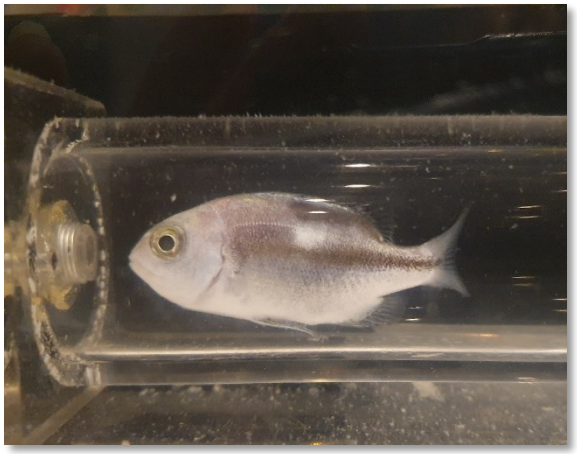
Thermal physiology of juvenile red roman seabream, *Chrysoblephus laticeps* after long-term exposure to low pH conditions



Thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

By

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29/08/2023

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Preface

This thesis consists of a general introduction (Chapter 1), general methods (Chapter 2), two research chapters (Chapters 3 and 4) and a general discussion and conclusion (Chapter 5).

Additional outputs completed, or in the process of completion during this thesis:

Conference presentations:

- Southern African Marine Science Symposium, 2022.
- Departmental seminar series, 2022.

Papers arising during this thesis:

- **Allison C,** Winkler AC, Childs A-R, Muller C, Potts WM. Can social media platforms be used to foster improved environmental behaviour in recreational fisheries? *Fisheries Research* **258:**106544. DOI:https://doi.org/10.1016/j.fisheries.2022.106544.

Financial support was received from the following sources during this thesis:

Travel funding:

- Rhodes University MSc travel grant.

Personal funding:

- The Henderson Scholarship

Contributions of others during this thesis:

Technical and Statistical support

- Dr Cuen Muller

Ethics statement:

This research project, of which this thesis is apart, received research ethics approval from Rhodes University Animal Ethics Committee (DIFS152018) under the project titled: "Fisheries-induced-evolution on fish thermal physiology – implications for climate change adaptation'.

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Acknowledgements

To my parents, Peter and Pam Allison, who have, with unwavering presence, supported me throughout this journey. You have given in great abundance all the love, nurturing and wisdom that I needed to grow, but in the same breath, you have always offered me the space and freedom I needed to develop into my own person. You have taught me that life is brief and precious and so it is a fundamental duty to grow, learn and love as much as we can while we are here. I have cherished every step of this journey – including the trip ups, the amble wonderings, getting lost, getting lost again and then finding my way back to the greatest love of my life, which is the ocean and all that goes with it. Your guidance into this venture has given me a life with great purpose, but at the same time, a life that has been, above all else, great fun. I love you both to the moon and back.

To Josi, Ben, and Jono. I have learnt so much from each one of you, and you have, in your own way, at different times along the road, offered the generosity of your, love, support, and friendship - the most precious gifts. I am so grateful for each one of you. To all the people I met along the way, at coffee shops, on the road, or in the water, who have offered small kindnesses on the days when it was hard to write, you have made the process infinitely more enjoyable. Thank you.

To my supervisors Dr Amber Childs and Prof. Warren Potts, your commitment and passion have inspired me every step of the way, and you have both shaped me into the scientist that I am. Dr Cuen Muller, thank you for your patience and help in the creation of this thesis, particularly the practical elements and the statistics. All three of you have been incredible educators.

To my dog, Kit, a constant companion, the greatest adventurer, love you, boy.

Declaration

I, Caitlin Allison, hereby declare that the work described in this thesis was carried out in the Department of Ichthyology and Fisheries Science, Rhodes University, under the supervision of Professor Warren Potts, Dr Amber Childs, and Dr Cuen Muller. The components of this thesis comprise original work by the author and have not been submitted to any other university.

Signed: 

Date: _29/08/2023_____

Abstract

Climate change has caused a combination of effects on the physiology of fishes. Of particular concern are the effects of thermal variability and ocean acidification. Organismal energy budgets change throughout ontogeny and research into the metabolic scope during early life stages is particularly useful in identifying potential bottlenecks. The first part of this thesis aimed to assess the absolute aerobic scope (AAS, described as the difference between the maximum and standard metabolic rates) of individual juveniles from a protected population of the endemic, commercially important seabream, *Chrysoblephus laticeps*, across a range of ecologically relevant temperatures (T = 11, 14, 18, 22˚C) under present-day conditions (pH = 8.03, $pCO_2 \approx 420$ µatm) using intermittent flow respirometry. The second component sought to investigate how long-term exposure (from fertilisation to juvenile, ~100 days exposure) to high-pCO_2/hypercapnic conditions (pH = 7.63, $pCO_2 \approx 1400$ µatm), would affect the AAS of juvenile *C. laticeps* over a range of temperatures. Lower pH conditions were predicted to cause a decrease in the AAS of treatment animals due to additional energetic costs of acid-base regulation. The findings of the first data chapter demonstrated that juvenile *C. laticeps* reared under current CO_2 conditions are tolerant to a wide range of thermal conditions, and individuals with a broad aerobic scope will be the best suited to coping with enhanced thermal variability. In contrast to the expected outcomes of the second data chapter, juvenile *C. laticeps* reared under high pCO_2 conditions displayed greater AAS at high and low temperatures when compared with specimens from high pH conditions. Whilst a high degree of individual phenotypic variation was observed in the metabolic response of both groups, this was reduced at the lower and upper extreme temperatures for high pH and low pH animals respectively. Notably, the variation in treatment animal's SMR was significantly diminished across all temperatures tested, compared to only a localised reduction in the SMR of high pH animals at cold temperatures. This may be indicative of compensatory pathways affecting energy restructuring and thermally-governed physiological trade-offs under hypercapnia. Given these results, juvenile *C. laticeps* appear to be more resilient to ocean acidification than anticipated, potentially owing to intrapopulation metabolic phenotypic diversity. This is likely attributed to the parental lineage originating in the Tsitsikamma MPA, which is thought to boast greater phenotypic diversity as a consequence of the refuge that these conservation areas offer from exploitation. Owing to the restriction imposed by the availability of surviving, captive-reared juveniles, the sample size used in this study was relatively low. However, owing to the

repeated-measures nature of this research the sample size was sufficient to offer suitable statistical power for the polynomial mixed model used in the analysis. Future research should incorporate both physiological and behavioural responses to multiple environmental stressors to better understand covariation between these two traits, and to detect any behavioural trade-offs that might arise through compensation. In addition, these trials should be repeated using offspring from outside of the MPA to compare whether the same level of resilience and metabolic phenotypic diversity would be present in an exploited population.

CHAPTER 1

General introduction: a brief conceptual background

1.1.1 Global climate change

We are currently living through a profound period of climate instability, comparable to the late Palaeocene era where enormous carbon build-up caused rapid ocean acidification (OA), global warming, and mass extinctions of deep-sea organisms and coral reef species across the seas (Jackson 2010). Today, the impacts of climate change are a result of the effects of anthropogenic activities, arising from increased use of coal energy and industrial expansion (Karl and Trenberth, 2003). Coastal zones, as densely inhabited regions, are particularly vulnerable to climate change (Harley et al. 2006). Our modern ocean is subject to many stressors, including global warming (IPCC 2013, Johnson and Lyman 2020), OA (Riebesell and Gattus 2015), hypoxia (Deutsch et al. 2011), sea-level rise (Wang et al. 2012, Cazenave et al. 2018), extreme weather events (e.g., storms, cold upwellings and heat waves; Bromirski et al. 2003), changes to Earth's ocean circulation (Timmermann et al. 1999, Pisias et al. 2001, Yang et al. 2020), and direct human impacts, such as pollution (Lu et al. 2018) and overexploitation (Galbraith et al. 2002).

Besides the diverse range, the complex interplay between stressors within ecosystems (He and Silliman 2019), are disrupting the suite of biotic and abiotic components of ecosystems and communities which rely on them (Pallewatta 2010). For example, sea level rise combined with anthropogenic subsidence in coastal zones enhances the risk of flooding (Wang et al. 2012), while ocean warming, and eutrophication interact to exacerbate harmful algal blooms (Paerl and Scott, 2010), and over-exploitation reduces resilience to climate-induced disturbances by removing physiologically and behaviourally fit phenotypes (Harley et al. 2006, Duncan et al. 2019a). Even so, the greatest threat that marine organisms are confronted with is perhaps the interaction between ocean warming and OA (Guscelli et al. 2019).

The carbonate chemistry - and the resultant pH of the marine environment - is dynamic in nature, particularly in the coastal zone where temporal and spatial variations exist as a result of both natural and anthropogenic inputs (Waldbusser and Sulisbury 2014). The current pH of seawater (~ 8.1 units) is determined by the relative proportion of the products of dissociation

of dissolved inorganic carbon (DIC). The composition of 0.5% aqueous CO_2 by mass, is typically made up of ~89% bicarbonate (HCO_3^-), and ~11% carbonate ions (CO_3^{2-}) (Waldbusser and Sulisbury 2014). Global OA has become a monolithic issue owing to the rate of absorption of atmospheric CO_2 where a large proportion of which is anthropogenic in origin (Guinotte and Fabry 2008, Doney et al. 2009, Hoegh-Guldberg et al. 2014). Absorption is exceeding the rate at which other processes (such as weathering, mixing in the water column, and transport/delivery systems) can provide compensatory HCO_3^- and CO_3^{2-} to buffer against the climbing carbonic acid (H_2CO_3) content in the water column (Waldbusser and Sulisbury 2014). The result is an alteration in the thermodynamic stability of calcium carbonate ($CaCO_3$) and a decrease in both CO_3^{2-} and pH (Honisch et al. 2012, Zeebe 2012). Coastal regions typically have unique spatial and temporal pH conditions due to unique combinations of drivers at each site along the coast (Kapsenberg and Hofmann 2016). For example, the variability in pH along coastal waters is the flux of 0.30 units has been documented along the Algoa Bay off the East coast of South Africa, with an average pH of 8.10 units (Edworthy et al. 2022). These environments are further exposed to multiple processes that can disrupt the balance in carbonate processes and exacerbate changes caused by global OA in coastal waters (Waldbusser and Sulisbury 2014). These processes are loosely defined as either 1.) chemical or, 2.) hydrological, where the former alters the amplitude and species of the various acid-base compounds, and the latter changes alkalinity with salinity, which in turn contributes to the total HCO_3^- concentration and the sensitivity to acid-base reactions (Waldbusser and Sulisbury 2014). Along coastal waters, there are three primary influences affecting coastal pH including: upwelling of lower pH water (Fabry et al. 2008, Hauri et al. 2013a), terrestrial freshwater inflow (Feely et al. 2009, Wang et al. 2014, Vargas et al. 2016, Carstensen and Duarte 2019) and biological activity in the form of algal bloom events or eutrophic conditions (Sunda and Cai 2012). An example of the variability in pH along coastal waters is the flux of 0.30 units has been documented along the Algoa Bay off the East coast of South Africa, with an average pH of 8.10 units (Edworthy et al. 2022). The gradual change in baseline pH will also likely cause an increase in the occurrence of extreme events (Hauri et al. 2013a,b), for example the large-scale shift in CO_2 content in the California Current system has contributed to the increased duration, severity and frequency of extreme events (Harris et al. 2013, Hauri et al. 2013a,b).

Whilst atmospheric CO_2 is an oceanwide driver affecting the global average pH and OA, recent literature has highlighted the importance of the habitat-specific, dynamic, localised drivers that

affect the carbonate systems of the spatial and temporal matrix that makes up the coastal zone (Hofmann et al. 2011, Barton et al. 2012, Duarte et al. 2013, Gobler and Talmage 2013). Contributors that influence pH and carbonate chemistry in the nearshore environment include events such as: cold upwellings, algal blooms and freshwater inflow (Fabry et al. 2008, Feely et al. 2009, Sunda and Cai 2012, Edworthy et al. 2022). In areas that are prone to seasonal cold upwellings, acidic bottom waters are brought to the surface over the continental shelf during these events (Fabry et al. 2008, Hauri et al. 2013a). Algal blooms and episodic eutrophication in estuaries and shallow bays are biological drivers of spatial and temporal variability of pH, where the decomposition of this plant matter leads to increased CO_2 and decreased oxygen (O_2) content from bacterial respiration (Sunda and Cai 2012). Finally, freshwater input from rivers and groundwater seeps adjacent to coastal zones may also influence carbonate chemistry and pH (Feely et al. 2009, Wang et al. 2014, Vargas et al. 2016, Carstensen and Duarte 2019), by diluting the alkalinity of seawater, and inadvertently reducing the buffering potential (Carstensen and Duarte 2019). The influx of nutrients also enhances the productivity of organic matter and thus contributes to excess respiration and acidification (Carstensen and Duarte 2019).

Understanding the dynamic response of animal populations and ecosystem services to reduced pH conditions is a priority (Riebesell and Gattus, 2015) if we are to preserve the remaining biological wealth and in turn, socio-economic welfare (Harley et al. 2006) of our oceans and coastal waters in particular. Organismal-level physiological ecology is an urgent requisite for our understanding of larger spatio-temporal effects as it is the changes at the molecular, cellular, and whole-animal levels that translate into changes to population-level dynamics such as survival, behaviour, growth, and reproduction (Pörtner 2001, Bozinovic and Pörtner 2015, Whitney et al. 2016).

1.1.2 The impact of stressors on fish physiology and the role of physiological research

Environmental stressors can alter fish physiology by disrupting immunological, neuroendocrinological, ionoregulatory, cardiorespiratory, osmoregulatory, and reproductive processes (Whitney et al. 2016). First hypothesised by Fry (1947, 1971) nearly 70 years ago, and since expanded upon by theories such as the metabolic theory of ecology (Brown et al. 2004, Whitfield 2004, Price et al. 2012), was the idea that environmental factors influenced organisms' physiology and behaviour through metabolism (Claireaux and Chabot 2016). Metabolic rate establishes the tempo of life by converting nutrients and stored energy into

useable energy needed to sustain activity (Brown et al. 2004, Nelson 2016). This energy, derived under steady state conditions and stored as Adenosine Triphosphate (ATP), is assumed to be the product of aerobic metabolism, and therefore, oxygen uptake is a useful proxy for metabolic rate (Nelson 2016). Traditionally we are interested in the two margins of oxygen uptake: the minimum (the basal or standard) and maximum oxygen uptake (Lefevre et al. 2017). The standard metabolic rate (SMR; Chabot et al. 2016) describes the energy needed for maintenance processes for homeostasis without activity, digestion, growth, or reproductive processes (Brett 1962, Frappell and Butler 2004). While the maximum metabolic rate (MMR, Norin and Clark 2016) is an estimate of the maximum rate of oxygen supply to support aerobic requirements beyond those of maintenance costs (Claireaux and Chabot 2016). Whilst methodologies may vary between different species and life stages (i.e., flow-through respirometry, intermittent flow respirometry and static-respirometry methods; Muir and Niimi 1972, Duthie 1982, Bushnell et al. 1984, Steffensen 1989, Svendsen et al. 2016, Muller et al. 2021) metabolic rate may be quantified by measuring the oxygen consumption of an organism by taking continuous measurements of dissolved oxygen (DO) content inside of a respirometer over time (Clark et al. 2013a). The aerobic scope (the difference between MMR and SMR) can then be calculated to determine whole-animal fitness and performance (Fry 1947, Claireaux and Lefrançois 2007, Pörtner and Knust 2007, Farrell et al. 2008, Pörtner et al. 2008, Munday et al. 2009a, Nilsson et al. 2009, Pörtner 2010, Clark et al. 2011, Donelson et al. 2012, Munday et al. 2012a, Pörtner, 2012). Despite the variety of means with which to examine the physiological response of fish to environmental stress, metabolic rate is still one of the most commonly employed physiological parameters in modern literature.

1.1.2 Temperature and the theory of OCLTT

Temperature (Magnuson et al. 1979) and DO (Farrell and Richards 2009) shape the physiology and behaviour of ectotherms in aquatic environments, forming two pragmatic margins of whole-animal performance (Fry 1947, 1971, Whitney et al. 2016). This defines an organism's thermal tolerance and in turn, helps to shape the distribution and abundance of a species (Gaston 2003). It is therefore unsurprising that ocean warming is a substantial threat to marine biodiversity (Kleypas et al. 1999, Doney et al. 2009, Harvey et al. 2013), and thermal metabolic studies offer a way to observe and describe the effects of temperature-related stress on fish physiology. The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesises that the evolution of biochemical and physiological capacities of aquatic ectotherms, and thus

fitness-related performance (e.g., growth, reproduction, and locomotion), is such that aerobic scope is optimised within a given thermal range (T_{optAS}), while performance decreases at higher and lower temperatures (Fig.1.1.A; Pörtner et al. 2017). In other studies however, it has been shown that aerobic scope continues to increase until lethal thermal limits are approached, after which aerobic scope declines rapidly (Fig. 1.1B; Clark et al. 2011, Healy and Schulte 2012). This phenomenon may be described by the Boltzmann factor or the Van't Hoff-Arrhenius relation which demonstrates an increasing rate of biological activity (or the rate of reaction) with temperature as: $e^{-E/kT}$ where E is the activation energy, k is Boltzmann's constant, and T is absolute temperature in K (Boltzmann 1872, Arrhenius 1889, Duarte 2007). Regardless of its shape, an aerobic scope curve provides an ideal physiological framework to test the impacts of environmental stressors across a thermal gradient.

The OCLTT physiological framework is useful in identifying species, populations and life history stages that are most vulnerable to climate change (Williams et al. 2008, Huey et al. 2012) and this can contribute to climate change adaptive management strategies (Paukert et al. 2016). For example, a metanalysis estimating the latitudinal patterns of warming-induced changes in metabolic rates of terrestrial ectotherms was conducted using high-frequency temperature data (1961 to 2009) and mass-normalised metabolic rates for a variety of ectotherms. It was found that warming has had the biggest absolute impact on the metabolic rates of organisms occupying tropical and north temperate regions with the most abrupt increases in metabolic rates observed in these regions (Dillon et al. 2010). In addition, a study conducted on ocean pout (*Macrozoarces americanus*), lumpfish (*Cyclopterus lumpus*), and shorthorn sculpin (*Myoxocephalus Scorpius*) over the course of their ontogeny showed that the limited scope for aerobic activity during their early life makes this stage particularly vulnerable to climate change (Killen et al. 2007). This is owing to the reduced ability of young fishes to 'multitask' metabolically demanding processes, leaving little in the way of energetic reserve for homeostatic maintenance during times of environmental or nutritional stress (Killen et al. 2007).

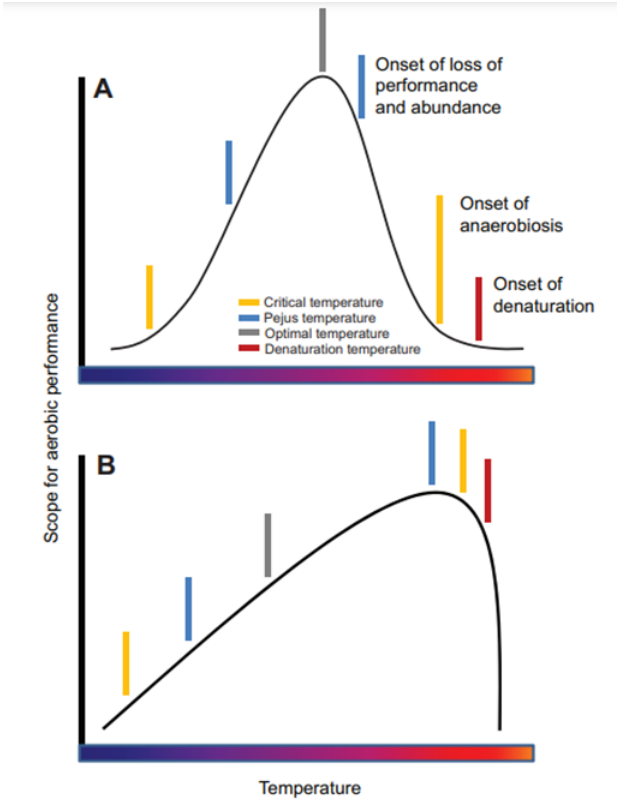

Figure 1. 1: Performance curves describing the relationship between aerobic scope of fishes and temperature (taken from Clark et al. 2013a). A. Is adapted from Pörtner and Farrell (2008) which assumes that the maximal aerobic scope coincides with T_{opt}, whilst B. which is adapted from Clark et al. (2011) assumes that T_{opt} increases with temperature until an organism nears their upper critical thermal limit.

It is important to note that there has been significant dispute over the generality of the OCLTT theory and the validity of AS research on ectotherms (Overgaard et al. 2012, Clark et al. 2013a, Clark et al. 2013b, Ern et al. 2014, Norin et al. 2014). The findings of Gräns et al. (2014, discussed by Jutfelt et al. 2014) contested the core principle on which the OCLTT theory is founded, which stipulates that tissue oxygen limitation is the primary process leading performance reductions at high temperatures. They demonstrated that reduced AS was not linked to the decline in growth observed at high temperatures in Atlantic halibut *Hippoglossus hippoglossus*, and the thermal windows for growth and AS differ. They instead propose that there is no single physiological mechanism explaining ectothermic thermal tolerance, and the limiting factor will depend on a suite of biological and environmental factors that may include: the rate of temperature change, species, lifestyle and physiological condition. Another study, not refuting the OCLTT theory but offering a different cellular mechanism, reveals that the high degree of interspecific variability in heart mitochondrial composition, function and

associated enzymes may be synonymous with thermal adaptation (Hunter-Manseau et al. 2019). The thermal sensitivity of cold-adapted species may be governed by gate keepers of fatty acid oxidation - carnitine palmitoyltransferase (CPT), whilst the mechanism of thermal impairment for warm-water species could be the impact of temperature on complex IV (CIV; Hunter-Manseau et al. 2019). This hints at the role of additional biological components affecting an organism's ability to adapt or acclimatise to thermal disturbances above and beyond the scope of the OCLTT theory.

Juxtaposing the above contradicting opinions on the OCLTT theory, Yao and Somero (2014) argued that the OCLTT theory is well-supported by a broad range of studies. Significant correlations were observed between reduced hypoxia tolerance and acute warming in the summer flounder (*Paralichthys dentatus*; Capossela et al. 2012), and positive correlations between hypoxia tolerance and the critical thermal maximum (CT_{max}) were observed in the Atlantic salmon (*Salmo salar*; Anttila et al. 2013). Unsuccessful migrations of adult sockeye salmon *Oncorhynchus nerka* have also been linked to the impacts of extreme warming on their aerobic scope (Farrell et al. 2008). Additional support for the theory has been observed in other taxa, such as invertebrates, where the sea cucumber *Apostichopus japonicus*, showed a significant reduction in oxygen consumption when exposed to a 6° C increase in temperature (Dong et al. 2011), and the Mediterranean bivalve *Modiolus barbatus* underwent metabolic depression and employed anaerobic metabolism when exposed to thermal stress (Anestis et al. 2008).

1.1.5 Rationale of study

Research on the effects of low pH conditions on fish have found changes in growth, neurosensory and behavioural endpoints, metabolism, otolith growth, mitochondrial function, and survival (Heuer and Grosell 2014, Brauner et al. 2019, Lefevre 2019, Munday et al. 2019). However, the responses vary considerably between taxa and life history stages (Wittmann and Pörtner 2013). This extends to disparities observed between closely related species, even among same-species populations separated geographically (Thor and Dupont 2017). Knowledge of the response of fishes to low pH is also not equally represented across climatic zones, as most of the literature pertaining to the response of fishes are from tropical areas (Wittmann and Pörtner 2013). Whilst it is also typically understood that early life stages are often more sensitive to high CO_2-induced stress (Munday et al. 2019, Heuer and Grosell 2014, Brauner et al. 2019), there is a paucity in the literature addressing the response of these life

history stages to low pH. The available literature, however, has documented widely varied effects on development. For instance, growth, survival, skeletal development, and otolith calcification were unaffected by CO_2 treatment in juvenile spiny damselfish (*Acanthochromis polyacanthus*; Munday et al. 2011), and this also had no detectable effect on egg survival, and size at hatching in the orange clownfish (*Amphiprion percula*; Munday et al. 2009b). On the other hand, significant tissue damage was observed in larval Atlantic cod (*Gadus morhua*; Frommel et al. 2012). Another topic that has been under-addressed is how the combined effects of thermal stress and acidification will affect marine ichthyofauna (Sala et al. 2000, Fabry et al. 2008), given that these drivers are unlikely to operate independently (Halpern et al. 2007). The few contemporary studies that have examined the effects of low pH over a range of temperatures have shown that thermal variability may moderate, counter, or exacerbate the effects of low pH (Laubenstein et al. 2019). For example, temperature had contrasting and interacting effects on behavioural lateralisation in reef fishes (Domenici et al. 2014). Thermal variability typically referred to in the referenced literature pertains to the shift in temperature within a specific region or over a specific time period from the mean thermal environment of any given organism, rather than the spatial heterogeneity in thermal gradients with depth, latitude and longitude. In the context of this study, thermal variability refers to fluctuations in ecologically relevant temperatures that can vary drastically over different spatial and temporal scales (Bates et al. 2018). Typically, these changes vary over weeks, hours and minutes, rather than changes that unfold over years and decades (Bates et al. 2018).

Several studies have noted that the severity of the response of fishes to high CO_2 stress is largely dictated by the duration of exposure (Shaw et al. 2013). Despite this, there is a deficiency in long-term studies on the response of fishes, although invertebrate research is rich with examples (particularly for predictions below the RCP8.5 CO_2 scenario at 936 ppm; Wittmann and Pörtner 2013). Much of the literature to date has focused on the short-term response of fish to low pH as a single driver (Riebesell and Gattuso 2015), and only 30 % of studies that have reported significant effects associated with high CO_2 exposure were longer than 10 days (Heuer and Grosell 2014). Examples of such studies include: research pertaining to the acid-base regulation and respiration in the gulf toadfish, *Opsanus beta* (Exposure = 24 h, pCO_2 = 1,000 and 1,900 µatm; Esbaugh et al. 2012), the auditory response of juvenile clownfish, *Amphiprion percula,* T =17-20 days, pCO_2 = 600, 700 and 900 µatm; Simpson et al. 2011), and the predator-prey interactions between the damselfish, *Pomacentrus*

amboinensis and the dottyback, *Pseudochromis fuscus* (T = 7 days, pCO_2 = 880 µatm; Allan et al. 2013).

Very little research has been conducted on the response of fish to OA in South Africa. One study was done looking at the short-term (five days acclimation period) response of pre-flexion larval blacktail (*Diplodus capensis*) to five pH treatments (7.27-8.02; incorporating both local variability and future projected OA scenarios). No metabolic reaction and no change in feeding was observed, suggesting that short-term exposure to near future OA conditions had no effect on survival or fitness in this species (Edworthy 2020). On the other hand, a medium-term study (from egg to 29 DAH) looking at the response of dusky kob (*Argyrosomus japonicus*) reared in a control (pCO_2 = 327.50 ± 80.07 µatm at pH 8.15), intermediate (pCO_2 477.40 ± 59.46 µatm at pH 8.03) and high pCO_2 treatment (pCO_2 910.20 ± 136.45 µatm at pH 7.78), showed that the rate of growth and skeletal development in high pCO_2 treatment fish was significantly slower (resulting in 47.2% smaller body size) from the onset of metamorphosis (Erasmus 2017). In addition, none of the high pCO_2 treatment fish survived past 26 DAH (Erasmus 2017).

Whilst short-term and medium-term studies are useful for looking at the general sensitivity of species to future CO_2 conditions, studies with longer durations help to identify the long-term acclimatisation and/or adaptation potential of populations (Sunday et al. 2014). For example, extended exposure to high pCO_2 in the bivalve *Mytilus edulis* (Gazeau et al. 2007) and a closely related species (*Mytilus galloprovincialis*; Michaelidis et al. 2005) mitigated the negative effects on calcification observed under short-duration experiments, demonstrating considerable acclimation to future CO_2 conditions (Berge et al. 2006, Thor and Dupont 2017). Similar findings have been observed in fish, where juvenile anemonefish (*Amphiprion melanopus*) whose ancestors had been exposed to a combination of high temperatures and reduced pH projected for 2100 showed an increase in metabolic rate and decreases in length, weight, condition and survival, only during short-term experiments (Miller et al. 2012). There is thus an urgent need to explore the response of fish populations under long-term exposure to end-of-century pCO_2 conditions, particularly early life-stages, so that we can garner a realistic understanding of population-level responses across practical timelines.

Given the scarcity of modern climate change/OA literature that takes into consideration individual variation, this study seeks to address this paucity by including a repeated measures element to the research. While the majority of studies aim to predict the response of fishes by

examining the physiological response at the population level, individual phenotypic variation must be considered (Guscelli et al. 2019). There is, a growing recognition of the prevalence of phenotypic, and behavioural variation in populations, and how this relates to ecosystem-level consequences (Ward et al. 2016). Historically, mean values have been used in isolation to predict and comprehend the response of biological units to environmental factors, which Bennett (1987) termed "the tyranny of the Golden Mean".

The scarcity of studies that consider inter-individual variation means that little is known about the determinative nature of intraspecific physiological variability in defining the response of a species to global change (Small et al. 2015, Walther et al. 2010). Contemporary literature alludes to the role that phenotypic diversity may play in the resilience and thus persistence of populations under habitat loss and degradation, exploitation, changes in food supply, as well as climate-driven warming and OA (Ward et al. 2016). Ultimately, individuals are the source of population- and ecosystem-level changes and understanding the variation in individual-level responses are pivotal to the effective management of these populations (Ward et al. 2016). Experimental approaches that prioritise intraspecific variability in the design will likely arrive at a more accurate and comprehensive postulate for a population-level response (Guscelli et al. 2019). For example, in a study looking at the response of individual sea urchins (*Heliocidaris erythrogramma*) to OA, reproductive success was found to vary distinctly between individuals, suggesting that in some, fertilisation will be enhanced in acidified conditions (Schlegel et al. 2012). If this trait is heritable, there will be a strong selection potential for these individuals, demonstrating the idea that enriched inter-individual variability is thought to buffer against population and species-level extinctions (Forsman and Wennersten, 2016). In this case, ecological and evolutionary implications of individual variation are not being eclipsed by the mean response. In the context of natural selection, individual variation in performance should be repeatable, demonstrate differential fitness, and should be heritable (Endler 1986, Marras et al. 2010). To evaluate variation in thermal metabolic performance individuals should be tested repeatedly over the breadth of their thermal window (Killen et al. 2021). Considering variability in the response of different metabolic phenotypes underlies a population's response to environmental stress, and offers adaptability to changing environments (Ricklefs and Wikelski 2002, Winemiller 2005), it is fundamental that this be integrated into physiological, climate-related research.

1.1.5 The coastal waters of South Africa

The 3650 km coastline of South Africa has distinct thermal spatial variation in the coastal waters which is a product of the dominant warm Agulhas Current along the East Coast and the cold Benguela Current on the West Coast (Fig. 1.2; Bolton et al. 2004, Anderson et al. 2009, Griffiths et al. 2010). This results in pronounced interannual and decadal thermal variability along the coast (Hutchings et al. 2009). The East Coast is showing signs of warming (Rouault et al. 2010) with a 1.5° C increase in temperature of the Agulhas Current since the 1980s. This is largely attributed to the intensification of the current which is driven by the increase in westerly trade winds in the southern Indian Ocean (Rouault et al. 2010). On the other hand, seasonal cooling of surface temperatures in nearshore zones along the south and southeast coasts have been documented. This is attributed to the same forces of change driven by increased westerly trade winds, causing an increase in dynamic upwelling in certain areas (Rouault et al. 2010). A nearly 0.5° C decrease in temperature per decade has been observed between January to August along the West Coast, and to a lesser degree, along the Sout Coast as well (Rouault et al. 2011). Future changes will likely depend on the future of the El Niño-Southern Oscillation (ENSO), which is a prominent driver of wind, rainfall and sea surface temperature in this region (SST; Rouault et al. 2010, Dufois and Rouault 2012, Philippon et al. 2012). The cooler water temperatures along the West Coast are owing to the intensification of widespread cold upwelling events, particularly along the southern part of this region, whilst the northern part of the system has experienced a decrease in upwelling (Veitch et al. 2010; Lamont et al. 2017). Increased upwelling events have also been observed in the dynamic upwelling cells off Gqeberha and Port Alfred between May to August (Rouault et al. 2011).

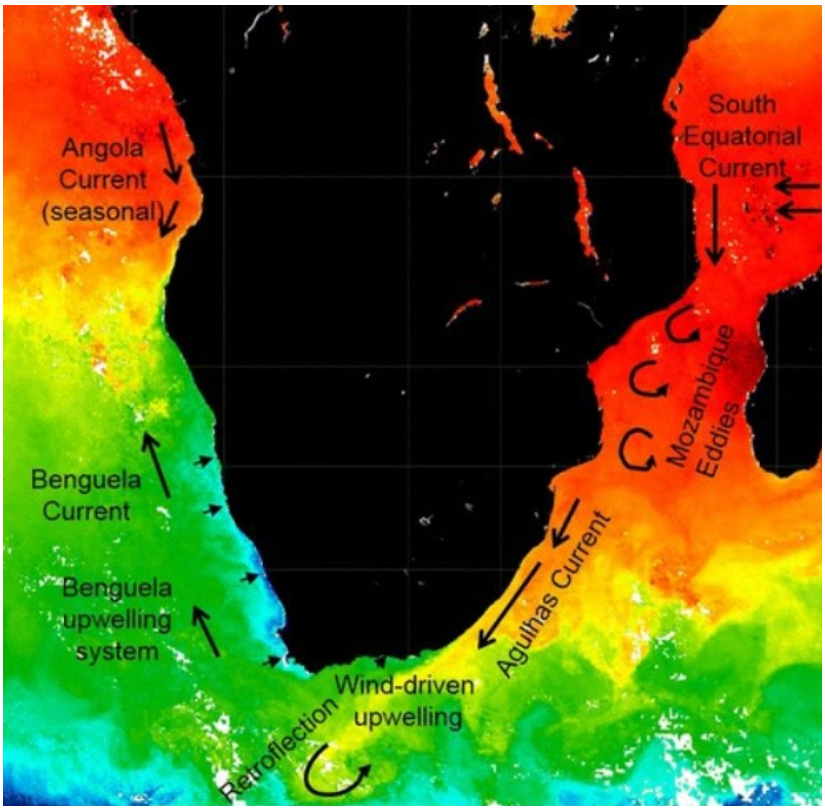

Figure 1. 2: Taken from Potts et al. (2015) this map shows the satellite sea surface temperature (February 2009), where red indicates warm water associated with Angola, Agulhas and South Equatorial currents as well as the retroflection of the Agulhas Current at the bottom tip of the continent. Blue indicates the location of cold upwelling cells, and green shows the wind-driven upwelling along the south coast and the Benguela Current region

Owing to the combination of dynamic inputs in coastal waters and global OA, coastal regions may experience higher acidification that far exceed predictions made about OA from atmospheric CO_2 uptake in isolation (Duarte et al. 2013). Coastal upwellings have been associated with a reduction in surface water pH (pH <7.8) which is attributed to CO_2 that has been remineralised at depth being brought to the surface during these events (Fabry et al. 2008, Feely et al. 2008, Hauri et al. 2013a). Upwelling events occur along *C. laticeps'* distribution on the south-east coast of South Africa as well as along the inshore boundary of the east coast Agulhas current (Duncan et al. 2019b, Walker 1986, Schumann 1987), and along the major capes of the south coast (Goschen and Schumann 1995). The latter are wind-driven and intermittent whilst the permanent Eastern-boundary upwelling zones are driven by large-scale ocean circulation and trade winds (Chavez and Messié 2009). The *El Niño*–Southern

Oscillation (ENSO) phase state plays a major part in the occurrence of extreme intermittent upwelling events, where the *La Niña* phase prompts more frequent and intense upwellings along the south coast when compared to *El Niño* conditions (Duncan et al. 2019b). As ENSO phase state has become more acute and varied, extreme upwelling events have become more intense with time along South Africa's south coast (Duncan et al. 2019b). These events have caused acute hypercapnia off the West and South coasts of South Africa (Moloney et al. 2014, Knapp et al 2016, Dziergwa et al. 2019). The decay of algal blooms which proliferate during nutrient-rich upwellings leads to hypercapnic hypoxia, and the occurrence of both is projected to increase in frequency with ongoing climate change (Knapp et al. 2016). Over the course of the first thorough nearshore pH-monitoring study in Africa (Algoa Bay on the east coast), coastal pH variability, in addition to the corelates of pH variability (salinity, temperature, and biological activity) were monitored, and substantial spatial and temporal differences (0.46 pH units) were observed along the study sites (Edworthy et al. 2022). Biological activity corresponded with offshore coastal pH, and salinity showed a negative correlation with inshore coastal pH (Edworthy et al. 2022). A finding worth mentioning was the potential role that macroalgae played through biological modulation (Edworthy et al. 2022). The uptake of inorganic carbon via photosynthesis (Krause-Jensen and Duarte 2016, McNicholl et al. 2019, Roleda and Hurd 2019) caused the surrounding water to have a higher-than-average pH (8.33, range = 0.25), which could offer potential OA refugia for sensitive coastal species (Noisette and Hurd 2018, Xiao et al. 2021). This study offered insight into the natural variability in pH that coastal species experience across space and time, providing a glimpse into the physiological conditioning and inherent biological toolkit that these species possess.

Any changes in pH and thermal variability that have been projected along the coastline of South Africa is likely to elicit heterogenous responses in coastal species considering the numerous coastal habitats and biogeographic zones (Fig. 1.3; Potts et al. 2015). The response of coastal species, as outlined by Potts et al. (2015) will largely depend on the varied life history styles, as well as which biogeographic zone they occupy, and the guild that they fall into, based on their life history strategy (i.e., resident, migratory, estuarine-dependent, and catadromous; Potts et al. 2015). The impacts of OA on skeletogenesis and olfaction may cause a reduction in the survival of eggs and larvae of all coastal fishes in this region. Resident species, which are normally exposed to fluctuations in temperature at various time scales, are very often eurythermic in cool-temperate, warm-temperate and sub-tropical zones. Despite this, changes in thermal regimes may impact the life history of resident species to a greater extent, as they

lack the capacity to track their preferred temperatures as migratory species do. Ultimately, altered thermal regimes and changes in local pH (not to mention sea-level rise, as well as changes in rainfall and current speed) are thought to alter the assemblage of coastal fishes, with important socio-economic consequences for the 750,000 recreational, 24,700 commercial, and 29,000 subsistence line fishers reliant on these resources (McGrath et al. 1997, Branch and Clark 2006).

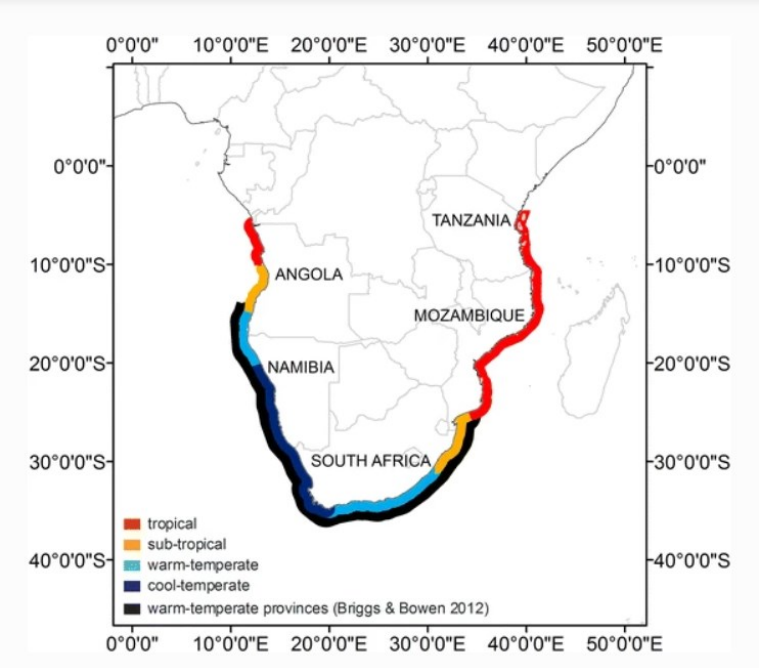

Figure 1. 3: Taken from Potts et al. (2015), this map shows the various biogeographic zones identified along the coastal region of Southern Africa, as outlined by Briggs and Bowen (2012) and Whitfield (2005), with alterations by Potts et al. 2015.

1.1.7 Model species Chrysoblephus laticeps

Chrysoblephus laticeps (*Valenciennes,* 1830) is a commercially important, endemic sparid species (Fig. 1.4; Crawford and Crous 1982, Buxton 1984) with a core distribution between False Bay in the Western Cape and the Kei River in the Eastern Cape (Griffiths and Wilke 2002), occupying both the cool-temperate and warm-temperate south coast of South Africa (Götz and Kerwath 2013). Their primary dispersal occurs during their egg and larval phases, where they form part of the pelagic biota for up to 30 days (Davis 1996). There is little

information on this species following its recruitment and settlement on reef habitats. However, they are highly resident during their adult phase, with 95 % of their displacements occurring within a travel range length (TRL) of 13 km according to one mark re-capture study (Griffiths and Wilke, 2002). Another study of this kind showed a 61% recapture rate within 50 m of the initial tagging position (Kerwath et al. 2007). *Chrysoblephus laticeps* is a protogynous species with females attaining sexual maturity between year 2.5-3.5 when they reach 172-184 mm fork length (FL; Buxton 1993a, Götz 2005). Sex change from female to male typically occurs between 270-370 mm FL (~7 years; Buxton 1993a). The maximum size recorded was 512 mm FL (Buxton 1987), and the oldest fish caught to date was aged at 19 years (Götz 2005). Under moderate to high fishing pressure, sex ratios can be skewed with fewer males (Buxton 1993b) however, in areas with reduced exploitation sex ratio is largely equal and can be maintained through a shift in size/age-at sex-change (Götz et al. 2008). Breeding and spawning occurs from October through to February in both the Eastern and Western Cape where pairs take part in elaborate courtship displays (Buxton 1990, Götz 2005), and resident males defend a territory with a harem of females (Provost and Jensen, 2015). Adult *C. laticeps* occupy deeper inshore and offshore reefs with varied profiles up to 100m in depth (Buxton and Smale 1984, Buxton 1987, Götz 2005, Götz et al. 2008), whilst juveniles inhabit more shallow subtidal reefs up to 30m (Penrith 1972, Buxton and Smale 1984, Buxton 1987).

Due to their limited capacity for movement during their adult-phase and the variable thermal environment along their distribution, it is unsurprising that this species is eurythermic and therefore able to survive *in situ* temperatures between 9 and 22°C and rapid thermal changes (Skeeles 2019). This broad thermal tolerance most likely makes them more resilient to climate change than equatorial resident species experiencing stable thermal environments (Malcolm et al. 2007, James et al. 2012, Rummer et al. 2014). Their protogynous hermaphroditic life history strategy (Buxton 1992) makes them theoretically vulnerable to loss of genetic diversity through increased genetic drift (Hauser and Carvalho 2008) and decreased effective population size (Hartl and Clark 1997). This also makes them particularly vulnerable to overfishing (Buxton 1988) as they are exploited along the length of their distribution by both the recreational and commercial boat-based linefishery (Smale and Buxton 1985, Mann 2013). Considering their commercial value, high site fidelity and vulnerability to exploitation, understanding the response of this species, particularly early-stage *C. laticeps,* to the projected increase in thermal disturbances is important for making predictions on their future stock status, and creating effective management plans.

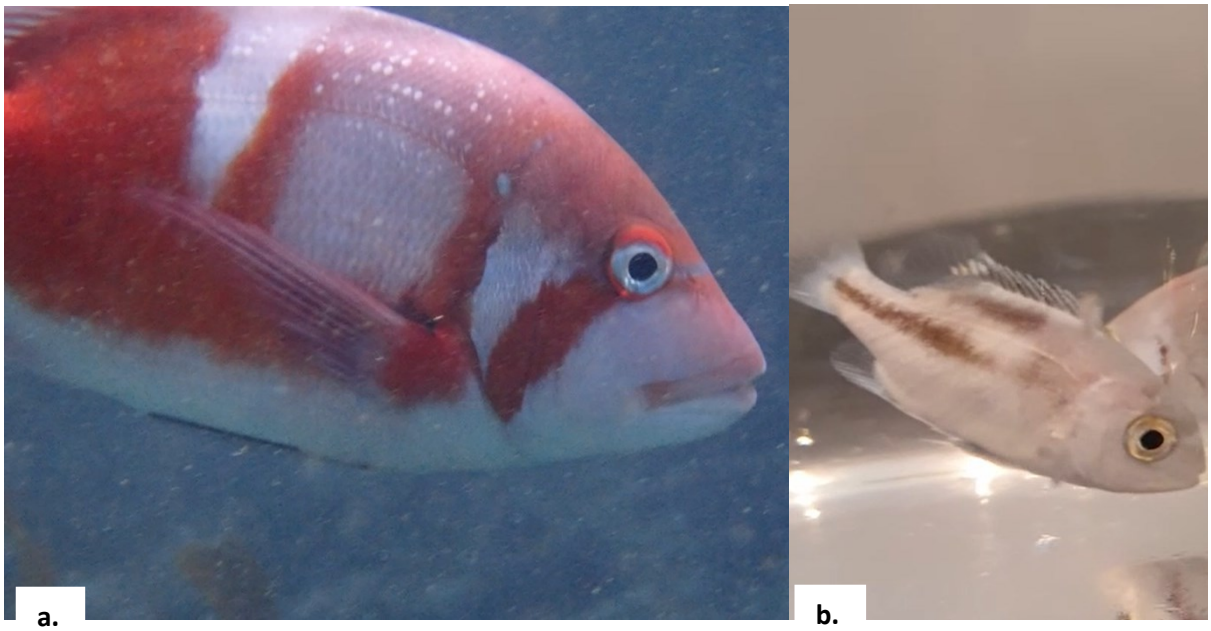

Figure 1. 4: The study species, *Chrysoblephus laticeps*, a.) in their adult phase and, b.) their juvenile phase.

1.2. Aim of the current research

There is a dearth of research on the long-term impacts of ocean acidification on marine organisms and less on coastal fishes. However, Muller et al. (2021) compared the metabolic response of *C. laticeps* that were exposed to future pH conditions from their egg to flexion larval phase. On completion of their research, the remaining larvae were maintained in the same low and high conditions for an additional two months. This provided an unprecedented opportunity to understand the physiological response of juvenile *C. laticeps* to long-term (whole life) exposure to OA conditions. The aim of this study is therefore to compare the thermal physiology of juvenile *C. laticeps* reared in high (pH = 8.03) and low (pH= 7.63) CO_2 conditions. To do this, the aerobic scope of each juvenile was assessed at a range of biologically relevant temperatures in a repeated measures design using intermittent-flow respirometry.

CHAPTER 2

General methodology

2.1.1 Fish capture, spawning, and rearing

All experiments were performed on *C. laticeps* which had been hatched and reared within laboratory conditions up until the early juvenile stage. Sexually reproductive adult *C. laticeps* were captured on the 12th December 2020 at the Tsistikamma National Park (ca. 34°01'16"S 23°50'45"E), by hook and line fishing from a ski boat on shallow reefs (<25m depth; Fig. 2.1). Captured fish were immediately vented with an 18-gauge hypodermic needle, inserted into the swim bladder, to prevent barotrauma related stress or injury. They were briefly maintained in one of two 250 L water drums which were aerated by continuous water exchange. Once the target number of mature adults had been captured, identified by size as four males and fourteen females, the fish were brought to the nearby Storm's River slipway where two 6000 L tanks (Fig. 2.2) had been positioned with full flow-through water supply. Within two hours of returning to shore, all fish were weighed to the nearest gram, injected with 0.5 ml. kg^{-1} Aquaspawn (a synthetic gonadotropin shown to induce rapid ovulation; Fig. 2.2), and returned to the tanks which had been covered with shade cloth to keep low lighting conditions. After 48 hours, gametes were stripped from fish by applying light pressure to the abdomen as eggs and sperm were collected in separate containers from 12 females (400 – 830g) and three males (1300 – 1460g), respectively (Fig. 2.3). Once all fish had been stripped, small quantities of sperm and fresh seawater were added to the eggs while being continuously mixed with a fine-bristled brush for 10 minutes. The solution was thereafter disinfected with 0.27 mL formaldehyde, flushed and strained with fresh seawater, separated into Ziplock bags and stored in a polystyrene cooler for transport back to the NRF-SAIAB Aquatic Ecophysiology Research Platform (AERP) Laboratory at Rhodes University in Makhanda, South Africa. On arrival at the laboratory, the floating fertilised eggs were separated from sinking, unfertilised eggs and enumerated by drawing ten 2 mL samples and then distributed into eight 75 L rearing tanks at 80 eggs L^{-1} or 6000 eggs per tank. For further details see Muller (2022).

From the third day after hatching, prior to yolk and oil reserves being depleted, larvae were fed with rotifers up until post-flexion at 30 days after hatching. Rotifers (*Brachionus plicatilis*), which were cultured using an enrichment diet (ORI-ONE, Skretting Stavanger, Norway) were added three times per day to maintain densities of at least 10 mL^{-1}. From 26 days after hatching,

483 newly hatched brine shrimp were added to tanks and maintained at densities of at least 5 mL^{-1}.
484 From 40 days after hatching, early juveniles were fed finely chopped sardine along with brine
485 shrimp. From 49 days after hatch, only finely chopped sardine was fed until satiation every
486 morning up until the completion of the study.

Figure 2. 1: a.) The process of fish capture using a hook and line off of a ski-boat, and b.) Adult roman seabream, *Chrysoblephus laticeps*, caught from a ski-boat inside the Tsitsikamma Marine Protected Area to be used for spawning of the individuals used in the current study.

Figure 2. 2: The holding tank used to keep adult *Chrysoblephus laticeps* following capture in the Tsitsikamma Marine Protected Area.

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Figure 2. 3: Adult roman seabream, *Chrysoblephus laticeps*, being stripped 48-hrs following the injection of synthetic gonadotropin hormone.

2.1.2 Husbandry and water chemistry

Fish were reared for three months post-hatching until the aerobic trials of the current study began. Fish husbandry and respirometry experiments were both carried out in the NRF-SAIAB AERP laboratory. The rearing facility comprised of a recirculating aquaculture system with a holding tank (maximum capacity of 6000 L) feeding into eight 75 L fibreglass tanks (Fig. 2.4). A marine recirculating system with ozone treatment, degassing, and mechanical and biological filtration was used, with partial (approximately 25% per week) seawater renewal, as per Muller et al. (2021). The photoperiod within the housing facility was set to automatically switch between a daily 14-hour light (of which 4-hours are low-light, morning and evening, achieved using 3-foot, 8000 K lights while full-light was supplemented with 5-foot, 6000 K lights for daylight conditions) and 10-hour dark period.

Figure 2. 4: Juvenile roman seabream, *Chrysoblephus laticeps*, in the fibreglass holding tanks

2.1.3 Respirometry set-up

Intermittent flow respirometry chambers were used to measure oxygen consumption rates of juveniles for all respirometry trials. The system design included a water bath (540 mm x 400 mm x 250 mm) in which four Plexiglass respirometry chambers were submerged, each with a volume of 250 ml (Fig. 2.5). Chambers were suitably sized to accommodate the size, shape and routine activity of the fish being tested without inducing confinement related stress. The sealed chambers also allowed for adequate mixing during both flush and measurement cycles while oxygen ingress and egress with the water bath was not detectable. Two flow loops cycled water through the chambers to alternately flush each chamber with fresh, oxygen saturated seawater from the water bath while the other formed a closed measurement loop. The oxygen content of the water (ml/L) was measured with a 4-channel oxygen sensing system (FireSting, PyroScience GmbH, Germany) with fibre optic sensors (1 m) and flow-through cells attached to each chamber by 4mm Tygon© tubing. Water flow through the system was propelled by a peristaltic pump with cycling between measurement and flush phases being controlled by magnetic isolation valves operated by an Arduino© with a timer. The Pyro Oxygen Logger software (PyroScience, Denmark) was used to monitor and record the oxygen readings from the four channels. All the sensors were calibrated using a 1-point calibration (where the 0% value from the sensor code and the 100% value from a manual calibration were taken) and oxygen measurements were taken by logging the oxygen content of each chamber in millilitres per litre. Two aquarium heaters, attached to an STC-1000 temperature controller, were placed into the water bath and were used to maintain the temperature at 18˚C or to increase the temperature during thermal trials. A chiller (Hailea, HS-28A) was connected to the water bath and used to reduce temperatures where necessary. A pH/CO_2 controller (Tunze 7070.200, Aquarientechnik GmbH, Penzberg, Austria) fitted with a pH sensor (Tunze 7070.110) was used to monitor the pH and maintain experimental conditions when respirometry was carried out on treatment specimens. Two air stones and an air pump were used to ensure oxygen saturation within the water bath, and separators made from black fluted plastic boards were placed between each chamber to avoid visual stress during the trials.

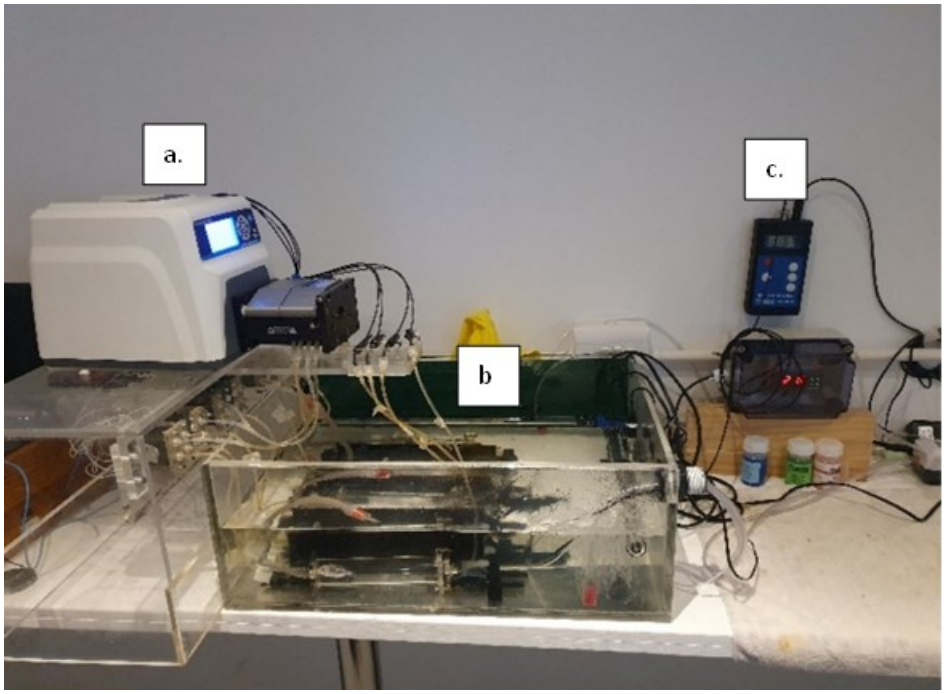


Figure 2. 5: The equipment used to carry out respirometry trials, consisting of (a) the peristaltic pump and PyroScience oxygen logger and sensors, (b) the water bath with the four chambers submerged, and (c) the pH and temperature controllers.

524 The water bath and respirometers were filled at the start of each trial with fresh seawater which
525 had been UV-sterilised and filtered to 1-micron. Seawater was collected on a spring high tide
526 from the Kariega Estuary mouth near Kenton on Sea and was stored in a 5 000 l PVC tank with
527 continuous circulation through a sand filter. Specimens used in each trial were fasted for 24
528 hours prior to carrying out respirometry and were collected from their respective tanks using
529 clean beakers and transferred into a petri dish with millimetric paper glued underneath to be
530 measured (Fig. 2.6). This was done by taking a photo of each fish individually from directly
531 above the petri dish and counting the number of blocks. After taring the scale, the petri dish
532 with the fish inside was weighed to get their wet weight (g). Once placed into the trial
533 chambers, they were given a period of one to four-hours for acclimation to respirometry
534 chambers and altering of temperature. Each trial was run overnight in darkness for a 12-hour
535 period. The oxygen measuring cycle was set to an automated 5-minute measuring and 15-
536 minute flushing periods for 11, 14 and 18 °C, whilst a 3-minute measuring and 18-minute

flushing period was used for the 22 °C trials. A dilute solution of ethanol (10 %) and mechanical methods were used to clean the chambers and tubes between trials to avoid high levels of microbial build-up. Due to the rapid growth of the juveniles over the course of the trial, the original chambers were replaced with new chambers (340 ml volume) for the final three trials at the end of the experiment.

Figure 2. 6: The process of measuring and weighing each individual using a petri dish and millimetric paper glued underneath.

2.1.4 Standard Metabolic Rate (SMR) and Maximum Metabolic Rate (MMR) and rate of oxygen consumption

Prior to the start of the trials, a preliminary test was conducted to examine whether there was a significant difference in diurnal and nocturnal oxygen consumption, and thus metabolic activity. A circadian respirometry trial was run for 22 hours, spanning late afternoon, evening, over overnight and early morning, and the following formula was used to calculate the rate of oxygen consumption in each chamber: $MO_2 = \frac{\frac{O_{2\,(start)} - O_{2\,(end)}}{T} \times V}{W}$

where MO_2 is the rate of oxygen consumption (mg $O_2^{-1}.g^{-1}.hr^{-1}$), $O_{2\,(start)}$ (mg $O_2^{-1}.l^{-1}$) is the oxygen reading at the start of the measurement cycle, $O_{2\,(end)}$ is the oxygen reading at the end

549 of the measurement period, T is the time (h), V is the respirometry chamber volume, and W is
550 the mass of the fish (Schwieterman et al. 2019).

551 A paired t-test was then used to test whether there was a significant difference in diurnal and
552 nocturnal rates from a total of 52 measurement cycles, once the assumptions of independence,
553 normality, and no extreme outliers had been checked. No significant difference was found
554 between diurnal and nocturnal metabolic activity (DF = 1; p-value = > 0.05), which allowed
555 the longer SMR trials to be run over night for the duration of the experiments.

556 Once the trial fish were fasted, measured and acclimated to the thermal conditions of each trial,
557 they were placed in the respirometers and the rate of oxygen consumption for each fish during
558 each trial was calculated from the oxygen concentration readings (ml^{-1}. O_2^{-1}) recorded
559 automatically every 15 seconds in each chamber (when the solenoid valves were closed and
560 there was no water movement during the five minute measurement phase). Fish were housed
561 in the respirometers for 12 hours for the SMR measurement. SMR was then determined as the
562 lowest observable oxygen use rates, representative of basal energetic metabolism requirements
563 using the procedure described in the data analyses section below.

564 Once each SMR trial had been run overnight, individual fish were transferred to a circular
565 container (75 L^{-1}) filled half-way with experimental water, where fish were chased for 10
566 minutes until exhausted. This was followed by 15 seconds of air exposure to ensure that the
567 fish was maximally fatigued (see Clark et al. 2013a), before being placed back into their
568 chamber. The maximum O_2 consumption rate was measured during an hour long
569 flushing/measuring cycle. The MMR was recorded as the single greatest decline in oxygen
570 during the measurement phase.

2.1.5 Experimental design

571 Both SMR and MMR trials for each individual were carried out at each experimental
572 temperature (10, 14, 18 or 22° C), which were chosen because they represent the thermal range
573 that *C. laticeps* would typically experience in the wild (Skeeles 2019). An ecologically relevant
574 temperature change of 1° C /half an hour was implemented (or 0.1° C/ 3 minutes, adjusted
575 manually) as that corresponds with the thermal change during an intense upwelling or
576 downwelling event (Skeeles 2019). Prior to the start of the trials, each fish was tagged using
577 bi-colour combinations of Visible Elastomer Implant (VIE) tags (Northwest Marine
578 Technology, USA) to serve as a method for identification owing to the repeated measures

nature of the study (Fig. 2.7). This was accomplished by injecting the implant subdermally, as an anterior tag just below the dorsal fin, and as a posterior tag above the caudal peduncle. Fish were given weeks to recover from the stress of tagging and sufficient time (minimum of four days) was also allowed between each trial so that the stress response elicited during one trial did not cause a carry-over effect for the subsequent trial.

Figure 2. 7: Fish were tagged at the posterior portion below the dorsal and the anterior part of the dorsal near the caudal peduncle using VIE tags to distinguish between individuals for the repeated measures element of the study.

Data analyses

The SMR data first had to be filtered by eliminating the first and last two hours of measurements and those readings below 70% oxygen saturation (5.0 mg $O_2.l^{-1}$) to ensure the inclusion of absolute resting rates. The mass-specific and temperature-specific SMR for each fish was calculated using the quantile of the lowest 20 % of the O_2 data (Chabot et al. 2016). The rate of oxygen decline over the course of each measuring period was automatically calculated using the RespR package (Harianto et al. 2019) in R version 4.1.2 (R Core Team 2018). This included the selection of the linear sections of decline in the data, representative of organismal oxygen consumption during each measuring period (Carey et al. 2016, Chu and

Gale 2017). The slope of the fitted linear model of oxygen decline against time was used to get the rate by running a rolling regression on these regions with a fixed time or "width" specified across each measured replicate. Preliminary evaluations of repeatability of the metabolic rate estimates were conducted on an earlier life stage of the same group of fish (during their larval phase) and the variability between replicates for one individual were similar; in other words, the estimates exhibited moderate-to-good agreement values (Muller, 2021). This accounts for any technical variability that would subjugate biological variability in the experimental design.. This accounts for any technical variability that would subjugate biological variability in the experimental design. Owing to the short duration of the MMR trials the MMR for each fish at each temperature was calculated manually in the RespR package.

Once the rate for each metabolic metric was computed, this was adjusted by subtracting the background respiration from the list of rates (Rogers et al. 2016). The background rate for SMR trials was calculated by subtracting a rate of oxygen decline from the control chamber at the start of a trial and at the end of a trial, which would account for the microbial growth over the 12-hour duration and the corresponding increase in microbial respiration. An average background rate showing a snapshot of the microbial biomass respiration was calculated for MMR trials owing to the short duration of these trials. The slope of the decline of one of the measuring cycles in the blank chamber was inspected and the rate of the decline was calculated. In cases where the background rate in the blank chambers of trials were unable to be recorded owing to human error or equipment malfunction, the average temperature-specific background rate was used to adjust rates. The third and final step, applicable to all three metabolic metrices, was converting the adjusted rates into the final usable rates with the following units: "$mg^{-1}.hr^{-1}.g^{-1}$".

CHAPTER 3

Assessing the thermal performance curve of lab-reared juvenile red roman seabream Chrysoblephus laticeps

An adult roman seabream, *Chrysoblephus laticeps*, photographed in its natural habitat in the Tsitsikamma Marine Protected Area.

3.1 Introduction

Increased thermal variability is projected along the coast of South Africa (Duncan et al. 2019a, van der Walt et al. 2021), within the range distribution of *C. laticeps*. Whilst the south coast is an area that is already characterised by localised, extreme sea temperature variability (Schumann et al. 1995), an increase in the frequency and intensity of upwellings (Rhein et al. 2013, Rouault et al, 2010, Rouault et al. 2011, Schlegel et al. 2017, Goschen and Schumann 1995) and heatwaves (Schlegel et al. 2017) has been projected. Warming, arising from the intensification of the Agulhas Current, and incipient ocean warming (Rouault et al. 2010) have also been observed. Whilst organisms that reside in highly variable thermal environments are frequently exposed to a wide range of temperatures, it has been shown that a similar sympatric, resident species, *Boopsoidea inornata,* has critical thermal limits that lie close to the extremes experienced in their habitat (Allison et al. 2021). In the case of *B. inornata,* the lower and upper

627 thermal stress limits (identified by opercular beat rates and loss of equilibrium) occurred at an
628 average of 9 °C and 25 °C, respectively, with the lower critical thermal limit (CT_{min}) estimated
629 to be 7.8 °C and the upper thermal limit (CT_{max}) estimated to be 30 °C for the species (Allison
630 et al. 2021). Compared with *in situ* temperatures, *B. inornata* is more susceptible to small
631 reductions in the minimum temperature than warming events. Therefore, the consequences of
632 exposure to both thermal extremes need to be explored for *C. laticeps*. The primary
633 physiological mechanisms underlying a fish's response to heat stress or cold shock largely
634 involve two fundamental neuroendocrine pathways: the brain–sympathetic–chromaffin cell
635 (BSC) axis and the hypothalamic-pituitary-interrenal (HPI) axis, responsible for the production
636 and release of catecholamines and cortisol, respectively (Wendelaar Bonga, 1997). The first is
637 thought to be responsible for making energy substrates readily available, which in turn
638 facilitates behavioural responses by supplying energy to muscles and other tissues (Fabbri and
639 Moon, 2016). The ubiquitous action of cortisol-mediated changes is then responsible for the
640 mobilisation and reallocation of these energy molecules, enabling an adaptative response
641 (Faught et al. 2016, Sadoul and Vijayan 2016).

642 Changes to energy budgets for extended periods of time under chronic or repeated thermal
643 stress may compromise acid–base regulation, feeding, behaviour, as well as metabolism
644 (Pörtner and Peck 2010, Rosa et al. 2012), and can cause long-term changes in immune
645 function, growth, or reproductive success in ways that often affect species fitness and survival
646 (Alfonso et al. 2021). These tertiary organismal changes may cascade into population-level
647 disruptions, altering size structure, distribution, and the trophic composition within ecological
648 communities (Perry et al. 2005, Dulvy et al. 2008, Graham et al. 2008, MacNeil et al. 2010).
649 As temperature determines the rate of physiological processes and dictates cellular structural
650 integrity, it thus shapes the boundaries of physiological performance (Hochachka and Somero
651 2002), and is one of the most formidable abiotic factors that alters the energy budget of
652 organisms (Yurista 1999, Anacleto et al. 2018)

653 It is well documented that the impacts of thermal stress on metabolic processes are species-
654 specific but also differ across various life stages (Wittmann and Pörtner 2013). Successive
655 development stages have varying requirements (ranging from habitat occupation, feeding,
656 behaviour, physiological constraints, and varied thermal niches) and will therefore be affected
657 dissimilarly by the impacts of climate change (Madeira et al. 2020). As a result, eco-physiology
658 studies across complex life history stages are imperative for predicting the impacts of climate
659 change on fishes. However, up to now, few studies have examined the capacity for adaptation

660 in early life history stages to a changing thermal environment when compared with adults (Yao
661 and Somero, 2014). Of the early life stages, more research has been conducted on the earliest
662 life stages (eggs and larvae), while comparatively little has been done on the juvenile life
663 stages, except for research focussing on salmonids (e.g. Cutts et al. 2002, Reid et al. 2011, Van
664 Leeuwen et al. 2011a, 2011b, Oligny-Hébert et al. 2015). Nevertheless, in cases where whole
665 life histories have been examined, the window of thermal tolerance is generally narrower for
666 eggs and larvae, relatively broad in juveniles, and constrained again during the adult phase
667 (Pörtner and Farrell 2008, Truebano et al. 2018).

668 Theoretically, the drivers of the tolerance of the different life stages of fishes to extreme
669 temperatures is related to the constraints in oxygen supply capacity, which is linked to body
670 size (Pörtner and Peck 2010). It is thought that the ontogenetic development of tissue functional
671 capacity results in different preferred thermal ranges between life stages (Dahlke et al. 2020,
672 Pörtner et al. 2017, Pörtner and Farrell 2008). This may translate into stage-specific thermal
673 responses (Truebano et al. 2018) and thus ontogenetic shifts in thermal tolerance (Pörtner and
674 Farrell, 2008, Rijnsdorp et al. 2009). In a Sparid example, the vulnerability of *Sparus aurata*
675 to heat stress was evaluated using estimates of mortality rates, tissue pathology and the ability
676 to engage the cellular stress responses (Madeira et al. 2020). Here, larvae were more vulnerable
677 than adults, lacking acclimation capacity, while adults were more vulnerable than juveniles
678 with lower plasticity and damage to brain, muscle, and liver tissues under heat stress (Madeira
679 et al. 2020). Considering the pronounced changes in thermal variability projected along the
680 coast of South Africa (Potts et al. 2015), with particular focus on the warm and cool-temperate
681 regions in which *C. laticeps* resides (Götz and Kerwath 2013), an understanding of the impact
682 of these changes on the juveniles of the species is required. This is necessary for making
683 predictions on likely outcomes in different climate scenarios. For example, determining the
684 physiological and metabolic impacts on critical life history stages, from larvae to juveniles,
685 will assist in our understanding of potential bottlenecks for surviving offspring.

686 In addition to the vastly different responses to environmental stress that exist between taxa and
687 life history stages, variation in the tolerance of individual phenotypes within a population has
688 been documented for a number of environmental parameters, including hypoxia, temperature,
689 salinity, ammonia, as well as CO_2-induced changes in pH (Munday et al. 2009a, b, Killen et al.
690 2012). As this confers differential mortality amongst individuals, it may also enforce strong
691 selective pressures, impacting both ecological and evolutionary processes as the abundance
692 and/or performance of phenotypes shift (Schindler et al. 2010, Munday et al. 2012). A potential

outcome of this selective 'filtering' applied through differing metabolic constraints on whole-animal performance may lead to a homogenised group of phenotypes, also known as directional selection or "portfolio dampening" (Helmuth 2009, Holt and Jørgensen 2014). Failing to account for inter-individual variation in physiological studies is an oversight that may lead to the failure in accounting for the cryptic and sub-lethal effects caused by climate change, as individual trait diversity is a critical component of ecosystem structure and functioning (Ward et al. 2016).

Aerobic scope is one of the most commonly employed physiological performance metrics used to examine the physiological response of fish to environmental stress (Overgaard et al. 2012, Clark et al. 2013a, Clark et al. 2013b, Ern et al. 2014, Norin et al. 2014). This is despite the contentious nature surrounding Pörtner's OCLTT theory (Pörtner et al. 2004), on which AS is based in the context of Fry's thermal performance curve (Fry 1947). The general application of the OCLTT theory in ectotherm research is still disputed, and instead, alternative mechanisms explaining ectothermic thermal performance, such as the rate of temperature change, species, lifestyle and physiological condition, are suggested to play a role beyond the scope of the OCLTT theory. Whilst it is important to be mindful of alternative theories, evidence from a wide range of studies has been offered in support of this theory (Anestis et al. 2008, Farrell et al. 2008, Dong et al. 2011, Capossela et al. 2012, Yao and Somero 2014). The aim of this chapter is to estimate the aerobic scope of individual juvenile *C. laticeps* across a range of ecologically relevant temperatures to better understand the effects that the increase in frequency and intensity of cooling (Goschen and Schumann 1995, Duncan et al. 2019b) and warming (Schlegel et al. 2017) events predicted within their distribution range will have on the juvenile phase of this species. It is anticipated that their AAS thermal performance curve will follow a characteristic bell-shaped curve, as performance becomes constrained on either side of the optimum thermal range once fish are exposed to pejus temperatures (Pörtner et al. 2017).

3.2 Methods and materials

Details of the adult spawning, larval rearing, husbandry, tagging and respirometry set-up are available in Chapter 2. Fifteen three-month-old (94 day-old) juvenile *C. laticeps* were randomly chosen, individually marked using elastomer tags (see description of tagging in chapter 2) and separated into two 75 L fibreglass tanks and maintained at a temperature of 18°

C. Oxygen concentrations were maintained above 90% saturation using air diffusers in each tank, and percentage oxygen, temperature and pH (National Bureau of Standards scale) were measured daily with a Hanna HI 98194 multi-parameter meter. Fish were fed a pilchard and dried rotifer mixture to satiation once-daily in the morning.

On the days of the respirometry trials, three individually marked fish were removed from the tank before feeding to ensure that they had been fasted (for 24 hours) and introduced into the experimental respirometry chambers (see Chapter 2). Fish were allowed to acclimate to their new environment (Fig. 3.1) for one hour before the temperature was either maintained at 18° C, increased to 22° C using an aquarium heater or decreased to 11 ° C or 14 ° C using a chiller. The temperature used in the cooling trials was originally reduced to 10° C which proved to be the thermal limit at which physiological functioning became unfeasible, identified by the critical, sub-lethal effects and mortalities observed in the trial subjects. The subsequent cooling trials were increased to 11° C and the data from the previous 10° C trials were omitted. Owing to time restrictions, the critical thermal limits of the species (particularly the juveniles) were not able to be formally established, and so temperatures were chosen based on previous thermal metabolic trials conducted on adult *C. laticeps* as well as *in situ* thermal data (Duncan 2018, Skeeles 2019, Bailey 2022). Temperatures were increased and decreased at a rate of 1° C every half an hour. This rate of change was selected based on the most rapid observed changes over a six-month period at the original adult capture site (Skeeles, 2019). The SMR and MMR of each individual was measured using the methods described in Chapter 2, after which fish were removed from their chambers and placed into a beaker of water taken from the water bath of the respirometer. Water from their holding tank (18° C) was slowly added in small increments to the beaker until the risk of thermal shock had been neutralised before reintroducing them back into their holding tanks.

Respirometry experiments for half the individuals were first conducted at 18° C, and for the rest of the animals, experiments started with 22° C trials. To ensure that the order of the thermal treatment did not have an impact on the results, half of the fish were then tested at 14° C followed by 11° C whilst the other half were first tested at 11° C and then 14° C. A minimum of four days was allowed between trials to ensure no residual stress accrued from the previous trials which would influence the results of the subsequent trials. Fish from low pH conditions were taken from tanks 5 and 6, whilst fish from high pH conditions were taken from tanks 3, 4 and 8. In addition, each individual was rotated between the experimental chambers for each thermal trial in order to mitigate the effect of chamber on the metabolic endpoints. Muller's

(2021) study was the precursor to the current study in which the same metabolic experiment was run on the same group of fish during their larval phase. The various static respirometer microplates were also alternated during the separate trials however, the metabolic rates derived from individual oxygen consumption data during their larval phase were consistent and repeatable.

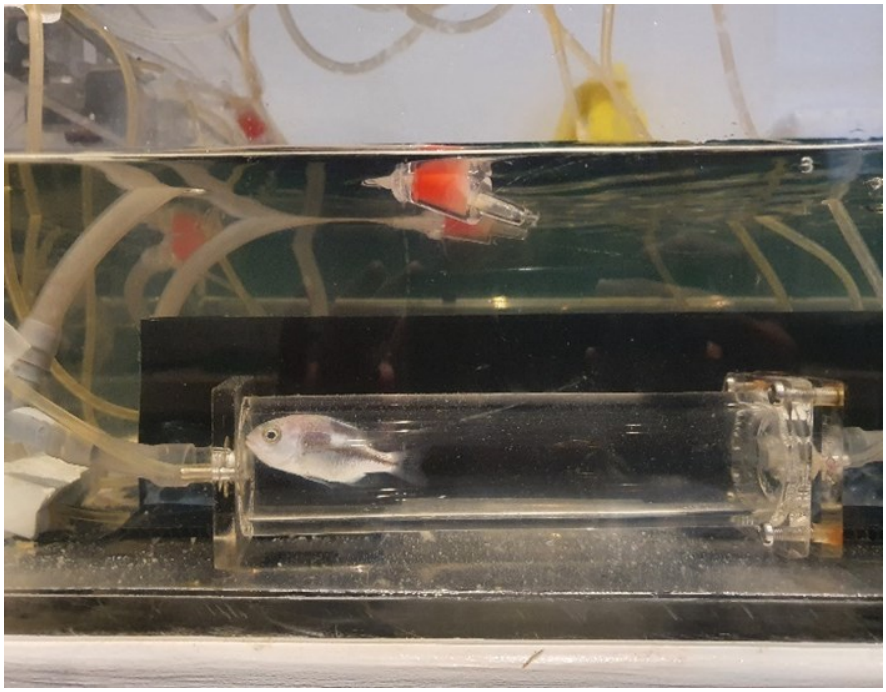


Figure 3. 1: A juvenile Chrysoblephus laticeps individual inside one of the experimental chambers during their thermal acclimation period.

3.2.2 Statistical analysis:

Once the mass-specific SMR and MMR for high pH animals was calculated (see Chapter 2 for methods) at each temperature, the absolute aerobic scope (AAS) was determined for each animal by subtracting the SMR from the MMR. The rates were then plotted to visualise the spread of the data. Plots of individual fish's SMR, MMR and AAS were created using only individuals with values for each metric at three or more temperatures so that the response of the individual across temperatures could be gauged. A linear mixed effects model was used to predict the effect of temperature on the metabolic rate estimates, using the lme4 package (Bates et al. 2015), and lmerTest‖ package (Kuznetsova et al. 2017) to account for data

homoscedasticity (all model assumptions were checked using diagnostic plots, see below) (Bolker et al. 2009, Zuur et al. 2009) and repeated measures of individual fish (Zuur 2009, Harrison et al. 2018). For each model, the effect of temperature on metabolic rates (SMR, MMR and AAS) were modelled using a second order polynomial relationship between metabolic rates (response variable) and temperature (fixed effect), while fish ID and tank number were included as the random effect structure, whereby fish ID was nested within each tank.

Metabolic rate data was log(x + 1) transformed to meet parametric assumptions. Residual diagnostics were carried out for hierarchical regression models using the "DHARMa" package (Hartig 2022) and Akaike Information Criterion (AIC) was used to determine the best fit model. The α level was set to $p < 0.05$ for all analyses.

The daily change in length and weight of each individual was calculated using the daily growth rate formula:

$$g = \frac{(xt2 - xt1)}{T}$$

where *xt1/xt2* is the change in the growth metric being measured, either weight or length, measured at time 1 or 2, and *T* is total days passed since the last measurement.

3.3 Results

The average weight of the fish used in the trials was 2.77 g at the start and 3.75 g by the end of the trials (n = 20 with replacement fish). The average length was 51.48 mm at the start and 56.50 mm by the end of the trials.

The average SMR for the group (Table 3.1) was lowest at the minimum experimental temperature, and increased with each subsequent temperature, with the highest average SMR occurring at the upper extreme temperature. The average MMR (Table 3.1) increased in a linear fashion until 18° C, after which the average MMR plateaued at high temperatures. Finally, the group's average AAS (Table 3.1) was lowest at the minimum experimental temperature, peaked at the acclimation temperature, and declined at the upper extreme temperature.

Table 3. 1: The average standard metabolic rate (SMR, mg $O_2^{-1}.g^{-1}.hr^{-1}$; and standard deviation), maximum metabolic rate (MMR, mg $O_2^{-1}.g^{-1}.hr^{-1}$) and absolute aerobic scope (AAS, mg $O_2^{-1}.g^{-1}.hr^{-1}$) for juvenile *Chrysoblephus laticeps* four ecologically relevant temperatures.

	Temperature (° C)			
	11	**14**	**18**	**22**
SMR	0.07 (± 0.03)	0.17 (± 0.11)	0.36 (± 0.24)	0.45 (± 0.16)
MMR	0.26 (± 0.10)	0.43 (± 0.11)	0.73 (± 0.28)	0.75 (± 0.24)
AAS	0.19 (± 0.09)	0.2602 (± 0.13)	0.37 (± 0.24)	0.30 (± 0.15)

There was a significant positive linear relationship between temperature and SMR (polynomial mixed model; p-value <0.05; Table 3.2; Fig. 3.2) but no significant non-linear relationship between temperature and SMR (polynomial mixed model; p-value = 0.42). Despite this, there was large individual variability in SMR, particularly at 18° C (Fig. 3.3). When examined at an individual level, fish 2 and 14, were maintained a very high SMR at 22° C but had a very low SMR at 18° C. The SMR of others (such as fish 1, 3, 7 and 8) increased from 11° C until 18° C and then declined at 22° C (Fig. 3.3).

Table 3. 2: Results of the polynomial linear mixed effects model analyses of metabolic rates (mg $O_2^{-1}.g^{-1}.hr^{-1}$) of juvenile *Chrysoblephus laticeps* in response to four ecologically relevant temperatures (° C). Temperature (1) represents the linear relationship between temperature and the metabolic metrices, whilst Temperature (2) represents the non-linear relationship between temperature and the metabolic metrices. Significant values reported in bold.

Effect	Estimate (intercept)	Std. Error	d.f.	t-value	P-value

SMR	Intercept	0.24	0.02	1.94	13.32	**0.01**
Pseudo-R² 0.49	Temperature (1)	0.80	0.12	33.05	6.55	**<0.01**
AIC - 45.34	Temperature (2)	-0.10	0.12	34.99	-0.81	0.42
	Random effects	**Variance**	**Std. Dev.**			
	ID:tank (Intercept)	<0.01	<0.01			
	Tank	<0.01	<0.01			
	Residual	<0.01	0.12			
MMR	Intercept	0.58	0.03	1.94	17.63	**<0.01**
Pseudo-R² 0.54	Temperature (1)	1.37	0.22	33.06	6.14	**<0.01**
AIC 7.51	Temperature (2)	-0.45	0.23	35.00	-1.99	0.05
	Random effects	**Variance**	**Std. Dev.**			
	ID:tank (Intercept)	**<0.01**	<0.01			
	Tank	**<0.01**	<0.01			
	Residual	**0.04**	0.22			
AAS	Intercept	0.29	0.03	1.94	10.85	**0.01**
Pseudo-R² 0.14	Temperature (1)	0.34	0.18	33.03	1.95	0.06
AIC - 13.41	Temperature (2)	-0.32	0.18	35.00	-1.85	0.08
	Random effects	**Variance**	**Std. Dev.**			
	ID:tank (Intercept)	<0.01	<0.01			
	Tank	<0.01	<0.01			
	Residual	0.03	0.17			

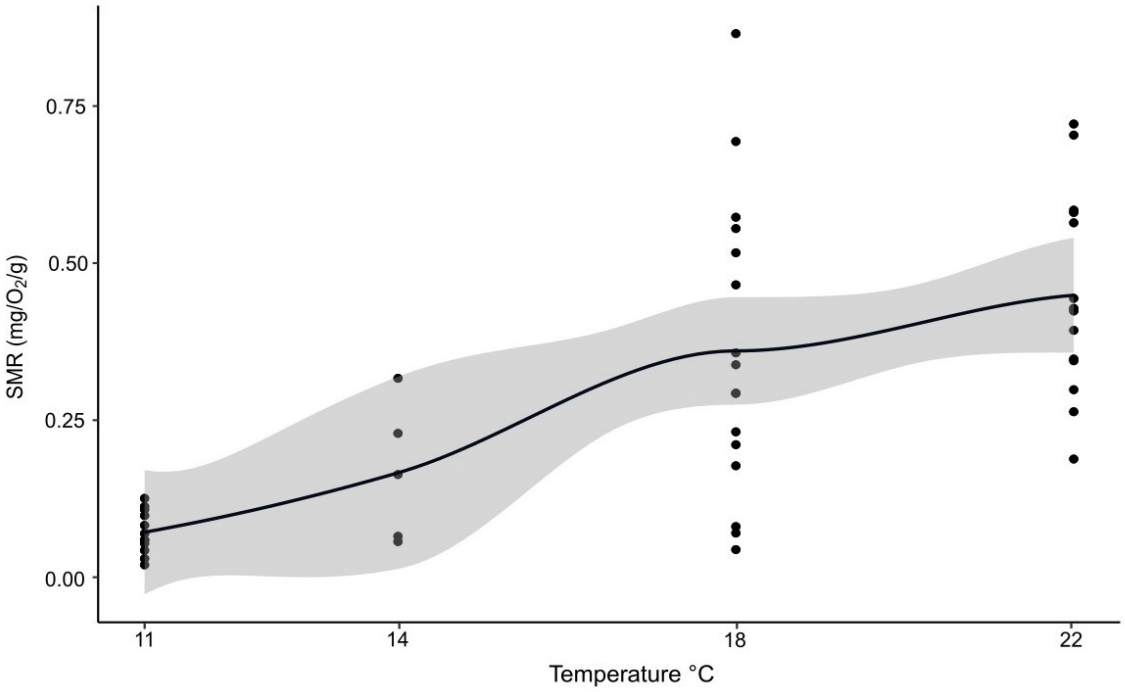


Figure 3. 2: The standard metabolic rate (SMR) of juvenile *Chrysoblephus laticeps* at four ecologically relevant temperatures (° C).

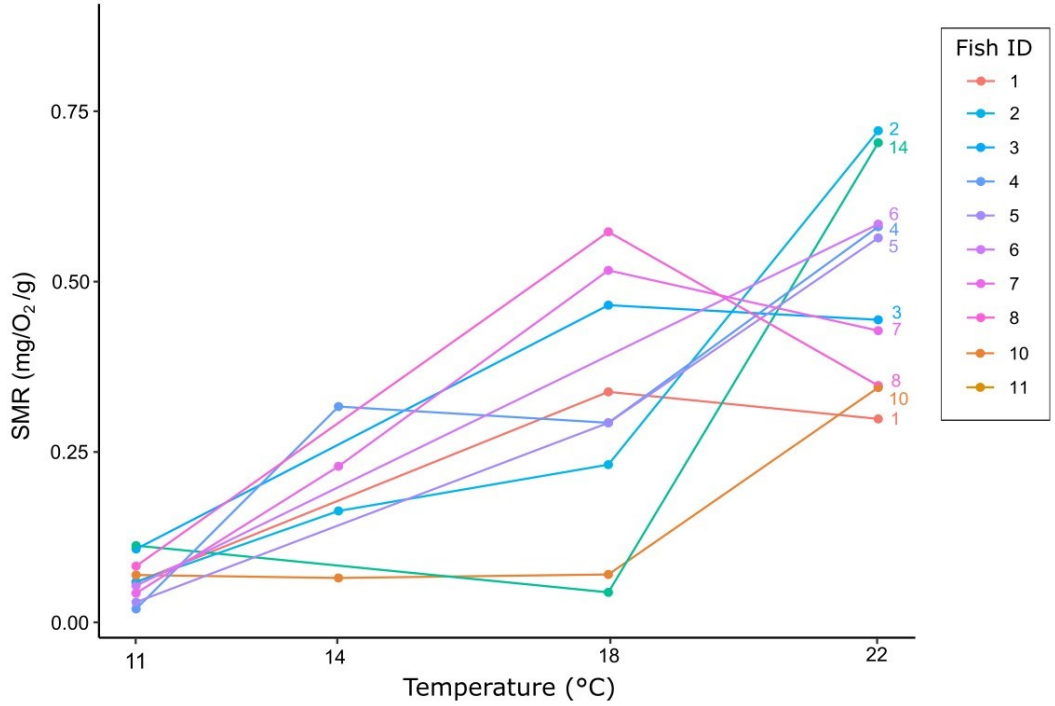


Figure 3. 3: The standard metabolic Rate (SMR) of individual juvenile *Chrysoblephus laticeps* at four ecologically relevant temperatures. This includes individual fish with values at three or more temperatures.

In line with SMR, there was a positive significant linear relationship between temperature and MMR (polynomial mixed model; p-value <0.05; Fig. 3.4, Table 3.2), and no significant non-linear relationship between temperature and MMR (polynomial mixed model; p-value = 0.05; Table 3.2), where the average MMR plateaued from 18° C. Individual variability in MMR was lower when compared with the SMR. However, fish 7 had an extremely high MMR at 18° C (Fig. 3.5). The rest of the individuals also showed a clear increase from 11° C until 18° C, after which a dichotomous response was observed where some individuals had an MMR that increased at extreme high temperatures, and others showed a decrease (Fig. 3.5). This meant that the variability around the mean MMR response was higher at 22° C (Fig. 3.5).

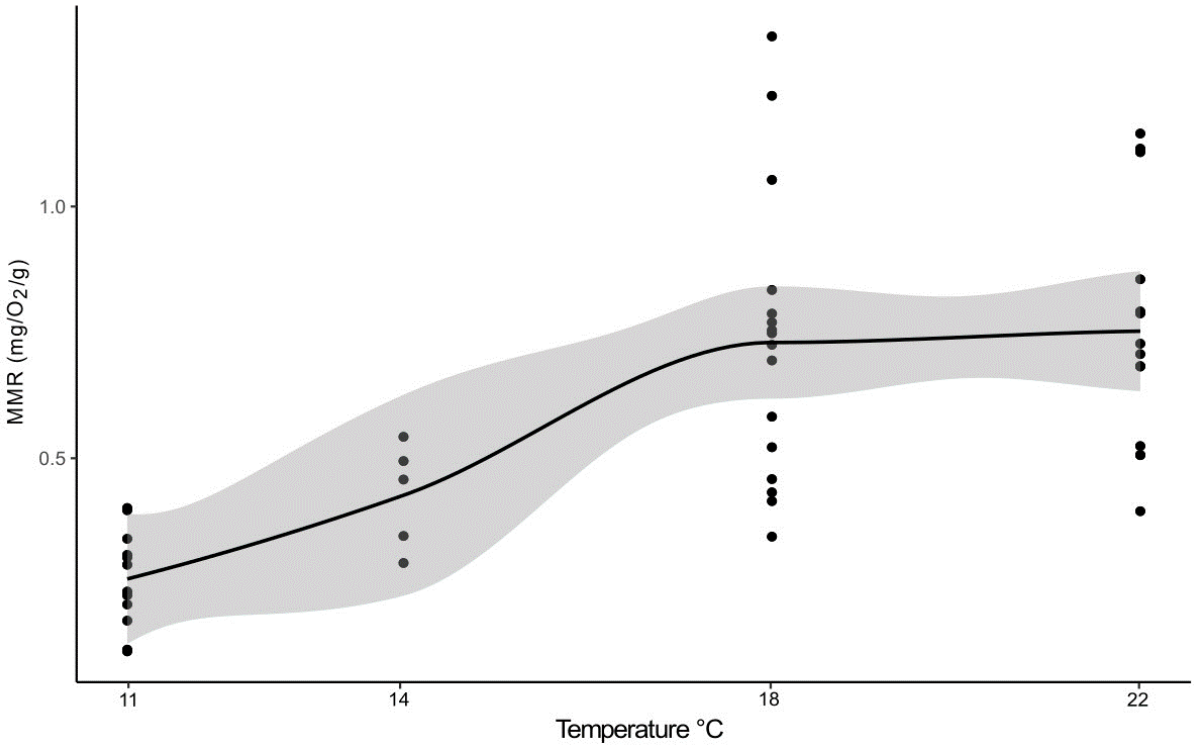


Figure 3. 4: The maximum metabolic rate (MMR) of juvenile *Chrysoblephus laticeps* at four ecologically relevant temperatures.

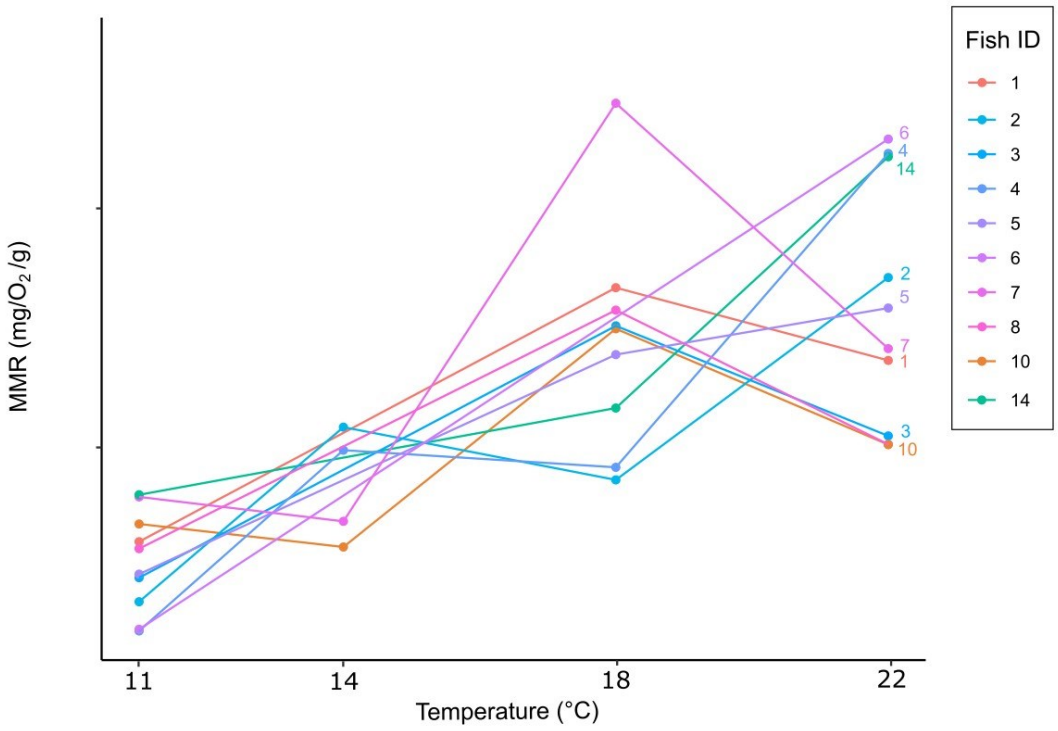


Figure 3. 5: The maximum metabolic rate (MMR) of individual juvenile *Chrysoblephus laticeps* at four ecologically relevant temperatures. This includes individual fish with values at three or more temperatures.

There was no significant linear or non-linear relationship between AAS and temperature (polynomial mixed model; p-value = 0.06 and 0.08; Table 3.2; Fig. 3.6). There was a high degree of variability in AAS at 18° C and 22° C (Fig. 3.7). At an individual level, aerobic performance of fish number 7 and 10 was high at their acclimation temperature (18° C) and at 11° C, but moderate at 22° C (Fig. 3.7). Others, such as fish 4, had a low aerobic performance at low and moderate temperatures, but high performance at 22° C (Fig. 3.7). The aerobic performance of the remaining individuals peaked at 18° C with declines in performance on either side of this temperature (Fig. 3.7).

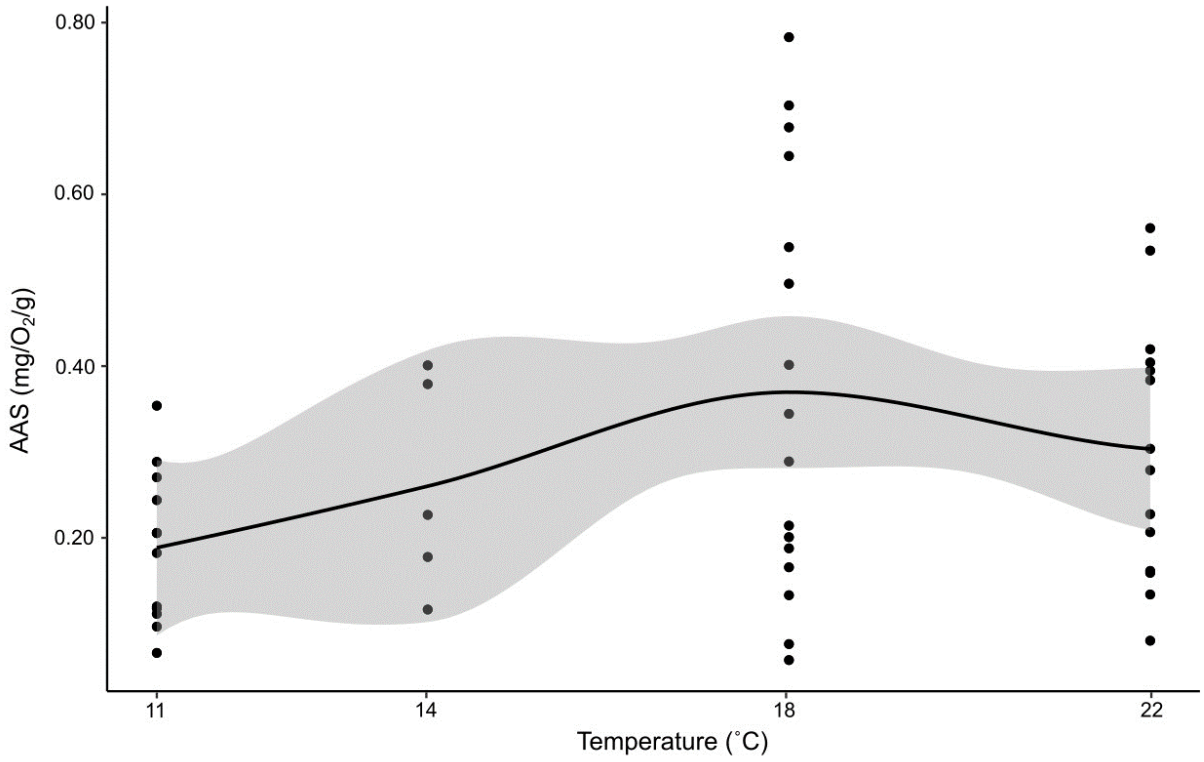


Figure 3. 6: The absolute aerobic scope (AAS) of juvenile *Chrysoblehus laticeps* at four ecologically relevant temperatures.

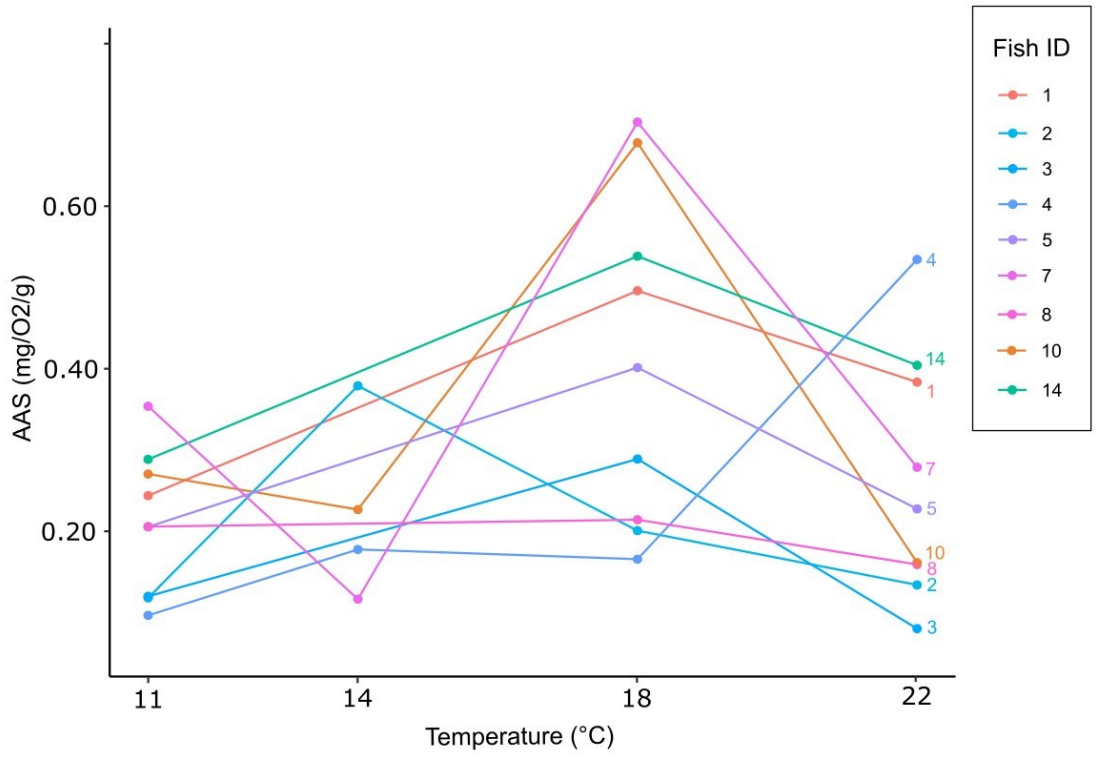


Figure 3. 7: The absolute aerobic scope (AS) of individual juvenile *Chrysoblephus laticeps* at four ecologically relevant temperatures. This includes individual fish with values at three or more temperatures.

3.4 Discussion

Juvenile *C. laticeps* appear to have a broad thermal performance bell-shaped curve, with an increase in performance from low temperatures (11° C) to a peak around 18° C and a decline above this temperature. This broad tolerance to varied thermal conditions is not unexpected in South Africa's, resident, temperate coastal species, as they frequently experience fluctuations in temperature (Mataboge 2021). Fishes with broad aerobic capacity are thought to be better equipped to deal with a highly variable thermal environment (Pörtner et al. 2004, Claireaux and Lefrancois 2007, Killen et al. 2017), as they can maintain AS across a range of tolerated temperatures (Healy and Schulte 2012). The metabolic thermal performance of adult *C. laticeps* from the same marine protected area (MPA; the Tsitsikamma National Park; Duncan 2018) resembled that of the juveniles in this study tested under a similar thermal range (8 – 24° C). The characteristic bell-shaped curve (Pörtner et al. 2017) was also recorded for thses adults, where metabolic rates increased with temperature until a point was reached (± 20° C), after which they declined (Duncan 2018). The similar thermal physiology is unusual as ontogenetic changes in the width of thermal tolerance windows typically occurs as juveniles have a wider thermal tolerance than adults owing to the higher performance capacity associated with smaller body size (Pörtner and Peck 2010). Larger individuals have a more narrow thermal window, as oxygen supply capacity decreases in relation to demand. (Pörtner and Peck 2010). This has been observed in many species such as sole, *Solea solea* (Rijnsdorp et al. 2009) and Atlantic cod, *Gadus morhua* (Pörtner et al. 2017). The similarity between juvenile and adult thermal performance curves may be attributed to their occupancy of similar habitats, unlike estuarine dependent species that have spatially distinct, stage-specific dissimilarities in habitat use (Adams et al. 2006).

The variability around the average AAS was reduced at cold temperatures in the current study but was distinctly high at 18 and 22° C. The latter was true for adults of the species, where metabolic rates differed in performance at control (16 ˚C) and upper experimental temperatures (21 ˚C and 24 ˚C). The largely consistent reduction in response at cold temperatures observed

in the juveniles tested in this study may be attributed to their occupancy of a habitat that frequently experiences temperatures that near their lower limit. For reference, adult *C. laticeps* were found to be particularly susceptible to extreme cooling from increased cold upwellings as "cold shock" (Donaldson et al. 2008) was induced at 8˚ C, and reduced growth and aerobic scope were observed (Duncan et al. 2018). Temperature was clearly an important predictor of metabolic activity in the study group of juvenile *C. laticeps,* seen in the response of the group's SMR and MMR, which ultimately dictates whole animal performance across the thermal spectrum (considering $AAS = MMR - SMR$; Nati et al. 2016).

The group's SMR increased linearly with increasing temperature, with the highest average SMR occurring at 22° C (0.4489 ± 0.161 mg O_2^{-1} g^{-1} $h^{-}1$; Table 3.1). The response of the group's SMR to temperature follows a familiar pattern originally observed in Fry (1947) and Fry and Hart (1948), where SMR increases exponentially (as was the case in adults of the population; Duncan 2018), or in this case linearly with increasing temperature. This trend is defined largely by the increase in oxygen demand with increasing temperature. This is well described by Pörtner and Knust (2007) in the theory of OCLTT which stipulates that the thermally determined mismatch between oxygen demand and supply is the first mechanism to restrict animal performance. Therefore, as temperature increases, so too does the concomitant metabolic costs associated with baseline metabolism (Pörtner and Knust 2007).

A high degree of variability in SMR at optimal and high temperatures was observed in juvenile *C. laticeps*. Diversity in the group's metabolic response means that the population may be better equipped to deal with the current and projected increase in environmental variability (Bailey 2022). Some of the heterogenous subsets of a group may be better suited to coping with different types of environmental conditions than others (Kussell and Leibler 2005). For example, one school of thought suggests that a reduced SMR offers an increase in surplus AS available for coping with environmental stressors, but this heightens baseline metabolic costs and lowers the calibre of an organism's MMR (Killen 2014). The advantages however, of a higher SMR in similar temperate and arctic juveniles of resident and/or territorial species such as the juvenile salmon, have been the correspondence of higher aggression and dominance which confers a greater competitive advantage over access to food and resources, albeit with higher maintenance costs (Priede 1985, Metcalfe et al. 1995, Cutts et al. 1999). Considering *C. laticeps* is a resident species and males are highly territorial, (Provost and Jensen 2015), this may be similarly beneficial. Which phenotype confers an advantage under future thermal variability, however, remains to be seen but the presence of both is biological kindling in the

form of differential plasticity for the accommodation of environmental change (Norin et al. 2016).

Standard metabolic rates are also plastic in nature and are able to be reduced in response to chronic warming (Sandblom et al. 2016), and as an adaptive response to limited food availability, as seen in species such as individual brown trout (*Salmo trutta*; Auer 2016), juvenile salmon (*Salmo salar;* Reid et. al. 2011) and a cyprinid species (*Carassius auratus*; Zeng et al. 2017). This adds an additional layer to the complexity regarding the variability observed in the group's SMR. Studies done on additional taxa such as amphibians (Hervant et al. 2001), insects (Roark and Bjorndal 2009), reptiles (McCue 2007), mammals (Ostrowski et al. 2006), and birds (Wiersma et al. 2005) also show that individuals within a population can increase their SMR when food is abundant and decrease it when food levels decline. Having been reared in an unrestricted food environment, the juvenile *C. laticeps* used in this study may have had SMR values that were higher than what they would have been in a food-limited natural environment. This apparent capacity of *C. laticeps* for metabolic and phenotypic flexibility is likely to have important consequences for fitness and their ability to cope with a changing environment, as found by Nussey et al. (2007) and Bolnick et al. (2011) using analytical frameworks and models incorporating individual/intraspecific variation.

The linear increase and plateau of MMR with increasing temperature in juvenile *C. laticeps*, has often been reported due to the limitations imposed by temperature on oxygen transport and supply systems (Fry and Hart 1948, Farrell et al. 2009; Pörtner, 2010, Pörtner et al. 2017, Seibel and Deutsch, 2020). In contrast to the theory of OCLTT (Pörtner et al. 2017), Seibel and Deutsch (2020) suggest that the limitation on MMR at high temperature results from something other than oxygen supply capacity (α, μmol O_2 h^{-1} g^{-1} kPa^{-1}). It is stipulated that α matches MMR across a species' temperature and size range at the prevailing environmental oxygen partial pressure (P_{O2}), as there are strong selective pressures acting on the oxygen supply system to meet the maximum oxygen demand (consistent with the theory of 'symmorphosis'; Weibel et al. 1991, Suarez 1998, Lindstedt and Conley 2001). They instead attribute the loss of performance at high temperatures, in many species, to the failure of muscle oxidative performance or the failure of "metabolic machinery" to make use of the oxygen supplied. Whilst it has been suggested that there is substantial individual variation in the thermal sensitivity of MMR (Norin et al. 2015), and the magnitude of variation in the response of MMR is thought to be similar to SMR (Norin and Malte 2011, 2012, Killen et al. 2012, Metcalfe et al. 2016, Norin et al. 2015), in this study, there was relatively little variation in the group's

MMR over the range of temperatures tested. There was, however, a slight increase in variability at high temperatures. This suggests that the reduction in oxygen saturation at high temperatures may have been the cause of the difference in aerobic performance observed in the group's MMR, as noted in adult *C. laticeps* (Bailey 2021), owing to varied thermal sensitivities of different metabolic phenotypes (Norin et al. 2016).

Whilst the shape of the juvenile aerobic scope thermal performance curve was similar to adult *C. laticeps* (Bailey 2022), maintenance costs and resource allocation will vary from adults of the species owing to the partitioning of energy to growth (Truebano et al. 2018). In juvenile fish there will be an additional cost imposed by the oxygen requirements of growth (Post and Lee 1996), and the mode of activity will often differ from adults as juveniles undergo short periods of activity followed by rest (Post and Lee 1996). High energetic demands are typically associated with thifs type of locomotion during foraging and predator avoidance as aerobic metabolism increases following burst-type anaerobic activity (Killen et al. 2007). This is expected to limit energy allocation to homeostatic maintenance during times of limited food and environmental stress (Killen et al. 2007). Despite this, similar performance curves have also been observed between the adults and juveniles of coho salmon *Oncorhynchus kisutch.* The aerobic performance curve of adult coho salmon has been shown to be maintained across the suite of ecologically relevant temperatures likely to be experienced at any point in their life cycle (Raby et al. 2016), and which was maximized at warmer temperatures in those with particularly challenging migratory conditions (Lee et al. 2003, Farrell 2007, Farrell et al. 2008, Eliason et al. 2011). The findings of two other studies showed that the aerobic scope of juvenile coho salmon was similar in that AAS was maintained or increased slightly, except when temperatures exceeded the ecologically relevant range (Casselman et al. 2012), showing no obvious peak at an optimum temperature (Sungalia 2018). This is despite juveniles of this species having very different selective pressures (Casselman et al. 2012). The similar performance curves observed between juvenile *C. laticeps* and the adults from the same population may be because they occupy similar habitats. Whilst some adult *C. laticeps* may reside on deeper reefs (both inshore and offshore) up to 100m in depth (Buxton and Smale 1984, Buxton 1987, Götz 2005, Götz et al. 2008), there is large overlap between adults and juveniles on shallow subtidal reefs up to 30m (Penrith 1972, Buxton and Smale 1984, Buxton 1987). Therefore, since both life stages are exposed to a similar, high degree of thermal variability along their distribution, it is likely that they will be similarly suited to coping with changing temperatures. Considering a thermal range between 9 and 22° C has been recorded

along their distribution (μ = 18° C; Skeeles 2019), this thermal history may be shaping the broad thermal performance curves of both stages.

The bell-shaped curve of the AAS thermal performance curve for juvenile *C. laticeps* is dictated by the different responses of the SMR and MMR at higher temperatures. According to the OCLTT theory, AAS is reduced at high temperatures as MMR does not increase at the same pace as SMR once temperatures exceed the species' optimal temperatures (Farrell et al. 2009, Farrell 2016, Lefevre 2016). In other words, at high temperatures, the physiological limit is reached for MMR, whilst SMR continues to increase (Fry 1947, Fry and Hart 1948). It has been suggested that this disparity exists due to the limitations imposed by the maximum heart rate (Farrell et al. 2009). This impacts the delivery of blood to swimming muscles which causes acidemia and hyperkalemia in the blood resulting from muscles having to work glycolytically (Farrell et al. 2009). This results in a feedback loop that further impairs heart function, compromising the oxygen transport system as warming continues (Norin et al. 2014). It should be mentioned that not all species will demonstrate the bell-shaped performance curve and instead MMR may continue to increase until lethal temperatures are reached (Jutfelt et al. 2018, Lefevre 2016, Nati et al. 2016). In this study, the decline in AAS after 18° C suggests that the maximum temperature for the species is not much higher than 22° C, which has been reflected in the findings from the breakpoint analysis using heart rate loggers by Skeeles et al. (2020), which shows that the species maxima is near 25° C. The naturally high degree of thermal variability along their distribution is projected to increase with time and contextualising the metabolic response of juvenile *C. laticeps* with these changes will help us to understand the potentially complex population-level implications.

The projected increases in the frequency and intensity of localised cooling events from increased coastal upwelling (Goschen and Schumann 1995) in the habitat range of *C. laticeps* is expected to have a major impact on the species. Cold shock has been reported in *C. laticeps* at temperatures of 8 ˚C (Duncan et al. 2019a). This causes a reduction in ventilatory oxygen supply mechanisms and a transition to anaerobic metabolism and leads to a build-up of harmful Reactive Oxygen Species (ROS) due to the slowing of lactate clearing pathways (Pörtner et al. 2004). The detrimental nature of extreme cooling events is reflected in the distinctly reduced variation in all three metabolic responses at extreme low temperatures. The uniform reduction in performance may indicate a reduced affinity for coping with extreme cold events. This is despite occupying habitats that frequently experience temperatures that near their thermal

minimum (Bailey 2022). The effects of this drastic temperature change can be likened to the effects that this would have on a sympatric, closely-related sparid species, *Boopsoidea inornate*, which has lower and upper thermal stress limits at an average of 9° C and 25° C, and a CT_{min} and CT_{max} of 7.8° C and 30° C respectively, based on measurmets of opercular beat rates (Allison et al. 2021). Given the frequency and magnitude of upwelling events along parts of their overlapping distribution, cooling may be worse than warming for these highly resident species.

Whilst the mean annual sea-surface temperature may be decreasing along areas of the south coast, localized areas of warming have also been identified (Lima and Wethey 2012). The relatively broad performance curves observed in juvenile *C. laticeps* and other temperate species (with gentle slopes in performance curves as opposed to narrow, steep slopes in tropical species) suggests that there may be a reduced effect on the species. This and a growing body of literature (Dillon et al. 2010, Paaijmans et al. 2013, Vasseur et al. 2014) suggests that temperate organisms are better equipped to deal with the projected increase in thermal variability, as they are frequently exposed to marked thermal fluctuations. However, the reduction in performance at high temperatures, which is synonymous with Shelford's law of tolerance stipulating that whole animal AS decreases at the onset of thermal limits at low and high *pejus* thresholds (Shelford 1931), may suggest that performance may be reduced with warming temperatures. Whilst the thermal maxima for adults of the species were estimated to be between 22° C and 25° C (Skeeles et al. 2020), indicating a propensity for tempered performance under increased warming, juvenile *C. laticeps* populations may be more resilient to warming events than anticipated. This can be attributed to the individual variation in the metabolic phenotypes at optimal and upper temperatures in this study.

The potential for a population to adapt to the current and projected thermal changes rests on the individual genetic and resultant phenotypic makeup of a population (Sunday et al. 2014). Ultimately, selection arising from new environmental conditions acts on the phenotypes presented in a population and alters the average phenotype according to which best confers the greatest fitness under the new conditions (Sunday et al. 2014). It is the variability of the gene pool that is thought to largely determine the plasticity of phenotypic response (Haugen and Vøllestad 2000, Jensen et al. 2008, Baumann and Conover 2011, Hutchings 2011). One of the benefits of this study was the element of repeated measures, which allowed for the observation of variability in the response of different metabolic phenotypes across the thermal spectrum of

temperatures tested (Killen et al. 2021). This offered insight into the ecologically applicable whole-animal response. In this study, there was a high degree of individual variability in physiological performance, particularly at optimum and high temperatures. Bailey (2022) also found a high degree of individual variation in an exploited population of adult *C. laticeps* at higher temperatures. However, it is possible that the increased variability in the juvenile *C. laticeps* in this study may be attributed to the genotype, as these individuals were spawned from adults from a longstanding MPA. This is supported by the findings in Duncan et al. (2019a) which showed that the protected population of adults in the same MPA had more aerobic scope phenotypic diversity as well as higher performance aerobic scope phenotypes. This diversity was, however, reduced at extreme cold temperatures (8° C), which was also reflected in all three metabolic indices of the current study. Bailey (2022) also found a reduction in metabolic phenotypic variability at lower temperatures in adult *C. laticeps* from an exploited area and suggested that the species was occupying habitats with temperatures nearing their thermal minima. The uniform reduction in performance, particularly for the response of the group's SMR but also the response of their MMR, which conveys the reduced variability in AAS, may indicate a maladaptive potential to the increased extreme cooling events projected along their distribution.

Owing to the combined effects of dynamic environmental factors and the effects that these have on the physiology of ectotherms, it is important to address the role that acclimation temperatures have on the outcome of metabolic studies. A potential caveat of this study was the stable acclimation temperature (18° C) at which the group of juvenile *C. laticeps* had been exposed to since hatching. Whilst this is the ecologically relevant average temperature that this population would be exposed to, the diel thermal variation found in the wild is absent, and exposure to daily changes or alternatively, slightly cooler or warmer average temperatures would likely impact their metabolic response. There have been numerous studies detailing the importance of acclimation temperatures to the physiological and behavioural response of fish to artificially simulated environmental stressors. For example, the cyprinid (*Tor putitora*) has the ability to adapt to higher acclimation temperatures by altering its haemato-biochemical variables, whilst its thermal limits and rate of oxygen consumption are dependent on its thermal history (Akhtar et al 2013). A study on juvenile Atlantic salmon (*Salmo salar*) showed that those exposed to daily fluctuations at 20 ± 2.5° C had an increased SMR (33.7%) compared to those acclimated at a constant temperature of 20° C (Oligny-Hébert et al. 2015). In contrast, shorthorn sculpin (*Myoxocephalus socalize*) exposed for extended periods to a higher-than-

usual acclimation temperature (16° C) demonstrated the capacity for thermal compensation in their SMR such that their AS was partly restored, and the specific dynamic action of feeding was no longer metabolically constrained (Sandblom et al. 2014). It may then be important for future research to incorporate a pilot study using in-field bio-loggers and/or biotelemetry studies to register the diel thermal variability an organism would typically be exposed to, to incorporate the dominant thermal spectrum, much like the diel photoperiod simulated during husbandry. In addition to this, biotelemetry would be an important tool, used in conjunction with laboratory studies, in conceptualising how often individuals near their MMR, and what this means for their maximum aerobic capacity within the confines of their thermal gradient (Metcalfe et al. 2016).

Ensuing the completion of the trials, further investigation into literature pertaining to the effects of food availability revealed that differing feeding regimes have an impact on intra-individual variation in metabolic performances, which should be considered in trials of this nature (Millidine et al. 2009, Reid et. al. 2011, Auer et al. 2014, Auer 2016, Zeng et al. 2017). Whilst the design of this study addressed the effects of post-feeding metabolism by fasting individuals for 24 hours prior to the start of the trials, future metabolic studies should look to incorporate varied feeding regimes to account for the within-organism disparities in maintenance costs arising from resource availability. In a study examining the ties between somatic growth rates and individual variation in both SMR and AS of brown trout (*Salmo trutta*), it was found that the links between individual variation and fitness vary under differing food availability and are context-dependent (Auer et al. 2014). Disproportionate intra-individual food consumption in communal holding tanks may impact the individual variability in metabolic phenotypes (Van Leeuwen et al. 2011a), owing to the flexible nature of SMR and the potential consequences for fitness (Nussey et al. 2007; Bolnick et al. 2011). Whilst the element of repeated measures within the same individual across a thermal gradient offers a clear assessment of individual variation in performance, a relatively large sample size is typically required to give precise estimates of this condition (Martin et al. 2011, van de Pol 2012, Allegue et al. 2017). Ultimately, this study was limited by the availability of surviving juvenile *C. laticeps*, and whilst the integrity of the data is uncompromised, the statistical power would have been enhanced by a larger sample size.

The following caveats were technical limitations of the study design, that in each case were overcome. Owing to this study being the first intermittent flow respirometry to be conducted on juvenile *C. laticeps,* the timing of measuring and flushing periods was decided upon based

on relevant literature on the juveniles of other species. The initial measuring period at high temperatures proved to be too long, to the detriment of the fish's condition, which required a change from five minutes to three minutes. A second element of the experimental process that required revision was the cleaning method of the pipes feeding into each chamber in the water bath. The method of sterilisation of submerging the equipment in a 0.01 % concentration of bleach and water was insufficient to completely clear the pipes of all microbial growth, which led to a build-up and thus enhanced microbial respiration. This was immediately corrected by employing a more rigorous, mechanical method of sterilisation and this resolved the issue.

Considering the pronounced and increasing thermal variably throughout the distribution of *C. laticeps*, and the changes in organismal energy budgets throughout ontogeny, research into the metabolic scope during the juvenile life stage is particularly useful in identifying potential bottlenecks. The findings of this chapter, however, not only suggest that the juvenile life stage of *C. laticeps* is resilient to a broad range of temperatures, but also that there is a great deal of individual variability in the sampled population, which may suggest that the population may be able to adapt to future thermal change. Individuals possessing a broad aerobic scope will be the best suited to maintain physiological functioning within the frame of enhanced thermal variability. However, since thermal change will not be the only driver, the thermal performance of juvenile *C. laticeps* that are subjected to other stressors, such as a decline in ocean pH should be tested. This is the focus of Chapter 4.

CHAPTER 4

The effects of ocean acidification on the aerobic scope of juvenile red roman seabream (Chrysoblephus laticeps)

4.1 Introduction

In addition to changes in dissolved oxygen (DO; Vaquer-Sunyer and Duarte 2008) and general ocean warming resulting from climate change (Gattuso *et al*. 2015), ocean acidification (OA) has been identified to be a ubiquitous, compounding factor arising as a direct consequence of CO_2 accumulation in the atmosphere (Doney et al. 2009). The ocean's role as a major carbon sink has led to the assimilation of nearly 30 % of the total anthropogenic carbon dioxide (CO_2) emitted since the Industrial Revolution (Sabine et al. 2004, Field et al. 2014). Atmospheric CO_2 has increased from 278 ppm to over 400 ppm since the pre-industrial era (Doney et al. 2009). The proposed trajectory for 2100, if there is no action to curb green-house gas emissions, is an atmosphere with a CO_2 concentration that could reach 1000 ppm (IPCC 2019). The resultant decrease in global ocean pH is expected to be 0.3 to 0.4 units from current levels (Feely et al. 2004). The global marine environment will thus be more acidic than it has been in the last 400 000 years (Feely et al. 2004) and is expected to impact life in all parts of the ocean, from the deep sea to near-shore environments, including coastal estuaries (Orr et al. 2005, Feely et al. 2009).

Fish typically have a well-developed acid-base regulatory system (Pörtner et al. 2004b) however, high-CO_2-induced hypercapnia has been shown to impact the physiological and behavioural responses of marine ichthyofauna. Behavioural changes include increased levels of boldness and altered predator-prey behaviour (Cripps et al. 2011, Ferrari et al. 2011a), changes in lateralisation and consequently spatial orientation, reactivity and group coordination (Domenici et al. 2012, 2014, Jutfelt et al. 2013), as well as compromised learning ability or decision-making (Ferrari et al. 2012). There have also been reports of changes in sensory functions (Simpson et al. 2011) with important consequences for homing behaviour and settlement (Munday et al. 2009b, Devine et al. 2012, see Briffa et al. 2012, Heuer and Grosell 2014, Clements and Hunt 2015, Nagelkerken and Munday 2016, Cattano et al. 2018, Munday et al. 2019a, for reviews on behavioural changes). A range of these behavioural changes have

been attributed to the impaired functioning of neurotransmitter receptors as a result of changes in acid-base status (Nilsson et al. 2012, Hamilton et al. 2014, Heuer and Grosell 2014). Behavioural changes may translate into community-level disruptions through altered predator-prey interactions, dispersal and recruitment, as well as habitat selection and settlement (Nagelkerken and Munday 2016). Ocean acidification has also been linked to physiological impacts such as severe tissue damage (Frommel et al. 2012), reduced survival and growth in early life-stages (Baumann et al. 2012, Pimentel et al. 2014, Edworthy 2017), reduced swimming performance (Hamilton et al. 2017), and negative effects on metabolic rates (Pimentel et al. 2014). It has been shown that marine eggs and larvae may be more susceptible to OA as organs which facilitate regulatory processes, such as the gills, are yet to develop (Baumann et al. 2013, Frommel et al. 2012). Undeveloped gills and inadequate acid-base regulation (Falk-Petersen 2005) may reduce olfaction and sensory abilities (Munday et al. 2009c), induce changes in behaviour (Munday et al. 2010), inhibit otolith growth (Checkley et al. 2009), affect tissue/organ structures in larval fish (Frommel et al. 2012), as well as alter survival and hatching success in eggs (Chambers et al. 2013).

Physiological and behavioural changes in response to CO_2-induced hypercapnia are thought to be elicited by compensatory acid-base regulation (Flannery 2018). Carbon dioxide is a small, uncharged molecule which acidifies the internal pH of fish by rapidly diffusing across cell membranes where it is catalysed by carbonic anhydrase and converted into carbonic acid (H_2CO_3) as internal pCO_2 levels increase (Ishimatsu et al. 2005). Subsequently, extracellular (pH_e) and intracellular pH (pH_i) decreases leading to acidosis which requires compensatory adjustments (Brauner et al. 2019). For fish, these adjustments may occur by the retention and/or uptake of bicarbonate (HCO_3^-) while increasing acid secretion (Heuer and Grosell 2014). Initially, the net compensatory accumulation of plasma HCO_3^- concentrations occurs through transferal from intracellular to extracellular spaces followed by an increased uptake of bicarbonate from the environment while simultaneously increasing bicarbonate retention (Toews 1983). Once internal pH stabilises, HCO_3^- may be returned to intracellular compartments and both pH_e and pH_i are compensated for by seawater-derived HCO_3^- (Toews 1983). Acid-base regulation primarily occurs at the gills and the transport of HCO_3^- and H^+ across the gill epithelium is facilitated by the exchange of counterions to sustain the electroneutrality of internal fluids (Ishimatsu et al. 2005). Sodium (Na^+) exchange brought about by Na^+/H^+ ion exchangers are involved in this process (Claiborne et al. 2002), and recently, chloride (Cl^-) ions have been found to act as main counterions, with chloride cells

playing a part in acid-base regulation (Hayashi et al. 2004, Ishimatsu et al. 2005, Heuer and Grosell 2014). Na^+ and Cl^- are actively secreted through chloride cells to counteract the diffusion of ions into body fluids to restore pH (Zadunaisky 1984).

The energetic costs associated with compensatory acid-base regulation can have effects on fish metabolism, which may translate into issues concerning growth, swimming, reproduction and feeding (Heuer and Grosell 2014). The general premise suggests that the metabolic costs of coping with hypercapnia, which include adjustments to acid-base regulation, cardiorespiration and osmoregulation, would cause a reduction in the AS of fishes by increasing SMR (loading stress), and conferring reduced fitness (Munday et al. 2009a, Enzor et al. 2013, Heuer and Grossell 2014). Furthermore, the acidification of internal conditions from high CO_2 is suggested to limit oxygen transport which imposes aerobic constraints on MMR (limiting stress), thus further reducing AS (Munday et al. 2009a, Heuer and Grosell 2014, Flannery 2018). Whilst this has been the case in certain species (Munday et al. 2009a, Enzor et al. 2013), there has been no effect on the AS of others (including tropical and temperate species; Melzner et al. 2009, Couturier et al. 2013, Miller et al. 2012), and some show increased AS (Rummer et al. 2013a). Even among sympatric and phylogenetically similar species, tolerance to lowered pH levels is highly varied in fish (Ferrari et al. 2011a, Hamilton et al. 2017). However, there is general agreement that the impacts of reduced pH on metabolism are compounded when fish are exposed to thermal extremes (Pörtner and Farrell 2008). Therefore, the evaluation of the physiological response of fishes to hypercapnia should be conducted across a range of thermal contexts.

Since the persistence of populations and the maintenance of their performance under acidifying conditions may be accomplished through acclimation or adaptation (Sunday et al. 2014), long-term exposure to low pH conditions is also necessary to fully understand the impacts of OA on organisms. Acclimation is achieved through phenotypic plastic adjustments in the behaviour, morphology or physiology of an individual that can preserve fitness in a stressful environment (Angilletta 2009, Sunday et al. 2014). Adaptation is driven by the intense selection under new environmental conditions, which shifts the average phenotype toward a new fitness peak (Sunday et al. 2014). The inherent genetic variability of the population is thought to largely determine the plasticity of phenotypic response across the phylogeny of species (Haugen and Vøllestad 2000, Jensen et al. 2008, Baumann and Conover 2011, Hutchings 2011). Plasticity can be summarised graphically across an environmental gradient as a change in phenotype, which has been termed the reaction norm (Fig. 4.1; Scheiner 1993, Hutchings et al. 2007).

Variation in the slope, shape, or intercept of the reaction norm indicates genetic differentiation, and potential adaptation (Lande 2009, Chevin et al. 2010). It is important to note that acclimation can interact with genetic adaptation, either hindering (Sunday et al. 214) or assisting in adaptation (e.g., genetic assimilation; Pigliucci et al. 2006).

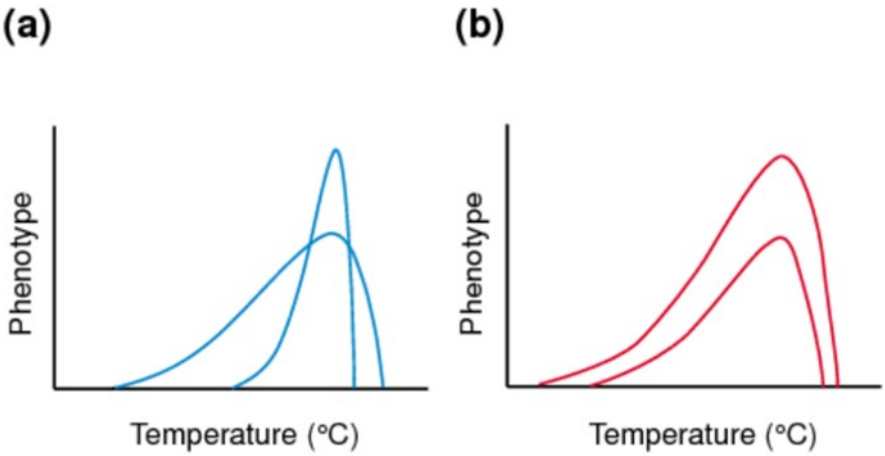


Figure 4. 1: Examples of the genetic variation for reaction norms across a thermal gradient. In this case, including trade-offs that may exist for the selection of a particular reaction norm where different genotypes show (a) a specialist–generalist trade-off and (b) an allocation or an acquisition trade-off. Taken from Angilletta et al. (2003).

Since climate change acts on individual-level processes which accumulate and translate into population-level effects, quantifying individual differences is necessary to appreciate the gravity of change occurring in ecological systems (Ward et al. 2016). Genetic variability in a population is the component on which selection acts, likely against phenotypes susceptible to OA, selecting for the rapid fixation of alleles that allow for successful reproduction under hypercapnia leading to adaptation (Schlegel et al. 2012). Whilst estimates of genetic variation in ocean acidification impact studies require complex mating or multi-generational designs (Sunday et al. 2011), inter-individual phenotypic variation can be estimated, and the acclimation potential (the phenotypical plasticity of physiological, morphological and behavioural responses that preserve fitness under new environmental conditions) can be assessed (Angilletta 2009). Wide variability in response phenotypes among individuals of six different species has been documented under moderate hypercapnia ($CO_2 \approx 700$ μAtm; Ferrari

et al. 2011b, Munday et al. 2010, Munday et al. 2012a, Munday et al. 2012b). Sufficient extant individual variation on which selection can act may help to ameliorate the effects of OA and climate change on fish populations (Schlegel et al. 2012). The existence of potential metabolic/energetic trade-offs from adaptation (or acclimation) to OA must also be quantified to ascertain if hypercapnic tolerance is limiting an organism's ability to cope with additional environmental stressors, thus compromising overall fitness (Heuer and Grossell 2014). Whilst longer evolutionary experimental designs are required to assess selection and responses across full lifespans of organisms (Sunday et al. 2014), estimates of phenotypic variation, particularly of fitness traits, may prove valuable for identifying the vulnerability of species to falling ocean pH.

Research pertaining to the response of early life stages to climate-related stressors, such as OA and thermal change, is pivotal to our understanding of population-level dynamics, particularly in commercially valuable species. Stressors acting on early ontogeny likely have important repercussions for fish populations, which are dependent on the successful recruitment of early life stages into the fishery (Houde 1997). There have been a few studies to date (*Solea senegalensis*; 75 days exposure, Olivera et al. 2022; juvenile walleye pollock, *Theragra chalcogramma*, cumulative exposure of 28 weeks, Hurst et al. 2012; Atlantic cod, *Gadus morhua*, 4-12 months, Melzner et al. 2009; juvenile spotted wolffish, *Anarhichas minor,* 10 weeks, Foss et al. 2003) that have examined the long-term effects of OA on fish, but research looking at extended exposure is limited. A meta-analysis of the literature found that negative impacts associated with OA, such as reduced growth and mortality, were observed in short-term studies but were reduced or absent in long-term studies (Cattano et al. 2018). The long-term response of the early life stages of fish has been identified as a critical knowledge gap requiring further long-term and multi-generational experiments to test for adaptation potential in fish under high CO_2 conditions. Research examining the long-term impacts of reduced pH on fish throughout the early stages of their ontogeny is particularly scarce. This study seeks to address this paucity of knowledge by incorporating long-term exposure to high CO_2-induced hypercapnia from egg to juvenile phase of the endemic, commercially important coastal seabream, *C. laticeps.*

Information on the impacts of OA on coastal fishes across thermal contexts is essential given the projected increase in OA and thermal variability of coastal waters (Bates et al. 2018). Comparably, the literature on open ocean pH is a great deal more extensive than localised pH

variability in coastal waters, particularly in Africa (Edworthy et al. 2022). The coastal environment shows a high degree of spatial and temporal variability in pH conditions owing to a combination of drivers and coastal processes (e.g., freshwater influx; Vargas et al. 2016; biological activity; Krause-Jensen and Duarte 2016) that influence pH and carbonate chemistry of different regions (Kapsenberg and Hofmann 2016). In a study by Edworthy et al. (2022) a monthly variability of ~0.46 pH unit (range = 8.43 – 7.98) was observed in Algoa Bay, South Africa. This is not unusual in coastal areas (Hofmann et al. 2011, Kapsenberg et al. 2015) as seasonal and inter-annual fluctuations in pH can change by up to 1.4 and 1.6 units respectively (Carstensen and Duarte 2019). Compared with the average global surface open ocean pH (8.07 ± 0.02) the average coastal pH values of the same area (8.05 – 8.1) are lower (Edworthy et al. 2022).

Despite the high pH variability in the coastal ocean of the southern coast of South Africa, few studies have examined the consequences of OA in South Africa. However, Edworthy (2017) and Erasmus (2017) quantified the physiological response and skeletal development of the early life stages of the dusky kob, *Argyrosomus japonicus,* a coastal Sciaenid species. They found that high CO_2 exposure caused significant reductions in growth, and skeletogenesis, with increased rates of mortality. *Argyrosomus japonicus* is, however, a migratory species (Griffiths 1996) and is able to move to coastal areas where reductions in pH are reduced (for example out of upwelling zones). One South African study has however examined the response of a coastal resident fish to reduced pH. Dziergwa et al. (2019) examined growth rates and denticle composition and structure in the puffadder dogshark, *Haploblephus edwardsii* in response to acute and chronic exposure to low pH conditions. They found that denticle corrosion had occurred under chronic exposure, which was speculated to compromise skin protection and hydrodynamics. However, no studies have examined the response of a resident teleost species, and none have considered juvenile fish that have been exposed throughout their lives to future OA conditions. Moreover, few studies have used repeated measures to examine the individual metabolic response to extended exposure to hypercapnia. Finally, due to the significant variation in response to OA observed among a diverse number of taxa (Kroeker et al. 2010), it is critical to collect data from a range of different species, particularly those of commercial importance.

4.1.2 Aims and objectives of this chapter

The aim of this chapter was to investigate how long-term exposure to experimental OA conditions, from egg to juvenile stage (three months), would affect the performance of juvenile *C. laticeps* by comparing the thermal performance curves of individuals from low ($pCO_2 \approx$ 1400 µatm, pH = 7.63) pH conditions with those reared in high pH conditions ($pCO_2 \approx 420$ µatm, pH = 8.03, Chapter 3). Lower pH conditions were projected to cause a decrease in absolute aerobic scope of treatment animals, particularly at thermal extremes due to additional energetic costs of acid-base regulation. This is based on the premise that an increase in SMR (Munday et al. 2009a, Enzor et al. 2013, Heuer and Grossell 2014) and a constraint imposed on MMR through limited oxygen transport under hypercapnic conditions reduces AS (Munday et al. 2009a, Heuer and Grosell 2014, Flannery 2018).

4.2 Methods and materials

4.2.1 Husbandry and water chemistry

The adult spawning, larval rearing, husbandry, tagging and respirometry set-up is described in Chapter 2. Prior to the introduction of fertilised eggs, rearing tanks were randomly assigned to one of two pH treatments: (1) low pCO_2 conditions (pH = 8.03; $pCO_2 \approx 420$ µatm); and (2) hypercapnic or high pCO_2 treatment (pH = 7.63; $pCO_2 \approx 1400$ µatm) conditions projected for 2100 with a total change of -0.4 pH units (Orr et al. 2005). Tanks 3, 4 and 8 housed fish exposed to low pCO_2 conditions whilst tanks 5 and 6 housed the high pCO_2 treatment fish. The remaining tanks 1 and 2 held replacement fish in the case of stress-induced mortalities. High pH treatment conditions had been determined from surface water samples which were collected on a monthly basis in Algoa Bay, Gqeberha, between June 2018 and January 2020 (30-60 m depth; mean pH = 8.03 ±0.07 SD; mean pCO_2 = 423.93 µatm ±84.6 SD; Edworthy, 2022). The temperature in the seven tanks was maintained at 19˚C using aquarium heaters. From the two assigned high CO_2 treatment holding tanks, which received the same CO_2 treatment, five fish were used from tank 5, and 12 fish were used from tank 6.

CO_2 was administered to the two low pH holding tanks using the same method of introduction during their larval phase (Muller et al. 2021). Using pH electrodes (Tunze 7070.110) fitted to pH/CO_2 controllers on each tank (Tunze 7070.200, Aquarientechnik GmbH, Penzberg, Austria), CO_2 was bled into low pH tanks using diffusers and needle valves to ensure a slow rate of diffusion. Electrodes were calibrated regularly using a buffer solution (Tunze 7040.130).

Ammonium, nitrate and nitrite were measured weekly using a JBL Testlab test kit and maintained at levels near zero with regular water changes. Oxygen concentrations were maintained above 90% saturation using air diffusers in each tank, and percentage oxygen, temperature, and pH (National Bureau of Standards scale) were measured daily with a Hanna HI 98194 multi-parameter meter. Total alkalinity (TA) was measured twice weekly with a total alkalinity mini-titrator (Hanna Hi 84531).

4.2.2 Experimental application and procedure

The same method was used to introduce CO_2 into the filtered and sterilised seawater used in the thermal trials when testing low pH animals. A line bleeding CO_2 was introduced into the water bath and the pH monitor was set to 7.6 units, which ensured the CO_2 being released into the water maintained this pH. CO_2 treatment trials were alternated with high pH trials every few days in order to mitigate any differences that may arise between high pH and low pH animals from slight changes in the progression of their development. Prior to the start of each trial, fish were weighed and measured as per Chapter 3. Upon transferral of each trial fish into their assigned respirometry chamber, experimental water was the same temperature as their acclimation temperature (18° C). The experimental design in this chapter is the same for low pH animals as it was for high pH animals and details of the procedure can be found in Chapter 3. The process for measuring SMR and MMR is described in Chapter 2.

4.2.3 Statistical analyses

The mass-specific SMR and MMR was calculated for treatment animals at each temperature, and the AAS was then determined for each individual by subtracting the SMR from the MMR. A polynomial mixed effects model was used to predict the effect of temperature and treatment on the metabolic rate estimates, using the lme4 (Bates et al. 2015) and ―lme4 package (Kuznetsova et al. 2017). In each model, metabolic rates (SMR, MMR and AAS) were incorporated as the response variable, temperature (and its quadratic form) and treatment, and the interaction between the two, were included as fixed effects, while tank number and fish ID were included in the random effect structure, with fish ID nested in tank. The SMR data was log (X + 1) transformed in order to suit parametric assumptions.

Each model was run three times: Once with data including all viable measurements from each individual at each temperature, the second model used data that only included individuals with measurements at three or more temperatures (Appendix Table A.1), and the third model had data that omitted outliers with metabolic rates that had been calculated with averaged background respiration rates. The outputs from each showed the same trend in significance for all metabolic metrices and so the outputs from model 3 were included for comparability with Chapter 3.

The average daily growth rate (DGR) of each individual fish was calculated using data from Table 4.1, with the following equation:

$$DGR = \frac{(x_2\text{-}x_1)}{t}$$

Where x_2 = the next consecutive weight (g) or length (mm) measurement, x_1 = the previous weight or length measurement subtracted to get the change in weight or length and dividing this by t = the number of days since the last measurement. Following a test for normality, a Mann-Whitney U test was used to establish whether there was a difference between the average daily growth rate for both weight and length between high pH and low pH fish.

An asymptotic test (Feltz and Miller, 1996) and the modified signed-likelihood ratio test (Krishnamoorthy and Lee, 2014) were used to compare the variability of SMR, MMR and AAS between individuals across temperatures for the high pH and low pH using the R package *cvequality* (Version 0.1.3; Marwick and Krishnamoorthy 2019).

4.3 Results

The average weight of the high pH fish used in the trials was 2.77 g at the start and 3.75 g by the end of the trials, whilst the average weight of the treatment fish was 3.00 g at the start and 3.37 g at the end of the trials (n = 20 and n = 17 respectively, with replacement fish). The average length of the high pH fish was 51.48 mm at the start and 56.50 mm by the end of the trials, whilst the average length for low pH fish was 55.14 mm at the start and 57.08 mm at the end of the trials. Both Mann Whitney tests showed that there was no significant difference between the high pH and low pH group's daily change in weight (W = 941.5, p-value = 0.169) and length (W = 1561, p-value = 0.299).

Table 4. 1: The average daily change in weight (g) and length (mm) for each individual fish from the high pH and low pH groups according to measurements taken before each trial. This was calculated by working out the average daily growth rate (DGR) between each trial to then get the overall average DGR of each fish used in the trials. Those fish that only had one measurement recording owing to stress-limited mortalities were omitted from the table and analysis.

High pH fish ID	Average daily change in length (mm)	Average daily change in weight (g)	Treatment fish ID	Average daily change in length (mm)	Average daily change in weight (g)
C1	0.446	0.043	**T16B**	0.342	0.038
C2	0.417	0.061	**T18B**	0.463	0.031
C3	0.488	0.106	**T19**	0.329	0.034
C4	0.251	0.053	**T20**	0.547	0.048
C5	0.251	0.014	**T21**	0.243	0.165
C6	0.377	0.056	**T22**	0.335	0.030
C7	0.309	0.012	**T22B**	0.268	0.011
C8	0.240	0.022	**T23**	0.419	0.027
C9	0.424	0.016	**T23B**	0.171	0.015
C9B	0.330	0.039	**T24**	0.802	0.044
C10	0.312	0.026	**T24B**	0.358	0.054
C11	0.470	0.024	**T25**	0.729	0.064
C11B	0.377	0.020	**T26**	0.399	0.160
C11C	0.999	0.173	**T27**	0.274	0.037
C12	0.815	0.098	**T28**	0.366	0.032
C12B	0.263	0.027	**T29**	0.399	0.024
C13C	0.905	0.029	**T30**	0.384	0.032
C14	0.416	0.040			
C15B	0.378	0.015			
μ	**0.446**	**0.046**		**0.402**	**0.050**
STDV	**0.220**	**0.041**		**0.162**	**0.044**

The model outputs from the first two models are summarised in Table A.1 (Appendix) and show the same trend in significance for SMR (Table 4.3), MMR (Table 4.4), and AAS (Table 4.5) as the chosen model used in the analysis.

The average SMR of high pH (μ_c) and low pH (μ_t) fish were both highest at 22° C, and lowest at 11° C respectively (Table 4.2). The average MMR of low pH animals was higher than the high pH group at low temperatures, and lower than the high pH group at high temperatures

(Table 4.2). Finally, the average AAS of low pH animals was higher than the high pH animals at both low and high temperatures respectively (Table 4.2)

Table 4. 2: The average (and standard deviation) standard metabolic rate (SMR), maximum metabolic rate (MMR) and absolute aerobic scope (AAS) for juvenile *Chrysoblephus laticeps* reared under high pH (pH = 8.03, pCO2 ≈ 420 µatm) and low pH (pH = 7.63; pCO_2 ≈ 1400) CO_2 conditions at the four ecologically relevant temperatures.

	Temperature (° C)			
	High pH group			
	11	**14**	**18**	**22**
SMR	0.07 (± 0.03)	0.17 (± 0.11)	0.36 (± 0.24)	0.45 (± 0.16)
MMR	0.26 (± 0.10)	0.43 (± 0.11)	0.73 (± 0.28)	0.75 (± 0.24)
AAS	0.19 (± 0.09)	0.26 (± 0.13)	0.37 (± 0.24)	0.30 (± 0.15)
	Low pH group			
SMR	0.12 (± 0.08)	0.10 (± 0.04)	0.25 (± 0.06)	0.25 (± 0.11)
MMR	0.47 (± 0.20)	0.46 (± 0.18)	0.62 (± 0.16)	0.67 (± 0.22)
AAS	0.35 (± 0.17)	0.36 (± 0.15)	0.37 (0.16)	0.42 (± 0.22)

The SMR of the high pH group followed the typical pattern of an increasing trend with temperature (Fig. 4.2). However, while the pattern for the low pH group was similar until 18° C, there was a marked decline in SMR between this temperature and 22° C (Fig. 4.2). Despite this decline, the mixed model showed a significant linear (p-value = < 0.05; Table 4.3) relationship between temperature and SMR, but no non-linear relationship between temperature and SMR (p-value = 0.34; Table 4.3). Treatment was also not a significant predictor of SMR (p-value = 0.07; Table 4.3). There was, however, a significant linear interaction between temperature and treatment (p-value = 0.03; Table 4.3), but with no significant non-linear reaction between temperature and treatment (p-value = 0.84; Table 4.3). This is despite the decline in SMR of the low pH fish at temperatures above 18° C (Fig. 4.3). The response of individual fish's SMR appears to be uniformly lower in the low pH group than

that of individuals in the high pH group, with a significant difference in variability occurring at 18° C (Fig. 4.3; Table 4.6).

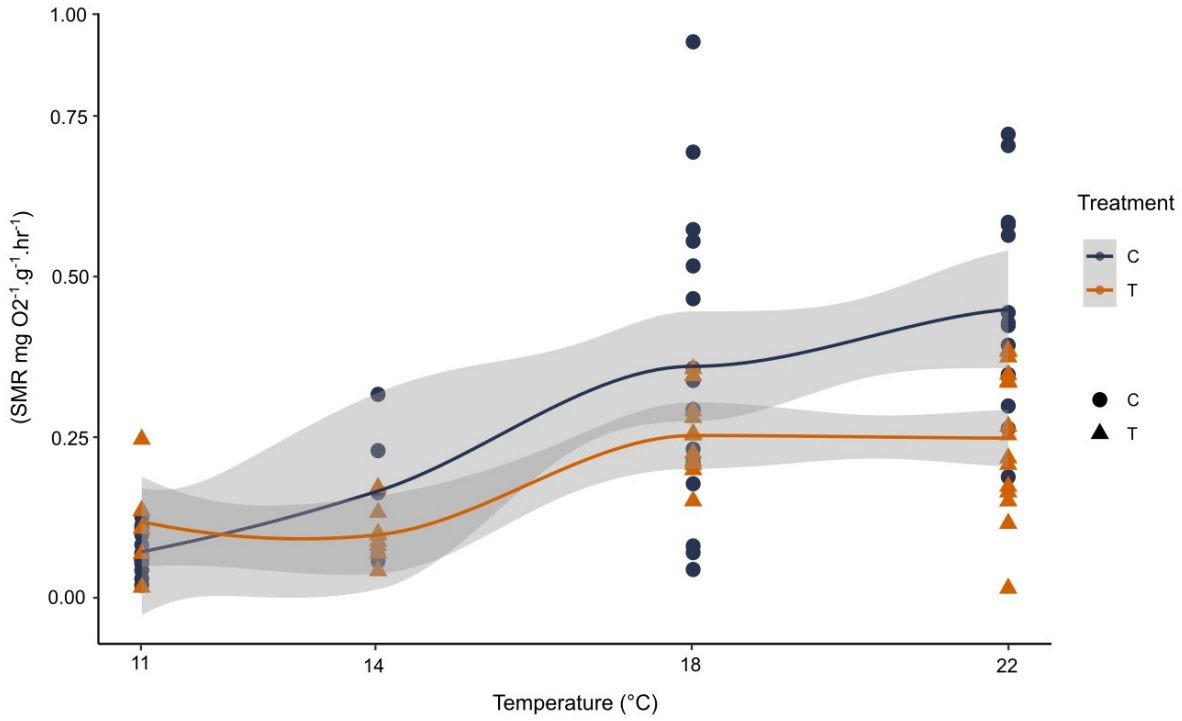


Figure 4. 2: The standard metabolic rate (SMR) of high pH (blue circles; indicating fish that were reared under CO_2 conditions where; *pH* = 8.03, $pCO_2 \approx 420$ µatm) and low pH (orange triangles; indicating CO_2 conditions projected for 2100; pH = 7.63; $pCO_2 \approx 1400$ µatm) juvenile *Chrysoblephus laticeps* measured at four ecologically relevant temperatures.

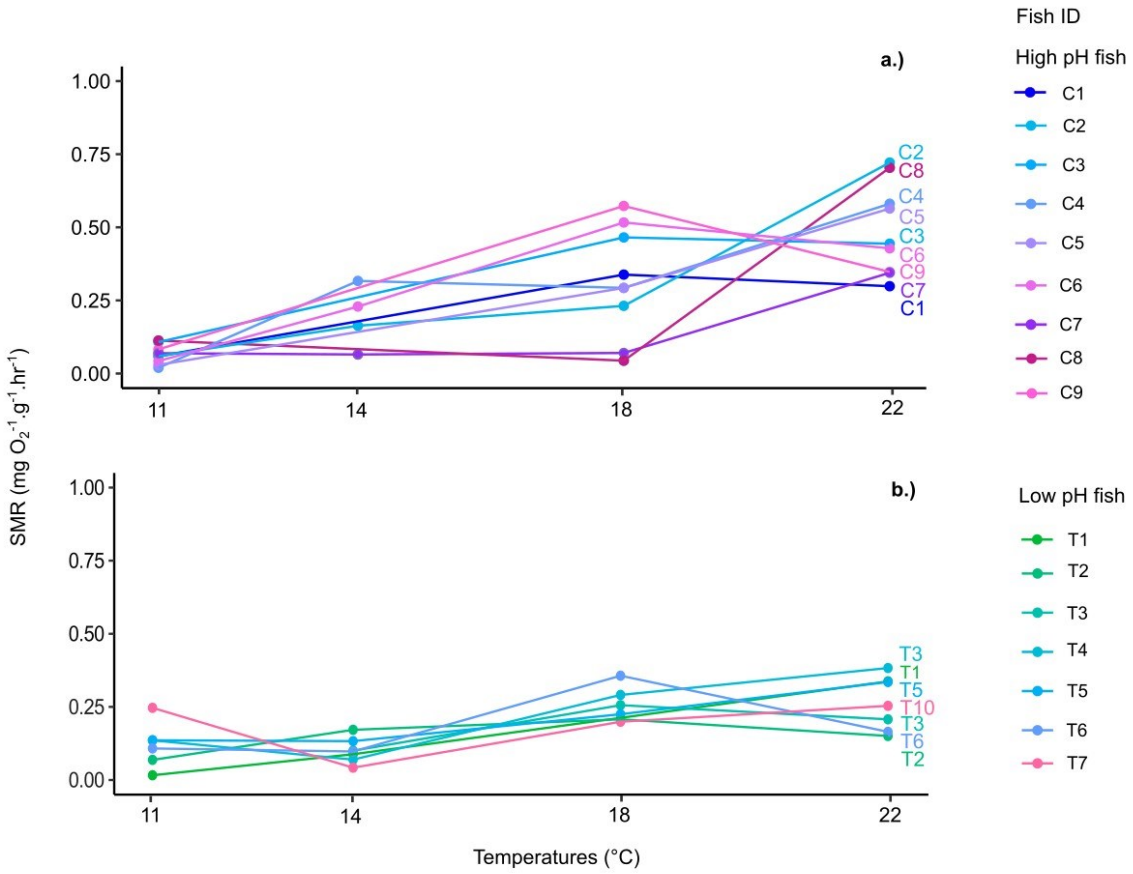


Figure 4. 3: The standard metabolic rate (SMR) of individual juvenile *Chrysoblephus laticeps* for a.) high pH fish that were reared under CO_2 conditions where; pH = 8.03, pCO_2 ≈ 420 µatm and b.) low pH animals indicating CO_2 conditions projected for 2100 (pH = 7.63; pCO_2 ≈ 1400 µatm) with measurements at three or more of the ecologically relevant experimental temperatures.

Table 4. 3: Results of the polynomial mixed effects model (GLM) analysis of the standard metabolic rates (SMR) of juvenile *Chrysoblephus laticeps* in response to temperature (° C) and CO_2 treatment projected for 2100 (pH = 7.63; $pCO_2 \approx 1400$). Temperature (1) represents the linear relationship between temperature and the metabolic metrices, whilst Temperature (2) represents the non-linear relationship between temperature and the metabolic metrices. Significant values reported in bold.

	Effect	Estimate (intercept)	Std. Error	d.f.	t-value	P-value
SMR (mg $O_2^{-1}.g^{-1}.hr^{-1}$)	**Fixed effects:**					
	Intercept	0.25	0.02	29.56	16.50	**<0.01**
	Temperature (1)	1.06	0.14	62.06	7.57	**<0.01**
Pseudo-R^2 0.48	Temperature (2)	-0.14	0.14	65.39	-0.97	0.34
	Treatment	-0.08	0.02	25.79	-3.30	0.07
AIC	Temperature (1): Treatment	-0.57	0.21	69.10	-2.66	**0.01**
-116.66	Temperature (2): Treatment	0.04	0.21	62.04	0.20	0.84
	Random effects:	**Variance**	**Std. Dev.**			
	ID	<0.01	<0.01			
	Tank	<0.01	<0.01			
	Residual	0.01	0.10			

There was a positive significant linear and non-linear relationship between temperature and MMR (p-value = < 0.05 and p-value = 0.03; Table 4.5), although both the low pH and high pH levelled-off above 18° C (Fig. 4.4). Whilst the mean MMR of low pH animals was higher at low temperatures and lower than the high pH group at the higher temperatures, low pH was not a significant predictor of MMR (p-value = 0.63; Table 4.4). There was however a significant negative linear relationship between MMR and the interaction with temperature and

treatment (p-value = 0.03; Table 4.4), but there was no significant non-linear interaction between temperature and low pH treatment (p-value = 0.17; Table 4.4). Variability in MMR was highest at 18° C, particularly for the high pH fish, while the variability was lowest at the lowest temperature (Fig. 4.5). The variation in the response of the MMR of individual fish in the low pH group was similar by inspection to that of individuals in the high pH group and no significant difference in variability was found at any of the experimental temperatures (Fig. 4.5; Table 4.7).

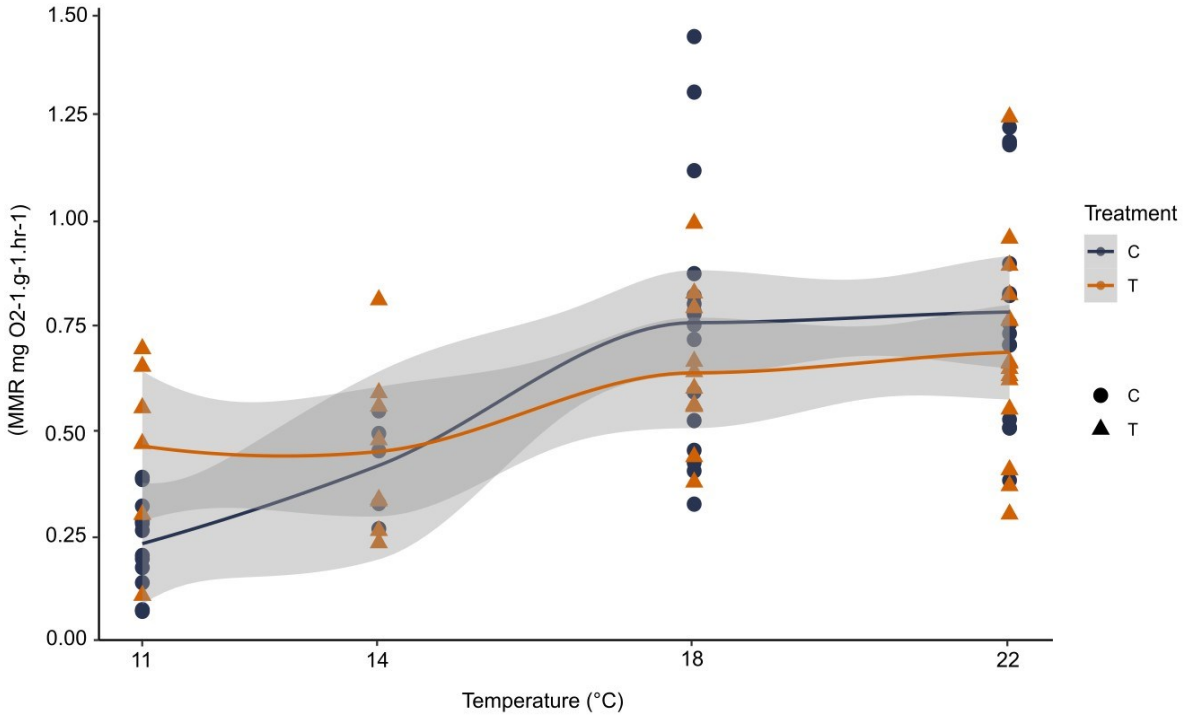


Figure 4. 4: The maximum metabolic rate (MMR) of high pH (blue circles; indicating fish that were reared under CO_2 conditions where; pH = 8.03, $pCO_2 \approx 420$ µatm) and low pH (orange triangles; indicating CO_2 conditions projected for 2100; pH = 7.63; $pCO_2 \approx 1400$ µatm) juvenile *Chrysoblephus laticeps* measured at four ecologically relevant temperatures.

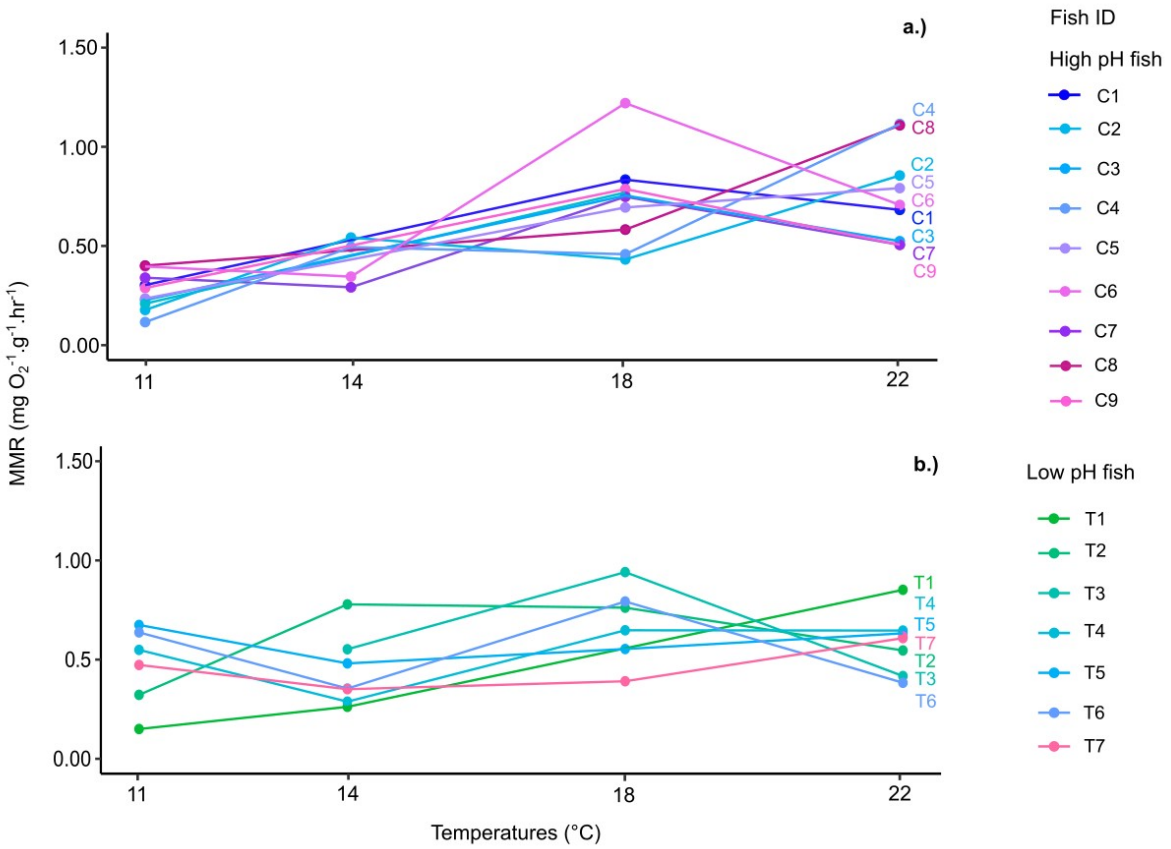


Figure 4. 5: The maximum metabolic rate (MMR) of individual juvenile *Chrysoblephus laticeps* for a.) high pH fish that were reared under CO_2 conditions where; pH = 8.03, $pCO_2 \approx$ 420 µatm and b.) low pH fish reared under CO_2 conditions projected for 2100 (pH = 7.63; $pCO_2 \approx$ 1400 µatm) with measurements at three or more of the ecologically relevant experimental temperatures.

Table 4. 4: Results of the polynomial mixed effects model analyses of maximum metabolic rates MMR) of juvenile C*hrysoblephus laticeps* in response to temperature and CO_2 treatment projected for 2100 (pH = 7.63; $pCO_2 \approx 1400$). Temperature (1) represents the linear relationship between mperature and the metabolic metrices, whilst Temperature (2) represents the non-linear relationship between temperature and the metabolic metrices. Significant values reported in bold.

	EFFECT	ESTIMATE (INTERCEPT)	STD. ERROR	D.F.	T-VALUE	P-VALUE
MMR **(MG O_2^{-1}.G^{-1}.HR^{-1})**	**Fixed effects:**					
	Intercept	0.60	0.04	3.22	16.75	**<0.01**
	Temperature (1)	1.75	0.28	61.80	6.25	**<0.01**
	Temperature (2)	-0.61	0.28	65.41	-2.18	**0.03**
PSEUDO-R^2 **0.40** ***AIC*** **-2.45**	Treatment	-0.03	0.06	2.58	-0.54	0.63
	Temperature (1): Treatment	-0.93	0.43	68.62	-2.17	**0.03**
	Temperature (2): Treatment	0.59	0.43	61.95	1.39	0.17
	Random effects:	**Variance:**	**Std. Dev**			
	ID	<0.01	<0.01			
	Tank	<0.01	0.03			
	Residual	0.04	0.21			

The mean AAS of the fish in the high pH group assumed a normal bell-shaped curve, with a peak at 18° C (Fig. 4.6). In contrast, the mean AAS of fish in the low pH group remained relatively stable but increased slightly between 18° C and 22° C (Fig. 4.6). The results from the mixed model showed that there was no linear or non-linear relationship between temperature and AAS (p-value = 0.10 and p-value = 0.07; Table 4.5), nor was there a significant linear or non-linear interaction between temperature and treatment (p-value = 0.67 and p-value p-value = 0.16; Table 4.5). Individual variability within the low pH and high pH groups were

similar across the experimental temperatures and the results from both the asymptotic test and the modified signed-likelihood ratio for each temperature showed that there was no significant difference in the variation of AAS rates between the high pH and low pH groups (Table 4.7).

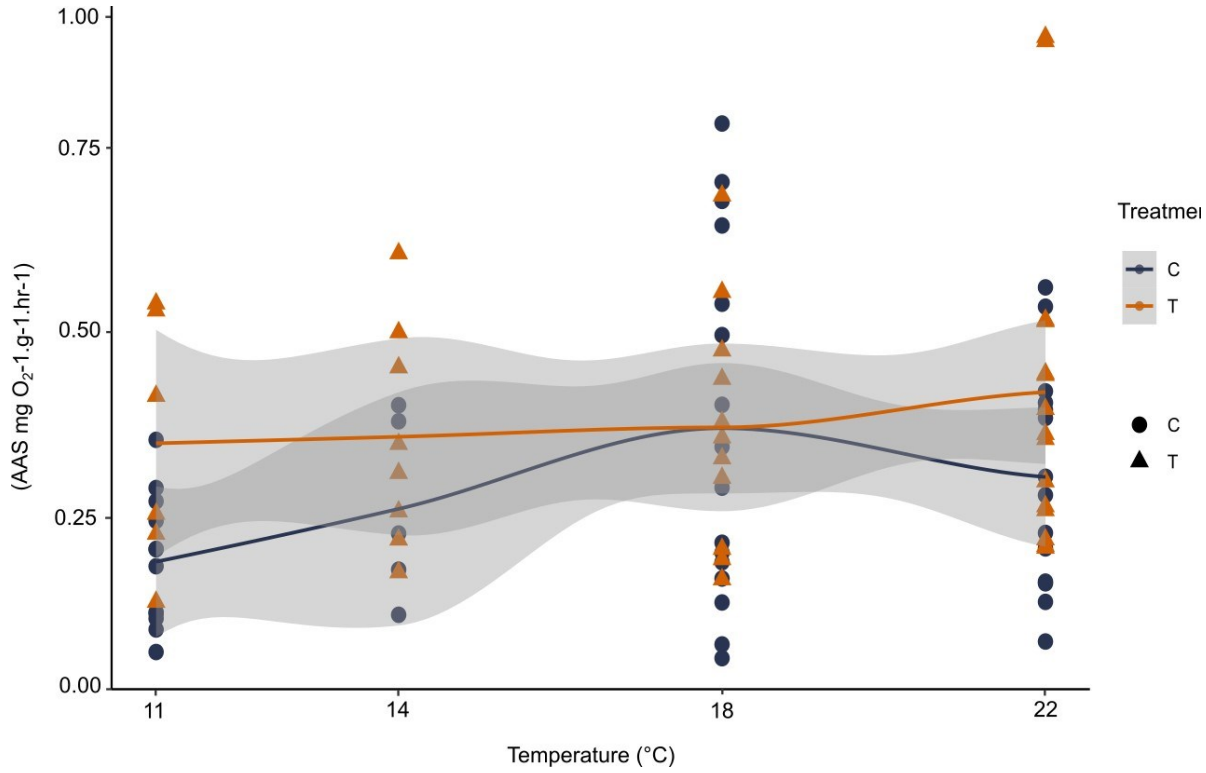


Figure 4. 6: The absolute aerobic scope (AAS) of high pH (blue circles; indicating fish that were reared under CO_2 conditions where; pH = 8.03, $pCO_2 \approx 420$ µatm) and low pH (orange triangles; indicating CO_2 conditions projected for 2100; pH = 7.63; $pCO_2 \approx 1400$ µatm) juvenile *Chrysoblephus laticeps* measured at four ecologically relevant temperatures.

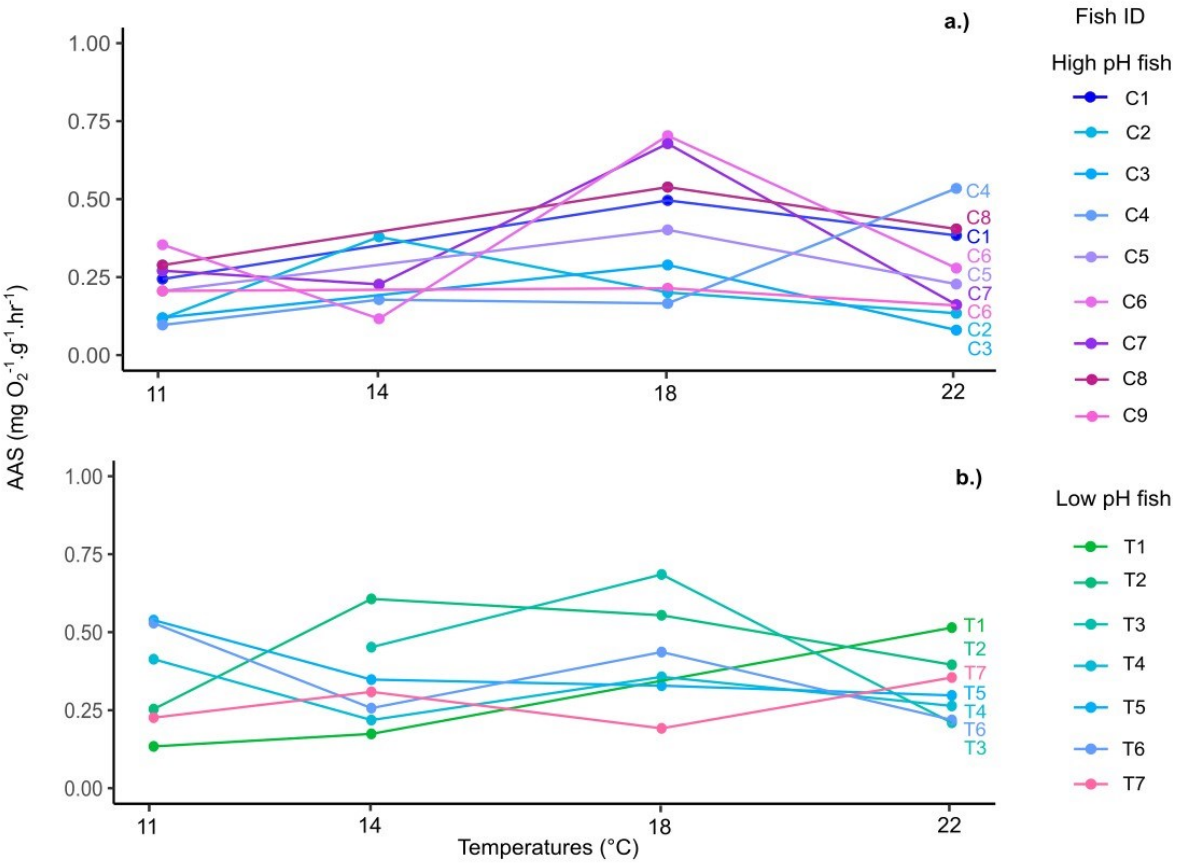


Figure 4. 7: The absolute aerobic scope (AAS) of juvenile *Chrysoblephus laticeps* for a.) high pH fish that were reared under CO_2 conditions where; pH = 8.03, $pCO_2 \approx 420$ µatm and b.) low pH fish reared under CO_2 conditions projected for 2100 (pH = 7.63; $pCO_2 \approx 1400$ µatm) with measurements at three or more of the ecologically relevant experimental temperatures.

Table 4. 5: Results of the polynomial mixed effects model analyses of the absolute aerobic scope (AAS) of juvenile *Chrysoblephus laticeps* in response to temperature and CO_2 treatment projected for 2100 (pH = 7.63; $pCO_2 \approx 1400$). Temperature (1) represents the linear relationship between temperature and the metabolic metrices, whilst Temperature (2) represents the non-linear relationship between temperature and the metabolic metrices. Significant values reported in bold.

	Effect	**Estimate (intercept)**	**Std. Error**	**d.f.**	**t-value**	**P-value**
AAS (mg O_2^{-1}.g^{-1}.hr^{-1})	**Fixed effects:**					
	Intercept	0.30	0.03	3.17	8.71	**<0.01**
Pseudo-R^2	Temperature (1)	0.40	0.24	61.69	1.67	0.10
	Temperature (2)	-0.44	0.24	65.39	-1.83	0.07
0.17	Treatment	0.08	0.05	2.71	1.45	0.25
AIC	Temperature (1): Treatment	-0.16	0.36	68.54	-0.43	0.67
-27.87	Temperature (2): Treatment	0.52	0.36	61.90	1.43	0.16
	Random effects:	**Variance**	**Std. Dev.**			
	ID	<0.01	<0.01			
	Tank	<0.01	0.04			
	Residual	0.03	0.18			

Table 4. 6: Results from the asymptotic test and the modified signed-likelihood ratio test for the standard metabolic rate (SMR), maximum metabolic rate (MMR) and absolute aerobic scope (AAS) at each temperature. Both tests of variation were carried out to examine whether there was a significant difference in variation between the metabolic metrices between the high pH and low pH treatment groups of juvenile *Chrysoblephus laticeps*. Significant results are highlighted in bold.

	Temperature (° C)			
	11	**14**	**18**	**22**
SMR				
D-AD	0.517	0.930	5.487	0.507
p-value	0.472	0.335	**0.019**	0.476
MSLRT	0.322	0.660	6.751	0.510
p-value	0.571	0.416	**0.009**	0.475
MMR				
D-AD	0.123	0.735	1.583	0.004
p-value	0.726	0.391	0.208	0.947
MSLRT	0.025	0.880	1.747	-0.006
p-value	0.875	0.348	0.186	1
AAS				
D-AD	0.004	0.073	1.465	0.033
p-value	0.951	0.787	0.226	0.855
MSLRT	-0.012	0.011	1.585	0.019
p-value	1	0.917	0.208	0.889

4.4. Discussion

One of the meaningful findings of this study was the interaction between treatment and temperature, which was a significant predictor of SMR and MMR. This indicates that a significant difference in these metrics exists between the low pH group (raised under CO_2 conditions; $pCO_2 \approx 1400$ µatm, pH = 7.63) and the high pH group (reared under current day CO_2 conditions; $pCO_2 \approx 420$ µatm, pH = 8.03) of juvenile *C. laticeps* at certain temperatures, particularly at extreme low and extreme high temperatures. The linear increase in the high pH group's SMR with temperature and the plateau of the corresponding MMR at high temperatures resulted in a typical bell-shaped AAS performance curve in the high pH group, described by Pörtner et al. (2017). In contrast, low pH animals had a higher, more stable AAS curve across temperatures, except at extreme high temperatures where it began to increase. This was caused by a mostly lower SMR in the low pH animals, particularly at high environmentally-relevant temperatures, and an MMR that was higher at extreme low experimental temperatures, but which was lower at the upper extreme temperatures. As with the high pH group, individual phenotypic variation was present in the low pH animals, but importantly, the variability in the response of their SMR was significantly different at their rearing temperature of 18° C. Developing organisms are characteristically plastic owing to the ongoing processes of growth, differentiation, and organogenesis (Del Giudice 2015, Moczek 2015, Beaman et al. 2016, Bavis and MacFarlane 2016, Rundle and Spicer 2016). This plasticity is often examined in the light of the advantages that it confers under environmental stress (Pelster and Burggren 2018), and as an acclimatory response, it needs to be considered in the context of energetic, metabolic or physiological trade-offs (Pelster and Burggren 2018). Whilst the costs associated with phenotypic plasticity might be measured by compensatory increases in oxygen consumption, ATP turnover, or decreased growth or fecundity (Relyea 2007, Callahan et al. 2008), there were no significant differences in the daily change in weight and length between the high pH and low pH group. This was surprising owing to the notably lower SMR in low pH animals and the aforementioned reduction in variability at 18° C. Processes such as growth are anticipated to be stunted under hypercapnia as a limited energy flux means that metabolic power and energy allocation goes towards maintenance requirements, as this takes priority and growth, reproduction or storage are negatively impacted (Wieser et al. 1988, Rombough 1994, Kooijman 2010). These findings suggest that long-term exposure to low pH conditions has a significant impact on the physiology of juvenile *C. laticeps* in a thermally variable

environment. However, these differences did not appear to have an impact on the growth of the species.

There appeared to be a significant reduction in the SMR of low pH fish at high temperatures when compared with those in the high pH group. Theoretically, fish respond to stressors, such as low pH through compensatory mechanisms, which are expected to impose additional energetic costs (Heuer and Grosell 2016, Kreiss et al. 2015), and result in an increase in resting metabolism. In this study, the significant relationship between the interaction of temperature and treatment with SMR and the lower SMR between the low pH and high pH groups at higher temperatures, not only suggests that hypercapnia affects the physiology of juvenile *C. laticeps* at extreme temperatures, but also that the effect did not align with the theory around the energetics associated with compensatory mechanisms. This finding is not unique. For example, there was a nonsignificant, but noteworthy decline in the SMR of Polar cod, *Boreogadus saida* and Atlantic cod, *Gadus morhua*, under hypercapnic conditions at high temperatures (16° C; Kunz et al. 2016). Whilst it is expected that changes to internal pH may compromise oxygen transport, particularly for those life stages or species with high metabolic demands (Pörtner et al. 2005), hypercapnia has been shown to cause an enhancement of oxygen delivery to tissues in some species (Rummer and Brauner 2011, Rummer et al. 2013b). The 'Root effect' prompts a pH-dependent decrease in the O_2 carrying capacity of red blood cell haemoglobin in ray-finned fishes (Root 1931, Pelster and Weber 1991), but there is evidence to suggest that Root haemoglobins improve O_2 delivery to tissues during acidotic stress (Rummer et al. 2015). Recently, plasma-accessible carbonic anhydrase (CA) activity is thought to short-circuit red blood cell pH regulation in muscle capillaries under mild hypercapnia, causing haemoglobin to unload O_2 in the tissue to meet O_2 demands (Rummer and Brauner 2011, Randall et al. 2014, Rummer et al. 2015, Alderman et al. 2016, Hannan and Rummer 2018, Harter et al. 2019). This could significantly enhance O_2 delivery during periods of hypercapnic stress (Rummer et al. 2015). This has been reported in Euroepean sea bass, *Dicentrarchus labrax,* which showed an increase in haemoglobin-O_2 affinity when exposed to reciprocal changes in O_2 and CO_2 (Montgomery et al. 2019). This mechanism may account for the lower SMR in low pH animals at high temperatures particularly and the absence of any significant disparity in the growth of high pH and low pH animals. Considering low pH fish had been exposed to hypercapnic conditions since fertilisation, it is likely that acclimation during their early ontogeny had occurred as a result of phenotypic plasticity. The long-term exposure to hypercapnic conditions

may have resulted in a new steady state in the low pH fish where, facilitation via the Root effect may have enhanced oxygen delivery to the tissues, potentially reducing their SMR.

An alternative explanation for the low SMR at higher temperatures may be metabolic rate suppression (otherwise known as metabolic depression) and was observed in the larval stage of *C. laticeps* from an exploited population exposed to the same CO_2 treatment (Muller 2022). Identified as the metabolic scope for survival (Hochachka and Somero 2002) the phenomenon occurs at the cellular and biochemical level signifying a reduction in the ATP-consuming processes contributing to SMR (any anabolic process that consumes ATP. e.g., urea synthesis, gluconeogensis, or RNA synthesis, could plausibly be downregulated to reduce cellular metabolic rates and extend an organism's survival time; Navas et al. 2010). However, this explanation is unlikely because of the similar growth patterns observed between fish in the low pH and high pH groups in this study.

Another explanation for the significantly lower SMR of low pH fish at higher temperatures may relate to selectivity. The juveniles that were included in this research were spawned from adults from a longstanding MPA and subjected to intense selection before the experiment. This may suggest that the observed metabolic response by the low pH fish and apparent tolerance to high CO_2 represents a shift in the reaction norm that goes beyond acclimation to yield a measurable selection response (Crozier and Hutchings 2014). Having been reared under artificially maintained hypercapnic conditions in isolation, with the absence of daily fluctuations in temperature, predator presence and resources sparsity, it is likely that low pH juveniles experienced radical and rapid selection for individuals that were best adapted to coping with hypercapnia. The surviving *C. laticeps* in the low pH group demonstrated uniformly lower SMR and reduced individual variability (which was significantly lower than the high pH group at 18° C). This finding may suggest that that the outcome of this intense selection was the survival of individuals that were able to reduce their SMR under hypercapnic conditions and was likely facilitated by the mechanisms discussed above.

If selectivity did result in a shift in the reaction norm of these juvenile *C. laticeps*, it may suggest that the phenotypic plasticity of these surviving individuals allowed for their should persistence under a new phenotypic optimum, but whether this is adaptive plasticity with the power of adaptive divergence remains in question (Ghalambor et al. 2007). The presence of high variation under strong selective pressures may be grounds for adaptation (Barrett et al. 2011) to OA in this population. However, without quantifying the effects of multiple selective

pressures from additional stressors (Pörtner et al. 2001, Pörtner and Farrell 2008, Anttila et al. 2013) this would be difficult to extrapolate under natural conditions (Crozier and Hutchings 2014). It is important to note that no single phenotype will demonstrate superior fitness across all environmental conditions (Moran 1992, Ghalambor et al. 2007). Considering substantial phenotypic plasticity in the trait responses, studies should integrate methods (such as whole genome sequencing) that are capable of detecting evolutionary changes arising from selection, including extended evaluations of selection coefficients and adjustments in reaction norms (Crozier and Hutchings 2014).

The MMR of *C. laticeps* from the high pH group assumed a normal pattern of increase with temperature and a levelling-off above their thermal optimum. In contrast, low pH group fish had a higher MMR at low temperatures, and a similar pattern after that, although the mean MMR was lower than the high pH group. The significant interaction between treatment and temperature suggested that the differences in MMR at thermal extremes were significant and emphasises the dynamic interplay between thermal stress and hypercapnia. The mechanism driving the reduced MMR of low pH fish at higher temperatures should be explored. Esbaugh (2018) suggested that the combination of exercise and OA antagonises acid–base stress on red blood cells through pH_i-mediated impairment in blood oxygen binding affinity. However, Crespel et al. (2019) showed that juvenile sea bass, *Dicentrarchus labrax*, exposed to moderate hypercapnia (pH = 7.7) and high hypercapnia (pH = 7.5) from 2 DAH until 1.5 years had higher MMR than high pH fish, which the authors attributed to heavier gill systems, as well as enhanced haemoglobin and mean corpuscular haemoglobin concentration, indicating an improved compensatory oxygen extraction and transport capacity (Crespel et al. 2019). There was, however, a reduced maximal heart rate under warming conditions. These results may suggest that higher MMR at cooler temperatures in low pH juvenile *C. laticeps*, can be attributed to improved compensatory oxygen extraction and transport capacity. However, heart rate experiments will be necessary to provide confirmation.

An alternative explanation for the low MMR of the low pH fish at high temperatures may be because MMR is more limited by temperature than SMR. This is a basic premise of the OCLTT theory (Pörtner 2010) and is particularly true for animals coping with additional environmental stressors as compensatory mechanisms draw energy away from the reserves of energy allocation used in additional stress responses (Heuer and Grossell 2014).

It is possible that food availability was not high enough to facilitate a high MMR for fish in the low pH group. However, a study looking at the flexibility in SMR and MMR of juvenile brown trout, *Salmo trutta*, in response to food availability found that SMR increased with increasing food without any corresponding change in MMR (Auer et al. 2016). This has been attributed to the effect that food intake has on organ mass, which is thought to contribute to whole organism SMR (Armstrong and Bond 2013), whilst skeletal muscle is believed to be the primary contributor to MMR (Weibel et al. 2004).

Although there was no significant difference between the AAS of the low pH and high pH groups in this study, there was a difference in the shape of the AAS curve, with the high pH group assuming the typical bell-shaped curve and the low pH group a positive linear trend. These differences can be attributed to the unusual SMR and MMR curves of low pH animals and particularly the anomalous findings at extreme temperatures.

The higher AAS for fish in the low pH group was surprising as it has been suggested that hypercapnia causes a reduction in the AS of fish (Pörtner and Farrell 2008) due to reduced tissue functional capacity or by an elevation in SMR from 'loading stress' (that is adjustments in acid-base regulation, osmoregulation, and cardiorespiration) and a decrease in MMR caused by limiting stress (or reduced oxygen uptake and delivery, Brett 1958, Heuer and Grosell 2014). The combined effects of temperature and OA have been seen to cause a further reduction in AS in some studies, and thus lowered overall fitness (Munday et al. 2009a, Schulte 2015). Despite this, a higher AS has been observed in several other studies involving; spiny damselfish (*Acanthochromis polyacanthus,* 17 days exposure, ΔpH = - 0.3 units; Rummer et al. 2013a), Atlantic halibut (*Hippoglossus hippoglossus*; 14–16 weeks exposure, ΔpH = - 0.4 units; Gräns et al. 2014), and juvenile Senegalese sole (*Solea senegalensis*; 75 days exposure, ΔpH = - 0.3 units; ΔT = +4° C; Oliveira et al. 2022). Enhanced maximum cardiac flow-generating capacity was also observed in Atlantic halibut with higher AS following acclimation to low pH, thus improving oxygen transport (Gräns et al. 2014). The generality of oxygen delivery compensation is still unknown as other studies, such as Michaelidis et al. (2007), demonstrate a switch from aerobic to anaerobic metabolisms in gilthead bream (*Sparus aurata*), dictated by enzymatic activity under hypercapnic conditions (ΔpH = - 0.75 of a unit from 8.05 to 7.3). While it is possible that heightened cardiac capacity and a switch to anaerobic respiration may have driven the elevated AAS of low pH fish in this study, additional experiments, such as those using heart rate loggers may be necessary to understand the physiological basis of the differences in the AAS of the high pH and low pH group.

It should be noted that higher AAS is generally thought to suggest resilience, as this indicates greater surplus energy available for non-life sustaining activities compared to those with lower AAS (Fry 1947). However, this may not necessarily be the case as any further changes in temperature and pH could push individuals dealing with hypercapnic stress beyond a tipping point from where they may not recover. This rests largely on the premise that, while basal energy requirements and resting metabolic rates are typically plastic, maximum metabolic capacities and upper thermal limits are considerably less flexible (Sandblom et al. 2016), which may temper the AAS of this species and cap the potential for resilience.

No significant difference was observed in the variability of the metabolic response for MMR, and AAS, as well as SMR at 11, 14 and 22° C between high pH and low pH animals in this study. However, a significant difference in the variability of the SMR was observed at 18° C. When compared to adult *C. laticeps* exposed to current day CO_2 conditions, phenotypic variation was reduced at lower temperatures, which was more prominent in the juveniles reared in high pH conditions in the current study and in adults of the same species (Bailey 2022). Considering the progeny used in this study were descendants of adults caught and spawned in an MPA, the concurrent impact of overfishing, which acts as a further authority over individual variation due to fisheries-induced evolution, may be an important factor that is missing in the current context. Duncan (2018) found that protected populations of adult *C. laticeps* have higher metabolic phenotypic diversity and a greater number of high-performance phenotypes compared to exploited populations. Similarly, when fishing pressure was considered together with hypercapnia, it was shown that larval *C. laticeps* from a protected population showed no evidence of metabolic depression or growth disparities under low pH conditions compared to progeny from an unprotected population (Muller 2022). Muller (2022) concluded that the selective removal of adult high-performance phenotypes has direct consequences on offspring and is predicted to lessen the buffering potential of a population to climate change. Considered within the context of this study, the distribution of metabolic performance phenotypes in juvenile low pH animals was still suitably varied, except in the metabolic response of their SMR. The latter is likely owing to the plastic and flexible nature of this metabolic metric (Sandblom et al. 2016), which potentially aided in their ability to cope with a new environmental regime (Nussey et al. 2007, Bolnick et al. 2011), and which may have been the actor on which the intense and rapid selection throughout their ontogeny could have played. The significant difference in variability in the response of the groups' SMR at 18° C, the temperature at which both groups were reared, attests to this theory and demonstrates that the

uniformity of the response of low pH animals' SMR at this temperature is the outcome of substantial selective pressures homogenising the response owing to the survival of those that are fittest under the artificially simulated selective pressures.

A characteristic of this study which may have influenced the thermal metabolic performance of both high pH and low pH animals was that of the constant acclimation temperature (18° C). Acclimation temperatures have been seen to have a weighted effect on the metabolic response of fish. The extent of the acclimation response in ectotherms is demonstrated through previous exposure to either high or low temperatures. The findings of Reemeyer and Rees (2020) showed that fish acclimated at high temperatures (23° C) and tested for time until loss of equilibrium (LOE) at the same temperature under hypoxic conditions did significantly better than fish acclimated at moderate temperatures (15° C) and exposed to high temperatures. However, fish acclimated and tested at 23° C were still less tolerant of hypoxia than those acclimated and tested at 15° C (Reemeyer and Rees 2020). The considerable benefits of plastic hypoxia tolerance in response to thermal acclimation may support the notion that acclimation temperatures have important consequences for response and plasticity to compounding environmental stressors. Additionally, constant acclimation temperatures omit the diel temperature fluctuations that fish typically experience in their natural environment (Bertolo et al. 2011). In a study on *S. salar,* diel temperature fluctuations in addition to mean acclimation temperature influenced their metabolic response, and in turn, the mean temperature impacted their responses to diel temperature fluctuations (Oligny-Hébert et al. 2015). Juvenile spiny damselfish (*Acanthochromis polyacanthusthat*) that were exposed to sudden warming had increased resting oxygen consumption rates, but the response was compensated for when they were acclimated to high temperatures during their development (Donelson et al. 2012). Contrary to popular literature, Healy and Schulte (2012) demonstrated that acclimation had little effect on the thermal breadth of aerobic scope. Their findings did however suggest that fish acclimated to high temperatures showed clear optimum AS performance between this temperature range, as opposed to that of unacclimated individuals who had constant AS across the whole thermal range with acute exposure to temperature. Future metabolic studies on *C. laticeps* should therefore attempt to incorporate varied thermal acclimation protocols, perhaps including diel variations if experimentally feasible, to better predict the physiological response of fish to environmental stressors.

An additional factor that may have impacted the metabolic response of juvenile *C. laticeps* was the feeding regime. Animals were fasted prior to the start of each trial in order to test the

absolute minimum maintenance energy required for homeostasis during SMR (excluding digestion and additional somatic processes; Brett 1962, Frappell and Butler 2004). However, all fish were fed communally once a day, *ad libitum,* until satiation was observed for all individuals, with no variation in feeding regime. Food availability and diet are often disregarded as important factors that can influence metabolic and acclimation responses (Hardison et al. 2021). Despite this, feeding regimes have been found to significantly impact the metabolic rates of *S. salar*, where a greater feed ration caused an increase in oxygen consumption as a direct consequence of increased specific dynamic action (SDA; Forsberg 1997). This increase in oxygen consumption may be attributed to the rise in metabolism resulting from digestion (Chabot et al. 2016), which comprises 60–80% of the maximum rate of organismal oxygen consumption (Alsop and Wood 1997, Metcalfe et al. 2016). Feeding regimes therefore regulate trait-specific thermal performance which could create diet trade-offs for organisms occupying thermally variable environments with the added pressure of lowered pH (Hardison et al. 2021). Food availability and feeding regimes need to be considered in the context of their environmentally orientated performance to comprehend connections between metabolic rates and fitness, as well as the persistence of metabolic phenotypic variation (Auer et al. 2015a). Therefore, further research looking at the effects of multiple stressors on the metabolic response of fish should look to incorporate varied feeding regimes, particularly during early stages of developmental (Vagner et al. 2019).

This study was the first of its kind that used repeated measures to examine the long-term metabolic effects of exposure to high CO_2 over the course of juvenile fish ontogeny, from fertilisation until ~ 100 DAH. Treatment was not a significant predictor of SMR, MMR and AAS in this study however, there was a significant interaction between temperature and treatment, and this was attributed to the different responses of fish at their thermal extremes. A high degree of individual phenotypic variation was observed in the metabolic response of both groups, with no difference in variability recorded between the SMR, MMR and AAS of the high pH and low pH group, except for SMR at 18° C. This is likely attributed to the progeny of this study descending from adult *C. laticeps* from a protected population with heightened phenotypic diversity (Duncan 2018). Although the juveniles used in this study were more resilient than anticipated to the enhanced thermal variability, increased upwelling, and anticipated reductions in local pH conditions, it is likely that the higher AAS of the low pH group is owing to abnormalities in their thermal physiology caused by the interaction of hypercapnia and thermal extremes. Specifically, both the SMR and MMR of low pH animals

were higher at low temperatures and lower at high temperatures. It is hypothesised that the strong selective pressures from early exposure to hypercapnia have led to the survival of individuals that were able to plastically lower their SMR in response to hypercapnic conditions, or those that had an innately lower SMR which survived past their larval phase and had the ability to cope with high CO_2. This may be indicative of compensatory pathways affecting energy restructuring and thermally governed physiological trade-offs under hypercapnia, where energy metabolism can be rearranged through acclimation to reduce SMR and/or maintain AS under mild environmental stress (Shumway and Koehn 1982, Sangiao-Alvarellos et al. 2005, Kidder et al. 2006). Future research should look to incorporate both physiological and behavioural responses to better understand covariation between these two traits, and to look for behavioural trade-offs that might arise by means of compensatory mechanisms. A genetic component, such as whole genome mapping with an identification of the genomic variants that underpin the physiological differences would benefit studies of this kind. A genetic component, such as whole genome mapping with an identification of the genomic variants that underpin the physiological differences would benefit studies of this kind. This would provide confirmation on whether physiological changes occurring in response to hypercapnia are driven by selection pressure and may ultimately cause a shift in the genetic norm response of the population and potentially promote rapid adaptation.

CHAPTER 5

General discussion and conclusion

The first principal objective of this thesis was to determine the metabolic physiology of juvenile *C. laticeps* reared under high pH conditions (pH = 8.03, $pCO_2 \approx 420$ µatm) across a range of ecologically-relevant temperatures. The high pH group showed a peak in AAS (0.3696 ± 0.241 mg O_2^{-1} g^{-1} hr^{-1}; Fig. 4.6) at their acclimation and rearing temperature (18° C), either side of which their AAS declined. The group's SMR scaled in a linear fashion with increasing temperature (Fig. 4.2), whilst MMR increased with temperature before plateauing at 18° C (Fig. 4.4). These findings are in the domain of expected outcomes as the same trends have been observed in a variety of fish species (Fry 1947, Claireaux and Lefrançois 2007, Pörtner and Farrell 2008, Lefevre 2016), resulting in the typical unimodal metabolic performance curve (Neubauer and Andersen 2019). The performance curves of juvenile *C. laticeps* are also similar to the adults of the species (Duncan 2018, Bailey 2022), which is likely attributed to their occupancy of similar habitats, as there is a large degree of overlap between adults and juveniles on shallow subtidal reefs up to 30m (Penrith 1972, Buxton and Smale 1984, Buxton 1987).

The second key component sought to investigate how long-term exposure, (from shortly after fertilisation to early juvenile, stage ~100 days exposure) to high-pCO_2/hypercapnic conditions (pH = 7.63, $pCO_2 \approx 1400$ µatm) would affect the AAS of juvenile *C. laticeps* over the same thermal range. Contrary to predictions of a generally lower AAS across the thermal range due to increased ionoregulatory requirements, the AAS of low pH animals was higher than that of the high pH group at all temperatures, despite the non-significant effect of treatment (Fig. 4.6; p-values = 0.25; Table 4.5). This can be attributed to the acclimation response reflected in the lower SMR of low pH animals except at low temperatures when compared with the those reared under high pH conditions (Fig. 4.2). Whilst a high degree of individual phenotypic variation was observed in the metabolic response of both groups, this was a nonsignificant reduction at the lower and upper extreme temperatures for high pH and low pH (Fig. 4.6) animals respectively. No significant difference was observed in average daily growth rates (weight: W = 941.5, p-value = 0.169; and length: W = 1561, p-value = 0.299), and the coefficient of variation showed negligible differences in the variation of the metabolic response of SMR, MMR and AAS between the high pH and low pH groups, except for SMR at 18° C (Table 4.7).

Comparing the metabolic response of juvenile *C. laticeps* raised under high CO_2 conditions to that of the baseline data from the high pH, it can be said that mild hypercapnic stress elicited a degree of energy restructuring (e.g., energy used for compensatory ionoregulation under hypercapnia may redirect limited energy reserves from other processes such as maintenance; Kooijman 2010) in developing individuals of this species, influencing their metabolic response to thermal change. Exposure to hypercapnic conditions was shown to have stage-specific effects on the metabolism of *C. laticeps* during the early developmental stages (Muller 2022). At the earliest developmental stages, hatchlings and early-preflexion larvae did not show adverse effects under hypercapnic conditions but prior to the onset of flexion (DAH 13 – 21) coinciding with development of the gills, minimum oxygen consumption rates were significantly greater in the low pH group when compared with the high pH group larvae (Muller 2022). Across the suite of temperatures chosen for the current study, except at the lower extreme thermal boundary, low pH animals had a significantly lower SMR than high pH animals (Fig. 4.2). This contradicts expectations that fish under hypercapnic stress will have a higher SMR and maintenance costs as energy is sdrawn away from storage, development, growth and activity (Sokolova 2013), towards life-sustaining processes. It offers promising insight into the potential acclimation of juvenile *C. laticeps* to high CO_2 stress by means of phenotypic plasticity (Pelster and Burggren 2018).

As was the case in the current study, Muller (2022) showed that although the metabolic rates of larval flexion *C. laticeps* reared under hypercapnic conditions were greater than the group reared under high pH conditions, the aerobic scope was not significantly different between the two groups (Muller 2022). He also found no differences in the length of fish in the low pH and high pH groups and attributed this to the phenotypic plasticity of the larvae as they were able to modify their energetic budgets to maintain normal growth and development rates (Muller 2022). This suggests that sufficient energy was allocated to growth and development in high CO_2-treated fish despite additional compensatory costs associated with hypercapnic stress. This is likely owing to unrestricted food availability, as was the case in the current study, as the author also suggested that mortality may have been higher for low pH specimens if food was limited due to the significantly elevated metabolic rates (Muller 2022). Several studies on larval growth and development have noted a decoupling between growth and metabolism for larval stages of other species, which is telling of compensatory energy allocation towards growth and away from other metabolic processes during development, particularly at this life history stage (Wieser et al. 1988, Rombough 1994, Moyano et al. 2018). Muller (2022)

described the observed metabolic adjustments using the energy-limited model (Sokolova 2013; Fig 5.1), which suggests that reduced AS is a reflection of a progressive decline in condition associated with increasing levels of environmental stress but is mediated through adaptation or acclimatisation (Muller 2022). While it is possible that the decoupling of growth and metabolism may have continued into the juvenile phase for *C. laticeps*, the nature of this long-term experiment may have facilitated strong selection for particular phenotypes and the acclimation of individuals. *laticeps*, the nature of this long-term experiment may have facilitated strong selection for particular phenotypes and the acclimation of individuals.

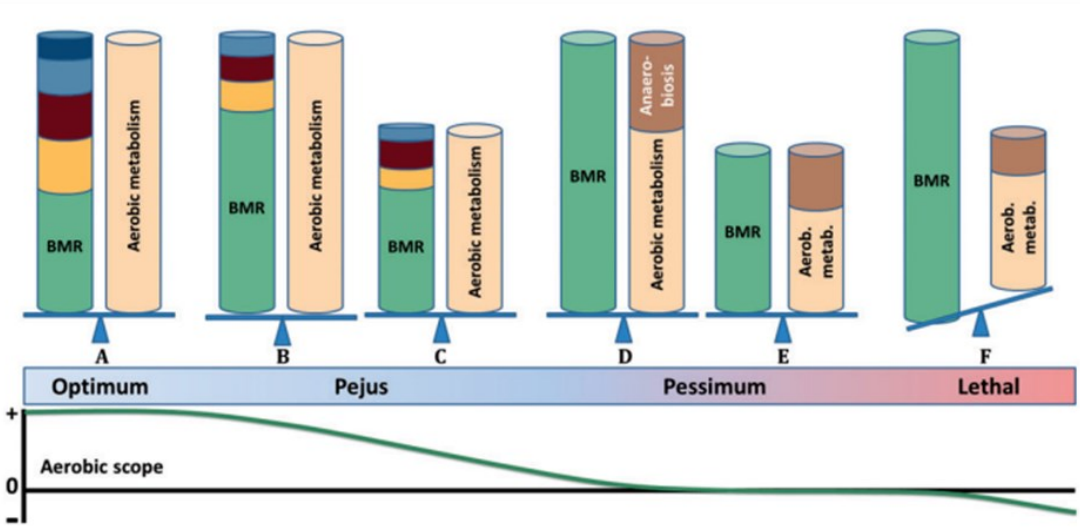


Figure 5. 1: Taken from Sokolova (2013) demonstrating the metabolic adjustments outlined in the energy-limited framework for stress tolerance where energy demanding functions including basal maintenance (green), activity (yellow), reproduction and maturation (dark red), growth and development (light blue) and deposition of energy reserves (dark blue) are represented inside each cylinder. The derivation of energy ATP is either aerobic (tan) or anaerobic (brown) and the contribution of the latter increases with increasing severity/length of exposure to stress. The degree of detriment that the exposure to a stressor causes in an organism is grouped into optimum (opaque blue), pejus (light blue), *pessimum* (light purple) and lethal (pink). The anticipated response of an organism to the various degrees of environmental stress is shown by the green line at the bottom of the graph.

The differences in physiological profiles, yet similarity in the growth of the high pH and low pH groups in this study deserve some attention. Sokolova's (2013) theory of energy limited

tolerance to stress covers outcomes that are opposite to the metabolic findings of the current research wherein AS is decreased under stress, which is accompanied by a reduction in growth (Fig. 5.1). However, the bioenergetic constraints dictating the physiological response of an organism to environmental stressors still offers a framework to conceptualise the metabolic response of the individuals within this study. Originally defined by Sokolova et al. (2012), the framework integrates the effects of multiple stressors with various cellular mechanisms and bridges the gap between physiological and ecological consequences of environmental stress (Sokolova 2013).

The OCLTT theory (Pörtner 2012) and the DEB model (Kooijman 2010) are the foundations on which the Sokolova et al. (2012) framework was built. The suitability of the framework balances on the acknowledgement that energetic trade-offs exist to maintain and maximise fitness (Kooijman 2010). Whilst environmental stress does impact energy allocation, assimilation and conversion due to compensatory mechanisms employed to maintain homeostasis, acclimation is possible (Willmer et al. 2000, Hochachka and Somero 2002). This can be accomplished through adjustments to energy assimilation, mitochondrial functionality and abundance, enzyme activity, membrane composition, as well as ion and gas transport (Willmer et al. 2000; Hochachka and Somero 2002). As seen with shifts in thermal tolerance windows under various thermal acclimation regimes (Sommer et al. 1997, van Dijk et al. 1999, Pörtner 2002, Sommer and Pörtner 2002; Sokolova and Pörtner 2003, Schröer et al. 2009) - as well as metabolic readjustments occurring under long-term acclimation to altered salinity - energy metabolism can be rearranged through acclimation to reduce SMR and/or conserve AS under moderately stressful conditions (Shumway and Koehn 1982, Sangiao-Alvarellos et al. 2005, Kidder et al. 2006). Under *pejus* and *pessimum* environmental conditions, it is stipulated that metabolism switches to partial anaerobiosis to support essential maintenance costs during time-limited survival (Sokolava 2013; Fig. 5.1), perhaps justifying the further reduction in SMR of low pH animals at high temperatures in the current study.

It is unclear whether additional acclimatory adjustments occurred during the development of these *C. laticeps*, as further research into mechanisms such as changes in cardio-respiratory function and digestion (Fitzgibbon et al. 2007) or improved compensatory oxygen extraction and transport capacity (Crespel et al. 2019) is needed. Regardless, this study suggests that individuals with a lower SMR (innately so or resulting from an acclimatory response/plastic reduction in SMR in response to low pH) were able to cope with and survive the long-term exposure to high CO_2 conditions. This outcome is likely due to the high mortality during the

early developmental stages which may have selectively removed individuals with alternative metabolic phenotypes that were less tolerant to hypercapnia. While this may indicate that there was shift in the reaction norm of this surviving population that this species has the potential for adaptation under strong selection (Lande 2009, Chevin et al. 2010), further investigations over lifespans and generations, in addition to the incorporation of multiple stressors and varied feeding regimes will be necessary. However, it is likely that the plasticity in metabolic trait responses interacted with the strong selection pressure imposed by high CO_2 stress, providing the first insight into the potential selection response (Crozier and Hutchings 2014) of this life stage of *C. laticeps.* Contextualising this response within the climate-induced changes expected along their distribution is then important for gaining perspective on these results.

The geographic distribution of *C. laticeps,* according to a minimum metabolic index (ϕ; the ratio of oxygen supply to demand across temperatures) threshold of 2.9, is limited in extent by low oxygen in the west and by high temperatures in the east (Duncan 2018). The expansion of the west coast cool temperate ecology, and the sub-tropical ecology in the east is anticipated to constrain potential distributional shifts of warm and cool temperate fauna along South Africa's coast (Potts et al 2015, Whitfield et al. 2016). However, the projected range contraction of *C. laticeps*, will likely not impact the core range (Fig. 5.2.a) of the species as this is expected to persist up until 2100 (Duncan 2018). Within this distribution, however, is the increase in the intensity and frequency of upwelling events which imposes demanding physio-chemical conditions and frequent low pH episodes around these upwelling centres (Broitman et al. 2018). These changes will no doubt have an impact on juvenile *C. laticeps* and this study has provided some evidence to understand some of these likely impacts.

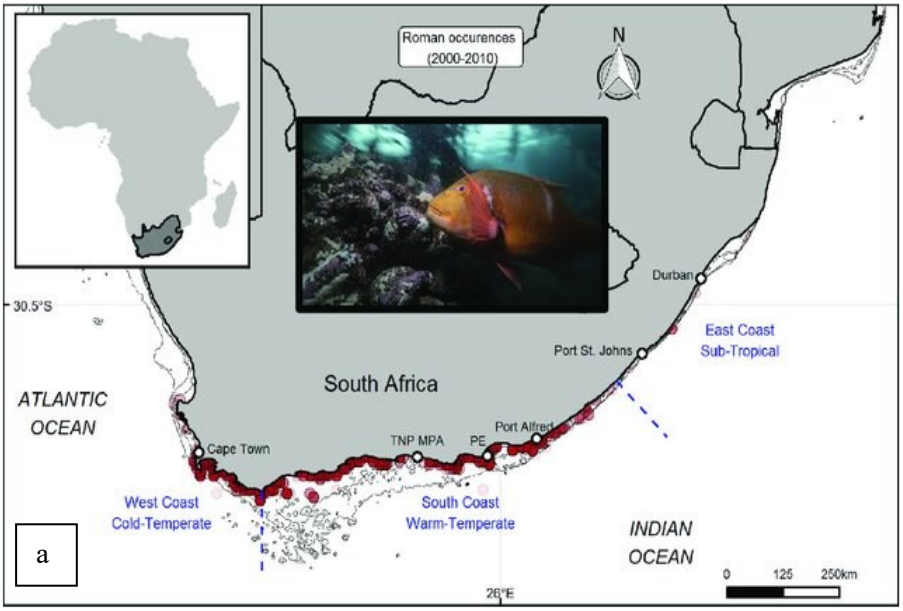
Juveniles in the high pH group exhibited a reduced scope of metabolic diversity at cooler temperatures, which was dissimilar to the response of adults exposed to current-day CO_2 conditions (Bailey 2022). Individuals did not exhibit significant reductions in physiological performance at temperatures resembling cold upwellings (10 ˚C; Goschen and Schumann 1995). *Chrysoblephus laticeps* occupies a shallow-water habitat (Laubenstein 2019), that routinely encounters cold upwellings. This means that they frequently experience temperatures nearing their lower limit of thermal tolerance however, *C. laticeps* has been shown to enter cold shock at 8 ˚C (Duncan et al. 2019a, Bates et al. 2019). This potentially contributed to the uniform response that high pH juvenile *C. laticeps* exhibited, which is likely to be inherited from their parental lineage. This study showed that juveniles developing in an acidified ocean may have the means to cope with the stress of hypercapnia and cooling due to their higher

MMR (Fig. 4.4) and AAS (Fig. 4.6). This goes against predictions made about impeded whole-animal performance under increased CO_2 environments (Pörtner and Farrell 2008) which is likely attributed to an acclimatory response. There may, however, be a narrowing of the thermal performance window of the species, particularly at the upper thermal boundary.

An important insight arising from this study was the existence of individual variation in the phenotypic response, which offered a look into the cryptic/fine-scale response of the population. The phenotypic diversity in the metabolic response of low pH animals, despite the strong selection pressure arising from the application of the treatment since hatching, is anticipated to contribute to the resilience and thus persistence of this population under OA (Ward et al. 2016). The presence of variable phenotypes is likely arising from the parental site capture being the Tsitsikamma MPA, as it has been shown that the protected population of adult *C. laticeps* in the same MPA had more aerobic scope phenotypic diversity as well as higher performance aerobic scope phenotypes than those of an exploited population (Duncan 2019a). This was attributed to the selective removal of high-performance aerobic scope phenotypes by the commercial linefishery in exploited areas (Duncan 2019a). The diminished diversity and absence of high-performance aerobic scope phenotypes may translate into population level consequences when faced with future environmental thermal variability (Bernhardt and Leslie 2013). When one considers that the progeny of an exploited population of larval *C. laticeps* (Gqeberha, South Africa) exposed to the same CO_2 treatment as the low pH animals in the current research experienced a period of metabolic and growth depression during their preflexion stages (Muller 2022), it is likely that it is the offspring from a protected population that may provide the resilience to environmental stressors. This likely indicates that physiological perturbation caused by fisheries-induced evolution (FIE) from passive fisheries has important consequences for sensitive developmental stages exposed to environmental stress, and the removal of high-performance phenotypes can reduce resilience of a population to deal with environmental stress (Muller 2022).

Based on the difference in the response of *C. laticeps* larvae from exploited and unexploited populations to OA and the adaptive potential of protected population juveniles observed in this study, protecting components of the populations from the negative impacts of exploitation will be critical. In South Africa there are a number of established MPAs which have the potential to preserve phenotypic diversity and broaden the response plasticity of a population (Roberts et al. 2017). Due to the extensive dispersal of eggs and larvae of this species, these MPAs will export these offspring to adjacent areas (Gell and Roberts 2003, Kerwath et al. 2013). The

recent declaration of 20 new MPAs (Fig. 5.2.b) by the Department of Environmental Affairs (DEA) in May of 2019, is said to offer at least some protection to 90 % of all habitat types and the spatial protection around South Africa's coast has reached 5.4 % (DFFE, 2021). This is promising for the population of *C. laticeps* as they have been found to respond well to the protection that MPAs provide (Kerwath et al. 2013), owing to the high residency of this species (Kerwath et al. 2007). However, understanding the dispersal patterns of the eggs and larvae from the existing MPAs will be important to assess whether these areas are appropriately placed to ensure broad dispersal of these highly valuable phenotypes.



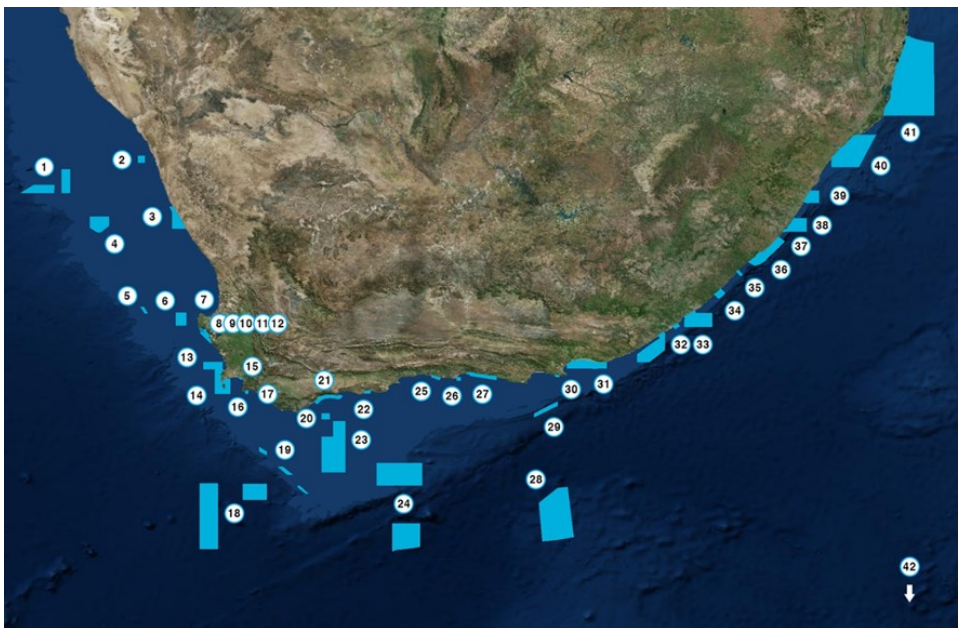


Figure 5. 2: a.) The known distribution of *Chrysoblephus laticeps*, taken from Duncan et al. (2020), and b.) The 41 MPAs along the coast of South Africa.

Whilst the use of oxygen consumption measurements is now a well-established method used in the scientific literature to quantify the response of metabolism to environmental stress, this method of measurement only estimates the response of aerobic metabolism and disregards the benefaction of anaerobic metabolism to ATP turnover and metabolic rate (Navas et al. 2010). This could result in metabolic rates being underestimated under environmental stress owing to the activation of anaerobic ATP generation through substrate-level phosphorylation. Aerobic scope quantification could offer a more reliable estimate of metabolic rate if end products of substrate-level phosphorylation (e.g. lactate) were measured and incorporated in future research (Navas et al. 2010). In addition, metabolic traits can respond differently to extrinsic (environmental) and intrinsic (population and species-specific) factors, varying even further under the interaction of these factors (Archer et al. 2020). The flexible nature of SMR and the observed sensitivity of this metric to food availability, showing reductions under food-limited conditions (Naya et al. 2007, Auer et al. 2015b, Zeng et al. 2018, Langer et al. 2018) and increases when food is abundant (Van Leeuwen et al. 2011b), demonstrates the importance of incorporating varied feeding regimes into trials of this nature. The benefits of food-induced SMR plasticity may also be temperature dependent, as seen in the common carp (*Cyprinus carpio*; Zeng et al. 2018), highlighting the need for combined stressors, such as hypercapnia and food restriction, to be tested against the flexibility of SMR (and the corresponding AS). This would paint a more accurate picture of the response of these populations to future environmental conditions under climate change, particularly in developing life stages of commercially important species (Vagner et al. 2019).

Repeated measures experiments are widely useful, as periodically measuring key traits offers a more definitive evaluation of within-organism change across time, whilst simultaneously enhancing the statistical power for detecting changes (Guo et al. 2013). Larger sample sizes are required for fewer repeated measures in order to obtain the same level of power however, the optimal combination of these two needs to be calculated for a realistic, cost- and time-effective study design (Dang et al. 2008). Whilst the sample size in the current research was dictated by surviving laboratory-reared juveniles at the commencement of the experiments, it offered sufficient numbers for appropriate statistical power. Finally, in the absence of experimentally tested thermal tolerance information (and critical thermal limits; CTL) for *C. laticeps* and the juveniles of this species, pilot studies to determine this information would have helped to identify these species-specific critical biological thresholds. Despite the temperatures

chosen for this study being ecologically relevant and representative of cold upwellings and heatwaves, this would allow for a more accurate examination of their metabolic response to extreme *pejus* and *pessimum* temperatures.

Future research should look to incorporate both physiology and behaviour in metabolic studies that are evaluating the effects of multiple stressors, as studies have shown that the absence in response of AAS to CO_2 treatments may not be paralleled by the absence in a behavioural response, and behaviours such as predator avoidance may be significantly impaired (Nagelkerken and Munday 2016). In other words, trade-offs may exist which constrain individuals along a maximal performance ridge (Sunday et al. 2014), where AS may be maintained, or an ecologically appropriate response may be elicited, but not both (Laubenstein 2019). In addition, the usefulness of research focusing on individual phenotypic variation would be enhanced if both behavioural and physiological traits were considered, as observations of covariation between behavioural and physiological traits would help researchers understand the processes behind these relationships, as well as whole animal responses to change (Careau et al. 2008, Davis et al. 2017, Biro et al. 2018). Considering the fish used in this study were progeny from a protected population and demonstrated high phenotypic variation and an element of conferred resilience - research should incorporate the effects of exploitation as a covariate on the metabolic response of fish to environmental stressors. Already the importance of fisheries-induced evolution (FIE) in shaping population-level response to environmental stressors has been demonstrated in a number of metabolic studies (Duncan 2018, Bailey 2022, Muller 2022). Finally, Kroeker et al. (2013) suggests that caution should be employed when extrapolating results (e.g., species response and abundance) from single-species laboratory experiments, as the responses of a species become more variable under multi-species assemblages when they are exposed to reductions pH, owing to indirect and unconsidered effects.

Considering the ubiquitous changes in thermal conditions and reduced pH in our ocean, as well as the projected increase in thermal variability, cold upwellings, and localised changes in pH along the distribution of *C. laticeps,* the key findings of this research offer insight into the response of this commercially important, endemic species during a critical stage of development.

1.) There is evidence to suggest that juvenile *C. laticeps* are more resilient to high CO_2 induced hypercapnia than anticipated, largely attributed to the apparent phenotypic plasticity that has

facilitated a capacity for acclimation. The significant difference in variability in the response of the groups' SMR at 18° C (Table 4.7) may signify that the overall reduction and homogenisation of metabolic rates at this temperature is likely the result of rapid selection over their ontogeny. This is ascribed to the long-term exposure (from fertilisation until ~ 100 DAH) during a critical and largely plastic period of development. Whilst the mechanism of the observed metabolic response requires further investigation and remains unresolved in the literature to date, a proverbial reshuffling of the bioenergetic budget has occurred in order to meet the demands of acid-base balance compensation and the maintenance of growth. The limited energy framework (Sokolova et al. 2012, Sokolova 2013) highlights the flexible nature of energy resource allocation and the importance of bioenergetics in the stress response of organisms.

2.) In line with the bioenergetic changes employed to accommodate what could be a new steady state under mild chronic stress, energetic, metabolic and physiological trade-offs exist due to the limited and finite nature of energy. A reduced affinity to cope with heat stress at the upper extreme temperatures was observed, indicating a 'downward' shift in the thermal performance window of juvenile *C. laticeps* under hypercapnic conditions. Whilst their affinity to cope with cold *pejus* temperatures remains intact (up until the 11° C threshold), anticipating a proficiency for coping with the thermal aspect of increased cold upwelling, warming may prove to be a problem for this species with simultaneous reductions in environmental pH.

3.) A high degree of individual variability was observed in the metabolic phenotypic response, both in the high pH and low pH group, *albeit* reduced at low and high temperatures for high pH and low pH fish respectively. One can anticipate that the variant metabolic disposition arises from the phenotypic variation expected of the maternal and paternal lines, safeguarded in the Tsitsikamma MPA. This demonstrates a robust buffering potential against climate change stressors, such as hypercapnia, owing to the existence of variable performance phenotypes, and proves once again the immense importance of protected areas to the persistence of species under immense anthropogenic and environmental pressure.

4.) Under sufficiently high hypercapnic selection pressure across their early ontogeny, and in the presence of abundant food supply, individuals with a lower SMR appear to be more tolerant to high CO_2 conditions. These individuals are suited to coping with this specific environmental stressor, but whether this outcome can be replicated under natural conditions remains to be seen.

5.) Long-term studies of this nature are of vital importance if we are to get a coherent perspective on the response of populations to climate change, as different life history stages have differing metabolic and physiological responses to environmental stressors. Considering that the strength of the biological response is often governed by the length and severity of an organism's exposure to a certain stressor, experiments that incorporate lengthened exposure times offer an opportunity to witness the activation of a species' acclimation potential, if this is in the realm of their physiological capacity under ecologically relevant conditions. The response of ectothermic marine populations varies widely between taxa and life history stages, and as demonstrated by the outcomes of this study, we need to be cognisant of the unforeseen and enlightening ways in which fishes can accommodate progressive changes in their environment.

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Appendix:

Table A. 1: Results of the polynomial linear mixed effects model analysis of the standard metabolic rates (SMR), maximum metabolic rate (MMR) and absolute aerobic scope (AAS) of juvenile *Chrysoblephus laticeps* in response to temperature and CO_2 treatment projected for 2100 (pH = 7.63; $pCO_2 \approx 1400$). Significant values reported in bold.

	Effect	Estimate (intercept)	Std. Error	d.f.	t-value	P-value
Model 1: data with all viable measurements for each individual and each temperature						
SMR (mg $O_2^{-1}.g^{-1}.hr^{-1}$)	**Fixed:**					
	Intercept	0.25	0.02	3.42	14.22	**<0.01**
	Temperature	1.07	0.16	6.69	65.77	**<0.01**
Pseudo-R^2 0.39	Treatment	-0.06	0.03	2.17	-2.10	0.16
AIC *-100.48*	Temperature: Treatment	-0.59	0.24	71.94	-2.40	**0.02**
MMR (mg $O_2^{-1}.g^{-1}.hr^{-1}$)	**Fixed:**					
	Intercept	0.60	0.04	3.24	14.42	**<0.01**
	Temperature	1.84	0.30	65.43	6.19	**<0.01**
Pseudo-R^2 *0.36*	Treatment	-0.01	0.06	2.64	-0.16	0.88
AIC *5.40*	Temperature: Treatment	-0.99	0.45	71.70	-2.21	**0.03**
AAS (mg $O_2^{-1}.g^{-1}.hr^{-1}$)	**Fixed:**					
	Intercept	0.30	0.03	3.26	9.26	**<0.01**

Pseudo-R^2 0.12 **AIC** -32.47	Temperature	0.46	0.24	65.48	1.92	**0.06**
	Treatment	0.07	0.05	2.59	1.48	0.25
	Temperature: Treatment	-0.21	0.36	71.74	-0.57	**0.57**
Model 2: data with individual fish that had viable measurements at three or more temperatures						
SMR (mg $O_2^{-1}.g^{-1}.hr^{-1}$) **Pseudo-R^2** 0.62 **AIC** *-91.70*	**Fixed:**					
	Intercept	0.23	0.02	1.73	13.12	**0.01**
	Temperature	0.96	0.11	39.80	8.48	**<0.01**
	Treatment	-0.06	0.03	1.55	-2.38	0.18
	Temperature: Treatment	-0.52	0.17	40.30	-3.03	**<0.01**
MMR (mg $O_2^{-1}.g^{-1}.hr^{-1}$) ***Pseudo-R^2*** *0.40* ***AIC*** *-4.29*	**Fixed:**					
	Intercept	0.56	0.04	1.73	14.04	**0.01**
	Temperature	1.49	0.26	39.80	5.80	**<0.01**
	Treatment	-0.02	0.06	1.55	-0.28	0.81
	Temperature: Treatment	-0.98	0.39	40.30	-2.51	**0.02**
AAS (mg $O_2^{-1}.g^{-1}.hr^{-1}$) **Pseudo-R^2** 0.08	**Fixed:**					
	Intercept	0.29	0.03	1.97	8.47	**0.02**
	Temperature	0.26	0.21	39.78	1.26	0.26
	Treatment	0.07	0.05	1.80	1.36	0.33

AIC -25.76	Temperature: Treatment	-0.27	0.32	40.27	-0.86	0.40