ECOLOGICAL CONSEQUENCES OF NON-NATIVE FISH INVASION IN EASTERN CAPE HEADWATER STREAMS

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Abstract

The introduction, spread and concomitant impacts of non-native species are a global problem. Fish are among the most widely introduced vertebrate groups, with their impacts affecting multiple levels of organisation- from individuals, populations and communities, to entire ecosystems. In South Africa, the largest perceived threat to range-restricted endemic headwater stream fishes is said to be invasion by non-native fishes, however, as is the case elsewhere, invasive impacts are often a case of risk perception rather than actual risk analysis. Two range-restricted headwater species, the Eastern Cape redfin *Pseudobarbus afer* and the Border barb *Barbus trevelyani* are redlisted by the International Union for the Conservation of Nature (IUCN) as 'Endangered', primarily due to invasion by non-native fishes.

To investigate invasions in South Africa, and provide a quantitative estimate of the impact of non-native fishes on the two imperilled endemics, *P. afer* and *B. trevelyani*, the overall aims of this thesis were to: (A) Provide a literature review on non-native fish invasions in South Africa; (B) Using two case studies on the headwaters of the perennial Keiskamma and episodic Swartkops River systems, investigate the naturalisation-invasion continuum to provide a holistic view of the invasion process in these variable environments. The specific thesis objectives were: (1) Reviewing current knowledge of invasive impacts of non-native fishes in South Africa; (2) Investigating invasibility of headwater stream environments by non-native fishes; (3) Determining the establishment success of non-native fishes, (4) Assessing the spatial and temporal impacts of invasion; (5) Understanding mechanisms responsible for non-native fish impacts; (6) Investigating the threat of non-native fish invasion on the genetic diversity of two the two headwater fishes, *P. afer* and *B. trevelyani*.

Results from the literature review of fish invasions (Chapter 1) showed that South Africa has a long history of non-native fish introductions, spanning two and a half centuries. Currently, 55 species have been introduced or translocated. Many of these introduced species have become fully invasive (36%). Their impacts also span multiple levels of biological organisation. There was a general paucity of studies on fish invasions (38 studies), however, of those conducted, reviewed studies placed emphases on invasive impacts (25 studies) and the transport, introduction, establishment and spread stages of the invasion process were largely ignored.

The two study systems, the Swartkops and Keiskamma Rivers, were heavily invaded and numbers of introduced species surpassed that of natives (Chapter 2, 3 and 5). Headwater streams had varying invasibility and a number of non-native species were successfully established (Chapter 2, 3, 5 and 6). The remainder of the invasions were casual incursions into headwater streams from source populations in mainstream and impoundment environments which were invasion hotspots. Irrespective of establishment, four predatory invaders (largemouth bass *Micropterus salmoides*, smallmouth bass *M. dolomieu,* brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*) impacted heavily on native fish communities (Chapter 3, 4 and 5). Two broad types of invasion were documented, top down invasion by non-native *O. mykiss* and *S. trutta* and upstream invasion by *M. salmoides* and *M. dolomieu* (Chapter 3 and 5). Their impacts included changes in community structure, extirpation from invaded stream reaches resulting in contracted distribution, and isolation and fragmentation of native fish populations. The impacts of non-native predatory fishes were particularly acute for *P. afer* and *B. trevelyani*.

Where non-native predatory fish occurred, *P. afer* and *B. trevelyani* had been extirpated (Chapter 3 and 5). As a result both native species exhibited contracted distributions (>20% habitat loss due to invasion). Upstream invasion by centrarchids isolated and fragmented *P. afer* populations into headwater refugia, while top down invasion by salmonids excluded *B. trevelyani* from invaded, more pristine stream reaches, by forcing the species into degraded unsuitable lower stream reaches. Predation also disrupted population processes such as adult dispersal for *P. afer*, and centrarchid-invaded zones acted as demographic sinks, where adults dispersing through invaded reaches were rapidly depleted. While the Mandela lineage of *P. afer* exhibited little within or between drainage genetic structuring, *B. trevelyani* was >4% divergent between drainages, and up to 2% divergent between streams within the Keiskamma River system (Chapter 7). The distribution of genetic diversity for *B. trevelyani* also indicated that the loss of diversity was imminent without immediate conservation interventions.

This thesis has provided conclusive evidence that native fishes are vulnerable to invasion and that non-native predatory fishes have significant impacts on native fishes in Eastern Cape headwater streams. If management and conservation measures are implemented, the unwanted introduction and spread of non-native fishes may be restricted, allowing native fishes opportunities for recovery.

Acknowledgements

Firstly, I would like to thank my mom and dad who taught me how through hard work and perseverance anything is possible. Dad, I now believe there is no such word as “can’t”. I would also like to thank my two sisters Bridget and Sara for their endless encouragement. Ten years on and I am finally ready to launch! I love you all very much.

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I am extremely fortunate to have had the opportunity to undertake my research on fishes of the headwater streams of the Swartkops River system within the Groendal Wilderness Area and the Keiskamma River system in the Amatola Mountains. This PhD has been an amazing journey.

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CHAPTER 1: A review of current knowledge, risk and impacts associated with non-native freshwater fish introductions in South Africa

1.1 Introduction

Globally freshwaters are relied upon to fulfil a multitude of essential services: directly by providing drinking water, irrigation for crops and power generation; and indirectly through recreation and fish as a source of food. This reliance of humanity on freshwaters results in their unsustainable use (Cucherousset and Olden 2011; Palmer 2010). Signs of fatigue are emerging from these fragile habitats and freshwaters contain more declining and extinct species than either terrestrial or marine environments (Johnson et al. 2008). Freshwater biodiversity is therefore the overriding conservation priority (Dudgeon et al. 2006). Major threats facing freshwater biota are overexploitation, water pollution, flow modification, destruction or degradation of habitat and invasion by non-native species (Dudgeon et al. 2006). The introduction and spread of non-native species resulting in homogenization of the Earths' biota (Clavero and García-Berthou 2006; Rahel 2007) has been dubbed "one of the least reversible human-induced global changes" (Kolar and Lodge 2002).

Invasion has been defined as a number of steps or stages that an introduced species has to traverse within the framework for biological invasions or range expansion process (Blackburn et al. 2011; Richardson et al. 2011; Richardson et al. 2000). The steps or stages involve four major processes: transport, introduction, establishment and spread (Blackburn et al. 2011; Richardson et al. 2000). A species can reach a recipient environment from the donor community either intentionally or accidently. Globally, economic activity has been cited as the primary driver of intentional fish introduction and spread (Gozlan et al. 2010) and the major vectors for transportation are aquaculture (51%), ornamental fish trade (21%), sport fishing (12%) and fisheries (7%) (Gozlan 2008). The introduction rate of non-native fishes has doubled in the last 30 years due to globalisation (Gozlan et al. 2010), and the world's freshwaters are heavily invaded (Strayer 2010).

Although these introductions have often achieved their desired economic objectives (Gozlan 2008), subsequent invasions and the resultant homogenization of biota (Clavero and García-

Berthou 2006; Rahel 2007) are considered “one of the least reversible human-induced global changes” (Kolar and Lodge 2002). Whether the impacts of these introductions are positive or negative is context dependent (Gozlan 2008; Vitule et al. 2012; Ricciardi et al. 2013) and has been the source of much debate (e.g. Gozlan 2008; Vitule et al. 2009). Research on the impacts of non-native fishes is therefore important for developing solutions to a difficult conservation problem (Cucherousset and Olden 2011; Richardson and Ricciardi 2013).

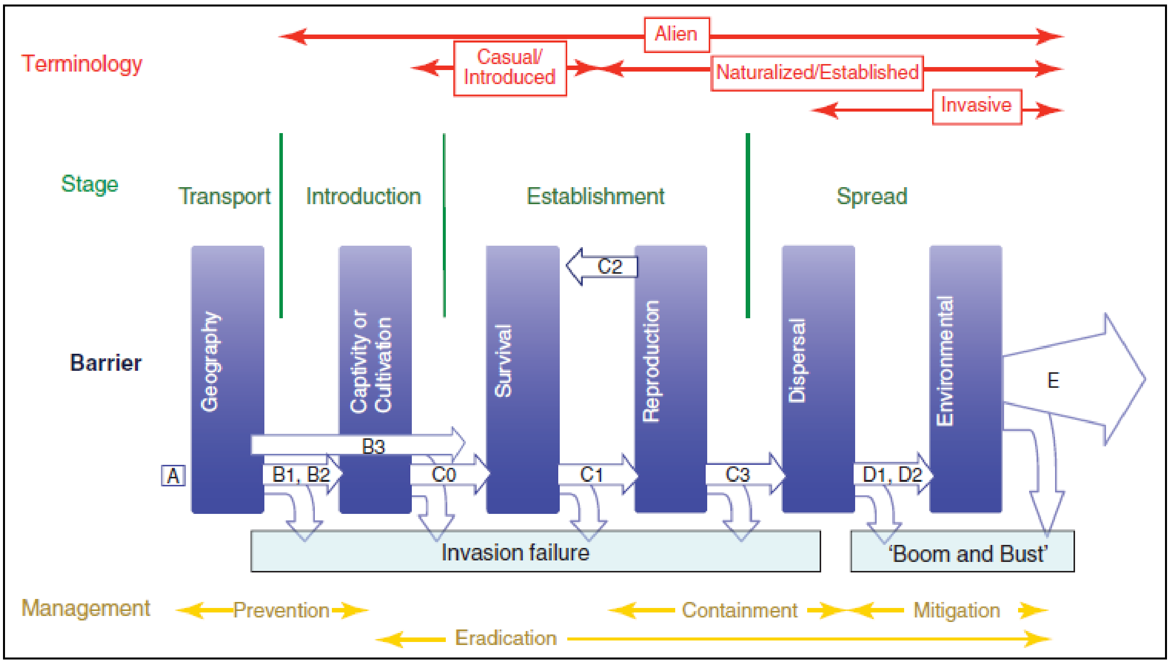


Figure 1.1 The invasion process and the stages needed to be overcome by an introduced species within the unified framework for biological invasions. Taken from Blackburn et al. (2011).

Impacts of introduced non-native species on recipient ecosystems can span multiple levels of biological organisation ranging from genes to ecosystems (Cucherousset and Olden 2011; Ribeiro and Leunda 2012). Impacts can be severe, as Clavero and Garcia-Berthou (2005) demonstrated by analysing the causes of extinction for 680 fish species reported as extinct by the International Union for the Conservation of Nature (IUCN). Of the extinct species, 170 had assigned causes and 20% of those were directly attributable to impacts by non-native species (Clavero and Garcia-Berthou 2005). A classic example of the extreme magnitude of a single species’ impacts was the introduction of Nile perch *Lates niloticus* into Lake Victoria, which is thought to have caused the disappearance of ~200 endemic cichlid species (Witte et al. 1992). Less noticeable but significant sub-lethal impacts, such as suppression of growth and reproduction (Ayala et al. 2007; Fraser and Gilliam 1992) and the inhibition of nutrient cycling between interconnected ecosystems (Baxter et al. 2004) are also major threats. For example, Baxter et al. (2004) showed that in streams invaded by rainbow trout *Oncorhynchus mykiss*, they out-competed native Dolly Varden charr *Salvelinus malma*, causing a shift in *S. malma* foraging from terrestrial invertebrates to invertebrate algal grazers. This foraging shift resulted in an increased algal biomass and decreased invertebrate biomass emerging from the stream to the adjacent forest, and consequently a 65% reduction in the density of riparian specialist spiders (Baxter et al. 2004). Research on the impacts of non-native fishes is increasing as recognition of their major impacts creates a demand for solutions to a difficult conservation problem. Although there is extensive accumulation of literature on fish invasions, Cucherousset and Olden (2011) highlight that it is trivial in comparison to what still needs to be learnt, particularly in poorly studied geographical regions.

In South Africa, one of six global fish invasion hotspots (Leprieur et al. 2008), the problem is extensive and non-native fishes are common components of fish assemblages in all major river systems (van Rensburg et al. 2011). Two significant publications in the mid 1980s assembled literature on non-native aquatic species in South Africa. The first was a review on faunal invasions of aquatic ecosystems of southern Africa by Bruton and Van As (1986), the second an ‘Atlas of Alien and Translocated Indigenous Aquatic Animals in southern Africa’ (de Moor and Bruton 1988). Both these publications greatly enhanced the knowledge of aquatic invasions of South Africa by summarising an extensive body of grey and peer reviewed literature into a usable format.

The major vectors for introductions of non-native fishes in South Africa were found to be primarily associated with recreational angling, aquaculture, conservation translocations, ornamental fish trade, inter-basin water transfer schemes (IBT’s) and bio-control (Bruton and Van As 1986). These introductions were not benign, and impacts on native species include direct predation, ecosystem alterations, hybridisation and the transfer of associated parasites (Bruton and Van As 1986; van Rensburg et al. 2011). Early impacts were predominantly inferred from grey literature. These impacts included direct predation where *O. mykiss* and largemouth bass *Micropterus salmoides* were implicated in the reduction or local extinction of small minnows (fiery redfin *Pseudobarbus phlegethon*, Berg River redfin *P. burgi*, Maloti minnow *P. quathlambae*, Breede River redfin *P. burchelli*, Clanwilliam redfin *Barbus calidus*, Border barb *B. trevelyani*, Treur River barb *B. treurensis*), Cape kurper *Sandelia capensis*, rock catlet *Austroglanis gilli* and kneria *Kneria auriculata*. Translocated redbreast tilapia *Tilapia rendalli* were implicated as the cause of decreased macrophyte densities where introduced. Competition between native and non-native fishes for food and space were documented, for example, the dietary overlap between introduced *Oncorhynchus mykiss* and native *P. quathlambae* and Eastern Cape rocky *Sandelia bainsii* and non-native *M. salmoides.* Hybridisation and genetic introgression potential was also recognised if introduced fishes such as Israeli tilapia *Oreochromis aureus* and Nile tilapia *O. niloticus* hybridize with the native Mozambique tilapia *Oreochromis mossambicus.* Upon reviewing the literature on impacts of invasive fishes cited in Bruton and Van As (1986), however, it became evident that the examples of invasive impacts from early literature were mostly based on casual observations. For example, statements in survey reports such as: "*What was very apparent, however, was that nowhere where there was an established population of exotics could endemic species be found*" (Gaigher 1973; p76), when referring to an ichthyofaunal survey of the Olifants River system, Western Cape, were cited as proof of impacts.

While these studies are valuable, there is an increased need for empirical research on all facets of the invasion process by conducting field and experimental studies on donor and recipient ecosystems to inform non-native species management and develop effective legislation (van Rensburg et al. 2011). Previous observational studies provide a platform upon which to build and direct future research on aquatic invasions in South Africa. It is therefore apt that 27 years after the Bruton and Van As (1986) review, an update on the introduction, status and impacts of non-native fishes is provided. This chapter attempts to review the introduction, establishment and spread of non-native fishes in South Africa, with emphasis on the current knowledge of invasive impacts and research gaps.

1.2 Materials & Methods

An extensive literature search was conducted for the period 1988 – present so as not to repeat what has already been summarised in previous invasion reviews (Bruton and Van As 1986; de Moor and Bruton 1988), and focus on recent advances in the field. All publications including any aspect of the Blackburn et al. (2011) unified framework for biological invasions (transport, introduction, establishment and spread) or documenting ecological impacts were included (Figure 1.1). For the purpose of this chapter, alien species are defined as those that have been introduced from outside the political boundaries of South Africa. Extralimital species have been translocated from their native drainages to other drainages, or within their native drainage to areas where they did not previously occur, such as above waterfalls for conservation purposes. Both alien and extralimital fishes will henceforth be referred to as non-native fishes.

The most up-to-date database of species distributions, the '*Atlas of southern African freshwater fishes,*' was used as a baseline for fish species presence/absences (Scott et al. 2006). Where data were considered deficient, additional updated records from the national fish collection distributions database were acquired (housed at the South African Institute for Aquatic Biodiversity). Publications with updated species lists between 2006 and 2013 were also reviewed and in some cases expert opinion was sought (for example, established researchers were consulted for up-to-date information on certain drainages). The status of each introduced or translocated species within South Africa was evaluated using the criteria outlined in Blackburn et al. (2011) and Table 1.1. Due to incomplete data from numerous drainages, 11 major drainages representative of the aquatic eco-regions of South Africa (Skelton 2001) with the most reliable data were analysed as examples of the introduction, establishment and spread of non-native species.

Table 1.1 Criteria from Blackburn et al. (2011) for categorising invasions which were applied in this review to classify the stage of invasion for all fish species introduced into South Africa.

Category	Description
A	Not transported beyond limits of native range
B1	Individuals transported beyond limits of native range, and in captivity or quarantine (i.e. individuals provided with conditions suitable for them, but explicit measures of containment are in place)
B2	Individuals transported beyond limits of native range, and in cultivation (i.e. individuals provided with conditions suitable for them but explicit measures to prevent dispersal are limited at best)
B3	Individuals transported beyond limits of native range, and directly released into novel environment
C0	Individuals released into the wild (i.e. outside of captivity or cultivation) in location where introduced, but incapable of surviving for a significant period
C1	Individuals surviving in the wild (i.e. outside of captivity or cultivation) in location where introduced, no reproduction
C2	Individuals surviving in the wild in location where introduced, reproduction occurring, but population not self-sustaining
C3	Individuals surviving in the wild in location where introduced, reproduction occurring, and population self-sustaining
D1	Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction
D2	Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction
E	Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence

1.3 Results and Discussion

1.3.1 Literature review

A review of literature for the period 1988-2013 revealed that only 38 studies have investigated invasive fishes within the framework of steps that comprise a biological invasion (Blackburn et al. 2011). The majority of these reviewed studies focussed on impacts (66%), while only 34% considered the introduction, establishment or spread stages. The greater focus on investigating invasive impacts is most likely due to the extensive period that most invasive species have been established in South Africa. In the last decade, however, there has been a considerable increase in the number of studies on fish invasions (Figure 1.2) mirroring the global increase in awareness of the invasive species problem (Davis et al. 2011).

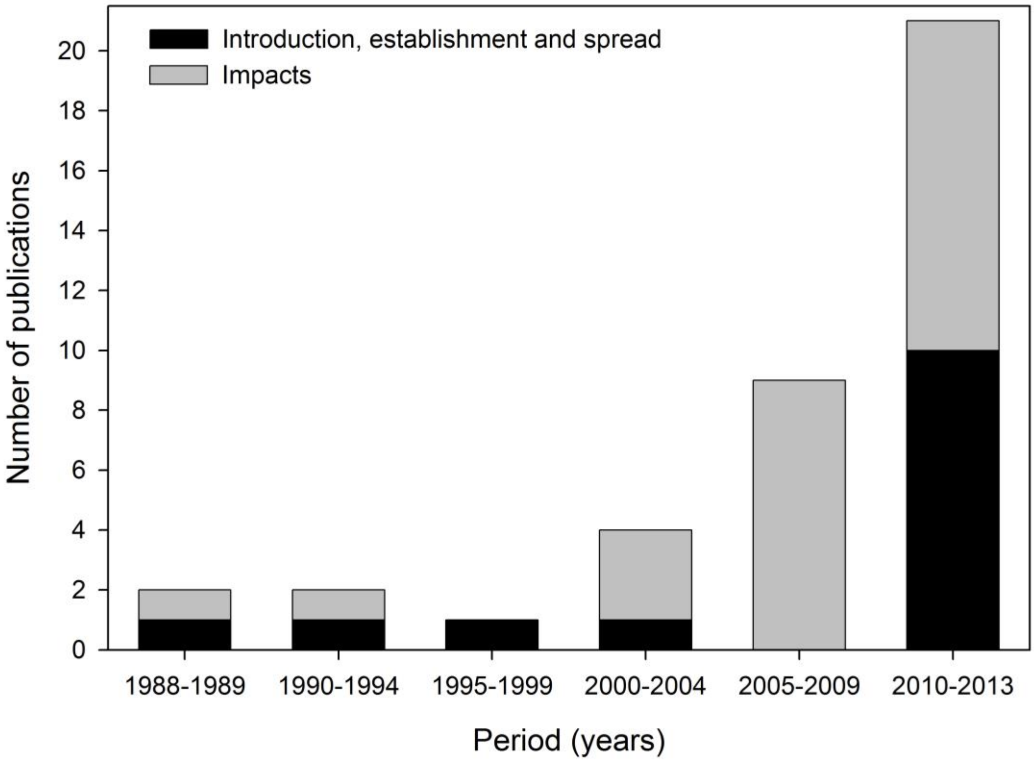


Figure 1.2 Temporal trends and focal aspect of publications within the naturalisation-invasion continuum (Blackburn et al. 2011) in South Africa for the period 1988-2013.

1.3.2 Introduction, establishment and spread

1.3.2.1 Introduction phase

The high introduction rate and spread of introduced and translocated extralimital fishes in South Africa confirms its status as a fish invasion hotspot (Leprieur et al. 2008) (Figure 1.3). To date, 55 species (28 alien, 27 extralimital) have been introduced into or translocated within South African freshwater ecosystems (Table 1.2).

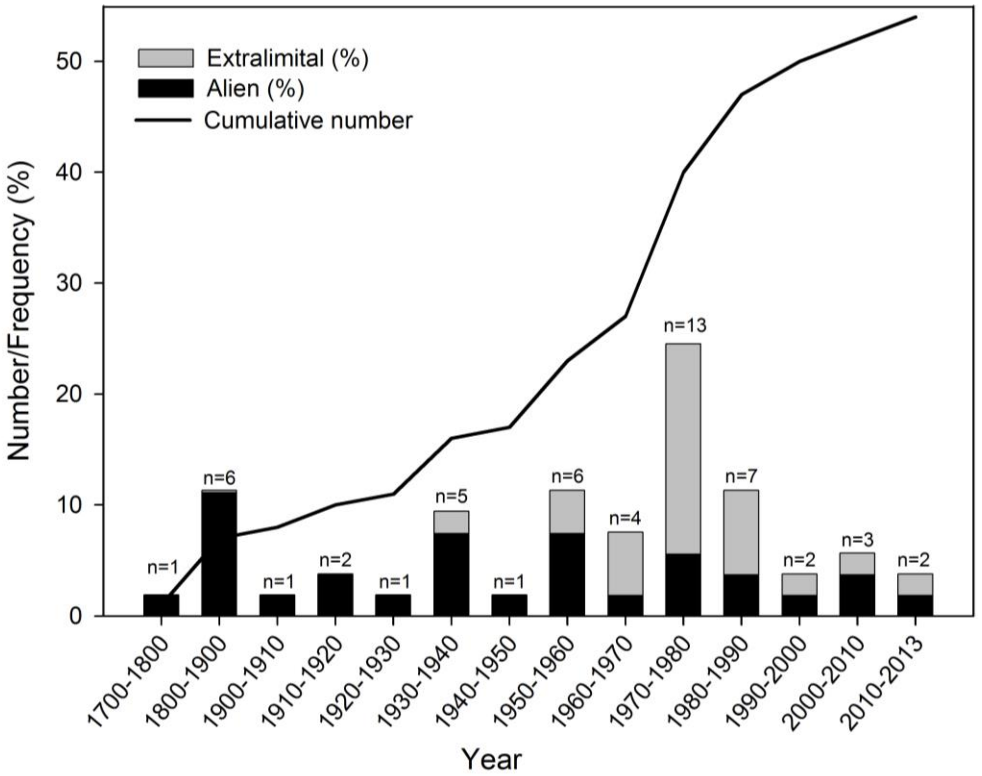


Figure 1.3 The number and rate of non-native and translocated fish introductions in South Africa from the early 1700s until present (only first time records of introductions into the wild were included).

The number of introduced species in South Africa exceeds that reported for Portugal, the Azores and Madeira Islands (Ribeiro et al. 2009), and Spain (Elvira and Almodóvar 2001) but is less than that reported for California, USA (Marchetti et al. 2004c). In South Africa the high number stems from a long history of non-native fish introductions dating back from the first introduction of goldfish *Carassius auratus* for ornamental purposes in 1726 (de Moor and Bruton 1988), to the most recent record of the giant pangasius *Pangasius sanitwongsei* in 2012, an illegal import thought to be an accidental release from the ornamental fish trade (Mäkinen et al. 2013).

Due to the lack of adequate technologies for effective fish transportation, the rate of introductions was initially low (Bruton and Van As 1986), but from 1900 onwards an average of four species were introduced or translocated per decade (Figure 1.3). All early introductions were of aliens imported into South Africa, but as biodiversity concerns began to surface in the 1960s (McCafferty et al. 2012) the first native species were translocated for conservation reasons (Figure 1.3). This was followed by an insurgence of conservation-related introductions in the 1970s and 1980s. From the 1990’s onwards, the introduction rate has slowed.

Table 1.2 A list of the non-native (alien and extralimital) species introduced and translocated in South Africa between 1726 and 2013 (ANG = angling; CON = conservation; AQU = aquaculture; ORN = ornamental; IBT = Inter-basin water transfer; BCT = Bio-control). Invasion category defined according to (Blackburn et al. 2011).

Species	**Date**	**Vector**	**Origin**	**Status**	**Category**
[2]*Carassius auratus*	1726	ORN	Alien	Established	E
[2]*Cyprinus carpio*	1859	ANG	Alien	Established	E
[2]*Salvelinus fontinalis*	1890	ANG	Alien	Failed	F
[2]*Salmo trutta*	1892	ANG	Alien	Established, widespread	E
[2]*Salmo salar*	1896	ANG	Alien	Failed	F
[2]*Tinca tinca*	1896	ANG	Alien	Established, localised	C3
[2]*Oncorhynchus mykiss*	1897	ANG	Alien	Established, widespread	E
[2]*Oreochromis aureus*	1910	AQU	Alien	Failed	F
[2]*Poecilia reticulata*	1912	ORN	Alien	Established, localised	E
[2]*Perca fluviatilis*	1915	ANG	Alien	Established, localised	C3
[2]*Micropterus salmoides*	1928	ANG	Alien	Established, widespread	E
[2]*Gambusia affinis*	1936	BCT	Alien	Established, widespread	E
[2]*Oreochromis mossambicus*	1936	AQU	Extralimital	Established, widespread	E
[2]*Micropterus dolomieu*	1937	ANG	Alien	Established, widespread	E
[2]*Lepomis macrochirus*	1939	ANG	Alien	Established, widespread	E
[2]*Micropterus punctulatus*	1940	ANG	Alien	Established, widespread	E
[1]*Pseudocrenilabrus philander*	1941	BCT	Extralimital	Established	B3
[2]*Tilapia sparrmanii*	1941	ANG	Extralimital	Established, widespread	E
[2]*Tilapia rendalli*	1952	BCT	Extralimital	Established, widespread	E
[2]*Labeobarbus aeneus*	1953	ANG	Extralimital	Established	E
[2]*Oreochromis niloticus*	1955	AQU	Alien	Established	D2
[2]*Sarotherodon galilaeus*	1959	AQU	Alien	Failed	F
[2]*Tilapia zilli*	1959	AQU	Alien	Failed	F
[2]*Serranochromis robustus*	1960	ANG	Alien	Failed	F
[2]*Labeobarbus natalensis*	1964	CON	Extralimital	Established	C3
[2]*Ctenopharyngodon idella*	1967	BCT	Alien	Established	E
[2]*Barbus gurneyi*	1970	ANG	Extralimital	Failed	F
[2]*Pseudobarbus burchelli*	1970	CON	Extralimital	Unknown	B3
[2]*Xiphophorus hellerii*	1974	ORN	Alien	Established, localised	D2
[2]*Austroglanis sclateri*	1975	IBT	Extralimital	Uncertain	C1
[2]*Barbus anoplus*	1975	IBT	Extralimital	Established, widespread	E
[5]*Chetia brevis*	1975	CON	Extralimital	Established, localised	C3
[2]*Clarias gariepinus*	1975	IBT	Extralimital	Established, widespread	E
[2]*Hypophthalmichthys molitrix*	1975	AQU	Alien	Established	D2
[2]*Labeo capensis*	1975	IBT	Extralimital	Established	D2
[2]*Labeo umbratus*	1975	IBT	Extralimital	Established	E
[2]*Mugil cephalus*	1975	AQU	Extralimital	Failed	C1
[2]*Myxus capensis*	1975	AQU	Extralimital	Failed	C1
[2]*Notobranchius orthonatus*	1975	CON	Extralimital	Failed	F
[2]*Notobranchius rachovii*	1976	CON	Extralimital	Established, localised	C3
[2]*Barbus treurensis*	1977	CON	Extralimital	Established	C3
[2]*Kneria auriculata*	1981	CON	Extralimital	Established	C3
[2]*Oreochromis andersoni*	1982	AQU	Alien	Failed	F
[2]*Sandelia capensis*	1982	ANG	Extralimital	Established, localised	C3
[2]*Micropterus floridanus*	1984	ANG	Alien	Established	E
[2]*Labeobarbus capensis*	1985	CON	Extralimital	Established, localised	C3
[2]*Protopterus annectens brieni*	1987	CON	Extralimital	Established, localised	F
[2]*Gilchristella aestuaria*	1990	ANG	Extralimital	Established, localised	E
[3]*Sandelia bainsii*	1995	CON	Extralimital	Uncertain	B3
[4]*Barbus calidus*	1998	CON	Extralimital	Established, localised	C3
[4]*Barbus serra*	1998	CON	Extralimital	Established, localised	C3
[8]*Pterygoplichthys disjunctivus*	2000	ORN	Alien	Established, localised	D2
[6]*Xiphophorus maculatus*	2006	ORN	Alien	Uncertain	B3
[9]*Pangasius sanitwongsei*	2012	ORN	Alien	Uncertain	B3
[7]*Hydrocynus vittatus*	2012	ANG	Extralimital	Established, localised	C3

The leading cause of introductions in South Africa is angling (35%) followed by the translocation of fishes for conservation purposes (20%) and aquaculture (17%) (Figure 1.4 A). Accidental escapees or releases of fish from the ornamental fish trade (11%), transfer via inter basin water transfer schemes (IBTs) (9%) and fish imported as bio-control agents (7%) were also contributors. These vectors for introduction are not unique to South Africa since sport fishing, aquaculture and the ornamental fish trade are also major global introductory pathways (Copp et al. 2007; Gozlan 2008; Ribeiro et al. 2009).

1.3.2.1.1 Angling

Initially, the large number of angling-motivated introductions were a result of state-supported formal stocking programs as South Africa due to the perceived paucity of suitable native angling species (de Moor and Bruton 1988). This practice continued until the 1980s (*sensu* McCafferty et al. 2012), which ensured that high propagule pressure (number of repeated introductions), a major determinant of establishment success (Copp et al. 2007; Duggan et al. 2006; Leprieur et al. 2008), maximised their chances of establishment. The perceived lack of suitable angling species prompted the introduction of numerous globally esteemed species such as largemouth bass *M. salmoides*, smallmouth bass *M. dolomieu*, common carp *Cyprinus carpio*, brown trout *Salmo trutta* and rainbow trout *O. mykiss*. A massive recreational fishery subsequently developed, largely targeting non-native fishes (McCafferty et al. 2012), with an estimated 1.5 million participants (Leibold and Van Zyl 2008). This large recreational fishery resulted in the further spread of suitable angling species and the introduction of associated non-native fodder fish such as bluegill sunfish *Lepomis macrochirus* and the extralimital banded tilapia *Tilapia sparrmanii* (de Moor and Bruton 1988). The extensive establishment of non-native sport fishes in South Africa has reduced pressures for further new introductions. The recent translocation of tigerfish *Hydrocynus vittatus* (O’Brien et al. 2012), however, indicates that angling is still a major vector for the spread of fish, but less so for the import of new species.

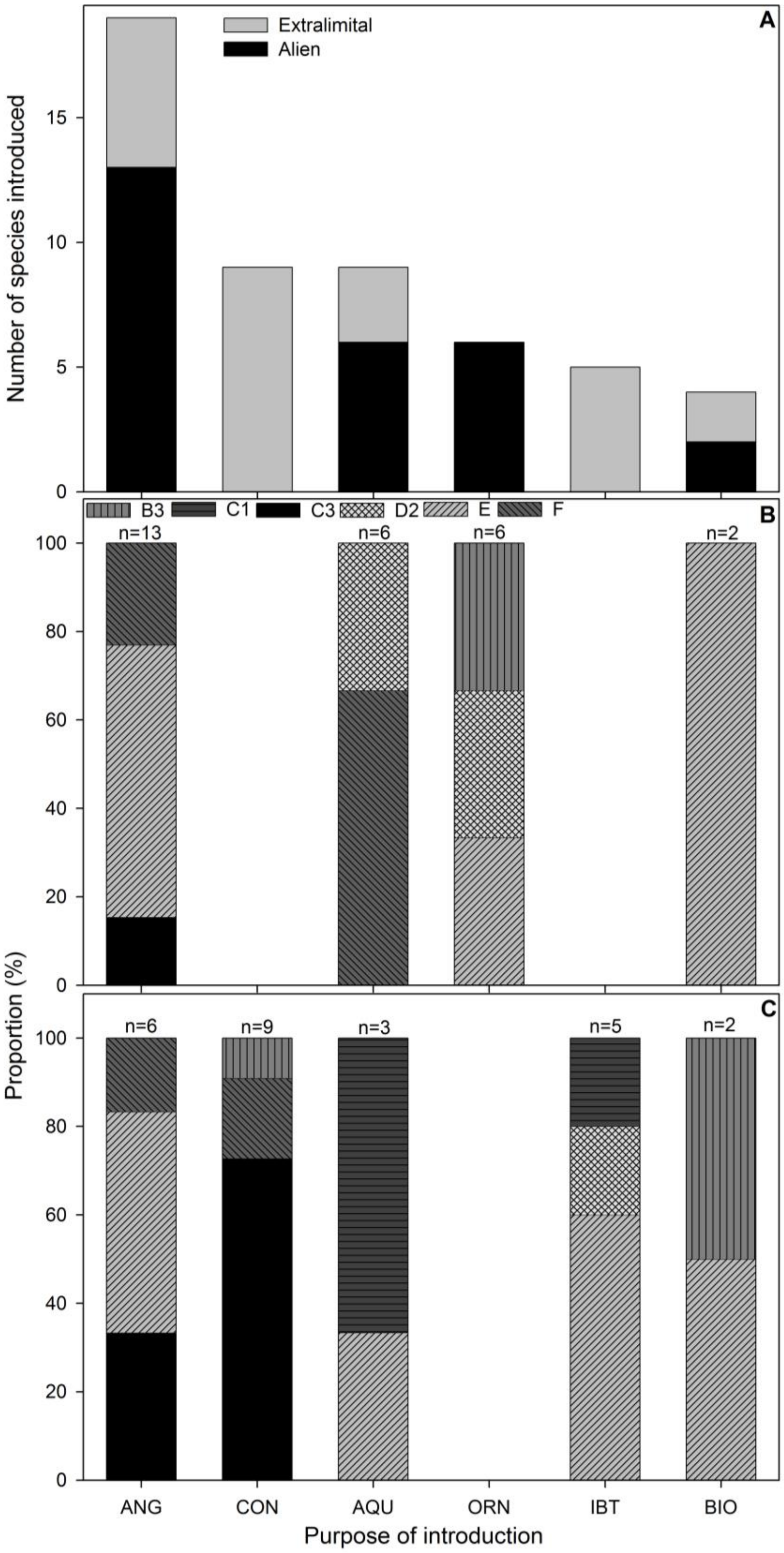


Figure 1.4 (A)The primary purpose for the introduction or translocation of alien and extralimital fishes in South Africa (ANG = angling; CON = conservation; AQU = aquaculture; ORN = ornamental; IBT = Inter-basin water transfer; BCT = Bio-control). Categorisation of the status of fishes introduced into South Africa for each of the five vectors using the criteria from Blackburn et al. (2011) for alien (B) and extralimital fishes (C). An additional category was included for failed (F) introductions.

1.3.2.1.2 Ornamental fish trade

Although it is the second-most import vector for fish introductions into the wild in South Africa, surprisingly, the ornamental fish trade has not resulted in larger numbers of introductions as reported from either England (Copp et al. 2007), the United states or Canada (Duggan et al. 2006). Currently South Africa permits the import and sale of ~1600 freshwater fish species (Anon. 1994). The ornamental fish trade has resulted in the most recent introductions, including *P. sanitwongsei* (Mäkinen et al. 2013) and the vermiculated sailfin *Pterigoplichthys disjunctivus* (Jones et al. 2013). These introductions are cause for concern and underlie the potential risks associated with the importation of new species via this vector. Due to consistent imports, and the possible release of fish by aquarists, the potential for introductions via this vector is high. To compound this, globally the ornamental species trade is a generally unregulated industry (Padilla and Williams 2004). This was exemplified in South Africa in a recent study using DNA barcoding to verify that reported common names corresponded to species on the permitted species list. Van der Walt (2012) demonstrated that from a random sample of 120 aquarium trade fish species, 19 were not on the permitted species list, resulting in a misidentification rate of 15%. Positively identifying species is also complicated by hybridisation between congeners, as demonstrated by Mäkinen et al. (2013) for *P. disjunctivus*, or confusion over common names (Van der Walt 2012). Both forms of misidentification illustrate the lack of control in the ornamental fish trade and the risk of further unwanted introductions via this vector.

1.3.2.1.3 Aquaculture

The introduction of non-native species for aquaculture is a highly contentious issue. Currently *O. mykiss* are the mainstay of South Africa’s freshwater aquaculture sector (van Rensburg et al. 2011), their introduction has resulted in negative impacts on native fishes in South Africa (Woodford and Impson 2004) and elsewhere (Crowl et al. 1992). In developing countries, economic pressure often dictates management decisions (Pelicice et al. 2013), therefore the import of new popular aquaculture species or the spread of currently restricted species such as Nile tilapia *Oreochromis niloticus* in South Africa is likely. Brazil for example has recently allowed non-native fish cage culture in any hydroelectric reservoir of the country, which will facilitate the introduction and spread of some of the world’s worst invasives (Pelicice et al. 2013). *Oreochromis niloticus* was introduced into South Africa for aquaculture in 1955 and is thought to be confined to the Limpopo River system and small coastal river systems in the Kwa-Zulu Natal Province, although their current status in the latter is unconfirmed (de Moor and

Bruton 1988; van Rensburg et al. 2011). *Oreochromis niloticus* is a popular aquaculture species that is also highly invasive (Weyl 2008) and has had devastating impacts through competition or hybridisation with native congenerics (Canonico et al. 2005; Firmat et al. 2013; Moralee et al. 2000). Another recently recognised issue is that of accurately identifying the tilapiine species that are being cultured. D'Amato et al. (2007) found that individuals identified taxonomically as *O. mossambicus,* when analysed genetically, turned out to carry mtDNA of several introduced *Oreochromis* species.

1.3.2.1.4 Introductions for conservation

In South Africa, more native species have been translocated, than aliens introduced. Ironically, the large number of translocations for conservation purposes may in many cases be attributed to counteracting their extirpation by non-native fish predation from core distributions within their native range (Engelbrecht et al. 2001; Impson and Tharme 1998; Kleynhans 1985). Three imperilled native fishes, *B. treurensis* (Limpopo River system), Clanwilliam sawfin *B. serra* and *B. calidus* (Olifants River system) were translocated within their native river systems to above waterfalls that originally marked the upper limit of natural fish distribution. Another endangered fish, the anabantid *S. bainsii,* has been translocated to sanctuaries within its native range to avoid the threat of imminent extinction (Cambray 1997). No mention is made of any risk assessments conducted to assess their impacts in fishless zones, which may be extensive (Knapp et al. 2001). Although the authors of the translocation studies had good intentions, many amphibians and invertebrates require fishless zones for their persistence (Adams et al. 2001; Knapp et al. 2007; Knapp et al. 2001). For example, after the eradication of introduced salmonids from previously fishless lakes, the mountain yellow-legged frog, *Rana muscosa*, significantly increased in abundance and partly reversed formerly observed declines (Knapp et al. 2007).

1.3.2.1.5 Inter-basin water transfer schemes (IBT's)

South Africa is a water-scarce country, and 26 Major IBTs have been constructed (Slabbert 2007) to stabilise water supplies (Ashton 2007). There is little information on fish introductions associated with IBTs. Literature on IBT-facilitated introductions deals almost entirely with the Orange/Fish IBT, which resulted in the transfer of five fish species from the Orange/Vaal to the Great Fish River system (smallmouth yellowfish *Labeobarbus aeneus*, African sharptooth catfish *Clarias gariepinus*, Orange River mudfish *Labeo capensis*, rock catlet *Austroglanis* *sclateri*, moggel *Labeo umbratus*) (Laurenson et al. 1989; Laurenson and Hocutt 1986). Genetically distinct populations of *L. umbratus* also occur in both systems and there is concern that translocated *L. umbratus* will hybridise with native populations (Ramoejane 2011). Concern was also expressed that previously isolated morphologically distinguishable *B. anoplus* populations from the Orange and Fish Rivers would hybridise via IBT introductions (de Moor and Bruton 1988). This has already been demonstrated for *L. umbratus* (Ramoejane 2011).

1.3.2.1.6 Bio-control

Only three fish species have been introduced for bio-control: mosquitofish *Gambusia affinis,* grass carp *Ctenopharyngodon idella* and redbreast tilapia *Tilapia rendalli* (Bruton and Van As 1986; de Moor and Bruton 1988). These introductions were all prior to 1967. The threat of new introductions via this vector is therefore considered negligible; however, further spread of these species within South Africa may pose threats to native biota.

1.3.2.2 Establishment

Successful establishment of non-native fishes after introduction is an important (Gozlan 2008), but often omitted (e.g. Leprieur et al. 2008; Marr et al. 2013) consideration in assessing fish invasions (Blackburn et al. 2011). Only 21% (11) of all introductions were reported to have failed and although this establishment rate seems high (79%), establishment rates were similar to those found in Portugal, the Azores, the Madeira Islands and Spain (Elvira and Almodóvar 2001; Ribeiro et al. 2009). Determining the establishment rates of fishes relies on accurate introduction data, which are often unavailable and failed introductions are not often reported (Ribeiro and Leunda 2012). While recognising the limitations due to inaccurate introduction data, such as inflated establishment estimates, the data are the most accurate currently available. Overall, establishment rates in South Africa were high for all vectors responsible for introductions: the highest was for IBTs (80%), angling (79%), bio-control (75%), conservation (73%), ornamental purposes (67%) and lowest was for aquaculture (33%). According to the Blackburn et al. (2011) criteria, 20 fishes (37%) are considered fully invasive (E). Introductions for angling were responsible for the highest proportion (55%) of invaders (E) with IBTs (15%), bio-control (15%) and aquaculture (5%) constituting the remainder (Figure 1.4 B). Translocation of fishes for conservation purposes has not resulted in any species fully invasive species (E), but populations are predominantly self-sustaining where released (C3) (Figure 1.4 C).

The mechanisms responsible for the high establishment rates of introduced fishes into South Africa may be a reflection of high human interest in the species, which also often mask underlying biological characteristics determining establishment success (Marchetti et al. 2004b). These mechanisms include: specifically chosen species with proven establishment rates and prior invasion success elsewhere (Marchetti et al. 2004b; Ribeiro et al. 2007; Ruesink 2005); high propagule pressure (Copp et al. 2007; Duggan et al. 2006; Leprieur et al. 2008); and a species' physiological tolerances (Marchetti et al. 2004b).

The introduced sport fishes *M. salmoides*, *O. mykiss* and *C. carpio* fit the profile since they are popular species with high human interest (Marchetti et al. 2004c) that have been spread and established globally. In South Africa intentional stocking programmes were conducted by government institutions and angling organisations (McCafferty et al. 2012) using species that were carefully chosen and imported to fill specific niches (van Rensburg et al. 2011). Intensive stocking regimes also resulted in high propagule pressure, further increasing chances of successful establishment. For example, after salmonids (*O. mykiss* and *S. trutta*) had been introduced into mountain streams, three centrarchid fishes were imported to fill specific niches not occupied by salmonids. *Micropterus salmoides* were introduced for lentic water and the lower reaches of rivers; *M. dolomieu* for the swifter, warm water lotic habitats and spotted bass *M. punctulatus* for larger more turbid environments. Considering these fishes were chosen according to their abilities to successfully establish elsewhere, their success in South Africa is not surprising. High rates of establishment for intentionally introduced sport fishes are consistent with Ruesink (2005), who documented that intentionally introduced fishes were more likely (384/506 = 76%) to establish.

High propagule pressure is most probably the mechanism responsible for high establishment rates from IBT-linked introductions. Regular water releases from IBTs create a corridor for fish translocation (Snaddon et al. 1998) which ensures a fairly regular propagule supply from donor to recipient systems. This is evident when examining establishment of fishes translocated from the Orange River system to the Great Fish River system by IBT. Those fish species abundant in Lake Gariep in the vicinity of the IBT tunnel intake have also established in the Great Fish River (e.g. *C. gariepinus*, *L. aeneus*, *L. capensis*; (Ellender et al. 2012c)), while those that were rare in the lake (e.g. *A. sclateri,* largemouth yellowfish *Labeobarbus kimberleyensis*) have not (Laurenson and Hocutt 1986; Weyl et al. 2009). The resultant low propagule pressure into the

Great Fish River system may therefore inhibit their successful establishment. A study on the drivers of establishment for fishes introduced into the Sundays River system irrigation network dams, indicated that propagule pressure was a significant determinant of establishment success (Woodford et al. 2013).

Ornamental fishes that have established in South Africa include *C. auratus*, guppy *Poecilia reticulata*, swordtail *Xiphophorus helleri*, platy *X. maculatus* and *P. disjunctivus*. The abovementioned species are all common and popular aquarium species, significant determinants of establishment success in Canadian and United States waters (Duggan et al. 2006). Although hundreds of species are currently being imported into South Africa, introductions into the wild are mainly facilitated by aquarists releasing unwanted pets. It is therefore impossible to quantify the number of species that have been released into the wild.

Fishes introduced for conservation purposes were predominantly translocated within the same river system but into areas where they did not previously occur, such as above waterfalls that would have originally limited their natural distribution. Fishes were also often stocked in previously fishless reaches of the river systems without other fish competitors or predators. Despite both low propagule size and number (Simberloff 2009) (often only a single introduction event), establishment rates were high (73%). As these areas were often geographically close and had conditions similar to their native range, this may explain the high establishment rates.

Countrywide distribution data have shown that broader scale distribution and establishment of non-native fishes may be a reflection of where they have been introduced rather than their actual physiological tolerances. For example, of the physiologically relevant environmental factors limiting the distribution of freshwater fishes, temperature is probably the most important. With the exception of salmonids (*S. trutta* and *O. mykiss;*), which generally prefer temperatures <20°C (Boughton et al. 2007; Edwards et al. 1979; Forseth et al. 2009), many of the fully invasive species in South Africa have broad physiological tolerances. North American centrarchids for example, tolerate temperatures between 4 °C and 30 °C (Warren 2009), *C. carpio* (2-40.6 °C;(Koehn 2004)), *G. affinis* (5- >35 °C;(Pyke 2005)). Their distribution in South Africa is not a reflection of these documented tolerances. On a finer scale, however, within River systems where introduced, their distribution may be a better reflection of their physiological tolerances or preference.

Determining establishment success on a countrywide scale may be misleading as finer scale studies investigating system and stream scale establishment rates are rare. Records of failed introductions are also scarce, which hampers accurately determining establishment rates (Ribeiro and Leunda 2012). This indicates that at drainage basin scale, establishment rates are probably closer to what was described as the ‘tens’ rule, where 10% of introduced organisms establish and 10% of those then become pests (Ruesink 2005; Williamson and Brown 1986). For example, Woodford et al. (2013) demonstrated that while an irrigation network resulted in the transport of nine fish species from donor to recipient environments, only five species successfully established. This establishment was a result of high propagule pressure and reproductive guild: benthic spawners (*C. carpio* and *C. gariepinus*) were less successful than live bearers (mosquitofish *Gambusia affinis*), pelagic spawners (estuarine roundherring *Gilchristella aestuaria* and river goby *Glossogobius callidus*) and mouth brooders (*O. mossambicus*) in irrigation ponds where water levels fluctuated daily (Woodford et al. 2013).

Due to the variable rates of spread, introduced species may take decades to fulfil their invasive potential (Strayer 2010). An assessment of the establishment of *L. aeneus* translocated from the Orange River system to the Great Fish River via IBT revealed that the species had not established eight years after their introduction (Laurenson et al. 1989). A follow-up study 30 years later confirmed that *L. aeneus* had subsequently established, and indicated that there was an extensive ‘lag’ phase between their initial introduction and establishment (Weyl et al. 2009). Even if a non-native fish species becomes established, this does not necessarily mean that it is able to establish in all parts of the river system. Establishment comparisons between populations in the mainstream Great Fish River and the Glen Melville reservoir, an off-stream impoundment, indicated that *L. aeneus* was only established in the Great Fish River and that persistence in the impoundment was due to continued recruitment from the Great Fish River (Weyl et al. 2009). Similarly, the *O. niloticus* introduction into the Limpopo River system has yet to fulfil its potential for establishment and spread in South Africa (Zengeya et al. 2013a). In the Blindekloof stream, a headwater tributary of the Swartkops River system, Eastern Cape, South Africa, Ellender et al. (2011) demonstrated that only four (*M. salmoides*, *M. dolomieu*, *C. gariepinus*, *T. sparrmanii*) out of six non-native species recorded from the river system had managed to invade the stream, and that only one of these, *T. sparrmanii*, had successfully established.

While many freshwater fishes are stenohaline and unable to invade estuaries, some non-native freshwater fishes have managed to establish in estuarine or brackish water environments. In the

Wilderness Lakes system, a series of interconnected estuarine lakes, two euryhaline non-natives (*O. mossambicus* and *G. affinis*) were established and *C. carpio* were in the early stages of invasion (Olds et al. 2011). *Micropterus salmoides*, which had been recorded 15 years previously, was absent from 2009 and 2010 surveys (Olds et al. 2011).

A prolific invader, *O. niloticus* has displayed extensive invasive potential outside its documented physiological tolerances. An ecological niche model developed to predict the invasive potential of *O. niloticus* indicated its potential for spread, since the environmental conditions in their native and introduced ranges were not congruent (Zengeya et al. 2013a). This implies that the species displays the ability to occupy habitats outside its documented habitat preferences (Zengeya et al. 2013a). A qualitative risk assessment model for the Limpopo River system also indicated that mainstream habitats and the lower reaches of tributaries were at high risk of invasion (Zengeya et al. 2013b). Low risk areas were predominantly associated with low temperature (8-12 °C) and high altitudes which were unsuitable for *O. niloticus* (Zengeya et al. 2013b).

1.3.2.3 Spread

There is a paucity of drainage-specific distribution data for South Africa, and those that do exist lack the spatial resolution for system-wide assessments (de Moor 1996). The most current distributions data are available in the Atlas of southern African freshwater fishes (Scott et al. 2006), however, inconsistencies and the lack of accurate data for the various drainages of South Africa hampers analyses. An example of this is that in many instances only native fishes were collected and their specimens added to fish collections during past ichthyological surveys, while non-native species were ignored or discarded (E.R Swartz pers. comm.). This severely constrains documenting the spread of introduced fishes in South Africa. On analysing finer scale patterns of establishment from 11 drainages with fairly accurate species distribution data, it was evident that 13 species have established in three or more of these drainages in South Africa (Table 1.3). Data on the spread of non-native fishes from their initial introduction sites may be scarce. However, using two common angling species as an indication of the potential to spread, *C. carpio* and *M. salmoides* now inhabit every major river system in South Africa (van Rensburg et al. 2011). The invasive potential of *C. carpio* was illustrated in a study on their life history and population dynamics in Lake Gariep, South Africa’s largest impoundment (Winker et al. 2011). Compared to populations in their native range, introduced *C. carpio* matured earlier and grew faster but had high mortality rates, traits indicative of rapid population growth potential (Winker et al. 2011). In the case of *M. salmoides*, in the 10 years following their introduction, they spread into five major catchments on the east coast of South Africa from the Clanwilliam/Olifants drainage on the west coast to the upper Incomati system on the east coast, a distance of >1500 km (de Moor 1996). This further illustrates the fervour with which people moved fish between drainages during early introduction phases. The extent to which non-native fishes are spread between drainages is also a function of time (Copp et al. 2007), and all currently widespread fishes have been established in South Africa for longer than 35 years (Table 1.2, Table 1.3).

Table 1.3 The distribution of non-native fishes that occur in three or more of the major drainages where reliable species presence/absence data were available in South Africa (*extralimital species).

	Berg	**Breede**	**Fish**	**Incomati**	**Keiskamma**	**Limpopo**	**Olifants**	**Orange**	**Pongolo**	**Sundays**	**Swartkops**
*L. umbratus**			*x*			*x*				*x*	
*L. aeneus**			*x*			*x*				*x*	
S. trutta					*x*		*x*	*x*			
*C. gariepinus**	*x*		*x*		*x*					*x*	*x*
G. affinis	*x*		*x*	*x*		*x*		*x*		*x*	
M. dolomieu	*x*	*x*		*x*		*x*	*x*				*x*
M. punctulatus	*x*		*x*	*x*		*x*	*x*				*x*
O. mykiss	*x*	*x*		*x*	*x*		*x*	*x*			
L. macrochirus	*x*	*x*	*x*		*x*	*x*	*x*	*x*			
*T. sparrmanii**	*x*	*x*	*x*		*x*		*x*			*x*	*x*
*O. mossambicus**	*x*	*x*	*x*		*x*		*x*	*x*		*x*	*x*
C. carpio	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*
M. salmoides	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*

1.3.3 Invasive impact studies in South Africa

Despite the large number of introduced and translocated species, research on the invasive impact of fishes in South Africa is in its infancy. In the 24 years since the previous invasions review by Bruton & Van As (1986), only 25 studies demonstrating the impact of non-native fish species on recipient ecosystems have been published. Included in this list are two perspective papers that document observational evidence on the impact of salmonids (*S. trutta* and *O. mykiss*) and *C. gariepinus* on native fishes in South Africa (Cambray 2003a; Cambray 2003b). While there has been increased interest in documenting invasive impacts, it was only between the years 2000 and 2013 that research in this field gained momentum (Figure 1.2). Research has predominantly focussed on competitive or predatory impacts at individual and population levels, with some research on genetic impacts.

1.3.4 Genetic impacts, hybridisation and introgression

Human-mediated hybridisation is the leading cause of global biodiversity loss (Muhlfeld et al. 2009). Hybridisation is defined as the mating between individuals from two genetically distinct populations and introgression results when the offspring are fertile and backcross to parental populations (Allendorf et al. 2013). The level and type of impact therefore depends on the viability of offspring. If offspring are viable it may result in a hybrid swarm and eventual genomic extinction (Muhlfeld et al. 2009). In South Africa, studies on the impact of *O. niloticus* introductions into the Limpopo River system indicate extensive hybridisation and introgression with native *O. mossambicus* (D’Amato et al. 2007; Moralee et al. 2000). Further complicating the matter, there were also non-native *O. mortimeri-andersoni* mtDNA specimens, pointing toward the presence of a hybrid swarm (D’Amato et al. 2007). Phylogeographic analysis of *O. mossambicus* within their native range recognised three lineages: a Zambezi basin lineage; a Malawian lineage and a southern lineage (including South African coastal estuarine populations) (D’Amato et al. 2007). These historically isolated lineages may be under threat as individuals sequenced from aquaculture facilities in the Limpopo basin grouped with the Zambezi and Malawian lineages, indicating extensive translocations and a threat of hybridisation with native lineages (D’Amato et al. 2007). Efforts should be made to preserve these unique lineages and the introduction of any *O. mossambicus* into these regions should be prevented, otherwise the long-term genetic integrity of *O. mossambicus* is likely to be further compromised (D’Amato et al. 2007). Hybridisation is recognised as a primary threat to *O. mossambicus* and they are consequently IUCN redlisted as ‘Near Threatened’ (Cambray and Swartz 2007).

Threats to the genetic integrity of a species may also result from a breakdown of biogeographical barriers, resulting in mixing of previously isolated populations of the same species or between congenerics. For example, the genetic integrity of *L. umbratus* is being threatened in numerous southern coastal river populations by introductions of congeners via IBTs (Ramoejane 2011). The natural distribution of *L. umbratus* encompasses the Vaal and upper Orange River systems and the Gouritz, Gamtoos, Sundays, Great Fish, Buffalo and Nahoon River systems on the east coast (Skelton 2001). Genetic analyses have indicated that each of these river systems harbours unique genetic diversity (Ramoejane 2011). Via the Orange Fish tunnel IBT, which also links the Great Fish and Sundays River systems, Orange River *L. umbratus* and its congener *L. capensis* were translocated from the Orange River system into the Fish River. There is therefore the threat of mixing within *L. umbratus*, and additionally *L. umbratus* x *L. capensis* hybrids have been documented (Ramoejane 2011). The 25 other IBTs in South Africa also provide vectors for mixing previously isolated populations or species.

1.3.5 Competition and predation

Impacts of non-natives on native fishes at the individual level include: alterations in behaviour; suppression of vital rates such as growth and reproduction (Fraser and Gilliam 1992); and morphological changes in response to invader presence/absence (Latta et al. 2007). Few studies have addressed impacts at the individual level in South Africa. However, in the upper Berg River where *P. burgi* co-occur with non-native *O. mykiss*, *P. burgi* juveniles exhibited predator avoidance along a depth gradient, only occupying shallow littoral habitats (Woodford and Impson 2004). In the Driehoeks River (Olifants River system) another small endemic, the Cape galaxias *Galaxias zebratus* occupied deeper more complex habitats in the presence of *M. salmoides*, than in non-invaded sites (Shelton et al. 2008). These studies were primarily descriptive, however the mechanisms responsible for the observed patterns are most probably related to the threat of predation by introduced fishes. A major bottleneck in quantifying behavioural impacts is that in most cases native and non-native fish species do not occur sympatrically (Ellender et al. 2011; Weyl et al. 2013; Woodford et al. 2005). Predation generally results in local extirpation of native fishes, and therefore individual level sub-lethal impacts are difficult to document due to lack of co-occurrence between native and non-natives.

At small spatial scales in the Blindekloof stream (Swartkops River system), Skelton (1993) and Ellender et al. (2011) recorded the complete absence of native fishes from stream reaches invaded by *M. salmoides*, and similar trends were observed from the nearby Wit River (Sundays River system) (Traas 2009). Similar within-stream patterns have been observed in invaded rivers throughout the Cape Floristic Region (CFR) (Lowe et al. 2008; Weyl et al. 2013; Woodford et al. 2005). There is evidence that vulnerability to invasion is also size and species specific. In the Rondegat River, a headwater tributary of the Olifants River system, three studies consistently recorded the same patterns, where four out of five native species were absent at sites invaded by *M. dolomieu*, and the fifth, *Labeobarbus capensis*, were only present as large adults (Lowe et al. 2008; Weyl et al. 2013; Woodford et al. 2005). In the upper Berg River, Woodford & Impson

(2004) investigated predation and spatial interactions between *O. mykiss* and three native species (*G. zebratus*, *P. burgi* and Cape kurper *Sandelia capensis*). The study documented low levels of native fish predation by *O. mykiss* (only *G. zebratus* were recorded from *O. mykiss* stomachs) and possible predator avoidance behaviour by *G. zebratus* (Woodford and Impson 2004). Non-native fishes have also been shown to prey on estuarine fishes, and in the Kowie River system, *M. salmoides* preyed on three native estuarine species, Cape mooney *Monodactylus falciformis*, flathead mullet *Mugil cephalus* and freshwater mullet *Myxus capensis* (Weyl and Lewis 2006).

At a system scale Clark et al. (2009), working in the Berg River, noted range contractions from historical distribution data for three endemic native fishes (*P. burgi, G. zebratus* and *S. capensis*) which were now limited to non-invaded stream reaches often above natural barriers, such as waterfalls, which inhibit non-native fish dispersal. While a suite of non-native fishes have been introduced into the Berg River system (*O. mykiss, M. dolomieu, M. punctulatus, M. salmoides, O. mossambicus, T. sparrmanii, C. gariepinus,* bluegill sunfish *Lepomis macrochirus*, *C. carpio, G. affinis*), disentangling impacts of non-native species from other anthropogenic stressors, such as pollution and water abstraction, was considered unrealistic (Clark et al. 2009). However, given the invasive nature of the non-native fishes present in the Berg River system, and the limitation of native fish distributions to non-invaded stream reaches, impacts on native fishes are inferred. Such findings highlight the low degree of biotic resistance of native stream fishes to invasion. This vulnerability to invasion displayed by native fishes is also reflected by other biota.

In two streams invaded by salmonids (*O. mykiss and S. trutta*) (Mobovaneni and Sterkspruit streams) of the uKhahlamba Drakensberg Park World Heritage site Karssing et al. (2012) demonstrated major differences in the abundance of tadpoles of the Natal cascade frog *Hadromophryne natalensis* above and below *O. mykiss* and *S. trutta* invasion barriers (Karssing et al. 2012). In both cases tadpole abundances were greatly reduced (4.7 and 15.7 times lower) in salmonid invaded zones of the Mobovaneni and Sterkspruit Rivers respectively (Karssing et al. 2012).

Some studies have investigated possible mechanisms of community level competition between native and non-native species. In the Tyume River, a Keiskamma River system headwater tributary, the endangered *S. bainsii* occurred sympatrically with *O. mykiss* in the upper reaches and with *M. salmoides* in the lower reaches (Mayekiso and Hecht 1988). High degrees of dietary overlap were observed between *S. bainsii* and both *O. mykiss* and *M. salmoides* (Mayekiso and

Hecht 1988). Low abundances in the lower reaches were ascribed to competition with *M. salmoides*, however, predation was more probably the cause, but overall population level impacts were not detectable (Mayekiso and Hecht 1988).

In the Limpopo River system, stomach content analyses revealed high levels of dietary overlap between invasive *O. niloticus* and native *O. mossambicus*, however, stable isotope analyses, which provides increased accuracy on long-term dietary carbon and nitrogen assimilation, indicated strong selective resource partitioning (Zengeya et al. 2011). It was therefore unclear whether *O. niloticus* would be a strong competitor in the Limpopo River system (Zengeya et al. 2011). In a broader scale study on the Sundays and Great Fish River systems, Kadye and Booth (2012c) indicated that *C. gariepinus* exhibited trophic breadth and plasticity throughout ontogeny, feeding predominantly on aquatic invertebrates at smaller sizes with an increased proclivity for piscivory at larger sizes (Kadye and Booth 2012a). Three native species formed part of its diet, *O. mossambicus* in the lower Great Fish River system, *L. umbratus* from Glen Melville reservoir and *B. pallidus* in the Sundays River. The authors predicted that dietary breadth and the opportunistic nature of *C. gariepinus* could impact heavily on invaded communities (Kadye and Booth 2012a; Kadye and Booth 2012b).

Four studies have attempted to document the impact of non-native fishes on invertebrate communities. The first was conducted on the Rondegat River in the Western Cape, where Lowe et al. (2008) described the impact of *M. dolomieu* invasion on the invertebrate community. Shifts in invertebrate assemblage were noted and certain grazing taxa showed reduced abundances, whereas all other taxa exhibited abundance increases in areas invaded by *M. dolomieu* (Lowe et al. 2008). Similar community level effects were observed in *M. salmoides*-invaded zones from the Wit River in the Eastern Cape (Weyl et al. 2010). Both studies postulated that top down control on invertebrates was decreased as native invertebrate fish predators had been extirpated by centrarchids, and therefore overall invertebrate predator biomass was reduced in invaded stream reaches. The impact of salmonids (*O. mykiss* and *S. trutta*) on invertebrate communities was investigated by comparing paired sites separated by waterfalls, with invaded sites below and non-invaded sites above waterfalls (Rivers-Moore et al. 2013). Shifts in community structure were noted between invaded and non-invaded sites, but confounding factors such as waterfalls acting as ecotones and sampling during high flows resulting in high levels of suspended sediments, rendered inconclusive results (Rivers-Moore et al. 2013). In an attempt to understand the relationship between invasive *C. gariepinus* and aquatic invertebrates, Kadye & Booth

(2012b) conducted multiple Before-After Control-Impact (MBACI) design experiments with and without *C. gariepinus,* in invaded and non-invaded tributaries of the Great Fish River system. Macro invertebrates showed little response to predators in the *C. gariepinus*-invaded Koonap River, while in the non-invaded Brak River, there was decreased species richness, diversity and biomass, postulated to be a result of predator naivety (Kadye and Booth 2012c).

1.3.6 Introductions of associated parasites and diseases

The introduction of novel parasites and diseases into environments can be particularly severe, as fish and novel parasite communities have not co-evolved, and hosts do not posses immune responses to infection (Gozlan 2008). Numerous non-native fish parasites have been introduced in South Africa (Bruton and Van As 1986; de Moor and Bruton 1988; Picker and Griffiths 2011). The primary vector fish species for parasite/disease introductions has been *C. carpio,* which are suspected to have introduced seven species (*Ichthyobodo necator, Chilodonella cyprini, C. hexasticha, Apiosoma piscicola, Trichodina acuta, T. nigra* and *Trichodinella epizootica*). Grass carp *Ctenopharyngodon idella* were also implicated in the introduction of *Bothriocephalus acheilognathi* (Bruton and Van As 1986). Currently a major threat for new introductions into South Africa is via species imported for the ornamental fish trade. Eight million fishes are imported annually, and the industry is largely unregulated (Mouton et al. 2001). A pilot study on the health of fish imported for the ornamental fish trade revealed that, of the four species tested (*C. auratus, C. carpio, P. reticulata* and cardinal tetras *Cheirodon axelrodi*), one harmful bacterium (*Mycobacterium fortuitum*), and numerous external parasites (*Trichodina mutabilis, I. multifiliis,* ciliophorans of the genus *Tetrahymena*, and monogeneans of the genera *Dactylogyrus* and *Gyrodactylus*) were present (Mouton et al. 2001). *Trichodina mutabilis* had not previously been recorded in South Africa (Mouton et al. 2001).

The impacts of introduced parasites/diseases in South Africa may be serious. Mass mortalities of native and non-native fishes have been attributed to five introduced parasite species (*C. hexasticha*, *Ichthyophthirius multifiliis, Argulus japonicus*, *Bothriocephalus acheilognathi, Trichodina acuta*) (Bruton and Van As 1986). Contemporary studies have documented individual and population level prevalence, abundance and mean intensity. For example, high prevalence and abundance of *B. acheilognathi* was recorded from two native species, *L. aeneus* and *L. kimberleyensis*, in the Vaal Dam and a translocated *L. aeneus* population in Glen Melville Dam on the Great Fish River system. The native longfin eel *Anguilla mossambica* has also been infected by the non-native parasitic gill monogenean *Pseudodactylogyrus anguillae* (Christison and Baker 2011; Parker et al. 2011). High prevalence and intensity of *P. anguillae* on *A. mossambica* were recorded from the Great Fish River system (Parker et al. 2011). Another non specific non-native fish louse *A. japonicus* infested (maximum of 87 individuals per fish) all collected fishes from the Witbank Dam on the Olifants River system (Avenant-Oldewage 2001). Despite recognition that high prevalence and abundance of introduced parasites and diseases pose to native fish communities, studies have failed to highlight population level impacts.

1.4 Conclusions

Aquatic environments in South Africa are invaded by a number of non-native fish species. Increasingly their impacts on invaded systems are being recognised and there has been a recent resurgence of studies investigating fish invasion, starting in 2000 and peaking between 2010 and 2013. Literature investigating these invasions has predominantly focussed on the impacts of non-native species, while relatively little research has been done on their introduction, establishment and spread. This focus on impacts may cause an extensive knowledge gap on the root cause of all invasions: the introduction phase. One of the primary measures suggested for managing biological invasion is the prevention of new introductions (Copp et al. 2005; Padilla and Williams 2004). In many cases, once a species has been introduced its removal is logistically unfeasible or even impossible (Copp et al. 2005; Gozlan et al. 2010). Therefore, investigating the transport and introduction phases is vitally important to impede new introductions and curb the further spread of non-native fishes (Clavero and García-Berthou 2006; Duggan et al. 2006).

Among the array of species introduced, established and spread throughout South Africa, are numerous highly invasive fishes that have had significant impacts on fish, amphibian and invertebrate communities. Documented impacts span multiple levels of biological organisation, but research has tended to focus on individual and population level impacts. Research on genetic level impacts needs increasing attention, specifically as the translocation of native species is high (Tweddle et al. 2009). Both descriptive and mechanistic studies were conducted using observational and experimental techniques. Numerous studies present descriptive evidence while others provide mechanisms that may cause impact of non-native fishes on native biota, but very few studies attempt to quantify these impacts at the system scale. As in other parts of the world, there are two major issues concerning quantifying impacts: firstly, a major stumbling block for researchers is the lack of accurate baseline pre-invasion data with which to compare current status (Ribeiro and Leunda 2012); and secondly, disentangling the impacts of other confounding factors such as river regulation, pollution and siltation is difficult and sometimes impossible (Didham et al. 2007).

Similar taxonomic and geographical biases were observed for South African impact studies to those documented in international invasive fish literature (Cucherousset and Olden 2011). Less than 50% of fully invasive fish species had been the subject of an impact study. Most studies focussed on impacts of the centrarchids *M. salmoides* and *M. dolomieu* on native biota. The remainder of the studies were also focussed on other global pests, *O. mykiss* and *C. gariepinus, O. niloticus* and *G. affinis*. This is not surprising as these are among the most widespread invaders in South Africa with the largest perceived impacts. Two highly invasive species, *C. carpio* and *L. macrochirus,* are widespread in South Africa, but little or no information exists on their impacts on invaded ecosystems. Regional bias was also observed as studies were predominantly focussed on areas in the vicinity of academic institutions: CFR studies were conducted around the universities of Cape Town and Stellenbosch; and Eastern Cape studies in the vicinity of Rhodes University and the South African Institute for Aquatic Biodiversity.

In conclusion, studies on non-native fish invasion should attempt to provide a more holistic overview of the invasion process, from their introduction, establishment, spread and impacts. There has also been a lack of studies on ecosystem scale impacts. Continued emphasis should also be placed on describing mechanisms responsible for the observed patterns in impact studies. It is suggested that future studies be less geographically and taxonomically biased. Describing the process of invasion and quantifying impacts will provide conservation managers with information necessary to manage current invasions and curb future introductions.

1.5 Thesis motivation and rationale

Introductions of non-native fishes are often a matter of risk perception rather than real risk analysis (Gozlan 2008) and perceived effects reflect human opinion rather than empirical evidence of ecological impact (Garcia-Berthou 2007; Ribeiro and Leunda 2012). As was demonstrated in a review of South African fish invasions literature, introduced non-native fishes can affect native biodiversity via a number of mechanisms such as predation, competition, hybridization, habitat modification and transmission of novel diseases (*sensu* Gozlan et al. 2010).

Regions characterised by low species diversity but high degrees of endemism such as the Cape Floristic Region (CFR) of South Africa contain fishes that are particularly sensitive to invasion (Linder et al. 2010; Marr et al. 2010). Ironically the CFR has also been listed as one of the six global freshwater fish invasion hotspots (Leprieur et al. 2008) and currently there are more non-native than endemic species (Marr et al. 2010). Conservation of these endemic fish assemblages is therefore a major challenge facing managers, researchers and officials (Marr et al. 2010). Documented impacts in the CFR, such as local extirpations of native species by non-native fish predation, are common (Ellender et al. 2011; Weyl et al. 2013; Woodford et al. 2005). The primary threat to all 24 of the range-restricted CFR endemic fishes is non-native fish invasion (van Rensburg et al. 2011; Tweddle et al. 2009). However, generally in South Africa, there is a paucity of information on the impact of introduced non-native species.

Range-restricted South African endemic headwater stream fishes are increasingly being threatened by human-mediated impacts such as habitat destruction and invasion by non-native fishes. Two Eastern Cape River systems characterised by similar low fish diversity but high endemism are the Swartkops and Keiskamma River systems (Mayekiso and Hecht 1988; Skelton 1993) where the Eastern Cape redfin *Pseudobarbus afer* and the Border barb *Barbus trevelyani* are redlisted by the International Union for the Conservation of Nature (IUCN) as ‘endangered’ primarily due to these factors (Cambray 2007; Swartz and Impson 2007). Both systems contain imperilled native fishes that are said to be threatened by non-native fish introductions (Mayekiso and Hecht 1988; Skelton 1993).

1.6 The Swartkops River system

The Endangered *P. afer* is a small endemic freshwater minnow species, and the genetically distinct 'Mandela' lineage is limited to the headwater streams of three river systems in the Eastern Cape, South Africa (Swartz et al. 2009; Swartz et al. 2007; Swartz and Impson 2007). Currently invasion by non-native fishes is considered the primary threat to the future survival of the species (Kadye and Booth 2012d; Swartz and Impson 2007). Despite two of the three populations being situated in formal protected areas (Addo Elephant National Park and Groendal Wilderness Area), there is no protection from non-native fish invasion because invasions originate in mainstream source/donor environments. Effective conservation of the species is currently constrained by the paucity of knowledge regarding the biggest perceived threat: non-native fish invasion. In particular, the persistence of *P. afer* populations in headwater streams of the Swartkops River system is threatened through invasion by non-native fishes (Skelton 1993; Swartz and Impson 2007).

Specifically, two non-native centrarchid species *Micropterus dolomieu* and *M. salmoides* have been implicated in the decline and local extinction of *P. afer* in the Swartkops River system (Skelton 1993). Both centrarchids have been shown to induce major impact on the fish communities in invaded ecosystems through direct predation, resulting in local extirpations, change in species composition and assemblage structures (MacRae & Jackson 2001; Woodford et al. 2005; Lowe et al. 2008; Shelton et al. 2008; Lawrence et al. 2012). *Micropterus salmoides* and *M. dolomieu* have been established in the Swartkops River system for at least 50 years. Current knowledge on the impact of centrarchids invasions on *P. afer* populations in this system is limited to an assessment by Skelton (1993), which found a lack of co-occurrence between *P. afer* and centrarchids in a short section of a single stream (Blindekloof stream), and postulated that centrarchid invasion results in the rapid extirpation of native fishes in invaded reaches. This study, however, lacked larger scale spatial and temporal components necessary to draw meaningful inferences of relationships between stream fishes and their habitat (Fausch et al. 2002).

1.6.1 Eastern Cape redfin *Pseudobarbus afer*

Pseudobarbus afer (Peters, 1864) is a small cyprinid that attains 110 mm in length (Figure 1.5; Skelton 2001). It is olive brown above and white below and as the name suggests, it has bright red fins. During the breeding season, fin colours of the males intensify and they develop large white conical tubercles on the head (3-4 on either side of the snout) (Cambray 1994; Skelton 2001).

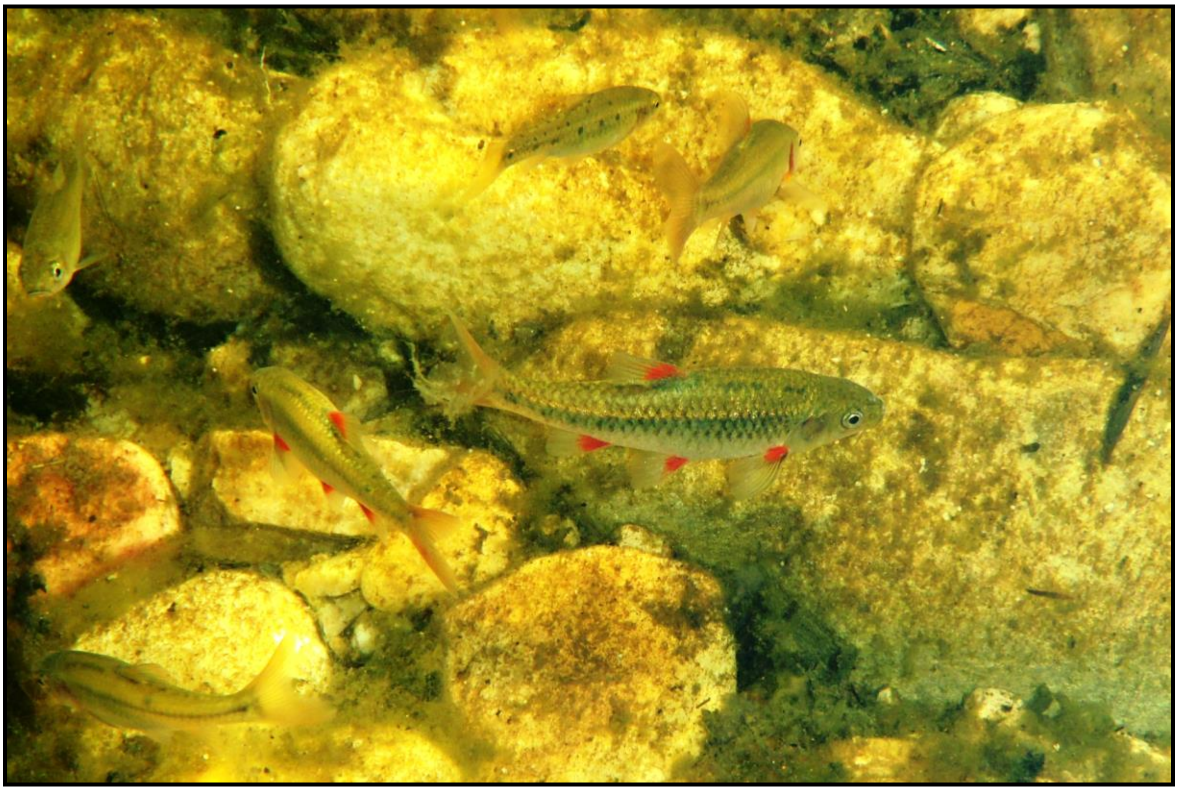


Figure 1.5 Eastern Cape redfins *Pseudobarbus afer* and goldie barbs *Barbus pallidus* shoaling in the Blindekloof stream, a headwater tributary of the Swartkops River system within the Groendal Wilderness Area, Eastern Cape, South Africa.

Pseudobarbus afer is endemic to headwater streams of eastward flowing coastal river systems in the Eastern Cape, South Africa. It is the most widespread redfin in the Cape Floristic Region, distributed from the Klein Brak River near Mossel Bay to the Sundays River system outside Port Elizabeth (Swartz et al. 2007). Recent evidence has identified the existence of a 'species complex' consisting of four major lineages, the 'Forest' (Klein Brak River –Tsitsikamma River), 'Krom' (Krom River), 'St Francis' (Swart River-Gamtoos River) and 'Mandela' (Baakens, Swartkops and Sundays Rivers) (Swartz et al. 2007) (Figure 1.6). Phylogenetic analyses have indicated that divergence between the Mandela lineage and other redfin lineages may be sufficient for the Mandela lineage to be described as a separate species (Swartz et al. 2007). The Mandela lineage of *P. afer* is limited to the headwater streams in the Sundays, Swartkops and Baakens Rivers (Swartz et al. 2007).

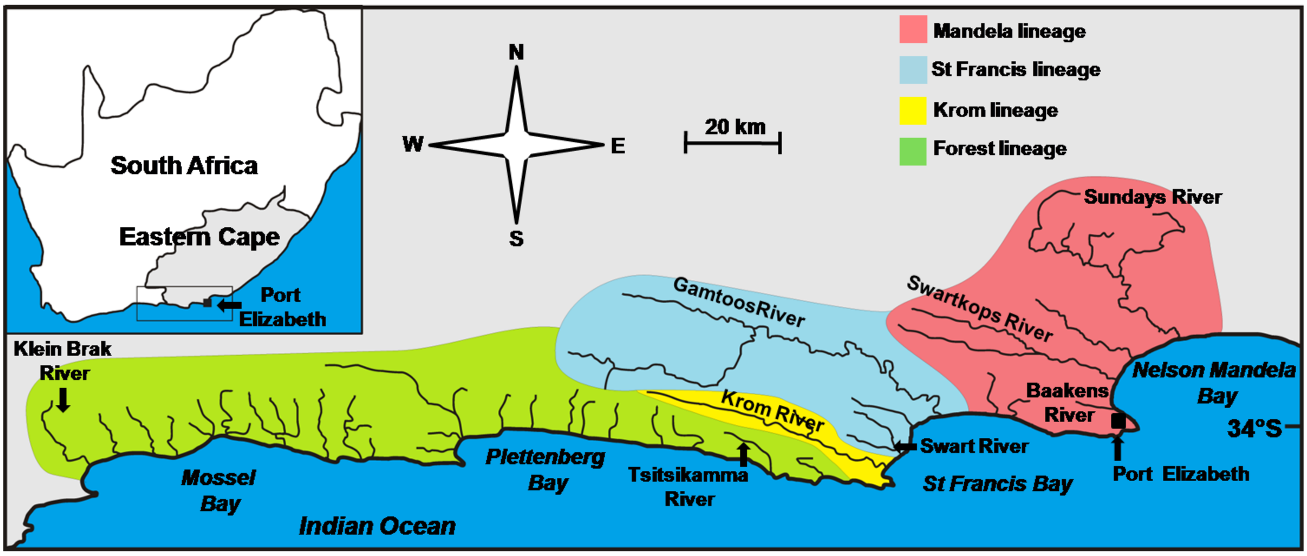


Figure 1.6 The distribution of the four *Pseudobarbus afer* lineages ('Forest': Klein Brak River –Tsitsikamma River, 'Krom': Krom River, 'St Francis': Swart River-Gamtoos River, 'Mandela': Baakens, Swartkops and Sundays Rivers) in coastal river systems along the south-eastern coast of South Africa (map adapted from Swartz et al. (2007)).

Pseudobarbus afer inhabits pools, riffles and runs in the upper reaches of pristine headwater tributary streams which are episodic or intermittent in nature. This means that these streams experience large fluctuations in flow, temperature and water quality to which *P. afer* has adapted. During wet periods the pools in these headwater tributaries are large and crystal clear. When it rains the streams begin to flow and *P. afer* rapidly disperse into areas that were previously dry. As the stream dries and the amount of available habitat contracts, so the *P. afer* are once again confined to a few refuge pools that are fed by groundwater and consequently don't dry up.

For a small-bodied fish species, *P. afer* grows fairly slowly, reaching a maximum age of 5-6 years and length of 110 mm (Cambray and Hecht 1995). Maturity is reached at approximately 40mm (2-3 years old) and *P. afer* have a protracted spawning season lasting from November to March (Cambray 1994). The cue for spawning is an increase in flow associated with episodic rainfall events (Cambray 1994). The unpredictable rainfall patterns and flow variability result in redfins adapting to optimise survival under these conditions. Each female has a number of different sizes of eggs in the ovaries, which indicates serial spawning (where each female may spawn multiple times in a spawning season) allowing a number of opportunities to spawn, therefore increasing the chances of survival in such a dynamic environment (Cambray 1994). During spawning, *P. afer* move out of pool habitats into riffles and deposit non-adhesive eggs on the bottom of the stream in cobble habitat where the eggs fall into cracks and spaces between the cobbles (Cambray 1994). The eggs hatch after two days, and at this time the larvae are photophobic (avoid light) and rely on their yolk sac for nutrition. After about 5 days they exhibit positive phototaxis (light attraction) and swim out of the interstitial spaces and crevices between cobbles and enter the water column from where they disperse passively downstream (Cambray 1994). Feeding typically starts approximately 10 days after hatching. *Pseudobarbus afer* are omnivorous, feeding mainly from the stream bottom on algae and aquatic insects (Cambray 1994; Skelton 2001).

1.6.1.1 Conservation status

The most recent assessment of *P. afer* considered only the Sundays, Swartkops and Baakens populations (Mandela lineage). *Pseudobarbus afer* is listed as endangered by the International Union for the Conservation of Nature (IUCN), a classification assigned to a species that is considered to face a very high risk of extinction in the wild (IUCN 2001; Swartz and Impson 2007). Justification for the status is that the species has an area of occupancy <10 km^2, fewer than 10 populations and available habitat is shrinking due to non-native fish invasion (Swartz and Impson 2007).

1.6.1.2 Threats

The primary threat to the persistence of *P. afer* populations is considered to be invasion by non-native fishes (Kadye and Booth 2012d; Swartz and Impson 2007). Despite two of the three populations being situated in formal protected areas (Addo Elephant National Park, Groendal Wilderness Area), the connectivity of river systems between areas within and outside formal parks is a major threat to *P. afer*, as non-native species introductions are more likely to occur outside these areas. Currently invasions by predatory *M. salmoides* and *M. dolomieu* threaten populations within both the above-mentioned protected areas (Kadye and Booth 2012d; Skelton 1993). In areas inhabited by *M. salmoides*, all *P. afer* have been extirpated (Skelton 1993) resulting in a decreasing area of occupancy (Swartz and Impson 2007).

1.6.2 *Micropterus salmoides* & *M. dolomieu*: Biology, ecology and documented impacts

1.6.2.1 Biology & Ecology

Micropterus salmoides and *M. dolomieu* are large-bodied (>500 mm TL) long-lived species attaining ages greater than 10 years (Beamesderfer and North 1995; Carlander 1977; Warren 2009). In *M. salmoides* maturity is reached early (1-5 years), depending on the prevailing abiotic conditions, while in *M. dolomieu* it is slightly delayed (2-7 years) (Carlander 1977; Warren 2009). Both species construct nests and spawning is initiated at temperatures exceeding 15 °C (Warren 2009). Spawning migrations have been recorded for *M. dolomieu* where individuals move from lakes or impoundments into inflowing tributaries to spawn, but this is the exception rather than the norm (Warren 2009). Movement data for both *M. salmoides* and *M. dolomieu* indicate that they are able to move considerable distances (Warren 2009).

Micropterus salmoides shows a preference for lentic habitats such as slower flowing rivers, lakes and ponds, while *M. dolomieu* prefer clear, cool lotic environments in small and large rivers and rocky shorelines of lakes and impoundments (Warren 2009). Due to the lentic habitat preference of *M. salmoides*, their establishment in streams with variable flow regimes limits their dispersal abilities (Almeida et al. 2012; Bernardo et al. 2003; Skelton 1993). The construction of impoundments in invaded river systems, however, facilitates establishment, dispersal and persistence of non-native fishes in invaded river systems (Almeida et al. 2012; Moyle et al. 2003). In contrast, *M. dolomieu* displays more of an affinity for flowing waters (Warren 2009) and has successfully established in headwater streams of the Cape Floristic Region (CFR) (Weyl et al. 2013; Woodford et al. 2005).

Micropterus salmoides and *M. dolomieu* are piscivorous top predators but display large dietary breadth, preying on organisms from aquatic and terrestrial invertebrates, reptiles, amphibians, crustaceans and small terrestrial mammals and birds (Skelton 1993; Warren 2009; Weyl and Hecht 1999; Weyl and Lewis 2006; Weyl et al. 2010).

1.6.2.2 Documented spread and impacts

The centrarchids *M. dolomieu* and *M. salmoides* are popular angling species that have been translocated widely in the USA and introduced globally, including Europe, Asia, South and Central America and Africa (Lever 1996; Robbins and MacCrimmon 1974). Where introduced, both species have been documented to induce major impact on the fish communities in invaded ecosystems by predation (Almeida et al. 2012), causing changes in species composition and assemblage structures (Godinho and Ferreira 2000; Lawrence et al. 2012; MacRae and Jackson 2001). On the Iberian Peninsula in Spain and Portugal, an area with similar climate to the Swartkops River system, direct predation by introduced centrarchids is the primary threat imposed on native species (Almeida et al. 2012; *sensu* Ribeiro & Leunda 2012).

Micropterus salmoides and *M. dolomieu* support a massive recreational fishery in the USA and Canada (Quinn and Paukert 2009; Schramm and Hunt 2007). As a result of their angling popularity, prowess and the lack of suitable game fish species in South African freshwaters, *M. salmoides* and *M. dolomieu* were introduced in 1928 and 1937 respectively and were subsequently spread widely (de Moor & Bruton 1988; *sensu* McCafferty et al. 2012). Due to their wide physiological tolerances (Warren 2009), they have successfully established throughout South Africa. The negative impact of centrarchids on native biota was recognised as early as 1967, when Dr Rex Jubb, a pioneer of ichthyology in South Africa, walked up the Jan Dissels River, an Olifants River headwater tributary in the Western Cape and remarked that they searched in vain for the “small redfin Barbus” but instead *M. dolomieu* had “taken possession of the stream” (Jubb 1967). There is little information on centrarchid invasions in South Africa. The few studies that have been undertaken documented local impact on a stream reach scale, which has limitations as these data can only be used as an inference of system scale impacts. Centrarchid impacts on native Cape Floristic Region (CFR) ichthyofauna include an impact of *M. salmoides* on habitat selection and adult abundance of *G. zebratus* (Shelton et al. 2008), extirpation of four out of five native species, and only large *L. capensis* recorded from stream reaches invaded by *M. dolomieu* (Woodford et al. 2005) and extirpation of *P. afer* from stream reaches invaded by *M. salmoides* (Skelton 1993). Despite the lack of data on impacts of centrarchid invasion in South Africa, indications are that they pose a major threat to native biodiversity (Shelton et al. 2008; Skelton 1993;

Woodford et al. 2005) the magnitude of which has an urgent need to be quantified to prioritize future conservation efforts.

1.7 Keiskamma River system

The late 1800s and early 1900s saw a major drive to introduce salmonids into South Africa (McCafferty et al. 2012), and a large effort was made to establish *O. mykiss* and *S. trutta* in the headwater reaches of the Keiskamma and Buffalo River systems (Hey 2008). There is also evidence suggesting that after their introduction, the salmonids *O. mykiss* and *S. trutta* had an impact on *B. trevelyani* populations through direct predation (Gaigher 1975; Jubb 1967). An early pioneer and inland fisheries officer, Sydney Hey, who was responsible for numerous early trout introductions into the Keiskamma River system, commented “These tiny minnow-like fish, by the way, serve as useful fodder fish for the trout, so much so that they have disappeared entirely from many streams where they were once plentiful” (Hey 2008). In another case *B. trevelyani* was thought extinct in the Buffalo River system until Jubb (1967) found 11 *B. trevelyani* when examining a large *O. mykiss* stomach. The suggested impact however needs to be investigated at system scale to make realistic inferences on whether the impact of salmonids threatens the persistence of the species and how to ensure their effective conservation.

1.7.1 Border barb *Barbus trevelyani*

1.7.1.1 Biology & ecology

Barbus trevelyani is a small (max 100 mm FL) shoaling barb species (Figure 1.7). It is easily identified by its translucent grey/brown colour and conspicuous black strip along the entire midline of the body ending in a triangular spot at the base of the tail (Skelton 2001).

Figure 1.7 Photograph of the Border barb, *Barbus trevelyani* from the Mnyameni River, a Keiskamma River headwater tributary, Eastern Cape, South Africa (Photo credit: Craig Garrow).

The species favours pools and riffles in clear rocky streams, and is very habitat specific, mostly being confined to pristine forested areas of the stream (Cambray 2007; Cambray 1985; Gaigher 1975). The diet of *B. trevelyani* is varied and consists of terrestrial and aquatic insects, predominantly mayflies as well as seeds and algae (Gaigher 1975). *Barbus trevelyani* displays life history characteristics typical of a small-bodied fish, having evolved in stable perennial headwater stream environments (Cambray 1994; Cambray and Hecht 1995; Gaigher 1975). Maturity is reached at the end of the first year in males but delayed until the third year for females at lengths of 65-75mm FL (Gaigher 1975). Growth is slow, with males and females attaining lengths of 90-100 mm FL in 6 years (Gaigher 1975). Spawning, between September and December, is thought to take place in the midstream over gravel or rocky substrate (Gaigher 1975). The eggs of *B. trevelyani* are sticky and demersal, adhering to each other and hatching after 2.5-3.5 days (Cambray 1985). After hatching the larvae cluster on the substrate and after 11 days begin actively feeding (Cambray 1985). Demersal eggs and larvae make these fragile life history stages extremely vulnerable to physical changes in the stream environment, such as suffocation by excessive silt loads in the stream (Cambray 2007).

Barbus trevelyani is endemic to two Eastern Cape river systems, the Keiskamma and Buffalo systems (Figure 5.1). Information on *B. trevelyani* in these river systems is poor, and limited to a few studies on their biology and ecology (Cambray 2007; Cambray 1996b; Cambray 1985; Gaigher 1975) in the Tyume River, a Keiskamma River tributary.

1.7.1.2 Conservation status and threats

Information on the current status of the species is limited (Cambray 1996b). It is currently listed as Endangered by the International Union for the Conservation of Nature (IUCN), and major threats to the species are a decline in habitat extent and quality as well as invasion by alien fish species (Cambray 2007). The biology and ecology of the species has been reasonably well documented, but the available information is predominantly limited to the Tyume River. Due to overgrazing and poor land use practices in the lower Tyume River, which has resulted in excessive siltation of the stream, *B. trevelyani* has a limited distribution as the species favours clear, perennial unsilted mountain streams. This has resulted in competition between *B. trevelyani* and *O. mykiss* for food and impact through direct predation by *O. mykiss* (Gaigher 1975). It is therefore vital to understand the biology, ecology and documented impacts of invasive salmonids to better understand their impact on *B. trevelyani*.

1.7.2 *Oncorhynchus mykiss* & *Salmo trutta*:

1.7.2.1 Biology and ecology

Their habitat preferences and physiological tolerances are specific, limiting them to clear, cool perennial streams and lakes, but they can also inhabit estuaries (Kottelat and Freyhof 2007). *Salmo trutta* and *O. mykiss* are primarily freshwater species but both may exhibit a facultative anadromous life history under suitable conditions (Kottelat and Freyhof 2007; Page and Burr 1991). Both species attain large sizes, but generally in streams range from 200-300 mm SL (Gaigher 1975; Kottelat and Freyhof 2007). These two salmonids are predatory by nature, feeding on a wide variety of aquatic and terrestrial invertebrates, and while *O. mykiss* displays some proclivity for piscivory, at larger sizes *S. trutta* becomes increasingly piscivorous (Gaigher 1975; Maitland 2004; Mayekiso and Hecht 1988). *Oncorhynchus mykiss* and *S. trutta* exhibit a plastic life history strategy but generally reach maturity between 2 and 4 years and are capable of undertaking spawning migrations (Kottelat and Freyhof 2007; Page and Burr 1991).

1.7.2.2 Documented impacts

Oncorhynchus mykiss and *S. trutta* are among the most widely introduced species globally (Fausch 2007; Townsend 1996) and form part of the eight worst global invasive freshwater fishes (Lowe et al. 2000). A large body of literature attests their invasive impact in recipient ecosystems. These impacts are particularly severe as they can span multiple biological domains (Dunham et al. 2004), ranging from hybridisation and competition with congenerics (Allendorf et al. 2001), alteration of population structure (Townsend and Crowl 1991) and recruitment (Woodford and McIntosh 2010) habitat fragmentation of native fishes (Crowl et al. 1992), extirpation through direct predation (Townsend 1996) and even extinction of the New Zealand Grayling *Prototroctes oxyrhynchus* (McDowall 1996). In South Africa, although there is little information on the impacts of salmonids on stream fishes, numerous inferences have been made suggesting significant impact (Gaigher 1975; Jubb 1967). A single study by Woodford & Impson (2004) documented predation of *O. mykiss* on the native galaxiid *Galaxias zebratus*.

1.8 Research approach and thesis outline

Invasion has been defined as a number of steps or stages that an introduced species has to traverse in the framework for biological invasions or range expansion process (Blackburn et al. 2011; Richardson et al. 2011; Richardson et al. 2000). The primary thread of this study was investigating the invasion process within the unified framework for biological invasions proposed by Blackburn et al. (2011) including the transport, introduction, establishment and spread stages. The Swartkops and Keiskamma River systems are charactersied by low species diversity but contain imperilled endemic fishes highly threatened by non-native fish invasion. The two systems are characterised by different climatic conditions and also different suites of invaders. This thesis therefore provides a broad overview on invasion in these varying environments. An attempt was then made to quantify the invasive impacts for two IUCN redlisted (Endangered) headwater fishes (Cambray 2007; Swartz and Impson 2007), the *P. afer* and *B. trevelyani*.

This thesis is split into two case studies. The first summarises invasion and impacts of non-native fish in the episodic Swartkops River system, the other in the perennial Keiskamma River system. Complimentary chapters were compiled addressing the following major themes: Reviewing current knowledge on invasive impacts in South Africa (Chapter 1), investigating invasibility of headwater stream environments (Chapter 2, 3, 4, 5, 6), determining the establishment of non-native species (Chapter 2, 3, 5, 6), assessing the spatial and temporal impacts of invasion (Chapter 3, 4, 5), mechanisms responsible for impacts (Chapter 4) and the threat of non-native invasion on the genetic diversity of two headwater fishes (Chapter 7). This thesis attempts to provide a multi-faceted, quantitative estimate on individual, population and system scale invasive impacts. The results from this research are directly applicable to requirements for effective management of non-native species, and for ensuring the conservation and persistence of imperilled native species.

1.8.1 Case study 1: Swartkops River system

Chapter 2 assessed the invasibility of a representative headwater stream and the re-invasion and establishment of non-native fishes in the Blindekloof stream after their eradication in 1989, from source populations in the mainstream Swartkops River. As a result of this eradication and because the entire stream is situated within the Groendal Wilderness Area, where direct introductions were unlikely because of access control, subsequent occurrences of non-native fishes are most probably a result of upstream invasions from the mainstream Swartkops River. The Blindekloof stream therefore provided a unique opportunity to investigate whether largemouth bass *M. salmoides* would reinvade after its removal in 1989, and whether the more recently introduced non-native species would invade headwater streams. Although the publication was co-authored, all the analyses and writing are my own. The co-authors contributed to conceptualising the study and providing useful comments to improve the manuscript for publication. The chapter has been published. See:

Ellender BR, Weyl OLF, Swartz ER (2011) Invasion of a headwater stream by non-native fishes in the Swartkops River system, South Africa. African Zoology 46, 39–46.

Chapter 3 investigated invasion dynamics in headwater streams of the Swartkops River system. Baseline and post flood survey data allowed community comparisons between invaded and non-invaded stream reaches under variable flow scenarios. The overall distribution of fishes in the headwater streams of the Swartkops River system were also used to quantify invaded and non-invaded stream reaches and estimate the proportion of stream habitat lost to *P. afer* through invasion by centrarchids. From these data, community and system scale impacts were examined. In addition, the *P. afer* populations of three streams (Fernkloof, Waterkloof and Blindekloof Streams) are isolated by the presence of three invasive non-native fishes in the lower reaches of the Blindekloof stream and in the mainstream Kwa-Zunga. The unpredictable catastrophic flood during June 2011 provided the opportunity to test the vulnerability of three fragmented and isolated headwater *P. afer* populations to a major stochastic disturbance and to discuss the role of an above average rainfall in context of facilitating or inhibiting non-native fish invasions in headwater streams.

Chapter 4: Flood redistribution of *P. afer* also provided an additional opportunity to investigate temporal population dynamics in invaded stream reaches where they were previously extirpated by centrarchids but have now recolonised after a major flooding event, in relation to non-invaded reference sites. The Blindekloof stream was monitored for six months over the *P. afer* spawning season to assess invasive impacts on recruitment and dispersal.

1.8.2 Case Study 2: Keiskamma River system

Chapter 5 assessed the factors influencing the distribution and abundance of fishes in the upper Keiskamma River system. Data were used to investigate the impacts of two of the major threats to *B. trevelyani* continued survival: non-native fishes and habitat degradation. Specifically, distribution, abundance and community assemblage data were assessed in headwater streams and impoundments and related to current status and future threats on *B. trevelyani*. The overall distribution of *B. trevelyani* and salmonids in the headwater streams of the Keiskamma River system were also used to quantify invaded and non-invaded stream reaches and estimate the proportion of stream habitat lost to invasion by salmonids.

Chapter 6: Two salmonids *O. mykiss* and *S. trutta* were introduced into the upper Keiskamma River system in the late 1890s and early 1900s and are proposed to have had an impact on native fishes. Both species are temperature limited and it is vitally important to record temperature regimes in these streams to better explain what limits the distribution of salmonids and relate this to the threat they pose to native imperilled headwater fishes. Temperature loggers were placed throughout the upper reaches of the Keiskamma river system. Using temperature and salmonid distribution data, the thermal range of salmonids in this system is discussed. Their current distribution in the upper Keiskamma River system also reflects their invasive ability and may lead to predictions on future invasion potential in other river systems.

Chapter 7: In South Africa non-native fish invasion has resulted in many cases of headwater species having highly fragmented distribution and being limited to isolated headwater refugia. The aim of this chapter is therefore to assess the current distribution of genetic diversity in the Swartkops and Keiskamma River systems. The results will be used to infer the potential impact of non-native fish invasion and to develop effective conservation measures for the two endangered minnows *P. afer* and *B. trevelyani* in the Swartkops and Keiskamma River systems, Eastern Cape, South Africa.

Chapter 8: In the general discussion, an overview of the invasion process in the variable environments of the Swartkops and Keiskamma River systems, as well as a quantification of impacts is provided and contrasted between the two headwater minnows, *P. afer* and *B. trevelyani*. A future prognosis for *P. afer* and *B. trevelyani* is also discussed in the context of resistance and resilience to invasion by non-native fishes and other threats. Management and conservation recommendations for *P. afer* and *B. trevelyani* are then examined.

1.8.3 Sampling method selection

Due to “Endangered” IUCN red list status of *P. afer*, an effort was made to choose the least destructive sampling method that would have little or no effect on the sampled population. As snorkelling transects are an established method for sampling stream fishes (Cunjak & Power 1986; Thurow & Schill 1996; Woodford et al. 2005) this method was employed as widely as possible. Due to the documented negative impacts of electrofishing on sampled fish populations, Underwater Video Analysis (UWVA) was investigated as an alternative non-destructive method for sampling imperilled stream fishes (Ellender et al. 2012a). Due to the variability in the physical habitat characteristics of each stream, three methods were employed during this study: backpack electrofishing, snorkelling transect surveys and underwater video analysis. After the initial sampling methods suitability studies (Chapter 3) in the smaller streams (Fernkloof, Waterkloof, Vyeboomkloof, Nounekkloof) where the water depth was generally <1 m, electrofishing was chosen as the primary sampling method. In the larger streams where pools depths were >1 m (Blindekloof, Chaseskloof, Kwa-Zunga) snorkelling transect surveys were used. In some instances in the larger streams electrofishing was conducted along the margins of the larger pools as a supplementary method. Specific methodologies are provided where necessary.

1.8.4 Supporting manuscripts

Two additional manuscripts emanate from work conducted during field surveys for this study. They provide supporting information on sampling methodology for imperilled headwater fishes and additional information on sampled non-native fish species. These data were collected and analysed during this study although the information presented does not directly relate to the thread of the thesis, the references are provided as background information.

Ellender BR, Becker A, Weyl OLF, Swartz ER (2012a) Underwater video analysis as a non-destructive alternative to electrofishing for sampling imperilled headwater stream fishes. Aquatic Conservation: Marine and Freshwater Ecosystems 22: 58-65.

Ellender BR, Taylor GC, Weyl OLF (2012b) Validation of growth zone deposition rate in otoliths and scales of flathead mullet *Mugil cephalus* and freshwater mullet *Myxus capensis* from known age fish. African Journal of Marine Science 34(3): 455-458.

1.8.5 Data collection, permitting and ethical clearance

Understanding any biological system requires an in depth knowledge on the structure and function of the study system. Snapshot studies, or being presented data that have already been collected, often result in erroneous conclusions due to lack of observational information. My approach was to spend as much time in the field as possible to maximise exposure to the study system and its fauna. For that reason all surveys were undertaken personally with the help of various field assistants. This involved logistical organisation, permitting applications and personally undertaking electrofishing, gillnetting, fyke-netting, day and night snorkelling surveys and underwater video analyses.

In many instances the sampled areas were extremely remote, which involved carrying sampling gear long distances through rugged terrain. I feel that spending extensive periods of time on the streams of the Swartkops and Keiskamma River system has increased my understanding of the functioning of the two study systems and provides a better understanding of the mechanisms responsible for the observed patterns.

Figure 1.8 The Chaseskloof stream on the left and the Blindekloof stream on the right, two remote Swartkops River system headwater streams which were accessed by hiking.

The sampling methods and protocols for this research were approved by the ethics committee of the South African Institute for Aquatic Biodiversity (SAIAB) and Eastern Cape Parks Board scientific services. Permits were issued by the Eastern Cape Department of Economic Development and Environmental Affairs (DEDEA) (Permit #: CRO 16/10CR, CRO 17/10) and the Eastern Cape Parks Board.

CHAPTER 2: Testing the invasibility of a headwater stream by non-native fishes in the Swartkops River system, South Africa

2.1 Introduction

Freshwater ecosystems, especially the headwaters of rivers, are key areas for the conservation of aquatic biodiversity because although they are characterised by low species diversity, they have a high degree of endemism (Abell et al. 2007). The fish communities that inhabit these headwater environments are often vulnerable to negative impacts of non-native predatory invaders (Weyl et al. 2013). Determining the ability of non-native fishes to invade headwater streams is therefore of high importance. Invasibility can be defined as the ability of a species to invade and survive in a recipient ecosystem (*sensu* Richardson et al. 2011). This can be mediated by the biotic and abiotic characteristics of the receiving river system (biotic and abiotic resistance hypotheses) as well as the biology and ecology of the invading fish species.

The invasibility of headwater streams in South Africa from downstream sources has been poorly documented. This is especially true in the Swartkops River system, Eastern Cape, South Africa, where the native headwater fish fauna comprises the genetically distinct Mandela lineage (Swartz et al. 2009) of the Endangered (Swartz and Impson 2007) Eastern Cape redfin, *Pseudobarbus afer*, goldie barb *Barbus pallidus*, Cape kurper *Sandelia capensis*, river goby *Glossogobius callidus* and the obligate catadromous longfin eel *Anguilla mossambica* and the giant mottled eel, *Anguilla marmorata*. As a result of intentional stocking and illegal introductions, the mainstream Swartkops River has been invaded by at least five non-native species (Table 2.1). The largemouth bass *Micropterus salmoides*, smallmouth bass *Micropterus dolomieu* and common carp *Cyprinus carpio* were already reported from this system prior to the 1970s. More recent additions are the African sharptooth catfish *Clarias gariepinus*, first collected in 1997 but caught by anglers since 1985 (B. Kurten, records officer Eastern Cape Bank Anglers Association pers. comm.), and the banded tilapia *Tilapia sparrmanii* collected during a survey in 2009.

In a study undertaken on the Blindekloof stream, a Swartkops headwater tributary, Skelton (1993) reported that *M. salmoides* was the only non-native species present, and had invaded 2.4 km upstream. In pools occupied by the non-native predatory *M. salmoides*, native fishes

(*S. capensis*, *G. callidus* and *P. afer*) were greatly reduced in abundance and in some cases extirpated. *Micropterus salmoides* was subsequently eradicated from the stream by conservation authorities.

Table 2.1 The first record and general comments on the introduction of non-native species within the freshwater reaches of the Swartkops River, below the Groendal Dam, and the Blindekloof stream including this study (Barrow 1971; Jubb 1971; Jubb 1965; de Moor and Bruton 1988; Scott et al. 2006; Skelton 1993).

Species	**Swartkops**		**Blindekloof**	
	First record	**Comments**	**First record**	**Current status**
Clarias gariepinus	1985	Reported from anglers catches since 1985, now common to below the Groendal Dam.	1997 (SAIAB 54628)	Recorded in 2010, neither common nor abundant.
Cyprinus carpio	1960s	Common target species for anglers since the 1960s but no official introduction date available.	-	-
Micropterus dolomieu	1965	Common angling species in Groendal Dam less common in the mainstream.	2010	First recorded in 2010, neither common nor abundant.
Micropterus salmoides	1935	Stocked into Groendal Dam.	1987 (SAIAB 27271)	Eradicated after 1987 but recorded again in 1997 and 2010. Neither common nor abundant.
Salmo trutta	1960s	No introduction date available, stocked into the upper catchment streams in the 1960s but did not establish.	-	-
Tilapia sparrmanii	2009	First recorded in 2009, common in mainstream.	2010	Common and abundant.

As a result of this eradication, and because the entire stream is situated within the Groendal Wilderness Area where direct introductions were unlikely because of access control, subsequent occurrences of non-native fishes in the Blindekloof stream are most probably a result of upstream invasions from the mainstream Swartkops River (Figure 2.1). This provided the opportunity to answer the following questions: (1) Would *M. salmoides* reinvade the Blindekloof stream after its removal in 1989 and; (2) Whether the Blindekloof stream would be invaded by the other non-native fishes found in the mainstream.

2.2 Materials and Methods

2.2.1 Study Site

The Blindekloof stream is a tributary of the Swartkops River, and is situated northwest of Uitenhage in the Grootwinterhoek Mountains in the Eastern Cape, South Africa (Figure 2.1). The source of the Blindekloof stream lies in the Grootwinterhoek Mountains and the stream is approximately 11 km long. The entire catchment is situated within the Groendal Wilderness Area, which was demarcated as State Forest for the protection of the indigenous forest and water resources early in the 19th century and is therefore unaffected by anthropogenic influences and relatively pristine. The geology of the area consists of quartzitic Table Mountain Group sandstones (Maclear 2001).

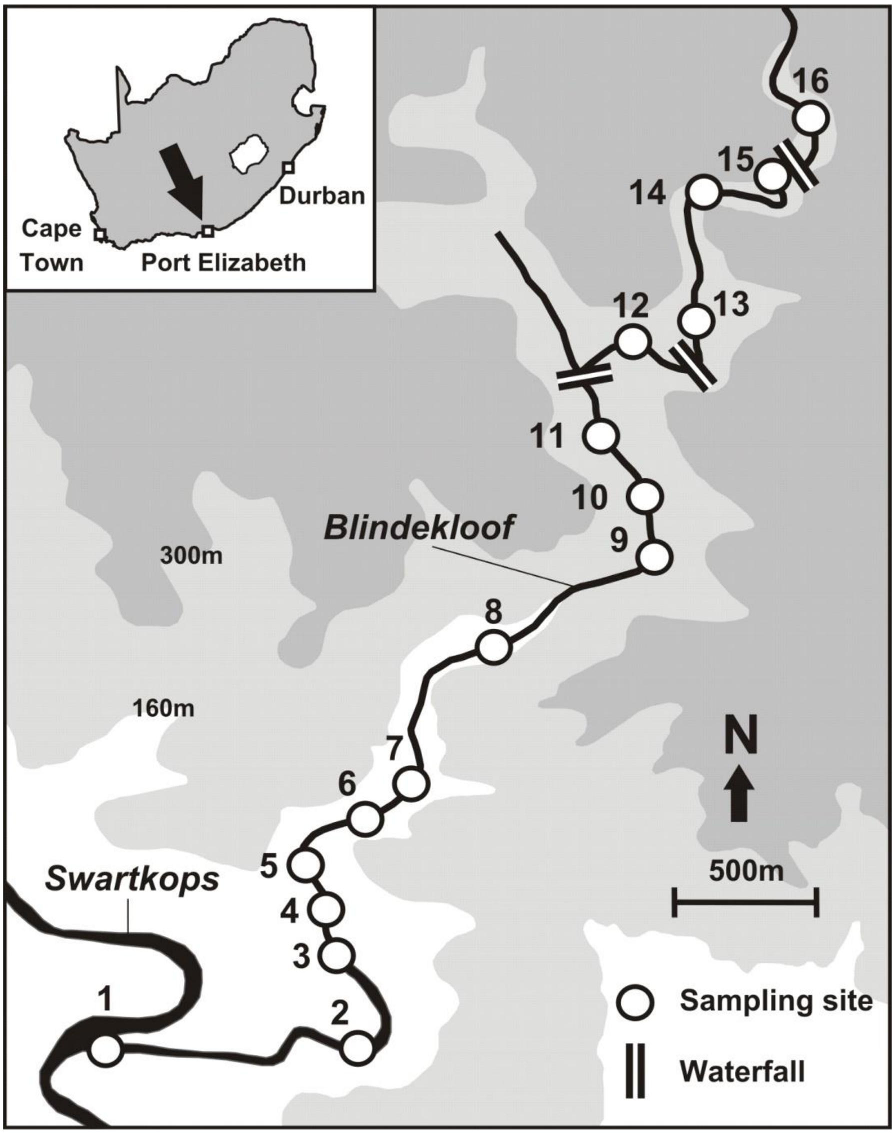


Figure 2.1 The location of the Blindekloof stream and the position of the sampling sites from site 1 at the Swartkops/Blindekloof confluence to site 16 in the upper reaches of the Blindekloof stream.

Typical in-stream habitat in the upper reaches was characterised by open canopy pools, with bedrock, large unconsolidated boulder, cobble and pebble substrates. The middle reaches were similar, but the canopy was predominantly closed. In the lower reaches of the stream the streambed was characterised by medium and small unconsolidated cobbles, pebbles and gravel. Pools in the Blindekloof stream are predominantly isolated on the surface, but there is permanent subsurface flow (Skelton 1993). This stream can be classified as episodic as it is fed by both precipitation and groundwater, and surface flow follows only after sustained heavy rain (Roux et al. 2002), an event that occurs on average 1.2 times/year in an erratic and unpredictable pattern (Skelton 1993). The stream gradient is high in the upper reaches with a number of natural physical barriers (waterfalls and cascades).

2.2.2 Sampling

Sampling took place during April/May 2010. Sixteen sampling sites, from the Blindekloof/Swartkops confluence area to the last point accessible by hiking, about 9 km upstream, were surveyed by snorkelling and electrofishing (Figure 2.1). Snorkel surveys were conducted using a modified zigzag method (Woodford et al. 2005). Pass one was initiated at the tail end of the pool, with the observer swimming upstream, zigzagging to cover as much of the pool as possible. Pass two was a repeat of pass one but in a downstream direction. During each pass, all fish seen were identified to species level and counted. In pools with extensive shallow areas (<0.5 m deep), snorkelling was ineffective and backpack electrofishing (SAMUS-725GN) was performed as a supplementary sampling method. Due to the episodic nature of the Blindekloof stream, pool sizes are dynamic and fluctuate seasonally. A simple method was therefore used to obtain an estimate of surface area (m^2), by calculating the product of the maximum width and length (m) of each pool. Oxygen saturation (%) and temperature were measured using an OxyGuard oxygen probe. Conductivity and pH were measured using a Hanna HI98129 Combo pH and Electrical Conductivity meter. Turbidity (NTU) was measured using a Hanna HI 98703 Turbidimeter.

2.3 Results

Water quality reflected the intact catchment and nutrient-poor geology. The water was very clear (mean ± standard deviation: 0.44 ± 0.25 NTU), well oxygenated (84.7 ± 15.9% saturated) with low conductivity (136.9 ± 16.1 $\mu s.cm^{-1}$) and near neutral pH (range: 6.75-7.64).

Fish distribution and their relative abundances are summarised in Figure 2.2 and Table 2.2. Eight fish species were recorded, of which four were non-native. The highest point where fish were recorded was site 12, below which 10 pools contained fish (Table 2.2). In sites containing fish, the relative species composition was dependent on sampling site (10 sites × 8 species contingency table; χ^2 test of independence: $\chi^2 = 778$, df = 77, $p < 0.05$). In sampling sites where they occurred, native species were common. *Barbus pallidus* was recorded in only the lower to middle reaches of the stream (sites 1-6, Figure 2.2) at densities of 0.2 to 5.2

fish/100 m^2. The more cryptic *G. callidus* was recorded throughout the system although it was more abundant in the middle and upper reaches (0.1-2.2 fish/100 m^2). *Sandelia capensis* was present only in the middle reaches (sites 6-8) at densities of 0.1-2.2 fish/100 m^2. *Pseudobarbus afer* was recorded from the middle to the upper reaches (sites 6-12), with abundance increasing from 0.3 fish/100 m^2 in the middle reaches to up to 126.5 fish/100 m^2 in the upper reaches. Non-native species were patchy in their distribution (Table 2.2). *Clarias gariepinus* was recorded at sites 1 and 6. The two centrarchids, *M. salmoides* and *M. dolomieu*, were sampled only from site 9 where they were the only fish in the pool. *Tilapia sparrmanii* was fairly abundant in the lower and middle reaches (0.1-4.6 fish/100 m^2) and was recorded as far upstream as site 8.

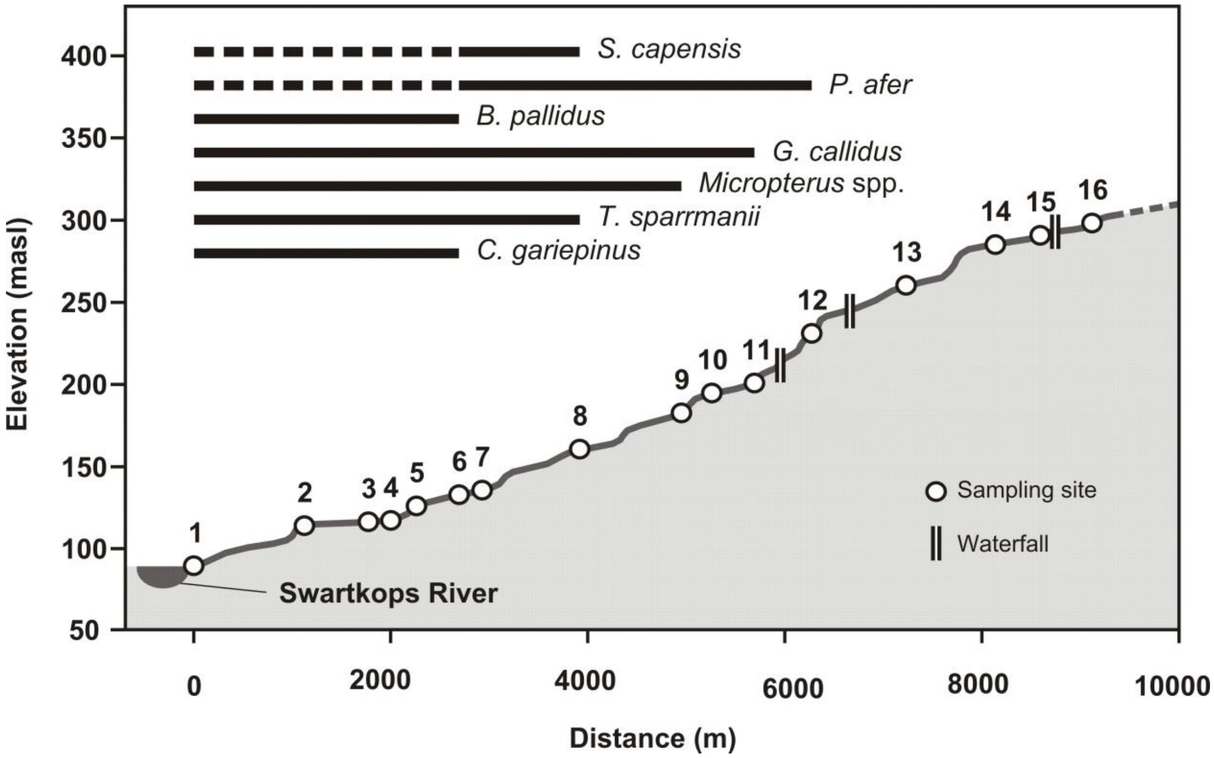


Figure 2.2 The maximum penetration of the native and non-native fishes in the Blindekloof stream in relation to the stream gradient and natural barriers (the dotted bar indicates where a species has been extirpated).

Table 2.2 The sites, sampling methods, pool sizes (m^2), distribution, abundance (fish/100 m^2) and relative abundance by site (%) of fishes in the Blindekloof stream from site 1 which is the Swartkops/Blindekloof confluence to site 16 in the upper reaches of the Blindekloof stream (* = non-native fishes) (EF = Electrofishing; S = Snorkelling).

		Lower reaches				**Middle reaches**					**Upper reaches**						
Species	**Site**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
	Surface area (m^2)	**97**	**1260**	**658**	**125**	**1250**	**2100**	**45**	**1250**	**2000**	**900**	**450**	**34**	**374**	**150**	**48**	**221**
	Method	**EF**	**S**	**S**	**EF**	**S**	**S**	**EF**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
B. pallidus	**Fish/100m^2**	5.2	0.2	3.6	-	0.2	0.5	-	-	-	-	-	-	-	-	-	-
	%	62.5	8.3	44.4	-	8.6	14.3	-	-	-	-	-	-	-	-	-	-
P. afer	**Fish/100m^2**	-	-	-	-	-	0.3	-	0.3	-	-	2.7	126.5	-	-	-	-
	%	-	-	-	-	-	8.5	-	17.4	-	-	54.8	100.0	-	-	-	-
G. callidus	**Fish/100m^2**	1.0	-	-	-	-	0.1	2.2	0.4	-	-	2.2	-	-	-	-	-
	%	12.5	-	-	-	-	2.1	33.3	21.7	-	-	45.2	-	-	-	-	-
S. capensis	**Fish/100m^2**	-	-	-	-	-	0.1	2.2	1.0	-	-	-	-	-	-	-	-
	%	-	-	-	-	-	2.1	33.3	56.5	-	-	-	-	-	-	-	-
T. sparrmanii*	**Fish/100m^2**	1.0	1.3	4.6	-	2.6	2.4	2.2	0.1	-	-	-	-	-	-	-	-
	%	12.5	91.7	55.6	-	91.4	72.3	33.3	4.4	-	-	-	-	-	-	-	-
M. salmoides*	**Fish/100m^2**	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
	%	-	-	-	-	-	-	-	-	80.0	-	-	-	-	-	-	-
M. dolomieu*	**Fish/100m^2**	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
	%	-	-	-	-	-	-	-	-	20.0	-	-	-	-	-	-	-
C. gariepinus*	**Fish/100m^2**	1.0	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-
	%	12.5	-	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-

2.4 Discussion

Despite the absence of two introduced species, *C. carpio* and *Salmo trutta*, the current study largely supports the hypothesis that the Blindekloof stream would be invaded by all non-native fishes introduced into the mainstream. *Salmo trutta* failed to successfully establish in the Swartkops River system and has not been recorded since the 1960s, while *C. carpio* has never been recorded within the Groendal Wilderness Area, although it contributes significantly to anglers' catches from the Swartkops River below the Groendal Wilderness Area (B. Kurten, records officer Eastern Cape Bank Anglers Association pers. comm.). The reinvasion of the Blindekloof stream by *M. salmoides* after its removal in 1989 (Skelton 1993), and the invasion by an additional three non-native species was, however, fairly rapid.

By 1997, *M. salmoides* had reinvaded the Blindekloof stream and during the current survey it was sampled from further upstream than previously recorded. In the upper Swartkops system, *M. salmoides* was introduced into the Groendal Dam, upstream of the Swartkops/Blindekloof confluence, in 1935 and subsequently spread downstream (Skelton 1993). *Micropterus salmoides* have subsequently established in the Swartkops River system and a reinvasion of the Blindekloof stream was inevitable because no mitigating measures were implemented after its removal in 1989. The invasive impacts of *M. salmoides* in South Africa are predominantly limited to mainstream rather than headwater environments (de Moor and Bruton 1988), although the species has invaded headwater streams (de Moor and Bruton 1988; Shelton et al. 2008; Skelton 1993). Despite this, its impact on the fish populations in individual pools is significant, with both Skelton (1993) and this study reporting the complete absence of other fishes in pools where *M. salmoides* was present. Periodic invasion into the reaches occupied by vulnerable native fishes, however, is likely to result in their extirpation in invaded pools, particularly for *S. capensis* and *P. afer* that have limited distributions within the Blindekloof stream. The invasion process may be dynamic and determined by rainfall, but after favourable recruitment conditions *M. salmoides* may provide a significant threat to native fishes.

This study also documents the first record of an additional centrarchid, *M. dolomieu*, in the Blindekloof stream. While the exact date of introduction is unknown, *M. dolomieu* has been caught by anglers in the Swartkops River system since 1965 (Brian Clark, Chairman: Eastern

Cape Freshwater Fish Conservancy pers. comm.). This species is one of the most successful invaders in Western Cape streams (Impson et al. 2002; Lowe et al. 2008; Woodford et al. 2005). In the Rondegat River, a headwater tributary of the Olifants River in the Western Cape, *M. dolomieu* extirpated five native fish species, and the juveniles of a sixth species, Clanwilliam yellowfish *Labeobarbus capensis*, were absent from invaded sections of the river (Woodford et al. 2005). As *M. dolomieu* is now in the Blindekloof stream, it may have a similar impact on the native fishes.

Clariid catfishes are well suited to establishing in new environments due to airbreathing adaptations, omnivorous diet, ability to move overland and their habit of burrowing during droughts (Cambray 2003a). Although little information exists on the effects of *C. gariepinus* invasion on native species, this species has been implicated as the primary cause for the decline of two native Eastern Cape species, smallscale redfin *Pseudobarbus asper* in the Gamtoos River system and Eastern Cape rocky *Sandelia bainsii* in the Tyume River (Cambray 2003a). Only two *C. gariepinus* individuals were recorded from the Blindekloof stream, and both co-occurred with native species. These findings indicate that *C. gariepinus* has not yet fully established in the Blindekloof stream. Low abundance and limited distribution was however also characteristic of the other two large predatory species, *M. dolomieu* and *M. salmoides*, which were neither abundant nor widespread. This may be a result of the episodic nature of the Blindekloof stream being unfavourable to large predators.

The temporal flow regimes, fluctuations in pool size and resultant limitations in prey availability, particularly during dry periods when pools are small, may make this environment marginal for large predators such as *M. salmoides*, *M. dolomieu* and *C. gariepinus*. In addition, during low flows, larger individuals in diminishing pools may become increasingly vulnerable to predation by the Cape clawless otter *Aonyx capensis*, and piscivorous birds, which may inhibit their successful establishment in the stream. Such low abundance was already noted by Skelton (1993), who observed that individual pools were inhabited by few *M. salmoides*. The occurrence of *M. salmoides* in this stream is therefore likely to depend on invasions of individuals from downstream. In contrast to the large predators, the smaller generalist, *T. sparrmanii* was both abundant and widespread in the lower and middle reaches of the Blindekloof stream and appears to have established successfully in the stream.

Tilapia sparrmanii has been widely translocated in South Africa as fodder fish for both *M. salmoides* and *M. dolomieu*, including Eastern Cape rivers (de Moor and Bruton 1988). Studies on its invasive potential are limited, although it has been suggested that the species competes with native fishes for food resources and possibly preys on juvenile native fish (de Moor and Bruton 1988). The successful establishment of *T. sparrmanii* in the Blindekloof stream may be due to its wide habitat tolerance and generalist nature. The species prefers quiet or standing waters, typical of the pools on the Blindekloof stream. *Tilapia sparrmanii* is predominantly macrophagous, but may feed on small invertebrates and in some cases even small fish (Skelton 2001; Zengeya and Marshall 2007). There may be food resource competition between *T. sparrmanii* and the two native species *P. afer* (diet of filamentous algae and invertebrates) and *S. capensis* (diet of terrestrial and aquatic invertebrates as well as small fish) (Skelton 2001). The invasive potential of *T. sparrmanii* is, however, still inconclusive and needs to be investigated further.

Predation by, and competition with, non-native fishes is considered a primary threat to native fishes in southern Africa (Tweddle et al. 2009). The limited distribution of *P. afer* and *S. capensis* make them particularly vulnerable to invasion by non-native fishes. The *P. afer* population is predominantly limited to a 1.7 km section of the stream that is not invaded. The steep gradient and physical barriers (waterfalls >2 m) seem to preclude this stream section from invasion. A similar situation exists in the Western Cape where native fishes are predominantly confined to the headwaters and smaller upper catchment streams, often as a result of waterfalls that prevent the spread of invasive fishes upstream (Gaigher et al. 1980; Lowe et al. 2008). *Sandelia capensis*, however, was recorded only from the middle reaches of the Blindekloof stream, and is afforded no protection by natural barriers (waterfalls) to invasion, as *P. afer* is. Although the Blindekloof *P. afer* and *S. capensis* populations are situated within a protected area, they are afforded little protection from invasion by non-native fishes, as invasion originates from the mainstream, which acts as a reservoir and donor population for invasion. This highlights the ineffectiveness of terrestrial reserves as refuges for fishes.

The findings from this study concur with available literature on invasion. The time period for successful invasion and establishment in the Blindekloof stream after initial introduction was both variable (Peterson et al. 2005) and governed by the ecological requirements and biological characteristics of the non-native fish species (Gozlan et al. 2010; Moyle and Light 1996; Strange et al. 1992; Weyl et al. 2009). Although due to specific ecological requirements and biological characteristics, a particular species may not successfully establish in any environment (Gozlan et al. 2010; Moyle and Light 1996; Strange et al. 1992; Weyl et al. 2009), this chapter of the thesis also indicates that the invasive potential of a species may not necessarily be limited to cases of successfully established species, but that its periodic invasion into unfavourable environments can still impact on native fishes.

In conclusion, while present, *M. salmoides*, *M. dolomieu* and *C. gariepinus* are not widespread or abundant within the Blindekloof stream, as this episodic stream may not conform to their specific habitat requirements. The biologically smaller generalist *T. sparrmanii* on the other hand, is more capable of establishing in the Blindekloof stream, and therefore may provide a greater persistent threat to the native fishes in future. Although this study indicates that the abundance and distribution of *P. afer* and *S. capensis* were negatively affected by the presence of non native fishes, it is uncertain whether these invasions will cause their extinction in Blindekloof stream. These results therefore indicate that a detailed study of the specific effects of the non-native fish invasion is required to better understand the invasion process and its effects on native fish in order to formulate future conservation strategies and to predict consequences of future invasions.

CHAPTER 3: Investigating fish invasions in episodic streams: understanding the spatio-temporal fish community dynamics pre- and post-flooding

3.1 Introduction

The ecological impact of biological invasions is the most poorly understood aspect of the invasion process (Kulhanek et al. 2011; Parker et al. 1999). This is further complicated by the context dependent nature of impacts, with very few rules on specific effects due to non-native species introductions (Ricciardi and Atkinson 2004; Williamson 1996). According to Kulhanek et al. (2011), even in instances where species have documented impacts elsewhere, only the type and direction of the expected impact can be inferred, but the size of the impact cannot be accurately predicted. Headwater streams are particularly susceptible to the impacts of introduced species, as they are characterised by low species diversity, but high degrees of endemism (Abell et al. 2007).

The impacts of non-native fishes on headwater stream fish assemblages have been relatively well documented in two Mediterranean-climate regions, the Iberian Peninsula (Portugal and Spain) and the Cosumnes River, California, USA (Bernardo et al. 2003; Collares-Pereira et al. 1999; Godinho and Ferreira 2000; Moyle et al. 2003; Moyle and Williams 1990). In the Raia stream, a tributary of the Tagus River in Portugal, for example, the presence of largemouth bass *Micropterus salmoides* was the best biotic predictor of native fish species assemblage structure, with small cyprinids being absent from reaches invaded by *M. salmoides* (Godinho and Ferreira 2000). This finding is consistent with other studies of fish assemblages in the region (Bernardo et al. 2003). In the intermittently flowing streams of the Guadiana River system, predation by non-native fish was a major contributing factor to the decline of the small cyprinid *Anaecypris hispanica*, which is consequently at high risk of extinction (Collares-Pereira et al. 1999). Similarly, since the introduction of non-native species to the Cosumnes River basin California (USA), there has been a gradual disappearance of native fish. In some areas invasive redeye bass *Micropterus coosae* represented >90% of fish collected (Moyle et al. 2003; Moyle and Williams 1990).

While impacts of non-native fishes in both regions are severe, abiotic resistance limits their establishment through the maintenance of natural flow regimes which has prevented non-native fishes from total domination and allows native species to maintain populations in non-invaded stream reaches (Bernardo et al. 2003; Moyle and Light 1996). In these regions prone to high seasonal flow variability, non-native fish abundances fluctuate and impacts vary according to the prevailing conditions, displaying high relative abundances during low flow scenarios, with native species recovering under high flow conditions (Bernardo et al. 2003; Moyle et al. 2003).

High magnitude floods form part of natural flow regimes and are an integral component, and in many cases considered a dominant organizing factor in stream ecology (Lytle and Poff 2004; Resh et al. 1988). Picket and White (1985) in Resh et al. (1988) define a flood disturbance as any relatively discrete event in time that disrupts ecosystem, community, or population structure, and that changes resources, availability of substratum, or the physical environment. Despite varied opinions on whether deterministic processes such as competition and predation, or stochastic factors such as droughts and floods are the defining factors structuring stream ecosystems (Grossman et al. 1982; Resh et al. 1988; Schlosser 1982; Schlosser and Ebel 1989), some authors aptly highlight the fact that it would be simplistic to consider that fish assemblages are regulated by a single process (Bernardo et al. 2003; Grossman et al. 1982).

The evolution of fish in environments with seasonally predictable floods has resulted in adaptations that minimize the exposure of certain vulnerable life history stages to perturbation, but maximise growth and reproduction when conditions are favourable (Franssen et al. 2006; Gasith and Resh 1999; Labbe and Fausch 2000; Lytle and Poff 2004; Resh et al. 1988). Unpredictable, infrequent and catastrophic floods, however, can have major impacts on affected fish communities (Matthews 1986; Nislow et al. 2002; Resh et al. 1988). The impacts of these floods include an immediate change in abundance of fishes (Magalhaes et al. 2003; Matthews 1986; Nislow et al. 2002; Pires et al. 2008), recruitment failure (Letcher and Terrick 1998) and alteration in composition of the entire fauna (Matthews 1986; Nislow et al. 2002). Habitats particularly susceptible to disturbance are headwater streams as they have small catchments and are easily influenced by relatively minor changes in local conditions (Meyer et al. 2007). Impacts of floods on headwater stream habitats include slope failures, bank erosion, substrate scouring, and loss of habitat and biota (Resh et al. 1988). Flooding can play a dual role in the invasion process: in some cases natural flow regimes play a major role in invasion resistance (Moyle and Light 1996; Poff 1997), in others an increase in flow associated with floods can facilitate non-native fish invasion of stream habitats.

Despite the documented impacts of floods on stream fish communities (Letcher and Terrick 1998; Matthews 1986; Nislow et al. 2002; Pires et al. 2008; Resh et al. 1988), fishes also display long-term resilience and have been shown to return to equilibrium fairly rapidly following catastrophic flood events (Dolloff et al. 1994; Matthews 1986). Following a major disturbance, under natural conditions headwater stream fishes are able to recolonise disturbed stream reaches from undisturbed refuge habitats (Dolloff et al. 1994; Matthews 1986). In these instances a number of factors facilitate the return of disturbed stream reaches to pre-disturbance conditions, for example, the presence of metapopulations in unaffected refuges which provide source populations for recolonisation (Medeiros and Maltchik 2001). Increasingly, however, the high incidence of headwater stream fish populations isolated from the rest of the stream network due to anthropogenic factors (dams, weirs) or biological barriers (invasive fishes) threatens the persistence of the these populations (Fausch et al. 2009). Such isolation and fragmentation of stream fish populations makes them vulnerable to catastrophic extinction (Hildebrand and Kershner 2000).

Recent research on fish communities in intermittent headwater streams indicates that the persistence of fishes in these environments may be dependent on dispersal between complementary habitats for reproduction, feeding, rearing and to avoid disturbance (Franssen et al. 2006; Labbe and Fausch 2000; Schlosser and Angermeier 1995). Isolation of headwater stream populations may inhibit dispersal opportunities, threatening their long-term persistence (Fausch et al. 2009). South African endemic headwater stream fishes are increasingly being isolated in small fragmented headwater refuges due to upstream invasion by non-native fishes (Woodford et al. 2005). This is particularly relevant in the headwaters of the Kwa-Zunga River where fragmentation of native Eastern Cape redfin *Pseudobarbus afer* by centrarchid fishes has already been demonstrated for the Blindekloof stream (Chapter 2). Stream invasions in the Kwa-Zunga River headwaters have predominantly been casual (they do not form self-replacing populations in the invaded region and whose persistence depends on repeated introductions from mainstream sources (Richardson et al. 2011)), with only one species establishing successfully in one stream (Chapter 2). According to Skelton (1993), the unpredictable flow regimes and stream size retard the invasion process. Despite their casual nature, these invasions have extirpated native fishes in invaded reaches of the stream (Skelton 1993).

The centrarchid invasion of the headwater tributaries of the Kwa-Zunga River, upper Swartkops River system, presented a unique prospect to research invasive impacts within a relatively pristine river system, without the confounding effects of major habitat alteration and anthropogenic influences. In addition, an unpredictable, catastrophic flood during June 2011 provided an opportunity to contrast community assemblages under varying flow scenarios (base flow and post flood). This was done by:

- Quantifying invaded and non-invaded stream reaches in order to estimate the proportion of stream habitat lost to *P. afer,* a high conservation priority species, through invasion by centrarchids during base-flow conditions.
- Comparing factors influencing the community assemblage structure in the headwater streams between base-flow and post flood conditions.
- Testing the impact of the flood on abundance and distribution of different *P. afer* size classes.
- Discussing the role of the flood in context of facilitating or inhibiting non-native fish invasions in headwater streams.

3.2 Materials and Methods

3.2.1 Study Area

3.2.1.1 Habitat characteristics

The study was conducted on six first- and second-order (Strahler 1957) headwater tributary streams (Blindekloof, Chaseskloof, Nounekkloof, Fernkloof, Waterkloof and Vyeboomkloof) of the Kwa-Zunga River (third-order), one of two major tributaries of the Swartkops River system within the Groendal Wilderness Area, Eastern Cape, South Africa (Figure 3.1). The Swartkops River system is a relatively short coastal river system forming part of the

Uitenhage Artesian Basin, South Africa's most important groundwater artesian basin (Maclear 2001). Its headwaters originate in the Grootwinterhoek Mountains at an altitude of 1000 metres above sea level, flowing for 110 km before the river enters the Indian Ocean near the city of Port Elizabeth (Figure 3.1) (see Chapter 2 for a physical habitat description). The geology of the Grootwinterhoek Mountains consists primarily of quartzitic Table Mountain Group sandstones (Maclear 2001).

The headwater streams draining into the Kwa-Zunga River are free flowing, but the mainstream was dammed in 1933 to create the 96 ha Groendal Dam (Figure 3.2). The stream catchments are pristine and unaffected by anthropogenic influence because they are located in the Groendal Wilderness Area (proclaimed for the protection of indigenous forest and water resources). The Kwa-Zunga River and its catchments are listed as a National Freshwater Ecosystem Priority Area (NFEPA, Nel et al. 2011). These NFEPA areas were identified on a national level to provide effective conservation of habitats most representative of South Africa's freshwater ecosystems and to ensure the sustainable use of water resources (Nel et al. 2011). This section of the Swartkops River system has also been identified as a fish sanctuary, a designation assigned to a river essential for protecting threatened or near threatened freshwater fish (Nel et al. 2011).

3.2.2 Metadata

3.2.2.1 Rainfall and flow

Long term hydrological and meteorological data were obtained from the Department of Water Affairs hydrology section (DWAF 2012). The situations of the recording stations are presented in Figure 3.1. Flow data were obtained from the gauging weir at Wincanton on the Elands River (M1H004), a Swartkops River system headwater tributary for the period 1965/04/06-2012/01/05 (Figure 3.1). These data were used to illustrate rainfall, flow variability and the magnitude of the catastrophic flood during June 2011. Rainfall data were obtained from station M1E001 at Groendal Dam (1950/02/16-2006/01/11) and supplemented with data from the nearby station M1E002 at Uitenhage (2006/11/30-2012/01/05).

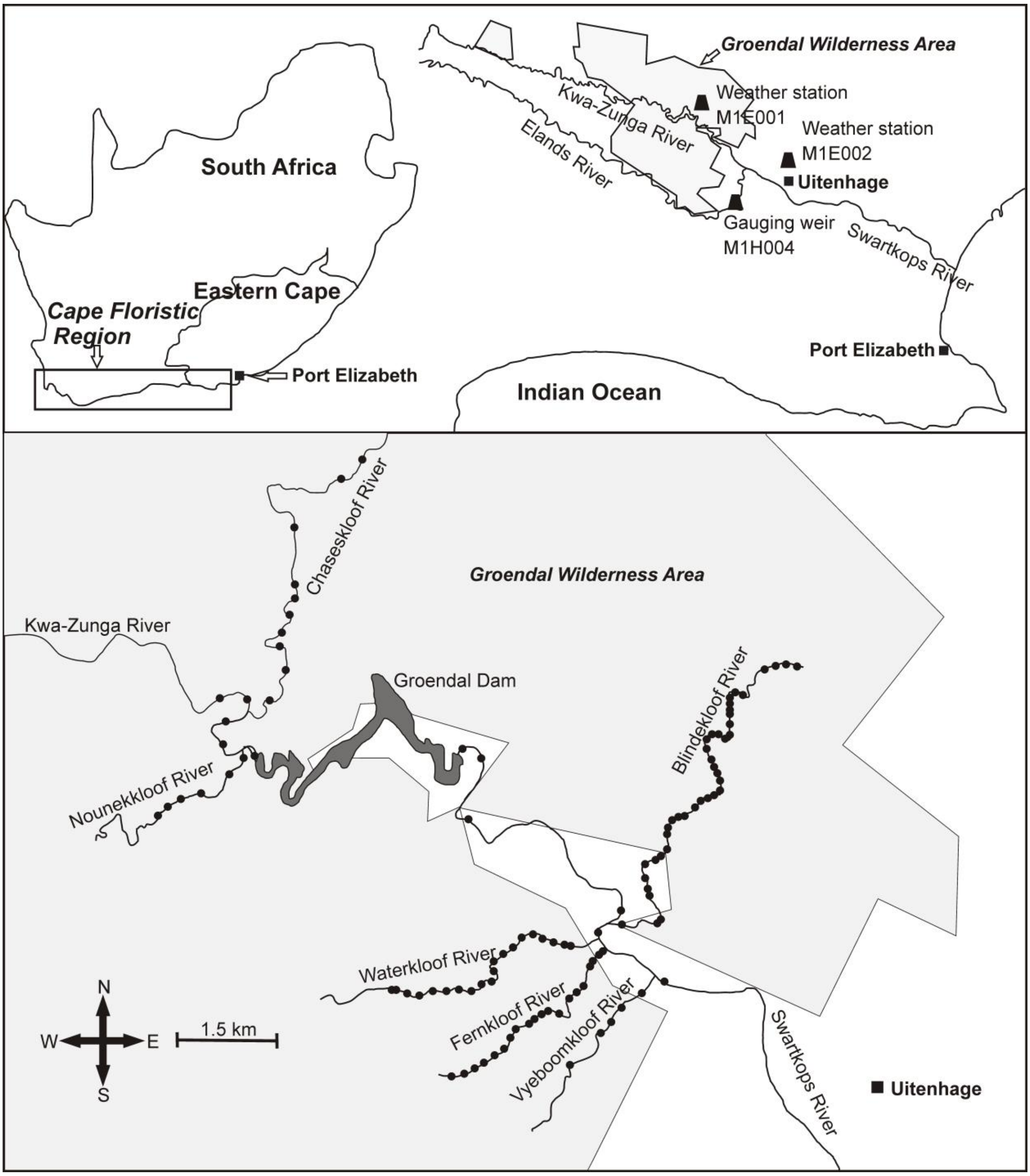


Figure 3.1 The situation of the Swartkops River system headwater streams and sites sampled during this study within the Groendal Wilderness Area, Eastern Cape, South Africa. The position of the gauging weir (M1H004) on the Elands River and the weather station (M1E001) at Groendal Dam are also noted. The Swartkops River system is the eastern-most system of the Cape Floristic Region (CFR).

Figure 3.2 The Groendal Dam constructed in 1933 on the Kwa-Zunga River, a Swartkops River system headwater tributary within the Groendal Wilderness Area, Eastern Cape, South Africa.

3.2.3 Field surveys

3.2.3.1 Habitat characteristics

At each sampling site, temperature, conductivity and pH were measured using a Hanna HI98129 Combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, USA). Turbidity (NTU) was measured using a Hanna HI 98703 turbidimeter (HANNA Instruments Inc.). To estimate pool volume and habitat diversity within each pool, the length of the pool was measured, followed by four to six equally spaced width measurements. On each width transect, three depths were measured, the outer two were each 0.2 m from the left- and right-hand stream bank and the third measurement taken midstream. At each depth measurement, the habitat type was recorded. Canopy cover was estimated as a percentage of total cover and bankside vegetation type was recorded.

3.2.3.2 Sampling techniques

Due to variability in physical characteristics of the surveyed streams, such as size and mean depth, two survey methods were employed: snorkelling surveys and backpack electrofishing.

To ensure consistency and standardised methods of data collection, all electrofishing and snorkelling surveys were undertaken by the same individual (BRE). Sites where the average depth of the stream was <1 m were sampled by single pass electrofishing. Electrofishing was conducted using a Samus 725G backpack electrofisher, attached to a 12 V battery with settings standardized at the duration of 0.3 ms and a frequency of 80 Hz. The pass was conducted from the downstream side (tail) of the pool in an upstream direction, covering the entire length of each pool. Upon completion of the pass, fish were identified to species level, measured, counted, and released. Block nets were not used as pools were mostly isolated from each other.

Snorkel surveys were conducted in streams where the depth of the sampled pools was >1 m, using a zigzag method (Chapter 2). Pass one was initiated at the tail end of the pool, with the observer swimming upstream, zigzagging to cover as much of the pool as possible. Pass two was a repeat of pass one but in a downstream direction. During each pass, all fish seen were identified to species level and counted.

3.2.3.3 Surveys

Two surveys were conducted: (1) A baseline distribution survey; (2) A post-flood distribution survey. The baseline survey (69 sites, 6 streams) was undertaken by electrofishing and day snorkelling during 2010 to determine overall distribution of fishes in the Kwa-Zunga headwater tributaries within the Groendal Wilderness Area. A flooding event in June 2011 prompted a post flood survey in November 2011. The post-flood survey employed electrofishing in two non-invaded streams (Fernkloof and Waterkloof). On each stream, 10 sites were sampled by electrofishing before the major flooding event, and these sites were then re-sampled in November 2011. Due to flood scouring and filling changing the position of pools within the two streams in the post-flood survey, the nearest pool to the pre-flood sample site was electrofished. On the Blindekloof stream, pools were too deep to electrofish effectively and a post-flood snorkel survey was conducted at 38 sites by day and night snorkelling to compare distribution and relative fish abundance to pre flood surveys conducted in Chapter 3.

3.2.4 Data analysis

3.2.4.1 Rainfall

Spearman Rank Order Correlation was used to test for a relationship between rainfall and flow in the Swartkops River system headwater streams at a significance of $P < 0.05$. According Picket and White (1985) in Resh et al. (1988), disturbance was defined as "any relatively discrete event in time that is characterized by a frequency, intensity, and severity outside a predictable range, and that disrupts ecosystem, community, or population structure and changes resources or the physical environment". These criteria were applied to peak flow data for the Swartkops River system to verify the magnitude of the flood in June 2011.

3.2.4.2 Overall distribution (base flow)

To describe overall distribution patterns, the lengths of stream occupied by *P. afer* and centrarchids *M. dolomieu* and *M. salmoides* was quantified. This was then split into invaded and non-invaded stream reaches. Of the invaded stream reaches, the length of stream where *P. afer* was absent was then estimated.

Multivariate analyses are commonly applied to characterise fish assemblages and determine the influence of environmental variables on their distribution (Godinho and Ferreira 2000; Morán-López et al. 2006; Smith and Kraft 2005). To determine the factors influencing the distribution of fishes in the upper Swartkops River system, data from 69 sites covering mainstream and tributary sites within the Groendal Wilderness Area were analysed. The necessity for using a multi-method approach for overall fish distribution surveys resulted in a lack of standardised catch data between streams. Therefore, for overall distribution data presence/absence data were analysed. For analyses, data were separated into two matrices. The first consisted of the presence/absence data by site, and the second was the environmental data by site. Environmental data were standardised with z transformation by transforming the original distribution to a mean of 0 and standard deviation of 1. Preliminary analyses using detrended correspondence analysis (DCA), indicated turnovers of >2 on the first axis, therefore unimodal ordination methods were chosen for analysis of overall distribution data in the form of correspondence analysis (CA, species data only) and canonical correspondence analysis (CCA, species-environmental relation) (ter Braak 1995). Initially 11 environmental variables were used in the CCA: canopy cover, altitude, instream barriers, maximum depth, habitat diversity, surface area, volume, pH, temperature, turbidity and conductivity. Environmental variables with variance inflation factors (VIF) >10 were then removed from further analyses due to multicollinearity with other variables (ter Braak and Smilauer 1998). A forward stepwise procedure was then used to determine the best predictor variables. The significance of these variables' contribution to the ordination was tested using 999 Monte Carlo simulations at the $P < 0.05$ level. To test the statistical significance of the selected environmental variables on the species presence/absence model, Monte Carlo permutation tests were used. The total variation in assemblage composition between the contribution of physical variables and chemical variables was then partitioned using a partial CCA as follows: (1) Calculating the total variation of all the environmental variables selected from the stepwise procedure outlined above (best predictor variables); (2) Variation accounted for by the best predictor physical environmental variables after partialling out the influence of physico-chemical variables (pure physical); (3) Variation accounted for by the best predictor physico-chemical variables after partialling out the influence of the physical variables (pure physico-chemical), and; (4) Unexplained variation.

3.2.4.3 Post flood distributions

To investigate the post flood factors influencing the presence/absence of fishes in three representative headwater streams (Blindekloof, Fernkloof, Waterkloof), multivariate analyses were conducted using species (presence/absence) and environmental data from the 54 sites sampled (day and night snorkelling data were pooled for the Blindekloof stream). Preliminary analyses using detrended correspondence analysis indicated turnovers of <2 on the first axis for presence/absence data, therefore redundancy analysis (RDA), a linear ordination model was chosen to analyse factors influencing fish distribution after the flood. Initially the following 14 environmental variables were used in the RDA: canopy cover, surface area, volume, turbidity, temperature, stream reach, habitat diversity, % boulders, % cobbles, % leaf litter, % aquatic macrophytes, % bedrock, % gravel and stream name was added as a covariable. Environmental variables with variance inflation factors (VIF) >10 were then removed from further analyses due to multicollinearity with other variables (ter Braak and Smilauer 1998). A forward stepwise procedure was then used to determine the best predictor variables. The significance of these variables contribution to the ordination was tested using 999 Monte Carlo simulations at the $P < 0.05$ level. To test the statistical significance of the selected environmental variables on the species abundance model, Monte Carlo permutation tests were used. All multivariate analyses were performed using *CANOCO v 4.5* (ter Braak and Smilauer 2002).

3.2.4.4 Impact of floods on *P. afer*

To investigate the impact of a major flooding event on *P. afer* in headwater streams, pre- and post-flood fish density (based on electrofishing catch per unit effort, expressed as fish m^{-3}) estimates were compared in two streams (Fernkloof, Waterkloof) using a main effects ANOVA at a significance level of $P \leq 0.05$. Specifically, fish density was tested for longitudinal trends (upper versus lower reaches), as well as flooding impact (pre- versus post-flood) and an interaction between the two factors. Due to complications resulting from differences in sampling months, with the pre-flood survey occurring at the end of the spawning season, and the post-flood survey at the beginning of the spawning season (Cambray 1994), *P. afer* juveniles (<40 mm) and adults (>40 mm) were analysed separately.

To test the vulnerability of the two *P. afer* size classes to the flooding event, the frequency of occurrence (number of sites per stream reach where each size class was present expressed as a percentage of all sites in that reach) by stream reach (upper and lower reaches) was tested for significance by reach and pre- and post-flooding using a chi-squared test of independence. All analyses were undertaken using MS Excel 2007, Microsoft® and Statistica 10.0, StatSoft®.

3.3 Results

3.3.1.1 Habitat characteristics

Typical in-stream habitat was characterized by isolated pools varying in volume from (mean ± SD) 15.9 ± 9.3 m^3 to 372.5 ± 425.6 m^3 on the Vyeboomkloof and Blindekloof streams respectively and 4206.6 ± 5145.5 m^3 on the mainstream Kwa-Zunga River. Substrates were dominated by cobbles (Range: Kwa-Zunga = 32.2 ± 25.9% - Blindekloof = 58.3 ± 17.9%), large unconsolidated boulders (Nounekkloof = 5.0 ± 5.1% - Blindekloof = 15.5 ± 19.1%) and bedrock (Waterkloof = 0% - Vyeboomkloof = 14.7 ± 21.3%) (Table 3.1). Sand, mud, aquatic macrophytes, algae and terrestrial vegetation constituted the remainder (Table 3.1). Stream bank vegetation in the upper reaches of the Blindekloof and Chaseskloof stream consisted of

Mesic Grassy Fynbos (Campbell 1985), and in the middle and lower reaches Tropical Coastal Forest (Acocks 1975) which is limited to the riverine valleys. The Fernkloof, Nounekkloof, Waterkloof and Vyeboomkloof are characterised by closed canopy Tropical Coastal Forest throughout the reaches where fish were distributed (Figure 3.3). During sampling periods, streams were generally cool (Nounekkloof = 15.3 ± 0.2 °C-Chaseskloof = 20.5 ± 2.6 °C), well oxygenated (Kwa-Zunga = 7.7 ± 0.9 mgl^{-1}-Waterkloof = 9.1 ± 0.5 mgl^{-1}), had near neutral pH (Waterkloof = 7.1 ± 0.3-Nounekkloof = 7.7 ± 0.0) and low conductivity (Chaseskloof = 114.5 ± 8.8 µS cm-1-Vyeboomkloof = 395.8 ± 6.7 µS cm-1). For an indication of temporal ranges in water quality see Chapter 4.

3.3.1.2 Rainfall and flow

According to the criteria outlined in Resh et al. (1988), the June 2011 flood was classified as a major disturbance (1245 m^{-3}s^{-1}) (Figure 3.4). Mean annual rainfall (mean ± SD) for the period 1950/02/16-2012/01/05 was 621 ± 212 mm.yr^{-1} (Table 3.2). During the period on record (1950/02/16-2012/01/05), rainfall was variable, falling in an unpredictable, erratic pattern in all months of the year, with the largest proportion falling during March (11.1%) and the lowest in May (4.9%) (Figure 3.5, Figure 3.6). There was a highly significant correlation between rainfall and peak flow (Spearman r = 0.389; $Z = 9.45$; $P < 0.05$). Peak flow between April 1965 and May 2012 was low (mean ± SD: 30.3 ± 202.3 m^{-3}s^{-1}) and was predominantly <25 m^{-3}s^{-1} (89%), with a further 8.7% <500 m^{-3}s^{-1}. During the 47 years on record, there were only four peak flows exceeding 1000 m^{-3}s^{-1} (Figure 3.7). Despite permanent subsurface flow, Swartkops River system headwater tributaries are primarily episodic in nature. The tributaries are fed by both precipitation and groundwater, and surface flow follows only after sustained heavy rain (Roux et al. 2002), an event that occurs on average 1.2 times/year in an erratic manner (Skelton 1993).

Table 3.1 Summary of the physico-chemical and habitat characteristics (mean ± SD) for sampling sites on six surveyed headwater streams and the mainstream Kwa-Zunga River, headwater tributaries of the Swartkops River system within the Groendal Wilderness Area, Eastern Cape, South Africa (* indicates where quantitative data were unavailable and qualitative estimates were provided) (Bk = Blindekloof; Fk = Fernkloof; Wk = Waterkloof; Vbk = Vyeboomkloof; Ck = Chaseskloof; Nnk = Nounekkloof; K-Z = Kwa-Zunga).

	Bk	**Fk**	**Wk**	**Vbk**	**Ck**	**Nnk**	**K-Z**
Length (m)	35.6±18.5	10.8±3.2	11.9±3.8	7.7±2.4	21.7±10.9	15.6±5.3	66.4±42.4
Width (m)	8.6±4.1	3.7±0.9	5.5±4.9	3.2±0.8	8.5±7.9	5.8±1.3	21.7±16.7
Depth (m)	0.9±0.5	0.7±0.4	0.8±0.6	0.6±0.2	-	0.7±0.2	2.4±1.7
Canopy cover (%)	8.2±17.7	46.0±23.0	33.5±24.8	28.0±19.2	6.7±10.0	30.0±25.3	2.0±6.0
Surface Area (m^2)	345±314	40±17	78±108	25±10	104±188	97±28	1580±1481
Volume (m^3)	372±425	34±35	120±295	15±9	345±1025	109±58	4206±5145
Cobbles (%)	58.3±17.9	54.0±10.9	46.7±28.3	36.0±17.9	50*	32.8±18.2	32.2±25.9
Leaf litter (%)	0.5±1.5	23.3±8.5	28.0±13.9	34.7±23.6	5*	20.6±12.9	4.4±7.7
Bedrock (%)	11.7±13.2	0.7±1.5	0±0	14.7±21.3	10*	4.4±9.4	0±0
Boulder (%)	15.5±19.1	10.0±2.4	15.3±19.4	4.7±10.4	14*	5.0±5.1	6.7±8.8
Sand (%)	7.4±9.0	0±0	0±0	0±0	0*	0.6±1.4	0±0
Mud (%)	0±0	3.3±7.5	0±0	1.3±2.9	5*	2.8±6.8	0±0
Aquatic macrophytes (%)	5.7±7.9	8.0±10.9	2.0±4.5	8.0±16.1	15*	8.9±9.4	53.3±29.6
Algae (%)	0±0	0±0	0±0	0±0	0*	20.0±25.9	3.3±5.8
Terrestrial vegetation (%)	0.1±0.8	0.7±1.5	7.3±10.1	0±0	1*	5.0±3.5	0±0
pH	7.3±0.2	7.2±0.3	7.1±0.3	7.3±0.1	7.2±0.4	7.7±0.0	7.7±0.3
Temperature (°C)	19.1±1.7	15.9±0.4	16.1±1.8	16.6±0.6	20.5±2.6	15.3±0.2	17.7±1.4
Turbidity (NTU)	0.4±0.2	0.3±0.1	0.4±0.1	0.8±0.3	0.6±0.3	0.2±0.0	0.7±0.5
Oxygen concentration (mg l^{-1})	8.7±0.8	8.5±0.7	9.1±0.5	8.1±0.6	8.1±0.6	8.6±0.7	7.7±0.9
Oxygen saturation (%)	90.0±14.8	93.1±7.2	95.5±6.8	83.2±7.0	91.5±3.9	88.9±6.8	85.6±8.3
Total dissolved solids (ppm)	65±25	146±6	125±4	196±2	57±4	113±2	85±13
Conductivity (µS cm^{-1})	129±17	293±14	253±9	395±6	114±8	230±4	172±26

Figure 3.3 (A) The Chaseskloof catchment, a typical upper Swartkops headwater tributary with the upper slopes characterised by Mesic Grassy Fynbos and the riparian vegetation by Tropical Coastal Forest; (B) Tropical Coastal Forest on the Blindekloof stream.

Table 3.2 Summary flow and rainfall statistics from the gauging weir at Wincanton (M1H004; 1965/04/06-2012/01/05) on the Elands River, a Swartkops River system headwater tributary and rainfall measured at Groendal Dam (M1E001; 1950/02/16-2006/01/11), supplemented with data from Uitenhage (M1E002; 2006/11/30-2012/01/05).

Month	**Peak flow (max) ($m^{-3}s^{-1}$)**	**Peak flow (mean ± SD) ($m^{-3}s^{-1}$)**	**Rainfall (mm) (mean ± SD)**	**%**
Jan	86.9	5.4 ± 18.6	57.0 ± 46.7	9.2
Feb	16.2	1.0 ± 2.9	53.2 ± 38.9	8.6
Mar	472.3	15.1 ± 73.8	69.2 ± 57.4	11.1
Apr	368.2	10.9 ± 56.7	55.5 ± 47.8	8.9
May	173.1	8.7 ± 32.3	34.4 ± 36.6	5.5
Jun	1245.4	35.9 ± 195.7	30.5 ± 36.1	4.9
Jul	835.5	22.6 ± 130.4	35.0 ± 50.7	5.6
Aug	2729.0	116.7 ± 470.6	51.7 ± 60.2	8.3
Sep	997.3	48.8 ± 205.8	49.7 ± 57.6	8.0
Oct	225.7	11.7 ± 45.3	61.9 ± 54.9	10.0
Nov	2581.0	77.8 ± 400.6	65.6 ± 54.2	10.5
Dec	118.5	8.9 ± 28.6	58.5 ± 46.9	9.4
Annual			620.7 ± 212.3	

Figure 3.4 A low level bridge at the confluence of the Blindekloof stream and Kwa-Zunga River illustrating the extent of damage caused by the floodwaters during June 2011.

Figure 3.5 Images illustrating the episodic nature of the Swartkops River system headwater tributaries within the Groendal Wilderness Area. Pools in the lower and middle reaches of the Blindekloof stream during high (A & D) and low (B & C) flow periods.

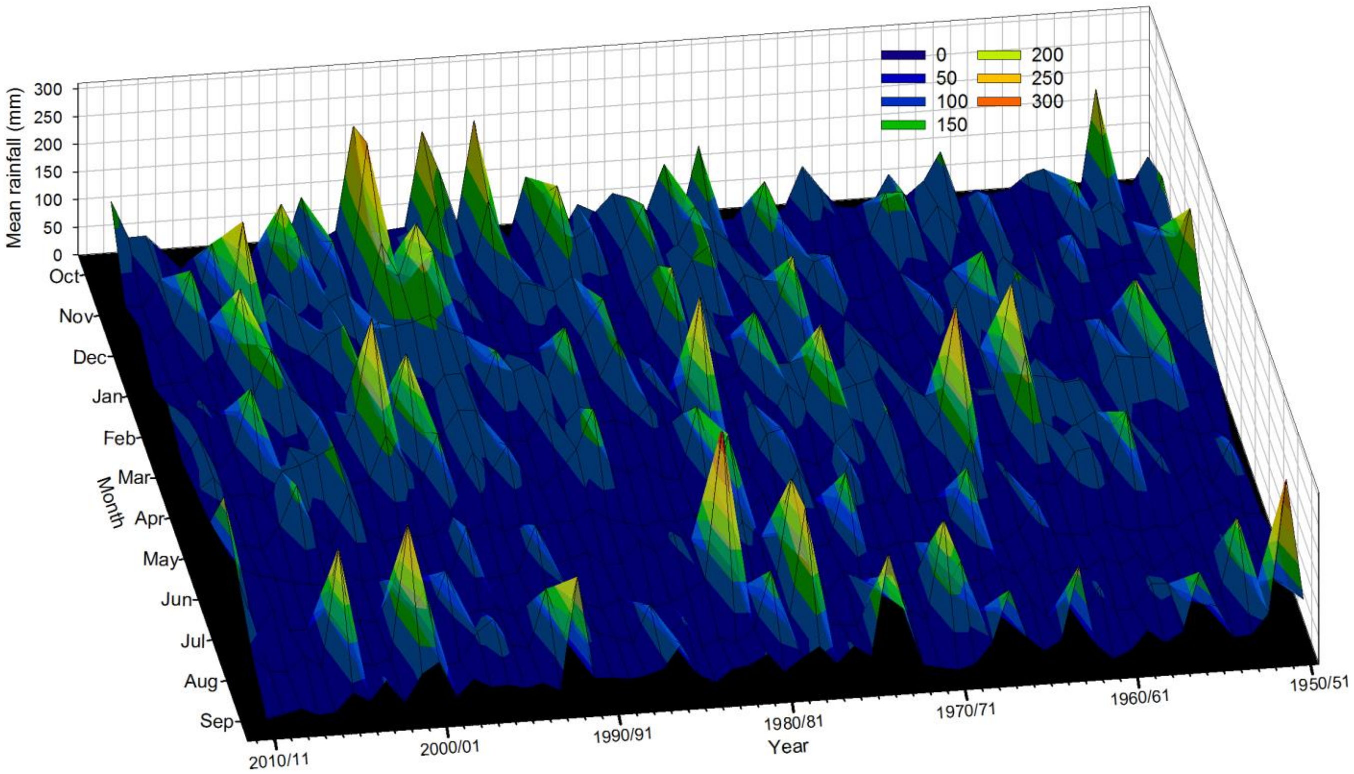


Figure 3.6. Total monthly rainfall (mm) for the Groendal Wilderness Area recorded from the Groendal Dam (M1E001) for the period (1950/02/16-2006/01/11), supplemented with data from the nearby station M1E002 at Uitenhage (2006/11/30-2012/01/05), illustrating the unpredictable rainfall patterns and the episodic nature of the Kwa-Zunga River headwater tributaries.

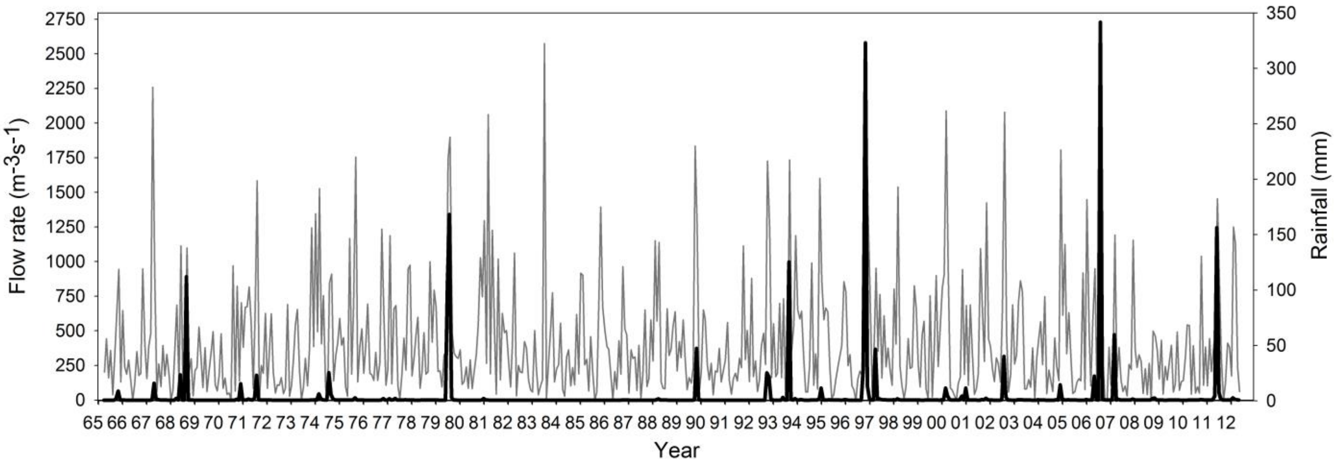


Figure 3.7 Monthly peak flows at the Wincanton gauging weir on the Elands River and total monthly rainfall at Groendal Dam (1950/02/16-2006/01/11) and at Uitenhage (2006/11/30-2012/01/05) illustrating rainfall and flow variability in the headwater tributaries of the Swartkops River system (flow data are missing from April 1981-September 1986).

3.3.1.3 Fish fauna of the Swartkops River system

The native fish fauna in the headwaters of the Swartkops River system is depauperate, consisting of six species: two predatory Anguillid eels, the longfin eel *Anguilla mossambica* and giant mottled eel *Anguilla marmorata*, two Cyprinid minnows, the goldie barb *Barbus pallidus* and *P. afer* (both invertivores), the Anabantid Cape kurper *Sandelia capensis* (invertivore/piscivore) and the Gobiid river goby *Glossogobius callidus* (zooplanktivore/invertivore) (Figure 3.8) (Skelton 2001). Of the six native fishes, one (*P. afer*) has been assessed by the International Union for the Conservation of Nature (IUCN) as 'Endangered', while the rest are either 'Least Concern' (*B. pallidus*, *G. callidus* and *A. marmorata*), 'Data Deficient' (*Sandelia capensis*) or have not been assessed (*A. mossambica*) (Table 3.3). Of the four non-native fishes, three are predators: *M. salmoides* (piscivore/invertivore), smallmouth bass *Micropterus dolomieu* (piscivore/invertivore) and African sharptooth catfish *Clarias gariepinus* (zooplanktivore/invertivore/piscivore) and the fourth, the omnivorous banded tilapia *Tilapia sparrmanii* (Table 3.3). Of the four introduced species, two are extralimital introductions (*C. gariepinus*, *T. sparrmanii*), i.e. they are native to other parts of South Africa but not to the Swartkops River system, and *M. salmoides* and *M. dolomieu* are alien.

Figure 3.8 Fishes of the upper Swartkops River system within the Groendal Wilderness Area (A = *Barbus pallidus*; B = *Pseudobarbus afer*; C = *Glossogobius callidus*; D = *Sandelia capensis*; E = *Anguilla mossambica*; F = *Anguilla marmorata*; G = *Micropterus dolomieu**; H = *Micropterus salmoides**; I = *Clarias gariepinus*;* J = *Tilapia sparrmanii**) (*non-native).

Table 3.3 Swartkops River system headwater stream fish fauna and their native distributions and maximum recorded size (de Moor and Bruton 1988; Scott et al. 2006; Skelton 2001). For native species the letters in brackets indicate IUCN redlist status, (NA = Not Assessed; DD = Data Deficient; LC = Least Concern; E = Endangered; IUCN 2001). Reproductive (Balon 1990) and trophic guild (Skelton 2001) classifications are also included.

Taxon	**Max size (mm SL)**	**Common name**	**Reproductive guild**	**Trophic guild**	**Native distribution**
Native species Cyprinidae					
Pseudobarbus afer (E) (Peters, 1864)	110	Eastern Cape redfin	Rock and gravel spawners with benthic larvae	Invertivore	Mossel Bay-Port Elizabeth, Eastern Cape, South Africa
Barbus pallidus (LC) A. Smith, 1841	70	Goldie barb	Plant spawners	Invertivore	Eastern Cape coastal Rivers Great Fish-Krom and the Vaal River system
Anabantidae					
Sandelia capensis (DD) (Cuvier, 1831)	200	Cape kurper	Benthic spawning rock guarders	Invertivore/ Piscivore	Eastern & Western Cape coastal rivers, Algoa Bay-Cape Flats
Gobiidae					
Glossogobius callidus (LC) (Smith, 1937)	120	River goby	Non-guarding pelagic spawner	Zooplanktivore/ Invertivore	East coast rivers, Mozambique-Swartvlei Western Cape
Anguillidae					
Anguilla mossambica (NA) Peters, 1852	1200	Longfin eel	Non-guarding pelagic spawner	Invertivore/ Piscivore	East coast rivers, Kenya-Cape Agulhas, Madagascar & Western Indian Ocean islands
Anguilla marmorata (LC) Quoy & Gaimard, 1824	1850	Giant mottled eel	Non-guarding pelagic spawner	Invertivore/ Piscivore	South East Africa-Eastern Cape, South Africa, Madagascar & adjacent islands
Non-native species Centrarchidae					
Micropterus salmoides (Lacepède, 1802)	600	Largemouth bass	Rock and gravel nesters	Piscivore/ Invertivore	Central USA (freshwaters of lower Great Lakes, Mississippi River system, Florida and the Atlantic coast to Virginia)
Micropterus dolomieu (Lacepède, 1802)	550	Smallmouth bass	Rock and gravel nesters	Piscivore/ Invertivore	Eastern Canada & the USA (Minnesota and southern Quebec to the Tennessee River system in Alabama and west to eastern Oklahoma)
Cichlidae					
Tilapia sparrmanii A. Smith, 1840	230	Banded tilapia	Rock and gravel nesters	Omnivore	Congo-Southern Africa
Oreochromis mossambicus (Peters, 1852)	400	Mozambique tilapia	Mouth brooder	Algivore/ iliophage	East coastal rivers from the lower Zambezi system south to the Bushman's River system, Eastern Cape
Clariidae					
Clarias gariepinus (Burchell, 1822)	1400	African sharptooth catfish	Non-guarding benthic spawner on plants	Zooplanktivore/ Invertivore/ Piscivore	Pan-African into eastern Europe

3.3.1.4 Overall distribution

Eight species were recorded from the surveyed streams, four native (*P. afer*, *S. capensis*, *B. pallidus*, *G. callidus*) and four non-natives (*M. salmoides*, *M. dolomieu*, *T. sparrmanii*, *C. gariepinus*). Fish occupied 19.2 km of stream, of which 12.7 km (65%) was non-invaded and 6.9 km (35%) had been invaded by non-native fishes (*T. sparrmanii*, *C. gariepinus*, *M. salmoides*, *M. dolomieu*). Of the 6.9 km of invaded headwater streams, native fish had been extirpated from 2.4 km, which equates to a loss of 12% available habitat. Of those sites where *P. afer* occurred (29 sites), they co-occurred with all other species (>60% with *S. capensis*; <11% with *G. callidus*, *B. pallidus*, *C. gariepinus* and *T. sparrmanii*), but not with either *M. salmoides* or *M. dolomieu*. The distribution of fishes is summarised in Table 3.4. In the non-invaded stream reaches, *P. afer* was widespread, inhabiting five of the six surveyed streams (Blindekloof, Waterkloof, Fernkloof, Nounekkloof and Chaseskloof). Centrarchids had invaded only short segments in the lower reaches of the Blindekloof and Chaseskloof streams.

Table 3.4 Overall distribution records for fishes from the mainstream Kwa-Zunga (Above = above Groendal Dam; Below = below Groendal Dam) and headwater streams (U = upper reaches; L = lower reaches) of the Swartkops River system within the Groendal Wilderness Area, Eastern Cape, South Africa (* indicates non-native species) (*Pa = Pseudobarbus afer; Bp = Barbus pallidus; Sc = Sandelia capensis; Gc = Glossogobius callidus; Am = Anguilla mossambica; Ama = Anguilla marmorata; Ms = Micropterus salmoides; Md = Micropterus dolomieu; Ts = Tilapia sparrmanii; Cg = Clarias gariepinus*).

	Kwa-Zunga		**Blindekloof**		**Chaseskloof**		**Fernkloof**		**Waterkloof**		**Nounekkloof**		**Vyeboomkloof**	
	A	B	U	L	U	L	U	L	U	L	U	L	U	L
Pa	0	1	1	1	1	1	1	1	1	1	1	1	0	0
Bp	0	1	0	1	0	0	0	0	0	0	0	0	0	1
Sc	0	1	1	1	1	1	1	1	1	1	0	1	1	1
Gc	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Am	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Ama	0	1	1	1	0	0	0	0	0	0	0	0	0	0
*Ms**	1	1	0	1	0	1	0	0	0	0	0	0	0	0
*Md**	1	1	0	1	0	1	0	0	0	0	0	0	0	0
*Ts**	0	1	0	1	0	0	0	1	0	0	0	0	0	0
*Cg**	0	1	1	1	0	0	0	1	0	1	0	0	0	0

The CA identified three distinct assemblages (native: *P. afer* and *S. capensis*; mixed: *B. pallidus*, *G. callidus*, *C. gariepinus* and *T. sparrmanii*; centrarchid: *M. dolomieu* and *M. salmoides*) explaining 54.1% of the variance in the species data on the first two axes (Table 3.5, Figure 3.9). The *P. afer* and *S. capensis* assemblage had high association with the *B. pallidus*, *G. callidus*, *C. gariepinus* and *T. sparrmanii* assemblage, but no association with the

M. dolomieu or *M. salmoides* assemblage (Figure 3.9). The CCA explained 70.3% of the overall species-environment relation on the first two axes (Table 3.5). The CCA’s explanatory variables (altitude, surface area, temperature and pH) significantly accounted for 27.9% of the species-environmental variance, leaving 72.1% unexplained (Monte Carlo permutation $P < 0.05$, of both the first axis and trace) (Table 3.5). The partitioning of the CCA indicated that the physical variables explained a significant amount of variance (altitude and surface area; 16.5%), while the variance explained by the physico-chemical (pH and temperature; 13%) variables on their own was insignificant, with a shared variance of 1.6% (Table 3.5, Figure 3.10. A and B).

Table 3.5 Summary statistics from Correspondence Analysis (CA, species data only) and Canonical Correspondence Analysis (CCA, species and environmental data) showing the contribution of species and environmental variation to overall assemblages for the distribution data from the headwater tributary streams of the Kwa-Zunga River within the Groendal Wilderness Area. Only environmental variables with a significant contribution to assemblage variation are listed.

Statistic	**Axis 1**	**Axis 2**	
Correspondence Analysis			
Eigenvalue	1.00	0.80	
Cumulative percentage variance of species data	28.5	51.4	
Canonical Correspondence Analysis			
Statistic			
Eigenvalue	0.40	0.31	
Species–environment correlation	0.64	0.65	
Cumulative percentage variance of species data	11.3	20.0	
Cumulative percentage variance of species-environment relation	39.6	70.3	
Weighted correlations			
Altitude	0.47	-0.27	
Surface Area	0.22	0.11	
Temperature	-0.07	0.14	
pH	0.29	0.53	
Total inertia			3.51
Sum of all canonical eigenvalues (full CCA)			1.00
Canonical eigenvalue (physical)			0.58
Canonical eigenvalue (physico-chemical)			0.46

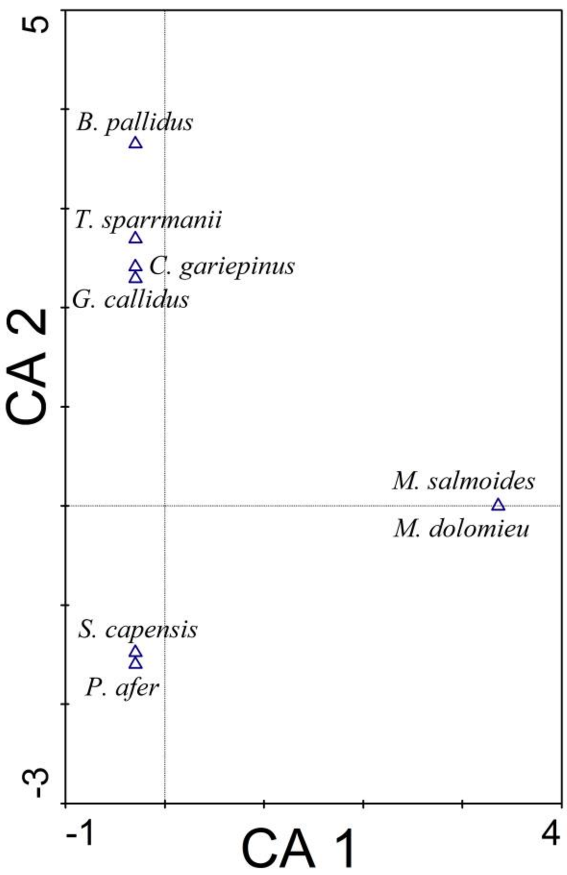


Figure 3.9 Correspondence Analysis ordination biplot of the overall species data from the headwater tributary streams of the Kwa-Zunga River within the Groendal Wilderness Area.

For the partial CCA (physical variables), CC Axis 1 suggested an association between *M. salmoides* and *M. dolomieu* with altitude whereas *P. afer* and *S. capensis* were consistent throughout a range of altitudes (Figure 3.10). The CC Axis 2 suggested a strong positive association between *B. pallidus*, *T. sparrmanii*, *G. callidus* and *C. gariepinus* with pool surface area, with *P. afer* and *S. capensis* showing little preference (Figure 3.10). For the physico-chemical variables, CC Axis 1 suggested an association between *B. pallidus*, *T. sparrmanii*, *G. callidus* and *C. gariepinus* with temperature and CC Axis 2, suggested that *M. dolomieu* was associated with pH, whereas *P. afer, S. capensis* and *M. salmoides* distributions were consistent with a range of temperature and pH conditions.

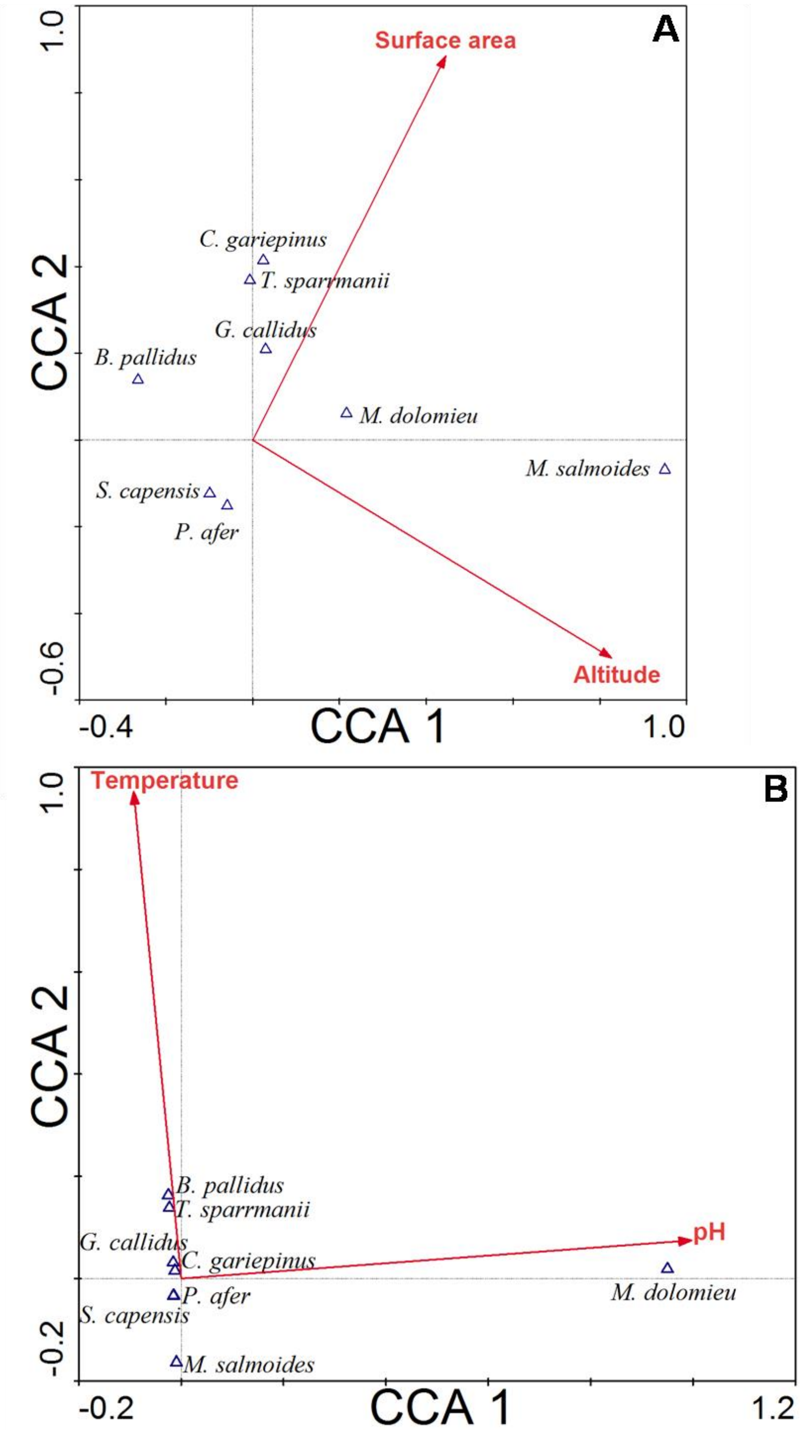


Figure 3.10 Canonical correspondence ordination biplot of species and environmental variables for: (A) Physical explanatory variables after partialling out the effect of physico-chemical variables; (B) Physico-chemical variables after partialling out the effect of physical variables for the overall distribution data of fishes from headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area.

3.3.1.5 Post-flood distribution

The PCA identified two assemblages, one strictly native (*P. afer* and *S. capensis*) and the other mixed (*B. pallidus, T. sparrmanii, G. callidus, C. gariepinus, A. mossambica, A. marmorata, M. dolomieu*), explaining 59.6% of the variation in species data on the first two axes (Table 3.6, Figure 3.11 A).

Table 3.6 Summary statistics from Principal Components Analysis (PCA) and Redundancy Analysis (RDA) for species presence/absence data showing the contribution of species and environmental variation to assemblages from the Blindekloof stream, a headwater tributary of the Kwa-Zunga River within the Groendal Wilderness Area. Only environmental variables with a significant contribution to assemblage variation are listed.

Statistic	**Axis 1**	**Axis 2**	
Principal Component Analysis (presence/absence)			
Statistic			
Total sum of squares in species data			52.91
Total standard deviation in species data TAU			0.33
Eigenvalue	0.404	0.165	
Cumulative percentage variance of species data	40.4	56.9	
Redundancy Analysis (presence/absence)			
Statistic			
Eigenvalue	0.171	0.041	
Species–environment correlation	0.775	0.534	
Cumulative percentage variance of species data	19.5	24.2	
Cumulative percentage variance of species-environment relation	77.7	96.3	
Weighted correlations			
Volume	0.376	0.369	
Reach	-0.603	0.335	
Habitat	0.491	0.204	
Canonical eigenvalue			0.22

The RDA explained 96.3% of the species environment relation on the first two axes (Table 3.6). The RDA explanatory variables (stream reach, volume, habitat diversity) significantly accounted for 22% of the species-environmental variance, leaving 78% unexplained (Monte Carlo permutation P <0.05) (Table 3.6, Figure 3.11 B). On the first axis there were strong positive associations between *B. pallidus, T. sparrmanii, G. callidus, C. gariepinus, A. mossambica, A. marmorata* and *S. capensis* with the variables habitat diversity and pool volume and a negative association with stream reach. *Micropterus dolomieu* was positively associated with pool volume. *Pseudobarbus afer* was ubiquitous throughout the sampled streams and not strongly associated with any of the investigated variables.

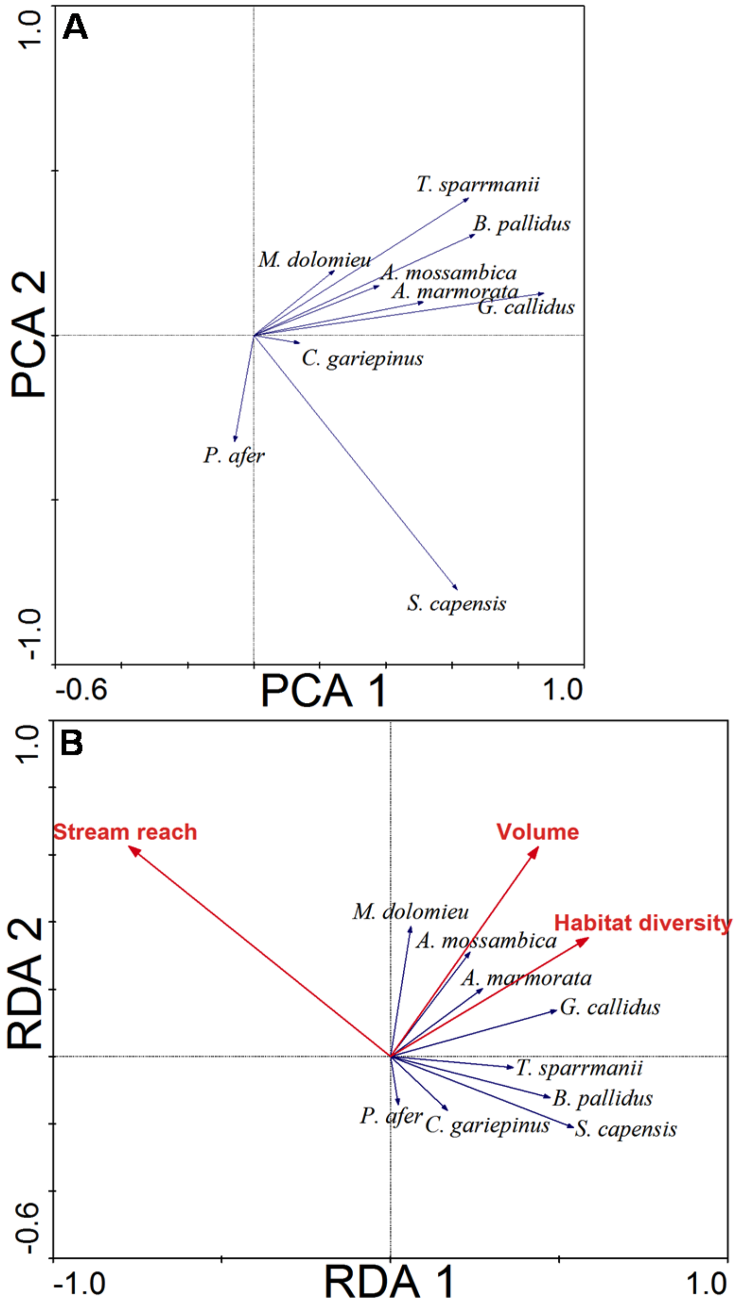


Figure 3.11 (A) Principal Components Analysis (PCA) ordination biplot of species presence/absence data from 54 sites on the Blindekloof, Fernkloof and Waterkloof streams; (B) Redundancy Analysis ordination biplot of species and environmental variables for abundance data (density: fish m^{-3}) from 38 sites on the Blindekloof stream, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area.

3.3.1.6 Impacts of floods on *P. afer*

The distribution and abundance of fishes from the Fernkloof and Waterkloof (water quality summarised in Table 3.7) streams pre- and post-floods are summarised in Table 3.8. Three species were shared between the Fernkloof and Waterkloof streams, two native (*P. afer*, *S. capensis*) and the non-native *C. gariepinus*. An additional non-native, *T. sparrmanii*, was only recorded in the Fernkloof stream. The distributional range of *P. afer* or *S. capensis* in the Fernkloof and Waterkloof streams did not change after the major flood (Table 3.8). Some notable changes were however observed for the non-native species. Prior to the flood, a single *C. gariepinus* was only recorded in the lower reaches of the Fernkloof stream, but was absent after the flood, however, another non-native *T. sparrmanii* was recorded at a single site. In the Waterkloof stream after the flood, one *C. gariepinus* was recorded for the first time. The relative abundance of *P. afer* was generally higher after the flood. There were no significant pre- and post-flood longitudinal or interactive effects (stream reach: upper and lower reaches; disturbance: pre- and post-flood) in density for *P. afer* in the Waterkloof stream (pre-flood mean ± se: 1.24 ± 0.33 fish m^{-3}; post-flood: 2.43 ± 0.40 fish m^{-3}).

There was, however, a significant increase in post-flood *P. afer* density in the Fernkloof stream (pre-flood: 1.33 ± 0.49 fish m^{-3}; post-flood: 6.53 ± 2.12 fish m^{-3}) (Figure 3.12, Table 3.9). The frequency of occurrence and length distributions for *P. afer* pre and post flood are summarised in Table 3.10 and Figure 3.13. The frequency of occurrence for juvenile (<40 mm) and adult *P. afer* was independent of stream reach and they were subsequently grouped by stream (Table 3.10). The frequency of occurrence of juvenile and adult *P. afer* was independent of flooding for the Waterkloof (pre- versus post-flood: $\chi^2 = 0.027$, df = 1, $P = 0.87$) and Fernkloof streams (pre- versus post-flood: $\chi^2 = 0.044$, df = 1, $P = 0.83$) (Table 3.10).

In the Waterkloof stream *P. afer* size distributions were similar between stream reaches and pre- and post-flooding (pre-flood lower mean, range: 43.7, 22-74 mm FL; pre-flood upper: 40.7, 16-71 mm FL; post-flood lower: 42.8, 21-67 mm FL; post-flood upper: 47.2, 28-81 mm FL). In the Fernkloof stream the mean length of *P. afer* was greater in the lower reaches but similar before and after the flood (pre-flood lower: 53.0, 11-96 mm FL; pre-flood upper: 43.0, 24-72 mm FL; post-flood lower: 52.0, 30-87 mm FL; post-flood upper: 46.3, 21-100 mm FL) (Figure 3.13).

The distributions of fishes in the Blindekloof stream pre- and post-flood are summarised in Table 3.11. The native *P. afer*, *S. capensis*, *G. callidus*, *A. marmorata* and *A. mossambica* were distributed throughout the stream, while *B. pallidus* was limited to lower stream reaches. The non-native *T. sparrmanii* was widespread in the lower stream reaches while *C. gariepinus* was only recorded at a single site and *M. dolomieu* in the middle reaches at three sites. In the Blindekloof stream some major distribution changes were noted after the flood. The native *P. afer*, *G. callidus* and *S. capensis* dispersed downstream following the flood and were recorded from sites where they were previously absent. Similar changes were not noted for non-native fishes; however, *M. salmoides* was not recorded post-flood. Prior to the flood, native fishes did not co-occur with centrarchids, but after the flood *P. afer*, *G. callidus* and *S. capensis* were sampled from pools containing *M. dolomieu.*

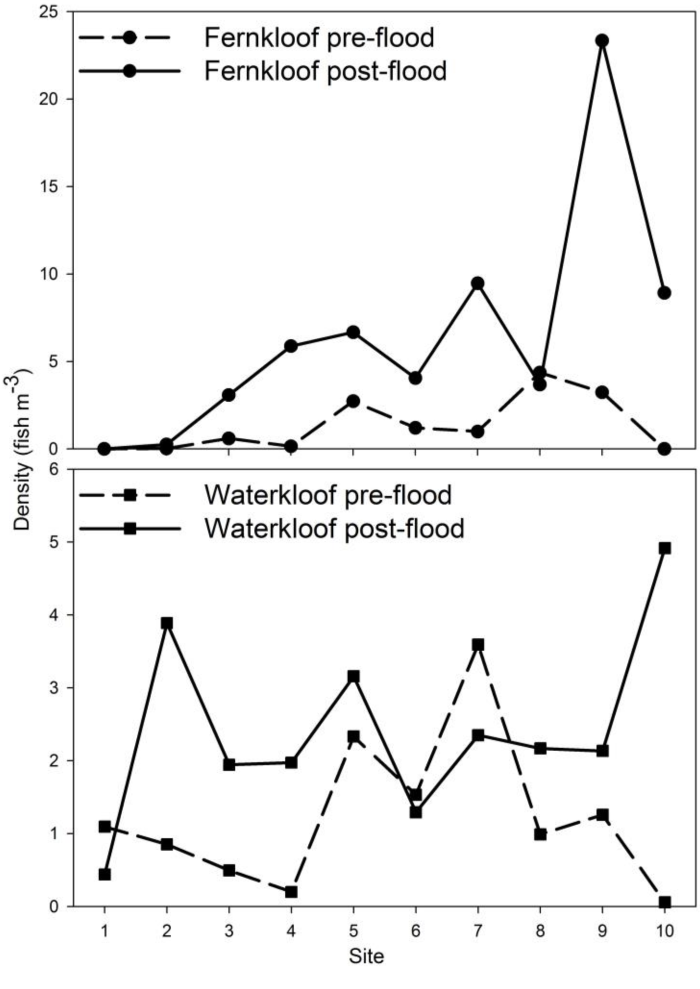


Figure 3.12 Pre- and post-flood longitudinal abundance trends for *Pseudobarbus afer* from the Waterkloof and Fernkloof streams, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area.

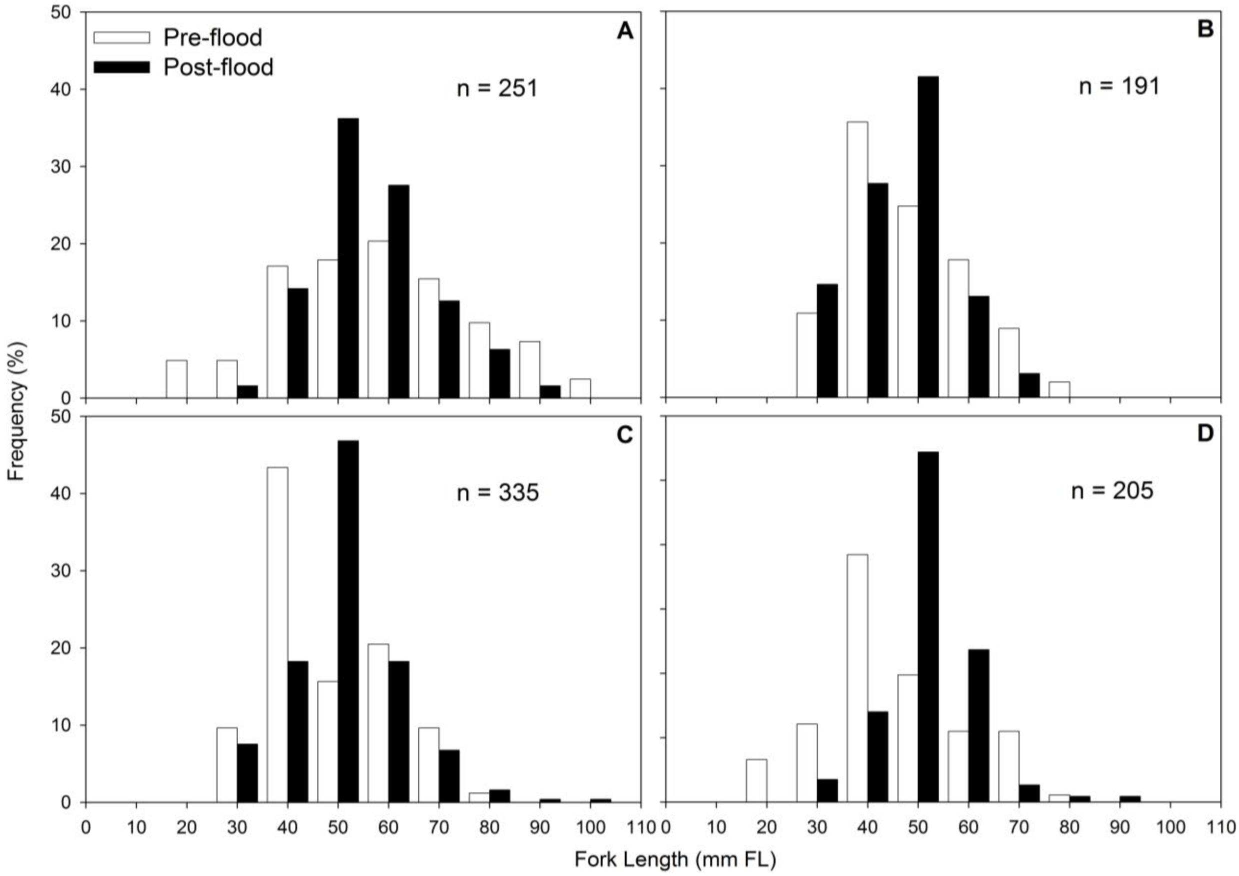


Figure 3.13 Pre- and post-flood length frequency distributions for *Pseudobarbus afer* from the upper and lower reaches of the Fernkloof (A = lower; C = upper) and Waterkloof (B = lower; D = upper) streams, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area.

Table 3.7 The pre- (Prfl) and post-flood (Pofl) water quality variables (Temp = temperature; Turb = turbidity; Cond = conductivity), from site 1 in the lower reaches to site 10 in the upper reaches of the Fernkloof and Waterkloof streams, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area (*non-native species).

	Fernkloof						**Waterkloof**					
	Temp (°C)		**Turb (NTU)**		**Cond (μs/cm)**		**Temp (°C)**		**Turb (NTU)**		**Cond (μs/cm)**	
Site	Prfl	Pofl	Prfl	Pofl	Prfl	Pofl	Prfl	Pofl	Prfl	Pofl	Prfl	Pofl
1	16.5	21	0.5	0.17	302	230	17.4	15.9	0.41	0.3	267	212
2	16.2	21.1	0.43		303	221	17.6	16	0.63	0.43	263	214
3	16.2	21.4	0.28	0.15	301	222	17.6	16	0.54	0.47	257	209
4	15.8	19.6	0.29	0.19	301	222	17.9	15.7	0.44	0.53	259	209
5	16.2	19.2	0.35	0.41	301	220	18.2	15.7	0.28	0.21	250	209
6	16.1	19.2	0.21	0.34	296	218	15.2	16.1	0.18	0.53	256	207
7	16.1	16.8	0.37	0.32	304	214	15.5	15.9	0.47	0.6	250	206
8	15.7	16.5	0.43	0.17	291	219	13.7	16.5	0.23	0.33	245	200
9	15.4	16.4	0.2	0.28	275	215	14.3	16.8	0.32	0.34	254	202
10	15.4	17.3	0.18	0.22	260	213	14	17.3	0.31	0.33	234	216

Table 3.8 The stream zones (Lower reaches, Upper reaches), sites (10 sites), distribution, density (fish m^{-3}), relative abundance of fishes by site (%) pre (Prfl) and post flood (Pofl), from site 1 in the lower reaches to site 10 in the upper reaches of the Fernkloof and Waterkloof streams, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area (*non-native species).

	Pseudobarbus afer		***Sandelia capensis***		***Clarias gariepinus***		***Tilapia sparrmanii***	
Fernkloof	Prfl	Pofl	Prfl	Pofl	Prfl	Pofl	Prfl	Pofl
1	0	0.10(10)	0.15(100)	0.93(90)	0	0	0	0
2	0.03(33)	0.36(68)	0.03(33)	0.20(32)	0.03(33)	0	0	0
3	0.38(73)	4.62(86)	0.14(27)	0.77(14)	0	0	0	0
4	1.22(91)	7.67(88)	0.12(27)	1.02(12)	0	0	0	0
5	2.15(80)	11.11(88)	0.53(20)	1.48(12)	0	0	0	0
6	1.20(75)	4.40(96)	0.40(25)	0	0	0	0	0.17(4)
7	0.99(71)	9.87(100)	0.39(29)	0	0	0	0	0
8	4.36(100)	4.60(100)	0	0	0	0	0	0
9	3.23(100)	27.89(100)	0	0	0	0	0	0
10	0	11.25(100)	0	0	0	0	0	0
Waterkloof								
1	0.40(100)	0.44(75)	0	0.15(25)	0	0	0	0
2	0.39(100)	5.21(96)	0	0.21(4)	0	1 observed	0	0
3	0.44(71)	2.55(95)	0.18(29)	0.12(5)	0	0	0	0
4	0.40(100)	2.23(96)	0	0.09(4)	0	0	0	0
5	0.10(89)	3.16(91)	0.12(11)	0.30(9)	0	0	0	0
6	1.53(78)	2.59(67)	0.44(22)	1.29(33)	0	0	0	0
7	3.59(85)	2.69(100)	0.63(15)	0	0	0	0	0
8	0.99(87)	3.47(94)	0.15(13)	0.22(6)	0	0	0	0
9	1.26(100)	5.40(100)	0	0	0	0	0	0
10	0.06(100)	5.60(100)	0	0	0	0	0	0

Table 3.9 A summary of pre and post flood longitudinal (upper and lower stream reaches) and disturbance (per and post flood) main effects ANOVA results for *Pseudobarbus afer* density (fish m^{-3}) from the Waterkloof and Fernkloof streams, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area.

	Df	**Ms**	***F***	**p**
Fernkloof stream				
Site	9	28.19	1.46	>0.05
Pre-/Post-	1	135.44	7.01	<0.05
Site*Pre-/Post-				
Error	9	19.33		
Waterkloof stream				
Site	9	1.16	0.72	>0.05
Pre-/Post-	1	7.04	4.39	>0.05
Site*Pre-/Post-				
Error	9	1.60		

Table 3.10 The frequency of occurrence (% of sites containing *P. afer*) of juvenile (<40 mm) and adult (>40 mm) *Pseudobarbus afer* pre- and post-flooding separated in the lower and upper reaches of the Fernkloof and Waterkloof streams, headwater tributaries of the Kwa-Zunga River within the Groendal Wilderness Area.

	Juvenile (<40 mm)	**Adult (>40 mm)**	**Mean length (range) mm FL**
Waterkloof stream pre-flood			
Lower reaches	100%	100%	43.7 (22-74)
Upper reaches	100%	100%	40.7 (16-71)
Waterkloof stream post-flood			
Lower reaches	100%	100%	42.8 (21-67)
Upper reaches	80%	100%	47.2 (28-81)
Fernkloof stream pre-flood			
Lower reaches	40%	60%	53.0 (11-96)
Upper reaches	80%	80%	43.0 (24-72)
Fernkloof stream post-flood			
Lower reaches	80%	80%	52.0 (30-87)
Upper reaches	100%	100%	46.3 (21-100)

Table 3.11 Pre- (PRFL = shaded dark grey) and post-flood (POFL = shaded light grey) distribution of fishes in the Blindekloof stream from site 1 at the confluence with the Kwa-Zunga River to site 39 at the limit of fish distribution in the upper reaches (*x* = species not recorded; 1 = species recorded; √ = site sampled; - = site not sampled) (* = non-native fishes).

Sample site		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Pre-flood		√	-	√	√	√	-	√	√	√	-	-	-	-	-	-	√	-	-	-	-	-	-	√	-	-	√	-	-	-	-	√	-	-	-	-	-	-	-	√
Post-flood		-	√	√	√	√	√	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
P. afer	PRFL	x	-	x	x	x	-	x	1	x	-	-	-	-	-	-	1	-	-	-	-	-	-	x	-	-	x	-	-	-	-	1	-	-	-	-	-	-	-	1
	POFL	-	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	x	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B. pallidus	PRFL	1	-	1	1	x	-	1	1	x	-	-	-	-	-	-	x	-	-	-	-	-	-	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	1	1	1	x	-	1	1	1	1	x	1	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
G. callidus	PRFL	1	-	x	x	x	-	x	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-	x	-	-	x	-	-	-	-	1	-	-	-	-	-	-	-	x
	POFL	-	1	x	1	1	1	-	1	1	1	1	1	1	1	x	1	1	1	1	1	x	1	1	x	1	1	1	x	1	1	x	x	x	x	x	x	x	x	x
S. capensis	PRFL	x	-	x	x	x	-	x	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	-	-	-	1	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	x	1	1	1	1	1	1	1	1	1	1	1	x	x	x	x	x
*T. sparrmanii**	PRFL	1	-	1	1	x	-	1	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	x	1	1	x	-	1	1	1	1	1	1	x	x	x	x	x	x	x	x	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
*M. salmoides**	PRFL	x	-	x	x	x	-	x	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-	1	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
*M. dolomieu**	PRFL	x	-	x	x	x	-	x	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-	1	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1	x	x	1	1	x	x	x	x	x	x	x	x	x	x	x	x
*C. gariepinus**	PRFL	1	-	x	x	x	-	x	1	x	-	-	-	-	-	-	x	-	-	-	-	-	-	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	x	x	x	x	-	x	x	1	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
A. marmorata	PRFL	x	-	x	x	x	-	x	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	x	x	x	x	-	1	x	x	x	x	1	x	x	x	1	x	x	x	x	1	x	x	x	1	x	1	x	x	x	x	x	x	x	x	x	x	x
A. mossambica	PRFL	x	-	x	x	x	-	x	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-	x	-	-	x	-	-	-	-	x	-	-	-	-	-	-	-	x
	POFL	-	x	x	x	x	x	-	1	1	x	x	x	x	x	1	x	x	x	x	x	1	x	1	x	x	1	1	x	x	x	x	1	x	x	x	x	x	x	x

3.4 Discussion

This study demonstrates that under base flow conditions, stream reaches invaded by centrarchids in the Kwa-Zunga River headwater streams were devoid of all small native fishes, a finding consistent with other headwater stream centrarchid invasions (Moyle et al. 2003; Shelton et al. 2008; Woodford et al. 2005). In the headwaters of the Swartkops River system, the three distinct assemblages identified under base flow conditions (native = *P. afer, S. capensis*; mixed = *B. pallidus, G. callidus, T. sparrmanii, C. gariepinus*; centrarchid = *M. salmoides, M. dolomieu*) were expected as biotic (predation, competition) and abiotic features of streams over any spatial scale (stream reach or stream order) typically result in variable species composition of fish assemblages (Angermeier and Winston 1999; Jackson et al. 2001; Smith and Kraft 2005). There was considerable overlap between the mixed and native assemblages, but neither co-occurred with the centrarchid assemblage. Although there were associations between the three assemblages and both physical (surface area and altitude) and physico-chemical (pH and temperature) factors, separation on an environmental gradient did not adequately explain the absence of both the mixed and native assemblage in centrarchid invaded zones, and it is postulated to be controlled by predation (Godinho and Ferreira 2000; MacRae and Jackson 2001; Schlosser 1987).

Piscivory can be a major contributor to the variation and structure of stream fish assemblages (Jackson et al. 2001; Moyle et al. 2003). The predatory nature of *M. dolomieu* and *M. salmoides* has resulted in total extirpation of all fishes from centrarchid invaded zones. Similar results have been reported from other studies on invaded river systems where their introduction has resulted in two distinct assemblages, one dominated by small native fishes and the other by large-bodied centrarchids (Godinho and Ferreira 2000; MacRae and Jackson 2001). In centrarchid-dominated assemblages, the number of other species present is also directly related to habitat complexity and heterogeneity, providing refuge from predation (MacRae and Jackson 2001; Moyle et al. 2003; Schlosser 1987; Tonn and Magnuson 1982). Kwa-Zunga headwater tributaries are episodic and predominantly isolated on the surface. During dry periods (> 95% of the time) native species are therefore confined with predatory centrarchids in invaded pools. For fishes inhabiting episodic environments, their persistence relies on refugia not prone to desiccation (Magoulick and Kobza 2003), but if these are occupied by invasive predatory fishes (as was the case on the Blindekloof stream), the persistence of the species is severely compromised. This has been demonstrated in systems with seasonal rainfall fluctuations, such as the Cosumnes River, California, USA (Moyle et al. 2003) and the Guadiana River Basin in Portugal (Collares-Pereira et al. 1998), where seasonal droughts results in the extirpation of native fishes from isolated pools inhabited by centrarchids.

The dispersal of fish after the flood in June 2011 resulted in the ‘meltdown’ of the distinct assemblages observed during base flow conditions. Although there was still separation between the mixed assemblage and the native assemblage on an environmental gradient, contrary to what was found in a stable state situation, *M. dolomieu* now co-occurred with other species. The role of environmental stochasticity in restructuring stream ecosystems is well recognised (Bernardo et al. 2003; Moyle and Light 1996; Schlosser 1991). This indicates that invasion in the Swartkops River headwater tributaries most probably experience dynamic equilibrium, as proposed by Huston (1979), where persistence is not defined by the competitive ability of a species, but by the influence of the environment on the outcome of species interactions (Poff et al. 1997; Resh et al. 1988; Schlosser 1991). It therefore follows that invasive impacts are dynamic, and pools inhabited by centrarchids act as demographic sinks (Dias 1996; Pulliam and Danielson 1991). Native fishes re-disperse into centrarchid-invaded stream reaches during periods of high flow. These are then depleted over time and return to baseline conditions where invaded reaches are again devoid of all native fishes. The limited periods of prolonged flow, however, preclude their migration back upstream into headwater refugia, as typically the mainstream Kwa-Zunga will also act as a demographic sink (Dias 1996; Pulliam and Danielson 1991) for individuals dispersed from the headwater streams. Following high flows, these streams are rapidly isolated (streams flow for approximately 4 days following heavy rainfall), effectively trapping *P. afer* in invaded stream reaches, making them susceptible to predation.

The invasion of the Kwa-Zunga River headwater tributaries was casual/sporadic, whereby centrarchids invade upstream from mainstream habitats but have not established self-replacing populations in the headwater streams. Their presence depends on repeated introduction of propagules/individuals from an established mainstream source (Richardson et al. 2000). Invasion is a dynamic process and demographic and environmental stochasticity often result in fluctuating invasive populations, either casual or established (Fausch 2008;

Lawrence et al. 2012; Sax and Brown 2000). Invasions also involve interaction between the invasibility of the recipient ecosystem and the ability of invaders to reach these areas (Leung and Mandrak 2007). The episodic nature of Kwa-Zunga River headwater streams, alternating between flowing and connected, and a disconnected state, creates an abiotic filter (Poff 1997) which may affect invasibility by limiting propagule pressure from mainstream source populations. Another factor contributing to the limited establishment success may be the pristine nature of the catchment and the resultant maintenance of natural flow regimes (Moyle & Light 1996; Moyle et al. 2003), as the major flooding in June 2011 resulted in *M. salmoides* being flushed out of the Blindekloof stream. The disappearance of *M. salmoides* from the Blindekloof stream following the flood is not surprising as the species prefers slow-flowing lentic ecosystems (*sensu* Warren 2009). The finding is consistent with results from other studies on stream invasions showing that abiotic resistance (Bernardo et al. 2003; Moyle and Light 1996; Skelton 1993) limits the establishment of *M. salmoides* in episodic streams. In the Guadiana River basin on the Iberian Peninsula in Portugal, the abundance of centrarchids, including *M. salmoides*, was directly related to extreme flow periods, with lowest abundance following floods (50% decrease) and highest abundance during stable low flow conditions (Bernardo et al. 2003).

Despite the limited length of headwater streams where centrarchids have invaded and extirpated *P. afer* (~ 12%), the presence of predatory centrarchids in the mainstream and the lower- to middle-reaches of the Blindekloof and Chaseskloof streams has created an impenetrable barrier to between-stream (all six surveyed streams are isolated as a result) and, in some cases (Blindekloof), within-stream dispersal. This has created numerous isolated and fragmented headwater stream *P. afer* populations that are confined to stream reaches not invasible by centrarchids. The consequences of isolation and fragmentation include a decreased resistance to catastrophic events, and genetic bottlenecks due to the loss of migration routes (Fausch et al. 2009). Genetically isolated populations can have lower viability and fecundity due to inbreeding depression, ultimately resulting in the extinction of that population (Couvet 2002). Stream habitats are also prone to stochastic environmental effects such as droughts or floods, and a loss of connectivity between habitats limits opportunity for recolonisation following such events (Fausch et al. 2009).

Flood-related disturbances typically elicit a variety of responses by fishes in stream environments, the nature of which are related to the predictability, magnitude/intensity and duration of the event (Lytle and Poff 2004), as well as the specific adaptations of the fishes to the abiotic conditions of that environment (Franssen et al. 2006; Lytle and Poff 2004). Results from this study exemplify this situation, with native and non-native species showing varied responses to flood disturbance. In the Fernkloof and Waterkloof streams, *P. afer* demonstrated resilience to a major flooding event, most probably related to their evolution in stochastic environments (Bernardo et al. 2003; Dolloff et al. 1994; Magalhaes et al. 2003; Pires et al. 2008). In both small streams there were no longitudinal trends in abundance before or after the flood, but overall abundance post-flood in the Fernkloof stream was higher. This may have been the result of the inability to sample the exact sites before and after the flood due to some pools filling up with flood deposition. The nearest pool to the pre-flood site was sampled, which may have resulted in sampling a refuge pool where post-flood abundances were high due to collection of fishes from that stream segment. Good recruitment during the 2010/2011 spawning season might also have led to the increase in abundance, as during this period rainfall was higher than in the preceding two spawning seasons. Increased recruitment during periods of good flow was recorded by Franssen et al. (2006) for intermittent prairie stream fishes in north-eastern Kansas, USA, due to an increase in available habitat. However, the *P. afer* length frequency data (Figure 3.13) from the Fernkloof stream does not support this and no noticeable recruitment pulse was observed after the flood.

The vulnerability of stream fishes to flood disturbance is not always clearly evident from overall abundance trends, but in some cases certain life history stages of particular species may be more vulnerable to disturbance than others (Gasith and Resh 1999; Letcher and Terrick 1998; Lytle and Poff 2004). In New England streams in the USA, a massive localized flood caused an age-0 year-class failure in brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta*, and a large decrease in abundance of Atlantic salmon *Salmo salar* (Letcher and Terrick 1998). Similar results have been recorded for other species after catastrophic flood events (Dolloff et al. 1994; Harvey 1987; Matthews 1986; Nislow et al. 2002).

This was not the case for *P. afer* as there were no significant changes in the occurrence of either juvenile or adult *P. afer* in the Waterkloof and Fernkloof streams from pre-flood occurrences. Both life history stages were distributed throughout both streams from their confluence with the mainstream Kwa-Zunga River in the lower reaches, to the limit of fish distribution in the upper reaches. The susceptibility of juvenile fishes to flood displacement and mortality has been documented to decrease rapidly with a small increase in length (Harvey 1987). *Pseudobarbus afer* may show similar trends in changing vulnerability with size. In the nearby Wit River, a headwater tributary of the Gamtoos River system, Cambray (1994) reported that late free embryos and early larvae drift out of the areas where they were spawned. This passive dispersal was also recorded during this study (see Chapter 4), and data indicated that *P. afer* <15 mm TL disperse downstream during high flows, resulting in susceptibility to displacement during floods. The flooding event took place 3-5 months after the peak spawning season of *P. afer* (Cambray 1994), allowing time for young of the year fish to grow out of the most vulnerable life history stages. However, data suggests flood facilitated dispersal of adult *P. afer* from the Blindekloof stream. *Pseudobarbus afer* increased their range downstream, moving 1.6 km further in 2011 than recorded in 2010 (Chapter 2). Notably, *P. afer* also dispersed into pools invaded by *M. dolomieu* that were previously devoid of native fishes. Other native species also showed longitudinal shifts in distribution after the flood.

In conclusion, *P. afer* displayed resilience to a major flooding event most probably related to their evolution in river systems characterised by environmental stochasticity (Dolloff et al. 1994; Magalhaes et al. 2003; Pires et al. 2008). Indications were that responses to the flood were species- and stream-specific. The distribution of centrarchids in the Kwa-Zunga River headwater tributary streams, and the vulnerability of *M. salmoides* to displacement by floods, indicate that current environmental variability is in effect managing centrarchid invasions and providing protection for the imperilled native species. The long-term persistence of *P. afer* in the isolated headwater refugia is therefore not threatened by flood disturbances of the observed magnitude and intensity.

There is a need to identify factors affecting population viability for imperilled species (Dunham et al. 1997). Understanding headwater populations requires viewing a system in a continuous way to understand how processes at different scales interact and influence stream fishes and their habitat (Fausch et al. 2002). While this study documented impact at different spatial (system and stream) and temporal scales (base flow and high flow), it has also highlighted the importance of understanding the contribution of landscape level factors such as dispersal and environmental stochasticity (Labbe and Fausch 2000) to the persistence of *P. afer* populations. The consequences of invasion by centrarchids isolating and fragmenting *P. afer* headwater stream populations also need to be investigated further. This would enhance our knowledge of the process that results in the observed pattern where non-native predators result in extirpation of native fishes. Chapter 4 will therefore investigate finer scale temporal impacts of non-native fishes on population processes of native fishes, such as adult dispersal and juvenile recruitment, to further understand mechanisms responsible for impacts.

CHAPTER 4: Invasive impacts of *Micropterus dolomieu* on a small native stream fish

4.1 Introduction

The greatest threat to biodiversity worldwide is the fragmentation and destruction of natural habitats (Hanski 1998; Moilanen and Hanski 1998). For terrestrial biota, fragmented populations can maintain connectivity via numerous alternative pathways in a two dimensional environment. Stream fishes, however, are particularly sensitive to fragmentation as connectivity between fragmented populations relies on a single dimension along the stream network (Cote et al. 2009). Despite this, little information exists on the importance of connectivity between populations in aquatic systems (Cote et al. 2009). Although stream and river networks have been extensively fragmented by impoundments, weirs and water abstraction, a contributory driver of this fragmentation has been cited as invasion by non-native fishes (Crowl et al. 1992; Moyle et al. 2003). Strong evidence for this has been documented in Australasia, where non-overlapping distributions between native galaxiids and introduced salmonids, due to predation and competition, result in highly disjunct native species ranges (Crowl et al. 1992). Similar results have been recorded in the Cosumnes River, California, USA, where native fishes have been extirpated by non-natives, and native fishes mostly persist above invasion barriers (Moyle et al. 2003). There is, however, a general lack of understanding on how this impacts on the demographic processes of native fishes, such as dispersal and recruitment.

According to the restricted movement paradigm, stream fishes are relatively sedentary and are able to complete their life cycles within a small stretch of stream (20-50 m) (Gerking 1959). However, while some species may conform to this paradigm, extensive data collected for stream fishes now demonstrate that numerous species-specific movement characteristics are exhibited (Labbe and Fausch 2000; Rodriguez 2002; Smithson and Johnston 1999). For example, within-species behavioural polymorphisms have been observed, with some individuals displaying a greater propensity to move than others (Smithson and Johnston 1999). For instance, stream fishes in the Ouachita highlands showed variable movement patterns, with the majority being recaptured at the capture site, while between 12% and 33% of individuals, depending on species, moved greater distances (Smithson and Johnston 1999). This is most probably due to the spatial and temporal heterogeneity of stream habitats resulting in stream fishes exhibiting complex life cycles and habitat use patterns (Schlosser and Angermeier 1995). Increasingly, the important role of connectivity between complimentary feeding and breeding habitats in maintaining population persistence has also been recognised (Labbe and Fausch 2000). Stream fishes exhibit ontogenetic changes in distributions, with certain life history stages requiring different conditions than others for favourable growth and survival (Grossman and Freeman 1987; Schlosser 1991). Not only can fragmentation potentially isolate complementary habitats, but long term persistence may also depend on recolonisation ability between populations in the case of stochastic events such as droughts or floods which result in local extinction of fishes (Lowe and Likens 2005).

In South Africa, the endpoint of invasions by predatory fishes into headwater streams has been well documented. Numerous examples exist where these invasions typically lead to complete extirpation of native fishes in invaded zones, and headwater stream fishes are increasingly being isolated in small fragmented headwater refuges due to invasion by non-native fishes (Clark et al. 2009; Weyl et al. 2013; Woodford et al. 2005). One such case is the Swartkops River system, where all six surveyed populations of *P. afer* were isolated in headwater refugia due to the presence of predatory centrarchids further downstream and in mainstream environments (Chapter 2 and 3). Data from two non-invaded Kwa-Zunga tributary reference streams, the Fernkloof and Waterkloof streams, suggest that *P. afer* abundance does not change longitudinally from their upper distribution limit, to their confluence with the mainstream (Chapter 3). This however, is not the case from two invaded streams, the Chaseskloof and Blindekloof streams. In the Chaseskloof stream, *P. afer* were totally absent in the lower stream reaches invaded by centrarchids. In the Blindekloof stream, centrarchids occupy a short segment in the middle reaches where *P. afer* were absent; their abundance is highest in the upper reaches and lowest in the centrarchid-invaded middle reaches (Chapter 3). The mechanism responsible for these observed impacts on *P. afer* distribution and abundance patterns, and the temporal consequences of isolation and fragmentation, however, remain unclear.

Both adult and larval *P. afer* have been documented to disperse downstream during periods of increased flow. Longitudinal downstream movement has been noted for *P. afer* on the Blindekloof stream, as after high flows on the Blindekloof stream (Chapter 3), it was noted that *P. afer* had increased its range by 1.6 km from that found in Chapter 2, and subsequent to the flood *P. afer* was distributed throughout the centrarchid-invaded stream reaches (Chapter 3). Cambray (1994) demonstrated the ability of larval *P. afer* to disperse and noted that approximately five days after hatching; larvae enter the water column and passively disperse downstream. This implies that isolated headwater populations are not confined to specific stream reaches and do in fact exhibit dispersal ability. Low levels of genetic differentiation between all six surveyed stream populations in tributaries of the Kwa-Zunga River concur with evidence of dispersal and historical mixing (Chapter 7). Ecological data therefore suggest that centrarchid invasion may disrupt population processes such as dispersal, between previously connected populations. It also follows that centrarchid impacts on *P. afer* are not static, but may be dynamic in nature depending on the prevailing environmental conditions (Chapter 3).

A high flow period (following the flood described in detail in Chapter 3) resulting in continuous longitudinal connectivity between the upper and lower stream reaches, provided an opportunity to investigate spatio-temporal population dynamics in the Blindekloof stream concurrent to the spawning season of *P. afer* (Cambray 1994). Fifteen representative sites, covering invaded and non-invaded stream reaches from site 1 in the lower reaches, to site 15 in the upper reaches (upper limit of fish distribution), were monitored over a six month period. To provide an understanding of the possible mechanisms responsible for the observed pattern that native and non-native fish do not co-occur under baseline conditions, the following questions were addressed: (1) during high flow periods, do *P. afer* abundances remain lower in centrarchid-invaded sites compared to non invaded reference sites; (2) do *P. afer* abundances decrease over time in invaded zones; (3) does the presence of centrarchids in the middle stream reaches impact on longitudinal population processes such as dispersal or recruitment; (4) what role extended periods of high flow play in the invasion process?

4.2 Materials and Methods

4.2.1 Study Area

The study was conducted on the Blindekloof stream, a headwater tributary of the Kwa-Zunga River, one of two major tributaries of the Swartkops River system within the Groendal Wilderness Area (GWA), Eastern Cape, South Africa (Chapter 3, Figure 3.1).

4.2.2 Field surveys

Following a major flood in 2011, *P. afer*, which had previously not co-occurred with centrarchids, re-colonised invaded stream reaches (described in Chapter 3). Monthly snorkel surveys (November 2011-April 2012) were then undertaken to document possible changes in the range and relative abundance of native fishes in invaded stream reaches. The Blindekloof stream was divided into three invasion states according to the presence/absence of centrarchids (smallmouth bass *Micropterus dolomieu*). Sites 1-5 represented the ‘mixed zone’ (no *M. dolomieu*, but other non-native species present), sites 6-9 the ‘invaded zone’ (*M. dolomieu* present) and sites 10-15 the ‘non-invaded zone’ (only native species present) (Figure 4.1). Despite the episodic nature of the Blindekloof stream, pools are fed by groundwater and so surface area and volume remained fairly constant throughout the study. It was therefore assumed that effort at each monitoring site was also constant and comparisons between snorkel counts would be sufficient to reflect any temporal abundance changes.

Snorkel surveys were conducted using the zigzag method as described in Chapter 3. Pass one was initiated at the tail end of the pool, with the observer swimming upstream, zigzagging to cover as much of the pool as possible. Pass two was a repeat of pass one but in a downstream direction. During each pass, all fish seen were identified to species level, counted, and placed into one of four predetermined length classes (0-15 mm, 15-30 mm, 30-60 mm, >60 mm). To estimate fish length, the snorkeler carried a slate with a ruler attached to it, where the snout and tail of the fish are aligned with the ruler (Cunjak and Power 1986). To avoid measuring the same individual twice, length was estimated only on the first snorkel pass. Two native predatory eels are found in the headwaters of the Swartkops River, the giant mottled eel *Anguilla marmorata* and the longfin eel *Anguilla* *mossambica*. Both eel species are nocturnal and in order to get a representative sample of native predator relative abundance it was necessary to undertake night snorkelling (night snorkelling was omitted from the April 2012 survey due to equipment failure). Identical methods were used during the day and at night except that fish lengths were not estimated at night. A 400 lumen SL4 eLED® dive torch (Underwater Kinetics, California USA) was used for observations. For those sites sampled during the day and at night, snorkelling was undertaken during the same 24 hr period (Thurow and Schill 1996). Night snorkelling commenced a minimum of 45 minutes after sunset.

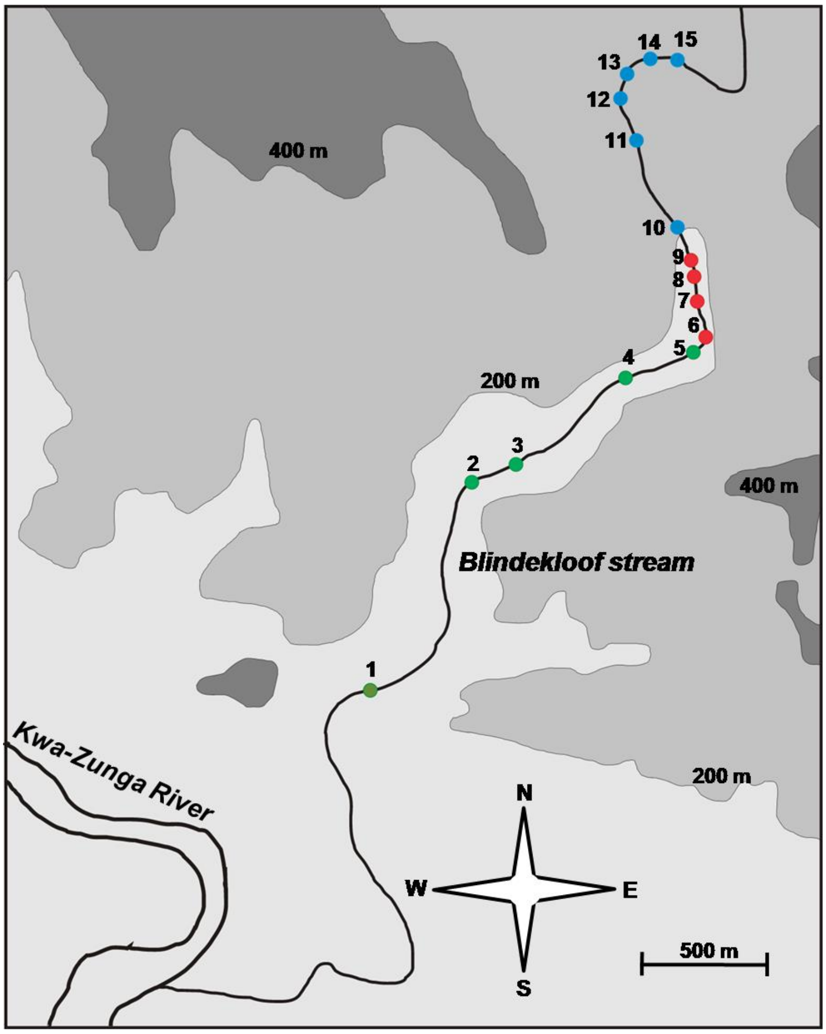


Figure 4.1 Monitoring sites from site 1 in the lower reaches to site 15 in the upper reaches of the Blindekloof stream, a Kwa-Zunga River headwater tributary within the Groendal Wilderness Area. Colours illustrate the three zones surveyed (Green = mixed zone; Red = invaded zone; Blue = non-invaded zone).

During previous field surveys it was noted that the African sharptooth catfish *Clarias gariepinus* was particularly visible as the species hunted along pool margins at night (BRE pers. obs.). In addition to snorkelling transects, spotlighting observations were conducted on the margins of pools as observers were moving between snorkelling sites. All pools sampled by night snorkelling were accessed on foot. This involved walking on the stream bank from the uppermost site (15) to the lowermost site (1) over two consecutive nights. The route followed was consistent over the monitoring period. Spotlighting involved scanning the edges of the pool and recording the number of *C. gariepinus* observed. To track possible temporal shifts in fish distribution, day and night snorkelling and spotlighting surveys were undertaken for five months (November 2011-March 2012).

At each sampling site, temperature, conductivity and pH were measured using a Hanna HI98129 Combo pH and electrical conductivity meter (HANNA Instruments Inc., Woonsocket, USA). Turbidity (NTU) was measured using a Hanna HI 98703 turbidimeter (HANNA Instruments Inc.). In addition, to monitor temporal water thermal regimes, two HOBO Pendant® temperature/light data loggers were placed in the Blindekloof stream (one at the Blindekloof/Kwa-Zunga River confluence and the other at site nine, Figure 4.1) for the period 11/12/2011-09/12/2012.

4.2.3 Data analysis

Fifteen monitoring sites on the Blindekloof stream were investigated over the 6 month monitoring period (mixed zone = 4 sites; invaded zone = 5 sites; non-invaded zone = 6 sites, Figure 4.1) to determine whether *M. dolomieu* would have a significant impact on overall *P. afer* temporal abundance (after flood redistribution, Analysis 1), recruitment (Young of year = YOY *P. afer* = <30 mm (Cambray 1994)) (Analysis 2) and adult abundance (Adult *P. afer* = >30 mm (Cambray 1994)) in invaded and non invaded zones (Analysis 3). Only first-pass snorkelling data were used in the analyses as this allowed for the necessary size class comparison. First-pass data were considered representative as there was a highly significant correlation between the first and second passes (Spearman r = 0.908, *P* <0.001). These data were analysed using Linear Mixed Models in SPSS version 16.0 (IBM, Armonk, NY, USA). Linear Mixed Models have become increasingly popular to analyse longitudinal data with repeated effects as they can include continuous covariates; accommodate designs with missing data; accommodate unbalanced designs; provide the option to choose between various alternative covariance structures and include various random effects (West 2009). Abundance analyses were undertaken using restricted maximum likelihood models in SPSS. Fixed effects for all three analyses were specified as stream zone (mixed zone, invaded zone and non-invaded zone), sample months (1-6) were added as a covariate and site (1-15) was included as a random effect. Analysis 1 overall *P. afer* abundance data (6 months) (repeated covariance type = Diagonal). Analysis 2 used the number of *P. afer* young of the year (YOY) (repeated covariance type = Compound Symmetry: Heterogeneous). Analysis 3 used adult *P. afer* abundance (repeated covariance type = Scaled Identity). Main treatment effects were analysed using least significant difference (LSD) pairwise comparisons.

To discount the impact of the native predatory eels on *P. afer* abundance, the frequency of occurrence (% of sites within each stream zone where *A. mossambica* and *A. marmorata* were recorded) was tested for uniform distribution between stream zones using a χ^2 test of independence. The five months of day and night snorkelling data on the Blindekloof stream were analysed for temporal changes in the frequency of occurrence (% of sites where non-native species were recorded) of non-natives fishes to test for an increase in stream invasibility during periods of high flow. Analyses were performed in MS Excel 2007, Microsoft®.

4.3 Results

Water quality was consistent throughout the sampling period. Conductivity was generally low (range: 97.2-104.5 µS cm-1), pH was near neutral (6.2-7.9), water was clear (0.2-0.5 NTU) and warm (19.0-22.0 °C) (Table 4.1). Temporal water temperature in the Blindekloof stream followed a sinusoidal, seasonal pattern with the lowest temperatures recorded during July (Blindekloof upper: 10.8 ± 0.7 °C; Blindekloof/Kwa-Zunga River confluence: 12.6 ± 0.5 °C) and the highest during January (Blindekloof upper: 22.3 ± 1.0 °C; Blindekloof/Kwa-Zunga River confluence: 24.3 ± 0.9 °C) (Figure 4.2).

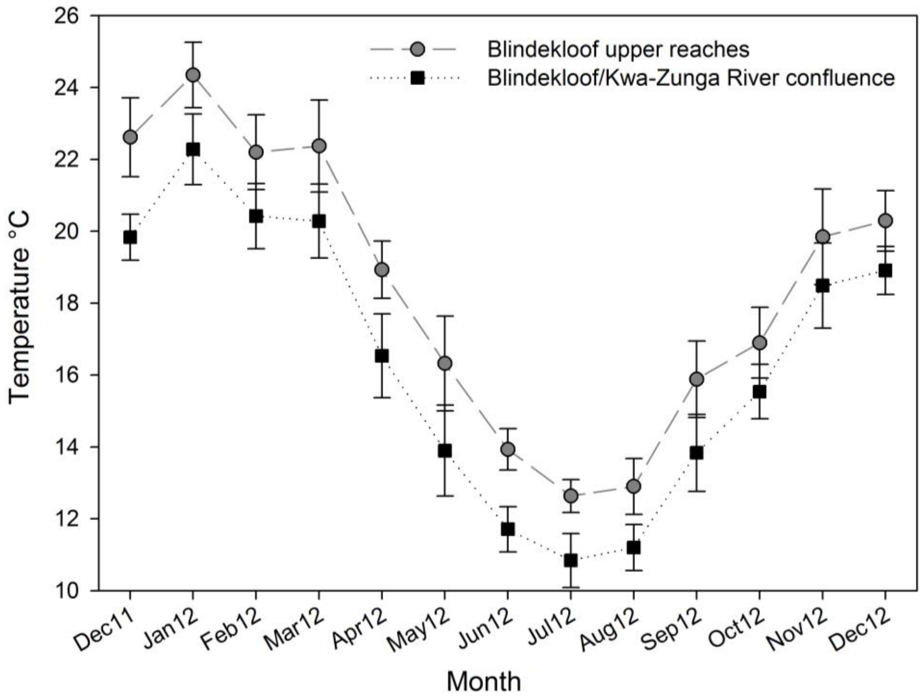


Figure 4.2 Temperature regime for the upper Blindekloof stream and the confluence of the Blindekloof stream and the Kwa-Zunga River for the period 11/12/2011-09/12/2012.

The overall abundance, relative abundance and distribution of fishes from the Blindekloof stream monitoring sites is summarised in Table 4.1. Nine species were recorded, six native (*P. afer*, Cape kurper *Sandelia capensis*, goldie barb *Barbus pallidus*, river goby *Glossogobius callidus*, *A. marmorata*, *A. mossambica*) and three non-natives (*M. dolomieu*, *banded tilapia Tilapia sparrmanii*, *C. gariepinus*). The 'mixed zone' was characterised by an assemblage consisting of the native species *P. afer*, *B. pallidus*, *G. callidus*, *S. capensis*, *A. mossambica* and *A. marmorata* and the non-native species *T. sparrmanii* and *C. gariepinus* (Table 4.1). The 'invaded zone' consisted of five sampling sites, three of which were inhabited by *M. dolomieu* and two sites in between by native fishes (Table 4.1). In the 'non-invaded zone' only *P. afer*, *S. capensis*, *G. callidus*, *A. mossambica* and *A. marmorata* were recorded (Table 4.1).

The frequency of occurrence of the native predators *A. mossambica* and *A. marmorata* was not significantly dependent on stream zone (*A. marmorata*; χ2 test of independence: χ2 = 2.81, d.f. = 10, *P* > 0.05; *A. mossambica*: χ2 = 0.61, d.f. = 10, *P* >0.05).

Table 4.1 The stream zones (mixed, invaded, non-invaded), sites (15 sites, including volume and water quality), distribution, mean abundance (fish pass^{-1}) and relative abundance of fishes by site (%) from site 1 in the lower reaches to site 15 in the upper reaches, which were monitored for 6 months (day and night snorkelling: November 2011-April 2012) on the Blindekloof stream, a headwater tributary of the Kwa-Zunga River within the Groendal Wilderness Area (*non-native species).

	mixed zone				**invaded zone**					**non-invaded zone**					
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
P. afer	131.2±35.5	78.3±10.5	67.3±12.7	42.6±5.3	12.3±8.0	23.5±9	38.7±13.1	39.8±19.1	52.6±17.2	39±10.7	70.2±8	128±17.8	97.6±20.2	126.5±16.4	54.8±12.7
%	58.1	69.9	69.1	43.6	83.6	67.8	80.0	89.6	87.7	91.7	98.0	99.8	100.0	99.7	99.3
B. pallidus	45.8±23.2	3.6±1.8	2.1±0.9	2.2±0.8	0±0	0±0	0.1±0.1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
%	18.0	2.4	1.6	1.8	-	-	0.1	-	-	-	-	-	-	-	-
S. capensis	33.4±8	26.4±3.4	25.7±2.1	47.4±4.2	0.2±0.1	8.5±2.1	6.1±1.1	1.2±0.7	4.5±3.8	2.7±1	1.3±0.6	0±0	0±0	0±0	0±0
%	12.7	17.8	19.4	39.4	0.4	12.4	6.8	1.3	4.3	3.1	1.1	-	-	-	-
*T. sparrmanii**	9.9±3.1	1±0.6	0.1±0.1	0.2±0.2	0.1±0.1	0.1±0.1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
%	3.6	0.6	0.0	0.1	0.1	0.1	-	-	-	-	-	-	-	-	-
G. callidus	4.8±1.3	2.1±0.8	2±0.6	5.2±1.6	0.4±0.2	2.5±1.1	3.4±0.6	2.1±0.8	1.7±0.7	0.7±0.5	0±0	0±0	0±0	0±0	0±0
%	1.8	1.3	1.4	3.7	0.7	3.3	3.7	2.3	1.6	0.7	-	-	-	-	-
*C. gariepinus**	0.3±0.2	0±0	0±0	0±0	0±0	0±0	0±0	0.1±0.1	0.3±0.2	0±0	0.1±0.1	0±0	0±0	0±0	0±0
%	0.1	-	-	-	-	-	-	0.1	0.3	-	0.1	-	-	-	-
*M. dolomieu**	0±0	0±0	0±0	0±0	1.2±0.2	0±0	0±0	0.4±0.2	0.1±0.1	0±0	0±0	0±0	0±0	0±0	0±0
%	-	-	-	-	2.0	-	-	0.4	0.1	-	-	-	-	-	-
A. marmorata	0.3±0.2	0.4±0.3	0.3±0.2	0.1±0.1	0.4±0.2	0±0	0±0	0.3±0.2	0±0	0.2±0.1	0±0	0±0	0±0	0.1±0.1	0±0
%	0.1	0.3	0.2	0.1	0.7	-	-	0.3	-	0.2	-	-	-	0.1	-
A. mossambica	0.3±0.2	0.3±0.2	0±0	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1	0.6±0.3	0.9±0.3	0±0	0±0	0.2±0.2	0±0	0.3±0.3	0.4±0.2
%	0.1	0.2	-	0.1	0.3	0.1	0.1	0.7	0.8	-	-	0.1	-	0.2	0.4
Water quality															
pH (range)	6.2-7.3	6.4-7.8	6.7-7.6	6.7-8.0	6.1-7.7	7.1-8.0	6.5-7.9	6.7-7.8	7.1-7.9	6.7-7.7	6.8-7.5	6.6-7.7	7.1-7.7	6.9-7.3	7.0-7.3
Conductivity (µS cm^{-1})	104.5±7.9	100.8±7.9	100.7±8.3	103.0±7.9	97.3±7.3	97.5±7.6	85.3±14.5	98.8±8.2	98.2±8.3	101.2±8.1	97.2±8.3	97.8±8.0	101.3±8.8	102.5±9.1	102.5±8.6
Turbidity (NTU)	0.3±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.5±0.1	0.4±0.1	0.4±0.0	0.4±0.1	0.4±0.1	0.4±0.0	0.4±0.1	0.4±0.1	0.5±0.1	0.4±0.1	0.5±0.1
Temperature (°C)	20.9±1.0	21.1±1.2	21.7±1.2	22.6±1.5	21.6±0.6	22.0±1.1	22.0±0.9	21.4±1.1	20.8±1.0	19.7±1.5	19.6±1.0	20.1±1.1	19.6±1.1	19.0±0.9	19.5±0.8
Volume (m^3)	1513.6	207.6	166.4	270.9	574.9	138.7	493.0	1268.5	887.2	77.1	67.4	82.6	21.4	180.4	285.2

Overall *P. afer* abundances did not differ significantly between stream zones ($F_{2,23.96}$=3.13, P>0.05) and abundances were highest in the non-invaded zone (mean ± se; 79.8 ± 11.7 fish), lowest in the invaded zone (40.9 ± 12.8 fish) and increased in the mixed zone (63.0 ± 14.3 fish) (Figure 4.3 A; Analysis 1). Overall *P. afer* abundances differed significantly by sample month ($F_{1,34.56}$=33.55, P<0.05), but no significant interactions between sample month and stream zone were observed. Abundance in all three stream zones increased throughout the monitoring period, with lowest abundance in November 2012 (non-invaded zone: 59.2 ± 20.0 fish; invaded zone: 23.6 ± 7.9 fish; mixed zone: 27.8 ± 11.3 fish pass^{-1}) and highest abundances in March 2012 (Non-invaded zone: 118.5 ± 24.6 fish pass^{-1}; invaded zone: 70.4 ± 10.3 fish; mixed zone: 121.0 ± 29.0 fish) (Figure 4.3 A).

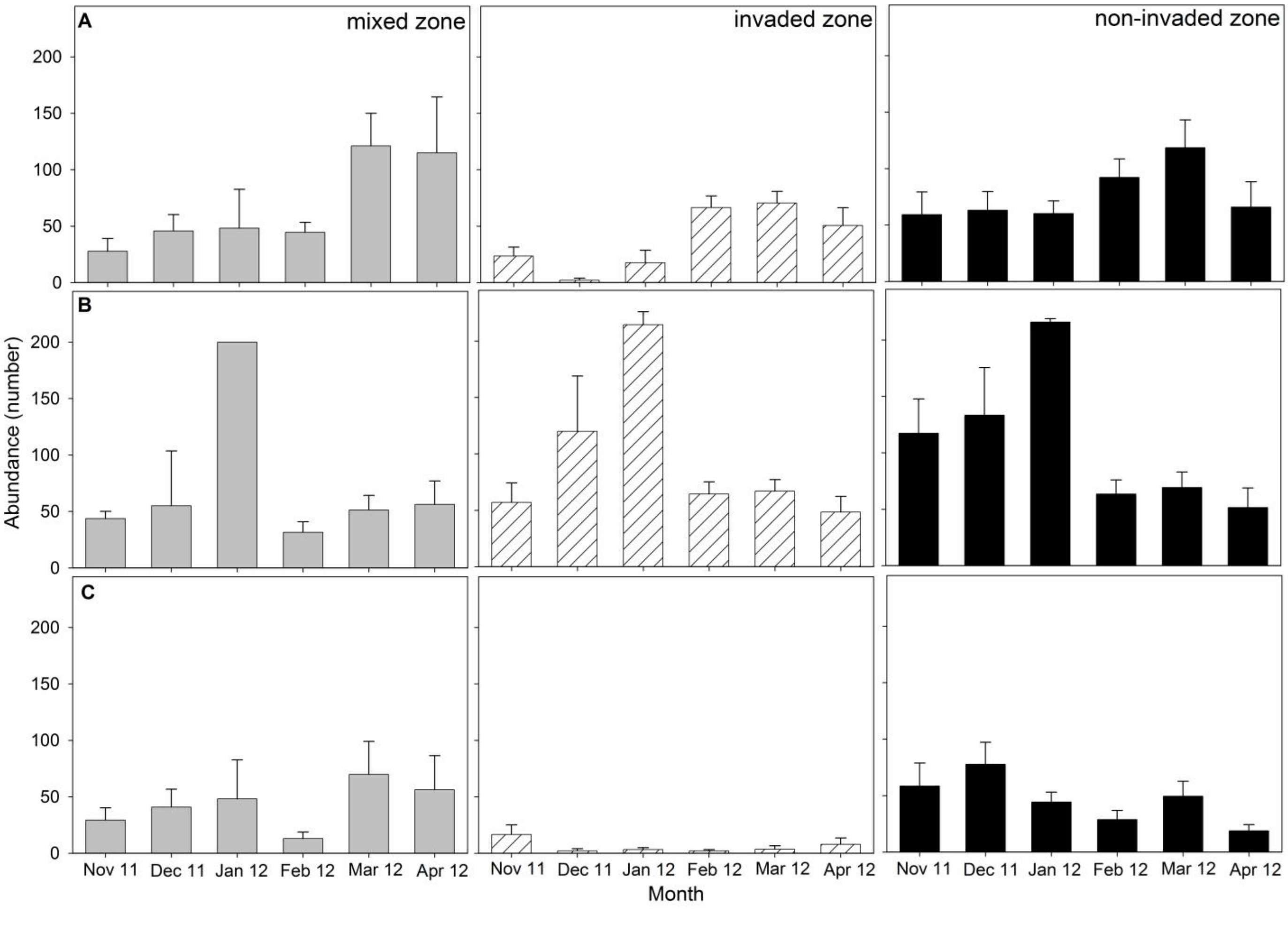


Figure 4.3 Abundance (mean ± SE) of the Eastern Cape redfin *Pseudobarbus afer* for: A) Mean overall abundance by zone (fish pass^{-1}, Analysis 1); B) Young of the year (YOY) abundance (fish, Analysis 2); C) Adult abundance (fish, Analysis 3).

Changes in YOY abundance were significantly explained by stream zone ($F_{2,16.61}$=5.08, P <0.05), and an interaction between stream zone and sample month ($F_{2,28.48}$=3.72, P <0.05). The mixed zone (25.3 ± 13.9 fish; P<0.05) was significantly lower than the invaded (63.4 ± 12.4 fish; P>0.05) and non-invaded zones (66.0 ± 11.3 fish; P >0.05), which were not significantly different from each other (Figure 4.3 B; Analysis 2). Young of the year abundances were low during November 2012 (77.6 ± 15.5 fish pass^{-1}), increasing monthly and peaking in January 2012 (211 ± 4.1 fish pass^{-1}), thereafter steadily decreasing (Figure 4.3 B).

Analysis of adult *P. afer* abundances indicated that stream zone ($F_{2,40.05}$=6.51, P <0.05), and sample month and stream zone interactions ($F_{2,72}$=5.76, P <0.05) were significant factors influencing adult abundance (Figure 4.3 C; Analysis 3). Adult *P. afer* abundances differed significantly between all zones (P <0.05), with lowest abundances recorded in the invaded (5.8 ± 10.2 fish), higher in the mixed (42.9 ± 11.4 fish) and highest in the non-invaded zone (46.3 ± 9.3 fish). Significant stream zone and sample month interactions were only observed for the mixed and non-invaded zones (P <0.05). In the non-invaded zone, there was a negative relationship between *P. afer* abundance and sample month, with abundance decreasing from November 2012 (58.5 ± 20.3 fish) to April 2012 (18.8 ± 5.4 fish), while in the mixed zone, the relationship between *P. afer* abundance and sample month was positive, with abundance increasing from November 2012 (29.2 ± 10.9 fish) to April 2012 (56.2 ± 30.1 fish) (Figure 4.4 C).

At sites within the invaded zone where *M. dolomieu* was consistently recorded, *P. afer* abundance decreased over time (Figure 4.4 A) compared to those pools where *M. dolomieu* was not recorded (Figure 4.4 B). During months of decreasing rainfall (November 2011-January 2012) *P. afer* abundance decreased to non detectable levels in *M. dolomieu*-inhabited sites (November = 4.3 ± 1.5 fish-February = 0 fish). Thereafter, high rainfall in February and March result in *P. afer* again dispersing into these sites at very low abundances (1 ± 1 fish). In sites where *M. dolomieu* was absent, abundance increased over time toward the end of the sampling period, from 6.5 ± 2.5 fish in January to 18.5 ± 11.5 fish in April. There was also a lag effect in the dispersal of adults into the invaded zone, with adults appearing two months after high rainfall during February and March 2012.

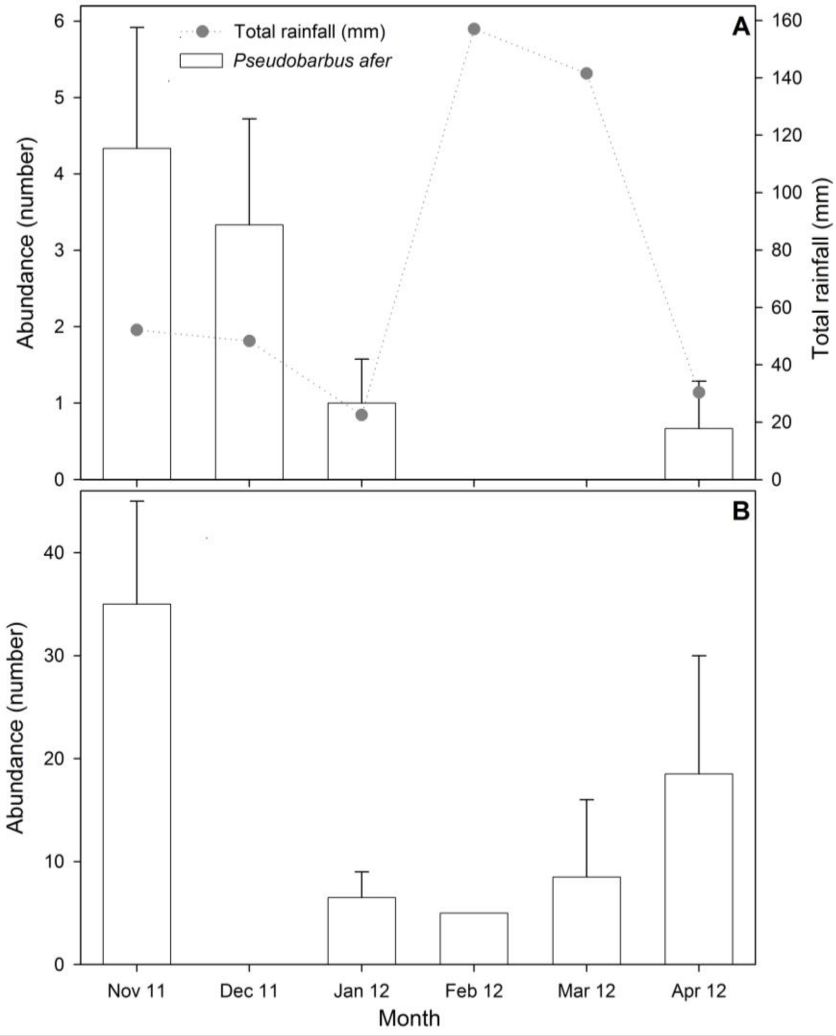


Figure 4.4 Adult *Pseudobarbus afer* abundance in the invaded zone over the six month monitoring period in the Blindekloof stream, a Kwa-Zunga headwater tributary within the Groendal Wilderness Area in; A) within the sites where *Micropterus dolomieu* was consistently recorded (n = 3)(rainfall is overlain) and; B) sites where *M. dolomieu* was not recorded (n = 2).

The penetration of non-native fishes in the Blindekloof stream remained fairly constant, but *C. gariepinus* progressively invaded upstream during the six month post-flood monitoring period (Table 4.2). In November 2011, *C. gariepinus* had penetrated 2.5 km upstream from the confluence of the Blindekloof stream and mainstream Kwa-Zunga River. By December 2011 they had moved 5 km upstream, and a maximum of 5.2 km by February 2012, where they remained for the duration of the study. The number of observed *C. gariepinus* recorded also increased from one individual observed in November 2011 to eight individuals observed in March 2012.

Table 4.2 The number and movement of *Clarias gariepinus* observed between November 2011 and March 2012 in the Blindekloof stream, a headwater tributary of the Kwa-Zunga River within the Groendal Wilderness Area.

Month/year	**Distance from confluence(km)**														
	2	2.25	2.50	2.75	3	3.25	3.50	3.75	4	4.25	4.50	4.75	5	5.25	Total
Nov 11’			1												1
Dec 12’	1														1
Jan 12’															0
Feb 12’	1								2				1		4
Mar 12’	2						1	1	2				1	1	8

4.4 Discussion

In situations where centrarchid invasion results in the total extirpation or exclusion of native fishes from invaded zones, impacts beyond loss of habitat become difficult to quantify. An above average rainfall year, however, provided an opportunity to corroborate stable state findings (Chapter 2 and Chapter 3) after *P. afer* recolonised centrarchid-invaded stream reaches, that were previously devoid of small native fishes. Over the monitoring period in the Blindekloof stream, overall abundances of *P. afer* in pools inhabited by *M. dolomieu* were not significantly lower than in the non-invaded zone in the upper stream reaches, as well as in the mixed zone in the lower reaches. There were, however, size specific differences in temporal abundance between stream zones. The maintenance of *P. afer* in the invaded zone was most probably a result of dispersal into invaded stream reaches from non-invaded source populations. Similar metapopulation dynamics, where the maintenance of native fishes in invaded zones is due to immigration from non-invaded source populations, have been observed for Canterbury galaxias *Galaxias vulgaris* in salmonid invasions of New Zealand (Woodford and McIntosh 2011; Woodford and McIntosh 2010). Specifically, *G. vulgaris* only persisted in reaches invaded by salmonids (*Oncorhynchus mykiss* and *Salmo trutta*) with nearby invader-free sources (Woodford and McIntosh 2011).

In the Blindekloof stream, YOY *P. afer* increased in abundance over the sampling period, as this corresponds to the spawning season for this species (Cambray 1994). Despite the extremely low abundance of adult *P. afer* in the invaded zone, YOY *P. afer* abundances did not differ significantly between invaded and non-invaded zones. This is contrary to findings by Godinho and Ferreira (2000), who found that in the Raia stream in Portugal, juvenile abundance of native fishes was significantly lower in stream reaches invaded by non-native

M. salmoides and pumpkinseed *Lepomis gibbosus*. The low abundance of *M. dolomieu* in invaded zones and the massive recruitment of YOY *P. afer* into the invaded zone probably resulted in a lag period between the influx and any noticeable changes in abundance due to predation over the relatively short duration of the study. In the mixed zone similar increases in YOY *P. afer* abundances were observed, but they were significantly lower than in the invaded and non-invaded zones. Two possible causes for this are: a decreased adult abundance in the mixed zone, resulting in poor recruitment, or variable reproductive potential in stream habitats due to physical or environmental factors making them less favourable in these respects than other stream reaches (Labbe and Fausch 2000).

Adult *P. afer* abundance differed significantly between stream zones, with the lowest abundance in the invaded zone, intermediate in the mixed zone and high in the non-invaded zone. *Pseudobarbus afer* adults also displayed temporal dynamics in their abundance changes between zones. In the Blindekloof stream, the invaded zone may therefore be acting as a filter resulting in reduced abundances of *P. afer* adults downstream, as this longitudinal decrease in abundance was not observed from the non-invaded Fernkloof and Waterkloof streams (Chapter 3). The impacts of other non-native fishes such as *T. sparrmanii* and *C. gariepinus* on *P. afer* recruitment in the mixed zone can also not be discounted. There were, however, indications of adult dispersal from the upper to lower stream reaches. In the non-invaded zone in the upper stream reaches there was a negative relationship between abundance and sample month, and concomitantly the opposite was true for the mixed zone in the lower stream reaches, where there was an accumulation over time. Despite the net movement of *P. afer* adults downstream, which continually flushed individuals through the invaded zone, by the end of the study adult *P. afer* was once again rare in the invaded zone, and absent from the pool with the highest abundance of *M. dolomieu.* In pools where *M. dolomieu* was consistently recorded, *P. afer* abundance decreased rapidly over time to undetectable levels (3 months). A high rainfall month resulted in adult *P. afer* being observed again in the invaded zone. Within the invaded zone, but at sites where *M. dolomieu* was not recorded, the opposite was true, and *P. afer* abundance increased over time to >15 times that recorded at invaded sites.

Whether *P. afer* naturally displays source-sink population dynamics (Dias 1996; Pulliam and Danielson 1991) where the upstream reaches act as a source (good habitat with positive population growth) and downstream reaches a demographic sink (poor quality habitat with negative population growth) is unknown. Alternatively, *P. afer* could be washed downstream and as the stream dries up move back upstream into refuge habitats before the onset of the dry season. This was shown by Labbe & Fausch (2000) for the Arkansas darter *Etheostoma cragini* in two intermittent Colorado plains streams. Many studies have shown that broad scale processes such as disturbance, dispersal, and habitat patch mosaic structure can influence populations as much as local environmental factors (Angermeier and Winston 1999; Angermeier and Winston 1998; Labbe and Fausch 2000; Schlosser 1991). The ability to disperse between refuge pools and habitats prone to seasonal drying was shown to be vital for rearing young of the year *E. cragini*, which highlights the importance of connectivity at much finer spatial scales than previously considered (Fausch et al. 2002; Labbe and Fausch 2000; Schlosser 1991). The barrier created by the presence of centrarchids (Chapter 3) in certain stream segments may inhibit dispersal of *P. afer* between important habitats. This seems most likely to impact adult *P. afer*, which exhibited the highest vulnerability to centrarchid invasion.

A factor omitted from overall distribution surveys (Chapter 3) is that of native versus non-native predatory impact. Separating the impact of non-native centrarchids and native predatory eels, *A. mossambica* and *A. marmorata*, on the *P. afer* population is complex, and some sort of natural predation pressure on *P. afer* has to be realistically assumed, as both eels are reported to eat fish (Bruton et al. 1987). For this reason, night snorkelling was included in monthly Blindekloof monitoring surveys in an attempt to elucidate native and non-native predatory impacts. Both eel species were found to be evenly distributed throughout the non-invaded, invaded and mixed zones, therefore although their impact cannot be considered negligible, it was considered consistent throughout the sampled stream reaches.

Anguilla marmorata and *A. mossambica* are ubiquitous in headwater streams inhabited by *P. afer*. Having co-evolved in these headwater streams could explain why *P. afer* may possibly also be impacted less by native predators, than by novel non-native predators. Naïvety by native fishes to introduced predators has been shown to impact heavily on native fishes (Cox and Lima 2006; Whitlow et al. 2003). Observations of fish position in the water column may provide some insight into this. While observing *P. afer* behaviour during day and night snorkelling surveys conducted on the Blindekloof stream it was apparent that at night, *P. afer* was predominantly suspended in the water column, well off the bottom of the stream while during the day, individuals were observed swimming in the water column and hiding in cracks and under rocks. At night eels on the other hand were hunting on the bottom of the stream, examining cracks and crevices while they systematically covered a pool.

Temporal longitudinal movement was also noted in the Blindekloof stream during a period of prolonged flow (about five months) and *C. gariepinus* managed to penetrate 5 km up the Blindekloof stream. *Clarias gariepinus* is a hardy pioneer species capable of long distance movements in response to an increase in flow. Van Der Waal (1997) reported that *C. gariepinus* penetrated >150 km up the seasonally dry Motloutse River from the mainstream Limpopo River in southern Botswana. The ability of *C. gariepinus*, a piscivorous predator with documented impacts on fish communities (Kadye and Booth 2012b) to invade Swartkops River system headwater stream environments is a major cause for concern. This also indicates that stream segments previously considered as refuge habitats may in fact be invasible. During periods of low rainfall, the surface isolation and contraction of habitat in these headwater streams into numerous refuge pools during a dry phase, may make *P. afer* extremely vulnerable to local extirpation, as was observed by their absence at invaded sites during stable state conditions (Chapter 2 and Chapter 3).

In conclusion, evidence suggests that the invasion of *M. dolomieu* in the Blindekloof stream may not disrupt YOY dispersal and recruitment, however, the *M. dolomieu*-invaded zone acted as an effective filter reducing adult abundance, and hence also recruitment downstream. It remains to be seen whether *P. afer* can indefinitely maintain the current dendritic metapopulations (Chapter 3) that characterise its distribution due to centrarchid invasions. The only way to accurately determine long term impact would be to establish whether these populations can maintain themselves, or whether they rely on inputs from others within the stream network. To effectively conserve small native stream fishes, a multilevel understanding of population processes at multiple scales (from reach to system) is needed to prioritise efforts for remediation and rehabilitation (Labbe and Fausch 2000; Schlosser and Angermeier 1995). A future threat may be the observed flood-facilitated invasion by non-native *C. gariepinus* into previously non-invaded headwater refuges.

CHAPTER 5: Impacts of non-native fish invasion and habitat degradation on an endangered headwater stream fish

5.1 Introduction

Freshwater ecosystems are among the most endangered habitats in the world (Dudgeon et al. 2006) and the decline and extinction of species in freshwater environments is increasing at an alarming rate (Collares-Pereira and Cowx 2004). The primary drivers of decline and extinction are anthropogenic disturbances in the form of species introductions and translocations, impoundment of rivers, pollution, habitat degradation and overexploitation (Collares-Pereira and Cowx 2004). An increased understanding of the complexity of these multiple stressors on freshwater ecosystem is imperative for successful management as challenges facing these environments are almost always multivariate in nature (Ormerod et al. 2010).

Many examples of species under threat being impacted by multiple stressors exist. In the Gaudiana basin in Portugal, the small cyprinid *Anaecyprus hispanica* is considered to be under threat from habitat alteration and destruction through flow regulation (especially by damming), pollution and eutrophication, water and sand/silt extraction, and dispersal of non-native fish species (Collares-Pereira et al. 2000). In a recent review on the ecological impacts of non-native freshwater fish species, Cucherousset and Olden (2011) highlight the fact that a major knowledge gap exists in the interaction of pressures such as habitat degradation and invasive species on native biota. The challenge of quantifying the impacts and interactions of multiple stressors lies in untangling the effects of different stressors to confidently infer their impact and provide suggestions for mitigation (Downes 2010).

In South Africa the situation is no different. Fish fauna are threatended by a multitude of anthropogenically induced stressors (Marr et al. 2009; Skelton et al. 1995). In the Eastern Cape, South Africa, the Keiskamma River system is home to two endangered fish species, the Border barb *Barbus trevelyani* and the Eastern Cape rocky *Sandelia bainsii*. The primary threat to both species has been cited as the introduction of non-native fishes and secondary impacts on the extent and quaility of available habitat due to habitat degradation and modification have been identified (Cambray 1996a; Gaigher 1975; Mayekiso and Hecht 1988). Research on these two species focused on a single stream within the upper Keiskamma River system (Gaigher 1975; Mayekiso and Hecht 1988). There is insufficient information on systemwide impacts to ensure effective conservation and management.

This chapter attempts to investigate the ecological consequences of non-native fish invasion in the headwaters of the Keiskamma River system in the Eastern Cape of South Africa, where habitat degradation and non-native fish invasion are the two major threats to the persistence of the imperilled Keiskamma River system fishes (Cambray 1996b; Gaigher 1975). Specifically the project aims to: (1) Assess factors influencing the distribution and abundance of fishes in the headwaters of the Keiskamma River system; (2) Assess establishment and relative abundance of headwater impoundment fish assemblages which often act as source populations for upstream invasion and (3) Quantify the invasive impact of non-native fishes on indigenous fishes in stream ecosystems.

5.2 Materials and Methods

5.2.1 Study Area

5.2.1.1 Habitat characteristics

The study was conducted on perennial headwater tributaries (Tyume = 5th order; Amatele = 2nd order; Wolf = 3rd order; Cata = 3rd order; Mnyameni = 2nd order; Gwiligwili = 2nd order; Rabula = 3rd order; upper Keiskamma = 3rd order; Gxulu = 4th order), and impoundment (Binfield Dam, Sandile Dam, Cata Dam) habitats of the Keiskamma River system, Eastern Cape, South Africa (Figure 5.1 and Figure 5.2). Stream order classifications were designated according to the method outlined in Strahler (1957).

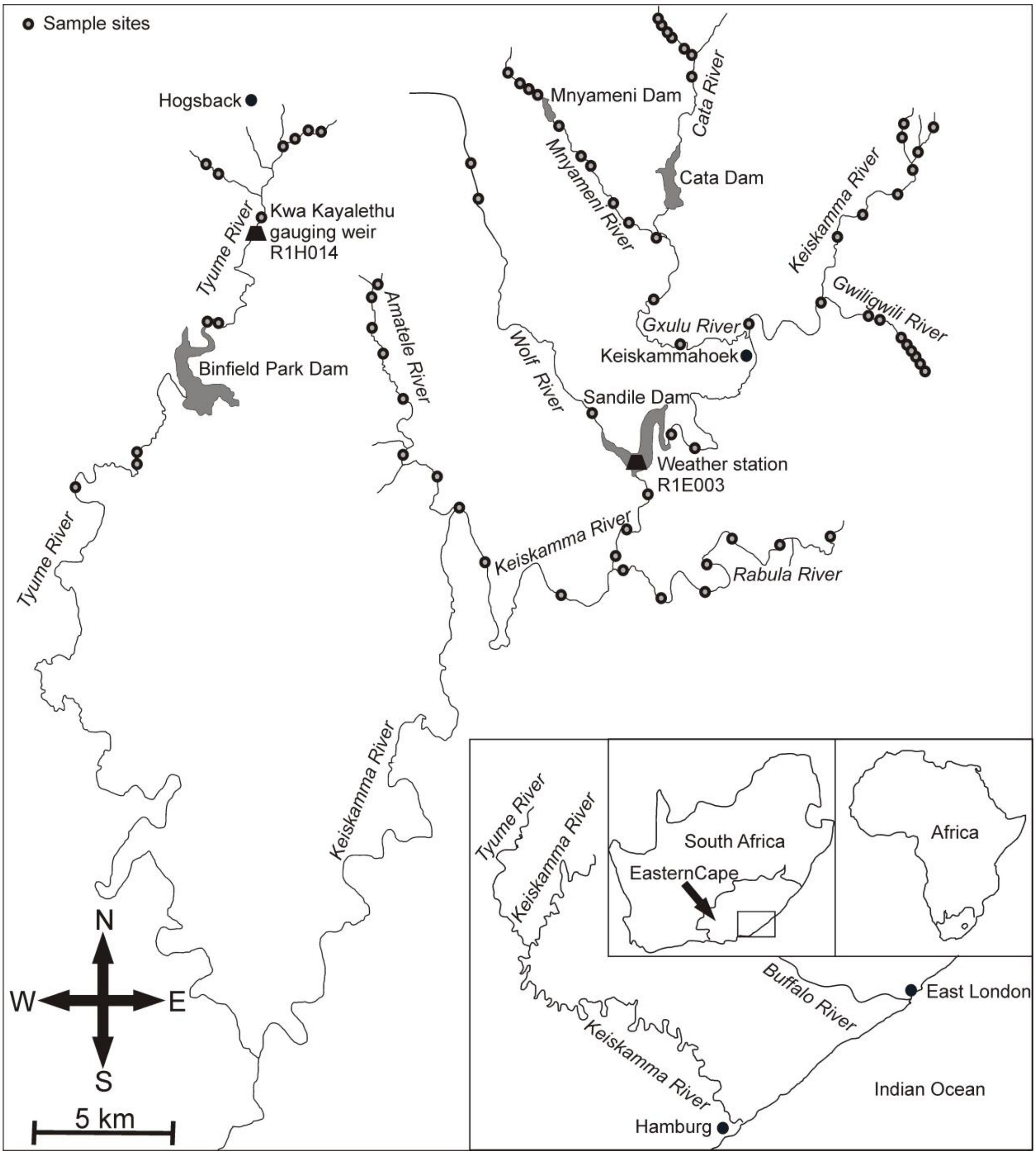


Figure 5.1 A map of the upper Keiskamma River system, Eastern Cape, South Africa, showing the position of the weather station (R1E003) at Sandile Dam and the gauging weir (R1H014) at Kwa Kayalethu on the Tyume River.

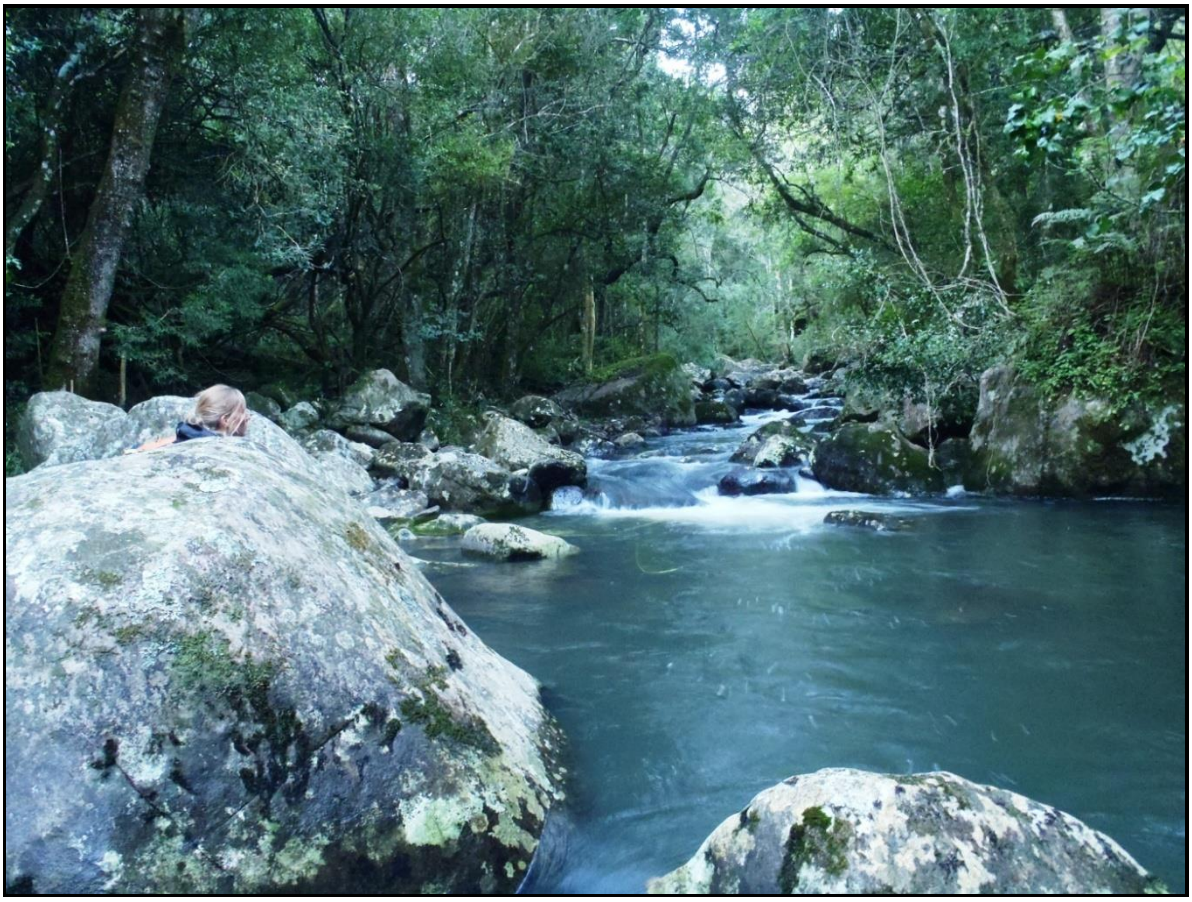


Figure 5.2 A riffle, run and pool on the Wolf River typical of headwater streams of the upper Keiskamma River system, Eastern Cape, South Africa.

The headwaters of the Keiskamma River system are situated in the Amatola region of the Eastern Cape. They drain off the Winterberg mountains, originating at an altitude of 1900 metres above sea level in montane grassland, and flow over the escarpment through indigenous mist-belt forests and savannah (thornveld or sourveld) vegetation in the coastal plateau grassland which extends to the base of the escarpment (Mhangara et al. 2012). The Keiskamma River catchment covers 2745 km^2 and the headwaters of the Keiskamma River originate in the region of Hogsback, flow for 263 km and enter the Indian Ocean south of East London at the settlement of Hamburg (Mhangara and Kakembo 2012) (Figure 5.1). The geology of the region is characterised by the Karoo supergroup underlain by the Beaufort Group consisting of shales, mudstone and sandstones (*sensu* Mhangara & Kakembo 2012).

The Keiskamma River catchment is characterised by scattered rural villages that undertake subsistence and small-scale commercial agriculture (Figure 5.3 A). The Keiskamma River catchment has been heavily modified through various anthropogenic practices such as poor land use (overgrazing, erosion; Mhangara & Kakembo 2012; Mhangara et al. 2012), water regulation and abstraction (impoundments and weirs Mhangara & Kakembo 2012; Mhangara et al. 2012), pollution (sewage; Fatoki, Gogwana & Ogunfowokan 2003) and the commercial cultivation of non-native plants which have spread through the catchment (Mexican pine *Pinus patula* and black wattle *Acacia mearnsii*; Mhangara & Kakembo 2012; Mhangara et al. 2012) and compromise riparian zone and stream bank integrity (Figure 5.3 B). In a study on soil erosion risk in the Keiskamma River catchment, Mhangara et al. (2012) estimated that 35% of the catchment was prone to extremely high soil loss. The highest levels of modification are found lower down the catchment in the coastal plateau grassland, while the escarpment region was relatively intact (Mhangara et al. 2012).

Figure 5.3 A) Scattered rural villages with Sandile Dam in the background characteristic of the upper Keiskamma River system. B) Overgrazing and removal of riparian vegetation resulting in erosion compromising the integrity of the river bank in the lower reaches of the Cata River.

South Africa is a water scarce country and consequently the mainstream Keiskamma River and its tributaries have been extensively dammed by impoundments and weirs to provide water for agricultural and domestic purposes. Four headwater impoundments are situated within the study area, Binfield Dam, Cata Dam, Mnyameni Dam and Sandile Dam. The characteristics of the impoundments are summarised in Table 5.1.

Table 5.1 The physical characteristics and construction dates of the four headwater impoundments of the Keiskamma River system, Eastern Cape, South Africa.

	Altitude (m)	**Catchment** (km^2)	**Surface Area** (ha)	**Capacity** (m^3)	**Construction Date** (year)	**Max depth** (m)
Binfield Dam	665	113	180	36830	1987	14
Cata Dam	775	57	86	12100	1980	14
Mnyameni Dam	900	19	18	2050	1975	12
Sandile Dam	590	353	146	30960	1983	21

5.2.2 Metadata

5.2.2.1 Rainfall and flow

Long term hydrological and meteorological data were obtained from the Department of Water Affairs hydrology section (DWAF 2012) and the situations of the recording stations are presented in Figure 5.1. These data were used to illustrate rainfall and flow variability in the headwaters of the Keiskamma River system. Flow data were obtained from the gauging weir at Kwa Kayalethu on the Tyume River (R1H014), a Keiskamma River system headwater tributary, for the period 1985/11-2012/09 (Figure 5.1). Rainfall data were obtained from station R1E003 at Sandile Dam (1985/11-2012/09) (Figure 5.1).

5.2.3 Field surveys

5.2.3.1 General

At each sampling site a full habitat assessment was undertaken to profile the physical (depth, mean wetted width, volume and habitat characteristics) and chemical (water quality) characteristics according to the standard protocol outlined in Chapter 3. Numerous complementary sampling methods were employed to get a representative sample of fish communities in both impoundments, mainstream and headwater stream habitats, the primary methods included gillnetting, fyke netting and backpack electrofishing. Angling and underwater video analysis were used as supplementary sampling methods where needed.

5.2.3.2 Electrofishing

Two pass backpack electrofishing was undertaken according to standard protocols described in Ellender et al. (2012a). The first pass was conducted from the downstream side (tail) of the pool in an upstream direction, covering the entire length of each pool. After the pass, fish caught were placed in a bucket with water. The second pass was identical to the first, but in a downstream direction. Upon completion of the pass, fish were identified to species level, measured, counted, and released. Catch per unit effort (CPUE) was expressed as fish m^3.

5.2.3.3 Underwater video analysis

In two streams (Cata, Wolf) high flow events resulted in extremely low conductivity (< 20 µS cm^{-1}) which decreased the catchability of fishes using electrofishing, so underwater video analysis was used as described in Ellender et al. (2012a). Relative abundance was expressed using the MaxN index, where relative abundance is defined as the maximum number of individuals for each species present in the field of view at the same time (Ellender et al. 2012a).

5.2.3.4 Gillnetting

Three impoundments (Binfield Dam, Cata Dam, Sandile Dam) were surveyed seasonally (Winter: 7/07/2011-15/07/2011; Summer: 21/03/2012-26/03/2012) using a multifilament experimental gillnet fleet. Each impoundment was surveyed over two consecutive nights per season and six gillnet fleets were set nightly. The gillnet fleet was 35 m long and comprised of seven randomly positioned panels (5 m long × 2.75 m deep) with stretch meshes of 35, 45, 57, 73, 93, 118 and 150 mm hung at a 50 % height : width ratio. Gillnet fleets were set overnight (ca. 1800-0600 hours), parallel to the shoreline at a depth of approximately 3 m to ensure that all mesh sizes were set in a similar depth. All fish caught in gillnets were separated by species and mesh size and weighed. Gillnet CPUE was expressed as fish (number).net $night^{-1}$.

5.2.3.5 Fyke netting

Fyke nets were used as a supplementary sampling method and set in mainstream and impoundment environments in areas >1 m depth that were unsuitable for electrofishing. Double-ended fyke nets with an 8 m guiding net and a first ring diameter of 550 mm with a 10 mm stretched mesh size were used. A mesh guard with 70 × 65 mm apertures was inserted in the valve of the first ring to avoid mortality of by-catch such as Cape clawless otters *Aonyx capensis* and native freshwater terrapins *Pelomedusa subrufa*. Fyke nets were set overnight and retrieved the following morning (ca. 1800-0600 hours). In each impoundment >10 fyke net sets were undertaken and >15 fyke net sets were undertaken in the mainstream Keiskamma River. Fyke net CPUE was expressed as fish (number).net night^{-1}.

5.2.4 Data analysis

5.2.4.1 Overall distribution

To describe overall distribution patterns, the extent of occurrence (EOO) and area of occupancy (AOO) for *B. trevelyani* and the salmonids *O. mykiss* and *S. trutta* was quantified according to criteria outlined by the IUCN (2001). According to the IUCN (2001), EOO is defined as “the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence” and the AOO is defined as “the area within its 'extent of occurrence' which is occupied by a taxon”. To quantify AOO, the distance of stream occupied by *B. trevelyani* and salmonids was estimated for each stream and then multiplied by the mean wetted width of the stream to approximate area coverage. The AOO was then quantified for invaded and non-invaded stream reaches. Of the invaded stream reaches, the distance of stream habitat lost to *B. trevelyani* was then estimated.

To compare similarity of fish species assemblages between headwater streams, impoundments and mainstream environments, Jaccard’s Index was applied. A chi-squared homogeneity of proportions test was used to test whether proportions of native and non-native species were dependant on environment (headwater streams, mainstream and impoundments). As impoundments have been identified as habitats that facilitate the invasion of riverine and stream habitats upstream and downstream (Adams et al. 2001), the establishment of non-native fishes in the headwater impoundments was assessed using two of the criteria outlined in Weyl et al. (2009):

1) Presence of juvenile and adult size classes from length frequency distributions (as there is no active stocking in Keiskamma headwater impoundments the presence of juveniles was taken as an indication of successful spawning).

2) Wide distribution indicated by a high frequency of occurrence (% of sites sampled containing a particular species).

3) If the species had been recorded in the system prior to this assessment in available literature or museum distribution records, and no evidence of periodic stocking existed it was also considered established.

To determine the factors influencing the distribution of fishes in the upper Keiskamma River system, data from 69 sites, covering mainstream and tributary sites, were analysed using multivariate methods in *CANOCO v 4.5* (ter Braak and Smilauer 2002). For analyses, data were separated into two matrices. For overall distribution data, the first consisted of the presence/absence data by site and the second environmental data by site.

Environmental data were standardised with *z* transformation by transforming the original distribution to a mean of 0 and standard deviation of 1. Preliminary analyses using detrended correspondence analysis (DCA) indicated turnovers of >2 on the first axis, therefore unimodal ordination methods were chosen for analysis of overall distribution data in the form of correspondence analysis (CA, species data only) and canonical correspondence analysis (CCA, species-environmental relation) (ter Braak 1995). Initially, 14 environmental variables (altitude, canopy cover, volume, % silt, % bedrock, % boulders, % gravel, % leaf litter, % terrestrial vegetation, % aquatic macrophytes, pH, temperature, turbidity and conductivity) were used in the CCA. Variables with the highest variance inflation factors (VIF) were removed individually from further analyses due to multicollinearity with other variables and the CCA was re-run. This process was continued until all the selected variables that had a VIF of >10 were removed (ter Braak and Smilauer 1998). A forward stepwise procedure was then used to determine the best predictor variables. The significance of these variables contribution to the ordination was tested using 999 Monte Carlo simulations at the $P < 0.05$ level. To test the statistical significance of the selected environmental variables on the species presence/absence model, Monte Carlo permutation tests were used.

To investigate the primary factors influencing the presence/absence of *B. trevelyani*, Classification Trees were used. Classification Trees are becoming increasingly popular to model ecological data (De' ath and Fabricius 2000) in particular species distributions in aquatic environments (Kadye and Booth 2012b; Olden and Jackson 2002). The non-parametric nature of Classification Trees and lack of pre-defined assumptions of data distribution are well suited to ecological data (De' ath and Fabricius 2000; Olden and Jackson 2002). Classification trees explain the variation of a single response variable (*B. trevelyani* presence/absence) by numerous explanatory variables, which can be either categorical (bankside vegetation: native/non-native; habitat quality: poor/good/excellent; salmonids: present/absent; habitat diversity: low/medium/high; stream reach: lower/middle/upper) or continuous (altitude, % canopy cover, volume, % silt, % bedrock, % boulder, % gravel, % cobble, % leaf litter, % terrestrial vegetation, % aquatic macrophytes, pH, temperature, turbidity, conductivity) (Breiman et al. 1984; De' ath and Fabricius 2000; Olden and Jackson 2002). Habitat quality was defined according to the following criteria: poor = habitats that were excessively silted, had compromised bankside integrity, which was indicated by heavily eroded or denuded banks; good: where there was either alien vegetation encroachment and low siltation and reasonable bankside integrity; excellent: habitats where only native vegetation was present, the riparian zone was intact and as a result siltation was minimal. The overall data or parent node is then split to construct a simple tree by pre-defined splitting criteria (define the stopping condition). Each split results in two mutually exclusive child nodes, homogenous groups with the lowest misclassification rate (misclassification cost), characterised by the mean value of the response variable, the group size and the value of the response variable that defines it (Breiman et al. 1984; De' ath and Fabricius 2000). To optimise tree size, a sequence of nested trees is created, each of which is the best for its size. The best tree is then chosen as the one with the lowest misclassification rate but the best predictive power (De' ath and Fabricius 2000).

Analysing data using Classification Trees involves a number of steps: (1) Specifying criteria to ensure predictive accuracy: for this study misclassification costs were set as equal and the

Gini measure of node impurity was used; (2) Determining splitting criteria and when to stop splitting: the splitting criteria or stopping rule was set to prune the tree on misclassification error; (3) Selecting the best tree: cross validation was used to select the tree with the lowest misclassification cost using V-fold cross validation and the 1-SE rule (Breiman et al. 1984; De' ath and Fabricius 2000). Classification was performed using Statistica 10.0, StatSoft®.

As one of the aims of the study was to investigate the impact of the invasive salmonids *O. mykiss* and *S. trutta*, the presence/absence of salmonids was added as an explanatory categorical variable. Altitude was a significant explanatory variable identified by the CCA to structure fish assemblages; therefore only sites below the maximum altitude where *B. trevelyani* was recorded (872 metres above sea level) were included in the analysis to avoid biasing results through false negatives.

5.3 Results

5.3.1.1 Habitat characteristics

The habitat and physico-chemical characteristics of the upper Keiskamma River system are summarised in Table 5.2. Typically, instream habitat consists of pools dominated by bedrock, boulder and cobble substrates in the upper stream reaches and open canopy pools in the lower reaches. Riparian vegetation was characterised by closed canopy tropical mist belt forest in the upper stream reaches and savannah in the lower reaches. Mean wetted width of the streams ranged from 1.7 ± 0.1 m in the upper Keiskamma River to 8.3 ± 2.3 m in the Gxulu River. The water quality variables pH (mean ± SD; Gwiligwili: 8.21 ± 0.18-Gxulu: 9.6 ± 0.1) and temperature (Cata: 16.2 ± 1.5 °C-Gxulu: 22.2 ± 2.7 °C) were similar between streams. Conductivity was variable but generally low, ranging from 12.0 ± 0.0 μS cm^{-1} in the Wolf River to 93.5 ± 10.6 μS cm^{-1} in the upper Keiskamma River. Turbidity varied between rivers with the Cata River (3.5 ± 1.7 NTU) being the least turbid while the Rabula River (60.3 ± 73.3 NTU) was the most turbid.

Table 5.2 Summary of the habitat and physico-chemical characteristics (mean ± SD) for 78 sites sampled on nine surveyed headwater streams and the mainstream Keiskamma River sampled during March 2011.

	Amatele (n = 8)	**Cata (n = 6)**	**Gwiligwili (n = 8)**	**Gxulu (n = 3)**	**Lower Keiskamma (n = 12)**	**Mnyameni (n = 8)**	**Upper Keiskamma (n = 2)**	**Rabula (n = 7)**	**Tyume (n = 12)**	**Wolf (n = 2)**
Pool length (m)	11.4 ± 6.9	10.1 ± 8.3	14.5 ± 4.6	17.7 ± 6.8	12.9 ± 5.5	13.2 ± 3.8	5.5 ± 2.1	15.5 ± 8.3	17.2 ± 8.0	12.3 ± 0.4
Width (m)	5.5 ± 1.5	5.2 ± 0.8	3.5 ± 0.9	8.3 ± 2.3	6.5 ± 4.2	4.4 ± 0.6	1.8 ± 0.1	5.0 ± 1.1	4.9 ± 1.5	5.6 ± 3.5
Depth (m)	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.2	0.4 ± 0.2	0.6 ± 0.2
Surface area (m^2)	61.9 ± 40.2	55.7 ± 52.2	52.3 ± 24.5	146.6 ± 65.2	89.7 ± 70.7	57.0 ± 15.1	9.8 ± 4.4	77.3 ± 45.6	80.8 ± 31.8	68.4 ± 41.1
Volume (m^3)	16.7 ± 9.8	22.3 ± 27.5	13.7 ± 10.4	35.6 ± 15.5	22.9 ± 18.0	19.4 ± 6.8	2.2 ± 1.2	17.8 ± 12.9	28.9 ± 15.5	39.7 ± 17.0
Canopy Cover (%)	32.5 ± 42.3	44.2 ± 19.6	33.8 ± 28.3	5.0 ± 8.7	16.7 ± 24.7	37.5 ± 32.4	40.0 ± 56.6	30.7 ± 24.9	15.8 ± 18.7	0 ± 0
Silt (%)	1.4 ± 3.9	1.6 ± 3.9	21.6 ± 24.8	1.9 ± 3.2	19.3 ± 30.9	0 ± 0	46.7 ± 18.9	3.7 ± 6.7	7.9 ± 14.1	3.3 ± 4.7
Bedrock (%)	11.6 ± 24.7	0 ± 0	8.7 ± 16.0	0 ± 0	8.2 ± 19.3	0.7 ± 1.9	0 ± 0	42.5 ± 34.4	21.4 ± 26.3	36.7 ± 51.9
Boulders (%)	31.8 ± 29.7	8.6 ± 11.8	37.5 ± 22.2	19.3 ± 17.6	37.9 ± 27.6	47.9 ± 13.2	40.0 ± 9.4	15.7 ± 17.9	26.6 ± 30.3	6.7 ± 9.4
Gravel (%)	23.5 ± 13.0	3.2 ± 7.8	24.7 ± 20.1	42.1 ± 34.2	23.2 ± 23.9	12.0 ± 12.5	13.3 ± 9.4	19.3 ± 15.6	23.8 ± 20.9	0 ± 0
Cobbles (%)	31.0 ± 31.9	85.1 ± 17.9	2.2 ± 4.3	35.2 ± 33.5	5.2 ± 6.6	39.4 ± 20.3	0 ± 0	14.5 ± 23.1	20.9 ± 20.2	46.7 ± 47.1
Leaf Litter (%)	0.7 ± 1.9	0.8 ± 1.9	3.3 ± 9.4	1.6 ± 2.8	0 ± 0	0 ± 0	0 ± 0	4.3 ± 7.4	0 ± 0	3.3 ± 4.7
Terrestrial Vegetation (%)	0 ± 0	0.8 ± 1.9	1.9 ± 3.9	0 ± 0	0.6 ± 1.9	0 ± 0	0 ± 0	0 ± 0	1.5 ± 3.9	3.3 ± 4.7
Aquatic Macrophytes (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5.7 ± 17.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
pH	8.9 ± 0.2	8.9 ± 0.1	8.2 ± 0.2	9.6 ± 0.1	9.2 ± 0.2	9.2 ± 0.2	8.9 ± 0.04	8.8 ± 0.2	9.1 ± 0.2	8.7 ± 0.4
Temperature (°C)	20.5 ± 1.1	16.2 ± 1.5	20.6 ± 2.4	22.2 ± 2.7	19.2 ± 1.9	18.2 ± 3.5	16.8 ± 0.9	20.9 ± 0.8	19.0 ± 1.5	20.7 ± 0.9
Turbidity (NTU)	5.4 ± 1.7	3.5 ± 1.7	7.8 ± 3.5	9.7 ± 0.8	22.7 ± 18.4	8.7 ± 1.4	6.0 ± 3.2	60.3 ± 73.3	17.9 ± 11.0	4.5 ± 0.2
TDS (ppm)	29.5 ± 6.6	12.7 ± 4.3	16.8 ± 3.1	37.7 ± 10.1	41.3 ± 19.3	22.1 ± 3.9	46.0 ± 5.7	34.6 ± 4.1	26.1 ± 15.0	6.0 ± 0.0
Conductivity ($\mu S\ cm^{-1}$)	59.0 ± 13.2	25.7 ± 8.3	33.9 ± 7.6	74.3 ± 22.9	81.2 ± 40.3	44.8 ± 7.9	93.5 ± 10.6	69.1 ± 7.6	52.9 ± 31.4	12.0 ± 0.0

5.3.1.2 Rainfall and flow

Rainfall (mean ± SD) ranged from a minimum of 22.6 ± 22.7 mm in July to 89.6 ± 68.8 mm in February. There is a large disparity between the rainfall patterns on the escarpment and in the coastal plateau zone, with the escarpment receiving approximately 1900 mm/annum and the coastal plateau 600 mm/annum (DWAF 2004). Mean annual rainfall at Sandile Dam (situated at the base of the escarpment) for the period 1985/11-2012/09 was 714.7 ± 214.7 mm. Although the Keiskamma River catchment is situated at the transition between winter and summer rainfall regions, 69.9% of the rainfall fell in summer (October-March) (Figure 5.4). The Tyume River was perennial and flowed consistently throughout the year (Figure 5.5). Mean monthly runoff (mean ± SD) ranged from 0.6 ± 0.6 mil.m^3 in July to 3.1 ± 5.2 mil.m^3 in November. Mean monthly runoff during the period 1985/11-2012/09 was 1.5 ± 2.2 mil.m^3 (Figure 5.5).

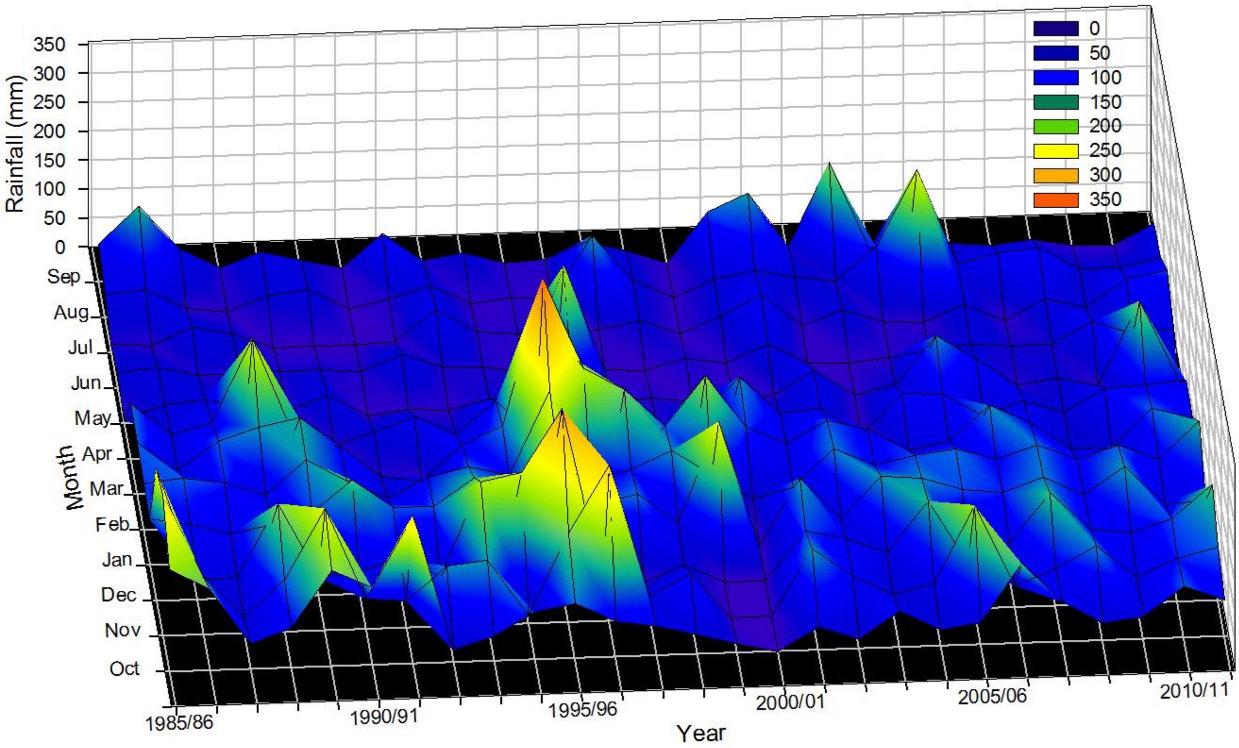


Figure 5.4 Total monthly rainfall (mm) from station R1E003 at Sandile Dam (1985/11-2012/09) as a representative of rainfall variability and seasonality (Austral summer: December, January and February; Austral winter: June, July and August) in the upper Keiskamma River system, Eastern Cape, South Africa.

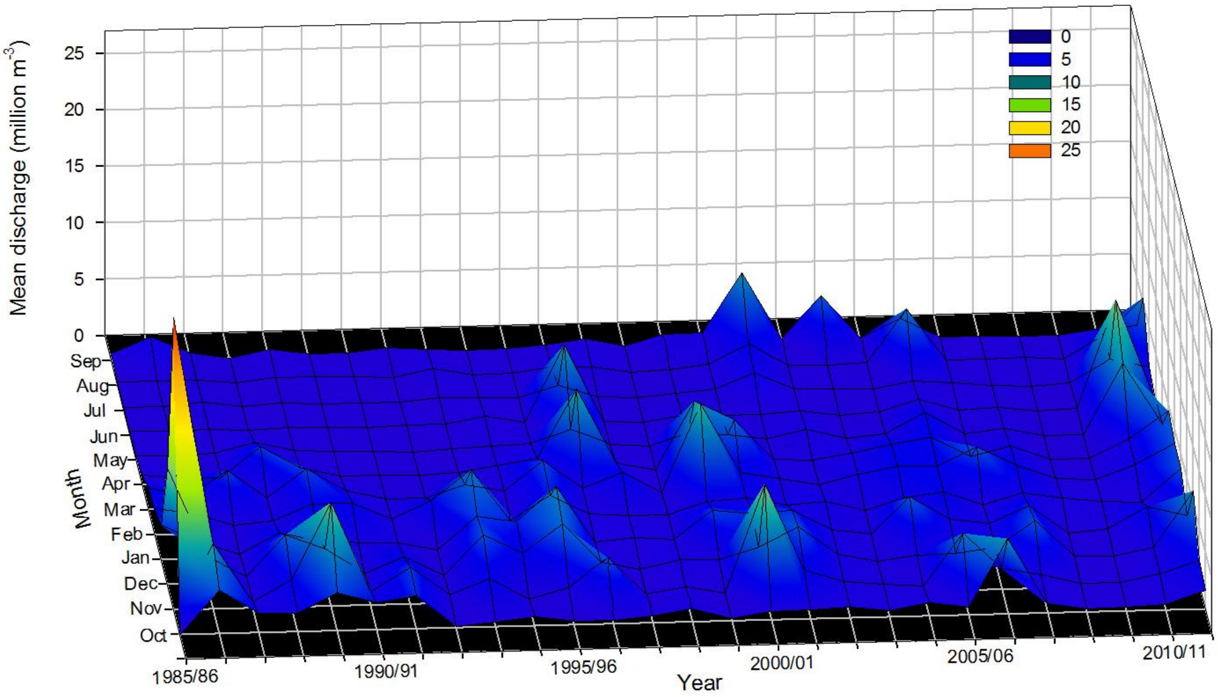


Figure 5.5 Mean monthly discharge (million m^{-3}) from the gauging weir at Kwa Kayalethu on the Tyume River (R1H014) for the period 1985/11-2012/09 as a representative of rainfall variability and seasonality (Austral summer: December, January and February; Austral winter: June, July and August) in the upper Keiskamma River system, Eastern Cape, South Africa.

5.3.1.3 Fish fauna of the Keiskamma River system

Typical of headwater streams, the fish fauna of the upper Keiskamma River system is depauperate, naturally consisting of only six species, two small minnows; *B. trevelyani* and chubbyhead barb *Barbus anoplus*, *S. bainsii*, moggel *Labeo umbratus*, freshwater goby *Glossogobius callidus* and the longfin eel *Anguilla mossambica* (Table 5.3). It is unlikely that the giant mottled eel *Anguilla marmorata* was historically present in the Keiskamma River headwaters as it generally occurs below 250 metres above sea level (Jubb 1961).

Since the late 1800s 12 non-native species have been introduced into the Keiskamma River system, ten of which were recorded during this study (Table 5.4). The earliest introductions were of rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta* and Atlantic salmon *Salmo salar* between 1898 and 1903. *Oncorhynchus mykiss* and *S. trutta* have established self-sustaining populations in some headwater streams, while no further records of *S. salar* exist. Of the six native species, five have been assessed and assigned a conservation status on the Red List of Threatened Species by the International Union for the Conservation of Nature (IUCN). *Barbus trevelyani* and *S. bainsii* are listed as ‘Endangered’; *L. umbratus, G. callidus* and *B. anoplus* are listed in the 'Least Concern' category and *A. mossambica* has not been assessed (IUCN 2001) (Table 5.3). The upper Keiskamma River system is of high conservation priority due to the 'Endangered' status of *B. trevelyani* and *S. bainsii*. The Keiskamma River headwaters are listed as a National Freshwater Ecosystem Priority Area (NFEPA), which is a national strategy aiming at conserving South Africa's freshwater ecosystems and ensuring sustainable use of water resources (Nel et al. 2011). Specifically the headwater tributaries are designated fish sanctuaries for the protection of endangered and critically endangered species, in this case *B. trevelyani* and *S. bainsii* (Nel et al. 2011).

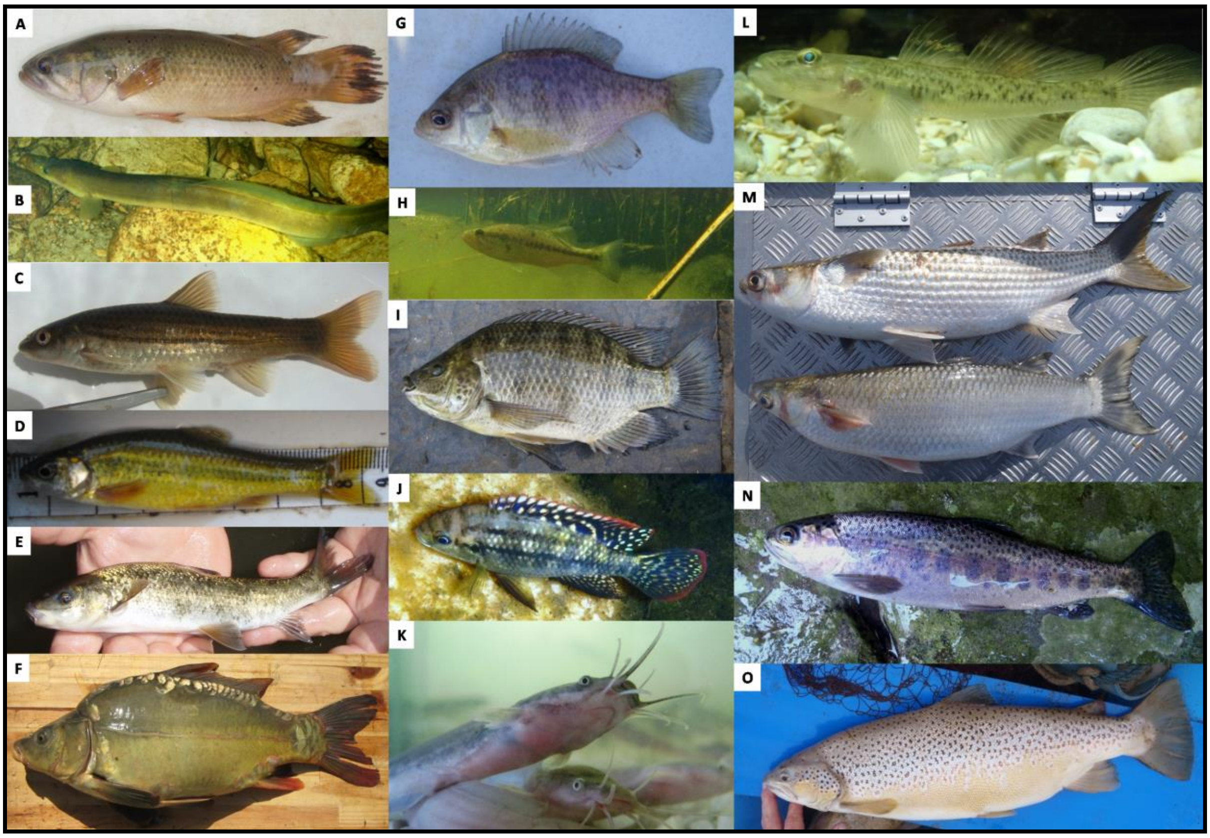


Figure 5.6 Photographs of the fish species recorded during this study on the upper Keiskamma River system, Eastern Cape, South Africa (A = *Sandelia bainsii*, B = *Anguilla mossambica*, C = *Barbus trevelyani*, D = *Barbus anoplus*, E = *Labeo umbratus*, F = *Cyprinus carpio**, G = *Lepomis macrochirus**, H = *Micropterus salmoides**, I = *Oreochromis mossambicus**, J = *Tilapia sparrmanii**, K = *Clarias gariepinus**, L = *Glossogobius callidus*, M = *Mugil cephalus** (top) and *Myxus capensis** (bottom), N = *Oncorhynchus mykiss**, O = *Salmo trutta** (* = non-native fishes).

Table 5.3 Keiskamma River system headwater stream fish fauna and their native distributions and maximum recorded size (Gaigher 1975; de Moor and Bruton 1988; Scott et al. 2006; Skelton 2001). For native species the superscript letters indicate IUCN redlist status, (NA = Not Assessed; DD = Data Deficient; LC = Least Concern; E = Endangered; IUCN 2001) (* non-native species).

Taxon	**Max size (mm SL)**	**Common name**	**Native distribution**
ANABANTIDAE			
Sandelia bainsii^{E} Castelnau, 1861	260	Cape kurper	Buffalo, Nahoon, Keiskamma, Great Fish and Kowie River systems, Eastern Cape
ANGUILLIDAE			
Anguilla mossambica^{NA} Peters, 1852	1200	Longfin eel	East coast rivers, Kenya-Cape Agulhas, Madagascar & Western Indian Ocean islands
CYPRINIDAE			
Barbus trevelyani^{E} Gunther, 1877	110	Border barb	Keiskamma and Buffalo River systems in the Eastern Cape, South Africa
Barbus anoplus^{LC} Weber, 1897	120	Chubbyhead barb	Highveld Limpopo-upland KwaZulu Natal, Transkei, middle and upper Orange River, larger coastal river systems of the Eastern and Western Cape (Olifants, Gouritz, Gamtoos, Sundays, Great Fish
Labeo umbratus^{LC} (A. Smith, 1841)	500	Moggel	Orange/Vaal River system; Eastern Cape: Gouritz, Gamtoos, Sundays, Great Fish, Keiskamma, Buffalo; Mpumalanga: Olifants to Limpopo River systems)
**Cyprinus carpio* Linnaeus, 1758	660	Common carp	Central Asia to the Black Sea and the Danube in Europe
CENTRARCHIDAE			
**Lepomis macrochirus* Rafinesque, 1819	200	Bluegill sunfish	Eastern and central North America
**Micropterus salmoides* (Lacepède, 1802)	600	Largemouth bass	Eastern North America from the Gulf of Mexico to southern Canada
CICHLIDAE			
**Oreochromis mossambicus* (Peters, 1852)	400	Mozambique tilapia	East coastal rivers from the lower Zambezi system south to the Bushman's River, Eastern Cape, South Africa
**Tilapia sparrmanii* A. Smith, 1840	230	Banded tilapia	Congo-Southern Africa
CLARIIDAE			
**Clarias gariepinus* (Burchell, 1822)	1400	African sharptooth catfish	Pan-African into eastern Europe
GOBIIDAE			
Glossogobius callidus^{LC} (Smith, 1937)	120	River goby	East coast rivers, Mozambique-Swartvlei Western Cape
MUGILIDAE			
**Mugil cephalus* Linnaeus, 1758	540	Flathead mullet	Cosmopolitan distribution occurring in all warm and temperate seas, estuaries and rivers
**Myxus capensis* (Valenciennes, 1836)	370	Freshwater mullet	Kosi system in northern KwaZulu Natal to Palmiet estuary in the south-western Cape
SALMONIDAE			
**Oncorhynchus mykiss* (Walbaum, 1792)	660	Rainbow trout	Rivers of the Pacific coast of North America from northern Mexico to Alaska
**Salmo trutta* Linnaeus, 1758	750	Brown trout	Europe and the Atlas Mountains of Morocco in north-east Africa

Table 5.4 The first record and general comments on the introduction of non-native fishes into the Keiskamma River system, Eastern Cape, South Africa (South African Institute for Aquatic Biodiversity, National Fish Collection voucher specimen: SAIAB 120466[4]; Gaigher 1975[2]; SAIAB 14969[3]; Mayekiso 1986[6]; de Moor & Bruton 1988[1]; Ellender et al. 2012b[5]).

Species	First record	Comment	Current status
L. macrochirus	1970[4]	Recorded in low abundances during 1972/73[2]. Introduction date and origin unknown	Abundant in Binfield Dam, not sampled elsewhere
M. salmoides	1984	Reported from ichthyofaunal surveys at the confluence of the Tyume and Keiskamma Rivers in 1984[6]	Sampled from Binfield and Sandile Dams where they were abundant
M. dolomieu	1984	Reported from ichthyofaunal surveys at the confluence of the Tyume and Keiskamma Rivers in 1984[6]	Not sampled
S. trutta	1903[1]	Introduced widely into Keiskamma River streams for angling and established in headwaters of the Tyume and Cata Rivers[1]	Sampled in the Cata River where they were established
O. mykiss	1903[1]	Introduced widely into Keiskamma River streams for angling and established in headwaters. Stocking of the Tyume River continued until the early 1980s	Self sustaining populations sampled from the upper reaches of the Tyume, Amatele, Wolf and Mnyameni Rivers
C. carpio	1981[3]	Museum record from the Keiskamma River	Sampled in Binfield Dam where they are established
T. sparrmanii	1972[2]	Recorded in low abundances, reported to have been introduced from a farm dam in the catchment during flooding	Sampled in low abundances from Binfield Dam
M. capensis	2000[5]	Introduced into Binfield Dam to enhance fisheries	Sampled in high abundances from Binfield Dam but not established
M. cephalus	2000[5]	Introduced into Binfield Dam to enhance fisheries	Sampled in high abundances from Binfield Dam but not established
C. gariepinus	1985[1]	Escaped from Fort Hare University aquaculture ponds during floods	A single individual sampled from the Keiskamma River below Sandile Dam
S. salar	1898[1]	8000 fingerlings introduced in the Keiskamma River in 1898 and 7000 into the Rabula River in 1899	No record since original introduction
O. mossambicus	2012	No previous record found above the Keiskamma estuary where they naturally occur	Two individuals sampled from Sandile Dam

5.3.1.4 Overall fish assemblages

Sixteen species were recorded from gillnet, fyke net, electrofishing and underwater video surveys on the upper Keiskamma River system headwater streams and impoundments (Figure 5.1). Ten species were non-native and only six species native (

Table 5.5). Similarity between all assemblages was low, with the greatest likeness between the headwater streams and the mainstream Keiskamma River (0.55), and the lowest between the mainstream Keiskamma River and Binfield Dam (0.07). Overall, lentic and lotic assemblages were dissimilar (0.37). The proportion of native/non-native species did not differ between headwater stream and mainstream environments (χ^2 = 0.141, df = 1, P >0.05) but was significantly different between headwater streams and impoundments (χ^2 = 4.848, df = 1, P <0.05) and mainstream environments and impoundments (χ^2 = 3.646, df = 1, P = 0.05), with lotic habitats being dominated by native species and lentic habitats by non-native species.

Table 5.5 Comparisons between lentic and lotic fish assemblages using Jaccard's Index of similarity and the presence/absence of fishes from headwater streams, mainstream and impoundments of the upper Keiskamma River system, Eastern Cape, South Africa.

	Headwater streams	**Keiskamma River**	**Binfield Dam**	**Sandile Dam**	**Cata Dam**
Headwater streams					
Keiskamma River	0.55				
Binfield Dam	0.14	0.07			
Sandile Dam	0.33	0.33	0.16		
Cata Dam	0.25	0.14	0.11	0.14	
Native species					
Anguilla mossambica	1	1	1	1	1
Barbus trevelyani	1	1	0	0	0
Barbus anoplus	1	1	0	0	0
Glossogobius callidus	1	0	0	0	0
Labeo umbratus	1	1	0	1	0
Sandelia bainsii	1	1	0	1	0
Non-native species					
Clarias gariepinus	0	1	0	0	0
Cyprinus carpio	0	0	1	0	0
Lepomis macrochirus	0	0	1	0	0
Micropterus salmoides	0	0	1	1	0
Mugil cephalus	0	0	1	0	0
Myxus capensis	0	0	1	0	0
Oncorhynchus mykiss	1	0	0	1	0
Oreochromis mossambicus	0	0	0	1	0
Salmo trutta	1	0	0	0	1
Tilapia sparrmanii	0	0	1	0	0
Total	**8**	**6**	**8**	**6**	**2**

5.3.2 Impoundments

5.3.2.1 Binfield Dam

5.3.2.1.1 Gillnets

Binfield Dam gillnet catches were dominated by the non-native species *M. cephalus* (summer: 56.3%; winter: 48.8%), *L. macrochirus* (summer: 34.5%; winter: 35.4%), *M. salmoides* (summer: 5.0%; winter: 4.9%), *C. carpio* (summer: 1.7%; winter: 1.2%), *T. sparrmanii* (summer: 2.5%) and *M. capensis* (winter: 9.8%). *Mugil cephalus* and *L. macrochirus* were the most dominant species in summer (mean ± se; *M. cephalus*: 5.6 ± 1.1 fish net night^{-1}; *L. macrochirus*: 3.4 ± 1.8 fish net night^{-1}) and winter (*M. cephalus*: 3.3 ± 1.4 fish net night^{-1}; *L. macrochirus*: 2.4 ± 0.7 fish net night^{-1}). *Micropterus salmoides* was recorded in both seasons, ranging in abundance from 0.5 ± 0.2 fish net night^{-1} in summer to 0.3 ± 0.1 fish net night^{-1} in winter. *Cyprinus carpio* was caught in low abundances (summer: 0.2 ± 0.1 fish net night^{-1}; winter: 0.1 ± 0.1 fish net night^{-1}). *Tilapia sparrmanii* was only sampled in summer (0.3 ± 0.3 fish net night^{-1}) and *M. capensis* in winter (0.7 ± 0.4 fish net night^{-1}) (Table 5.6).

5.3.2.1.2 Fyke nets

Lepomis macrochirus and *A. mossambica* were the only two species represented from fyke net catches at mean abundances of (mean ± se: 6.0 ± 2.2 fish net night^{-1}) and (1.5 ± 0.7 fish net night^{-1}) respectively (Table 5.7).

5.3.2.2 Sandile Dam

5.3.2.2.1 Gillnets

Six species were recorded from Sandile Dam gillnet catches, three native (*L. umbratus*, *S. bainsii*, *A. mossambica*) and three non-native (*M. salmoides*, *O. mykiss*, *O. mossambicus*). The most abundant species was *L. umbratus* (summer: 96.8%; winter: 98.8%), ranging in abundance from mean ± se: 35.4 ± 2.7 fish net night^{-1} in summer to 20.9 ± 3.9 fish net night^{-1} in winter. *Micropterus salmoides* was the second most dominant species (summer: 2.5%; winter: 0.4% but was caught in low abundances (summer: 1.0 ± 0.4 fish net night^{-1}; winter 0.1 ± 0.1 fish net night^{-1}). Incidental catches were made of *S. bainsii* (0.1 ± 0.1 fish net night^{-}

^{1}) and *O. mossambicus* (0.2 ± 0.1 fish net night^{-1}) in summer and *O. mykiss* (0.2 ± 0.1 fish net night^{-1}) in winter (Table 5.6).

5.3.2.2.2 Fyke nets

Fyke net catches were dominated by *L. umbratus* (mean ± se: 3.1 ± 0.8 fish net night^{-1}), followed by *M. salmoides* (0.5 ± 0.1 fish net night^{-1}) and *A. mossambica* (0.4 ± 0.1 fish net night^{-1}) (Table 5.7).

5.3.2.3 Cata Dam

5.3.2.3.1 Gillnets

A single species, *S. trutta*, was the only species recorded from gillnet catches at low abundances during winter (mean ± se: 0.2 ± 0.2 fish net night^{-1}), and no fishes were caught during summer (Table 5.6).

5.3.2.3.2 Fyke nets

Anguilla mossambica was the only fish species recorded from fyke net catches (mean ± se: 0.5 ± 0.1 fish net night^{-1}) (Table 5.7).

5.3.2.4 Mnyameni Dam

A local community project runs a recreational fishery targeting *O. mykiss* on the Mnyameni Dam, and gillnets were therefore not allowed to be set. Two fyke nets were set overnight and no catch was recorded. Hook and line angling sampled only *O. mykiss*.

Table 5.6 Catch per unit effort (CPUE), relative abundance, frequency of occurrence (FO%) of the fish species sampled (gillnets) and physico-chemical characteristics from Binfield Dam, Cata Dam and Sandile Dam, headwater impoundments of the Keiskamma River system, Eastern Cape, South Africa (* = non-native fishes).

	Binfield Dam		**FO (%)**	**Cata Dam**		**FO (%)**	**Sandile Dam**		**FO (%)**
	Summer n=12	Winter n=12		Summer n=12	Winter n=12		Summer n=12	Winter n=12	
Sandelia bainsii	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0	0.1 ± 0.1	0 ± 0	4.2
%							0.2		
*Oncorhynchus mykiss**	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0	0 ± 0	0.2 ± 0.1	8.3
%								0.8	
*Salmo trutta**	0 ± 0	0 ± 0	0	0 ± 0	0.2 ± 0.2	4.2	0 ± 0	0 ± 0	0
%					100.0				
Labeo umbratus	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0	35.4 ± 2.7	20.8 ± 3.9	95.8
%							96.8	98.8	
*Micropterus salmoides**	0.5 ± 0.2	0.3 ± 0.1	37.5	0 ± 0	0 ± 0	0	1.0 ± 0.4	0.1 ± 0.1	29.2
%	5.0	4.9					2.5	0.4	
*Oreochromis mossambicus**	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0	0.2 ± 0.1	0 ± 0	8.3
%							0.5		
*Cyprinus carpio**	0.2 ± 0.1	0.1 ± 0.1	12.5	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0
%	1.7	1.2							
*Lepomis macrochirus**	3.4 ± 1.8	2.4 ± 0.7	66.7	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0
%	34.5	35.4							
*Tilapia sparrmanii**	0.3 ± 0.3	0 ± 0	4.2	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0
%	2.5								
*Mugil cephalus**	5.6 ± 1.1	3.3 ± 1.4	75.0	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0
%	56.3	48.8							
*Myxus capensis**	0 ± 0	0.7 ± 0.4	12.5	0 ± 0	0 ± 0	0	0 ± 0	0 ± 0	0
%		9.8							
Temperature	21.9 ± 0.7	11.2 ± 1.2		19.9 ± 0.9	9.7 ± 0.5		22.2 ± 0.3	10.1 ± 0.9	
pH	8.1 ± 0.5	9.1 ± 0.1		8.7 ± 0.4	9.3 ± 0.2		8.1 ± 0.1	8.9 ± 0.2	
Conductivity	73.2 ± 4.4	74.6 ± 3.1		65.1 ± 2.8	64.0 ± 6.0		89.9 ± 3.9	74.1 ± 1.8	
Turbidity	17.6 ± 1.3	54.2 ± 1.4		31.5 ± 3.8	55.2 ± 2.7		23.5 ± 1.1	104.8 ± 3.5	

Table 5.7 Relative abundance (fish net night^{-1}) and frequency of occurrence (%) for fyke nets set in the headwater streams and impoundments of the Keiskamma River system, Eastern Cape, South Africa (* = non-native fishes).

	Binfield Dam (n=12)	**Cata Dam** (n=35)	**Gxulu River** (n=3)	**Keiskamma River** (n=34)	**Rabula River** (n=2)	**Sandile Dam** (n=30)	**Tyume River** (n=5)
Barbus trevelyani	0 ± 0	0 ± 0	0.67 ± 0.33	0 ± 0	0 ± 0	0 ± 0	1.4 ± 0.9
%	0	0	12.5	0	0	0	0
Sandelia bainsii	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.2	0 ± 0	0 ± 0	5.8 ± 2.4
%	0	0	0	9.7	0	0	72.5
Lepomis macrochirus*	6.0 ± 2.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
%	80.0	0	0	0	0	0	0
Barbus anoplus	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
%	0	0	25.0	0	0	0	0
Micropterus salmoides*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.1	0 ± 0
%	0	0	0	0	0	12.0	0
Labeo umbratus	0 ± 0	0 ± 0	3.3 ± 1.7	3.5 ± 0.7	0.5 ± 0.5	3.1 ± 0.8	0 ± 0
%	0	0	62.5	70.3	100.0	77.8	0
Anguilla mossambica	1.5 ± 0.7	0.5 ± 0.1	0 ± 0	0.9 ± 0.2	0 ± 0	0.4 ± 0.1	0.8 ± 0.8
%	20.0	100.0	0	19.4	0	10.2	10.0
Clarias gariepinus*	0 ± 0	0 ± 0	0 ± 0	0.03 ± 0.03	0 ± 0	0 ± 0	0 ± 0
%	0	0	0.6	0	0	0	0

5.3.2.5 Establishment

5.3.2.5.1 Impoundments

Length frequency histograms and frequency of occurrence (FO) for non-native species from the three headwater impoundments are presented in Figure 5.7 and Table 5.6. *Micropterus salmoides* was considered established in Sandile Dam and Binfield Dam, with a wide size range present (Sandile Dam: 175-494 mm FL ; Binfield Dam: 132-503 mm FL) in gillnet and anglers catches, and the species was represented at >25% of sites. *Lepomis macrochirus* was considered established in Binfield Dam as it had high FO (66.7%), and adults and juvenile were represented from gillnet catches (89-178 mm FL). Neither of the two mugilids spawn in freshwater (Whitfield 1998); *M. cephalus* had high FO (75%) and *M. capensis* low FO (12.5%), but for both species the population had a narrow size and age distribution, consisting only of large old individuals from the original stocking (Ellender et al. 2012b) and were therefore not considered established. Broad size ranges of *C. carpio* were sampled (434-

666 mm FL) but the species had low FO (12.5%). From these data it is unclear whether the species is established. Only two *T. sparrmanii* were caught during the study period, but the species has previously been recorded from Binfield Dam and is most probably established (Mayekiso and Hecht 1988). Two *O. mossambicus* and *O. mykiss* were recorded from Sandile Dam gillnet catches in summer and winter respectively. *Oreochromis mossambicus* may still be in the early establishment phase, while *O. mykiss* is probably not established. Both species are likely vagrants from the established headwater stream populations. In Cata Dam *S. trutta* was not established as the species was infrequently caught (4.2%) and represented by two large individuals (>500 mm FL). *Oncorhynchus mykiss* from Mnyameni dam was considered established as catches from hook and line angling ranged in size from 200 mm FL to 350 mm FL. In addition, no stocking has been conducted for more than 20 years. As juvenile *O. mykiss* (< 50 mm TL) was observed (BRE pers. obs.) immediately upstream of the Mnyameni Dam it is assumed that the impoundment receives recruits from the inflowing stream.

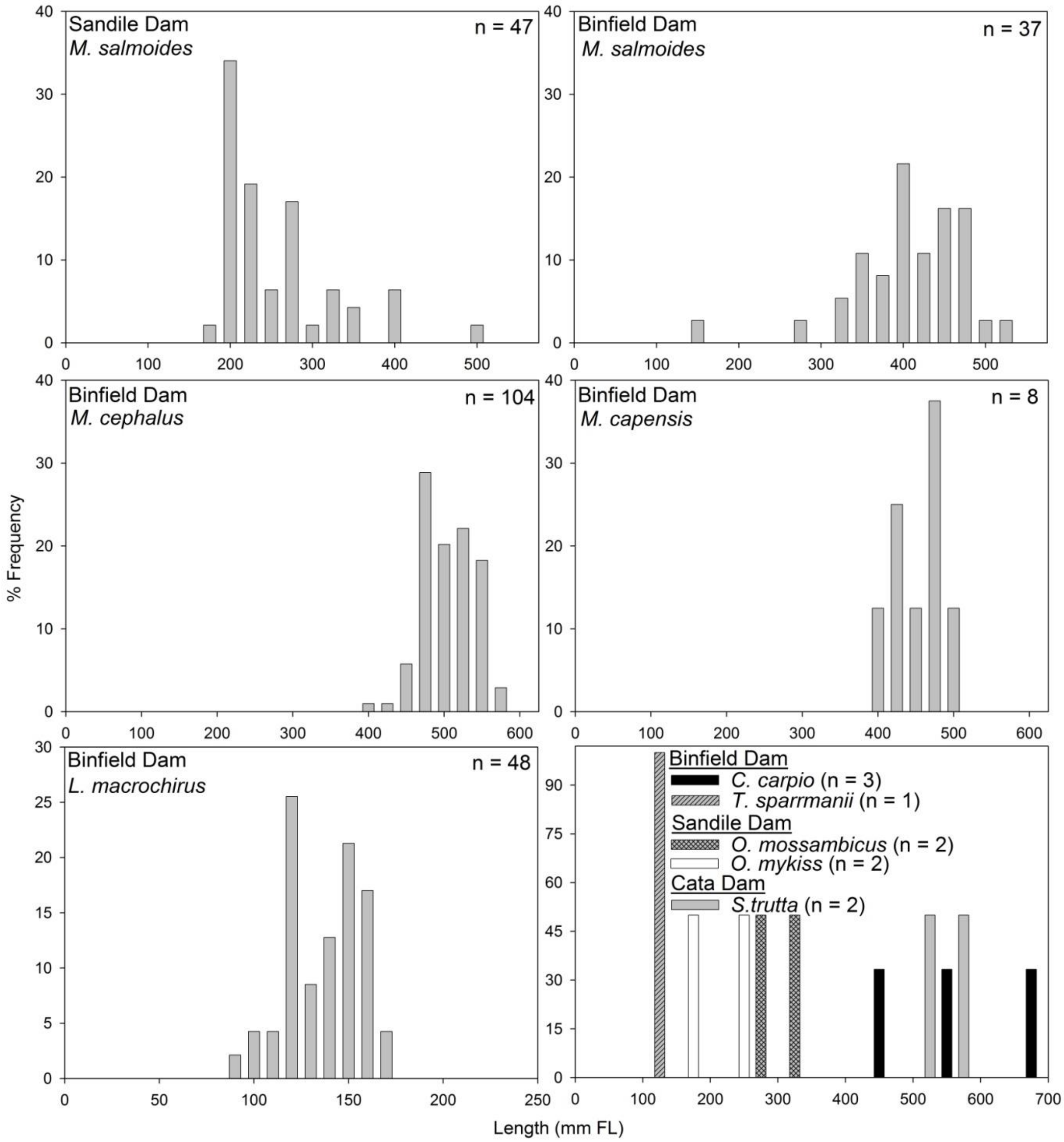


Figure 5.7 Length frequency histograms for non-native species from Binfield Dam, Cata Dam and Sandile Dam, headwater impoundments of the Keiskamma River system, Eastern Cape, South Africa.

5.3.2.5.2 Headwater stream and mainstream environments

Oncorhynchus mykiss and *S. trutta* were the only non-native species recorded from headwater streams. *Oncorhynchus mykiss* was established in the Tyume, Amatele, Mnyameni and Wolf Rivers, while *S. trutta* were established in the Cata River. Only one *C. gariepinus* was caught in the mainstream Keiskamma River. *Clarias gariepinus* has established in the lower Tyume

(Mayekiso and Hecht 1988) and is probably in the early establishment phase in the upper Keiskamma River.

5.3.3 Streams and mainstream

5.3.3.1.1 Electrofishing and underwater video analysis

Nine species were recorded from headwater stream and mainstream habitats, six native (*B. trevelyani, B. anoplus, G. callidus, L. umbratus, S. bainsii, A. mossambica*) and three non-native (*C. gariepinus, O. mykiss, S. trutta*). All nine species had highly fragmented distributions (Table 5.8). *Barbus trevelyani* was the most widespread and abundant species (six out of nine surveyed streams and the mainstream Keiskamma River) ranging from the low abundances in the Tyume stream (0.01 ± 0.02 fish m^{-3}), to the highest in the Gwiligwili stream (0.4 ± 0.2 fish m^{-3}). *Barbus anoplus* was also widespread but only abundant in the Rabula stream (0.2 ± 0.1 fish m^{-3}). *Sandelia bainsii* was only recorded from the mainstream Keiskamma River (0.01 ± 0.01) fish m^{-3}), Tyume (0.03 ± 0.02 fish m^{-3}) and Amatele (0.1 ± 0.02 fish m^{-3}) Rivers at low abundances. *Glossogobius callidus* was recorded at a single locality on the Tyume River. *Salmo trutta* was the only recorded fish species from the Cata River (0.01 ± 0.01 fish m^{-3}). *Oncorhynchus mykiss* was recorded in the Amatele (0.03 ± 0.01 fish m^{-3}), Mnyameni (0.1 ± 0.03 fish m^{-3}), Tyume (0.04 ± 0.02 fish m^{-3}) and Wolf (0.03 ± 0.01 mMaxN) Rivers.

5.3.3.1.2 Stream fish length frequencies

Size ranges of fishes sampled from headwater streams are summarised in Figure 5.8. A broad size range of *B. trevelyani* (15-110 mm FL) and *B. anoplus* (28-102 mm FL) was sampled from headwater streams. *Sandelia bainsii* from riverine habitats ranged from 32 mm FL to 157 mm FL. The *O. mykiss* stream populations consisted of relatively small individuals ranging in size from 85 mm FL to 243 mm FL.

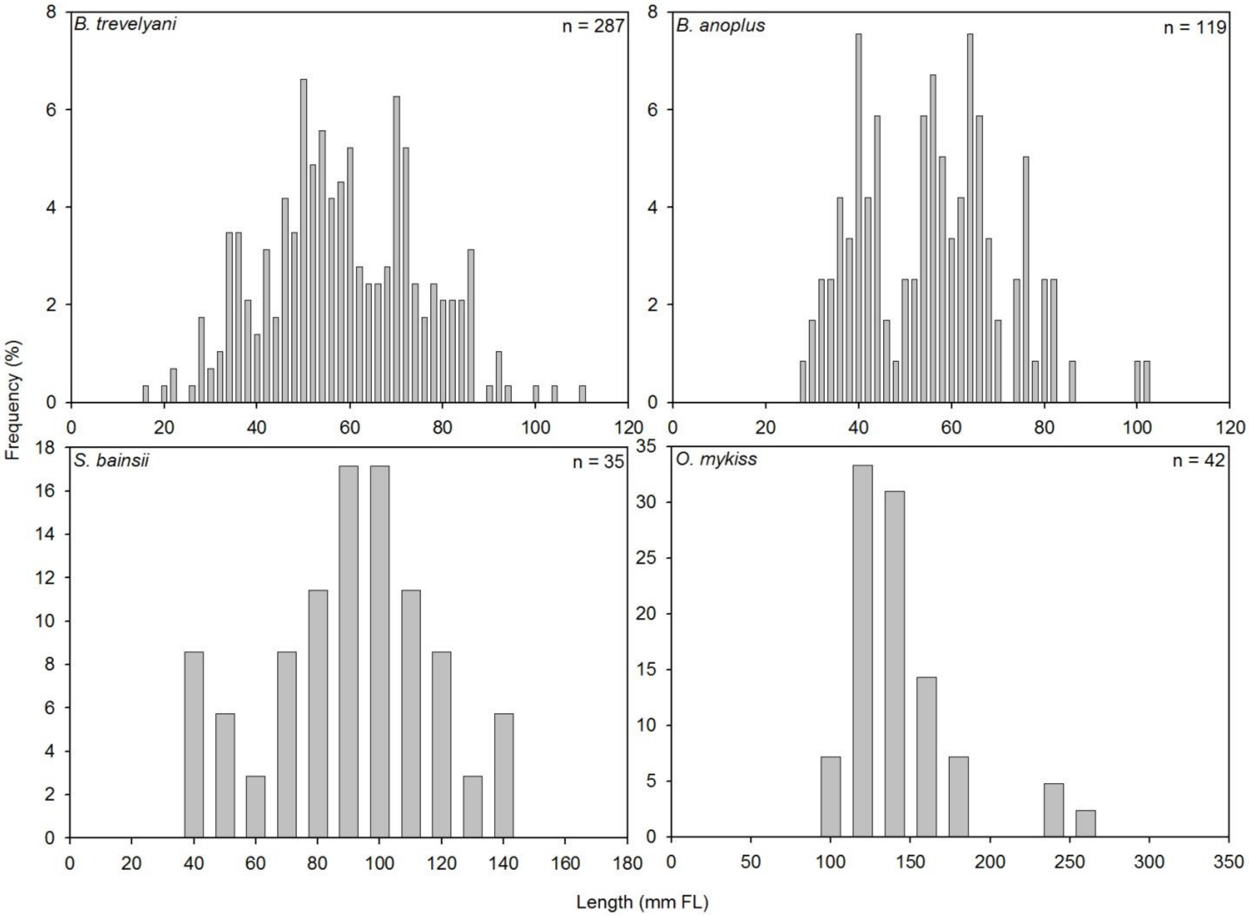


Figure 5.8 Length frequency histograms of *Barbus trevelyani*, *Barbus anoplus*, *Sandelia bainsii* and *Oncorhynchus mykiss* sampled from the headwaters of the Keiskamma River system, Eastern Cape, South Africa.

Table 5.8 The distribution, abundance and relative abundance of fishes sampled from nine headwater tributary streams and the mainstream Keiskamma River using backpack electrofishing (fish m^{-3}) and underwater video analysis (mMaxN) (* = non-native fishes).

	Amatele (fish m^{-3})	**Cata** (mMaxN)	**Gwiligwili** (fish m^{-3})	**Gxulu** (fish m^{-3})	**Lower Keiskamma** (fish m^{-3})	**Mnyameni** (fish m^{-3})	**Upper Keiskamma** (fish m^{-3})	**Rabula** (fish m^{-3})	**Tyume** (fish m^{-3})	**Wolf** (mMaxN)
Barbus trevelyani	0.1 ± 0.05	0 ± 0	0.4 ± 0.2	0.2 ± 0.1	0.05 ± 0.02	0.2 ± 0.1	0 ± 0	0.2 ± 0.1	0.03 ± 0.02	0 ± 0
%	43.8	0.0	98.0	100.0	38.6	72.3	0.0	41.7	24.4	0.0
Barbus anoplus	0.07 ± 0.03	0 ± 0	0.004 ± 0.004	0 ± 0	0.07 ± 0.03	0.004 ± 0.004	0.02 ± 0.02	0.3 ± 0.2	0 ± 0	0 ± 0
%	22.5	0.0	1.0	0.0	47.4	1.5	100.0	57.4	0.0	0.0
Sandelia bainsii	0.07 ± 0.02	0 ± 0	0 ± 0	0 ± 0	0.01 ± 0.008	0 ± 0	0 ± 0	0 ± 0	0.03 ± 0.02	0 ± 0
%	20.2	0.0	0.0	0.0	8.8	0.0	0.0	0.0	31.1	0.0
Glossogobius callidus	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.01 ± 0.01	0 ± 0
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0
Oncorhynchus mykiss*	0.03 ± 0.01	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.06 ± 0.03	0 ± 0	0 ± 0	0.04 ± 0.02	1.0 ± 1.0
%	10.1	0.0	0.0	0.0	0.0	26.2	0.0	0.0	35.6	100.0
Salmo trutta*	0 ± 0	1.0 ± 1.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
%	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anguilla mossambica	0 ± 0	0 ± 0	0.004 ± 0.004	0 ± 0	0.002 ± 0.002	0 ± 0	0 ± 0	0.004 ± 0.004	0.002 ± 0.002	0 ± 0
%	0.0	0.0	1.0	0.0	1.8	0.0	0.0	0.9	2.2	0.0
Labeo umbratus	0.01 ± 0.004	0 ± 0	0 ± 0	0 ± 0	0.005 ± 0.005	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
%	3.4	0.0	0.0	0.0	3.5	0.0	0.0	0.0	0.0	0.0

5.3.3.1.3 Fyke nets

Four fish species were represented from Keiskamma River fyke net catches. Catches were dominated by *L. umbratus* (mean ± se: 3.5 ± 0.0.7 fish net night^{-1}) followed by *A. mossambica* (0.9 ± 0.2 fish net night^{-1}) and *S. bainsii* (0.5 ± 0.2 fish net night^{-1}). Only a single *C. gariepinus* (0.03 ± 0.03 fish net night^{-1}) was recorded (Table 5.7). *Labeo umbratus* also dominated catches from the Gxulu River (3.3 ± 1.7 fish net night^{-1}) with lower abundances of *B. anoplus* (1.3 ± 1.3 fish net night^{-1}) and *B. trevelyani* (0.7 ± 0.3 fish net night^{-1}). *Sandelia bainsii* and *B. trevelyani* were the two most abundant species from Tyume River fyke net catches (*S. bainsii*: 5.8 ± 2.4 fish net night^{-1}; *B. trevelyani*: 1.4 ± 0.9 fish net night^{-1}) while *A. mossambica* (0.8 ± 0.8 fish net night^{-1}) was represented at low abundances. *Labeo umbratus* was the only species represented at low abundance in fyke net catches from the Rabula River (0.5 ± 0.5 fish net night^{-1}) (Table 5.7).

5.3.3.1.4 Barbus trevelyani range

In the upper Keiskamma River system *Barbus trevelyani* occupied a geographical range (EOO) of 355.2 km^2 (Centroid/Midpoint: S-32°40'31.0137", E027°05'34.7003"; Bounding box maximum: S-32°36'16.3488", E027°13'45.7429"; Bounding box minimum: S-32°45'45.5559", E026°55'07.9985"). Of the 158 km of streams surveyed, *B. trevelyani* occurred in 56.0 km of stream and had an AOO of 0.3 km^2 while salmonids occupied 37.6 km and an AOO of 0.2 km^2. Due to lack of co-occurrence between salmonids and *B. trevelyani*, this equates to approximately 24% habitat loss of the total stream area surveyed due to salmonids. A further 39% of stream is currently uninhabited by *B. trevelyani.*

5.3.3.1.5 Headwater fish assemblages

Correspondence analysis indicated three distinct assemblages, the native assemblage (*B. trevelyani, B. anoplus, L. umbratus* and *A. mossambica*) primarily separated from *S. trutta* on the first axis and *O. mykiss* on the second axis, indicating high co-occurrence between native species, some co-occurrence between native species and *O. mykiss* but none between native species and *S. trutta* (Figure 5.9).

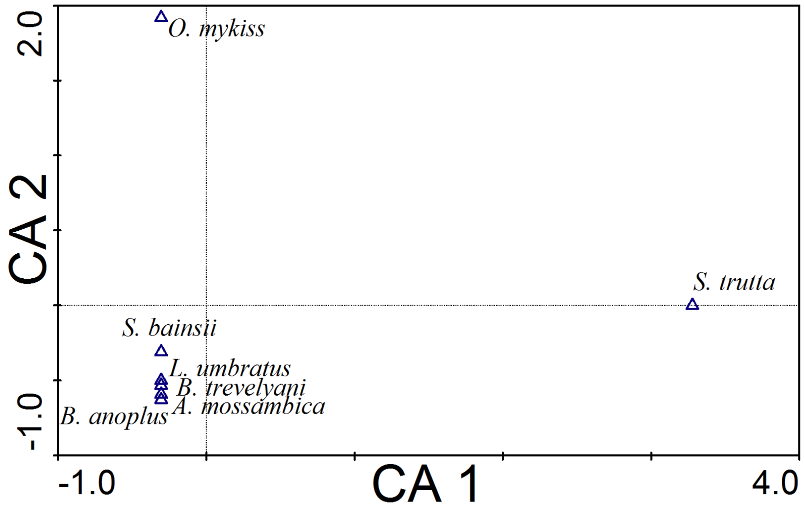


Figure 5.9 Correspondence Analysis (CA) ordination biplot of species presence/absence data from 60 sites sampled in the headwaters of the Keiskamma River system, Eastern Cape, South Africa.

From the overall distribution data the CCA identified two distinct assemblages separating out on an environmental gradient, the native assemblage consisting of *B. trevelyani*, *B. anoplus*, *L. umbratus* and *A. mossambica* and two non-native species, *O. mykiss* and *S. trutta* explaining 90.3% of the species-environment relation on the first two axes (Figure 5.10; Table 5.9). The CCA explanatory variables altitude, temperature and % silt accounted for 23.3% of the species-environmental variance, leaving 77.3% unexplained (Monte Carlo permutation P <0.05, of both the first axis and trace). The two salmonids *O. mykiss* and *S. trutta* were positively associated with altitude and negatively associated with temperature. The native assemblage showed a negative association with % silt and positive association with temperature. *Barbus anoplus* was positively associated with silt and showed some tendency to persist in disturbed habitats.

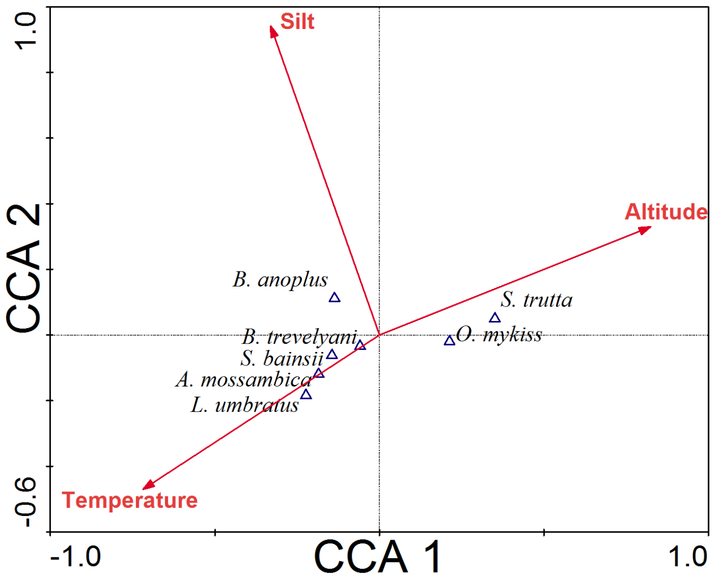


Figure 5.10 Canonical correspondence ordination biplot of species and environmental variables for the overall presence/absence distribution data of fishes from headwaters of the Keiskamma River system, Eastern Cape, South Africa.

The classification tree for *B. trevelyani* presence/absence had four child nodes with two splits. The first split was between sites where *SALMONIDS* were present (15) and absent (45), with a greater proportion of *SALMONID*-free sites containing *B. trevelyani* (Figure 5.11). The second explanatory variable was *%SILT*. Sites with >40.9% silt had a lower proportion of presences for *B. trevelyani* than those sites with <40.9% silt. Overall misclassification rate was 23.3%.

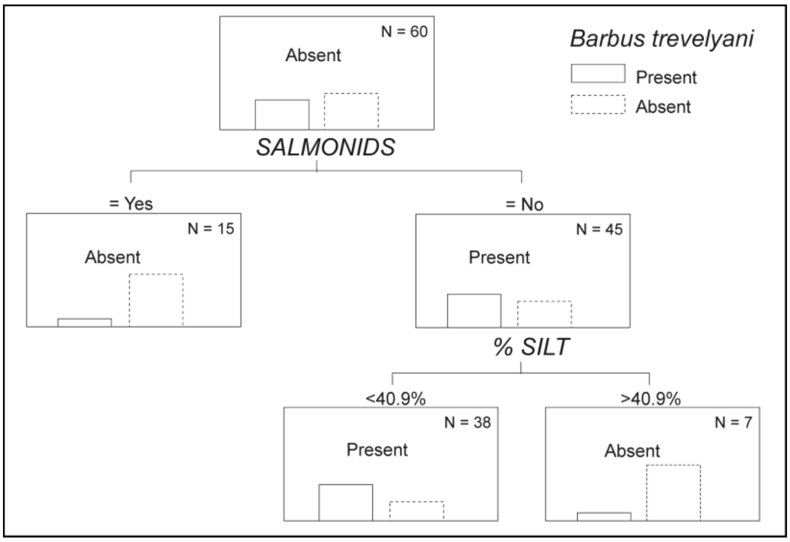


Figure 5.11 The Classification tree for *Barbus trevelyani* presence/absence in relation to the significant predictors non-native salmonids and % silt from 60 sites sampled in the headwaters of the Keiskamma River system, Eastern Cape, South Africa.

Table 5.9 Summary statistics from Correspondence Analysis (CA) and Canonical Correspondence Analysis (CCA) for presence/absence data showing the contribution of species and environmental variation to assemblages (only environmental variables with a significant contribution to assemblage variation are listed) from the headwaters of the Keiskamma River System, Eastern Cape, South Africa.

Statistic	Axis 1	Axis 2
Correspondence analysis		
Eigenvalue	1.000	0.942
Cumulative percentage variance of species data	31.3	60.9
Canonical Correspondence Analysis		
Statistic		
Eigenvalue	0.579	0.092
Species–environment correlation	0.787	0.432
Cumulative percentage variance of species data	18.2	21.0
Cumulative percentage variance of species-environment relation	78.0	90.3
Weighted correlations		
Altitude	0.649	0.143
%Silt	-0.260	0.408
Temperature	-0.566	-0.204
Total inertia		3.190
Sum of all canonical eigenvalues (full CCA)		0.743

5.4 Discussion

The acceleration in the introduction rate of non-native species into freshwaters is a global concern (Cucherousset and Olden 2011), and in many regions the number of introduced species has surpassed that of the natives (Marr et al. 2010; Moyle et al. 2003; Ribeiro et al. 2009). This has resulted in non-native species dominance and biotic homogenisation of freshwaters, often at the expense of unique native species (Rahel 2007). This trend is reflected in the Keiskamma River system headwaters, where 62.5% of fishes were non-native. There was also a low degree of similarity between impoundments, mainstream and headwater stream fish assemblages. Proportion of native and non-native fishes were dependent on environment, with headwater stream and mainstream assemblages dominated by native species, but impoundments were invasion hotspots in the system, containing >70% of non-natives. These results concur with those of Moyle et al. (2003) and Godinho and Ferreira (2000) in the Cosumnes River, California, USA and the Guadiana basin in Portugal, respectively, where non-native fishes were associated with dams and impoundments.

In invaded river systems, the position and establishment of species in the stream network is dependent on the abiotic tolerances of the introduced species and biotic resistance of the invaded fish community (Moyle and Light 1996; Townsend 1996). The establishment of certain species can, however, also be facilitated by suitable habitat within the stream network, such as impoundments (Godinho and Ferreira 2000; Moyle et al. 2003), which are not reflective of prevailing stream conditions but are an anthropogenically altered habitat. Since the angling-motivated introduction of salmonids (*Salmo salar*, *O. mykiss, S. trutta*) in the late 1890s numerous other species have been introduced into the system. Only *O. mykiss* and *S. trutta* had established successfully in headwater stream environments. This high degree of invasion resistance by the Keiskamma headwater streams may result from a number of abiotic factors, anthropogenic and natural in origin.

There are a number of physical features such as impassable barriers that limit upstream dispersal of non-native fishes, as was the case with Binfield Dam, where a waterfall (<1 m at full dam level) inhibited upstream invasion. Species such as *M. salmoides, L. macrochirus, T. sparrmanii, C. carpio* and *O. mossambicus* also favour lentic habitats and the perennial flow regime of the Keiskamma River system headwater streams may be unsuitable, limiting their establishment in stream or river habitats. A study documenting before and after impoundment (Lake Texoma) fish assemblages in Buncombe Creek, Marshall, Oklahoma, Lienesch, Lutterschmidt & Schaefer (2000) noted that *M. salmoides*, *L. macrochirus* and *C. carpio* only persisted in the headwater stream in a large permanent pool. The lack of suitable habitat in the Tyume River above and below Binfield Dam may also preclude their establishment in the stream environment and explain their exclusivity to the impoundment. Contrary to findings from other studies (e.g. Godinho and Ferreira 2000; Adams et al. 2001), impoundments did not seem to facilitate invasion of headwater stream environments as there was little evidence of upstream or downstream dispersal of non-native fishes from Keiskamma impoundments.

As opposed to the high levels of abiotic resistance exhibited by Keiskamma River system headwater streams, native assemblages inhabiting these streams show little biotic resistance to invasion, and overlap between native and non-native species was low. Only *S. trutta, O. mykiss* and the native *A. mossambica* occurred in the upper reaches of the Tyume, Amatele, Mnyameni and Cata Rivers. Data from this study suggest that the absence of other native fishes in the upper reaches of the invaded upper Keiskamma River system streams is most likely to be a result of exclusion by the salmonids. Co-occurrence between *B. trevelyani*, *S. bainsii* and *O. mykiss* was also only observed at a single site.

Multivariate analyses of species and environmental relation identified three variables explaining species-environmental associations, altitude, temperature and silt. On the first axis the primary separation was on an altitudinal gradient, with both salmonids occurring at high altitudes, *B. trevelyani* and *B. anoplus* occurring at a range of altitudes and *S. bainsii*, *L. umbratus* and *A. mossambica* associated with lower altitudes. Separation of fish assemblages on an altitudinal gradient is expected as altitude may be a surrogate for numerous other variables such as temperature, rainfall, soils, geology and channel form (Rashleigh et al. 2009). On the second axis the native assemblage was positively associated and non-natives negatively associated with temperature, while all species except *B. anoplus* were negatively associated with habitats with a heavily silted substratum. Salmonids are ubiquitous in cooler headwater stream segments (Moyle et al. 2003) while *S. bainsii* and *L. umbratus* are more mainstream species, which reflects their association with higher temperatures. Separation of species assemblages on an environmental gradient did not, however, sufficiently account for the absence of native fishes from areas inhabited by salmonids.

These results are further supported by the classification tree, which indicated that the presence/absence of *B. trevelyani* was primarily negatively influenced by the presence of salmonids in the upper reaches and high silt percentage in the lower stream reaches. Direct predation by salmonids on *B. trevelyani* has been documented (Jubb 1967), and the consequent exclusion from large intact stream segments by salmonids has resulted in major habitat loss. The geography of headwater stream invasions is vitally important when attempting to elucidate the invasive impact of non-native species on native biodiversity (Adams et al. 2001). The introduction of salmonids into the upper reaches of headwater streams, in many instances above the natural distribution of fishes in the system, allows for downstream dispersal, and in some cases over major barriers that would inhibit upstream dispersal (Adams et al. 2001). In contrast to the typical South African invasion scenario where invasion originates from source populations in mainstream environments (see Chapter 3), in the Keiskamma River system salmonids were stocked in the headwaters often above waterfalls (Hey 2008). Salmonids then spread downstream to as far as their physiological and environmental tolerances allow (see Chapter 6), and, due to lack of overlap between salmonids and native fishes, limited *B. trevelyani* to lower stream reaches. Compounding the habitat loss in the upper reaches is the limitation of *B. trevelyani* to degraded stream reaches, as the species showed a negative association with silt.

The negative association of most species with silt indicates that Keiskamma headwater fishes are highly vulnerable to the habitat degradation which is commonplace below the escarpment zone of the Keiskamma River system (Mhangara and Kakembo 2012). Effects of fine sediment in lotic environments on fishes include: reduced growth, increased susceptibility to disease, clogging of the gills resulting in death, smothering of eggs inducing reproductive failure and lowered visibility resulting in decreased feeding efficiency and decreased prey abundance (decreased primary productivity) (Bruton 1985; Wood and Armitage 1997). It is unclear from the available data exactly what mechanisms exclude Keiskamma River fishes from degraded areas but it is most likely to be a combination of the above factors. The stenoecious nature of *B. trevelyani* resulting from the specific habitat requirements makes this species particularly vulnerable (Gaigher 1975). Another major consequence of the non-native fish invasion and impoundment of the upper Keiskamma system is the isolation and fragmentation of headwater stream fish populations, as inter-population dispersal boosts ecological resilience to local extinctions due to opportunities for recolonisation from unaffected stream reaches (Lowe and Likens 2005). The ‘multiple stressor’ effect (Ormerod et al. 2010) of various anthropogenic factors such as invasive species introductions, habitat degradation and modification (weirs and impoundments) also act synergistically to compound impacts on *B. trevelyani*.

In conclusion, there were multiple consequences of invasion and human-induced habitat degradation and modification that detrimentally affected *B. trevelyani* populations. The primary impact is range restriction and exclusion from invaded reaches by salmonids, forcing *B. trevelyani* into degraded unsuitable stream reaches. Impoundments containing predatory non-native fish probably act as sinks inhibiting *B. trevelyani* dispersal, which is also impacted by impoundment walls acting as impassable barriers resulting in fragmented and isolated distribution patterns. Looming threats to *B. trevelyani* include the development of fisheries based on non-native species in the catchment and continued habitat degradation through bad land use practices such as overgrazing. Development is inevitable, and therefore, providing information to ensure the transfer of sufficient information to conserve biodiversity is essential (Downes 2010). Possible solutions lie in reconciling the socio economic benefits of development against the importance of maintaining biodiversity.

CHAPTER 6: Does temperature limit the invasive potential of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* in the upper Keiskamma River system?

6.1 Introduction

In the context of invasion biology and ecology, the invasive potential of a non-native fish species is variable and governed by its ecological requirements and biological characteristics (Garcia-Berthou 2007; Olden et al. 2006). Temperature is an especially important abiotic factor determining the distribution of two of the most widely introduced salmonids: rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* (Crawford and Muir 2007; Fausch 2007; Townsend 1996), species typically associated with cooler environments (Eaton and Scheller 1996; Ebersole et al. 2001; Jonsson and Jonsson 2009; Wehrly et al. 2007). Temperature regulates a variety of critical biological processes in salmonids such as metabolic rates, physiology, growth, reproduction and behaviour (Boughton et al. 2007; Ebersole et al. 2001; Edwards et al. 1979; Forseth et al. 2009; Jonsson and Jonsson 2009; Kaeding 1996). A study on the relationship between stream thermal refugia and *O. mykiss* abundance by Ebersole et al. (2001) found that daily maximum ambient stream temperature was the only variable significantly correlated to *O. mykiss* density. Temperature regimes are therefore one of the major determinants for the successful establishment and invasive potential of introduced salmonids (Fausch 2007).

The thermal tolerances of *O. mykiss* and *S. trutta* have been well documented (Ebersole et al. 2001; Elliott and Elliott 2010; Forseth et al. 2009; Jonsson and Jonsson 2009; Wehrly et al. 2007). Temperatures for optimal growth of the two species were variable and dependant on a variety of biotic and abiotic factors, and ranged from 13 to 17 °C (Boughton et al. 2007; Edwards et al. 1979; Forseth et al. 2009). They also share similarities in their preferred range (<20 °C) (Boughton et al. 2007; Edwards et al. 1979; Forseth et al. 2009) and upper thermal limits (~24 °C) (Eaton et al. 1995). The temperature tolerances of fish populations are related to their genetic makeup as well as their thermal history or acclimation (Carline and Machung 2001). It has been suggested that salmonids are able to increase their upper thermal limits through acclimation, but recent evidence has refuted this (Elliott and Elliott 2010).

Oncorhynchus mykiss inhabiting rivers prone to temperatures exceeding 29 °C shows the same lethal limits as hatchery-reared fish under optimal conditions (Kaya 1977 in Elliott and Elliott 2010). The species tolerance of habitats prone to high temperatures was later shown to be a result of cool thermal refugia existing within the stream network (Kaeding 1996).

Salmonids were introduced into the upper Keiskamma River system in the late 1890s and early 1900s and are proposed to have an impact on native fishes. Due to their documented thermal limitations, it is vitally important to recorded temperature regimes in these streams to better explain what limits the distribution of salmonids and relate this to the threat they pose to native imperilled headwater fishes. In their introduced range *O. mykiss* and *S. trutta* do not exhibit acclimation to local temperatures, and display similar thermal tolerances to those in their native range (Elliott and Elliott 2010). It is therefore hypothesized that the salmonids *O. mykiss* and *S. trutta* would occupy a distributional range in the Keiskamma River system falling within their documented thermal tolerance range (Elliott and Elliott 2010). It also follows that current distribution of these two fishes in the upper Keiskamma River system reflect their invasive ability and may lead to predictions on future invasion potential in other river systems.

6.2 Materials and Methods

6.2.1 General

Thirty one temperature loggers (27 HOBO Pendant® temperature/light data loggers, 4 HOBO water temperature Pro v2 data loggers, Onset®) were placed at sites throughout the upper reaches of the Keiskamma River system (518-1074 metres above sea level), including headwater streams, mainstream environments and impoundments (Figure 6.1).

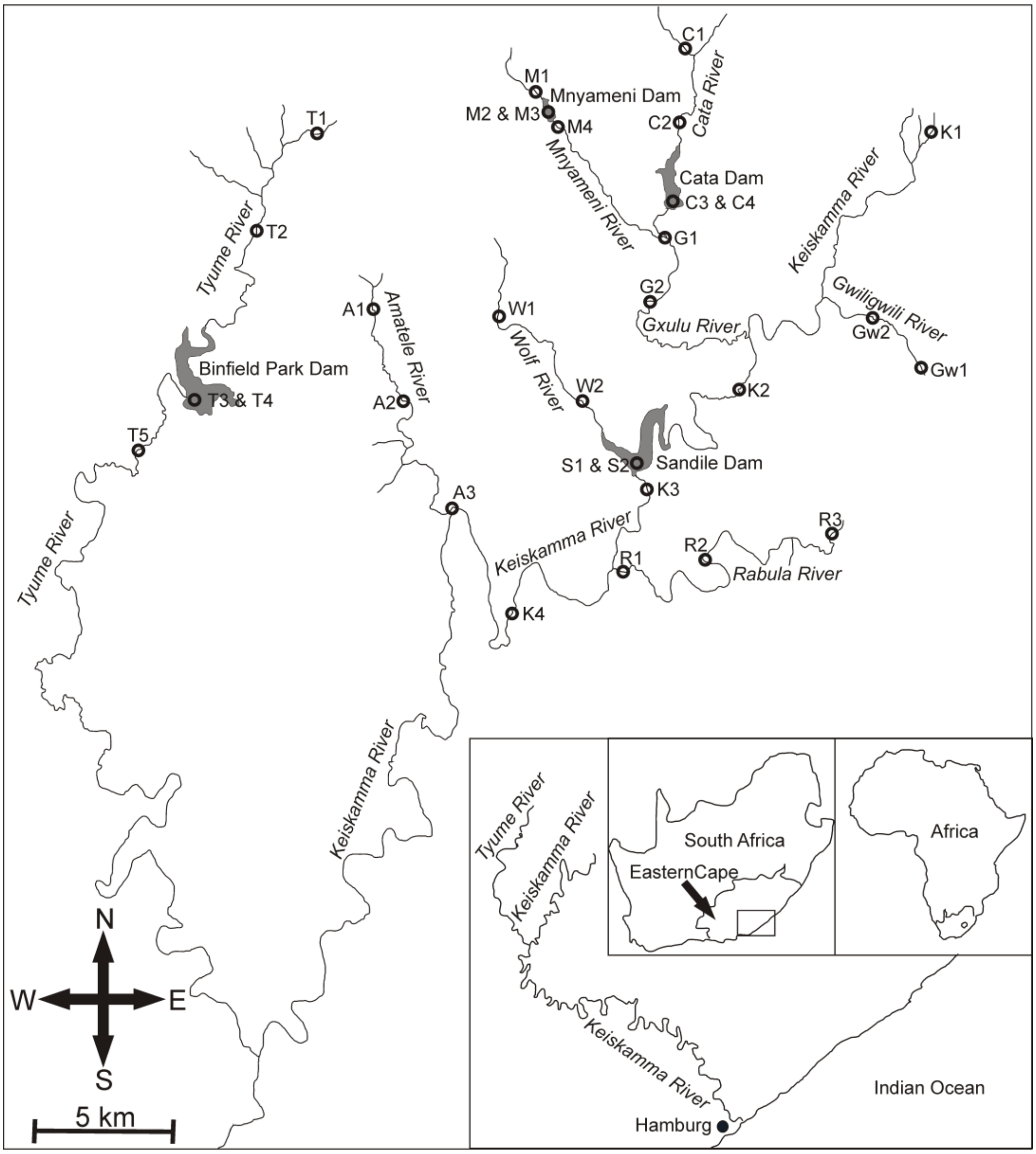


Figure 6.1 Site codes and location of the temperature loggers distributed throughout the upper reaches of the Keiskamma River system, Eastern Cape, South Africa.

These units are waterproof and are widely used to record water temperatures (Rivers-Moore et al. 2005; Rivers-Moore and Jewitt 2004). In river/stream habitats (lotic) loggers were attached to bolts drilled into large boulders which were placed on the stream bottom. Loggers ended up approximately 200-300 mm off the bottom, midstream. In some instances to avoid tampering by people or loss during floods this was altered and loggers were placed out of sight behind boulders or next to the stream bank under overhanging trees. In impoundments (lentic) loggers were attached to a rope at depths of 300 mm (shallow logger) and 2000 mm (deep logger) below the water surface. Temperatures were recorded hourly for the period 18/07/2011-10/12/2012 and loggers were downloaded approximately every three months.

6.2.2 Data analysis

Due to equipment failure, vandalism and floods, some loggers (n = 8) were either lost, stopped logging or had data gaps due to removal from the stream for extended periods. At one site no temperature data were downloaded (T2). The periods logged at each site are summarised in Table 6.1. Hourly temperature data were used to calculate daily means, ranges and absolute minimum and maximum temperatures for all sites, but for the analyses only sites with a full year's data were included. Seven-day moving averages were plotted for all sites where at least one successful download was undertaken.

A full years' (18/07/2011-18/07/2012) data were used for the following analyses: (1) Hourly temperature recordings were used to investigate the proportion of time (%) in each 1 °C interval for the study period, using frequency histograms (Rivers-Moore and Jewitt 2004). (2) Heating rate was summarised using cumulative degree day curves for temperatures greater than 20°C. (3) The following critical temperature threshold ranges of the non-native salmonids *O. mykiss* and *S. trutta* were explored: (TT1= the temperature of optimum growth for *S. trutta* and *O. mykiss* (<17 °C) (Boughton et al. 2007; Edwards et al. 1979; Forseth et al. 2009), TT2 = the temperature at which both species have been shown to seek out thermal refugia where temperatures are lower than the prevailing stream condition (20 °C) (Ebersole et al. 2001) and TT3 = the maximum weekly average temperature tolerance (>24 °C) (Eaton et al. 1995). The resulting three threshold ranges were <20 °C (TT1), 20 – 24 °C (TT2) and >24 °C (TT3). The ultimate upper incipient lethal temperature (UUILT) limit for salmonids is most often based on their ability to tolerate temperatures over a seven-day period (Eaton et al. 1995). Thermal thresholds were therefore calculated based on a seven-day moving average of the maximum daily water temperature using histograms of the frequency of exceedance (Rivers-Moore et al. 2005).

6.3 Results

Temperature logging period, site codes, position in the catchment and temperature ranges are summarised in Table 6.1. As expected, stream temperatures exhibited a seasonal sinusoidal pattern with the lowest winter temperatures recorded from June to August and summer peaks from December to February (Figure 6.2). Seven-day moving averages indicated that in all streams temperature increased gradually as downstream distance increases, and sites lower down in the catchment and impoundments had consistently higher temperatures than sites at higher elevations (Figure 6.2). Absolute minimum temperatures ranged from 3.8 °C (T1), 4.8 °C (M1) and 5.0 °C (C1) in the upper stream reaches to 9.9 °C (C3), 10.6 °C (T3) and 10.7 °C (T4) in the lower reaches and impoundments.

Table 6.1 The location, altitude, logging period, map code, salmonid presence/absence (p/a) and establishment (Est.) of the Hobo temperature loggers distributed throughout the upper reaches of the Keiskamma River system between 18/07/2011 and 10/12/2012 (*****Sites without a full year's data used in the analyses (18/07/2011-18/07/2012).

Map #	Site	Altitude	GPS Co-ordinates		Logging period	Absolute range	Mean SD	Salmonid p/a	Est.
A1	Amatele (forest)	671	S32 40 01.9	E26 59 18.0	18/07/2011-10/12/2012	5.35-22.33	13.4 ± 3.54	1	Yes
A2	Amatele (middle)	604	S32 41 41.8	E27 00 02.5	18/07/2011-10/12/2012	5.96-23.97	14.35 ± 3.91	0	No
A3	Amatele (lower)	504	S32 43 50.1	E27 01 21.8	18/07/2011-10/12/2012	5.96-28.75	16.09 ± 4.8	0	No
C1	Cata (forest)	874	S32 34 45.9	E27 07 36.5	18/07/2011-10/12/2012	5.04-21.95	13.03 ± 3.46	1	Yes
C2	**Cata (lower)***	**778**	**S32 36 32.2**	**E27 07 20.6**	**18/07/2011-24/03/2012**	**5.86-27.86**	**15.99 ± 4.25**	**1**	**No**
C3	Cata (shallow)	771	S32 37 50.1	E27 07 06.2	18/07/2011-10/12/2012	9.88-27.85	17.31 ± 4.39	1	No
C4	Cata (deep)	771	S32 37 50.1	E27 07 06.2	18/07/2011-10/12/2012	9.83-25.23	16.88 ± 4.08	1	No
G1	**Cata/Mnyameni confluence***	**717**	**S32 38 27.3**	**E27 06 43.5**	**18/07/2011-10/01/2012**	**6.67-29.45**	**16.02 ± 4.35**	**0**	**No**
G2	Gxulu (middle)	681	S32 39 44.1	E27 06 31.6	18/07/2011-10/12/2012	5.76-30.15	16.75 ± 4.78	0	No
Gw1	Gwiligwili (forest)	706	S32 40 35.5	E27 13 15.9	18/07/2011-10/12/2012	5.45-21.76	14.04 ± 3.54	0	No
Gw2	**Gwiligwili (lower)***	**683**	**S32 40 06.0**	**E27 12 24.7**	**18/07/2011-24/03/2012**	**6.37-22.53**	**15.77 ± 3.58**	**0**	**No**
K1	Keiskamma (upper)	839	S32 36 32.4	E27 13 51.1	18/07/2011-10/12/2012	6.06-23.2	13.95 ± 3.55	0	No
K2	Keiskamma (middle)	614	S32 42 41.3	E27 07 43.3	18/07/2011-20/10/2012	8.98-25.32	16.2 ± 3.65	0	No
K3	Keiskamma (below Sandile Dam)	551	S32 43 31.3	E27 06 26.9	18/07/2011-10/12/2012	8.98-26.49	14.61 ± 3.16	0	No
K4	Keiskamma (lower)	518	S32 45 35.0	E27 04 07.0	18/07/2011-10/12/2012	7.18-26.59	16.4 ± 4.55	0	No
M1	Mnyameni (forest)	938	S32 35 35.6	E27 03 34.6	18/07/2011-10/12/2012	4.83-22.24	12.61 ± 3.57	1	Yes
M2	Mnyameni (shallow)	912	S32 35 59.4	E27 03 52.3	18/07/2011-10/12/2012	8.05-26.33	16.22 ± 4.58	1	Yes
M3	Mnyameni (deep)	912	S32 35 59.4	E27 03 52.3	18/07/2011-10/12/2012	8.2-25.67	16.06 ± 4.37	1	Yes
M4	Mnyameni (below dam)	903	S32 36 10.7	E27 04 00.3	18/07/2011-10/12/2012	6.78-25.81	15.38 ± 4.61	0	No
R 1	Rabula (forest)	673	S32 44 22.1	E27 12 07.1	18/07/2011-10/12/2012	6.17-22.91	14.07 ± 3.61	0	No
R 2	Rabula (middle)	590	S32 44 55.7	E27 07 57.0	18/07/2011-10/12/2012	6.67-25.13	15.23 ± 4.38	0	No
R 3	Rabula (lower)	559	S32 45 08.3	E27 05 51.2	18/07/2011-10/12/2012	7.28-26.78	15.84 ± 4.59	0	No
S1	**Sandile (deep)***	**593**	**S32 43 09.3**	**E27 06 06.5**	**18/07/2011-02/10/2011**	**10.16-22.33**	**12.98 ± 2.66**	**0**	**No**
S2	**Sandile (shallow)***	**593**	**S32 43 09.3**	**E27 06 06.5**	**18/07/2011-02/10/2011**	**10.26-23.2**	**12.93 ± 2.49**	**0**	**No**
T1	**Tyume (forest)***	**1074**	**S32 36 25.5**	**E26 57 44.7**	**18/07/2011-22/03/2012**	**3.79-23.2**	**12.82 ± 3.85**	**1**	**Yes**
T2	**Tyume (middle)***	**712**	**S32 38 20.6**	**E26 56 11.1**	**-**	**-**	**-**	**0**	**No**
T3	Binfield (shallow)	661	S32 41 40.4	E26 54 23.0	18/07/2011-10/12/2012	10.55-30.86	18.66 ± 4.76	0	No
T4	Binfield (deep)	661	S32 41 40.4	E26 54 23.0	18/07/2011-10/12/2012	10.65-26	17.56 ± 4.16	0	No
T5	**Tyume (below Binfield Dam)***	**599**	**S32 42 42.9**	**E26 53 00.2**	**18/07/2011-22/03/2012**	**8.88-29.05**	**18.45 ± 4.65**	**0**	**No**
W1	Wolf (forest)	806	S32 39 45.5	E27 02 34.6	18/07/2011-10/12/2012	5.14-22.05	12.9 ± 3.7	1	Yes
W2	Wolf (lower)	631	S32 41 45.1	E27 04 45.6	18/07/2011-10/12/2012	5.45-24.55	14.24 ± 4.05	1	Yes

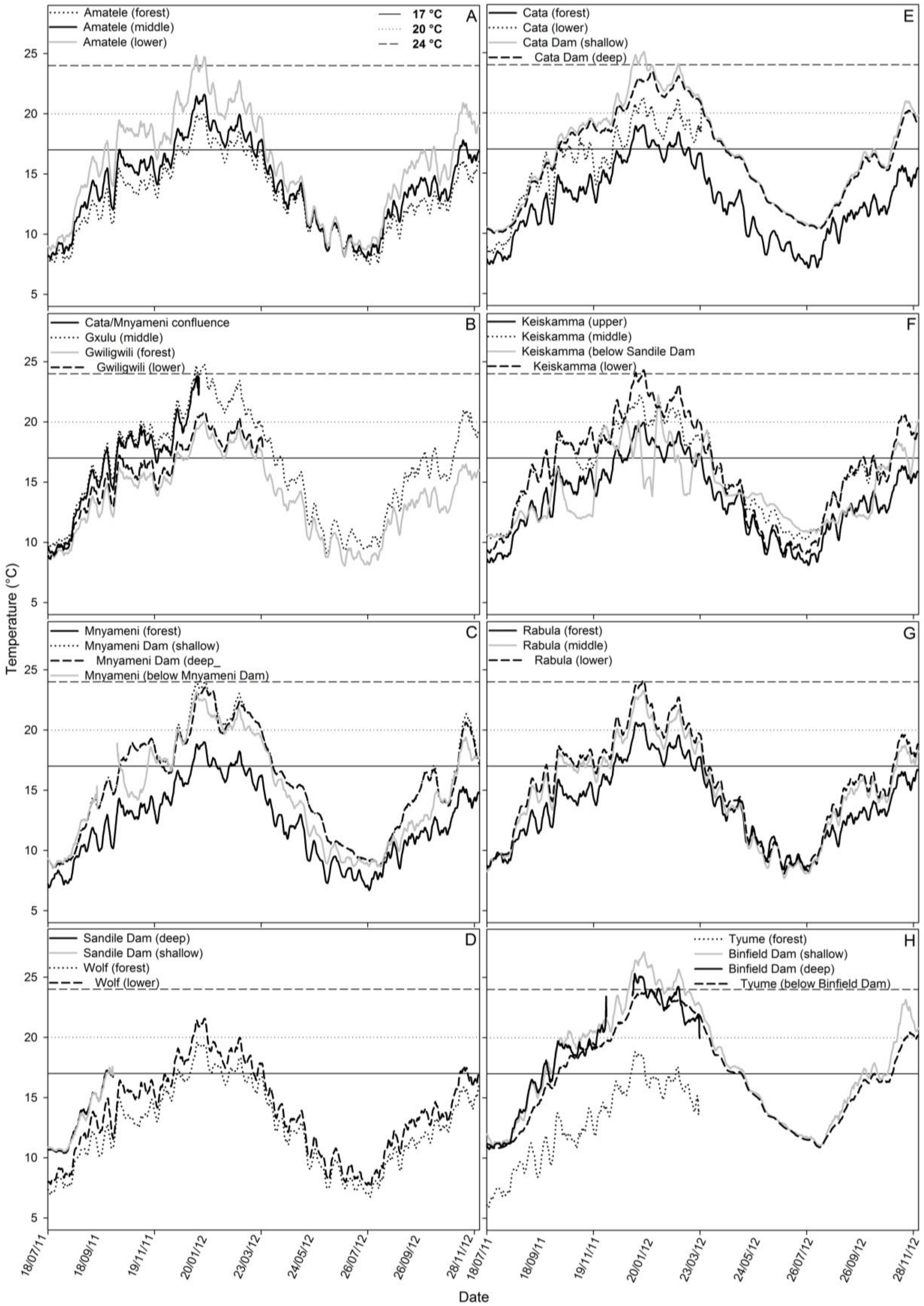


Figure 6.2 Seven-day moving average plots for all sites on the upper Keiskamma River system where at least one successful download was undertaken (18/07/2011-10/12/2012).

Absolute maximum temperatures were also lower in the upper reaches (Gw1 = 21.7 °C, C1 = 21.9 °C, W1 = 22.1 °C) and higher in the lower stream reaches and impoundments (A3 = 28.8 °C, T3 = 30.9 °C G2 = 30.2 °C). Frequency histograms showed similar trends, with the greatest proportion of time above the 20 °C threshold in the lower river reaches and impoundments (Rivers = A3, G2, C3 &4, K2, 3 & 4; W2; R2 &3, Impoundments = C3 & 4; M 3 & 4; T 3 & 4) (Figure 6.3).

The Mnyameni Dam was characterised by epilimnetic (surface) outflow and had larger differences between temperatures upstream (mean ± se; M1 = 12.6 ± 3.6 °C) and immediately downstream (M4 = 15.4 ± 4.6 °C) than the Sandile Dam. Sandile Dam releases hypolimnetic (bottom/deep) water which exhibited narrow ranges and was on average cooler downstream (K3 = 14.6 ± 3.2 °C) than upstream (K2 = 16.2 ± 3.7 °C; W2 = 14.2 ± 4.1 °C) of the impoundment. Heating rate in impoundments was slowest at M3 on the Mnyameni Dam (99 degree days) and highest at T3 on Binfield Dam (187 degree days) (Figure 6.4). In lentic habitats the number of days over the 20 °C thermal threshold for salmonids ranged from 3 degree days at site C1 on the Cata River to 145 degree days at A3 on the Amatele River (Figure 6.4).

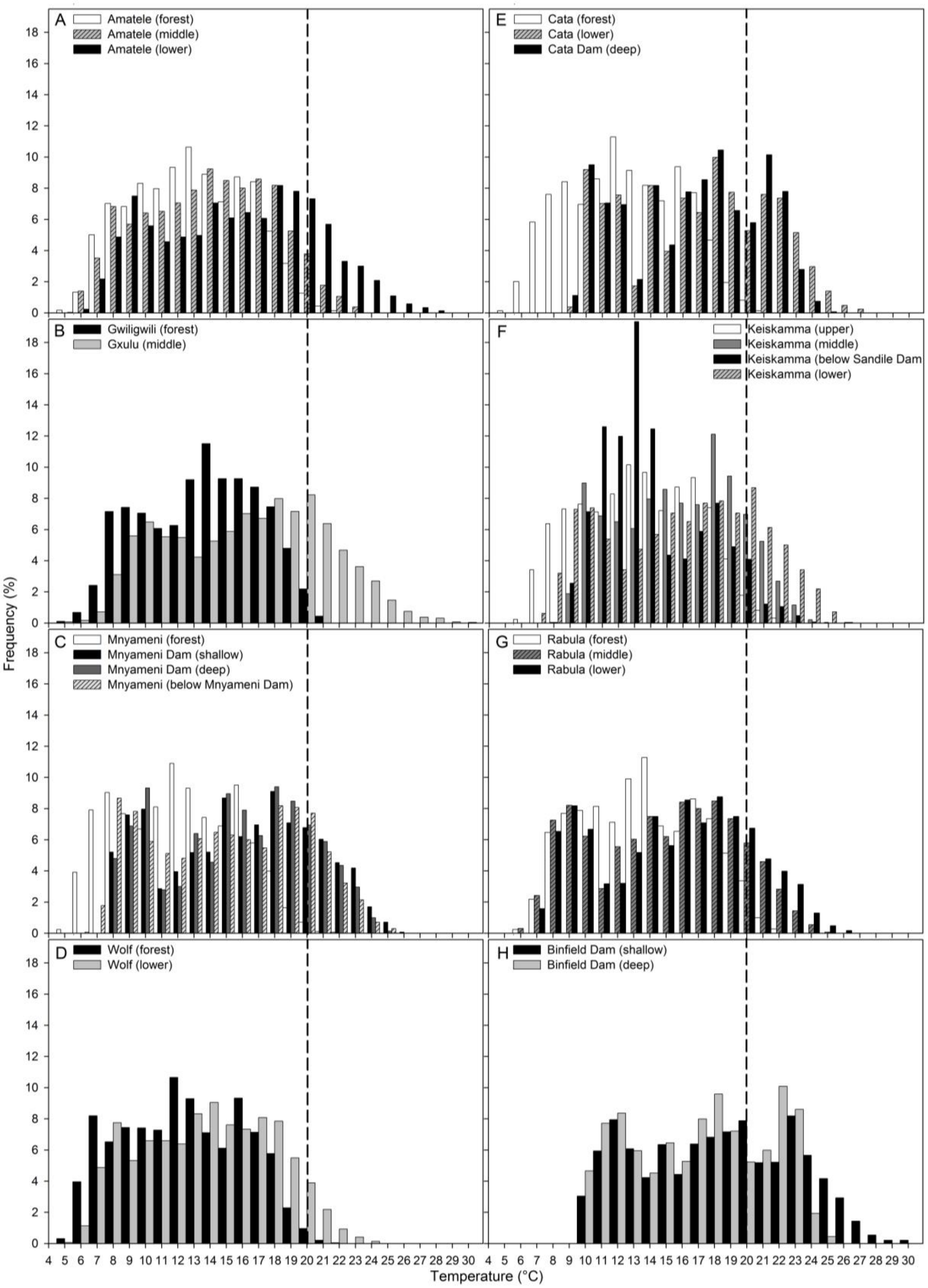


Figure 6.3 Hourly frequency plots of time spent in each one-degree temperature class for the range of temperatures experienced in the rivers and dams of the upper Keiskamma River system, Eastern Cape, South Africa (18/07/2011-10/12/2012). The dashed line at 20 °C indicates the temperature above which salmonids actively seek out cooler thermal refugia (Elliott and Elliott 2010).

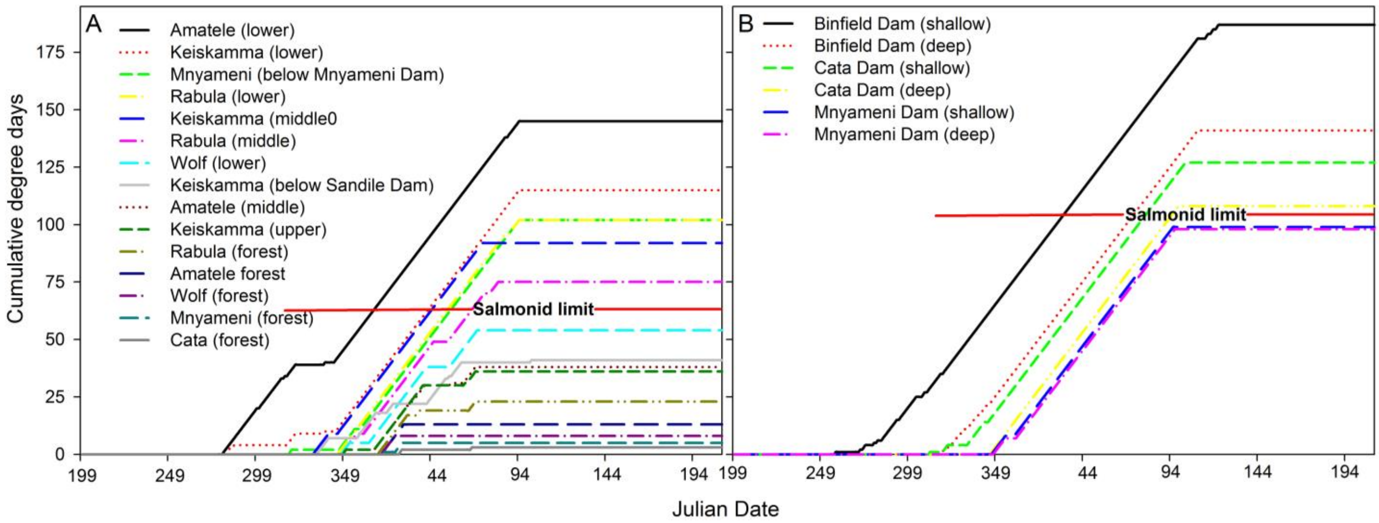


Figure 6.4 Cumulative number of days above the TT1 = 20 °C based on seven-day moving averages for the rivers (A) and impoundments (B) of the upper Keiskamma River system, Eastern Cape, South Africa (18/07/2011-18/07/2012).

Oncorhynchus mykiss was established in the Tyume, Amatele, Wolf and Mnyameni Rivers and *S. trutta* was only established in the Cata River. Salmonids were generally established at higher altitude sites with the lowest mean annual temperatures (M1 = 12.6 ± 3.6 °C, W1 = 12.9 ± 3.7 °C, C1 = 13.0 ± 3.5 °C, A1 = 13.4 ± 3.5 °C). The frequency of temperatures within the threshold ranges at sites where salmonids were present all had frequencies of >85 % in the preferred range (TT1), <15% in TT2 and never exceeded TT3 (Figure 6.5). Salmonids only occurred at sites with the least proportion of time >20 °C. In rivers and streams where salmonids were established the heating rate (the number of degree days >20 °C) was low and the total number of degree days above the 20 °C threshold did not exceed 55 degree days (W2 = 54 degree days). Impoundments facilitated tolerance of higher heating rates of up to 99 degree days (M3 & 4). This was further influenced by the position of the impoundment in the catchment and the Mnyameni Dam, despite being at an altitude of >900 metres above sea level, the epilimnetic outflow resulted in absence of *O. mykiss* immediately downstream.

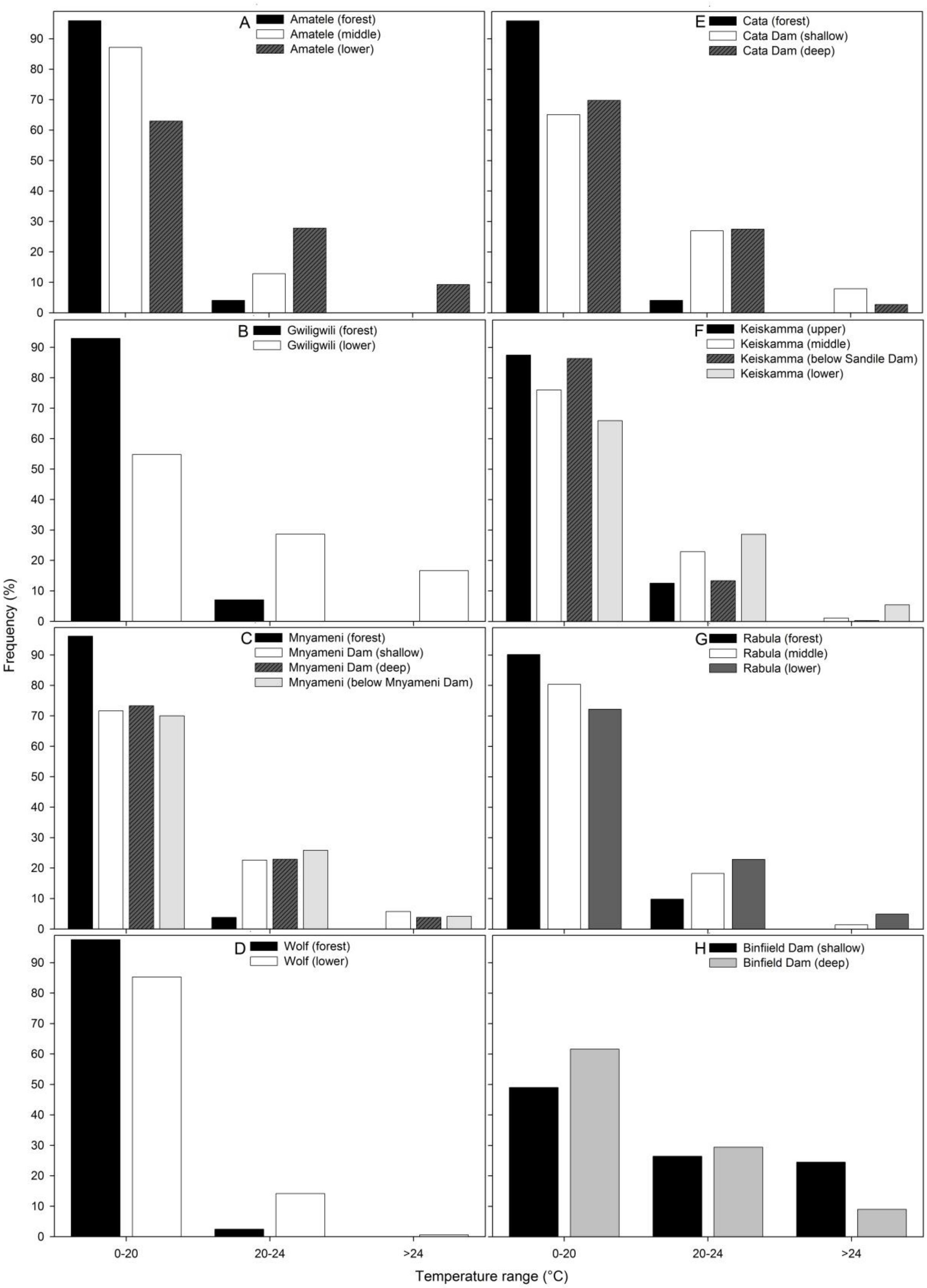


Figure 6.5 The frequency (%) of time spent in each of the three temperature ranges based on three threshold temperatures (TT1 = 17 °C, TT2 = 20 °C, TT3 = 24 °C) for *Salmo trutta* and *Oncorhynchus mykiss* in the rivers and dams of the upper Keiskamma River system, Eastern Cape, South Africa.

6.4 Discussion

Upper Keiskamma River system thermal ranges were extreme, with absolute minima as low as 3.8 °C and maxima of up to 30.9 °C. Both non-native salmonids *O. mykiss* and *S. trutta* are coldwater species (Eaton and Scheller 1996) which was reflected by their distribution patterns, showing similar thermal preference and upper tolerances to streams in their native (Eaton and Scheller 1996) and introduced ranges (Wehrly et al. 2007). Salmonids in the upper Keiskamma River system were limited to lotic sites with low frequencies of hourly temperatures >20° C, specifically they favoured stream reaches with TT1 >85%. This is consistent with findings from other studies which show that both species grow optimally between 13 °C and 17 °C (Boughton et al. 2007; Edwards et al. 1979; Forseth et al. 2009), and at 20 °C actively seek thermal refugia in areas of the stream where temperatures were 3-8 °C below ambient conditions (Ebersole et al. 2001). Interestingly, despite being introduced throughout the Keiskamma River upper reaches, *S. trutta* only ever established in the Cata River, while *O. mykiss* established in the Tyume, Amatele, Wolf and Mnyameni Rivers.

On comparison of sympatric and allopatric populations of *O. mykiss* and *S. trutta* in southern Appalachian mountain streams, sympatric populations exhibited competitive exclusion of *O. mykiss* by *S. trutta*, resulting in reduced population densities and growth (Gatz et al. 1987). This study did not however examine the role of temperature preferences or requirements in the outcome of competitive interactions. On average the Cata River was one of the cooler rivers and had the lowest heating rate in the system (3 degree days >20°C during the study period). In this study the establishment of allopatric populations suggests some competitive interaction between *O. mykiss* and *S. trutta*, but also abiotic influence. Under optimal conditions *S. trutta* may have been able to outcompete and exclude *O. mykiss* from the Cata River, but in the other Rivers that experienced higher temperatures *O. mykiss* may have had the survival advantage, as was observed by Bear et al. (2007), who documented the dominance of *O. mykiss* over westslope cutthroat trout *Oncorhynchus clarkii lewisi* at temperatures >20 °C. Despite no noticeable local adaptations or acclimation to increased temperatures, changes in salmonid distribution within the Keiskamma River system since their introduction have been noted.

The headwaters of the Keiskamma River system have been highly fragmented by the creation of impoundments and irrigation weirs throughout the system. Prior to the construction of impoundments and abstraction weirs in the system, salmonids were distributed throughout the Keiskamma River headwaters (Hey, 2008). Current salmonid distribution has largely contracted from their historic range and they are now limited to the headwaters of streams above impoundments. Impoundments are documented to modify natural flow and temperature regimes, resulting in highly altered downstream conditions (Dallas 2008; Tomasson et al. 1985). Due to increasing water demands for agricultural and domestic use in the 1970s and 1980s, four impoundments were constructed in the headwaters of the Keiskamma River system (Binfield Dam, Cata Dam, Mnyameni Dam, Sandile Dam). These impoundments subsequently rendered some stream segments unfavourable to salmonids due to changes in the thermal regime of the stream below the impoundments. The current absence of *O. mykiss* in the lower reaches of the Mnyameni River below the Mnyameni Dam supports this.

Historically, *O. mykiss* occurred in the Mnyameni River from the upper reaches all the way to the town of Keiskammahoek (Hey 2008). The outlet of the Mnyameni Dam is on the surface and as a result, warm epilimnetic water overflows into the stream below the dam. On average, temperatures in the pool directly below Mnyameni dam were 3 ºC warmer than the stream above the dam. Consequently the heating rate and degree days >20 ºC were 20 times higher below the dam than at the upstream site, despite being only 1.3 km apart. Contrasting patterns were observed above and below the Sandile Dam, where the downstream temperatures were lower than at upstream sites due to hypolimnetic releases. Position in the catchment (low altitude) and limited covering riparian vegetation may result in rapid heating to beyond the thermal tolerance of salmonids at the site 7.5 km downstream of Sandile Dam. If for some reason the depth of water releases from Mnyameni Dam were to change, and conditions downstream became suitable for salmonids, the imperilled *Barbus trevelyani* population currently finding refuge in this section of the river would be under severe threat. Impoundments aren’t the only factor that may have altered stream thermal regimes. Other factors such as degraded riparian zones, due to bad land use practices reducing land cover, may also promote generally higher temperatures in the catchment (Mhangara et al. 2012; Mhangara and Kakembo 2012), making the mainstream and lower reaches on the rivers unsuitable for salmonids.

In conclusion, temperature limits the invasive potential of salmonids in the upper Keiskamma River system. Unsuitable temperatures are a result of natural (downstream increases) and anthropogenic influences (impoundment construction, land use patterns). Data on the invasive species' thermal ranges is vital for multiple purposes. Firstly, it can be used to provide information on potential spread and invasion of headwater environments, and secondly the data can be used to advise on the threats and impacts of future salmonid introductions in headwater environments. Due to economic and social pressures, intentional stocking of salmonids in headwaters streams and impoundments is inevitable. While these headwater salmonid introductions have a major economic benefit in some regions (du Preez and Lee 2010) they can also cause significant impacts on recipient fish communities (Dunham et al. 2004; Townsend 1996). The data and analyses in this chapter have therefore provided vital information on the invasive potential of *O. mykiss* and *S. trutta*, which can be used for future non-native species management in South Africa. Particularly relevant is the finding that salmonids did not seem to display local acclimation and therefore future predictions may be based primarily on pre-existing thermal tolerance data. It must however be noted that salmonids are highly mobile and capable of extensive migrations (Meka et al. 2003), and the lack of temporal abundance data at the selected sites precludes analyses of finer scale distributions in relation to prevailing stream thermal regimes. Information on the occupied thermal range of *O. mykiss* and *S. trutta* provides important information to describe the distribution patterns of fishes in the upper Keiskamma River system in Chapter 5.

CHAPTER 7: Can non-native fish invasion impact on the genetic diversity of two imperilled headwater minnows?

7.1 Introduction

Invasive impact has been defined as a measurable loss of diversity or a change in ecosystem functioning (Gozlan et al. 2010). It is therefore necessary to quantify exactly what is meant by biodiversity before assessments of the potential impacts of non-native fishes can be done effectively. Major concern has been raised in the literature on the appropriate taxonomic level to focus biodiversity conservation efforts, as traditional taxonomy fails to recognise underlying population level genetic diversity (Avise 1989; Moritz 1994; Waples 1991). It has been widely recognised that simply conserving a species is insufficient and that including genetic diversity in conservation plans is vital to maximise their evolutionary fitness and long-term survival (Allendorf et al. 2013; Alves et al. 2001; Wofford et al. 2005). Effectively conserving biological diversity therefore requires the identification of the fitting conservation units that include an appropriate level of genetic diversity of a species (Allendorf et al. 2013).

Commonly used designations for protecting population level diversity are Evolutionary Significant Units. These were first proposed by Ryder (1986) as a means to prioritise preservation of adaptive genetic variation of a species in *ex-situ* breeding programs, for example large mammals in zoos. This concept was further developed as a means for including molecular genetics analyses for the management of biotic diversity (Avise 1989). There has been extensive debate on what constitutes an Evolutionary Significant Unit (Crandall et al. 2000; Fraser and Bernatchez 2001). For the purpose of this study the definitions of Moritz (1994) were applied for two defined conservation units, an Evolutionary Significant Unit and Management Unit. According to Moritz (1994) “Evolutionary Significant Units should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci” while Management Units “are populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles”. These conservation units have since been used by researchers to guide conservation efforts for numerous threatened stream fishes (Alves et al. 2001; Henriques et al. 2010; Sousa et al. 2010). This designation is particularly useful for species exhibiting highly fragmented distribution as a result of human-mediated activities such as the introduction and spread of non-native fishes and habitat modification and degradation (Alves et al. 2001; Henriques et al. 2010; Sousa et al. 2010; Stockwell et al. 1998). For example, on investigation of genetic relationships between four extant populations of White Sands pupfish *Cyprinodon tularosa*, Stockwell et al. (1998), discovered that two of the four extant populations had been introduced. The remaining two, however (Malpais Spring and Salt Creek), each constituted Evolutionary Significant Units and the loss of either would result in a significant loss of genetic diversity. This led the authors to prioritise conservation efforts for the Malpais Spring population which was the more threatened (Stockwell et al. 1998).

The Olifants River in South Africa is a system where non-native fish predation is the primary cause of the decline of native fishes (Lowe et al. 2008; Weyl et al. 2013). In this system, within-drainage patterns of genetic structuring varied between two of the threatened cyprinid redfin minnow species (fiery redfin *Pseudobarbus phlegethon* and Clanwilliam redfin *Barbus calidus*). This was postulated to be as a result of niche preference, behavioural and morphological differences (Swartz et al. 2004). *Pseudobarbus phlegethon* showed a major genetic divergence between tributaries draining into the Olifants and Doring catchments of the Olifants River system (Swartz et al. 2004). In contrast, *B. calidus* did not show differentiation, despite occurring in both catchments, often in sympatry with *P. phlegethon*. This indicates that isolation and fragmentation of these populations by non-native fish predation will have a greater impact on *P. phlegethon*, than on *B. calidus* genetic diversity, if one of the Evolutionary Significant Units were lost.

In the Eastern Cape Province of South Africa, two headwater stream species, Border barb *Barbus trevelyani* and Eastern Cape redfin *Pseudobarbus afer* are impacted by non-native fish invasion (Chapters 2, 3, 4 and 5). These invasions have caused local extinctions, resulting in restricted and fragmented distributions (see Chapter 3 and Chapter 5). In some cases entire streams are devoid of native fishes (e.g. Wolf and Cata Rivers in the Keiskamma River system; Chapter 5), or populations are limited to tiny headwater refugia (Blindekloof River in the Swartkops River system; Chapter 2 and Chapter 3). *Pseudobarbus afer* also shows major differentiation between currently isolated river systems (Swartz et al. 2009; Swartz et al. 2007). Continued invasive impacts may therefore cause significant loss of genetic diversity in *P. afer*. If *B. trevelyani* follows similar genetic patterns it might also be vulnerable to significant loss of genetic diversity due to invasion.

Knowledge of the geographic distribution of genetic diversity of native fishes will enable better quantification of non-native fish impact. For the purpose of the present study, the mitochondrial cytochrome *b* gene region was chosen as it is rapidly evolving and has been used successfully in *P. afer* to identify historically isolated lineages (equivalent to Moritz (1994) Evolutionary Significant Units) and to infer phylogenetic relationships between these lineages (Swartz et al. 2009; Swartz et al. 2007). The aims of this chapter were therefore to identify historically isolated lineages in *P. afer* (additional sites sampled to those collected by Swartz et al. (2007, 2009)) and *B. trevelyani*, and to map current distribution of genetic diversity. These data will then be used to infer the potential genetic impact non-native fish invasion may have on the two threatened minnows *P. afer* and *B. trevelyani* should further invasion of tributaries occur in the Swartkops and Keiskamma River systems.

7.2 Materials and Methods

7.2.1 Sampling

Specimens of *B. trevelyani* (n = 34) were collected from the Tyume, Amatele, Keiskamma, Mnyameni, Gwiligwili and Rabula Rivers, headwater tributaries of the Keiskamma River system. *Pseudobarbus afer* (n = 63) was collected from the Waterkloof, Fernkloof, Chaseskloof, Nounekkloof and Blindekloof Rivers, headwater tributaries of the Swartkops River system. Numbers and localities of fish sampled are presented in Appendix 1. The primary sampling method was electrofishing, supplemented with samples collected by dip-netting. Pieces of muscle tissue (from voucher specimens) or fin clips (from released fish) removed from the fish were placed in 95% ethanol in the field and stored in a -20 °C freezer upon return to the laboratory. Sample sizes were kept to a minimum (samples consisted predominantly of fin clips) due to the conservation status of both species (IUCN listed as Endangered) and voucher specimens (four individuals per stream) were lodged in the national fish collection at the South African Institute for Aquatic Biodiversity.

7.2.2 DNA extraction, amplification and sequencing

DNA was extracted from a subsample of collected tissue using two methods. Initially samples were extracted using the Wizard® Genomic DNA purification kit (Promega, USA) and were then eluted in a buffer solution. Due to low nucleic acid concentrations obtained using this method, samples were then extracted using the NucleoSpin® Tissue kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to manufacturer-specified protocols. Concentrations of nucleic acid from the extracted product were calculated using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific Inc.©). If nucleic acid concentration were <2 ng/µl the sample was re-extracted. A fragment of the mitochondrial DNA cytochrome *b* gene region was amplified (Polymerase Chain Reaction: PCR) using the primers GluF (5’ - AAC CAC CGT TGT ATT CAA CTA CAA - 3’) and ThrR (5’ - ACC TCC GAT CTT CGG ATT ACA AGA CCG - 3’). Amplification (PCR) of the cytochrome *b* gene region were conducted using a 96 Well Veriti Thermal Cycler (Applied Biosystems by Life Technologies, Carlsbad, California) with the reaction mixture (20 µl total) of 10 µl KAPA ready mix (Kapa Biosystems©), 0.5 µl of each primer (GluF and ThrR), 3 µl of DNA and 6 µl oh H_2O. Polymerase chain reactions (PCR) were undertaken according to Swartz et al. (2009) as follows: an initial denaturation of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C and 45 s at 72 °C and a final extension of 5 min at 72 °C. Polymerase Chain Reaction products were sent to Macrogen Inc. (Seoul, Korea) for purification (Montáge PCR filter units, Millipore) and sequencing (Applied Biosystems 3730xl DNA Analyser, Applied Biosystems by Life Technologies, Carlsbad, California).

7.2.3 Data analysis

Sequences were checked, cleaned and trimmed using SEQMAN ver 7.2.1 (DNASTAR, Lasergene, Seqman Pro). Sequences were then aligned using ClustalX 2.0.11 (Larkin et al. 2007) and the number of haplotypes that were shared or unique were calculated using DnaSP 5.10 (Librado and Rozas 2009). To estimate the best fit model for nucleotide substitution, which allows for the calculation of probabilities of change between nucleotides along the branches of a phylogenetic tree jModelTest was used with Akaikes Information Criterion (AIC) (Posada 2008). Maximum likelihood analyses were then run in PAUP* (Swofford 2003) using the models suggested by jModelTest. Bayesian analyses were then performed in MrBayes (Huelsenbeck and Ronquist 2001). Data collected during the present study were complemented with published sequences of closely related *P. afer* and *B. trevelyani* individuals used for phylogeny construction and outgroups (summarised in Table 7.1 and Table 7.2) (Machordom and Doadrio 2001; Swartz et al. 2009; Tsigenopoulos et al. 2002).

Table 7.1 Details of the outgroup and sequences from other closely related cyprinids used to construct the phylogeny for *Pseudobarbus afer* (Machordom and Doadrio 2001[1]; Swartz et al. 2009[3]; Tsigenopoulos et al. 2002[2]).

Species	River	System	Genbank #
P. afer[3]	Blindekloof	Swartkops	
P. afer[1]	Blindekloof	Swartkops	AF287449
P. afer[1]	Swartkops	Swartkops	AF287450
P. afer[2]	Blindekloof	Swartkops	AF180851
P. afer[3]	Kaaimans	Kaaimans	
P. afer[3]	Klein Brak	Klein Brak	
P. afer[3]	Klein Brak	Klein Brak	
P. afer[3]	Kaboega	Sundays	
P. afer[3]	Wit	Sundays	
P. afer[3]	Krom	Krom	
P. afer[3]	Kabeljous	Kabeljous	
P. afer[3]	Ys	Ys	
P. afer[3]	Tsitsikamma	Tsitsikamma	
P. asper[3]	Groot	Gamtoos	
P. phlegethon[3]	Breekrans	Olifants	
P. phlegethon[3]	Driehoeks	Olifants	
P. phlegethon[3]	Noordhoeks	Olifants	
P. phlegethon[1]	Noordhoeks	Olifants	AF287452
P. phlegethon [3]	Rondegat	Olifants	

Table 7.2 Details of the outgroup and sequences from other closely related cyprinids used to construct the phylogeny for *Barbus trevelyani* (Machordom and Doadrio 2001[1]; Tsigenopoulos et al. 2002[2]**)**.

Species	River	System	Genbank #
Pseudobarbus burchelli[1]	Breede	Breede	AF180848
Barbus andrewi[1]	Breede	Breede	AF180843
Barbus serra[2]	Olifants	Olifants	AF287446
Barbus serra[2]	Olifants	Olifants	AF287447
Barbus serra[1]	Stellenbosch		AF180844
Barbus erubescens[1]	Twee	Olifants	AF180845
Barbus calidus[1]	Noordhoeks	Olifants	AF180846
Barbus calidus[2]	Olifants	Olifants	AF287422
Barbus calidus[2]	Noordhoeks	Olifants	AF287423
Barbus trevelyani[1]	Buffalo	Buffalo	AF180847

7.3 Results

7.3.1 Pseudobarbus afer

Analyses of the mtDNA cytochrome *b* region for 63 *P. afer* using 675 bp sequences revealed six haplotypes with four variable sites. All localities within the Swartkops River system shared a common haplotype (S1) and two haplotypes, S6 and S7, were exclusive to Nounekkloof and Blindekloof respectively (Table 7.3). Haplotype S2 (Blindekloof, Waterkloof), S3 (Chaseskloof, Fernkloof), S4 (Blindekloof, Fernkloof, Swartkops and Nounekkloof) and S5 (Fernkloof and Waterkloof) were shared among localities (Table 7.3). The two haplotypes from the Sundays River system (Su8 and Su9) were both exclusive (Table 7.3).

Table 7.3 Haplotype frequencies for 63 *Pseudobarbus afer* from the Swartkops River and two from the Sundays River systems, Eastern Cape, South Africa (*supplementary data obtained from Swartz et al. 2009).

		Swartkops River						**Sundays River**	
Haplotype	**N**	**Blindekloof (6)**	**Chaseskloof (6)**	**Fernkloof (22)**	**Swartkops (4)**	**Nounekkloof (6)**	**Waterkloof (19)**	**Kaboega (1)**	**Wit (1)**
S1	40	4	5	15	2	3	11		
S2	5	1					4		
S3	6		1	5					
S4	9	1		1	2	2			
S5	4			1			3		
S6	1					1			
S7	1	1*							
Su8	1							1*	
Su9	1								1*

jModeltest (Posada 2008) indicated that the TIM1+G model of nucleotide substitution best fitted the data. Maximum likelihood calculated sequence divergences for within the Swartkops River system and between the four major *P. afer* lineages (Swartz et al. 2009) are summarised in

Table 7.4 and Figure 7.1. Localities sampled from the Swartkops River system during this study grouped within the *P. afer* Mandela lineage (Figure 7.2). Sequence divergences within the Swartkops River system were low (0.15-0.44%). Within the Mandela Lineage of *P. afer*, minor structuring was evident between the Swartkops and Sundays River systems (0.15-0.74%). There were high levels of sequence divergence between the Swartkops River system *P. afer* and the Forest, Krom and St Francis lineages (4.89-6.67%). Sequence divergence between *P. afer* and *P. phlegethon* was high (4.89-5.63), but lower than that of *P. afer* and *P. asper* from the Groot River (8.30-8.74%). Bayesian posterior probability support for the phylogeny was 100% for the Mandela, Forest and *P. phlegethon* lineages and low for the St Francis lineage (58%).

Table 7.4 Summary of maximum likelihood obtained sequence divergences (%) for *Pseudobarbus afer* and the closely related *Pseudobarbus phlegethon* and *Pseudobarbus asper* within and between river systems.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1. *P. afer*	0.28						
Swartkops River	(0.15-0.44)						
2. *P. afer*	0.47						
Sundays	(0.15-0.74)	0.59					
3. *P. afer*	6.40	6.52	2.02				
Forest Lineage	(6.07-6.67)	(6.22-6.82)	(0.30-2.82)				
4. *P. afer*	5.08	5.33	5.11				
St Francis Lineage	(4.89-5.19)	(5.19-5.48)	(4.89-5.33)				
5. *P. afer*	5.35	5.48	5.93				
Krom Lineage	(5.19-5.48)	(5.33-5.63)	(5.63-6.37)	4.00			
6. *P. asper*	8.57	8.74	8.89				
Groot River	(8.30-8.74)	(8.59-8.89)	(8.30-9.33)	7.70	8.44		
7. *P. phlegethon*	5.26	5.28	5.14	4.84	5.83	8.94	1.09
Olifants River	(4.89-5.63)	(5.04-5.78)	(4.44-5.93)	(4.59-5.19)	(5.63-6.07)	(8.59-9.48)	(0.74-1.48)

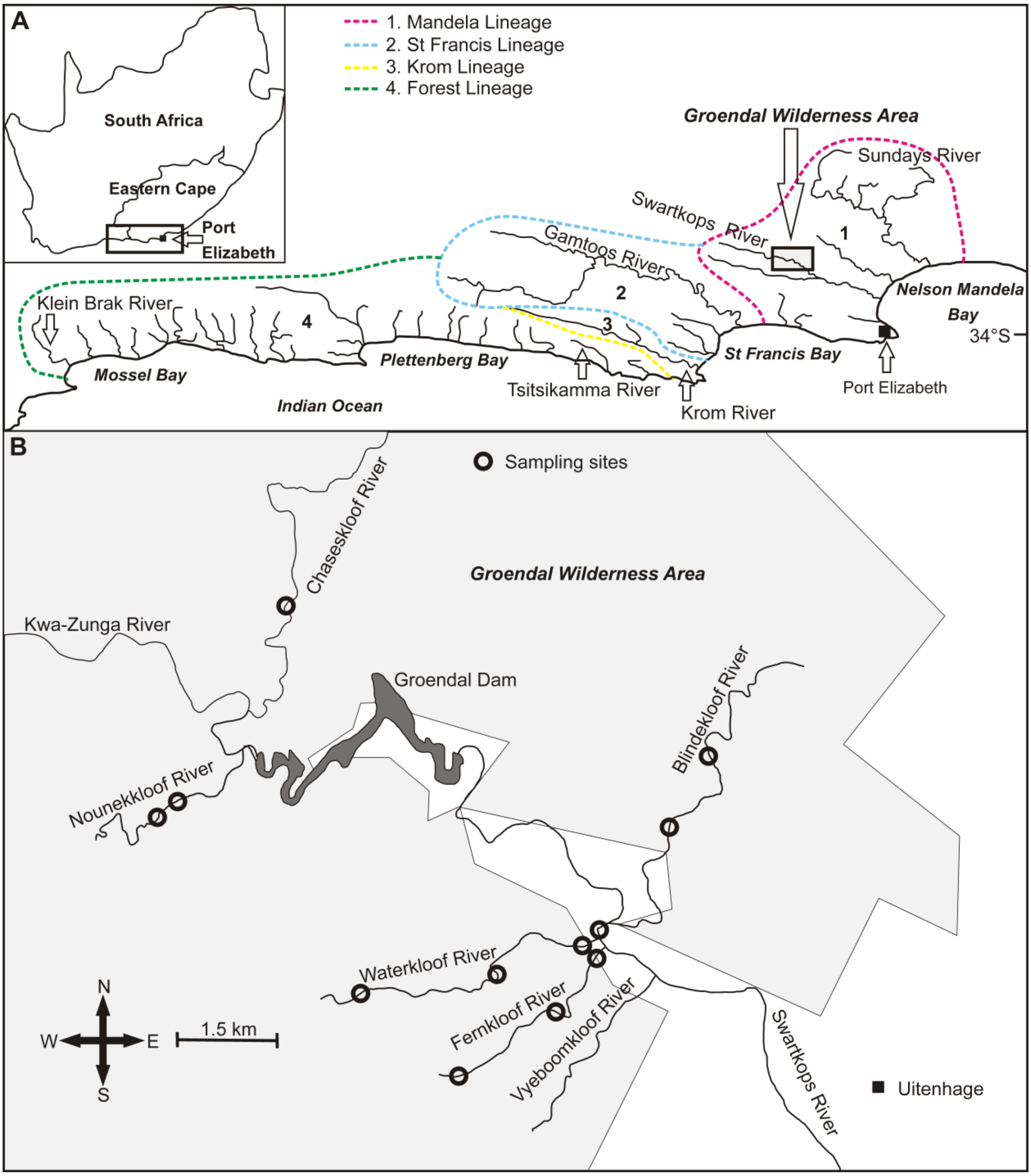


Figure 7.1 A) A map of the *Pseudobarbus afer* lineages comprising the species complex (Swartz et al. 2009; Swartz et al. 2007). B) Localities and streams sampled during this study within the upper Swartkops River system, Eastern Cape, South Africa.

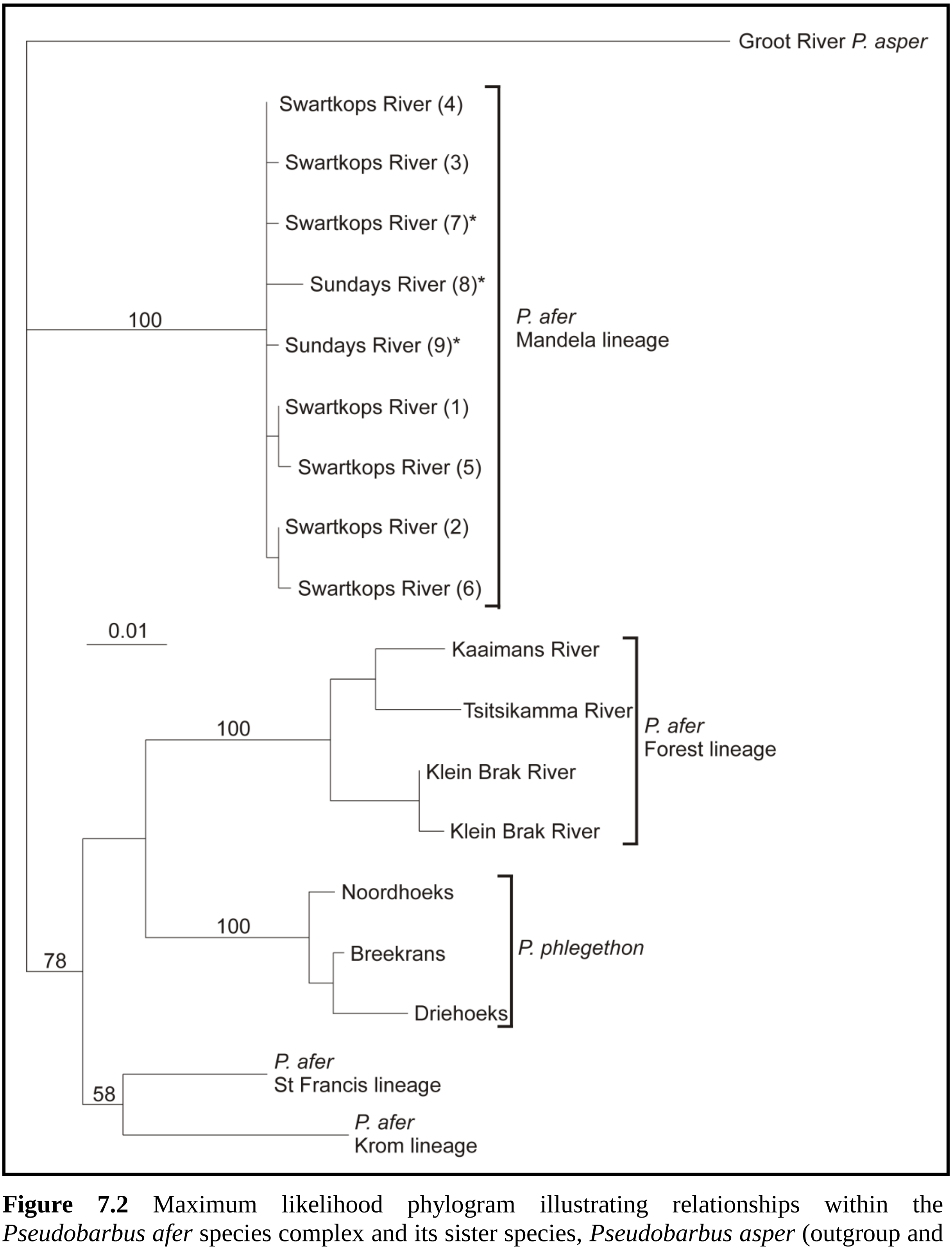


Figure 7.2 Maximum likelihood phylogram illustrating relationships within the *Pseudobarbus afer* species complex and its sister species, *Pseudobarbus asper* (outgroup and published sequences from Machordom and Doadrio 2001; Tsigenopoulos et al. 2002; Swartz et al. 2009). Bayesian posterior probabilities are reported above the branches.

7.3.2 *Barbus trevelyani*

Thirty-four *B. trevelyani* sequences of 702 bp from the Keiskamma River system were analysed, consisting of 14 haplotypes and 27 variable sites. Haplotype frequencies for *B. trevelyani* from the Keiskamma and Buffalo River systems are summarised in Table 7.5. Haplotype K3 was shared among all localities except the Tyume River and haplotype K1 was shared between the Rabula and Keiskamma Rivers. The remaining 12 Keiskamma River system haplotypes were exclusive. Haplotype B15 from the Buffalo River was also exclusive.

Table 7.5 Haplotype frequencies for 34 *Barbus trevelyani* sampled from the Keiskamma River and one from the Buffalo River, Eastern Cape, South Africa. (*supplementary data obtained from Tsigenopoulos et al. 2002).

Haplotype	***N***	**Tyume (10)**	**Amatele (5)**	**Mnyameni (5)**	**Gwiligwili (5)**	**Keiskamma (6)**	**Rabula (3)**	**Buffalo (1)**
K1	2					1	1	
K2	1					1		
K3	12		2	4	3	1	2	
K4	1				1			
K5	1					1		
K6	1		1					
K7	1					1		
K8	4	4						
K9	1		1					
K10	6	6						
K11	1			1				
K12	1					1		
K13	1		1					
K14	1				1			
B15								1*

The TIM1+G model of nucleotide substitution was recommended by jModeltest (Posada 2008). Sequence divergences and the *B. trevelyani* maximum likelihood phylogram are presented in Table 7.6 and Figure 7.3. Structuring within *B. trevelyani* was evident and three lineages were identified by maximum likelihood analyses, a Buffalo River lineage and two lineages within the Keiskamma River system, KEISKAMMA WIDE lineage (Keiskamma, Rabula, Amatele, Gwiligwili and Mnyameni Rivers) and the TYUME lineage (Tyume River). Sequence divergence between the Buffalo River and TYUME was high (3.85-3.98%), but lower than between Buffalo River lineage and KEISKAMMA WIDE lineage (4.13-4.84%). Sequence divergences were lowest within the TYUME lineage (0.14%) and highest for the KEISKAMMA WIDE lineage (0.14-1.28%). Sequence divergence was, however, high between the KEISKAMMA WIDE and TYUME lineages ranging from 1.43-2.14% (Figure 7.4). Divergences between *B. trevelyani* and other closely related cyprinids ranged from 11.11% (*B. calidus*) to 13.83% (*P. burchelli*). Bayesian posterior probability support was generally high with 100% support for *B. trevelyani*, 74% for the Keiskamma River system, 76% for the KEISKAMMA WIDE lineage and 95% for TYUME lineage.

Table 7.6 Summary of maximum likelihood obtained sequence divergences (%) for *Barbus trevelyani* within its distribution range and between other closely related South African cyprinids (Machordom and Doadrio 2001; Tsigenopoulos et al. 2002).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. *P. burchelli* Breede River								
2. *B. andrewi* Breede River	13.96							
3. *B. serra* Olifants River	13.30 (13.11-13.39)	2.33 (2.28-2.42)	0.43					
4. *B. callidus* Olifants River	11.92 (11.82-11.96)	8.69 (8.55-8.83)	9.02 (8.69-9.40)	0.47 (0.14-0.71)				
5. *B. erubescens* Twee River	12.54	8.55	8.45 (8.41-8.55)	4.08 (3.85-4.42)				
6. *B. trevelyani* Buffalo River	12.82	13.53	13.30 (13.11-13.39)	11.59 (11.40-11.82)	12.11			
7. *B. trevelyani* Keiskamma River KEISKAMMA WIDE	13.05 (12.82-13.68)	12.62 (12.25-13.11)	12.67 (12.25-13.25)	11.49 (11.11-11.82)	12.04 (11.82-12.39)	4.33 (4.13-4.84)	0.57 (0.14-1.28)	
8. *B. trevelyani* Keiskamma River TYUME	13.82 (13.82-13.83)	12.68 (12.68-12.69)	12.73 (12.54-12.83)	11.78 (11.68-11.84)	12.11 (12.11-12.12)	3.92 (3.85-3.98)	1.78 (1.43-2.14)	0.14

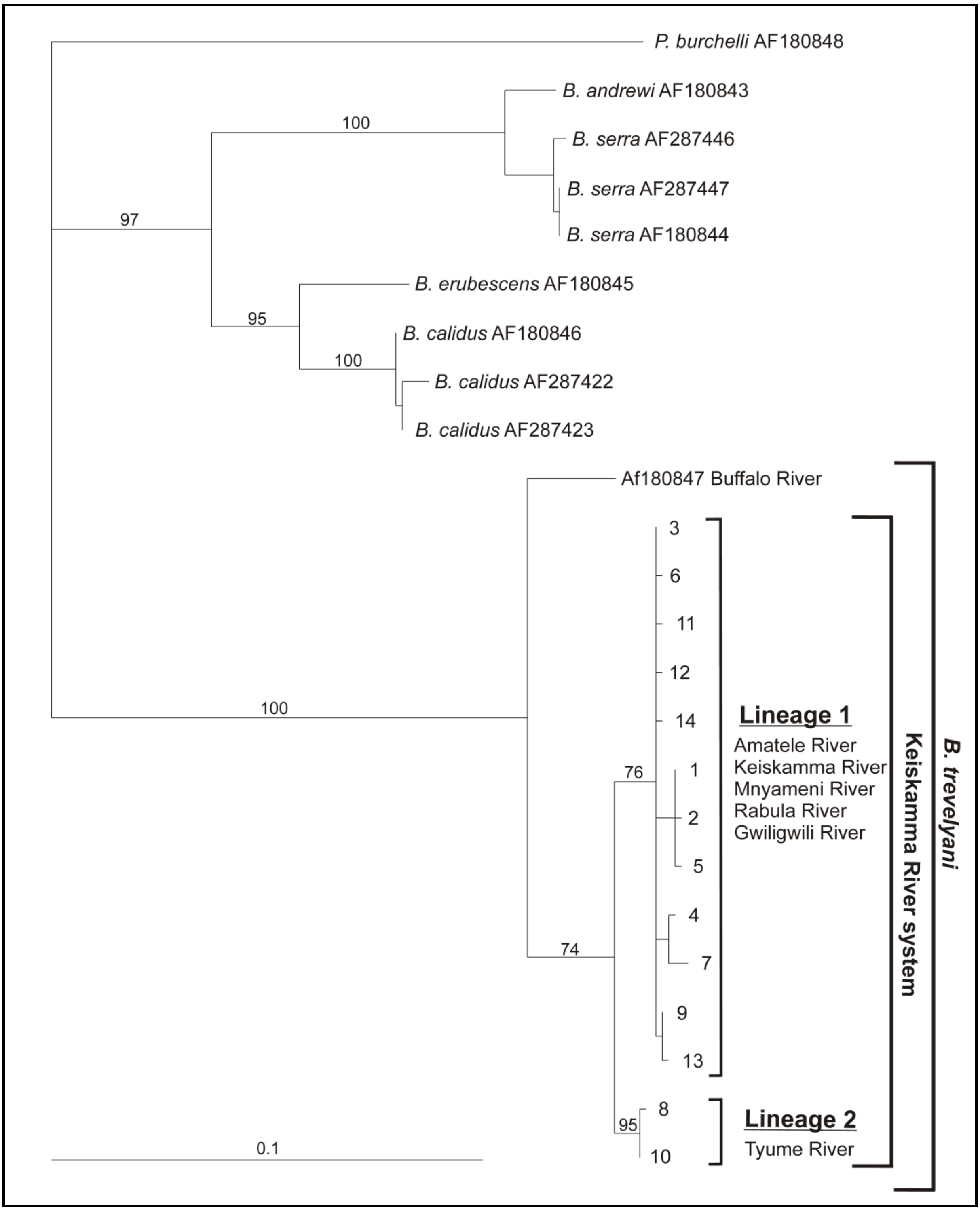


Figure 7.3 Maximum likelihood phylogram illustrating relationships within *Barbus trevelyani* from the Keiskamma and Buffalo River system and between other closely related South African cyprinids (Machordom and Doadrio 2001; Tsigenopoulos et al. 2002). Bayesian posterior probabilities are reported above the branches.

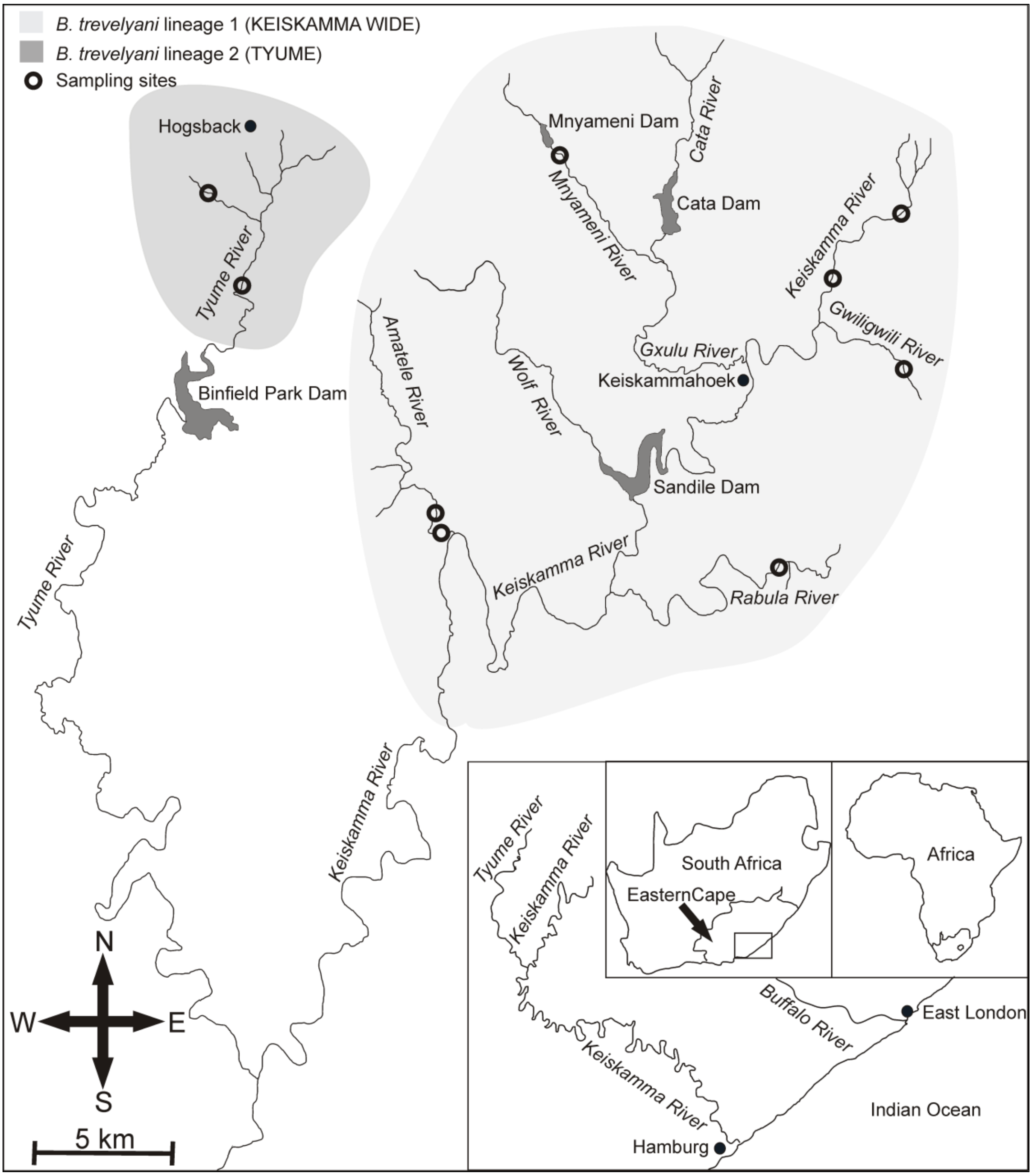


Figure 7.4 A map of the upper Keiskamma River system illustrating the sampling sites and the two lineages (KEISKAMMA WIDE and TYUME) of *Barbus trevelyani* within this system.

7.4 Discussion

7.4.1 Genetic structuring and divergence

Similar patterns of genetic structuring were evident for *P. afer* (Swartz et al. 2009; Swartz et al. 2007) and *B. trevelyani* with significant genetic divergences between currently isolated river systems. All the *P. afer* samples collected fell within the Mandela Lineage as proposed by (Swartz et al. 2009; Swartz et al. 2007). *Pseudobarbus afer* show extensive structuring between drainages and constituted a species complex with four major lineages, the Mandela Lineage, Forest Lineage, Krom Lineage and St Francis Lineage (Figure 7.1). Within the Mandela Lineage, *P. afer* also exhibited minor structuring between the Swartkops and Sundays Rivers (up to 0.74%) and each was represented by unique haplotypes. The Swartkops and Sundays populations have been isolated since at least the Last Glacial Maximum about 18000 years ago, when the two river systems possibly shared a common confluence (Swartz et al. 2007). Despite this relatively recent isolation and the minor divergence between haplotypes, the contribution of the two river systems to the overall genetic diversity is nonetheless significant and worthy of a separate conservation Management Unit.

The low levels of genetic differentiation and sharing of one haplotype among all *P. afer* populations from the upper Swartkops River tributaries suggest one Management Unit for this region (according to Moritz (1994)). These results are not entirely surprising due to the small geographic distances between these populations (all within 15 km along the watercourse). The continuous distribution of *P. afer* from the upper reaches of the tributary streams to the confluence with the mainstream Swartkops River, sporadic occurrence of the species in the mainstream and the lack of natural barriers that can prevent gene flow between populations supports this designation as a single Management Unit. Temporal abundance data from the Blindekloof River presented in Chapter 4 also support this inference as there was a net movement of adult *P. afer* downstream during high flow periods, which illustrate the ability of the species to disperse throughout the stream network.

In contrast to the results found for *P. afer*, *B. trevelyani* did not show extensive divergence between only river systems, but also within the Keiskamma River system. Each of the three lineages identified constituted important conservation units: the Buffalo, KEISKAMMA WIDE and TYUME lineages, consistent with the Moritz (1994) definition for an Evolutionary Significant Unit. No haplotypes were shared between the three lineages, and divergence between the Buffalo River *B. trevelyani* and those from the Keiskamma River system was extremely high (4%). This high level of divergence corresponds to other species level divergences, as observed between *P. afer* and *P. phlegethon,* two relatively closely related cyprinids also included in this study (see Swartz et al. 2009; Figure 7.3). From the reconstruction of paleo river systems it seems that the Keiskamma and Buffalo River systems may not have shared a common confluence during the Last Glacial Maximum (E. Swartz pers. comm.), which could have caused the relatively long period of isolation between *B. trevelyani* from the two river systems. In contrast, the Swartkops and Sundays River *P. afer* populations showed <1% genetic divergence. These two river systems may have shared a common confluence during the low sea levels of the Last Glacial Maximum (Swartz et al. 2009). The two lineages within the Keiskamma River system showed less divergence, but the >2% divergence still suggests that KEISKAMMA WIDE and TYUME have been isolated from each other for a long time. Despite suspected relatively recent gene flow between streams of the KEISKAMMA WIDE lineage, unique haplotypes were recorded from a number of streams, indicating that there may also be minor structuring within this lineage warranting further investigation.

7.4.2 Potential impact of non-native species on genetic diversity and conservation implications

Non-native fish invasion of the Swartkops and Keiskamma River systems has resulted in isolated and fragmented *P. afer* and *B. trevelyani* populations (see Chapter 3 and Chapter 5). The most severe impacts are likely to come from the *B. trevelyani* TYUME lineage (Tyume River). Within the Keiskamma River system, current findings highlight the need for immediate management interventions to conserve the *B. trevelyani* TYUME lineage from extinction. The TYUME lineage is not abundant (least abundant of all stream populations; Chapter 5) and is limited to ~10 km of the Tyume River in areas highly degraded by overgrazing, cultivation of the riparian zone and consequent erosion (Chapter 5). The comparatively pristine upper reaches of the Tyume River are also inhabited by non-native *Oncorhynchus mykiss* that prevent *B. trevelyani* from occupying these areas (Chapter 5). The absence of *B. trevelyani* from the Cata and Wolf Rivers (only occupied by *S. trutta* and *O. mykiss*, respectively) suggests that non-native salmonids have the ability to extirpate entire *B. trevelyani* populations and may prevent recolonisation in areas where they currently occur. With minor structuring and unique genetic diversity in the streams of the *B. trevelyani* TYUME lineage, the threat of local extinctions and a loss of historically evolved genetic diversity may be high. The high levels of genetic divergence between the Buffalo and

Keiskamma River systems and within the Keiskamma River systems also indicate that a taxonomic revision of *B. trevelyani* will be necessary.

For the Swartkops *P. afer* populations and the KEISKAMMA WIDE lineage of *B. trevelyani*, isolating populations that experienced recent gene flow may restrict vital connectivity. Lack of gene flow between these stream populations can lead to general effects of inbreeding if population size is too small, which can result in lower viability and survival (Couvet 2002; Morita and Yamamoto 2002; Vrijenhoek 1998; Vrijenhoek et al. 1985). In Camp Creek, a small headwater stream in western Oregon, fragmentation and isolation of cutthroat trout *Oncorhynchus clarki clarki* resulted in reductions of genetic diversity (Wofford et al. 2005). Each *P. afer* and *B. trevelyani* headwater stream population in the Swartkops and Keiskamma River systems is limited to short stream segments in areas with limited invasibility by non-native fishes. Unfortunately data on effective population sizes are unavailable, but it is likely that these populations are now more vulnerable than when they occupied larger segments of stream network prior to invasion. Restoring connectivity and gene flow between stream populations needs to be weighed against removing barriers to dispersal which currently protect *P. afer* and *B. trevelyani* from non-native fish invasion.

The genetic results cannot reject the possibility that *P. afer* occupied mainstream habitats in the Swartkops River system, and that its current absence indicates extirpation from these habitats in invaded reaches. Distribution data from this system support such a possibility (Chapter 2 and Chapter 3). In areas occupied by non-native centrarchids, all native species are absent and environmental factors did not adequately explain their absences (Chapter 3). Although *P. afer* was most probably extirpated from invaded stream reaches, the lack of genetic structuring and numerous abundant *P. afer* populations within the Swartkops River system suggest that this lineage is not under any immediate threat of extinction.

It must be noted that the current classification of conservation units is based on an overview of the genetic diversity and structuring in the two species. Further, the results are based on only the mtDNA cytochrome *b* gene region and can therefore provide limited conclusions and inferences. Other factors such as genetic bottlenecks, founder effects and drainage history may be responsible for the observed genetic patterns (Leary et al. 1993). Further investigation is desirable using additional techniques of analysis and a range of different genetic markers (Vrijenhoek 1998).

In conclusion, while it is difficult to quantify the impact of non-native fishes on genetic diversity, the present study suggests that non-native species have the potential to cause losses of genetic diversity in the near future, especially in *B. trevelyani*. The different divergence levels among lineages of these two headwater fishes highlight the species-specific and system-specific nature of potential impacts. These data have provided preliminary results with which to prioritise conservation efforts and have identified knowledge gaps to direct future research.

CHAPTER 8: General discussion

8.1 Overview

The human-aided transport of species has broken previous biogeographical barriers (Rahel 2007), and in the process, has accelerated introduction rates to unprecedented levels (Ricciardi 2007). There is huge potential for introduced species to impact negatively on recipient ecosystems (Cucherousset and Olden 2011; Gozlan et al. 2010; Ricciardi et al. 2013). According to Ricciardi et al. (2013) impact can be defined as measurable change to the properties of an ecosystem by a non-native species. These impacts can affect multiple levels of organisation from individuals (mortality and growth or reproduction), populations (abundance and genetics), communities (species richness, evenness, composition and trophic structure) to entire ecosystems (physical habitat, nutrient cycling, contaminant cycling and energy flow) (Cucherousset and Olden 2011; Ricciardi et al. 2013). Recent debate in the literature argues about the actual severity and proportion of harmful impacts attributed to non-native species introductions (Gozlan 2008; Sagoff 2005; Simberloff 2005). In some instances, impact may be minimal, in others, severe (Ricciardi et al. 2013). It must be noted, however, that zero impact cannot be inferred from an absence of negative biotic interactions alone (Trexler et al. 2000). An introduced species must at least use some resources, whether space or foods, which were previously available to native species in the recipient ecosystem. Impacts are also situation-specific and often generalisations about species impacts are not meaningful (Ricciardi et al. 2013). The only way to define impacts is by undertaking studies to provide empirical evidence.

Non-native fish introductions in South Africa typify the global spread of non-native fishes (Rahel 2007), with a history of introductions spanning two and a half centuries. Studies on the actual impacts of these non-native fish introductions are, however, rare. As is the case in other regions, impacts in South Africa are often a case of risk perception, rather than actual risk analysis (Garcia-Berthou 2007; Gozlan 2008; Ribeiro and Leunda 2012). A literature review (Chapter 1) demonstrated that to date in South Africa, 55 fishes have been introduced or translocated. The impacts of these introductions were particularly severe and spanned multiple levels of biological organisation. Reviewed studies emphasised invasive impacts, but the transport, introduction, establishment and spread stages of the invasion process were largely neglected (Chapter 1). It must, however, be noted that only 26 studies on impact were conducted, which in itself is an indication of the paucity of studies on non-native fish invasions in South Africa.

To investigate the consequences of non-native fish introductions, Trexler et al. (2000) suggest that experimental studies over a range of environments are needed to understand biotic interactions and predict impacts. To enhance knowledge on invasions in South Africa, this thesis used experimental data from a range of environments. In addition, to deal with further knowledge gaps on invasions, as suggested by Cucherousset and Olden (2011), this thesis: (1) Addressed invasion in a poorly studied geographical area; (2) Encompassed multiple levels of biological organisation; (3) Investigated synergistic impacts between invasive species and other stressors such as habitat degradation; (4) Alluded to the evolutionary responses that make South African headwater species vulnerable to invasions. This thesis is the first that I am aware of in South Africa that attempts to provide a multi-faceted, quantitative estimate on invasive impacts of non-native fishes.

The thesis had two specific objectives. Firstly, to provide a literature review on non-native fish invasions in South Africa. Secondly, using two case studies on the headwaters of the Keiskamma and Swartkops River systems, to investigate the naturalisation-invasion continuum and provide a holistic view of the invasion process. Specifically, the impact of invasion by two centrarchids (largemouth bass *Micropterus salmoides* and *M. dolomieu*) and two salmonids (rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*) on two IUCN redlisted as Endangered (Cambray 2007; Swartz and Impson 2007), native minnow species, Eastern Cape redfin *Pseudobarbus afer* in the Swartkops River system and Border barb *Barbus trevelyani* in the Keiskamma River system were investigated.

This chapter of the thesis provides an overview of the invasion in two variable Eastern Cape headwater environments. The vulnerability of *P. afer* and *B. trevelyani* to invasion is discussed, a long term prognosis for native fishes is provided and potential management interventions suggested.

8.2 The unified framework for biological invasions

The invasion status and impacts of non-native fishes in the Swartkops and Keiskamma River systems are summarised in Figure 8.1. An assessment of the two study systems, the Swartkops and Keiskamma Rivers, showed that these were heavily invaded and numbers of introduced species surpassed those of natives (Chapter 2, 3and 5). Headwater streams had varying invasibility and establishment success, depending on the invading species and the environment (Chapter 2, 3, 4, 5 and 6). Many invasions were casual incursions into headwater streams from source populations in mainstream and impoundment environments which were invasion hotspots (Chapter 2, 3 and 5). Irrespective of establishment success, four predatory invaders (*M. salmoides*, *M. dolomieu*, *S. trutta* and *O. mykiss*) impacted heavily on native fish communities.

Although within the Blackburn et al. (2011) framework for biological invasions a species passes through successive stages from introduction to eventual establishment, impacts can happen at any stage after introduction (Ricciardi et al. 2013). For this reason it is important firstly to understand impacts and commonalities within the two river systems, and then to investigate at which stages these impacts are mediated (by biotic or abiotic interactions and processes). This would provide a greater understanding of the invasion process between the variable environments of the episodic Swartkops and perennial Keiskamma River systems. A key aim of studies such as this one, on non-native species invasions and impacts, is also to develop predictions and generalisations which help to effectively manage invasions (Blackburn et al. 2011).

Invasive impacts and commonalities between the Keiskamma and Swartkops River system are summarised as follows: Invasion results in changes to species assemblage compositions (distinct assemblages in the Swartkops and Keiskamma River systems with and without non-native fishes) (Chapter 3 and Chapter 5); There was no overlap in native fishes and non-native predator distributions (no local co-occurrence but regional, system scale co-occurrence) (Chapter 2, 3 and 5); Native fishes experienced habitat loss due to lack of co-occurrence (>20% for both *P. afer* and *B. trevelyani*) (Chapter 2, 3 and 5); Habitat degradation was also responsible for a contraction of *B. trevelyani* range, which was compounded by non-native species impacts (Chapter 5); Native fish populations were isolated and fragmented (Swartkops *P. afer* is isolated in headwater refugia, while

Keiskamma *B. trevelyani* is isolated in the non-invaded middle stream reaches) (Chapter 3 and Chapter 5); Predation disrupted population processes such as adult dispersal for *P. afer* (centrarchid-invaded zones also act as demographic sinks where adults dispersing through invaded reaches were rapidly depleted) (Chapter 4); Data on the distribution of genetic diversity for the endangered native fish *Barbus trevelyani* also indicated an imminent loss of diversity (>2% divergence from other populations) (Chapter 7). *Pseudobarbus afer* on the other hand exhibited low between-stream diversity (Chapter 7).

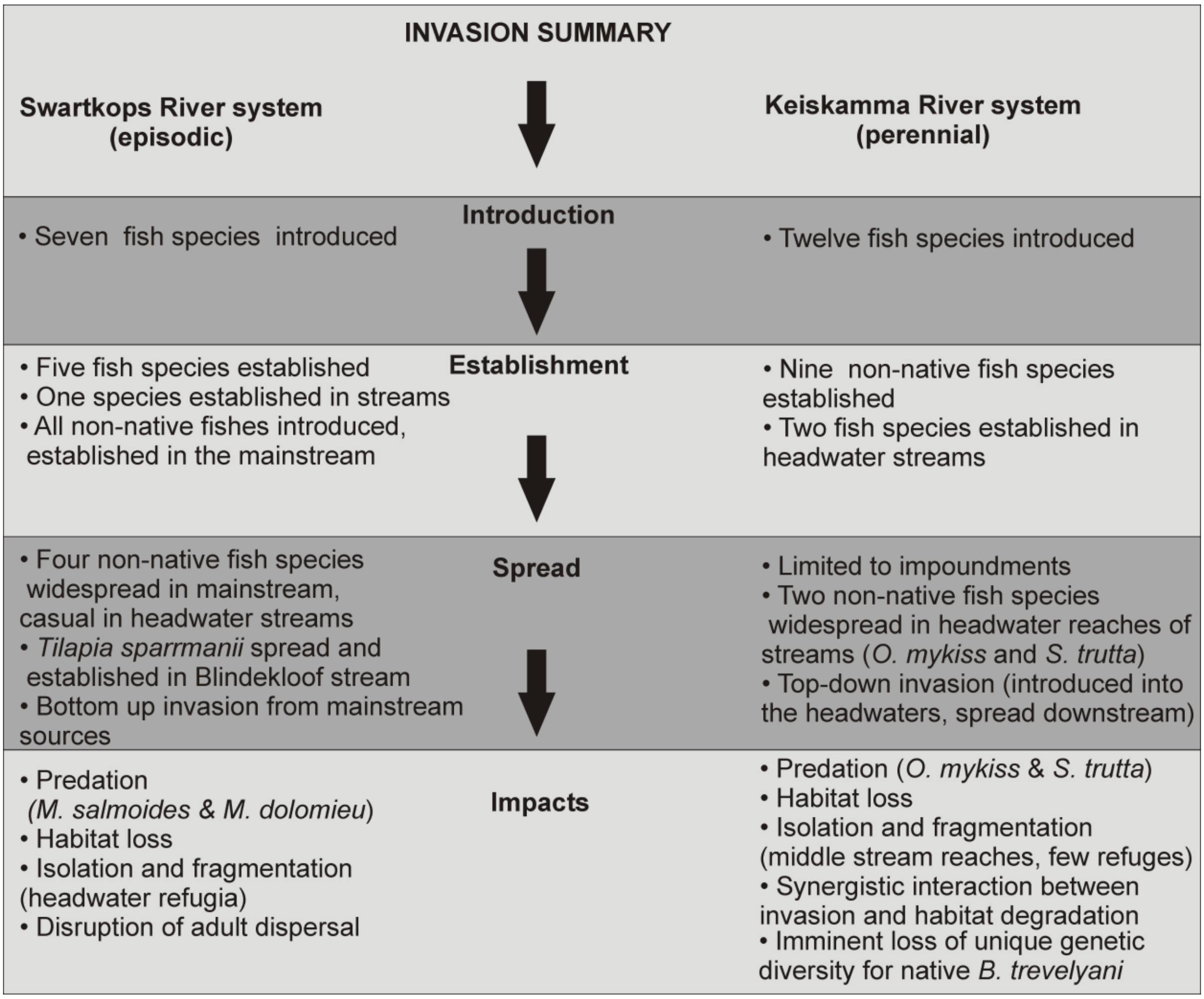
It is evident from the list of impacts that non-native predatory fishes in the Keiskamma and Swartkops River systems have induced a measurable change to the properties of an ecosystem as defined by Ricciardi et al. (2013). While non-native fishes have many impacts on native fishes, co-occurrence at regional scales (within a river system) indicates that other factors may be responsible for mediating system scale impacts. As non-native predatory centrarchids and salmonids had limited distributions in the study systems, their establishment and spread may be hampered by biotic or abiotic factors (Moyle and Light 1996). Determining factors responsible for successful establishment is a complex task that is encompassed by the biotic and abiotic resistance hypotheses. The biotic resistance hypothesis proposes that competitive interactions, novel pathogens and complex fish assemblages limit the establishment success of an invader (Baltz and Moyle 1993; Lodge 1993; Ricciardi and Mottiar 2006). The abiotic resistance hypothesis suggests that a species physiological tolerances limit their establishment to suitable environment conditions (Bernardo et al. 2003; Moyle and Light 1996; Ricciardi and Mottiar 2006).

As native fishes exhibited little biotic resistance to non-native fish invasions (Chapter 2, 3, 4 and 5), the hypotheses related to abiotic resistance (Ricciardi et al. 2013) may best describe the invasion process in the study systems. This involves explaining the role of environmental heterogeneity and disturbance in facilitating or inhibiting invasions and subsequent impacts. While this hypothesis may be relevant, another major determinant of establishment success is propagule pressure, which, when above a certain threshold, results in establishment even if the biotic and abiotic resistances are high (Simberloff 2009). Intentional stocking of non-native fishes has not occurred in more than 30 years, so the role of propagule pressure in facilitating establishment can only be surmised.

Abiotic resistance may limit invasibility and the extent of impact by centrarchids and salmonids in the Keiskamma and Swartkops River systems. The focal invaders in this thesis (*O. mykiss, S. trutta, M. salmoides,* and *M. dolomieu*) had all been established in their respective river systems for at least 60 years. Their current distribution patterns therefore reflect their invasive ability and the native species distributions may be indicative of the extent of impact.

The initial step in an invasion is to overcome geographic barriers, and be transported and introduced into a novel recipient ecosystem (Blackburn et al. 2011). The number of species, subspecies or genotypes introduced into an environment is termed colonisation pressure (Ricciardi et al. 2013). Both systems had high colonisation pressure; however, the number of species introduced into the Keiskamma River system (Chapter 5) surpassed that of the Swartkops River system (Chapter 2 and 3). Initial introductions into both systems were of predatory fishes for angling purposes (Swartkops River system: *M. salmoides* and *M. dolomieu*; Keiskamma River system: *O. mykiss* and *S. trutta*). The introduction rate of non-native fishes can also be influenced by the extent of urban development in an area, and shows a strong association with the presence of dams (Marchetti et al. 2004a). Four headwater impoundments, a more developed catchment, and open access to the area are probably all contributory factors to the heavy invasion of the Keiskamma river system (Chapter 5). Although the upper Swartkops River system is situated in a formally protected area, invasions originating downstream can still invade into upstream protected areas (Chapter 2). It does however provide some sort of protection or barrier to local introductions. Initially, however, the successful invasion of fishes is dependent on their establishment in recipient ecosystems (Kolar and Lodge 2002).

The invasibility of headwater environments and establishment of non-native fishes was highly variable. Both Eastern Cape river systems displayed almost no biotic resistance, however the stochastic nature of the Swartkops River system inhibited widespread invasion, with only a single stream being extensively invaded (Blindekloof) and the remainder of the invasions were casual. Although Eastern Cape headwater streams were invasible and the Blindekloof stream was invaded by four of the six non-native species present in the Swartkops River system headwaters (Chapter 2), establishment was highly variable (Chapter 3). Establishment depended on the prevailing environmental conditions within the recipient river system as well as the physiological tolerances of the introduced species. For example, *M. salmoides*, which prefers lentic ecosystems, was not recorded from the Blindekloof stream after a major flood (Chapter 3); however, this provided an opportunity for African sharptooth catfish *Clarias gariepinus* to extend its range upstream (Chapter 4). Headwater streams in the Eastern Cape conformed to prevailing theories predicting that in highly variable stochastic environments which maintain natural flow regimes, invading species would encounter abiotic filters (Lytle and Poff 2004; Poff 1997) that would limit establishment success (Moyle and Light 1996). In the Keiskamma River system, impoundments were invasion hotspots dominated by non-native species (disturbed habitats are prone to invasion (Ricciardi et al. 2013)) while headwater streams were not invaded to the same extent (Chapter 5). The maintenance of natural flow regimes in the headwaters of the Keiskamma River system limited invasion of headwater stream reaches. This did not, however, inhibit the establishment of salmonids in these areas, as these conditions conform to their specific habitat preferences. Their distribution was limited to headwater stream reaches above impoundments, as in the lower impounded reaches salmonids were excluded due to unusually rapid warming, facilitated by outflows of surface waters from impoundments (Chapter 5 and 6).



INVASION SUMMARY	Swartkops River system (episodic)	Keiskamma River system (perennial)
Introduction	• Seven fish species introduced	• Twelve fish species introduced
Establishment	• Five fish species established • One species established in streams • All non-native fishes introduced, established in the mainstream	• Nine non-native fish species established • Two fish species established in headwater streams
Spread	• Four non-native fish species widespread in mainstream, casual in headwater streams • *Tilapia sparrmanii* spread and established in Blindekloof stream • Bottom up invasion from mainstream sources	• Limited to impoundments • Two non-native fish species widespread in headwater reaches of streams (*O. mykiss* and *S. trutta*) • Top-down invasion (introduced into the headwaters, spread downstream)
Impacts	• Predation (*M. salmoides & M. dolomieu*) • Habitat loss • Isolation and fragmentation (headwater refugia) • Disruption of adult dispersal	• Predation (*O. mykiss* & *S. trutta*) • Habitat loss • Isolation and fragmentation (middle stream reaches, few refuges) • Synergistic interaction between invasion and habitat degradation • Imminent loss of unique genetic diversity for native *B. trevelyani*

Figure 8.1 A summary of the framework for biological invasions (Blackburn et al. 2011), including the impacts of non-native fishes for the upper Swartkops and Keiskamma River systems, Eastern Cape, South Africa.

8.3 What makes *P. afer* and *B. trevelyani* so vulnerable to invasion?

At the system scale, impacts may fall into a subset of the trophic position hypothesis, which suggests that uncontrolled predator effects will be strong in insular systems where communities are naïve, native populations have limited distributions and refugia are limited (Ricciardi et al. 2013). These considerations seem generally applicable to invasion scenarios in South African headwater streams where native fishes are highly vulnerable to predation by non-native fishes (Clark et al. 2009; Kleynhans 1985; Weyl et al. 2013). Both *B. trevelyani* and *P. afer* are also range restricted headwater specialists.

The ecological and evolutionary success of a species is dependent on its ability to avoid predation (Palma and Steneck 2001). Predation was postulated to be the primary mechanism impacting native fishes in the Keiskamma (Chapter 5) and Swartkops River systems (Chapter 2, 3 and 4). Piscivorous fishes are gape-limited (Nowlin et al. 2006) and can consequently only select prey fishes that fall with their gape size limitations. This was evident from salmonid-invaded streams in New Zealand, where only Canterbury galaxias *Galaxias vulgaris* adults persisted (Woodford and McIntosh 2010). Similar results were observed from the Rondegat stream in the Western Cape, South Africa, where Clanwilliam yellowfish *Labeobarbus capensis* only persisted as adults in stream reaches invaded by *M. dolomieu* (Weyl et al. 2013). The two native minnows *P. afer* and *B. trevelyani* are small fusiform-bodied fishes with maximum sizes seldom exceeding 100 mm TL- well within the gape limitations of large salmonids (*O. mykiss* and *S. trutta*) and centrarchids (*M. salmoides* and *M. dolomieu*). It has been demonstrated experimentally that *M. salmoides* preferentially selected fusiform-shaped fathead minnows *Pimephales promelas* over deeper bodied pumpkinseeds *Lepomis gibbosus* (prey size range 20-89 mm SL) (Hambright 1991). Similarly, only large *G. vulgaris* (>90 mm TL) persisted in salmonid-invaded New Zealand streams (Woodford and McIntosh 2010). As a result, all life history stages of *P. afer* and *B. trevelyani* may be vulnerable to predation.

Habitat segregation/avoidance can also dampen invader predatory impacts (MacRae and Jackson 2001). In the episodic Swartkops River system pools become isolated on the surface during low rainfall periods and *P. afer* is then confined with invasive centrarchid predators, negating any opportunity for avoidance (Chapter 3). For *B. trevelyani*, in the Keiskamma River system, being a riffle/run inhabitant means that its habitat preferences largely overlap those of the salmonids *O. mykiss* and *S. trutta* (Chapter 5). Lack of predator avoidance behaviour may also be explained by the lack of evolutionary experience with functionally different predatory species (Cox and Lima 2006; Ricciardi et al. 2013). For example, the nocturnal behaviour observed for *P. afer* was to suspend in the water column (Chapter 4). This corresponds to when native predatory eels, longfin eel *Anguilla mossambica* and giant mottled eel *A. marmorata* (demersal predators) were most active, hunting for prey in benthic cracks and crevices. The lack of experience with the functionally different benthopelagic predators *M. salmoides* and *M. dolomieu* may also enhance impacts (Cox and Lima 2006). Nocturnal activity has also been recorded for *M. dolomieu* and *M. salmoides* (Demers et al.

1996), and *P. afer* suspended in the water column may increase their vulnerability to predation by novel predators.

8.4 Invasive impacts: top down versus bottom up invasions

The patterns observed of the invasions in the Swartkops and Keiskamma River systems were nearly identical (always resulting in no local co-occurrence between native and non-native fishes), although the invaders occupied different stream reaches according to their physiological tolerances, invasive ability and initial stocking locations. While the extent of stream network invasible from downstream invasion sources is determined by the geography of the stream network and barriers inhibiting upstream dispersal, headwater stocking results in source populations which are able to invade most downstream habitats, including native fish refugia (Adams et al. 2001). In the Swartkops River system, which is characterised by episodic flows, centrarchids were stocked into the Groendal Dam, a mainstream impoundment. From there they established up and downstream of the impoundment and spread into headwater streams (Chapter 3). They have therefore only penetrated as far upstream as their physiological tolerances, preferences, jumping ability or the environment would allow (Figure 8.2). The episodic nature of the system also limited invasion opportunity (propagule pressure) into headwater streams. As a result, *P. afer* persists in isolated and fragmented headwater refugia, situated in stream reaches not invasible to centrarchids (Chapter 3).

In the perennial Keiskamma River system, salmonids, on the other hand, were stocked into several headwater streams, often above natural barriers that may have inhibited upstream dispersal. The perennial flow regime allows constant downstream dispersal opportunities. This resulted in *O. mykiss* and *S. trutta* dispersing as far downstream as their physiological tolerances (temperature limitations) would allow (Figure 8.2) (the downstream invasion barrier here was the 20 degree thermal barrier that limited downstream distribution, see Chapter 6). Due to damming of the Keiskamma River system, thermal regimes have also changed from their pristine state and after the construction of impoundments (resulting in an increased heating rate) the downstream area invasible has decreased (Chapter 6). The impacts of this downstream invasion pattern are particularly severe as *B. trevelyani* is forced to occupy unfavourable (due to habitat degradation in the lower stream reaches) lower stream reaches (Chapter 5). This results in the invasion front moving in an upstream direction in the Swartkops River system and a downstream direction in the Keiskamma River system. The pressures exerted by invasion on native species are therefore unique in each system (Figure 8.2).

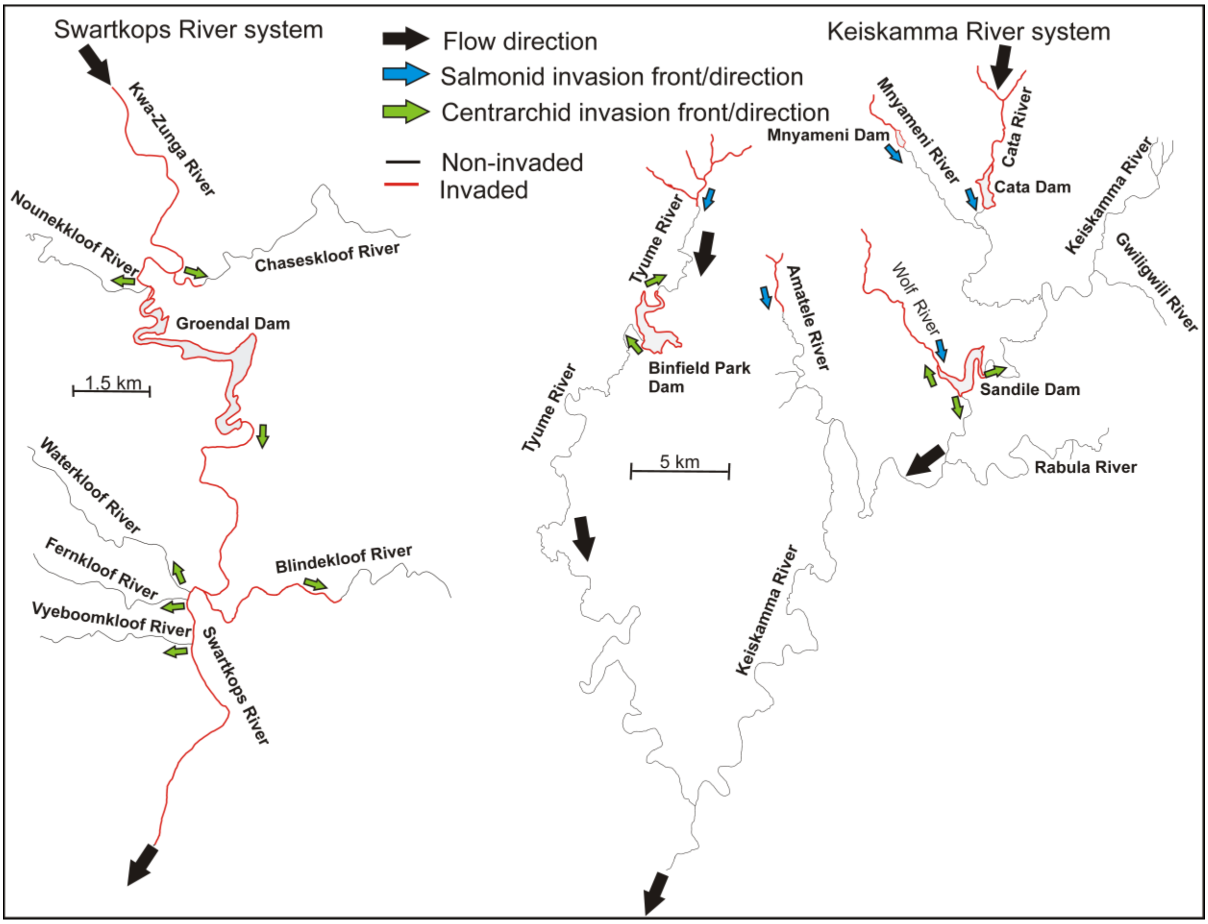


Figure 8.2 Contrasting invasion patterns of the Swartkops (bottom up) and Keiskamma River (top down and bottom up) systems due to initial stocking locations, physiological tolerances and invasive ability.

8.5 Long-term prognosis: resistance and resilience

Biotic acceptance can be defined as situations where natural ecosystems accommodate the establishment and coexistence of non-native species (Richardson et al. 2011). The opposite is true for the biotic resistance hypothesis, which suggests that resident species resist invasion and establishment by non-native species (Richardson et al. 2011). In general, headwater fish communities of the Swartkops and Keiskamma river systems displayed little biotic acceptance (because acceptance implies that native and non-native species can co-occur) or biotic resistance (as the non-native species have successfully established) (Chapter 3, 4 and 5). In this case, the presence of a non-native predator almost always resulted in the absence of all native fish, as long as they fell into the potential prey category (large native fishes persisted: *A. marmorata*, *A. mossambica* and *L. umbratus*). Following from this, a long term prognosis for a species or community (considering impacts and remediation efforts) depends on the vulnerability of a species to invasion (resistance) and its resilience (ability to recover following invasion). The interaction of these two factors provides vital information on the response of a native species to non-native fishes, if suitable management measures are put in place to control non-native fish invasions.

Both *Pseudobarbus afer* in the Swartkops River system (Chapter 2, 3 and 4), and *B. trevelyani* in the Keiskamma River system (Chapter 5), displayed low resistance to invasions, and in invaded areas impacts were severe, mostly in the form of local extirpations. Both species, however, also showed potential to be highly resilient to recovery. Source populations of *P. afer* in non-invaded stream segments above invaded stream reaches dispersed downstream and, following a flooding event during an above average rainfall year, resulted in recolonisation of stream reaches where *P. afer* was previously absent (Chapter 3). Temporal assessment of the *P. afer* population dynamics in the Blindekloof stream during the spawning season indicated that the species may display high resilience to invasions. Larval abundances were high, and did not differ between invaded and non-invaded stream reaches which were previously devoid of native fishes (Chapter 4). High flows following rainfall facilitated the dispersal of *P. afer* into these invaded stream reaches from non-invaded source populations in the upper stream reaches (Chapter 4). *Barbus trevelyani* showed similar resilience in its recolonisation of the Mnyameni River below Mnyameni Dam after impoundment construction that resulted in the creation of unfavourable conditions for *O. mykiss* (Chapter 5). This evidence was also recently corroborated on the Rondegat stream, a headwater tributary of the Olifants River system, Western Cape, South Africa. Following South Africa's first non-native fish eradication exercise, native fishes rapidly recruited into (one year post-treatment) previously invaded stream reaches (O. Weyl unpubl. data). These high levels of resilience indicate that despite their low levels of resistance to invasions, if managers and conservationists are able to eradicate non-native fishes or rehabilitate degraded stream reaches, recolonisation by native species may be rapid. This recolonisation potential also hinges on the presence of source populations to provide recruits from non-invaded stream reaches.

8.6 Management and conservation recommendations

The native Keiskamma and Swartkops River system fishes are impacted by multiple stressors (for example habitat degradation and non-native fish invasion) that threaten their persistence in headwater stream environments. In the Swartkops River system, which is relatively pristine and situated in a formally protected area, threats to *P. afer* predominantly originate from impacts of non-native fishes (Chapter 2, 3 and 4). In the Keiskamma River system, however, both non-native fish invasion and habitat degradation act synergistically, and impact heavily on *B. trevelyani* populations (Chapter 5). In this situation, Strayer (2010) suggests that a possible solution lies in managing non-native fishes and other stressors (in this case habitat degradation) as a group of closely linked problems, rather than managing each separately. It is also worthwhile to note that for the purpose of remediation, it is difficult to define impoundments as a desired conservation state. Firstly, they are man-made lentic structures that are not representative of habitats that would naturally occur or of prevailing environmental conditions under lotic conditions. Secondly, it has been globally recognised that impoundments are invasion hotspots where reintroductions are highly likely (Johnson et al. 2008). For these reasons only riverine/stream environments were considered as conservation priorities in this thesis.

Due to the extent of invasions and penetration of invasive fishes into areas of biodiversity concern in the Swartkops and Keiskamma River systems, the management and control of non-native species in these systems is deemed necessary. Options for managing non-native fish species were recently reviewed by Britton et al. (2012) and include no action, control and containment of the species within defined spatial areas, and eradication. Globally, remediation attempts are gaining in popularity, mostly in developed countries but generally focussed on small geographical areas (Britton et al. 2012). In New Zealand, the first stream rehabilitation project was undertaken recently (Pham et al. 2013). The project involved the treatment of two streams with rotenone to eradicate *S. trutta*, followed by the re-introduction of the native banded kokopu *Galaxias fasciatus* (Pham et al. 2013). Indications were that one year post treatment there was successful recruitment of *G. fasciatus* juveniles in the treatment streams (Pham et al. 2013). Currently in South Africa, initiatives aimed at remediating the impacts of non-native species are also in their infancy. The recent implementation of the CAPE Alien Fish Eradication Project is the first attempt at a large scale fish eradication and rehabilitation project (Marr et al. 2012). The pilot phase of the study involves eradication of non-native fishes from four rivers (Western Cape: Krom, Rondegat and Suurvlei Rivers; Eastern Cape: Krom River) using the piscicide rotenone (Marr et al. 2012). The initial implementation involved the application of rotenone to a 2 km stream reach invaded by *M. dolomieu* in the Rondegat River, a headwater tributary of the Olifants River system. The pilot phase was successful, with no *M. dolomieu* detected post treatment (Weyl et al. 2013). This treatment therefore paves the way for other remediation projects in areas where native fishes are threatened by non-native fish invasion.

8.6.1 Swartkops River system

All of the sampled *P. afer* populations in the upper Swartkops River system fall within the Groendal Wilderness Area. As a result, this part of the system is relatively pristine and free of major anthropogenic stressors such as habitat degradation and pollution. The primary threat to fishes in the upper Swartkops River system is invasion by non-native fishes (Chapter 2, 3 and 4). The mainstream Kwa-Zunga River and the lower reaches of the Blindekloof and Chaseskloof streams are heavily invaded and *P. afer* is absent from these reaches (Chapter 2 and 3). As a result, *P. afer* populations are fragmented, and they maintain strongholds, solely in the upper non-invaded stream reaches (Chapter 3). *Pseudobarbus afer* is also widespread and abundant within these headwater refugia (Chapter 3). Due to lack of genetic structuring (no unique alleles) observed between the headwater populations, together they constitute a single management unit (Chapter 7). The importance of this connectivity for population maintenance is uncertain. It is also therefore questionable whether eradication of non-native fishes is a necessary management intervention to maintain long-term *P. afer* persistence.

Currently the eradication of non-native fishes from a number of the Swartkops River system headwater streams may be feasible due to low abundances and the casual nature of the invasions (Chapter 3). It is however unlikely that the mainstream Kwa-Zunga River and the Groendal Dam, both invasion sources, could be rehabilitated. This means that as was shown by Skelton (1993) after *M. salmoides* were eradicated from the Blindekloof stream, without the construction of a barrier to prevent re-invasion, eradication only provides a temporary solution. This sentiment was also expressed by Marr et al. (2012), who stated that choosing an area where reintroductions are highly unlikely is vital to ensure eradication success. This is also not the most pressing issue as only 12% of the headwater stream habitat (35% including mainstream reaches) was invaded by centrarchids. Restoring connectivity between currently isolated populations is a more pressing issue but would require the removal of non-native fishes from the source populations in the mainstream Kwa-Zunga River and Groendal Dam. This is not currently possible. Currently, *P. afer* is afforded some protection via maintenance of natural flow regimes (Chapter 3). This environmental stochasticity, and instream barriers, provides protection for 88% of the surveyed stream populations from invasion.

8.6.2 Keiskamma River system

Fish communities of the Keiskamma River system are impacted by multiple stressors, including non-native fishes and habitat degradation and fragmentation (Chapter 5). While striving to achieve the ultimate conservation goal of habitat rehabilitation, non-native fish eradication and restoring connectivity between fragmented metapopulations, a pragmatic approach is most probably going to yield the best results. The Keiskamma River catchment supports extensive small scale agricultural activities, fisheries based on non-native fishes and provides potable water to a network of scattered rural villages and larger settlements in the region. It is therefore highly unlikely that complete restoration of the upper Keiskamma stream network is feasible. It must also be noted that although restoring connectivity between dendritic metapopulations promotes the maintenance of genetic diversity, enhances migratory fish populations and allows fish access between complementary habitats to meet life history needs, maintenance of fragmented populations also limit invasion opportunities, and in many instances protects native fishes (Rahel 2013). Prioritising attainable short- and long-term conservation goals is therefore vital.

Currently the stream reaches constituting the most “intact” (least degraded or impacted by overgrazing resulting in siltation) segments are situated in the headwater reaches of the streams which are occupied by invasive salmonids (Chapter 5). These are also the most logistically feasible reaches on which to focus conservation efforts in the short term. As this catchment supports numerous subsistence and small-scale agricultural activities the feasibility of habitat restoration in the lower reaches should rather be considered a long-term goal. In some instances, such as the Cata and Mnyameni streams, community initiatives are centred on recreational angling for *S. trutta* and *O. mykiss*, and the eradication of non-native species in these areas may not be feasible and perhaps not altogether desirable.

To prioritise conservation efforts for *B. trevelyani*, a scoring system was developed. A low score indicates a highly stressed stream and therefore a high priority zone (Table 8.1). The logistic feasibility was also included to provide immediate high priority areas (Table 8.1).

Table 8.1 Prioritising conservation efforts for *Barbus trevelyani* in the upper Keiskamma River system via a scoring system (low % = high priority; high % = low priority) using quantitative data presented in Chapter 5 (Invasion state: 1 = widespread, 2 = localised, 3 = non-invaded; Habitat state: 1 = degraded, 2 = intermediate, 3 = relatively pristine; Genetic status: 1 = mixed , 2 = unique; Abundance: 1 = low , 2 = medium , 3 = high; Distribution: 1 = localised, 2 = intermediate, 3 = widespread).

River/stream	**Invasion state**	**Habitat state**	**Genetic status**	**Abundance**	**Distribution**	**%**	**Remediation feasibility**	**Priority**
Tyume	2	1	1	1	1	43	high	1
Ngobozana	3	1	2	1	1	57	low	
Lower Keiskamma	2	2	2	1	2	64	low	
Amatele	2	3	2	2	1	71	high	2
Gwiligwili	3	1	2	3	1	71	high	3
Gxulu	3	2	2	3	2	86	low	
Mnyameni	2	3	2	3	3	93	high	4
Rabula	3	2	2	3	3	93	low	
Wolf	1	3	na	absent	absent	na	na	na
Cata	1	3	na	absent	absent	na	na	na

From the data presented in Table 8.1 it is clear that three high priority areas exist within the system. The highest priority is the Tyume River. The *B. trevelyani* population from this stream represents a unique genetic lineage, constituting an Evolutionary Significant Unit (Chapter 7). Abundance of *B. trevelyani* is also very low (least abundant of all stream populations) and its distribution is limited. Furthermore, the area of the stream (~10 km) where *B. trevelyani* persists is highly degraded by overgrazing, cultivation of the riparian zone and consequent erosion. In addition, the non-native *O. mykiss* inhabits the more pristine upper stream reaches and prevents *B. trevelyani* from occupying these areas (Chapter 5). Remediation and rehabilitation of the Tyume River is likely to succeed, since a natural barrier exists just above Binfield Dam which would prevent upstream invasion of non-native fishes. It is therefore recommended that *O. mykiss* be eradicated from the upper intact stream reaches, allowing *B. trevelyani* access to these reaches. Due to the small, confined *O. mykiss* population, manual eradication (electrofishing) would be potentially feasible. This would need to be implemented in conjunction with a riparian zone rehabilitation program. Public awareness and community engagement would be key in this situation as the rural villagers situated in close proximity to the stream use the land for grazing and irrigation.

Although the Ngobozana and lower Keiskamma streams have low scores and are in need of rehabilitation, logistic feasibility is questionable. Therefore, the Amatele and Gwiligwili streams are the next feasible priority streams. The upper reaches of the Amatele stream are invaded by *O. mykiss* (Chapter 5). Its eradication (manual: electrofishing or small meshed gillnets) in these reaches would increase the possible range occupied by *B. trevelyani* in a relatively pristine stream environment. The Gwiligwili stream is not invaded; however, habitat rehabilitation in the lower reaches would benefit *B. trevelyani*. The lower Mnyameni stream (below Mnyameni Dam) is also a priority as non-native vegetation has already been removed from the stream banks through a community development program. Maintenance of this effort would provide a vital refuge function for *B. trevelyani*. If these suggested conservation measures were to succeed, an additional status evaluation would need to be conducted in other streams to provide information on further rehabilitation efforts. A long-term monitoring program would also be useful as a tool to temporally assess the status of *B. trevelyani* in the upper Keiskamma system. It is assumed that other imperilled fishes, for instance Eastern Cape rocky *Sandelia bainsii*, inhabiting the upper Keiskamma River system, will also benefit, with *B. trevelyani* as a conservation 'surrogate/indicator species' on which to base rehabilitation efforts.

8.7 Future research

From South African invasions literature, it is clear that the introduction, spread and impacts of non-native fishes continues unhindered. To address this problem, the first step would be to undertake a risk assessment, using the fish invasiveness scoring kit (FISK) (Copp et al. 2009) for the main invaders in South Africa, and identify the potential risks of species likely to be introduced in the near future. This will help guide policy makers and managers curb unwanted introductions and identify species likely to become invasive and spread in the future.

One of the most effective ways to prevent the spread of non-native species is by early detection. Recent technological advances now allow researchers to test water and detect species at low densities using environmental DNA (eDNA: sloughed tissues suspend in the aqueous environment allowing researchers to collect samples in cases where the species are not detectable using traditional methods) (Jerde et al. 2011). This has been verified as a suitable method for detecting unwanted aquarium imports (Collins et al. 2013). These new technologies may provide more effective means for monitoring invasions. Currently, however, there are no structures in place to monitor any freshwater environments over a longer time scale.

Past research has been undertaken predominantly as once-off assessments. This provides little temporal resolution on the status of imperilled native fishes. A formal long-term monitoring program of headwater stream environments is a logistically difficult challenge. The recent proclamation of National Freshwater Ecosystem Priority Areas (NFEPAs), which provide spatial priorities for conserving South Africa’s freshwater ecosystems (Nel et al. 2011), however, provides a starting point for monitoring areas of high biodiversity concern. Within thes areas, fish sanctuaries have been proposed to protect threatened or near-threatened native fishes (Nel et al. 2011). Long-term monitoring of these fish sanctuaries would serve three important functions: (1) it would give an indication or detection of new introductions into areas of biodiversity concern and also monitor current invasion fronts, providing insight into temporal population dynamics of invasive species; (2) longer-term responses of rare native species to invasions are lacking and these would provide vital information on their distributions; (3) information on the temporal status of native fishes in relation to their major threats (habitat degradation and non-native fish invasions) is vital for effective conservation.

A widespread impact of non-native fish invasions in South Africa is the isolation of imperilled headwater stream fish populations. The consequences of this isolation are unclear. Population viability analyses of these dendritic metapopulations, fragmented by non-native fish invasions and river impoundment are vital to quantifying temporal impacts and prioritise long-term solutions. Investigating the role of connectivity in maintaining dendritic metapopulations of native fishes is also a key research question that needs answering (Labbe and Fausch 2000). Knowledge on how much stream area is required to sustain a species indefinitely and to prevent local extinctions is also needed (Lowe and Likens 2005). Studies on fine- and coarse-scale movements of *P. afer* and *B. trevelyani* would provide insight into this.

Once system scale distribution, abundance and impacts of non-native fishes have been defined, impact frameworks can be applied to contrast impact among species or between systems. The Parker et al. (1999) framework would be particularly useful. This framework proposes that overall impact (I) is a function of the range size R (in m^2) of a species, its average abundance per unit area across that range (A, in numbers, biomass, or other relevant measure per m^2), and E, the effect per individual or per biomass unit of the invader ($I = R \times A \times E$) (Parker et al. 1999). This would allow managers to prioritise areas where conservations efforts are most required.

8.8 Conclusions and perspectives

This thesis has provided conclusive evidence that non-native predatory fishes have significant impacts on native fishes in Eastern Cape headwater streams. The synergistic interactions between non-native fish invasion and habitat degradation, which exacerbated impacts, also underlie threats faced by headwater fishes. Native fishes are afforded some level of protection by abiotic factors such as environmental heterogeneity and environmental stochasticity, which are maintained by natural rainfall and flow regimes. Considering a growing global human population and increased pressure on natural resources, threats to these fishes and systems will become more acute in the future. A possible solution may lie in promoting the ecosystems services concept, which promotes functioning aquatic ecosystems as natural assets that provide vital services to society such as clean drinking water, recreational opportunities, nutrient removal and biodiversity (Cowx and Portocarrero Aya 2011; Lowe and Likens 2005). A paradigm shift which educates society on the importance of maintaining intact aquatic ecosystems (Cowx and Portocarrero Aya 2011), especially headwater stream environments which are the source of life (Lowe and Likens 2005), may provide a platform to ensure that native fishes persist.

References

Abell R, Allan J, Lehner B (2007) Unlocking the potential of protected areas for freshwaters. Biological Conservation 134:48–63.

Acocks JPH (1975) Veld types of South Africa. Memoirs of the Botanical Survey of South Africa 40:1–128.

Adams SB, Frissell CA, Rieman BE (2001) Geography of invasion in mountain streams: Consequences of headwater lake fish introductions. Ecosystems 4:296–307.

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution 16:613–622.

Allendorf FW, Luikart GH, Aitken SN (2013) Conservation and the genetics of populations, 2nd ed. 624.

Almeida D, Almodóvar A, Nicola GG, et al. (2012) Trophic plasticity of invasive juvenile largemouth bass *Micropterus salmoides* in Iberian streams. Fisheries Research 113:153–158.

Alves MJ, Coelho H, Collares-Pereira MJ, Coelho MM (2001) Mitochondrial DNA variation in the highly endangered cyprinid fish *Anaecypris hispanica*: importance for conservation. Heredity 87:463–473.

Angermeier PL, Winston MR (1999) Characterizing fish community diversity across Virginia landscapes : prerequisite for conservation. Ecological Applications 9:335–349.

Angermeier PL, Winston MR (1998) Local vs . regional influences on local diversity in stream fish communities of Virginia. Ecology 79:911–927.

Anon. (1994) Fresh water fish species for aquarium purpose only: species allowed for importation into RSA.

Ashton PJ (2007) Riverine biodiversity conservation in South Africa: current situation and future prospects. Aquatic Conservation: Marine and Freshwater Ecosystems 17:441–445.

Avenant-Oldewage A (2001) *Argulus japonicus* in the Olifants River system – possible conservation threat? South African Journal of Wildlife Research 31:59–63.

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. Trends in Ecology & Evolution 4:279–81.

Ayala JR, Rader RB, Belk MC, Schaalje GB (2007) Ground-truthing the impact of invasive species : spatio-temporal overlap between native least chub and introduced western mosquitofish. Biological Invasions 9:857–869.

Balon EK (1990) Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. Guelph Ichthyology Reviews 1:1–48.

Baltz DM, Moyle PB (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. Ecological Applications 3:246–255.

Barrow I (1971) Freshwater fish of the Swartkops River. In: The Swartkops Estuary, An Ecological Survey, pp. 33–34. The Swartkops Trust, Port Elizabeth.

Baxter C V., Fausch KD, Murakami M, Chapman PL (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. Ecology 85:2656–2663. doi: 10.1890/04-138

Beamesderfer RCP, North JA (1995) Growth, natural mortality, and predicted response to fishing for largemouth bass and smallmouth bass populations in North America. North American Journal of Fisheries Management 15:688–704.

Bear EA, McMahon TE, Zale A V (2007) Comparative thermal requirements of westslope cutthroat trout and rainbow trout: Implications for species interactions and development of thermal protection standards. Transactions of the American Fisheries Society 136:1113–1121.

Bernardo JM, Ilhéu M, Matono P, Costa AM (2003) Interannual variation of fish assemblage structure in a Mediterranean river: implications of streamflow on the dominance of native or exotic species. River Research and Applications 19:521–532.

Blackburn TM, Pyšek P, Bacher S, et al. (2011) A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26:333–339.

Boughton DA, Gibson M, Yedor R, Kelley E (2007) Stream temperature and the potential growth and survival of juvenile *Oncorhynchus mykiss* in a southern California creek. Freshwater Biology 52:1353–1364.

Breiman L, Friedman JH, Olshen RA, Stone CG (1984) Classification and Regression Trees. Wadsworth International Group, ont, CA, USA

Britton JR, Gozlan RE, Copp GH (2012) Managing non-native fish in the environment. Fish and Fisheries 12:256–274.

Bruton MN (1985) The effects of suspensoids on fish. Hydrobiologia 125:221–241.

Bruton MN, Van As JG (1986) Faunal invasions of aquatic ecosystems in southern Africa, with suggestions for their management. In: MacDonald IAW, Kruger FJ, Ferrar AA (eds) The Ecology and Management of Biological Invasions in Southern Africa. Oxford University Press, pp 47–61

Bruton MN, Bok AH, Davies MTT (1987) Life history styles of diadromous fishes in inland waters of southern Africa. American Fisheries Society Symposium 1:104–121.

Cambray J (2007) *Barbus trevelyani*. In: In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. www.iucnredlist.org. Accessed 14 Nov 2012

Cambray J, Swartz E (2007) *Oreochromis mossambicus*. In: IUCN 2012 IUCN Red List of Threatened Species Version 20122 <www.iucnredlist.org> Downloaded on 17 April 2013.

Cambray JA (1997) Captive breeding and sanctuaries for the endangered African anabantid *Sandelia bainsii* , the Eastern Cape rocky. Education 168:159–168.

Cambray JA (2003a) The need for research and monitoring on the impacts of translocated sharptooth catfish , *Clarias gariepinus*, in South Africa. Environmental Biology of Fishes 28:191–195.

Cambray JA (2003b) The global impact of alien trout species - a review; with reference to their impact in South Africa. African Journal of Aquatic Science 28:61–67.

Cambray JA (1994) The comparative reproductive styles of two closely related African minnows (*Pseudobarbus afer* and *P. asper*) inhabiting two different sections of the Gamtoos River system. Environmental Biology of Fishes 41:247–268.

Cambray JA (1985) Early development of an endangered African barb, *Barbus trevelyani* (Pisces: Cyprinidae). Revista de Biologia Tropical 18:51–60.

Cambray JA (1996a) Threatehed fishes of the world: *Barbus trevelyani* Gunther, 1877 (Cyprinidae). Environmental Biology of Fishes 47:202.

Cambray JA (1996b) Threatened fishes of the world: *Barbus trevelyani* Günther, 1877 (Cyprinidae). Environmental Biology of Fishes 47:202.

Cambray JA, Hecht T (1995) Comparison of the growth of two closely related redfin minnows, *Pseudobarbus afer* (Peters 1864) and *P. asper* (Boulenger 1911) (Pisces, Cyprinidae), in the Gamtoos River system, South Africa. Journal of African Zoology 109:349–376.

Campbell BM (1985) A classification of the mountain vegetation of the fynbos biome. Memoirs of the Botanical Survey of South Africa 50:1–21.

Canonico GC, Arthington A, Mccrary JK, Thieme ML (2005) The efects of introduced tilapias on native biodiversity. Ecosystems 483:463–483.

Carlander KD (1977) Handbook of freshwater fishery biology. Volume 2. Life history data on centrarchid fishes of the United States and Canada. Iowa State University Press, Ames

Carline RF, Machung JF (2001) Critical thermal maxima of wild and domestic strains of trout. Transactions of the American Fisheries Society 130:1211–1216.

Christison KW, Baker GC (2011) First record of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) (Monogenea) from South Africa. African Zoology 42:279–285.

Clark BM, Impson D, Rall J (2009) Present status and historical changes in the fish fauna of the Berg River, South Africa. Transactions Of The Royal Society Of South Africa 64:142–163.

Clavero M, Garcia-Berthou E (2005) Invasive species are a leading cause of animal extinctions. Trends in Ecology & Evolution 20:110.

Clavero M, García-Berthou E (2006) Homogenization dynamics and introduction routes of invasive freshwater fish in the Iberian Peninsula. Ecological Applications 16:2313–2324.

Collares-Pereira MJ, Cowx IG (2004) The role of catchment scale environmental management in freshwater fish conservation. Fisheries Management and Ecology 11:303–312.

Collares-Pereira MJ, Cowx IG, Ribeiro F, et al. (2000) Threats imposed by water resource development schemes on the conservation of endangered fish species in the Guadiana River Basin in Portugal. Fisheries Management and Ecology 7:167–178.

Collares-Pereira MJ, Cowx IG, Rodrigues JA, et al. (1999) The status of *Anaecypris hispanica* in Portugal: Problems of conserving a highly endangered Iberian fish. Biological Conservation 88:207–212.

Collares-Pereira MJ, Pires AM, Coelho MM, Cowx IG (1998) Towards a conservation strategy for *Anaecypris hispanica*, the most endangered non-migratory fish in Portuguese streams. Cowx, I.G. (Ed.), . Fishing Ne. In: Cowx IG (ed) Stocking and Introduction of Fish. Fishing News Books, Blackwell Science, Oxford, pp 437–499

Collins RA, Armstrong KF, Holyoake AJ, Keeling S (2013) Something in the water: biosecurity monitoring of ornamental fish imports using environmental DNA. Biological Invasions 15:1209–1215.

Copp GH, Bianco PG, Bogutskaya NG, et al. (2005) To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21:242–262.

Copp GH, Templeton M, Gozlan RE (2007) Propagule pressure and the invasion risks of non-native freshwater fishes: a case study in England. Journal of Fish Biology 71:148–159.

Copp GH, Vilizzi L, Mumford J, et al. (2009) Calibration of FISK, an invasiveness screening tool for nonnative freshwater fishes. Risk Analysis 3:457–467.

Cote D, Kehler DG, Bourne C, Wiersma YF (2009) A new measure of longitudinal connectivity for stream networks. Landscape Ecology 24:101–113.

Couvet D (2002) Deleterious effects of restricted gene flow in fragmented populations. Conservation Biology 16:369–376.

Cowx IG, Portocarrero Aya M (2011) Paradigm shifts in fish conservation: moving to the ecosystem services concept. Journal of Fish Biology 79:1663–1680.

Cox JG, Lima SL (2006) Naivete´ and an aquatic–terrestrial dichotomy in the effects of introduced predators. Evolution 21:674–680.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15:290–295.

Crawford SS, Muir AM (2007) Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870–2007. Reviews in Fish Biology and Fisheries 18:313–344.

Crowl TA, Townsend CR, McIntosh AR (1992) The impact of introduced brown and rainbow trout on native fish: the case of Australasia. Reviews in Fish Biology and Fisheries 2:217–241.

Cucherousset J, Olden JD (2011) Ecological impacts of non-native freshwater fishes. Fisheries 36:215–230.

Cunjak RA, Power G (1986) Winter habitat utelization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Canadian Journal of Fisheries and Aquatic Sciences 43:1970–1981.

D'Amato ME, Esterhuyse MM, Van Der Waal BCW, et al. (2007) Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. Conservation Genetics 8:475–488.

Dallas H (2008) Water temperature and riverine ecosystems : An overview of knowledge and approaches for assessing biotic responses , with special reference to South Africa. Water SA 34:393–404.

Davis MA, Chew MK, Hobbs RJ, et al. (2011) Don't judge species on their origins. Nature 474:153–154.

De'ath G, Fabricius KE (2000) Classification and regression trees: A powerful yet simple technique for ecological data analysis. Ecology 81:3178–3192.

Demers E, McKinley RS, Weatherley AH, McQueen DJ (1996) Activity patterns of largemouth and smallmouth bass determined with electromyogram biotelemetry. Transactions of the American Fisheries Society 125:434–439.

De Moor IJ (1996) Case studies of the invasion by four alien fish species (*Cyprinus carpio, Micropterus salmoides, Oreochromis macrochir* and *O. mossambicus*) of freshwater ecosystems of southern Africa. Transactions Of The Royal Society Of South Africa 51:233–255.

De Moor IJ, Bruton MN (1988) Atlas of alien and translocated indigenous aquatic animals of southern Africa. South African National Scientific Programmes Report 144:78–133.

Dias PC (1996) Sources and sinks in population biology. Trends in Ecology & Evolution 11:326–330.

Didham RK, Tylianakis JM, Gemmell NJ, et al. (2007) Interactive effects of habitat modification and species invasion on native species decline. Trends in ecology & evolution 22:489–96.

Dolloff CA, Flebbe PA, Owen MD (1994) Fish habitat and fish populations in a southern Appalachian watershed before and after Hurricane Hugo. Transactions of the American Fisheries Society 123:668–678.

Downes BJ (2010) Back to the future: little-used tools and principles of scientific inference can help disentangle effects of multiple stressors on freshwater ecosystems. Freshwater Biology 55:60–79.

Dudgeon D, Arthington AH, Gessner MO, et al. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Society 81:163–182.

Duggan IC, Rixon CAM, MacIsaac HJ (2006) Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biological Invasions 8:377–382.

Dunham JB, Pilliod DS, Young MK (2004) Assessing the consequences of nonnative trout in headwater ecosystems in western North America. Fisheries 29:18–26.

Dunham JB, Vinyard GL, Rieman BE (1997) Habitat fragmentation and extinction risk of lahontan cutthroat trout. North American Journal of Fisheries Management 17:1126–1133.

Du Preez M, Lee DE (2010) The contribution of trout fly fishing to the economy of Rhodes, North Eastern Cape, South Africa. Development Southern Africa 27:241–253.

DWAF (2012) Department of Water Affairs. In: Department of Water Affairs and Forestry River station database.

DWAF (2004) Amatole-Kei Area Internal Strategic Perspective WMA 12. 04 August 2004 Report.

Eaton JG, McCormick JH, Goodno BE, et al. (1995) A field information-based system for estimating fish temperature tolerances. Fisheries 20:10–18.

Eaton JG, Scheller RM (1996) Effects of climate in warming on fish thermal habitat in streams of the United States. Limnology and Oceanography 41:1109–1115.

Ebersole JL, Liss WJ, Frissell CA (2001) Relationship between stream temperature, thermal refugia and rainbow trout *Oncorhynchus mykiss* abundance in arid-land streams in the northwestern United States. Ecology of Freshwater Fish 10:1–10.

Edwards RW, Densem JW, Russell PA (1979) An assessment of the importance of temperature as a factor controlling the growth rate of brown trout in streams. Journal of Animal Ecology 48:501–507.

Ellender BR, Becker A, Weyl OLF, Swartz ER (2012a) Underwater video analysis as a non-destructive alternative to electrofishing for sampling imperilled headwater stream fishes. Aquatic Conservation: Marine and Freshwater Ecosystems 22:58–65.

Ellender BR, Taylor GC, Weyl OLF (2012b) Validation of growth zone deposition rate in otoliths and scales of flathead mullet *Mugil cephalus* and freshwater mullet *Myxus capensis* from fish of known age. African Journal of Marine Science 34:455–458.

Ellender BR, Weyl OLF, Swartz ER (2011) Invasion of a headwater stream by non-native fishes in the Swartkops River system , South Africa. African Zoology 46:39–46.

Ellender BR, Weyl OLF, Winker H (2012c) Age and growth and maturity of southern Africa's largest cyprinid fish, the largemouth yellowfish *Labeobarbus kimberleyensis*. Journal of Fish Biology 81:1271–1284.

Elliott JM, Elliott JA (2010) Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. Journal of Fish Biology 77:1793–817.

Elvira B, Almodóvar A (2001) Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. Journal of Fish Biology 59:323–331.

Engelbrecht GD, Mulder PFS, Mpherwane SK (2001) Conservation genetics of the vulnerable Treur River barb, *Barbus treurensis*. African Journal of Aquatic Science 26:39–44.

Fatoki OS, Gogwana P, Ogunfowokan AO (2003) Pollution assessment in the Keiskamma River and in the impoundment downstream. Water SA 29:183–188.

Fausch KD (2008) A paradox of trout invasions in North America. Biological Invasions 10:685–701.

Fausch KD (2007) Introduction, establishment and effects of non-native salmonids: considering the risk of rainbow trout invasion in the United Kingdom*. Journal of Fish Biology 71:1–32.

Fausch KD, Rieman BE, Dunham JB, et al. (2009) Invasion versus isolation: trade-offs in managing native salmonids with barriers to upstream movement. Conservation Biology 23:859–70.

Fausch KD, Torgersen CE, Baxter C V, et al. (2002) Landscapes to riverscapes: Bridging the gap between research and conservation of stream fishes. BioScience 52:483–498.

Firmat C, Alibert P, Losseau M, et al. (2013) Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. PloS one 8:1–12.

Forseth T, Larsson S, Jensen a J, et al. (2009) Thermal growth performance of juvenile brown trout *Salmo trutta*: no support for thermal adaptation hypotheses. Journal of Fish Biology 74:133–49.

Franssen NR, Gido KB, Guy CS, et al. (2006) Effects of floods on fish assemblages in an intermittent prairie stream. Freshwater Biology 51:2072–2086.

Fraser DF, Gilliam JF (1992) Nonlethal impacts of predator invasion: facultative suppression of growth and reproduction. Ecology 73:959–970.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology 10:2741–2752.

Gaigher CM (1973) The Clanwilliam River: it is not yet too late. Piscator 88:75–78.

Gaigher IG (1975) The ecology of a minnow, *Barbus trevelyani* (Pisces: Cyprinidae) in the Tyume River, Eastern Cape. Annals of the Cape Provincial Museum of Natural History 11:1–19.

Gaigher IG, Hamman KCD, Thorne S (1980) The distribution, status and factors affecting the survival of indigenous freshwater fishes in the Cape Province. Koedoe 23:57–88.

Garcia-Berthou E (2007) The characteristics of invasive fishes: what has been learned so far? Journal of Fish Biology 71:33–55.

Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics 30:51–81.

Gatz AJ, Sale MJ, Loar JM (1987) Habitat shifts in rainbow trout: competitive influences of brown trout. Oecologia 74:7–19.

Gerking SD (1959) The restricted movement of fish populations. Biological Reviews 34:221–242.

Godinho FN, Ferreira MT (2000) Composition of endemic fish assemblages in relation to exotic species and river regulation in a temperate stream. Biological Invasions 2:231–244.

Gozlan RE (2008) Introduction of non-native freshwater fish: is it all bad? Fish and Fisheries 9:106–115.

Gozlan RE, Britton JR, Cowx IG, Copp GH (2010) Current knowledge on non-native freshwater fish introductions. Journal of Fish Biology 44:751–786.

Grossman GD, Freeman MC (1987) Microhabitat use in a stream fish assemblage. Journal of Zoology 212:151–176.

Grossman GD, Moyle PB, Whitaker JO (1982) Stochastcity in structural and functional characteristics of an Indian stream fish assemblage. The American Naturalist 120:423–454.

Hambright KD (1991) Experimental analysis of prey selection by largemouth bass: role of predator mouth width and prey body depth. Transactions of the American Fisheries Society 120:500–508.

Hanski I (1998) Metapopulation dynamics. Nature 396:41–49.

Harvey BC (1987) Susceptability of young-of-the-year fishes to downstream displacement by flooding. Transactions of the American Fisheries Society 116:851–855.

Henriques R, Sousa V, Coelho MM (2010) Migration patterns counteract seasonal isolation of *Squalius torgalensis*, a critically endangered freshwater fish inhabiting a typical Circum-Mediterranean small drainage. Conservation Genetics 11:1859–1870.

Hey S (2008) The rapture of the river - The autobiography of a South African fisherman, 2nd ed. Platanna Press

Hildebrand RH, Kershner JL (2000) Conserving inland cutthroat trout in small streams: How much stream is enough? North American Journal of Fisheries Management 20:513–520.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:745–755.

Huston M (1979) A general hypothesis of species diversity. The American Naturalist 113:81–101.

Impson ND, Bills R, Cambray JA (2002) A conservation plan for the unique and highly threatened freshwater fishes of the Cape Floristic Kingdom. In: Collares-Periera MJ, Cowx IG, Coelho MM (eds) Conservation of Freshwater Fishes: Options for the Future. Fishing News Books, Blackwell Science, United Kingdom, pp 432–440

Impson ND, Tharme R (1998) Translocation of indigenous freshwater fishes for conservation and flyfishing: the Boontjies River, Western Cape as a case study. Piscator 130:87–92.

IUCN (2001) IUCN redlist categories and criteria version 3.1. Online at: http://www.iucnredlist.org (accessed 5/11/2012).

Jackson DA, Peres-Neto PR, Olden JD (2001) What controls who is where in freshwater fish communities — the roles of biotic , abiotic , and spatial factors. Canadian Journal of Fisheries and Aquatic Sciences 58:157–170.

Jerde CJ, Mahon AR, Chadderton WL, Lodge DM (2011) “Sight-unseen” detection of rare aquatic species using environmental DNA. Conservation Letters 00:1–8.

Johnson PT, Olden JD, Vander Zanden MJ (2008) Dam invaders: impoundments facilitate biological invasions into freshwaters. Frontiers in Ecology and the Environment 6:357–363.

Jones RW, Weyl OLF, Swartz ER, Hill MP (2013) Using a unified invasion framework to characterize Africa’s first Loricariid catfish invasion. Biological Invasions DOI 10.1007/s10530–013–0438–7.

Jonsson B, Jonsson N (2009) A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. Journal of Fish Biology 75:2381–447.

Jubb (1971) Some interesting Eastern Cape freshwater fishes. Eastern Cape Naturalist 43:7–9.

Jubb RA (1967) Freshwater fishes of southern Africa. 248.

Jubb RA (1965) Freshwater fishes of the Cape Province. Annals of the Cape Provincial Museums 4:1–72.

Jubb RA (1961) The freshwater eels (*Anguilla* sp.) of Southern Africa: an introduction to their identification and biology. Annals of the Cape Provincial Museum of Natural History 1:15–48.

Kadye WT, Booth AJ (2012a) Integrating stomach content and stable isotope analyses to elucidate the feeding habits of non-native sharptooth catfish *Clarias gariepinus*. Biological Invasions 14:779–795.

Kadye WT, Booth AJ (2012b) An invader within an altered landsacape: One catfish, two rivers and an inter-basin water transfer scheme. River Research and Applications. DOI: 10.1002/rra.2599.

Kadye WT, Booth AJ (2012c) Detecting impacts of invasive non-native sharptooth catfish, *Clarias gariepinus*, within invaded and non-invaded rivers. Biodiversity and Conservation 21:1997–2015.

Kadye WT, Booth AJ (2012d) Inter-seasonal persistence and size-structuring of two minnow species within headwater streams in the Eastern Cape, South Africa. Journal of Applied Ichthyology 28: 791–799.

Kaeding LR (1996) Summer use of coolwater tributaries of a geothermally heated stream by rainbow and brown trout *Oncorhynchus mykiss* and *Salmo trutta*. American Midland Naturalist 135:283–292.

Karssing RJ, Rivers-Moore NA, Slater K (2012) Influence of waterfalls on patterns of association between trout and Natal cascade frog *Hadromophryne natalensis* tadpoles in two headwater streams in the uKhahlamba Drakensberg Park World Heritage Site, South Africa. African Journal of Aquatic Science 37:102–112.

Kleynhans CJ (1985) The future of rare fish species in the Transvaal. Fauna & Flora 42:30–32.

Knapp RA, Boiano DM, Vredenburg VT (2007) Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). Biological conservation 135:11–20.

Knapp RA, Corn PS, Schindler DE (2001) The Introduction of non-native fish into wilderness lakes: good intentions, conflicting mandates and unintended consequences. Ecosystems 4:275–278.

Koehn JD (2004) Priority management actions for alien freshwater fish species in Australia. Environmental Research 38: 457-472.

Kolar CS, Lodge DM (2002) Ecological predictions and risk assessment for alien fishes in North America. Science 298:1233–1236.

Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. 646.

Kulhanek SA, Ricciardi A, Leung B (2011) Is invasion history a useful tool for predicting the impacts of the world ' s worst aquatic invasive species ? Ecological Applications 21:189–202.

Labbe TR, Fausch KD (2000) Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecological Applications 10:1774–1791.

Larkin MA, Blackshields G, Brown NP, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

Latta LC, Bakelar JW, Knapp RA, Pfrender ME (2007) Rapid evolution in response to introduced predators II: the contribution of adaptive plasticity. BMC Evolutionary Biology 7:21.

Laurenson LJB, Hocutt CH (1986) Colonisation theory and invasive biota: the Great Fish River, a case history. Environmental Monitoring and Assessment 6:71–90.

Laurenson LJB, Hocutt CH, Hecht T (1989) An evaluation of the success of invasive fish species of the Great Fish River. Journal of Applied Ichthyology 1:28–34.

Lawrence DJ, Olden JD, Torgersen CE (2012) Spatiotemporal patterns and habitat associations of smallmouth bass (*Micropterus dolomieu*) invading salmon-rearing habitat. Freshwater Biology 1929–1946.

Leary RF, Allendorf FW, Forbes SH (1993) Conservation genetics of bull trout in the Columbia and Klamath River drainages. Conservation Biology 7:856–865.

Leibold M, Van Zyl CJ (2008) The economic impact of sport and recreational angling in the Republic of South Africa, 2007: Extensive report. In: Development Strategies International (Pty.) Ltd (eds.). Report of project to scientifically determine the overall economic impact and strate. 49.

Leprieur F, Beauchard O, Blanchet S, et al. (2008) Fish invasions in the world's river systems: when natural processes are blurred by human activities. PLoS Biology 6:0404–0410.

Letcher BH, Terrick TD (1998) Maturation of male age-0 Atlantic salmon following a massive , localized flood. Journal of Fish Biology 53:1243–1252.

Leung B, Mandrak NE (2007) The risk of establishment of aquatic invasive species: joining invasibility and propagule pressure. Proceedings of the Royal Society B: Biological Sciences 274: 2603-2609.

Lever C (1996) Naturalized fishes of the world. 408.

Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Lienesch PW, Lutterschmidt WI, Schaefer JF (2000) Seasonal and long-term changes in the fish assemblage of a small stream isolated by a reservoir. The Southwestern Naturalist 45:274–288.

Linder HP, Johnson SD, Kuhlmann M, et al. (2010) Biotic diversity in the Southern African winter-rainfall region. Current Opinion in Environmental Sustainability 2:109–116.

Lodge DM (1993) Lodge 1993 invasions and lessons for ecology.pdf. Trends in Ecology & Evolution 8:133–136.

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world’s worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN) 12.

Lowe S, Woodford D, Impson D, Day J (2008) The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. African Journal of Aquatic Science 33:51–62.

Lowe WH, Likens GE (2005) Moving headwater streams to the head of the class. BioScience 55:196–197.

Lytle DA, Poff NL (2004) Adaptation to natural flow regimes. Trends in Ecology & Evolution 19:94–100.

Machordom A, Doadrio I (2001) Evolutionary history and speciation modes in the cyprinid genus Barbus. Proceedings: Biological Sciences 268:1297–306.

Maclear LGA (2001) The hydrogeology of the Uitenhage Artesian Basin with reference to the Table Mountain Group Aquifer. Water SA 27:499–506.

MacRae PSD, Jackson DA (2001) The influence of smallmouth bass (*Micropterus dolomieu*) predation and habitat complexity on the structure of littoral zone fish assemblages. Canadian Journal of Fisheries and Aquatic Sciences 58:342–351.

Magalhaes MF, Schlosser IJ, Collares-Pereira MJ (2003) The role of life history in the relationship between population dynamics and environmental variability in two Mediterranean stream fishes. Journal of Fish Biology 63:300–317.

Magoulick DD, Kobza RM (2003) The role of refugia for fishes during drought: a review and synthesis. Freshwater Biology 48:1186–1198.

Maitland PS (2004) Keys to the freshwater fish of Britain and Ireland, with notes on their distribution and ecology. 248.

Mäkinen T, Weyl OLF, van der Walt K-A, Swartz ER (2013) First record of an introduction of the giant pangasius, *Pangasius sanitwongsei* Smith 1931, into an African river. African Zoology

Marchetti MP, Light T, Moyle PB, Viers JH (2004a) Fish invasions in California watersheds: testing hypotheses using landscape patterns. Ecological Applications 14:1507–1525.

Marchetti MP, Moyle PB, Levine R (2004b) Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. Freshwater Biology 646–661.

Marchetti MP, Moyle PB, Levine R (2004c) Alien fishes in California watersheds: Characteristics of successful and failed invaders. Ecological Applications 14:587–596. doi: 10.1890/02-5301

Marr S, Sutcliffe L, Day J, et al. (2009) Conserving the fishes of the Twee River, Western Cape, South Africa: revisiting the issues. African Journal of Aquatic Science 34:77–85.

Marr SM, Impson ND, Tweddle D (2012) An assessment of a proposal to eradicate non-native fish from priority rivers in the Cape Floristic Region, South Africa. African Journal of Aquatic Science 37:131–142.

Marr SM, Marchetti MP, Olden JD, et al. (2010) Freshwater fish introductions in mediterranean-climate regions: are there commonalities in the conservation problem? Diversity and Distributions 16:606–619.

Marr SM, Olden JD, Leprieur F, et al. (2013) A global assessment of freshwater fish introductions in mediterranean-climate regions. Hydrobiologia. DOI:10.1007/s10750-013-1486-9.

Matthews WJ (1986) Fish faunal structure in an Ozark stream: Stability, persistence and a catastrophic flood. Copeia 1986:388–397.

Mayekiso M (1986) Some aspects of the ecology of the Eastern Cape rocky *Sandelia bainsii* (Pisces: Anabantidae) in the Tyume River, Eastern Cape, South Africa. 130.

Mayekiso M, Hecht T (1988) Conservation status of the anabantid fish *Sandelia bainsii* in the Tyume River, South Africa. South African Journal of Wildlife Research 18:101–108.

McCafferty JR, Ellender BR, Weyl OLF, Britz P (2012) The use of water resources for inland fisheries in South Africa. Water SA 38:327–344.

McDowall RM (1996) Threatened fishes of the world: *Prototroctes oxyrhynchus* Giinther , 1870. Environmental Biology of Fishes 46:60.

Medeiros ESF, Maltchik L (2001) Fish assemblage stability in an intermittently flowing stream from the Brazilian semiarid region. Austral Ecology 26:156–164.

Meka LM, Knudsen EE, Douglas DC, Benter RB (2003) Variable migratory patterns of different adult rainbow trout life history types in a southwest Alaska watershed. Transactions of the American Fisheries Society 132:717–732.

Meyer JL, Strayer DL, Wallace JB, et al. (2007) The contribution of headwater streams to biodiversity in river networks. Journal of the American Water Resources Association 43:86–103.

Mhangara P, Kakembo V (2012) An object-based classification and fragmentation analysis of land use and cover change in the Keiskamma catchment , Eastern Cape , South Africa. World Applied Sciences Journal 19:1018–1029.

Mhangara P, Kakembo V, Lim KJ (2012) Soil erosion risk assessment of the Keiskamma catchment, South Africa using GIS and remote sensing. Environmental Earth Sciences 65:2087–2102.

Moilanen A, Hanski I (1998) Metapopulation dynamics: effects of habitat quality and landscape structure. Ecology 79:2503–2515.

Moralee RD, van der Bank FH, van der Waal BCW (2000) Biochemical genetic markers to identify hybrids between the endemic *Oreochromis mossambicus* and the alien species, *O. niloticus* (Pisces: Cichlidae). Water Research Commission 26:263–268.

Morán-López R, Da Silva E, Pérez-Bote JL, Corbacho Amado C (2006) Associations between fish assemblages and environmental factors for Mediterranean-type rivers during summer. Journal of Fish Biology 69:1552–1569.

Morita K, Yamamoto S (2002) Effects of Habitat Fragmentation by Damming on the Persistence of Stream-Dwelling Charr Populations. Conservation Biology 16:1318–1323.

Moritz C (1994) Defining “Evolutionarily Significant Units” for conservation. Trends in Ecology & Evolution 9:373–375.

Mouton A, Basson L, Impson D (2001) Health status of ornamental freshwater fishes imported into South Africa: a pilot study. Aqaurium Sciences and Conservation 3:313–319.

Moyle PB, Crain PK, Whitener K, Mount JF (2003) Alien fishes in natural streams: fish distribution, assemblage structure, and conservation in the Cosumnes River, California, U.S.A. Environmental Biology of Fishes 68:143–162.

Moyle PB, Light T (1996) Fish Invasions in California: Do abiotic factors determine success? Ecology 77:1666–1670.

Moyle PB, Williams JE (1990) Biodiversity loss in the temperate zone: Decline of the native fish fauna of California. Conservation Biology 4:275–284.

Muhlfeld CC, Kalinowski ST, McMahon TE, et al. (2009) Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters 5:328–331.

Nel JL, Strydom WF, Maherry A, et al. (2011) Atlas of freshwater ecosystem priority areas in South Africa: Maps to support sustainable development of water resources. Water Research Commission 61.

Nislow KH, Magilligan FJ, Folt CL, Kennedy BP (2002) Within-basin variation in the short-term effects of a major flood on stream fishes and invertebrates. Journal of Freshwater Ecology 17:305–318.

Nowlin WH, Drenner RW, Guckenberger KR, et al. (2006) Gape limitation, prey size refuges and the top–down impacts of piscivorous largemouth bass in shallow pond ecosystems. Hydrobiologia 563:357–369.

O'Brien GC, Bulfin JB, Husted A, Smit NJ (2012) Comparative behavioural assessment of an established and a new tigerfish *Hydrocynus vittatus* population in two man-made lakes in the Limpopo River catchment, southern Africa. African Journal of Aquatic Science 37:253–263.

Olden JD, Jackson DA (2002) A comparison of statistical approaches for modelling fish species distributions. Freshwater Biology 47:1976–1995.

Olden JD, Poff NL, Bestgen KR (2006) Life-history strategies predict fish invasions and extirpations in the Colorado River basin. Ecological Monographs 76:25–40.

Olds AA, Smith MKS, Weyl OLF, Russell IA (2011) Invasive alien freshwater fishes in the Wilderness Lake System, a wetland of international importance in the Western Cape Province, South Africa. African Zoology 46:179–184.

Ormerod SJ, Dobson M, Hildrew AG, Townsend CR (2010) Multiple stressors in freshwater ecosystems. Freshwater Biology 55:1–4.

Padilla DK, Williams SL (2004) Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Fronteirs in Ecology and the Environment 2:131–138.

Page LM, Burr BM (1991) A field guide to freshwater fishes of North America north of Mexico. 432.

Palma AT, Steneck RS (2001) Does variable coloration in juvenile marine crabs reduce risk of visual predation. Ecology 82:2961–2067.

Palmer MA (2010) Beyond infrastructure. Nature 467:534–535.

Parker D, Weyl OLF, Taraschewski H (2011) Invasion of a South African *Anguilla mossambica* (Anguillidae) population by the alien gill worm *Pseudodactylogyrus anguillae* (Monogenea). African Zoology 46:371–377.

Parker IM, Simberloff D, Lonsdale WM, et al. (1999) Impact : toward a framework for understanding the ecological effects of invaders. Biological Invasions 3–19.

Pelicice FM, Vitule JRS, Junior DPL, et al. (2013) A serious new threat to Brazilian freshwater ecosystems: the naturalisation of nonnative fish by decree. Conservation Letters. DOI: 10.1111/conl.12029.

Peterson MS, Slack WT, Woodley CM (2005) The occurrence of non-indigenous nile tilapia *Oreochromis niloticus* (linnaeus) in coastal Mississippi , USA : Ties to aquaculture and thermal effluent. Wetlands 25:112–121.

Pham L, West D, Closs GP (2013) Reintroduction of a native galaxiid (*Galaxias fasciatus*) following piscicide treatment in two streams: response and recovery of the fish population. Ecology of Freshwater Fish 22:361–373.

Picker M, Griffiths CL (2011) Alien & invasive animals: a South African perspective. 240.

Pires AM, Magalhães MF, Moreira Da Costa L, et al. (2008) Effects of an extreme flash flood on the native fish assemblages across a Mediterranean catchment. Fisheries Management and Ecology 15:49–58.

Poff NL (1997) Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391–409.

Poff NL, Allan JD, Bain MB, et al. (1997) The natural flow regime a paradigm for river conservation and restoration. BioScience 47:769–784.

Posada D (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Pulliam HR, Danielson BJ (1991) Sources , sinks , and habitat selection: a landscape perspective on population dynamics. The American Naturalist 137:S50–S66.

Pyke GH (2005) A Review of the biology of *Gambusia affinis* and *G. holbrooki*. Reviews in Fish Biology and Fisheries 15:339–365.

Quinn S, Paukert C (2009) Centrarchid fisheries. In: Cooke SJ, Philipp DP (eds) Centrarchid Fishes: Diversity, Biology and Conservation. Wiley-Blackwell, pp 312–330

Rahel FJ (2007) Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. Freshwater Biology 52:696–710.

Rahel FJ (2013) Intentional fragmentation as a management strategy in aquatic systems. BioScience 65:362–372.

Ramoejane M (2011) *The genetic integrity of* Labeo capensis *and* L. umbratus *(Cyprinidae) in South Africa in relation to inter-basin water transfer schemes*. MSc Thesis, Grahamstown: Rhodes University, 125 pp.

Rashleigh B, Hardwick D, Roux D (2009) Fish assemblage patterns as a tool to aid conservation in the Olifants River catchment (East), South Africa. Water SA 35:517–524.

Resh VH, Brown A V, Covich AP, et al. (1988) The role of disturbance in stream ecology. Journal of the North American Benthological Society 7:433–455.

Ribeiro F, Collares-Pereira MJ, Moyle PB (2009) Non-native fish in the fresh waters of Portugal, Azores and Madeira Islands: a growing threat to aquatic biodiversity. Fisheries Management and Ecology 16:255–264.

Ribeiro F, Elvira B, Collares-Pereira MJ, Moyle PB (2007) Life-history traits of non-native fishes in Iberian watersheds across several invasion stages: a first approach. Biological Invasions 10:89–102.

Ribeiro F, Leunda PM (2012) Non-native fish impacts on Mediterranean freshwater ecosystems: current knowledge and research needs. Fisheries Management and Ecology 19:142–156.

Ricciardi A (2007) Are modern biological invasions an unprecedented form of global change? Conservation Biology 21:329–336.

Ricciardi A, Atkinson SK (2004) Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. Ecology 781–784.

Ricciardi A, Hoopes MF, Marchetti MP, Lockwood JL (2013) Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs 83:263–282.

Ricciardi A, Mottiar M (2006) Does Darwin’s naturalization hypothesis explain fish invasions? Biological Invasions 8:1403–1407.

Richardson DM, Ricciardi A (2013) Misleading criticisms of invasion science: a field guide. Diversity and Distributions 19, 1461–1467.

Richardson DM, Pyšek P, Carlton JT (2011) A compendium of essential concepts and terminology in invasion ecology. Fifty years of invasion ecology The legacy of Charles Elton. Wiley-Blackwell, Oxford, pp 409–420.

Richardson DM, Pyšek P, Rejmánek M, et al. (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6:93–107.

Rivers-Moore NA, Fowles B, Karssing RJ (2013) Impacts of trout on aquatic macroinvertebrates in three Drakensberg rivers in KwaZulu-Natal, South Africa. African Journal of Aquatic Science 38:93–99.

Rivers-Moore NA, Jewitt GPW (2004) Intra-annual thermal patterns in the main rivers of the Sabie Catchment, Mpumalanga, South Africa. Water SA 30:445–452.

Rivers-Moore NA, Jewitt GPW, Weeks DC (2005) Derivation of quantitative management objectives for annual instream water temperatures in the Sabie River using a biological index. Water SA 31:473–482.

Robbins WH, MacCrimmon HR (1974) The blackbass in America and overseas. 196.

Rodriguez MA (2002) Restricted movement in stream fish: the paradigm is incomplete, not lost. Ecology 83:1–13.

Roux D, Moor F De, Cambray J, Barber-James H (2002) Use of landscape-level river signatures in conservation planning: a South African case study. Conservation Ecology 6:6.

Ruesink JL (2005) Global analysis of factors affecting the outcome of freshwater fish introductions. Conservation Biology 19:1883–1893.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. Trends in Ecology & Evolution 1:9–10.

Sagoff M (2005) Do non-native species threaten the natural environment. Journal of Agricultural and Environmental Ethics 18:215–236.

Sax DF, Brown JH (2000) The Paradox of Invasion. Global Ecology and Biogeography 9:363–371.

Schlosser IJ (1987) The role of predation in age- and size-related habitat use by stream fishes. Ecology 68:651–659.

Schlosser IJ (1991) Fish ecology: a landscape perspective. BioScience 41:704–712.

Schlosser IJ (1982) Fish community structure and function along two habitat gradients in a headwater stream. Ecological Monographs 52:395–414.

Schlosser IJ, Angermeier PL (1995) Spatial variation in demographic processes of lotic fishes: conceptual models, empirical evidence, and implications for conservation. American Fisheries Society Symposium 17:392–401.

Schlosser IJ, Ebel KK (1989) Effects of flow regime and Cyprinid predation on a headwater stream. Ecological Monographs 59:41–57.

Schramm HL, Hunt KM (2007) Issues, benefits, and problems associated with fishing tournaments in inland waters of the United States: A survey of fishery agency Administrators. 32:234–243.

Scott LEP, Skelton PH, Booth AJ, et al. (2006) Atlas of southern African freshwater fishes. Smithiana Monograph 2:1–303.

Shelton JM, Day JA, Griffiths CL (2008) Influence of largemouth bass, *Micropterus salmoides*, on abundance and habitat selection of Cape galaxias, *Galaxias zebratus*, in a mountain stream in the Cape Floristic Region, South Africa. African Journal of Aquatic Science 33:201–210.

Simberloff D (2009) The role of propagule pressure in biological invasions. Annual Reviews in Ecology, Evolution and Systematics 40:81–102.

Simberloff D (2005) Non-native species do threaten the natural environment! Journal of Agricultural and Environmental Ethics 595–607.

Skelton PH (2001) A Complete Guide to the Freshwater Fishes of Southern Africa. 395.

Skelton PH (1993) On bass in the Blindekloof - the impact of an alien predator on a wilderness stream. The Naturalist 37:27–27.

Skelton PH, Cambray JA, Lombard A, Benn GA (1995) Patterns of distribution and conservation status of freshwater fishes in South Africa. South African Journal of Zoology 30:71–81.

Slabbert N (2007) The potential impact of an inter-basin water transfer on the Modder and Caledon River systems. MSc Thesis, Bloemfontein: University of the Free State, 215 pp.

Smith TA, Kraft CE (2005) Stream fish assemblages in relation to landscape position and local habitat variables. Transactions of the American Fisheries Society 134:430–440. doi: 10.1577/T03-051.1

Smithson EB, Johnston CE (1999) Movement patterns of stream fishes in a Ouachita Highlands stream: an examination of the restricted movement paradigm. Transactions of the American Fisheries Society 128:847–853.

Snaddon CD, Wishart MJ, Davies BR (1998) Some implications of inter-basin water transfers for fiver ecosystem functioning and water resources management in southern Africa. Aquatic Ecosystem Health and Management 1:159–182.

Sousa V, Penha F, Pala I, et al. (2010) Conservation genetics of a critically endangered Iberian minnow: evidence of population decline and extirpations. Animal Conservation 13:162–171.

Stockwell CA, Mulvey M, Jones AG (1998) Genetic evidence for two evolutionarily significant units of White Sands pupfish. Animal Conservation 1:213–225.

Strahler AN (1957) Quantitative analysis of watershed geomorphology. Transactions, American Geophysical Union 38:913–920.

Strange EM, Moyle PB, Foin TC (1992) Interactions between stochastic and deterministic processes in stream fish community assembly. Fisheries (Bethesda) 3:1–15.

Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55:152–174.

Swartz ER, Flemming AF, Mouton PLFN (2004) Contrasting genetic patterns and population histories in three threatened redfin species (Cyprinidae) from the Olifants River system, western South Africa. Journal of Fish Biology 64:1153–1167.

Swartz ER, Impson ND (2007) *Pseudobarbus afer*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1.

Swartz ER, Skelton PH, Bloomer P (2009) Molecular phylogenetics and evolution phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. Molecular Phylogenetics and Evolution 51:75–84.

Swartz ER, Skelton PH, Bloomer P (2007) Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redfins (*Pseudobarbus afer* and *Pseudobarbus phlegethon* , Cyprinidae) across multiple river systems in South Africa. Journal of Biogeography 2086–2099.

Swofford DL (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. (Sinauer Associates, Sunderland, MA).

Ter Braak CJF (1995) Ordination. In: Jongman R, ter Braak C, Van Tongeren O (eds) Data Analysis in Community and Landscape Ecology. Wageningen: Pudoc, pp 91–173

Ter Braak CJF, Smilauer P (1998) CANOCO Reference Manual and User's Guide to CANOCO for Windows: Software for Canonical Community Ordination (version 4).

Ter Braak CJF, Smilauer P (2002) CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). 500.

Thurow R, Schill DJ (1996) Comparison of day snorkeling, night snorkeling and electrofishing to estimate bull trout abundance and size structure in a second-order Idaho stream. North American Journal of Fisheries Management 16:314–323.

Tomasson T, Bruton MN, Hamman KCD (1985) The demography and management of large cyprinids in a reservoir on the Orange River, South Africa. Fisheries Research 3:279–308.

Tonn WM, Magnuson JT (1982) Patterns in the Species Composition and Richness of Fish Assemblages in Northern Wisconsin Lakes. Ecology 63:1149–1166.

Townsend CR (1996) Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. Biological Conservation 78:13–22.

Townsend CR, Crowl TA (1991) Fragmented population structure in a native New Zealand fish: an effect of introduced brown trout? Oikos 61:347.

Traas GRL (2009) The conservation and management of freshwater fishes in the Greater Addo Elephant National Park. MSc Thesis, Grahamstown: Rhodes University, 157 pp.

Trexler JC, Loftus WF, Jordan F, et al. (2000) Empirical assessment of fish introductions in a subtropical wetland: an evaluation of contrasting views. Natural History 2:265–277.

Tsigenopoulos CS, Rab P, Naran D, Berrebi P (2002) Multiple origins of polyploidy in the phylogeny of southern African barbs (Cyprinidae) as inferred from mtDNA markers. Heredity 88:466–473.

Tweddle D, Bills R, Swartz E, et al. (2009) The status and distribution of freshwater fishes. Chapter 3 In: The Status and Distribution of Freshwater Biodiversity in Southern Africa.IUCNRed List of Threatened Species. Regional Assessment, (eds) W.R.T. Darwall, K.G. Smith, D. Tweddle & P.H. S.

Vrijenhoek RC (1998) Conservation genetics of freshwater fish. Journal of Fish Biology 53:394–412.

Vrijenhoek RC, Douglas ME, Meffe GK (1985) Conservation genetics of endangered fish populations in Arizona. Science 229:400–402.

Van Der Waal BCW (1997) Some observations on the fish life in a seasonal sand river. Southern African Journal of Aquatic Sciences 23:95–102.

Van der Walt K-A (2012) Species identification in South Africa's ornamental trade – how accurate are the names? Honours Thesis, Grahamstown: Rhodes University, 46.

Van Rensburg BJ, Weyl OLF, Davies SJ, et al. (2011) Invasive vertebrates of South Africa. In: Pimentel D (ed) Biological invasions: Economic and environmetal costs of alien plant, animal, and microbe species. Taylor & Francis Group, pp 325–380.

Vitule, JRS, Freire CA, Simberloff D (2009) Introduction of non-native freshwater fish can certainly be bad. Fish and Fisheries 10: 98-108.

Vitule JRS, Freire CA, Vazquez DP, et al. (2012) Revisiting the potential conservation value of non-native species. Conservation Biology 26: 1153-1155.

Waples RS (1991) Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the endangered species act. Marine Fisheries Reviews 53:11–22.

Warren ML (2009) Centrarchid identification and natural history. In: Cooke SJ, Philipp DP (eds) Centrarchid Fishes: Diversity, Biology and Conservation. Wiley-Blackwell, pp 375–533

Wehrly KE, Wang L, Mitro M (2007) Field-Based estimates of thermal tolerance limits for trout: Incorporating exposure time and temperature fluctuation. Transactions of the American Fisheries Society 136:365–374.

West BT (2009) Analyzing longitudinal data with the Linear Mixed Models procedure in SPSS. Evaluation & the Health Professions 32:207–228.

Weyl OLF (2008) Rapid invasion of a subtropical lake fishery in central Mozambique by Nile tilapia, *Oreochromis niloticus* (Pisces: Cichlidae). Aquatic Conservation: Marine and Freshwater Ecosystems 18:839–851.

Weyl OLF, Ellender BR, Woodford DJ, Jordaan MS (2013) Native fish distributions and immediate impact of rotenone treatment on alien fish abundance in the Rondegat River, Cape Floristic Region, South Africa. African Journal of Aquatic Science 38:201–209.

Weyl OLF, Hecht T (1999) A successful population of largemouth bass, *Micropterus salmoides*, in a subtropical lake in Mozambique. Environmental Biology of Fishes 54:53–66.

Weyl OLF, Stadtlander T, Booth AJ (2009) Establishment of translocated populations of smallmouth yellowfish, *Labeobarbus aeneus* (Pisces: Cyprinidae), in lentic and lotic habitats in the Great Fish River system , South Africa. African Zoology 44:93–105.

Weyl OLR, Lewis H (2006) First record of predation by the alien invasive freshwater fish *Micropterus salmoides* L. (Centrarchidae) on migrating estuarine fishes in South Africa. African Zoology 41:294–296.

Weyl PS, de Moor FC, Hill MP, Weyl OLF (2010) The effect of largemouth bass *Micropterus salmoides* on aquatic macro-invertebrate communities in the Wit River, Eastern Cape, South Africa. African Journal of Aquatic Science 35:273–281.

Whitfield AK (1998) Biology and ecology of fishes in southern African estuaries. 223.

Whitlow WL, Rice NA, Sweeney C (2003) Native species vulnerability to introduced predators: testing an inducible defense and a refuge from predation. Biological Invasions 5:23–31.

Williamson M (1996) Biological Invasions. 256.

Williamson M, Brown KC (1986) The analysis and modelling of British invasions. Philosophical Transactions of the Royal Society of London B314:505–522.

Winker H, Weyl OLF, Booth AJ, Ellender BR (2011) Life history and population dynamics of invasive common carp, *Cyprinus carpio*, within a large turbid African impoundment. Marine And Freshwater Research 62: 1270-1280.

Witte F, Goldschmidt T, Wanink J, et al. (1992) The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environmental Biology of Fishes 34:1–28.

Wofford JEB, Gresswell RE, Banks MA (2005) Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. Ecological Applications 15:628–637.

Wood P, Armitage P (1997) Biological effects of fine sediment in the lotic environment. Environmental Management 21:203–17.

Woodford DJ, Hui C, Richardson DM, Weyl OLF (2013) Propagule pressure drives establishment of introduced freshwater fish: quantitative evidence from an irrigation network. Ecological Applications in press.

Woodford DJ, Impson ND (2004) A preliminary assessment of the impact of alien rainbow trout (*Oncorhynchus mykiss*) on indigenous fishes of the upper Berg River , Western Cape Province , South Africa. African Journal of Aquatic Science 29:107–111.

Woodford DJ, Impson ND, Day JA, Bills IR (2005) The predatory impact of invasive alien smallmouth bass *Micropterus dolomieu* (Teleostei: Centrarchidae), on indigenous fishes in a Cape Floristic Region mountain stream. African Journal of Aquatic Science 30:167–173.

Woodford DJ, McIntosh AR (2010) Evidence of source – sink metapopulations in a vulnerable native galaxiid fish driven by introduced trout. Ecological Applications 20:967–977.

Woodford DJ, McIntosh AR (2011) Location of demographic sources affects the distributions of a vulnerable native fish in invaded river networks. Freshwater Biology 56:311–324.

Zengeya TA, Booth AJ, Bastos ADS, Chimimba CT (2011) Trophic interrelationships between the exotic Nile tilapia, *Oreochromis niloticus* and indigenous tilapiine cichlids in a subtropical African river system (Limpopo River, South Africa). Environmental Biology of Fishes 92:479–489.

Zengeya TA, Marshall BE (2007) Trophic interrelationships amongst cichlid fishes in a tropical African reservoir (Lake Chivero, Zimbabwe). Hydrobiologia 592:175–182.

Zengeya TA, Robertson MP, Booth AJ, Chimimba CT (2013a) Ecological niche modeling of the invasive potential of Nile tilapia *Oreochromis niloticus* in African river systems: concerns and implications for the conservation of indigenous congenerics. Biological Invasions 23:DOI:10.1007/s10530–012–0386–7.

Zengeya TA, Robertson MP, Booth AJ, Chimimba CT (2013b) A qualitative ecological risk assessment of the invasive Nile tilapia, *Oreochromis niloticus* in a sub-tropical African river system (Limpopo River, South Africa). Aquatic Conservation: Marine and Freshwater Ecosystems 23:51–64.

Appendices

Appendix 1 Details of sequenced *Pseudobarbus afer* collected from the Swartkops River system and *Barbus trevelyani* collected from the Keiskamma River system, Eastern Cape, South Africa.

Species	River System	River	S	E	Field tube no.	Sequence no.
Pseudobarbus afer	Swartkops	Blindekloof	33 42 15.4	25 18 05.9	BE2010-266	MB7351
Pseudobarbus afer	Swartkops	Blindekloof	33 42 15.4	25 18 05.9	BE2010-264	MB7352
Pseudobarbus afer	Swartkops	Blindekloof	33 42 15.4	25 18 05.9	BE2010-259	MB7353
Pseudobarbus afer	Swartkops	Blindekloof	33 41 36.0	25 18 37.3	BE2010-293	MB7355
Pseudobarbus afer	Swartkops	Blindekloof	33 41 36.0	25 18 37.3	BE2010-261	MB7356
Pseudobarbus afer	Swartkops	Blindekloof	33 41 36.0	25 18 37.3	MZ2010-270	MB7358
Pseudobarbus afer	Swartkops	Chaseskloof	33 40 12.4	25 14 11.3	BE2010-098	MB5465
Pseudobarbus afer	Swartkops	Chaseskloof	33 40 12.4	25 14 11.3	BE2010-089	MB5474
Pseudobarbus afer	Swartkops	Chaseskloof	33 40 12.4	25 14 11.3	BE2010-096	MB5475
Pseudobarbus afer	Swartkops	Chaseskloof	33 40 12.4	25 14 11.3	BE2010-100	MB5476
Pseudobarbus afer	Swartkops	Chaseskloof	33 40 12.4	25 14 11.3	BE2010-072	MB5477
Pseudobarbus afer	Swartkops	Chaseskloof	33 40 12.4	25 14 11.3	BE2010-086	MB5485
Pseudobarbus afer	Swartkops	Fernkloof	33 43 27.2	25 17 04.0	BE2010-037	MB5482
Pseudobarbus afer	Swartkops	Fernkloof	33 43 05.2	25 17 22.1	BE2010-296	MB5632
Pseudobarbus afer	Swartkops	Fernkloof	33 43 05.2	25 17 22.1	BE2010-257	MB5637
Pseudobarbus afer	Swartkops	Fernkloof	33 43 05.2	25 17 22.1	BE2010-273	MB6800
Pseudobarbus afer	Swartkops	Fernkloof	33 43 05.2	25 17 22.1	BE2010-258	MB6812
Pseudobarbus afer	Swartkops	Fernkloof	33 43 05.2	25 17 22.1	BE2010-285	MB6813
Pseudobarbus afer	Swartkops	Fernkloof	33 43 05.2	25 17 22.1	BE2010-023	MB5469
Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-028	MB5479
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-058	MB5630
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-076	MB5633
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-060	MB5634
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-081	MB5635
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-078	MB5636
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-038	MB6805
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-284	MB6809
Pseudobarbus afer	Swartkops	Fernkloof	33 43 38.1	25 16 44.3	BE2010-064	MB6811
Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-056	MB5626
Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-095	MB5628
Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-099	MB5639
Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-295	MB6795
Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-298	MB6802

Pseudobarbus afer	Swartkops	Fernkloof	33 43 58.4	25 16 18.1	BE2010-297	MB6808
Pseudobarbus afer	Swartkops	Swartkops	33 42 57.8	25 17 22.0	BE2010-002	MB5466
Pseudobarbus afer	Swartkops	Swartkops	33 42 57.8	25 17 22.0	BE2010-004	MB5470
Pseudobarbus afer	Swartkops	Swartkops	33 42 57.8	25 17 22.0	BE2010-003	MB5473
Pseudobarbus afer	Swartkops	Swartkops	33 42 57.8	25 17 22.0	BE2010-001	MB6807
Pseudobarbus afer	Swartkops	Nounekkloof	33 41 56.2	25 12 54.8	BE2010-034	MB5471
Pseudobarbus afer	Swartkops	Nounekkloof	33 41 56.2	25 12 54.8	BE2010-049	MB5472
Pseudobarbus afer	Swartkops	Nounekkloof	33 41 56.2	25 12 54.8	BE2010-043	MB5478
Pseudobarbus afer	Swartkops	Nounekkloof	33 41 56.2	25 12 54.8	BE2010-044	MB5483
Pseudobarbus afer	Swartkops	Nounekkloof	33 41 51.6	25 13 02.5	BE2010-039	MB6804
Pseudobarbus afer	Swartkops	Nounekkloof	33 41 51.6	25 13 02.5	BE2010-021	MB6806
Pseudobarbus afer	Swartkops	Waterkloof	33 43 00.4	25 17 00.9	BE2010-009	MB5467
Pseudobarbus afer	Swartkops	Waterkloof	33 43 00.4	25 17 00.9	BE2010-010	MB5468
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-012	MB5480
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-262	MB5627
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-294	MB5631
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-057	MB5638
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-283	MB5641
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-289	MB5642
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-291	MB6796
Pseudobarbus afer	Swartkops	Waterkloof	33 43 17.0	25 16 20.3	BE2010-013	MB6803
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-026	MB5484
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-274	MB5629
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-288	MB5640
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-286	MB5643
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-079	MB6797
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-071	MB6798
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-252	MB6801
Pseudobarbus afer	Swartkops	Waterkloof	33 43 23.9	25 15 37.6	BE2010-292	MB6810
Barbus trevelyani	Keiskamma	Amatele	32 43 50.2	27 01 22.5	BE2010-127	MB5510
Barbus trevelyani	Keiskamma	Amatele	32 43 50.2	27 01 22.5	BE2010-139	MB5512
Barbus trevelyani	Keiskamma	Amatele	32 43 12.6	27 00 51.0	BE2010-117	MB5516
Barbus trevelyani	Keiskamma	Amatele	32 43 50.2	27 01 22.5	BE2010-110	MB5520
Barbus trevelyani	Keiskamma	Amatele	32 43 50.2	27 01 22.5	BE2010-108	MB6788
Barbus trevelyani	Keiskamma	Gwiligwili	32 40 45.4	27 13 22.5	BE2010-137	MB5513
Barbus trevelyani	Keiskamma	Gwiligwili	32 40 45.4	27 13 22.5	BE2010-144	MB5514
Barbus trevelyani	Keiskamma	Gwiligwili	32 40 45.4	27 13 22.5	BE2010-112	MB5518
Barbus trevelyani	Keiskamma	Gwiligwili	32 40 45.4	27 13 22.5	BE2010-134	MB5521
Barbus trevelyani	Keiskamma	Gwiligwili	32 40 45.4	27 13 22.5	BE2010-123	MB6791

Barbus trevelyani	Keiskamma	Keiskamma	32 38 25.3	27 11 26.1	BE2010-116	MB5509
Barbus trevelyani	Keiskamma	Keiskamma	32 37 09.2	27 13 26.8	BE2010-115	MB5511
Barbus trevelyani	Keiskamma	Keiskamma	32 38 25.3	27 11 26.1	BE2010-113	MB5515
Barbus trevelyani	Keiskamma	Keiskamma	32 38 25.3	27 11 26.1	BE2010-141	MB5517
Barbus trevelyani	Keiskamma	Keiskamma	32 38 25.3	27 11 26.1	BE2010-135	MB6787
Barbus trevelyani	Keiskamma	Mnyameni	32 36 10.7	27 04 00.3	OW10B-493	MB6782
Barbus trevelyani	Keiskamma	Mnyameni	32 36 10.7	27 04 00.3	OW10B-498	MB6783
Barbus trevelyani	Keiskamma	Mnyameni	32 36 10.7	27 04 00.3	OW10B-447	MB6784
Barbus trevelyani	Keiskamma	Mnyameni	32 36 10.7	27 04 00.3	OW10B-417	MB6785
Barbus trevelyani	Keiskamma	Mnyameni	32 36 10.7	27 04 00.3	OW10B-488	MB6815
Barbus trevelyani	Keiskamma	Rabula	32 44 23.8	27 11 19.9	BE2010-131	MB5508
Barbus trevelyani	Keiskamma	Rabula	32 44 23.8	27 11 19.9	BE2010-107	MB6790
Barbus trevelyani	Keiskamma	Rabula	32 44 23.8	27 11 19.9	BE2010-105	MB6792
Barbus trevelyani	Keiskamma	Rabula	32 44 23.8	27 11 19.9	BE2010-128	MB6814
Barbus trevelyani	Keiskamma	Tyume	32 37 13.0	26 55 08.0	BE2010-124	MB5519
Barbus trevelyani	Keiskamma	Tyume	32 37 13.0	26 55 08.0	BE2010-132	MB5644
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	BE2010-090	MB5645
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	OW10B-426	MB6779
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	OW10B-440	MB6780
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	OW10B-414	MB6781
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	BE2010-092	MB6789
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	BE2010-063	MB6793
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	BE2010-070	MB6794
Barbus trevelyani	Keiskamma	Tyume	32 38 22.6	26 56 09.9	OW10B-410	MB6816