Understanding the unintended spread and impact of alien and invasive fish species – development of management guidelines for South African inland waters

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

Invasive alien species are recognised globally as direct drivers of biodiversity and ecosystem service loss that pose substantial threats to fragile ecosystems and threatened and endangered species. In South Africa, this recognition led to the promulgation of the National Environmental Management: Biodiversity Act (Act 10 of 2004) and the subsequent supporting Alien Invasive Species Regulations (RSA 2013 and amended in 2014). These regulations provide a legal framework for the future management of invasive alien species, including freshwater fishes, within the borders of South Africa. The Alien Invasive Species regulations now recognise 3 distinct categories of invasive fish species each with prescribed management obligations.

RATIONALE

In South Africa some invasive alien fish species such as bass, carp and trout threaten biodiversity and sensitive ecosystems in some areas but are important contributors to the national economy elsewhere. It is also recognised that in many cases alien species are so well established that their eradication would not be feasible. As a result, some alien fishes should be actively managed to limit further impacts on biodiversity, as well as to maximise social and economic benefits from the resource in areas where they are already established. This will require a national management plan that will guide eradication in areas of biodiversity concern (e.g. protected areas) and permit their use in others.

The rationale for this project was to use case studies that exemplify the issues surrounding the understanding of alien fish invasion processes and impacts in order to collate key lessons learned that would inform a management decision framework. The key processes for the various case studies were:

- Sundays River: Obtaining increased understanding of processes of transport and establishment of invasive alien species especially through water transfer infrastructure;
- Groot Marico catchment: Understanding interspecific impacts and conservation implications for native species; and
- Other case studies: Determining invasion state, assessing impacts over time in unstable systems, determining best practices for eradication operations.

OBJECTIVES AND AIMS

The overarching objectives of this study were:

- To conduct two comprehensive case studies that exemplify the South African situation regarding the spread and impacts of alien invasive fishes in aquatic ecosystems; and
- To use the results of these case studies to develop a decision support framework to prioritise areas for alien fish eradication efforts within river systems and better manage the impacts of human mediated transfer of alien fish.

CASE STUDY 1: ASSESSING IMPACTS OF LARGEMOUTH BASS IN A NEAR-PRISTINE ECOSYSTEM

The catchment of the Groot Marico River is characterised by deeply incised gorges that are fairly inaccessible and unsuitable for large-scale agriculture or development, and therefore uniquely un-impacted compared to most other rivers in the North West Province or in South African. The Groot Marico is also relatively unique in the South African context in that it originates in a series of dolomitic eyes. The dolomitic eyes discharge into tributaries that confluence to form the Groot Marico main stem. Studies conducted during the 1980s and 1990s identified the significant conservation value of the dolomitic eye ecosystems and their associated biota. Based on those assessments the tributaries of the Groot Marico River were identified as biodiversity features of special ecological significance. As a result, these streams are prioritised in the provincial conservation plan and the entire upper catchment is classified as a Freshwater Priority Area (FEPA) and Fish Support Area. This study aimed to assess the impact of Largemouth bass (*Micropterus salmoides)*, an invasive alien fish species, on the relatively unimpacted fish community in the Groot Marico catchment.

Based on this assessment the endemic Marico barb (*Barbus motebensis)* was the only species that was strongly negatively correlated with the presence of *M. salmoides.* The density of *B. motebensis* showed significant differences between invaded and un-invaded pools within the elevations at which *M. salmoides* occurred. The remainder of the fish species either showed no correlation or were positively correlated with the presence of *M. salmoides*. The absence of *M. salmoides* from the upper reaches of the Groot Marico catchment was attributed to the increased elevation, increasing gradient and barriers to upstream migration. With the exception of the Sterkstroom/ Polkadraaispruit and a seemingly isolated population in the Marico eye (Kaaloog se Loop) *M. salmoides* was absent from the tributaries.

The assessment of trophic interactions between alien and indigenous fish species did not show any discernable difference between invaded and un-invaded sites that could be attributed to the presence of *M. salmoides*. The only observed evidence of the potential impact of the invasive *M. salmoides* on indigenous fish communities was the absence of *B. motebensis* from river section where *M. salmoides* occurred.

The results of the genetic assessment identified two distinct lineages (the Draai and Eastern lineages) of *B. motebensis* suggesting historical isolation between these populations. There were also significant levels of differentiation between tributary populations of *Amphilius uranoscopus* resulting in the identification of two lineages (the Ribbok and Western lineages). Priority areas for conservation were identified based on the current conservation status of the species (*B. motebensis* being Vulnerable (VUL) on the IUCN Red List), the number of Evolutionary Significant Units for each species as well as the overall genetic diversity of the species. In total, four tributary populations were identified as conservation priorities areas, these were the Draaifontein, Van Straatens, Ribbokfontein and Kaaloog se Loop tributaries.

CASE STUDY 2: UNDERSTANDING THE ROLE OF WATER-TRANSFER INFRASTRUCTURE IN ENABLING INVASION

This case study assessed the processes of invasion using a natural experiment provided by the irrigation water transfer infrastructure of the Sundays River valley, a citrus-growing area with a complex canal network that supplies water from an Inter-Basin Water Transfer (IBWT) scheme to approximately 400 small off-channel impoundments. A year-long study linked the number of propagules (immigrating fish) being supplied by the irrigation network to 30 water storage ponds of varying ages, to assess the patterns and processes of fish invasion through water transfer infrastructure. The project showed that high propagule size in the canals corresponds to rapid colonisation of the ponds, provided the introduced fish species is capable of reproducing within the environment of the pond. Although we set out to assess what management strategies could mediate the transport of fish through the canals, we determined that this was impossible, as no change in management strategy or modification of water-transfer infrastructure was likely to result in decreased transfer of fish propagules or meaningful improvements to the conservation status of the freshwater ecosystems. We did, however, identify an on-going invasion of the adjacent Addo Elephant National Park, which was likely enabled through water discharges from the irrigation network. While prevention of this invasion is no longer possible, we describe how an assessment of the risk of invasion and subsequent investment into infrastructure to prevent it (i.e. a weir placed on the tributary upstream of the canal discharge point) may have historically prevented the invasion. We conclude the case study by emphasising the importance of considering the implications of organism transfers when designing inter-basin or intra-basin water transfer schemes, and in investing in preventative measures prior to water transfer infrastructure becoming operational.

OTHER CASE STUDIES

We use three case studies to explore other aspects of fish invasions, impacts and management not explicitly covered by the two main case studies summarised above. We describe the use of a unified framework to define invasion state in assessing the status of an invasion of a loricariid catfish in the Richards Bay area of KwaZulu-Natal. We emphasise the benefits of using the framework to establish the extent of the invasion and to prioritise management options. We assessed multiple species invasions in the Swartkops River system in the Eastern Cape, which consisted of natural expansions up a seasonally flowing tributary (the Blindekloof stream) by alien fish species from a common invasion source (the Swartkops main stream). This study showed that alien species vary in their ability to colonise new stream reaches and to have an impact on native fish fauna, so that management plans must be constructed on a species-specific basis to take these variations into account. Finally, we describe the successful eradication of alien fish from a mountain stream (the Rondegat River) using the piscicide rotenone. We describe the processes and procedures that were followed to secure approval for the project, which included a comprehensive EIA and significant engagement with angling user groups. We describe the successful aftermath of the operation, which saw the rapid recovery of native fish in the treated reach following the eradication of the alien predator.

DEVELOPING A DECISION SUPPORT TOOL FOR MANAGING INVASIVE FISH IN SOUTH AFRICA

We initially describe and collate the major findings, implications and lessons learned emerging from the case studies above for the assessment, management and prevention of fish invasions and their impacts. These include an assessment that the major pathways for fish invasions within South Africa today are the aquarium trade and illegal movement of fish by angler, which activities will need to be more carefully regulated in the future. We assess the options available for mitigating the impacts on invasive fish on native biota, which realistically are limited to targeted eradications in logistically feasible habitats with high conservation priority. We also examine the lessons learned during the successful Rondegat rehabilitation project, which included choosing a river that is logistically feasible to treat, ensuring that barriers to re-invasion by the alien fish are breach-proof but do not negatively affect other biota (i.e. native migratory fish), using a standardised operating procedure to ensure human safety and successful eradication of target species, as well as community engagement to minimise the risk of future illegal re-introduction of the invasive species.

With these general principals in place, we then describe a decision support framework for managing invasive fish in South Africa. The framework is written in the form of a decision

tree that enables systematic prioritisation of discreet populations of invasive fish for active management or eradication. The framework asks the following questions in sequence:

1. Is the population established?
2. Is the population invasive?
3. Does the population have socio-economic value?
4. Is eradication feasible?

A positive response to question 4 results in a recommendation to “eradicate” the target population, while a positive response to question 3 results in a recommendation to “manage against impacts and further spread”. Negative responses to Questions 2 and 4 also result in the recommendation to “manage against impacts and further spread”.

We illustrate the use of the framework with examples from the Sundays, Marico, Nseleni and Swartkops case studies. In the Sundays example, we demonstrate that while alien fish populations within the irrigation network itself are unmanageable, the invasive catfish population in the Coerney River can be managed, and that the decision tree recommends “manage against impacts and further spread” because eradication is deemed not feasible within the stream. We similarly investigate three discreet populations of bass in the Groot Marico catchment, and determine through the decision tree that only the population in the Marico Eye is an appropriate target for eradication, due to its small geographic extent and its proximity to a vulnerable stream reach (the Kaaloog se Loop stream) that could easily be invaded with human assistance. We similarly demonstrate that the Loricariid catfish in the Nseleni and Centrarchid basses in the Swartkops cannot be eradicated for logistical reasons.

We conclude this chapter and the report by emphasising that the framework can only be an effective tool for invasive fish management if there are conservation practitioners available to pursue the management goals it suggests. This will require increased employment of aquatic scientists within provincial government, and increased funding for tackling aquatic conservation issues at a national scale. We also highlight the need for improved engagement between government and the users of invasive fish species, as only through dialogue and cooperation can the desired management outcomes for invasive fishes be achieved within South Africa.

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LIST OF ACRONYMS

AENP	Addo Elephant National Park
ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
CBD	Convention on Biological Diversity
CCA	Canonical Correspondence Analysis
COP	Conference of Parties
DAFF	Department of Agriculture Fisheries and Forestry
DNA	Deoxyribonucleic acid
DWAF	Department of Water Affairs and Forestry
EIA	Environmental Impact Assessment
ESU	Evolutionary Significant Unit
FEPA	Freshwater Ecosystem Priority Areas
FL	Fork Length
FOSA	Federation of South African Flyfishers
GWA	Groendal Wilderness Area
IBWT	Inter-Basin Water Transfer
IRI	Index of Relative Importance
ISMP	Invasive Species Management Programme
IUCN	International Union for Conservation of Nature
LSRWUA	Lower Sundays River Water Users Association
Masl	Meters Above Sea Level
NEM:BA	National Environmental Management: Biodiversity Act
NFEPA	National Freshwater Ecosystem Priority Areas
NRF	National Research Foundation

NWDACE	North-West Department of Agriculture, Conservation and Environment
PCR	Polymerase Chain Reaction
POM	Particulate Organic Matter
RSA	Republic of South Africa
SABAA	South African Bass Angling Association
SAFBAF	South African Freshwater Bank Angling Federation
SAIAB	South African Institute of Aquatic Biology
SEA	Standard Ellipse Areas
SRIN	Sundays River Irrigation Network
VUL	Vulnerable
WMA	Water Management Area
WRC	Water Research Commission

1 THE THREAT OF ALIEN AND INVASIVE FISHES IN SOUTH AFRICA

1.1 A history of introductions to the country

A review of fish introductions and their impacts in South Africa is provided in Ellender & Weyl (2014). This review, conceptualised and developed during the current project phase was published in the open access journal Aquatic Invasions. A short synopsis follows.

In South Africa intentional fish introductions have occurred to establish food fishes, create new fisheries and to control plants and invertebrates. Additional accidental introductions are linked to escape from aquaculture facilities and the pet trade[1] (Table 1). Of 28 alien fish species that were introduced into South African waters, only six failed to establish (Table 2) and there is evidence of self-sustaining populations of 22 alien fishes (Ellender & Weyl, 2014).

In addition to species that are alien to the geographic borders of South Africa, there are introduction records for 27 South African species into waters outside of their native ranges. Such introductions have resulted in the establishment of extra-limital populations of several species. These species are alien to the river where introduced and have severe impacts on recipient environments. Such species include the African Sharptooth catfish (*Clarias gariepinus)*, which is spreading rapidly through the Eastern and Western Cape where it threatens native fishes and has had negative impacts on recreational angling in several impoundments. Another species is the Orange River mudfish (*Labeo capensis)*, a species that was introduced into the Eastern Cape through the Orange-Fish River inter basin water transfer scheme. In the Eastern Cape this species hybridises with, and threatens the genetic integrity of native Moggel (*Labeo umbratus)*. As these species are native to South Africa they are often difficult to manage. As a result they will require special attention in emerging legislation.

[1] In the South African context it is important to differentiate between the pet trade and other modes of introduction. This is because South Africa currently permits the import and sale of ~1600 freshwater fish species in the pet trade. These fish are introduced for keeping in aquaria and because they their physiological requirements seldom match the environmental conditions when they escape captivity. As a result, of the 28 alien species with introduction records into the wild, only 5 were linked to the pet trade.

Table 1: A list of alien fish species with evidence for introduction into the wild in South Africa between 1726 and 2013; region of origin; the associated introduction vector and their current status in South Africa (after Ellender & Weyl, 2014).

Date	Common name	Species	Origin	Vector	Status
1726	Goldfish	*Carassius auratus*	Asia	Pet trade	Established, localised
1859	Common carp	*Cyprinus carpio*	Asia	Fisheries	Established,
1890	Brook trout	*Salvelinus fontinalis*	North America	Fisheries	Failed
1892	Brown trout	*Salmo trutta*	Europe	Fisheries	Established,
1896	Atlantic salmon	*Salmo salar*	Europe	Fisheries	Failed
1896	Tench	*Tinca tinca*	Europe	Fisheries	Established, localised
1897	Rainbow trout	*Oncorhynchus mykiss*	North America	Fisheries	Established,
1910	Israeli tilapia	*Oreochromis aureus*	Africa	Aquaculture	Failed
1912	Guppy	*Poecilia reticulata*	Central America	Pet trade	Established, localised
1915	Perch	*Perca fluviatilis*	Europe	Fisheries	Established, localised
1928	Largemouth bass	*Micropterus salmoides*	North America	Fisheries	Established,
1936	Mosquitofish	*Gambusia affinis*	Central America	Bio-control	Established,
1937	Smallmouth bass	*Micropterus dolomieu*	North America	Fisheries	Established,
1939	Bluegill sunfish	*Lepomis macrochirus*	North America	Fisheries	Established,
1940	Spotted bass	*Micropterus*	North America	Fisheries	Established,
1955	Nile tilapia	*Oreochromis niloticus*	Africa	Aquaculture	Established, spreading
1959	Mango tilapia	*Sarotherodon galilaeus*	Africa	Aquaculture	Failed
1959	Redbelly tilapia	*Tilapia zillii*	Africa	Aquaculture	Failed
1960	Nembwe	*Serranochromis*	Africa	Fisheries	Failed
1967	Grass carp	*Ctenopharyngodon*	Asia	Bio-control	Established
1974	Swordtail	*Xiphophorus hellerii*	Central America	Pet trade	Established, localised
1975	Silver carp	*Hypophthalmichthys*	Asia	Aquaculture	Established
1982	Threespot tilapia	*Oreochromis andersoni*	Africa	Aquaculture	Failed
1984	Florida bass	*Micropterus floridanus*	North America	Fisheries	Established
2000	Vermiculated Sailfin	*Pterygoplichthys*	South America	Pet trade	Established, localised
2006	Southern Platyfish	*Xiphophorus maculatus*	Central America	Pet trade	Uncertain
2012	Highfin pangasius	*Pangasius*	Asia	Pet trade	Uncertain

Table 2: A list of alien fish species that have established wild populations in South Africa; their NEMBA listing; invasion state, known impacts, their current status and invasion category defined according to (Blackburn et al., 2011). Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence (after Ellender & Weyl, 2014).

Species	A	State	Impact	SA evidence	Uses	Value of naturalised populations
C. auratus	NL	Localised in urban dams.	Feeding action increases turbidity	Never assessed	Important aquaculture species (pet trade)	None
C. carpio	1a	Established in rivers and dams in 14/17 assessed river basins in SA.	Bottom grubbing behaviour impacts on water quality.	Only anecdotal evidence.	Important recreational and subsistence angling species. Harvested commercially in the Free State.	Food security and economic opportunities.

Species	A	State	Impact	SA evidence	Uses	Value of naturalised populations
S. trutta	1a	Widespread at higher altitudes, stocked in dams	Predation and competition	Demonstrated impacts on fish and amphibian distributions in Eastern Cape and KZN	Important recreational fisheries species that is cultured for stocking.	Economic opportunities linked to recreational angling.
T. tinca	NL	Localised, established in the Breede River only	Bottom grubbing may increase turbidity.	Never assessed	None	None
O. mykiss	1a	Established, widespread but limited to high altitude rivers and impoundments.	Predation and competition	Demonstrated impacts on fish and amphibian distributions in Eastern Cape, Western Cape and KZN	Most important freshwater aquaculture species in South Africa and important in recreational fisheries.	Economic opportunities linked to recreational angling.
P. reticulata	NL	Established, localised mainly in urban rivers and ponds.	Potential competition with native species.	Never assessed	Important aquaculture species (pet trade)	None
P. fluviatilis	P	Established, extremely localised and possibly extinct.	Competition with and predation on native species.	Never assessed	None	None
M. salmoides	1a	Established, widespread and occurrence records exist from all major rivers.	Competition with and predation on native species.	Demonstrable impacts on native fish populations in Eastern Cape and Limpopo.	Important in recreational and subsistence fisheries.	Food security and economic opportunities linked to recreational angling.
G. affinis	P	Established, widespread	Competition with and predation on native species.	Never assessed	None	None
M. dolomieu	1a	Established, widespread	Competition with and predation on native species.	Alterations of insect and native fish communities in streams.	Important in recreational and subsistence fisheries.	Economic opportunities linked to recreational angling.
L. macrochirus	1a	Established, widespread	Competition with and predation on	Not assessed	Minor angling species.	None

Species	A	State	Impact	SA evidence	Uses	Value of naturalised populations
			native species.			
M. punctulatus	1a	Established, widespread	Competition with and predation on native species.	Not assessed	Minor angling species.	None
O. niloticus	1a	Established, spreading	Hybridisation with native Mozambique tilapia, competition with native fishes for food and habitat.	Hybridises with native Mozambique tilapia and competes with native tilapias for resources.	Emerging aquaculture species.	None
C. idella	1a	Established	Denudation through heavy grazing on aquatic macrophytes.	Never assessed	By catch in subsistence and recreational fisheries where established	Subsistence fisheries.
X. hellerii	NL	Established, localised	Competition with and predation on native species.	Never assessed	Important aquaculture species (pet trade)	None
H. molitrix	1a	Established	Competition with native fishes for phyto and zooplankton.	Not assessed	By catch in subsistence and recreational fisheries where established	Subsistence fisheries.
M. floridanus	1a	Established	Competition with and predation on native species.	Not assessed directly but similar to those of *M. salmoides* with which it hybridises.	Important in recreational and subsistence fisheries.	Food security and economic opportunities linked to recreational angling.
P. disjunctivus (Weber)	1a	Established, localised	Potential competition with native fishes interferes with commercial fisheries.	Low trophic position	Important import in the pet trade. No longer produced locally.	None

1.2 Known pathways of invasion

The most successful and widespread species in South Africa are those that were stocked to create opportunities for fisheries (Ellender & Weyl, 2014). These introductions into natural (rivers) or man-made (dams) water bodies were, until the mid-1980s, facilitated by government-supported stocking programs whereafter private stocking, both permitted and illegal, has ensured the continued spread alien fishes, particularly those that are of interest to anglers (e.g. *M. salmoides*, *S. trutta)* and *C. carpio)*) (Ellender & Weyl, 2014). Many introductions resulted in the desired economic outcomes, i.e. the establishment of fisheries for food and recreation that contribute to national policy objectives of poverty eradication, food security and economic empowerment at national and local levels. While the magnitude of these contributions has not been formally assessed at a national level, local-scale assessments indicate that they are considerable. Alien fishes are also essential in aquaculture development, a recognised growth sector for the Department of Agriculture Fisheries and Forestry (DAFF). Currently farmed species include the following listed species: *O. mykiss*, *O. niloticus*, *C. carpio*, Grass carp (*Ctenopharyngodon idella)*, *M. salmoides*, Atlantic salmon (*Salmo salar)* and many aquarium species (e.g. *C. auratus*, *P. reticulata*.) Escapes from captivity have contributed to the establishment of Silver carp (*Hypophthalmichthys molitrix)*, *O. niloticus,* and Grass carp in South African rivers.

1.3 Known global impacts

Invasive alien species are one of the most important direct drivers of biodiversity loss and ecosystem service changes, and constitute the greatest threat to fragile ecosystems (Ricciardi, 2007). There is a broad scientific consensus that invasive species are likely to play a crucial and devastating role in species extinction (Hulme, 2003; Vitousek *et al*., 1996). At the 2010 Convention on Biological Diversity (CBD) Conference of Parties (COP10) it was stated that invasive species are the second biggest driving force of species extinction, after the effects of human activity (habitat loss, overexploitation, and pollution).

Few studies have addressed impacts at the individual level in South Africa[2]. Those that have, present evidence of severe impacts on native biota. Impacts are most severe where

[2] The state and impact of aquatic invasions in South Africa are not well documented because of a lack of national capacity to undertake aquatic assessments. As a result information on distribution and impact is limited to results of *ad hoc* research projects funded through competitive research support channels (e.g. Water Research Commission, NRF). This has resulted in a situation where assessments are driven by researcher interests rather than national information needs. As a result,

native fishes have not evolved with native predatory fishes (e.g. Weyl *et al*., 2014). In streams in the Cape Floristic Region for example, alien fishes have resulted in range restrictions and fragmentation of populations of endemic fishes many of which now only occur in non-invaded stream reaches often above natural barriers such as waterfalls which inhibit non-native fish dispersal (Ellender *et al*., 2011; Weyl *et al*., 2013). Other biota are also effected and in the uKhahlamba Drakensberg Park World Heritage site, the abundance of tadpoles of the Natal cascade frog (*Hadromophryne natalensis)* was up to 15 times lower in stream reaches that had been invaded by Brown trout and Rainbow trout than in non-invaded reaches (Karssing *et al*., 2012). Changes in invertebrate community structure have been documented following Bass (*Micropterus* spp.) introductions in several localities (e.g. Lowe *et al*., 2008; Weyl *et al*., 2010). In the Rondegat River in the Western Cape these changes in invertebrate communities have been shown to result in changes in ecosystem functioning (Lowe *et al*., 2008).

Alien fish can also impact on water quality. The common carp, for example, are aggressive invaders that are widely considered as a pest species and has been linked to habitat alterations brought about by increased turbidity which results from its bottom grubbing feeding behaviour. This increased turbidity not only impacts on native ecosystems by increasing nutrient loading, smothering of fish eggs and decreasing the efficiency sight-feeding predators, but also increases the cost of water purification for human consumption (see De Moor & Bruton, 1988).

A severe impact of alien fish introductions is hybridisation with native biota. In South Africa, studies on the impact of *O. niloticus* introductions into the Limpopo River system indicate extensive hybridisation and introgression with native *Oreochromis mossambicus* (Mozambique tilapia) (Firmat *et al*., 2013). Hybridisation is recognised as a primary threat to *O. mossambicus* and they are consequently listed on the IUCN Red List as 'Near Threatened' (Van der Waal & Bills, 2000).

One of the impacts of alien fishes on the environment and specific the indigenous fishes that are often neglected is the effect that their parasites might have on the indigenous fish populations. It is well recorded in South Africa and abroad that the biggest pathological and subsequent mortality of freshwater fishes caused by parasites are mainly when they are infected by alien parasites. For example, the Asian tapeworm (*Bothriocephalus acheilognathi)*, and Fish louse (*Argulus japonicas)* both introduced to South Africa via there

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Information is often limited to isolated case studies that are not geographically relevant for management advice.

alien host the Common carp, has serious pathological effects on indigenous fishes and various records of mortality of indigenous fish caused by these parasites exist (see Ellender & Weyl, 2014 for review).

1.4 National Environmental Management: Biodiversity Act and the obligation to manage alien and invasive fishes

Following the democratic dispensation of 1994 and the progressive new legislation that has come in its wake, South Africa as a nation is becoming more proactive in its attitude and reactions to the threat posed by invasive species on its natural biodiversity heritage. Fundamental to this new stance was the promulgation of the National Environmental Management: Biodiversity Act (Act 10 of 2004) and the subsequent publication of its supporting Alien Invasive Species Regulations (RSA 2013 and amended in 2014)3. These new regulations provide a legal framework for the future management of invasive alien species, including freshwater fishes, within the borders of South Africa. In the case of freshwater fish species, there are now 3 distinct categories under which a species may fall, although at the time of this report, some species are listed under more than one category in order to enable more practical management. The categories are as follows:

- 1a – Prohibited in South Africa: These species do not currently occur within South Africa's borders, and should not be granted permission for importation. Should they be found within the borders, they will be managed as for category 1b;
- 1b – Require control via a management plan: These species are established within South Africa, and may not be moved outside of their current established range within the country. They may not be bought or sold but may be caught and released within their current distribution without a permit. These species can be controlled once a formal management plan is developed on a species- and area-specific basis; and
- 2 – Management via permit. These species require a permit for purchase, sale, movement and stocking within catchments in which they currently occur. They may not be moved to catchments in which they do not occur.

1.5 Aims and objectives of this study

Control of alien fish is recognized as difficult worldwide. In South Africa, alien fishes are so well established in the lower reaches of rivers and in impoundments that their eradication is often not possible (Van Rensburg *et al*., 2011). In addition, many alien fishes in South Africa

[3] Whilst these regulations are yet to be formally promulgated, and are subject to change, they do provide a legal context to the objective of this assessment, which is to provide a decision support framework for the identification and subsequent management of invasive fish populations, specific to individual populations within single catchments (the most practical scale at which to manage invasive fishes).

are potential conflict species where they threaten biodiversity conservation but are also important contributors to the national economy. Unfortunately there has never been a proper peer-reviewed study undertaken at national level on these economic benefits, although available data from case-studies support international evidence that recreational fisheries based on alien fishes such as bass, carp and trout contribute significantly to the national economy. As a result, some alien fishes should be actively managed to limit further impact, as well as to maximise social and economic benefits from the resource in areas that are already invaded. This will require a national management plan that will guide eradication in areas of biodiversity concern (e.g. protected areas) and permit their use in others. It is clear that in some areas the major impact (local biodiversity loss) has already occurred and that it is not economically desirable to remove them (Van Rensburg *et al*., 2011). In these areas, populations should be managed for maximum economic benefits, e.g. through sport fishing. In other areas, however, river rehabilitation through removal of alien fish is a desirable conservation action, for example to improve local ecosystem function or to expand the range of threatened species in the catchment. The aims of this study were therefore:

- To conduct two comprehensive case studies that exemplify the South African situation regarding the spread and impacts of alien invasive fishes in aquatic ecosystems;
- Use the results of these case studies to develop a decision support framework to prioritise areas for alien fish eradication efforts within river systems and better manage the impacts of human mediated transfer of alien fish;

In the Sundays River Irrigation scheme case study:

- Determine the invasion history of 30 impoundments connected to the irrigation scheme;
- Identify key factors that dictate successful invasion of an impoundment, including stocking history, physico-chemical characteristics and community composition within the impoundment;
- Identify invasion pathways from the Sundays River irrigation scheme into the Sundays River catchment that could explain current invasions in the Addo Elephant National Park; and
- Develop a decision support model that provides situation-appropriate strategies to mitigate the spread of alien fish through utilisation of the Sundays River irrigation scheme by water resource users.

In the Groot Marico case study:

- Assess the ichthyofaunal distribution in the Groot Marico catchment with specific emphasis on *M. salmoides;*

- Assess ecological interactions between the native and alien fishes, with an emphasis on assessing the vulnerability of indigenous fishes to predation by *M. salmoides*;
- Assess the genetic diversity of selected indigenous fish species in the Groot Marico catchment to determine whether indigenous fishes comprise one population or are genetically distinct populations separated by natural barriers; and
- Develop a management strategy for eradication of invasive alien fish species in the Groot Marico catchment with specific emphasis on *M. salmoides*.

2 CASE 1: ASSESSING IMPACTS OF LARGEMOUTH BASS IN A NEAR-PRISTINE ECOSYSTEM

PK Kimberg, DJ Woodford, OLF Weyl, TP Msezane, KA Van der Walt, ER Swartz, CT Chiminba, T Zengeya

2.1 The Groot Marico River Catchment

The project area consists of the Groot Marico sub-management area upstream and including Marico Bosveld Dam and comprises quaternary catchments A31A and A31B (Figure 1).

The Groot Marico River originates on the plateau south of the town of Groot Marico. The upper reaches are dominated by a number of dolomitic eyes and tributaries that cut through the mountains south of the town. This creates deeply incised gorges that are fairly inaccessible for agricultural development and are therefore relatively un-impacted (NWDACE, 2007).

The upper catchment of the Groot Marico the landscape is generally flat to gently rolling due to the Malmani dolomites. The dolomite is intruded by numerous dolerite dykes that have effectively sub-divided the dolomite into a series of compartments, which may or may not be hydraulically linked. Groundwater is widespread, especially in chert rich horizons and karst zones (DWAF, 2009 b).

A study by the J.L.B. Smith Institute (now the South African Institute of Aquatic Biology SAIAB) during the late 1980s and early 1990s identified the dolomitic eye ecosystems and their biota of extremely significant conservation value (Skelton *et al*., 1994). Subsequently, the tributaries in the catchment of the Groot Marico River have been identified as biodiversity features of special ecological significance and have also been included in the targeted river reaches for ensuring that 20% of all types of aquatic ecosystems are conserved (NWDACE, 2007; Figure 1).

- Kaaloog se Loop originating from the Marico dolomitic eye and downstream aquatic ecosystem (Kaaloog se Loop) has been identified as a special feature due to its high level of biodiversity (Smith-Adao *et al.,* 2006).
- The Rietspruit tributary originates from a dolomitic eye with a perennial stream and wetland area in the upper reaches, and then flows through a deeply incised gorge area (NWDACE, 2007). The Rietspruit and its associated dolomitic eye have been identified as biodiversity special features due to its pristine state (Smith-Adao *et al.,* 2006).
- Ribbokfontein se Loop and its associated wetlands have been identified as biodiversity special features due to the high level of habitat diversity (Smith-Adao *et al.,* 2006).

- The Draaifontein has been described as biodiversity special feature due to the high level of habitat and invertebrate species diversity as well as the presence of geographically isolated fish species (Smith-Adao *et al.,* 2006).
- Van Straatensvlei has been identified as a biodiversity species feature due to its habitat diversity, isolated fish populations and pristine state (Smith-Adao *et al.,* 2006).

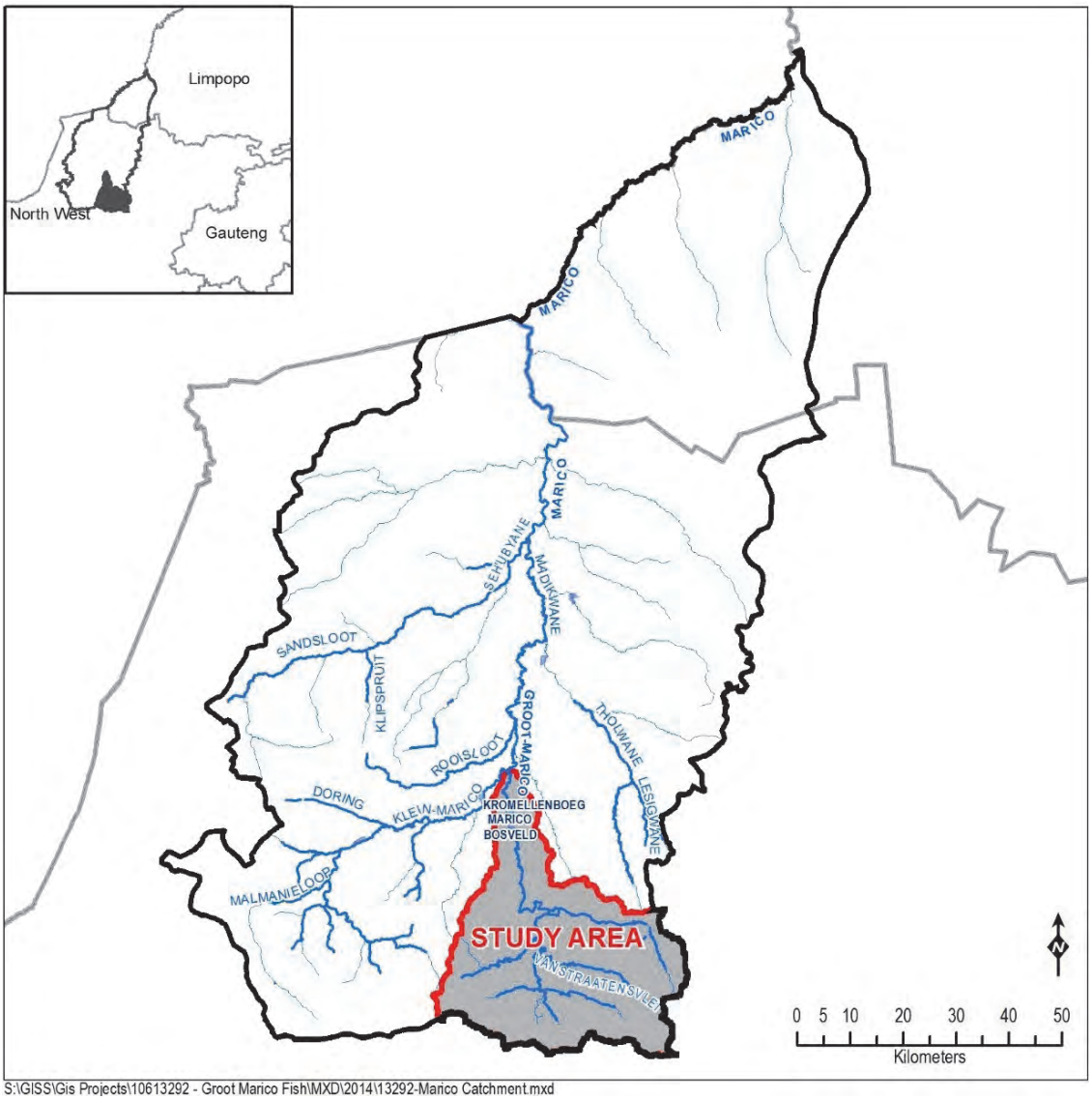

Figure 1: Map showing the location of the study area comprising the upper reaches of the Groot Marico catchment and its tributaries within the Marico River catchment.

2.1.1 Fish species diversity

Based on an assessment of historical data, 19 indigenous and 3 introduced fish species have been recorded in the upper reaches of the Groot Marico catchment (Table 3) (Kleynhans *et al*., 2007; SAIAB, 2011; DWAF, 2009, 2010). Of these, Smith-Adao *et al.,* 2006) listed four indigenous fish species as being of special conservation concern in the

Systematic Freshwater Conservation Plan for the Crocodile (West) and Marico WMA namely: *Barbus motebensis* (Marico barb); *Chetia flaviventris* (Canary kurper); *Amphilius uranoscopus* (Stargazer catfish); and *Chiloglanis pretoriae* (Shortspine suckermouth).

Barbus motebensis is endemic to the Crocodile (West) and Marico WMA and is only know from 10 locations in the upper reaches of the Marico and Crocodile rivers most of which are threatened by impacts such as water abstraction and predation from introduced species such as *M. salmoides* (Engelbrecht & Bills, 2007). *Barbus motebensis* is listed as VUL on the IUCN Red List of Threatened Species due to its restricted geographical range (Engelbrecht & Bills, 2007).

Chiloglanis pretoriae is sensitive to habitat, flow and water quality modification which has meant that is has disappeared from streams draining urban areas (Smith-Adao *et al.*, 2006). Skelton (2001) identified *C. pretoriae* as an indicator species in river conservation studies.

According to the North-West Province Freshwater Conservation Plan *A. uranoscopus* is a habitat specialist with a low fecundity and is not associated with any intensive form of development (Smith-Adao, *et al*., 2006). The Groot Marico River and its tributaries are considered to be very important for the conservation of this species (Smith-Adao, *et al*., 2006).

Chetia flaviventris is a South African endemic with a fragmented distribution in the upper reaches of the Limpopo catchment (Skelton, 2001).

Special mention must be made of the population of *T. sparrmanii* in the Marico Eye also represents a genetically unique population of conservation importance (Skelton *et al*., 1994).

Table 3: Indigenous and introduced fish species expected in the upper reaches of the Groot Marico River catchment (Kleynhans et al., 2007; SAIAB, 2011; DWAF, 2009, 2010, Skelton, 2001).

Scientific name	Common name	Distribution
Anguilla mossambica	Longfin eel	East Coast rivers from Kenya south to Cape Agulhas, also Madagascar and other western Indian Ocean islands
Amphilius uranoscopus	Stargazer catfish	Okavango and Zambezi systems, east coast rivers. Also widespread in Central and East Africa
Barbus brevipinnis	Shortfin barb	Sabie-Sand system in Mpumalanga and

Scientific name	Common name	Distribution
		tributaries of the Phongolo River in KwaZulu-Natal
Barbus motebensis	Marico barb	Headwater tributaries of the Marico, Crocodile and Steelpoort branches of Limpopo River system
Barbus paludinosus	Straightfin barb	Widespread in east coastal rivers from East Africa south to the Vungu, KwaZulu-Natal, also southern Congo tributaries, Quanza Angola and Orange River system
Barbus trimaculatus	Threespot barb	Widespread from Ruvuma Tanzania to Umvoti in KwaZulu-Natal, also Orange, Cunene and Zambian Congo systems
Barbus unitaeniatus	Longbeard barb	Widely distributed in southern Africa from the Zambian Congo system and the Cunene, Okavango and Zambezi south to the Phongolo
Chetia flaviventris	Canary kurper	Tributaries of the Limpopo in South Africa, Botswana and Zimbabwe
Chiloglanis pretoriae	Shortspine suckermouth	Incomati, Limpopo, middle and lower Zambezi, Pungwe and Buzi systems
Clarias gariepinus	Sharptooth catfish	Most widespread fish species in Africa, from West Africa to the Nile in the north and the Orange River system in the south
Labeo cylindricus	Redeye labeo	Widespread from East Coast African rivers south through the Zambezi system and coastal drainages to the Phongolo
Labeo molybdinus	Leaden labeo	Middle and lower Zambezi south to the Tugela system
Labeobarbus marequensis	Largescale Yellowfish	Widely distributed from the middle and lower Zambezi south to the Phongolo system

Scientific name	Common name	Distribution
Labeobarbus polylepis	Smallscale Yellowfish	Southern tributaries of the Limpopo, also Incomati and Phongolo systems
Mesobola brevianalis	River sardine	Cunene, Okavango, upper Zambezi and east coastal rivers from the Limpopo to the Umfolozi
Micralestes acutidens	Silver robber	Cunene, Okavango, Zambezi and east coast rivers south to the Phongolo
Oreochromis mossambicus	Mozambique tilapia	East coast rivers from the lower Zambezi system to the Bushman's system, Eastern Cape
Pseudocrenilabrus philander	Southern mouthbrooder	From the Orange River and southern KwaZulu-Natal northwards throughout Southern Africa
Tilapia sparrmanii	Banded tilapia	Orange River and KwaZulu-Natal south coast northwards to upper reaches of southern Congo tributaries, Lake Malawi and Zambezi
Alien		
Cyprinus carpio	Carp	Natural distribution includes Central Asia and Europe. Widespread throughout southern Africa
Micropterus salmoides	Largemouth bass	Natural distribution is in central and eastern North America. Widely introduced into Southern African impoundments and rivers
Oncorhynchus mykiss	Rainbow trout	Natural range is the Pacific coast of North America. Widely introduced into east coast rivers of Southern Africa

2.1.2 National Freshwater Ecosystem Priority Area (NFEPA) Status

In an attempt to better conserve aquatic ecosystems, South Africa has recently categorised its river systems according to set ecological criteria (i.e. ecosystem representation, water yield, connectivity, unique features, and threatened taxa) to identify Freshwater Ecosystem

Priority Areas (FEPAs) (Driver *et al.,* 2011) The FEPAs are intended to be conservation support tools and envisioned to guide the effective implementation of measures to achieve the National Environment Management Biodiversity Act (NEM:BA) biodiversity goals (Nel *et al.,* 2011).

The Groot Marico catchment in the North West Province has been classified as a FEPA because (Nel *et al.,* 2011):

- The upper reach of the Groot Marico is the only free-flowing river in the North West Province;
- The catchment contains unique landscape features of special ecological significance including dolomitic eyes and wetlands (Roux, 2010); and
- The catchment contains fish species of special conservation concern.

These unique landscape features include dolomitic eyes, which are groundwater fed aquifers that arise from fractures at the contact zones between the underlying dolomite intrusions and sedimentary or igneous rock (Wellington, 1995). The permanent water flow provided by these aquifers to headwater tributaries of the river is of special ecological significance in this arid region. As a result, these streams are prioritised in national-level conservation plans (e.g. Provincial River Health Programme Progress Report, 2011) and are FEPA-listed as fish sanctuary areas (Nel *et al.,* 2011).

2.1.3 *Micropterus salmoides* invasion

The introduction date of *M. salmoides* into the Marico Eye is uncertain. According to Skelton *et al.,* 1994) *M. salmoides* was 1st introduced into the Molopo Eye near the town of Ottoshoop in the late 1920s and thereafter maintained by the Transvaal Department of Nature and Environmental Conservation until 1979. It is possible that similar efforts were undertaken by the conservation authorities of the day to introduce and establish *M. salmoides* in the Marico Eye. According to Skelton *et al.,* 1994), *M. salmoides* was introduced into the Marico Eye to reduce the indigenous fish populations.

Marico-Bosveld Dam is an earth-fill type dam on the Groot Marico main stem that delineates the downstream boundary of the Groot Marico project area. The dam was constructed in 1933 to supply water for irrigation purposes (DWA, 2014). As with the Marico Eye the date of the original introduction of *M. salmoides* into Marico-Bosveld is uncertain, it may have been shortly after construction of the dam as part of the same stocking program by the Transvaal Department of Nature Conservation.

The origin of *M. salmoides* population in the Groot Marico main stem upstream of Marico-Bosveld Dam may stem from the following sources: fish that have washed down from the Marico Eye population; fish that might have migrated upstream into the Groot Marico main-stem from Marico-Bosveld Dam; and fish that have been obtained from the 2 above mentioned locations or elsewhere and introduced into the catchment by local landowners or anglers.

2.2 Objectives

Given the well-known impacts of *Micropterus* species on other ecosystems in Southern Africa (Gratwicke & Marshall, 2001; Woodford *et al.,* 2005; Ellender *et al.,* 2011), the presence of *M. salmoides* in relatively unimpacted FEPA such as the Groot Marico represents a conservation concern and an opportunity to both assess the threat and provide management recommendations within a system where confounding conservation threats to native fish such as land-use are limited. In the Groot Marico case study we assess the ichthyofaunal distribution in the Groot Marico catchment with specific emphasis on *M. salmoides*; assess the ecological interactions between native and invasive fishes, with an emphasis on the vulnerability of key species to predation by *M. salmoides*; assess the genetic diversity of selected indigenous fish species in the Groot Marico catchment to determine whether indigenous fishes comprise one population or are genetically distinct populations separated by natural barriers; and develop a management strategy for eradication of invasive alien fish species in the Groot Marico catchment with specific emphasis on *M. salmoides*.

2.3 Methodology

2.3.1 Assessing fish distributions

A total of ninety four (94) sites were sampled in the upper Groot Marico catchment during surveys conducted in March and November 2012 (details of sampling sites are provided in Appendix 1). Sites were sampled in the main stem of the Groot Marico River as well as its tributaries. A map of the project area showing location of sampling sites is provided in Figure 2.

Alien invasive fish species were recorded at 17 of the 94 sites, and 77 sites were uninvaded. Sampling focussed primarily on sites in the upper catchment, in areas where the presence of *M. salmoides* was uncertain, rather than the lower reaches of the project area where its presence was well established and confirmed.

a) Habitat sampling

Sampled habitats included riffle, run and pool habitats. At each 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 turbiditimeter (HANNA Instruments Inc.). To estimate pool surface area and volume, the length (± 0.1 m) of each pool was measured followed by between three and five (depending on habitat), equally spaced, width measurements (± 0.1 m). On each width transect, three depths (± 0.1 m) were measured, the outer two were each 0.2 m from the left- and right-hand river bank and the third measurement taken midstream.

b) Fish sampling

Six (6) sampling methods were utilised during the surveys, these included: snorkel transects, backpack electrofishing, seine netting, angling, fyke netting and bank based visual observation. These techniques were selected to adequately represent different habitats and species preferences, allowing an assessment of the distribution, species composition, population structure and relative abundance in the fish community.

Habitat type and site characteristics determined the sampling method employed at each site. While electrofishing was limited to shallower sites (< 1 m deep), snorkelling was applied in a wide range of habitats. Other methods were used incidentally in areas too deep or too turbid for electrofishing or snorkel surveys.

Snorkel transects were conducted following the method described by Ellender *et al.,* 2011) whereby the number of fish are enumerated during two consecutive snorkel passes and averaged to give an estimate for the number of fish present in the pool. During these fish counts the length of the fish encountered was also recorded into categories (e.g. >15, 15-30 and >30 cm for *L. marequensis;* >2, 2-5 and > 5 cm for Barbus sp.). Length was only estimated on the first pass to avoid measuring the same fish twice. Electrofishing was conducted both upstream and downstream into a fine meshed block net.

Two portable electrofishers were used during the survey. A Samus© 725G backpack electrofisher was used and the settings for the electrofisher were standardised at 200 V, duration 0.3 ms, and a frequency of 90 Hz. A Smith-Root LR24 electrofisher was also used with the following settings: 200 V, duration 0.1 ms and a frequency of 120 Hz.

Due to the large size and diversity of habitats three pass electrofishing was not feasible. Rather each site was fished methodically until no further fish were detected. All fish captured were placed in separate buckets where after they were identified to species level, counted,

measured to the nearest mm fork length (FL) and released. The total number of fish sampled was taken as the representative of the number of fish in the sampling site.

Due to the inherent species and size selectivity of the various sampling methods and the variability of the sampling sites, catch rates and species composition data could not be compared directly between sites. As a result, all catches from all sampling gears were grouped and only the presence/absence data were used to assess fish species distributions.

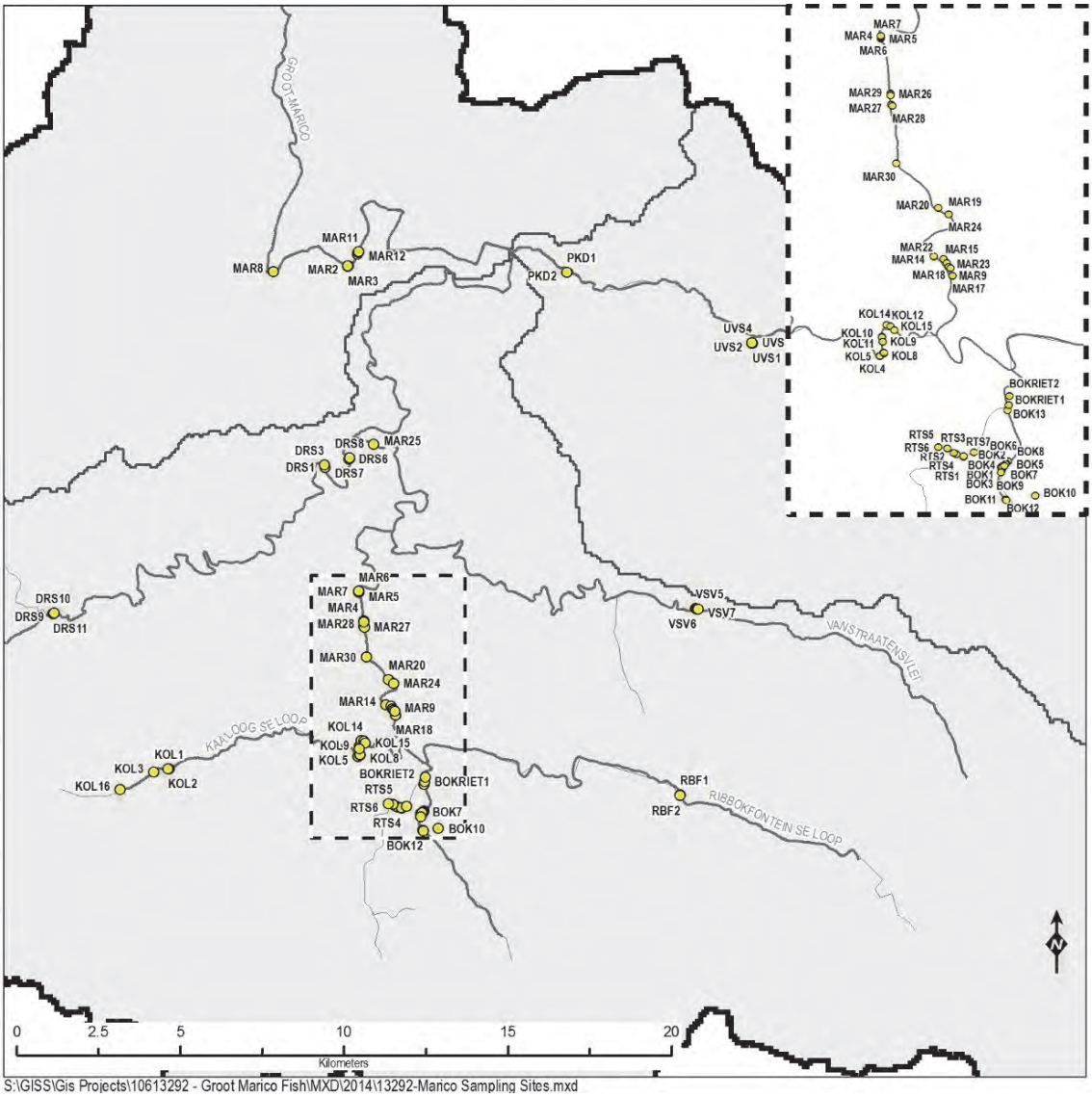

Figure 2: Map of the Groot Marico catchment showing sites sampled during the March and November 2012 surveys.

c) Data analysis

Survey data was analysed by means of multivariate procedures using Canoco version 4.5. This is due to the community-based nature of the data which makes classical univariate assumptions invalid. Non-parametric multivariate analysis of community data, based on among sample similarity matrices, draws inferences only from its ranks. These methods lack

model assumptions and have a general validity of application. In contrast to univariate analyses (ANOVA, regression) multivariate procedures consider each taxon to be a variable and the presence/ absence of each taxon to be an attribute of a site or time. Subtle changes in community composition across sites, which are generally masked when the characteristics of a site are combined into a single index value, are more likely to be detected by multivariate procedures. Spatial trends in community composition can therefore be displayed by means of multivariate methods of data analysis.

Due to the inherent species and size selectivity of the various sampling methods and the variability of the sampling sites, catch rates and species composition data could not be compared directly between sites. As a result, only the presence/absence data was used in the Canoco assessment.

Canoco allows for the selection of the driving variables or environmental data that is intended to be overlaid onto the CCA (Ter Braak & Smilauer, 2002). In this case the environmental data that was included was:

- Elevation (masl);
- Site length (m);
- Average width (m);
- Average depth (cm);
- Surface area of site (m^2);
- Invasion status (*M. salmoides* present/ absent); and
- Habitat type (pool/ riffle/ run, chute).

Micropterus salmoides was removed from the fish data in order to avoid autocorrelation with the invasion status parameter. Fish species with low occurrences (< 5) were also removed from the fish assemblage as these incidental records can skew the community responses to environmental variables (Ter Braak & Smilauer, 2002). Species that were removed from the observed fish assemblage due to low abundances and occurrences were: *O. mossambicus; B brevipinnis; C. flaviventris;* and *C. carpio.*

2.3.2 Trophic interactions

The comparison of foodweb interactions among fish populations in invaded and non-invaded river sections in the Groot Marico River system attempted to whether there were differences in the trophic structure of food webs between invaded and non-invaded sections of the Groot Marico, and if there was any evidence of niche shift by indigenous species as the results of the presence of invaders. To do this, two complimentary methods (gut content and stable isotope analyses) were used in the present study to determine diet and trophic interactions

between invasive and indigenous fish species of the Groot Marico River. Gut content analysis was used to identify possible food sources while stable isotopes were used to assess food assimilated over long periods of time (Katzenberg & Saunders, 2007).

Fishes, sampled between March and November 2012 from the invaded main stem of the Groot Marico River and the non-invaded Kaaloog se Loop tributary were used for the study. Fish were euthanized with an overdose of clove oil and then fixed in 5% formalin before storage in 70 % ethanol. To obtain an estimate of the overall food spectrum available to individual fish species, specimens from both invaded and non-invaded sampling sites were combined for gut content analysis, but to differentiate food niches between the two sampling sites (invaded and non-invaded sites) stable isotope samples were collected separately for fish specimens from the two respective sampling sites.

a) Stomach content analysis

Stomach contents were analysed using the methods of Zengeya & Marshall (2007); the contents of each stomach were suspended in 100 ml of water per gram of stomach contents and examined under an inverted microscope. Each item in the diet was then identified to the lowest possible taxonomic level after which the food items were then combined into broader taxonomic categories for quantitative comparisons (e.g. cyanophyta, green algae, diatoms, periphyton, fine detritus, plant detritus, macrophytes, zooplankton and macrofauna). The contribution of each food category in each gut was estimated using a modification of the methods used in Platell & Potter (2001) by evenly spreading all contents from each gut in the counting cell chamber and examining under a microscope. To determine the importance of each prey group, an index of relative importance (IRI) was calculated for each prey item (Hyslop, 1980) as follows:

IRI = (%N = %V) X (%F)

Where prey abundance (%N) = the number of individuals as the proportion of all prey items, (%F) = the number of stomachs containing a specific prey item as a percentage of all sampled stomachs, and the volume of each prey item (%V) = percentage of the total volume of all stomach contents). The IRI values for each prey category were then standardized by expressing each value as a proportion (percentage) of the sum of all IRI values for all prey items (%IRI). Diet diversity estimated by index of niche breadth (Levin, 1968), dietary overlap between size classes, among and between species was calculated from a niche overlap co efficient (Pianka, 1974). Indices may range from zero (indicate no dietary overlap or specialized feeding behaviour) to 1.0 (indicating an even use of resources or complete

overlap) with values > 0.6 being considered to represent a biologically significant overlap (Langton, 1982).

b) Stable isotope analysis

Stable isotopes of nitrogen and carbon are increasingly being used to characterize changes in energy flow and structure of aquatic food webs related to non-native fish introductions (Zambrano *et al*., 2010; Zengeya *et al*., 2011; Kadye & Booth, 2011). Transfer of ^{13}C between trophic levels remains relatively the same (≤ 1‰), therefore large differences of ^{13}C between consumers can indicate different food sources or that their food webs are based on different primary producers (De Niro & Epstein, 2001). In contrast, δ¹%N is enriched (≥3.4‰) from each trophic level and differences in δ¹%N ratios among organisms can therefore indicate trophic position within a given food web (Pasquad *et al*., 2011).

To estimate the isotopic baseline of the food web in the river system, various samples were collected from potential fish food sources that included phytoplankton, microbes (biofilm), particulate organic matter (POM), detritus insects and from small aquatic fauna such crabs. Samples were then over-dried at 60°C and analysed at Rhodes University, Department of Botany (Iso Environmental cc), Grahamstown, South Africa. Isotope ratios for carbon and nitrogen were quantified as deviations relative to isotopic standards as follows:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample}/ R_{standard}) - 1] \times 1000$$

Where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$. The standards were Vienna Pee Dee Belemnite limestone for $\delta^{13}C$ (Craig, 1957) and atmospheric nitrogen for $\delta^{15}N$ (Ehleringer & Rundel, 1989).

Stable isotope data was checked for normality and homogeneity of variances and transformed using the box-cox transformation. To test for differences in stable isotope ratios among fish species in each respective river section, a one factor analysis of variance (ANOVA) was used with species as a fixed factor. The Bayesian mixing model (Jackson *et al.*, 2011) was used to estimate the relative contribution of various food sources that are likely to be utilised by fish in the two river systems. Fractionation between the resources and consumers was set at 2.3 ± 0.28‰ for $\delta^{15}N$ and 0.4 ± 0.17‰ for $\delta^{13}C$ after the meta-analysis of McCutchan *et al.,* 2003). Six quantitative population matrixes of isotope data available from the package *Stable Isotope Ellipses in R* (SIBER) were used describe the trophic structure and niche overlap among fish species in Kaaloog se Loop and Groot Marico rivers (Figure 2). The stable isotope values were bootstrapped to 10 000 iterations.

The matrixes were first proposed by Layman *et al.,* 2007) and further developed by Jackson *et al.,* 2011) to correct for small sample sizes. In brief the modifications proposed by Jackson

et al., 2011) are: the total area (TA) as proposed by Layman *et al.,* 2007) encompasses a convex hull drawn around the most extreme outliers in a given isotope bi-plot. This inherently makes it susceptible to changes in sample size, as convex hull area is likely to increase with increasing sample size. To counteract this sample size effect, Jackson *et al.,* 2011*)* proposed the use of standard ellipse areas (SEA) which are constructed using variance and covariance of the bivariate isotope to contain 40% of the data, which represents the core isotopic niche of each fish population that is not affected by sample size. The resultant SEA is then corrected (SEA_C) to minimize bias caused by small sample sizes using the following correction factor:

1. $(SEA_C) = SEA \times [(n-1)/ (n-2)]$

Furthermore, SEA_C was used to estimate the degree of isotopic overlap among fish species and hence give a quantitative measure of the dietary similarity among fish populations.

2.3.3 Genetic assessments

Three native freshwater species *A. uranoscopus*, *C. pretoriae* and *B. motebensis*, have high local conservation importance and *B. motebensis* is endemic to the catchment and is IUCN-listed as VUL. The main objective of this study is to contribute towards the effective conservation of these three species in the Groot Marico River system by assessing their genetic structure to determine whether tributary populations of the three species comprise of one genetic population or whether they are divided into genetically distinct subpopulations, in order to prioritise areas for conservation. The central null hypothesis was that there is no genetic differentiation between tributary populations (i.e. panmixia) of *B. motebensis*, *A. uranoscopus* and *C. pretoriae* in the Groot Marico catchment, North West Province.

Genetic samples were collected during the March and November 2012 surveys. Samples were grouped to represent a total of 15 sampling reaches representative of the catchment (Figure 3). In total, 80 individuals per species were collected, targeting at least 10 individuals per population from a total of eight populations (seven tributaries and the Groot Marico main stem) and across the study area. Samples were collected by electrofishing and specimens were euthanized using an overdose of clove oil. A sample of muscle tissue was removed for genetic evaluation and the remainder of the specimens served as voucher specimens. For the genetic evaluation, mitochondrial (ND2, cyt *b*) and nuclear (S7) genes were used. Genetic techniques used were DNA extraction, polymerase chain reaction (PCR), purification and sequencing. From the 240 individuals collected, 123 sequences for *B. motebensis,* 111 sequences for *A. uranoscopus* and 103 sequences for *C. pretoriae* were analysed across all three genes. Statistical analysis included looking at cleaned sequences

in order to obtain models using MODELTEST (version 3.06). Population structuring and phylogeographic analysis was performed in Arlequin (version 2000), TCS (version 1.2.1) and PAUP*. A detailed description of the methods is available in Van der Walt (2014) the abstract of which is provided in Appendix 2.

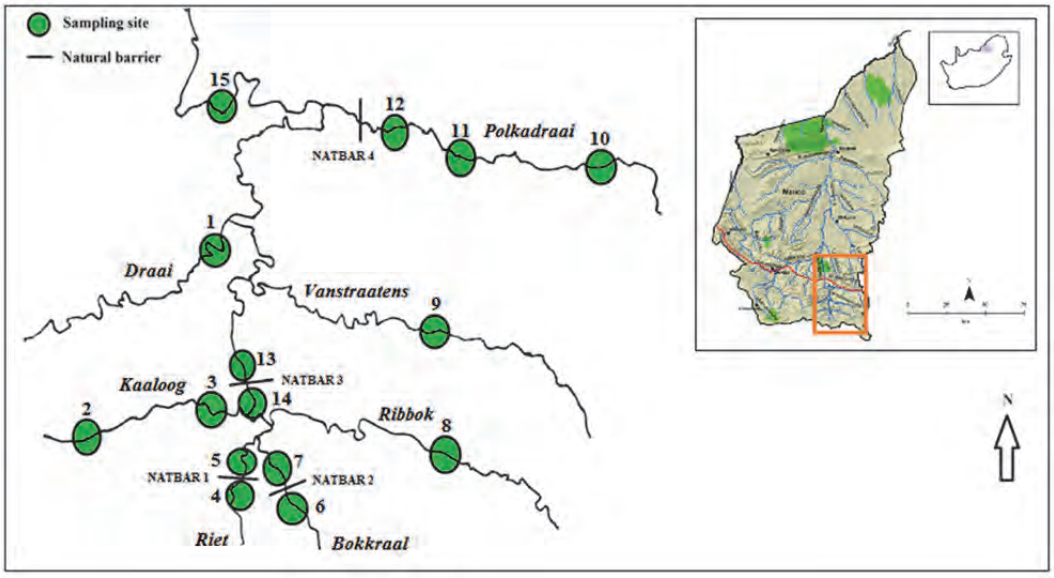

Figure 3: Map illustrating the tributaries and 15 sampling sites (green dots) in which all three species were collected and analysed across the upper Groot Marico catchment.

Figure Notes: (1 = Lower Draaifonteinspruit; 2 = Upper Kaaloog se Loop; 3 = Lower Kaaloog se Loop; 4 = Above Rietspruit waterfall; 5 = Below Rietspruit waterfall; 6 = Above Bokkraal waterfall; 7 = Below Bokkraal waterfall; 8 = Upper Ribbokfontein; 9 = Middle Van Straatens; 10 = Upper Polkadraai; 11 = Middle Polkadraai; 12 = Lower Polkadraai; 13 = Groot Marico at Sonop; 14 = Below Groot Marico waterfall; 15 = Groot Marico at Riverstill).

2.4 Results and Discussion

2.4.1 Distributions and relative abundances of fish species

A total of 14 indigenous and 2 alien fish species were recorded in the Groot Marico catchment during the March and November 2012 surveys. A list of the fish species recorded is provided in Table 4. Fish species that have previously been recorded in the Groot Marico catchment that were not sampled during the March and November 2012 surveys included: *A. mossambica*; *B. trimaculatus*; *B. unitaeniatus*; *L. cylindricus*; and *M. acutidens.*

Table 4: List of indigenous and introduced fish species recorded in the upper reaches of the Groot Marico catchment during the March and November 2012 surveys as well as the number of individuals and the number of sites each species was recorded at during the surveys.

Species	Common name	No of sites	No of individuals
Amphilius uranoscopus	Stargazer catfish	35	311
Barbus motebensis	Marico barb	54	2329
Barbus paludinosus	Straightfin barb	9	281
Clarias gariepinus	Sharptooth catfish	7	12
Chiloglanis pretoriae	Shortspine suckermouth	23	604
Labeobarbus marequensis	Largescale yellowfish	60	1881
Labeobarbus polylepis	Smallscale yellowfish	9	123
Pseudocrenilabrus philander	Southern mouthbrooder	13	162
Tilapia sparrmanii	Banded tilapia	22	298
Barbus brevipinnis	Shortfin barb	1	2
Chetia flaviventris	Canary kurper	2	4
Labeo molybdinus	Leaden labeo	4	10
Mesobola brevianalis	River sardine	1	17
Oreochromis mossambicus	Mozambique tilapia	3	4
INTRODUCED ALIENS			
Micropterus salmoides	Largemouth bass	16	32
Cyprinus carpio	Carp	3	6

Labeobarbus marequensis was the most widespread fish species in the project area and was recorded at 60 of the sampling sites (Table 4). *Barbus motebensis* was the most abundant fish species in the project area (n = 2329) and was recorded at 54 sites (Table 4).

The least abundant fish species during the March 2012 survey was *B. brevipinnis* (n = 2) which was only collected at a single site in the upper reaches of Kaaloog se Loop (Table 4). Other species that were collected in low abundances included *C. flaviventris* (n = 4) which was only recorded at 2 sites in the main stem of the Groot Marico River and *O. mossambicus* (n = 4) which was only recorded at 3 sites (Table 4).

Micropterus salmoides (Largemouth bass) were collected at 16 of the 94 sampling sites including sites in the main stem of the Groot Marico River, Sterkstroom/ Polkadraaispruit and a seemingly isolated population in the Marico eye at the top of Kaaloog se Loop. *Cyprinus carpio* was only recorded at 3 sites in the catchment and appears to be present in low abundances (Table 4).

The distribution of some species provides opportunities for further assessments.

a) *Barbus motebensis*

Barbus motebensis was present in all of the tributaries except the Sterkstroom/ Polkadraaispruit (Figure 4). It was also recorded in the main stem of the Groot Marico River upstream of the invaded zone (Figure 4). *Barbus motebensis* inhabits slow flowing sections and shallow pools of small streams (Engelbrecht & Bills, 2007). It is usually found in association with banks and marginal vegetation which makes it sensitive to predation from *M. salmoides* due to the overlap in preferred habitat (Engelbrecht & Bills, 2007). The density of *B. motebensis* showed a significant difference between invaded and un-invaded pools. The absence of *B. motebensis* from pools in the invaded zone confirms the sensitivity of this species to predation from *M. salmoides*. The fork lengths of *B. motebensis* ranged between 15 mm and 85 mm with most individuals measured at 30 to 40 mm fork lengths.

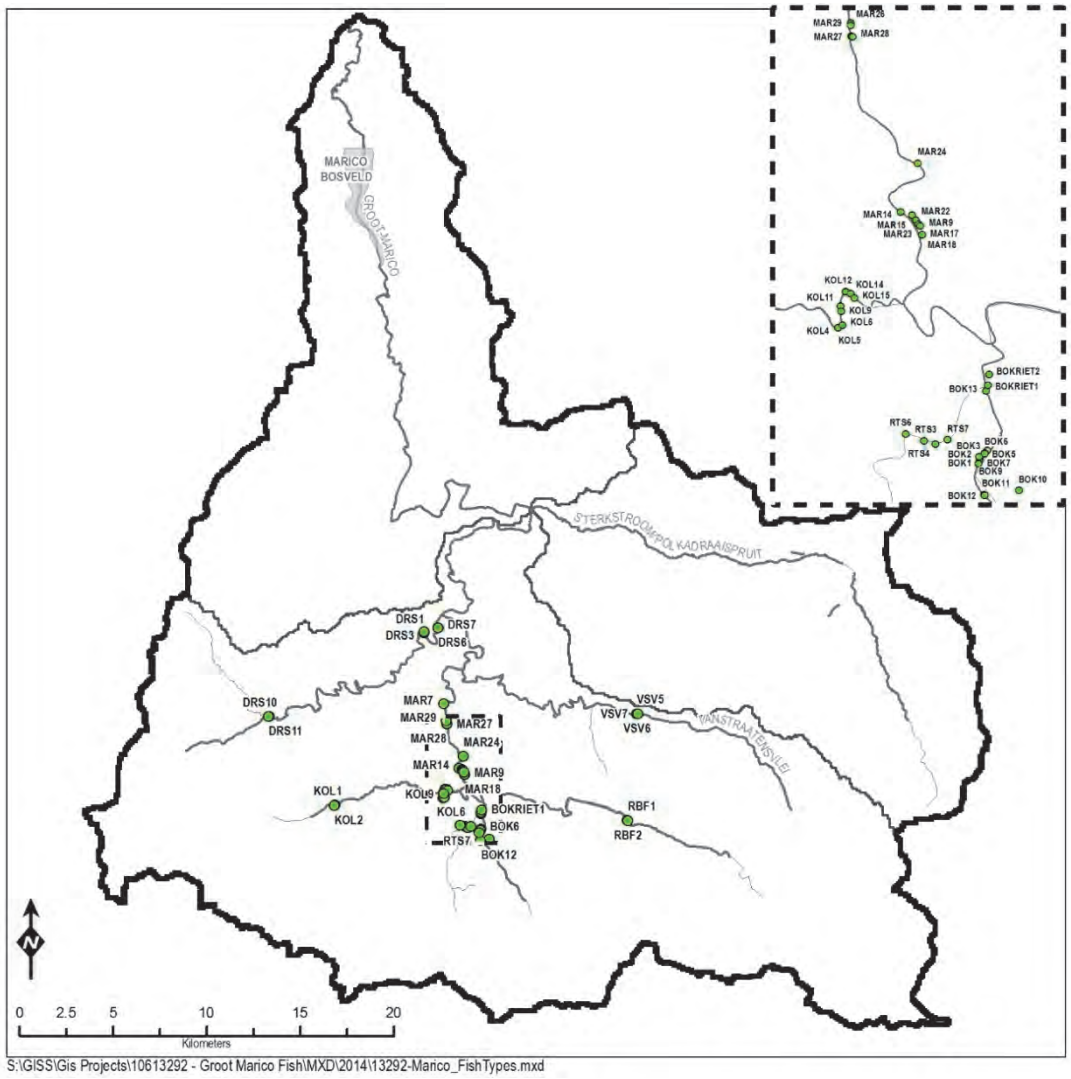

Figure 4: Distribution of *B. motebensis* in the Groot Marico catchment based on the results of the March and November 2012 surveys.

b) Amphilius uranoscopus

Amphilius uranoscopus was recorded at 35 of the 94 sampling sites including sites in the invaded zone (Figure 5). It was recorded in the main stem of the Groot Marico River as well as all the tributaries with the exception of Ribbokfontein se Loop (Figure 5). This species inhabits clear, fast flowing rocky reaches where it feeds on benthic macroinvertebrates that are grazed off rock surfaces (Skelton, 2001).

The highest densities of *A. uranoscopus* were recorded in cascades and un-invaded riffle habitats (Figure 5). A small decrease in density was noted in invaded riffle habitats. No difference was noted between invaded and un-invaded run habitats. This may be because run habitats are not ideal habitats for *M. salmoides*.

Fork lengths ranged from 40 mm to 185 mm with the majority of the individuals measured at 65 mm.

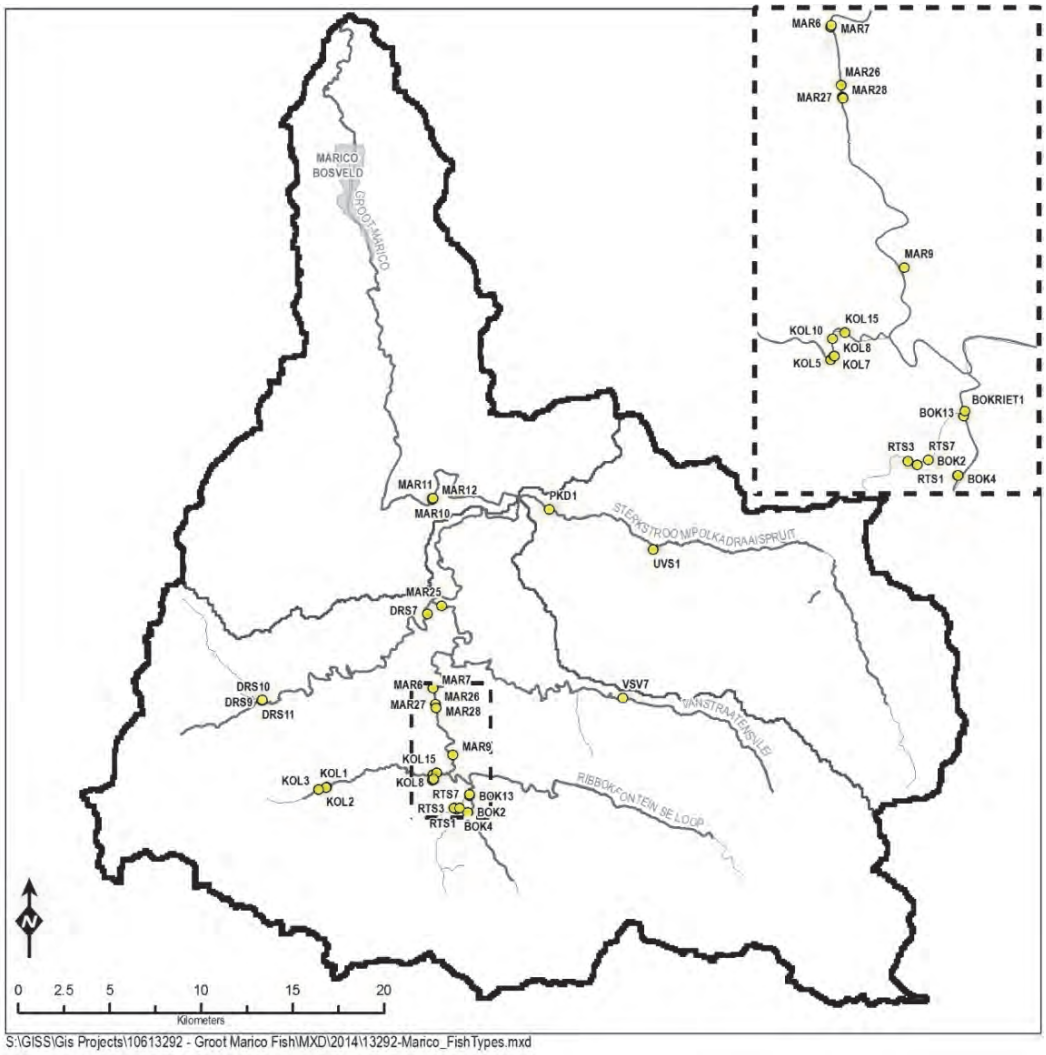

Figure 5: Distribution of *A. uranoscopus* in the Groot Marico catchment based on the results of the March and November 2012 surveys.

c) Chiloglanis pretoriae

Chiloglanis pretoriae was recorded at 23 sites in the Groot Marico and its tributaries (Figure 6). *Chiloglanis pretoriae* was mostly recorded in the lower reaches of the tributaries and did not extend as far upstream into the tributaries as *A. uranoscopus.* The highest densities of *C. pretoriae* were recorded in riffle habitats in the invaded zone (Figure 6). It is likely that the density of *C. pretoriae* is unrelated to the presence or absence of *M. salmoides* but rather indicative of ideal habitats for this species within the invaded zone. *C. pretoriae* inhabits shallow, fast flowing rocky habitats such as riffles and runs (Skelton, 2001). Its habitat preference therefore largely shields it from predation by *M. salmoides.* In the longer term however the presence of *M. salmoides* may begin to have an increased impact as individuals that migrate through pools between riffles are exposed to predation. Fork lengths ranged from 30 mm to 80 mm with the majority of the individuals having fork lengths of 55 mm (Figure 6).

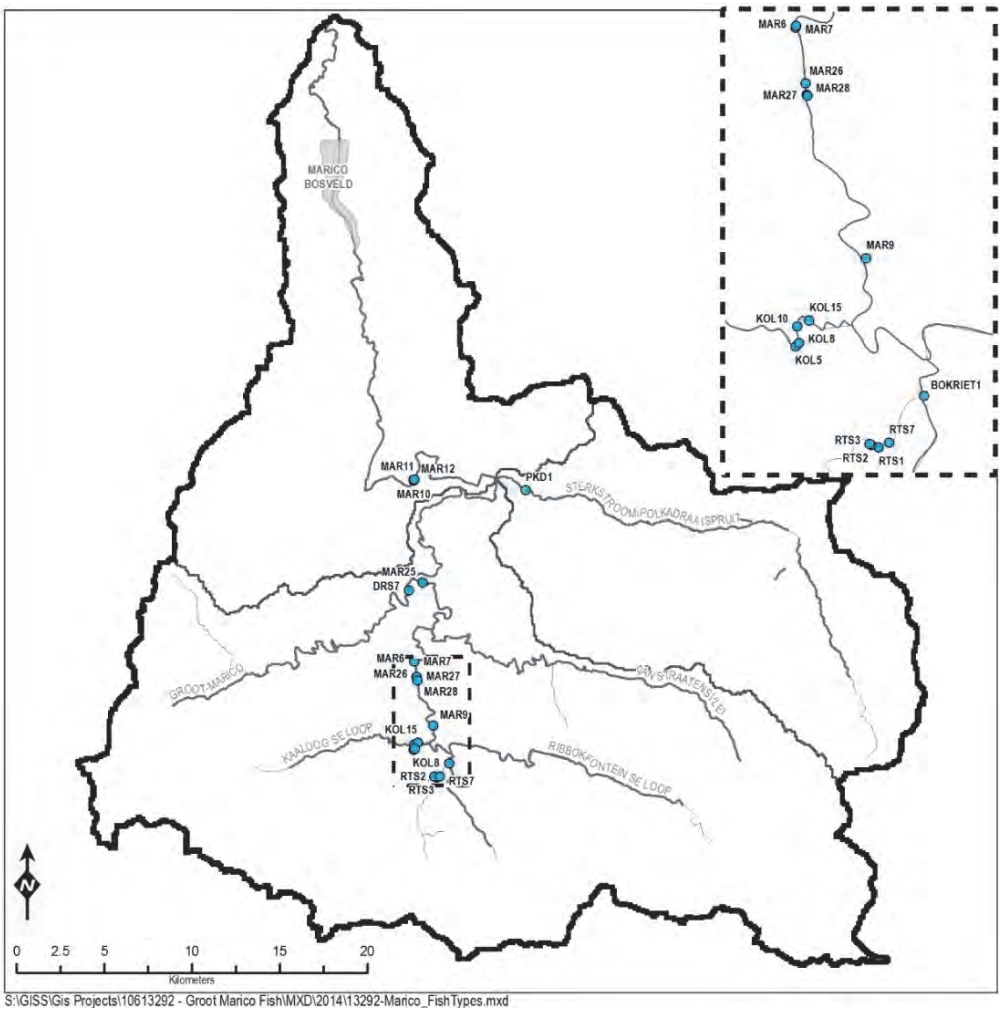

Figure 6: Distribution of *C. pretoriae* in the Groot Marico catchment based on the results of the March and November 2012 surveys.

d) *Labeobarbus marequensis*

Labeobarbus marequensis was the most widespread fish species in the Groot Marico catchment and was recorded at 60 sampling sites (Figure 7). It was present in the main stem of the Groot Marico River as well as in the tributaries, both in and out of the invaded zone (Figure 7).

Densities of *L. marequensis* showed little variation between invaded and un-invaded habitats. The highest *L. marequensis* densities were recorded in cascades and riffles. As was the case with *A. uranoscopus* and *C. pretoriae*, this species' preference for fast flowing habitats and larger adult size partially shields it from predation by *M. salmoides*. The *L. marequensis* forks lengths ranged from 15 mm to 265 mm. The majority of the individuals had fork lengths of 70 to 75 mm.

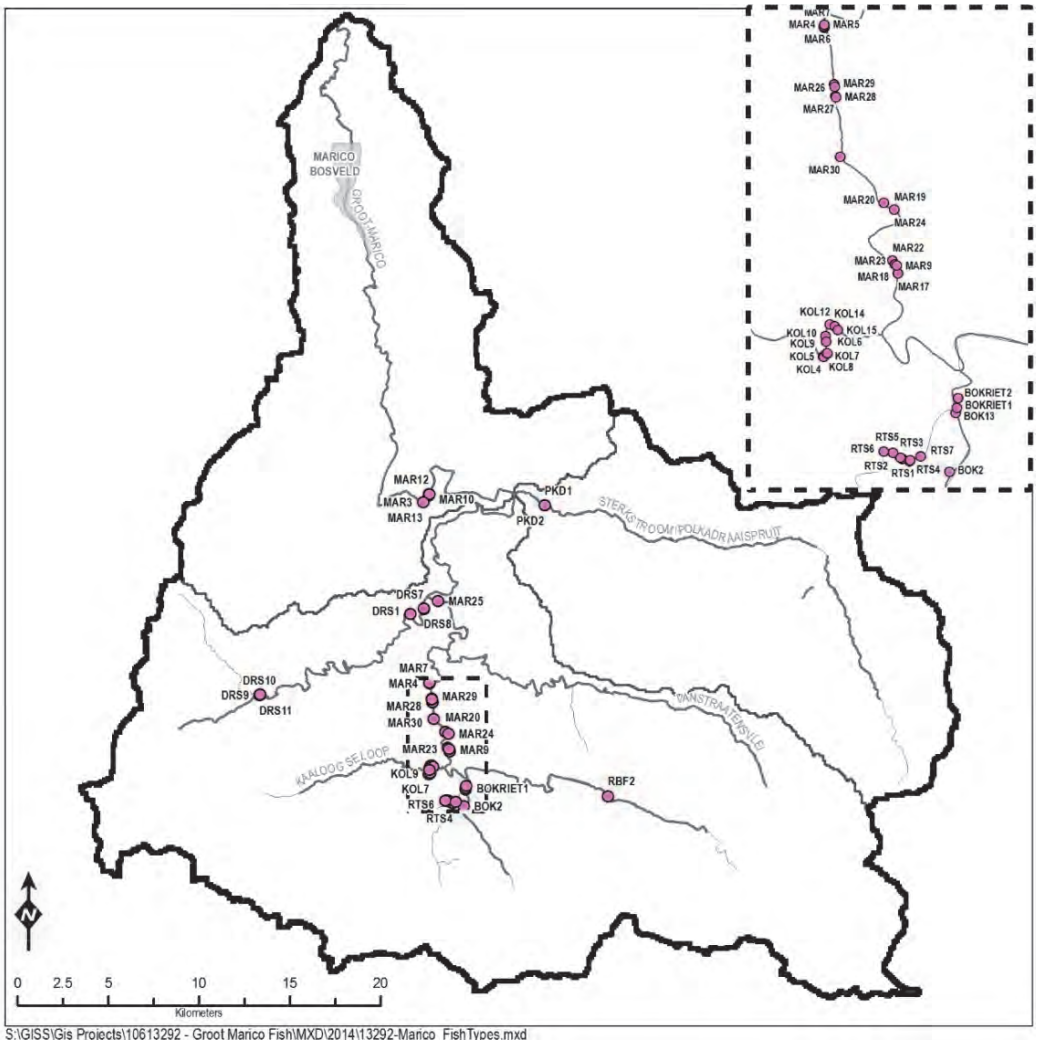

Figure 7: Distribution of *L. marequensis* in the Groot Marico catchment during the March and November 2012 surveys.

e) Extent of *M. salmoides* penetration into the catchment

Micropterus salmoides was recorded at 16 sites in the Groot Marico catchment, primarily in the main stem of the Groot Marico River and in the Sterkstroom/ Polkadraaispruit (Figure 8). With the exception of the Sterkstroom/ Polkadraaispruit and a seemingly isolated population in the Marico eye (Kaaloog se Loop) *M. salmoides* was absent from the tributaries. This species has moved approximately 1 km upstream into the Draaifonteinspruit but a migration barrier in the form of a road bridge has prevented it from moving any further upstream. *Micropterus salmoides* was absent from the uppermost reaches of the Groot Marico River main stem (Figure 8). The highest *M. salmoides* densities were recorded in pool habitats with some individuals recorded in riffle and run habitats. Fork lengths ranged from 50 to 275 mm with the majority of the individuals measured at 135 mm.

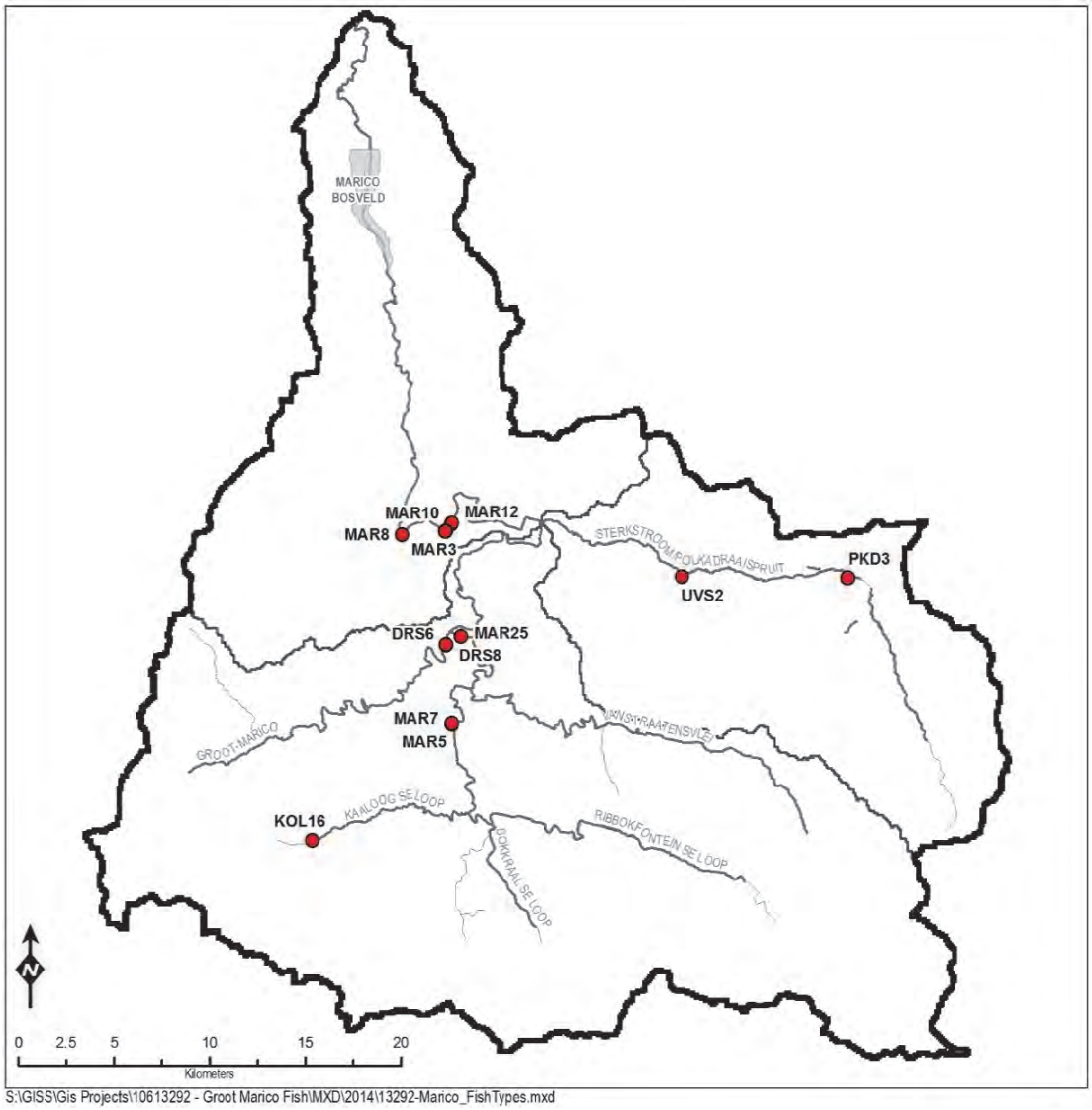

Figure 8: Distribution of *M. salmoides* in the upper reaches of the Groot Marico catchment based on results of the March and November 2012 surveys.

f) Community structure and habitat associations

Table 5 presents the statistical significance of the various habitat parameters driving the presence/ absence of indigenous fish species. Based on this assessment the average width, invasion status (*M. salmoides* present/ absent) and habitat type (pool/ riffle/ run) parameters were found to be statistically significant ($p < 0.05$) in terms of the presence/ absence of indigenous fish species whereas the site length and average depth were not significant ($p > 0.05$) and were excluded from the Canoco ordination. Although the p-value of the elevation (masl) parameter exceeded 0.05 it was included in the assessment for the insight it provided into the distribution of *B. motebensis* in particular (Table 5).

Table 5: Statistical significance of habitat parameters driving the presence/ absence of indigenous fish species for use in Canoco ordination.

Habitat parameter	p-value
Width	0.002
Invaded	0.002
Habitat	0.002
Elevation	0.098
Site length	0.218
Depth	0.516

Figure 9 presents the results of the CCA ordination. Based on the ordination Leaden labeo (*L. molybdinus)* was positively correlated with the presence of *M. salmoides* (Figure 9). This species was only recorded in the lower reaches of the Groot Marico main stem at sites where *M. salmoides* was also present.

Three species namely *M. brevianalis*, *C. gariepinus,* and *C. pretoriae* were positively correlated with invaded reaches of the catchment and showed positive correlation with specific habitat types (Figure 9). *C. pretoriae* was primarily associated with riffle habitats (Figure 10).

Mesobola brevianalis was only recorded at a single riffle habitat in the Groot Marico main stem. *Clarias gariepinus* was primarily recorded in pool habitats in the invaded section of the Groot Marico main stem, although it was also recorded with *B. motebensis* in the upper reaches of Van Straatensvlei. *Amphilius uranoscopus* also showed positive correlation to specific habitat types with increased densities recorded in riffle habitats (Figure 10). This species prefers rocky habitats with flowing water where it grazes invertebrates from the rock surfaces (Skelton, 2001). It showed no positive or negative correlation with the presence of *M. salmoides* suggesting that it is largely unaffected by the presence of this species

(Figure 9). This may be attributed to the different habitat preferences of the 2 species. Although *M. salmoides* is typically associated with pool habitats some individuals were recorded in riffle habitats as well (Figure 10).

Tilapia sparrmanii and *B. paludinosus* showed a positive correlation with increasing average habitat width indicating that these species typify the fish communities in the larger, lower reaches of the project area (Figure 9). These species didn't show any correlation with the presence of *M. salmoides* (Figure 9).

The presence of *L. polylepis* was positively correlated with the presence of *M. salmoides* as well as the increasing average width of the sampling sites indicating that this species characterises the fish communities in the larger sites on the Groot Marico main stem (Figure 9). All the above mentioned species were negatively correlated with the elevation habitat parameter confirming that these species characterise the fish communities in the lower reaches of the catchment which coincides with the *M. salmoides* invasion. *Labeobarbus marequensis* did not show strong positive or negative correlation with any of the habitat parameters (Figure 9). The small cichlid species Southern mouthbrooder (*Pseudocrenilabrus philander*) similarly showed very little correlation with the recorded habitat parameters including the presence of *M. salmoides* (Figure 9).

Barbus motebensis was the only species that was positively correlated with elevation and strongly negatively correlated with the presence of *M. salmoides* (Figure 9). *Barbus motebensis* was recorded in all of the tributaries except the Sterkstroom/ Polkadraaispruit which is invaded by *M. salmoides*. The Sterkstroom/ Polkadraaispruit differs from the other tributaries in that is has a relative low gradient across its length. *B. motebensis* was also recorded in the main stem of the Groot Marico River upstream of the invaded zone (Figure 4). *Barbus motebensis* inhabits slow flowing sections and shallow pools of small streams (Engelbrecht & Bills, 2007). Figure 10 shows the densities of this species in pool habitats in the uninvaded reaches of the Groot Marico catchment. It is usually found in association with banks and marginal vegetation which makes it very sensitive to predation from *M. salmoides* (Engelbrecht & Bills, 2007). The density of *B. motebensis* showed a significant difference between invaded and un-invaded pools within the elevations at which *M. salmoides* occurred (Figure 11). Figure 9 and Figure 11 suggest that the absence of *M. salmoides* from the upper reaches of the Groot Marico catchment can be attributed to the increased elevation and associated barriers to upstream movement in the upper reaches of the Groot Marico catchment.

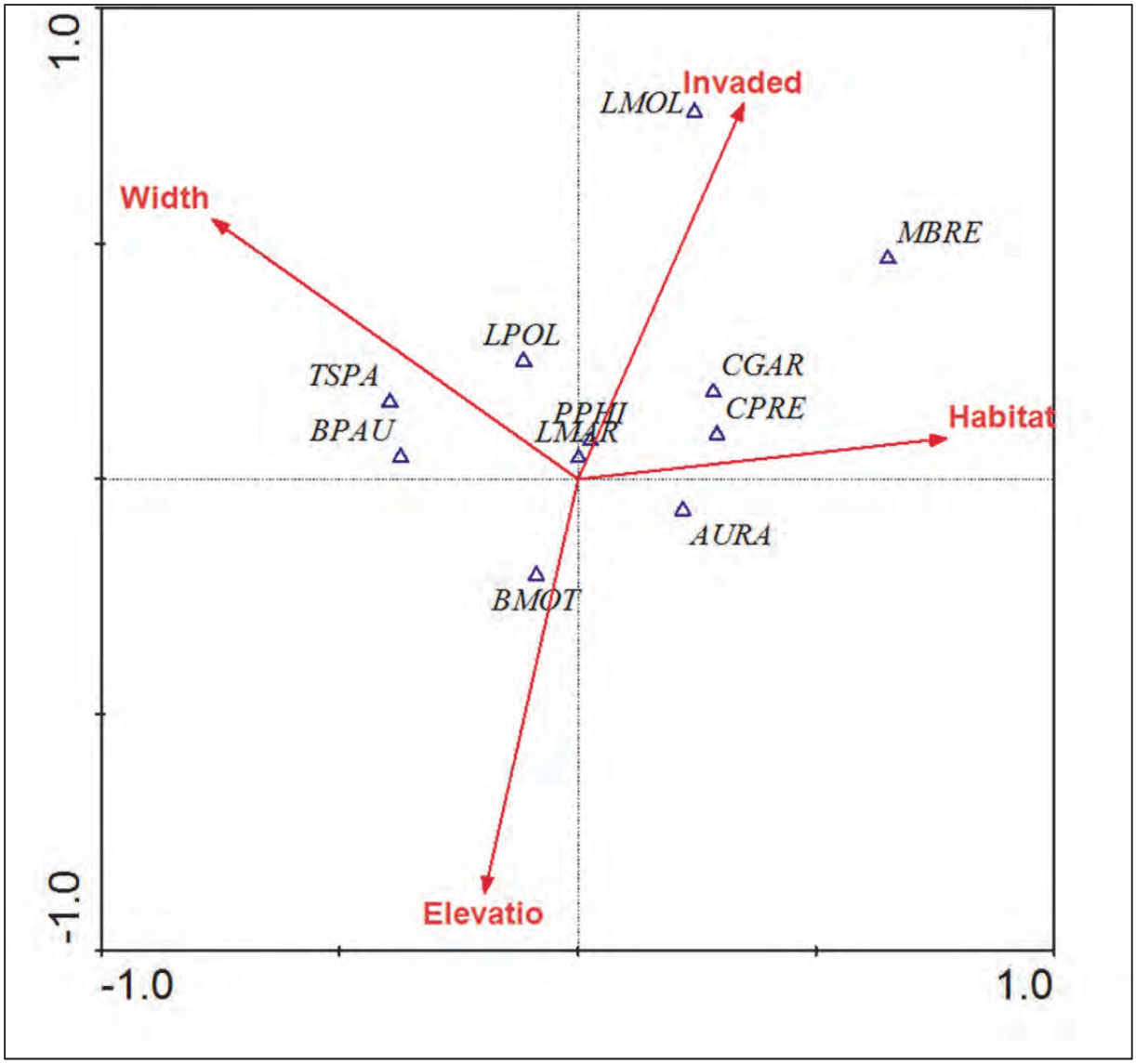

Figure 9: Canonical Correspondence Analysis (CCA) ordination showing the correlation between fish species and habitat variables in the upper reaches of the Groot Marico catchment.

Figure Notes: The approximated correlation is positive when the angle is acute and negative when the angle is larger than 90°. The distance between the sampling sites in the diagram approximates the dissimilarity of the variables as measured by their Euclidean distance.

Figure 11 shows the differences in densities of the small cyprinid species *B. motebensis* in the invaded and uninvaded reaches of the catchment. Although the *B. motebensis* densities continued to increase with increasing elevation beyond the invaded zone, the species was not abundant in the highest elevations. This suggests that *B. motebensis* is an upper-foothill specialist rather than a headwater specialist, and that the positive association with gradient in the CCA is more attributable to the impact of *M. salmoides* on this species than its affinity for higher elevation reaches. Based on this assessment it can be concluded that

B. motebensis may never have occurred in large abundances in the lower reaches of the Groot Marico main stream, but it is nonetheless likely that these low-elevation populations have been extirpated by *M. salmoides*.

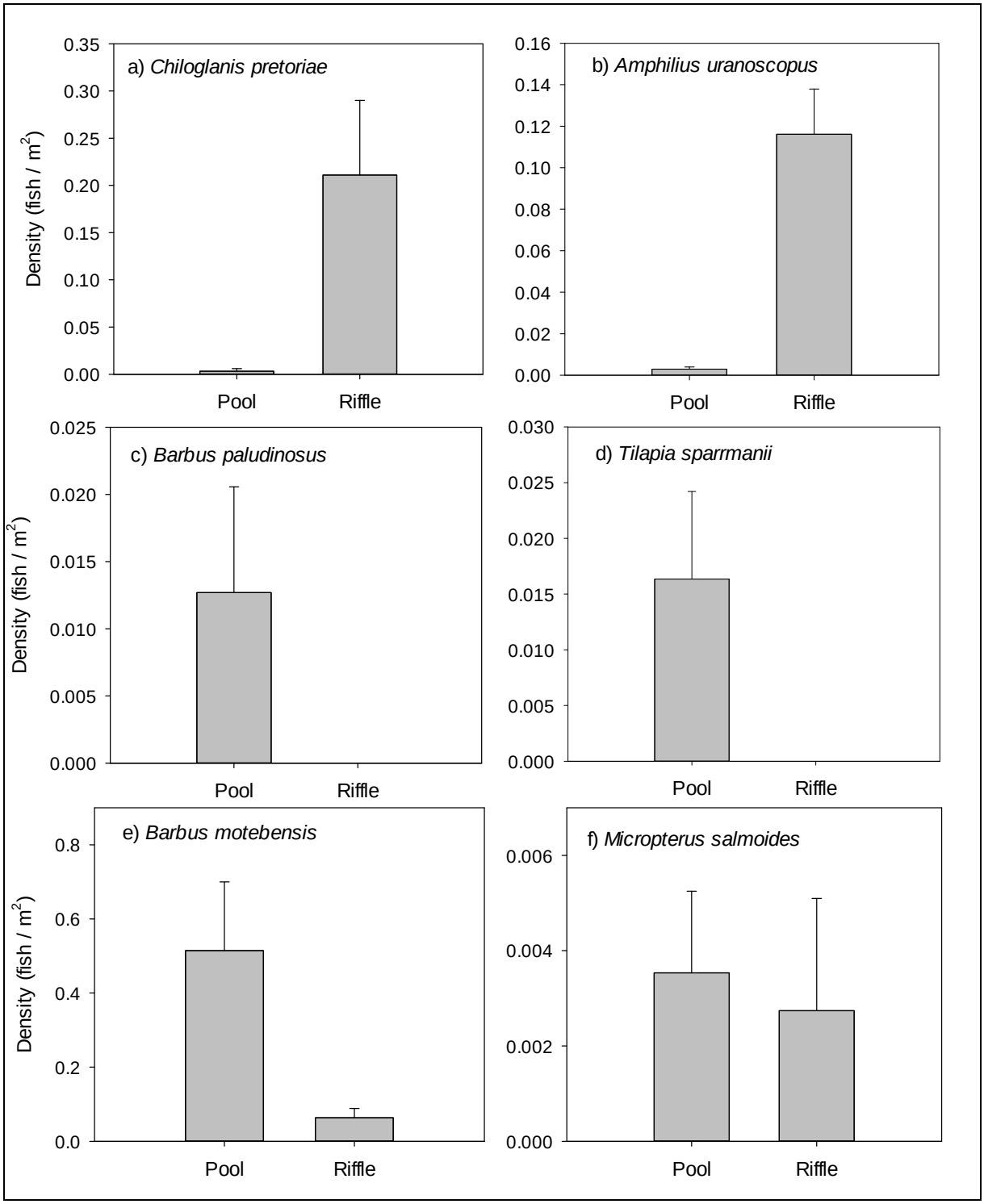

Figure 10: Compound figure showing densities of a) *C. pretoriae*, b) *A. uranoscopus*, c) *B. paludinosus*, d) *T. sparrmanii*, e) *B. motebensis* and f) *M. salmoides* in two major habitat classes (riffle, pool).

Figure 12 shows the distributions of *B. motebensis* and *M. salmoides* in the Groot Marico catchment. The lack of overlap between the species can be attributed to the elimination of *B. motebensis* from the invaded zone and highlights the sensitivity of this species to predation by *M. salmoides.* The impact of predation by *M. salmoides* on small cyprinid species has been shown in various studies worldwide (Takamura, 2007; Jackson, 2002). The results of the Canoco ordination indicate that all species apart from *B. motebensis* are resilient to *M. salmoides* predation.

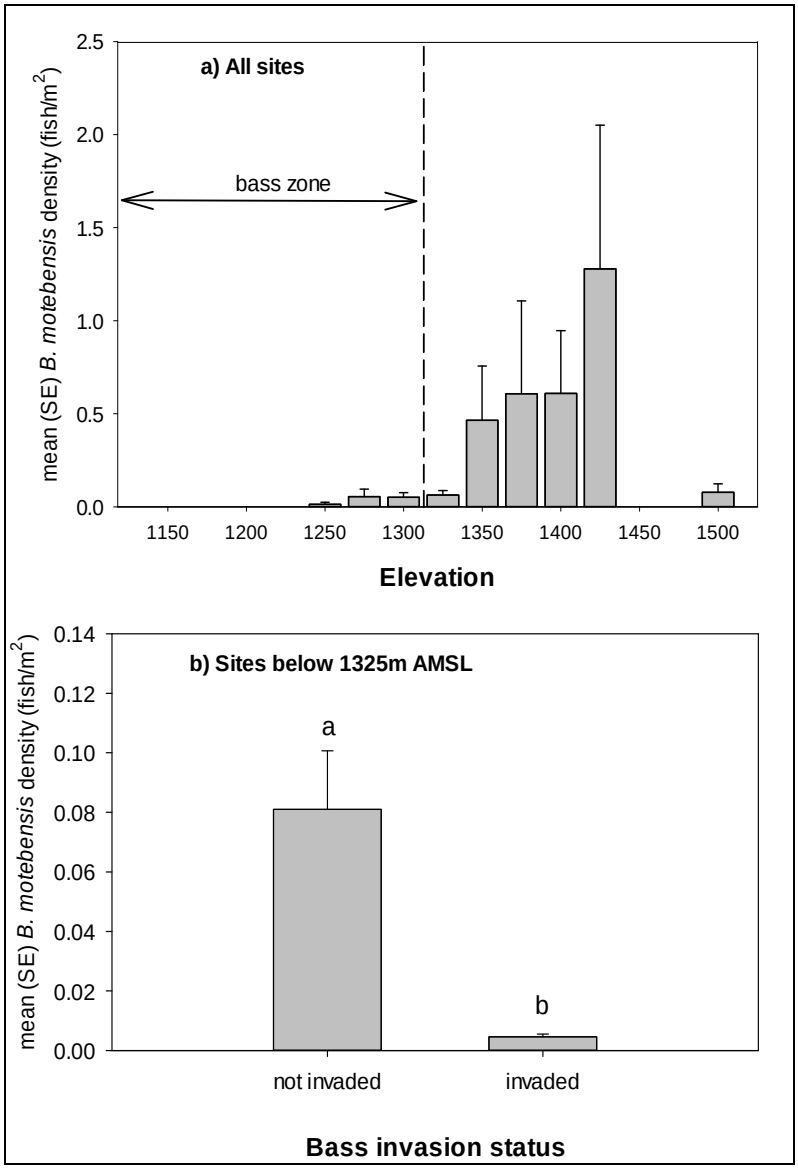

Figure 11: Graph showing a) the relative densities of *B. motebensis* along an elevation gradient and b) the relative densities of *B. motebensis* at bass-invaded and bass-free sites within streams at elevations where *M. salmoides* were able to penetrate the Groot Marico and its tributaries (the "bass zone").

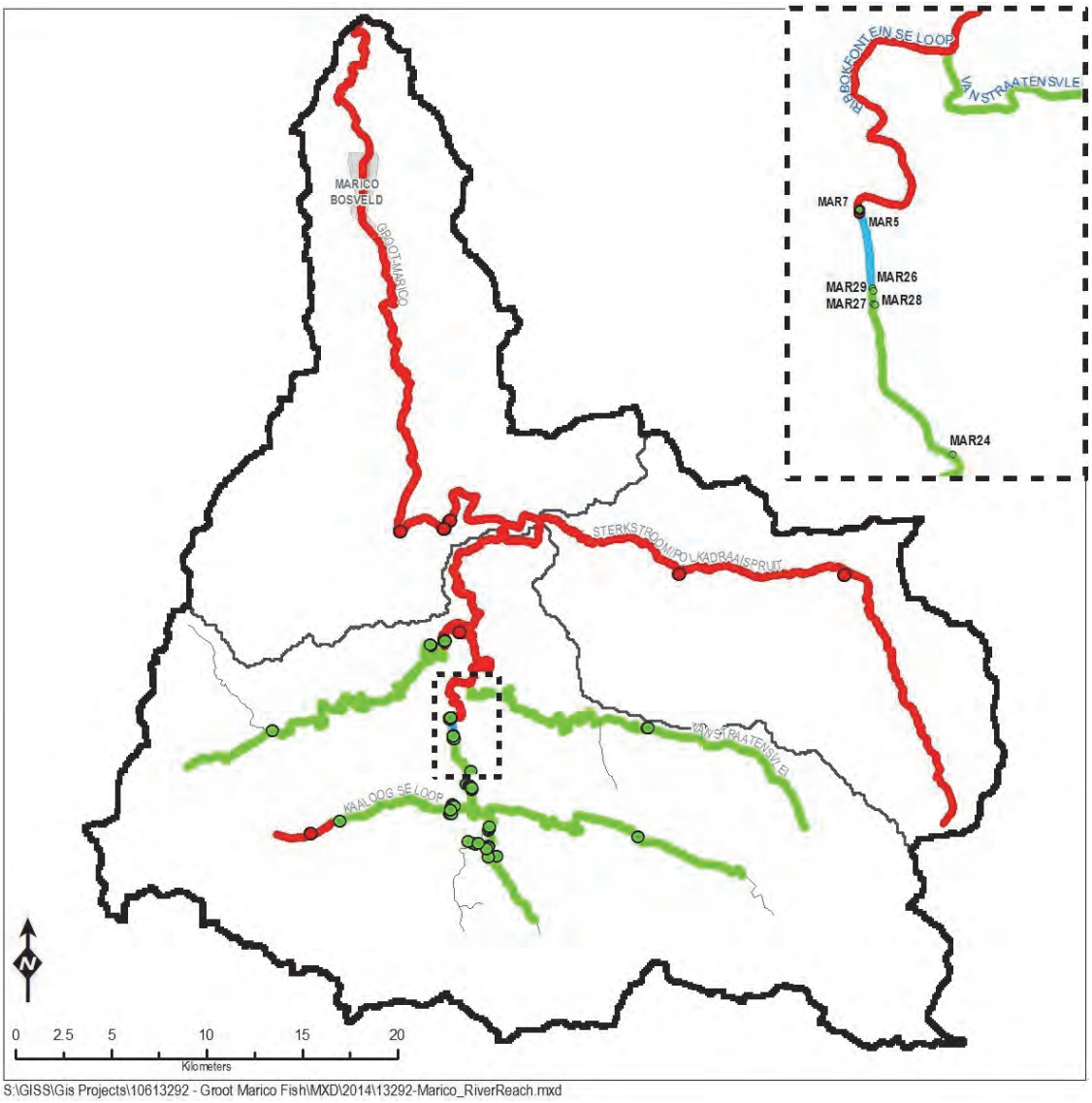

Figure 12: Distribution of *B. motebensis* and *M. salmoides* in the Groot Marico catchment showing the distributions of the 2 species.

Figure Notes: Green river reaches denotes the distribution of B. motebensis and red those of M. salmoides. The upper reaches of the Groot Marico and main stem are largely free of M. salmoides with the exception of the population in the Marico Eye at the top of Kaaloog se Loop. The inset highlighting the blue river reach shows the limited area of overlap between the 2 species.

2.4.2 Assessing trophic interactions between invasive and indigenous species

a) Gut content analysis

Of the 15 fish species known to occur in the Groot Marico River system eight were assessed namely: *A. uranoscopus*, *L. marequensis*, *C. pretoriae*, *B. motebensis*, *L. polylepis*, *T. sparrmanii*, *C. gariepinus* and *M. salmoides*. Stomach content analysis, summarised in Table 6, revealed significant differences in diet among six species from the Groot Marico River

(ANOSIM, Global R = 0.026, P < 0.05). Assessments of niche overlap among species () demonstrated four major feeding groups:

(1) *Amphilius uranoscopus* which had the lowest niche breadth (0.28) and dietary overlap with other species (Table 7), its diet consisting largely of Oligochaete worms (> 50%) and insects (Hemiptera, Odonata, and Ephemeroptera) (Table 6);

(2) Omnivorous species (*B. motebensis*, *C. pretoriae, L. marequensis* and *L. polylepis*) that fed mainly on algae and insects and plant material (Figure 13). The niche overlap among the omnivorous species was high (>0.90) but low with the remainder of the other species (Table 7). Among the second group, *C. pretoriae* and *L. polylepis* had highest niche breadth (>0.41), and their diet largely consisted of algae and mayflies (Ephemeroptera) (Table 7). The remainder (*B. motebensis* and *L. marequensis*) had the lowest niche breadth (<0.39) with algae and insect orders such as Diptera, Ephemeroptera and Odonata being the dominant food types;

(3) *Clarias gariepinus* whose diet comprised mainly of insects (> 78% of total diet) with the insect orders Odonata (32%) and Ephemeroptera (29%) being the main diet components. As expected *C. gariepinus* had a wide niche (0.46), but low dietary overlap (< 0.56) with all other fish species (Table 7); and

(4) Carnivore, *M. salmoides* was only assessed using percentage numbers as various data sources were used to increase sample size, it was therefore impossible to calculate % V, %F and %IRI and it fed mainly on insects (Ephemeroptera, Coleoptera and Hemiptera), crabs and fish.

Table 6: Gut content analysis of six indigenous and one alien invasive fish species.

Food group	***A. uranoscopus***	***B. motebensis***	***C. gariepinus***	***C. pretoriae***	***L. marequensis***	***L. polylepis***	***M. salmoides***
Algae	1.0	68.3	29.6	0.0	32.8	29.6	0.0
Cyanobacteria	0.0	1.2	0.7	0.0	7.2	0.7	0.0
Diatoms	0.0	0.0	0.7	0.0	0.0	0.7	0.0
Plant matter	0.0	1.2	0.0	8.2	7.2	0.0	0.0
Seeds	0.0	0.0	0.0	16.3	0.0	0.0	0.0
Zooplankton	0.0	3.0	2.8	0.0	0.0	2.8	0.0
Coleoptera	0.0	0.6	4.2	4.1	4.0	4.2	4.1
Diptera	2.6	10.8	15.5	18.4	6.4	15.5	0.0
Ephemeroptera	13.5	3.6	26.8	18.4	14.4	26.8	18.4

Food group	*A. uranoscopus*	*B. motebensis*	*C. gariepinus*	*C. pretoriae*	*L. marequensis*	*L. polylepis*	*M. salmoides*
Hemiptera	21.4	0.0	0.0	0.0	2.4	0.0	2.0
Mantodea	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Mites	0.0	0.0	0.7	0.0	0.0	0.7	6.1
Monoligastrida	47.9	6.6	4.2	0.0	4.8	4.2	0.0
Odonata	10.4	3.0	5.6	22.4	12.8	5.6	2.0
Orthoptera	0.0	0.0	0.0	0.0	0.0	0.0	6.1
Plecoptera	0.0	0.0	1.4	0.0	0.0	1.4	0.0
Protists	0.0	0.0	1.4	0.0	0.0	1.4	0.0
Trichoptera	1.6	1.2	2.8	6.1	8.0	2.8	0.0
Crabs	0.0	0.0	0.0	0.0	0.0	0.0	28.6
Shrimps	1.6	0.6	3.5	0.0	0.0	3.5	20.4
Fish	0.0	0.0	0.0	0.0	0.0	0.0	8.2
Frog	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Unidentified	0.0	0.0	0.0	6.1	0.0	0.0	0.0

Table 7: Niche overlap among of six fish species sampled in Groot Marico River, South Africa. The most significant overlaps (>0.90) are shown in bold.

	A. uranoscopus	***C. pretoriae***	***C. gariepinus***	***B. motebensis***	***L. marequensis***
L. polylepis	0.20	**0.94**	0.38	**0.93**	**0.91**
L. marequensis	0.24	**0.97**	0.52	**0.91**	
B. motebensis	0.23	**0.95**	0.49		
C. gariepinus	0.34	0.56			
C. pretoriae	0.33				

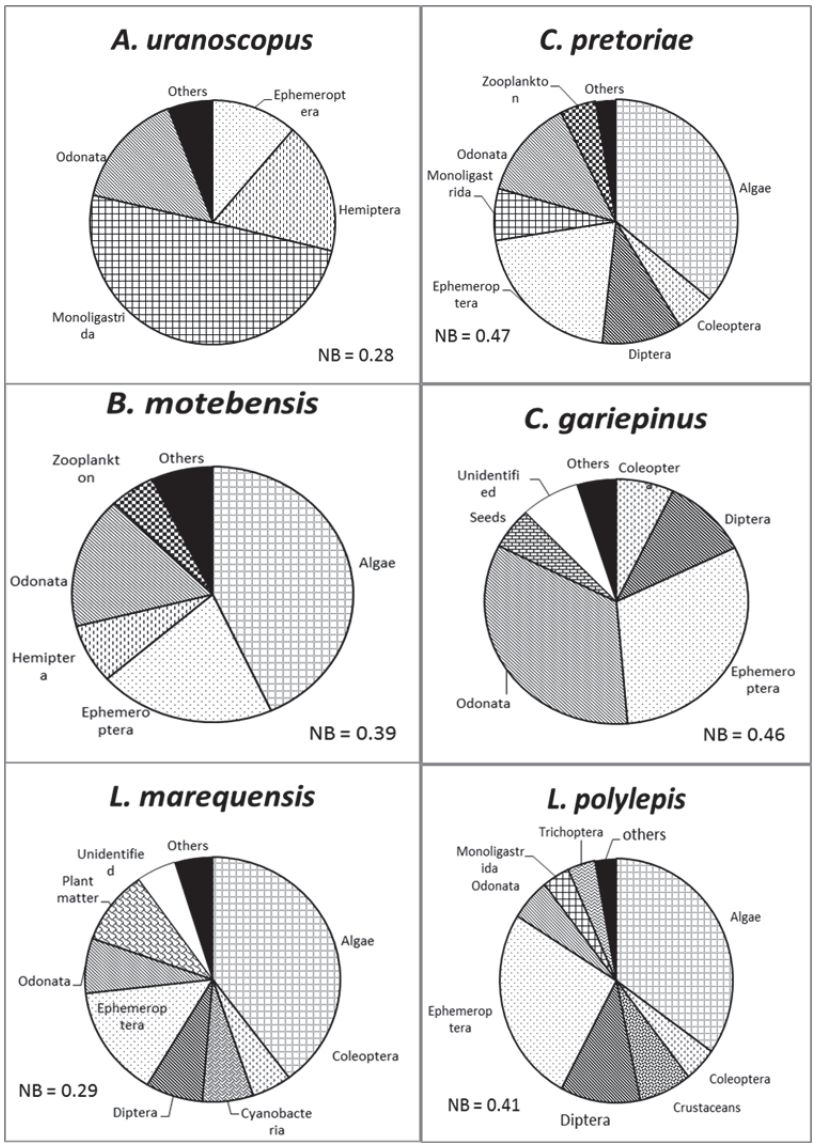

Figure 13: The proportion by percentage (%IRI) of food items in the diet of six fish species sampled in the Groot Marico, North-West Province, South Africa. NB; index of niche breadth and food items that contributed < 5% were combined and presented as others.

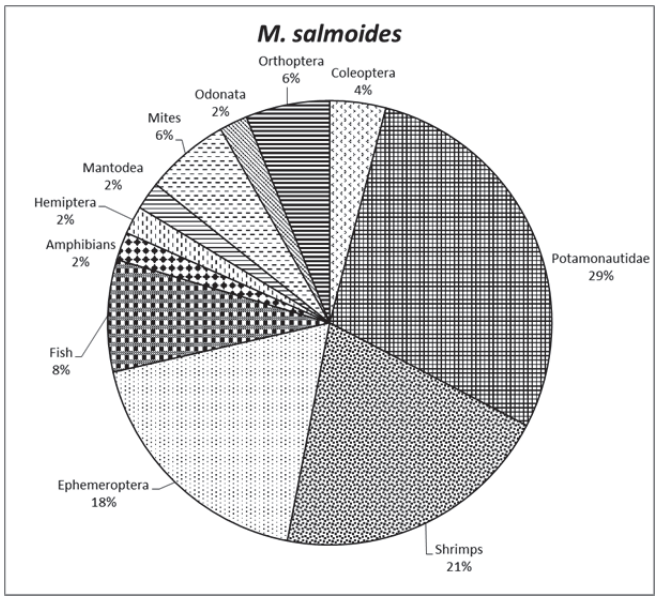

Figure 14: The proportion by percentage (%N) of food items in the diet of *M. salmoides* sampled in the Groot Marico, Northwest Province, South Africa.

b) Isotope analysis

In the non-invaded Kaaloog se Loop there were significant differences in isotopic values for $\delta^{13}C$ (ANOVA, $F_{(9, 96)} = 38.24$, $P = 0.00$) and $\delta^{15}N$ (ANOVA, $F_{(9, 96)} = 48.77$, $P = 0.00$) among fish species and isotopic baseline components (insects, algae, detritus and small aquatic fauna) (Table 8). The basal food web components (detritus and particulate organic matter) had depleted δ15N values (≤ 5.23‰) and $\delta^{13}C$ range (-12.86 to -24.53‰), while detritus was enriched in $\delta^{13}C$ but depleted in $\delta^{15}N$ values (Figure 15). Aquatic insects had wide $\delta^{15}N$ values (3.47-10.51‰) that were significantly different from all the food web components with the exception of *T. sparrmanii* and detritus. The remainder of the fish species formed the last feeding group (secondary consumers) that had similar enriched $\delta^{15}N$ values (8.52-11.67‰) and a narrow $\delta^{13}C$ (-19.07 to -23.08‰.).

In the invaded zone, there were also significant differences in isotopic values for $\delta^{13}C$ (ANOVA, $F_{(11, 76)} = 17.169$, $P = 0.00$) and $\delta^{15}N$ (ANOVA, $F_{(11, 76)} = 50.12$, $P = 0.00$) among fish species and isotopic baseline components (insects, algae, detritus and small aquatic fauna) in the invaded main stem of the Groot Marico (Figure 15). The basal food web components had a wide range of $\delta^{15}N$ values (4.69-10.78‰), with detritus been the most $\delta^{15}N$‰ depleted while biofilm and algae were significantly $\delta^{15}N$‰ enriched (range 10-51-10.78‰). The macrophagous fish species *T. sparrmanii* had a low $\delta^{15}N$ (≤ 10.00‰). Insects, crabs and the omnivore's *C. gariepinus* and *L. marequensis* crabs had intermediate δ15N values (10.74-12.81‰).

The apex species in the food wed were most enriched in $\delta^{15}N$ (mean $\delta^{15}N > 13.40‰$) and consisted of *C. pretoriae*, *A. uranoscopus* and *M. salmoides* (Figure 15).

Table 8: The Mean (δ13C and δ15N)values (± SD) of stable isotopes and total length of fish species and food sources collected from an non-invaded river section of the Kaaloog se Loop, an upper tributary of the Groot Marico River, and invaded river section in the main stem of the Groot Marico River, Northwest Province, South Africa.

Site	Species	$\delta^{13}C$	$\delta^{15}N$	Length (mm)
	A. uranoscopus	-21.58 ± 0.5	10.90 ± 0.4	81.96 ± 26.0
	B. motebensis	-21.47 ± 0.5	10.50 ± 0.4	52.30 ± 5.7
	C. gariepinus	-20.86 ± 0.4	10.21 ± 0.3	256.00 ± 48.5
	C. pretoriae	-21.97 ± 0.7	11.02 ± 0.2	47.67 ± 5.0
	L. marequensis	-21.32 ± 1.0	10.09 ± 0.7	115.00 ± 72.2
Kaaloog se Loop	*T. sparrmanii*	-19.31 ± 0.3	8.76 ± 0.3	79.50 ± 21.9
	Insects	-23.21 ± 1.168	7.56±1.7	
	POM	-24.47 ± 0.8	5.04 ± 0.4	
	Biofilm	-14.80 ± 2.03	5.12 ± 1.0	
	Detritus	-24.37 ± 0	5.59 ± 0	
	A. uranoscopus	-24.06 ± 0.9	13.93 ± 0.5	96.00 ±16.3
	C. gariepinus	-22.07 ± 1.7	12.31 ± 1.5	247.67 ±58.6
	C. pretoriae	-22.35 ± 0.7	14.22 ± 0.4	62.80 ± 7.8
	L. marequensis	-21.60 ± 1.0	12.01 ± 1.1	85.65 ± 48.9
	T. sparrmanii	-21.10 ± 1.0	9.33 ± 0.3	50.53 ± 15.3
Mainstem	*M. salmoides*	-21.32 ± 0.7	13.43 ± 0.6	226.48 ± 97.6
	Biofilm	-18.94 ± 0.32	10.64 ± 0.19	
	Detritus	-24.08 ± 2.26	5.71 ± 1.4	
	Insects	-24.14 ± 1.64	10.52 ± 1.1	
	Algae	-21.89 ± 0	9.56 ± 0	
	POM	-26.55 ± 0	8.63 ± 0	
	Potamonautidae	-21.87 ± 0.2	12.22 ± 0.8	

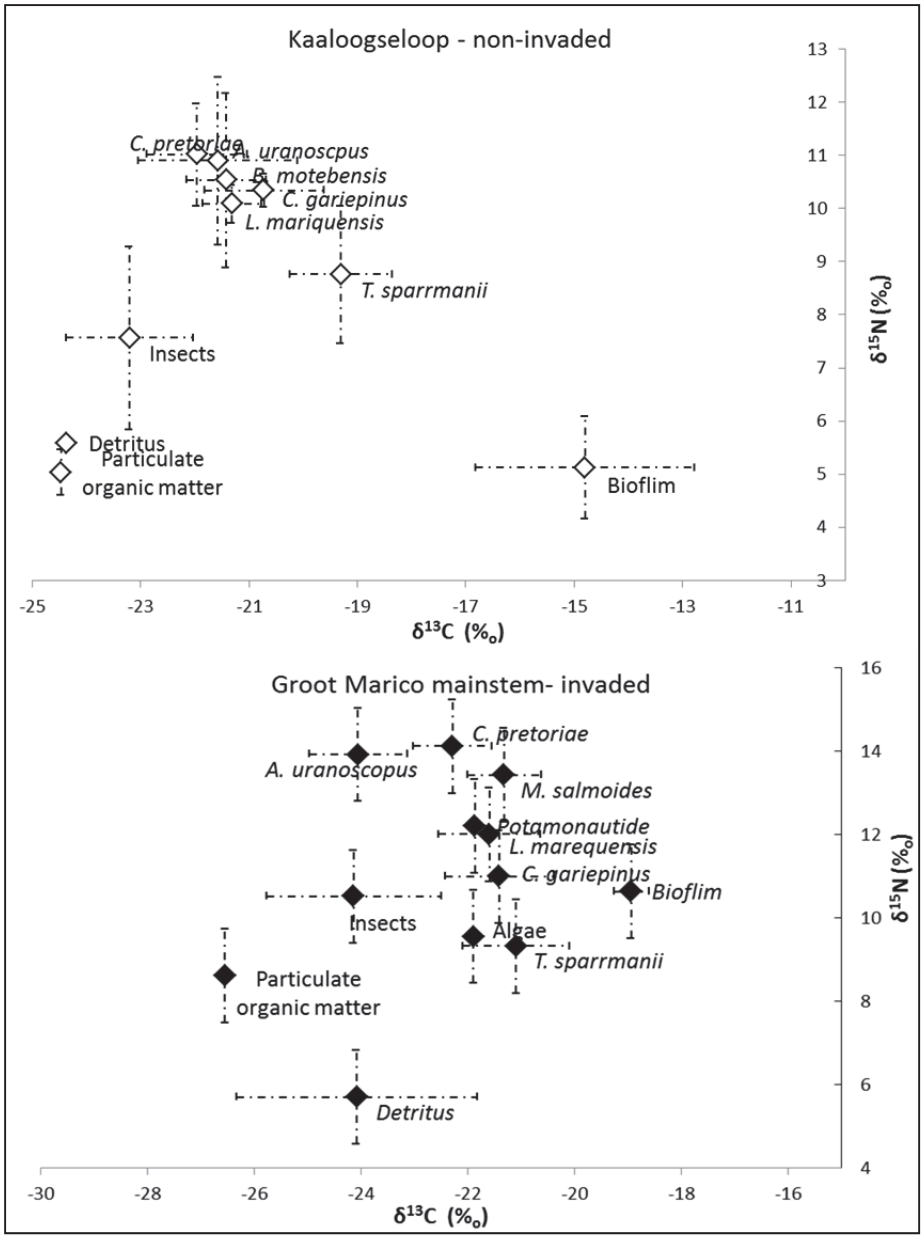

Figure 15: Stable isotope bi-plots (δ13C and δ15N) of fish species and possible food resources (biofilm, particulate organic matter (POM) and detritus) collected from non-invaded and invaded reaches of the Groot Marico catchment. Each point represents a mean and error bars represent standard deviations.

c) Mixing models

Application of the mixing model in invaded and non-invaded situations indicated a largely insectivorous diet for *A. uranoscopus* and *B. motebensis* where insects accounted for more 40% of the species respective diets, followed by detritus and particulate organic matter with biofilm contributing small amounts (Figure 16). The diets of the omnivorous species *C. gariepinus*, *C. pretoriae* and *L. marequensis* were more diverse with insects and detritus being significant dietary components while *T. sparrmanii* consumed significantly greater

proportions of biofilm in addition to POM, insects and detritus relative to the other fish species.

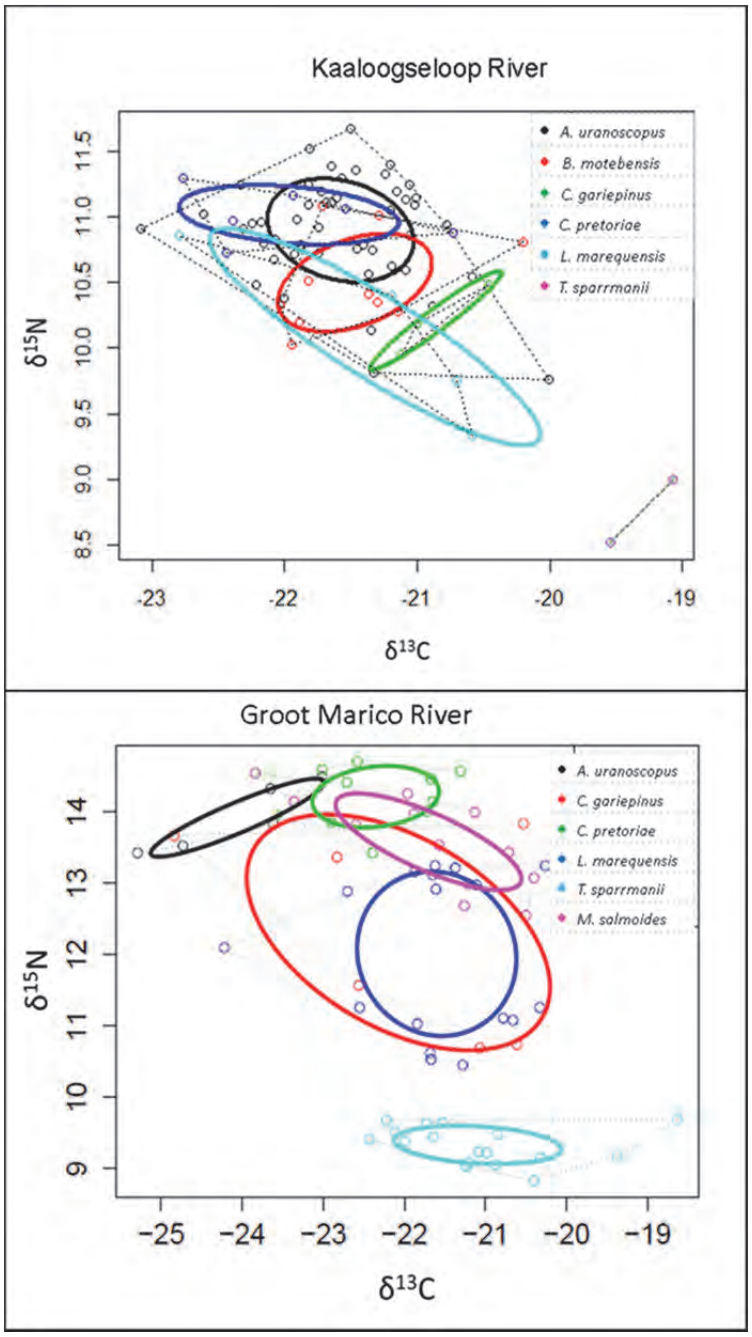

Figure 16: Stable isotope bi-plots (δ13C and δ15N) of fish species collected from the non-invaded Kaaloog se Loop and the invaded Groot Marico River main stem. Dashed lines enclose the standard ellipse area (SEA) (Jackson et al., 2011), while the solid lines which are colour-coded to represent different fish species, enclose the corrected SEA (Jackson et al., 2011).

The food web analysis showed that fishes of the Groot Marico system feed mainly on aquatic insects but there was also evidence of subtle differences in feeding preferences that

suggest niche partitioning. *Tilapia sparrmanii's* isotopic niche was different to all the other fish species and might reflect its tendency to feed on vegetative detritus that was not consumed by the other species. There were differences in the food niches of fish in non-invaded and invade river sections that might be related to environmental differences between the two river sections and partially due to the presence of the invasive *M. salmoides.* The difference in food web structures might be related to changes in ecosystem processes and biological communities along river gradients. The only observed evidence of the possible impact of the invasive *M. salmoides* on indigenous fish communities was the absence of *B. motebensis* from river section where *M. salmoides* occurred. To further understand the interactions between native and invasive fish in the Groot Marico system further studies need to be conducted preferably with larger sample sizes to validate the findings of this study and other aspects of the biology of fish in system such as species abundance and composition in relation to habitat availability and presence of invasive species.

2.4.3 Using population genetics to prioritise conservation areas

Results indicated that for *B. motebensis* the null hypothesis that all populations were genetically identical could be rejected as there were two distinct lineages (the Draai and Eastern lineages) that demonstrated significant divergence in both the ND2 and S7 genes, suggesting historical isolation. The low divergence in the mitochondrial cytochrome *b* gene ($0\% < D < 0.8\%$) suggests that this isolation is fairly recent and is probably not comparable to species level differentiation. Relaxed molecular clock dating would be required to determine the isolation period between the tributary populations. The null hypothesis was also rejected for *A. uranoscopus* as there were also significant levels of differentiation between tributary populations resulting in the identification of two lineages (the Ribbok and Western lineages). However, for *C. pretoriae,* the null hypothesis could not be rejected as there was no genetic differentiation between tributary populations, i.e. one panmictic population.

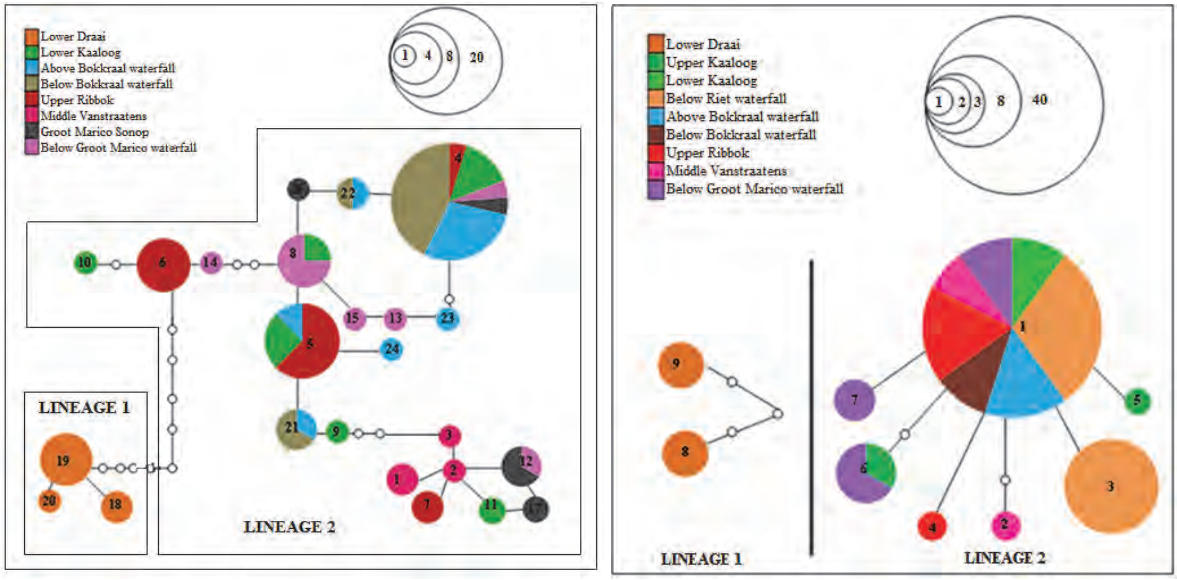

Figure 17: (A) Parsimony network with 95% plausible set of mitochondrial ND2 allele connections (numbered circles) constructed with the program TCS 1.2.1 (Clement *et al.*, 2000) for *B. motebensis*.

Figure Notes: The size of the circles indicates the relative frequency of the alleles (total N= 68). Small open white circles that are not numbered indicate missing alleles. Each line in the network represents one mutational change. Lineages are indicated by rectangular lined boxes. Lineage 1 is referred to as the Draai lineage. Lineage 2 is referred to as the Southern lineage. Within the Southern lineage, the Van Straatens tributary does not share any of its alleles with other populations (alleles 1, 2 and 3). Genetic distances within the Draaifontein tributary (0.1% < D < 0.3%) show low divergence. Whereas, the genetic distance within the Southern lineage (0.1% < D < 2%) and between the Draai and Southern lineage (0.9% < D < 1.9%) show high divergence. (B) Parsimony network for the S7 nuclear gene alleles (numbered circles) of B. motebensis. Allele1 is the central allele. The size of the circles indicates the relative frequency of the alleles. Small open white circles that are not numbered indicate missing alleles. Each line in the network represents one mutational change. The thick line represents a separation within the parsimony network, indicating the two different lineages. Genetic distances showed that within lineage divergence (0% < D < 1%) was much lower than between lineage divergence (9% < D < 11%).

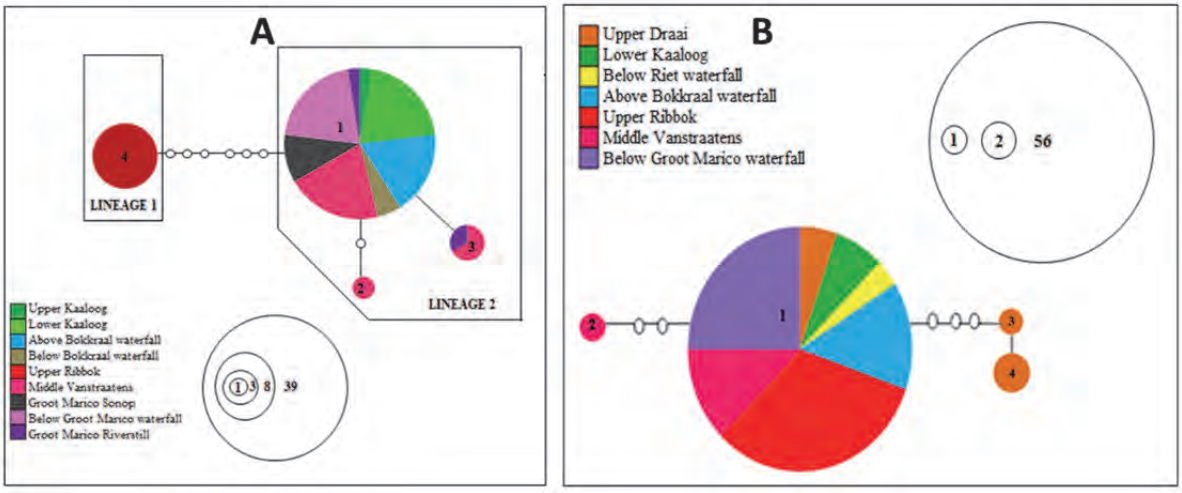

Figure 18: Parsimony network with 95% plausible set of mitochondrial ND2 allele connections (numbered circles) constructed with the program TCS 1.2.1 (Clement *et al.*, 2000) for *A. uranoscopus*.

Figure Notes: The size of the circles indicates the relative frequency of the alleles (total N=51) (see Table 4). Small open circles that are not numbered indicate missing alleles. Each line in the network represents one mutational stage. Lineages are indicated by rectangular lined boxes. Lineage 1 is referred to as the Ribbok lineage. Lineage 2 is referred to as the Western lineage. The Western lineage consists of the Kaaloog, Bokkraal and Van Straatens tributaries and the Groot Marico main stem. Genetic distances within the Ribbok lineage were 0%. Within the Western lineage, genetic distances indicate that divergence was low (0.2% < D < 0.5%) and between the Ribbok lineage and the Western lineage, the divergence was relatively high (1.3% < D < 1.7%). (B) Parsimony network for the S7 nuclear gene alleles (numbered circles) of A. uranoscopus. Allele 1 is the central allele. The size of the circles indicates the relative frequency of the alleles. Small open white circles that are not numbered indicate missing alleles. Each line in the network represents one mutational change.

Therefore, due to each species showing different genetic structuring within the tributary populations, more than one priority area for conservation needs to be implemented. These priority areas of conservation were therefore evaluated based on the current conservation status of the species (*B. motebensis* being VUL on the IUCN Red List), the number of Evolutionary Significant Units (ESUs) for each species and the overall genetic diversity of all three species in the Groot Marico catchment. In total, four tributary populations were identified as conservation priorities areas, these were the Draaifontein, Van Straatens, Ribbokfontein and Kaaloog se Loop tributaries.

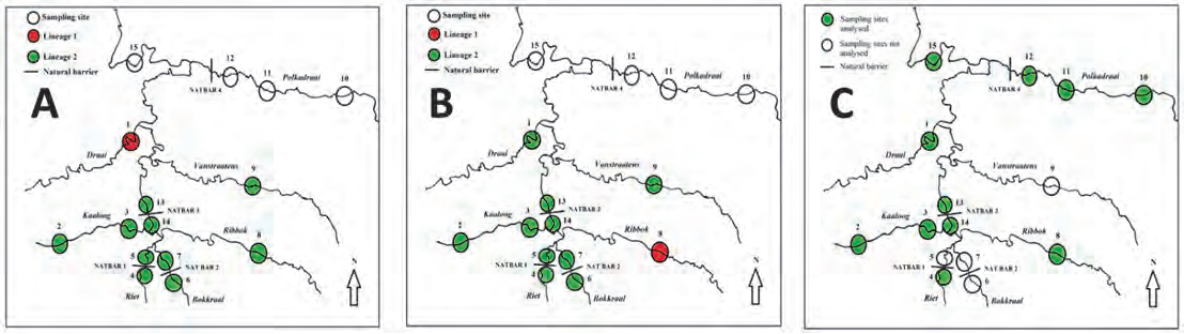

Figure 19: (A) *B. motebensis*; (B) *A. uranoscopus* and (C) *C. pretoriae* genetic distribution across the upper Groot Marico catchment. For *B. motebensis* (A) Genetic analysis revealed 2 major genetic lineages.

Figure Notes: The first lineage (Draai lineage, red) only occurs in the Draaifontein. The second lineage (Southern lineage, green) occurs in Kaaloog se Loop, Rietspruit, Bokkraal, Ribbokfontein and Van Straatens tributaries as well as the Groot Marico main stem. For A. uranoscopus (B), genetic analysis revealed 2 main lineages. The first lineage (Ribbok lineage, red) only occurs in the Ribbokfontein tributary. The second lineage (Western lineage, green) occurs in the Draaifontein, Kaaloog, Rietspruit, Bokkraal and Van Straatens tributaries as well as the Groot Marico main stem. There are 4 major natural barriers in the study area, indicated by perpendicular lines (NATBAR = natural barrier).

a) *Barbus motebensis*

The Draaifontein, Van Straatens and Kaaloog se Loop tributaries were selected as priority areas for *B. motebensis* (*B. motebensis* is considered to be the most vulnerable of all three species). The Draaifontein tributary was selected due to the *B. motebensis* population within the tributary showing isolation from the remaining populations. In order to conserve *B. motebensis* from the Southern lineage, the Van Straatens and Kaaloog se Loop tributaries were selected. Reasons for selecting these two specific tributaries within the Southern lineage were that the Van Straatens tributary had unique alleles (three Evolutionary Significant Units) for *B. motebensis* and the Kaaloog tributary had high genetic diversity (H_D = 0.889, ND2 gene) when compared to the other tributary populations.

b) *Amphilius uranoscopus*

The Ribbokfontein and Van Straatens tributaries were selected as priority areas for the conservation of *A. uranoscopus*. The Ribbokfontein tributary was selected as it showed isolation from the rest of the tributary populations, as seen with the Draaifontein tributary (*B. motebensis*) and the Van Straatens tributary was selected to represent the Western lineage as it had the highest diversity for both genes (ND2 and S7). The Ribbokfontein tributary has the highest prioritisation when compared to the Van Straatens tributary.

c) *Chiloglanis pretoriae*

Chiloglanis pretoriae occurs within the Draaifontein, Van Straatens, Ribbokfontein and Kaaloog se Loop tributaries, therefore by prioritising these tributaries for conservation, *C. pretoriae* will in turn be conserved.

3 CASE 2: UNDERSTANDING THE ROLE OF WATER-TRANSFER INFRASTRUCTURE IN ENABLING INVASION EXPERIMENTAL PROCEDURES

DJ Woodford, OLF Weyl, C Hui, DM Richardson

3.1 The Orange Fish Sundays Inter-basin Water Transfer Scheme (IBWT)

The Orange-Fish-Sundays IBWT links the waters of the Orange-Vaal catchment to the Great Fish River via a tunnel between the Gariep Dam and the Teebus Rivers, and the Great Fish to the Sundays Catchment via a canal originating in the Little Fish River that empties into Darlington Dam on the Sundays River (Figure 20). The link between the Orange-Vaal and Sundays catchments was first completed in 1975, while the Fish and Sundays catchments were first joined in 1978.

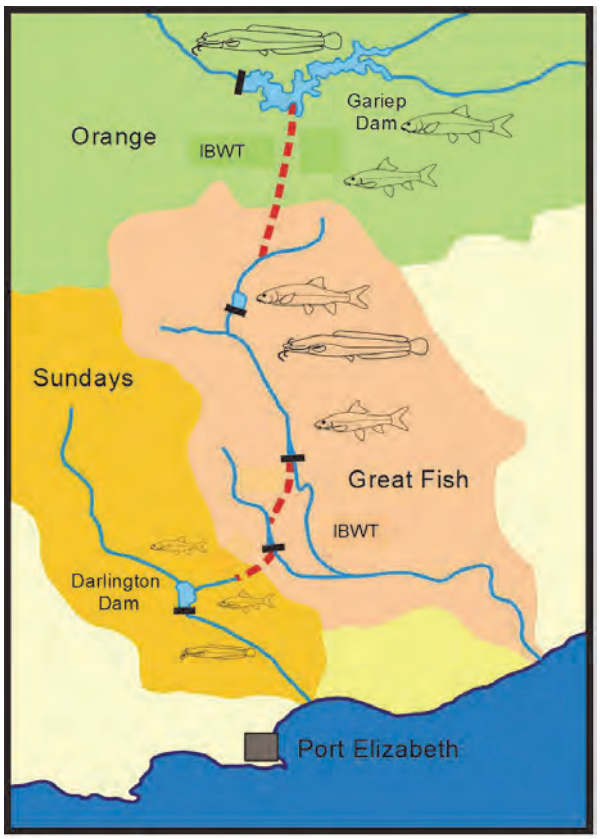

Figure 20: Schematic map of the Orange-Fish-Sundays Inter-basin water transfer scheme showing the connection between the three river basins.

The IBWT has resulted in the transfer of many organisms between the three catchments, with potentially detrimental results (Laurenson & Hocutt, 1985). One particularly worrying impact has been the replacement of native simuliid blackfly species *Simulium adersi* and *S. nigritarse* by the Orange-Vaal native *S. chutteri* in the middle reaches of the Fish River

(O'Keefe and De Moor, 1988). *Simulium chutteri* is a pest species that forms blood-feeding swarms that can severely damage cattle herds, causing economic impacts in the lower Orange River (Snaddon & Davies, 1998). Furthermore, five species of fish have been introduced into both the Great Fish and Sundays catchments by the IBWT: these are the Smallmouth yellowfish (*Labeobarbus aeneus*), *C. gariepinus*, *L. capensis*, the Banded tilapia (*Tilapia sparrmanii*) and *O. mossambicus* (Laurenson & Hocutt, 1985; Weyl *et al*., 2008). In the Sundays River, these species have colonised the majority of the middle reaches of the main-stem downstream of Darlington Dam, and have in some cases also colonised the tributaries which flow from the Addo Elephant National Park (AENP), which are home to threatened native fish species.

3.2 The Sundays Catchment and Addo Elephant National Park

The Sundays River flows through many distinctive bioregions and provides vastly contrasting habitats for fish, although its fish fauna has in several places been homogenised by the introduction of fish through the IBWT (Weyl *et al.,* 2008). The headwaters of the upper Sundays River flow through semi-arid Noorsveld and Karoo and are naturally seasonal upstream of Darlington Dam (Figure 21). Little or no fish survey work has been done on these ephemeral upper reaches, but detailed fish data exist for Darlington Dam and the river and tributaries downstream within the Addo Elephant National Park (AENP; Weyl *et al.,* 2008, 2009a, 2010; Table 9). Weyl *et al.,* 2010) list 13 native and eight alien fish species from the Sundays River and its tributaries (Table 9), most occur in Darlington Dam and in the lower river.

The introduced *C. carpio* and Mosquitofish (*Gambusia affinis*) are both highly abundant in the system starting at the dam, as is *L. umbratus* and Estuarine roundherring (*Gilchristella aestuaria*). The *L. umbratus* in Darlington Dam are likely a mix of native Sundays River fish and introduced Orange-Vaal stock that were brought in by the IBWT. *G. aestuaria* is native to the lower, tidally influenced reaches of the Sundays River, but after a recent introduction into Darlington Dam now occurs in great abundance throughout the Sundays River. The Flathead mullet (*Mugil cephalus*) and the diadromous migratory Longfin eel (*Anguilla mossambica*) have also been recorded in the dam. In the lower reaches below major migration barriers, estuarine species such as Freshwater mullet (*Myxus capensis*) and Cape mooney (*Monodactylus falciformis*) are present. These fishes cannot penetrate upstream of the first major irrigation weir on the Sundays River main-stem and are restricted to the river below Kirkwood.

The majority of the eastern tributaries flow through the AENP (Figure 21) and while still naturally seasonal do provide some permanently flowing habitats for native fishes in certain upper reaches within the protected areas of the park (Russell, 1998; Weyl *et al.,* 2008).

Table 9: Checklist for freshwater fishes in the Addo Elephant National Park. After Weyl et al., 2010).

Species	**Category**	**Locality and year in which the species was last recorded**		
		Sundays River	**Tributaries**	**Darlington Dam**
Indigenous				
Longfin eel *Anguilla mossambica*	Obligate catadromous	2009	2009	2009
Mottled eel *Anguilla marmorata*	Obligate catadromous	2009	-	-
Shortfin eel *Anguilla bicolor bicolor*	Obligate catadromous	1958	-	-
Chubbyhead barb *Barbus anoplus*	Primary freshwater	1996	2007	-
Goldie barb *Barbus pallidus*	Primary freshwater	2009	2009	-
Moggel *Labeo umbratus*	Primary freshwater	2009	2009	2009
Eastern Cape redfin *Pseudobarbus afer*	Primary freshwater	-	2009	-
Freshwater mullet *Myxus capensis*	Facultative catadromous	2009	-	-
Flathead mullet *Mugil cephalus*	Facultative catadromonous	-	-	2009
Estuarine round-herring *Gilchrestella Aestuaria*	Estuarine & Freshwater	2009	2009	2009
Cape moony *Monodactylus falciformis*	Facultative catadromous	2009	-	-
River goby *Glossogobius callidus*	Estuarine & Freshwater	2009	2009	2009
Freshwater goby *Awaous aeneofuscus*	Facultative catadromous	1983	-	-
Alien				
Sharptooth catfish	Alien primary freshwater	2009	2009	2009

Species	Category	Locality and year in which the species was last recorded		
		Sundays River	Tributaries	Darlington Dam
Clarias gariepinus				
Smallmouth yellowfish *Labeobarbus aeneus*	Alien primary freshwater	2009	2009	2009
Orange river mudfish *Labeo capensis*	Alien primary freshwater	2009	2009	-
Largemouth bass *Micropterus salmoides*	Alien primary freshwater	-	2009	-
Mozambique tilapia *Oreochromis mossambicus*	Alien primary freshwater	2009	2009	2009
Mosquitofish *Gambusia affinis*	Alien primary freshwater	2009	2009	2009
Common carp *Cyprinus carpio*	Alien primary freshwater	2009	-	2009
Banded tilapia *Tilapia sparrmanii*	Alien primary freshwater	2009	-	-

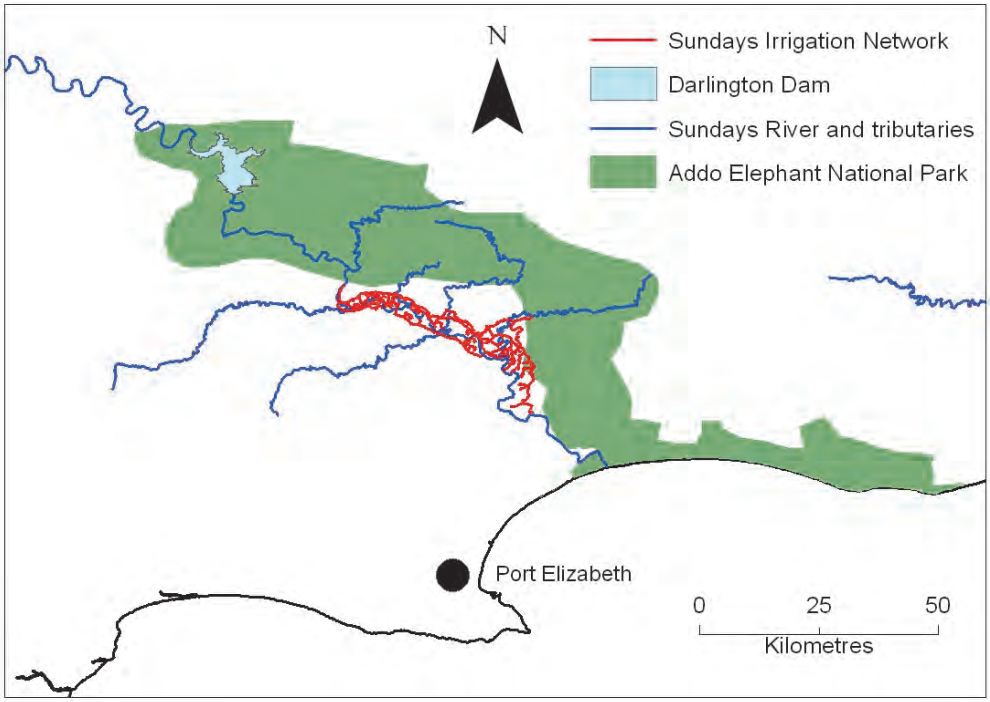

Figure 21: Map of the Sundays River and its associated tributaries, four of which flow through the Addo Elephant National Park. Water from the Orange-Vaal catchment enters the system at Darlington Dam, after which it is circulated through the Sundays Irrigation Network marked in red before spilling back into the Sundays River.

The three major tributaries within the AENP, the Uie, Wit and Krom/Coerney, all support populations of native *P. afer*, River goby (*Glossogobius calidus*) and the Goldie barb (*Barbus pallidus*) in their upper reaches (Weyl *et al.,* 2008). *Anguilla mossambica* also occurs in the Uie River (Weyl *et al.,* 2008). *Clarias gariepinus* have invaded far up the Uie and Krom/Coerney rivers, and are currently prevented from further upstream invasion by natural barriers in these tributaries, as well as artificial barriers such as the Slagboom Dam on the Wit River.

3.3 The Sundays River Irrigation Network (SRIN)

The Sundays River Irrigation Network (SRIN), which was first commissioned in 1911, and largely completed by 1962 (Van Vuuren, 2009), comprises a network of branching, gravity fed concrete canals that distribute water originating from a single off-take canal at Korhaansdrift Weir to approximately 300 individual small irrigation ponds in the Sundays River Valley, stretching from Korhaanspoort in the Zuurberg mountains in the north-west to Barkley Bridge south of Addo in the south-east (Figure 21). Because new ponds have been added to the network over time as citrus farms were established and expanded, the network now provides a natural experiment, wherein multiple novel habitats of varying age (the ponds) are all fed by a single immigration source for fish (the canal network). This setup allows fundamental hypotheses about alien fish introductions, establishment, and community dynamics to be tested.

3.4 Methodology

Fish distribution within the system was determined from surveys conducted between September 2007 and December 2010 (summarised in Woodford *et al.,* 2013). Different sampling methods and equipment were used to sample the full range of habitats in the river, reservoirs and weirs (see Woodford *et al.,* 2013 for full list of methods and gears). Because each sampling method has inherent species and size selectivity, catch rate and species composition data could not be compared directly between sites or gears. As a result, all catches from all sampling gears were grouped and only the presence/absence data were used to assess fish species distributions in the five river reaches.

3.4.1 Sampling the SRIN

a) Irrigation canal surveys

To establish the species composition and introduction rates entering the irrigation network, we deployed fyke and drift nets near the source of the main canal 200 m downstream of the off-take weir at Korhaansdrift, at the northernmost point of the canal network (Figure 22).

Sampling took place over six days in January and three days each in February, April, June, August, September and November 2012.

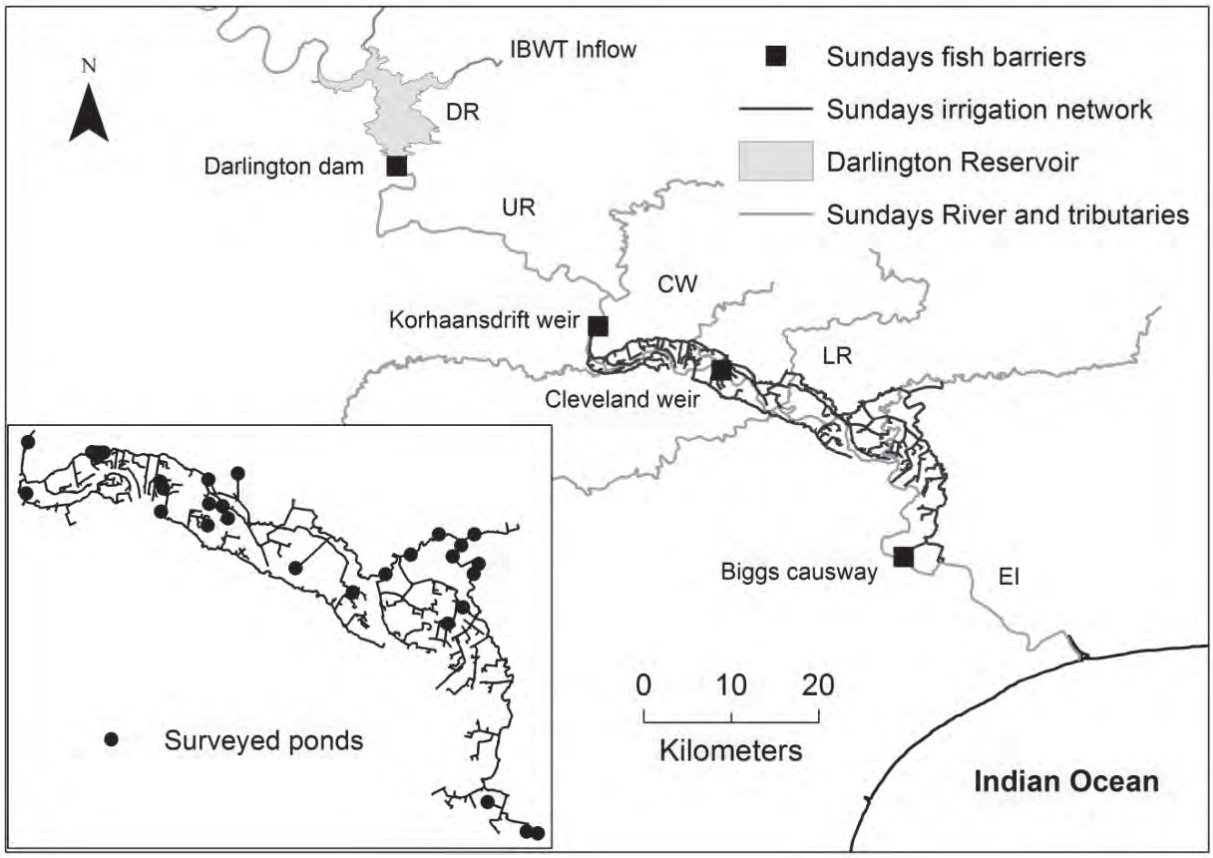

Figure 22: The Sundays River and its associated tributaries.

Figure Notes: Water from the Orange-Vaal catchment enters the system at Darlington Dam, after which it is circulated through the Sundays irrigation network before spilling back into the Sundays River. Barriers to upstream fish movement are labelled with squares on the main stem. Inset shows localities of 30 sampled irrigation ponds across the network. Faunal zones along the Sundays River (excluding the irrigation network) are labelled as follows: within Darlington reservoir (DR); river upstream of the off-take canal at Korhaansdrift (UR); river upstream of Cleveland weir in the middle reaches (CW); lower river between Cleveland weir and the Biggs causeway (LR); and below Biggs causeway in the estuarine-influenced zone (EI).

These sampling dates captured seasonal variation in fish movement, with the extended January sample targeting the time of year when maximum larval drift in most species was expected. Drift nets (WP2 or bongo nets with mouth diameter of 60 cm and mesh size 0.5 mm) were suspended in the water column below a bridge. Sampling lasted two hours and was conducted morning, midday, early evening, and at night, to capture diel fluctuations in fish drift. Fyke nets were also deployed below bridges in the first two kilometers of the main canal. Each fyke net had upstream and downstream facing traps with a 20 mm mesh size and a mouth area of 0.5 m^2. In determining the number of fish propagules entering the

network, we only assessed fish captured in the downstream trap of each fyke, as it was considered to be filtering water flowing downstream in a similar manner to the drift nets.

b) Irrigation pond surveys

Surveys of 30 irrigation ponds (Figure 22) were conducted over two periods; a winter survey on 18 ponds in July 2011 and a summer survey of a further 12 ponds in January 2012. Selected ponds represented a range of ages from a number of hours since filling to 30 years of continuous inundation, and had no history of stocking by landowners. Site selection also ensured a range of both new and old ponds located at varying distances along the irrigation network from the source at Korhaansdrift. The location of ponds along the irrigation network was determined using a GIS model of the network provided by the Lower Sundays River Water Users Association (LSRWUA, Sunland, SA). Pond area was measured on the ground and confirmed using geo-referenced aerial photographs where possible (pond size ranged from 1089m^2 to 18900m^2). Mean depth was measured along a transect through the center of the pond. We collected physico-chemical measurements using a YSI multi-probe Sonde. Maximum and minimum pond temperatures were captured by a submerged temperature logger that recorded two-hourly temperatures for 12 months in a representative pond. Information on the age of each pond was provided by the landowners and the LSRWUA.

Fish were collected using a 30 m × 2 m seine with 12 mm mesh wings and an 8 mm mesh codend. We used between two and five seine samples per dam, depending on the surface area. For each sample, the net was deployed in a semi-circle near the center of the pond by an inflatable boat and retrieved into a corner or the center of a dam wall. The sampling effort for each sample was calculated as the area covered by the seine pull, estimated as the semi-circular area within the net plus the area between the net and the shore at the beginning of retrieval. All fish retrieved were identified and the first 100 of each species were measured to the nearest mm.

3.4.2 Surveying Addo Elephant National Park (AENP)

In November 2012, fish surveys were conducted in the three major tributaries of the Sundays River that drained the AENP. These were the Krom River (a seasonally intermittent tributary of the ephemeral Coerney River), the Wit River (seasonally intermittent), the ephemeral Uie and its seasonally intermittent tributaries, the Groot Uie and Klein Uie. Surveys were conducted at the end of a wet winter that had broken a six year drought in the Sundays catchment, wherein the majority of these tributaries had dried up and all fish were reduced to isolated refuge pools. The aim of the surveys was to document the re-invasion of these tributaries by *C. gariepinus*, which were found downstream of natural and artificial

barriers in the Uie and Wit Rivers and were present in the lower reaches of the Krom in 2007 (Weyl *et al.,* 2008, 2010). Sampling was conducted at multiple localities on each tributary stream, using a combination of electrofishing, seine netting, overnight fyke netting, with gill nets used in pools too deep for the other techniques. Due to the variability of species-specific capture efficacy among the gears, we assessed species presence/absence only.

3.4.3 Data Analyses

a) Characterizing invasion pathways into the ponds

To confirm whether Korhaansdrift weir could be considered as the only source of immigration into the ponds, we compiled an assembly list based on species presence at each sampled region of the Sundays River, Darlington reservoir, the Korhaansdrift intake, and the ponds themselves. We compared the similarity of the fish communities across these sites to determine whether known and assumed upstream barriers to fish movement were characterized by dissimilarities in the fish assemblages. We employed Jaccard's coefficient of similarity to compare the difference between fish communities based on the presence/absence of shared species (see Woodford *et al.,* 2013 for full method).

b) Assessing Propagule Pressure and Establishment Rates

Propagule pressure (the number of introduced individuals) for each species entering the irrigation network at the off-take was determined by estimating the mean number of fish entering the canal network per m^3 of water filtered by the drift and fyke nets. The overall mean propagule pressure for each species collected in each sampling month was then weighted by the volume of water purchased for a representative monitoring pond in the corresponding month, to ensure overall mean propagule pressure reflected actual volumes of water (and thus fish densities) entering the ponds throughout the annual cycle.

We assessed fish establishment patterns in the ponds in two ways. First, to characterize overall establishment success for each species, we calculated the probability of capture from all seine passes within a pond ($P_{capture}$). Comparison of mean $P_{capture}$ across all ponds indicated whether or not a species consistently established. Second, we assessed overall rates of establishment by plotting Pcapture for each species against pond age, fitting an exponential increase toward the maximum $P_{capture}$ (=1):

$$y = 1 - e^{-b \cdot x} \quad (1)$$

where *y* represents predicted $P_{capture}$, *x* pond age, and *b* the rate of increase towards the maximum $P_{capture}$. This rate of increase provided a fitted analogue to the global rate of establishment for the species across all ponds. This establishment rate was then used to

distinguish species that established quickly versus slowly. The effect of propagule pressure on establishment success was determined by comparing the log-transformed propagule pressures of species that did and did not establish in the ponds using a Student t-test. The relationship between propagule pressure and the establishment rate was also examined.

Because the environmental and reproductive adaptations of freshwater fish can affect their ability to establish in new ecosystems (Olden and Poff, 2006), we compared critical life-history characteristics of all species found within the irrigation ponds. These included age at maturity, reproductive and environmental guilds (after Balon, 1975) and feeding guilds, which were derived using diet preferences summarized by Skelton (2001). We also compared the native temperature ranges of each species to those recorded in the ponds, as temperature tolerance may also affect establishment success (Kolar and Lodge, 2002).

3.5 Results and Discussion

3.5.1 The pathways of invasion into and through the Sundays River catchment

Assessment of fish community composition throughout the Sundays River main-stem indicates that barriers to upstream movement act as significant filters of fish diversity, preventing the co-occurrence of several key species in various regions of the system. Twenty-one fish species were recorded from surveys in Darlington reservoir, the irrigation network, and four distinct zones in the Sundays River main-stem that were separated by known barriers to upstream fish movement (Table 10, Figure 22).

The furthest downstream zone, representing sites in the estuarine influenced reaches below the Biggs causeway, contained five species unique to the zone. These species were all marine-spawning species that seldom enter purely freshwater environments. Six species occurred in the Sundays River parallel to the irrigation network, but not in the network itself nor in the river upstream of Korhaansdrift weir, the off-take point for the network (Figure 22).

Table 10: Distribution of species in the Sundays River system from Darlington reservoir.

Species	Group	Faunal zones						
		DR[1]	UR[2]	IO[3]	IP[4]	CW[5]	LR[6]	EI[7]
Cyprinus carpio	A	√	√	√	√	√	√	√
Gambusia affinis	A	√	√	√	√	√	√	√
Oreochromis mossambicus	A	√	√	√	√	√	√	√
Tilapia sparrmanii	A					√	√	√
Clarias gariepinus	O	√	√	√	√	√	√	√
Labeo capensis	O	√	√		√			
Labeobarbus aeneus	O	√	√	√	√			

Species	Group	Faunal zones						
		DR[1]	UR[2]	IO[3]	IP[4]	CW[5]	LR[6]	EI[7]
Barbus pallidus	N					√	√	√
Gilchristella aestuaria	N	√	√	√	√	√		
Glossogobius callidus	N	√	√	√	√	√	√	√
Labeo umbratus	N	√	√	√	√		√	
Anguilla marmorata	E						√	√
Anguilla mossambica	E	√	√	√	√	√	√	√
Argyrosomus japonicas	M							√
Lithognathus lithognathus	M							√
Liza trimaculatus	M							√
Monodactylus falciformis	M						√	√
*Mugil cephalus**	M	√					√	√
Myxus capensis	M						√	√
Pomadasys commersonii	M							√
Rhabdosargus holubi	M							√

Notes: Species are categorized as: Alien to the system (A); alien Orange River fauna (O); native freshwater spawning fish (N); catadromous eel (E); and marine spawning fishes (M). The presence of Mugil cephalus in the Darlington reservoir is a result of stocking and not unaided penetration of upstream barriers. [1.] Darlington reservoir (DR); [2.] River upstream of the off-take canal at Korhaansdrift (UR); [3.] Inside the off-take canal (IO); [4.]Inside the irrigation ponds (IP); [5.] River upstream of Cleveland weir in the middle reaches (CW); [6.] Lower river between Cleveland weir and the Biggs causeway (LR); [7.] Below Biggs causeway in the estuarine-influenced zone (EI).

Comparisons of fish communities in various reaches of the Sundays River using Jaccard's coefficient revealed two major discontinuities in community similarity. Fish communities within the Darlington reservoir, the upper Sundays River, the Korhaansdrift off-take and the irrigation ponds were significantly similar to each other but not to the other sampled communities, whereas the estuarine-influenced fish community was significantly different from this group of similar fish communities (Woodford *et al.,* 2013). These patterns indicated that fish were only entering the irrigation network at the Korhaansdrift off-take, and that fish species only occurring downstream of this barrier on the main-stem did not have a pathway into the ponds.

Previous surveys in the Sundays River tributaries had identified key barriers to invasion on the Groot Uie and Coerney rivers that ought to be impenetrable to dispersing fish like *C. gariepinus* (Weyl *et al.,* 2010). However, the presence of *C. gariepinus* in the lower Krom River, prior to their extirpation as a result of the drought (the water hole they were recorded

in was fished to depletion and subsequently dried up), indicated that the Coerney River barrier (Figure 23) had somehow been bypassed.

Figure 23: The causeway on the Coerney River presumed to be an upstream barrier to fish movement, particularly dispersal of *C. gariepinus* from the Sundays River into the upper Coerney River.

This prompted an investigation into the management of flows within the canals of SRIN, and the discovery of “reject canals” and “dump valves”. These canals and sluices are placed sporadically throughout the SRIN, and are utilised by the LSRWUA to periodically empty sections of the canals in order to perform maintenance. One of these valves is located at a spot where the main canal tunnels under the Coerney River, and empties directly into a pool on the stream, kept wet all year round presumably by return flows from the surrounding irrigated orchards (Figure 24). Located several kilometres upstream from the Coerney causeway barrier, this permanently inundated pool represents the most likely point of invasion for *C. gariepinus* into the Krom River.

Figure 24: LSRWUA water manager Tinus van Vuuren stands over the discharge pipe that allows Orange River water from the irrigation network to spill into the Coerney River, creating a point source for fish invasions.

3.5.2 Patterns of establishment within the SRIN

a) Propagules in the canal network

Sampling in the source of the irrigation network downstream of the Korhaansdrift offtake revealed significant fluctuations in the abundance of key fish species entering the network (Figure 25). *Glossogobius callidus* was the most abundant species entering the network, and displayed a large spike in larval fish immigration in November, where densities in the canal were almost an order of magnitude higher than any other fish species. The next most common species, *Gambusia affinis*, was most abundant in January, while *Gilchristella aestuaria* was most abundant in winter and spring (Figure 25). The remaining species were relatively scarce, occurring at very low densities either in the bongo nets or in the fyke nets (Table 11).

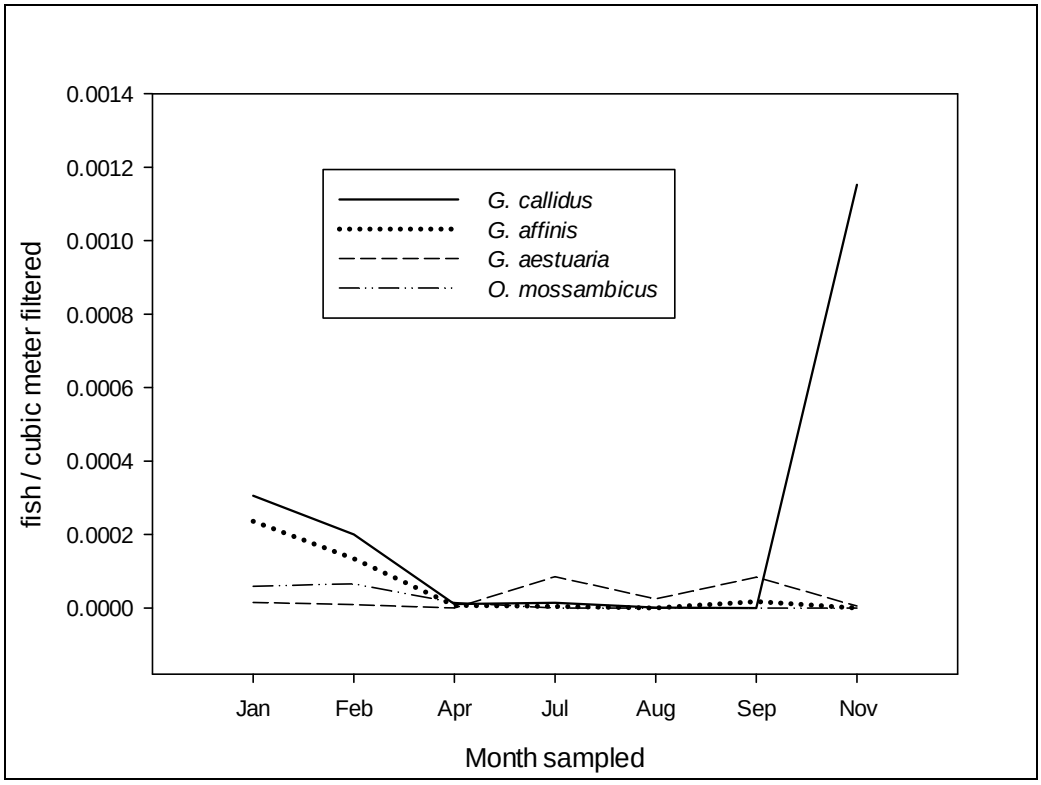

Figure 25: Seasonal densities of common fish species entering the irrigation network at Korhaansdrift.

Table 11: Catch per unit effort in WP2 drift nets and downstream fyke nets, set in the irrigation off-take canal.

Species	$P_{capture}$	Weighted mean density ($m^3 \times 10^6$)	St. dev	N	Mean size (mm)
Drift net data					
G. callidus	0.53	244.14	732.52	412	27
G. affinis	0.33	81.49	219.05	157	23
G. aestuaria	0.27	23.02	56.79	98	49
O. mossambicus	0.19	19.24	51.02	60	54
C. carpio	0.01	4.33	38.79	2	101
A. mossambica	0.01	0.80	10.21	1	102*
L. umbratus	0.01	0.46	5.83	1	139*
Fyke net data					
L. umbratus	0.14	1.77	7.33	52	169
O. mossambicus	0.03	0.21	1.16	8	178
C. gariepinus	0.02	0.13	1.03	5	314
L. capensis	0.01	0.03	0.38	1	273*
A. mossambica	0.02	0.10	0.84	7	489

Notes: Data are standardized to number of fish per cubic meter filtered by the net, and weighted according to the relative volumes recorded entering a monitored pond each month over 12 months. $P_{capture}$ = probability of capture, i.e. the probability that a sample contained a species; n = absolute number of individuals collected.

b) Patterns of establishment in the irrigation ponds

When mean $P_{capture}$ of fish species across all ponds was compared, four species grouped together with a relatively high mean $P_{capture}$, four species grouped together with a relatively low mean $P_{capture}$, and one (*C. carpio*) grouped with neither, having a mean $P_{capture}$ of 0.43 (Figure 26).

All species with high mean $P_{capture}$ had a $P_{capture}$ of 1 in the majority ponds older than 10 years, indicating that population establishment generally occurred in the first decade after pond filling. *Cyprinus carpio* establishment was inconsistent over time, while the remaining species all had low mean $P_{capture}$ that corresponded to random, low density incidence across the range of ponds (Woodford *et al.,* 2013).

In the four species where population establishment generally occurred, the exponential rate of establishment (*b* in equation (1)) varied from 1.11 y^{-1} in *G. callidus* to 0.41 y^{-1} in *G. aestuaria* (Figure 26). *Glossogobius callidus* established fastest, with $P_{capture}$ = 1 being attained in all but one pond older than three years. *Cyprinus carpio* in contrast established slowly at a rate of 0.07 y^{-1} (Figure 26) and was absent in many ponds older than 10 years. Propagule pressure was significantly higher for the four establishing species together with *C. carpio* when compared to that of the remaining non-establishing species captured at the off-take ($t = 4.04$, $df = 7$, $p < 0.005$). When the rate of population establishment based on pond survey $P_{capture}$ was plotted against propagule pressure for all species (Figure 26), the interspecific relationship closely fitted a saturation response curve ($F_{(2,7)} = 836.74$; $p < 0.0001$; adjusted $R^2 = 0.99$).

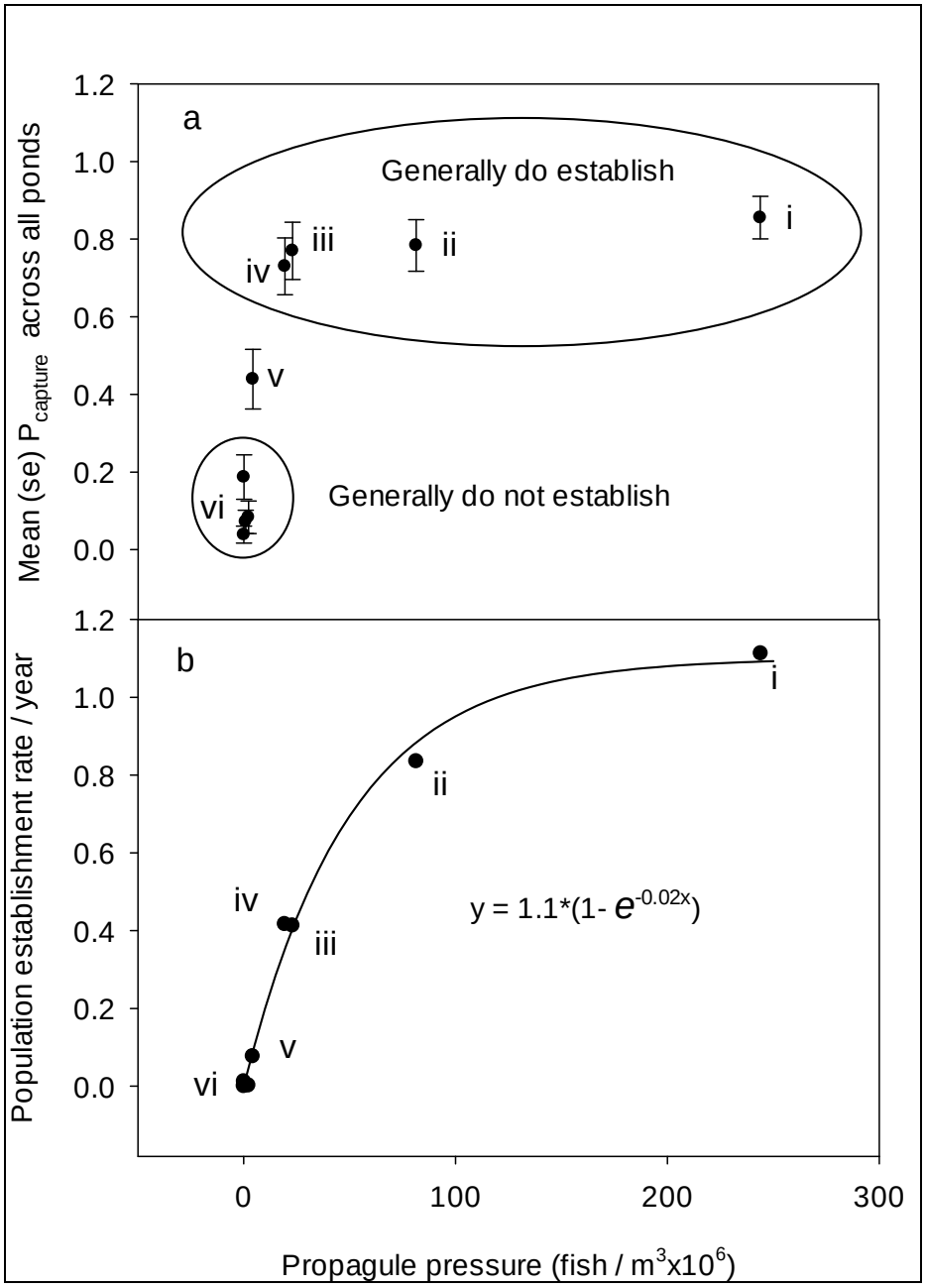

Figure 26: Relationships between propagule pressure at the network immigration source and a) mean $P_{capture}$ for each species across all surveyed ponds and b) rates of population establishment for each species.

Figure Notes: Differences in mean $P_{capture}$ illustrate two colonization types in the fish fauna: Those that regularly established populations in most ponds, and those that did not establish but were recorded incidentally. Four species, G. callidus (i), G. affinis (ii), G. aestuaria (iii) and O. mossambicus (iv), generally established. One species, C. carpio (v), had an intermediate $P_{capture}$, grouping neither with the establishing species nor the non-establishing species. The catadromous eel A. mossambica (vi) and three other species did not establish in any ponds. Figure reproduced from Woodford et al., 2013).

The measured temperature range in the study ponds (10-31°C) fell within the thermal tolerance of all the native species present in the system, as well as the introduced *O. mossambicus* (7-37°C), *G. affinis* (4-38°C), *C. carpio* (4-36°C) and *C. gariepinus* (8-35 °C) (De Moor and Bruton, 1988). Temperature was thus not a likely filtering factor affecting establishment success within the ponds. The species displaying successful establishment in the ponds mostly had reproductive strategies that did not require specific habitats to enable spawning. While *G. affinis* is a live bearer (Pyke, 2005), *G. aestuaria* is a pelagic spawning clupeid and *O. mossambicus* is a mouth-brooder (Weyl and Hecht, 1998). Although we suspect that *G. callidus* lays its eggs on coarse substrates, the larvae become pelagic upon hatching (Strydom and Neira, 2006). These spawning strategies indicate limited reliance on habitat suitability for reproductive success. In contrast, less successful fish like *C. carpio*, *C. gariepinus*, *L. umbratus* and *L. aeneus* all require substrate (vegetation or clean cobbles) for egg laying and post-hatching recruitment (Bruton, 1979; Crivelli, 1981; Potts *et al*., 2006; Weyl *et al*., 2009b). Thus, dependence on spawning substrate appears to act as a critical filter to establishment success within the ponds.

c) Drivers of establishment success and rate

The patterns of establishment for the majority of species surveyed in the ponds reflect a combined effect of propagule pressure originating from the irrigation network and the reproductive strategies of the individual species. Establishing species in the irrigation network received significantly more propagules than incidental species, and establishment rates displayed a saturation response curve to propagule pressure within the ponds (Figure 26). This response indicates not only that relative interspecific rates of establishment were driven by propagule size, but that the two fastest establishing species (*G. callidus* and *G. affinis*) received potentially thousands more colonists than necessary to maximize their establishment rate. These findings support the notion that on-going propagule pressure acts as a key driving force in overcoming the demographic and environmental resistance barriers that allow introduced fish to reach the establishment stage (Marchetti *et al.,* 2004). Furthermore, those species with life-history strategies that prevent population establishment in the ponds (*A. mossambica*, *L. aeneus*) are powerful indicators of the effect of continuous propagule pressure on range expansion without establishment. Every single species introduced from the Sundays River into the irrigation network was recorded downstream in the ponds, regardless of how scarce they were at the immigration source. In the context of the invasion framework of Blackburn *et al.,* 2011), every species ever transported along the SRIN reached the introduction stage within the environments of the ponds.

3.5.3 On-going invasions in the AENP

Surveys on the Krom River (upstream of the confluence with the Coerney River) revealed that *C. gariepinus*, which had previously been extirpated from the river by drought, had colonised at least half of the river flowing within the Addo Elephant National Park (Figure 27). This is of concern, in that there are no known barriers to upstream fish movement that separate the furthest upstream record of *C. gariepinus* with the upper distribution limit of the endangered *P. afer*. The survey results mean that catfish have the theoretical ability to occupy all *P. afer* habitat within the Krom River.

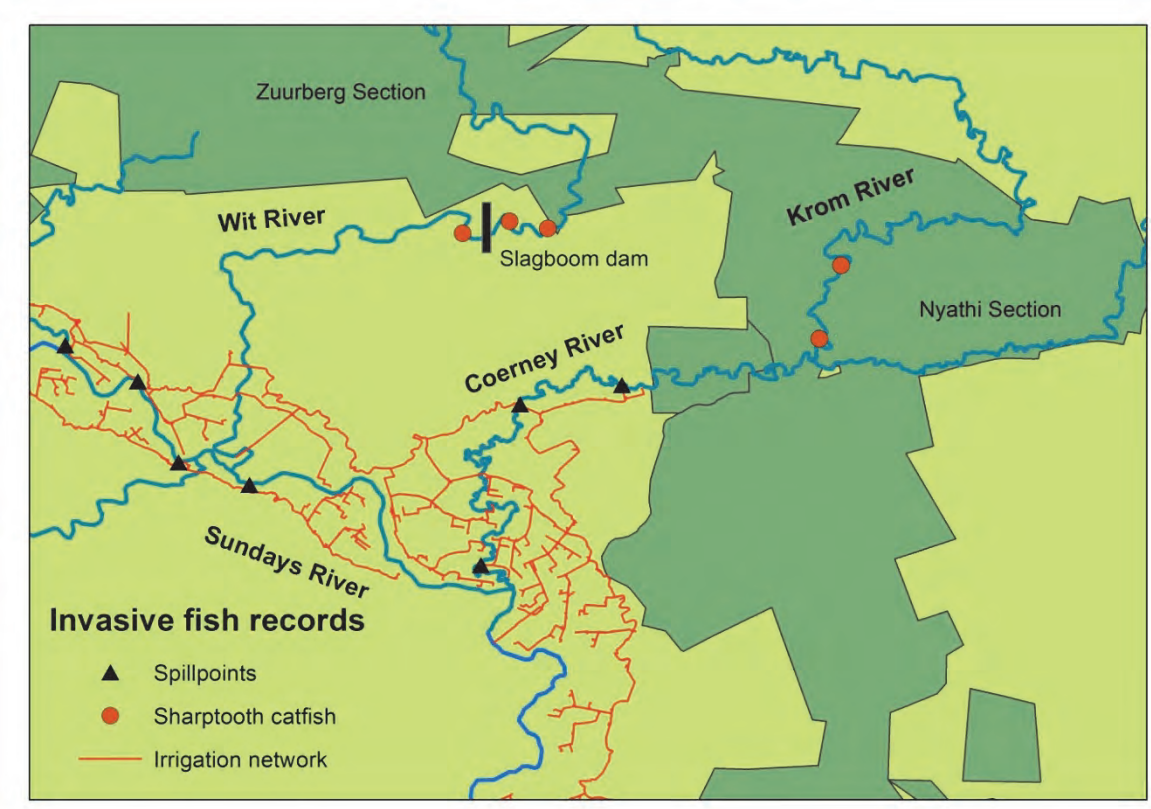

Figure 27: Map of the Sundays River catchment showing new records of introduced *C. gariepinus* in the Wit and Krom rivers.

Figure Notes: On the Wit River, C. gariepinus have been moved upstream of Slagboom Dam, making the entire upper catchment vulnerable to invasion. The two irrigation network spill-points located on the Coerney River are the most likely source of C. gariepinus invasions in the Coerney/Krom.

The native *L. umbratus*, which almost certainly also originated from the canal network, was also found well up the system and has successfully colonised the Krom River following the ending of the drought. It is unclear whether this species' presence here should be classed as extra-limital, or whether it would have historically migrated up the Krom from the Sundays River prior to the construction of the causeway barrier on the Coerney. What is clear is that

the water provided by the irrigation network, both through canal emptying and through return flows from orchards, has provided drought refugia in the lower Coerney River upstream of the causeway. These refugia apparently enabled *C. gariepinus*, *L. umbratus*, *G. callidus* and the Goldie barb *Barbus pallidus* to persist in the Coerney River during the drought and consequently to rapidly re-colonise the Krom River when the floodwaters returned.

On the Wit River, which does not receive water directly from the canal network but nonetheless flows into the Sundays River, which acts as a source of invasion from downstream, we detected range expansions of *C. gariepinus* relative to historical records. *C. gariepinus*, which had previously only been recorded downstream of Slagboom Dam (Figure 27), were found upstream of the reservoir in the upper Wit River. This range expansion appears to have been human-mediated, as catfish could not have climbed the wall unassisted. In this case, anglers associated with the South African Police Service training facility, which abuts the reservoir above Slagboom Dam, are the most likely vector responsible for *C. gariepinus* overcoming this barrier to upstream migration.

3.5.4 Management Recommendations

a) Current management options in the Sundays River valley

When Weyl *et al.,* 2008, 2009a, 2010) assessed the then-current status of alien and native fishes in the AENP, their determination was that the Sundays River main-stem was in many ways a lost cause for alien fish management. In particular, they pointed out that the Sundays River was so altered by flows of Orange-Fish-Sundays IBWT origin and its associated biota, that the main-stem could not be considered a conservation priority. In sharp contrast, the presence of threatened *P. afer* in all three major tributaries of the Sundays River, made these streams critical biodiversity conservation priority areas, where the further incursion of alien fishes was to be prevented if at all possible (Weyl *et al.,* 2010). These findings and recommendations remain valid following the breaking of the drought and the reconnection of the tributaries to the main-stem in 2011.

Today, the incursion of *C. gariepinus* upstream of Slagboom Dam on the Wit River and Gwarrie Pan on the Krom River are the most alarming developments post flooding, and require a management response by the AENP. At a minimum, on-going monitoring of *C. gariepinus* and *P. afer* densities and distributions are required to better characterise whether or not negative biotic interactions are occurring. Beyond assessment of the new situation, options for conservation interventions should be explored, in the event that *C. gariepinus* is found to threaten the conservation of these Redfin minnow populations, which are all genetically unique populations (Weyl *et al.,* 2010).

The recent successful eradication of *M. dolomieu* from the Rondegat River in the Western Cape (Weyl *et al.,* 2013) has provided a case study for how *C. gariepinus* could be managed in the Wit River, though there are several logistical challenges that make eradication extremely difficult. Firstly, it is unclear what the current upper limit of *C. gariepinus* on the Wit River is, and whether there are natural barriers upstream of this point that could mediate further incursion. There are three low causeways on the Wit River between the current know uppermost locality of *C. gariepinus* and Narina Camp, which currently do not act as effective barriers. One or more of these structures could however be upgraded to block upstream movement of *C. gariepinus*, if it is determined that the species has not already bypassed them. It is therefore imperative that intensive surveys using traps, gill nets and baited long-lines be conducted as soon as possible to establish with more certainty the new limits of invasion within the Wit River, so that the value of barrier upgrades can be assessed.

Hypothetically, should *C. gariepinus* be found only downstream of these causeways, there would be justification to pursue a programme of chemical eradication. This will however be a complex undertaking, particularly since Slagboom Dam and the river immediately upstream are on private land rather than the park, meaning AENP will need to negotiate with landowners on the location, construction and cost of a lower barrier near the inflow of the Slagboom Dam reservoir. It is thus up to the landowners and AENP to manage alien fishes in the river upstream of the reservoir, and work on permanent solutions that will restrict alien fish to this water body.

In the case of the Coerney and Krom rivers, the issue of restricting upstream penetration of *C. gariepinus* is even more problematic, as the species already co-occurs with redfins in much of the lower Krom River. This situation may change however if drought conditions return. In particular, if the Krom River reverts to its previous state of multiple disconnected pools, the distribution of *C. gariepinus* may become easier to ascertain and reduce through physical methods (fishing to depletion with gill, seine and fyke nets). The long-term prognosis for the river is troubling, however, as the Coerney River in particular is a very low gradient floodplain stream with a geomorphology that makes permanent barriers extremely difficult to construct within the borders of the AENP. Partial barriers outside the park fence, such as the Zuurberg Road (R355) crossing on the Coerney River, could be upgraded to prevent future migration of *C. gariepinus* into AENP, or a completely new weir could be constructed further downstream. Such actions would however only be appropriate if *C. gariepinus* were to first be successfully eradicated from the park upstream. Even then, barrier construction would need to be co-ordinated with landowners and provincial government (in the case of construction on the R355). Ultimately, the costs and benefits of

such an action would need to be weighed against the conservation threat posed by *C. gariepinus* within the Nyathi Section of the park, which at present remains unclear.

b) Hypothetical historical best practice solutions

The most striking insight gained through conducting research on the irrigation network has been how inevitable invasions become once an inter-basin transfer scheme becomes active. These canal networks act as invasion corridors, which provide multiple opportunities for species to be transported to the receiving river system (Wilson *et al.,* 2009). What is more disconcerting is that the international literature contains very few examples of methods preventing the transfer of fish along these corridors (Rahel, 2007). The only method that has shown any success is electric barriers, but these are extremely expensive, vulnerable to fluctuations in power supply, and ultimately may not be practical in the South African context.

In the case of the Sundays River, the invasion of the Coerney/Krom catchment by *C. gariepinus* is an excellent illustrative example. When the species was first introduced into the catchment via the IBWT at Darlington Dam in 1978, it was immediately able to move downstream until it occupied the entire Sundays River main-stem, where it established viable populations (Figure 28). The road causeway on the lower Coerney River (Figure 23) would have prevented the initial invasion of this tributary from the main-stem, if it were not for the SRIN.

At the same time that populations were establishing in the main-stem, the fish entering the irrigation network would have been deposited in any of the 300+ farm dams, where population establishment was generally rare. The failure to establish populations in the ponds appears to be driven by *C. gariepinus*' requirement for submerged aquatic vegetation on which to lay its eggs, which is rare in the majority of these irrigation dams. While flow is directed entirely into the dams over the majority of the year, once or twice a year the main canal is emptied via spillpoints (Figure 24, Figure 27) into the Sundays and Coerney Rivers, with two spillpoints on the Coerney (Figure 27) located upstream of the barrier causeway (Figure 23). This process allows the catfish to bypass the barrier and move up into the AENP, where they now co-occur with the threatened *P. afer*.

The only plausible solution that may have prevented the penetration of catfish into the national park would have been to place a second weir on the Coerney, upstream of the spillpoints (Figure 28), prior to the commissioning of the Fish-Sundays inter-basin link in 1978. This barrier would have ensured that only by direct human-mediated introductions into the Nyathi Section (a highly unlikely scenario once it was incorporated into the AENP), could this species have entered the Krom River. Unfortunately, once the first flows started

depositing excess water from the irrigation network into the lower Coerney River, the invasion of the national park was inevitable.

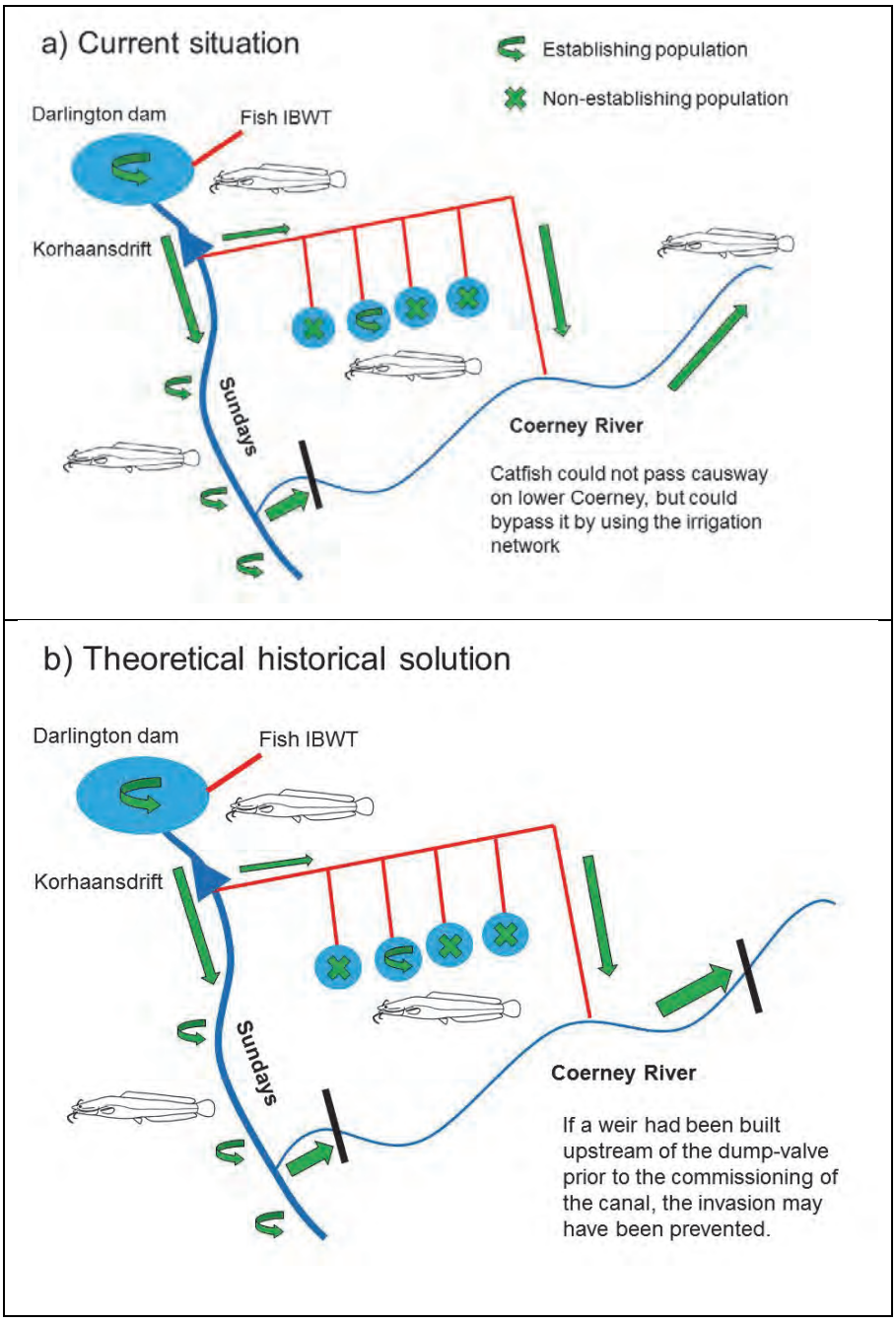

Figure 28: Schematic representation of the invasion of the Sunday River and irrigation network by *C. gariepinus*, showing a) the current situation including the invasion of the Coerney/Krom catchment, and b) a hypothetical historical scenario that might have prevented that invasion.

3.6 Conclusions

The Sundays River Irrigation Network has proven to be an excellent natural experiment for studying the patterns and processes of alien fish establishment, and for examining how fish use irrigation infrastructure to disperse over time. The ability of the irrigation network to

provide a continuous supply of propagules to the receiving environments downstream underscores the danger posed by IBWT schemes to fish conservation. The global implications of this introduction pathway are most troubling when compared to other pathways such as angler introductions and the pet trade. Whereas these pathways tend to be good for few, highly desired species used for ornamentation or recreation (Richardson *et al.,* 2003, Duggan *et al.,* 2006), IBWTs represent a pathway that is indiscriminate, providing continuous propagule pressure for any species moving from the donor catchment to the receiving catchment. In this way, undesirable species can arrive in receiving catchments, and without any further human intervention will establish and eventually invade if they possess appropriate life-history strategies. IBWTs effectively break down the biogeographic barriers between entire fish communities, making them a powerful driver of landscape-scale fish community homogenization (Rahel, 2007).

In the case of this particular irrigation network, there appear to be few real options for future remedial action to prevent new invasions. All species originally imported from the Orange River to the Sundays River by the IBWT are now established or at least present in the main-stem, and many are continuing to penetrate up into sensitive tributaries with minimal human intervention. The role of the irrigation network as a pathway for invasions is no longer as relevant today as it was in the early years of the irrigation scheme. Rather, the main role the irrigation infrastructure plays today, particularly in mediating catfish invasions, is through the provision of drought refugia. An extended drought that lasted from 2006 to 2010 resulted in much of the Coerney/Krom River system drying up. Only return flows from the orchards fed by the irrigation network allowed permanent pools to persist in the lower Coerney River, which meant that even after catfish were extirpated from the AENP by the drought, they were able to rapidly recolonize when flows returned in the winter of 2011. These synergies between different mechanisms controlling habitat availability and invasion pathways need long-term monitoring to be properly understood, and demonstrate that systems as complex as the Sundays cannot be described using once-off “snapshot” surveys.

As South Africa becomes an increasingly water-stressed country, the pressure to construct new water transfer schemes to supply water-poor areas from water-rich areas is likely to increase. Our findings suggest that the implications of such schemes for fish introductions need to be considered from the outset and measures to mediate these impacts be designed into the scheme before they are made operational to have a positive effect on aquatic conservation values.

4 OTHER CASE STUDIES: DETERMINING THE THREAT AND IMPLEMENTING REHABILITATION

OLF Weyl, BR Ellender, DJ Woodford

4.1 The Nseleni River: Assessing Africa's first armoured catfish invasion

The South American armoured catfish family Loricariidae includes more than 700 nominal species. Some are popular aquarium fishes in the global pet trade. Releases by aquarists and escape from fish farms have resulted in several armoured catfish invasions in Central and North America, Asia and now, Africa. Armoured catfishes were first reported from a lake near the town of Empangeni in 2000. This was followed by the formal collection of specimens from the Mthlathuse River in 2004 and from the adjacent Nseleni River system in 2007. This indicated the need for a fuller assessment because documented impacts of armoured catfish invasions include destabilisation of river banks by their breeding burrows. The subsequent collaboration between the South African Institute for Aquatic Biodiversity, Ezemvelo KZN Wildlife and Rhodes University, and research paper "*Using a unified invasion framework to characterize Africa's first loricariid catfish invasion*" (Jones *et al.*, 2013), is an excellent example of how fish invasions should be characterised.

An important step in developing alien invasive species management strategies is determining their extent and traits. A unified framework such as that proposed by Blackburn *et al.,* 2011), which combines previous stage-based and barrier models, and provides a terminology and categorisation for populations at different points in the invasion process, was therefore applied. According to the framework, the invasion process can be divided into a series of four stages (transport, introduction, establishment and spread). Each of these stages is confounded by barriers (geography, captivity, survival, reproduction, dispersal and environmental) that need to be overcome before passing on to the next invasion stage. Blackburn *et al.,* 2011) also suggest management actions for each invasion stage. These include preventing introductions, containment once introduced, mitigating spread once established in the wild and eradication when feasible. The aim of the current study was to assess the loricariid population in the Nseleni River and use the Blackburn *et al.,* 2011) unified framework to determine invasion stage and to identify appropriate management actions (Table 12).

Table 12: Categorisation of the *P. disjunctivus* invasion in South Africa using the Blackburn et al., 2011) unified framework.

Stage	Category	Management action	*P. disjunctivus* in South Africa
Transport	A. Not transported beyond limits of native range.	Prevention	Native to South America, Loricariid catfishes have been introduced into South Africa via the pet trade since at least since the late 1970s.
GEOGRAHICAL BARRIER			
Introduction	B1. Individuals transported beyond limits of native range and explicit measures of containment are in place.	Prevention and/or eradication	Wild specimens identified as *P. disjunctivus*, a native to the Madeira River drainage of the Amazon basin in Brazil and Bolivia and invasive in Mexico, the USA, the Philippines and Taiwan.
Introduction	B2. Individuals transported beyond limits of native range but measures to prevent dispersal are limited at best.		
CAPTIVITY BARRIER			
Introduction	B3. Individuals transported beyond limits of native range, and directly released into the novel environment.	Prevention and/or eradication	First reported from Lake Mpangeni in the year 2000. Introduction into the wild was either intentional release by aquarists of unwanted pets or by escape from an urban pond.
Introduction	C0. Individuals released into the wild in the location where introduced but are incapable of surviving for a significant period.	Eradication	
SURVIVAL BARRIER			
Establishment	C1. Individuals surviving in the wild in the location	Containment and/or	Interviews with fishers in 2011 indicated continued survival in

Stage	Category	Management action	*P. disjunctivus* in South Africa
	where introduced but no reproduction.	eradication	Lake Mpangeni.
Establishment	C2. Individuals surviving in the wild in location where introduced, reproduction occurring, but population is not self-sustaining		
REPRODUCTION BARRIER			
Establishment	C3. Individuals surviving in the wild in location where introduced, reproduction occurring, and the population is self-sustaining.	Containment and/or eradication	In 2004, two juvenile specimens were collected from the Mhlathuze River downstream of Lake Mpangeni.
DISPERSAL BARRIER			
Spread	D1. Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction	Mitigation and/or eradication	The collection of an adult specimen from the Nseleni River in 2007 demonstrated spread using an inter basin water transfer to breach the dispersal barrier.
Spread	D2. Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction.		Juvenile and spawning capable adult fish are present in the Nseleni River population.
ENVIRONMENTAL BARRIER			
Spread	E. Fully invasive species,	Mitigation and/or	Further dispersal is likely if

Stage	Category	Management action	*P. disjunctivus* in South Africa
	with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence.	eradication	natural dispersal barriers between catchments are breached.

Notes: The invasion process is divided into four stages, each of which is confounded by barriers that need to be overcome before passing onto the next invasion stage. Invasions are categorised (A-E) according to criteria within each invasion stage and management actions for each invasion stage are suggested.

To assess the status of the population fish surveys using valve traps, fyke nets, gill nets, electrofishing and habitat traps were conducted. The research team also interviewed local community members who were encouraged to report and donate specimens. The information from interviews and specimens collected during surveys and from donations were then used to identify the invading species and its invasion stage according to unified invasion framework criteria (see Blackburn *et al.,* 2011).

Over two years of surveys, we collected 346 specimens including 12 mm-long juveniles, and adults measuring almost 50 cm. Identification required both taxonomic keys and DNA barcoding because selection in the pet trade has resulted in invading populations exhibiting a wide variety of colour patterns that are not found in natural populations. Specimens were identified as Vermiculated sailfin *P. disjunctivus* (Weber, 1991), a native to the Madeira River drainage of the Amazon basin in Brazil and Bolivia and the main nuisance species in Mexico, USA, Philippines and Taiwan.

Interestingly, *P. disjunctivus* is not on South Africa's draft list of permitted fish species under the National Environmental Management: Biodiversity Act (Act 10 of 2004), and is likely that during import it was identified as a closely related permitted species. Accidental or intentional misidentifications are common in the pet trade and often result in the importation of invasive organisms. On escape from captivity, this fish established first in the Mthlathuse River and then spread to the Nseleni River via an inter-basin water transfer (Figure 29). The researchers characterised the invasion as D2: "*self-sustaining population in the wild with individuals surviving and reproducing a significant distance from their original point of introduction*". Containing this invasion will require educating the public on the dangers of fish introductions and discouraging the trade in loricariid species with proven invasion histories

and their look-alike species. In addition, the assessment conducted on the Nseleni River resulted in the inclusion of *P. disjunctivus* in the NEMBA: Alien Species regulations published in 2014.

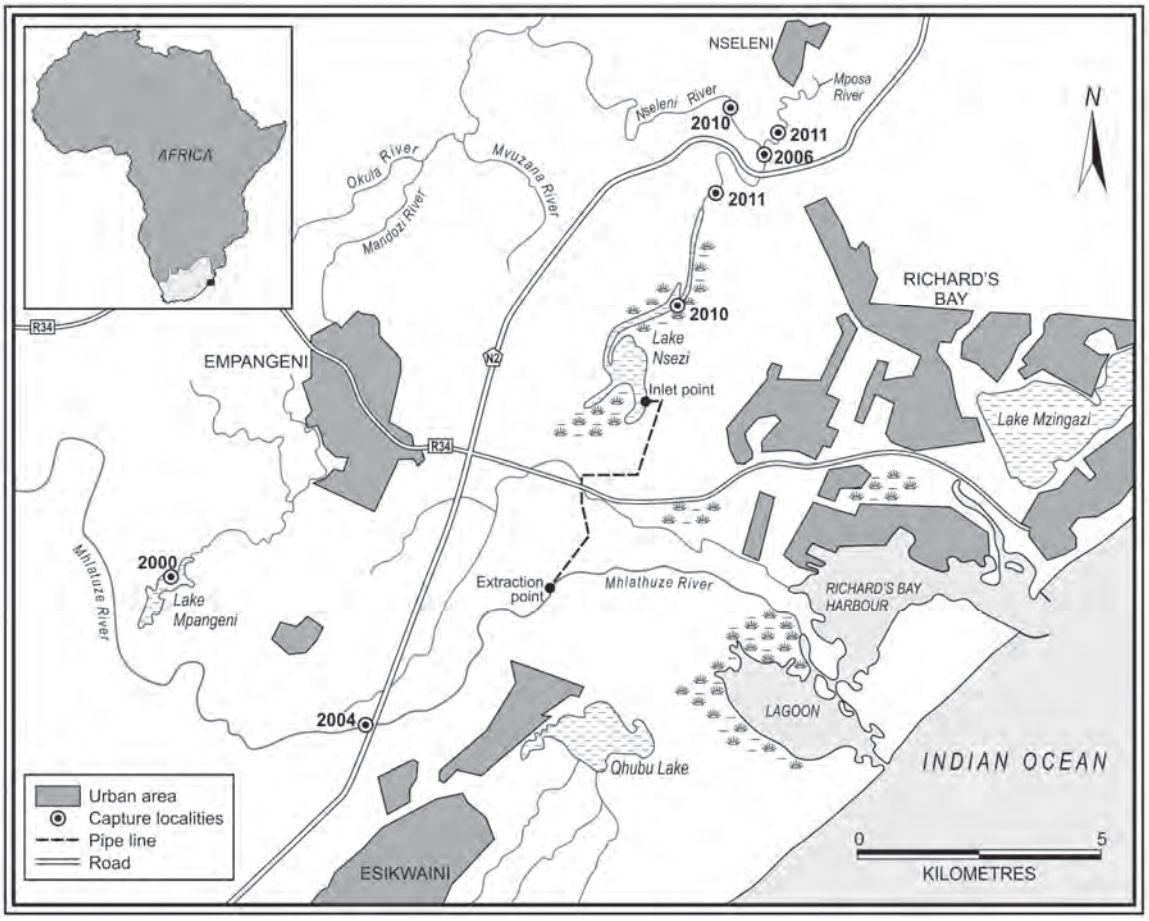

Figure 29: Invasion history of P. disjunctivus in the Richards Bay area of KwaZulu-Natal, South Africa. Dates next to capture localities indicate first records (after Jones et al., 2013).

4.2 The Swartkops River: Invasion of the Blindekloof stream

Due to connectivity of the headwater streams to mainstream environments and dams, any non-native fish introduced into these environments provides a potential threat for invasion of these headwater streams which often provide refugia for range restricted imperilled native fishes. As a result of intentional stocking and illegal introductions, the mainstream Swartkops River has been invaded by at least five non-native species. In a previous study undertaken on the Blindekloof stream, a Swartkops River headwater tributary in 1989, *M. salmoides* were the only non-native fish recorded and were subsequently eradicated from the stream. Due to 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 likely a result of upstream invasions from the mainstream Swartkops River. The Blindekloof stream therefore provided a unique

opportunity to investigate whether *M. salmoides* would reinvade after its removal in 1989 and if the more recently introduced non-native fishes would invade headwater streams. Sixteen sampling sites, from the Blindekloof/ Swartkops confluence area 9 km upstream, were surveyed by snorkelling, electrofishing and a visual assessment (Figure 30).

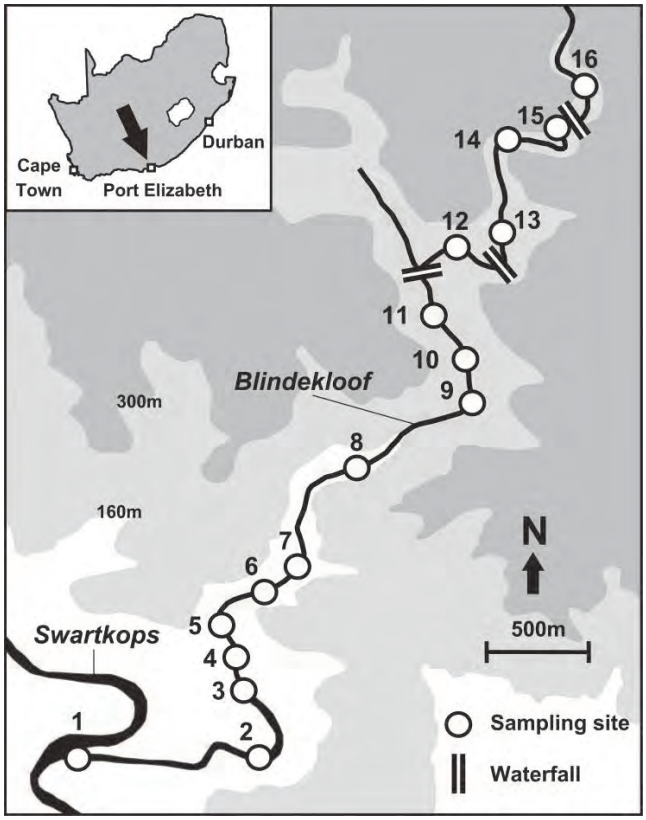

Figure 30: The location of the Blindekloof stream and the position of the sampling sites from site 1 at the Swartkops/Blindekloof confluence to site 16 in the upper reaches of the Blindekloof stream.

Of five non-native fishes in the mainstream four (*M. salmoides*, *M. dolomieu*, *T. sparrmanii* and *C. gariepinus* had invaded the Blindekloof stream and only *T. sparrmanii* had successfully established. Imperilled native fishes (*P. afer*, Cape kurper *Sandelia capensis*, Goldie barbs *Barbus pallidus* and the River goby *Glossogobius callidus*) were limited to sites where *M. salmoides* and *M. dolomieu* were absent (Figure 31). The findings from this study indicate that invasion success in headwater streams is species specific, the time period for successful establishment in the Blindekloof stream after initial introduction was both variable and probably governed by the habitat requirements and the biological characteristics of the non-native fish species. Indications are that where present, non-native predators extirpate all native fishes, however, this requires further investigation.

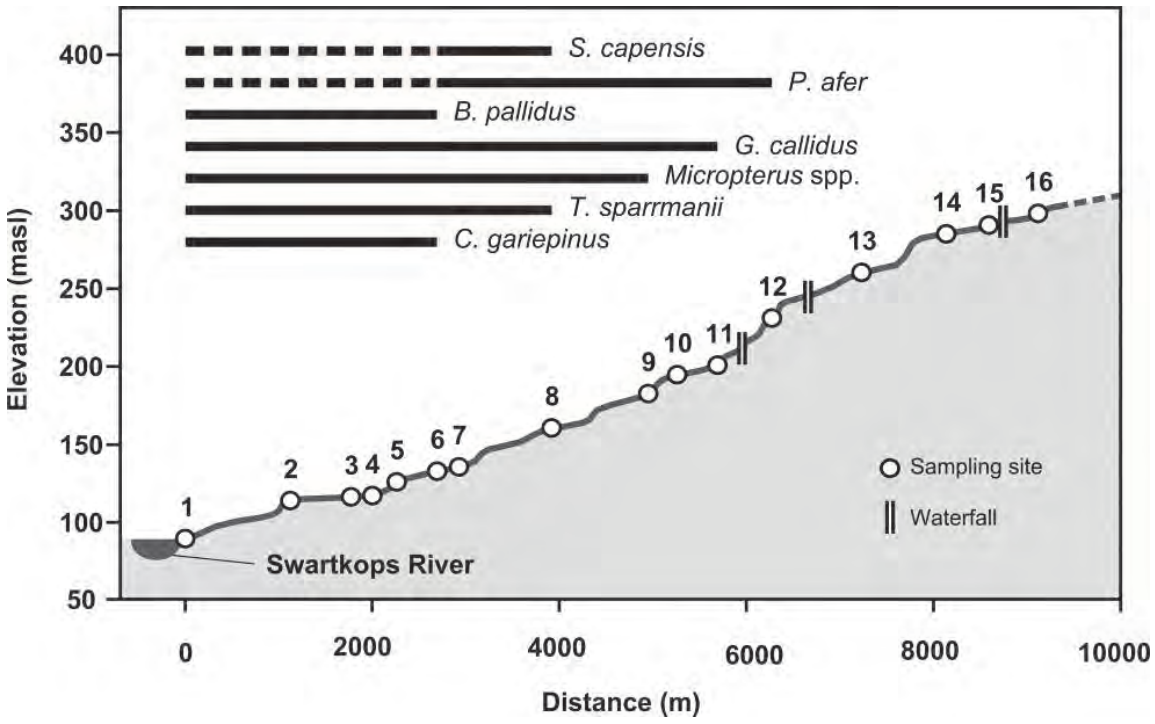

Figure 31: The distribution of the native and non-native fishes in the Blindekloof stream in relation to the stream gradient and natural barriers (solid bar indicates where a species is present and the dotted bar indicates where a species has been extirpated).

4.3 The Rondegat River

In February 2012 CapeNature rehabilitated the Rondegat River, a small perennial tributary of the Olifants River in the north-western Cape Floristic region by removing *M. dolomieu* using the piscicide rotenone (Woodford *et al*., 2013). The research and treatment of this river are well documented (see Marr *et al*., 2012, Weyl *et al*., 2013, Weyl *et al.,* 2014). The Rondegat is recognised as one of South Africa's Freshwater Ecosystem Priority Areas and contains a conservation-worthy assemblage of threatened fish species and regionally endemic freshwater fishes (Impson *et al*., 2013). The lower reaches of the river from the dam up to a waterfall barrier had been invaded by *M. dolomieu*, a predatory fish that extirpated two species of redfin as well as the Clanwilliam rock catfish (Figure 32). The rotenone treatment was preceded by an environmental impact assessment (EIA) to verify that the rehabilitation projects was justified, that the Rondegat River should be the first pilot, and that the choice of rotenone was appropriate (Marr *et al*., 2012).

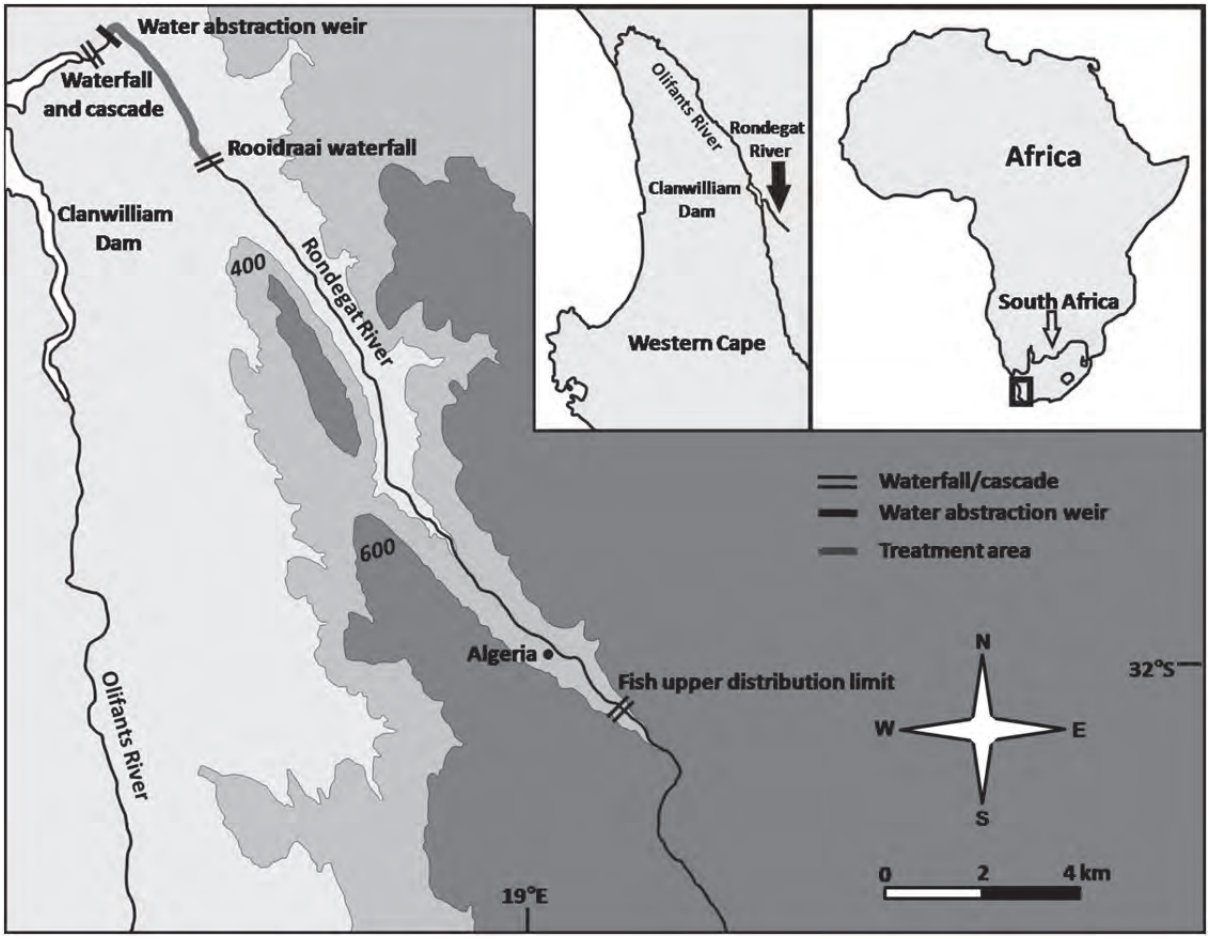

Figure 32: Rondegat River treatment area.

Following the EIA, several organisations collaborated to initiate the Rondegat pilot project (for review see Weyl *et al.,* 2014). Funding was allocated from the Department of Environmental Affair's Working for Water Programme to simultaneously fund alien plant control and fish eradication, and the Citrusdal Irrigation Board was appointed to manage the plant control operation. The provincial conservation authority (CapeNature) took responsibility for the fish eradication. The South African Institute for Aquatic Biodiversity carried out pre- and post-treatment monitoring of aquatic fauna with funding from the Water Research Commission. CapeNature also focused on raising awareness by organising meetings with riparian landowners and water users, and by providing regular progress reports to stakeholders (Impson *et al*., 2013). The project employed international best practice in piscicide treatments, and was further guided by on-site advice from experts from the USA and Norway and the target area was first treated on 28 February 2012 by dispensing rotenone at seven treatment stations.

Eight backpack sprayers treated side channels and pools. At the end of the treatment area, immediately downstream of the weir, rotenone was de-activated using potassium permanganate. During the 2012 rotenone operation, 470 smallmouth bass and 139

Clanwilliam yellowfish were collected. According to standard operating procedure, a second treatment was conducted on 13 March 2013, and no further bass were collected, indicating that the prime objective had been met (Figure 33). During the 2013 treatment, ~3000 young-of-year (<10 cm) native fishes were collected from the treatment area, including Clanwilliam yellowfish, fiery and Clanwilliam redfins and Clanwilliam rock catlets (Weyl *et al*., 2014). These fish were absent from the treatment area prior to bass removal and their presence 1 year later suggests that a large number of native fishes were previously consumed by bass, and also that native fishes are likely to rapidly recolonise areas where bass are eradicated. Preliminary results of monitoring the aquatic invertebrate community response to the rotenone treatment indicated that invertebrate biomass and diversity is also recovering rapidly after treatment (Woodford *et al.,* 2013).

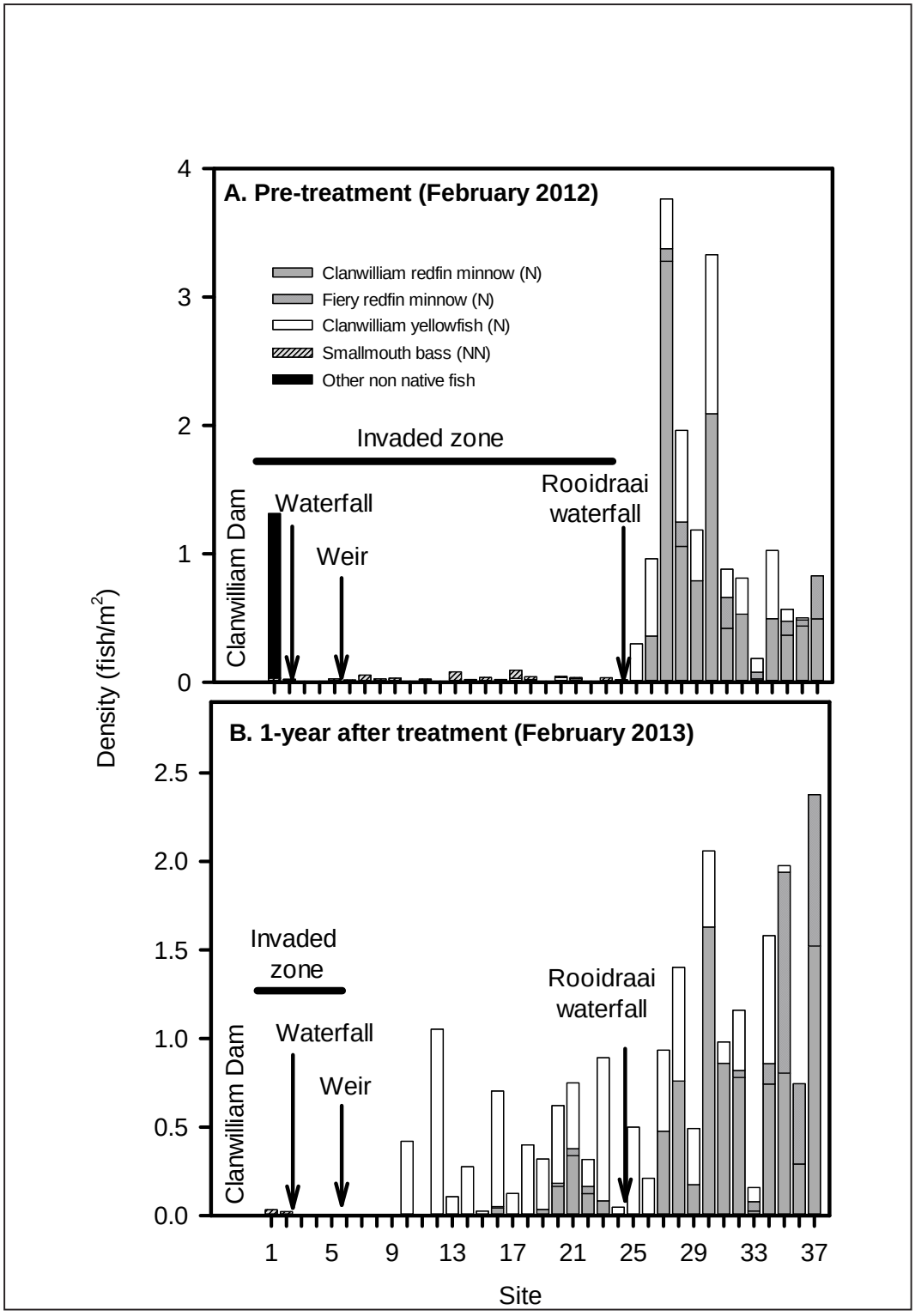

Figure 33: Fish density in invaded and non-invaded survey sites of the Rondegat River determined from snorkel surveys conducted in (A) February 2012 immediately before the rotenone treatment and (B) February 2013 one year after the first treatment.

Figure Notes: Potential barriers to upstream migration of M. dolomieu are the lower waterfall, weir and the Rooidraai waterfall. N = native; NN = non-native.

5 DEVELOPING A DECISION SUPPORT TOOL FOR MANAGING INVASIVE FISH IN SOUTH AFRICA

DJ Woodford, OLF Weyl, PK Kimberg

5.1 General Principals for managing invasive fish

5.1.1 Movement and establishment of fish

The review by Van Rensberg *et al.,* 2011) identified several causes for the introduction of freshwater fish species into South Africa, including angling, aquaculture, biocontrol and the pet trade. Today, the pet trade is the single largest vector for new introductions into South Africa, with new records continuing to appear. The most recent confirmed introduction was the Giant pangasius catfish *Pangasius sanitwongsei* (Mäkinen, 2013), which was demonstrated through genetic barcoding analysis to have arrived in the country via the pet trade. Movement of species already established within South African continue into new catchments through active introductions (generally illegal stockings by anglers) and passive transport through inter-basin water transfer schemes (Woodford *et al.,* 2013).

The regulation of the aquarium trade is a highly complex subject, which is hampered by the large species pool being actively dispersed in the global market. A significant impediment to successful management is the fact that many species are inconsistently or incorrectly labelled when imported into South Africa, rendering the exclusion of potential problem species or those listed as prohibited extremely difficult (Mäkinen, 2013). The movement of fish into new catchments by anglers is somewhat simpler to regulate, and is covered by the proposed NEM:BA invasive alien species regulations. All recognised invasive sport fish species are categorised as either 1b or 2, both of which prohibit the stocking of fish in catchments where they have not been documented to occur. Of course, enforcement of such regulations will depend strongly on the co-operation of organised angling bodies, such as the Federation of South African Flyfishers (FOSA), the South African Bass Angling Association (SABAA), and the South African Freshwater Bank Angling Federation (SAFBAF) as the Department of Environmental Affairs will have limited capacity for local enforcement of the regulations, and will depend heavily on peer reporting and enforcement for implementation to be effective.

The regulation of passive transport through water transfer infrastructure is even harder, as the responsible human entities are either government departments or water user associations who's mandate it is to ensure the pathways of invasion (water canals) remain open and do not disrupt the supply of water to their constituents (Koehn and

Mackenzie, 2004). It is therefore difficult to envision a scenario in which these entities willingly take on the expense of modifying their infrastructure to mitigate against the transport of fish between catchments. This point is however rendered moot by the fact that in most cases within South Africa, the homogenisation of the fish communities linked by the inter-basin transfer scheme has already been completed, and the impacts practically irreversible.

5.1.2 Mitigating against interspecific impacts

Five stages of biological invasion can be identified namely: pre-introduction, introduction, naturalization, expansion and saturation (Clout & Williams, 2009). Generally eradication of an invasive species is only possible prior to naturalization (Clout & Williams, 2009). Once a species has become established the potential for eradication diminishes with the exception of small isolated populations. Naturalization refers to the point where an organisms is self-sustaining and reproducing consistently (Clout & Williams, 2009). When eradication of an invasive species becomes unfeasible, containment and control become the alternatives (Clout & Williams, 2009). In some cases invasive species have socio-economic value which will preclude eradication as an option. Examples include the bass and trout fishing or timber industries in South Africa.

Containment refers to keeping an invasive species under control or within a previously defined geographically confined area whereas control refers to the management of the impact of an invasive species without necessarily trying to limit its range. In some cases both measures might be required. In the context of the *M. salmoides* invasion in the Groot Marico catchment, eradication from the entire catchment may be classified as unfeasible due to the extent of the invaded habitat and the state of the *M. salmoides* invasion. The species is well established, reproducing and has reached saturation, i.e. it appears to occupy all reaches downstream of barriers to further spread. Furthermore complete eradication of the species from the catchment would have serious socio-economic impacts, as Marico Bosveld Dam in particular is a popular bass fishing destination.

Although *M. salmoides* appears to have had a limited impact on the overall fish community in the Groot Marico catchment, the sensitivity and conservation importance of *B. motebensis* necessitates the use of some measures of containment and control. Containment measures could include targeted eradications from key localities of conservation concern, such as the *M. salmoides* population in the Marico Eye, which is directly upstream of Kaaloog se Loop. Although *M. salmoides* has so far failed to penetrate this tributary naturally, the proximity of the eye provides an easy opportunity for future human-mediated spread. Even a few individuals occupying the pools in this tributary and would likely severely deplete the resident

B. motebensis population, making complete elimination of *M. salmoides* from this tributary catchment desirable.

Another containment measure would consist of ensuring that property owners in the upper catchment do not introduce *M. salmoides* into dams that could potentially spill into the tributaries. This principle is expressly stated in the NEM:BA invasive species regulations, where all category 1b species, including *M. salmoides*, may not be introduced into water bodies where they do not already occur (RSA 2014). Nonetheless, an education campaign to inform local landowners and stakeholders of threat posed by bass to the receiving environment would greatly aid local stakeholder buy-in to the regulations, and ultimately their enforcement. Apart from containment, active control measures such as targeting the abundance of bass might also be considered in order to limit the potential impact of *M. salmoides* on indigenous fish communities. Control measures may include angling pressure and harvesting of *M. salmoides* during specific times of the year such as the start of the spawning season (usually October-September). Again, education of the local population would reduce the likelihood of further illegal introductions of *M. salmoides* being made into invaded reaches of the catchment.

5.1.3 Features of a successful eradication programme

The Rondegat River rehabilitation programme provides several important examples of the kinds of challenges facing managers who seek to eradicate specific populations of invasive fishes, as well as best practice methods that should be employed. In the case of the Rondegat, the main challenge was in setting a precedent for operations of this kind, which had never before been attempted in South Africa. The project was, in the years leading up to implementation, highly controversial among certain sectors of the recreational angling community, as it was seen as a threat to established fisheries based on alien species (Marr *et al.,* 2012). As a result, several angling groups, particularly trout fishermen, whose sport is centred on small mountain streams like the Rondegat, led efforts to challenge the initiative in the media (Marr *et al.,* 2012, Weyl *et al.,* 2014). CapeNature, the implementing organisation, was also challenged to find funding for the operation, as piscicide operations were not part of their core funding. CapeNature solved both these problems by engaging in a full environmental impact assessment, led by independent consultants, to determine the feasibility and desirability of using rotenone for river rehabilitation. The successful conclusion of the EIA unlocked funding from the World Bank's Global Environment Facility, and convinced concerned stakeholders such as angling groups to support the operation (Weyl *et al.,* 2014).

This process also set a precedent for independent assessment of state-run eradication programmes, as apart from the initial EIA, pre- and post-treatment monitoring was carried out by a research team from the South African Institute for Aquatic Biodiversity, using funding independently awarded by the Water Research Commission (projects K8-922 and K5-2261). The independently funded, independently administered monitoring programme adhered to international best practice, and assured that the positive results of the programme would be incontrovertible. An added bonus of this approach was that the scientists involved in the monitoring could raise the profile of the successful operation by publishing the results of the monitoring in the international scientific literature (see Woodford *et al.,* 2013, Weyl *et al.,* 2014).

One of the reasons that the EIA found the Rondegat River to be an appropriate subject for what was in essence a pilot programme to assess whether rotenone could be used in South Africa was that it presented very few logistical challenges. The section of river targeted for rotenone operations was only 4 km long, featured relatively easy bank-side access throughout, and was small (less than 1 m^3/s discharge in summer) meaning relatively little rotenone was needed for a successful operation. Another critical aspect of the stream was the existence of permanent barriers to upstream movement for fish. The treatment reach on the Rondegat was bounded downstream by two separate barriers, a concrete weir and a bedrock cascade, which ensured that bass would not be able to re-invade the treated reach without human assistance. When assessing alien fish management options, eradication should only be considered if there is a negligible likelihood of the alien species naturally re-colonising the invaded reach. The existence of physical barriers to upstream movement are thus a fundamental prerequisite for eradication operations, and if a barrier downstream of the targeted stream reach does not exist, it will need to be created prior to operations beginning. In the case of the Rondegat, the existing downstream barrier was a concrete weir that was damaged on one side and did not represent a complete barrier under all flow conditions. CapeNature were thus obligated to repair the weir with additional basket gabion structures as part of the operation, which added significantly to the overall operating budget. When choosing a stream in which to attempt eradication, the requirement for additional or upgraded barriers will need to be factored into the overall budget of the implementing entity.

The installation of artificial barriers to upstream movement raises the question as to whether the creation of such a structure will have negative impacts on the native species present in the river. Species with a marine component to their life history (diadromous fishes) often require migration from estuaries to headwater streams to complete their life cycles, and can be excluded from a river if barriers to migration occur too close to the estuary (Rolls, 2011). Even non-migratory species occurring upstream of barriers can be threatened by those

barriers, which could restrict gene flow and increase risk to stochastic extinction events if the population is too small (Fausch *et al.,* 2009). The decision to create a barrier to enable alien fish eradication must thus be weighed against the need of the native fish to access the river downstream of the barrier and to link with conspecific populations in other parts of the catchment. In the case of the Rondegat, these issues were not a concern, as the only diadromous species known to inhabit mountain streams in South Africa, *A. mossambica*, does not occur in the Olifants-Doring catchment where the Rondegat River is situated (Skelton, 2001). Furthermore, the vulnerable redfin minnows and Rock catfish that did inhabit the upper Rondegat River had been entirely extirpated by the bass downstream, while the population of yellowfish occurring below the barrier was considered a sink population that depended on the upstream population to persist (Woodford *et al.,* 2005). The weir could thus be upgraded to be a total barrier to migration and still improve the conservation of the fish species upstream.

Another critical lesson learned through the implementation of the Rondegat rehabilitation was the way to effectively build capacity for a sustainable capability within the implementing agency. In the years leading up to the operation the leader of the implementation, Dean Impson of CapeNature, attended a training course in the United States on the safe and effective use of rotenone for fisheries management, and later arranged for the leader of that course, American Fisheries Society fish management chemicals subcommittee member Brian Finlayson, to travel to South Africa and assist in training the CapeNature staff involved in the operation. The utilisation of international expertise again ensured international best practice, with the AFS Rotenone Standard Operating Procedures Manual (Finlayson *et al.,* 2010) being used to guide the safety procedures, minimum effective dosage, and due diligence to confirm success (e.g. use of sentinel fish, follow-up treatment in 2013). All these actions improved the chances of success of the operation.

An added bonus of this procedure, was that CapeNature was able to create a dedicated team trained in rotenone operations within their organisation, which is now available to participate in future fish eradications with significantly lower costs (e.g. person-hours dedicated to training) than those associated with the Rondegat rehabilitation. If alien fish eradication is to be attempted in other parts of the country, the responsible managing agency will need to invest in similar levels of training and capacity building during the first operation, creating competent teams that can increase their productivity and efficiency over time.

Finally, it is critical to re-emphasise the importance of community buy-in regarding eradication operations. The on-going debate around the proposed NEM:BA alien species

regulations, where certain stakeholder groups are vigorously opposing the legislation that will enable active management of invasive fish populations, highlights the danger of conservation agencies attempting to manage these species in a socio-economic vacuum. As articulated in the Decision Support Framework below, only alien fish populations with little or no socio-economic impact will be appropriate targets for eradication. The Rondegat provides a wonderful example of this, in that while the adjacent population of smallmouth bass in Clanwilliam Dam is one of the most economically important populations of this species in the country, the population in the Rondegat River had no fishery value at all, and thus no users who would object to their removal. Nonetheless, the threat of disgruntled members of the public taking it upon themselves to illegally re-introduce an eradicated species to a rehabilitated stream is far too serious to be ignored by conservation managers.

5.2 The decision support tree

5.2.1 Description and explanation of the Decision Support Framework

The current proposed alien species regulations under NEM:BA are crafted for each listed invasive species to take it's known invasiveness and socio-economic value into account (RSA 2013 and amended in 2014). Invasive fishes are listed as either Category 1b or 2, the latter category referring to species whose utilisation and transport are controlled by permit. Category 1b species may not be transported under any circumstances, and must be controlled under a management plan in conservation priority areas. There is, however, no immediate obligation on government agencies or landowners to control or eradicate Category 1b species *in situ*, but rather an obligation to prevent the expansion of the species outside its current recorded range. Nonetheless, the landowner or the relevant government custodian of the invaded water body do have the authority to control or remove Category 1b species from the area provided an Invasive Species Management Programme is first developed for the species within the area in question. While each invaded area will present unique management challenges, any management programme should be guided by an overall prioritisation strategy that can balance conservation risk and logistical feasibility to determine the most appropriate management intervention for a given species in a given area.

The summarised options available to managers of invasive species produced by the IUCN Global Invasive Species Programme (Wittenberg & Cock, 2001) provides a fundamental decision tree for all alien species management decisions. By adapting these options to include aspects of invasion pathways, actions, and priorities unique to invasive fish already occurring within South African water bodies, we arrived at an umbrella decision support

framework that can in turn be adapted to various scenarios involving Category 1b invasive fishes (Figure 34).

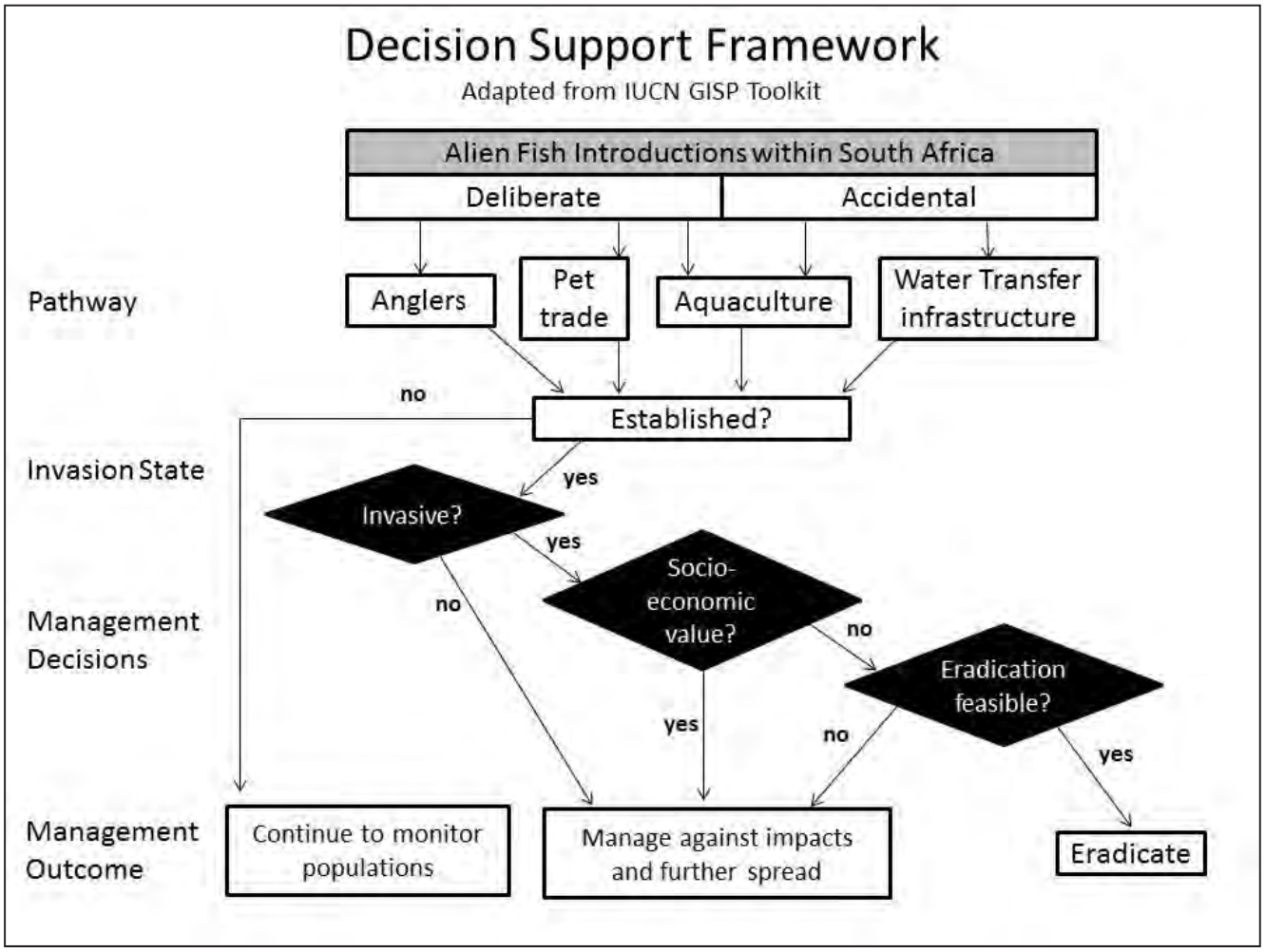

Figure 34: Umbrella decision support framework for managing alien freshwater fish species within South Africa.

The decision support framework can be used to assess:

1) The invasion status of the species within a given area, and

2) The preferred management outcome for the species, which can then be codified in an area- and species-specific Invasive Species Management Programme.

This decision tree prioritises the order in which management questions are asked, in order to arrive at a feasible management strategy for a single species in a geographically restricted area. Criteria by which managers should evaluate the answer to each question are presented in Table 13.

Table 13: List of criteria for assigning decisions within the decision support framework.

Decision step	Decision support criteria
Step 1: Is the species established?	• For criteria to assess alien population establishment state, refer to categories in Table 12. • Assessment should be made explicitly regarding the population under assessment, rather than the species within the borders of South Africa as a whole. • If field data are insufficient to confidently define the spatial extent of the target population, a sequentially expanded spatial scale should be used until managers have high confidence that the maximum dispersal range of the population is covered by management decisions: 1. Water body containing alien species up to known barriers to fish movement. 2. Catchment in which population is known to occur. 3. Adjacent catchments where inter-basin transfer is known to occur or is at a high risk of occurring. 4. If the species is listed as C3 or higher (Table 12), Step 1 response is YES. 5. If the species is listed as B1 or higher and has a history of negative environmental impacts outside its range in other regions, Step 1 response is YES on the basis of environmental risk.
Step 2: Is the species invasive?	• For criteria to assess alien population invasiveness, refer to categories in Table 12 • If the species is listed as C3, but no evidence of negative environmental impact in the target area or elsewhere exists, Step 2 response is NO on the basis of environmental risk. • If the species is listed as D1 or higher, Step 2 response is YES. • If Step 1 was answered YES on basis of the species having a history of negative environmental impacts outside its range in other regions, Step 2 response is YES on the basis of environmental risk.

Decision step	Decision support criteria
Step 3: Does the species have socio-economic value?	• As with previous steps, Step 3 must be answered at the scale of the managed population (see Step 1 criteria). • If there is no evidence of recreational, commercial, or artisanal exploitation of the managed population, Step 3 response is NO. • If a known recreational, commercial, or artisanal fishery exists based on the assessed population, managers should consider whether eradication of the population would significantly affect the users of the fishery. If, through fishery assessment and/or user engagement, it can be determined that eradication of the population would not severely affect users, and that eradication would be sustainable in terms of user compliance with the outcome (i.e. restocking by users is unlikely), Step 3 response is NO. • If assessment determines that eradication of the population would severely affect users, and that such action would be associated with a high risk of user non-compliance, Step 3 response is YES.
Step 4: Is eradication feasible?	• Managers should consider logistical, legal, and financial implications of eradication when assessing the feasibility of an eradication operation. The following criteria should be addressed: o Availability of barriers to reinvasion o Feasibility of total fish community eradication o Feasibility of targeted alien species removals o Size of treated area and associated cost implications o Availability of trained and/or accredited personnel • Availability of barriers to invasion is a prerequisite for attempting eradication. If pathways for reinvasion exist, infrastructure for creating a barrier that does not impinge on the conservation status of adjacent fish communities must be applied to the pathway. If this cannot be achieved, Step 4 response is NO.
Step 4 continued	• If significant numbers of native fish co-occur with the managed population, eradication using piscicides is likely to be unfeasible, and Step 4 response is NO. An exception would be if the invasive species poses a significant conservation threat to the managed area and the co-occurring native species are of low conservation

Decision step	Decision support criteria
	concern or are well represented in adjacent areas. • If piscicides are unfeasible due to co-occurrence with native fish, targeted manual removals can be considered. It should be pointed out that few case studies of successful targeted removals occur and this is likely to be a high-risk course of action. • Piscicides and their associated logistical and human resource requirements are very costly and managers will need to carefully assess their capacity to attempt eradication. A cost benefit analysis of total eradication versus more conservative mitigation actions will need to be carefully considered in all cases. • Availability of trained, accredited operators to carry out rotenone operations is an import consideration. Managers should consider licensing requirements and pre-operations training of implementing teams when planning logistics and budget for eradication operations.

The first two questions on the decision support tree relate to the invasion status of the species in a given area (Figure 34). While several theoretical frameworks exist to determine invasion states, we recommend that managers use the Unified Framework for Biological Invasions (Blackburn *et al.,* 2011) which is starting to become a *de facto* international standard for characterising the stage of biological invasions. The framework in particular requires evidence of successful reproduction at the site to characterise the population as established/naturalised, and evidence of further, unaided spread to characterise it as invasive. These criteria are generally assessed through repeated field data collection at key times of the year, matched to the reproductive cycle of the species. Examples of the application of the framework to South African fish invasions are provided in Jones *et al.,* 2013) who used the unified invasion framework to characterize Africa's first loricariid catfish invasion and Mäkinen *et al.,* 2013) who used the framework to evaluate the invasion state of Giant pangasius, *Pangasius sanitwongsei* in the Breede River. If a Category 1b species is not yet established, but occurs incidentally within the water body, then developing an Invasive Species Management Programme is a likely to be a low priority. Follow-up monitoring of the incursion is nonetheless recommended (Figure 34), so that should a population establish in the future, it can be detected. In the case of incidental alien species that are not listed under NEMBA (e.g. Giant pangasius), while there is no legal obligation for control it is nonetheless recommended that a risk assessment be carried out for the species, to inform future management decisions (Mäkinen *et al.,* 2013).

Once a species is determined as being established but not (or at least not yet) invasive, the framework dictates that a Management Programme should focus on containment of the population within its current range, and mitigation of any negative impacts it may be having on native species (Figure 34). The specific approaches taken to achieve this will depend on availability of field data on observed impacts, as well as an assessment of current dispersal pathways out of the containment area. The management approach will also be dictated by the logistical reality of the situation. The rehabilitation of the Rondegat River, where an established *M. dolomieu* population was eradicated from a physically isolated 4 km reach (Weyl *et al.,* 2014), provides an example of a logistically feasible containment strategy. The overall extent of established smallmouth bass populations in the Olifants-Doring Catchment is too large for complete eradication to be considered. However, their experimental removal from a discreet reach of river where they were no longer invasive locally (the invasion front had not moved for 20 years), was the first of a series of planned small-scale interventions that will serve to mitigate the negative impacts and spread of smallmouth bass within the greater catchment.

If the species is shown to be invasive and in the process of expanding its range, containment becomes the first priority, after which strategies for *in situ* population control can be explored. At this point of the decision making process, it is important that the socio-economic impact of the species be taken into account, as attempts to control or eradicate the species may have negative consequences for some local stakeholders, and thus run the risk of being opposed by those stakeholders. The long process taken to approve and eventually successfully implement the removal of Smallmouth bass from the Rondegat River in the Western Cape, demonstrated the necessity for buy-in from stakeholders such as landowners and anglers (Marr *et al.,* 2012, Weyl *et al.,* 2014).

Finally, if the species is deemed to have negative impacts on the environment and is of no significant socio-economic value, then the practicality of eradication can be explored (Figure 34). The decision on whether or not to attempt eradication will be informed by the size of the affected area, the choice of method (e.g. piscicides, de-watering, manual removals), and the associated cost and logistical requirements. If eradication is deemed impractical, the management strategy falls back to containment and mitigating the negative impacts of the invasive species.

To illustrate how managers should use the decision support system to prioritise conservation interventions involving alien invasive fishes, the case studies previously described in this report are examined in turn and the framework used to determine practical management outcomes in each case.

5.2.2 Case Study 1 example: Managing *Clarias gariepinus* in the upper Coerney River

The scenario of on-going invasions in the Sundays River Catchment, Eastern Cape, demonstrates how spatially explicit the problem and its potential solutions can be within a system that has both natural and man-made invasion pathways. The Sundays River main stream and lowland tributaries, including the lower 20 km of the Coerney River, represent habitats where the process of invasion has climaxed and is practically irreversible (Weyl *et al.,* 2010). The reason for this is that the source of propagules into the system, the Orange-Fish-Sundays IBWT scheme, is unlikely to ever be modified to block the transport of fishes within the water it carries, because there are no conservation or legal imperatives to do so. The constant influx of new introductions renders any population control strategies in the Sundays River futile and thus the area is unsuitable for an Invasive Species Management Programme (ISMP). Specifically, there is no conservation incentive to manage the effect of the irrigation network as an invasion pathway, as the introduced species being transported in it are established both at the sources and destinations of the canals. In terms of the NEMBA legislation, the restricted activity within the Act written to deal with IBWT (the transfer or introduction of species from one catchment/sub-catchment in which it occurs, to another catchment/sub-catchment in which it does not occur) no longer applies to the Sundays River main stream.

The current *C. gariepinus* invasion in the upper Coerney River nonetheless still provides an appropriate example of how a Management Plan could be formed using the Decision Support Framework. The upstream penetration of *C. gariepinus* in the river has been mediated by the intermittent flow conditions in the Coerney catchment, which have made the pathway for upstream invasion available for relatively brief periods of time only. The challenge for managers now is to characterise the current state of invasion in order to develop a management plan (Table 14).

Using the framework (Figure 34) and associated decision making criteria (Table 14), the first question to ask is “are catfish established in the upper Coerney River”. The geographic extent of the population in question (the management unit), marked by range extent, is the Coerney River from the permanently wetted pool at the irrigation network outflow (spill-point) that marks the invasion source to the uppermost record of catfish in the Coerney River, in this case a stream reach of approximately 30 km. We exclude the lower Coerney River, extending downstream from the spill point to the causeway near the confluence with the Sundays River, because for the majority of time, these reaches are naturally dry and only provide fish habitat for short periods after heavy rain. To answer the question of establishment requires population monitoring to reveal whether multiple year classes are

present in the river, indicating repeated successful spawning events. While more than one size class were recorded in the Coerney River, they fall into a narrow band that suggest subsequent incursions into the stream by differing, single cohorts of fish, rather than successful spawning within the stream (Ellender et al., in press). Despite the lack of evidence for successful spawning, it must be recognised that *C. gariepinus* is an emerging invader with documented negative impacts on stream biota in other regions (Vitule et al., 2008; Kadye and Booth, 2012). Thus, on the basis of environmental risk, this question as well as the follow-up question "are catfish invasive in the upper Coerney River" shall be answered YES.

The next question the framework asks is "does the invasive population have socio-economic value". As the invasion is taking place within a National Park where exploitation of living resources is prohibited, the answer is NO. Thus the manager must ask "is eradication practical". Here the answer is less clear. Catfish populations are currently patchy and their upper limit in the system is not known. Worse, they co-occur with endangered native fishes (Eastern Cape redfin), making eradication using piscicides undesirable, at least in the upper reaches of the invasion front. Finally, there is still no physical barrier in the lower Coerney River that would prevent re-invasion from the downstream spill-point should an eradication programme be attempted. The pragmatic answer to the eradication question at this point in time must therefore be NO, meaning the stated goal of the ISMP for *C. gariepinus* in the upper Coerney River would be to "manage against impacts and further spread".

Table 14: Decision support framework applied to *C. gariepinus* in the Coerney River.

Decision step	**Decision with supporting criteria**
Step 1: Is the species established?	YES, due to environmental risk posed by the species.
Step 2: Is the species invasive?	YES, due to environmental risk posed by the species.
Step 3: Does the species have socio-economic value?	NO. Location in a protected area precludes any utilisation of the population.
Step 4: Is eradication feasible?	NO. The lack of a downstream barrier to re-invasion and co-occurrence with threatened native fish preclude eradication by piscicides.

5.2.3 Case Study 2 example: Choosing management strategies for two discreet *M. salmoides* populations in the Groot Marico catchment

Micropterus salmoides have been present in the Groot Marico Catchment for several decades (Skelton *et al.,* 1994), and are today patchily distributed in the mainstream and two tributaries (see Deliverable 14). For management purposes, the bass can be divided into three discreet populations within the upper catchment, each separated by barriers to upstream or downstream dispersal. The entire upper catchment is a FEPA and Fish Support Area (Nel *et al.,* 2011). Under the NEM:BA invasive species regulations, this makes all the *M. salmoides* within the Groot Marico catchment Category 1b Invasive Species (RSA 2013 and amended in 2014). While an Invasive Species Management Strategy will encompass all *M. salmoides* occurring within the FEPA, the Decision Support Framework should be used individually on each discreet population within the area. Here we describe the decision making process for two of the three populations.

The first population occurs in the main stream, between the Marico Bosveld Dam and a waterfall barrier downstream of the confluence between the Marico and Kaaloog se Loop, the lower reaches of the Draaifonteinspruit (up to a causeway barrier), and the lower reaches of the Polkadraaispruit (downstream of the Twyfelspoort Dam). This population co-occurs with several native species within the Marico River, and has a steady source of propagules in the Marico Bosveld reservoir, as well as in-stream breeding habitats such as large pools formed by weirs and causeways at multiple localities along the Marico River. Using the Decision Support Framework, multiple age classes throughout the Marico River show the population here to be “established”, so that the answer to “are *M. salmoides* established in the lower Groot Marico River” is YES. Although the population is restricted in its current distribution by the aforementioned barriers to upstream migration, it clearly survives in multiple reaches that are a long distance upstream of the likely first point of introduction, Marico Bosveld Dam, giving the population an invasion state categorisation of D2. Thus the second question, are *M. salmoides* invasive in the lower Groot Marico River” is YES. The species appears to have little socio-economic impact in the majority of its distribution within the FEPA, although there is some recreational use, particularly on Marico Bosveld Dam. However, given the high conservation value of the FEPA, managers may want to engage with local recreational users, to determine the likelihood of restocking of bass occurring within the region should eradication be attempted. Assuming local stakeholder buy-in to the process of bass eradication can be achieved, the question “Do *M. salmoides* in the lower Groot Marico River have socio-economic value” could be answered NO. Unfortunately, due to the size of the management area, as well as the widespread co-occurrence of *M. salmoides* with multiple native species, the answer to “is eradication

feasible” is also clearly NO. The desired management goal for this bass population therefore is “manage against impacts and further spread”.

The second population we assess is restricted to the Marico dolomitic eye, a deep spring-fed pool that forms the source of the Kaaloog se Loop tributary (Table 15). The presence of multiple year classes within the eye indicates successful breeding although bass appear to have been prevented from expanding outside of the eye by dense riparian vegetation at the outflow. These data make the invasion state of the population C3. Given the known invasiveness of the species, and its proximity to both human activity centres (Marico Eye is adjacent to a campsite built to accommodate snorkelers and divers in the eye) and the sensitive Kaaloog se Loop stream, the risk of fish from this population being transported to and becoming invasive within Kaaloog se Loop is high. The answers to both the “established” and “invasive” questions on the decision tree are thus YES based on environmental risk. Bass are not fished for within the eye, although they do add variety to the species being viewed by snorkelers and divers. Given the large numbers of native banded tilapia present in the eye, the negative impact of removing bass on the recreational diver is likely to be negligible, thus the answer to “do bass in the Marico Eye have socio-economic value” is NO. Eradication using piscicides is ruled out due to the presence of native fish of conservation value (the banded tilapia population is geographically isolated from other populations in the catchment and is suspected to be genetically distinct). Nonetheless, the high water clarity and limited size of the habitat, together with the small size of the bass population, makes manual removal methods such as spear-fishing practical. Eradication can therefore be considered at least potentially feasible, and the desired management goal for the Marico Eye bass population should thus be “eradicate”.

Table 15: Decision support framework applied to the bass in the Marico Eye.

Decision step	**Decision with supporting criteria**
Step 1: Is the species established?	YES. Establishment was demonstrated by sampling multiple length (age) cohorts in the population.
Step 2: Is the species invasive?	YES, due to environmental risk posed by the species.
Step 3: Does the species have socio-economic value?	NO. There is no direct utilisation of the bass population in the eye.
Step 4: Is eradication feasible?	YES. The spall geographic extent of the eye and the small population size of bass makes manual removal a potential option.

5.2.4: Case study 3 example: Managing *P. disjunctivus* in the Nseleni River

The South American armoured catfish family Loricariidae includes more than 700 nominal species. Some are popular aquarium fishes in the global pet trade. Releases by aquarists and escape from fish farms have resulted in several armoured catfish invasions in Central and North America, Asia and now, Africa. In South Africa, armoured catfishes were first reported from a lake near the town of Empangeni in 2000. This was followed by the formal collection of specimens from the Mthlathuse River in 2004 and from the adjacent Nseleni River system in 2007. This was cause for an assessment because documented impacts of armoured catfish invasions include the destabilisation of river banks resulting from the breeding-burrows that armoured catfishes excavate. This assessment is described in detail in Jones et al., 2013) and the findings of this survey can be utilised directly in the assessment framework (Table 16).

Establishment (step 1) was demonstrated through the sampling of 346 specimens including 12 mm long juveniles and adults measuring almost 50 cm. The fish are considered to be invasive (step 2) because they first escaped from captivity, established in the Mthlathuse River and then spread to the Nseleni River via an inter-basin water transfer. Jones et al. (2013) categorised this invasion as D2: "*self-sustaining population in the wild with individuals surviving and reproducing a significant distance from their original point of introduction*". Although the fish are valuable in the aquarium trade their presence in the wild has no socioeconomic value (step 3) and although eradication would be desirable, the Mthlathuse and Nseleni Rivers are just too big for this to be feasible. For this reason the preferred management goal is "manage against impacts and further spread". Containing this invasion will require educating the public on the dangers associated with fish introductions and discouraging the trade in Loricariid species with proven invasion histories.

Table 16: Decision support framework applied to the Loricariid catfish invasion in the Nseleni River.

Decision step	Decision with supporting criteria
Step 1: Is the species established?	YES. Establishment was demonstrated by sampling multiple length (age) cohorts in the population.
Step 2: Is the species invasive?	YES. Using objective criteria, the loricariid invasion was categorised as stage “D2” invasion.
Step 3: Does the species have socio-economic value?	NO. The economic value of this species is based on their use in the aquarium trade and not on harvest from the wild.
Step 4: Is eradication feasible?	NO. The Nseleni and Mthlathuze Rivers are too large for eradication of *P. disjunctivus* to be logistically or economically feasible.

5.2.5: Case study 4 example: Managing *Micropterus salmoides* and *M. dolomieu* in Groendal Nature Reserve

Micropterus salmoides were first introduced in to upper Swartkops River system in 1935 and *M. dolomieu* in 1965 (Ellender *et al.,* 2011). The upper Swartkops River system is split into two major tributaries, the Elands and Kwa-Zunga rivers. As the Elands River catchment is situated on private land, the feasibility of conservation/management interventions is low. As a result the Kwa-Zunga River was chosen as a case study due to its catchment being almost entirely situated within an access-controlled protected area, the Groendal Wilderness Area. The Kwa-Zunga River and its catchments are listed as a FEPA (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 species (Nel *et al.,* 2011). Given that the invasion status and management options available for both *M. dolomieu* and *M. salmoides* are essentially identical, we considered them together in this example (Table 17).

The point of centrarchid introduction was the Groendal Dam (situated on the Kwa-Zunga River mainstem) which facilitated their spread both upstream and downstream of the impoundment. They have consistently been recorded in the Groendal Wilderness Area for over 50 years and can now be considered fully established in the Kwa-Zunga River mainstem but have only penetrated two of the headwater tributaries, Blindekloof Stream below and Chaseskloof Stream above the impoundment (Ellender, 2013). In light of this

evidence they are classified as D2, and we answer both the establishment and invasive questions in the framework YES. Despite some targeted angling on the Groendal Dam in the past, due to current access controls this no longer happens. Centrarchids therefore do not provide significant enough economic benefit to the region to justify their presence in a FEPA Fish Sanctuary (Nel *et al.,* 2011). The risk of re-introduction is also low due to being situated in an access controlled wilderness area. In the Blindekloof Stream, centrarchids have been documented to extirpate native Eastern Cape redfin minnows *Pseudobarbus afer* from invaded stream reaches. For all these reasons, we answer the question of socio-economic value with NO.

Eradication of centrarchids from the Blindekloof Stream using piscicides is not possible due to the presence of threatened native fishes. Manual eradication has been successfully undertaken before, however, in the absence of measures mitigating the chances of reinvasion by centrarchids such as barrier weirs this is not practical. For example, during a study of the Blindekloof Stream in 1987 all *M. salmoides* were manually eradicated, however, a follow-up survey in 2010 confirmed that *M. salmoides* and an additional invader *M. dolomieu* had re-invaded the stream (Skelton, 1993; Ellender et al., 2011). The new invasion of *Clarias gariepinus* in the Blindekloof Stream (Ellender et al., in press) poses an additional threat and uncertainty regarding invasive species management. Even if centrarchids were eradicated from the Groendal Wilderness Area (GWA), their reinvasion from downstream sources outside of the GWA is highly likely. Thus the answer to feasibility of eradication is NO and the desired management goal should be “manage against impacts and further spread”.

Table 17: Decision support framework applied to the bass in Groendal nature reserve.

Decision step	**Decision with supporting criteria**
Step 1: Is the species established?	YES, due to environmental risk posed by the species.
Step 2: Is the species invasive?	YES, due to environmental risk posed by the species.
Step 3: Does the species have socio-economic value?	NO. Location in a protected area precludes any utilisation of the population.
Step 4: Is eradication feasible?	NO. The lack of downstream and upstream barriers to re-invasion precludes eradication by piscicides.

5.3 Conclusion

The decision support framework presented here is a support tool which has the potential to improve the efficiency of alien fish management practices across South Africa. It can, however, only work if there are sufficient human resources to implement management strategies. The framework is thus only the starting point for developing sound invasive fish management programmes with a high probability of success. At the time of publishing the NEMBA regulations on invasive species have only just become law, and it remains to be seen whether conservation practitioners in South Africa will be able to enforce the management of invasive fishes as set out in the law. The biggest obstacle to this challenge is the lack of aquatic expertise currently employed by provincial governments, a situation that will need to be remedied if the many threats to South Africa's aquatic ecosystems are to be addressed. Nonetheless, the authors concur that this decision support framework provides a simple and systematic way to prioritise future conservation interventions involving invasive freshwater fishes, and that the lessons learned from the case studies presented in this report represent a wealth of information that can inform management best practices for native and alien fishes within South Africa.

Moving forward, there is an increasing need to balance the needs and wishes of stakeholders that utilise invasive fishes within South Africa with the need to protect our national biodiversity heritage, which many highly utilised invasive species threaten. The management of these "conflict species" will only be possible if all stakeholders work together to ensure a mutually acceptable management state can be achieved in invaded catchments. For the correct management goal to be chosen, it will be critical to expand our knowledge and understanding of invasive species impacts, as they may be more subtle than expected (e.g. *M. salmoides* in the Groot Marico catchment) or else unknown due to limited field data (e.g. *P. disjunctivus* in the Nseleni River). Only through targeted research on invasive species in areas of high conservation concern will our understanding of invasions and their impacts be improved, allowing for more effective management strategies that benefit both the biota and the people of South Africa.

6 LIST OF REFERENCES

Adams, S.B., C.A. Frissell, and B.E. Rieman. 2001. Geography of invasion in mountain streams: consequences of headwater lake fish introductions. Ecosystems 4: 296-307.

Balon, E. 1975. Reproductive guilds in fishes: a proposal and definition. Journal of the Fisheries Research Board of Canada 32: 821-864.

Blackburn, T.M., P. Pyšek, S. Bacher, J.T. Carlton, R.P. Duncan, V. Jarošík, J.R.U. Wilson, and D.M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in ecology & evolution 26: 333-339

Bruton, M.N. 1979. The breeding biology and early development of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias* (Clarias). Transactions of the Zoological Society of London 35: 1-45.

Clement, M., D. Posada, and K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657-1659.

Clout, M.N., and P.A. Williams. 2009. Invasive Species Management: A Handbook of Techniques. Oxford University Press Inc., New York

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. *Geomica et Cosmochimica Acta* **12:** 133-149Firmat, C., Alibert, P., Losseau, M., Baroiller, J.F & Schliewn, U.K. 2013. Successive Invasion Mediated Interspecific Hybridisations and Population Structure in the Endangered Cichlid *Oreochromis mossambicus. PLoS ONE 10:* e63880. doi: 10.1371/journal.pone. 00633880.

Crivelli, A.J. 1981. The biology of the common carp, *Cyprinus carpio* L. in the Camargue, southern France. Journal of Fish Biology 18: 271-290.

De Moor, I., and M.N. Bruton. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report 144.

De Niro, M.J., and M.J. Epstein. 1981. Influence of the diet on the distribution of nitrogen isotopes in animals*. Geochimica et Cosmochimica Acta***45**: 341-351

DWA. 2014. Department of Water Affairs. List of dams. http://www.dwaf.gov.za/iwqs/gis_apps/dam/dams/index.htm

DWAF. 2009b. Department of Water Affairs and Forestry. Intermediate Reserve Determination Study for Selected Water Resources (Rivers, Wetlands and Groundwater) in the Crocodile (West) and Marico Management Areas (Report compiled for Department of Water Affairs by Zitholele Consulting)

DWAF. 2010. Department of Water Affairs and Forestry. Marico River Catchment Reserve Determination Specialist Report: Present Ecological State – Fish (Report compiled for Department of Water Affairs by Zitholele Consulting)

Driver, A., J.L. Nel, K. Snaddon, K. Murray, D.J. Roux, L. Hill, E.R. Swartz, J. Manual, and N. Funke. 2011. *Implementation manual for freshwater ecosystem priority areas.* WRC Report No. 1801/1/11. Pretoria: Water Research Commission

Duggan, I.C., C.A.M. Rixon, and H.J. MacIsaac. 2006. Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biological invasions 8: 377-382.

Ehleringer, J.E., and P.W. Rundell. 1989. Stable isotopes: History and Instrumentation. In: Stable Isotopes in Ecological Research. Springer-Verlag New York Inc. USA

Ellender, B.R., and O.L.F. Weyl. 2014. In press. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions* **9**.

Ellender, B.R., O.L.F. Weyl, and E.R. Swartz. 2011. Invasion of a headwater stream by non-native fishes in the Swartkops River system, South Africa. *African Zoology* 46(1): 39-46.

Engelbrecht, J., and R. Bills. 2007. *Barbus motebensis*. The IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>.

Finlayson, B., R. Schnick, D. Skaar, J. Anderson, L. DeMong, D. Duffield, W. Horton and J. Steinkjer. 2010. Planning and standard operating procedures for the use of rotenone in fish management. American Fisheries Society, Bethesda, Maryland.

Firmat, C., P. Alibert, M. Losseau, J-F. Baroiller, and U.K. Schliewen. 2013 Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. Plos One 8: 1-12, http://dx.doi.org/10.1371/journal.pone.0063880

Gratwicke, B., and B.E. Marshall. 2001. The relationship between the exotic predators *Micropterus salmoides* and *Serranochromis robustus* and native stream fishes in Zimbabwe. J. Fish Biol. 58:68-75.

Hyslop, E.J. 1980. Stomach content analysis a review of methods and their application. *Journal of Fish Biology* **17:** 411-429

Impson, N.D., B.W. van Wilgen, and O.L.F. Weyl. 2013. Coordinated approaches to rehabilitating a river ecosystem invaded by alien plants and fish. S. Afr. J. Sci. 109:1-4.

Jackson, D.A. 2002. Ecological Effects of Micropterus Introductions: The Dark Side of Black Bass. Black Bass: Ecology, Conservation and Management. American Fisheries Symposium 31. Bethesda, Md 724pp.

Jackson, L.W., R. Inger, C. A. Parnell and S. Bearhop. 2011. Comparing isotopic niche widths among and within communities: SIBER-Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology doi: 10.1111/j.1365-2656.2011.01806.

Jones, R.W., O.L.F. Weyl, E.R. Swartz, and M.P. Hill. 2013. Using a unified invasion framework to characterize Africa's first loricariid catfish invasion. Biological Invasions 15: 2139-2145.

Kadye, T., and T.J. Booth. 2011. Integrating stomach content and stable isotope analysis to elucidate the feeding of non-native Sharptooth catfish *Clarias gariepinus*. *Biological Invasions*

Karssing, R.J., N.A. Rivers-Moore, and K. Slater. 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, http://dx.doi.org/10.2989/16085914.2012.666381

Katzenberg, M.A., and S.R. Saunders. 2007. *Stable Isotope Analysis: A Tool for Studying Past Diet, Demography and Life History* in: Biological Anthropology of the Human Skeleton (Second Edition). John Wiley and Sons Inc. Hoboken, New, Jersey, USA.

Kleynhans, C.J., M.D. Louw, and J. Moolman. 2007. Reference frequency of occurrence of fish species in South Africa. Report produced for the Department of Water Affairs and Forestry (Resource Quality Services) and the Water Research Commission

Koehn, J.D., and R.F. Mackenzie. 2004. Priority management actions for alien freshwater fish species in Australia. New Zeal. J. Mar. Freshw. Res. 38:457-472.

Kolar, C.S., and D.M. Lodge. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology & Evolution 16: 199-204.

Langton, R.W. 1982. Diet overlap between the Atlantic cod *Gardus mohua*, Silver hake, *Merluccius bilinearias* and fifteen other northwest Atlantic finfish. Fisheries Bulletin 80: 745-759.

Laurenson, L.B.J., and C.H. Hocutt. 1985. Colonisation theory and invasive biota: the Great Fish River, a case study. Environmental Monitoring and Assessment 6: 71-90.

Laymann, C.A., Arrington, D.A., Montana, G.C & Post, D.M. 2007. *Ecology* **88:** 42-48

Levin, R. 1968. Evolution in changing environments. Princeton Univ. Press, Princeton, N.J. 120p

Lowe, R.J., D.J. Woodford, N.D. Impson, and J.A. Day. 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

Mäkinen, T., O.L.F. Weyl, K.A. van der Walt, and E.R. Swartz. 2013. First record of an introduction of the Giant pangasius, *Pangasius sanitwongsei* Smith 1931, into an African river. African Zoology 48: 388-391.

Marchetti, M.P., T. Light, P.B. Moyle, and J.H. Viers. 2004. Fish invasions in California watersheds: testing hypotheses using landscape patterns. Ecological Applications 14: 1507-1525.

Marr, S.M., N.D. Impson, and D. Tweddle. 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.

McCutchan, J.H., Lewis, W.M., Kendall, C & McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulphur. *Oikos* **102:** 378-390

Nel, J.L., A. Driver, W.F. Strydom, A. Maherry, C. Petersen, L. Hill, D.J. Roux, S. Nienaber, H. van Deventer, E.R. Swartz, L.B. Smith-Adao. 2011. Atlas of freshwater ecosystem priority areas in South Africa: Maps to support sustainable development of water resources. Water Research Commission, WRC Report NO. TT 500/11, South Africa

NWDACE. 2007. North West Provincial Department of Agriculture Conservation and Environment. Habitat Integrity Assessment Report of Selected Rivers in the North West Province.

Olden, J., and N. Poff. 2006. Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. Ecological Monographs 76: 25-40.

Pasquad, M., Pillet, M., David, V., Sautor, B. & Ellie, P. 2011. Determination of fish trophic levels in an estuarine system. *Estuarine, Coastal and Shelf Science* **86:** 237-246

Pianka, E.R. 1974. Niche overlap and diffuse competition. Proceedings of the National Academy of Sciences of the United States of America. 71; 2141-2145

Platell, M.E., and I.C. Potter. 2001. Partitioning of food resources amongst 18 abundant benthic carnivorous fish species in marine waters on the lowest coast of Australia. Journal of Experimental Marine Biology and Ecology 261: 31-54

Potts, W., A. Booth, T. Hecht, and T. Andrew. 2006. The life history and fishery potential of *Labeo umbratus* (Teleostei: Cyprinidae) in five small South African reservoirs. African Journal of Aquatic Science 31: 37-41.

Provincial River Health Programme Progress report (2011) Compiled by: Roux H. Department: Economic Development, Environment, Conservation and tourism, North West Provincial Government, Republic of South Africa, 74 pp.

Pyke, G.H. 2005. A Review of the Biology of *Gambusia affinis* and *G. holbrooki*. Reviews in Fish Biology and Fisheries 15: 339-365.

Rahel, F.J. 2007. Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. Freshwater Biology 52: 696-710.

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

Richardson, D.M., J.A. Cambray, R.A. Chapman, W.R.J. Dean, C.L. Griffiths, D.C. Le Maitre, D.J. Newton, and T.J. Winstanley. 2003. Vectors and pathways of biological invasions in South Africa – past, present and future. Pages 292-349 in Ruiz GM and Carlton JT, editors. Invasive Species: vectors and management strategies. Island Press, Washington, D.C.

Rolls, R.J. 2011. The role of life-history and location of barriers to migration in the spatial distribution and conservation of fish assemblages in a coastal river system. Biol. Conserv. 144:339-349.

Roux, H. 2010. *A comparison between regional and multivariate classification of biomonitoring sites based on aquatic macroinvertebrate assemblages in the Marico River catchment.* PhD Thesis. 60 pp

RSA (Republic of South Africa). 2013. National Environmental Management: Biodiversity Act (10/2004): Alien and Invasive Species Regulations. Government Gazette, South Africa 506 (33683).

Russell, I.A. 1998. Freshwater fish of the Addo Elephant National Park. Southern African Journal of Aquatic Sciences 24: 57-70

Skelton, E.R., A.J. Ribbink, and V. Twentyman-Jones (eds). 1994. The conservation of dolomitic ecosystems in the Western Transvaal, South Africa. JLB Smith Institute of Ichthyology, Grahamstown.

Skelton, P.H. 2001. A complete guide to the freshwater fishes of southern Africa. Struik Publishers, South Africa.

Smith-Adao, L.B., J.L. Nel, D.J. Roux, L. Schonegevel, D. Hardwick, G. Maree, L. Hill, H. Roux, C.J. Kleynhans, J. Moolman, C. Thirion, and C. Todd. 2006. A Systematic Conservation Plan for the Crocodile (West) and Marico Water Management Area. CSIR Report Number CSIR/NRE/ECO/ER/2006/0133/C compiled for the Department of Water Affairs and Forestry.

Snaddon, C.D., and B.R. Davies. 1998. A preliminary assessment of the effects of a small South African inter-basin water transfer on discharge and invertebrate community structure Regulated Rivers: Research & Management 14: 421-441

SAIAB. 2011. South African Institute for Aquatic Biodiversity. Information Portal – Fish Collection Database (http://saiab.ac.za/infoportal/database.html)

Strydom, N.A., and F.J. Neira. 2006. Description and ecology of larvae of *Glossogobius callidus* and *Redigobius dewaali* (Gobiidae) from temperate South African estuaries. African Zoology 41: 240-251.

Takamura, K. 2007. Performance as a fish predator of Largemouth bass (*Micropterus salmoides* (Lacepe `de)) invading Japanese freshwaters: a review. Ecol Res 22: 940-946

Ter Braak, C.J.F., and P. Smilauer. 2002. Canoco Reference Manual and Canodraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Ithaca, NY: Microcomputer Power, 500pp.

Van der Waal, B.C.W., and R.I. Bills. 2000. *Oreochromis niloticus* (Teleostei: Cichlidae) now in the Limpopo River System. South African Journal of Science **96:**47-48

Van Der Walt, K.A. 2014. Prioritising Native Fish Populations for Conservation Using Genetics in the Groot Marico Catchment, North-West Province, South Africa. M.Sc. Thesis, Rhodes University, Grahamstown.

Van Rensburg, B.J., O.L.F. Weyl, S.J. Davies, N.J. van Wilgen, D. Spear, C.T. Chimimba, and F. Peacock. 2011 Invasive vertebrates of South Africa. In: Pimentel D, editor. Biological invasions: Economic and environmental costs of alien plant, animal, and microbe species. CRC Press, Boca Raton, Florida, pp 326-378

Van Vuuren, L. 2009. Darlington Dam – SA's troubled lake. Water Wheel Jul/Aug 20: 24-28.

Vitousek, P.M., C.M. D'Antonio, L.L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. American Scientist 84: 468-478

Wellington, J.H. 1995. *Southern Africa a Geographical Study, Volume 1: Physical Geography*. Cambridge University Press, London, United Kingdom

Weyl. O.L.F., A.J. Booth, and G.R.L. Traas. 2008. Distribution and abundance of fish in the Sundays River Catchment of the Addo Elephant National Park. Sundays River Freshwater Fish Research Project: Report Number 2. Report prepared for SAN-Parks, 31p

Weyl, O.L.F., A.J. Booth, G.R.L. Traas, and E.R. Swartz. 2009a. Task 2: Determination of critical conservation areas in the Sundays river system for indigenous fish species, Sundays River Freshwater Fish Research Project, Project ID: PO64438, Report No.4, 31p

Weyl, O.L.F., A.J. Booth, H. Winker, B.R. Ellender, and G.R.L Traas. 2010. TASK 5: Management recommendations for freshwater fishes in the Sundays River system, Addo Elephant National Park. Report prepared for SAN-Parks: Sundays River Freshwater Fish Research Project. Project ID: PO64438, 56p

Weyl, O.L.F., B.R. Ellender, D.J. Woodford, and M.S. Jordaan. 2013. Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of rotenone treatment in an invaded reach. African Journal of Aquatic Science. 38: 201-209

Weyl, O.L.F., B. Finlayson, N.D. Impson, D.J. Woodford, J. Steinkjer. 2014. In press. Threatened Endemic Fishes in South Africa's Cape Floristic Region: A New Beginning. Fisheries.

Weyl, O.L.F., and T. Hecht. 1998. The biology of *Tilapia rendalli* and *Oreochromis mossambicus* (Pisces: Cichlidae) in a subtropical lake in Mozambique. South African Journal of Zoology 33: 178-188.

Weyl, O.L.R., T. Stadtlander, and A.J. Booth. 2009b. 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 1976:93-105.

Wilson, J.R.U., E.E. Dormontt, P.J. Prentis, A.J. Lowe, and D.M. Richardson. 2009. Something in the way you move: dispersal pathways affect invasion success. Trends in ecology & evolution 24: 136-44.

Wittenberg, R., and M.J.W. Cock. (eds). (2001). Invasive alien species: a toolkit of best prevention and management practices. CAB International, Wallingford, Oxon, UK.

Woodford, D.J., C. Hui, D.M. Richardson, O.L.F. Weyl. 2013. Propagule pressure drives establishment of introduced freshwater fish: quantitative evidence from an irrigation network. Ecological Applications 23: 1926-1937.

Woodford, D.J., N.D. Impson, J.A. Day, and I.R. Bills. 2005. The predatory impact of invasive alien Smallmouth bass, *Micropterus dolomieu* (Teleostei: Centrarchidae), on indigenous fishes in a Cape Floristic Region mountain stream. African J. Aquat. Sci. 30:167-173.

Zambrano, L., E. Valiente, and M.J. van der Zanden. 2010. Stable isotope variation of a highly heterogenous shallow freshwater system. Hydrobiologia 646: 327-336

Zengeya, T.A., and B.E. Marshall. 2007. Trophic interrelationships among cichlid fishes in a tropical African reservoir (Lake Chivero, Zimbabwe). Hydrobiologia 592: 175-182

Zengeya, T.A., A.J. Booth, A.D.S. Bastos, and C.T. Chimimba. 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

APPENDIX 1: Site Information

Breakdown of Groot Marico sites surveyed during the March 2012 and November 2012 sampling surveys per river reach, GPS coordinates, sampling method, sampling date and invasion status

River	Site	GPS-S	GPS-E	Habitat	Sampling date	Sampling method	Invaded
Bokkraal	BOK1	25.79623	26.44899	Pool	2012-03-21	Electrofishing	No
	BOK10	25.79993	26.45379	Pool	2012-03-21	Seine	No
	BOK11	25.80043	26.44956	Run	2012-03-21	Electrofishing	No
	BOK12	25.8005	26.44964	Riffle	2012-03-21	Electrofishing	No
	BOK13	25.78782	26.44979	Riffle	2012-03-23	Electrofishing	No
	BOK2	25.7958	26.44903	Pool	2012-03-21	Electrofishing	No
	BOK3	25.79583	26.44892	Pool	2012-03-21	Electrofishing	No
	BOK4	25.79583	26.44905	Chute	2012-03-21	Electrofishing	No
	BOK5	25.79501	26.44991	Pool	2012-03-21	Electrofishing	No
	BOK6	25.79534	26.44961	Riffle	2012-03-21	Electrofishing	No
	BOK7	25.79542	26.44958	Pool	2012-03-21	Electrofishing	No
	BOK8	25.79569	26.44934	Chute	2012-03-21	Electrofishing	No
	BOK9	25.79663	26.44888	Pool	2012-03-21	Snorkelling	No
Bokkraal & Rietspruit confluence	BOKRIETSPRUIT1	25.78707	26.45001	Riffle	2012-03-23	Electrofishing	No
	BOKRIETSPRUIT2	25.78581	26.45013	Pool	2012-03-23	Snorkelling	No
Draaifonteinspruit	DRS1	25.7008	26.42272	Pool	2012-03-26	Snorkelling	No
	DRS10	25.74089	26.3481	Riffle	2012-03-24	Electrofishing	No
	DRS11	25.74097	26.34798	Pool	2012-03-24	Electrofishing	No
	DRS2	25.70033	26.42262	Pool	2012-03-26	Snorkelling	No
	DRS3	25.7002	26.42253	Pool	2012-03-26	Snorkelling	No

River	Site	GPS-S	GPS-E	Habitat	Sampling date	Sampling method	Invaded
	DRS4	25.70137	26.42319	Riffle	2012-03-24	Electrofishing	No
	DRS5	25.70059	26.42264	Riffle	2012-03-24	Electrofishing	No
	DRS6	25.69854	26.42924	Pool	2012-03-25	Electrofishing	Yes
	DRS7	25.6984	26.42935	Riffle	2012-03-25	Electrofishing	No
	DRS8	25.69825	26.42938	Pool	2012-03-25	Electrofishing	Yes
	DRS9	25.74079	26.34834	Pool	2012-03-25	Electrofishing	No
Kaaloog se Loop	KOL1	25.78352	26.37984	Pool	2012-03-22	Electrofishing	No
	KOL10	25.77734	26.43205	Riffle	2012-03-22	Electrofishing	No
	KOL11	25.77744	26.43204	Pool	2012-03-22	Seine	No
	KOL12	25.77571	26.43266	Pool	2012-03-22	Snorkelling	No
	KOL13	25.77569	26.43301	Chute	2012-03-22	Electrofishing	No
	KOL14	25.77592	26.43327	Pool	2012-03-22	Snorkelling	No
	KOL15	25.77645	26.43375	Pool	2012-03-22	Snorkelling	No
	KOL16	25.78928	26.36646	Pool	2012-03-20	Snorkelling	Yes
	KOL2	25.78358	26.37953	Riffle	2012-03-22	Electrofishing	No
	KOL3	25.78449	26.37571	Pool	2012-03-22	Electrofishing	No
	KOL4	25.78009	26.4317	Pool	2012-03-22	Snorkelling	No
	KOL5	25.78012	26.43176	Riffle	2012-03-22	Electrofishing	No
	KOL6	25.77976	26.43227	Pool	2012-03-22	Snorkelling	No
	KOL7	25.77968	26.43222	Pool	2012-03-22	Electrofishing	No
	KOL8	25.77962	26.43231	Chute	2012-03-22	Electrofishing	No
	KOL9	25.77807	26.43211	Pool	2012-03-22	Snorkelling	No
Groot Marico main-stem	MAR1	25.6445	26.42876	Pool	2012-03-24	Angling	Yes
	MAR10	25.64223	26.43154	Pool	2012-03-24	Electrofishing	Yes
	MAR11	25.64177	26.4318	Riffle	2012-03-24	Electrofishing	No
	MAR12	25.64165	26.43193	Run	2012-03-24	Electrofishing	Yes

River	Site	GPS-S	GPS-E	Habitat	Sampling date	Sampling method	Invaded
	MAR13	25.64559	26.42888	Pool	2012-03-24	Electrofishing	Yes
	MAR14	25.76597	26.43933	Pool	2012-03-29	Snorkelling	No
	MAR15	25.76637	26.44074	Pool	2012-03-29	Snorkelling	No
	MAR16	25.76105	26.44253	Pool	2012-03-29	Snorkelling	No
	MAR17	25.76877	26.44198	Pool	2012-03-29	Snorkelling	No
	MAR18	25.76877	26.44198	Pool	2012-03-29	Snorkelling	No
	MAR19	25.76004	26.44147	Pool	2012-03-29	Snorkelling	No
	MAR2	25.64551	26.42913	Pool	2012-03-24	Electrofishing	Yes
	MAR20	25.75914	26.44001	Riffle	2012-03-29	Snorkelling	No
	MAR21	25.6445	26.42876	Pool	2012-03-26	Observation	Yes
	MAR22	25.76697	26.44118	Pool	2012-03-29	Electrofishing	No
	MAR23	25.76747	26.4415	Pool	2012-03-29	Snorkelling	No
	MAR24	25.76004	26.44147	Pool	2012-03-29	Electrofishing	No
	MAR25	25.69443	26.43608	Riffle	2012-03-27	Electrofishing	Yes
	MAR26	25.74292	26.43325	Pool	2012-11-22	Snorkelling	No
	MAR27	25.74453	26.43335	Riffle	2012-11-22	Electrofishing	No
	MAR28	25.74464	26.43348	Riffle	2012-11-22	Electrofishing	No
	MAR29	25.74322	26.43327	Pool	2012-11-22	Snorkelling	No
	MAR30	25.75285	26.43407	Pool	2012-11-22	Snorkelling	No
	MAR3	25.64555	26.42891	Riffle	2012-03-24	Electrofishing	Yes
	MAR4	25.73518	26.4319	Pool	2012-03-27	Electrofishing	No
	MAR5	25.73518	26.4319	Pool	2012-03-27	Snorkelling	Yes
	MAR6	25.73499	26.43183	Riffle	2012-03-27	Electrofishing	No
	MAR7	25.73481	26.43185	Pool	2012-03-27	Electrofishing	Yes
	MAR8	25.64706	26.40857	Pool	2012-03-27	Electrofishing	Yes
	MAR9	25.76769	26.44179	Riffle	2012-03-29	Electrofishing	No
Polkadraaispruit	PKD1	25.6472	26.48914	Riffle	2012-03-26	Electrofishing	No

River	Site	GPS-S	GPS-E	Habitat	Sampling date	Sampling method	Invaded
	PKD2	25.64732	26.48903	Run	2012-03-26	Electrofishing	No
	PKD3	25.66714	26.61758	Pool	2012-03-26	Electrofishing	Yes
	PKD4	25.66729	26.61786	Riffle	2012-03-26	Electrofishing	No
	PKD5	25.66751	26.61788	Pool	2012-03-26	Electrofishing	No
	PKD6	25.66762	26.61807	Pool	2012-03-26	Electrofishing	No
Ribbokfontein	RBF1	25.79067	26.52009	Pool	2012-03-27	Electrofishing	No
	RBF2	25.7909	26.52016	Pool	2012-03-27	Electrofishing	Yes
Rietspruit	RTS1	25.79444	26.44356	Run	2012-03-23	Electrofishing	No
	RTS2	25.79406	26.44256	Chute	2012-03-23	Electrofishing	No
	RTS3	25.79387	26.44224	Riffle	2012-03-23	Electrofishing	No
	RTS4	25.79428	26.44362	Pool	2012-03-23	Snorkelling	No
	RTS5	25.79323	26.44126	Pool	2012-03-23	Snorkelling	No
	RTS6	25.79306	26.44001	Pool	2012-03-23	Snorkelling	No
	RTS7	25.79376	26.44505	Riffle	2012-03-23	Electrofishing	No
Uitvlugtspruit	UVS1	25.6668	26.53992	Riffle	2012-03-26	Electrofishing	No
	UVS2	25.66673	26.53988	Pool	2012-03-26	Electrofishing	Yes
	UVS3	25.66654	26.53986	Pool	2012-03-26	Electrofishing	No
	UVS4	25.66654	26.53986	Pool	2012-03-26	Snorkelling	No
Van Straatensvlei	VSV5	25.7396	26.53446	Pool	2012-03-26	Electrofishing	No
	VSV6	25.73963	26.5247	Pool	2012-03-26	Electrofishing	No
	VSV7	25.73964	26.52501	Riffle	2012-03-26	Electrofishing	No

Appendix 2: K.A. Van der Walt 2014 M.Sc. Thesis Abstract

The Groot Marico catchment in the North West Province is a Freshwater Ecosystem Priority Area (FEPA) because it represents unique landscape features with unique biodiversity that are considered to be of special ecological significance. Three native freshwater species *Amphilius uranoscopus*, *Chiloglanis pretoriae* and *Barbus motebensis*, have high local conservation importance and *B. motebensis* is endemic to the catchment and is IUCN-listed as vulnerable. The main objective of this study is to contribute towards the effective conservation of these three species in the Groot Marico River system by assessing their genetic structure to determine whether tributary populations of the three species comprise of one genetic population or whether they are divided up into genetically distinct subpopulations, in order to prioritise areas for conservation. The central null hypothesis was that there is no genetic differentiation between tributary populations (i.e. panmaxia) of *B. motebensis*, *A. uranoscopus* and *C. pretoriae* in the Groot Marico catchment, North West Province.

In total, 80 individuals per species were collected, targeting at least 10 individuals per population from a total of eight populations (seven tributaries and the Groot Marico main stem) and across the study area. Samples were collected by electrofishing and specimens were euthanized using an overdose of clove oil. A sample of muscle tissue was removed for genetic evaluation and the remainder of the specimens served as voucher specimens. For the genetic evaluation, mitochondrial (ND2, cyt *b*) and nuclear (S7) genes were used. Genetic techniques used were DNA extraction, polymerase chain reaction (PCR), purification and sequencing. From the 240 individuals collected, 123 sequences for *B. motebensis,* 111 sequences for *A. uranoscopus* and 103 sequences for *C. pretoriae* were analysed across all three genes. Statistical analysis included looking at cleaned sequences in order to obtain models using MODELTEST (version 3.06). Population structuring and phylogeographic analysis was performed in Arlequin (version 2000), TCS (version 1.2.1) and PAUP*.

Results indicated that for *B. motebensis* the null hypothesis could be rejected as there were two distinct lineages (the Draai and Eastern lineages) that demonstrated significant divergence in both the ND2 and S7 genes, suggesting historical isolation. The low divergence in the mitochondrial cytochrome *b* gene ($0\% < D < 0.8\%$) suggests that this isolation is not very old and is probably not comparable to species level differentiation. The null hypothesis was also rejected for *A. uranoscopus* as there were also significant levels of differentiation between tributary populations resulting in the identification of two lineages (the

Ribbok and Western lineages). However, for *C. pretoriae,* the null hypothesis could not be rejected as there was no genetic differentiation between tributary populations and consisted of one panmictic population.

Therefore, due to each species showing different genetic structuring within the tributary populations, more than one priority area for conservation needs to be implemented. These priority areas of conservation where therefore evaluated based on the current conservation status of the species (*B. motebensis* being vulnerable on the IUCN Red List), the number of Evolutionary Significant Units for each species and the overall genetic diversity of all three species in the Groot Marico catchment. In total, four tributary populations were conservation priority areas, these were the Draaifontein, Van Straatensvlei, Ribbokfontein and Kaaloog se Loop tributaries.

The Draai, Vanstraatens and Kaaloog tributaries were selected as priority areas for *B. motebensis* (*B. motebensis* is considered to be the most vulnerable of all three species). The Draai tributary was selected due to the *B. motebensis* population within the tributary showing isolation from the rest of the tributary populations and in order to conserve *B. motebensis* from the Southern lineage, the Vanstraatens and Kaaloog tributaries were selected. Reasons for selecting these two specific tributaries within the Southern lineage were that the Vanstraatens tributary had unique alleles (three Evolutionary Significant Units) for *B. motebensis* and the Kaaloog tributary had high genetic diversity (*HD* = 0.889, ND2 gene) when compared to the other tributary populations.

The Ribbok and Vanstraatens tributaries were selected as priority areas for the conservation of *A. uranoscopus*. The Ribbok tributary was selected as it showed isolation from the rest of the tributary populations, as seen with the Draai tributary (*B. motebensis*) and the Vanstraatens tributary was selected to represent the Western lineage as it had the highest diversity for both genes (ND2 and S7). The Ribbok tributary has the highest prioritisation when compared to the Vanstraatens tributary.

Chiloglanis pretoriae occurs within the Draai, Vanstraatens, Ribbok and Kaaloog tributaries, therefore by prioritising these tributaries for conservation, *C. pretoriae* will in turn be conserved.