The thermal tolerances and preferences of native fish in the Cape Floristic Region: towards understanding the effect of climate change on native fish species

by

Jody-Lee Reizenberg

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Supervisors: Dr Helen Dallas, Dr Jeremy Shelton, and Dr Cecile Reed

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J. Reizenberg (13 March 2017)

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"In an age where man has forgotten his origins and is blind even to his most essential needs for survival, water along with other resources has become the victim of his indifference."

- *Rachel Carson,* Silent Spring (1962 pp39*)*

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ABSTRACT

Global climate change models indicate that a rise in temperature and reduction in rainfall in the Western Cape Province of South Africa is inevitable and unavoidable. Within the Western Cape lies the Cape Floristic Region (CFR); a biodiversity hotspot with high levels of endemism. This includes its freshwater fish assemblage. Whereas the current greatest threats to native fish biodiversity are habitat degradation and invasion by non-native species, predicted climate change is likely to further impact fish communities negatively. As a master abiotic variable in aquatic ecosystems; temperature influences the fitness, behaviour, and life-histories of aquatic biota. Thermal alteration may therefore affect sensitive fish species. The upper thermal limit, determined via the critical thermal method, has been validated as a measure of thermal sensitivity. To better understand the impacts of climate change on the native fish of the CFR, upper thermal limits (critical thermal maxima/CT_{max}) were determined for seven native species of freshwater fish. Thermal preferences were also determined for five of these species using the acute gradient tank approach to elucidate thermal habitat preferences. Species that were identified by the IUCN as vulnerable, endangered, or critically endangered were selected from the four main families of native fish in the CFR (Anabantidae, Austroglanidae, Cyprinidae, and Galaxiidae), from four Rivers. Overall, Cape galaxias (*Galaxias zebratus*), Breede River redfin (*Pseudobarbus burchelli*), Berg River redfin (*Pseudobarbus burgi*), Clanwilliam redfin (*Pseudobarbus calidus*), and fiery redfin (*Pseudobarbus phlegethon)* were found to be most sensitive to increased temperature (CT_{max}= 29.8-32.7ºC). Clanwilliam rock-catfish (*Austroglanis gilli*) and Cape kurper (*Sandelia capensis*) were found to be moderately sensitive (CT_{max}= 33.0-35.3ºC). Similar trends were found using the thermal preference approach as CT_{max} and thermal preference were found to correlate well. The results were related to *in-situ* water temperature, which influenced both parameters. Thermal tolerances and preferences of all the native species exceed that of invasive salmonids (*Onchorynchus mykiss* and *Salmo trutta*). However, non-native centrarchids (*Micropterus* spp.) are more thermally tolerant, indicating an increase in threat by warm adapted non-natives. These data suggest that species interactions and distributions are likely to undergo substantial changes in response to elevated water temperature.

KEY WORDS: Cape Floristic Region, climate change, critical thermal limits, thermal preference

CHAPTER 1: General introduction

1.1 Introduction

Climate change projections indicate that the Cape Floristic Region (CFR) of South Africa, a Mediterranean system, will become drier and hotter, driving a temperature increase in lotic systems of that region (New, 2002). Global climate change models predict a decrease in mean annual precipitation by up to 40mm and summer maximum air temperature increase of 4-6ºC per decade with a corresponding increase in water temperature (Dallas and Rivers-Moore, 2014). In a region that is already water scarce (receiving only 60% of the world average in rainfall), rivers in the CFR will be amongst the most impacted ecosystems as water withdrawals escalate, dissolved oxygen decreases, and pollutants become more toxic (Zucchini and Nenadić, 2006; Ficke et al., 2007; King and Pienaar, 2011; Filipe et al., 2013). According to Vitousek et al., (1997) more than half of all the worlds accessible surface water is already being utilized. Further reduction in discharge and thermal alteration may affect both temporal and spatial interactions of freshwater species (Ormerod, 2009). As arid regions become drier and demands for water escalate, up to 75% of local fish biodiversity (in rivers with reduced discharge) may be headed towards extinction by the year 2070 (Xenopoulos et al., 2005).

The CFR has been identified as a global biodiversity hotspot (Myers et al., 2000), and a priority biogeographic unit for freshwater conservation (Abell et al., 2008; Nel, 2011). With reference to climate change, Dallas and Rivers-Moore (2014) recognise the region not only as a biodiversity hotspot, but a 'hotspot for concern'. This concern is due to high levels of endemism and low species diversity which are attributable to an ancient history of geographic isolation of the CFR's catchments (Linder et al., 2010). Due to these restricted ranges and specific habitat requirements, the invasion by non-native species has severely affected native populations. As a result, the conservation statuses of most of the region's endemic fish is vulnerable, threatened, or critically endangered (Tweddle et al., 2009). While the current vulnerability of fish biodiversity are due to habitat alteration and invasion by non-native fish (Weyl et al., 2014), the added effect of climate change may further impact fish communities in the future.

In the northern hemisphere, the effects of global climate change on biodiversity have been well documented (Parmesan, 2006), and the data suggest a general decline in reproductive fitness across taxa (Heino et al., 2009). However, this is not the case for the global south: In South Africa, the effect of temperature on the spawning of cyprinids has been documented by Paxton and King (2009), but no studies have been undertaken to determine the effects of climate change on fish *per se*. A likely scenario is that the distribution ranges of the more thermally sensitive fish species will be modified with increasing water temperature (Dallas, 2008). To this end, Ellender et al., (2017) promote future studies focussing solely on climate change effects on freshwater fish: given the paucity of such studies in the CFR and in South Africa on the whole.

Thermal data are required to set water temperature thresholds that will ensure conservation of sensitive fish species. Chronic exposure data for freshwater invertebrates have been used in the past by Dallas and Rivers-Moore (2012) and Rivers-Moore et al., (2013) to determine Maximum Weekly Allowable Temperature (MWAT) for freshwater ecosystems in the CFR. In South Africa, the determination of these thresholds not only promotes conservation, but is mandated by national legislature. The National Water Act (Act No. 36 of 1998) acknowledges temperature as an important abiotic water quality variable of the ecological Reserve, which is the minimum quantity and quality of freshwater required to maintain ecological integrity and satisfy societal needs (Palmer et al., 2007). The benefits of determining thermal requirements for native fish are therefore threefold: it promotes the maintenance of the ecological Reserve, informs conservation by elucidating real biological thresholds (and thus sensitive species), and assists towards understanding the ecological consequences of climate change. To date, the lack of physiological information on CFR's fish species has inhibited the setting of biological thresholds, and the assessment of projected climate change impacts on freshwater fish distribution (Ellender et al., 2017).

1.2 Freshwater fish and climate change in the Cape Floristic Region

1.2.1 The state of the region's native freshwater fish: past and present

There are currently 33 identified species of freshwater fish in southern Africa, with the majority of endemic species occurring in the mountain streams of the southwestern Cape (Skelton, 2001). The CFR holds 21 recognised species of native fish, 17 of which are endemic to the region (Weyl et al., 2014; Ellender et al., 2017).

The presence of non-native fish species is one of the current greatest threats to native fish (Ellender and Weyl, 2014). According to Skelton (2001), 22 fish species have been introduced to southern Africa; 20 of which have become established in the CFR (Weyl et al., 2014). To date, more than half of the CFR's endemic species are endangered according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Tweddle et al., 2009). Predation by, and competition with, non-native species, as well as habitat alteration, are largely responsible for the state of the region's freshwater fish populations (Weyl et al., 2014). The year 1859 saw the first introduction of a non-native fish species, the common carp (*Cyprinus carpio*), into the freshwaters of South Africa, with brown and rainbow trout

(*Salmo trutta* and *Oncorhynchus mykiss* respectively) being successfully introduced in the 1890s (van Rensburg et al., 2011). The repercussions of non-native species introductions were beginning to manifest in the latter part of the 1920s and 1930s when additional species, such as large and smallmouth bass (*Micropterus salmoides* and *Micropterus dolomieu* respectively), were added to South Africa's freshwater ecosystems (Skelton, 2001). A general decline in the abundance of native fish species was documented in response to this impact (Weyl et al., 2014). In the 1970s, Skelton (1977) released the South African Red Data book for freshwater fish identifying the status of known species at the time, and the dire straits of the Cape's freshwater fish finally drew attention from conservation authorities (McCafferty et al., 2012), with governmental support for non-native fish hatcheries in the CFR officially ceasing in the 1990s (Weyl et al., 2014).

By the turn of the 21st century a new conservation agenda was adopted; the Cape Conservation Plan for Aquatic Ecosystems (Impson et al., 2002). Whereas before the 1990s there was no legislature curbing the introduction of non-native angling species, the regulations promulgated under National Environmental Management: Biodiversity Act (Act No. 10 of 2004) in 2013 prohibited the introduction of any non-native species without a non-native species management program. Under this law, governmental departments and management authorities (e.g. CapeNature and South African Institute for Aquatic Biodiversity) are liable to enforce eradication plans, as well as implement monitoring and control measures (Weyl et al., 2014).

At present, freshwater fish conservation has a strong focus on control and eradication of non-native species, but climate change and increased water withdrawals, especially in the dry season, equally threaten native fish populations (Skelton, 2001). The pollution and eutrophication of rivers coupled with human demands for freshwater have intensified (King and Pienaar, 2011), thus modifying and fragmenting habitats. Dams and weirs have also been shown to inhibit migration of fish, thereby restricting the range and population size of certain species (Paxton and King, 2009).

1.2.2 Native freshwater fish of the Cape Floristic Region

Although the CFR has one of the highest proportions of endemic plant species in the world (Cowling et al., 2003), the fish fauna of the region is characterized by low species diversity (an average of two to four species per river system) (Linder et al., 2010). This has led to the region being recognized as an 'arena' for evolutionary diversification (Linder et al., 2010). Within this geographically isolated region, evolution has shaped the adaptations of the families that are present today. The freshwater fish fauna in the CFR consists of four main families (Linder et al., 2010), namely Anabantidae, Austroglanidae, Cyprinidae, and

Galaxiidae. Of these families, only the genera *Sandelia* (Anabantidae), *Pseudobarbus* (Cyprinidae), and *Galaxias* (Galaxiidae) are widespread.

The genus *Galaxias* is thought to be a relict group of species from the break-up of Gondwanaland 180-135 million years ago (Linder et al., 2010). Close relatives of this southern faunal assemblage in New Zealand and South America allude to the connection of the landmasses before the splitting of the supercontinent (Skelton, 2001). Only one described species in the genus, *Galaxias zebratus,* is found in Africa, with an extreme level of intra-specific divergence (Wishart et al., 2006). Molecular studies show that *G. zebratus* consists of at least five distinct lineages with subsequent unpublished work suggesting that the genus is a species complex (Wishart et al., 2006). Although the distribution of the species stretches from the Gamtoos River system in the east to the Olifants system in the extreme west, the effect of non-native species have affected population size and distribution. *G. zebratus* is the preferred prey for most non-native fish species due to its small size (Skelton, 2001; Shelton et al., 2015), resulting in extreme competition for habitat and resources (Shelton et al., 2008).

The most species-rich (and most revised) genus of freshwater fish in the CFR, *Pseudobarbus,* belongs to the Cyprinid family (Swartz et al., 2009). *Pseudobarbus* is a more recent African lineage compared to the Galaxiidae (Linder et al., 2010), and its evolutionary history has made for important discoveries with regards to drainage history in the CFR. Generally, only one species of *Pseudobarbus* occurs in each river system (Chakona et al., 2013). The reason for this is attributed to allopatric speciation. Using molecular and morphological data for the *Pseudobarbus* species, Swartz et al., (2009) proved that the river systems in the CFR were last connected before the Last Glacial Maximum (LGM) (18 000 years ago). The subsequent isolation of these systems facilitated genetic divergence in the genus (Linder et al., 2010). Since the genera *Pseudobarbus*, *Barbus*, and *Enteromius* are under revision and additions are being made with every genetic study (Skelton, 2016), the number of species in the genus is still debatable.

The Anabantidae are widely distributed in the CFR, and includes the ubiquitous *Sandelia capensis*. This species has been described as hardy; being able to use its accessory breathing organ to survive in rivers that are oxygen-poor (Skelton, 2001). The hardy nature of the species is responsible for its persistence in many of the CFR's systems, but other species have not responded similarly. A supposedly hardy species only occurring in the western CFR (Olifants system), *Austroglanis gilli* (family Austroglanidae), has had its populations severely impacted by the presence of non-native bass (Skelton, 2001). This is largely due to the inability of species to escape the river systems in which invasive species have been introduced.

Following the LGM, the main families of freshwater fish in the CFR have remained largely restricted as a result of the geological formation of the Cape Fold Belt. Isolated populations are particularly vulnerable to unnatural impediments to their ancient life histories and consequently 11 of the region's species are listed as critically endangered on the IUCN Red List of Threatened Species (Weyl et al., 2014).

1.2.3 Thermal requirements of fish

Water temperature and flow are recognised as master abiotic variables in aquatic ecosystems, controlling the biological rhythms of aquatic fauna from invertebrates to fish (Dallas, 2008). Since aquatic organisms are sensitive to temperature change and rely on thermal cues to instigate life-history events, the behaviour and life-history of freshwater biota may be significantly influenced by thermal alteration (Vannote and Sweeney, 1980). Vannote and Sweeney (1980) noted that all aquatic species have a range of temperatures at which metabolism, growth, fitness, and reproduction are favourable; this is known as the Optimal Thermal Regime (OTR) (Vannote and Sweeney, 1980). Outside the OTR, behaviour and life history become mismatched. In the CFR, freshwater fish are particularly sensitive to temperature-mediated effects given their restricted ranges, geographical isolation, and life histories that have been adapted to regional conditions over millennia (Chakona et al., 2013). At the community level, temperature is known to affect aquatic community structure (Vannote and Sweeney, 1980), but has also been identified as having a pivotal role in maintaining niche differentiation in lotic assemblages (Magnuson et al., 1979). This regulates distribution patterns and diversity of species at the large scale (Vannote and Sweeney, 1980).

Within a species, specific thermal optima are inherited, but genetic and physiological plasticity within a species allows recent thermal history to influence thermal preference and tolerance (Johnson and Kelsch, 1998). Long and short-term acclimation (months to days respectively) are known to influence the thermal optima and preferences of fish. Generally, physiological adaptations to elevated temperatures cause a proportional increase in both thermal tolerance and preference. Notwithstanding thermal history, differences in temperature requirements may also be attributable to ontogeny. Temperature influences the vital functions of an organism throughout its life, and as such thermal constraints are likely to differ at every life stage. McCauley and Huggins (1979) put forward the hypothesis that young fish select higher temperatures than do older con-specifics, confirming that from inception to death, thermal requirements differ significantly. Coutant (1987) refers to this range as the species' 'thermal niche', and describes it as a dynamic entity that undergoes contraction and expansion through the course of an organism's development. Developing embryos have little or no capacity for behavioural thermoregulation, making them more stenothermic than adults, thereby resulting in a narrower thermal niche (Burt et al., 2011).

Adults on the other hand have a greater capacity for behavioural thermoregulation and can thus exist in a wider thermal niche. Thermal requirements at various life stages are therefore partly influenced by evolution, and partly by recent thermal history/acclimation.

In terms of population dynamics, climate-induced changes in temperature, and to a lesser extent photoperiod, will have considerable impacts on fish mortality and fecundity. While mortality is a direct result of unfavourable temperature, fecundity and reproduction is more complex. Bobe and Labbé (2010) suggest that the development of eggs and the number of viable offspring produced in a spawning season will diminish as a result of climatic warming, and thermal stress may thereby influence the ability of a population to reproduce optimally (Burt et al., 2011). Indeed, experimental studies have consistently shown significant differences between eggs incubated at different temperatures, with upper and lower temperature treatments having considerably less successful hatches (where fry are normal and viable) (Kelley, 1968). Ultimately, temperature is one of the most important abiotic determinants of vital life history events and survival at any life stage (Rivers-Moore et al., 2013). Abstraction, inter-basin transfer, removal of riparian vegetation, effluents that are thermally anomalous, and climate change in certain regions are some of the main contributors to the general decline in thermally suitable habitats, and are the greatest threat to all life stages (Dallas and Rivers-Moore, 2014).

With respect to non-native species, temperature is an important abiotic factor determining the success of invaders (Moyle and Light, 1996; Britton et al., 2010). Based on at least four species, Moyle and Light (1996) found that locally adapted non-native species performed better than native fish. Provided that temperature remains favourable, non-native species will continue to dominate habitats and determine the presence and abundance of native fish (more so, warm-adapted species such as tilapia and bass). A study by Woodford et al., (2005) found that predation by smallmouth bass *(Micropterus dolomieu*), was responsible for abundance and absence of native fish (more so than habitat quality). The findings of Shelton et al., (2008) corroborate this, in that the presence of largemouth bass (*Micropterus salmoides*) influenced microhabitat selection in the native *G. zebratus.* Climate change could thus indirectly dictate non-native fish density and thus their impact on native fish. Whether native fish abundance is directly or indirectly influenced by climate change, it is clear that species distribution will undergo change based on which species are more or less vulnerable to regional warming.

1.2.4 Fish biodiversity, distribution, and climate change in the Cape Floristic Region

The world's Mediterranean regions are located in southwestern Australia, central Chile, coastal California, around the Mediterranean Basin, and in the Western Cape of South Africa (Filipe et al., 2013).

Driven by large subtropical high pressure ocean cells shifting towards the poles in summer and towards the equator in winter, the climate in these regions is characterised by cool wet winters and warm dry summers (Gasith and Resh, 1999). Mediterranean regions are expected to experience some of the most drastic effects of climate change as temperature increases and precipitation decreases at unprecedented rates (Lawrence et al., 2010; Filipe et al., 2012). In the Western Cape of South Africa particularly, the reduction in rainfall and concomitant reduction in the natural flow of rivers will become increasingly difficult to manage. Water withdrawals are expected to escalate on already-reduced flows, threatening the sustainability of ecosystems in the face of climate change (King and Pienaar, 2011).

In what Woodwell (1990) refers to as the 'Earth in Transition', climate change and its instability have led to the biotic impoverishment of freshwater ecosystems, irrevocably altering them. Freshwater fish distributions have shifted over decades, centuries, and millennia in response to climate change (Heino et al., 2009), but current rates of thermal alteration are unprecedented. The CFR being a Mediterranean region will likely undergo considerable changes in the taxonomic composition of species assemblages (Midgley et al., 2003). In the southwestern Cape in particular there may be significant taxonomic homogenisation within the freshwater fish community, as sensitive and specialist species may be weeded out (Marr et al., 2010). Filipe et al., (2013) note that if the Intergovernmental Panel on Climate Change (IPCC) global circulation models are correct, freshwater ecosystems in Mediterranean regions will be substantially affected by the loss of sensitive species- despite their adaptability and resilience. Coupled with other anthropogenic disturbances, climate warming can be expected to trigger a rise in extinction rates worldwide (Frazee et al., 2003).

In Mediterranean regions, native fish species are outnumbered by non-native fish (Marr et al., 2010). In the CFR specifically, Marr et al., (2012) estimate that non-native fish have invaded more than 90% of mainstem river habitat. A potential positive outcome of climate warming in the CFR would be the thermal exclusion and range restriction of stenothermic cold-adapted non-native species. Indeed, in the Temperate North, ranges of cold-adapted species are expected to shrink as climate warms with a corresponding proliferation of warm-adapted species- altering species assemblages (Buisson et al., 2008; Britton et al., 2010). Similarly, habitat preferences in the high altitude populations of fish in the CFR may experience range restrictions. Coastal populations of Cape Kurper (*Sandelia capensis*) and Cape Galaxias (*Galaxias zebratus*) were found to exhibit wider habitat tolerances relative to certain non-native species (Cowling et al., 2003), suggesting that they may experience an increase in fitness in the future.

Expected temperature rise may also impede the reproductive fitness of non-native fish. Rainbow trout (*Onchoryncus mykiss*) for instance require temperatures of 10-17°C to successfully ovulate and spawn

(Samarin et al., 2008). Shelton et al., (2015) showed that the impacts of non-native species (like trout) on native fish abundance are density dependent. Native species would thus benefit from the thermal elimination of trout. However, not all non-native species are cold-adapted, and the level of threat by warm adapted centrarchids (*Micropterus* spp.) may be problematic for native species.

1.2.5 Determination of thermal requirements: history and methodology

Thermal experiments have been used the world-over to determine the thermal limits for a plethora of aquatic organisms (Lutterschmidt and Hutchison, 1997). The discipline emerged and developed rapidly within the aquaculture sciences due to a rapid increase in human population consumption of freshwater fish as a protein source (Ficke et al., 2007). Since fish species grow at differential rates and at different temperatures, the determination of thermal optima was necessary to maximise yield (Kellogg and Gift, 1983; Cuenco et al., 1985; Austreng et al., 1987). As climate change intensified across the globe, and threatened to endanger natural populations, thermal studies on wild freshwater fish grew in popularity (Ficke et al., 2007). The objective of these studies was to determine which temperatures species could tolerate (thermal maxima), and which temperatures were most favourable to their life histories (thermal preferences). Two common tools for the determination of thermal tolerance and thermal preference are the Critical Thermal Method (CTM), and the thermal preference experiment (Dallas and Ross-Gillespie, 2015).

Within the broader literature, CTM may refer to a parameter or method (Becker and Genoway, 1979). For the purposes of this study, the critical thermal method/CTM (the method) is used to determine the critical thermal maximum (CT_{max}) (the parameter). CTM experiments are static, and determine acute exposure effects by ramping temperature at a constant rate until a predetermined physiological threshold has been reached. The temperature change is generally achieved by means of a thermostat-controlled water-bath or circulating heater allowing for a precise rate of change (Dallas and Ross-Gillespie, 2015).

Thermal preference experiments are choice experiments, by which the preferred temperature of a group of fish can be determined. The temperature most frequently gravitated towards in an open hetero-thermal chamber is interpreted as the preferred temperature. This chamber may be vertical or horizontal, with the gradient achieved by means of a heating element on one end and a cooling element at the other (Dallas and Ross-Gillespie, 2015). The theory and technical underpinnings of these experiments are elaborated on in detail in Chapter 2.

To make informed predictions about the potential impacts of climate warming on already-threatened freshwater fish populations, a solid understanding of thermal sensitivity is necessary. Established experimental protocols for evaluating tolerance and preference are the best way to elucidate the effects of elevated water temperature on fitness and survival, and to establish environmental water temperature guidelines for freshwater ecosystems (Dallas and Rivers-Moore, 2012). These experiments may be undertaken in a laboratory or in the field, but the desired parameters, logistical constraints, and resources determine the choice of experiment (Dallas and Ross-Gillespie, 2015).

1.3 Objectives and structure of the thesis

This project forms part of a broader Water Research Commission project no. K5-2337 "Assessing the effect of global climate change on native and non-native fish in the Cape Floristic Region." The overall aim of the broader project was to assess the vulnerability of freshwater fish of the Cape Floristic Region with respect to climate change. This dissertation focussed on the experimental component of the broader project for the Western Cape study. To better understand the impacts of climate change on the fish of the CFR, experiments were undertaken to determine thermal limits (via the Critical Thermal Method) and thermal preferences (via acute gradient tank experiments). These methods have been used before to elucidate the thermal sensitivity and preferred temperature of wild populations. Native species from the main freshwater fish families in the CFR that are listed by the IUCN as vulnerable or endangered were selected for experimentation (species are listed in Chapter 2, Table 2.1).

To disseminate the findings of this research, the dissertation is structured as follows:

- A review of previous methodologies for the determination of thermal tolerance and preference, and a description of the method adapted for this study (Chapter 2).
- The first data chapter (Chapter 3) aims to compare thermal tolerances of seven native fish in the CFR and discusses thermal limits in light of climate change.
- The second data chapter (Chapter 4) aims to compare the thermal preferences of five native fish and relates this data to thermal tolerance data for the determination of thermal sensitivity.
- The thesis concludes with a brief synthesis of the findings, and recommendations for future research (Chapter 5).

CHAPTER 2: A methodology for thermal experiments

2.1 Introduction

The complex nature of habitat selection and physiological plasticity in animals confounds attempts to design experiments in which an organism’s temperature requirements are isolated (Beitinger and Fitzpatick 1979). Moreover, acclimation state, age, and body size of an organism have been shown to influence parameters such as thermal tolerance and preference (Reynolds, 1977; McCauley and Huggins, 1979; Kita et al., 1996; Sogard and Olla, 1996; Despatie et al., 2001; Golovanov, 2006). Due to these inconsistencies, experimental methodologies for the determination of thermal tolerance and preference have remained largely unstandardised. Notwithstanding differences in the experimental approach, experimental data are still more appropriate for characterizing a species’ thermal niche as compared to field data (Todd et al., 2008). Field data have been used in the past to determine thermal tolerance of fish, but these data do not account for the ability of fish to detect minute changes in temperature and thus seek out cooler conditions thereby avoiding stress (Eaton et al., 1995). Furthermore, field data are confounded by biotic interactions: for instance the effect of food resources or predation on the location, abundance, and temperature selection of prey species. In this regard, laboratory experiments facilitate controlled exposure and the assessment of biological responses that are more accurate (Todd et al., 2008). So, to understand the effect of temperature fluctuation on a species, controlled experiments that isolate a single parameter are required. Moreover, there are no alternatives to using live animals to determine these parameters (Lutterschmidt and Hutchison, 1997), necessitating the standardisation of safe experimental protocol involving endangered species.

Dallas and Ross-Gillespie (2015) refer to thermal experiments as the toolbox within which lie the instruments to ‘measure’ thermal sensitivity of organisms; this chapter describes two such tools and their application to this study. Biological temperature thresholds/resistance (one), and optimal temperature (two) are completely different parameters, both of which are equally important to understand the ecological significance of temperature-mediated effects in freshwater ecosystems. The following is a review of the theory behind each of these experimental methods.

2.1.1 Thermal tolerance

Thermal tolerance refers to the sensitivity of an organism to temperature extremes (both upper and lower limits). An indicator of thermal tolerance is the upper thermal limit, also referred to as the Critical Thermal Maximum (CT_{max}), is expressed as a temperature threshold in degrees Celsius (ºC). To determine CT_{max}, the critical thermal method is used; this involves placing fish in a water bath where temperature is increased at a constant rate until an end-point (CT_{max}) is reached. This method is non-destructive to the organism as the end point of the experiment is the temperature at which the first sign of thermal stress is observed (Paladino et al., 1980). Thermal stress is indicated by a specific behaviour, usually associated with a species. Thermal maxima have been studied extensively for a wide range of aquatic taxa, and have been shown to provide an ecologically useful indication of thermal sensitivity to elevated water temperature (Paladino et al., 1980; Dallas and Rivers-Moore, 2012).

With reference to thermal stress, Beitinger (1990) notes that behavioral reactions of fish serve as biomarkers of stress. These bio-markers are measureable, and have evolved as an adaptive feature which contributes to the evolutionary fitness of the species (Beitinger, 1990). The loss of ability to remain upright, followed by the onset of muscular spasms (or tremors) is indicative of this stress marker, either of which are widely used as experimental end-points for fish (Becker and Genoway, 1979; Luttershmidt and Hutchinson, 1997). Once the behavior/bio-marker is shown, the organism is removed from the experiment to recover. If the organism recovers, the temperature at which it was removed is recorded as the CT_{max} for that individual (Dallas and Ketley, 2011).

For the behavioural biomarker to be visible, an organism will have approached its physiological tolerance limit. Since body temperature is environmentally determined for ectotherms, fish seek out microhabitats in which water temperature is most favourable for their internal body temperature, thereby optimizing the metabolic rate. In the absence of suitable thermal refugia, the most sensitive tissues dictate the thermal limit of the organism (given that behavioural thermoregulation becomes impossible). Brett (1956) found that nerve tissues of fish showed signs of inactivation before that of the other somatic tissues, suggesting that the most sensitive tissues are those of the central nervous system. Damage to these tissues in fish is what results in the loss of ability to remain upright (LRR), followed by the onset of muscular spasms (OS) (Becker and Genoway, 1979; Luttershmidt and Hutchinson, 1997). Beyond these bio-markers, damage to the nerve tissues and muscle are too severe to recover.

There are other methods to determine thermal sensitivity which do not rely on bio-markers as end points. The Incipient Lethal Temperature (ILT) method is used to elucidate Incipient Upper Lethal Temperature (IULT)/ LT_{50} which is the temperature survived by 50% of the population for 96 h (Beitinger and Bennet, 2000; Dallas, 2016). In the CFR both CTM and ILT methods have been used to elucidate the effect of acute and chronic exposure on freshwater invertebrates (Dallas and Ketley, 2011; Dallas and Rivers-Moore, 2012; Dallas, 2016). IULT is more appropriate when determining the effect of chronic exposure as it is used to determine the Maximum Weekly Allowable Temperature (MWAT) of a freshwater ecosystem (Dallas, 2016), but this method is based on lethality and this is not ethical with respect to endangered species.

There are additional reasons which promote the use of CT_{max} instead of IULT. Firstly, CTM data are easy to compare with simple statistics (Beitinger et al., 2000). Furthermore, a much smaller sample size is required for the CTM as compared to ILT. It is also important to note here that there is a subtle but clear difference between the terminology and underpinnings of the IULT and CT_{max}: IULT is not an equivalent to CT_{max} (Becker and Genoway, 1979), as IULT is an indication of thermal resistance, while CT_{max} is an indication of thermal tolerance (Beitinger et al., 2000). Prior to any commencement of experiments, it must be clear which data are being obtained and why, as this will inform which method is opted for. Generally, where species abundance is high (such as with macroinvertebrates), ILT is more useful. However, when sample sizes are small, abundance is low, and conservation status is critical, the CTM is more appropriate.

Luttershmidt and Hutchinson (1997) suggest that there are four criteria which are necessary to satisfy the experimental design of the critical thermal method. These are (1) that experimental subjects should be small and uniform in size, (2) that the loss of ability to maintain an upright should be interpreted as the endpoint of the experiment, (3) that death temperatures should be included as the lethal thermal maximum, and (4) that there should be linearity in the rate of temperature change.

While common protocol and criteria are necessary for the comparison of data, there are pitfalls and limitations to the suggestions mentioned. First, and most importantly, lethal temperatures may not be attainable; especially when handling endangered species. The rate of change and end-point of the experiment however can and must be consistent with the criteria. Mora and Maya (2006) suggest that if the rate of temperature change is too rapid, the lag time between body and water temperature result in an overestimation of CT_{max}. Similarly, a rate that is too gradual may result in an underestimation of CT_{max} as re-acclimation may occur (Beers and Sidell, 2011). To this, Becker and Genoway (1979) found that a change of 1°C/minute was too high to mimic internal body temperature, while a rate of 0.3°C/minute was sufficient to avoid an acclimation effect. Using this 0.3°C/minute rate of change, the endpoint should be the loss of righting response which occurs prior to the onset of spasms (Becker and Genoway, 1979). For endangered fish, this minimizes stress while still producing valid data. Finally, concerning the size and life stage of fish, CT_{max} significantly differed between juveniles and adults for the Angelfish, *Pterophyllum scalare* (Perez et al., 2003). To negate this effect of ontogeny, size should be standardised, and no young of year or old individuals should be used.

Other causes of methodological inconsistency are the number, duration, and temperature of acclimation treatments. These protocols differ significantly between studies, and may have a considerable effect on CT_{max}. Previous studies on marine and freshwater fish provide support for the use of at least one or more acclimation treatments (Deacon et al., 1987; Richardson et al., 1994; Kita et al., 1996; Diaz and Buckle, 1999; Perez et al., 2003), over a period of 24 hours to 30 days (Richardson et al., 1994; Perez et al., 2003). As opposed to cold-adapted species, fish that inhabit tropical or subtropical environments are able to tolerate more abrupt changes in temperature (given that they experience their thermal extremes over a short period of time) (Perez et al., 2003). Theoretically, warm-adapted fish require less acclimation time than those that are evolutionarily cold-adapted. However Reynolds and Casterlin, (1979) suggest that acclimation is not a requirement if fish are used immediately upon collection and reliable habitat temperature data is available.

2.1.2 Thermal preference

Another parameter for consideration is thermal preference. Neill (1979) defines thermal preference as the behavioural avoidance of temperatures that are unfavourable to an organisms ‘life processes’. Since fish seek out temperatures that match the most efficient operation of their metabolism (Richardson et al., 1994), behavioural thermoregulation ensures that an organism is able to maximize its time spent in favourable thermal habitats (Neill, 1979; Kelsch and Neill, 1990). This thermoregulatory behaviour can either be predictive, or reactive. The former will result in the fish moving into an alternative microhabitat in search of a more suitable temperature based on individual experience or evolutionary instinct. For the latter, the fish will move in an undirected manner until the net movement becomes the thermal preferendum; a behaviour also influenced by recent thermal experience of the individual (Neill, 1979). It is this exploratory behaviour that lead to Jobling (1981) adapting the concept of the ‘final preferendum zone’ rather than a distinct temperature at which fitness is optimised.

There are two ways in which preference can be determined experimentally; viz. acute and gravitational methods, both of which involve the use of gradient tanks to create a heterothermal environment. Thomas et al., (1963) presented some of the first methodology addressing the use of gradient tanks to determine the preferred temperature of small aquatic organisms. When using the acute method, fish are placed in a gradient tank and ‘preferred’ and ‘avoided’ temperatures are observed and recorded at predetermined time intervals (e.g. every 10 minutes). Since the acute method is influenced by thermal history (acclimation), this should last no longer than two hours to avoid re-acclimation (Reynolds and Casterlin, 1979). Gradient tanks are typically 2-4m long, with a range of at least 8ºC (McCauley and Huggins, 1979; Deacon et al., 1987; Sogard and Olla, 1996; Diaz and Buckle, 1999; Perez et al., 2003). The gradient tank can be split into thermal sections depending on the range determined by field information for that species (Diaz and Buckle, 1999). The thermal section that has the highest percentage frequency of fish over time is interpreted as the thermal preference of the group (Richardson et al., 1994). Unlike the acute method, measured less than two hours after placement in the gradient, the gravitational method is independent of acclimation. If fish are left for a sufficient period of time (usually 24-96 hours); they will re-acclimate and gravitate towards the final preferendum (Jobling, 1981). This final preferendum is the temperature at which the acclimation temperature and thermal preference are equal, and points to the temperature that would ultimately be favoured by the species regardless of age or seasonality (Reynolds and Casterlin, 1979). For climate change research (such as the current study), preference determined via the acute approach would be favourable, as these studies are concerned with the effect of temperature over the summer extremes when flow is reduced and water temperature is at its peak.

Group sizes used per replicate (i.e. each trial of a thermal preference experiment) vary considerably between studies (ranging from 2-30 individuals) (Deacon et al., 1987; Richardson et al., 1994; Despatie et al., 2001; Perez et al., 2003). In a heterothermal environment, the behaviour and position of a school may be influenced by temperature (Ferguson, 1958; Niwa, 1998). This complicates the interpretation of experimental data with respect to preferenda and warrants preliminary studies on the species to rule out its effect. While Richardson et al., (1994) used fish densities observed in the field as a guide for experimental group size in the laboratory; assumptions of this kind can be false. In a computer simulation model, Viscido et al., (2005) showed almost no influence of neighbour-fish with less than four fish, but that 8-16 fish were required to maintain a cohesive school in the computer simulated species (Viscido et al., 2005). Thus, finding the minimum number of fish where schooling is induced is not straightforward and requires complex mathematical models, field data, and preliminary tests (Niwa, 2004). It can be concluded that schooling may influence the thermal preference of fish, but that behavioural observations and preliminary experiments of various group sizes (from one to ten or more) are required to quantify this effect if any.

2.2 Aims

In South Africa, thermal experiments of this nature (especially those using the thermal preference approach) have rarely been attempted in the field given the lack of appropriate protocol and the technical expertise required to develop them. These data however, are necessary to recommend temperature regimes for waterways (Richardson et al., 1994). This chapter describes a robust and complete study design for determining thermal tolerance of native fish in the field (from collection to release). A methodology for the determination of preferred temperature is also presented. These methodologies have been tailored to field studies in South Africa.

2.3 Methodology

2.3.1 Selection and description of sites

Selection of sites was based on species presence within the CFR, accessibility, availability of hourly water temperature data, and alignment with other components within the overall project. The rivers selected were the Amandel, Berg, Driehoeks, and Rondegat Rivers.

Description of study region and sites

The 87 900 km^2 CFR is a Mediterranean region stretching from the south-western to the south-eastern region of South Africa (33-35ºS and 18-22ºE) (Cowling et al., 2003). The region experiences cool wet winters, and dry hot summers. During summer, the greatest extremes in diurnal air temperature are experienced, but winter air temperature is considerably cooler with 66% of the mean average rainfall (>1 200mm) received between April to September (Chase and Meadows, 2007). The significant increase in winter rainfall is responsible for intense fluvial action over this season, followed by a summer drying event- thus shaping the spatial and temporal dynamics of riverine biota (Gasith and Resh, 1999).

Mediterranean regions are typically dominated by steep topographical relief, influencing the physical features and hydrology of their rivers (Gasith and Resh, 1999). In the CFR, the Cape Fold Belt (CFB) provides this relief in the form of steep anticlinal mountain ranges (Day et al., 1979) (444-903masl from this study). The sandstone geology of the CFB influences water chemistry as mountain streams in the region are naturally oligotrophic due to the leaching of nutrient-poor sandstone (Day et al., 1979). Mountain streams are also typically clear-brown in colour due to the humic substances leached from decaying Fynbos (the regions predominant vegetation type) (Day et al., 1979). Humic substances, or tannins, cause a low pH and TDS (<0.6) relative to other rivers in South Africa (Davies and Day, 1998).

In the CFR, mountain streams are less disturbed, but water quality and quantity in lower reaches are affected by anthropogenic disturbance to varying degrees. Descriptions of physical habitat, various indicators of water quality, and the current greatest threats for each of the sites are listed in Table 2.1. These results are based on State of Rivers Reports, using the grading system from those reports: Poor, Fair, Good, and Natural (River Health Programme, 2004; River Health Programme, 2006; River Health Programme, 2011).

Table 2.1*:* Site descriptions and major threats (**IHI**= Index of Habitat Integrity, **GI**= Geo-morphological Index, **RVI**= Riparian Vegetation Index, **SASS**= South African Scoring System, **FAII**= Fish Assemblage Integrity Index, **WQ**= Water Quality, **EI & SR**= Ecological Importance and Sensitivity Rating , **ES**= Eco-status)

River	**Physical Habitat**			**Water Quality and Ecological Sensitivity**					**Major Threats***
	IHI	GI	RVI	SASS	FAII	WQ	EI&SR	ES	
Amandel	Good-Fair	Good	Good	Good	Good	N/A	Importance: Very High Sensitivity: High	Good	Abstraction downstream, invasion
Berg (upper)	Natural-Fair	Natural	Poor	Natural	Good	Natural	N/A	N/A	Pollution, degradation, alien vegetation, inter-basin transfer, river modification (all downstream of site)
Driehoeks	Good	Good	Fair	Good	Good	Good	Importance: Very High Sensitivity: High	Good	Invasion (predation)
Rondegat	Natural	Fair	Good	Natural	Natural	Natural	Importance: Very high Sensitivity: Very High	Good	Invasion

**Physical habitat, water quality, ecological sensitivity, and major threats according to the River Health Program: State of Rivers reports (2004-2011)*

2.3.2 Selection and description of species

Target species were selected from four major endemic fish families in the CFR: Anabantidae, Austroglanidae Cyprinidae, and Galaxiidae (Figure 2.1, Table 2.2). From the family Anabantidae, *Sandelia capensis* was selected from the Amandel and Berg Rivers. From the Austroglanidae, *Austroglanis gilli* was selected from the Rondegat River. *Pseudobarbus burchelli*, *Pseudobarbus burgi*, *Pseudobarbus calidus*, and *Pseudobarbus phlegethon* (family Cyprinidae) were selected from the Amandel, Berg, Rondegat, and Driehoeks Rivers respectively. *Galaxias zebratus* (family Galaxiidae) was sourced from two populations in the Driehoeks River. Geographical coordinates of the collection sites are provided in Table 2.2.

Before the study commenced, ethical approval was obtained from the CapeNature and University of Cape Town Animal Ethics Committee (2015) for collection and experimentation (30-50 individuals per experiment). Presence of the desired species were verified by literature, personal communications, and pilot studies. For ethical reasons, the conservation status and the population size of the species were also taken into account prior to collection (For the conservation statuses of each species refer to Table 2.2).

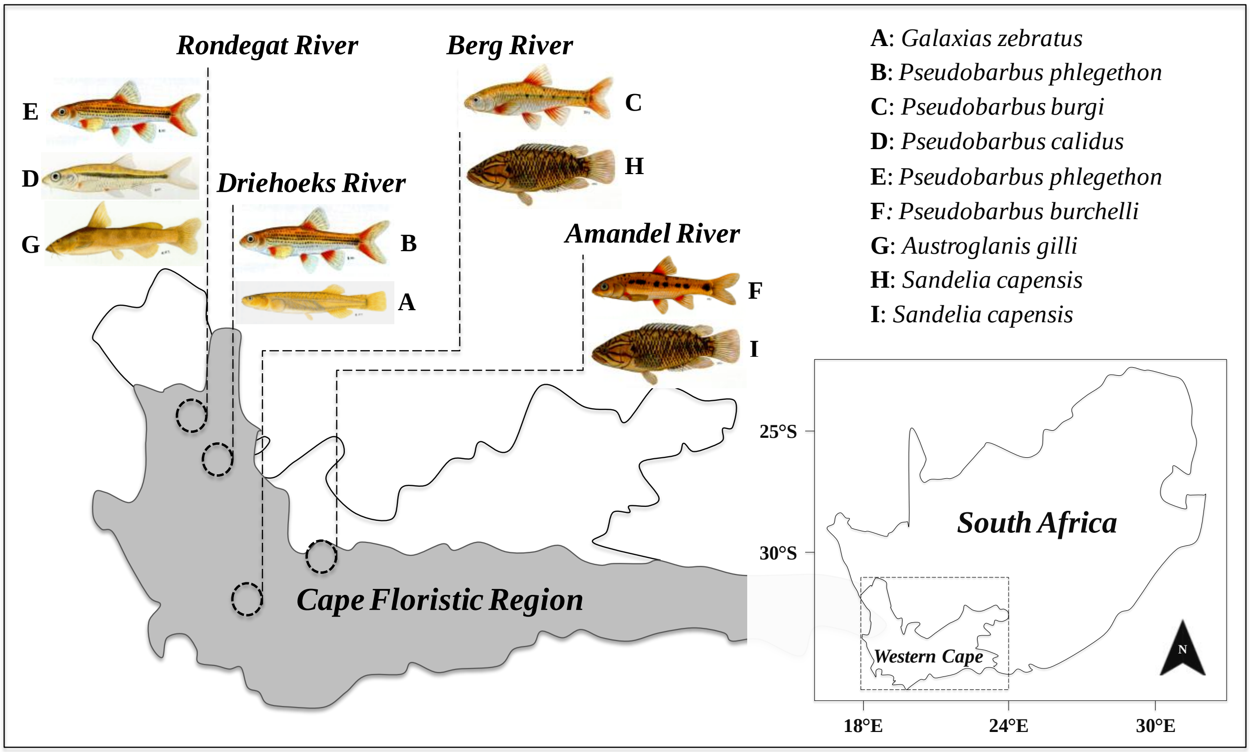


Figure 2.1: Map showing geographical location of study sites and selection of species within the Cape Floristic Region (*fish images courtesy of SAIAB*)

Table 2.2: Source location and experiments undertaken for each species

Scientific name	Common name	River/Source	Catchment	Coordinates of collection sites	Altitude (masl)	Conservation status*	Experiments	
							Tolerance	Preference
Austroglanis gilli Barnard (1943)	Clanwilliam rock-catfish	Rondegat	Olifants/Doring	-32.362955, 19.044911	452	Vulnerable	x	x
Pseudobarbus calidus Barnard (1938)	Clanwilliam Redfin	Rondegat	Olifants/Doring	-32.362955, 19.044911	452	Endangered	x	x
Galaxias zebratus Castelnau (1861)	Cape Galaxias	Driehoeks (1)	Olifants/Doring	-32.430056, 19.175561	909	Near Threatened	x	x
		Driehoeks (2)		-32.431450, 19.148493	903			
Pseudobarbus burchelli Smith (1841)	Breede River Redfin	Amandel	Breede	-33.503815,19.486673	444	Endangered	x	x
Pseudobarbus burgi Boulinger (1911)	Berg River Redfin	Upper Berg	Berg	-33.955372, 19.073266	268	Critically Endangered	x	x
Pseudobarbus phlegethon (Doring Lineage) Barnard (1938)	Fiery Redfin	Rondegat	Olifants/Doring	-32.375680, 19.065614	520	Endangered	x	
Pseudobarbus phlegethon *(*Olifants Lineage) Barnard (1938)	Fiery Redfin	Driehoeks	Olifants/Doring	-32.434625, 19.180826	906	Endangered	x	
Sandelia capensis Cuvier (1831)	Cape Kurper	Amandel	Breede	-33.504439, 19.487910	448	Data Deficient	x	x
		Upper Berg	Berg	-33.955372, 19.073266	268			

**Conservation status according to Darwall et al., (2009) IUCN Red List of threatened species*

2.3.3 Collection and husbandry of fish

Pilot studies were conducted in May 2015 to determine the suitability of sites and species for collection and experimentation respectively. Fieldwork and experiments were undertaken during spring and summer low-flows: September-December 2015. Individuals were collected using 4mm-mesh fyke nets set overnight on the night before experiments were run, as well as with hand nets on the day the experiments were run. The nets were emptied between 8am and 10am daily depending on accessibility to sites, and fish were transferred to aerated buckets containing river water at stream temperature (portable air compressors provided sufficient oxygen for each bucket). These buckets were transported in cooler-boxes to the experimental location (transportation time varied from 30 minutes to one hour depending on the distance from the experimental location). River water from each site was used in the experiments, and replenished daily to prevent deteriorating water quality and disease. Following the completion of each experiment, fish were kept overnight in aerated buckets at stream temperature in a dark room to recover before being released at the collection site the following day. Fish were identified by visual inspection in the field and in the lab individuals were further identified under a dissecting microscope using Skelton (2001). The IUCN status and experiments undertaken for each species are listed in Table 2.2.

2.3.4 Acquisition of water temperature data

Hourly water temperature data were recorded in the Amandel and Berg Rivers for a one-year period encompassing the experimental period (2015-2016). These data were recorded on pre-programmed waterproof Hobo UTB1-001 TidBit V2 loggers (Onset Computer Corporation, 2008) and secured in pools where fish were present. These loggers were placed in the thalweg of the pools at depths less than 1m. According to Dallas and Rivers-Moore (2011), thermal stratification only occurs at depths greater than 1m. For the Driehoeks River, data from, 2005-2006 (Paxton, 2008) were used, while for the Rondegat River, data from 2010 were used (Dallas and Rivers-Moore, 2012). Use of data collected prior to this study (2015 -2016) assumes stationarity of water temperature data from one year to another. These data were obtained to characterise the rivers in terms of their thermal signatures (i.e. annual fluctuation in temperature). This allows for the interpretation of thermal tolerance and preference with respect to habitat temperature.

One week prior to the commencement of experiments, Dallas iButtons were installed at the Driehoeks and Rondegat sites to ensure that the recent thermal histories of the fish were known for all sites.

To differentiate between the thermal histories of the species, each river's thermal signature was expressed in terms of annual thermal metrics (indicators of thermal alteration). The metrics used for disaggregating thermal time series data are represented in Table 2.3. The indicators of thermal alteration methodology of Rivers-Moore et al., (2012) were used to calculate these metrics. These analyses are further explained in Chapter 3.

Table 2.3: Temperature metrics for disaggregating thermal time series (from Rivers-Moore et al., 2012).

Annual descriptive statistics		Mean + Std. Dev. of annual temperature Annual coefficient of Variability (% CV) Predictability (Colwell 1974) Mean of daily range Mean of annual minima, maxima Degree days (annual)
Group 1	Monthly magnitudes	Jan – Dec mean, minimum and maximum temperatures
Group 2	Magnitude and duration of annual extreme water temperature conditions	7-day mean 7, 30 & 90-day minima 7, 30 & 90-day maxima Mean daily minimum Maximum daily range
Group 3	Timing - Julian date of maximum and minimum metrics (thermal triggers)	Date of onset of longest exceedance of minimum threshold Date of onset of longest exceedance of maximum threshold
Group 4	Frequency and duration (successive days of event above or below a threshold)	Minimum temperature threshold (count and duration) Maximum temperature threshold (count and duration)

2.3.5 Thermal tolerance experiment

Fish were placed in a room at the experimental facility (field laboratory) at stream temperature for 24 hours prior to commencement of the experiment. This was to minimize stress on the fish. For the duration of the holding period, the fish were not fed. The holding temperature ranged from 18.0-24.5ºC depending on the location and the local air temperature on the day of the experiment (Hobo^{TM} V2 temperature loggers were used to record water temperature during this period). Holding temperatures relative to stream temperature are presented in Appendix A. These temperatures were all between the rivers' daily mean and daily maximum temperatures recorded during the week when experiments were conducted. Of note are the comparatively cool ambient temperature for one of the days at the Driehoeks and Rondegat sites, where holding temperature exceeded daily maximum temperature for one day each. In all cases, however, the ambient holding temperature was correlated with the mean daily stream temperature (correlation co-efficient = 0.83) and seldom exceeded the maximum daily stream temperature. In this sense, the experiment allowed for acclimatization whereby the organism responds to their natural climate or environment, rather than acclimation to a fixed, consistent temperature attainable in a temperature control environment.Natural light was maintained throughout the experimental period.

After the holding period, three to six fish were placed in a mesh basket submerged in a water bath (Figure 2.2) fitted with a circulating heater (Julabo^{TM}). Three to six fish were selected per trial. For smaller (<11cm total length) and less active species no more than six adult fish were selected for one trial, whereas for larger (>11cm total length) and/or more active species, up to three adult fish were used per trial. After a 30 minute control period, where temperature in the water bath equaled holding temperature, the temperature was increased at a constant rate of 0.3°C/min (Perez et al., 2003). When an individual showed signs of thermal stress (i.e the biomarker), it was removed from the water bath and placed in an aerated recovery chamber at the holding temperature. These biomarkers were determined during the pilot study using three individuals of each species. Following recovery, each individual was measured to one decimal place (Total Length (TL), cm). Both length and CT_{max} were recorded (see Appendix D). Trials were repeated until the CT_{max} had been determined for all the individuals. Where possible, a total of 30 individuals were used for the experiment.

Beers and Sidell (2011) identified a period of hyperactivity prior to loss of righting response. This was interpreted as the point of thermal reactivity (PTR) (Dallas and Ketley, 2011), and these behaviours were also recorded for each species.

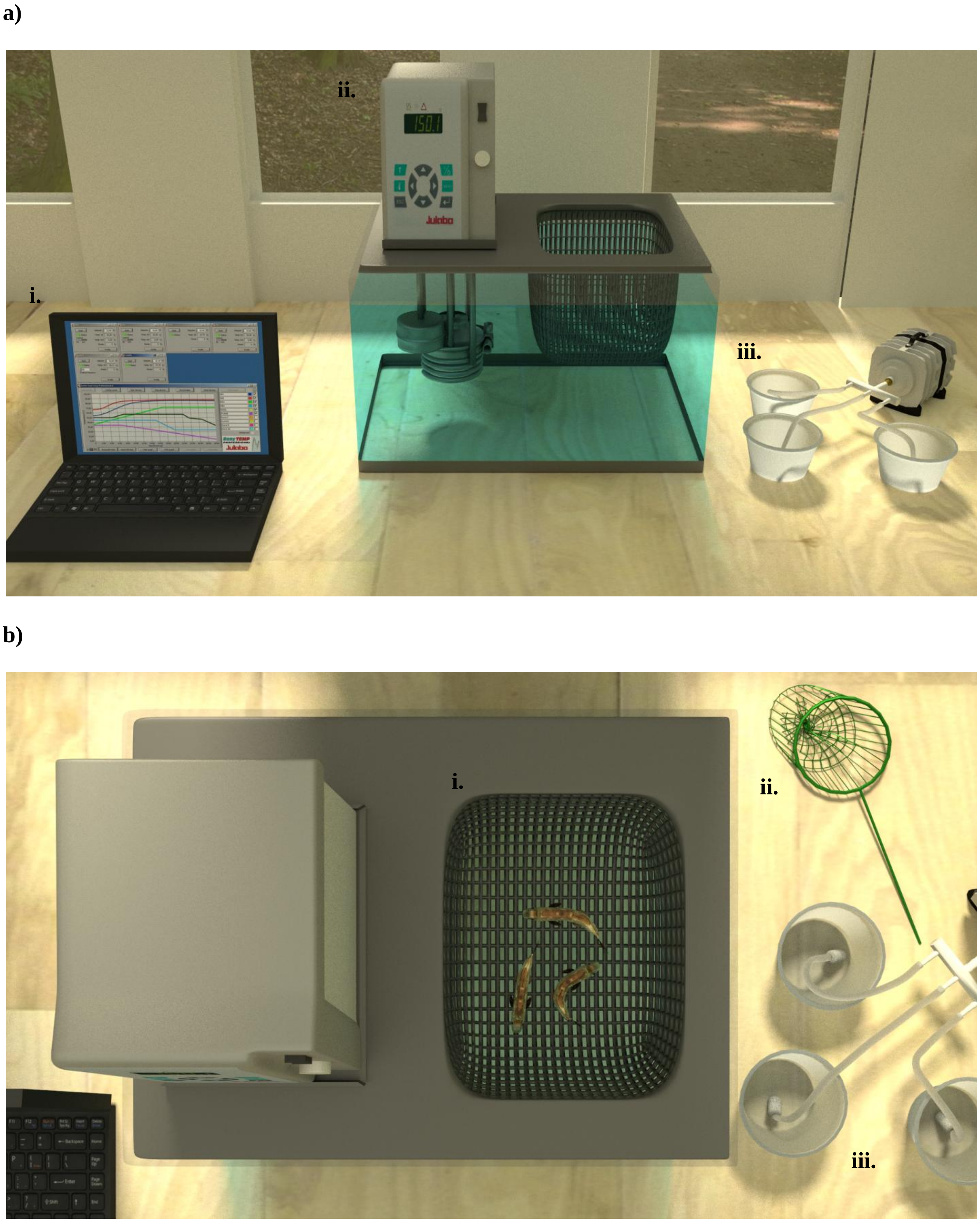


Figure 2.2: Diagram showing **a**) the experimental setup for the Critical Thermal Method **i**. Laptop with Julabo software to control temperature **ii**. Julabo circulating heater connected to laptop **iii**. Recovery

chambers, and **b**) a CTM experiment underway **i**. Mesh basket in which fish are placed **ii**. Net used to remove fish when biomarker observed **iii**. Recovery chambers supplied with oxygen and filled with cold water

2.3.6 Thermal preference experiment

For each species of fish, 10-30 individuals were placed in the gradient tanks upon collection (Figure 2.3), and allowed 30 minutes to acclimatize with no thermal gradient. Fish were not exposed to an acclimation period on the assumption that they were acclimatized to the ambient stream thermal regime. The thermal gradient tanks, designed by Dallas et al., (2015) were constructed from PVC piping (3m length, 125mm width, 87mm height). The tanks were filled with river water to achieve 60mm depth, and each tank was fitted with perforated tubing attached to an air compressor and secured to the bottom of the tank, that aerated the water along the length of the tank. This ensured that a dissolved oxygen (DO) concentration of 75-85% was maintained throughout the tank (this was tested with a Crison multimeter in the laboratory and a Hanna multimeter in the field laboratory). The maintenance of this concentration was important to negate effect of decreased DO at the warm pole, thereby removing the influence from temperature. The tanks were fitted with 100 Watt heaters at the warm pole, and at the cold pole, copper coils fed with cold water (circulated through a freezer) via a pump system, were inserted into the water. Mesh barriers were fitted between the heaters and coolers to prevent the fish from coming into contact with the apparatus. The net length of the tank was 2.25m, which was subdivided into ten 25cm sections. Throughout the experiment, Hobo^{TM} Tidbit V2 loggers recorded temperature at ten-minute intervals; one logger per section. In the control tanks, one logger was placed in every second section (on the assumption that temperature was constant throughout). After the initiation of the experiment, a 60% shade cloth was used to cover the apparatus. This was used to add habitat/structural complexity (i.e cover to which the fish are accustomed in the wild) and to minimize the stress on the fish. The cover also ensured that fish were not seeking out the edges of the tank for cover. The mesh size was also suitable for visual clarity.

Following the introduction phase of 30 minutes, the experiment was initiated by switching on the heating and cooling systems (establishment phase). The gradient was gradually established over three hours, after which, a two hour experimental period followed (experimental phase). The tanks were checked at 10 minute intervals during the establishment and experimental phase. For each observation, the number/frequency of fish in each 25cm thermal section was recorded. Temperature in each section was recorded every 10 minutes with pre-programmed Hobo Tidbit V2 temperature loggers. In total 31 observations were made from the start to the end of the experiment. To determine thermal preference, three treatment tanks (A-C) were paired with three control tanks (D-F). The use of control tanks was to discount stress effects on fish. In the control tanks, temperature remained constant for the duration of the experiment. These controls were used to evaluate mortalities associated with experimental conditions. In the treatment tanks, a thermal gradient of ±15 to 30ºC was established. Each experiment consisted of three trials. The experiment was replicated three times where possible to increase the total number of trials to nine.

The experimental apparatus and setup are shown in Figure 2.3.

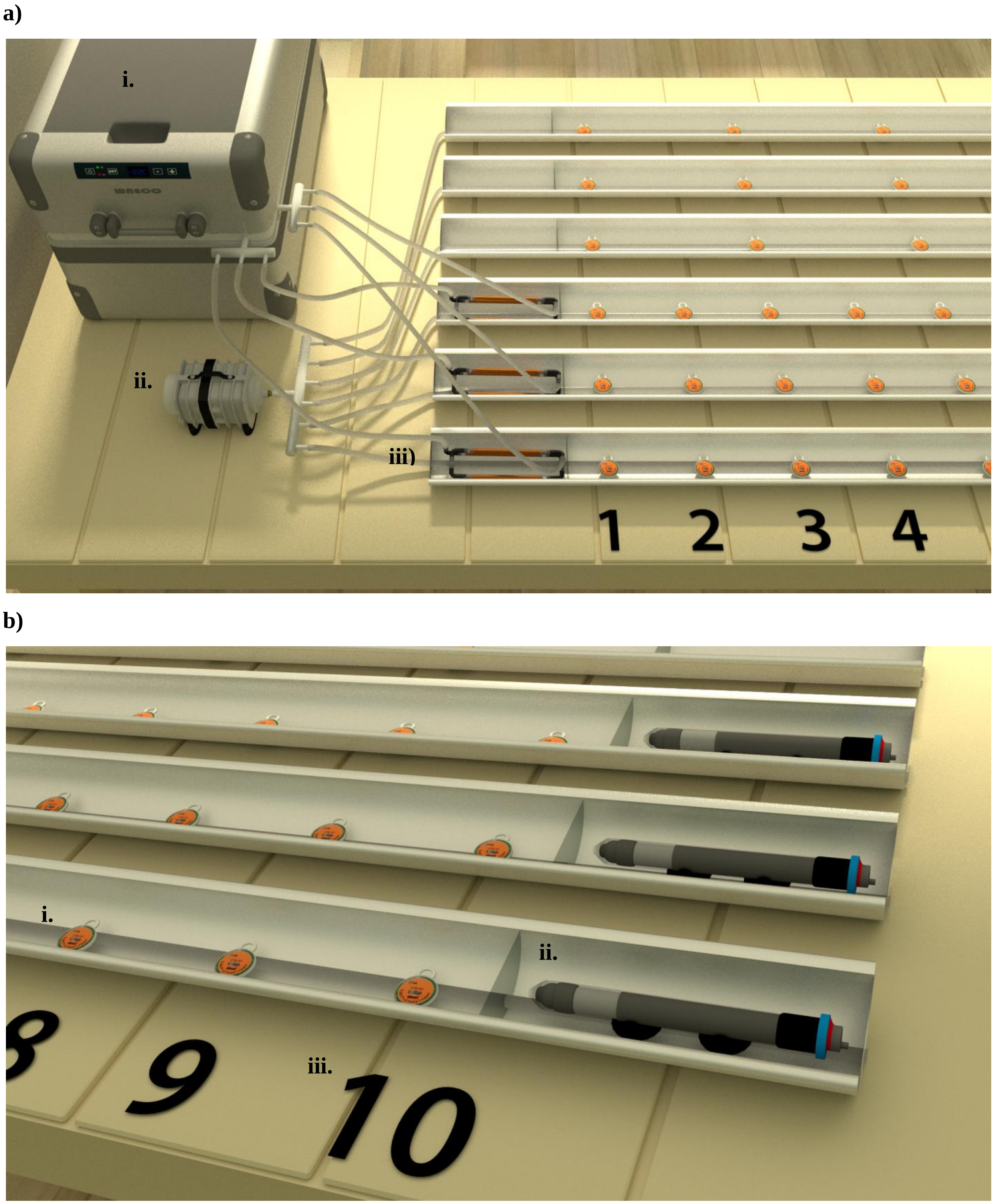


Figure 2.3: Diagram showing **a**) the experimental setup for the cold pole of the thermal preference experiment **i**. Cooler with pump inserted to supply cold water to copper coil **ii**. Pipes along the length of the apparatus supplied with oxygen from air compressor **iii**. Copper coil fed with cold water inserted at

cold pole to maintain cold temperature, and **b**) the warm pole **i**. Temperature loggers **ii**. Heaters used to maintain temperature at the warm pole **iii**. Numbers denoting the thermal section (1-10) for observations

2.3.7 Statistical analyses

Analyses of experimental data were performed in R version 3.2.3 (2015-12-10) - "Wooden Christmas-Tree" Copyright (C) 2015 The R Foundation for Statistical Computing. The following packages were installed for the analyses: ‘doBy’, ‘phsych’, and ‘lattice’. For detailed descriptions of analyses, refer to the relevant methodologies in Chapter 3, and Chapter 4.

CHAPTER 3: Thermal tolerances of select native fish in the Cape Floristic Region

3.1 Introduction

The term ‘Critical Thermal Maximum’ was first used by Cowles and Bogert (1944) to define the measurement of sublethal thermal maxima (Beitinger et al., 2000). The Critical Thermal Maximum (CT_{max}) is the upper temperature at which physiological signs of heat stress are observed in animals. It provides an estimate of thermal tolerance, and is the point at which behavioral thermoregulation is impeded and the tissues of the organism begin to respond negatively (Paladino et al., 1980). If exposure to CT_{max} is prolonged, heat stress is sustained by the animal and this may results in mortality. In fish, visible physiological symptoms of heat stress are generally a loss of righting response followed by the onset of muscular spasms with subtle differences between species (Lutterschmidt and Hutchison, 1997). Experiments in the laboratory allow for the determination of this threshold, with the thermal stress being short-lived. This can be beneficial to the organism as it induces heat hardening (Beitinger et al., 2000). In nature however, if thermal maxima are exceeded, organisms are not expected to recover. At both the cellular and molecular level, heat-shock proteins and tolerance-genes prohibit recovery beyond the species’ threshold (Iwama et al., 1999; Fangue et al., 2006), while at the organismal level damage to the central nervous systems, muscular tissues and vital organs are the lethal factors (Sänger, 1993; Eliason et al., 2011). Significant mortalities have been documented in migratory populations confronted with thermal barriers (Eliason et al., 2011), and restricted populations experiencing thermal pollution from industry (Coutant and Brook, 1970).

The experimental determination of thermal tolerance allows for an evaluation of thermal sensitivity and is thus useful for understanding the potential effects of regional climate warming on native fish. Using a fish temperature database for the United States of America, Eaton et al., (1995) found that field-information-based systems for estimating thermal tolerance often underestimated thermal limits. This necessitates the acquisition of experimental data which are more reliable as a physiological limit (Lutterschmidt and Hutchison, 1997). Much of the primary experimental data on thermal tolerance of fish from the northern Hemisphere were acquired prior to the 1960s (Heino et al., 2009), however, thermal tolerance data are not available for South African fish. Such data are particularly important given the predictions of global climate change on the freshwater ecosystems of the CFR (Dallas and Rivers-Moore, 2014). Ranking thermal sensitivities of native CFR fish will support more focused conservation on the most vulnerable species, particularly in Freshwater Ecosystem Priority Areas (FEPAs; Nel et al., 2011). This will also assist toward revision of the IUCN status of native fish.

3.2 Aims and Hypotheses

The aims of this chapter were to:

- Experimentally determine and compare the upper thermal limits of select native fish in the Cape Floristic Region using the Critical Thermal Method.
- Identify thermally sensitive species within the context of regional climate change.
- Provide experimental data on thermal tolerance to assist with the establishment of water temperature criteria.

The main hypotheses were:

- Upper thermal limits were expected to differ amongst species.
- Signs of thermal stress were expected to differ between the species.

3.3 Methodology

3.3.1 Water temperature and indicators of thermal alteration

Using one year of hourly water temperature data for each site, annual temperature metrics were calculated using the Indicators of Thermal Alteration (ITA) approach (Table 2.3) (Rivers-Moore et al., 2012; Rivers-Moore et al., 2013). ITA were used to characterise and compare thermal signatures of the four rivers by converting sub-daily water temperature data to daily data (mean, minimum, maximum and range). From these data, thermal metrics may be calculated to describe water temperatures with respect to magnitude and duration of thermal events. In so doing, the fishes thermal histories could be correlated to the experimental parameters.

Seven-Day moving averages (i.e. moving average of temperature of the seven preceding days) of daily mean, minimum and maximum temperatures were generated from hourly water temperature data for each river. In addition, the magnitude and duration of annual extreme water temperature conditions were calculated, where the Mean_7 is the maximum 7-day moving average of mean temperatures over a one-year period. Similarly, the Max_7 is the maximum 7-day moving average of maximum temperatures, and the Min_7 is the minimum 7-day moving average of minimum temperatures, over a one-year period. This allows for one to establish the most extreme period of thermal alteration (be it a 7, 30 or 90-day period) over an annual cycle.

From annual data, monthly thermal metrics were also calculated using the ITA method. However, to derive metrics for the week prior to experiments, mean, maximum and minimum water temperature (ºC) were calculated from logger data for seven days prior to the last catch.

3.3.2 Comparison of upper thermal limits amongst species, genera, and sites

The CTM (see Chapter 2) was applied to the target species (refer to Table 2.2), to estimate upper thermal limits and evaluate interspecific differences in thermal maxima. Differences in upper thermal limits amongst species within a genus, and within a species amongst sites were also examined. A total of 30 individuals were used where possible (sample sizes are indicated in Table 3.2) and the median CT_{max} was calculated for each species. The data were tested for normality using Shapiro-Wilk and Kolmogerov-Smirnov normality tests. Both tests showed the assumptions for parametric tests were violated, and thus a Kruskal-Wallis (non-parametric equivalent of a one-way Analysis of Variance) was used to compare CT_{max} between species, genera, and sites.

3.3.3 Methodological standards: experimental endpoint and sample size

To determine an appropriate sample size and behavioural end point, preliminary experiments were carried out on the selected species. Sample size varied between experiments (from three to six), and the final sample sized was informed by visual clarity (observation of stress response for all the individuals), the size of the fish, and the level of stress shown in the experimental apparatus (Table 3.4).

3.4 Results

3.4.1 Thermal history: water temperature

Temperature metrics showed weekly, monthly, and annual variability in the thermal signatures of the four rivers (Table 3.1, Figure 3.1). Full year metrics showed that the Amandel River was the warmest river overall, with mean, minimum, and maximum temperature exceeding the other rivers. The Berg River was found to be cooler. Mean Annual Temperature (MAT ± Standard Deviation) was lowest in the Driehoeks (15.1 ± 5.1ºC). Variability was highest in the Driehoeks and lowest in the Rondegat. The Amandel, Berg and Rondegat had similar 7-day moving averages of daily mean (24.6ºC, 24.2ºC and 24.0ºC respectively) compared to the Driehoeks (23.7ºC). The 7-day moving averages of daily maximum were highest in the Amandel and Berg Rivers (31.6ºC and 29.6ºC, respectively) and lowest in the Driehoeks River (24.8ºC).

Monthly averages (Table 3.1) were lowest in winter (June to August: 8.4ºC to 11.1ºC) and warmest in late summer (January and February: 18.8 ºC to 23.0ºC) and early autumn (March: 18.5 ºC to 21.0ºC). The Driehoeks was the coolest and least variable river, while the Berg and Amandel were warmer and more variable rivers. The Amandel, Berg and Rondegat experienced the highest averages, maximum temperatures, and the greatest range over the summer months (Figure 3.1).

Mean, minimum, and maximum temperature from the week preceding experiments (Table 3.1) showed that the Amandel and Berg Rivers were the warmest rivers with weekly maximum temperatures of 26.5ºC and 28.3ºC respectively. The Driehoeks and Rondegat had the lowest weekly and monthly mean temperatures of 17.3ºC to 18.3ºC. The Berg River had the highest 7-day moving averages of daily maximum temperature (26.3ºC).

Table 3.1: Thermal statistics for the Amandel, Berg, Driehoeks and Rondegat Rivers (**SD**= Standard deviation, **Min**= Minimum temperature, **Max**= Maximum temperature, **7-Day MA Mean**= Seven day moving average of daily mean, **7-Day MA Max**= Seven day moving average of daily maximum)

	River	**Mean (ºC)**	**SD**	**Min (ºC)**	**Max (ºC)**	**Range**	**7-Day MA Mean**	**7-Day MA Max**
Week preceding experiments	Amandel	21.5	na	18.7	26.5	7.8	na	na
	Berg	22.1	na	16.0	28.5	10.5	na	na
	Driehoeks	17.3	na	12.6	23.8	10.6	na	na
	Rondegat	18.3	na	15.1	21.9	6.8	na	na
Month preceding experiments	Amandel	18.3	na	15.2	25.1	8.1	19.9	24.4
	Berg	19.1	na	12.9	27.8	10.5	21.5	26.3
	Driehoeks*	17.4	na	12.5	22.4	4.3	20.1	21.9
	Rondegat**	17.3	na	13.3	23.3	5.8	18.6	21.4
One year	Amandel	17.4	4.8	15.6	20.5	14.31	24.6	31.6
	Berg	16.6	4.4	14.3	19.9	13.41	24.2	29.6
	Driehoeks*	15.1	5.1	14.0	16.2	4.24	23.7	24.8
	Rondegat**	17.9	3.6	16.3	20.5	8.91	24.0	28.3

** 2005-2006 data from Paxton (2008), ** 2010 data from Dallas & Rivers-Moore (2012)*

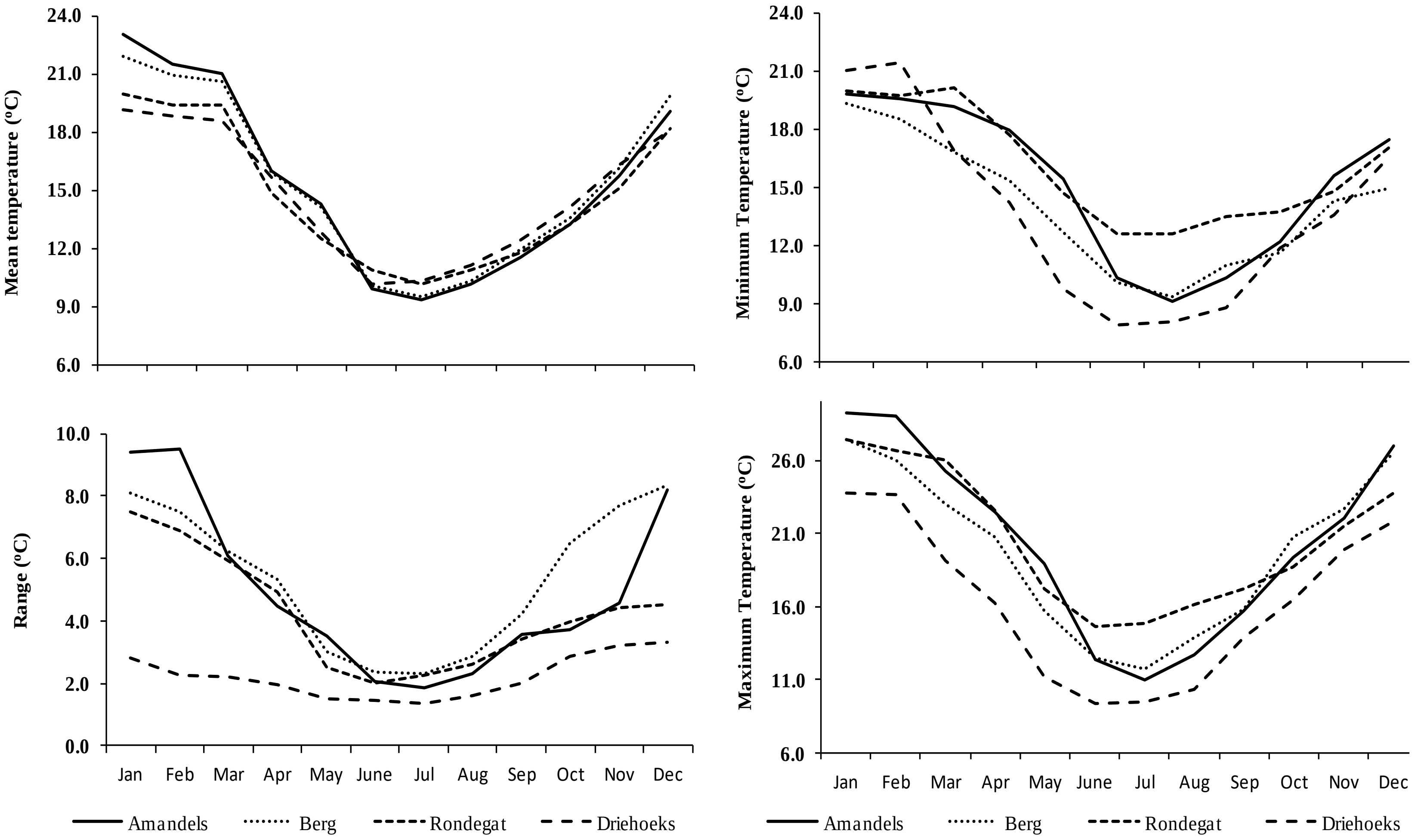


Figure 3.1: Monthly averages for mean, minimum, maximum temperatures, and temperature ranges (°C) for the target-rivers over a one-year period (Amandel, 2015-2016; Berg, 2015-2016; Driehoeks, 2005-2006; Rondegat, 2010)

3.4.2 Thermal tolerance between species, genera and sites

There was a significant difference in CT_{max} amongst the species (Kruskal-Wallis chi-squared = 248, df = 201, p= 0.013) (Figure 3.2). Of the seven species examined, *G. zebratus* was the most thermally sensitive with a CT_{max} of 29.80 ⁰C. *S. capensis* was most thermally tolerant with a CT_{max} of 34.75⁰C and 35.27⁰C in the Amandel and Berg Rivers respectively (Table 3.2). *Pseudobarbus calidus*, *G. zebratus*, *P. burchelli*, *P. burgi*, and *P. phlegethon* were highly thermally sensitive, while *A. gilli*, and *S. capensis* were moderately thermally sensitive.

There was no significant difference in CT_{max} amongst the species within the genus *Pseudobarbus* (p>0.05) (Figure 3.3a). There was also no significant difference (p>0.05) in CT_{max} amongst fish within the same species between sites (*P. phlegethon* **-** Rondegat and Driehoeks; *S. capensis* – Berg and Amadels) (Figures 3.3b and 3.3c respectively). Furthermore, no significant intra-site differences were found (p>0.05) (Figure 3.4a-d). The results of inter-species and inter-site Kruskal-Wallis tests are presented in Table 3.3.

Table 3.2: Median CT_{max} and holding temperatures for seven species of native CFR freshwater fish

River	**Species**	**Code**	**CT_{max} (⁰C)**	***n***	**Mean total length (cm)***	**Holding temperature (⁰C)**
Rondegat	*Austroglanis gilli*	G	33.03	16	12.02	20.70
Driehoeks	*Galaxius zebratus*	A	29.80	32	4.89	19.30
Amandel	*Pseudobarbus burchelli*	F	32.75	32	8.28	23.50
Berg	*Pseudobarbus burgi*	C	32.13	41	7.97	24.30
Rondegat	*Pseudobarbus calidus*	D	32.40	23	7.36	20.90
Driehoeks	*Pseudobarbus phlegethon*	B	30.30	27	6.90	18.00
Rondegat	*Pseudobarbus phlegethon*	E	32.57	26	6.22	20.70
Amandel	*Sandelia capensis*	H	34.75	30	8.15	24.40
Berg	*Sandelia capensis*	I	35.27	39	8.52	24.50

**Adults and sub-adults selected (for Total Length (cm) and CT_{max} correlations, see Appendix D)*

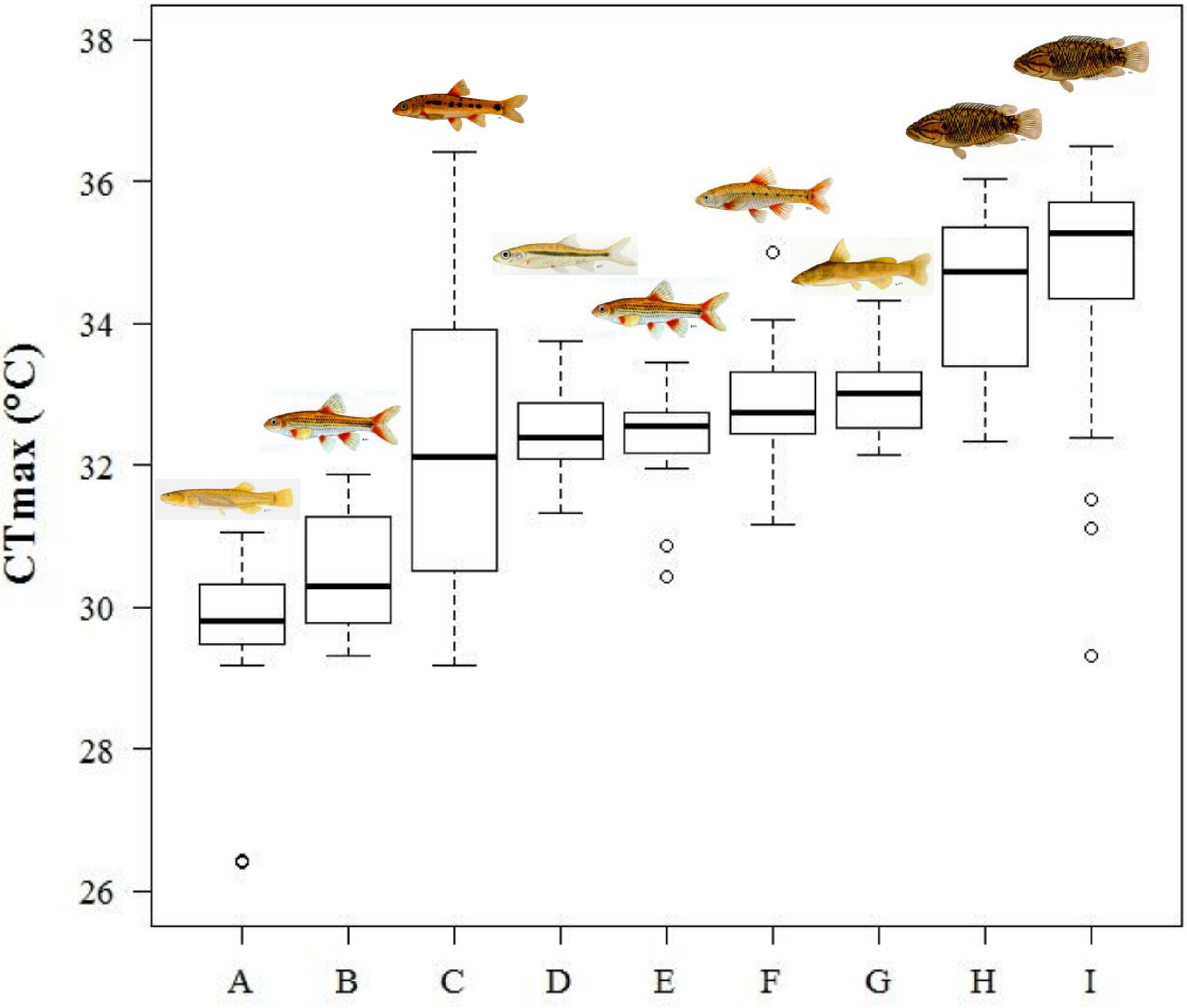


Figure 3.2: Boxplot showing median, quartiles, and range of CT$_{max}$ for all species from least tolerant to most tolerant

(**A**: *Galaxias zebratus*, **B**: *Pseudobarbus phlegethon* (Driehoeks), **C**: *Pseudobarbus burchelli*, **D**: *Pseudobarbus calidus*, **E**: *Pseudobarbus phlegethon* (Rondegat), **F**: *Pseudobarbus burgi*, **G**: *Austroglanis gilli*, **H**: *Sandelia capensis* (Amandel), **I**: *Sandelia capensis* (Berg))

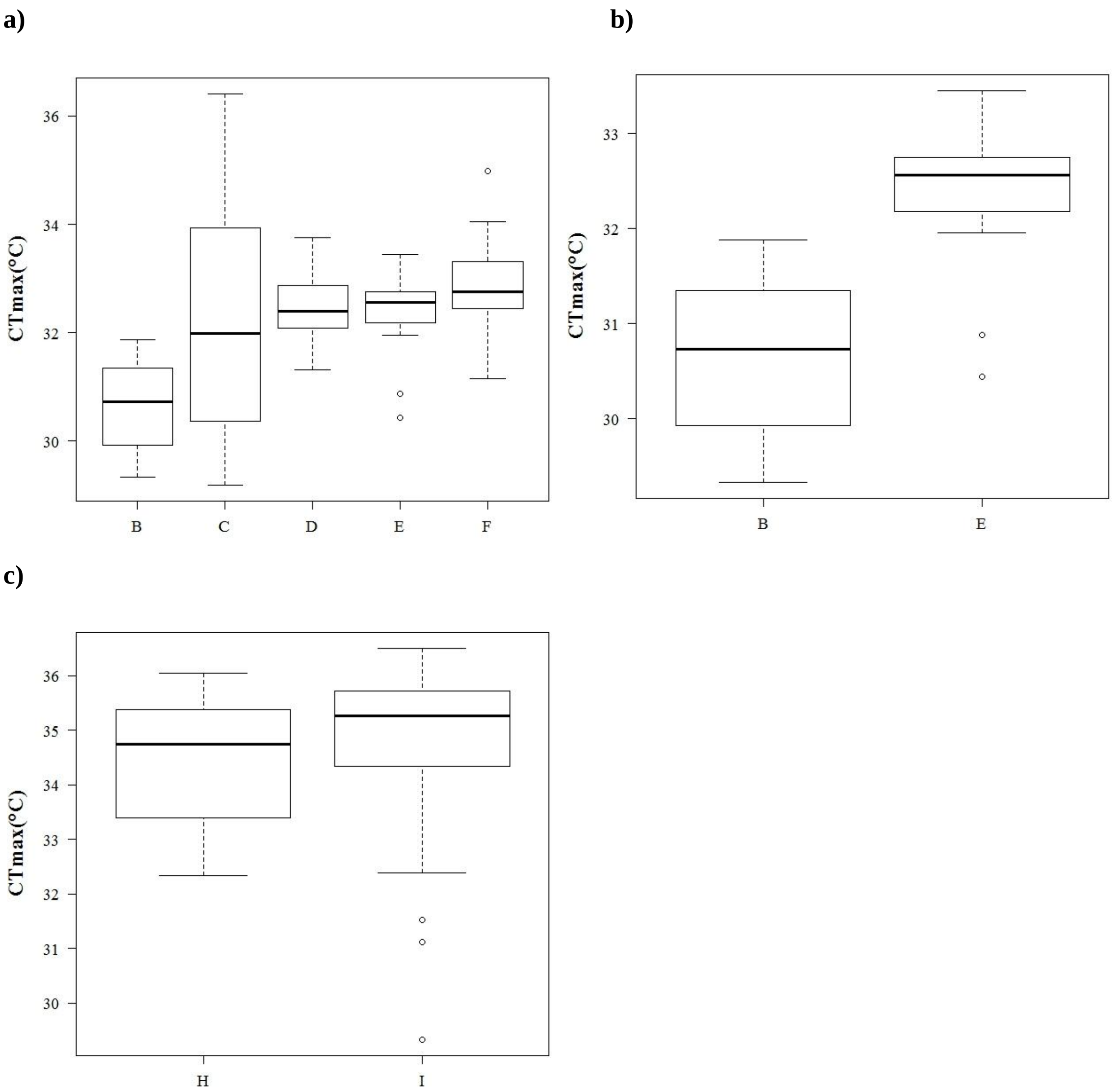


Figure 3.3: Boxplot showing median, quartiles, and range of thermal tolerances between **a)** All five selected species within the genus *Pseudobarbus*, **b)** *Pseudobarbus phlegethon* from two rivers, and **c)** *Sandelia capensis* from two rivers

(**B**: *Pseudobarbus phlegethon* (Driehoeks), **C**: *Pseudobarbus burchelli*, **D**: *Pseudobarbus calidus*, **E**: *Pseudobarbus phlegethon* (Rondegat), **F**: *Pseudobarbus burgi*, **H**: *Sandelia capensis* (Amandel), **I**: *Sandelia capensis* (Berg))

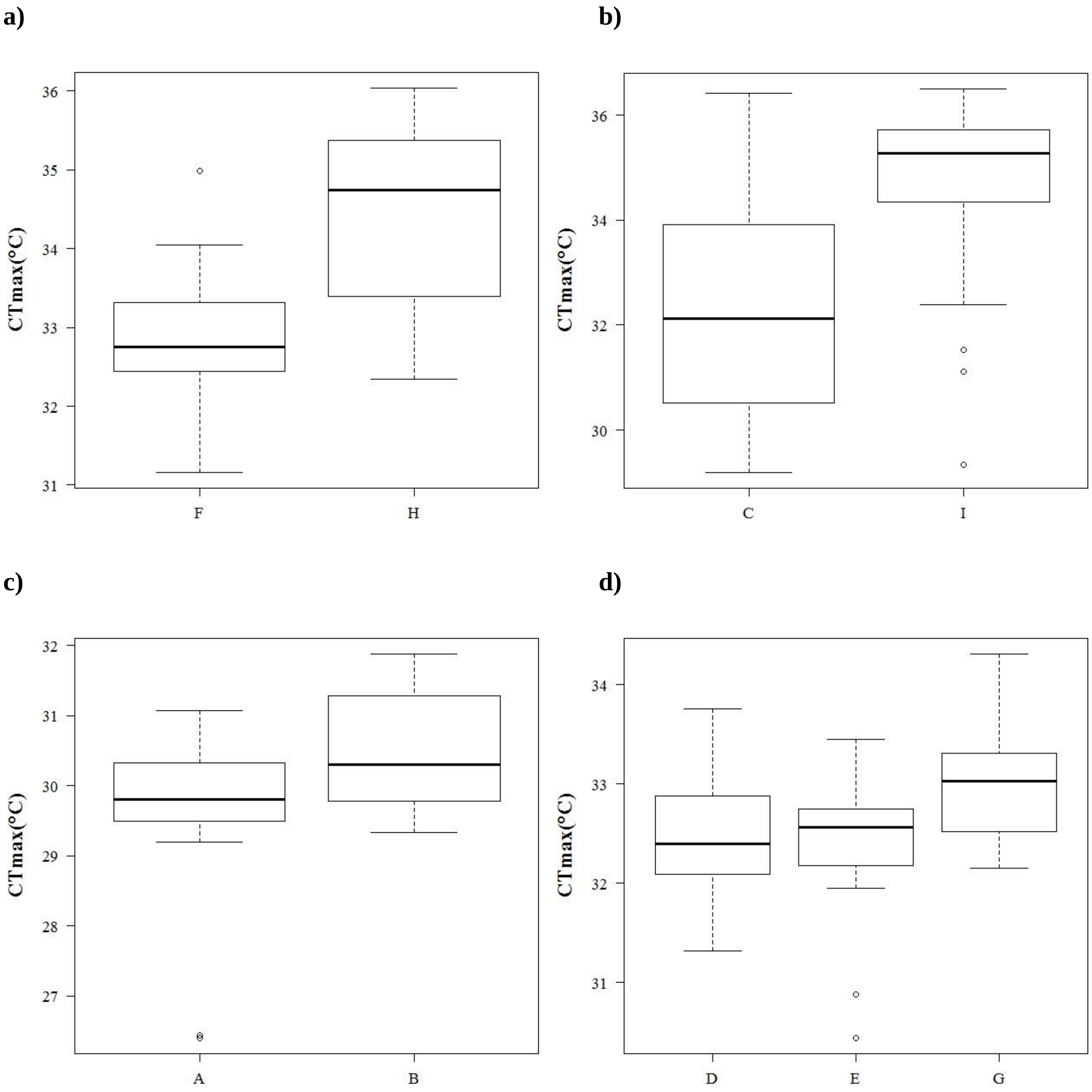


Figure 3.4: Boxplot showing median, quartiles, and range of thermal tolerances between species in **a)** Amandel River, **b)** Berg River, **c)** Driehoeks River, and **d)** Rondegat River

(**A**: *Galaxias zebratus*, **B**: *Pseudobarbus phlegethon* (Driehoeks), **C**: *Pseudobarbus burchelli*, **D**: *Pseudobarbus calidus*, **E**: *Pseudobarbus phlegethon* (Rondegat), **F**: *Pseudobarbus burgi*, **G**: *Austroglanis gilli*, **H**: *Sandelia capensis* (Amandel), **I**: *Sandelia capensis* (Berg))

Table 3.3: Results of inter-species and inter-site Kruskal-Wallis (K-W) tests between species and within sites

	Species/Genus	**Sites**	***p***	**DF**	**K-W test statistic**
Species/Genus	*Pseudobarbus* spp.	Amandel, Berg, Driehoeks, Rondegat	>0.05	118	131
	Pseudobarbus phlegethon	Driehoeks, Rondegat	>0.05	44	48
	Sandelia capensis	Amandel, Berg	>0.05	58	65.99
Sites	*Pseudobarbus burchelli*, *Sandelia capensis*	Amandel	>0.05	54	61
	Pseudobarbus burgi, *Sandelia capensis*	Berg	>0.05	71	76.04
	Galaxius zebratus *Pseudobarbus phlegethon*	Driehoeks	>0.05	45	54.04
	Austroglanis gilli, *Pseudobarbus calidus*, *Pseudobarbus phlegethon*	Rondegat	>0.05	51	56.80

3.4.3 Methodological standards: experimental endpoint and sample size

Behaviour within the control phase was different for each species, but during the experimental phase behavioural signs of stress (i.e. increased activity followed by the loss of righting response) were common to all species (Table 3.4). The end point of the experiment for each individual was determined by the loss of righting response, where the individual lost complete ability to remain upright. These endpoints were determined for four genera. By observing control behaviour in various group sizes, it was established from visual observations (Table 3.4) that three to six individuals could be used in the experiment depending on size, species, visibility of the stress response, and level of stress shown.

Table 3.4: Ethogram of behavioural responses from pilot study of four genera of fish used in the thermal tolerance experiment (**PTR**= Point of Thermal Reactivity from Dallas and Ketley (2011), **CT$_{max}$**= Critical Thermal maximum, **LRR**= Loss of Righting Response from Betinger and Lutteschmidt (2011))

Genus/Species	**Observed behaviour**		**Endpoint (CT$_{max}$)**
	Control	**Experiment**	
Austroglanis gilli	Remained at the base of the basket in a cohesive group.	Initially, no change. At PTR fish became more active. Swimming along the sides of the mesh basket.	First signs of disorientation/LRR. Underside became visible.
Galaxias zebratus	Remained at the base of the basket, fins extended at rest, no cohesive group. Intermittent swimming into mesh.	Initially no change. At PTR, still remained at bottom of basket but pectoral fins continuously moving. Close to CT$_{max}$ S-shape position assumed, fin movement increased.	First signs of disorientation/LRR. Rapid movement of the tailfin.
Pseudobarbus spp.	Swam back and forth in a cohesive group. Periodic surfacing.	Initially, increased movement and spreading of pectoral fins. At PTR decreased movement but still maintaining a cohesive group. Close to CT$_{max}$, surfacing increased and became more erratic. Group fissure.	First signs of disorientation/LRR.
Sandelia capensis	Very active, surfacing regularly but maintained a cohesive group.	Initially, increased movement. At PTR, movement of pectoral fins and mouth more rapid. Close to CT$_{max}$ individuals morbid.	First signs of disorientation/LRR Alternating between spasms and nose-down position.

3.5 Discussion

3.5.1 Biotic effects: the species effect

Differences in the breadth and magnitude of thermal ranges are evident between different species of ectotherms (Somero and Dahlhoff, 2008). Thermal magnitude is a measure of an organism's tolerance to temperature and can be expressed as CT_{max}: a critical thermal maximum temperature above which mortality is imminent. Thermal breadth is a measure of the thermal range of an organism, with stenothermic species having narrow thermal ranges, whereas eurythermic species have broad thermal ranges (Moore, 1940). These two groups can further be divided on the basis of warm or cold-adaptedness such that four types of 'thermal identities' can be described: warm-adapted stenotherms, cold-adapted stenotherms, warm-adapted eurytherms, and cold-adapted eurytherms (Moore, 1940).

Eurythermic species tend to have greater phenotypic plasticity, or thermal rigour, thus the ability to tolerate temperature fluctuations (Setchell, 1920). Relative to terrestrial exotherms, aquatic organisms are generally more stenothermic, and thus more susceptible to thermal change. In this study, the range in CT_{max} between the species did not exceed 10ºC: far below the 19ºC suggested by Moore (1940) as the range above which an exotherm can be classified as eurythermic. Therefore, all of the species in this study can be considered as stenothermic although their thermal breadths differed; it is simply the degree of stenothermy relative to each other.

In terms of evolutionary advantages, there are trade-offs in physiology with respect to thermy. Somero (2010) suggests that amongst the major losers of climate change will be extreme stenotherms and warm adapted eurytherms as they already live close to their thermal maxima. As the least stenothermic species, *P. burgi* and *S. capensis* may be more likely to withstand temperature fluctuations associated with climate change. On the other hand, *A. gilli*, *P. calidus*, *G. zebratus*, and the remainder of the genus *Pseudobarbus* (*P. burchelli and P. phlegethon,*) are expected to be less tolerant. It must also be noted that the thermal breadth may expand or contract depending on the life stage of the organism, and that these groupings of sensitivity are based on thermal maxima only. There are a range of temperatures below the CT_{max} which are favourable to the organism, and ideally a CT_{min} would need to be determined to encompass the full range of temperatures that are tolerable. Furthermore, temperature tolerance should be determined for all life stages of the organism from the egg stage to adulthood to encompass the entire thermal breadth of the species.

Pörtner et al., (2006) noted that there are tradeoffs in thermal adaptation. Warm-adapted stenotherms have traded eurythermy to maximize the upper limits of environmental temperature. In so doing they are able to optimize their fitness at higher temperatures. Such tradeoffs may manifest in the distribution of the species. If a habitat is favourable, and fitness is maximized, the species may opt for a stenothermic strategy rather than occupy a wide geographic range and reduce fitness (Gilchrist, 1995). This study showed that *Galaxias* spp. and most *Pseudobarbus* spp. (the most stenothermic species) can be expected to survive in pockets/thermal refugia rather than extend their distribution ranges. For species whose physiologies allow a wider distribution strategy, some form of thermal compensation must occur to counteract movement into less-optimal habitat (Pörtner et al., 2006). Other physiological attributes must compensate to maintain a metabolic rate that is favourable, thus requiring more energy. This compensation may be at the cellular level or even manifest itself in the reproductive success of the individual. Ultimately these biochemical compensations could set ecological patterns (Pörtner et al., 2006), and in fact may have shaped the ecology of the systems in the CFR over evolutionary time.

Mediterranean regions are the most vulnerable to climate change (Filipe et al., 2013) as freshwater biota will be pushed to the point of their physiological resilience. Somero and Dahlhoff (2008) note that processes at the physical and molecular/biochemical level are responsible for these limits, and the establishment of biogeographical patterns observed today. Using field distribution and temperature data, previous studies have been able to pinpoint thermal maxima as a limiting factor for fish abundance and spatial patterns (Ostrand and Wilde, 2001). Common examples of this effect are the latitudinal increase in tolerance breadth, and the conservation of thermal tolerances amongst species or genera (Sunday et al., 2011). The latter is a result of orthologs: genes evolved from a common ancestral gene, and which retain their function through time and evolution. These genes may be responsible for the conservation of thermal maxima within the three major genera in the CFR, namely *Galaxias*, *Pseudobarbus*, and *Sandelia* from different sites. This is a 'species effect', where evolutionary history has fixed the thermal limit. As the species in the CFR have only been isolated since the last glacial maximum (18 000 years ago), it is possible that thermal limits were set before or within this period, where water temperatures may have been 5-6ºC cooler than current temperatures (based on ambient temperature estimations) (Meadows and Baxter, 1999). If these orthologs evolved during this time, then the species may currently be pushing the bounds of their optimum habitat temperatures. If thermal tolerance evolved before the last glacial maximum, then it is possible that the species are predisposed to tolerate higher temperatures. The thermal tolerance of the closely related *Galaxias* species *Galaxias maculatus* from New Zealand was found to be between 30.5-35.4ºC (Simons, 1986). This exceeds the thermal limit of *G. zebratus* suggesting that there has already been divergence in the thermal tolerance since the splitting of Gondwana when the species diverged. Genetic sequencing and/or experiments on other known galaxiids in the greater Table Mountain region would elucidate whether this is the case. Notwithstanding this fact, there is still a difference in thermal maxima amongst the three major genera, suggesting that thermal niches already existed at the time (since they have evolved in sympatry). Fish communities may have already been restricted by thermal ranges during these speciation events, given that there were no invasive species to restrict their movement. It is therefore plausible that temperature influenced the distribution of fish since the last glacial maximum, more so than other habitat parameters.

3.5.2 Abiotic effects: the site effect

The lack of significant difference of CT_{max} within sites (henceforth 'site effect') may be indicative of an abiotic effect on the fish communities. Having already discussed how biology, physiology and genes determine thermal maxima in section *3.5.1*, this effect will explain how long and short-term environmental change (temperature) may have determined the thermal maxima of fish in the CFR.

Climate and geography have had significant effects on fish distributions and divergences within the CFR. Chakona et al., (2013) propose three hypotheses whereby the current genetic diversity in the genera *Galaxias*, *Pseudobarbus,* and *Sandelia* can be explained. (1) The Refugia Hypothesis suggests that during the Miocene-Pliocene marine transgression (15-2.6 million years ago), species were restricted which resulted in the isolation of populations and subsequent allopatric speciation. (2) The Paeleoriver hypothesis which suggests that the Last Glacial Maximum (18 000 years ago) resulted in the confluence of certain drainage basins into a 'Paleoriver', thus allowing for the dispersal of fish once again. Evidence supporting this Hypothesis is the occurrence of the three main genera across these systems. During the LGM temperatures were significantly cooler (5-6ºC) and wetter than at present (Meadows and Baxter, 1999). (3) Inter-drainage dispersal occurred in more recent geological time when pluvial periods were common (up until the Holocene about 8000-6000 years ago). The Holocene was considerably drier and warmer than the LGM, with the current Mediterranean climate and patterns only having been established for 5 000 years (from the second half of the Holocene) (Meadows and Baxter, 1999). This gave the fauna approximately 5 000 generations to adapt to the current thermal regime. For species that spawn annually (Skelton, 2001), this is a relatively long succession time. If there still has not been a differentiation in thermal maxima of different populations after 5 000 years, the rate of climate change may exceed the rate of evolution, and this may lead to an increase in mortality. Chevin et al., (2010) note that the current rate of human-induced environmental change may exceed organisms' adaptive genetic and developmental capacity. Furthermore, populations have evolved demographic mechanisms to cope with environmental change, but these mechanisms are now being pushed to their limits (Chevin et al., 2010).

Although adaptation occurs on evolutionary timescales, it also occurs over relatively short periods of time i.e punctuated evolution (months to years) (Heino et al., 2009). In this study, thermal tolerance was well correlated with stream temperature, where species from the warmer and more variable habitats such as the Amandel River had higher tolerance than those from colder and less variable rivers. The Driehoeks for instance is the coldest of the rivers, with *G. zebratus* showing the lowest CT_{max}. Furthermore, water temperature data from 2005 compared to the same period in 2015 shows that the Driehoeks system is currently experiencing greater extremes in temperature which may already be exerting a selection pressure on the cold-adapted stenotherms. Although the Berg River was the least thermally variable site, *P. burgi* and *S. capensis* had the highest CT_{max} within their genus and species respectively; but this may be attributable to higher water temperatures the week preceding experiments.

Habitat structure can limit fish distribution even when the thermal requirements do not. For instance, an individual can only migrate if it has both access to new habitats and if the new habitat is physiologically tolerable (Filipe et al., 2013). More so than climate change, water withdrawals may impact sensitive species. In South Africa, this has been one of the greatest impediments to ecological sustainability (Richter et al., 2003). Loss of habitat by water withdrawal can result in a loss of connectivity that is required for the movement of individuals and gene-flow. This would result in small isolated populations with low genetic diversity. As habitat requirements for each species are very specific in terms of depth and flow, changes in hydrology as a result of climate change could be more devastating than temperature alone (Filipe et al., 2013). A decrease in volume and increase in evaporation rates are expected in the CFR with seasonal changes likely to occur as well. With reference to the Amandel River, where abstraction and reduced flow already result in the drying-out of suitable habitats, this is a highly probable scenario. *P. burchelli* for instance may thus be more sensitive than their counterparts based on the level of threat from indirect climate-induced effects.

Here it is also important to note that CT_{max} represents extreme physiological limits of fish, while chronic exposure temperature would be 1-4 ºC lower (Beitinger et al., 2000). Longer term thermal limits (96-hour Incipient Lethal Upper Temperature - ILUT), are required to derive MWAT thresholds for the ecosystem. Dallas and Ketley (2011) showed that there was a significantly positive linear relationship between ILUT and median CT_{max} based on ten species of aquatic invertebrates. Applying their formula to the fish from this study, CT_{max} data revealed that predicted ILUTs ranged from 24.5-30.8ºC. Examination of these limits (CT_{max} and predicted ILUT) in relation to the magnitude of annual extreme water temperature conditions, specifically the 7-day moving average of daily mean (Mean_7) and maximum (Max_7) temperatures, showed that CT_{max} values were higher than both Mean_7 and Max_7 for all species and rivers. In contrast, predicted ILUT values were higher than Mean_7 for all species and rivers, but not Max_7, which was 2.4ºC higher for *P. burgi* in the Berg River and 3.7 and 1.4ºC *for P. burchelli* and *S. capensis* in the Amandel River respectively. This suggests that for some species thermal limits may be exceeded for periods of time over the hottest months, namely January and February.

3.5.3 Distribution and potential future implications

Potential effects of climate change on native and non-native fish

The current and potential abundance and distribution of native and non-native fish in response to warming will no doubt have a considerable effect on native species as invasive species are the main driver for the distribution boundaries of native fish in the CFR (Cambray, 2003). In the northern hemisphere, the data and literature on the thermal limits of that region's native species are relatively comprehensive. Within southern Africa however, experimental thermal data are comparatively rare, rendering it difficult to compare thermal tolerances of African invasive species with those in the CFR (see Table 3.5). Nevertheless, there is general consensus that of the biotic factors that currently affect native fish communities, predation by non-native fish is one of the major influencers of fish distribution (Jackson et al., 2001; Darwall et al., 2009; Marr et al., 2012, Weyl et al., 2013; Ellender et al., 2017). Thus, a shift in distribution of predator species may have an equal or greater effect on native fish in the near future than climate change *per se*. Less sensitive African species such as the Mozambican tilapia (*Oreochromis mossambicus*) which has the highest CT_{max} of all invasive species (Table 3.5), can be expected to compete with native fish as climate warms. Assuming that the physical structure of the habitat remains suitable, less tolerant European species may be effectively excluded from previously favourable habitats, thus allowing for reclamation by native fish. If the physical characteristics of the habitat are no longer suitable however, then both groups will be excluded, fragmenting river habitat such that native species remain restricted and thus do not benefit.

Two of the most prolific and iconic invasive species in the CFR are rainbow trout (*Oncorhyncus mykiss*) and bass (*Micropterus* spp.). Simon and Townsend (2003) describe trout as the most efficient energy converters, enabling them to successfully and easily invade most habitats. However, as cold/cool adapted fish, the thermal limits of *O. mykiss* are well below the limits of native fish from this study (29.8-35.27ºC)

(Table 3.5). As a highly thermally sensitive species, their distributions are thus likely to recede in the face of climate change. In fact, Eaton and Scheller (1996) suggest that at least 50% of the current habitat of salmonids will be reduced in response to expected temperature increase. This includes brown trout (*Salmo trutta*) whose thermal tolerance is 22-26.4ºC. Conversely, species that are less thermally sensitive such as bluegill sunfish (*Lepomis macrochirus*) (37.3ºC), common carp (*Cyprinus carpio*) (40.6ºC), mosquitofish (*Gambusia affinis*) (37.3ºC), and Mozambique tilapia (*Oreochromis mossambicus*) (43.3ºC) are likely to increase their distribution ranges if regional climate intensifies. The high thermal tolerance of *M. salmoides* (36.4ºC) should be of particular concern given that the thermal limit of its preferred prey species, *G. zebratus* (Shelton et al., 2008), is well below its own, thus intensifying predation and habitat competition.

Table 3.5: Thermal tolerances (CT_{max}) of native and non-native species in the Cape Floristic Region

Species	**CT_{max} (°C)**	**Reference/Source**
Native		
Austroganlis gilli	33.0	*This study*
PseudoPseudobarbus calidus	32.4	*This study*
Galaxias zebratus	29.8	*This study*
Pseudobarbus burchelli	32.8	*This study*
Pseudobarbus burgi	32.1	*This study*
Pseudobarbus phlegethon	30.3-32.6	*This study*
Sandelia capensis	34.8-35.3	*This study*
Non-native*		
Carassius auratus	30.8-43.6	Fry et al., (1942); Hoyland et al., (1979), Lutterschnidt and Hutchison (1997), Ford and Beitinger (2005)
Clarius gariepinus	na	na
Ctenopharyngoden idella	na	na
Cyprinus carpio	35.7-40.6	Black (1953); (Meuwis and Heuts (1957); Horoszewics (1973)
Gambusia affinis	36.9-37.3	Hart (1952), Lutterschmidt and Hutchison (1997)
Labeo capensis	na	na
Labeobarbus auneus	na	na
Lepomis macrochirus	30.4 -37.3	Hickman and Dewey (1973); Banner and van Arman (1973); Cherry et al., (1977), Lutterschmidt and Hutchison (1997)
Oncorhyncus mykiss	29.0	Chen et al., (2015)
Oreochromis auneus	na	na
Oreochromis mossambicus	41.6-43.3	Zaragoza et al., (2008)
Micropterus dolomieu	28.3-35.0	Cherry et al., (1977), Lutterschmidt and Hutchison (1997)
Micropterus floridanus	39.4	Guest (1985)
Micropterus punculatus	36.0	Cherry et al., (1977)
Micropterus salmoides	30.6-36.4	Hart (1952), Lutterschmidt and Hutchison (1997)
Perca fluviatilis	31.4-33.0	Alabaster and Downing (1966); Horoszewics (1973); Willemsen (1977)
Pseudocrenilabrus philander	na	na
Salmo trutta	23.0-26.4	Bishai (1960); Alabaster and Downing (1966)
Tilapia sparmanii	na	na
Tinca tinca	na	na

**Predominant non-native species in the CFR from Weyl et al., (2014)*

Distribution of aquatic species in response to warming

It is important to consider entire ecosystems when drawing inferences on climate-related effects on species. Thus far, community interactions have been discussed, but changes in trophic interactions may also drive community structure and abundance i.e. which species thrive and where. In this regard, there are top-down and bottom-up effects. Considering first the bottom up effects, the ecosystem is controlled by primary production and inputs. This concept has been well-described by Vannote et al., (1980) in the River Continuum Concept: Aquatic autochthonous and riparian allochthonous inputs determine invertebrate community structure, thereby determining the community structure of all higher trophic levels. In response to temperature increase, changes in the distribution and performance of aquatic macrophytes have been recorded (Barko et al., 1986). Barko et al., (1986) suggest that macrophytes will be most affected by changes in day length as photoperiod is crucial to photosynthesis. Growth and chlorophyll production can be expected to increase, thereby increasing the primary productivity of the ecosystem. However, if temperatures exceed the thermal limits beyond which plant tissues cease to function normally, it is likely that range shifts may occur. Anderson (1969) noted that the study of climate effects on aquatic plants was lacking. Although that study was published more than 40 years ago, to date literature on life history requirements of aquatic plants in the CFR is sparse at best. Notwithstanding trophic significance, in-stream vegetation also has structural importance, offering refuge to many species including *G. zebratus*; in its absence, small native fish are more prone to predation (Shelton et al., 2008). Similarly, shifts in the distribution of riparian vegetation could alter the amount of allocthonous input in mountain streams, and the loss of shading vegetation may increase diurnal water temperature (Davies, 2010).

There is also evidence that climate change may drive a shift in the distribution and performance of freshwater invertebrates (Dallas, 2008; Dallas and Ketley, 2011; Dallas, 2016). An alteration in invertebrate community is likely to affect freshwater fish in the CFR, given that the primary diet of these species consists of grazing invertebrates (Skelton, 2001). Non-native species further intensify competition for resources as salmonids are known to cause trophic cascades by removing grazing invertebrates (Simon and Townsend, 2003). This releases algae from top-down grazing; increasing algal biomass (Simon and Townsend, 2003). With the exception of those fish which feed primarily on algae, the native fish of the region will experience strong selection pressure to outperform salmonids (*Salmo trutta* and *Oncorhyncus mykiss*) if climate change hasn’t already impacted said species.

Dallas and Ketley (2011) published the reference thermal tolerance data for aquatic invertebrates in the CFR. From this, it is possible to draw inferences on potential trophic impacts of climate change. Grazing invertebrates from that study (families Heptageniidae, Teloganodidae, and Notonemouridae) had thermal tolerances below 33ºC (i.e. highly thermally sensitive). Should the abundance and distribution of these species shift, biological interactions could be disturbed, potentially altering fish community structure and prevalence. If the thermal limits of aquatic invertebrates are considered as a limiting factor, then the data by Dallas and Ketley (2011) could be used in sequential equation modeling (a form of multiple linear regression modeling) to determine to what extent this will influence the fish community. The benefit of sequential equation modeling is that it assists in determining feedbacks, allowing variables to function as both predictors and outcomes (Alsterberg et al., 2013). This removes the limitations of dependent and independent variables. Major advances have been made in terms of data acquisition in the CFR, but this data should now be used to move away from mere inferences towards statistical approaches which substantiate the effect of biotic and abiotic variables in relation to the entire ecosystem. These approaches have already been used in marine studies on climate change, and can be successfully applied to freshwater ecosystems.

3.6 Conclusion

3.6.1 Synthesis of findings

There are several main findings in response to the hypotheses set out at the beginning of this chapter, including (1) The magnitude and breadth of thermal sensitivity was found to differ between the species, indicating that varying responses to climate change are likely; (2) there was no difference in upper thermal limits within species and genera from different sites, suggesting that thermal tolerance has been influenced by evolutionary history (there is a species effect); (3) temperature metrics revealed variability in the thermal signatures of the rivers, but there was no difference in upper thermal limits between species within sites; suggesting preceding thermal conditions at a site, i.e. the thermal history has an effect on thermal tolerance; (4) both the site and species effect indicate that long and short term thermal history is likely to influence thermal tolerance (either genetically, or plastically/phenotypically).

These findings are discussed in terms of ecosystem impacts-especially with regards to invasive species, and how this may inform future research and conservation. Since there are clear differences in the thermal limits of the non-native and native species. Certain native species with low thermal thresholds (i.e. *G.*

zebratus) may be more vulnerable to climate change, and hardier species (e.g. *S. capensis* and *A. gilli*) less so. Under predicted climate change scenarios for the CFR, stenothermic cold-adapted species like the salmonids are likely to be more negatively impacted than native species, while the more eurythermal warm-adapted tropical species the tilapia and bass are likely to perform better. The interactions between native and non-native species may be considerably affected; however the exact outcome remains speculative. Climate change will have synergistic effects on freshwater ecosystems in ways that have not yet been observed or quantified.

3.6.2 Recommendations

Water temperature records together with chronic and acute experimental data is considered the standard for any climate-related research on aquatic biota. Unfortunately the data is neither easy nor cheap to produce. With data loggers, there is an initial cost, as well as the logistical constraints of suitable attachment sites. South Africa still lacks the scientific infrastructure to make these data easily attainable, but it is required to set biological thresholds for management of thermal regimes. Furthermore, varying methodologies and funding limitations are problematic for data acquisition, thus making the setting of water temperature thresholds difficult at best. Moreover, whether the required data is experimentally-derived, or determined via models or field data, it is difficult, if not impossible to ensure that there is consistency between studies unless there is collaboration between institutions.

Table 3.5 shows that experimental data (i.e. thermal maxima) for a number of the main non-native ‘concern’ species in the CFR has not yet been determined or published. Albeit so, thermal research in South Africa has made considerable progress over the past decade, with some of the seminal work on thermal tolerance published on thermal limits of aquatic invertebrates by Dallas and Ketley (2011) and Dallas and Rivers-Moore (2012). These studies also revealed the need for chronic exposure data in order to comfortably set thermal limits for aquatic biota. Since the species in this study are both endangered and native, an alternative method was opted for; the thermal preference experiment which is discussed in Chapter 4. While these data do not assist towards setting MWAT thresholds for ecosystems, they do provide insight into the thermal requirements of native fish.

Sunday et al., (2011) promoted the standardisation of critical thermal methodology, especially with respect to acclimation temperature and comparable data. Fish respond to abiotic changes over short periods of time (day to day, month to month, or year to year), and Somero and Dahlhoff (2008) refer to this as phenotypic adaptation. This form of acclimation/phenotypic adaptation has been shown to influence critical thermal limits and thermal sensitivity for almost all taxa that have been studied

(Beitinger et al., 2000). In terms of duration, Perez et al., (2003) suggest a 30 day acclimation period, but I argue that this is not necessary if water temperature data are available for sites. The water temperature data in this study were used to interpret thermal history and response to CT_{max}. In so far as short term acclimation temperatures go (i.e acclimatization); the fish were not exposed to an acclimation period, but rather a holding period. This was to ensure minimal stress was incurred by the fish, and this holding temperature did not exceed the 7-Day moving average for each site (See Appendix B).

Finally, with regards to site and species selection, Somero (2010) suggests that latitudinal effects on single species must be studied when establishing thermal limits. In the CFR this can easily be undertaken as the extent of the region stretches both northward and eastward. Future research should thus be aimed towards thermal tolerance data acquisition (not only for native species, but non-native species as well). Such data for African invasive fish, such as the tilapia and cichlid species for example, would assist in unravelling the potential effects of climate change on the relationship between native and non-native species. On the physical selection of species for experimentation, the results from this study (Appendix C and D) suggest that only mature individuals and no young of year are selected.

Ultimately, the methods proposed here will assist towards three main objectives: the acquisition of reference experimental data that is comparable, identifying priority species for conservation (also informing the IUCN statuses of native fish), and encouraging collaborative research across South Africa.

CHAPTER 4: Thermal preferences of select native fish in the Cape Floristic Region

4.1 Introduction

Thermal preference is the environmental temperature chosen by an organism, at which the organism's fitness is optimised (Jobling, 1981). In experimental terms, it is the range of temperatures at which animals congregate in a free-choice situation. In a seminal paper, Cowles and Bogert (1944) found that in ecotherms/poikilotherms, preferred temperature was achieved by means of behavioural thermoregulation. Whilst studying desert lizards, Bogert (1949) noticed that the animals migrated between areas of shade and full sunlight depending on their body temperature and energy requirements. Habitat or microhabitat selection as a means of behavioural thermoregulation thus regulates the body temperature, metabolism, and level of activity of the ectotherm (Bogert, 1949; Sänger, 1993). Bryan et al., (1990) refer to this as the Maximum Power Principle; an organism will seek out the environment in which it can best optimise its flow of energetic resources and reach its physiological optima (Jobling, 1981).

Although it is relatively easy to determine the preferred temperature of a fish in the laboratory (where the number of interacting variables may be controlled), resource selection in animals can be complicated by environmental heterogeneity. In nature, and as previous studies have shown, thermal preference is influenced by thermal history, availability of oxygen, ontogeny, infection, social interactions, and food rationing to name a few (Reynolds, 1977; McCauley and Huggins, 1979; Kita et al., 1996; Sogard and Olla, 1996; Despatie et al., 2001; Golovanov, 2006). Temperature can be viewed as an ecological resource, but temperature is not the only ecological resource available to an animal (Magnuson et al., 1979; Manly et al., 2007), and there is evidence that this has driven niche selection and sympatric speciation (Ohlberger et al., 2008). Ultimately, in a natural aquatic habitat, a suite of abiotic variables including (but not restricted to) temperature, flow, and structural complexity of the habitat, influence/determine where the organism will choose to exist (Crowder and Cooper, 1982).

When favourable conditions are not accessible to an organism, biological trade-offs begin to occur. By a process called physiological compensation (Huey, 1991), there is a reallocation of energy/fitness by the organisms for the acquisition of a resource. This is also known as a fitness cost (Gilchrist, 1995). Ectotherms generally opt for behavioural thermoregulation as a means to avoid physiological compensation as this incurs the least energetic expense. For example, a fish may choose to remain in a habitat where temperature is outside of its optimal/preferred range if it can exploit an abundant resource such as food. At this point the muscle tissues of the animal begin to compensate for the change (Sänger, 1993). At cold temperatures the number red muscle fibres may increase thus allowing for increased oxygen supply (Sänger, 1993), whereas in warm temperatures collagen and myosin muscle fibres denature (Ofstad et al., 1993).

For freshwater fish specifically, fluctuation in environmental temperature is much more intense than for marine fish: summer water temperature may rise to 30ºC and in winter drop well below 4ºC thus exposing them to extremes in temperature and testing their thermal resilience (Sänger, 1993). Under these conditions, both behavioural thermoregulation and physiological compensation are necessary. For ecologists to predict how further fluctuation or extremes in temperature may manifest in fish communities, both thermal tolerance and preference data are required. This will indicate not only their thermal limits, but their habitat requirements as well. Prior to this study, thermal preference data did not exist for native fish in South Africa. These data will assist toward understanding the overall impacts of climate change on already-vulnerable native freshwater fish communities.

4.2 Aims and Hypotheses

The aims of this chapter were to:

- Characterise and compare the thermal preferences of native fish in the CFR.
- Provide reference data on thermal preferences of native fish in the CFR.
- Examine the suitability of the thermal preference experiment as a method for the determination of thermal sensitivity.
- Evaluate the applicability of experimental design for future studies on endangered fish in South Africa.

The main hypotheses were:

- Significant differences were expected amongst species
- A positive linear relationship is expected between thermal preference and thermal tolerance.

4.3 Methodology

4.3.1 Thermal preference between species

Prior to the commencement of the study, a schooling pilot-experiment was conducted. The objective of the schooling experiment was to determine whether group size influenced thermal preference, thereby also determining an appropriate sample size for the preference experiment. In the pilot study, the behaviours of groups and single individuals of *G. zebratus*, *P. burchelli*, and *S. capensis* were observed during a control and experimental phase (without and with a temperature gradient respectively). Differences were then tested between the thermal preferences of groups (five individuals) vs single fish using *P. burgi* as a test species. Both the pilot study and final experiments were conducted and analysed in the same manner.

The thermal gradient was incrementally established over three hours (establishment phase), after which, a two-hour experimental period followed. The tanks were checked at 10 minute intervals during the establishment and experimental phase. Thermal Preference (ºC) for each species was calculated using the statistical approach described in Richardson et al., (1994). For each observation, the number of fish in each 25 cm section (representing different thermal environments) was recorded. Based on the findings of the pilot study, five individuals were placed in each of the tanks. Where the number of fish collected was insufficient, the number of fish in the experiment was reduced to four (these data can be found in Appendix E). Over a period of five hours, 31 observations were made for each experiment (T1-T31). The frequency of the fish at each thermal section was pooled for the final two hours of the experiment (T20-T31). This was converted into a percentage cumulative frequency for each Tank (A-C). The 50% (median) of the percentage cumulative frequency was then interpolated in ‘R’. The median values of each trial were then pooled to derive an overall preferred median temperature for the species. Where the coldest temperature in the experiment did not equal zero percentage cumulative frequency (0%), two standard deviations were subtracted from the coldest temperature to plot the cumulative frequency curve. This standard deviation was calculated from temperature logger data for that section.

The experiments were replicated at least three times for each species. QQplots and Shapiro-Wilk tests revealed that the data were not normally distributed. Therefore a linear model was then used to compare preferences between the groups and single individuals. The linear model is a flexible method for fitting strongly skewed non-parametric data (Miao et al., 2009). In total 46 experimental trials were undertaken on six species to evaluate differences in thermal preferences between them (one of the species occurred in two sites, thus seven datasets were collected in total).

4.3.2 Thermal tolerance and thermal preference

The correlation between thermal preference and thermal tolerance was evaluated to investigate whether a relationship existed between these variables i.e. whether thermal preference could also be used as a measure of thermal sensitivity. Using median CT_{max} and median preference for each species, a linear model was applied to the data.

4.4 Results

4.4.1 Determination of schooling effect and sample size

Differences in behaviour were observed between groups and single fish (as indicated in Table 4.1). A difference in the selection of temperature was also observed between the groups and single fish (Figures 4.1-4.4). For *S. capensis*, single fish displayed lethargic behaviour, often settling in a section of the gradient tank for the duration of the experiment. *Pseudobarbus* spp. on the other hand displayed agitated behaviours when left alone, and often escaped the experimental apparatus. When three fish were used, similar antisocial and lethargic behaviours were observed in certain species. Five fish generally showed schooling behaviour, but were less stressed.

No significant difference was found in the thermal preference of single fish (four replicates) compared with a group of five fish (two replicates) of *P. burgi* but the group selected a higher preferred temperature with less variation (residual standard error: 4.39 on 4 degrees of freedom, multiple $R^2 = 0.19$, adjusted $R^2 = -0.01$, $F_{1,4} = 0.94$, $p = 0.385$) (Figure 4.3).

Table 4.1: Ethogram of behavioural responses for individual and groups of fish in preliminary preference experiment

Species	**Single**		**Group size = 3**		**Group size = 5**	
	Control	**Experiment**	**Control**	**Experiment**	**Control**	**Experiment**
Galaxias zebratus	Individual rested in a fixed position at the bottom of the tank unless disturbed with a jet of water.	Individual remained in the initial position throughout.	No schooling behaviour observed. Brief periods of movement.	No schooling behaviour observed. Brief periods of movement.	*Individuals initially excited, swimming between poles. Formation of groups remained at either pole.	*No movement of individuals. Aggregation of individuals in the coldest sections with none/one/two isolated in other sections.
Pseudobarbus burchelli	Erratic behaviour, often jumping out of the experimental apparatus.	Erratic behaviour persisted throughout; individual swam between warm and cold poles continuously.	Initial erratic behaviour, one or more individuals jumping out of apparatus, periods of movement and schooling.	Schooling behaviour observed. Frequent fissure and recombination of group.	*Initial erratic behaviour, groups of 2-3 maintained.	*Schooling behaviour observed, consistent groups of 2-3 individuals and aggregation at the preferred temperature
Pseudobarbus burgi	Erratic behaviour, often jumping out of the experimental apparatus.	Erratic behaviour persisted throughout; individual swam between warm and cold poles continuously.	No data	No data	*Initial erratic behaviour, groups of 2-3 maintained.	*Schooling behaviour observed, consistent groups of 2-5 individuals and aggregation at the preferred temperature
Sandelia capensis	Individual remained in a fixed position unless disturbed with a jet of water.	Remained in the initial position for most of the experimental phase, often settling in warmest or coldest section.	No schooling behaviour observed. Brief periods of movement, but returned to initial position.	No schooling behaviour observed. Very little movement. Individuals remained in initial position.	*No schooling behaviour observed. Brief periods of movement and grouping.	*No schooling behaviour observed. Aggregation at the preferred temperature.

**From actual experiments*

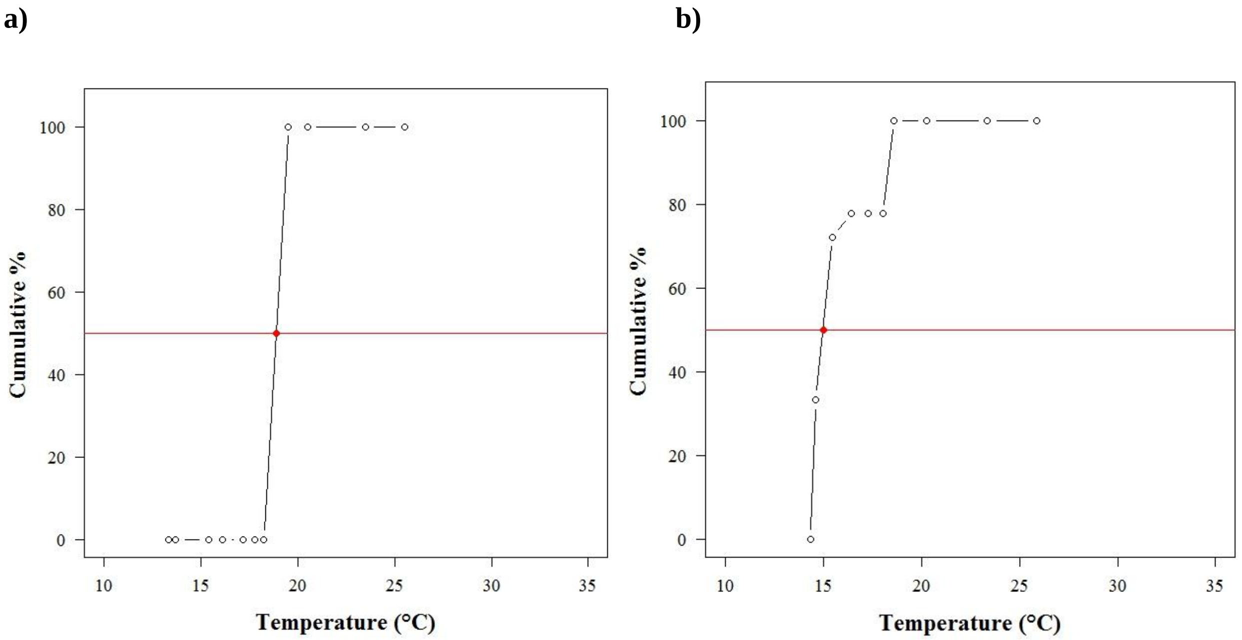


Figure 4.1: Median thermal preference for *Galaxias zebratus* from the Amandel River **a)** single, and **b)** group of three fish

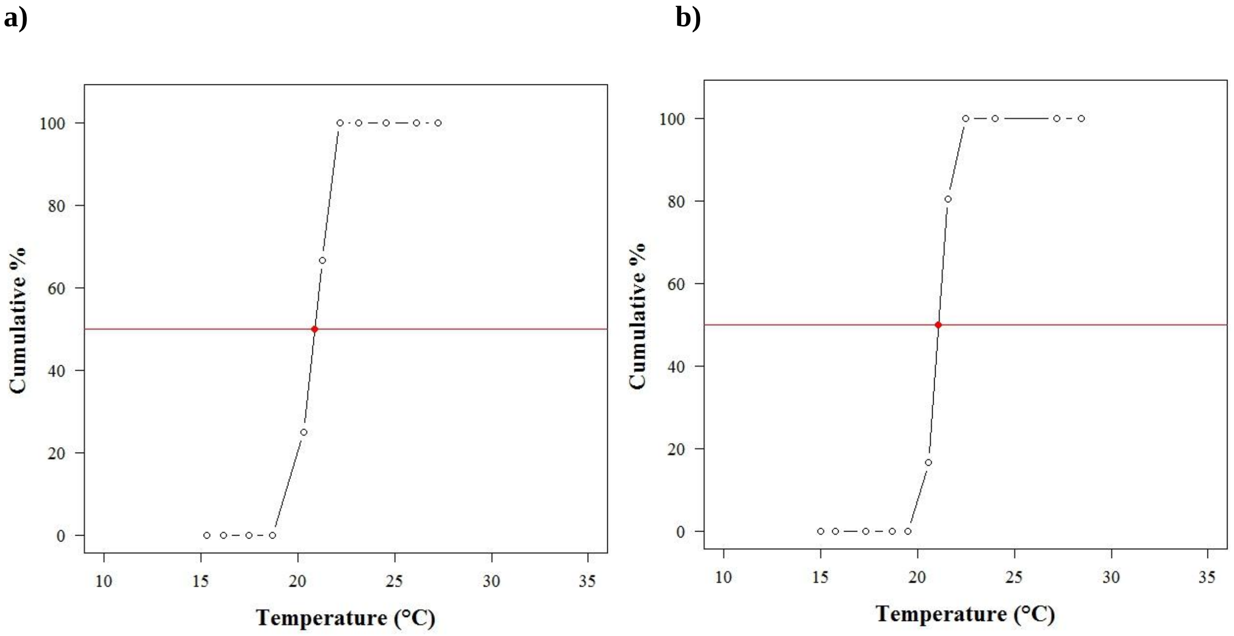


Figure 4.2: Median thermal preference for *Pseudobarbus burchelli* from the Amandel River **a)** single, and **b)** group of three fish

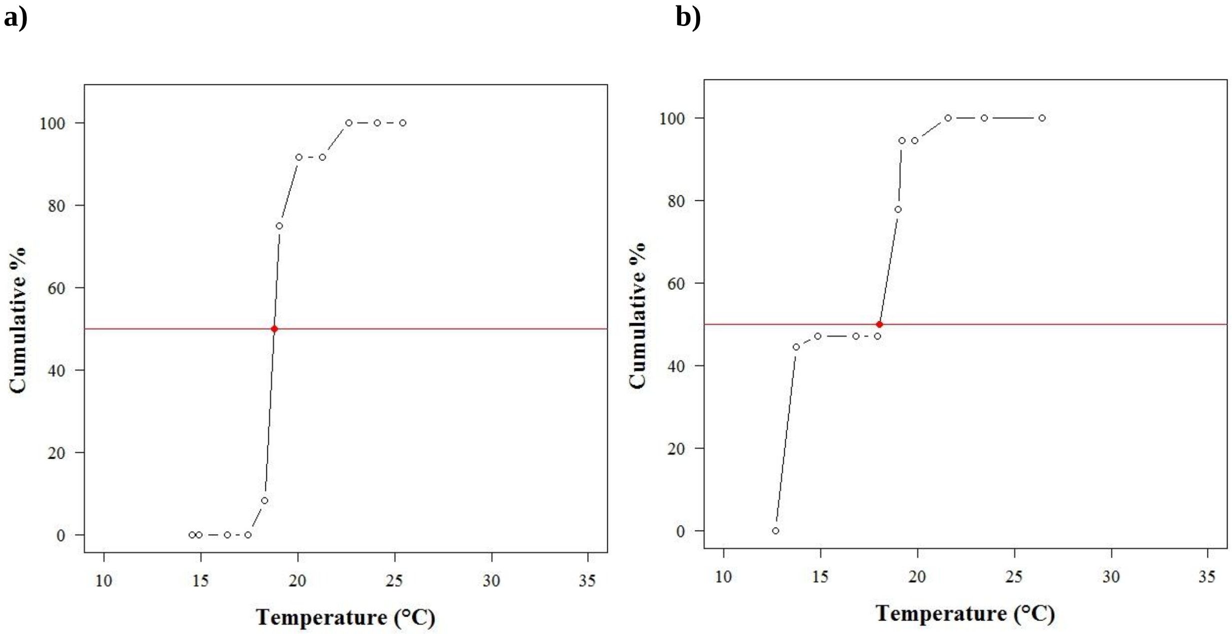


Figure 4.3: Median thermal preference for *Sandelia capensis* from the Amandel River **a)** single, and **b)** group of three fish

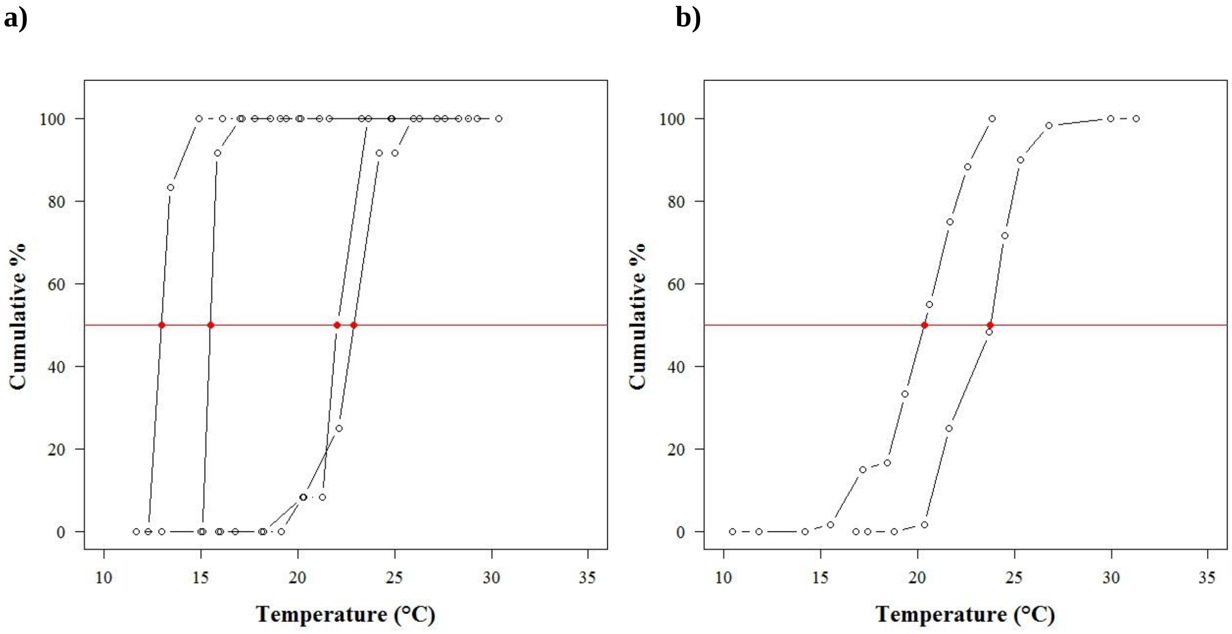


Figure 4.4: Median thermal preferences for *Pseudobarbus burgi* (Berg River), **a**) single, and **b**) group of five fish (four and two replicates respectively)

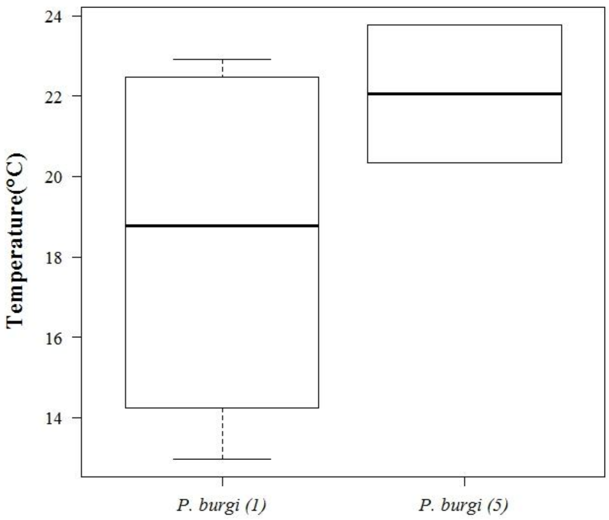


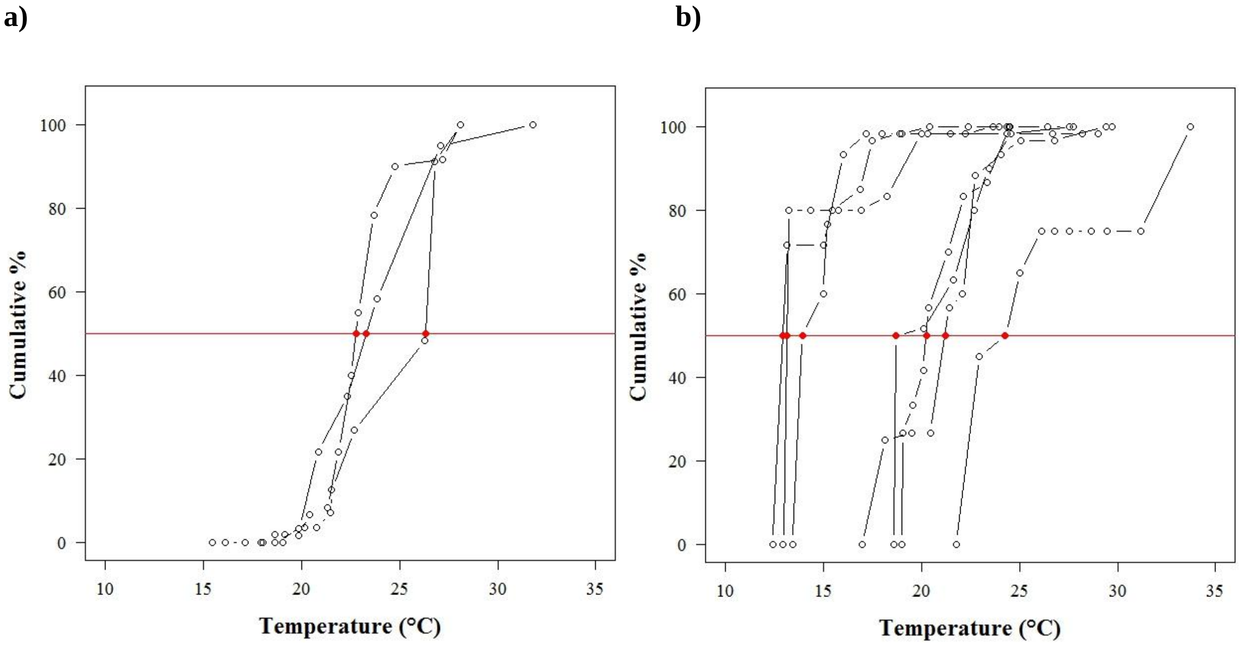
Figure 4.5: Boxplot showing median, quartiles, and range of thermal preference of single and group (5 fish) of *Pseudobarbus burgi*

4.4.2 Thermal preference between species, genera and sites

A significant difference in thermal preference was found amongst the species using the linear model **(**residual standard error: 2.56 on 39 degrees of freedom, multiple R^2= 0.59, adjusted R^2= 0.53, $F_{6, 39}$= 9.47, p< 0.001) (Table 4.2, Figure 4.6, Figure 4.7). *Galaxias zebratus* showed a preference for the coolest temperature, and *S. capensis* showed a preference for the warmest temperature. A significant difference in thermal preference was also found between species and genera from different sites. *S. capensis* displayed a significantly lower thermal preference in the Amandel vs the Berg River (Figure 4.8). Differences were also observed among members within the genus *Pseudobarbus* with *P. calidus* showing the preference for the coolest temperature, and *P. burgi* showing a preference for the warmest temperature (Table 4.2, Figure 4.8). Inter-site differences in thermal preference were not significantly different (Table 4.3, Figure 4.9).

Table 4.2: Median Thermal preferences of six species of native CFR freshwater fish

River	**Species**	**Code**	***n***	**Preference (ºC)**	**Preference Range (ºC)**
Rondegat	*Austroglanis gilli*	G	14	23.3	22.8-26.3
Rondegat	*Pseudobarbus calidus*	D	60	22.3	18.2-23.3
Driehoeks	*Galaxias zebratus*	A	35	18.7	12.9-24.3
Amandel	*Pseudobarbus burchelli*	F	45	23.5	19.5-26.4
Berg	*Pseudobarbus burgi*	C	29	27.0	25.9-28.4
Amandel	*Sandelia capensis*	H	30	23.9	17.3-25.5
Berg	*Sandelia capensis*	I	15	27.7	26.8-28.0



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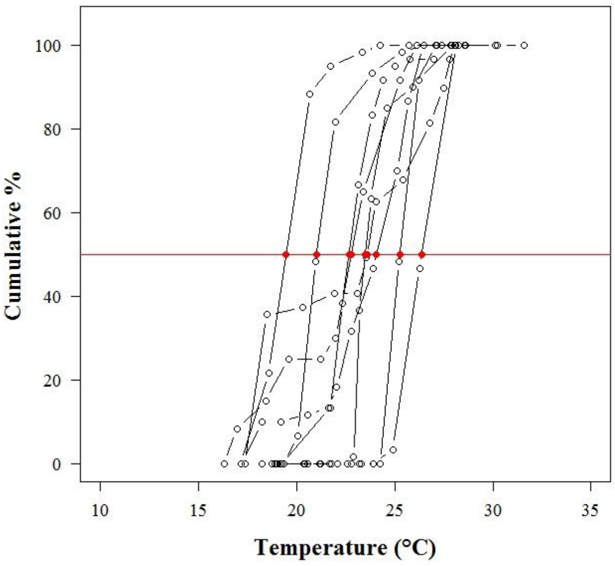
Cumulative %
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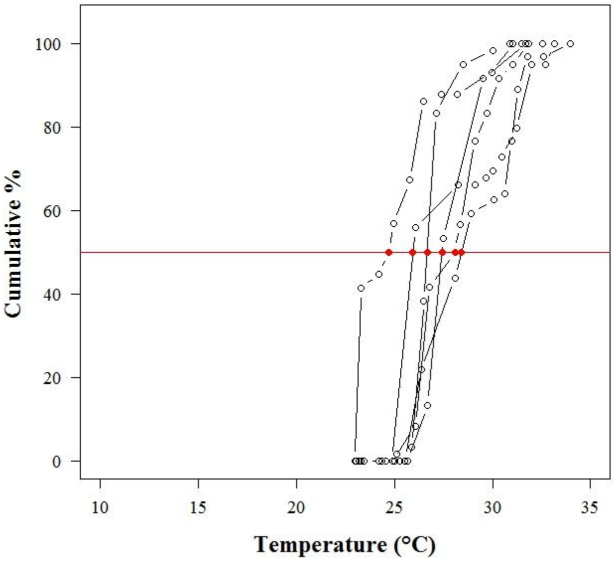
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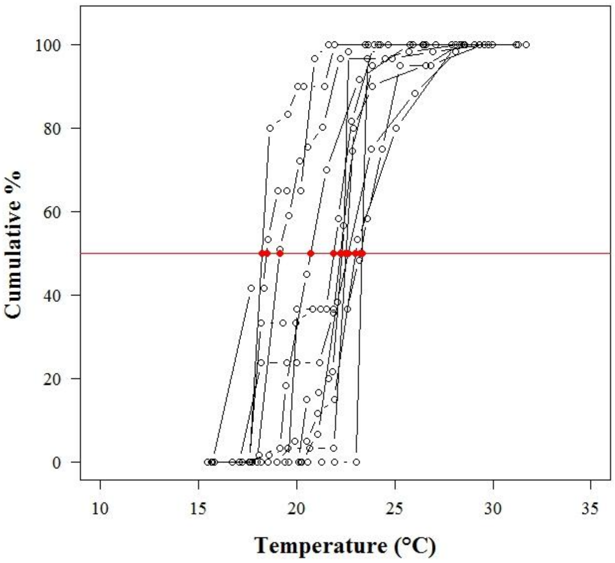
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Temperature (°C)

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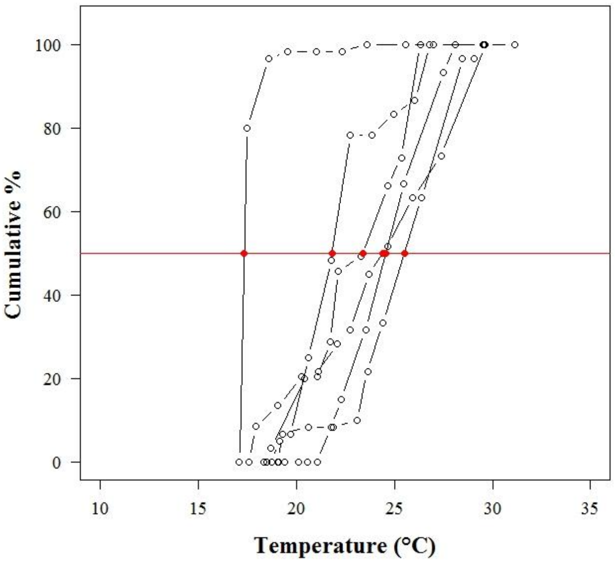
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Temperature (°C)

g)

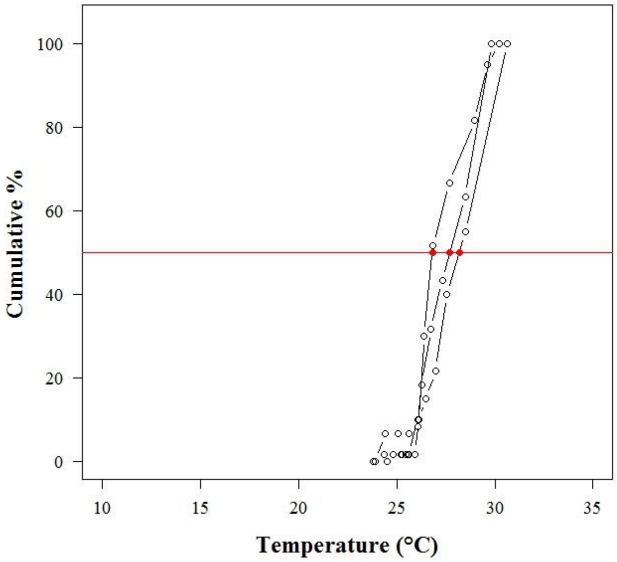


Figure 4.6: Median preferences for all trials of each species **a**) *Austroglanis gilli* (Rondegat River) **b**) *Galaxius zebratus* (Driehoek River) **c**) *Pseudobarbus burchelli* (Amandel River) **d**) *Pseudobarbus burgi* (Berg River) **e**) *Pseudobarbus calidus* (Rondegat River) **f**) *Sandelia capensis* (Amandel River) **g**) *Sandelia capensis* (Berg River)

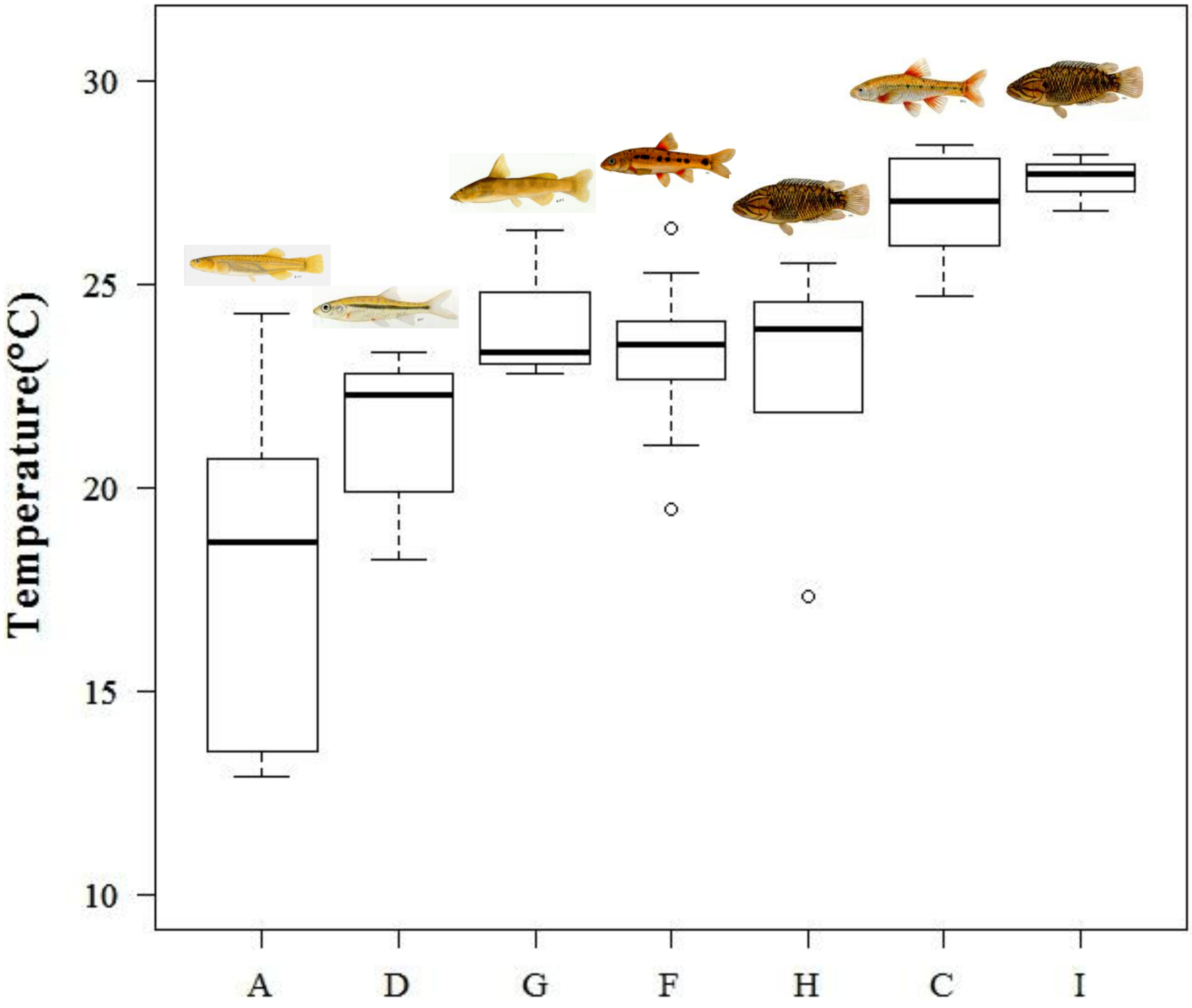


Figure 4.7: Boxplot showing median, quartiles, and range of thermal preference of all species from coolest to warmest temperature preference

(**A**: *Galaxias zebratus*, C: *Pseudobarbus burgi*, **D**: *Pseudobarbus calidus*, **F**: *Pseudobarbus burchelli*, **G**: *Austroglanis gilli*, **H**: *Sandelia capensis* (Amandel), **I**, *Sandelia capensis* (Berg))

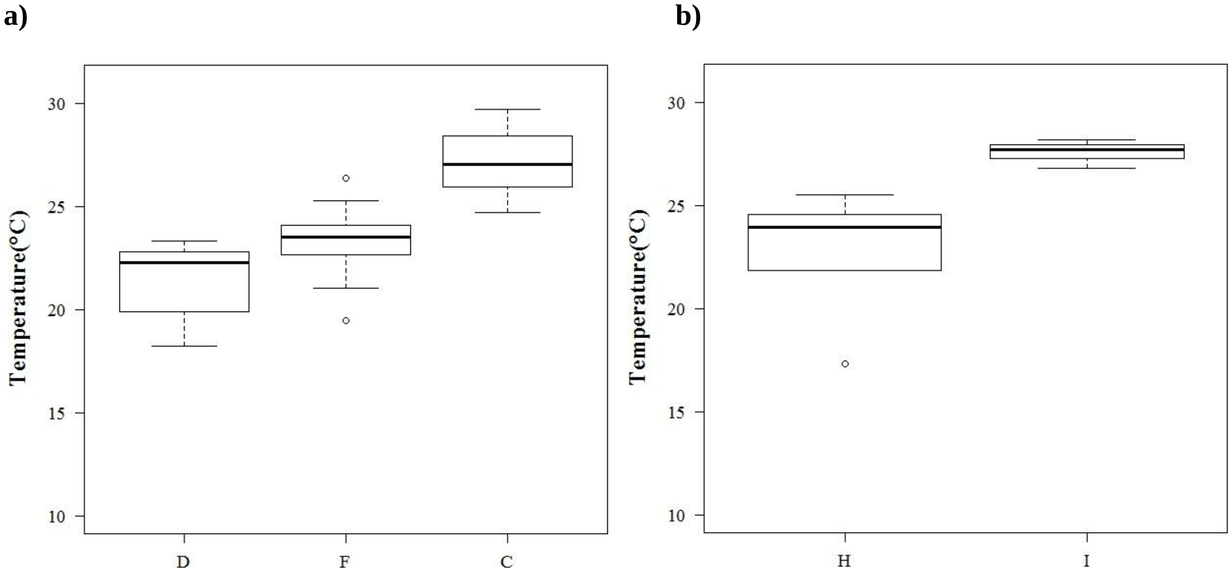
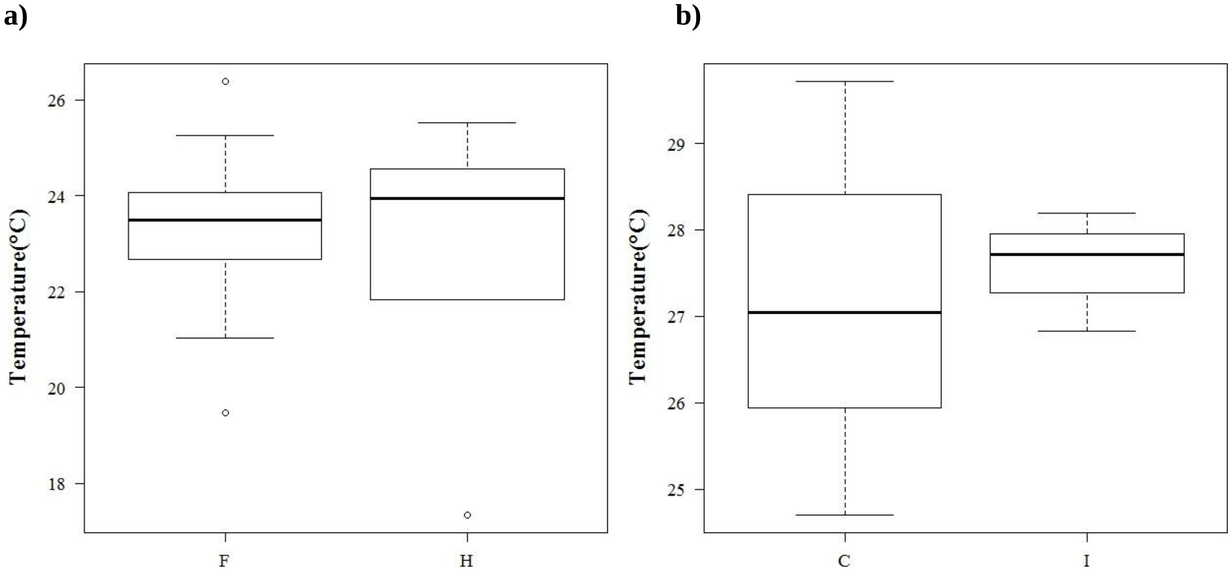


Figure 4.8: Boxplot showing median, quartiles, and range of thermal preferences within species and genera from different sites **a)** *Pseudobarbus* spp., and, **b)** *Sandelia capensis*
(**C**: *Pseudobarbus burgi*, **D**: *Pseudobarbus calidus*, **F**: *Pseudobarbus burchelli*, **H**: *Sandelia capensis* (Amandel), **I**, *Sandelia capensis* (Berg))



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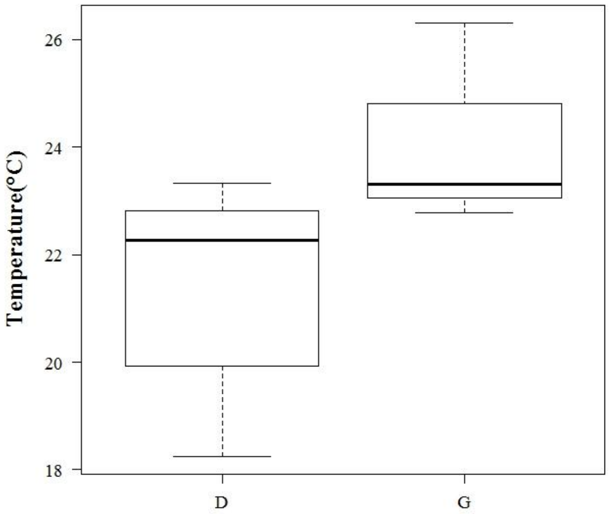

Figure 4.9: Boxplot showing median, quartiles, and range of thermal preferences of species within sites **a)** Amandel River **b)** Berg River, and **c)** Rondegat River

(**C**: *Pseudobarbus burgi*, **D**: *Pseudobarbus calidus*, **F**: *Pseudobarbus burchelli*, **G**: *Austroglanis gilli*, **H**: *Sandelia capensis* (Amandel), **I**, *Sandelia capensis* (Berg))

Table 4.3: Results of linear models comparing inter-species, and inter-site thermal preferences of CFR species

	Species/Genus	**Sites**	***p***	**DF**	***F***
Species/Genus	*Pseudobarbus* spp.	Amandel, Berg, Rondegat	<0.01	2, 24	17.41
	Sandelia capensis	Amandel, Berg	<0.05	1, 7	6.90
Sites	*Pseudobarbus burchelli*, *Sandelia capensis*	Amandel	>0.05	1, 13	0.068
	Pseudobarbus burgi, *Sandelia capensis*	Berg	>0.05	1, 7	0.16
	Austroglanis gilli, *Pseudobarbus calidus*	Rondegat	<0.05	1, 13	4.85

4.4.3 Seasonal effects on thermal preference

Preferences between spring and summer were found to be significantly different in *P. burgi* (Figure 4.10-11), with summer preference being significantly higher than spring preference in the species (residual standard error: 1.53 on 7 degrees of freedom, multiple R^2= 0.77, adjusted R^2= 0.73, $F_{1,\ 7}$= 23.15, p= 0.002).

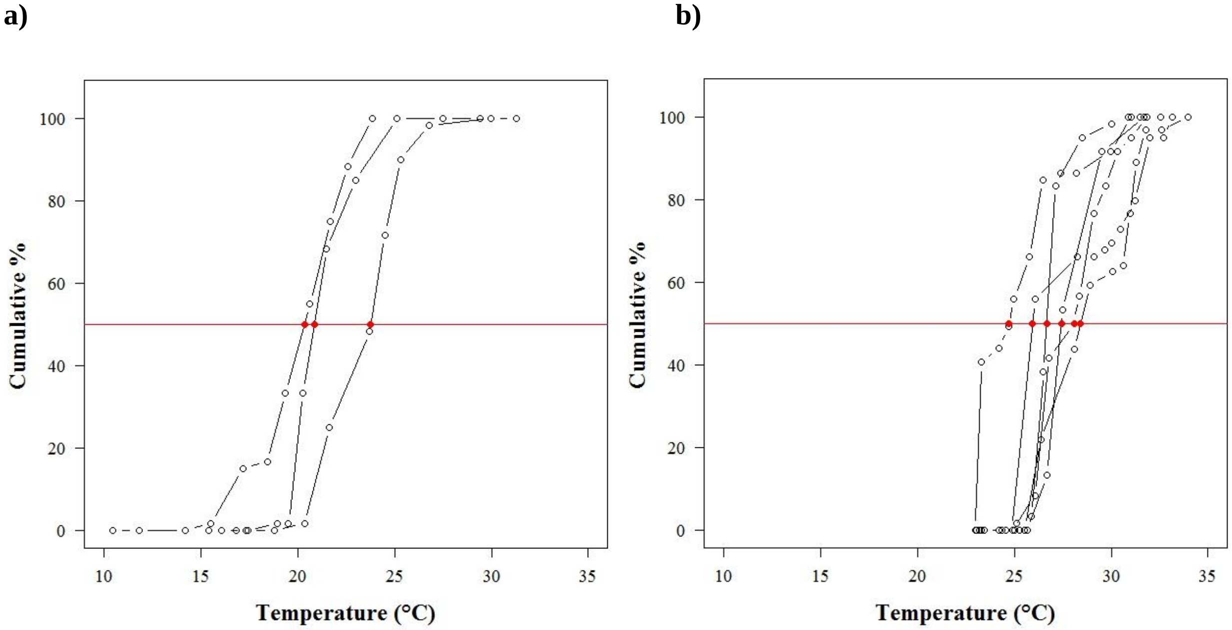

Figure 4.10: Thermal preferences of *Pseudobarbus burgi* from the Berg River in **a)** spring, and **b)** summer

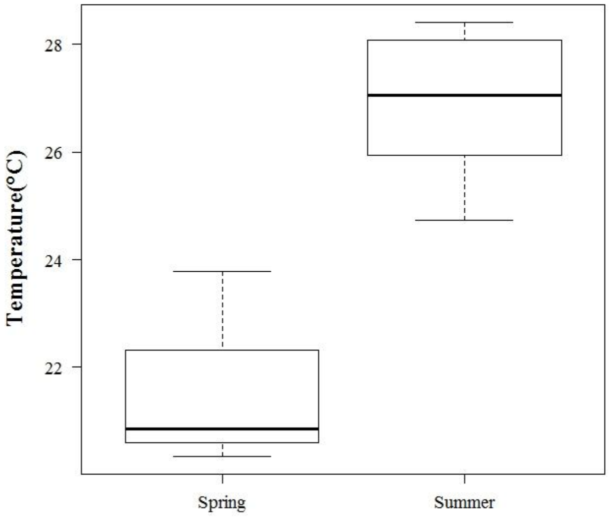

Figure 4.11: Boxplot showing median, quartiles, and range of thermal preference for *Pseudobarbus burgi* from the Berg River in spring and summer

4.4.4 Correlates of thermal sensitivity

A positive linear relationship was found between CT_{max} and preferred temperature including all species in analyses (Table 4.4, Figure 4.12a). During the week of experiments, thermal data for the Berg River, and for the control groups, showed extreme warming. When Berg River species (*P. burgi* and *S. capensis*) were excluded from the analyses, the relationship was found to be stronger (Table 4.4, Figure 4.12b).

Table 4.4: Results of linear models showing relationship between thermal tolerance and thermal preference

Species	**Multiple R^2**	**s^2**	**p**	**DF**	**F**
Austroglanis gilli, Galaxias zebratus, Pseudobarbus burchelli, Pseudobarbus burgi, Pseudobabarbus calidus, Sandelia capensis (Amandel), *Sandelia capensis* (Berg)	0.52	1.36	0.066	1, 5	5.48
Austroglanis gilli, Galaxias zebratus, Pseudobarbus burchelli , Pseudobarbus calidus, Sandelia capensis (Amandel)	0.88	0.72	0.018	1, 3	21.47

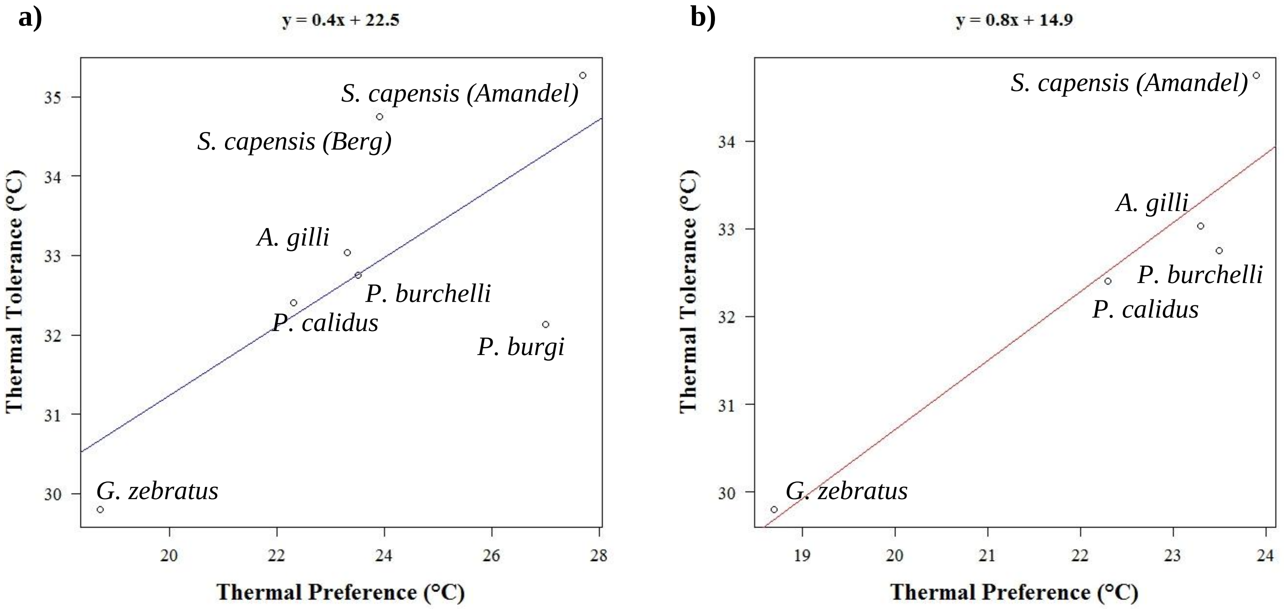

Figure 4.12: Plot showing the relationship between thermal preference and thermal tolerance for **a)** all six species, and **b)** five species (*P. burgi* and *S. capensis* (Berg) removed)

4.5 Discussion

4.5.1 Effects of spatial and temporal changes in temperature

The fluctuation of temperature over space and time is natural and different species have evolved to exploit these changes to varying degrees (Gilchrist, 1995). Spatial variation in habitat characteristics (including temperature) may influence the breadth and magnitude of thermal preference in fish, as they exhibit phenotypic plasticity to the immediate environment or habitat. While there were differences in the magnitude and breadth of thermal preferences observed between some of the species examined in this study, species and genera from different sites did not show similar thermal preferences (i.e. inter-stream difference). At the species level, *S. capensis* showed a significant difference in thermal preference, while at the genus level *Pseudobarbus* spp. also selected significantly different preferred temperatures. This suggests an adaptation to the site/habitat's thermal regime. In fact, Freidenburg and Skelly (2004) showed that in response to different habitat selection pressures ectotherms were found to locally adapt and undergo rapid speciation, supporting this statement. Since there are a range of metrics that are specific to the different sites (the thermal 'signature'; see Table 3.1, Chapter 3) the species may be responding to the thermal breadth within the site. Whether this is speciation, or phenotypic plasticity would need to be determined by genetic studies.

Although thermal preferences were higher for *S. capensis* (Amandel and Berg Rivers) than for *Pseudobarbus* spp., no difference in thermal preference was found within sites for the genus, suggesting that the species within the genus exploit similar thermal niches. This selection or partitioning of thermal preferences between species within a community is commonly called thermal niche partitioning (Magnuson et al., 1979). Gilchrist (1995) takes this idea a step further, suggesting that fish communities form thermal guilds. If the main genera of freshwater fish in the CFR do in fact belong to a thermal guild, then competition for thermal resources may be occurring within the group, rather than with other genera. Referring to temperature as an ecological resource, Magnuson et al., (1979) liken the selection of preferred temperature in fish to the selection of more 'traditional ecological resources' such as food and favourable spawning sites. In this regard, tradeoffs between the acquisition of resources may manifest and could explain the difference in preference within the Rondegat River. The significant difference found in temperature selection of *A. gilli* and *P. calidus* within the site suggest that niche partitioning may be at play in the Rondegat River. Given that there are no longer non-native species present at the sampling site following their removal (Marr et al., 2012), this is not unusual. Notwithstanding temperature, *A. gilli* and *P. calidus* also exploit different biotic ecological resources such as food type. *A. gilli*, may be optimising its foraging by spending time in a warmer microhabitat than that of *P. calidus*, thus maximising its own fitness. Similarly, *P. calidus* may be exploiting optimal habitat by favouring lower temperatures.

Only one study within this chapter examines the temporal effects of temperature on species; however studies concerning the topic are plentiful. Thermal history can have a significant effect on the selection of preferred temperature by fish (Cherry et al., 1977), and this has been shown by comparing the temperature metrics and species preference in this study (Table 3.1, Chapter 3). Since temperature data existed for the sites, thermal history was known, and it was thus possible to elucidate what the true preferences of the species were at the time of collection (Considering that fish were taken from their natural habitat and experimented on immediately). Reynolds and Casterlin (1979) first alluded to this idea, but until now this has not been tested. Taking fish directly from a known environment avoids multiple acclimation temperatures (and associated stress) and is indicative of the true thermal preference in response to the environmental temperature at the time. Furthermore, this also removes complexity in the experimental design as the organisms were neither stressed; rationed; kept in unnatural group sizes; nor exposed to any other unnatural phenomena that would have been experienced in laboratory acclimation.

More so than any other climate-induced change, seasonal changes are likely to affect species in freshwater ecosystems. Organisms are strongly influenced by thermal cues, and these cues can initiate or prohibit important life history events such as spawning, migration, emergence (in macroinvertebrates), and reproduction (Vannote and Sweeney, 1980). This study showed that there is a significant difference in spring and summer preferred temperature of *P. burgi*, suggesting an influence of thermal history. This corroborates the findings of significant site effect on thermal preference of species. However, ontogeny should be considered together with temporal effects, as life history is strongly influenced by seasonal cues, and changes in preferences may not be temperature-mediated alone, but a function of the life stage (McCauley and Huggins, 1979; Clark and Green., 1991).

Coutant et al., (1984) found that spring-summer temperature selection of juvenile striped bass was significantly higher than winter and autumn temperature (viz 24-27ºC and 20-25ºC respectively). However, Haro (1991) found that in Atlantic eels (genus *Anguilla*), preferences were not significantly different between life-history phases. Haro (1991) further proved that acclimation temperatures, photoperiods, illumination regimes, or sexual maturation state had no effect on the thermal preference. These findings suggest that there may or may not be an effect of ontogeny or life-history stage, warranting further investigation for native species in the CFR.

4.5.2 Species interactions in response to warming

Thermal preference is a ‘plastic’ response to habitat temperature, and should be used with caution to determine species distribution boundaries. However, these data can be used to elucidate what is likely to happen in the microhabitat selection of both native and non-native species (thermal selection of different species under climate change scenarios). Theoretically, niche differentiation would reduce competition as species ‘share’ resources to maximise their own fitness (Magnuson et al., 1979). However, non-native species may not have similar thermal requirements to native species, and thus do not compete directly for thermal resources. Non-native species, while competing for habitat, may indirectly eliminate the preferred temperature resource from utilization by native species, and may thereby influence the distribution patterns of native fish. At present it is difficult to predict thermal microhabitat partitioning. Of the 20 species listed by Weyl et al., (2014) as the main established non-native fish species in the CFR, thermal preference data are available for 13 species (Table 4.5). If such data do exist for the other seven species, they have either not been published or have not been released in peer-reviewed articles. Drawing inferences on temperature selection in fish communities based on an incomplete dataset is tentative as interactive effects of data-deficient species may be significant and these must be acknowledged.

Table 4.5: Thermal preferences of native and main non-native species in the Cape Floristic Region

Species	**Preference (^{0}C)**	**Reference/Source**
Native		
Austroglanis gilli	23.3	*This study*
Pseudobarbus calidus	22.3	*This study*
Galaxias zebratus	18.7	*This study*
Pseudobarbus burchelli	23.5	*This study*
Pseudobarbus burgi	27	*This study*
Sandelia capensis	23.9-27.7	*This study*
Non-native*		
Carassius auratus	28-30	Roy and Johansen (1970); Reynolds et al., (1978); Reynolds and Casterlin (1979e)
Clarius gariepinus	30	Britz and Hecht (1987)
Ctenopharyngoden idella	na	na
Cyprinus carpio	29-32	Pitt et al., (1956); Neill and Magnuson (1974); Reynolds and Casterlin (1977)
Gambusia affinis	28-31	Bacon et al., (1967); Winkler (1979)
Labeo capensis	na	na
Labeobarbus auneus	na	na
Lepomis macrochirus	30.9-32.3	Ferguson (1958); Beitinger (1974); Neill and Magnuson (1974); Cherry et al., (1975); Cherry et al., (1977); Reynolds and Casterlin (1978e)
Oncorhyncus mykiss	12.7-16.1	Schurmann et al., (1991)
Oreochromis auneus	na	na
Oreochromis mossambicus	32.2	Stauffer (1986)
Micropterus dolomieu	28-31.3	Ferguson (1958); Cherry et al., (1975); Cherry et al., (1977); Reynolds and Casterlin (1978b)
Micropterus floridanus	8-32.0	Koppelman et al., (1988)
Micropterus punculatus	30.8-32.1	Cherry et al., (1975); Cherry et al., (1977)
Micropterus salmoides	28.5-32	Fergusen (1958); Neill and Magnuson (1974); Reynolds et al., (1976); Reynolds and Casterlin (1978b); Venables et al., (1978)
Perca fluviatilis	23.7-26.8	Jobling (1981)
Pseudocrenilabrus philander	na	na
Salmo trutta	14.3-17.6	Fergusen (1958); Cherry et al., (1977)
Tilapia sparmanii	na	na
Tinca tinca	na	na

** Predominant non-native species in the CFR from Weyl et al., (2014)* Much lower thermal preferences were observed for non-native species, *Salmo trutta* and *Oncorhyncus mykiss* (Table 4.5), than for native species. In this sense, there is no competition for thermal resources, and it is likely that thermal niche partitioning already drives some of the physical habitat partitioning. This could be beneficial to native species if water temperature increases as it will ‘force’ non-native species into narrower thermal niches. Similarly, non-native species with very low thermal preferences can be expected to impact more strongly on native fish communities in cold systems as it increases the thermal preference breadth. For instance if *S. trutta* and *O. mykiss* establish and proliferate in systems where species like *G. zebratus* are abundant, it may directly impact fish abundance and community structure in these sites (such as the Driehoeks River). This is not an effect of climate change *per se*, as this may already be impacting native populations in cold systems. On the other hand, warm adapted species such *Clarius gariepinus* and *M. salmoides* could be a major threat as regional climate change drives temperature increases in freshwater bodies. *C. gariepinus* has a higher thermal preference than the most tolerant native species from this study, *S. capensis* (Table 4.5). It is likely that this species and other non-native African species would impose the greatest competition on native fish.

Boscarino et al., (2007) showed that avoidance and selection of temperature was based on the presence of the prey species within a vertical gradient tank. Predators may move out of their zone of comfort to maximise the amount of food they can consume. In that study, predators were found in a thermal zone 10ºC greater than their real thermal preference. Predator-prey interactions thus determine where fish position themselves in a habitat or thermal gradient. Jackson et al., (2001) suggest that predator species may actually migrate to follow prey species migration. Should the prey species (be it fish or invertebrates) migrate; the predator will be required to leave its preferred habitat. For native fish, the movement of preferred invertebrate species as a result of climate change may influence their distribution, while the distribution of non-natives will be determined by the presence of natives. De Staso and Rahel (1994) used a laboratory stream design to compare the competitive advantage of two trout species to different temperatures. This approach would identify which species are likely to perform better under different water temperature scenarios, but will require the stocking of such ‘streams’ with native and non-native species, and would require ethical clearance as a high rate of mortality is likely.

4.5.3Thermal experiments on endangered fish: correlates of thermal sensitivity

Two factors require consideration with regards to the type of experiments opted for in the determination of thermal sensitivity: firstly, the IUCN status of the target species, and secondly the appropriateness of the methodology for elucidating the desired parameter. Careful consideration should be given to the conservation status of fish on which experiments are to be undertaken to minimise mortality and stress on the organisms. However, there are few methods besides the thermal preference experiments, which do not require lethal methodology to elucidate chronic exposure thresholds.

In terms of appropriate thermal methodology, the LT_{50} experiment has long been used as a standard for determining the effects of chronic exposure on aquatic organisms (Zitko, 1979). LT_{50} is simply explained as the median survival time (in days) of a population until 50% of the population dies (Zitko, 1979). LT_{50} data for freshwater invertebrates in the CFR obtained from studies like Dallas and Rivers-Moore (2013) have been used to determine MWAT (Maximum weekly Allowable Temperature) metrics for rivers/sites. This is critical for conservation given regional climate change predictions. Previous studies have found a strong correlation between LT_{50} and CT_{max} (Dallas and Ketley, 2011), thus the two complement each other in determining the overall sensitivity of organisms. However, a critique of this method, especially with regards to native and endangered fish, is that it results in a high mortality rate (Beitinger et al., 2000). For this reason, an alternative approach is required.

Thermal preference has also been found to correlate with CT_{max}. As opposed to the LT_{50}, thermal preference gives an indication of optimum temperature, given a range of temperatures. In this regard, the LT_{50} experiment does not account for slight variation in temperature- as there are only a certain number of treatment temperatures and it does not give the organism an opportunity to exploit microhabitats. While such data are useful for determining population survival, it cannot give an indication of the preferred temperature of the species. If the focus is on survival alone, (i.e. which temperatures a population can tolerate for it to remain viable for sufficiently long), then the LT_{50} is more appropriate.

Using data for eight species, Richardson et al., (1994) confirmed that thermal sensitivity (CT_{max}) and preferred temperature were strongly correlated and either parameter could thus be determined via the regression equation developed for that region’s fish. In this study, the aim was not to determine thermal sensitivities, as these have already been determined in Chapter 3. The primary aim of the preference experiment was to determine thermal microhabitat/resource selection and to speculate on how climate change may influence this selection in the future. Nevertheless, this method has been shown to correlate well with thermal tolerance and therefore could also be used as a measure of thermal sensitivity. In this study data were obtained for seven species (from eight populations). While Jobling (1981) used 38 species, Richardson et al. (1994) found that the same regression line could be used for eight species, and the correlation was stronger using fewer species. This supports the validity of the thermal tolerance and preference regressions derived from this study.

4.6 Conclusion

4.6.1 Synthesis of findings

Thermal preferences differed between different populations of the same species, indicating that thermal history may have had significant effect on thermal preference. As temperature metrics showed a clear difference in the thermal signatures of the sites, the effect of the site (thermal history) was evident. Seasonal differences in thermal preference of *Pseudobarbus* spp. supports the suggestion of an acclimation effect attributable to site temperature, as there was a significant difference in thermal preference between seasons (higher thermal preference selected in a warmer season). This could however also be attributable to an ontogenetic effect.

Thermal preference did not differ between unrelated species within a site, suggesting that native species occupy similar thermal niches and may be behaving as a thermal guild (with respect to resource partitioning). The thermal preferences obtained for non-native species suggest that non-native species form a cool-adapted thermal guild compared to native species. Finally, the strong correlation between thermal preference and thermal tolerance suggest that thermal preference could be used as an indicator of thermal sensitivity, and may also be used to determine thermal tolerance via linear regression.

4.6.2 Recommendations

Future research should have a stronger focus on temporal changes in thermal preference as this would assist towards understanding the life history and biology of freshwater fish of which little has been published (Ellender et al., 2017). For instance, juveniles may be more or less susceptible to temperature alteration. Brandt (1980) found that adults and juveniles selected different water temperatures to reduce competition. It is possible that native fish behave similarly; however, additional experiments are required to confirm whether thermal preferences actually differ at the different life stages. Furthermore, genetic studies would assist toward identifying whether preference is in fact a plastic response, thereby indicating how rigid/resilient species may be to expected climate change.

In a study on New Zealand's freshwater fish, Richardson et al., (1994)'s found that preferred temperatures based on laboratory experiments correlated well with occurrence of *G. maculatus* in the field. However, the remainder of the species (eight) from that study were observed occurring at 4-10ºC lower than their preferred temperature in the laboratory (Richardson et al., 1994). This may be the case with the CFR fish given the minimum temperatures recorded for the sites (Table 3.1, Chapter 3). It is apparent that the fish were exposed to temperatures far below their preferred temperature in the laboratory. It is therefore possible that the preferred temperature range has a lower bound that has not been quantified. Real preferred temperatures may be lower when there are compounding variables acting upon species in the wild. These factors will need to be investigated to determine the temporal change in thermal requirements of native fish.

Finally, since this methodology has been successfully implemented in this study to produce the first real data for thermal preference, it is recommended that these protocols are replicated for the remaining native fish in South Africa. This will ensure that preference data are comparable. Although the limitations of the field study (in both design and execution) have been mentioned, it is also recommended that the methodologies be further tested and refined- bearing in mind the logistical feasibility and safely of the test species which is of primary importance.

CHAPTER 5: Synthesis and conclusion

5.1 Synthesis of thesis

5.1.1 Freshwater resources and climate change: a recap

Climate change is not an abnormal phenomenon. Since the beginning of geological time, the planet has undergone changes in its celestial cycles, dictating the distribution of life on earth. In the Anthropocene era, the earth has undergone some of its most drastic changes as anthropogenic disturbance and carbon emissions have increased exponentially since the industrial revolution; so much so that natural climate patterns have been altered. This rate of change in the earth's atmosphere is unprecedented. The IPCC reports that the world has not experienced such rapid change in over 1 000 years (Heino et al., 2009).

The exact effects of climate change in South Africa are region-specific, with the east coast of South Africa expected to experience an increase in precipitation and extreme flooding events (Dallas and Rivers-Moore, 2014). The western CFR is a Mediterranean region. These regions are typically dry over summer months, but an expected increase in drought and decrease in precipitation threaten to reduce the availability of freshwater resources over the critical season. This will have massive implications for agriculture and society, but equally important; freshwater biota. Since the life-histories of freshwater biota (timing of reproduction, spawning, migration, and growth) are closely coupled with seasonal changes in temperature and flow, the increase in magnitude and duration of extreme thermal and hydrological events will affect the fitness of aquatic organisms (including fish).

5.1.2 Implications for freshwater fish in the CFR

The least conservative estimated 4-6ºC increase in water temperature may affect freshwater fish diversity as sensitive species suffer an increase in mortality and decrease in fecundity. Range shifts may also occur in species that have the potential to migrate to thermal refugia (Magoulick and Kobza, 2003). Heino et al., (2009) predict a 500km latitude shift in species distribution in response to doubling of CO_2, and 680km shift with a 4ºC increase in temperature. North-south range shifts or isolation of freshwater species have already been documented due to temperature increase and water withdrawals, and may continue as habitats are gradually disturbed from their natural states (Magoulick and Kobza, 2003). Species from this study may be prohibited from range shifts as they already occupy the upper reaches of their respective rivers (with the coolest temperatures). More so than thermal refuge however, these fish are likely to seek refuge from habitat loss. The effect of water withdrawals and the concomitant decrease in environmental flows may act synergistically to increase water temperature and reduce water levels.

Indirect effects of temperature on native species may also be caused by the movement or disappearance of prey species. Similarly, shifts in more basal trophic components such as benthic algae and invertebrates may have an impact on biological diversity in the CFR. Species physiologies have been shaped by such temperature changes over millennia, shifting their distributions and facilitating their genetic and phenotypic adaptation. However, Heino et al., (2009) note that it is the rate of change that will determine species diversity and the winners and losers of climate change (Somero, 2010).

The main findings from the Chapters 3 and 4 suggest that there will be climate change winners and losers as proposed by Somero (2010). The key determinant of fitness will be physiological resilience. The species that were selected for this study had already been identified by the IUCN as vulnerable, endangered, and critically endangered. However, no published data existed on their thermal tolerances or habitat preferences with respect to temperature. Overall, the loser-species (species that was found to be most sensitive) included *G. zebratus.* The genus *Pseudobarbus* on the whole was also found to be thermally sensitive. Given that this is the most species rich genus in the CFR, and that there is already low diversity in the region's ichthyofauna, this is the group requiring focussed conservation attention. As each species is typically catchment- specific, small isolated populations will be under the harshest selection pressure. Additionally, non-native fish in the region intensify the level of threat experienced by these species. More hardy winner-species *A. gilli* and *S. capensis* were found to be moderately sensitive. This does not however, necessarily negate the threat of climate change on these species as water withdrawal is expected to increase, eliminating and fragmenting preferred habitats.

Similar results were found using the thermal preference approach. CT_{max} and thermal preference were found to correlate well. In this regard, it is advisable to make use of the acute gradient tank approach as it is less stressful on the organism and is a more realistic indication of both temperature optima (preference) and sub-optima (avoided temperatures). The sample size required to produce statistically significant results is however the shortfall of the experiment. The critical thermal method requires far fewer individuals and fewer logistical complications.

Regarding laboratory acclimation prior to experiments, a lengthy acclimation period is not recommended. Given that native fish of the CFR are classified as vulnerable, threatened, or critically endangered (Tweddle et al., 2009), and that the fish have already been exposed to their natural thermal regime, eliminating an acclimation period provides a more appropriate representation of the two parameters (tolerance and preference). An acclimation effect was evident in temperature preference of *P. burgi* between seasons. Moreover, preference was found to correlate with mean daily water temperature for the week of experiments. This affirmed that a range of temperatures are favourable by the organism based on recent thermal history and season. Since both experiments (thermal tolerance and preference) were conducted over the warmest period of the year, during summer low flows, the maximum tolerance and preference in an annual cycle were represented.

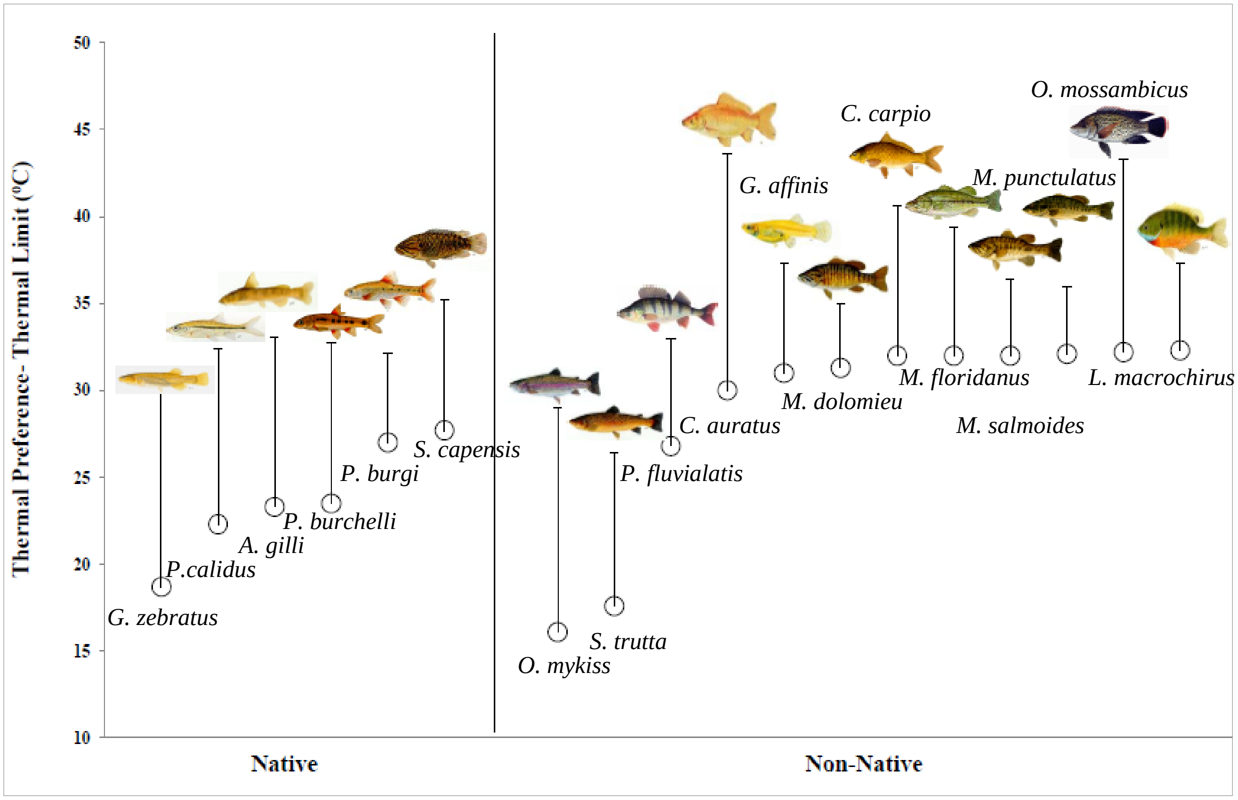

Figure 5.1: Diagram showing known maximum thermal preferences of native and main non-native fish in the Cape Floristic Region (error bars represent the range between the maximum thermal preference and maximum thermal tolerance recorded for the species) (*fish images courtesy of SAIAB*)

Adding another dimension of direct and indirect threat to the survival of native species are the presence and distribution of non-native fish. Figure 5.1 shows the thermal preferences and maximum tolerances (represented as error bars) for both native and non-native fish in the CFR.Thermal tolerances and preferences were found to exceed two of the most prolific invasive non-native species rainbow and brown trout (*O. mykiss* and *S. trutta* respectively). However, highly invasive species, large and smallmouth bass (*M. dolomieu* and *M. salmoides*), were found to be more thermally tolerant. These data suggest that the level of threat to native fish may increase in future as water temperature escalates. Notwithstanding loss of habitat, increased water temperature may thus influence species interactions and ultimately species distributions. The data shown in Figure 5.1 however do not represent all the non-native species as many of these are data deficient. These data (and those that are absent) will be more crucial to interpreting interactive effects of temperature in the future. To this end, modeling distribution and population dynamics are necessary, but require data for all species in the CFR.

5.2 Conclusions and recommendations

To understand and evaluate the ecological consequences of elevated water temperature on the native freshwater fish in the CFR, thermal experiments are required. Two experimental approaches were used in this study, both of which have their respective benefits for elucidating temperature requirements of fish. This study has produced the reference thermal tolerance and preference data for seven native fish in the CFR, and has examined the link between *in-situ* water temperature and these parameters.

The data produced in this study allowed native fish to be ranked in terms of their thermal sensitivity and associated likely vulnerability to regional climate change. It is advisable to strengthen conservation efforts in the CFR for the most sensitive species by allocating fiscal and physical resources to maintaining suitable habitat conditions for native fish. To do so, the current non-native eradication plans should be directed towards catchments where climate warming impacts are expected to be most severe. The severity of climate change may also be minimised by more prudent water allocation to agriculture. Water abstraction will require more stringent monitoring and management. The reported illegal tampering with water meters for instance thwart management objectives in water-stressed catchments. Habitat restoration may include physical restoration or alteration of habitat to improve flow and minimise diurnal temperature extremes. Habitat restoration may also include the restoration efforts that allow the recolonisation of native species, such as was successful in the Rondegat River. On the whole the CFR requires more protected areas specifically designed to safeguard native species and to act as repositories for vulnerable and critically endangered species.

Further research is warranted on the early life stages of native fish (from the egg to juvenile stage). This would improve understanding on the potential effects of thermal alteration on population dynamics and overall fitness of different species. Since temperature is expected to affect reproductive strategies of fish (Angilletta et al., 2006), the reproductive fitness of adults and egg sensitivity to temperature (Bobe and Labbé, 2010), require further examination at different temperature scenarios. As thermal history was shown to vary spatially and temporally, further research examining the relationship between thermal history and both parameters are also necessary. These data can be linked to field studies to improve the overall understanding of how temperature affects fish in their natural habitats. The benefits of field studies are invaluable as they can also expose potential distribution corridors or barriers. Terrestrial conservation has long since moved away from the old-fashioned style of protected areas to protected corridors, allowing for climate-induced range shifts. This kind of predictive management will be required to manage freshwater fish populations in the CFR. Predictive theory however requires both field and laboratory data as (1) the suitability of the physical habitat needs to be assessed, and (2) tolerant, sensitive, specialist, and generalist species need to be identified (Chevin et al., 2010), respectively.

Finally, continued long term monitoring of *in-situ* water temperature is, and will continue to be, the holy grail of freshwater research. Hourly water temperature data are necessary to monitor long term change in freshwater ecosystems, and are required to produce thermal thresholds of concern. To continue collecting experimental and field data, logistical support and cooperation from institutions such as the South African Institute for Aquatic Biodiversity (SAIAB), CapeNature, Freshwater Research Centre, and universities within the greater CFR will be required.

Ultimately, the CFR holds a unique group of aquatic fauna that are highly endemic and specialised to the regions unique freshwater ecosystems. Climate change threatens biological diversity, the degree to which has not yet been fully realised. Now, there is evidence of the thermal stress likely to be caused by climate change on native species. Beyond this thesis, these data must be disseminated in an appropriate forum to effect change in freshwater conservation. Freshwater research requires a cross-disciplinary approach (of the biological and social sciences) to have an impact on the conservation of sensitive aquatic biota throughout the CFR. The objective of these studies is not only to secure freshwater ‘oases’ for native fish (Heino et al., 2010), but to ensure the longevity of South Africa’s freshwater ecosystems for future generations.

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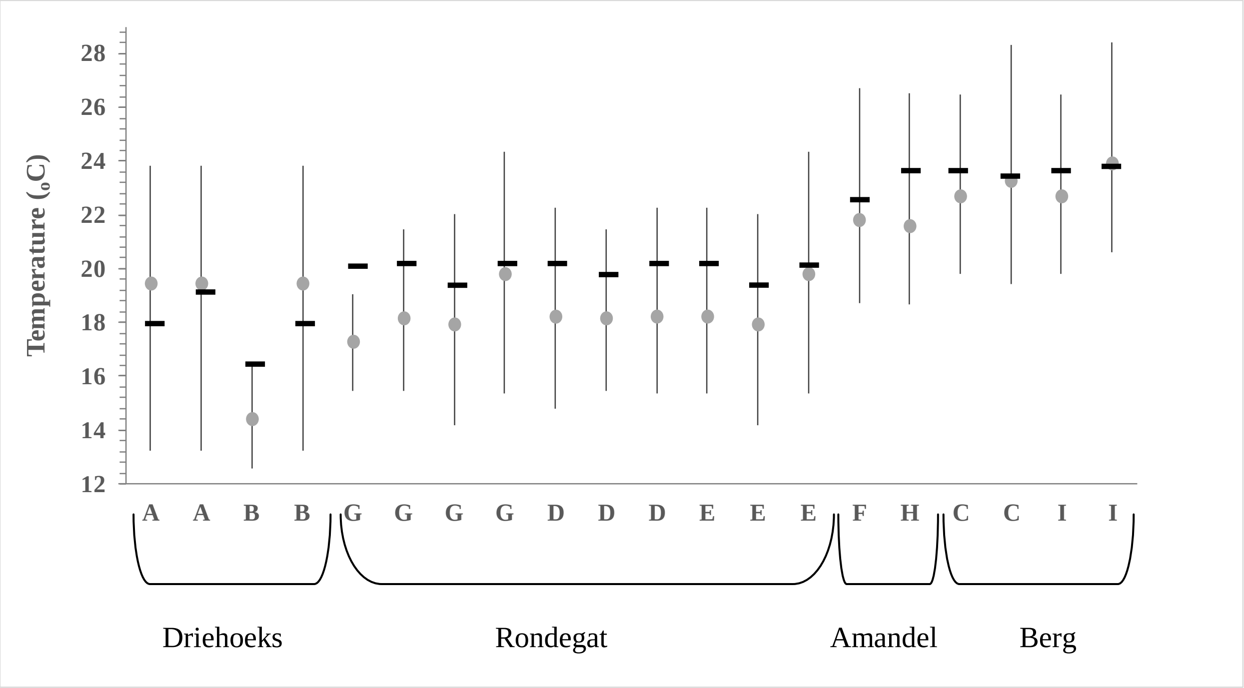
APPENDICES

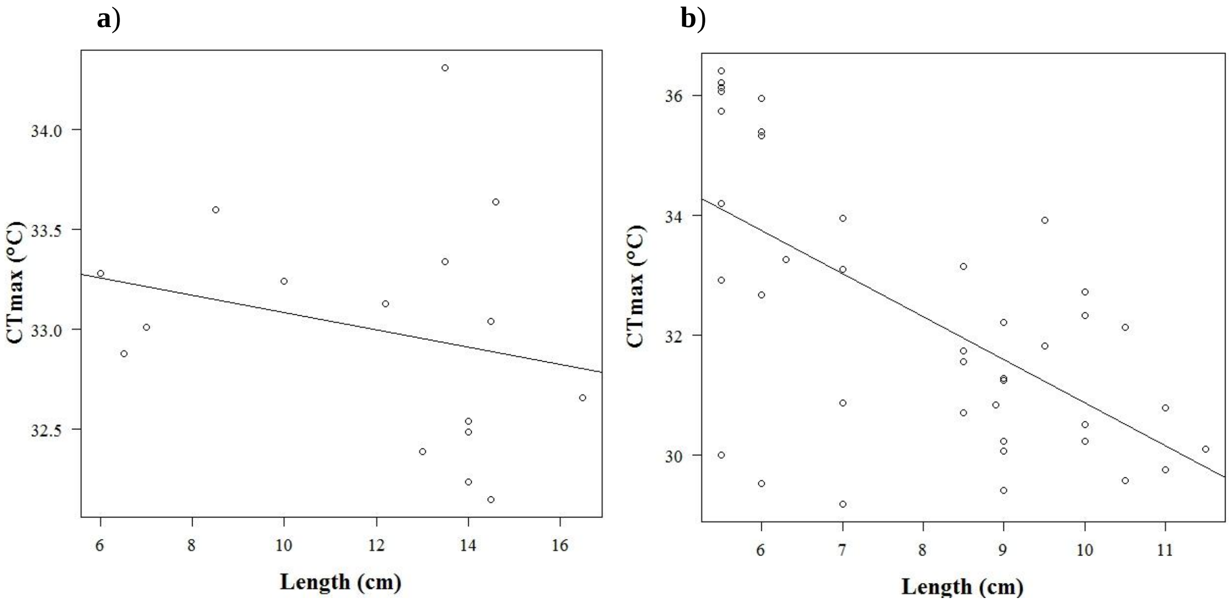
Appendix A: Median CT$_{max}$ for each holding temperature of eight species, parentheses denote sample size

Species	**River**	**Code**	**Date**	**CT$_{max}$ (^{0}C)**		**Holding temperature (^{0}C)**	**Holding temperature (S^2)**
A. gilli	Rondegat	G	11-11-2015	32.54	(3)	20.90	1.70
			13-11-2015	32.39	(3)	21.00	1.20
			15-11-2015	33.04	(7)	20.10	1.00
			16-11-2015	33.60	(3)	21.00	0.40
G. zebratus	Driehoeks	A	23-11-2015	29.69	(13)	18.70	0.80
			24-11-2015	29.85	(19)	19.90	0.20
P. burchelli	Amandel	F	10-12-2015	32.75	(32)	23.50	1.40
P. burgi	Berg	C	04-12-2015	31.50	(4)	24.40	1.30
			06-12-2015	32.22	(37)	24.20	0.70
P. calidus	Rondegat	D	12-11-2015	31.95	(9)	21.00	0.80
			13-11-2015	32.67	(8)	20.60	1.60
			14-11-2015	32.87	(6)	21.20	1.20
P. phlegethon	Driehoeks	B	22-11-2015	30.73	(20)	17.30	0.90
			23-11-2015	29.64	(7)	18.70	0.80
P. phlegethon	Rondegat	E	14-11-2015	32.60	(9)	21.20	1.20
			15-11-2015	32.26	(6)	20.10	1.00
			16-11-2016	32.55	(11)	21.00	0.40
S. capensis	Amandel	H	09-12-2015	34.75	(30)	24.40	0.80
S. capensis	Berg	I	04-12-2015	34.46	(6)	24.40	1.30
			05-12-2015	35.39	(33)	24.60	0.70

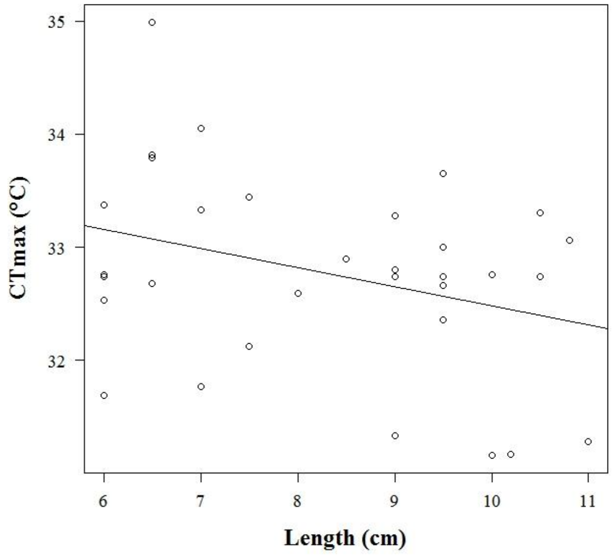
**Fish were retained in aerated holding buckets for 24h prior to commencement of experiments.*



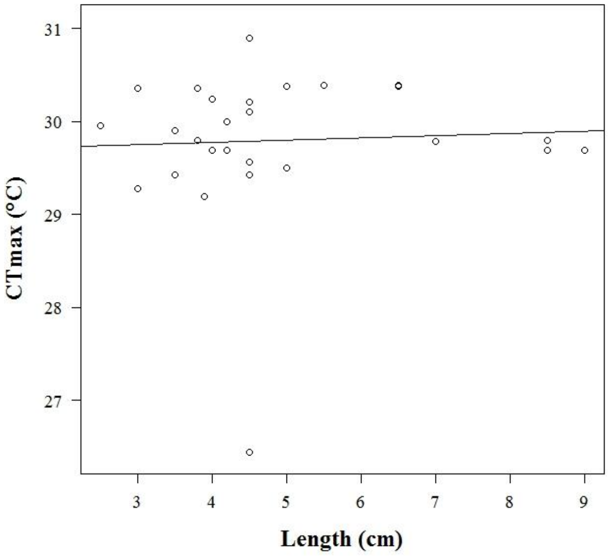
Appendix B: Daily Mean (●), minimum (lower bar) and maximum (upper bar) river temperatures at each site for the period preceding and including the experimental period. The holding temperature for the 24 hours preceding each experiment is indicated (▬). Species indicated by code.



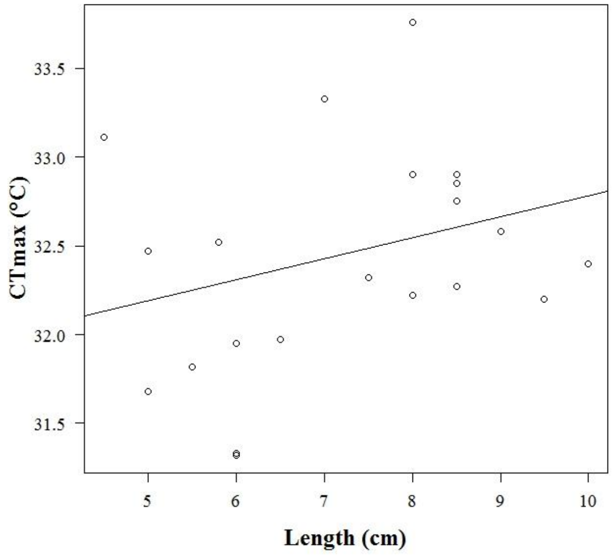
c)


35
34
33
32
CTmax (°C)
6
7
8
9
10
11
Length (cm)

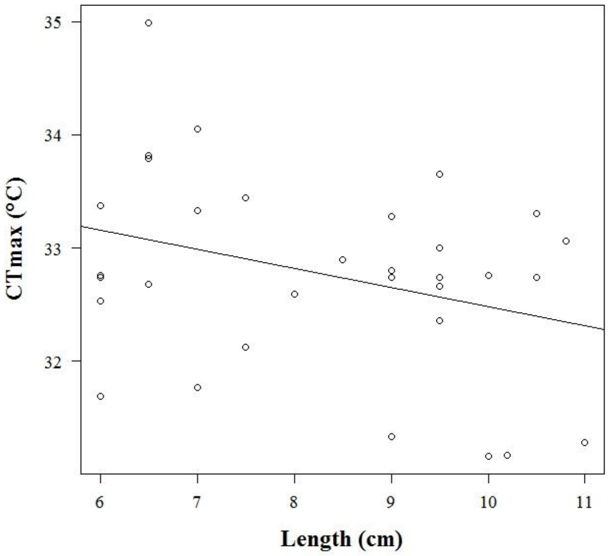
d)


31
30
29
28
27
CTmax (°C)
3
4
5
6
7
8
9
Length (cm)

e)


33.5
33.0
32.5
32.0
31.5
CTmax (°C)
5
6
7
8
9
10
Length (cm)

f)


35
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CTmax (°C)
6
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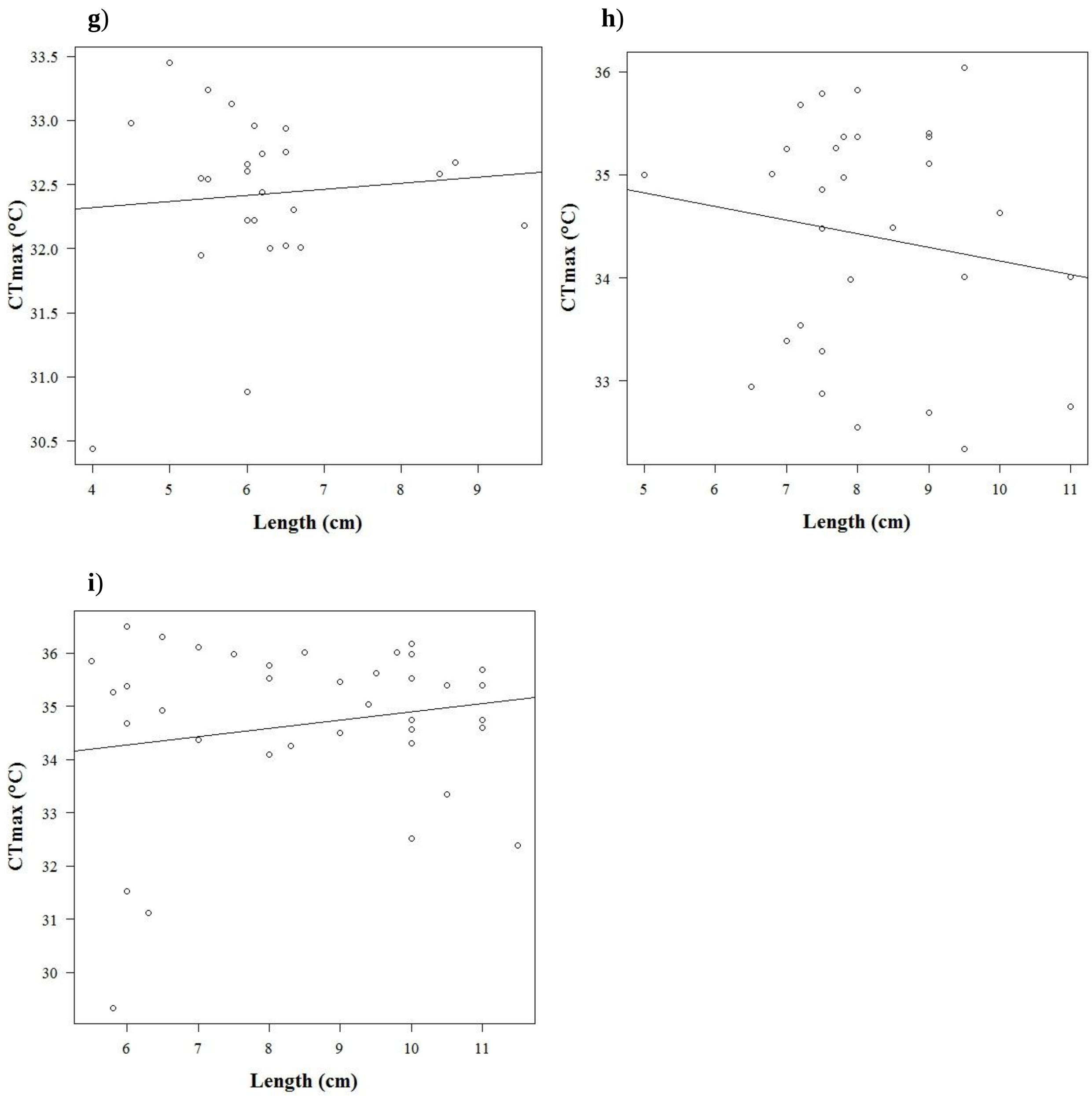
10
11
Length (cm)



Appendix C: Total Length (cm) and CT_{max}(ºC) correlations for **a**) *A. gilli*, **b**) *G. zebratus*, **c**) *P. burchelli*, **d**) *P. burgi*, **e**) *P. calidus*, **f**) *P. phlegethon* (Driehoeks), **g**) *P. phlegethon* (Rondegat), **h**) *S. capensis* (Amandel), **i**) *S. capensis* (Berg)

Appendix D: Statistical results for Total Length and CT_{max} correlations

Species	**Mean Length (TL)(cm)**	**Minimum Length (cm)**	**Maximum Length (cm)**	**SD**	***p***	***r***
S.capensis (A)	8.2	5.0	11.0	1.3	0.42	-0.15
P.burchelli	8.3	6.0	11.0	1.7	0.07	-0.33
S.capensis (B)	8.5	5.5	11.5	1.9	0.26	0.18
P.burgi	8.0	5.5	11.5	2.0	0.00	-0.62
P.phlegethon (D)	6.9	6.0	8.2	0.7	0.51	0.14
G.zebratus	4.9	2.5	9.0	1.7	0.80	0.05
B.calidus	7.4	4.5	10.0	1.7	0.17	0.30
A.gilli	12.0	6.0	16.5	3.3	0.36	-0.25
P.phlegethon (R)	6.2	4.0	9.6	1.2	0.67	0.09

Appendix E: Median preference by experiment for six species in alphabetical order

Species	**River**	**Date**	**Tank**	***n***	**Median**	**Total *n***	**Preference (^{0}C)**
Austroglanis gilli	Rondegat	11-11-15	A	4	26.3	14	23.3
			B	5	23.3		
		13-11-15	A	5	22.8		
Pseudobarbus calidus	Rondegat	10-11-15	A	5	22.6	60	22.3
			B	5	20.7		
			C	5	18.5		
		11-11-15	C	5	23.0		
		12-11-15	A	5	23.3		
			B	5	22.3		
			C	5	23.3		
		13-11-15	B	5	18.2		
			C	5	21.9		
		14-11-15	A	5	22.5		
			B	5	19.1		
			C	5	22.2		
Galaxias zebratus	Driehoeks	21-11-15	A	5	13.1	35	18.7

			B	5	13.9		
			C	5	12.9		
		22-11-15	A	5	18.7		
			B	5	20.2		
			C	5	21.2		
		23-11-15	C	5	24.3		
Pseudobarbus burchelli	Amandel	10-12-15	A	5	23.6	45	23.5
			B	5	22.7		
			C	5	19.5		
		12-12-15	A	5	24.1		
			B	5	21.0		
			C	5	22.8		
		08-12-15	A	5	23.5		
			B	5	26.4		
			C	5	25.3		
Pseudobarbus burgi	Berg	21-22-015	A	5	26.7	29	27.0
			B	4	24.7		
			C	5	27.4		
		05-12-15	A	5	25.9		
			B	5	28.1		
			C	5	28.4		
Sandelia capensis	Amandel	11-12-15	A	5	23.4	30	23.9
			B	5	21.8		
			C	5	17.3		
		09-12-15	A	5	25.5		
			B	5	24.6		
			C	5	24.4		
Sandelia capensis	Berg	04-12-15	A	5	26.8	15	27.7
			B	5	27.7		
			C	5	28.0		