

**Tolerating climate change:
A study on the influence of thermal history on thermal
tolerance of *Galaxias zebratus* in rivers of the Cape
Peninsula, South Africa**

by

Toni Olsen (OLSTON002)

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



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Signed by candidate

04 July 2018

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Abstract

Global climate change models predict a reduction in rainfall and rise in air temperature for the Cape Peninsula of South Africa's Cape Floristic Region (CFR). The CFR is a biodiversity hotspot renowned for its high level of floral endemism, but the high level of endemism also applies to the region's freshwater fish assemblage. Whereas the current threats to endemic freshwater fish include habitat modification, water abstraction, pollution and impacts of non-native species, climate change is predicted to further exacerbate negative impacts on fish communities. The endemic CFR fish species, Cape Galaxias, *Galaxias zebratus* Castelnau, 1861, is widespread throughout the region, and occurs in both non-perennial and perennial rivers, and wetlands. The species is thought to be a relict group of ancient species originating from the break up of Gondwanaland 180 – 135 million years ago. Endemic CFR freshwater fish, like *G. zebratus*, may be sensitive to the thermal regime of their environment and may thus be influenced by climate warming. The most commonly used experimental approach for determining the effect of elevated temperature on freshwater biota is the Critical Thermal Method (CTM). The CTM determines the upper thermal tolerance limit or critical thermal maximum (CT_{max}) of a species. Thermal history is the range of temperatures experienced by an organism in its natural habitat over time and this may be an important factor determining the thermal tolerance of species. The aim of this study was to evaluate the influence of thermal history, reflecting a stream's thermal profile, on upper thermal tolerance limits of *G. zebratus*. We hypothesised that *G. zebratus* from warmer sites would have a higher CT_{max} than individuals at cooler sites. To examine the influence thermal history has on the thermal tolerance of *G. zebratus*, hourly water temperature data were collected and the CT_{max} values were determined for fish ($n=30$ per site) from 10 different sites in rivers of the Cape Peninsula. The CT_{max} values from all sites for the November-December experimental period ranged from 30.00°C to 32.45°C. CT_{max} values for all sites from the January-February experimental period ranged from 31.29°C to 33.42°C. Upper thermal tolerance limits of *G. zebratus* increased from the November-December experiments to the January-February experiments. Regression analyses show that *G. zebratus* upper thermal tolerance limits are significantly influenced by its thermal history as characterised by the seven day moving average of daily mean (Mean_7) two weeks preceding the experiments, implying that changes to the thermal regime will influence the thermal tolerance of *G. zebratus*. The resultant regression equation allows *G. zebratus* CT_{max} to be predicted by thermal history based on Mean_7, providing valuable information to set thermal limits of *G. zebratus* and guide future research. This is the first study on the thermal ecology of *G. zebratus* in the CFR and in Africa. The data not only enhance understanding of the thermal ecology of the species, but also further our understanding of their potential vulnerability to climate change.

Key Words: Cape Peninsula, Cape Floristic Region, climate change, freshwater fish, *Galaxias zebratus*, upper thermal tolerance limit, thermal history

Chapter 1: Introduction

1.1 The impact of climate change on Mediterranean regions

Compared to other climate regions of the world, Mediterranean regions are likely to experience the drastic effects of climate change as rainfall decreases and temperatures increase (Filipe et al., 2012). According to climate projections, this region's climate system will become hotter and drier in the future resulting in elevated temperatures of freshwater systems (New, 2002). Summer air temperature maxima are predicted to increase by 4 to 6°C in the intermediate future (2046 – 2065) and mean annual rainfall is predicted to decrease by 20 to 40mm in the more distant future (2081-2100) (Schulze, 2011). This may be translated into a ~2 °C increase in mean water temperature and a ~20% decrease in flow (Dallas & Rivers-Moore 2014). Regions of the world characterised by a Mediterranean type climate include western Australia, central Chile, coastal California, areas around the Mediterranean Basin and the Cape Floristic Region (CFR) of South Africa (Filipe et al., 2012). Intensified climate change will significantly impact vulnerable ecosystems of Mediterranean regions (IPCC, 2007). A Mediterranean climate is characterised by mild to cool, wet winters and hot, dry summers, which differs from other southern African countries that receive summer rains and experience dry winters (Filipe et al., 2012; Midgley et al., 2005). This weather pattern is driven by subtropical high-pressure cells moving towards the poles in summer and towards the equator in winter (Filipe et al., 2012). In the CFR of South Africa particularly, a reduction in rainfall and the associated reduction in natural flow of rivers will become increasingly challenging to manage (King & Pienaar, 2011). Furthermore, expected increases in water withdrawals for domestic and agricultural purposes on already reduced flows may compound impacts on freshwater ecosystems in the region in the face of climate change (King & Pienaar, 2011).

1.2 The impact of climate change on Mediterranean rivers and their biota

Watercourses of Mediterranean regions are influenced by predictable disturbance events such as floods and droughts (Filipe et al., 2012). Despite aquatic organisms having the ability to withstand and recover rapidly from natural disturbances, climate change-induced changes are predicted to negatively impact freshwater biota (Filipe et al., 2012). Subsequently, temperate regions will become more Mediterranean-like and thus experience further effects on the inhabiting biota (Filipe et al., 2012). The effects of climate change on biodiversity have been well documented in the northern hemisphere, with a general trend of decreased fitness across taxa with increasing temperature (Parmesan, 2006). However, the effects of climate change on biodiversity in the southern hemisphere remain relatively poorly studied (Parmesan, 2006). A decade ago Dallas (2008) predicted that more thermally sensitive fish would modify their distribution range with elevated water temperatures. In South Africa, the effects of temperature on fish spawning have been documented by Paxton and King

(2009), but despite the recent work of Dallas et al. (2017), Reizenberg (2017) and Shelton et al. (2018), published data on the effect of climate change on native freshwater fish remain relatively sparse. According to Filipe et al. (2012), there is increasing interest in determining the impacts of climate change on Mediterranean stream's biodiversity. However, it is difficult to disentangle climate change effects from natural climate variation.

Over the past five decades changes to the hydrological cycle in Mediterranean regions have been credited to climate change (Filipe et al., 2012). Climate change has the ability to induce environmental changes to watercourses, such as hydrological modifications, changes in wildfire intensity and frequency, and land use change (Filipe et al., 2012). Drastic shifts in hydrological patterns of aquatic ecosystems are predicted, which are likely to experience a constant decline in annual volume of runoff (Boix-Fayos et al., 1998). Together with seasonal changes, climate change is likely to modify the inter-annual variability in stream flow in Mediterranean regions (Boix-Fayos et al., 1998). The occurrence, and magnitude of extreme droughts or floods will depend on the land use activities; for example, canalising streams will intensify flood events (Opperman et al., 2009).

Climate change can drastically alter the local habitat of Mediterranean streams due to increased water temperature, reduced dissolved oxygen, decrease in stream flow and disturbed reach connectivity (Filipe et al., 2012). Distribution ranges of aquatic species may change because of dispersal ability and physiological tolerances (Filipe et al., 2012). Distributional shifts are the most commonly studied response to climate change (Parmesan, 2006), and climate change has caused freshwater fish distributions to shift measurably (Heino et al., 2009). The observed distributional shifts in Mediterranean stream biota is poleward and upward (Otero et al., 2011), and species range shifts can lead to alteration in species richness, community structure and reproductive capacity (Filipe et al., 2012). Forecasts established for freshwater fish, suggest the survival of future populations will depend on their location along the upstream-downstream gradient of the system (Filipe et al., 2012). For example, communities located at midstream locations, and at medium elevations, may experience major changes including an increase in species reassembly and species richness due to arrival of non-native species and local extinctions (Buisson et al., 2010).

Recent climate change has caused changes in life history traits in terrestrial, marine and freshwater species (Filipe et al., 2012; Parmesan, 2006). Such changes include shifts in seasonal activity due to environmental factors (phenology) (Filipe et al., 2012; Parmesan, 2006). Recent data of changes in life history traits in macroinvertebrates, benthic algae and fish found in Mediterranean rivers reflect climate-induced changes such as drought and reduced stream flow (Filipe et al., 2012). Studies on the effect of climate change on life history traits in Mediterranean freshwater biota are limited to three of the five Mediterranean regions including Australia, California and the Mediterranean Basin (Filipe et

al., 2012). According to Filipe et al. (2012), there are no recorded extinctions of Mediterranean stream biota that can be attributed entirely to climate change, though many species that were widespread in the past, have become increasingly range-restricted and thus more susceptible to extinction. Due to expected loss of suitable habitats, extinction will be most felt amongst highly endemic species and those that are geographically isolated (Filipe et al., 2012).

Climate change and its unpredictability have led to the impoverishment of life in freshwater systems and permanently modified freshwater ecosystems (Woodwell, 1990). The CFR of South Africa is a Mediterranean region and likely to experience major alterations to freshwater ecosystem structure and function, with corresponding impacts on species assemblages with the increasing threat of climate change (Midgley et al., 2003). As a result, the International Union for the Conservation of Nature (IUCN) has red-listed many endemic species in Mediterranean regions (including the CFR) as Endangered or Critically Endangered (Ellender et al., 2017). Assuming that the Intergovernmental Panel on Climate Change (IPCC) global circulation model predictions are accurate, Mediterranean regions can expect a substantial loss of sensitive species, despite their resilience and adaptability (Frazee et al., 2003). In short, current anthropogenic disturbance, coupled with climate warming is predicted to trigger a drastic increase in extinction rates worldwide (Frazee et al., 2003).

1.3 The vulnerability of the Cape Peninsula to climate change

The Cape Peninsula forms the south-western tip of the CFR in the Western Cape of South Africa (Cowling et al., 1996). The CFR spans a distance of 87 892km² from the south-eastern to the south-western area of South Africa (33-35°S and 18-22°E) (Cowling et al., 2003). The Cape Peninsula is an area spanning 2461km² of varied climate, rugged scenery and developing metropolis of Cape Town (Pasquini et al., 2015). The CFR is considered a global biodiversity hotspot, and in the context of climate change, 'a hotspot for concern' (Cowling et al., 2003; Dallas & Rivers-Moore, 2014). Concern around the regions' biodiversity arises because of its combination of high species endemism (a result of ancient geographic isolation of the CFR river catchments (Linder et al., 2010)), and ongoing anthropogenic stresses on the regions' ecosystems. The Cape Peninsula is characterised by extremely variable annual rainfall, nutrient poor soils and high topographical heterogeneity (Cowling et al., 1996). The Cape Peninsula experiences a Mediterranean climate (Cowling et al., 1996), receiving the majority of its rainfall in the winter months, followed by dry summers (Midgley et al., 2005). South Africa is a water scarce country and receives only 60% of the global average in rainfall (Dallas & Rivers-Moore, 2014; Filipe et al., 2012; King & Pienaar, 2011), and 65% of the country, (specifically the arid and semi-arid interior and western areas), receives on average less than 500 mm of rainfall per annum (Dallas & Rivers-Moore, 2014). During the winter months, the high-pressure systems in the southern Atlantic and Indian Ocean shift northward and so do the westerly waves

(Midgley et al., 2005). Due to the shift in high-pressure cells that typically block the path of frontal systems over the southern African landmass, the Peninsula is more exposed to frontal systems in winter (Midgley et al., 2005). Regular drought conditions are created in this region due to the variation in position of the high-pressure cells and westerly waves in the winter months, influencing seasonal and annual rainfall (Midgley et al., 2005).

Currently, the CFR is facing the worst drought on record, with the six major dams that supply water to the area being recorded at 22.50% of their collective capacity on 23 March 2018 (near the end of summer) (City of Cape Town, 2018). The period between October 2016 and September 2017 was the driest period in approximately 100 years (Conradie, 2018), with 2017 being the driest year, followed by 2015 as the second driest year on record (Conradie, 2018). The period 2015 - 2017 was the driest three-year period in the dam catchment area going back to the records from the 1930s and these periods would naturally occur every few hundreds of years (Conradie, 2018). According to climate models climate change is expected to cause a drying trend over Mediterranean climate zones in the southern hemisphere, a trend already evident in south-western and southern Australia, and the CFR (Conradie, 2018). Data from the past 40 years has shown that the CFR has dried significantly, and longer-term data (pre 1980) show that the CFR has actually gotten slightly wetter (Conradie, 2018). Therefore, the recent drying trend could potentially be attributed to long-term natural variability. However, if one only looks at the mountain catchment areas of the CFR from which the dams are fed, a drying trend is observed even in long-term data (Conradie, 2018). This highlights the difficulty in separating climate change from long-term climate variability. Another aspect of the water crisis to consider is the increase in formal residential and agricultural water consumption that occurs during periods of high temperatures and low rainfall, as occurred during the first summer of the drought in 2014/2015 (Conradie, 2018). In summary, a range of factors have contributed to the Cape Town water crisis and the extent to which climate change is involved requires more research (Conradie, 2018).

A major consequence of the drought in Cape Town is the lack of rainfall contributing to the flow of rivers and wetlands of the Cape Peninsula. River flows reflect rainfall events, with local variation dependent on factors such as geology, vegetation, soils and slope (Murray et al., 2009). Midgley et al. (2005) predict climate change will lead to sea level rise; loss of species in this biodiversity hotspot; tremendous decline in water supply; enhanced frequency and intensity of wildfires; as well as negative social impacts including health problems due to air pollution, damage to property after extreme flooding events, impacts on forestry, fisheries and agriculture. The water resources in the area are already highly stressed and there is little scope for development and implementation of alternative methods of water management (Midgley et al., 2005). A water deficit exists in some catchments where the Ecological Reserve has been incorporated into studies. The Ecological Reserve (henceforth 'the Reserve') governs the principles behind water resource management, and is defined

as the quality and quantity of water required to provide a basic water supply for all people and to conserve aquatic ecosystems to ensure ecologically sustainable development and use of water resources (King & Pienaar, 2011). Water demands continue to grow from the City of Cape Town, agricultural areas and coastal towns (Midgley et al., 2005). The already stressed water supply situation is extremely vulnerable to periodic droughts such as that which the region is currently experiencing (Midgley et al., 2005). A reduction in water in rivers may have a drastic impact on the estuaries and wetlands associated with the regions' river systems (Midgley et al., 2005). Estuaries are very productive and serve as nurseries for fish, and rely on freshwater inputs to maintain a certain salinity profile and for removing sediment (Midgley et al., 2005). With an expected 0.3m sea level rise due to ocean thermal expansion in the next few decades, saltwater could intrude into coastal aquifers, and consequent flooding coupled with extreme storm events and coastal erosion will be experienced (Midgley et al., 2005).

As is the case throughout the CFR, rivers on the Peninsula are impacted by water abstraction, pollution, physical habitat modification and invasive species. According to de Moor and Day (2013) of these threats, the increasing human demand for freshwater is the major threat to native freshwater biotas. In the lowlands of the CFR, land is mostly modified for urban developments and agriculture (de Moor & Day, 2013). Water storage and abstraction has huge impacts on the CFR's rivers. Virtually all potential dam sites in the CFR have been utilised causing the lower reaches of rivers to have a lower than natural discharge rate (de Moor & Day, 2013). Water pollution varies according to the surrounding land uses. Large industries are generally concentrated to urban areas, such as Cape Town, the largest urban area in the CFR. However, regulations covering the discharge of pollutants are relatively effective in Cape Town (de Moor & Day, 2013). The major pollutant is inadequately treated effluent, which is often the case for rivers situated below wastewater treatment plants in Cape Town (de Moor & Day, 2013).

In summary, the consequences of climate change resulting from a drier and warmer climate are likely to cause gradual impoverishment of species richness in this internationally acclaimed biodiversity hotspot (Midgley et al., 2005). However, projections for the effects of climate change on species are highly uncertain due to lack of knowledge of species tolerance (Midgley et al., 2005). Terrestrial and freshwater species loss in the CFR is predicted to be as high as 30% for the worst-case scenario, as a response to warming and drying, and indirectly by shifting fire regimes and presence of non-native species (Midgley et al., 2005).

1.4 Endemic freshwater fish biodiversity of the CFR and Cape Peninsula

To date, there are 21 formally described native freshwater fishes in the CFR, of which 17 are endemic to the region (Ellender et al., 2017; Skelton, 2001). However, DNA-based studies recommend revision of the taxonomic status of almost all CFR fishes studied thus far (Chakona et al., 2013a; Waters & Cambray, 1997; Wishart et al., 2006). Current genetic work indicates that at least 42 genetically distinct fish lineages exist within the CFR – double the present number of species - with species level divergence, suggesting several species within the genera *Galaxias*, *Sandelia* and *Pseudobarbus* (Chakona et al., 2013a; Wishart et al., 2006). Based on the geographical distribution of endemic freshwater fish there are eight main catchments areas in the CFR, namely, (1) Breede and adjacent rivers, (2) Gourits, (3) Berg, (4) Olifants, (5) western Agulhas, (6) Swartkops, Sundays and adjacent rivers, (7) Gamtoos and adjacent rivers, and (8) south coastal rivers (Chakona et al., 2013b). The greatest threat to native fish in the CFR is the presence of non-native fish species (Ellender et al., 2017). Non-native fish reduce ranges of the CFR endemic fish species and homogenise fish communities of all Mediterranean regions (de Moor & Day, 2013). Humankind has introduced 22 species of non-native fish and of these, 20 have become established in the CFR (Marr et al., 2012). Sport enhancement has been the main reason for the introduction of non-native fish, specifically in areas with predator-poor native fish assemblages, and in South Africa negative consequences and negative impacts on native fish populations have been recorded as early as the 1920s (Weyl et al., 2014; Skelton, 2001). Freshwater fishes of the CFR represent the characteristics of old, well established mountain fauna, with a high level of endemism (89%), relatively inflexible in life history strategies, geographically isolated distributions and a low resilience to anthropogenic disturbance (Marr et al., 2012). The current state of freshwater fish is dictated by habitat modification and competition and predation by non-native fish (Weyl et al., 2014). Sixty per cent of endemic freshwater fish species in South Africa's CFR region are classified as either Endangered or Critically Endangered (Ellender et al., 2017).

There has been a strong focus in freshwater fish conservation on managing non-native fish populations and impacts in the CFR (Weyl et al., 2014), however with intensifying climate change and an increase in water withdrawals, particularly in the dry season, attention should be given to these threats on native fish populations (Shelton et al., 2018). Due to arid regions becoming drier and hotter; and water demands escalating every year; about 75% of native fish species (in rivers with reduced discharge) may go extinct by 2070 (Xenopoulos et al., 2005). This is a serious problem as fish are a critically important component of ecological functioning and biodiversity in rivers and wetlands (Murray et al., 2009). Habitats are increasingly becoming modified, fragmented and polluted due to increase in anthropogenic pressures in freshwater ecosystems (King & Pienaar, 2011). Paxton and King (2011) reported that dams and weirs have restricted fish migration, impacting population

size and habitable ranges for particular freshwater fish species. Factors heightening vulnerability to extinction of CFR endemic freshwater fish include high endemism, limited geographical distribution and isolation in headwaters. These factors increase susceptibility of fish to human-linked stressors such as changes in temperature and flow linked to climate change including pollution, water abstraction, impacts of non-native species, and habitat modification (Ellender et al., 2017). Ellender et al. (2017) identified a need for studies on the impacts of climate change of freshwater fish in the CFR and South Africa as a whole.

Geomorphological and geological processes, sea level rise, climate changes and human impacts influence natural river fauna and flora. The Cape Peninsula has experienced taxonomic homogenisation within the freshwater fish assemblage as specialist and sensitive species have been eliminated (Marr et al., 2010). Surprisingly, the rivers and wetlands of the Cape Peninsula are populated with a low diversity of native freshwater fish (Murray et al., 2009). Two primary native fish species have been documented from this area, including the Cape galaxias, *Galaxias zebratus* Castelnau, 1861, and Cape kurper, *Sandelia capensis* Cuvier, 1831 (Murray et al., 2009). *Galaxias zebratus* is a widespread species in the perennial and non-perennial rivers, and permanent wetlands of the area (Murray et al., 2009). *Galaxias zebratus* is found in rivers with good water quality and with few or no non-native fish (Murray et al., 2009). Molecular studies have shown *G. zebratus* is part of a species complex comprising of at least 10 deeply divergent lineages (Chakona et al., 2018). Populations that are genetically unique are found in Klaasjagers, Hout Bay, Lourens, Silverstroom and Diep Rivers. *Sandelia capensis* is relatively large (<12cm) and found in the lower reaches of rivers (Murray et al., 2009). *Sandelia capensis* is not as genetically diverse as *G. zebratus* (Murray et al., 2009). Populations have been found in the Diep, Liesbeek and Hout Bay Rivers (Murray et al., 2009).

1.5 *Galaxias zebratus*

The family Galaxiidae display a Gondwanian distribution, in that it is limited to relatively cool regions within the southern hemisphere (Barnard, 1943). Galaxioid fishes are the most speciose of the freshwater fishes with more than 50 recognised species (McDowall, 2006). Representatives are found in western and eastern Australia, New Caledonia, Lord Howe Island, Tasmania, the Chatham, New Zealand, Auckland and the Campbell Islands, Argentina, Chile, the Falkland Islands and South Africa (McDowall, 2006). In New Zealand and Australia the group is most diverse (McDowall, 2006). The genus *Galaxias* from the Galaxiidae family is thought to be a relict group of species originating from the break up of Gondwanaland, 180 – 135 million years ago (Linder et al., 2010). Gondwana was the supercontinent of the southern hemisphere, which broke up into Australia, New Zealand, South America, Antarctica, India and Africa according to the theory of plate tectonics (Linder et al., 2010).

Evidence of the *Galaxias* genus having Gondwanian distribution lies with the discovery of close relatives in New Zealand and South America, alluding to the fact that landmasses were once connected as a supercontinent (Skelton, 2001). McDowall (1973) argues for marine dispersal as the explanation for galaxiid distribution. Galaxiidae fishes exhibit two different life history strategies, some species are diadromous and others not (McDowall, 2006). Diadromy is widely represented within the group and involves reciprocal, usually obligatory migration between freshwater and the ocean (McDowall, 2006). The galaxiids either distributed by continental drift or shift in ocean currents (Cambray et al., 1995). Southern Africa possesses one unique and ancient species, *G. zebratus*, which is limited to the south-western Cape region (Barnard, 1943).

Galaxias zebratus was once thought to be the sole species of the family Galaxiidae to occur in Africa (Chakona et al., 2018). The similarities found between extant and fossil species suggest that *G. zebratus* has a conservative morphology, with a slow rate of morphological evolutionary change (Wishart et al., 2006). According to Skelton (2001) *G. zebratus* occupied the widest range of all freshwater fishes restricted to the CFR, spanning over 20 isolated river systems from the Gamtoos in the eastern CFR to the Olifants system on the west coast of the CFR. Recent molecular studies suggest that *G. zebratus* is in fact a species complex comprising of approximately 10 deeply divergent lineages (Chakona et al., 2018; Wishart et al., 2006). This raises conservation concern because only one of these lineages is widespread across the CFR and the other lineages have smaller population sizes and narrower ranges (Chakona et al., 2013a). The initiation of molecular methods for identifying lineages and species within groups has advanced our understanding of galaxiid biodiversity; and ecological studies combined with these techniques are providing remarkable evidence for previously unknown diversity (McDowall, 2006).

There is some level of genetic variation among *G. zebratus* populations found in rivers of the Cape Peninsula, and data presented by Wishart et al. (2006) suggests the presence of five different species. The drainage of the Cape Fold Mountains is considered to play a major role in the distribution of *G. zebratus* (Barnard, 1943). The populations found on Table Mountain are genetically distinct from those in the nearby Eerste River described by Waters and Cambray (1997), suggesting a period of isolation and independent evolution around five million years ago (Wishart et al., 2006). The patterns of distribution in the Cape Peninsula represent east/west-flowing streams historically connected during periods of low sea level (Wishart et al., 2006). Periods of low sea level were significant in promoting river capture adjacent to the Cape region's western escarpment (Dingle & Hendey, 1984), and providing opportunity for gene flow by reconnecting previously submerged drainage (Wishart et al., 2006). Periods of increased sea level would reduce population size, erode variability and therefore accentuate the degree of genetic divergence (Wishart et al., 2006).

Galaxias zebratus is found along the Western Cape coastal strip for approximately 600km, from the Keurbooms River (south coast) to the Olifants-Doring system (Albany Museum records in Wishart et al., 2006). *Galaxias zebratus* is tolerant of alkaline and acidic waters, and prefers gentle currents and riffles with sheltered banks near heads of pools (McDowall, 2006; Wishart et al., 2006). This species is primarily confined to lowland areas and occupies a vast range of habitats from acidic mountain streams in the Cape Fold Mountains to alkaline wetlands on the Cape Flats (Barnard, 1943). Within the Cape Fold Mountain, the evolution of drainage has had a strong influence on the distribution of fishes (Barnard, 1943).

Like most galaxiids, *G. zebratus* are small (maximum total length (TL) = 75mm), fusiform, elongated and have cryptic habits (McDowall, 2006; Waters & Cambray, 1997). *Galaxias zebratus* has a small terminal mouth suggesting it is a drift feeder species (McDowall, 2006). Terrestrial invertebrates feature relatively strongly in the diet of *G. zebratus*, further supporting the opinion that they are insectivorous, drift feeders (McDowall, 2006). Due to their small size and breeding patterns, it has been noted that *G. zebratus* is the ideal food for non-native, predatory species like rainbow trout (*Oncorhynchus mykiss*) (McDowall, 2006). There is little information about *G. zebratus* life history, but it is known that they are non-diadromous (i.e. live in freshwater throughout their life cycle) and mostly live in streams and rivers, although occasionally they can be found in wetlands, and on shores of ponds and lakes (McDowall, 2006).

Galaxias zebratus has been identified by the IUCN as being data deficient (IUCN, 2018), though certain distinct lineages could be considered Critically Endangered due to their severely fragmented populations, restricted geographical range, and continuous decline as a result of habitat modification, loss of mature adults and restricted area of occupancy (Chakona et al., 2018). The conservation status of *G. zebratus* is predicted to change once the taxonomy of populations in the CFR is better resolved (McDowall, 2006). However, the clear decline in *G. zebratus* populations in the CFR is alarming and worrying given that it is South Africa's sole freshwater fish link to Gondwanaland (McDowall, 2006). *Galaxias zebratus* is threatened by multiple impacts including habitat degradation (i.e. pollution, siltation and water abstraction), modifications to hydrology, and impacts of non-native species (Chakona et al., 2018). Climate change is also predicted to severely impact stream flow and habitat diversity due to reduced river discharge as a result of reduced precipitation and increased evapotranspiration (Palmer et al., 2007). The impacts of climate change will be further exacerbated by an increase in human demand for water, which may alter flow regimes through excessive abstraction of surface water and the use of aquifers that sustain base flow during the dry season (Chakona et al., 2018). Almost all native freshwater fish are vulnerable to climate change and the associated human impacts in the CFR (Shelton et al., 2018). However, there is a critical knowledge gap about the

biology and ecology of native fishes such as, *G. zebratus*, which limits an understanding of climate change impacts in this region (Shelton et al., 2018).

1.6 Thermal variation of rivers

Thermal characteristics of riverine ecosystems in nature are dependent on climatological (e.g. air temperature, cloud cover, wind speed); hydrological (e.g. source of water, rate of flow or discharge); regional (e.g. altitude and latitude); and structural features (e.g. aspect and slope, riparian vegetation) (Dallas, 2008). At catchment scale, temperature variation is driven by climate, geography, topography and vegetation (Poole et al., 2001). At river scale, variation in temperature occurs longitudinally down a river system, with lowland areas typically warmer than headwaters (Dallas, 2008). At site scale, differences in temperature are determined laterally across the site and in relation to habitat (Poole et al., 2001).

Different temperature profiles are found in different habitats (Dallas, 2008). Seasonal climate lotic systems exhibit seasonal (annual) and daily (diel) temperature patterns (Ward, 1985). Hourly temperature is lowest at night or early morning and highest temperatures occur during mid to late afternoon, but this depends on the size of the river and season (Ward, 1985). Rivers that are small and heavily canopied, and rivers that are deep and large exhibit the least daily range in water temperature. However, rivers that are shallow, with minimal canopy, thus exposed to direct sunlight, and braided streams have greater ranges in daily water temperature (Ward, 1985). In terms of seasonal temperatures, highest water temperatures occur in the summer and lowest in the winter. When determining the ecological responses of aquatic organisms to change in temperature, it is essential to understand temporal and spatial trends in lotic systems (Dallas, 2008). Aquatic organisms are ectothermic therefore their presence and success are highly dependent on environmental temperature (Arscott et al., 2001). Thus, temperature is considered the key environmental factor structuring aquatic communities (Barrantes et al., 2017; Dallas, 2008).

1.7 Thermal requirements of freshwater organisms

Flow and water temperature are the key abiotic factors in aquatic ecosystems (Caissie, 2006; Dallas, 2008; Rivers-Moore et al., 2013). These factors influence the behaviour of aquatic organisms including fish and invertebrates (Dallas 2008). Water temperature fluctuates in space and time, therefore shaping the seasonal and geographical distribution of fish (Aigo et al., 2014). Aquatic organisms are highly sensitive to changes in temperature as they rely on thermal cues for life-history events, such as spawning (Vannote & Sweeny, 1980). Therefore, alteration to thermal regimes influence aquatic organisms behaviour and life histories (Vannote & Sweeney, 1980). Vannote and

Sweeney (1980) describe the Optimal Thermal Regime (OTR) as the range of temperatures at which reproduction, fitness, growth and metabolism is favourable for aquatic organisms.

Freshwater fish in the CFR are expected to be sensitive to thermal effects due to geographical isolation, limited range and life histories adapted to regional conditions over many centuries (Chakona et al., 2013a). However, fish have evolved physiological, biochemical and behavioural responses to live in environments with fluctuating temperatures (Beitinger & Lutterschmidt, 2011; Dabruzzi et al., 2012). The first response is behavioural thermoregulation and is characterised as avoidance and preference responses (Wallman & Bennett, 2006). Usually fish living in thermally heterogeneous habitats select a preferred temperature in which their metabolic processes and functions perform with efficiency (Fry, 1971). The second defence against extreme temperatures is the physiological response and is characterised by readjustments in tolerance endpoints, usually occurring over a course of hours to weeks (Dabruzzi et al., 2012). The evolutionary history of species and populations partly determine temperature tolerances and preferences (Beitinger & Bennett, 2000). However, these can also be modified during growth and development by environmental factors such as salinity, oxygen, predators and food availability (Beitinger & Lutterschmidt, 2011). The third and most extensive response to changing thermal conditions involves the biochemical alterations of metabolic pathways or synthesis of new molecules (e.g. isozymes or allozymes), which usually takes days to weeks to accommodate (Dabruzzi et al., 2012; Ficke et al., 2007). At the underlying biochemical and physiological level, traits depicting performance include functions such as nerve conduction velocity, heart rate and enzyme activity (Schulte et al., 2011). Metabolic pathways are plastic so can adjust with a changing environmental condition (Ficke et al., 2007) affecting their ability to reproduce, grow and maintain homeostasis (Barrantes et al., 2017). Standard metabolic rates of ectotherms double with every 10°C increase in temperature, however deviations from the norm may enhance energy budgets of fishes living in thermally heterogeneous environments (Dabruzzi et al., 2012). Temperature changes will impact aquatic community structure at a community level, but has also been known to dictate niche diversity in lotic systems (Magnuson et al., 1979). At a large scale this maintains species diversity and patterns of distribution (Vannote & Sweeney, 1980).

Despite thermal optima characteristics being inherited, species exhibit plasticity that allows recent thermal histories to determine thermal tolerance and preference (Johnson & Kelsch, 1988). Thermal history is the range of temperatures experienced by an organism in its natural habitat over time, either short term (weeks) or long term (months). Usually, the physiological adaptations to increased temperatures causes an increase in thermal preference and tolerance. Coupled with thermal history, ontogeny (development of organism from egg to mature adult) also influences species thermal requirements. McCauley and Huggins (1979) hypothesised in their study that younger fish selected higher temperatures compared to their older con-specifics, reiterating that throughout the fish life

cycle, thermal requirements may differ. Coutant (1987) describes this as a thermal niche. A thermal niche is a dynamic entity that undergoes change as an organism develops (Coutant, 1987). Embryos are incapable of behavioural thermoregulation and thus have narrow thermal niches, whereas adults have a greater capacity for thermoregulation and have wider thermal niches (Burt et al., 2011). Therefore, thermal requirements at different life stages are influenced by thermal acclimation as well as evolution.

Mortality is a direct effect of elevated temperature, but thermal effects on reproduction and fertility is known to be more complex. Bobe and Labbé (2010) propose that due to climate warming, the number and viability of offspring produced during a spawning season will diminish and the resultant thermal stress will reduce the ability for optimal reproduction. Kelley (1968) confirmed that experiments incubating eggs at upper and lower thermal extremes significantly affected less successful hatches. Temperature is the main factor determining riverine organisms critical life history events and survival at different life history stages (Rivers-Moore et al., 2013). Other factors contributing to a reduction in thermally favourable habitats include removal of riparian vegetation, abstraction, effluents with unpredictable temperatures and climate change in particular regions (Dallas and Rivers-Moore, 2014).

1.8 Determining thermal tolerance

Thermal experiments have been used globally to determine thermal limits for a range of aquatic life (Lutterschmidt & Hutchinson, 1997). This discipline emerged and developed quickly from aquaculture sciences because of an increase in human demand for freshwater fish as a source of food (Ficke et al., 2007). In order to maximise yield, it is essential to determine the thermal optima of fish as species grow at different rates and at different temperatures (Austreng et al., 1987). Thermal studies in freshwater fish have grown in popularity as climate change has intensified globally (Ficke et al., 2007). The main aim of these studies was to determine the thermal optima (thermal maximum) that species could tolerate and which temperatures were more preferable for their life histories (thermal preference). Commonly used experimental methods used to determine thermal tolerance are the Critical Thermal Method (CTM) and the Incipient Lethal Temperature Method (ILT), which is equivalent to the LT_{50} method (Dallas, 2016; Dallas & Ketley, 2011; Ficke et al., 2007; Lutterschmidt & Hutchinson, 1997).

For the purpose of this study, the CTM is used to determine the critical thermal maximum (CT_{max}), which is the upper thermal endpoint or limit. Thermal tolerance is described as the sensitivity of an organism to upper and lower temperature extremes. The upper thermal limit is an indicator of thermal tolerance and is known as the CT_{max} , expressed in degrees Celsius. The CTM is static and involves placing the fish in a water bath fitted with a circulating heater and increasing the temperature at a

constant rate until the end point or physiological thresholds have been reached (CT_{max}). A thermostat controlled water bath or circulating heater allows for the specific change in water temperature (Dallas & Ross-Gillespie, 2015). At the end point the fish start displaying signs of thermal stress (Paladino et al. 1980). Thermal stress is indicated by behaviour that is generally species specific. The loss of ability to remain upright (loss of righting response) followed by muscular spasms is suggestive of end point markers in fish (Becker & Genoway, 1979; Lutterschmidt & Hutchinson, 1997). This CTM has been used on numerous aquatic taxa and is useful to determine the thermal sensitivity of organisms with elevated water temperatures (Dallas & Rivers-Moore, 2012).

There are alternate methods to determine thermal sensitivity, however these do not rely on bio-markers as end points. Incipient Upper Lethal Temperature (ILUT) is determined by the Incipient Lethal Temperature (ILT) method. ILUT/LT₅₀ is considered the temperature survived by 50% of the population (Beitinger & Bennet, 2000; Dallas, 2016). Both CTM and ILT method have been used in the CFR to elucidate chronic and acute exposure to freshwater invertebrates (Dallas, 2016; Dallas & Ketley, 2011; Dallas & Rivers- Moore, 2012). However, IULT is more applicable 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). ILT is a method based on lethality, therefore it is not ethical to use for endangered species (Dallas, 2016).

Furthermore, there are additional reasons to motivate the use of CT_{max} rather than IULT. Firstly, results from CTM are easier to compare with simple statistics (Beitinger & Bennet, 2000). Secondly, a smaller sample size is required for CTM compared to the ILT method. It is essential to note that IULT is not an equivalent to CT_{max} (Becker & Genoway, 1979). IULT is an indication of thermal resistance, whilst CT_{max} indicates thermal tolerance (Beitinger & Bennet, 2000). Thirdly, IULT is more time consuming than CTM. Overall, it is more appropriate to use CTM when abundance is low, sample sizes are small and conservation status is critical.

Fish are ectotherms, thus their presence and success within a habitat depend largely on environmental temperature (Barrantes et al., 2017). Water temperature can indirectly affect viability of populations at high latitudes through limitations to body size as a result of limited food during winter, and directly by thermal lethality (Fry, 1971). Therefore, thermal history can be predicted to significantly influence thermal tolerance of fish. Information about CT_{max} of galaxiid species is rare, nevertheless Barrantes et al. (2017) found that *Galaxias platei* from Tierra del Fuego, South Patagonia, has a good ability to acclimate to different temperatures. Furthermore, it was deduced that changes to their acclimation temperature significantly impact CT_{max} of *G. platei*. Similarly, the goldfish, *Carassius auratus*, upper and lower thermal tolerances were measured at constant acclimation temperatures. CT_{max} was significantly linearly related to acclimation temperature (Ford & Beitinger, 2005). Acclimation

temperature explained 90% of the variance in temperature tolerance of *C. auratus* (Ford & Beitinger, 2005). The thermal tolerance and oxygen consumption of the Indian, *Anabas testudineus*, was also found to be dependent on acclimation temperatures (Sarma et al., 2010). These studies support that thermal history or acclimation temperatures significantly influence CT_{max} of fish.

Determining thermal thresholds of freshwater fish is a key element in understanding the potential impacts climate change will have on already threatened freshwater fish populations and to ensure their effective conservation. The determination of these thresholds in South Africa is not just to encourage biodiversity conservation, but is mandated by South African national legislature: the National Water Act (RSA, 1998) recognises water temperature as an essential abiotic water quality parameter of the Reserve (Palmer et al., 2007). Tested experimental protocols, such as the Critical Thermal Method, are valuable in elucidating the effects of increased temperatures on fitness and survival and to develop environmental water temperature guidelines for freshwater ecosystems (Dallas & Rivers-Moore, 2012). Due to the lack of up to date physiological and distributional data on CFR endemic fish, setting biological thresholds and evaluating the potential impacts of climate change on freshwater fish distribution is limited (Ellender et al., 2017). The incomplete and inconclusive state of knowledge on the thermal biology of *G. zebratus* and the importance of this endemic species conservation, led to the pursuit of this study. While various current studies aim to predict distributional shifts of species with elevated environmental temperatures, the intention of this study is to determine the potential effects of climate change on *G. zebratus*, a key species from Cape Peninsula rivers, through its physiological (thermal tolerance range) response.

1.9 Research aim

The aim of this study was to evaluate the influence of thermal histories on thermal tolerance of *G. zebratus*, in rivers of the Cape Peninsula of the CFR, South Africa. The study experimentally determined and compared the upper thermal limits of *G. zebratus* from sites with different thermal characteristics using the Critical Thermal Method (Dallas & Rivers-Moore, 2012; Dallas & Ross-Gillespie, 2015; Lutterschmidt & Hutchinson, 1997). The findings of the study broadened our understanding of the factors responsible for variation in fish thermal tolerances, and supported *G. zebratus* conservation by determining its biological thresholds and thermal requirements.

1.10 Objectives

1. Characterise and compare thermal profiles of different sites in rivers of the Cape Peninsula using established, biologically relevant thermal metrics (Dallas & Rivers-Moore, 2012; Rivers-Moore et al., 2013).
2. Characterise and compare CT_{max} of *G. zebratus* from sites with different thermal profiles.
3. Determine whether any differences in *G. zebratus* CT_{max} among sites are related to differences in thermal characteristics (as characterised by seven day moving average of daily mean and seven day moving average of daily maximum temperatures) of those sites.

1.11 Hypotheses

1. CT_{max} of *G. zebratus* individuals will differ among sites with different thermal profiles (as characterised by their seven day moving average of daily mean and seven day moving average of daily maximum temperatures (see section 2.4) (Dallas & Rivers-Moore, 2012; Rivers-Moore et al., 2013)).
2. Individuals of *G. zebratus* from warmer sites will have higher CT_{max} values than individuals from cooler sites.

Chapter 2: Methods

2.1 Study sites

The rivers selected for this study were the Liesbeek, Silvermine, Klawersvlei, Diep, Schusters and Disa Rivers. Ten sites were selected overall based on presence of *G. zebratus*, access and the presence of a reasonable logger attachment point (see attachment details in section 2.4 below). This information was gained via literature, pilot studies, site visits and personal communications. Three sites were selected along the Liesbeek, three along the Silvermine, one on the Disa, one on the Diep, one on the Klawersvlei, and one on the Schusters River (Figure 1). These sites were selected to incorporate as wide a range of river thermal profiles as possible.

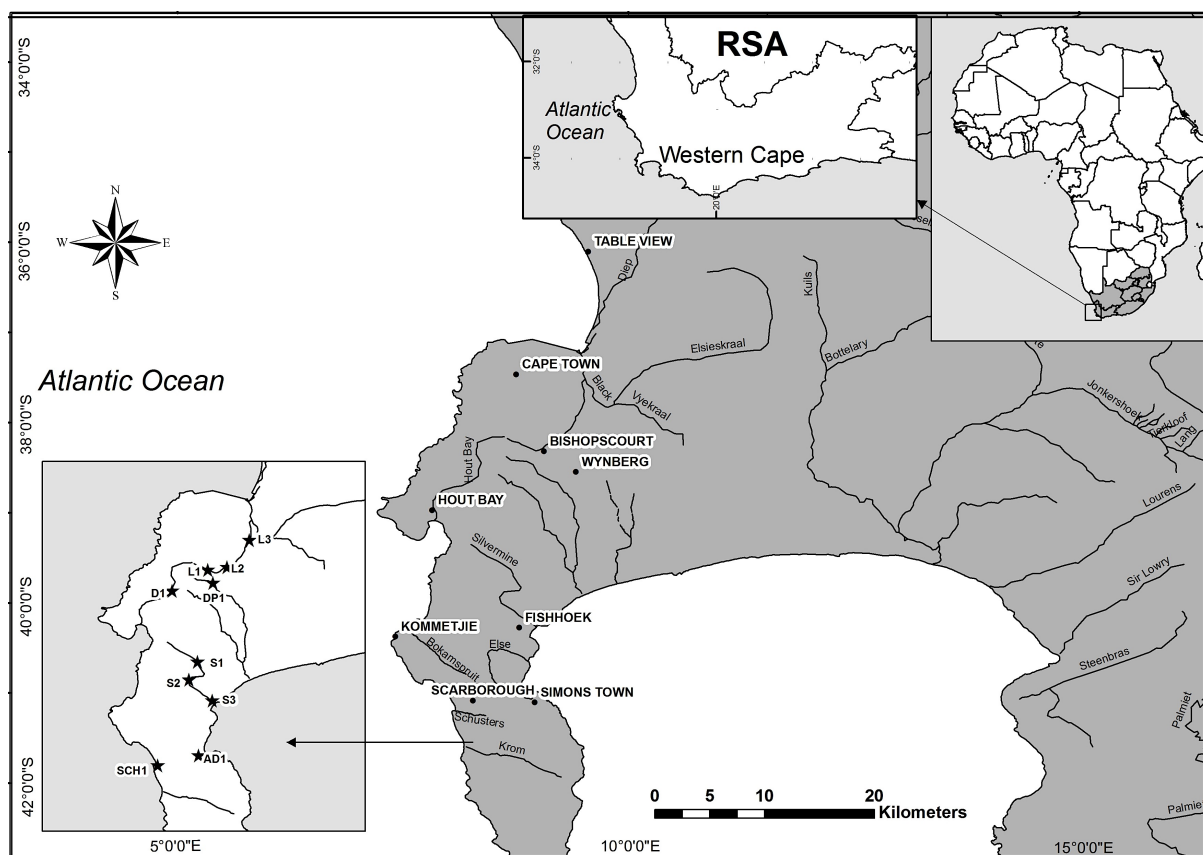


Figure 1: Map of the 10 study sites on the Cape Peninsula, South Africa. **L1**=Upper Liesbeek River, **L2**=Middle Liesbeek River, **L3**=Lower Liesbeek River, **S1**=Upper Silvermine River, **S2**=Middle Silvermine River, **S3**=Lower Silvermine River, **AD1**=Klawersvlei River, **SCH1**= Schusters River, **D1**=Disa River, **DP1**=Diep River (map courtesy of Tumisho Ngobela)

The study sites are located on the Cape Peninsula of the Western Cape, South Africa. The rivers and wetlands of the Cape Peninsula are complex systems, like all freshwater systems around the world, which have been shaped by the influence of hydrology, geology and ecology (Murray et al., 2009).

The influences of hydrology, geology and ecology are primarily hierarchical and it is mostly the geology and geomorphology shaping catchments, and provides the template for ecology and hydrology to apply their influence (Murray et al., 2009). The current arrangement of rivers and wetlands of the Cape Peninsula reflect past and present climate change and sea level oscillation; geology and geomorphology; interactions between hydrology, vegetation and sedimentation; and human interference including water abstraction, channel engineering and land use changes in catchments (Murray et al., 2009).

The Cape Peninsula has a Mediterranean-type climate (Cowling et al., 1996). River flows are strongly seasonal, reflecting the seasonal rainfall pattern (Murray et al., 2009). Most of the flow occurs during the winter months (i.e. June to September), and flows tend to be lowest in the west and highest in the east of the Peninsula (Murray et al., 2009). The relief of the Peninsula plays an important role in the manner that the frontal systems deposit their moisture over land, as it characterises the runoff into river systems at the basin level, and affects geomorphology and hydraulics of the rivers (Murray et al., 2009). The mean annual rainfall on the Peninsula is 600 to 800mm, but small-scale (e.g. among catchments) variability is high, and linked to local topographic characteristics (Murray et al., 2009).

Steep topographical relief is a common feature of Mediterranean regions and largely influences the physical features and hydrology of rivers (Gasith & Resh, 1999). The Cape Peninsula forms part of the Cape Folded Belt, which comprises erosion resistant, quartzitic sandstone mountains with valleys and plains underlain with soft shale (Deacon et al., 1992). The coastal margin is characterised by calcareous and young siliceous sediments (Deacon et al., 1992). The Cape Supergroup sandstones and shale are the predominant rocks of the Folded Belt, and on the Peninsula the Graafwater and Peninsula Formations represent the Cape Supergroup (Deacon et al., 1992). Many rivers on the Peninsula are organised by the contours of the Table Mountain Group rocks. For example, the course of the Liesbeek River is determined by the north-northeastern fault zone that runs through Kirstenbosch to Constantia Nek (Murray et al., 2009). Rivers of the Cape Peninsula are usually narrow and steep due to underlying Table Mountain Group rocks (Murray et al., 2009). Mountain streams of the Peninsula are generally oligotrophic as a result of leaching from nutrient poor sandstone of the Cape Folded Belt (Day et al., 1979). Decaying Fynbos (the regions predominant vegetation) causes mountain streams to be clear-brown in colour due to leaching of humic substances called tannins (Day et al., 1979). These tannins often result in rivers being acidic with a very low pH (Day et al., 1979).

In the Cape Peninsula, upper mountain streams are less disturbed compared to in the lower reaches where water quality and quantity are highly impacted by anthropogenic disturbance at varying degrees (Murray et al., 2009). The sources of rivers in the Cape Peninsula are often boggy areas with

acidic soil, rich in organic material (Murray et al., 2009). Generally, mountain streams of the Cape Peninsula are characterised by narrow, steep channels, swift flows and waterfalls, with substrata of bedrock and boulders (Murray et al., 2009). The riparian zone of mountain streams is usually highly vegetated forming a canopy over the stream and resulting in cooler water temperatures compared to sections further down the river course (Vannote et al., 1980). Middle reaches are characterised by widening channels, decreased bed gradient, reduced flow velocities, and substrata of bedrock, sand or cobbles (Vannote et al., 1980). There are usually trees growing in the riparian zone, but due to the wider channel, the canopy is open (Murray et al., 2009). The lowland sections of rivers have a very shallow gradient, with stony reaches alternating with areas of deposition (Vannote et al., 1980). Reed beds and a few trees can be found on the riparian zone resulting in sparse canopy, and thus higher water temperatures than those of the mountain streams (Murray et al., 2009). As the lowland reaches of rivers in the Cape Peninsula flow through urbanised and industrial areas, they suffer a range of human-induced threats. The main threat is the level of pollution that enters the system in the lower reaches either through direct dumping or stormwater runoff (River Health Programme, 2005). Other impacts include water abstraction, habitat modification (e.g. canalisation) and infestation of non-native plants (River Health Programme, 2005).

The Liesbeek River

The Liesbeek River originates on Table Mountain above the Kirstenbosch Botanical Gardens (Murray et al., 2009). The Liesbeek River is perennial and is approximately 10km long, with much of the lower section (downstream of the suburb Bishopscourt) canalised (Murray et al., 2009). Although urbanisation is the major cause of pollution in the Liesbeek River, it still maintains characteristics of the natural river and holds educational and recreational value. The upper Liesbeek site (L1) was located in Kirstenbosch Gardens in a pool surrounded by boulders. L1 was shaded due to the dense riparian vegetation. The middle Liesbeek site (L2) was located in the “River Garden” in Bishopscourt. L2 had less vegetated canopy than L1 and this site was more susceptible to anthropogenic disturbance, such as pollution from the stormwater drains. The lower Liesbeek site (L3) located in Mowbray has been canalised. L3 had no vegetated canopy, and the flow was shallow and uniform. L3 was highly susceptible to pollution and other anthropogenic disturbance due to the urban surroundings. (Figure 2, Table 1, Appendix A).



Figure 2: The Liesbeek River, a) Upper Liesbeek River (L1) located in the Kirstenbosch Gardens, b) Middle Liesbeek River (L2), c) the canalised lower Liesbeek River (L3)

The Silvermine River

The Silvermine River sources in the Steenberg Mountains at the southern tip of Constantiaberg Mountain and then flows across the Steenberg Plateau towards the east coast (Murray et al., 2009). The upper Silvermine River catchment is entirely enclosed within the boundaries of the Table Mountain National Park (Reinecke et al., 2008), and it then flows south through the Silvermine Valley until the Fish Hoek coastal plain and urbanised area. The river then enters the sea at False Bay in the suburb of Clovelly (Murray et al., 2009). The Silvermine River is a 12km long, perennial river, although the upper reaches do not receive surface flow in the summer months. The upper Silvermine site (S1) was located in the Silvermine Nature Reserve in a pool shaded by overhanging vegetation. The middle Silvermine site (S2) was located 1km from the Sunbird Centre. This site predominately comprised of flat sheets of bedrock and had very little canopy. The lower Silvermine site (S3) was located in the Silvermine Wetlands. S3 was a very deep pool, highly vegetated with bulrushes and very susceptible to pollution (Figure 3, Table 1, Appendix A)

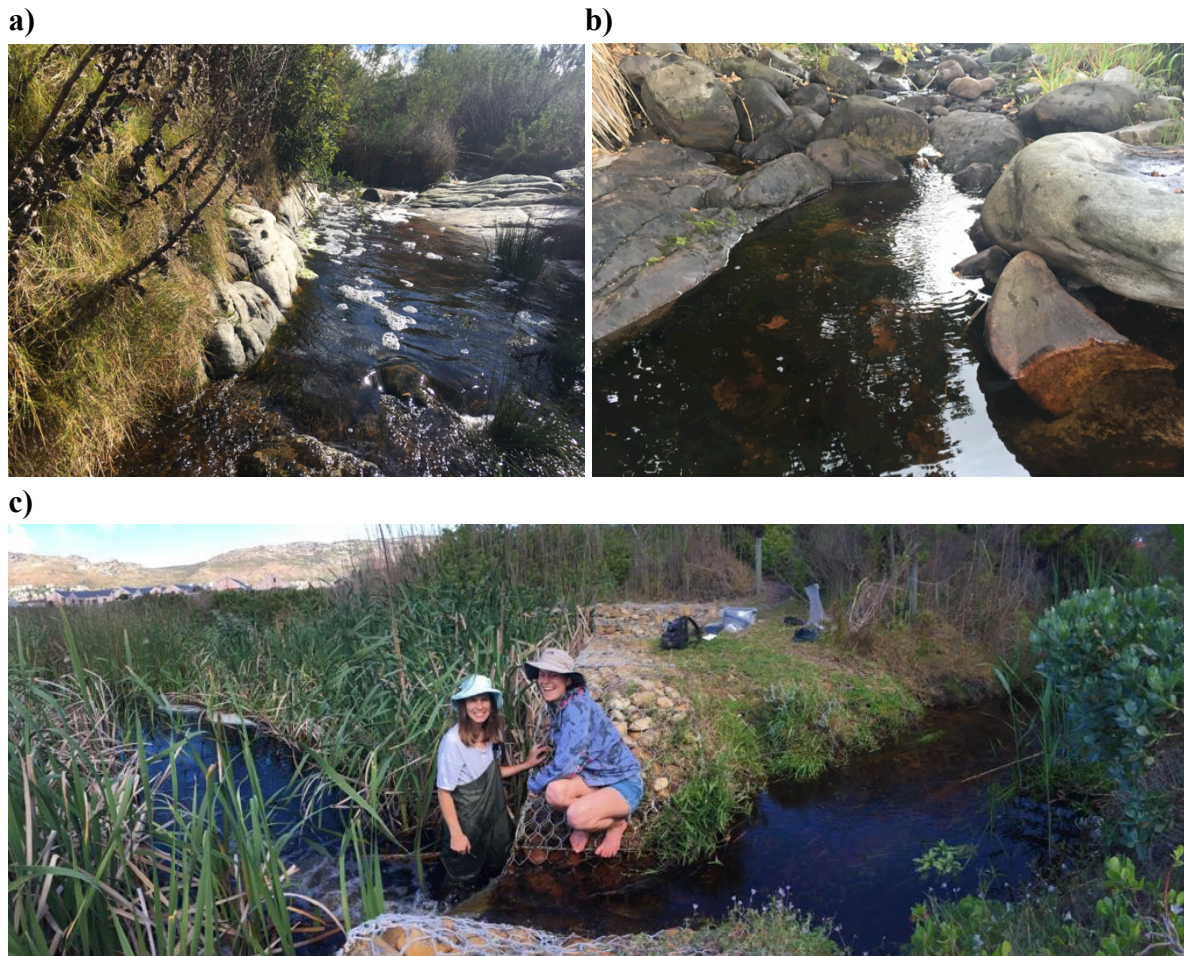


Figure 3: The Silvermine River, a) upper Silvermine (S1) located in the Silvermine Nature Reserve, b) middle Silvermine (S2), c) myself and field assistant, Lily Bovim, attaching a logger in the lower Silvermine (S3) (image courtesy of Jeremy Shelton)

The Schusters River

The Red Hill and Wildeschutzbrand areas inland from Scarborough drain into the upper Schusters River (Murray et al., 2009). Other than road runoff, no other development impacts the Schusters. The lower reach of the river, where the SCH1 site was located, consists of a large wetland that has been significantly modified due to infilling to place the Good Hope Nature Reserve fence and increasing housing developments in the Scarborough area (Murray et al., 2009). The river enters the sea through a small lagoon just south of Scarborough (Murray et al., 2009). The Schusters River is only about 5km in length and is non-perennial (Figure 4, Table 1, Appendix A).



Figure 4: Measuring water quality in the Schusters River (SCH1) (image courtesy of Lily Bovim)

The Diep River

The Diep/Sand River system drains the southern end of Table Mountain and the area just south of Wynberg Hill (Murray et al., 2009). The upper reaches flow through interlinked open public spaces in the suburb of Constantia and represent a fairly natural state (with the exception of riparian zones dominated by exotic ornamental plants in some stretches), but the lower reaches below the M3 freeway are in relatively poor condition (Murray et al., 2009). The Diep River has non-perennial flow and is approximately 14km in length. The Diep River site (DP1) was located in the Constantia Greenbelt in a pool shaded with overhanging vegetation (Figure 5, Table 1, Appendix A).



Figure 5: Diep River (DP1)

The Disa River

There are two main streams of the Hout Bay River, the Disa stream and the Orange Kloof stream (Murray et al., 2009). These streams source on top of Table Mountain and flow down through Orange Kloof Nature Reserve (Murray et al., 2009). The Disa River is perennial and 12km in length (Figure 6, Table 1, Appendix A). The Disa River site (D1) was located in the Orange Kloof Nature Reserve just upstream from the crossing bridge. Riparian vegetation provided the site with shade and there was little anthropogenic disturbance (Figure 6, Table 1, Appendix A).



Figure 6: Logger installation in the Disa River (D1) (image courtesy of Jeremy Shelton)

The Klawersvlei River

The perennial Klawersvlei River emerges from the mountains behind Miller's Point and then flows in a northwesterly direction along the plateau above Simons Town (Murray et al., 2009). It then flows east towards a large waterfall at Admirals Kloof before entering the sea at Simons Town railway station (Murray et al., 2009). The Klawersvlei site (AD1), located below the waterfall, was completely shaded by large boulders. There was little anthropogenic disturbance at the site (Figure 7, Table 1, Appendix A).

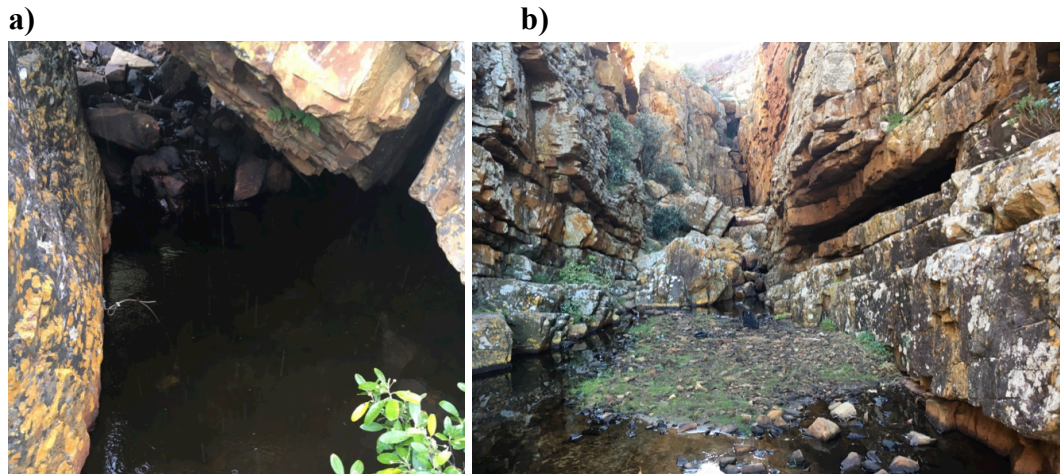


Figure 7: a) Logger installed in the Klawersvlei River, b) Admirals Kloof waterfall

Table 1: Site coordinates, elevation, substratum and land use of the sites

Site	River	Latitude	Longitude	Elevation (m)	Substratum	Land use
L1	Liesbeek	33° 59' 7.61"S	18° 25' 49.15"E	172	Rocky	Nature Reserve
L2	Liesbeek	33° 58' 56.92"S	18° 27' 4.85"E	52	Rocky/sandy	Residential
L3	Liesbeek	33° 57' 8.48"S	18° 28' 34.77"E	10	Canalised	Residential/industrial
S1	Silvermine	34° 5' 15.72"S	18° 25' 7.32"E	314	Rocky, mostly bedrock	Nature Reserve
S2	Silvermine	34° 6' 27.3"S	18° 24' 33.81"E	89	Rocky, mostly bedrock	Nature Reserve
S3	Silvermine	34° 7' 50.73"S	18° 26' 6.67"E	9	Sandy/muddy	Residential/public open space
SCH1	Schusters	34° 12' 8.96"S	18° 22' 28.37"E	4	Sandy/muddy	Residential/public open space
DP1	Diep	34° 0' 0.88"S	18° 26' 8.78"E	90	Rocky/sandy	Residential/public open space
D1	Disa	34° 0' 31.92"S	18° 23' 26.76"E	98	Rocky	Nature Reserve
AD1	Klawersvlei	34° 11' 30"S	18° 25' 11.7"E	176	Rocky	Public open space

2.2 Study species

Galaxias zebratus is the only formally described species from the genus found in South Africa, and has a high level of intra-specific divergence (Wishart et al., 2006). Molecular studies show that the species represents a species complex that could potentially contain 10 genetically distinct lineages (Chakona et al., 2018). The species is elongate and rarely grows to more than 70mm in length (McDowall, 2006). This cryptic species is known to occur in river systems of the Cape Peninsula in open pools or riffles (McDowall, 2006). From personal observation adult *G. zebratus* tend to hide out underneath rock ledges or amongst vegetation, while the juveniles are more exposed and swim on the outskirts and in the middle of slow flowing pools. Prior to the onset of this study, presence of *G. zebratus* at all 10 sites was confirmed through pilot site visits. All fish used in this study were adults

and sub-adult *G. zebratus* (TL >30mm). Prior to the commencement of the study, ethical clearance was acquired from the University of Cape Town Animal Ethics Committee (2018) (approval number 2017/V19/Dallas) and Cape Nature (permit number CN44-31-2893) for collection and experimentation on 30 individuals per site.



Figure 8: Adult *Galaxias zebratus* (image courtesy of Geoff Spiby)

2.3. Collection and care of fish

Fieldwork and experiments were undertaken during early summer (November-December 2017) and mid/late summer (January-February 2018). Overall, the experiments ran for a total of 20 days, with each site being sampled on a separate day. Individuals were collected in the morning using SASS nets with 1mm mesh on a square (30cm²) aluminium frame, and with hand nets with 5mm mesh. Fifteen individuals were captured from each site and transported carefully in buckets with aerated river water (using battery-operated airstones) in cooler boxes, fitted with ice bricks to keep the water cool and reduce stress, to the experimental laboratory at the Freshwater Research Centre in Kommetjie. Transportation time varied between 15 - 30 minutes depending on the distance of the site from the experimental location. Dechlorinated, aerated tap water was used in the experiments. Following the completion of each experiment, fish were transported in buckets containing their original aerated river water in cooler boxes to the location from where they were collected, and released on the same day.



Figure 9: Collection of fish using a SASS net (Images courtesy of Lily Bovim)

2.4 Collection of water temperature data

Hourly water temperature ($^{\circ}\text{C}$) data were recorded at all 10 sites for a period of six months (October 2017 to April 2018). The data were recorded on pre-programmed waterproof Hobo UTB1-001 TidBit V2 loggers (Onset Computer Corporation, 2008), which were securely attached to a large boulder or bedrock in a metal housing connected to a bolt drilled into a rock with a steel cable in pools where fish were present. These loggers were placed in position where they received flow into the pool and depths of the pools were less than one meter. The hourly water temperature data were used to characterise the sites in terms of their temperature fluctuations over time (i.e. thermal signature). To differentiate between the thermal histories at the 10 sites, each site's thermal signature was expressed as monthly thermal metrics or 'Indicators of Thermal Alteration' (ITA), based on Rivers-Moore et al. (2012). The ITA were used to determine thermal signatures of all 10 sites by converting sub-daily water temperature data into daily metrics (i.e. minimum, mean, maximum and range). These data allowed a relationship to be tested between *G. zebratus* upper thermal tolerance limits at each site and the environmental temperatures of their habitat over a period of time. For each site, seven day moving averages of daily mean (Mean_7) and maxima (Max_7) (i.e. moving averages of temperature of the seven previous days) were calculated and used to determine magnitude and duration of monthly extremes in water temperature.

2.5 Thermal tolerance/ CT_{max} experiments

Individuals were kept in aerated buckets at their stream temperature at the air-conditioned experimental facility at the Freshwater Research Centre for a period of time depending on which of the three daily experiments they would be used for. A holding period did not exceed four hours. The fish were not fed for the duration of the holding period, and the holding temperature in the buckets

ranged between 14.63°C and 20.74°C depending on the site and ambient air temperature on the day of experiments (see Appendix B). Holding temperatures were kept between each sites' daily minimum and maximum recorded during the two weeks preceding the experiment. The holding temperature correlated with mean daily stream temperature for the two weeks preceding the experiment ($r=0.86$). Hobo™ V2 temperature loggers were used to measure the holding temperature in each bucket. The fish were not exposed to an acclimation period on the assumption that they were acclimatized to their natural site thermal regime (thermal history), rather than a fixed temperature generated by a temperature-controlled environment (Reizenberg, 2017). Short and long-term acclimation (days to months respectively) state of an organism is known to influence the upper thermal limit (McCauley & Huggins, 1979).

For each experiment, five individuals were placed in a mesh basket inside a water bath fixed with a circulatory heater (Julabo™). Five individuals were selected per trial and each trial was repeated three times for every site ($n=15$). Each trial had a 30-min control period, where fish were placed in water at their holding temperature (i.e. temperature of the stream from where they were collected) (Appendix B). After the control period, temperature increased at a constant rate of 0.3°C /min to avoid an acclimation effect (Perez et al., 2003). Mora and Maya (2006) suggest if the rate of temperature change is too rapid the lag time between body and water temperature leads to an overestimation of CT_{max} . Similarly, a rate that is too gradual results in an underestimation of CT_{max} . Once signs of thermal stress or loss of righting response (LRR) were expressed (Beitinger & Lutteschmidt, 2011), the CT_{max} was recorded and the individual was removed from the water bath using a small hand net and placed in aerated recovery buckets. Once all individuals in the water bath had reached their thermal endpoint and removed from the waterbath, their total length (TL; mm) was measured. After complete recovery, all five individuals were placed together in an aerated bucket containing their original river water until they were returned to their site. Trials were repeated three times for each experiment day so that a total of 15 individuals had been experimented on. There were two sites where less than 15 individuals were captured; the Schusters River site (SCH1) ($n=11$) and the lower Silvermine River site (S3) ($n=10$).

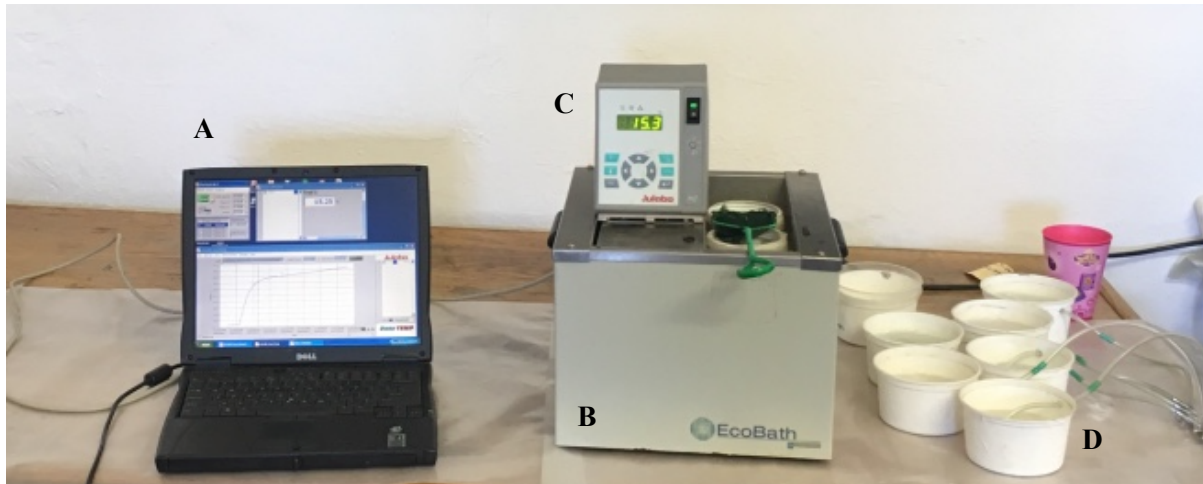


Figure 10: Experimental setup for the Critical Thermal Method, a) Laptop with Julabo software installed to control the experiment, b) Water bath containing a mesh basket in which the fish are placed, c) Circulatory heater (Julabo™) connected to the laptop, d) Recovery buckets filled with cold water and fitted with air stones.

2.6 Statistical analyses

Analyses of experimental data were performed in RStudio version 1.1.447 (2016). The “PMCMR” (Pohlert, 2014), “ggplot2” (Wickham, 2009), “devtools” (Wickham et al., 2018), “FactoMineR” (Le et al., 2008), “factoextra” (Kassambara & Mundt, 2017) and “ggpubr” (Kassambara, 2017) packages were installed for analyses. Simple linear regression analysis was used to test relationships between thermal history metrics and CT_{max} values for the fish at each site. Assumptions of normality were evaluated with Shapiro-Wilk tests. The results from the Shapiro-Wilk tests showed that the assumptions of normality were not met ($p= 0.001$); therefore a non-parametric Kruskal-Wallis test was used to compare CT_{max} and thermal metrics between sites. Principle components analysis was run on the metrics to substantiate which sites group best with each other. Metrics used were the mean, standard deviation, maximum, minimum, range, Mean_7 and Max_7 for each site from October 2017 to April 2018. The correlation coefficient (r) was determined to test for a relationship between total length of individuals and the median CT_{max} .

Chapter 3: Results

3.1 Water temperature: Thermal history

Weekly and monthly variability in thermal signatures of all 10 sites is summarised in the temperature metrics presented in Table 2, Table 3 and Figure 11. Over the six-month period (October to April) or period of temperature logger submersion, the metrics showed that site L3 was the warmest on average with a mean of 19.76°C. The coolest site on average was L1 with a mean of 16.35°C. Site SCH1 had the highest variability, whilst site S3 had the lowest variability over the six-month period (Table 3).

Max_7 were highest at site L3 (27.66°C) and lowest at site DP1 (19.27°C). Correspondingly, site L3 (23.01°C) had the highest Mean_7 and site DP1 (18.49°C) had the lowest Mean_7. Monthly averages were highest in mid-summer (January) and lowest in spring (October) (Table 2, Figure 11).

Mean, maximum and minimum temperatures from two weeks preceding the November-December 2017 experiments showed that sites L3 and S2 reached the warmest temperatures with maximum temperatures of 24.94°C and 24.77°C respectively (Table 2). Sites AD1 and S1 reached the lowest temperatures with minimum temperatures of 12.82°C and 13.04°C, respectively (Table 2). Site L3 had the highest Max_7 and Mean_7 (22.51°C and 19.22°C respectively) and site L1 had the lowest Max_7 and Mean_7 (16.90°C and 16.25°C respectively). Site S2 had the most variability during this two-week period (Table 2).

Mean, maximum and minimum temperatures from two weeks preceding the January-February 2018 experiments showed that sites L3 and SCH1 reached the highest temperatures with maximum temperatures of 29.46°C and 26.62°C respectively (Table 3). The coolest temperatures reached were at L1 and S1 with minimum temperatures of 15.06°C and 15.82°C respectively (Table 3). Site L3 had the highest Max_7 (26.91°C) and site DP1 had the lowest Max_7 (18.49°C) (Table 3). Site SCH1 had the highest Mean_7 (22.48°C) and site DP1 had the lowest Mean_7 (17.83°C). Site L3 had the most variability during this two-week period (Table 3).

Table 2: Thermal statistics for all 10 sites for the November-December 2017 experiments (**SD**= standard deviation, **Max**= Maximum temperature, **Min**= Minimum temperature, **Max_7**= Seven day moving average of daily maximum, **Mean_7**= Seven day moving average of daily mean)

	Site	Mean (°C)	SD	Max (°C)	Min (°C)	Range	Max_7 (°C)	Mean_7 (°C)
Two weeks preceding CT _{max} experiments	S1	16.58	0.87	20.51	12.82	7.69	18.81	16.84
	S2	17.95	1.20	24.77	13.50	11.27	22.19	18.09
	S3	17.85	0.76	20.94	15.27	5.67	19.75	18.37
	L1	15.82	0.93	18.84	13.40	5.44	16.90	16.25
	L2	17.04	1.04	21.58	13.67	7.91	19.47	17.06
	L3	18.60	1.12	24.94	14.86	10.08	22.51	19.22
	DP1	16.03	0.53	18.56	14.39	4.17	17.68	16.39
	D1	16.67	0.97	20.32	13.71	6.61	19.22	17.13
	AD1	16.73	0.95	22.71	13.04	9.67	20.23	16.89
	SCH1	18.97	0.57	23.21	15.70	7.51	21.35	19.12
One month preceding CT _{max} experiments	S1	16.54	1.25	22.44	12.05	10.39	21.00	17.67
	S2	17.98	1.19	24.85	12.12	12.73	22.87	18.47
	S3	17.74	0.62	20.94	15.27	5.67	19.75	18.39
	L1	15.27	1.13	18.84	12.15	6.69	16.90	16.26
	L2	17.31	0.96	21.89	13.33	8.56	20.17	17.92
	L3	18.50	1.06	24.94	14.31	10.63	22.91	19.28
	DP1	16.23	0.67	18.70	14.17	4.53	18.16	16.91
	D1	16.07	1.06	20.32	12.92	7.40	19.22	17.13
	AD1	16.80	1.08	22.71	12.41	10.30	20.23	17.67
	SCH1	18.54	1.32	23.81	14.43	9.38	22.23	19.37
Two months preceding CT _{max} experiments	S1	15.66	1.65	22.44	10.71	11.73	21.00	17.67
	S2	16.55	1.82	24.85	11.35	13.50	22.87	18.48
	S3	17.08	1.26	22.23	13.14	9.09	19.75	18.49
	L1	14.18	1.70	18.84	10.32	8.52	17.35	16.30
	L2	16.16	1.53	21.89	11.69	10.20	20.17	17.94
	L3	17.52	1.58	24.94	12.87	12.07	22.91	19.23
	DP1	15.54	1.08	18.70	12.87	5.83	18.16	16.91
	D1	15.23	1.43	20.32	11.83	8.49	19.22	17.15
	AD1	16.32	1.92	22.71	11.32	11.39	20.23	18.90
	SCH1	17.08	1.94	23.81	12.92	10.89	22.23	19.37

Table 3: Thermal statistics for all 10 sites for the January-February 2018 experiments (**SD**= standard deviation, **Max**= Maximum temperature, **Min**= Minimum temperature, **Max_7**=Seven day moving average of daily maximum, **Mean_7**= Seven day moving average of daily mean)

	Site	Mean (°C)	SD	Max (°C)	Min (°C)	Range	Max_7 (°C)	Mean_7 (°C)
Two weeks preceding CT _{max} experiments	S1	18.26	0.93	22.87	15.82	7.05	20.16	18.63
	S2	21.11	1.37	26.38	16.63	9.75	24.85	21.22
	S3	19.35	0.71	21.15	17.84	3.31	19.99	19.47
	L1	17.86	1.11	26.38	15.06	11.32	21.82	17.92
	L2	21.01	0.88	25.56	17.23	8.33	24.51	21.41
	L3	22.12	1.26	29.46	17.03	12.43	26.91	22.27
	DP1	17.78	0.55	19.63	15.99	3.64	18.49	17.83
	D1	19.23	0.66	22.47	16.51	5.96	21.75	19.50
	AD1	19.78	0.74	21.7	16.84	4.86	21.12	20.14
	SCH1	22.46	1.20	26.62	19.15	7.47	23.92	22.48
One month preceding CT _{max} experiments	S1	17.78	0.85	22.87	15.37	7.50	20.16	18.63
	S2	20.67	1.16	26.38	16.06	10.32	24.85	21.22
	S3	18.78	0.77	21.15	17.12	4.03	19.98	19.47
	L1	17.39	1.00	26.38	14.51	11.87	23.07	17.97
	L2	21.37	1.00	27.11	17.23	9.88	25.19	22.01
	L3	22.19	1.22	29.46	17.03	12.43	27.67	23.01
	DP1	17.46	0.57	19.63	15.56	4.07	18.74	17.83
	D1	19.54	0.73	23.55	16.51	7.04	22.13	20.15
	AD1	19.85	0.76	23.59	15.89	7.70	21.58	20.30
	SCH1	21.39	1.49	26.62	17.56	9.06	23.92	22.51
Two months preceding CT _{max} experiments	S1	17.35	0.79	21.72	12.82	8.90	19.50	18.33
	S2	19.96	1.47	28.87	13.50	15.37	26.12	21.22
	S3	18.38	0.83	21.15	15.27	5.88	19.99	19.47
	L1	16.50	1.50	26.38	12.15	14.23	23.07	17.97
	L2	20.66	1.31	27.11	16.25	10.87	25.19	22.00
	L3	21.35	1.43	29.46	16.44	13.02	27.66	23.01
	DP1	17.08	0.91	20.98	14.17	6.81	19.27	17.94
	D1	18.84	1.14	23.55	15.25	8.30	22.13	20.15
	AD1	19.15	1.17	23.59	14.75	8.84	21.58	20.30
	SCH1	20.33	1.78	26.62	15.29	11.33	23.92	22.51
Duration temperature logger was submerged in water (six months)	S1	16.70	1.73	26.97	10.71	16.25	22.73	19.47
	S2	19.00	2.13	28.87	11.35	17.52	26.12	21.79
	S3	18.34	1.49	21.20	13.14	8.06	20.86	20.23
	L1	16.35	2.15	26.38	10.15	16.23	22.84	18.95
	L2	18.89	2.23	27.11	11.69	15.43	25.19	21.99
	L3	19.76	2.10	29.46	12.87	16.59	27.66	23.01
	DP1	16.57	1.49	20.98	12.10	8.89	19.27	18.49
	D1	17.27	2.03	25.62	11.83	13.79	22.36	20.15
	AD1	17.93	1.85	23.59	11.32	12.27	21.58	20.32
	SCH1	19.36	2.46	30.16	11.69	18.48	27.42	22.76

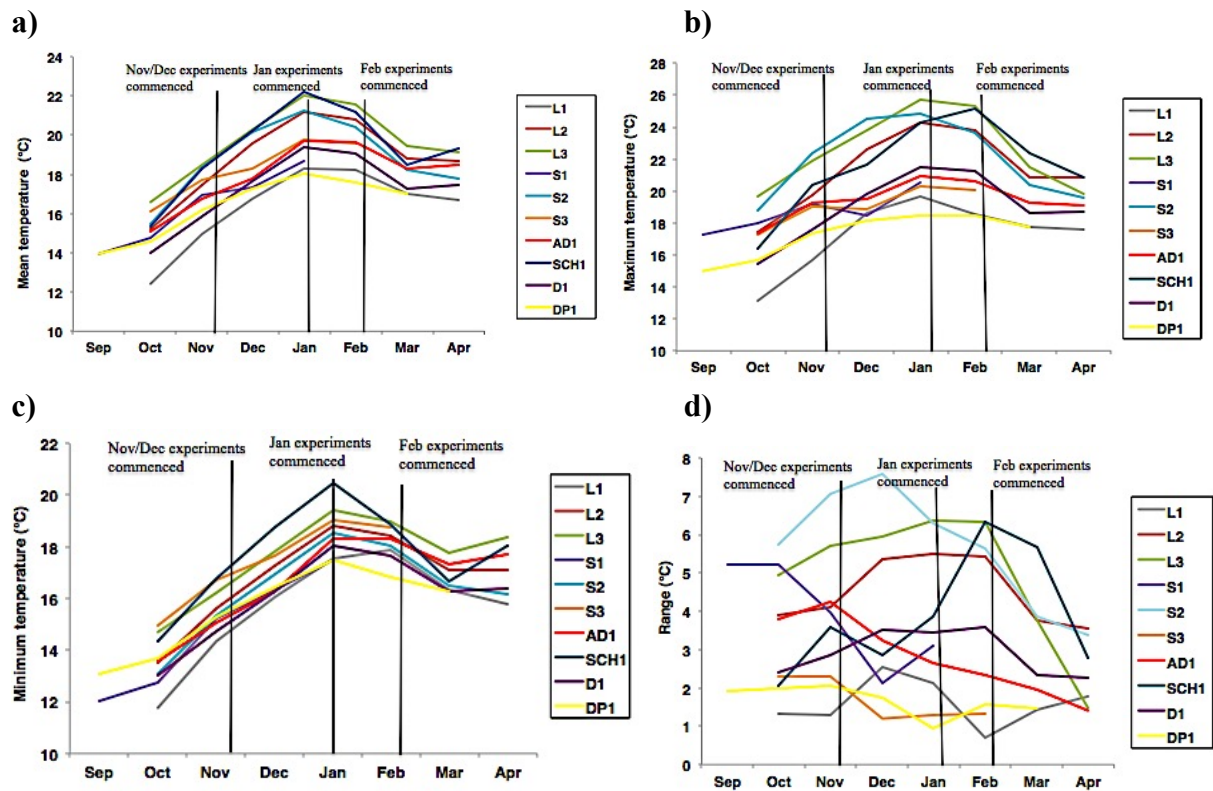


Figure 11: Monthly averages for mean (a), maximum (b), minimum (c) temperatures and temperature ranges (d) for all 10 target sites over a six-month period/period of logger submersion

The principle components analysis shows that the first principle component explains 68.80% of the total variation and 27.00% of the variation is explained by the second principle component (Figure 12). So the first two principle components explain 95.80% of the total variation. The first component correlates highly with the Max_7 metric, while the Min metric correlates almost perfectly with the second component. The sites that are grouped are AD1 and D1 (Group 1). Sites L3, L2, S2 and SCH1 form a group (Group 2) and sites S1 and L1 (Group 3) are also grouped slightly. The remaining sites (i.e. DP1 and S3) do not have a strong membership with any of the other groups (Figure 12). The groups can be characterised by how warm versus cool they were. Thermal groups are represented along gradients of selected thermal metrics (i.e. Min, Mean, Max, Mean_7, Max_7, Standard Deviation (SD) and Range over the six-month period). Group 2 was the warmest group comprising sites in the middle and lower reaches, whilst Group 3 was the coolest group comprising largely upland river sites, and Group 1 was an intermediate thermal group.

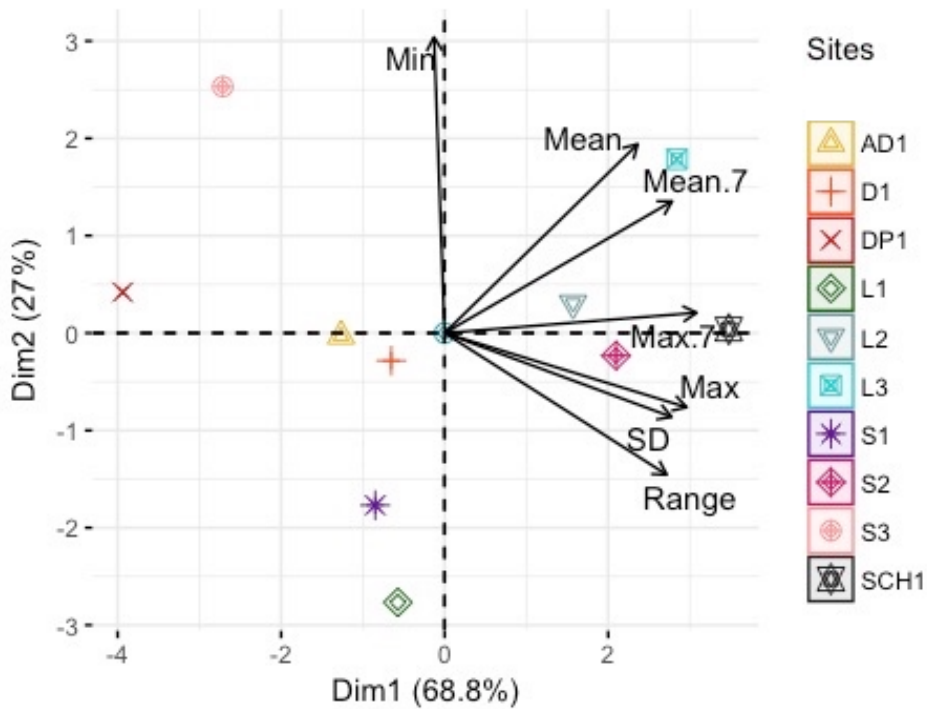


Figure 12: Principle component analysis of the water temperature logger sites (PC axes 1-2) showing selected thermal metrics, which were best correlated with the sites

3.2 Upper thermal limits of *Galaxias zebratus* between sites

The CT_{max} values from all sites for the November-December experimental period ranged from 30.00°C (at site SCH1) to 32.45°C (at site L3). CT_{max} values for all sites from the January-February experimental period ranged from 31.29°C (at site S3) to 33.42°C (at site L3) (Appendix B). There was a significant difference in *G. zebratus* CT_{max} values across the 10 sites for the November-December 2017 experiments, the January-February 2018 experiments and both experiment periods combined (Kruskal-Wallis chi-squared = 63.62, $df = 9$, $p < 0.001$; Kruskal-Wallis chi-squared = 89.61, $df = 9$, $p < 0.001$; Kruskal-Wallis chi-squared = 94.92, $df = 9$, $p < 0.001$ respectively) (Table 4). *Galaxias zebratus* tolerated the highest temperatures at sites L3 and S2 during the November-December 2017 experiments with CT_{max} values of 32.45°C and 32.00°C respectively (Figure 13 and Appendix B). *Galaxias zebratus* from site L3 and L2 during the January-February 2018 experiments were most thermally tolerant with CT_{max} values of 33.42°C and 33.13°C respectively (Figure 13 and Appendix B). During the November-December experiments, *G. zebratus* had the lowest CT_{max} at site SCH1 (30.00°C). During the January-February experiments, *G. zebratus* were most thermally sensitive at site S3 (31.29°C) (Figure 13 and Appendix B). Outliers are evident at L2 and L3 during the January-February experiments, and at S2 during the November-December experiments (Figure 13). The outliers could potentially be individuals that selected a cooler habitat, for example within the dense

vegetation at the site or at the bottom of the pool, and did not experience a similar thermal history to the other individuals, hence influencing CT_{max} values.

Table 4: Results of inter-site Kruskal-Wallis (K-W) tests between all 10 sites for both experimental periods and for both experimental periods combined

Experiment period	p	df	K-W test statistic
November-December 2017	<0.001	9	63.62
January-February 2018	<0.001	9	89.61
Both experimental periods	<0.001	9	94.92

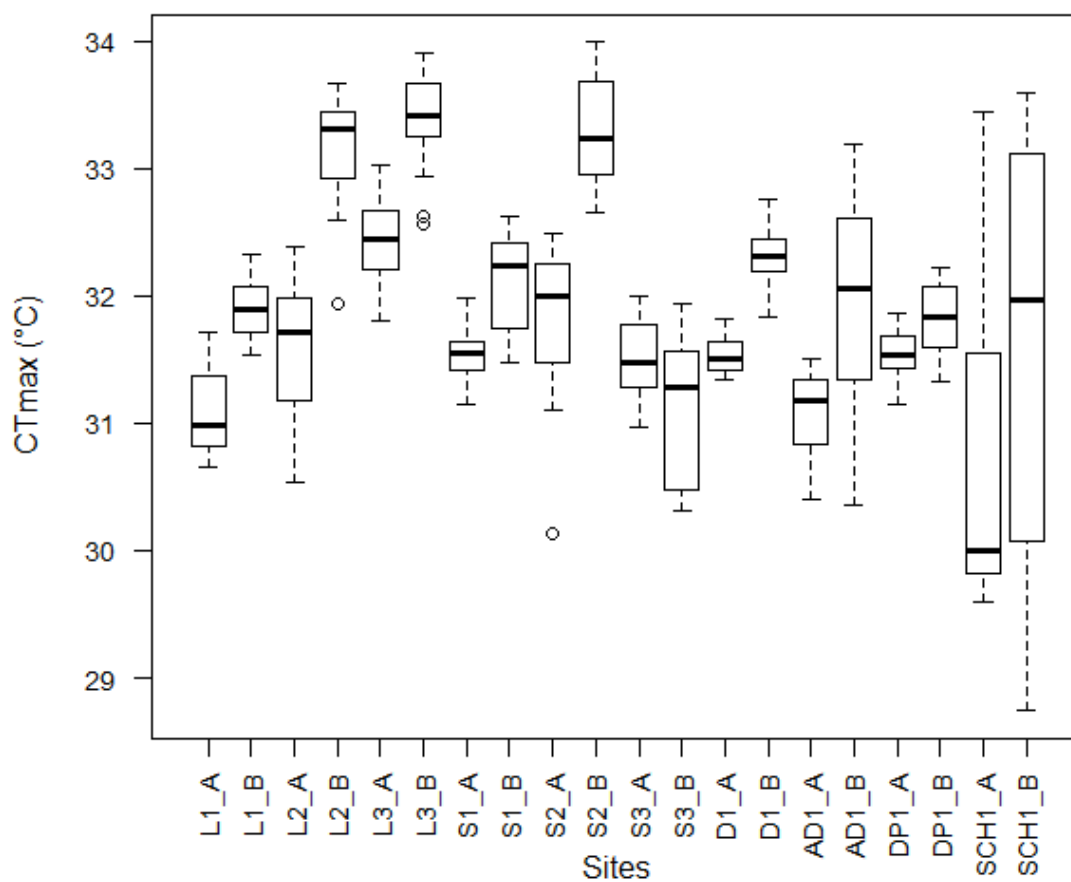


Figure 13: Boxplot showing median, upper and lower quartiles and range of CT_{max} ($^{\circ}C$) for *G. zebratus* for all 10 sites from the November-December 2017 (A) and January-February 2018 (B) experimental period

3.3 Regression analyses between CT_{max} and thermal history (including non-riverine sites, SCH1 and S3)

Linear regression was run twice on the data; the first time included all 10 sites; the second (see 3.4) excluded SCH1 and S3. SCH1 and S3 are non-riverine sites, and therefore may have differed from the other typical riverine sites in terms of their hydrology and water chemistry. SCH1 has estuarine system properties, particularly in summer when river inflow is minimal, and S3 is a wetland site with still waters.

Regression analysis found a very weak, but significant relationship between CT_{max} values from the November-December 2017 experiments and thermal history (TH) based on Max_7 two months preceding the experiments ($F_{1,8} = 0.40$; $p > 0.005$). The following model defines the relationship:

$$CT_{max} = 0.07 (TH) + 29.92$$

The R^2 value for the model indicates that only 6.72% of the variability in the critical thermal maxima is explained by changes in TH (see Figure 14 and Appendix C).

Using the same TH metric based on Max_7 two months preceding experiments, a significant relationship was found between CT_{max} values from the January-February 2018 experiments and TH ($F_{1,8} = 16.69$; $p < 0.005$), being defined by the following model:

$$CT_{max} = 0.21 (TH) + 27.65$$

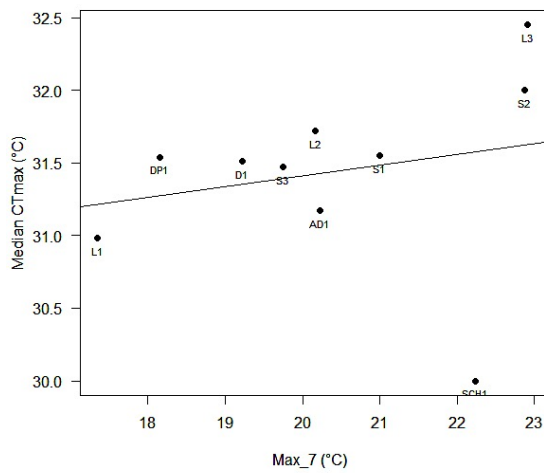
The R^2 value for the model shows that 67.60% of the variability in the critical thermal maxima is explained by changes in the thermal history. This is the strongest relationship determined for this set of analyses. CT_{max} values are notably dependent on TH (see Figure 14 and Appendix C).

Regression analysis found a weak, but significant relationship between CT_{max} values from the November-December and January-February 2018 experiments combined and TH based on Max_7 two months preceding the experiment ($F_{1,18} = 16.94$; $p < 0.005$), being defined by the following model:

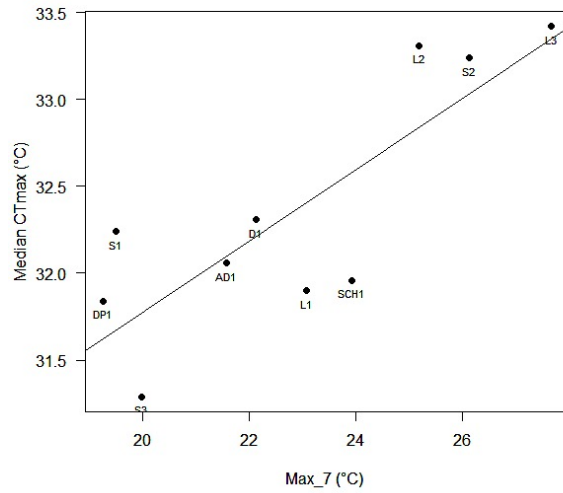
$$CT_{max} = 0.21 (TH) + 27.32$$

The R^2 value for the model shows that 50.49% of the variability in the critical thermal maxima is explained by changes in thermal history (see Figure 14 and Appendix C).

a)



b)



c)

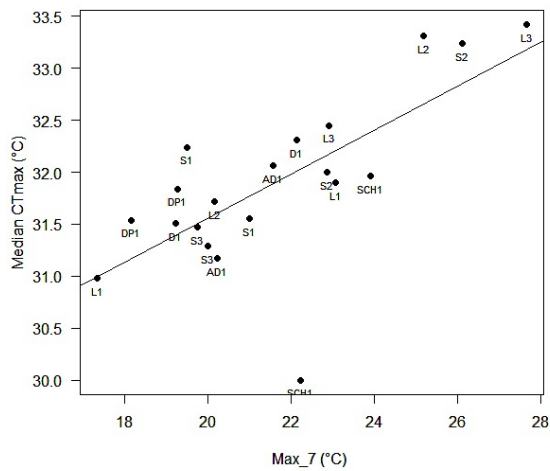


Figure 14: Linear regression between median CT_{max} and Max_7 two months preceding experiments for all sites from the November-December 2017 experiments (a), January-February 2018 experiments (b) and both experimental periods combined (c)

3.4 Regression analyses between CT_{max} and thermal history (excluding non-riverine sites, SCH1 and S3)

Regression analysis found a highly significant relationship between CT_{max} values from the November-December 2017 experiments and thermal history based on Mean_7 two weeks preceding the experiment ($F_{1,6}=27.67$; $p<0.005$), being defined by the following model:

$$CT_{\max} = 0.43 (TH) + 24.26$$

The R^2 value for the model shows that 82.18% of the variability in the critical thermal maxima is explained by changes in thermal history (see Figure 15 and Appendix D).

Regression analysis found a significant relationship between CT_{\max} values from the January-February 2018 experiments and thermal history based on Mean_7 two weeks preceding the experiment ($F_{1,6}=34.82$; $p<0.005$), being defined by the following model:

$$CT_{\max} = 0.37 (TH) + 25.22$$

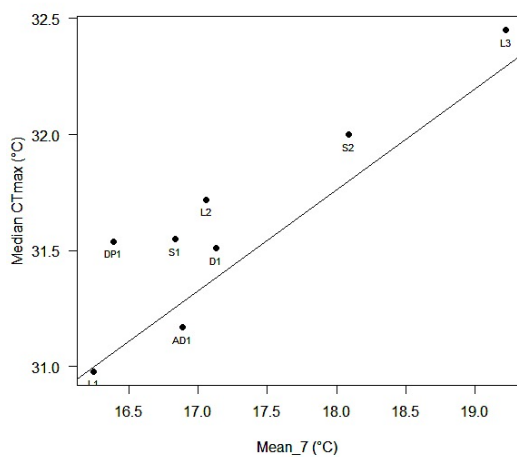
The R^2 value for the model shows that 85.30% of the variability in the critical thermal maxima is explained by changes in the thermal history (see Figure 15 and Appendix D).

Using the TH metric based on Mean_7 two weeks preceding the experiment for the November-December 2017 and January-February 2018 experiments combined, a strong relationship was found between CT_{\max} and TH ($F_{1,14} = 135.10$; $p<0.005$). The following model defines the relationship:

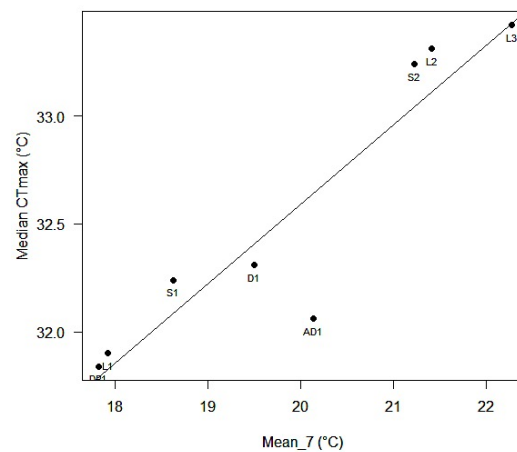
$$CT_{\max} = 0.37 (TH) + 25.27$$

The R^2 value for the model indicates that 90.61% of the variability in the critical thermal maxima is explained by changes in TH. This is the strongest relationship determined for this set of analyses. CT_{\max} values are notably dependent on TH (see Figure 15 and Appendix D).

a)



b)



c)

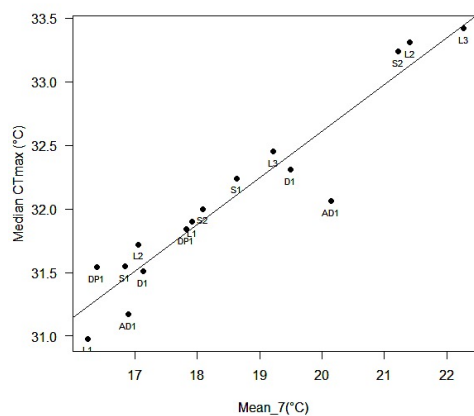


Figure 15: Linear regression between median CT_{max} and Mean_7 two weeks preceding experiments from sites excluding SCH1 and S3 from the November-December 2017 experiments (a), January-February 2018 experiments (b) and both experimental periods combined (c)

3.5 Correlations between *Galaxias zebratus* size and CT_{max} values

The correlation coefficients (r) reflect that there is no strong correlation between mean total length (mm) of *G. zebratus* and CT_{max} values from all 10 sites (Table 5). Site L3 has a moderate, negative relationship. Sites S1, S2, AD1, SCH1 and D1 have weak, positive relationships. Sites L2, S3, DP1, L1 have weak, negative relationships. A significant relationship was found between mean size and CT_{max} at sites S1, L3 and DP1 ($p < 0.05$). No significant relationship ($p > 0.05$) was found at the remaining sites, namely S2, L2, AD1, S3, SCH1, D1 and L1 (Table 5).

Table 5: Statistical results for Total Length (TL) and CT_{max} correlations

Site	Mean TL (mm)	Min TL (mm)	Max TL (mm)	SD	p	r
S1	45.00	35.00	63.00	6.43	0.044	0.37
S2	40.43	30.00	50.00	4.68	0.329	0.18
L2	46.67	34.00	62.00	7.66	0.980	-0.01
AD1	47.93	35.00	62.00	7.17	0.061	0.35
L3	47.77	35.00	71.00	8.58	<0.001	-0.61
S3	40.84	31.00	53.00	6.12	0.570	-0.12
SCH1	34.35	29.00	42.00	3.66	0.483	0.14
DP1	50.50	41.00	75.00	8.04	0.011	-0.46
D1	45.87	38.00	60.00	5.01	0.907	0.02
L1	52.23	40.00	71.00	7.57	0.141	-0.28

3.6 Behavioural responses of *Galaxias zebratus* and sample size

Based on observational data, behaviour of *G. zebratus* during the control phase and experimental phase were the same for individuals from all 10 sites and for both experimental periods. Behavioural signs of stress included an increase in activity followed by disorientation and loss of righting response (LRR). The loss of righting response represented the end point for all individuals and was determined when an individual lost the ability to remain upright (Table 6). During a trial experiment and visual observation it was determined that five individuals would be experimented on at one time based on their size, level of stress presented and visibility of their stress response.

Table 6: Behavioural responses of *G. zebratus* from all 10 sites during both experimental periods (November-December 2017 and January-February 2018) (CT_{max} = Critical Thermal Maximum, **PTR**= Point of Thermal Reactivity (Dallas & Ketley, 2011), **LRR**= Loss of Righting Response (Beitinger & Lutterschmidt, 2011))

Species	Observed behaviour		End Point (CT_{max})
	Control	Experiment	
<i>Galaxias zebratus</i>	Individuals remained at the side and base of the mesh basket. Sometimes two or three individuals lay on top of one another attached to the side of the basket. Fins were extended at rest and some individuals intermittently darted into the side of the basket.	Initially there was no change in behavioural response to temperature increase. However at PTR, fin and tail movement increased and their body formed an S shape close to their CT_{max} . Individuals would swim around the base of the basket rapidly. Their gills extended wider. Individuals would stick their snouts out of the water. Some individuals, particularly larger ones (>50mm), would jump out of the water to escape the basket.	First signs of LRR (disorientation). Individuals exhibited rapid tailfin movement. Gills extended wider.

Chapter 4: Discussion

The data produced allows *G. zebratus* in different rivers of the Cape Peninsula to be ranked in terms of their thermal sensitivity and associated vulnerability to regional climate change. The significant difference in *G. zebratus* upper thermal tolerance limits between the 10 sites suggests that there may be an abiotic influence on the CT_{max} of *G. zebratus*. Regression analyses proved that there is a strong relationship between thermal history and CT_{max} of *G. zebratus* at the study sites. Riverine sites that reached warmer temperatures had *G. zebratus* with higher upper thermal limits than *G. zebratus* from sites with cooler temperatures. The following sections shall discuss the biotic (i.e. species effect) and abiotic (i.e. site effect) influences determining upper thermal tolerance limits of *G. zebratus*.

4.1 Evolutionary history of *Galaxias zebratus*: the species effect

The measure of an organism's tolerance to temperature can be expressed as CT_{max} . This is the critical thermal maximum temperature, above which mortality will almost definitely occur (Beitinger et al., 2000). The CT_{max} reflects the absolute extreme physiological endpoint of the fish and the prolonged exposure temperature would be 1-4°C lower (Beitinger et al., 2000). Temperature is the key environmental factor influencing the physiological activity of ectotherms such as fish (Beitinger et al., 2000). Most fish perform their vital functions in a relatively narrow range of water temperatures between freezing point and 40°C (Beitinger et al., 2000). Species may have a wide or narrow thermal range depending on environmental conditions, with eurythermic species having a wide thermal range, while stenothermic species have a narrow thermal range (Moore, 1940). Four types of thermal identities may be used to describe these two groups of stenothermy and eurythermy, namely cold-adapted stenotherms, warm-adapted stenotherms, cold-adapted eurytherms and warm-adapted eurytherms (Moore, 1940).

Aquatic species are usually more stenothermic than ectothermic terrestrial species because thermal fluctuations on land are much greater than in water (Moore, 1940). Eurythermic species have a greater ability to tolerate temperature fluctuations due to their phenotypic plasticity (Moore, 1940; Somero, 2010). An ectothermic organism can be described as eurythermic if the range of temperatures it can withstand is 19°C (Franklin et al., 2001; Moore, 1940; Somero, 2010). Therefore, *G. zebratus* in this study can be considered stenothermic because the range of *in situ* water temperature for all sites did not exceed 19°C (see Table 3), although temperature ranges differed between sites. This means that the degree of stenothermy within *G. zebratus* individuals differed between each site.

CT_{max} information about other galaxiid species is scarce, although available literature documents high tolerances (measured as IULT) between 28.00°C and 31.70°C for five species from New Zealand (Richardson et al., 1994). Reizenberg (2017) recorded median CT_{max} of *G. zebratus* from the Driehoeks River to be 29.85°C. The closely related *Galaxias maculatus* from New Zealand (McDowall, 2006), has a thermal tolerance range measured as IULT, of 30.50 to 35.40°C (Simons, 1986). *Galaxias platei* found in Patagonia exhibited CT_{max} values between 25.58°C and 30.78°C (Barrantes et al., 2017). The range of CT_{max} found in this study was 30.00°C to 33.42°C. The differences in upper thermal tolerance limits between and within *Galaxias* species, suggests the breaking up of Gondwanaland may have caused a divergence in upper thermal tolerance limits when the species separated. Results from genetic studies on *G. zebratus* in different river systems of the Cape Peninsula would be able to further support this idea. However, there is still evidence of differences in thermal limits within the *Galaxias* genera (i.e. between species of *Galaxias*) since they evolved via sympatric speciation (McDowall, 2006). During these speciation events thermal ranges may have already limited communities of *G. zebratus* due to the fact that invasive species were not around to do so. Therefore, temperature may have been responsible for the distribution of *G. zebratus* rather than other habitat factors.

With the current threat of climate change, it is essential to determine which animals are most at risk and physiological studies predict the impacts of climate change by determining which species presently live closest to their thermal maximum (CT_{max}), which species are able to modify their thermal tolerance through acclimatisation and adaptation, and which physiological systems set the upper thermal tolerance limits for these species (Somero, 2010). According to Somero (2010), it is the stenotherms and cold-adapted eurytherms that will be most threatened by climate change. *Galaxias zebratus* are considered stenothermic, and therefore are expected to be vulnerable to climate change, while bearing in mind that this sensitivity categorisation of *G. zebratus* is based on thermal maxima and thermal ranges may expand or contract depending on life stages of the fish. In order to decipher the full range of temperatures tolerable by *G. zebratus*, a CT_{min} would need to be established. Moreover, in order to encompass the entire thermal range of the species, thermal tolerance should be determined for all life history stages from the egg to adult.

There are some tradeoffs between the stenothermic and eurythermic strategies. For example, warm-adapted stenotherms compromise their ability to occupy a wide geographic range to become eurythermic, but in doing so maximise their fitness at higher temperatures (Pörtner et al., 2006). If the conditions of a habitat are stable and fitness is optimised, a species may select a stenothermic strategy, rather than eurythermy which allows for wide distribution, but at the cost of reduced fitness and losing the ability to survive large fluctuations in local temperature (Gilchrist, 1995). Biochemical trade-offs or compensations may in fact have set ecological patterns and thus influenced the ecology

of river systems in the CFR and Cape Peninsula over time (Pörtner et al., 2006). In order for species with the ability to occupy a wide range of habitats, a thermal compensation must occur to prevent the occupancy of less-optimal environments (Pörtner et al., 2006). Similarly, physiological traits must compensate to maintain a favourable metabolic rate, which requires more energy. These physiological compensations can manifest into reproductive success of individuals or occur at the cellular level (Pörtner et al., 2006).

Our observational data show that certain sites namely, S1 and DP1 dried up during summer, resulting in isolated pools, which eventually also dry up, yet *G. zebratus* are able to repopulate the dry riverbed each year. The ability of *G. zebratus* to withstand this drying up of stream and avoid desiccation may be attributable to the fish insulating itself beneath the dry riverbed with a 'water bubble' - as seen in other freshwater fishes (Greenwood, 1986; Hecht et al., 1988) and invertebrates (Robson et al., 2011). The CFR endemic galaxiid lineage, *Galaxias* 'nebula', has a widespread distribution across isolated river systems in the CFR (Chakona et al., 2011). Field observations suggest that *G.* 'nebula' can abandon dry pools and move over damp soil to colonise nearby refugia overland, which explains the widespread distribution pattern of this lineage (Magellan, 2015; Chakona et al., 2011). Moreover, the New Zealand galaxiid, *Neochanna burrowsius*, has been well documented to burrow aestivate (Eldon, 1979). The ability to perform gaseous exchange through the skin is the key adaptation (Urbina et al., 2014). *Neochanna burrowsius* is one of the most prominent cutaneous respiring fish, which meets up to 43% of its oxygen requirements through the skin (Urbina et al., 2014). Like all members of the Galaxiidae family, *N. burrowsius*, is characterised by a small body size, lack of scales and slender body form (Urbina et al., 2014). The capacity for oxygen uptake through the skin has also been postulated for the unusual emersion behaviour (leaping from the water under conditions of severe hypoxia) of *Galaxias maculatus* (Urbina et al., 2014). The ability of cutaneous respiration may be present in *G. zebratus* and would explain their ability to repopulate dried up pools. With regard to freshwater invertebrates, there are relatively few studies of drought refuge use by stream invertebrates (Robson et al., 2011). Although the following refuges have been identified for a number of taxa: desiccation resistant eggs or pupae (e.g. Ephemeroptera (mayflies)); aestivation in dry sediment (e.g. Hydrudinea (leeches)); aestivation in moist sediment (e.g. Amphipods); and flying adults move to perennial waters/pools (e.g. Anisoptera (dragonflies)) (Robson et al., 2011). The hypothesis that *G. zebratus* aestivates beneath the dry riverbed is yet to be studied for the species in the CFR and should be examined in future studies.

Mediterranean regions are at risk from the impacts of climate change and freshwater life will be pushed to the limit of their physiological resilience (Filipe et al., 2012). Processes at molecular and physiological level influence these limits and the biogeographical pattern trends evident today (Somero & Dalhoff, 2008). Previous studies using temperature and empirical distribution data

obtained from the field have consistently identified thermal maxima as the limiting factor restricting fish distribution and abundance (Ostrand & Wilde, 2001). Species found at higher latitudes are expected to have wider tolerance ranges than species closer to the equator because seasonal variation is much greater at higher latitudes (Sunday et al., 2011). Some species or genera have the ability to preserve their thermal tolerance through genes, known as orthologs, that evolve from an ancestral gene, which maintains their function through evolution and time (Sunday et al., 2011). Orthologs may be responsible for determining the thermal maxima in *G. zebratus* from different sites. Since the last glacial maximum (LGM) almost 18000 years ago, *G. zebratus* in the Cape Peninsula have been isolated from other *Galaxias* species (Wishart et al., 2006). Based on ambient temperature approximations by Meadows and Baxter (1999) water temperatures may have been 5-6°C cooler and wetter during the LGM than today. It is possible that thermal maxima may have been set before or during the LGM. If thermal maxima were set before the LGM then thermal tolerance would be higher for species. In cases where evolutionary history has set the thermal limits, it is known as the species effect.

The crux of what causes a difference in thermal tolerance is physiological mechanisms at the biochemical level (Ficke et al., 2007). Fish from warmer streams develop a biochemical mechanism, which presumably is not developed or expressed in fish from cooler streams (Ficke et al., 2007). All fish have a thermal range bounded by the CT_{max} on the upper end and CT_{min} on the lower end (Fry, 1971). However acclimation to higher-than-ambient or near-lethal temperatures allows fish to adjust their critical thermal range, but the rate and magnitude of thermal acclimation is limited (Taniguchi & Nakano, 2000). Fish are able to closely couple their metabolic rates with water temperature (Ficke et al., 2007). Allocation pathways are plastic to allow fish to change according to their surrounding environmental conditions (Ficke et al., 2007). As temperatures increase more energy is dedicated to respiration (Ficke et al., 2007). At the cellular level, proteins are susceptible to heat damage, therefore in warmer streams repairing proteins is an important part of maintenance metabolism (Ficke et al., 2007). Due to the minimum metabolic requirements of fish being met before energy can be dedicated to reproductive or somatic growth, rising stream temperatures may hinder the ability of fish to reproduce, grow and maintain homeostasis (Sogard and Spencer, 2004). Thermal ranges are species-specific and we can expect fish populations experiencing changes to thermal regimes to achieve a new equilibrium, ruled predominantly by the energy costs associated with a new thermal environment and interspecific interactions (Ficke et al., 2007). Therefore, physiologically, *G. zebratus* from warmer streams may be using their energy differently to *G. zebratus* from cooler streams to adapt to the rising temperatures and reach equilibrium. The significant inter-site difference in *G. zebratus* upper thermal tolerance limits suggests that there may be more of an abiotic influence rather than a species or biotic effect determining the thermal tolerance of *G. zebratus*.

4.2 Looking at the abiotic factors: the site effect

Having discussed how physiology, genes and biology can influence a species' thermal limits, the following section discusses how long- and short-term temperature fluctuations (thermal history) could influence the thermal maxima of *G. zebratus* from rivers of the Cape Peninsula.

Median CT_{max} values for adults and sub-adult *G. zebratus* ranged from 30.00°C to 33.42°C; values that fall well above the natural water temperatures recorded at all 10 sites. Existing literature states that upper temperature tolerances of most fish species exceed temperatures of their natural habitats (Mundahl, 1990), therefore evidence of heat deaths of fishes in nature are very rare (Matthews et al., 1982). However, under some environmental conditions, for example reduction in water movement, high ambient temperatures and exposure to strong solar radiation, water temperatures may have the potential to exceed maximum thermal tolerances of fishes, particularly in shallow waters (<20cm) (Matthews et al., 1982). Under these conditions two approaches can be taken to enhance survival. Firstly, a physiological response can occur, where fish actually increases thermal tolerance or, secondly, a behavioural response can occur where fish select and redistribute to cooler habitats (Matthews et al., 1982).

Indeed, local geography and climate appear crucial factors determining the distribution of *G. zebratus* in the CFR (Chakona et al., 2013a; Chakona et al., 2018). According to Chakona et al. (2013a), three hypotheses could potentially account for the genetic diversity observed within the *Galaxias* genus. The first is the Refugia Hypothesis. During the Miocene-Pliocene marine transgression (15 – 2.6 million years ago), species' ranges were restricted leading to isolated populations and allopatric speciation (Chakona et al., 2013a). The second hypothesis is the Paeleoriver hypothesis. This hypothesis states that the Last Glacial Maximum (LGM) (18000 years ago) resulted in a paeleoriver consisting of a confluence of certain drainage basins. This allowed for the fish to disperse once again. Evidence lies in the presence of three primary genera across these systems. During the LGM air temperatures were 5 - 6°C cooler and weather was wetter than today (Meadows & Baxter, 1999). Thirdly, inter-drainage dispersal occurred in more recent geological time (i.e. during the Holocene 8000-6000 years ago). The Holocene was a period much warmer and drier than the LGM. Mediterranean climates were only established in the second half of the Holocene about 5000 years ago (Meadows & Baxter, 1999). Therefore living organisms had 5000 years to adapt to the present-day thermal regime. If after 5000 years there still has not been a differentiation in thermal maxima of different populations, the rate of climate change may be faster than the rate of evolution and this could result in an increase in mortality. In short, the rate of anthropogenic environmental change may exceed organism's adaptive capacity (Chevin et al., 2010), and the mechanisms evolved to cope with this enhanced environmental change are now being strained (Chevin et al., 2010).

Adaptation can occur on evolutionary timescales or over short periods of time (months to years) (Heino et al., 2009). A strong relationship was found between *G. zebratus* upper thermal limits and thermal history of the streams in this study. *Galaxias zebratus* from warmer riverine sites, such as the lower Liesbeek (L3), middle Liesbeek (L2) and middle Silvermine (S2) had higher CT_{max} values than at the cooler riverine sites such as the Upper Liesbeek (L1) and Diep (DP1). Linear regression was run twice on the data; the first time included the Schusters site (SCH1) and the lower Silvermine site (S3) (see 3.3); the second excluded these two sites (see 3.4). This is justified because SCH1 has estuarine system properties (electrical conductivity (EC) in December and January, 638 $\mu\text{S}/\text{cm}$ and 1332 $\mu\text{S}/\text{cm}$ respectively) (see Appendix A), especially in summer when river inflow is minimal, while S3 is classified as a wetland site comprising slow flowing or still water.

The inflow of river water is most crucial in estuarine systems due the riverine systems role in the hydrodynamic and sedimentary processes within individual systems (Whitfield, 1992). Temperatures in estuaries are likelier to reflect riverine conditions during floods, and during drought or normal river flows, temperatures tend to reflect marine temperatures (Whitfield, 1992). A thermocline may exist at the SCH1 site marked by an abrupt change in temperature above or below a certain layer. The pool where fish were caught was 1.5m in depth. According to Dallas and Rivers-Moore (2011), a thermocline forms in rivers with a stream depth greater than 1m. Differences in the vertical thermal profile would highlight the impact both the riverine and marine systems have on these estuarine sites (Whitfield, 1992). *Galaxias zebratus* from SCH1 were interpreted as outliers, and therefore removed from analysis because the likelihood of the site having a thermocline unlike the other river sites is quite high. The salinity of the water derived from the EC measurements at the site also differed significantly from the other river sites (see Appendix A).

Wetlands do not drain the landscape and consist of slow flowing or still waters, which accumulate a large amount of organic matter and soil particles (Murray et al., 2009). Site S3, a wetland site, faces the threat of invasion of *Typha*, although a native species, due to excessive nutrients in the stormwater runoff (City of Cape Town, 2010). This leads to regular control measures and the mechanical removal of *Typha*, and thus preferred habitat for *G. zebratus* at the site. Despite, the ability of wetlands to cleanse the water column passing through by causing particles (including bacteria) to drop out of suspension, a noticeable decline in water quality at the S3 site has been recorded, potentially due to the surrounding residential developments and highly polluted stormwater runoff (Murray et al., 2009; City of Cape Town, 2010). *Galaxias zebratus* from S3 were interpreted as outliers due to the restricted flow, level of organic matter (total dissolved solids (TDS) was measured as 425 ppm in December and 449 ppm in January) and controlled vegetation removal, ultimately making the *G. zebratus* habitat of S3 different to those of the riverine sites (see Appendix A).

The fish from SCH1 and S3 behaved in a noticeable stressed manner upon collection and throughout the experimental period. There is reason to believe that the thermal signal (i.e. CT_{max}) of *G. zebratus* from these two sites was likely over-ridden by other stresses once placed in the experiment water, such as lack of dissolved oxygen, concentration of TDS or EC (Whitfield, 1992). With these two sites excluded from the regression analysis, the relationship between CT_{max} and thermal history became much stronger ($R^2=90.61\%$). Therefore, the results offer evidence that thermal history does indeed influence upper thermal limits of *G. zebratus* in rivers of the Cape Peninsula. The regression equation, $CT_{max} = 0.37 (TH) + 25.27$, can predict CT_{max} of *G. zebratus* using thermal history based on Mean_7. Furthermore, the regression equation is useful to set upper thermal limits of *G. zebratus* and guide future research. Typical riverine sites that reached warmer temperatures had individuals with a higher CT_{max} than those from riverine sites with relatively cooler temperatures. This study also showed that the most robust thermal history metric to use to generate the equation for the relationship between CT_{max} and thermal history is the Mean_7 two weeks preceding the experiment.

In some situations habitat composition can restrict fish movement even when thermal factors do not. For example, an individual *G. zebratus* can only move to a new habitat if it is accessible and is physiologically favourable. In addition to climate change, water abstraction can also highly impact sensitive freshwater fish species like *G. zebratus*. Water abstraction can lead to loss of habitat connectivity and therefore restricted movement of individuals and reduced gene flow within the population, which could impact negatively on *G. zebratus* genetic diversity. Alterations in hydrology as a result of climate change and other human impacts may be more harmful to species than temperature alone (Filipe et al., 2012). In the Cape Peninsula and CFR as a whole, evaporation rates are expected to increase in addition to the decrease in water volume (Midgely et al., 2005). With reference to the Upper Silvermine River, where flow is restricted as a result of the reservoir upstream being an overflow or overfall dam, this section of the river dries up completely in mid-summer (January/February) and leaves no surface water habitat for the fish and other freshwater biota. *Galaxias zebratus* from this site could be considered more impacted than their conspecifics due to the high level of anthropogenic impacts coupled with indirect climate change impacts.

4.3 Future implications for Galaxias zebratus and their ecosystem

One of the major future implications is that the impacts of climate change on *G. zebratus* are likely to be patchy, affecting some river's *G. zebratus* populations more than others. When looking at the effects of climate change on *G. zebratus* it is essential to consider the entire ecosystem. Indirect climate change effects on food webs in addition to direct thermal impacts on *G. zebratus* may occur. Biotic and abiotic forces can influence stream fish populations. For a long time, ecologists have

understood that phenotypic differences among species play a pivotal role in structuring ecological communities (Start et al., 2018). Modifications to an ecosystem's species composition is rarely attributed to a single trait, but rather a suite of traits that act independently or in conjunction with other species environmental responses to environmental differences, biotic interactions and stressors (Start et al., 2018). Furthermore, species that are dissimilar with regard to certain traits are more likely to coexist because there is less competition (Start et al., 2018).

Abiotic forces, such as thermal warming have the ability to impact entire food webs and specific trophic interactions (Gilman et al., 2010). Modifications to trophic interactions are as important to discuss as changes in the *G. zebratus* assemblage structure because they may impact abundance and structure of the community. With trophic interactions, top down and bottom up effects are considered. In ecosystems there are primary productions and inputs. Vannote et al. (1980) coined the River Continuum Concept (RCC) where all biological components and processes change according to a longitudinal downstream continuum. Headwaters differ from middle and lower reaches in that they are steep, narrow and densely shaded by riparian vegetation. Fish would have bodies that allow them to move with ease in fast flowing water and mouthparts suitable to feed on collectors and grazers (Vannote et al., 1980). The middle reaches of rivers are more exposed to sunlight and serve as habitats for larger fish because there is more food to eat and larger, sheltered pools to inhabit (Vannote et al., 1980). Lower reaches are very exposed to sunlight and fish here are generally adapted to slow flowing waters, and feed on zooplankton in the water column or feed on detritus on the bottom of the channel (Vannote et al., 1980).

For *G. zebratus*, in addition to direct thermal stress, there could be other indirect impacts of climate change – either through changes in bottom up pathways (production) or top down forces (predation) or abiotic effects (habitat change due to changes in temperature and precipitation). An increase in temperatures will result in a change in distribution and functioning of aquatic macrophytes (Barko et al., 1986). Primary production of ecosystems is expected to increase due to enhanced growth and chlorophyll production. Although if temperatures rise above the thermal limit where plant tissues cannot function normally, a likely consequence would be a shift in range. There are limited studies on the effect of climate change on aquatic vegetation as noted by Anderson (1969) almost 50 years ago. Currently, literature on life history needs of aquatic plants is really rare. Despite trophic significance of aquatic plants, in-stream vegetation serves as refuge for many aquatic organisms such as *G. zebratus* (Shelton et al., 2008). Range shifts of riparian vegetation could result in loss of allochthonous energy input into mountain streams and loss of shading vegetation leads to increase in diurnal water temperatures (Davies, 2010).

A changing climate may drive range shifts and functioning of freshwater invertebrates (Dallas, 2008; Dallas & Ross-Gillespie, 2015). Given that grazing invertebrates are the primary food source for freshwater fish, a change in invertebrate community would impact the freshwater fish assemblage (Skelton, 2001). Dallas and Ketley (2011) have published reference thermal tolerance data for freshwater invertebrates including grazing invertebrates from the families Teloganodidae, Notonemouridae and Heptageniidae, which were shown to have an upper thermal limit of less than 33.00°C, which means they are highly thermally sensitive. If the abundance and distribution of these taxa shift, biological interactions may be disturbed, which may ultimately impact on the prevalence and abundance of freshwater fish communities. To appreciate the full extent of climate change impacts on *G. zebratus* in the Peninsula, future studies should consider the range of biotic (top-down and bottom-up) and abiotic consequences.

4.4 Experimental strengths, shortcomings and solutions

The standardisation of the critical thermal maxima methodology among studies is encouraged, particularly with regard to creating comparable data sets and acclimation temperatures (Sunday et al., 2011). Fish display phenotypic adaptation to temperature, meaning they can respond to abiotic changes over a short period of time (i.e. over days or months). According to Beitinger et al. (2000), this phenotypic adaptation is likely to influence thermal endpoints (CT_{max}) and the thermal sensitivity of individuals from all taxa that have been studied. That a laboratory acclimation period in this study was not included could be a weakness of this experimental approach; a 30-day acclimation period is suggested by Perez et al. (2003). However, acclimation is not necessary if fish are used immediately upon collection and adequate site temperature data is available (Reynolds and Casterlin, 1979), like in this study. Furthermore, the water temperature data collected in this study were used to infer thermal history and observe how the thermal history influenced the upper thermal limits of *G. zebratus* from different sites. The fish were not subjected to an acclimation period, but rather a short holding period (1-4 hours) before they were placed in the water bath. This holding period intended to reduce the stress of the fish and did not exceed the Mean_7 for each site (see Appendix B). Future studies of this nature are recommended to use site temperature data (acclimatization) to determine thermal history, as it is a more realistic representation of the sites' thermal profile than laboratory controlled acclimation.

When establishing thermal limits, the latitudinal effects on single species should be taken into account (Somero, 2010). The extent of the CFR region stretches both eastward and northward so this can easily be undertaken. Future research should focus on obtaining thermal tolerance data on native and non-native species, which would further understanding of how climate change may influence the

interaction between native and non-native CFR fishes. The results from this study suggest that juveniles should be treated separately in experimentation, as juveniles of other fish species have been shown to select different water temperatures than adults (Brandt, 1980). Only adults, or sub-adults if no adults are available, were used in experiments. Studies on the thermal tolerances of aquatic invertebrates in South Africa by Dallas and Ketley (2011), Dallas and Rivers-Moore (2012) and Dallas (2016) further highlighted the need for chronic exposure data to accurately set thermal limits for aquatic biota; and highlighted the need for further examination of thermal tolerance within a species amongst sites with different thermal regimes. According to Dallas and Rivers-Moore (2012), the challenge lies in linking the experimental data of upper thermal tolerance limits to *in situ* water temperature data, in a manner that reflects the potential consequences of alteration of thermal regime on aquatic biota in reality.

Sample size is another aspect of this experimental approach that requires consideration. A power analysis is suggested to determine the sample size required to detect an effect of a given size with a given degree of confidence. In this study a total of 30 individual fish were experimented on per site over both experimental periods (total n= 291). Barrantes et al. (2017) experimented on 79 *G. platei* juveniles in their CT_{max} work and Reizenburg's (2017) study used 32 *G. zebratus* per site in the CTM. Sample size choice is dependent on sample variation, which will differ among studies.

Similarly, the thermal metric used to infer a relationship between upper thermal tolerance limits and thermal history should be standardised. This study showed that Mean_7 two weeks preceding experiments to be the most robust thermal metric to depict a relationship between CT_{max} and thermal history. However, other studies, such as Barrantes et al. (2017), infer relationships based on specific acclimation temperatures (i.e. 2, 4, 10 and 16°C). It is thus important that future studies declare whether acclimation or *in situ* thermal history has been used to infer relationships, and in the case where *in situ* thermal history is used, the thermal metric (i.e. Mean_7 two weeks preceding experiments) must be standardised.

Spatial and temporal limitations are evident in this study: hourly water temperature data were recorded for a total of six months for each site, and experiments were only performed over one summer. *Galaxias zebratus* from 10 sites were experimented on and some of the target rivers had only one site. It would be valuable to record long-term hourly water temperature data (> one year) and repeat the experiments over another summer to assess the temporal repeatability of my findings. Due to high levels of pollution, particularly in the lower reaches, and lack of logger attachment sites, only one site could be used on some of the target rivers (i.e. Schusters, Disa, Diep and Klawersvlei Rivers). Ideally, an upper, middle and lower site would be allocated to each target river to encompass a wider range of thermal profiles for each river system.

The use of CTM rather than IULT or another chronic exposure method is a shortcoming of this study. CTM is useful to provide information on acute tolerance, but not chronic tolerance, like IULT and other chronic exposure methods (Dallas, 2016; Dallas & Rivers-Moore, 2012). This makes the data less realistic in terms of establishing future climate change impacts. CTM was used in this study because sample sizes were small and there were time constraints. Repeating this study using chronic exposure methods are required to more accurately comment on expected impacts of climate change on *G. zebratus* in the Cape, as they elucidate thermal resistance (Beitinger & Bennet, 2000) and this is more valuable for a real life climate change scenario. However, Dallas and Ketley (2011) found a significant positive linear relationship between estimated IULT and CT_{max} in their study on ten thermally sensitive aquatic macroinvertebrates. Though, no such study has been extrapolated for *G. zebratus*, likely because no distinct endpoint has been determined for the species.

Furthermore, genetic studies are required for the Cape Peninsula *G. zebratus* to confirm the number of genetically-distinct lineages within the species in the study area. Wishart et al. (2006) identified genetically distinct lineages of *G. zebratus*, alluding to the presence of five discernable species in rivers of the Peninsula. According to Reizenberg (2017) different species within a genus tolerate temperature differently. If multiple species within the *Galaxias* genus occur on the Peninsula, then this kind of study may be required to determine interspecific differences in CT_{max} . Given the numerous threats to existing populations, accurate assessment of the conservation status and mapping of the lineages is required, even before they are formally described, to guide the development and implementation of adequate management and conservation strategies.

Chapter 5: Conclusion

5.1 Overview of the key findings

The main findings of my study were: (1) upper thermal limits of *G. zebratus* from sites with different thermal profiles, as characterised by the thermal metrics, differed; (2) individuals from warmer sites had higher CT_{max} values than did individuals from cooler sites suggesting that *G. zebratus* from warmer sites are hardier than those from cooler sites; (3) CT_{max} values for all sites, except S3, were higher during the January-February 2018 experiments than the November-December 2017 experiments, indicating that the temperature increase that occurred over one/two months prior to sampling had a noticeable effect on *G. zebratus* upper thermal limits; (4) both the site and species effect indicate that thermal history (characterised by Mean_7 two weeks preceding the experiments) influences the upper thermal limits of *G. zebratus* either phenotypically or genetically (thermal history metric used was Mean_7, two weeks preceding the experiment to define the relationship equation); and (5) the observed *G. zebratus* CT_{max} differed to numbers recorded for other galaxiid species and localities. *Galaxias zebratus* from the Driehoeks River has an observed median CT_{max} of 29.85°C (Reizenberg, 2017). The Patagonian, *Galaxias platei*, exhibited CT_{max} values between 25.58°C and 30.78°C (Barrantes et al., 2017). *Galaxias zebratus* from this study exhibited a CT_{max} range of 30.00°C to 33.42°C, slightly higher than *G. zebratus* from the Driehoeks and the range of *G. platei*.

The effects of climate change in South Africa are expected to be region specific. The Cape Peninsula in the south-western corner of South Africa's CFR, is typically dry over the summer months and now, with expected increase in drought and reduction in rainfall, freshwater resources are becoming increasingly limited in this region. This could have substantial implications for society and agriculture, but also consequences for freshwater biodiversity. The life histories of many freshwater species are tightly coupled with the seasonality of temperature and flow; and an increase in magnitude and timing of hydrological and thermal events could impact the life histories and fitness of freshwater organisms throughout the region (Dallas & Rivers-Moore, 2014). Thermal experiments are an important step towards evaluating the ecological implications of elevated water temperature on aquatic organisms such as fish (Barrantes et al., 2017; Dallas, 2008). In response to this need, mine is the first study documenting the behavioural responses of *G. zebratus* populations from the rivers of the Cape Peninsula to temperature variation. The data presented are valuable not only for advancing knowledge on the thermal ecology of this species in this specific region, but also for predicting their vulnerability to short-and long-term climate change.

5.2 National challenges and recommendations for future freshwater climate change research

For any standard climate-related research on aquatic biota records of water temperature and acute, long-term experimental data are required. However, South Africa currently lacks the scientific infrastructure to collect spatially comprehensive water temperature data for rivers. Such data are expensive to collect and challenging to acquire: the cost of the data loggers is the initial cost, followed by difficulty of attachment at sites. However, the major issue is the general lack of infrastructure from relevant authorities and lack of capacity to implement management schemes. In order for management of thermal regimes to be successful these data are needed in order to set the biological thresholds to prevent ecological extinctions and conserve species impacted by climate change. Another risk factor in most peri-urban streams in South Africa, is the vulnerabilities of fixed scientific infrastructure to vandalism and theft. Furthermore, funding and differences in methodologies among studies are obstacles to obtaining these data. What is needed is partnerships between institutions such as the South African Institute for Aquatic Biodiversity (SAIAB), Freshwater Research Centre, Cape Nature and universities within the greater CFR, as it is difficult to ensure consistency between studies as some data could be collected experimentally and other data could be derived from models. Governmental departments, international institutions and relevant authorities need to be made aware of the importance of these data in order to provide financial assistance and capacity to implement such management schemes.

Future research is necessary on the early life stages of *G. zebratus*. This would provide information on the potential impacts of thermal alteration on population dynamics and overall fitness of the species. Since temperature is predicted to alter reproductive strategies of fish, egg sensitivity to changes in temperature, and adult reproductive fitness (Bobe & Labbé, 2010), further examination is required at different temperature scenarios. Thermal history varies both spatially and temporally; therefore further research is necessary to examine the relationship between thermal history and both space and time parameters. The data suggests that *G. zebratus* are good at developing higher upper thermal tolerances if they are exposed to warmer temperatures for a period of time (three to six months). A valuable future study would be to acclimate the fish in the laboratory to some high temperatures (i.e. $>25^{\circ}\text{C}$) and then record the CT_{max} .

The data obtained from this study could be linked to field studies to enhance understanding of how changes in temperature impact *G. zebratus* in their natural habitat. Field studies are instrumental as they can expose potential distribution corridors or barriers. In terrestrial conservation, management strategies have shifted from old-fashioned protected areas, to protected corridors to account for climate-induced range shifts. A similar predictive approach is necessary to manage freshwater fish populations in the CFR. However, this kind of predictive approach requires laboratory and field data

as (a) sensitive, tolerant, generalist and specialist species need to be identified and (b) the adequacy of the physical habitat needs to be assessed (Chevin et al., 2010) respectively.

Finally, the CFR holds a distinctive group of aquatic biota that are highly specialised and endemic to the regions unique freshwater ecosystems. Climate change is known to threaten biological diversity, however the degree to which has not been fully recognised. Now, there is confirmation of thermal stress likely to be caused by climate change on a native fish species. Beyond this thesis, results from this study will contribute to promoting the conservation of *G. zebratus* in the local rivers of the Cape Peninsula through providing reference data of the thermal tolerance of *G. zebratus* that can be used in comparative studies and promote collaborative freshwater research across South Africa and internationally. The objective of this study is not only to secure *G. zebratus* refuges, but also to ensure the longevity of South Africa's freshwater ecosystems for future generations.

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Appendices

Appendix A: Water quality data from all 10 sites measured on the day of experiments using a hand-held multi-parameter probe (Hanna HI98194) (EC= Electrical Conductivity, TDS= Total Dissolved Solids, DO = Dissolved Oxygen)

November - December 2017 Experiments					January - February 2018 Experiments			
Site	pH	EC ($\mu\text{S/cm}$)	TDS (ppm)	DO (%)	pH	EC ($\mu\text{S/cm}$)	TDS (ppm)	DO (%)
S1	4.64	172.00	86.00	83.70	4.76	220.00	110.00	37.20
S2	4.69	250.00	125.00	60.50	6.12	391.00	197.00	na
L2	6.46	192.00	96.00	103.20	6.68	191.00	96.00	84.30
AD1	6.22	425.00	213.00	82.80	6.52	470.00	235.00	64.80
L3	6.28	221.00	110.00	101.80	6.49	259.00	128.00	78.50
S3	6.65	851.00	425.00	39.40	6.7	898.00	449.00	na
SCH1	5.6	638.00	319.00	60.10	6.44	1332.00	676.00	na
DP1	6.48	163.00	82.00	100.70	6.34	304.00	154.00	72.00
D1	5.33	137.00	69.00	123.20	6.27	132.00	66.00	95.30
L1	6.15	84.00	42.00	86.50	5.23	132.00	61.00	na

* na = DO probe faulty at the time of measurement

Appendix B: Mean holding temperatures, median CT_{\max} values and mean total length for all 10 sites from both November-December 2017 and January-February 2018 experimental periods

River	Site code	Experiment date	n	CT_{\max} ($^{\circ}\text{C}$)	Holding temperature ($^{\circ}\text{C}$)	Mean total length (mm)
Liesbeek	L1	08/12/2017	15	30.98	16.36	54.67
Liesbeek	L2	28/11/2017	15	31.72	15.37	48.27
Liesbeek	L3	01/12/2017	15	32.45	16.83	52.20
Silvermine	S1	24/11/2017	15	31.55	14.63	43.07
Silvermine	S2	27/11/2017	15	32.00	16.00	40.53
Silvermine	S3	04/12/2017	15	31.47	16.22	39.33
Diep	DP1	06/12/2017	15	31.54	16.52	49.13
Disa	D1	07/12/2017	15	31.51	17.42	46.00
Klawersvlei	AD1	30/11/2017	15	31.17	16.67	43.73
Schusters	SCH1	05/12/2017	11	30.00	18.66	33.64
Liesbeek	L1	22/01/2018	15	31.90	18.93	49.80
Liesbeek	L2	20/02/2018	15	33.31	19.63	45.27
Liesbeek	L3	21/02/2018	15	33.42	20.42	41.33
Silvermine	S1	19/01/2018	15	32.24	17.36	46.93
Silvermine	S2	23/01/2018	15	33.24	20.21	40.33
Silvermine	S3	24/01/2018	10	31.29	19.49	43.10
Diep	DP1	18/01/2018	15	31.84	17.06	51.87
Disa	D1	19/02/2018	15	32.31	18.33	45.73
Klawersvlei	AD1	16/02/2018	15	32.06	19.80	52.13
Schusters	SCH1	25/01/2018	15	31.96	20.74	34.87

Appendix C: Regression analyses between CT_{max} values and thermal history (TH) for November-December, January-February and both experiments combined including non-riverine sites (SCH1 and S3). Thermal history metrics used were the Mean_7 two months, one month and two weeks preceding each experiment; Max_7 two months, one month and two weeks preceding each experiment; and the absolute maximum (Abs Max) two months, one month and two weeks preceding each experiment

TH	Nov - Dec experiments			Jan – Feb experiments			Combined (both experiments)		
	<i>p</i>	R ²	Equation	<i>p</i>	R ²	Equation	<i>p</i>	R ²	Equation
Mean_7 2 months	0.953	0.001	=-0.14(x) + 31.68	0.044	0.42	= 0.25(x) + 27.32	0.003	0.39	=0.27(x) + 26.70
Mean_7 1 month	0.905	0.002	=0.03(x)+ 30.93	0.041	0.43	=0.25(x) + 27.21	0.002	0.42	=0.28(x) + 26.59
Mean_7 2 weeks	0.942	0.001	=0.02(x) + 31.16	0.068	0.36	=0.25(x) + 27.27	0.003	0.39	=0.27(x) + 26.88
Max_7 2 months	0.547	0.07	=0.07(x) + 29.92	0.003	0.68	=0.21(x) +27.65	0.001	0.51	=0.21(x) + 27.32
Max_7 1 month	0.530	0.05	=0.07(x) + 29.93	0.005	0.66	=0.21(x) + 27.53	0.001	0.47	=0.21(x) + 27.32
Max_7 2 weeks	0.496	0.06	=0.09(x)+ 29.72	0.004	0.67	=0.23(x) + 27.31	0.001	0.50	=0.23(x) + 27.1
Abs Max 2 month	0.550	0.05	=0.06(x) + 30.05	0.008	0.60	=0.18(x) + 27.92	0.001	0.46	=0.18(x) + 27.62
Abs Max 1 month	0.561	0.04	=0.06(x) + 30.11	0.017	0.53	=0.17(x) + 28.05	0.002	0.42	=0.18(x) + 27.71
Abs Max 2 weeks	0.488	0.06	=0.07(x) + 29.88	0.034	0.45	=0.16(x) + 28.58	0.003	0.39	=0.17(x) + 27.92

Appendix D: Regression analyses between CT_{max} values and thermal history (TH) for November-December, January-February and both experiments combined excluding non-riverine sites (SCH1 and S3). Thermal history metrics used were the Mean_7 two months, one month and two weeks preceding each experiment; Max_7 two months, one month and two weeks preceding each experiment; and the absolute maximum (Abs Max) two months, one month and two weeks preceding each experiment

TH	Nov – Dec experiments			Jan – Feb experiments			Combined (both experiments)		
	<i>p</i>	R ²	Equation	<i>p</i>	R ²	Equation	<i>p</i>	R ²	Equation
Mean_7 2 months	0.090	0.40	=0.29(x) + 26.48	0.002	0.82	=0.31(x) + 26.22	<0.001	0.80	=0.35(x) + 25.53
Mean_7 1 month	0.003	0.79	=0.44(x) + 23.90	0.002	0.83	=0.32(x) + 26.10	<0.001	0.89	=0.36(x) + 25.35
Mean_7 2 weeks	0.002	0.82	=0.43(x) + 24.26	0.001	0.85	=0.37(x) + 25.22	<0.001	0.91	=0.37(x) + 25.26
Max_7 2 months	0.013	0.67	=0.19(x) + 27.84	0.005	0.76	=0.19(x) + 28.13	<0.001	0.79	= 0.23(x) + 27.21
Max_7 1 month	0.012	0.68	=0.18(x) + 28	0.005	0.79	=0.20(x) + 27.97	<0.001	0.78	=0.23(x) + 27.15
Max_7 2 weeks	0.017	0.64	=0.19(x) + 27.97	0.001	0.84	=0.22(x) + 27.56	<0.001	0.83	=0.24(x) + 26.97
Abs Max 2 month	0.043	0.52	=0.14(x) + 28.62	0.014	0.66	=0.17 (x)+ 28.27	<0.001	0.72	=0.191 (x) + 27.578
Abs Max 1 month	0.043	0.52	=0.14(x) + 28.62	0.025	0.60	=0.17(x) + 28.35	<0.001	0.68	=0.20(x) + 27.53
Abs Max 2 weeks	0.043	0.52	=0.14(x) + 28.70	0.037	0.54	=0.16(x) + 28.77	0.001	0.63	=0.19(x) + 27.78