IMPACT OF *ONCORHYNCHUS MYKISS*, *SALMO TRUTTA* AND *CLARIAS GARIEPINUS* ON AQUATIC COMMUNITIES WITHIN MAGOEBASKLOOF AREA, LIMPOPO PROVINCE, SOUTH AFRICA

by

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DEDICATION

“To Kutshembha Wanga Hlungwani, my hope”

DECLARATION

I declare that **IMPACT OF *ONCORHYNCHUS MYKISS*, *SALMO TRUTTA* AND *CLARIAS GARIEPINUS* ON AQUATIC COMMUNITIES WITHIN MAGOEBASKLOOF AREA, LIMPOPO PROVINCE, SOUTH AFRICA** is my own work and that all the sources that I have used have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

_____________________________ __________________

Hlungwani Hlulani Archiebold (Mr) Date

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ABSTRACT

Fish assemblages in relation to environmental variables within the Broederstroom and Debengeni Rivers were investigated. Both rivers were characterized by coarse substrates (pebble and gravel), temperatures below 20°C and moderate depth. Trout dominated fish assemblages in terms of numbers caught and was only distributed at higher altitude sites >1400 m (a.s.l). Coarse substrates, temperatures below 15°C, flow rate, depth and riparian cover were the variables shown important for the distribution of trout by multivariate analysis. The confinement of the trout to higher altitude and lack of optimal habitat variables at lower altitude sites contributed to the assertion that the area is marginal for trout distribution. It was therefore inferred that the marginality of the area is the possible explanation for trout failure to self-sustain its population, therefore dependent on the continuous restocks by the local hatchery.

The continuous restocks of small size trout in the Broederstroom River prompted a subsequent study where trout's impact on macroinvertebrate communities was evaluated through surveys and field experiments. The ability of small trout to utilize macroinvertebrates made them suitable candidates to evaluating their impact in the area. Aquatic invertebrates were found to be the main food source for the trout in the area. Taxa such as Gomphidae and Potamonautidae were the most frequent food items from the analyzed stomachs of trout. However, observations from both field surveys and experiments showed that trout is a weak regulator of macroinvertebrate diversity in the area, since there were no significant differences (ANOVA, $P<0.05$) in the diversity of invertebrates from trout invaded and uninvaded sites.

Trout being a weak regulator of macroinvertebrate diversity in the area, it prompted surveys to the Ebenezer Dam to determine its competitive interactions with native predatory species. If the introduced species is a more efficient predator than the native predator species, it may affect changes in the structure of the habitat and food resource. Trout in the Ebenezer Dam was found to be selective to habitat variables whilst *C. gariepinus* was cosmopolitan to all habitat categories. The catfish also had a broader food preference than trout and the diversity of the food items was significantly different (ANOVA, $P<0.05$) between the two species. Unfortunately, the interspecific food overlap between trout and the catfish could not be determined in Ebenezer Dam, because of the small sample size of trout but food selection between

them was evident. It was then concluded that the native catfish has a wider niche and it is a more efficient predator than the introduced trout. This observation contributed further to the assertion that the area is marginal for trout to thrive.

Due to trout selection of habitat variables, it became prudent to carry out another study where the past climate and land use changes were analyzed to determine their effect on the habitat that could have affected the distribution of trout in the area. Future projections were also made to determine possible future impacts of climate change on the distribution of trout in the area. The effects of climate and land use change resulted in warmer water temperature, altered riparian cover and altered stream flow patterns. The changes could have influenced the confinement of trout to higher altitude catchments. The projected maximum temperatures by 2050 shows an increase from 2014 with a decline in precipitation. If these projections are to be the same for water temperature and flow regimes, coupled with current land uses in the area, they will continue to affect the distribution of trout negatively.

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CHAPTER 1:

GENERAL INTRODUCTION

1.1 INTRODUCTION

The understanding of factors that control the structure of biological communities has long been a prominent focus in freshwater ecosystems. Historically, biological communities were seen to be influenced by only habitat variables (Gaigher, 1973; Gornman & Karr, 1978; Vila-Gispert *et al.*, 2002; Kadye *et al*., 2008). However, recent studies have shown that the introduction of non-native fish species also influence biological communities (Marr *et al*., 2012; Kadye *et al*., 2013; Shelton *et al*., 2014; Shelton *et al*., 2015). Non-native fish introductions have resulted in numerous establishment of invasive fishes globally (Figure 1.1), contributing to shaping the structure of biological communities through a combination of predation and competition (Dudgeon *et al*., 2006; Leprieur *et al*., 2008; Gozlan, 2008). The introductions are higher in freshwater ecosystems in developing countries as the introductions continue without environmental monitoring studies (Crowl *et al*., 1992; Lintermans, 2000; McDowall, 2006; Vitule *et al*., 2009; Habit *et al*., 2010). The impacts associated with these introductions have led to many developed countries to give it serious attention, but their impacts in countries like South Africa remain largely unknown (Cambray, 2003; Ellender & Weyl, 2014; Shelton *et al*., 2015).

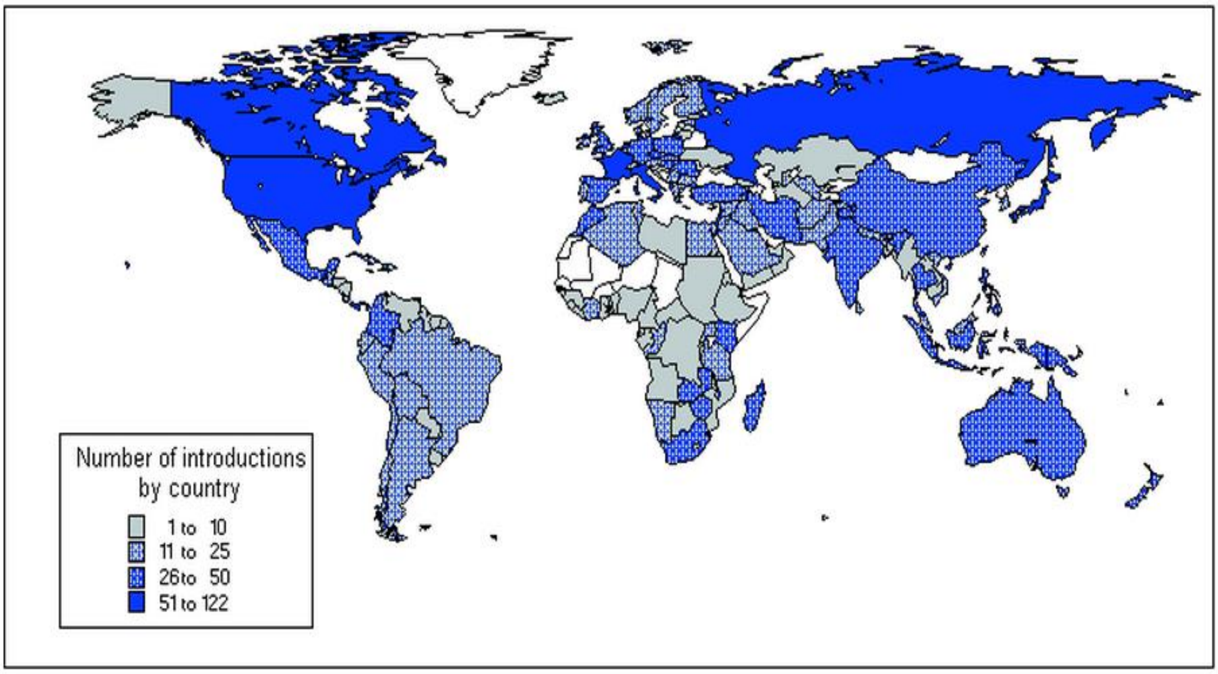

Figure 1.1: Worldwide distribution of non-native freshwater fish (Leprieur *et al.,* 2008).

In South Africa, 41 fish species have been introduced into local freshwater systems. Sixteen of these fishes are introduced from outside southern Africa and are referred to as non-native, whereas 25 were translocated, having been introduced from elsewhere within southern Africa (de Moor & Bruton, 1998; Ellender & Weyl, 2014). Due to the number of non-natives that have established populations in South Africa, it is considered to be one of fish invasion hotspots (Leprieur *et al.,* 2008). As a result, the establishment of non-native species such as bass and trout have been reported to be a threat to fish and macroinvertebrate biodiversity in many rivers and impoundments (de Moor & Bruton, 1988). For example, in the Western Cape Province of South Africa, bass and trout threaten many range-restricted and genetically distinct populations of minnows such as the redfins *Pseudobarbus* spp. and other endemic species shown in Table 1.1 (Swartz *et al.*, 2004; Tweddle *et al.,* 2009; Darwall *et al.,* 2009; Marr *et al*., 2012; Shelton *et al.*, 2015).

Table 1.1: Summary of some endangered fish species threatened by the presence of trout and bass in Western Cape Province, South Africa (Darwall *et al.*, 2009)

Species	Conservation status
Pseudobarbus afer (Peters, 1864)	Endangered
Pseudobarbus tenuis (Barnard, 1938)	Near threatened
Pseudobarbus asper (Boulenger, 1911)	Endangered
Pseudobarbus burchelli (Smith, 1841)	Critically endangered
Pseudobarbus burgi (Boulenger, 1911)	Endangered
Pseudobarbus phlegethon (Barnard, 1938)	Endangered
Sandelia capensis (Cuvier, 1831)	Data deficient
Galaxias zebratus (Castelnau, 1861)	Data deficient
Barbus andrewi (Barnard, 1937)	Endangered
Barbus serra (Peters, 1864)	Endangered
Barbus calidus (Barnard, 1938)	Vulnerable
Barbus erubescens (Skelton, 1974)	Critically endangered
Labeobarbus capensis (A. Smith, 1841)	Vulnerable
Labeo seeberi (Gilchrist & Thompson, 1911)	Endangered
Austroglanis barnardi (Skelton, 1981)	Endangered
Austroglanis gilli (Barnard, 1943)	Vulnerable

Trout is widely introduced globally due to its popularity as an angling fish, with only the common carp (*Cyprinus carpio*) and Nile perch (*Lates niloticus*) being more widely introduced (Welcomme, 1988). Outside its native range, trout have been introduced to at least 97 countries, and to every continent except Antarctica (Leprieur *et al.,* 2008).

1.1.1 Trout impacts in South Africa

It has long been suspected that the impacts of trout in South Africa are predominantly negative based on international literature (Cambray, 2003b; Leprieur *et al.,* 2008). However, little work have been done to quantitative assessments of trout impacts on native biota in southern Africa (Woodford & Impson, 2004; Karssing *et al.,* 2012; Kadye *et al.*, 2013; Shelton *et al*., 2015). Considering that many species of South African native fish and macroinvertebrates have evolved in the absence of predatory fish such as trout, it seems likely that they will be vulnerable to predation by the introduced trout. It is therefore prudent to investigate the impacts of trout on a local scale.

In response to the threat posed by trout, most countries have implemented legislation prohibiting new introductions and some have developed adaptive management strategies to identify and reduce the impact of the trout (Shelton *et al.*, 2014). In South Africa, invasive species such as trout are regulated through the alien and invasive species regulations of the National Environmental Management: Biodiversity Act (NEM: BA; RSA 2004). Both trout species, *Oncorhynchus mykiss* and *Salmo trutta,* are currently listed in section 71(3) category 2, which states that a permit must be obtained for activities involving live trout. These activities include:

- That trout may be farmed or stocked in protected areas for freshwater aquaculture facilities, and may only be stocked in rivers, lakes and wetlands, but only with a permit
- Trout may be kept in dams without a permit, provided this is not in a protected area; is not an aquaculture facility; and trout already legally occur in the catchment.
- Fly-fishers and others may catch and release trout in the same waters (including in protected areas) without a permit.

- Most importantly, trout may not be introduced into a discrete catchment system in which they do not occur.

A legal argument in South Africa has been put forward by the anti-regulation lobby that trout should not be listed as an invasive species because the current act considers invasive species to either be a cause of economic harm or a cause of harm to human health (Shelton *et al*., 2015. The implications of such a law is that indigenous aquatic fauna could then be decimated or driven to extinction and the law should not intervene, unless the economy or human wellbeing has been proven to be negatively affected (Shelton *et al*., 2014).

Formal scientific studies, however, see no merit in the legal argument presented by the anti-regulation lobby (Shelton *et al.*, 2015). Trout have self-sustaining populations in many waters and they are predating on the local aquatic fauna. Formal studies that have been done in South Africa (Simberloff & Von Holle, 1999; Swartz *et al.*, 2004; Tweddle *et al*., 2009; Strayer, 2010; Ellender & Weyl, 2014; Shelton *et al*., 2014; Shelton *et al.*, 2015) also have indicated that trout not only compete for limited resources with indigenous species but also predates on local species and macroinvertebrates (Shelton *et al*., 2014). No study in South Africa has shown the opposite, therefore, this together with international literature confirms that trout should be classified as an invasive species (Raleigh *et al*., 1984; Kawaguchi & Nakano, 2001; Dudgeon *et al.,* 2006; Baxter *et al.*, 2007; Leprieur *et al.,* 2008; Ricciardi & MacIsaac, 2011).

1.1.2 Introduction of trout in South Africa

In the past, British colonists in the Cape and the then Natal Province realized that in the absence of suitable indigenous angling species, many mountain streams would be suitable for the introduction of trout (de Moor & Bruton, 1988). The first reported attempt to import ova was in 1875, but it was in 1890 that brown trout ova were successfully imported to Natal from Scotland. After hatching, the young trout were stocked into the headwaters of the Mooi, Bushmans and Umgeni rivers. In 1892, brown trout ova were also imported successfully into the Cape where they were hatched in some brewery ponds in Newlands, Cape Town (McCafferty *et al*., 2012).

These efforts prompted the building of hatcheries in Jonkershoek and at Pirie near King Williams Town in the Eastern Cape. By 1897, locally bred brown trout were being produced and the first batches of rainbow trout ova were imported (Cambray, 2003a). Widespread stocking of suitable rivers followed from these and other importations. Until the 1980s, both trout species were bred mainly by nature conservation authorities for sport-fishing purposes. However, it remains unclear as to which populations are established or restocked by angling societies and hatcheries. For example, in the Berg River in the Western Cape, despite repeated stocking, there have been no reported catches since 1986 (Clark & Ratcliffe, 2007).

In the early 1960s, conservation organizations gradually became aware that trout appeared to be having a negative impact on native aquatic species in southern Africa (de Moor & Bruton, 1988). This led to policy changes in the 1980s, and the focus of conservation organizations halted the production and promotion of non-native fish species and shifted efforts towards the conservation of threatened native aquatic species (de Moor & Bruton, 1988). In addition to the concerns over the direct effects of trout on the native fish (Ellender & Weyl, 2014), there has been mounting concern that trout may also affect other components such as macroinvertebrates (de Moor & Bruton, 1988; Cambray, 2003b, Shelton *et al*., 2015).

Trout is a species of significant economic value in South Africa (Bainbridge *et al.,* 2005). They are farmed commercially for food production and recreationally as an angling species. Commercially, trout are farmed and sold locally and exported as a high value food source (Bainbridge *et al.,* 2005). The recreational trout industry sustains a considerable industry of aquaculture, tourist operators, professional guides and accommodation establishments (Bainbridge *et al.,* 2005; du Preez & Hosking, 2010), and is an important source of income and job creation in South Africa (du Preez & Hosking, 2010). In Limpopo Province, the Magoebaskloof area is the only area where trout occurs. Due to its popularity as an angling fish, a hatchery and an angling association known as the Haenertsburg Trout Association (HTA) has also been formed (DWA, 2013).

1.1.3 Interactions between trout and native species

The catfish is probably the most widely distributed fish in Africa (Skelton, 2001). It has been widely introduced in many parts of the world through aquaculture practices and is being cultivated in fish farms throughout Europe, Asia and South America (Lal *et al*., 2003; Vitule *et al*., 2006; Bhakta & Bandyopadhyay, 2007; Radhakrishnan *et al*., 2011). In these regions, it has successfully established within freshwater ecosystems as escapees from aquaculture facilities and through deliberate angler introductions (Cambray, 2005; Vitule *et al*., 2009).

The catfish's ecological impacts are numerous, as the fish has generalist feeding habits and an active predator (Kadye & Booth, 2012). It is likely to have a negative impact on macroinvertebrates and fishes wherever present (Skelton, 2001; Kadye & Booth, 2012). It is also likely to directly compete for resources used by indigenous and non-native species. Trout is very sensitive to changes in water quality whereas the catfish is more tolerant. It is therefore important to investigate and understand the key seasonal water quality changes that affect the distribution and interactions between these species.

1.1.4 Impact of climate change on the distribution of fish species

Abiotic factors coupled with the global climate change are affecting biological communities (FAO, 2009; Jones *et al*., 2013). Studies have projected an expansion of species range for some species and the possible extinction of others due to climate change (Jones *et al.*, 2013). Such range shifts raise concern for species whose long-term persistence are already threatened by other human disturbances such as introduction of predatory species. Studies have attempted to assess the effects of future climate changes on aquatic communities using a multi-model approaches (Field *et al*., 2007; Santiago *et al*., 2015). For this reason, little is known in South Africa, especially with regard to the effects of climate change on introduced cold water fish species in marginal areas such as Magoebaskloof.

1.1.5 Impacts of land-use changes on aquatic biodiversity

Changes in land use are the direct and indirect consequence of human actions to secure essential resources (Beeson & Doyle, 1995). These actions includes the burning of areas to enhance the availability of land for agriculture, resulting in the extensive clearing (deforestation) and management of earth's terrestrial surface (Hudak & Wessman, 1998). Industrialization has encouraged the concentration of human populations within urban areas (urbanization) and the depopulation of rural areas, accompanied by the intensification of agriculture in the most productive lands and the abandonment of marginal lands (Maktav *et al*., 2005). Diversity of aquatic life is often reduced dramatically by changes in land use, when land is transformed from a primary forest to a farm. The loss of forest species and detoriation of water quality due to increased runoff within deforested areas are immediate (Hudak & Wessman, 1998; Maktav *et al*., 2005).

1.1.6 Rationale and objectives of the present study

Magoebaskloof is a mountainous area, ranging from approximately 800 m to 2160 m above sea level (a.s.l). The forest in the area has small perennial streams that flow down the mountains in branches (DWA, 2013). High rainfall and deep soils make it ideal for the planting of timber with a canopy height between 15 and 30 m which is currently the major land use. The climate is mild and the prevailing climate conditions include frequent mist, occasional wind and hail in the mountains (DWA, 2013). The area is an ecological sensitive area, prone to soil erosion due to the nature of steep river embankments. As a result, high turbidity levels during the rainy season are experienced (DWA, 2013).

Considering the topography of the Magoebaskloof area, the streams provide different habitats for fish. Therefore, it is important to determine the habitat preferences of the fishes in the area as Kadye and Moyo (2008) showed that fish species are consistently associated with their habitat types. This nature of investigation is appropriate since no comparative study on fish species and habitat association has been carried out in Magoebaskloof area. Other studies elsewhere suggest that associations can also be a result of factors such as predation and morphological adaptations of fish to the physical conditions of the rivers (Gaigher, 1973; Vadas & Orth, 2000; Bond & Lake, 2003). With the presence of trout in the area, it is important to identify and determine if trout is not one of the causal factors underlying the distribution patterns of the fish in the area.

This study is one of the first aimed at examining whether trout has self-sustaining populations in Magoebaskloof area since no historical published data exists. Studies have shown internationally and locally that trout poses a threat to the survival of the indigenous freshwater fauna (Rinne & Alexander, 1995; McDowall, 2003; Cambray, 2003b; McDowall & Allibone, 2004; Kadye *et al*., 2013, Shelton *et al*., 2015). Considering the marginality of Magoebaskloof area, there is also a need to investigate whether trout presence has negative impacts as in other systems where it thrives (Bradford *et al.,* 1998; Suding *et al*., 2004; Meissner & Moutka, 2006; Karssing *et al.*, 2012).

The outcome will aid environmental managers to come up with meaningful decisions as Marr *et al*. (2012) shown that there is a need of studies on environmental monitoring in South Africa, as they will help establish methods to eradicate non-native fish from critical biodiversity areas. The findings of the present study will therefore, add to the establishment of methods to the control of trout in South Africa by giving information on whether trout has established or is solely sustained through restocking in the area. Such knowledge is essential for preventing new introductions and curbing the further spread of established non-native fishes as stated by the National Environmental Management: Biodiversity Act (NEM: BA) regulations.

1.2 DISSERTATION LAYOUT

The impact of *Oncorhynchus mykiss*, *Salmo trutta* and *Clarias gariepinus* on aquatic communities within Magoebaskloof area, Limpopo Province, South Africa was assessed. There are seven chapters in this dissertation. Chapter 1 identifies the research problem and gives an outline of previous attempts to solve the problem.

Chapter 2

Relevant literature on fish distributions in relation to environmental variables, impacts of trout on fish and macroinvertebrates, interactions of trout and other fish, impacts of climate and land use changes are reviewed.

Chapter 3

This chapter focuses on fish assemblages in relation to environmental variables, growth of trout and habitat preferences of trout within the Broederstroom and Debengeni Rivers.

Chapter 4

The effect of trout on native fish and macroinvertebrate diversity in the Magoebaskloof area were assessed in this chapter.

Chapter 5

This chapter focuses on the possible interactions between the trout and the native African sharptooth catfish.

Chapter 6

Chapter 6 focuses on predicting the potential impacts of climate and land use changes on the introduced trout in the Magoebaskloof area.

Chapter 7

This chapter provides specific recommendations that could be implemented to curb further introductions of trout, as well as recommendations for further research to be conducted on the subject.

CHAPTER 2:

LITERATURE REVIEW

2.1 An overview of non-native fish in freshwater ecosystems

The global spread of non-native fish for angling and aquaculture has led to a large number of invasions in freshwater systems (Rahel, 2002; Dudgeon *et al.,* 2006; Leprieur *et al.,* 2008; Ricciardi & MacIsaac, 2011). Species such as trout have invaded and established self-sustaining populations in many streams globally (Leprieur *et al.,* 2008), with the consequences of their introduction shown to extend beyond the boundaries of aquatic ecosystems. For example, trout-induced changes in aquatic invertebrate abundance and the corresponding flux of invertebrates from aquatic to terrestrial systems has been shown to influence the abundance of riparian consumers in streams (Nakano *et al.,* 1999; Baxter *et al.*, 2004; Baxter *et al.*, 2007).

In South Africa, trout has established populations in the Drakensberg Escarpment and in the Cape Floristic Region of the Western Cape Province. Numerous resorts and fly fishing ventures have evolved into a huge business in areas like the KwaZulu-Natal where dams and rivers are stocked with trout by the Natal Trout Association (Picker & Griffiths, 2011). Limpopo Province is characterized by warm temperatures, with an annual average of 28°C. The fish species in the province are dominated by warm water species such as tilapia and catfish. However, trout has been introduced and is restocked annually for angling by the Haenertsburg Trout Association (HTA). At present, there is no published data on the presence and impacts associated with the trout in the area. This motivated the conception of this study to be the first to aim on defining the establishment state and ecological impacts of the trout in the area.

2.2 Species profiles

2.2.1 *Salmo trutta*

The family salmonidae includes trout, salmon and charr (Skelton, 2001). Salmonidae have been introduced to southern Africa in the past, but only trout have survived and established populations in some high altitude rivers. *Salmo trutta,* commonly known as Brown trout, derives from the Latin word “*trutta*”, meaning "trout". Behkne (2007) noted that the brown trout was the first species of trout described in the “1758 edition of *Systema Naturae*” by Swedish zoologist Carl Linnaeus. Brown trout is classified as follows:

Order: Salmoniformes

Family: Salmonidae

Genus: *Salmo*

Species: *Salmo trutta* (Linnaeus 1758)

Distribution and biology of brown trout

Brown trout is a cold water tolerant fish that naturally occurs in Europe and western Asia. It has been introduced to almost all continents including Africa (Figure 2.1).

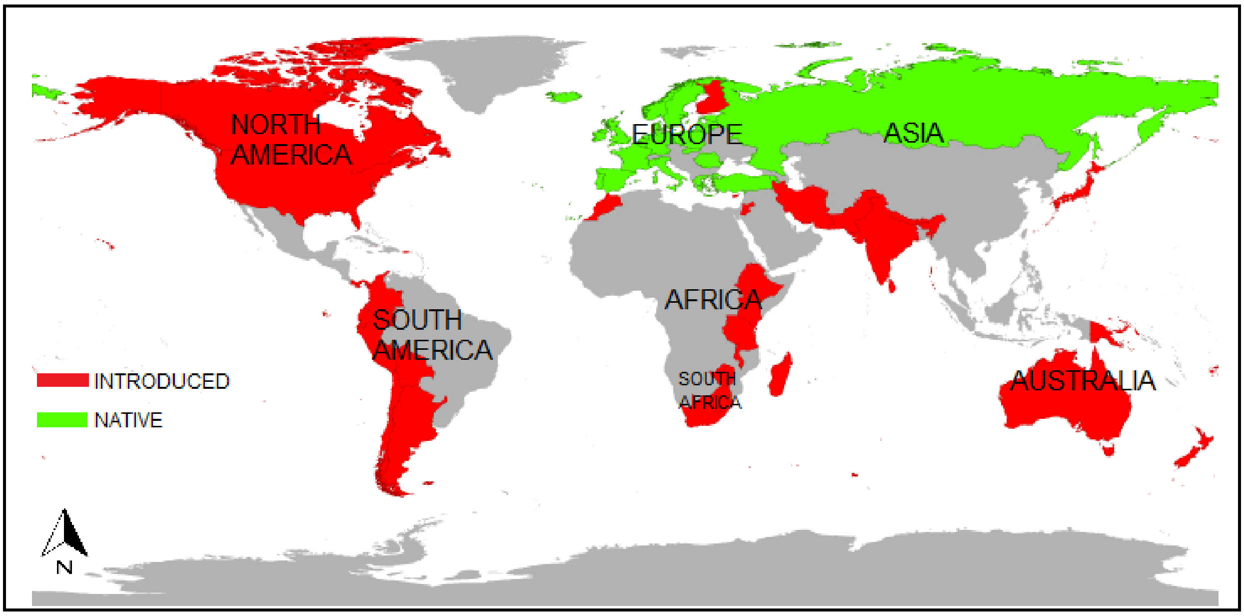

Figure 2.1: Native (green) and introduced (red) ranges of brown trout globally (GISD, 2012).

Brown trout has a fusiform body shape (a streamlined body plan often characteristic of fast-moving fish) which is silver to brown, with small scales and covered with large red spots (Figure 2.2). In South African dams, this species can grow up to 75 cm in length and over 6 kg in mass, whereas in rivers, specimens are found to be smaller (Picker & Griffiths, 2011). Magoebaskloof area in Limpopo Province is regarded a marginal area for brown trout, and the growth performance of this species is unknown in this area.

Figure 2.2: *Salmo trutta* (Skelton, 2001).

In its juvenile stage, brown trout feeds on plankton, whereas adults feed on aquatic and terrestrial invertebrates, small fish and frogs. It feeds at all levels in the water column. Under optimal conditions, brown trout can obtain lengths of 18 cm after one year and 25 cm after two years (Skelton, 2001). Brown trout males become sexually mature between one and three years of age and females between two and four years (Vandeputte, 2008). Their predatory feeding habits qualifies them to be invasive species that can lead to negative impacts on the ecosystems that they are introduced into.

In the wild, spawning takes place in gravel beds in the rivers or streams in areas of high altitude. A shallow depression known as a redd is created in the gravel prior to spawning. Females are capable of producing between 1,600 and 1,900 eggs/kg body weight (Alp *et al.,* 2010), therefore the brood size is size dependent, but is on average 10,000 eggs (Froese & Pauly, 2011). Once spawning is complete, the eggs are covered with gravel and left unguarded (Freyhoff, 2011) until they hatch approximately 3 weeks later. Brown trout live in cool waters (below 21°C), but require water temperatures of less than 16°C for breeding (Skelton, 2001).

Populations of brown trout can be found in several mountain rivers in South Africa (Figure 2.3). However, Picker and Griffiths (2011) made no mention on the presence of trout in Magoebaskloof in Limpopo Province in their trout distribution study in South Africa. Preliminary observations of the present study confirmed the presence of this species at the Broederstroom River in Magoebaskloof area. It is not clear if the populations are self-sustaining or sustained through the restocking's by the HTA. Studies on physico-chemical variables and habitat preference are therefore necessary to determine the factors that affect their distributions and establishment in the area.

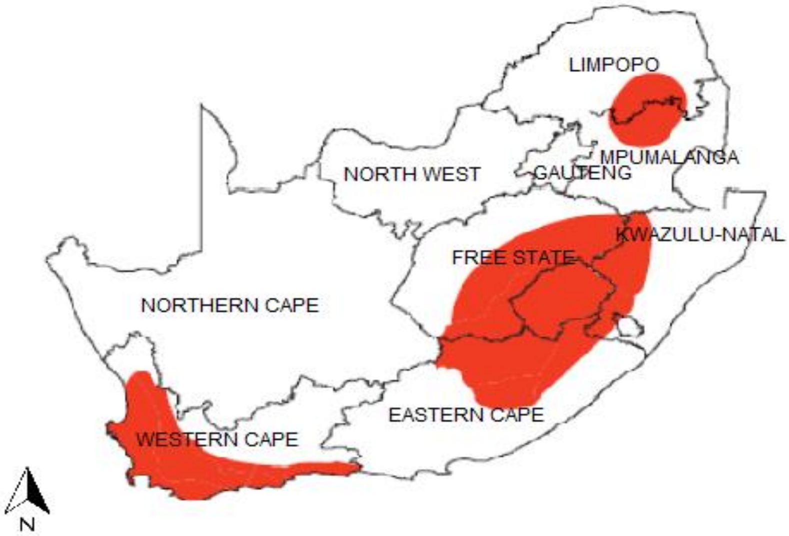

Figure 2.3: Map of range of *Salmo trutta* introduced within South Africa (Picker & Griffiths, 2011).

2.2.2 *Oncorhynchus mykiss*

Oncorhynchus mykiss, commonly known as rainbow trout was originally named by German naturalist and taxonomist Johann Julius Walbaum in 1792, based on the type of specimens from the Kamchatka Peninsula in Siberia. Walbaum's original species name, *mykiss*, was derived from the local Kamchatkan name used for the fish "mykizha". The name of the genus is from the Greek "*onkos*" which means hook and "*rynchos*" which means nose, in reference to the hooked jaws of males in the mating season. Until mid-1980's, the genus *Salmo* included both the brown and rainbow trout. In 1989, morphological and genetic studies indicated that trout of the Pacific basin were genetically closer to Pacific salmon, *Oncorhynchus* species than to the *Salmo*. Thus, rainbow trout was moved into the genus *Oncorhynchus*. Walbaum's name had precedence, so the species name *O. mykiss* became the scientific name of the rainbow trout. Rainbow trout is classified as follows:

Order: Salmoniformes

Family: Salmonidae

Genus: *Oncorhynchus*

Species: *Oncorhynchus mykiss* (Walbaum 1792)

Distribution and biology of rainbow trout

Rainbow trout is native to North America from Alaska to Mexico (Figure 2.4). It requires high oxygenated cold water. As a result, it is commonly found in fast flowing streams and dams situated at high altitudes (Picker & Griffiths, 2011). It has been widely introduced to almost all continents.

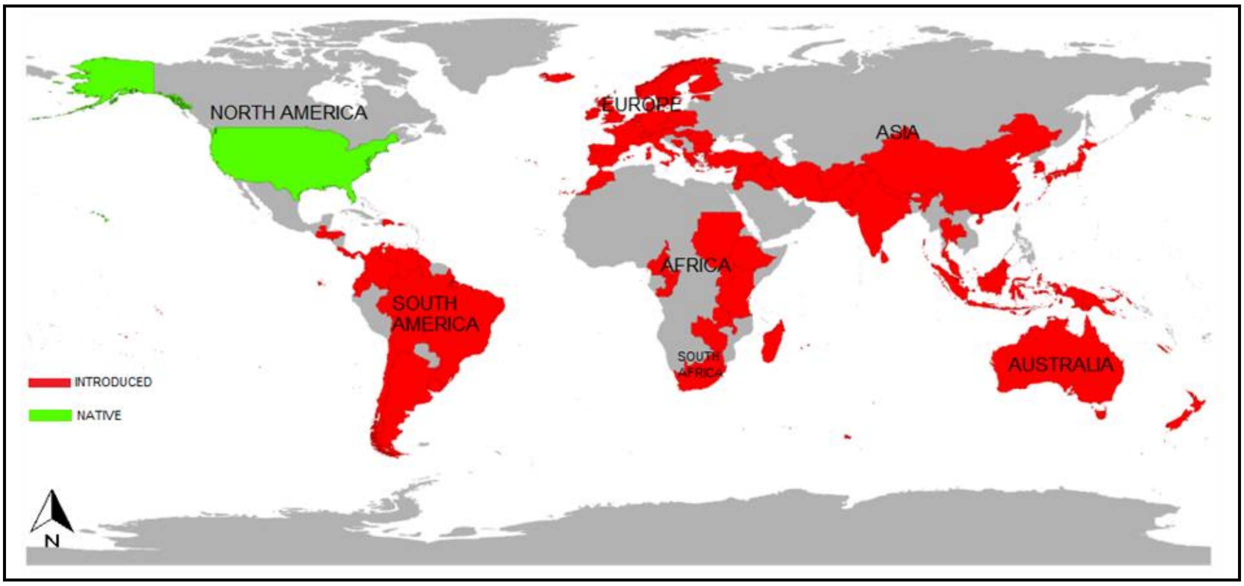

Figure 2.4: Native (green) and introduced (red) ranges of rainbow trout globally (GISD, 2012).

Rainbow trout has a fusiform body shape which is blue to olive green, with a pink coloration along the lateral line. In freshwater, these fish are yellow-green or brown with dark spots on the body, dorsal fin and tail. They have a pink or red band extending along the body (Figure 2.5). In salt water or brackish water, rainbow trout are silvery with the top half of the fish being darker and has dark spots above the lateral line. The fins and dorsal surface of the body is covered in small black spots (FAO, 2005-2012).

Figure 2.5: *Oncorhynchus mykiss* (Skelton, 2001).

Rainbow trout is an opportunistic feeder, predating on both terrestrial and aquatic invertebrates, small fish and fish eggs. In the wild, it feeds on freshwater shrimps, which contain carotenoid that are responsible for the fish's pink flesh. In aquaculture, this pink colour is produced artificially by feeding the fish synthetic pigments (FAO, 2005-2012). Under optimal conditions, rainbow trout in South Africa reportedly grow to 15 to 18 cm in the first year, reaching 26 cm after two years (Skelton, 2001). The growth parameters of rainbow trout in the rivers and dams in Magoebaskloof area are currently unknown.

Rainbow trout only spawn once a year in the wild, usually between June and September in South Africa. Spawning takes place in rocky or gravel habitats. The females are capable of producing 2000 eggs/kg body weight. Once spawning is complete, the eggs are left unguarded until they hatch approximately 4-7 weeks later (Skelton, 2001). Rainbow trout tolerate water temperatures of between 0 and 26°C. However, growth and reproduction are preferred at temperatures between 9 and 14°C (FAO, 2005-2012) while optimal spawning temperatures range from 10 and 13°C. Dissolved oxygen levels appear to be a major limiting factor for the distribution of rainbow trout, with 2.5 mg/l being the minimum tolerated concentration (Rowe & Chisnall, 1995).

There are a large number of self-sustaining populations of rainbow trout in high altitude river areas of South Africa (Figure 2.6). Formal scientific studies by de Moor and Bruton (1988) and Picker & Griffiths (2011) haven't reported the presence of rainbow trout in Magoebaskloof area. However, preliminary observations have confirmed the presence of this species in this area.

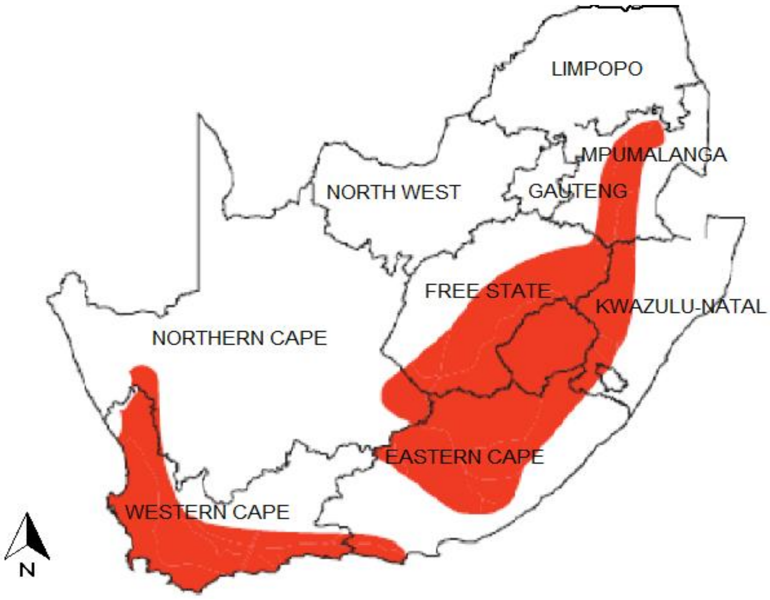

Figure 2.6: Map of range of *Oncorhynchus mykiss* introduced within South Africa (Picker & Giffiths, 2011).

2.2.3 *Clarias gariepinus*

Unlike non-native species that have been introduced into local systems, there are native species (such as catfish) that also predates on macroinvertebrates and smaller fish. The African sharptooth catfish is arguably the most latitudinally distributed freshwater fish that naturally occur from the Middle East to the Orange River in South Africa (Skelton, 2001). This species has been widely introduced in many parts of the world through aquaculture (Radhakrishnan *et al*., 2011). Although their impact in introduced areas is unknown, concern has been raised over its predation and competition impact on local biota (Khan & Panikkar, 2009; Kadye & Booth, 2012). In the Eastern Cape Province, South Africa, the catfish was translocated primarily through the Orange/Fish inter-basin water transfer (IBWT) scheme, which was completed in 1976 (Cambray & Jubb, 1977).

The African sharptooth catfish has since spread into several other river and impoundments through secondary IBWT schemes, angler introductions and to a lesser extent, aquaculture (Cambray, 2005). Translocated catfish from the Orange River system have established in many Eastern Cape rivers that include the Great Fish, Sundays, Kouga, Swartkops, Keiskamma, Buffalo, Nahoon and Mtata Rivers, and in many reservoirs (de Moor & Bruton, 1988; Kadye & Booth, 2012). The catfish is indigenous in Magoebaskloof area. However, its interaction with introduced fish in the area have not been recorded before.

The African sharptooth catfish is a catfish species of the family Clariidae, which are air-breathing catfishes. They have a scale-less, elongated body with long dorsal and anal fins. Their colour varies dorsally from dark to light brown and is often spotted with shades of olive and grey while the underside is a pale cream to white (Skelton, 2001). The genus *Clarias* was reassessed in the 1980s, which resulted in several widespread species being synonymized whereby *Clarias capensis* of southern Africa, *Clarias mossambicus* of central Africa and *Clarias lazera* of West and North Africa came to be known as *Clarias gariepinus* (Teugels, 1986).

The African sharptooth catfish is classified as follows:
Order: Siluriformes
Family: Clariidae
Genus: *Clarias*
Species: *Clarias gariepinus* (Burchell 1822)

Distribution and biology of the African sharptooth catfish

According to Skelton (2001), it is probably the most widely distributed fish in South Africa (Figure 2.7). Its natural distribution is as far south as the Orange River system in the west and the Umtamvuna River in the east of South Africa.

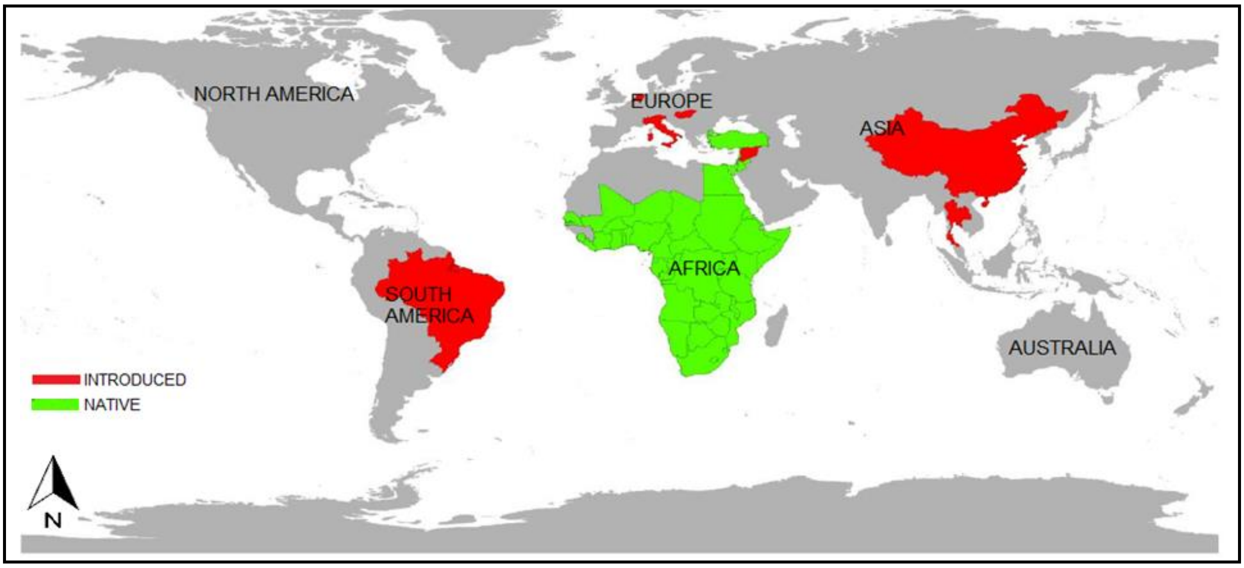

Figure 2.7: Native (green) and introduced (red) ranges of *Clarias gariepinus* globally (GISD, 2012).

The native range of *C. gariepinus* covers most of the African continent with the exception of Maghreb, Upper and Lower Guinea, and the Cape provinces of South Africa (Figure 2.7 and 2.8).

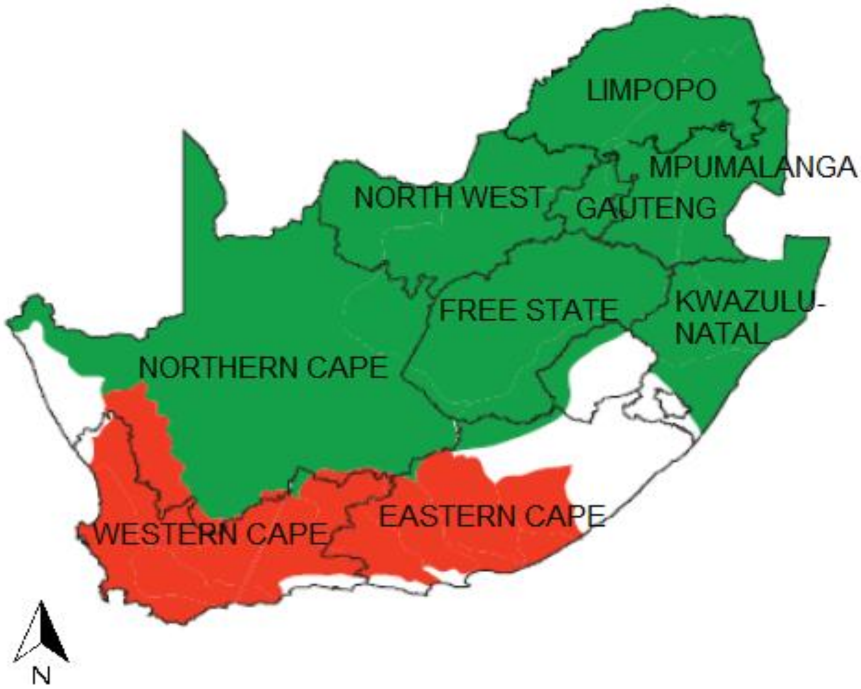

Figure 2.8: Map of native and introduced range of *Clarias gariepinus* within South Africa (Picker & Giffiths, 2011).

This species favours floodplains, slow flowing rivers, lakes and dams (Skelton, 2001). *Clarias gariepinus* tolerates high turbidity and low dissolved oxygen levels, and is often the last or only fish species found in remnant pools of drying rivers. *Clarias gariepinus* is considered to be omnivorous displaying both scavenging and predatory feeding behaviour. It is known to have a varied diet consuming fruits and seeds, all types of aquatic invertebrates and small vertebrates, small mammals and even plankton (Skelton, 2001). Older and larger individuals show a specific dietary shift towards smaller fish. It prefers inactive foods which it detects with its sensory barbells (Figure 2.9) before securing them with its fine teeth prior to gulping (Skelton, 2001).

Clarias gariepinus display rapid growth rate depending on conditions and habitat (Skelton, 1993). Individual specimen have been recorded to reach 200 mm standard length within a year (Skelton, 2001). In females, the growth rate decreases after 3 years resulting in the males reaching larger sizes (Skelton, 2001). Individuals of this species are known to live for eight or more years. Shoals of this species migrate upstream or to the shores of still water bodies prior to breeding (de Moor & Bruton, 1988). Spawning and egg laying takes place at night often after the spring first rains. Eggs usually adhere to recently submerged vegetation, either aquatic or terrestrial vegetation that has recently been submerged as a result of seasonal water level rise (Skelton, 2001). Hatching of the eggs occurs soon after spawning, usually after 24 to 36 hours. There is no parental care of the young.

Figure 2.9: *Clarias gariepinus* (Skelton, 2001).

Clarias gariepinus can endure harsh conditions as they are able to tolerate very low oxygen concentrations and even survive for considerable periods out of water, due to the use of a suprabranchial organ (Skelton, 2001). This organ is a large paired chamber with branches above the gill arches that is specifically adapted for air breathing (Maina & Maloiy, 1986). This organ allows the species to move over land at night in order to seek larger water bodies. Water temperatures between 8 and 35°C, salinities of 0 to 10‰ and a wide pH range are all tolerated. High growth rates are favoured at 30°C (Britz & Hecht, 1987). The ability of the fish to be able to tolerate these extreme conditions allows it to survive even in moist sand or in burrows with an air-water interface (Skelton, 1993). Although native to certain regions of South Africa, a recent study by Kadye and Booth (2012) shows the need to monitor the ecological impacts associated with this species.

2.3 Fish assemblages in relation to environmental variables

Little work on fish assemblages in relation to environmental variables has been undertaken in southern Africa (Kadye *et al*., 2008; Darwall *et al.,* 2009; Ellender & Weyl, 2014). The occurrence of fish species at specific locations can be influenced by habitat factors such as the nature of the substrate (Kadye & Magadza, 2008), depth and flow rate (Vadas & Orth, 2000; Kadye *et al*., 2008).

Due to the topography of the Magoebaskloof area, streams descend along an altitude gradient, thereby providing different habitats. It is seen important to determine the habitat preferences of the fishes in the area as Kadye *et al.* (2008) reported that fish species are consistently associated with their habitat types. These authors also reported that species richness increases downstream along an altitude gradient. Observations by Humpl and Pivnicˇka (2006) are in agreement with those of Kadye *et al.* (2008). No comparative study on fish species and habitat association has been carried out in Magoebaskloof area. Other studies suggest that associations are a result of factors such as predation and morphological adaptations of fish to the physical conditions of the rivers (Gaigher, 1973; Vadas & Orth, 2000; Bond & Lake, 2003; Kadye *et al*., 2008). With the continuous annual stocking of trout in Magoebaskloof area, it is important to identify the fish assemblages and determine causal processes underlying the distribution patterns of the fishes in the area.

2.4 Factors affecting the structure of biological communities in aquatic systems

Understanding the factors regulating the structure of biological communities has long been a prominent focus in ecology. Lindeman (1942) described communities as distinct sets of functionally similar organisms called “trophic levels”, with each successive level dependent on the level below it as a source of energy. In their view, resource availability is the major factor constraining the structure of communities. Nearly two decades later, Hairston *et al.* (1960) proposed the complementary hypothesis that the “top-down” influence of predators is the major force shaping biological communities. They reasoned that by suppressing herbivores, predators indirectly release plants from herbivory, allowing an increase in their abundance to occur. In this way, predators are capable of shaping the structure of entire communities through a combination of direct and indirect top-down effects.

For several decades, ecologists debated the applicability of these two apparently opposing models. The debate inspired vigorous research into the factors driving community structure, and compelling examples of both bottom-up and top-down control surfaced in the literature (Hunter & Price, 1992). It is now widely accepted that these processes are not exclusive, but rather are complementary, opposing forces that act simultaneously to shape the structure of biological communities (Terborgh & Estes, 2010). Bottom-up processes are inherent in every system and determine the flow of resources within a given community, while top-down forces act upon the stage set by bottom-up processes, and dictate the distribution of resources among different trophic levels (Terborgh & Estes, 2010). In recent years, the primary focus of community ecologists have shifted towards trying to understand the factors and mechanisms responsible for variation in bottom-up and top-down effects within and among systems (Power, 1990), and in particular, on how human-related perturbations modify the nature and outcome of bottom-up and top-down effects (Estes *et al.,* 2011).

Modification of biological communities by introduction of fish species

Introduction of non-native fish species can alter the structure and function of biological communities by modifying bottom up and/or top-down forces in a system (Österblom *et al.,* 2007). Predatory fish are at the top of the food web, they can regulate the structure and function of biological communities below them through a combination of direct and indirect effects. They directly affect their prey by reducing their abundance or by changing their behaviour. This can indirectly influence other components of the communities to which the prey are linked as food or competitors (Allan & Castillo, 2007). This phenomenon is known as a “trophic cascade”, which Pace *et al.* (1999) have defined as a process in which there are reciprocal predator to prey effects that alter the abundance, biomass or productivity of a population, community or trophic level across more than one level in a food web.

Impacts of invasive fish on indigenous fish communities

The impact of introduced fish on fish communities have been studied in some areas globally. In Australia, alteration in the distribution of the mountain and climbing galaxias, due to predation by trout, has been reported (McDowall, 2003; McDowall & Allibone, 2004). In many areas the galaxias species was absent due to the presence of trout. In most cases, *Galaxias vulgaris* were only found above waterfalls large enough to prevent trout migration. Predation is the most likely mechanism for the observed decline in distributions between the native fish and trout in Australia. McDowall (2003) reported that the addition of trout has had negative impacts on some galaxias and other native species in New Zealand, particularly several galaxiids. The population of *Galaxias gracilis* declined severely when rainbow trout were introduced (McDowall & Allibone, 2004). These authors recommended that trout stocking in one lake be stopped and that *G. gracilis* be reintroduced. Similar negative impacts were reported in the United States of America, where a decline of native fishes has resulted from stocking of non-native species, principally rainbow and brown trout in Arizona (Rinne & Alexander, 1995). Rinne and Alexander (1995) provided both laboratory and field proof of the negative impact of introduced trout on native species. Results of field enclosure (cages) and laboratory studies suggested that introduced rainbow and brown trout were effective predators on the native little Colorado spinedace (*Lepidomeda vittata*).

The impacts of trout on native biotas have also been noted in Africa (Cambray, 2003b; Kadye *et al*., 2013, Shelton *et al*., 2015). However, much work still needs to be done to quantify and understand the true extent of the trout impacts. For example, just five scientific studies have been published in southern Africa, three in South Africa, one in Zimbabwe and the other in Malawi. Woodford and Impson (2004) studied sympatric populations of trout and three species of native fish (Berg River redfin *Pseudobarbus burgi*, Cape kurper *Sandelia capensis* and Cape galaxias *Galaxias zebratus*) in a single headwater stream in the south-western Cape and found some evidence that trout consume one the native *G. zebratus* and that native fish displayed avoidance behaviour to areas occupied by trout.

In KwaZulu-Natal, Karssing *et al.* (2012) studied populations of tadpoles of the native Natal cascade frog *Hadromophryne natalensis* in habitats above and below waterfalls which acted as physical barriers to trout invasions in two headwater streams (one containing *O. mykiss* and the other *S. trutta*). The findings revealed the presence of trout to be the likely cause of abrupt declines in amphibian abundance downstream of the waterfalls. Shelton *et al.* (2015) reported that the introduced *O. mykiss* have invaded many headwater streams in the Cape Floristic Region (CFR) and depleted or eliminated native fish populations. However, the question of whether trout invasions also have consequences for lower trophic levels in these systems has not been addressed. Their findings indicate that trout invasions have changed the structure and function of benthic communities in these streams.

In Zimbabwe, a study by Kadye *et al.* (2013) reports that trout influenced both the distribution and abundance of the *Amphillius* catfish species that occupied shallow reaches possibly to avoid predation from trout that occurred in the deeper habitats. Their study also showed that most macroinvertebrate taxa were more abundant in exclusion treatments than inclusion treatments. By contrast, within trout-free reaches, most macroinvertebrates either did not differ between treatments or were generally more abundant in exclusion than inclusion treatments. This suggests that the macroinvertebrate communities responded differently within invaded and non-invaded reaches. By influencing distribution and abundance of native biota, non-native trout may have wider ecological effects, such as influencing trophic interrelationships within invaded habitats.

In a survey conducted at 24 sites along streams situated on the Nyika Plateau, Malawi, Kadye and Magadza (2008) found that the feeding behaviour and habitat preferences of the native mountain catfish *Amphilius uranoscopus* appeared to be influenced by the presence of trout. These findings indicate that trout may well be preying upon (and negatively impacting) native fish and amphibian populations, and thereby possibly altering native fish behaviour in southern Africa. Considering that many species of South African native fish have evolved in isolation and in the absence of large predatory fish, it seems likely that they will be especially vulnerable to predation by introduced trout (Cox & Lima, 2006).

The feeding biology of *Clarias gariepinus* is well known and literature suggests that *C. gariepinus*, the dominant component of native fish assemblages in many South African streams (Skelton, 2001), are primarily euryphagous feeders that consume mostly aquatic invertebrates, algae and detritus and predates on fish (de Moor & Bruton, 1988). Based on the study by Kadye and Booth (2012), trout and native *C. gariepinus* may have similarities in the functional role that they perform in Magoebaskloof area streams and dams, and thus may influence community structure and behaviour.

In South Africa, studies have primarily focused on competitive or predatory impacts at individual and population levels, instead of investigating habitats that are preferred by these fishes. Efforts should now be directed towards understanding the environmental variables and habitats that affect the distribution of trout. This can be very crucial in predicting future impacts and establishment of the trout. The negative impact of trout on South African aquatic biodiversity has been evident for some time. The threat trout pose to indigenous fish and macroinvertebrate is of importance, especially now that so many of South African species are included in the IUCN list of threatened species (Ellender & Weyl, 2014; Shelton *et al*., 2015).

Impact of invasive fish species on macroinvertebrates

Benthic aquatic macroinvertebrates are widely used as indicators in environmental impact studies. Their differential response to different categories of disturbances makes them excellent candidates for impact studies (Rosenberg & Resh, 1993). In predation impact studies, especially by invasive fish, aquatic macroinvertebrates are highly susceptible and can exhibit responses ranging from simple interactions such as reduction or local extinction of populations, to complex interactions such as trophic cascading (Flecker & Townsend, 1994; Nyström *et al*., 2003; Williams *et al.,* 2003).

Experimental studies indicate that when invasive predators become established, they tend to remove the most vulnerable macroinvertebrate taxa and the resultant community either shows little response to predation related disturbances (Meissner & Moutka, 2006), or it becomes highly unstable (Angeler & Moreno, 2007). Community instabilities in predator mediated communities are a consequence of a transition to a new alternative stable state during both the disturbance and post-disturbance periods (Suding *et al*., 2004). Such communities are often dominated by macroinvertebrate taxa that can either seek alternative refuge or quickly recolonize from adjacent habitats (Miller & Crowl, 2006), or are not preferred by the predator (Meissner & Moutka, 2006). Therefore, the presence of invasive non-native predators can be likened to press disturbances that persistently influence community structure and function. Press disturbances represent environmental perturbations caused by a sustained impact that continuously disrupts community structure and composition (Lake, 2000; Parkyn & Collier, 2004).

Consequences of trout introductions in California have been reported, where mountain yellow-legged frog (*Rana muscosa*) and some aquatic invertebrates have been rendered extinct. In the alpine area of the Californian Sierra Nevada, introduced trout affected the structure and composition of faunal assemblages in High Sierra lakes (Bradford *et al.,* 1998). The large and mobile, conspicuous taxa, including tadpoles, large-bodied micro-crustacean zooplankton and many macroinvertebrates were rare or absent from lakes containing trout (Bradford *et al.,* 1998). In England, Healey (1984) reported a significant change in species composition of stream invertebrates, most notably a sharp decline in dragonfly nymphs (*Lates sponsa*), some dytiscid larvae and a water beetle after brown trout was introduced into a moorland stream.

Keyse *et al.* (2006) reported that trout predates primarily on macroinvertebrates, thus affecting the abundance thereof and food web interaction. Walls *et al.* (1990) has shown that for prey to survive and reproduce under intense predation the prey species either develop means to decrease its vulnerability to predation by camouflaging themselves in order to blend in with their surrounding habitat or by being reclusive (Cambray, 2003b). Alternatively, predation may force prey to relocate to other habitats that have limited resources that, in turn, may lead to a reduction in the reproduction and growth (Keyse *et al*., 2006).

Shelton *et al*. (2015) has shown the impact of the introduced rainbow trout on benthic invertebrates in South Africa. The trout have invaded many headwater streams in the Cape Floristic Region (CFR). Their findings, together with comparisons of environmental conditions between invaded and uninvaded sites, indicate that trout invasions have changed the structure and function of benthic communities in these streams. With trout introduced in Magoebaskloof area, it is hypothesized that the impact on macroinvertebrates will be small because the area is marginal for the trout species.

2.5 Methods used in examining the impact of non-native invasive fish

A variety of approaches have been used for assessing non-native invasive species impacts. These methods includes predictive studies based on information on similar invasions elsewhere; comparative and correlational studies that take advantage of natural variations in non-native invasive species presence/abundance; dietary studies and experimental manipulations (Park, 2004; Kadye & Booth, 2012; Kadye *et al.,* 2013; Shelton *et al.,* 2015). Each method offers exclusive insights into the nature and extent of impacts and has specific strengths and weaknesses which need to be recognized when choosing which one to use (Park, 2004; Shelton *et al.*, 2015).

Monitoring an invasion in action is an ideal way to study the effects of a non-native invasive species on a recipient system, but unfortunately most studies only begin long after the impacts have already occurred (Lintermans, 2000; Shelton *et al*., 2015). When pre-invasion data are not available and studying an invasion in action is not an option, various alternative techniques can be employed to infer invader impacts. One possibility is to conduct comparisons among systems with and without the introduced predator and to use differences in native species abundance to infer impacts (Marr *et al*., 2012; Shelton *et al*., 2014). The present study will employ this technique, since no historical data is available for the study area. The major weakness of this approach is that it does not take into account other factors that may influence the abundance of the native species, such as variation in habitat characteristics among the sites being compared (Townsend & Crowl, 1991). Furthermore, there is usually a large degree of natural variation among freshwater systems making it difficult, in many cases, to separate the predator impact from the environment (Meissner, 2000). However, these problems can by partly overcome by incorporating variation in other environmental factors into analyses, and assessing the influence of environmental factors alongside that of the introduced predator (Townsend & Crowl, 1991).

The identification of mechanisms behind patterns observed in the field requires experiments that enable the factor of interest to be manipulated, while controlling for all other potential sources of variation (Meissner, 2000; Park, 2004). Unfortunately, to achieve an understanding over potentially confounding variables the experiments often have to be conducted on small spatial scales (Cooper & Dudley, 1988; Shelton *et al*., 2015). Surveys, on the other hand, can be conducted on large spatial scales, and thus incorporate natural levels of environmental heterogeneity. Because surveys and experiments offer contrasting advantages and insights into non-native predator impacts, the use of these approaches in complementary roles has been strongly advocated by several authors (Cooper & Dudley, 1988; McIntosh *et al.,* 2002; Kats & Ferrer, 2003; Kadye & Booth, 2012; Kadye *et al.*, 2013; Shelton *et al*., 2015). Strong associations between experimental and survey results is generally considered strong evidence for an impact (Meissner, 2000; Park, 2004; Shelton *et al*., 2015). In this study, a combination of comparative and experimental approaches to investigate the impact of the trout on native macroinvertebrates and their structure in the Broederstroom and Debengeni Rivers in the Magoebaskloof area will be used.

Recent studies have primarily focused on the impacts of non-native fishes (Woodford *et al*., 2005; Lowe *et al.,* 2008; Weyl *et al.,* 2013), while relatively little work has been done on their habitat associations, spread and interaction with native species. This focus on impacts may result in inadequate information on the establishment and introduction phases of non-native fishes. This study aims to improve the knowledge and understanding about their impacts in an area regarded marginal for the trout species. This will build on our limited knowledge about trout impacts on South African systems. Trout has only been introduced into the Broederstroom River and not the Debengeni River in Magoebaskloof area, which presents a distinctive opportunity to investigate trout impacts on macroinvertebrates by means of broad-scale comparative field surveys and small-scale, controlled field experiments.

2.6 Effects of climate change on fish distribution

Since Magoebaskloof area is a marginal area for trout, the effect of climate change on its future distribution should be known. Freshwater fisheries and aquaculture of many countries are likely to be affected by the climate change (FAO, 2009). The possible effects of climate change on fisheries and aquaculture could be the warming of streams which will result in oxygen poor water. Trout being a cold water fish species and sensitive to poor water quality, changes in water temperatures and oxygen levels in marginal areas like Magoebaskloof can lead to them being confined to high altitudes only and can negatively affect their establishment. A comprehensive report by Handisyde *et al*. (2006) using geographic information system (GIS) model identified the most overall vulnerable countries and areas susceptible to future climate change in Europe. Studies of this nature are necessary to predict the future of aquaculture and fisheries in South Africa. Effect of climate change on the fisheries of South Africa as a whole should also be considered in future studies.

River flow regimes, including long-term average flows and seasonality play an important role for freshwater ecosystems. Climate change affects freshwater ecosystems not only by increased temperatures but also by altered river flow regimes (Doll & Zhang, 2010). Doll and Zhang (2010) quantified the impact of climate change on a global scale. They reported four climate change scenarios based on two global climate models and two greenhouse gas emissions scenarios. Their study showed that by the year 2050, climate change may have impacted ecologically relevant river flow characteristics more strongly than dams and water withdrawals have up to now. However, little work has been carried out on a local scale.

Jones *et al.* (2013) has shown that climate change will have an effect on the threatened species in the UK waters. Their projections assessed the changes in habitat suitability in selected candidate protected areas around the UK under future climatic scenarios. The result shows that adverse consequences of climate change on the habitat suitability of protected areas are projected to be small. Jones *et al*. (2012) also estimated the biological and economic impacts of climate change on freshwater fisheries in the United States. Based on their model, the spatial distribution of cold water fisheries is projected to being replaced by warm or cool water and high-thermally tolerant, lower recreational priority fisheries.

Despite Africa's fast-growing human population and the associated impacts on natural resources, it is one of the least studied continents in terms of ecosystem dynamics and climate variability (Hély *et al*., 2006). Climate change is already having an impact on the dynamics of African biomes and its rich biodiversity. Species composition and diversity are expected to change due to individual species response to climate change conditions (Erasmus *et al*., 2002). The projected rapid rise in temperature combined with other stresses, such as the destruction of habitats from land use, could easily disrupt the connectedness among species, transforming existing communities and showing variable movements of species through ecosystems, which could lead to numerous localized extinctions (Jones *et al*., 2013).

Climate change may also affect species range, which could have profound impacts on species population size. For example, in South Africa a modeling study found that a reduction in the range of a species is likely to have an increased risk in local extinction climate change (Erasmus *et al*., 2002). The authors suggest that this may be due to the positive inter-specific relationship between population size and range size; if range size decreases, there is likelihood that there will be a rapid decline in population size. Additionally, this relationship could be exacerbated if climate change restricts the range of a species to just a few key sites and an extreme weather event occurs, thus driving up extinction rates even further (Erasmus *et al*., 2002). Species ranges will probably not shift in cohesive and intact units and are likely to become more fragmented as they shift in response to changing climate (Channel & Lomolino, 2000).

2.7 Effects of land use/cover changes on fish distribution

Knowledge about land use/land cover together with climate change has become important to overcome the problem of biogeochemical cycles, loss of productive ecosystems, biodiversity, deterioration of environmental quality, loss of agricultural lands, destruction of wetlands, and loss of fish and wildlife habitat (Hudak & Wessman, 1998). Technologies such as satellite remote sensing are required to help outline how these changes will affect fish distributions and how to mitigate the changes in future (Maktav *et al*., 2005). These technologies provide data to study and monitor the dynamics of natural resources for environmental management. This study will employ these technologies to outline how the changes in land use and cover affects the distribution of trout in Magoebaskloof area.

CHAPTER 3:

FISH ASSEMBLAGES IN RELATION TO ENVIRONMENTAL VARIABLES WITHIN THE BROEDERSTROOM AND DEBENGENI RIVERS OF MAGOEBASKLOOF AREA, LIMPOPO PROVINCE, SOUTH AFRICA

3.1 INTRODUCTION

Fish assemblages are influenced by both biotic and abiotic factors (Vila-Gispert *et al.,* 2002; Kadye *et al.*, 2008; Shelton *et al*., 2014). Biotic factors such as the introduction of predatory fish play a role in influencing fish assemblages as they disrupt the biota of their recipient ecosystems directly through predation and competition (Kadye *et al.,* 2008). Fish assemblages can also be defined by abiotic factors such as the nature of the substrate, depth and flow rate (Vadas & Orth, 2000; Bond & Lake, 2003; Kadye & Moyo, 2007; Kadye *et al*., 2008). The establishment of predatory fish such as trout and the continual destruction of habitats needs serious attention in countries like South Africa since they are negatively affecting fish assemblages without environmental monitoring studies.

Little work has been done on biotic and abiotic factors that affect the distribution and establishment of trout species in South Africa, especially in areas such as Magoebaskloof, Limpopo Province. The streams in Magoebaskloof area descend along an altitude gradient, thereby providing different habitats for trout. However, the continuous annual restocking's in the area raises questions on the suitability of the area for the trout. It was then seen important to identify fish assemblages around the area and determine the causal biotic and abiotic processes underlying the distribution patterns of trout and other fish species in the area.

Trout was introduced into the Broederstroom River in the early 19th century and is restocked annually for sport fishing by the Haenertsburg Trout Association (HTA) (DWA, 2013). However, there is no historical published data on its establishment in this area. This study is one of the first to examine the effect of abiotic factors such as environmental conditions on the growth, establishment and self-sustainability of trout in an area regarded to be marginal for cold water species such as trout.

The main objective of this chapter was to determine the effect of environmental variables on fish assemblages and distribution patterns of trout within the Broederstroom in Magoebaskloof area.

3.2 METHODS AND MATERIALS

3.2.1 Study area

The Magoebaskloof area, commonly known as "The land of the silver mist" is a mountainous area in Limpopo Province. It is situated at the north eastern tip of the Drakensberg mountain range. The mountains and valleys of the area are regularly masked in a soft mist. This mist belt has resulted in the lush afro-montane forests that make the area a welcome green oasis in the Limpopo bushveld (Figure 3.1). The indigenous forest in the area is linked by streams that flow through rocks and pools. The Wood-Bush complex, the largest indigenous forest in Limpopo Province is found in the area (Mucina & Rutherford, 2006).

Figure 3.1: The afro-montane forest in Magoebaskloof Wood-Bush area.

Geology and climate

Magoebaskloof area ranges in altitude from approximately 800 to 2160 m (a.s.l.). The shallow underlying soil is mostly lithosols (DWA, 2013). The area is characterized by granite, mudstone and sandstone. The climate is cool with frequent mist, occasional wind, hail and frost in winter (Mucina & Rutherford, 2006). The temperature patterns follow a distinct seasonal trend with higher temperatures occurring in the summer period. The colder months are June and July (Figure 3.2a).

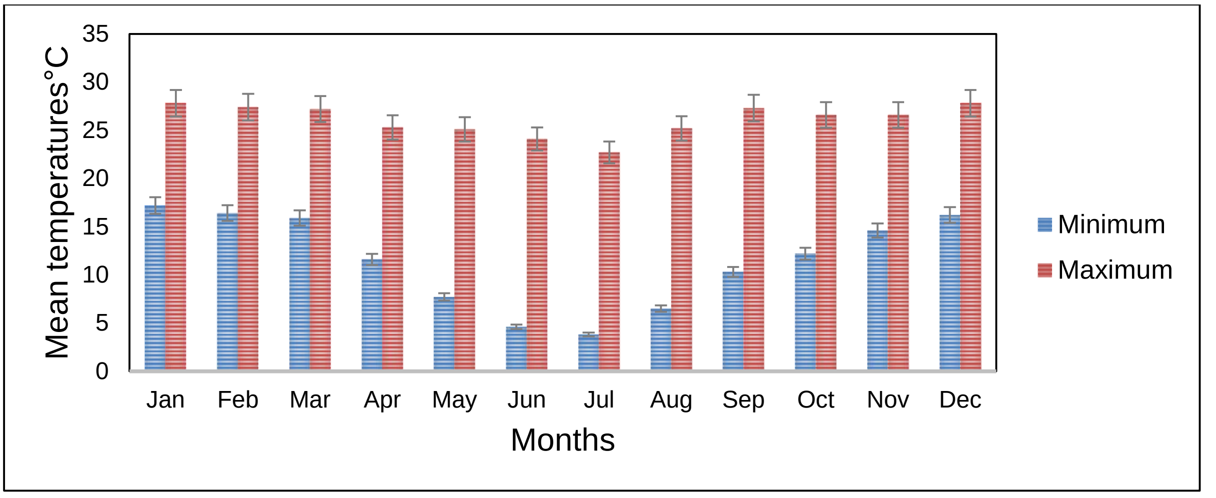

Figure 3.2a: A bar graph depicting mean monthly air temperature for the Magoebaskloof area. (2014 data obtained through consultation with the South African Weather Services).

The mountainous topography at the area results in a much higher rainfall with the mean average precipitation varying between 700 to 1500 mm (Figure 3.2b). The rainfall patterns follow a distinct seasonal trend with the majority of the rainfall occurring in the summer period, which begins from October to March. The peak rainfall months are January and February. The dry season begins in May.

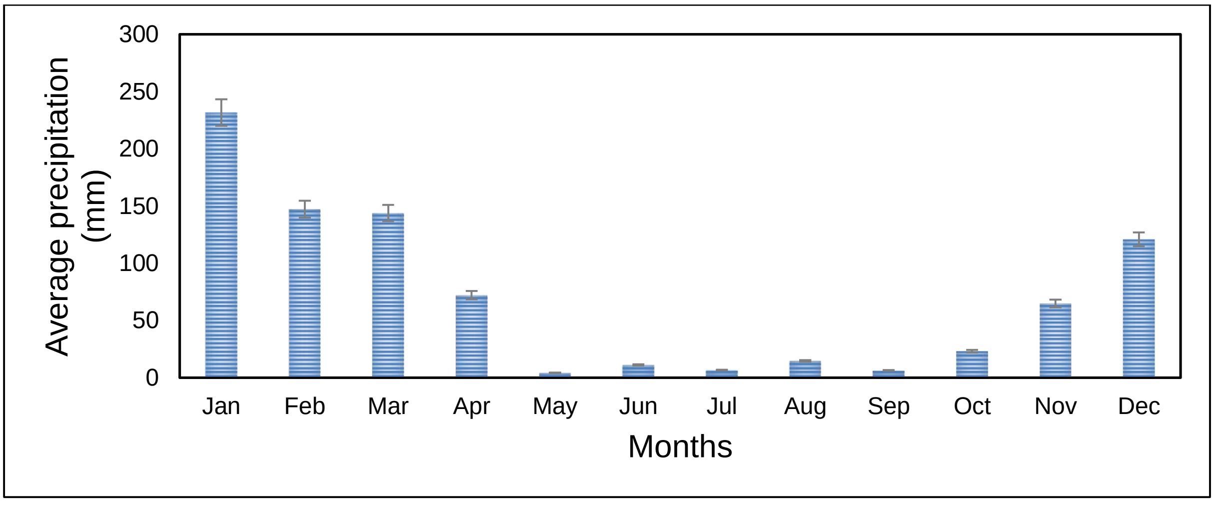

Figure 3.2b: A bar graph depicting mean monthly rainfall for the Magoebaskloof area. (2014 data obtained through consultation with the South African Weather Services).

Land use and vegetation

The catchment of Magoebaskloof area, particularly the Wood-Bush complex is a highly productive agricultural area with mixed farming that includes forestry and agricultural plantations, cattle ranching and game farming (Mucina & Rutherford, 2006). Agriculture typically consists of small-scale farming by rural communities and large scale commercial farming. High rainfall and deep soils make the area ideal for the planting of timber with a canopy height between 15 and 30 m (Figure 3.3). About 45% (more than 20 000 ha) of the total area comprises plantations with <5% consisting of afro-montane grassland (DWA, 2013). Climate in this area is the fundamental determinant of the natural distribution of most forestry species. It is also the overriding strategic factor influencing species choice for commercial forestry (Mucina & Rutherford, 2006).

Figure 3.3: A typical site showing the canopy height and patches of shrubs.

Besides the dominant plantations, current land uses in the area include the production of avocados, kiwi fruit, potatoes, macadamia nuts and dairy products. The area appears to be an eco-tourism destination in recent years. The forest host popular birding, hiking and mountain biking routes, sailing and other watersport. The upper catchments are dominated by pines and eucalyptus plantations whilst the foothill zones contain tea estates.

3.2.2 Site selection

The origins of the Broederstroom and Debengeni Rivers are at approximately >1600 m a.s.l. of the Drakensberg Escarpment. They descend to feed into the Ebenezer and Magoebaskloof Dams respectively (Figure 3.4). The Magoebaskloof Wood-Bush complex forms part of a catchment supporting the Dap Naude, Ebenezer, Tzaneen and Magoebaskloof Dams. These dams supply water to Polokwane and Tzaneen.

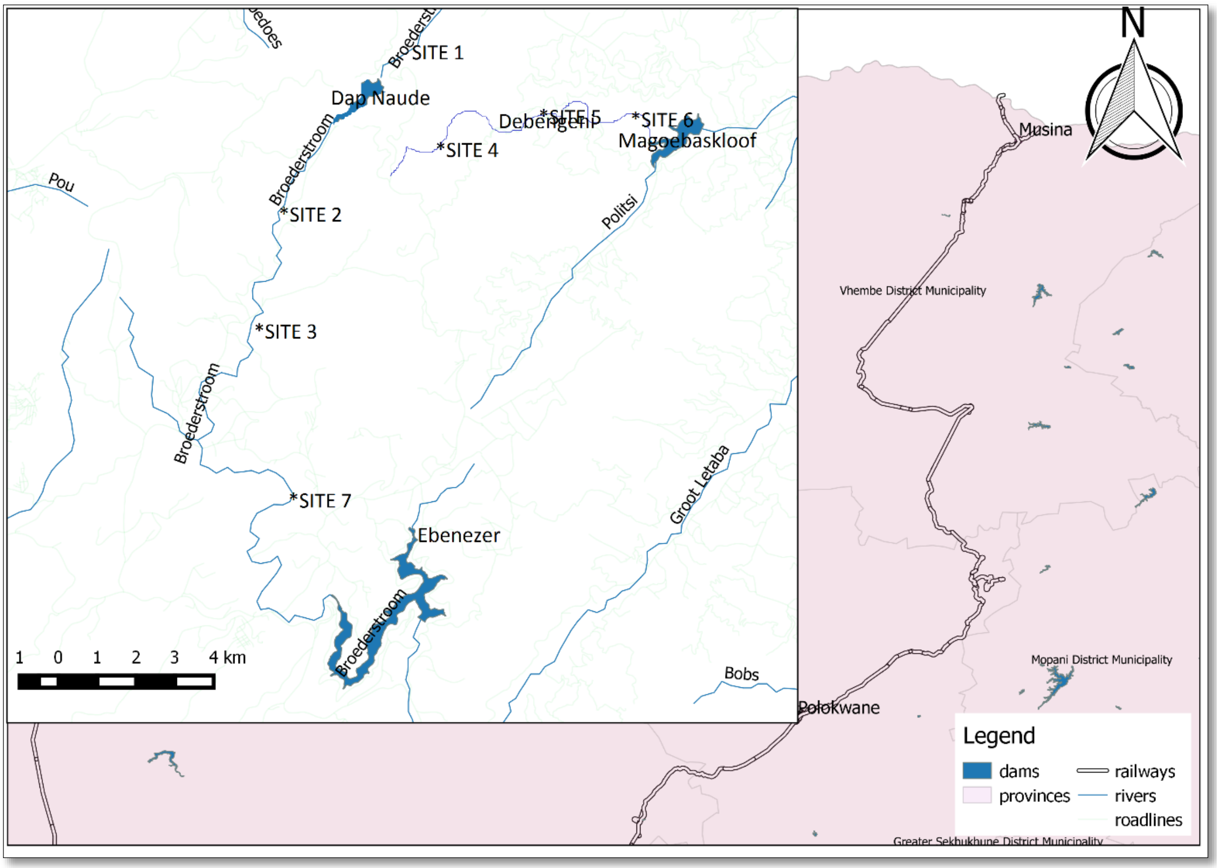

Figure 3.4: The Magoebaskloof area catchment with rivers and sampling sites.

The Debengeni and Broederstroom Rivers are in the same catchment and have similar environmental variables. Trout is present in the Broederstroom but not in Debengeni River. Since distinct zones along a river are defined by gradient and the nature of the river bed, prior to sampling, the rivers were divided into zones which constituted the upper, intermediate and lower reaches. Adjacent to the rivers are forests of timber plantations with most parts of the rivers shaded. Water is cool and clear as coloration by organic compounds is absent and well oxygenated due to features such as waterfalls and fast flowing rapids. Seven sites were selected and chosen along an altitude gradient in both rivers. A total of four sites in the trout invaded Broederstroom River and three in the un-invaded Debengeni River were sampled (Figure 3.4). For recording of coordinates and altitude, a handheld GPS (Model: GARMIN *etrex* Legend) was used.

Sites description

Site 1, 2, 3 and 7 are in the Broederstroom River whereas site 4, 5 and 6 are in the Debengeni River (Figure 3.4). Sites were accessible in the Broederstroom River as opposed to the Debengeni River which has steep river embarkments hence, only three sites in Debengeni River were sampled as opposed to the four in the Broederstroom River.

- Site 1 (S 23°48'02.3"; E 29°58'38.9")

The site is at 1557 m a.s.l., dominated by rock, pebbles, and sand and shielded with marginal vegetation. The water is cold, clear and well oxygenated as it receives water from a waterfall. From the site, the river feeds into the Dap Naude Dam (Figure 3.5).

- Site 2 (S 23°49'20.3"; E 29°57'29.6")

Site 2 is characterized by slow to moderate flow, the site has pools and riffles. The site is surrounded by a picnic area just after Dap Naude Dam at 1526 m a.s.l. The site is partially covered with patches of marginal vegetation and the substrate is dominated by sand and pebbles. It is shaded with exotic trees that runs along the river banks.

- Site 3 (S 23°49'45.8"; E 29°57'08.5")

Slow flow and deep pools characterize the site. Adjacent to the site are pine tree plantations and patches of marginal vegetation. The substrate is dominated by sand, mud and gravel. It is at an altitude of 1459 m a.s.l.

- Site 4 (S 23°48'23.0"; E 30°01'15.3")

Situated at the Ditlokwa hut at 1240 m a.s.l., the site is dominated by cobbles with patches of sand and less marginal vegetation. The flow of the site is moderate to fast due to the prevailing cobbles and shallow riffles.

- Site 5 (S 23°48'51.9"; E 30°01'44.8")

Situated at 1020 m a.s.l., the site is above the Debengeni falls and it feeds into the Debengeni waterfalls. It is dominated by sand, mud and cobbles and characterized by varying flow rates, slow in the pools with mud and sand substrate, and fast in the riffles with rock substrate. Marginal vegetation is scarce.

➢ Site 6 (S 23°48’63.3”; E 29°01’54.1”)

The site is situated below the Debengeni waterfalls at 840 m a.s.l. and is characterized by fast flowing waters resulting from the rock substrate and water from the waterfalls. The site is partially shaded by trees and low lying shrubs.

➢ Site 7 (S 23°56’19.1”; E 29°56’50.7”)

The site is characterized by mud, sand, pebble and rock substrates. It situated at 1365 m a.s.l. just before the mouth of the Ebenezer Dam. It has scarce marginal vegetation with some patches of algae. The water at this site is turbid as opposed to the other sites.

Figure 3.5a: The main habitat types at Broederstroom and Debengeni Rivers.

Figure 3.5b: The main habitat types at Broederstroom and Debengeni Rivers.

3.2.3 Habitat assessment

Habitats were assessed based on the nature of the bottom substrate, pool variety and riparian vegetation conditions at stream reaches were scored. At each site, five transects were set up to measure physical habitat variables. Measurements were taken at three points along each transect.

Each habitat was classified following studies by Gorman and Karr (1978), Schlosser (1982), and Kadye and Moyo (2008). Four depth classes, five substrate classes, three velocity classes and three riparian cover classes were identified such that:

- Depth:
  - 0-5 cm (Very shallow)
  - 5-20 cm (Shallow)

- 20-50 cm (moderate)
- > 50 cm (Deep)

➢ Substrate:
- Mud (<0.05 cm)
- Sand (0.05-2 cm)
- Gravel (2-10 cm)
- Pebble (10-30 cm)
- Rock (30-50 cm)

➢ Flow:
- Slow (<0.1 m/s)
- Moderate (0.2-0.3 m/s)
- Fast (>0.3 m/s)

➢ Riparian cover
- <40% (Scarce)
- 40-70% (Moderate)
- >70% (Shaded)

Stream flow rate was measured at three points along each transect using a PASCO Scientific PS-2000 flow-meter.

3.2.4 Fish sampling

Fish were collected with an electrofisher from 100 m section of the rivers. Electrofishing (LR-24, Smith Root Company, U.S.A) was conducted in an upstream direction for 30 minutes set at 300-800 volts (Figure 3.6). Each sampling site was divided into three sections, each of which was then blocked using a fine meshed net at either end before sampling. Fish data was expressed as species richness (number of fish collected at each site) and relative abundance (catch per unit effort, calculated as number of fish caught per minute).

Fish caught were identified following guidelines of Skelton (2001). Total length and standard length in centimeters and mass in grams of the fish were recorded using a measuring board and a weighing balance (A & D Company, model FX2000i) respectively. Specimens not easily identifiable were placed into 10% formalin and later preserved in 70% ethanol and returned to the laboratory (Aquaculture Research Unit) for identification.

Figure 3.6: Fish sampling by means of a backpack electrofisher.

3.2.5 Ageing *Oncorhynchus mykiss* and *Salmo trutta*

Scales were removed above the lateral line, anterior to the dorsal fin from each fish, using tweezers and placed in individually marked envelopes. In the laboratory, the scales were soaked in distilled water and cleaned. Once dry, the scales were mounted on Bells and Howell ABR-IV microfiche reader (Figure 3.7) and the annuli counted.

Figure 3.7: A Bells and Howell ABR-IV microfiche reader.

The criteria used in the identification of annuli was as follows:

- Areas where circuli fuse or form anastomoses (Payne, 1976; Barger, 1990).
- Clear zones that were devoid of circuli (Cohen, 1991).
- Areas where circuli crowded together.

The assumption used for identification of annuli was that a true annulus can be traced completely around the scale and generally exhibits crossing over in the posterior portion of the lateral fields. Age was assigned to each fish based on the number of complete annuli.

Determination of growth

Past version 2.17 software was used to calculate parameters for the generalized von Bertalanffy growth function (Von Bertalanffy, 1938) for length (*L*) at age (*t*) and *K* (Pauly, 1982) for *O. mykiss* and *S. trutta* within the Broederstroom River. The Von Bertalanffy growth model is defined as:

$$L_t = L_\infty [1 - e^{-K (t-t_0)}]$$

Where: L_t = length at time t

K = growth coefficient

L_∞ = average size the stock of fish would reach if they were allowed to grow indefinitely

t_0 = hypothetical age of the fish at zero length

3.2.6 Physical and chemical parameters

Dissolved oxygen, pH, conductivity, salinity and temperature were measured *in situ* using a handheld multi parameter instrument (YSI meter MPS 556). Turbidity was measured with a WTW turbidity meter (Model: Turb 430 IR/T) (Figure 3.8).

Figure 3.8: YSI meter MPS 556 and WTW turbidity meter.

Water samples were collected in 1L bottles at the surface of the water column for laboratory analysis of ammonia, orthophosphate, total dissolved solids, nitrate and nitrite and total alkalinity concentrations. Ammonia, orthophosphate, nitrate and nitrite and total alkalinity were analysed following the methods in the Hach DR3900 spectrophotometer manuals (Figure 3.9).

Figure 3.9: Hach DR3900 spectrophotometer used for water analysis.

Total dissolved solids (TDS)

The total dissolved solids (TDS) were determined using the method adapted from Eaton *et al*. (1995). A sample of 100 ml was filtered through a filtration apparatus where a glass fibre filter (Whatman, 1.6 µm) was used. After draining the water sample, 10 ml of distilled water was filtered for three minutes to allow complete drainage. The filter paper was then removed and placed in a clean glass petri-dish and dried at 120°C.

The TDS was calculated as follows:

$$\text{Total dissolved solids (mg/l)} = \frac{(\mathrm{A}-\mathrm{B})\times 1000}{\text{Sample volume,ml}}$$

Where: A = weight of dried filter paper + dish (mg)

B = weight of dish (mg)

3.2.7 Data analysis

Normality and homogeneity of variance for all the data was tested using the Shapiro-Wilk and Levene's test respectively. Detrended correspondence analysis (DCA) was performed using the presence-absence data to determine the pattern of species distribution between all sampled sites in both the Broederstroom and Debengeni Rivers. DCA is an indirect gradient ordination that extracts axes to explain the relationship between species and sampling units without constraining this relationship to a set of environmental variables. The analyses was carried out using CANOCO version 5 (Ter Braak & Šmilauer, 2012).

To summarize the relationship between species, using the presence-absence data and the habitat and physical variables, a direct gradient analysis method, canonical correspondence analysis (CCA), was performed for all sampled sites of both the Broederstroom and Debengeni Rivers together. CCA is a multivariate constrained ordination technique that extracts major gradients among combinations of explanatory variables in a dataset. CCA was used to analyse fish and physico-chemical parameters relationship to identify parameters potentially influencing fish assemblages within the Broederstroom and Debengeni Rivers. A Monte Carlo permutation test was performed to assess the statistical significance of the environmental parameters (Heino, 2000). The environmental factors used were habitat variables, pH, water temperature, conductivity, flow rate and depth. CCA was carried out on fish collected at the sites over the sampling period. The analysis was carried out using CANOCO version 5 (Ter Braak & Šmilauer, 2012).

The preferred habitats by *O. mykiss* and *S. trutta* caught in site 1, 2 and 3 (Broederstroom River) were assessed by calculating the standardized selection ratio (SSR). Manly *et al*. (1993) SSR represents the probability that an individual will use a particular habitat type, taking into account the different resource availability. Higher values of SSR indicate a strong preference for the selected resource which in this case are both physical and chemical variables (values between 0 and 1). Manly *et al's* (1993) index, $\hat{W}i$, was used:

$$\hat{W}i = {}^{Oi}/_{\pi i}$$

where Oi is the proportion of available resource units in category i and πi is the proportion of available resource units in category i.

The standardized selection ratio:

$$Bi = \sum_{i=1}^{n} \hat{W}i$$

was used to estimate the probability of each habitat category.

Logistic regressions were used to test the most influential habitat variables associated with each trout species in the Broederstroom River. This method is suitable where the analysis involves the relationship between continous and non continous variables. In this case, the presence and absence of individual species (response variable) was tested against the frequencies of the habitat categories (predictor variables). Chi square goodness-of-fit tests were used to assess the fit of the model. The significance of each coefficient (b) was evaluated by Wald statistic. Odds ratios were used to evaluate the relationship between the predictor and response variable. The odds ratios are the multiplicative factor by which the odds of a habitat occupied change when the variable in question increases by one unit (Quinn & Kenough, 2002). If the value is greater than 1, the odds are increased and if the value is less than 1, the odds are decreased.

3.3 RESULTS

3.3.1 Habitat structure in the Broederstroom and Debengeni Rivers

Site 1 to 3 and 7 were characterized by moderate depths whereas site 4 to 6 were characterized by very shallow depths (Table 3.1). Coarse substrates (rock, pebble and gravel) were the dominant substrate types in most sites. The percentage of riparian cover was dominant at site 2 to 5 (Table 3.1). The highest altitude was at Site 1 (1557 m a.s.l.) and the lowest altitude at site 6 (840 m a.s.l.).

Table 3.1: Habitat composition (%) of all sampling sites in the Broederstroom and Debengeni Rivers recorded in February 2014

	Broederstroom River				Debengeni River		
Sites	Site 1	Site 2	Site 3	Site 7	Site 4	Site 5	Site 6
Altitude (m)	1557	1526	1459	1365	1240	1020	840
Substrate (%)							
Rock	60	50	0	40	55	20	70
Pebble	20	30	0	30	30	10	10
Gravel	10	5	0	5	10	0	0
Sand	10	15	60	10	5	50	20
Mud	0	0	40	15	0	20	0
Depth (cm)							
Very shallow	15	5	0	5	40	35	24
Shallow	30	15	0	35	20	40	56
Moderate	50	40	30	10	40	25	15
Deep	5	40	70	50	0	0	5
Flow (m/s)							
Slow	10	50	75	65	5	80	15
Moderate	85	45	25	13	85	15	78
Fast	5	5	0	22	10	5	7
Riparian cover (%)							
Scarce	50	5	0	85	15	0	48
Moderate	45	80	10	12	75	15	42
Shaded	5	15	90	3	10	85	10

3.3.2 Fish assemblages in the Broederstroom and Debengeni Rivers

Six species, representing four families, were recorded in both the Broederstroom and Debengeni Rivers (Table 3.2). *Oncorhynchus mykiss* dominated the fish assemblages in terms of numbers caught followed by *Amphilius uranoscopus*, *S. trutta* and *A. natalensis*. Other fish species recorded are *Micropterus salmoides* and *Oreochromis mossambicus*. *Amphilius natalensis* and *A. uranoscopus* only occurred in the Debengeni River. *Oncorhynchus mykiss* and *S. trutta* occurred at the high altitude sites and were only caught at site 1, 2 and 3. *Micropterus salmoides* and *O. mossambicus* were at site 7 (Table 3.2).

Table 3.2: Fish species sampled in the Broederstroom and Debengeni Rivers from February 2014 to February 2015. * indicates that the species is non-native in the area

Family	Species	River						
		Broederstroom				Debengeni		
Sites		1	2	3	7	4	5	6
Salmonidae	*Oncorhynchus mykiss**	68	31	12				
	*Salmo trutta**	1	28	16				
Amphilidae	*Amphilius uranoscopus*					17	9	36
	Amphilius natalensis					7	9	19
Centrarchidae	*Micropterus salmoides**				7			
Cichlidae	*Oreochromis mossambicus*				2			

**Site 1 to 3 and 7 are at Broederstroom River and site 4 to 6 are in Debengeni River*

3.3.3 Relative abundance of fish caught in the Broederstroom and Debengeni Rivers

Relative abundance (CPUE) was highest in winter for both *O. mykiss* and *S. trutta* with a CPUE of 0.62±0.14 and 0.23±0.03 respectively (Table 3.3). *Amphilius uranoscopus* and *A. natalensis* CPUE increased from autumn to spring, with a decrease in summer (Table 3.3). *Micropterus salmoides* and *O. mossambicus* were not caught during autumn and winter (Table 3.3). There were no significant differences in CPUE amongst the seasons (ANOVA, $P<0.05$).

Table 3.3: The relative abundance (CPUE) for fish sampled in the Broderstroom and Debengeni Rivers from February 2014 to February 2015

Fish species	Autumn	Winter	Spring	Summer
Oncorhynchus mykiss	0.19±0.14	0.62±0.14	0.29±0.09	0.21±0.11
Salmo trutta	0.11±0.09	0.23±0.03	0.18±0.07	0.09±0.03
Amphilius uranoscopus	0.06±0.02	0.08±0.03	0.33±0.05	0.24±0.19
Amphilius natalensis	0.02±0.01	0.04±0.01	0.15±0.06	0.09±0.04
Micropterus salmoides	-	-	0.16±0.00	0.03±0.00
Oreochromis mossambicus	-	-	0.06±0.00	-

3.3.4 Monthly length frequency of trout in the Broederstroom River

Oncorhynchus mykiss had high mean total lengths of 18.5±5.43, 20.2±6.00 and 15.1±1.68 cm during February, June and December 2014 respectively. Fry and fingerlings with mean length of 4.8±1.11 cm were caught during August and gradually increased to 10.6±1.69 cm in October (Figure 3.10). Mean lengths (TL) of *S. trutta* was highest in June and lowest in December (Figure 3.11). There were no *S. trutta* fry and fingerlings recorded in all sampled months.

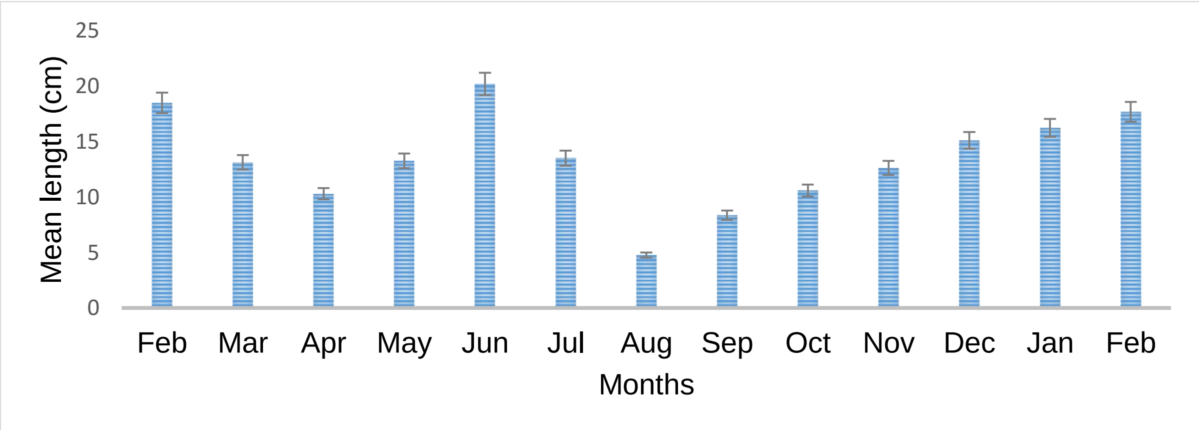

Figure 3.10: The mean length of *Oncorhynchus mykiss* from samples taken monthly from February 2014 to February 2015 in the Broederstroom River.

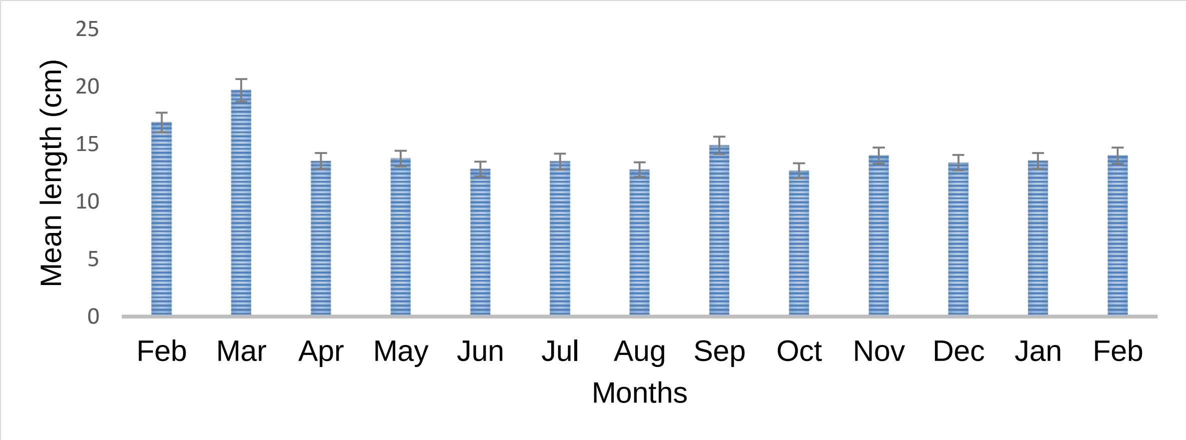

Figure 3.11: The mean length of *Salmo trutta* from samples taken monthly from February 2014 to February 2015 in the Broederstroom River.

3.3.5 Age determination of trout in the Broederstroom River

The maximum observed length of *O. mykiss* was 25.4 cm (TL) for a 4 year old. The growth model for *O. mykiss* was L_∞ = 26.7 cm, *K* = 1.049 and t_0 = -0.616 (Figure 3.12). The maximum observed length for *S. trutta* was 24 cm (TL) for a 3 year old. The growth model for *S. trutta* was L_∞ = 24.1, *K* = 1.672 and t_0 = -1.012 (Figure 3.13).

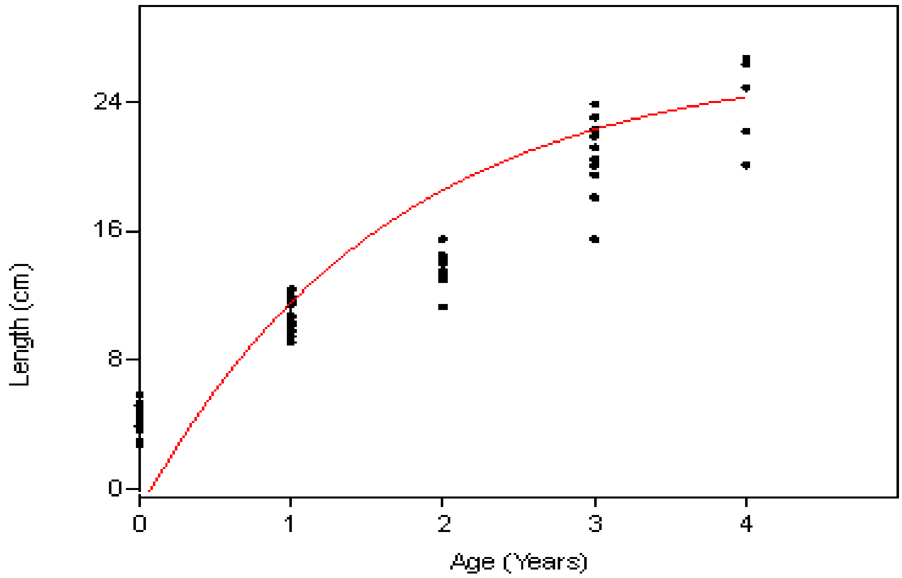

Figure 3.12: Length at age data (L_t= 26.7 [1- $e^{-1.049\ (t+0.616)}$]) for *Oncorhynchus mykiss* in the Broederstroom River from February 2014 to February 2015.

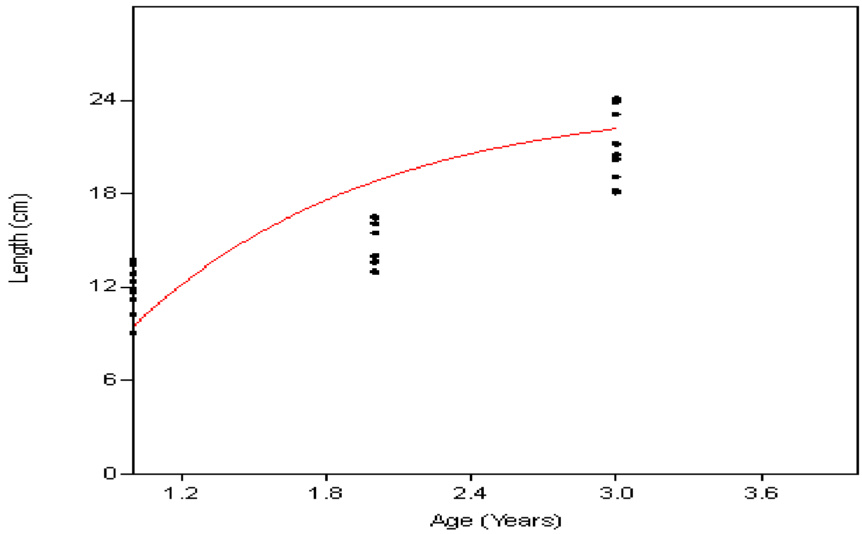

Figure 3.13: Length at age data (L_t= 24.1 [1- $e^{-1.672\ (t+1.012)}$]) for *Salmo trutta* in the Broederstroom River from February 2014 to February 2015.

3.3.6 Physico-chemical parameters affecting fish assemblages in the Broederstroom and Debengeni Rivers

The physico-chemical parameters parameters that were used to investigate fish assemblages and distribution in both rivers are listed in Table 3.4. Depth fluctuated across the different sites. There were significant differences of depth between sites (ANOVA, $P<0.05$). Site 4 was the shallowest and site 2 the deepest (Table 3.4). There were no significant differences (ANOVA, $P<0.05$) in total dissolved solids across all sites (Table 3.4). The lowest temperature was recorded at site 1. The temperature of the area is defined by an altitude gradient of the area. Ammonia levels ranged between 0.02 and 0.05 mg/l across all sites (Table 3.4). Flow fluctuated across all sites but showed no significant differences, with the highest recorded at site 3 and lowest at site 5. There were no significant differences of nitrate, nitrite, alkalinity and orthophosphate across all sites (Table 3.4).

Table 3.4: The mean±SD of physico-chemical parameters sampled from February 2014 to February 2015 analyzed for fish distributions against TWQR of trout across all sites in the Broederstroom and Debengeni Rivers. Different superscripts in a row indicate statistically significant differences (P<0.05, ANOVA)

Variable	Magoebaskloof area							TWQR
	Broederstroom River				Debengeni River			
Sites	Site 1	Site 2	Site 3	Site 7	Site 4	Site 5	Site 6	
Min-Max temp (°C)	7.4-17.5	8.1-17.8	8.6-19.2	12.2-21.5	11.8-19.8	10.3-19.8	13.1-22.9	
Mean temp (°C)	10.5±0.51^{a}	11.6±1.20^{ab}	11.2±3.25^{ab}	19.3±2.61^{b}	12.1±0.71^{ab}	13.6±2.87^{ab}	20.4±2.06^{ab}	8-22°C
Depth (cm)	33±10.3^{ab}	44±17.2^{a}	27±8.4^{ab}	32±14.8^{ab}	19±10.7^{b}	30±15.0^{ab}	25±17.5^{ab}	-
TDS (mg/l)	0.03±0.02^{a}	0.05±0.02^{a}	0.04±0.02^{a}	0.04±0.02^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0-12 mg/l
Flow (m/s)	0.25±0.18^{a}	0.19±0.18^{a}	0.36±0.25^{a}	0.26±0.22^{a}	0.26±0.16^{a}	0.17±0.09^{a}	0.26±0.22^{a}	>0.05 m/s
Turbidity NTU	3.85±2.48^{a}	2.92±2.15^{a}	4.74±0.85^{b}	5.94±2.68^{b}	3.48±2.33^{a}	4.04±1.39^{a}	3.96±0.53^{a}	<25 NTU
Conductivity mS/cm	0.03±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.04±0.00^{a}	0.04±0.00^{a}	-
pH	7.21-8.84	6.13-8.60	6.68-8.41	5.59-8.63	7.01-8.26	6.61-8.83	6.55-8.10	6.0-8.5
DO (mg/l)	5.26±1.56^{a}	4.52±1.32^{ab}	4.68±1.95^{ab}	4.22±1.26^{bc}	3.99±0.33^{abc}	3.84±0.21^{abc}	2.99±0.31^{c}	Near saturation
Ammonia (mg/l)	0.05±0.01^{a}	0.05±0.02^{a}	0.05±0.02^{a}	0.04±0.01^{a}	0.03±0.02^{ab}	0.04±0.03^{ab}	0.02±0.02^{b}	<0.1 mg/l
Nitrate (mg/l)	0.65±0.15^{ab}	0.74±0.09^{a}	0.66±0.09^{ab}	0.63±0.14^{a}	0.51±0.14^{b}	0.56±0.01^{a}	0.45±0.01^{b}	<2 mg/l
Nitrite (mg/l)	0.02±0.01^{a}	0.01±0.00^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	<0.1 mg/l
Alkalinity ($CaCo_3$)	9.86±2.83^{ab}	7.69±0.73^{b}	8.71±0.99^{ab}	9.3±2.13^{a}	8.95±0.10^{ab}	10.65±3.5^{a}	8.46±1.08^{ab}	6-400 mg/l
Orthophosphate (mg/l)	0.18±0.07^{a}	0.12±0.07^{a}	0.12±0.11^{a}	0.12±0.09^{a}	0.14±0.01^{a}	0.12±0.09^{a}	0.13±0.10^{a}	<0.5 mg/l

*TWQR= Target water quality range for cold water trout (FAO, 2012)

3.3.7 Multivariate analysis of fish assemblages and environmental variables in the Broederstroom and Debengeni Rivers

The first and second axis accounted for 56.7% of the variation in fish assemblage (Table 3.5). The first axis explains the spatial distribution of species downstream from site 1. The species that was positively associated with the second axis is *S. trutta*. The second axis, on the other hand, exhibited a temporal gradient in the species distribution (Figure 3.14). The first axis is positively associated with *A. uranoscopus, A. natalensis, M. salmoides* and *O. mossambicus*. These species were clustered in the first axis. Low species diversity was observed where *S. trutta* and *O. mykiss* are distributed (Figure 3.14).

The Monte Carlo permutation test for the CCA ordination confirmed a significant variation using the relative abundance data. Axis 1, 2 and 3 accounted 58.8% of the variation of the species data (Figure 3.15, Table 3.5). Axis 1 represented positive loadings of temperature and turbidity and negative loadings of ammonia, nitrate and moderate pools (Table 3.6). The fish species positively associated with this gradient are *A. natalensis*, *A. uranscopus, M. salmoides and O. mossambicus.* Axis 2 had positive loadings of deep pools and slow flow rates. The species associated with this gradient is *S. trutta*. Axis 3 had positive loadings of temperature, moderate flow rates and rock substrates. The species associated with this gradient is *O. mykiss* (Table 3.6).

Table 3.5: Eigenvalues of the correlation matrix of the species-environment relation

Statistic	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	1.0000	0.377	0.049	0.003
Explained variation (cumulative)	41.2	56.7	58.8	58.9
Pseudo-canonical correlation	0.000	0.000	0.000	0.000
Total variation				2.426

Table 3.6: The correlation matrix of fish species and physico-chemical variables

Variable	Axis 1	Axis 2	Axis 3
Altitude	-0.8511	-0.5066	-0.3499
Temperature	0.5704	0.3761	0.5894
Very shallow	0.6612	-0.0117	0.1471
Shallow	0.6909	-0.0717	0.2990
Moderate	-0.8223	-0.7350	-0.2580
Deep	-0.3365	0.5065	-0.1375
Slow flow	-0.0598	0.6053	-0.6025
Moderate flow	-0.0439	-0.6231	0.5725
Fast flow	0.6567	0.0971	0.3445
Scarce	0.3307	-0.5066	0.3445
Moderate	-0.2946	-0.0534	0.2772
Shaded	0.0472	0.4826	-0.5240
Pebble	-0.1925	-0.2147	-0.0019
Mud	-0.0317	0.4366	-0.3704
Gravel	-0.4787	-0.8089	-0.0358
Sand	0.0150	0.5278	-0.4016
Rock	0.2014	-0.4099	0.5521
Conductivity	0.6471	0.6367	0.2602
Ammonia	-0.8610	-0.4383	-0.5778
Nitrate	-0.8219	-0.2418	-0.4746
Nitrite	-0.3394	-0.5430	-0.3715
Alkalinity	0.0447	-0.6275	-0.4636
Orthophosphate	-0.3847	-0.9672	0.0021
Turbidity	0.4143	0.0896	-0.0456

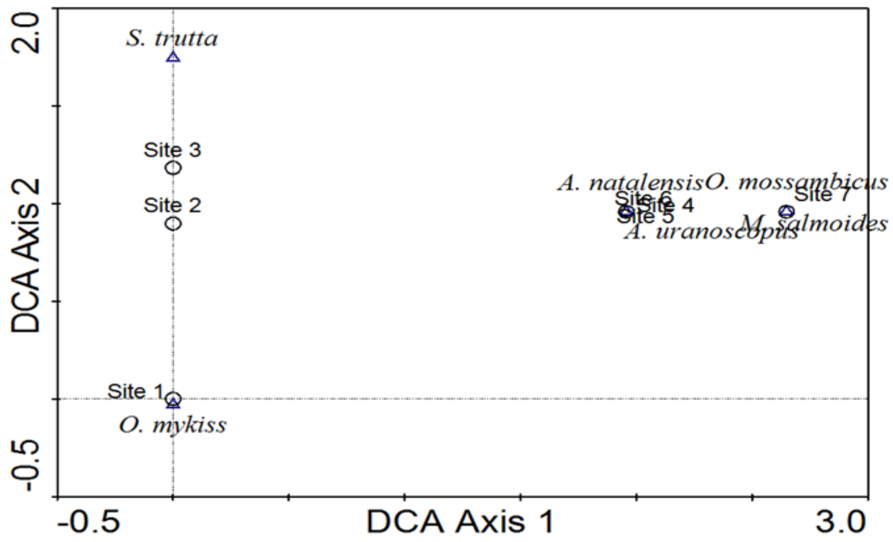

Figure 3.14: Detrended correspondence analysis (DCA) based on the species presence-absence data in the Broederstroom and Debengeni Rivers.

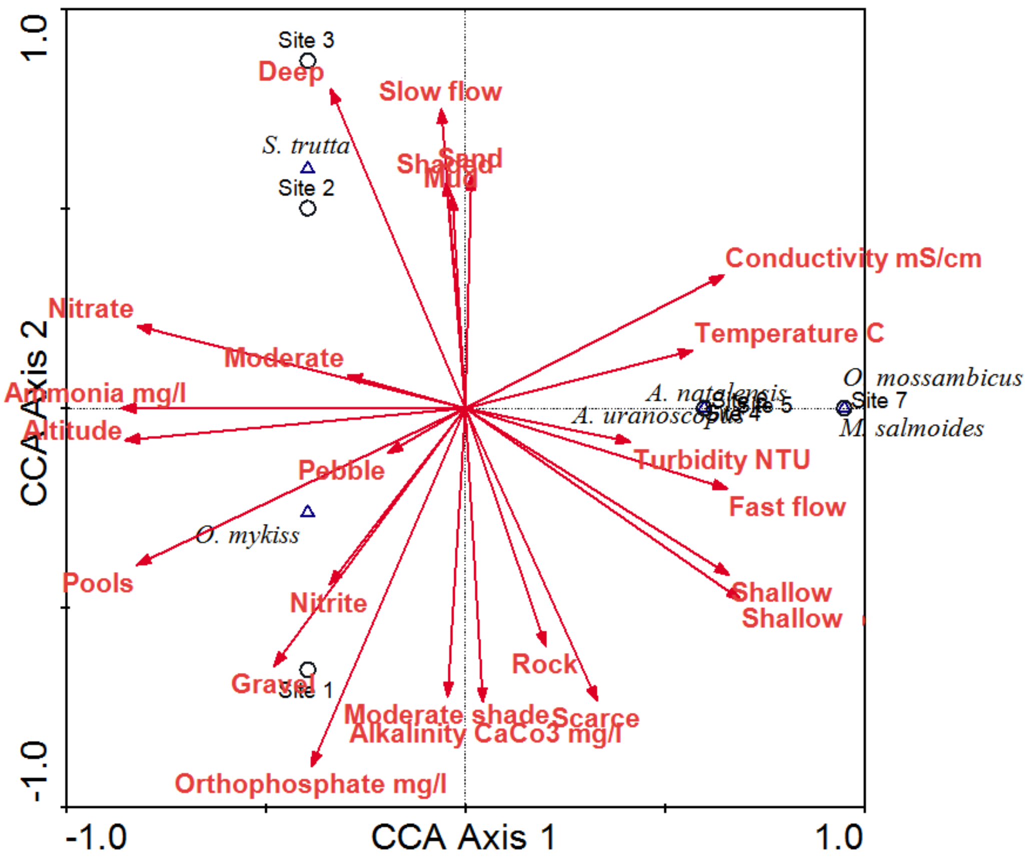

Figure 3.15: CCA plot of the relationship between habitat variables and fish species caught in the Broederstroom and Debengeni Rivers.

3.3.8 Standardized selection ratio (SSR) for trout in the Broederstroom River

CCA showed the relationship between fish species and environmental variables. The variables were further tested using SSR to show which of the habitat variables were most utilized by trout. Both *O. mykiss* and *S. trutta* utilized gravel than mud and sand (Table 3.7). *Oncorhynchus mykiss* utilized almost all the depths compared to *S. trutta* which preferred deep pools (Table 3.7). Temperatures below 15°C were the most preferred with no capture at temperatures above 18°C (Table 3.7). *S. trutta* preferred slow flow rates compared to fast flow rates by *O. mykiss*. *Salmo trutta* preferred shaded habitats, whereas *O. mykiss* was caught in almost all riparian covers (Table 3.7).

Table 3.7: Estimation of selection indices for the occurrence of *Oncorhynchus mykiss* and *Salmo trutta* on different habitats portions within the Broederstroom River

Habitat	Selection index ($\hat{W}i$)		Standardized index (SSR) ($\boldsymbol{Bi}$)	
	O. mykiss	*S. trutta*	*O. mykiss*	*S. trutta*
Substrate				
Mud	0	1.05	0	0.144
Sand	1.011	1.488	0.179	0.204
Gravel	2.868	3.807	0.508	0.522
Pebble	0.862	0.952	0.153	0.131
Rock	0.907	0	0.161	0
Total	5.648	7.297	1	1
Depth (m)				
Very shallow	2.269	0	0.388	0
Shallow	2.32	1.147	0.396	0.349
moderate	0.815	0.518	0.139	0.158
Deep	0.452	1.621	0.077	0.493
Total	5.856	3.286	1	1
Temperature (°C)				
<15	0.974	1.119	0.47	0.646
15-18	1.09	0.614	0.528	0.354
18-21	0	0	0	0
Total	2.064	1.733	1	1
Flow rate (m/s)				
Slow	0.636	1.88	0.057	0.578
Moderate	0.760	0.222	0.068	0.068
Fast	9.727	1.152	0.875	0.34
Total	11.123	3.254	1	1
Riparian cover (%)				
Scarce	2.162	0.289	0.605	0.105
Moderate	1	0.469	0.28	0.169
Shaded	0.409	2.008	0.115	0.726
Total	3.571	2.766	1	1

3.3.9 Logistic regression for trout in the Broederstroom River

SSR showed the most utilized variables. Logistic regression (P<0.05) was then done to highlight which variables from the utilized are most influential to the presence of trout. *Oncorhynchus mykiss* was influenced by temperature (<15°C) and fast flow rate (Table 3.8). *Salmo trutta* was influenced by depth (deep pools), slow flow rate, temperature (<15°C) and riparian cover (P<0.05). Depth and riparian cover had no influence on *O. mykiss* (Table 3.8). Temperature and coarse substrates (gravel and pebble) were the common variables influencing the presence of both *O. mykiss* and *S. trutta.*

Table 3.8: Logistic model results for the relationship between habitat variables and *Oncorhynchus mykiss* and *Salmo trutta* at the Broederstroom River

Species	Variable	b	*SE*	Wald	Sig	Odds ratio
O. mykiss	Coarse substrate	-0.847	0.379	4.995	0.025	0.429
	Temperature (<15°C)	2.131	0.787	7.335	0.004	4.853
	Fast flow rate	-2.108	1.112	3.596	0.038	0.122
S. trutta	Coarse substrate	-1.444	0.540	7.146	0.008	0.236
	Depth (deep pools)	1.580	0.582	7.355	0.003	4.843
	Slow flow rate	-0.761	0.404	3.550	0.050	0.467
	Temperature (<15°C)	1.794	0.576	9.698	0.002	6.016
	Riparian cover (%)	-2.108	1.112	3.596	0.054	0.122

**The Wald statistic tests the significance of each coefficient (b). Overall goodness of fit for O. mykiss (X^2= 9.337; r^2=0.04) and S. trutta (X^2=10.101; r^2= 0.427)*

3.4 DISCUSSION

Six species representing the salmonidae, amphilidae, centrachidae and cichlidae families were recorded. The fish species were numerically dominated by the salmonid, *O. mykiss. Amphilius uranoscopus* was second and *S. trutta* third in dominance. It is important to note that except *A. uranoscopus*, the two other dominant species are non-native in the area. Lévêque (1997) and Jackson *et al*. (2001) reported that fish assemblages are influenced by environmental conditions of the river that they are morphologically and physiologically adapted to. In this study, trout is considered to be well adapted to the environmental conditions at site 1 to 3 as trout is known to prefer high oxygen levels and cold water temperatures (Raleigh *et al*., 1984). This study agrees with the descriptions by Raleigh *et al*. (1984), since trout was distributed at sites with high oxygen levels because of fast flow rate and cold temperatures.

The presence of *Amphilius* species in Debengeni River may be due to the absence of trout which is known to predate on this species. Furthermore, the low fish diversity in both the Broederstroom and Debengeni Rivers can be attributed to the altitude and temperature regimes of the Magoebaskloof area. Water bodies in Limpopo Province are dominated by warm water fish such as cichlids (Skelton, 2001). Most cichlids such as tilapias are warm water fish species that thrives at an optimum temperature of 28°C. Hence, a cichlid recorded in this study (*O. mossambicus*) was localized at low altitude sites where water temperatures were greater than 14°C and turbid when compared to the sites at higher altitudes where temperatures were less than 13°C.

Oncorhynchus mykiss had higher mean lengths than *S. trutta* in the Broederstroom River. Factors influencing fish growth in an area includes among others; environmental variables and food availability (Kadye *et al.*, 2013). This result implies that *O. mykiss* might be self-sustaining its populations as different length groups were observed. However, the data is limited in scope and difficult to give meaningful conclusions on the self-sustainability of *O. mykiss* in the area. *Salmo trutta* could be solely sustained through the restocking's by the Haenertsburg trout association (HTA) as their mean lengths are dominated by mostly cohorts without juveniles to show natural recruitment. Headwaters are usually the domain of small trout, except when spawning adults may be present (Picker & Griffiths, 2011). Typically, in the Broederstroom River, similar trend as described by Picker and Griffiths (2011) was observed. Small trout were encountered as opposed to large ones at the headwaters of the Broederstroom River except during the winter months. The relative abundance (CPUE) of trout also increased during the winter months. This coincides with the onset of moderate flow rates and colder water temperatures in this region. This indicates that the breeding activities may be a function of these environmental ques.

The growth of both *O. mykiss* and *S. trutta* is low in the Broederstroom River. The Von-Bertalanffy growth curve fitted the observed length-at-age data fairly well. The theoretical asymptotic length differed only slightly with the largest length recorded for each species. Trout generally exhibit a rapid linear growth rate in the first year of life. Skelton (2001) reported that under optimal natural conditions, trout in South Africa can obtain lengths of 18 cm in one year and 25 cm after two years. However, this study showed a different result to the one reported by Skelton (2001) as trout in the Broederstroom River grew up to 22 cm in 3 years. Annual rings on the scales are often laid with a change in season (Moyo & Fernando, 1999), with most of their young life spent in the hatchery, seasonal laying of the rings on the scales is not as efficient as when they are in a natural environment. More work needs to be done to validate the formation of the first annulus by collecting and analyzing scales from recruits at the hatchery against the naturally recruited stock from the river to validate the number of annulus formed per year.

The asymptotic length (L_∞) of both *O. mykiss* and *S. trutta* is low in the Broederstroom River. Little is known about the L_∞ of *O. mykiss* and *S. trutta* in South African rivers. However, Nthimo (2000) has reported *O. mykiss* to have an L_∞ of 52.6 cm in a lentic system in Katse Dam, Lesotho. Trout in lotic systems are smaller than those in lentic system (Picker & Griffiths, 2011). This implies that the L_∞ of *O. mykiss* in the Katse Dam cannot be compared to *O. mykiss* in the Broederstroom River since they are in different systems. However, Arslan *et al.* (2007) reported L_∞ of *S. trutta* in a lotic system in Turkey to be 33.27 cm which is higher than that of *S. trutta* and *O. mykiss* in Broederstroom River. Regarding the same age classes, trout in the Broederstroom River are smaller than those reported by Arslan *et al*. (2007).

The Broederstroom River is dominated by rocky substrates which is a limiting factor to trout reproduction. Literature has shown that trout prefers gravel and sand to reproduce (Picker & Griffiths, 2011; Skelton, 2001). No historical data is available on the parameters that affect natural reproduction of trout in marginal areas like Magoebaskloof. However, studies elsewhere show that environmental variables are important determinants of trout reproduction (Manning & Kime, 1985; Taranger & Hansen, 1993; Vikingstad *et al*., 2008). The Broederstroom River fall within the water quality range for trout to reproduce. However, the factors that could be contributing to the shortfall in their reproduction could be the short winter period in the area. The shortness of winter and long summer in the area doesn't give the trout enough time to be well conditioned for spawning. Therefore, further studies on the reproduction phases and gonad maturation in the Broederstroom River are needed to safely conclude that this is a marginal area for the trout to reproduce. This study has shown through SSR that gravel is utilized by the trout in the river. The lack of adequate gravel beds in the rocky dominated Broederstroom River could be the limiting factor for trout reproduction in the river. Gravel beds are crucial for the formation of redd and incubation of eggs.

Turbidity and total dissolved solids (TDS) are within the range of trout in the Broederstroom River. The catchment around the Broederstroom River is dominated by plantations, which retain soil erosion and wash off. This probably explains the low turbidity and TDS levels in the river, which in turn favours the survival of the trout in the river. No trout catches were recorded at sites where plantations were scarce and or absent, which resulted in high turbidity levels such as site 7. Shaw and Richardson (2001) have also shown that abundance of trout declined in simulations with higher turbidity regimes.

The Broederstroom and Debengeni Rivers are characterized by low conductivity. The low conductivity could be due to the underlying granite surface, which over time have been leached due to high rainfall activity and no longer produce a lot of ions. The low conductivity values also reflect low rates of chemical weathering in the catchment soils (Enge & Kroglund, 2010). The soils in the area are dominated by clay. This could be the reason of the low rates of chemical weathering in the area as they are used up by the soils. CPUE provides a measure of population density in water bodies. The low CPUE in the area could be a function of the low conductivity.

This observation is not unique as it is consistent with the findings by Enge and Kroglund (2010) in Norway and should be evaluated as a fish-restricting element in other high mountain areas too.

Physico-chemical parameters were important determinants of trout distribution in the Broederstroom River. The presence and abundance of fish in rivers depends on many factors (Skelton, 2001), water quality being one. The amount of dissolved oxygen, pH, ammonia, nitrate and nitrite, alkalinity and orthophosphates levels can all influence the habitat suitability of water for fish (FAO, 2005-2012). When changes in land-use occur in river catchments, or pollutants enter waterways, one or more of these key variables can change such that the water quality deteriorates and distribution of fish is affected (FAO, 2005-2012). *Oncorhynchus mykiss* and *S. trutta* are among the more sensitive freshwater fish species (Skelton, 2001). When oxygen levels drop below 2 mg/l, trout become stressed and emigrate to more suitable waters (FAO, 2005-2012). But, when the pH drops below 4 or ammonia levels increase beyond 0.5 mg/l, mortality occurs (FAO, 2005-2012).

The Broederstroom River presented habitat associations that differed for *O. mykiss* and *S. trutta*. This was confirmed using multivariate statistical analysis. Raleigh *et al.* (1984) described the optimal river habitats for both *O. mykiss* and *S. trutta* as clear and cold water, silt free coarse habitats, vegetated stream banks, abundant instream cover and a relatively stable flow. This study generally confirmed this description of trout's habitat association. Both species in this study preferred habitats with coarse substrates, low temperatures (<15°C) and silt free. The exception was that *S. trutta* was associated with shaded habitats as opposed to *O. mykiss* which was caught in almost all sampled riparian cover. The importance of riparian cover in the functional ecology of streams is documented. It provides, among other functions, shade and a supply of invertebrate food items (Growns *et al*., 2003), with *S. trutta* an opportunistic predator relying on fish, aquatic and terrestrial invertebrates (Raleigh *et al*., 1984; Kawaguchi & Nakano, 2001). Riparian cover was a significant variable for the distribution of *S. trutta* in this study since it provided shade and an adequate influx of invertebrates.

Flow rate, temperature and depth are significant contributors to the presence of *O. mykiss* and *S. trutta* in the Broederstroom River. CCA, SSR and logistic regression indicates and agrees that *S. trutta* was significantly associated with slow flow rates and deep habitats, compared to *O. mykiss* which preferred fast flow and almost all depths. These observations are consistent with the descriptions of trout's habitat by Raleigh *et al.* (1984) and Skelton (2001). Both *O. mykiss* and *S. trutta* were significantly associated with low temperatures and was the common variable associated with both *O. mykiss* and *S. trutta* as both were not recorded at temperatures above 18°C.

Magoebaskloof area is marginal for *O. mykiss* and *S. trutta*. As a result they can only be distributed at higher altitudes. Therefore, this study agrees with the hypothesis that the area is marginal for the trout species to thrive. The low growth of both *O. mykiss* and *S. trutta* also confirms that the area is marginal. The factors that could be contributing to the area to be marginal are factors such as the nature of the bottom substrate. The Broederstroom River does not have enough sand and gravel and multivariate analysis has shown that gravel is an important substrate for these species. The physico-chemical parameters might be within the target range for trout but the high turbidity levels during rainy seasons at low altitude sites also make the area marginal for the trout.

CHAPTER 4:

IDENTIFYING THE IMPACTS OF TROUT ON MACROINVERTEBRATES WITHIN A MARGINAL BROEDERSTROOM RIVER, LIMPOPO PROVINCE FROM SURVEYS AND EXPERIMENTS

4.1 INTRODUCTION

The human-assisted spread of trout around the world for angling and aquaculture has led to a large number of invasions in freshwater systems (Ricciardi & MacIsaac, 2011). In many cases, trout have had strong impact on native fish and community structure of macroinvertebrates (Eby *et al.*, 2006). In South Africa, trout have been widely introduced for angling purposes and annual restocking's continue in areas such as Magoebaskloof, Limpopo Province. Trout are aggressive, opportunistic predators that feed primarily on drifting aquatic and terrestrial invertebrates, but become increasingly piscivorous as they grow. As a result of their biology, they alter the structure and function of benthic communities in streams globally and locally (Buria *et al*., 2010; Shelton *et al*., 2015). In many cases, trout have been shown to deplete populations of smaller native fish through predation and/or competition for space and resources (Cambray, 2003b). This then leads to a decrease in the aquatic and terrestrial abundance of invertebrates (Simon & Townsend, 2003).

Aquatic invertebrates are widely used as indicators in environmental impact studies. Their ubiquity and differential response to different categories of disturbances makes them excellent candidates for predation studies (Rosenberg & Resh, 1993; Shelton *et al*., 2015). In predation impact studies, especially by non-native fish, aquatic invertebrates are highly susceptible and exhibit responses ranging from simple interactions such as reduction or local extinction of populations, to complex interactions such as trophic cascading (Flecker & Townsend, 1994; Nyström *et al*., 2003; Williams *et al*., 2003; Shelton *et al*., 2015). Experimental studies also indicate that when predators become established, they tend to remove the most vulnerable macroinvertebrate taxa and the resultant community shows little response to predation related disturbances (Meissner & Moutka, 2006; Shelton *et al*., 2014).

In Limpopo Province, the Broederstroom River is a habitat to a number of macroinvertebrate taxa. Trout was first introduced in the area in the early 1900s and are supplemented annually by the HTA. Some studies elsewhere have shown that although macroinvertebrates are widespread, negative responses in diversity and abundance are likely where their habitat is invaded by trout (Kadye & Magadza, 2008; Shelton *et al*., 2015). It was therefore, hypothesized that the impact on macroinvertebrates by trout will not be severe in the Broederstroom River since the area is considered to be marginal for trout as shown in chapter 3.

In this chapter, the impact of trout on macroinvertebrates in the Broederstroom River was assessed. The composition of benthic invertebrate using short-term fish inclusion and exclusion experiments was investigated. The main objective of this study was to determine the impact of trout on macroinvertebrate diversity and abundance within mountain streams of Magoebaskloof area.

4.2 METHODS AND MATERIALS

4.2.1 Study systems and data collection

In this study, sites that had similar riparian vegetation and physico-chemical parameters were selected from the Broederstroom River which has ambient populations of trout, and a neighbouring Debengeni River which is trout free. This was done to make sure that the study sites were similar and no variables other than trout presence differed amongst them. Three sites (Figure 4.1) were selected in each river as outlined in Chapter 3 (3.2.2).

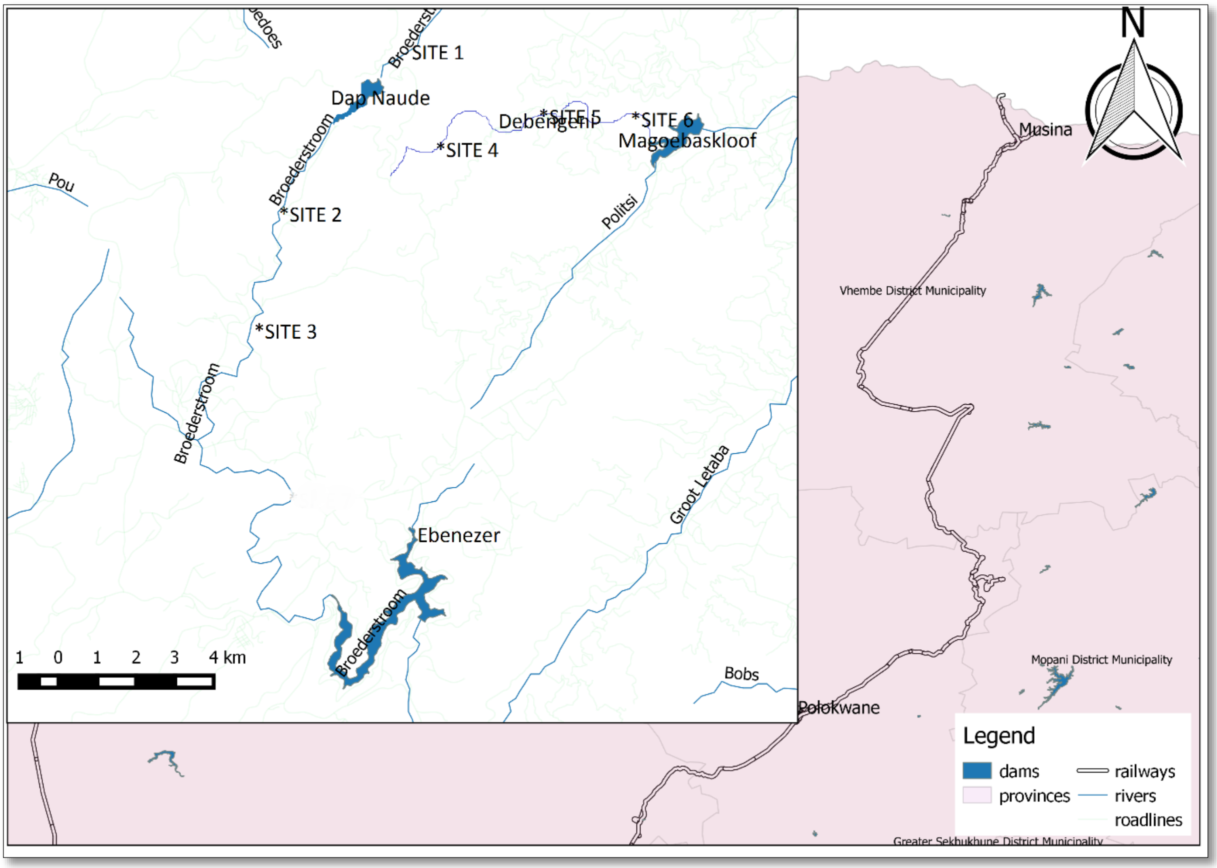

Figure 4.1: Map of the sites at Broederstroom River which is trout invaded and the Debengeni River which is trout free.

4.2.2 Fish sampling

Each sampling site was divided into three reaches, each of which was then blocked by a fine meshed net at either end before sampling. Three transects were set perpendicular to the direction of flow to measure physical habitat variables within each reach. The measurements made were depth and substrate types at three points along each transect. Habitat assessment and fish capturing were done as outlined in Chapter 3 (3.2.3).

4.2.3 Stomach content analysis

Trout (*O. mykiss* and *S. trutta*) caught from February 2014 to February 2015 were sacrificed by severing the spinal cord, then assigned to different standard length groups (2-5, 5-10, 10-15, 15-20 and >20 cm). The stomach from each fish was then removed through dissection of the abdomen (Figure 4.2). Each stomach was awarded an index of fullness from 0 to 1. An empty stomach scored 0; 0.25 for a quarter full; 0.5 half full; 0.75 three quarters full and 1 for a full stomach and grouped seasonally. The removed stomachs were then put in 10% formalin in individually marked sample bottles. In the laboratory, each stomach was opened and the entire gut contents were placed in a petri dish containing 10 ml distilled water (Figure 4.3).

Figure 4.2: Dissection and removal of stomach for content analysis.

Figure 4.3: Gut contents placed in a petri dish containing 10 ml distilled water.

Only the portion between the esophagus and the small intestine were used from the removed stomach. This is because digestion is less advanced on these portions and food items were identifiable. The contents were viewed under a light microscope at 50X magnification. The frequency of occurrence was calculated for each identified food item as done by Hyslop (1980):

$$\% \text{ Occurrence} = \frac{\text{Number of stomachs with food items}}{\text{Total number of stomachs analyzed}}$$

The diversity of food items among different size groups was determined using the Shannon Weiner diversity index (Shannon and Weaver, 1949):

$$H' = n \log n - \sum (f_i \log f_i)$$

Where: H'= Shannon's diversity index

n= total number of frequencies

f_i= occurrence frequency of the item

The overlap of food items among the categorized size groups was measured using the Schoener's index of similarity (Colwell & Futuyma, 1971) based on percentage frequency of occurrence:

$$C_{jk} = 1 - {}^{1}/_{2} \sum_{I} /P_{ij} - P_{ik}$$

Where: C_{jk} = resource (food type) overlap between species j and k

P_{ij} and P_{ik} = proportions of the i^{th} resource used by species j and k

Overlap in diet between species j and k is complete when C_{jk}=1 and is absent when C_{jk}=0.

4.2.4 Macroinvertebrate sampling and identification

To compare the impact of trout on the diversity and abundance of macroinvertebrates, three sites in Broederstroom River and three in Debengeni River were sampled. The samples were collected in replicates monthly from February 2014 to February 2015. Sampling was done as outlined by South African Scoring System, Version 5 (SASS-5) sampling protocol (Dickens & Grayham, 2002).

Approximately 20 m to 25 m of river length were sampled at each site. Kick and sweep sampling techniques were employed using a SASS net (Figure 4.4).

Figure 4.4: Macroinvertebrate sampling using a SASS net.

Samples were collected and analysed separately from the three pre-defined SASS-5 biotope groups (i.e. stones in current; marginal and aquatic vegetation and sand in current), where present. As per the protocol, stones-incurrent (SIC) were sampled for two minutes, and gravel, sand and mud (GSM) for a total of one minute, while 2 m of marginal vegetation were sampled for two minutes. Sampling of each biotope was conducted over the whole sampling area available. At each sampling site, macroinvertebrate samples collected from each of the biotope groups were placed in separate sampling trays for sorting and identification. Plastic, white-coloured trays, approximately 30 × 45 cm in size with a depth of 10 cm, were used. After adding river water from the site to each tray, and carefully removing debris, the macroinvertebrates collected from each biotope group were added to 5*l* buckets and preserved in 10% formalin for identification. In the laboratory, uncertain family-level identifications were confirmed using optical microscopes. Identification was undertaken to the family level, using a photographically illustrated identification guide (Gerber & Gabriel, 2002) for aquatic invertebrates of South African rivers.

4.2.5 Macroinvertebrate experiment

To compare macroinvertebrate responses to the presence of trout, an experiment in the headwater sites (siite 1, 2, 4 and 5) was conducted. Cage experiments were replicated within sites that had comparable physico-chemical parameters. This was considered as the most rigorous approach available to offset the potential for the responses to be confounded by small-scale prey movements and historical effects of trout presence. At each site, a completely randomized experimental design with two treatments: (1) fish inclusion and (2) fish exclusion were used. Each treatment had three replicate cages. Cages, each measuring 1 m in length, width and height, respectively, were constructed on steel frames. The cages were fitted with fine gauze mesh (3 mm) and placed at depths of 0.3–0.7 m with homogenous pebble substrate that was the dominant substratum.

All cages were deployed in July 2014 and inclusion cages were stocked with five trout of between 10 and 16 cm total length (this size group was chosen because small size trout are insectivores compared to large size trout which are piscivorous (Shelton *et al*., 2015)) and were sampled in September and November 2014. During sampling, pebbles were picked and washed with stream water into a sampling bucket and then filtered through a macroinvertebrate net. The organisms were preserved in 10% formalin and later 10% ethanol. In the laboratory, they were sorted and identified to family level for most taxa using a stereomicroscope. Gerber and Gabriel's (2002) was used to identify macroinvertebrates.

4.2.6 Data analysis

The diversity of food items among different size groups and diversity of the different taxa were determined using the Shannon Weiner diversity index (Shannon & Weaver, 1949). One way analysis of variance (ANOVA, $P<0.05$) was used to test for significant differences in total macroinvertebrate abundance between the sites. The Shannon-Weiner diversity index was also used to determine the diversity of macroinvertebrates in sampled sites and in exclusion and inclusion experiments. The Shannon-Weiner diversity index was then subjected to ANOVA ($P<0.05$) to determine if there were any statistically significant size related shifts in the food types.

4.3 RESULTS

4.3.1 Fish assemblages in the Broederstroom and Debengeni Rivers

The common mountain catfish *Amphilius uranoscopus* dominated the native fish assemblage at uninvaded sites (4 to 6). In contrast, rainbow trout (*O. mykiss*) was the dominant species at invaded sites (1 to 3), making up 71% of the fish assemblage by numbers at the invaded sites (Table 4.1).

Table 4.1: Fish species sampled across the sites within the Broederstroom and Debengeni Rivers from February 2014 to February 2015

Family	Species	River					
		Broederstroom			Debengeni		
Sites		1	2	3	4	5	6
Salmonidae	*Oncorhynchus mykiss**	68	31	12			
	*Salmo trutta**	1	28	16			
Amphilidae	*Amphilius uranoscopus*				17	9	36
	Amphilius natalensis				7	9	19

** indicates that the species is non-native in the area*

4.3.2 Establishing the impact of trout through stomach content analysis

A total of 156 stomachs of trout (*O. mykiss* and *S. trutta*) were analyzed from February 2014 to February 2015. Majority of *O. mykiss* stomachs had food, however, 39.6% of them were empty. A similar trend was observed for *S. trutta* with 37.8% empty (Table 4.2). The fullness of the stomachs varied with season. Feeding intensity was higher in winter and lower in summer (Figure 4.5).

Table 4.2: Categorization of stomach fullness of both *Oncorhynchus mykiss* and *Salmo trutta* in the Broederstroom River sampled from February 2014 to February 2015

Fullness	Number of samples		Percentage (%)	
	O. mykiss	*S. trutta*	*O. mykiss*	*S. trutta*
0	44	17	39.6	37.8
0.25	28	11	25.2	24.4
0.5	19	9	17.1	20.0
0.75	13	6	11.7	13.3
1	7	2	6.3	4.4
Total	111	45	100	100

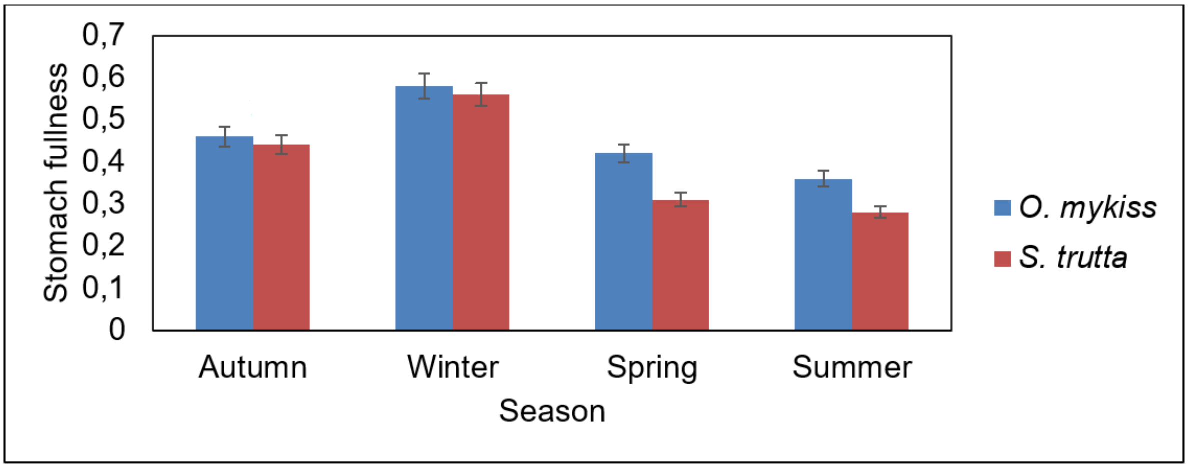

Figure 4.5: Seasonal stomach fullness of *Oncorhynchus mykiss* and *Salmo trutta.*

The food items found in the stomachs were categorized into four groups excluding unidentified items. These were aquatic invertebrates (which comprised of odonates, zooplankton, diptrans, chironomids, moths and Potamonautidae), terrestrial insects (Pentatomidae), fish eggs and detritus (Table 4.3). Aquatic invertebrates were dominated by odonates such as Gomphidae (Figure 4.6) which was the most frequent food item in the category followed by Potamanautidae. The diversity of food items increased with increasing fish size, the highest score was 1.43 for trout >20 cm in length but did not differ significantly with the other length groups (Table 4.3).

Table 4.3: Food types expressed as % frequency of occurrence in the stomachs of the different length groups of *O. mykiss* and *S. trutta* in the Broederstroom River

Length groups (Standard length)	2-5 cm	5-10 cm	10-15 cm	15-20 cm	>20 cm
No. of fish stomachs assessed	15	25	29	22	14
Food types					
Detritus	20.00	16.00	6.89	4.55	14.29
Terrestrial invertebrates	0.00	0.00	13.79	36.36	42.86
Fish eggs	0.00	0.00	10.34	31.81	35.71
Aquatic invertebrates	60.00	72.00	100	100	100
Unidentified items	0.00	16.00	27.58	45.45	35.71
Shanon-Weinner index (H’)	0.57[a]	0.82[a]	1.13[a]	1.30[a]	1.43[a]

The dietary overlap was highest (98%) between the 2-5 and 5-10 cm length group (Table 4.4). The lowest dietary overlap (42%) was between the 15-20 cm and >20 cm length groups. The overlap decreased with an increasing size group.

Table 4.4: Values of food overlap (Schoener's index of similarity) for trout in Broederstroom River

Length (cm)	5-10	10-15	15-20	>20
2-5	0.98			
5-10		0.93		
10-15			0.45	
15-20				0.42

4.3.3 Establishing the impact of trout through macroinvertebrate composition

Thirty nine macroinvertebrate taxa belonging to sixteen groups were recorded (Table 4.5 and Figure 4.6). Thirty one taxa were recorded in the Broederstroom River compared to thirty four in the Debengeni River. In comparison, within the Broederstroom River, dragon flies (Gomphidae) and crustaceans (Potamonautidae) were less abundant when compared to Debengeni River (Table 4.5). However, there is no significant difference in diversity of macroinvertebrates between the sites (Table 4.5). The Broederstroom River (Site 1 to 3) had less total macroinvertebrate abundance compared to the Debengeni River (Site 4 to 6). Site 2 had the lowest abundance and site 6 the highest.

Table 4.5: Macroinvertebrate taxa abundance (No.m^{-2}) in sites for all samples and substrates from the Broederstroom and Debengeni River, Limpopo Province, South Africa

		Broederstroom River			Debengeni River		
Group	Taxa	1	2	3	4	5	6
PORIFERA					4	1	3
TURBELLARIA		9	3	14	8	10	11
ANNELIDA	Oligochaeta	24	33	28	7	3	6
CRUSTACEA	Potamonautidae	30	13	27	101	43	88
HYDRACARINA						2	
PLECOPTERA	Perlidae	7	9	5	1	1	
EMPHEMEROPTERA	*Baetidae* sp.	43	78	130	88	104	93
	Leptophleblidae	5	2				
	Tricorythidae	5	8	4		2	
ODONATA	Chlorolestidae	2	4		2		
	Coenagrionidae					3	
	Lestidae	1	1	1	2	1	1
	Aeshnidae	34	12	44	68	100	83
	Corduliidae				3		4
	Gomphidae	9	18	40	78	88	74
	Lebellulidae	1	7			3	
LEPIDOPTERA	Pyralidae	13	8	4	2	4	1
HEMIPTERA	Naucoridae	6	8	2	1		
	Notonectidae	3	4	2			
	Veliidae	1	3	5	1	3	2
TRICHOPTERA	Hydroptilidae					2	
	Hydropsychidae sp.	33	21	16			
	Leptoceridae	13	11	17	21	9	24
	Pisulidae	16	11	20			
	Sericostomatidae						2
COLEOPTERA	Dytiscidae	8	12	5	15	6	16
	Helodidae	14	6	8			
	Elmidae				1	2	
	Hydrophilidae	4	8	7	1		
DIPTERA	Athericidae	1	3		18	14	29
	Chironomidae	34	21	40	18	16	33
	Culcidae					2	2
	Psychodidae	4	1	3			
	Simulidae	13	4	9	16	14	15
	Tipulidae	7			7	6	
GASTROPODA	Lymnaeidae					3	
PELECYPODA	Corbiculidae	1		3			
	Sphaeridae	2	3				
	Unionidae					2	
OTHER	Tadpoles		19	25	55	66	59
	Frogs		3		2	4	8
	Spiders					4	2
H'		2.9[a]	3.1[a]	2.6[a]	2.5[a]	2.5[a]	2.9[a]
TOTAL ABUNDANCE		466	334	459	520	518	556

**H' values with different superscripts in a row are significantly different (ANOVA, $P<0.05$)*

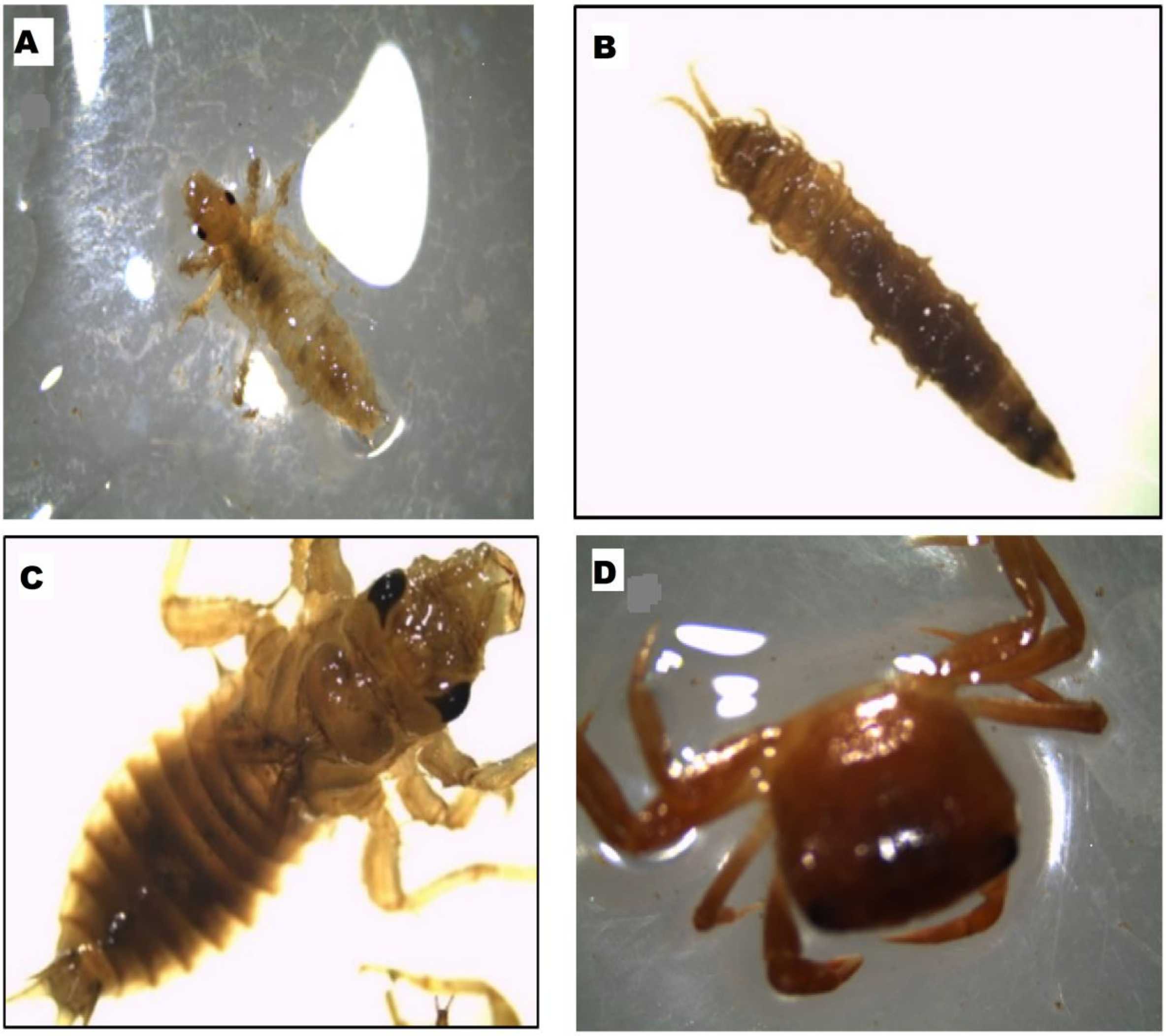

Figure 4.6: Some of the macroinvertebrates taxa targeted by trout. (A) Aeshnidae, (B) Athericidae, (C) Gomphidae and (D) Potamonautidae.

4.3.4 Establishing the impact of trout through inclusion and exclusion experiments

A total of 18 macroinvertebrate taxa, representing ten orders were collected from the experimental cages (Table 4.6). Aeshnidae and Gomphidae were more abundant in exclusion treatments compared to inclusion treatments. By contrast Emphemeroptera (*Baetidae* sp), Hydroptilidae and Chironomidae were more abundant within the inclusion treatments compared to exclusion treatments (Table 4.6). Collectively, exclusion treatments had higher abundance of macroinvertebrates than inclusion treatments, however, there is no significant difference in diversity and abundance of macroinvertebrates between the treatments.

Table 4.6: Mean±SD macroinvertebrate abundance ($No.m^{-2}$) in experimental cages sampled in September and November 2014

Groups	Taxa	September		November	
		Exclusion	Inclusion	Exclusion	Inclusion
TURBELLARIA		4.33±1.25	2.00±0.00	4.33±1.24	-
ANNELIDA	Oligochaeta	5.67±3.85	-	-	-
CRUSTACEA	Potamonautidae	-	-	11.3±2.49	3.00±1.00
PLECOPTERA	Perlidae	-	-	-	4.50±1.50
EMPHEMEROPTERA	*Baetidae* sp.	9.00±5.00	21.3±5.74	10.0±5.89	32.7±9.39
ODONATA	Chlorolestidae	4.50±1.50	-	-	-
	Lestidae	-	-	2.50±1.50	-
	Aeshnidae	15.3±2.49	5.50±2.50	9.00±2.94	3.33±0.94
	Gomphidae	26.0±13.1	-	13.3±2.49	6.00±1.63
	Lebellulidae	0.33±0.47	0.67±0.47	5.00±2.00	-
TRICHOPTERA	Hydroptilidae	-	5.33±3.39	-	-
	Hydropsychidae	-	-	-	2.00±1.00
	Leptoceridae	3.67±1.69	-	4.33±2.05	-
COLEOPTERA	Dytiscidae	-	-	7.00±3.26	-
	Hydrophilidae	-	-	-	8.67±4.78
DIPTERA	Athericidae	5.00±1.00	9.00±1.00	-	6.33±2.49
	Chironomidae	15.00±2.16	18.3±1.69	14.3±1.24	11.0±1.41
	Culcidae	3.00±0.82	1.50±0.50	-	-
OTHER	Tadpoles	8.33±6.84	12.0±0.00	17.7±3.68	7.33±4.99
H'		2.17[a]	1.90[a]	2.26[a]	1.97[a]
TOTAL ABUNDANCE		8.34±6.88[a]	8.40±7.00[a]	8.97±4.62[a]	8.49±8.48[a]

4.4 DISCUSSION

Trout (*O. mykiss* and *S. trutta*) was the only species recorded at site 1, 2 and 3, whereas *Amphilius* spp*.* were recorded at site 4, 5 and 6. The absence of trout at site 4, 5 and 6 was used to explain the presence of *Amphilius* spp. at these sites since both trout and *Amphilius* spp. prefers the same environmental conditions such as temperature (as shown in chapter 3). Having no historical data in the area, it is assumed that the presence of *Amphilius* spp. at site 4, 5 and 6 implies that *Amphilius* spp. could have been present in the Broederstroom River (site 1, 2 and 3) before the introduction of trout (although no evidence of this was determined in this study), because literature has shown that trout predates on *Amphilius* spp. (Kadye *et al*., 2013; Shelton *et al*., 2015).

The feeding intensity of trout is high in winter and low in summer in the Broederstroom River. This usually happens when fish are introduced outside their optimal ranges, where a series of biotic and abiotic factors affect the fish in different ways (Kadye *et al*., 2013). Literature have shown that when abiotic factors such as temperature and dissolved oxygen are not favourable for trout, it is stressed (Skelton, 2001; Kadye & Magadza, 2008). When stressed, its feeding activity is reduced. Trout is a cold water fish and introduced outside its natural range in the Broederstroom River (Site 1, 2 and 3), it is likely to get stressed by the changes in abiotic factors such as temperature when the season changes. This was evident since the feeding intensity was higher in winter, implying that conditions such as temperature were favourable than in summer. This observation is consistent with the report by Rasool *et al.* (2012) that variations of stomach fullness and feeding intensity of trout in the upper streams of the Kashmir valley (India) are affected by seasonal changes.

The feeding overlaps which occurred between different size groups suggest a degree of intra-specific competition. The highest overlap was observed between the 2 - 5 and 5 - 10 cm size groups. This implies that although fish >2 cm had already started including other food items in their diet, aquatic invertebrates such as chironomids, moths and zooplankton were still a major component. The low overlap coefficient on the 15 - 20 and >20 cm confirms the assertion that as trout grows, its diet becomes more specialized and focused to larger macroinvertebrates. Their ability to utilize large macroinvertebrates is because of their streamlined and elongate bodies and evenly distributed fins (Skelton, 2001) that provide stability and maneuverability, which is important for swimming and actively seeking prey.

The diversity of food items increased with increasing fish size. The common and frequent food item found in all length groups is aquatic invertebrates. This is explained by the dominance of small size trout in the Broederstroom River which are known to be insectivores as shown by Shelton *et al*. (2015) that small size trout are insectivores as opposed to large trout which are piscivorous. Fish undergo a size related dietary shift as they all start feeding on zooplankton and later become specialized (Moyo & Fernando, 2009) hence the length group 2 to 5 cm fed on aquatic invertebrates such as zooplankton and later shifted to large invertebrates such as Gomphidae. Trout in the Broederstroom River started feeding on large aquatic invertebrates such as Gomphidae and Athericidae when they were between 5 and 10 cm length group.

There is no significant difference in macroinvertebrate diversity at all sites sampled, however, abundance differed between the sites. For example, high abundance of odonates and potamonautidae were recorded at site 4, 5 and 6 compared to site 1, 2 and 3. Odonates and potamonautidae are usually the most vulnerable group in invaded habitats since they feed on the surfaces of stones and/or on food particles suspended in the water column (Cummins *et al*., 2005). Their feeding behaviour could have likely rendered them vulnerable to trout predation as it overlaps with trout preferred habitats at site 1, 2 and 3 (see chapter 3 for trout's preferred habitats). Samways (1994) also noted that in South Africa, the abundance of odonates appears to be negatively affected by the presence of trout.

Aeshnidae and Gomphidae were more abundant in exclusion treatments compared to inclusion treatments. The high abundance of these taxa in exclusion treatments can be explained by the absence of trout in the treatment. Studies have shown that the most common response of large macroinvertebrates such as Aeshnidae and Gomphidae to the presence of predation is a decline in their densities (Kadye & Magadza, 2008; Johnson *et al*., 2009; Shelton *et al*., 2015). This study's observation also show that trout targets the most conspicuous odonates such as Aeshnidae since their large body sizes and activity makes them visible prey. Marzano *et al*.

(2003) have also shown that the size and biology of odonates such as Aeshnidae and Gomphidae renders them the most vulnerable group to predation by non-native species. This observation is also confirmed by the stomach content analysis results.

Baetids and caddis flies (Hydroptilidae) were more abundant within the inclusion treatments compared to exclusion treatments. These taxa are relatively not vulnerable to trout predation because of their small body sizes. Observations by Shelton *et al*. (2015) mirrors these observations, where *Baetis* sp. were abundant at sites with trout in the Cape Floristic Region (CFR). The lifestyle and morphology of these macroinvertebrates plays a role in their invulnerability, For example, trichoptrans were abundant in inclusion treatments because of their hard plant and sand grain case. In addition, the presence of trout at site 1, 2 and 3 may induce macroinvertebrate morphological adaptations, such as dominance of small-bodied morphs. Lebelluidae and chironomidae also showed resilience to the presence of trout. Their resilience could be attributed to both their lifestyle because they are less active benthic dwelling deposit feeders.

Observations from this study concur with most studies on impacts of introduced trout (Kadye *et al*., 2013; Shelton *et al*., 2015). Most studies have shown that whenever trout is introduced, it either wipes out certain taxa of macroinvertebrates or negatively affect their diversity. This study showed that the presence of trout affects the abundance of macroinvertebrates negatively although no significant differences in diversity of aquatic invertebrates was observed between the rivers. In summary, it can be said that trout in the Broederstroom River are weak regulators of macroinvertebrate diversity but strong regulators of macroinvertebrate abundance. The overall conclusion on whether trout has small or severe impact patterns in the area cannot be meaningful since the data is limited in scope.

CHAPTER 5:

INTERACTION BETWEEN A NON-NATIVE COLD WATER *ONCORHYNCHUS MYKISS* AND NATIVE WARM WATER *CLARIAS GARIEPINUS* IN THE EBENEZER DAM, SOUTH AFRICA

5.1 INTRODUCTION

Ebenezer Dam is an earth-fill type of dam on the Groot Letaba River in Magoebaskloof area, Limpopo Province. It was established in 1959 and its primary purpose is for municipal and industrial usage. Trout have been introduced in the dam and continuous restocking by the Haenertsburg Trout Association (HTA) occurs annually. Trout introduction often poses threats to indigenous species and community stability in natural environments (Cambray, 2003b). The consequences of the introductions in the receiving environment also depends on how trout alters the macroinvertebrates structure and indigenous fish assemblage. Work elsewhere have underlined the role of competitive interactions in the dynamics of freshwater communities, however, little is known in southern Africa (Flecker & Townsend, 1994). In freshwater systems, competitive interactions are triggered by the introduction of fish species that compete with the native fauna (Simon & Townsend, 2003). Therefore, the introduction of trout in Ebenezer Dam could have triggered competitive interactions with native species.

The ecological relationship of a species with the aquatic community is partly influenced by its habitat preferences and feeding patterns. If the introduced fish species is a more efficient predator than the indigenous predator species, it may affect changes in the structure of the habitat and food resource (Dorgeloh, 1995). Trout prefers cold oxygenated water habitats and animal protein food, whereas indigenous species found in Ebenezer Dam such as *Clarias gariepinus* have a wide-range of habitats and food (Beauchamp, 1990; Dorgeloh, 1995; Kadye & Magadza, 2008; Kadye & Booth, 2012; Kadye *et al*., 2013; Shelton *et al.*, 2015).

Little work on interaction between trout and *C. gariepinus* have been conducted in South Africa and none in Magoebaskloof area. Therefore, the objective of this chapter was to outline the degree of interaction between introduced trout and the indigenous *C. gariepinus* in Ebenezer Dam.

5.2 METHODS AND MATERIALS

5.2.1 Study area

The Ebenezer Dam (Figure 5.1) is situated near Haenertsburg Village about 60 km to the east of Polokwane in Limpopo Province, South Africa. The dam is built on the Great Letaba River with a surface area of 386.2 hectares and a mean depth of 20 m (DWA, 2013). The surroundings of the Ebenezer Dam is densely forested and mountainous. The dam water is mainly used for domestic, agricultural, livestock and recreational purposes. Farms and plantations are the dominant land use around the dam. The dam is a popular dam often visited by locals and visitors for picnics, camping, birding, fishing and boating. Anglers at the dam catch largemouth and smallmouth bass, rainbow trout, common carp, tilapia and catfish.

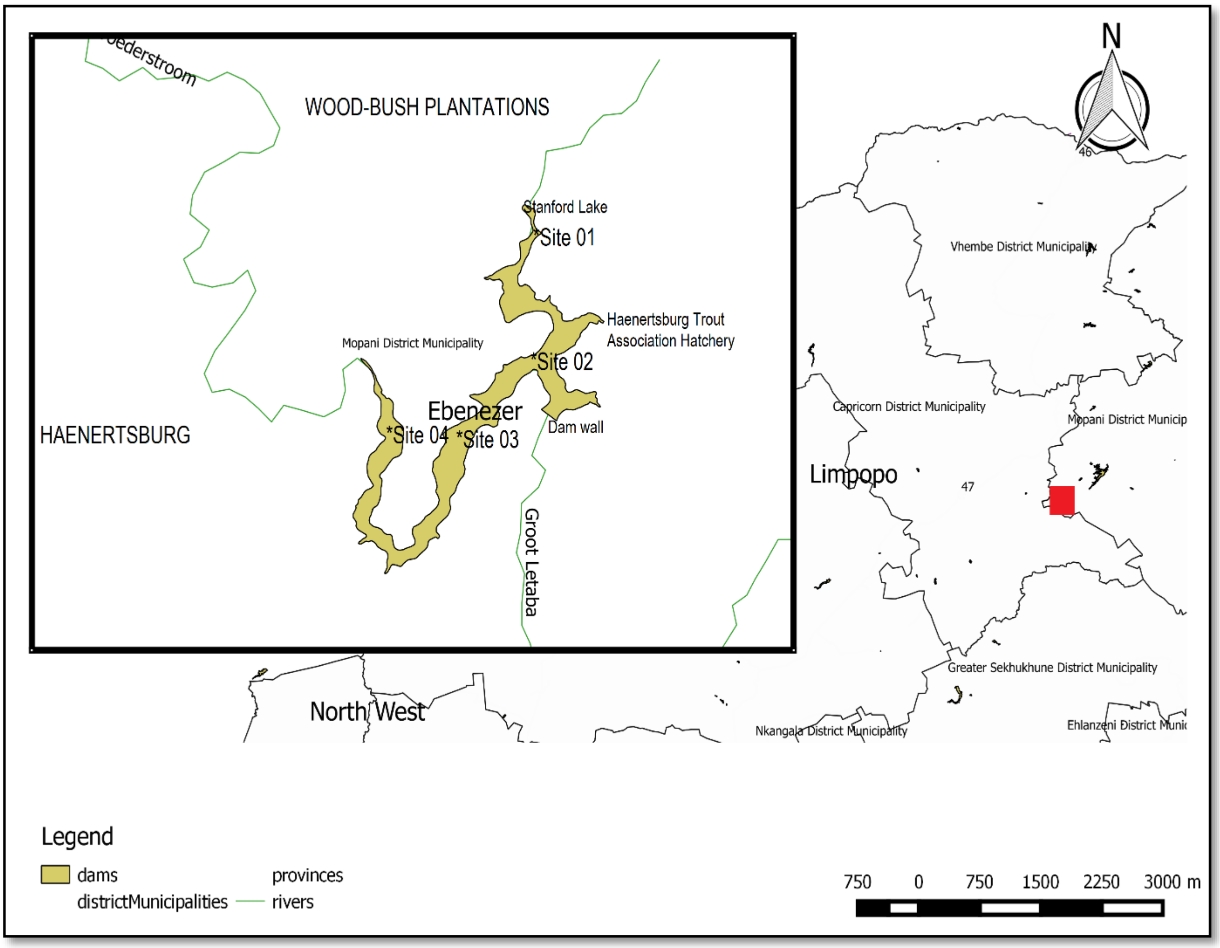

Figure 5.1: Map of Ebenezer Dam indicating the sampling sites (1 to 4).

5.2.2 Sampling sites

Four sites were selected in the Ebenezer Dam (Figure 5.1 and 5.2). Site 1 is at the water fall where the Stanford Lake feeds into the Ebenezer Dam. The water fall is about 8 meters long. The site is dominated by rocks beneath the water fall. Site 2 is situated towards the dam wall. The banks at the site are mostly rocks with low lying shrubs of afro-montane grassland. Site 3 is in the middle of the dam. The banks close to the site are characterized by picnic sites and has *Cyperus sexangularis* marginal vegetation submerged in water. Site 4 is at the inflow where the Broederstroom River feeds into the dam and is characterized by turbid water compared to site 1 to 3 (Figure 5.2).

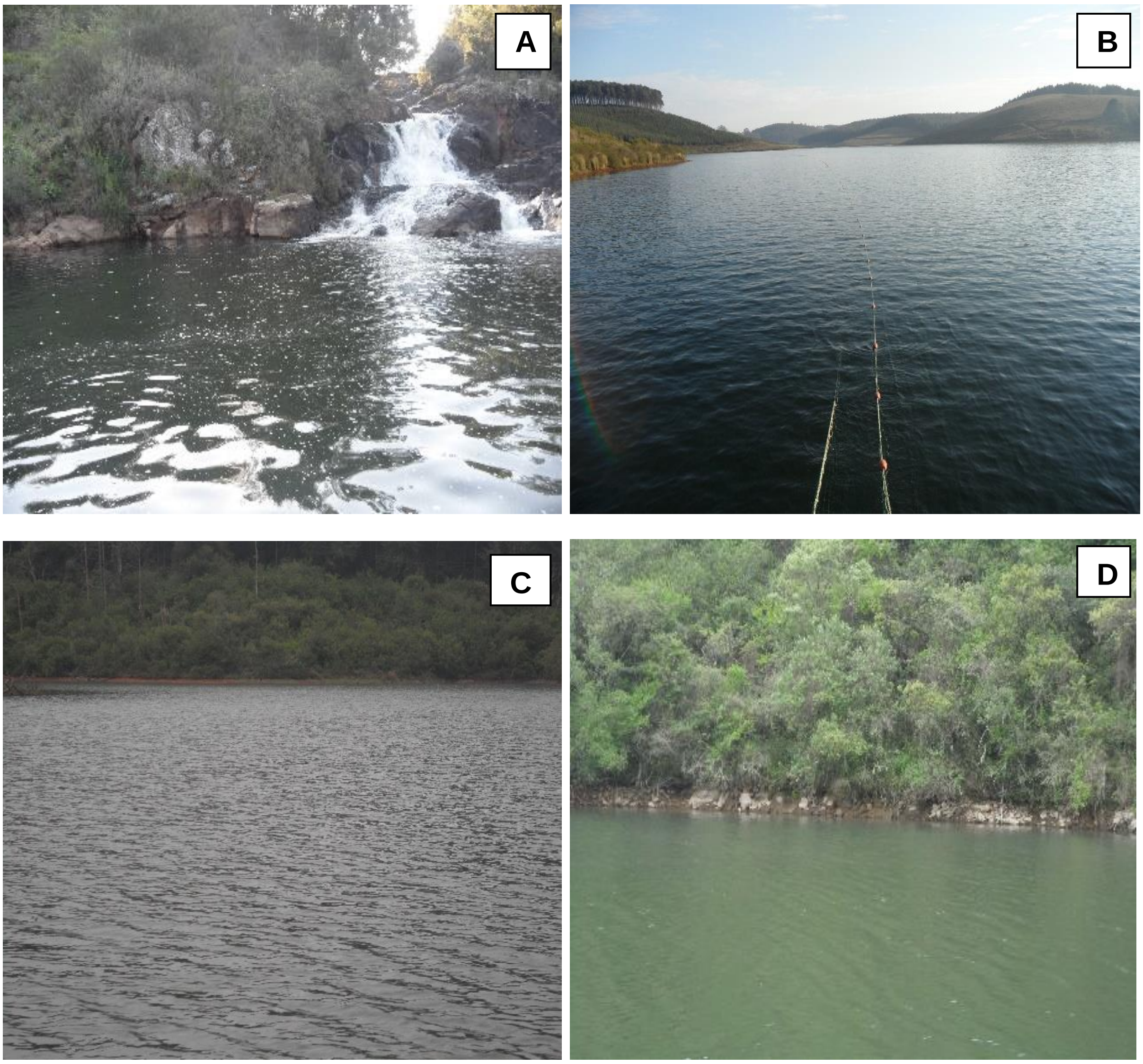

Figure 5.2: (A) Site 1, (B) Site 2, (C) Site 3 and (D) site 4 at the Ebenezer Dam.

5.2.3 Physico-chemical parameters of Ebenezer Dam

Water samples were taken monthly (March 2014 to February 2015) at all sampling sites. This was done in the morning at 08:00 hours. At each site, dissolved oxygen, pH, conductivity, salinity and temperature were measured using an YSI meter from surface to 12 meters (Model MPS 556). Turbidity was measured with a WTW turbidity meter (Model: Turb 430 IR/T). Temperature, depth and dissolved oxygen were categorized to determine the most preferred ranges by *Clarias gariepinus* and *Oncorhynchus mykiss*. Temperature had four categories (12-13, 14-15, 16-17 and >18°C), depth had three (2-4, 4-6, and >6 m) and Dissolved oxygen two (2-3 and 3-4 mg/l).

Water samples from each site were collected at 0.5 m below the surface and analyzed for ammonia, orthophosphate, nitrate and nitrite concentrations following the methods in the Hach DR3900 spectrophotometer manuals. The total dissolved solids (TDS) was determined using the method explained in Chapter 3 (3.2.6).

5.2.4 Fish sampling at Ebenezer Dam

Top-set and bottom-set multifilament nylon gill-nets consisting of 5 panels (10 m long x 2 m deep) with stretched mesh sizes of 44 mm, 60 mm, 75 mm, 100 mm and 144 mm. The nets were set at dusk, left overnight and retrieved the following morning at sunrise (Figure 5.3). Seine nets and electro-shocking were also used at the banks of the dam. Selective angling gears were also used to catch trout and catfish (Figure 5.3). All fish caught were identified and measured as explained in chapter 3.

Figure 5.3: (A) Collecting fish from gill nets and (B) Angling gears used to catch fish.

5.2.5 Stomach content analysis

Oncorhynchus mykiss and *C. gariepinus* were sacrificed by placing them on ice. The stomach from each fish was then removed through dissection of the abdomen. Stomach content analysis was then done following procedures as outlined in Chapter 4 (4.2.3).

5.2.6 Data analysis

Diversity of food items found through stomach content analysis for both *O. mykiss* and *C. gariepinus* were determined using the Shannon Weiner diversity index (Shannon & Weaver, 1949). The Shannon-Weiner diversity index was then subjected to one-way analysis of variance (ANOVA, $P<0.05$) to determine if there were any statistically significant in diversity of the food items between the species.

5.3 RESULTS

5.3.1 Fish species composition at Ebenezer Dam

Seven species, representing five families, were caught at the Ebenezer Dam (Table 5.1). The species were dominated by *Clarias gariepinus* in terms of numbers caught. *Micropterus salmoides* was the second dominant species followed by *Oreochromis mossambicus*, *Cyprinus carpio* and *Micropterus dolomieu* respectively. *Oncorhynchus mykiss* and *Tilapia rendalli* were the least species caught in terms of numbers respectively (Table 5.1).

Table 5.1: Number of fish species sampled across the sites at Ebenezer Dam from March 2014 to February 2015. * indicates that the species is non-native at Ebenezer Dam

		Sites			
Family	Species	1	2	3	4
Salmonidae	*Oncorhynchus mykiss**	2	4	2	
Centrarchidae	*Micropterus salmoides**	3	9	2	6
	*Micropterus dolomieu**	3	2	4	
Cichlidae	*Oreochromis mossambicus*				11
	Tilapia rendalli				2
Cyprinidae	*Cyprinus carpio**	2	3	4	
Claridae	*Clarias gariepinus*	18	16	11	9

5.3.2 Physico-chemical properties of water in Ebenezer Dam

Site 4 had higher TDS and turbidity levels compared to site 1 to 3. Site 3 had low conductivity compared to the other sites (Table 5.2). Salinity, ammonia, nitrate, nitrite and orthophosphate were uniform across all sites (Table 5.2).

Table 5.2: The mean±SD of water quality parameters of Ebenezer Dam sampled from March 2014 to February 2015

	Site 1	Site 2	Site 3	Site 4
TDS (mg/l)	0.27±0.25	0.26±0.25	0.29±0.20	0.47±0.19
Turbidity (NTU)	1.77±0.28	1.91±0.33	1.94±0.33	2.89±0.58
Conductivity (mS/cm)	0.04±0.06	0.04±0.06	0.03±0.00	0.04±0.01
pH (range)	7.04-8.61	7.01-8.43	7.01-8.88	7.04-8.65
Salinity (‰)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Ammonia (mg/l)	<0.2±0.04	<0.2±0.05	<0.2±0.04	<0.2±0.02
Nitrate (mg/l)	0.19±0.21	0.17±0.14	0.20±0.20	0.24±0.24
Nitrite (mg/l)	<0.1±0.02	<0.1±0.03	<0.1±0.02	<0.1±0.02
Total alk. as $CaCo_3$ (mg/l)	10.82±1.33	9.90±1.79	11.35±1.75	12.31±1.51
Orthophosphate (mg/l)	<0.2±0.02	<0.2±0.02	<0.2±0.02	<0.2±0.02

Temperature and dissolved oxygen profiles in Ebenezer Dam

The average temperature was relatively uniform throughout the water column in winter and spring. In autumn and summer, however, surface temperature were higher with gently drops from 5 to 8 m depths (Figure 5.4).

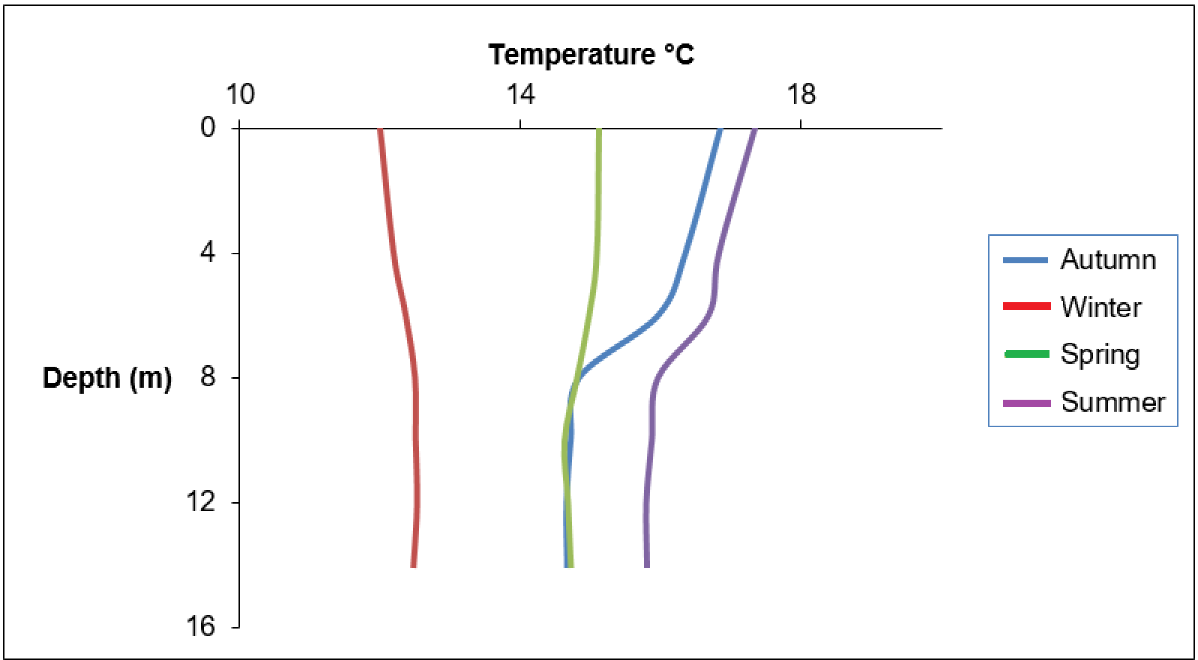

Figure 5.4: Seasonal temperature depth profiles taken at the middle of the dam from March 2014 to February 2015.

Dissolved oxygen (DO) was well distributed throughout the water column in winter. Spring and summer had DO levels that fluctuated from surface to bottom. Autumn levels decreased with the increase in depth (Figure 5.5).

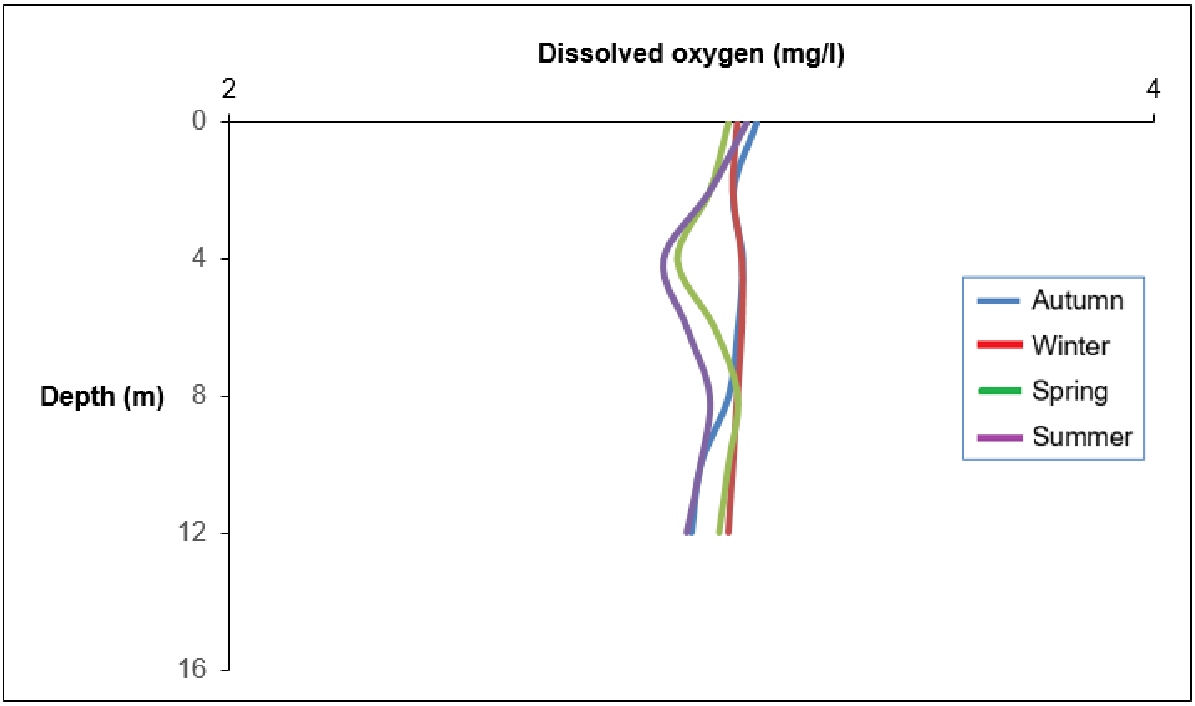

Figure 5.5: Seasonal dissolved oxygen profiles taken at the middle of the dam from March 2014 to February 2015.

5.3.3 *Clarias gariepinus* and *Oncorhynchus mykiss* habitat preferences at Ebenezer Dam

Figure 5.6 shows the number of records of each species in each habitat category. *Clarias gariepinus* preferred all temperature categories, whilst *O. mykiss* preferred temperatures below 17°C. *Oncorhynchus mykiss* preferred dissolved oxygen levels above 3 mg/l, whereas *C. gariepinus* was cosmopolitan to all dissolved oxygen levels. *Oncorhynchus mykiss* preferred deeper depths compared to *C. gariepinus* (Figure 5.6). *Clarias gariepinus* in the Ebenezer Dam had broader habitat preference than *O. mykiss* and the interactions between *O. mykiss* and *C. gariepinus* depend on the preferences of habitat variables at Ebenezer Dam.

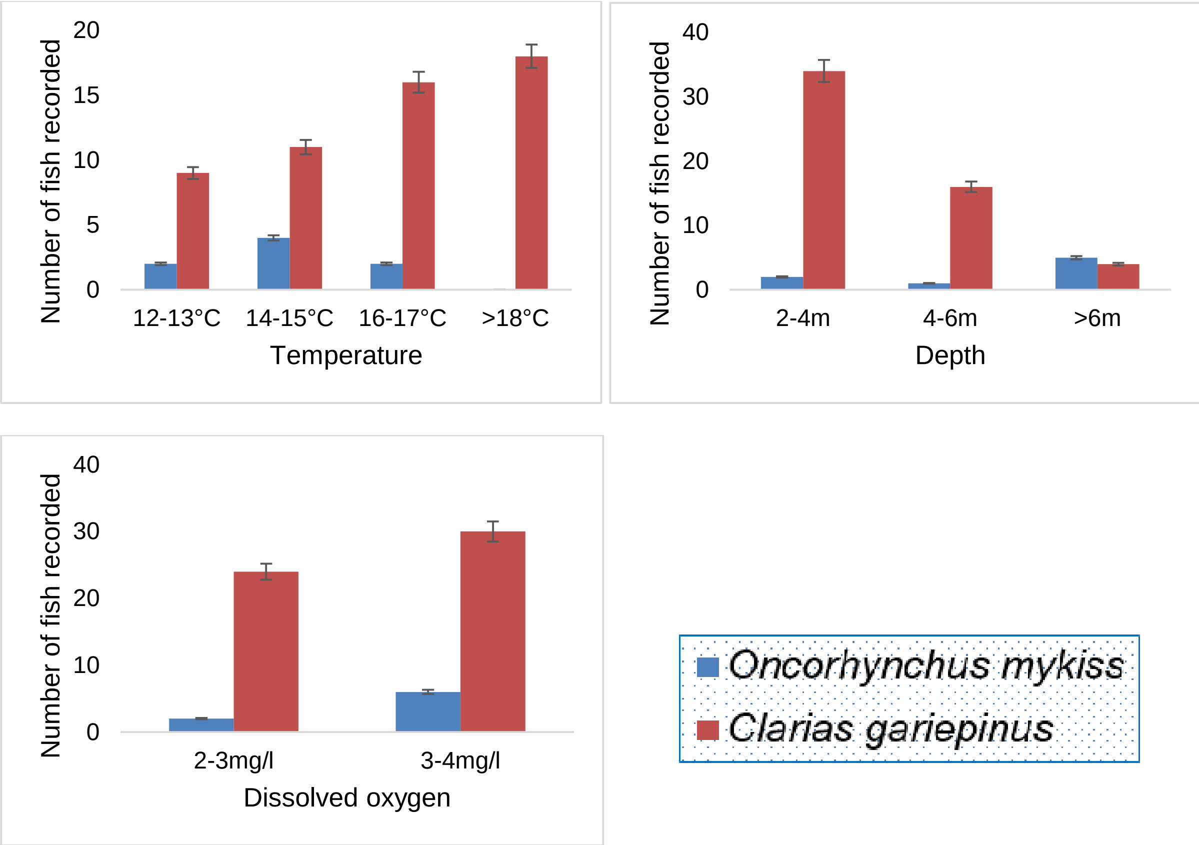

Figure 5.6: Number of *Oncorhynchus mykiss* and *Clarias gariepinus* recorded in each habitat category at Ebenezer dam from March 2014 to February 2015.

5.3.4 *Clarias gariepinus* and *Oncorhynchus mykiss* food preferences at Ebenezer Dam

Fifty four stomachs of *C. gariepinus* were analyzed, of which sixteen were empty. Stomach content analysis revealed food items belonging to five groups excluding unidentified items. These were detritus, terrestrial and aquatic invertebrates, plant material and fish remains. Amongst the groups, detritus and plant material were the common ones (Table 5.3).

Eight *O. mykiss* stomachs were analyzed for food content, two were empty. The analysis revealed two groups of food items excluding unidentified items. These were terrestrial and aquatic invertebrates. Aquatic invertebrates were the most dominant food item. The aquatic invertebrates were dominated by crustaceans, dipterans and zooplankton (Table 5.3). The diversity of the food items is significantly different between *C. gariepinus* and *O. mykiss*.

Table 5.3: Food types expressed as % frequency of occurrence recorded on the stomach of *Clarias gariepinus* and *Oncorhynchus mykiss* at Ebenezer Dam from March 2014 to February 2015

Species	*Clarias gariepinus*	*Oncorhynchus mykiss*
No. of fish stomachs assessed	38	6
Detritus	69.3	0.00
Terrestrial invertebrates	27.1	66.6
Aquatic invertebrates	100	100
Plant material	89.5	0.00
Fish remains	32.3	0.00
Unidentified items	100	100
H’	1.68^{a}	1.09^{b}

**H’ values with different superscripts in a row are significantly different (ANOVA, P<0.05)*

5.4 DISCUSSION

Fish caught at Ebenezer are numerically dominated by *C. gariepinus* (African sharptooth catfish) and *M. salmoides.* The fish species of most systems found in Limpopo are dominated by cosmopolitan species that tolerates poor water quality such as tilapia and catfish (Skelton, 2001), hence the dominance of the catfish at the dam is not suprising. Although the environmental conditions of Ebenezer Dam are different to other systems in Limpopo, the dominance of the catfish can also be attributed to its biology. Its ability to tolerate wide range of environmental variables makes it a hardy species able to thrive in different conditions (Kadye & Booth, 2012).

The competitive interaction between trout and catfish depends on habitat variables such as temperature at Ebenezer Dam. For example, this study has shown that temperature have a large impact on their competitive interaction since trout preferred water temperatures below 17°C, whereas the catfish was cosmopolitan to all sampled temperatures. Based on this observation, together with the known biology of trout, colder temperatures seem to broaden the habitat for trout at Ebenezer Dam (Dorgeloh, 1995). This observation therefore, leads to an agreement with the previous chapters that Magoebaskloof area is marginal and cannot provide proper habitat conditions for trout especially when temperatures increases.

Dissolved oxygen levels in the Ebenezer Dam affects the distribution and interaction of trout with other species. The results have shown that the distribution of trout is positively associated with dissolved oxygen levels >3 mg/l. This implies that dissolved oxygen levels <3 mg/l at the Ebenezer are not favoured by trout and thereby affects its interactions with the catfish which is positively associated with all dissolved oxygen levels at the dam. Studies have shown that low dissolved oxygen levels stresses trout and when stressed (Raleigh, 1985; Skelton, 2001; Kadye & Magadza, 2008), its activity is reduced. With the catfish not stressed by changes in oxygen levels due to its ability to utilize atmospheric oxygen, it is likely to be active regardless of the oxygen levels.

Trout preferred deeper depths compared to the catfish. The Ebenezer Dam could be oligotrophic (unproductive) since it has low algal biomass and nutrients which allows deeper light penetration and less decomposition (Although this study didn’t determine the algal biomass, the assertion is based on visual observation), leading to less consumption of oxygen by decomposition (DWA, 2013). Therefore it is thought not suprising that DO concentrations have shown an increase with depth below the thermocline. This could be a possible explanation of the low numbers of trout at shallow depths since oxygen levels were favourable at deeper depths due to the unproductive profile of the dam (DWA, 2013). However, this observation differs with a couple of reports that have shown that trout are often caught at the surface since they are drift feeders that aims at drifting invertebrates and fish at shallow depths towards the banks of the dams (Dorgeloh, 1995). However, more in depth studies on the productivity of the dam should be done to confirm this assertion.

The diversity of the food items is significantly different between the catfish and trout. The catfish did not only have a broader preference on temperature and oxygen, but also on food. The Shanon-Weiner index showed that the catfish had a more diverse diet than trout, implying a broad niche in terms of food. However, this could also be linked to the habitat conditions in the dam as being not optimal for trout. Therefore, the less diversity of food items could be because of the stress caused by the depth, temperature and oxygen levels. Dorgeloh (1995) reported similar results in Sterkfontein Dam in the Tugela-Vaal catchment, South Africa, that the interaction of trout and the catfish can only be large when temperature and oxygen are favourable, for example, during winter months. Unfortunately, the interspecific food overlap between trout and the catfish could not be determined in Ebenezer Dam, because of the small sample size of trout but food selection between them is evident.

In conclusion, the difference in habitat requirements between trout and catfish decreases the competitive interaction between the two species in Ebenezer Dam. The catfish could have also reduced competition strength by extending its habitat because of its wide-range of habitat and food preferences (Kadye & Booth, 2012). Trout and catfish interactions in Ebenezer Dam should be placed within a framework of ecological studies to determine long-term interactions.

CHAPTER 6:

PREDICTING THE IMPACT OF CLIMATE AND LAND USE CHANGES ON THE DISTRIBUTION AND ESTABLISHMENT OF TROUT IN MAGOEBASKLOOF AREA, LIMPOPO PROVINCE, SOUTH AFRICA

6.1 INTRODUCTION

The past 100 years have seen significant changes in the global climate that was likely caused by anthropogenic greenhouse gas emissions (Jones *et al.*, 2013). Mean global surface temperature has increased by approximately 0.1°C per decade since the late 1950s and is projected to be 1.4 to 2.1°C by 2050 (Deutsch *et al*., 2011). Climate change has been observed to be having a profound effect on both aquatic and terrestrial biodiversity (Root *et al*., 2003). This trend is expected to continue, with associated changes in species compositions (Parmesan & Yohe, 2003) and distributions (Hobday *et al*., 2006). Concern over the impact of climate change in the aquatic environment is also increasing, with longer-term shifts in mean environmental conditions and climatic variability moving outside the bounds within which adaptations in aquatic communities have previously been associated (Jones *et al*., 2013).

Global air temperatures are expected to continue to increase throughout the 21st century (Meehl *et al*., 2000). This increase in air temperatures will result in higher stream temperatures, potentially altering the thermal suitability of streams for freshwater fish (Christensen *et al*., 2007; Field *et al*., 2007). These projected changes in temperature and precipitation are anticipated to alter the behaviour of the hydrologic cycle, and could lead to impacts in fish habitats that could adversely affect their distribution and survival (Jones *et al*., 2013). The changes in abundances and distributions that result from these climate changes may severely impact the biological and environmental functioning of ecosystems or food webs (Root *et al*., 2003). The effects of climate change on species that are confined to a unique geographic area such as trout are of particular concern. Trout species are frequently restricted to relatively small areas and population sizes and have highly specific habitat requirements (Picker & Griffiths, 2011).

Of late, there has been an increase in studies attempting to assess how climate change might impact freshwater fish species (Cheung *et al.*, 2010; Harborne & Mumby, 2011; Sumaila *et al*., 2011) and how conservation goals and actions should adapt in a changing climate (Thuiller *et al*., 2006; Fuller *et al*., 2008; Hagerman *et al*., 2010; Hu & Jiang, 2011). However, little work has been done in South Africa with regard to the impacts of climate change on the abundance and distribution of fish species in freshwater ecosystems.

Climate change together with human induced environmental changes such as land use are of concern because of deterioration of the environment (Hudak & Wessman, 1998; Tziztiki *et al*., 2012). Knowledge about land use/land cover has become important to overcome the problem of biogeochemical cycles, loss of productive ecosystems, biodiversity, deterioration of environmental quality, loss of agricultural lands, destruction of wetlands, and loss of fish and wildlife habitat (Hudak & Wessman, 1998). Technologies such as satellite remote sensing and Geographical Information Systems (GIS) are required to help outline how these changes will affect fish distributions and how to mitigate the changes in future (Maktav *et al*., 2005). These technologies gives projections to study and monitor the dynamics of natural resources for environmental management. This study employed these technologies to outline how the changes in land use and cover affects the distribution of trout in an area regarded as marginal for this species.

The objective of this chapter was to demonstrate the impact changes in climate and land use may affect the distribution of trout in Magoebaskloof area. The chapter will assess the past climate and land use changes and compute the projected changes in the area using global climate models (GCMs) and GIS.

6.2 METHODS AND MATERIALS

6.2.1 Study area’s profile

The Magoebaskloof area (Figure 6.1) is situated in Limpopo Province of South Africa. The average air temperatures in Magoebaskloof reach around 21 to 26°C in January and fall to about 11°C in July as shown in chapter 3 (3.2.1).

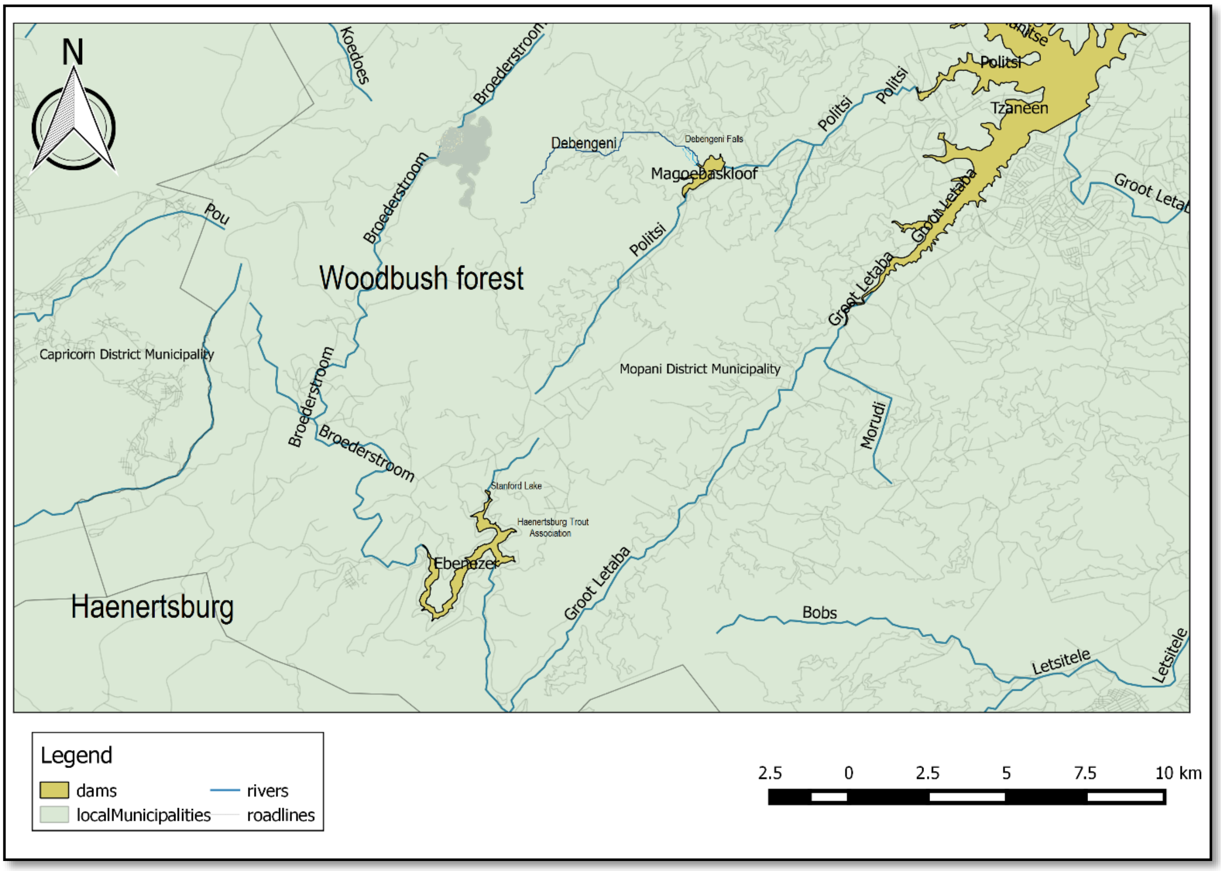

Figure 6.1: Magoebaskloof area catchment.

The area receives summer rain and a pronounced dry spell during winter. The streams in the area originate in the Drakensberg Escarpment, the banks are of gentle slope. Riparian vegetation is sparse. The area is categorized by mountains with high to moderate relief, northeastern mountain grassland and afromontane forest.

6.2.2 Data collection

Six sampling sites were chosen along an altitude gradient in the Broederstroom River as outlined in chapter 3 and four were chosen at the Ebenezer Dam as outlined in chapter 5. Temperature and fish capturing were done as shown in chapter 3 and 5. Temperature and precipitation data of the Magoebaskloof area for the past 40 years (1975 to 2014) were obtained from the South African Weather Services. Topographic map data (1:50 000 scale) prepared in 1966, 1973, 1994 and 2008 were obtained from the Department of Rural Development and Land Reform (National Geo Spatial Information), Government of South Africa, Mowbray. The maps were converted to digital mode using by digitizing QGIS software.

6.2.3 Data analysis

Time-series analysis was used to detect changes in air temperature and precipitation for the past 40 years. Mann-Kendall trend test was used to show the trend in temperature and precipitation changes. Both maximum and minimum mean temperature values were analyzed and produced graphs. Trend lines were drawn and changes were worked out from the fitted curves. Linear regression analysis was used to forecast the minimum and maximum temperature and precipitation by 2050. Global climate model (GCM) scenario generator MAGICC and SCENGEN were used to project rainfall and temperature climate change scenarios by 2053. Emission scenarios A1 (a reference scenario A1-BAIM) and B2 (a policy scenario B2-MES) were used for the projections.

The land use maps pertaining of two different periods (1966 and 2008) were used for post classification comparison, which facilitated the estimation of changes in the land use category. Post classification comparison is the most commonly used quantitative method of change detection. This method involves independently produced spectral classification results from different data sets, followed by a pixel-by-pixel or segment-by-segment comparison to detect changes in the classes (grasslands, plantations, agriculture and buildings).

6.3 RESULTS

6.3.1 Fish distributions in relation to altitude and temperature

Trout is widespread at high altitudes and was caught at altitudes above 1400 m a.s.l. Site 1 had the lowest temperature and site 6 the highest. Temperature increased with a decrease in altitude (Table 6.1).

Table 6.1: The distribution of fish in relation to altitude and temperature in the Broederstroom River from February 2014 to February 2015

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Altitude (m a.s.l)	1557	1526	1459	1393	1380	1365
Mean Temp. (°C)	10.5±0.51	11.2±3.25	11.6±1.20	16.3±2.61	17.8±0.71	19.1±2.61
Min-Max (°C)	7.4-17.5	8.1-17.8	8.6-19.2	10.3-19.8	11.8-19.8	12.2-21.5
O. mykiss	X	X	X	-	-	-
S. trutta	X	X	X	-	-	-
M. salmoides	-	-	-	-	X	X
O. mossambicus	-	-	-	-	-	X

(X) Indicates presence and (-) indicates absence

Temperature remained consistently the same in site 1 to 3 at Ebenezer Dam (Table 6.2). Highest temperature was recorded at site 4. Warm water *M. salmoides* and *C. gariepinus* were widespread at all sampled temperatures, whilst *O. mykiss* was localized at temperatures below 17 °C (Table 6.2).

Table 6.2: Number of fish species sampled across the sites at Ebenezer Dam. * indicates that the species is non-native at Ebenezer Dam

Site	1	2	3	4
Mean Temp. (°C)	16.8±1.66	16.6±1.38	16.9±1.38	18.1±1.29
Min-Max Temp. (°C)	12.3-18.7	12.5-18.9	12.5-19.5	13.3-20.7
*Oncorhynchus mykiss**	X	X	X	-
*Micropterus salmoides**	X	X	X	X
*Micropterus dolomieu**	X	X	X	-
Oreochromis mossambicus	-	-	-	X
Tilapia rendalli	-	-	-	X
*Cyprinus carpio**	X	X	X	-
Clarias gariepinus	X	X	X	X

(X) Indicates presence and (-) indicates absence

6.3.2 Changes in past temperatures that might have affected the distribution of trout in Magoebaskloof area

Figure 6.2a provides the time series plot of the annual minimum mean air temperatures for the past 40 years in Magoebaskloof area. The computed p-value is greater than the significance level (α=0.05), showing no trend in the series (Table 6.3). Figure 6.2b gives the time series plot of annual mean maximum air temperatures. The computed p-value for maximum is lower than the significance level (α=0.05), showing a trend in the series (Table 6.3). The Sen's slope is positive, showing a 2°C increase in maximum air temperatures from 24.3±2.53°C in 1975 to 26.1±1.55°C in 2014. The highest temperature was 27.1±4.31°C recorded in 1992, followed by 26.5±3.79°C in 2003 (Figure 6.2b). The projected minimum and maximum mean temperatures by 2050 will be 11.80 and 26.98°C respectively, showing a 0.39 and 2.04°C increase.

Table 6.3: Mann-Kendall trend test for minimum and maximum mean annual temperatures from 1975 to 2014 at the Magoebaskloof area

	Minimum temperature	Maximum temperature
Sen's slope	0.005	0.033
Kendall's	0.063	0.437
p-value (two tailed)	0.576	< 0.0001
Confidence interval	-0.292, 0.232	-0.246, 0.286
Projected change	0.39°C	2.04°C

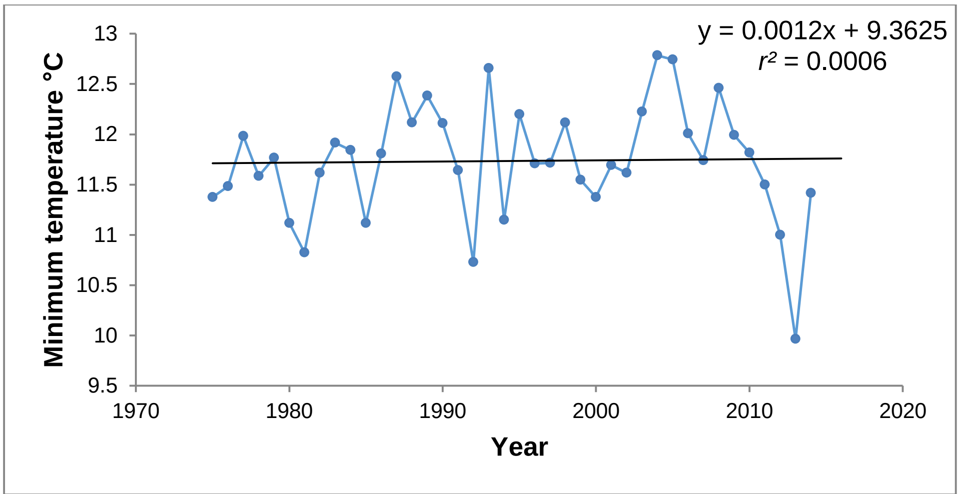

Figure 6.2a: Observed mean annual minimum temperatures for the past 40 years of Magoebaskloof area.

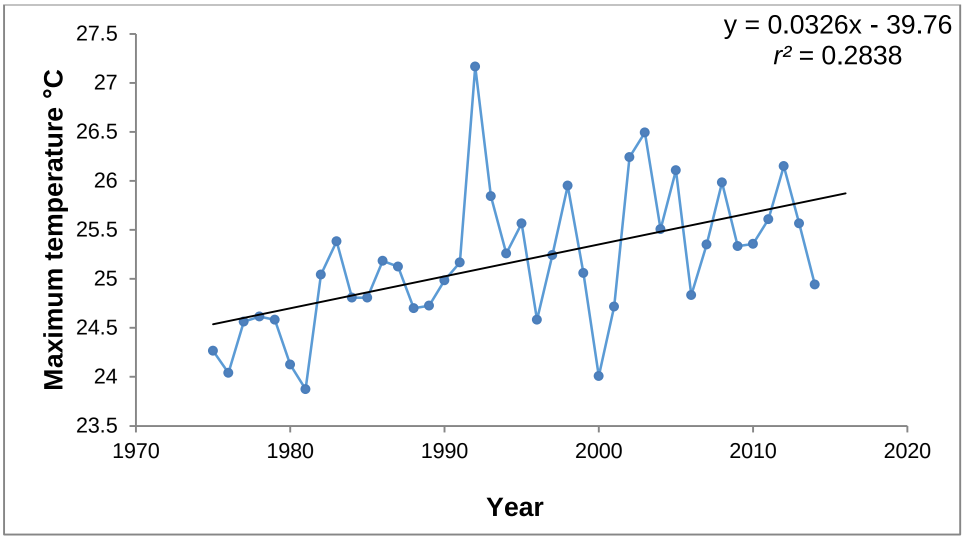

Figure 6.2b: Observed mean annual maximum temperatures for the past 40 years of Magoebaskloof area.

6.3.3 Changes in precipitation that might have affected the distribution of trout in Magoebaskloof area

Precipitation has fluctuated in the past 40 years in Magoebaskloof area. The computed p-value is greater than the significance level (α=0.05). Across the years, Sen's slope shows a decrease in precipitation from 1975 to 2014 (Table 6.4 and Figure 6.3). The projected precipitation by 2050 will be 36.65%, indicating a decline in precipitation by 1.49% from 2014.

Table 6.4: Mann-Kendall trend test for mean annual precipitation (%) for the past 40 years at the Magoebaskloof area, Limpopo Province, South Africa

	Precipitation (%)
Sen's slope	-0.11
Kendall's	-0.090
p-value (two tailed)	0.424
Confidence interval	-3.923, 4.360
Projected change	-1.49

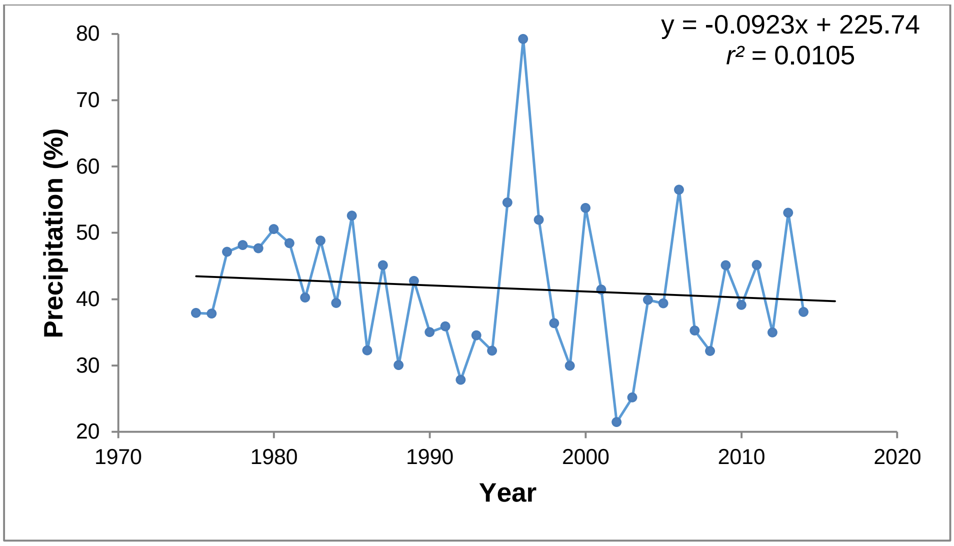

Figure 6.3: Observed precipitation (%) for the past 40 years of Magoebaskloof area.

6.3.4 Projected changes in temperature and precipitation that may affect trout in Magoebaskloof area

The climate projections presented in Table 6.5 consist of two main scenarios generated using SCENGEN model. All of South Africa is projected to be warmer by 2053. The warming is to be greater than the global annual mean warming (Table 6.5 and Figure 6.4). The model projects an increase in the annual temperature of more than 1.91°C and a 7.3% change in precipitation over the northern interior regions of South Africa where Magoebaskloof area is situated.

Table 6.5: Forecasted changes in average annual temperature and precipitation in Magoebaskloof area according to emission scenario A1 and B2 by 2053

	Temperature		Precipitation (%)	
	A1-BAIM	B2-MES	A1-BAIM	B2-MES
Global range	0.02 to 4.61	0.01 to 3.88	-27.0 to 45.0	-22.7 to 37.9
Global mean dT	1.69°C	1.42°C	1.69°C	1.42°C
Projected change	2.27°C	1.91°C	8.7%	7.3%

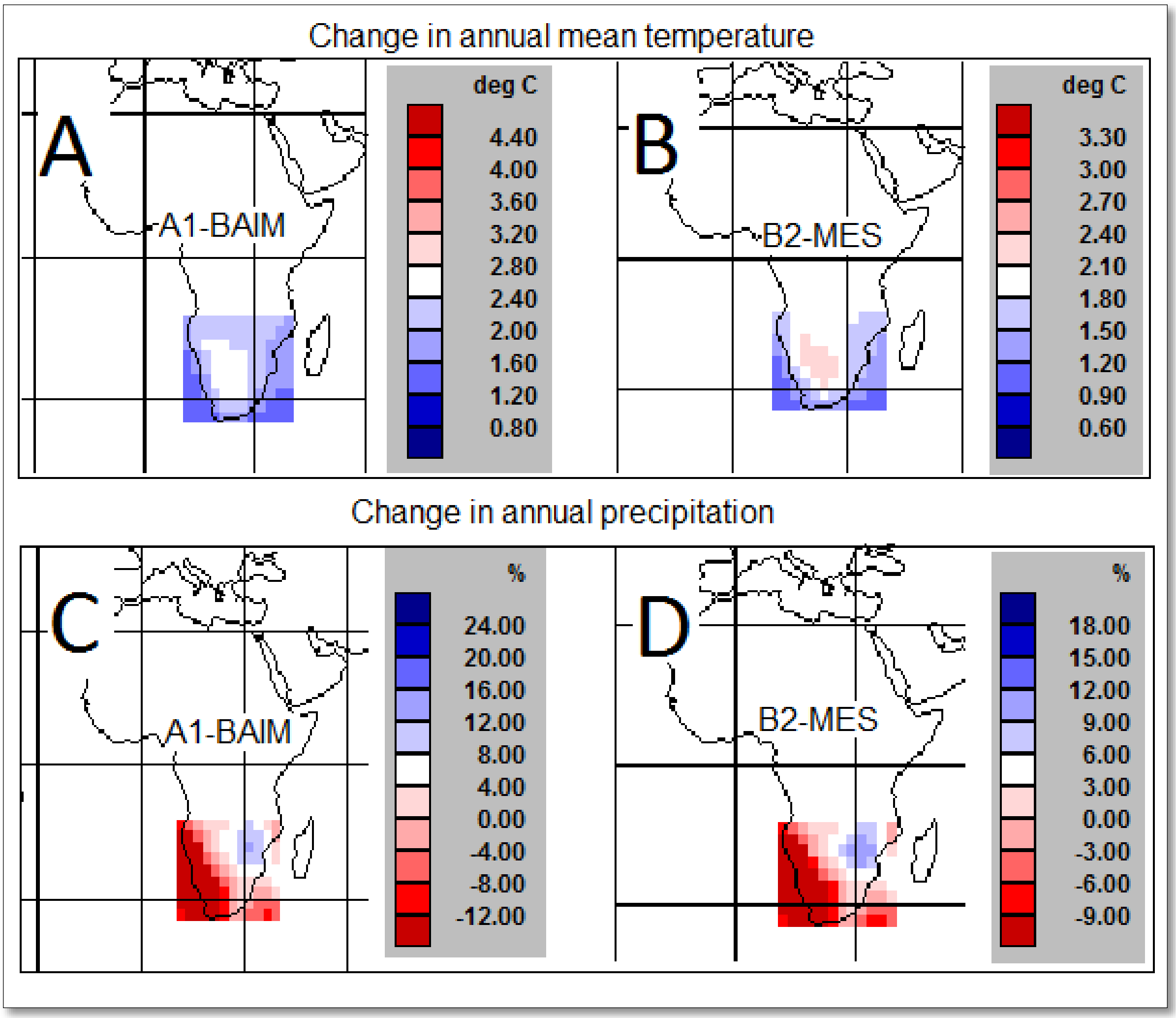

Figure 6.4: Forecasted changes in average annual temperature (A and B) and precipitation (C and D) in South Africa using SCENGEN scenario A1-BAIM and B2-MES by 2053.

6.3.5 Patterns of land use/cover changes around Magoebaskloof area that affected distribution of trout

Plantations have largely increased in the past years (1966 to 2008) in Magoebaskloof area (Figure 6.5). Grasslands have decreased during this period, leading to a decrease of marginal vegetation and canopy covering the Broederstroom River. The upper catchment (>1400 m a.s.l) of the area have had a large amount of afro-montane grass-land converted into timber plantations and other urban development activities such as buildings and agriculture (Table 6.6). The vegetation around the water areas has been largely replaced by plantations. The land consisting of roads, mostly link roads, joining the Haenertsburg village settlement and barren land with or without scrub and sandy area is largely broadened. Mining activities are absent from the study area (Figure 6.5).

Table 6.6: Land use/land cover changes from 1966 to 2008 in Magoebaskloof area. (−) indicates decrease

	Area, km^2		Change km^2
Class name	1966	2008	Change
Grasslands	152.69	95.87	- 56.82
Plantations	78.35	112.46	34.11
Agriculture	25.23	45.14	19.91
Buildings	4.14	18.07	13.93

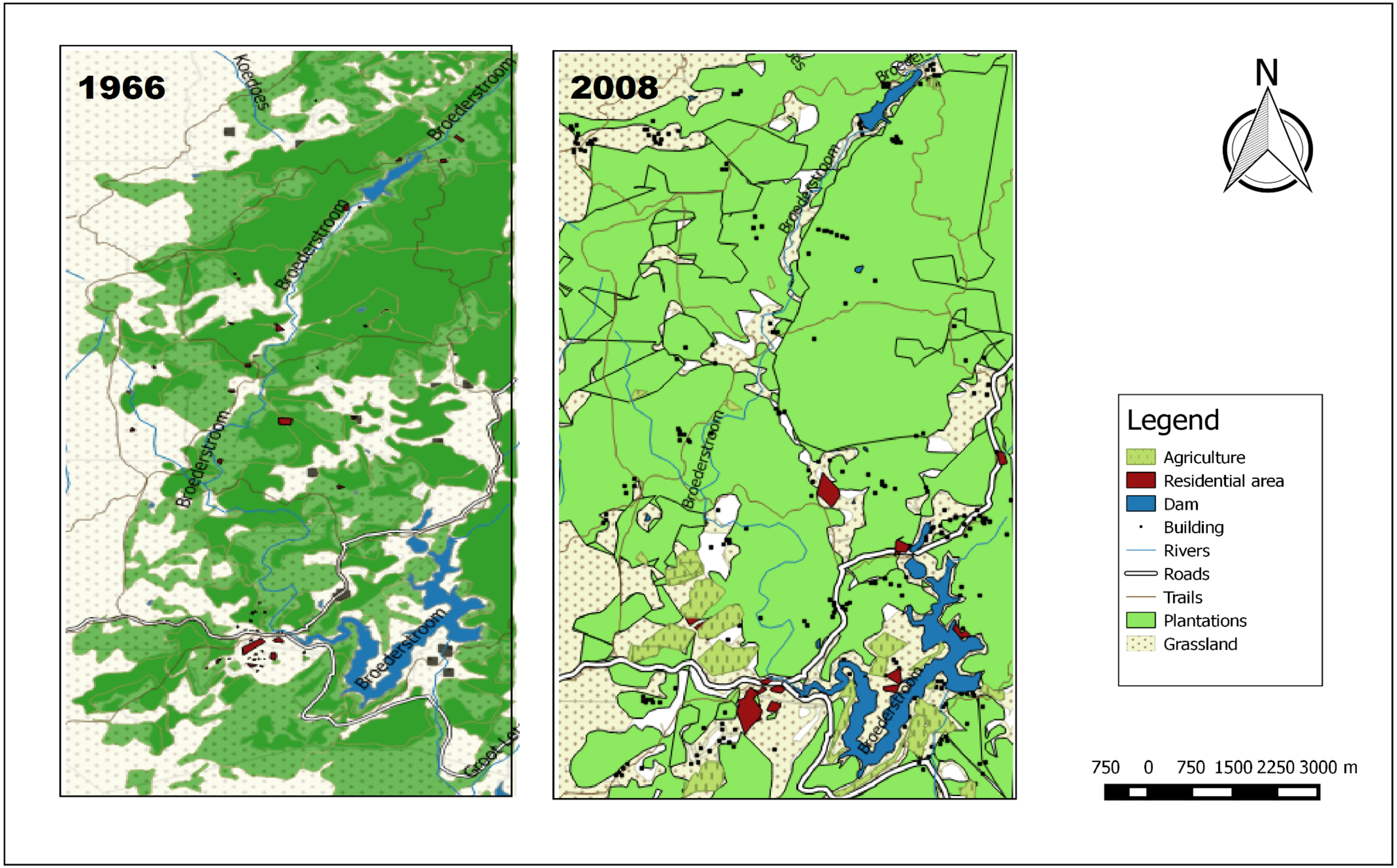

Figure 6.5: Time series maps from 1966 to 2008 showing land use/cover changes within Magoebaskloof area.

6.4 DISCUSSION

Fish distributions in the Broederstroom River are defined by altitude and temperature gradients. Temperatures less than 13°C were recorded at altitudes above 1400 m a.s.l., and temperatures greater than 13°C at altitudes below 1400 m. Literature has shown that fish have an optimal range of temperature preferences that favours their optimal survival. It is therefore not suprising that trout at the Broederstroom River was only confined at altitudes above 1400 m a.s.l where temperatures were favourable, whilst bass (*M. salmoides*) and tilapia (*O. mossambicus*) were distributed below 1400 m a.s.l., where temperature favoured their distributions. The distribution of fish in the Ebenezer Dam also shows that fish are distributed where temperature favours them. For example, trout and tilapia were only caught where temperatures favoured them.

Trend and linear regression analysis have shown a significant increase in annual maximum air temperatures in Magoebaskloof area, suggesting that the atmospheric temperatures have warmed up by 2°C in the past forty years and will warm up by 2°C in 2050. If water temperatures at the Broederstroom River have had the same increase as air temperatures in the past years, then species distributions can be assumed to have been affected by the changes. For example, the warming at lower altitudes have favoured the establishment of bass opposed to cold water trout. Therefore, the changes can be used as an explanation of why trout is confined only at altitudes above 1400 m, where apart from the temperature changes, the conditions remain favourable for trout.

The current trout establishment and distribution status in the Broederstroom River could be a result of the 2°C temperature increase (However, lack of historical data on their distribution in the area makes it impossible to emphasize this assumption). Raleigh *et al*. (1984) and Skelton (2001) have shown that warm temperatures are associated with reduced and delayed reproduction in trout species. The temperature regimes of the areas they are introduced to, controls the specific timing of their spawning. This implies that the 2°C temperature increase in Magoebaskloof area could have negatively affected the timing of spawning for trout and survival of their eggs, hence their populations are supplemented by the annual restocking's by the HTA.

The effects of the significant changes in temperature in Magoebaskloof area are likely to have favoured indigenous species. The fish species that dominate freshwater systems in Limpopo Province are mainly warm water species such as *C. gariepinus*, *T. rendalli* and *O. mossambicus* (Skelton, 2001). The increase in temperature due to climate change in areas such as Magoebaskloof can increase the suitability of the area to favour warm water species which often have limited capacity to thrive in cold waters. Skelton (2001) has shown that indigenous species in Limpopo thrives in warm water environments. The past increases in temperature in Magoebaskloof area could be seen to have favoured warm water species.

Annual precipitation has decreased in Magoebaskloof area in the past forty years and is projected to continue to decrease. Flow rate and depth of streams are dependent on the amount of annual precipitation. Kadye and Magadza (2008) have shown that flow rate and depth are important variables in the distribution and assemblage structure of fish in freshwater ecosystems. Several other studies also supports their findings (Taylor, 2000; Humpl & Pivnicˇka, 2006; Kadye *et al*., 2008). Studies have shown that trout prefers moderate to fast flowing waters, therefore their establishment outside their natural range is controlled by the amount of precipitation of the area. The decrease in precipitation in Magoebaskloof area have led to reduction in flow rates and depth at the Broederstroom River. This may lead to the destruction of trout habitat.

Projected temperature changes on annual basis using SCENGEN shows that all of South Africa is projected to warm by 2053 and is to be greater than the global annual mean warming. Climate change is primarily influenced by the total stock of greenhouse gases (GHGs) in the atmosphere. Jones *et al*. (2013) indicated that historically developed countries and economies in transition are responsible for about 75% of the total global stock of GHGs. South Africa is highly vulnerable to climate change as its economy is heavily reliant on climate-sensitive sectors like agriculture and forestry. Magoebaskloof area is projected to have an increase in the annual temperature of more than 1.91°C and a 7.3% change in precipitation by 2053. If this air temperature change is to be the same with water temperature, the trout populations in the area will only be confined to high altitude reaches and will be sustained though the annual restocking by the HTA. The current annual mean water temperature of the area only favours sustainability of this species at high altitudes reaches.

Plantations, agriculture, and urban practices have singly or in combination, impaired the riparian vegetation and bank stability. The impairment of vegetation and bank stability then resulted in increased sediment erosion, thereby affecting instream habitat. Historically, much of the land in the Woodbush Forest (sampling sites) was dominated by afro-montane grasslands with an established riparian community, which maintained cooler water temperatures, provided food and cover for fish, and stabilized stream banks. Vegetated banks commonly have less bank erosion and offer a diverse macroinvertebrate influx as food source for fish (Beeson & Doyle, 1995), current conditions, however, are greatly altered in the area. Most sites lack a riparian community, average canopy cover is less than 50%, with most of this in isolated reaches. There is also substantial bank erosion in the sampled sites, which may be partially due to the reduction of riparian vegetation and agricultural practices, mostly at altitudes below 1400 m a.s.l. Lack of a riparian community resulted in warm water temperatures at altitudes below 1400 m a.s.l. of the Broederstroom River. These warm temperatures may partially explain the lack of trout, which are present at altitudes above 1400 m a.s.l.

Turbidity levels of the Broederstroom River are a result of previous land use changes. Turbid waters are accelerated by human activities that increase inputs of runoff, primarily sediment and debris to surface waters, thereby causing excessive sediment in the streams. This causes a wide swing in water clarity and oxygen concentrations, because clean water retain oxygen better than turbid water and turbid water does not sustain trout life (Skelton, 2001). Turbid waters also alters physical habitat for trout by slowing the flow of water. This has occurred in the South Fork of the Palouse River in Washington where it resulted in dissolved oxygen concentrations decreasing below the level required for trout (Kellogg *et al*., 1994). This could be the explanation altitudes (<1400 m a.s.l.) at the Broederstroom River are not sustaining trout life since trout is sensitive to changes of turbidity levels.

In conclusion, the projections demonstrate that climate and land use changes had and might continue to have effects on the distribution and establishment of trout at the Broederstroom River, Magoebaskloof area. The effects are a result of the changes causing warmer water temperature, altered riparian covers and altered stream flow patterns. The changes will influence the distribution of trout in only high altitudes. Rahel and Olden (2008) have shown that cold water species may be unable to persist under new climate conditions due to climate change. The projections of this study agrees with the findings by Rahel and Olden (2008) that trout will not tolerate the changes in Magoebaskloof area. However, future in-depth studies on climate and land use/cover change in the area needs to be undertaken to explain the true extent of the effects of these changes on aquatic fauna.

CHAPTER 7:

GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSION

Key habitat variables sustaining trout populations in Magoebaskloof area

Temperature, substrate, depth and flow rate are the main habitat variables required to sustain trout in headwater sites in the Broederstroom River. These habitat variables were only optimum for trout at high altitudes, resulting in its confinement to higher altitude sites. This observation confirmed the assertion that the area is marginal for trout. However, regardless of the area's marginality, trout is continuously stocked by the HTA. Evidence of trout's negative impacts has been mounting in South African streams (Cambray, 2003a; Ellender & Weyl, 2014; Shelton *et al*., 2015). This shows that there is a need to understand the factors controlling trout abundance and distribution. Hence, the findings of this study have shown through multivariate analyses that trout could potentially inhabit many headwater streams in southern Africa. Therefore, studies need to look towards developing habitat suitability models for trout and use them to predict possible scenarios for future trout invasions. The understanding of these models will help curb future and continuous stocking of not only trout but other invasive species.

Impact of trout in Magoebaskloof area

The diversity of macroinvertebrates in Magoebaskloof area is not affected by the presence of trout. However, their abundance is affected by the trout with groups such as Aeshnidae and Gomphidae vulnerable to trout predation. Studies continue to show the consequences associated with trout outside its native range (Shelton *et al*., 2015). However, it can be assumed that *Amphilius* sp. would have been present in the Broederstroom River, based on its similar altitude as Debengeni River. Given that the Debengeni River and other tributaries in the area are still trout free, it can be proposed that the primary goal of trout management in the area and elsewhere in marginal areas should not necessarily be to remove the trout from the streams but to rather prevent new trout introductions where they do not occur.

A secondary objective would then be to monitor the quantity of the stocking densities to the streams that trout already occur. Anglers also need to be given workshops on the impacts associated with the trout to curb deliberate introductions to parts of the areas that doesn't contain trout. Therefore, the key to mitigating future trout impacts in marginal areas such as Magoebaskloof, lies not in constraining the development of the trout avenues, but rather in guiding its expansion in a direction that minimizes new introductions.

Implications for fish conservation in South Africa

The world is currently in a global biodiversity crisis since the rate of extinctions has increased in recent times, especially in freshwater systems (Shelton *et al.*, 2015). This situation demands the identification of biologically important areas and the protection of species and ecosystems (Brooks *et al.,* 2006). Many approaches exist for prioritizing areas for conservation, and the criteria most commonly incorporated include endemic species richness, total biodiversity and a measure of habitat modification (Wilson *et al.,* 2007). Once identified like the Cape Floristic Region in the Western Cape Province of South Africa, the areas can then be set aside as protected areas. However, protection of biologically important areas often clashes with human interests and activities such as sport fishing (Margules & Pressey, 2000). South Africa should therefore adopt frameworks focusing on conservation efforts like the biodiversity hotspot concept developed by Myers *et al.* (2000). Biodiversity hotspots are areas that support exceptionally high concentrations of unique species, but that are also under serious threat from human-related activities. Freshwater biodiversity in South Africa is under threat from many factors including habitat loss, climate change and most notably invasions by non-native species. Therefore, effective conservation of stream environments rest on the ability to mitigate future introduction of invasive species.

In Magoebaskloof and other South African catchments, invasive species such as trout were originally stocked into larger tributaries, and from there spread into many of the smaller headwater tributaries (de Moor & Bruton, 1988). Fortunately, many headwater streams still remain trout-free, and these act as reserves for native aquatic biodiversity. From a conservation perspective, it is therefore important that future studies should be aimed to understand what stands between remaining trout-free streams and new trout invasions.

Climate change effects on fish assemblages in Magoebaskloof area

The impacts of climate change are not going to be uniform in Magoebaskloof and across the whole of South Africa. It is therefore vital to understand the distribution patterns of fish not only in Magoebaskloof but across the different latitudes of South Africa's climatic regions. The understanding will help in development of models and policies that will curb destruction of freshwater habitats due to the climate changes. Trout is a cold water fish, any increase in temperature in its habitat will have significant influence to its distribution. The projections of this study demonstrate that climate change coupled with land use changes had and will continue to have effects on the distribution of fish assemblages in Magoebaskloof area. The effects are a result of the changes causing warmer water temperature, altered riparian covers and altered stream flow patterns. The changes will influence the confinement of trout to only high altitude catchments. Studies have shown how climate change might affect freshwater fish species and how conservation goals and actions should adapt in a constantly changing climate worldwide (Daw *et al*., 2011). However, little is known in southern Africa regarding the impacts of climate change on the survival and distribution of fish in freshwater ecosystem, specifically coldwater fish that are introduced in marginal areas that are characterized by warm temperatures such as Limpopo Province.

If the consequences of climate change are not properly addressed, the future of trout in Magoebaskloof area will solely be sustained through the restocking's by the HTA. The marginality of the area will also worsen and only the distribution of warm water fish will be favoured in the area. Fish assemblages will also be affected not only in marginal areas but in the whole of southern Africa as Rahel and Olden (2008) indicated that most freshwater fish species may be unable to persist under new climate conditions due to climate change.

Recommendations for the management of trout and other invasive species

The results presented in study represents quantitative assessment of the impact of trout in a marginal area in South Africa. The study revealed that, not only do trout reduce native fish populations, but that it is a weak regulator of invertebrate diversity in marginal areas. These findings contribute to the conceptual understanding of trout impacts in marginal systems. The approach of combining broad-scale field surveys with small-scale controlled experiments to evaluate specific hypotheses about invasive species impacts is novel in the context of invasive fish research in South Africa, and holds promise for quantifying impacts of other invaders in other systems in the country. The findings of the study can be incorporated into management plans, and used to refine existing legislation regarding the conservation of freshwater biodiversity in trout invaded areas in South Africa that are in line with the National Environmental Management: Biodiversity Act (NEM:BA; RSA 2004). Magoebaskloof area also need to adopt new management practices if trout is to thrive in the area. This could be achieved through negotiations with landowners to adopt better practices of riparian vegetation, plantations and water obstruction. These practices will help improve access to the rivers for monitoring. It will also help in the improvement of water quality which currently limits trout on the lower stretches of the Broederstroom River.

CHAPTER 8:

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