 2010; Fulton, 2011; Doney *et al.*, 2012).

Thus far, experimental and theoretical research of thermal tolerance has improved the predictions of species composition and richness in a region. Generally, these studies suggest that marine ectotherms in tropical (nearer to the equator) or polar latitudinal ranges, where climate is relatively stable (with minimal seasonal variation), are considered to have narrow thermal breadths of tolerance and are highly sensitive to temperature change (Cheung *et al.*, 2009; Pörtner and Peck, 2010; Nguyen *et al.*, 2011; Sunday *et al.*, 2012; Payne and Smith, 2017). In contrast, marine ectotherms inhabiting temperate regions, which are characterized by seasonal variability (Cheung *et al.*, 2009; Sunday *et al.*, 2011, 2012), generally have broader thermal breadths of tolerance and are less sensitive to temperature changes. The predicted poleward shift of tropical species in response to warming is expected to result in increases in the richness of species in the temperate regions (Cheung *et al.*, 2009; Hoegh-Guldberg and Bruno, 2010; Doney *et al.*, 2012).

Distributional shifts of marine ectotherms are frequently predicted by developing thermal performance curves (TPCs) (Deutsch *et al.*, 2008; Sunday *et al.*, 2012; Schulte, 2015; Sinclair *et al.*, 2016; Speers-Roesch and Norin, 2016). These performance curves, also referred to as Fry aerobic scope curves (derived from the oxygen- and capacity-limited thermal tolerance hypotheses, i.e. OCLTT, and is the difference between standard and maximum metabolic rates), are assumed to be a proxy for fitness. Performance curves represent thermal tolerance or physiological performance of organism's across a range of environmental conditions, such

2

as temperature, but they also provide further insight into the differentiation between the thermal range of active survival through temperature-dependent performance and the range of time-limited passive tolerance to temperature extremes (Fry, 1947; Pörtner, 2001, 2002, 2010, 2012; Pörtner and Knust, 2007; Pörtner and Peck, 2010; Payne *et al.*, 2016; Sinclair *et al.*, 2016) (Figure 1.1). These curves also explain the transitions between those ranges (Pörtner and Peck, 2010). Performance curves imply that physiological performance rises slowly with temperature up to a maximum/peaked level, referred to as the optimum temperature (T_{OPT}), and declines rapidly at higher and lower temperatures, where physiological performance usually reaches zero at the upper and lower critical temperatures (CT_{max} and CT_{min}, i.e. T_{CRIT}) (Pörtner and Knust, 2007; Pörtner, 2012; Clark *et al.*, 2013; Payne *et al.*, 2016; Sinclair *et al.*, 2016) (Figure 1.1). Beyond T_{OPT}, and prior to CT_{max} and CT_{min}, are pejus (getting worse) temperatures (T_{PEJ}), which reflect the reduction in aerobic/metabolic scope (the top ~10% of the T_{OPT} curve), limiting the energy available for activity, growth and other vital rates (Pörtner and Knust, 2007) (Figure 1.1).
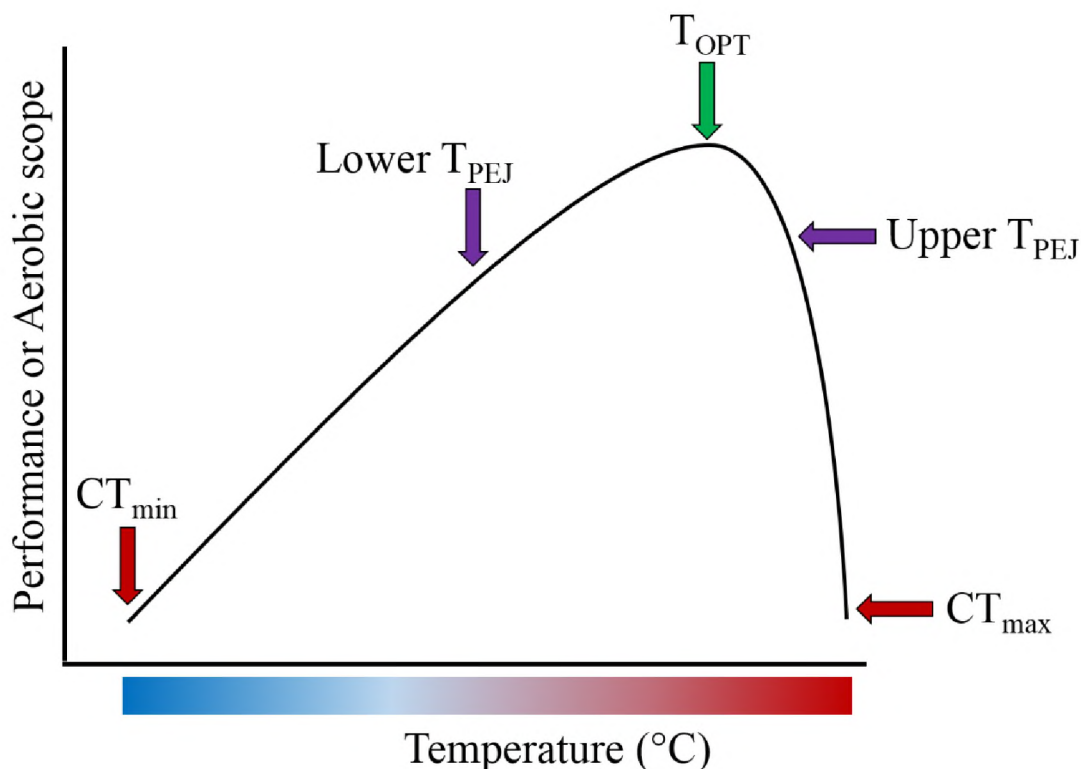
Figure 1.1. Hypothetical thermal performance curve (TPC) for marine ectotherms redrawn from Sinclair *et al.* (2016) and Speers-Roesch and Norin (2016). TPCs are usually left-skewed such that the distance between the optimal temperature (T_{OPT}) for any given performance trait is closer to the maximum critical temperature (CT_{max}/ upper T_{CRIT}) than the minimum critical temperature (CT_{min}/lower T_{CRIT}).

There is a general expectation that optimal (T_{OPT}) and critical (CT_{min} and CT_{max}) values will align with the environmental temperatures a marine ectotherm experiences within its distributional range, otherwise referred to as the climate variability hypothesis or Rapaport's Rule (see Stevens, 1989; Gaston *et al.*, 1998) (Payne *et al.*, 2016). This is a result of marine ectotherms having the capacity to phenotypically acclimate and adapt to thermal change (Angilletta and Angilletta, 2009). Some marine ectotherms may, however, tolerate greater temperature extremes than those to which they are exposed, or may respond behaviourally to avoid critical extreme temperatures in their thermal environment (Kordas *et al.*, 2011; Sunday *et al.*, 2012). Despite this, the identification of the thermal optimum and the critical temperatures of marine ectotherms will nonetheless provide valuable information about the degree of thermal specialization and the width of the thermal range (upper and lower T_{PEJ}) of a species in relation to its biogeographical distribution (Lannig *et al.*, 2004). Frederich and Pörtner (2000) and Pörtner (2001) proposed that an understanding of the width of the thermal pejus window (thermal range between lower T_{PEJ} and upper T_{PEJ}) is relevant for predicting future distributions as metabolic and oxygen limitation at cold or warm temperatures sets in prior to function failure, which finally develops beyond critical temperatures (CT_{max} and CT_{min}).

A range of methods and experimental approaches to estimate appropriate physiological parameters to make predictions have been used. Some of the common methods used include the dynamic method, the measurement of maximum heart rate (f_{Hmax}) and respirometry. The dynamic method determines the critical thermal tolerance limits (CT_{max} and CT_{min}) by either increasing or decreasing water temperature at a specific rate until an endpoint is reached, which is commonly a loss of equilibrium (fish) or righting response (macro-invertebrates). The measurement of f_{Hmax} and respiration commonly determines T_{OPT} and T_{PEJ}. Cardiac function is an important determinant of thermal tolerance and performance because, for a number of marine ectotherms, maximum heart rate has been shown to decrease quickly from the upper T_{PEJ} and begin to collapse as critical thermal limits are reached (Stillman and Somero, 1996; Hochachka and Somero, 2002; Stillman, 2003). In turn, around the upper T_{PEJ}, oxygen supply (respiration) is also impaired, contributing to a reduction in aerobic scope (Pörtner, 2002; Pörtner and Knust, 2007; Pörtner and Farrell, 2008). A mechanistic connection thus exists between all three experimental approaches (Stillman, 2002, 2003). The hierarchy of optimal, upper and lower pejus and critical temperatures and the scope between these thermal margins awaits quantification for many marine ectothermic organisms, especially comparatively, for

marine ectotherms from stable and unstable environments (regions and habitats) (Pörtner, 2001).

Evidence suggests that the rate of thermal change in the ocean is not uniform. Considerable regional differences occur in the rate of temperature change, with localized areas of increased warming or thermal variability commonly referred to as "climate change hotspots" (Hobday and Pecl, 2014; Pecl *et al.*, 2014; Vergés *et al.*, 2014). In their global review, Hobday and Pecl *et al.* (2014) identified 24 discrete climate change hotspots (Figure 1.2a) many of which are associated with western boundary currents (WBCs). They suggested that these areas would provide ideal laboratories for assessing impacts and evaluating adaptation options for marine ecosystems, fisheries and aquaculture. Ecological and environmental hotspots can enhance regional adaptation and their identification can facilitate the advancement of adaptation science globally (Giorgi, 2006; Diffenbaugh *et al.*, 2008; Frusher *et al.*, 2014), just as the identification of biodiversity hotspots has focused conservation efforts in the ocean (e.g. Worm *et al.*, 2003; Renema *et al.*, 2008; Tittensor *et al.*, 2010). Ramírez *et al.* (2017) identified six hotspots of marine biodiversity, where the impacts of climate change are also the highest (there is also considerable overlap between biodiversity hotspots and climate change hotspots), potentially threatening the survival of many marine ectotherms (Figure 1.2b). One of these threatened hotspots is the south-eastern coastline of Africa (Figure 1.2b).
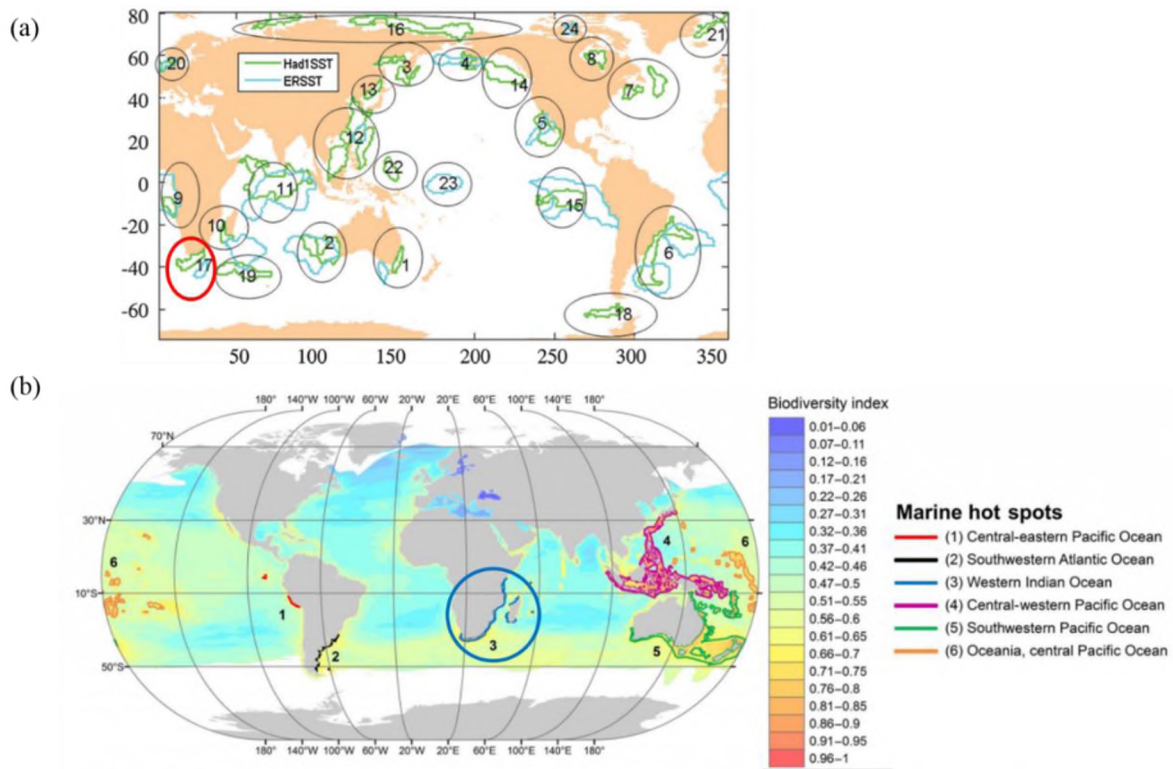
Figure 1.2. Map of ocean warming and biodiversity showing (a) 24 discrete climate change hotspots and (b) six biodiversity hotspots globally. The red circle in (a) and the blue circle in (b) indicate the south-eastern coastline of Africa's climate and biodiversity overlapping hotspots, which is relevant for this study. Map adapted from Hobday and Pecl (2014) (a) and Ramírez *et al.* (2017) (b).

The south-eastern coast of southern Africa is a complex hotspot as it hosts marine fauna from four bioregions, including: 1) the south-western Cape bioregion which extends from Cape Columbine to Cape Point; 2) the Agulhas bioregion which extends from Cape Point to the Mbashe River; 3) the Natal bioregion which extends from the Mbashe River to Cape Vidal; and 4) the Delagoa bioregion which extends from Cape Vidal into Mozambique (Lombard *et al.*, 2004) (Figure 1.3). Under this classification, the south-western Cape bioregion is cool-temperate, the Agulhas bioregion warm-temperate, the Natal bioregion subtropical and the Delagoa bioregion tropical (Whitfield, 2005; Griffiths *et al.*, 2010) (Figure 1.3).
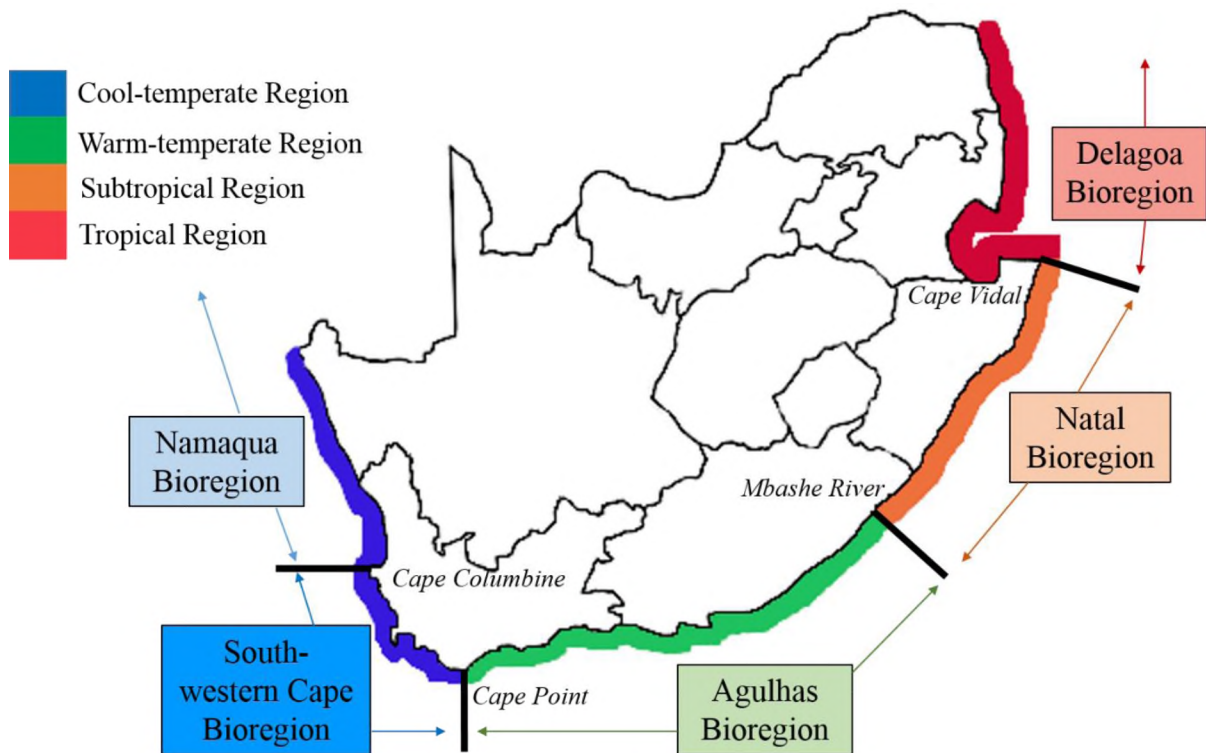
Figure 1.3. Map illustrating the five inshore bioregions for various fauna and flora (Namaqua Bioregion, South-western Cape Bioregion, Agulhas Bioregion, Natal Bioregion, and Delagoa Bioregion) as defined by the South African National Spatial Biodiversity Assessment report (Lombard *et al.*, 2004) and the four biogeographical regions (warm-temperate, cool-temperate, subtropical and tropical) as defined by Whitfield (2005) and Griffiths *et al.* (2010).

Within the Agulhas bioregion, the warm-temperate south-east and south coasts of South Africa are characterized by a large annual thermal range when compared with the cool-temperate and subtropical coasts (south-western Cape and Natal bioregions). This is a result of the retention and cooling of the Agulhas Current's water on the Agulhas Bank; the presence of current-driven upwelling cells (e.g. Port Alfred cell) along the shoreward side of the Agulhas Current in this coastal section (Maree *et al.*, 2000; Roberts, 2005; Lutjeharms, 2007); and the effects of embayments and capes throughout the region (Schlegel *et al.*, 2017). In addition, analysis of inshore (*in situ*) and offshore (optimally interpolated SST – OISST; Reynolds *et al.*, 2007) temperature data spanning a 21 year time series (*in situ* – 40 years; OIST – 33 years) indicates that warm (marine heat waves – MHWs) and cold (marine cold spells – MCSs) events along the warm-temperate region are more intense and longer in duration than those along the cool-temperate and subtropical regions (Schlegel *et al.*, 2017). Furthermore, MHWs have been increasing every decade in this warm-temperate region, while MCSs have been decreasing,

with exceptions near Port Elizabeth and north of the Cape Peninsula where the count of MCSs is increasing very near to the coast (Schlegel *et al.*, 2017). This suggests that the warm-temperate region, particularly near Port Elizabeth, may experience increases in warm and cold events (that are on par with the intensity and duration of similar events known to have ecological effects elsewhere in the world) and that the development of some coastal MCSs may be attributed to an increase and intensification of upwelling events (Miranda *et al.*, 2013; Schlegel *et al.*, 2017).

Upwelling events are defined by periodic occurrences of cold water of deep origin brought to the surface near the coast, causing maximum drops in SST of ~10.0 °C within a few hours, followed by a slower relaxation over several days (Ganachaud *et al.*, 2010). The warm-temperate south-east coast of South Africa experiences localized upwelling from the Port Alfred permanent upwelling cell and easterly or westerly winds during spring and summer (Rouault *et al.*, 1995). In the Tsitsikamma region on the south-east coast of South Africa, for example, SST can drop from 24.0 °C to 12.0 °C in less than 12 hours as a result of upwelling (Duncan *et al.*, 2019). Recent climate modelling consensus predicts that within this region, the frequency and intensity of upwelling is increasing, which in turn will drive increases in sea temperature variability (Duncan *et al.*, 2019). Although mean sea surface temperatures are increasing (1.5 °C SST increase of the Agulhas Current since the 1980s), there has been seasonal cooling associated with an increase in the frequency of upwelling (Rouault *et al.*, 2010). In addition to an increase in upwelling and temperature variability, this region is also experiencing changes in air temperature and sea level rise (Mead *et al.*, 2013). In South Africa, mean annual air temperature increased by 0.13 °C per decade between 1960 and 2003 (Kruger and Shongwe, 2004) and sea level on average has risen by 1.48 mm.y^{-1} along the warm-temperate south coast from 1957 to 2006 (Mather *et al.*, 2009).

Marine associated biodiversity within this warm-temperate region comprises a mixture of species with different biogeographic affinities including tropical species at the edge of their distribution, warm-water endemic species, cool-water endemic species, temperate species and widespread species (Harrison and Whitfield, 2006; Figure 1.4). Extreme increases or decreases in temperature associated with climate change may result in mortality for tropical and cool-water endemic species, respectively, if their pejus and critical thermal limits are reached (Rijnsdorp *et al.*, 2009; Horta e Costa *et al.*, 2014). This is particularly likely if they are unable to escape extreme MCSs or MHWs (Dulvy *et al.*, 2008).
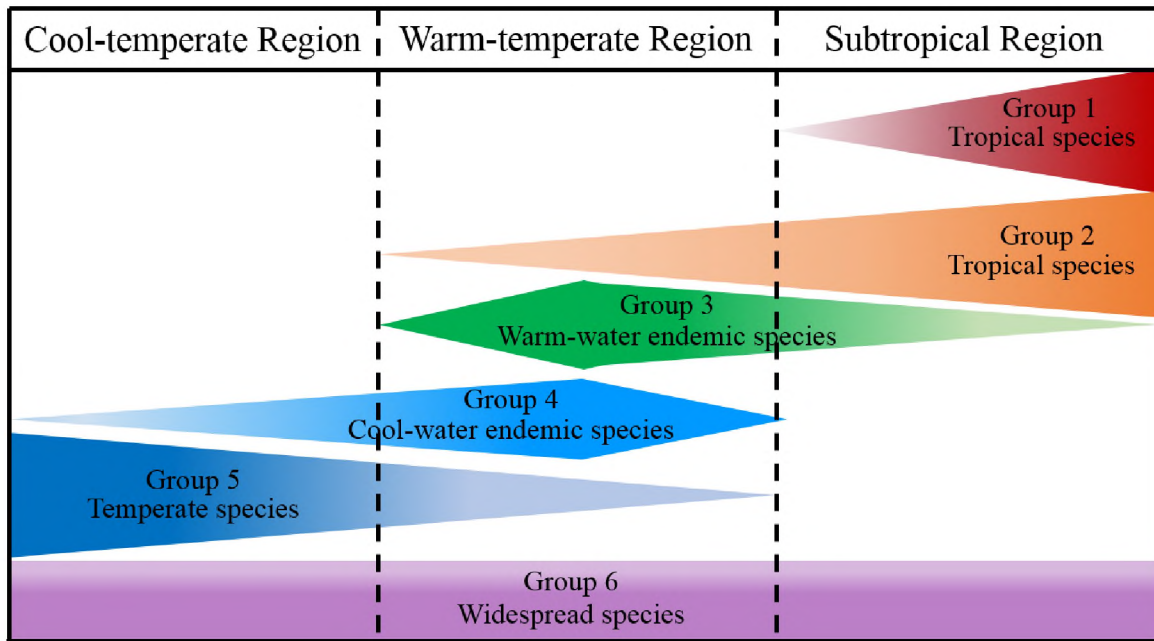
Figure 1.4. South African estuarine fish faunal groupings based on temperature preferences in accordance with their biogeographical region (warm-temperate, cool-temperate, subtropical) affinities (modified from Harrison and Whitfield, 2006).

Shallow habitats, such as rocky intertidal habitats and estuaries have little thermal inertia and wide daily and seasonal amplitudes (Madeira *et al.*, 2012a). It has been suggested that research into climate change in the marine environment should focus on these shallow habitats along with their associated fauna, because marine species in estuaries and rock pool habitats may be the first to reflect changes in temperature (Madeira *et al.*, 2012a). Marine ectotherms inhabiting intertidal and supratidal shallow habitats, in particular, are thought to be living close to their lower and especially upper thermal tolerance limits (Stillman and Somero, 1996; Vinagre *et al.*, 2018). As such, even small changes in temperature could result in dramatic changes in community structure in these shallow habitats, putting them at risk to the effects of climate change (Bertness *et al.*, 1999; Vinagre *et al.*, 2018). Furthermore, shallow intertidal macro-invertebrate organisms from rock pools and estuaries, unlike subtidal and demersal (open sea) fish, experience variations in body temperature between immersion and emersion, and, as such, both air and seawater temperature can affect their physiological functions and biogeographic distributions (Helmuth *et al.*, 2006a; Tagliarolo and McQuaid, 2015). Species with different biogeographic and habitat affinities as well as faunal groupings, therefore, may respond differently to climate change, and, as such, this south-east temperate region may serve as a

natural laboratory providing an ideal setting to assess these particular marine ectotherms' thermal tolerance and performance.

1.2 RATIONALE, AIM OF THE STUDY, AND STRUCTURE OF THE THESIS

Recent literature has emphasized the importance of examining the physiological limits of species at levels relevant to that experienced in their native habitat, otherwise there may be mismatch between species range (realized niche) and the range predicted by physiological tolerance (physiological niche) (Helmuth *et al.*, 2002; Sánchez-Fernández *et al.*, 2012). In South Africa, there is increasing evidence of rising water temperatures and increased thermal variability (Rouault *et al.*, 2010; Lima and Wethey, 2012; Schlegel *et al.*, 2017), but limited information on the thermal performance of marine ectothermic fishes (Marais, 1978; Kemp, 2009; Duncan, 2018) and macro-invertebrates (Baldanzi *et al.*, 2015; Fusi *et al.*, 2015; Mostert 2015; Tagliarolo and McQuaid 2015, 2016) from the warm-temperate region and especially from shallow habitats.

Different shallow habitats in this region include several estuaries and a number of rock pools and gullies along the adjacent intertidal rocky shores (Mucina *et al.*, 2006). There are two major types of estuaries in this region, namely permanently open and temporarily open/closed which comprise different zones (Whitfield 2019). Within these shallow habitats, a large diversity of ectothermic fish (e.g. Beckley, 1985; Harrison, 2002; Whitfield, 2005; Harrison and Whitfield, 2006; James *et al.*, 2007) and macro-invertebrates (e.g. McQuaid and Branch, 1984; Teske and Wooldridge, 2004; Richoux and Froneman, 2007; Griffiths *et al.*, 2010; Richoux *et al.*, 2014) are present. Estimating the physiological limits of selected marine ectotherms (fish and macro-invertebrates) in these shallow habitats and comparing the resulting information with available and predicted temperature data may be important to examine intra-species tolerance in order to gauge the local vulnerability and adaptability of different species. It may also provide a platform for localized thermal tolerance and performance research in South Africa. The overall aim of this thesis is, thus, to quantify and compare the thermal tolerance and performance of a range of coastal marine ectotherms (taxonomic, habitat and biogeographical) to predict what potential effects climate change would have on them.

To achieve this, the thesis is presented in six chapters. The study area is described in Chapter two. This second chapter synthesises the important morphological and biological details of the

habitats (estuarine, subtidal, gully and intertidal rock pools) and marine ectotherms (fish and macro-invertebrates) of focus, as well as air and water temperature variability for the various shallow habitats within this warm-temperate region. This second chapter, thus, provides important baseline information for the remaining data chapters.

Chapter three reports on the exploration of the upper and lower thermal tolerance limits (CT_{max} and CT_{min}) of juvenile fish and adult macro-invertebrates from different habitats (estuarine, rock pool, and subtidal gully) in both summer and winter, and compares these thermal limits to the summer maximum and winter minimum habitat temperatures measured *in situ*.

The focus of Chapter four is on the measurement of the thermal tolerance limits of a model fish species, *Diplodus capensis*, at the juvenile and adult life stages. The thermal performance of juveniles and adults was also assessed using static respirometry on the juveniles and using implantable heart rate loggers on the adults to measure maximum heart rate (f_{Hmax}) under conditions of acute warming.

Chapter five provides further information on the effects of cardiac activity under increasing thermal stress for two intertidal macro-invertebrate species, the brown mussel *Perna perna* and the estuarine crab *Parasesarma catenatum*, when submersed in seawater and compared between summer and winter using non-invasive infrared technology.

The final chapter, Chapter six, provides a synthesis of what has been evaluated in this thesis and discusses the global relevance of the findings. In doing so, it outlines the observed effects of temperature change on the thermal tolerance and performance of several fish and macro-invertebrate species from different habitats in the warm-temperate climate change hotspot, and provides information on appropriate methodology for different species and life stages using a multi-scale, multi-method approach.

1.3 PERMISSION FOR SPECIMEN COLLECTION AND ETHICAL CLEARANCE

All specimen collections and experimental trials were carried out by me in person, with assistance from fellow students. The sampling methods, experimental setup and experimental protocols for working with fish and macro-invertebrates were researched, developed and built by me and were approved by the ethics committee of the South African Institute for Aquatic Biodiversity (SAIAB) (REF#: 2016/02) and Rhodes University (DIFS van der Walt 2017).

Permits for collection and transport of fish and macro-invertebrates for research purposes were issued by the Department of Environmental Affairs (DEA), Republic of South Africa and are listed: Permit No's. RES2017/26; RES2018/26.

CHAPTER TWO: STUDY AREA AND STUDY SPECIES

2.1 STUDY AREA

2.1.1 Coastal zone and climate variability

The coastal zone is the interface between land (continental shelf) and water (low tide mark) and presents 7.6% of the world's ocean habitat (Yool and Fasham, 2001). Coastal zones are important because they provide vital ecosystem services, such as habitat and food, for a host of marine organisms along with commercial and recreational opportunities for human populations (Potts *et al.*, 2015). Coastal zones are therefore considered to be one of the most ecologically and socio-economically important systems on the planet (Harley *et al.*, 2006). Globally, coastal zones are responsible for generating high levels of productivity that support over 90% of the global fish catches (Pauly *et al.*, 2002). In addition, coastal zones provide a diverse range of habitats such as rocky coasts, beaches, barrier islands, reefs, atolls, estuaries and tidal flats that make these areas important for biodiversity, with a majority of described marine taxa found in this ocean zone (Ray, 1991).

Of these coastal habitats, rocky shores and estuaries are considered the most dynamic and are characterized by rapidly fluctuating parameters (Richardson *et al.*, 2006; Vinagre *et al.*, 2018). Intertidal environments, such as rocky shores, are distinctive, highly variable habitats which can range from a few meters to hundreds of kilometres of unbroken rock-based substrate (Horn and Martin, 2006). In South Africa, most rocky shores can be divided into four zones occupied by distinctive species, i.e. Littorina Zone, Upper Balanoid Zone, Lower Balanoid Zone and the Infratidal Zone (Branch and Branch, 2018). Few species live in the Littorina Zone, on the highest part of the shore. This zone is occupied mostly by littorinid periwinkles (Branch and Branch, 2018). The Upper Balanoid Zone lies at the mid-tide mark, where animals such as limpets and barnacles are present (Branch and Branch, 2018). Further down the shore, in the Lower Balanoid Zone, different species of algae, limpets, and mussels are supported (Branch and Branch, 2018). Lastly, at the bottom of the shore, algal beds form an obvious band called the Infratidal Zone (Branch and Branch, 2018). This zone is the most diverse and consists of mixed beds of algae, red bait and isolated corals (Branch and Branch, 2018). Bio-physical drivers that determine where a species can live on the lower shore include wave action and

13

species interactions. On the higher shore, temperature and water loss (desiccation) play a major role in determining the survival and abundance of organisms (Branch and Branch, 2018).

Rock pools within these zones comprise of indentations and depressions within the rock substratum that retain water during low tide, acting as a refuge from most predators (low depth excludes large consumers) or nursery area for intertidal marine organisms or transient larvae and juveniles of marine fish species (Zander *et al.*, 1999; Dias *et al.*, 2014, 2016; Vinagre *et al.*, 2016, 2018). Pools are usually patchily distributed along the intertidal rocky shores, highly variable both spatially and temporarily like the adjacent intertidal rocky shore (Martins *et al.*, 2008; Firth *et al.*, 2013). Even though rock pools have been acknowledged as important microhabitats, there is some debate as to whether they represent a "true intertidal habitat", as marine organisms are submerged for the full tidal cycle and are thus not subject to the full range of environmental fluctuations seen in adjacent habitats (Huggett and Griffiths, 1986; Underwood *et al.*, 1991). There is nevertheless clear evidence that, like the surrounding rocky shore habitat and unlike subtidal habitats such as gullies (generally covered by water at all states of the tide and have a higher degree of water turnover and mixing; Purchase, 2017), conditions in rock pool habitats are highly regulated by the tidal cycle and environmental variability (temperature, salinity, oxygen and pH) (Branch and Branch, 2018) (Figure 2.1).

Further factors that influence the rock pools environment are latitude, the intertidal height along the shore (location), wave exposure, degree of shading, volume and depth of the pools, duration of isolation from the ocean at low tide, the degree of low tide exposure (air temperature, solar radiation intensity, wind activity and humidity), freshwater input, precipitation, and evaporation (Ganning, 1971; Daniel and Boyden, 1975; Morris and Taylor, 1982; Huggett and Griffiths, 1986; McMahon, 1990; Metaxas and Scheibling, 1993; Kemp, 2009; Little *et al.*, 2009) (Figure 2.1). Water temperatures in tropical/low latitude rock pools, for instance, can reach maximums of up to 40.0 °C, whereas in temperate/high latitudes rock pools temperatures can drop below freezing during cooler months (Vinagre *et al.*, 2018). Rock pools higher along the intertidal rocky shore coast (high-shore rock pools) are exposed to greater external environmental extremes such as temperature, salinity, and oxygen compared with those lower on the shore (low-shore rock pools) which are flushed by waves more frequently. This indicates a degree of isolation (driven by tides) that is key for physical environment changes as well as species diversity (Gibson, 1972; Huggett and Griffiths, 1986) and survival (i.e. mass mortalities

of different marine ectotherms due to desiccation can occur higher on the rocky shore) (Little *et al.*, 2009; Firth *et al.*, 2015; Vinagre *et al.*, 2016) (Figure 2.1).
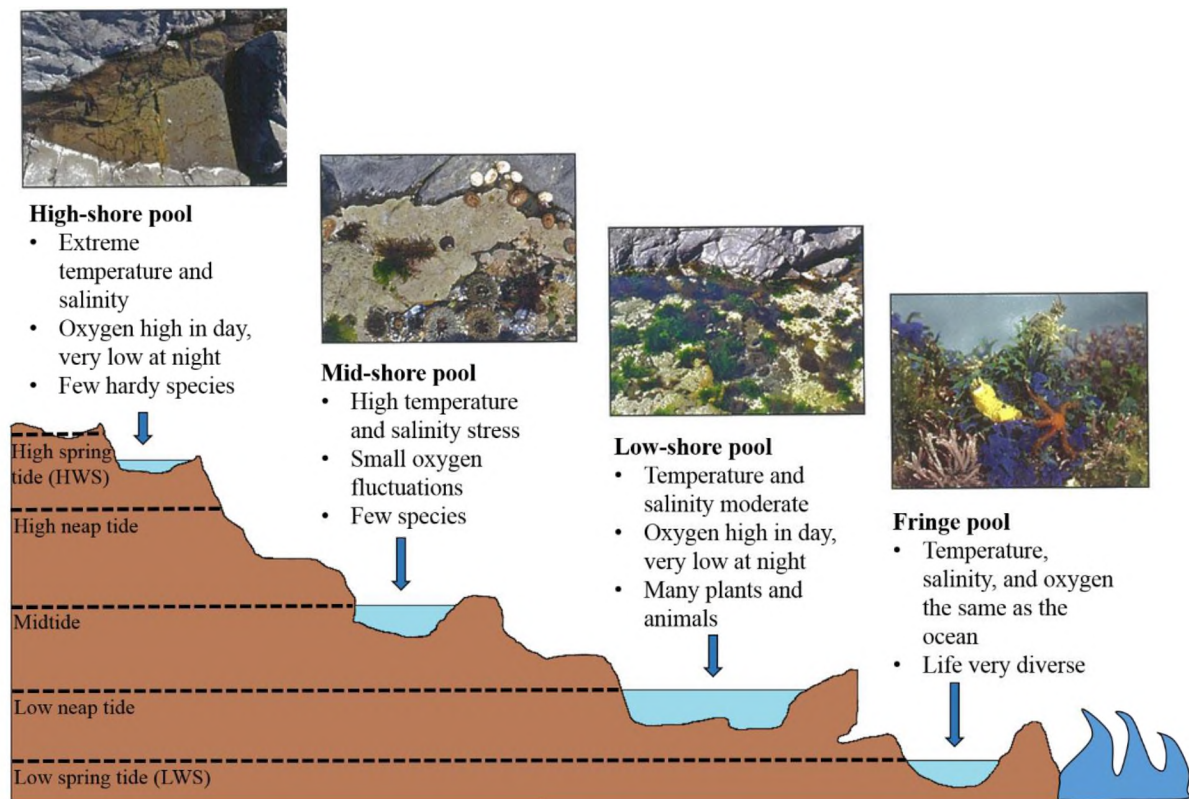
Figure 2.1. Characteristics of the rock pools in terms of temperature, salinity, oxygen, and species diversity according to the position along the shoreline (redrawn from Branch and Branch, 2018).

Estuaries are also dynamic shallow habitats where marine and fresh waters meet and, as such, are characterized by fluctuating environmental conditions (e.g. Harrison and Whitfield, 2006). More recently, Whitfield and Elliott (2011) globally define estuaries as "a semi-enclosed coastal body of water which is connected to the sea either permanently or periodically, has a salinity that is different from that of the adjacent open ocean due to freshwater inputs, and includes a characteristic biota". They are mostly shallow, calm and turbid environments which are strongly influenced by tidal action, freshwater inflow, wind, wave action, rainfall as well as physical environmental variations in air and water temperature, salinity, dissolved oxygen, and turbidity (James *et al.*, 2013). Changes in the environmental conditions within an estuary may be fairly predictable, or alternatively, they may be caused by short- and/or long-term

unpredictable climatic fluctuations, all of which may modify the physical and biological characteristics (James *et al.*, 2013). This in turn may place considerable physiological demands on fish and invertebrates occupying these systems (Whitfield, 1999), who rely heavily on them for food resources, protection from predators, and as nursery areas (Beck *et al.*, 2001; Martinho *et al.*, 2009; Sheaves *et al.*, 2015), which may ultimately affect their abundance and distribution (Flint, 1985; Desmond *et al.*, 2002).

Of the environmental fluctuating conditions influencing estuaries, temperature and salinity have been found to be major factors affecting the abundance and distribution of fish species in South African estuaries (Harrison and Whitfield, 2006). The temperature regime of individual estuarine systems is directly related to biogeography (i.e. tropical, subtropical, warm-temperate and cool-temperate regions) (Heydorn and Tinley, 1980). For example, estuaries within tropical regions may have higher water temperatures in comparison to estuaries from warm-temperate and cool temperate regions (Whitfield, 2019). Temperature variability can also differ greatly depending on whether an estuary is permanently open to the sea or temporarily open/closed (Whitfield, 2019). During the closed phase, solar heating and evaporative cooling of the estuarine water body are the major determinants of the prevailing temperature regime (Whitfield, 2019). Floods and droughts also influence the temperature regime in estuaries with temperatures reflecting riverine conditions during floods and the sea having a stronger influence during normal river flows/tides or droughts (Whitfield, 2019). Salinity, another important physical variable of estuaries, also varies greatly biogeographically and plays an important role in characterizing individual estuaries and describing transition zones within a particular system, such as being either oligohaline (0.5 – 4.9 units), mesohaline (5.0 – 17.9 units), polyhaline (18.0 – 29.9 units), euhaline (30.0 – 39.9 units) or hypersaline (> 40.0 units) (Whitfield, 2019).

2.2 SAMPLING LOCATION

The permanently open Kariega Estuary and adjacent intertidal rocky low-shore, in the form of intertidal rock pool and subtidal habitats, were selected as the locations in which to identify thermal variability patterns and to see how these patterns may impact the performance of selected juvenile fish and adult macro-invertebrates along the south-east warm-temperate coastal zone of South Africa.

2.2.1 Kariega Estuary

The Kariega Estuary (33°40'46.6″ S, 26°40'57.9″ E), situated between Port Alfred and Port Elizabeth, enters the sea at Kenton-on-Sea (Paterson and Whitfield, 2000) (Figure 2.2). The estuary is 18 km in length and can be subdivided into lower, middle and upper transition reaches and drains a catchment of 686 km^2 (Grange and Allanson, 1995) (Figure 2.2). The main channel averages 2.3 m in depth and has an average width of 110 m in the mouth region, narrowing to 40 – 60 m in its upper reaches, with a water depth of 1 – 4 m (James and Harrison, 2010) (Figure 2.2).
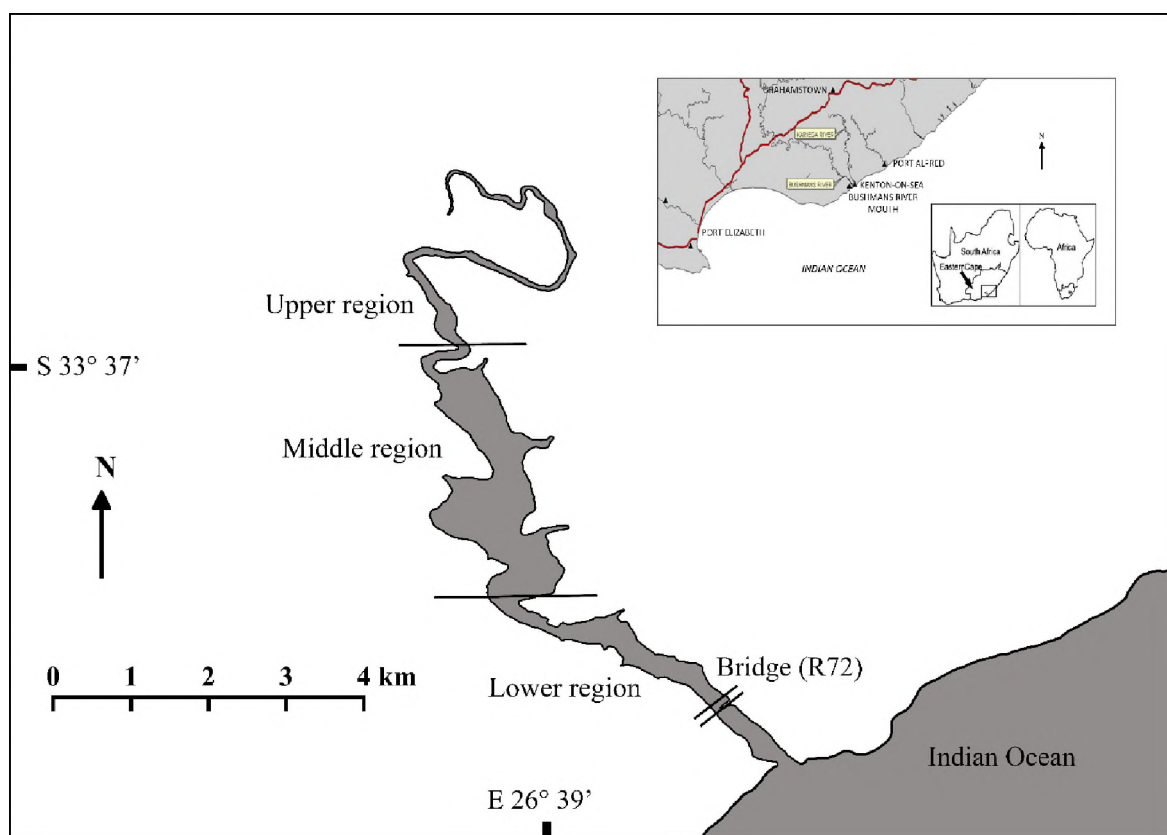
Figure 2.2. Map of the Kariega Estuary, showing the head, upper, middle and lower reaches and adjacent coastline situated between Port Alfred and Port Elizabeth, in the Eastern Cape, South Africa.

The estuary has a spring tidal range of approximately 1.6 m in the lower reaches (Grange and Allanson, 1995; Grange et al., 2000). The estuary receives negligible mean annual runoff of ± 15 x 10^6 m^3, which is attributed to the combined effect of the semi-arid climate of the Eastern

Cape, the small catchment area, three large dams (Settlers, Moss and Assegai) and numerous weirs within the catchment that impound much of the runoff (Hodgson, 1987; Allanson and Read, 1995; Grange and Allanson, 1995). With very little riverine influence, the estuary is marine dominated and is well-mixed with no salinity stratification of the water column at any stage of the tidal cycle (Grange and Allanson, 1995; Whitfield and Paterson, 2003). During extended periods of no river flow (times of drought), hypersaline conditions can occur in the upper reaches of the estuary (45 units), with a reverse salinity gradient in the system. During low flow conditions salinity is usually uniformly marine (35 units) along the length of the estuary (Nodo *et al.*, 2018). Water temperatures in the estuary do not deviate significantly from the natural state, since considerable tidal exchange of marine water in the lower and middle reaches prevents adverse warm conditions developing in these regions (Allanson and Read, 1995). As a result of the marine influence, there is low turbidity in the lower reaches of the Kariega Estuary (>10 NTU) (Bailey and James, 2013). The sediment in the upper reaches is dominated by coarse material and silt, the middle reaches by sand and fine sediment particles, and the lower reaches by sand and a small clay and mud component (Richardson *et al.*, 2006).

For this study, the lower reaches of the Kariega Estuary extending from the mouth (33° 40' 58.92" S, 26° 41' 10.90" E) to 5.27 km upstream (33° 36' 53.72" S, 26° 39' 15.93" E) were sampled and temperature data from the lower, middle and upper reaches were evaluated (Figure 2.3).
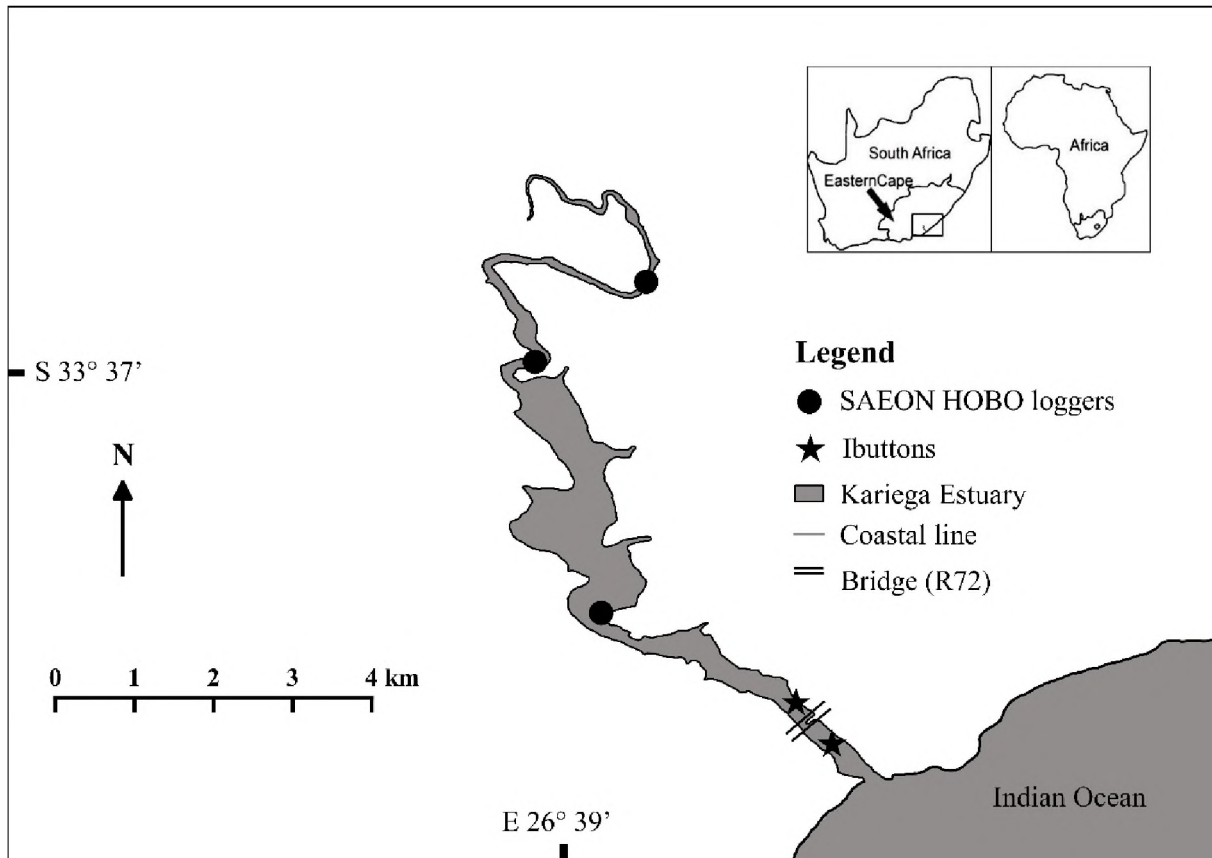
Figure 2.3. Map of the Kariega Estuary showing locations of SAEON HOBO loggers in the upper, middle and lower reaches (round circles) and Ibutton thermal loggers (black stars) in the lower reach.

Atmospheric temperature recorded from the closest weather station (Port Alfred) between 2012 and 2018 from the South African Weather Service indicated a mean monthly minimum temperature of 6.0 °C (R^2 = 0.01) and a mean monthly maximum temperature of 27.0 °C (R^2 = 0.00) (Figure 2.4a). Between 2017 and 2018, the minimum temperature recorded was 0.0 °C and the maximum temperature recorded was approximately 35.0 °C (Figure 2.4b). Within this period, temperature reached 0.0 °C on two separate occasions (28 June and 19 July 2017) and temperature was above 35.0 °C on five separate occasions (28 February 2017, 22 March 2017, 30 March 2017, 4 September 2017, 3 October 2017).
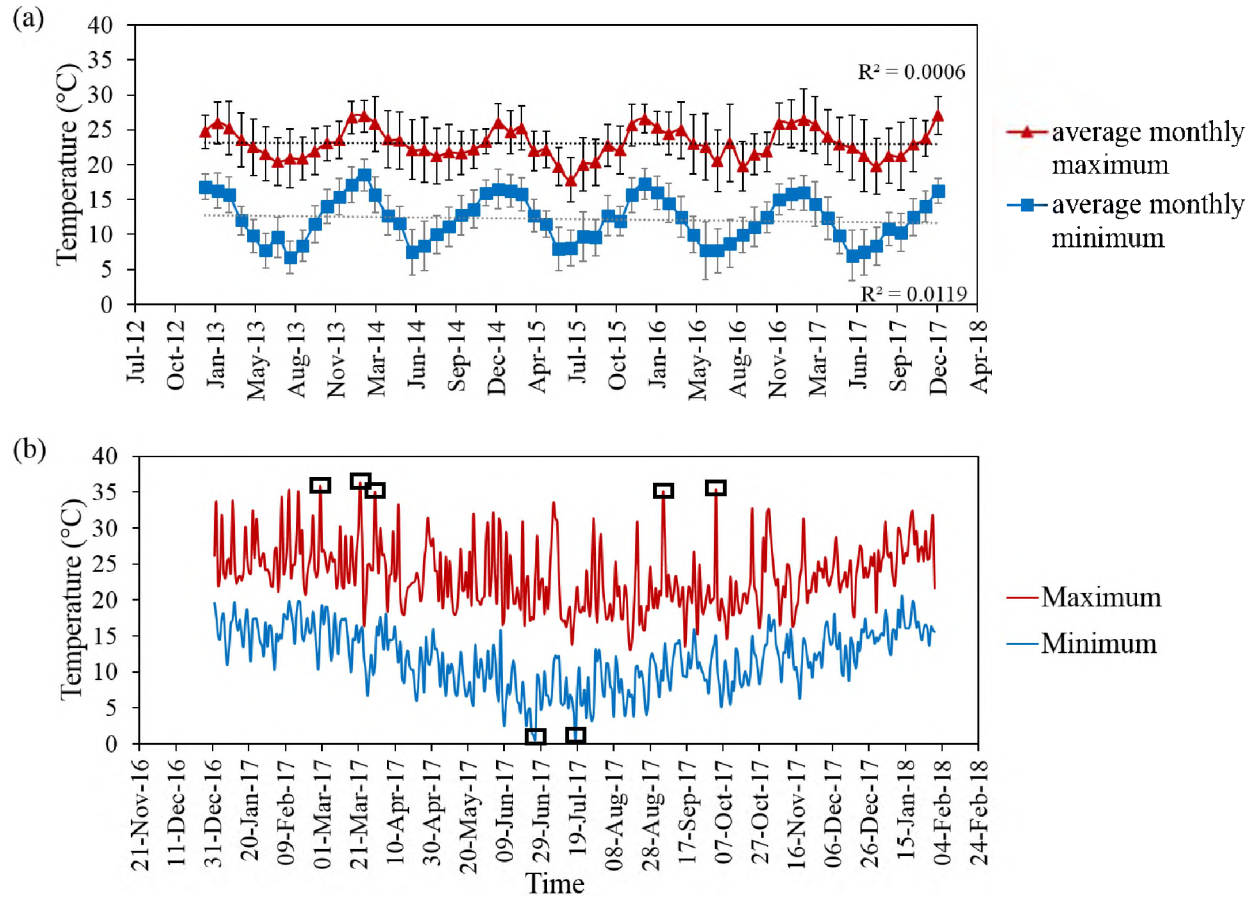
Figure 2.4. Atmospheric temperature data measured from the Port Alfred station showing (a) the mean monthly maximum and minimum air temperatures (°C) for the years 2013 – January 2018 (South African Weather Service, *in litt.*) and (b) the maximum and minimum air temperatures recorded in the sampling period, 2017 and 2018. In Figure (a), regression lines are indicated by a black dotted line for the average monthly maximum temperatures and a grey dotted line for the average monthly minimum temperatures. In Figure (b), black outlined squares indicate dates in 2017 where temperatures reach 0.0 °C and 35.0 °C.

20

2.2.2 Rocky shore coastal environment

Adjacent to the warm-temperate Kariega Estuary and extending to the Bushman's Estuary (±3.0 km to the west) are sections of rocky coastline (Figure 2.2). This rocky coastline consists of three major geological units. The oldest of these units are the sandstone and shale deposits of the Bokkeveld Group, which are over-lain with sandy limestone and marine deposits made up of the Alexandria Formation, and above this unit are later tertiary to quaternary deposits of the Aeolian beach and dune sands (Fraser, 2003). The Aeolianite or dune rock also forms a number of wave-cut rocky platforms or promontories, which often shield or surround gullies and a number of rock pools. Within this rocky coastline, Sydney's Hope Gully (33° 41' 43.92" S, 26° 39' 56.07" E) and a low-shore intertidal rock pool (33° 41' 44.46" S, 26° 39' 57.84" E) were evaluated for this study (Figure 2.5, Figure 2.6).

Sydney's Hope Gully is situated 250 m from the mouth of the Bushman's Estuary (Figure 2.5). The subtidal gully has a maximum depth of 2.5 m at low tide, a maximum width of 21.3 m and a maximum length of 64 m with one end open to the sea at both low and high tide (Figure 2.6). Although the gully is exposed to tidal regimes, it always has a large volume of water entering it from the seaside (Figure 2.6).

The low-shore rock pool situated close to Sydney's Hope Gully has a depth range of 0.5 – 0.8 m at low tide, a maximum width of 7.4 m and a length of 15 m (Figure 2.5, Figure 2.6). The west and south sides of the rock pool are covered by mussel beds and the north and east sides are vertically surrounded by wave-cut rocky walls or ridges, providing shaded shelter to the area (Figure 2.6). Furthermore, the pool is exposed to semi-diurnal tide cycles of immersion (high tide) and emersion (low tide). Salinity in both the low shore rock pool and Sydney's Hope Gully, during the sampling period (2017 and 2018) always measured 35.0.
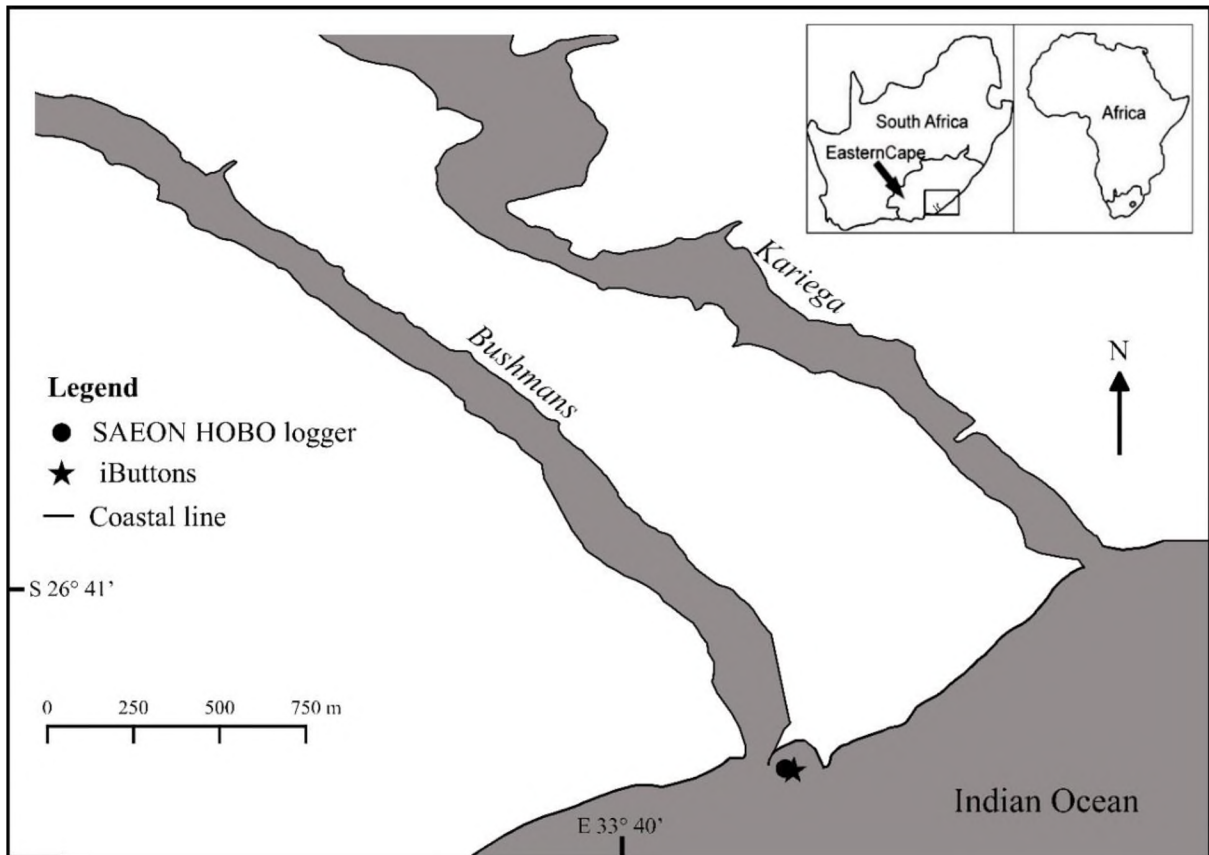
Figure 2.5. Map of the adjacent coastline showing the location of the SAEON HOBO and Ibuttons thermal loggers placed within the Sydney's Hope Gully and low-shore rock pool where intertidal fish and macro-invertebrates were collected.

Figure 2.6. Low-shore rock pool (left) and Sydney's Hope Gully (right).

2.3 THERMAL VARIABILITY

2.3.1 Kariega Estuary

Temperature data were provided by the Algoa Bay Sentinel Site for LTER of the South African Environmental Observation Network (SAEON). Data were obtained from HOBO temperature loggers deployed at three stations [upper (33° 36' 53.72" S, 26° 39' 15.93" E), middle (33° 37' 32.99" S, 26° 38' 21.36" E) and lower (33° 39' 36.03" S, 26 ° 38' 53.84" E)] in the Kariega Estuary since November 2012 (Figure 2.3). In addition, two constantly immersed Ibuttons (ColdChain ThermoDynamics, Fairbridge Technologies) were placed in the lower reaches close to the mouth of the Kariega Estuary (0.6 m depth on low tide and 1.2 m depth on high tide) (33° 40' 40.45" S, 26° 40' 47.19" E; 33° 40' 20.10" S, 26° 40' 29.50" E) (Figure 2.3) to assess temperature fluctuations in shallow water. The Ibuttons recorded temperature every hour and were retrieved every six months over the one-year sampling period (2017). Unfortunately, one of the Ibuttons (Ibutton 2) was lost in a large tidal event and temperature data was only available from the beginning to the middle of 2017.

Water temperatures in the estuary showed clear seasonal trends between 2013 and 2018. Lowest temperatures were recorded in autumn and winter months (March - August), and highest temperatures in spring and summer (September - February) (Figure 2.7). Daily water temperature variability was greatest in the lower reaches (Figure 2.7a) compared with the middle (Figure 2.7b) and upper (Figure 2.7c) reaches. In winter, average water temperatures were highest in the upper (16.1 °C) and lower (16.1 °C) reaches followed by the middle reaches (15.8 °C) (Figure 2.7) presumably as a factor of depth. The coldest temperatures, however, recorded over the five-year period, occurred from the upper reaches (10.9 °C – 3 August 2015) followed by the middle (11.7 °C – 28 July 2015) and upper (12.2 °C – 22 June 2014) reaches, most likely influenced by colder air temperatures (Figure 2.7).

In summer, average water temperatures were highest in the upper reaches (27.1 °C) followed by the middle reaches (26.6 °C) and the lower reaches (23.9 °C) (Figure 2.7). The hottest temperature recorded in the estuary over the five-year period was from the middle reaches (31.9 °C) and the upper reaches (31.1 °C) in February of 2014 followed by the lower reaches in December 2015 (30.1°C) (Figure 2.7). Alternatively, the coldest temperatures recorded were

from the lower reaches compared to the middle and upper reaches of the Kariega Estuary, with temperatures in the lower reaches reaching minimum temperatures of 14.6 °C in January 2018, 15.6 °C in February 2016, and 14.9 °C in December 2014 (Figure 2.7, Figure 2.8). Low minimum temperatures in summer in the lower reaches are associated with the movement of upwelled water into the estuary on the high tide.

In the lower reaches of the Kariega Estuary, shallow Ibutton temperature data for the sampling period of 2017-2018 matched relatively closely to the temperature data collected using the SAEON HOBO logger, especially for Ibutton 2 (Figure 2.7a). Ibutton 1, however, situated close to the mouth of the Kariega Estuary, had greater variations in temperature and demonstrated lower temperature records possibly as a result of the movement of cold upwelled water into the mouth of the estuary (Figure 2.7a).
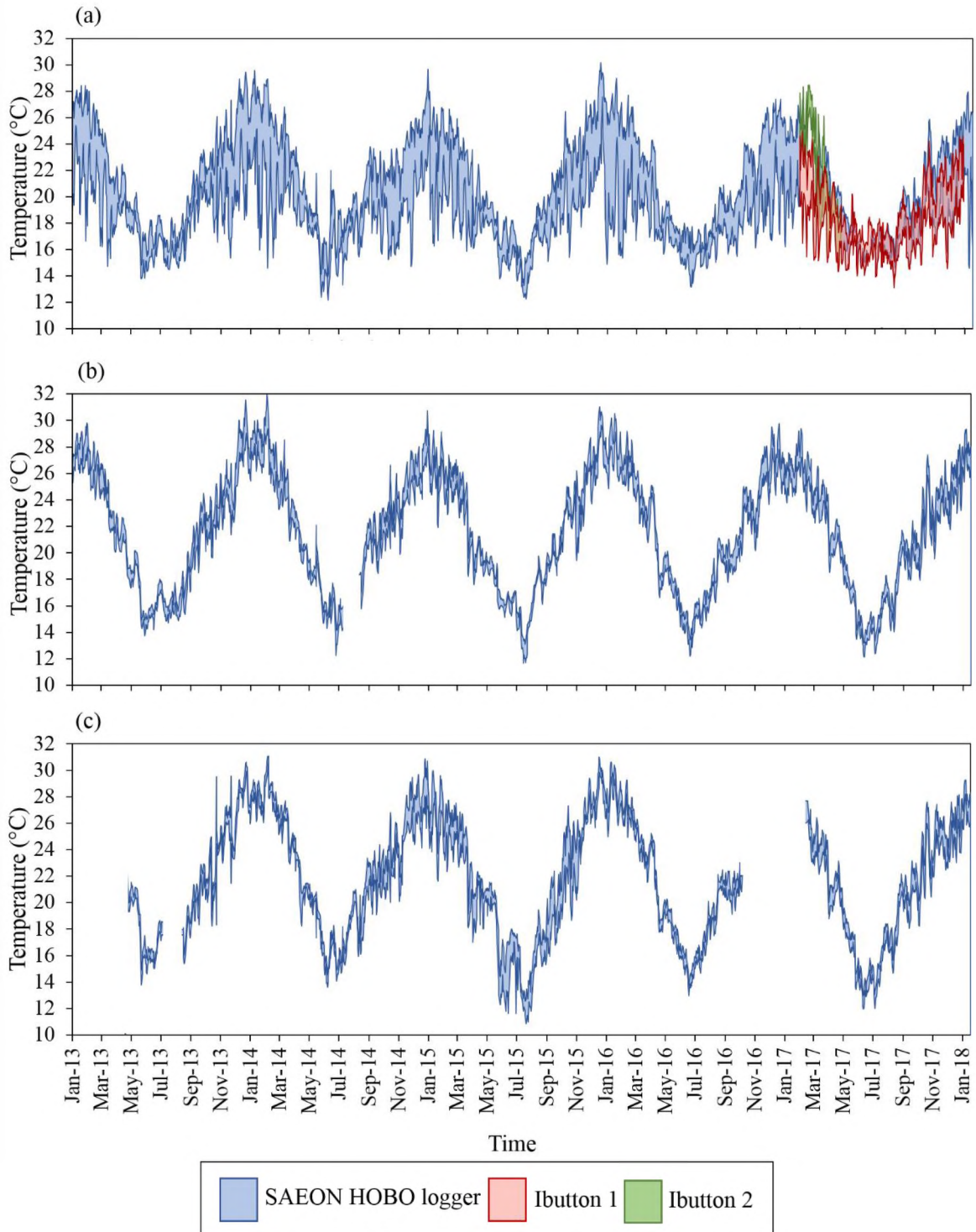
Figure 2.7. Local estuarine water temperatures (daily maximum and minimum recordings) recorded from the (a) lower reaches, (b) middle reaches and (c) upper reaches of the Kariega Estuary from 2013 to 2018 using SAEON (Elwandle Node) HOBO thermal loggers (blue). Shallow estuarine water temperatures (daily maximum and minimum recordings) recorded from the lower reaches (a) of the Kariega Estuary from February 2017 to January 2018 using Ibutton 1 (red) and from February to May 2017 (green) using Ibutton 2 are included.
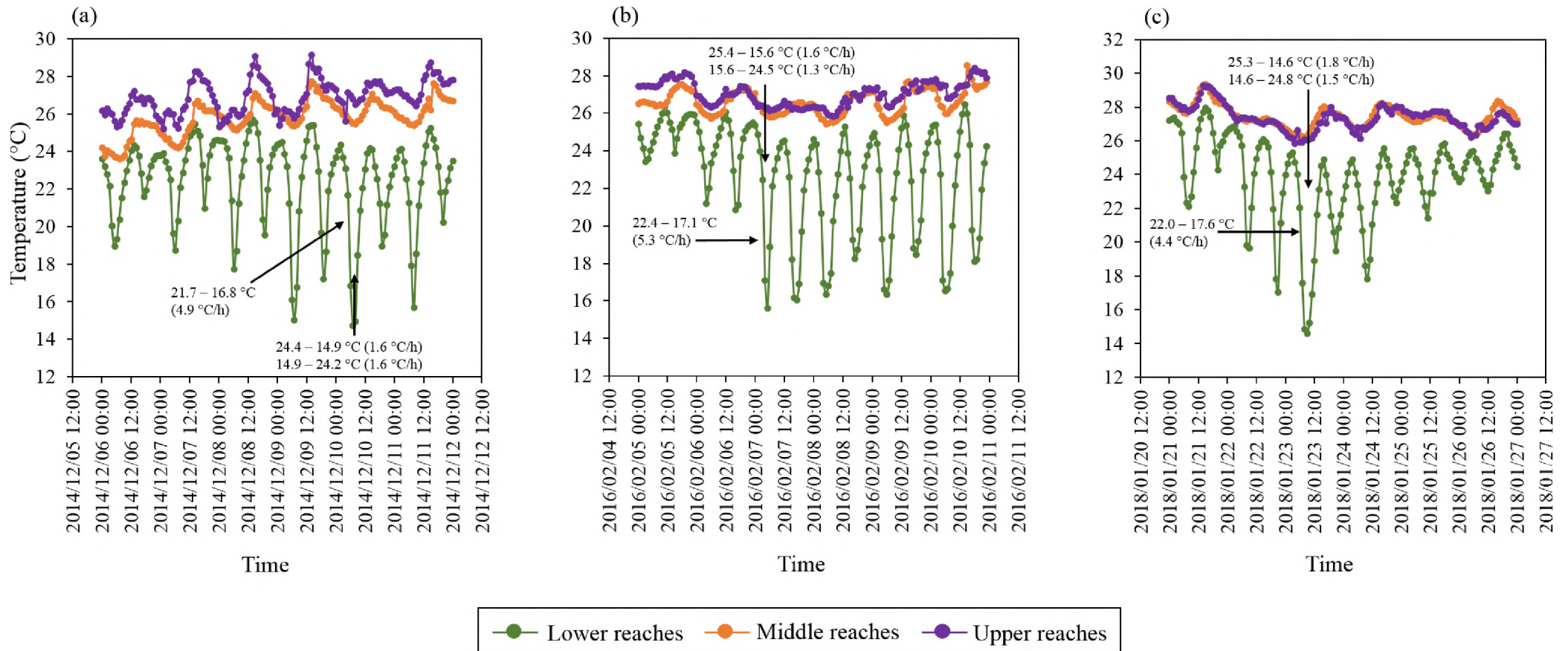
Figure 2.8. Hourly estuarine temperature data from the lower (green), middle (orange) and upper (purple) reaches of the Kariega Estuary for (a) December 2014, (b) February 2016 and (c) January 2018 indicating upwelling events and extreme drops in temperature with black arrows and the range and rate of temperature change over a period of eight days.

2.3.2 Low-shore rock pool and gully

Hourly temperature data between 2013 and 2018 were provided by the Algoa Bay Sentinel Site for LTER of the South African Environmental Observation Network (SAEON) from a HOBO logger located in Sydney's Hope Gully (33° 41' 43.92" S, 26° 39' 56.07" E) (Figure 2.5). To obtain temperature measurements from the intertidal low-shore rock pool, two Ibuttons (33° 41' 44.46" S, 26° 39' 57.84" E; and 33° 41' 29.17" S, 26° 40' 32.29" E) were deployed (Ibutton 1: 0.5 m depth on low tide and 0.9 m depth on high tide; Ibutton 2: 0.8 m depth on low tide and 1.2 m depth on high tide) (Figure 2.5). The Ibuttons were set to record hourly temperature from the beginning of 2017 until January 2018. Ibutton temperature data was retrieved every six months. Unfortunately, one of the Ibuttons was lost due to sand deposition and temperature data was irretrievable at the end of 2017, with temperature data from the beginning to the middle of the year successfully retrieved.

Water temperatures within Sydney's Hope Gully and the adjacent intertidal low-shore rock pool showed seasonal variation, however, coldest daily water temperature values were recorded in summer and not in winter, especially for Sydney's Hope Gully, as is evident in the three obvious sharp decreases in temperature during January/February 2013, 2014, 2016 and 2018 and December 2014 (Figure 2.9, Figure 2.10). These drops in temperature during summer months are a result of upwelling events.

When comparing daily temperature recorded data between Sydney's Hope Gully and the adjacent low-shore rock pool, Sydney's Hope Gully was more variable as a result of the gully having an open connection to the sea (Figure 2.9). In summer, in Sydney's Hope Gully, an average temperature of 19.0 °C, a minimum temperature of 12.8 °C (7 February 2016) and a maximum temperature of 25.1 °C (3 January 2016) was recorded (Figure 2.9). For winter, in Sydney's Hope Gully, an average temperature of 16.5 °C, a minimum temperature of 13.2 °C (22 August 2017) and a maximum temperature of 20.0 °C (2 July 2014) was recorded (Figure 2.9). During the sampling period (2017-2018) in the low-shore rock pool, however, an average temperature of 18.9 °C, a minimum temperature of 13.8 °C (10 December 2017) and a maximum temperature of 23.7 °C (4 January 2018) was recorded for Ibutton 1 in summer (Figure 2.9). In winter, Ibutton 1 had an average temperature of 16.4 °C, a minimum temperature of 13.5 °C (23 August 2017) and a maximum temperature of 18.2 °C (2 June 2017) (Figure 2.9). For Ibutton 2, an average temperature of 17.3 °C, a minimum temperature of 13.5

°C (30 July 2017) and a maximum temperature of 22.9 °C (14 February 2017) occurred between February and the end of July 2017 in the low-shore rock pool habitat (Figure 2.9).
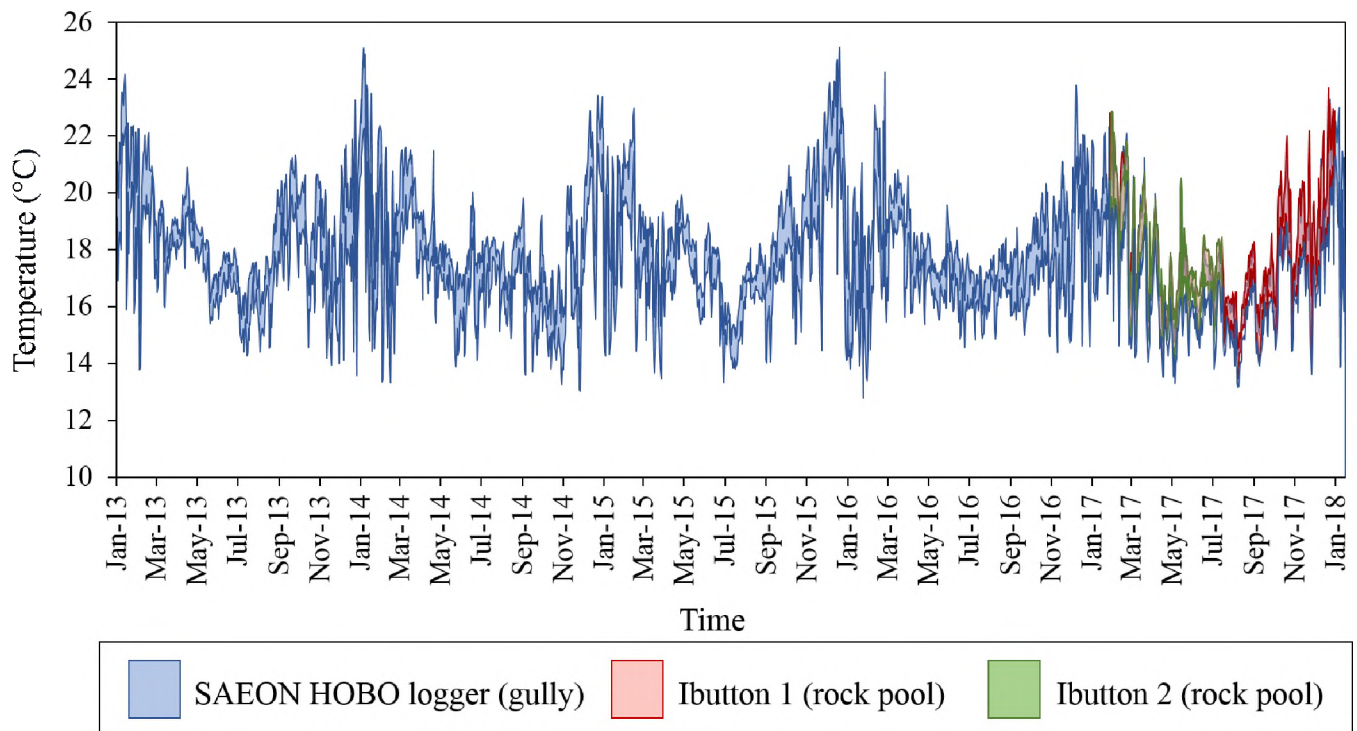
Figure 2.9. Local rocky shore water temperatures (daily maximum and minimum recordings) recorded from Sydney's Hope Gully between 2013 and 2018 using SAEON (Elwandle Node) HOBO loggers (blue) and a shallow low-shore rock pool habitat between 2017 and 2018 using Ibutton 1 (red) and Ibutton 2 (green).
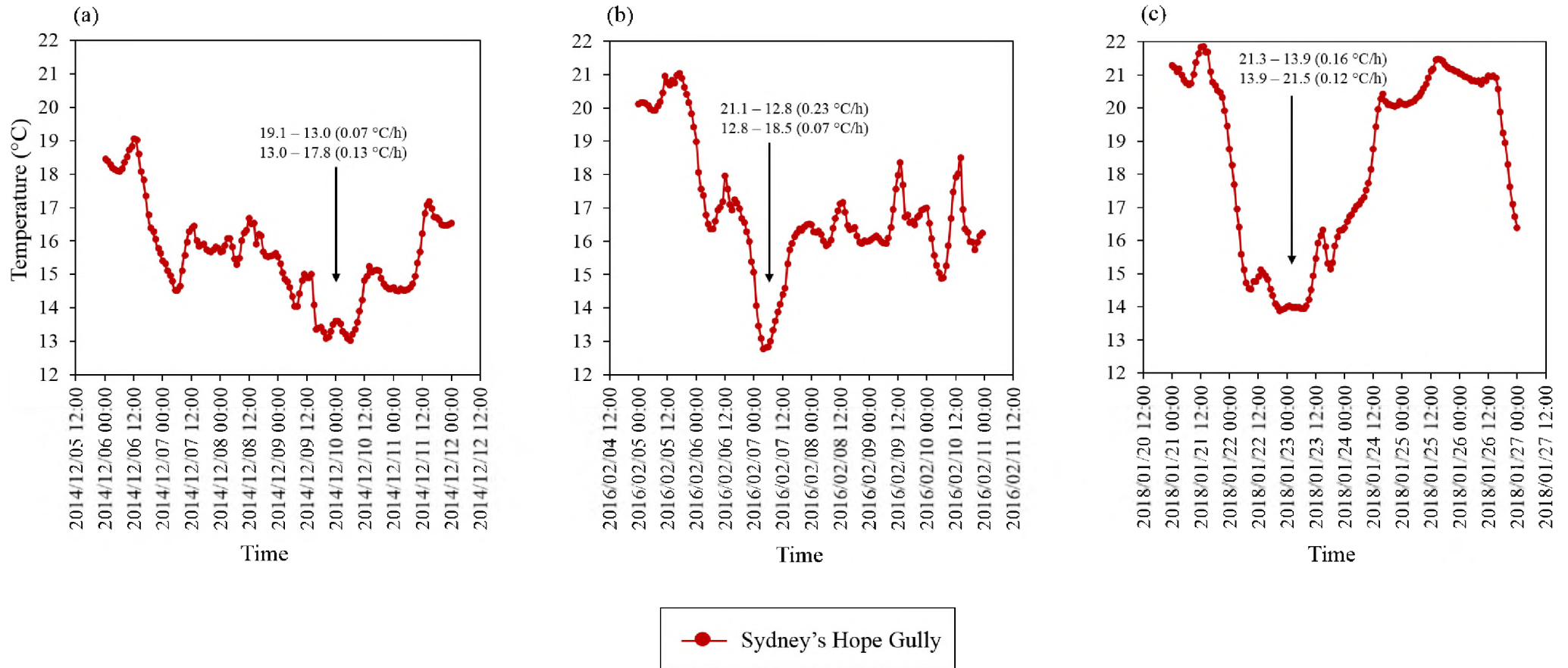
Figure 2.10. Hourly sea temperature data from Sydney's Hope Gully for (a) December 2014, (b) February 2016 and (c) January 2018 indicating upwelling events and extreme drops in temperature with black arrows and the range and rate of temperature change over a period of eight days.

2.4 STUDY SPECIES

The study area is located in the centre of the warm-temperate region and as such contains marine ectothermic fish and macro-invertebrate species with overlapping northern and southern range affinities (temperate, cool-water, warm-water and tropical species) as well as widespread species. Harrison and Whitfield (2006) classified estuarine fish species into several groups based on their distribution and relationship to temperature and salinity (refer to Figure 1.4 in Chapter 1). These include tropical species that extend their distribution into subtropical estuaries in South Africa (Group 1, Figure 1.4, Table 2.1). A second group of tropical species extend their distribution from subtropical estuaries into warm-temperate estuaries (Group 2, Figure 1.4, Table 2.1). The third group was classified as warm-water endemic species that are found in warm-temperate and subtropical estuaries (Group 3, Figure 1.4, Table 2.1). The fourth group comprises cool-water endemic species that occur in both cool-temperate and warm-temperate estuaries that are uncommon in subtropical estuaries (Group 4, Figure 1.4, and Table 2.1). The fifth group comprises temperate species that occur in the eastern Atlantic region and extend their distribution around the South African coast (Group 5, Figure 1.4, and Table 2.1). The sixth group comprises widespread species that occur in estuaries throughout South Africa (Group 6, Figure 1.4, and Table 2.1). Griffiths *et al.* (2010) classified macro-invertebrates into five bioregions based on their spatial patterns of species richness and endemism (refer to Figure 1.3 in Chapter 1).

For the purpose of this study, the Harrison and Whitfield (2006) classification system for estuarine fish species was adopted for fish and macro-invertebrates found in the lower reaches of the Kariega Estuary and rocky intertidal nearshore (Table 2.1). Where possible, species from these different groups were targeted as well as species that were present, abundant and easily accessible in each of the different habitats (estuary, gully, rock pool) (Table 2.1). Only species from Group 1 were not caught as these species were not obtained in sufficient numbers. The most abundant fish species were secondary resident/transient species that occurred as juveniles in rock pools and estuaries. In contrast, most macro-invertebrates were resident species that were present as both juveniles and adults.

Table 2.1. Study species for the present study including their biogeographic distribution, life stage, environment and sampling area in which they were collected.

Biogeographic affinity	Species	Illustration	Taxonomic group	Life stage	Environment	Habitat	Sampling area
Group 2 Tropical	*Chaetodon marleyi*		Fish	Juvenile	Subtidal, littoral	Shallow coastal rocky waters, reefs, rock pools, estuaries	Estuary
Group 2 Tropical	*Kuhlia mugil*		Fish	Juvenile	Subtidal, bentho-pelagic, demersal	Shallow coastal waters, reefs, rock pools	Rock pool
Group 3 Warm-water endemic	*Chelon dumerili*		Fish	Juvenile	Demersal, littoral	Shallow coastal waters, estuaries, lagoons, tidal rivers	Estuary
Group 3 Warm-water endemic	*Rhabdosargus holubi*		Fish	Juvenile	Demersal, littoral	Inshore shallow rocky and sandy waters, lagoons, estuaries	Estuary
Group 4 Cool-water endemic	*Chelon richardsonii*		Fish	Juvenile	Demersal, pelagic	Offshore shallow coastal open waters, estuaries	Estuary
Group 5 Temperate	*Sarpa salpa*		Fish	Juvenile	Bentho-pelagic/ Demersal	Inshore shallow rocky and sandy waters, surf zone, rock pools, estuaries	Rock pool/Gully
Group 5 Temperate	*Diplodus capensis*		Fish	Juvenile and Adult	Demersal, littoral	Inshore rocky coastal waters, surf zone, rock pools, estuaries	Rock pool, gully (juveniles) Sandy beach surf zone (adults)
Group 2 Tropical	*Clibanarius virescens*		Invertebrate	Adult	Intertidal/ Subtidal	Inshore rocky shallow waters, sandy beaches, rock pools, estuaries	Estuary
Group 6 Widespread	*Parasesarma catenatum*		Invertebrate	Adult	Intertidal/ Subtidal	Inshore rocky shallow waters, estuaries, saltmarshes, mangroves	Estuary
Group 6 Widespread	*Upogebia africana*		Invertebrate	Adult	Intertidal/ Subtidal	Inshore rocky shallow waters, estuaries	Estuary
Group 6 Widespread	*Perna perna*		Invertebrate	Adult	Intertidal	Inshore rocky shallow waters, rock pools, estuaries	Rock Pool
Group 6 Widespread	*Parechinus angulosus*		Invertebrate	Adult	Intertidal	Inshore rocky shallow waters, rock pools, estuaries	Rock pool
Group 6 Widespread	*Palaemon peringueyi*		Invertebrate	Adult	Intertidal/ Subtidal	Inshore rocky areas, rock pools, estuaries	Rock pool

*Different colours represent the biogeographic distributions groupings in accordance to Figure 1.4 in Chapter 1, adapted from Harrison and Whitfield (2006) (Tropical – orange, Warm-water endemic – green; Temperate – dark blue; Cool-water endemic – light blue, widespread - purple). This table was also constructed based on the following references: Branch and Branch (1981, 2018); Kensley (1981); McQuaid and Branch (1984); Hugget and Griffiths (1986); Smith and Smith (1986); Thomson (1986); de Villiers *et al.* (1999); Randall and Randall, 2001; Heemstra and Heemstra (2004); Richoux and Froneman (2007); Muller (2011); Wait and Schoeman (2012); Emmerson (2016); Tagliarolo and McQuaid (2015, 2016).

The tropical fish species (Group 2) selected for this study included the doublesash butterflyfish *Chaetodon marleyi* (Regan, 1921) from the lower reaches of the Kariega Estuary and the barred flagtail *Kuhlia mugil* (Forster, 1801) from the rock pool habitat (Table 2.1). Both species are considered marine stragglers (Whitfield, 2019) and are found in shallow coastal waters where juveniles predominantly inhabit rock pools and lower reaches of estuaries seasonally (mainly summer), while adults mostly occur in rocky reef areas (Heemstra and Heemstra, 2004). *Chaetodon marleyi* belongs to the family Chaetodontidae (attains 200 mm TL) and are indigenous to South Africa, occurring from Lamberts Bay in the Northern Cape to Maputo in southern Mozambique where spawning takes place mostly between May and November (Vine, 1998; Heemstra and Heemstra, 2004). *Kuhlia mugil* belongs to the family Kuhliidae (attains 200 mm TL) and occurs throughout the tropical and subtropical Indo-Pacific, Red Sea and Oman to Cape Agulhas (Heemstra and Heemstra, 2004). Both species are considered stenothermal and stenohaline as adults, as they can only tolerate a narrow range of temperature and salinity in nearshore marine rocky or coral reefs. As juveniles, both species may be considered more eurythermal and euryhaline, as a result of them mostly occurring in shallow habitats such as rock pools and estuaries. Thus, they are able to tolerate a broader range of temperatures and salinity fluctuations, reducing the risk of predation and make use of a wider site range for settlement (Vine, 1998).

Warm-water endemic fish species (Group 3) included the groovy mullet *Chelon dumerili* (Steindachner, 1870) and the Cape stumpnose *Rhabdosargus holubi* (Steindachner, 1881) collected from the lower reaches of the Kariega Estuary and are both considered marine estuarine-dependent species (Whitfield, 2019) (Table 2.1). *Chelon dumerili* (max length = 400 mm TL) belongs to the Mugilidae family (Whitfield, 1998). Juveniles are normally abundant in estuaries, with the adults occurring in both the estuarine and nearshore marine environment (Wallace, 1975; Whitfield, 1998; Whitfield, 2019). *Chelon dumerili* distribution extends from Senegal to Namibia and False Bay to Mozambique (Whitfield, 1998; Harrison, 2008). *Rhabdosargus holubi* is commonly found inhabiting shallow coastal rocky shore waters as adults (max length = 450 mm TL) and exclusively estuaries as juveniles (< 140 mm TL) (Whitfield, 1998; Whitfield, 2019). They are considered endemic to southern Africa, with distribution extending from St Helena Bay, Western Cape to Maputo, southern Mozambique (Whitfield, 1998; Whitfield, 2019). Both species are common in warm-temperate and subtropical estuaries but uncommon in cool-temperate estuaries as a result of their preference

for warmer waters (Harrison and Whitfield, 2006). These species are eurythermal and strongly euryhaline as juveniles allowing them to inhabit the entire estuary (Whitfield, 1998). *Chelon dumerili* further exhibits these eurythermal and euryhaline qualities as adults, unlike *R. holubi*, using entire estuaries as a refuge throughout all life stages.

The only cool-water endemic fish species (Group 4) selected for this study was the southern mullet *Chelon richardsonii* (Smith, 1846) belonging to the Mugilidae family and which is considered a marine estuarine-opportunist (Whitfield, 2019) (Table 2.1). Juveniles and adults are commonly found in cool-temperate and warm-temperate estuaries but are most abundant in the nearshore marine environment and are considered euryhaline (Whitfield, 1998; Whitfield, 2019). They are endemic to southern Africa, with their distribution extending from Cunene River, southern Angola to St Lucia, northern KwaZulu Natal (Whitfield, 1998; Whitfield, 2019).

The temperate fish species (Group 5) included blacktail *Diplodus capensis* (Smith, 1844) and strepie *Sarpa salpa* (Linnaeus, 1758) collected from the rocky shore habitat (Table 2.1). Both species belong to the family Sparidae and are both considered marine estuarine-opportunist species. Adults (> 150 mm FL) of both species predominantly occur in the coastal surf zone, usually close to the inshore rocky areas while juveniles (25 - 100 mm TL) of both species are abundant in tide pools, gullies, reefs, sandy beach surf-zones and the lower reaches of estuaries (seagrass beds) (Christensen and Winterbottom, 1981; Beckley 1985; Smale and Buxton, 1989). *Diplodus capensis* is endemic to southern Africa (Potts *et al.*, 2014). Within southern Africa, there are two disjunct populations of *D. capensis*, one along the eastern seaboard extending from Cape Point to southern Mozambique (Smith and Heemstra, 1991; Mann, 1992) and the other from Namibia to southern Angola (Richardson, 2010). *Sarpa salpa* is distributed throughout the Mediterranean and parts of the eastern Atlantic around the South African coast to southern Mozambique (Fischer and Bianchi, 1984; Smith and Heemstra, 1991; Heemstra and Heemstra, 2004). Both species have evolved to be tolerant of fluctuating diel and seasonal temperature regimes, with a wide thermal tolerance (Heemstra and Heemstra, 2004; Potts *et al.*, 2014) and prefer cooler water temperatures (winter months) for spawning (Mann and Buxton, 1998; Potts *et al.*, 2014).

The widespread macro-invertebrates (group 6) collected from the low-shore rock pool habitat included the shrimp *Palaemon peringueyi* (Stebbing, 1915), the Cape sea urchin *Parechinus*

angulosus (Leske, 1778) and the brown mussel *Perna perna* (Linnaeus, 1758) (Table 2.1). *Palaemon peringueyi* (Arthropoda: Crustacea) occurs along the southern African coastline from Walvis Bay on the west coast to Kosi Bay on the east coast, although it tends to be more common toward the south-east and south coasts (de Villiers *et al.*, 1999). *Palaemon peringueyi* has a marine adult phase and a juvenile phase that can either be completed in nursery habitats of estuaries or intertidal rock pools (Emmerson, 1985; Emmerson, 1986; de Villiers *et al.*, 1999). Within estuarine systems, the highest densities of *P. peringueyi* can be found within eelgrass beds (*Zostera capensis*) (Emmerson, 1986; Wooldridge, 1999). Furthermore, within estuaries, *P. peringueyi* forms a major component of the hyperbenthos of the lower and middle reaches of both permanently open and intermittently open systems (Bernard and Froneman, 2004; Froneman, 2004; Kemp and Froneman, 2004), but, however, is almost completely absent from the upper reaches of these estuaries (Kibirige and Perissinotto, 2003; Bernard and Froneman, 2004). This suggests that this absence from the upper reaches of estuaries may reflect physiological constraints to temperature and salinity (Robertson, 1984; de Villiers *et al.*, 1999). Salinity extremes and lower temperatures may be tolerated, however, for limited periods only while temperatures greater than 30.0 °C tend to be lethal (Robertson, 1984; de Villiers *et al.*, 1999).

Parechinus angulosus (Echinodermata) is considered the most widespread of the southern African echinoids with a distribution extending from Lüderitz (just north of the Orange River mouth) to Umhlali in northern KwaZulu Natal (Branch *et al.*, 2002; Day and Branch, 2002). They commonly inhabit intertidal rock pools, estuaries, reefs and kelp beds (Muller, 2011) forming very dense, sometimes continuous, populations of up to 90 animals m^2 (Fricke, 1978). This high population density and herbivorous feeding pattern (Greenwood, 1975) make them key consumers in the ecology of kelp bed communities (Fricke, 1978, 1980; Stuart and Field, 1981; Branch *et al.*, 2002). Adults of *P. angulosus* can attain up to 60 mm total length (age of four - seven years) and vary in colour (Muller, 2011). Individuals within the same rock pool can display three distinct colour forms, namely pink, purple and red. Whether these colour polymorphisms represent distinct evolutionary lineages is currently unknown (Muller, 2011).

Perna perna (Mollusca) is native to South Africa and is abundant in upper and lower mussel zones (Bownes and McQuaid, 2006) along the entire east and south coasts, but less abundant, if not absent, along the west coast due to intense competition with the invasive mussel *Mytilus*

galloprovincialis (Lamarck, 1819) (Berry, 1978; van Erkom Schurink and Griffiths, 1993; Branch and Steffani, 2004) (Table 2.1). Two genetic lineages of *P. perna* with different geographic distributions have been identified, i.e. west and east (Zardi *et al.*, 2007b). The western lineage includes individuals from both Namibia and the south coast of South Africa while the eastern lineage includes individuals from the south-east and east coasts of South Africa (Zardi *et al.*, 2007b). The east and west lineages do, however, overlap for 200 km on the south-east coast of South Africa (convergence region) (Zardi *et al.*, 2011). *Perna perna* are highly sedentary, long-lived (five to six years attaining a maximum size of 200 mm TL) and exhibit external fertilization (Bayne, 1976; Suchanek, 1985; Underwood and Fairweather, 1989; Levitan, 1995; Underwood and Keough, 2001; McQuaid and Lindsay, 2000). The habitat in which they occur predominantly is highly dynamic due to strong wave action, currents and temperature extremes (both air and water) typical of the intertidal rocky shore (Berry, 1978; van Erkom Schurink and Griffiths, 1992) which allows them to tolerate a wide range of fluctuating temperatures (Tagliarolo and McQuaid, 2015).

From the lower reaches of the Kariega Estuary, the tropical yellow-banded hermit crab *Clibanarius virescens* (Krauss, 1843) (Group 2), the widespread crab *Parasesarma catenatum* (Ortmann, 1897) (Group 6) and the widespread mud prawn *Upogebia africana* (Ortmann, 1894) (Group 6) were collected for this study (Table 2.1). *Clibanarius virescens* (Arthropoda: Crustacea) is one of the most abundant hermit crabs in southern Africa and in the Indo-West Pacific region with a tropical distribution extending from Cape Recife in the Eastern Cape (Wait and Schoeman, 2012) to northern Mozambique (de Grave and Barnes, 2001). They are associated mostly with mid-shore rocky zones (Branch and Branch, 1981; Barnes, 1997) and frequently make use of gastropod shells for protection. Wait and Schoeman (2012) identified that *C. virescens* commonly make use of *Burnupena cincta* and *Burnupena pubescens* gastropod shells in Cape Recife.

Parasesarma catenatum (Arthropoda: Crustacea) endemic to southern Africa is a small estuarine crab that has a temperate-subtropical widespread distribution which extends from the Breede River in the Western Cape through the Eastern Cape to KwaZulu Natal (Branch and Grindley, 1979; Kensley, 1981) and southern Mozambique (Macnae and Kalk, 1962; Kalk, 1995) (Table 2.1). It is one of the most common estuarine crabs along the east and south-east coast of South Africa that is abundant along the length of estuaries (Taylor and Allanson, 1993).

35

Although this crab is mobile and moves according to low and high-water tides along the entire tidal gradient, it commonly inhabits mid-tidal zones among saltmarshes or mangroves, being either in *Spartina* (Day, 1974) or *Arthrocnemum* (Branch and Grindley, 1979; Vorsatz, 2009). This crab is rarely found above the high-water spring tide mark, as it shows a preference for saturated substrates and is capable of living and being active underwater (Emmerson, 2016). It is a detritivore and is not considered to be a burrowing crab, but rather uses very shallow holes that do not penetrate to water level because of the excellent respiratory and water-saving adaptations of these crabs (Emmerson, 2016). In the Kariega Estuary, this crab is dominant with a mean density of 47 crabs.m^{-2} in the lower and middle reaches (Hodgson, 1987; de Villiers *et al.*, 1999) and has been considered an indicator species due to its central importance in the carbon flow of estuaries (Morant and Quinn, 1999).

In southern Africa, one of the most widespread and abundant endemic intertidal estuarine species is the burrowing mud prawn *U. africana* (Arthropoda: Crustacea) (Day, 1964; Day, 1981) (Table 2.1) and is distributed from Langebaan Lagoon in the Western Cape to Inhambane in northern Mozambique (Cretchley, 1996). *Upogebia africana* is particularly abundant in many permanently open east and south Cape estuaries of southern Africa (Hill, 1967; Day, 1981; Hodgson, 1987; Hanekom *et al.*, 1988; Martin, 1988; Whitfield, 1988), as temperature may be a major factor limiting their distribution further north of the east coast (too warm) and west coast (too cold) (Hill, 1967). They commonly inhabit U-shaped burrows constructed by themselves (Hill, 1971; Schaefer, 1970) in the intertidal zone, found mostly among beds of eelgrass (*Z. capensis*) and are considered filter-feeders (Stamhuis *et al.*, 1998). They have been considered "promoter" species (Reise, 1985) because of their capacity to increase sediment oxygenation and mineralization (Hines and Jones, 1985), and by controlling community structure of soft bottom estuarine habitats (Virnstein, 1977; Posey *et al.*, 1991; Wynberg and Branch, 1994). They are popularly exploited, however, as a bait organism (Hanekom and Erasmus, 1988; Cretchley, 1996).

CHAPTER THREE: THERMAL TOLERANCE OF MARINE VERTEBRATES AND INVERTEBRATES IN WARM-TEMPERATE ESTUARINE AND INTERTIDAL ENVIRONMENTS

3.1 INTRODUCTION

A first step towards understanding the vulnerability of marine ectotherms to extreme temperatures is to determine which taxa currently live near their upper and lower thermal limits (thermal tolerance) and whether they have the ability to acclimatize to changing temperatures (Stillman, 2003; Somero, 2010, 2012). Thermal tolerance studies have focused on identifying and quantifying the thermal thresholds through two different experimental approaches, i.e. the static (incipient lethal) and dynamic (critical thermal) methods (e.g. Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997a, 1997b; Currie *et al.*, 1998).

The static or incipient lethal temperature (ILT) method determines the lower and upper thermal limits based on the temperature at which 50% of a sample of organisms die when acclimated and subjected to a range of temperatures (Fry, 1947; Beitinger and Bennett, 2000). The dynamic or critical thermal methodology (CTM) is characterized by the determination of the critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}), which involves subjecting organisms to a constant temperature change until the upper and lower sub-lethal limits (i.e. a loss of equilibrium or the onset of muscular spasms for vertebrates and a loss of righting response for macro-invertebrates) are reached (Cowles and Bogert, 1944; Lowe and Vance, 1955; Cox, 1974). The dynamic method has largely replaced the static method as it has many advantages in determining thermal tolerance. It requires fewer animals and experiments are shorter than with the ILT method; partial acclimation during trials is allowed; it is sub-lethal rather than lethal (i.e. animals are able to recover and survive, hence it is more ethical); it is effective across many species and individuals; it has been shown to explain the thermal distributions of many species; and it is comparable to natural conditions (i.e. good for aquatic systems where the properties of water change less abruptly than in air) (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997a; Beitinger *et al.*, 2000; Sunday *et al.*, 2012). Overall, the dynamic method is highly reliant, but is dependent on the implementation of an appropriate and taxon-tailored experimental protocol (Becker and Genoway, 1979).

Recent physiological studies using coastal temperate marine ectotherms subjected to the dynamic method (CT_{max}) showed that the thermal niches of individual species are important in identifying their thermal limits (Mora and Ospína, 2001; Mora and Maya, 2006; Madeira *et al.*, 2012a, 2012b, 2013, 2014a, 2014b; Vinagre *et al.*, 2013, 2015). These thermal niches can be biogeographically and/or habitat dependent (Somero 2002, 2010). Madeira *et al.* (2012a), for instance, demonstrated that critical thermal limits (CT_{max}) differed among crabs, shrimp and fish that occupied different biogeographic distributions found within a single temperate intertidal/subtidal habitat.

In addition, when determining the thermal limits for various fish and crustaceans from variable shallow habitats such as a temperate estuary and adjacent coastline (rock pools), where environmental variables change over a short space and time frame, intertidal and supratidal species have higher CT_{max} values than subtidal species (Madeira *et al.*, 2012a), suggesting that intertidal species are able to cope better with thermal shock (Madeira *et al.*, 2012a, 2012b, 2014a, 2014b, Vinagre *et al.*, 2013, 2015). It is not certain, however, whether intertidal and subtidal marine ectotherms in a temperate environment will be able to tolerate extreme climate events, such as marine heatwaves (MHWs) and cold spells (MCSs) that are predicted to increase in intensity, frequency and duration (Vinagre *et al.*, 2015; Shultz *et al.*, 2016) and, when comparing the two habitats (intertidal and subtidal), which one would be more vulnerable.

The resilience of different marine ectotherms to climate change, furthermore, may also be broadly categorized according to their taxonomy and limited by their mobility (Peck *et al.*, 2009; Somero, 2010; Tittensor *et al.*, 2010), with mobile species (e.g. fish) having lower thermal tolerance than sessile/sedentary species (e.g. macro-invertebrates) (Somero, 2010). This is because vagile species can avoid unfavourable thermal conditions while sessile species are forced to experience and hence withstand harsh conditions (e.g. Hiddink and ter Hofstede, 2008; Huang *et al.*, 2015; Messmer *et al.*, 2017).

Studies that concurrently test the effect of warming rate on CT_{max} and even more so the cooling rate on CT_{min} over various marine ectotherms belonging to different taxonomic groups and thermal niches (biogeographical and habitat) are still lacking (Vinagre *et al.*, 2015). Within this framework, the aim of this research was to: 1) determine the thermal limits (CT_{max} and CT_{min}) of seven fish (three rocky shore species, four estuarine species) and six macro-invertebrate

species (three rocky shore species, three estuarine species) in the warm-temperate region of South Africa to further determine differences in thermal scope based on taxonomic grouping, biogeography and habitat affinity; and 2) relate these thermal limits to current and projected water temperatures in an estuary, low-shore intertidal rock pool and subtidal gully between summer and winter.

3.2 MATERIALS AND METHODS

3.2.1 Field sampling

Fish and macro-invertebrates were collected from the lower reaches of the Kariega Estuary, adjacent low-shore rock pool and subtidal gully (refer to Chapter 2 for details of the sampling localities and study species) (Table 3.1). Collections were done during the summer months (January, February, and December) of 2017 and 2018 and during the winter months (June, July, and August) of 2017 (Table 3.1). Temperature and salinity measurements and sampling localities are detailed in Chapter Two. Sampling techniques ranged from collection by hand (*P. angulosus*, *P. perna*) to dip nets (*P. peringueyi*), modified plastic nets (*C. marleyi*), cast nets (rock pool and gully juvenile fish), a 30 m x 1.7 m x 15 mm bar mesh seine net with a 5 mm bar mesh purse (juvenile estuarine fish), and prawn pumps for adult *U. africana*.

3.2.2 Experimental setup

After collection, all specimens were transported to the NRF-SAIAB Aquatic Ecophysiology Research Platform (AERP) laboratory at the Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, in sealed aerated containers filled either with sea water or estuarine water from the collection site.

3.2.2.1 Fish

Up to 24 fish per species were caught on each sampling trip. Half of these fish were then randomly transferred into treatment and control recirculating systems for the first experiment, when CT_{max} was determined (Figure 3.1). The remaining half were transferred to an acclimation recirculating system, ready for the next experiment, to determine CT_{min} (Figure 3.1). Acclimation temperatures varied between 16.0 and 23.0 °C depending on the ambient

temperature during collection (Table 3.1). The treatment and control recirculating systems each consisted of three 90 L holding tanks, a 90 L filter tank (filter socks and biofilter beads), and a 90 L return tank (protein skimmer and return pump) (Figure 3.1). The acclimation recirculating system consisted of four 90 L holding tanks, a 90 L filter tank, and a 90 L return tank (Figure 3.1). Preliminary observations revealed high stress levels when smaller individuals were placed in tanks with larger individuals (which displayed aggressive behaviour towards smaller individuals) during pre-acclimation. All fish were thus placed into tanks according to similarity in length (mm SL) and weight (g). There was no observed difference in survival between male and females and, as such, individuals were not separated by sex. For all recirculating systems and experiments, salinity was maintained between 35 and 36 (to mimic natural conditions found at the study sites, refer to Chapter 2) and dissolved oxygen varied between 95% and 100% (dependant on temperature).
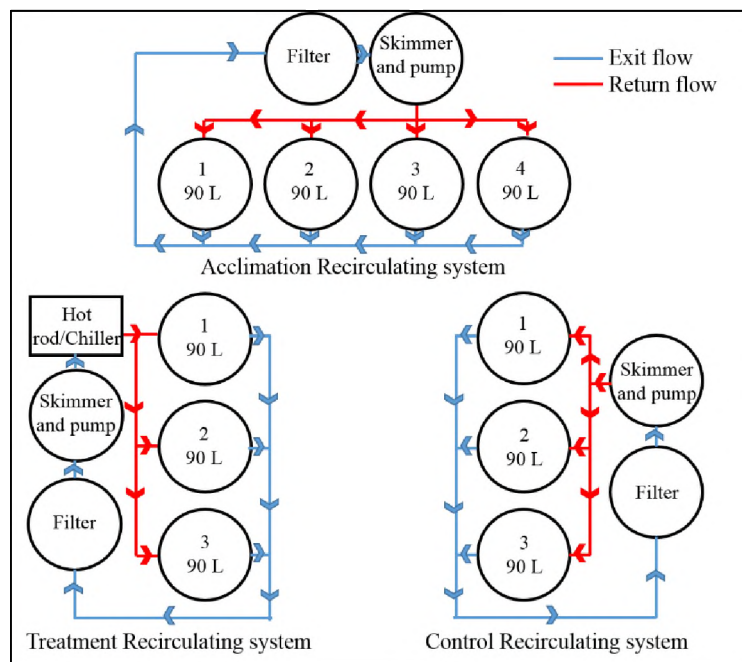
Figure 3.1. Experimental setup for fish and macro-invertebrate critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) experiments. The blue lines indicate the flow of water leaving the 90 L holding tanks and flowing into the 90 L filter tank (filter socks and biofilter beads) and then into the 90 L return tank (protein skimmer and return pump). Water was then either heated or cooled by a hot rod or chiller before being returned, indicated by the red lines, to the 90 L holding tanks.

3.2.2.2 Macro-invertebrates

Seventy-two individuals per species were placed in 100 ml plastic containers (one individual per container with a diameter and height of 10 cm) and covered with 1 mm fine mesh material using an elastic band (Figure 3.2). Half of these containers were then randomly placed into the treatment and control recirculating systems (six macro-invertebrate containers per species x three treatment/control tanks = 36 individuals) for the CT_{max} experiment (Figure 3.1). The remaining half were kept in the acclimation recirculating system (nine macro-invertebrate containers per species x four holding tanks = 36 individuals) (Figure 3.1) until required for the following CT_{min} experiment, where they were randomly transferred to the treatment and control recirculating systems. Animals were isolated individually to avoid competition/predation and any artefactual influence on CT_{max} by conspecifics (Paganini *et al.*, 2014). Crabs and mud prawns were sexed into male and female. Tanks contained nine male crabs: nine female crabs. For the mud prawns, only males were used as most females sampled were carrying eggs. It was not possible to sex hermit crabs, mussels and sea urchins prior to the experiment. As a result, gender could only be attributed to each specimen at the end of each thermal endpoint trial.

Figure 3.2. Cape sea urchins *Parechinus angulosus* placed individually in 100 ml plastic containers (diameter and height of 10 cm) and covered with 1 mm fine mesh material using an elastic band (mesh material and elastic bands seen next to plastic containers in image).

3.2.3 Acclimation

Animals in the treatment and control tanks were left to acclimate for a maximum of 36 hours at the water temperature in which they were collected. Field temperature at the time of the collection was measured consistently (every six hours) using a hand-held multi-parameter probe (Aqualytic water parameter, United Scientific, Germany) (Table 3.1). A 36-hour acclimation period was selected based on the findings of Mora and Maya (2006) and conveniently this time period was also appropriate to maintain a 12 L: 12 D photoperiod and setup of the experiments that followed. Marine and estuarine organisms were not fed during the acclimation period.

Table 3.1. Taxonomic group, habitat, biogeographic affinity, sample size, mean standard length (SL), weight (g) and acclimation/field temperatures for different species in the present study according to season (winter and summer) and treatment (CT_{max} and CT_{min}).

Species	Taxonomic group	Habitat	Biogeographic affinity	Season and treatment	Sample size	Length (mm) mean ± SD	Range (mm)	Weight (g) mean ± SD	Range (g)	Acclimation/field temperature (°C)
Diplodus capensis	Fish	Rocky shore	Temperate	Summer CT_{max}	12	63.11 ± 18.53	43-100	12.18 ± 16.16	3-53	20.0
				Winter CT_{max}	12	87.33 ± 17.01	60-100	9.42 ± 10.75	3-30	16.0
				Summer CT_{min}	12	50.92 ± 7.13	44-63	3.28 ± 1.66	1-5	20.0
				Winter CT_{min}	12	72.33 ± 1.15	52-73	4.39 ± 1.85	2-6	16.0
Sarpa salpa	Fish	Rocky shore	Temperate	Summer CT_{max}	6	61.33 ± 3.21	59-73	2.30 ± 0.52	2-4	20.0
				Winter CT_{max}	12	80.00 ± 7.21	74-88	4.82 ± 1.24	4-6	18.0
				Summer CT_{min}	6	74.33 ± 2.52	65-82	3.56 ± 0.08	3-6	20.0
				Winter CT_{min}	12	81.00 ± 8.19	66-90	5.19 ± 2.11	4-8	18.0
Kuhlia mugil	Fish	Rocky shore	Tropical	Summer CT_{max}	6	27.33 ± 6.12	19-33	0.19 ± 0.15	0-0.3	20.0
				Winter CT_{max}	6	24.83 ± 3.13	22-30	0.09 ± 0.09	0-0.2	18.0
				Summer CT_{min}	6	25.50 ± 5.09	19-32	0.15 ± 0.14	0-0.3	20.0
				Winter CT_{min}	6	29.00 ± 5.25	24-37	0.25 ± 0.15	0-0.5	18.0
Palaemon peringueyi	Invertebrate	Rocky shore	Widespread	Summer CT_{max}	36	22.53 ± 7.91	3-42	0.15 ± 0.14	0-0.5	21.0
				Winter CT_{max}	36	22.28 ± 4.68	7-33	0.55 ± 0.30	0-2	16.0
				Summer CT_{min}	36	24.75 ± 6.67	13-36	0.15 ± 0.15	0-1	21.0
				Winter CT_{min}	36	26.17 ± 6.88	12-38	0.46 ± 0.39	0-1	16.0
Parechinus angulosus	Invertebrate	Rocky shore	Widespread	Summer CT_{max}	36	39.83 ± 4.61	26-49	13.95 ± 4.35	7-27	21.0
				Winter CT_{max}	36	40.69 ± 3.60	35-50	31.56 ± 7.76	20-54	16.0
				Summer CT_{min}	36	39.54 ± 3.92	31-48	13.19 ± 4.40	4-22	21.0
				Winter CT_{min}	36	42.72 ± 4.33	34-52	15.85 ± 4.08	8-25	16.0

Perna perna	Invertebrate	Rocky shore	Widespread	Summer CT$_{max}$
				Winter CT$_{max}$
				Summer CT$_{min}$
				Winter CT$_{min}$
Chelon dumerili	Fish	Estuarine	Warm-water	Summer CT$_{max}$
				Winter CT$_{max}$
				Summer CT$_{min}$
				Winter CT$_{min}$
Rhabdosargus holubi	Fish	Estuarine	Warm-water	Summer CT$_{max}$
				Winter CT$_{max}$
				Summer CT$_{min}$
				Winter CT$_{min}$
Chelon richardsonii	Fish	Estuarine	Cool-water	Summer CT$_{max}$
				Winter CT$_{max}$
				Summer CT$_{min}$
				Winter CT$_{min}$
Chaetodon marleyi	Fish	Estuarine/ subtidal	Tropical	Summer CT$_{max}$
				Summer CT$_{min}$
Clibanarius virescens	Invertebrate	Estuarine	Tropical	Summer CT$_{max}$
				Winter CT$_{max}$
				Summer CT$_{min}$
				Winter CT$_{min}$

36	79.31 ± 12.27	28-95	18.61 ± 6.02	8-36	21.0
36	70.81 ± 8.00	57-90	30.64 ± 9.04	13-54	16.0
36	76.75 ± 8.17	59-93	17.21 ± 5.19	8-30	21.0
36	79.61 ± 8.09	63-93	20.18 ± 6.15	11-37	16.0
12	83.50 ± 18.92	60-111	6.45 ± 6.03	1-23	22.0
12	116.08 ± 29.06	80-150	11.87 ± 8.54	4-24	16.0
12	135.67 ± 22.63	70-180	20.10 ± 8.62	2-36	22.0
12	97.25 ± 20.04	68-132	6.60 ± 4.29	0-16	16.0
12	64.42 ± 18.08	31-85	8.06 ± 7.35	2-27	22.0
12	67.50 ± 27.22	43-117	6.78 ± 7.14	0-12	16.0
12	60.75 ± 11.82	45-88	2.81 ± 1.21	1-5	22.0
12	65.67 ± 34.30	40-116	13.45 ± 16.40	1-47	16.0
12	54.00 ± 5.20	44-60	1.04 ± 0.25	0-1	18.0
12	118.25 ± 9.12	106-132	12.92 ± 2.50	9-13	18.0
12	65.33 ± 15.31	44-83	2.50 ± 2.02	0-4	18.0
12	86.25 ± 24.04	61-123	6.98 ± 5.57	0-20	18.0
28	33.47 ± 9.49	18-45	1.65 ± 1.66	0-5	23.0
25	48.07 ± 7.65	30-61	2.33 ± 1.72	0-5	23.0
36	21.28 ± 5.60	12-35	0.41 ± 0.29	0-1	21.0
36	20.94 ± 9.26	5-48	0.76 ± 0.85	0-4	17.0
36	21.58 ± 5.63	12-32	0.91 ± 1.59	0-10	21.0
36	14.25 ± 3.50	8-22	0.49 ± 0.33	0-1	17.0

44

Parasesarma catenatum	Invertebrate	Estuarine	Widespread	Summer CT_{max}
				Winter CT_{max}
				Summer CT_{min}
				Winter CT_{min}
Upogebia africana	Invertebrate	Estuarine	Widespread	Summer CT_{max}
				Winter CT_{max}
				Summer CT_{min}
				Winter CT_{min}

36	18.03 ± 3.15	11-24	1.66 ± 0.85	0-5	21.0
36	18.33 ± 3.27	11-23	1.55 ± 0.92	0-4	17.0
36	19.33 ± 3.49	13-29	1.84 ± 1.09	0-4	21.0
36	17.89 ± 3.13	11-22	1.69 ± 1.07	0-4	17.0
36	36.18 ± 6.12	24-48	0.57 ± 0.29	0-1	21.0
36	46.78 ± 8.45	30-73	1.13 ± 0.58	0-3	17.0
36	37.39 ± 5.46	24-47	0.87 ± 1.67	0-11	21.0
36	42.36 ± 7.08	30-58	2.59 ± 2.04	1-8	17.0

45

3.2.4 Dynamic method (CT$_{max}$ and CT$_{min}$)

The dynamic method used to determine thermal tolerance was the critical thermal maximum (CT$_{max}$) (upper thermal limit) and critical thermal minimum (CT$_{min}$) (lower thermal limit). Once the acclimation period was completed, each treatment tank was exposed either to a constant rate of temperature increase (CT$_{max}$) or decrease (CT$_{min}$) by 1.0 °C. hour^{-1}, using a combination of a submerged hot rod (heater reaching 100.0 °C, Metalquip, The Hot Rod) or chiller (Hailea, HS-90 A), or cold finger (Lauda ETLC-30, Germany) and the temperature control room air conditioner. This heating rate of 1°C.hour^{-1} was also selected based on the findings of Mora and Maya (2006) who found that increasing or decreasing from an intermediate heating rate of ~1.0 °C.hour^{-1} as opposed to rapid or slow heating rates was successful for several marine ectotherms, resulting in this heating rate being preferred in thermal tolerance studies. The vitality of the animals was checked hourly until the experimental specimens reached either their upper or lower thermal endpoint respectively, after which the specimens were placed in a recovery tank.

The endpoint of each experiment (CT$_{max}$ and CT$_{min}$) in fish was measured by a loss of equilibrium (LOE) (Cowles and Bogert, 1944; Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997a, 1997b). Behaviour of the fish was noted every hour during the experiments. Fish behaviour indicative of temperature stress included aggressive behaviour (fish chasing or biting another fish), disorientated swimming, gulping at the water surface and swimming into the sides of the tank (van der Vyver *et al.*, 2013).

The endpoint of each experiment (CT$_{max}$ and CT$_{min}$) in macro-invertebrates was measured by a loss of righting response according to the methods of Collin *et al.* (2016), in which animals were stimulated with a plastic tweezer and flipped upside down until they were able to right themselves in an upright position (*P. angulosus, P. catenatum*). The righting response was measured by recording the time it took for the individual to return to its upright position using a stopwatch. Measurements were taken every second hour for both treatment and control specimens throughout the experiments. For *P. angulosus* a maximum period of 20 minutes (Collin *et al.*, 2016) and for *P. catenatum* a maximum period of one minute was allowed for righting response to occur (Hopkin *et al.*, 2006). The CT$_{max}$ / CT$_{min}$ was considered to be the highest/lowest temperature at which the animal was unable to right itself. The endpoint for *P. perna* was measured by a loss of gaping (Anestis *et al.*, 2007). Here, the posterior mantle edge

or siphons were rubbed gently with a pair of plastic tweezers. If the tactile stimulation did not elicit a valve closure response, the mantle edges and siphons were more vigorously probed. If this more vigorous tactile stimulation still did not elicit valve closure, the individual was considered to have reached its endpoint. The endpoint for *C. virescens* was measured by the complete emersion of crab from its shell, according to Bertness (1981). When stimulated with tweezers, if it did not retreat back into its shell, it was considered to have reached its endpoint. The endpoint for *U. africana* and *P. peringueyi* was marked by a collapse of the animal when they rolled over onto their sides. Sometime after collapse, the time varying with the degree of stress, the scaphognathite (thin leaf-like appendage of the second maxilla of decapod crustaceans) beat ceased (Hill and Allanson, 1971).

3.2.5 Physical measurements

During CT_{max} and CT_{min} experimental trials, temperature (°C), oxygen (%) and pH were monitored every hour with a hand-held multi-parameter probe (Aqualytic water parameter, United Scientific, Germany). The temperatures in the tanks were also measured continuously using Ibuttons. Salinity was measured every hour with a hand-held refractometer (Hannah Instruments, Germany). At the beginning, during (every fourth hour), and end of the experimental trials, water quality measurements (nitrate and ammonia) were taken (Salifert Test Kit).

To prevent any additional handling stress, the length and weight of all specimens were recorded at the end of the trials using a slide calliper (to the closest 0.01 mm) and a scale (AEADAM, PGL 2002, 0.01 g) respectively. Weight was recorded as living/whole weight (g), with no roe removed. No soft tissues were removed, but rather the organism was weighed as a whole (g). Standard length (mm, SL) and wet weight (g) were measured for all fish (Eme *et al.*, 2011). For the mussel, *P. perna*, the total shell length (mm TL) was measured from the posterior margin of the shell to the anterior tip of the umbo (McMahon and Ussery, 1995). Shell weight was measured by draining the mantle cavity water. For the sea urchin, *P. angulosus*, the maximum total height (mm TL) and maximum total width (mm TL) were recorded (Siikavuopio *et al.*, 2006; Peck *et al.*, 2009; Collin *et al.*, 2016). *Palaemon peringueyi* and *U. africana* were measured using total length (mm TL), which started at the tip of the rostrum and extended to the tip of the tail (Hill and Allanson, 1971; Allan *et al.*, 2006) and whole wet weight was further recorded (g). For *P. catenatum*, the widest part of the body (width of carapace) was

measured from the tip of the spine to the other tip of the spine (mm TL) of the carapace (Cuculescu *et al.*, 1998; Hopkin *et al.*, 2006) and whole wet weight was also recorded (g). For *C. virescens*, each hermit crab was removed from its shell by heating the apex with a flame and measured from apex to siphonal canal (cephalothorax length) with callipers (nearest 0.1 mm) and wet weight was measured (g) (Bertness, 1981). Fish and macro-invertebrate individuals were then euthanized using an overdose (> 0.2 ml. L^{-1}) of clove oil.

3.2.6 Data analysis

The upper (CT_{max}) and lower (CT_{min}) thermal limits for each species were calculated as the arithmetic mean of the collective thermal points at which the endpoint is reached using the following equation:

$$CT_{max} / CT_{min} \ (species) = \sum (Tend\text{–}point \ n)/n$$

where Tend-point is the temperature at which the endpoint was reached for each individual, and n stands for sample size. To determine intraspecific variability of CT_{max} and CT_{min} for each season, the coefficient of variation (in percentage) was calculated for each species:

$$\%CV = (SD/Mean_{\ CTmax/CTmin}) \times 100$$

Thermal scope or thermal resistance range was estimated for each species ($CT_{max} - CT_{min}$) for summer and winter (Pörtner and Farrell, 2008). Upper (CT_{max} – acclimation temperature) and lower (acclimation temperature – CT_{min}) breadth in tolerance for each species across seasons was also calculated to determine which season each species may be more at risk of exceeding their critical thermal limits (Shultz *et al.*, 2016). A small breadth in tolerance indicates that a species may be at risk to warmer or cooler temperatures in a given season (Shultz *et al.*, 2016). Thermal safety margins (TSM) of each species across summer (summer CT_{max} – summer maximum environmental temperature) and winter months (winter minimum environmental temperature - winter CT_{min}) were also calculated. Positive TSM values indicate that environmental temperatures do not exceed tolerance limits. Negative values indicate that present-day environmental temperatures currently exceed tolerance limits.

T-tests were performed for each fish and macro-invertebrate species to compare CT_{max}, CT_{min}, thermal scope, upper breadth in tolerance, and lower breadth in tolerance between seasons

(summer and winter). In addition, two-way repeated measures ANOVA tests of *P. angulosus'* righting response for CT_{max} (summer and winter) and CT_{min} (summer and winter) between treatment and control groups; temperature intervals; and treatment and control groups versus temperature intervals were performed. Significant results were further tested using Holm-Sidak post hoc comparisons. All data analysis was performed in SigmaPlot 12.5 except where otherwise specified. Normality of distributions was tested using a Shapiro-Wilk test and homoscedasticity was tested using the Levene's test. When the normality and homogeneity assumptions were not satisfied, Mann-Whitney U tests were performed in place of the parametric tests.

3.3 RESULTS

3.3.1 Rocky shore species

3.3.1.1 Fishes

3.3.1.1.1 Diplodus capensis

The CT_{max} (34.9 °C summer, 33.2 °C winter) and the CT_{min} (8.0 °C summer, 6.5 °C winter) of the temperate blacktail *D. capensis* differed significantly between seasons (Figure 3.3a, Table 3.2). Intraspecific variability (% CV) was low for CT_{max} for both summer (1.6%) and winter (0.6%), and intraspecific variability for CT_{min} was higher for summer (2.5%) than winter (0.9%) (Table 3.4). The thermal scope in tolerance between seasons for both upper (14.5 °C summer, 17.2 °C winter) and lower (12.0 °C summer, 9.5 °C winter) thermal scope was significantly different (Table 3.2, Table 3.5). The maximum summer environmental temperature of 25.1 °C in the low shore rock pool did not exceed the summer CT_{max} of *D. capensis* (34.5 °C), resulting in a positive upper TSM of 9.4 °C (Table 3.5). The minimum winter environmental temperature of 13.2 °C was higher than the winter CT_{min} (6.5 °C) of *D. capensis*, resulting in a positive lower TSM of 6.7 °C (Table 3.5). The CT_{max} was higher than the maximum temperature predicted for 2100 (Figure 3.3a).

3.3.1.1.2 Sarpa salpa

The CT_{max} of the temperate strepie *S. salpa*, differed significantly between seasons, with CT_{max} approximately 1.5 °C higher in summer (33.9 °C) than in winter (32.4 °C) (Figure 3.3b, Table 3.2). The CT_{min} differed significantly between seasons, with CT_{min} 0.7 °C higher in summer (7.8 °C) than in winter (7.1 °C) (Figure 3.3b, Table 3.2). Intraspecific variability for CT_{min} was very low for both seasons (0% for summer and winter) compared with CT_{max} for both seasons (1.1% summer, 0.8% winter) (Table 3.4). Thermal scope for *S. salpa* was significantly higher in summer (26.1 °C) than in winter (25.3 °C) (Table 3.2, Table 3.5). The summer CT_{max} (33.9 °C) was higher than the maximum summer environmental temperature of 25.1 °C, resulting in a positive upper TSM of 8.8 °C (Table 3.5). The winter CT_{min} (7.1 °C) was lower than the minimum winter environmental temperature of 13.2 °C, resulting in a positive lower TSM of 6.1 °C (Table 3.5). The maximum temperature predicted for 2100 was lower than the CT_{max} (Figure 3.3b).

3.3.1.1.3 Kuhlia mugil

The CT_{max} (37.8 °C summer, 37.1 °C winter), CT_{min} (8.7 °C summer, 8.2 °C winter), thermal scope (29.1 °C summer, 28.9 °C winter), upper breadth in tolerance (17.8 °C summer, 19.1 °C winter) and the lower breadth in tolerance (11.3 °C summer, 9.8 °C winter) of the tropical barred flagtail *K. mugil* did not differ significantly between seasons (Table 3.2, Table 3.5). Intraspecific variability was very low for CT_{max} (0.3%) in summer and winter (0.6%), and CT_{min} (1.8%) in summer was low compared with CT_{min} (2.5%) in winter (Table 3.4). The maximum summer environmental temperature of 25.1 °C did not exceed the summer CT_{max} of *K. mugil* (37.8 °C), resulting in a very high positive upper TSM of 12.7 °C (Table 3.5). The minimum winter environmental temperature (13.2 °C) was higher than the winter CT_{min} (8.2 °C), resulting in a positive lower TSM of 5.0 °C (Table 3.5). CT_{max} (37.8 °C summer, 37.1 °C winter) was higher than the maximum temperature (26.9 °C) predicted for 2100 (Figure 3.3c).

3.3.1.2 Macro-invertebrates

3.3.1.2.1 Palaemon peringueyi

The CT_{max} and CT_{min} of the widespread caridean shrimp *P. peringueyi* differed significantly between seasons, with CT_{max} 0.8 °C higher in summer (35.7 °C) than in winter (34.9 °C); and CT_{min} 1 °C lower in winter (3.7 °C) than in summer (4.7 °C) (Figure 3.3d, Table 3.2). Intraspecific variation for CT_{max} (1.4%) in summer was similar to the CT_{max} (1.5%) in winter, and the same trend was observed for CT_{min} (4.1% summer, 4.2% winter) (Table 3.4). For both upper and lower breadth in tolerance there was a significant difference between seasons, with upper breadth in tolerance 4.2 °C higher in winter (18.9 °C) than in summer (14.7 °C); and lower breadth in tolerance 4°C higher in summer (16.3 °C) than in winter (12.3 °C) (Table 3.2, Table 3.5). The winter CT_{min} (3.7 °C) was lower than the minimum winter environmental temperature of 13.2 °C, resulting in a positive lower TSM of 9.5 °C (Table 3.5). The CT_{max} was higher than the maximum temperature predicted for 2100 (Figure 3.3d).

3.3.1.2.2 Parechinus angulosus

For the widespread and sedentary Cape sea urchin *P. angulosus*, CT_{max} (31.3 °C summer, 27.0 °C winter), CT_{min} (7.9 °C summer, 4.5 °C winter), thermal scope (23.4 °C summer, 22.5 °C winter), upper breadth in tolerance (10.3°C summer, 11.0 °C winter) and lower breadth in tolerance (13.1 °C summer, 11.5 °C winter) were all significantly different between seasons (p < 0.01), with summer being higher than winter, except for the upper breadth in tolerance where winter was higher than summer (Figure 3.3e, Table 3.2, Table 3.5). *Parechinus angulosus* had a low intraspecific variation for CT_{min} in summer (2.7%) and a high CT_{min} (3.4%) in winter (Table 3.4). CT_{max} intraspecific variation was low for both summer (0.7%) and winter (0%) (Table 3.4). The maximum summer environmental temperature of 25.1 °C in the rock pool did not exceed the summer CT_{max} (31.3 °C) of *P. angulosus*, resulting in a positive upper TSM of 6.2 °C (Table 3.5). The minimum winter environmental temperature of 13.2 °C was higher than the winter CT_{min} (4.5 °C) for *P. angulosus*, resulting in a positive lower TSM of 8.7 °C (Table 3.5). The CT_{max} for winter (27 °C) was at the maximum temperature predicted for summer in 2100 (26.9 °C) (Figure 3.3e).

3.3.1.2.3 Perna perna

For the widespread and sedentary brown mussel *P. perna*, CT_{max} (38.9 °C summer, 37.9 °C winter), CT_{min} (4.3 °C summer, 4.0 °C winter), thermal scope (34.6 °C summer, 34.0°C winter), upper breadth in tolerance (17.9 °C summer, 21.9 °C winter) and lower breadth in tolerance (16.7°C summer, 12.0 °C winter) were all significantly different between seasons ($p < 0.01$), with summer being higher than winter, except for the upper breadth in tolerance, where winter was higher than summer (Figure 3.3f, Table 3.2). Intraspecific variability for CT_{min} in summer (3.4%) and winter (4.7%) was high and intraspecific variability for CT_{max} in summer (0.5%) and winter (0.6%) was low (Table 3.4). The maximum summer environmental temperature of 25.1 °C did not exceed the summer CT_{max} (38.9 °C), resulting in a positive upper TSM of 13.8 °C (Table 3.5). The minimum winter environmental temperature of 13.2 °C was 9.2 °C higher than the winter CT_{min} (4 °C), resulting in a positive lower TSM (Table 3.5). CT_{max} was higher than the maximum temperature predicted for 2100 (Figure 3.3f).
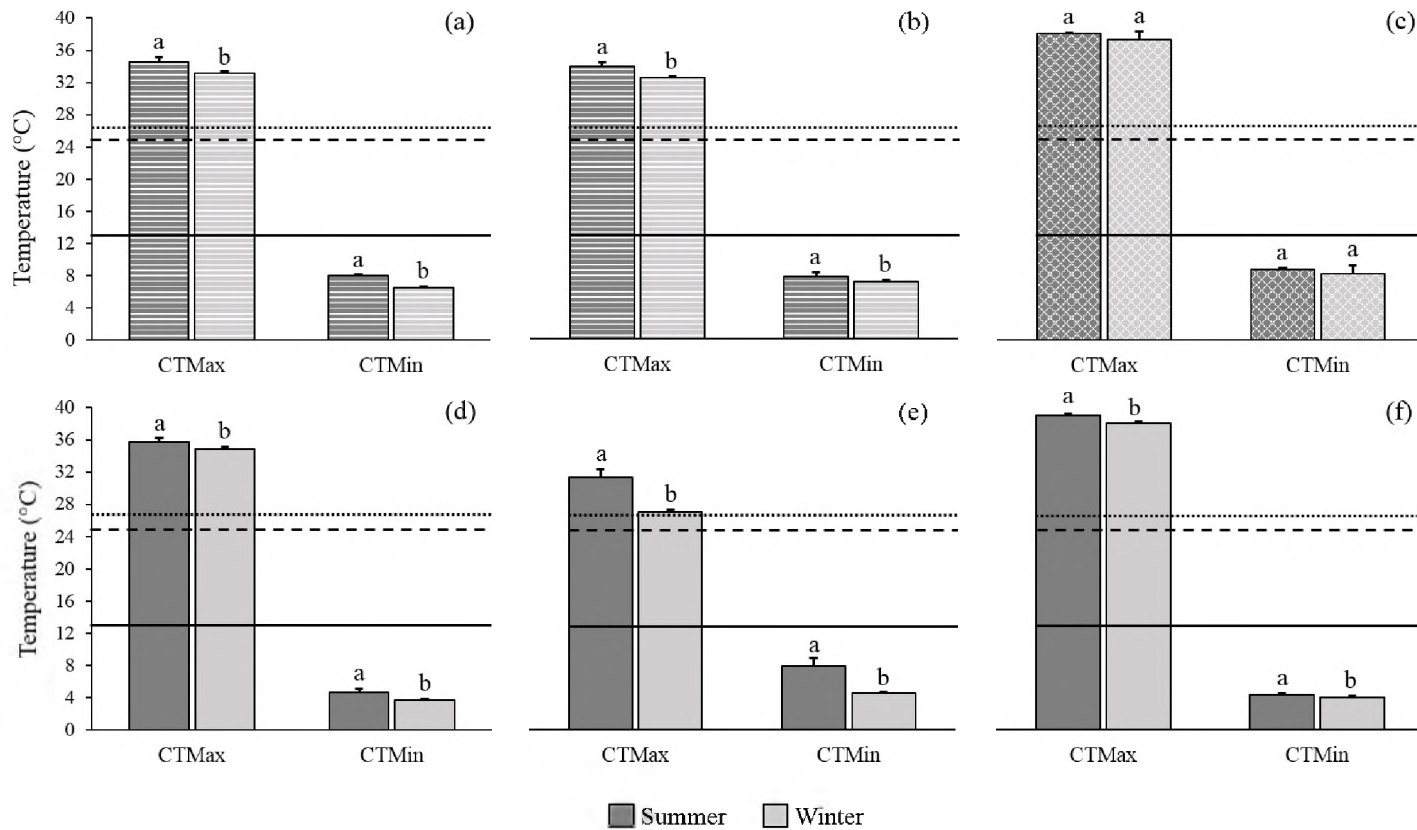
Figure 3.3. Critical thermal limits of rocky shore fish species: (a) *Diplodus capensis*, (b) *Sarpa salpa,* (c) *Kuhlia mugil*; and macro-invertebrates: (d) *Palaemon peringueyi*, (e) *Parechinus angulosus*, (f) *Perna perna* acclimated in laboratory conditions in the summer (20.0 - 21.0 °C) and winter (16.0 - 18.0°C). Letters (a) and (b) denote significant differences in CT_{max} / CT_{min} between seasons for each species. Error bars represent ± SD. A solid horizontal line indicates minimum water temperature recorded in winter (13.2 °C). A dashed horizontal line indicates maximum water temperature recorded in summer (25.1 °C). A dotted horizontal line represents potential maximum water temperatures in the rock pool in 2100 [maximum summer temperatures plus the Inter-Governmental Panel on Climate Change (IPCC, 2014) decadal increase in sea surfaces temperatures (0.2 °C per decade x 9 decades = 1.8 °C)]. Horizontal line bar graphs represent temperate biogeographic affinity; diamond bar graphs represent tropical biogeographic affinity, and no patterned bar graphs represent widespread distributions.

53

Table 3.2. Results of Mann-Whitney U tests comparing the seasonal variation (summer vs winter) in thermal scope ($CT_{max} - CT_{min}$), CT_{max}, upper breadth in tolerance (CT_{max} – acclimation temperature), CT_{min}, and lower breadth in tolerance (acclimation temperature – CT_{min}) of rocky shore fish and macro-invertebrate species. Significant differences indicated in bold when $p < 0.05$.

Variable	Season	*Diplodus capensis*			*Sarpa salpa*			*Kuhlia mugil*			*Palaemon peringueyi*			*Parechinus angulosus*			*Perna perna*		
		N	Mann Whitney U	*p*	N	Mann Whitney U	*p*	N	Mann Whitney U	*p*	N	Mann Whitney U	*p*	N	Mann Whitney U	*p*	N	Mann Whitney U	*p*
Thermal scope	summer	6	11.50	0.31	3	0.00	**0.02**	3	2.00	0.10	18	113.00	0.12	18	0.00	**< 0.01**	18	31.50	**< 0.01**
	winter	6			6			3			18			18			18		
CT_{max}	summer	6	0.00	**< 0.01**	3	0.00	**0.02**	3	0.00	0.10	18	31.50	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			3			18			18			18		
Upper breadth in tolerance	summer	6	0.00	**< 0.01**	3	1.00	**0.05**	3	0.00	0.10	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			3			18			18			18		
CT_{min}	summer	6	0.00	**< 0.01**	3	0.00	**0.02**	3	0.00	0.10	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	36.00	**< 0.01**
	winter	6			6			3			18			18			18		
Lower breadth in tolerance	summer	6	0.00	**< 0.01**	3	0.00	**0.02**	3	0.00	0.10	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			3			18			18			18		

3.3.2 Estuarine species

3.3.2.1 Fishes

3.3.2.1.1 Chelon dumerili

The CT_{max} and CT_{min} of the warm-water groovy mullet *C. dumerili* differed significantly between seasons, with CT_{max} 2 °C higher in summer (37.7 °C) than in winter (35.6 °C); and CT_{min} 4 °C higher in summer (9.3 °C) than in winter (5.3 °C) (Figure 3.4b, Table 3.3). Intraspecific variability of CT_{min} in summer (1.1%) was lower than in winter (2.9%), and intraspecific variability of CT_{max} for both summer (1.1%) and winter (1.8%) was both relatively low (Table 3.4). Thermal scope for *C. dumerili* was significantly greater in winter (30.3 °C) than in summer (28.4 °C) (Table 3.3, Table 3.5). In addition, there were significant differences in upper (15.7 °C summer, 19.6 °C winter) and lower (12.7 °C summer, 10.7 °C winter) breadth in tolerances between seasons for this species (Table 3.3, Table 3.5). The summer CT_{max} (37.6 °C) exceeded the maximum summer environmental temperature of 30.1 °C in the estuarine habitat, resulting in a positive upper TSM of 7.6 °C (Table 3.5). Furthermore, the winter CT_{min} (5.3 °C) was below the minimum winter environmental temperature of 12.2 °C, resulting in a positive TSM of 6.9 °C (Table 3.5).

3.3.2.1.2 Rhabdosargus holubi

For the warm-water Cape stumpnose *R. holubi*, the CT_{max} was significantly higher in summer (35.6 °C) than in winter (32.3 °C), with the winter CT_{max} relatively close to the maximum summer temperature predicted for 2100 (31.9 °C) (Figure 3.4c, Table 3.3). Moreover, CT_{min} was significantly higher in summer (8.1 °C) than in winter (5.8 °C) (Figure 3.4c, Table 3.3). Intraspecific variations of CT_{min} and CT_{max} in winter (2.3%; 2.2%, respectively) were higher than in summer (0.0%; 0.4%, respectively) (Table 3.4). The thermal scope was significantly higher in the summer (27.5 °C) than in winter (26.5 °C) (Table 3.4, Table 3.5). Additionally, the upper breadth in tolerance in summer (17.6 °C) was 3.3 °C, significantly higher than in winter (14.3 °C) and the lower breadth in tolerance in winter (12.2 °C) was 2.3 °C, significantly higher than in summer (9.9 °C) (Table 3.3, Table 3.5). The maximum summer environmental temperature of 30.1 °C did not exceed the summer CT_{max} (35.6 °C), resulting in a positive upper

TSM of 5.5 °C (Table 3.5). The minimum winter environmental temperature of 12.2 °C was above the winter CT_{min} (5.8 °C), resulting in a positive lower TSM of 6.3 °C (Table 3.5).

3.3.2.1.3 Chelon richardsonii

The CT_{max} and CT_{min} of the cool-water southern mullet *C. richardsonii* were significantly higher in summer (34.9 °C CT_{max}, 5.7 °C CT_{min}) than in winter (34 °C CT_{max}, 4.6 °C CT_{min}) (Figure 3.4a, Table 3.4). Intraspecific variation for CT_{min} in summer (1.0%) and winter (1.8%) was very similar and intraspecific variation for CT_{max} was lower in winter (0.2%) than in summer (0.4%) (Table 3.4). Moreover, the upper breadth in tolerance was significantly higher in winter (18.0 °C) than in summer (12.9 °C) and the lower breadth in tolerance was significantly higher in summer (16.4 °C) than in winter (11.4 °C) (Table 3.3, Table 3.5). The maximum summer environmental temperature of 30.1°C did not exceed the summer CT_{max} (34.9 °C), resulting in a positive upper TSM of 4.8 °C (Table 3.5). The CT_{max} for both seasons (34.9 °C summer, 34.0 °C winter) was relatively close to the maximum temperature predicted for 2100 (31.9 °C) (Figure 3.4a).

3.3.2.1.4 Chaetodon marleyi

The tropical doublesash butterflyfish *C. marleyi* was only caught during summer and had a CT_{max} of 35.2 °C and a CT_{min} of 11.2 °C, with CT_{min} being higher than the other species (Figure 3.4d). Intraspecific variation for CT_{max} in summer was high (1.7%) and intraspecific variation for CT_{min} in summer was very high (3.6%) (Table 3.4). The maximum summer environmental temperature of 30.1 °C did not exceed the summer CT_{max}, resulting in a positive upper TSM of 5.1 °C (Table 3.5). The minimum winter environmental temperature of 12.2 °C was one degree below the summer CT_{min} (Figure 3.4d).

3.3.2.2 Macro-invertebrates

3.3.2.2.1 Clibanarius virescens

The CT_{max} for the tropical yellow-banded hermit crab *C. virescens* was 0.3 °C significantly higher in winter (38.6 °C) than in summer (38.3 °C), and CT_{min} was 1.6 °C significantly higher in summer (5.6 °C) than in winter (4.0 °C) (Figure 3.4e, Table 3.3). Intraspecific variation for

CT_{min} in winter was high (4.4%) compared with summer, which was low (0.9%); and intraspecific variation for CT_{max} in winter (0.4%) was higher than in summer (0.1%) by 0.3% (Table 3.4). The thermal scope (32.7 °C summer, 34.6 °C winter), upper breadth in tolerance (17.3 °C summer, 21.6 °C winter) and lower breadth in tolerance (15.4 °C summer, 13.03 °C winter) were significantly different between seasons (Table 3.3, Table 3.5). The maximum summer environmental temperature of 30.1 °C did not exceed the summer CT_{max} of 38.3 °C and the minimum winter environmental temperature of 12.2 °C was above the CT_{min} of 4 °C, resulting in a positive upper and lower TSM of 8.2 °C (Table 3.5). The CT_{max} was above the maximum temperatures predicted for 2100 (31.9 °C) and CT_{min} was below the minimum temperatures observed during the study period (Figure 3.4e).

3.3.2.2.2 Parasesarma catenatum

For the widespread crab *P. catenatum*, CT_{max} (39.8 °C summer, 39.2 °C winter), CT_{min} (6.0 °C summer, 4.9 °C winter), thermal scope (33.8 °C summer, 34.3 °C winter), upper breadth in tolerance (18.8 °C summer, 22.2 °C winter) and lower breadth in tolerance (15.0 °C summer, 12.1 °C winter) were all significantly different between seasons ($p < 0.01$), with summer being higher than winter, except for thermal scope and upper breadth in tolerance where winter was higher than summer (Figure 3.4f, Table 3.3, Table 3.5). Intraspecific variation during summer and winter was very low for both CT_{max} (0.3% summer, 0.4% winter) and CT_{min} (0.0% summer, 1.7% winter) (Table 3.4). The CT_{max} in summer (39.8 °C) exceeded the maximum summer environmental temperature of 30.1 °C, resulting in a positive upper TSM of 9.7 °C (Table 3.5). The maximum temperature predicted for 2100 (31.9 °C) was lower than this species CT_{max} (39.8 °C summer, 39.2 °C winter) (Figure 3.4f).

3.3.2.2.3 Upogebia africana

For the widespread estuarine mud prawn *U. africana*, CT_{max} (38.3 °C summer, 36.2 °C winter), CT_{min} (6.0 °C summer, 4.5 °C winter), thermal scope (32.3 °C summer, 31.7 °C winter), upper breadth in tolerance (17.3 °C summer, 19.2 °C winter) and lower breadth in tolerance (15.0 °C summer, 12.5 °C winter) were all significantly different between seasons ($p < 0.01$) (Figure 3.4g, Table 3.3, Table 3.5). Intraspecific variation for both CT_{min} and CT_{max} in winter (2.0% CT_{min}, 0.9% CT_{max}) was higher than in summer (0.0% CT_{min}, 0.1% CT_{max}) (Table 3.4). The

maximum summer environmental temperature of 30.1 °C did not exceed the summer CT_{max} (38.3 °C) and the minimum winter environmental temperature of 12.2 °C was above the CT_{min} (4.5°C), resulting in a positive upper TSM of 8.2 °C and a positive lower TSM of 7.6 °C (Table 3.5).
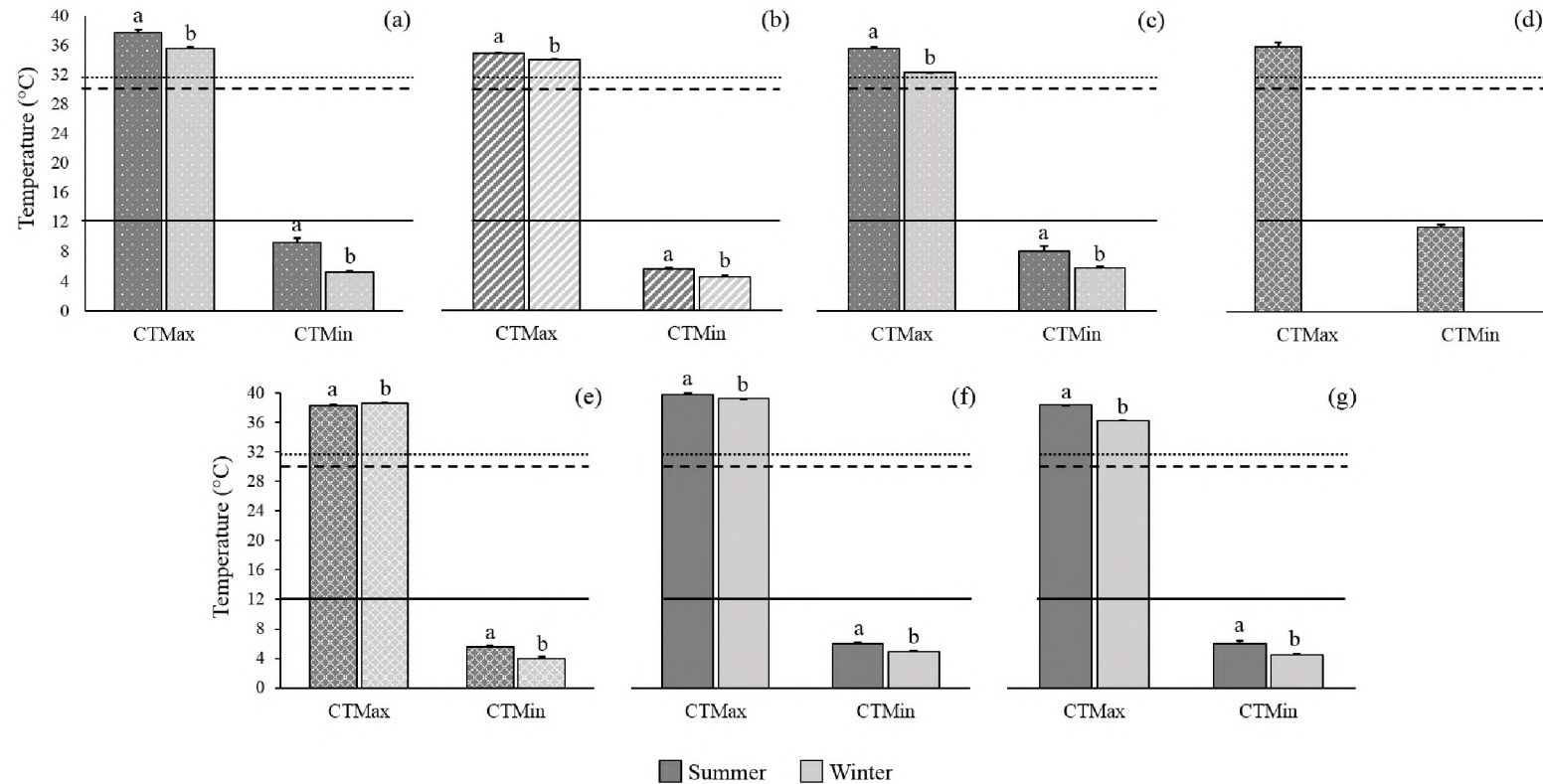
Figure 3.4. Critical thermal limits of estuarine fish species: (a) *Chelon dumerili*, (b) *Chelon richardsonii*, (c) *Rhabdosargus holubi*, (d) *Chaetodon marleyi*; and macro-invertebrates: (e) *Clibanarius virescens*, (f) *Parasesarma catenatum*, (g) *Upogebia africana* acclimated in laboratory conditions in the summer (18.0 – 23.0 °C) and winter (16.0 - 18.0 °C). Letters (a) and (b) denote significant differences in CT_{max} and CT_{min} between seasons for each species. Error bars represent ± SD. A solid horizontal line indicates minimum water temperature in winter (12.2 °C). A dashed horizontal line indicates maximum water temperature in summer (30.1 °C). A dotted horizontal line represents potential maximum water temperatures in the estuary in 2100 [maximum summer temperatures plus the Inter-Governmental Panel on Climate Change (IPCC, 2014) decadal increase in sea surface temperatures (0.2 °C per decade x 9 decades = 1.8 °C)]. Dotted bar graphs represent warm-water biogeographic affinity; diagonal bar graphs represent cool-water biogeographic affinity; diamond bar graphs represent tropical biogeographic affinity, and no patterned bar graphs represent widespread distributions.

59

Table 3.3. Results of Mann-Whitney U tests comparing the seasonal variation (summer vs winter) in thermal scope ($CT_{max} - CT_{min}$), CT_{max}, upper breadth in tolerance (CT_{max} – acclimation temperature), CT_{min}, and lower breadth in tolerance (acclimation temperature – CT_{min}) of estuarine fish and macro-invertebrate species. Significant differences indicated in bold when $p < 0.05$.

Variable	Season	*Chelon dumerili* N	Mann Whitney U	*p*	*Chelon richardsonii* N	Mann Whitney U	*p*	*Rhabdosargus holubi* N	Mann Whitney U	*p*	*Clibanarius virescens* N	Mann Whitney U	*p*	*Parasesarma catenatum* N	Mann Whitney U	*p*	*Upogebia africana* N	Mann Whitney U	*p*
Thermal scope	summer	6	0.00	**< 0.01**	6	9.50	0.18	6	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			6			18			18			18		
CT_{max}	summer	6	0.00	**< 0.01**	6	0.00	**< 0.01**	6	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			6			18			18			18		
Upper breadth in tolerance	summer	6	0.00	**< 0.01**	6	0.00	**< 0.01**	6	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			6			18			18			18		
CT_{min}	summer	6	0.00	**< 0.01**	6	0.00	**< 0.01**	6	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			6			18			18			18		
Lower breadth in tolerance	summer	6	0.00	**< 0.01**	6	0.00	**< 0.01**	6	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**	18	0.00	**< 0.01**
	winter	6			6			6			18			18			18		

Table 3.4. The intraspecific variability of CT_{max} and CT_{min} for both summer and winter given by the coefficient of variation (in percentage) for a range of rocky shore and estuarine fish and macro-invertebrate species.

Species	Summer		Winter	
	CT_{max} CV (%)	CT_{min} CV (%)	CT_{max} CV (%)	CT_{min} CV (%)
Diplodus capensis	1.6	2.5	0.6	0.9
Sarpa salpa	1.1	0.0	0.8	0.0
Kuhlia mugil	0.3	1.8	0.6	2.5
Palaemon peringueyi	1.4	4.1	1.5	4.2
Parechinus angulosus	0.7	2.7	0.0	3.4
Perna perna	0.5	3.4	0.6	4.7
Chelon dumerili	1.1	1.1	1.8	2.9
Chelon richardsonii	0.4	1.0	0.2	1.8
Rhabdosargus holubi	0.4	0.0	2.2	2.3
Chaetodon marleyi	1.7	3.8	-	-
Clibanarius virescens	0.1	0.7	0.4	4.4
Parasesarma catenatum	0.3	0.0	0.4	1.7
Upogebia africana	0.1	0.0	0.9	2.1

Table 3.5. Seasonal observations in thermal scope (°C), upper and lower breadth in tolerance (°C), and upper and lower thermal safety margins (TSM) (°C) for rocky shore and estuarine fish and macro-invertebrate species.

species	Summer				Winter			
	Thermal scope	Upper breadth in tolerance	Lower breadth in tolerance	Upper TSM	Thermal scope	Upper breadth in tolerance	Lower breadth in tolerance	Lower TSM
Diplodus capensis	26.5	14.5	12.0	9.4	26.7	17.2	9.5	6.7
Sarpa salpa	26.1	13.9	12.2	8.8	25.3	14.4	10.9	6.1
Kuhlia mugil	29.1	17.8	11.3	12.7	28.9	19.1	9.8	5.0
Palaemon peringueyi	31.0	14.7	16.3	10.6	31.2	18.9	12.3	9.5
Parechinus angulosus	23.4	10.3	13.1	6.2	22.5	11.0	11.5	8.7
Perna perna	34.6	17.9	16.7	13.8	34.0	21.9	12.0	9.2
Chelon dumerili	28.4	15.7	12.7	7.6	30.3	19.6	10.7	6.9
Chelon richardsonii	29.3	12.9	16.3	4.8	29.4	18.0	11.4	7.6
Rhabdosargus holubi	27.5	17.6	9.9	5.5	26.5	14.3	12.2	6.3
Chaetodon marleyi	24.0	12.2	11.8	5.1	-	-	-	-
Clibanarius virescens	32.7	17.3	15.4	8.2	34.6	21.6	13.0	8.2
Parasesarma catenatum	33.8	18.8	15.0	9.7	34.3	22.2	12.1	7.3
Upogebia africana	32.3	17.3	15.0	8.2	31.7	19.2	12.5	7.6

3.3.3 Fish behaviour

Approximately three hours prior to fish reaching their upper and lower thermal limits (characterized by disorientated swimming and a loss of equilibrium), all fish species began displaying aggressive behaviour, which included biting and chasing the other individuals in the tank (Table 3.6). Roughly by the second last hour prior to reaching their thermal limits for both CT_{max} and CT_{min} experiments (summer and winter), all fish species, in addition to displaying aggressive behaviour, began showing signs of disorientation by swimming into the sides of the tank and coming to the surface of the tank where increased gulping was observed (Table 3.6). For the last hour of the CT_{max} experiments, all fish species displayed disorientated swimming, increased gulping at the water surface, and finally a loss of equilibrium at the surface (Table 3.6). Furthermore, within the last hour of the experiments, it was observed that all specimens for both mullet species shed more scales from the epidermal layer of the skin than the other fish species, and some individuals, particularly *C. dumerili*, would jump out from the treatment tanks (Table 3.6). During the last hour of the CT_{min} experiments, all specimens of all fish species almost went into a state of dormancy, where swimming was minimal and was limited to the bottom of the tank where they would finally lose equilibrium (Table 3.6).

3.3.4 Macro-invertebrate righting response

Righting response was measured for *P. catenatum* and *P. angulosus*, while the other macro-invertebrates such as *P. perna*, *P. peringueyi*, *U. africana* and *C. virescens* were checked hourly for their endpoint behaviours. For *P. catenatum*, during each measurement (every two hours), specimens were able to right themselves within two seconds of being flipped upside down (treatment and control). When the specimens within the treatment plastic containers were unable to right themselves after a minute, they were considered to have reached their upper and lower thermal limits. These observations were consistent for both CT_{max} (summer and winter) and CT_{min} (summer and winter). For *P. angulosus*, for both CT_{max} (summer and winter) and CT_{min} (summer and winter) treatments (n =18), the time it took the specimens to right themselves during each measurement (every two hours) increased with both an increase and decrease in temperature. For the control specimens (n = 18), however, time remained fairly consistent and low (approximately three minutes) for each measurement (Figure 3.5). In addition, the results of the two-way repeated measures ANOVA for CT_{max} (summer and winter) and CT_{min} (summer and winter) between treatment and control groups; temperature intervals;

63

and treatment and control groups versus temperature intervals all revealed significant differences in righting response time for *P. angulosus* (Figure 3.5, Table 3.7).

Table 3.6. Behavioural observations and the associated temperatures of rocky shore and estuarine fish species with the last three hours of either CT_{max} or CT_{min} experiments for both summer and winter.

Species	Hour prior to limit	Behaviour at CT_{max}	Summer temperature (°C)	Winter temperature (°C)	Behaviour at CT_{min}	Summer temperature (°C)	Winter temperature (°C)
Diplodus capensis	3	AG	32.0	31.0	AG	6.0	5.0
	2	AG, DS	33.0	32.0	AG, DS	7.0	6.0
	1	DS, IG, LOE	34.5	33.2	SD, LOE	8.0	6.5
Sarpa salpa	3	AG	32.0	30.0	AG	6.0	5.0
	2	AG, DS	33.0	31.0	AG, DS	7.0	6.0
	1	DS, IG, LOE	33.9	32.4	SD, LOE	7.8	7.1
Kuhlia mugil	3	AG	36.0	35.0	AG	7.0	6.0
	2	AG, DS	37.0	36.0	AG, DS	8.0	7.0
	1	DS, IG, LOE	37.8	37.1	SD, LOE	8.7	8.2
Chelon dumerili	3	AG	35.0	34.0	AG	7.0	3.0
	2	AG, DS	36.0	35.0	AG, DS	8.0	4.0
	1	DS, IG, LS, LOE	37.7	35.6	SD, LS, LOE	9.3	5.3
Chelon richardsonii	3	AG	33.0	32.0	AG	4.0	3.0
	2	AG, DS	34.0	33.0	AG, DS	5.0	4.0
	1	DS, IG, LS, LOE	34.9	34.0	SD, LS, LOE	5.6	4.5
Rhabdosargus holubi	3	AG	34.0	30.0	AG	6.0	4.0
	2	AG, DS	35.0	31.0	AG, DS	7.0	5.0
	1	DS, IG, LOE	35.6	32.3	SD, LOE	8.1	5.8
Chaetodon marleyi	3	AG	33.0	-	AG	9.0	-
	2	AG, DS	34.0	-	AG, DS	10.0	-
	1	DS, IG, LOE	35.2	-	SD, LOE	11.2	-

AG = Aggressive behaviour; DS = disorientated swimming; IG = Increased gulping; LS = loss of scales; SD = State of dormancy; LOE = Loss of equilibrium
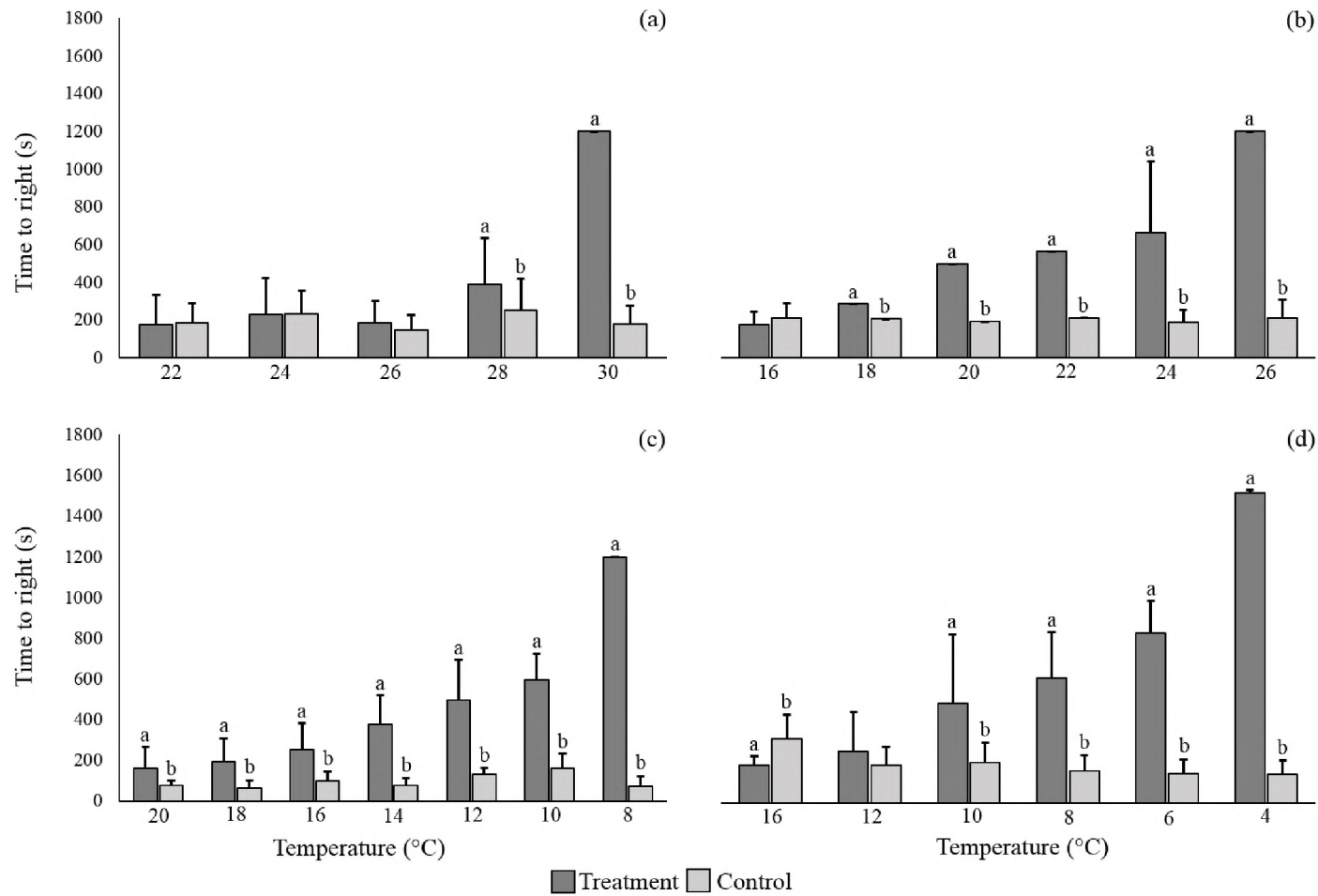
Figure 3.5. Righting response of *Parechinus angulosus* to two-hour temperature intervals for (a) summer CT_{max}, (b) winter CT_{max}, (c) summer CT_{min}, and (d) winter CT_{min} (n =18). Error bars indicate SD and letters (a) and (b) indicate significant difference ($p < 0.05$) between treatments and controls.

Table 3.7. Two-way repeated measures ANOVA tests of *Parechinus angulosus* righting response for CT_{max} (summer and winter) and CT_{min} (summer and winter) between groups (treatment and control); temperature intervals (2 hours); and groups and temperature intervals. Holm-Sidak post hoc tests used. Significant differences indicated in bold when $p < 0.05$.

	Summer CT_{max}			Winter CT_{max}			Summer CT_{min}			Winter CT_{min}		
	DF	F	p	DF	F	p	DF	F	p	DF	F	p
Group (Treatment/control)	1	84.26	**< 0.01**	1	431.77	**< 0.01**	1	404.39	**< 0.01**	1	194.31	**< 0.01**
Temperature interval	4	90.40	**< 0.01**	5	87.11	**< 0.01**	6	164.72	**< 0.01**	5	111.94	**< 0.01**
Group (Treatment/control) x Temperature interval	4	94.16	**< 0.01**	5	86.31	**< 0.01**	6	157.60	**< 0.01**	5	160.96	**< 0.01**
Error	136			170			204			170		
Total	179			215			251			215		

3.4 DISCUSSION

Major results from the study using the dynamic method on several fish and macro-invertebrate species indicated that there were differences in thermal tolerance according to taxonomic grouping, habitat affinity and biogeography between summer and winter. Taxonomically, widespread adult macro-invertebrates generally presented higher CT_{max}, lower CT_{min}, higher upper and lower breadths in tolerance, higher upper and lower thermal safety margins, higher thermal scopes and higher intraspecific variability for CT_{min} than the fish. Thermal tolerance results according to habitat indicated that marine ectotherms (fish and macro-invertebrates) from the intertidal estuarine habitat had higher thermal tolerances than marine ectotherms from the intertidal rocky low-shore habitat (intertidal rock pool and subtidal gully) for both summer and winter. Biogeographically, tropical (Group 2) and warm-water endemic juvenile fish species had the highest thermal tolerance (CT_{max}), however, tropical species demonstrated the lowest thermal resilience (high CT_{min}) to decreases in temperature.

Adult macro-invertebrates generally presented a higher thermal tolerance and thermal scope compared with the juvenile fish species in this warm-temperate region for both summer and winter. This trend may be a result of their widespread distributions and limited mobility, allowing them to tolerate a wide range of temperatures. A widespread distribution suggests that species need to function over a wide scope of temperatures (Spicer and Gaston, 1999; Madeira *et al.*, 2012a). Most macro-invertebrates are not as mobile as fish and many are sedentary, if not sessile, with limited capacities of moving to more favourable conditions, hence often living closer to their upper and lower thermal limits in intertidal environments (Madeira *et al.*, 2012a). As a result, many benthic macro-invertebrates have evolutionarily adapted to a sedentary lifestyle through advanced ventilation and circulation for physiological acclimation and behavioural thermoregulation (Thompson *et al.*, 2002; Anestis *et al.*, 2007; Somero, 2010). For instance, macro-invertebrates from the low rocky shore (e.g. Mollusca) and living at the interface of marine and terrestrial systems, such as adjacent coastlines and estuaries (e.g. Crustacea), make use of the above mechanisms to allow them to cope with the stresses associated with alternating tidal immersion and emersion (air exposure) and extreme daily temperatures during low tides (see Newell, 1979; Davidson and Pearson, 1996; Karsten *et al.*, 1996; Pörtner, 2002; Helmuth *et al.*, 2002, 2006; Kelly *et al.*, 2012). These adaptations to an intertidal lifestyle hence fit well with the highest CT_{max} and lowest CT_{min} endpoints and the

highest thermal scopes, breadths in tolerance and safety margins for both summer and winter for the sedentary brown mussel *P. perna* inhabiting the rocky shores and the mobile crab *P. catenatum* from the lower reaches of the Kariega Estuary.

The sedentary *P. perna* can behaviourally thermoregulate through gaping (opening and closing of shell valves) and acclimating physiologically by depressing its metabolism (Marshall and McQuaid, 1993; Nicastro *et al.*, 2010; Tagliarolo and McQuaid, 2015). Previous studies also suggest that gaping behaviour allows *P. perna* to avoid accumulating metabolites during hypoxia and enables gas exchange so that aerobic respiration can be maintained in air (Coleman and Trueman, 1971; McMahon, 1988; Marshall and McQuaid, 1993). However, gaping increases water losses and the risk of desiccation (Nicastro *et al.*, 2010) and, according to Marshall and McQuaid (1993), *P. perna* appear to have little control over the loss of mantle water. Their preferred mechanism to avoid extreme environmental temperatures and hypoxia in air and water is metabolic depression (Tagliarolo and McQuaid, 2015). Metabolic depression is a state characterized by a 60 – 100% reduction in the basal metabolic rate, which extends the time that an animal can survive on stored fuel supplies during periods of environmental stress (Guppy *et al.*, 1994; Guppy and Withers, 1999). The relatively air-intolerant *P. perna* exhibits metabolic depression by closing their valves (or delaying gaping) and delaying bradycardia in order to activate anaerobic pathways in air (Widdows *et al.*, 1979; Shick *et al.*, 1986; McMahon, 1988). Tagliarolo and McQuaid (2015) further demonstrated that in water, *P. perna* (found along the east coast) can maintain higher metabolic rates through metabolic depression and can cope with thermal stress under conditions of acute temperature variability. The mechanism of metabolic depression for *P. perna*, may account for its high thermal tolerance and scope in this study.

The high thermal tolerance and broad thermal scope of the mobile *P. catenatum* found in the intertidal estuarine habitat for this study may be a result of it successfully using the mechanism of behavioural thermoregulation as well as depressing its metabolism under thermally challenging periods (Newell and Branch, 1980). Although this species has a broader thermal scope when compared to the other sedentary estuarine macro-invertebrates *C. virescens* and *U. africana*, this result is not surprising, as *P. catenatum* spends a substantial amount of time exposed to air during low tides on the exposed mud flats actively moving around in search for food, unlike *U. africana* and *C. virescens*. As a result, to thermoregulate its body temperature,

which can fluctuate by 25.0 °C or more in a matter of hours as demonstrated by Stillman and Somero (1996) using other intertidal crabs, it makes use of bimodal breathing (breathing in both air and water) (Emmerson, 2016). In air, *P. catenatum* recycles water within its branchial chambers by pumping water over the grooved and hairy branchiostegite surfaces or "chest" (Alexander and Ewer, 1969). *Parasesarma catenatum* is also able to avoid thermal stress during low tide air exposure by retreating frequently into their burrows which are normally 15-20 cm deep, with one or more side entrances (Alexander and Ewer, 1969). These burrows do not penetrate the groundwater table, and are usually occupied by up to six crabs (Alexander and Ewer, 1969; Zoutendyk and Bickerton, 1988; Emmerson, 2016). Besides sheltering in burrows, *P. catenatum* may move to higher ground to prevent submersion and here they shelter amongst roots or tree trunks of mangrove trees (Fratini *et al.*, 2005; Porri, pers. comm.).

In contrast, *Upogebia africana* predominantly burrows in mud and is submersed in water, while *C. virescens* rarely leaves the water (Bertness, 1981; Taylor, 1982; Wait and Schoeman, 2012). As such, these animals cope with heat stress in water (rather than both in air and water) using their mud burrow and shell (and to some extent mobility; Reese, 1969) respectively. The *Uca* burrows used by *U. africana* can be several degrees cooler than exposed mud flats (Edney, 1961), allowing them to feed continuously in a thermally protected environment (Hill and Allanson, 1971). During high tide, when *P. catenatum* may become submersed unavoidably, it may decrease its aerobic performance by depressing its metabolism with its advanced ventilation and circulation, widening the boundaries of its thermal tolerance to cope with fluctuating water temperature extremes (e.g. De Pirro *et al.*, 1999).

The Cape sea urchin *P. angulosus*, also from the intertidal low-shore rock pool habitat, had the lowest CT_{max}, the highest CT_{min} and the narrowest upper and lower breadth in tolerance compared with all other macro-invertebrates in this study. This finding may be a result of this marine ectotherm being unable to depress its metabolism, to cope with maximum temperature extremes, as reported by Guppy *et al.* (1994) who stated that in the face of environmental stress, all major invertebrate phyla, with the exception of Echinodermata, make use of metabolic depression. Adult echinoderms have a poor ability to regulate ion concentration in their extracellular fluids (Stickle and Diehl, 1987) and are considered to be hypometabolic (abnormally low metabolic rate) (Melzner *et al.*, 2009), as they have low respiratory rates (Lawrence and Lane, 1982; Shick, 1983). Their oxygen uptake is dependent mostly on

nutritional state, size, ambient temperature, oxygen tension, seasonality, salinity and pH, particularly more than other ectotherms (e.g., Hiestand, 1940; Farmanfarmaian, 1966; McPherson, 1968; Sabourin and Stickle, 1981; Lawrence and Lane, 1982; Brockington and Clarke, 2001; Talbot and Lawrence, 2002; Wood *et al.*, 2008, 2010, 2011; Christensen *et al.*, 2011). This may explain why in this study *P. angulosus* may be able to tolerate low temperatures in winter, but not extreme temperatures (hot and cold events) in summer. This finding is similar to those for the temperate purple sea urchin *Strongylocentrotus purpuratus*, which could not tolerate temperatures higher than 25.0 °C and could not withstand temperatures as high as 23.5 °C for long periods of time (Farmanfarmaian and Giese, 1963).

Among the fish species investigated, transient marine fish species found in estuaries had a greater thermal scope in general than the low rocky shore transient fish species, being able to tolerate higher temperatures in summer. This suggests that juvenile marine fishes will not be directly affected by temperature increases occurring below their CT_{max} endpoint in the Kariega Estuary. To support this, Eme *et al.* (2011) found that juvenile squaretail mullet *Chelon vaigiensis* (Quoy and Gaimard, 1825) and crescent terapon *Terapon jarbua* (Forsskål, 1775) captured from shallow seagrass estuarine nursery habitats around Hoga Island in Indonesia had CT_{max} endpoints of ~45.0 °C and ~44°C when acclimated from 37.0 °C. For resident rock pool species, however, this trend may not be the same, as resident rock pool fish species tend to demonstrate decreased temperature sensitivity and euryhaline characteristics (Gibson, 1972; Zander *et al.*, 1999). For example, Kemp (2009) demonstrated that the banded goby *Caffrogobius caffer* (Günther, 1874) is capable of acclimatizing to extreme thermal variability and hypo- and hypersaline conditions, allowing it to penetrate the upper intertidal zone if required, unlike the transient juvenile *D. capensis*, which do not have the adaptive scope to cope with extended thermal stress and hypersaline conditions, especially in mid- to high-shore rock pools.

Although juvenile warm-water endemic and the temperate *D. capensis* fishes are able to tolerate low temperatures in winter they are less tolerant of cold temperatures (lower thermal tolerance) in summer. For example, *R. holubi*, *C. dumerili* and *D. capensis* had lower CT_{min} endpoints in winter (5.8, 5.3 and 6.5 °C) than in summer (8.1, 9.3 and 8.0 °C). This may suggest that for these juvenile fish species, a metabolic/physiological trade-off between thermal tolerance to colder temperatures in summer and growth/development may be taking place.

Pörtner and Gutt (2016) refer to this strategy as a low cost of stenothermal versus high cost of eurythermal cold adaptation. For example, temperate eurythermic fish species may shift their thermal scopes between seasons to adjust their standard metabolic rates, from low cost during winter stenothermy, to high cost during spring cold eurythermy and lower cost in the summer when cold temperatures are generally excluded from this thermal scope (Wittman *et al.*, 2008). As a result of losing tolerance to cold temperatures in summer, this excess energy turnover may allow eurythermic organisms to invest more energy into growth during the warmer time of the year (Pörtner and Gutt, 2016). Within this warm-temperate study region, where intermittent upwelling occurs, the juvenile warm-water endemic estuarine-dependent marine fish species may benefit from this strategy, as they are less likely to be influenced by upwelling in estuaries due to their broad eurythermic and euryhaline physiology, allowing them to escape unfavourable thermal conditions further upstream. This strategy, however, may ultimately be detrimental for the juvenile temperate estuarine-opportunist *D. capensis*, which has limited habitat choices as a result of its physiology (not strongly euryhaline as compared with *R. holubi*), requiring it to inhabit nearshore rocky habitats (rock pools and gullies) and the lower reaches of estuaries, which may be more exposed to drastic drops in temperatures during upwelling.

Biogeographically, for this study, the highest CT_{max} thermal endpoints were observed for the tropical juvenile *K. mugil* (Group 2) followed by the juvenile warm water endemic estuarine-dependent marine fish species (*C. dumerili* and *R. holubi* Group 3) and the tropical *C. marleyi* (Group 2). The lowest CT_{max} thermal endpoints were observed for the only juvenile cool-water endemic estuarine-opportunist *C. richardsonii* (Group 4). This data supported Madeira *et al.* (2012a) who found that cool-water fish species (with a northern distribution) had critical thermal maxima on average 1.1 °C below the maximum habitat temperature compared with the warm-water species (with a southern distribution) who had critical thermal maxima on average 3.5 °C above the maximum habitat temperature, making cool-water species more vulnerable to extreme increases in temperature.

Fish species with the highest CT_{min} were the tropical marine straggler species *C. marleyi* followed by *K. mugil* in the nearshore coastal environment showing that they had the lowest tolerance of cool temperatures. Figueira and Booth (2010) identified cool winter temperatures as a major factor preventing the establishment of tropical fishes such as chaetodons in the

temperate regions of Australia. In the present study, *C. marleyi* was found only in the lower reaches of the Kariega Estuary during the summer months and, based on its narrow cold safety margins (5.0 °C), it is likely that they cannot survive the cold winter temperatures in this estuary as a result of thermal stress, starvation and predation (Hurst, 2007). In a study on a related *Chaetodon* species in the western North Atlantic, McBride and Able (1998) found that these fishes died from hypo-thermal conditions at the onset of winter in temperate waters. If water temperatures do increase in this warm-temperate region to within the tolerance range of *C. marleyi* as a result of climate change, however, it may allow for them to occur over winter. Figueira and Booth (2010) and Booth *et al.* (2018) also identified that winter water temperatures in Australia are increasing at a rate above the global average, allowing tropical transient species such as Chaetodontidae, Acanthuridae (surgeonfishes) and Pomacentridae (damselfishes) to recruit into these temperate waters and expand their range, potentially becoming more resident. Interestingly, the cool-water endemic *C. richardsonii* were more tolerant of low temperatures than the temperate species (*D. capensis* and *S. salpa*) and this may be due to the centre of their distribution being the cool-temperate region of South Africa where summer upwelling is persistent.

When examining the fish species in this study at the level of family, it appears that Kuhliidae, followed by Sparidae, Mugilidae and Chaetodontidae, are the most thermally tolerant family in this warm-temperate region. Kuhliidae, even though tropically distributed (Indo-pacific and Eastern Pacific) (Floeter *et al.*, 2008), seem to be highly thermally tolerant and this may be a result of this family mostly inhabiting dynamic rock pool habitats (Heemstra and Heemstra, 2004). Sample sizes, however, need to be increased significantly for Kuhliidae to assess whether this high thermal tolerance does exist across seasons. Sparidae are considered to have evolved in the subtropical and temperate regions (Floeter *et al.*, 2008) and along the South African coast they are distributed from the south Benguela region (Namibia and southern Angola) to the Agulhas region. As a result, Sparidae can tolerate a wide range of temperatures and salinities (Blaber, 1973), as well as occupy various habitats or niches (Heemstra and Heemstra, 2004). For example, the genus *Diplodus* is thought to have originated in the subtropical Cape Verde Islands (North Atlantic Ocean) (Summerer *et al.*, 2001) and, according to Vinagre *et al.* (2010) who studied them between the temperate and subtropical waters of Portugal, they can colonize various habitats further north and south due to their wide thermal tolerance. Mugilidae are able to tolerate extreme temperatures, as many species within this

family are tropical and are able to tolerate temperatures exceeding 40.0 °C (Menasveta, 1981; Mora and Ospína, 2001; Rajaguru, 2002; Eme and Bennett, 2009). Tropical Chaetodontidae, originating from the western Indian Ocean and diversifying into the Atlantic and Eastern Pacific (Fessler and Westneat, 2007), are highly tolerant of warm temperatures in tropical and warm-temperate regions. Their diversification into cooler waters (mid-Atlantic), however, has shown that this family eventually perish because of declining winter temperatures (McBride and Able, 1998).

Intraspecific variability for this study was found to be highest for the CT_{min} thermal tolerances of mostly the intertidal sedentary macro-invertebrates *P. angulosus*, *P. perna* and *P. peringueyi* as opposed to their CT_{max} thermal tolerances in this warm-temperate region. This high CT_{min} intraspecific variability could be a result of phenotypic plasticity, natural selection and genetic diversity which may serve as drivers of individual variation and population differentiation as proposed by Tibblin *et al.* (2016). Phenotypic plasticity, defined as the ability of individual genotypes to produce different traits when exposed to altered environmental conditions (Pigliucci, 2005, Pigliucci *et al.*, 2006), can change rapidly within a lifetime and over a few generations, especially across populations from dissimilar habitats (Des Roches *et al.*, 2018). Populations with greater genetic diversity, specifically in unstable environments, will have greater resilience to environmental fluctuations, thus increasing their survival, growth and fecundity compared with those with lower genetic diversity, specifically in stable environments (Aguirre and Marshall, 2012). The increased intraspecific variation for CT_{min} thermal tolerances in macro-invertebrates found in the intertidal rocky shore habitat suggests that they display high genetic diversification to buffer the cold temperatures more, especially in this region where the impacts of summer intermittent upwelling are expected to irregularly intensify.

According to Lutterschmidt and Hutchison (1997a), the sequence of events for fish during an acute temperature increase is agitated behaviour, loss of equilibrium (LOE), onset of spasms (OS), heat rigor, coma, and death. For this study, fish behavioural changes such as disorientated swimming, aggressive behaviour, and a state of dormancy were evident for all species in this study prior to or very close to them reaching their critical upper and lower thermal limits, which was displayed by a loss of equilibrium. No muscular spasms, heat rigor, heat comas or death were observed as individuals of each species were removed prior to this in this study. Van der

74

Vyver *et al.* (2013), also working on *R. holubi*, found that behavioural changes such as disorientated swimming and a loss of equilibrium were also only evident when the critical thermal endpoint was reached. Most individuals of the mobile species increased their responses to the increase in temperature by increasing their swimming speed, hence increasing disorientation. This was not surprising as several authors (e.g. Taylor *et al.*, 1997; Peck *et al.*, 2009; Breau *et al.*, 2011) have observed increases in swimming speed for temperate fauna exposed to warmer temperatures. In the natural environment, an increase in swimming performance in response to sustained thermal stress may be detrimental, making fish behave differently to oncoming fishing gear, and thus more vulnerable to capture (Pörtner and Peck, 2010). Increased swimming performance also may increase their energetic requirements (food intake), and if in a thermally stressful environment, food resources may too be limited, and the mortality of these fishes may increase (Pörtner and Peck, 2010).

In contrast to the rapid swimming observed at warmer temperatures, most individuals of the mobile species went into a state of "dormancy" (remaining near the bottom of the tank with limited movement) as the temperature approached their CT_{min}. This mechanism is thought to be a behavioural strategy that fish use to reduce energetic expenditures and may be necessary for maintaining energetic costs and recycling waste products at low temperatures (Pörtner *et al.*, 2006; Almeida *et al.*, 2015). This dormant behaviour can, however, have negative consequences such as increased mortality by predation and indirect effects on population intrinsic growth rate (Almeida *et al.*, 2015). It has been inferred, nonetheless, that climate driven changes in temperature can modify the behaviours of ectothermic organisms (Munday *et al.*, 2009), which may alter migrations to feeding grounds, as already seen for some temperate marine species (Pörtner and Peck, 2010). Although these trials were run over a short period of time to simulate an extreme temperature event, the results do show the potential impacts of increasing or decreasing temperatures on ectothermic fish's behaviour.

For the macro-invertebrates, the righting response method used in this study proved successful in estimating CT_{max} and CT_{min}. Specimens of *P. catenatum* remained on their back for the full one minute period without any signs of righting when approaching the upper and lower thermal endpoints, as demonstrated by Cuculescu *et al.* (1998) and Hopkin *et al.* (2006). Korhonen and Lagerspetz (1996) suggested that CT_{max} measured by a loss of righting response is an ecologically relevant index of the upper thermal tolerance of decapod crustaceans, as it is

probable that it measures the tolerance of that part of the crab's nervous system which controls the righting reflex. For *P. angulosus*, it was evident that the righting time response increased with both an increase and decrease in temperature. This type of bidirectional response has been reported for tropical (*Lytechinus variegatus*; Collin *et al.*, 2016) and temperate sea urchins (*Strongylocentrotus purpuratus*; Farmanfarmaian and Giese, 1963).

In conclusion, this study revealed that, for most species, with the exception of *C. marleyi* and *P. angulosus*, average critical maximum and minimum thermal tolerance endpoints were comfortably above the maximum summer and below the minimum winter environmental temperatures recorded in each habitat within this warm-temperate region. This suggests that water temperatures would have to increase or decrease substantially before deleteriously impacting any of the studied species. Extreme cold events, such as cold spells and summer upwelling, that are expected to increase in intensity, frequency and duration as a result of climate change, however, may significantly impact these species due to rapid decreases in temperature over a short period. To better explain the underlying physiological changes of these marine ectotherms, a comprehensive physiological phenotypic assessment of their upper and lower thermal performances needs to be investigated alongside their upper and lower thermal tolerances (CT_{max}/CT_{min}). In addition, these thermal performance assessments can provide the optimal (T_{OPT}) and pejus (T_{PEJ} – getting worse) temperatures to mark the transition from one temperature range into the next, to better understand the effects of climate change based on an organism's survival with a reduced scope for aerobic activity rather than the extreme evaluation of an organism's death.

CHAPTER FOUR: COMBINING THE DYNAMIC METHOD, STATIC RESPIROMETRY AND MAXIMUM HEART RATE TO UNDERSTAND THE THERMAL PHYSIOLOGY OF JUVENILE AND ADULT DIPLODUS CAPENSIS (SMITH 1844)

4.1 INTRODUCTION

A range of methods have been used to understand the thermal physiology of fishes. Each method has its own associated limitations and biases and may be more appropriate for certain species or for different life stages of a single species. Estimating thermal tolerance and performance of a species through its life history may therefore require the use of multiple approaches. Some of the most common techniques used to understand the thermal physiology of fishes are the dynamic method (critical upper and lower thermal endpoints, i.e. CT_{max} and CT_{min}), respirometry, and the measurement of maximum heart rate (f_{Hmax}). These techniques stem from the pioneering physiological studies of thermal performances in fishes by Fry (1947), the recent concept of "oxygen- and capacity-limited thermal tolerance" (Pörtner and Knust, 2007; Pörtner and Farrell, 2008), and the recent finding that rate transition temperatures for heart rate improve the understanding of the upper thermal tolerance (CT_{max}) that controls the biogeographic distributions of a species (Somero, 2010; Tepolt and Somero, 2014).

Critical thermal endpoints (CT_{max} and CT_{min}) determine the tolerance of a species to extreme maximum and minimum temperatures and these limits are relatively simple to measure (Cowles and Bogert, 1944; Lowe and Vance, 1955; Cox, 1974), irrespective of size (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997a, 1997b; Beitinger and Bennett, 2000; Sunday *et al.*, 2012). This method is also affordable, does not require specialized equipment, and is rapid (experiments last days, rather than weeks or months) (Lutterschmidt and Hutchison, 1997a). Although knowledge of the thermal tolerance range (temperature between CT_{max} and CT_{min}) of an organism is important, understanding the thermal dependence of an organism's performance may be more valuable from an ecological perspective (Fry, 1947). This is because the thermal limits of an organism's functional performance are narrower than its critical thermal endpoints (Farrell, 2009).

77

A common method of determining the functional thermal performance of an individual organism over a range of activities is measuring oxygen utilization, i.e. respirometry (Rummer et al., 2016). Respirometry techniques which include closed-system, open-system, static and intermittent methods are commonly used to measure the rate of oxygen consumption (MO_2), which usually increases in a regular manner with increasing temperature, and to estimate the metabolic performance on a range of life stages of an organism at rest or while executing different locomotory activities (Clark et al., 2013; Rummer et al., 2016). Two important measures, the maximum metabolic rate (MMR) and the standard metabolic rate (SMR), are used to define the upper and lower limits of an organism's capability to metabolize energy. The MMR of an individual is the maximum amount of energy that can be metabolized aerobically and can be determined by measuring an organism's MO_2 during or immediately after exhaustive exercise (Norin and Clark, 2016). The SMR refers to the minimum amount of energy required for survival and maintenance and does not include energy spent on voluntary muscular movements, digestion or absorption (Krogh, 1914; Chabot et al., 2016) and is determined by measuring MO_2 in a post-absorptive, resting state (Nelson and Chabot, 2011; Chabot et al., 2016).

Aside from the MMR and SMR, other metabolic rate metrics include routine metabolic rate (RMR), which is the mean metabolic rate of fish in a resting state but exhibiting minor activity in a respirometer (e.g. swimming to maintain position) (Winberg, 1960; Brett, 1962; Nelson and Chabot, 2011), and active metabolic rate (AMR), which is the mean metabolic rate of an active fish while swimming at a continued, constant speed (Lefrançois and Claireaux, 2003; Chabot et al., 2016). When activity is known to be very limited, such as small fin movements to maintain position in the respirometer, authors have used the terms "low routine", "resting", or "fasting" metabolic rates (Blaxter, 1989; Plaut, 2000).

Metabolic scope, also referred to as aerobic scope, is the difference between MMR and SMR and represents the maximum amount of energy available for any energy consuming activity (e.g. swimming, feeding, and reproducing) beyond maintenance at ecologically relevant temperatures (Brett, 1964; Fry, 1947; Farrell, 2009). When using RMR, the difference between the final and initial rates of aerobic metabolism is called the "relative scope of activity" or the relative aerobic scope (RAS) (Wieser, 1985; Wieser and Forstner, 1986). Aerobic scope/relative aerobic scope is generally maximized within a given temperature range (termed

T_{OPT}) to optimize fitness related performance, while performance diminishes as scope decreases at pejus (T_{PEJ} – top 10% of the T_{OPT} curve) and higher and lower temperatures (CT_{max} and CT_{min}, i.e. T_{CRIT} –bottom 90% of the T_{OPT} curve) (Clark *et al.*, 2013). In other words, an elevated SMR/RMR without a corresponding increase in MMR will reduce T_{OPT} and T_{PEJ}, thus decreasing CT_{max} (Clark *et al.*, 2013) and increasing CT_{min}, narrowing the thermal range and increasing thermal vulnerability. In this same instance, an increase in temperature of 10.0 °C beyond T_{OPT} will double the rate of oxygen consumption (Cossins and Bowler, 1987), referred to as the Q_{10} temperature coefficient (Schmidt-Nielsen, 1997), resulting in an abrupt decrease in metabolism (Q_{10} for aerobic scope > 2.0) (Sidhu *et al.*, 2014).

Accordingly, these respirometry performance indicators, particularly T_{OPT} and T_{PEJ}, provide valuable information regarding the capability of organisms, fish in this instance, to manage changing temperatures (Clark *et al.*, 2013; Norin and Clark, 2016). Although respirometry can be used for all life stages to measure physiological performance, it is considered more time consuming and requires specialized equipment and acute attention to detail. For instance, for respirometry experiments to be effective, respirometer design and function to relative mass, acclimation period, experimental duration and the number of measurements all need to be carefully considered (Peck *et al.*, 2009; Clark *et al.*, 2013; Peck and Moyano, 2016).

An alternative technique that is considered quicker than respirometry trials (days versus weeks; Casselman *et al.*, 2012) and that has been associated as a limiting factor for upper thermal tolerance (CT_{max}) is the measurement of maximum heart rate function (Stillman and Somero, 1996; Stenseng *et al.*, 2005; Steinhausen *et al.*, 2008; Somero, 2010; Eliason *et al.*, 2011; Casselman *et al.*, 2012; Anttila *et al.*, 2013, 2014). Maximum heart rate (f_{Hmax}) in fishes stops increasing at a temperature just beyond the optimal temperature (T_{OPT}), which in turn limits the amount of oxygen that can be supplied to tissues above the routine needs (Fry, 1947; Steinhausen *et al.*, 2008; Farrell, 2009; Eliason *et al.*, 2011, 2013), termed the Arrhenius breakpoint temperature (T_{AB}) (Casselman *et al.*, 2012; Anttila *et al.*, 2013, 2014; Sidhu *et al.*, 2014). Thus, for fishes *in vivo* (when swimming freely) and *in vitro* (pharmacologically stimulated), the rate transition temperatures T_{AB} and T_{QB} (when Q_{10} decreases abruptly) for f_{Hmax} can be indirectly comparable to that of $T_{OPT}AS$ for the same species (Anttila *et al.*, 2014) and provides an indication/verification of where a species preference to temperature lies relative to its upper thermal tolerance. In addition, further increases in temperature eventually

leads to an arrhythmic heartbeat (T_{ARR}), a cardiac collapse that occurs just below the upper critical temperatures (CT_{max} and T_{CRIT}) (Casselman *et al.*, 2012; Anttila *et al.*, 2013, 2014).

The f_{Hmax} has been measured primarily using exterior electrodes whereby an electrocardiogram (ECG) is recorded (Casselman *et al.*, 2012). Although this methodology has been proven to be successful, there are limitations to using this equipment for marine fishes, mainly due to the technical difficulties of placing electrodes in salt water (Skeeles *et al.*, accepted). Leadless micro heart rate loggers developed by Star-Oddi have only recently been used successfully in marine fishes (Bjarnason *et al.*, 2017; Skeeles *et al.*, accepted). These loggers simultaneously record heart rate and internal body temperature, which are both important in determining T_{AB} (Bjarnason *et al.*, 2017; Skeeles *et al.*, accepted). One limitation with these micro-heart rate loggers (2.5 cm), however, is that they are too large to implant in juveniles, which limits their utility to adults of larger species. Additionally, the heart rate technology is relatively new (particularly in marine species), and due to it being quicker than respirometry (quicker laboratory turnover time), it may be important to better understand and compare the relationship between heart rate metrics in adults first (T_{AB} and T_{OPT}; T_{CRIT} and CT_{max}) to assess the adaptability for juveniles in future studies. A good candidate species to test this is the eurythermal temperate blacktail fish species *Diplodus capensis* (refer to Chapter 2).

The aim of this chapter is to employ multiple methods to examine physiological metrics of juveniles and adults of the temperate ectothermic fish *D. capensis* exposed to acute increases and decreases in temperature. To do this, upper (CT_{max}) and lower (CT_{min}) thermal limits for both juvenile and adult *D. capensis* were determined along with the RMR and MMR of juvenile *D. capensis* at five test temperatures using static respirometry, and maximum heart rate (f_{Hmax}) of adult *D. capensis* under conditions of acute heating using Star-Oddi micro heart rate loggers.

4.2 MATERIALS AND METHODS

4.2.1 Dynamic method

The methods for the determination of critical thermal endpoints for juvenile *D. capensis* are detailed in Chapter Three. To obtain preliminary estimates of CT_{max} for adult *D. capensis*, six adults (190 - 310 mm TL) were collected in summer (December – February 2018) from a sandy beach surf zone (33° 89' 33.6" S 26° 29' 81.5" E) using rod and line. After collection, adults were transported back to the AERP laboratory in Grahamstown in aerated containers containing seawater from the study site.

Three adults were transferred to one 500 L cylindrical recirculating tank connected to a heat pump (AquaHeat 9.2 kW pump). Fish were acclimated for a minimum of 36 hours at the field temperature of 20.0 °C with a 12h L: 12h D photoperiod. Fish were not fed during this acclimation period. After acclimation, the dynamic method using the critical thermal maximum (CT_{max}) was determined by increasing the water temperature by a constant rate of 1.0 °C.hour^{-1} until an endpoint was reached (loss of equilibrium) (Lutterschmidt and Hutchison, 1997a). The same experimental procedure was repeated to determine the critical thermal minimum (CT_{min}) for the remaining three adults by decreasing the water temperature by a constant rate of 1.0 °C. hour^{-1} until an endpoint was reached (loss of equilibrium). Once the upper and lower endpoints were reached, adults were weighed (g) and measured (mm TL) and euthanized.

4.2.1.1 Data analysis

The CT_{max} and CT_{min} thermal limits for adult *D. capensis* were calculated as the arithmetic mean of the collective thermal points at which the endpoint is reached using the following equation:

$$CT_{max} / CT_{min} \text{ (species)} = \sum \text{ (Tend–point n)/n}$$

where Tend-point is the temperature at which the endpoint was reached for any given individual.

4.2.2 Static respirometry

Thirty juvenile blacktail, *D. capensis* (25 - 45 mm SL), were collected in summer (December – February 2018) using a range of gears (cast net, dip net, and seine net) from a series of habitats surrounding the Kariega Estuary (33° 36' 53.72" S, 26° 39' 15.93" E) where this species occurs as juveniles (rock pools, mouth and lower reaches of the estuary). After collection, juveniles were transported back to the AERP laboratory in Grahamstown in aerated containers. Individuals were then randomly placed into five 90 L cylindrical recirculating tanks at the field temperature of 20.0 °C. Each tank consisted of six individuals placed into separate floating 100 ml plastic containers (diameter =10 cm, height = 10 cm) and covered with 1 mm fine mesh material using an elastic band. This separation of individuals was done to reduce initial MO_2 stress for transference to the respirometer chambers for the experimental trials (McKenzie *et al.*, 2007; Dupont-Prinet *et al.*, 2013) and to lag metabolic activity to levels that may occur in its natural habitat (Clark *et al.*, 2013; Chabot *et al.*, 2016). All individuals were then left to acclimate for a minimum period of 36 hours with a 12h L:12h D photoperiod before experimental trials began in order to re-set metabolic stability. Juveniles were kept in a given tank for a maximum of 11 days to complete all experimental trials and, as a result of such a short holding period (and appropriate fasting period), fish growth was not considered a significant factor (Chabot *et al.*, 2016). During this holding period, temperature (°C), oxygen (%), pH, salinity, ammonia, nitrate and nitrite were measured using a hand held multi-parameter probe (Aqualytic water parameter, United Scientific, Germany); a refractometer (Hannah Instruments, Germany) and Salifert Test Kits for each tank at 12:00 every second day prior to the experimental trials.

Experimental trials consisted of using static respirometry to measure oxygen consumption of juvenile *D. capensis* at five test temperatures: 9.0 °C; 13.0 °C; 18.0 °C; 28.0 °C and 34.0 °C using a flow-through system. The minimum water temperature of 9.0 °C was selected to represent the lower thermal limit that this species can tolerate in summer (CT_{min} = 8.0 °C; refer to Chapter 3), while the maximum water temperature of 34.0 °C was selected to represent the upper thermal limit that this species can tolerate in summer (CT_{max} = 35.0 °C; refer to Chapter 3). Juvenile *D. capensis* in the tank assigned to the temperature being tested were starved 12 hours prior to experimentation, and the remaining juveniles for the rest of the experiments (30 individuals) were fed at libitum. Each individual *D. capensis* was subjected to only one thermal

treatment. The static respirometry protocols were adapted from Chen *et al.* (2015) and Boucher *et al.* (2018) applying the recommendations of Clark *et al.* (2013), Chabot *et al.* (2016) and Peck and Moyano (2016). Respirometry equipment was validated by assessing suitable respirometer volumes, oxygen ingress and drift associated with bacterial background respiration through preliminary pilot trials. Seawater was sterilized by being autoclaved and filtered using a water filtration system with 20 um filters. Glass respirometry chambers with a volume of 45 ml were used to accommodate approximately 45 mm fish (sufficiently large enough to prevent confinement stress, but small enough to reduce activity; Chabot *et al.*, 2016). Calculations to determine the chambers' water displacement as a result of the inclusion of the fishes' mass was also done for each test temperature (Clark *et al.*, 2013; Chabot *et al.*, 2016) so that respirometry measurements could be adjusted to a specific unit. This displacement volume also corrects and determines the actual volume of water in the chambers.

For oxygen consumption measurements, 20 L of sea water in a water bath was circulated from the chambers through flow-through cells containing a contactless fibre-optic oxygen sensor (Pyroscience e.K., Aachen, Germany) using a peristaltic pump (flow rate = 700 ml/min); the oxygen concentration (mg. L^{-1}) was recorded using red flash dye technology and readings were transmitted via a fibre-optic cable connected to a Firesting oxygen reader (Pyroscience e.K., Aachen, Germany). Oxygenated water in the chambers remained uniformly mixed by looping through the flow-through cells and two submersible pumps were also placed in the water bath to minimize temperature stratification and to provide uniform oxygen distribution. The system consisted of four respirometry chambers per trial, of which three contained individual fish and one a blank control with no fish.

For each test temperature, three starved fish in their plastic containers were randomly selected from their respective tanks, and individually transferred into the three respirometric chambers and left to further acclimate for one hour. Fish were then acutely exposed to one of five new test temperatures from the acclimation temperature of 20.0 °C at a rate of 1.0 °C. hour^{-1} by warming or cooling, using a submersible heater (EHEIM Jagger Heater 300W) or chiller (Hailea, HS-90 A). Once the particular test temperature was reached (time period ranged from 2 – 11 hours) fish were left to acclimate for a further one hour period before RMR was measured (Chen *et al.*, 2015). Juvenile *D. capensis*, even when stationary, tend to exhibit minor spontaneous activity (small fin movements to maintain position in respirometer chambers),

and, consequently, measurements of SMR are closer to RMR (Fry, 1947), and therefore RMR was measured in this study for approximately 10 minutes every fifteen seconds. Following this, each fish was removed from the respirometer chambers and returned to its 10 cm diameter circular plastic container containing aerated water at the relevant test temperature, where it was exercised to exhaustion over a roughly five-minute period by combining chasing and gentle tail pinching until the fish was unresponsive to touch. The fish was immediately returned to the respirometer chamber (< 1 min) which was sealed and flushed with oxygen-saturated water for one minute followed by measurements of oxygen consumption for approximately five minutes every fifteen seconds to determine the MMR (Chen et al., 2015). Oxygen concentration was allowed to decrease by no more than 25% of saturated seawater at each test temperature for both RMR and MMR (Clark et al., 2013; Chabot et al., 2016). Each test temperature experiment was repeated on the remaining individuals to gather a sample size of six individuals per temperature (in accordance with Chen et al., 2015). Trials were conducted during daylight hours after acclimation (when in a state or period of minimal inactivity; Chabot et al. 2016) for this species as Sparidae are predominantly diurnal at the post-larval and juvenile life stage (Whitfield 1989; Requena et al. 1997; Figueiredo et al. 2005). Aerobic experiments were then completed in 10 days (two days x five test temperatures). After each experimental trial, fish were removed from the chamber, euthanized by an overdosed concentration of clove oil (> 0.2 ml. L^{-1}) and placed on filter paper to blot dry excess water in order to be weighed individually to the nearest 0.001 g using a scale (aeADAM, PGL 303, d = 0.001 g).

4.2.2.1 Data analysis

Oxygen consumption rates for RMR and MMR were determined for each individual fish at each of the five test temperatures. Oxygen concentration readings below 75% saturation were excluded from the calculations. A least square linear regression of oxygen concentration (mg. L^{-1}) over time was performed for each individual (n = 6) for each of the test temperatures for both RMR and MMR to generate a best-fit curve. Residual analysis was first applied to determine whether the data were suitable for use in a linear regression model (performed in Statistica 13). The linear regression models were then used to calculate the oxygen consumption for each individual and blank control chamber at each test temperature for both RMR and MMR using the following equation:

$$V_{O2} = ([O_2] t_0 - [O_2] t_1) \times V / (T \times M_b)$$

84

where V_{O2} is oxygen consumption rate (mg.O_2.kg^{-1}.h^{-1}); *[O2]* t_0 is oxygen concentration at time zero (mg.O_2.L^{-1}); *[O2]* t_1 is oxygen concentration at time t_1 (mg.O_2.L^{-1}) and V is the respirometer volume minus the volume of the experimental animal (L). Animal volume (L) was assumed to be the same as the value of animal mass (kg); $T = t_1 - t_0$ (h) (overall time), and M_b is the body mass of the experimental animal (kg) (Chen *et al.*, 2015). The average oxygen consumption values for the blank chambers were then subtracted from the final calculated oxygen consumption values for each individual at each test temperature to account for background respiration in the sea water.

For the range of water test temperatures examined (9.0 – 34.0 °C), Q_{10} values were calculated as follows:

$$Q10 = \left(\frac{K_2}{K_1}\right)^{10/(T_2 - T_1)}$$

where K_2 is the metabolic rate at temperature T_2 and K_1 is the metabolic rate at temperature T_1. The Q_{10} values were determined for juvenile *D. capensis* juveniles based on mean RMR and MMR values for each test temperature (Kemp, 2009).

Relative aerobic scope (RAS) was then determined using the following equation:

Relative Aerobic Scope (RAS) = MMR – RMR

where mean routine metabolic rate (RMR) is subtracted from the maximum metabolic rate (MMR) (Fry, 1971; Post and Lee, 1996; Rombogh, 2006; Killen *et al.*, 2007; Boucher *et al.*, 2018). Relative aerobic scope was calculated in place of aerobic scope as SMR could not be measured due to minor spontaneous activity (Boucher *et al.* 2018) of juvenile *D. capensis*.

Three separate one-way ANOVA tests were performed for RMR, MMR and RAS respectively to test for differences between the five test temperatures (five levels, fixed) where relevant. Significant results were further tested using Tukey's post hoc comparisons. Furthermore, variation (variance) within individuals (n = 6) for each test temperature was evaluated for both RMR and MMR. All data analysis was performed in SigmaPlot 12.5 unless otherwise stated. Normality of distributions were tested using a Shapiro-Wilk test and homoscedasticity was tested using the Levene's test. When the normality and homogeneity assumptions were not

satisfied, Kruskal-Wallis one-way ANOVA's and Dunn's post hoc comparisons were performed in place of the parametric tests. Statistical significance for all analyses was set at $\alpha=0.05$.

4.2.3 Maximum heart rate (f_{Hmax})

Thirty adult blacktail *D. capensis* (270 - 340 mm TL) were collected in summer from the Port Alfred surf zone (33° 89' 33.6" S 26° 29' 81.5" E) using rod and line. After collection, adult *D. capensis* were transported to the AERP laboratory in Grahamstown in a sealed aerated tank and were then transferred to a 5900 L indoor cylindrical recirculating tank set at the field/acclimation temperature of 18.0 °C and were acclimated for a minimum of 36 hours (according to the methods of Mora and Maya, 2006) prior to the first experiment. They remained in this tank for the experimental period, which lasted two weeks, with a photoperiod 12h L: 12h D. Fish were fed a mixed diet of squid (*Loligo reynaudii*) and sardine (*Sardinops sagax*) every other day and starved 36 hours prior to experiments. Salinity was kept constant at 35 and checked every second day along with temperature, pH and oxygen. Water quality measurements of nitrate, nitrite and ammonia were also taken (Salifert Test Kit) every second day prior to when experimental trials were conducted. If these water quality parameters were found to be high, a partial water change was conducted whereby a quarter of the seawater was removed and replaced with filtered rainwater.

4.2.3.1 Pilot study for the stimulation of f_{Hmax}

Eight adults (270 – 320 mm TL) were used to validate the action of isoproterenol to stimulate maximum heart rate (as per the methods of Casselman *et al.*, 2012), ensuring that maximum heart rate would be stimulated throughout the four-hour experiment and to test the optimal position of the heart rate logger. One individual was tested at a time and was collected from the 5900 L indoor cylindrical recirculating tank and placed into a 250 L rectangular aerated tank for two hours where temperature was raised by 1.0 °C/h to acclimate to a temperature of 20.0 °C from 18.0 °C. Following this, the individual was then anaesthetized in a solution of 2-phenoxyethanol ($C_8H_{10}O_2$; 0.2 mL L^{-1}) until it was unresponsive and the opercular movements decreased significantly (Summerfelt and Smith, 1990; Keene *et al.*, 1998; Mylonas *et al.*, 2011). The individual was immediately transferred to a scale (aeADAM, PGL 10001, d = 0.1 g) and rapidly weighed (g) and transferred to an operating trough for surgery where the

anaesthetic solution was applied continuously over the gills during the procedure in order to maintain respiration and anaesthesia.

The surgery entailed inserting a heart rate logger (DST micro HRT, 8.3 mm X 25.4 mm, 3.3 g, Star-Oddi, Iceland, http://www.star-oddi.com), which was pre-programmed to measure the heart rate every 15 seconds for a minute (four readings) followed by one minute of recording ECG data at 200 Hz. This programmable setting was continuous for the four-hour period. The heart rate logger was attached with two sutures (Clinisut® silk suture; 3-0). An incision of approximately 2.5 cm was made directly below the origin of the left pectoral fin (Figure 4.1). This location allowed the heart rate logger to be situated immediately posterior to the pericardium membrane, which is essential for the successful functioning of the logger. The heart rate logger was inserted into the cavity, with the two circular electrodes positioned either sideward (facing away from the body wall to make direct contact with the muscular tissue once sealed) (n = 4) or upwards (facing towards the body wall) (n = 4). The incision was stitched using the two sutures attached to the heart rate logger and coagulating antiseptic wound gel was applied. All surgical instruments used were sterilized with ethanol.

Figure 4.1. The position of the incision made on the individual blacktail, *D. capensis* for the insertion of the heart rate logger.

After surgery, the fish was immediately placed into a rectangular 250 L tank, which formed part of a 800 L recirculating system that was dosed with 2-phenoxyethanol (0.2 ml. L^{-1}), in a weighted foam sling that kept the fish suspended in an upright position (Figure 4.2). The 800 L recirculating system was aerated with air stones, one in the sump and one in the rectangular 250 L tank. Temperature was maintained at 20.0 °C and monitored using an Ibutton temperature logger placed at the bottom of the tank. The tank had two inflows, one to regulate water circulation and temperature, and the other to maintain fish respiration. The respiratory inflow comprised of a plastic aquarium pipe that was fitted with a PVC pipe nozzle placed into the mouth of the fish (Figure 4.2). The flow rate of this respiratory inflow was kept constant at 1 $L.min^{-1}$.

Figure 4.2. The position of an individual blacktail (*Diplodus capensis*) maintained throughout experiments in a weighted sling with a respiratory inflow valve.

Anaesthetized fish were stabilized at the acclimation temperature (20 ° C) for one hour in this position in order to record the basal heart rate. After the one-hour period, the fish was removed from the sling and intraperitoneally injected with a solution of atropine sulphate (Sigma-Aldrich; 1.2 $mg.kg^{-1}$) to inhibit vagal tonus to the heart, as well as a saline solution of isoproterenol (Sigma-Aldrich; 1.2 $\mu g.kg^{-1}$) to stimulate cardiac adrenergic β-receptors (Casselman *et al.*, 2012; Chen *et al.*, 2015). The fish was then returned to the sling and the

88

heart rate of the individual was recorded using the heart rate logger for a further three hours. After the three-hour period, the experiment was terminated, the individual removed from the tank, and euthanized using a lethal dose (0.5 ml. L^{-1}) of 2-phenoxyethanol. The heart rate logger was then recovered, rinsed, dried and placed in the communication box in order to retrieve the heart rate data from the experiment. The remaining seven *D. capensis* individuals were then tested following the same procedure. The only variable that differed, however, was the logger position, either sideward (n = 4) or upwards (n = 4).

4.2.3.2 Estimation of f_{Hmax} indicators

The experiments followed the methodology for the pilot study described above. Fourteen adult *D. capensis* were used for these experiments. Prior to the estimation of f_{Hmax} experiments, individual *D. capensis* were placed into a rectangular 250 L tank and the temperature was lowered from 18.0 °C to 14.0 °C within a two-hour period (2.0 °C/h decrease) using a chiller (Hailea, HS-90 A). Each individual was then anaesthetized and taken for surgery to insert the heart rate logger sideward (the optimal position). The individual was then placed in a different rectangular 250 L tank which formed part of a 800 L recirculating system that was dosed with 2-phenoxyethanol (0.2 ml. L^{-1}) in a sling and was left to acclimate at 14.0 °C for a further hour in an anaesthetized state. Five minutes after the intraperitoneal injections, the heart rate logger was set to start recording and the water temperature was raised using an AquaHeat 9.2 kW pump at 7.0 °C/h from 14.0 to 30.0 °C. At the end of the three-hour experiment, the individual fish was removed from the sling and tank and a blood sample was taken from the caudal vein using an 80 μl capillary tube. Fish were then euthanized, and the logger was retrieved. The sacrificed fish then underwent a full biological evaluation whereby gonad maturity stages were assigned macroscopically following Griffiths *et al.* (2002) (refer to Appendix A for macroscopic maturity criteria). The capillary tube was then placed into a capillary centrifuger (EinsSci E-C14-H24P, Spellbound Laboratory Solutions, South Africa) at 6000 g for 10 minutes at 25 °C to separate the red blood cells from the blood plasma to analyse blood haematocrit.

4.2.3.3 Data analysis

The Star-Oddi heart rate logger returns heart rates (BPM) validated using a quality index (QI) (QI 0 = great; QI 1 = good; QI 2 = fair; QI 3 = poor). Heart rates were filtered to use only

values with a QI of zero and binned into 0.25 °C increments. Clear heart rate outliers were removed from the analysis if they fell outside the 95% confidence interval of the mean heart rate for that respective temperature increment. If the heart rate loggers gave false f_{Hmax} recordings for an individual, it was excluded from the analysis. A trial on an individual was considered successful if readings with a QI of zero spanning across at least 80% of the 0.25 °C temperature increments were found and if there was a uniform increase of heart rate with temperature when plotting the corresponding graph.

For the f_{Hmax} analysis, T_{max} (the temperature at which f_{Hmax} reached its maximum absolute value), T_{ARR} (the temperature at which f_{Hmax} first began to decrease rapidly after f_{Hmax} plateaued and results in cardiac arrhythmia), T_{AB} (the first Arrhenius breakpoint temperature) and T_{QB} (the breakpoint temperature for Q_{10} of f_{Hmax}) were determined from the responses of individual fish. The T_{AB} was calculated using piecewise linear regression models (Quasi-Newton estimation) fitted to the Arrhenius plot [natural logarithm of the heart rate (ln (f_{Hmax})) against the inverse of temperature in Kelvin (1000. K^{-1})] in order to locate the intersecting breakpoint between two regression lines (STATISTICA, v. 12, Statsoft). Heart rate data for temperature bins from 14 °C to the temperature corresponding to maximum f_{Hmax} were used for the T_{AB} analysis (Ferreira *et al.*, 2014). T_{QB} (Incremental Q_{10}) for f_{Hmax} of individual fish was determined for every 1.0 °C increase using the following equation (Ferreira *et al.*, 2014):

$$Q10 = \left(\frac{f_{H2}}{f_{H1}}\right)^{10/(T_2 - T_1)}$$

where f_{H1} and f_{H2} are heart rates at first T_1 and second T_2 temperatures, respectively. The Q_{10} breakpoint (T_{QB}) is the temperature at which the incremental Q_{10} drops below 2.0. This is because a Q_{10} value of 2.0 is regarded as a regular rate of change of routine metabolism with temperature for fish (Drost *et al.*, 2014). The Q_{10} breakpoint was therefore estimated by finding the linear equation of the two consecutive points above and below 2.0 and calculating the temperature at which the two lines intersect (Quasi-Newton estimation, STATISTICA, v. 12, Statsoft).

4.3 RESULTS

4.3.1 Dynamic method

The summer CT_{max} of adult *D. capensis* (32.0 °C) was lower than the juvenile CT_{max} (35.0 °C) (Table 4.1). The summer CT_{min} for both adults (8.4 °C) and juveniles (8.0 °C) were, however, similar (Table 4.1).

Table 4.1. Sample size, sample size at thermal limit, mean standard length (SL), mean weight (g), and CT_{max} and CT_{min} thermal limits for juvenile and adult blacktail, *Diplodus capensis*

	Adult CT_{max}	Juvenile CT_{max}	Adult CT_{min}	Juvenile CT_{min}
Sample size	3	18	3	18
Sample size at thermal limit	3	9	3	9
Length (mm SL) mean ±SD	223.30 ± 3.51	70.83 ± 22.21	286.71 ± 2.08	50.21 ± 6.11
Range (mm SL)	190 – 260	41-111	270 - 310	42-63
Weight (g) mean ±SD	173.87 ± 95.73	16.98 ± 16.33	430.60 ± 108.29	2.57 ± 1.30
Range (g)	85.4 - 275.5	3-53	318 - 534	1 - 5
Field temperature (°C)	20.0	20.0	20.0	20.0
Thermal limit (°C) mean ± SD	32.0 ± 0.0	35.0 ± 0.4	8.4 ± 0.0	8.0 ± 0.2

4.3.2 Static respirometry

The RMR in juvenile *D. capensis* increased across the test temperatures 9.0 °C; 13.0 °C; 18.0 °C and 28.0 °C and decreased slightly between the test temperatures of 28.0 °C and 34.0 °C (Figure 4.3). For test temperatures, 9.0, 13.0 and 34.0 °C, three individuals were removed from the analysis as a result of high stress related oxygen consumption readings. The RMR peaked at 28.0 °C (0.63 ± 0.22 $mg.O_2.kg^{-1}.h^{-1}$), with the largest variation around the mean (0.05 ± 0.22 $mg.O_2.kg^{-1}.h^{-1}$; n = 6) (Figure 4.3). The lowest RMR was obtained at 9.0 °C (0.24 ± 0.03 $mg.O_2.kg^{-1}.h^{-1}$) with a variation around the mean of 0.00± 0.03 $mg.O2.kg^{-1}.h^{-1}$; n = 3), followed by 13.0 °C (0.38 ± 0.04 $mg.O_2.kg^{-1}.h^{-1}$; variance = 0.00 ± 0.04 $mg.O_2.kg^{-1}.h^{-1}$; n =3), 18.0 °C (0.57 ± 0.08 $mg.O_2.kg^{-1}.h^{-1}$; variance = 0.01 ± 0.08 $mg.O_2.kg^{-1}.h^{-1}$; n = 6) and 34.0 °C (0.56 ± 0.02 $mg.O_2.kg^{-1}.h^{-1}$; variance = 0.00 ± 0.02 $mg.O_2.kg^{-1}.h^{-1}$; n = 3) (Figure 4.3). The RMR values for juvenile *D. capensis* differed significantly with temperature (ANOVA; F (4, 20) = 5.31, p = 0.006), with a Tukey's post hoc test revealing a significant difference between the minimum

test temperature (9.0 °C) and the 28.0 °C test temperature (Figure 4.3). The $Q_{10\,(9-34)}$ value for RMR of juvenile *D. capensis* was 1.40.

For MMR measurements, three individuals at the 9.0 °C and 13.0 °C test temperatures were removed as a result of low stress related oxygen consumption readings. The MMR was highest at 28.0 °C (1.01 ± 0.29 mg.O_2.kg^{-1}.h^{-1}; n = 6) and lowest at 9.0 °C (0.29 ± 0.02 mg.O_2.kg^{-1}.h^{-1}; n =3) (Figure 4.3). The variation around the mean increased with temperature and was greatest at 28.0 °C (variance = 0.08 ± 0.29 mg.O_2.kg^{-1}.h^{-1}) (Figure 4.3). The MMR was significantly different among four test temperatures (Kruskal-Wallis ANOVA; H (3) = 13.53, p = 0.004) (Figure 4.3). Dunn's post- hoc tests revealed that there were significant differences in the MMR of *D. capensis* between 9.0 and 28.0 °C (Figure 4.3). No MMR data was obtained for juvenile blacktail at 34.0 °C due to a loss of equilibrium of individuals after chasing. For MMR, the Q_{10} $_{(9-28)}$ value was 1.94 respectively, higher than RMR value of 1.40, reported above.

RAS was lowest at the 9.0 °C (0.05 ± 0.05 mg.O_2.kg^{-1}.h^{-1}; n = 3) and 13.0 °C (0.05 ± 0.04 mg.O_2.kg^{-1}.h^{-1}; n = 3) test temperatures and increased slightly for the 18.0 °C (0.13 ± 0.08 mg.O_2.kg^{-1}.h^{-1}; n = 3) test temperature (Figure 4.3). The greatest RAS was observed for the 28.0 °C (0.38 ± 0.29 mg.O_2.kg^{-1}.h^{-1}; n = 3) test temperature illustrating that an increase in RAS occurred with an increase in temperature (Figure 4.3). The RAS was significantly different among the four test temperatures (Kruskal-Wallis ANOVA; H (3) = 10.67, p = 0.014). RAS for the 34.0 °C test temperature was unattainable as a result of fish loosing equilibrium after chasing for the MMR measurements (Figure 4.3).
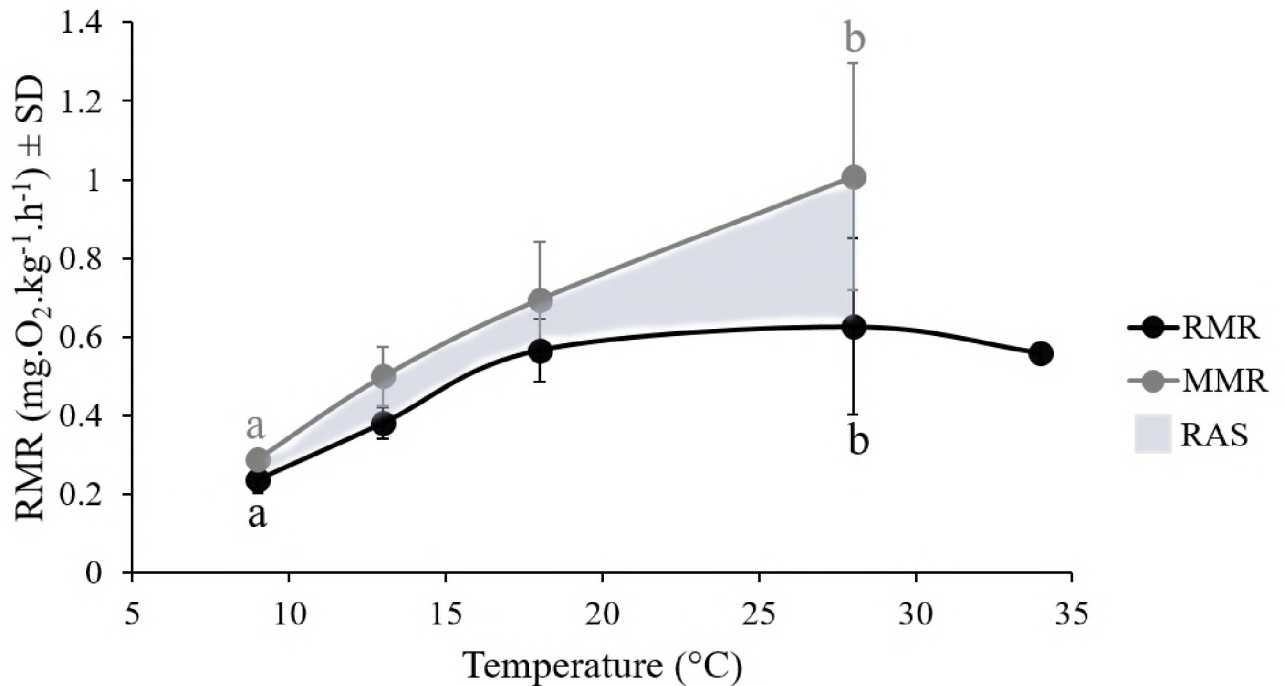
Figure 4.3. Routine metabolic rate (RMR), maximum metabolic rate (MMR) and relative aerobic scope (RAS) of juvenile *D. capensis* at different test temperatures (9.0 °C, 13.0 °C, 18.0 °C, 28.0 °C, 34.0 °C). Each point is a mean; error bars indicate ± SD. RMR is indicated in black, MMR is indicated in grey and RAS is indicated in a shaded light blue-grey. Different letters (a) and (b) represent significant differences in RMR and MMR between test temperatures ($p < 0.05$). MMR and RAS for the 34.0 °C test temperature was not measured as individuals lost equilibrium during the chasing period.

4.3.3 Maximum heart rate

4.3.3.1 Pilot study for the stimulation of f_{Hmax}

The heart rate logger efficacy was low with high variability in the QI, except for individual two, seven and eight (Figure 4.6), where QI was primarily zero (corresponding to "great quality" results - see section 4.2.3.3). Of the eight individuals included in the pilot study, three trials were successful (> 70% "great quality" results) (Figure 4.6 ii, vii, viii). The heart rate logger in two of these (Figure 4.6 vii, viii) was positioned sideward, while the third was positioned upwards (Figure 4.6 ii). The sideward positioning was preferred as the heart rate logger was in direct contact with the body wall, making heart rate detection higher. Therefore, this sideward positioning was the chosen position of the logger deployment for the temperature

ramping protocol (estimation of f_{Hmax} indicators). Following the injections of the pharmaceutical stimulants for individuals seven and eight, the average heart rate increased from 94 beats.min^{-1} to 111 beats.min^{-1} and from 88 beats.min^{-1} to 104 beats.min^{-1}, respectively. These both remained at f_{Hmax} for the duration of the four-hour experimental period (Figure 4.7). For individual two, whose heart rate logger was positioned upwards, average heart rate rose from 93 beats.min^{-1} to 111 beats.min^{-1} following the pharmaceutical stimulant injections and remained approximately at 111 beats.min^{-1} for the duration of the four-hour experimental period (Figure 4.7). The effects of the pharmaceutical stimulants were observed within two to eight minutes of the injection and stabilized after approximately 20 minutes for individual two and individual eight. For individual seven, however, the effects of the pharmaceutical stimulants and the stabilization thereof was immediately observed (Figure 4.7). For individual three, the battery of the heart rate logger was depleted after an hour and thus the base heart rate was only recorded prior to the injections of the pharmaceutical stimulants.
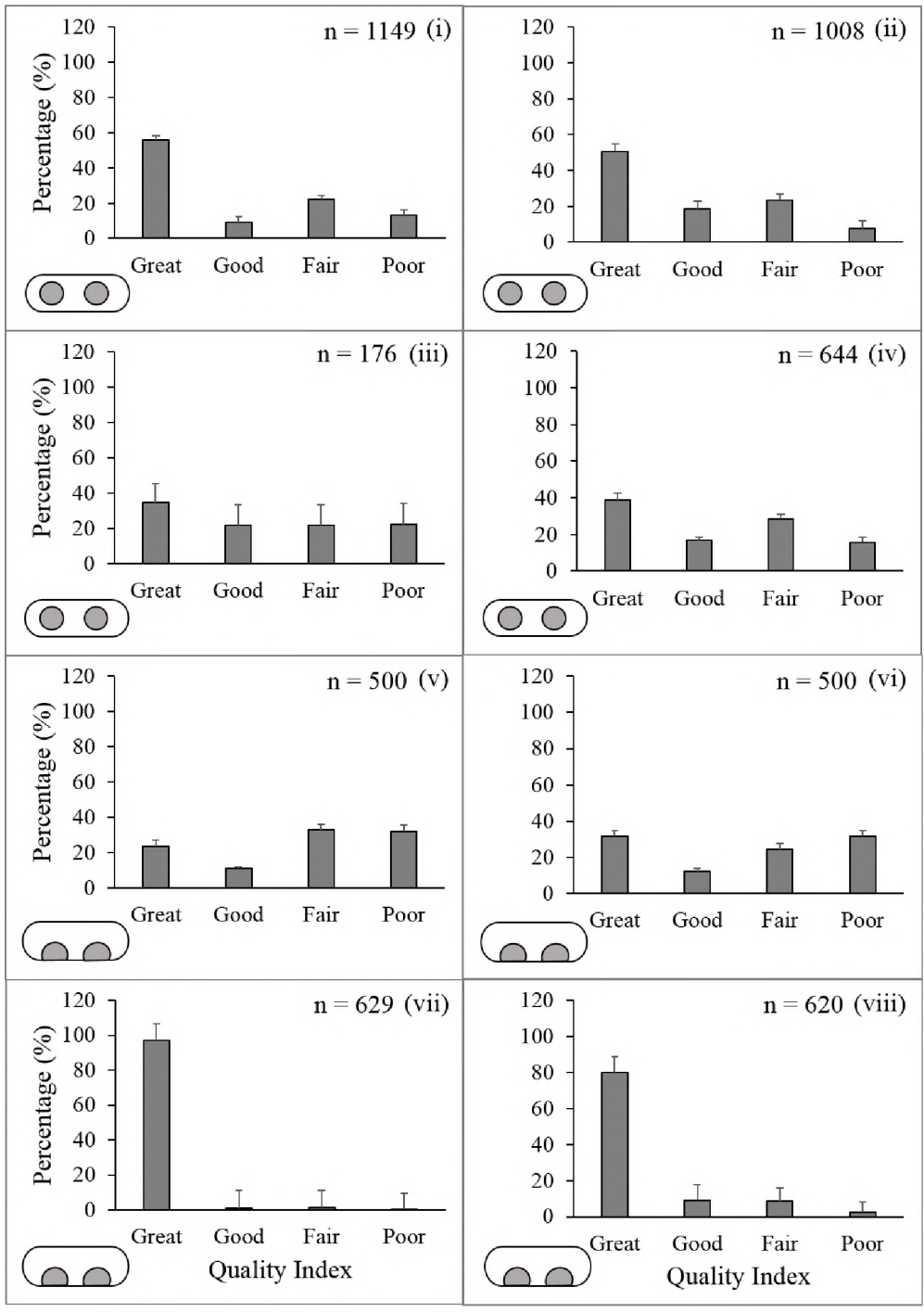
Figure 4.4. Percentage of great, good, fair and poor quality index (QI) values for the stimulation of f_{Hmax} heart rate detections (by Star-Oddi heart rate loggers positioned either sidewards or upwards from a top view) of eight (i - viii) adult *Diplodus capensis* individuals.
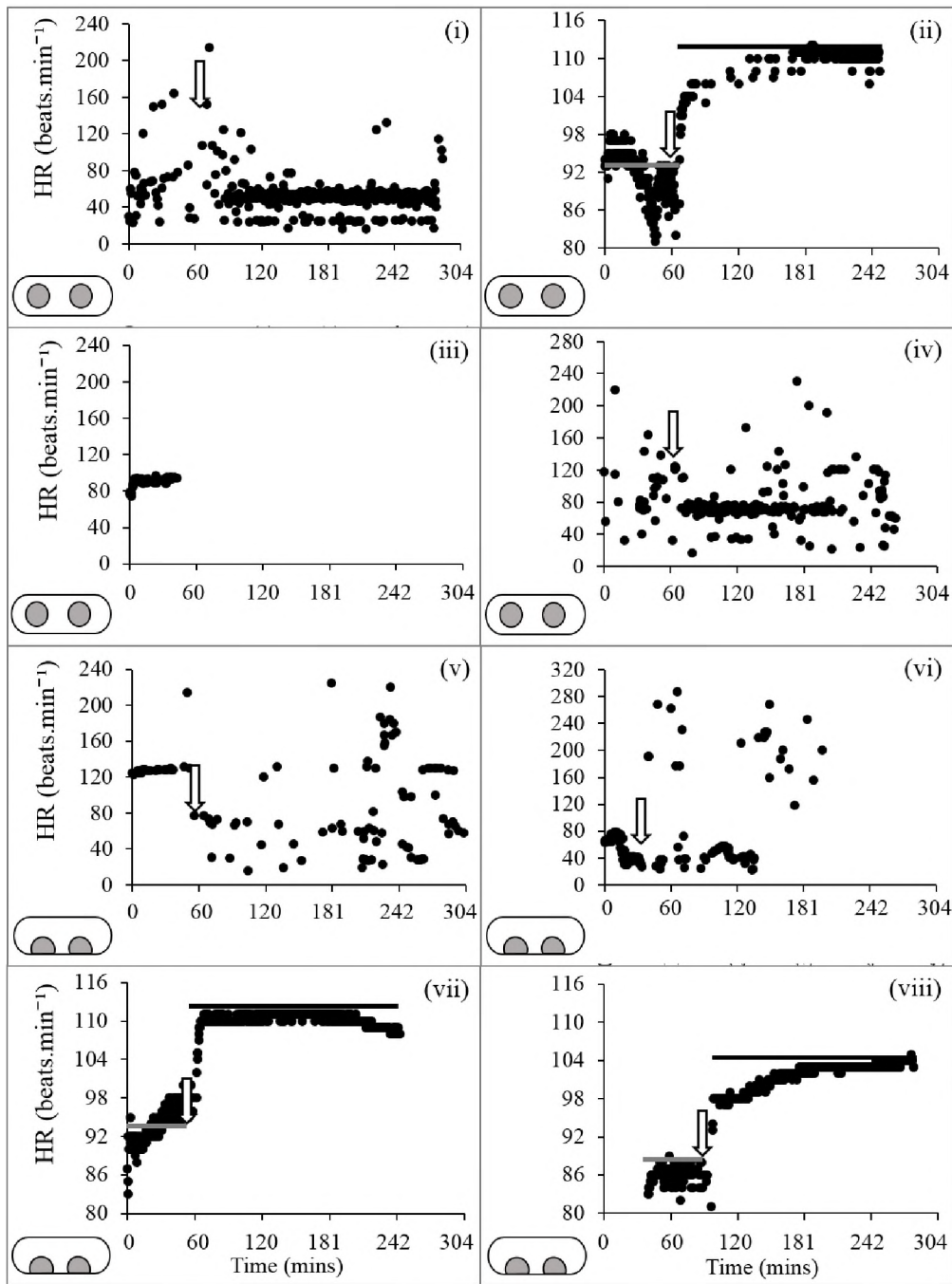
Figure 4.5. Raw heart rates (HR) of eight individual adult *Diplodus capensis* (i) – (viii) before and after the addition of heart rate stimulant pharmaceuticals with the exception of individual (iii) whose heart rate logger stopped recording before stimulant injections. (i) = female (0.35 kg); (ii) = male (0.37 kg); (iii) = female (0.36 kg); (iv) = female (0.38 kg); (v) = female (0.34 kg); (vi) = male (0.47 kg); (vii) = male (0.62 kg); (viii) = female (0.47 kg). The black and white arrows indicate the time at which the drugs were administered. Grey lines represent the routine heart rate (f_H) prior to injections. The black solid line represents the maximum heart (f_{Hmax}) following the injections.

4.3.3.2 Effect of temperature on f_{Hmax}

For the stimulation of the effect of temperature on f_{Hmax} for 14 *D. capensis* individuals, 12 individuals (with the heart rate logger positioned sideward) provided interpretable results with a high heart rate logger efficacy, whereby the desired QI of zero was attained ~95% throughout each trial (Figure 4.8).
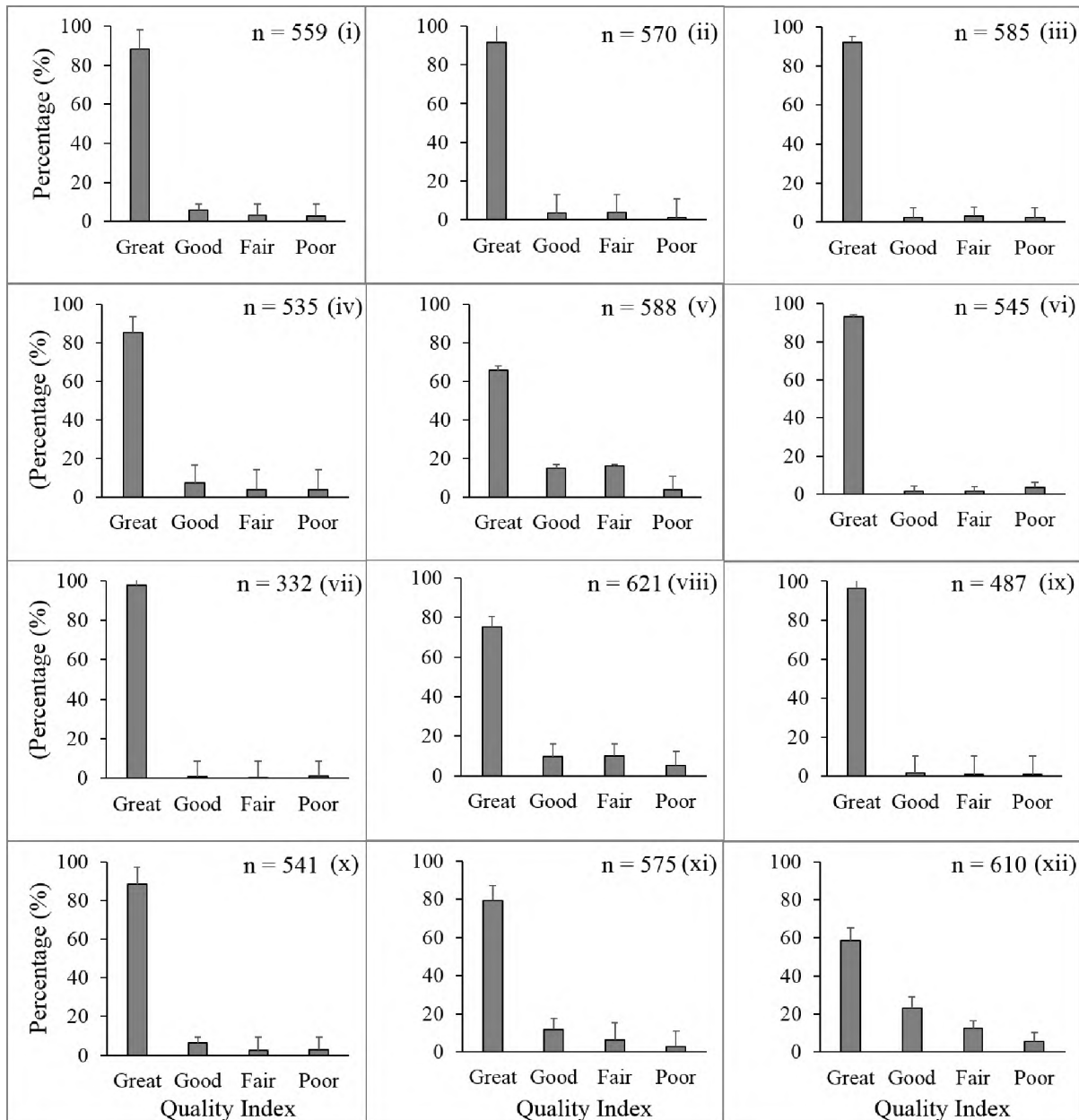
Figure 4.6. Percentage of great, good, fair and poor quality index (QI) values for f_{Hmax} heart rate detections (by Star-Oddi heart rate loggers positioned sideward) of 12 (i - xii) adult *Diplodus capensis* individuals.

The f_{Hmax} of all twelve adult *D. capensis* increased with increasing temperature, with an average peak of 152 beats.min^{-1} ($\pm$ 17 SD) observed at 28.0 $\pm$ 1.7 °C (Figure 4.9, Table 4.2). The highest f_{Hmax} was generally followed by a plateau and decline in heart rate, which signified the beginning of cardiac arrhythmia (T_{ARR}) (Figure 4.9). The average T_{ARR} was 28.3 °C $\pm$ 1.7 °C SD (Table 4.2).
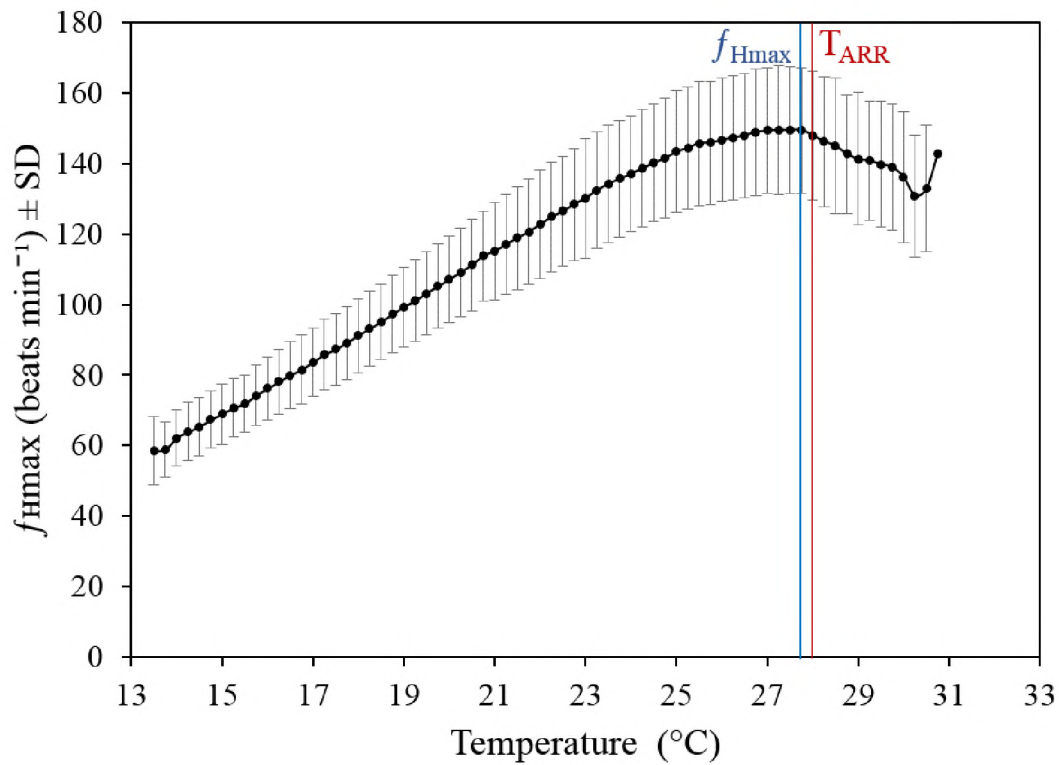
Figure 4.7. Average change in maximum heart rate (f_{Hmax}) of adult *Diplodus capensis* in response to increasing water temperature from 14.0 °C. Each point represents a mean; $\pm$ SD (n = 12). Blue vertical line represents the average maximum heart rate (f_{Hmax} = 152 beats.min^{-1} at 28.0 °C). Red vertical line represents the average arrhythmic temperature (T_{ARR} = 28.3 °C).

Table 4.2. Biological information, f_{Hmax} index values for individual blacktail, *Diplodus capensis* in response to acute warming. f_{Hmax} values include the cardiac arrhythmia temperature (T_{ARR}), Arrhenius break temperature (T_{AB}) and Q_{10} breakpoint temperature (T_{QB}).

	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10	Fish 11	Fish 12	Average	SD
Sex	Female	Female	Female	Female	Male	Female	Female	Female	Female	Female	Female	Female		
Reproductive Stage	Spent	Mature	Mature	Mature	Spent	Mature	Mature	Mature	Mature	Mature	Mature	Mature		
	Stage 4	Stage 5	Stage 2	Stage 2	Stage 4	Stage 2	Stage 2	Stage 2	Stage 2	Stage 2	Stage 2	Stage 2		
Mass (kg)	0.65	0.46	0.81	0.66	0.57	0.56	0.64	0.48	0.34	0.42	0.31	0.23	0.49	0.17
Length (cm TL)	33.0	33.0	34.0	32.0	30.0	30.0	30.0	34.0	30.0	30.0	30.0	30.0	31.0	2.0
Relative ventricular mass (%)	2.0	1.4	2.4	2.1	1.9	1.9	2.1	1.4	1.1	1.4	1.0	0.8	1.6	0.5
Haematocrit (%)	26.3	17.9	20.4	10.6	20.0	15.1	23.8	21.0	33.9	16.1	26.3	25.0	22.8	7.3
Highest f_{Hmax} (beats.min^{-1})	150	141	145	145	156	150	162	115	188	158	157	161	152	17
T at highest f_{Hmax} (°C)	30.0	29.5	29.5	26.0	27.8	28.8	25.5	30.5	28.0	26.5	27.3	26.8	28.0	1.7
T_{ARR} (°C)	30.3	29.8	29.8	26.3	28.0	29.0	25.8	30.8	28.3	26.8	27.5	27.0	28.3	1.7
T_{AB} (°C)	20.5	20.4	21.3	23.4	21.3	20.5	20.4	21.0	20.2	19.9	20.6	19.8	20.8	1.0
T_{QB} (°C)	22.0	19.0	20.2	19.6	21.4	21.3	20.8	21.7	21.9	19.9	21.0	22.5	20.9	1.1

The individual piecewise linear regression models returned an average Arrhenius break point temperature (T_{AB}) of 20.8 °C ± 1.0 °C SD (Figure 4.10, Table 4.2). The Q_{10} breakpoint (T_{QB}) was similar to the T_{AB}, ranging from 19.0 to 22.5 °C with an average of 21.0 °C ± 1.0 °C SD (Figure 4.11, Table 4.2).
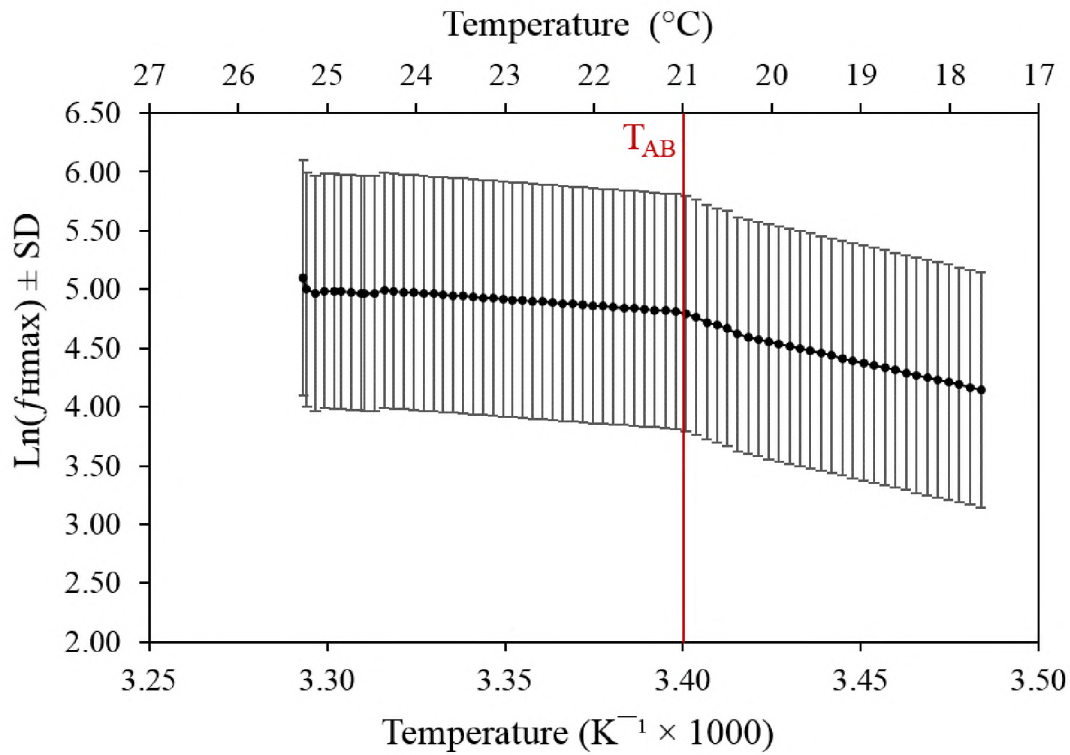
Figure 4.8. Average Arrhenius plots of the natural log of maximum heart rate (ln (f_{Hmax})) against the inverse temperature in Kelvin (1000 K^{-1}) of adult *Diplodus capensis*. Each point is a mean; error bars indicate ± SD (n = 12). The red vertical line represents the average Arrhenius breakpoint temperature (T_{AB} = 20.8 °C).
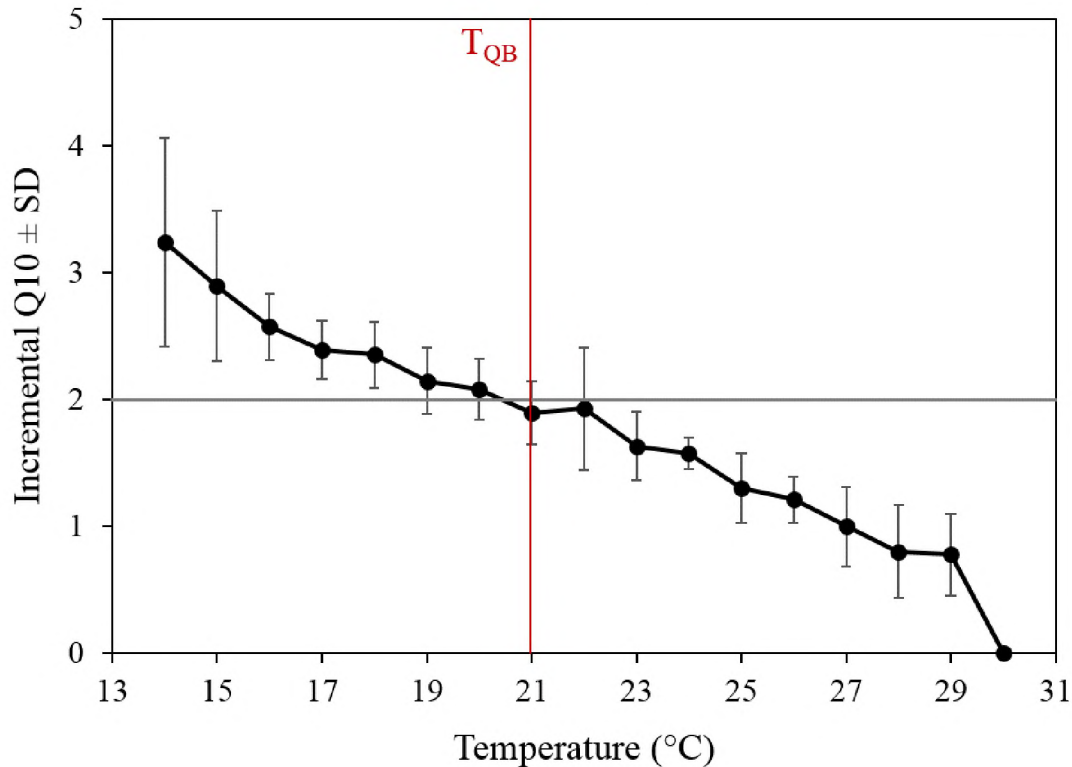
Figure 4.9. Average incremental Q_{10} analysis of f_{Hmax} for 1.0 °C increments of adult *Diplodus capensis*. Each point is a mean; error bars indicate ± SD (n = 12). The grey horizontal solid line represents the average Q_{10} breakpoint (Q_{10} < 2.0) and the red vertical line represents the incremental Q_{10} breakpoint temperature (T_{QB} = 21.0 °C).

4.4 DISCUSSION

To understand the physiological changes of a model species *D. capensis* to increasing temperatures, a comprehensive multi-method physiological assessment of their thermal tolerance and performance was undertaken at two life stages using the dynamic method for juveniles and adults, static respirometry for juveniles, and maximum heart rate for adults under conditions of acute warming. Results for the use of the dynamic method indicated that the upper critical thermal limits (CT_{max}) for juvenile *D. capensis* (35.0 °C) were higher than for the adults (32.0 °C), while lower critical thermal limits (CT_{min}) did not differ (juveniles = 8.0 °C; adults = 8.4 °C). Existing information on thermal physiology within fish species show that the windows of thermal tolerance in juveniles are wider than in larval and adult life stages (Pörtner, 2006; Pörtner and Farrell, 2008). This is because, during the larval phase, the central organs

101

responsible for regulating physiology are not yet fully developed (Pörtner *et al.*, 2006) and their small body size, low metabolic scope and low energy reserves may negatively affect their survival as temperature increases (Brewer, 1976; Rijnsdorp *et al.*, 2009). During the adult phase, oxygen supply capacity in relation to demand decreases as a result of them investing more metabolic energy into spawning, potentially making them more thermally sensitive to temperature change (Pörtner and Peck, 2010). During the juvenile phase, however, thermal windows widen in line with rising performance capacity at small body size making them less thermally sensitive to temperature increases (Pörtner and Peck, 2010). Results from this study for *D. capensis* are therefore consistent with the literature in that juveniles are more thermally tolerant to increases in temperature than their adult counterparts.

For juvenile *D. capensis*, using static respirometry to measure thermal tolerance and performance, results indicated that both routine (RMR) and maximum (MMR) oxygen consumption rates increased with test temperature from an acclimated temperature of 20.0 °C, and rapidly decreased beyond the 28.0 °C test temperature, where individual variance was highest. A study conducted by Kemp (2009) on juvenile *D. capensis* from the same region found that RMR increased with increasing water temperature and intraspecific variability was also at its highest at 28.0 °C. RMR for this study, however, was lower compared with Kemp's (2009) results, possibly due to the smaller sample size used for the trials. In addition, high individual variability for the juvenile *D. capensis* at the 28.0 °C test temperature could be at its highest compared with the other test temperatures due to slight differences in size and weight (see Calder, 1987). In a similar study, Pirozzi and Booth (2009) found that at 26.0 °C, "extra-small" individuals (60.4±0.9 g) of a coastal marine estuarine-dependent species, the dusky kob *Argyrosomus japonicus* (Temminck and Schlegel, 1844), had the highest RMR oxygen consumption rates compared to all other size classes tested (including adults).

A study conducted on temperate region juveniles (6.5 ± 0.24 g) of the related sparid species, the gilthead seabream *Sparus aurata* (Linnaeus, 1758), reported that their highest oxygen consumption rates were 28.0 °C for RMR and MMR, with a rapid decrease thereafter (Requena *et al.*, 1997). Although their research followed a comparatively different heating rate (1.0 °C.day⁻¹) to this study (1.0 °C.h⁻¹), it suggests that oxygen consumption rates, predominantly MMR (juveniles of *D. capensis* at 34.0 °C test temperature for this study lost equilibrium), decrease rapidly beyond 28.0 °C for these two related species that occupy similar

biogeographic niches – i.e. temperate regions, where temperatures are naturally not greater than 30.0 °C. The native range of *S. aurata* is the warm-temperate Mediterranean coast which is subject to wide seasonal variation (Requena *et al.*, 1997). Its distribution also overlaps with the congener of *D. capensis*, namely *D. sargus* (Floeter *et al.*, 2008). Furthermore, after an exposure period of 12 days at 28.0 °C, juvenile *S. aurata* showed a further reduction in daily RMR and MMR oxygen consumption rates, coupled with an increase in mortality (Requena *et al.*, 1997). This suggests that sparid juveniles may not be able to acclimate to and survive sea temperatures greater than 28.0 °C for extended periods of time (e.g. marine heatwaves) within temperate regions where temperatures are approaching those greater than 30.0 °C (Kemp, 2009). Extrapolation across genera within the Sparidae, however, needs to be verified by extending thermal tolerance experiments to multiple species. Nevertheless, evidence from this study for juvenile *D. capensis* demonstrated this, with further support of a CT_{max} of 35.0 °C obtained from the dynamic method and as a consequence, oceanic temperature changes may negatively affect its metabolic processes, in turn influencing its vertical, seasonal and latitudinal distribution (Gibson, 1982).

When assessing the $Q_{10\,(9-34\,°C)}$ for RMR and the $Q_{10\,(9-28\,°C)}$ for MMR of juvenile *D. capensis*, MMR (1.94) oxygen consumption rate increased by a half in energy demand to the RMR (1.40) oxygen consumption rate. This suggests that juvenile *D. capensis* may not be able to compensate for the loss of metabolic energy with an increase in temperature under increased levels of activity. This sensitivity may have accounted for the loss of equilibrium experienced by all individuals at the 34.0 °C test temperature for MMR. To support this, Kemp (2009), using RMR, showed that *D. capensis* with a $Q_{10\,(14-28\,°C)}$ of 2.07 was more temperature sensitive than *Caffrogobius caffer* (Günther, 1874) which had a $Q_{10\,(14-28\,°C)}$ of 1.83 because *C. caffer* is able to metabolically adapt to temperature in the dynamic intertidal environment and demonstrated low levels of spontaneous activity under thermal stress, unlike *D. capensis*. In addition, Requena *et al.* (1997) also demonstrated that *S. aurata* exhibited an increased metabolic response to temperature for MMR [$Q_{10\,(20-28\,°C)} = 2.7$].

The use of internal heart rate loggers successfully measured maximum heart rate on adult *D. capensis* (86% success rate). It was also possible to identify the average T_{AB} (20.8 °C), T_{QB} (21.0 °C) and T_{ARR} (28.3 °C) over a simulated warming event. The high success rate for heart rate logger efficacy for this study, when compared with similar studies on sparids (Skeeles *et*

al., accepted; Muller *et al.*, accepted), may most likely be attributed to optimal placement (directly below the origin of the pectoral fin, posterior to the pericardium membrane) and positioning (sideward results > upward results) of the logger, identified during pilot studies. Skeeles *et al.* (accepted) examining thermal performance of the endemic red roman *Chrysoblephus laticeps* (Valenciennes, 1830) placed the loggers further back in line with the origin of the pelvic fin and positioned the loggers upwards which may have resulted in a lower success rate being obtained (60%).

The successful maximum heart rate performance indices identified for adult *D. capensis* (T_{AB} = 20.8 °C; T_{QB} = 21.0 °C; T_{ARR} = 28.3) were comparable to values of heart indices obtained for *C. laticeps* (T_{AB} = 19.1 °C; T_{QB} = 19.1 °C; T_{ARR} = 25.5 °C) over an acute warming event (Skeeles *et al.*, accepted). This similarity in results from the two studies could be due to all the specimens being exposed to the same temperature ramping range (14.0 – 30.0 °C) and procedure, both species having similar temperate distributions (*D. capensis* is, however, more coastal compared to *C. laticeps*) and/or belonging to the same family (Sparidae), whereby specific genetic adaptations may allow this family to have similar thermal tolerance responses (Schaefer and Ryan, 2006).

When comparing maximum heart rate thermal indices to the CT_{max} for adult *D. capensis*, CT_{max} was nearly 4.0 °C higher that T_{ARR} (28.3 °C), 11.2 °C higher than T_{AB} (20.8 °C) and 11.1 °C higher than T_{QB} (20.9 °C). This comparison, functionally, suggests that due to the large difference between T_{AB}/T_{QB} and CT_{max}, adult *D. capensis* have a wide thermal window (~11.0 °C) in which f_{Hmax} can increase, which is typical of a eurythermic nature (Sidhu *et al.* 2014). However, even though this window is wide, there is still the possibility that the effective physiological response may not cope with an increase in temperature. In addition, due to the small difference between T_{ARR} and CT_{max} (3.7 °C), f_{Hmax} is potentially unable to keep up with the Q_{10} effect of warming on tissue oxygen demand (Sidhu *et al.* 2014), if the Q_{10} for aerobic scope is > 2.0 (Taylor *et al.*, 2005), and may indicate that adult *D. capensis* may be vulnerable to further temperature increases.

Interestingly, the T_{QB} and T_{AB} was similar for the adult *D. capensis*. This is not unusual in eurythermic fish species. For example, Sidhu *et al.* (2014) demonstrated that for eurythermal *Danio* species there was a 0.5 °C difference between T_{AB} and T_{QB}, consistent with this study (0.1 °C difference) and the other adult sparid species *C. laticeps* (0.1 °C difference) (Skeeles

et al., accepted). This similarity between T_{QB} and T_{AB} (despite very different methods of estimation) for eurythermal species thus provides some verification of a mechanistic relationship between the two indices, suggesting that, regardless of species, with an increase in temperature beyond T_{AB}/T_{OPT}, metabolism begins to decrease at the same point at which the oxygen demand becomes excessive (Q_{10} for aerobic scope > 2.0). Functionally, this suggests that maximum heart rate can increase for adult *D. capensis* (and other eurythermic species) at higher temperatures, but metabolic cost and energy expenditure to maintain this may determine its upper bound. In the case of stenothermic species, metabolism beyond T_{AB}/T_{OPT} may begin to decrease before or after the oxygen demand becomes excessive resulting in a larger difference between T_{AB} and T_{QB} (Chen *et al.*, 2015; Logan and Buckley, 2015).

The high metabolic cost incurred by eurythermic fish to acclimate to a broad thermal range may also explain why the margin between T_{ARR} and CT_{max} is narrow for adult *D. capensis*. Beyond T_{ARR}, which in this instance may alternatively serve as the upper T_{PEJ}, the transition to CT_{max} may be narrow, resulting in a rapid decline in oxygen, causing oxidative stress, a heat shock response, anaerobic metabolism and finally the denaturation of heat shock proteins (Pörtner *et al.*, 2017) at lesser extremes of temperature for adult *D. capensis* (32.0 °C) compared to juveniles of the same species. This observed narrower pattern in adults is unlike that observed by organisms experiencing extreme temperatures (e.g. macro-invertebrates) who have narrow active thermal ranges that lower their metabolic costs allowing them to tolerate anaerobically extreme temperatures by protective mechanisms (metabolic depression, e.g. Tomanek and Somero, 2002) (Pörtner *et al.*, 2017).

The metabolic costs of withstanding and inhabiting warmer waters beyond T_{ARR} (> 28.0 °C) for adults of this species are high and, therefore, may reduce the energy available for reproduction at higher temperatures. Many of the species belonging to the family Sparidae are serial spawners and have adopted a bet-hedging spawning strategy (asynchronous spawning) over an extended season to account for variability in egg and larval survival (Robertson, 1991). *Diplodus capensis* extends its spawning season up to five months during summer, where temperatures may be cooler as a result of upwelling events (Mylonas *et al.*, 2011). If water temperatures are too high, however, there is unlikely to be sufficient egg development and spawning events. This may explain why adult *D. capensis* does not spawn at temperatures

higher than 20.0 °C in summer as examined by Potts *et al.* (2014) which further coincides with the Arrhenius breakpoint temperature for this study (T_{AB} = 20.8 °C).

To improve results of using this multi-method approach, recommendations to the study need to be discussed. Firstly, sample sizes for the adult *D. capensis* CT_{max} and CT_{min} experiments, as well as the static respirometry experiments for the juvenile *D. capensis* need to be increased. Future work should evaluate when is the best time of day to conduct RMR and MMR measurements as this has not been established for this species as yet. Secondly, finer scale size intervals for the juveniles in the respirometry experiments need to be done to possibly identify refined ontogenetic levels of thermal tolerance. Lastly, static respirometry experimental trials on adult *D. capensis* need to be included as well as the larval life stages of *D. capensis* to understand the metabolic physiology of this species through the main life stages.

In conclusion, when evaluating the results of the multi-method approach between juvenile and adult *D. capensis*, juvenile *D. capensis* had a higher thermal tolerance and optimal temperature compared with the adults, suggesting that there are ontogenetic differences in thermal tolerance for this species. This may be a result of water temperatures being more variable in nursery habitats (estuaries and intertidal rocky shore habitats) than in the adjacent marine coastal zone. Thermal performance appears to decrease above 28.0 °C for both life stages. This suggests that if ocean temperatures in this temperate region increase rapidly above 28.0 °C as a result of projected climate or heatwaves, survival of both life stages for this species may be limited. This further implies that for this family and similar warm-temperate fish species, ocean warming may result in sub-optimal conditions.

In addition, the use of the multi-method proved successful, allowing for a more refined representation of extreme tolerance limits and pejus temperatures for both juvenile and adult *D. capensis* as opposed to just using one method. Furthermore, maximum heart rate measurements using internal Star-Oddi heart rate loggers showed much promise (reliable thermal tolerance and performance indices, quick turn over time and cost effective) and should be considered in future studies to quantify the thermal physiology of marine eurythermal species. Due to the large size of the heart rate loggers, however, the use of complimentary techniques such as exterior electrodes or infrared technology (see Chapter 5) should be developed for the measurement of maximum heart rate on earlier life stages.

CHAPTER FIVE: CRITICAL THERMAL MAXIMA (CT$_{MAX}$) OF MUSSELS *PERNA PERNA* AND CRABS *PARASESARMA CATENATUM* ASSESSED USING HEART RATE MEASUREMENTS

5.1 INTRODUCTION

Intertidal macro-invertebrates are ectotherms and, as such, their body temperatures are directly influenced by external climatic factors (Helmuth *et al.*, 2002; Helmuth *et al.*, 2006). Ambient temperature, therefore, has effective direct impacts on their internal processes (physiology) (Jørgensen *et al.*, 2017) and may be the most influential external factor affecting their distribution and survival (Somero, 2002, 2005). In comparison to fish species, who have a greater locomotory capacity (in their juvenile and adult life stages) and are able to colonize different habitats to escape temperature extremes (Fangue and Bennett, 2003; Taylor *et al.*, 2005; Madeira *et al.*, 2012a), intertidal macro-invertebrates are often sedentary or sessile, forced to tidal regimes of immersion and emersion and extreme daily temperature variations. As a result, they often live close to their upper and lower thermal limits and rely mostly on morphological, physiological and behavioural adaptation in order to survive (Underwood, 1979; Pörtner and Farrell, 2008; Prusina *et al.*, 2014; Huang *et al.*, 2015). As a result, measuring the thermal tolerance and performance of intertidal macro-invertebrates from different intertidal habitats may help us understand how different species are adapted to their present day environments and to predict what the effects of projected climate change may be on their physiology.

Previous thermal tolerance studies performed on intertidal macro-invertebrates show that heart rate measurement is a fairly simple and practical technique to monitor the physiological responses under acute heating in order to determine their upper critical thermal limits (CT$_{max}$) (Stillman and Somero, 1996; Somero, 2002, 2010; Jørgensen *et al.*, 2017). This is because under acute thermal stress, the dependence on anaerobic metabolism leads to toxic build-up of anaerobic by-products (e.g. lactate) and a short supply of ATP to ion motive pumps which results in a loss of homeostatic control, degeneration of ion gradients, and eventually organ failure (e.g. heart failure) (Pörtner *et al.*, 1999; Frederich and Pörtner, 2000; Pörtner, 2002). Subsequently, heart failure has been proposed as a proximate death in some macro-invertebrate

species (e.g. crustaceans, molluscs, gastropods), and the onset of cardiac failure has been used as an estimate of CT_{max} (Stillman and Somero, 1996; Somero, 2002, 2010).

One of the first methods used to determine the onset of cardiac failure in intertidal macro-invertebrates was the measurement of changes in circulatory structures with electrical impedance. This method requires electrodes to be implanted into the pericardial cavity and although it is invasive, it is still frequently employed (Helm and Trueman, 1967; Burnett *et al.*, 2013). A more recent alternative uses an infrared light emitting diode that generates an electric signal that is electronically amplified and filtered (Depledge and Andersen, 1990; Chelazzi *et al.*, 1999). This method has been applied extensively in macro-invertebrate physiological research as it is non-invasive, allows for multiple cardiac activities to be measured simultaneously, functions in both air and water, and is able to record cardiac activity continuously for long periods of time (Burnett *et al.*, 2013).

A number of heart rate studies assessing the physiological responses to thermal stress using these above methods have been conducted on crustaceans and molluscs (e.g. DeFur and Mangum, 1978; Stillman and Somero, 1996; Chelazzi *et al.*, 1999; Santini *et al.*, 1999; Frederich and Pörtner, 2000; Rovero *et al.*, 2000; Stenseng *et al.*, 2005; Braby and Somero, 2006; Marshall *et al.*, 2011a, 2011b; Tagliarolo and McQuaid, 2015). Generally, these studies indicated that heart rate during heating ramps revealed an Arrhenius/Q_{10} breakpoint temperature below which heart rate increased linearly with temperature, and above which heart rate declined until the onset of cardiac failure, i.e. CT_{max} for a variety of crustaceans and molluscs (e.g. De Pirro *et al.*, 1999; Nicholson, 2002; Jørgensen *et al.*, 2017). This trend, however, varies depending on the heating rate used (speed of ramping ranging from 4.0 °C. h^{-1} to 10.0 °C. h^{-1}) (e.g. Stillman and Somero, 1996; Stillman, 2002; Braby and Somero, 2006; Jones *et al.*, 2009; Kuo and Sanford, 2009; Marshall *et al.*, 2011a; Tagliarolo and McQuaid, 2015), acclimation temperature (e.g. Pickens, 1965; Hicks and McMahon, 2002; Stenseng *et al.*, 2005), vertical distribution along the intertidal shore (low-shore, mid-shore, high-shore) (e.g. Pickens, 1965; Chelazzi *et al.*, 2001; Dong and Williams, 2011), and latitude/biogeographic region (e.g. Pickens, 1965; Logan *et al.*, 2012; Tagliarolo and McQuaid, 2015). In addition, both molluscs and crustaceans demonstrated a physiological strategy of either metabolic depression (bradycardia) or metabolic elevation (tachycardia) during emersion and/or immersion when thermally stressed close to their Arrhenius breakpoint temperatures

(e.g. Helm and Trueman, 1967; MacMillen and Greenaway, 1978; Stillman and Somero, 1996; De Pirro *et al.*, 1999; Guppy and Withers, 1999; Chelazzi *et al.*, 2001; Dong and Williams, 2011; Marshall *et al.*, 2011a; Prusina *et al.*, 2014; Tagliarolo and McQuaid, 2015).

Metabolic depression is a reduction in metabolic rate to below the normal resting value (Guppy and Withers, 1999; refer to Chapter 3). The extent of metabolic depression can vary remarkably for different species, from a minor lowering to 80% of resting, to commonly 5 – 40% of resting, to complete absence of measurable metabolism (i.e. cardiac arrest or acardia) and can last as long as a few hours, overnight, a season, or years (Guppy *et al.*, 1994; Guppy and Withers, 1999; Storey and Storey, 2004). Metabolic depression in some intertidal macro-invertebrates can be temperature independent, whereby energy is conserved by a neutral insensitive relationship between metabolism and temperature (Newell, 1969; Brown and Da Silva, 1979; Newell and Branch, 1980; Marshall and McQuaid, 1992; 1993; Boutet *et al.*, 2009; Marshall and McQuaid, 2011; Marshall *et al.*, 2011a) compared with the positive relationship proposed for all ectotherms in accordance with fundamental thermodynamics (Gillooly *et al.*, 2001; Dillon *et al.*, 2010). Furthermore, depressed temperature insensitive metabolism suggests that the relationship between energy gain and resting loss is different to that of the theoretical contexts for thermal adaptation (Marshall *et al.*, 2011a). The best examples of macro-invertebrates that display this zone of energy-conserving, temperature-insensitive metabolisms are bivalves and marine gastropods (Guppy *et al.*, 1994). Conversely, metabolic elevation, indicates a high metabolic rate above the normal resting value as a result of increasing temperatures (deFur and Mangum, 1978; Chelazzi *et al.*, 2001). Thermally induced metabolic elevation is thought to occur by nervous stimulation of thermoreceptors and the direct effect of temperature on the cardiomyocytes (Pickens, 1965; Trueman and Lowe, 1971) and may be achieved by means of oxidizing accumulated acids produced during anaerobiosis (repaying and oxygen debt) (Nicholson, 2002).

Other studies have also evaluated whether there are any differences in heart rate (T_{AB}/Q_{10}) under acute heating between seasons. Studies on intertidal limpets have indicated that there are no differences between summer and winter maximum heart rates suggesting an absence of seasonal metabolic compensation (Marshall and McQuaid, 1994). In contrast, a study on an intertidal crab *Pachygrapsus marmoratus* (Fabricius, 1787) found that summer heart rate

values were lower than winter heart rate values at corresponding temperature and body size, suggesting seasonal metabolic compensation (De Pirro *et al.*, 1999).

The aim of this chapter is to report on the test conducted on whether heart rate performance provides supporting evidence for other thermal tolerance methods, and to compare the physiological responses to increasing temperatures before critical temperatures are reached within intertidal macro-invertebrates occupying different habitats between summer and winter. To do this, non-invasive infrared technology combined with the dynamic method (CT_{max}) was used to measure the cardiac activity of two macro-invertebrates, namely the sedentary brown mussel *Perna perna* from the intertidal low-rocky shore and the motile crab *Parasesarma catenatum* from the lower reaches of the Kariega Estuary, until upper critical temperatures were reached.

5.2 MATERIALS AND METHODS

5.2.1 Study sites, macro-invertebrate species and collection

Adult mussels *P. perna* of 40 – 120 mm TL, from the mussel beds found on the intertidal low-shore rock pool adjacent to the Kariega and Bushman's estuaries, and adult crabs *P. catenatum*, of 15 – 25 mm TL from the salt marshes in the lower reaches of the Kariega Estuary were collected by hand and placed in plastic buckets containing aerated water from the capture site. Collections of both species were carried out in winter and summer. *Perna perna* were collected in July 2017 (winter; n = 8), August 2017 (winter; n = 12) and January 2018 (summer; n = 20). *Parasesarma catenatum* were collected in July 2017 (winter; n = 20) and January 2018 (summer; n = 20). *Perna perna* and *P. catenatum* were not separated by sex as there was no difference in thermal endpoints (CT_{max} and CT_{min}) between females and males for both summer and winter (Chapter 3).

5.2.2 Experimental setup and acclimation conditions

Immediately after collection, the specimens were transported to the AERP laboratory in Grahamstown. In the laboratory, *P. perna* and *P. catenatum* individuals were gently cleaned of any visible epibiont and acclimated for a minimum period of 36 hours in a temperature-controlled room with 12 h L/12 h D photoperiod before experimental trials began in order to

reset metabolic stability. Both adult *P. perna* and *P. catenatum* were kept in two 90 L tanks, each containing approximately eight to ten individuals at any given time for a maximum of seven days to complete all experimental trials for both seasons. Temperature (°C), oxygen (%), pH (hand-held multi-parameter probe, United Scientific, Germany) and salinity (refractometer, Hannah Instruments, Germany) were monitored every second day before trials commenced. Seawater was continuously aerated using an air compressor. Mussels were fed using a commercial phytoplankton mix (PhytoGreen-M, Brightwell Aquatics) while crabs were fed moistened cat pellets once over 12 hours. The animals were starved for 24 hours prior to experimentation. Each individual was subjected to one experimental trial.

5.2.3 Heart rate ramping procedure

The heart rate experiments were carried out by applying a non-invasive methodology developed by Depledge and Andersen (1990) and modified by Chelazzi *et al.* (1999) and Burnett *et al.* (2013), which used an infrared (IR) light emitting diode (LED; 5 mm,623 nm, 2 V, 10 mA). This diode generates an electric signal that is amplified, filtered and coupled with a phototransistor detector (IR sensor) (Burnett *et al.*, 2013) (Figure 5.1). At the beginning of each trial, three IR sensors were glued on to the shell or carapace of three individuals. For *P. perna*, the sensors were glued next to the mid-dorsal posterior hinge area, which corresponds best to the position of the heart (Burnett *et al.*, 2013; Tagliarolo and McQuaid, 2015, 2016) (Figure 5.1), and for *P. catenatum* the sensor was glued onto the centre of the posterior cephalothorax (markings on the carapace), which is dorsal to the heart (Burnett *et al.*, 2013) (Figure 5.1). The appendages of *P. catenatum* were bound prior to the attachment of the IR sensors to eliminate movement throughout the trial (Stillman and Somero, 1996).
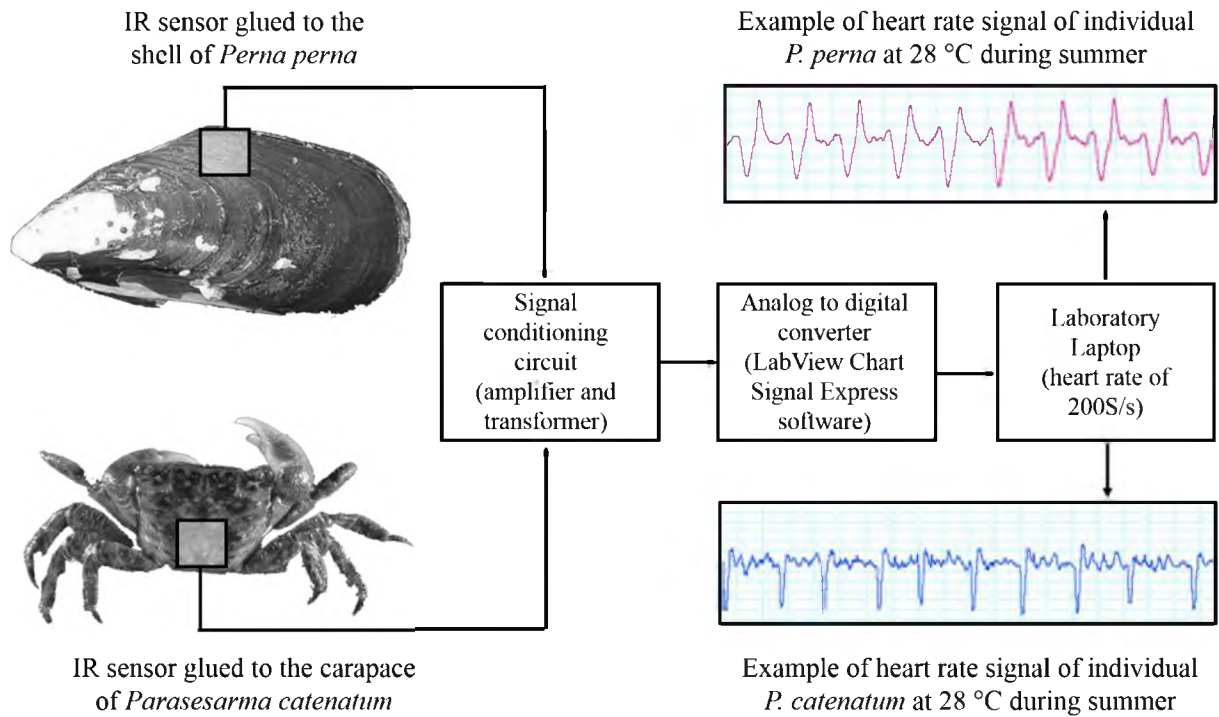
Figure 5.1. Flowchart of the heartbeat signal from the attachment of the IR sensor for *P. perna* and *P. catenatum* to the data logging device (modified from Burnett *et al.*, 2013).

Once the glue was dry and the IR sensor was securely attached, the three individuals were each placed in separate 100 ml containers containing seawater and an air stone and floated in a programmable water bath (GP 200, Grant Instruments) filled with distilled water (Figure 5.2) at the field/acclimation temperature (23 °C for summer and 17 °C for winter for both *P. perna* and *P. catenatum*) and left undisturbed for 15 minutes to allow recovery from handling before the start of each experimental trial. Preliminary tests showed that the heart rate signal stabilized 10 to 15 min after handling for both *P. perna* (in accordance with Tagliarolo and McQuaid, 2015, 2016) and *P. catenatum*.

Figure 5.2. Experimental setup for measuring heart rate activity with an increase in temperature for mussels *Perna perna* and crabs *Parasesarma catenatum* (left) whereby infrared sensors were attached to mussels/crabs and placed in 100 ml aerated containers floated in a programmable water bath (right).

At the start of each experimental trial, the three individuals were initially left to acclimate for a period of one hour at the field/acclimation temperature. Following this, temperature was then increased from the field/acclimation temperature for both species (23 °C for summer; 17 °C for winter) at a rate of 1 °C. hour^{-1} (0.02 °C. min^{-1}, in relation to the heating procedure in Chapter 3; Mora and Maya, 2006) until the CT_{max} endpoints (identified as the open gaping of mussels and a lack of righting response for crabs) were reached. Heart rate signals were recorded by the other end of the IR sensors which were connected to a recording system that included an amplifier and transformer (Powerlab 4/30, AdInstruments, Dunedin, New Zealand) and which converted voltage waveform data into visual data using the LabView Chart Signal Express (version 7.0) (http://www.ni.com/labview/signalexpress/) software with a sampling rate of 200S/s (Figure 5.1). The temperature in the water bath was monitored using a T-type thermos-couple probe connected to the recording system. Each experimental trial lasted until the CT_{max} endpoint was reached. The test individuals were then removed, measured (total length, TL in mm), weighed (whole weight in grams) and euthanized using an overdose (> 0.2 ml. L^{-1}) of clove oil. Trials were repeated on separate occasions until a sample size of $\geq$ 20 individuals per species and per season was attained.

5.2.4 Heart rate data analysis

Heart rates (HR) of individuals for both *P. perna* and *P. catenatum* were monitored continuously throughout the experimental trials using LabView software and were determined/calculated using the following steps: 1) inter-beat intervals were automatically extracted from the raw signal using global threshold detection criteria (identifies and classifies beats into a normal beat or into classes of beats based on the ECG waveform shape and temporal positions relative to surrounding beats; de Chazal and Reilly, 2003); 2) inter-beat intervals were smoothed (100 ms), filtered (2-3 Hz), auto levelled (normalize at a window of seven seconds and noise levels eliminated to 0V) and adjusted to detect minimum two-sided peak heights of 2 S.D every 10 seconds; 3) manual identification of obvious inaccurate inter-beat intervals was performed to eliminate possible errors due to movement if gone undetected by customized detection settings (step 2); and 4) remaining accurate inter-beat intervals were averaged per minute (Burnett *et al.*, 2013; Tagliarolo and McQuaid, 2015, 2016).

Heart rates (beats.min^{-1}) of individuals were then binned into 0.5 °C temperature increments, and the median heart rate value within each temperature bin was used. The maximum heart rate (HR_{max}) and corresponding temperature (T at HR_{max}) were then recorded. In addition, the temperature at which heart rate first began to decrease rapidly after HR_{max} referred to as the temperature of cardiac arrhythmia (T_{ARR}), for each individual was recorded. Arrhenius breakpoint temperatures, which represent a loss of protein function (Pörtner, 2002), were then determined (T_{AB}) from Arrhenius plots for each individual. Arrhenius plots were constructed by plotting the natural logarithm of the heart rate (ln (HR)) against the inverse of temperature in Kelvin (1000. K^{-1}). Piecewise linear regression models (Quasi-Newton estimation, STATISTICA, v. 12, Statsoft) were then fitted to the data in order to determine the breakpoint temperatures (Stillman and Somero, 1996; Tagliarolo and McQuaid, 2015; Jørgensen *et al.*, 2017). For each individual, three Arrhenius breakpoint temperatures were identified: 1) the temperature that initiated temperature-insensitive metabolism (T_{BP1}); 2) the upper limit for temperature-insensitive metabolism (T_{BP2}); and 3) the final Arrhenius breakpoint temperature (T_{AB}), above which heart rate declines with further heating (Marshall *et al.*, 2011a). Incremental Q_{10} temperature values for HR of individuals were determined for every 1 °C increase using the following equation (Aagaard, 1996; Huang *et al.*, 2015):

$$Q10 = \left(\frac{f_{H2}}{f_{H1}}\right)^{10/(T_2 - T_1)}$$

where f_{H1} and f_{H2} are heart rates at first T_1 and second T_2 temperatures, respectively. From these incremental Q_{10} values, a Q_{10} breakpoint value was determined (where two lines intersect) for each individual, using piecewise linear regression models (Quasi-Newton estimation, STATISTICA, v. 12, Statsoft). Three incremental Q_{10} temperature values which had the same value as the calculated Q_{10} breakpoint value that either fell above or below the curve were then identified as: 1) the temperature that initiated temperature sensitivity of a physiological process due to an increase by 10.0 °C (T_{QB1}); 2) the upper limit for temperature sensitivity of a physiological process due to an increase by 10.0 °C (T_{QB2}); and 3) the final incremental Q_{10} temperature value (T_{QB}), above which heart rate collapses with further warming.

Differences in HR_{max}, arrhythmia (T_{ARR}), breakpoints (T_{BP1}, T_{BP2}, T_{AB}) and incremental Q_{10} (T_{QB1}, T_{QB2}, T_{QB}) values between summer and winter were tested using t-tests. These analyses were performed in SigmaPlot 12.5. Normality of distributions were tested using a Shapiro-Wilk test and homoscedasticity was further tested using the Levene's test. When normality and homogeneity assumptions were not met, Mann-Whitney U tests were performed in place of the parametric tests. Statistical significance for all analyses was set at $\alpha=0.05$.

5.3 RESULTS

5.3.1 Mussels (*Perna perna*)

5.3.1.1 Methodological efficacy

In summer, measurements from 17 *P. perna* individuals showed a heart rate (metabolism) that was either positively or neutrally (positive gradient, however, the value is small) related to an increase in temperature (Figure 5.3). The heart rate of one individual had a negative relationship with an increase in temperature and two more were considered unsuccessful as a result of irregular or extremely high/low heart rates, thought to be a result of a technical malfunction (Figure 5.4). In winter, measurements from 16 individuals had a heart rate that was either positively or neutrally related to an increase in temperature (Figure 5.5). Three individuals, conversely, were considered unsuccessful as they both had irregular heart rate recordings and a negative relationship with an increase in temperature, while one individual was unsuccessful as a result of irregular heart rate recordings (Figure 5.6). As a result, for both summer and winter, individuals with a heart rate that was neutrally or positively related with an increase in temperature were used for the evaluation of breakpoint temperatures (T_{AB}/T_{QB}) due to a larger sample size.
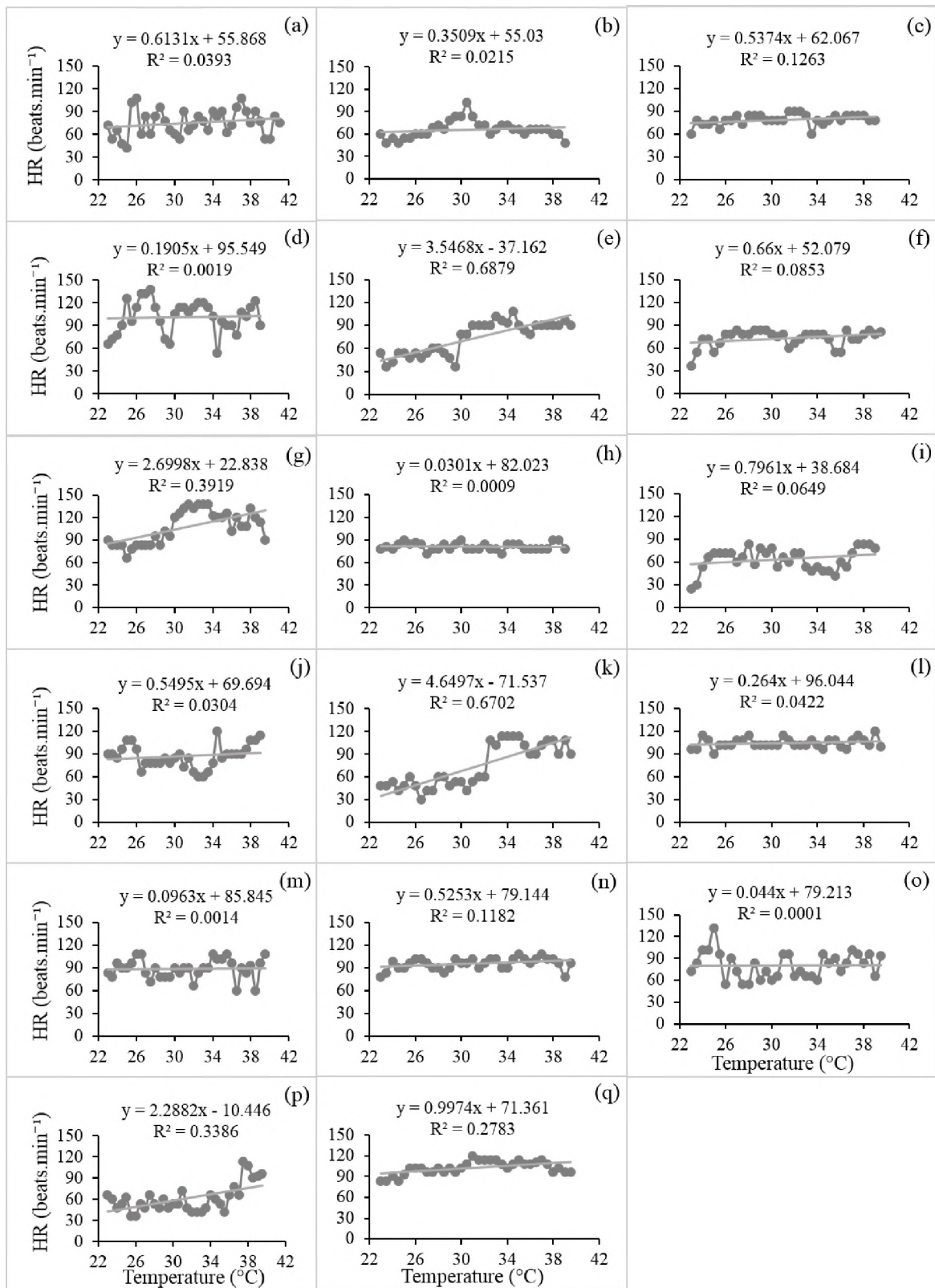
Figure 5.3. Heart rates (HR - binned into 0.5 °C temperature increments) of 17 individual mussels *Perna perna* in summer (a) – (q) that had a positive or neutral response to an increase in water temperature from 23 °C until an endpoint was reached (open gaping mussels). Linear regression represented by a light grey solid trend line.

117
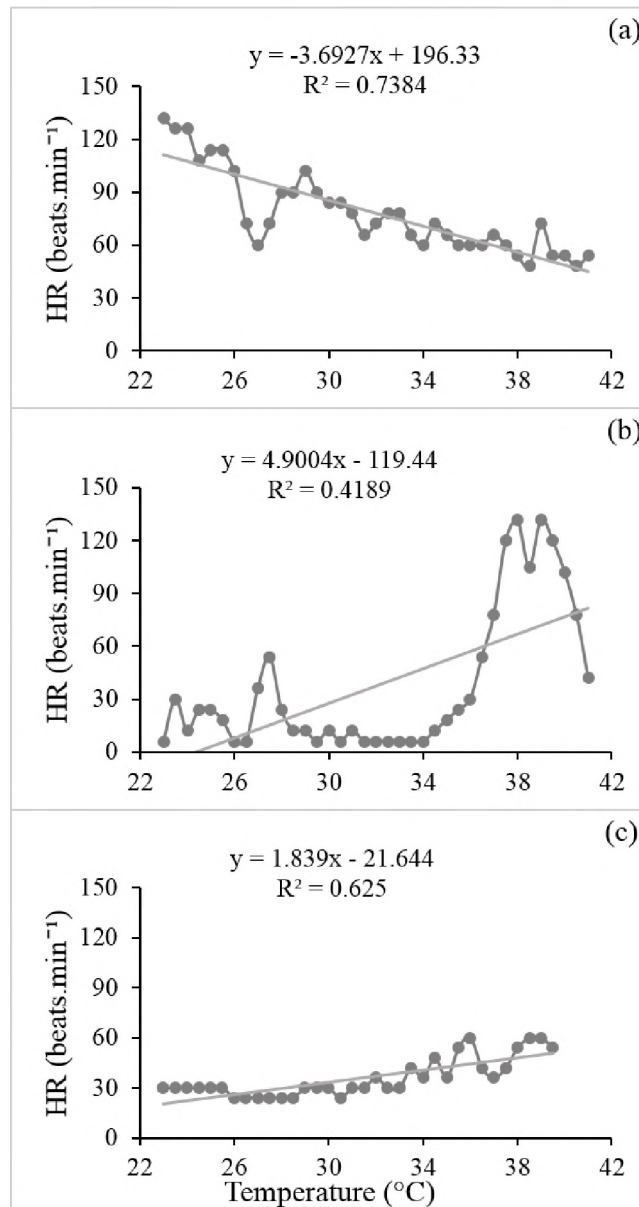
Figure 5.4. Heart rates (HR - binned into 0.5 °C temperature increments) of three individual mussels *Perna perna* in summer (a) – (c) that had unsuccessful/irregular heart rate recordings (b and c) or a negative response to an increase in water temperature (a) from 23 °C until an endpoint was reached (open gaping mussels). Linear regression represented by a light grey solid trendline.
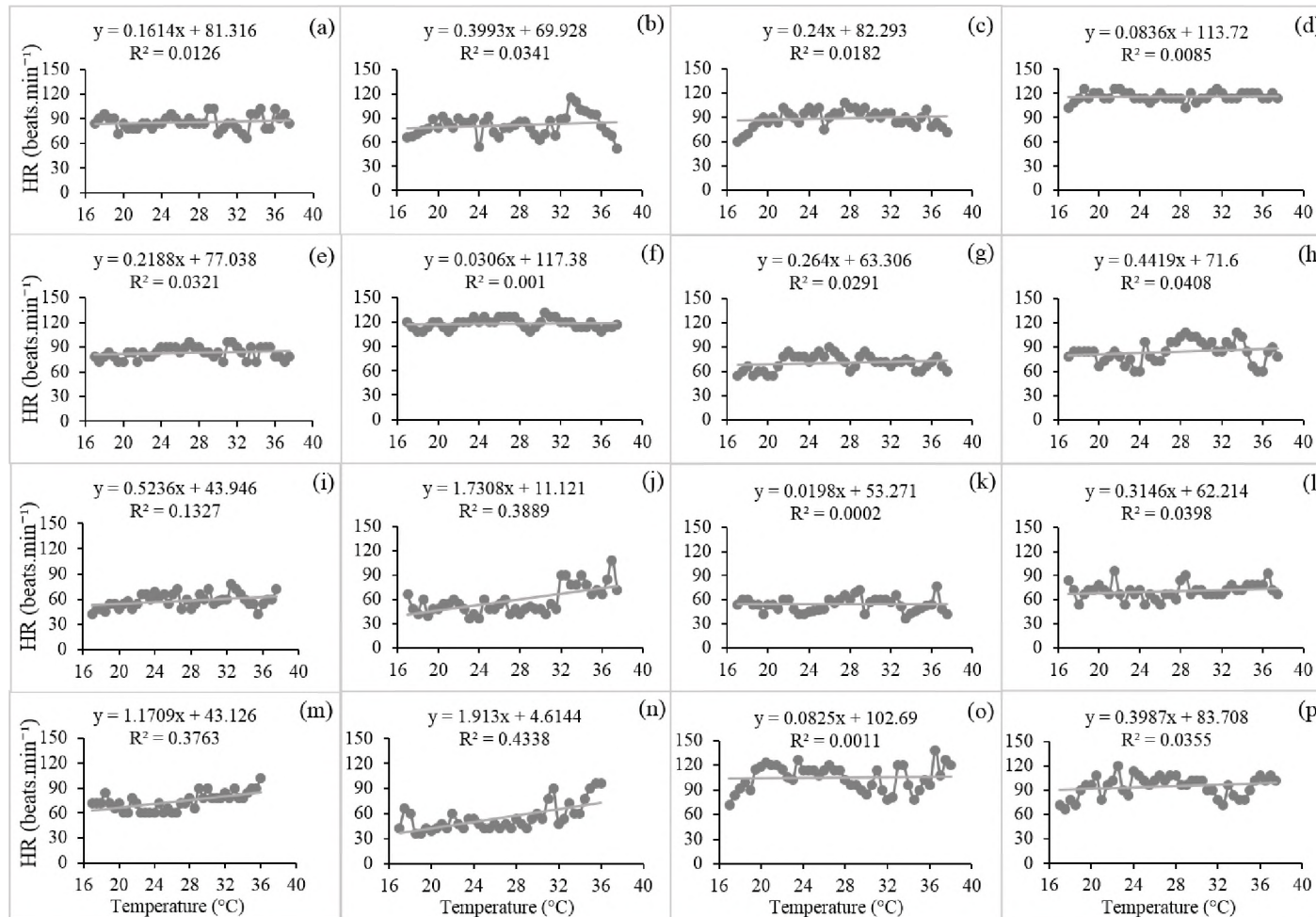
Figure 5.5. Heart rates (HR - binned into 0.5 °C temperature increments) of 16 individual mussels *Perna perna* in winter (a) – (p) that had a positive or neutral response to an increase in water temperature from 17.0 °C until an endpoint was reached (open gapping mussels). Linear regression represented by a light grey solid trendline.
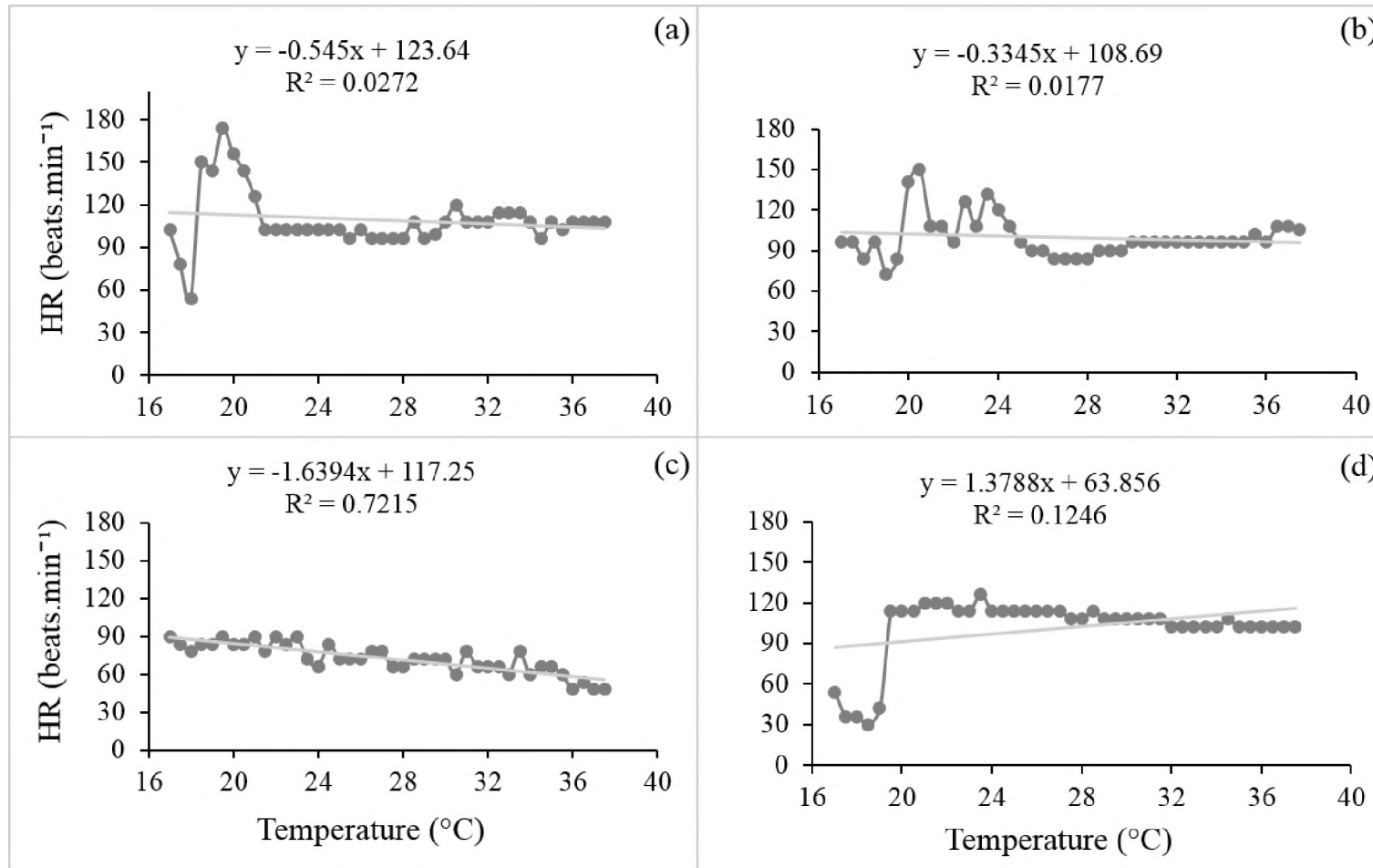
Figure 5.6. Heart rates (HR - binned into 0.5 °C temperature increments) of four individual mussels *Perna perna* in winter (a) – (d) that had unsuccessful/irregular heart rate recordings and a negative response to an increase in water temperature (a – c) or just unsuccessful heart rate recordings (d) from 17.0 °C until an endpoint was reached (open gapping mussels). Linear regression represented by a light grey solid trendline.

5.3.1.2 Effect of temperature ramping on HR

Heart rates of *P. perna* in response to an increase in water temperature resulted in high individual variation for summer and winter (Figure 5.7). Heart rate peaked between a range of $66 - 132$ beats.min^{-1} and $60 - 138$ beats.min^{-1} at temperatures ranging between 37.0 to 39.0 °C in summer and 35.5 to 37.0 °C in winter (Figure 5.7, Table 5.1, Table 5.2). Heart rates of individuals declined at temperatures above the maximum heart rate (Figure 5.7). The temperature at maximum heart rates (T at HR_{max}) were significantly different between summer and winter (Mann-Whitney U-test; U (31) = 9.0, p = < 0.01). Temperatures that triggered cardiac arrhythmia (T_{ARR}) ranged between 37.5 to 39.5 °C in summer (Figure 5.7, Table 5.1) and 36.0 to 37.5 °C in winter (Figure 5.7, Table 5.2). The summer and winter T_{ARR} differed significantly from one another (Mann-Whitney U-test; U (31) = 9.0, p = < 0.01) with higher values in summer.

Individual piecewise linear regression models for the 17 *P. perna* for summer gave an initial temperature insensitive metabolism breakpoint temperature (T_{BP1}) that ranged between $24.4 - 27.5$ °C, an upper limit for temperature insensitive metabolism breakpoint temperature (T_{BP2}) between $29.0 - 33.0$ °C, and a final Arrhenius breakpoint temperature (T_{AB}; heart rate declines with further heating) between $34.0 - 37.0$ °C (Figure 5.8, Table 5.1). For winter, individual piecewise linear regression models for 16 *P. perna* gave a T_{BP1} that ranged between $20.0 - 24.0$ °C, T_{BP2} between $25.0 - 30.5$ °C and a T_{AB} between $33.0 - 36.0$ °C (Figure 5.8, Table 5.2). Between summer and winter, the T_{BP1} (T-test; T (31) = 13.1, p = < 0.01), T_{BP2} (T-test; T (31) = 4.0, p = < 0.01), and T_{AB} (Mann-Whitney U-test, U (31) = 55.0, p = < 0.01) were significantly different, with summer being higher than winter for all breakpoint indicators.

The incremental Q_{10} for HR of *P. perna* showed high individual variation and oscillated with an increase in temperature for both seasons and then decreased sharply around 38.0 °C for summer and 37.0 °C for winter (Figure 5.9). For summer, incremental Q_{10} values for the 17 *P. perna* yielded a T_{QB1} range between 24.0 - 28.0 °C, a T_{QB2} between $29.0 - 32.0$ °C and a T_{QB} between $33.0 - 37.0$ °C (Figure 5.9, Table 5.1). For winter, incremental Q_{10} values for the 16 *P. perna* yielded a T_{QB1} range between $20.0 - 23.0$ °C, a T_{QB2} between $24.0 - 31.0$ °C and a T_{QB} between $33.0 - 36.0$ °C (Figure 5.9, Table 5.2). Between summer and winter, the T_{QB1} (Mann-Whitney U-test; U (31) = 0.0, p = <0.01), T_{QB2} (T-test; T (31) = 3.1, p = < 0.01), and

$_{\text{TQB}}$ (Mann-Whitney U-test; $U(31) = 80.5$, $p = 0.04$) was significantly different, with summer being higher than winter.

Figure 5.7. Heart rates (HR) of individual mussel *Perna perna* in response to an increase in water temperature from 23.0 °C in (a) summer and 17.0 °C in (b) winter. Solid black lines represent the average HR for summer (n = 17) and winter (n = 16). Blue shaded blocks represent the range of temperatures occurring at the maximum heart rate (T at HR_{max}) for *P. perna* individuals for both summer (37.0 – 39.0 °C) and winter (35.5 – 37.0 °C). Red shaded blocks represent the range of arrhythmic temperatures (T_{ARR}) for *P. perna* individuals for both summer (37.5 – 39.5 °C) and winter (36.0 – 37.5 °C).
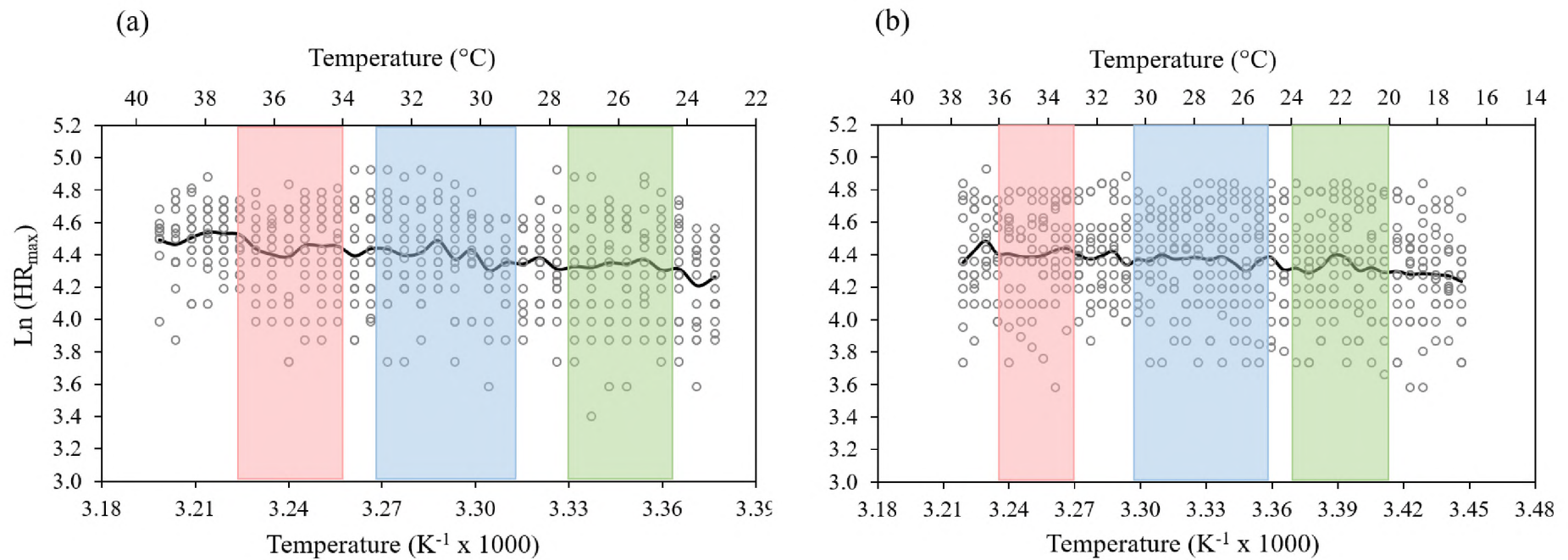
Figure 5.8. Arrhenius plots of the natural log of heart rate (ln (HR)) against the inverse temperature in Kelvin (1000. K^{-1}) for the mussel *Perna perna* for (a) summer and (b) winter. Solid black lines represent the average Ln (HR$_{max}$) for summer (n = 17) and winter (n = 16). Green shaded blocks represent the range of the first temperatures that initiated a temperature insensitive metabolism (T$_{BP1}$) for summer (24.5 – 27.5 ⁰C) and winter (20.0 – 24.0 ⁰C). Blue shaded blocks represent the range of the upper limit temperatures for a temperature insensitive metabolism (T$_{BP2}$) for summer (29.0 – 33.0 ⁰C) and winter (25.0 – 30.5 ⁰C). Red shaded blocks represent the range of the final Arrhenius breakpoint temperatures (T$_{AB}$) for summer (34.0 – 37.0 ⁰C) and winter (33.0 – 36.0 ⁰C).

124
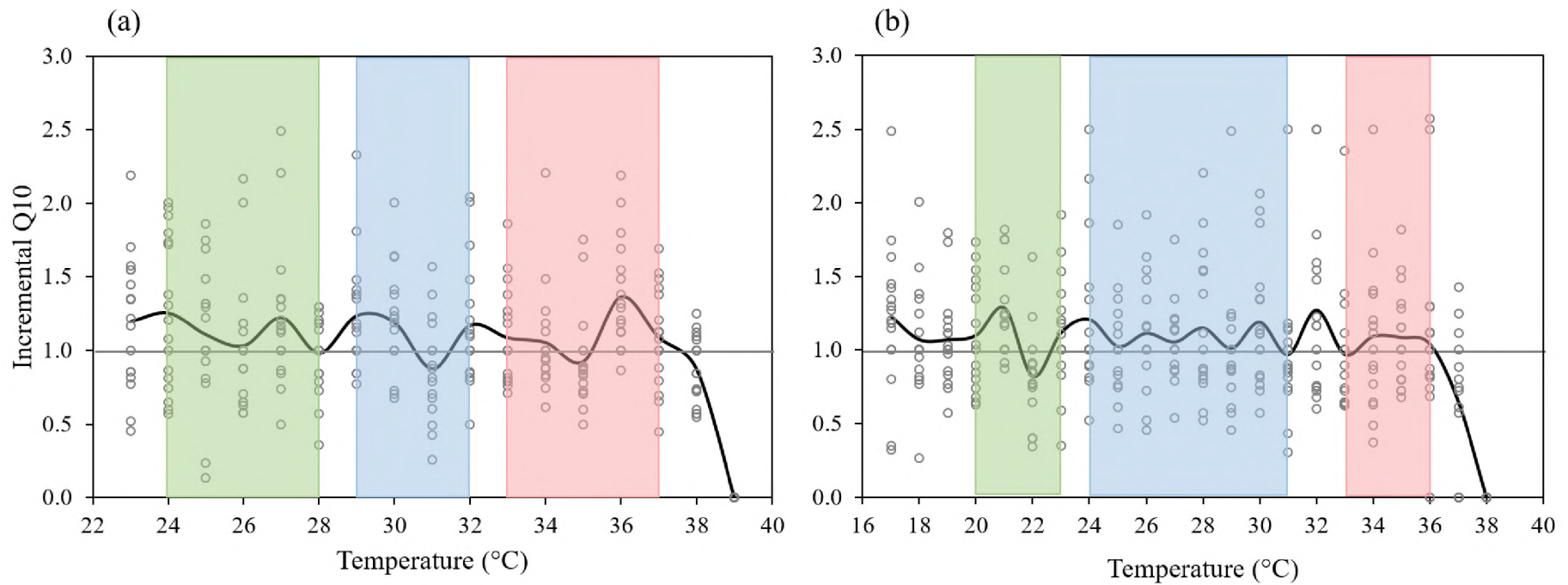
Figure 5.9. Incremental Q_{10} of heart rates (HR) for 1.0 °C increments for *Perna perna* for both (a) summer and (b) winter. Solid black lines represent the average incremental Q_{10} for summer (n = 17) and winter (n = 16). Green shaded blocks represent the range of the first temperatures that initiated temperature sensitivity of a physiological process due to an increase of 10.0 ºC (T_{QB1}) for summer (24.0 – 28.0 ºC) and winter (20.0 – 23.0 ºC). Blue shaded blocks represent the ranges of the upper limits for temperature sensitivity of a physiological process due to an increase by 10.0 ºC (T_{QB2}) for summer (29.0 – 32.0 ºC) and winter (24.0 – 31.0 ºC). Red shaded blocks represent the ranges of the final incremental Q_{10} temperature values (T_{QB}), above which heart rate collapses with further warming for summer (33.0 – 37.0 ºC) and winter (33.0 – 36.0 ºC). Horizontal grey solid line represents the average Q_{10} breakpoint temperature ($Q_{10} < 1.0$).

Table 5.1. Mass, length and HR values for individual mussels *Perna perna* in response to acute warming for summer. HR index values include the Arrhenius breakpoint temperatures (T_{BP1}, T_{BP2}, T_{AB}), the Q_{10} breakpoint temperatures (T_{QB1}, T_{QB2}, T_{QB}), HR_{max} (beats.min^{-1}) and cardiac arrhythmia temperature (T_{ARR}) beyond T_{AB} and T_{QB}.

perna perna	mass (g)	Length (mm TL)	T_{BP1}	T_{BP2}	T_{AB}	T_{QB1}	T_{QB2}	T_{QB}	Q_{10} breakpoint	HR_{max} (beats.min^{-1}) beyond T_{AB}	T at HR_{max} (beyond T_{AB})	T_{ARR}
1	12.90	48	25.0	30.5	36.0	27.0	30.0	35.0	1.0	90	38.5	39.0
2	14.43	62	27.0	33.0	35.5	26.0	30.0	36.0	0.9	66	37.5	38.0
3	9.36	52	26.0	31.0	36.0	28.0	32.0	37.0	1.0	84	38.0	38.5
4	15.56	62	25.5	30.0	35.0	25.0	31.0	35.0	1.0	123	38.5	39.0
5	11.96	52	25.5	29.5	36.0	26.0	31.0	37.0	1.1	96	39.0	39.5
6	9.69	45	25.5	31.5	36.0	26.0	30.0	34.0	1.0	84	38.5	39.0
7	14.14	59	25.0	29.5	36.0	26.0	31.0	36.0	1.0	132	38.0	38.5
8	9.02	41	27.0	30.5	36.0	25.0	31.0	36.0	1.0	90	38.5	39.0
9	20.26	69	27.0	30.5	36.0	25.0	29.0	33.0	1.1	84	38.5	39.0
10	25.30	77	26.5	30.0	35.0	27.0	32.0	36.0	1.0	108	38.0	38.5
11	14.44	58	26.5	31.5	36.0	25.0	29.0	34.0	1.1	108	38.0	38.5
12	12.95	60	25.0	30.5	36.5	26.0	29.0	37.0	1.0	120	39.0	39.5
13	6.27	42	27.5	32.0	37.0	27.0	30.0	34.0	1.0	96	39.0	39.5
14	18.31	69	25.0	29.0	36.0	26.0	31.0	35.0	1.0	108	37.0	37.5
15	14.89	53	26.0	30.5	36.0	27.0	32.0	35.0	1.2	102	37.0	37.5
16	14.41	53	25.5	30.5	35.5	24.0	29.0	34.0	1.2	114	37.5	38.0
17	19.69	65	24.5	30.0	34.0	26.0	29.0	35.0	1.0	114	37.0	37.5
Average	14.33	57	25.9	30.6	35.8	26.0	30.4	35.2	1.0	101	38.1	38.6
SD	4.68	10	0.9	1.0	0.7	1.0	1.1	1.2	0.1	17	0.7	0.7

Table 5.2. Mass, length and HR values for individual mussels *Perna perna* in response to acute warming for winter. HR index values include the Arrhenius breakpoint temperatures (T_{BP1}, T_{BP2}, T_{AB}), the Q_{10} breakpoint temperatures (T_{QB1}, T_{QB2}, T_{QB}), HR_{max} (beats.min^{-1}) and cardiac arrhythmia temperature (T_{ARR}) beyond T_{AB} and T_{QB}.

Perna perna	mass (g)	Length (mm TL)	T_{BP1}	T_{BP2}	T_{AB}	T_{QB1}	T_{QB2}	T_{QB}	Q_{10} breakpoint	HR_{max} (beats.min^{-1}) beyond T_{AB}	T at HR_{max} (beyond T_{AB})	T_{ARR}
1	8.08	49	20.0	30.0	35.5	22.0	31.0	36.0	1.0	96	37.0	37.5
2	17.58	60	24.0	30.5	36.0	22.0	28.0	34.0	1.1	72	36.5	37.0
3	8.66	48	21.0	25.0	36.0	20.0	24.0	36.0	1.0	100	35.5	36.0
4	11.44	55	21.0	30.5	34.0	20.0	31.0	34.0	1.0	120	37.0	37.5
5	20.86	66	22.0	30.0	34.0	21.0	29.0	33.0	1.0	90	35.5	36.0
6	13.00	56	21.5	29.5	34.0	22.0	31.0	34.0	1.0	114	37.0	37.5
7	12.13	50	21.0	28.5	35.5	22.0	29.0	33.0	1.0	78	36.5	37.0
8	17.37	67	21.0	26.5	34.5	21.0	30.0	35.0	1.0	90	37.0	37.5
9	13.69	54	21.5	28.0	34.5	21.0	27.0	35.0	1.1	60	36.5	37.0
10	22.47	78	21.0	26.0	36.0	21.0	27.0	35.0	1.2	108	37.0	37.5
11	32.06	95	22.5	29.5	33.0	23.0	27.0	33.0	1.0	76	36.5	37.0
12	27.28	81	21.0	30.5	36.0	21.0	31.0	35.0	1.0	93	36.5	37.0
13	22.85	68	21.5	28.0	34.0	23.0	29.0	33.0	1.0	102	36.0	36.5
14	20.86	66	21.5	27.0	34.0	22.0	26.0	33.0	1.3	96	35.5	36.0
15	31.60	74	22.5	29.5	34.0	20.0	29.0	34.0	1.1	138	36.5	37.0
16	16.15	59	23.0	29.0	35.0	22.0	29.0	36.0	1.0	108	37.0	37.5
Average	18.51	64	21.6	28.6	34.8	21.4	28.6	34.3	1.1	96	36.5	37.0
SD	7.46	13	1.0	1.7	1.0	1.0	2.0	1.1	0.1	19	0.6	0.6

5.3.2 Crabs (*Parasesarma catenatum*)

5.3.2.1 Methodological efficacy

In summer, measurements from 15 *P. catenatum* individuals showed a heart rate (metabolism) that was either positively or neutrally related to an increase in temperature (Figure 5.10). Heart rates of two individuals had a negative relationship with an increase in temperature, two individuals were considered unsuccessful as a result of irregular heart rate recordings, and one individual had both irregular heart rate recordings and a negative relationship with temperature (Figure 5.11). In winter, measurements from 18 individuals showed a heart rate that was either positively or neutrally related to an increase in temperature (Figure 5.12) and two individuals had a heart rate that was negatively related to an increase in temperature (Figure 5.13). As a result, for both summer and winter, individuals with a heart rate that was neutrally or positively related with an increase in temperature were used for the evaluation of breakpoint temperatures (T_{AB}/T_{QB}) due to a larger sample size.
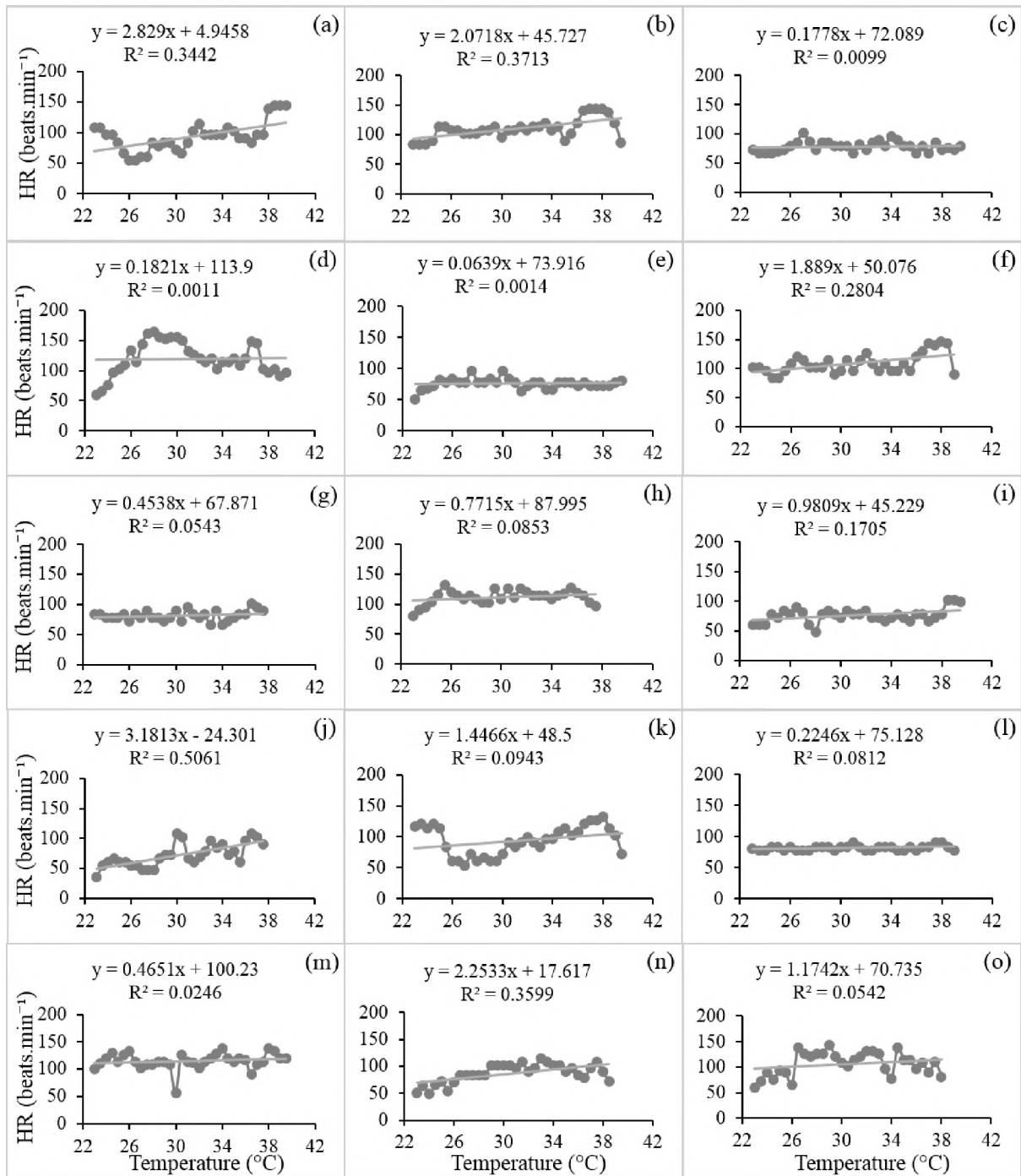
Figure 5.10. Heart rates (HR - binned into 0.5 °C temperature increments) of 15 individual crabs *Parasesarma catenatum* in summer (a) – (o) that had a positive or neutral response to an increase in water temperature from 23.0 °C until an endpoint was reached (no righting response). Linear regression represented by a light grey solid trendline.
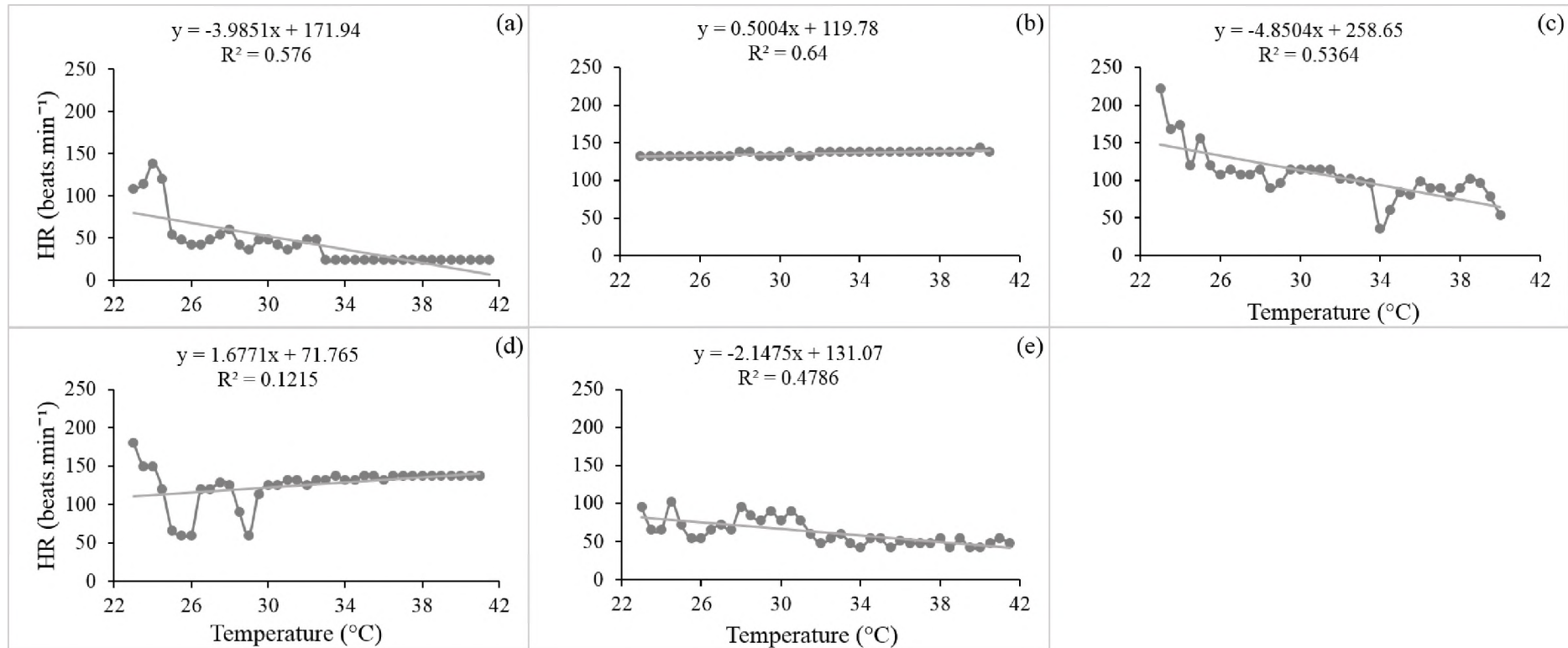
Figure 5.11. Heart rates (HR - binned into 0.5 °C temperature increments) of five individual crabs *Parasesarma catenatum* in summer (a) – (e) that had unsuccessful/irregular heart rate recordings and a negative response to an increase in water temperature (a), unsuccessful/irregular heart rate recordings (b and d) or heart rates negatively related (c and e) to an increase in water temperature from 23.0 °C until an endpoint was reached (no righting response). Linear regression represented by a light grey solid trendline.
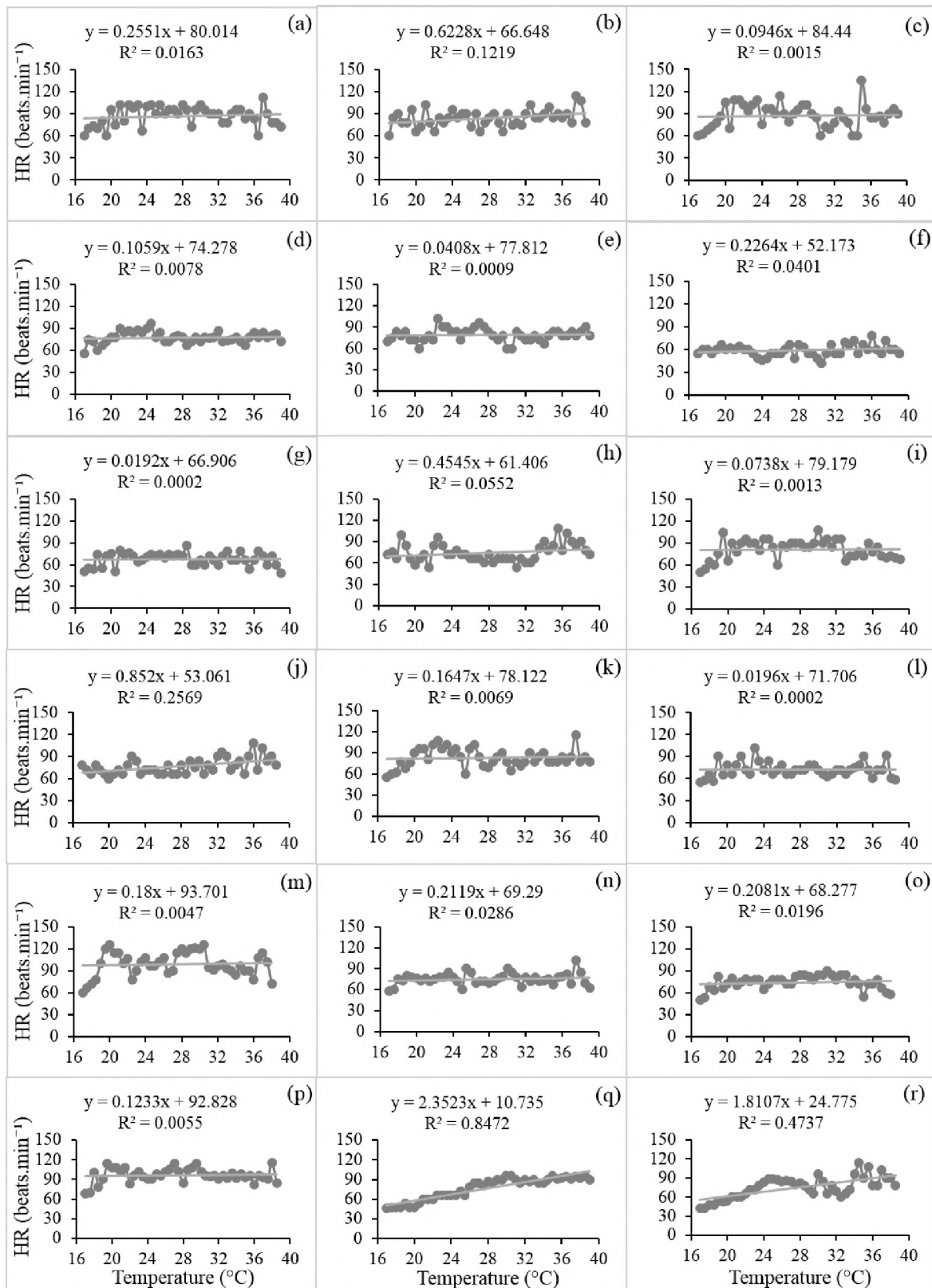
Figure 5.12. Heart rates (HR - binned into 0.5 °C temperature increments) of 18 individual crabs *Parasesarma catenatum* in winter (a) – (r) that had a positive or neutral response to an increase in water temperature from 17°C until an endpoint was reached (no righting response). Linear regression represented by a light grey solid trendline.

131
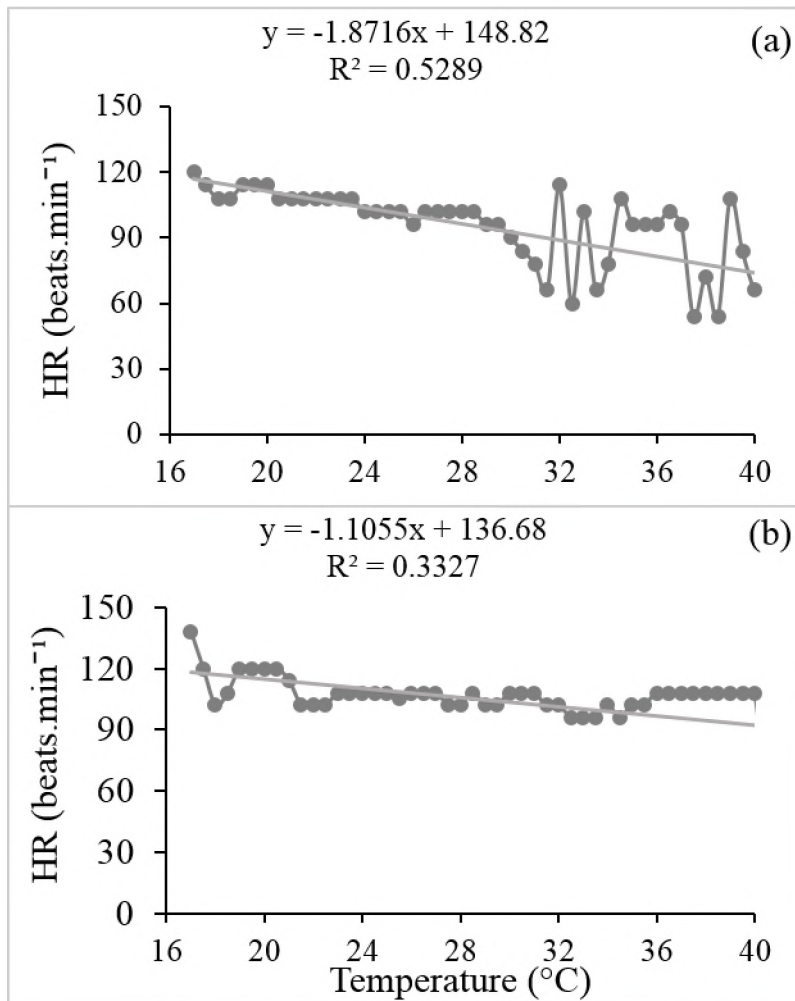
Figure 5.13. Heart rates (HR - binned into 0.5 °C temperature increments) of two individual crabs *Parasesarma catenatum* in winter (a) and (b) that had a negative response to an increase in water temperature from 17.0 °C until an endpoint was reached (no righting response). Linear regression represented by a light grey solid trendline.

5.3.2.2 Effect of temperature ramping on HR

Heart rates of *P. catenatum* in response to an increase in water temperature resulted in high individual variation (Figure 5.14). Heart rate peaked between a range of 75 - 144 beats.min^{-1} and 60 - 115 beats.min^{-1} between 37.0 – 39.0 °C and 34.5 - 38.5 °C in summer and winter, respectively (Figure 5.14, Table 5.3, 5.4). Temperature at maximum heart rate (HR$_{max}$) of the individuals did differ significantly between summer and winter (Mann-Whitney U-test; U (31) = 63.0; p = 0.01). Temperatures that triggered cardiac arrhythmia (T$_{ARR}$) ranged between 37.5 - 39.5 °C in summer and this was significantly different (Mann-Whitney U-test; U = (31) = 62.0, p = 0.01) to T$_{ARR}$ in winter which ranged between 35.0 – 39.0 ± 1.2 °C SD) (Figure 5.14, Table 5.3, 5.4).

Individual piecewise linear regression models for the 15 *P. catenatum* for summer gave an initial temperature insensitive metabolism breakpoint temperature (T$_{BP1}$) that ranged between 25.0 - 28.5 °C, an upper limit for temperature insensitive metabolism breakpoint temperature (T$_{BP2}$) between 28.5 - 31.5 °C, and an Arrhenius breakpoint temperature (T$_{AB}$; heart rate declines with further heating) between 34.5 - 37.0 °C (Figure 5.15, Table 5.3). For winter, individual piecewise linear regression models for 18 *P. catenatum* gave a T$_{BP1}$ that ranged between 20.0 - 24.5 °C, T$_{BP2}$ between 27.0 – 31.5 °C and a T$_{AB}$ between 33.5 - 37.0 °C (Figure 5.15, Table 5.4). Between summer and winter, the T$_{BP1}$ (T-test; T (31) = 11.1, p = < 0.01) and T$_{BP2}$ (T-test; T (31) = 3.31, p = < 0.01) was significantly different, with summer being higher than winter. For T$_{AB}$, however, there was no significant difference between summer and winter (T-test; T (31) = 1.29, p = 0.21).

The incremental Q$_{10}$ for HR$_{max}$ of *P. catenatum* showed high individual variation and oscillated with an increase in temperature for both seasons and then decreased sharply around 37.0 °C for both summer and winter (Figure 5.16). For summer, 15 *P. catenatum* gave a T$_{QB1}$ that ranged between 25.0 - 28.0 °C, a T$_{QB2}$ between 29.0 - 33.0 °C and a T$_{QB}$ between 33.0 - 38.0 °C (Figure 5.16, Table 5.3). For winter, 18 *P. catenatum* gave a T$_{QB1}$ that ranged between 20.0 - 24.0 °C, a T$_{QB2}$ between 28.0 - 32.0 °C and a T$_{QB}$ between 33.0 - 37.0 °C (Figure 5.16, Table 5.4). Between summer and winter, T$_{QB1}$ (Mann-Whitney U-test; U (31) = 0.00, p = <0.01) was significantly different, with summer being higher than winter. For both T$_{QB2}$ (T-test; T (31) =

1.63, $p = 0.11$) and $_{TQB}$ (Mann-Whitney U-test; $U(31) = 112.0$, $p = 0.39$), however, there was no significant difference between summer and winter.
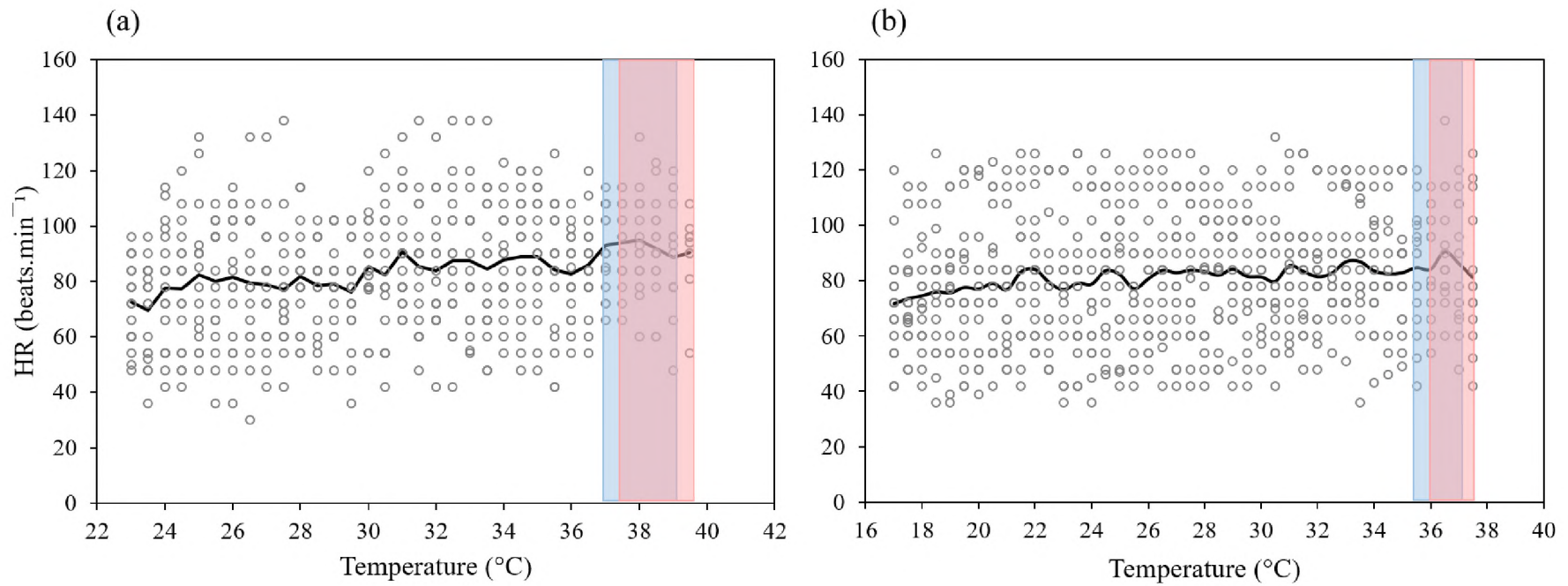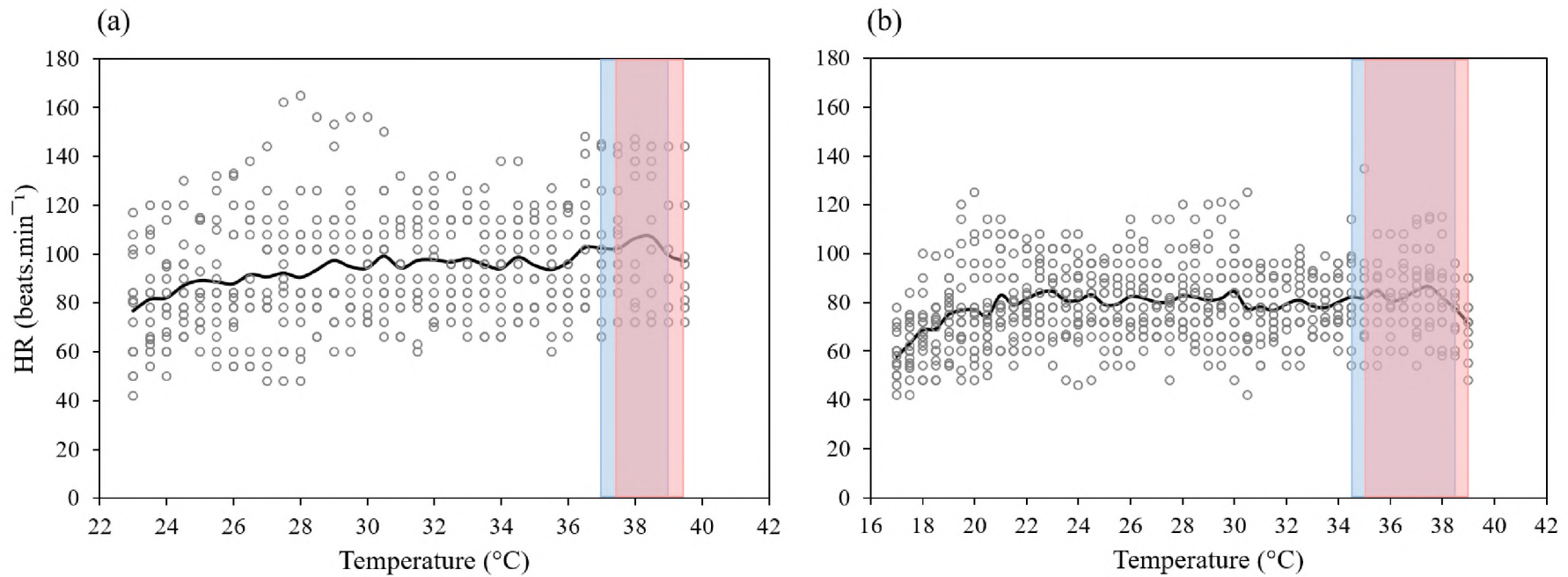
Figure 5.14. Heart rates (HR) of individual crab *Parasesarma catenatum* in response to an increase in water temperature from 23 °C in (a) summer and 17 °C in (b) winter. Solid black lines represent the average HR for summer (n = 15) and winter (n = 18). Blue shaded blocks represent the range of temperatures occurring at the maximum heart rate (T at HR_{max}) for *P. catenatum* individuals for both summer (37.0 – 39.0 °C) and winter (34.5 – 38.5 °C). Red shaded blocks represent the range of arrhythmic temperatures (T_{ARR}) for *P. catenatum* individuals for both summer (37.5 – 39.5 °C) and winter (35.0 – 39.0 °C).
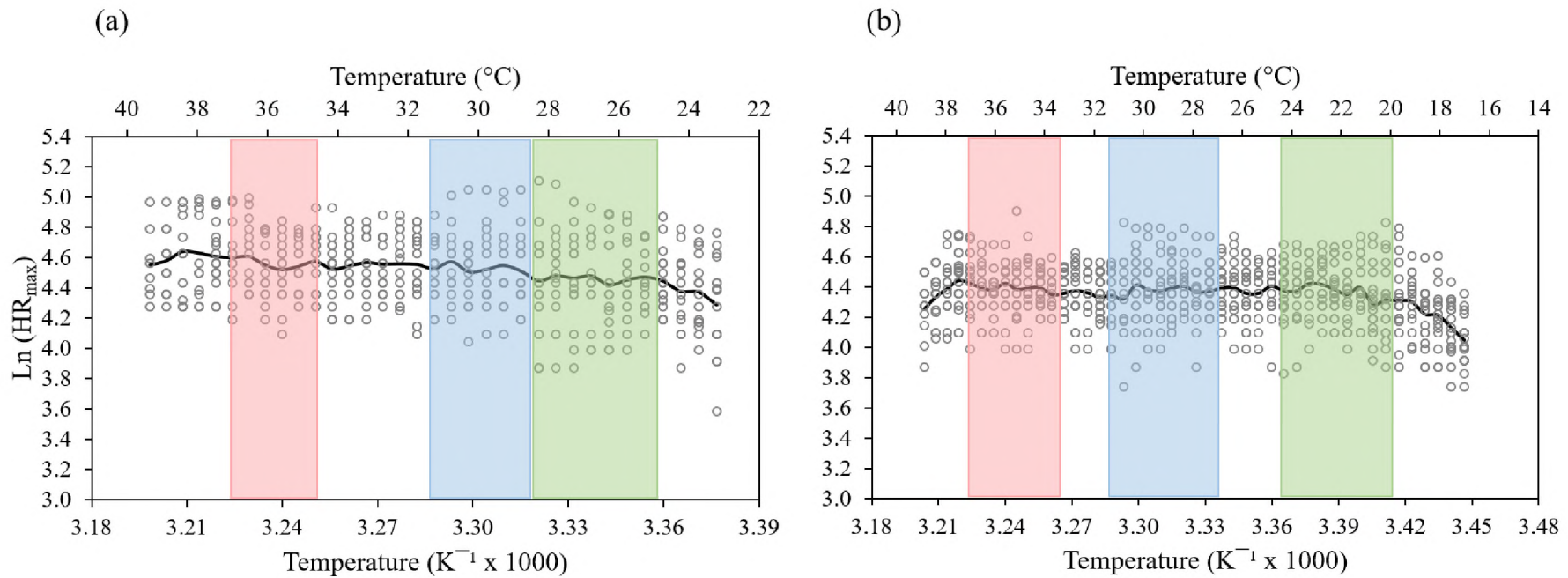
Figure 5.15. Arrhenius plots of the natural log of heart rate (ln (HR)) against the inverse temperature in Kelvin (1000. K^{-1}) for the crab *Parasesarma catenatum* for (a) summer and (b) winter. Solid black lines represent the average Ln (HR_{max}) for summer (n = 15) and winter (n = 18). Green shaded blocks represent the range of the first temperatures that initiated a temperature insensitive metabolism (T_{BP1}) for summer (25.0 – 28.5 °C) and winter (20.0 – 24.5 °C). Blue shaded blocks represent the range of the upper limit temperatures for a temperature insensitive metabolism (T_{BP2}) for summer (28.5 – 31.5 °C) and winter (27.0 – 31.5 °C). Red shaded blocks represent the range of the final Arrhenius breakpoint temperatures (T_{AB}) for summer (34.5 – 37.0 °C) and winter (33.5 – 37.0 °C).
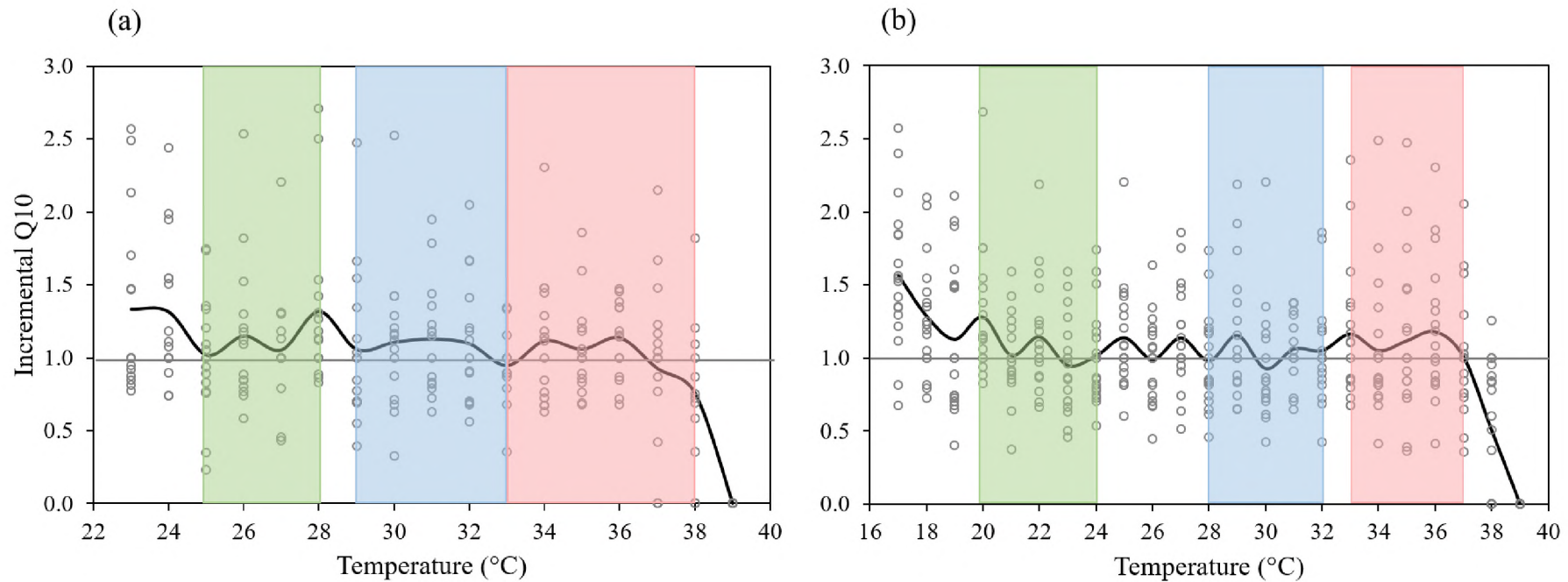
Figure 5.16. Incremental Q_{10} of heart rates (HR) for 1.0 °C increments for *Parasesarma catenatum* for both (a) summer and (b) winter. Solid black lines represent the average incremental Q_{10} for summer (n = 15) and winter (n = 18). Green shaded blocks represent the range of the first temperatures that initiated temperature sensitivity of a physiological process due to an increase of 10.0 °C (T_{QB1}) for summer (25.0 – 28.0 °C) and winter (20.0 – 24.0 °C). Blue shaded blocks represent the ranges of the upper limits for temperature sensitivity of a physiological process due to an increase by 10.0 °C (T_{QB2}) for summer (29.0 – 33.0 °C) and winter (28.0 – 32.0 °C). Red shaded blocks represent the ranges of the final incremental Q_{10} temperature values (T_{QB}), above which heart rate collapses with further warming for summer (33.0 – 38.0 °C) and winter (33.0 – 37.0 °C). Horizontal grey solid line represents the average Q_{10} breakpoint temperature ($Q_{10} < 1.0$).

Table 5.3. Mass, length and HR values for individual crabs *Parasesarma catenatum* in response to acute warming for summer. HR index values include the Arrhenius breakpoint temperatures (T_{BP1}, T_{BP2}, T_{AB}), the Q_{10} breakpoint temperatures (T_{QB1}, T_{QB2}, T_{QB}), HR_{max} (beats.min^{-1}) and cardiac arrhythmia temperature (T_{ARR}) beyond T_{AB} and T_{QB}.

Parasesarma catenatum	mass (g)	Length (mm TL)	T_{BP1}	T_{BP2}	T_{AB}	T_{QB1}	T_{QB2}	T_{QB}	Q_{10} breakpoint	HR_{max} (beats.min^{-1}) beyond T_{AB}	T at HR_{max} (beyond T_{AB})	T_{ARR}
1	6.22	23	25.0	31.0	36.0	28.0	33.0	38.0	1.0	144	39.0	39.5
2	2.16	17	26.0	31.0	35.5	27.0	31.0	37.0	1.0	120	39.0	39.5
3	1.83	17	26.0	30.5	36.5	25.0	31.0	36.0	1.0	75	38.5	39.0
4	1.88	16	26.5	32.5	36.0	26.0	29.0	35.0	1.1	90	39.0	39.5
5	4.13	19	25.5	31.0	35.5	25.0	31.0	35.0	1.1	78	39.0	39.5
6	2.62	19	26.0	30.5	35.0	26.0	30.0	37.0	1.0	144	38.5	39.0
7	2.18	17	25.5	29.5	35.0	27.0	30.0	33.0	1.0	96	37.0	37.5
8	3.42	20	26.5	30.0	34.5	27.0	30.0	35.0	1.0	102	37.0	37.5
9	4.26	19	27.0	30.0	36.5	26.0	30.0	35.0	1.1	102	39.0	39.5
10	4.45	20	28.5	31.5	35.5	27.0	31.0	36.0	1.3	102	37.0	37.5
11	2.08	15	25.5	31.5	35.5	28.0	32.0	35.0	1.0	102	39.0	39.5
12	2.61	18	25.5	31.5	36.5	26.0	31.0	34.0	0.9	84	38.5	39.0
13	2.28	18	28.0	32.0	37.0	28.0	31.0	35.0	1.0	120	39.0	39.5
14	2.21	18	25.5	28.5	36.0	27.0	31.0	35.0	1.1	90	38.0	38.5
15	2.01	17	26.0	30.5	36.0	27.0	30.0	35.0	1.1	110	37.5	39.0
Average	2.96	18.20	26.2	30.8	35.8	26.7	30.7	35.4	1.0	104	38.3	38.8
SD	1.27	1.93	1.0	1.0	0.7	1.0	1.0	1.2	0.1	21	0.8	0.8

Table 5.4. Mass, length and HR values for individual crabs *Parasesarma catenatum* in response to acute warming for winter. HR index values include the Arrhenius breakpoint temperatures (T_{BP1}, T_{BP2}, T_{AB}), the Q_{10} breakpoint temperatures (T_{QB1}, T_{QB2}, T_{QB}), HR_{max} (beats.min^{-1}) and cardiac arrhythmia temperature (T_{ARR}) beyond T_{AB} and T_{QB}.

Parasesarma catenatum	mass (g)	Length (mm TL)	T_{BP1}	T_{BP2}	T_{AB}	T_{QB1}	T_{QB2}	T_{QB}	Q_{10} breakpoint	HR_{max} (beats.min^{-1}) beyond T_{AB}	T at HR_{max} (beyond T_{AB})	T_{ARR}
1	2.29	16	23.5	29.0	36.0	21.0	31.0	35.0	1.1	112	37.0	37.5
2	1.29	13	22.5	31.5	37.0	24.0	32.0	35.0	1.1	108	38.0	38.5
3	1.21	12	20.5	29.5	35.5	20.0	28.0	36.0	1.1	96	38.5	39.0
4	2.65	17	20.5	28.0	35.5	21.0	29.0	36.0	1.0	82	38.5	39.0
5	3.30	16	21.5	29.5	34.5	21.0	31.0	35.0	1.0	90	38.5	39.0
6	2.81	17	22.5	31.0	35.5	21.0	31.0	34.0	1.0	60	38.5	39.0
7	3.43	20	20.5	29.0	36.0	21.0	31.0	35.0	1.0	60	38.5	39.0
8	2.84	19	21.0	28.0	36.0	22.0	29.0	35.0	1.0	78	38.5	39.0
9	2.47	19	20.0	31.5	36.5	22.0	31.0	37.0	1.0	72	37.0	37.5
10	3.27	19	20.0	29.5	36.5	21.0	30.0	36.0	1.0	108	36.0	36.5
11	3.22	17	21.5	30.0	34.5	21.0	30.0	36.0	1.0	115	37.5	38.0
12	4.07	20	22.0	30.0	35.5	20.0	29.0	36.0	1.1	92	37.5	38.0
13	3.46	18	23.0	27.0	35.5	20.0	31.0	34.0	1.1	114	37.0	37.5
14	2.86	18	24.5	31.5	34.5	23.0	31.0	34.0	1.0	102	37.5	38.0
15	3.23	20	21.5	27.0	34.5	22.0	28.0	33.0	1.0	78	36.5	37.0
16	3.55	18	22.5	28.0	36.5	20.0	29.0	34.0	1.1	115	38.0	38.5
17	5.23	22	21.0	28.5	34.0	21.0	30.0	35.0	1.0	96	35.0	35.5
18	4.42	20	23.0	29.0	33.5	24.0	31.0	34.0	1.1	114	34.5	35.0
Average	3.09	17.83	21.8	29.3	35.4	21.4	30.1	35.0	1.0	94	37.4	37.9
SD	0.97	2.50	1.3	1.4	1.0	1.2	1.2	1.0	0.1	19	1.2	1.2

5.4 DISCUSSION

To understand the physiological responses of two intertidal macro-invertebrates, the brown mussel *P. perna* (Mollusca) and the crab *P. catenatum* (Arthropoda), to increasing temperatures from different habitats between summer and winter, heart rate performance was assessed using infrared technology. This approach, overall, indicated that both *P. perna* and *P. catenatum* from the intertidal rocky low-shore and the intertidal lower reaches of the Kariega Estuary, when submersed in water, had heart rates that were mostly positively or neutrally related to an increase in water temperature up until the final Arrhenius breakpoint temperature (T_{AB}), upon which heart rate began to fluctuate, reaching its maximum heart rate (HR_{max}) and further declined beyond cardiac arrhythmia (T_{ARR}) until its CT_{max}. Beyond T_{ARR}, however, some individuals of both species did increase their heart rate until CT_{max} was reached, and this individual variability may have been a result of size variation (van Erkom Schurink and Griffiths, 1992; Gillooly *et al.*, 2001) or phenotypic plasticity (Stenseng *et al.*, 2005; Sarà and De Pirro, 2011; Logan *et al.*, 2012).

Nevertheless, these positive or neutral trends in heart rate with increasing temperature for this study demonstrated that immersed *P. perna* and *P. catenatum* exhibited the physiological mechanism of temperature insensitive/independent depression, especially, within a particular temperature zone [initial (T_{BP1}) to upper (T_{BP2}) temperature insensitive metabolism zone] up until T_{ARR} in order to cope under a period of acute temperature ramping (1.0 °C.h^{-1}). Previous studies that have demonstrated the use of temperature insensitive metabolic depression include Boutet *et al.* (2009) working on vent mussels *Bathymodiolus azoricus* (Costel and Comtet, 1999), who recorded that an increase in temperature triggered anaerobiosis (metabolic shutdown); and Marshall *et al.* (2011a) working on the intertidal snail *Echinlittorina malaccana* (Philippi, 1847), who showed that to overcome high body temperature (23.0 - 50.0 °C) when exposed in air, these snails depress resting metabolism between 35.0 and 46.0 °C. Metabolic elevation, by a few *P. catenatum* individuals was used, especially upon reaching the onset of cardiac failure. Selected intertidal macro-invertebrates (e.g. fiddler crabs), may thus rely on increasing their metabolism after remaining in their burrows during high tide or withstanding anaerobiosis for 24 hours by actively moving around during low tide to feed under thermally increased conditions (Teal and Carey, 1967, Vernberg and Vernberg, 1968). Previous studies on barnacles inhabiting different habitats in higher shore regions out of water are

exposed to high oxygen tensions, and can have a higher metabolic rate than those on the lower intertidal zone to cope with more variable environmental conditions (Augenfeld, 1967).

For *P. perna*, its temperature insensitive metabolism, demonstrated by three Arrhenius breakpoint temperatures (T_{BP1}, T_{BP2} and T_{AB}), three incremental Q_{10} breakpoint temperatures (T_{QB1}, T_{QB2} and T_{QB}) and cardiac arrhythmia (T_{ARR}), was significantly higher in summer compared with winter. In addition, the heart rates of *P. perna* individuals increased slightly beyond T_{BP2}, peaked beyond T_{AB} and decreased beyond T_{ARR} for summer and winter, which is possibly related to the abandonment of metabolic depression above T_{AB} (Marshall *et al.*, 2011a). These results consistently suggest that shifts in physiological traits can occur in *P. perna* which may enable this species to survive extreme temperature variations, especially associated with intertidal habitats (Hicks and McMahon, 2002) and may allow for seasonal metabolic compensation.

Many intertidal molluscs such as *P. perna* have little or no capacity for temperature acclimation due to the dynamic and extreme environment they inhabit. Instead, they have developed the capacity for acute thermal metabolic depression within a portion of their normal ambient temperature range (Bayne *et al.*, 1976; Newell *et al.*, 1976; McMahon and Russel-Hunter, 1977; Newell, 1979; Griffiths and Griffiths, 1987) which may be dependent on the rate of heating and the state of aestivation (Marshall *et al.*, 2011a). The results of this study, including the temperature insensitive metabolism zone induced between 24.5 – 33.0 °C in summer and 20.0 – 30.5 °C in winter (according to their T_{BP1} and T_{BP2} Arrhenius breakpoints), of *P. perna* submersed in water supports this notion. Similarly, research conducted on *P. perna* by Tagliarolo and McQuaid (2015) in the same region as this study identified a similar upper breakpoint temperature (T_{AB}) of 30.5 ± 3.1 °C when submersed in water, confirming their ability to maintain acute thermal depression and high upper critical thermal limits, especially during low tide when they are emerged to increased temperatures. The temperature insensitive metabolic depression for *P. perna* prevents wasteful metabolic expenditures (e.g. gaping) over short (tidal/diurnal) and long term (seasonal) ambient temperature fluctuations (McMahon and Ussery, 1995) and possibly provides a better chance of survival during very low winter and high summer temperatures. Reduced metabolism allows these animals to go into a state of aestivation under adverse environmental conditions as a means of extending their rest period to improve survival over fitness (Brown *et al.*, 2004; Marshall and McQuaid, 2011).

Interestingly, the Q_{10} breakpoint values for *P. perna*, based on heart rates with increasing temperature, ranged from 0.9 to 1.2 for summer and winter in this study. Bayne (1976) recorded similar Q_{10} breakpoint values (based on acute thermal regulation of oxygen consumption rate – VO_2) in Brazilian specimens of *P. perna* across a range of 1.2 -1.5, while van Erkom Schurink and Griffiths (1992) recorded Q_{10} breakpoint values of 1.5 in South African *P. perna*. Values of Q_{10} less than 1.8 in the upper half of the temperature range signifies a reduction in temperature sensitivity with increasing temperature (deFur and Mangum, 1978; De Wachter and McMahon, 1996) which further may indicate that *P. perna* from this study is displaying temperature insensitive metabolic depression in both summer and winter.

For *P. catenatum*, the temperature insensitive metabolic depression zone under temperature ramping was induced between 25.0 -31.5 °C in summer and 20.0 – 31.5 °C in winter according to their initial (T_{BP1}) and upper (T_{BP2}) Arrhenius breakpoints. These initial and upper Arrhenius, as well as Q_{10} (T_{QB1} and T_{QB2}) breakpoint temperatures, were significantly higher in summer than in winter. The final Arrhenius (T_{AB}) and Q_{10} (T_{QB}) breakpoint temperature (T_{AB}), however, did not differ between summer and winter. These results suggest that the temperature insensitive metabolic depression zone (T_{BP1} –T_{BP2}; T_{QB1} – T_{QB2}) for this species may vary according to seasonal water temperatures in the intertidal mudflats of the lower reaches of the Kariega Estuary in this region, but the final breakpoint temperature (T_{AB}), which is indicative of cardiac failure, may potentially be genetically fixed. Thermal tolerance and performance of marine crabs can be subject to phenotypic alteration within a thermal range (i.e. metabolic depression zone) (Cuculescu *et al.*, 1998). However, beyond this thermal range (i.e. beyond T_{AB}) survival is limited because of the irreversible thermal history of individuals and parents of a particular species and their evolutionary selection (Tomanek and Somero, 1999; Schaefer and Ryan, 2006).

For several crustaceans, average incremental Q_{10} temperatures range between 1.5 at high and 4.0 at low temperatures (deFur and Mangum, 1978; Burton *et al.*, 1980; Depledge, 1984; Zainal *et al.*, 1992; De Wachter and Wilkens, 1996). The average incremental Q_{10} temperature of *P. catenatum* for this study were 1.0 for both summer and winter during temperature ramping. This suggests that, due to a low incremental Q_{10} value of 1.0 for both summer and winter (Q_{10} < 1.5), this species has a reduced sensitivity to temperature as shown in other crabs, such as the widespread *Cancer magister* (Dana, 1852) ranging from Alaska to Mexico in shallow coastal

habitats (De Wachter and McMahon, 1996; De Wachter and Wilkens, 1996) and the widespread *Callinectes sapidus* (Rathbun, 1896) ranging from the western Atlantic Ocean to the gulf of Mexico in intertidal estuarine habitats (Burton *et al.*, 1980). These results further supports the contention that intertidal crabs may be well adapted to conserving energy in both summer and winter (De Pirro *et al.*, 1999).

P. catenatum was observed to have a higher thermal tolerance and cardiac performance compared to *P. perna* when exposed to temperature ramping, irrespective of it being mobile. A possible explanation for this could be that *P. catenatum* endures higher summer maximum water temperatures (and air temperatures) in the lower reaches of the Kariega Estuary (30.1 °C) compared with the adjacent low shore rock pool habitat inhabited by *P. perna* which was on average 5.0 °C cooler (25.1 °C) (refer to Chapter 2). Other possible explanations could be attributed to metabolic trade-offs and distinct cardiac properties between these species.

In terms of metabolic trade-offs, *P. perna* may have a lower thermal tolerance and cardiac performance than *P. catenatum*, potentially as a result of investing more metabolic energy into byssal thread production for attachment to the substrate in order to withstand increased wave action on the intertidal rocky shore rather than investing metabolic energy into maximum heat resilience. The process of byssal thread production can be energetically expensive, forming 8 to 15% of a mussel's monthly energy expenditure (e.g. Griffiths and King, 1979) and as a result, energy requirements for tolerating maximum temperatures may be minimized (Zardi *et al.*, 2007a).

In terms of cardiac performance, *P. catenatum* has cardiac myofibers which differ largely from those in bivalves, being striated rather than smooth, which have been shown to aid in lowering its thermal sensitivity (deFur and Mangum, 1978) and providing an evolutionary advantage in coping with thermal extremes. In addition, the short distance between the heart fibres in *P. catenatum* compared to *P. perna* is a very important factor which may limit the contractibility of the muscle in the heart, as well as other features of excitation contraction coupling (deFur and Mangum, 1978). In terms of temperature ramping or external thermal stress in this study, these characteristics of *P. catenatum* heart fibres were evident, enabling it to decrease its heart rate and metabolism (metabolic depression) in order to lower energy demand while still being functionally mobile.

143

Overall, the heart rate index T at HR_{max} for both species correlated well with the thermal tolerance index CT_{max} for summer and winter (see Chapter 3). In addition, for both species, the onset of time-dependent cardiac collapse was initiated around T_{BP2}, as expected, and was well below the thermal limit (CT_{max}) and the temperature that maximizes metabolism (T_{AB}). This finding suggests that temperature related cardiac failure between T_{BP2} and the T_{AB} is caused by an inability to generate energy to meet the cellular demand (Marshall et al., 2011a). Such indications of thermal discrepancy, however, would not have been identified if a multi-method approach to evaluate cardiac performance under a CT_{max} ramping protocol was not incorporated. The inclusion of ranges of T_{ARR}, three T_{AB} and three T_{QB} therefore provided fundamental information about the underlying physiology and thermal sensitivity of these intertidal ectothermic species. In addition, the quantification of the upper sub-lethal pejus temperatures (T_{PEJ}), optimum temperature preferences (T_{OPT}) and T_{CRIT}, rather than just the CT_{max} using respirometry, will additionally improve predictions on the physiological effects of seasonal temperature change and projected climate change scenarios (Casselman et al., 2012; Jørgensen et al., 2017).

To further improve results for this study, limitations to using the infrared technology on active animals need to be considered. For instance, during a few experimental trials, *P. catenatum* chelae and walking legs would often break free of their binds and for *P. perna* the gaping behaviour proved problematic in detecting accurate heartbeat signals. In addition, technical issues, including faulty infrared sensors, arose resulting in irregular heart rate measurements during some experimental trials. As such, better and stronger material that would cause no harm or damage should be used for binding *P. catenatum*. For *P. perna*, the option of decreasing the frequency and duration of gaping behaviour is difficult as it varies between species and temperature (Rodland et al., 2009), and with ramping this is unavoidable. The only solution, therefore, is to remove from the analysis the irregular heartbeats (indicated by high average frequencies in Hz) caused by gaping behaviour. Technical equipment, such as infrared sensors, should also be replaced frequently. Furthermore, to improve future work using *P. perna* and *P. catenatum*, heart rate needs to be compared between the laboratory and the field (see Tagliarolo and McQuaid, 2016), and heart rate should be monitored in air as well as in the water (see Tagliarolo and McQuaid, 2015).

In conclusion, both *P. perna* and *P. catenatum*, in their respective habitats, in this warm-temperate region, will not be vulnerable to the current and projected increases in water temperature. This is a result of both species having the ability to physiologically depress their metabolism in water, irrespective of season, to conserve energy to offset lifelong constraints on energy gain and the fact that both species are currently able to tolerate temperatures well beyond temperatures measured in water. When comparing both species' vulnerability to projected increases in extreme events (e.g. heatwaves) due to climate change, however, it is most likely that *P. perna* from the intertidal rocky low-shore habitat would be more vulnerable than *P. catenatum* from the estuarine habitat, as they are commonly sedentary. *Perna perna*, furthermore, found on slightly higher shores, may also be considered more vulnerable as they would be exposed to increased effects of desiccation and prolonged emersion during low tide.

CHAPTER SIX: GENERAL DISCUSSION

Many of the world's coastal marine ecosystems and the services they provide are under growing threat from increases in mean ocean temperature, alterations to ocean climate, and weather variability because of anthropogenic climate change (e.g. Harley *et al.*, 2006; Hoegh-Guldberg and Bruno, 2010; Wernberg *et al.*, 2011, 2012; Thornton *et al.*, 2014). Predicting the impacts of these changes on marine species' biological responses at ecological, physiological and evolutionary levels and, perhaps most importantly, predicting which marine species or populations are most vulnerable, has thus become a key research focus (e.g. IPCC, 2014; Boyd *et al.*, 2018).

To contribute to narrowing this knowledge gap, this thesis aimed to systematically quantify and compare the thermal tolerance and performance of a range of marine ectotherms using a multi-scale (different taxonomic, biogeographic and ontogenetic groups from different coastal habitats) and multi-method physiological approach (dynamic method, static respirometry and maximum heart rate experiments) within a South African warm-temperate coastal climate change hotspot to available and projected *in situ* temperature data. This was also undertaken to gauge the vulnerability of each species across summer and winter seasons.

Major findings of this thesis indicated that when all coastal marine ectotherm's thermal tolerances (CT_{max} and CT_{min}) were considered together with the *in situ* temperature data collected, summer CT_{max} endpoints largely increased compared with winter, and winter CT_{min} endpoints decreased compared with summer, highlighting the plasticity in thermal scope across seasons, especially for the widespread macro-invertebrates (with the exception of *P. angulosus*) (Table 6.1). Upper and lower thermal safety margins in summer, however, were narrower than in winter, especially for the temperate, warm-water endemic and tropical juvenile fish species (Table 6.1). This suggests that, within this warm-temperate region, these juvenile fish species may be more vulnerable to temperature variability in summer than in winter, potentially as a result of extreme summer heatwaves and upwelling if they do not find thermal refuge.

Table 6.1. Levels of vulnerability (Green = low vulnerability; Orange = medium vulnerability; Red = high vulnerability) of selected coastal marine ectotherms to temperature variability in the form of extreme events (summer marine heatwaves, summer upwelling and winter cold spells) as a result of climate change according to their thermal tolerance (CT_{max} and CT_{min}) and performance (T_{OPT}/T_{AB}; T_{PEJ}/T_{ARR}) in relation to their environmental water temperatures between taxonomic groups, biogeographic affinities, habitats and seasons.

Species	Illustration	Taxonomic group	Biogeographic affinity	Habitat sampling area	CT_{max} (T_{OPT}/T_{AB}; T_{PEJ}/T_{ARR}) ↑ Temperature (°C) Summer	↑ Temperature (°C) Winter	CT_{min} (T_{OPT}/T_{PEJ}) ↓ Temperature (°C) Summer	↓ Temperature (°C) Winter	Water temperature (°C) Summer Max	Summer min	Water temperature (°C) Winter Max	Winter Min
Diplodus capensis juveniles		Fish	Temperate (Group 5)	Rock pool/Gully	34.5 (T_{OPT} = 25; T_{PEJ} = 27.5)	33.2	8	6.5	25.1	12.8	20.0	13.2
Diplodus capensis adults		Fish	Temperate (Group 5)	Surf zone	32 (T_{AB} = 20.8; T_{ARR} = 28.3)		8.4		30.1	14.6	20.8	12.2
Sarpa salpa		Fish	Temperate (Group 5)	Rock pool/Gully	33.9	32.4	7.8	7.1	25.1	12.8	20.0	13.2
Kuhlia mugil		Fish	Tropical (Group 2)	Rock pool	37.8	37.1	8.7	8.9	25.1	12.8	20.0	13.2
Palaemon peringueyi		Macro-invertebrate	Widespread (Group 6)	Rock pool	35.7	34.9	4.7	3.7	25.1	12.8	20.0	13.2
Parechinus angulosus		Macro-invertebrate	Widespread (Group 6)	Rock pool	31.3	27	7.0	4.5	25.1	12.8	20.0	13.2
Perna perna		Macro-invertebrate	Widespread (Group 6)	Rock pool	38.9 (T_{AB} = 31.6; T_{ARR} = 38.6)	37.9 (T_{AB} = 28.7; T_{ARR} = 36.9)	4.3	4.0	25.1	12.8	20.0	13.2
Chelon dumerili		Fish	Warm-water (Group 3)	Lower estuarine	37.7	35.6	9.3	5.3	30.1	14.6	20.8	12.2
Chelon richardsonii		Fish	Cool-water (Group 4)	Lower estuarine	34.9	34	5.7	4.6	30.1	14.6	20.8	12.2
Rhabdosargus holubi		Fish	Warm-water (Group 3)	Lower estuarine	35.6	32.3	8.1	5.8	30.1	14.6	20.8	12.2
Chaetodon marleyi		Fish	Tropical (Group 2)	lower estuarine (subtidal)	35.2		11.2		30.1	14.6	20.8	12.2
Clibanarius virescens		Macro-invertebrate	Tropical (Group 2)	Lower estuarine	38.3	38.6	5.6	4.0	30.1	14.6	20.8	12.2
Parasesarma catenatum		Macro-invertebrate	Widespread (Group 6)	Lower estuarine	39.8 (T_{AB} = 30.8; T_{ARR} = 38.1)	39.2 (T_{AB} = 28.7; T_{ARR} = 37.3)	6.0	4.9	30.1	14.6	20.8	12.2
Upogebia africana		Macro-invertebrate	Widespread (Group 6)	Lower estuarine	38.3	36.2	6.0	4.5	30.1	14.6	20.8	12.2

Heatwaves are usually defined as "periods of abnormally and uncomfortably hot weather invoked during summer" (Glickman, 2000) that exceed the acclimation capacity of animals (Gutschick and BassiriRad, 2003) and often result in large-scale shifts in the distributions, phenological changes, changes in ecosystem structure and, in extreme cases, high levels of mortality in marine species (Perry *et al.*, 2005; Lima *et al.*, 2007; Beaugrand *et al.*, 2008; Wernberg *et al.*, 2011). Recent evidence has suggested that heatwaves in the ocean are becoming longer and more frequent (e.g. Lima and Wethey, 2012; deCastro *et al.*, 2014; Frölicher *et al.*, 2018; Oliver *et al.*, 2018). In South Africa, Schlegel *et al.* (2017) found that marine heatwaves have been increasing every decade in all coastal regions. This suggests that the fauna along the south-eastern coast will be increasingly susceptible to marine heatwaves.

The results of the thermal tolerance experiments in this study suggest that marine estuarine opportunist fish such as the temperate *D. capensis* (both juvenile and adult), temperate juvenile *S. salpa* and cool-water endemic juvenile *C. richardsonii* and the juveniles of the marine estuarine-dependent warm-water endemic *R. holubi* may be negatively influenced by predicted increases in heatwaves (Figure 6.1, Figure 6.2, Table 6.1). Surprisingly, the juvenile tropical marine straggler *C. marleyi* may also be impacted, with its summer CT_{max} endpoint being relatively low compared with the other juvenile tropical marine straggler species *K. mugil* (Figure 6.2, Table 6.1). The only widespread macro-invertebrate that may be negatively impacted by heatwaves is *P. angulosus* (Figure 6.1), with a low summer CT_{max} endpoint compared with the other macro-invertebrates which all had very high summer CT_{max} endpoints (Table 6.1). As a permanently open estuary with attributes akin to an arm of the nearshore ocean, the Kariega Estuary can serve as a thermal refuge for thermally stressed species. However, the extent of its utility may depend on the biogeography, biology and habitat preference (ecology) of the affected species.
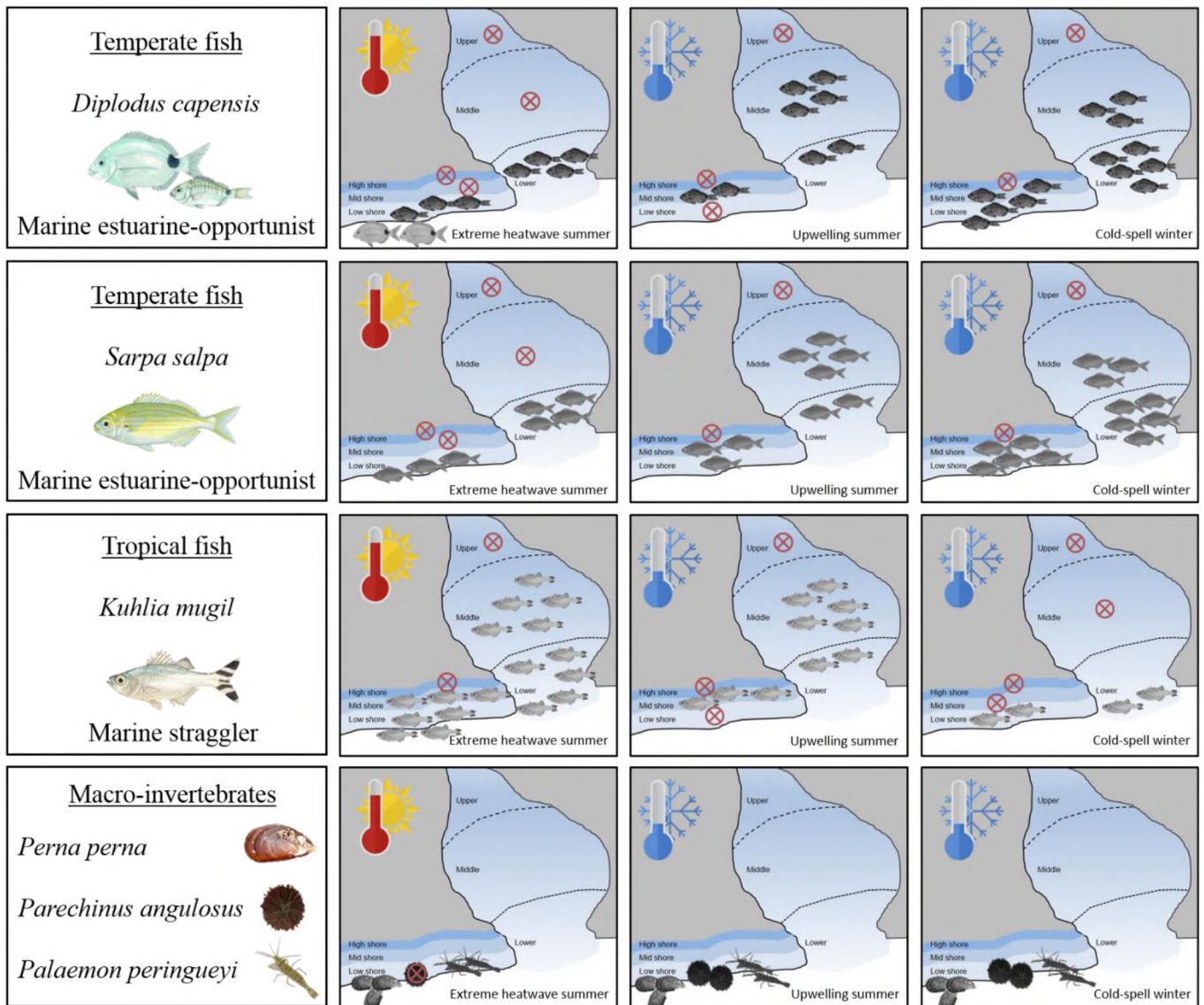
Figure 6.1. Schematic representation of where marine ectotherms collected from the intertidal low-shore rocky habitat within this warm-temperate study region would seek thermal refuge under the predicted effects of summer extreme heatwaves, summer upwelling and winter cold spells as a result of climate change.
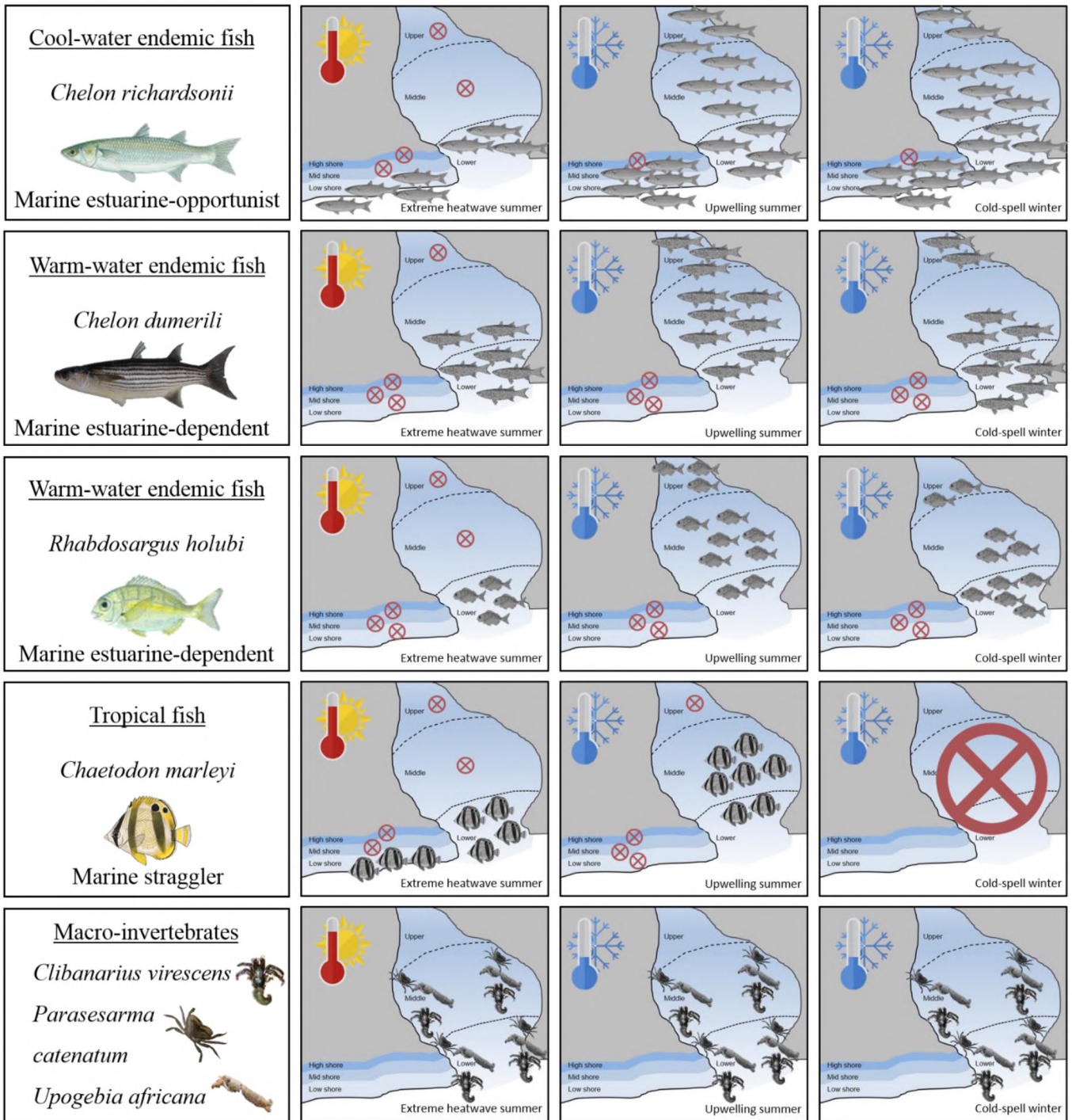
Figure 6.2. Schematic representation of where marine ectotherms collected from the intertidal lower reaches of the estuarine habitat within this warm-temperate study region would seek thermal refuge under the predicted effects of summer extreme heatwaves, summer upwelling and winter cold spells as a result of climate change.

For instance, as a result of their thermal tolerance, biogeography, biology and habitat preference, *D. capensis, S. salpa, C. richardsonii* and *C. marleyi* may be restricted to the low-shore rocky habitat and the lower reaches of the Kariega Estuary as a thermal refuge during heatwave events (Figure 6.1, Figure 6.2), while *R. holubi* may be restricted to the lower reaches of the Kariega Estuary (Figure 6.2). When measuring the thermal performance of juvenile and adult *D. capensis*, it was found that they are negatively impacted at temperatures beyond 28.0 °C (based on the average upper T_{PEJ} for juveniles (27.3 °C) and the T_{ARR} (28.3 °C) for adults; refer to Chapter 4). In addition, their relatively low summer CT_{max} endpoints are already close to current temperatures recorded in the middle and upper reaches of the estuary and this limits the utility of these areas as thermal refuges for the species. In terms of *D. capensis* and *S. salpa* biology and habitat preference, they are not strongly euryhaline in comparison to *R. holubi* and as juveniles they are rarely found to occur in the middle and upper reaches of any estuary, with adult *D. capensis* further found only briefly to occupy the lower reaches of an estuary opportunistically and during spawning (Whitfield, 1998, 2019). Kemp (2009) also indicated that juvenile *D. capensis* would not be able to tolerate higher salinities found in the mid- and upper-shore rocky habitats.

Rhabdosargus holubi cannot survive in water temperatures higher than 34.0 °C (this study, van der Vyver *et al.*, 2013). *Rhabdosargus holubi*, which occurs exclusively as juveniles in estuaries only (Whitfield, 1998, 2019) may, thus, be restricted to the lower reaches of the estuary during a summer heatwave. *Chelon richardsonii*, being the only cool-water endemic species which prefers cooler waters (James *et al.*, 2016), may not be able to tolerate extreme increases in temperature in the middle and upper reaches of the estuary and the mid-shore rock pools. Other environmental preferences may also restrict the utility of certain habitats as a refugia. For example, *C. marleyi*, prefer clear water conditions, which may restrict them to the lower reaches of the estuary where turbidity is lower compared with the middle and upper reaches (Vine, 1998). The mobility of the species will also have a bearing on its response to heatwave conditions. As a sedentary species, *P. angulosus*, which predominantly occurs in the low-shore rocky habitat, will not be able to move to a suitable thermal refuge during heatwaves and it is therefore unlikely to survive. This is not unusual for sedentary species. For example, Farmanfarmaian and Giese (1963) found that the purple sea urchin *Strongylocentrotus purpuratus* relied on a broad thermal tolerance to survive thermal extremes.

Cold upwelling events in summer can lower temperatures drastically within a few hours (Ganachaud *et al.*, 2010) and can cause mass mortalities of marine species (Whitfield, 2019). For example, locally, within the mouth region of the Storms River Estuary along the south-east coast, mortality was observed in 14 marine fish species following an upwelling event which resulted in sudden water temperature drop from 21.0 °C to 11.0 °C (Hanekom, 1989). Recently, Duncan *et al.* (2019) found that within the Tsitsikamma region, SST can drop from 24.0 °C to 12.0 °C in less than 12 hours as a result of summer upwelling. This may explain why large shoals (200 – 3000 individuals) of marine fish frequently take refuge in estuaries along the Tsitsikamma coast when upwelling occurs (Whitfield, 2019). For this study region, the permanently open Kariega Estuary may serve as a good thermal refuge due to its atypical estuarine properties (uniformly marine), especially considering that summer upwelling is predicted to increase in frequency, duration and intensity for this region, driving increases in SST variability (Duncan *et al.*, 2019).

Of the species studied in this thesis, thermal tolerance results indicate that juvenile *D. capensis* and *K. mugil* collected from the low-shore rocky habitat (rock pools and gullies) would be most vulnerable to the effects of current and predicted summer upwelling, as a result of them having high CT_{min} endpoints in summer, potentially eliminating them from this habitat and lowering their abundance in the lower reaches of the Kariega Estuary (Figure 6.1). As a result, juvenile *D. capensis* and *K. mugil* may be forced to seek thermal refuge in the middle reaches of the Kariega Estuary and the mid-shore of the rocky habitat temporarily to escape low nearshore temperatures (Figure 6.1). The upper reaches of the Kariega Estuary and the high-shore rocky habitat would be excluded as a thermal refuge for these species as a result of their biology and habitat preference whereby both species may be unable to tolerate increased salinities (high-shore rocky habitat) and turbidity (estuarine habitat) and no preferred habitat structure may be provided, especially for *K. mugil* (Figure 6.1). Within the same habitat, even though juvenile *S. salpa* and *P. angulosus* also presented high CT_{min} endpoints in summer (Table 6.1), they may not be affected by summer upwelling. This may be a result of juvenile *S. salpa* not needing to use the strategy described by Pörtner and Gutt (2016), unlike juvenile *D. capensis*, where a trade-off between growth and thermal tolerance to colder temperatures in summer takes place, as its winter CT_{min} endpoint is similar to its summer CT_{min} endpoint, not losing cold water adaptability in summer (Table 6.1). Additionally, temperatures within the rock pool habitat are

unlikely to drop below *P. angulosus* CT$_{min}$, even though this species may lose thermal adaptability to cold temperatures in summer (Table 6.1).

Within the estuarine habitat, the only species from this study that may be vulnerable to current and predicted increases in summer upwelling is the tropical subtidal juvenile *C. marleyi*. This is because this species had the highest summer CT$_{min}$ endpoint of all species studied (Table 6.1). In terms of thermal refuge, *C. marleyi* may have to recruit into the middle reaches of the estuary where temperatures will be warmer however, thermal refuge will not be extended to the upper reaches of the estuary due to limited habitat choices or to the rocky shore habitat, which will be highly influenced by nearshore cold temperatures (Figure 6.2). Although other marine-dependent species such as *C. dumerili* and *R. holubi* had high summer CT$_{min}$ endpoints, it is likely that they will be less influenced by summer upwelling events as they have the ability to move into the middle and upper reaches of the Kariega Estuary to seek thermal refuge as a result of their euryhaline physiology (Figure 6.2). The only fish species from this study that will most likely be tolerant of the current and predicted increases in summer upwelling in the estuarine and rocky shore habitats is the cool-water marine estuarine-opportunist *C. richardsonii* as a result of its low CT$_{min}$ endpoints (Figure 6.2, Table 6.1).

Globally, winter marine cold spells (MCSs) that follow the passage of cold fronts are predicted to become less frequent under future climate scenarios, but there are examples of them becoming more frequent in some localities (e.g. Gershunov and Douville, 2008; Matthes *et al.*, 2015) resulting in mass fish (Gunter, 1941, 1951; Holt and Holt, 1983) and invertebrate (Gunter, 1951; Crisp, 1964) kills. In this study region, Schlegel *et al.* (2017) showed that along the south-east coast, particularly near Port Elizabeth, MCSs are increasing very near the coast. Although results from this study indicate that temperature variability in summer may have more of an effect on juvenile fish species (heatwaves and upwelling), winter cold spells may too have an effect, especially for the tropical marine stragglers occurring in this region.

When exposed to extreme cold events, juvenile *K. mugil* may not be able to cope due to their high winter CT$_{min}$ endpoints compared with the other fish species studied (Table 6.1). Individuals that may survive the initial drop in temperature, however, may seek thermal refuge in the lower reaches of the Kariega Estuary and the adjacent low-shore rocky habitat, which likely may be cold but not as cold as the middle and upper reaches of the estuary and the mid- and high-shore rocky habitat as well as the nearshore sea (Figure 6.2). Similarly, Boucek and

Rehage (2014), when evaluating a subtropical estuarine fish community in South Florida after a severe cold front, found that tropical euryhaline and non-native species decreased in abundance significantly, leaving the temperate species to dominate the community. The findings from this study suggest that juvenile *C. marleyi* will not survive MCSs. However, this is not surprising as the species has not been present within the Kariega Estuary during winter months anyway (Figure 6.2). McBride and Able (1998) recorded similar findings for other Chaetodontidae in a temperate environment. However, if increases in average annual temperatures occur as a result of climate change, it is possible that vulnerable tropical species may begin overwintering in this region. This will increase the abundance and overall survival and possibly result in the tropicalization of the community (Figueira and Booth, 2010; Horta e Costa *et al.*, 2014; Vergés *et al.*, 2014). Although the results suggest that the remaining warm-water marine estuarine-dependent species (*C. dumerili* and *R. holubi*) from this study will not be influenced by winter cold spells, their abundance during these events may increase in the lower reaches where temperatures may be warmer compared with the middle and upper reaches (Figure 6.2), while the cool-water *C. richardsonii* abundance throughout the estuary will remain unaffected (Figure 6.2).

The resilience of macro-invertebrates, with the exception of *P. angulosus*, to temperature variability in the form of summer heatwaves and upwelling and winter cold spells, as shown by their broad thermal scopes and high intraspecific variability across seasons in this study (refer to Chapter 3; Figure 6.1, Figure 6.2; Table 6.1), could be attributed to their widespread distributions and their ability to physiologically depress their metabolism by lowering their heart rate (Chapter 5) (Thompson *et al.*, 2002; Chen and Stillman, 2012; Paganini *et al.*, 2014; Gunderson *et al.*, 2016). As a result, this allows them to cope with extreme cold and hot temperatures associated with alternating immersion and emersion of the intertidal environment (see Newell, 1979; Davidson and Pearson, 1996; Karsten *et al.*, 1996). In terms of the predicted effects associated with climate change (heatwaves, upwelling and cold spells), this taxonomic group may have a physiological advantage over coastal fishes. Similar results have also been found for macro-invertebrates in other warm-temperate regions by Madeira *et al.* (2012a, 2016) and Vinagre *et al.* (2018), who showed that fishes' CT_{max} endpoints were narrower than macro-invertebrates', depending on their latitude and habitat. In addition, Morley *et al.* (2016), assessing thermal sensitivity and critical limits of macro-invertebrates from the tropical Ascension Island, temperate New Zealand and Antarctic found that with physiological

154

acclimation/compensation in response to seasonal increases in temperature, tropical and temperate macro-invertebrates can have higher thermal tolerance limits than annual maximum water temperature, possibly averting the effects of climate change.

Overall, the findings of this study suggest that permanently open intertidal estuaries, particularly the atypical Kariega Estuary, may provide good thermal refugia for euryhaline marine ectotherms against the effects of extreme climate events compared with the intertidal rocky shore pools and gullies. This is because the middle and upper reaches can provide thermal refugia for vulnerable euryhaline fish species when the marine environment and lower reaches of the estuary are impacted by MCSs and MHWs (refer to Chapter 2; Figure 6.1, Figure 6.2). Since rock pools are poorly connected and patchy, the movement of organisms to more benign thermal environments may be restricted. Furthermore, as a result of other projected climate change factors at play, such as sea level rise, gullies and rock pools may be further eliminated as a thermal refuge all together (Mather *et al.*, 2009) as they will become fully submerged and disappear/be altered resulting in a "coastal squeeze" which will influence other marine ectotherms survival such as macro-invertebrates. A possible habitat re-allocation may then follow with marine ectotherms moving into nearby areas such as estuaries in the intertidal rocky shore (Pethick, 1993).

One possible aspect that was not included as part of this multi-method approach in this thesis was that of the biochemical responses to changes in temperature. Marine ectotherms, especially fish, react to a temperature stressor which produces a physiological response that in turn increases stress hormones (cortisol, catecholamines, glucose levels; Barton and Iwama, 1991) and alters the actions and functions of various heat shock proteins (Hightower, 1991; Iwama *et al.*, 1999; Goligorsky, 2001). The measurement of stress hormones, such as cortisol and heat shock proteins, in combination with thermal tolerance and performance measurements, can increase the level of confidence in determining marine ectotherms sub-lethal limits (pejus temperatures). Additionally, as heat shock proteins vary according to species (Basu *et al.*, 2002; Nakano and Iwama, 2002), developmental stage (Lele *et al.*, 1997; Santacruz *et al.*, 1997; Martin *et al.*, 2001) and season (Fader *et al.* 1994), knowledge of their role will improve intra-specific predictions of thermal vulnerability, especially in the context of climate change (Roessig *et al.*, 2004; Chadwick *et al.*, 2015).

Another aspect that was beyond the scope of this study was the response to multiple stressors and comparing field and laboratory-based studies. The resistance of individual marine ectotherms to one stressor, such as changes in temperature, may be lowered in the face of multiple stressors, such as changes in temperature and pH, which may result in a particular ecosystem suffering from diversity loss, in turn compromising ecosystem functioning and resilience to further change (e.g. acidification of coral reefs; Brierley and Kingsford, 2009) (Cull et al., 2015). To date, most research on multiple stressors, as indicated by Wernberg et al. (2012), Bozinovic and Pörtner (2015) and Deutsch et al. (2015), is lacking and the research that has been conducted has been restricted to laboratory studies, e.g. Ackerman et al. (2000), Vijayan et al. (2000) and Basu et al. (2001) (Cull et al., 2015).

In conclusion, given the importance of understanding the responses of marine ectotherms to anthropogenic climate change, this study should serve as a starting point for future physiological thermal tolerance research on South African marine ectotherms and highlights the importance of understanding the complexity of impacts in other geographic climate change hotspots. The use of a multiple method approach in this study further assisted in identifying the different effects of temperature on the marine ectotherms of focus and highlighted different aspects of vulnerability and resistance. The study further highlighted that understanding the role of thermal variability on marine ectotherms' tolerance and performance may be more important than just the responses to mean predicted SST changes in various habitats of this warm-temperate climate change hotspot, which is emerging as an important avenue of research globally (see Bates et al., 2018). Finally, this research provides valuable perceptions of physiological aspects affecting population dynamics over a local scale in a climate change and biodiversity hotspot and lays the foundations for predicting population dynamics and species distributions based on physiological sensitivity to temperature in several fish and macro-invertebrate species.

REFERENCES

Aagaard A. 1996. In situ variation in heart rate of the shore crab *Carcinus maenas* in relation to environmental factors and physiological condition. *Marine Biology* 125: 765–772.

Ackerman PA, Forsyth RB, Mazur CF, Iwama GK. 2000. Stress hormones and the cellular stress response in salmonids. *Fish Physiology and Biochemistry* 23: 327–336.

Aguirre JD, Marshall DJ. 2012. Genetic diversity increases population productivity in a sessile marine invertebrate. *Ecology* 93: 1134–1142.

Alexander SJ, Ewer DW. 1969. A comparative study of some aspects of the biology and ecology of *Sesarma catenata* Ort. and *Cyclograpsus punctatus* M. Edw., with additional observations on *Sesarma meinerti* De Man. *Zoologica Africana* 4: 1-35.

Allan EL, Froneman PW, Hodgson AN. 2006. Effects of temperature and salinity on the standard metabolic rate (SMR) of the caridean shrimp *Palaemon peringueyi*. *Journal of Experimental Marine Biology and Ecology* 337: 103–108.

Allanson BR, Read GHL. 1995. Further comment on the response of eastern cape province estuaries to variable freshwater inflows. *Southern African Journal of Aquatic Science* 21: 56–70.

Almeida JR, Gravato C, Guilhermino L. 2015. Effects of Temperature in Juvenile Seabass (*Dicentrarchus labrax* L.) Biomarker Responses and Behaviour: Implications for Environmental Monitoring. *Estuaries and Coasts* 38: 45–55.

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: R911–R921.

Angilletta MJ, Angilletta MJ. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford, United Kingdom. 289 pp.

Anttila K, Casselman MT, Schulte PM, Farrell AP. 2013. Optimum Temperature in Juvenile

Salmonids: Connecting Subcellular Indicators to Tissue Function and Whole-Organism Thermal Optimum. *Physiological and Biochemical Zoology* 86: 245–256.

Anttila K, Couturier CS, Øverli Ø, Johnsen A, Marthinsen G, Nilsson GE, Farrell AP. 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nature Communications* 5: 1–6.

Augenfeld JM. 1967. Respiratory metabolism and glycogen storage in barnacles occupying different levels of the intertidal zone. *Physiological Zoology* 40: 92–96.

Bailey SE, James NC. 2013. Fish sampling in the marine-dominated Kariega Estuary, South Africa, using a demersal otter trawl: day/night effects. *African Journal of Aquatic Science* 38: 115–120.

Baldanzi S, Weidberg NF, Fusi M, Cannicci S, McQuaid CD, Porri F. 2015. Contrasting environments shape thermal physiology across the spatial range of the sandhopper *Talorchestia capensis*. *Oecologia* 179: 1067–1078.

Barnes DKA. 1997. Ecology of tropical hermit crabs at Quirimba Island, Mozambique: distribution, abundance and activity. *Marine Ecology Progress Series* 154: 133-142.

Barton B., Iwama G. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* 1: 3–26.

Basu N, Nakano T, Grau EG, Iwama GK .2001. The effects of cortisol on heat shock protein 70 levels in two fish species. *General and comparative endocrinology* 124: 97–105.

Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM, Iwama GK. 2002. Heat shock protein genes and their functional significance in fish. *Gene* 295: 173–183.

Bates A, Helmuth B, Burrows M, Duncan M, Garrabou J, Guy-Haim T, Lima F, Queiros A, Seabra R, Marsh R, et al. 2018. Biologists ignore ocean weather at their peril. *Nature* 560: 299–301.

Bayne BL. 1976. The biology of mussel larvae. In: Bayne BL (ed.), *Marine mussels: their ecology and physiology*. Cambridge University Press, Cambridge, United Kingdom. pp 81-120.

Beaugrand G, Brander KM, Alistair Lindley J, Souissi S, Reid PC. 2003. Plankton effect on cod recruitment in the North Sea. *Nature* 426: 661–664.

Beaugrand G, Edwards M, Brander K, Luczak C, Ibanez F. 2008. Causes and projections of abrupt climate-driven ecosystem shifts in the North Atlantic. *Ecology Letters* 11: 1157–1168.

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, et al. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51: 633–641.

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

Beckley LE. 1985. The fish community of East Cape tidal pools and an assessment of the nursery function of this habitat. *South African Journal of Zoology* 20: 21–27.

Beitinger T, Bennett WA. 2000. Quantification of the role of accclimation temperature in temperature tolerance of fishes. *Environmental Biology of Fishes* 58: 277–288.

Beitinger TL, Bennett WA, McCauley RW. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental biology of fishes* 58: 237–275.

Bernard ATF, Froneman PW. 2004. Population dynamics of the caridean shrimp *Palaemon peringueyi* in a temperate temporarily open/closed southern African Estuary. *Estuarine, Coastal and Shelf Science* 63: 73-81.

Berry PF. 1978. Reproduction, growth and production in the mussel, *Perna perna* (Linnaeus) on the east coast of South Africa. *South African Association of Marine Biology Research* 48: 1-28.

Bertness MD. 1981. Predation, physical stress and the organization of a tropical rocky intertidal hermit crab community. *Ecology* 62: 411–425.

Bertness MD, Leonard GH, Levine JM, Bruno JF. 1999. Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. *Oecologia* 120: 446–450.

Bjarnason A, Bjarkadóttir AV, Gunnarsson, A. 2017. *Leadless heart rate loggers minimize impact of surgery and remove housing constraints.* NC3Rs Cardiovascular Showcase 2018, London.

Blaber SJ. 1973. Temperature and salinity tolerance of juvenile *Rhabdosargus holubi* [Steindachner (Teleostei: Sparidae)]. *Journal of Fish Biology* 5: 593-598.

Blaxter K. 1989. *Energy metabolism in animals and man.* Cambridge University Press, Cambridge, United Kingdom. 336 pp.

Booth DJ, Beretta GA, Brown L, Figueira WF. 2018. Predicting success of range-expanding coral reef fish in temperate habitats using temperature-abundance relationships. *Frontiers in Marine Science* 5: 1–7.

Boucek RE, Rehage JS. 2014. Climate extremes drive changes in functional community structure. *Global Change Biology* 20: 1821–1831.

Boucher MA, Baker DW, Brauner CJ, Shrimpton JM. 2018. The effect of substrate rearing on growth, aerobic scope and physiology of larval white sturgeon *Acipenser transmontanus*. *Journal of Fish Biology* 92: 1731–1746.

Bounama C, Von Bloh W, Franck S. 2007. How rare is complex life in the Milky Way? *Astroiology* 7: 745-756.

Boutet I, Tanguy A, Le Guen D, Piccino P, Hourdez S, Legendre P, Jollivet D. 2009. Global depression in gene expression as a response to rapid thermal changes in vent mussels. *Proceedings of the Royal Society B: Biological Sciences* 276: 3071–3079.

Bownes SJ, McQuaid D. 2006. Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? *Journal of Experimental Marine Biology and Ecology* 338: 140-152.

Boyd PW, Collins S, Dupont S, Fabricius K, Gattuso J-P, Havenhand J, Hutchins DA, Riebesell U, Rintoul MS, Vichi M, et al. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change - a review. *Global Change Biology* 24: 2239–2261.

Bozinovic F, Pörtner HO. 2015. Physiological ecology meets climate change. *Ecology and Evolution* 5: 1025–1030.

Braby CE, Somero GN. 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *The Journal of Experimental Biology* 209: 2554–2566.

Branch GM, Branch ML. 1981. *The living shores of southern Africa*. Struik Publishers, Cape Town. 272 pp.

Branch GM, Griffiths CL, Branch ML, Beckley LE. 2002. *Two Oceans: A guide to the marine life of southern Africa* (5th edn). David Philip, Cape Town. 360 pp.

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

Branch G, Branch M. 2018. *Living shores: interacting with southern Africa's marine ecosystems*. Struik Nature, Cape Town, South Africa. 320 pp.

Breau C, Cunjak RA, Peake SJ. 2011. Behaviour during elevated water temperatures: Can physiology explain movement of juvenile Atlantic salmon to cool water? *Journal of Animal Ecology* 80: 844–853.

Brett JR. 1962. Some considerations in the study of respiratory metabolism in fish, particularly salmon. *Journal of the Fisheries Research Board of Canada* 19: 1025-1038.

Brett JR. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada* 21: 1183-1226.

Brewer GD. 1976. Thermal tolerance and resistance of the northern anchovy, *Engraulis mordax*. *Fisheries Bulletin* 74: 433-445.

Brierley AS, Kingsford MJ. 2009. Impacts of climate change on marine organisms and ecosystems. *Current Biology* 19: R602–R614.

Brockington S, Clarke A. 2001. The relative influence of temperature and food on the metabolism of a marine invertebrate. *Journal of Experimental Marine Biology and Ecology* 258:87–99.

Brown AC, Da Silva FM. 1979. The effects of temperature on oxygen consumption in *Bullia digitalis* Meuschen (Gastropoda, Nassaridae). *Comparative Biochemistry and Physiology* 62: 573-576.

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

Burnett NP, Seabra R, de Pirro M, Wethey DS, Woodin SA, Helmuth B, Zippay ML, Sara G, Monaco C, Lima FP. 2013. An improved noninvasive method for measuring heartbeat of intertidal animals. *Limnology and Oceanography-Methods* 11: 91–100.

Burton DT, Richardson LB, Moore CJ. 1980. Cardiac frequency compensation response of adult blue crabs (*Callinectes sapidus* Rathbun) exposed to moderate temperature increases. *Comparative Biochemistry and Physiology Part A: Physiology* 65: 259-263.

Calder WA. 1987. Scaling energetics of homothermic vertebrates: an operational allometry. *Annual Reviews in Physiology* 49: 107-120.

Casselman MT, Anttila K, Farrell AP. 2012. Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. *Journal of Fish Biology* 80: 358–377.

Chabot D, Steffensen JF, Farrell AP. 2016. The determination of standard metabolic rate in

fishes. *Journal of Fish Biology* 88: 81–121.

Chadwick JG, Nislow KH, McCormick SD. 2015. Thermal onset of cellular and endocrine stress responses correspond to ecological limits in brook trout, an iconic cold-water fish. *Conservation Physiology* 3: 1–12.

Chelazzi A, Chelazzi G, Williams OGA, Gray PDR. 1999. Field and laboratory measurement of heart rate in a tropical limpet, *Cellana grata*. *Journal of the Marine Biological Association of the United Kingdom* 79: 749–751.

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

Chen X, Stillman JH. 2012. Multigenerational analysis of temperature and salinity variability affects on metabolic rate, generation time, and acute thermal and salinity tolerance in *Daphnia pulex*. *Journal of Thermal Biology* 37: 185–194.

Chen Z, Snow M, Lawrence CS, Church AR, Narum SR, Devlin RH, Farrell AP. 2015. Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Journal of Experimental Biology* 218: 803–812.

Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, Pauly D. 2009. Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* 10: 235–251.

Christensen MS, Winterbottom R. 1981. A correction factor for, and its application to, visual censuses of littoral fish. *South African Journal of Zoology* 16: 73–79.

Christensen AB, Nguyen HD, Byrne M. 2011. Thermotolerance and the effects of hypercapnia on the metabolic rate of the ophiuroid *Ophionereis schayeri*: inferences for survivorship in a changing ocean. *Journal of Experimental Marine Biology and Ecology* 403:31-38.

Clark TD, Sandblom E, Jutfelt F. 2013. Review: Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *The Journal of Experimental Biology* 216: 2771–2782.
163

Coleman N, Trueman ER. 1971. The effect of aerial exposure on the activity of the mussels *Mytilus edulis* L. and *Modiolus modiolus* (L.). *Journal of Experimental Marine Biology and Ecology* 7: 295–304.

Collin R, Yu K, Chan K. 2016. The sea urchin *Lytechinus variegatus* lives close to the upper thermal limit for early development in a tropical lagoon. *Ecology and Evolution* 6: 5623–5634.

Cossins AR, Bowler K. 1987. *Temperature Biology of Animals*. Chapman and Hall, London.

Cowles RB, Bogert CM. 1944. Preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History* 83: 261–296.

Cox DK. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. In: Gibbons JW, Sharitz RR (eds), *Thermal Ecology*. National Technical Information Service, CONF-730505, Springfield, VA. pp 158-163.

Crashaw LI, O'Connor CS. 1997: Behavioural compensation for long-term thermal change. In: Wood CM, McDonald DG (eds), *Global warming: implications for freshwater and marine fish*. Cambridge University Press, Seminar Series-Society for Experimental Biology (61), Cambridge, United Kingdom. pp 351-371.

Cretchley R. 1996. *Exploitation of the bait organism Upogebia africana (Crustacea: Anomura) in the Knysna Estuary*.MSc thesis, Rhodes University, Grahamstown, South Africa.

Crisp DJ. 1964. The Effects of the Severe Winter of 1962-63 on Marine Life in Britain. *Journal of Animal Ecology* 33: 165–210.

Cuculescu M, Hyde D, Bowler K. 1998. Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. *Journal of Thermal Biology* 23: 107–110.

Cull F, Suski CD, Shultz A, Danylchuk AJ, O'Connor CM, Murchie KJ, Cooke SJ. 2015. Consequences of experimental cortisol manipulations on the thermal biology of the checkered puffer (*Sphoeroides testudineus*) in laboratory and field environments. *Journal of Thermal Biology* 47: 63–74.

Currie RJ, Bennett WA, Beitinger TL. 1998. Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environmental Biology of Fishes* 51: 187–200.

Daniel MJ, Boyden CR. 1975. Diurnal variations in physico-chemical conditions within intertidal rock pools. *Field Studies* 4: 161-176.

Davidson IR, Pearson GA. 1996. Stress tolerance of intertidal seaweeds. *Journal of Phycology* 32: 197-211.

Day EG, Branch GM. 2002. Influences of the sea urchin *Parechinus angulosus* (Leske) on the feeding behaviour and activity rhythms of juveniles of the South African abalone *Haliotis midae* Linn. *Journal of Experimental Marine Biology and Ecology* 276: 1-17.

Day JH. 1964: The origin and distribution of estuarine animals in South Africa. *Ecological studies in South Africa, Monographiae Biologicae* 24: 159-173.

Day JH. 1974. *Marine life on South African shores*. A.A. Balkema publications, Cape Town, South Africa. 329 pp.

Day JH. 1981. The estuarine fauna. In: Day JH (ed.), *Estuarine ecology with particular reference to southern Africa*. A. A. Balkema Publications, Cape Town, South Africa. pp 147-178.

deCastro M, Gomez-Gesteira M, Costoya X, Santos F. 2014. Upwelling influence on the number of extreme hot SST days along the Canary upwelling ecosystem. *Journal of Geophysical Research: Oceans* 119: 2183–2200.

deFur PL, Mangum CP. 1978. The effects of environmental variables on the heart rates of invertebrates. *Comparative Biochemistry and Physiology* 62A: 283–294.

De Grave S, Barnes DKA. 2001. Ecology of tropical hermit crabs (Crustacea Decapoda) at Quirimba Island, Mozambique: a multivariate assemblage perspective. *Tropical Ecology* 14: 197-209.

de Chazal P, Reilly RB 2003. Automatic classification of ECG beats using waveform shape and heart beat interval features. *2003 IEEE International Conference on Acoustics, Speech, and Signal Processing, 2003. Proceedings. (ICASSP '03).* 2: 269–72.

De Pirro M, Cannicci S, Santini G. 1999. A multi-factorial experiment on heart rate variations in the intertidal crab *Pachygrapsus marmoratus. Marine Biology* 135: 341–345.

Depledge MH. 1984. Cardiac activity in the intertidal crab *Hemigrapsus samguineus* (De Haan). *Asian Marine Biology* 1: 115-123.

Depledge MH, Andersen BB. 1990. A computer-aided physiological monitoring system for continous, long-term recording of cardiac activity in selected invertebrates. *Comparative Biochemistry and Physiology* 96: 473–411.

Desmond JS, Deutschman DH, Zedler JB. 2002. Spatial and temporal variation in estuarine fish and invertebrate assemblages: Analysis of an 11-year data set. *Estuaries* 25: 552–569.

Des Roches S, Post DM, Turley NE, Bailey JK, Hendry AP, Kinnison MT, Schweitzer JA, Palkovacs EP. 2018. The ecological importance of intraspecific variation. *Nature Ecology and Evolution* 2: 57–64.Deutsch C, Ferrel A, Seibel B, Pörtner HO, Huey RB. 2015. Climate change tightens a metabolic constraint on marine habitats. *Science* 348: 1132–1135.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences* 105: 6668–6672.

de Villiers C, Hodgson AN, Forbes A. 1999. Studies of estuarine macroinvertebrates. In: Allanson, BR, Baird D. (eds), *Estuaries of South Africa.* Cambridge University Press, Cambridge, United Kingdom. pp 167-208.

De Wachter B, McMahon BR. 1996. Temperature effects on heart performance and regional hemolymph flow in the crab *Cancer magister. Comparative Biochemistry and Physiology* 114: 27–33.

De Wachter B, Wilkens JL. 1996. Comparison of temperature effects on heart performance of

the dungeness Crab , *Cancer magister* , in vitro and in vivo. *Biological Bulletin* 190: 385–395.

Dias M, Roma J, Fonseca C, Pinto M, Cabral HN, Silva A, Vinagre C. 2016. Intertidal pools as alternative nursery habitats for coastal fishes. *Marine Biology Research* 12: 331–344.

Dias M, Silva A, Cabral HN, Vinagre C. 2014. Diet of marine fish larvae and juveniles that use rocky intertidal pools at the Portuguese coast. *Journal of Applied Ichthyology* 30: 970–977.

Diffenbaugh NS, Giorgi F, Pal JS. 2008. Climate change hotspots in the United States. *Geophysical Research Letters* 35: 1–5.

Dillon ME, Wang G, Huey RB. 2010. Global metabolic impacts of recent climate warming. *Nature* 467: 704–706.

Doney SC, Ruckelshaus M, Emmett Duffy J, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, et al. 2012. Climate change impacts on marine ecosystems. *Annual Review of Marine Science* 4: 11–37.

Dong Y-W, Williams GA. 2011. Variations in cardiac performance and heat shock protein expression to thermal stress in two differently zoned limpets on a tropical rocky shore. *Marine Biology* 158: 1223–1231.

Drost HE, Carmack EC, Farrell AP. 2014. Upper thermal limits of cardiac function for Arctic cod *Boreogadus saida*, a key food web fish species in the Arctic Ocean. *Journal of Fish Biology* 84: 1781–1792.

Dulvy NK, Rogers SI, Jennings S, Stelzenmüller V, Dye SR, Skjoldal HR. 2008. Climate change and deepening of the North Sea fish assemblage: a bitoic indicator of warming seas. *Journal of Applied Ecology* 45: 1029–1039.

Duncan MI. 2018. *A physiological study on a commercial reef fish to quantify the relationship between exploitation and climate change resilience*. PhD thesis, Rhodes University, Grahamstown, South Africa.

Duncan MI, Bates AE, James NC, Potts WM. 2019. Exploitation may influence the climate resilience of fish populations through removing high performance metabolic phenotypes. *Scientific Reports* 9: 11437: 1–10.

Dupont-Prinet A, Pillet M, Chabot D, Hansen T, Tremblay R, Audet C. 2013. Northern shrimp (*Pandalus borealis*) oxygen consumption and metabolic enzyme activities are severely constrained by hypoxia in the Estuary and Gulf of St. Lawrence. *Journal of Experimental Marine Biology and Ecology* 448: 298–307.

Edney EJ. 1961. The water and heat relationships of fiddler crabs (Uca spp.). *Transactions of the Royal Society of South Africa* 36: 71-91.

Edwards M, Richardson AJ. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* 430: 881–884.

Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP. 2011. Differences in thermal tolerance among Sockeye Salmon populations. *Science* 332: 109–112.

Eliason EJ, Clark TD, Hinch SG, Farrell AP. 2013. Cardiorespiratory performance and blood chemistry during swimming and recovery in three populations of elite swimmers: Adult sockeye salmon. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 166: 385–397.

Eme J, Bennett WA. 2009. Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. *Journal of Thermal Biology* 34: 220–225.

Eme J, Dabruzzi TF, Bennett WA. 2011. Thermal responses of juvenile squaretail mullet (*Liza vaigiensis*) and juvenile crescent terapon (*Terapon jarbua*) acclimated at near-lethal temperatures, and the implications for climate change. *Journal of Experimental Marine Biology and Ecology* 399: 35–38.

Emmerson WD. 1985. Seasonal abundance, growth and production of *palaemon pacificus* (Stimpson) in Eastern Cape tide pools, *South African Journal of Zoology* 20: 221-231.

Emmerson WD. 1986. The ecology of *Palaemon pacificus* (Stimpson) associated with *Zostra capensis*, Setchell. *Transactions of the Royal Society of South Africa* 46: 79-97.

Emmerson WD. 2016. *A guide to and checklist for, the Decapoda of Namibia, South Africa and Mozambique* (Vol. 3). Cambridge Scholars Publishing. 711 pp.

Fader S, Yu Z, Spotila J. 1994. Seasonal variation in heat shock proteins (hsp70) in stream fish under natural conditions. *Journal of Thermal Biology* 19: 335–341.

Fangue NA, Bennett WA. 2003. Thermal tolerance responses of laboratory-acclimated and seasonally acclimatized Atlantic stingray, *Dasyatis sabina*. *Copeia* 2003: 315–325.

Farmanfarmaian A. 1966. The respiratory physiology of the echinoderms. In: Boolootian RA (ed) *Physiology of echinodermata*. Interscience Publishers, USA

Farmanfarmaian A, Giese AC. 1963. Thermal tolerance and acclimation in the western purple sea urchin, *Strongylocentrotus purpuratus*. *Physiological Zoology* 36: 237–243.

Farrell AP. 2009. Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology* 212: 3771–3780.

Fessler JL, Westneat MW. 2007. Molecular phylogenetics of the butterflyfishes (*Chaetodontidae*): Taxonomy and biogeography of a global coral reef fish family. *Molecular Phylogenetics and Evolution* 45: 50–68.

Figueira WF, Booth DJ. 2010. Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Global Change Biology* 16: 506–516.

Figueiredo M, Morato T, Barreiros JP, Afonso P, Santos RS. 2005. Feeding ecology of the white seabream, *Diplodus sargus*, and the ballan wrasse, *Labrus bergylta*, in the Azores. *Fisheries Research* 75: 107–119.

Firth LB, Thompson RC, White FJ, Schofield M, Skov MW, Hoggart SPG, Jackson J, Knights AM, Hawkins SJ. 2013. The importance of water-retaining features for biodiversity on artificial intertidal coastal defence structures. *Diversity and Distributions* 19: 1275–1283.

Firth LB, Mieszkowska N, Grant LM, Bush LE, Davies AJ, Frost MT, Moschella, PS, Burrows, MT, Cunningham PN, Dye SR, Hawkins SJ. 2015. Historical comparisons reveal multiple drivers of decadal change of an ecosystem engineer at the range edge. *Ecology and Evolution* 5: 3210–3222.

Flint RW. 1985. Long-term estuarine variability and associated biological response. *Estuaries* 8: 158–169.

Floeter SR, Rocha LA, Robertson DR, Joyeux JC. 2008. Atlantic reef fish biogeography and evolution. *Journal of Biogeography* 35: 22–47.

Francour P, Boudouresque CF, Harmelin JG, Harmelin-Vivien ML, Quignard JP. 1994. Are the mediterranean waters becoming warmer? Information from biological indicators. *Marine Pollution Bulletin* 28: 523–526.

Fraser C. 2003. *Coastal dune dynamics and management at the bushman' s river mouth ,Kenton-on-sea*. MSc thesis, Rhodes University, Grahamstown, South Africa.

Fratini S, Vannini M, Cannicci S, Schubart CD. 2005. Tree-climbing mangrove crabs: A case of convergent evolution. *Evolutionary Ecology Research* 7: 219–233.

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: 1531–1538.

Fricke AH. 1978. Kelp grazing by the common sea urchin *Parechinus angulosus* Leske in False Bay, Cape. *African Zoology* 14: 143-148.

Fricke AH. 1980. Aspects of population structure of *Parechinus angulosus* Leske, around the Cape Peninsula. *South African Journal of Zoology* 15: 177-185.

Frölicher TL, Fischer EM, Gruber, N. 2018. Marine heatwaves under global warming. *Nature* 560: 360.

Froneman PW. 2004. Zooplankton community structure and biomass in a southern African temporarily open/closed estuary. *Estuarine, Coastal and Shelf Science* 60: 125-132.

Frusher SD, Hobday AJ, Jennings SM, Creighton C, D'Silva D, Haward M, Holbrook NJ, Nursey-Bray M, Pecl GT, van Putten EI. 2014. The short history of research in a marine climate change hotspot: From anecdote to adaptation in south-east Australia. *Reviews in Fish Biology and Fisheries* 24: 593–611.

Fry FEJ. 1947. Effects of the environment on animal activity. *Publications of the Ontario Fisheries Research Laboratory* 55: 1-62.

Fry FEJ. 1971. The effect of environmental factors on the physiology of fish. In: Hoar WS, Randall DJ. (eds), *Fish physiology*, (vol. 6). Academic Press, New York and London. pp 1-99.

Fulton EA. 2011. Interesting times: Winners, losers, and system shifts under climate change around Australia. *ICES Journal of Marine Science* 68: 1329–1342.

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 populations to global warming. *Oikos* 124: 784–795.

Ganachaud A, Vega A, Rodier M, Dupouy C, Maes C, Marchesiello P, Eldin G, Ridgway K, Le Borgne R. 2010. Observed impact of upwelling events on water properties and biological activity off the southwest coast of New Caledonia. *Marine Pollution Bulletin* 61: 449–464.

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

Gaston KJ, Blackburn TM, Spicer JI. 1998. Rapoport's rule: Time for an epitaph? *Trends in Ecology and Evolution* 13: 70–74.

Gershunov A, Douville HE. 2008. Extensive summer hot and cold extremes under current and possible future climatic conditions: Europe and North America. *Climate Extremes and Society* 9780521870: 74–98.

Gibson RN. 1972. The vertical distribution and feeding relationships of intertidal fish on the Atlantic coast of France. *Journal of Animal Ecology* 41: 189–207.

171

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

Giorgi F. 2006. Climate change hot-spots. *Geophysical Research Letters* 33: 1–4.

Goligorsky MS. 2001. The concept of cellular 'fight-or-flight'reaction to stress. *The American Physiological Society* 280: F551–F561.

Grange N, Allanson BR. 1995. The influence of freshwater inflow on the nature, amount and distribution of seston in estuaries of the Eastern cape, South Africa. *Estuarine, Coastal and Shelf Science* 40: 403–420.

Grange N, Whitfield AK, de Villiers CJ, Allanson BR. 2000. The response of two South African east coast estuaries to altered river flow regimes. *Aquatic Conservation: Marine and Freshwater Ecosystems* 10: 155–177.

Greenwood PJ. 1975. Population dynamics and ecological energetics of *Parechinus angulosus* at Robben Island and False Bay, South Africa. MSc thesis, University of Cape Town, South Africa.

Griffiths CL, King JA. 1979. Energy expended on growth and gonad output in the ribbed mussel *Aulacomya ater*. *Marine Biology* 53: 217–222.

Griffiths CL, Griffiths RJ. 1987. Bivalvia. In: Pandian TJ, Vemberg FJ (eds), *Bivalvia through Reptilia* (Vol. 2). Academic Press, New York. pp 1-88.

Griffiths MH, Wilke C, Penney AJ, Melo Y. 2002. Life history of white stumpnose *Rhabdosargus globiceps* (Pisces: Sparidae) off South Africa. *South African Journal Of Marine Science* 24: 281–300.

Griffiths CL, Robinson TB, Lange L, Mead A. 2010. Marine biodiversity in South Africa: An evaluation of current states of knowledge. *PLoS ONE* 5: e12008.

Gunderson AR, Stillman JH. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B: Biological Sciences* 282: 1–8.

Gunderson AR, Armstrong EJ, Stillman JH. 2016. Multiple stressors in a changing world : The need for an improved perspective on physiological responses to the dynamic marine environment. *Annual Review of Marine Science* 8: 357–378.

Gunter G. 1941. Death of fishes due to cold on the Texas coast , January , 1940. *Ecology* 22: 203–208.

Gunter G. 1951. Destruction of fishes and other organisms on the south Texas coast by the Cold Wave of January 28-February 3, 1951. *Ecology* 32: 731–736.

Guppy M, Withers P. 1999. Metabolic reduction in animals: Physiological perspectives and biochemical generalisations. *Biological Reviews* 74: 1–40.

Guppy M, Fuery CJ, Flanigan JE. 1994. Biochemical principles of metabolic depression. *Comparative Biochemistry and Physiology - Part B: Biochemistry and* 109: 175–189.

Gutschick VP, BassiriRad H. 2003. Extreme events as shaping physiology, ecology, and evolution of plants: Toward a unified definition and evaluation of their consequences. *New Phytologist* 160: 21–42.

Hanekom N, Baird D, Erasmus T. 1988. A quantitative study to assess standing biomasses of macrobenthos in soft substrate of the Swartkops Estuary, South Africa. *South African Journal of Marine Science* 6: 163-174.

Hanekom N. 1989. A note on the effects of a flood of medium intensity on macrobenthos of soft substrata in the Swartkops Estuary, South Africa. *South African Journal of Marine Science* 8: 349–355.

Harley CD., Hughes AR, Hultgren KM, Miner BG, Sorte CJ., Thornber CS, Rodriguez LF, Tomanek L, Williams SL. 2006. The impacts of climate change in coastal marine systems. *Ecology Letters* 9: 228–241.

Harrison TD. 2002. Preliminary assessment of the biogeography of fishes in South African estuaries. *Marine and Freshwater Research* 53: 479–490.

Harrison TD, Whitfield AK. 2006. Temperature and salinity as primary determinants

influencing the biogeography of fishes in South African estuaries. *Estuarine, Coastal and Shelf Science* 66: 335–345.

Harrison IJ. 2008. Mugilidae. In: Stiassny MLJ, Teugels GG, Hopkins CD (eds), *The fresh and brackish waters of Lower Guinea, West-Central Africa* (Vol. 2). Collection Faune et Flore tropicales 42. Institut de Rechereche pour le Développement, Paris, France, Muséum National d'Histoire Naturelle, Paris, France, and Musée Royal de l'Afrique Centrale, Tervuren, Belgium. pp 450-471.

Heemstra P, Heemstra E. 2004. *Coastal fishes of southern Africa*. National Inquiry Service Centre (NISC) and South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown. 488 pp.

Helm MM, Trueman ER. 1967. The effect of exposure on the heart rate of the mussel, *Mytilus edulis* L. *Comparative Biochemistry and Physiology* 21: 171–177.

Helmuth B., Harley CD, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA. 2002. Climate change and latitudinal patterns of intertidal thermal stress. *Science* 298: 2002–2004.

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

Heydorn AEF, Tinley KL. 1980. Estuaries of the Cape, Part 1-Synopsis of the Cape coast - Natural features, dynamics and utilization. CSIR Research Report 380, Stellenbosch, South Africa.

Hicks DW, McMahon RF. 2002. Respiratory responses to temperature and hypoxia in the nonindigenous brown mussel, *Perna perna* (*Bivalvia : Mytilidae*), from the Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* 277: 61–78.

Hiddink JG, ter Hofstede R. 2008. Climate induced increases in species richness of marine fishes. *Global Change Biology* 14: 453–460.

Hiestand W. 1940. Oxygen consumption of *Thyone briareus* (Holothuroidea) as a function of oxygen tension and hydrogen ion concentration of surrounding medium. *Transactions of the Wisconsin Academy of Sciences, Arts and Letters* 32:167–174.

Hightower LE, 1991. Heat shock, stress proteins, chaperones and proteotoxicity. *Cell* 66: 191–197.

Hill BJ. 1967. Contributions to the ecology of the Anomuran mud prawn *Upogebia africana* (Ortmann). PhD thesis, Rhodes University, Grahamstown, South Africa.

Hill BJ. 1971. Osmoregulation by an estuarine and a marine species of *Upogebia* (Anomura, Crustacea). *Zoologica Africana* 6: 229–236.

Hill BJ, Allanson BR. 1971. Temperature tolerance of the estuarine prawn *Upogebia africana* (Anomura, Crustacea). *Marine Biology* 11: 337–343.

Hines ME, Jones GE. 1985. Microbial biogeochemistry and bioturbation in sediments of Great Bay, New Hampshire. *Estuarine, Coastal and Shelf Science* 20: 729-742.

Hobday AJ, Pecl GT. 2014. Identification of global marine hotspots: Sentinels for change and vanguards for adaptation action. *Reviews in Fish Biology and Fisheries* 24: 415–425.

Hochachka PW, Somero GN. 2002. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press, Oxford, United Kingdom. 466 pp.

Hodgson AN. 1987. Distribution and abundance of the macrobenthic fauna of the Kariega estuary. *South African Journal of Zoology* 22: 153–162.

Hoegh-Guldberg O, Bruno JF. 2010. Review: The impact of climate change on the world's marine ecosystems. *Science* 328: 1523–1528.

Holt S, Holt G. 1983. Cold death of fishes at Port Aransas, Texas: January 1982. *The Southwestern Naturalist* 28: 464–466.

Hopkin RS, Qari S, Bowler K, Hyde D, Cuculescu M. 2006. Seasonal thermal tolerance in marine Crustacea. *Journal of Experimental Marine Biology and Ecology* 331: 74–81.

Horn MH, Martin KL. 2006. Rocky intertidal zone. In: Allen LG, Pondella DJ, Horn MH (eds), *The ecology of marine fishes: California and adjacent waters*. University of California Press, Berkeley, California. pp 205-226.

Horta e Costa B, Assis J, Franco G, Erzini K, Henriques M, Gonçalves EJ, Caselle JE. 2014. Tropicalization of fish assemblages in temperate biogeographic transition zones. *Marine Ecology Progress Series* 504: 241–252.

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

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

Hurst TP. 2007. Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71: 315–345.

IPCC. 2014. Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. P. (Core Writing Team, RK Pachauri, LA Meyer (Eds), IPCC, Geneva, Switzerland. 151 pp.

Iwama GK, Vijayan MM, Forsyth R, Ackerman PA. 1999. Heat shock proteins and physiological stress in fish. *American Zoologist* 39: 901–909.

James NC, Cowley PD, Whitfield AK, Lamberth SJ. 2007. Fish communities in temporarily open/closed estuaries from the warm- and cool-temperate regions of South Africa: A review. *Reviews in Fish Biology and Fisheries* 17: 565–580.

James NC, Harrison TD. 2010. A preliminary survey of the estuaries on the southeast coast of South Africa, Cape Padrone – Great Fish River, with particular reference to the fish fauna. *Transactions of the Royal Society of South Africa* 65: 149–164.

James NC, Van Niekerk L, Whitfield AK, Potts WM, Götz A, Paterson AW. 2013. Effects of climate change on South African estuaries and associated fish species. *Climate Research* 57: 233–248.

James NC, Whitfield AK, Harrison TD. 2016. Grey mullet (*Mugilidae*) as possible indicators of global warming in South African estuaries and coastal waters. *Marine Environmental Research* 122: 188–195.

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

Jørgensen LB, Overgaard J, MacMillan HA. 2017. Paralysis and heart failure precede ion balance disruption in heat-stressed European green crabs. *Journal of Thermal Biology* 68: 186–194.

Kalk M. 1995. *A natural history of Inhaca Island Mozambique*. Witwatersrand University Press, Johannesburg, South Africa. 395 pp.

Karsten U, Koch S, Kirst GO, West JA. 1996. Physiological responses of the eulittoral macroalga *Stictosiphonia hookeri* (*Rhodomelaceae, Rhodophyta*) from Argentina and Chile: Salinity, light and temperature acclimation. *European Journal of Phycology* 31: 361–368.

Kelly MW, Sanford E, Grosberg RK. 2012. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society B* 279: 349–356.

Kemp JOG, Froneman PW. 2004. Recruitment of ichthyoplankton and macrozooplankton during overtopping events into a temporarily open/closed southern African estuary. *Estuarine, Coastal and Shelf Science* 61: 529–537.

Kemp JOG. 2009. Effects of temperature and salinity on resting metabolism in two South African rock pool fish: the resident gobiid *Caffrogobius caffer* and the transient sparid *Diplodus sargus capensis*. *African Zoology* 44: 151–158.

Kensley B. 1981. On the zoogeography of southern African Decapod Crustacea, with a distributional checklist of the species. *Smithsonian Contributions to Zoology* 338: 1-64.

Kibirige I, Perissinotto R. 2003. The zooplankton community of the Mpenjati Estuary, a South African temporarily open/closed system. *Estuarine, Coastal and Shelf Science* 58: 727–741.

Killen SS, Costa I, Brown JA, Gamperl AK. 2007. Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B* 274: 431–438.

Kordas RL, Harley CDG, O'Connor MI. 2011. Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. *Journal of Experimental Marine Biology and Ecology* 400: 218–226.

Korhonen IA, Lagerspetz KY. 1996. Heat shock response and thermal acclimation in *Asellus aquaticus*. *Journal of Thermal Biology* 21: 49–56.

Kruger AC, Shongwe S. 2004. Temperature trends in South Africa: 1960-2003. *International Journal of Climatology* 24: 1929–1945.

Kuo ESL, Sanford E. 2009. Geographic variation in the upper thermal limits of an intertidal snail: Implications for climate envelope models. *Marine Ecology Progress Series* 388: 137–146.

Lannig G, Bock C, Sartoris FJ, Pörtner HO. 2004. Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L., studied by magnetic resonance imaging and on-line venous oxygen monitoring. *American journal of physiology. Regulatory, integrative and comparative physiology* 287: R902–R910.

Lawrence JM, Lane JM. 1982. The utilization of nutrients by post- metamorphic echinoderms. In: Jangoux M, Lawrence JM (eds), *Echinoderm Nutrition*. A.A. Balkema, Rotterdam.

Lefrançois C, Claireaux G. 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Marine Ecology Progress Series* 259: 273–284.

Lele Z, Engel S, Krone PH. 1997. Hsp47 and hsp 70 gene expression is differentially regulated in a stress- and tissue-specific manner in zebrafish embryos. *Developmental Genetics* 21:

123–133.

Levitan DR. 1995. The ecology of fertilization in free-spawning invertebrates. In: Mc Edward
L (ed.), *Ecology of marine invertebrate larvae*. CRC Press, Boca Raton. pp 123-156.

Lima FP, Ribeiro PA, Queiroz N, Hawkins SJ, Santos AM. 2007. Do distributional shifts of
northern and southern species of algae match the warming pattern? *Global Change
Biology* 13: 2592–2604.

Lima FP, Wethey DS. 2012. Three decades of high-resolution coastal sea surface temperatures
reveal more than warming. *Nature Communications* 3: 704.

Little C, Williams GA, Trowbridge CD. 2009. *The biology of rocky shores*. Oxford University
Press, Oxford, United Kigdom. 356 pp.

Logan CA, Kost LE, Somero GN. 2012. Latitudinal differences in *Mytilus californianus*
thermal physiology. *Marine Ecology Progress Series* 450: 93–105.

Logan CA, Buckley BA. 2015. Transcriptomic responses to environmental temperature in
eurythermal and stenothermal fishes. *The Journal of experimental biology* 218: 1915–
1924.

Lombard AT, Strauss T, Harris J, Attwood C, Hutchings L. 2004. South African National
Spatial Biodiversity Assessment Technical Report Volume 4: Marine Component.
Pretoria.

Lowe CH, Vance VJ. 1955. Acclimation of the critical thermal maximum of the reptile
Urosaurus Ornatus. *Science* 122: 73–74.

Lutjeharms JRE. 2007. Three decades of research on the greater Agulhas Current. *Ocean
Science* 3: 129–147.

Lutterschmidt WI, Hutchison VH. 1997a. The critical thermal maximum: history and critique.
Canadian Journal of Zoology 75: 1561–1574.

Lutterschmidt WI, Hutchison VH. 1997b. The critical thermal maximum: data to support the

onset of spasms as the definitive end point. *Canadian Journal of Zoology* 75: 1553–1560.

MacMillen RE, Greenaway, P. 1978. Adjustments of energy and water metabolism to drought in an Australian arid-zone crab. *Physiological Zoology* 51: 230–240.

Macnae WK, Kalk M. 1962. The ecology of the mangrove swamps at Inhaca Island, Mozambique. *The Journal of Ecology* 50: 19-34.

Madeira D, Narciso L, Cabral HN, Vinagre C. 2012a. Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. *Journal of Sea Research* 70: 32–41.

Madeira D, Narciso L, Cabral HN, Vinagre C, Diniz MS. 2012b. HSP70 production patterns in coastal and estuarine organisms facing increasing temperatures. *Journal of Sea Research* 73: 137–147.

Madeira D, Narciso L, Cabral HN, Vinagre C, Diniz MS. 2013. Influence of temperature in thermal and oxidative stress responses in estuarine fish. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 166: 237–243.

Madeira D, Narciso L, Cabral HN, Diniz MS, Vinagre C. 2014a. Role of thermal niche in the cellular response to thermal stress: Lipid peroxidation and HSP70 expression in coastal crabs. *Ecological Indicators* 36: 601–606.

Madeira D, Vinagre C, Costa PM, Diniz MS. 2014b. Histopathological alterations, physiological limits, and molecular changes of juvenile *Sparus aurata* in response to thermal stress. *Marine Ecology Progress Series* 505: 253–266.

Madeira D, Vinagre C, Narciso L, Diniz M. 2016. Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms. *Ecological indicators* 62: 317–327.

Mann BQ. 1992. Aspects of the biology of two inshore sparid fishes (*Diplodus sargus capensis* and *Diplodus cervinus hottentotus*) off the south-east coast of South Africa. MSc thesis, Rhodes University, Grahamstown, South Africa.

Mann BQ, Buxton CD. 1998. The reproductive biology of *Diplodus sargus capensis* and *D. cervinus hottentotus* (Sparidae) off the south-east Cape coast, South Africa. *Cybium* 22: 31-47.

Marais JFK. 1978. Routine oxygen consumption of *Mugil cephalus*, *Liza dumerili* and *L. richardsoni* at different temperatures and salinities. *Marine Biology* 50: 9–16.

Maree RC, Whitfield AK, Booth AJ. 2000. Effect of water temperature on the biogeography of South African estuarine fishes associated with the subtropical/warm temperate subtraction zone. *South African Journal of Science* 96: 184–188.

Marshall DJ, McQuaid CD. 1992. Relationship between heart rate and oxygen consumption in the intertidal limpets *Patella granularis* and *Siphonaria oculus*. *Comparative Biochemistry and Physiology* 103: 297–300.

Marshall DJ, McQuaid CD. 1993. Differential physiological and behavioural responses of the intertidal mussels, *Choromytilus meridionalis* (Kr.) and *Perna perna* L., to exposure to hypoxia and air: a basis for spatial separation. *Journal of Experimental Marine Biology and Ecology* 171: 225–237.

Marshall DJ, McQuaid CD. 1994. Seasonal and diel variations of in situ heart rate of the intertidal limpet *Siphonaria oculus* Kr. (Pulmonata). *Journal of Experimental Marine Biology and Ecology* 179: 1–9.

Marshall DJ, McQuaid CD. 2011. Warming reduces metabolic rate in marine snails: Adaptation to fluctuating high temperatures challenges the metabolic theory of ecology. *Proceedings of the Royal Society B: Biological Sciences* 278: 281–288.

Marshall DJ, Dong Y, McQuaid CD, Williams GA. 2011a. Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. *The Journal of Expermental Biology* 214: 3649–3657.

Marshall DJ, McQuaid CD, Williams GA. 2011b. Non-climatic thermal adaptation: implications for species' responses to climate warming. *Biology letters* 7: 160.

Martinho F, Dolbeth M, Viegas I, Teixeira CM, Cabral HN, Pardal MA. 2009. Environmental

effects on the recruitment variability of nursery species. *Estuarine, Coastal and Shelf Science* 83: 460–468.

Martin AP. 1988. Birds of the Swartkops Estuary and its environs and the effects of developments and bait exploitation. *South African National Scientific Programmes Report* 156: 86-94.

Martin CC, Tang P, Barnardo G, Krone PH. 2001. Expression of the chaperonin 10 gene during zebrafish development. *Cell Stress Chaperones* 6: 38-43.

Martins GM, Thompson RC, Hawkins SJ, Neto AI, Jenkins SR. 2008. Rocky intertidal community structure in oceanic islands: Scales of spatial variability. *Marine Ecology Progress Series* 356: 15–24.

Mather AA, Garland GG, Stretch DD. 2009. Southern African sea levels: Corrections, influences and trends. *African Journal of Marine Science* 31: 145–156.

Matthes H, Rinke A, Dethloff K. 2015. Corrigendum: Recent changes in Arctic temperature extremes: Warm and cold spells during winter and summer. *Environmental Research Letters* 10: 114020.

McBride RS, Able KW. 1998. Ecology and fate of butterflyfishes, Chaetodon spp., in the temperate, western North Atlantic. *Bulletin of Marine Science* 63: 401–416.

McKenzie DJ, Steffensen JF, Korsmeyer K, Whiteley NM, Bronzi P, Taylor EW. 2007. Swimming alters responses to hypoxia in the Adriatic sturgeon *Acipenser naccarii*. *Journal of Fish Biology* 70: 651–658.

McMahon RF. 1988. Respiratory response to periodic emergence in intertidal molluscs. *Integrative and Comparative Biology* 28: 97–114.

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

McMahon, R.F.; Ussery TA. 1995. Thermal tolerance of zebra mussels (*Dreissena polymorpha*) relative to rate of temperature increase and acclimation temperature.

McMahon RF. 1988. Respiratory response to periodic emergence in intertidal molluscs. *Integrative and Comparative Biology* 28: 97–114.

McPherson BC. 1968. Feeding and oxygen uptake of the tropical sea urchin, *Eucidari tribuloides* (Lamarck). *Biological Bulletin* 132:308–321.

McQuaid CD, Branch GM. 1984. Influence of sea temperature, substratum and wave exposure on rocky intertidal communities: an analysis of faunal and floral biomass. *Marine Ecology Progress Series* 19: 145–151.

McQuaid CD, Lindsay TL. 2000. Effect of wave exposure on the growth and mortality rates of the mussel *Perna perna*: bottom up regulation of intertidal populations. *Marine Ecology Progress Series* 206: 147-154.

Mead A, Griffiths CL, Branch GM, McQuaid CD, Blamey LK, Bolton JJ, Anderson RJ, Dufois F, Rouault M, Froneman PW, et al. 2013. Human-mediated drivers of change - impacts on coastal ecosystems and marine biota of South Africa. *African Journal of Marine Science* 35: 403–425.

Melzner F, Gutowska MA, Langenbuch M, Dupont S, Lucassen M, Thorndyke MC, Bleich M, Pörtner HO. 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: Pre-adaptation through lifestyle and ontogeny? *Biogeosciences* 6: 2313–2331.

Menasveta P. 1981. Lethal temperature of marine fishes of the Gulf of Thailand. *Journal of Fish Biology* 18: 603-607.

Messmer V, Pratchett MS, Hoey AS, Tobin AJ, Coker DJ, Cooke SJ, Clark TD. 2017. Global warming may disproportionately affect larger adults in a predatory coral reef fish. *Global Change Biology* 23: 2230–2240.

Metaxas A, Scheibling RE. 1993. Community structure and organization of tidepools. *Marine Ecology Progress Series* 98: 187–198.

Miranda PMA, Alves JMR, Serra N. 2013. Climate change and upwelling: Response of Iberian upwelling to atmospheric forcing in a regional climate scenario. *Climate Dynamics* 40: 2813–2824.

Mora C, Maya MF. 2006. Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. *Journal of Thermal Biology* 31: 337–341.

Mora C, Ospína AF. 2001. Tolerance to high temperatures and potential impact of sea warming on reef fishes of Gorgona Island (tropical eastern Pacific). *Marine Biology* 139: 765–769.

Morant P, Quinn N. 1999. Influence of man and management of South African estuaries. In: Allanson, BR, Baird D (eds), *Estuaries of South Africa*. Cambridge University Press, Cambridge, United Kingdom. pp 289-320.

Morley SA, Bates AE, Lamare M, Richard J, Nguyen KD, Brown J, Peck LS. 2016. Rates of warming and the global sensitivity of shallow water marine invertebrates to elevated temperature. *Journal of the Marine Biological Association of the United Kingdom* 96: 159–165.

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

Mostert BP. 2015. *Assessing the impact of climate change on mangrove crabs: the role of ontogenetic macrophysiology and settlement in the persistence of central and marginal populations*. PhD thesis, Rhodes University, Grahamstown, South Africa.

Mucina L, Adams JB, Knevel IC, Rutherford MC, Powrie LW, Bolton JJ. 2006. Coastal vegetation of South Africa. *The vegetation of South Africa, Lesotho and Swaziland. Sterilitza* 19: 650–583.

Muller CM. 2011. *The phylogeographic population structure of the Cape sea urchin, Parechinus angulosus*. MSc thesis, Stellenbosch Univeristy, Stellenbosch, South Africa.

Muller C, Skeeles MR, Childs A, van der Walt K, Winkler AC, Duncan MI, James NC, Potts WM. accepted. Implantation, orientation and validation of a commercially produced heart rate logger in a typical Perciform, *Pachymetopon grande* (Sparidae). *Conservation Biology*.

Munday PL, Crawley NE, Nilsson GE. 2009. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology*

Progress Series 388: 235–242.

Munday PL, McCormick MI, Nilsson GE. 2012. Impact of global warming and rising CO_2 levels on coral reef fishes: What hope for the future? *Journal of Experimental Biology* 215: 3865–3873.

Mylonas CC, Zohar Y, Pankhurst N, Kagawa H. 2011. Reproduction and broodstock management. In: Pavlidis M, Mylonas C (eds), *Sparidae: biology and aquaculture of gilthead sea bream and other species*. Wiley-Blackwell, Oxford.

Nakano K, Iwama GK. 2002. The 70-kDa heat shock protein response in two intertidal sculpins, *Oligocottus maculosus* and *O. snyderi*: Relationship of hsp70 and thermal tolerance. *Comparative Biochemistry and Physiology* 133: 79–94.

Nelson JA, Chabot D. 2011. General energy metabolism. In: Farrell AP (ed), *Encyclopedia of Fish Physiology: from genome to environment* (Vol. 3). Academic Press, San Diego, California. pp 1566-1572.

Newell RC. 1969. *The biology of intertidal animals*. American Elsevier, New York, USA. 555 pp.

Newell RC. 1979. *Biology of intertidal animals* (3rd edn). Marine Ecological Survey Ltd, Faversham, Kent, United Kingdom. 781 pp.

Newell RC, Armitage KB, Roy A. 1976. An analysis of factors affecting the oxygen consumption of the isopod *Ligia oceanica*. *Physiological Zoology* 49: 109-137.

Newell RC, Branch GM. 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. *Advances in Marine Biology* 17: 329–396.

Nguyen KDT, Morley SA, Lai CH, Clark MS, Tan KS, Bates AE, Peck LS. 2011. Upper temperature limits of tropical marine ectotherms: Global warming implications. *PLoS ONE* 6: e29340.

Nicastro KR, Zardi GI, McQuaid CD, Stephens L, Radloff S, Blatch GL. 2010. The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. *BMC*

Ecology 10: 1–11.

Nicholson S. 2002. Ecophysiological aspects of cardiac activity in the subtropical mussel *Perna viridis* (L.) (*Bivalvia: Mytilidae*). *Journal of Experimental Marine Biology and Ecology* 267: 207–222.

Nodo P, James NC, Childs A-R, Nakin MD V. 2018. Response of demersal fish assemblages to an extreme flood event in a freshwater-deprived estuary in South Africa. *Marine and Freshwater Research* 69: 253–266.

Norin T, Clark TD. 2016. Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology* 88: 122–151.

Oliver ECJ, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander L V., Benthuysen JA, Feng M, Sen Gupta A, Hobday AJ, et al. 2018. Longer and more frequent marine heatwaves over the past century. *Nature Communications* 9: 1–12.

Paganini AW, Miller NA, Stillman JH. 2014. Temperature and acidification variability reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes cinctipes*. *Journal of Experimental Biology* 217: 3974–3980.

Paterson AW, Whitfield AK. 2000. The ichthyofauna associated with an intertidal creek and adjacent eelgrass beds in the Kariega Estuary, South Africa. *Environmental Biology of Fishes* 58: 145–156.

Pauly D, Christensen V, Guénette S, Pitcher T., Sumaila UR, Walters CJ, Watson R, Zeller D. 2002. Towards sustainability in world fisheries. *Nature* 418: 689–695.

Payne N, Smith J, Van Der Meulen D, Taylor M, Watanabe Y, Takahashi A, Marzullo T, Gray C, Cadiou G, Suthers I. 2016. Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Functional Ecology* 30: 903–912.

Payne NL, Smith JA. 2017. An alternative explanation for global trends in thermal tolerance. *Ecology Letters* 20: 70–77.

Peck LS, Clark MS, Morley SA , Massey A, Rossetti H. 2009. Animal temperature limits and ecological relevance: Effects of size, activity and rates of change. *Functional Ecology* 23: 248–256.

Peck MA, Moyano M. 2016. Measuring respiration rates in marine fish larvae: Challenges and advances. *Journal of Fish Biology* 88: 173–205.

Pecl G, Hobday A, Frusher S, Sauer WH, Bates A. 2014. Ocean warming hotspots provide early warning laboratories for climate change impacts. *Revisions in Fish Biological Fisheries* 24: 409–413.

Perry AL, Low PJ, Ellis JR, Reynolds JD. 2005. Climate change and distribution shifts in marine fishes. *Science* 308: 1912–1915.

Pethick J. 1993. Shoreline adjustments and coastal management : Physical and biological processes under accelerated sea-level rise. *The Geographical Journal* 159: 162–168.

Pickens PE. 1965. Heart rate of mussels as a function of latitude, intertidal height, and acclimation temperature. *The university of Chicago Press Journals* 38: 390–405.

Pigliucci M. 2005. Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology and Evolution* 20: 481–486.

Pigliucci M, Murren CJ, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology* 209: 2362–2367.

Pirozzi I, Booth MA. 2009. The effect of temperature and body weight on the routine metabolic rate and postprandial metabolic response in mulloway, *Argyrosomus japonicus*. *Comparative Biochemistry and Physiology* 154: 110–118.

Posey MH, Dumbauld BR, Armstrong DA. 1991. Effects of a burrowing mud shrimp, *Upogebia pugettensis* (Dana), on abundances of macro-infauna. *Journal of Experimental Marine Biology and Ecology* 148:283-294.

Plaut I. 2000. Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline Cyprinodontid, *Aphanius dispar*, acclimated to a wide range of salinities.

Physiological and Biochemical Zoology 73: 590–596.

Pörtner HO, Hardewig I, Peck LS. 1999. Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternula elliptica*. *Comparative Biochemistry and Physiology-A Molecular and Integrative Physiology* 124: 179–189.

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

Pörtner HO. 2002. Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology* 132: 739–761.

Pörtner HO. 2006. Climate-dependent evolution of Antarctic ectotherms: An integrative analysis. *Deep-Sea Research Part II* 53: 1071–1104.

Pörtner HO, Bennett AF, Bozinovic F, Clarke A, Lardies MA, Lucassen M, Pelster B, Schiemer F, Stillman JH. 2006. Trade-Offs in Thermal Adaptation: The Need for a Molecular to Ecological Integration. *Physiological and Biochemical Zoology* 79: 295–313.

Pörtner HO, Knust R. 2007. Climate Change affects marine fishes through the oxygen Limitation of thermal tolerance. *Science* 315: 95–97.

Pörtner HO, Farrell AP. 2008. Physiology and Climate Change. *Science* 322: 690–692.

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

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

Pörtner H, Gutt J. 2016. Impacts of climate variability and change on (Marine) Animals: Physiological underpinnings and evolutionary consequences. *Integrative and Comparative Biology* 56: 31–44.

Pörtner HO, Bock C, Mark FC. 2017. Oxygen- and capacity-limited thermal tolerance : bridging ecology and physiology. *Journal of Experimental Biology* 220: 2685–2696.

Post JR, Lee AL. 1996. Metabolic ontogeny of teleost fishes. *Canadian Journal of Fish Aquatic Science* 53: 910–923.

Potts WA, Götz A, James NC. 2015. Review of the projected impacts of climate change on coastal fishes in southern Africa. *Reviews in Fish Biology and Fisheries* 25: 603–630.

Potts WM, Booth AJ, Richardson TJ, Sauer WHH. 2014. Ocean warming affects the distribution and abundance of resident fishes by changing their reproductive scope. *Revisions in Fish Biological Fisheries* 24: 493–504.

Prusina I, Sarà G, Pirro M De, Dong Y-W, Han G-D, Glamuzina B, Williams GA, De Pirro M, Dong Y-W, Han G-D, et al. 2014. Variations in physiological responses to thermal stress in congeneric limpets in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology* 456: 34–40.

Purchase DB 2017. *Patterns of distribution, abundance and community composition of rock pool fishes in Goukamma Nature Reserve, South Africa.* MSc thesis, University of Cape Town, Cape Town, South Africa.

Rajaguru S. 2002. Critical thermal maximum of seven estuarine fishes. *Journal of Thermal Biology* 27: 125–128.

Ramírez F, Afán I, Davis LS, Chiaradia A. 2017. Climate impacts on global hotspots of marine biodiversity. *Science Advances* 3: 1–8.

Ray GC. 1991. Coastal-zone biodiversity patterns - Principles of landscape ecology may help explain the processes underlying coastal diversity. *BioScience* 41: 490–498.

Reese ES. 1969. Behavioral adaptations of intertidal hermit crabs. *American Zoologist* 9: 343–

355.

Reise K. 1985. *Tidal Flat Ecology: An experimental approach to species interactions*. Springer Verlag, Berlin-Heidelberg. 191 pp.

Renema W, Bellwood DR, Braga JC, Bromfield K, Hall R, Johnson KG, Lunt P, Meyer CP, McMonagle LB, Morley RJ, O'Dea A. 2008. Hopping hotspots: global shifts in marine biodiversity. *Science* 321: 654-657.

Requena A, Fernandez-Borrás J, Planas J. 1997. The effects of a temperature rise on oxygen consumption and energy budget in gilthead sea bream. *Aquaculture International* 5: 415–426.

Reynolds RW, Smith TM, Liu C, Chelton DB, Casey KS, Schlax MG. 2007. Daily high-resolution-blended analyses for sea surface temperature. *Journal of Climate* 20: 5473–5496.

Richardson N, Whitfield AK, Paterson AW. 2006. The influence of selected environmental parameters on the distribution of the dominant demersal fishes in the Kariega Estuary channel, South Africa. *African Zoology* 41: 89–102.

Richardson TJ. 2010. The taxonomy, life-history and population dynamics of blacktail, *Diplodus capensis* (Perciformes: Sparidae), in southern Angola. MSc thesis, Rhodes University, Grahmastown, South Africa, 157 pp.

Richoux NB, Froneman PW. 2007. Assessment of spatial variation in carbon utilization by benthic and pelagic invertebrates in a temperate South African estuary using stable isotope signatures. *Estuarine, Coastal and Shelf Science* 71: 545–558.

Richoux NB, Vermeulen I, Froneman PW. 2014. Stable isotope ratios indicate differential omnivory among syntopic rocky shore suspension-feeders. *Marine Biology* 161: 971–984.

Rijnsdorp AD, Peck MA, Engelhard GH, Molmann C, Pinnegar JK. 2009. Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science* 66: 1570–1583.

Roberts MJ. 2005. Chokka squid (*Loligo vulgaris reynaudii*) abundance linked to changes in

South Africa's Agulhas Bank ecosystem during spawning and the early life cycle. *ICES Journal of Marine Science* 62: 33–55.

Robertson DR. 1991. The role of adult biology in the timing of spawning of tropical reef fishes. In: Sale PF (ed), *The ecology of fishes on coral reefs*. Academic Press, San Diego, pp 356–386.

Robertson WD. 1984. Aspects of the ecology of the shrimp *Palaemon pacificus* (Stimpson) (Decapoda, Palaemonidae) in the Bushman's River Estuary. MSc thesis, Rhodes University, Grahamstown, South Africa.

Rodland DL, Schöne BR, Baier S, Zhang Z, Dreyer W, Page NA. 2009. Changes in gape frequency, siphon activity and thermal response in the freshwater bivalves *Andonta cygnea* and *Margaritifera falcata*. *Journal of Molluscan Studies* 75: 51-57.

Roessig JM, Woodley CM, Cech JJ, Hansen LJ. 2004. Effects of global climate change on marine and estuarine fishes and fisheries. *Reviews in Fish Biology and Fisheries* 14: 251–275.

Rouault M, Lee-Thorp AM, Ansorge I, Lutjeharms JR. 1995. Agulhas Current air-sea exchange experiment. *South African Journal of Science* 91: 493–496.

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: 237–246.

Rovero F, Hughes RN, Whiteley NM, Chelazzi G. 2000. Estimating the energetic cost of fighting in shore crabs by noninvasive monitoring of heartbeat rate. *Animal Behaviour* 59: 705–713.

Rummer JL, Binning SA, Roche DG, Johansen JL. 2016. Methods matter: Considering locomotory mode and respirometry technique when estimating metabolic rates of fishes. *Conservation Physiology* 4: 1–13.

Sabourin TD, Stickle WB. 1981. Effects of salinity on respiration and nitrogen excretion in two species of echinoderms. *Marine Biology* 65: 91–99.

Sánchez-Fernández D, Aragón P, Bilton DT, Lobo JM. 2012. Assessing the congruence of thermal niche estimations derived from distribution and physiological data. A test using diving beetles. *PLoS ONE* 7: e48163.

Santacruz H, Vriz S, Angelier N. 1997. Molecular Characterization of a heat shock cognate cDNA of zebrafish, hsc70, and developmental expression of the corresponding transcripts. *Developmental Genetics* 21: 223–233.

Santini G, De Pirro M, Chelazzi G. 1999. In Situ and laboratory assessment of heart rate in a Mediterranean Limpet using a noninvasive technique. *Physiological and Biochemical Zoology* 72: 198–204.

Sarà G, De Pirro M. 2011. Heart beat rate adaptations to varying salinity of two intertidal Mediterranean bivalves: The invasive *Brachidontes pharaonis* and the native *Mytilaster minimus* adaptation of intertidal bivalves at varying salinity. *Italian Journal of Zoology* 78: 193–197.

Schaefer N. 1970. The functional morphology of the foregut of three species of Decapod Crustacea: *Cyclograpsus punctatus* Milne Edwards, *Diogenes brevirostris* Stimpson and *Upogebia africana* Ortmann. *African Zoology* 5: 309–326.

Schaefer J, Ryan A. 2006. Developmental plasticity in the thermal tolerance of zebrafish *Danio rerio*. *Journal of Fish Biology* 69: 722–734.

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

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

Schulte PM. 2015. The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *The Journal of Experimental Biology* 218: 1856–1866.

Sheaves M, Baker R, Nagelkerken I, Connolly RM. 2015. True value of estuarine and coastal nurseries for fish: Incorporating complexity and dynamics. *Estuaries and Coasts* 38: 401–

414.

Shick JM. 1983. Respiratory gas exchange in echinoderms. In: Jangoux M, Lawrence JM (eds), *Echinoderms studies* 1. Balkema, Rotterdam.

Shick JM, Gnaiger E, Widdows J, Bayne BL, de Zwaan A. 1986. Activity and metabolism in the mussel *Mytilus edulis* L. during intertidal hypoxia and aerobic recovery. *Physiological Zoology* 59: 627-642.

Shultz DA, Zuckerman ZC, Suski CD. 2016. Thermal tolerance of nearshore fishes across seasons : implications for coastal fish communities in a changing climate. *Marine Biology* 163: 1–10.

Sidhu R, Anttila K, Farrell AP. 2014. Upper thermal tolerance of closely related *Danio* species. *Journal of Fish Biology* 84: 982–995.

Siikavuopio SI; Christiansen JS; Dale T. 2006. Effects of temperature and season on gonad growth and feed intake in the green sea urchin (*Strongylocentrotus droebachiensis*). *Aquaculture* 225: 389–394.

Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S, Dong Y, Harley CDG, Marshall DJ, Helmuth BS, et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters* 19: 1372–1385.

Skeeles MR, Winkler AC, Duncan MI, James NC, van der Walt K, Potts WM. accepted. The use of implantable heart rate loggers in determining breakpoints of marine fish during an acute warming event. *Journal of thermal biology*.

Smale MJ, Buxton C. 1989. The subtidal gully fish community of the Eastern Cape and the role of this habitat as a nursery area. *African Zoology* 24: 58–67.

Smith MM, Heemstra PC. 1991. *Smith's Sea Fishes* (1st edn). Southern Book Publishers, Johannesburg, 1047 pp.

Somero GN. 2002. Thermal physiology and vertical zonation of intertidal animals: optima,

limits, and costs of living. *Integrative and comparative biology* 42: 780–789.

Somero GN. 2005. Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in zoology* 2: 1–9.

Somero GN. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *The Journal of experimental biology* 213: 912–920.

Somero GN. 2012. The physiology of global change: Linking patterns to mechanisms. *Annual Review of Marine Science* 4: 39–61.

Speers-Roesch B, Norin T. 2016. Ecological significance of thermal tolerance and performance in fishes: new insights from integrating field and laboratory approaches. *Functional Ecology* 30: 842–844.

Spicer JI, Gaston KJ. 1999. *Physiological diversity and its ecological implications.* Blackwell Science, Oxford, United Kingdom. 241 pp.

Stamhuis EJ, Schreurs CE, Videler JJ. 1998. Burrow architecture and turbative activity of the thalassinid shrimp *Callianassa subterranean* from central North Sea. *Marine Ecology Progress Series* 154: 155-163.

Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, Farrell AP. 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *Journal of Experimental Biology* 211: 3915–3926.

Stenseng E, Braby CE, Somero GN. 2005. Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (Genus *Tegula*): Implications for vertical zonation. *Biological Bulletin* 208: 138–144.

Stevens GC. 1989. The latitudinal gradient in geographical range : How so many species coexist in the tropics. *The University of Chicago Press for the Americal Society of naturalists* 133: 240–256.

Stickle WB, Diehl WJ. 1987. Effects of salinity on echinoderms. In: Jangoux M, Lawrence JM (eds), *Echinoderm studies* (Vol 2), A.A. Balkema, Rotterdam.

Stillman JH. 2002. Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, Genus *Petrolisthes*. *Integrative and Comparative Biology* 42: 790–796.

Stillman JH 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301: 65.

Stillman JH, Somero GN. 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (Genus *Petrolisthes*): Correlation of physiology, biochemistry and morphology with vertical distribution. *The journal of experimental Biology* 199: 1845–1855.

Storey KB, Storey JM. 2004. Metabolic rate depression in animals: Transcriptional and translational controls. *Biological Reviews of the Cambridge Philosophical Society* 79: 207–233.

Stuart V, Field JG. 1981. Respiration and ecological energetics of the sea urchin *Parechinus angulosus*. *South African Journal of Zoology* 16: 91-95.

Suchanek TH. 1985. Mussels and their role in structuring rocky shore communities. In: Moore PG, Seed R (eds), *The ecology of rocky coasts*. Hodder and Stoughton, London. pp 70-96.

Summerer M, Hanel R, Sturmbauer C. 2001. Mitochondrial phylogeny and biogeographic affinities of sea breams of the genus *Diplodus* (*Sparidae*). *Journal of Fish Biology* 59: 1638–1652.

Sunday JM, Bates AE, Dulvy NK. 2011. Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences* 278: 1823–1830.

Sunday JM, Bates AE, Dulvy NK. 2012. Thermal tolerance and the global redistribution of animals. *Nature Climate Change* 2: 686–690.

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

535: 145–159.

Tagliarolo M, McQuaid CD. 2016. Field measurements indicate unexpected, serious underestimation of mussel heart rates and thermal tolerance by laboratory studies. *PLoS ONE* 11: e0146341.

Talbot T, Lawrence JM. 2002. The effect of salinity on respiration, excretion, regeneration and production in *Ophiophragmus filograneus* (Echinodermata: Ophiuroidea). *Journal of Experimental Marine Biology and Ecology* 275:1–14.

Taylor PR. 1982. Environmental resistance and the ecology of coexisting hermit crabs: thermal tolerance. *Journal of Marine Biology and Ecology* 57: 229–236.

Taylor D, Allanson B. 1993. Impacts of dense crab populations on carbon exchanges across the surface of a salt marsh. *Marine Ecology Progress Series* 101:119-129.

Taylor EW, Egginton S, Taylor SE, Butler PJ. 1997. Factors which may limit swimming performance at different temperatures. In: McDonald DG, Wood CM (eds), *Global warming: Implications for freshwater and marine fish*. Cambridge University Press, Cambridge, United Kingdom. pp 105-133.

Taylor J, Cook M, Kirkpatrick A, Galleher S, Eme J, Bennett WA. 2005. Thermal tactics of air-breathing and non air-breathing Gobiids inhabiting mangrove tidepools on Pulau Hoga, Indonesia. *Copeia* 4: 885-892.

Teal JM, Carey FG. 1967. Division of comparative physiology and biochemistry, society for integrative and comparative biology. *Physiological Zoology* 40: 83–91.

Tepolt CK, Somero GN. 2014. Master of all trades: Thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. *Journal of Experimental Biology* 217: 1129–1138.

Teske PR, Wooldridge TH. 2004. Affinities of some common estuarine macroinvertebrates to salinity and sediment type: empirical data from Eastern Cape estuaries, South Africa. *African Zoology* 39: 183–192.

Thompson RC, Crowe TP, Hawkins SJ. 2002. Rocky intertidal communities : past environmental changes, present status and predictions for the next 25 years. *Environmental Conservation* 29: 168–191.

Thomson JM. 1986. Mugilidae. In: Daget J, Gosse JP, Thys van den Aufenaerde DFE (eds), *Check-list of freshwater fishes of Africa (CLOFFA)* (Vol. 2). ISBN, Brussels, MRAC, Tervuren and Orstom, Paris. pp 344-349.

Thornton P, Ericksen P, Herrero M, Challinor AJ. 2014. Climate variability and vulnerability to climate change : A review. *Global Change Biology* 20: 3313–3328.

Tibblin P, Forsman A, Borger T, Larsson P. 2016. Causes and consequences of repeatability, flexibility and individual fine-tuning of migratory timing in pike. *Journal of Animal Ecology* 85: 136–145.

Tittensor DP, Mora C, Jetz W, Lotze HK, Ricard D, Berghe EV, Worm B. 2010. Global patterns and predictors of marine biodiversity across taxa. *Nature* 466: 1098–1101.

Tomanek L, Somero GN. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: Implications for limits of thermotolerance and biogeography. *The Journal of Experimental Biology* 202: 2925–2936.

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*): Implications for regulation of hsp gene expression. *Journal of Experimental Biology* 205: 677–685.

Trueman ER, Lowe GA. 1971. The effect of temperature and littoral exposure on the heart rate of a bivalve mollusc, I*sognomum alatus*, in tropical conditions. *Comparative Biochemistry and Physiology -- Part A: Physiology* 38: 555–564.

Underwood AJ. 1979. The ecology of intertidal gastropods. *Advances in Marine Biology* 16: 111-210.

Underwood AJ, Fairweather PG. 1989. Supply-side ecology and benthic marine assemblages. *Trends in Ecology and Evolution* 4: 16-20.

Underwood AJ, Kingsford MJ, Andrew NL. 1991. Patterns in shallow subtidal marine assemblages along the coast of New South Wales, Australia. *Australian Journal of Ecology* 16: 231-250.

Underwood AJ, Keough MJ. 2001. Supply-side ecology: the nature and consequences of variations in recruitment of intertidal organisms. In: Bertness MD, Gaines SD, Hay ME (eds), *Marine community ecology*. Sinauer Associates, Inc. Publishers, Sunderland. pp 183-200.

van der Vyver JSF, Kaiser H, Potts WM, James N. 2013. Using blood plasma cortisol concentration and fish behaviour to determine temperature avoidance in the estuarine-dependent fish species *Rhabdosargus holubi* (steindachner, 1881) (*Sparidae*). *Journal of Applied Ichthyology* 29: 1275–1278.

van Erkom Schurink C, Griffiths CL. 1992. Physiological energetics of four South African mussel species in relation to body size, ration and temperature. *Comparative Biochemistry and Physiology -- Part A: Physiology* 101: 779–789.

van Erkom Schurink C, Griffiths CL. 1993. Factors affecting relative rates of growth in four South African mussel species. *Aquaculture* 109: 257-273.

Vergés A, Steinberg PD, Hay ME, Poore AG, Canpbell AH, Ballesteros E, Heck KL, Booth DL, Coleman MA, Feary DA, et al. 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society B: Biological Sciences* 281: 20140846.

Vernberg WB, Vernberg FJ. 1968. Physiological diversity in metabolism in marine and terrestrial crustacea. *Integrative and Comparative Biology* 8: 449–458.

Vijayan MM, Campbell T, Strutt E, Sathiyaa, R. 2000. *Cortisol modulates heat shock protein expression in primary cultures of rainbow trout hepatocytes*. Proceedings of 4th International Symposium on Fish Endocrinology, Seattle, Washington.

Vinagre C, Cabral HN, Costa MJ. 2010. Relative importance of estuarine nurseries for species of the genus *Diplodus* (*Sparidae*) along the Portuguese coast. *Estuarine, Coastal and Shelf Science* 86: 197–202.

Vinagre C, Dias M, Roma J, Silva A, Madeira D, Diniz MS. 2013. Critical thermal maxima of common rocky intertidal fish and shrimps — A preliminary assessment. *Journal of Sea Research* 81: 10–12.

Vinagre C, Leal I, Mendonça V, Flores AAV. 2015. Effect of warming rate on the critical thermal maxima of crabs , shrimp and fish. *Journal of Thermal Biology* 47: 19–25.

Vinagre C, Leal I, Mendonça V, Madeira D, Narciso L, Diniz MS, Flores AAV. 2016. Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms. *Ecological Indicators* 62: 317–327.

Vinagre C, Mendonça V, Cereja R, Abreu-Afonso F, Dias M, Mizrahi D, Flores AAV. 2018. Ecological traps in shallow coastal waters-Potential effect of heat-waves in tropical and temperate organisms. *PLoS ONE* 13: 1–17.

Vine NG 1998. *Aspects of the biology of the doublesash butterflyfish, Chaetodon marleyi*. MSc thesis, Rhodes University, Grahamstown, South Africa.

Virnstein RW. 1977. The importance of predation by crabs and fishes on benthic infauna in Chesapeake Bay. *Ecology* 58: 1199-1217.

Vorsatz JP. 2009. Ecological role of estuarine brachyuran crabs in mangrove and salt marsh estuaries, Eastern Cape, South Africa, PhD thesis, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa.

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: 203–214.

Wallace JH. 1975. The estuarine fishes of the east coast of South Africa: I Species composition and length distribution in the estuarine and marine environments. II. *Seasonal abundance*

and migrations. Investigational Report, Oceanographic Research Institute, Durban 40: 1-72.

Wernberg T, Russell BD, Moore PJ, Ling SD, Smale DA, Campbell A, Coleman MA, Steinberg PD, Kendrick GA, Connell SD. 2011. Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming. *Journal of Experimental Marine Biology and Ecology* 400: 7–16.

Wernberg T, Smale DA, Thomsen MS. 2012. A decade of climate change experiments on marine organisms : Procedures, patterns and problems. *Global Change Biology* 18: 1491–1498.

Whitifield AK. 1988. The fish community of the Swartvlei Estuary and the influence of food availability on resource utilization. *Estuaries* 11: 160-170.

Whitifield AK. 1998. *Biology and ecology of fishes in southern African estuaries* (Vol. 2). Ichthyological Monograph, JLB Smith Institute of Ichthyology, Grahamstown, South Africa. 223 pp.

Whitfield AK. 1999. Ichthyofaunal assemblages in estuaries: A South African case study. *Reviews in Fish Biology and Fisheries* 9: 151–186.

Whitfield AK, Paterson AW. 2003. Distribution patterns of fishes in an Eastern Cape estuary and river with particular emphasis on the ebb and flow region. *Water SA* 29: 61–68.

Whitfield AK. 2005. Preliminary documentation and assessment of fish diversity in sub-Saharan African estuaries. *African Journal of Marine Science* 27: 307–324.

Whitfield A, Elliott M. 2011. *Ecosystem and Biotic Classifications of Estuaries and Coasts.* Elsevier Inc.

Whitfield AK. 2019. *Fishes of southern african estuaries: from species to systems* (4th edn). Grahamstown: Smithiana Monograph, South African Intitute for Aquatic Biodiversity (SAIAB).

Widdows J, Fieth P, Worrall CM. 1979. Relationships between seston, available food and

feeding activity in the common mussel *Mytilus edulis*. *Marine Biology* 50: 195–207.

Wieser W. 1985. Developmental and metabolic constraints of the scope for activity in young rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* 118: 133–142.

Wieser W, Forstner H. 1986. Effects of temperature and size on the routine rate of oxygen consumption and on the relative scope for activity in larval cyprinids. *Journal of Comparative Physiology B* 156: 791–796.

Winberg GG. 1960. Rate of metabolism and food requirements of fishes. *Fisheries Research Board of Canada Translation Series* 194: 202.

Wood HL, Spicer JI, Widdicombe S. 2008. Ocean acidification may increase calcification rates—butatacost. *Proceedings of the Royal Society* 275:1767–1773.

Wood HL, Spicer JI, Lowe DM, Widdicombe S. 2010. Interaction of ocean acidification and temperature; the high cost of survival in the brittlestar *Ophiura ophiura*. *Marine Biology* 157:2001–2013.

Wood HL, Spicer JI, Kendall MA, Lowe DM, Widdicombe S. 2011. Ocean warming and acidification; implications for the Arctic brittlestar *Ophiocten sericeum*. *Polar Biology* 34:1033-1044.

Wooldridge TH. 1999. Estuarine zooplankton community structure and dynamics. In: Allanson BR, Baird D (eds), *Estuaries of South Africa*. Cambridge University Press, Cambridge, United Kingdom. pp 141-166.

Worm B, Lotze HK, Myers RA. 2003. Predator diversity hotspots in the blue ocean. *Proceedings of the National Academy of Sciences* 100: 9884–9888.

Wynberg RP, Branch GM. 1994. Disturbance associated with bait collecting for sandprawns (*Callianassa kraussi*) and mudrawns (*Upogebia africana*): Long term effects on the biotta of intertidal sandflats. *Journal of Marine Research* 52: 523-558.

Yool A, Fasham JR. 2001. An examination of the " continental shelf pump " in an open ocean general circulation model. *Global Biochemical Cycles* 15: 831–844.

Zainal KAY, Taylor AC, Atkinson RJA. 1992. The effect of temperature and hypoxia on the respiratory physiology of the squat lobster, *Munida rugose* and *Munida sarsi* (Anomura, Galatheidae). *Comparative Biochemistry and Physiology Part A: Physiology* 101: 557-567.

Zander C, Nieder J, Martin K. 1999. Vertical distribution patterns. In: Horn MH, Martin KLM, Chotkowski MA (eds), *Intertidal Fishes: Life in two worlds* (Vol. 1). Academic Press. pp 26-48.

Zardi GI, McQuaid CD, Nicastro KR. 2007a. Balancing survival and reproduction: Seasonality of wave action, attachment strength and reproductive output in indigenous *Perna perna* and invasive *Mytilus galloprovincialis* mussels. *Marine Ecology Progress Series* 334: 155–163.

Zardi GI, McQuaid CD, Teske PR, Barker NP. 2007b. Unexpected genetic structure of mussel populations in South Africa: Indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. *Marine Ecology Progress Series* 337: 135–144.

Zardi GI, Nicastro KR, McQuaid CD, Hancke L, Helmuth B. 2011. The combination of selection and dispersal helps explain genetic structure in intertidal mussels. *Oecologia* 165: 947-958.

Zoutendyk P, Bickerton I. 1988. Burrow identification of some estuarine organisms. *South African Journal of Zoology* 23: 235–238.

APPENDIX A

Classification and description of macroscopic gonad maturity stages of *Rhabdosargus globiceps* used to identify sexual maturity for adult *Diplodus capensis* in Chapter 4. Taken from Griffiths *et al.* (2002).

Stage	Macroscopic Description
1: Inactive	Sexual organs small. Ovaries appear translucent, yellow to pinkish sacs with no visible eggs. Testis thin and transparent to greyish-white
2: Active	Ovary swells and increases in length to 90% of visceral cavity. Colour changes to opaque orang/yellow and eggs are visible to naked eye. Testis become thicker and beige to white in colour. Sperm is present in the main sperm duct, but not in the tissue.
3: Ripe	Ovary is swollen and amber in colour owing to the presence of a substantial proportion of hydrated/transparent eggs. Testis is swollen and white. It is easily ruptured if lightly pinched, and perm is present in the sperm duct as well as tissue.
4: Spent	Ovary substantially smaller and flaccid with large lumen. It is reddish orange in colour with few eggs visible. Spent testes were not identifiable.