THE EFFECT OF WATER TEMPERATURE ON THE DISTRIBUTION OF THE EASTERN CAPE REDFIN MINNOW, *PSEUDOBARBUS AFER* (Peters, 1864)

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Abstract

The main objective of this MSc study was to disentangle whether temperature or the presence of non-native fish was limiting the distribution of *Pseudobarbus afer* (Peters, 1864) in the Blindekloof stream, Eastern Cape, South Africa. The aims were to: i) describe the thermal regime of the Blindekloof stream; ii) conduct experiments to determine the preferred and critical temperatures of *P. afer* and; iii) use snorkel surveys to contextualise the distribution of both native and non-native fishes in the Blindekloof stream to assess whether the downstream distribution of *P. afer* was likely to be influenced by temperature or by the presence of non-native, predatory fishes.

To describe the thermal regime, year-long temperature data from four long term monitoring sites in the Blindekloof stream were collected using Hobo temperature loggers and analysed in order to better understand the thermal profile, the thermal variation and the rate of temperature change in the stream. The warmest temperatures were recorded in late December 2015 (absolute maximum of 29.4 °C). The coolest water temperatures were recorded in early August 2015 (absolute minimum of 9.5 °C). There is both seasonal and diel variation in temperature with mean, minimum, maximum, 7 day mean, 7 day maximum and temperature ranges differing significantly between sites. With knowledge of the thermal regime of a monitored reach of the Blindekloof stream, the thermal tolerance and preference of *P. afer* were investigated.

The thermal tolerance of *P. afer* was investigated using the Critical Thermal Method (CTM) which uses non-lethal endpoints (the loss of equilibrium). At low acclimatization temperatures (11.9 ± 0.7 °C), the mean CT_{max} of *P. afer* was found to be 29.9 ± 0.7 °C, while at a higher acclimatization temperature (19.9 ± 0.1°C), the mean CT_{max} was 35.1 ± 0.6°C. Custom-built thermal choice tanks were used to investigate the thermal preference of *P. afer* in both summer and winter. The preferred median temperatures for the summer experiments ranged from 22.4 – 29.3 °C while the winter preferred median temperatures ranged from 18.5 – 23.1 °C. The thermal tolerance of *P. afer* was compared to the thermal regime of the stream and the results suggest that temperature is not limiting the distribution of *P. afer*.

Snorkel surveys were used to determine the distribution of fishes in the Blindekloof stream. Analysis of the distribution data suggests that, in the absence of non-native predatory species, native fishes have the potential to inhabit pools throughout the system right to the confluence, thus predatory fishes and not temperature was limiting *P. afer* distributions.

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Chapter 1 General Introduction

Climate change, which is expected to alter air temperature and precipitation, amongst other things, is a widely accepted global phenomenon which many recognise as inevitable, and thus, research has started to focus on the possible effect of climate change on ecosystems (Sharma *et al.*, 2007; Rahel & Olden, 2008; Fenoglio *et al.*, 2010; Moyle *et al.*, 2013). Certain regions have been identified as hotspots for climate change, with some ecosystems being viewed as more vulnerable than others (Kundzewicz *et al.*, 2008; Strayer & Dudgeon, 2010; Ellender *et al.*, 2017). While much of the initial research focused on the impacts of climate change on terrestrial ecosystems, 2005 to 2015 was declared the International Decade for Action – "Water for Life" and there have been a resultant increased number of studies focusing on the projected impacts of climate change on freshwater ecosystems (Dudgeon *et al.*, 2006; Filipe, 2013).

FRESHWATER ECOSYSTEMS

Freshwater ecosystems can be viewed as islands of freshwater in a sea of land (Faulks *et al.*, 2010). Due to their small size and isolated nature, these ecosystems are particularly vulnerable to change with a low potential for resilience (Meyer *et al.*, 2007). Furthermore, they are influenced by all activities within their catchment area, and inevitably exposed to various changes and stressors (Cummins, 1977; Skelton *et al.*, 1995; Meyer *et al.*, 2007). Despite only making up 0.01% of the earth's water, freshwater ecosystems are diverse in nature and thus not all aquatic ecosystems are expected to face the same stressors in light of climate change (Dudgeon *et al.*, 2006).

Many of the species found within freshwater ecosystems have limited or no ability to disperse should their current environment change and become unfavourable; the water temperature and availability within these systems are directly linked to the climate (Barnett *et al.*, 2005; Woodward *et al.*, 2010). As the projected climate change impacts differ, some freshwater species are likely to be "winners" and others "losers" in response to climate change and different biota within ecosystems are expected to be affected in different manners (Petchey *et al.*, 1999; Dallas & Rivers-Moore, 2014). These unique freshwater ecosystems are a conservation priority, particularly when considering the increased anthropogenic threats that they are under which include overexploitation, water pollution, flow modification, destruction or degradation of habitat and the invasion of non-native species (Dudgeon *et al.*, 2006). Climate change, which is expected to alter air temperature, precipitation and evaporations, is therefore expected to exacerbate flow modification and lead to habitat alteration, and on a smaller scale may worsen water pollution (for example: mobilise absorbed pollutants such as metals) (Dallas & Rivers-Moore, 2014).

In order to successfully and effectively conserve and protect freshwater ecosystems and their native biota, reserves should envelope the entire river catchment (Skelton *et al.*, 1995). However, the protection of entire catchment areas is unfortunately often not practical because of their large areas, the reliance of humans on freshwater for life and the complex nature of entire catchments. Therefore, to successfully protect these ecosystems focal areas must be identified (Abell *et al.*, 2007). These focal areas, termed critical management zones, include headwater streams, which are characterised by low species diversity but high levels of endemism (Abell *et al.*, 2007).

HEADWATERS

Headwater streams are defined as “channels that occur at the fringe of any fluvial network” (Richardson & Danehy, 2007). Richardson & Danehy (2007) note that studies often neglect headwater streams due to insufficient understanding of their importance and the ecosystem services they provide. The Cape Fold Ecoregion (CFE), in the south-western region of South Africa, is rich in headwater streams which provide the last remaining refuges for many of South Africa’s small-bodied fishes (Ellender *et al.*, 2014) and have thus been identified as one such focal point. The CFE streams are characteristically cool and clear containing a highly endemic and distinct fish fauna (Skelton, 2001; Tweddle *et al.*, 2009). The majority of these endemic fishes (60%) are International Union for Conservation of Nature (IUCN) Red Listed as either Endangered or Critically Endangered (Ellender *et al.*, 2017). This region is therefore important for the conservation of freshwater fishes and the areas of protection priority should aim to incorporate these vulnerable fish communities throughout their distribution and as much of the broader habitat as possible.

These headwater streams are vulnerable to climate change, particularly those on the eastern boundary of the CFE, such as the Blindekloof stream, which are episodic in nature and are therefore likely to experience wide ranges in temperature and flow. Understanding the interaction between fishes and their physiological tolerances is increasingly important to understand the risk that climate change poses to imperilled species.

Water temperature is arguably the most biologically important property of both lotic and lentic freshwater ecosystems as many fundamental physiological functions, such as metabolic rate and reproduction, are temperature dependent (Wotton, 1995; Webb, 1996; Lessard & Hayes, 2003; Caissie, 2006). Specifically, in lotic systems, water temperature may be highly variable and affected by factors such as flow regime (Sinokrot & Gulliver, 2000; Caissie, 2006). Describing the temperature regimes is therefore essential for an understanding of what fishes within these systems are currently able to tolerate and to predict their responses to the consequent shift in thermal regimes linked to climate change.

The interpretation of the thermal results can only be as accurate as the equipment used to record the data, further assuming that the scale of observation is sufficient for the scale of the driving process (Rivers-Moore *et al.*, 2004). Thus, while daily maximum water temperature is regarded as an important thermal measure of ecological significance (Rivers-Moore *et al.*, 2004), this measure alone is not enough to provide a detailed description of the complex nature of thermal regimes within a system. Similarly, the use of mean temperatures may fail to adequately capture extreme thermal events which might be responsible for ecosystem stress, therefore it is not only mean or maximum temperatures but rather the variability which is important in such studies (Rivers-Moore *et al.*, 2013a). Therefore, an in-depth, comprehensive understanding of the thermal characteristics of the stream occupied by native biota is important to understand the current thermal environment inhabitable by these species, and thus better predict the effects of projected climate change scenarios.

THREATS TO HEADWATER ECOSYSTEMS AND THEIR ENDEMIC FAUNA

Ellender *et al.* (2011) pointed out that due to the complex nature of headwater ecosystems, it is difficult to disentangle the various threats that drive fish distributions. The most recognised threat to headwater biodiversity is species invasion, but multiple other stressors, and the manner in which they interact, also impact headwater ecosystems and are highlighted in Figure 1.1 (Dudgeon *et al.*, 2006). Figure 1.1 further highlights the difficulty of disentangling the threats to freshwater biodiversity. In light of climate change, these ecosystems are expected to face added thermal stresses (Dallas *et al.*, 2015, Ellender *et al.*, 2017). Dallas *et al.* (2015) noted that freshwater ecosystems are identified as highly vulnerable to global climate change. In southern African, which is already a critical region of water stress and where freshwater ecosystems face a number of stressors, climate change is expected to exacerbate the stresses (Dallas *et al.*, 2015). Understanding how water temperatures will change, along with how vulnerable the biota is to these changes, will allow one to better predict how climate change will impact these critical ecosystems (Dallas *et al.*, 2015). In order to predict how resilient or vulnerable the biota may be to thermal stress, thermal thresholds need to be investigated and thus thermal preference and tolerances need to be identified. This, with an understanding of the streams' present thermal signature, will enable a better understanding of current thermal stress as well as predicting how this may change over time (Dallas *et al.*, 2015).

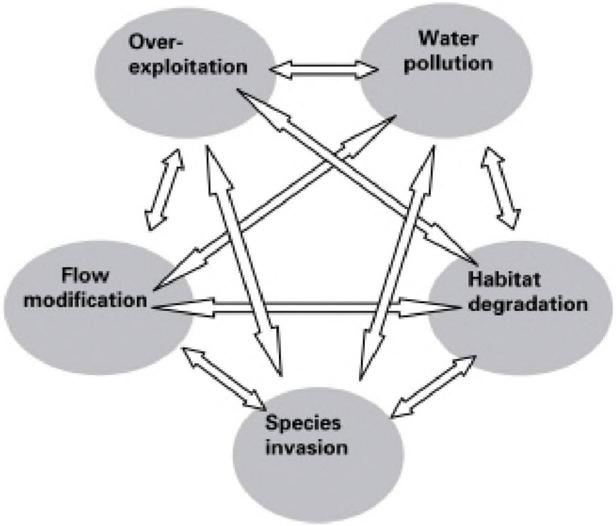

Figure 1.1 To show the major threats to freshwater biodiversity and their interactions (taken from Dudgeon *et al.* 2006).

The primary threat to biota inhabiting otherwise pristine headwater ecosystems is the presence of non-native fish species (Swartz & Impson, 2007; Ellender *et al.*, 2011; Ellender & Weyl, 2014; Kadye & Booth, 2014; Ellender & Weyl, 2015). Non-native fish species were introduced into South Africa for a variety of reasons including aquaculture, the pet trade, angling and as biological control agents (Ellender & Weyl, 2015). Non-native fish invasions pose a major threat to native, headwater species (Jackson *et al.*, 2001; Ellender *et al.*, 2011). Direct threats include predation on native fishes and competition for resources such as habitat or food and therefore, in some instances, the presence of non-native fishes has completely extirpated native fishes (Jackson *et al.*, 2001; Ellender & Weyl, 2014) and as such the availability of habitat for native species is shrinking (Swartz & Impson, 2007). These invasions may also have subtle influences on the ecosystem, such as altering the behaviour of native fishes but they may also have far-reaching influences on the entire food web (Kadye & Booth, 2012).

Non-native fish introductions are a global phenomenon with far-reaching consequences, for example: the introduction of *Clarias gariepinus* (Burchell, 1822), the African sharptooth catfish, is of particular concern in Brazil (Vitule *et al.*, 2006). *Clarias gariepinus* were first introduced as an aquaculture species in Brazil but have subsequently spread and potentially formed self-sustaining, established populations (Vitule *et al.*, 2006). The situation is cause for concern as, comparable to the CFE, several of the rivers in the Atlantic Forest of South America are inhabited by endemic fishes which are therefore endangered (Vitule *et al.*, 2006).

Similarly, a study by Ellender *et al.* (2014) found that *C. gariepinus* had invaded three monitored headwater streams in the CFE. The invasions were however "casual" as *C. gariepinus* failed to establish self-sustaining populations in the headwaters but the invasion potential was still cause for concern (Ellender *et al.*, 2014). This study highlights that the invasion potential of these headwaters by the predatory *C. gariepinus* may pose a threat to endemic fish fauna (Ellender *et al.*, 2014).

Highly endemic species are more vulnerable to habitat changes associated with global climate change due to their restricted distribution (Meyer *et al.*, 2007; Moyle *et al.*, 2013). Abell *et al.* (2007) highlight how due to the diverse nature of these systems, terrestrial management strategies will not successfully conserve freshwater ecosystems. To successfully protect endemic species and their suited habitat one must understand their distribution and population structures as well as the drivers thereof (Kadye & Booth, 2012; Ellender *et al.*, 2017).

Both exogenous factors, such as the presence of non-native fishes, and endogenous factors, such as physiological tolerances of a species, play a role in fish distributions (Abell *et al.*, 2007; Kayde & Booth, 2012). Fish distributions have previously been determined by hydrographic and geomorphological history (Skelton *et al.*, 1995). Presently, along with man-induced effects (exogenous), temperature, physio-chemical parameters and natural phenomena such as floods may alter populations and distributions of native fishes (Shuter *et al.*, 1980; Grossman & Freeman, 1987; Jackson *et al.*, 1992; Jackson *et al.*, 2001). Once aware of this, key areas for conservation may be highlighted and every effort to conserve these focal points can be made.

STUDY SPECIES

The focal species of this study is the Eastern Cape redfin minnow, *Pseudobarbus afer* (Peters, 1864) which is listed as Endangered on the IUCN Red List of Threatened Species. *Psuedobarbus afer* is a highly endemic fish species with distributions on the eastern border of the CFE. This listing as endangered is a classification which suggests the species is at high risk of extinction in the wild (Swartz & Impson 2007).

Pseudobarbus afer is a small-bodied stream fish (110 mm standard length) in the family Cyprinidae (Chakona & Skelton, 2017). *Pseudobarbus* species are characterised by the bright red colouration at the base of their fins (Skelton, 1988; Skelton, 2001; Chakona & Skelton, 2017). *Pseudobarbus* species are limited to the upper reaches of pristine headwater tributaries in the CFE (Swartz *et al.*, 2007; Chakona & Skelton, 2017). Many of the species are therefore restricted in their distribution (Chakona & Skelton, 2017). Investigating *P. afer* in these variable headwater habitats may provide valuable insights into their tolerances at their distribution limits within a system.

A recent revision by Chakona and Skelton (2017) indicates that what was once described as four lineages of *P. afer* (Swartz *et al.*, 2007) are different species and have consequently been described as such, with a co-occurrence of at least two species in the Gamtoos River System. The current study focused on *P. afer*. *Pseudobarbus afer* are limited in distribution to the headwaters of only three Eastern Cape, eastward flowing, coastal river systems, namely the Baakens, Swartkops and Sundays River systems (Swartz *et al.*, 2007; Ellender, 2013). Chakona and Skelton (2017) found that the suggested likely original locality of *P. afer* is the Swartkops River System. Despite there being no evidence of *P. afer* populations in the mainstem, Chakona (pers. comm.) suggests that the distribution in various headwaters may be indicative of the species at least using the mainstem as a medium for dispersal. While literature highlights that *P. afer* do not co-occur with predatory non-native fishes such as those of the genus *Micropterus*, it is not possible to infer whether the mainstem was inhabited by self-sustaining, established populations of *P. afer* prior to invasion (Ellender *et al.*, 2011; Ellender, 2013).

HABITAT

Psuedobarbus afer can presently be observed in pools, riffles and runs of these headwater streams, but their distribution is limited to only the pristine upper reaches of these three headwaters (Ellender, 2013). These streams are known to fluctuate in flow, which in turn causes fluctuations in both temperature and physico-chemistry, all of which *P. afer* has adapted to (Ellender, 2013). In dry months, pools fed by groundwater serve as a refuge (Ellender, 2013; Kayde & Booth, 2014) and limit the distribution of *P. afer* while periods of rainfall (wet periods) result in higher flow, more connectivity of pools and a rapid dispersal of *P. afer* into previously dry areas (Ellender, 2013).

MORPHOLOGY, AGE AND GROWTH

Pseudobarbus afer individuals are olive brown dorsally and creamy white ventrally with red colouring at the base of their fins (Figure 1.2). Maturity is reached at approximately 40 mm (Skelton, 2001). *Pseudobarbus afer* is a relatively slow growing species (ages of five to six years) (Cambray & Hecht, 1995) and therefore only reach maturity at three to four years of age (Ellender, 2013).

Figure 1.2 Appearance of mature *Pseudobarbus afer* with red colouring at the base of the fins (©NRF SAIAB, illustration by Dave Voorvelt).

REPRODUCTIVE STRATEGY

During the breeding season (summer), which runs from November to March (Cambray, 1994a; Ellender, 2013), males can be distinguished by the intensified red colouring of the fins and the presence of large, white, conical tubercles on their heads (Cambray, 1994a; Chakona & Skelton, 2017). While Cambray (1994a) stated that *P. afer* breed in summer in response to an increase in flow, a study by Ellender and Weyl (2015) found that the ratio of sampled juvenile to adult *P. afer* was independent of high flows, suggesting that other environmental factors, such as temperature, may act as a spawning cue to this species. This may be the case as temperature (coupled with photoperiod) has been shown to act as a spawning cue for other cyprinid species, such as *Notemigonus crysoleucas*, the golden shiner of North America (de

Vlaming, 1975). In a study by de Vlaming (1975) warmer temperature regimes have been linked to gonadal development and ultimately spawning in this species.

THREATS

Pseudobarbus afer are believed to have been widely distributed in the Sundays, Swartkops and Baakens River systems but populations have declined, both in distribution and in abundance, as a result of habitat availability and interactions with non-native predatory species (Ellender *et al.*, 2011; Chakona & Skelton, 2017). The species is limited to a 56 km^2 area of occupancy (Chakona & Skelton, 2017). While further threats to this species include the degradation of suitable habitat and the deterioration of water quality (Chakona & Skelton, 2017), Tweddle *et al.* (2009) highlight that the primary threat to this and many other freshwater fishes in southern Africa is the invasion by non-native species.

The populations of *P. afer* limited to the Baakens system are of utmost concern due to the catchment being heavily urbanized, thus further anthropogenic degradation of the system and the widespread invasions, particularly of *Tilapia sparrmanii*, A. Smith, 1840 (Chakona & Skelton, 2017). Thus studies focused on improved understanding of this imperilled species and its tolerances are becoming increasingly important.

In the Blindekloof stream, a headwater tributary of the Swartkops River System where *P. afer* is known to occur, a *Micropterus* species invasion threatened this species' distribution (Skelton, 2000). Where *Micropterus* were present, all native species were absent (Skelton, 2000). That is, due to predation pressures, no native species in the Blindekloof stream appear to be able to coexist with *Micropterus* (Skelton, 2000; Ellender *et al.*, 2011). This species has not successfully established in this stream and thus their distribution is limited (Ellender *et al.*, 2011). This is hypothesised to be due to the low flow and oligotrophic nature of the stream (Skelton, 2000). However, in summer months where juveniles may be present and in times of high flow, the invasion front may shift and it is therefore critical that the distribution is continually monitored (Skelton *et al.*, 1995).

Two of the three river systems inhabited by *P. afer* are within declared protected areas. Some, such as the Blindekloof stream, are remote and therefore Ellender *et al.* (2015) propose that in these areas the presence of non-native species can seldom be attributed to direct introductions by man. The primary origin of these invasions is therefore suggested to be incursions from mainstream source populations (Ellender *et al.*, 2011; 2015). Thus, it is evident that in order to effectively manage and conserve these endemic species, one must understand processes that drive changes or aid the introduction of non-native species.

AIMS AND OBJECTIVES

The need to better understand thermal tolerances and the dependence of *P. afer* on environmental triggers for critical processes and their distribution is highlighted by the fact that the susceptibility of a species to climate change is likely to depend on biological traits of the species (Dallas & Rivers-Moore, 2014). Such understanding also enables one to better predict *P. afer* resilience to climate change and their persistent distribution. That is, changes in climate or habitat may not affect all species or all life stages of a species in the same manner (Jobling, 1995; Dallas, 2008; Heino *et al.*, 2009; Barrantes *et al.*, 2017). A review by Desta *et al.* (2012) states that species with specific habitat requirements, narrow environmental tolerances or those which have a dependence on specific environmental triggers are likely to be more susceptible to climate change.

The aim of this study was to build on the knowledge of the endangered *P. afer* in relation to its habitat, particularly with regard to temperature. Furthermore, this thesis aimed to develop an understanding of factors limiting the distribution of *P. afer* and their resilience to spatial and temporal variability of these headwater ecosystems, to which they are endemic.

This chapter served as a general introduction aimed to introduce the topic of freshwater ecosystems, the threats they face and particularly to highlight the vulnerability of endemic headwater biota to these threats as well as to introduce the focal species of this thesis, *P. afer*.

In Chapter 2, the thermal regime of a monitored stretch of the Blindekloof stream was determined and describe, hypothesising that the thermal regime of the Blindekloof stream would be complex in nature, with both daily and seasonal fluctuations.

In Chapter 3, the preferred and critical maximum temperatures of *P. afer* from the Fernkloof headwater tributary were experimentally determined. I hypothesised that: i) *Pseudobarbus afer* will have an acute thermal preference closely linked to that of the stream temperature, ii) due to the episodic nature of the system, *P. afer* will have a broad thermal tolerance, therefore even in light of current climate change predictions this species is expected to survive and persist as the stream temperatures are not expected to exceed their upper critical temperature.

In Chapter 4, the distribution of both native and non-native fishes in the monitored headwater streams was contextualised in the knowledge of the thermal regime of the Blindekloof to assess whether the downstream distribution of *P. afer* and the upstream invasion fronts were likely to be influenced by temperature. I hypothesised that i) community composition would be significantly altered by the presence of non-native fishes, ii) *P. afer* would not co-occur with non-native predatory species such as *Micropterus* species in the downstream environment and therefore, iii) it is the presence of non-native species which limit the lower distribution of *P. afer* rather than temperature.

Finally, in Chapter 5, I contextualise the findings of all three data chapters in order to determine whether the stream temperature encompassed the thermal preference of *P. afer*, did not exceed the thermal tolerance of *P. afer* and thus allow one to disentangle whether it was temperature or non-native fishes which were limiting the distribution of *P. afer*.

Chapter 2 Study area and thermal regime of the Blindekloof stream

INTRODUCTION

Freshwater aquatic environments are under increasing pressures from habitat loss, water pollution, over-exploitation, flow modification, species invasion and global climate change (Dudgeon *et al.*, 2006; Woodward *et al.*, 2010). Global climate change is predicted to negatively impact freshwater resources due to, amongst other factors, the altered precipitation and thus flow regimes (Filipe *et al.*, 2013). Furthermore, streams are expected to be more sensitive to change than lentic systems due to the direct relatedness of factors such as runoff on lotic ecosystems (Sala *et al.*, 2000).

In their review, Sala *et al.* (2000) suggest land-use, climate and biotic exchange are the primary drivers of stream biodiversity. However, Sala *et al.* (2000) also highlight that each of these drivers influences different stream environments to varying degrees. It is accepted that thermal regimes of freshwater ecosystems vary across space and time with spatial variations observable at the global, national and catchment scale (Smith, 1968; Steele, 1982; Webb, 1996). The thermal regime of a river is a dynamic measure of complex interactions between various factors, such as the volume of water, the geology and riparian shading (Olden & Naiman, 2010). Therefore, not all regions of freshwater are equally sensitive to thermal drivers, such as solar radiation and surface friction (Rivers-Moore *et al.*, 2004).

Mediterranean climatic regions, defined by cool wet, winters and hot, dry summers (Gasith & Resh, 1999; Reason & Rouault, 2005), have been identified as particularly vulnerable regions for climate change (Filipe *et al.*, 2013). Of particular interest in this thesis is the Cape Fold Ecoregion, a Mediterranean system which stretches from the Western Cape into the Eastern Cape of South Africa (Filipe *et al.*, 2013; Ellender *et al.*, 2017). The CFE predominantly follows a winter rainfall pattern with ambient temperatures ranging from as low as -10 °C in winter months to as high as 40 °C in summer (Bradshaw & Cowling, 2014). The CFE is not only a hotspot for climate change but is also a region of high endemism, particularly in plants but also in stream fishes (Tweddle *et al.*, 2009; de Moor & Day, 2013; Ellender *et al.*, 2017). In the CFE, for example, global climate change predictions suggest that ambient temperatures will increase, resulting in increased evaporation and surface runoff will decrease due to decreased precipitation (Dallas & Rivers-Moore, 2014). Specifically, the mean annual air temperature is expected to increase by 2 - 6 °C and the precipitation decrease by up to 40 mm per decade (Dallas & Rivers-Moore, 2014).

Many chemical and physical (and therefore biological) properties of water are temperature dependent (Rivers-Moore *et al.*, 2004). Water temperature influences major physiological processes such as metabolic and growth rates as well as reproduction of fishes (Coutant, 1987; Bernatzerder & Britz, 2007). With this in mind, studies suggest that how different species within these systems will respond to such changes will depend on factors such as their behavioural and physiological adaptability and tolerances (Caissie, 2006). In general, stream biota are expected to experience change in distributional ranges as suitable habitat may become fragmented with an increase in frequency and intensity of extreme climate events such as floods, droughts and fires (Filipe *et al.*, 2013).

In the Eastern Cape Province of South Africa, on the western boundary of the CFE, climate change scenarios, for example an increase in droughts and floods (Gbetibouo & Ringler, 2009), are likely to increase the pressures already faced by many native fishes. Many native fishes' distributional ranges have previously been fragmented by human activities such as water abstraction and invasion by non-native predatory species (Ellender *et al.*, 2017). Studies which aid the understanding of the threat faced by native fish assemblages under climate change predictions are particularly pivotal in the CFE, which is already a water scarce region with a predicted extinction of up to 75% of endemic fish fauna by 2070 (Ficke *et al.*, 2007; Filipe *et al.*, 2013; Dallas *et al.*, 2017).

One such endemic species is *Pseudobarbus afer* (Peters, 1864). *Pseudobarbus afer* have a limited distribution in the headwaters of the Swartkops, Sundays and Baakens Rivers (see Chapter 1; Chakona & Skelton, 2017). With such a limited distribution, one of the strongholds of *P. afer* distribution is in the headwater streams of the Swartkops River which lie within the Groendal Nature Reserve (Ellender *et al.*, 2011). As such, the Swartkops system provides an ideal opportunity to study and understand the vulnerability of indigenous fishes in the CFE and the threat posed by non-native fishes under predicted climate change conditions. The Swartkops River and its headwaters provide a suite of environmental scenarios which allow for studies on possible natural factors which limit the distribution of native and non-native species.

This study focused on describing the thermal environment of the Blindekloof stream, one of the headwaters of the Swartkops River. The thermal regime of the stream was described by using hourly water temperature data which were recorded from four monitoring sites over the period of one year. The hourly temperatures provided information on daily maxima and minima at each of the sites, which allowed for daily ranges to be calculated. The hourly data were also averaged in order to provide daily mean temperatures.

To develop an understanding of the ecosystem and the relationship between the biotic and abiotic factors requires a comprehensive understanding of the study area. By using the hourly thermal data collected from the Blindekloof stream, this chapter aimed to characterise the thermal regime of the Blindekloof stream by describing the magnitude and duration of diurnal and seasonal fluctuations of water temperatures.

It was hypothesised that the temperature would vary significantly across the monitored sites of the Blindekloof stream. Furthermore, it was expected that the warmest temperatures would be recorded at the lowest site, closest to the confluence, while the site closest to the source would be the coolest.

MATERIAL AND METHODS

STUDY AREA

This study was conducted on the Blindekloof stream, a headwater tributary of the Swartkops River. The Swartkops River basin is situated near the western boundary of the Eastern Cape Province of South Africa (Figure 2.1).

SWARTKOPS RIVER

The Swartkops catchment (Figure 2.1) covers an area of approximately 1360 km^2 (Scharler & Baird, 2003). The land cover of the upper Swartkops catchment area is predominantly native forest, and forms a large area of wilderness region of the Greater Baviaans Kloof Reserve (Scharler & Baird, 2003). Small, isolated patches of the Swartkops catchment region are used for agriculture (Scharler & Baird, 2003) and the lower reaches of the catchment are heavily polluted due to urbanised activities which include salt works, clay mining and a sewage treatment plant (Scharler & Baird, 2003) finally discharging in Algoa Bay, near Port Elizabeth (Figure 2.1).

The Swartkops catchment area falls within the Fynbos and Albany Thicket Biomes (Mucina & Rutherford, 2011). The upper reaches of the catchment area fall within the Eastern Fynbos Renosterveld region of the Fynbos Biome while the lower reaches of the mainstem vegetation is classified as Albany thicket (Mucina & Rutherford, 2011).

The geology of the catchment area is characterised by both quartzitic sandstone, from the Table Mountain Group of the Cape Super Group, and conglomerate of subordinate sandstones, lenticular sandstones and clay stones from the Enon Formation of the Uitenhage Group (Shone, 2006; Thamm & Johnson, 2006). Quartzite is a sedimentary rock composed of sand grains which recrystallize to form a very hard rock (McCarthy & Rubidge, 2005). The Uitenhage Group, the largest of these sedimentary deposits, was formed from localised sediment deposits related to active faults, (McCarthy & Rubidge, 2005), while the gravel sediment deposits form the conglomerates found in the Enon Formation (McCarthy and Rubidge, 2005).

THE BLINDEKLOOF STUDY SITE

Heavy rains only occur in the Swartkops catchment basin on average 1.2 times a year (Table 2-1), with no seasonality but rather in an erratic and unpredictable manner (Skelton, 1993). The unpredictable nature of the rainfall results in the Blindekloof stream being classified as an episodic stream that it is fed by both precipitation and groundwater. Surface flow in the Blindekloof stream is intermittent, only occurring after heavy rains (Roux *et al.*, 2002; Ellender *et al.*, 2011). Consequently, because of the episodic nature of the Blindekloof stream, there are large fluctuations in surface flow, and associated parameters such as physico-chemistry with expectant fluctuations in water temperature. The large, permanent pools in the Blindekloof stream subsequently remain isolated for extended periods but with permanent subsurface flow (Skelton, 1993; Ellender *et al.*, 2011, 2017). These isolated pools serve as refuge pools for native fish fauna.

The Blindekloof stream has its entire catchment in the Groendal Wilderness Area and has its source in the Grootwinterhoek Mountains (Ellender *et al.*, 2011), connecting to the mainstem of the Swartkops River in the Groendal Wilderness Area. The Groendal Wilderness Area, which was demarcated as state forest in the 19th century, aimed to preserve indigenous forest and water resources (Ellender & Weyl, 2015).

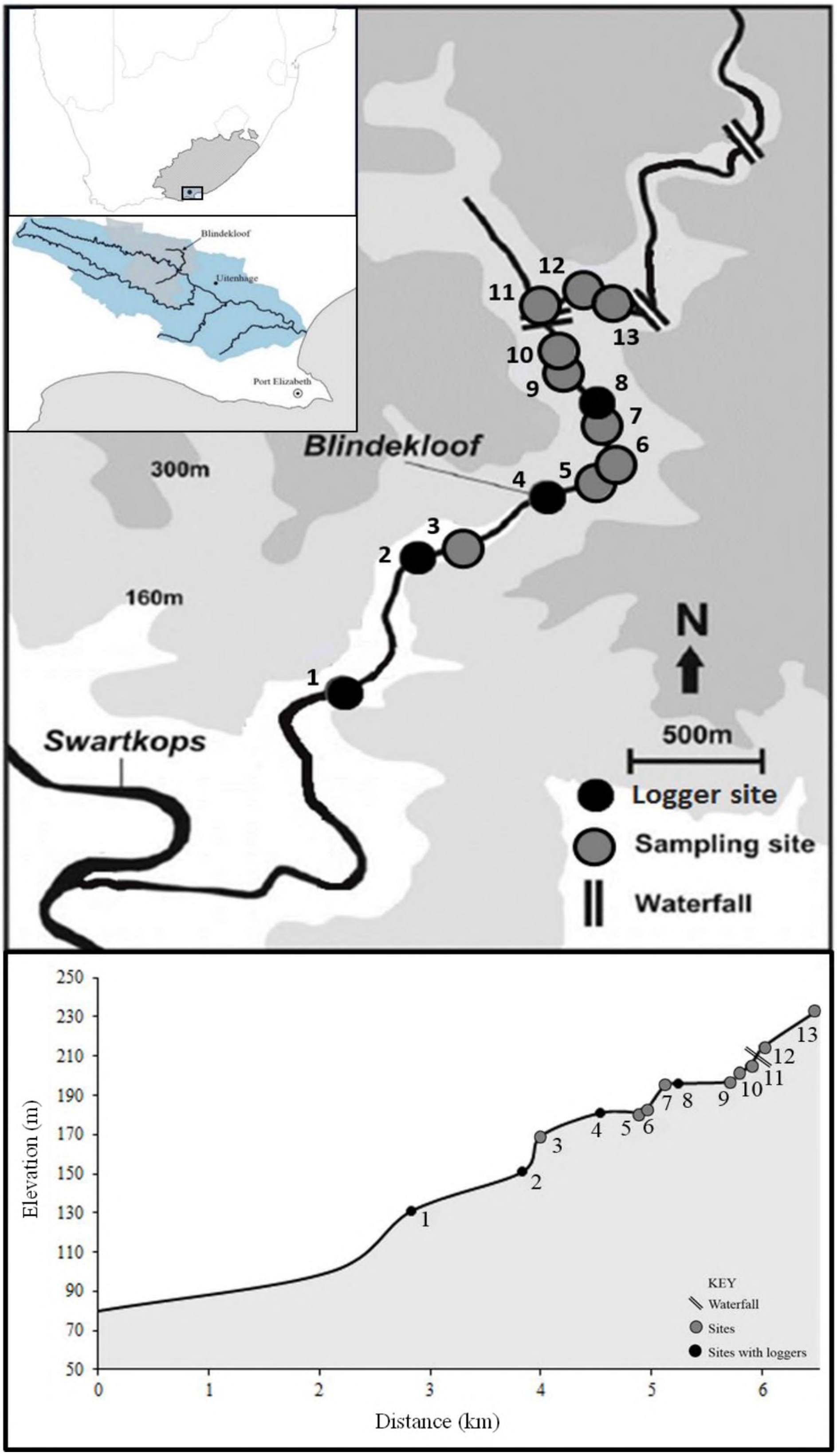

Figure 2.1 The position and elevation profile of long-term monitoring sites used to investigate the distribution of fishes in the Blindekloof stream and sites where temperature loggers have been retrieved in the Blindekloof stream, upper Swartkops River system (modified from Ellender *et al.*, 2011).

The Blindekloof stream is approximately 11 km long from its source to the confluence of the mainstem of the Swartkops River. Within this reach, 13 long-term monitoring sites (Figure 2.1) encompassing approximately 7 km of the Blindekloof stream, were identified and used to monitor spatial and temporal shifts in fish distribution. Figure 2.1 also indicates where the logger data were retrieved from. The Blindekloof stream (as well as the upper reaches of the Sundays River and Baakens River) is an important habitat for *P. afer* (Swartz *et al.*, 2007, 2009). The mainstem of the Swartkops River has documented populations of non-native fish species, namely *Micropterus dolomieu* (Lacepède, 1802), *Micropterus salmoides* (Lacepède, 1802), *Clarias gariepinus* (Burchell, 1822) and *Tilapia sparrmanii* A. Smith, 1840. Opportunistic invasions have been previously documented in the Blindekloof stream because there is no barrier to prevent upstream invasion from the source populations in the upper reaches of the Swartkops mainstem (Ellender *et al.*, 2011).

HABITAT / GRADIENT

The 11 km stretch of river was divided into an upper (Sites 10 – 13), middle (Sites 6 – 9) and lower (Sites 1 – 5) reach (see Fig 2.1). In the upper reaches, the habitat was characterised by boulders, bedrock and some pebbles with open canopy pools. Similarly, pools found in the middle reaches were characterised by the same substrate types as those found in the upper reaches but generally exhibited more closed canopies, which include overhanging trees and cliffs. Lastly, the lower reaches were characterised by small cobbles and gravel with a primarily closed canopy. Figure 2.2 highlights the typical instream habitat from two of the snorkelled pools. The stream gradient is high in the upper reaches (Figure 2.1) with one small waterfall between Sites 11 and 12.

Figure 2.2 Typical instream characteristics of snorkelled pools in the Blindekloof stream showing the boulders, bedrock and pebbles as well as the typical canopy cover.

DATA COLLECTION

HABITAT DATA COLLECTION

Each site comprised of a single pool. Pool width (four or six width transects were measured dependent on the size of the pool) and length were measured using a measuring tape (m), and three depths were recorded at each width transect. Surface area of each pool was calculated by multiplying the maximum pool length by the average pool width (Table 2-3).

During three independent sampling events (December 2015, November 2016 and February 2017) conductivity (µS / cm), total dissolved solids (ppm), pH and immediate temperature (°C) were measured at each of the sites using a Hanna HI98129 Combo pH and Electrical Conductivity meter (Table 2-3).

TEMPERATURE

Hourly water temperatures were recorded using Hobo Water Temp Pro v2 loggers. Nine loggers were deployed in July 2015. Five of these loggers were retrieved in November 2016. One of the loggers was however faulty and the data were not used in this study, therefore only data from four loggers were used. The data were collected from Site 1, 2, 4 and 8, with the upper most loggers not being retrieved as they were not found. The water temperature loggers were programmed to record hourly temperature and for the analyses, hourly data recorded from midnight on 04 July 2015 to 23h00 on 03 July 2016 were used. An hourly logging interval was used as it is considered adequate for measuring biologically meaningful water temperatures (Rivers-Moore *et al.*, 2004). The loggers were attached to a chain by a bolt which was bolted to rock on the river bed. The loggers were suspended in the water column by the metal chain.

Hourly water temperature data from the Hobo loggers were used to describe the diurnal temperature ranges, and compare these between pools, specifically the period of the annual minimum (winter) and maximum (summer), and the daily temperature fluctuations for these periods were graphed.

As this thesis has a biological focus, the degree days were calculated for each site. The degree days is reflective of the cumulative temperature experienced by organisms above a threshold temperature (for this study 9.46 ° C was used) (Dallas *et al.*, 2017). The daily degree day is calculated by subtracting the threshold temperature from the calculated daily mean temperature and the total degree days are calculated by summing the differences for the year (Dallas *et al.*, 2017).

The Indicators of Thermal Alteration method (ITA) (Rivers-Moore *et al.*, 2012; Rivers-Moore *et al.*, 2013a) was used to characterise and compare thermal signatures of the four sites using one year of hourly data, by converting sub-daily water temperature data to daily data (mean, minimum, maximum and range). From the data, thermal metrics were calculated to describe water temperatures with respect to magnitude of water temperatures and duration of thermal events. These metrics were the maximum daily temperature (the maximum temperature recorded at each site daily), minimum daily temperature (the minimum temperature recorded at each site daily), mean daily temperature (the average of the hourly temperatures at each site daily), daily temperature range (the maximum minus the minimum recorded temperature at each site daily), a 7-day moving average of the daily mean (Mean_7) (the average of the mean for seven days at each site) and a 7-day moving average of the daily maximum temperature (Max_7) (the 7-day mean of the daily maximum temperature at each site).

A Shapiro-Wilk test for normality was run on all metrics. A Friedman test was conducted on the non-normally distributed data (Dytham, 2011). Thereafter, Bonferroni Post-hoc tests were conducted to determine which sites differ significantly from one another in terms of each of the thermal metrics (Dytham, 2011).

RESULTS

HABITAT DATA

Hydrological data from a gauging weir at Wincanton on the Elands River (M1H004), a Swartkops River headwater tributary, and rainfall from Uitenhage (M1E002) were obtained from the Department of Water Affairs hydrology section. These data are used to illustrate mean rainfall for each month over a ten year period (Table 2-1). Furthermore, to illustrate the erratic rainfall pattern, monthly rainfall recorded between January 2015 and December 2016 were graphed (Figure 2.3). Flow variability was also graphed (Figure 2.4).

Table 2-1 Summary of rainfall statistics from Uitenhage (M1E002; 2006/11/30-2016/11/30).

Month	**Mean rainfall (mm) (mean ± SD)**
Jan	73.9 ± 70.4
Feb	45.5 ± 27.4
Mar	50.9 ± 38.3
Apr	23.1 ± 9.4
May	52.7 ± 38.5
Jun	56.1 ± 35.6
Jul	44.0 ± 31.7
Aug	18.2 ± 17.1
Sep	54.3 ± 49.7
Oct	50.1 ± 40.4
Nov	27.7 ± 18.0
Dec	26.7 ± 26.2
Annual	523.3 ± 147.7

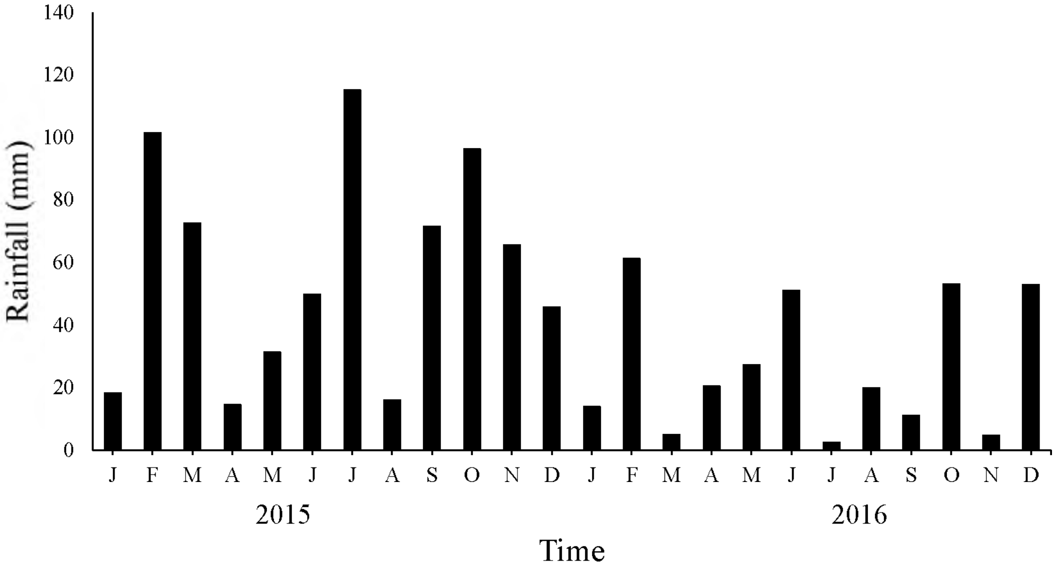

Figure 2.3 Monthly rainfall recorded from Uitenhage (M1E002) between January 2015 and December 2016.

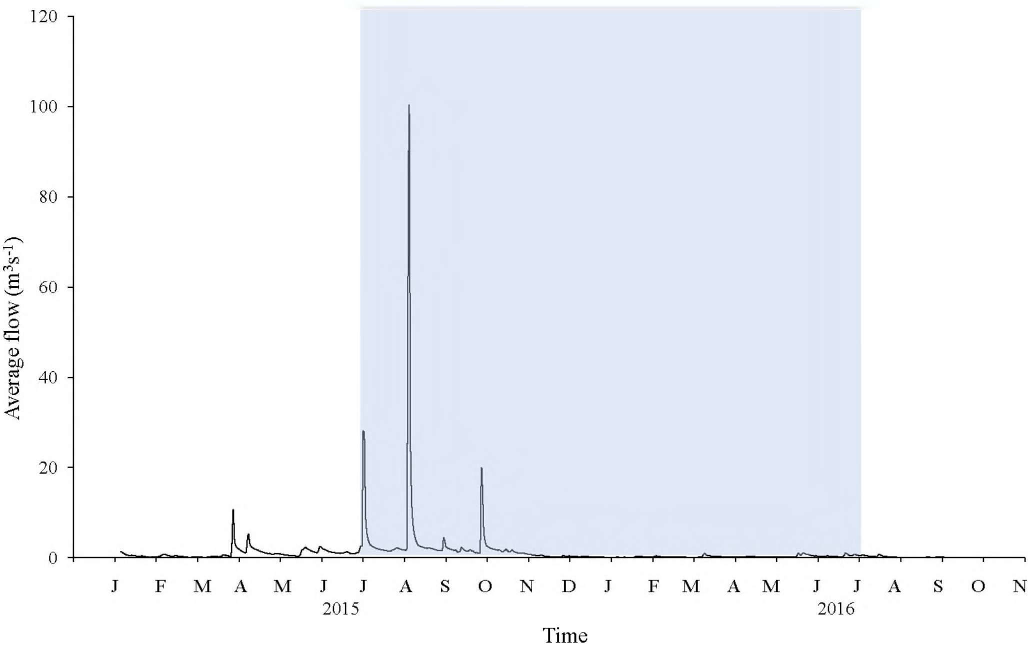

Figure 2.4 Average daily flow (m^3s^{-1}) obtained from the gauging weir at Wincanton on the Elands River (M1H004), a Swartkops River headwater tributary, for the period between 01 January 2015 and 01 December 2016 (sampling period shaded).

Ambient temperature data recorded at Uitenhage were supplied by the South African Weather Service (0034763 X) (Table 2-2). The preference experiments were conducted in summer (February) 2016 (mean minimum of 15.9 ± 3.0 and mean maximum of 29.0 ± 4.1) and winter (July) 2016 (mean minimum of 6.4 ± 3.6 and mean maximum of 20.8 ± 4.6).

Table 2-2 Monthly mean minimum and maximum ambient temperatures (°C) from Uitenhage (South African Weather Service station 0034763 X) recorded between 01 January 2016 and 31 December 2016.

Month	Mean monthly minimum (°C)	Mean monthly maximum (°C)
January	17.3 ± 3.6	29.3 ± 3.0
February	15.9 ± 3.0	29.0 ± 4.1
March	14.8 ± 3.3	27.3 ± 5.0
April	12.0 ± 3.7	27.6 ± 5.0
May	9.3 ± 3.0	25.1 ± 4.2
June	7.2 ± 4.6	22.8 ± 4.5
July	6.4 ± 3.6	20.8 ± 4.6
August	7.9 ± 3.9	25.0 ± 6.4
September	9.0 ± 3.3	21.9 ± 3.7
October	9.9 ± 3.9	25.2 ± 3.3
November	12.3 ± 2.8	25.8 ± 3.3
December	14.2 ± 2.9	29.4 ± 4.3

The pool size data, along with the habitat data collected using the Hanna HI98129 are summarised in Table 2-3. It is evident from these recordings that the pool sizes vary in a random fashion amongst sites, the largest being Site 1 (surface area of 1595.7 m^2) while the smallest was Site 11 (surface area of 56.7 m^2). The measured physico-chemical parameters; pH, total dissolved solids and conductivity, were fairly consistent (Table 2-3). The pH ranged from 5.34 at Site 6 to 6.84 at Site 13. The total dissolved solids (ppm) ranged from a mean of 116.7 ± 0.6 at Site 10 to 131.7 ± 0.6 at Site 1. The physico-chemical parameters reflect the oligotrophic nature of the water although the conductivity values are higher than those recorded by Ellender *et al.* (2011), where the mean conductivity was 136.9 ± 16.1 µs / cm. This may be due to the concentration of ions or water temperature at these sites during the low flow period during which these measurements were recorded.

TEMPERATURE DATA

The hourly temperatures recorded for the four sites were plotted and the graph illustrates the difference in temperature for the year at each site (Figure 2.5). The thermal pattern was fairly consistent for all four sites but the thermal metrics were still tested for homogeneity between sites.

From the graph (Figure 2.5) it is evident that the warmest temperatures are experienced in mid-summer, between late December and early January (mean Max_7 of the four sites for January 2016 is 24.83 ± 0.91 °C (Figure 2.6e), with a maximum Max_7 recording of 28.18 °C recorded at Site 2 in December 2015 (Figure 2.7e) and an absolute maximum temperature recording of 29.37 °C recorded at site 2 at 16h00 on 31 December 2015 (Figure 2.7c)). The coolest temperatures were recorded in August, in late-winter (mean minimum of the four sites for August 2015 was 11.44 ± 0.86 °C (Figure 2.6b) with an absolute minimum temperature recording of 9.46 °C at site 8 at 09h00 on 02/08/2015 (Figure 2.7b)). Four days were selected from each of these periods and graphed to illustrate the diurnal variation in temperature (Figure 2.5). The greatest thermal range across sites was observed in summer (mean thermal range of 1.97 ± 0.96 °C) and the lowest thermal range across sites was observed in winter (mean thermal range of 0.85 ± 0.46 °C (Figure 2.6f)).

Table 2-3 The site location (and those from which temperature loggers were retrieved*), pool sizes (m^2) and physico-chemical parameters of sample sites along the Blindekloof stream.

Site Number	Latitude	Longitude	Max Length (m)	Avg. Width (m)	Surface Area (m^2)	Mean Depth (m)	Distance from main stem (km)	Elevation (m above sea level)	pH Range	Mean TDS (ppm)	Mean Conductivity (µs / cm)	Mean Temperature (°C)
1*	33°42'18.90"S	25°17'55.68"E	100.0	16.0±6.2	1595.7	0.9±0.6	2.8	131	5.60-5.66	131.7±0.6	236.3±0.6	24.3±0.1
2*	33°41'53.45"S	25°18'8.03"E	32.0	7.6±1.4	241.8	0.9±0.6	3.8	151	5.58-5.60	125.0±0.0	249.7±0.6	22.8±0.1
3	33°41'56.17"S	25°18'14.26"E	35.3	7.3±3.3	258.2	0.6±0.5	4.0	169	5.43-5.49	124.0±1.0	247.7±0.6	22.2±0.1
4*	33°41'47.08"S	25°18'27.94"E	50.0	7.7±1.2	386.3	0.7±0.5	4.5	181	5.50-5.10	121.0±0.0	242.7±0.6	24.1±0.1
5	33°41'44.81"S	25°18'38.63"E	44.0	12.5±7.0	548.7	1.0±0.7	4.9	181	5.49-5.96	119.7±0.6	240.0±0.0	23.7±0.2
6	33°41'43.12"S	25°18'38.88"E	40.0	12.2±3.5	486.7	1.0±0.7	4.9	182	5.34-5.79	120.3±0.6	240.3±0.6	25.1±0.1
7	33°41'36.06"S	25°18'37.30"E	43.0	11.6±2.7	498.2	1.2±0.9	5.1	195	5.47-5.53	117.7±0.6	237.0±0.0	23.8±0.1
8*	33°41'34.37"S	25°18'37.26"E	49.0	8.8±4.8	429.8	2.1±0.9	5.2	196	5.62-5.73	119.3±1.2	239.0±0.0	22.9±0.3
9	33°41'22.67"S	25°18'29.52"E	25.2	4.9±1.8	124.6	0.6±0.5	5.7	197	6.12-6.34	117.7±0.6	233.7±2.3	23.2±0.7
10	33°41'21.66"S	25°18'28.91"E	31.5	6.1±1.2	192.7	0.4±0.2	5.8	202	5.98-6.12	116.7±0.6	233.0±1.0	22.8±0.2
11	33°41'18.06"S	25°18'27.58"E	12.0	4.7±0.6	56.7	0.4±0.2	5.9	205	6.11-6.32	118.7±0.6	237.0±2.6	24.8±1.0
12	33°41'15.76"S	25°18'30.17"E	51.0	4.7±2.2	240.4	0.8±0.4	6.0	215	6.34-6.43	122.7±06	245.7±0.6	23.4±0.1
13	33°41'12.66"S	25°18'34.13"E	49.2	4.8±1.2	234.8	1.2±0.8	6.5	233	6.57-6.84	122.3±0.6	245.7±0.6	22.8±0.3

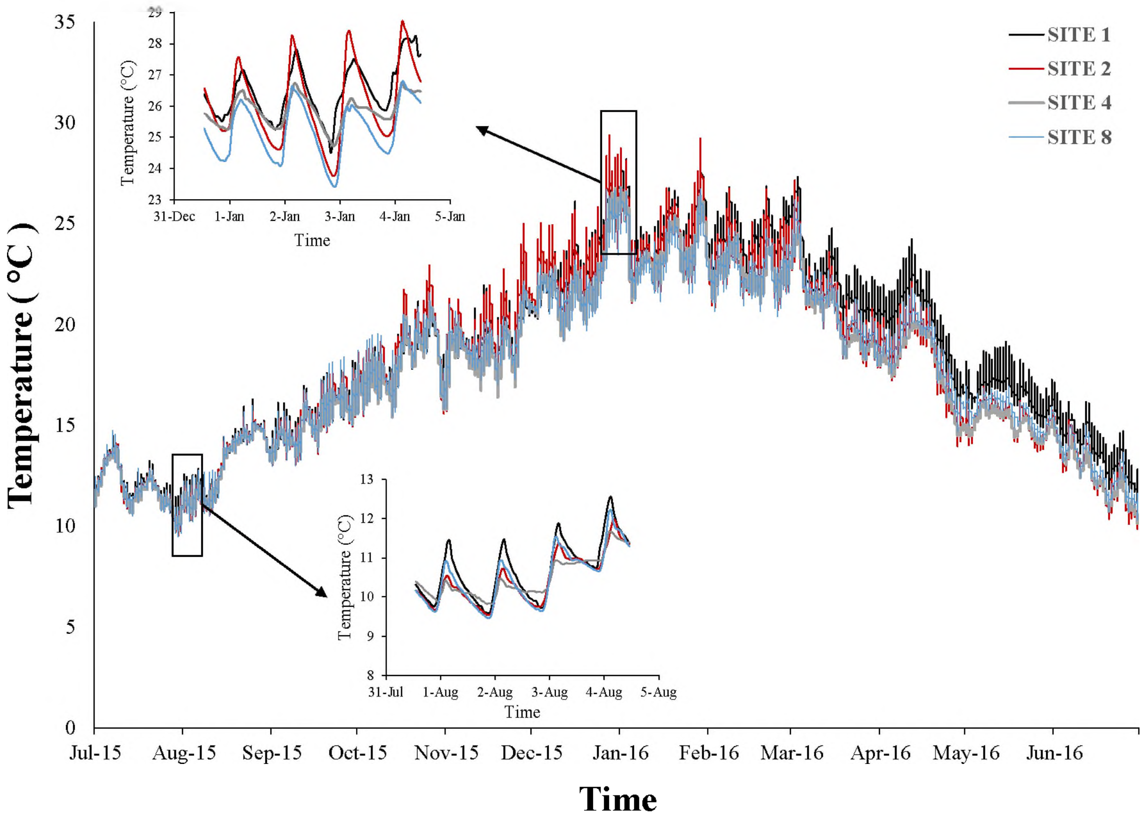

Figure 2.5 Graph to show water temperature data collected from all four sites on the Blindekloof stream measured hourly throughout a year. The data follows both a seasonal and diurnal pattern and highlights the daily thermal fluctuations during the hottest and coldest recorded four days.

The total degree days of each site are summarised in Table 2-4. The degree days observed are not unexpected as the threshold temperature used to calculate degree days was 9.46 °C (Figure 2.8 (b)). From the degree days the lowest site, Site 1, is the warmest while one of the middle sites, Site 4, is the coolest with a 363 °C difference in degree days between the warmest and coldest sites over a one-year period.

Table 2-4 Total degree days for each site monitored on the Blindekloof stream calculated from 4 July 2015 to 3 July 2016.

	Site 1	**Site 2**	**Site 4**	**Site 8**	**Mean**
Total Degree Days	3336	3111	2973	3049	3117 ± 156

From the thermal metrics it is evident that the temperatures observed at the different sites tend to follow the same seasonal pattern, however the degree of similarity varies between metrics and over time (Figure 2.7).

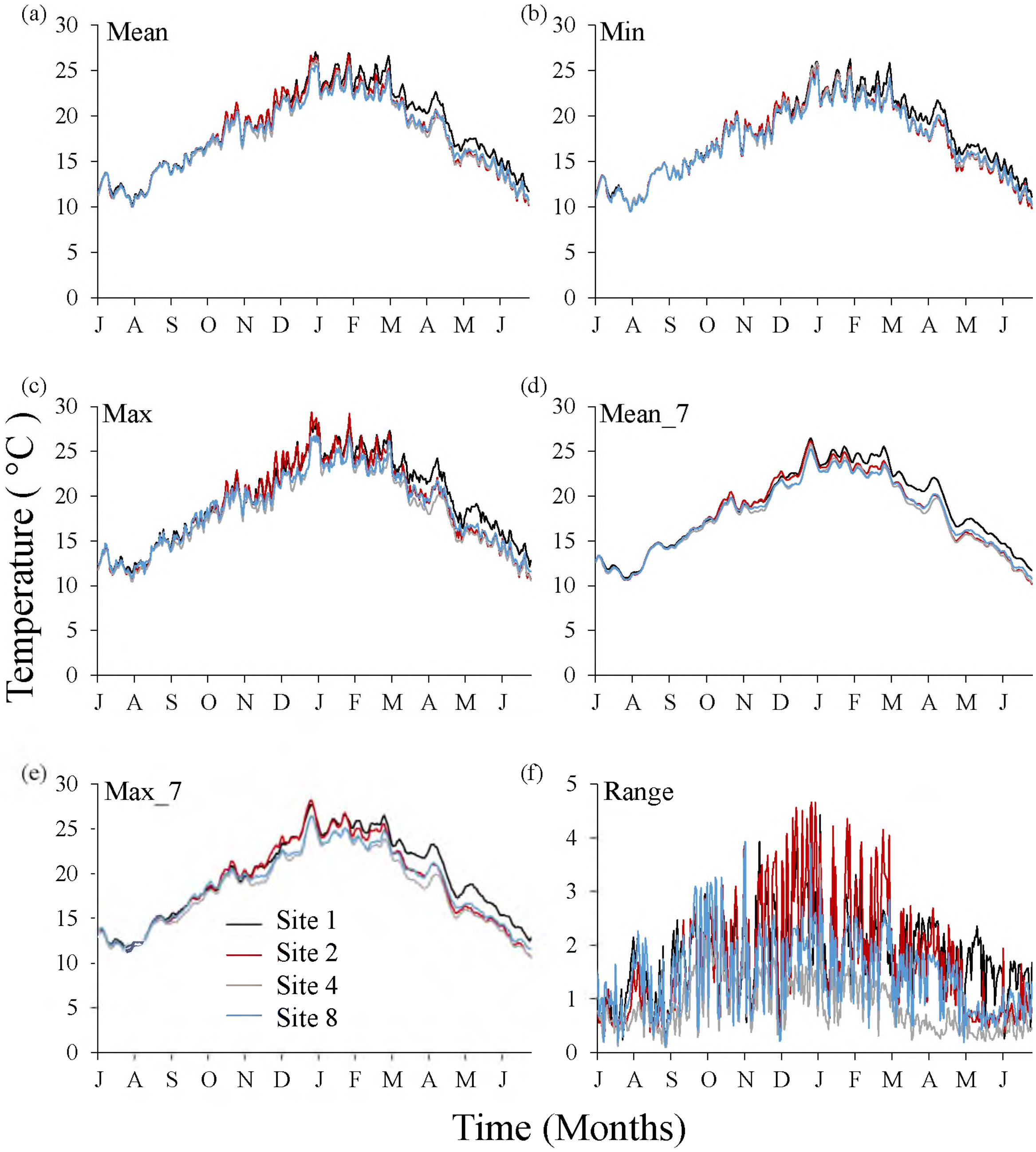

Figure 2.6 Thermal data from each of four sites monitored in the Blindekloof stream from 04 July 2015 to 03 July 2016 to show thermal variation between sites within the different metrics; (a) mean daily temperature, (b) daily minimum, (c) daily maximum, (d) Mean_7, (e) Max_7, and (f) daily thermal range.

The results of a Shapiro Wilk test for normality showed that the thermal metrics (mean, minimum, maximum, mean_7, max_7 or range) were non-normally distributed (p-value < 0.05). The results from the nonparametric paired test, a Friedman test, suggested that all metrics (mean, minimum, maximum, mean_7, max_7 and range) differed significantly between sites (p < 0.05). The summary of the data from the metrics is presented in Figure 2.7 to illustrate how each of the metrics differ between sites.

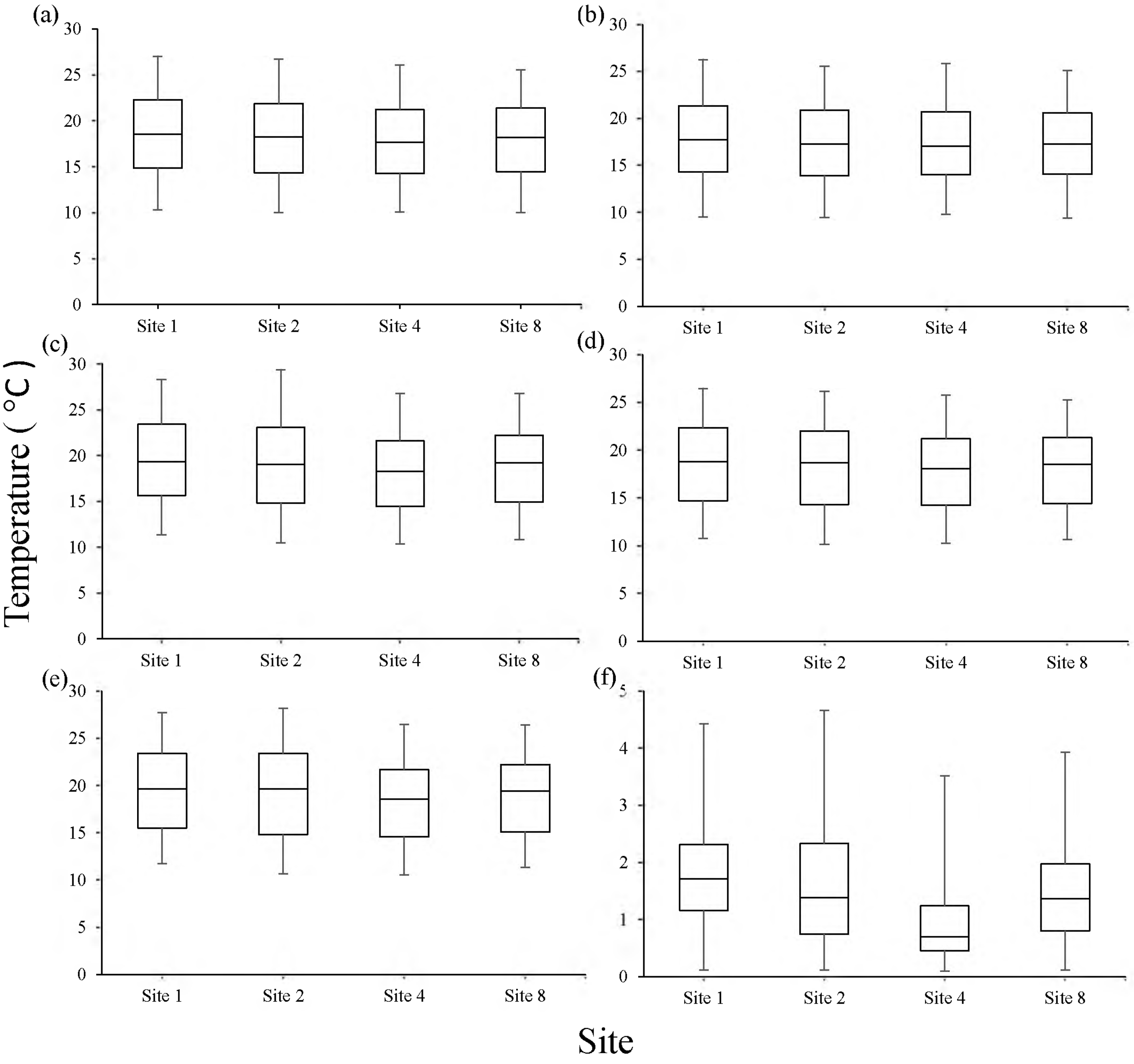

Figure 2.7 Boxplots depicting the variation in the thermal data for each of the four sites on the Blindekloof stream in each of the metrics; (a) mean daily temperature, (b) daily minimum, (c) daily maximum, (d) Mean_7, (e) Max_7, (f) daily thermal range.

To determine which of the sites differ significantly from one another in each of the metrics, a post-hoc test was conducted. The results from the Bonferroni Post-hoc test show which sites differ significantly from each other and are summarised in Table 2-5.

Table 2-5 Summary of the statistic results for the thermal metrics showing which sites differ significantly (NS = Not Significant).

	Site 1	**Site 2**	**Site 4**	**Site 8**
Mean				
Site 1				
Site 2	NS			
Site 4	**<0.05**	NS		
Site 8	NS	NS	NS	
Minimum				
Site 1				
Site 2	NS			
Site 4	NS	NS		
Site 8	NS	NS	NS	
Maximum				
Site 1				
Site 2	NS			
Site 4	**<0.001**	NS		
Site 8	**<0.05**	NS	NS	
Mean_7				
Site 1				
Site 2	NS			
Site 4	**<0.01**	NS		
Site 8	NS	NS	NS	
Max_7				
Site 1				
Site 2	NS			
Site 4	**<0.001**	**<0.05**		
Site 8	**<0.05**	NS	NS	
Range				
Site 1				
Site 2	**<0.01**			
Site 4	**<0.001**	**<0.001**		
Site 8	**<0.001**	NS	**<0.001**	

DISCUSSION

Stream temperature in the Blindekloof stream followed a seasonal and diurnal pattern. There was also evidence supporting the hypothesis that the thermal regime of the system was complex and varied across the monitored reach of the headwater as the metrics differed significantly between sites. The daily temperature range between sites differed most. Most metrics followed the same trend, which is not unexpected as the distance between Site 1 and Site 8 is only 2.4 km. Therefore, many of the factors influencing the thermal regime of a stream, such as air temperature, humidity, precipitation and geology (Figure 2.8) were likely to be consistent throughout the monitored reach. However, factors such as pool size, altitude and canopy cover may account for the differences between sites in the metrics. These variations may be explained by the fact that thermal regimes differ at both the local and catchment scale (Smith, 1968; Steele, 1982; Webb, 1996), with longitudinal shifts and the largest expected range in temperature, recorded at Site 2, often observed in the middle reaches (Vannote & Sweeney, 1980).

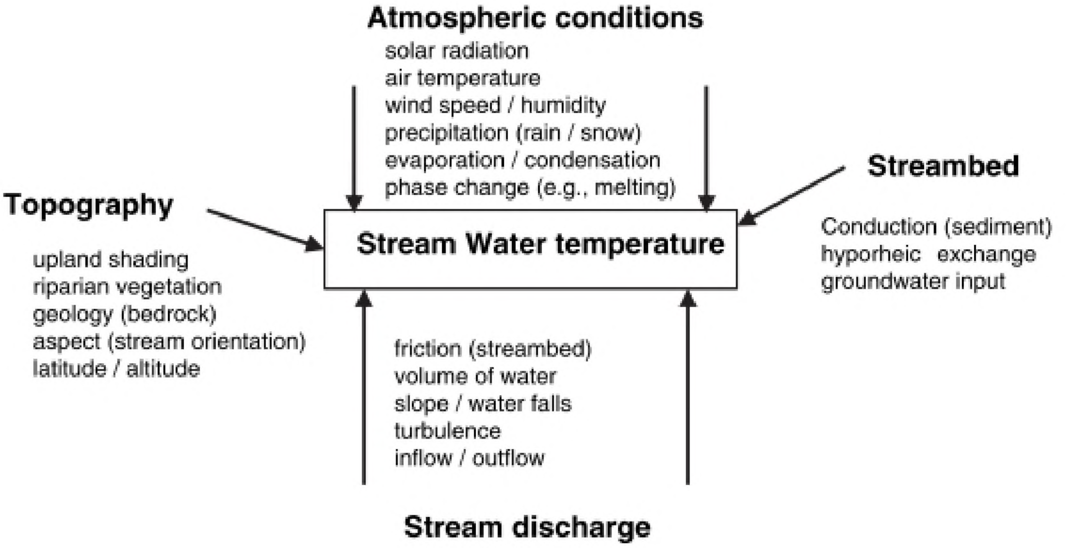

Figure 2.8 Factors influencing the water temperature within a river system (taken from Caissie, 2006).

Caissie (2006) states that in general, due to the fact that the thermal regime of headwaters is closely linked to groundwater, the daily thermal range is smaller for small headwater streams than for large rivers, which are more susceptible to the influence of meteorological conditions.

Wehrly *et al.* (2003) classify streams as either stable (< 5 °C), moderate (5 °C to <10 °C) or extreme (≥ 10 °C) due to their temperature fluctuations. With the data collected from the Hobo temperature loggers, the daily range metric presented in this chapter and the definitions of streams according to their daily temperature fluctuation (Wehrly *et al.*, 2003), the Blindekloof stream can be classified as a stable stream (Figure 2.9) as the recorded daily temperature range varied between 0.1 and 4.7 °C.

As thermal regimes differ within and between systems they are difficult to classify because numerous factors, such as atmospheric conditions and stream discharge, play a role in determining stream temperature. Streams have typically been classified as "equatorial", "tropical" or "temperate" (Caissie, 2006) or cold, cool and warm according to their thermal regimes. Cold is defined as a stream with mean temperatures below 19 °C, cool has observable mean temperatures between 19 - 22 °C and warm streams have a mean temperature equal to or greater than 22°C (see Figure 2.9; Wehrly *et al.*, 2003). The Blindekloof stream had previously been classified as a temperate stream (Skelton *et al.*, 1995). With the data collected from the Hobo temperature loggers, the metrics presented in this chapter and the definitions of streams according to their thermal regimes, the Blindekloof stream can best be classified as a cold stream as it has an observable annual mean temperature of 18.0 ± 4.3 °C.

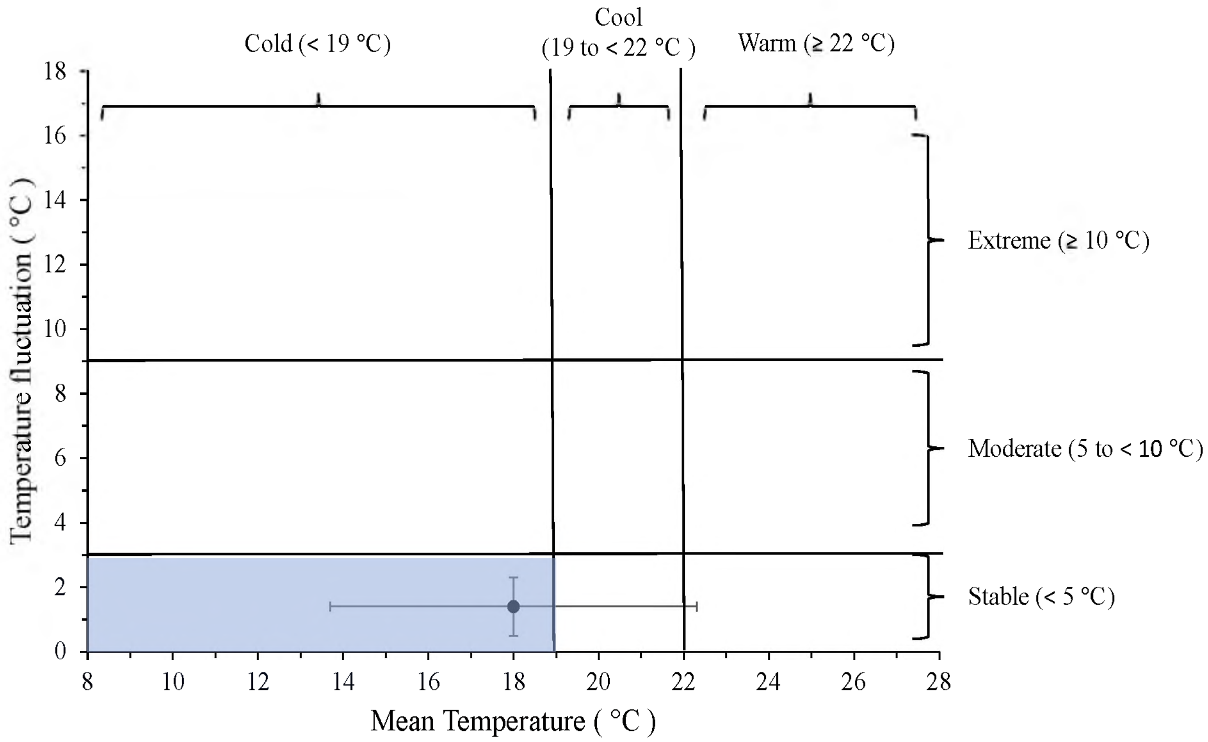

Figure 2.9 The intersection of thermal category boundaries (solid lines) defined as cold (< 19 °C), cool (19 °C to < 22 °C) or warm (≥ 22 °C) mean temperatures and stable (> 5 °C), moderate (5 °C to < 10 °C) or extreme (≥ 10 °C) temperature fluctuation and the position of the Blindekloof stream (shaded) as a stable, cold water system (modified from Wehrly et al., 2003).

The Blindekloof stream thermal regime as descried in this chapter fits into the broad range of cold, temperate Australian streams such as those cited by Lake *et al.* (1985). Lake *et al.* (1985) compared the annual temperature ranges of temperate Australian systems and observed annual temperature ranges of between 7.5 °C (Olga River) and 21.6 °C (Murrumbidgee River). In these temperate Australian systems, the highest cited mean summer maximum was 26.0 °C (Wellington River) and the lowest cited winter minimum was 0.0 °C (Waterfall Creek) (Lake *et al.*, 1985). The recorded temperatures on the Blindekloof stream, compared to these temperature recordings, would lead to the classification of the Blindekloof stream as a temperate stream. The temperature range in the Blindekloof is similar (absolute of 19.1 °C) but the lowest minimum observed is considerably higher than the majority of those cited by Lake *et al.* (1985).

Locally, the recorded temperatures and their seasonal pattern are typical of streams in the CFE. Despite being episodic, in comparison to other stream temperatures in the CFE, the Blindekloof stream displayed similar temperature trends, for example Dallas & Rivers-Moore

(2012) recorded hourly water temperature in 13 CFE streams and observed annual daily means ranging from as low as 11.3 ± 0.5 °C (Window Gorge, a perennial stream) to 21.0 °C (Palmiet, also a perennial stream). The Blindekloof stream fits within the range of these thermal extremities recorded from the CFE and displays a thermal trend most similar to that of the Duiwnhoks which has an annual daily mean of 18.7 ± 0.5 with an annual minimum of 9.8 °C (9.46 °C in the Blindekloof stream) and an annual maximum 27.0 °C (29.37 °C in the Blindekloof stream).

In the Blindekloof stream, the maximum mean heating rate in November 2015 was 0.46 °C/hour. Other studies such as that done in Oregon, USA suggest that in some small, exposed streams the rate of change may be as high as 3.3 °C/hour (Brown, 1969; Ward & Stanford, 1982). This heating rate is likely to be due to flow (Figure 2.8) as episodic streams have an especially complex thermal regime (Chapin *et al.*, 2014). Due to the high variability in flow and consequent thermal fluctuations, studies mapping and understanding these variations have become increasingly important, particularly in the USA where nearly 60% of streams are categorised as episodic (Chapin *et al.*, 2014).

As stream temperature is an important driving environmental factor in influencing biotic assemblages, the thermal metrics presented in this chapter can be used to aid understanding of native fish thermal ranges based on their distributions and any potential invasion debt. As *P. afer* occurs within the monitored pools (see Chapter 4), the thermal regime of the Blindekloof stream as described in this chapter provides empirical evidence of the thermal ranges of native fishes. It is therefore hypothesised that this species has a thermal tolerance greater than the stream temperatures recorded. However, it is important to investigate these tolerances as in light of global climate change, the thermal regime of these regions is predicted to shift, getting warmer and drier (Dallas & Rivers-Moore, 2014).

It is also important to consider the thermal preferences and tolerances of any potential invaders in order to understand the risk these non-native species pose to environments such as the Blindekloof stream. Understanding the thermal structure of the system and how it varies is becoming increasingly important as it aids in understanding how resilient or susceptible environments and the species which inhabit these environments are to global climate change, and this understanding provides the opportunity to establish effective conservation and management strategies (Jones *et al.*, 2013). It is hypothesised that fishes which inhabit variable environments are likely to be more resilient and thus able to better withstand the resultant environmental response to global climate change (Filipe *et al.*, 2013). Conversely, there are many species which have already been limited in distribution, due to environmental changes, which may have little ability to further adapt and global climate change therefore poses a greater risk to such species (Filipe *et al.*, 2013). Following this knowledge of the Blindekloof stream temperature regime, in the next chapter I aim to assess the thermal tolerance and preference of *P. afer*.

Chapter 3 Thermal tolerance and preference of *Pseudobarbus afer*

INTRODUCTION

The lack of physiological information on South African native and endemic fishes has been highlighted as the primary factor making it difficult to predict how climate change will impact the different endemic fishes (Ellender *et al.*, 2017). Effective conservation of these endemic species depends on understanding many key aspects that temperature may influence - such as their ecology, life history, distribution and physiology (Ellender *et al.*, 2017). With water temperature considered an important abiotic factor affecting fishes (Beitinger & McCauley, 1990; Dabruzzi *et al.*, 2013; Barrantes *et al.*, 2017), it is important that studies on endemic species investigate their thermal tolerances and preferences.

As poikilotherms, fishes are sensitive to temperature and will, where possible, select environments where temperature regimes are optimal for physiological functions (Crawshaw, 1977). The thermal tolerances and optima are species specific and may be influenced by a range of factors including thermal history, acclimation temperature, life history and behaviour (Beitinger & Bennett, 2000; Golovanov, 2006; Barrantes *et al.,* 2017). While many studies have documented the thermal preference and tolerance of northern hemisphere fishes (for example, Feldmeth *et al.* 1974; Johnson, 1976; Stauffer, 1980; Stauffer, 1981; Carveth *et al.* 2006; Jones *et al.* 2013), very few studies have been undertaken on fishes in the southern hemisphere, with none focusing on the physiological tolerances of freshwater fishes before the recent WRC report (Dallas *et al.*, 2017). With this gap in knowledge there is a need to investigate these in order to better understand the physiological tolerances of native fishes.

Armour (1991) highlights how studies assessing the thermal tolerances of a species are crucial in understanding a species' susceptibility to change, particularly those which inhabit environments where the thermal regime is likely to shift or be altered by human mediated impacts such as thermal discharges or stream modification. Further motivation for such studies is that by investigating the thermal tolerance and preference of a species, this knowledge may aid in conservation practices as it allows one to develop species specific thermal regimes for protecting fishes (Armour, 1991; Richardson *et al.* 1994). The thermal experimental data can inform species specific maximum weekly allowable temperatures

(MWAT) which may inform and thus guide conservation practices and priority conservation areas (Armour, 1991; Rivers-Moore *et al.*, 2013b). The MWAT is a measurement of a species specific thermal threshold and is calculated using the species specific optimal and incipient lethal temperatures (Rivers-Moore *et al.*, 2013b). With knowledge of the thermal preference, tolerance and MWAT, the species can then be categorised as either a cold-, cool- or warm-water species (Armour, 1991; Heino *et al.* 2009).

The thermal tolerance of a species is generally investigated using either the incipient lethal temperature (ILT) or the critical thermal method (CTM) (Becker and Genoway, 1979). Both methods include time and temperature as primary test variable and are determined experimentally (Becker & Genoway, 1979), but the ILT is determined by placing fishes, acclimated to various temperatures, into water at a constant temperature and is thus an abrupt temperature change until mortality occurs (Becker & Genoway, 1979; Beitinger *et al.* 1999). The CTM requires a linear change in water temperature in which the fish are placed, resulting in a behavioural stress response, namely the loss of equilibrium, which is the predetermined endpoint (Becker & Genoway, 1979). The CTM is more appropriate for use with endangered species because ILT, which requires death of the test individual (Becker & Genoway, 1979; Beitinger *et al.*, 1999; Dallas & Ketley, 2011) is not recommended for endangered species (Beitinger *et al.*, 1999).

The CTM was defined by Cox (1974) and amended by Becker and Genoway (1979: 225) as:

"The critical thermal maxima (minimum) is both an experimental method and an obtained parameter. The CTM method is a means of quantifying the upper (lower) thermal tolerance of poikilotherms by raising (lowering) the temperature from ambient acclimation level at a constant rate, so that no significant time lag occurs between temperatures of the external media and internal tissue of test organisms. The CTM parameter is the temperature (calculated arithmetic mean) where the test organism loses ability to escape from conditions that promptly lead to its death, determined by the CTM method. The CTM temperature is followed by the lethal thermal maximum (minimum) temperature (calculated arithmetic mean) representing apparent death of the test organism, determined by continuing the linear temperature rise."

For the CTM, the critical thermal endpoint (CTE) is an observable behavioural stress response, and one commonly used for fishes is the loss of equilibrium (LOE) (Becker &

Genoway, 1979). This is appropriate as with such disorganised locomotion, fishes will have lost the ability to escape adverse conditions which would promptly result in death (Ernst *et al.*, 1984).

According to Bernatzerder and Britz (2007), thermal preference can be classified as either acute or final. The acute thermal preference is determined over short time periods and is influenced by acclimation temperature, while the final temperature preference is determined over the long term and is where individuals of a given species will "ultimately congregate" (Jobling, 1981; Bernatzerder & Britz 2007). Studies have shown that acclimation temperature is important in determining final thermal preference (Hall *et al.* 1978; Barila & Stauffer, 1979; Shingleton *et al.* 1981). For example, in a study by Cherry *et al.* (1975), results showed that with a decrease in acclimation temperature, the temperature avoided decreased too, thus pointing to the conclusion that acclimation temperature does in fact significantly alter thermal regimes of species. This study investigated the acute thermal preference as stream temperature was treated as acclimation temperature.

AIMS AND OBJECTIVES

This chapter investigated the upper thermal tolerance and the thermal preference of *Pseudobarbus afer* (Peters, 1864) in order to better understand the physiological requirements of these species. It was hypothesised that i) thermal tolerance would vary between seasons; ii) thermal tolerance would be size-dependent; iii) the thermal preference of *P. afer* would vary between seasons; iv) *P. afer* would aggregate at their preferred temperature, and thus finally that; v) as *P. afer* would aggregate at their preferred temperature in the experimental tanks, the distribution of the observation between the control and experimental tanks would differ.

MATERIAL AND METHODS

Psuedobarbus afer individuals were collected from the Fernkloof, a second-order headwater tributary of the Kwa-Zunga River which is one of the major tributaries of the Swartkops River (Figure 2.1). The Fernkloof Headwater is an episodic headwater with its entire catchment situated in the Groendal Wilderness Area, with isolated surface pools fed by groundwater (Ellender & Weyl, 2015). The substrate is characterised by bedrock, large boulders and some pebbles. The Fernkloof environment can be described as pristine, with a predominantly closed canopy, an abundance of marginal vegetation, high profile banks and narrow valleys.

Conductivity (µS / cm), total dissolved solids (ppm), pH and instantaneous temperature (°C) were measured for each pool using a Hanna HI98129 Combo pH and Electrical Conductivity meter during each of the sampling events (February 2016, 2017 as Summer, July 2016 as Winter) (Table 3-1).

Table 3-1 Mean water chemistry measurements (mean ± SD) from collection sites of test individuals in both summer and winter.

	pH Range	Conductivity (µS / cm)	TDS (ppm)	Temperature (°C)
Summer	6.54 – 6.95	273 ± 67	137 ± 33	19.9 ± 0.1
Winter	5.34 – 6.60	339 ± 5	170 ± 3	11.9 ± 0.7

EXPERIMENTAL DESIGN

For the thermal experiments, fish were collected by means of a small push seine net from the Fernkloof (research permit CRO 27/16CR; CRO 28/16CR; ethics clearance 25/4/1/7/5/2016-05).

For each run of the tolerance and preference experiments, 36 individuals of *P. afer* were collected. *Pseudobarbus afer* individuals were selected for size (sub-adult) and fitness (no visually observable parasites) as recommended by Becker and Genoway (1979). Upon seining, the fish were placed (using dip nets to minimize handling) in aerated 10 L buckets filled with stream water collected *in situ* and transported to the field laboratory.

Both thermal tolerance and thermal preference experiments were run on 30 individuals in field laboratories near to the site of collection to minimise transport time and stress. The experiments were conducted in water from the river and all equipment was washed with disinfectant and then rinsed with river water between trials. Test individuals were retained for 24 hours after completion of experiments to recover before returning them to the site of collection.

THERMAL TOLERANCE

Collected *P. afer* were kept in aerated buckets at the field laboratory for a 24 hour period prior to the experiment to allow for sufficient recovery from collection and transport stress. The water in the buckets was exchanged for fresh stream-collected water every eight hours.

A single thermal experiment was conducted using five *P. afer* individuals and replicated six times using new individuals for each experimental run. *Pseudobarbus afer* individuals were placed in a mesh basket which was submerged in a water bath filled with fresh river water which was continually aerated to ensure oxygen saturation levels remained above 70 % throughout the experiment (Dallas *et al.*, 2015) (Figure 3.1). The water bath was fitted with a Julabo circulating heater and fish were allowed to acclimate for 30 minutes prior to conducting the experiment.

The water heating rate is a critical factor in the CTM experimental design and while increasing rates of change range from 0.1°C / minute to 1.0°C / minute, the standardised rate of increase is 0.3 °C / minute (Becker & Genoway, 1979; Beitinger *et al.*, 1999; Barrantes *et al.*, 2017). According to Beitinger *et al.* (1999), the rate of change in water temperature should be linear, and slow enough to ensure the fishes' core temperatures do not significantly lag behind the water temperature, but rapid enough to ensure the fish do not acclimate during the experiment. The water temperature was increased at the standardized, accepted rate of 0.3 °C / minute for all experimental replicates (Beitinger *et al.*, 1999).

The fish were continually observed during each experiment and the temperature at which LOE was observed was recorded as the CT_{max} (°C). Loss of equilibrium was defined as the loss of dorso-ventral orientation followed by the inability of a fish to return to an upright position (Becker & Genoway, 1979). Once observed, each individual fish exhibiting LOE was removed from the water bath using a dip net, measured to the nearest millimetre (fork length) and placed in a bucket containing aerated water at the original acclimation temperature. Once recovered, each fish was put into a new bucket with aerated water where they were kept for a further 24 hours to ensure recovery and subsequently returned to the collection site.

As death was not a valid CTM criterion, if an individual did not recover after being subjected to the experimental procedure, that particular experimental replicate data were not used (see Becker & Genoway, 1979; Beitinger *et al.*, 1999). Each thermal experiment was repeated until data from 30 individuals were recorded; the warm bath was washed between each trial and filled with fresh river water.

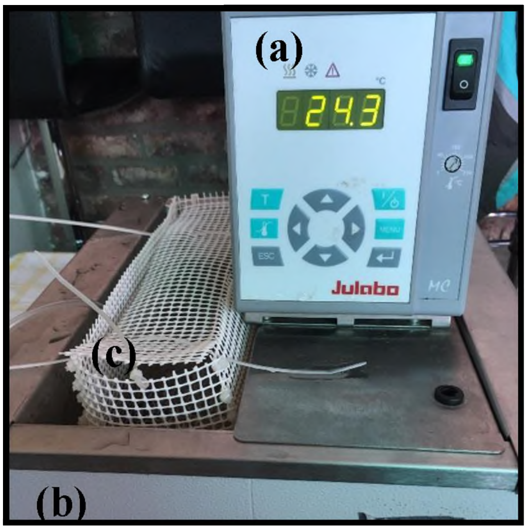

Figure 3.1 Experimental apparatus as set up to conduct the thermal tolerance experiment showing the Julabo circulating heater (a), the warm bath (b) and the mesh cage (c) in which the test individuals were placed for observation during the experiment.

THERMAL PREFERENCE DESIGN

The thermal preference experiments were conducted in thermal gradient tanks (six tanks were used for each experiment; three serving as a control with no thermal gradient and three serving as the experiment with a thermal gradient) constructed from PVC gutters (3 m length, 125 mm width, 87 mm height; design as described by Dallas *et al.* 2015) (Figure 3.2).

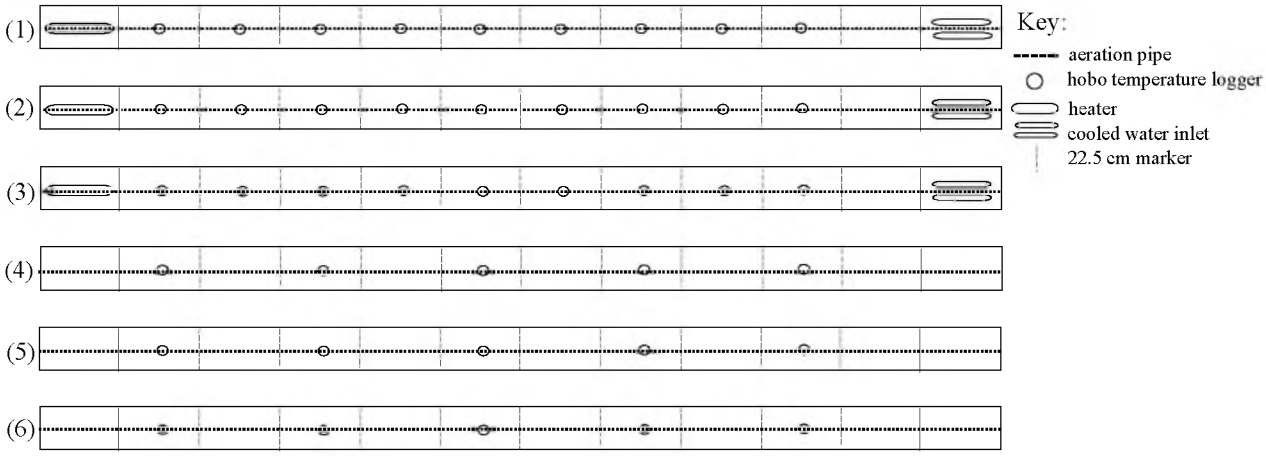

Figure 3.2 Schematic representation of the thermal preference experimental design with three experimental tanks (1-3) and three control tanks (4-6).

The temperature in the control tanks remained constant throughout the experiment and served to evaluate any mortalities associated with the experimental conditions not related to temperature. The tanks were each fitted with perforated tubing along the bottom, attached to an air compressor which served to aerate the water along the length of the tanks. This also served to prevent vertical thermal stratification (Richardson *et al.*, 1994).

In the experimental tanks, 100W aquarium heaters were fitted at one end of the tank while at the opposite end, copper coils were fitted that were pumped with cold water from a portable freezer. Plastic mesh barriers were fitted at each end to separate the heating and cooling apparatus from the tank area where fish were placed to ensure that the fish did not come into direct contact with the heating and cooling apparatus (Richardson *et al.*, 1994; Dallas *et al.*, 2015). Plastic mesh barriers were also fitted in the control tanks as procedural controls.

The remaining length of the tank (2.25 m) was subdivided into 10 equal sections (22.5 cm). A Hobo UTB1-001 TidBit V2 logger, programmed to record the temperature at 10 minute intervals, was placed in each subdivided section in the experimental tanks, and in every second section in the control tanks (on assumption that temperature was constant throughout). The tanks were filled with river water to a depth of 60 mm at the start of each experimental replicate.

Five *P. afer* were placed in each of the tanks and the entire experimental setup was covered with shade cloth to mimic habitat cover and to prevent fish from escaping vertically. All fish were left to acclimate to these tanks for 30 minutes during which time, no thermal gradient was created. Thereafter, a thermal gradient was gradually established over the course of three hours by turning on the heaters and starting the pumps. This was termed the establishment phase. While the fish were observed at 10 minute intervals during this establishment phase (T1 – T19), and any fatalities recorded and replaced, these data were not used in the data analysis. After the establishment phase, the experiment was run for two hours, during which each experimental replicate was observed at 10 minute intervals and the number of fish in each section was recorded (T20 – T31).

Upon termination of the experiment, all fish were placed in aerated water-filled buckets for 24 hours to ensure recovery before returning to the collection site.

The experiment was run for three consecutive days on 30 individuals in summer and winter. The experiment was run from mid-morning to mid-afternoon to ensure standard natural environmental conditions for each of the runs.

DATA ANALYSIS

THERMAL TOLERANCE

The hypothesised size dependent effect was assessed by correlating CT_{max} and fish length, expressed as fork length. As there was no significant effect of size, the recorded CT_{max} data were tested for normality using a Shapiro-Wilk and Equal Variance (Brown-Forsythe) test. Given that both tests showed the CT_{max} data to be normally distributed, to test the hypothesis that there is a significant effect of seasonal acclimatization on CT_{max}, a Welsh two-sample test was used to compare mean CT_{max} between seasons.

THERMAL PREFERENCE

Thermal preference was calculated using the method described by Richardson *et al.* (1994) whereby only the fish position observations made during the experimental period were used for the seasonal comparison (observations T20 – T31). This was done by averaging the T20 – T31 temperatures recorded by each water temperature logger and thus calculating a corresponding mean temperature for each cell. The number of observations per cell were then related to the mean temperature of that cell and converted into a percentage cumulative frequency for each of the recorded temperatures.

To determine the preferred temperature of *P. afer,* the median of the percentage cumulative frequency for each replicate was interpolated (nine values for summer and nine values for winter; Table 3-2) (modified from the R Core Team, 2015 in Dallas *et al.*, 2017). To determine whether thermal preference varied between seasons, median preference data were first tested for normality and as they were non-normally distributed, a linear model was used to compare *P. afer* preference in summer and winter.

To test the hypothesis that *P. afer* will aggregate at their preferred temperature, a Kolmogorov-Smirnov test for goodness of fit was used. A Kolmogorov-Smirnov test requires the data to be continuous but makes no assumption about the distribution of the data (Dytham, 2011). A Kolmogorov-Smirnov test, used to test the probability that two sets of data follow the same distribution (Dytham, 2011), was conducted between the frequency distribution of the control and experimental tanks.

RESULTS

THERMAL TOLERANCE

The results of a linear model suggest that there is no significant effect of fish size on the CT_{max} of *P. afer* ($R^2 = 0.004$; $p > 0.05$; $n = 60$) (Figure 3.3).

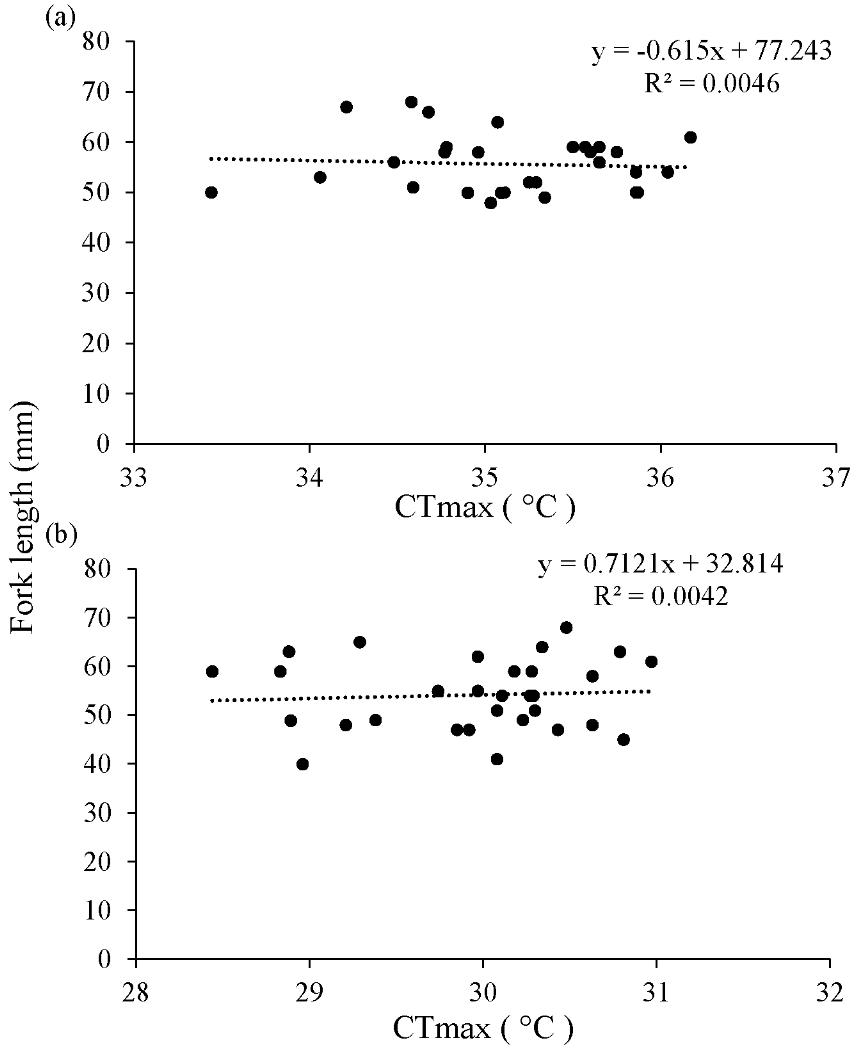

Figure 3.3 Critical thermal maximum and fork length (mm) of *Pseudobarbus afer* when acclimated to (a) 19.9 ± 0.1 °C and (b) 11.9 ± 0.7 °C.

The results of a Welch two-sample t-test show that there is a significant difference in CT_{max} of *P. afer* between different stream acclimation temperatures ($P < 0.05$). The recorded CT_{max} was significantly higher on individuals from the stream (acclimation) temperature at 19.9 ± 0.1 °C (summer) than 11.9 ± 0.7 °C (winter) (Figure 3.4). The mean observed CT_{max} for individuals acclimated to 19.9 ± 0.1 °C was 35.1 ± 0.6 °C while the mean observed CT_{max} for individuals acclimated to 11.9 ± 0.7 °C was 29.9 ± 0.7 °C.

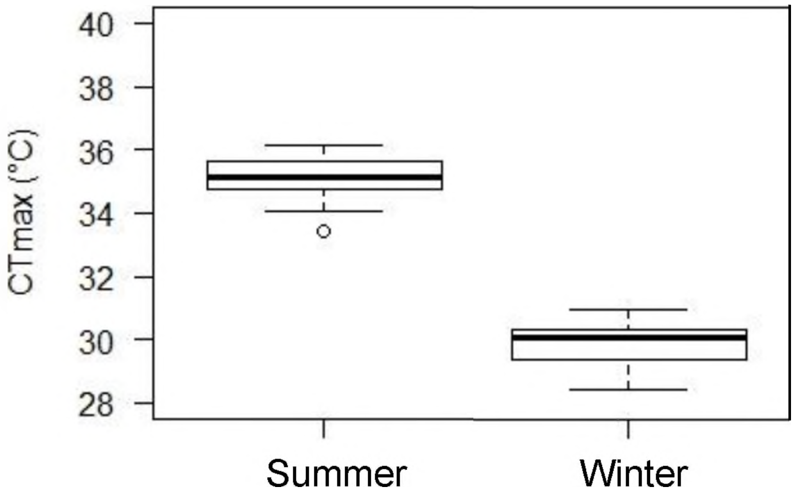

Figure 3.4 Boxplot depicting the observed CT_{max} (°C) of *Pseudobarbus afer* acclimated to 19.9 ± 0.1 °C (summer) and 11.9 ± 0.7 °C (winter).

THERMAL PREFERENCE

The thermal gradients established throughout the experimental tanks are displayed in Figure 3.5. In the thermal gradient tanks during the summer experimental phase, where ambient temperature ranged between 15.9 ± 3.0 °C and 29.0 ± 4.1 °C (Table 2-2), the maximum recorded temperature in the tank during the experimental phase was 32.4 ± 0.3 °C while the minimum recorded temperature in the tank during the experimental phase was 17.7 ± 1.3 °C and therefore the temperature difference and thermal gradient within a gutter was a maximum of 14.7 °C. In winter, where ambient temperature ranged between 6.4 ± 3.6 °C and 20.8 ± 4.6 (Table 2-2) during the experimental phase, the maximum recorded temperature in the tank was 26.1 ± 0.5 °C and the minimum recoded temperature in the tank was 14.0 ± 0.2 °C and the temperature gradient within a gutter was a maximum of 12.1 °C.

The interpolated (median) preferred temperatures (Figure 3.6) in summer had a mean of 26.29 ± 2.50 °C and a range of 7.17 °C while in winter the mean preferred temperature was 17.07 ± 1.82 ° and a range of 5.69 °C (Figure 3.6, Table 3-2). The results suggest that thermal preference differed significantly between summer (acclimatization temperature of 19.9 ± 0.1 °C) and winter (acclimatization temperature of 11.9 ± 0.7 °C) (R^2= 0.64, adjusted R^2= 0.62, F1, 16= 28.72, p <0.001). The median thermal preference in summer, with warmer ambient and water temperatures, was significantly higher than that observed in winter (Figure 3.7).

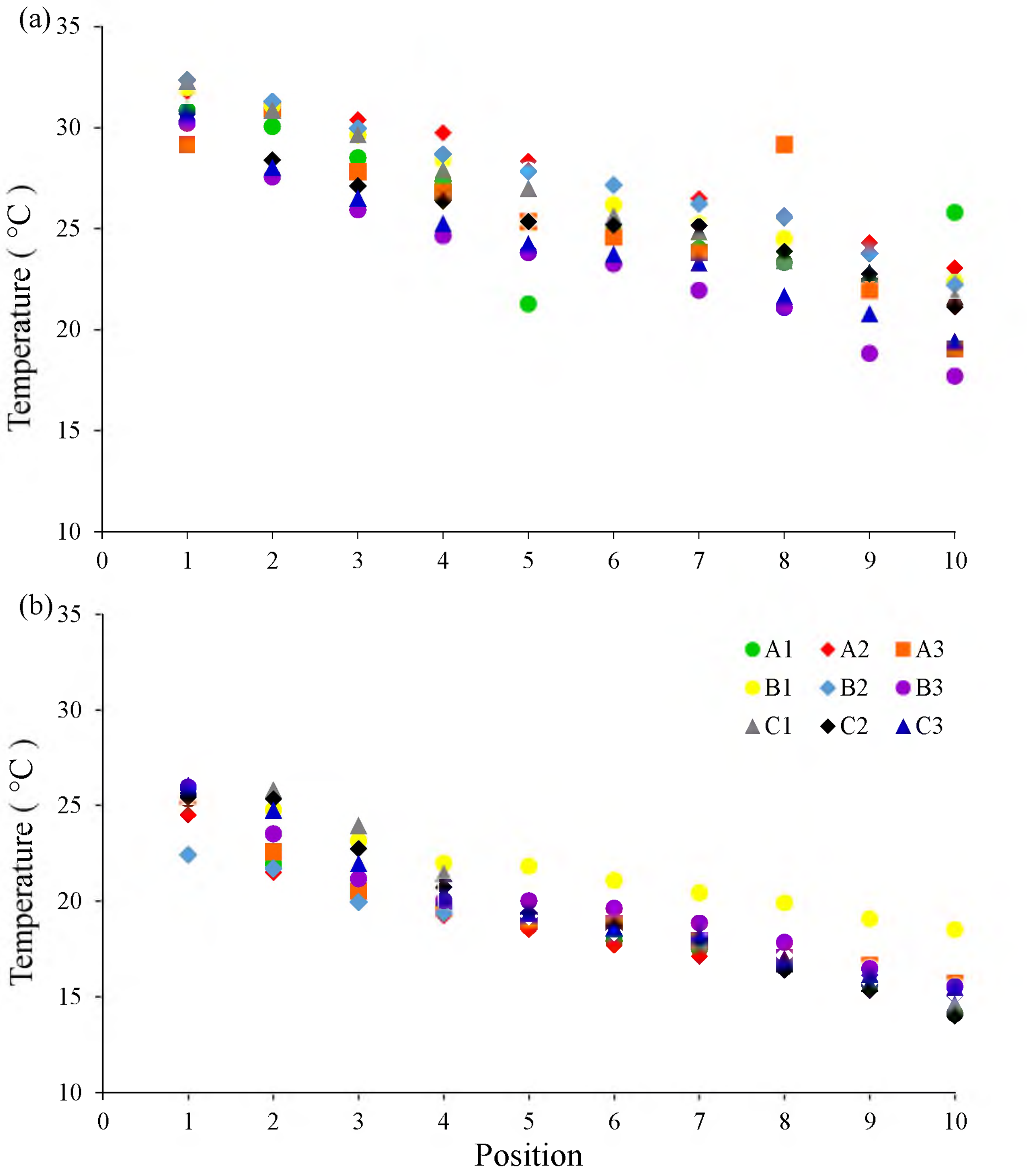

Figure 3.5 The mean observed temperature in each cell of the experiment tank in (a) summer where ambient temperature ranged between 15.9 ± 3.0 °C and 29.0 ± 4.1 °C, and (b) winter where ambient temperature ranged between 6.4 ± 3.6 °C and 20.8 ± 4.6, showing the thermal gradient created in each run of the experiment.

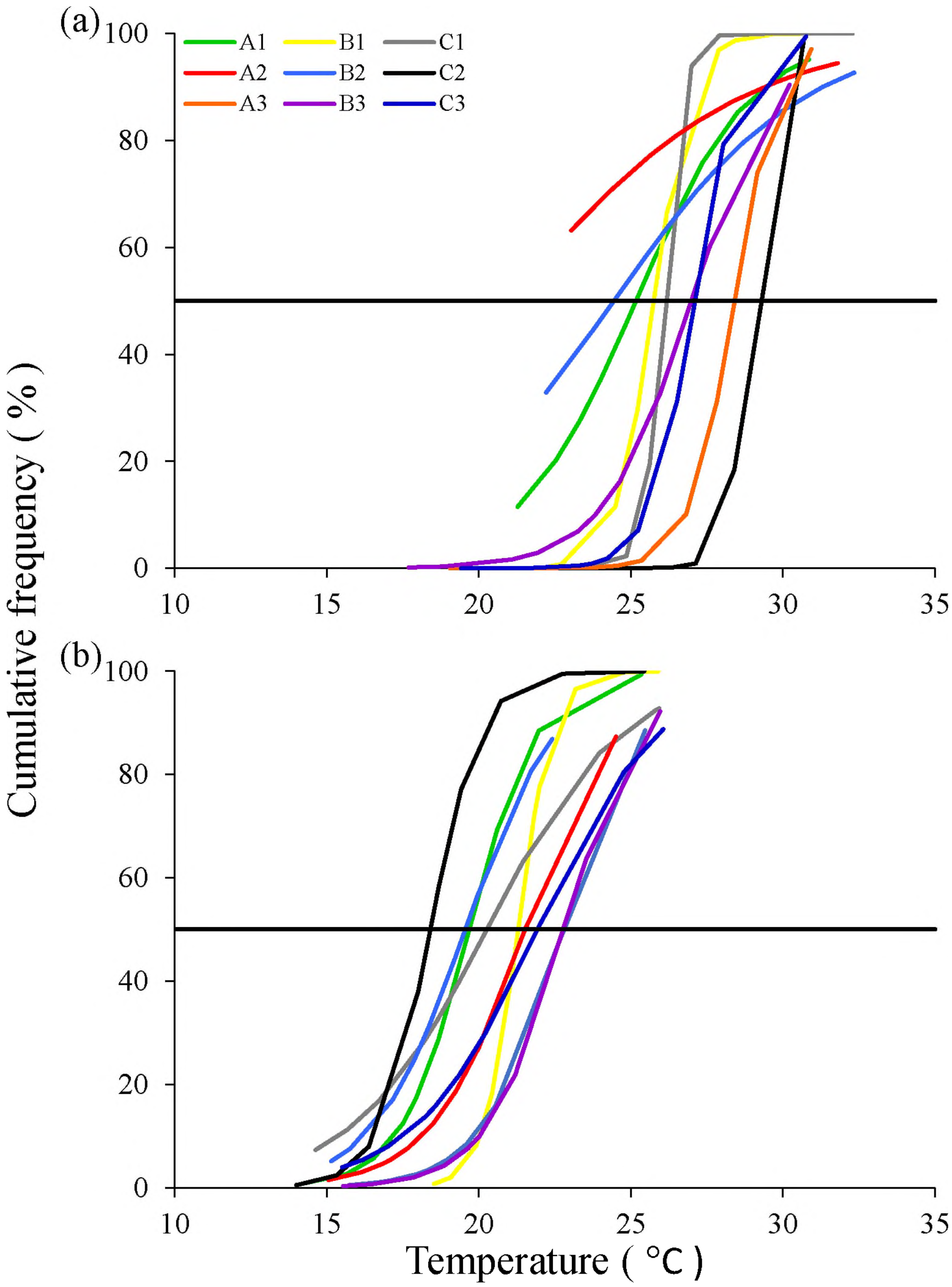

Figure 3.6 The cumulative frequency (%) and median (50%) preferred temperature (°C) of *Psuedobarbus afer* observed in each of the nine trials when acclimation temperature was (a) 19.9 ± 0.1 °C and (b) 11.9 ± 0.7 °C.

Table 3-2 Observed median preferred temperature (°C) of *Pseudobarbus afer* for each run of the experiment.

Tank	Summer (°C)	Winter (°C)
A1	24.54	16.36
B1	26.00	20.69
C1	26.19	17.92
A2	22.09	15.00
B2	23.68	17.19
C2	29.26	18.90
A3	28.53	15.69
B3	27.20	15.80
C3	29.09	16.12
Mean	26.29 ± 2.50	17.07 ± 1.82

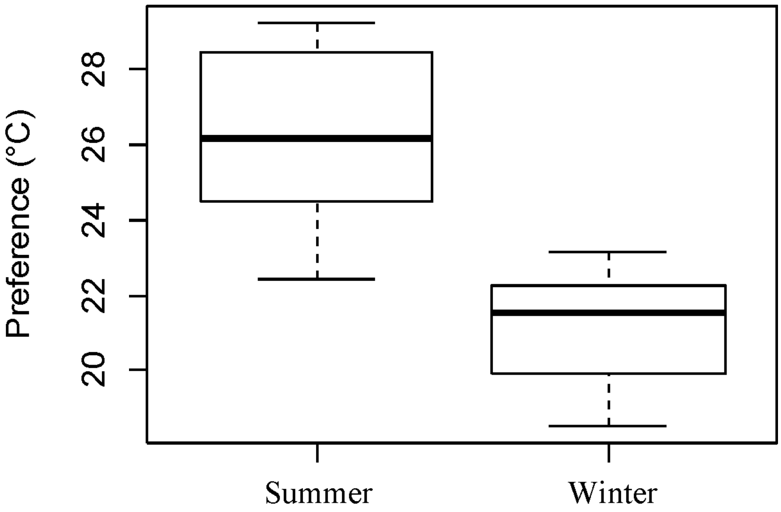

Figure 3.7 Boxplot depicting the observed median thermal preferences (°C) of *Pseudobarbus afer* in summer where acclimation temperature was (a) 19.9 ± 0.1 °C and winter when acclimation temperature was (b) 11.9 ± 0.7 °C.

The observed distribution of *P. afer* in both the control and experimental tanks are displayed in Figure 3.8. There were no observed mortalities in the control. During the summer experimental phase an end-effect was observed in the control tanks (evident in Figure 3.8). This is probably explained by the experimental design whereby the excess shade cloth was folded over double at the ends and may have resulted in preferred cover. After the observation during sampling, this was corrected for in the winter trials and it is evident that the distribution in the winter controls is random.

From the results of a two-sample Kolmogorov-Smirnov test (D (358) = 0.1537, $P < 0.05$) it is evident that the distribution of fish position control and experimental differed significantly (Figure 3.8).

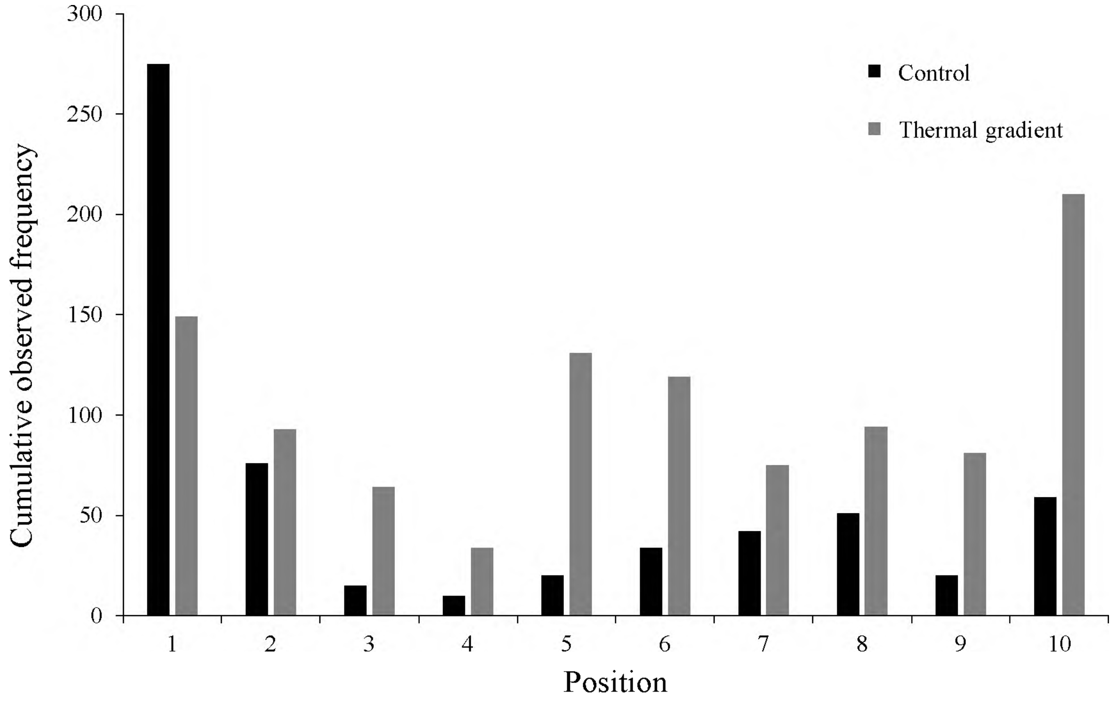

Figure 3.8 Showing the distribution of the observed cumulative frequency of *Pseudobarbus afer* position in the thermal gradient and control tanks.

DISCUSSION

From the results of the thermal experiments one can conclude that the thermal tolerance of *P. afer* was not significantly related to size of the individual but that the thermal tolerance was significantly influenced by seasonal acclimatization. Similarly, the thermal preference differed significantly due to seasonal acclimatization. From the results of this study, it was evident, from both the thermal experiments, that there was a seasonal acclimatization effect. It is therefore important, in order to make these results comparable to similar studies, that either fish are acclimated to a set of standard temperatures (where possible) or that the acclimation effect is corrected for. This could be done by, for example, standardizing when the experiments are conducted (standardizing seasonality and therefore acclimatization temperature).

Although some studies have shown that acclimation temperature does not influence preferred temperature (e.g. McCauley & Tait, 1970) others have shown that acclimation is an important determinant for final thermal preference (Barila & Stauffer, 1979; Hall *et al.*, 1979; Stauffer *et al.*, 1980; Shingleton *et al.*, 1981). For example, in a study by Shingleton *et al.* (1981), fish which were acclimated to 6, 12 or 18 °C had a final thermal preference higher than those temperatures, while fish acclimated to 24, 30 or 33 °C preferred temperatures lower than their acclimation temperatures. Results from a study by Coutant *et al.*, (1984) found that juvenile striped bass, *Morone saxailis* (Walbaum, 1792) had a higher thermal preference in summer (24 – 27 °C) than in autumn (20 – 25 °C). Similarly, *P. afer* displayed a thermal preference higher than the stream temperature in each of the experimental runs, with a mean preference of 26.3 ± 2.5 °C when stream temperature was 19.9 ± 0.1 °C and a mean preference of 17.1 ± 1.8 °C when stream temperature was 11.9 ± 0.7 °C.

It is important to understand acclimatization effect on both the thermal tolerances and preferences of native fishes in order to better understand and predict the likely responses of these fishes to elevated water temperatures in the CFE, a predicted scenario of climate change (Dallas & Rivers-Moore, 2014). These results suggest that *P. afer* displays thermal plasticity. That is, *P. afer* is able to tolerate a wide range of temperatures with a significant acclimatization effect and thus possible ability to acclimate which may, in part, be due to the ability to adapt to habitat variability as the Blindekloof stream is an episodic stream. These data are the first on thermal tolerances and preferences of *P. afer*.

As climate change is expected to increase the invasion potential of ecosystems (Heino *et al.*, 2009), it is also important to contextualise these findings in the known thermal tolerance and preference of other native and non-native species, particularly those which pose an invasion threat such as those from the *Micropterus* genus. It is accepted that thermal tolerance and preference are species specific and influenced by factors such as thermal history and life history (Beitinger & Bennett, 2000; Golovanov, 2006; Barrantes *et al.*, 2017) therefore literature was reviewed to find the thermal tolerance and preferences of species which pose invasion threats and species which are closely linked to the target species, *P. afer* (Figure 3.9).

From the literature, the investigated thermal preference of *Pseudobarbus* species range between 22.3 °C (*P. calidus* Barnard, 1938) and 27.0 °C (*P. burgi* Boulenger, 1911) (Reizenberg, 2017), with *P. afer* displaying a mean thermal preference slightly lower than that of *P. burgi* (26.29 °C). Many common, non-native species, *C. gariepinus* (Burchell, 1822) (30 °C), *M. dolomieu* (Lacepéde, 1802) (31.3 °C), *M. salmoides* (Lacepéde, 1802) (32.0 °C), *M. punctulatus* (Rafinesque, 1819) (32.1 °C), *O. mossambicus* (Peters, 1852) (32.2 °C) and *C. carpio* Linnaeus, 1758 (32.0 °C) display a thermal preference higher than that of native CFE *Pseudobarbus* species (Figure 3.9) (Reynolds & Casterlin, 1977; Reynolds & Casterlin, 1978; Stauffer, 1986; Britz & Hecht, 1987).

Pseudobarbus afer displayed the greatest CT_{max} of *Pseudobarbus* species (35.1 °C) (Figure 3.9). The CT_{max} of *P. afer* is only slightly higher than that of *M. dolomieu* (35.0 °C) (Lutterschmidt & Hutchison, 1997) but is lower than all other non-native species (Black, 1953; Cherry *et al.*, 1977; Lutterschmidt & Hutchison, 1997; Zaragoza *et al.*, 2008) (Figure 3.9). This suggests that *P. afer* populations are more at risk to the possible warming effect of global climate change than the non-native species which inhabit the Swartkops and those which have previously been recorded in the Blindekloof stream. However, as acclimation temperature (thermal history) significantly influenced thermal tolerance and preference in this study, it is suggested that future studies may want to re-examine the CT_{max} and preference of all recorded species within this system in order to make the results more comparable by standardizing thermal history and the methods.

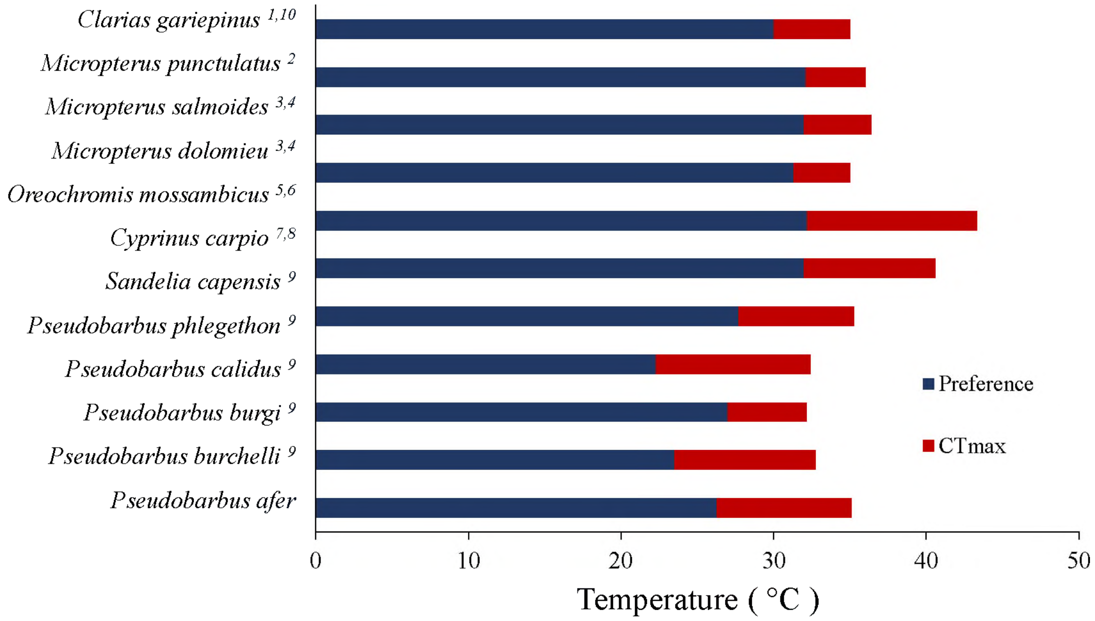

Figure 3.9 Comparison of the thermal preference and tolerance of various native; *P. afer* (this study), *P. burchelli*, *P. burgi*, *P. calidus*, and *S. capensis* (Reizenberg, 2017) and non-native species; *C. carpio* ([7]Reynolds & Casterlin, 1977; [8]Black, 1953), *O. mossambicus* ([5]Stauffer, 1986; [6]Zaragoza *et al.*, 2008), *M. dolomieu* and *M. salmoides* ([3]Reynolds & Casterlin, 1978; [4]Lutterschmidt & Hutchison, 1997), *M. punctulatus* ([2]Cherry *et al.*, 1977) and *C. gariepinus* ([10]Safriel & Bruton, 1984; [1]Britz & Hecht, 1987).

The CT_{max} provides important understanding of what specific species are able to tolerate thermally. The method employed has numerous advantages such as the practicality, the advantage of not using fatality as an end point (important for endangered species) and it is economical in terms of both equipment and time (Becker & Genoway, 1979). It is argued that CT_{max} is of greatest ecological significance in river systems which are exposed to water stress and thus great thermal fluctuations (Dallas & Rivers-Moore, 2012) such as the episodic Blindekloof and Fernkloof Headwaters.

However, while CTM is an accepted measure of thermal tolerance in fishes, in reality it is unlikely that fishes will experience the rapid changes in temperatures that they are exposed to during CTM experiments due to the relative thermal inertia of water (Hutchinson, 1961; Becker & Genoway, 1979). This is supported in that the maximum heating rate observed in the Blindekloof stream as described in the previous chapter is far from a rate of 18 °C / hr (maximum daily range observed was 4.66 °C). This is important to consider as thermal tolerance is affected by factors such as the heating rate (as well as acclimation temperature) (Dallas, 2008). The CTM is therefore a standardized approach to investigating the thermal tolerances of fishes in order to provide species specific information which is then comparable between species (Becker & Genoway, 1979; Richardson *et al.*, 1994; Beitinger *et al.*, 1999; Barrantes *et al.*, 2017).

This study investigated the acute thermal preference of *P. afer*, but literature argues that temperature preference is better described as a range rather than a specific temperature as fish are seldom static and thus do not remain at only one temperature (Reynolds & Casterlin, 1979; Bernatzeder & Britz, 2007). The preferred temperature may also be skewed due to competing factors such as competition, habitat preference and food (Coutant, 1987). Another limitation of the experimental design, and thus the findings, is that species responses to thermal regimes may be related to their ontogeny (Coutant, 1987; Richardson *et al.*, 1994; Jobling, 1995; Barrantes et al, 2017) and therefore, for a more holistic understanding, the thermal tolerance of various life stages should be investigated.

It is important to note that thermal preference, where a species fitness, food conversion rate and hence growth rate is optimal, is of most worth to aquaculturists (Bernatzeder & Britz, 2007). However, the knowledge of thermal preference does allow us to categorise the species as either a cold-, cool- or warm- water species (Heino *et al.*, 2009). Heino *et al.* (2009) define warm water species to have a summer thermal preference between 27 - 31 °C, cool water species to have a summer thermal preference between 21 - 25 °C and cold water species to have a summer thermal preference between 11 – 15 °C. Thus, the investigated summer thermal preference of *P. afer* suggests it is on the boundary of a warm water species. Furthermore, as many human activities (such as water discharge and the building of dams) impact the thermal regime of a stream (Olden & Naiman, 2009), it is becoming increasingly important to understand the thermal preference and tolerance of a species.

By understanding the thermal tolerance of *P. afer* and the thermal regime of the Blindekloof stream, one can better hypothesise whether temperature is in fact limiting native fish distributions across the river scope. This was investigated in the next chapter.

Chapter 4 The longitudinal distribution of fishes in the Blindekloof stream

INTRODUCTION

Freshwater ecosystems are considered some of the most vulnerable ecosystems as they face multiple stressors such as overexploitation, habitat degradation, flow modification, water pollution and non-native invasions (Dudgeon *et al.*, 2006; Abell *et al.*, 2008; Ellender *et al.*, 2017). The primary threat faced by native headwater fishes is the introduction of non-native predatory fishes (Swartz & Impson, 2007; Tweddle *et al.*, 2009; van Rensberg *et al.*, 2011; Kadye & Booth, 2012; Ellender *et al.*, 2017). The effect of these multiple stressors is expected to be exacerbated as the predicted climate change scenarios act synergistically with these stressors (Dudgeon *et al.*, 2006; Jackson *et al.*, 2016). In particular, freshwater fish fauna is expected to decrease in diversity as a result of these human mediated impacts which leads to the degradation of freshwater ecosystems (Dudgeon *et al.*, 2006; Moyle *et al.*, 2013).

Meyer *et al.* (2007) classified headwater fishes into three broad categories: fishes which are headwater specialists; fishes which are generalists and may move into headwaters should mainstream conditions be unfavourable, and species which rely on the headwaters as spawning or nursery habitats. The Cape Fold Ecoregion is home to many range-restricted headwater specialist freshwater fish species which, due to their limited locality, are considered a conservation priority as there is an ever-present threat of extinction (Ellender *et al.*, 2017). In a review by Darwall *et al.* (2009) it is noted that 60% of native freshwater species in the CFE are IUCN Red Listed as endangered or critically endangered.

The conservation status of native freshwater fishes may, in some instances, be linked to the presence of non-native fishes, however Ellender *et al.*, (2011) points out that it is often difficult to uncouple the various factors which interact to drive fish distributions. On the global scale, many freshwater ecosystems are now inhabited by more non-native than native species and all major rivers in South Africa are invaded by non-native species (Leprieur *et al.*, 2008; Strayer, 2010; van Rensberg *et al.*, 2011). Furthermore, in a review by Ellender and Weyl (2014), it was documented that 55 non-native species (28 are alien species while the other 27 species are extralimital) had been introduced into South African freshwater systems.

Not all non-native species successfully establish and the invasion occurs in three stages: (i) introduction, (ii) dispersal of these species within a system and finally, (iii) the subsequent spread of these introduced species (Leprieur *et al.*, 2008; Blackburn *et al.*, 2011). Once a species has been introduced beyond its native range and has established, it is often near impossible to eradicate (Copp *et al.*, 2005; Gozlan *et al.*, 2010). Thus invasion rates are likely to continue to rise (Strayer, 2010). It is particularly true as ecosystem management is incredibly complex. Invasive species management has been broadly categorised into three actions: (i) prevention, (ii) eradication and, (iii) impact reduction (Woodford *et al.*, 2016). In many instances, the most successful strategy will be to inhibit further invasions, as preventing further spread - and thus more far-reaching, irreversible consequences - is imperative to the survival of many threatened fishes (van der Walt *et al.*, 2016). To do this one needs to better understand what factors determine the distribution of non-native fishes in river networks.

The introduction and invasion of non-native freshwater fishes is not observable only in South Africa. A global example of the impact of predatory species on native fish assemblages is that of brown trout, *Salmo trutta* Linnaeus, 1958, in New Zealand (Townsend & Crowl, 1991; Townsend, 1996). *Salmo trutta* were first introduced for angling but have spread and established in many New Zealand waters (Townsend, 1996). Anecdotal evidence suggests that trout impacts on native fishes, such as those in the family Galaxiidae, were rapid and severe (Townsend, 1996). Studies from these regions suggest that many endemic fishes are now limited in distribution to locations which are inaccessible and thus unoccupied by *Salmo trutta* (Eldon, 1979; Main *et al.*, 1989; Townsend, 1996).

One of the most constant, predictable factors influencing invasion fronts is natural barriers, which may prevent upstream movement of species and thus limit the reaches which non-native species are able to invade (Rahel, 2007; van der Walt *et al.*, 2016). Instream barriers may include physical features of the stream such as waterfalls, chutes or cascades. In a study by van der Walt *et al.* (2016) it was observed that natural barriers limited the upstream movement of *Micropterus dolomieu* Lacepède, 1802 and *Micropterus punctulatus* (Rafinesque, 1819). This study focused on 41 tributaries of the Olifants-Doorn River (ODR), classifying instream barriers (waterfall, chute or cascade) and investigating the relationship of these barriers and the extent of invasion (van der Walt *et al.*, 2016), concluding that instream barriers are a conservation tool as they restrict the upstream distribution of non-native fishes. Furthermore, the connectivity of suitable habitats may also influence invasion fronts. An example is the Blindekloof stream, which is an episodic system and the upstream movement of larger predatory fishes may thus be hindered by lack perennial of connectivity (Ellender *et al.*, 2011; van der Walt *et al.*, 2016).

Ellender *et al.* (2014) noted that other factors limiting invasion potential and establishment may include low propagule pressure and the nature of the receiving environment, for example the Blindekloof stream's episodic character. While *Micropterus* species have been highlighted as the primary invasion concern in the Blindekloof stream (Skelton, 1993; Ellender *et al.*, 2011), more recent studies suggest that the extralimital African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) may also pose a threat in the Blindekloof stream (Ellender *et al.*, 2014). The study by Ellender *et al.* (2014) aimed to investigate the invasion potential of *C. gariepinus* in three headwater tributaries including the Blindekloof stream. The monitored streams were within protected areas and thus it is likely that the invasions were the result of a source population in the mainstem (Ellender *et al.*, 2014). The effects of non-native fishes vary and may include predation on native fauna assemblages and interspecific competition for resources such as habitat or food (Townsend, 1996; Ellender *et al.*, 2014).

Moyle and Light (1996) listed various other factors which impact invasions including, but not limited to, the diversity of the receiving community- in complex systems, the invaders may have more competition and the effects of successful invasions may be less noticeable in an intricate system; the level of human activity and impact on the system; the receiving environments variability, the severity thereof and the predictability. It is therefore evident that freshwater invasions pose the primary threat to endemic (often vulnerable) fishes and that freshwater systems are complex, with a range of factors which influence and aid invasions. Understanding these fronts is crucial for assessing the risk associated with invasions and identifying key conservation areas; climate change is expected to exacerbate the effect of invasions.

The shifts in invasion fronts have consequent shifts on native fish distributions, as it has been reported that in invaded reaches of the Blindekloof stream, small-bodied native fishes, including the IUCN Red listed endangered *Pseudobarbus afer* (Peters, 1864) were absent (Ellender *et al.*, 2011, 2017). Due to a number of variables such as environmental changes, non-native species may take years to reach their invasion potential (Strayer, 2010).

This chapter investigates fish distributions in the Blindekloof stream over time. It was hypothesised that i) the upstream invasion of *Micropterus* species in the Blindekloof stream was limited by instream barriers; ii) the upstream invasion of *Micropterus* species was further limited in the Blindekloof stream by temperature; iii) the downstream distribution of native fishes, particularly *P. afer,* was limited by temperature, and iv), the downstream distribution of native fishes, particularly *P. afer,* was limited by the presence of non-native fishes.

METHODS AND MATERIALS

STUDY AREA

The physical characteristics have been described in Chapter 2. The native fish fauna of the Blindekloof stream includes the Eastern Cape redfin, *Pseudobarbus afer*; Goldie barb, *Enteromius pallidus*, A. Smith, 1841; the river goby, *Glossogobius callidus* (Smith, 1937); the Cape kurper, *Sandelia capensis* (Cuvier, 1831), African longfin eel, *Anguilla mossambica*, Peters, 1852 and; the giant mottled eel, *A. marmorata*, Quoy & Gaimard, 1824 (Ellender, 2013). Non-native fishes which have been documented in the Swartkops River include largemouth bass, *Micropterus salmoides* (Lacepède, 1802); smallmouth bass, *M. dolomieu*; the banded tilapia, *Tilapia sparrmanii* Smith, 1840; the African sharptooth catfish, *Clarias gariepinus*; and common carp, *Cyprinus carpio* Linnaeus, 1758. All non-native fishes but *C. carpio* have also been documented in the Blindekloof stream. However, as snorkel surveys for this study were undertaken during the day, data were not collected for *Anguilla* species or for *C. gariepinus*.

Ellender *et al.* (2011) stated that these non-native fish invasions are likely to be the result of opportunistic invasions from a mainstem source population, which penetrate up the stream during the intermittent periods of flow and are then unable to move back downstream once the pools become isolated. The most threatening of these invaders are those from the *Micropterus* genus, but as they have not established in the Blindekloof stream, their occupancy in the river is variable (Ellender *et al.*, 2017).

The longitudinal distribution of fishes, both native and non-native, was investigated in the Blindekloof stream. The data were collected from the thirteen long-term monitoring sites as highlighted in Figure 2.3. The habitat data for each of the sites are summarised in Table 2-3.

DATA COLLECTION

Fish distribution was evaluated by undertaking snorkel surveys in the 13 sites using a two-pass snorkel method. This method makes use of a zigzag pattern, with the observer starting at the bottom of the pool and swimming upstream in a zigzag manner so as to cover the greatest area of the pool (Woodford *et al.*, 2005; Ellender *et al.*, 2011). In this initial pass, a count of all species observed was recorded in three different size (total length) brackets; (i) individuals less than 30mm (fry), (ii) individuals between 30 and 60 mm and, (iii) those greater than 60mm. This is done as establishment is indicated by the ability to breed and have self-sustaining populations, thus if there is evidence of breeding it is likely that fish are established. The second pass was a repeat of the first, but this time the observer swam in a downstream direction and only recorded counts of species (not accounting for size). The sampling took place in December 2015 and again in February 2017.

DATA ANALYSIS

To determine whether non-native fish distributions were limited by natural barriers such as waterfalls, *in situ* observations of such barriers were recorded and are shown on the map (Figure 2.3; Ellender *et al.*, 2011). The invasion fronts were then recorded using snorkel surveys and analysed in light of any instream barriers.

With the knowledge of the instream thermal regime in the river reach (Chapter 2), literature was reviewed to determine both the thermal preference and thermal tolerance of *Micropterus* species in order to investigate whether temperature was in fact limiting the upstream invasion.

Similarly, with the results of the thermal preference and tolerance experiments conducted on *P. afer* (Chapter 3), and the knowledge of the thermal regime of the monitored stretch of the Blindekloof stream (Chapter 2), it was investigated whether the downstream distribution of *P. afer* was limited by temperature.

Ellender *et al.* (2011) observed that in the presence of non-native *Micropterus* species, many native fishes were absent, and therefore the more recent data were reviewed for similar observations which may support the hypothesis that the downstream distribution of native fishes is driven by the presence of non-native fishes.

The species densities were tested for significant differences over time and between sampling reaches (previously invaded and not invaded) using a PERMENOVA add-in in PRIMER. The species density data were log (x + 1) transformed to normalise the large densities observed, particularly in the 2017 survey. A non-metric multidimensional scaling (NMDS) analysis (based on the Euclidean distance) was used to visualise the difference in community structure between survey years and between the previously invaded and non-invaded reaches of the Blindekloof stream. The significance of the NMDS clusters was tested by permutation MANOVA (PERMANOVA) and similarity percentage (SIMPER) analyses using PRIMER software.

RESULTS

While no *Micropterus* were recorded in the snorkel surveys of 2015 and 2017 (Table 4-1, Figure 4.1), in 2010 the fish did not invade to the waterfall barrier and it is therefore unlikely that instream barriers are limiting the upper distribution of non-native fishes. It is evident that *P. afer* densities were lower in previously invaded reaches of the Blindekloof stream than the *P. afer* densities recorded in the uninvaded reaches during all sampling events (Figure 4.1; Figure 4.2).

No *Micropterus* species have been recorded in the Blindekloof stream since the 2011 flooding event and their absence from the lower reaches may explain the ability of *P. afer* populations to re-establish in the previously invaded reaches (Figure 4.2). It is however noteworthy that despite *P. afer* persistence in the lower reaches of the Blindekloof stream, their density has decreased in the previously invaded reach between the 2015 survey and the 2017 survey (Figure 4.2).

Table 4-1 The pool surface area (100 m^2) and the distribution and abundance (fish / 100 m^2) of fishes by site in the 13 monitoring sites of the Blindekloof stream in (a) 2010 (Ellender *et al.*, 2011), (b) 2015 and (c) 2017.

	Species	Lower reaches					Middle reaches				Upper reaches			
	Site	1	2	3	4	5	6	7	8	9	10	11	12	13
	Surface Area (100 m^2)	14.26	2.62	2.72	3.62	5.94	3.34	4.45	5.25	4.79	0.93	0.70	1.38	0.99
(a)	*E. pallidus*	5.2	0.2	3.6	-	0.2	-	-	-	-	-	-	-	-
	P. afer	-	-	-	-	-	-	0.3	-	-	126.5	-	-	-
	G. callidus	1.0	-	-	-	-	2.2	0.4	-	-	-	-	-	-
	S. capensis	-	-	-	-	-	2.2	1.0	-	-	-	-	-	-
	*T. sparrmanii**	1.0	1.3	4.6	-	5.6	2.2	0.1	-	-	-	-	-	-
	*M. salmoides**	-	-	-	-	-	-	-	0.1	-	-	-	-	-
	*M. dolomieu**	-	-	-	-	-	-	-	0.1	-	-	-	-	-
(b)	*E. pallidus*	7.0	1.7	1.1	-	0.1	1.3	-	-	0.1	-	-	-	-
	P. afer	10.9	36.3	40.4	51.1	20.6	17.9	21.9	11.0	32.9	61.6	128.4	188.0	78.0
	G. callidus	0.7	1.0	1.7	0.6	0.8	0.1	0.2	0.1	0.6	4.3	-	-	-
	S. capensis	0.6	8.6	8.3	10.4	2.7	12.7	2.6	4.8	2.66	9.1	0.7	-	-
	*T. sparrmanii**	0.6	0.8	-	-	0.1	-	-	-	-	-	-	-	-
	*M. salmoides**	-	-	-	-	-	-	-	-	-	-	-	-	-
	*M. dolomieu**	-	-	-	-	-	-	-	-	-	-	-	-	-
(c)	*E. pallidus*	-	-	-	-	-	-	-	-	-	-	-	-	-
	P. afer	-	17.2	20.2	22.8	10.8	10.5	18.0	17.0	20.9	62.7	476.5	191.3	116.2
	G. callidus	-	1.9	0.4	3.5	0.3	1.8	1.8	0.1	-	-	-	-	-
	S. capensis	3.7	13.4	23.5	4.1	1.8	5.1	6.4	0.4	0.2	-	-	-	-
	*T. sparrmanii**	-	21.4	24.7	2.8	0.3	-	-	-	-	-	-	-	-
	*O. mossambicus**	-	-	-	4.1	-	-	-	-	-	-	-	-	-
	*M. salmoides**	-	-	-	-	-	-	-	-	-	-	-	-	-
	*M. dolomieu**	-	-	-	-	-	-	-	-	-	-	-	-	-

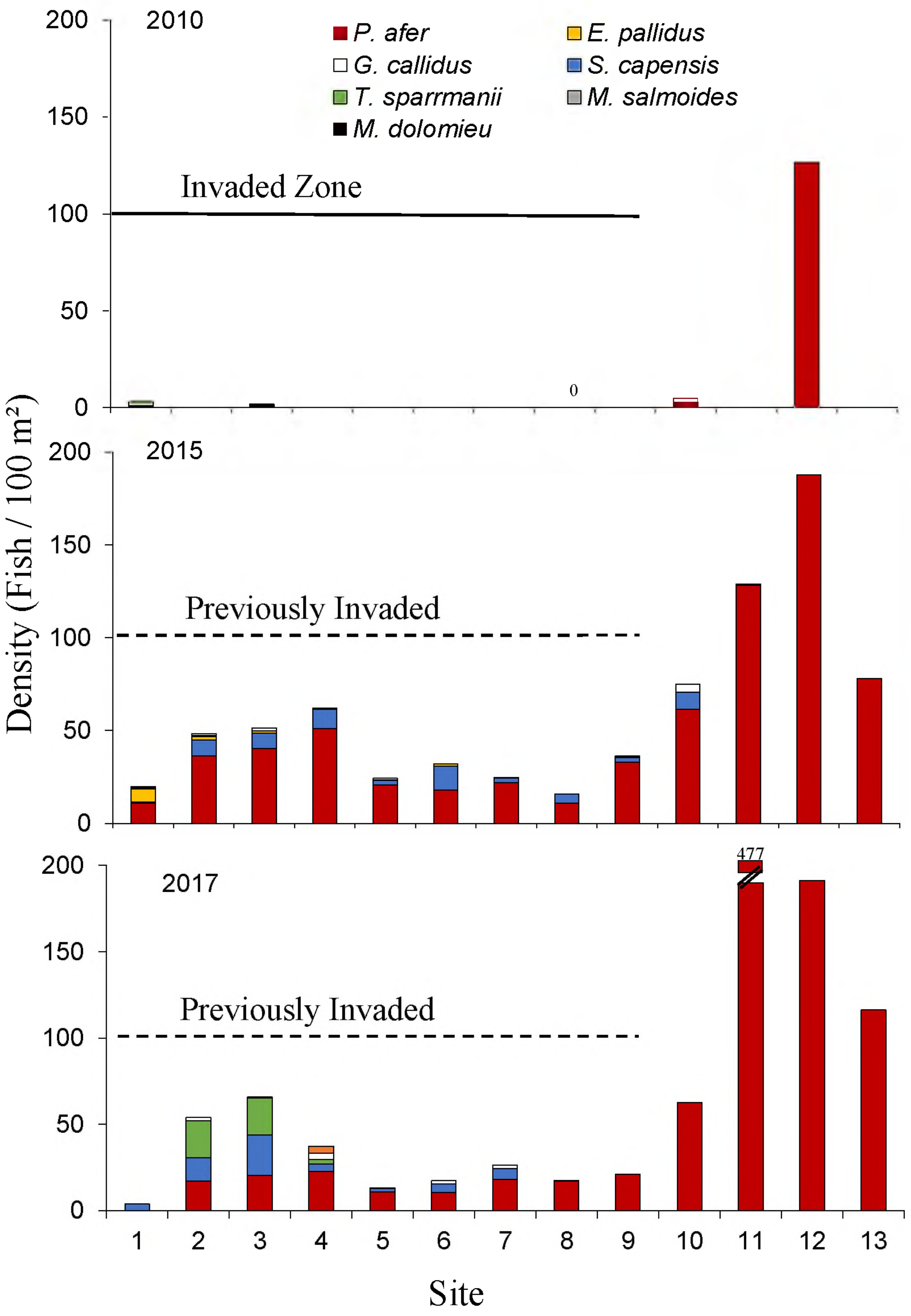

Figure 4.1 The density (fish / 100 m^2) of all species at each site recorded during snorkel surveys (2010, 2015 and 2017) in previously invaded and non-invaded reaches of the Blindekloof stream (2010 snorkel data taken from Elleder *et al.*, 2011).

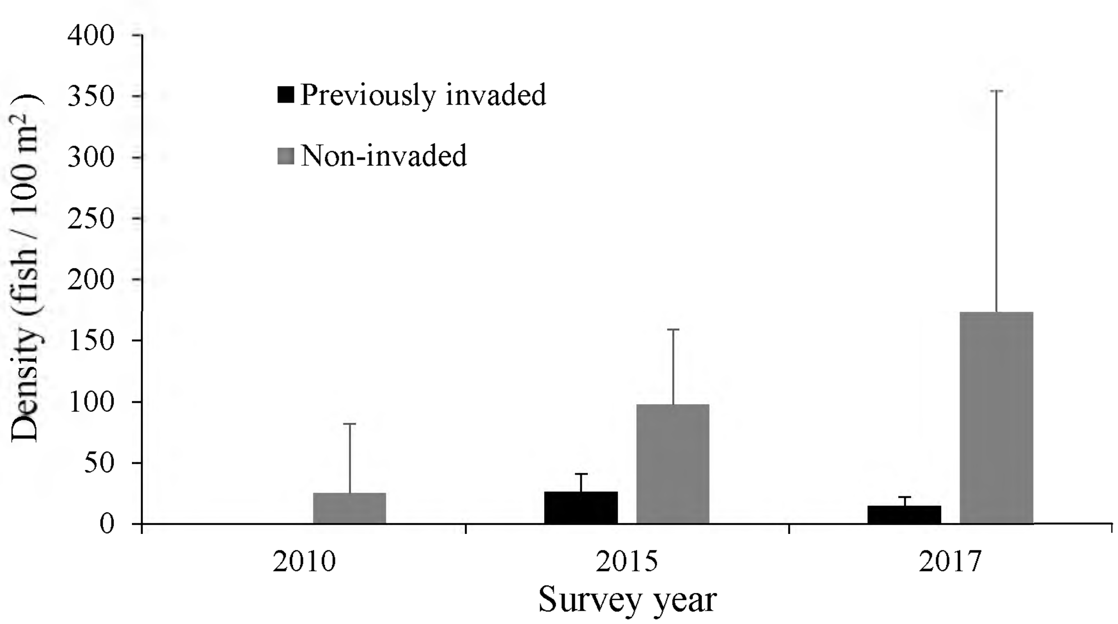

Figure 4.2 Average *Pseudobarbus afer* density in reaches of the Blindekloof stream previously invaded (represented in black) by *Micropterus* species (2010, data from Ellender *et al.*, 2011) and non-invaded reaches (represented in grey).

The 2-dimensional stress value (0.08) suggests that the NMDS plot gives a good representation (≤ 0.1) with little risk of misinterpretation of the data (Figure 4.3) (Clarke & Warwick, 2001; Botha *et al.*, 2016). The results of a PERMDISP test, testing for homogeneity of dispersion (Anderson *et al.*, 2008), suggest that survey year did not significantly influence the dispersion from the centroid ($F = 3.30$; $p > 0.01$). Similarly, the results of a PERMDISP suggest that survey reach (previously invaded and non-invaded) did not significantly influence the dispersion from the centroid ($F = 4.47\ e^{-2}$; $p > 0.05$). The results of a PERMANOVA suggest that the species composition did however differ significantly between years (Pseudo-$F = 18.50$; $p < 0.001$) and between reaches (Pseudo-$F = 13.40$; $p < 0.001$). The results of a post-hoc test suggest that the fish communities differed significantly between 2010 and 2015 surveys ($t = 5.94$; $p < 0.001$) and between 2010 and 2017 surveys ($t = 4.56$; $p < 0.001$).

According to the results of the SIMPER analysis, *P. afer* contributed 61.1 % of the variability in the fish assemblage between the previously invaded and non-invaded reaches in 2010, with *T. sparrmanii* and *E. pallidus* contributing 16.8 % and 13.2 % respectively. In the 2015 survey, *S. capensis*, *P. afer* and *E. pallidus* contribute 38.5 %, 32.0 % and 17.5 % of the variation between the previously invaded and non-invaded reaches. *Pseudobarbus afer*, *T. sparrmanii* and *S. capensis* account for 42.2 %, 26.1 % and 22.6 % respectively in the 2017 survey variation between the previously invaded and not invaded reaches. *Pseudobarbus afer* contributes the most to the variation in both the previously invaded and non-invaded reaches with a contribution 46.3 % and 88.7 % respectively. From these results it is evident that, except for *T. sparrmanii*, it is the abundance of native fish species (predominantly *P. afer*) which account for the majority of the variation in fish community structure in the Blindekloof stream.

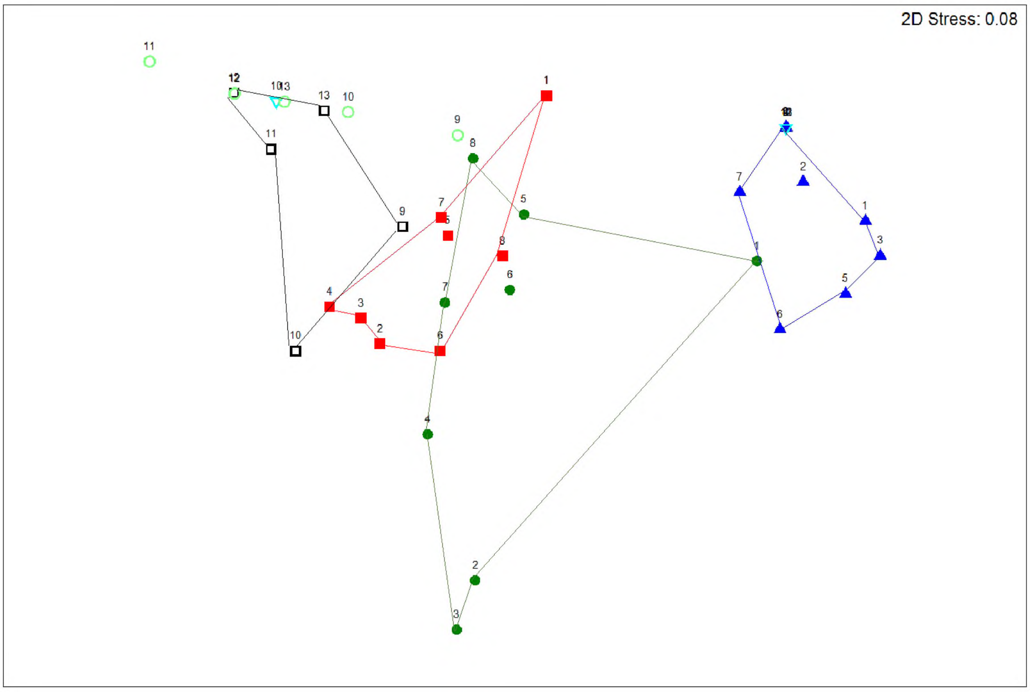

Figure 4.3 Non-metric multidimensional scaling (NMDS) analyses based on fish density in each of the pools (numbers) in 2010 in the previously invaded (▲) and not invaded (▽) reaches, the fish density in 2015 in the previously invaded (■) and not invaded (□) reaches, and the fish density in 2017 in the previously invaded (●) and not invaded (○) reaches of the Blindekloof stream.

DISCUSSION

The distribution data provided evidence that invasion fronts in the Blindekloof stream are fluid in time and space (Ellender & Weyl, 2015). The data collected in 2015 and 2017 are in line with the findings of Ellender *et al.* (2011), where it was stated that the non-native *Micropterus* species have not established self-sustaining populations following an upstream invasion of the Blindekloof stream from the mainstem. *Micropterus salmoides* had previously invaded and subsequently been actively removed by authorities (Skelton, 1993; Ellender *et* *al.*, 2011) but as no mitigation measures were put in place to avoid invasion, the Blindekloof had the potential for reinvasion.

The results of Ellender *et al.* (2011) show that the *Micropterus* species were neither abundant nor widespread when they reinvaded the Blindekloof stream, and these researchers suggest that this may be due to the episodic nature of the system which is unfavourable to such large predators. While this remains likely, the *Micropterus* species had only invaded to Site 8 which was below the waterfall, lending itself to the conclusion that it is unlikely that instream barriers are driving the distribution of these species, but that habitat suitability may have a significant influence.

One such environmental factor to consider is water temperature as it is accepted that temperature is a driving force in fish distributions (e.g. Coutant, 1987; Richardson *et al.*, 1994; Beitinger *et al.*, 1999). The distribution of fishes was therefore contextualised in light of the stream temperature regime (Chapter 2) and the known thermal preference and tolerance of non-native species (literature) in order to investigate whether temperature was limiting the upstream distribution of *Micropterus* species. In a review by Jobling (1981) it is stated that *Micropterus salmoides* has a final preferred temperature of 28.5 – 32.0 °C and a lethal temperature of 36.4 °C. Similarly, *Micropterus dolomieu* has a final preferred temperature of 28.0 – 31.3 °C and a lethal temperature of 35.0 °C (Figure 3.9). From the thermal temperatures recorded in the Blindekloof stream (Chapter 2), it is evident that while the thermal preference of both species was reached during the hottest periods (maximum Max_7 of 28.2 °C and an absolute maximum of 29.4 °C), the recorded stream temperature did not approach the CT_{max} of these species. Furthermore, the hottest recorded temperatures were below the invasion front and therefore it is unlikely that temperature was limiting the upstream distribution of *Micropterus* species. The data suggest that, while the temperature regime of the stream is within the tolerance of *Micropterus* species, the suitable habitat and pool size for larger non-native fishes is limited and patchy.

Having investigated the thermal tolerance and preference of *P. afer,* as reported in the previous chapter, it was found that, season dependent, the thermal tolerance of the species was between 29.9 and 35.1 °C, while the thermal preference, also season dependent, ranged between a median of 16.4 and 26.2 °C. While the maximum temperatures recorded in the Blindekloof stream approach the thermal tolerance of the species as observed in winter, the site at which the maximum temperature was recorded is within their distribution range. As acclimatization temperature had a significant effect on the thermal tolerance and preference of this species, and the maximum recorded temperature is 5.73 °C lower than the summer CT_{max}, it is unlikely that temperature was limiting the downstream distribution of *P. afer*. Furthermore, as *P. afer* and other native fishes establish downstream in the absence of non-native predatory species, it is unlikely that temperature is the driving factor of their distribution in the Blindekloof stream.

The patchy, non-permanent distribution of *Micropterus* species in the Blindekloof stream may explain how small native species are able to persist in the previously invaded reaches of the river. It is however evident that, in the presence of *Micropterus* species, non-native fishes were absent. This is supported by the results of the PERMANOVA analyses which suggest a significant difference in fish assemblages between sampling events where *Micropterus* species were present and not present (i.e. significant difference between 2010 and 2015, and between 2010 and 2017) but not between sampling events when *Micropterus* species were absent (between 2015 and 2017). Given the significant impacts of *Micropterus* presence on native fish assemblages in this study, most noticeably *P. afer* (SIMPER results) and on small-bodied native fishes elsewhere (van der Walt *et al.*, 2016; Dallas *et al.*, 2017), native fish populations are at risk if there are no barriers to limit the invasion by non-native fishes.

Ellender *et al.* (2011) observed that in the presence of *Micropterus* species, many native fishes were absent (Table 4-1) and therefore it is likely that the presence of non-native fishes was driving the longitudinal distribution of native fishes. This finding is consistent with data on the distribution of other redfin species in the CFE in the presence of non-native predators (van der Walt *et al.*, 2016). van der Walt *et al.* (2016) observed that in the Olifants-Doring River system non-native *Micropterus* species invaded perennial tributaries, also from a mainstem source populations, but that their invasion potential was limited by a physical barrier. In line with the findings of this study, van der Walt *et al.* (2016) observed that where these fish were present, native small-bodied minnows were absent but that native minnow were abundant above the invasion front. Dallas *et al.*, (2017) highlights how in the Bos River system, *Pseudobarbus swartzi* (a recently described species previously recorded as *P. afer* (Chakona & Skelton, 2017)) are also absent where *Micropterus* species were present.

Importantly however, when non-native predatory species are absent native fishes re-establish throughout the system (Table 4-1). Thus the range of many native species seems to have increased. *Enteromius pallidus* were subsequently recorded in the lower to middle reaches in 2015, *S. capensis* which were previously limited to the middle reaches (Ellender *et al.*, 2010) have also extended their distribution and, most noticeable, *P. afer* which were recorded in only two pools below the invasion front (Ellender *et al.*, 2011) were present in all pools in 2015 and all but the lowest pool in 2017. Importantly, *Oreochromis mossambicus* (Peters, 1852) was observed in the most recent data collection. While *O. mossambicus* were observed at only one site (Table 4-1), the size structure of the individuals observed suggests that they were able to breed and thus form self-sustaining populations. While many of the native species were still observed at this site as well, suggesting the ability to co-occur, this further highlights how fluid invasion fronts may be, changing over time and further motivating why they need to be monitored.

The ability of CFE native fishes to re-establish after non-native fishes were removed has also been documented elsewhere, for example Weyl *et al.* (2013). In this study, a 4km reach of the Rondegat River in the Cederberg (western region of the CFE) was rotenoned in an attempt to remove *M. dolomieu* which had established in the system, having dire consequences on native fish abundance and distributions, including a *Pseudobarbus* species, *P. phlegethon* (Barnard 1938) (Weyl *et al.*, 2013). Having successfully removed *M. dolomieu*, native fish assemblages which persisted above the invaded reach were able to recolonise the previously invaded stretch of the Rondegat River (Weyl *et al.*, 2014).

Of concern though is that rising temperatures may facilitate the invasion of headwaters by non-native fishes (Heino *et al.*, 2009). With invasion fronts being fluid in both space and time, and due to the lack of natural barriers to invasion in this particular system, it is difficult to predict re-invasion rates, success and thus long-term effects of non-native fishes. Testament to this fact is a recording of a new invasion in this stream by *O. mossambicus*. While this non-native species was recorded in only one pool sampled, both adults and juveniles were present and this attests to establishment of this species. However, unlike in the presence of *Micropterus* species, native fish populations still persisted in this site.

The observable decline of native fish diversity in the presence of *Micropterus* species, along with the unpredictable invasion potential, is cause for concern and, due to the endemic nature of many of the native species, should be a priority for conservation (Arthington, 2012; Moyle *et al.*, 2013).

Chapter 5 General discussion and concluding remarks

SYNTHESIS OF FINDINGS

This thesis aimed to uncouple what factors may be limiting the downstream distribution of *Pseudobarbus afer* (Peters, 1964). Ellender (2013) highlighted that it is difficult to quantify impacts and potential invasion as it is often near impossible to disentangle the various factors influencing aquatic ecosystems. To address this, I first characterised the thermal regime of the Blindekloof stream and demonstrated that the thermal regime is typical of a cold, stable stream (Chapter 2). I then investigated the thermal tolerance and preference of *P. afer* with varying seasonal acclimatization in order to investigate the physiological tolerance of the species (Chapter 3). It was evident that the species displays some thermal plasticity, with acclimatization temperature significantly influencing both the thermal tolerance and the thermal preference of the species. From the thermal preference results, *P. afer* was best described as a species on the lower boundary of a warm water species (defined to have a summer thermal preference of between 27 and 31 °C by Heino *et al.* (2009) and the average recorded summer thermal preference for *P. afer* was 26.3 ± 2.5 °C). Lastly, snorkel surveys demonstrated that *P. afer* were able to persist in the lower reaches of the Blindekloof stream in the absence of *Micropterus* species. The results presented in this thesis therefore suggest that it is likely to be non-native predatory fishes which limit the distribution of *P. afer* rather than water temperature.

Literature states that *P. afer* populations are limited to headwaters of only three rivers in the Cape Fold Ecoregion of South Africa (Chakona & Skelton, 2017). Thus not only is this species highly endemic but also deserving of its IUCN Red List status of endangered (Swartz & Impson, 2007). Results of this study contribute to a better understanding of the thermal regime of reaches of the Blindekloof stream, one of the stronghold distributions of *P. afer*. Furthermore, the results of this study also shed light on the thermal tolerance and preference of this species and how these may be influenced by acclimation temperature. Lastly, the results of this study document the ability of this species to recover after heavy impact from non-native fishes. This was done by assessing their distribution and relative abundance in the Blindekloof stream over time.

The results of the thermal data collected from the Blindekloof stream showed that the stream is complex, with both a seasonal and diel effect on water temperature. It was also found that the water temperatures (mean, minimums, maximums, ranges, mean_7 and max_7) differed significantly between sites. It is expected that water temperature of ecosystems vary at the catchment scale but the results of this study are indicative of significant differences on a scale smaller than 4 km.

The results of the thermal experiments demonstrated that seasonality had a significant influence on both thermal tolerance and thermal preference. This may be attributable to the fact that, as literature suggests, the thermal history and acclimation temperature significantly impact the thermal preference and tolerance of a species (Jobling, 1981; Beitinger & Bennett, 2000; Golovanov, 2006; Bernatzeder & Britz, 2007; Barrantes *et al.*, 2017). When comparing the observed distribution of *P. afer* between the control and experimental tanks it was found that the distributions differ significantly, therefore lending itself to the conclusion that a species will move to favourable temperatures as expected (Bernatzeder & Britz, 2007). *Pseudobarbus afer* were found to aggregate at temperatures ranging from the lowest winter median of 15.0 °C to the maximum summer median of 29.3 °C. This puts this species in the range of either a cool- (winter results) or warm- (summer results) species according to Heino *et al.* (2009).

While it is accepted that factors such as fitness, life stage and size may influence the thermal tolerance of a species (Becker & Genoway, 1979; Dallas, 2008), it was found that there was no significant correlation between the size of *P. afer* individuals and their thermal tolerance. The investigation of the species' thermal tolerance and preference allow one to better understand their thermal sensitivity, which in turn aids the knowledge of the probable effects of changes in water temperature on the species (Dallas & Ketley, 2011).

Of interest to note is that, in summer there was a confounding effect of "shade" on the distribution of *P. afer* in the control gutters and hence an observable "end-effect". To uncouple the potential of an end-effect, further experiments would be required. Excluding the possibility of an end-effect, the observations in the experimental tanks suggest that favourable temperature is a stronger driving factor on fish distributions than other favourable conditions such as that of "habitat". This is supported in literature by the claim that temperature is the overriding driving factor behind fish distributions in freshwater ecosystems (for example see Beitinger *et al.*, 1999; Jackson *et al.*, 2001; Barrantes *et al.*, 2017). With this knowledge in mind, as well as data on the thermal regime of the monitored stretch of the Blindekloof stream, it was possible to investigate the longitudinal distribution of fishes in the Blindekloof stream in order to try to untangle whether temperature was likely to be limiting both native and non-native fish distributions in the Blindekloof stream.

Given that temperature is a crucial abiotic factor in freshwater ecosystems, driving ecosystem functions and their biotic assemblages, the effect of water temperature on fish distribution was investigated in this study. Using the knowledge of the thermal regime of the monitored stretch of the Blindekloof stream, literature was reviewed on the thermal tolerance and preferences of non-native species which have previously been documented in the Blindekloof stream. This understanding, along with accompanying distribution data from snorkel surveys, allowed for interpreting whether invasions have been limited due to temperature. Figure 5.1 shows the mean temperature of the four sites where temperature loggers were retrieved from the Blindekloof stream, along with the thermal tolerance of various species. The Figure highlights that the thermal tolerance, cited from Jobling (1981), of *Micropterus* species is considerably higher than the mean stream temperature and therefore that the upstream invasion of non-native *Micropterus* species is not likely to be attributable to stream temperature.

Similarly, when the thermal tolerance of *P. afer* is superimposed on the mean stream temperature over the four sites (Figure 5.1), one can conclude it is unlikely that temperature was limiting the downstream distribution of this species, as the thermal tolerance of *P. afer* is similar to that of *Micropterus* species. The absence of *P. afer* in the presence of *Micropterus* species as documented by Ellender *et al.* (2011) may therefore be attributed to the predation pressure and therefore, ultimately, the downstream distribution of *P. afer* may be a response to non-native fish presence.

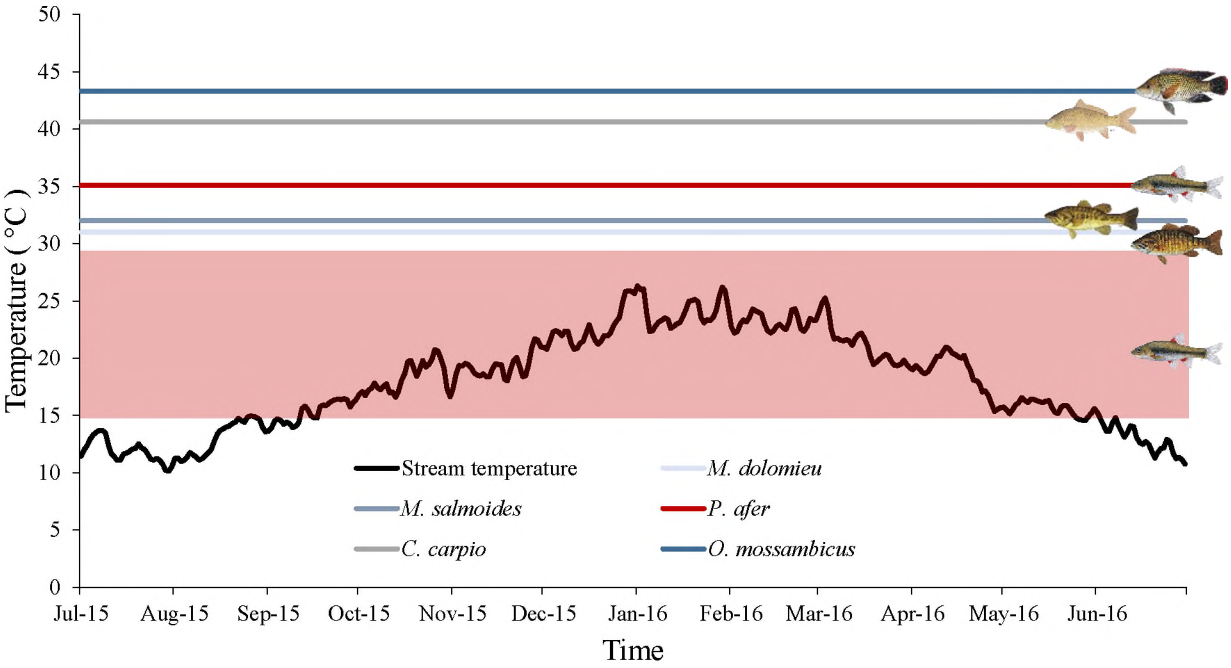

Figure 5.1 Blindekloof stream mean temperature as recorded for the four sites over a year (04 July 2015 - 03 July 2016) in relation to the thermal preference range of *Pseudobarbus afer* as investigated in summer and winter (highlighted in pink) and the CT$_{max}$ of *Micropterus* species (Jobling, 1981), the CT$_{max}$ of *Oreochromis mossambicus* (Zaragoza *et al*., 2008) and the mean CT$_{max}$ of *Pseudobarbus afer* as observed in summer (illustrations ©NRF SAIAB, illustrated by Dave Voorvelt).

It is important to remember that *P. afer* are already limited in distribution and therefore in order to effectively conserve this species, it is imperative that the distribution of both native and non-native fishes in the streams they inhabit are continually monitored. This information will aid an understanding of the threat posed by non-native fish invasions and allow for proactive interventions if necessary.

In conclusion, the recorded stream temperature of the Blindekloof stream is encompassing of the thermal preference of *P. afer*, and the investigated tolerance of the species is above the maximum temperatures recorded in the stream. It is therefore likely that the limiting factor to *P. afer* distribution is the predation of non-native fishes. Further support of this is that, in the absence of non-native species, *P. afer* are able to establish populations in the previously invaded reaches. These invasions therefore pose ominous threats and it is of utmost importance that these invasions are monitored. This is especially true in this system where the threat is ever present due to mainstem source populations and the lack of barriers preventing headwater invasions.

RECOMMENDATIONS FOR FUTURE RESEARCH

Temperature loggers have been replaced at Site 1, Site 4 and Site 8. This will enable further, more comprehensive descriptions of the thermal regime of the Blindekloof stream over time. This will allow one to continue to monitor the annual thermal regime of the Blindekloof stream. With the knowledge that *P. afer* is limited in distribution to headwaters of only three rivers, future studies could focus on describing the thermal regime of all systems. Such studies would provide an understanding of the various habitats this species inhabits in order to investigate whether the different populations inhabit streams which display a significant difference in temperature regime.

Having successfully used the methods described in Chapter 3 (Dallas *et al.* 2015) to investigate the thermal tolerance and preference of *P. afer* from the Blindekloof stream, future studies could investigate whether thermal history significantly influences the tolerance and preference of *P. afer*. By investigating the thermal tolerance and preference of *P. afer* individuals from different streams (which may display different thermal regimes) individuals may have different thermal histories and one can test whether thermal history significantly influences thermal preference and tolerance. Furthermore, future studies could aim to investigate the thermal tolerance and preference of other native fishes such as *Enteromius pallidus* A. Smith, 1841 and *Glossogobius callidus* (Smith, 1937).

Additionally, as the methods employed successfully investigated the thermal tolerance and preference of sub-adult *P. afer* individuals, future research on other life stages (both egg and juvenile) would provide valuable insight to the thermal tolerance of the species as a whole and thus improve the understanding of the vulnerability of the species to predicted warming due to global climate change. Similarly, as water temperature is also known to influence important functions such as fecundity and spawning (Vanote & Sweeney, 1980; Beitinger *et al.*, 1999; Dallas and Ketley, 2011), future studies should aim to investigate the maximum temperatures at which a species can reproduce. That is, although this study investigated the CT_{max} of sub-adult *P. afer*, this provides no insight on whether the species would be able to reproduce at these elevated temperatures. Such studies would provide insight into not only whether a species is able to survive these temperatures but rather about whether populations would persist in elevated water temperatures over time.

Although studies have investigated the thermal tolerance and preference of many of the non-native fishes which are known to occur in the Swartkops River (for example, Black, 1953; Cherry *et al.*, 1977; and others highlighted in the review by Jobling, 1981), none have been conducted on these species in South Africa. As thermal history is known to influence thermal tolerance and preference of a species (Beitinger & Bennett, 2000; Golovanov, 2006; Barrantes *et al.*, 2017) future studies may aim to investigate the thermal tolerance and preference of non-native fishes acclimatized within systems such as the Swartkops, to better understand their potential spread, establishment and thus current invasion debt.

While CT_{max} of *P. afer* was investigated in this study because climate change predictions suggest the CFE is likely to become warmer and drier (Dallas & Rivers-Moore, 2014), studies which investigate the Critical Thermal Minimum may provide valuable insight on the range of temperatures *P. afer* are able to withstand. Similarly, CT_{min} temperatures could also be conducted on other native fish species, particularly those which inhabit cooler streams.

Furthermore, Cambray (1994a; 1994b) observed that *P. afer* spawn after increased water flow and are known to spawn in summer months (Cambray 1994a). However, Cambray (1994a) conducted his study on *P. afer* populations from the Gamtoos River, which have subsequently been re-described as a genetically distinct species (Chakona & Skelton, 2017). Future studies could therefore aim to investigate whether *P. afer* do spawn in response to increased flow. Such information would provide valuable insight into the environmental cues on which *P. afer* rely for important physiological processes and how projected climate change predictions, such as reduced flow, may influence this species.

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